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## The phylogeny of coleoid cephalopods inferred from molecular evolutionary analyses of the cytochrome c oxidase I, muscle actin, and cytoplasmic actin genes

David Bruno Carlini

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THE PHYLOGENY OF COLEOID CEPHALOPODS INFERRED FROM MOLECULAR  
EVOLUTIONARY ANALYSES OF THE CYTOCHROME C OXIDASE I, MUSCLE  
ACTIN, AND CYTOPLASMIC ACTIN GENES

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A Dissertation

Presented to

The Faculty of the School of Marine Science

The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of

Doctor of Philosophy

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by

David Bruno Carlini

1998

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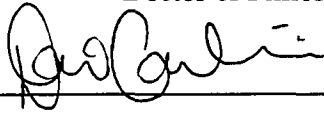
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## APPROVAL SHEET

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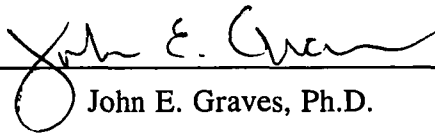
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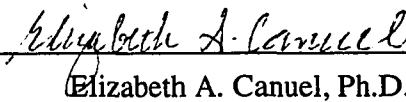
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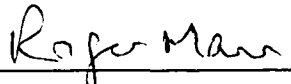
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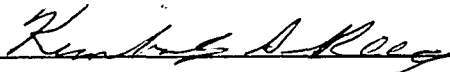
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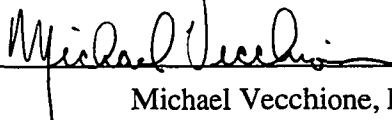
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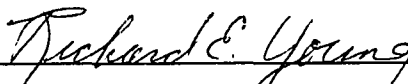
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## ABSTRACT

Although the fossil record of early cephalopods is rich and demonstrates the dominance of the group in Paleozoic times, the mainly soft-bodied coleoids (Cephalopoda: Coleoidea) are poorly represented. Therefore, little is known of the evolutionary history of coleoids through paleontology and current classifications of the subclass are based primarily on the morphology of extant representatives. There is substantial disagreement among the various higher-level classifications of the Coleoidea. This incongruence can be attributed to the difficulty in obtaining comparative material for morphological studies, the paucity of information regarding ancestral character states, and the lack of objective criteria used in constructing phylogenetic relationships in many of the previous studies. A molecular phylogenetic analysis of the Coleoidea was therefore warranted.

Phylogenetic relationships within the Coleoidea were constructed using molecular sequence data from one mitochondrial and two nuclear genes: cytochrome *c* oxidase I (COI) and two unlinked actin genes (Actin I and Actin II, respectively). A 657 base-pair portion of the COI gene was examined for 55 coleoid taxa encompassing a broad spectrum of diversity in the subclass. The COI gene exhibited the most rapid evolutionary rate among the three genes examined, yet the gene was informative for determining deep as well as shallow-level relationships within the Coleoidea. Eighty-two sequences from a 784 base-pair portion of three paralogous actin genes were obtained from 44 terminal taxa. The Actin I gene (38 taxa) was highly conserved and provided information for determining deep-level relationships. The Actin II gene (32 taxa) was intermediately conserved, exhibited a broad range of sequence divergence, and was informative for inferring deep and shallow-level relationships. The evolution of the actin gene family in cephalopods was compared to that in other molluscs, protostomes, and deuterostomes. Analyses of actin gene family evolution provided evidence that the Actin I gene encodes a muscle-type of actin, and that the Actin II gene encodes a cytoplasmic actin. These analyses also supported at least two independent derivations of muscle-type actins during the evolution of the protostome lineage. Analyses of the COI, Actin I, and Actin II genes did not provide phylogenetic stability in the inference of intermediate-level relationships, particularly those among many families in the diverse suborder Oegopsida. The codon usage patterns of selected taxa, expressed as the frequency of occurrence of each codon per amino acid, were determined and compared within and among the three genes.

With regard to higher-level phylogenetic relationships, the following conclusions were drawn from the results of phylogenetic analyses: 1) the cephalopod subclass Coleoidea is monophyletic; 2) the order Octopoda is monophyletic and is sister group to the monotypic order Vampyromorpha; 3) the Decapodiformes, consisting of the orders Teuthoidea and Sepioidea, is monophyletic; 4) the orders Teuthoidea and Sepioidea are polyphyletic; 5) the teuthoid suborders Myopsida and Oegopsida are monophyletic and polyphyletic, respectively; 6) the Myopsida and the oegopsid families Ctenopterygidae and Bathyteuthidae are more closely related to the sepioid families Spirulidae, Sepiidae, and Sepiolidae, than they are to other teuthoid groups.

THE PHYLOGENY OF COLEOID CEPHALOPODS INFERRED FROM MOLECULAR  
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ACTIN, AND CYTOPLASMIC ACTIN GENES

## GENERAL INTRODUCTION

The Cephalopoda are the most complex class of molluscs. In light of their special adaptations related to bioluminescence, buoyancy, crypsis, feeding, intelligence, speed, and vision, they are generally considered to be among the most highly evolved marine invertebrates. There are more than 700 extant species of cephalopods, divided into two subclasses, five orders, 47 families, and 139 genera (Sweeney and Roper, 1998). Cephalopods are important components of oceanic communities and are the target of many international commercial fisheries, yet knowledge of their evolution is embarrassingly scant. They represent the only invertebrate taxon to occupy the nektonic habitat, directly competing with many vertebrate groups at high trophic levels (Packard, 1972). An increased resolution of cephalopod relationships is clearly needed and would bring stability and clarification to their classification. This knowledge would facilitate further research in diverse topics such as physiology, ecology, fisheries management, and evolutionary relationships below the familial level.

### Morphological Systematics of the Coleoidea

The cephalopods diverged from a monoplacophoran ancestor in the late Cambrian period (Salvini-Plawen, 1980). With the exception of the Nautiloidea, all extant cephalopods are members of the subclass Coleoidea, which are distinct from the Nautiloidea and other subclasses (†Orthoceratoidea, †Actinoceratoidea, †Endoceratoidea, and †Ammonoidea) in several ways, most notably the reduction and internalization or complete loss of shell (Teichert, 1988). The Nautiloidea is represented today by a single genus, *Nautilus*, which consists of at least 6 species

†Belemnitida, †Phragmoteuthida, †Belemnoteuthida, Sepioidea, Teuthoidea, Octopoda, and Vampyromorpha (Jeletzky, 1966; Teichert, 1988). Although the fossil record of early cephalopods is rich and demonstrates the success of the group in Paleozoic times, the mainly soft-bodied coleoid cephalopods are poorly represented. Therefore, little is known of the evolutionary history of coleoids through paleontology and current classifications of the group are based primarily on the morphology of living representatives. Three of the most prominent classifications of the extant coleoid cephalopods are presented in Figure 1. Unless otherwise noted, this dissertation will follow the classification scheme of Voss (1977), because it remains the most commonly used classification scheme of coleoid cephalopods used by biologists. For reference, some general anatomical features of coleoid cephalopods are illustrated in Figure 2.

Of the four orders of extant coleoid cephalopods, the ordinal status of Sepioidea is perhaps the most controversial. The order consists of five groups which have been assigned various taxonomic ranks (referred to hereafter as families). These families are united by similarities in fin morphology and position, possession of retractile tentacular stalks, the simple form of mantle and funnel cartilages (Sepiadariidae excepted), presence of a branchial canal, and benthic habitat preference. The Spirulidae, which are represented by a single mesopelagic species (*Spirula spirula*) lacking a radula and possessing a coiled internal shell, is clearly distinct from the other sepioids. Several characters do not support the placement of the Idiosepiidae within the Sepioidea. Unlike other members of the order, tentacular development in idiosepiids is delayed (Natsukari, 1970) and they lack accessory nidamental glands. Furthermore, the Idiosepiidae possess a thin gladius (Hylleberg and Nateewathana, 1991). Differences between the other 3 families (Sepiidae, Sepiolidae, and Sepiadariidae) are considered important enough by some researchers to raise their taxonomic ranks above the familial level (Fioroni, 1981; Clarke, 1988a; Khromov, 1990; Boletzsky, 1995). Establishing the relationships among families of the Sepioidea, and the

demonstration or refutation of sepioid monophyly remains one of the most significant problems in coleoid phylogeny (Donovan, 1977).

The Order Teuthoidea comprises two suborders, the Myopsida and Oegopsida. The myopsids inhabit neritic waters and are the most well known squids. They are represented by one family, the Loliginidae, which includes the commercially important and speciose genus *Loligo* and seven other genera. Myopsid squids are distinguished from oegopsid squids primarily by the presence of a corneal membrane covering the distal eye chamber. The myopsids share this character with the Sepioidea, along with the presence of accessory nidamental glands, unpaired oviducts, and tentacle pockets. Myopsid affinities with sepiids (the true cuttlefish), in particular, have been suggested by several researchers (Young, 1977; Berthold and Engeser, 1987) although the possible convergent evolution of the corneal membrane has been suggested (Naef, 1923; Clarke, 1988a).

The oegopsid squids have the highest familial-level diversity of all coleoid groups, the possible result of a Tertiary radiation (Donovan and Toll, 1988). Of the 25 families now recognized, 15 are monogeneric, and 7 of those are monotypic. Phylogenetic relationships of the various oegopsid families remain largely unknown due the difficulty in obtaining sufficient comparative material and lack of cladistic analyses. Few attempts have been made to elucidate relationships within the Oegopsida, none of which used a rigorous, repeatable method of phylogenetic reconstruction (Toll, 1982; Hess, 1987; Clarke, 1988a). Toll (1982) constructed a phylogeny based on overall similarities among the families with respect to a single suite of characters, gladius morphology, where the potential for convergence is high given the design constraints required by the pelagic habitat. The study by Hess (1987) is similarly descriptive. He examined an array of characters related to a single structure, the spermatophore, and his hypothesis of oegopsid relationships is based on subjective interpretations of overall similarity in spermatophore morphology. Clarke (1988a) did not use cladistic methods to arrive at his hypothesis of relations between the various oegopsid families. His "cladogram", based mainly on analysis of statolith, beak,

hook, and gladius characteristics, did not include a map of the character state transformations occurring along each branch, and is in little agreement with Toll (1982). Descriptive accounts of the various oegopsid families, which document the wide geographical distribution and numerical abundance of ommastrephids (Clarke, 1966; Roper et al., 1984), are in accordance with Toll (1982), Donovan (1977), and Donovan and Toll (1988). These studies suggest that the Ommastrephidae are the likely root stock of modern oegopsid squids based on the unique gladius morphology of ommastrephids. However, if the similarities in the gladius of ommastrephids and fossil teuthoids represent a derived rather than ancestral condition, the ommastrephids may not represent basal oegopsids (Young et al., 1998). The time of origin of the teuthoid squids remains unknown, but most estimates date their emergence to the Triassic or Lower Jurassic (Donovan, 1977).

As the name indicates, the Decapodiformes, comprising the orders Sepioidea and Teuthoidea, are united by their possession of ten arms. However, since the ten-armed state is plesiomorphic in coleoids, it should not be used to designate a monophyletic subgroup (Berthold and Engeser, 1987). Because the sepioids and teuthoids share other traits, some of which may be apomorphic such as suckers with constricted stalks and horny rings, hectocotylyzation of the ventral arm pair, and fusion of the kidneys, the Decapodiformes are generally considered to be monophyletic. Although Young and Vecchione (1996) described several potentially synapomorphic characters for the group, they could only find a single polarizable character to unite the decapods, the modification of the fourth arm pair into tentacles.

The order Octopoda consists of two suborders: Cirrata and Incirrata. The three families of cirrate octopods are also known as the finned octopods and possess cirri on the arms. Most cirrate octopods live just on or just above the ocean floor in the deep sea and have many primitive characters (Voss, 1988). Incirrate octopods are much more diverse than their cirrate counterparts and are represented by 8 families, 33-35 genera, and 165-180 species, with about half those species being members of the familiar genus *Octopus* (Nesis,

1987). Of the 8 incirrate families only one, the Octopodidae, are benthic. Synapomorphies which define the incirrate octopods include loss of internal shell, loss of cirri, loss of funnel valve, and lack of protective egg membranes.

*Vampyroteuthis infernalis*, an inhabitant of the bathypelagic realm, is the sole living representative of the order Vampyromorpha (=vampire squids). The phylogenetic position of the Vampyromorpha is problematic as *Vampyroteuthis* is in many ways intermediate between the decapods and octopods. Whether the Vampyromorpha should be aligned more closely to the octopods (Young, 1977; Engeser, 1990), decapods (Fioroni, 1981), or distinct from both groups (Clarke, 1988a) has been a matter of debate until recently (Young and Vecchione, 1996), where the monophyly of the Octopoda plus Vampyromorpha clade (= Octopodiformes) was well supported.

The evolution of modern forms of coleoid cephalopods may be due to their freedom from the nearshore benthic habitat and concomitant reduction in competition and predation from teleost fishes (Packard, 1972; O'Dor and Webber, 1986; Aronson, 1991). The importance of teleost fishes in driving the evolution of coleoid traits such as the loss of chambered shell, rapid growth, semelparity, development of a fusiform body and a lens eye is one of the most widely accepted theories in the cephalopod literature. Given the purportedly great selective pressure driving the evolution of these features which allowed for the invasion of pelagic habitats, the potential for convergent evolution in the Coleoidea is considerable. As taxa that subsequently reinvaded the benthos are derived from pelagic ancestors, convergence is not restricted to pelagic forms alone (Clarke, 1988b; Young et al., 1998). In light of this convergence, it is not surprising that there is substantial disagreement in the various higher-level classifications of the Coleoidea (Voss, 1977; Berthold and Engeser, 1987; Clarke, 1988a; Khromov, 1990; Doyle et al., 1994; Young and Vecchione, 1996). The lack of congruence in higher-level classifications can also be attributed to the difficulty of obtaining comparative material, the paucity of information

regarding ancestral characters, and the general lack of objective criteria used in constructing phylogenetic relationships.

With the exception of Young and Vecchione (1996), serious methodological problems underlie the conclusions of these higher-level morphological studies. Even if an objective criterion such as parsimony is specified, there is no description of how the parsimony algorithm was implemented, how many trees were obtained, whether the tree represents a consensus of multiple trees, etc. Characters that support the phylogenetic hypothesis are described and mapped onto a tree, and a discussion or indication of homoplastic change is conspicuously neglected. To date, most morphological phylogenetic studies that have employed an explicit method of constructing relationships of coleoids have focused on lower-level relationships, such as those among genera within a family (Voss and Voss, 1983; Roeleveld, 1988; Voight, 1993; Anderson, 1996). Only recently have morphological studies attempted to determine relationships among different families using cladistic methodologies (Voight, 1997; Young and Harman, 1998).

#### Molecular Systematics of the Coleoidea

The few phylogenetic studies of coleoid cephalopods using molecular sequence data published to date have defined the reconstruction method employed and have focused on higher-level relationships within the group (Bonnaud et al., 1994, 1997). The definition of an objective function using molecular data is a necessary consequence of several factors. These include the much larger number of characters used in molecular analyses and in the unclear relationships among the 4 possible character states (nucleotides).

The change in systematic focus from descriptive accounts of species, genera, or families toward an increased interest in understanding relationships among families, suborders, and orders, may reflect a growing trend in cephalopod systematics. Undoubtedly, this change in phylogenetic focus is also related to the nature of the data, where an understanding of the relationships between alternate character states is perceived to be unnecessary. The only



analytical requirement for molecular studies is that all the terminal taxa included in the study possess the gene of interest (i.e., that the molecular characters used in the analysis are homologous). To the extent that this is the only requirement necessary to obtain meaningful results from molecular studies, it is an easy condition to satisfy.

For a rigorous phylogenetic analysis of morphological characters, however, the requirements are much stiffer: characters must be polarizable, and an understanding of the relationships between states of multistate characters is highly valuable and sometimes compulsory. For example, Young and Vecchione (1996) eliminated half of the morphological characters they surveyed due to lack of sufficient knowledge about character evolution. Therefore, when undertaking an objective phylogenetic study that seeks to determine relationships among many morphologically disparate families, the perceived requirements of molecular character data are much easier to satisfy. However, an increased awareness of the problems associated with analyzing molecular data without regard to the patterns and processes of molecular evolution is a currently emerging paradigm in the molecular phylogenetic literature (Huelsenbeck and Rannala, 1997). Current molecular phylogenetic studies involve much more attention to the underlying assumptions of analytical methods and the potential for error in phylogeny estimation than at any time in the past.

The few molecular phylogenetic studies that have been conducted on cephalopods to date have focused on mitochondrial genes. Bonnaud et al. (1994) examined decapod relationships using a ~500 bp portion of the 16S rRNA gene. The outgroup taxon in the study was *Octopus* and the ingroup included representatives from 7 oegopsid families (13 taxa), myopsid squids (3 taxa), and 3 sepioid families (11 taxa). The results of Bonnaud et al. (1994) did not support the monophyly of the Sepioidea. The Sepiolidae grouped outside of the remaining decapods, supporting the recommendation that their taxonomic status be raised to ordinal rank (Fioroni, 1981; Clarke, 1988). *Spirula* did not cluster with any of the sepioids included in their study, instead it nested within oegopsid clades. The 5

sepiid taxa represented formed a monophyletic group in the neighbor-joining distance tree but not in the parsimony analysis. To explain the failure of the 16S data to unequivocally support the monophyly of the Sepiidae, a morphologically well-defined family, Bonnaud et al. (1994) proposed two hypotheses: 1) either the evolutionary rate of the 16S rDNA is more rapid in the Sepiidae than in other coleoid groups or, 2) a Mesozoic emergence of the sepiids rather than a Cenozoic emergence as was suggested previously by paleontological evidence (Teichert, 1988). Bonnaud et al. (1994) considered the second hypothesis more likely. Their study also did not support the monophyly of the Teuthoidea, Myopsida or Oegopsida. The conclusions of Bonnaud et al. must be tempered with a consideration of the taxonomic sampling. Pertinent to the conclusions about sepioid relationships, no representatives of two of the 5 families of the sepioids (Idiosepiidae, Sepiadariidae) were included. This is important because Khromov (1990) has suggested a close relationship between the sepiadariids and sepiolids. He considered the Sepiolidae, Sepiadariidae, and Idiosepiidae to be more closely related to each other than to the Sepiidae and Spirulidae. Naef (1923) also proposed a close relationship between the sepiadariids and the sepiolids. Relationships among the few oegopsid families included in the study were highly unstable across the 2 methods of analysis employed. Bootstrap analysis of the neighbor-joining distance tree did not support any of the oegopsid interfamily relationships. The main conclusion Bonnaud et al. drew from the 16S study was that a gene with a slower evolutionary rate was necessary to investigate the higher level phylogeny of the decapods.

The second molecular study conducted by the same group used a 500 bp fragment of the mitochondrial cytochrome *c* oxidase subunit III gene (COIII) to examine coleoid relationships (Bonnaud et al., 1997). Similar to the 16S study, taxonomic sampling was proportionately greater for the Sepioidea. The taxa represented in the COIII study included 2 octopods, *Vampyroteuthis*, 2 oegopsid families (3 taxa), myopsid squids (3 taxa), and 4 sepioid families (7 taxa). The monophyly of the Decapodiformes was well supported in all analyses. The position of *Vampyroteuthis* was equivocal, only one of the neighbor-joining

distance trees presented supported placement of *Vampyroteuthis* with the Octopoda. The additional sepioid family included in the COIII study was the Idiosepiidae, which consistently placed with one of the two oegopsid families. However, the sepiadariids were not included in the analysis, so their conclusions concerning the placement of *Idiosepius* within the Oegopsida must be regarded with this in mind. Quite different from the results of the 16S study, the Sepiolidae did not emerge basal to the remaining decapods. The difference in the placement of the sepiolids between the two studies may be due to the differences in the substitution patterns of the two genes, although a more likely explanation for the difference is the inclusion of additional non-decapod taxa in the COIII study. The position of *Spirula* was highly unstable across the four trees presented. The monophyly of the myopsid squids was strongly supported, although their position within the decapods was also unstable. The COIII study also concluded that a more conserved gene was necessary to allow a more accurate assessment of deeper-level decapod relationships.

The results of Bonnaud et al. (1994, 1997), though enlightening in some respects, leave room for additional molecular studies of the Coleoidea. Taxonomic sampling of the Oegopsida and Octopoda was inadequate for determining relationships within those groups. To perform a rigorous test of the monophyly of the Sepioidea, representatives of all five constituent families should be considered. The main method of phylogenetic analysis employed in both studies, neighbor-joining of uncorrected distances, though repeatable and explicitly defined, has been shown to be quite inconsistent when compared to other available methods (Huelsenbeck, 1995). Assumptions about the nature of the data were made but never tested. For example, the 16S and COIII data were assumed to be saturated due to some anomalous relationships obtained in the analyses of the two genes, but evidence for saturation (i.e., plots of pairwise divergences demonstrating a decrease in the proportion of transition substitutions with increasing sequence divergence) was not presented in either study. Clade support was tested by neighbor-joining bootstrap analysis, a method which artificially inflates bootstrap proportions and has been considered an

inappropriate use of the nonparametric bootstrap (Swofford et al., 1996). Perhaps most importantly, neither study used a nuclear gene to examine phylogenetic relationships among coleoid cephalopods. As nuclear and mitochondrial genes possess unique evolutionary histories, the conclusions drawn from phylogenetic analysis of recombining, biparentally-inherited nuclear genes are likely to differ from the conclusions based on phylogenetic analysis of non-recombining, maternally-inherited mitochondrial genes.

### Phylogenetic Analysis

In this dissertation, a rigorous phylogenetic analysis of coleoid cephalopods will be conducted. A variety of currently accepted phylogenetic reconstruction algorithms will be employed. These include unweighted parsimony, weighted parsimony, and maximum likelihood analyses. While a detailed discussion of the many methods phylogenetic reconstruction is beyond the scope of this general introduction (see Swofford et al., 1996), a brief discussion of the methods used in this study is warranted.

The parsimony method is the most commonly used approach to reconstruct phylogeny. The parsimony method selects a tree that minimizes the number of evolutionary changes (i.e., steps) required by the characters to produce the tree. At a given tree length, the most parsimonious tree includes the greatest number of homologous character changes and fewest number of homoplastic character changes. There are three types of homologies: shared general homologies (symplesiomorphies), shared derived homologies (synapomorphies), and unique homologies (autapomorphies). In parsimony, only synapomorphies provide evidence for common ancestry. Symplesiomorphies, autapomorphies, and homoplasies (false homologies due to convergences or parallelisms) do not provide evidence for common ancestry and are therefore not informative in constructing phylogenetic relationships. The homology status of each character is determined through comparisons with the character states possessed by one or more organisms outside the group of interest (outgroups). Unweighted parsimony analysis

assigns an equal cost to changes in all characters. Weighted parsimony analysis generally assigns a greater cost to more drastic changes in character states and also assigns a greater cost to changes in less variable characters. For molecular sequence data, an example of a more drastic character change is a transversion, involving a substitution of a purine base for a pyrimidine base or vice-versa. In weighted parsimony, transversional changes may be assigned a greater cost (e.g., more than one step for a single change) than transitional changes, those involving substitutions between purines or between pyrimidines.

To date, the maximum likelihood method of phylogenetic reconstruction has been used less frequently than parsimony methods. Maximum likelihood is a probabilistic approach to phylogeny and is mainly restricted to the analysis of molecular data sets because it requires a probabilistic model of character change. For molecular sequence data, the probabilistic models commonly involve the estimation of three components of DNA substitution: 1) base frequencies, 2) substitution rates of different substitution classes (e.g., transition and transversion substitution rates), and 3) site-specific substitution rates which account for among-site rate heterogeneity. Given a probabilistic model of DNA substitution, the probability of change from any given nucleotide to another can be calculated. For each character on a given tree, the likelihoods for all possible character states at all nodes on the tree are calculated and summed. The full likelihood of the tree is calculated as the products of the likelihoods of each character. The maximum likelihood tree is that tree which has the greatest probability of occurring under the assumed model of DNA substitution.

In the unweighted parsimony analyses, clade support will be assessed using the nonparametric bootstrap (Felsenstein, 1985) and the Bremer support index (Bremer, 1988). Weighted parsimony analyses will assess clade support through the nonparametric bootstrap technique alone. The nonparametric bootstrap is a statistical method based on repeated random sampling with replacement from the original data set to provide a new set of pseudoreplicate data matrices. Parsimony searches are conducted on each of the

pseudoreplicate data matrices to provide an estimate of the precision of phylogenetic estimation for a particular data set. Bootstrap values represent the proportion of pseudoreplicate data sets that support the clades defined by the most parsimonious tree. The Bremer support index indicates the number of extra steps required to collapse a branch in consensus. Bremer support values are obtained by successively evaluating subparsimonious trees and determining which clades remain supported in consensus. The monophyly of the Sepioidea will be tested using a powerful new technique, the parametric bootstrap, the details of which are discussed in Chapter 1 (Huelsenbeck and Hillis, 1996; Huelsenbeck et al., 1996).

A more thorough investigation of phylogenetic relationships, using mitochondrial and nuclear gene sequences from a wider array of coleoid families, and employing a variety of current analytical methods of phylogenetic inference, is necessary to increase our understanding of relationships among coleoid cephalopods. The poor fossil history of the group, paucity of clearly polarizable morphological characters, and lack of sufficient comparative study material required for morphological studies renders a molecular approach to their phylogeny attractive. Reconstructing phylogenetic relationships from a single suite of characters (molecules included) is an inherently flawed approach. As a leading cephalopod systematist recently put it, "I think it is hardly possible to construct a non-contradictory (taxonomic) system of cephalopods based on any single system of characters." (Nesis, 1995). Phylogenetic analyses will be conducted on three independent molecular data sets derived from the mitochondrial and nuclear genomes. Finally, analyses of the combined data sets will be conducted and compared to the results obtained in analyses of the individual data sets.

#### Phylogenetic Hypotheses Tested

The end result of a phylogenetic analysis of a single data set is a hypothesis of phylogenetic relationships, not a proof of phylogenetic relationships. In this context, an *a*

*priori* statement of hypotheses to be tested in this study is somewhat untenable. However, the results from analyses of multiple independent data sets, using different methods of phylogenetic reconstruction, and the results from analyses of combined data sets provides a means by which phylogenetic hypotheses can be tested. In this dissertation, the following four phylogenetic hypotheses will be tested by comparing the trees derived from analysis of different data sets and through different methods of phylogenetic reconstruction: 1) the Octopoda and Vampyromorpha are sister groups (i.e., monophyly of the Octopodiformes); 2) the two octopod suborders, Incirrata and Cirrata, are monophyletic groups; 3) the Decapodiformes (Sepioidea + Teuthoidea) is a monophyletic group; 4) the Sepioidea, as defined by Voss (1977), is a monophyletic group.

Figure 1. Three prominent classifications of the coleoid cephalopods. For heuristic purposes, the taxonomic scheme of Voss (1977) will be used throughout this dissertation. Note the differences among the three classifications with respect to the placement of the five sepioid "families", the placement of the Myopsida, and the placement of the Vampyromorpha.



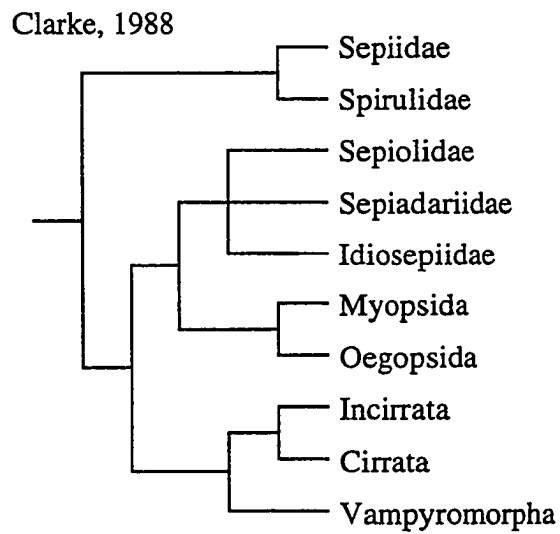
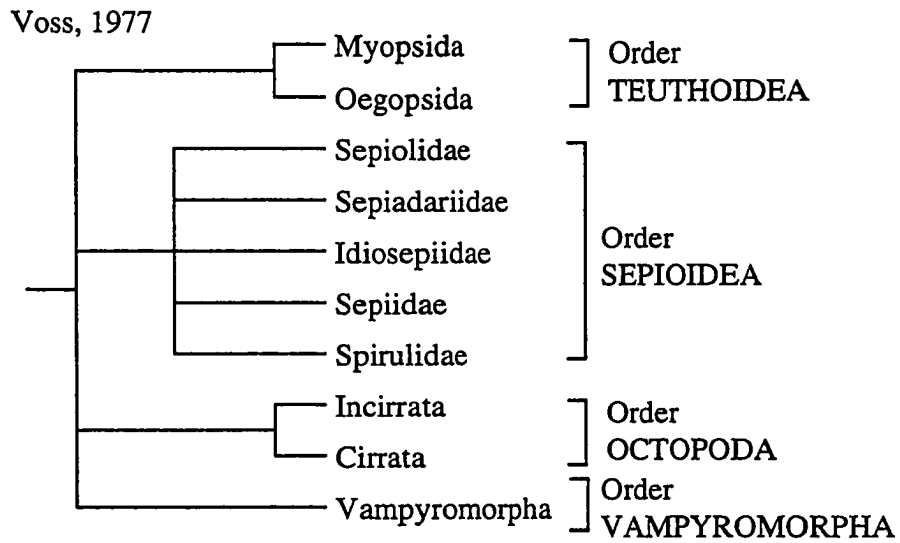
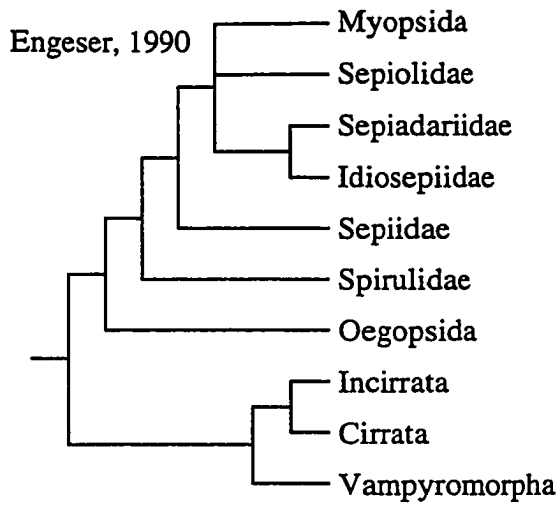
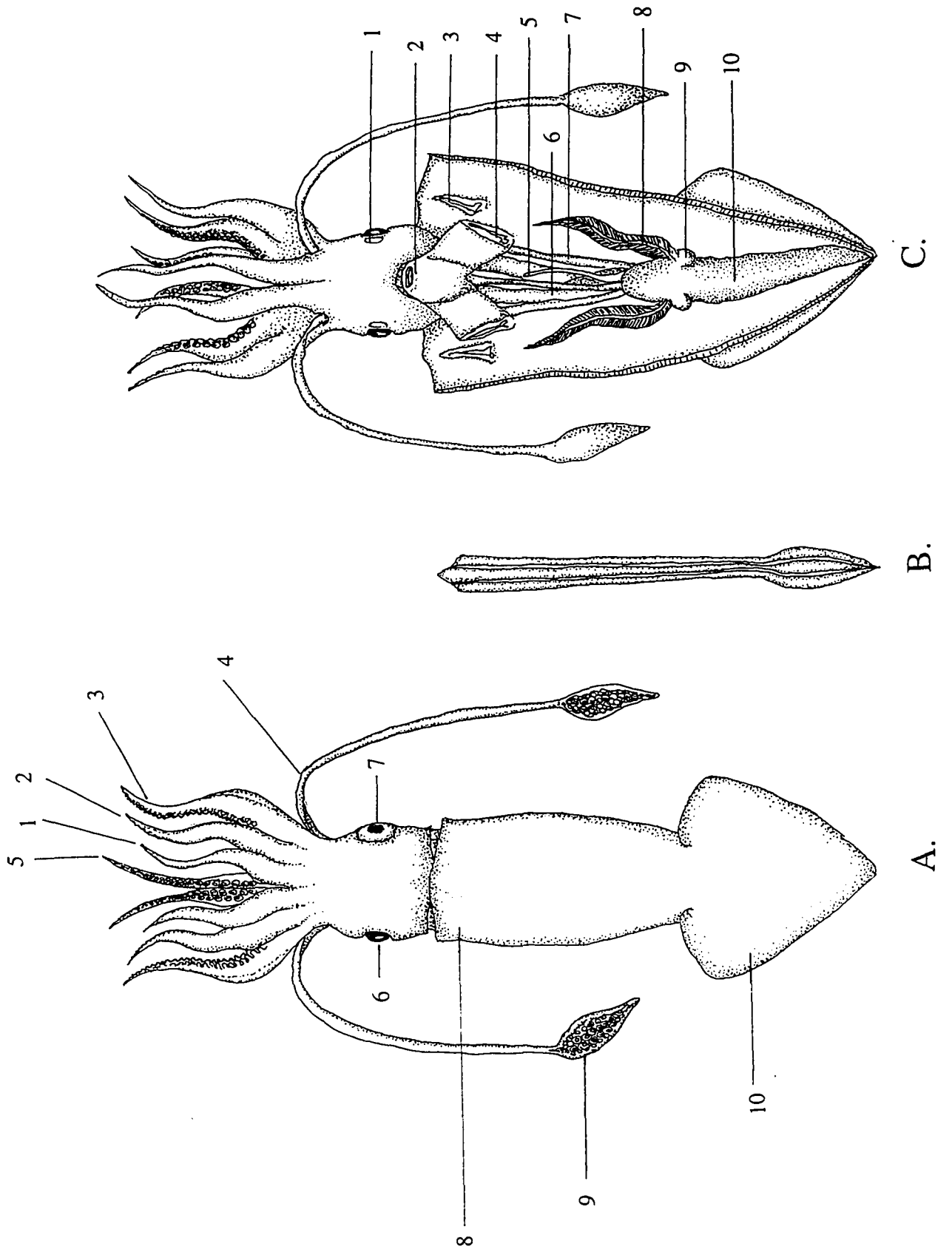


Figure 2. An illustration of some external and internal anatomical features of a generalized squid. **A.** Dorsal view depicting external anatomy: 1-First arm (dorsal arm); 2-Second arm (dorsolateral arm); 3-Third arm (ventrolateral arm); 4-Tentacular stalk of fourth arm (tentacle); 5-Fifth arm (ventral arm); 6-Oegopsid-type eye; 7-Myopsid-type eye with corneal membrane; 8-Mantle; 9-Tentacular club; 10-Fin. **B.** Gladius (pen) removed from the animal. **C.** Ventral view of a dissected squid depicting some simplified internal anatomical features: 1-Ventral (subocular) photophore; 2-Funnel; 3-Mantle component of locking cartilage; 4-Funnel component of locking cartilage; 5-Anus; 6-Cephalic retractor muscle; 7-Funnel retractor muscle; 8-Gill; 9-Branchial heart; 10-Visceral mass.



CHAPTER 1. PHYLOGENETIC RELATIONSHIPS OF COLEOID CEPHALOPODS  
INFERRED FROM THE MOLECULAR EVOLUTION OF THE MITOCHONDRIAL  
CYTOCHROME C OXIDASE I GENE

## INTRODUCTION

In this chapter the results of a phylogenetic analysis of the coleoid cephalopods based on molecular sequence data from the mitochondrial cytochrome *c* oxidase subunit I (COI) gene will be reported. The COI gene codes for the first subunit of the cytochrome *c* oxidase protein complex, which is composed of a total of 13 subunits in mammals (Kadenbach et al., 1983). The three heaviest subunits (I-III) are encoded by mitochondrial DNA; a variety of smaller subunits are encoded in the nucleus. The cytochrome *c* oxidase protein complex is located on the inner mitochondrial membrane of eukaryotic cells and the catalytic function of the enzyme is to couple the oxidation of reduced cytochrome *c*, a single electron donor, with the reduction of molecular oxygen to water, a four-electron reaction. Through catalysis of this reaction, cytochrome *c* oxidase establishes a proton gradient across the inner mitochondrial membrane (i.e., a "proton pump"), leading to a difference in the electrochemical potential of protons across the two sides of the membrane. It is this potential which is used to drive the synthesis of adenosine 5'-triphosphate (ATP) from adenosine 5'-diphosphate (ADP) and inorganic phosphate ( $P_i$ ) (Babcock and Wikström, 1992). The reaction catalyzed by cytochrome *c* oxidase plays a fundamental role in aerobic life and is responsible for the reduction of approximately 90% of all respired oxygen to water (Blenkinsop et al., 1996). The first subunit of the protein complex contains two heme groups and a copper center, functioning as the catalytic core of the enzyme complex. Hence, the protein structure of subunit I is the most conserved of all cytochrome oxidase subunits (Iwata et al., 1995; Collman et al., 1997). It follows that the COI gene is among the most conserved protein-coding genes in the mitochondrial genome of metazoans (Brown, 1985).

Though the amino acid sequence of the COI protein is highly conserved, silent changes in the nucleotides (i.e. mainly third position substitutions) are just as common as are found in other mitochondrial genes with lower levels of amino acid conservation. COI nucleotide and amino acid sequences have been used in several studies focused on resolving relationships between taxa that have diverged over 100 m.y.a. (Folmer et al., 1994; Cummings et al., 1995; Zardoya and Meyer, 1996). At the same time, third codon position nucleotides of the COI gene are highly variable and have proved informative in resolving taxa that have diverged more recently, such as among congeners (Van Syoc, 1994; Spicer, 1995; Palumbi, 1996).

A number of studies have compared the phylogenetic performance of the different mitochondrial genes in recovering "known" phylogenies, for example the phylogeny of the vertebrate classes (Cao et al., 1994; Cummings et al., 1995; Russo et al., 1996; Zardoya and Meyer, 1996). In general, the performance of the COI gene was better than most other mitochondrial genes in recovering the expected phylogeny or a close variation thereof (Cao et al., 1994; Russo et al., 1996; Zardoya and Meyer, 1996). Conveniently, these studies also employed a variety of analytical methods (parsimony, maximum likelihood, and neighbor-joining) to determine which reconstruction method obtained the most accurate results (i.e., recovered the "true" phylogeny). Phylogenetic analysis of the complete mitochondrial genome recovered the "true" phylogeny, that phylogeny expected based on morphological, paleontological, and other molecular evidence, no matter which reconstruction methodology was used. In cases where the COI gene did not consistently recover the expected phylogeny across different reconstruction methodologies, maximum likelihood analysis of the COI gene yielded the expected phylogeny (Cummings et al., 1995).

The selection of an appropriate gene, the first step taken when conducting a phylogenetic research project, is perhaps the most critical phase in the design and outcome of a particular phylogenetic study (Graybeal, 1994). Obviously, how well a gene will

perform for a given phylogenetic problem cannot be evaluated *a priori*. The gene selection process is difficult for cephalopods because they and their relatives (e.g. gastropods) are poorly represented in the Genbank and EMBL sequence databases. Thus, although the COI gene appeared to be the best candidate in the mitochondrial genome for phylogenetic analysis of the Coleoidea, many of the criteria for which the gene was selected were based on patterns observed in analyses of vertebrate evolution. It is well known that the patterns of molecular evolution are often taxon specific (e.g., evolutionary rate, base composition, substitution bias), therefore it is not entirely appropriate to assume that the patterns observed in vertebrate taxa would also apply to cephalopods.

From the results of the studies cited above it appeared that the COI gene was the best mitochondrial gene candidate for examining coleoid relationships. The results of Bonnaud et al. (1994) suggested that a more conserved gene than the mitochondrially encoded 16S gene was necessary for establishing higher-level relationships within the Coleoidea. Other genes that performed well in comparative studies (ND4, ND5, Cytochrome *b*) were shown to be less conserved than the COI and 16S genes. An alternative would be to use the 12S rRNA gene which exhibits a similar level of conservation as the COI gene in vertebrates (Cummings et al., 1995). However, for practical and empirical reasons, the COI gene was selected for phylogenetic analysis of the Coleoidea. The practical reason for choosing the COI gene over the 12S gene was the availability of primers. Folmer et al. (1994) had recently designed "universal" primers that were demonstrated to amplify the COI gene from a diverse array of metazoan taxa, including *Loligo pealei* and *Octopus* sp.

The COI gene was also chosen over the 12S gene because it is a protein-coding gene, facilitating alignment and translation into a putative amino acid sequence. In case the degree of conservation was not great enough, it was hoped that the deduced amino acid sequence of the COI protein would provide additional information at deep-level divergences. Furthermore, highly variable third codon position sites in the nucleotide sequences would provide information for recent divergences without introducing errors in

alignment. Highly variable sites in rRNA genes are frequently omitted from analyses due to alignment ambiguities in loop regions, as was evidenced in the 16S study of cephalopods (Bonnaud et al., 1994). In addition, rRNA genes have been shown to violate the assumption of character independence required in all methods of phylogenetic analysis (Swofford et al., 1996). As highly variable loop regions are frequently discarded from analyses, the less variable but easy to align stem regions of rRNA genes account for the majority of informative change. However, stem region characters in rRNA genes are not independent, as a mutation in one base must result in a corresponding mutation in the pairing base in the opposite strand in the stem region (Mindell and Honeycutt, 1990; Hillis and Dixon, 1991). The result of nonindependence leads to inflated clade support since there is less conflict among characters in their support of any given clade due to the compensatory substitutions in opposite strands of stem regions.

The obvious division of the COI gene into 3 character partitions, according to positions in the codon, is also an attractive attribute for maximum likelihood analysis. Rather than approximating evolution rate variation across sites from a discrete gamma distribution of rates, biologically meaningful rate categories corresponding to codon positions can be designated prior to the analysis (Yang, 1994b). The definition of rate categories prior to likelihood analysis also minimizes the computational time required in the analysis and decreases the variance in other parameter estimates when multiple parameters are estimated simultaneously (Huelsenbeck and Rannala, 1997). Since the errors associated with each parameter estimate are higher for more complicated models (i.e., those which must simultaneously estimate more parameters) than for simpler models, the accuracy of the estimated phylogeny may be improved by estimating fewer parameters at once (Kuhner and Felsenstein, 1994). Although the use of a discrete gamma model with three discrete rate categories would also involve the estimation of the same number of rate categories, a fourth parameter  $\alpha$ , the shape parameter, must be estimated under this model.



In this chapter the results of phylogenetic analyses of a 657 bp fragment of the COI gene from 55 cephalopod taxa will be presented and discussed. Patterns of substitution will be explored through pairwise comparisons among a subset of the COI sequences. The subset of taxa used in sequence diagnostics will correspond to the same taxa for which the nuclear gene sequences were also available for analyses (26 taxa). Maximum parsimony, maximum likelihood, and weighted parsimony analyses will be conducted on the COI data set. Clade support for the parsimony trees will be assessed through bootstrap analysis and Bremer support analysis. Lastly, the monophyly of the Sepioidea will be statistically tested using the parametric bootstrap.

## MATERIALS AND METHODS

### Taxonomic Sampling

A portion of the cytochrome *c* oxidase I (COI) gene was sequenced for 55 cephalopod taxa representing a broad spectrum of diversity in the class. The classification, following Voss (1977), and source of the specimens used in this study are given in Table 1. Included are taxonomic representatives from each of the five families of the order Sepioidea, two genera from the suborder Myopsida, 23 families of the suborder Oegopsida, four families from the suborder Incirrata, three families from the suborder Cirrata, a representative from the monotypic order Vampyromorpha, and a member of the subclass Nautiloidea was also included as an outgroup. Tissue samples from specimens were stored in either 70% ethanol (-20°C) or tissue storage buffer (0.25M ethylenediamine tetraacetate [EDTA], 20% dimethyl sulfoxide [DMSO], saturated NaCl, pH 8.0) (Seutin et al., 1991) until DNA extractions were performed.

### DNA Extraction, PCR Amplification, and Cloning

A modification of a protocol designed explicitly for extracting DNA from mollusc tissue (Winnepenninckx et al., 1993) was used for extracting DNA from cephalopod specimens. A small amount (approximately 0.1g) of muscle tissue from the mantle, fin, arm, or tentacle of preserved specimens was finely diced with a sterile razor blade and placed in a microfuge tube containing 500 µl of isolation buffer (50mM EDTA, 50mM Trishydroxymethyl aminomethane [Tris], 150mM NaCl, pH 8.0), 60 µl of 10% sodium dodecyl sulfate [SDS], 10 µl of 10 mg/ml ribonuclease A, and 10 µl of 25 mg/ml proteinase K and was incubated overnight at 37°C. The following morning 10 µl of

hexadecyltrimethylammoniumbromide [CTAB] buffer (10% w/v CTAB, 0.7M NaCl) was added to the samples, which were then incubated for 20 min. at 65°C and allowed to cool to room temperature. Once cool, 350 µl of saturated NaCl was added and the tubes were vortexed at high speed for 15 min. The suspension was extracted once with phenol, once with phenol:chloroform:isoamylalcohol (25:24:1), and once with chloroform:isoamylalcohol (24:1) using wide bore pipette tips during transfer of the aqueous phase. High molecular weight DNA was precipitated with 2 volumes of 100% ethanol and collected by either spooling or centrifugation at 4°C. The DNA was washed once with 70% ethanol, dried in a vacuum concentrator, resuspended in 50 µl of sterile TE (10mM Tris, 1mM EDTA, pH 8.0), and stored at 4°C.

Metazoan COI primers, sequences LCO1490 and HCO2198 (Folmer et al., 1994), were ordered from Life Technologies (Gaithersburg, MD). The polymerase chain reaction (PCR) was used to amplify a 657 bp portion (excluding primer sequences) of the mitochondrial COI gene using the BRL PCR Reagent System (Life Technologies). A typical 50 µl amplification consisted of the following reagents: 5-10 ng template DNA, 20mM TrisHCl (pH 8.4), 50mM KCl, 1.5mM MgCl<sub>2</sub>, 50 pmoles of each primer, 0.2mM of each dNTP, and 1.25 units of *Taq* DNA polymerase. An MJ Research PTC-200 (Watertown, MA) thermocycler was used to conduct 40 cycles of the following temperature profile: 94°C for 1 minute, 45-47.5°C (depending on the sample) for 1 min., and 72°C for 2 min. A final extension step at 68°C for 7 minutes followed the 40 cycles of amplification.

COI PCR products were cloned into a plasmid vector using the Original TA Cloning® Kit with pCR™2.1 (Invitrogen Corp., San Diego, CA). Plasmid DNA from transformant colonies was isolated and digested with *Eco*R1 (Life Technologies) to check for presence of the 710 bp COI insert. Transformant colonies containing the 710 bp insert were grown overnight in 3 ml liquid growth media containing 100 µg/ml ampicillin. Plasmid DNA was isolated from the overnight cultures using a standard alkaline lysis protocol (Sambrook et al., 1989) or through use of the PERFECTprep® plasmid purification system kit

(5 Prime->3 Prime, Inc., Boulder, CO).

### Manual Sequencing

Approximately 5 µg of plasmid DNA containing the COI insert were manually sequenced in both directions. The M13 Reverse Primer (New England BioLabs, Beverly, MA ) and the T7 Promoter sequence (Life Technologies) were used to prime upstream and downstream of the insert site, respectively. Manual sequencing was accomplished by Sanger's (1977) dideoxy chain-termination method using the Sequenase® Version 2.0 Sequencing Kit (United States Biochemical, Cleveland, OH) along with radiolabeled [ $\alpha$ - $^{35}$ S]-dATP (New England Nuclear, Boston, MA). Sequencing reactions were electrophoresed on a 6% polyacrylamide gel for 7 hours at 80 W. Following electrophoresis, the gel was transferred to 3 MM chromatography paper, vacuum-dried, and exposed to autoradiography film. The film was developed 24-72 hours following the initial exposure and read by eye and recorded in a standard 5 X 5 quad ruled composition book.

After obtaining about 400 base pairs of sequence data for the COI gene fragment of 12 taxa, an internal cephalopod-specific COI sequencing primer was designed with the aid of the computer program PC/Gene (Intelligenetics Inc., Geneva, Switzerland). The internal primer, designated LCO1648 following the nomenclature of Folmer et al. (1994), was then used in sequencing reactions to obtain sequence data for the internal region of the cloned COI gene fragment. The sequence of the LCO1648 primer, which begins 158 bp downstream of the LCO1490 primer, is as follows: 5'-ta gtt ata cct att ata att gg-3'.

### Automated Sequencing

The concentration of plasmid DNA was determined by fluorometric analysis using a DyNA Quant™ 200 fluorometer (Amersham Pharmacia Biotech, Buckinghamshire, England). Approximately 300 fmol of template DNA was used in each sequencing reaction

along with 1.5 pmol of IRD800 fluorescent-labeled M13 Forward or M13 Reverse primer (LI-COR, Inc., Lincoln, NE). The Thermo Sequenase® fluorescent-labeled primer cycle sequencing kit with 7-deaza-dGTP (Amersham Pharmacia Biotech, Buckinghamshire, England) was used in all cycle sequencing reactions. Denatured samples were loaded onto a 4% Long Ranger™ acrylamide gel (FMC Bioproducts, Rockland, ME) and run on a LI-COR model 4000L automated DNA sequencer. DNA sequences were read by computer from the image file created by the 4000L DNA sequencer using the Base ImagIR version 2.3 software package.

#### Data Analysis

*Alignment.* The COI sequence of the outgroup *Katharina* sp. (Mollusca: Polyplacophora) was downloaded from GenBank [KSU56845]. DNA sequences were aligned by eye with the aid of the *Katharina* sp. homologous sequence and compiled in MacClade 3.0 (Maddison and Maddison, 1992) or Gene Jockey II (Biosoft, Cambridge, UK). It was not necessary to introduce gaps into the aligned sequences as there were no insertion/deletion events or alignment ambiguities, a finding consistent with the results obtained by Folmer et al. (1994), where no gaps were introduced in the alignment of COI sequences from diverse metazoan phyla.

*Sequence Characteristics.* MacClade 3.0 was used to assigning codon positions to the nucleotide data, to translate nucleic acid sequences into amino acid sequences, and to generate various assumption sets (weight and character inclusion sets, transition or transversion type sets) used in later analyses. To reduce the total number of possible pairwise comparisons between taxa, a subset of 26 taxa from the COI data set were used to calculate patristic distance under the various assumption sets (all characters included, first and second codon position characters only, third codon position characters only, transversional or transitional substitutions only) in PAUP\* (Swofford, 1996). The base

frequencies at each codon position for each of the 26 taxa were determined and a chi-square test was employed to test for significant heterogeneity among taxa with respect to the frequency of bases at different codon positions. The 26 taxa chosen for these analyses, hereafter referred to as the restricted data set, represented the taxa for which all three genes (COI, Actin I, and Actin II) had been sequenced, thus facilitating comparisons between the different data sets.

*Phylogenetic Analyses.* Maximum parsimony analysis of the aligned nucleotide and deduced amino acid sequences was conducted using the heuristic tree-search option in PAUP\* [Versions 4.0d60 through 4.0d64] (Swofford, 1996) with 50 random sequence addition replicates. The consistency and retention indices (CI and RI, respectively) were calculated in PAUP\*. The CI is a ratio representing the sum of the minimal number of individual character changes divided by the observed number of changes. The CI represents the amount of homoplasy in the data and ranges from 0 (all characters are homoplastic) to 1 (no homoplastic characters). Because autapomorphic characters can artificially inflate the CI, the RI was also calculated. The RI is the ratio representing the difference between the maximum number of extra changes and observed number of extra changes divided by the maximum number of extra changes. The RI has the same range as the CI, with an RI value of 1 representing a data set with no homoplastic and no autapomorphic characters. Support for clades within phylogenetic trees was tested using the heuristic bootstrap search command (1000 replicates) in PAUP\*. A second measure of clade support, the Bremer decay index (Bremer, 1988), was also determined for each clade in the most-parsimonious tree using the software program TreeRot (Sorenson, 1996).

Preliminary maximum likelihood analyses were conducted on the restricted data set of 26 taxa to determine the most appropriate model of base substitution to be used in analysis of the entire data set. The strategy used to test models of substitution was similar to that described in Huelsenbeck and Crandall (1997). A hierarchy of likelihood ratio tests

(LRTs) was conducted starting with the most constrained model, which assumes equal base frequencies, equal probabilities of all possible base substitutions, and no rate heterogeneity across sites (Jukes and Cantor, 1969). The second model examined in the likelihood search allowed for unequal base frequencies but retained equal probabilities of base substitutions and no rate heterogeneity across sites (Felsenstein, 1981). The log likelihood of the tree obtained under the Jukes and Cantor model (JC69) was compared with the log likelihood of the tree generated under the Felsenstein model (F81) in a LRT to determine if the difference in likelihoods was statistically significant. Since the JC69 model is a nested case of the F81 model, the significance of the LRT statistic was compared to chi square distribution with 3 degrees of freedom.

The substitution model of Hasegawa, Kishino, and Yano (1985) was then tested, which allows for unequal base frequencies and considers two rate categories of base substitution corresponding to transitions and transversions (Hasegawa, Kishino, and Yano, 1985). The F81 model was used as the null model in next LRT as it is a nested case of the Hasegawa, Kishino, and Yano model (HKY85). The F81 log likelihood was compared to the log likelihood of the tree obtained under the HKY85 model and the significance determined as described for the preceding test.

The fourth model of substitution tested was the HKY85 model allowing for rate heterogeneity across sites (HKY85+). In this model substitution rates are categorized into 3 classes corresponding to first, second, and third codon positions. The fit of this model was tested using the HKY85 model as the null hypothesis in the LRT. The most complicated model of substitution, the general time-reversible model (GTR), was not examined due to the excessive amount of computational time required to generate a likelihood tree. The GTR model allows for unequal base frequencies and 6 different substitution rate categories, corresponding to the 6 possible reversible character state transformations for nucleotide sequence data. Although the GTR model is the most parameter-rich model and therefore is likely to result in a tree with the highest likelihood,

the error associated with each parameter estimate is greater, including the error associated with estimates of tree topology (Huelsenbeck and Rannala, 1997). Parameter values obtained in the HKY85+ likelihood analysis of the restricted data set were fixed in the subsequent maximum likelihood analysis of the entire data set.

Transversional weighting schemes have been shown to be an efficient means of accounting for superimposed changes at highly variable positions without discounting potentially informative substitutions in parsimony analyses (Huelsenbeck, 1995). The transition:transversion ratio (TI:TV) estimated in the HKY85+ maximum likelihood analysis was used to construct an assumption set for transversionally-weighted parsimony analyses. A step matrix was defined in which weight of transversions was scaled so that the average number of transversions would approximate the average number of transitions (i.e., perfect weighting sensu Huelsenbeck, 1995). For example, given a TI:TV ratio of 3.5, transversions would be assigned a weight of 7 steps while transitions would be assigned a weight of 2 steps. A heuristic (25 random addition replicates) parsimony search of the weighted data was conducted. Bremer support indices of the weighted data, however, were not calculated as support values across nodes and between different trees is not comparable for weighted data. Although it is also possible to differentially weight characters with respect to their codon positions, giving less weight to more variable third codon position characters, this method of weighting was not employed due to the fact that the majority of the phylogenetically informative sites were at the third codon position.

*Test of Sepioid Monophyly.* A maximum likelihood tree for the constrained data, where the Sepioidea was constrained to be monophyletic, was generated in the same manner as described for the unconstrained data described above. Model parameters were estimated in a successive approach and then fixed in the following searches to obtain the maximum likelihood tree assuming a HKY85+ model of sequence evolution. The maximum likelihood tree obtained was then used to generate 50 simulated data sets under the same



model parameters using the computer program SeqGen 1.04 (Rambaut and Grassly, 1997). The simulated data sets were analyzed (heuristic searches with 10 random addition replicates) to generate a null distribution of most-parsimonious tree length differences, calculated as the difference in parsimony tree length under the null (Sepioidea monophyly) and alternate (unconstrained) hypotheses for each of the 50 simulated data sets. The tree length difference for the actual data was then compared to the null distribution to determine if the actual tree length difference was statistically significant. The proportion of replicates in which the actual tree length difference was exceeded by the tree length difference derived from the simulated data sets represented the significance level of the test.

## RESULTS

### Sequence Variation and Divergence

The multiple alignment of nucleotide sequences of the COI gene of cephalopods is presented in Figure 3. The 657 base pair fragment of the COI gene from 55 species comprised 350 variable characters (53.3%). Of the 350 variable characters, 96 (27.4%) were first codon position bases (25 of which were parsimony uninformative), 36 (10.3%) were second codon position bases (24 of which were parsimony uninformative), and 218 (62.2%) characters were third codon position bases (four of which were parsimony uninformative).

Comparisons of sequence divergences within and among the major groups of cephalopod taxa are presented in Table 2. As expected, mean sequence divergences determined from all possible pairwise comparisons within groups were less than mean sequence divergences resultant from pairwise comparisons among groups. For example, the mean sequence divergence (+/- standard deviation) among the Oegopsida was 17.72+/- 1.63% whereas comparisons between the Oegopsida and other groups ranged from 19% to approximately 26%. However, pairwise comparisons among Coleoid groups did not differ appreciably (e.g., Oegopsida vs. Myopsida = 19.74+/-1.16%, Oegopsida vs. Sepioidea = 19.13+/-1.62%). Mean sequence divergences for all comparisons between *Nautilus* and other groups were significantly greater than mean divergences among the various coleoid groups.

Figure 4 presents the sequence divergences for pooled first and second position nucleotides and third position nucleotides plotted as a function of total uncorrected sequence divergence for all possible comparisons between the 26 taxa in the restricted COI

data set. Substitutions at third codon position nucleotides account for a greater proportion of the variation in the COI gene at lower total sequence divergences than at greater sequence divergences. At greater total sequence divergences, there was an increased contribution of first and second position substitutions to the total sequence divergence. Because third position substitutions did not account for as great a proportion of sequence divergence at higher levels of total sequence divergence, these data provide evidence for saturation at third codon positions where the incursion of superimposed substitutions can mask the actual total number of substitutions that have taken place. As has been demonstrated by other studies using mitochondrial protein-coding gene sequences, third codon position characters were clearly the most variable (Kocher and Carleton, 1997). The percentage of total sequence divergence accounted for by transversional and transitional substitutions are plotted as a function of total uncorrected percent sequence divergence for all possible sequence comparisons of the restricted data set in Figure 5. As sequence divergence increased, the percentage of substitutions decreased for third codon position characters, and increased for the pooled first and second codon position characters. There did not appear to be a clear relationship between the percentage of transitional or transversional substitutions and sequence divergence for comparisons of the unweighted data. In general transitions accounted for the majority of substitutions across the entire spectrum of uncorrected sequence divergence, but transversions also made a significant contribution to the total sequence divergence across the entire range of divergence.

There were differences with respect to the degree of variation within third codon characters with 30.6% of third codon characters being twofold variable (exhibited two character states), 27.9% were threefold variable, and 41.5% were fourfold variable. There was also significant heterogeneity in base composition at third codon position characters (chi square = 434.0, df = 75,  $p < 0.001$ ) whereas chi square values for base composition bias at first and second codon characters (41.37 and 1.56, respectively) were not significant (df = 75,  $p > 0.99$ ). The frequencies of the four bases was highly unequal at all

codon positions, with cytosines the rarest at first positions (Figure 6), all purines rare at second positions (Figure 7), and guanines extremely rare at third positions (Figure 8).

### Phylogenetic Relationships

*Unweighted Parsimony.* A heuristic search (1000 random addition replicates) of the equally weighted data yielded the single most parsimonious tree depicted in Figure 9. For this tree and all parsimony trees discussed below, bootstrap values for nodes supported by over 50% of 1000 heuristic bootstrap replicate searches are indicated below supported nodes. The Bremer decay values are given above each node. The branch lengths are drawn proportional to the number of unambiguous changes occurring along each branch. The tree generated from equal weights analysis supports the monophyly of the Coleoidea, Decapodiformes, Octopodiformes, Octopoda, Sepiolidae, Myopsida, and various families represented by more than one taxon (Oegopsida: Enoploteuthidae, Cranchiidae, Gonatidae, Ommastrephidae, Onychoteuthidae; Incirrata: Bolitaenidae; Sepioidea: Sepiidae). The monophyly of the Sepioidea, Teuthoidea, Oegopsida, Incirrata, and Cirrata was not supported by unweighted parsimony analysis of the COI data. Other taxonomic groupings not supported by maximum parsimony analysis of the unweighted data set include the Cycloteuthidae (as including *Discoteuthis*) and the Pholidoteuthidae (as including *Lepidoteuthis*). The Lycoteuthidae, Pyroteuthidae and Ancistrocheiridae are somewhat aligned with each other but separate from the Enoploteuthidae. The majority of the relationships determined from analysis of the equally weighted data set were not supported by bootstrap analysis. Bootstrap analysis did provide support for the monophyly of several groups including the Coleoidea, Decapodiformes, Sepiolidae, Sepiidae, Gonatidae, Ommastrephidae, Onychoteuthidae, Loliginidae, and Bolitaenidae. Bootstrap analysis also supported an association between *Octopus* and *Hapalochlaena*, and between *Stauroteuthis* and *Cirrothauma*. Bremer support values were in relative agreement with bootstrap support values, but they also provided some information for the support of nodes not supported in

at least 50% of the bootstrap replicates. For instance, moderately strong Bremer support was found for the node defining the (*Mastigoteuthis Pholidoteuthis*) clade although bootstrap support for the association was lacking. Other clades which received moderate to strong Bremer support (>5) without bootstrap support were (*Brachioteuthis Octopoteuthis*), (*Spirula (Thysanoteuthis (Alluroteuthis (Gonatidae)))*), (*Bathyteuthis (Chtenopteryx (Ommastrephidae))*), (*Ancistrocheirus (Pyroteuthis (Loliginidae))*), (*Abralia Enoploteuthis*), (*Teuthoidea + Spirula*), (*Idiosepius Sepioloidea*), (*Argonauta (Octopus Hapalochlaena)*), (*(Graneledone Vitreledonella)(Cirrata + Bolitaenidae)*), and (Octopoda).

*Parsimony on Deduced Amino Acid Sequences.* A heuristic parsimony search of the inferred COI amino acid sequences yielded 3416 equally parsimonious trees. Although the nucleotide sequences demonstrated significant variability, the amino acid sequences were highly conserved (only 19.6% of the characters were phylogenetically informative). Within major lineages, phylogenetically informative variability in the amino acid sequences was virtually nonexistent, resulting in the generation of many equally parsimonious trees. The strict consensus of the 3416 equally parsimonious trees generated from the amino acid data split the coleoids into two major groups, the Decapodiformes and the Octopodiformes (Octopoda+Vampyromorpha). The amino acid data also supported the monophyly of the Cirrata, a result not obtained in the nucleotide data analysis. Bootstrap analysis of the amino acid data was not conducted due to the small number of informative characters and the large amount of time required to complete a single search replicate. The amino acid data, although of limited use in constructing relations within the octopods and decapods, was useful in strongly confirming placement of *Vampyroteuthis* with the octopods, and in demonstrating the monophyly of the two major groups of coleoid cephalopods.

*Maximum Likelihood Analyses.* Initial likelihood analyses were conducted on the restricted nucleotide data set to estimate parameters used in the subsequent analysis of the

comprehensive COI data set. It was not possible to estimate parameters from searches of the entire data set due to excessive time required by maximum likelihood analyses. The time required to complete heuristic searches of the restricted data set ranged from a few hours (under the JC69 model) to over a week (under the HKY85+ model). The HKY85+ substitution provided the best fit to the data. However, it is quite probable that a more general model such as the general time-reversible model, which allows for six separate reversible substitution categories corresponding to all 12 possible substitution types, would have provided a better fit to the data as is frequently the case for parameter-rich models (Huelsenbeck et al., 1997). Substitution parameters obtained in the analysis of the restricted data set under the HKY85+ model were then fixed in the subsequent search of the comprehensive data set. Log likelihoods, substitution parameters, and the results of likelihood ratio tests obtained in analyses of the restricted data set are presented in Table 3.

The maximum likelihood tree from a single heuristic search of the comprehensive data set under the HKY85+ model is depicted in Figure 10. The tree supports the monophyly of the Coleoidea, Decapodiformes, Octopodiformes, Octopoda, Sepiolidae, Myopsida, and various families represented by more than one taxon (Oegopsida: Cranchiidae, Gonatidae, Ommastrephidae, Onychoteuthidae; Incirrata: Bolitaenidae; Sepioidea: Sepiidae). The monophyly of the Sepioidea, Teuthoidea, Oegopsida, Incirrata, and Cirrata was not supported by maximum likelihood analysis of the COI data. Similar to the parsimony results, the monophyly of the following interfamilial groups was supported:

(*Pholidoteuthis Chiroteuthis Mastigoteuthis*), (*Alluroteuthis* (Gonatidae)), (*Psychroteuthis Histiotheuthis*), (*Bathyteuthis (Chtenopteryx (Ommastrephidae))*); (*Argonauta (Octopus Hapalochlaena)*), (*Stauroteuthis Cirrothauma*), and (*Grimpoteuthis Opisthoteuthis*). Some of the main differences found between the parsimony and likelihood analyses include the placement of *Spirula*, *Thysanoteuthis*, *Idiosepius*, enoploteuthids, *Ancistrocheirus*, *Pyroteuthis*, *Lycoteuthis*, *Brachiotheuthis*, *Octopoteuthis*, *Lepidoteuthis*. These differences could be the result of the different methodologies and assumptions in the two techniques of

phylogenetic reconstruction. Alternately, they might be due to different peaks found in tree space, with more space explored in the parsimony analysis (1122 random addition replicates) than in likelihood analysis (a single simple stepwise addition replicate).

*Weighted Parsimony.* A transition:transversion ratio of 3.496 was obtained in the maximum likelihood analysis under the HKY85+ model of substitution. Therefore, transversional substitutions were weighted 3.5X greater than transitional changes in the weighted parsimony analysis to account for the greater frequency of transitions. The most parsimonious tree obtained in weighted parsimony analysis is presented in Figure 11. Clades supported in both the parsimony and likelihood analyses were also generally supported in parsimony analysis of the transversionally weighted data. Substantial differences between the weighted parsimony analysis and prior analyses include placement of *Vampyroteuthis* outside of the remaining coleoids, support for the monophyly of the cirrate octopods, placement of *Pholidoteuthis* with *Lepidoteuthis* instead of with *Chiroteuthis* and *Mastigoteuthis*,

*Test of Sepioid Monophyly.* The tree derived from maximum likelihood analysis of the COI data constraining the monophyly of the Sepioidea was used to generate 50 simulated data sets. The substitution parameters under the HKY85+ model of evolution that were used to obtain the original tree were used to generate the simulated data sets. The tree length difference obtained in parsimony searches of the actual COI data set is compared to null distribution of tree length differences obtained by parsimony searches of the simulated data sets in Figure 12. The observed tree length difference, 16 steps, falls well outside of the null distribution of simulated tree length differences ( $p \ll 0.01$ ). This result statistically validates the conclusion that the COI data do not support the monophyly of the Sepioidea. If the failure of the COI data to support the monophyly of the Sepioidea were due to stochastic variation in the COI data alone, or to some systematic bias generated by

tree topology, the observed tree length difference would have occurred within the range of tree length differences obtained in parsimony searches of the simulated data sets (Huelsenbeck and Hillis, 1996; Huelsenbeck et al., 1996).



## DISCUSSION

### Sequence Variation and Divergence

Uncorrected pairwise sequence divergences between coleoid taxa ranged from <1% between congeners to >22% in comparisons between octopod and decapod taxa. The range of sequence divergences between coleoid taxa is comparable to the range of COI sequence divergences found in higher-level analyses of other protostome groups (Harasewych et al., 1997; Black et al., 1997). Slightly less than two-thirds of the variation was restricted to third codon position nucleotides, suggesting that homoplasy at third codon position characters is quite likely. The plot of pooled first and second codon position substitutions and third codon position substitutions against total sequence divergence also indicated saturation at the third position as the relative contribution of third position substitutions declined within increasing sequence divergence (Figure 4). The plot of percent transitions and transversions against total uncorrected sequence divergence did not provide clear evidence for transitional saturation although there appeared to be a slight decrease in the proportion of transitions as sequence divergence increased (Figure 5). These plots indicate that it may be advisable to employ some method to correct for masked multiple substitution events at third codon position nucleotides.

Analysis of base composition at the three codon positions demonstrated significant heterogeneity among taxa in base frequencies at third codon positions (Figure 8). The use of a maximum likelihood method of phylogeny reconstruction using a model which accounts for unequal base frequencies, unequal probabilities of character transformations, and unequal rates of substitution across the three codon positions is probably most appropriate for these data.

### Phylogenetic Relationships

Previous investigations of coleoid systematics have attempted to determine relationships within the Octopoda, Sepioidea, and Myopsida through phylogenetic analysis of morphological and molecular character data. Most recently, Young and Vecchione (1996) conducted a higher-level analysis of the coleoid cephalopods. Consistent with Young and Vecchione (1996), and as was independently determined in an earlier study by Berthold and Engeser (1987), the COI results confirm that the coleoids can be divided into two main lineages, the Octopodiformes (Octopoda+Vampyromorpha) and the Decapodiformes (Sepioidea+Teuthoidea).

Within the Octopodiformes parsimony and likelihood analyses supported a sister group relationship between the Vampyromorpha and Octopoda, a result that was also consistent with Young and Vecchione (1996). Results within the Octopoda differed from those obtained by Young and Vecchione and the scenario proposed by other researchers (Naef, 1923; Robson, 1932; Berthold and Engeser, 1987; Voss, 1988; Voight, 1993, 1997) wherein the Cirrata are sister taxon to the Incirrata, and both suborders are considered monophyletic. The Incirrata were found to be polyphyletic, as the cirrates consistently grouped within the incirrate clade regardless of how the data were treated (parsimony, weighted parsimony, or maximum likelihood). Furthermore, the monophyly of the cirrates was not supported in the equally weighted parsimony and maximum likelihood analyses of the nucleotide data, where *Stauroteuthis* and *Cirrothauma* grouped with the bolitaenids. Weighted parsimony analysis of the nucleotide data and parsimony analysis of the deduced amino acid sequences supported the monophyly of the cirrates although in both cases the cirrate clade was not found to be the sister group to the incirrate clade. The cirrates have been considered primitive, having diverged from the incirrates early in the evolution of the Octopoda. As the maximum likelihood analysis also failed to support a sister group relationship between the cirrates and incirrates, the placement of the cirrates cannot be

explained as an artifact of the longer period for which the COI gene evolved in the supposedly older cirrate lineage.

The use of maximum likelihood techniques in phylogenetic analyses was initially spurred by the need to avoid the problems associated with sampling taxa with widely differing substitution rates or divergence times (Felsenstein, 1978; Swofford et al., 1996). Although it is tempting to conclude that the placement of *Stauroteuthis* and *Cirrothauma* with the bolitaenids was due to the attraction between long branches subtending each of the two clades, the maximum likelihood tree (Figure 10) did not support such a conclusion. Convergent evolution of the COI gene between bolitaenids and cirrates cannot be ruled out, although it is difficult to envision why similar selective pressures would be exerted on the evolution of the COI gene in cirrates and bolitaenids but not in other incirrates.

In Voight's (1997) recent cladistic analysis of morphology to determine relationships within the Octopoda, the bolitaenids emerged basal to the remaining incirrates. Perhaps an early divergence of both the cirrates and bolitaenids is responsible for the unexpected result obtained in analyses of the COI data. Taxonomic sampling of the bolitaenids in this study was limited, and the inclusion of additional bolitaenid (or bolitaenoids such as the monotypic *Idioctopodidae* and *Amphitretidae*) is needed to clarify relationships within the Octopoda. Also contrary to the findings of Voight (1997) was the placement of *Bathypolypus*, *Graneledone* outside of the octopodids and the relatively distant placement of *Vitreledonella* and *Argonauta*, which would be expected to cluster together. *Argonauta* always clustered with the shallow water octopodids and *Vitreledonella*, and although its placement was somewhat unstable across the 3 different analyses, always emerged near the bolitaenids and cirrates. The lack of other argonautoid families (e.g. *Alloposidae*, *Ocythoidea*, *Tremoctopodidae*) in the representation of octopod diversity may have contributed to this anomalous result that was not supported by bootstrap analyses. The placement of the deep water octopodids *Bathypolypus* and *Graneledone* apart from the

shallow water octopodids also runs contrary to expectations based on cladistic analysis of morphology (Voight, 1997).

Although the monophyly of the Decapodiformes is well supported by the COI data, the validity of the order Sepioidea as defined by Voss (1977) was not confirmed. Several studies have rejected the monophyly of the order, although the way they have divided the Sepioidea has differed substantially (Fioroni, 1981; Berthold and Engeser, 1987; Clarke, 1988; Khromov, 1990; Bonnaud et al., 1994). *Spirula* did not group with any of the sepioids but clustered with various oegopsid taxa, a result concordant with the results of Bonnaud et al. (1994). However, placement of *Spirula* within the oegopsid squids was unstable as it varied depending on the method of phylogeny reconstruction and was not supported in bootstrap analyses. Parsimony analysis of the nucleotide data supports an affinity between the Sepiadariidae and Idiosepiidae and does not support placement of the *Idiosepius* within the Oegopsida, as suggested by Bonnaud et al. (1997). It is important to recognize that many of the differences between the present study and previous molecular studies may be the result of very different taxonomic sampling schemes and types of analyses conducted. A greater proportion of oegopsid representatives was included in this study whereas Bonnaud et al. (1994; 1997) included a greater proportion of sepioid taxa in their work. Parsimony analysis of the amino acid data suggests a relationship between *Idiosepius* and the myopsid squids, although only two unambiguous amino acid character changes define the (*Idiosepius* Myopsida) clade. Likelihood analysis also did not support inclusion of *Idiosepius* within the Sepioidea and found *Sepioloidea*, a member of the family Sepiadariidae, to be most closely related to the Sepiolidae. The monophyly of the Sepiolidae was well supported in all of the analyses, however, their rank and position within the Decapodiformes is not clear. Clarke (1988) and Bonnaud et al. (1994, 1997) recommend raising the sepiolids to ordinal rank. While results from equally weighted parsimony analysis of the COI nucleotide data set supported a distinction between the sepiolids and other sepioid taxa, results from the weighted parsimony analysis, parsimony

analysis of the amino acid data, and likelihood analysis supported a sister-group relationship between the sepiolids and the sepiadariids. As the status of the Sepioidea is likely to remain a matter of debate for some time to come, making recommendations regarding the taxonomy of the 5 sepioid families would be premature. Such taxonomic revisions should await the results of additional molecular and morphological phylogenetic studies.

Two other lines of evidence also refute the monophyly of the Sepioidea. The results of pairwise comparisons among COI sequences within and among major groups of cephalopod taxa (Table 2) showed that the Sepioidea exhibited the greatest average within-group sequence divergence. The test of monophyly also indicated that the failure to support the monophyly of the Sepioidea was not due to stochastic variation in the data (Figure 12). The observed parsimony tree length difference between the analysis where the Sepioidea was constrained to be monophyletic, and the unconstrained analysis was significantly greater than the tree length differences of the simulated data sets. This test of monophyly has recently been used to confirm or refute the monophyly of various taxa (Huelsenbeck et al, 1996; Van Den Bussche et al., in press). This method has also been used to test if the infection of a dental patient with the HIV virus was transmitted through the dentist alone or through multiple sources (Hillis et al., 1996).

The monophyly of the Myopsida was strongly supported in all the analyses but definition of the sister group to the Myopsida proved problematic. The COI data did not support a close relationship between *Chtenopteryx* and the myopsids as was suggested by studies based on morphological evidence (J.Z. Young, 1991) and allozymes (Brierley et al., 1996), both of which concluded that *Chtenopteryx* is a bathypelagic myopsid squid. The COI nucleotide data placed myopsids as either a basal teuthoid group clustering with enoploteuthid taxa (equally weighted parsimony) or as sister taxon to the Sepiidae (likelihood, weighted parsimony). In either case, the monophyly of the Teuthoidea was not supported, although the monophyly of the Teuthoidea was also refuted by the

placement of *Spirula* within the Oegopsida. The COI data suggest an early divergence of the myopsids from most of the other teuthoid taxa. The results of Bonnaud et al. (1994; 1997) also suggest an early divergence of the myopsids, placing them as a sister group to Oegopsida+*Idiosepius* (COIII) or Oegopsida+*Spirula* (16S) clades.

The results of parsimony and likelihood analyses indicate that the suborder Oegopsida, as defined by Voss (1977), is polyphyletic. All analyses placed *Spirula* with the oegopsids, however, other findings also precluded oegopsid monophyly. Parsimony analysis of the equally weighted and transversionally weighted nucleotide data placed the myopsids within a clade of enoploteuthid-like families. Maximum likelihood analysis placed the Enoploteuthidae along with *Idiosepius* outside of the remaining Decapodiformes.

Few studies have attempted to resolve relationships within the Oegopsida, none of which has used molecular sequence data or a rigorous, testable methodology for inferring phylogenetic relationships (Toll, 1982; Hess, 1987; Clarke, 1988). Clarke's analysis of relationships within the group was based on subjective interpretation of overall morphological similarity for a variety of characters. Toll (1982) used an array of morphological characters all related to the same structure, the gladius, to determine relationships among the Oegopsida. Similar to Toll, Hess (1987) examined an array of morphological characters all related to the same structure, in this case the spermatophore. Clarke's study divided the oegopsid squids into two major clades, with the cranchiids grouping outside of one clade and the gonatids grouping outside of the second clade. Toll placed the Thysanoteuthidae and Ommastrephidae outside of all the remaining oegopsid squid families, which were divided into two clades. The two remaining clades in Toll's summary of phylogenetic relationships consisted of the Gonatidae, Onychoteuthidae, Enoploteuthidae, Lycoteuthidae, Histioteuthidae and Psychroteuthidae in one clade, and the remaining oegopsid families in the other clade. Hess' study divided the Teuthoidea into two main groups, one of which contained the myopsids, Ctenopterygidae, Brachioteuthidae, Ommastrephidae, Enoploteuthidae, Gonatidae, Octopoteuthidae, and

Onychoteuthidae. The second major group was further divided into two main clades and consisted of the remaining oegopsid families.

Intra-oegopsid family relationships from analysis of the COI gene were generally not supported by bootstrap analysis and varied somewhat according to the type of analysis. The parsimony analyses and maximum likelihood analysis agreed in placing the Enoploteuthidae outside of the remaining oegopsids, with the Cranchiidae and enoploteuthid-like families (Ancistrocheiridae, Lycoteuthidae, and Pyroteuthidae) also diverging before other oegopsid families. An early enoploteuthid divergence is most consistent with Hess' results, in which the ancistrocheirids and pyroteuthids were treated as enoploteuthid subfamilies and the Lycoteuthidae were found to be quite different. Relationships among the enoploteuthid families are also most consistent with Hess' results since the two enoploteuthid taxa always clustered, as did *Ancistrochierus* and *Pyroteuthis*, although the placement of *Lycoteuthis* was unstable. A detailed morphological study of the enoploteuthid families found the Lycoteuthidae and Pyroteuthidae to be most closely related based on the placement and structure of their photophores (Young and Harman, 1998). Clarke considered the Ancistrocheiridae to be distinct from the Enoploteuthidae and Pyroteuthidae, which he regarded as closely related. Toll found all three families to be closely related but relationships among them were left unresolved and the Lycoteuthidae were placed basal to this trichotomy. The COI results suggest that the pyroteuthids, ancistrocheirids, and lycoteuthids are closely related, but clearly distinct and somewhat distant from the enoploteuthids.

After divergence of the enoploteuthids, the branching orders of the trees derived from the parsimony, weighted parsimony, and likelihood analyses differ substantially. The equally weighted parsimony data indicate that the next clade to diverge consists of the Ommastrephidae, Bathyteuthidae, and Ctenopterygidae. The monophyly of this clade was also supported in the weighted parsimony and likelihood analyses, although both of these analyses placed the clade well within the Oegopsida. None of the previous studies of

oegopsid relationships suggested a close relationship between these 3 families. Bootstrap support for this clade was lacking, however, a Bremer support value of 7 indicates that this clade is more stable than most other oegopsid clades. The two ommastrephid genera included in the COI analysis, *Ommastrephes* and *Sthenoteuthis*, grouped together no matter how the data were analyzed and were supported by strong bootstrap and Bremer support values.

The next clade to branch off in the parsimony analysis consisted of the Histioteuthidae and Psychroteuthidae and the Onychoteuthidae. The Histioteuthidae and Psychroteuthidae were found to be closely related no matter how the data were analyzed and a close relationship between them is supported by gladius morphology (Toll, 1982). Likelihood analysis placed the Onychoteuthidae elsewhere but the two onychoteuthid genera included in the analysis, *Onychoteuthis* and *Moroteuthis*, always grouped together and were weakly supported in bootstrap analysis. The onychoteuthids were not placed with the gonatids, as would be expected based on the results of Toll (1982) and Hess (1987).

The Chiroteuthidae and Mastigoteuthidae consistently grouped together although bootstrap support for a close relationship between the families was lacking. A clade of "chiroteuthid families" has been suggested (Young, 1991; Young et al., 1998), which consists of the Chiroteuthidae, Mastigoteuthidae, and Joubiniteuthidae and is based primarily on tentacle morphology. This clade was supported in weighted parsimony analysis, however, *Joubiniteuthis* grouped just outside of this clade in the equally weighted analysis and was somewhat distant to this clade in the likelihood analysis. *Pholidoteuthis* also grouped with the chiroteuthid families in the equally weighted parsimony analysis and also in likelihood analyses, although so did *Lepidoteuthis* and *Octopoteuthis*. A close relationship between the Lepidoteuthidae and Octopoteuthidae to each other and to "chiroteuthid families" has also been found by morphological studies (Clarke, 1988; Young, 1991). Equally weighted parsimony analysis did not support such an association



between Pholidoteuthis, Lepidoteuthis, and Octopoteuthis although likelihood and weighted parsimony analyses did.

*Alluroteuthis* consistently grouped with the gonatids, and both types of parsimony analyses indicated a relationship between this clade and *Thysanoteuthis*. Bootstrap support for these relationships was lacking and Bremer support was weak. Morphological evidence for a relationship between these families is lacking. The monophyly of the three gonatid taxa included in the COI analysis was strongly supported by bootstrap analysis and moderately supported by Bremer analysis of the equally weighted data.

The weighted parsimony and likelihood analyses found the cycloteuthids and *Brachioteuthis* to be related. Interestingly, the two cycloteuthid taxa (*Cycloteuthis* and *Discoteuthis*) were not monophyletic in any of the analyses. Of the decapod families represented by more than one taxon in phylogenetic analysis of the COI gene, the Cycloteuthidae were the only family whose monophyly was not strongly supported. The equally weighted data placed *Brachioteuthis* with *Octopoteuthis* and the cycloteuthids with *Lepidoteuthis* and *Architeuthis*. The placement of *Architeuthis* was very unstable, differing greatly across the three methods of phylogenetic analysis employed.

Many of the relationships within the oegopsid squids were not entirely consistent between the three methods of phylogenetic analysis employed, and bootstrap and Bremer analyses of the COI data did not lend support to many of the oegopsid nodes. Therefore, the determination of phylogenetic relationships within the Oegopsida remains a problem to be solved by further study. Although some relationships among 2-3 families were stable across all analyses, placement of these larger groups in relation to other such family groups was unstable across analyses (e.g., placement of the (*Bathyteuthis* (*Chtenopteryx* (Ommastrephidae))) clade).

The failure to conclusively determine phylogenetic relationships among oegopsid families may be due to the use of an inappropriate gene for constructing family-level relations in the group. The COI sequence is highly conserved at the amino acid level and at

first and second codon position characters at the nucleotide level. However, third codon position characters that may be informative in determining relationships between taxa which have diverged more recently, were highly variable and homoplastic change may have masked phylogenetic signals at the interfamilial level. Alternately, the lack of resolution within the Oegopsida may be the result of non-dichotomous branching events (Hoelzer and Melnick, 1994). Perhaps the ancestral oegopsid taxon gave rise to several new families in a very short period of time or simultaneously, yielding a polytomous branching pattern. Several life-history traits of oegopsid squids are characteristic of species which are likely to produce polytomous branching patterns in inferred phylogenies, including cryptic speciation (Smith et al., 1981; Brierley et al., 1993; Yeatman and Benzie, 1993), rapid evolutionary rate (Bonnaud et al., 1994; O'Dor, 1995) and cosmopolitan distributions (O'Dor, 1988). However, poor resolution of branching order deep in the tree is a common result in phylogenetic analyses of mitochondrial sequences. Lack of resolution at basal nodes is frequently attributed to rapid radiation when, in fact, the results of power analyses have demonstrated that not enough data have been gathered in many published studies to detect even a 10% difference in divergence times (Kocher and Carleton, 1997).

The COI results, particularly in reference to the oegopsid squids, must be interpreted with caution as bootstrap and Bremer analyses failed to lend substantial support for many clades. Although bootstrap support does not necessarily confirm that a certain relationship is "true," lack of bootstrap support does indicate instability of the data set. The results of parsimony, weighted parsimony and likelihood analyses also differed with respect to relationships among the oegopsid squids. Analysis of pairwise sequence divergences at different codon positions and for transitions versus transversions, combined with analysis of base composition make it clear that correction for superimposed change and biases in base composition should be attempted. However, imposition of a weighting scheme derived from a global transition:transversion ratio is likely to influence relationships among recently diverged taxa in a different way than it influences relationships among taxa which

diverged long ago. This is currently a problem in both weighted parsimony and likelihood analyses. The same argument applies to base frequencies and rate variation among sites in likelihood analyses. Finally, the likelihood analysis employed was computationally intensive such that only a single heuristic search was conducted, adding taxa in order of occurrence. Parsimony analysis of the equally weighted data for 55 taxa indicated that at least 100 random addition replicates were necessary to obtain the shortest heuristic tree. Thus, the maximum likelihood tree obtained in analysis of the COI data is quite probably not the optimal tree as sufficient exploration of tree space was prohibited by the time required in a single likelihood search (over 1 week).

With respect to higher-level relationships, the following conclusions can be drawn from the COI data: 1) the Coleoidea, Octopodiformes, Decapodiformes, and Octopoda are monophyletic groups; 2) the Vampyromorpha and Octopoda are sister taxa; 3) the Sepioidea, as including the 5 families Spirulidae, Sepiolidae, Sepiidae, Sepiadariidae, and Idiosepiidae, is polyphyletic; 4) *Spirula* is more closely related to the Teuthoidea than it is to the Sepioidea; and 5) the Oegopsida, as currently defined, is polyphyletic.

TABLE 1. CLASSIFICATION OF CEPHALOPOD TAXA INCLUDED IN THIS STUDY (VOSS, 1977)

| Classification                   | <sup>a</sup> Source | <sup>b</sup> Collection Number |
|----------------------------------|---------------------|--------------------------------|
| Phylum MOLLUSCA                  |                     |                                |
| Class CEPHALOPODA                |                     |                                |
| Subclass NAUTILOIDEA             |                     |                                |
| Family Nautilidae                |                     |                                |
| <i>Nautilus pompilius</i>        | RY                  | Waikiki Aquarium               |
| Subclass COLEOIDEA               |                     |                                |
| Order SEPIOIDEA                  |                     |                                |
| Family Sepiidae                  |                     |                                |
| <i>Sepia officinalis</i>         | MV&RY               |                                |
| <i>Sepia pipara</i>              | AR&MN               | ANU4vii95                      |
| Family Sepiolidae                |                     |                                |
| Subfamily Heteroteuthinae        |                     |                                |
| <i>Stoloteuthis leucoptera</i>   | MV                  | ALB9402.14.18                  |
| <i>Heteroteuthis hawaiiensis</i> | DC                  | Hokusei Maru 1996              |
| Subfamily Rossinae               |                     |                                |
| <i>Rossia palpebrosa</i>         | MV                  | ALB9402.19.27                  |
| Family Spirulidae                |                     |                                |
| <i>Spirula spirula</i>           | MV&RY               |                                |
| Family Sepiadariidae             |                     |                                |
| <i>Sepioloidea lineolata</i>     | AR&MN               | ANU5vii95                      |
| Family Idiosepiidae              |                     |                                |
| <i>Idiosepius pygmaeus</i>       | JS                  |                                |
| Order TEUTHOIDEA                 |                     |                                |
| Suborder MYOPSIDA                |                     |                                |
| Family Loliginidae               |                     |                                |
| <i>Loligo opalescens</i>         | DC                  |                                |
| <i>Loligo pealei</i>             | SH                  | NOAA/Chapman #957              |
| <i>Sepioteuthis australis</i>    | AR&MN               | ANU4vii1995                    |
| Suborder OEGOPSIDA               |                     |                                |
| Family Ancistrocheiridae         |                     |                                |
| <i>Ancistrocheirus lesueuri</i>  | RY                  | Hokusei Maru 1994              |
| Family Architeuthidae            |                     |                                |
| <i>Architeuthis</i> sp.          | TS                  | F 78297                        |
| Family Bathyteuthidae            |                     |                                |
| <i>Bathyteuthis abyssicola</i>   | DC                  | Hokusei Maru 1996              |
| Family Brachioteuthidae          |                     |                                |
| <i>Brachioteuthis beani</i>      | MV                  | JSL3749                        |
| Family Chiroteuthidae            |                     |                                |
| <i>Chiroteuthis veranyi</i>      | MV                  | Hatteras 94, Trawl 13          |
| Family Ctenopterygidae           |                     |                                |
| <i>Ctenopteryx sicula</i>        | RY                  | Hokusei Maru 1994              |
| Family Cranchiidae               |                     |                                |
| <i>Cranchia scabra</i>           | RY                  | Hokusei Maru 1994              |
| <i>Liocranchia valdiviae</i>     | RY                  | New Horizon 1993               |
| Family Cycloteuthidae            |                     |                                |
| <i>Cycloteuthis sirventi</i>     | RY                  | Hokusei Maru 1994              |
| <i>Discoteuthis laciniosa</i>    | RY                  | Hokusei Maru 1994              |

TABLE 1 (Continued). CLASSIFICATION OF CEPHALOPOD TAXA INCLUDED IN THIS STUDY (VOSS, 1977)

| Classification                    | <sup>a</sup> Source | <sup>b</sup> Collection Number |
|-----------------------------------|---------------------|--------------------------------|
| Family Enoploteuthidae            |                     |                                |
| <i>Abralia</i> sp.                | MV                  | Hatteras 94, Trawl 20          |
| <i>Enoploteuthis reticulata</i>   | DC                  | Hokusei Maru 1996              |
| Family Gonatidae                  |                     |                                |
| <i>Gonatus berryi</i>             | DC                  | Hokusei Maru 1996              |
| <i>Gonatus onyx</i>               | DC                  | NOAA/DSJordan9606              |
| <i>Gonatopsis borealis</i>        | DC                  | NOAA/DSJordan9606              |
| Family Histioteuthidae            |                     |                                |
| <i>Histioteuthis hoylei</i>       | DC                  | Hokusei Maru 1996              |
| Family Joubiniteuthidae           |                     |                                |
| <i>Joubiniteuthis portieri</i> .  | DC                  | Hokusei Maru 1996              |
| Family Lepidoteuthidae            |                     |                                |
| <i>Lepidoteuthis grimaldii</i>    | RY                  | Hokusei Maru 1994              |
| Family Lycoteuthidae              |                     |                                |
| <i>Lycoteuthis lorigera</i>       | MV&RY               | South Africa Museum            |
| Family Mastigoteuthidae           |                     |                                |
| <i>Mastigoteuthis magna</i>       | MV                  | JSL3750                        |
| Family Neoteuthidae               |                     |                                |
| <i>Alluroteuthis antarctica</i>   | MV                  | Polar Stern 1997               |
| Family Octopoteuthis              |                     |                                |
| <i>Octopoteuthis nielsenii</i>    | RY                  | Hokusei Maru 1994              |
| Family Ommastrephidae             |                     |                                |
| <i>Ommastrephes barramii</i>      | DC                  | Hokusei Maru 1996              |
| <i>Sthenoteuthis oualaniensis</i> | RY                  | Hokusei Maru 1994              |
| Family Onychoteuthidae            |                     |                                |
| <i>Onychoteuthis compacta</i>     | DC                  | Hokusei Maru 1996              |
| <i>Moroteuthis knipovitchi</i>    | MV                  | Polar Stern 1997               |
| Family Pholidoteuthidae           |                     |                                |
| <i>Pholidoteuthis adami</i>       | MV                  | F/V Contender 1994             |
| Family Psychroteuthidae           |                     |                                |
| <i>Psychroteuthis glacialis</i>   | MV                  | Polar Stern 1997               |
| Family Pyroteuthidae              |                     |                                |
| <i>Pyroteuthis addolux</i>        | BS                  | NHI-95-161                     |
| Family Thysanoteuthidae           |                     |                                |
| <i>Thysanoteuthis rhombus</i>     | DC                  | Hokusei Maru 1996              |
| Order OCTOPODA                    |                     |                                |
| Suborder CIRRATA                  |                     |                                |
| Family Cirroteuthidae             |                     |                                |
| <i>Cirrothauma murrayi</i>        | BS                  | NHI-95-291                     |
| Family Stauroteuthidae            |                     |                                |
| <i>Stauroteuthis syrtensis</i>    | MV                  | F/V Contender 1995             |
| <i>Grimpoteuthis glacialis</i>    | MV                  | Polar Stern 1997               |
| Family Opisthoteuthidae           |                     |                                |
| <i>Opisthoteuthis sp.2</i>        | MV&RY               | South Africa Museum            |
| Suborder INCIRRATA                |                     |                                |
| Family Argonautidae               |                     |                                |
| <i>Argonauta nodosa</i>           | TS                  | MOV-F 75026                    |

TABLE 1 (Continued). CLASSIFICATION OF CEPHALOPOD TAXA INCLUDED IN THIS STUDY (VOSS, 1977)

| Classification                   | <sup>a</sup> Source | <sup>b</sup> Collection Number |
|----------------------------------|---------------------|--------------------------------|
| Family Bolitaenidae              |                     |                                |
| <i>Eledonella pygmaea</i>        | RY                  | New Horizon 1993               |
| <i>Japatella diaphana</i>        | DC                  | Hokusei Maru 1996              |
| Family Octopodidae               |                     |                                |
| Subfamily Octopodinae            |                     |                                |
| <i>Octopus tetricus</i>          | TS                  | MOV-F 78082                    |
| <i>Hapalochlaena maculosa</i>    | TS                  | MOV-F 78078                    |
| Subfamily Bathypolypodinae       |                     |                                |
| <i>Bathypolypus arcticus</i>     | MV&RY               | ALB9402.1.1                    |
| Subfamily Graneledoninae         |                     |                                |
| <i>Graneledone verrucosa</i>     | MV&RY               | F/V Contender 1994             |
| Family Vitreledonellidae         |                     |                                |
| <i>Vitreledonella richardi</i>   | RY                  | New Horizon 1996               |
| Order VAMPYROMORPHA              |                     |                                |
| Family Vampyroteuthidae          |                     |                                |
| <i>Vampyroteuthis infernalis</i> | DC                  | Hokusei Maru 1996              |

<sup>a</sup>Source code: AR = Amanda Reid; BS = Brad Seibel; DC = David Carlini; JS = Jayson Semmens; MN = Mark Norman; MV = Michael Vecchione; RY = Richard Young; SH = Scott Herke; TS = Timothy Stranks. The genus and species of each sample was determined by the source indicated.

<sup>b</sup>In cases where collection numbers are not available, the oceanographic cruise from which the sample was obtained is listed instead.

Figure 3. Multiple alignment of nucleotide sequences of the cytochrome *c* oxidase I gene of cephalopods. Nucleotide positions relative to the 3' end of the LCO1498 primer (Folmer et al., 1994) are indicated at the top of each page. Positions with identical nucleotides are shown as a dot (.), and positions with unknown nucleotide characters are indicated as a question mark. The genus and in some cases the species name of the taxa are given to the left of each sequence. The cytochrome *c* oxidase nucleotide sequences are arranged alphabetically by taxon (Oegopsida, Myopsida, Sepioidea, Incirrata, Cirrata, Vampyromorpha) with the outgroup taxon *Nautilus* at the bottom.

|                   |             |             |                |               |               |               |               |              |                 |                 |
|-------------------|-------------|-------------|----------------|---------------|---------------|---------------|---------------|--------------|-----------------|-----------------|
| Abralia           | ACACTTATT   | TCATTTTGG   | TATCTGATCG     | GGATTATTAG    | GAACATCCTT    | AGACTAATG     | ATTGCMACCG    | AATTAGTCA    | ACCAGGTTCT      | TTACTTAACG      |
| Alluroteuthis     | ...T.A...   | ...T.C...   | ...C.T...      | ...G.T...     | ...C.T...     | ...G.A...     | ...C.A...     | ...C.C...    | ...A.A...       | ...G.A...       |
| Ancistrocheirus   | ...G...     | ...T.C...   | ...G.A...      | ...T...       | ...G.C.T.A... | ...T...       | ...G...       | ...G.A...    | ...C.C.T.A...   | ...T...         |
| Architeuthis      | ...A.C...   | ...T.C...   | ...T.G.A...    | ...C.TC...    | ...C...       | ...T.T...     | ...T.T...     | ...A...      | ...G.A...       | ...T.A...       |
| Bachyeteuthis     | ...C...     | ...T.C.C... | ...T.G.A...    | ...T.T.T...   | ...T.A...     | ...C...       | ...C...       | ...A...      | ...G.A...       | ...T.A.T...     |
| Brachyoteuthis    | ?????????   | ????        | ...T.G.A...    | ...C.TC...    | ...G.T...     | ...T.T...     | ...T.T...     | ...A...      | ...A.G...       | ...C.T.A.T...   |
| Chiroteuthis      | ...A.C...   | ...T...     | ...G.A...      | ...G...       | ...T.T...     | ...T.T...     | ...C...       | ...C...      | ...A...         | ...A.T...       |
| Chtenopteryx      | ...T.A.C... | ...T...     | ...G.A...      | ...T...       | ...T.A...     | ...T.T...     | ...G...       | ...A...      | ...T.A...       | ...A.T...       |
| Cranchia          | ...T.G.C... | ...T...     | ...G.A...      | ...T...       | ...C.T...     | ...T.A...     | ...C.T...     | ...C.T...    | ...C.G...       | ...C.C.T.A...   |
| Cyclotheuthis     | ...A...     | ...T...     | ...G...        | ...C.C...     | ...C.T.TC...  | ...A...       | ...C.C.T...   | ...C.C...    | ...T.C.A...     | ...C.T.A...     |
| Discoteuthis      | ...A...     | ...C...     | ...G.A...      | ...C.C.T...   | ...T.T.TC...  | ...A...       | ...C...       | ...A...      | ...A.C.C...     | ...T...         |
| Enoploteuthis     | ...C...     | ...T...     | ...G.A...      | ...C...       | ...T...       | ...A...       | ...T.T...     | ...C...      | ...C...         | ...T.A.T...     |
| Gonatopsis        | ...T.A...   | ...T.C...   | ...T.G.A...    | ...C...       | ...G.T.TC...  | ...A...       | ...G.T...     | ...C.G...    | ...T...         | ...C.A.T...     |
| Gonatus_berryi    | ...T.A...   | ...T.C...   | ...T.G.A...    | ...T.C.C...   | ...C.C...     | ...A...       | ...T...       | ...G.G...    | ...T.C.C...     | ...C.A...       |
| Gonatus_onyx      | ...T.A...   | ...T.C...   | ...T.G.A...    | ...T.C.C...   | ...C.C...     | ...A...       | ...T...       | ...G.G...    | ...T.C.C...     | ...C.A...       |
| Hioteuthis        | ...T.A...   | ...T.C...   | ...T.G.A...    | ...T.C.C...   | ...C.C...     | ...A...       | ...T...       | ...G.G...    | ...T.C.C...     | ...C.A...       |
| Joubiniteuthis    | ...T.A...   | ...T.C...   | ...T.G.A...    | ...T.C.C...   | ...C.C...     | ...A...       | ...T...       | ...G.G...    | ...T.C.C...     | ...C.A...       |
| Lepidoteuthis     | ...T.A.C... | ...T...     | ...G.A...      | ...T.C...     | ...T.T...     | ...G.G...     | ...C.T.T...   | ...C...      | ...T.A...       | ...C.C.T.A...   |
| Liocranchia       | ...T.A.C... | ...T...     | ...G.A...      | ...T.C...     | ...T.T...     | ...T.A...     | ...T.T...     | ...G...      | ...A.A...       | ...C.T.T.A...   |
| Lycoteuthis       | TT.A...     | ...T.C...   | ...T.G.A...    | ...T...       | ...T.A...     | ...T.A...     | ...T.T...     | ...C...      | ...TT...        | ...C.T.A.T...   |
| Mastigoteuthis    | ...A...     | ...T...     | ...G.A...      | ...G...       | ...C.T...     | ...T...       | ...T...       | ...C...      | ...C.A...       | ...T.C.C.C...   |
| Moroteuthis       | ...T.A.C... | ...T.C...   | ...T.G.A...    | ...G...       | ...C.T...     | ...T...       | ...T...       | ...C...      | ...A.A...       | ...A.C.A.A...   |
| Ommastrephes      | ...T.A...   | ...T...     | ...G.A...      | ...C.C...     | ...G.T.TC...  | ...C...       | ...A.C.T.T... | ...C...      | ...T.A.C.C.T... | ...G.T...       |
| Onychoteuthis     | ...T.A.C... | ...T.C...   | ...T.G.T...    | ...T...       | ...T.T...     | ...T.A...     | ...T.T...     | ...A...      | ...G.A...       | ...A.A...       |
| Pholidoteuthis    | ...A...     | ...T...     | ...G.T.GG.T... | ...T.G...     | ...C.TC...    | ...T.GT...    | ...A.C.T.T... | ...C.C...    | ...T.C...       | ...C.T.A.T...   |
| Psychroteuthis    | ...T.A.C... | ...C.C...   | ...T.G.A...    | ...T.C...     | ...T...       | ...TT...      | ...A.C.T.T... | ...C.A...    | ...C.A...       | ...A.T...       |
| Pyroteuthis       | ...T.A.C... | ...C...     | ...T.G.T...    | ...T.GC.T...  | ...T.A...     | ...T.A...     | ...T.A...     | ...C...      | ...A.C.T.T.A... | ...T...         |
| Stenoteuthis      | ...T.A.C... | ...T...     | ...GG.A...     | ...C.TC...    | ...G.C.C...   | ...T.A...     | ...C.T...     | ...C...      | ...C.G.G...     | ...A.A...       |
| Thysanoteuthis    | ...A...     | ...T...     | ...G.T.GG.T... | ...T.G...     | ...C.TC...    | ...T.GT...    | ...A.C.T.T... | ...C.C...    | ...T.C...       | ...C.T.A.T...   |
| Octopoteuthis     | ...A...     | ...C...     | ...T.G.C...    | ...T...       | ...T...       | ...T.A...     | ...T.A...     | ...C...      | ...C.A...       | ...A.T...       |
| Loligo_opalescens | ?????????   | ??          | ...A.C...      | ...G.C.G...   | ...T.A...     | ...T.G.A...   | ...C.T.A...   | ...G.A...    | ...T.C.C...     | ...T.A.T...     |
| Loligo_pealei     | ...T.A...   | ...T.A...   | ...T.G.A...    | ...G...       | ...T.T.A...   | ...G.T.G.A... | ...T.A...     | ...C.T.AA... | ...T.A.A...     | ...T.A.T...     |
| Sepioteuthis      | ...A...     | ...C...     | ...T.G.A...    | ...T.G...     | ...T.T.A...   | ...T.G.A...   | ...C...       | ...G...      | ...A.A...       | ...C.T.A.T...   |
| Heteroteuthis     | TT.A...     | ...T...     | ...C...        | ...A.TC.C...  | ...A...       | ...GT.G.A...  | ...T...       | ...AA...     | ...T.A.A...     | ...C.G.T.A.T... |
| Idiosepius        | ...T...     | ...T...     | ...G.T...      | ...T.G...     | ...T.T.T...   | ...T.T...     | ...A.C...     | ...G...      | ...A.A...       | ...T.T...       |
| Rossia            | ?????????   | ?????????   | ?????????      | ?????????     | ?????????     | ?????????     | ?????????     | ?????????    | ?????????       | ?????????       |
| Sepia_officinalis | ...T.A...   | ...T...     | ...A...        | ...T...       | ...C.C.A...   | ...C...       | ...T.T...     | ...A...      | ...C.A.A...     | ...T.A.T...     |
| Sepia_opipara     | ...A.C...   | ...G.A...   | ...A.T...      | ...A...       | ...T.C.GC...  | ...T.AC...    | ...G.A...     | ...G.AA...   | ...T.A.A...     | ...C.T.A.T...   |
| Sepioloidea       | ...A.C...   | ...G.A...   | ...C...        | ...GC.T...    | ...T.AC...    | ...T.AC...    | ...G.A...     | ...G.AA...   | ...T.A.A...     | ...C.T.A.T...   |
| Spirula           | ...T.A.C... | ...A.T...   | ...G.A...      | ...C...       | ...T.T...     | ...T.T...     | ...A...       | ...A...      | ...A.A...       | ...T.A.T...     |
| Stoloteuthis      | ?????????   | ?????????   | ?????????      | ?????????     | ?????????     | ?????????     | ?????????     | ?????????    | ?????????       | ?????????       |
| Argonauta         | ...T.A...   | ...T...     | ...A...        | ...C.C.C...   | ...T...       | ...T...       | ...A...       | ...A...      | ...A.C...       | ...T.A.T...     |
| Bathypolypus      | ?????????   | ?????????   | ?????????      | ?????????     | ?????????     | ?????????     | ?????????     | ?????????    | ?????????       | ?????????       |
| Eledonella        | ...C.T.A... | ...T...     | ...C...        | ...A...       | ...T...       | ...TC...      | ...T...       | ...A...      | ...A...         | ...C.T...       |
| Graneledone       | ...T.A...   | ...T...     | ...C...        | ...A...       | ...C...       | ...T...       | ...A...       | ...A...      | ...A...         | ...C.T...       |
| Hapalochlaena     | ...A...     | ...T...     | ...A...        | ...T.CC.T...  | ...T.C.A...   | ...T.T...     | ...A...       | ...A...      | ...A.C...       | ...T.T...       |
| Japattella        | ...T.A.C... | ...T...     | ...C...        | ...A...       | ...T.C...     | ...T.T...     | ...A.C.C.T... | ...A.A...    | ...G.G.A...     | ...T.T...       |
| Octopus           | ...A.C...   | ...T...     | ...A...        | ...T.A...     | ...C.T...     | ...T.T...     | ...A...       | ...C...      | ...G.C.C.C...   | ...A.T...       |
| Vitreledonella    | TT.A...     | ...T...     | ...A...        | ...T.G.G...   | ...C...       | ...A...       | ...T.A...     | ...C...      | ...T.A...       | ...A.T...       |
| Cirrochauma       | ...T.A...   | ...T...     | ...A...        | ...T.C...     | ...AC...      | ...T.A...     | ...C.G...     | ...G.C...    | ...C.C.A...     | ...C.T.A.T...   |
| Grimpoteuthis     | ...T.A...   | ...T...     | ...A...        | ...T...       | ...C.A...     | ...T.A...     | ...T.A...     | ...C.T...    | ...A...         | ...C.C.T.A.T... |
| Opisthoteuthis    | ...T.A.C... | ...T...     | ...A...        | ...T.C.C.T... | ...C.A...     | ...T.A...     | ...T.A...     | ...A...      | ...A...         | ...T.A.T...     |
| Stauroteuthis     | ...T.A...   | ...T.C...   | ...A...        | ...T.C.C.T... | ...C.A...     | ...T...       | ...A...       | ...A...      | ...A...         | ...C.T.A.T...   |
| Vampyroteuthis    | TT.A...     | ...T...     | ...A...        | ...T.A...     | ...T...       | ...TT...      | ...T.A...     | ...C.T.A...  | ...A.C...       | ...T.A.T...     |
| Nautilus          | ...T...     | ...C...     | ...K.T.A.C...  | ...A.T.G.C... | ...T.G.C...   | ...T.T.T.A... | ...T.A...     | ...A.A...    | ...T.AA...      | ...C.TT.AGGA... |



|                   | 110        | 120        | 130        | 140        | 150         | 160        | 170         | 180        | 190        | 200          |
|-------------------|------------|------------|------------|------------|-------------|------------|-------------|------------|------------|--------------|
| Abrella           | ARGATCAGCT | TTACAATGTA | GTATGAGTCT | CTCATGAGTT | TATPATTAATT | TCTTTTATAG | TAAATACCAAT | CATAAATGGA | GGATTTGGGA | ACTGATTAAGT  |
| Alluroteuthis     | .C.C.A.A.  | A..T.....  | .G.C.A.A.  | C.C.....   | .....T.G    | .....T     | .....T      | .....T     | .....T     | .....C...    |
| Ancistrocheirus   | .C.A.A.    | A..T.....  | .T.....    | C.....     | .....CT     | .....T     | .....T      | .....T     | .....T     | .....T       |
| Bathyteuthis      | .C.A.A.    | C..T.....  | .T.....    | C.....     | .....T      | .....T     | .....T      | .....T     | .....G     | .....T       |
| Brachioteuthis    | .AT.A.     | A.....     | A.C.....   | .....      | .....C      | .....T     | .....T      | .....T     | .....T     | .....T       |
| Chiroteuthis      | .AT.A.     | A..T.....  | A.A.C.     | C.C.C.T.   | .....       | F.G.C.T    | .....T      | .....T     | .....T     | .....T       |
| Chtenopteryx      | .AT.A.     | T.....     | T.....     | C.C.T.     | .....       | T.....     | .....T      | .....T     | .....T     | .....T       |
| Cranchia          | .T.C.      | .....      | .....      | .....      | .....       | .....      | .....       | .....      | .....      | .....        |
| Cycloteuthis      | .C.C.A.T.  | A..T.....  | T.A.....   | .....      | .....       | CT         | .....       | .....      | .....      | .....        |
| Discoteuthis      | .C.A.A.    | A..T.....  | A.....     | C.....     | .....       | .....      | .....       | .....      | .....      | .....        |
| Enoploeteuthis    | .C.A.A.    | A..T.....  | .G.A.....  | .....      | .....       | .....      | .....       | .....      | .....      | .....        |
| Gonatopsis        | .C.A.A.    | .....T     | T.A.C      | C.....     | .....       | T.C        | .....       | .....G     | .....      | .....T.C.T.  |
| Gonatus_berryi    | .C.A.A.    | T.C.T      | C.A.C      | C.....     | .....       | T.T        | .....       | .....      | .....      | .....T.C.T.  |
| Gonatus_onyx      | .A.C       | .....T     | T.A.C      | C.....     | .....       | T.T        | .....       | .....      | .....      | .....T.C.T.  |
| Histioteuthis     | .A.A.T     | .....T     | A.A.C      | C.C.C.T.   | .....       | T.T        | .....       | .....      | .....      | .....T       |
| Joubiniteuthis    | .A.        | T.....     | T.....     | C.C.       | .....       | T          | .....       | .....      | .....      | .....T       |
| Lepidoteuthis     | .A.        | A.T.C.C    | A.A.       | A.....     | .....       | T          | .....       | .....      | .....      | .....T       |
| Liocranchia       | .A.        | T.....     | A.....     | .....      | .....       | T          | .....       | .....      | .....      | .....T.GC    |
| Lycoteuthis       | .C.A.A.    | A..T.....  | T.C.TA     | .....      | .....       | CC.T       | .....       | .....      | .....      | .....T       |
| Mastigoteuthis    | .AT.A.     | A.....     | A.C        | C.....     | .....       | CT         | .....       | .....      | .....      | .....T       |
| Moroteuthis       | .A.        | A.....     | A.A.C      | C.G.       | .....       | T.C        | .....       | .....      | .....      | .....T       |
| Ommastrephes      | .A.A.      | A..C.....  | .....      | G.C.T.     | .....       | T          | .....       | .....      | .....      | .....T.C     |
| Onychoteuthis     | .C.C.A.    | C.T.....   | A.....     | C.....     | .....       | T.C        | .....       | .....      | .....      | .....T       |
| Pholidoteuthis    | .C.C.A.    | A..T.....  | T.A.C      | C.....     | .....       | T.CT       | .....       | .....      | .....      | .....T.C.C.  |
| Psychroteuthis    | .C.A.T.    | A..T.....  | T.T.C      | C.....     | .....       | T          | .....       | .....      | .....      | .....T.C.C.  |
| Pyroteuthis       | .C.A.      | .....G     | T.A        | .....      | .....       | T          | .....       | .....      | .....      | .....C.A     |
| Schenoteuthis     | .A.A.      | A..T.....  | C.....     | A.....     | .....       | T          | .....       | .....      | .....      | .....C.A     |
| Thysanoteuthis    | .C.C.A.    | A.T.C      | .....      | T.T.C      | .....       | T.CT.G     | .....       | .....      | .....      | .....C.T.    |
| Octopoteuthis     | .C.C.A.T.  | A..T.....  | A.....     | C.C.       | .....       | CC         | .....       | .....      | .....      | .....T       |
| Loligo_opalescens | .AT.A.     | A.....     | T.....     | A.....     | .....       | T          | .....       | .....      | .....      | .....T.C     |
| Loligo_pealei     | .AT.A.     | A.....     | T.....     | A.C.T.C    | .....       | T          | .....       | .....      | .....      | .....T.G     |
| Sepioteuthis      | .AT.A.     | A..T.....  | T.G        | .....      | .....       | T          | .....       | .....      | .....      | .....G.A     |
| Heteroteuthis     | .T.A.T     | .....      | T.....     | .....      | .....       | T          | .....       | .....      | .....      | .....G.G     |
| Idiosepius        | .C.A.T.    | A..T.....  | T.A.A      | C.C.       | .....       | T          | .....       | .....      | .....      | .....T       |
| Rossia            | .C.A.T.    | A.T.C      | .....      | A.C.T      | .....       | T          | .....       | .....      | .....      | .....T.G     |
| Sepia_officinalis | .C.C.A.    | .....T     | A.....     | C.T.       | .....       | T          | .....       | .....      | .....      | .....C.T.    |
| Sepia_opipara     | .A.        | C.T.C.T    | A.T.A      | .....      | .....       | T          | .....       | .....      | .....      | .....T.C.T.  |
| Sepioloida        | .C.A.T.    | A.T.C      | .....      | A.C        | .....       | T          | .....       | .....      | .....      | .....T       |
| Spirula           | .AT.A.     | A..T.....  | T.C        | C.C.       | .....       | T          | .....       | .....      | .....      | .....T       |
| Stoloteuthis      | .C.A.T.    | A..T.....  | A.G        | A.C        | .....       | CG         | .....       | .....      | .....      | .....G       |
| Argonauta         | .C.A.A.    | A..T.....  | A.T.A      | C.CT       | .....       | T          | .....       | .....      | .....      | .....T       |
| Bathypolypus      | .A.A.      | A..T.....  | G.A.T.A    | A.C        | .....       | T          | .....       | .....      | .....      | .....T.GC.T. |
| Eledonella        | .C.A.T.    | A..T.....  | A.T        | C.C        | .....       | T          | .....       | .....      | .....      | .....T       |
| Granelledone      | .A.A.      | A..T.....  | A.T.A      | C.C        | .....       | T          | .....       | .....      | .....      | .....T       |
| Hapalochlaena     | .AT.A.     | T.....     | A.T.A      | C.CC       | .....       | T          | .....       | .....      | .....      | .....T       |
| Japatella         | .AT.A.     | T.C        | A.T.T      | C.CT       | .....       | T          | .....       | .....      | .....      | .....T.C.A   |
| Octopus           | .A.A.      | A..T.....  | A.T.A      | C.C        | .....       | T          | .....       | .....      | .....      | .....T       |
| Vitreledonella    | .AT.A.     | T.....     | A.T.A      | C.C        | .....       | T          | .....       | .....      | .....      | .....T       |
| Cirrothauma       | .C.A.      | .....T     | A.C.A.C    | .....      | .....       | CG.A       | .....       | .....      | .....      | .....T       |
| Grimpoteuthis     | .A.        | .....C     | A.T.A      | .....      | .....       | CG.C.MM    | .....       | .....      | .....      | .....T       |
| Opisthoteuthis    | .AT.A.     | T.....     | A.T.A      | .....      | .....       | T          | .....       | .....      | .....      | .....G       |
| Stauroteuthis     | .C.A.T.    | A..T.....  | A.T.A      | C.C        | .....       | T          | .....       | .....      | .....      | .....T       |
| Vampyroteuthis    | .AT.A.     | T.....     | A.T.C.A    | .....      | .....       | CT         | .....       | .....      | .....      | .....T       |
| Nautilus          | .C.A.T.    | A..T.....  | A.C.T.A    | C.CC       | .....       | G          | .....       | .....      | .....      | .....T       |

210 220 230 240 250 260 270 280 290 300

|                    |            |            |            |            |            |            |            |            |            |           |
|--------------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|-----------|
| Abrealia           | TCCCTTAATA | TTAGGAGCTC | CTGATATAGC | TTTTCCCGGT | ATAAATAATA | TAAATCTCTG | GTTATTAAGT | CCATCTTTAA | CTATACTACT | AGGCTCCCA |
| Alluroteuthis      | A.T.       | C.C.C.C.A. | A.A.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | T.A.A.A.A. | A.A.C.G.C  | A.A.C.G.C  | A.C.T.G.   | .T.T.     |
| Ancistrocheirus    | C.AC.      | C.G.A.     | G.A.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | A.A.A.A.A. | A.A.A.A.A. | C.C.TT.    | G.C.T.    |
| Architeuthis       | A.C.       | A.A.A.A.A. | A.A.A.A.A. | A.A.A.A.A. | A.A.A.A.A. | A.A.A.A.A. | A.A.A.A.A. | A.A.A.A.A. | A.C.T.     | .T.T.     |
| Bathyteuthis       | C.T.       | G.C.A.     | G.A.C.A.A. | A.C.A.A.A. | C.C.A.A.A. | T.A.C.A.A. | A.A.C.C.T  | C.A.A.     | T.C.       | .T.T.     |
| Brachyteuthis      | TC.T.      | C.A.A.A.A. | C.A.A.A.A. | C.A.A.A.A. | C.A.A.A.A. | T.A.C.T.T. | A.C.T.C.T. | T.         | A.C.C.T.   | C.A.T     |
| Chiroteuthis       | A.T.       | G.A.A.A.A. | A.A.A.A.A. | A.A.A.A.A. | A.A.A.A.A. | T.A.A.A.A. | A.A.A.A.A. | A.A.A.A.A. | A.T.       | .T.T.C    |
| Chtenopteryx       | T.         | G.C.A.A.A. | A.A.A.A.A. | C.A.A.A.A. | G.A.A.A.A. | T.A.A.A.A. | A.A.C.C.C. | C.         | C.T.T.     | TA.A.T.C  |
| Cranchia           | C.         | A.A.A.A.A. | C.A.A.A.A. | C.A.A.A.A. | C.A.A.A.A. | T.A.A.A.A. | T.A.A.A.A. | T.A.A.A.A. | C.T.T.F.   | A.A.T.    |
| Cycloteuthis       | C.T.       | G.C.A.A.A. | A.A.A.A.A. | C.A.A.A.A. | C.A.A.A.A. | T.A.A.A.A. | A.C.TC.    | C.         | A.C.C.     | C.C.T.    |
| Discoteuthis       | A.         | G.C.A.A.A. | C.A.A.A.A. | C.A.A.A.A. | C.A.A.A.A. | T.A.A.A.A. | A.C.C.T.   | C.A.A.     | A.C.TT.    | .T.T.     |
| Enoploteuthis      | T.         | G.C.A.A.A. | C.T.A.A.A. | C.T.A.A.A. | T.T.T.T.C. | T.A.A.A.A. | A.A.A.A.A. | A.A.A.A.A. | C.T.       | T.T.A.    |
| Gonatopsis         | A.         | C.C.A.A.A. | C.T.A.A.A. | C.T.A.A.A. | T.T.T.T.C. | T.A.A.A.A. | A.A.A.A.A. | A.A.A.A.A. | A.C.TT.    | .T.T.     |
| Gonatus_berryi     | A.         | C.C.A.A.A. | C.T.A.A.A. | C.T.A.A.A. | T.T.T.T.C. | T.A.A.A.A. | A.A.A.A.A. | A.A.A.A.A. | A.C.TT.    | .T.T.     |
| Gonatus_onyx       | T.         | C.C.A.A.A. | C.T.A.A.A. | C.T.A.A.A. | T.T.T.T.C. | T.A.A.A.A. | A.A.A.A.A. | A.A.A.A.A. | A.C.TT.    | .T.T.     |
| Histioteuthis      | T.         | C.C.A.A.A. | C.T.A.A.A. | C.T.A.A.A. | T.T.T.T.C. | T.A.A.A.A. | A.A.A.A.A. | A.A.A.A.A. | A.C.TT.    | .T.T.     |
| Joubiniteuthis     | A.T.       | G.A.A.A.A. | A.C.A.A.A. | A.C.A.A.A. | C.C.A.A.A. | T.A.C.T.T. | A.C.TC.T.  | C.C.C.     | T.TT.      | .A.T.G    |
| Lepidoteuthis      | A.TC.      | C.G.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | .C.TC.T.   | .T.C.G.    | .T.C.G.    | C.T.T.     | T.T.G     |
| Liocranchia        | A.T.       | C.C.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | .C.TC.T.   | .T.C.G.    | .T.C.G.    | A.C.T.     | A.C.T.    |
| Lycoteuthis        | T.         | G.A.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | .C.TC.T.   | .T.C.G.    | .T.C.G.    | C.C.C.     | A.T.T     |
| Mastigoteuthis     | G.T.       | A.A.A.A.A. | A.A.A.A.A. | A.A.A.A.A. | A.A.A.A.A. | T.T.A.A.A. | T.T.A.A.A. | T.A.A.A.A. | C.         | T.A.      |
| Moroteuthis        | G.C.T.     | C.G.A.A.A. | A.A.A.A.A. | A.A.A.A.A. | A.A.A.A.A. | T.A.A.A.A. | A.C.C.C.C  | A.C.C.C.C  | C.TT.      | C.T.      |
| Ommastrephes       | G.G.C.G.C. | A.A.A.A.A. | A.A.A.A.A. | A.A.A.A.A. | A.A.A.A.A. | .G.C.T.    | .G.C.T.    | .G.C.T.    | C.C.C.     | T.T.T     |
| Onychoteuthis      | G.AC.T.G   | T.A.A.A.A. | T.A.A.A.A. | T.A.A.A.A. | T.A.A.A.A. | T.T.A.A.A. | T.A.A.A.A. | T.A.A.A.A. | A.T.       | T.A.A.T.  |
| Pholidoteuthis     | C.T.       | G.A.A.A.A. | C.T.A.A.A. | C.T.A.A.A. | C.T.A.A.A. | T.A.A.A.A. | .C.TC.T.C  | C.         | T.GT.      | C.        |
| Psychroteuthis     | A.T.       | T.A.A.A.A. | C.C.T.A.A. | C.C.T.A.A. | C.C.T.A.A. | T.A.A.A.A. | A.A.C.T.   | A.A.C.T.   | A.T.       | A.A.T     |
| Pyroteuthis        | G.T.G.G    | T.A.A.A.A. | C.C.T.A.A. | C.C.T.A.A. | C.C.T.A.A. | T.A.A.A.A. | A.A.A.A.A. | A.A.A.A.A. | CC.        | C.A.C     |
| Sthenoteuthis      | T.         | G.A.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | T.A.A.A.A. | A.C.C.C.T. | T.CC.      | T.T.       | T.T.T     |
| Thysanoteuthis     | TC.        | C.G.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | T.A.A.A.A. | A.C.C.T.   | T.A.C.C.T. | A.C.T.     | A.A.T     |
| Octopoteuthis      | A.C.       | C.C.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | T.A.A.A.A. | T.A.A.A.A. | T.A.A.A.A. | A.C.T.     | A.A.T     |
| Lolligo_opalescens | A.T.       | T.A.A.A.A. | A.C.A.A.A. | A.C.A.A.A. | A.C.A.A.A. | T.A.A.A.A. | A.C.C.C.   | T.C.       | TA.A.      | .G        |
| Lolligo_pealei     | A.         | G.C.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | T.A.A.A.A. | A.C.C.T.A  | T.A.C.C.   | C.T.TT.    | A.A.      |
| Sepioteuthis       | C.TC.      | G.A.A.A.A. | A.C.T.A.A. | A.C.T.A.A. | A.C.T.A.A. | T.A.A.A.A. | T.A.A.A.A. | T.A.A.A.A. | C.TT.      | C.T       |
| Heteroteuthis      | G.T.       | G.A.A.A.A. | A.C.T.A.A. | A.C.T.A.A. | A.C.T.A.A. | T.A.A.A.A. | A.C.T.A.   | A.T.C.     | T.G.       | .T.       |
| Idiosepius         | A.T.       | C.A.A.A.A. | A.C.A.A.A. | A.C.A.A.A. | A.C.A.A.A. | T.A.A.A.A. | A.C.TC.T.  | G.         | A.T.       | C.A.      |
| Rossia             | C.G.       | C.A.A.A.A. | C.T.A.A.A. | C.T.A.A.A. | C.T.A.A.A. | T.A.A.A.A. | A.C.T.C    | T.CC.      | CC.        | CT.       |
| Sepia_officinalis  | C.T.       | T.C.A.A.A. | T.A.A.A.A. | T.A.A.A.A. | T.A.A.A.A. | T.A.A.A.A. | T.A.A.A.A. | T.A.A.A.A. | CC.TT.     | T.C.G.C   |
| Sepia_opipara      | T.         | C.C.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | T.A.A.A.A. | A.A.A.A.A. | A.A.A.A.A. | C.T.       | T.C.A.T   |
| Sepioloidea        | A.A.       | A.A.A.A.A. | A.C.A.A.A. | A.C.A.A.A. | A.C.A.A.A. | T.A.A.A.A. | A.C.C.     | G.C.       | T.T.       | G.T.      |
| Spirula            | A.T.       | C.C.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | T.A.A.A.A. | A.C.C.T.   | T.C.T.     | .ATA.      | TG.       |
| Stoloteuthis       | G.TC.      | C.A.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | T.A.A.A.A. | A.C.C.C    | T.A.A.     | A.C.       | T.A.T.    |
| Argonauta          | TC.T.      | A.A.A.A.A. | A.A.A.A.A. | A.A.A.A.A. | A.A.A.A.A. | T.A.A.A.A. | A.C.T.     | TG.        | C.T.TT.    | A.AG.     |
| Bathypolypus       | C.AC.T.    | C.C.A.A.A. | C.C.T.A.A. | C.C.T.A.A. | C.C.T.A.A. | T.A.A.A.A. | A.C.T.     | T.         | A.C.T.T.   | A.T.AG.   |
| Eledonella         | A.A.       | C.C.A.A.A. | C.C.T.A.A. | C.C.T.A.A. | C.C.T.A.A. | T.A.A.A.A. | A.C.T.C    | T.         | C.C.T.     | TATT.TG.T |
| Graneladone        | A.A.       | C.A.A.A.A. | A.C.A.A.A. | A.C.A.A.A. | A.C.A.A.A. | T.A.A.A.A. | A.C.C.T.C  | T.         | CC.T.T.    | TA.A.AG.T |
| Hapalochlaena      | C.T.       | C.A.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | T.A.A.A.A. | A.C.C.T.A  | G.C.C.C.   | T.TT.      | T.A.TG.G  |
| Japatella          | C.T.       | C.A.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | T.A.A.A.A. | A.C.T.T.A  | A.C.C.C.   | C.C.T.     | CAT.TG.T  |
| Octopus            | T.         | C.A.A.A.A. | A.C.A.A.A. | A.C.A.A.A. | A.C.A.A.A. | T.A.A.A.A. | A.C.C.C    | T.C.       | C.T.T.     | TT.A.TG.  |
| Vitreledonella     | AC.        | A.A.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | T.A.A.A.A. | A.C.T.C    | T.         | AT.        | TATA.TG.C |
| Cirrothauuma       | T.         | C.C.G.A.A. | C.A.A.A.A. | C.A.A.A.A. | C.A.A.A.A. | T.A.A.A.A. | .C.TC..C   | T.CC.C.    | C.C.       | A.C.TG.C  |
| Grimptoteuthis     | C.         | G.A.A.A.A. | A.A.A.A.A. | A.A.A.A.A. | A.A.A.A.A. | T.A.A.A.A. | A.C.C.C    | T.A.C.T.   | C.T.       | T.TG.T    |
| Opisthoteuthis     | TC.T.      | C.T.C.C.   | A.A.A.A.A. | A.A.A.A.A. | A.A.A.A.A. | T.A.A.A.A. | A.C.TC.T.C | C.T.       | C.C.T.     | A.C.AG.T  |
| Stauroteuthis      | T.         | C.A.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | T.A.A.A.A. | A.C.T.G    | T.CC.C.    | CC.T.      | A.T.AG.C  |
| Vampyroteuthis     | C.T.       | G.A.A.A.A. | A.A.A.A.A. | A.A.A.A.A. | A.A.A.A.A. | T.A.A.A.A. | .CTC.T.    | .T.T.      | T.T.       | A.C.TG.T  |
| Nautilus           | G.C.T.G    | A.A.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | C.C.A.A.A. | T.A.A.A.A. | A.C.TC..C  | C.         | TGT.       | .T.T.G.C  |

|                   |            |            |             |            |            |              |             |            |           |            |
|-------------------|------------|------------|-------------|------------|------------|--------------|-------------|------------|-----------|------------|
|                   | 310        | 320        | 330         | 340        | 350        | 360          | 370         | 380        | 390       | 400        |
|                   | CCCGTCGAAA | GAGGAGCCGG | AACAGAGATGA | ACTGTTTATC | CTCCACTATC | TAGAAATTTA   | TCTCATCCGG  | GCCCATCCGT | AGATTAGCT | ATTTTCTCTT |
| Abrallia          | .T.        | .C.T.      | .G.         | .A.C.      | .C.T.      | .A.C.T.      | .A.C.T.     | .T.C.G.    | .C.       | .T.C       |
| Alluroteuthis     | .T.        | .C.T.      | .G.         | .A.C.      | .C.T.      | .A.C.T.      | .A.C.T.     | .T.C.G.    | .C.       | .T.C       |
| Ancestrocheirus   | .A.G.      | .G.T.      | .A.         | .A.        | .C.TT      | .A.T.C.T.    | .A.         | .T.F.A.    | .T.C.     | .T.AC      |
| Architeuthis      | .T.G.      | .G.A.      | .C.G        | .A.A.C.    | .CTT       | .C.C.C.C.    | .C.C.       | .C.A.      | .C.C.     | .AC        |
| Bathyteuthis      | .T.A.      | .A.        | .C.T.       | .A.A.      | .C.CT      | .C.          | .A.         | .A.A.      | .C.C.     | .T.CC      |
| Brachiotheuthis   | .T.A.      | .T.G.T.    | .A.         | .A.A.C.    | .C.CT.G.   | .C.A.C.T.    | .A.         | .A.T.A.    | .G.C.A    | .T.AC      |
| Chiroteuthis      | .T.A.      | .G.A.      | .A.         | .A.A.C.    | .C.TT      | .CC.C.A.     | .C.T.A.     | .A.T.A.    | .T.       | .T.C       |
| Chtenopteryx      | .A.T.      | .T.        | .G          | .A.G.      | .TT        | .T.C.T.      | .A.C.A.     | .A.T.      | .T.C      | .T.C       |
| Cyclotheuthis     | .T.A.      | .G.G.      | .A.         | .A.A.      | .C.T.      | .C.C.        | .T.C.A.     | .T.CC.     | .A        | .C.A.A     |
| Discoteuthis      | .A.A.      | .T.        | .T.         | .G.A.G.    | .C.        | .A.          | .A.T.A.     | .T.C.A.    | .C.T.A    | .C         |
| Enplotteuthis     | .T.A.      | .T.T.      | .A.         | .A.A.C.    | .TT        | .C.          | .T.T.       | .T.T.      | .C.G.     | .T.        |
| Gonatopsis        | .T.T.      | .A.        | .G.C.       | .A.C.      | .T.T.      | .C.          | .A.T.A.     | .T.C.      | .C        | .T.        |
| Gonatus_berryi    | .T.        | .G.A.      | .T.         | .A.C.C.    | .C.T.T.    | .C.C.        | .A.C.T.A.   | .T.        | .C        | .T.        |
| Gonatus_onyx      | .A.T.      | .G.A.      | .G.         | .C.C.      | .C.T.T.    | .T.          | .A.T.A.     | .T.C.      | .C        | .T.        |
| Histioteuthis     | .T.A.      | .A.        | .A.         | .A.G.C.    | .C.        | .A.C.A.      | .A.T.A.     | .T.CC.T.   | .T.       | .T.        |
| Joubiniteuthis    | .T.A.G.    | .G.        | .T.         | .C.C.      | .C.T.G.    | .A.F.        | .A.C.T.A.   | .C.T.A.    | .C        | .C         |
| Lepidoteuthis     | .A.        | .T.T.      | .T.         | .C.C.      | .C.TT      | .C.C.C.C.    | .A.T.C.A.   | .C.G       | .C.T.CC   | .T.C       |
| Liocranchia       | .G.T.G.    | .G.G.      | .G.A.       | .G.A.      | .T.T.      | .C.C.T.      | .A.T.A.     | .CC        | .T.C      | .C         |
| Lycoteuthis       | .T.        | .A.        | .A.         | .C.C.      | .CT        | .T.          | .C.T.T.     | .C.G       | .T.C      | .C         |
| Mastigoteuthis    | .T.A.      | .A.        | .C.         | .A.A.      | .C.TT      | .C.T.        | .A.A.C.A.   | .CC.T.A    | .T.C      | .C         |
| Moroteuthis       | .A.A.      | .A.        | .G.         | .A.C.      | .TT        | .G.          | .A.A.T.A.   | .CC.T.     | .T.C      | .C         |
| Ommastrephes      | .G.T.      | .G.A.      | .T.G        | .A.A.      | .C.        | .C.C.C.      | .A.T.A.     | .T.CC.     | .C        | .C         |
| Onychoteuthis     | .T.A.      | .A.        | .A.         | .A.C.      | .A.T       | .T.          | .A.C.T.A.   | .C.T.A     | .AC       | .C         |
| Pholidoteuthis    | .T.A.G.    | .G.        | .T.         | .A.A.C.    | .CT        | .C.T.        | .A.C.T.A.   | .G.C.T.A   | .T.GC     | .T         |
| Psychroteuthis    | .A.A.      | .T.        | .A.         | .A.C.      | .CTT       | .C.C         | .A.T.A.     | .C.T.      | .T.       | .T.        |
| Pyroteuthis       | .T.T.G.    | .T.        | .T.T.T.     | .A.A.C.    | .TT        | .C.C.C.T.    | .T.T.A.     | .C.T.      | .T.C      | .C         |
| Sthenoteuthis     | .T.A.      | .A.        | .A.         | .G.C.      | .TT        | .T.          | .A.A.A.     | .T.C       | .C        | .C         |
| Thysanoteuthis    | .A.A.      | .G.        | .T.         | .C.C.      | .T.F.      | .CC.         | .C.C.T.     | .T.A.      | .C        | .C         |
| Octopoteuthis     | .A.G.G.    | .G.A.      | .T.         | .A.A.C.    | .C.TT      | .CC.C.A.     | .A.T.       | .T.        | .G        | .G         |
| Loligo_opalescens | .T.T.      | .C.        | .T.         | .A.A.C.    | .A.C.      | .C.C.        | .T.T.A.     | .CC.C.C    | .AC       | .C         |
| Loligo_pealei     | .A.T.      | .G.G.      | .T.         | .A.C.      | .C.T.      | .T.CC.T.     | .C.C.C.     | .C.T.A.    | .CC.C.A   | .AC        |
| Sepioteuthis      | .T.T.      | .G.T.      | .T.T.G.     | .A.C.      | .CTT       | .A.T.CC.C.A. | .C.T.T.     | .CC.T.C    | .T.CC     | .C         |
| Heteroteuthis     | .A.A.      | .T.G.      | .C.         | .G.        | .TTT       | .T.A.T.      | .C.A.A.     | .A.T.T.    | .C        | .T.C       |
| Idiosepius        | .A.T.      | .T.        | .T.         | .C.        | .T         | .T.          | .C.A.A.C.A. | .T.        | .G.       | .G.        |
| Rossia            | .T.A.      | .A.        | .C.         | .A.A.C.    | .C.TT      | .A.T.        | .A.C.A.     | .T.C.A.    | .C        | .CC        |
| Sepia_officinalis | .A.T.      | .G.G.      | .T.         | .G.C.      | .C.TT      | .T.C         | .C.C.A.     | .C.C.A.    | .C        | .C         |
| Sepia_opipara     | ATPA.A.    | .G.T.      | .T.         | .A.        | .C.TT      | .C.          | .C.C.A.     | .C.A.      | .C.TC.    | .C         |
| Sepioloidea       | .A.T.      | .C.A.      | .T.         | .A.        | .T.T.      | .A.T.A.C.    | .C.A.       | .C.T.      | .C        | .C         |
| Spirula           | .T.T.      | .G.T.      | .T.         | .A.A.C.    | .A.CT      | .A.C.C.A.    | .C.C.A.     | .T.C.A.    | .T.C      | .CC        |
| Stoloteuthis      | .A.        | .C.A.      | .C          | .A.        | .TTT       | .A.T.        | .A.C.A.     | .A.T.      | .T.CC     | .C         |
| Argonauta         | .A.T.      | .C.A.      | .TG.        | .A.        | .G.T.T.    | .A.G.        | .ATA.       | .T.T.      | .A        | .C.T.C     |
| Bathypolypus      | .A.A.      | .G.T.      | .T.         | .A.C.C.    | .TT        | .T.CC.       | .G.C.ATA.   | .C.A.      | .T.C      | .C         |
| Eledonella        | .A.A.      | .A.        | .A.         | .A.A.C.    | .C.T.      | .G.C.A.      | .ATA.       | .T.T.      | .C.T.     | .T.C       |
| Granelledone      | .A.A.      | .T.        | .T.         | .A.        | .A.T.      | .G.A.ATA.    | .T.F.A.     | .T.        | .C.T.AC   | .C         |
| Hapalochlaena     | C.T.A.     | .T.T.A.    | .T.         | .A.        | .T.        | .G.C.CATA.   | .C.A.       | .T.C.      | .A        | .C         |
| Japatella         | .A.A.      | .A.        | .A.         | .C.C.      | .T.        | .A.G.C.C.    | .ATA.       | .T.        | .C.T.     | .T.C       |
| Octopus           | .A.        | .T.A.      | .T.C.       | .C.        | .T.T.      | .G.          | .ATA.       | .A.T.T.    | .T.C.A    | .AC        |
| Vitreledonella    | .A.A.      | .T.A.      | .T.         | .A.        | .C.T.T.    | .A.A.        | .C.ATA.     | .T.T.      | .C.A      | .C         |
| Cirrothamna       | .A.A.      | .T.C.A.    | .C.         | .G.C.      | .C.        | .A.A.        | .ATA.       | .C.C.T.    | .T.C      | .CC        |
| Grimpoteuthis     | .A.A.      | .A.        | .C.         | .A.        | .TT        | .KG.         | .ATA.CA.A.  | .C.T.T.    | .T.C      | .C         |
| Opisthoteuthis    | .A.A.      | .A.        | .T.         | .A.        | .TT        | .A.C.        | .ATA.CA.T.  | .T.T.      | .T.AC     | .C         |
| Stauroteuthis     | .A.A.      | .T.C.A.    | .T.         | .A.C.      | .C.T.      | .A.A.A.      | .A.T.       | .T.C.C.T.  | .C        | .CC        |
| Vampyroteuthis    | .A.A.T.    | .G.TT      | .T.         | .A.A.      | .A.T.TG.   | .C.G.        | .A.C.T.     | .A.C.T.    | .T.GC     | .C         |
| Nautilus          | .A.        | .A.        | .T.         | .A.A.C.    | .C.T.      | .AGAT.C.     | .G.         | .T.A.      | .A        | .T.AC      |

|                   |            |            |             |              |             |            |             |             |             |                 |
|-------------------|------------|------------|-------------|--------------|-------------|------------|-------------|-------------|-------------|-----------------|
|                   | 410        | 420        | 430         | 440          | 450         | 460        | 470         | 480         | 490         | 500             |
|                   | TGCATTTAGC | AGGTATCTCT | TCTATTTTAG  | GAGCAATTA    | CCTCATTTAA  | ACTATTTTAA | ATATPACGATG | AGAGGATTA   | CAAAATAGAAC | GTTTACCTTT      |
| Abralia           | A..C.....  | ..G.A....  | ..C..C....  | .....T....   | ..C....C..  | ..A.....   | .....G..G.. | .....G..... | .....G..... | .....A.....     |
| Allureteuthis     | C.....     | T.GG.G...  | ..C....C..  | .....T....   | ..T...T...C | ..A.....   | .....G..G.. | .....G..... | .....G..... | .....AC.....    |
| Ancistrocheirus   | T.....     | ..GG.A.... | ..C....C..  | .....T....   | ..T...T...C | ..A.....   | .....G..G.. | .....G..... | .....G..... | .....AC.....    |
| Bathyteuthis      | C.....     | ..AG.T.... | ..C....C..  | .....T....   | ..T...T...C | ..A.....   | .....G..G.. | .....G..... | .....G..... | .....AC.G.CC.   |
| Brachoteuthis     | T.CC.....  | ..AG.A.C   | ..C....C..  | .....T....   | ..T...T...C | ..A.....   | .....G..G.. | .....G..... | .....G..... | .....AC.....    |
| Charoteuthis      | A.CC.C...  | ..T.A....  | ..C..G....  | .....C....   | ..C....C..  | ..A.....   | .....T....  | .....T.C.   | .....A..... | .....C.....     |
| Chtenopteryx      | C.....     | ..G.T....  | ..C....C..  | .....T....   | ..T...T...C | ..C..C..   | .....C....  | .....C....  | .....C....  | .....A.....     |
| Cycloteuthis      | T.CC.T...  | ..AG...C   | ..C....C..  | .....T....   | ..T...T...C | ..A.CC..   | .....G..G.. | .....G..... | .....G..... | .....C.....     |
| Discoteuthis      | A.C.T...   | ..G...A..  | ..C..A..C.T | .....C....   | ..T...T...C | ..A.....   | .....G..G.. | .....G..... | .....G..... | .....AC.T....   |
| Enoplototeuthis   | A.W.....   | ..GG.W.M.T | ..C..C..W   | ..T.D.T..T   | ..T.A.CCK.. | .....      | .....G..G.. | .....G..... | .....G..... | .....AC.....    |
| Gonatopus         | A.....     | ..G.A.C.C  | ..C....C..  | .....T....   | ..T...T...C | ..A.....   | .....G..G.. | .....G..... | .....G..... | .....AC.G.CC.   |
| Gonatus_berryi    | A.....     | ..AG.T.C   | ..C....C..  | .....T....   | ..T...T...C | ..A.....   | .....G..G.. | .....G..... | .....G..... | .....GC.C.C.    |
| Gonatus_onyx      | A.....     | ..AG.C.A   | ..C....C..  | .....T....   | ..T...T...C | ..A.....   | .....G..G.. | .....G..... | .....G..... | .....G.G.AC.    |
| Histioteuthis     | T.....     | ..GG.G.C   | ..C..CC..   | .....G.T.... | ..T...T...C | ..A.....   | .....G..G.. | .....G..... | .....G..... | .....C.T.AC.    |
| Joubiniteuthis    | A.....     | ..G.G.T... | ..C..CC..   | .....G.T.... | ..T...T...C | ..A.....   | .....G..G.. | .....G..... | .....G..... | .....C.T.CC.    |
| Lepidoteuthis     | T.C.....   | ..GG.G...  | ..C....C..  | .....T....   | ..T...T...C | ..A.CC..   | .....G..G.. | .....G..... | .....G..... | .....GC.T.CC.   |
| Liocranchia       | C.CC.T...  | ..G....C   | ..C....C..  | .....T....   | ..T...T...C | ..A.C..C   | .....G..G.. | .....G..... | .....G..... | .....C.T.CC.    |
| Lycoteuthis       | A.C.....   | ..AG.A.C   | ..C..C..G   | .....C.C..T  | ..T...T...C | ..A.....   | .....G..G.. | .....G..... | .....G..... | .....CC.T...C   |
| Maatigoteuthis    | C.C.....   | ..T.A.T.A  | ..C....G..  | .....T....   | ..T...T...C | ..A.CC..   | .....G..G.. | .....G..... | .....G..... | .....C.T...C    |
| Moroteuthis       | T.C.T...   | ..G.T.C    | ..C..C..G   | .....G....   | ..T...T...T | ..A.C..C   | .....G..G.. | .....G..... | .....G..... | .....C.T...C    |
| Ommastrephes      | T.....     | ..C...T... | ..A...G...  | .....G....   | ..T...T...T | ..A.....   | .....G..G.. | .....G..... | .....G..... | .....AC.C.AC.   |
| Onychoteuthis     | C.C.....   | ..AG.C     | ..C....C..  | .....T....   | ..T...T...C | ..A.A..C   | .....G..G.. | .....G..... | .....G..... | .....AC.T...A   |
| Pholidoteuthis    | A.C.T...   | ..AG.T...  | ..C....C..  | .....T....   | ..T...T...C | ..A.CC..   | .....G..G.. | .....G..... | .....G..... | .....A.TT.A.    |
| Psychroteuthis    | T.C.....   | ..C.G.T... | ..C..G...   | .....T....   | ..T...T...C | ..A.C.T.C  | .....G..G.. | .....G..... | .....G..... | .....AC.C.AC.   |
| Pyroteuthis       | A.C.T...   | ..T...T... | ..G...G...  | .....T....   | ..T...T...C | ..C.CC..   | .....G..G.. | .....G..... | .....G..... | .....AC.CC.     |
| Sthenoteuthis     | T.CC.....  | ..G...G... | ..C..C..G   | .....T.C.C.. | ..T...T...T | ..C.T..    | .....G..G.. | .....G..... | .....G..... | .....AC.....    |
| Thysanoteuthis    | A.....     | ..C.A.G.C  | ..C....C..  | .....G....   | ..T...T...T | ..A.....   | .....G..G.. | .....G..... | .....G..... | .....AC.C.CC.   |
| Octopoteuthis     | T.C.....   | ..G.T.C    | ..C....C..  | .....G.T.... | ..T...T...C | ..A.....   | .....G..G.. | .....G..... | .....G..... | .....AC.CT...C  |
| Loligo_opalescens | C.CC...    | ..T...T... | ..G...T...  | .....G.T.... | ..T...T...C | ..CA...    | .....G..G.. | .....G..... | .....G..... | .....AC.TT.C.   |
| Loligo_pealei     | C.C.....   | ..T...T... | ..G.C.C.C   | .....T....   | ..T...T...G | ..A.....   | .....G..G.. | .....G..... | .....G..... | .....CC.....    |
| Sepioteuthis      | A.....     | ..G.A.G.C  | ..A..C..G   | .....T.C..   | ..T...T...C | ..A.....   | .....G..G.. | .....G..... | .....G..... | .....A.T.G.     |
| Heteroteuthis     | T.CC...    | ..C.A.G.C  | ..A..C..G   | .....T.C.C.. | ..T...T...T | ..C..AT..  | .....G..G.. | .....G..... | .....G..... | .....CC.....    |
| Idiosepius        | A.....     | ..GG.A.A   | ..A...G...  | .....C....   | ..T...T...C | ..C..CC..  | .....G..G.. | .....G..... | .....G..... | .....CC.....    |
| Rossia            | A.....     | ..G.T...   | ..C....G..  | .....T....   | ..T...T...C | ..C..C..C  | .....G..G.. | .....G..... | .....G..... | .....C.....     |
| Sepia_officinalis | T.CC...    | ..GG.A...  | ..C....G..  | .....T....   | ..T...T...C | ..C..CC..  | .....G..G.. | .....G..... | .....G..... | .....A.....     |
| Sepia_opipara     | A.....     | ..G.T...   | ..C....G..  | .....T....   | ..T...T...C | ..C..C..C  | .....G..G.. | .....G..... | .....G..... | .....C.....     |
| Sepioloidea       | T.CC...    | ..GG.A...  | ..C....G..  | .....T....   | ..T...T...C | ..A.....   | .....G..G.. | .....G..... | .....G..... | .....A.....     |
| Spirula           | T.C.....   | ..GG.A...  | ..C....G..  | .....T....   | ..T...T...C | ..A.C..C   | .....G..G.. | .....G..... | .....G..... | .....AG...AC.   |
| Stoloteuthis      | T.C.....   | ..GG.G.C   | ..A..C..G   | .....T....   | ..T...T...C | ..CC..     | .....G..G.. | .....G..... | .....G..... | .....A.....     |
| Argonauta         | T.....     | ..C.A.C    | ..A..C..G   | .....T....   | ..T...T...C | ..A.T..    | .....G..G.. | .....G..... | .....G..... | .....A.....     |
| Bathypolypus      | A.....     | ..A.T...   | ..C....C..  | .....T....   | ..T...T...C | ..A.A.T..  | .....G..G.. | .....G..... | .....G..... | .....A.....     |
| Eledonella        | T.CC...    | ..AG.A.C   | ..C....C..  | .....T....   | ..T...T...C | ..A..C..   | .....G..G.. | .....G..... | .....G..... | .....AC.T...C   |
| Graneledone       | A.....     | ..AG.A...  | ..A..C..G   | .....T....   | ..T...T...C | ..A.T..    | .....G..G.. | .....G..... | .....G..... | .....AC.....    |
| Hapalochlaena     | A.....     | ..T.A.A    | ..A..C..G   | .....T....   | ..T...T...C | ..A.T..    | .....G..G.. | .....G..... | .....G..... | .....AC.....    |
| Japatella         | T.C.....   | ..AG.A.C   | ..C..CC..   | .....T....   | ..T...T...C | ..A.....   | .....G..G.. | .....G..... | .....G..... | .....AC.T...C   |
| Octopus           | T.C.....   | ..A.T.A    | ..A..C..G   | .....T....   | ..T...T...C | ..A.T..    | .....G..G.. | .....G..... | .....G..... | .....AC.T...AC. |
| Vitreledonella    | T.....     | ..AG.T...  | ..C....C..  | .....T....   | ..T...T...C | ..A.....   | .....G..G.. | .....G..... | .....G..... | .....C.T...C    |
| Cirrothauma       | A.CC...    | ..G.G.C    | ..A..C..G   | .....T....   | ..T...T...C | ..A.A.T..  | .....G..G.. | .....G..... | .....G..... | .....CC.....    |
| Grimpoteuthis     | T.C.....   | ..C.A...   | ..A..G...G  | .....T....   | ..T...T...C | ..A.A.T..  | .....G..G.. | .....G..... | .....G..... | .....A.....     |
| Opisthoteuthis    | T.....     | ..AG.A...  | ..A..C..G   | .....T....   | ..T...T...C | ..A.A.T..  | .....G..G.. | .....G..... | .....G..... | .....A.....     |
| Stauroteuthis     | T.C.C...   | ..G.A.C    | ..A..C..G   | .....T....   | ..T...T...C | ..A.A.C..C | .....G..G.. | .....G..... | .....G..... | .....CC...C.    |
| Vampyroteuthis    | C.C.T...   | ..T.A.T    | ..C....G..  | .....T....   | ..T...T...C | ..A.....   | .....G..G.. | .....G..... | .....G..... | .....C.....     |
| Nautilus          | A.C.....   | ..T.A.G... | ..G..A..C   | .....T.A...  | .....C..C   | ..A.T..    | .....G..G.. | .....G..... | .....G..... | .....A.....     |

|                          | 510        | 520           | 530         | 540              | 550                | 560             | 570           | 580         | 590        | 600         |
|--------------------------|------------|---------------|-------------|------------------|--------------------|-----------------|---------------|-------------|------------|-------------|
| <i>Abraia</i>            | ATTGCTTGA  | TCAGTTTTA     | TTACTGCTAT  | TTTATTACTT       | TTATCTTAC          | CAGTTCGCG       | GGACCAATT     | ACTATACTGT  | TAACTGACCG | TAAATTTCAAC |
| <i>Alluroteuthis</i>     | ...C...    | ...T.A...     | ...A...     | ...C...          | ...TC.T...         | ...C.T...       | ...G...       | ...G...     | ...A...    | ...A...     |
| <i>Ancistrocheirus</i>   | ...A...    | ...A...       | ...AG...    | ...C.TC.T...     | ...C...            | ...T.G...       | ...G.T...     | ...A...     | ...T...    | ...A.C...   |
| <i>Bathyteuthis</i>      | ...C...    | ...C.A...     | ...C.A...   | ...C.C...        | ...C...            | ...T.A.G...     | ...A.G.T...   | ...A...     | ...TAC...  | ...A.C.T.T  |
| <i>Brachioteuthis</i>    | ...T...    | ...T.A...     | ...A...     | ...A.C.G...      | ...A.C.T...        | ...T.C.A...     | ...G...       | ...A...     | ...C...    | ...A.C.T.T  |
| <i>Chiroteuthis</i>      | ...T...    | ...T.C...     | ...A...     | ...C.TC.A...     | ...G.TC...         | ...T.C.A...     | ...C...       | ...G...     | ...A...    | ...A.C...   |
| <i>Chtenopteryx</i>      | ...T...    | ...T.C...     | ...A...     | ...C.TC.T.A...   | ...T...            | ...T.A.A...     | ...A...       | ...G.T...   | ...A.T...  | ...A...     |
| <i>Cranchia</i>          | ...T...    | ...T.C...     | ...A...     | ...C.T...        | ...A...            | ...AT.G...      | ...T.T...     | ...T...     | ...A...    | ...A...     |
| <i>Cycloteuthis</i>      | ...TC...   | ...C.C...     | ...A...     | ...CC.TC.T.A...  | ...C.T.AC...       | ...C.AT.A...    | ...A.G.C.C... | ...A...     | ...TC...   | ...C.A...   |
| <i>Discoteuthis</i>      | ...T...    | ...T.A.C...   | ...C.A...   | ...C...          | ...A...            | ...T.T.A...     | ...A...       | ...C...     | ...G...    | ...A...     |
| <i>Enoploteuthis</i>     | ...C...    | ...T.A...     | ...C...     | ...C...          | ...C.T...          | ...GT.G...      | ...C.C...     | ...T.C...   | ...T.A...  | ...T.T      |
| <i>Gonatopsis</i>        | ...T...    | ...T.A...     | ...C.C.A... | ...C.C.A.C...    | ...AC.T...         | ...T.C.A...     | ...C...       | ...T.W...   | ...A...    | ...TAC...   |
| <i>Gonatus_berryi</i>    | ...T...    | ...T.G...     | ...C.A...   | ...C.C...        | ...AC.T...         | ...T.T.A...     | ...C...       | ...AC...    | ...A.C...  | ...T...     |
| <i>Gonatus_onyx</i>      | ...T...    | ...T.A...     | ...A...     | ...C.T...        | ...C.T...          | ...T.T...       | ...G...       | ...T.A...   | ...A...    | ...T...     |
| <i>Histioteuthis</i>     | ...C...    | ...T.A...     | ...A...     | ...C.T.G...      | ...C...            | ...AT.A...      | ...A...       | ...C...     | ...T...    | ...A...     |
| <i>Joubiniteuthis</i>    | ...TA...   | ...C.C.C...   | ...A...     | ...C...          | ...C.A...          | ...T.T.A...     | ...A.G.C...   | ...T...     | ...A...    | ...T...     |
| <i>Lepidoteuthis</i>     | ...T...    | ...T.A...     | ...A...     | ...C...          | ...C.A...          | ...A.T.A...     | ...C...       | ...AC...    | ...T...    | ...A...     |
| <i>Liocranchia</i>       | ...G...    | ...T...       | ...C.A...   | ...C...          | ...TC.T...         | ...T.CT.A...    | ...C...       | ...TC...    | ...T...    | ...A...     |
| <i>Lycoteuthis</i>       | ...T...    | ...T...       | ...A.C...   | ...C.C.T...      | ...C...            | ...C.T.A...     | ...T...       | ...C...     | ...T...    | ...A.C.T... |
| <i>Nastigoteuthis</i>    | ...T...    | ...T...       | ...A...     | ...C...          | ...C...            | ...T.CT.A...    | ...A...       | ...C...     | ...T...    | ...A...     |
| <i>Moroteuthis</i>       | ...C...    | ...T.C...     | ...A...     | ...C.C...        | ...A...            | ...T.T.A...     | ...C...       | ...T.A...   | ...A...    | ...T...     |
| <i>Omastrephes</i>       | ...C...    | ...T.A...     | ...C...     | ...C.C.T...      | ...A.C.A...        | ...T.T.G...     | ...C.G.T...   | ...G.A...   | ...A...    | ...T...     |
| <i>Onychoteuthis</i>     | ...G...    | ...T.A...     | ...A...     | ...C.T...        | ...T...            | ...T.T.A...     | ...A...       | ...TC...    | ...T...    | ...A...     |
| <i>Pholidoteuthis</i>    | ...T...    | ...G.A.C...   | ...A...     | ...C...          | ...TC.T...         | ...G.G.T.A...   | ...A...       | ...GT.A...  | ...A...    | ...A...     |
| <i>Psychroteuthis</i>    | ...TA...   | ...T.A...     | ...C.A...   | ...C.C.A...      | ...C...            | ...T.A.A...     | ...A.G.T...   | ...A...     | ...A...    | ...T...     |
| <i>Pyroteuthis</i>       | ...C...    | ...C.A...     | ...C...     | ...G.T.A.C.T...  | ...C...            | ...T.T.A...     | ...A...       | ...TC...    | ...C...    | ...A.C.T.T  |
| <i>Sthenoteuthis</i>     | ...G...    | ...T...       | ...A...     | ...C.T...        | ...C...            | ...T.T.A...     | ...A...       | ...G.AC...  | ...C...    | ...G.T.T    |
| <i>Thysanoteuthis</i>    | ...T...    | ...T...       | ...A...     | ...C.C...        | ...C...            | ...T.A.A...     | ...T...       | ...A...     | ...A...    | ...T...     |
| <i>Octopoteuthis</i>     | ...T...    | ...T.A...     | ...A...     | ...C...          | ...C...            | ...AT.A...      | ...A.C...     | ...A...     | ...C...    | ...T...     |
| <i>Loligo_opealei</i>    | ...ATC...  | ...A...       | ...A...     | ...C...          | ...C.T...          | ...A.A.T.T...   | ...A...       | ...T...     | ...A...    | ...T...     |
| <i>Loligo_opealei</i>    | ...TC.G... | ...A.C...     | ...C...     | ...C.TC.C...     | ...C...            | ...AT.G...      | ...T.T...     | ...A...     | ...TAC...  | ...C.T...   |
| <i>Sepioteuthis</i>      | ...T...    | ...T...       | ...C...     | ...C.C.G...      | ...C...            | ...C.A.C.G.T... | ...C...       | ...T.A...   | ...A...    | ...T...     |
| <i>Heteroteuthis</i>     | ...TG...   | ...T...       | ...C...     | ...C.C.G...      | ...C...            | ...T.GT.A...    | ...A...       | ...T.A...   | ...A...    | ...T...     |
| <i>Idiosepius</i>        | ...G...    | ...T.A...     | ...A...     | ...GC.TT.A...    | ...T...            | ...A.T...       | ...T...       | ...T.A...   | ...A...    | ...T...     |
| <i>Rossia</i>            | ...TC...   | ...T...       | ...A...     | ...CC...         | ...A.C.G.C.T...    | ...T.CT.A...    | ...C...       | ...A...     | ...G.T...  | ...A...     |
| <i>Sepia_officinalis</i> | ...G...    | ...G.A...     | ...A...     | ...G...          | ...C.C.C.C.C...    | ...T.AT.A...    | ...A...       | ...A...     | ...A...    | ...T...     |
| <i>Sepia_opipara</i>     | ...T...    | ...A...       | ...C.C...   | ...C.T...        | ...T.A...          | ...T.A.A...     | ...A.G.T...   | ...T.A...   | ...A...    | ...T...     |
| <i>Sepioidea</i>         | ...T...    | ...T.A...     | ...C.A...   | ...C.C.T...      | ...C...            | ...T.AT.A...    | ...A.T...     | ...C...     | ...A...    | ...T...     |
| <i>Spirula</i>           | ...T...    | ...T.A...     | ...A...     | ...C...          | ...C...            | ...T.A.A...     | ...A...       | ...A...     | ...A...    | ...T...     |
| <i>Stoloteuthis</i>      | ...AT...   | ...T...       | ...C...     | ...C.T.A.C...    | ...T...            | ...T.GT.A...    | ...G...       | ...A...     | ...T.A...  | ...A...     |
| <i>Argonauta</i>         | ...C.T...  | ...T.C...     | ...A.G...   | ...A...          | ...T...            | ...T.A...       | ...T...       | ...TAC...   | ...A...    | ...T...     |
| <i>Bathypolypus</i>      | ...TG...   | ...T.C.A...   | ...A...     | ...C...          | ...AC...           | ...T.A.A...     | ...A...       | ...T...     | ...A...    | ...T...     |
| <i>Eledonella</i>        | ...T...    | ...T.AC...    | ...C.C.A... | ...C.T...        | ...T.A.C.T.TC.T... | ...A.A...       | ...T.C...     | ...A...     | ...TC...   | ...T.A.T... |
| <i>Granelone</i>         | ...T...    | ...T...       | ...A...     | ...C.T...        | ...T.A.C...        | ...T.A.A...     | ...T...       | ...A...     | ...TC...   | ...T...     |
| <i>Hapalochlaena</i>     | ...TA...   | ...T.A...     | ...C...     | ...C...          | ...C.C.AC...       | ...A...         | ...T.T...     | ...AC...    | ...A...    | ...T.T      |
| <i>Japatella</i>         | ...T...    | ...T.C...     | ...A.A...   | ...C.TC...       | ...C.T...          | ...AT.A...      | ...T...       | ...C...     | ...TC...   | ...C.A.T... |
| <i>Octopus</i>           | ...TA...   | ...T...       | ...C.A...   | ...C.A...        | ...A...            | ...A...         | ...T...       | ...T...     | ...A...    | ...T.T      |
| <i>Vitreledonella</i>    | ...T...    | ...T.A...     | ...A...     | ...C.TC...       | ...C...            | ...CT.A...      | ...A.T...     | ...C...     | ...T...    | ...A...     |
| <i>Cirrothauma</i>       | ...AT...   | ...C.AC.A...  | ...C...     | ...C...          | ...C.C...          | ...CA.C.T.A...  | ...T.C...     | ...TC...    | ...C.T...  | ...C.T...   |
| <i>Grimptoteuthis</i>    | ...TC...   | ...A...       | ...A...     | ...C.TT.A.C...   | ...TC.T...         | ...T.A.G...     | ...A...       | ...TC...    | ...T...    | ...C.T...   |
| <i>Opisthoteuthis</i>    | ...F...    | ...TC.C.A...  | ...C...     | ...CC.TC.TT.A... | ...C.T.TC.T...     | ...A.A.C.T.C... | ...A...       | ...T...     | ...V...    | ...K...     |
| <i>Stauroteuthis</i>     | ...C.T...  | ...CT.AC.A... | ...C...     | ...C.CC...       | ...A...            | ...T.C.A...     | ...A...       | ...C...     | ...A...    | ...A.C...   |
| <i>Vampyroteuthis</i>    | ...TA...   | ...A...       | ...A...     | ...AA...         | ...TA...           | ...T.A.T.A...   | ...T...       | ...A...     | ...T.A...  | ...A...     |
| <i>Nautilus</i>          | ...TA.G... | ...T...       | ...AAA...   | ...A.A...        | ...C...            | ...T.CT.A...    | ...A.G.C.C... | ...GT.AC... | ...A.T...  | ...CC...    |

|                   | 610        | 620        | 630        | 640        | 650        | 657               |
|-------------------|------------|------------|------------|------------|------------|-------------------|
| Abralia           | ACTACTTTTT | TTGACCCAAG | AGGAGGGGGA | GACCCATTTC | TTTATCAACA | CTTATTC           |
| Alluroteuthis     | .C.C.      |            | G.T.A.     | .A.T       | A.C.       | .C.T              |
| Ancistrocheirus   | .A.C.      |            | .G.A.G     | .T         | .G.C.      | .C.C.T            |
| Architeuthis      |            |            | G.T.       | .T.C.C.    | A.C.       | ?.....            |
| Bathyteuthis      | .C.C.      | C.         | .A.        | .C.T       | A.         | T.....T           |
| Brachioeteuthis   | .C.C.      | T.T.       | .G.A.G     | ?????????? | ?????????? | ????????          |
| Chiroteuthis      |            |            | T.         | .A.G       | .T.C.      | A.....TC.....T    |
| Chtenopteryx      | .A.C.      | T.T.       | T.G.A.     | .T.T.T     | A.         | T.....T           |
| Cranchia          | .A.C.      |            | G.G.A.G    | .T.T       | G.         | T.....T           |
| Cycloteuthis      |            | GG.        | G.G.A.G    | .C.T       | A.         | .C.....T          |
| Discoteuthis      | .C.        |            | T.         | .A.T       | A.         | TC.....           |
| Enoplateuthis     | .C.        | T.T.       | .A.T       | .T.C.      | .C.        | .C.....T          |
| Gonatopsis        | .A.        |            | GA         | G.G.A.T?   | WT         | A.C.....          |
| Gonatus_berryi    | .A.C.      | T.T.       | G.         | .A.C       | .CT        | A.C.....C.....    |
| Gonatus_onyx      | .A.        |            | G.         | .T.CT      | .G.C.      | .C.....T          |
| Histioteuthis     |            | T.T.       | G.G.T.G    | .T.CT      | A.         | .....T            |
| Joubiniteuthis    | .C.        |            | G.G.A.G    | .C.        | A.C.       | TC.....T          |
| Lepidoteuthis     | .C.C.      | C.T.       | .A.G       | .T.C.      |            | TC.....T          |
| Liocranchia       |            | A.T.T.     | G.         |            | T          | A.....TC.....T    |
| Lycoteuthis       | .C.C.C.    | T.         | .T.A.T     |            | T          | A.C.....T         |
| Mastigoteuthis    |            | T.         | G.         |            | C.         | .....             |
| Moroteuthis       | .C.        |            | G.         | .A.C       | .CT        | .G.....TC.....    |
| Ommastrephes      | .C.        |            | T.         | .G         | .T         | A.....T.....T     |
| Onychoteuthis     | .C.        |            |            |            | T          | A.C.....T.....T   |
| Pholidoteuthis    | .A.        | ??????     | ?????????? | ?????????? | ?????????? | ????????          |
| Psychroteuthis    |            | T.G.       | .G.A.G     | .T.        | .C.        | .....T.....T      |
| Pyroteuthis       | .A.G.      | T.         | .A.T       | .T         | A.         | .....T.....T      |
| Sthenoteuthis     | .A.        | T.         |            | .CT        | A.         | .....G.T          |
| Thysanoteuthis    | .C.        |            | T.A.G      | .C.        | CG.        | .C.....T          |
| Octopoteuthis     | .C.        |            | T.         | A.         |            | .C.....           |
| Loligo_opalescens | .C.        | C.         | T.         | .A.        | .T.        | .C.?????? ??????? |
| Loligo_pealei     | .C.C.      |            | G.A.       |            |            | A.....            |
| Sepioteuthis      |            | T.         | .A.G       |            | .C.        | T.....T           |
| Heteroteuthis     | .C.        | C.         | .G.        | .G         | .T         | A.C.....C.T..     |
| Idiosepius        | .A.A.      | C.T.T.     | G.         | .A.G       | .T.T       | A.C.....C.T.T     |
| Rossia            | .A.        |            | G.G.A.     | ?????????? | ?????????? | ????????          |
| Sepia_officinalis |            | C.T.       | G.         | .A.        | .T         | A.C.....G.T       |
| Sepia_opipara     |            | C.T.       | G.         | .A.T       | .C.        | .....C.....T      |
| Sepioloidea       | .A.        | C.T.       | T.G.A.     | .A.CT      | .G.C.      | .C.....           |
| Spirula           | .C.C.      |            | G.G.A.G    | .T.T       | A.C.       | .....T.....T      |
| Stoloteuthis      |            |            | G.A.       | ?????????? | ?????????? | ????????          |
| Argonauta         | .A.C.      | T.         | T.         | .A.        | .T         | A.C.....T.....    |
| Bathypolypus      | .A.        | T.T.       | T.??????   | ?????????? | ?????????? | ????????          |
| Eledonella        | .A.A.      |            | T.T.T      | .T.        |            | .C.....           |
| Graneledone       | .A.        |            | T.         | .A.        |            | .....TC.....T     |
| Hapalochlaena     | .C.C.      | T.         | .A.        | .T.T       | A.         | .....T            |
| Japatella         | .A.A.      |            | G.A.G      | .T.        |            | .C.....T          |
| Octopus           | .A.        |            | T.         | .A.        | .T.A.      | A.....TC.....T    |
| Vitreledonella    | .A.C.      | T.         | G.T.       |            |            | .C.....T          |
| Cirrothauma       | .A.A.C.    |            | G.T.G      | .T.CT      | A.C.       | TC.G...           |
| Grimpoteuthis     |            |            | T.         | .T.A.      | .T.        | .C.....T          |
| Opisthoteuthis    | .A.A.      | T.         | .C.T.      | .T.A.      | K.T.       | T...K.            |
| Stauroteuthis     | .A.        | T.T.       | G.T.A.T    | .A.T       | A.C.       | TC.....T          |
| Vampyroteuthis    | .C.        | T.T.       | .T.A.      |            | T          | A.C.....T.....T   |
| Nautilus          | .T.A.      | C.T.GC     | T.         | .A.        | .T.A.      | .....             |

TABLE 2. PAIRWISE COMPARISONS OF COI GENE UNCORRECTED SEQUENCE DIVERGENCES WITHIN AND AMONG MAJOR CEPHALOPOD TAXONOMIC GROUPS

|                       | Myopsida   | Oegopsida            | Sepioidea           | Incirrata           | Cirrata            | <i>Vampyroteuthis</i> |
|-----------------------|--|----------------------|---------------------|---------------------|--------------------|-----------------------|
| Myopsida              | <sup>a</sup> x 15.82<br><sup>b</sup> SD 2.21<br><sup>c</sup> N 3 |                      |                     |                     |                    |                       |
| Oegopsida             | x 19.74<br>SD 1.16<br>N 90                                       | 17.72<br>1.63<br>435 |                     |                     |                    |                       |
| Sepioidea             | x 19.77<br>SD 1.65<br>N 24                                       | 19.13<br>1.62<br>240 | 18.55<br>2.21<br>28 |                     |                    |                       |
| Incirrata             | x 21.04<br>SD 1.04<br>N 24                                       | 20.26<br>1.69<br>240 | 20.61<br>1.75<br>64 | 16.67<br>2.52<br>28 |                    |                       |
| Cirrata               | x 22.45<br>SD 1.26<br>N 12                                       | 21.25<br>1.46<br>120 | 21.22<br>1.26<br>32 | 18.66<br>1.65<br>32 | 18.08<br>1.60<br>6 |                       |
| <i>Vampyroteuthis</i> | x 21.51<br>SD 1.29<br>N 3  | 21.37<br>1.52<br>30  | 20.42<br>1.39<br>8  | 18.63<br>1.55<br>8  | 20.28<br>1.25<br>4 |                       |
| <i>Nautilus</i>       | x 26.23<br>SD 1.01<br>N 3  | 25.83<br>1.17<br>30  | 25.56<br>0.80<br>8  | 24.34<br>0.91<br>8  | 25.28<br>1.41<br>4 | 25.88<br>---<br>1     |

<sup>a</sup>x = mean % sequence divergence.

<sup>b</sup>SD = standard deviation of the mean.

<sup>c</sup>N = number of pairwise comparisons.

Figure 4. The sequence divergences for pooled first and second position nucleotides (open circles) and third position nucleotides (filled squares) plotted as a function of total uncorrected sequence divergence for all possible pairwise comparisons between the 26 taxa in the restricted COI data set. Substitutions at third codon position nucleotides accounted for a greater proportion of the variation in the COI gene at lower total sequence divergences. Third position substitutions occurred two to three times more frequently than pooled first and second codon position substitutions below 15% total sequence divergence. At greater total sequence divergences, third position substitutions occurred about one and a half times more frequently than pooled first and second position substitutions. Because third position substitutions did not account for as great a proportion of the total sequence divergence at higher sequence divergences, these data provide evidence for saturation at third codon positions where the incursion of multiple hits masks the total number of substitutions that have taken place. Therefore, third codon characters of the COI gene are not likely to be informative for determining relationships among highly diverged taxa. At low sequence divergences, relatively few substitutions occurred at first and second codon position nucleotides such that third position nucleotides are much more likely to be informative for relationships among more recently diverged taxa.



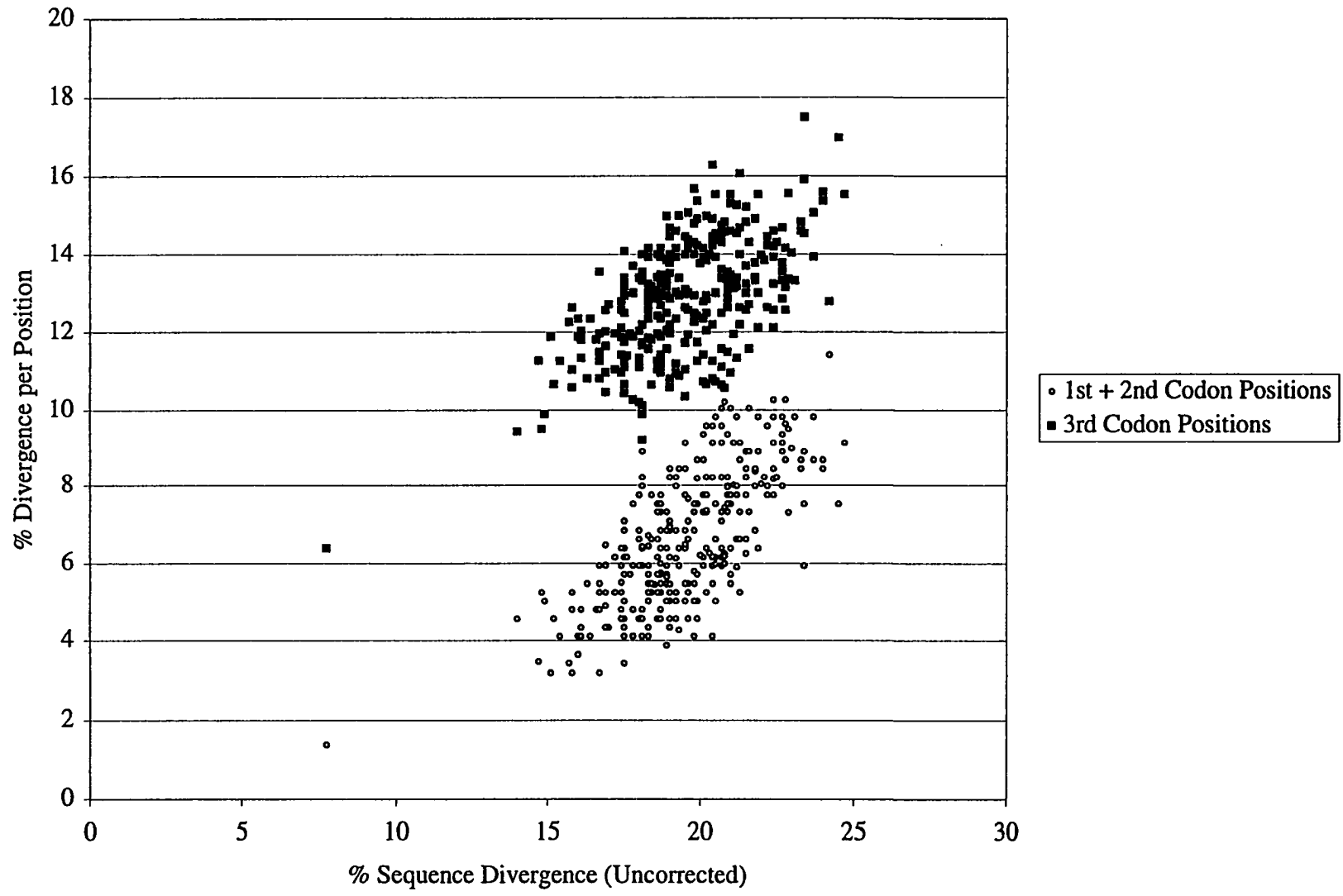


Figure 5. The percentage of total sequence divergence accounted for by transversional substitutions (open circles) and transitional substitutions (filled squares) plotted as a function of total uncorrected sequence divergence for all possible pairwise comparisons between the 26 taxa in the restricted COI data set. Although transitional substitutions accounted for the majority of substitutions (roughly 60-70% of the total) across the entire spectrum of uncorrected sequence divergence, transversional substitutions also made a substantial contribution to the total sequence divergence across the spectrum. Saturation in the COI data cannot be attributed to strictly transitional substitutions, transversional substitutions are also likely to be saturated at third codon position nucleotides.

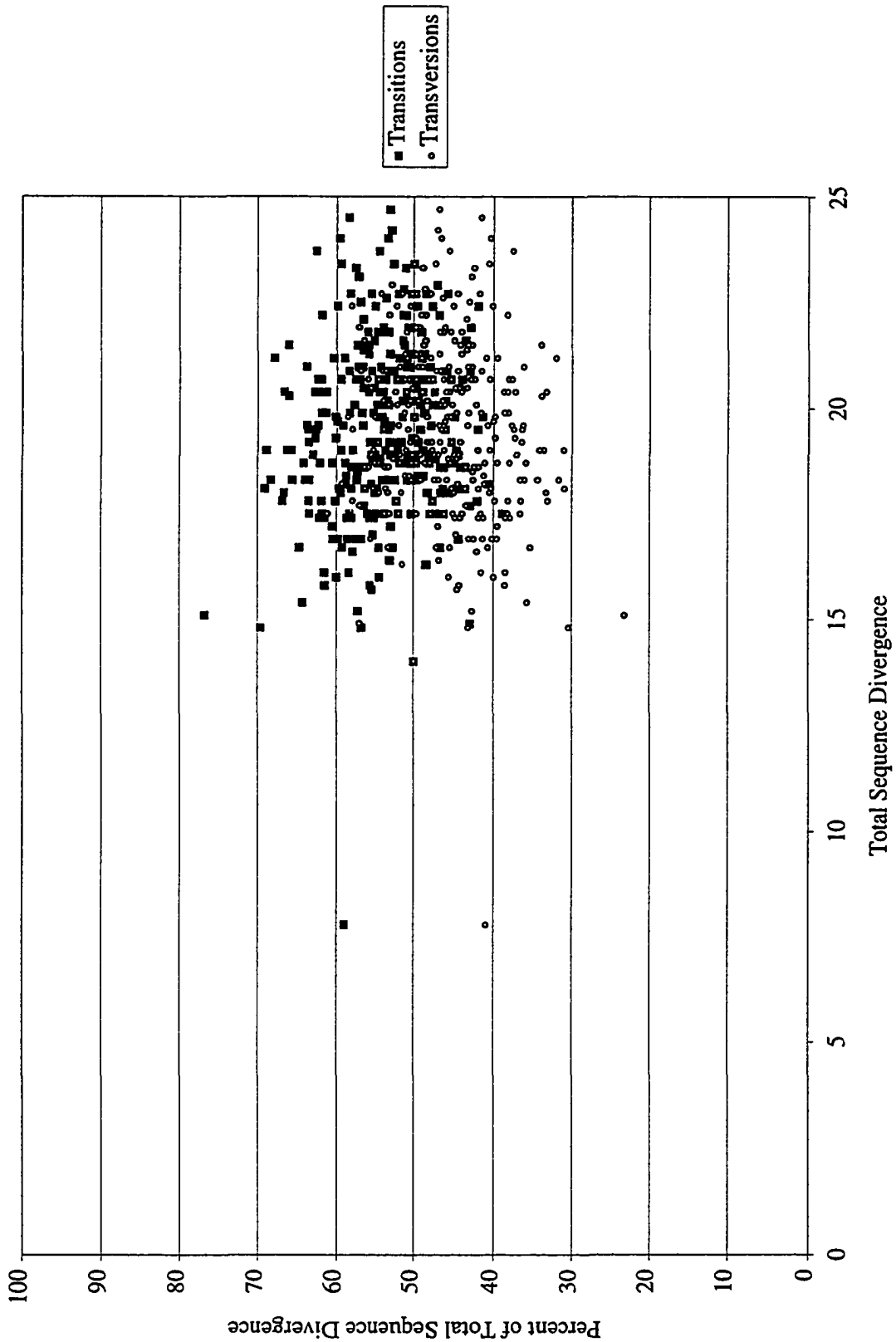


Figure 6. Base compositions at first codon positions in the COI gene for the 26 taxa in the restricted data set. The overall frequencies of the four bases were not equal (cytosines were the rarest), and a chi-square test did not demonstrate significant heterogeneity among taxa in first codon position base frequencies ( $\chi^2 = 41.37$ ,  $df = 75$ ,  $p > 0.995$ ).

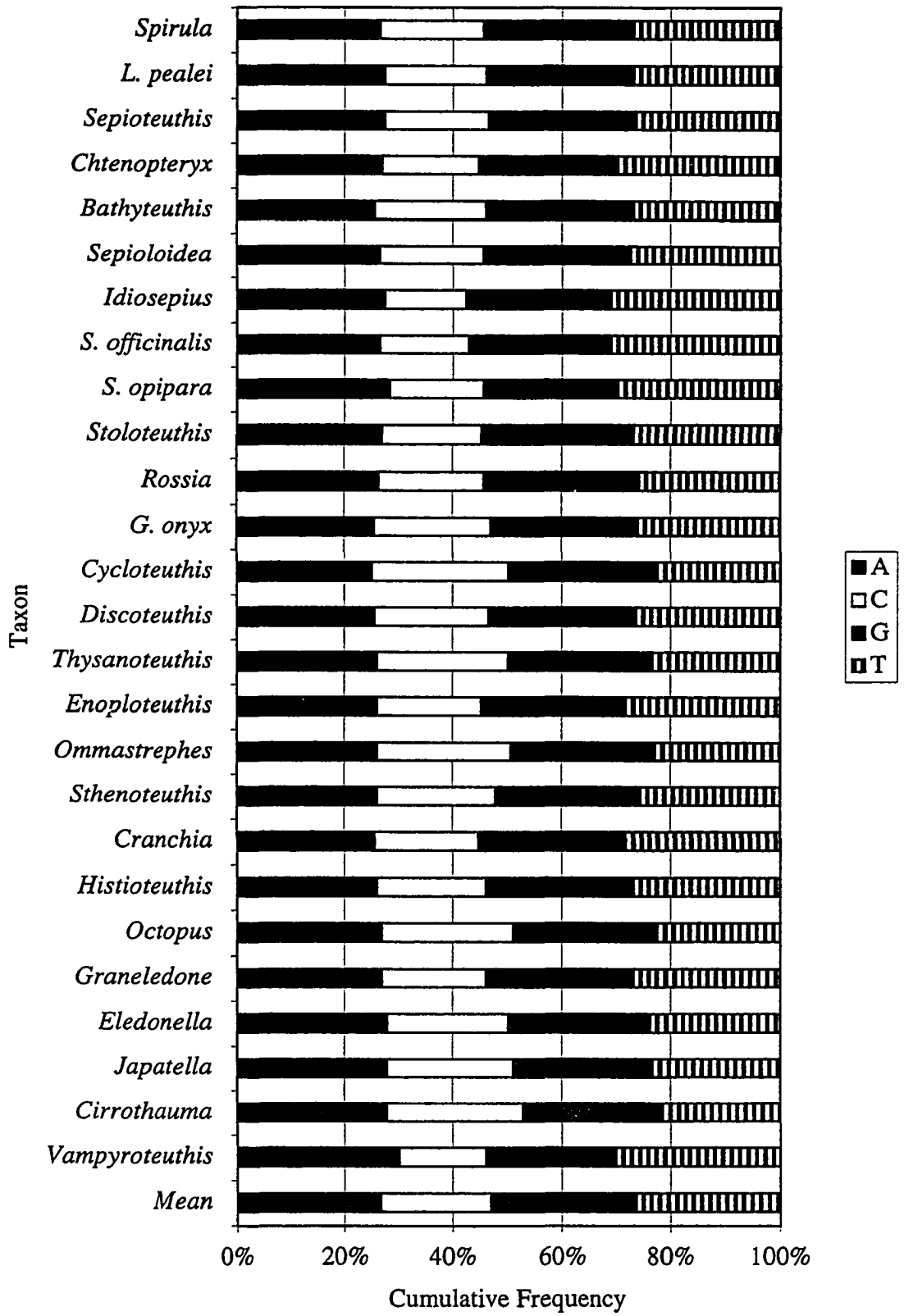


Figure 7. Base compositions at second codon positions in the COI gene for the 26 taxa in the restricted data set. The overall frequencies of the four bases were not equal (adenines were the rarest), and a chi-square test did not demonstrate significant heterogeneity among taxa in second codon position base frequencies ( $\chi^2 = 1.56$ ,  $df = 75$ ,  $p > 0.995$ ).

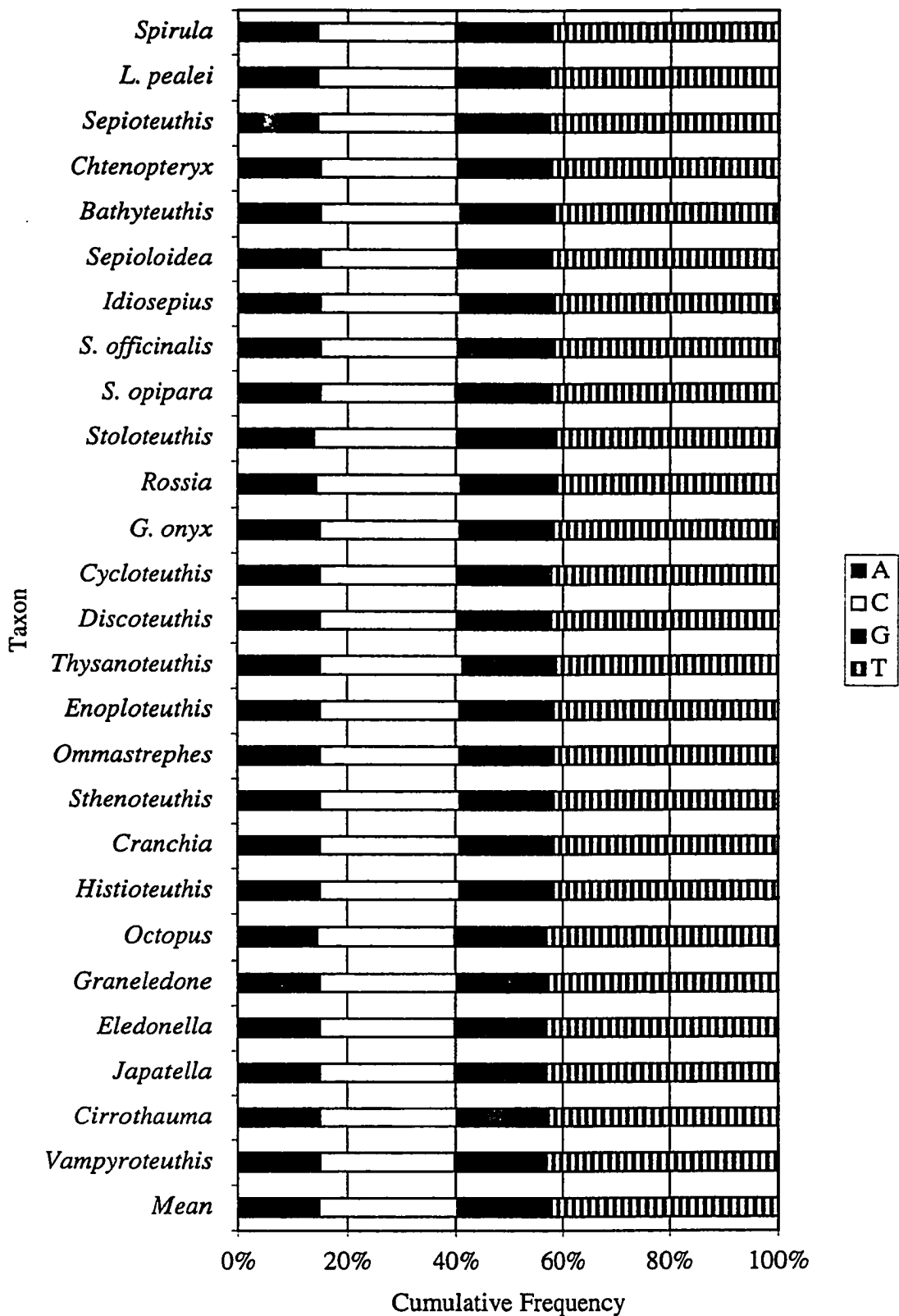


Figure 8. Base compositions at third codon positions in the COI gene for the 26 taxa in the restricted data set. The overall frequencies of the four bases were not equal (guanines were the rarest), and a chi-square test demonstrated significant heterogeneity among taxa in third codon position base frequencies ( $\chi^2 = 434.0$ ,  $df = 75$ ,  $p < 0.001$ ).



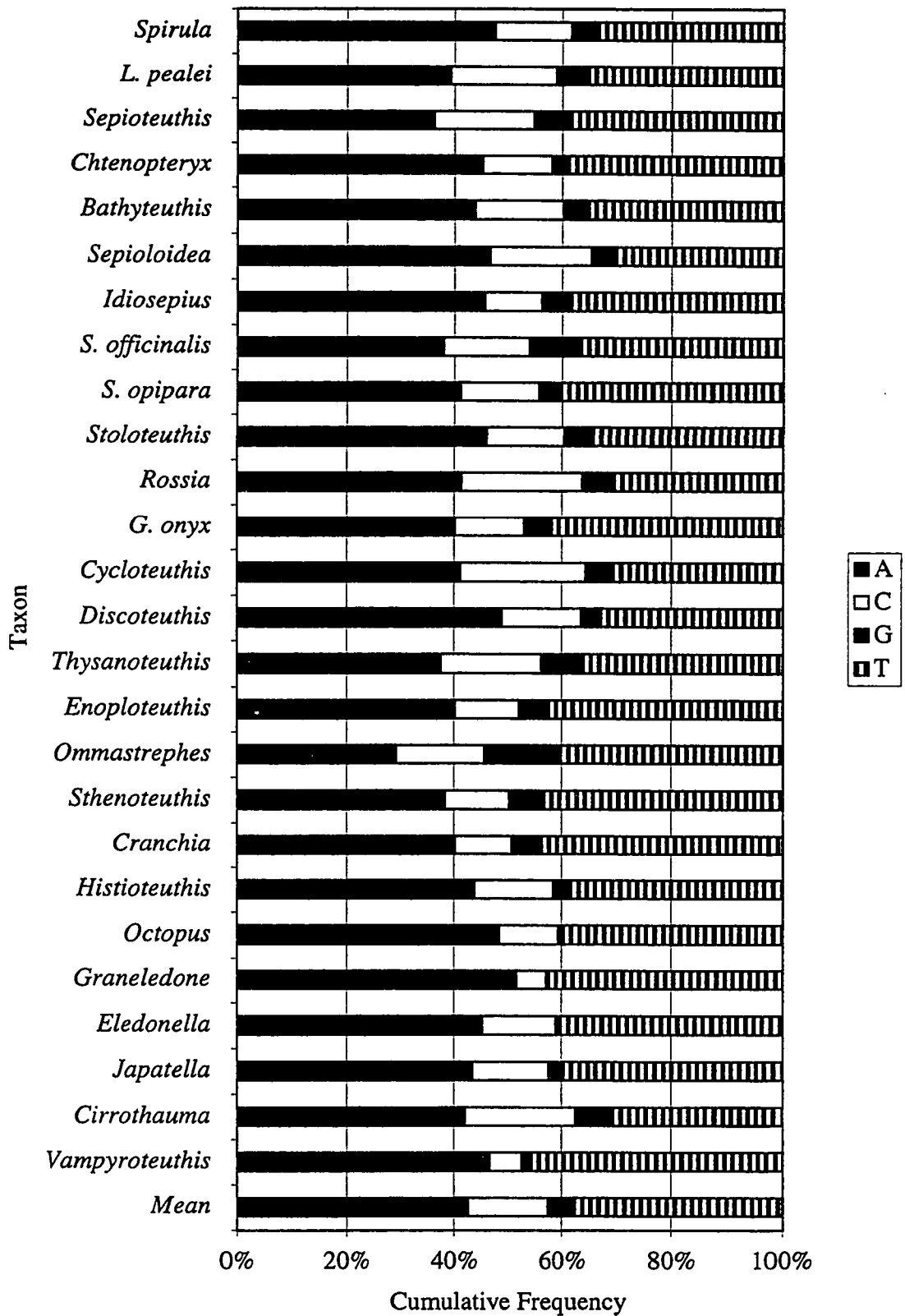


Figure 9. Most parsimonious tree obtained in a heuristic search (1000 random addition replicates) of the unweighted COI data set (TL = 3763; CI = 0.167; RI = 0.329). Branch lengths are drawn proportional to the number of character changes taking place between nodes. Bootstrap proportions are indicated as percentages below nodes and Bremer support values are indicated above nodes. Higher-level taxonomic designations are indicated in boldface to the right of each terminal taxon (C = Suborder Cirrata; I = Suborder Incirrata; M = Suborder Myopsida; O = Suborder Oegopsida; V = Order Vampyromorpha).

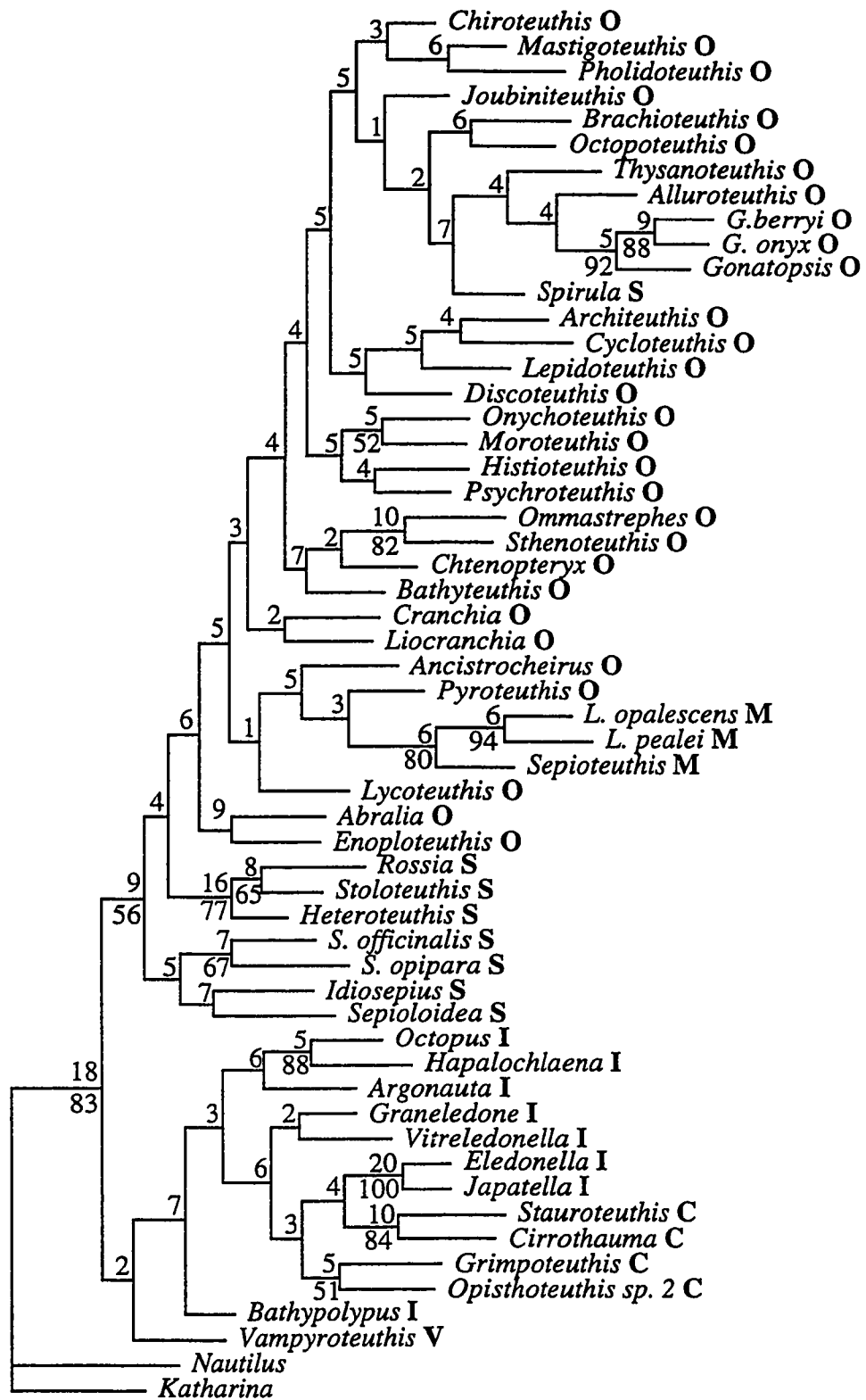


TABLE 3. RESULTS OF LIKELIHOOD RATIO TESTS OF SUBSTITUTION MODELS AND PARAMETER ESTIMATES FOR THE RESTRICTED COI DATA SET

| Null Hypothesis         | Models Compared   | ln L     | -2 log $\delta$ | df | P     | Parameter Estimates   |
|-------------------------|---|----------|-----------------|----|-------|---|
| Equal base frequencies  | H <sub>0</sub> : Jukes and Cantor (1969)                                      | -9400.31 | 425.08          | 3  | <0.01 | None  |
|                         | H <sub>1</sub> : Felsenstein (1981)   | -9187.77 |                 |    |       | <sup>a</sup> $\pi_A=0.29$ , $\pi_C=0.18$ ,<br>$\pi_G=0.05$ , $\pi_T=0.48$ |
| TI rate equals TV rate  | H <sub>0</sub> : Felsenstein (1981)   | -9187.77 | 418.06          | 1  | <0.01 | <sup>b</sup> TI:TV=3.496  |
|                         | H <sub>1</sub> : Hasegawa et al. (1985)                                       | -8978.74 |                 |    |       |   |
| Equal rates among sites | H <sub>0</sub> : Hasegawa et al. (1985)                                       | -8978.74 | 3059.5          | 1  | <0.01 | <sup>c</sup> $r_1=0.25$ , $r_2=0.02$ , $r_3=2.73$                         |
|                         | H <sub>1</sub> : Hasegawa et al. (1985)<br>with among-site rate heterogeneity |          | -7442.73        |    |       |   |

<sup>a</sup> $\pi_A$  = base frequency of adenines;  $\pi_C$  = base frequency of cytosines;  $\pi_G$  = base frequency of guanines;  $\pi_T$  = base frequency of thymines.

<sup>b</sup>TI:TV = Ratio of rates of transitional substitutions to transversional substitutions.

<sup>c</sup> $r_1$  = substitution rate at first codon position nucleotides;  $r_2$  = substitution rate at first codon position nucleotides;  $r_3$  = substitution rate at first codon position nucleotides.

Figure 10. Maximum likelihood tree generated from a heuristic search of the COI data assuming a Hasegawa, Kishino, and Yano (1985) model of substitution with site specific rates estimated according to the partitioned codon positions ( $-\ln L=14,708.77$ ). Branch lengths are drawn proportional to the probabilities of change occurring along each branch under the HKY85 model. Substitution parameters estimated in the likelihood search were as follows:  $\pi_A=0.286$ ,  $\pi_C=0.184$ ,  $\pi_G=0.047$ ,  $\pi_T=0.484$ ;  $TI/TV=3.496$ ;  $r_1=0.249$ ,  $r_2=0.017$ ,  $r_3=2.734$ . Higher-level taxonomic designations are indicated in boldface to the right of each terminal taxon (C = Suborder Cirrata; I = Suborder Incirrata; M = Suborder Myopsida; O = Suborder Oegopsida; V = Order Vampyromorpha).

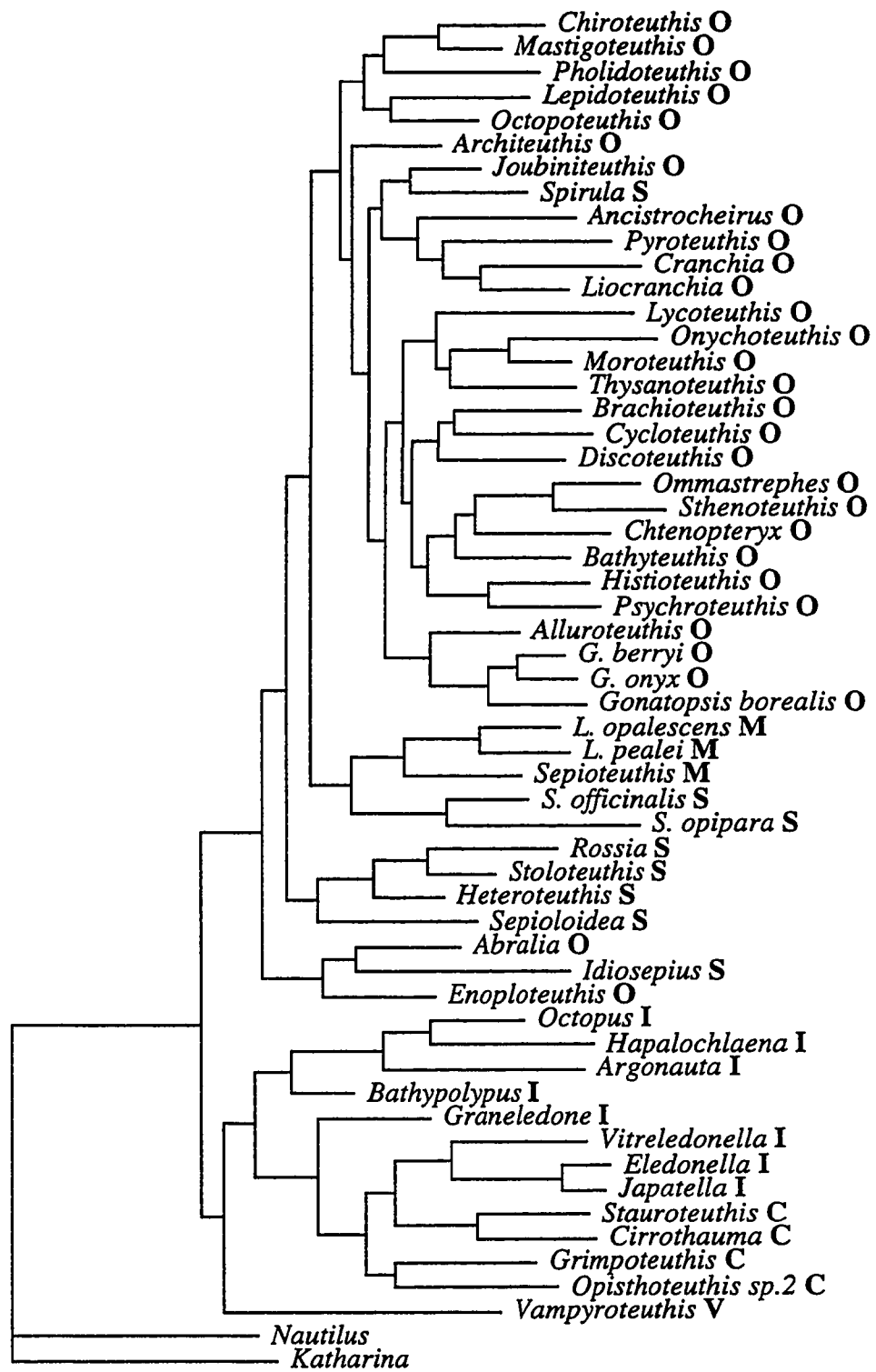


Figure 11. Most parsimonious tree obtained in a heuristic search (100 random addition replicates) of the weighted COI data set (TL = 15,364; CI = 0.148; RI = 0.363).

Transversion substitutions were assigned a weight of 7 steps and transitions were assigned a weight of 2 steps. Branch lengths are drawn proportional to the amount of change.

Bootstrap proportions are indicated as percentages below nodes. Higher-level taxonomic designations are indicated in boldface to the right of each terminal taxon (C = Suborder Cirrata; I = Suborder Incirrata; M = Suborder Myopsida; O = Suborder Oegopsida; V = Order Vampyromorpha).

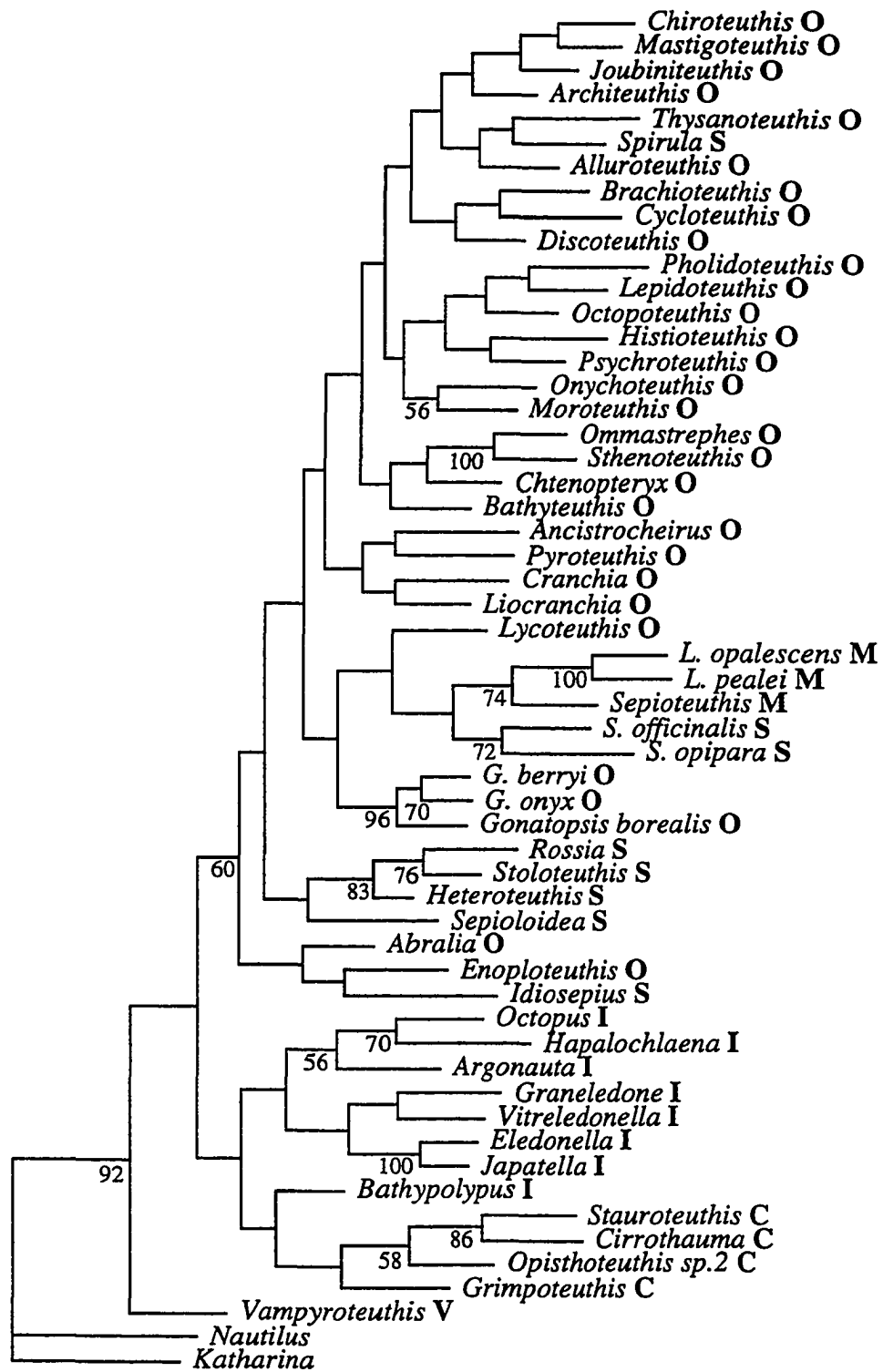
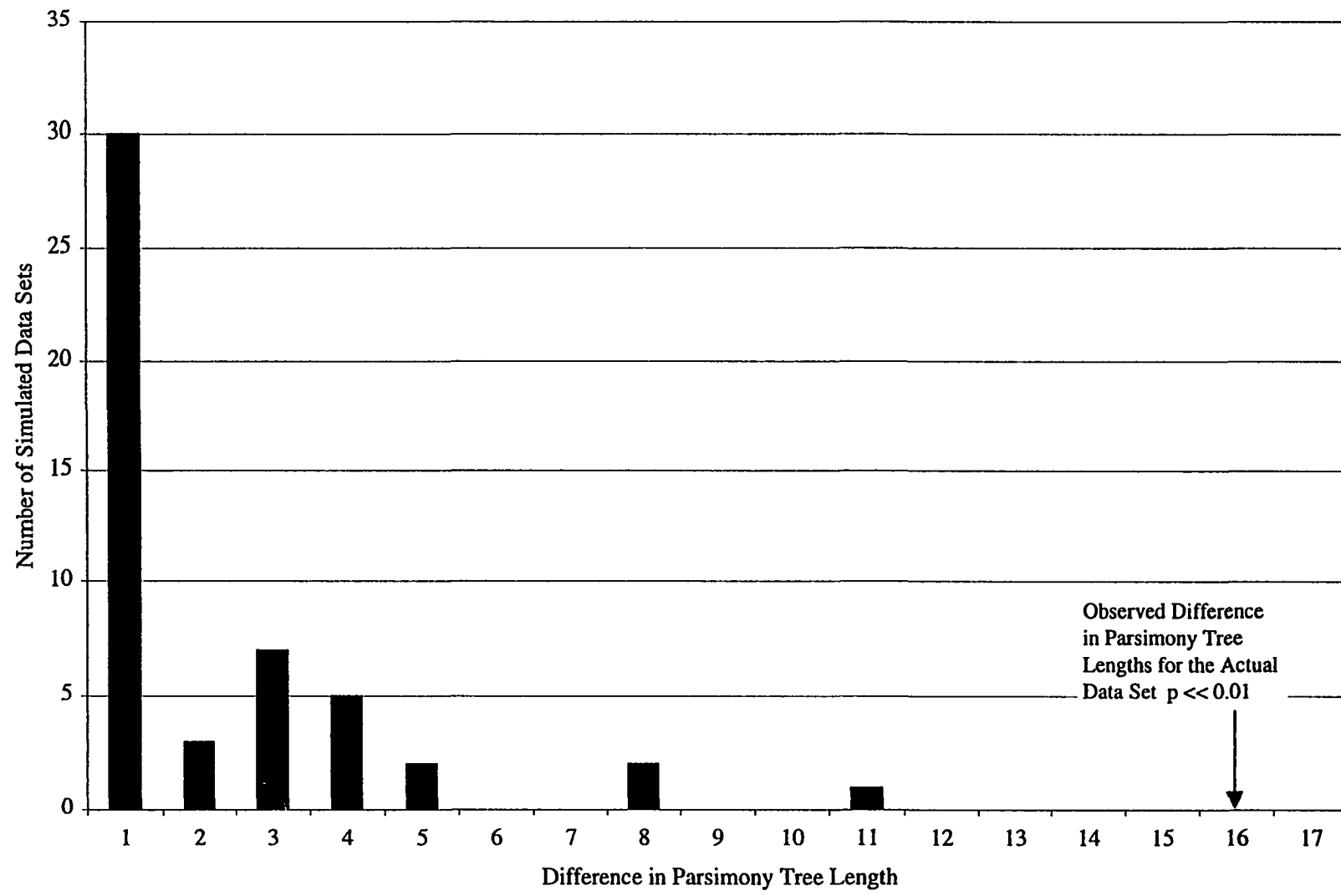




Figure 12. Results from parametric bootstrap analysis of the COI data set. The tree derived from maximum likelihood analysis of the COI data set constraining the monophyly of the Sepioidea was used to generate 50 simulated data sets. The substitution parameters under the HKY85+ model of evolution that were used to obtain the initial tree were also used to generate the simulated data sets ( $\pi_A=0.286$ ,  $\pi_C=0.184$ ,  $\pi_G=0.047$ ,  $\pi_T=0.484$ ;  $TI/TV=3.496$ ;  $r_1=0.249$ ,  $r_2=0.017$ ,  $r_3=2.7334$ ). Two rounds of parsimony analysis were conducted on each of the simulated data sets. The first parsimony search was conducted under the null hypothesis: constraint of sepioid monophyly. The second search was conducted with no constraints on the data. The differences in scores between the best tree derived from the constrained and unconstrained parsimony searches of each of the 50 simulated data sets was recorded and graphed to obtain the expected distribution under the null model. Each of the 50 sampled tree length differences fall below 12 steps, whereas for the observed data the tree length difference between the constrained and unconstrained searches was 16 steps. Therefore, a difference this great would be expected much less than 1% of the time if the null hypothesis were true, so the null hypothesis of sepioid monophyly is rejected at  $p \ll 0.01$ .



CHAPTER 2. PHYLOGENETIC RELATIONSHIPS OF COLEOID CEPHALOPODS  
INFERRED FROM THE MOLECULAR EVOLUTION OF TWO PARALOGOUS  
GENES FROM THE ACTIN GENE FAMILY

## INTRODUCTION

With the exception of the nuclear rRNA genes (5S, 18S, and 28S), there are very few nuclear genes that have been widely employed as phylogenetic markers (Harrison, 1991; Friedlander et al., 1992; Friedlander et al., 1994). However, it is now accepted that no single molecular sequence is sufficient to make reliable phylogenetic inferences. Stable hypotheses must be derived from the analysis of multiple sequences, either through congruence among the results from analyses of separate data sets (Mickey, 1978; Miyamoto and Fitch, 1995), or through the analysis of combined data sets (Kluge, 1989). It follows that the nuclear genome represents a virtually untapped source of phylogenetic information. The use of nuclear protein-coding genes in phylogenetics, though obviously warranted, has been impeded by several factors ostensibly unique to the nuclear genome. These factors include the problems associated with low gene copy number, distinguishing orthologs (homologous genes in different taxa) from paralogs (nonhomologous genes), concerted evolution, recombination, and insertion/deletion events. The difficulties presented by these factors are encountered in all phases of a phylogenetic study, but perhaps the most critical barrier to the widespread use of protein-coding nuclear genes in phylogenetics is in the design of reliable taxon-specific primers. Primer design is complicated by the lack of conservation across taxa in exon-intron organization of protein-coding genes, where the presence of introns in the primer annealing regions of primers designed for use in other taxa, or the presence of long introns within the target region can prevent amplification. Furthermore, the low copy number of nuclear protein-coding genes relative to rRNA genes or mitochondrial genes decreases the efficiency of PCR amplification.

In this chapter the results of a phylogenetic analysis of the coleoid cephalopods based on molecular sequence data from the protein-coding regions of two actin genes are reported. Actin is a ubiquitous protein in eukaryotic cells, and plays a crucial role in muscle contraction, cell motility, cytoskeletal structure, cell division, intracellular transport, and cell differentiation. Actin proteins are encoded by a multigene family in the nuclei of all animals, plants, and protozoa examined to date, but is encoded by only a single gene in yeast and prokaryotes (Hightower and Meagher, 1986). Related proteins of a multigene family encoded at separate loci (paralogous genes) are termed *isoforms*. The actin isoforms are all encoded by a set of structurally related genes, whose expression is spatially and temporally regulated, and which descended by duplication and divergence from common ancestral genes (Hightower and Meagher, 1986). The number of actin isoforms found in different taxonomic groups is quite variable. Mammals possess at least six different isoforms (Vandekerckhove and Weber, 1978); nine different isoforms have been characterized in teleost fishes (Venkatesh et al., 1996); the echinoderm genome contains at least eight nonallelic actin genes (Lee et al., 1984; Fang and Brandhorst, 1994); and insects have been shown to have at least six actin genes (Fyrberg et al., 1980). The actin gene family of plants is much larger than that of animals, comprising 8-44 genes depending on the specific taxa (Moniz de Sá and Drouin, 1996; Reece et al., 1992). The petunia genome contains over 100 actin genes, though most are thought to be pseudogenes (McLean et al., 1990).

The designation of different actin isoforms is somewhat confusing as there are no universal standards for reference; however, most researchers follow the terminology used for chordate actins. Chordate actin isoforms are divided into two main categories, muscle actins and cytoplasmic actins. The muscle actins are in turn divided into two types, each containing two members: striated muscle actins ( $\alpha$ -skeletal and  $\alpha$ -cardiac) and smooth muscle actins ( $\alpha$ -vascular and  $\gamma$ -enteric). In chordates there are also two cytoplasmic actin isoforms, designated  $\beta$  and  $\gamma$  actins (Herman, 1993). The distinction between muscle and

nonmuscle actins may be restricted to the complex metazoans since it is also observed in insects but not in nematodes. However, the muscle actins of arthropods differ from the muscle actins of deuterostomes to such an extent that two independent derivations of muscle actins probably occurred, once within the protostome lineage and once within the deuterostome lineage (Mounier, 1992). Invertebrate muscle actins are generally thought to be more similar to chordate cytoplasmic actins than to chordate muscle actins (Vandekerckhove and Weber, 1984).

Surprisingly little is known about the diversity, types, expression, and molecular evolution of actin genes in non-arthropod protostome phyla. To date, only two studies have attempted to determine the number of actin genes in molluscs, one in the sea hare *Aplysia californica* (DesGroseillers et al., 1994), the other from a sea scallop *Placopecten magellanicus* (Patwary, 1996). Although the results of this study were not entirely conclusive, the number of actin genes in the sea hare, as estimated through Southern blot analysis using sperm DNA as a probe, ranged between three and five copies per haploid genome. Southern blot data on the sea scallop suggested the presence of approximately 12 to 15 actin genes. Further analysis of actin gene evolution in molluscs is clearly warranted and would provide insight into the multiple origins of muscle actin isoforms, such as where along the protostome lineage the gene duplication event occurred that produced a muscle-type actin isoform.

The use of actin as a phylogenetic marker has been largely restricted to analyses of actin gene evolution (see preceding references) or in the analysis of distantly related taxa, such as relationships between phyla (Bhattacharya and Ehling, 1995; Reece et al., 1997). The evolutionary rate of the actin gene(s) has been considered too slow to determine relationships of taxa below the phylum/division level (Mounier, 1992). However, categorical designation of a gene or gene family as "highly conserved" is a vague and arbitrary term, as there are clear differences in the evolutionary rate of genes between taxa, and differences in the evolutionary rate of paralogous genes (Li, 1997). It is entirely

possible for two genes that have undergone very different patterns of evolution and have correspondingly different phylogenetic utilities, to show the same values of uncorrected percent sequence divergence. Therefore, use of uncorrected sequence divergences as the sole determinant of the phylogenetic utility of a particular gene can lead to the rejection of potentially informative candidate genes (Graybeal, 1994). In addition, many of the conclusions drawn from studies addressing the evolutionary rate of actin are pertinent to the evolutionary rates of the amino acid sequences, not of the nucleotide sequences. The synonymous substitution rate of actin genes can be quite high, in some cases up to 35 times the nonsynonymous substitution rate (Moniz de Sá and Drouin, 1996). While the use of synonymous substitutions is not generally appropriate for determining relationships at deep divergences due to saturation, there are exceptions, for example in the albumin gene and *c-myc* oncogene (Graybeal, 1994). Synonymous substitutions in highly conserved genes may also provide a wealth of information about lower-level relationships. This was demonstrated for the "highly conserved" elongation factor-1 $\alpha$  gene, in which synonymous substitutions were informative to reconstruct relationships within a moth subfamily that diverged less than 20 million years ago (Cho et al., 1995). Finally, the combined use of multiple paralogous actin genes increases the number of phylogenetically informative characters for both nonsynonymous and synonymous substitutions, so that resolution within Coleoidea may be obtained in a "brute force" approach.

This chapter will present and discuss the results from the phylogenetic analysis of a 784 bp fragment from three paralogous actin genes from 44 cephalopod taxa. The number of protein-coding actin genes present in the genomes of coleoid cephalopods will be estimated through phylogenetic analysis of 82 coleoid actin sequences. The amino acid sequences of three paralogous actin genes from each of seven will be aligned and analyzed with 30 amino acid sequences from an array of 30 metazoan taxa. Following the unequivocal demonstration that at least 3 paralogous actin genes had been cloned and sequenced for coleoids, the results from a more thorough analysis of two of the three paralogs will be

presented, including unweighted parsimony, maximum likelihood, and weighted parsimony analysis of the two data sets. The monophyly of the Sepioidea will be tested for both data sets using the parametric bootstrap technique.



## MATERIALS AND METHODS

### Taxonomic Sampling

A portion of the actin gene(s) was sequenced for 44 cephalopod taxa representing a broad spectrum of diversity within the class. The individual specimens used in generating the actin gene sequences for each taxon were identical to those used in generating the COI sequence data. Included are taxonomic representatives from each of the 5 "families" of the order Sepioidea, 2 genera from the suborder Myopsida, 19 families of the suborder Oegopsida, 3 families from the suborder Incirrata, 2 families from the suborder Cirrata, a representative from the monotypic order Vampyromorpha, and a member of the subclass Nautiloidea was also included as an outgroup. Tissue samples from specimens were stored in the same manner as described in Chapter 1.

### PCR Amplification, Cloning, and Sequencing

Two sets of degenerate primers were used to amplify actin gene(s) from cephalopods (Table 4). Initially, the Actin 480 and Actin 483 primer set was used to amplify a 623 bp fragment (excluding primer sequence) of the actin gene(s). After obtaining sequence data for several taxa using the Actin 480 and Actin 483 primers, a second set of primers, Actin 481 and Actin 482, was used to amplify a larger portion (784 bp) of the actin gene. Amplification conditions were similar for both pairs of primers. A typical 50  $\mu$ l amplification consisted of the following reagents: 5-10 ng template DNA, 20mM TrisHCl (pH 8.4), 50mM KCl, 1.5mM MgCl<sub>2</sub>, 50 pmoles of each primer, 0.2mM of each dNTP, and 1.25 units of *Taq* DNA polymerase. An MJ Research PTC-200 (Watertown, MA) thermocycler was used to conduct 40 cycles of the following temperature profile: 94°C for

1 minute, 45–46°C (depending on the sample) for 1 min., and 68°C for 2 min. A final extension step at 68°C for 7 min. followed the 40 cycles of amplification. PCR products were cloned and sequenced as described in Chapter 1.

Preliminary phylogenetic analyses conducted on the cephalopod actin sequences suggested that at least three paralogous cephalopod actin genes had been amplified and cloned. In order to obtain adequate taxonomic sampling for at least two of the paralogs (=isoforms), multiple clones from each species were digested with diagnostic restriction endonucleases prior to sequencing to avoid sequencing identical isoforms within the same species. Two restriction endonucleases, *SstI* and *BamHI* (Life Technologies, Gaithersburg, MD) were used to discriminate among the three isoforms, with one isoform having a *SstI* site at position 15 (Actin I), one isoform having neither site (Actin II), and the third isoform having a *BamHI* site at position 761 (Actin III).

#### Data Analysis

*Alignment*. In order to align and compare cephalopod actin sequences to other metazoan actin sequences, 30 actin sequences were downloaded from GenBank (Table 5). Cephalopod DNA sequences were aligned by eye with the aid of the downloaded sequences and compiled in MacClade 3.0 (Maddison and Maddison, 1992) or Gene Jockey II (Biosoft, Cambridge, UK). Introduction of gaps into the aligned cephalopod actin sequences was unnecessary as there were no insertion/deletion events or alignment ambiguities. Introduction of two gaps into the *S. spirula* actin clone #40 sequence, which had deletions at positions 433–438 and 737–739, was necessary. Both deletions were in frame, resulting in a loss of 3 and 1 amino acids, respectively, from the deduced amino acid sequence. The amino acid sequences of human  $\beta$  cytoplasmic and  $\alpha$  muscular actins were also downloaded from GenBank for a comparison of the diagnostic muscular and cytoplasmic amino acids in humans with those of invertebrate taxa.

*Sequence Characteristics.* MacClade 3.0 was used to assign codon positions to the nucleotide data, to translate the cephalopod nucleic acid sequences into amino acid sequences, and to generate various assumption sets (weight and character inclusion sets, transition or transversion type sets) used in later analyses. To reduce the total number of possible pairwise comparisons between taxa, the restricted data set, a subset of 26 overlapping taxa from two of the actin data sets, was used in the calculation of patristic matrices under various sets of assumptions. Uncorrected pairwise sequence divergences were also calculated for intraspecific comparisons of the Actin I and Actin II genes. A single factor ANOVA was used to test for a significant difference in the mean intraspecific sequence divergences of the Octopodiformes and Decapodiformes. The base frequencies at each codon position for each of the 26 taxa were determined and a chi-square test was employed to test for significant heterogeneity among taxa in base composition at the three codon positions.

*Phylogenetic Analyses.* Maximum parsimony analysis of the aligned nucleotide and deduced amino acid sequences was conducted using the heuristic tree-search option in PAUP\* (Swofford, 1996) with 50 random sequence addition replicates. Support for clades within phylogenetic trees was tested using the heuristic bootstrap search command (1000 replicates) in PAUP\*. A second measure of clade support, the Bremer decay index (Bremer, 1988), was also determined for each clade on the most-parsimonious tree using the software program TreeRot (Sorenson, 1996). Analysis of the entire actin data set (82 terminal "taxa") revealed the presence of three distinct actin paralogs. The inclusive actin data set was therefore partitioned into three data sets, one for each paralog. In addition, to explore the relationship between the three cephalopod actin isoforms and their relationship to other metazoan actin isoforms, a fourth actin data set was constructed. This data set consisted of the amino acid sequences from three cephalopod taxa for which all three

isoforms had been sequenced (= nine terminal “taxa”) along with actin amino acid sequences from the 30 metazoan taxa downloaded from GenBank.

Two of the three paralogous actin data sets were analyzed in detail. Analysis of the two actin data sets containing 38 (Actin I) and 32 (Actin II) taxa was conducted in the same manner as that described in Chapter 1. Briefly, both data sets were initially subjected to parsimony analysis. Following parsimony analysis, maximum likelihood analyses were conducted on the restricted data sets to determine the most appropriate model of substitution. The HKY85+ model was found to best fit the data and was used in likelihood analyses of the inclusive data sets. The transition:transversion ratio estimated via maximum likelihood analyses was then used to construct a perfect weighting step matrix for weighted parsimony analyses. Twenty-five random addition replicate heuristic parsimony searches of the weighted data were conducted, along with bootstrap analysis to determine support for various clades in the weighted parsimony analysis.

*Test of Sepioidea Monophyly.* A maximum likelihood tree for the constrained data, where the Sepioidea was constrained to be monophyletic, was generated for each of the two actin data sets in the same manner as described for the unconstrained data described above. Model parameters were estimated in a successive approach and then fixed in the following searches to obtain the maximum likelihood tree assuming a HKY85+ model of sequence evolution. The maximum likelihood tree obtained was then used to generate 50 simulated data sets for each of the two actin genes under the same model parameters using the computer program SeqGen 1.04 (Rambaut and Grassly, 1997). The simulated data sets for each gene were then used to generate a null distribution of most-parsimonious tree length differences, calculated as the difference in parsimony tree length under the null (Sepioidea monophyly) and alternate (unconstrained) hypotheses for each of the 50 simulated data sets. The tree length difference for the actual data was then compared to the null distribution to determine if the actual tree length difference was statistically significant.

The proportion of the replicates in which the tree length difference calculated using the actual data was exceeded for the simulated data represented the significance level of the test.

## RESULTS

### Actin Isoforms

The multiple alignment of nucleotide sequences of the actin genes of cephalopods is presented in Figure 13. Parsimony analysis of the 82 actin sequences yielded 360 equally parsimonious trees and revealed the presence of three distinct actin isoforms (Figure 14). The first isoform cloned and examined was arbitrarily designated Actin I. The second most common isoform was designated Actin II and the third isoform discovered was designated Actin III. The mean intraspecific nucleotide sequence divergence between the Actin I and Actin II isoforms of the Octopodiformes (21.11 $\pm$ 1.48%) was significantly greater than that for calculated for the Decapodiformes (18.02 $\pm$ 1.33%) (Figure 15). The sequences of all three actin isoforms (Actin I, Actin II, and Actin III) were obtained from seven cephalopod taxa. The amino acid sequences of three of these seven taxa, *Chtenopteryx*, *Sepia opipara*, and *Vampyroteuthis*, were analyzed with actin sequences downloaded from GenBank. Analysis of the deduced amino acids of cephalopod actins along with other metazoan actin protein sequences also revealed the presence of three distinct cephalopod actin isoforms (Figure 16). Of the three isoforms, Actin I was most closely related to the deuterostome muscle-type actins and exhibited the least variability. Actins II and III exhibited comparable levels of variation and clustered among the mollusc cytoplasmic actins. The three isoforms were phylogenetically analyzed separately thereafter except in the "total evidence" analyses. A comparison of the three cephalopod actin amino acid sequences with those of the human  $\beta$  cytoplasmic and  $\alpha$  skeletal actins and the cytoplasmic and muscle actin sequences from *Drosophila* and *Aplysia* is presented in Figure 17. Of the

three cephalopod actin isoforms, the Actin I sequence was most similar to the human, *Drosophila*, and *Aplysia* muscle actin amino acid sequences.

#### Actin I Sequence Variation and Divergence

The 784 base pair fragment of the Actin I gene from 38 species exhibited 292 variable characters (37.2%). Of the 292 variable characters, 51 (17.5%) were first codon position bases (31 of which were parsimony uninformative), 30 (10.3%) were second codon position bases (27 of which were parsimony uninformative), and 211 (72.3%) characters were third codon position bases (50 of which were parsimony uninformative). There was a total of 184 parsimony informative characters for the Actin I data set.

Comparisons of sequence divergences within and among the major groups of cephalopod taxa are presented in Table 6. Mean sequence divergences determined from all possible pairwise comparisons within groups were less than mean sequence divergences resultant from pairwise comparisons among groups. For example, the mean sequence divergence (+/- standard deviation) for all pairwise comparisons within the Oegopsida was 6.34+/-1.73%, whereas comparisons between the Oegopsida and other groups ranged from 7.10% to 10.57%. The Sepioidea exhibited the greatest within-group mean sequence divergence (6.82%) and the greatest standard deviation in within-group sequence comparisons (2.54%). However, pairwise comparisons among Coleoid groups did not differ appreciably (e.g., Oegopsida vs. Myopsida = 6.38%, Oegopsida vs. Sepioidea = 7.77+/-2.0%).

Figure 18 presents the sequence divergences for pooled first and second position nucleotides and third position nucleotides plotted as a function of total uncorrected sequence divergence for all possible comparisons between the 26 taxa in the restricted Actin I data set. Substitutions at third codon position nucleotides did not account for a greater proportion of the variation in the Actin I gene at lower total sequence divergences than at greater total sequence divergences. Because third position substitutions did not account for

a lesser proportion of sequence divergence at higher levels of total sequence divergence, these data do not provide evidence for saturation at third codon positions.

The percentage of total sequence divergence accounted for by transversions and transitions are plotted as a function of total uncorrected percent sequence divergence for all possible sequence comparisons of the restricted Actin I data set in Figure 19. Although transitions accounted for the majority of substitutions across the entire range of uncorrected sequence divergence (0 to 20%), transversions also made a significant contribution across the same range (0 to 20%). The relationship between the type of substitution and total uncorrected sequence divergence indicated that the Actin I data were not likely to be saturated with respect to transitions. Transition substitutions accounted for approximately the same proportion of total substitutions at low (~70% of the total at 5% divergence) and high (~65% of the total at 15% divergence) sequence divergences.

There was not significant heterogeneity in base composition at third codon position characters (chi square = 46.02, df = 75,  $p < 0.001$ ), and chi square values for base composition bias at first and second codon characters (3.31 and 0.99, respectively) were not significant (df = 75,  $p > 0.99$ ). The frequencies of the four bases were highly unequal at all codon positions, with cytosines the rare at first positions (Figure 20), guanines rare at second positions (Figure 21), and adenines quite rare at third positions (Figure 22).

#### Actin II Sequence Variation and Divergence

The 784 base pair fragment of the Actin II gene from 32 species consisted of 342 variable characters (43.6%). Of the 342 variable characters, 67 (19.6%) were first codon position bases (35 of which were parsimony uninformative), 33 (12.6%) were second codon position bases (28 of which were parsimony uninformative), and 242 (70.8%) characters were third codon position bases (50 of which were parsimony uninformative). There were a total of 244 parsimony informative characters for the Actin II data set.



Comparisons of sequence divergences within and among the major groups of cephalopod taxa are presented in Table 7. Mean sequence divergences determined from all possible pairwise comparisons within groups were less than mean sequence divergences resultant from pairwise comparisons among groups. For example, the mean sequence divergence (+/- standard deviation) within the Oegopsida was 7.55+/-1.87% whereas comparisons between the Oegopsida and other groups ranged from 9.39% to 18.38%. As was the case for the COI and Actin I genes, among the major groups of cephalopods, the Sepioidea exhibited the greatest within-group mean sequence divergence (9.37%) and the greatest standard deviation in within-group sequence comparisons (3.49%). Some pairwise comparisons among related Coleoid groups differed appreciably (e.g., *Vampyroteuthis* vs. Cirrata = 15.12+/-0.8%, *Vampyroteuthis* vs. Incirrata = 18.45+/-1.2%).

Figure 23 presents the sequence divergences for pooled first and second position nucleotides and third position nucleotides plotted as a function of total uncorrected sequence divergence for all possible comparisons between the 26 taxa in the restricted Actin II data set. Substitutions at third codon position nucleotides accounted for a greater proportion of the variation (roughly 75%) in the Actin II gene at lower total sequence divergences (~5%) than at higher total sequence divergences. At increased total sequence divergences (~20%), there was an increase in the contribution of first and second position substitutions (roughly 50%) to the total sequence divergence. Because third position substitutions did not account for as great a proportion of sequence divergence at higher levels of total sequence divergence, these data provide evidence for saturation at third codon positions. The incursion of superimposed substitutions at third codon position nucleotides may potentially mask the actual total number of substitutions that have taken place in the Actin II gene.

The percentage of total sequence divergence accounted for by transversions and transitions are plotted as a function of total uncorrected percent sequence divergence for all

possible sequence comparisons of the restricted Actin II data set in Figure 24. Although transitions accounted for the majority of substitutions across the entire range of uncorrected sequence divergence, the relative contribution of transversions to total sequence divergence increased with greater sequence divergence. The relationship between the type of substitution and total uncorrected sequence divergence indicated that the Actin II data are likely to be saturated with respect to transitions at greater sequence divergences.

There was significant heterogeneity in base composition at third codon position characters (chi square = 103.8, df = 75,  $p < 0.05$ ), whereas chi square values for base composition bias at first and second codon characters (3.48 and 1.90, respectively) were not significant (df = 75,  $p > 0.99$ ). The frequencies of the four bases were highly unequal at all codon positions, with cytosines the rarest at first positions (Figure 25), guanines rare at second positions (Figure 26), and adenines rare at third positions (Figure 27).

#### Phylogenetic Relationships-Actin I

*Unweighted Parsimony.* Parsimony analysis on the Actin I data set yielded 36 equally parsimonious trees of length 784. The strict consensus, along with bootstrap support values given below nodes and Bremer support values indicated above nodes, is depicted in Figure 28. The equally weighted data do not provide much resolution; however, the consensus tree is relatively robust as many of the peripheral nodes were supported by the majority of bootstrap replicates. As the Actin I gene exhibited little variability, Bremer support values were quite low for most nodes. The Actin I data supported the monophyly of the Octopoda, Incirrata, Decapodiformes, Sepiolidae, Sepiidae, and Myopsida. The two ommastrephids did not cluster, as *Ommastrephes* grouped with *Pyroteuthis* (with bootstrap and Bremer support). *Onychoteuthis* grouped just outside of the gonatids, *Cycloteuthis* and *Discoteuthis* were found to be related, as were *Chtenopteryx* and *Bathyteuthis*. Interestingly, the equally weighted parsimony data supported a clade consisting of

*Ctenopteryx*, *Bathyteuthis*, Myopsida, and Sepioidea, although Bremer support for the clade was weak and bootstrap support lacking.

*Parsimony on Deduced Amino Acid Sequences.* Parsimony analysis was conducted on the deduced amino acid sequences but only 10 characters were found to be phylogenetically informative. As there were so few informative characters, a heuristic search was not completed due to the generation of many equally parsimonious trees. When the search was aborted, a strict consensus of the trees revealed no phylogenetic structure with the exception of one clade, (*Cranchia Liocranchia*).

*Maximum Likelihood Analyses.* As described in the previous chapter, initial likelihood analyses were conducted on the restricted nucleotide data set to estimate parameters used in the subsequent analysis of the comprehensive data set. Log likelihoods, substitution parameters, and the results of likelihood ratio tests obtained in analyses of the restricted Actin I data set are presented in Table 8. As with the COI gene, of the four substitution models examined, the HKY85+ model provided the best fit to the data. Substitution parameter values obtained in the analysis of the restricted data set under the HKY85+ model were then fixed in the subsequent search of the comprehensive data set.

The maximum likelihood analysis from a single heuristic search of the comprehensive data set under the HKY85+ model yielded the tree depicted in Figure 29. The tree supports the monophyly of the Decapodiformes, Octopoda, Incirrata, Myopsida, and most families represented by more than one taxon (Oegopsida: Cranchiidae, Cycloteuthidae, Gonatidae; Incirrata: Octopodidae, Bolitaenidae; Sepioidea: Sepiidae, Sepiolidae). The monophyly of the Sepioidea, Teuthoidea, and Oegopsida, was not supported by maximum likelihood analysis of the Actin I data. Results obtained in the maximum likelihood analysis were largely congruent with the parsimony results. As in the parsimony analysis, the monophyly of the following interfamilial groups was supported: (Myopsida (*Spirula* (*Sepiidae*))), (*Sepioloidea* (*Sepiolidae*)), (*Ctenopteryx Bathyteuthis*), (*Lepidoteuthis*

*Octopoteuthis*), (*Onychoteuthis* (Gonatidae)), (*Ommastrephes Pyroteuthis*), and (Cirrata (Bolitaenidae Octopodidae)). Also consistent with the parsimony results, *Alluroteuthis*, followed by *Histioteuthis* and *Chiroteuthis*, were the first species found to branch off the decapod line.

*Weighted Parsimony.* A TI:TV ratio of 2.51 was obtained in the maximum likelihood analysis under the HKY85+ model of substitution. Therefore, transversional substitutions were weighted 2.5X greater than transitional changes in the weighted parsimony analysis to account for the greater frequency of transitions. A strict consensus of 44 equally parsimonious trees obtained in weighted parsimony analysis is presented in Figure 30. Clades supported in both the unweighted parsimony and likelihood analyses were also generally supported in parsimony analysis of the weighted data. The one substantial difference between the weighted parsimony analysis and prior analyses was in the placement of the (Sepiadariidae Sepiolidae) clade with the sepiids and *Spirula*. Weighting the data also caused the enoploteuthids to cluster, and as in the likelihood analysis, the (*Lepidoteuthis Octopoteuthis*) and ((*Pyroteuthis Ommastrephes*)(*Onychoteuthis* (gonatid))) clades were supported. Bootstrap analysis of the weighted data did not increase the total number of nodes supported in over 50% of the replicates, nor did weighting the data increase the proportion of replicates supporting those nodes.

*Statistical test of Sepioid Monophyly.* The tree length difference obtained in parsimony searches of the Actin I data set is compared to null distribution of tree length differences obtained by parsimony searches of the simulated data sets in Figure 31. The observed tree length difference, 3 steps, falls outside of the null distribution of simulated tree length differences ( $p \ll 0.01$ ). This result statistically confirms the conclusion that the Actin I data do not support the monophyly of the Sepioidea. The difference between the observed tree length difference and the range of tree length differences found in parsimony searches

of the simulated data sets is much less than that observed for COI. Although the difference remains significant, the highly conserved nature of the Actin I gene resulted in a small range of overall difference in tree lengths in the simulated data sets.

#### Phylogenetic Relationships-Actin II

*Unweighted Parsimony.* A heuristic search (25 random addition replicates) of the Actin II data set yielded 5 equally parsimonious trees of length 1217. Figure 32 shows the strict consensus tree constructed from the 5 equally parsimonious trees, along with bootstrap and Bremer support values given above and below the nodes, respectively. Parsimony analysis of the Actin II gene supported the monophyly of the Octopodiformes, Cirrata, Incirrata, Bolitaenidae, Decapodiformes, Cycloteuthidae, Ommastrephidae, Sepiolidae, Sepiidae, and Myopsida. Also supported was a close relationship between *Histioteuthis* and *Psychroteuthis* and also between *Ctenopteryx* and *Bathyteuthis*. Bootstrap analysis resulted in moderate to strong support for most of the monophyletic groups outlined above with Bremer decay indices also in general agreement. Clades which received moderate to strong Bremer support but which lacked bootstrap support were (*Idiosepius* (Ommastrephidae)), and the Decapodiformes exclusive of (*Gonatus*)(*Cycloteuthis* *Discoteuthis*).

*Parsimony on Deduced Amino Acid Sequences.* Analysis of the Actin II deduced amino acid sequences revealed only 18 phylogenetically informative characters. A heuristic search found 75 equally parsimonious trees of length 126. As there were so few informative characters, very little phylogenetic structure was revealed by the amino acid data. Each of the 75 trees supported a clade within the Octopoda consisting of (*Argonauta* (*Octopus* (*Bathypolypus Cirrothauma*))). Each of these clades was weakly supported, with no more than 3 synapomorphies occurring along any given branch of all 75 trees.

*Maximum Likelihood Analyses.* Table 9 presents log likelihoods, substitution parameters, and the results of likelihood ratio tests obtained in analyses of the restricted Actin II data set. As with the COI and Actin I genes, the HKY85+ substitution provided the best fit to the data for the four substitution models examined. Substitution parameters obtained in the analysis of the restricted data set under the HKY85+ model were then fixed in the subsequent search of the comprehensive data set.

The maximum likelihood analysis from a single heuristic search of the comprehensive data set under the HKY85+ model yielded the tree illustrated in Figure 33, rooted with *Vampyroteuthis*, as *Nautilus* grouped within the Decapodiformes. Although rooting the tree with *Vampyroteuthis* is not logically defensible, given the fact that the Decapodiformes+*Nautilus* are definitely not a monophyletic group, the tree was rooted in this manner for heuristic purposes. Rooting the tree with *Nautilus* results in a tree topology that is difficult to interpret, with the exception of the Octopodiformes, which become monophyletic with *Nautilus* as the root. The likelihood tree supports the monophyly of the Incirrata, Myopsida, (Sepiolidae+Sepiariidae) and various families represented by more than one taxon (Oegopsida: Cycloteuthidae; Incirrata: Bolitaenidae; Sepioidea: Sepiidae, Sepiolidae). The monophyly of the Octopodiformes, Octopoda, Cirrata, Sepioidea, Teuthoidea, and Oegopsida was not supported by maximum likelihood analysis of the Actin II data.

*Weighted Parsimony.* A transition:transversion ratio of 2.62 was obtained in the maximum likelihood analysis under the HKY85+ model of substitution. Therefore, transversional substitutions were weighted 2.5X greater than transitional changes in the weighted parsimony analysis to account for the greater frequency of transitions. The most parsimonious tree obtained in weighted parsimony analysis is presented in Figure 34. Weighted parsimony analysis supported the monophyly of the Octopodiformes, Decapodiformes, Cirrata, Incirrata, Myopsida, and various families (Incirrata:

Bolitaenidae; Oegopsida: Cycloteuthidae and Ommastrephidae; Sepioidea: Sepiidae and Sepiolidae). As found in the unweighted parsimony analysis, the Octopoda was not supported as the cirrate octopods appeared more closely related to *Vampyroteuthis* than to the incirrate octopods although bootstrap support for this relationship was weak. Clades supported in both the parsimony and likelihood analyses were also generally supported in parsimony analysis of the weighted data. The one substantial difference between the weighted parsimony analysis and prior analyses was the placement of the (Sepiadariidae Sepiolidae) clade with the sepiids, myopsids, and *Spirula*. Bootstrap analysis of the weighted data did not increase the total number of nodes supported in over 50% of the replicates, nor did weighting the data increase the proportion of replicates supporting those nodes.

*Test of Sepioid Monophyly.* The tree length difference obtained in parsimony searches of the Actin II data set, compared to null distribution of tree length differences obtained by parsimony searches of the simulated data sets is presented in Figure 35. The observed tree length difference, 38 steps, falls outside of the null distribution of simulated tree length differences ( $p < 0.01$ ). The large difference between the observed tree length difference and simulated tree length suggests that the failure of the Actin II gene data to support the monophyly of the Sepioidea is not due to stochastic variation in the data alone. This result statistically confirms the conclusion that the Actin II data do not support the monophyly of the Sepioidea.

## DISCUSSION

### The Actin Gene Family of Coleoid Cephalopods

The strict consensus of 360 equally parsimonious trees obtained in analysis of the 82 actin sequences clearly demonstrates the presence of at least 3 distinct forms of the actin gene in coleoid cephalopods. The degenerate primers used to amplify actin fragments were therefore amplifying multiple actin loci. Cloning and sequencing of amplified products does not guarantee that all actin isoforms were revealed. The high degeneracy of the Actin 481 and Actin 482 primers, which were designed from relatively conserved regions of the actin locus, renders the discovery of additional protein-coding actin loci in coleoid cephalopods unlikely unless other loci possess long intervening introns within the target region. Of the 44 taxa analyzed, 13 were represented by a single actin isoform, 24 were represented by at least two actin isoforms, and all three isoforms had been obtained for 7 taxa. Each of these isoforms clearly belonged to one of the three major clades determined through phylogenetic analysis of the nucleotide data. Therefore, it is unlikely that additional attempts to clone and sequence actin genes from cephalopods using the Actin 481 and Actin 482 primers would result in the discovery of a new isoform class. However, it is still possible that the degenerate primers used in this study preferentially amplified a distinct class of actin genes and that the use of other primers designed to amplify alternate isoforms would result in the discovery of additional cephalopod actin isoforms.

There is another way to test the hypothesis that preferential amplification of a distinct class of actin genes resulted in the incomplete representation of major types of actin isoforms in cephalopods. This method entails phylogenetic analysis of representative amino acid sequences of the three cephalopod actin isoforms along with known actin



isoform amino acid sequences from a diverse array of metazoan taxa downloaded from GenBank. To be most informative about the number and type of actin isoforms possessed by cephalopods, the sequences from non-cephalopodan taxa included in the analysis would preferably be derived from cDNA libraries so that the tissue in which the particular isoforms were expressed would be defined. If all three isoforms clustered together within a particular category of actin genes (e.g., all were cytoplasmic isoforms), then the argument that the PCR primers preferentially amplified only a subset of the actin loci in the genomes of cephalopods could be advanced. However, if the three isoforms grouped among different types of actin isoforms (e.g., some with cytoplasmic isoforms, some with muscle isoforms), the hypothesis of preferential amplification would not be defensible. In this case the prospect of discovering additional major classes of actin isoforms in cephalopods is unlikely, unless alternate actin loci possess large introns within the 784 bp region analyzed.

It is also possible that each of the 3 classes of actin isoforms found in this study may represent different subclasses of isoforms. Indeed studies have shown that distinct loci, determined through analysis of cDNA library clones, may possess identical protein sequences and nearly identical (>95%) nucleotide sequences (Wahlberg and Johnson, 1997). Thus, although the sequences may be nearly identical, they may not be homologous. Gene conversion has been invoked as the mechanism maintaining homogeneity among separate actin loci (Crain et al., 1987; Wahlberg and Johnson, 1997). Such phenomena could potentially have serious consequences for phylogenetic reconstruction as the comparison of nonhomologous sequences would render the results of phylogenetic analysis meaningless. Fortunately, if gene conversion has been a factor in the evolution of a seemingly homologous group of actin sequences, one would expect little to no concordance in the structure of gene trees derived from the analysis of different actin isoforms. Phylogenetic analysis of the entire actin data set of 82 terminal taxa (Figure 14) reveals that gene conversion is an unlikely scenario in the molecular evolution of

cephalopod actin isoforms because the gene trees are highly concordant at deeper divergences, where the actin genes are most informative in constructing phylogenetic relationships. Concordance is also evident in relationships among recently diverged taxa, such as those obtained for species in well-defined families (e.g. Bolitaenidae, Loliginidae, Sepiolidae) or congeners (e.g. *Sepia*). Differences in the gene trees are primarily restricted to relationships within the Decapodiformes, which are probably due to the conserved nature of actin sequences and the lack of resolving power of actin genes at intermediate levels of divergence.

Phylogenetic analysis of the entire actin data set revealed three distinct clades of actin genes within the Cephalopoda. Each clade was well-defined, and the possibility that each clade may consist of more than one gene lineage was excluded on the basis of the analysis of the entire actin data set. Subtle intraclade differences among purported paralogues may have been obscured by the large interclade differences in the comprehensive actin data set. Intraspecific comparisons of Actin I and Actin II nucleotide sequences could potentially reveal disjunct patterns of molecular evolution, indicating multiple actin lineages within each isoform. Although such comparisons would not provide rigorous proof that each isoform is itself composed of multiple gene lineages, the absence of obvious heterogeneity within lineages would be another means of substantiating that each isoform is the result of a single gene duplication event. Intraspecific actin isoform comparisons revealed that none of the 26 taxa considered departed significantly from the overall mean divergence between the isoforms (18.73%). Although individual taxa did not appear to depart significantly from the overall mean divergence between the Actin I and Actin II genes, a comparison between the mean intraspecific divergence within the Octopodiformes and within the Decapodiformes revealed that the Octopodiformes actin genes were significantly more divergent than decapod counterparts (single class ANOVA,  $F_s = 23.896$ ,  $p < 0.01$ ) (Figure 15).

The results from phylogenetic analysis of deduced amino acid sequences of cephalopod actin isoforms along with known actin isoforms from diverse taxa indicates that the cephalopod isoforms are not restricted to one class of actin genes (Figure 16). Actin I groups with the muscle-type actins of gastropods and bivalves while Actins II and III cluster with the bivalve and gastropod cytoplasmic isoforms. Although this method of analysis does not provide direct and conclusive evidence that cephalopods possess no more than 3 actin loci in their genomes, it provides some circumstantial evidence in favor of a three-locus hypothesis. If additional actin loci in cephalopods are identified in the future, they will probably not represent a major new class of actin isoform, instead they will likely be closely related to at least one of the three isoforms identified in this dissertation.

The finding that molluscan muscle actins actin sequence are more similar to the chordate and echinoderm muscle actins was unexpected; invertebrate muscle actins are generally regarded to be more similar to vertebrate cytoplasmic actins than to vertebrate muscle actins (Vandekerckhove & Weber, 1984; Kusakabe et al., 1997; Mounier and Sparrow, 1997). However, such a finding is not unprecedented as starfish (Kowbel and Smith, 1989) and *Drosophila* (Fyrberg et al., 1981) muscle actins display more amino acids characteristic of the vertebrate muscle actins than do nonmuscle actins. This unusual result probably relates to convergent evolution in the requirements of muscle contractile properties (Kusakabe et al., 1997). A comparison of selected amino acids from gastropod, cephalopod, and arthropod muscle and cytoplasmic actins with those known to be diagnostic for the  $\alpha$  skeletal and  $\beta$  cytoplasmic human actins also revealed that molluscan muscle actins are more similar to chordate actins than they are to arthropod actins (Figure 17). These results, taken together, suggest an independent derivation of the molluscan muscle-type actins from a cytoplasmic ancestral gene. If this were indeed the case, the muscle-type actins probably arose more than once in the protostome lineage. This supports the conclusions drawn by Mournier et al. (1992), who proposed that the ancestral arthropod muscle actin gene appeared after separation of molluscs and arthropods and that no muscle-specific actin gene

was present in ancestral protostomes. At the time, the existence of muscle-specific actin isoforms in molluscs had not been demonstrated and Mournier et al. (1992) drew their conclusions based on the assumption that molluscs did not possess muscle-specific actin genes. As the analyses described above are based on 70% of the coding regions of actin genes, it is possible that analyses containing the entire coding region from the actin genes may have produced a different result. However, the C-terminal end region of the actin polypeptide exhibits the majority of diagnostic amino acid replacements (Vandekerckhove and Weber, 1978) and the amino acids analyzed in this study are more representative of the C-terminal region, excluding only 15 amino acids of the mature actin protein in this region. It is unlikely that analysis of the remaining 15 amino acids would support the alternate hypothesis of a single origin for protostome muscle actin genes.

#### Sequence Variation and Divergence

For Actin I nucleotide sequences, uncorrected pairwise divergences among coleoid taxa ranged from ~1% in congeners to >11% in comparisons between octopod and decapod taxa. Actin II nucleotide divergences ranged from ~1% in congeners to >19% in comparisons between octopods and decapods. Comparisons between *Nautilus* and coleoids ranged from 18 to 22% for the Actin II gene. The range of interspecific nucleotide sequence divergences differed between the actin isoforms, with Actin II exhibiting more variation than Actin I. Another indication that the Actin II gene is more variable than the Actin I gene is evident in the comparison of the number of parsimony informative characters in each data set. The Actin I data comprised 184 parsimony informative characters in 38 taxa whereas the Actin II data comprised 244 parsimony informative characters in 32 taxa. The difference in the number of parsimony informative characters is less dramatic when the restricted data set of 26 taxa are considered: the Actin I sequences comprised 166 parsimony informative characters in comparison to 210 parsimony informative characters for the Actin II sequences. Though the number of parsimony

informative characters is bound to increase with the number of taxa included, the unexpected steeper decline in the number of parsimony informative characters for the Actin II data set is the result of excluding *Nautilus* from the analysis.

The relative contribution of first, second, and third codon position nucleotides to variable characters was similar between the Actin I and Actin II data sets. First, second, and third codon positions composed 17.5%, 10.3%, and 72.3% of variable characters in the Actin I data set, respectively; first, second, and third codon position nucleotides accounted for 19.6%, 9.6%, and 70.8% of variable characters in the Actin II data set, respectively. Although the overall rate of nucleotide sequence evolution is higher for the Actin II gene, the similarity in patterns of sequence evolution suggests that functional constraints in the evolution of the Actin I and Actin II proteins are nearly identical.

The plot of pooled first and second codon position substitutions and third codon position substitutions against total sequence divergence does not indicate saturation at the third position as the relative contribution of third position substitutions continues to increase linearly with increasing sequence divergence for the Actin I data (Figure 18). Likewise, the plot of percent transitions and transversions against total uncorrected sequence divergence does not demonstrate transitional saturation for the Actin I data (Figure 19). These patterns of nucleotide substitution are expected for the highly conserved Actin I gene. In contrast, the plot of pooled first and second codon position substitutions and third codon position substitutions against total sequence divergence for the Actin II data suggests that third codon position nucleotides are saturated at greater sequence divergences (Figure 23). The plot of percent transitions and transversions against total uncorrected sequence divergence demonstrates transitional saturation for the Actin II data (Figure 24).

Analysis of Actin I base composition at the three codon positions did not demonstrate significant heterogeneity among taxa in base frequencies at any of the codon positions. However, base frequencies at the three codon positions were quite different. The Actin II

data exhibited significant heterogeneity among taxa in base frequencies at third codon position characters. In addition, base frequencies were quite different from that assumed under a JC69 model. The use of a maximum likelihood method of phylogenetic reconstruction using a model which accounts for unequal base frequencies, unequal probabilities of character transformations, and unequal rates of substitution across the three codon positions is warranted for the Actin I and Actin II data.

### Phylogenetic Relationships

Due to the highly conserved nature of the Actin I gene, little resolution was provided in parsimony analysis which generated 36 and 44 equally parsimonious trees in the equally weighted and transversionally weighted analyses, respectively (Figures 28 and 30). The maximum likelihood analysis resulted in more resolution but the extremely short branch lengths leading to several deep nodes within the Decapodiformes amounts to the same interpretation of relationships as unresolved polytomies in strict consensus trees (Figure 29). The trees are rooted with *Vampyroteuthis* although the results from analysis of the COI gene clearly supported the monophyly of the Octopodiformes. This result was also supported in analysis of the Actin I gene as the branch leading to the Octopoda is attached to the base of the tree as an unresolved polytomy. The monophyly of the Decapodiformes was also strongly supported in all analyses of the Actin I gene. Parsimony analysis of the Actin II gene produced 5 equally parsimonious trees and weighted parsimony analysis produced a single most parsimonious tree. The Actin II data were less conserved than the Actin I data, and therefore provided more resolution of coleoid relationships. Like the Actin I and COI data, the monophyly of the Octopodiformes and Decapodiformes was strongly supported in parsimony analyses of the Actin II data. However, maximum likelihood analysis of the Actin II gene produced an anomalous result, with *Nautilus* grouping within the Decapodiformes. For this reason, the tree illustrated in Figure 33 is

rooted with *Vampyroteuthis* for heuristic purposes (rooting with *Nautilus* makes it very difficult to interpret phylogenetic relationships from the tree).

Another interesting result from analysis of the Actin II gene is the placement of *Vampyroteuthis* as sister taxon to the cirrates rather than as sister taxon to the Octopoda. The (*Vampyroteuthis* Cirrata) clade was well supported in bootstrap analysis of the equally weighted data; however, bootstrap analysis of the transversally weighted data provided weak support for the clade. In maximum likelihood analysis, the branch leading to this clade is among the longest of the subterminal branches, indicating relatively strong likelihood support for such a relationship.

Though taxonomic sampling of the Octopoda was limited, a close relationship between the bolitaenids and cirrates, inferred from analysis of the COI gene, was not supported in analyses of the Actin I and Actin II genes. Perhaps this is due to the fact that the Actin I and Actin II genes are more conserved than the COI gene, and therefore more informative about deep relationships. On the other hand, convergent evolution at the molecular level may be responsible for the COI gene results, whereas similar back mutations and/or selective pressures on cirrates and bolitaenids did not occur in the nuclear genes. Unweighted parsimony analysis of the Actin I and Actin II genes could not resolve the relationship between *Octopus*, *Graneledone*, and the bolitaenids. Transversally weighted parsimony analysis of the Actin I and Actin II genes placed *Graneledone* with the Bolitaenidae, with a moderate (Actin I) to high (Actin II) levels of bootstrap support. The results of maximum likelihood analysis of the Actin I and Actin II genes differed, Actin I placed *Graneledone* and *Octopus* together as would be expected based on morphological evidence, whereas Actin II again placed *Graneledone* with the bolitaenids.

The Actin II data set contained two additional incirrates, *Bathypolypus* and *Argonauta*, whose placements were unstable across the three different methods of analyses. No resolution among incirrates was provided in the unweighted parsimony analysis of the Actin II gene. The results of weighted parsimony analysis were similar to those obtained in

analysis of the COI gene with respect to placement of *Bathypolypus* basal to other incirrates, but differed in the placement of *Argonauta* with the (*Graneledone* Bolitaenidae) clade. Maximum likelihood analysis placed *Argonauta* outside of the remaining incirrates and *Bathypolypus* grouped with *Octopus*.

In the analysis of the COI gene, where taxonomic sampling of the Octopods is more extensive, *Graneledone* never clustered with the shallow water octopodids, nor did *Bathypolypus*. Furthermore, a close relationship between *Graneledone* and *Bathypolypus* was never obtained in analysis of any of the three actin genes, no matter what method of phylogenetic analysis was employed. This finding runs contrary to morphological designations, where the two taxa are placed within the Octopodidae, either in the subfamily Bathypolypodinae (Voss, 1977) or in separate octopodid subfamilies (Voss, 1988). In her cladistic analysis of Octopodid subfamilies, Voight (1993) found *Bathypolypus* and *Graneledone* to be closely related, although she avoided use of the subfamily category in octopodid classification due to insufficient knowledge of Octopodid relations. The results presented here support her assertion that our knowledge of octopodid relations is not yet at the level required for subfamilial designations. Indeed our classification of incirrates at the family level may be flawed: all incirrates may be octopodids. This is quite possible as the few characters that support the monophyly of the Octopodidae could be plesiomorphic states.

The Actin I gene provided little resolution of relationships within the Decapodiformes; decapod relationships were resolved in analysis of the Actin II gene, though widely unstable across trees derived from different reconstruction methods. In contrast to the results obtained in analysis of the COI gene, the sepioid families did not emerge basal to the remaining decapods in trees derived from either of the actin data sets. The monophyly of the Sepioidea was unsupported in analysis of the Actin I gene. However, if the Myopsida, *Chtenopteryx*, and *Bathyteuthis* were included, the group was found to be monophyletic in the equally weighted parsimony and likelihood analyses. Bootstrap support for such a



clade was lacking, and Bremer support was weak. Bremer support values obtained in analysis of the Actin I gene are not comparable to values obtained in analysis of the COI gene as fewer changes defined Actin I clades. Also, it is important to bear in mind that the Bremer support values of the Actin I data reflect the number of steps required to collapse a clade on the *consensus* tree, where many clades have already been collapsed. Therefore fewer steps are required to collapse the remaining clades. The monophyly of the Sepioidea was not supported in phylogenetic analysis of the Actin II gene. Furthermore, statistical tests of monophyly for the Actin I and Actin II data sets each rejected the monophyly of the Sepioidea (Figures 31 and 35).

A major difference in the results of the COI and Actin data sets is evidenced in the placement of *Spirula*. The COI data unequivocally placed *Spirula* well within an oegopsid clade and outside of the Sepioids. The Actin I data placed *Spirula* with the Sepiids in all analyses. Analyses of the Actin II data also placed *Spirula* with or near the sepiids, however, the placement of *Spirula* was unstable across the trees derived from different reconstruction methodologies. Similar to results of analysis of the COI gene, the myopsid squids tended to cluster with the sepioids. Analysis of the Actin I data supported a close relationship between the myopsids, sepiids, and *Spirula*. Parsimony analysis of the Actin II data also placed the myopsids with sepioids, although myopsid relationships to the (Sepiidae *Spirula*) clade or the Sepiolidae depended on how the data were weighted. Likelihood analysis of the Actin II gene placed the Myopsida outside of a large clade consisting of oegopsids, sepiids, and *Spirula*.

The placement of *Idiosepius* was unstable in analyses of both actin data sets. Although equally weighted parsimony and maximum likelihood analyses of the Actin I data placed *Idiosepius* in the sepioid, myopsid, *Chtenopteryx*, and *Bathyteuthis* clade, transversally weighted parsimony placed *Idiosepius* outside of the clade as sister taxon to *Sthenoteuthis*. The Actin II data consistently grouped *Idiosepius* with the Ommastrephidae, although the placement of this clade of three taxa varied across the different analyses. Equally weighted

parsimony analysis placed this clade as sister group to the sepiolids; transversally weighted parsimony analysis placed the (*Idiosepius* (Ommastrephidae)) clade basal to a large clade consisting of sepioids, myopsids, and some oegopsid taxa. Likelihood analysis placed *Idiosepius* and the ommastrephids outside most of the decapods excluding the sepiolids and *Enoploteuthis*. These results are consistent with the work of Bonnaud et al. (1997), where *Idiosepius* consistently grouped with the ommastrephid squid *Illex argentinus* rather than with the other sepioid taxa included in the analysis of COIII gene sequences. The placement of *Idiosepius* based on the actin data, surprisingly, is in better agreement with the results of Bonnaud et al. (1997) than are the results obtained in analysis of the mitochondrial COI gene.

*Sepioloidea*, a member of the family Sepiadariidae, consistently emerged basal to the Sepiolidae clade in all analyses of the Actin I and Actin II data sets. This relationship was also supported in bootstrap analyses of both data sets, whether or not the data were weighted. Although the results of parsimony analysis of the COI data supported a close relationship between *Sepioloidea* and *Idiosepius*, maximum likelihood placed *Sepioloidea* basal to the Sepiolidae. This result supports the relationships first described by Naef (1923), wherein the Sepiadariids were considered ancestral within the Sepiolidae and later by Khromov (1990), who considered the Sepiolidae, Sepiadariidae, and Idiosepiidae to be more closely related to each other than to the Sepiidae and Spirulidae.

Few relationships among the oegopsid families were conclusively determined through phylogenetic analysis of the actin data sets. A close relationship between *Chtenopteryx* and *Bathyteuthis* was found in all analyses of the Actin I data set and was supported by a moderate proportion of bootstrap replicates. This relationship was also supported in analysis of the equally weighted Actin II data; transversally weighted parsimony and likelihood analysis of the Actin II data did not support a close relationship, though they clustered near one another on both trees.

The Actin I gene, although incapable of resolving many relationships among oegopsid taxa, consistently supported a close relationship between the Onychoteuthidae and Gonatidae. This relationship was supported in a high proportion of bootstrap replicates and also by a high Bremer support value. A close relationship between these families, along with the Enoploteuthidae and Octopoteuthidae, was suggested by Naef (1923) based on the formation of hooks in all these families. Young and Harman (1998) argued that hooks probably arose independently in these families although their data shows unusual similarity in the structure of hooks (i.e., presence of “skirt”) between gonatids and onychoteuthids. The work of Toll (1982) and Hess (1987) also supported a close relationship between the Gonatidae and Onychoteuthidae. The COI data, however, did not support a close relationship between the two families. An Actin II sequence was not obtained from an onychoteuthid species so the relationship with gonatids cannot be compared across nuclear genes. In phylogenetic analyses of the Actin II sequences, *Gonatus onyx* tended to cluster with the Cycloteuthidae. Contrary to results obtained in the analysis of COI gene, the monophyly of the Cycloteuthidae was strongly supported in all analyses conducted on both actin data sets. Strong bootstrap support for cycloteuthid monophyly was obtained in parsimony analyses of the Actin II data set; moderate to low bootstrap support for monophyly was obtained in analyses of the Actin I data set.

As the remaining oegopsid taxa included in the analysis differed between the Actin I and Actin II data sets, each data set will be discussed separately with regard to these taxa. The Actin I data supported a basal emergence of *Alluroteuthis*, *Histioteuthis*, and *Chiroteuthis* in all three methods of phylogenetic analysis. This result was not supported in bootstrap analysis and, with the exception of *Chiroteuthis*, was weakly supported by Bremer support analysis of the unweighted data. The cranchiids were well defined in all analyses of the Actin I data and were supported by high bootstrap and Bremer values. Unexpectedly, a close relationship was obtained between *Ommastrephes* and *Pyroteuthis*, even though another ommastrephid was included in the data set, as were two enoploteuthids. This

relationship was found in all three analyses and supported by a moderate to high proportion of bootstrap replicates. Parsimony analysis of the transversionally weighted data related this clade to the (Gonatidae Onychoteuthidae) clade discussed above, but without bootstrap support. Although the unweighted Actin I data failed to establish a relationship between *Lepidoteuthis* and *Octopoteuthis*, weighted parsimony and likelihood analyses supported a relationship between the two families, consistent with the results obtained in maximum likelihood and weighted parsimony analysis of the COI gene. Further resolution of oegopsid families was not obtained in equally weighted parsimony analysis of the Actin I data. Transversionally weighted parsimony placed *Sthenoteuthis* with *Idiosepius* but this relationship was not supported in bootstrap analysis or in maximum likelihood analysis. Maximum likelihood analysis provided a small amount of additional resolution within the oegopsid families, placing *Thysanoteuthis* and enoploteuthids basal to the ((*Ommastrephes Pyroteuthis*) (*Onychoteuthis* (Gonatidae))) clade.

The unweighted Actin II data supported a close relationship between the Brachioteuthidae, Histioteuthidae, and Psychroteuthidae. Bootstrap and Bremer support was obtained for a close relationship between the latter two families. These relationships were not maintained in likelihood and weighted parsimony analyses. The placement of *Cranchia* basal to clades containing sepioids and myopsids in analysis of the Actin II data is somewhat similar to the placement of the cranchiids in weighted parsimony and likelihood analyses of the Actin I data. *Thysanoteuthis* placed within the clade containing the Sepiidae, Myopsida, and *Spirula* in the parsimony analyses. In the likelihood analysis, *Thysanoteuthis* diverged from a large clade just after the divergence of the myopsids but immediately prior to the divergence of *Spirula*. Weighted parsimony analysis placed *Thysanoteuthis* outside the (Myopsida Sepiolidae) clade. A relationship between *Thysanoteuthis* and the Myopsida, Sepiidae, Sepiolidae, or *Spirula* is not compatible with the results of any morphological study. The placement of *Thysanoteuthis* was not supported in bootstrap analysis and was inconsistent across the three methods of analysis.

Morphologists have differed in their conclusions regarding the position of the Thysanoteuthidae with some considering the family to be representative of an ancestral oegopsid taxon (Naef, 1923; Toll, 1982). Naef (1923) regarded the Thysanoteuthidae and Ommastrephidae to be closely related whereas Toll (1982) demonstrated that the Thysanoteuthidae are unique and not related to the Ommastrephidae in terms of gladius morphology. Hess (1987) considered the Thysanoteuthidae to be ancestral to a relatively derived clade of oegopsids comprising the Bathyteuthidae, Histioteuthidae, Lycoteuthidae, Cranchiidae, Neoteuthidae, and Architeuthidae. Analysis of the Actin II gene also resulted in instability regarding the position of *Enoploteuthis*. Parsimony analysis of the raw data put *Enoploteuthis* outside of a clade containing sepiolids, *Idiosepius*, Sepioloidea and the ommastrephids. Likelihood analysis placed *Enoploteuthis* outside of all the remaining decapods whereas weighted parsimony placed it basal to the (*Gonatus* (Cycloteuthidae)) clade. Neither of the results obtained using parsimony methods was supported in bootstrap analysis; however, the length of the branch separating *Enoploteuthis* from the remaining decapods is among the longest of subterminal branches that does not lead to confamilial species, which suggests that such a relationship is relatively stable in the likelihood analysis. In contrast to the results obtained in analysis of the Actin I sequences, the ommastrephids consistently grouped together in analysis of the Actin II data. However, the position of the Ommastrephidae was unstable across the different methods of analysis, as was discussed above in the discussion of *Idiosepius*.

With respect to higher-level relationships, the following conclusions can be drawn from analyses of the Actin I and Actin II data sets: 1) the Coleoidea, Octopodiformes, Decapodiformes, Incirrata, and Cirrata are monophyletic groups; 2) the Vampyromorpha and Octopoda are sister taxa; 3) the Sepioidea is polyphyletic; 4) the myopsid squids are more closely related to the Sepiidae and *Spirula* than to oegopsid squids; and 5) the Oegopsida, as currently defined, is polyphyletic.

TABLE 4. PRIMER PAIRS USED TO AMPLIFY CEPHALOPOD ACTIN GENES

| Primer Name | Primer Sequence                            | Strand | Region Amplified <sup>a</sup> | Size of Amplified Product<br>(Excluding Primer Sequences) |
|-------------|--|--------|-------------------------------|---|
| Actin 480   | aayggigaraaratgacicarathatgtt <sup>b</sup> | +      | 371-1100 (aa105-350)          | 623 base pairs  |
| Actin 483   | ccaiaciswrtayttickytciggigg                | -      |                               |   |
| Actin 481   | tgggaygayatggaraaratitggcaycayac           | +      | 295-1130 (aa80-360)           | 784 base pairs  |
| Actin 482   | tiswdatccacatytytgraigt                    | -      |                               |   |

<sup>a</sup>Relative to the complete nucleotide (aa = amino acid) sequence of *Aplysia californica* actin cDNA gene isolated from an abdominal ganglion cDNA library (EMBL Accession Number: X52868).

<sup>b</sup>IUPAC Codes for DNA: y = C or T; i = inosine; r = A or G; h = A, C, or T; s = C or G; w = A or T; k = G or T; d = A, G, or T.

TABLE 5. SPECIES, GENES, GENBANK/EMBL ACCESSION NUMBERS  
AND REFERENCES USED IN THE ANALYSIS OF ACTIN GENE EVOLUTION

| Abbreviated Name       | Species                    | Actin Type and Method of Characterization | Accession Number | Reference                                     |
|------------------------|----------------------------|---|------------------|---|
| <i>Aplysia</i> M       | <i>Aplysia californica</i> | Muscle cDNA gene                          | X52868           | DesGroseillers et al. (1990)                  |
| <i>Aplysia</i> C       | <i>Aplysia californica</i> | Neuron cDNA gene                          | U01352           | DesGroseillers et al. (1994)                  |
| <i>Artemia</i> 205M    | <i>Artemia</i> sp.         | Muscle-specific expression                | X52602           | Macias and Sastre(1990); Ortega et al. (1990) |
| <i>Artemia</i> 211M    | <i>Artemia</i> sp.         | “   | X52603           | “   |
| <i>Artemia</i> 403C    | <i>Artemia</i> sp.         | Cytoplasmic expression                    | X52605           | “   |
| <i>Biomphalaria</i>    | <i>B. glabrata</i>         | Uncharacterized                           | Z72387           | Unpublished                                   |
| <i>Bombyx</i> A1M      | <i>Bombyx mori</i>         | Muscle-specific expression                | X05185           | Mournier et al. (1987)                        |
| <i>Bombyx</i> A3C      | <i>Bombyx mori</i>         | Cytoplasmic expression                    | U49854           | Mange et al. (1997)                           |
| <i>Caenorhabditis</i>  | <i>C. elegans</i>          | Uncharacterized                           | J01042           | Files et al. (1983)                           |
| <i>C. gigas</i> C      | <i>Crassostrea gigas</i>   | cDNA from hemocytes                       | AF026063         | Unpublished                                   |
| <i>C. virginica</i> C  | <i>Crassostrea virg.</i>   | cDNA from gill tissue                     | X75894           | Unger and Roesijadi (1993)                    |
| <i>Drosophila</i> 5CC  | <i>D. melanogaster</i>     | Cytoplasmic expression                    | K00667           | Fyrberg et al. (1981)                         |
| <i>Drosophila</i> 42AC | <i>D. melanogaster</i>     | “   | K00670           | Fyrberg et al. (1981)                         |

TABLE 5 (Continued). LIST OF SPECIES, GENES, GENBANK/EMBL ACCESSION NUMBERS USED IN THE ANALYSIS OF ACTIN GENE EVOLUTION

| Abbreviated Name                | Species                      | Actin Type and Method of Characterization | Accession Number | Reference               |
|---------------------------------|------------------------------|---|------------------|-------------------------|
| <i>Drosophila</i> 57AM          | "                            | Muscle-specific expression                | K00673           | Fyrberg et al. (1981)   |
| <i>Drosophila</i> 87EM          | "                            | "   | K00674           | Fyrberg et al. (1981)   |
| <i>Fugu</i> $\alpha$ Anomalous  | <i>Fugu rubripes</i>         | Testis-specific expression                | U38962           | Venkatesh et al. (1996) |
| <i>Fugu</i> $\alpha$ Cardiac    | "                            | Heart-specific expression                 | U38959           | "                       |
| <i>Fugu</i> $\alpha$ Skeletal   | "                            | Muscle-specific expression                | U38850           | "                       |
| <i>Fugu</i> $\beta$ Cytoplasmic | "                            | Non-muscle expression                     | U37499           | "                       |
| <i>Halocynthia</i> M            | <i>Halocynthia roretzi</i>   | cDNA from larval muscle                   | D10887           | Kusakabe et al. (1991)  |
| <i>Halocynthia</i> C            | "                            | Cytoplasmic cDNA probe                    | D45164           | Kusakabe et al. (1997)  |
| <i>H. erythrogramma</i> C       | <i>Heliocidaris erythro.</i> | Cytoplasmic expression                    | U09633           | Hahn et al. (1995)      |
| <i>H. erythrogramma</i> M       | "                            | Muscle-specific expression                | U32348           | Unpublished             |
| <i>H. tuberculata</i> C         | <i>Heliocidaris tuber.</i>   | Cytoplasmic expression                    | U12272           | Hahn et al. (1995)      |
| <i>H. tuberculata</i> M         | "                            | Muscle-specific expression                | U32353           | Unpublished             |
| <i>Hydra</i>                    | <i>Hydra attenuata</i>       | Uncharacterized                           | M32364           | Fisher and Bode (1989)  |



TABLE 5 (Continued). LIST OF SPECIES, GENES, GENBANK/EMBL ACCESSION NUMBERS  
USED IN THE ANALYSIS OF ACTIN GENE EVOLUTION

| Abbreviated Name           | Species                      | Actin Type and Method of Characterization | Accession Number | Reference                 |
|----------------------------|------------------------------|---|------------------|---------------------------|
| <i>Molgula</i> AdultM      | <i>Molgula citrina</i>       | Muscle cDNA probe                         | L21915           | Swalla et al. (1994)      |
| <i>Placopecten</i> M       | <i>P. magellanicus</i>       | cDNA from adductor muscle                 | U55046           | Patwary et al. (1996)     |
| <i>S. purpuratus</i> IIbC  | <i>Strongylocentrotus p.</i> | 3'-UTR Cytoplasmic                        | M35323           | Schuler and Keller (1983) |
| <i>S. purpuratus</i> IIIbC | <i>Strongylocentrotus p.</i> | "   | M35324           | "                         |

Figure 13. Multiple alignment of nucleotide sequences of the actin genes of cephalopods. Positions relative to the 3' end of the Actin 482 primer are indicated at the top of each page. Positions with identical nucleotides are shown as a dot (.), positions with unknown nucleotide characters are indicated as a question mark (?), and positions containing inserted gaps (*Spirula* #40) to optimize the alignment are given a dash (-). The genus and in some cases the species name of the taxa are given to the left of each sequence, followed by the plasmid clone number or primer numbers 480/483 for those sequences obtained using nested Actin 480 and 483 primers only. The coleoid actin sequences are arranged alphabetically by taxon (Oegopsida, Myopsida, Sepioidea, Octopoda, Vampyromorpha) and grouped into blocks of orthologous sequences (Actin I, Actin II, Actin III).

|                      | 10         | 20         | 30         | 40         | 50         | 60         | 70         | 80         |
|----------------------|------------|------------|------------|------------|------------|------------|------------|------------|
| Abralia#3            | CTTCTACAAC | GAGCTCCGTG | TCGCCCCCGA | AGAGCACCCC | GTCCCTCTCA | CAGAGGCTCC | CTTGAACCCC | AAGGCTAACA |
| Alluroteuthis#6      | .....T     | .....A     | .T.....    | .....      | .T.....    | T.....     | .....C     | .....      |
| Bathyteuthis#1       | .....      | .....      | .....      | .....      | .....      | .....      | .....      | .....      |
| Chiroteuthis#12      | .....      | .....      | .....      | .....      | .....      | .....      | .....      | .....      |
| Ctenopteryx#41       | .....      | .....C     | .....      | .....      | .....T     | .....T     | .....      | .....A     |
| Cranchia#2           | .....      | .....      | .....      | .....      | .....      | .....      | .....      | .....      |
| Cycloteuthis#29      | .....      | .....      | .....      | .....      | .....      | .....      | .....      | .....      |
| Discoteuthis#11      | .....      | .....      | .....      | .....      | .....      | .....      | .....      | .....      |
| Enoplotеuthis#7      | .....      | .....      | .....      | .....      | A.....     | T.....     | A.....     | .....      |
| Gonatopsis#11        | .....      | .....      | .....A     | .....      | .....      | .....      | T.....     | .....      |
| Gonatus_onyx#32      | .....      | .....      | .....A     | .....      | T.....     | .....      | T.....     | .....      |
| Histioteuthis#49     | .....      | .....      | .....A     | .....      | .....      | .....      | .....      | .....C     |
| Lepidoteuthis#27     | .....      | .....      | .....      | .....      | .....      | .....      | .....      | .....      |
| Liocranchia#6        | .....      | .....      | .....      | .....      | .....      | .....      | .....      | .....      |
| Mastigoteuthis480483 | ?????????? | ?????????? | ?????????? | ?????????? | ?????????? | ?????????? | ?????????? | ?????????? |
| Octopoteuthis#5      | .....      | .....      | .....      | .....      | .....      | .....      | .....      | .....      |
| Ommastrephes#49      | .....      | .....      | .....T     | .....      | .....T     | .....      | A.....     | .....A     |
| Onychoteuthis#26     | .....      | A.....     | C.....     | T.....     | .....      | .....      | .....      | .....      |
| Pholidoteuthis#21    | .....      | .....      | .....      | .....      | .....      | .....      | .....      | .....      |
| Pyroteuthis#4        | .....T     | .....      | .....T     | .....      | A.....     | .....      | A.....     | .....      |
| Thysanoteuthis#44    | .....      | .....      | .....T     | .....T     | .....      | .....      | .....      | .....      |
| Sthenoteuthis#32     | T.....     | T.....     | .....      | .....      | A.....     | .....      | C.....     | A.....     |
| Loligo_pealei#27     | .....      | .....      | .....T     | .....      | .....      | C.....     | C.....     | A.....     |
| Sepioteuthis480483   | ?????????? | ?????????? | ?????????? | ?????????? | ?????????? | ?????????? | ?????????? | ?????????? |
| Heteroteuthis#21     | .....      | .....      | .....      | .....      | .....      | A.....     | C.....     | A.....     |
| Idiosepius#17        | .....      | .....      | .....      | .....      | .....      | A.....     | C.....     | A.....     |
| Rossia#4             | .....      | .....      | .....A     | .....      | .....      | A.....     | C.....     | A.....     |
| Sepia_officinalis#17 | T.....     | .....      | Sepia      | T.....     | T.....     | G.....     | TC         | A.....T    |
| Sepia_opipara#13     | .....      | .....      | .....T     | .....      | .....      | .....      | C.....     | .....      |
| Sepioloidea#32       | .....      | .....      | .....      | .....      | .....      | .....      | .....      | .....      |
| Spirula#22           | .....      | .....      | .....T     | .....      | .....      | .....      | C.....     | .....T     |
| Stoloteuthis#13      | .....      | .....      | .....      | .....      | .....      | .....      | .....      | .....      |
| Cirrothauma#14       | .....T     | A.....     | T.....     | A.....     | T.....     | C.....     | A.....     | C.....T    |
| Eledonella#6         | .....T     | .....      | A.....     | A.....     | T.....     | C.....     | A.....     | C.....C    |
| Graneledone#36       | .....T     | .....      | A.....     | A.....     | T.....     | C.....     | A.....     | C.....C    |
| Japatella#17         | .....T     | .....      | A.....     | A.....     | T.....     | C.....     | A.....     | C.....C    |
| Octopus#27           | .....T     | .....      | A.....     | A.....     | T.....     | C.....     | A.....     | AC         |
| Vampyroteuthis#44    | .....      | .....      | A.....     | A.....     | .....      | C.....     | .....      | C.....C    |
| Bathyteuthis#15      | T.....     | T.....     | T          | GA         | A.....     | T          | T.....     | A.....     |
| Brachioteuthis#4     | T.....     | T.....     | T          | GA         | A.....     | T.....     | CT         | G.....     |
| Ctenopteryx#09       | T.....     | T.....     | T          | GA         | A.....     | T.....     | T.....     | A.....     |
| Cranchia#1           | T.....     | T.....     | T          | GA         | A.....     | T.....     | A.....     | CT         |
| Cycloteuthis#61      | T.....     | T.....     | T          | GA         | A.....     | T.....     | AT         | G.....     |
| Discoteuthis#5       | T.....     | T.....     | T          | GA         | A.....     | T.....     | AT         | G.....     |
| Enoplotеuthis#5      | .....T     | .....T     | .....T     | .....T     | .....T     | .....T     | .....T     | .....T     |
| Gonatus_onyx#90      | T.....     | T.....     | T          | GA         | A.....     | T.....     | G.....     | T.....     |
| Histioteuthis#6      | T.....     | T.....     | T          | GA         | A.....     | T.....     | G.....     | T.....     |
| Ommastrephes#6       | T.....     | T.....     | T          | GA         | A.....     | T.....     | T.....     | A.....     |
| Psychroteuthis#14    | T.....     | T.....     | AT         | AA         | G.....     | T.....     | G.....     | AT         |
| Sthenoteuthis#21     | T.....     | T.....     | AT         | GA         | A.....     | T.....     | T.....     | AT         |
| Thysanoteuthis#31    | T.....     | T.....     | T          | GA         | A.....     | T.....     | A.....     | GT         |
| Loligo_pealei#23     | T.....     | T.....     | AT         | GA         | A.....     | T.....     | A.....     | GT         |
| Sepioteuthis#13      | T.....     | T.....     | T          | GA         | A.....     | T.....     | A.....     | GT         |
| Idiosepius#43        | T.....     | T.....     | AT         | G.....     | T.....     | T.....     | C.....     | AC         |
| Rossia480483         | ?????????? | ?????????? | ?????????? | ?????????? | ?????????? | ?????????? | ?????????? | ?????????? |
| Sepia_officinalis#5  | T.....     | T.....     | AT         | GA         | A.....     | T.....     | T.....     | A.....     |
| Sepia_opipara#35     | T.....     | T.....     | AT         | GA         | A.....     | T.....     | T.....     | A.....     |
| Sepioloidea#6        | ?????????? | ?????????? | ?????????? | ?????????? | ?????????? | ?????????? | ?????????? | ?????????? |
| Spirula#30           | .....T     | .....T     | .....T     | .....T     | .....T     | .....T     | .....T     | .....T     |
| Stoloteuthis#29      | T.....     | T.....     | T          | GA         | A.....     | T.....     | A.....     | CT         |
| Argonauta#5          | A.....     | .....      | T          | GA         | A.....     | T.....     | A.....     | GT         |
| Bathypolypus#5       | A.....     | .....      | T          | GA         | A.....     | T.....     | A.....     | GT         |
| Cirrothauma#20       | T.....     | .....      | T          | GA         | A.....     | T.....     | G.....     | A.....     |
| Eledonella#5         | A.....     | .....      | T          | GA         | A.....     | T.....     | T.....     | GT         |
| Graneledone#39       | A.....     | .....      | T          | GA         | A.....     | T.....     | T.....     | GT         |
| Japatella#16         | A.....     | .....      | T          | GA         | A.....     | T.....     | T.....     | GT         |
| Octopus#9            | A.....     | .....      | T          | GA         | A.....     | T.....     | T.....     | GT         |
| Stauroteuthis#22     | T.....     | .....      | AT         | GA         | A.....     | T.....     | G.....     | A.....     |
| Vampyroteuthis#21    | .....      | .....      | AT         | GA         | A.....     | T.....     | G.....     | A.....     |
| Nautilus#1           | T.....     | .....      | AT         | G.....     | T.....     | G.....     | A.....     | C.....     |
| Abralia#18           | .....      | .....      | T.....     | A.....     | G.....     | A.....     | T.....     | A.....     |
| Brachioteuthis#6     | .....      | .....      | T.....     | A.....     | G.....     | A.....     | T.....     | A.....     |
| Chiroteuthis#16      | .....T     | .....T     | .....G     | .....A     | G.....A    | T.....A    | G.....A    | .....A     |
| Ctenopteryx#3        | A.....     | T.....     | T.....     | A.....     | A.....     | T.....     | A.....     | G.....     |
| Histioteuthis#3      | .....      | .....      | T.....     | A.....     | A.....     | T.....     | A.....     | G.....     |
| Mastigoteuthis#22    | .....      | .....      | A.....     | T.....     | A.....     | T.....     | A.....     | G.....     |
| Onychoteuthis#33     | .....      | .....      | A.....     | T.....     | A.....     | T.....     | A.....     | G.....     |
| Idiosepius#06        | A.....     | T.....     | G.....     | T.....     | A.....     | G.....     | T.....     | C.....     |
| Sepia_officinalis#41 | .....T     | .....T     | .....G     | .....T     | .....A     | G.....A    | .....G     | .....T     |
| Sepia_opipara#23     | .....T     | .....T     | .....G     | .....T     | .....A     | G.....A    | .....G     | .....T     |
| Spirula#40           | .....T     | .....T     | .....G     | .....T     | .....A     | G.....A    | .....G     | .....T     |
| Vampyroteuthis#13    | T.....     | .....      | AT         | G.....     | T.....     | A.....     | G.....     | A.....     |

|                      | 90         | 100             | 110        | 120        | 130        | 140        | 150        | 160        |
|----------------------|------------|-----------------|------------|------------|------------|------------|------------|------------|
| Abrialia#3           | GGGAAAAGAT | GACCCAAATC      | ATGTTTGAAA | CCTTCAACGC | CCCAGCCATG | TATGTTGCCA | TCCAGGCTGT | CCTCTCCCTC |
| Alluroteuthis#6      | . . . . .  | . T . . . . .   | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Bathyteuthis#1       | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Chiroteuthis#12      | . . . . .  | . T . . . . .   | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Chtenopteryx#41      | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Cranchia#2           | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Cycloteuthis#29      | . . . . .  | . T . . . . .   | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Discoteuthis#11      | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Enoplateuthis#7      | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Gonatopsis#11        | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Gonatus_onyx#32      | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Histioteuthis#49     | . . . . .  | . T . . . . .   | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Lepidoteuthis#27     | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Liocranchia#6        | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Mastigoteuthis480483 | ?????????? | ??????????      | ?????      | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Octopoteuthis#5      | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Ommastrephes#49      | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Onychoteuthis#26     | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Pholidoteuthis#21    | . . . . .  | . T . . . . .   | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Pyroteuthis#4        | . . . . .  | . TGT . . . . . | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Thysanoteuthis#44    | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Sthenoteuthis#32     | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Loligo_pealei#27     | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Sepioteuthis480483   | ?????????? | ??????????      | ?????      | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Heteroteuthis#21     | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Idiosepius#17        | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Rossia#4             | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Sepia_officinalis#17 | . . . . .  | . T . . . . .   | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Sepia_opipara#13     | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Sepioloidea#32       | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Spirula#22           | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Stoloteuthis#13      | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Cirrothauma#14       | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Eledonella#6         | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Graneledone#36       | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Japatella#17         | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Octopus#27           | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Vampyroteuthis#44    | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Bathyteuthis#15      | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Brachioteuthis#4     | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Chtenopteryx#09      | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Cranchia#1           | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Cycloteuthis#61      | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Discoteuthis#5       | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Enoplateuthis#5      | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Gonatus_onyx#90      | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Histioteuthis#6      | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Ommastrephes#6       | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Psychroteuthis#14    | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Sthenoteuthis#21     | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Thysanoteuthis#31    | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Loligo_pealei#23     | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Sepioteuthis#13      | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Idiosepius#43        | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Rossia480483         | ?????????? | ??????????      | ?????      | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Sepia_officinalis#5  | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Sepia_opipara#35     | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Sepioloidea#6        | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Spirula#30           | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Stoloteuthis#29      | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Argonauta#5          | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Bathypolypus#5       | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Cirrothauma#20       | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Eledonella#5         | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Graneledone#39       | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Japatella#16         | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Octopus#9            | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Stauroteuthis#22     | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Vampyroteuthis#21    | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Nautilus#1           | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Abrialia#18          | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Brachioteuthis#6     | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Chiroteuthis#16      | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Chtenopteryx#3       | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Histioteuthis#3      | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Mastigoteuthis#22    | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Onychoteuthis#33     | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Idiosepius#06        | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Sepia_officinalis#41 | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Sepia_opipara#23     | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Spirula#40           | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |
| Vampyroteuthis#13    | . . . . .  | . . . . .       | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  | . . . . .  |

|                      | 170        | 180        | 190        | 200        | 210        | 220        | 230       | 240        |
|----------------------|------------|------------|------------|------------|------------|------------|-----------|------------|
| Abralia#3            | TACTGTTCTG | GTCGTACCAC | CGGTATCGTT | CTTGACTCCG | GTGATGGTGT | CACCCACACC | GTCCCATCT | ATGAAGGTTA |
| Alluroteuthis#6      | GC         |            |            |            |            |            | A.A       |            |
| Bathyteuthis#1       | GC         |            | T          | T          |            |            | A.A       |            |
| Chiroteuthis#12      | GC         |            |            |            |            | T          | A.A       |            |
| Chtenopteryx#41      | GC.C       |            | T          | T          |            | T          | A.A       |            |
| Cranchia#2           | GC         |            | T          |            |            | A          | A.A       |            |
| Cycloteuthis#29      | GC         |            | T          | T          | C          | T          | A.A       |            |
| Discoteuthis#11      | GC         |            | T          | T          |            | T          | A.A       |            |
| Enoploteuthis#7      | GC         |            |            |            |            |            | A         |            |
| Gonatopsis#11        | GC.C       |            |            | T          | A          |            | A         |            |
| Gonatus_onyx#32      | GC.C       |            |            | T          | A          |            | A         |            |
| Histioteuthis#49     | GC         |            | T          |            | A          | T          | A.A       |            |
| Lepidoteuthis#27     | GC         |            |            |            |            |            | A.A       |            |
| Liocranchia#6        | GC         |            | T          |            |            | A          | A.A       |            |
| Mastigoteuthis480483 | GC         |            |            |            |            |            | A.A       |            |
| Octopoteuthis#5      | GC         |            |            |            |            |            | A.A       |            |
| Ommastrephes#49      | GC         |            |            |            |            |            | A.A       |            |
| Onychoteuthis#26     | GC         |            | T          |            | A          | A          | A.T       |            |
| Pholidoteuthis#21    | GC         |            |            |            |            |            | A.A       |            |
| Pyroteuthis#4        | GC         |            | G          | C          | G          |            | A.A       |            |
| Thysanoteuthis#44    | GC         | GG         | T          |            |            |            | A.A       |            |
| Sthenoteuthis#32     | GCC.C      |            | A          |            |            |            | A.A       |            |
| Loligo_pealei#27     | GC         |            | T          |            |            |            | A.A       |            |
| Sepioteuthis480483   | GC         |            | T          |            |            |            | A.A       |            |
| Heteroteuthis#21     | GC.C       |            | T          |            |            | T          | A         |            |
| Idiosepius#17        | GC         |            | T          | T          | C          |            | A.A       |            |
| Rossia#4             | GC.C       |            | T          | C          |            | AT         | A         |            |
| Sepia_officinalis#17 | GC.A       | C.A        | T          | C          | T          | G          | A.A       |            |
| Sepia_opipara#13     | GC         | A          | T          | T          |            |            | A.A       |            |
| Sepioloides#32       | GC.C       | T          | T          |            |            |            | A.A       |            |
| Spirula#22           | GC         | T          | T          | C          | T          |            | A.A       |            |
| Stoloteuthis#13      | GC.A       |            | T          |            | A          |            | CA        |            |
| Cirrochauma#14       | GC         |            |            |            | C          | C          | A         |            |
| Eledonella#6         | GC         |            | T          |            | C          |            | T         |            |
| Granelledone#36      | GC         |            | T          |            | C          | A          | T         | A          |
| Japatella#17         | GC         |            | T          |            | C          |            | T         |            |
| Octopus#27           | GC         | A          | T          | T          | A          |            | T         | A          |
| Vampyroteuthis#44    | GC         | T          | T          | G          | C          | T          | A         |            |
| Bathyteuthis#15      | TGCC.C     | G          | T          | T          | C          | A          | G         | T          |
| Brachioteuthis#4     | TGCC.C     |            | T          | T          | C          | A          | G         | T          |
| Chtenopteryx#09      | TGCC.C     |            | T          | T          | C          | A          | G         | T          |
| Cranchia#1           | TGC.C      |            | T          | T          | C          | A          | G         | T          |
| Cycloteuthis#61      | TGGC.C     |            | T          | T          | G          | A          | G         | T          |
| Discoteuthis#5       | TGGC.C     |            | T          | T          | G          | A          | G         | T          |
| Enoploteuthis#5      | TGC.C      |            | T          | T          | G          | A          | G         | T          |
| Gonatus_onyx#90      | TGCA.C     |            | T          | T          | G          | A          | G         | T          |
| Histioteuthis#6      | GCC.C      |            | A          | T          | C          | A          | G         | T          |
| Ommastrephes#6       | GC.C       |            | A          | T          | C          | A          | G         | T          |
| Psychroteuthis#14    | TGCC.C     |            | T          | T          | C          | A          | G         | T          |
| Sthenoteuthis#21     | GC.C       |            | T          | T          | C          | A          | G         | T          |
| Thysanoteuthis#31    | TGC        |            | T          | T          | C          | A          | G         | T          |
| Loligo_pealei#23     | GCC.C      |            | T          | T          | C          | A          | G         | T          |
| Sepioteuthis#13      | TGC.C      |            | T          | T          | C          | A          | G         | T          |
| Idiosepius#43        | GC         |            | T          | T          | G          | A          | G         | T          |
| Rossia480483         | TGC.C      |            | T          | T          | C          | A          | G         | T          |
| Sepia_officinalis#5  | TGC.C      |            | T          | T          | C          | A          | G         | T          |
| Sepia_opipara#35     | TGC.C      |            | T          | T          | C          | A          | G         | T          |
| Sepioloides#6        | TGC        |            | T          | T          | C          | A          | G         | T          |
| Spirula#30           | GCC        |            | T          | T          | A          | G          | T         | T          |
| Stoloteuthis#29      | TGC.C      |            | T          | T          | C          | A          | G         | T          |
| Argonauta#5          | TGC        |            | T          | T          | A          | A          | G         | T          |
| Bathypolypus#5       | TGC.C      |            | G          | T          | T          | A          |           |            |
| Cirrochauma#20       | TGCC       | A          | T          | T          | T          | G          | T         | T          |
| Eledonella#5         | TGCA       | A          | A          | C          | A          | G          | T         | A          |
| Granelledone#39      | TGCA.C     |            | A          | C          | A          | G          | T         | A          |
| Japatella#16         | TGCA       | A          | A          | C          | A          | G          | T         | A          |
| Octopus#9            | TGC.C      |            | A          | T          | T          | A          |           |            |
| Stauroteuthis#22     | TGCC       | A          | T          | T          | C          | A          | G         | T          |
| Vampyroteuthis#21    | TGCA       | T          | T          | T          | C          | A          | G         | T          |
| Nautilus#1           | GCC.C      |            | G          | G          | C          | A          | G         | T          |
| Abralia#18           | GC.C       | A          | A          | T          | G          | C          | T         | A          |
| Brachioteuthis#6     | GC.G       | A          | G          | T          | G          | C          | T         | A          |
| Chiroteuthis#16      | GC.C       | A          | G          | T          | G          | C          | T         | A          |
| Chtenopteryx#3       | GC.G       | G          | A          | A          | T          | G          | C         | T          |
| Histioteuthis#3      | GC         | C.A        | T          | A          | T          | C          | C         | T          |
| Mastigoteuthis#22    | GC.C       | A          | G          | T          | G          | C          | T         | A          |
| Onychoteuthis#33     | GC.G       | A          | T          | T          | G          | C          | T         | A          |
| Idiosepius#06        | GCG        | A.A        | A          | A          | C          | C          | G         | C          |
| Sepia_officinalis#41 | GC.G       | A          | A          | A          | G          | C          | A         | A          |
| Sepia_opipara#23     | GC.G       | G          | A          | A          | T          | G          | C         | A          |
| Spirula#40           | GC.A       | A          | A          | A          | T          | G          | C         | T          |
| Vampyroteuthis#13    | TGC        |            | T          | A          | TT         | G          |           |            |

|                      | 250        | 260        | 270        | 280        | 290        | 300        | 310        | 320        |
|----------------------|------------|------------|------------|------------|------------|------------|------------|------------|
| Abralia#3            | TGCCCTTCCC | CACGCCATCC | TCCGCTCTGA | CTTGGCCCGA | CGTGATCTTA | CTGACTACCT | CATGAAGATC | TTTACTGAGC |
| Alluroteuthis#6      |            |            |            | T.C        |            |            |            | C...A      |
| Bathyteuthis#1       | T          |            |            |            |            |            |            | C.C.C      |
| Chiroteuthis#12      |            |            |            | T          |            |            |            | C.C.A      |
| Chtenopteryx#41      | T          |            |            | T          |            |            |            | C.C.C      |
| Cranchia#2           |            |            |            |            |            |            |            | C...A      |
| Cycloteuthis#29      |            |            |            |            | G          | C          | A.A        | C...A      |
| Discoteuthis#11      |            |            |            |            |            |            |            | C...A      |
| Enoploteuthis#7      |            |            |            | T          |            | T          |            | C...A      |
| Gonatopsis#11        | T          |            |            | T          |            | A.T        |            | C...A      |
| Gonatus_onyx#32      | T          |            |            | T          |            | A.T        |            | C.A...A    |
| Histioteuthis#49     | T          |            |            | T          |            |            |            | C...A      |
| Lepidoteuthis#27     |            |            | C          | T          |            |            |            | C...A      |
| Liocranchia#6        |            |            |            |            |            |            |            | C...A      |
| Mastigoteuthis480483 |            |            |            | T          |            |            |            | C...A      |
| Octopoteuthis#5      | T          | T          |            | T          |            |            | G          | C...A      |
| Ommastrephes#49      | T          |            |            | T.T        |            |            |            | C...A      |
| Onychoteuthis#26     | T.C        |            |            | T          |            | T          | A          | C...A      |
| Pholidoteuthis#21    |            |            |            | T          |            | T          |            | C...A      |
| Pyroteuthis#4        | T.C        | T          | A          | C.A        |            |            |            | C...C      |
| Thysanoteuthis#44    | C          |            |            |            |            |            |            | C...A      |
| Sthenoteuthis#32     |            |            |            |            |            |            |            | C...A      |
| Loligo_pealei#27     |            |            |            |            |            |            |            | C...C      |
| Sepioteuthis480483   |            |            |            |            |            | T          | ?          | C...C      |
| Heteroteuthis#21     |            |            |            |            |            |            |            | G.C        |
| Idiosepius#17        | C.T        | T          | T          |            |            | T          | A          | C.C.C.A    |
| Rossia#4             |            |            |            |            |            |            |            | G.C        |
| Sepia_officinalis#17 |            |            |            |            |            |            |            | C...C      |
| Sepia_opipara#13     |            |            |            |            |            |            |            | C...A      |
| Sepioloidea#32       |            | T          | T          |            |            |            |            | G.C        |
| Spirula#22           |            |            |            |            | C          |            |            | C...A      |
| Stoloteuthis#13      |            | A          |            |            |            |            |            | G.C        |
| Cirrothauma#14       | C          | T          | T.G        | T.T        | CT         | T          |            | C...C      |
| Eledonella#6         | C          | T          | T.G        | T.T        |            |            |            | C...C      |
| Graneledone#36       | C          | T          | T.G        | A          | T.T        | T          | T          | C...A      |
| Japatella#17         | C          | T          | T.G        | T.T        |            |            |            | C...C      |
| Octopus#27           | C          | T          | T.G        | T.T        |            |            |            | C...C      |
| Vampyroteuthis#44    | C          |            | T.G        | T.C        |            |            |            | C.C.C      |
| Bathyteuthis#15      | C          | T.T        | T.T.G      | T          | CT.G       | A.T        | G          | C.G.C.A    |
| Brachioteuthis#4     | C          | T.T        | T.T.G      | T          | CT.G       | A.T        | G          | C.C.C.A    |
| Chtenopteryx#09      |            | T          | T.G        | T          | CT.G       | A.T        | T          | G.C.A      |
| Cranchia#1           | C          | T.T        | T.T.G      | T          | CT.G       | A.T        | G          | C.G.C.A    |
| Cycloteuthis#61      |            |            | T.G        | T          | CT.G       | T          | G          | C.C.C      |
| Discoteuthis#5       |            |            | T.G        | T          | CT.G       | T          | G          | C.C.C      |
| Enoploteuthis#5      |            | T.T        | T.T.G      | T          | CT.G       | A.T        | G          | C...A      |
| Gonatus_onyx#90      | C          |            | T.G        | T          | CT.G       | A.T        | G          | C.C.C.A    |
| Histioteuthis#6      | C          | T          | T.G        | T          | CT.G       | A.T        | T          | C.G.C.A    |
| Ommastrephes#6       |            | T.A        | T.G        | T          | CT.G       | A.T        | G          | C.G.C.A    |
| Psychroteuthis#14    |            | T.T        | T.T.G      | T          | CT.G       | A.T        | G          | A.G.C.A    |
| Sthenoteuthis#21     | C          | T          | T.G        | T          | CT.G       | A.T        | T          | G.A        |
| Thysanoteuthis#31    | C          | T          | T.G        | T          | CT.G       | A.T        | T          | G.A        |
| Loligo_pealei#23     |            | T          | T.G        | T          | CT.G       | A          | G          | C.G...A    |
| Sepioteuthis#13      |            | T          | T.G        | T          | CT.G       | A          | T          | G...A      |
| Idiosepius#43        |            | T          | T.G        | T          | CT.A       | A          | T          | G...A      |
| Rossia480483         |            | T          | T.G        | T          | CT.A       | C          | T          | G...A      |
| Sepia_officinalis#5  | C          | T          | T.G        | T          | CT.G       | A          | T          | T...A      |
| Sepia_opipara#35     | C          | T          | T.G        | T          | CT.G       | A          | T          | T...A      |
| Sepioloidea#6        |            | T          | T.G        | T          | CT.G       | A          | T          | T...A      |
| Spirula#30           | C          | T          | T.G        | T          | CT.G       | A          | T          | G...A      |
| Stoloteuthis#29      | C          | T          | T.G        | T          | CT.G       | A          | T          | G...A      |
| Argonauta#5          |            | T.A        | T.T.A      | TC         | T.T.G      | T.G        | C          | T...G      |
| Bathypolypus#5       |            | T.G        | T.T.A      | TC         | T.T.T      | CT.G       | C          | T...G      |
| Cirrothauma#20       | C          | T.G        | T.T.A      | TC         | T.T.T      | CT.G       | C          | T...G      |
| Eledonella#5         |            | T.G        | T.T.A      | TC         | T.T.T      | CT.G       | A          | T...G      |
| Graneledone#39       |            | T.G        | T.T.A      | TC         | T.T.T      | CT.G       | A          | T...G      |
| Japatella#16         |            | T.G        | T.T.A      | TC         | T.T.T      | CT.G       | A          | T...G      |
| Octopus#9            | C          | T.G        | T.T.A      | TC         | T.T.T      | CT.G       | A          | T...G      |
| Stauroteuthis#22     | C          | GT.G       | T.T.T      | G          |            | T.C        | A.G        | A.T...T    |
| Vampyroteuthis#21    | C          | G          | T          | T          | T.C        | CT.G       | T          | T...T      |
| Nautilus#1           | C          | G          | T          | T          | T.C        | CT.G       | T          | T...T      |
| Abralia#18           | C          | T          | T          | AAA        | GT.G       | TC         | T          | T...G      |
| Brachioteuthis#6     | C          | T          | T          | AGA        | GT.G       | TC         | T          | T...G      |
| Chiroteuthis#16      | C          |            | G          | AAA        | GT.GA      | TC         | T          | T...G      |
| Chtenopteryx#3       | C          | A          | T          | AAA        | GT.G       | T          | T          | T...G      |
| Histioteuthis#3      | C          | T          | T          | AAA        | GT.G       | TC         | T          | T...G      |
| Mastigoteuthis#22    | C          | T          | T          | AAA        | GT.G       | T          | T          | T...G      |
| Onychoteuthis#33     | C          | T          | T          | AAA        | GT.G       | TC         | T          | T...G      |
| Idiosepius#06        |            | G          | T          | AAA        | G.G        | TC         | T          | T...G      |
| Sepia_officinalis#41 | C          | T          | A          | AGA        | G.C        | T          | T          | T...G      |
| Sepia_opipara#23     | C          | T          | A          | AAA        | G.G        | T          | T          | T...G      |
| Spirula#40           | CT         | C          | T          | A          | A          | C          | G          | TC         |
| Vampyroteuthis#13    | CA         | T          | G          | A          | CA         | A          | GA         | TC         |

|                      | 330                   | 340                   | 350                   | 360                   | 370                   | 380                   | 390                   | 400                               |
|----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------------------|
| Abralia#3            | GTGGTTATTC            | ATTCAACAACC           | ACCGCCGAGA            | GAGAGATYGT            | TCGTGACATC            | AAGGAGAAAT            | TGTGCTATGT            | TGCTCTTGAC                        |
| Alluroteuthis#6      | . . . . . C . . . . . | . . . . . C . . . . . | . . . . .             | . . . . . A . . . . . | . . . . .             | . . . . . C . . . . . | . . . . . T . . . . . | . . . . .                         |
| Bathyteuthis#1       | . . . . . T . . . . . | . . . . . C . . . . . | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . . C . . . . . T           |
| Chiroteuthis#12      | . . . . . C . . . . . | . . . . .             | . . . . .             | . . . . . A . . . . . | . . . . .             | . . . . . G . . . . . | . . . . .             | . . . . .                         |
| Chtenopteryx#41      | . . . . . C . . . . . | . . . . . T . . . . . | . . . . . C . . . . . | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . . C . . . . . T           |
| Cranchia#2           | . . . . .             | . . . . .             | . . . . .             | . . . . . A . . . . . | . . . . .             | . . . . .             | . . . . . C . . . . . | . . . . . A . . . . .             |
| Cycloteuthis#29      | . . . . .             | . . . . . C . . . . . | . . . . . G . . . . . | . . . . . T . . . . . | . . . . . C . . . . . | . . . . .             | . . . . .             | . . . . .                         |
| Discoteuthis#11      | . . . . .             | . . . . .             | . . . . .             | . . . . . A . . . . . | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Enoploteuthis#7      | . . . . . C . . . . . | . . . . .             | . . . . .             | . . . . . A . . . . . | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Gonatopsis#11        | . . . . .             | . . . . . T . . . . . | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Gonatus_onyx#32      | . . . . .             | . . . . . T . . . . . | . . . . .             | . . . . .             | . . . . . T . . . . . | . . . . .             | . . . . .             | . . . . .                         |
| Histioteuthis#49     | . . . . . C . . . . . | . . . . .             | . . . . . C . . . . . | . . . . .             | . . . . . A . . . . . | . . . . .             | . . . . .             | . . . . .                         |
| Lepidoteuthis#27     | . . . . .             | . . . . . T . . . . . | . . . . .             | . . . . . A . . . . . | . . . . .             | . . . . .             | . . . . . C . . . . . | . . . . . T . . . . .             |
| Liocranchia#6        | . . . . .             | . . . . .             | . . . . .             | . . . . . A . . . . . | . . . . . C . . . . . | . . . . .             | . . . . .             | . . . . . A . . . . .             |
| Mastigoteuthis480483 | . . . . . C . . . . . | . . . . .             | . . . . . C . . . . . | . . . . .             | . . . . . A . . . . . | . . . . .             | . . . . .             | . . . . .                         |
| Octopoteuthis#5      | . . . . .             | . . . . . T . . . . . | . . . . .             | . . . . . A . . . . . | . . . . .             | . . . . . C . . . . . | . . . . . T . . . . . | . . . . .                         |
| Ommastrephes#49      | . . . . . C . . . . . | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Onychoteuthis#26     | . . . . .             | . . . . . T . . . . . | . . . . .             | . . . . . A . . . . . | . . . . . C . . . . . | . . . . .             | . . . . .             | . . . . . T . . . . . G . . . . . |
| Pholidoteuthis#21    | . . . . . C . . . . . | . . . . .             | . . . . .             | . . . . . A . . . . . | . . . . . C . . . . . | . . . . .             | . . . . .             | . . . . . C . . . . . T . . . . . |
| Pyroteuthis#4        | . . . . . C . . . . . | . . . . . T . . . . . | . . . . .             | . . . . . A . . . . . | . . . . .             | . . . . .             | . . . . .             | . . . . . C . . . . .             |
| Thysanoteuthis#44    | . . . . .             | . . . . .             | . . . . .             | . . . . . G . . . . . | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Sthenoteuthis#32     | . . . . .             | . . . . . C . . . . . | . . . . .             | . . . . . A . . . . . | . . . . . C . . . . . | . . . . . T . . . . . | . . . . .             | . . . . .                         |
| Loligo_pealei#27     | . . . . . C . . . . . | . . . . .             | . . . . . C . . . . . | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . . C . . . . .             |
| Sepioteuthis480483   | . . . . .             | . . . . .             | . . . . .             | . . . . . A . . . . . | . . . . .             | . . . . .             | . . . . .             | . . . . . C . . . . .             |
| Heteroteuthis#21     | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . . C . . . . . | . . . . .             | . . . . .             | . . . . .                         |
| Idiosepius#17        | . . . . .             | . . . . . C . . . . . | . . . . .             | . . . . . T . . . . . | . . . . .             | . . . . . A . . . . . | . . . . . C . . . . . | . . . . .                         |
| Rossia#4             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . . C . . . . .             |
| Sepia_officinalis#17 | . . . . .             | . . . . .             | . . . . .             | . . . . . A . . . . . | . . . . . G . . . . . | . . . . .             | . . . . . A . . . . . | . . . . . C . . . . . C . . . . . |
| Sepia_opipara#13     | . . . . .             | . . . . .             | . . . . .             | . . . . . A . . . . . | . . . . . G . . . . . | . . . . .             | . . . . .             | . . . . . C . . . . .             |
| Sepioloidea#32       | . . . . .             | . . . . .             | . . . . .             | . . . . . T . . . . . | . . . . .             | . . . . .             | . . . . .             | . . . . . C . . . . .             |
| Spirula#22           | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . . C . . . . . T . . . . . |
| Stoloteuthis#13      | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Cirrothauma#14       | . . . . . C . . . . . | . . . . .             | . . . . .             | . . . . . A . . . . . | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Eledonella#6         | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Graneledone#36       | . . . . . A . . . . . | . . . . . C . . . . . | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Japatella#17         | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Octopus#27           | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Vampyroteuthis#44    | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Bathyteuthis#15      | . . . . . C . . . . . | . . . . . C . . . . . | . . . . . T . . . . . | . . . . . T . . . . . | . . . . . G . . . . . | . . . . .             | . . . . .             | . . . . .                         |
| Brachioteuthis#4     | . . . . . C . . . . . | . . . . . T . . . . . | . . . . . T . . . . . | . . . . . T . . . . . | . . . . . G . . . . . | . . . . .             | . . . . .             | . . . . .                         |
| Chtenopteryx#09      | . . . . . C . . . . . | . . . . . T . . . . . | . . . . . T . . . . . | . . . . . T . . . . . | . . . . . G . . . . . | . . . . .             | . . . . .             | . . . . .                         |
| Cranchia#1           | . . . . . C . . . . . | . . . . . C . . . . . | . . . . . T . . . . . | . . . . . T . . . . . | . . . . . G . . . . . | . . . . .             | . . . . .             | . . . . .                         |
| Cycloteuthis#61      | . . . . . C . . . . . | . . . . . C . . . . . | . . . . . T . . . . . | . . . . . T . . . . . | . . . . . G . . . . . | . . . . .             | . . . . .             | . . . . .                         |
| Discoteuthis#5       | . . . . . C . . . . . | . . . . . C . . . . . | . . . . . T . . . . . | . . . . . T . . . . . | . . . . . G . . . . . | . . . . .             | . . . . .             | . . . . .                         |
| Enoploteuthis#5      | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Gonatus_onyx#90      | . . . . . C . . . . . | . . . . . C . . . . . | . . . . . T . . . . . | . . . . . T . . . . . | . . . . . G . . . . . | . . . . .             | . . . . .             | . . . . .                         |
| Histioteuthis#6      | . . . . . C . . . . . | . . . . . C . . . . . | . . . . . T . . . . . | . . . . . T . . . . . | . . . . . G . . . . . | . . . . .             | . . . . .             | . . . . .                         |
| Ommastrephes#6       | . . . . . C . . . . . | . . . . . C . . . . . | . . . . . T . . . . . | . . . . . T . . . . . | . . . . . G . . . . . | . . . . .             | . . . . .             | . . . . .                         |
| Psychroteuthis#14    | . . . . . C . . . . . | . . . . . C . . . . . | . . . . . T . . . . . | . . . . . T . . . . . | . . . . . G . . . . . | . . . . .             | . . . . .             | . . . . .                         |
| Sthenoteuthis#21     | . . . . . C . . . . . | . . . . . C . . . . . | . . . . . T . . . . . | . . . . . T . . . . . | . . . . . G . . . . . | . . . . .             | . . . . .             | . . . . .                         |
| Thysanoteuthis#31    | . . . . . C . . . . . | . . . . . C . . . . . | . . . . . T . . . . . | . . . . . T . . . . . | . . . . . G . . . . . | . . . . .             | . . . . .             | . . . . .                         |
| Loligo_pealei#23     | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Sepioteuthis#13      | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Idiosepius#43        | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Rossia480483         | . . . . . A . . . . . | . . . . . C . . . . . | . . . . . T . . . . . | . . . . . T . . . . . | . . . . . G . . . . . | . . . . .             | . . . . .             | . . . . .                         |
| Sepia_officinalis#5  | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Sepia_opipara#35     | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Sepioloidea#6        | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Spirula#30           | . . . . . C . . . . . | . . . . . C . . . . . | . . . . . T . . . . . | . . . . . T . . . . . | . . . . . G . . . . . | . . . . .             | . . . . .             | . . . . .                         |
| Stoloteuthis#29      | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Argonauta#5          | . . . . . C . . . . . | . . . . . C . . . . . | . . . . . T . . . . . | . . . . . T . . . . . | . . . . . G . . . . . | . . . . .             | . . . . .             | . . . . .                         |
| Bathypolypus#5       | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Cirrothauma#20       | . . . . . C . . . . . | . . . . . C . . . . . | . . . . . T . . . . . | . . . . . T . . . . . | . . . . . G . . . . . | . . . . .             | . . . . .             | . . . . .                         |
| Eledonella#5         | . . . . . C . . . . . | . . . . . C . . . . . | . . . . . T . . . . . | . . . . . T . . . . . | . . . . . G . . . . . | . . . . .             | . . . . .             | . . . . .                         |
| Graneledone#39       | . . . . . C . . . . . | . . . . . C . . . . . | . . . . . T . . . . . | . . . . . T . . . . . | . . . . . G . . . . . | . . . . .             | . . . . .             | . . . . .                         |
| Japatella#16         | . . . . . C . . . . . | . . . . . C . . . . . | . . . . . T . . . . . | . . . . . T . . . . . | . . . . . G . . . . . | . . . . .             | . . . . .             | . . . . .                         |
| Octopus#9            | . . . . . C . . . . . | . . . . . C . . . . . | . . . . . T . . . . . | . . . . . T . . . . . | . . . . . G . . . . . | . . . . .             | . . . . .             | . . . . .                         |
| Stauroteuthis#22     | . . . . . C . . . . . | . . . . . C . . . . . | . . . . . T . . . . . | . . . . . T . . . . . | . . . . . G . . . . . | . . . . .             | . . . . .             | . . . . .                         |
| Vampyroteuthis#21    | . . . . . C . . . . . | . . . . . C . . . . . | . . . . . T . . . . . | . . . . . T . . . . . | . . . . . G . . . . . | . . . . .             | . . . . .             | . . . . .                         |
| Nautilus#1           | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Abralia#18           | . . . . . A . . . . . | . . . . . C . . . . . | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Brachioteuthis#6     | . . . . . G . . . . . | . . . . . C . . . . . | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Chiroteuthis#16      | . . . . . G . . . . . | . . . . . C . . . . . | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Chtenopteryx#3       | . . . . . G . . . . . | . . . . . C . . . . . | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Histioteuthis#3      | . . . . . G . . . . . | . . . . . C . . . . . | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Mastigoteuthis#22    | . . . . . G . . . . . | . . . . . C . . . . . | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Onychoteuthis#33     | . . . . . G . . . . . | . . . . . C . . . . . | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Idiosepius#06        | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Sepia_officinalis#41 | . . . . . G . . . . . | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Sepia_opipara#23     | . . . . . G . . . . . | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Spirula#40           | . . . . . A . . . . . | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |
| Vampyroteuthis#13    | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .             | . . . . .                         |

|                       | 410         | 420           | 430             | 440                | 450           | 460         | 470           | 480           |
|-----------------------|-------------|---------------|-----------------|--------------------|---------------|-------------|---------------|---------------|
| Abralia#3             | TTCGAAACAGG | AGATGGCCAC    | CGCCGGCTCA      | CCTTCTCTCC         | TTGAGAAGAG    | CTACGAGTTG  | CCCGATGGTC    | AAGTTATCAC    |
| Alluroteuthis#6       | .....A..... | .....         | .....T.....     | .....              | .....A.....   | .....T..... | .....A.....   | .....         |
| Bathyteuthis#1        | .....       | .....         | .....T.C.....   | .....              | .....         | .....       | .....G.A..... | .....         |
| Chiroteuthis#12       | .....       | .....T.....   | .....T.C.....   | .....              | .....G.....   | .....       | .....C.....   | .....A.....   |
| Chtenopteryx#41       | G.....      | A.....        | .....T.C.....   | .....              | .....         | .....       | .....         | .....A.....   |
| Cranchia#2            | .....       | .....         | .....CA.....    | .....T.....        | .....         | .....       | .....         | .....A.....   |
| Cycloteuthis#29       | C.....      | A.....T.....  | .....T.....     | .....T.....        | .....         | .....       | .....G.....   | .....         |
| Discoteuthis#11       | .....       | .....T.....   | .....           | .....T.....        | .....         | .....       | .....         | .....         |
| Enoplateuthis#7       | .....       | .....         | .....T.....     | .....T.....        | .....         | .....       | .....         | .....         |
| Gonatopsis#11         | .....       | .....         | .....T.....     | .....T.....        | .....         | .....       | .....C.....   | .....         |
| Gonatus_onyx#32       | .....       | .....         | .....T.....     | .....T.....        | .....         | .....       | .....C.....   | .....         |
| Histioteuthis#49      | .....       | .....T.....   | .....           | .....              | .....         | .....       | .....         | .....A.....   |
| Lepidoteuthis#27      | .....       | .....         | .....T.C.....   | .....              | .....         | .....       | .....         | .....         |
| Liocranchia#6         | .....       | A.....        | .....T.....     | .....T.C.A.....    | .....         | .....       | .....         | .....A.....   |
| Mastigoteuthis#480483 | .....       | .....T.....   | .....T.C.....   | .....              | .....G.....   | .....       | .....C.....   | .....A.....   |
| Octopoteuthis#5       | .....       | A.....        | .....T.....     | .....T.....        | .....         | .....       | .....C.....   | .....         |
| Ommastrephes#49       | .....       | .....         | .....T.C.T..... | .....              | .....C.....   | .....       | .....         | .....         |
| Onychoteuthis#26      | .....       | .....         | .....C.T.A..... | .....              | .....A.....   | .....       | .....C.....   | .....         |
| Pholidoteuthis#21     | .....       | .....         | .....T.C.....   | .....              | .....         | .....       | .....         | .....         |
| Pyroteuthis#4         | A.A.....    | .....         | .....T.C.A..... | G.....             | .....         | .....A..... | .....         | .....A.....   |
| Thysanoteuthis#44     | .....       | .....         | .....T.....     | .....T.....        | .....         | .....       | .....         | .....         |
| Sthenoteuthis#32      | .....       | A.....        | .....C.....     | .....              | .....         | .....       | .....         | .....G.....   |
| Loligo_pealei#27      | .....       | .....         | .....T.C.A..... | .....              | .....         | .....       | .....         | .....A.....   |
| Sepioteuthis#480483   | .....       | .....         | .....A.T.....   | .....              | .....         | .....       | .....T.....   | .....G.A..... |
| Heteroteuthis#21      | T.....      | A.....        | .....T.....     | .....              | .....C.A..... | .....       | .....         | .....A.....   |
| Idiosepius#17         | T.....      | T.....        | .....C.....     | .....              | .....A.....   | .....       | .....         | .....         |
| Rossia#4              | .....       | A.....        | .....T.....     | .....              | .....         | .....       | .....         | .....G.C..... |
| Sepia_officinalis#17  | G.....      | A.....        | G.T.....        | T.....             | C.....        | A.....      | .....         | G.A.....      |
| Sepia_opipara#13      | T.....      | .....         | T.T.....        | TT.....            | C.....        | .....       | .....         | A.....        |
| Sepioloidea#32        | T.....      | .....         | .....           | .....              | C.....        | .....       | T.....        | G.C.....      |
| Spirula#22            | .....       | .....         | .....T.A.....   | C.....             | .....         | .....       | .....         | A.....        |
| Stoloteuthis#13       | G.....      | A.....        | .....T.....     | .....              | .....C.....   | .....       | .....         | G.A.....      |
| Cirrothauma#14        | G.....      | A.....T.....  | .....T.C.T..... | .....              | A.....        | .....       | .....         | C.....        |
| Eledonella#6          | G.....      | A.....T.....  | .....T.C.T..... | .....              | A.....        | .....       | .....T.....   | G.....        |
| Graneledone#36        | T.....      | AC.....T..... | T.T.....        | T.T.C.T.....       | A.....        | .....       | .....         | T.....        |
| Japatella#17          | G.....      | A.....T.....  | C.T.C.....      | T.....             | A.....        | .....       | .....         | G.....        |
| Octopus#27            | G.....      | A.....T.....  | T.....          | T.C.T.....         | A.....        | T.....      | A.....        | T.....        |
| Vampyroteuthis#44     | G.....      | A.....T.....  | .....           | T.C.T.....         | .....         | A.....      | .....         | T.....        |
| Bathyteuthis#15       | C.....      | GA.....       | CAA.....        | A.T.C.T.C.T.....   | AT.....       | G.....      | .....         | A.....        |
| Brachioteuthis#4      | C.....      | A.....        | CAA.....        | A.T.C.T.C.....     | AT.....       | G.....      | .....         | C.....        |
| Chtenopteryx#09       | C.....      | A.....        | CAG.....        | A.T.C.T.C.T.....   | AT.....       | G.....      | .....         | A.....        |
| Cranchia#1            | T.....      | A.....        | CAA.....        | A.T.C.T.C.T.....   | TT.....       | G.....      | A.....        | T.....        |
| Cycloteuthis#61       | T.T.....    | .....         | CAG.....        | G.....             | C.C.T.C.....  | TT.....     | G.....        | A.....        |
| Discoteuthis#5        | T.C.....    | .....         | CAG.....        | T.T.C.C.T.C.....   | TT.....       | G.....      | .....         | C.....        |
| Enoplateuthis#5       | T.....      | A.....        | CAA.....        | T.C.T.C.T.....     | AT.....       | G.....      | A.....        | C.....        |
| Gonatus_onyx#90       | T.C.....    | A.....        | CAG.....        | .....              | C.T.A.....    | T.....      | GT.....       | G.....        |
| Histioteuthis#6       | C.....      | A.....        | CAA.....        | A.T.C.T.C.T.....   | AT.....       | G.....      | .....         | A.A.....      |
| Ommastrephes#6        | C.....      | A.....        | CAA.....        | A.T.C.T.C.T.....   | AT.....       | G.....      | .....         | T.....        |
| Psychroteuthis#14     | C.....      | A.....        | CAA.....        | G.T.C.T.C.T.....   | AT.....       | G.....      | .....         | A.A.....      |
| Sthenoteuthis#21      | C.....      | A.....        | CAA.....        | A.T.C.T.C.T.....   | AT.....       | G.....      | .....         | T.....        |
| Thysanoteuthis#31     | T.....      | A.....        | CAA.....        | A.T.C.T.C.T.....   | AT.....       | G.....      | .....         | T.....        |
| Loligo_pealei#23      | T.A.....    | A.....        | CAG.....        | T.C.C.T.C.T.....   | AT.....       | A.....      | .....         | T.A.....      |
| Sepioteuthis#13       | T.....      | A.....        | CAG.....        | A.T.C.T.C.T.....   | AT.....       | A.....      | .....         | T.A.....      |
| Idiosepius#43         | C.....      | A.....        | CAA.....        | .....              | T.AAG.....    | TT.....     | G.....        | A.....        |
| Rossia#480483         | T.C.A.....  | A.....        | CAA.....        | A.T.G.T.G.T.....   | AT.....       | G.....      | A.....        | A.....        |
| Sepia_officinalis#5   | C.....      | A.....        | CAA.....        | A.T.C.T.C.T.....   | AT.....       | G.....      | .....         | T.....        |
| Sepia_opipara#35      | C.....      | A.....        | CAA.....        | A.T.C.T.C.T.....   | AT.....       | G.....      | .....         | T.....        |
| Sepioloidea#6         | T.A.....    | A.....        | CAA.....        | A.A.....           | T.T.C.T.....  | GT.....     | G.....        | A.....        |
| Spirula#30            | T.....      | .....         | CAG.....        | A.T.C.T.C.T.....   | AT.....       | G.....      | .....         | A.A.....      |
| Stoloteuthis#29       | T.C.A.....  | A.....        | CAA.....        | A.T.G.T.G.T.....   | AT.....       | G.....      | .....         | A.A.....      |
| Argonauta#5           | T.....      | A.....        | CAA.....        | T.A.C.T.....       | A.TT.....     | A.....      | .....         | T.T.A.A.....  |
| Bathypolypus#5        | T.....      | A.....        | CAG.....        | T.A.C.C.T.C.A..... | TT.....       | G.....      | .....         | T.A.A.....    |
| Cirrothauma#20        | G.....      | .....         | CAA.....        | T.T.C.T.....       | A.A.T.....    | G.....      | .....         | T.A.....      |
| Eledonella#5          | A.....      | A.....        | CAG.....        | T.A.C.C.T.A.A..... | TT.....       | G.....      | .....         | T.A.A.....    |
| Graneledone#39        | A.....      | A.....        | ACAG.....       | T.A.C.T.T.A.A..... | TT.....       | G.....      | .....         | A.A.A.....    |
| Japatella#16          | A.....      | A.....        | CAG.....        | T.A.C.C.T.A.A..... | TT.....       | G.....      | .....         | T.A.A.....    |
| Octopus#9             | .....       | A.....        | CAG.....        | T.A.C.C.T.....     | A.TT.....     | G.....      | .....         | T.A.A.....    |
| Stauroteuthis#22      | T.....      | .....         | CAG.....        | T.T.T.C.T.....     | A.A.T.....    | G.....      | .....         | T.A.....      |
| Vampyroteuthis#21     | .....       | A.....        | CAG.....        | A.TT.C.T.C.....    | AA.....       | G.....      | .....         | T.A.....      |
| Nautilus#1            | G.A.....    | A.....        | CAG.....        | G.A.CAGC.....      | AGCAGT.....   | T.....      | .....         | C.A.....      |
| Abralia#18            | .....       | A.....        | AG.....         | AT.A.AA.....       | C.....        | G.....      | A.....        | A.A.....      |
| Brachioteuthis#6      | .....       | A.....        | A.....          | AT.G.AA.....       | T.G.....      | G.....      | A.....        | A.....        |
| Chiroteuthis#16       | .....       | CAC.....      | AGT.....        | GT.AAA.....        | G.....        | A.....      | .....         | A.....        |
| Chtenopteryx#3        | C.A.....    | A.....        | AG.....         | TT.T.AAGA.....     | T.A.GA.....   | A.....      | .....         | C.....        |
| Histioteuthis#3       | C.A.....    | A.....        | A.....          | AT.G.AA.....       | T.C.G.A.....  | A.....      | .....         | T.A.C.A.....  |
| Mastigoteuthis#22     | .....       | A.....        | A.....          | AT.G.AA.....       | T.G.G.A.....  | A.....      | .....         | T.A.C.T.....  |
| Onychoteuthis#33      | .....       | A.....        | A.....          | AT.G.AA.....       | T.G.G.A.....  | A.....      | .....         | T.A.C.T.....  |
| Idiosepius#06         | T.C.....    | A.....        | A.....          | T.T.AA.....        | T.....        | G.....      | A.....        | A.....        |
| Sepia_officinalis#41  | C.A.....    | A.....        | A.....          | T.A.AACA.....      | T.....        | GG.....     | G.....        | A.....        |
| Sepia_opipara#23      | C.A.....    | A.....        | A.....          | T.G.AACA.....      | T.....        | GG.....     | G.....        | A.....        |
| Spirula#40            | C.A.....    | A.....        | A.....          | TT.G.AACA.....     | T.....        | GG.....     | G.....        | A.....        |
| Vampyroteuthis#13     | .....       | A.....        | A.....          | TT.G.AACA.....     | T.....        | GG.....     | G.....        | A.....        |



|                      | 490           | 500           | 510            | 520              | 530           | 540         | 550         | 560         |
|----------------------|---------------|---------------|----------------|------------------|---------------|-------------|-------------|-------------|
| Abralia#3            | CATTGGTAAC    | GAGAGATTCA    | GGTGCCAGCA     | ATCATGTTTC       | CAGCCTTCCT    | TCTTGGGTAT  | GGAATCTGCT  | GGTATCCATG  |
| Alluroteuthis#6      | .....A.G..... | .....T.....   | .....A.....    | .....A.....      | .....A.....   | .....A..... | .....A..... | .....A..... |
| Bathyteuthis#1       | .....C.....   | .....G.....   | .....C.T.....  | .....A.....      | .....A.....   | .....A..... | .....A..... | .....A..... |
| Chiroteuthis#12      | .....G.....   | .....G.T..... | .....G.....    | .....A.....      | .....C.....   | .....C..... | .....C..... | .....C..... |
| Chtenopteryx#41      | .....G.A..... | .....G.....   | .....G.....    | .....A.....      | .....C.....   | .....C..... | .....C..... | .....C..... |
| Cranchia#2           | .....T.....   | .....G.....   | .....G.....    | .....A.....      | .....C.....   | .....C..... | .....C..... | .....C..... |
| Cycloteuthis#29      | .....C.....   | .....C.T..... | .....G.C.....  | .....CC.T.....   | .....A.....   | .....A..... | .....G..... | .....G..... |
| Discoteuthis#11      | .....C.....   | .....C.T..... | .....CC.T..... | .....A.....      | .....A.....   | .....G..... | .....G..... | .....G..... |
| Enoplateuthis#7      | .....C.....   | .....C.T..... | .....CC.T..... | .....A.....      | .....A.....   | .....G..... | .....G..... | .....G..... |
| Gonatopsis#11        | .....C.....   | .....C.T..... | .....CC.T..... | .....A.....      | .....A.....   | .....G..... | .....G..... | .....G..... |
| Gonatus_onyx#32      | .....C.....   | .....C.T..... | .....CC.T..... | .....A.....      | .....A.....   | .....G..... | .....G..... | .....G..... |
| Histioteuthis#49     | .....C.....   | .....G.....   | .....A.....    | .....A.....      | .....A.....   | .....A..... | .....A..... | .....A..... |
| Lepidoteuthis#27     | .....C.....   | .....G.T..... | .....T.....    | .....G.CC.T..... | .....A.....   | .....T..... | .....T..... | .....T..... |
| Liocranchia#6        | .....C.....   | .....G.....   | .....T.....    | .....C.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Mastigoteuthis480483 | .....A.G..... | .....T.....   | .....C.T.....  | .....T.....      | .....A.....   | .....A..... | .....A..... | .....A..... |
| Octopoteuthis#5      | .....C.....   | .....G.....   | .....T.C.....  | .....T.....      | .....A.....   | .....A..... | .....A..... | .....A..... |
| Ommastrephes#49      | .....C.....   | .....G.....   | .....C.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Onychoteuthis#26     | .....T.....   | .....G.....   | .....C.....    | .....G.CC.T..... | .....A.....   | .....T..... | .....T..... | .....T..... |
| Pholidoteuthis#21    | .....C.....   | .....A.G..... | .....T.....    | .....C.....      | .....G.T..... | .....A..... | .....A..... | .....A..... |
| Pyroteuthis#4        | .....C.....   | .....G.....   | .....C.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Thysanoteuthis#44    | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Sthenoteuthis#32     | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Loligo_pealei#27     | .....T.....   | .....G.....   | .....T.....    | .....A.....      | .....A.....   | .....A..... | .....A..... | .....A..... |
| Sepioteuthis480483   | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Heteroteuthis#21     | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Idiosepius#17        | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Rossia#4             | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Sepia_officinalis#17 | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Sepia_opipara#13     | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Sepioloidea#32       | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Spirula#22           | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Stoloteuthis#13      | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Cirrothauma#14       | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Eledonella#6         | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Graneledone#36       | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Japatella#17         | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Octopus#27           | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Vampyroteuthis#44    | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Bathyteuthis#15      | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Brachioteuthis#4     | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Chtenopteryx#09      | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Cranchia#1           | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Cycloteuthis#61      | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Discoteuthis#5       | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Enoplateuthis#5      | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Gonatus_onyx#90      | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Histioteuthis#6      | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Ommastrephes#6       | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Psychroteuthis#14    | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Sthenoteuthis#21     | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Thysanoteuthis#31    | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Loligo_pealei#23     | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Sepioteuthis#13      | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Idiosepius#43        | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Rossia480483         | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Sepia_officinalis#5  | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Sepia_opipara#35     | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Sepioloidea#6        | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Spirula#30           | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Stoloteuthis#29      | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Argonauta#5          | .....A.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Bathypolypus#5       | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Cirrothauma#20       | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Eledonella#5         | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Graneledone#39       | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Japatella#16         | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Octopus#9            | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Stauroteuthis#22     | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Vampyroteuthis#21    | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Nautilus#1           | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Abralia#18           | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Brachioteuthis#6     | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Chiroteuthis#16      | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Chtenopteryx#3       | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Histioteuthis#3      | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Mastigoteuthis#22    | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Onychoteuthis#33     | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Idiosepius#06        | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Sepia_officinalis#41 | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Sepia_opipara#23     | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Spirula#40           | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |
| Vampyroteuthis#13    | .....C.....   | .....G.....   | .....T.....    | .....G.T.....    | .....A.....   | .....A..... | .....A..... | .....A..... |

|                      | 570           | 580         | 590          | 600            | 610               | 620                | 630           | 640          |
|----------------------|---------------|-------------|--------------|----------------|-------------------|--------------------|---------------|--------------|
| Abralia#3            | AAACCACCTA    | CAACTCCATC  | ATGAAATGGC   | ATGTCGATAT     | CAGGAAAGAC        | TTGTACGCCA         | ACACTGTGCT    | GTCCGGAGGT   |
| Alluroteuthis#6      | .....T..      | ...T.....   | .....G....   | .....T.C..     | .....T.C..        | .....GT..          | .....AA..     | ...A.T..     |
| Bathyteuthis#1       | .G.....       | .....       | .....        | .....C..       | .....T.C..        | .....T..           | .....         | T.....       |
| Chiroteuthis#12      | .....         | .....       | .....G....   | .....T.C..     | .....T.C..        | .....              | .....         | T.....       |
| Ctenopteryx#41       | .G.....       | .....       | .....        | .....C..       | .....T.C..        | .....T.C..         | .....T..      | .....        |
| Cranchia#2           | .....A..T     | .....       | .....        | .....T.C..     | .....T.C..        | .....T.C..         | .....AG..     | .....C..     |
| Cycloteuthis#29      | .....         | .....A..    | .....T..     | .....C..       | .....T.C..        | .....T.C..         | .....T..      | .....        |
| Discoteuthis#11      | .....         | .....       | .....        | .....C..       | .....T.C..        | .....T.C..         | .....         | T.....       |
| Enoploteuthis#7      | .....T..      | .....       | .....        | .....C..       | .....T.C..        | .....T.C..         | .....         | T.....       |
| Gonatopsis#11        | .....T..      | .....       | .....G....   | .....T.C..     | .....T.C..        | .....T.C..         | .....C..CT    | .....        |
| Gonatus_onyx#32      | .....T..      | .....       | .....G....   | .....T.C..     | .....T.C..        | .....T.C..         | .....C..CT    | .....        |
| Histioteuthis#49     | .....T..      | .....       | .....G....   | .....T.C..     | .....T.C..        | .....T.C..         | .....         | T.....       |
| Lepidoteuthis#27     | .....         | .....       | .....        | .....C..       | .....T.C..        | .....T.C..         | .....T..      | .....        |
| Liocranchia#6        | .G...A..T     | .....       | .....        | .....T.C..     | .....T.C..        | .....T.C..         | .....T..T     | .....G..T..  |
| Mastigoteuthis480483 | .....         | .....       | .....        | .....T.C..     | .....A..T.C..     | .....T.C..         | .....T..      | .....        |
| Octopoteuthis#5      | .....T..      | .....       | .....        | .....C..       | .....T.C..        | .....T.C..         | .....         | T.....       |
| Ommastrephes#49      | .....T..      | .....       | .....        | .....T.C..     | .....T.C..        | .....T.C..         | .....A..CT    | .....        |
| Onychoteuthis#26     | .....T..      | .....       | .....        | .....T.C..     | .....T.C..        | .....T.C..         | .....A..      | .....        |
| Pholidoteuthis#21    | .....T..      | .....       | .....        | .....C..       | .....T.C..        | .....T.C..         | .....T..      | .....        |
| Pyroteuthis#4        | .....T..      | .....       | .....G....   | .....C..       | .....T.C..        | .....T.C..         | .....CT       | .....        |
| Thysanoteuthis#44    | .....         | .....       | .....        | .....C..       | .....T.C..        | .....T.C..         | .....         | .....        |
| Sthenoteuthis#32     | .....A..      | .....T..    | .....        | .....T.C..     | .....T.C..        | .....T.C..         | .....C..AT    | .....        |
| Loligo_pealei#27     | .G.....       | .....       | .....        | .....C..       | .....T.C..        | .....T.C..         | .....T..T     | .....T..     |
| Sepioteuthis480483   | .G.....       | G.....      | .....        | .....C..       | .....T.C..        | .....T.C..         | .....T..T     | .....        |
| Heteroteuthis#21     | .....         | .....       | .....        | .....C..       | .....T.C..        | .....T.C..         | .....T..T     | .....        |
| Idiosepius#17        | .G..A...      | .....       | .....        | .....C..       | .....T.C..        | .....T.C..         | .....         | .....        |
| Rossia#4             | .G.....       | .....       | .....T..     | .....C..       | .....T.C..        | .....T.C..         | .....         | .....        |
| Sepia_officinalis#17 | .G.....       | .....A..    | .....        | .....T.C..     | .....A..T.C..     | .....T.C..         | .....         | .....        |
| Sepia_opipara#13     | .G..A..A..    | .....       | .....        | .....C..       | .....T.C..        | .....T.C..         | .....T..T     | .....        |
| Sepioloidea#32       | .G..A..A..    | .....       | .....        | .....C..       | .....T.C..        | .....T.C..         | .....T..T     | .....        |
| Spirula#22           | .G.....       | .....       | .....        | .....A..C..    | .....T.C..        | .....T.C..         | .....T..T     | .....        |
| Stoloteuthis#13      | .G.....       | .....       | .....        | .....C..       | .....T.C..        | .....T.C..         | .....         | .....        |
| Cirrochauma#14       | .....A..      | .....       | .....G....   | .....T..       | .....T.C..        | .....T.C..         | .....         | CT.....      |
| Eledonella#6         | .....A..      | T.....      | .....        | .....T..       | .....T.C..        | .....T.C..         | .....         | CT.....T..   |
| Granelledone#36      | .G..A..A..    | .....       | .....        | .....T..       | .....T.C..        | .....T.C..         | .....         | CT.....      |
| Japatella#17         | .....A..      | T.....      | .....        | .....T..       | .....T.C..        | .....T.C..         | .....         | CT.....T..   |
| Octopus#27           | .G..A..A..    | .....       | .....        | .....T..       | .....T.C..        | .....T.C..         | .....         | CT.....T..   |
| Vampyroteuthis#44    | .....A..      | .....       | .....G..T..  | .....T.C..     | .....T.C..        | .....T.C..         | .....         | AT.....      |
| Bathyteuthis#15      | .G..A..T..    | .....T..    | .....        | .....C..       | .....T.C..        | .....T.C..         | .....A..      | .....        |
| Brachioteuthis#4     | .G..A..A..    | .....       | .....T..     | .....          | .....T.C..        | .....T.C..         | .....A..A..   | .....        |
| Ctenopteryx#09       | .G..A..A..    | .....T..    | .....G....   | .....          | .....T.C..        | .....T.C..         | .....C..      | T.....       |
| Cranchia#1           | .G..A..T..    | .....       | .....G..T..  | .....C..       | .....T.C..        | .....T.C..         | .....A..A..   | T.....       |
| Cycloteuthis#61      | .G.....       | .....T..A.. | .....G..T..  | .....G..C..    | .....G..T.C..     | .....T.C..         | .....A..      | T.....C      |
| Discoteuthis#5       | .G.....       | .....T..A.. | .....G..T..  | .....G..       | .....T.C..        | .....T.C..         | .....         | T.....       |
| Enoploteuthis#5      | .....T..      | .....T..    | .....G..T..  | .....C..       | .....T.C..        | .....T.C..         | .....C..AT    | T.....       |
| Gonatus_onyx#90      | .G.....       | .....T..    | .....G..T..  | .....C..       | .....T.C..        | .....T.C..         | .....C..A..   | T.....       |
| Histioteuthis#6      | .G.....       | .....T..    | .....G..T..  | .....T.C..     | .....T.C..        | .....T.C..         | .....T..A..   | T.....C      |
| Ommastrephes#6       | .G.....       | .....T..    | .....G..T..  | .....T.C..     | .....T.C..        | .....T.C..         | .....T..A..AT | T.....       |
| Psychroteuthis#14    | .G.....       | .....T..    | .....G..T..  | .....T.C..     | .....T.C..        | .....T.C..         | .....C..A..   | T.....       |
| Sthenoteuthis#21     | .....T..      | .....T..    | .....G..T..  | .....T.C..     | .....T.C..        | .....T.C..         | .....T..A..AT | T.....       |
| Thysanoteuthis#31    | .G.....       | T..T..A..   | .....G..T..  | .....C..       | .....T.C..        | .....T.C..         | .....T..      | T.....       |
| Loligo_pealei#23     | .....T..      | .....T..    | .....G..T..  | .....C..       | .....T.C..        | .....T.C..         | .....A..CT    | .....        |
| Sepioteuthis#13      | .....T..      | .....T..    | .....G..T..  | .....C..       | .....T.C..        | .....T.C..         | .....A..CT    | .....        |
| Idiosepius#43        | .....T..      | .....T..    | .....G..T..  | .....G..       | .....T.C..        | .....T.C..         | .....AT       | T.....T..    |
| Rossia480483         | .G..G..T..    | .....T..    | .....G..T..  | .....C..       | .....A..T.C..     | .....T.C..         | .....         | T.....       |
| Sepia_officinalis#5  | .....A..T..   | .....T..T.. | .....G..T..  | .....C..       | .....T.C..        | .....T.C..         | .....T..A..   | A.....T..    |
| Sepia_opipara#35     | .....A..T..   | .....T..T.. | .....G..T..  | .....C..       | .....T.C..        | .....T.C..         | .....T..A..   | A.....T..    |
| Sepioloidea#6        | .....T..      | .....T..    | .....G..T..  | .....C..       | .....A..T.C..     | .....T.C..         | .....A..TT    | .....C..     |
| Spirula#30           | .G..AG..T..   | .....T..    | .....G..T..  | .....T.C..     | .....A..T.C..     | .....T.C..         | .....A..      | .....        |
| Stoloteuthis#29      | .G..T..T..    | .....T..    | .....G..T..  | .....T.C..     | .....A..T.C..     | .....T.C..         | .....C..T..   | T..T..       |
| Argonauta#5          | .....A..A..   | .....T..A.. | .....T..     | .....T..       | .....T.C..        | .....T.C..         | .....AT       | T..T..       |
| Bathypolypus#5       | .G.....       | .....A..    | .....        | .....T..       | .....T.C..        | .....T.C..         | .....         | CT.....      |
| Cirrochauma#20       | .....T..      | .....T..A.. | .....T..     | .....          | .....G..T.C..     | .....T.C..         | .....T..T..   | A.....G..T.. |
| Eledonella#5         | .....G..T..   | .....T..A.. | .....        | .....T..       | .....T.C..        | .....T.C..         | .....AT       | T..T..       |
| Granelledone#39      | .....A..TTC.. | .....A..    | .....G..TC.. | .....A..       | .....T..          | .....T.C..         | .....C..AT    | T..T..       |
| Japatella#16         | .....G..T..   | .....T..A.. | .....        | .....T..       | .....T.C..        | .....T.C..         | .....AT       | T..T..       |
| Octopus#9            | .....T..      | .....A..    | .....T..     | .....T..       | .....T.C..        | .....T.C..         | .....AT       | T..T..       |
| Stauroteuthis#22     | .....T..      | GT..A..     | .....G..T..  | .....          | .....T.C..        | .....TT..T..       | .....C..A..   | A..G..T..C   |
| Vampyroteuthis#21    | .....GT..T..  | .....T..T.. | .....G..T..  | .....A..       | .....T.C..        | .....C..A..TT..T.. | .....         | A.....C      |
| Nautilus#1           | .....G..      | .....G..A.. | .....G..     | .....A..C..    | .....T..G..       | .....G..           | .....C..T..   | A..T..G      |
| Abralia#18           | .....A..      | .....T..T.. | .....G..     | .....C..A..G.. | .....C..T..T..    | .....              | .....CA..     | C..C         |
| Brachioteuthis#6     | .....A..G..   | .....T..T.. | .....G..T..  | .....C..C..    | .....C..A..G..    | .....C..C..T..     | .....C..CT    | .....        |
| Chiroteuthis#16      | .G..G..A..    | .....G..    | .....G..     | .....C..C..    | .....C..A..G..    | .....C..C..        | .....C..CT    | .....C..     |
| Ctenopteryx#3        | .....A..G..   | T.....G..   | .....T..C..  | .....C..C..    | .....C..T..G..    | .....C..A..        | .....T..A..   | C..C         |
| Histioteuthis#3      | .G..A..G..    | .....T..    | .....G..T..  | .....C..C..    | .....C..A..G..    | .....C..           | .....C..C     | .....C..     |
| Mastigoteuthis#22    | .....A..A..   | GGT..G..    | .....G..T..  | .....C..C..    | .....C..T..G..    | .....C..C..        | .....C..C..   | T.....       |
| Onychoteuthis#33     | .....A..G..   | .....T..    | .....G..T..  | .....T..C..    | .....C..T..G..T.. | .....C..C..        | .....C..C..   | .....        |
| Idiosepius#06        | .....A..      | .....G..A.. | .....G..T..  | .....G..       | .....TC..C..G..   | .....C..A..        | .....C..C..   | C..T..C      |
| Sepia_officinalis#41 | .....A..G..   | .....A..    | .....T..C..  | .....C..C..    | .....TC..A..      | .....C..C..G..     | .....C..C..   | T.....       |
| Sepia_opipara#23     | .....A..A..   | .....G..G.. | .....T..C..  | .....C..C..    | .....AC..A..      | .....C..C..G..     | .....C..C..   | T.....       |
| Spirula#40           | .....A..G..   | .....G..    | .....G..T..  | .....C..C..    | .....AC..A..G..   | .....C..T..G..     | .....C..C..   | T.....       |
| Vampyroteuthis#13    | .....A..      | T.....AG..  | .....TG..    | .....T..       | .....TC..T..      | .....C..           | .....T..A..TT | A..T..T..A   |

|                      | 650         | 660             | 670              | 680           | 690                 | 700           | 710                   | 720               |
|----------------------|-------------|-----------------|------------------|---------------|---------------------|---------------|-----------------------|-------------------|
| Abralia#3            | ACCACCATGT  | TCCCGGTAT       | TGCTGACAGG       | ATGCAGAAGG    | AAATCACATC          | CCTGGCCCCC    | AGCACCATGA            | AGATCAAGAT        |
| Alluroteuthis#6      | .....       | .....           | .....            | .....         | .....C..            | .....T..      | .....                 | .....             |
| Bathyteuthis#1       | .....       | .....           | .....            | .....         | .....               | .....         | .....                 | .....T.....       |
| Chiroteuthis#12      | .....       | .....           | .....            | .....         | .....               | .....A        | .....                 | .....             |
| Chtenopteryx#41      | .....       | .....           | .....            | .....         | .....TC..           | .....         | .....T..              | .....G.....       |
| Cranchia#2           | .....       | .....T.....     | .....            | .....A.....   | .....G.TG..         | .....T.....   | .....                 | .....             |
| Cycloteuthis#29      | .....       | .....           | .....C.A         | .....         | .....               | .....T.....   | .....                 | .....             |
| Discoteuthis#11      | .....       | .....           | .....            | .....         | .....T.....         | .....         | .....                 | .....             |
| Enoploteuthis#7      | .....       | .....           | .....            | .....A.....   | .....               | .....T.....A  | .....                 | .....             |
| Gonatopsis#11        | .....       | .....           | .....            | .....A.....   | .....               | .....T.T.T..  | .....                 | .....             |
| Gonatus_onyx#32      | .....       | .....           | .....            | .....A.....   | .....               | .....T.T.T..  | .....                 | .....             |
| Histioteuthis#49     | .....       | .....           | .....            | .....A.....   | .....               | .....T.....   | .....                 | .....T.....       |
| Lepidoteuthis#27     | .....       | .....           | .....            | .....         | .....               | .....         | .....                 | .....             |
| Liocranchia#6        | .....       | .....T.....     | .....            | .....A.....   | .....G.T.....       | .....         | .....                 | .....             |
| Mastigoteuthis480483 | .....       | .....           | .....            | .....         | .....               | .....         | .....                 | .....             |
| Octopoteuthis#5      | .....       | .....           | .....            | .....         | .....               | .....         | .....                 | .....             |
| Ommastrephes#49      | .....       | .....           | .....T.....      | .....A.....   | .....               | .....T.T..... | .....                 | .....             |
| Onychoteuthis#26     | .....       | .....           | .....A.....      | .....A.A..... | .....               | .....T.T.T..  | .....                 | .....             |
| Pholidoteuthis#21    | .....       | .....           | .....            | .....         | .....               | .....T.....   | .....                 | .....             |
| Pyroteuthis#4        | .....       | .....           | .....            | .....A.....   | .....TG.....        | .....T.T..... | .....                 | .....             |
| Thysanoteuthis#44    | .....       | .....           | .....            | .....A.....   | .....               | .....T.....   | .....                 | .....             |
| Sthenoteuthis#32     | .....       | .....           | .....C.T.....    | .....A.A..... | .....               | .....         | .....A.....           | .....             |
| Loligo_pealei#27     | .....       | .....           | .....A.....      | .....A.....   | .....               | .....         | .....                 | .....T.....       |
| Sepioteuthis480483   | .....       | .....T.....     | .....T.....      | .....         | .....               | .....         | .....                 | .....             |
| Heteroteuthis#21     | .....       | .....           | .....            | .....A.....   | .....               | .....T.....   | .....                 | .....             |
| Idiosepius#17        | .....       | .....           | .....            | .....A.....   | .....A.G.....       | .....         | .....                 | .....T.....       |
| Rossia#4             | .....A..... | .....           | .....            | .....A.A..... | .....               | .....T.....   | .....                 | .....             |
| Sepia_officinalis#17 | .....       | .....T.A.....   | .....C.....      | .....         | .....               | .....TT.....  | .....CT.G.....        | .....A.A.....     |
| Sepia_opipara#13     | .....       | .....T.....     | .....            | .....         | .....               | .....         | .....A.....           | .....             |
| Sepioloidea#32       | .....       | .....           | .....            | .....A.....   | .....               | .....         | .....                 | .....             |
| Spirula#22           | .....A..... | .....T.....     | .....            | .....A.....   | .....               | .....         | .....                 | .....             |
| Stoloteuthis#13      | .....       | .....           | .....            | .....A.....   | .....               | .....T.....   | .....                 | .....             |
| Cirrothauma#14       | .....       | .....           | .....C.T.....    | .....A.....   | .....               | .....T.....   | .....                 | .....             |
| Eledonella#6         | .....       | .....           | .....T.A.....    | .....A.....   | .....               | .....T.T..... | .....                 | .....             |
| Graneledone#36       | .....       | .....           | .....T.A.....    | .....A.....   | .....               | .....T.T..... | .....                 | .....A.....       |
| Japatella#17         | .....       | .....           | .....T.A.....    | .....A.....   | .....               | .....T.T..... | .....                 | .....             |
| Octopus#27           | .....       | .....           | .....T.A.....    | .....A.A..... | .....               | .....T.T..... | .....                 | .....A.....       |
| Vampyroteuthis#44    | .....       | .....           | .....C.T.....    | .....A.....   | .....               | .....T.T..... | .....                 | .....             |
| Bathyteuthis#15      | T.....      | .....A.....     | .....C.T.....    | .....         | .....T.TG..         | TT.....       | .....A.GC.....        | .....A.....       |
| Brachiotheuthis#4    | T.....      | .....A.....     | .....TC.T.....   | .....         | .....G..T.TG.       | TT.....       | .....A.GC.....        | .....A.....       |
| Chtenopteryx#09      | T.....      | .....A.C.....   | .....TC.T.....   | .....         | .....G..T.TG.       | TT.....       | .....A.GC.....        | .....A.....       |
| Cranchia#1           | T.....      | .....A.....     | .....TC.T.....   | .....         | .....G..T.TG.       | TT.....       | .....A.GC.....        | .....A.....       |
| Cycloteuthis#61      | T.....      | .....A.....     | .....TC.T.....   | .....         | .....G..T.TG.       | TT.....       | .....A.GC.....        | .....A.....       |
| Discoteuthis#5       | T.....      | .....A.....     | .....TC.T.....   | .....         | .....G..T.TG.       | TT.....       | .....A.GC.....        | .....A.....       |
| Enoploteuthis#5      | T.A.....    | .....A.....     | .....TC.T.....   | .....         | .....G..T.TG.       | TT.....       | .....A.GC.....        | .....A.....       |
| Gonatus_onyx#90      | T.....      | .....A.....     | .....TC.T.....   | .....         | .....G..T.TG.       | TT.....       | .....A.GC.....        | .....A.....       |
| Histioteuthis#6      | T.....      | .....A.....     | .....TC.T.....   | .....         | .....G..T.TG.       | TT.....       | .....A.GC.....        | .....A.....       |
| Ommastrephes#6       | T.....      | .....A.....     | .....C.T.....    | .....         | .....G..T.TG.       | TT.....       | .....T.A.GC.....      | .....A.....       |
| Psychroteuthis#14    | T.....      | .....A.C.....   | .....TC.T.....   | .....         | .....G..AT.C..      | TT.....       | .....A.GC.....        | .....A.....       |
| Sthenoteuthis#21     | T.....      | .....A.....     | .....C.T.....    | .....         | .....G..T.TG.       | TT.....       | .....T.A.GC.....      | .....A.....       |
| Thysanoteuthis#31    | T.....      | .....A.....     | .....C.T.....    | .....         | .....G..T.TG.       | TT.....       | .....A.GC.....        | .....A.....       |
| Loligo_pealei#23     | T..T.....   | .....A.....     | .....C.T.....    | .....         | .....G..T.TG.       | TT.....       | .....A.A.GC.....      | .....A.T.....     |
| Sepioteuthis#13      | T..T.....   | .....A.....     | .....C.T.....    | .....         | .....T.G.....       | TT.....       | .....A.A.GC.....      | .....A.T.....     |
| Idiosepius#43        | T..T.....   | .....T.....     | .....TC.T.....   | .....         | .....G..T.TG.       | TT.....       | .....T.G.GC.....      | .....A.....       |
| Rossia480483         | .....       | .....T.A.....   | .....TC.T.....   | .....         | .....G..T.TG.       | TT.....       | .....T.A.GC.....      | .....A.T.....     |
| Sepia_officinalis#5  | T.A..T..... | .....A.....     | .....C.T.....    | .....         | .....G..T.TG.       | TT.....       | .....A.GC.....        | .....A.T.....     |
| Sepia_opipara#35     | T.A..T..... | .....A.....     | .....TC.T.....   | .....         | .....G..T.TG.       | TT.....       | .....A.GC.....        | .....A.T.....     |
| Sepioloidea#6        | T.A.....    | .....A.....     | .....TC.T.....   | .....A.....   | .....G..T.TG.       | TT.....       | .....A.GC..G.....     | .....A.T.....     |
| Spirula#30           | T.A.....    | .....A.....     | .....C.T.....    | .....         | .....G..T.TG.       | T.....        | .....A.GC.....        | .....A.T.....     |
| Stoloteuthis#29      | T.T.T.....  | .....A.....     | .....C.T.....    | .....         | .....G..T.TG.       | TT.....       | .....A.A.GC.....      | .....A.T.....     |
| Argonauta#5          | T..A.....   | .....A.A.....   | .....TC.T.....   | .....A.....   | .....G.TG.....      | TT.....       | .....T.A.A.CCT.....   | .....T.....       |
| Bathypolypus#5       | .....       | .....           | .....T.A.....    | .....A.A..... | .....T.....         | TT.....       | .....T.T.....         | .....A.....       |
| Cirrothauma#20       | T..T.....   | .....           | .....C.C.....    | .....A.....   | .....T.GT.....      | TT.....       | .....T.T.A.CCA.....   | .....T.....       |
| Eledonella#5         | T..A.....   | .....A.A.....   | .....C.T.....    | .....         | .....GTG.....       | TT.....       | .....T.A.A.CCT.....   | .....T.A.....     |
| Graneledone#39       | G..A.....   | .....AT.A.....  | .....A.C.T.....  | .....         | .....GTG.....       | TT.....       | .....A.A.G.CCT.A..... | .....T.....       |
| Japatella#16         | T..A.....   | .....A.A.....   | .....C.T.....    | .....         | .....GTG.....       | TT.....       | .....T.A.A.CCT.....   | .....T.A.....     |
| Octopus#9            | T..A.....   | .....A.A.....   | .....C.T.....    | .....         | .....GCC.....       | TT.....       | .....T.A.A.CCT.T..... | .....T.....       |
| Stauroteuthis#22     | T..T.....   | .....A.....     | .....CAC.....    | .....         | .....G.T.GT.....    | TT.T.A.....   | .....CCA.T.....       | .....GT.....      |
| Vampyroteuthis#21    | T..T.....   | .....C.....     | .....C.T.....    | .....A.G..... | .....T.GTCA.....    | AT.....       | .....T.A.GA.....      | .....G.....       |
| Nautilus#1           | T.A..A..... | .....A.....     | .....C.A.....    | .....A.....   | .....G.TT.CG.....   | T.C.T.....    | .....GC.....          | .....             |
| Abralia#18           | .....       | .....A.A.....   | .....C.A.....    | .....A.....   | .....GG.TT.AG.....  | TT.T.....     | .....AG.....          | .....A.....G..... |
| Brachiotheuthis#6    | .....       | .....A.G.....   | .....C.....      | .....A.....   | .....GG.TT.GG.....  | TT.C.....     | .....AG.G.....        | .....G.....       |
| Chiroteuthis#16      | CA.....     | .....A.G.....   | .....A.....      | .....A.....   | .....G.TT.GG.....   | TAC.....      | .....A.AGG.G.....     | .....A.....G..... |
| Chtenopteryx#3       | .....       | .....AT.G.....  | .....A.....      | .....A.....   | .....G.TT.TG.....   | AT.....       | .....AG.A.....        | .....A.....G..... |
| Histioteuthis#3      | .....       | .....A.G.C..... | .....A.....      | .....A.....   | .....GG.TT.TGG..... | T.A.....      | .....AG.....          | .....G.....       |
| Mastigoteuthis#22    | .....       | .....A.G.A..... | .....A.....      | .....A.....   | .....GG.TT.GG.....  | TT.C.....     | .....AG.....          | .....G.....       |
| Onychoteuthis#33     | .....       | .....T.....     | .....A.....      | .....A.....   | .....GG.TT.TGG..... | AT.C.....     | .....AG.....          | .....G.....       |
| Idiosepius#06        | .....       | .....G.....     | .....AT.G.A..... | .....A.....   | .....GG.TT.TAG..... | T.T.A.....    | .....AG.G.....        | .....T.AG.....    |
| Sepia_officinalis#41 | .....       | .....A.....     | .....A.G.....    | .....T.A..... | .....A.A.....       | GG.TT.GG..... | .....T.T.....         | .....AG.A.....    |
| Sepia_opipara#23     | .....       | .....A.....     | .....A.....      | .....A.A..... | .....GG.TT.GG.....  | T.T.....      | .....AG.A.....        | .....T.G.....     |
| Spirula#40           | .....       | .....A.....     | .....G.....      | .....A.....   | .....GG.TT.GG.....  | T.C.....      | .....CG.A.....        | .....G.A.C.....   |
| Vampyroteuthis#13    | .....       | .....T.....     | .....C.C.....    | .....A.....   | .....GG.TT.CA.....  | .....A.A..... | .....AAT.A.....       | .....A.....       |

|                       | 730           | 740                     | 750             | 760           | 770             | 780                  | 784    |
|-----------------------|---------------|-------------------------|-----------------|---------------|-----------------|----------------------|--------|
| Abrolia#3             | CATTGCTCCC    | CCTGAGCGTA              | AATACTCCGT      | CTGGATGGT     | GGCTCCATCC      | TGGCTTCOCT           | CTCC   |
| Alluroteuthis#6       | ...C...T      | ...C...                 | ...             | A...          | ...             | ...T...              | ...    |
| Bathyteuthis#1        | ...C...       | ...C...                 | ...             | ...           | ...             | ...T...              | G...   |
| Chiroteuthis#12       | ...           | ...                     | ...             | ...           | ...             | ...T...              | ...    |
| Chtenopteryx#41       | ...C...       | ...A...                 | ...T...         | ...           | ...             | ...T...              | ...    |
| Cranchia#2            | ...           | ...C...C...             | ...             | ...           | ...             | ...T...              | G...T  |
| Cycloteuthis#29       | T...          | ...C...                 | ...             | ...           | ...             | ...T...              | ...    |
| Discoteuthis#11       | ...           | ...T...C...             | ...             | ...           | ...             | ...T...              | ...    |
| Enoplateuthis#7       | ...           | ...                     | ...             | ...           | ...             | ...T...              | ...    |
| Gonatopsis#11         | ...           | ...A...A...             | ...             | ...           | ...             | ...T...              | G...   |
| Gonatus_onyx#32       | ...           | ...T...A...A...         | ...             | ...           | ...             | ...T...              | G...   |
| Histioteuthis#49      | ...           | ...A...A...             | ...A...         | ...           | ...             | ...T...              | ...    |
| Lepidoteuthis#27      | ...           | ...C...                 | ...             | ...           | ...             | ...T...              | G...   |
| Liocranchia#6         | ...           | ...                     | ...             | ...           | ...             | ...T...              | G...T  |
| Mastigoteuthis#480483 | ...           | ...???                  | ...??????????   | ...?????????? | ...??????????   | ...??????????        | ...??? |
| Octopoteuthis#5       | ...           | ...T...C...             | ...             | ...           | ...             | ...T...              | ...    |
| Ommastrephes#49       | ...           | ...A...A...             | ...             | ...           | ...             | ...T...              | ...    |
| Onychoteuthis#26      | ...           | ...C...                 | ...             | ...A...       | ...             | ...T...              | G...   |
| Pholidoteuthis#21     | ...           | ...C...                 | ...             | ...           | ...             | ...T...              | G...   |
| Pyroteuthis#4         | ...           | ...T...C...             | ...             | ...           | ...             | ...T...              | ...    |
| Thysanoteuthis#44     | ...           | ...C...                 | ...             | ...T...       | ...             | ...T...              | ...    |
| Sthenoteuthis#32      | ...           | ...C...C...             | ...             | ...           | ...             | ...T...              | ...    |
| Loligo_pealei#27      | ...           | ...C...                 | ...             | ...           | ...             | ...T...              | G...   |
| Sepioteuthis#480483   | ...           | ...C...???              | ...??????????   | ...?????????? | ...??????????   | ...??????????        | ...??? |
| Heteroteuthis#21      | ...           | ...C...                 | ...T...         | ...           | ...             | ...T...              | G...T  |
| Idiosepius#17         | ...           | ...C...                 | ...             | ...           | ...             | ...T...              | ...    |
| Rossia#4              | ...           | ...C...T                | ...T...         | ...T...C      | ...T...         | ...T...              | G...   |
| Sepia_officinalis#17  | ...           | ...C...                 | ...T...         | ...           | ...             | ...T...              | G...   |
| Sepia_opipara#13      | ...           | ...C...C...             | ...             | ...           | ...             | ...T...              | G...   |
| Sepioloidea#32        | ...           | ...G...                 | ...             | ...C...       | ...             | ...T...              | G...   |
| Spirula#22            | ...           | ...C...T                | ...C...         | ...           | ...             | ...T...              | G...   |
| Stoloteuthis#13       | ...           | ...C...                 | ...             | ...T...       | ...             | ...T...              | G...   |
| Cirrothauma#14        | T...          | ...A...C...             | ...             | ...T...       | ...             | ...TT...             | T      |
| Eledonella#6          | T...          | ...A...A...             | ...             | ...           | ...             | ...T...              | ...    |
| Graneledone#36        | T...          | ...T...A...             | ...             | ...           | ...             | ...T...              | ...    |
| Japatella#17          | T...          | ...C...A...A...         | ...             | ...           | ...             | ...T...              | ...    |
| Octopus#27            | T...          | ...A...A...             | ...             | ...           | ...             | ...T...              | ...    |
| Vampyroteuthis#44     | ...           | ...C...C...             | ...T...A...     | ...T...       | ...             | ...T...              | ...    |
| Bathyteuthis#15       | ...           | ...A...A...A...G...     | ...T...A...     | ...T...       | ...T...T...     | ...T...A...          | T...   |
| Brachioleuthis#4      | ...           | ...C...A...A...A...G... | ...T...A...     | ...T...       | ...T...T...     | ...T...A...          | T...   |
| Chtenopteryx#09       | ...           | ...A...A...A...A...G... | ...T...A...     | ...T...       | ...T...T...T... | ...T...A...          | T...   |
| Cranchia#1            | ...           | ...A...A...A...A...G... | ...T...A...     | ...T...       | ...T...T...T... | ...T...A...          | T...   |
| Cycloteuthis#61       | ...           | ...A...A...A...A...G... | ...T...A...     | ...T...       | ...T...T...T... | ...T...A...          | T...   |
| Discoteuthis#5        | ...           | ...A...C...A...G...     | ...T...A...     | ...T...       | ...T...T...T... | ...T...A...          | T...   |
| Enoplateuthis#5       | ...           | ...A...A...A...G...     | ...T...A...     | ...T...       | ...T...T...T... | ...T...A...          | T...   |
| Gonatus_onyx#90       | ...           | ...A...A...A...G...     | ...T...A...     | ...T...       | ...T...T...T... | ...T...A...          | T...   |
| Histioteuthis#6       | ...           | ...A...A...A...G...     | ...T...A...     | ...T...       | ...T...T...T... | ...T...A...          | T...   |
| Ommastrephes#6        | ...           | ...A...A...A...G...     | ...T...A...     | ...T...       | ...T...T...T... | ...T...A...          | T...   |
| Psychroteuthis#14     | A...C...T     | ...A...A...G...         | ...T...A...     | ...T...       | ...T...T...T... | ...T...A...          | T...   |
| Sthenoteuthis#21      | ...           | ...A...A...G...         | ...T...A...     | ...T...       | ...T...T...T... | ...T...A...          | T...   |
| Thysanoteuthis#31     | ...           | ...A...A...G...         | ...T...A...     | ...T...       | ...T...T...T... | ...T...A...          | T...   |
| Loligo_pealei#23      | ...           | ...C...A...AA...G...    | ...T...A...     | ...T...       | ...T...T...T... | ...T...A...          | T...   |
| Sepioteuthis#13       | ...           | ...A...A...A...G...     | ...T...A...     | ...T...       | ...T...T...T... | ...T...A...          | T...   |
| Idiosepius#43         | T...C...A     | ...C...A...G...         | ...T...A...     | ...T...       | ...T...T...T... | ...T...A...          | T...   |
| Rossia480483          | ...           | ...???                  | ...??????????   | ...?????????? | ...??????????   | ...??????????        | ...??? |
| Sepia_officinalis#5   | ...           | ...TA...A...A...G...    | ...T...A...     | ...T...       | ...T...T...T... | ...T...A...          | T...   |
| Sepia_opipara#35      | ...           | ...A...A...A...G...     | ...T...A...     | ...T...       | ...T...T...T... | ...T...A...          | T...   |
| Sepioloidea#6         | ...           | ...A...A...A...G...     | ...T...A...     | ...T...       | ...T...T...T... | ...T...A...          | T...   |
| Spirula#30            | ...           | ...A...A...A...G...     | ...T...A...     | ...T...       | ...T...T...T... | ...T...A...          | T...   |
| Stoloteuthis#29       | ...           | ...A...A...AA...G...    | ...T...A...     | ...T...       | ...T...T...T... | ...T...A...          | T...   |
| Argonauta#5           | ...           | ...A...A...A...C...     | ...T...A...     | ...T...       | ...T...T...T... | ...T...A...TT...     | G...T  |
| Bathypolypus#5        | T...          | ...A...                 | ...             | ...           | ...             | ...T...              | ...    |
| Cirrothauma#20        | A...C...C...A | ...A...A...             | ...T...A...     | ...T...       | ...T...T...T... | ...T...A...          | G...A  |
| Eledonella#5          | ...           | ...A...A...             | ...A...A...     | ...T...       | ...T...T...T... | ...T...A...          | T...   |
| Graneledone#39        | ...           | ...TA...A...A...T...    | ...A...G...     | ...T...       | ...T...T...T... | ...T...A...          | G...   |
| Japatella#16          | ...           | ...A...A...             | ...A...A...     | ...T...       | ...T...T...T... | ...T...A...          | T...   |
| Octopus#9             | ...           | ...A...                 | ...T...A...     | ...T...G...   | ...G...         | ...T...              | G...   |
| Stauroteuthis#22      | A...C...C...A | ...A...A...C...         | ...T...A...     | ...T...       | ...T...T...T... | ...T...A...          | G...   |
| Vampyroteuthis#21     | T...C...A     | ...A...A...C...         | ...T...A...     | ...T...       | ...T...T...T... | ...T...A...T...      | G...T  |
| Nautilus#1            | ...           | ...T...C...             | ...T...G...     | ...           | ...             | ...T...A...          | G...   |
| Abrolia#18            | G...C...C...A | ...G...AA...A...        | ...T...         | ...           | ...A...TT...    | ...A...A...          | ...    |
| Brachioleuthis#6      | G...C...C...A | ...C...A...G...         | ...T...         | ...           | ...A...TT...    | ...A...G...          | ...    |
| Chiroteuthis#16       | G...C...G...A | ...G...AA...G...        | ...             | ...           | ...A...TT...    | ...A...G...          | T...   |
| Chtenopteryx#3        | G...A...G...C | ...AA...A...            | ...T...         | ...           | ...A...TT...    | ...A...G...T...      | T...   |
| Histioteuthis#3       | G...A...A...G | ...AA...A...            | ...             | ...           | ...A...TT...    | ...A...A...A...T...  | T...   |
| Mastigoteuthis#22     | G...G...G...A | ...G...AA...G...        | ...T...C...     | ...A...       | ...T...A...     | ...A...G...          | G...   |
| Onychoteuthis#33      | G...C...C...A | ...A...AA...A...        | ...A...         | ...           | ...A...TT...    | ...A...A...G...      | G...   |
| Idiosepius#06         | ...           | ...C...A...AA...G...    | ...T...         | ...A...       | ...G...T...     | ...C...T...G...A     | ...    |
| Sepia_officinalis#41  | G...G...G...A | ...G...A...A...         | ...G...T...T... | ...T...C...   | ...T...A...T... | ...A...G...G...T...  | T...   |
| Sepia_opipara#23      | G...A...A...G | ...A...A...             | ...T...T...     | ...T...       | ...G...TT...    | ...A...G...T...T...A | ...    |
| Spirula#40            | A...G...A...G | ...A...                 | ...T...T...     | ...T...       | ...A...TT...    | ...A...G...T...T...  | ...    |
| Vampyroteuthis#13     | A...C...A...A | ...A...                 | ...A...         | ...T...A...   | ...T...TT...    | ...A...A...T...G...A | ...    |

Figure 14. Strict consensus of 360 equally parsimonious trees generated by a heuristic search (10 random addition replicates) of the unweighted actin data set (TL=3076; CI=0.239; RI=0.658). The arbitrarily designated actin isoforms I, II, and III referred to throughout this study are indicated above the bases of the 3 major clades.

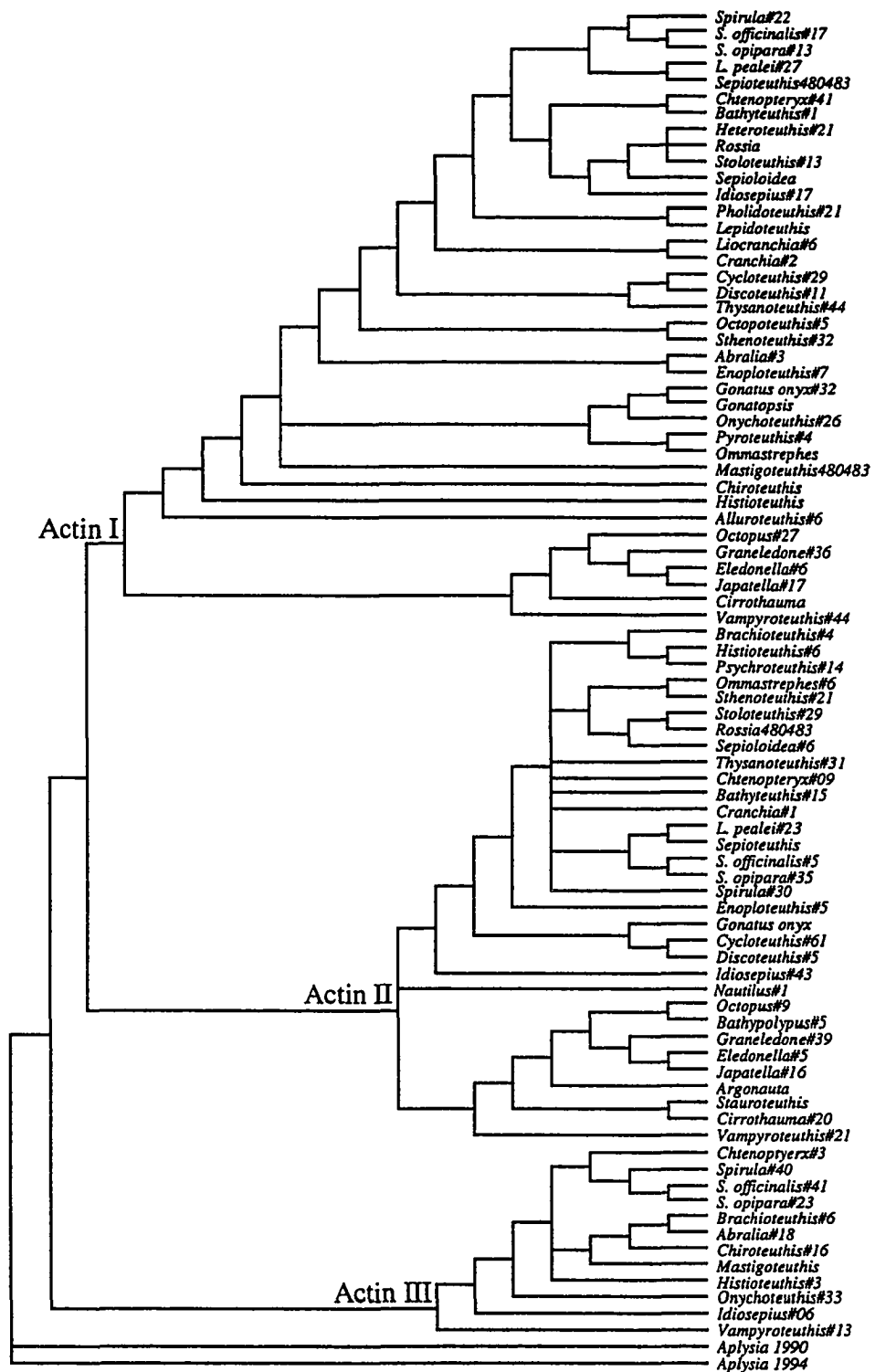


Figure 15. Results from intraspecific comparisons of the paralogous Actin I and Actin II genes for the 26 taxa in the restricted data set. The uncorrected nucleotide sequence divergence between the aligned Actin I and Actin II genes was calculated for each taxon. The mean ( $\pm$  standard deviation) uncorrected divergence between the two actin isoforms for the Octopodiformes, 21.11 $\pm$ 1.48%, was significantly greater than the mean divergence for the Decapodiformes, 18.02 $\pm$ 1.33% (single class ANOVA,  $F_s = 23.90$ ,  $p < 0.01$ ).

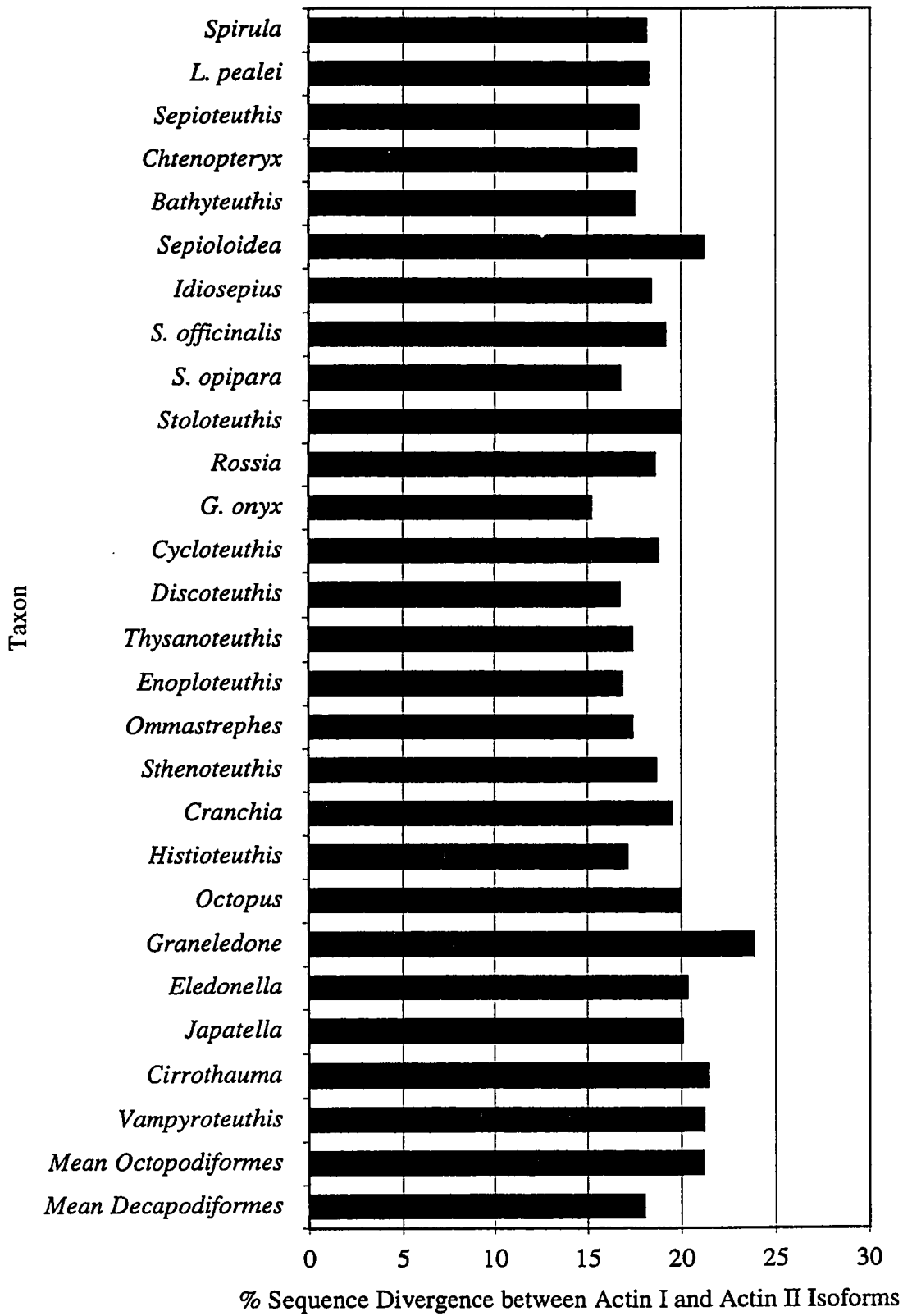




Figure 16. Neighbor-joining tree produced from the analysis of the deduced amino acid sequences of the Actin I, II, and III genes of *Chtenopteryx*, *Sepia opipara*, and *Vampyroteuthis* along with the actin genes from a diverse array of metazoan taxa. The GenBank Accession numbers and references from which the sequences were obtained are given in Table 5. Thick lines indicate clades supported by the strict consensus tree derived from parsimony analysis of the same data set. Bootstrap proportions (100 replicates) are indicated as percentages below nodes.

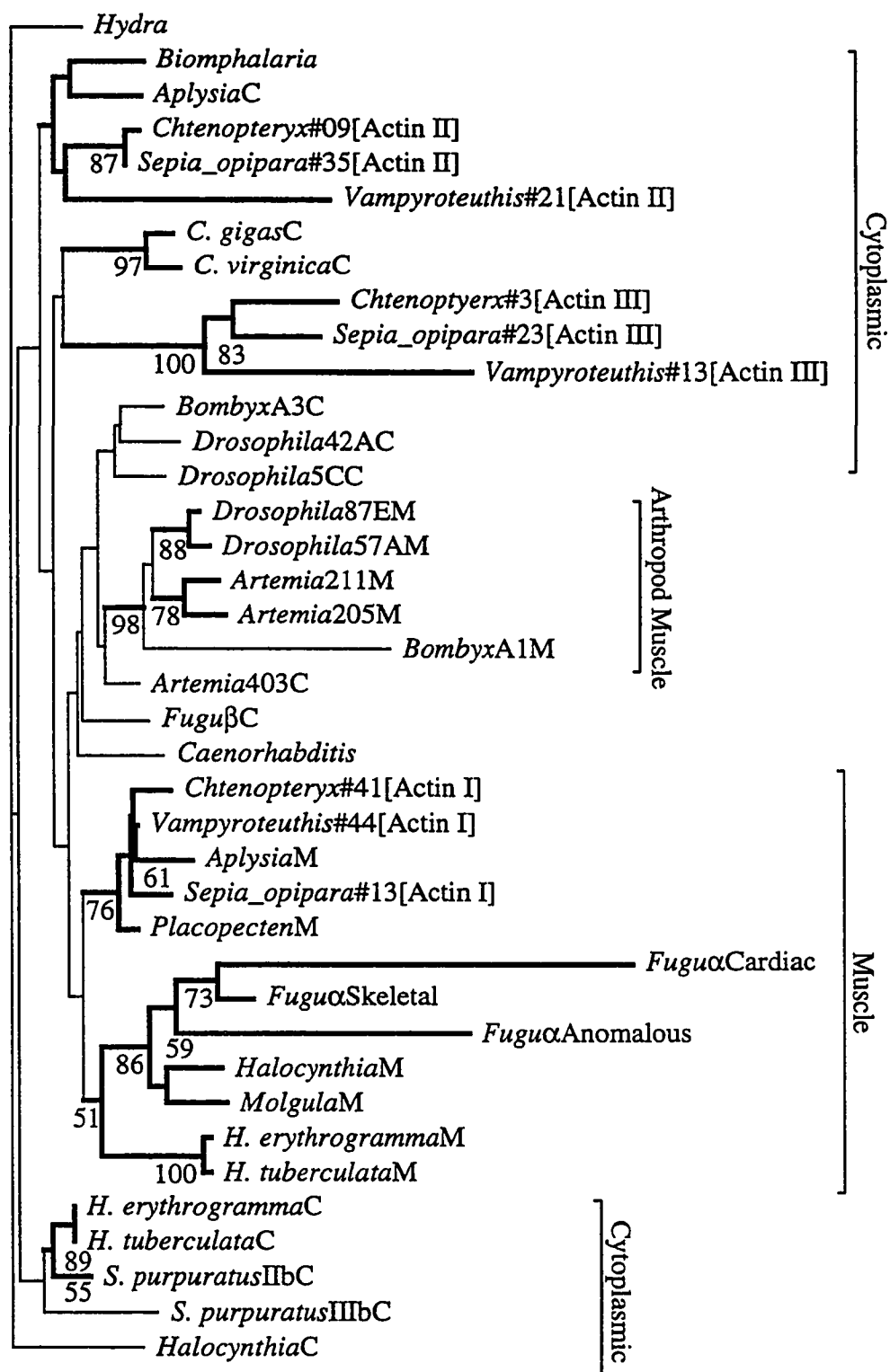


Figure 17. A comparison of cephalopod actin amino acid sequences with the amino acids of human  $\beta$  cytoplasmic (Nakajima-Iijima et al., 1985, GenBank Accession Number: M10277) and  $\alpha$  skeletal actins (Hanauer et al., GenBank Accession Number: J00068) and the cytoplasmic and muscle actins derived from *Drosophila* and *Aplysia* cDNA libraries. Only the positions that differ between the human cytoplasmic and skeletal muscle actin proteins are considered. The positions indicated above the sequences are relative to the cephalopod sequences, with position 14 corresponding to position 103 in the mature actin protein sequence in humans. For the *Drosophila*, *Aplysia*, and cephalopod actin sequences, only the residues that differ from the human cytoplasmic actin are indicated. Conservative or identical amino acid substitutions with the  $\alpha$  skeletal actin in humans are given in boldface (np = nonpolar amino acid; p = polar amino acid). The cephalopod Actin I isoform shares five residues with the human  $\alpha$  skeletal actin sequence, while the Actin II and Actin III isoforms share two residues with the  $\alpha$  skeletal actin sequence. Two of the five Actin I boldfaced residues involve conservative changes: 1) position 40 – alanine (cephalopod Actin I) and valine (human  $\alpha$  skeletal) are both nonpolar (hydrophobic) amino acids; 2) position 171 – serine (cephalopod Actin I) and threonine (human  $\alpha$  skeletal) are both polar (hydrophilic) uncharged amino acids. The actin III amino acid substitution at position 87 is nonconservative with respect to both the  $\beta$  and  $\alpha$  human actins. The *Aplysia* muscle actin is almost identical to the cephalopod Actin I sequence (one nonconservative substitution: Val->Tyr) for the 14 diagnostic residues in the region of actin sequences analyzed in this study. See Table 5 for GenBank Accession numbers and references for the *Drosophila* and *Aplysia* actin genes.

| <u>Amino Acid Position</u>                  | 14                | 40               | 64               | 73               | 87               | 112              | 136              | 171              | 178              | 183              | 190              | 198              | 208              | 210              |
|---|-------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| <u>Human <math>\beta</math> Cytoplasmic</u> | <sup>n</sup> PVal | <sup>p</sup> Thr | <sup>n</sup> Met | <sup>p</sup> Thr | <sup>n</sup> Leu | <sup>p</sup> Thr | <sup>p</sup> Gln | <sup>n</sup> Ala | <sup>n</sup> Leu | <sup>p</sup> Cys | <sup>n</sup> Phe | <sup>n</sup> Val | <sup>p</sup> Thr | <sup>n</sup> Leu |
| <u>Human <math>\alpha</math> Skeletal</u>   | <sup>p</sup> Thr  | <sup>n</sup> Val | <sup>n</sup> Leu | <sup>p</sup> Asn | <sup>n</sup> Met | <sup>n</sup> Val | <sup>p</sup> Asn | <sup>p</sup> Thr | <sup>n</sup> Ile | <sup>n</sup> Ala | <sup>p</sup> Tyr | <sup>n</sup> Ile | <sup>p</sup> Asn | <sup>n</sup> Met |
| <i>Drosophila</i> Cytoplasmic               |                   |                  | <sup>n</sup> Leu |                  |                  |                  |                  |                  |                  |                  | <sup>p</sup> Tyr |                  |                  |                  |
| <u><i>Drosophila</i> Muscle</u>             | <sup>p</sup> Ser  | <sup>n</sup> Leu |                  |                  |                  |                  |                  | <sup>p</sup> Ser |                  |                  | <sup>p</sup> Tyr | <sup>n</sup> Ile |                  | <sup>n</sup> Met |
| <i>Aplysia</i> Cytoplasmic                  |                   | <sup>n</sup> Leu |                  |                  | <sup>n</sup> Met |                  |                  |                  |                  | <sup>n</sup> Ala |                  |                  |                  |                  |
| <u><i>Aplysia</i> Muscle</u>                |                   | <sup>n</sup> Ala | <sup>n</sup> Leu |                  |                  |                  |                  | <sup>p</sup> Ser |                  | <sup>n</sup> Ala | <sup>n</sup> Val |                  |                  |                  |
| Cephalopod Actin I                          |                   | <sup>n</sup> Ala | <sup>n</sup> Leu |                  |                  |                  |                  | <sup>p</sup> Ser |                  | <sup>n</sup> Ala | <sup>p</sup> Tyr |                  |                  |                  |
| Cephalopod Actin II                         |                   |                  |                  |                  |                  |                  |                  |                  |                  | <sup>n</sup> Ala | <sup>p</sup> Tyr |                  |                  |                  |
| <u>Cephalopod Actin III</u>                 |                   |                  | <sup>n</sup> Leu |                  | <sup>p</sup> Gln |                  |                  |                  |                  |                  | <sup>p</sup> Tyr |                  |                  |                  |

TABLE 6. ACTIN I UNCORRECTED NUCLEOTIDE SEQUENCE DIVERGENCES FOR PAIRWISE COMPARISONS WITHIN AND AMONG MAJOR CEPHALOPOD TAXONOMIC GROUPS

|                       |                 | Myopsida | Oegopsida | Sepioidea | Octopoda |
|-----------------------|-----------------|----------|-----------|-----------|----------|
| Myopsida              | <sup>a</sup> x  | 6.38     |           |           |          |
|                       | <sup>b</sup> SD | ---      |           |           |          |
|                       | <sup>c</sup> N  | 1        |           |           |          |
| Oegopsida             | x               | 7.10     | 6.34      |           |          |
|                       | SD              | 1.67     | 1.73      |           |          |
|                       | N               | 44       | 275       |           |          |
| Sepioidea             | x               | 6.57     | 7.77      | 6.82      |          |
|                       | SD              | 1.70     | 1.99      | 2.54      |          |
|                       | N               | 17       | 176       | 28        |          |
| Octopoda              | x               | 10.95    | 10.57     | 11.78     | 5.07     |
|                       | SD              | 0.71     | 1.07      | 1.47      | 1.80     |
|                       | N               | 10       | 110       | 40        | 10       |
| <i>Vampyroteuthis</i> | x               | 8.98     | 8.93      | 10.03     | 7.65     |
|                       | SD              | 0.61     | 1.02      | 1.48      | 0.83     |
|                       | N               | 2        | 22        | 8         | 5        |

<sup>a</sup>x = mean % sequence divergence.

<sup>b</sup>SD = standard deviation of the mean.

<sup>c</sup>N = number of pairwise comparisons.

Figure 18. The sequence divergences for pooled first and second position nucleotides (open circles) and third position nucleotides (filled squares) plotted as a function of total uncorrected sequence divergence for all possible pairwise comparisons between the 26 taxa in the restricted Actin I data set. Because third position substitutions did not account for a greater proportion of the total sequence divergence at lower sequence divergences than at higher sequence divergences, these data do not provide evidence for saturation at third codon positions. Third codon characters of the Actin I were still predicted to be informative for determining relationships among highly diverged taxa as third position substitutions accounted for the great majority of variation in the gene. Since very few changes at first and second position nucleotides occurred across the entire spectrum of sequence divergence, they were not predicted to be of much use in constructing phylogenetic relationships within the Coleoidea.

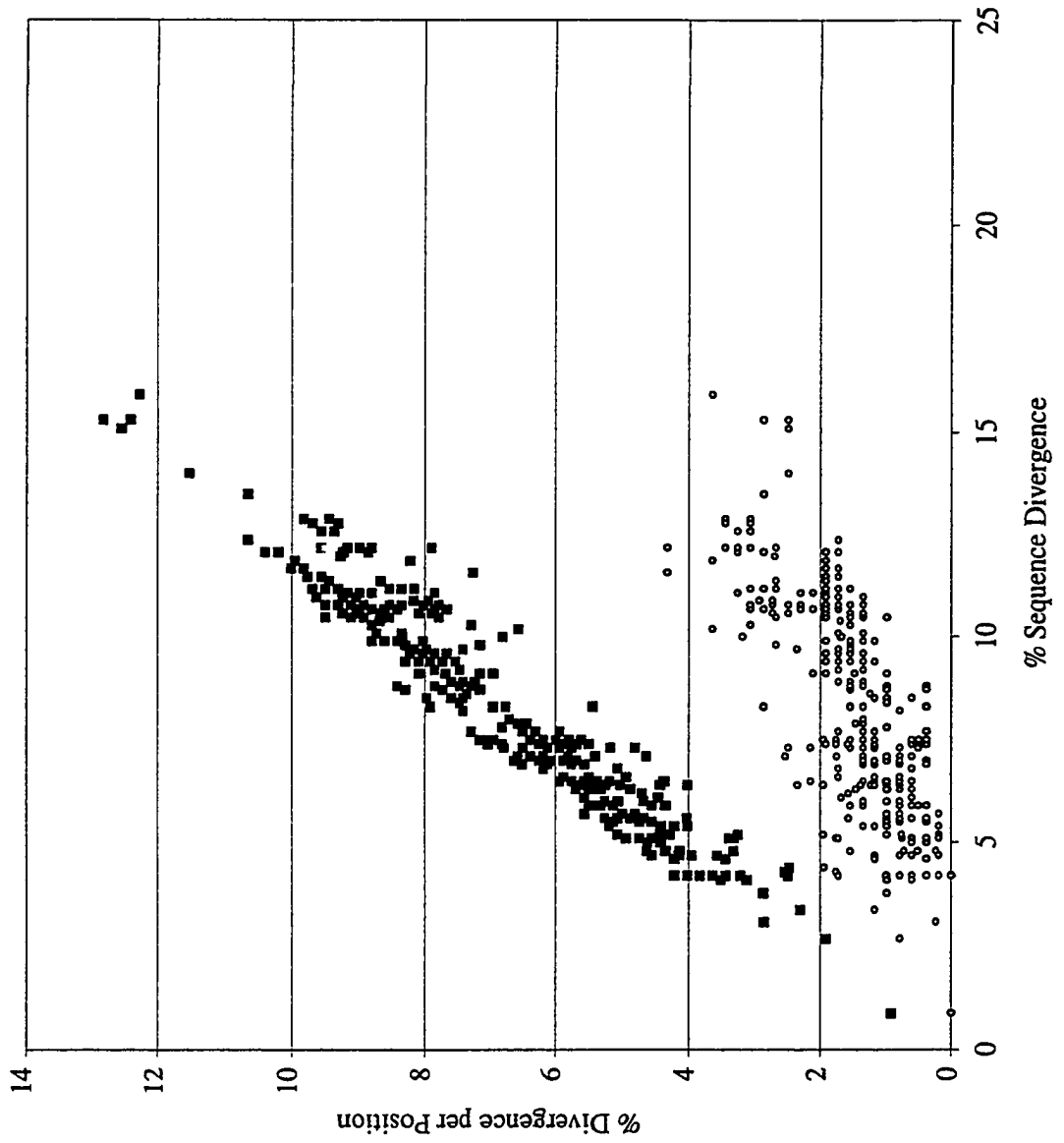


Figure 19. The percentage of total sequence divergence accounted for by transversional substitutions (open circles) and transitional substitutions (filled squares) plotted as a function of total uncorrected sequence divergence for all possible pairwise comparisons between the 26 taxa in the restricted Actin I data set. Although transitional substitutions accounted for the majority of substitutions across the entire spectrum of uncorrected sequence divergence, transversional substitutions also made a significant contribution to the total sequence divergence across the spectrum. The relationship between substitution type and total uncorrected sequence divergence indicated that the Actin I were not likely to be saturated with respect to transitional or transversional substitutions.



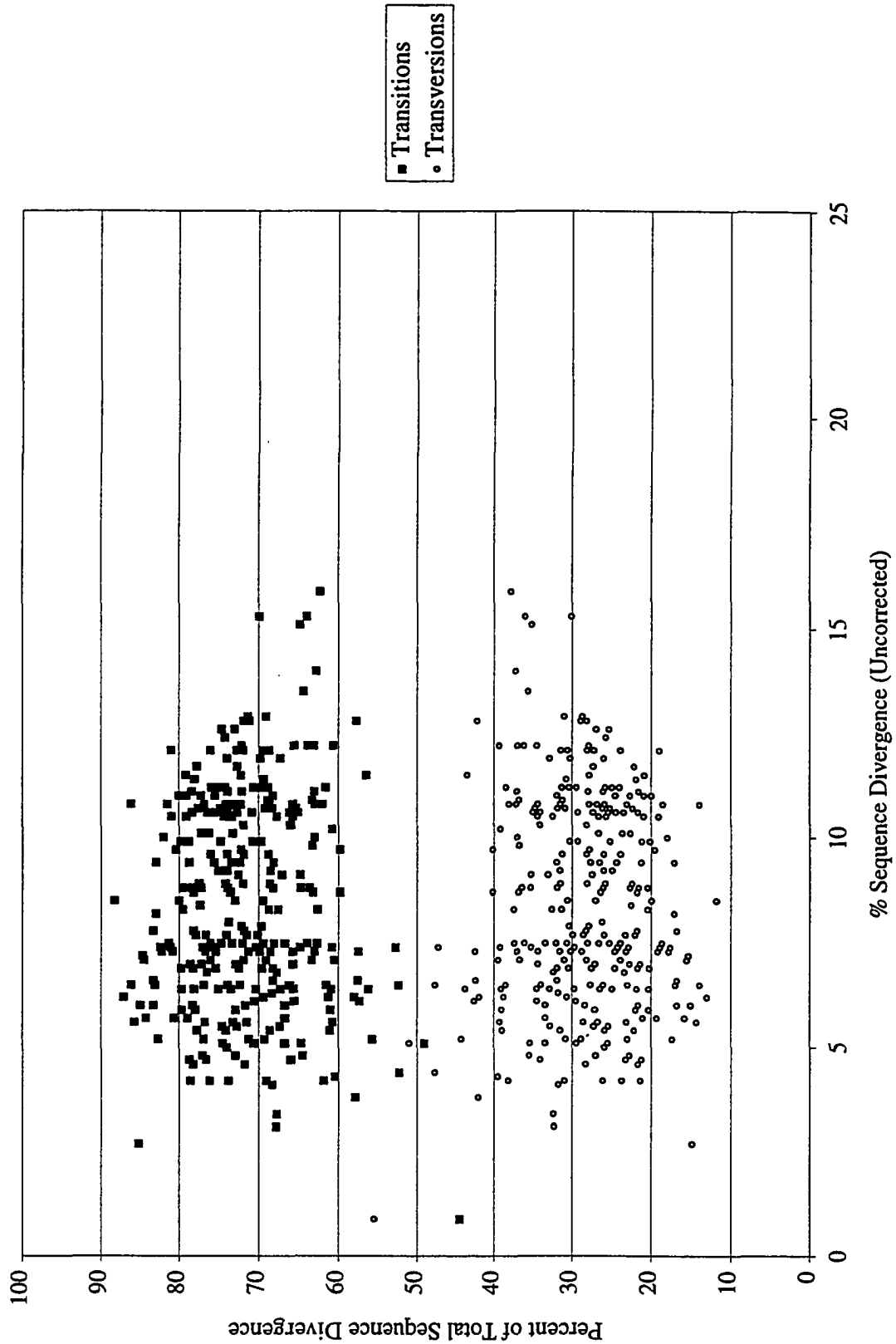


Figure 20. Base compositions at first codon positions in the Actin I gene for the 26 taxa in the restricted data set. The overall frequencies of the four bases were not equal (cytosines were the rarest), and a chi-square test did not demonstrate significant heterogeneity among taxa in first codon position base frequencies ( $\chi^2 = 3.31$ ,  $df = 75$ ,  $p > 0.995$ ).

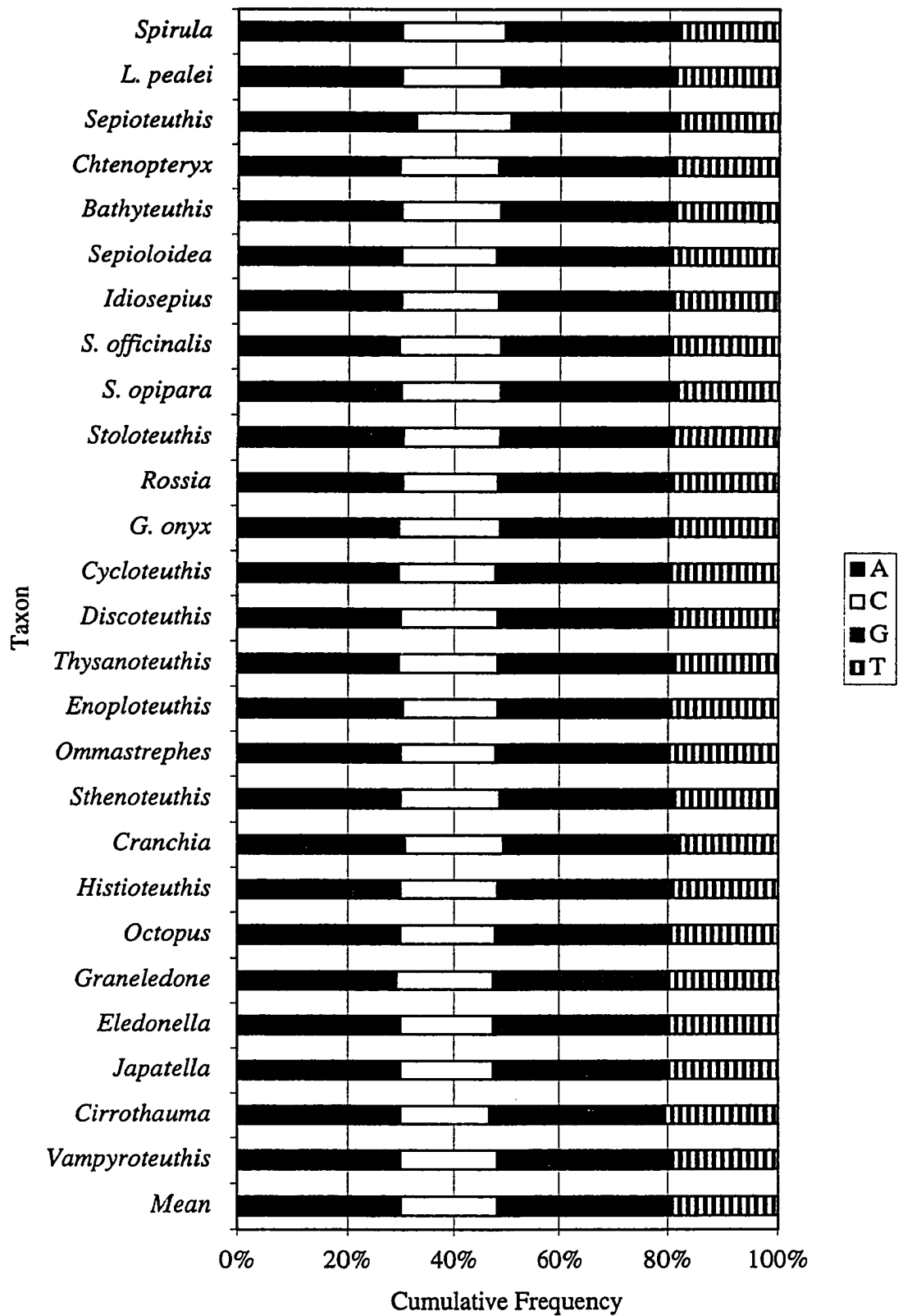


Figure 21. Base compositions at second codon positions in the Actin I gene for the 26 taxa in the restricted data set. The overall frequencies of the four bases were not equal (guanines were the rarest), and a chi-square test did not demonstrate significant heterogeneity among taxa in second codon position base frequencies ( $\chi^2 = 0.99$ ,  $df = 75$ ,  $p > 0.995$ ).

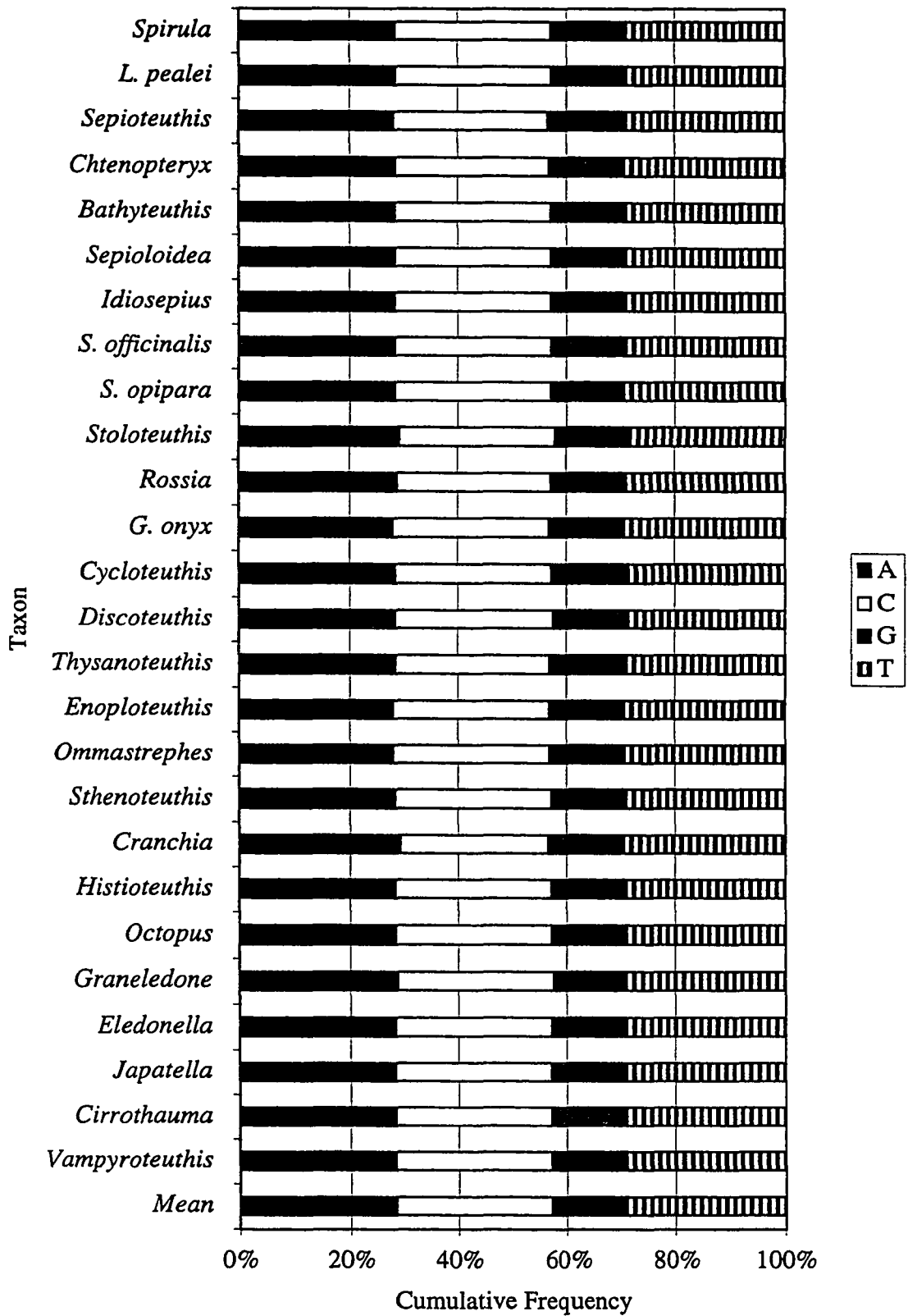


Figure 22. Base compositions at third codon positions in the Actin I gene for the 26 taxa in the restricted data set. The overall frequencies of the four bases were not equal (adenines were the rarest), and a chi-square test did not detect significant heterogeneity among taxa in third codon position base frequencies ( $\chi^2 = 46.02$ ,  $df = 75$ ,  $p > 0.995$ ).

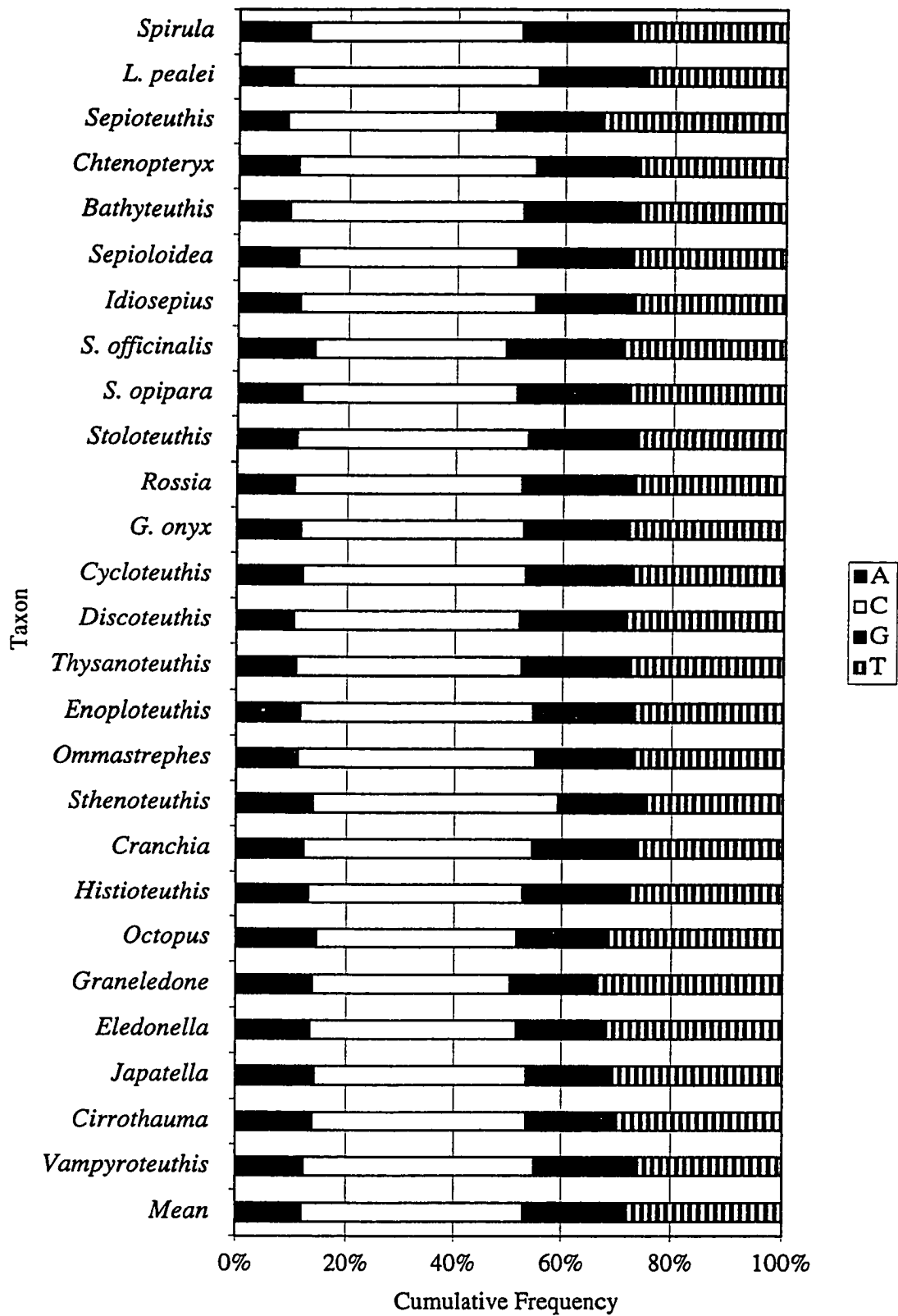


TABLE 7. PAIRWISE COMPARISONS OF THE ACTIN II GENE WITHIN AND AMONG MAJOR CEPHALOPOD TAXONOMIC GROUPS

|                       |                 | Myopsida | Oegopsida | Sepioidea | Incirrata | Cirrata | <i>Vampyroteuthis</i> |
|-----------------------|-----------------|----------|-----------|-----------|-----------|---------|-----------------------|
| Myopsida              | <sup>a</sup> x  | 2.551    |           |           |           |         |                       |
|                       | <sup>b</sup> SD | ---      |           |           |           |         |                       |
|                       | <sup>c</sup> N  | 1        |           |           |           |         |                       |
| Oegopsida             | x               | 8.33     | 7.55      |           |           |         |                       |
|                       | SD              | 1.70     | 1.87      |           |           |         |                       |
|                       | N               | 26       | 78        |           |           |         |                       |
| Sepioidea             | x               | 8.92     | 9.39      | 9.37      |           |         |                       |
|                       | SD              | 2.76     | 2.50      | 3.49      |           |         |                       |
|                       | N               | 14       | 91        | 21        |           |         |                       |
| Incirrata             | x               | 15.67    | 15.17     | 16.11     | 8.72      |         |                       |
|                       | SD              | 1.42     | 1.21      | 1.49      | 3.31      |         |                       |
|                       | N               | 12       | 66        | 42        | 15        |         |                       |
| Cirrata               | x               | 16.39    | 16.46     | 16.97     | 14.03     | 8.16    |                       |
|                       | SD              | 0.97     | 1.02      | 1.64      | 2.27      | ---     |                       |
|                       | N               | 4        | 26        | 14        | 12        | 1       |                       |
| <i>Vampyroteuthis</i> | x               | 18.37    | 17.65     | 19.31     | 18.45     | 15.12   |                       |
|                       | SD              | 0.36     | 0.49      | 1.73      | 1.18      | 0.81    |                       |
|                       | N               | 2        | 13        | 7         | 6         | 2       |                       |
| <i>Nautilus</i>       | x               | 18.88    | 18.38     | 19.94     | 20.64     | 21.68   | 22.19                 |
|                       | SD              | 0.36     | 0.81      | 1.61      | 0.92      | 0.72    | ---                   |
|                       | N               | 2        | 13        | 7         | 6         | 2       | 1                     |

<sup>a</sup>x = mean % sequence divergence.

<sup>b</sup>SD = standard deviation of the mean.

<sup>c</sup>N = number of pairwise comparisons.



Figure 23. The sequence divergences for pooled first and second position nucleotides (open circles) and third position nucleotides (filled squares) plotted as a function of total uncorrected sequence divergence for all possible pairwise comparisons between the 26 taxa in the restricted Actin II data set. Substitutions at third codon position nucleotides accounted for a greater proportion of the variation in the Actin II gene at lower total sequence divergences (roughly 90% of the total variation at 10% sequence divergence) than at greater total sequence divergences (roughly 50% of the total variation at 20% sequence divergence). At greater total sequence divergences, there was an increased contribution of first and second codon position substitutions to total sequence divergence. Because third position substitutions did not account for as great a proportion of the total sequence divergence at higher sequence divergences, these data provide evidence for saturation at third codon positions. The incursion of multiple hits in highly diverged taxa masks the total number of substitutions that have taken place. Therefore, third codon characters of the Actin II were not predicted to be informative for determining relationships among highly diverged taxa. At low sequence divergences, very few substitutions occurred at first and second codon position nucleotides such that third position nucleotides are predicted to be more informative for relationships among more recently diverged taxa.

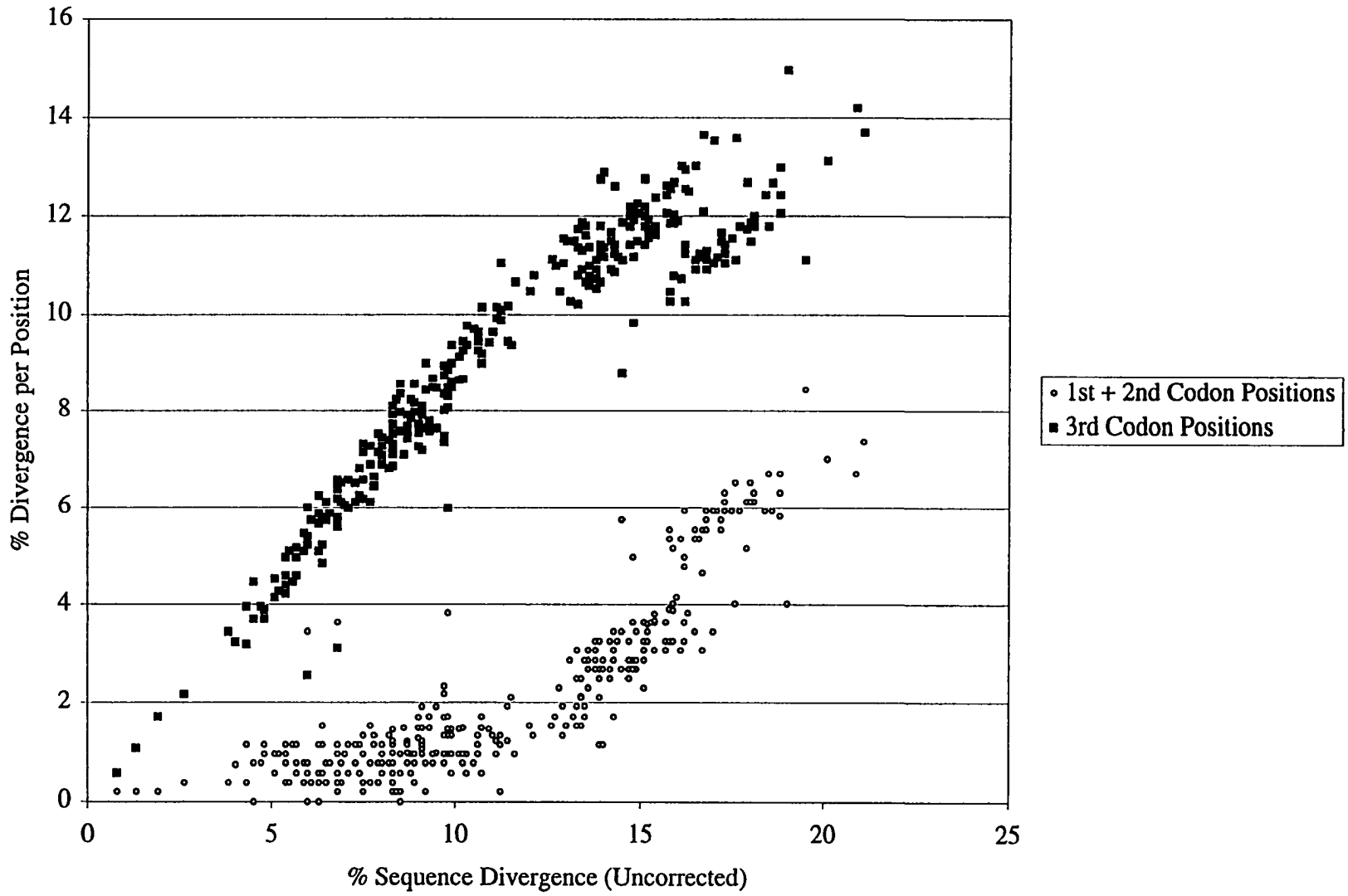
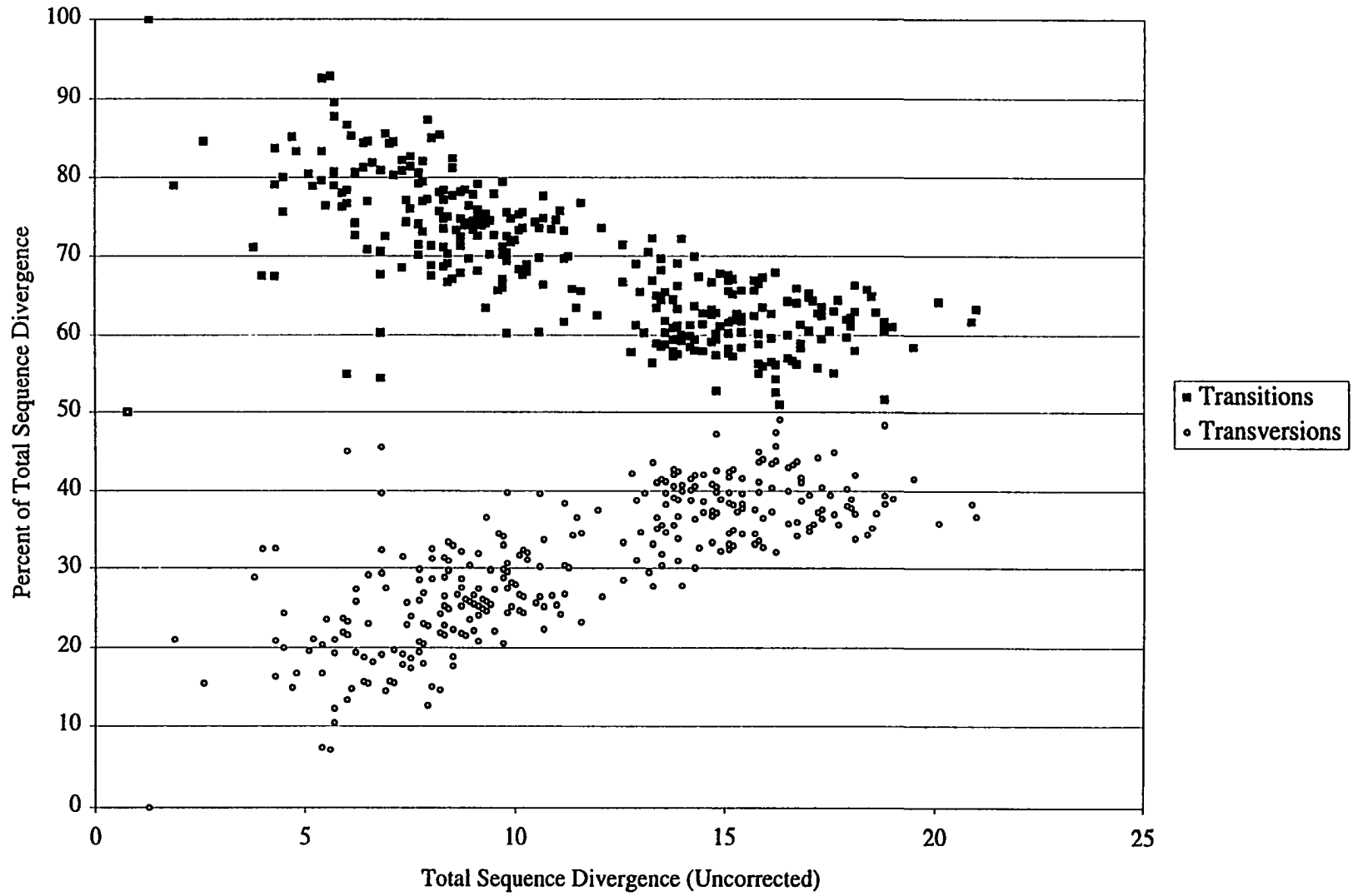
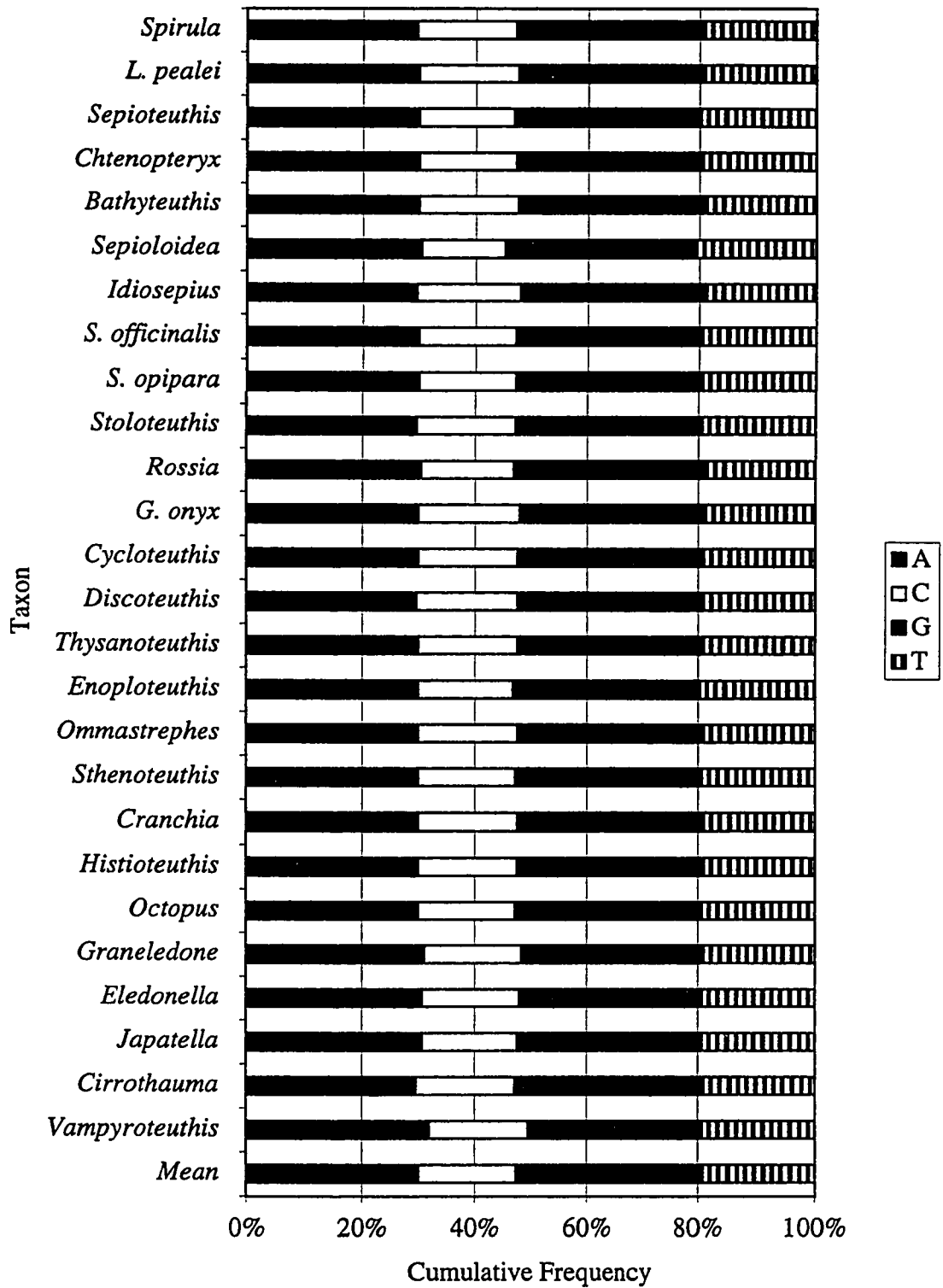


Figure 24. The percentage of total sequence divergence accounted for by transversional substitutions (open circles) and transitional substitutions (filled squares) plotted as a function of total uncorrected sequence divergence for all possible pairwise comparisons between the 26 taxa in the restricted Actin II data set. Although in general transitional substitutions accounted for the majority of substitutions across the entire spectrum of uncorrected sequence divergence, the relative contribution of transversional substitutions to total sequence divergence increased at greater sequence divergences. Because transitional substitutions did not account for as great a proportion of the total sequence divergence at higher sequence divergences, these data provide evidence for saturation in transitional substitutions, where the incursion of multiple hits masks the total number of transitional substitutions that have taken place among highly diverged taxa. Therefore, transitional substitutions in the Actin II gene were not likely to be informative for determining relationships among highly diverged taxa. At low sequence divergences, relatively few transversions occurred such that transitions are much more likely to be informative for resolving relationships among more recently diverged taxa.



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Figure 25. Base compositions at first codon positions in the Actin II gene for the 26 taxa in the restricted data set. The overall frequencies of the four bases were not equal (cytosines were rare), and a chi-square test did not demonstrate significant heterogeneity among taxa in first codon position base frequencies ( $\chi^2 = 3.48$ ,  $df = 75$ ,  $p > 0.995$ ).



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Figure 26. Base compositions at second codon positions in the Actin II gene for the 26 taxa in the restricted data set. The overall frequencies of the four bases were not equal (guanines were rare), and a chi-square test did not demonstrate significant heterogeneity among taxa in second codon position base frequencies ( $\chi^2 = 1.90$ ,  $df = 75$ ,  $p > 0.995$ ).

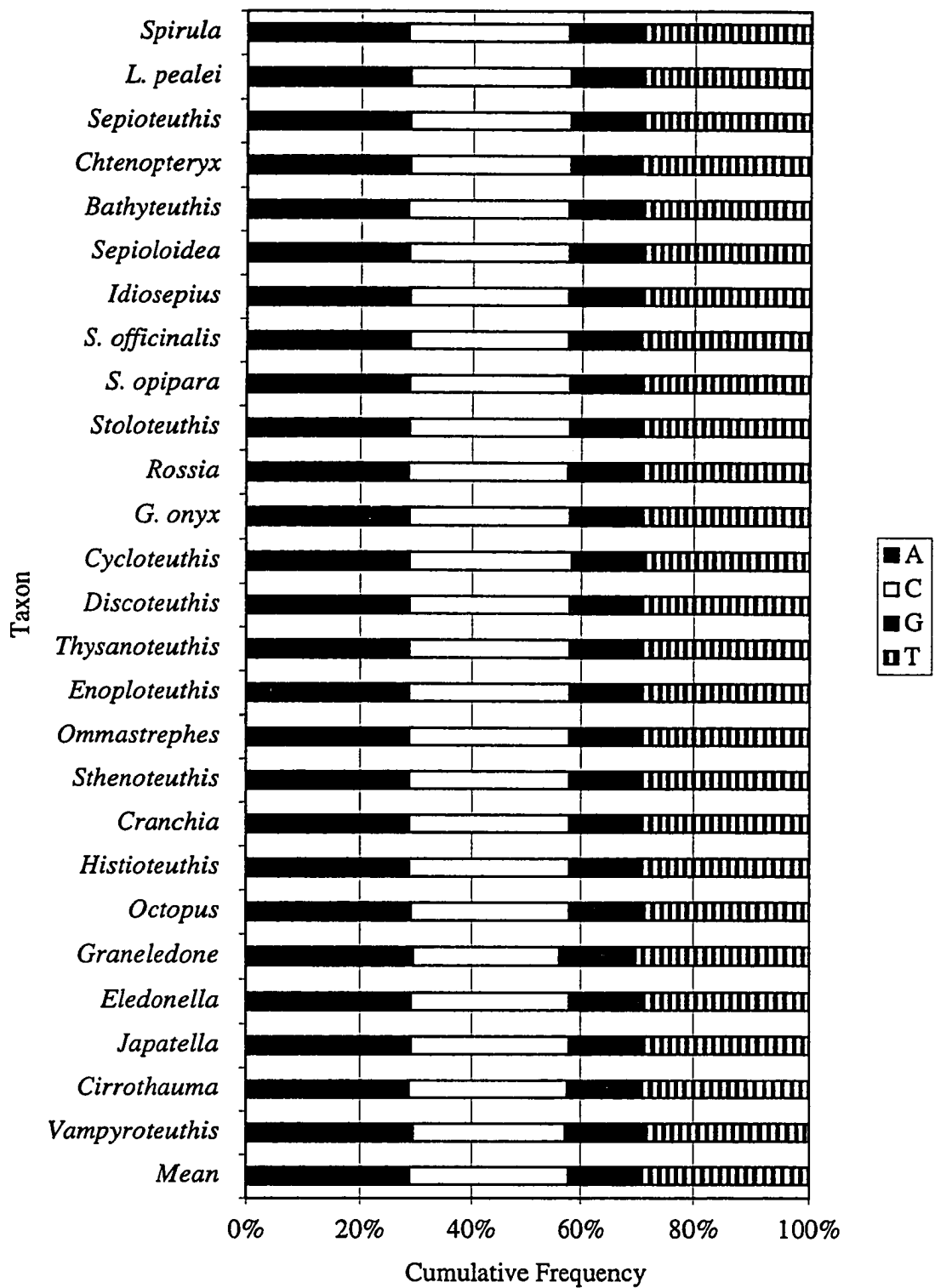




Figure 27. Base compositions at third codon positions in the Actin II gene for the 26 taxa in the restricted data set. The overall frequencies of the four bases were not equal (adenines were rare), and a chi-square test detected significant heterogeneity among taxa in third codon position base frequencies ( $\chi^2 = 103.8$ ,  $df = 75$ ,  $p < 0.05$ ).

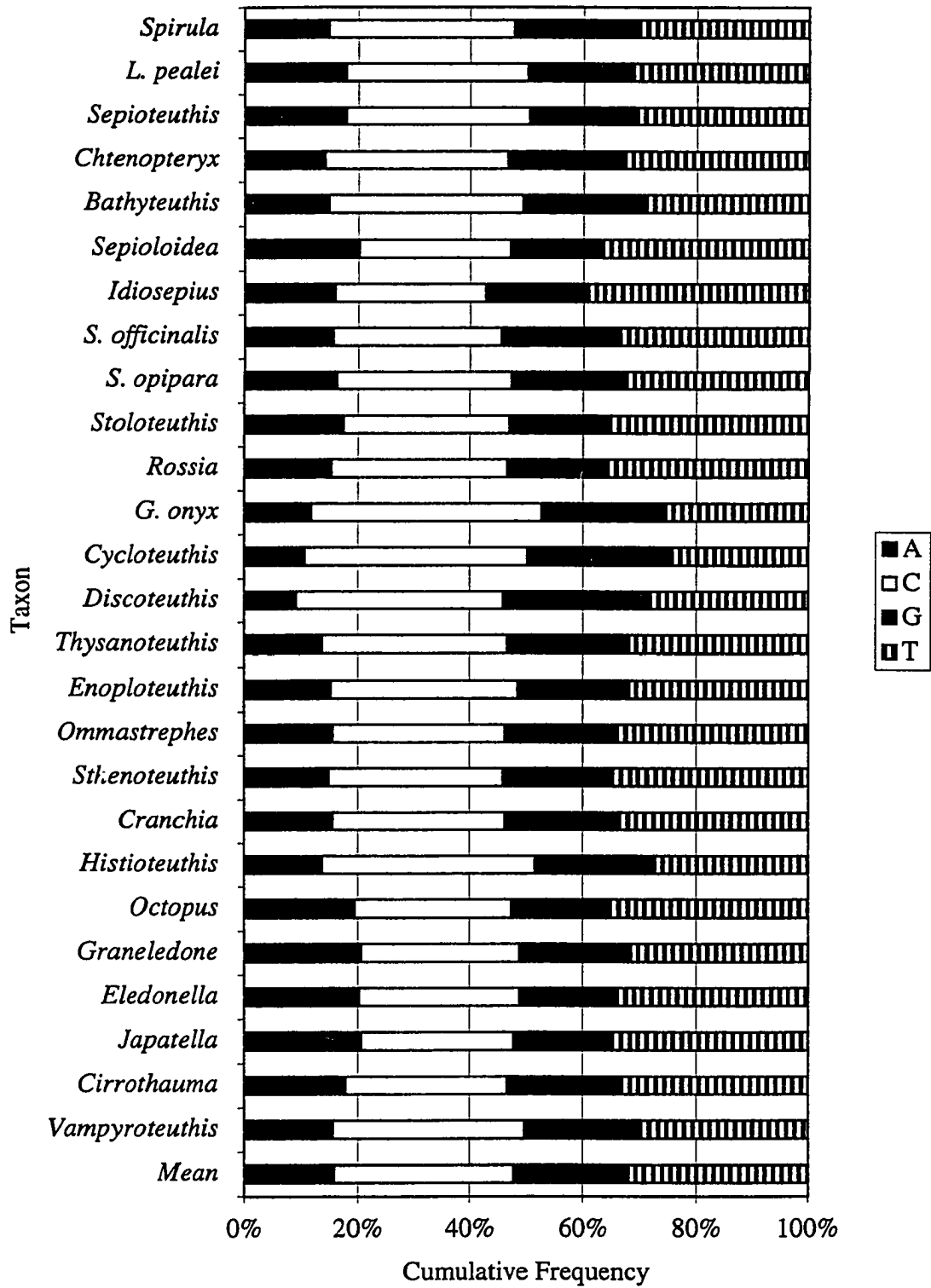


Figure 28. Strict consensus of 36 most parsimonious trees obtained in a heuristic search (100 random addition replicates) of the unweighted Actin I data set (TL = 784; CI = 0.414; RI = 0.530). Bootstrap proportions are indicated as percentages below nodes and Bremer support values are indicated above nodes. Higher-level taxonomic designations are indicated in boldface to the right of each terminal taxon (C = Suborder Cirrata; I = Suborder Incirrata; M = Suborder Myopsida; O = Suborder Oegopsida; V = Order Vampyromorpha).

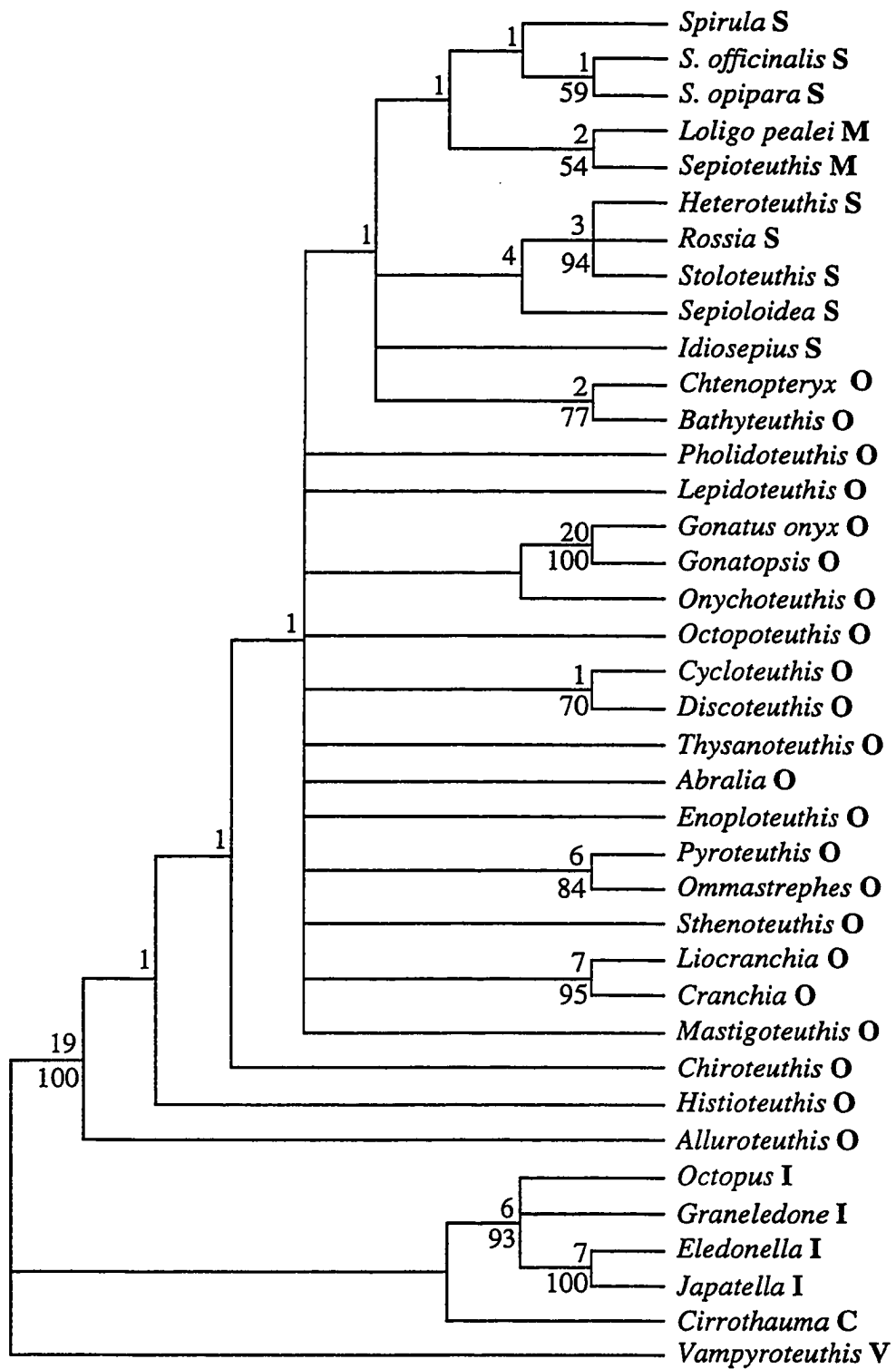


TABLE 8. RESULTS OF LIKELIHOOD RATIO TESTS OF SUBSTITUTION MODELS AND PARAMETER ESTIMATES FOR THE RESTRICTED ACTIN I DATA SET

| Null Hypothesis         | Models Compared   | ln L     | -2 log $\delta$ | df | P     | Parameter Estimates   |
|-------------------------|---|----------|-----------------|----|-------|---|
| Equal base frequencies  | H <sub>0</sub> : Jukes and Cantor (1969)                                      | -4985.02 | 92.70           | 3  | <0.01 | None  |
|                         | H <sub>1</sub> : Felsenstein (1981)   | -4938.67 |                 |    |       | <sup>a</sup> $\pi_A=0.24$ , $\pi_C=0.30$ ,<br>$\pi_G=0.18$ , $\pi_T=0.28$ |
| TI rate equals TV rate  | H <sub>0</sub> : Felsenstein (1981)   | -4938.67 | 389.38          | 1  | <0.01 | <sup>b</sup> TI:TV=2.514  |
|                         | H <sub>1</sub> : Hasegawa et al. (1985)                                       | -4743.98 |                 |    |       |   |
| Equal rates among sites | H <sub>0</sub> : Hasegawa et al. (1985)                                       | -4743.98 | 922.70          | 1  | <0.01 | <sup>c</sup> $r_1=0.30$ , $r_2=0.11$ , $r_3=2.58$                         |
|                         | H <sub>1</sub> : Hasegawa et al. (1985)<br>with among-site rate heterogeneity | -4282.63 |                 |    |       |   |

<sup>a</sup> $\pi_A$  = base frequency of adenines;  $\pi_C$  = base frequency of cytosines;  $\pi_G$  = base frequency of guanines;  $\pi_T$  = base frequency of thymines.

<sup>b</sup>TI:TV = Ratio of rates of transitional substitutions to transversional substitutions.

<sup>c</sup> $r_1$  = substitution rate at first codon position nucleotides;  $r_2$  = substitution rate at first codon position nucleotides;  $r_3$  = substitution rate at first codon position nucleotides.

Figure 29. Maximum likelihood tree generated from a heuristic search of the Actin I data assuming a Hasegawa, Kishino, and Yano (1985) model of substitution with site specific rates estimated according to the partitioned codon positions ( $-\ln L = 5455.93$ ). Branch lengths are drawn proportional to the probabilities of change occurring along each branch under the HKY85 model. Substitution parameters estimated in the likelihood search were as follows:  $\pi_A=0.242$ ,  $\pi_C=0.299$ ,  $\pi_G=0.177$ ,  $\pi_T=0.282$ ;  $TI/TV=2.514$ ;  $r_1=0.304$ ,  $r_2=0.109$ ,  $r_3=2.581$ . Higher-level taxonomic designations are indicated in boldface to the right of each terminal taxon (C = Suborder Cirrata; I = Suborder Incirrata; M = Suborder Myopsida; O = Suborder Oegopsida; V = Order Vampyromorpha).

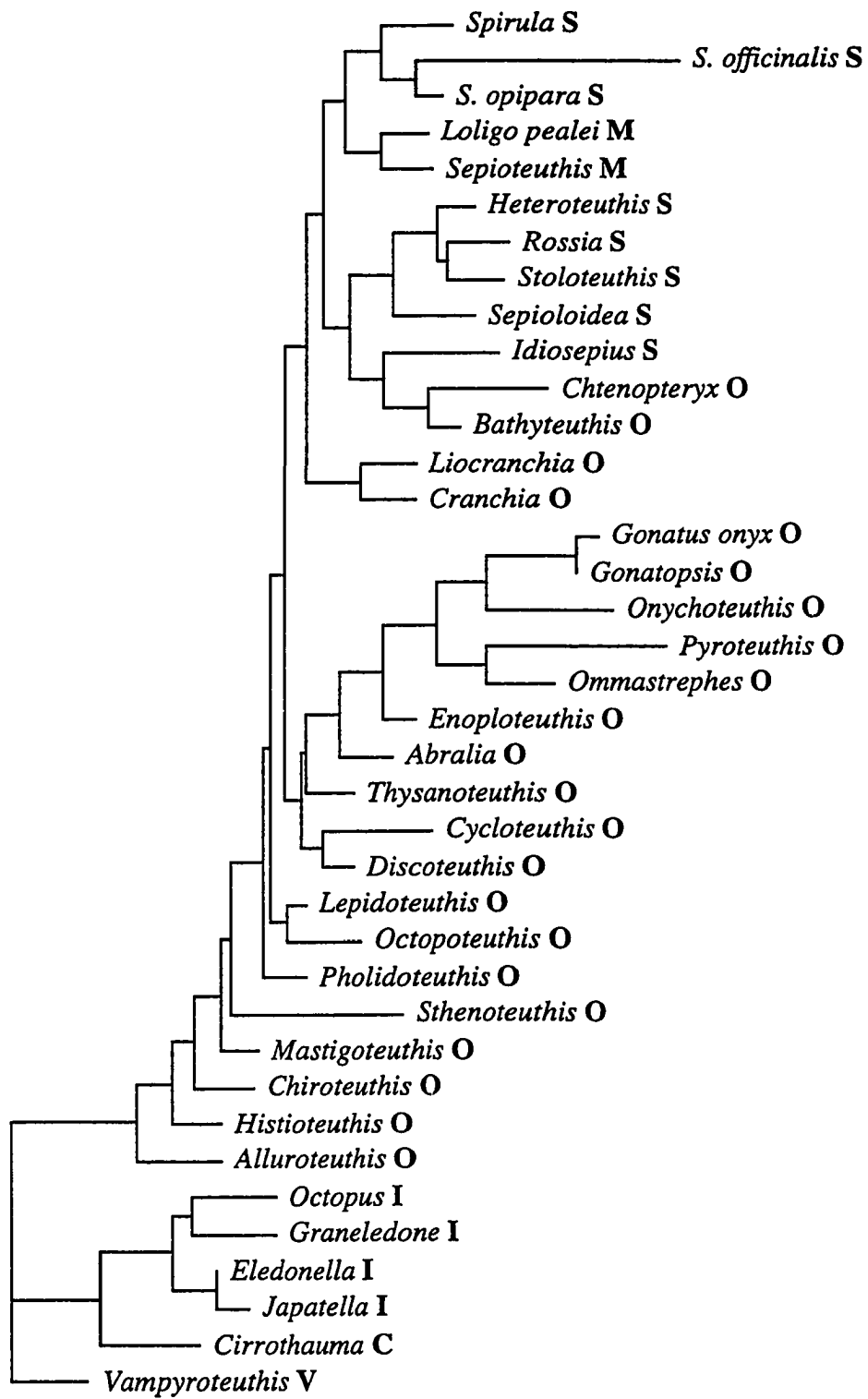


Figure 30. Strict consensus of 44 equally parsimonious trees obtained in a heuristic search (100 random addition replicates) of the weighted Actin I data set (TL = 2514; CI = 0.440; RI = 0.544). Transversion substitutions were assigned a weight of five steps and transitions were assigned a weight of two steps. Branch lengths are drawn proportional to the amount of change. Bootstrap proportions are indicated as percentages below nodes. Higher-level taxonomic designations are indicated in boldface to the right of each terminal taxon (C = Suborder Cirrata; I = Suborder Incirrata; M = Suborder Myopsida; O = Suborder Oegopsida; V = Order Vampyromorpha).



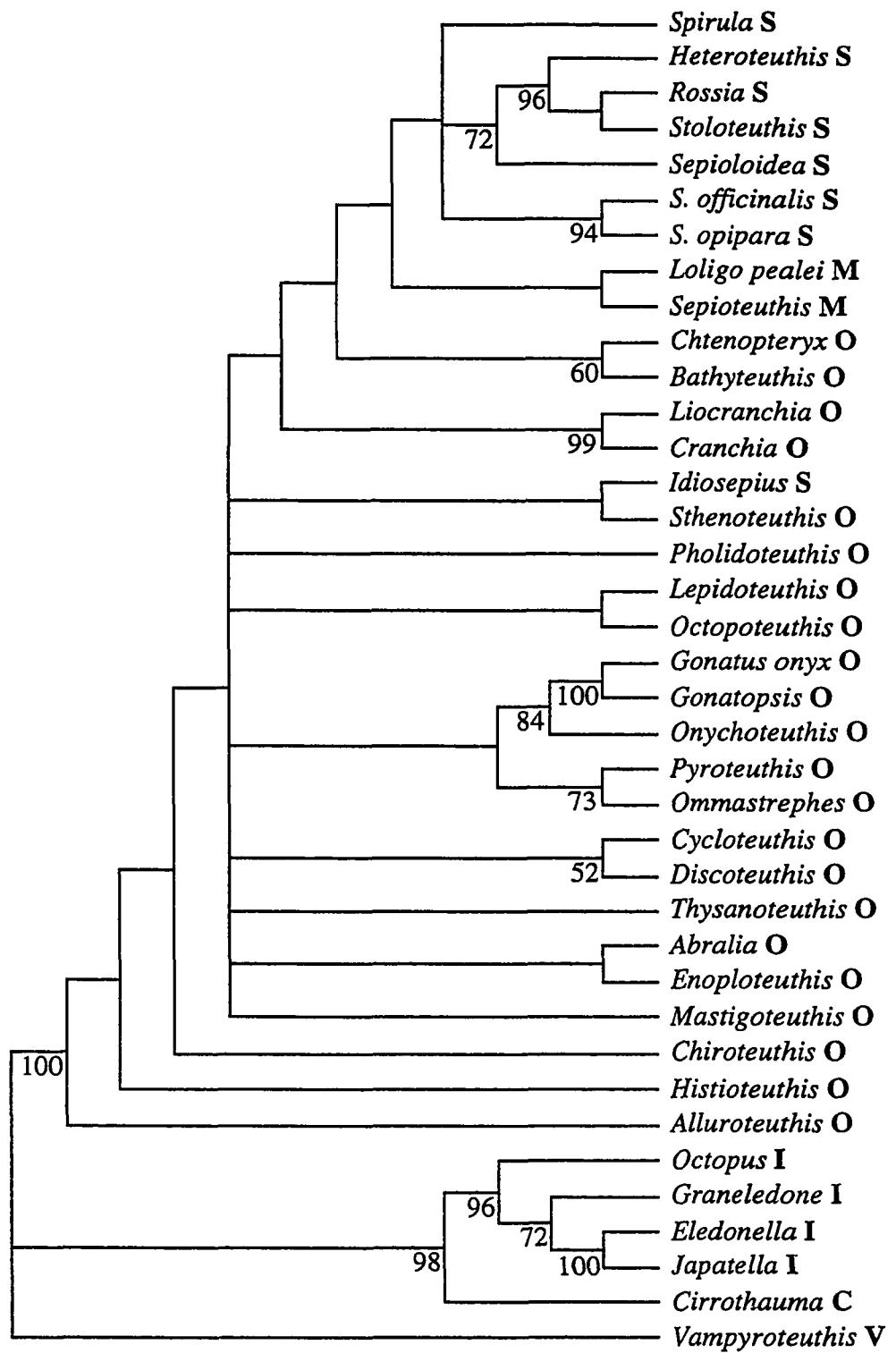


Figure 31. Results from parametric bootstrap analysis of the Actin I data set. The tree derived from maximum likelihood analysis of the Actin I data set constraining the monophyly of the Sepioidea was used to generate 50 simulated data sets. The substitution parameters under the HKY85+ model of evolution that were used to obtain the initial tree were also used to generate the simulated data sets ( $\pi_A=0.242$ ,  $\pi_C=0.299$ ,  $\pi_G=0.177$ ,  $\pi_T=0.282$ ; TI/TV=2.514;  $r_1=0.304$ ,  $r_2=0.109$ ,  $r_3=2.581$ ). Two rounds of parsimony analysis were conducted on each of the simulated data sets. The first parsimony search was conducted under the null hypothesis: constraint of sepioid monophyly. The second search was conducted with no constraints on the data. The differences in scores between the best tree derived from the constrained and unconstrained parsimony searches of each of the 50 simulated data sets was recorded and graphed to obtain the expected distribution under the null model. Forty-seven of the 50 sampled tree lengths resulted in a difference of 1 step, and the three remaining tree length differences were 2 steps ( $p=0.06$ ), whereas for the observed data the tree length difference between the constrained and unconstrained searches was 3 steps. Therefore, a difference this great would be expected less than 1% of the time if the null hypothesis were true, so the null hypothesis of sepioid monophyly was rejected at  $p < 0.01$ .

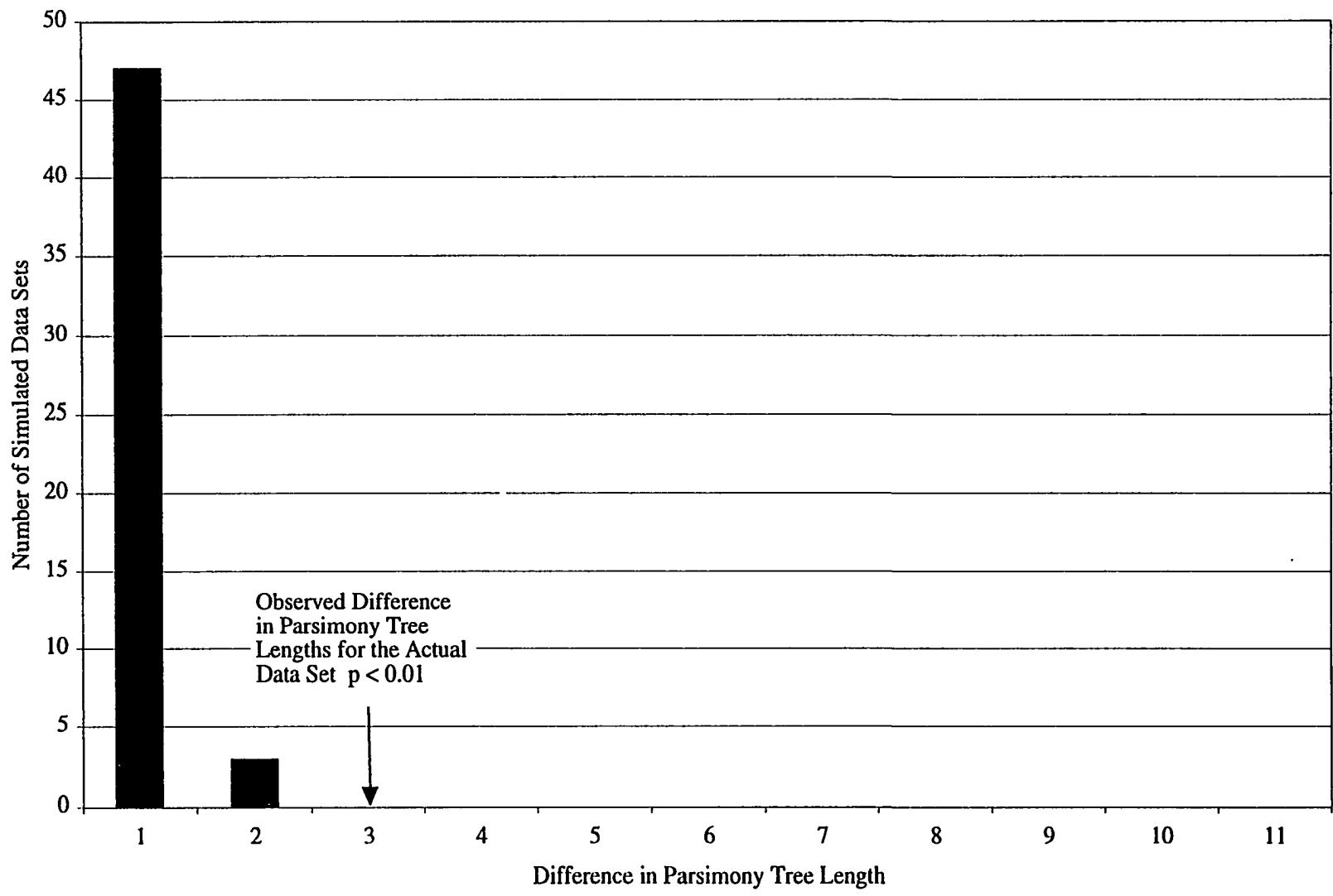


Figure 32. Strict consensus of five most parsimonious trees obtained in a heuristic search (100 random addition replicates) of the unweighted Actin II data set (TL = 1217; CI = 0.418; RI = 0.555). Bootstrap proportions are indicated as percentages below nodes and Bremer support values are indicated above nodes. Higher-level taxonomic designations are indicated in boldface to the right of each terminal taxon (C = Suborder Cirrata; I = Suborder Incirrata; M = Suborder Myopsida; O = Suborder Oegopsida; V = Order Vampyromorpha).

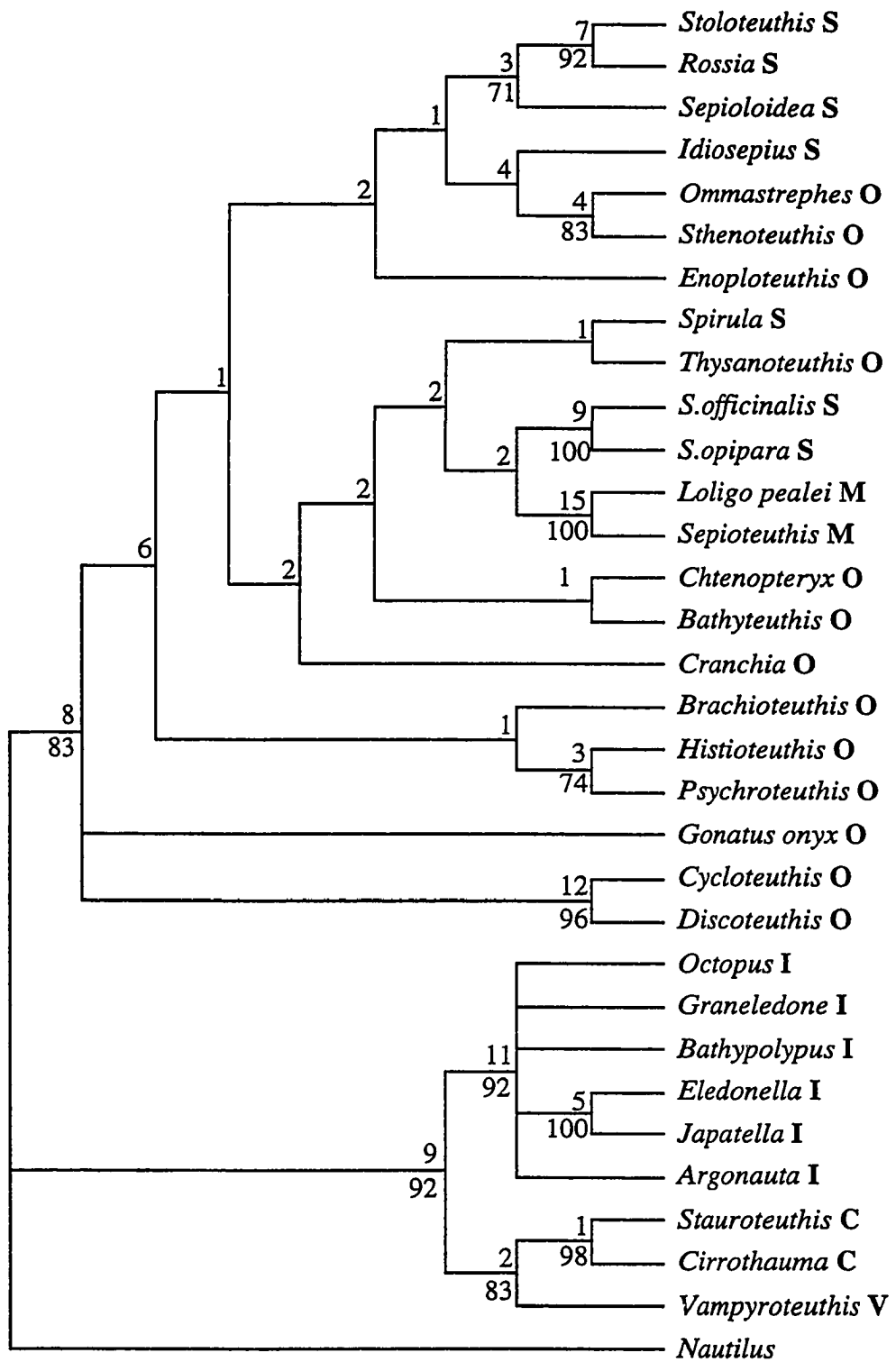


TABLE 9. RESULTS OF LIKELIHOOD RATIO TESTS OF SUBSTITUTION MODELS AND PARAMETER ESTIMATES FOR THE RESTRICTED ACTIN II DATA SET

| Null Hypothesis         | Models Compared   | ln L     | -2 log $\delta$ | df | P     | Parameter Estimates   |
|-------------------------|---|----------|-----------------|----|-------|---|
| Equal base frequencies  | H <sub>0</sub> : Jukes and Cantor (1969)                                      | -5902.88 | 94.16           | 3  | <0.01 | None<br><sup>a</sup> $\pi_A=0.24$ , $\pi_C=0.26$ ,<br>$\pi_G=0.19$ , $\pi_T=0.32$ |
|                         | H <sub>1</sub> : Felsenstein (1981)   | -5855.80 |                 |    |       |   |
| TI rate equals TV rate  | H <sub>0</sub> : Felsenstein (1981)   | -5855.80 | 458.82          | 1  | <0.01 | <sup>b</sup> TI:TV=2.619  |
|                         | H <sub>1</sub> : Hasegawa et al. (1985)                                       | -5626.39 |                 |    |       |   |
| Equal rates among sites | H <sub>0</sub> : Hasegawa et al. (1985)                                       | -5626.39 | 1289.12         | 1  | <0.01 | <sup>c</sup> $r_1=0.26$ , $r_2=0.08$ , $r_3=2.66$                                 |
|                         | H <sub>1</sub> : Hasegawa et al. (1985)<br>with among-site rate heterogeneity | -4981.83 |                 |    |       |   |

<sup>a</sup> $\pi_A$  = base frequency of adenines;  $\pi_C$  = base frequency of cytosines;  $\pi_G$  = base frequency of guanines;  $\pi_T$  = base frequency of thymines.

<sup>b</sup>TI:TV = Ratio of rates of transitional substitutions to transversional substitutions.

<sup>c</sup> $r_1$  = substitution rate at first codon position nucleotides;  $r_2$  = substitution rate at first codon position nucleotides;  $r_3$  = substitution rate at first codon position nucleotides.

Figure 33. Maximum likelihood tree generated from a heuristic search of the Actin II data assuming a Hasegawa, Kishino, and Yano (1985) model of substitution with site specific rates estimated according to the partitioned codon positions ( $-\ln L = 6395.63$ ). Branch lengths are drawn proportional to the probabilities of change occurring along each branch under the HKY85 model. Substitution parameters estimated in the likelihood search were as follows:  $\pi_A=0.240$ ,  $\pi_C=0.258$ ,  $\pi_G=0.186$ ,  $\pi_T=0.316$ ;  $TI/TV=2.619$ ;  $r_1=0.261$ ,  $r_2=0.076$ ,  $r_3=2.657$ . Higher-level taxonomic designations are indicated in boldface to the right of each terminal taxon (C = Suborder Cirrata; I = Suborder Incirrata; M = Suborder Myopsida; O = Suborder Oegopsida; V = Order Vampyromorpha).

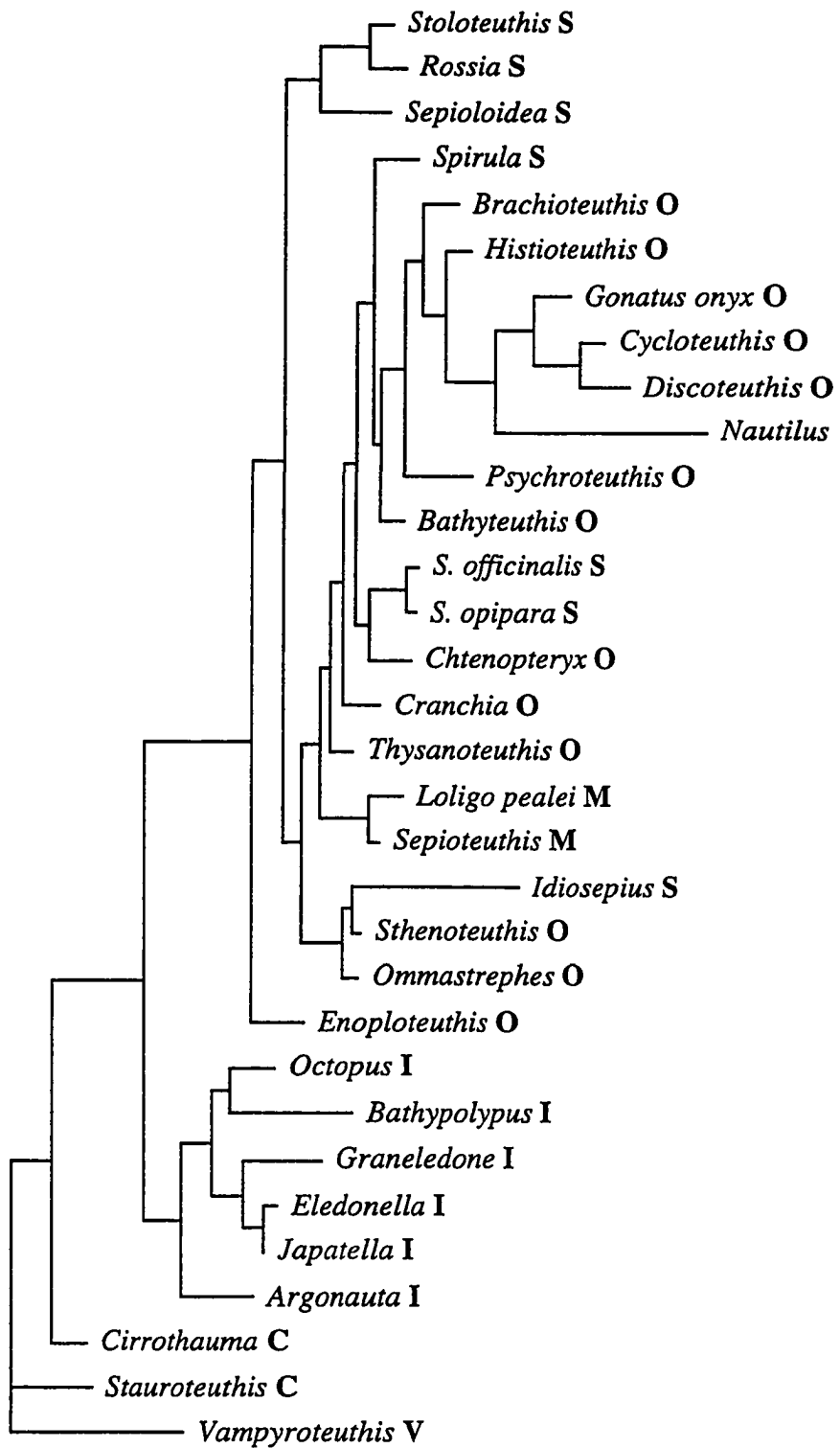




Figure 34. Most parsimonious tree obtained in a heuristic search (100 random addition replicates) of the weighted Actin II data set (TL = 3635; CI = 0.439; RI = 0.566). Transversion substitutions were assigned a weight of five steps and transitions were assigned a weight of two steps. Branch lengths are drawn proportional to the amount of change. Bootstrap proportions are indicated as percentages below nodes. Higher-level taxonomic designations are indicated in boldface to the right of each terminal taxon (C = Suborder Cirrata; I = Suborder Incirrata; M = Suborder Myopsida; O = Suborder Oegopsida; V = Order Vampyromorpha).

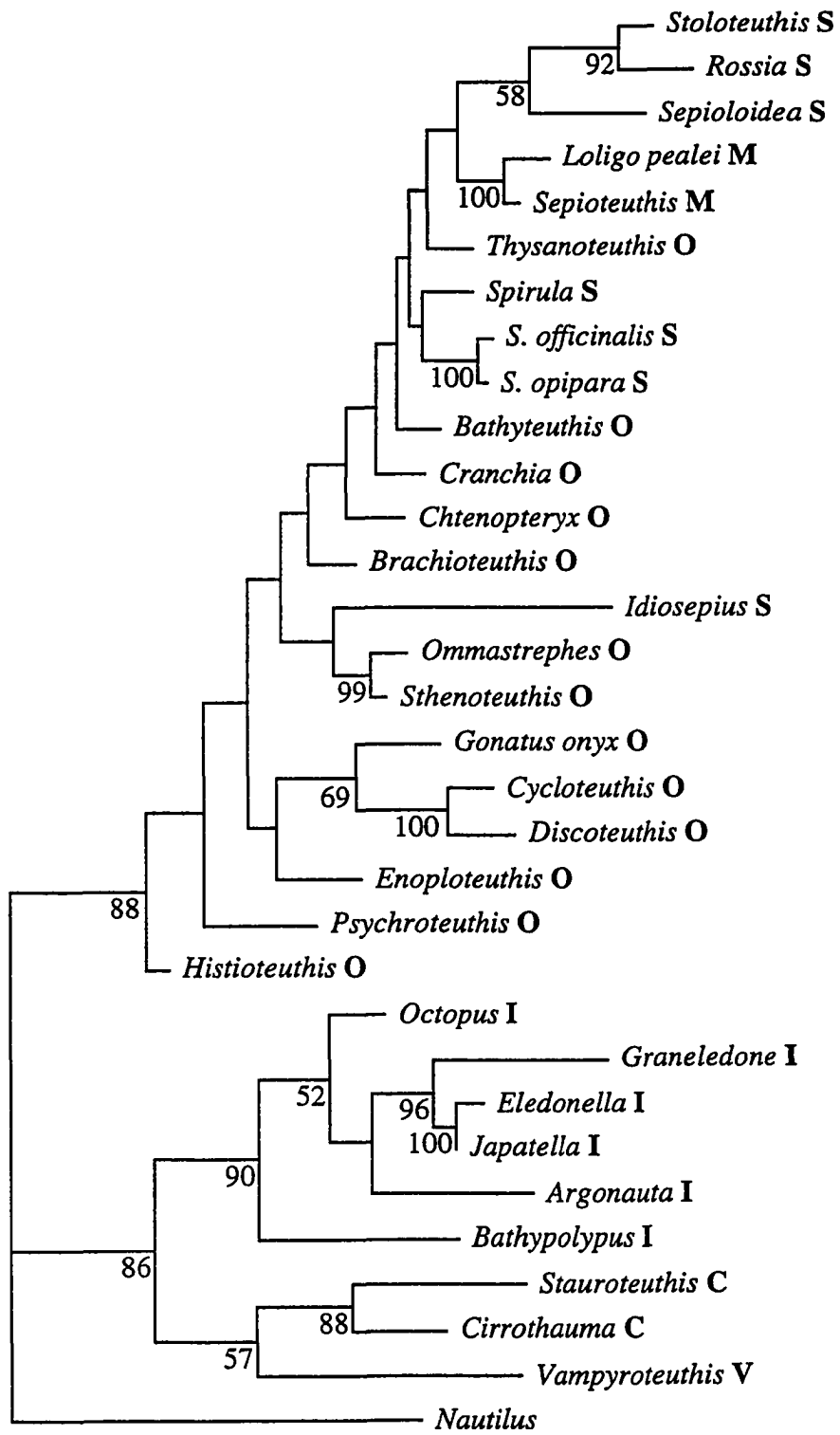
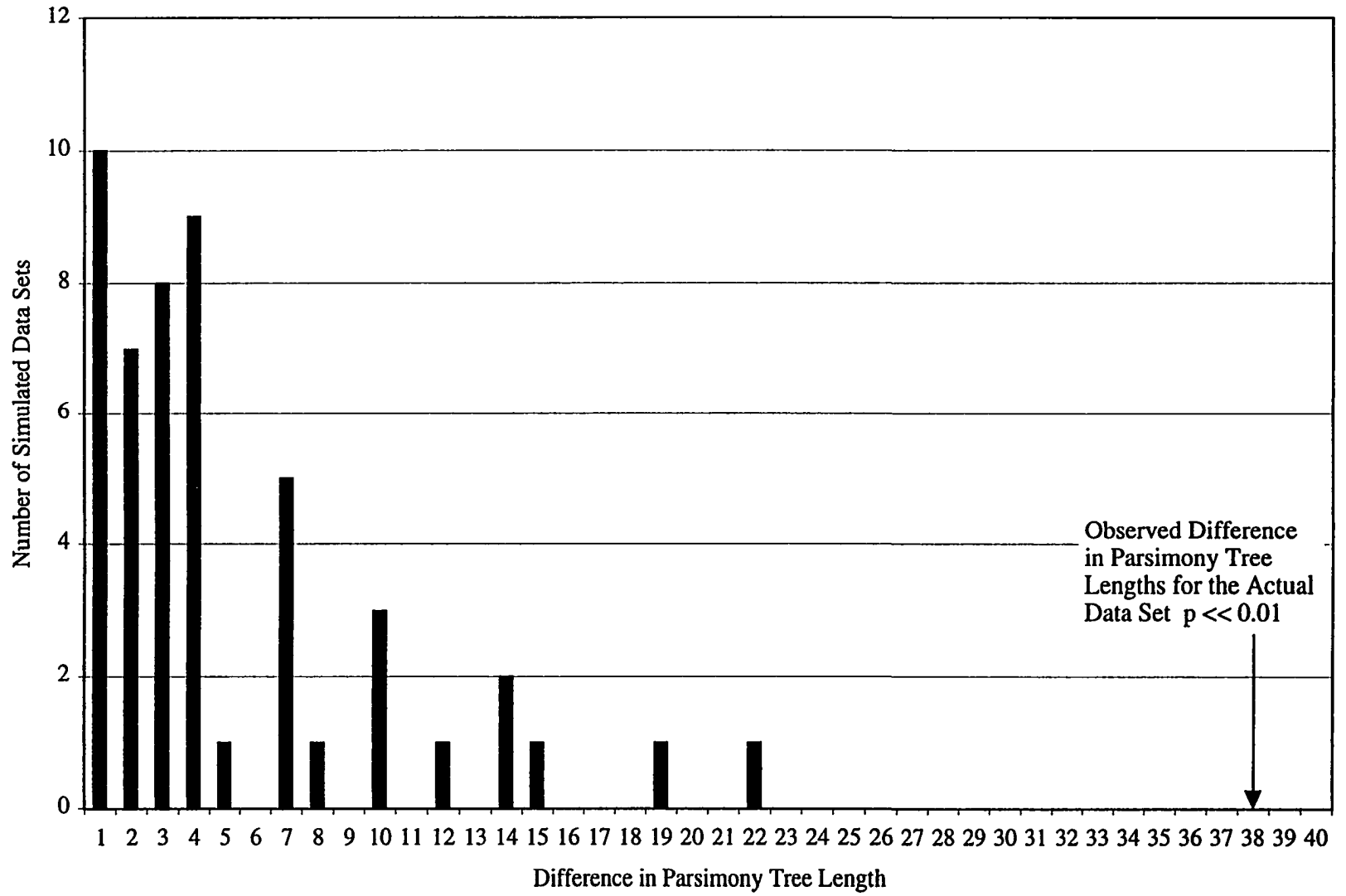


Figure 35. Results from parametric bootstrap analysis of the Actin II data set. The tree derived from maximum likelihood analysis of the Actin II data set constraining the monophyly of the Sepioidea was used to generate 50 simulated data sets. The substitution parameters under the HKY85+ model of evolution that were used to obtain the initial tree were also used to generate the simulated data sets ( $\pi_A=0.240$ ,  $\pi_C=0.258$ ,  $\pi_G=0.186$ ,  $\pi_T=0.316$ ; TI/TV=2.619;  $r_1=0.261$ ,  $r_2=0.076$ ,  $r_3=2.657$ ). Two rounds of parsimony analysis were conducted each of the simulated data sets. The first parsimony search was conducted under the null hypothesis: constraint of sepioid monophyly. The second search was conducted with no constraints on the data. The differences in scores between the best tree derived from the constrained and unconstrained parsimony searches of each of the 50 simulated data sets was recorded and graphed to obtain the expected distribution under the null model. Each of the 50 sampled tree length differences fall below 22 steps, whereas for the observed data the tree length difference between the constrained and unconstrained searches was 38 steps. Therefore, a difference this great would be expected much less than 1% of the time if the null hypothesis were true, so the null hypothesis of sepioid monophyly was rejected at  $p \ll 0.01$ .



CHAPTER 3. PHYLOGENETIC RELATIONSHIPS OF COLEOID CEPHALOPODS  
INFERRED FROM THE ANALYSIS OF COMBINED ACTIN I, ACTIN II, AND  
CYTOCHROME C OXIDASE I DATA SETS AND A COMPARISON OF THEIR  
PATTERNS OF MOLECULAR EVOLUTION

## INTRODUCTION

When multiple data sets are available for the investigation of a particular phylogenetic problem, a philosophical decision must be made as to whether the data sets should be analyzed independently (taxonomic congruence) or combined in the phylogenetic analysis (character congruence or total evidence). The taxonomic congruence approach involves conducting separate analyses on the individual data sets followed by comparison of the results and construction of a consensus tree that summarizes the relationships supported by the separate analyses (Mickey, 1978). The character congruence approach involves simultaneous analysis of the combined data sets (Kluge, 1989). A third approach, prior agreement, combines elements from both schools of thought. It is based on the premise that it is inappropriate to combine data sets which, when analyzed separately, result in strongly supported but conflicting trees or that give significantly heterogeneous results (Bull et al., 1993; de Queiroz, 1993). The prior agreement approach necessitates the use of a statistical test for heterogeneity among trees, such as the incongruence length difference test (Farris et al., 1994). If the results from separate analyses are not significantly incongruent, the data may then be combined in a total evidence analysis.

Advocates of taxonomic congruence argue that different sets of characters evolve under demonstrably different rules, or process partitions, *sensu* Bull et al. (1993). For example, two genes may exhibit different evolutionary rates, differ in base compositions, or differ in substitution properties. Miyamoto and Fitch (1995) outlined some general considerations for recognizing molecular character sets as independent process partitions. Genes that are considered independent process partitions must be unlinked, the gene products must not interact, the gene products must have separate functions and not be involved in a common

biochemical pathway, and there must be no epistatic interactions among the genes. Combining the data without accounting for different process partitions could lead to an erroneous result. Furthermore, combining the data could also mask non-stochastic heterogeneity, which may provide information about differences in the evolutionary histories of the partitioned data sets. In the taxonomic congruence perspective, the best estimates of the true phylogeny for a particular group are those that are supported by different lines of evidence, i.e. congruent trees obtained from analyses of independent data sets. Consensus techniques are commonly employed to summarize relationships supported in the separate analyses. The philosophical grounds for the taxonomic congruence approach are argued to be most similar to scientific hypothesis testing in general, where independent information can be used to test a particular hypothesis (Miyamoto and Fitch, 1995).

Proponents of the character congruence approach argue that the data partitions are arbitrary since they are artifacts of technology, and that there are no "natural classes" of data (Eernisse and Kluge, 1993). Secondly, results of separate analyses may all support an incorrect phylogeny, in which case a consensus derived from separate analyses would also support an incorrect phylogeny. The third argument erected in criticism of taxonomic congruence is leveled at consensus techniques. Most methods of constructing consensus trees (strict, majority rule, and Nelson) will collapse clades that differ in the placement of a single terminal taxon, even though they might share considerable structure with respect to the placement of the remaining taxa in the clade. The character congruence approach would provide information about the phylogenetic relationships among these taxa whereas such information would be lost in the consensus derived from separate analyses. On the other hand, a consensus derived from separate analyses might support an incorrect group that appeared on the trees resultant from separate analyses. A consensus approach might therefore reinforce confidence in an incorrect phylogenetic conclusion whereas analysis of the combined data sets might not support such a grouping (Barret et al., 1991). The

criticism raised by advocates of taxonomic congruence that character congruence ignores different process partitions is addressed through character weighting which, when employed correctly, accommodates for differences in the processes of character evolution (Chippindale and Wiens, 1994). For example, a weighting scheme which downweights characters from the more variable data set can accommodate for rate heterogeneity between the two data sets. Finally, proponents of character congruence do not agree with the assertion that the separate analysis of independent data sets is consistent with the universal method of scientific hypothesis testing. Rather, it is argued that the use of a larger combined data set increases the informativeness and explanatory power of the scientific hypothesis (Kluge, 1989; Eernisse and Kluge, 1993).

There is no question that systematists are interested in utilizing all the information available to most accurately reconstruct phylogenetic relationships; the issue is how best to utilize the information. Prior agreement recognizes that in some cases, combining data in a single analysis is warranted (for example, in the analysis of linked mitochondrial genes). However, when significantly incongruent trees are obtained in separate analyses, the data cannot be combined unless the cause of heterogeneity is identified and accommodated by a differential weighting scheme in parsimony analysis (Bull et al., 1993). A common criticism of the prior agreement approach is that no suggestions are made as to how to proceed if the data sets are significantly heterogeneous and the cause of heterogeneity cannot be determined. This apparent problem can be circumvented in part through the use of the maximum likelihood method of phylogeny estimation (Huelsenbeck et al., 1994). In likelihood analysis of the combined data set, substitution model parameters (base frequencies, transformation probabilities, and rate variation among sites) can be estimated for each gene although in practice such analyses are computationally intensive and time limiting. Another criticism leveled at the prior agreement approach is that the individual data sets are themselves assumed to be internally homogeneous, an assumption which is frequently violated (Siddall, 1997). In such cases, carrying the prior agreement approach



to the extreme would result in conducting separate analyses on the different character partitions in the individual data set (e.g., conduct separate analyses for third codon position characters and first and second codon position characters).

Despite all the attention to the issue of taxonomic versus character congruence, the opposing viewpoints still represent one of the most contentious issues in contemporary systematics. That the debate remains unsettled is best evidenced by the position of one prominent researcher who once argued strongly in favor of character congruence (Miyamoto, 1985), but who has recently abjured this view in favor of taxonomic congruence (Miyamoto and Fitch, 1995). In light of this unresolved controversy, many systematists present the results derived from both types of analysis, an approach taken in this chapter. Up until this time, the results presented have been obtained in analyses of the separate data sets. In this chapter the results obtained in analyses of the combined data sets will be presented. Parsimony analyses of the unweighted and weighted combined data will be conducted, as well as likelihood analyses of the combined data. As explained in the materials and methods section, the weighting scheme employed in weighted parsimony analysis was devised to follow the suggestions of Chippendale and Wiens (1994) that character weighting accommodates differences in evolutionary processes in multiple data sets. Parameter values under the HKY85+ model of substitution were estimated from likelihood analysis of the combined data sets. Due to computational limitations, separate parameter values were not estimated for each gene used in the combined analysis. The parameter estimates obtained in analyses of the combined data sets represent global optima and therefore are not optimized for each individual gene. However, several simulation studies have demonstrated the robustness of the likelihood method in obtaining the correct tree under suboptimal substitution model assumptions (Hillis et al., 1994; Huelsenbeck, 1995; Yang, 1996a; Huelsenbeck and Crandall, 1997). The results obtained in the unweighted parsimony, weighted parsimony, and maximum likelihood analyses of the

combined data sets will then be compared to the results obtained in the analyses of the individual data sets.

## MATERIALS AND METHODS

### Taxonomic Sampling

Taxonomic overlap between the 3 data sets was incomplete. COI sequences were determined from the greatest number of taxa, and all of the combined analyses were conducted on a subset of these 56 taxa. The following 4 possible combinations of the data were constructed using the PAUP\* editor to cut and paste individual sequences into the combined data sets: Actin I and Actin II (26 taxa); Actin I and COI (38 taxa); Actin II and COI (32 taxa); Actin I, Actin II, and COI (26 taxa). The alignments used in analyses of the individual data sets were also used to construct the combined data sets.

### Data Analysis

*Sequence Characteristics.* MacClade 3.0 was used to assign codon positions to the combined nucleotide data, to translate nucleic acid sequences into amino acid sequences, and to generate various assumption sets (weight and character inclusion sets, transition or transversion type sets) used in later analyses. For the 26 overlapping taxa, all possible uncorrected sequence divergences were calculated in comparisons of the 3 individual data sets along with the uncorrected sequence divergences for comparisons of the combined data set. Pairwise sequence divergences of the individual data sets were plotted as a function of the sequence divergence of the combined data set to compare the relative evolutionary rates of the 3 genes.

The frequencies of each of the 64 codons were calculated for each of 26 taxa for all 3 genes with the aid of spreadsheet software (McEwan and Gatherer, 1998). For each gene, a codon usage table was constructed by dividing the frequency of occurrence of each codon by the total number of codons specific for the same amino acid. For example, there are

four threonine codons: ACA, ACC, ACG, and ACT. The frequencies of amino acid codon use in a particular gene were calculated for the ACA codon by dividing the number of ACA codons found in a sequence by the total number of threonine codons found in a sequence: frequency of ACA codons = (# ACA codons / (# ACA codons + # ACC codons + # ACG codons + # ACT codons)). The average frequency of codon use was also calculated for the Oegopsida, Myopsida, Sepioidea, Octopoda, and for *Vampyroteuthis* for all three genes.

*Phylogenetic Analyses.* Maximum parsimony analysis of the aligned nucleotide and deduced amino acid sequences was conducted on each of the 4 data sets using the heuristic tree-search option in PAUP\* (Swofford, 1996) with 100 random sequence addition replicates. Support for clades within phylogenetic trees was tested using the heuristic bootstrap search command (1000 replicates) in PAUP\*. A second measure of clade support, the Bremer decay index (Bremer, 1988), was also determined for each clade on the most-parsimonious tree using the software program TreeRot (Sorenson, 1996).

To determine if the data partitions were significantly incongruent, a partition homogeneity test was conducted (Farris et al., 1994). This test was implemented in PAUP\* and involved randomly redistributing the characters in the original data partitions into an equal number of data partitions. A parsimony search was then conducted on each of the data partitions containing a number of characters randomly drawn from each of the original data partitions. The sum of most parsimonious tree lengths were stored for the redistributed data set. One hundred redistributed character sets were constructed and analyzed by unweighted parsimony. The distribution of sums of tree lengths for the randomly redistributed characters were then compared to the sum of tree lengths derived from the analyses of the original data partitions. The frequency with which the sum of tree lengths of the original data partitions exceeded the sum of tree lengths of the replicated data

sets consisting of randomly redistributed characters represented the significance level of the test.

The models of substitution were not tested in maximum likelihood analyses of the combined data sets. These tests were performed on the individual data sets and all resulted in supported the HKY85 model allowing for rate heterogeneity across sites (HKY85+) as the best model of sequence evolution. However, it was not possible to employ the HKY85+ model for the combined data in the same manner as was conducted in the analyses of the individual data sets for two reasons. The first problem encountered when attempting to employ the HKY85+ model of substitution was practical: maximum likelihood analysis of the combined data sets took much longer than analysis of the individual data sets. The difference in computational time requirements was not simply a factor of 2 or 3, as was the case for parsimony analyses. The number of calculations required by likelihood analyses are much more dependent on the number of characters than is parsimony, rising exponentially with an increase in the number of site patterns (Yang, 1996a).

The second problem was encountered when attempting to define a realistic model of substitution. Since the different genes used in this study are likely to perform different functions, the evolutionary processes governing substitution may have been very different. This is evidenced by the different estimates of nucleotide frequencies, the difference in transition:transversion rate estimates, and the difference in estimates of among site rate variation obtained in likelihood analyses of the individual data sets. Independent estimation of all model parameters for each gene in a combined data set would also lead to a practical problem of computational time, consequently increasing the variance in parameter estimates dramatically (Huelsenbeck and Hillis, 1997). The approach taken in this study was similar to that advocated by Yang (1996b), wherein estimates of base frequency and transition:transversion rate bias were obtained by treating the combined data as a single gene, while estimates of gene-specific rate heterogeneity were circumvented by applying a

discrete-gamma model of substitution rates among sites, with the use of 3 rate categories (Yang, 1994b). A critical property of gamma distribution is the shape parameter ( $\alpha$ ), which is related to the inverse of the extent of rate variation among sites. A shape parameter of infinity indicates no rate variation among sites whereas a shape parameter of 0 indicates that each nucleotide site has a substitution rate significantly different from all other sites. Yang (1996b) demonstrated that separate gamma distributions need not be used for each gene in the analysis of combined data sets when the combined data sets consist of similarly conserved (or unconserved) sequences. Yang advocated use of separate gamma models only for extreme cases, as would be the case for a combined data set consisting of a highly conserved protein coding gene and a pseudogene.

Unfortunately, calculation of the gamma shape parameter increased the amount of computational time required in likelihood analyses. The use of the HKY85+ model with gamma distributed rates (HKY85+ $\Gamma$ ) was restricted to maximum likelihood analyses of the two data sets which had the smaller number of taxa, Actin I Actin II, and Actin I, Actin II, COI. Maximum likelihood analyses of the Actin I, COI and Actin II, COI data sets was conducted using a Jukes Cantor (1969) model of substitution, which assumes equal base frequencies and equal probabilities of all substitution types and does not account for rate heterogeneity across sites. Although the JC69 model does not provide the best fit to the data, in simulation studies likelihood analysis under a JC69 model has been shown to outperform unweighted parsimony (Yang, 1996a).

The transition:transversion ratio estimated via maximum likelihood analyses of the individual data sets were then used to construct a perfect weighting step matrix for weighted parsimony analyses of the combined data sets. The step matrix used in weighted parsimony analysis of the combined Actin I and Actin II data set was TVs5:TIs2. When multiple step matrices were used in a single analysis (Actin I, COI; Actin II, COI; Actin I Actin II COI), the character weights for a particular gene were assigned the value equal to the largest step matrix value of the opposite gene to equalize character weights across

genes. For example, the step matrices used in analysis of the Actin I, COI data set were TVs5:TIs2 for the Actin I characters, and TVs7:TIs2 for the COI characters. The Actin I characters were then assigned a weight of 7 while the COI characters were assigned a weight of 5. Thus the total cost for a transversion in an Actin I character would be 35, while the cost of a transition would be 14; the total cost for a transversion in a COI character would also be 35, while that for a transition would be 10. The costs associated with Actin I and COI transversions are equal under this weighting scheme. Not scaling the character weights would have the effect of equating the costs of transitions in the two genes. Since the frequencies of transversions are more likely to be similar across different protein-coding genes than the frequencies of transitions, it is preferable to equate the costs of transversions, thereby downweighting the cost of transitions in the gene with the stronger transition bias (Chippendale and Wiens, 1994). One hundred random addition replicate heuristic parsimony searches of the weighted data were conducted, along with bootstrap analysis to determine support for various clades in the weighted parsimony analysis.

## RESULTS

### Sequence Variation and Divergence

The mean sequence divergences for all pairwise comparisons among the 26 taxa in the restricted data set differed for the three genes examined. The mean divergences and standard deviations for the Actin I, Actin II, and COI genes were 8.3 +/- 2.6%, 11.4 +/- 4.2%, and 19.5 +/- 2.1%, respectively. Pairwise comparisons were also made for the combined data of 2225 characters to examine the relative proportion of differences due to each gene. The sequence divergence for each gene is plotted as a function of total sequence divergence for all possible pairwise comparisons in Figure 36. Thus, the individual values on the Y-axis do not sum up to the value on the X-axis for each pairwise comparison because X-axis values represent sequence divergences obtained from pairwise comparisons of the combined data set. As expected from the mean sequence divergences listed above, the COI gene exhibited the greatest sequence divergences followed by the Actin II and Actin I genes, respectively. At low total sequence divergences, the COI gene accounted for the majority of total sequence divergence. At high total sequence divergences the relative contribution of the COI gene to total sequence divergence was substantially reduced. The slopes and change in slopes for each gene are informative about the relative importance of saturation in the data. The range of sequence divergence for each gene is also potentially informative. The Actin II gene showed the greatest range in sequence divergences while the Actin I and COI genes exhibited much less range in divergence. The range of sequence divergence may indicate at what level the gene of interest is informative and for these data suggest that the Actin II gene may perhaps be informative for a greater range of divergences among taxa than are the Actin I and COI genes.



## Codon Usage

The codon usage frequencies of 26 taxa for the COI gene are given in Table 9. The frequency of each codon was determined by summing the number of occurrences of the codon in the sequence divided by the total number of occurrences of codons specifying the same amino acid. In all 26 taxa, there was a strong and consistent bias against codons ending in guanine. This finding is consistent the results presented earlier in Figure 8, where guanines at third codon positions accounted for ~5% of the 4 possible character states. Although the chi-square test resulted in significant heterogeneity among taxa with respect to character states at third codon position characters, the pattern was not due to obvious differences in codon usage among well-defined taxonomic groups. However, it does appear that the bias against guanines at third codon positions is most extreme for the incirrate octopods (e.g., AGN and TCN serine codons). For a few amino acids, codon usage of some taxa appeared to differ significantly from others. For example, the threonine codon usage patterns for the Ommastrephidae were quite distinct from all other taxa.

Table 10 presents the codon usage frequencies for the Actin I gene. The bias against guanine at third codon positions was not quite as pronounced for the Actin I gene as it was for the COI gene, as the bias was limited primarily to fourfold degenerate amino acid codons. Twofold degenerate codons exhibited no such bias although there was an overall bias against purines at third positions for all fourfold and sixfold degenerate codons. Unlike the COI gene, none of the higher level taxonomic groups displayed a clear difference in codon usage patterns of the Actin I gene.

The codon usage frequencies for the Actin II gene are presented in Table 11. Similar to the Actin I codon usage patterns, there was a bias against purines at third positions, however, there was not a consistent pattern in preference for adenines or guanines at twofold, fourfold, and sixfold degenerate sites. For example, the fourfold degenerate threonine codons were biased against purines at the third position, but the bias was

stronger against guanines than it was against adenines. Inspection of alanine codons showed a bias against purines but in this case the bias is stronger against adenines.

For ease of comparison, the mean frequencies of codon use for the three genes for major groups of cephalopod taxa are presented in Table 12. When the codon usage data are presented in this format, codon usage patterns are easier to compare among taxonomic groups and among genes. Although the Actin I and Actin II genes code for related products whose amino acid sequences are quite similar, codon usage patterns for the two genes are quite different. The histidine and cysteine codons of all major cephalopod groups exhibited a preference for third position cytosines for the Actin I gene while thymines were preferred at third positions for Actin II arginine codons. Taxon specific differences in codon usage patterns were evident for lysine codons. Oegopsid Actin I lysine codons showed a slight preference for guanines while oegopsid Actin II lysine codons showed no such preference. Sepioid Actin I lysine codons also showed a slight preference for guanines while sepoid Actin II lysine codons exhibited a slight preference for adenines. On the other hand, both octopods and *Vampyroteuthis* showed a slight preference for third position guanines at both Actin I and Actin II gene lysine codons. Clear differences in codon usage patterns were evident between nuclear and mitochondrial genes, for example in the codon usage patterns of asparagine. Across all taxa, Actin I and Actin II genes showed a preference for third position cytosines while the converse pattern held for the COI asparagine codons of all taxa, where thymine was preferred.

#### Phylogenetic Analysis

*Actin I and Actin II Combined.* Parsimony analysis of the unweighted combined Actin I and Actin II data resulted in a single most parsimonious tree (Figure 37). The most parsimonious tree supports the monophyly of the Octopoda, Incirrata, Bolitaenidae, Decapodiformes, Myopsida, Cycloteuthidae, Ommastrephidae, Sepiolidae, and Sepiidae. Each of these taxonomic groups was also strongly supported by bootstrap and Bremer

indices. Other relationships supported in parsimony analysis of the Actin I and Actin II data include a (*Chtenopteryx Bathyteuthis*) clade and a (*Sepioloidea* (Sepiolidae)) clade, both of which received strong bootstrap and Bremer support. The partition homogeneity test indicated that the Actin I and Actin II data partitions were significantly incongruent ( $p < 0.01$ ).

Maximum likelihood analysis of the combined Actin I and II data under the JC69 model of substitution resulted in a tree identical to that obtained in the equally-weighted parsimony analysis. Likelihood analysis of the combined Actin I and II data under the HKY85 model of substitution allowing for rate heterogeneity across sites yielded a tree nearly identical to that obtained in the equally weighted parsimony and JC69 likelihood analyses (Figure 38). The only difference in the HKY85 tree is in the branching order of *Thysanoteuthis* and *Cranchia*, where *Thysanoteuthis* branches off prior to *Cranchia* in the HKY85 model as opposed to *Cranchia* branching off before *Thysanoteuthis* in the other two analyses. The difference in the log likelihoods between the JC69 and HKY85+ $\Gamma$  models was substantial (1333.31), indicating that the HKY85+ $\Gamma$  model provided a better fit to the data.

Parsimony analysis of the weighted Actin I and Actin II data resulted in a single most parsimonious tree very similar to that obtained in analysis of the equally weighted data (Figure 39). Two differences in tree structures were in the placement of the Cycloteuthidae and *Thysanoteuthis*. The monophyly of taxonomic groups supported in analysis of the equally weighted data was also supported in analysis of the weighted data. Bootstrap support also mirrored that obtained in the unweighted analysis with the exception of the (*Spirula* (Loliginidae Sepiidae)) clade which received weak bootstrap support in weighted parsimony analysis.

Parsimony analysis of the deduced amino acid sequences of the Actin I and II genes combined yielded 1080 equally parsimonious trees. The strict consensus of these trees consisted of just 3 clades: Decapodiformes, Octopodiformes, and (*Octopus Cirrothauma*).

The lack of resolution was due to the highly conserved nature of the actin proteins, where only 14 of 522 total characters were phylogenetically informative.

*Actin I and COI Combined.* Parsimony analysis of the combined Actin I and COI genes resulted in a single most parsimonious tree (Figure 40). The monophyly of major taxa supported in analysis of the equally weighted data include Octopoda, Bolitaenidae, Decapodiformes, Myopsida, Sepiidae, Sepiolidae, Enoploteuthidae, Cranchiidae, Cycloteuthidae, Gonatidae, and Ommastrephidae. In addition to these clades, other clades which were supported in both bootstrap and Bremer analyses were (Sepioloidea (Sepiolidae)), (*Chroteuthis Mastigoteuthis*), and (*Graneledone (Cirrothauma (Bolitaenidae))*). Some clades that were not supported in the majority of bootstrap replicates but that received Bremer support were (*Pholidoteuthis (Lepidoteuthis Octopoteuthis)*), (*Histioteuthis (Chroteuthis Mastigoteuthis)*), (*Chtenopteryx Bathyteuthis*), (*Alluroteuthis (Gonatidae)*), and (*Cycloteuthis Discoteuthis*). The partition homogeneity test indicated that the Actin I and COI data partitions were significantly incongruent ( $p < 0.01$ ).

Likelihood analysis of the combined Actin I and COI genes produced a tree dissimilar to that obtained in the unweighted parsimony analysis (Figure 41). The sepioid taxa no longer emerged basal to the remaining decapods, and *Idiosepius* clustered with the enoploteuthids rather than outside of the other sepioids. Interestingly, the tree produced in this analysis was one of the few that came close to supporting the monophyly of the Oegopsida. However, placement of *Idiosepius* with the enoploteuthids would render the Oegopsida, as currently defined, paraphyletic.

Parsimony analysis of the weighted data resulted in a single most parsimonious tree quite different in topology from that resulting from the unweighted data (Figure 42). The weighted data support the monophyly of the same major taxa supported in analysis of the equally weighted data. However, placement of major taxa differed substantially and is

more in accord with the results of the likelihood analysis. This is most evident in placement of Sepioid taxa which, with the exception of *Idiosepius*, tended to cluster together as a crown group along with the myopsids in the weighted analysis. Bootstrap analysis of the weighted data tended to support the same groups as were supported in bootstrap analysis of the equally weighted data. The retention index (RI) of the weighted tree (0.371) was slightly greater than the RI of the tree from the equally weighted data (0.349), indicating that the weighted tree structure is less influenced by homoplastic change.

Analysis of the deduced amino acid sequences of Actin I and COI data resulted in 1895 equally parsimonious trees. Of 480 characters, 58 were parsimony informative. The resulting consensus tree had more structure than the consensus tree generated from the Actin I and Actin II amino acid data. The monophyly of the Decapodiformes, Myopsida, Octopodidae, Bolitaenidae, Sepiidae, Sepiolidae+Sepiadariidae, and Cranchiidae were supported by the amino acid data. Also supported were clades consisting of (*Chiroteuthis Mastigoteuthis*), (*Pyroteuthis (Ommastrephes Onychoteuthis)*), (*Idiosepius* Loliginidae), and (*Spirula Histioteuthis*). Most of the resolution obtained in the analysis of the combined amino acid data can be attributed to the COI gene as changes in the Actin I amino acid sequences were extremely rare.

*Actin II and COI Combined.* Maximum parsimony analysis of the combined Actin II and COI genes yielded two equally parsimonious trees which differed in their placement of *Bathyteuthis*. The strict consensus tree (Figure 43) supports the monophyly of the Octopodiformes, Incirrata, Cirrata, Bolitaenidae, Decapodiformes, Myopsida, Cycloteuthidae, Ommastrephidae, Sepiidae, (*Histioteuthis+Psychroteuthis*) and Sepiolidae. The monophyly of each of these taxonomic groups was also supported in bootstrap and Bremer analyses. Other clades supported by moderate Bremer support values but which were not supported in bootstrap analysis were (*Brachioteuthis (Histioteuthis*

*Psychroteuthis*), (*Gonatus* (Cycloteuthidae)), and (*Cranchia* (Ommastrephidae)). The partition homogeneity test indicated that the Actin II and COI data partitions were significantly incongruent ( $p < 0.01$ ).

Maximum likelihood analysis of the combined Actin II and COI data produced a tree that was largely congruent with the strict consensus tree generated from parsimony analysis of the unweighted data (Figure 44). The topology within the Octopodiformes was identical to that obtained in the unweighted parsimony analysis, as was the placement of the myopsid squids with the Sepiolidae. A few substantial differences occur, including the placement of Sepioloidea, *Enoploteuthis*, and the ommastrephids.

A single most parsimonious tree resulted from analysis of the weighted Actin II and COI data (Figure 45). Bootstrap support was obtained for the monophyly of the same taxa as was obtained in the equally weighted analysis of the data set. The branching order within the Octopodiformes was identical to that obtained in the equally weighted analysis. With respect to the decapods, major differences were found between the equally weighted and the weighted analyses such as in the placement of *Idiosepius*, *Sepioloidea*, *Enoploteuthis*, *Cranchia* and the Sepiolidae. The tree obtained from parsimony analysis of the weighted data exhibited slighted lower homoplasy (RI = 0.415) than the 2 trees resultant from the equally weighted data (RI = 0.395).

Parsimony analysis of the deduced amino acid sequences of the combined Actin II and COI data set yielded 9836 equally parsimonious trees; the strict consensus of these trees provided little resolution, paralleling results obtained from other amino acid analyses. The Decapodiformes and Octopodiformes were found to be monophyletic as were the Ommastrephidae, Loliginidae, Sepiidae, Bolitaenidae. The Octopodiformes were divided into two clades with *Stauroteuthis* and *Vampyroteuthis* joined in one clade by a shared derived amino acid state in all 9836 trees. However, as with the other amino acid analyses, clades defined within the two major clades shared a single state whereas the two major clades were defined by 3-5 synapomorphies, depending on the tree.

*Actin I, Actin II, and COI Combined.* Analysis of the combined Actin I, Actin II, and COI data sets produced 2 equally parsimonious trees that differed only in their placement of *Gonatus onyx*. The strict consensus tree (Figure 46) supports the monophyly of the Octopoda, Incirrata, Bolitaenidae, Decapodiformes, Myopsida, Cycloteuthidae, Ommastrephidae, Sepiidae, and Sepiolidae. All of these clades were also highly supported in bootstrap and Bremer analyses. *Chtenopteryx* and *Bathyteuthis* were found to be related, as was *Sepioloidea* with the sepiolids. The partition homogeneity test indicated that the Actin I, Actin II, and COI data partitions were significantly incongruent ( $p < 0.01$ ).

Maximum likelihood analysis of the combined data set under the HKY85+ $\Gamma$  model of substitution yielded a tree (Figure 47) more similar to the weighted parsimony tree (Figure 48) than to the tree obtained in analysis of the equally weighted data (Figure 46). Parsimony analysis of the weighted data resulted in a single most parsimonious tree which supported the monophyly of the same groups described in the analysis of the equally weighted data. However, the tree structure was quite different when the data were weighted, particularly with respect to the placement of *Enoploteuthis*, *Idiosepius*, Cycloteuthidae, Sepiolidae, *Cranchia*, and *Thysanoteuthis*. Still supported is a close relationship between the Loliginids, Sepiids, and Spirula. The HKY85+ $\Gamma$  tree supported the monophyly of the Octopoda, Incirrata, Bolitaenidae, Decapodiformes, Myopsida, Cycloteuthidae, Ommastrephidae, Sepiidae, and Sepiolidae. As in the parsimony analyses, a close relationship between the loliginids, sepiids, and *Spirula* was supported. Unlike the parsimony results, the maximum likelihood tree also supported a relationship between *Idiosepius* and the well-defined (Sepiolidae *Sepioloidea*) clade. Like the weighted parsimony results, the *Chtenopteryx Bathyteuthis* clade was placed as sister group to the *Spirula*, Loliginidae, Sepiidae clade.

## DISCUSSION

### Sequence Variation and Divergence

The plot of sequence divergence for pairwise comparisons of each gene as a function of the corresponding pairwise comparisons for the average divergence of the combined data indicates that the three genes exhibit different evolutionary patterns. These differences are manifest in two ways: 1) The slope of the lines through data points was different for each of the genes; differences in the slopes correspond to differences in the ranges of sequence divergence obtained from pairwise comparisons for each gene, with a greater slope indicating a greater range in sequence divergences; 2) The relative positions of the data points for each of the three genes differed and represents the relative degrees of conservation of the three genes.

With respect to the slopes, the Actin I and COI genes appear to have the same range of sequence divergences whereas the Actin II data has a greater range of sequence divergences. Although one might conclude that the Actin II data are therefore likely to be more informative at a greater range of divergences than are the Actin I and COI data sets, the results of phylogenetic analyses of the individual data sets do not support this conclusion. The Actin II gene was not more informative than the COI gene throughout a range of divergences, if the degree of concordance among separate methods of phylogeny reconstruction and tree stability are taken as a measure of a gene's information content. Analyses of the Actin II data set were not concordant across different reconstruction methodologies, nor were a greater proportion of nodes supported by bootstrap and Bremer analyses of the Actin II data when compared to support for the COI data.



## Codon Usage

As expected, differences in patterns of codon usage were more significant in comparisons among genes than in comparisons among major taxa within a particular gene (Table 12). A bias was found in the codon usage for COI gene: codons with third position adenines or thymines were favored over those with guanines or cytosines. This is consistent with the general pattern found in the mitochondrial protein-coding genes of cephalopods (Bonnaud et al., 1997) and other metazoans (Brown, 1985; Frati et al., 1997). The octopods and *Vampyroteuthis* exhibited a stronger bias against third position guanine codons than the Oegopsida, Myopsida, or Sepioidea for 9 of the 13 amino acids which can be coded for by third position guanines. Of the four cases where the Octopodiformes did not exhibit the strongest bias against guanines for synonymous codons (glutamine, proline, valine, and TCN serines), the bias was not appreciably stronger for the other taxa and may be attributed primarily to sampling error. That the Octopoda and *Vampyroteuthis* shared this bias pattern provides additional evidence for a relationship between the two groups.

Codon usage patterns in the Actin I and Actin II genes were much more similar to each other than either was to the COI gene. However, significant differences in usage patterns of the two nuclear genes were observed in the codon usage patterns of 8 synonymous codons: arginine, isoleucine, histidine, proline, glutamic acid, valine, cysteine, and in the TCN serines. Codon bias among taxa (orthologous codon bias) has been repeatedly demonstrated; on the other hand, the subject of codon bias among paralogous genes in multigene families has received comparatively little attention. The effect of codon usage bias may have profound implications for phylogenetic analyses when different member genes of multigene families such as actin share the same bias. The bias patterns observed may be due to concerted evolution, selective constraints imposed on the actin genes by tRNA availability, or other factors (He and Haymer, 1995). In such cases, higher homology is found between multigene family members within a species (paralogs) as

opposed to genes between species (Hood et al., 1975; Dover, 1982; Dover and Tautz, 1986). This was clearly not the case for the two actin genes analyzed in this study. The average nucleotide sequence divergence for pairwise comparisons among the nucleotide sequences of the two isoforms within a species ( $18.73\pm\%$ ) was clearly greater than the average within gene nucleotide sequence divergence for comparisons among taxa ( $8.3\pm 2.6\%$  and  $11.4\pm 4.2\%$  for the Actin I and Actin II genes, respectively).

### Phylogenetic Relationships

*Actin I and Actin II Combined.* Parsimony analysis of the unweighted data and maximum likelihood analysis of the Actin I and Actin II data sets combined yielded nearly identical tree topologies, differing only in the basal branching order of *Cranchia* and *Thysanoteuthis* in one of the crown clades. Weighted parsimony analysis also generated a tree very similar to the likelihood tree with the exception that the Cycloteuthidae was placed as sister group to *Thysanoteuthis* rather than with *Gonatus*. The topology of the trees generated from analyses of the combined Actin I and Actin II data sets was the most stable of all the trees presented in this study. The tree stability was indicated by the proportion of nodes supported by high bootstrap and Bremer values and also in the degree of concordance among trees generated by different methods of phylogenetic analysis. Another indication of tree stability were the high CI (0.46) and RI (0.55) values for the unweighted parsimony tree. The CI and RI values were greater for this tree than in trees derived from any of the unweighted analyses of any data set analyzed alone, or for any of the analyses of combined data sets, excluding cases where multiple trees were generated in a single analysis. Such stability does not necessarily imply that the relationships described by the trees are "true"; rather, the stability indicates that similar forces operated on the molecular evolution of the Actin I and Actin II genes. However, the partition homogeneity test demonstrated that there was significant incongruence among the Actin I and Actin II data partitions.

Within the Octopoda, relationships obtained in analysis of the combined actin data sets mirrored relationships obtained in analyses of the separate data sets. Notably, *Graneledone* clustered with the Bolitaenidae rather than with *Octopus*. The finding that *Graneledone* is more closely related to the bolitaenids than it is to the octopodids was strongly supported by bootstrap and Bremer analyses and is consistent throughout this study: all analyses of the individual COI, Actin I, and Actin II data sets support this conclusion (but see discussion of Actin I and COI combined). Since neither of the actin data sets supported a close relationship between the bolitaenids and cirrates, it is not surprising that analysis of the combined data sets did not support such a relationship.

Within the Decapodiformes, the deep branches of the clade were not supported by bootstrap analysis and the low Bremer support values indicate instability. Because the Actin I data were weakly informative for relationships in this region of the tree, the relationships among decapod taxa were driven primarily by Actin II characters. The Actin II tree topology within the Decapodiformes was quite similar to the topology of the consensus tree obtained in unweighted parsimony analysis of the Actin II data set. Differences were mostly due to the absence of *Brachioteuthis* and *Psychroteuthis* in analysis of the combined actin data sets. One exception to this was in the placement of *Thysanoteuthis*, which was very unstable in analyses of the Actin II data alone. Analyses of the combined actin data sets consistently placed *Thysanoteuthis* outside of the ((*Ctenopteryx Bathyteuthis*) (*Spirula* (Sepiidae Loliginidae))) clade. The monophyly of this latter clade was supported in the results of all three methods of tree reconstruction. The clade was supported by a relatively weak Bremer value of five in parsimony analysis of the unweighted data; bootstrap analysis of the transversally weighted data provided weak support for the monophyly of this clade. In contrast, analysis of the Actin II data alone supported the clade with a Bremer value of 2; no support was obtained for monophyly of this clade in bootstrap analysis of the Actin II data. That Actin II characters were mainly responsible for tree topology within the Decapodiformes is also evidenced in the placement

of the (*Sepioloidea* (Sepiolidae)) clade. The Actin I data supported a relationship between this group and the sepiids, loliginids, *Spirula*, *Chtenopteryx*, *Bathyteuthis*, and *Idiosepius*. The Actin II data separated the (*Sepioloidea* (Sepiolidae)) from these other families and placed the clade with another consisting of *Idiosepius* and the Ommastrephidae. The combined data produced the same results as the Actin II data with respect to these relationships.

*Actin I and COI Combined.* The topology of the most parsimonious tree derived from analysis of the unweighted data was driven primarily by COI characters. This is evidenced in the basal placement of the sepioid families within the Decapodiformes (whereas the nuclear data places these families terminally in the Decapodiformes), the monophyly of the (Bolitaenidae Cirrata) clade, and in most of the relationships among the oegopsid families. However, the Actin I characters appeared to influence the relationship of *Sepioloidea* with the sepiolids, and the close relationship between *Chtenopteryx* and *Bathyteuthis*.

Weighted parsimony and maximum likelihood analyses resulted in quite different topologies from the unweighted analysis, and from each other. A greater proportion of relationships consistent with those found in analyses of the Actin I data were supported in the weighted parsimony and likelihood trees. For example, the consistent relationship between gonatid and onychoteuthid squids obtained in all analyses of the Actin I data was also obtained in the weighted parsimony and likelihood analyses. Parsimony analysis of the unweighted data revealed a relationship between the gonatids and *Alluroteuthis*, and placed *Onychoteuthis* with the Ommastrephidae, results consistent with analysis of the COI data. This finding suggests that the weighted parsimony and maximum likelihood methods, which assign more weight to transversal substitutions and avoid the problems of "long branch attraction" by considering change more likely to occur on long branches, are exerting greater influence on COI characters than Actin I characters. That these analyses would affect COI characters more profoundly than Actin I characters is not

surprising in light of the patterns of substitution found in plots of pairwise sequence divergences.

Increased discordance among trees produced from different methods of analysis of the combined data sets is also illustrated in relationships among the Octopoda. Relationships within the octopods were generally well supported in all analyses of the individual data sets. Bootstrap support was usually obtained for most clades, and different reconstruction methodologies supported similar conclusions. However, there were differences in the results obtained from analysis of the COI sequences and the Actin I and Actin II sequences. In the analysis of the Actin I and COI data combined, there were only 5 octopod taxa yet the 3 phylogenetic methods produced 3 incongruent topologies. Analysis of the Actin I data alone produced 2 incongruent topologies among the 3 methods, as did analysis of the COI data alone. Despite the fact that more information was available for constructing relations using the combined data set, analysis of the combined data resulted in decreased stability. Unweighted parsimony analysis of the combined data produced a topology consistent with the parsimony and likelihood results of the COI gene. Likelihood analysis of the combined data produced a result incongruent with all trees obtained in analyses of both the COI and Actin I data sets. Weighted parsimony analysis of the combined data yielded a topology congruent with parsimony and likelihood analysis of the Actin I gene. These results suggest that for coleoid cephalopods, increasing the number of characters used in phylogenetic analysis by combining information from nuclear and mitochondrial genes does not provide increased resolution or stability of phylogenetic trees. As all combinations of the data resulted in significant incongruence among the data partitions, the failure of combined data sets to provide increased resolution was not unexpected.

*Actin II and COI Combined.* In contrast to the results obtained from unweighted parsimony analysis of the combined Actin I and COI data sets, the tree topology of 2 most parsimonious trees obtained in unweighted parsimony analysis of the Actin II and COI data

sets combined was not influenced primarily by COI characters. This is evidenced in the distal placement of most sepioid families within the Decapodiformes, whereas these families emerged as basal decapods in the analyses of the COI gene. Other findings consistent with the Actin II results were support for the monophyly of the incirrates, whereas the incirrates were polyphyletic in analyses of the COI gene, and in some of the relationships among the oegopsid families: basal emergence of *Gonatus* with the Cycloteuthidae, the placement of *Brachioteuthis* outside the (*Histioteuthis Psychroteuthis*) clade, and the position of *Thysanoteuthis*. However, the COI characters appeared to influence the relationship of *Idiosepius* with *Sepioloidea*, and the lack of a close relationship between *Chtenopteryx* and *Bathyteuthis*.

The results of weighted parsimony and maximum likelihood analyses conducted on the combined Actin II and COI data set were identical with respect to relationships within the Octopodiformes. Bootstrap and Bremer analysis of the unweighted data and bootstrap analysis of the weighted data provided moderate to strong support for all nodes within the Octopodiformes with the exception of the node defining the ((*Octopus Argonauta*) (*Graneledone* (*Eledonella Japatella*))) clade. This clade was weakly supported by both measures of clade support in unweighted parsimony analysis. Analyses of the combined data maintained support for a close relationship between *Graneledone* with the bolitaenids obtained in analysis of the Actin II data. Analysis of the combined data also maintained support for the basal position of *Bathypolypus* within the Incirrata and a relationship between *Octopus* and *Argonauta* obtained in analysis of the COI data.

The placement of sepioid families was very unstable across the three methods of analysis: the sister group of each of the five families was found to be different in each of the three methods of analysis. In the separate analyses of the Actin II and COI data the results were also more discordant than in the separate analyses of the Actin I and COI data. However, a relationship was consistently obtained between the Loliginidae and the sepioid families.

*Actin I, Actin II and COI Combined.* Parsimony analysis of the unweighted data and maximum likelihood analysis of the Actin I, Actin II, and COI data sets combined yielded moderately similar tree topologies, differing in the placement of *Cranchia*, *Enoploteuthis*, *Gonatus*, *Idiosepius*, and *Thysanoteuthis*. Weighted parsimony analysis generated a tree very different from the unweighted parsimony and likelihood trees, one major difference being in the placement of the (*Sepioloidea* (Sepiolidae)) clade basal within the decapods and separate from the sepiids, *Spirula*, and Loliginidae. The phylogenetic relationships obtained from analyses of the combined Actin I, Actin II, and COI data sets were not as consistent among different methodologies as the results obtained through analysis of the combined actin data sets alone. Although a comparable proportion of nodes was supported by bootstrapping the unweighted data, the bootstrap values were lower for all nodes except that supporting the Ommastrephidae. Surprisingly, Bremer support values were much greater from the analysis of the Actin I and Actin II genes than for all 3 genes combined. In the analysis of all 3 genes (2225 characters), there were 646 parsimony informative characters in comparison to 376 parsimony informative characters in the analysis of the Actin I and Actin II genes (1568 characters).

As was determined in the other combined analyses, relationships within the Octopoda were consistent across methods, were supported in bootstrap and Bremer analyses, and were concordant with relationships determined from most of the previous analyses. Basal relationships within the decapods were unsupported in bootstrap analyses and instability in this region was also indicated by the very low Bremer support values. The results from analysis of the Actin I and Actin II genes consistently supported a sister-group relationship between the Loliginidae and Sepiidae, with *Spirula* emerging basal to this clade. The (*Chtenopteryx Bathyteuthis*) clade consistently followed directly outside of *Spirula*. Analysis of the Actin I, Actin II, and COI genes combined also supported such a relationship in the likelihood and weighted parsimony analyses, although the results of

unweighted parsimony analysis supported a sister-group relationship between *Spirula* and the Sepiidae with the Loliginidae emerging basal to this clade and the (*Chtenopteryx Bathyteuthis*) clade emerging further outside. The placement of *Idiosepius* was inconsistent across the three methods of tree reconstruction and was also very different from the results obtained in analyses of the Actin I and Actin II genes combined, where a relationship with the ommastrephid squids was always obtained. Instead, *Histioteuthis* grouped with the ommastrephid squids in the combined analyses of all 3 genes, although the relationship was not supported by bootstrap analysis and was weakly supported by the Bremer analysis. Another similarity in the results obtained in analysis of the actin genes alone and analysis of the actin genes with COI was in the early divergence of *Gonatus* and the Cycloteuthidae within the Decapodiformes. As in analysis of the Actin I and Actin II data, unweighted parsimony and likelihood analyses supported this relationship whereas parsimony analysis of the weighted data arrived at a different conclusion regarding the placement of the Cycloteuthidae.

Results from analyses of the combined data sets are difficult to generalize for two reasons. First, the number of analyses conducted (12) precludes a concise summary. Second, the results from the many analyses of the combined data sets were largely incongruent. The incongruence is best explained by differences in the evolutionary rates of the genes. Figure 49 graphically depicts the ln-likelihood scores obtained in likelihood analyses of the three genes under the four models of substitution employed. Although in all cases the HKY85+ model provided the best fit to the data, the relative improvement in likelihood scores with the introduction of additional free parameters is different among the three genes. The Actin I and Actin II genes exhibited similar patterns, although it is apparent that the evolutionary rate of the Actin II gene is significantly greater than the evolutionary rate of the Actin I gene. The COI gene exhibited an even greater evolutionary rate than the Actin II gene. When differences in sizes of the Actin and COI data sets



(784bp versus 657bp) are accounted for, the true difference between the evolutionary rates of the actin genes and the COI gene would most likely be magnified. The problems associated with the analysis of taxa with widely differing evolutionary rates have been recognized for two decades (Felsenstein, 1978). When data sets are combined, an additional source of error may be incorporated into the analysis, particularly when the evolutionary rates of the genes differ to a greater degree than do the evolutionary rates of the taxa.

Figure 36. The uncorrected sequence divergences for Actin I (open circles), Actin II (filled triangles) and COI (filled squares) genes plotted as a function of total uncorrected sequence divergence calculated from the combined data set for all possible pairwise comparisons between the 26 taxa. For any given pairwise comparison, the COI gene exhibited the greatest uncorrected sequence divergence, with a mean  $\pm$  standard deviation of  $19.5 \pm 2.1\%$ . In general, the Actin II data exhibited an intermediate level of uncorrected sequence divergence, with a mean  $\pm$  standard deviation of  $11.4 \pm 4.2\%$ . The Actin I gene was characterized by lowest levels of sequence divergence, with a mean  $\pm$  standard deviation of  $8.3 \pm 2.6\%$ . The slopes and change in slopes in the scatter plots of pairwise comparisons for each gene illustrate the relative importance of saturation in the three data sets. The slopes can be considered to be representative of the range in divergences in which each data set is informative. The Actin II data contain the greatest slope, and suggest that the Actin II data are informative for the broadest range of divergences among taxa. The slopes of the Actin I and COI data are comparable, and suggest that the Actin I and COI data are informative for deep and shallow divergences, respectively. The change in slope with increasing sequence divergence indicates saturation in the data. There appears to be a significant change in slope in the Actin II and COI data at approximately 14% sequence divergence, whereas the slope of the Actin I data remains constant throughout the range of sequence divergences, indicating that the Actin I data are not saturated.

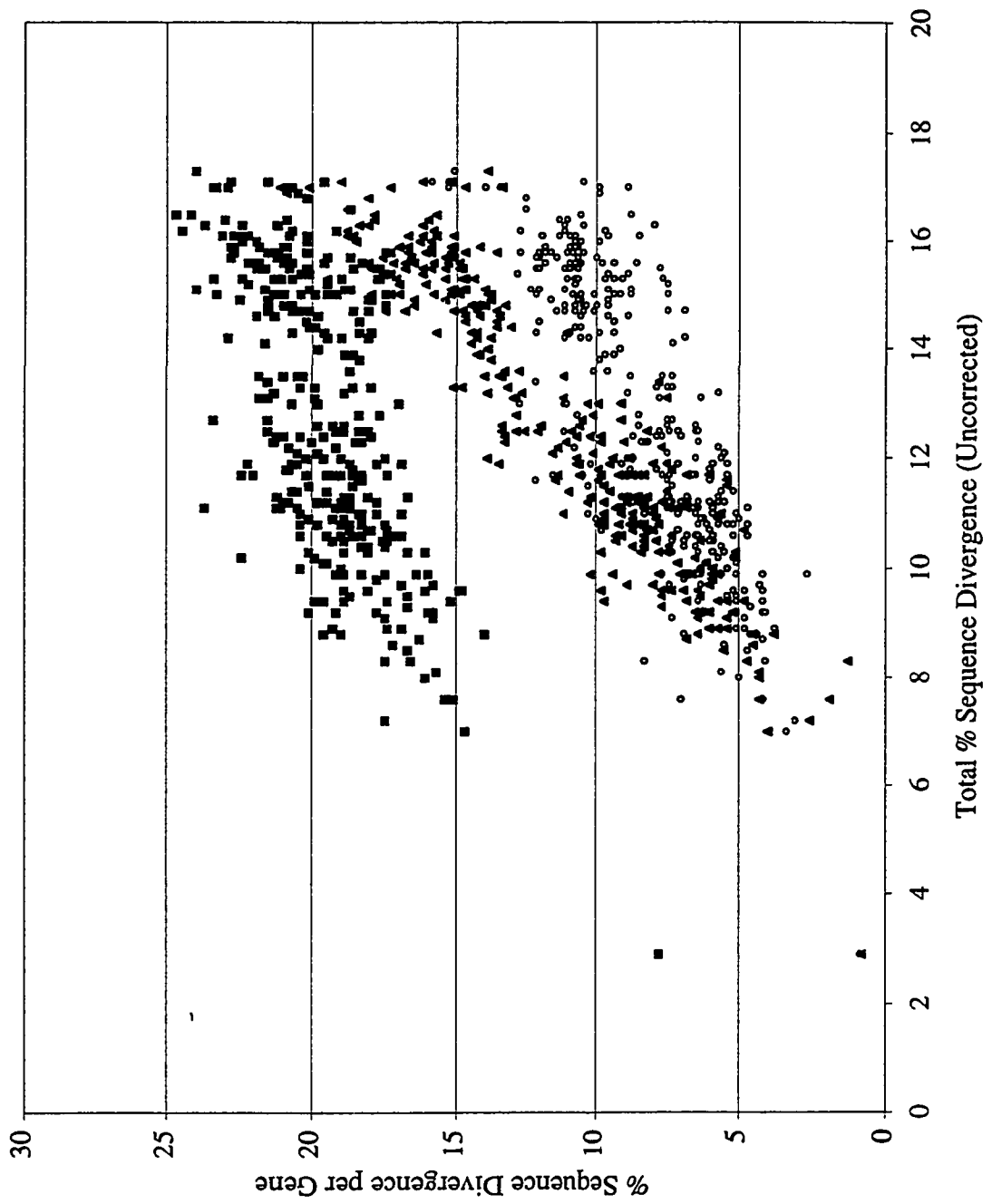


TABLE 10. FREQUENCIES OF COI AMINO ACID CODON USE FOR THE RESTRICTED DATA SET

| Amino Acid | Codon | Idiosepius | Rossia | S. officinalis | S. ophiopara | Sepioides | Spirula | Stoloteuthis | L. pealei | Sepioteuthis | Bathyeuthis | Chenopieryx | Cranchia | Cycloteuthis | Discoteuthis | Enphoteuthis | G. onyx | Histioeuthis | Ommastrephes | Sthenoteuthis | Thysanoteuthis | Eledonella | Graneldome | Japaltella | Ocotopus | Cirrotheuthis | Vampyroteuthis |      |
|------------|-------|------------|--------|----------------|--------------|-----------|---------|--------------|-----------|--------------|-------------|-------------|----------|--------------|--------------|--------------|---------|--------------|--------------|---------------|----------------|------------|------------|------------|----------|---------------|----------------|------|
| Lys        | AAA   | 1          | 1      | 1              | 1            | 1         | 1       | 1            | 1         | 1            | 0           | 0           | 0        | 0            | 0            | 0            | 0       | 0            | 0            | 0             | 0              | 0          | 0          | 0          | 0        | 0             | 0              |      |
| Lys        | AAG   | 0          | 0      | 0              | 0            | 0         | 0       | 0            | 0         | 0            | 0           | 0           | 0        | 0            | 0            | 0            | 0       | 0            | 0            | 0             | 0              | 0          | 0          | 0          | 0        | 0             |                |      |
| Asn        | AAC   | 0.2        | 0.3    | 0.3            | 0.1          | 0.3       | 0.2     | 0.4          | 0.6       | 0.3          | 0.2         | 0.4         | 0.4      | 0.2          | 0.2          | 0.2          | 0.7     | 0.3          | 0.4          | 0.2           | 0.6            | 0.3        | 0          | 0          | 0.1      | 0.2           | 0.1            |      |
| Asn        | AAT   | 0.8        | 0.7    | 0.7            | 0.9          | 0.7       | 0.8     | 0.6          | 0.4       | 0.7          | 0.8         | 0.6         | 0.6      | 0.8          | 0.8          | 0.8          | 0.3     | 0.7          | 0.6          | 0.8           | 0.4            | 0.7        | 0.9        | 0.9        | 0.8      | 0.8           | 0.9            |      |
| Thr        | ACA   | 0.73       | 0.38   | 0.36           | 0.27         | 0.36      | 0.33    | 0.46         | 0.43      | 0.07         | 0.43        | 0.43        | 0.43     | 0.64         | 0.43         | 0.57         | 0.5     | 0.33         | 0.14         | 0.14          | 0.36           | 0.57       | 0.47       | 0.57       | 0.47     | 0.36          | 0.38           |      |
| Thr        | ACC   | 0.07       | 0.23   | 0.14           | 0.33         | 0.14      | 0.2     | 0            | 0.07      | 0.14         | 0.14        | 0.36        | 0.36     | 0            | 0.21         | 0.07         | 0.21    | 0.27         | 0.07         | 0.29          | 0.29           | 0.14       | 0.07       | 0.07       | 0.21     | 0.19          | 0.17           |      |
| Thr        | ACG   | 0          | 0.08   | 0.07           | 0            | 0.07      | 0       | 0.08         | 0         | 0.14         | 0           | 0           | 0        | 0            | 0            | 0            | 0       | 0            | 0            | 0.07          | 0              | 0          | 0          | 0          | 0        | 0.06          | 0              |      |
| Thr        | ACT   | 0.2        | 0.31   | 0.43           | 0.4          | 0.43      | 0.47    | 0.46         | 0.5       | 0.64         | 0.43        | 0.21        | 0.21     | 0.36         | 0.36         | 0.36         | 0.29    | 0.4          | 0.79         | 0.5           | 0.36           | 0.29       | 0.47       | 0.36       | 0.43     | 0.38          | 0.39           |      |
| Ser        | AGA   | 0.17       | 0.16   | 0.1            | 0.16         | 0.09      | 0.18    | 0.21         | 0.16      | 0.17         | 0.22        | 0.17        | 0.17     | 0.18         | 0.28         | 0.22         | 0.11    | 0.22         | 0.06         | 0.22          | 0.22           | 0.26       | 0.12       | 0.25       | 0.11     | 0.12          | 0.27           |      |
| Ser        | AGC   | 0          | 0.05   | 0              | 0.05         | 0         | 0.06    | 0            | 0.00      | 0.00         | 0.00        | 0.00        | 0.00     | 0.00         | 0.00         | 0.00         | 0.11    | 0.00         | 0.17         | 0.00          | 0.06           | 0.00       | 0.00       | 0.00       | 0.00     | 0.00          | 0.00           |      |
| Ser        | AGG   | 0.06       | 0.05   | 0.1            | 0.05         | 0.09      | 0.06    | 0            | 0.05      | 0.00         | 0.06        | 0.06        | 0.06     | 0.00         | 0.00         | 0.00         | 0.06    | 0.06         | 0.06         | 0.00          | 0.00           | 0.00       | 0.00       | 0.00       | 0.00     | 0.00          | 0.00           |      |
| Ser        | AGT   | 0.06       | 0      | 0.1            | 0.05         | 0.09      | 0       | 0.05         | 0.05      | 0.11         | 0.00        | 0.06        | 0.06     | 0.00         | 0.00         | 0.00         | 0.00    | 0.00         | 0.00         | 0.06          | 0.00           | 0.11       | 0.18       | 0.10       | 0.11     | 0.06          | 0.07           |      |
| 2 Ser      | TCA   | 0.17       | 0.39   | 0.25           | 0.37         | 0.25      | 0.23    | 0.25         | 0.29      | 0.27         | 0.22        | 0.11        | 0.11     | 0.24         | 0.39         | 0.17         | 0.17    | 0.17         | 0.11         | 0.11          | 0.06           | 0.17       | 0.11       | 0.41       | 0.15     | 0.33          | 0.30           | 0.13 |
| 2 Ser      | TCC   | 0.06       | 0.25   | 0.2            | 0.1          | 0.2       | 0.18    | 0.14         | 0.20      | 0.22         | 0.22        | 0.00        | 0.00     | 0.18         | 0.06         | 0.17         | 0.27    | 0.22         | 0.22         | 0.22          | 0.17           | 0.05       | 0.00       | 0.15       | 0.11     | 0.23          | 0.07           |      |
| 2 Ser      | TCG   | 0.22       | 0      | 0.15           | 0            | 0.14      | 0       | 0            | 0.00      | 0.00         | 0.06        | 0.06        | 0.06     | 0.00         | 0.00         | 0.00         | 0.00    | 0.00         | 0.00         | 0.06          | 0.00           | 0.00       | 0.00       | 0.00       | 0.00     | 0.00          | 0.06           |      |
| 2 Ser      | TCT   | 0.27       | 0.05   | 0.1            | 0.21         | 0.1       | 0.3     | 0.29         | 0.20      | 0.22         | 0.27        | 0.56        | 0.56     | 0.35         | 0.27         | 0.39         | 0.27    | 0.33         | 0.39         | 0.39          | 0.39           | 0.47       | 0.30       | 0.35       | 0.27     | 0.12          | 0.40           |      |
| Met        | ATA   | 1          | 0.82   | 1              | 1            | 1         | 1       | 1            | 0.93      | 1            | 0.91        | 1           | 1        | 1            | 0.91         | 0.92         | 0.73    | 1            | 0.55         | 0.82          | 0.82           | 1          | 1          | 0.93       | 1        | 1             | 1              |      |
| Met        | ATG   | 0          | 0.18   | 0              | 0            | 0         | 0       | 0            | 0.07      | 0            | 0.09        | 0           | 0        | 0            | 0.09         | 0.08         | 0.27    | 0            | 0.45         | 0.18          | 0.18           | 0          | 0          | 0.07       | 0        | 0             | 0              |      |
| Ileu       | ATC   | 0.18       | 0.23   | 0.19           | 0.24         | 0.19      | 0.19    | 0.07         | 0.13      | 0.24         | 0.19        | 0.19        | 0.19     | 0.56         | 0.38         | 0.13         | 0.31    | 0.19         | 0.18         | 0.24          | 0.24           | 0.31       | 0.06       | 0.13       | 0.06     | 0.41          | 0.05           |      |
| Ileu       | ATT   | 0.82       | 0.77   | 0.81           | 0.76         | 0.81      | 0.81    | 0.93         | 0.88      | 0.76         | 0.81        | 0.81        | 0.81     | 0.44         | 0.63         | 0.88         | 0.69    | 0.81         | 0.82         | 0.76          | 0.76           | 0.69       | 0.94       | 0.87       | 0.94     | 0.59          | 0.95           |      |
| Gln        | CAA   | 1          | 1      | 1              | 1            | 1         | 1       | 1            | 1         | 1            | 1           | 0.67        | 0.67     | 1            | 1            | 1            | 1       | 1            | 1            | 1             | 1              | 1          | 1          | 1          | 1        | 1             | 1              |      |
| Gln        | CAG   | 0          | 0      | 0              | 0            | 0         | 0       | 0            | 0         | 0            | 0           | 0.33        | 0.33     | 0            | 0            | 0            | 0       | 0            | 0            | 0             | 0              | 0          | 0          | 0          | 0        | 0             | 0              |      |
| His        | CAC   | 0.75       | 1      | 0.25           | 0.25         | 0.75      | 0.75    | 0.67         | 1         | 0.5          | 0           | 0.25        | 0.25     | 0.5          | 0            | 0.25         | 0.25    | 0.75         | 0.5          | 0.25          | 0.75           | 0.5        | 0.25       | 0.25       | 0.5      | 0.25          | 0              |      |
| His        | CAT   | 0.25       | 0      | 0.75           | 0.75         | 0.75      | 0.25    | 0.33         | 0         | 0.5          | 1           | 0.75        | 0.75     | 0.5          | 1            | 0.75         | 0.75    | 0.25         | 0.5          | 0.75          | 0.25           | 0.5        | 0.75       | 0.75       | 0.5      | 0.75          | 1              |      |
| Pro        | CCA   | 0.38       | 0.08   | 0.14           | 0.27         | 0.14      | 0.5     | 0.15         | 0.38      | 0.29         | 0.21        | 0.43        | 0.43     | 0.21         | 0.29         | 0.21         | 0.07    | 0.29         | 0.14         | 0.21          | 0.27           | 0.36       | 0.43       | 0.36       | 0.64     | 0.29          | 0.29           |      |
| Pro        | CCC   | 0.15       | 0.38   | 0.14           | 0.2          | 0.14      | 0.14    | 0.15         | 0.23      | 0.07         | 0.36        | 0           | 0        | 0.5          | 0.14         | 0.07         | 0.29    | 0.21         | 0.29         | 0.14          | 0              | 0.14       | 0.14       | 0.21       | 0.07     | 0.43          | 0.07           |      |
| Pro        | CCG   | 0          | 0      | 0              | 0            | 0         | 0       | 0            | 0         | 0            | 0           | 0           | 0        | 0.07         | 0            | 0            | 0       | 0            | 0            | 0.07          | 0              | 0          | 0          | 0.07       | 0        | 0             | 0              |      |
| Pro        | CCT   | 0.46       | 0.54   | 0.71           | 0.53         | 0.71      | 0.36    | 0.69         | 0.38      | 0.64         | 0.43        | 0.57        | 0.57     | 0.21         | 0.57         | 0.71         | 0.64    | 0.5          | 0.57         | 0.57          | 0.67           | 0.5        | 0.43       | 0.36       | 0.29     | 0.64          | 0.64           |      |
| Arg        | CGA   | 0.8        | 0.4    | 0.8            | 0.6          | 0.8       | 0.8     | 1            | 0.6       | 0.6          | 1           | 0.8         | 0.8      | 0.6          | 1            | 0.6          | 0.8     | 0.4          | 0.4          | 0.4           | 0.8            | 0.8        | 0.4        | 0.6        | 1        | 0.6           | 0.8            |      |
| Arg        | CGC   | 0          | 0      | 0.2            | 0.2          | 0.2       | 0       | 0            | 0         | 0.4          | 0           | 0           | 0        | 0            | 0            | 0            | 0       | 0            | 0            | 0             | 0              | 0          | 0          | 0          | 0        | 0             | 0              |      |
| Arg        | CGG   | 0          | 0.2    | 0              | 0            | 0         | 0       | 0            | 0         | 0            | 0           | 0           | 0        | 0            | 0            | 0            | 0.2     | 0            | 0.2          | 0.2           | 0.2            | 0          | 0          | 0          | 0        | 0             | 0              |      |
| Arg        | CGT   | 0.2        | 0.4    | 0              | 0.2          | 0         | 0       | 0            | 0.4       | 0            | 0           | 0.2         | 0.2      | 0.2          | 0            | 0.4          | 0       | 0.4          | 0.4          | 0.4           | 0              | 0.2        | 0.4        | 0.2        | 0        | 0.2           | 0.2            |      |

TABLE 10 (Continued). FREQUENCIES OF COI AMINO ACID CODON USE FOR THE RESTRICTED DATA SET

| Amino Acid | Codon | Idiosepius | Rossia | S. officinalis | S. opipara | Sepioidae | Spirula | Stoloteuthis | L. pealei | Sepioteuthis | Bathyteuthis | Chenopteryx | Cranchia | Cycloteuthis | Discoteuthis | Enpioteuthis | G. onyx | Histioleuthis | Ommastrephes | Sthenoteuthis | Thysanoteuthis | Eledonella | Graneldone | Japallia | Octopus | Cirrotheuthis | Vampyroteuthis |
|------------|-------|------------|--------|----------------|------------|-----------|---------|--------------|-----------|--------------|--------------|-------------|----------|--------------|--------------|--------------|---------|---------------|--------------|---------------|----------------|------------|------------|----------|---------|---------------|----------------|
| Leu        | CTA   | 0.03       | 0.26   | 0.13           | 0.10       | 0.22      | 0.27    | 0.32         | 0.11      | 0.16         | 0.20         | 0.11        | 0.13     | 0.44         | 0.31         | 0.11         | 0.36    | 0.18          | 0.42         | 0.33          | 0.32           | 0.18       | 0.21       | 0.16     | 0.37    | 0.49          | 0.05           |
| Leu        | CTC   | 0.00       | 0.06   | 0.05           | 0.03       | 0.09      | 0.00    | 0.06         | 0.08      | 0.13         | 0.08         | 0.07        | 0.08     | 0.08         | 0.05         | 0.00         | 0.00    | 0.03          | 0.03         | 0.03          | 0.05           | 0.02       | 0.00       | 0.13     | 0.08    | 0.10          | 0.05           |
| Leu        | CTG   | 0.00       | 0.06   | 0.00           | 0.00       | 0.00      | 0.00    | 0.00         | 0.00      | 0.00         | 0.05         | 0.00        | 0.00     | 0.02         | 0.00         | 0.00         | 0.03    | 0.00          | 0.08         | 0.03          | 0.00           | 0.02       | 0.00       | 0.00     | 0.00    | 0.03          | 0.00           |
| Leu        | CTT   | 0.19       | 0.09   | 0.08           | 0.16       | 0.13      | 0.13    | 0.03         | 0.27      | 0.16         | 0.13         | 0.15        | 0.18     | 0.21         | 0.10         | 0.26         | 0.16    | 0.24          | 0.16         | 0.16          | 0.29           | 0.24       | 0.19       | 0.34     | 0.26    | 0.10          | 0.13           |
| 2Leu       | TGA   | 0.71       | 0.50   | 0.64           | 0.66       | 0.49      | 0.56    | 0.60         | 0.46      | 0.49         | 0.54         | 0.62        | 0.57     | 0.28         | 0.49         | 0.55         | 0.43    | 0.53          | 0.21         | 0.41          | 0.29           | 0.38       | 0.59       | 0.37     | 0.29    | 0.26          | 0.78           |
| 2Leu       | TGT   | 0.08       | 0.03   | 0.10           | 0.05       | 0.08      | 0.03    | 0.00         | 0.08      | 0.05         | 0.00         | 0.05        | 0.05     | 0.00         | 0.03         | 0.05         | 0.03    | 0.03          | 0.10         | 0.05          | 0.05           | 0.00       | 0.00       | 0.00     | 0.00    | 0.03          | 0.00           |
| Glu        | GAA   | 0.75       | 1      | 0.75           | 0.5        | 0.75      | 0.67    | 0.75         | 1         | 0.75         | 1            | 1           | 1        | 1            | 1            | 1            | 1       | 1             | 0.75         | 1             | 0.75           | 1          | 1          | 1        | 1       | 1             | 1              |
| Glu        | GAG   | 0.25       | 0      | 0.25           | 0.5        | 0.25      | 0.33    | 0.25         | 0         | 0.25         | 0            | 0           | 0        | 0            | 0            | 0            | 0       | 0             | 0.25         | 0             | 0.25           | 0          | 0          | 0        | 0       | 0             | 0              |
| Asp        | GAC   | 0.14       | 0.33   | 0.71           | 0.29       | 0.71      | 0.29    | 0.67         | 0.57      | 0.71         | 0.71         | 0.29        | 0.29     | 0.86         | 0.71         | 0.29         | 0.43    | 0.29          | 0.57         | 0.43          | 0.71           | 0.43       | 0.43       | 0.14     | 0.14    | 0.29          | 0.13           |
| Asp        | GAT   | 0.86       | 0.67   | 0.29           | 0.71       | 0.29      | 0.71    | 0.33         | 0.43      | 0.29         | 0.29         | 0.71        | 0.71     | 0.14         | 0.29         | 0.71         | 0.57    | 0.71          | 0.43         | 0.57          | 0.29           | 0.57       | 0.57       | 0.86     | 0.86    | 0.71          | 0.88           |
| Ala        | GCA   | 0.4        | 0.31   | 0.23           | 0.27       | 0.23      | 0.5     | 0.31         | 0.36      | 0.29         | 0.4          | 0.27        | 0.27     | 0.21         | 0.5          | 0            | 0.33    | 0.5           | 0.27         | 0.4           | 0.47           | 0.42       | 0.64       | 0.31     | 0.71    | 0.46          | 0.17           |
| Ala        | GCC   | 0.27       | 0.31   | 0.23           | 0.27       | 0.23      | 0.29    | 0.23         | 0.29      | 0.29         | 0.2          | 0.07        | 0.07     | 0.29         | 0.21         | 0.42         | 0.4     | 0.07          | 0.27         | 0             | 0.13           | 0.17       | 0          | 0        | 0       | 0             | 0              |
| Ala        | GCG   | 0          | 0      | 0.15           | 0          | 0.15      | 0       | 0            | 0         | 0            | 0            | 0           | 0        | 0.14         | 0            | 0            | 0       | 0             | 0.13         | 0             | 0              | 0          | 0          | 0        | 0       | 0             | 0              |
| Ala        | GCT   | 0.33       | 0.38   | 0.38           | 0.45       | 0.38      | 0.21    | 0.46         | 0.36      | 0.43         | 0.4          | 0.67        | 0.67     | 0.36         | 0.29         | 0.58         | 0.27    | 0.43          | 0.33         | 0.6           | 0.4            | 0.42       | 0.36       | 0.54     | 0.21    | 0.38          | 0.83           |
| Gly        | GGA   | 0.52       | 0.45   | 0.29           | 0.43       | 0.29      | 0.55    | 0.4          | 0.29      | 0.29         | 0.57         | 0.52        | 0.52     | 0.32         | 0.62         | 0.45         | 0.52    | 0.67          | 0.24         | 0.62          | 0.38           | 0.67       | 0.8        | 0.72     | 0.75    | 0.35          | 0.7            |
| Gly        | GGC   | 0          | 0.15   | 0.05           | 0.1        | 0.05      | 0       | 0.15         | 0.1       | 0.05         | 0.05         | 0.05        | 0.05     | 0.23         | 0.05         | 0.1          | 0.1     | 0             | 0.05         | 0             | 0.1            | 0          | 0          | 0        | 0       | 0.25          | 0              |
| Gly        | GGG   | 0.05       | 0.1    | 0.19           | 0.1        | 0.19      | 0.32    | 0.25         | 0.24      | 0.38         | 0.14         | 0.1         | 0.1      | 0.32         | 0.1          | 0.1          | 0.14    | 0.1           | 0.52         | 0.14          | 0.38           | 0.06       | 0          | 0.17     | 0.05    | 0.2           | 0.1            |
| Gly        | GGT   | 0.43       | 0.3    | 0.48           | 0.38       | 0.48      | 0.14    | 0.2          | 0.38      | 0.29         | 0.24         | 0.33        | 0.33     | 0.14         | 0.24         | 0.35         | 0.24    | 0.24          | 0.19         | 0.24          | 0.14           | 0.28       | 0.2        | 0.11     | 0.2     | 0.2           | 0.2            |
| Val        | GTA   | 0.5        | 0.38   | 0.45           | 0.4        | 0.45      | 0.54    | 0.33         | 0.58      | 0.42         | 0.36         | 0.27        | 0.27     | 0.42         | 0.5          | 0.64         | 0.27    | 0.42          | 0.4          | 0.2           | 0.3            | 0.64       | 0.54       | 0.46     | 0.25    | 0.45          | 0.63           |
| Val        | GTC   | 0          | 0.46   | 0.18           | 0          | 0.18      | 0       | 0            | 0.08      | 0.17         | 0.18         | 0           | 0        | 0.25         | 0.08         | 0.09         | 0.27    | 0.08          | 0            | 0.1           | 0              | 0.07       | 0.08       | 0.15     | 0.17    | 0             | 0              |
| Val        | GTG   | 0          | 0      | 0              | 0          | 0         | 0       | 0.25         | 0         | 0            | 0.09         | 0.18        | 0.18     | 0            | 0.08         | 0.27         | 0.09    | 0.17          | 0            | 0             | 0              | 0          | 0          | 0        | 0       | 0.18          | 0              |
| Val        | GTT   | 0.5        | 0.15   | 0.36           | 0.6        | 0.36      | 0.46    | 0.42         | 0.33      | 0.42         | 0.36         | 0.55        | 0.55     | 0.33         | 0.33         | 0            | 0.36    | 0.33          | 0.6          | 0.7           | 0.7            | 0.29       | 0.38       | 0.38     | 0.58    | 0.36          | 0.38           |
| STOP       | TAA   | 0          | 0      | 0              | 0          | 0         | 0       | 0            | 0         | 0            | 0            | 0           | 0        | 0            | 0            | 0            | 0       | 0             | 0            | 0             | 0              | 0          | 0          | 0        | 0       | 0             | 0              |
| STOP       | TAG   | 0          | 0      | 0              | 0          | 0         | 0       | 0            | 0         | 0            | 0            | 0           | 0        | 0            | 0            | 0            | 0       | 0             | 0            | 0             | 0              | 0          | 0          | 0        | 0       | 0             | 0              |
| Tyr        | TAC   | 0.25       | 0.33   | 0.5            | 0.25       | 0.5       | 0.75    | 0            | 0.5       | 0.25         | 0.5          | 0.25        | 0.25     | 0            | 0.25         | 0.75         | 0.5     | 0.25          | 0.25         | 0.5           | 0.75           | 0.5        | 0          | 0.75     | 0.25    | 0.5           | 0.2            |
| Tyr        | TAT   | 0.75       | 0.67   | 0.5            | 0.75       | 0.5       | 0.25    | 1            | 0.5       | 0.75         | 0.5          | 0.75        | 0.75     | 1            | 0.75         | 0.25         | 0.5     | 0.75          | 0.75         | 0.5           | 0.25           | 0.5        | 1          | 0.25     | 0.75    | 0.5           | 0.8            |
| Trp        | TGA   | 0.83       | 0.8    | 0.5            | 0.83       | 0.5       | 1       | 1            | 0.67      | 0.83         | 0.83         | 0.67        | 0.67     | 1            | 0.83         | 1            | 1       | 1             | 0.67         | 0.83          | 0.83           | 1          | 1          | 1        | 1       | 1             | 0.67           |
| Trp        | TGG   | 0.17       | 0.2    | 0.5            | 0.17       | 0.5       | 0       | 0            | 0.33      | 0.17         | 0.17         | 0.33        | 0.33     | 0            | 0.17         | 0            | 0       | 0             | 0.33         | 0.17          | 0.17           | 0          | 0          | 0        | 0       | 0             | 0.33           |
| Cys        | TGC   | 0          | 0      | 0              | 0          | 0         | 0       | 0            | 0         | 0            | 0            | 0           | 0        | 0            | 0            | 0            | 0       | 0             | 0            | 0             | 0              | 0          | 0          | 0        | 0       | 0             | 0              |
| Cys        | TGT   | 0          | 0      | 0              | 0          | 0         | 0       | 0            | 0         | 0            | 0            | 0           | 0        | 0            | 0            | 0            | 0       | 0             | 0            | 0             | 0              | 0          | 0          | 0        | 0       | 0             | 0              |
| Phe        | TTC   | 0.31       | 0.31   | 0.25           | 0.31       | 0.25      | 0.25    | 0.38         | 0.44      | 0.31         | 0.25         | 0.13        | 0.13     | 0.31         | 0.31         | 0.19         | 0.19    | 0.31          | 0.24         | 0.13          | 0.38           | 0.4        | 0.13       | 0.4      | 0.25    | 0.4           | 0.13           |
| Phe        | TTT   | 0.69       | 0.69   | 0.75           | 0.69       | 0.75      | 0.75    | 0.62         | 0.56      | 0.69         | 0.75         | 0.88        | 0.88     | 0.69         | 0.69         | 0.81         | 0.81    | 0.69          | 0.76         | 0.88          | 0.63           | 0.6        | 0.88       | 0.6      | 0.75    | 0.6           | 0.87           |

TABLE 11. FREQUENCIES OF ACTIN I AMINO ACID CODON USE FOR THE RESTRICTED DATA SET

| Amino Acid | Codon | Idiosepius | Rossia | S. officinalis | S. opipara | Sepioida | Spirula | Sotoluthis | L. pealei | Septoteuthis | Bathyteuthis | Chenoporyx | Cranchia | Cycloteuthis | Discoluthis | Enplotheuthis | G. onyx | Histioteuthis | Ommastrephes | Shenoteuthis | Thysanoteuthis | Eledone | Granelone | Japella | Ocropus | Cirrothaurus | Vampyroteuthis |
|------------|-------|------------|--------|----------------|------------|----------|---------|------------|-----------|--------------|--------------|------------|----------|--------------|-------------|---------------|---------|---------------|--------------|--------------|----------------|---------|-----------|---------|---------|--------------|----------------|
| Lys        | AAA   | 0.5        | 0.5    | 0.42           | 0.33       | 0.5      | 0.33    | 0.42       | 0.33      | 0.29         | 0.33         | 0.42       | 0.33     | 0.5          | 0.33        | 0.42          | 0.33    | 0.25          | 0.42         | 0.5          | 0.33           | 0.42    | 0.4       | 0.42    | 0.5     | 0.5          | 0.33           |
| Lys        | AAG   | 0.5        | 0.5    | 0.58           | 0.67       | 0.5      | 0.67    | 0.58       | 0.67      | 0.7          | 0.67         | 0.58       | 0.67     | 0.5          | 0.67        | 0.58          | 0.67    | 0.75          | 0.58         | 0.5          | 0.67           | 0.58    | 0.6       | 0.58    | 0.5     | 0.5          | 0.67           |
| Asn        | AAC   | 0          | 0.25   | 0.86           | 1          | 0.86     | 0.71    | 0.89       | 1         | 0.75         | 0            | 0          | 0        | 0            | 0.86        | 1             | 1       | 1             | 1            | 0            | 0.86           | 0.71    | 0.63      | 0.71    | 0.71    | 0.71         | 1              |
| Asn        | AAT   | 0          | 0.25   | 0.14           | 0          | 0.14     | 0.29    | 0.11       | 0         | 0.25         | 0            | 0          | 0        | 0            | 0.14        | 0             | 0       | 0             | 0            | 0            | 0.29           | 0.38    | 0.29      | 0.29    | 0.29    | 0            | 0              |
| Thr        | ACA   | 0.19       | 0.14   | 0.19           | 0.33       | 0.19     | 0.24    | 0.1        | 0.05      | 0.13         | 0.1          | 0.05       | 0.15     | 0.05         | 0.1         | 0.14          | 0.14    | 0.1           | 0.14         | 0.14         | 0.24           | 0.15    | 0.19      | 0.19    | 0.24    | 0.24         | 0.19           |
| Thr        | ACC   | 0.67       | 0.71   | 0.38           | 0.43       | 0.62     | 0.52    | 0.62       | 0.81      | 0.69         | 0.81         | 0.8        | 0.6      | 0.67         | 0.67        | 0.71          | 0.76    | 0.67          | 0.76         | 0.67         | 0.7            | 0.62    | 0.52      | 0.57    | 0.48    | 0.52         | 0.67           |
| Thr        | ACG   | 0          | 0      | 0.1            | 0          | 0        | 0       | 0          | 0         | 0            | 0            | 0          | 0        | 0.05         | 0           | 0             | 0       | 0             | 0            | 0            | 0              | 0       | 0         | 0       | 0       | 0            | 0              |
| Thr        | ACT   | 0.14       | 0.14   | 0.33           | 0.24       | 0.19     | 0.24    | 0.29       | 0.14      | 0.19         | 0.1          | 0.15       | 0.25     | 0.24         | 0.24        | 0.14          | 0.1     | 0.24          | 0.1          | 0.1          | 0.15           | 0.19    | 0.29      | 0.19    | 0.29    | 0.24         | 0.14           |
| Arg        | AGA   | 0.23       | 0.08   | 0.15           | 0.08       | 0.08     | 0.08    | 0.08       | 0.23      | 0            | 0.15         | 0.08       | 0.08     | 0.08         | 0.08        | 0.15          | 0.08    | 0.08          | 0.15         | 0.08         | 0.07           | 0.15    | 0.15      | 0.17    | 0.15    | 0.08         | 0.15           |
| Arg        | AGG   | 0.23       | 0.38   | 0.15           | 0.33       | 0.38     | 0.38    | 0.38       | 0.23      | 0.57         | 0.31         | 0.38       | 0.38     | 0.31         | 0.38        | 0.31          | 0.31    | 0.38          | 0.31         | 0.38         | 0.43           | 0.31    | 0.23      | 0.25    | 0.31    | 0.38         | 0.31           |
| 2 Arg      | CGA   | 0          | 0      | 0              | 0          | 0        | 0       | 0          | 0         | 0            | 0            | 0          | 0        | 0            | 0           | 0             | 0       | 0             | 0            | 0            | 0              | 0       | 0         | 0       | 0       | 0            | 0              |
| 2 Arg      | CGC   | 0          | 0      | 0.08           | 0          | 0        | 0       | 0          | 0         | 0            | 0            | 0          | 0        | 0            | 0           | 0             | 0       | 0             | 0            | 0            | 0              | 0       | 0.08      | 0       | 0       | 0            | 0              |
| 2 Arg      | CGG   | 0.54       | 0.54   | 0.62           | 0.58       | 0.54     | 0.46    | 0.54       | 0.54      | 0.43         | 0.54         | 0.46       | 0.46     | 0.54         | 0.54        | 0.54          | 0.54    | 0.54          | 0.54         | 0.46         | 0.5            | 0.54    | 0.54      | 0.58    | 0.46    | 0.54         | 0.54           |
| 2 Arg      | CGT   | 0          | 0      | 0.08           | 0          | 0.08     | 0       | 0          | 0         | 0            | 0            | 0          | 0        | 0.08         | 0           | 0             | 0.08    | 0             | 0            | 0            | 0              | 0       | 0.08      | 0       | 0.08    | 0            | 0              |
| Ser        | AGC   | 0.1        | 0.1    | 0.09           | 0.1        | 0.1      | 0.11    | 0.1        | 0.1       | 0.14         | 0.1          | 0.1        | 0.12     | 0.1          | 0.1         | 0.1           | 0.1     | 0.1           | 0.1          | 0.1          | 0.1            | 0.1     | 0.09      | 0.1     | 0.1     | 0.1          | 0.1            |
| Ser        | AGT   | 0          | 0      | 0              | 0.05       | 0        | 0       | 0          | 0         | 0            | 0            | 0          | 0        | 0            | 0           | 0             | 0       | 0             | 0            | 0            | 0              | 0       | 0         | 0.05    | 0       | 0            | 0              |
| 2 Ser      | TCA   | 0          | 0.1    | 0.22           | 0.05       | 0.05     | 0.11    | 0.15       | 0.1       | 0.07         | 0.1          | 0.12       | 0.05     | 0.14         | 0.1         | 0.1           | 0       | 0.15          | 0.05         | 0            | 0.15           | 0.05    | 0.09      | 0.05    | 0.05    | 0.05         | 0.05           |
| 2 Ser      | TCC   | 0.65       | 0.55   | 0.39           | 0.5        | 0.55     | 0.53    | 0.5        | 0.5       | 0.43         | 0.6          | 0.6        | 0.53     | 0.55         | 0.57        | 0.6           | 0.57    | 0.55          | 0.57         | 0.8          | 0.5            | 0.5     | 0.5       | 0.52    | 0.5     | 0.5          | 0.55           |
| 2 Ser      | TCG   | 0          | 0      | 0              | 0          | 0        | 0       | 0          | 0         | 0            | 0            | 0          | 0        | 0.05         | 0           | 0             | 0       | 0             | 0            | 0            | 0              | 0       | 0         | 0       | 0       | 0            | 0              |
| 2 Ser      | TCT   | 0.25       | 0.25   | 0.3            | 0.3        | 0.3      | 0.26    | 0.25       | 0.3       | 0.36         | 0.2          | 0.2        | 0.24     | 0.25         | 0.19        | 0.2           | 0.33    | 0.2           | 0.29         | 0.1          | 0.25           | 0.35    | 0.32      | 0.29    | 0.35    | 0.35         | 0.3            |
| Ileu       | ATA   | 0          | 0      | 0.05           | 0          | 0        | 0       | 0          | 0         | 0            | 0            | 0          | 0        | 0.1          | 0           | 0             | 0       | 0             | 0            | 0            | 0              | 0       | 0.05      | 0       | 0       | 0            | 0              |
| Ileu       | ATC   | 0.8        | 0.75   | 0.75           | 0.8        | 0.75     | 0.8     | 0.79       | 0.75      | 0.8          | 0.75         | 0.76       | 0.74     | 0.6          | 0.75        | 0.81          | 0.85    | 0.7           | 0.8          | 0.8          | 0.74           | 0.75    | 0.7       | 0.75    | 0.75    | 0.75         | 0.85           |
| Ileu       | ATT   | 0.2        | 0.25   | 0.2            | 0.2        | 0.25     | 0.2     | 0.21       | 0.25      | 0.2          | 0.25         | 0.24       | 0.26     | 0.3          | 0.25        | 0.19          | 0.15    | 0.3           | 0.2          | 0.2          | 0.26           | 0.25    | 0.25      | 0.25    | 0.25    | 0.25         | 0.15           |
| Met        | ATG   | 1          | 1      | 1              | 1          | 1        | 1       | 1          | 1         | 1            | 1            | 1          | 1        | 1            | 1           | 1             | 1       | 1             | 1            | 1            | 1              | 1       | 1         | 1       | 1       | 1            | 1              |
| Gln        | CAA   | 0.6        | 0.5    | 0.17           | 0.33       | 0.33     | 0.67    | 0.5        | 0.5       | 0            | 0.17         | 0.33       | 0.5      | 0.33         | 0.5         | 0.33          | 0.5     | 0.67          | 0.5          | 0.33         | 0.5            | 0.33    | 0.67      | 0.33    | 0.5     | 0.67         | 0.5            |
| Gln        | CAG   | 0.4        | 0.5    | 0.83           | 0.67       | 0.67     | 0.33    | 0.5        | 0.5       | 1            | 0.83         | 0.67       | 0.5      | 0.67         | 0.5         | 0.67          | 0.5     | 0.33          | 0.5          | 0.67         | 0.5            | 0.67    | 0.33      | 0.67    | 0.5     | 0.33         | 0.5            |
| Iliis      | CAC   | 0.5        | 0.75   | 0.75           | 0.75       | 0.67     | 0.75    | 0.75       | 0.75      | 0.67         | 0.75         | 0.75       | 0.75     | 0.75         | 0.75        | 0.75          | 0.75    | 0.75          | 0.75         | 0.75         | 0.75           | 0.75    | 0.5       | 0.5     | 0.5     | 0.5          | 0.75           |
| Iliis      | CAT   | 0.5        | 0.25   | 0.25           | 0.25       | 0.33     | 0.25    | 0.25       | 0.25      | 0.33         | 0.25         | 0.25       | 0.25     | 0.25         | 0.25        | 0.25          | 0.25    | 0.25          | 0.25         | 0.25         | 0.25           | 0.5     | 0.5       | 0.5     | 0.5     | 0.5          | 0.25           |
| Pro        | CCA   | 0.07       | 0.14   | 0.07           | 0.14       | 0.14     | 0.13    | 0.08       | 0.07      | 0.17         | 0.14         | 0.29       | 0.21     | 0.14         | 0.14        | 0.36          | 0.36    | 0.36          | 0.29         | 0.21         | 0.14           | 0.14    | 0.08      | 0.14    | 0.29    | 0.14         | 0.14           |
| Pro        | CCC   | 0.79       | 0.64   | 0.36           | 0.64       | 0.57     | 0.6     | 0.85       | 0.79      | 0.33         | 0.79         | 0.64       | 0.57     | 0.71         | 0.71        | 0.5           | 0.43    | 0.57          | 0.5          | 0.64         | 0.71           | 0.71    | 0.75      | 0.71    | 0.5     | 0.71         | 0.71           |
| Pro        | CCG   | 0          | 0      | 0.07           | 0          | 0.07     | 0       | 0          | 0         | 0            | 0            | 0          | 0        | 0.07         | 0           | 0             | 0       | 0             | 0            | 0            | 0              | 0       | 0         | 0       | 0       | 0            | 0              |
| Pro        | CCT   | 0.14       | 0.21   | 0.5            | 0.21       | 0.21     | 0.27    | 0.08       | 0.14      | 0.5          | 0.07         | 0.07       | 0.21     | 0.07         | 0.14        | 0.14          | 0.21    | 0.07          | 0.21         | 0.14         | 0.14           | 0.14    | 0.17      | 0.14    | 0.21    | 0.14         | 0.14           |

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TABLE 11(Continued). FREQUENCIES OF ACTIN 1 AMINO ACID USE FOR THE RESTRICTED DATA SET

| Amino Acid | Codon | Idiosepius | Rossia | S. officinalis | S. opipara | Sepioides | Spirula | Stoloteuthis | L. pealii | Sepioteuthis | Bathyeuthis | Chenopieryx | Cranchia | Cycloteuthis | Discoteuthis | Eupoloteuthis | G. onyx | Histioteuthis | Ommastrephes | Sthenoteuthis | Thyaneoteuthis | Eledonella | Graneldome | Japacella | Octopus | Cirrochaetasma | Vampyroteuthis |
|------------|-------|------------|--------|----------------|------------|-----------|---------|--------------|-----------|--------------|-------------|-------------|----------|--------------|--------------|---------------|---------|---------------|--------------|---------------|----------------|------------|------------|-----------|---------|----------------|----------------|
| Leu        | CTA   | 0          | 0      | 0.04           | 0          | 0.04      | 0       | 0            | 0         | 0            | 0           | 0           | 0        | 0.04         | 0            | 0             | 0.04    | 0             | 0            | 0             | 0              | 0.04       | 0          | 0.04      | 0       | 0              | 0              |
| Leu        | CTC   | 0.24       | 0.28   | 0.25           | 0.24       | 0.24      | 0.24    | 0.28         | 0.24      | 0.17         | 0.24        | 0.28        | 0.26     | 0.25         | 0.28         | 0.24          | 0.2     | 0.24          | 0.28         | 0.28          | 0.28           | 0.28       | 0.28       | 0.27      | 0.28    | 0.29           | 0.32           |
| Leu        | CTG   | 0.12       | 0.16   | 0.17           | 0.2        | 0.16      | 0.24    | 0.16         | 0.2       | 0.22         | 0.16        | 0.04        | 0.09     | 0.13         | 0.08         | 0             | 0.12    | 0.12          | 0            | 0.04          | 0.12           | 0.04       | 0.15       | 0.04      | 0.08    | 0.04           | 0.12           |
| Leu        | CTT   | 0.36       | 0.24   | 0.25           | 0.28       | 0.24      | 0.28    | 0.24         | 0.28      | 0.44         | 0.32        | 0.4         | 0.39     | 0.25         | 0.32         | 0.4           | 0.36    | 0.32          | 0.36         | 0.4           | 0.32           | 0.28       | 0.27       | 0.27      | 0.24    | 0.21           | 0.24           |
| 2 Leu      | TTA   | 0          | 0      | 0.04           | 0          | 0         | 0       | 0            | 0         | 0            | 0           | 0           | 0        | 0            | 0            | 0             | 0       | 0             | 0            | 0             | 0.08           | 0.04       | 0.12       | 0         | 0.04    | 0              | 0              |
| 2 Leu      | TTG   | 0.28       | 0.32   | 0.25           | 0.28       | 0.32      | 0.24    | 0.32         | 0.28      | 0.17         | 0.28        | 0.28        | 0.26     | 0.33         | 0.32         | 0.36          | 0.28    | 0.32          | 0.36         | 0.28          | 0.28           | 0.32       | 0.27       | 0.31      | 0.36    | 0.42           | 0.32           |
| Glu        | GAA   | 0.43       | 0.38   | 0.38           | 0.43       | 0.33      | 0.43    | 0.33         | 0.38      | 0.44         | 0.33        | 0.38        | 0.48     | 0.5          | 0.43         | 0.43          | 0.38    | 0.48          | 0.43         | 0.67          | 0.43           | 0.71       | 0.71       | 0.71      | 0.67    | 0.67           | 0.52           |
| Glu        | GAG   | 0.57       | 0.62   | 0.62           | 0.57       | 0.67      | 0.57    | 0.67         | 0.62      | 0.56         | 0.67        | 0.62        | 0.52     | 0.5          | 0.57         | 0.57          | 0.62    | 0.52          | 0.57         | 0.33          | 0.57           | 0.29       | 0.29       | 0.29      | 0.33    | 0.33           | 0.48           |
| Asp        | GAC   | 0.5        | 0.58   | 0.58           | 0.5        | 0.5       | 0.42    | 0.58         | 0.58      | 0.4          | 0.42        | 0.42        | 0.58     | 0.67         | 0.5          | 0.58          | 0.42    | 0.58          | 0.58         | 0.42          | 0.58           | 0.5        | 0.29       | 0.5       | 0.5     | 0.33           | 0.58           |
| Asp        | GAT   | 0.5        | 0.42   | 0.42           | 0.5        | 0.5       | 0.58    | 0.42         | 0.42      | 0.6          | 0.58        | 0.58        | 0.42     | 0.33         | 0.5          | 0.42          | 0.58    | 0.42          | 0.42         | 0.58          | 0.42           | 0.5        | 0.71       | 0.5       | 0.5     | 0.67           | 0.42           |
| Ala        | GCA   | 0          | 0      | 0.05           | 0          | 0         | 0       | 0.04         | 0         | 0            | 0           | 0           | 0.05     | 0            | 0            | 0             | 0       | 0             | 0            | 0             | 0              | 0          | 0          | 0         | 0       | 0              | 0.05           |
| Ala        | GCC   | 0.41       | 0.48   | 0.53           | 0.52       | 0.41      | 0.45    | 0.43         | 0.55      | 0.5          | 0.41        | 0.45        | 0.62     | 0.45         | 0.45         | 0.45          | 0.38    | 0.36          | 0.43         | 0.64          | 0.45           | 0.32       | 0.32       | 0.41      | 0.36    | 0.36           | 0.27           |
| Ala        | GCG   | 0          | 0      | 0              | 0          | 0         | 0       | 0            | 0         | 0            | 0           | 0           | 0        | 0            | 0            | 0             | 0       | 0             | 0            | 0             | 0              | 0          | 0          | 0         | 0       | 0              | 0              |
| Ala        | GCT   | 0.59       | 0.52   | 0.42           | 0.48       | 0.59      | 0.55    | 0.52         | 0.45      | 0.5          | 0.59        | 0.55        | 0.33     | 0.55         | 0.55         | 0.55          | 0.62    | 0.64          | 0.57         | 0.36          | 0.55           | 0.68       | 0.68       | 0.59      | 0.64    | 0.64           | 0.68           |
| Gly        | GGA   | 0.13       | 0.13   | 0.24           | 0.13       | 0.19      | 0.19    | 0.19         | 0.06      | 0.14         | 0.13        | 0.19        | 0.06     | 0.12         | 0.13         | 0.13          | 0.25    | 0.19          | 0.06         | 0.13          | 0.13           | 0.06       | 0.07       | 0.07      | 0.06    | 0.06           | 0.06           |
| Gly        | GGC   | 0.06       | 0.06   | 0.06           | 0          | 0.06      | 0.06    | 0.06         | 0.06      | 0            | 0.06        | 0.06        | 0.13     | 0.12         | 0.12         | 0.06          | 0.06    | 0.06          | 0.06         | 0.06          | 0.13           | 0.06       | 0.13       | 0.13      | 0.13    | 0.13           | 0.19           |
| Gly        | GGG   | 0          | 0      | 0              | 0          | 0         | 0       | 0            | 0         | 0            | 0           | 0           | 0        | 0            | 0            | 0             | 0       | 0             | 0            | 0             | 0              | 0          | 0          | 0         | 0       | 0              | 0              |
| Gly        | GGT   | 0.81       | 0.81   | 0.71           | 0.88       | 0.75      | 0.75    | 0.75         | 0.88      | 0.86         | 0.81        | 0.75        | 0.81     | 0.76         | 0.81         | 0.81          | 0.69    | 0.75          | 0.88         | 0.75          | 0.81           | 0.81       | 0.8        | 0.81      | 0.81    | 0.75           | 0.75           |
| Val        | GTA   | 0.08       | 0.08   | 0.15           | 0.15       | 0.08      | 0.23    | 0.08         | 0.15      | 0.25         | 0.15        | 0.14        | 0.23     | 0.15         | 0.08         | 0             | 0       | 0.23          | 0.08         | 0.23          | 0.07           | 0.08       | 0          | 0.08      | 0.08    | 0.08           | 0.23           |
| Val        | GTG   | 0.54       | 0.62   | 0.38           | 0.54       | 0.62      | 0.54    | 0.5          | 0.62      | 0.5          | 0.46        | 0.43        | 0.38     | 0.46         | 0.38         | 0.58          | 0.46    | 0.31          | 0.46         | 0.46          | 0.36           | 0.23       | 0.25       | 0.31      | 0.31    | 0.38           |                |
| Val        | GTG   | 0.08       | 0.08   | 0.15           | 0.15       | 0.08      | 0.08    | 0.08         | 0.08      | 0.13         | 0.08        | 0.07        | 0.08     | 0.08         | 0.08         | 0             | 0       | 0.08          | 0            | 0             | 0.07           | 0.08       | 0.08       | 0         | 0.08    | 0              | 0.08           |
| Val        | GTT   | 0.31       | 0.23   | 0.31           | 0.15       | 0.23      | 0.15    | 0.33         | 0.15      | 0.13         | 0.31        | 0.36        | 0.31     | 0.31         | 0.46         | 0.42          | 0.54    | 0.38          | 0.46         | 0.31          | 0.5            | 0.62       | 0.67       | 0.62      | 0.54    | 0.54           | 0.38           |
| STOP       | TAA   | 0          | 0      | 0              | 0          | 0         | 0       | 0            | 0         | 0            | 0           | 0           | 0        | 0            | 0            | 0             | 0       | 0             | 0            | 0             | 0              | 0          | 0          | 0         | 0       | 0              | 0              |
| STOP       | TAG   | 0          | 0      | 0              | 0          | 0         | 0       | 0            | 0         | 0            | 0           | 0           | 0        | 0            | 0            | 0             | 0       | 0             | 0            | 0             | 0              | 0          | 0          | 0         | 0       | 0              | 0              |
| STOP       | TGA   | 0          | 0      | 0              | 0          | 0         | 0       | 0            | 0         | 0            | 0           | 0           | 0        | 0            | 0            | 0             | 0       | 0             | 0            | 0             | 0              | 0          | 0          | 0         | 0       | 0              | 0              |
| Tyr        | TAC   | 0.67       | 0.5    | 0.5            | 0.54       | 0.5       | 0.42    | 0.42         | 0.67      | 0.38         | 0.58        | 0.58        | 0.55     | 0.62         | 0.58         | 0.64          | 0.55    | 0.67          | 0.73         | 0.67          | 0.67           | 0.58       | 0.75       | 0.58      | 0.58    | 0.83           | 0.75           |
| Tyr        | TAT   | 0.33       | 0.5    | 0.5            | 0.5        | 0.46      | 0.5     | 0.58         | 0.33      | 0.63         | 0.42        | 0.42        | 0.45     | 0.38         | 0.42         | 0.36          | 0.45    | 0.33          | 0.27         | 0.33          | 0.33           | 0.42       | 0.25       | 0.42      | 0.42    | 0.17           | 0.25           |
| Cys        | TGC   | 1          | 0.67   | 1              | 1          | 1         | 1       | 0.67         | 1         | 1            | 0.67        | 1           | 1        | 1            | 1            | 1             | 1       | 1             | 1            | 1             | 0.67           | 0.67       | 0.67       | 0.67      | 1       | 1              | 0.67           |
| Cys        | TGT   | 0          | 0.33   | 0              | 0          | 0         | 0.33    | 0            | 0         | 0            | 0.33        | 0           | 0        | 0.33         | 0            | 0             | 0       | 0             | 0            | 0.33          | 0              | 0.33       | 0.33       | 0.33      | 0       | 0              | 0.33           |
| Trp        | TGG   | 1          | 1      | 1              | 1          | 1         | 1       | 1            | 1         | 0            | 1           | 1           | 1        | 1            | 1            | 1             | 1       | 1             | 1            | 1             | 1              | 1          | 1          | 1         | 1       | 1              | 1              |
| Phe        | TTC   | 0.89       | 1      | 0.89           | 0.8        | 0.89      | 0.78    | 1            | 0.89      | 1            | 1           | 1           | 0.8      | 0.78         | 0.88         | 0.8           | 1       | 0.89          | 1            | 1             | 0.89           | 0.89       | 0.89       | 1         | 0.78    | 0.8            | 0.89           |
| Phe        | TTT   | 0.11       | 0      | 0.11           | 0.2        | 0.11      | 0.22    | 0            | 0.11      | 0            | 0           | 0           | 0.2      | 0.22         | 0.13         | 0.2           | 0       | 0.11          | 0            | 0             | 0.11           | 0.11       | 0.11       | 0         | 0.22    | 0.2            | 0.11           |

TABLE 12. FREQUENCIES OF ACTIN II AMINO ACID CODON USE FOR THE RESTRICTED DATA SET

| Amino Acid | Codon | Idiosepius | Rossia | S. officinalis | S. oippara | Sepioides | Spirula | Stoloteuthis | L. pealei | Septoteuthis | Babyloteuthis | Chtenopteryx | Cranchia | Cycloteuthis | Discoteuthis | Enopteuthis | G. onyx | Histiotenuthis | Ommastrephes | Sibonoteuthis | Thysanoteuthis | Eledonella | Gracledone | Japarella | Ociopus | Cirrothamna | Vampyroteuthis |
|------------|-------|------------|--------|----------------|------------|-----------|---------|--------------|-----------|--------------|---------------|--------------|----------|--------------|--------------|-------------|---------|----------------|--------------|---------------|----------------|------------|------------|-----------|---------|-------------|----------------|
| Lys        | AAA   | 0.56       | 0.8    | 0.5            | 0.5        | 0.78      | 0.5     | 0.56         | 0.56      | 0.5          | 0.56          | 0.44         | 0.56     | 0.38         | 0.38         | 0.5         | 0.5     | 0.44           | 0.55         | 0.55          | 0.5            | 0.55       | 0.56       | 0.5       | 0.46    | 0.5         | 0.33           |
| Lys        | AAG   | 0.44       | 0.2    | 0.5            | 0.5        | 0.22      | 0.5     | 0.44         | 0.44      | 0.5          | 0.44          | 0.56         | 0.44     | 0.63         | 0.63         | 0.5         | 0.5     | 0.56           | 0.45         | 0.45          | 0.5            | 0.45       | 0.64       | 0.5       | 0.54    | 0.5         | 0.67           |
| Asn        | AAC   | 0.7        | 0.5    | 0.73           | 0.73       | 0.57      | 0.73    | 0.7          | 0.7       | 0.78         | 0.7           | 0.8          | 0.7      | 0.82         | 0.82         | 0.78        | 0.73    | 0.8            | 0.75         | 0.75          | 0.78           | 0.75       | 0.91       | 0.78      | 1       | 0.83        | 0.9            |
| Asn        | AAT   | 0.3        | 0.5    | 0.27           | 0.27       | 0.43      | 0.27    | 0.3          | 0.3       | 0.22         | 0.3           | 0.2          | 0.3      | 0.18         | 0.18         | 0.22        | 0.27    | 0.2            | 0.25         | 0.25          | 0.22           | 0.25       | 0.09       | 0.22      | 0       | 0.17        | 0.1            |
| Thr        | ACA   | 0.15       | 0.06   | 0.3            | 0.3        | 0.26      | 0.32    | 0.2          | 0.2       | 0.26         | 0.3           | 0.3          | 0.3      | 0.1          | 0.1          | 0.1         | 0.1     | 0.25           | 0.35         | 0.3           | 0.15           | 0.2        | 0.24       | 0.2       | 0.15    | 0.1         | 0.09           |
| Thr        | ACC   | 0.4        | 0.47   | 0.35           | 0.35       | 0.42      | 0.42    | 0.45         | 0.5       | 0.53         | 0.45          | 0.5          | 0.45     | 0.65         | 0.6          | 0.65        | 0.8     | 0.65           | 0.4          | 0.45          | 0.4            | 0.35       | 0.41       | 0.35      | 0.45    | 0.45        | 0.5            |
| Thr        | ACG   | 0.05       | 0.06   | 0              | 0          | 0         | 0       | 0            | 0         | 0            | 0             | 0            | 0        | 0.05         | 0            | 0           | 0       | 0              | 0            | 0             | 0              | 0.05       | 0          | 0.05      | 0       | 0           | 0.05           |
| Thr        | ACT   | 0.4        | 0.41   | 0.35           | 0.35       | 0.32      | 0.26    | 0.35         | 0.3       | 0.21         | 0.25          | 0.2          | 0.25     | 0.2          | 0.3          | 0.25        | 0.1     | 0.1            | 0.25         | 0.25          | 0.45           | 0.4        | 0.35       | 0.4       | 0.4     | 0.45        | 0.36           |
| Arg        | AGA   | 0.17       | 0.4    | 0.2            | 0.2        | 0.27      | 0.27    | 0.25         | 0.27      | 0.27         | 0.2           | 0.22         | 0.3      | 0.27         | 0.18         | 0.27        | 0.33    | 0.27           | 0.3          | 0.3           | 0.3            | 0.27       | 0.31       | 0.27      | 0.25    | 0.25        | 0.25           |
| Arg        | AGG   | 0.08       | 0      | 0.1            | 0.1        | 0.09      | 0.09    | 0.08         | 0.09      | 0.09         | 0.11          | 0.11         | 0        | 0.09         | 0.09         | 0.09        | 0.08    | 0.09           | 0            | 0             | 0              | 0          | 0.08       | 0         | 0.08    | 0.08        | 0.08           |
| 2 Arg      | CGA   | 0          | 0      | 0              | 0          | 0.09      | 0       | 0.08         | 0         | 0            | 0.11          | 0            | 0        | 0.09         | 0            | 0.09        | 0.1     | 0              | 0            | 0             | 0              | 0.09       | 0.07       | 0.09      | 0.08    | 0.09        | 0              |
| 2 Arg      | CGC   | 0          | 0      | 0              | 0          | 0         | 0       | 0            | 0         | 0            | 0             | 0            | 0        | 0            | 0            | 0           | 0       | 0              | 0            | 0             | 0              | 0          | 0          | 0         | 0       | 0.09        | 0.14           |
| 2 Arg      | CGG   | 0          | 0      | 0              | 0          | 0         | 0       | 0            | 0         | 0            | 0             | 0            | 0        | 0            | 0            | 0           | 0       | 0              | 0            | 0             | 0              | 0          | 0          | 0         | 0       | 0           | 0              |
| 2 Arg      | CGT   | 0.75       | 0.5    | 0.58           | 0.64       | 0.55      | 0.64    | 0.54         | 0.58      | 0.58         | 0.67          | 0.6          | 0.64     | 0.54         | 0.64         | 0.64        | 0.55    | 0.6            | 0.64         | 0.64          | 0.55           | 0.64       | 0.47       | 0.64      | 0.54    | 0.55        | 0.43           |
| Ser        | AGC   | 0.06       | 0      | 0.11           | 0.11       | 0.06      | 0.09    | 0.06         | 0.09      | 0.09         | 0.11          | 0.11         | 0.08     | 0.09         | 0.09         | 0.09        | 0.06    | 0.09           | 0.08         | 0.08          | 0.08           | 0.05       | 0.05       | 0.05      | 0.06    | 0.06        | 0.08           |
| Ser        | AGT   | 0.03       | 0      | 0              | 0          | 0         | 0       | 0            | 0         | 0            | 0             | 0            | 0.03     | 0            | 0            | 0           | 0       | 0              | 0.03         | 0.03          | 0.03           | 0.05       | 0.03       | 0.05      | 0.03    | 0.03        | 0.08           |
| 2 Ser      | TCA   | 0          | 0      | 0              | 0          | 0         | 0       | 0            | 0         | 0            | 0             | 0            | 0        | 0            | 0            | 0           | 0       | 0              | 0            | 0             | 0              | 0          | 0.03       | 0         | 0       | 0.03        | 0              |
| 2 Ser      | TCC   | 0.23       | 0.41   | 0.36           | 0.39       | 0.33      | 0.4     | 0.38         | 0.4       | 0.4          | 0.39          | 0.36         | 0.31     | 0.37         | 0.37         | 0.43        | 0.41    | 0.43           | 0.33         | 0.33          | 0.39           | 0.32       | 0.32       | 0.32      | 0.26    | 0.34        | 0.32           |
| 2 Ser      | TCG   | 0.34       | 0.41   | 0.33           | 0.33       | 0.33      | 0.34    | 0.35         | 0.34      | 0.34         | 0.33          | 0.33         | 0.33     | 0.34         | 0.34         | 0.34        | 0.35    | 0.34           | 0.33         | 0.33          | 0.33           | 0.32       | 0.32       | 0.32      | 0.32    | 0.34        | 0.29           |
| 2 Ser      | TCT   | 0.34       | 0.18   | 0.19           | 0.17       | 0.27      | 0.17    | 0.21         | 0.17      | 0.17         | 0.17          | 0.19         | 0.25     | 0.2          | 0.2          | 0.14        | 0.18    | 0.14           | 0.22         | 0.22          | 0.17           | 0.26       | 0.27       | 0.26      | 0.31    | 0.2         | 0.24           |
| Ileu       | ATA   | 0.44       | 0.2    | 0.33           | 0.56       | 0.56      | 0.2     | 0.44         | 0.4       | 0.4          | 0.3           | 0.33         | 0.4      | 0.09         | 0.09         | 0.33        | 0.09    | 0.3            | 0.44         | 0.33          | 0.3            | 0.2        | 0.11       | 0.25      | 0.44    | 0.33        | 0.3            |
| Ileu       | ATC   | 0.22       | 0.4    | 0.22           | 0.22       | 0.22      | 0.3     | 0.22         | 0.3       | 0.3          | 0.3           | 0.22         | 0.3      | 0.36         | 0.36         | 0.22        | 0.36    | 0.3            | 0.22         | 0.22          | 0.3            | 0.3        | 0.33       | 0.13      | 0.22    | 0.22        | 0.2            |
| Ileu       | ATT   | 0.33       | 0.4    | 0.44           | 0.22       | 0.22      | 0.5     | 0.33         | 0.3       | 0.3          | 0.4           | 0.44         | 0.3      | 0.55         | 0.55         | 0.44        | 0.55    | 0.4            | 0.33         | 0.44          | 0.4            | 0.5        | 0.56       | 0.63      | 0.33    | 0.44        | 0.5            |
| Met        | ATG   | 1          | 1      | 1              | 1          | 1         | 1       | 1            | 1         | 1            | 1             | 1            | 1        | 1            | 1            | 1           | 1       | 1              | 1            | 1             | 1              | 1          | 1          | 1         | 1       | 1           | 1              |
| Gln        | CAA   | 0.5        | 0.67   | 0.63           | 0.6        | 0.71      | 0.6     | 0.73         | 0.64      | 0.58         | 0.55          | 0.55         | 0.64     | 0.36         | 0.4          | 0.5         | 0.38    | 0.55           | 0.5          | 0.4           | 0.6            | 0.6        | 0.5        | 0.6       | 0.57    | 0.67        | 0.4            |
| Gln        | CAG   | 0.5        | 0.33   | 0.38           | 0.4        | 0.29      | 0.4     | 0.27         | 0.36      | 0.42         | 0.45          | 0.45         | 0.36     | 0.64         | 0.6          | 0.5         | 0.62    | 0.45           | 0.5          | 0.6           | 0.4            | 0.4        | 0.5        | 0.4       | 0.43    | 0.33        | 0.6            |
| His        | CAC   | 0.25       | 0      | 0              | 0          | 0         | 0       | 0            | 0         | 0            | 0             | 0            | 0        | 0.33         | 0.25         | 0           | 0       | 0              | 0            | 0             | 0              | 0          | 0.17       | 0         | 0       | 0.17        | 0.33           |
| His        | CAT   | 0.75       | 1      | 1              | 1          | 1         | 1       | 1            | 1         | 1            | 1             | 1            | 1        | 0.67         | 0.75         | 1           | 1       | 1              | 1            | 1             | 1              | 1          | 0.83       | 1         | 1       | 0.83        | 0.67           |
| Pro        | CCA   | 0          | 0      | 0              | 0          | 0.14      | 0       | 0.13         | 0         | 0            | 0.14          | 0            | 0        | 0            | 0.13         | 0           | 0.14    | 0.14           | 0            | 0             | 0              | 0.13       | 0.13       | 0.13      | 0.13    | 0.13        | 0              |
| Pro        | CCC   | 0          | 0      | 0              | 0          | 0         | 0       | 0            | 0         | 0            | 0             | 0            | 0        | 0            | 0            | 0           | 0       | 0              | 0            | 0             | 0.14           | 0          | 0          | 0         | 0       | 0.13        | 0.25           |
| Pro        | CCG   | 0          | 0      | 0              | 0          | 0         | 0       | 0            | 0         | 0            | 0             | 0            | 0        | 0            | 0            | 0           | 0       | 0              | 0            | 0             | 0              | 0          | 0          | 0         | 0       | 0           | 0              |
| Pro        | CCT   | 1          | 1      | 1              | 1          | 0.86      | 1       | 0.88         | 1         | 1            | 0.86          | 1            | 1        | 1            | 0.88         | 1           | 0.86    | 0.86           | 1            | 1             | 0.86           | 0.88       | 0.88       | 0.88      | 0.88    | 0.88        | 0.75           |



TABLE 12 (Continued). FREQUENCIES OF ACTIN III AMINO ACID CODON USE FOR THE RESTRICTED DATA SET

| Amino Acid | Codon | Idiosepius | Rossia | S. officinalis | S. opipara | Sepiolidea | Spina | Stoloteuthis | L. pealei | Sepioteuthis | Bathyteuthis | Chenopiteryx | Cranchia | Cycloteuthis | Discoteuthis | Enoplateuthis | G. onyx | Histioteuthis | Ommastrephes | Sthenoteuthis | Thysanoteuthis | Eledonella | Granelone | Japaltella | Ocotopus | Cirrothauma | Vampyroteuthis |
|------------|-------|------------|--------|----------------|------------|------------|-------|--------------|-----------|--------------|--------------|--------------|----------|--------------|--------------|---------------|---------|---------------|--------------|---------------|----------------|------------|-----------|------------|----------|-------------|----------------|
| Leu        | CTA   | 0.3        | 0.15   | 0.26           | 0.26       | 0.29       | 0.19  | 0.28         | 0.31      | 0.3          | 0.21         | 0.24         | 0.24     | 0.1          | 0.08         | 0.29          | 0.13    | 0.2           | 0.21         | 0.26          | 0.19           | 0.31       | 0.31      | 0.36       | 0.37     | 0.27        | 0.3            |
| Leu        | CTC   | 0.07       | 0.08   | 0.14           | 0.14       | 0.1        | 0.14  | 0.16         | 0.17      | 0.18         | 0.14         | 0.1          | 0.1      | 0.1          | 0.1          | 0.1           | 0.1     | 0.11          | 0.17         | 0.17          | 0.14           | 0.11       | 0.13      | 0.09       | 0.12     | 0.11        | 0.16           |
| Leu        | CTG   | 0.13       | 0.19   | 0.21           | 0.21       | 0.1        | 0.26  | 0.19         | 0.17      | 0.2          | 0.23         | 0.24         | 0.24     | 0.29         | 0.33         | 0.2           | 0.29    | 0.24          | 0.26         | 0.21          | 0.28           | 0.11       | 0.11      | 0.11       | 0.12     | 0.23        | 0.27           |
| Leu        | CTT   | 0.22       | 0.27   | 0.16           | 0.16       | 0.24       | 0.16  | 0.14         | 0.14      | 0.15         | 0.19         | 0.22         | 0.21     | 0.16         | 0.17         | 0.22          | 0.17    | 0.18          | 0.14         | 0.14          | 0.16           | 0.18       | 0.16      | 0.18       | 0.16     | 0.16        | 0.16           |
| 2 Leu      | TTA   | 0.02       | 0.04   | 0.02           | 0.1        | 0.02       | 0.02  | 0.02         | 0.02      | 0.03         | 0            | 0            | 0        | 0.02         | 0            | 0             | 0.02    | 0             | 0.02         | 0             | 0              | 0.09       | 0.11      | 0.09       | 0.07     | 0           | 0.03           |
| 2 Leu      | TIG   | 0.26       | 0.27   | 0.21           | 0.21       | 0.17       | 0.23  | 0.21         | 0.19      | 0.15         | 0.23         | 0.2          | 0.21     | 0.33         | 0.31         | 0.2           | 0.29    | 0.27          | 0.19         | 0.21          | 0.23           | 0.2        | 0.18      | 0.18       | 0.16     | 0.23        | 0.08           |
| Glu        | GAA   | 0          | 0      | 0              | 0          | 0          | 0     | 0.07         | 0         | 0            | 0            | 0            | 0        | 0.17         | 0.11         | 0             | 0       | 0             | 0            | 0             | 0              | 0          | 0         | 0          | 0        | 0           | 0              |
| Glu        | GAG   | 1          | 1      | 1              | 1          | 1          | 1     | 0.93         | 1         | 1            | 1            | 1            | 1        | 0.83         | 0.89         | 1             | 1       | 1             | 1            | 1             | 1              | 1          | 1         | 1          | 1        | 1           | 1              |
| Asp        | GAC   | 0.5        | 0.75   | 0.5            | 0.25       | 0.33       | 0.33  | 0            | 0.5       | 0.5          | 0.33         | 0.25         | 0.25     | 0.29         | 0.33         | 0.33          | 0.5     | 0             | 0.25         | 0.33          | 0.2            | 0.5        | 0.33      | 0.5        | 0.33     | 0.5         | 0.27           |
| Asp        | GAT   | 0.5        | 0.25   | 0.5            | 0.75       | 0.67       | 0.67  | 1            | 0.5       | 0.5          | 0.67         | 0.75         | 0.75     | 0.71         | 0.67         | 0.67          | 0.5     | 1             | 0.75         | 0.67          | 0.8            | 0.5        | 0.67      | 0.5        | 0.67     | 0.5         | 0.73           |
| Ala        | GCA   | 0          | 0      | 0              | 0          | 0          | 0     | 0            | 0         | 0            | 0.05         | 0            | 0        | 0.06         | 0.07         | 0             | 0       | 0             | 0            | 0             | 0              | 0          | 0         | 0          | 0        | 0           | 0              |
| Ala        | GCC   | 0.67       | 0.67   | 0.61           | 0.57       | 0.65       | 0.69  | 0.74         | 0.57      | 0.55         | 0.5          | 0.57         | 0.55     | 0.5          | 0.6          | 0.59          | 0.53    | 0.6           | 0.67         | 0.68          | 0.55           | 0.56       | 0.54      | 0.56       | 0.52     | 0.55        | 0.36           |
| Ala        | GCG   | 0.1        | 0      | 0.09           | 0.1        | 0.05       | 0.06  | 0.11         | 0.1       | 0.09         | 0.11         | 0.1          | 0.14     | 0.19         | 0.07         | 0.18          | 0.11    | 0.1           | 0.11         | 0.11          | 0.1            | 0.16       | 0.17      | 0.16       | 0.14     | 0.18        | 0.21           |
| Ala        | GCT   | 0.24       | 0.33   | 0.3            | 0.33       | 0.3        | 0.25  | 0.16         | 0.33      | 0.36         | 0.32         | 0.33         | 0.32     | 0.25         | 0.27         | 0.23          | 0.37    | 0.3           | 0.22         | 0.21          | 0.35           | 0.28       | 0.29      | 0.28       | 0.24     | 0.27        | 0.43           |
| Gly        | GGA   | 0.33       | 0.33   | 0.25           | 0.25       | 0.18       | 0.22  | 0.13         | 0.22      | 0.25         | 0.21         | 0.27         | 0.18     | 0.27         | 0.33         | 0.18          | 0.07    | 0.14          | 0.13         | 0.13          | 0.17           | 0          | 0.13      | 0          | 0.08     | 0           | 0.08           |
| Gly        | GGC   | 0.17       | 0.17   | 0.08           | 0.08       | 0.27       | 0.22  | 0.38         | 0.22      | 0.13         | 0.14         | 0.09         | 0.09     | 0.13         | 0.2          | 0.18          | 0.21    | 0.21          | 0.33         | 0.33          | 0.17           | 0.22       | 0.25      | 0.22       | 0.38     | 0.36        | 0.23           |
| Gly        | GGG   | 0          | 0      | 0              | 0          | 0          | 0     | 0            | 0         | 0            | 0            | 0            | 0        | 0            | 0            | 0             | 0       | 0             | 0            | 0             | 0              | 0          | 0         | 0          | 0        | 0           | 0              |
| Gly        | GGT   | 0.5        | 0.5    | 0.67           | 0.67       | 0.55       | 0.56  | 0.5          | 0.56      | 0.63         | 0.64         | 0.64         | 0.73     | 0.6          | 0.47         | 0.64          | 0.71    | 0.64          | 0.53         | 0.53          | 0.67           | 0.78       | 0.63      | 0.78       | 0.54     | 0.64        | 0.69           |
| Val        | GTA   | 0          | 0      | 0              | 0          | 0          | 0     | 0            | 0         | 0            | 0            | 0            | 0        | 0            | 0            | 0             | 0       | 0             | 0            | 0             | 0              | 0          | 0         | 0          | 0        | 0           | 0              |
| Val        | GTC   | 0.55       | 0.5    | 0.36           | 0.36       | 0.45       | 0.2   | 0.36         | 0.39      | 0.41         | 0.18         | 0.38         | 0.36     | 0.23         | 0.36         | 0.31          | 0.2     | 0.23          | 0.31         | 0.33          | 0.36           | 0.4        | 0.44      | 0.4        | 0.38     | 0.45        | 0.33           |
| Val        | GTG   | 0.27       | 0      | 0.18           | 0.18       | 0.09       | 0.2   | 0.09         | 0.11      | 0.12         | 0.09         | 0.08         | 0        | 0.08         | 0.07         | 0.19          | 0.1     | 0.08          | 0.08         | 0.08          | 0.18           | 0.33       | 0.38      | 0.33       | 0.19     | 0.45        | 0.25           |
| Val        | GTT   | 0.18       | 0.5    | 0.45           | 0.45       | 0.45       | 0.6   | 0.55         | 0.5       | 0.47         | 0.73         | 0.54         | 0.64     | 0.69         | 0.57         | 0.5           | 0.7     | 0.69          | 0.62         | 0.58          | 0.45           | 0.27       | 0.19      | 0.27       | 0.44     | 0.09        | 0.42           |
| STOP       | TAA   | 0          | 0      | 0              | 0          | 0          | 0     | 0            | 0         | 0            | 0            | 0            | 0        | 0            | 0            | 0             | 0       | 0             | 0            | 0             | 0              | 0          | 0         | 0          | 0        | 0           | 0              |
| STOP       | TAG   | 0          | 0      | 0              | 0          | 0          | 0     | 0            | 0         | 0            | 0            | 0            | 0        | 0            | 0            | 0             | 0       | 0             | 0            | 0             | 0              | 0          | 0         | 0          | 0        | 0           | 0              |
| STOP       | TGA   | 0          | 0      | 0              | 0          | 0          | 0     | 0            | 0         | 0            | 0            | 0            | 0        | 0            | 0            | 0             | 0       | 0             | 0            | 0             | 0              | 0          | 0         | 0          | 0        | 0           | 0              |
| Tyr        | TAC   | 0.67       | 1      | 0.67           | 0.67       | 0          | 0.67  | 0.5          | 0.67      | 0.67         | 0.67         | 0.67         | 0.5      | 0.5          | 0.5          | 0.5           | 0.5     | 0.5           | 0.67         | 0.5           | 0.5            | 0.67       | 0.67      | 0.5        | 0.67     | 0           | 0              |
| Tyr        | TAT   | 0.33       | 0      | 0.33           | 0.33       | 1          | 0.33  | 0.5          | 0.33      | 0.33         | 0.33         | 0.33         | 0.33     | 0.5          | 0.5          | 0.5           | 0.5     | 0.33          | 0.5          | 0.5           | 0.33           | 0.33       | 0.5       | 0.33       | 1        | 1           | 1              |
| Cys        | TGC   | 0          | 0      | 0              | 0          | 0.5        | 0     | 0.25         | 0         | 0            | 0            | 0            | 0.5      | 0.5          | 0.33         | 0.5           | 0.5     | 0             | 0.5          | 0.5           | 0              | 0          | 0.5       | 0          | 0.67     | 0.5         | 0.4            |
| Cys        | TGT   | 1          | 1      | 1              | 1          | 0.5        | 1     | 0.75         | 1         | 1            | 1            | 1            | 0.5      | 0.5          | 0.67         | 0.5           | 0.5     | 1             | 0.5          | 0.5           | 1              | 1          | 0.5       | 1          | 0.33     | 0.5         | 0.6            |
| Trp        | TGG   | 1          | 1      | 1              | 1          | 1          | 1     | 1            | 1         | 0            | 1            | 1            | 1        | 1            | 1            | 1             | 1       | 1             | 1            | 1             | 1              | 1          | 1         | 1          | 1        | 1           | 1              |
| Phe        | TTC   | 0.78       | 1      | 1              | 0.89       | 0.91       | 1     | 0.78         | 0.86      | 1            | 1            | 1            | 1        | 0.89         | 0.88         | 0.9           | 0.88    | 1             | 0.89         | 0.9           | 1              | 0.91       | 0.77      | 0.91       | 1        | 0.89        | 1              |
| Phe        | TTT   | 0.22       | 0      | 0              | 0.11       | 0.09       | 0     | 0.22         | 0.14      | 0            | 0            | 0            | 0        | 0.11         | 0.13         | 0.1           | 0.13    | 0             | 0.11         | 0.1           | 0              | 0.09       | 0.23      | 0.09       | 0        | 0.11        | 0              |

TABLE 13. MEAN FREQUENCIES OF AMINO ACID CODON USE FOR MAJOR TAXONOMIC GROUPS OF CEPHALOPODS

| GENE       |       | ACTIN I   |         |           |          | ACTIN II       |           |         |           | COI      |                |           |         |           |          |                |
|------------|-------|-----------|---------|-----------|----------|----------------|-----------|---------|-----------|----------|----------------|-----------|---------|-----------|----------|----------------|
| Amino Acid | Codon | Oegopsida | Mypsida | Septoidea | Octopoda | Vampyroteuthis | Oegopsida | Mypsida | Septoidea | Octopoda | Vampyroteuthis | Oegopsida | Mypsida | Septoidea | Octopoda | Vampyroteuthis |
| Lys        | AAA   | 0.38      | 0.31    | 0.43      | 0.45     | 0.33           | 0.49      | 0.53    | 0.60      | 0.47     | 0.33           | 0.49      | 0.53    | 0.60      | 0.47     | 0.33           |
| Lys        | AAG   | 0.62      | 0.69    | 0.57      | 0.55     | 0.67           | 0.51      | 0.47    | 0.40      | 0.53     | 0.67           | 0.51      | 0.47    | 0.40      | 0.53     | 0.67           |
| Asn        | AAC   | 0.94      | 0.88    | 0.87      | 0.69     | 1.00           | 0.77      | 0.74    | 0.67      | 0.85     | 0.90           | 0.77      | 0.74    | 0.67      | 0.85     | 0.90           |
| Asn        | AAT   | 0.06      | 0.13    | 0.13      | 0.31     | 0.00           | 0.23      | 0.26    | 0.33      | 0.15     | 0.10           | 0.23      | 0.26    | 0.33      | 0.15     | 0.10           |
| Thr        | ACA   | 0.12      | 0.09    | 0.20      | 0.22     | 0.19           | 0.21      | 0.23    | 0.23      | 0.18     | 0.09           | 0.21      | 0.23    | 0.23      | 0.18     | 0.09           |
| Thr        | ACC   | 0.71      | 0.75    | 0.56      | 0.54     | 0.67           | 0.55      | 0.52    | 0.41      | 0.40     | 0.50           | 0.55      | 0.52    | 0.41      | 0.40     | 0.50           |
| Thr        | ACG   | 0.00      | 0.00    | 0.01      | 0.00     | 0.00           | 0.00      | 0.00    | 0.02      | 0.02     | 0.05           | 0.00      | 0.00    | 0.02      | 0.02     | 0.05           |
| Thr        | ACT   | 0.16      | 0.17    | 0.22      | 0.24     | 0.14           | 0.24      | 0.26    | 0.35      | 0.40     | 0.36           | 0.24      | 0.26    | 0.35      | 0.40     | 0.36           |
| Arg        | AGA   | 0.10      | 0.12    | 0.11      | 0.14     | 0.15           | 0.27      | 0.27    | 0.25      | 0.27     | 0.25           | 0.27      | 0.27    | 0.25      | 0.27     | 0.25           |
| Arg        | AGG   | 0.35      | 0.40    | 0.32      | 0.30     | 0.31           | 0.06      | 0.09    | 0.08      | 0.05     | 0.08           | 0.06      | 0.09    | 0.08      | 0.05     | 0.08           |
| 2 Arg      | CGA   | 0.02      | 0.00    | 0.01      | 0.02     | 0.00           | 0.04      | 0.00    | 0.02      | 0.08     | 0.00           | 0.04      | 0.00    | 0.02      | 0.08     | 0.00           |
| 2 Arg      | CGC   | 0.00      | 0.00    | 0.01      | 0.02     | 0.00           | 0.01      | 0.00    | 0.00      | 0.02     | 0.14           | 0.01      | 0.00    | 0.00      | 0.02     | 0.14           |
| 2 Arg      | CGG   | 0.51      | 0.49    | 0.55      | 0.53     | 0.54           | 0.00      | 0.00    | 0.00      | 0.00     | 0.00           | 0.00      | 0.00    | 0.00      | 0.00     | 0.00           |
| 2 Arg      | CGT   | 0.01      | 0.00    | 0.02      | 0.03     | 0.00           | 0.61      | 0.58    | 0.60      | 0.57     | 0.43           | 0.61      | 0.58    | 0.60      | 0.57     | 0.43           |
| Ser        | AGC   | 0.10      | 0.12    | 0.10      | 0.10     | 0.10           | 0.09      | 0.09    | 0.07      | 0.05     | 0.08           | 0.09      | 0.09    | 0.07      | 0.05     | 0.08           |
| Ser        | AGT   | 0.00      | 0.00    | 0.01      | 0.01     | 0.00           | 0.01      | 0.00    | 0.00      | 0.04     | 0.08           | 0.01      | 0.00    | 0.00      | 0.04     | 0.08           |
| 2 Ser      | TCA   | 0.09      | 0.09    | 0.10      | 0.06     | 0.05           | 0.00      | 0.00    | 0.00      | 0.01     | 0.00           | 0.00      | 0.00    | 0.00      | 0.01     | 0.00           |
| 2 Ser      | TCC   | 0.59      | 0.47    | 0.52      | 0.50     | 0.55           | 0.37      | 0.40    | 0.36      | 0.31     | 0.32           | 0.37      | 0.40    | 0.36      | 0.31     | 0.32           |
| 2 Ser      | TCG   | 0.00      | 0.00    | 0.00      | 0.00     | 0.00           | 0.34      | 0.34    | 0.35      | 0.32     | 0.29           | 0.34      | 0.34    | 0.35      | 0.32     | 0.29           |
| 2 Ser      | TCT   | 0.22      | 0.33    | 0.27      | 0.33     | 0.30           | 0.19      | 0.17    | 0.22      | 0.26     | 0.24           | 0.19      | 0.17    | 0.22      | 0.26     | 0.24           |
| Ileu       | ATA   | 0.01      | 0.00    | 0.01      | 0.01     | 0.00           | 0.27      | 0.40    | 0.39      | 0.27     | 0.30           | 0.27      | 0.40    | 0.39      | 0.27     | 0.30           |
| Ileu       | ATC   | 0.75      | 0.78    | 0.78      | 0.74     | 0.85           | 0.29      | 0.30    | 0.26      | 0.24     | 0.20           | 0.29      | 0.30    | 0.26      | 0.24     | 0.20           |
| Ileu       | ATT   | 0.24      | 0.23    | 0.22      | 0.25     | 0.15           | 0.44      | 0.30    | 0.35      | 0.49     | 0.50           | 0.44      | 0.30    | 0.35      | 0.49     | 0.50           |
| Met        | ATG   | 1.00      | 1.00    | 1.00      | 1.00     | 1.00           | 1.00      | 1.00    | 1.00      | 1.00     | 1.00           | 1.00      | 1.00    | 1.00      | 1.00     | 1.00           |
| Gln        | CAA   | 0.42      | 0.25    | 0.44      | 0.50     | 0.50           | 0.49      | 0.61    | 0.63      | 0.59     | 0.40           | 0.49      | 0.61    | 0.63      | 0.59     | 0.40           |
| Gln        | CAG   | 0.58      | 0.75    | 0.56      | 0.50     | 0.50           | 0.51      | 0.39    | 0.37      | 0.41     | 0.60           | 0.51      | 0.39    | 0.37      | 0.41     | 0.60           |
| His        | CAC   | 0.73      | 0.71    | 0.70      | 0.50     | 0.75           | 0.05      | 0.00    | 0.04      | 0.07     | 0.33           | 0.05      | 0.00    | 0.04      | 0.07     | 0.33           |
| His        | CAT   | 0.27      | 0.29    | 0.30      | 0.50     | 0.25           | 0.95      | 1.00    | 0.96      | 0.93     | 0.67           | 0.95      | 1.00    | 0.96      | 0.93     | 0.67           |
| Pro        | CCA   | 0.24      | 0.12    | 0.11      | 0.16     | 0.14           | 0.05      | 0.00    | 0.04      | 0.13     | 0.00           | 0.05      | 0.00    | 0.04      | 0.13     | 0.00           |
| Pro        | CCC   | 0.62      | 0.56    | 0.64      | 0.68     | 0.71           | 0.01      | 0.00    | 0.00      | 0.03     | 0.25           | 0.01      | 0.00    | 0.00      | 0.03     | 0.25           |
| Pro        | CCG   | 0.01      | 0.00    | 0.02      | 0.00     | 0.00           | 0.00      | 0.00    | 0.00      | 0.00     | 0.00           | 0.00      | 0.00    | 0.00      | 0.00     | 0.00           |
| Pro        | CCT   | 0.13      | 0.32    | 0.23      | 0.16     | 0.14           | 0.94      | 1.00    | 0.96      | 0.85     | 0.75           | 0.94      | 1.00    | 0.96      | 0.85     | 0.75           |

TABLE 13 (Continued). MEAN FREQUENCIES OF AMINO ACID CODON USE FOR MAJOR TAXONOMIC GROUPS OF CEPHALOPODS

| GENE       |       | ACTINI    |          |          |          | ACTINI II      |           |          |          | COI      |                |           |          |          |          |                |
|------------|-------|-----------|----------|----------|----------|----------------|-----------|----------|----------|----------|----------------|-----------|----------|----------|----------|----------------|
| Amino Acid | Codon | Oegopsida | Myopsida | Septidea | Octopoda | Vampyroteuthis | Oegopsida | Myopsida | Septidea | Octopoda | Vampyroteuthis | Oegopsida | Myopsida | Septidea | Octopoda | Vampyroteuthis |
| Leu        | CTA   | 0.01      | 0.00     | 0.01     | 0.02     | 0.00           | 0.20      | 0.31     | 0.25     | 0.32     | 0.30           | 0.26      | 0.14     | 0.19     | 0.28     | 0.05           |
| Leu        | CTC   | 0.26      | 0.21     | 0.25     | 0.27     | 0.32           | 0.12      | 0.18     | 0.12     | 0.11     | 0.16           | 0.04      | 0.11     | 0.04     | 0.07     | 0.05           |
| Leu        | CTG   | 0.08      | 0.21     | 0.17     | 0.07     | 0.12           | 0.26      | 0.19     | 0.18     | 0.14     | 0.27           | 0.02      | 0.00     | 0.01     | 0.01     | 0.00           |
| Leu        | CTT   | 0.35      | 0.36     | 0.27     | 0.25     | 0.24           | 0.18      | 0.15     | 0.19     | 0.17     | 0.16           | 0.18      | 0.22     | 0.11     | 0.23     | 0.13           |
| 2 Leu      | TTA   | 0.00      | 0.00     | 0.01     | 0.06     | 0.00           | 0.01      | 0.03     | 0.03     | 0.07     | 0.03           | 0.45      | 0.47     | 0.59     | 0.38     | 0.78           |
| 2 Leu      | TTG   | 0.30      | 0.23     | 0.29     | 0.34     | 0.32           | 0.24      | 0.17     | 0.22     | 0.19     | 0.08           | 0.04      | 0.07     | 0.05     | 0.01     | 0.00           |
| Glu        | GAA   | 0.45      | 0.41     | 0.39     | 0.69     | 0.52           | 0.03      | 0.00     | 0.01     | 0.00     | 0.00           | 0.95      | 0.88     | 0.74     | 1.00     | 1.00           |
| Glu        | GAG   | 0.55      | 0.59     | 0.61     | 0.31     | 0.48           | 0.97      | 1.00     | 0.99     | 1.00     | 1.00           | 0.05      | 0.13     | 0.26     | 0.00     | 0.00           |
| Asp        | GAC   | 0.52      | 0.49     | 0.52     | 0.42     | 0.58           | 0.28      | 0.50     | 0.38     | 0.43     | 0.27           | 0.51      | 0.64     | 0.45     | 0.29     | 0.13           |
| Asp        | GAT   | 0.48      | 0.51     | 0.48     | 0.58     | 0.42           | 0.72      | 0.50     | 0.62     | 0.57     | 0.73           | 0.49      | 0.36     | 0.55     | 0.71     | 0.88           |
| Ala        | GCA   | 0.00      | 0.00     | 0.01     | 0.00     | 0.05           | 0.02      | 0.00     | 0.00     | 0.02     | 0.00           | 0.33      | 0.33     | 0.32     | 0.51     | 0.17           |
| Ala        | GCC   | 0.46      | 0.53     | 0.46     | 0.35     | 0.27           | 0.58      | 0.56     | 0.66     | 0.55     | 0.36           | 0.19      | 0.29     | 0.26     | 0.11     | 0.00           |
| Ala        | GCG   | 0.00      | 0.00     | 0.00     | 0.00     | 0.00           | 0.12      | 0.10     | 0.07     | 0.16     | 0.21           | 0.02      | 0.00     | 0.04     | 0.00     | 0.00           |
| Ala        | GCT   | 0.53      | 0.48     | 0.52     | 0.65     | 0.68           | 0.29      | 0.35     | 0.27     | 0.27     | 0.43           | 0.45      | 0.40     | 0.37     | 0.38     | 0.83           |
| Gly        | GGA   | 0.14      | 0.10     | 0.17     | 0.06     | 0.06           | 0.19      | 0.24     | 0.24     | 0.04     | 0.08           | 0.49      | 0.29     | 0.42     | 0.66     | 0.70           |
| Gly        | GGC   | 0.08      | 0.03     | 0.05     | 0.14     | 0.19           | 0.19      | 0.18     | 0.20     | 0.29     | 0.23           | 0.07      | 0.08     | 0.07     | 0.05     | 0.00           |
| Gly        | GGG   | 0.00      | 0.00     | 0.00     | 0.00     | 0.00           | 0.00      | 0.00     | 0.00     | 0.00     | 0.00           | 0.19      | 0.31     | 0.17     | 0.10     | 0.10           |
| Gly        | GGT   | 0.78      | 0.87     | 0.78     | 0.80     | 0.75           | 0.62      | 0.60     | 0.56     | 0.67     | 0.69           | 0.24      | 0.34     | 0.34     | 0.20     | 0.20           |
| Val        | GTA   | 0.12      | 0.20     | 0.12     | 0.06     | 0.23           | 0.00      | 0.00     | 0.00     | 0.00     | 0.00           | 0.37      | 0.50     | 0.44     | 0.47     | 0.63           |
| Val        | GTC   | 0.43      | 0.56     | 0.53     | 0.30     | 0.31           | 0.30      | 0.40     | 0.40     | 0.41     | 0.33           | 0.10      | 0.13     | 0.12     | 0.09     | 0.00           |
| Val        | GTG   | 0.05      | 0.11     | 0.10     | 0.05     | 0.08           | 0.09      | 0.12     | 0.14     | 0.34     | 0.25           | 0.10      | 0.00     | 0.04     | 0.04     | 0.00           |
| Val        | GTT   | 0.40      | 0.14     | 0.24     | 0.60     | 0.38           | 0.61      | 0.49     | 0.45     | 0.25     | 0.42           | 0.44      | 0.38     | 0.41     | 0.40     | 0.38           |
| STOP       | TAA   | 0.00      | 0.00     | 0.00     | 0.00     | 0.00           | 0.00      | 0.00     | 0.00     | 0.00     | 0.00           | 0.00      | 0.00     | 0.00     | 0.00     | 0.00           |
| STOP       | TAG   | 0.00      | 0.00     | 0.00     | 0.00     | 0.00           | 0.00      | 0.00     | 0.00     | 0.00     | 0.00           | 0.00      | 0.00     | 0.00     | 0.00     | 0.00           |
| STOP       | TGA   | 0.00      | 0.00     | 0.00     | 0.00     | 0.00           | 0.00      | 0.00     | 0.00     | 0.00     | 0.00           | 0.39      | 0.38     | 0.37     | 0.40     | 0.20           |
| Tyr        | TAC   | 0.62      | 0.53     | 0.52     | 0.66     | 0.75           | 0.56      | 0.67     | 0.60     | 0.37     | 0.00           | 0.61      | 0.63     | 0.63     | 0.60     | 0.80           |
| Tyr        | TAT   | 0.38      | 0.48     | 0.48     | 0.34     | 0.25           | 0.44      | 0.33     | 0.40     | 0.63     | 1.00           | 0.00      | 0.00     | 0.00     | 0.00     | 0.00           |
| Cys        | TGC   | 0.91      | 1.00     | 0.91     | 0.80     | 0.67           | 0.30      | 0.00     | 0.11     | 0.33     | 0.40           | 0.00      | 0.00     | 0.00     | 0.00     | 0.00           |
| Cys        | TGT   | 0.09      | 0.00     | 0.09     | 0.20     | 0.33           | 0.70      | 1.00     | 0.89     | 0.67     | 0.60           | 0.24      | 0.41     | 0.39     | 0.37     | 0.20           |
| Trp        | TGG   | 1.00      | 0.50     | 1.00     | 1.00     | 1.00           | 1.00      | 0.50     | 1.00     | 1.00     | 1.00           | 0.22      | 0.30     | 0.23     | 0.16     | 0.10           |
| Phe        | TTC   | 0.91      | 0.95     | 0.89     | 0.87     | 0.89           | 0.94      | 0.93     | 0.91     | 0.90     | 1.00           | 0.23      | 0.38     | 0.29     | 0.32     | 0.13           |
| Phe        | TTT   | 0.09      | 0.06     | 0.11     | 0.13     | 0.11           | 0.06      | 0.07     | 0.09     | 0.10     | 0.00           | 0.77      | 0.63     | 0.71     | 0.69     | 0.87           |

Figure 37. Single most parsimonious tree obtained in a heuristic search (100 random addition replicates) of the unweighted Actin I and Actin II data sets combined for 26 taxa (TL = 1581; CI = 0.461; RI = 0.549). Branch lengths are drawn proportion to the number of character changes occurring along the branches. Bootstrap proportions are indicated as percentages below nodes and Bremer support values are indicated above nodes. Higher-level taxonomic designations are indicated in boldface to the right of each terminal taxon (C = Suborder Cirrata; I = Suborder Incirrata; M = Suborder Myopsida; O = Suborder Oegopsida; V = Order Vampyromorpha).

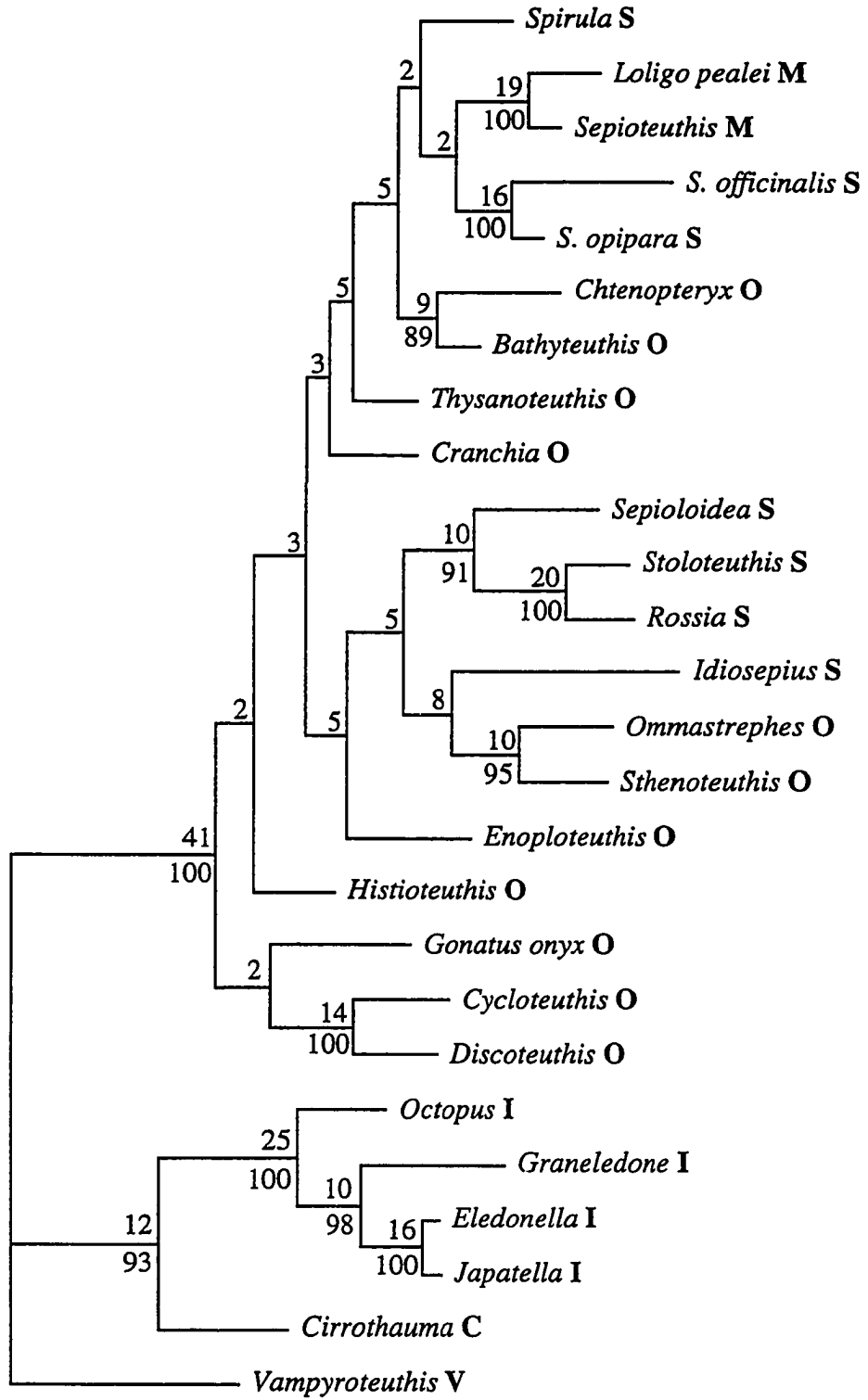


Figure 38. Maximum likelihood tree generated from a heuristic search of the combined Actin I and Actin II combined data assuming a Hasegawa, Kishino, and Yano (1985) model of substitution ( $-\ln L = 9761.48$ ) with gamma distributed rates. Branch lengths are drawn proportional to the probabilities of change occurring along each branch under the HKY85 model. Substitution parameters used in the likelihood search were as follows:  $\pi_A=0.253$ ,  $\pi_C=0.283$ ,  $\pi_G=0.176$ ,  $\pi_T=0.288$ ; TI/TV=2.703;  $\alpha=0.249$ ; proportion invariant sites = 0. Higher-level taxonomic designations are indicated in boldface to the right of each terminal taxon (C = Suborder Cirrata; I = Suborder Incirrata; M = Suborder Myopsida; O = Suborder Oegopsida; V = Order Vampyromorpha).

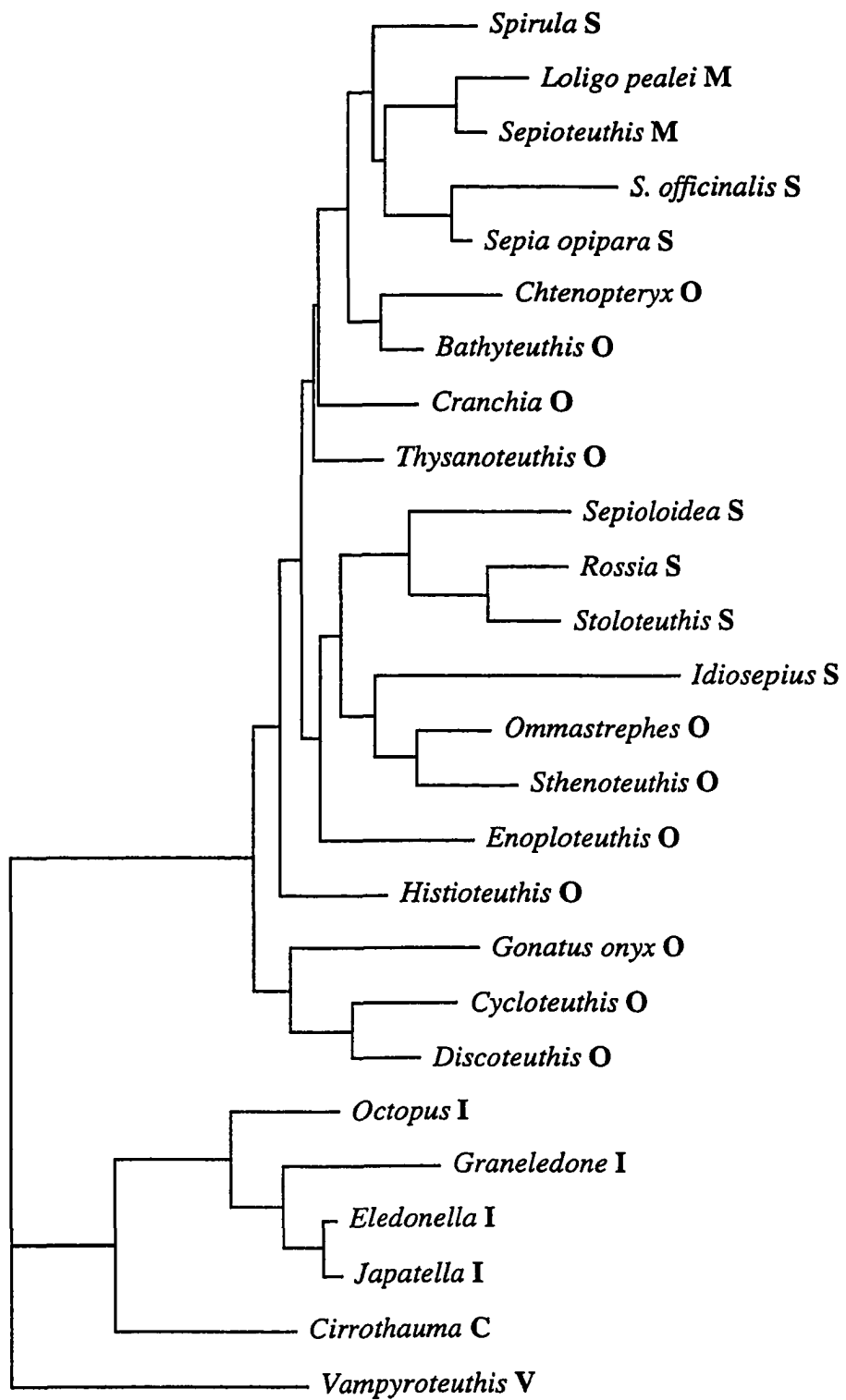


Figure 39. Maximum parsimony tree obtained in a heuristic search (100 random addition replicates) of the weighted Actin I and Actin II combined data sets (TL = 4529; CI = 0.489; RI = 0.564). Transversion substitutions were assigned a weight of five steps and transitions were assigned a weight of two steps. Branch lengths are drawn proportional to the amount of change. Bootstrap proportions are indicated as percentages below nodes. Higher-level taxonomic designations are indicated in boldface to the right of each terminal taxon (C = Suborder Cirrata; I = Suborder Incirrata; M = Suborder Myopsida; O = Suborder Oegopsida; V = Order Vampyromorpha).



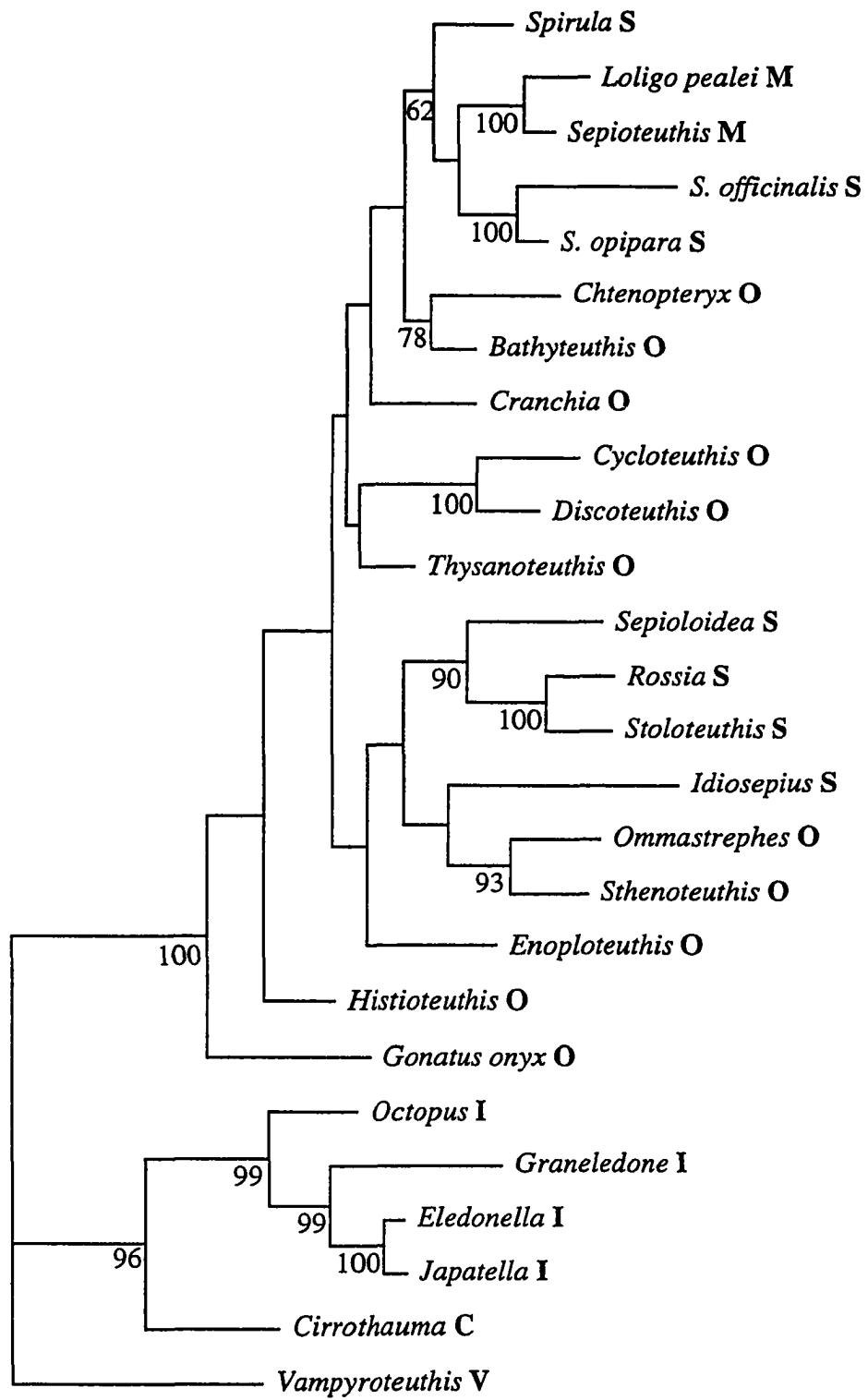


Figure 40. Most parsimonious tree obtained in a heuristic search (1000 random addition replicates) of the unweighted combined Actin I and COI data sets (TL = 3545; CI = 0.256; RI = 0.349). Branch lengths are drawn proportional to the number of character changes taking place between nodes. Bootstrap proportions are indicated as percentages below nodes and Bremer support values are indicated above nodes. Higher-level taxonomic designations are indicated in boldface to the right of each terminal taxon (C = Suborder Cirrata; I = Suborder Incirrata; M = Suborder Myopsida; O = Suborder Oegopsida; V = Order Vampyromorpha).

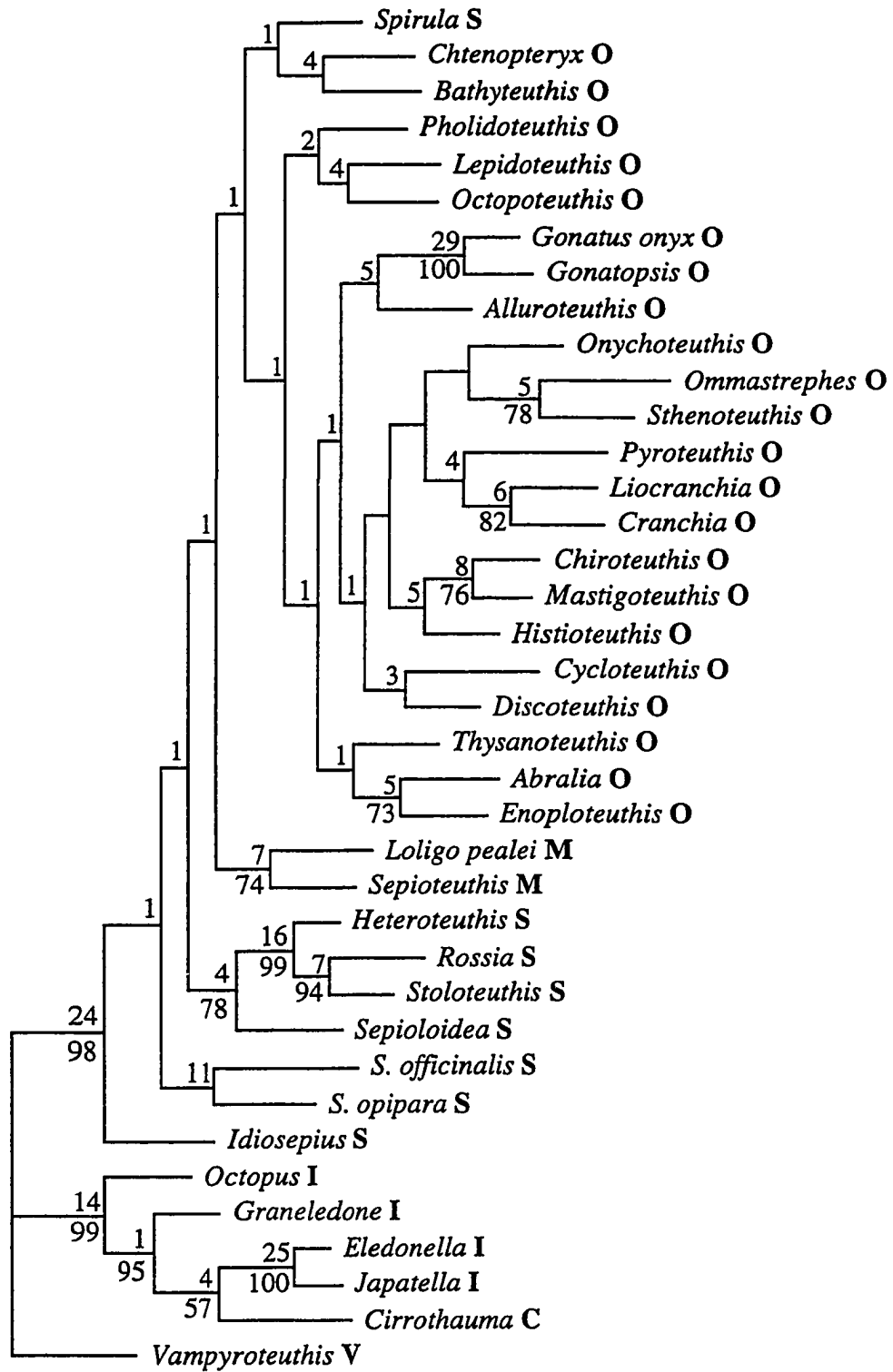


Figure 41. Maximum likelihood tree generated from a heuristic search of the combined Actin I and COI data assuming a Jukes Cantor (1969) model of substitution ( $-\ln L = 20,358.15$ ). Branch lengths are drawn proportional to probabilities of change under the Juke Cantor model. Higher-level taxonomic designations are indicated in boldface to the right of each terminal taxon (C = Suborder Cirrata; I = Suborder Incirrata; M = Suborder Myopsida; O = Suborder Oegopsida; V = Order Vampyromorpha).

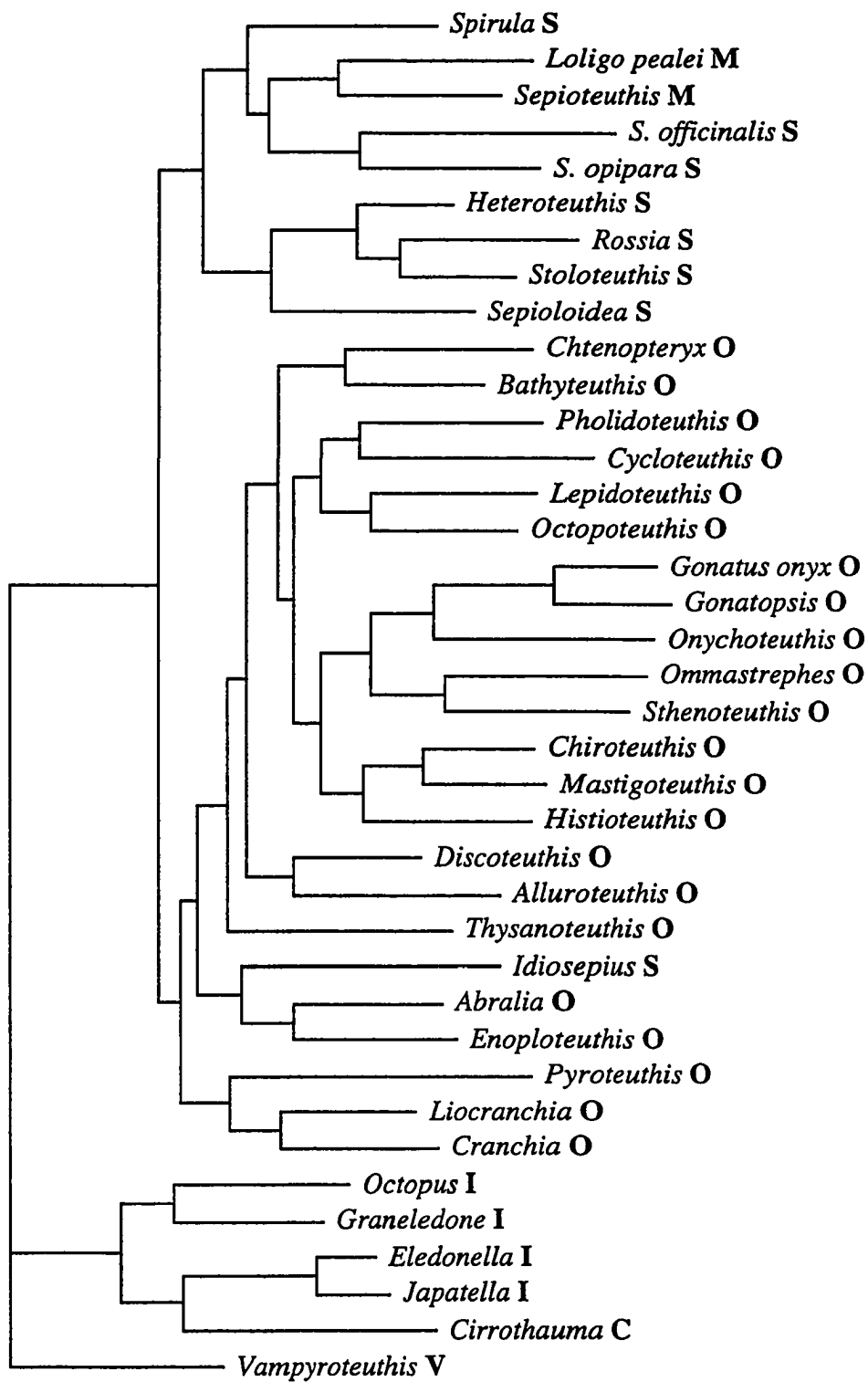


Figure 42. Maximum parsimony tree obtained in a heuristic search (100 random addition replicates) of the weighted Actin I and COI combined data sets (TL = 71,264; CI = 0.244; RI = 0.371). A transversional substitution was assigned a cost of 35 steps. The cost of transitional substitutions differed between the Actin I and COI portions of the combined character set with the assignment of a transitional cost of 14 steps in the Actin I characters and a cost of 10 steps in the COI characters. In this manner the appropriate transition:transversion ratios for each gene, as determined through maximum likelihood analyses of the individual gene data sets, were maintained for the Actin I and COI character partitions. Branch lengths are drawn proportional to the amount of change. Bootstrap proportions are indicated as percentages below nodes. Higher-level taxonomic designations are indicated in boldface to the right of each terminal taxon (C = Suborder Cirrata; I = Suborder Incirrata; M = Suborder Myopsida; O = Suborder Oegopsida; V = Order Vampyromorpha).

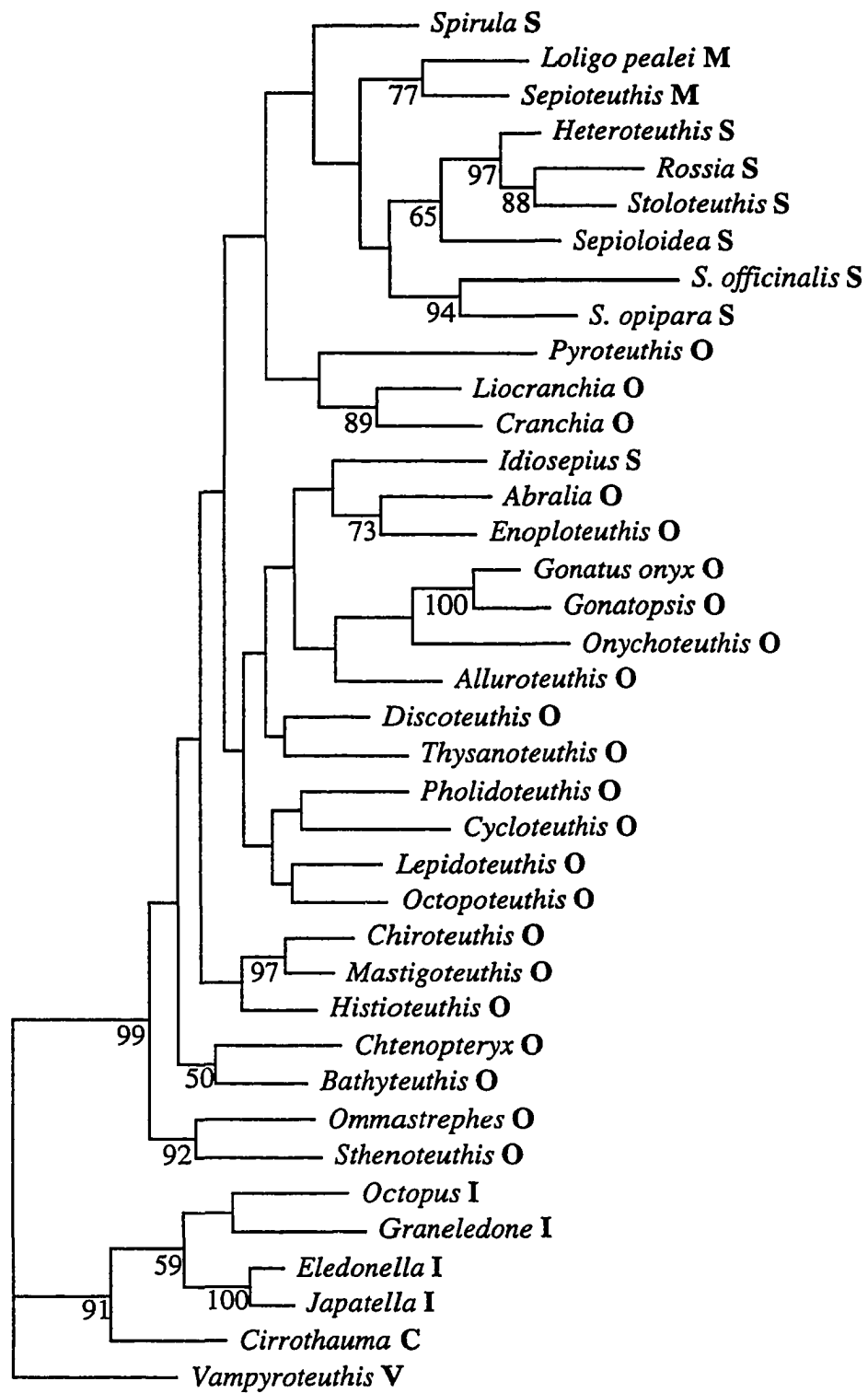


Figure 43. Strict consensus of two equally parsimonious tree generated from a heuristic search (100 random addition replicates) of the combined unweighted Actin II and COI data sets (TL = 3532; CI = 0.300; RI = 0.395). Bootstrap proportions are indicated as percentages below nodes and Bremer support values are indicated above nodes. Higher-level taxonomic designations are indicated in boldface to the right of each terminal taxon (C = Suborder Cirrata; I = Suborder Incirrata; M = Suborder Myopsida; O = Suborder Oegopsida; V = Order Vampyromorpha). A partition homogeneity test (Farris et al., 1994) was conducted to determine if the Actin II and COI characters were significantly incongruent. The results indicated that the two data partitions were significantly incongruent ( $p < 0.01$ ).





Figure 44. Maximum likelihood tree generated from a heuristic search of the combined Actin II and COI data assuming a Jukes Cantor (1969) model of substitution ( $-\ln L = 19,591.79$ ). Branch lengths are drawn proportional to the probabilities of change occurring along each branch under the JC69 model. Higher-level taxonomic designations are indicated in boldface to the right of each terminal taxon (C = Suborder Cirrata; I = Suborder Incirrata; M = Suborder Myopsida; O = Suborder Oegopsida; V = Order Vampyromorpha).

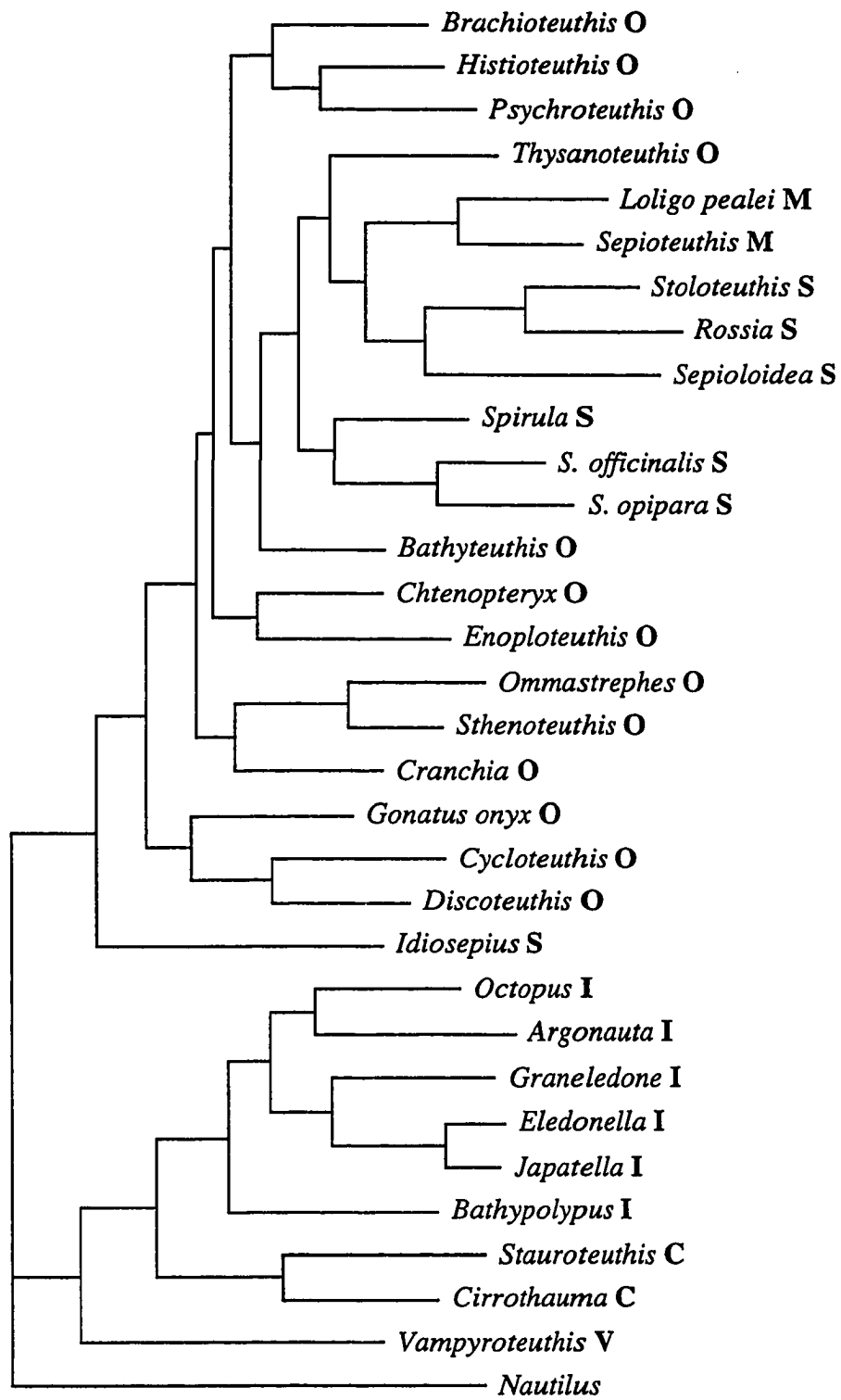


Figure 45. Maximum parsimony tree obtained in a heuristic search (100 random addition replicates) of the weighted Actin II and COI combined data sets (TL = 73,246; CI = 0.289; RI = 0.415). A transversional substitution was assigned a cost of 35 steps. The cost of transitional substitutions differed between the Actin II and COI portions of the combined character set with the assignment of a transitional cost of 14 steps in the Actin II characters and a cost of 10 steps in the COI characters. In this manner the appropriate transition:transversion ratios for each gene, as determined through maximum likelihood analyses of the individual gene data sets, were maintained for the Actin II and COI character partitions. Branch lengths are drawn proportional to the amount of change. Bootstrap proportions are indicated as percentages below nodes. Higher-level taxonomic designations are indicated in boldface to the right of each terminal taxon (C = Suborder Cirrata; I = Suborder Incirrata; M = Suborder Myopsida; O = Suborder Oegopsida; V = Order Vampyromorpha).

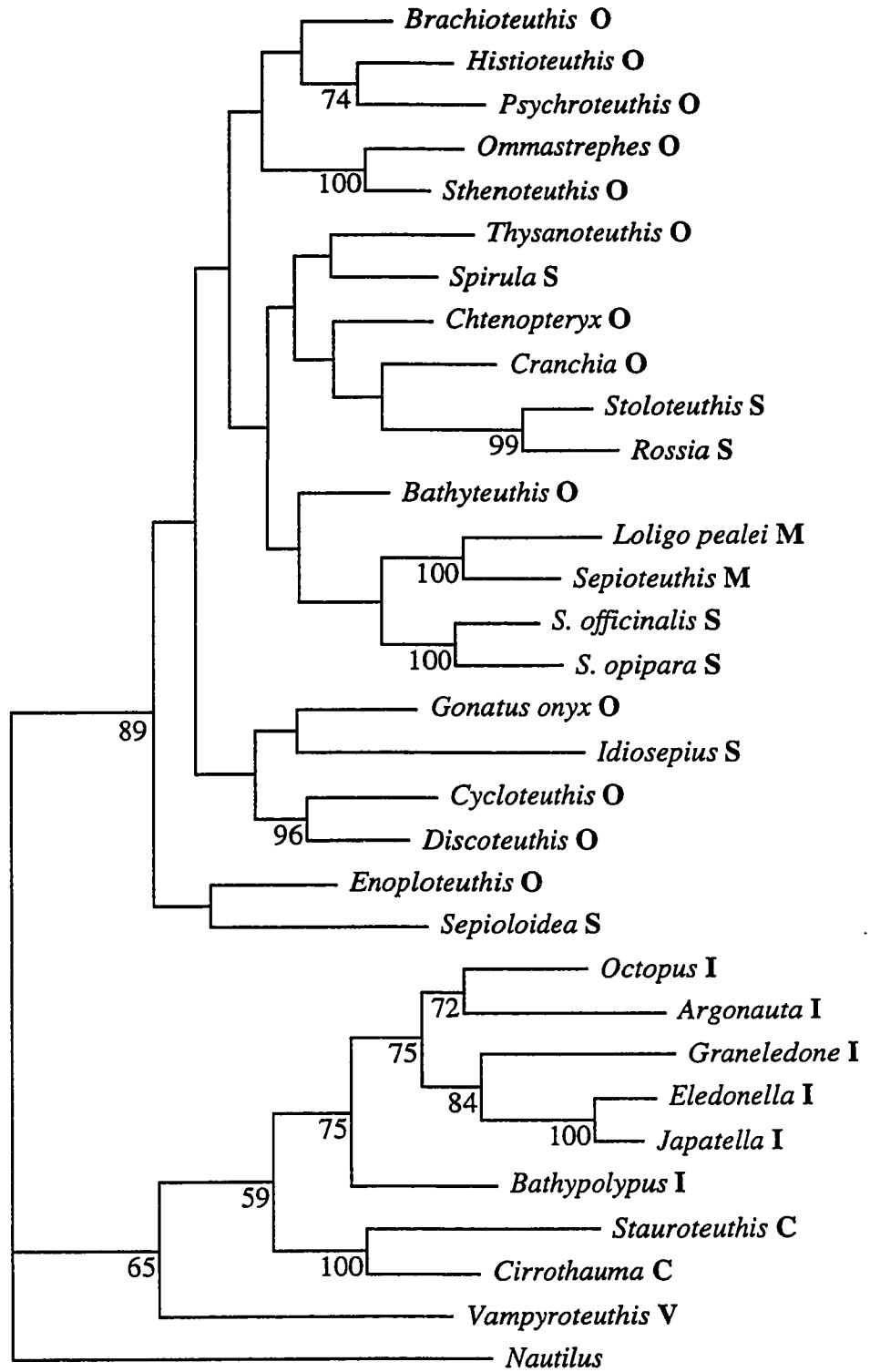


Figure 46. Strict consensus of two most parsimonious trees obtained in a heuristic search (100 random addition replicates) of the unweighted Actin I, Actin II, and COI data sets combined for 26 taxa (TL = 3476; CI = 0.356; RI = 0.411). Bootstrap proportions are indicated as percentages below nodes and Bremer support values are indicated above nodes. Higher-level taxonomic designations are indicated in boldface to the right of each terminal taxon (C = Suborder Cirrata; I = Suborder Incirrata; M = Suborder Myopsida; O = Suborder Oegopsida; V = Order Vampyromorpha). A partition homogeneity test (Farris et al., 1994) was conducted to determine if the Actin I, Actin II, and COI characters were significantly incongruent. The results indicated that the three data partitions were significantly incongruent ( $p < 0.01$ ).

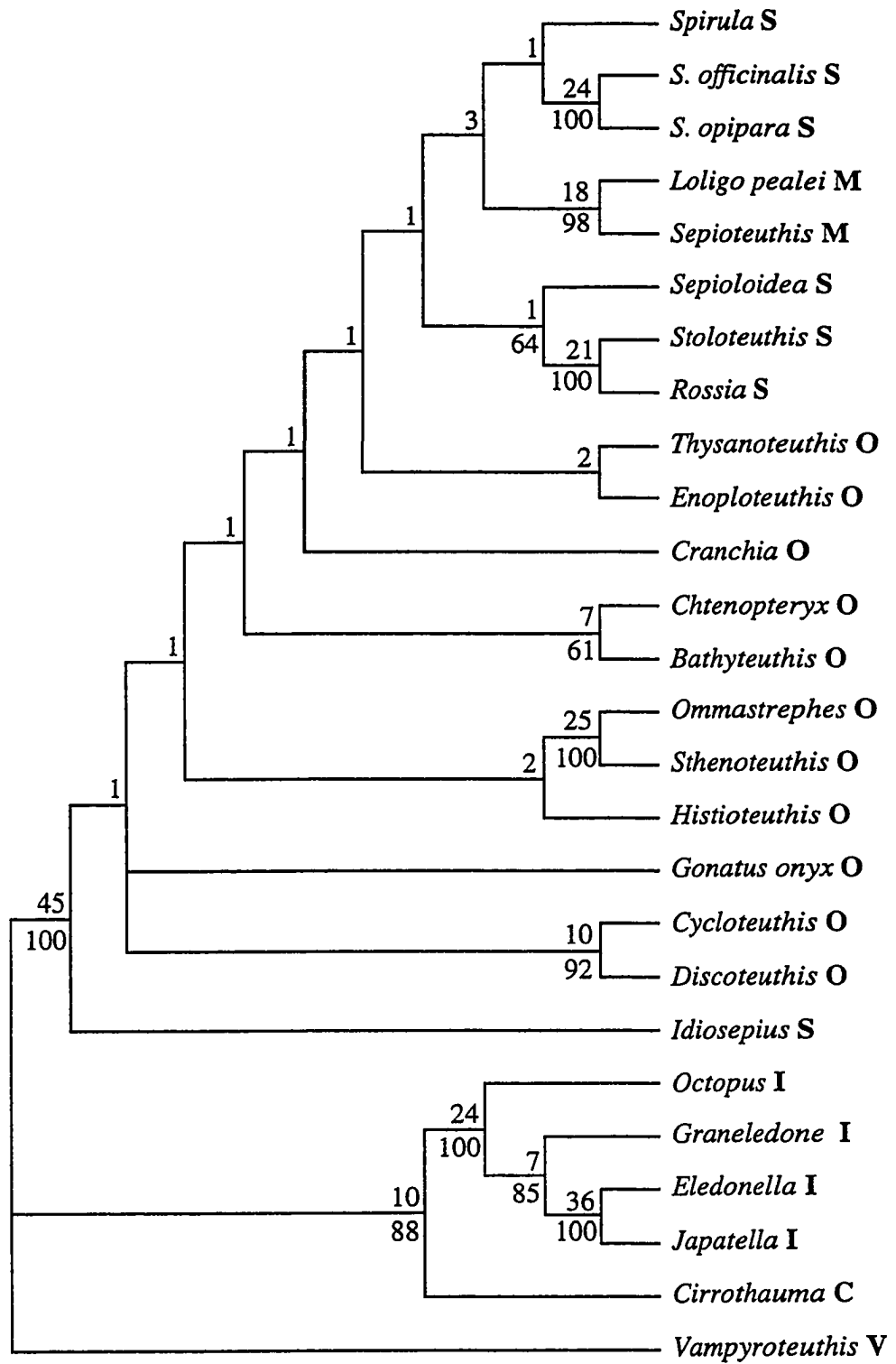


Figure 47. Maximum likelihood tree generated from a heuristic search of the combined Actin I, Actin II, and COI data assuming a Hasegawa, Kishino, and Yano (1985) model of substitution ( $-\ln L = 17,814.54$ ) with gamma distributed rates. Branch lengths are drawn proportional to the probabilities of change occurring along each branch under the HKY85 model. Substitution parameters used in the likelihood search were as follows:  $\pi_A=0.265$ ,  $\pi_C=0.253$ ,  $\pi_G=0.123$ ,  $\pi_T=0.359$ ; TI/TV=2.195;  $\alpha=0.239$ ; proportion invariant sites = 0. Higher-level taxonomic designations are indicated in boldface to the right of each terminal taxon (C = Suborder Cirrata; I = Suborder Incirrata; M = Suborder Myopsida; O = Suborder Oegopsida; V = Order Vampyromorpha).



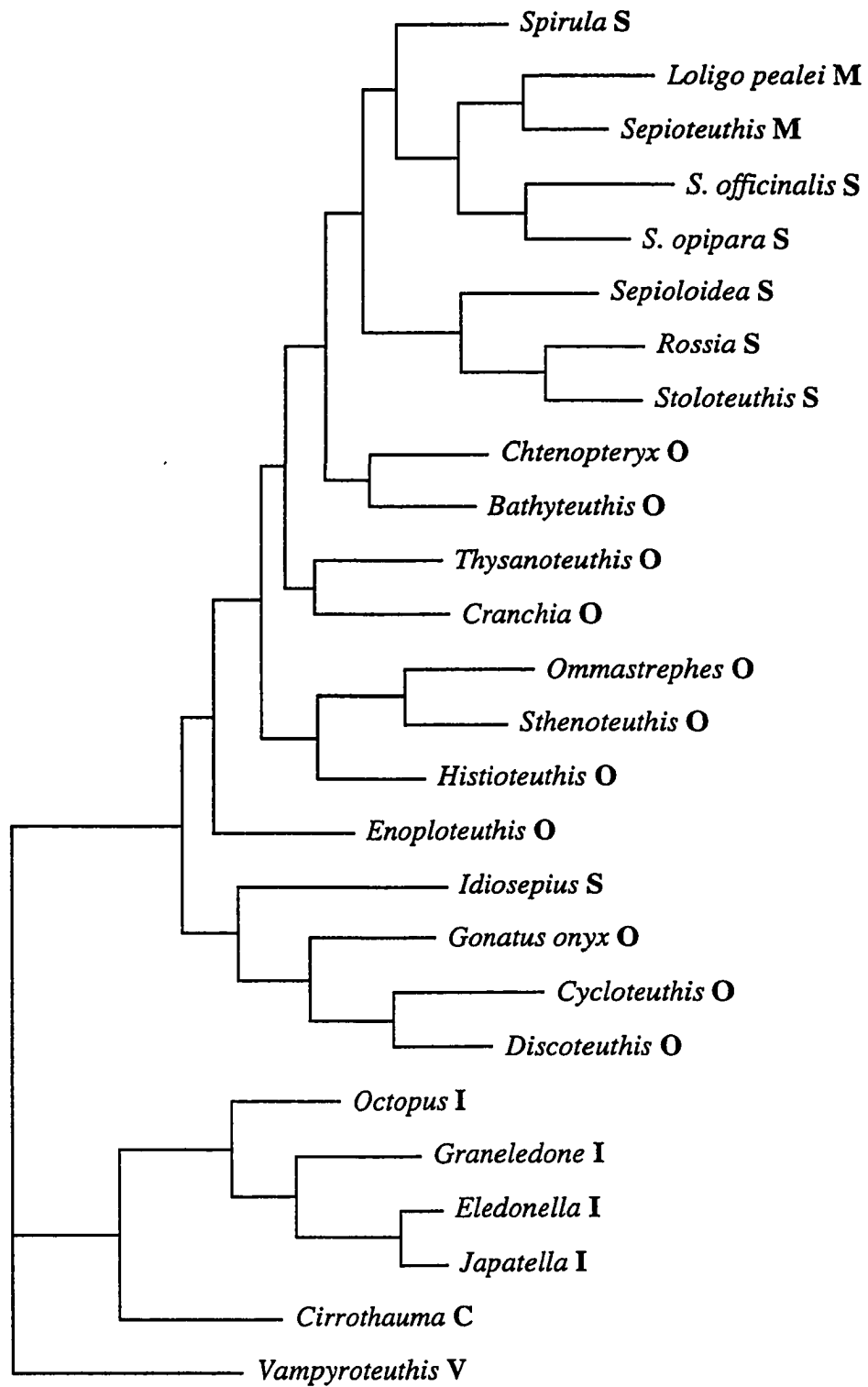


Figure 48. Maximum parsimony tree obtained in a heuristic search (100 random addition replicates) of the weighted Actin I, Actin II and COI combined data sets (TL = 70,568 CI = 0.351; RI = 0.424). A transversional substitution was assigned a cost of 35 steps. The cost of transitional substitutions differed between the Actin and COI portions of the combined character set with the assignment of a transitional cost of 14 steps in both actin gene characters and a cost of 10 steps in the COI characters. In this manner the appropriate transition:transversion ratios for each gene, as determined through maximum likelihood analyses of the individual gene data sets, were maintained for the Actin I, Actin II and COI character partitions. Branch lengths are drawn proportional to the amount of change. Bootstrap proportions are indicated as percentages below nodes. Higher-level taxonomic designations are indicated in boldface to the right of each terminal taxon (C = Suborder Cirrata; I = Suborder Incirrata; M = Suborder Myopsida; O = Suborder Oegopsida; V = Order Vampyromorpha).

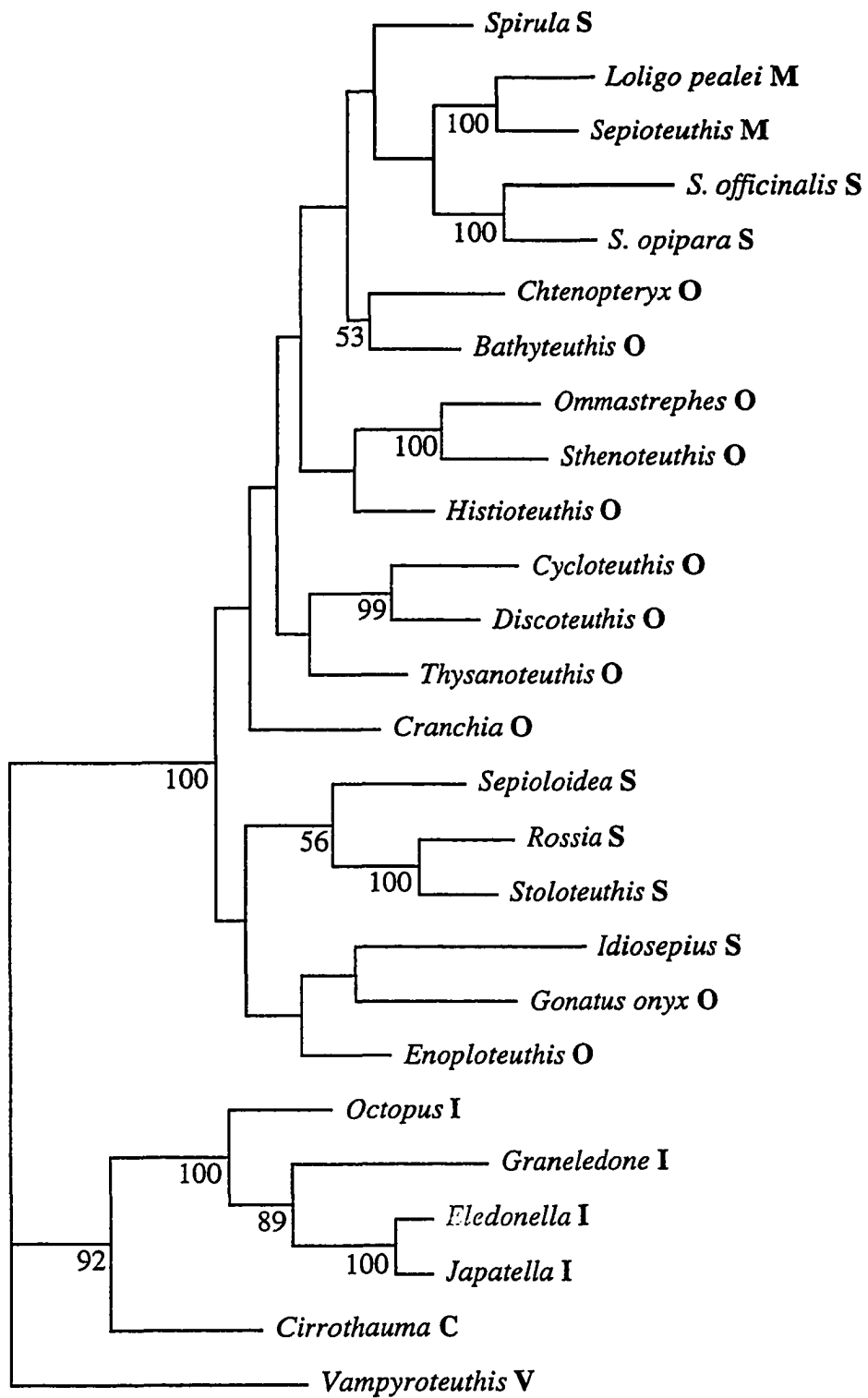
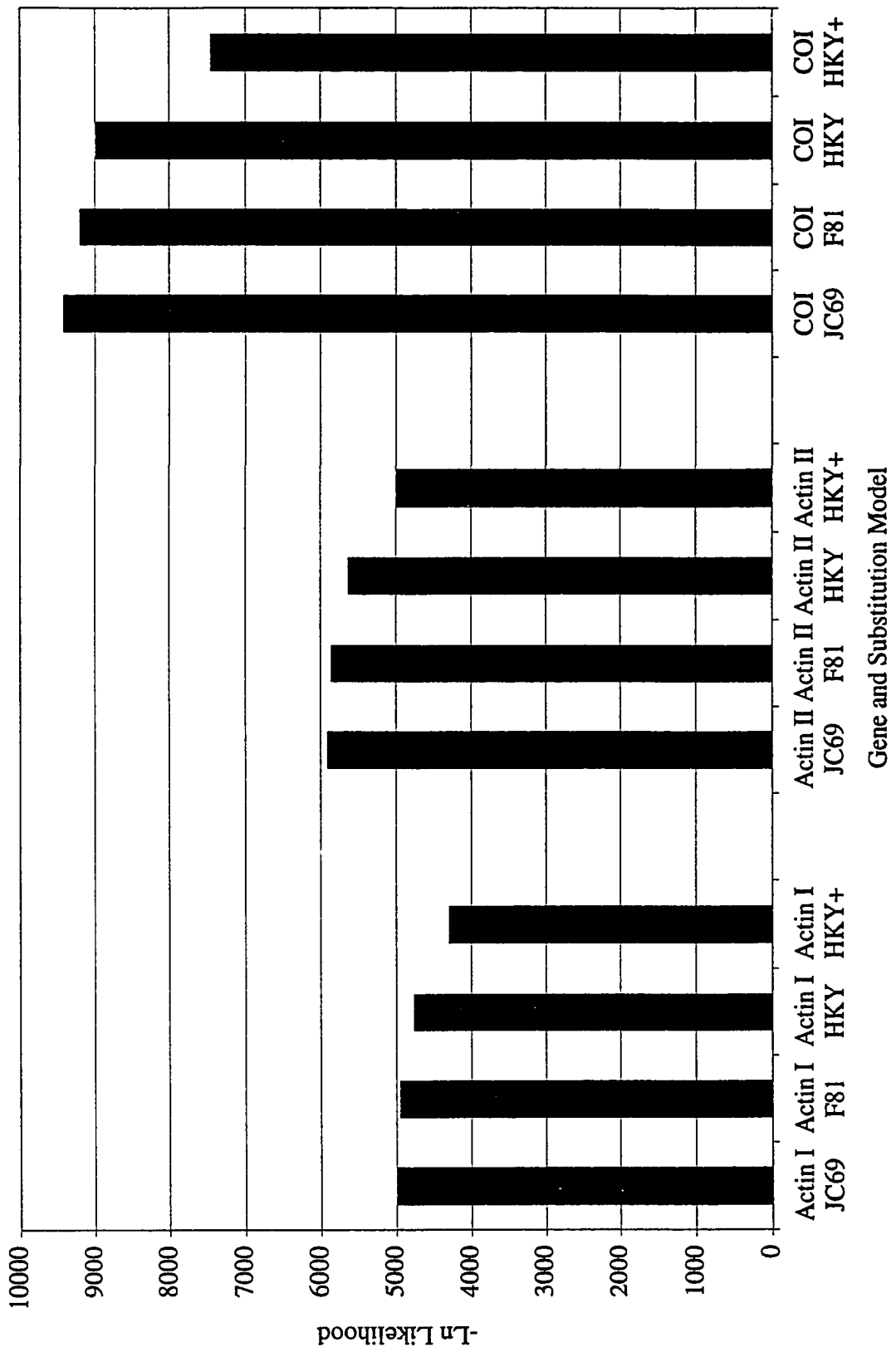


Figure 49. Results from maximum likelihood analyses of the restricted Actin I, Actin II, and COI data sets under four DNA substitution models. Likelihood ratio tests were conducted to determine the most appropriate model of sequence substitution. In all cases, the HKY85+ model (which allows for unequal base frequencies, two classes of substitution types, and rate heterogeneity across sites categorized by codon positions) provided the statistically significant best fit to the data among the models tested. For all three genes, accounting for rate heterogeneity across nucleotide sites resulted in the most dramatic improvement in fit to the data. Comparisons of likelihood values among the three genes illustrates their relative evolutionary rates: COI > Actin II > Actin I.



## CONCLUSIONS

With respect to the four phylogenetic hypotheses presented in the Introduction, the following can be concluded from the results presented in this dissertation:

- 1)The Octopodiformes is monophyletic and the Octopoda and Vampyromorpha are sister groups.
- 2)The two octopod suborders, Incirrata and Cirrata, are monophyletic groups.
- 3)The Decapodiformes (Sepioidea + Teuthoidea) is a monophyletic group.
- 4)The Sepioidea, as defined by Voss (1977), is not a monophyletic group.

### Octopodiformes and Decapodiformes

The monophyly of the Octopodiformes and Decapodiformes was well supported in all analyses of the 7 nucleotide data sets (COI, Actin I, Actin II, Actin I + Actin II, Actin I + COI, Actin II + COI, Actin I + Actin II + COI) and from analyses of the deduced amino acid sequences, except weighted parsimony analysis of the COI data set, where *Vampyroteuthis* emerged basal to all remaining coleoids. Bootstrap and Bremer support values for the monophyly of both groups was consistently stronger than support values for the monophyly of other higher-level groups. These results are in agreement with those obtained by Young and Vecchione (1996), where the monophyly of the Octopodiformes and Decapodiformes was demonstrated through cladistic analysis of morphological character data.

### Octopoda

Several recent studies and reviews have examined or discussed relationships within the order Octopoda (Voss, 1988; Voight, 1993; Voight, 1997; Young et al., 1998). To date there have been no molecular studies which have examined higher level relationships within the Octopoda, although one study has examined relationships among a few species in the large genus *Octopus* (de los Angeles et al., 1995). Voss (1988) did not consider pelagic octopods in his study of octopod relationships, therefore, only one of the 8 incirrate families, the Octopodidae, was included in his study, which also examined relationships among cirrates.

Three main clades were defined within the Octopodidae: 1) Octopodinae and Bathypolypodinae (to which the genera *Octopus*, *Hapalochlaena*, and *Bathypolypus* would be assigned in this study), 2) Eledoninae (no representatives included in this study), and 3) Graneledoninae (to which the genus *Graneledone* is assigned). Voss (1988) regarded the Eledoninae and Graneledoninae as sister taxa, as were the Bathypolypodinae and Octopodinae. Therefore, based on Voss' results one would predict the following relationships among the octopodids included in this study: ((*Bathypolypus* (*Octopus Hapalochlaena*))(*Graneledone*)). Voight (1993) performed a cladistic analysis of morphological characters to clarify relationships within the Octopodidae. Voight's (1993) conclusions would predict the following: ((*Octopus Hapalochlaena*)(*Graneledone Bathypolypus*)). Another study by Voight (1997) examined higher-level relationships within the order Octopoda. With respect to the taxa included in the present study, the following relationships among incirrate taxa included in this study would be predicted from Voight (1997): (Bolitaenidae ((*Vitreledonella Argonauta*)(Octopodidae))).

Although the COI data set contained a much better representation of octopod diversity than either of the actin data sets, the results obtained in analyses of the actin data sets lend further support to some of the conclusions drawn from the COI analyses. Analyses of the actin genes also provided good evidence to refute an anomalous result obtained in the COI study, namely that the Bolitaenidae are closely related to the Cirrata. However, other

apparently anomalous COI results were not refuted in analyses of the actin data sets. These include: 1) Placement of *Argonauta* with the Octopodidae rather than as sister taxon to *Vitreledonella* (COI: all analyses; Actin II: parsimony, weighted parsimony; Actin II and COI combined: all analyses); 2) a closer relationship of *Graneledone* with the Bolitaenidae than with the Octopodidae (Actin I: weighted parsimony; Actin II: likelihood, weighted parsimony; Actin I and Actin II combined: all analyses; Actin I and COI combined: parsimony; Actin II and COI combined: all analyses; Actin I, II, and COI combined: all analyses); 3) the basal emergence of *Bathypolypus* (COI: parsimony, weighted parsimony; Actin II: weighted parsimony; Actin II and COI combined: all analyses).

Naef (1923) proposed a relationship between *Argonauta* and the Octopodinae and he suggested that the pelagic argonautoid families were derived from the Octopodinae. This view was later refuted by Robson (1932) and more recently by Voight (1990), who considered morphological similarities such as two sucker rows and the presence of an ink sac in both groups to be ancestral characters or the result of convergence. Both the COI and Actin II data sets supported a relationship between *Argonauta* and the Octopodidae as suggested by Naef (1923).

The apparent relationship between *Graneledone* and the Bolitaenidae is probably due to inadequate taxonomic sampling. Analysis of the COI gene did not support a close relationship between *Graneledone* and the Bolitaenidae. Relative stability of the relationship in analyses of the combined data sets may not have occurred if a *Vitreledonella* actin sequence had been obtained.

The basal emergence of *Bathypolypus* cannot be ascribed to poor taxonomic sampling as this result is a much more dramatic deviation from expectations than that described for *Graneledone*. *Bathypolypus* is represented in the COI and Actin II data sets. In parsimony analyses of the COI data, *Bathypolypus* emerged basal to all octopods, however, maximum likelihood analysis of the COI data placed *Bathypolypus* within the Octopodidae (exclusive of *Graneledone*). In unweighted parsimony analysis of the Actin II data, the position of



incirrate families was not resolved but in weighted parsimony analysis *Bathypolypus* emerged basal to the incirrates. Maximum likelihood analysis of the Actin II data placed *Bathypolypus* with *Octopus*. Analysis of the combined Actin II and COI data consistently placed *Bathypolypus* basal to the incirrates; however, an unrealistic model of sequence evolution (JC69) was used in maximum likelihood analysis of the combined data. The basal position of *Bathypolypus* was unsupported when a parameter rich (i.e., HKY85+) model of sequence evolution was employed in phylogenetic analysis of the Actin II data. This provides evidence that the placement of *Bathypolypus* using parsimony methods, or under the JC69 model in maximum likelihood analysis, may be an artifact of the reconstruction methodology.

#### Sepioidea and Myopsida

None of the results from phylogenetic analyses of the 7 molecular data sets supported the monophyly of the Sepioidea. Several results which were consistent across most analyses contributed to the refutation of sepioid monophyly: 1) a close relationship between the Myopsida and Sepiidae, 2) placement of *Spirula* with oegopsid taxa in analyses including the COI data, 3) instability in the placement of *Idiosepius*, 4) mean sequence divergence within the Sepioidea was consistently greater than the mean sequence divergences within other major taxa, and 5) statistical tests of sepioid monophyly conducted on the COI, Actin I, and Actin II data sets rejected the monophyly of the Sepioidea with statistical significance.

Although the total number of sepioid taxa included in this study was small in comparison to the number of oegopsid taxa, the sepioids were the only group for which taxa of all families were included in the analyses of all 7 data sets. This permitted a relatively rigorous test of the monophyly of the group through phylogenetic analysis of each of the data sets using 3 different reconstruction methodologies. The taxonomic sampling within the Sepioidea also permitted the use of statistical tests of monophyly,

which are not relevant unless all representatives from the major groups within the taxon of interest are included. Though the results of such tests cannot be considered "proof" of a group's monophyly or nonmonophyly, the rejection of sepioid monophyly for 3 separate data sets reported here provides quite convincing evidence to refute the monophyly of the group.

The use of inappropriate models of DNA substitution can be a serious problem affecting the relevance of statistical tests of monophyly based on null distributions of tree length differences from simulated data sets. The use of an inappropriate model of DNA substitution in generating simulated data sets results in an increased frequency of rejecting the null hypothesis when the null hypothesis is in fact true-i.e., Type I error (Huelsenbeck et al., 1996). However, the assumption of an incorrect model was a serious problem only when the rates of sequence evolution were exceptionally high. In the simulation study, when the rates of sequence evolution were low, the use of an inappropriate model did not result in an increased frequency of rejecting the null hypothesis. Since a parameter rich model of sequence evolution was used to generate the simulated data sets in the present study, and since the rates of sequence evolution are not exceptionally high for the COI and Actin genes, the rejection of sepioid monophyly is probably not due to Type I error.

Taken alone, the rejection of sepioid monophyly is not a significant contribution to our understanding of coleoid evolution. The monophyly of the group has been questioned in the past by morphological studies (Fioroni, 1981; Clarke, 1988; Khromov, 1990) and in molecular studies (Bonnaud et al., 1994; 1997). A more important contribution to our understanding of coleoid evolution would be definitive conclusions regarding relationships of the 5 sepioid families to each other and to other coleoids. Unfortunately, such definitive statements are not possible based on the data presented in this dissertation. However, the following important conclusions can be drawn from the results of these phylogenetic analyses. The Sepiadariidae and Sepiolidae are sister taxa. Results from analyses of the actin data sets and most of the combined data sets support this conclusion. The results

from the maximum likelihood analysis of the COI data set also support this conclusion. A close relationship probably exists between the Myopsida, Sepiidae, and Spirulidae. Although the results from analyses of the COI gene placed *Spirula* with oegopsid squids, analyses of the Actin I gene and combined data sets generally placed *Spirula* with the Myopsida and Sepiidae. The Myopsida and Sepiidae were found to be sister groups in 11 of 21 analyses (three phylogenetic methods used for each data set multiplied by seven data sets) while *Spirula* was found to be sister group to the Sepiidae in 6 of 21 analyses. Engeser (1990) and Hass (1997) also suggested a relationship between the myopsid squids and the Sepioidea. Strong evidence for a sister-group relationship between the (Sepiidae *Spirula* Myopsida) clade and the (Sepiadariidae Sepiolidae) clade was lacking. The relationship between these two clades was usually complicated by the placement of oegopsid families such as the Ctenopterygidae and Bathyteuthidae within one of the two clades. In general, the two clades tended to cluster near one another in most of the analyses but a strict sister group relationship between the two clades was only obtained in analyses of the combined Actin I, Actin II, and COI data sets.

The position of the Idiosepiidae was especially unstable across different phylogenetic reconstruction methodologies and was also unstable across different data sets. Analysis of the actin data sets tended to place *Idiosepius* with the Ommastrephidae, a result consistent with the results of Bonnaud et al. (1997). However, parsimony analysis of the COI data placed *Idiosepius* with the Sepiadariidae. Analyses of the combined mitochondrial and nuclear data sets were not consistent in the placement of *Idiosepius*. Its relationship to the Sepioidea cannot be confirmed or refuted based on the results presented in this dissertation. The answer to this problem will have to await further molecular studies as it is not likely to come from morphological studies due to the secondary simplification associated with the small size attained by idiosepiids (Young et al, 1998).

Oegopsida

Although it is not possible to make conclusive statements about the deep branching order within the Oegopsida, the following relationships among families were reasonably stable through at least one of the three determinants of clade stability discussed above. A close relationship between the Histioteuthidae and Psychroteuthidae was obtained in all three analyses of the COI data set. An Actin I sequence for *Psychroteuthis* was not obtained so the relationship was not tested in analyses of the Actin I data set or in the analyses of combined data sets including Actin I sequences. Unweighted parsimony analysis of the Actin II data also indicated a close relationship between these families with moderate bootstrap support. The clade was not supported in weighted parsimony and likelihood analyses of the Actin II data set, although in the weighted parsimony analysis the two taxa diverged from an oegopsid clade, adjacent to one another. The three analyses of the combined Actin II and COI data sets all supported a (*Histioteuthis Psychroteuthis*) clade, which was supported in bootstrap and Bremer analyses in the parsimony trees. Morphologically, the two families share similar tentacular armature and are considered related (Young et al., 1998b).

The Bathyteuthidae and Ctenopterygidae were found to be closely related to each other in all analyses of the COI data, all analyses of the Actin I data (with bootstrap support), in the unweighted parsimony analysis of the Actin II data, in all analyses of the Actin I and Actin II, and Actin I and COI combined data, and in all analyses of the combined Actin I, Actin II, and COI data. These two families have both been considered to be related to the myopsid squids and also to one another by some researchers (Naef, 1923; Brierley et al., 1996; J.Z. Young, 1977; Anderson, 1996). Although the COI data did not support a close relationship between these families and the myopsid squids, the Actin I data consistently placed the families within the myopsid-sepioid line. The unweighted parsimony analysis of the Actin II data set also supported such a relationship, as did all analyses of the combined Actin I and Actin II data sets. Among the oegopsid families, the Ctenopterygidae and Bathyteuthidae appeared most closely related to myopsid squids and the Sepioidea.

A close relationship between the Chiroteuthidae and Mastigoteuthidae was supported in all analyses of the COI data set, and in all analyses of the combined Actin I and COI data sets (with bootstrap support). The taxa were not included in the Actin II data set, so a relationship between the two families was not evaluated in all analyses of data sets containing Actin II sequences. Morphologically, feeding tentacles and similar structure of the funnel-mantle locking apparatus and the size of arms IV support a close relationship between these families (Young, 1991). The Joubiniteuthidae, a purported member of the chiroteuthid family clade, did not consistently cluster with the chiroteuthid clade.

The Lepidoteuthidae and Octopoteuthidae were found to be related in weighted parsimony and maximum likelihood analyses of the COI data set and also in the weighted parsimony and likelihood analyses of the Actin I data set. All three methods of analysis of the combined Actin I and COI data set supported a close relationship between these families. Unweighted parsimony analysis of the COI or Actin I data sets did not support a close relationship between the families, but when the data were combined the unweighted parsimony analysis also supported a close relationship. This suggests that the results obtained by "correcting" the data by weighting or likelihood methods were corroborated in the analyses of the combined data set. The taxa were not included in the Actin II data set, so it is not possible to make conclusions based on analyses of data sets containing information from this gene. Both families lose their tentacles during development although their larval tentacles share similar clubs (Young et al., 1998) and have nearly identical beaks (Clarke, 1988). The genus *Pholidoteuthis* has been considered by some to be a member of the family Lepidoteuthidae (Voss, 1977; Nesis, 1987) due to similarities in the mantle surface, which has a particular type of scales in both groups. The weighted COI data supported a relationship between the Pholidoteuthidae, Lepidoteuthidae, and Octopoteuthidae as did the unweighted parsimony analysis of the combined Actin I and COI data set. Maximum likelihood and weighted parsimony analyses of the combined Actin I and COI data set also placed *Pholidoteuthis* near the (*Lepidoteuthis Octopoteuthis*)

clade. Perhaps *Lepidoteuthis* and *Octopoteuthis* derived from an ancestor similar to *Pholidoteuthis*, losing their adult tentacles sometime after the split from *Pholidoteuthis* but before they split from their common ancestor.

The placement of the Gonatidae was unstable across the different methods of analysis and also across different genes. The sister group to the gonatids was not consistent but three families may be related to gonatid squids. The COI data suggest a relationship between the gonatids and *Alluroteuthis*. The Actin I data support a relationship with the Onychoteuthidae with bootstrap support in the weighted parsimony analysis. The Actin II data supports a relationship with the Cycloteuthidae which was also supported by bootstrap analysis of the weighted data. Results from the analyses of the combined data sets are conflicting, as would be expected given the amount of incongruence among the individual data sets. However, the combined actin data sets and the Actin II and COI combined data set tended to place gonatids basal to the remaining decapods. Naef (1923) considered the gonatids to be near the base of the Oegopsida due to the armature in four series on the arms as in the sepiids.

The families Ancistrocheiridae, Enoploteuthidae, Lycoteuthidae, and Pyroteuthidae are considered to be closely related based on a number of morphological synapomorphies (Young and Harman, 1998). Within the clade, the Ancistrocheiridae are basal to the (Enoploteuthidae (Lycoteuthidae Pyroteuthidae)) clade (Young and Harman, 1998). The structure and distribution of photophores are very similar in the lycoteuthids and pyroteuthids, although they are quite different in regard to many other morphological features. The COI data did not support a close relationship between *Lycoteuthis* and *Pyroteuthis*. In fact, a closer relationship was obtained between *Pyroteuthis* and *Ancistrocheirus*. The three families did not cluster with *Abralia* and *Enoploteuthis* in any of the COI analyses. *Ancistrocheirus* and *Lycoteuthis* actin sequences were not obtained but the relationship between *Pyroteuthis* and the enoploteuthids was also tested with the actin data. An anomalous result was obtained in analyses of the Actin I data, where *Pyroteuthis*

placed with *Ommastrephes* with moderately high bootstrap support. This relationship was not supported in the analyses of the combined Actin I and COI data sets.

The monophyly of the oegopsid squids was not supported in any of the analyses conducted in this study, consistent with a recent conclusion that the taxon is "a phylogenetic void" (Young et al., 1998). Although relationships among a few families were supported in many of the analyses, the lack of stability in deep level relationships renders any conclusions about phylogenetic relationships of oegopsid families moot. The lack of stability in oegopsid relations was observed in three ways. Both measures of clade stability employed in this study, the nonparametric bootstrap and the Bremer support index, failed to lend strong support to most oegopsid clades. The results from analyses of the same data set using three different reconstruction methodologies were frequently discordant with respect to deep divergences within the Oegopsida. Analyses of different data sets were frequently discordant with respect to oegopsid relations, although this may have been due in part, to the different taxonomic compositions of the data sets.

Of the 25 total oegopsid families listed by Sweeney and Roper (1998), 23 were represented by at least one taxon in the analyses of the COI gene; samples were not obtained for the families Batoteuthidae and Promachoteuthidae. Of the 23 oegopsid families sampled, 17 were represented by a single species. For the Actin I gene and combined Actin I and COI data, a total of 17 oegopsid families were analyzed, 12 of which were represented by a single species. In the analyses of the Actin II gene and combined Actin II and COI data, 11 oegopsid families were considered of which nine consisted of a single species representative. Finally, in the analyses of the combined Actin I and Actin II data and in the analyses of the combined data for all three genes a total of nine oegopsid families were included, seven represented by a single species.

Most of the sampling of oegopsid taxa, therefore, consisted of a single species from each family so that part of the difficulty in constructing stable oegopsid relationships may have been due to the taxonomic sampling scheme. Taxonomic sampling has important

implications for tree stability and accuracy. Several studies have demonstrated that adding taxa can be a more important means of increasing the stability and accuracy of phylogenetic hypotheses than adding characters (Hendy and Penny, 1989; Lecointre et al., 1993; Graybeal, 1998; Poe, 1998). The problem of statistical inconsistency, the failure of phylogenetic methods even with an infinite number of characters, is attributed mainly to widely unequal branch lengths in phylogenetic trees (Felsenstein, 1978). Advocates of increased taxonomic sampling argue that consistency problems are alleviated by adding taxa to break up the long branches, thereby decreasing the variance in branch lengths in the resultant tree. Unfortunately, it was not possible to increase the sampling of several of the oegopsid families because of the rarity of additional species (Ctenopterygidae, Neoteuthidae, Pholidoteuthidae, Lycoteuthidae). In addition, further sampling within a family was not possible in monotypic families, of which there were several: Ancistrocheiridae, Joubiniteuthidae, Lepidoteuthidae, Psychroteuthidae, and Thysanoteuthidae. Moreover, there are practical limits to the number of taxa that can be included in a data set (e.g., several of the maximum likelihood searches employed in this study ran for over a week on the fastest personal computer available at the time (Macintosh® G3). Finally, the probability of finding the optimal tree in both parsimony and likelihood analyses using heuristic search methods decreases with an increase in the number of taxa included. For example, the number of possible unrooted bifurcating trees for 56 taxa is  $3.185 \times 10^{86}$ , adding one taxon increases the number of possible trees to  $3.472 \times 10^{88}$ , an increase of over 2 orders of magnitude.

The determination of phylogenetic relationships among the oegopsid families is likely to remain a major problem facing cephalopod systematics for some time to come for the reasons outlined above. Choosing an appropriate outgroup is also problematic, since the oegopsid squids, as currently defined, are polyphyletic. Therefore, it would be inadvisable to use non-oegopsid decapods as an outgroup for resolving relationships among the oegopsid squids until the monophyly of oegopsid subclades is conclusively demonstrated.



Until then the outgroup for morphological and molecular analyses of oegopsid relationships must be a member(s) of the Octopodiformes. This presents problems for both morphological and molecular data sets. Much of the morphological information regarding oegopsid relationships may be contained in the tentacles, structures that are altogether lacking in the Octopodiformes. Use of distant outgroups in molecular analyses are also problematic, as the definition of character polarity becomes difficult when all characters are constrained to only 4 states (Wheeler, 1990). The high family level diversity, combined with low species level diversity in most oegopsid families, presents a special challenge to taxonomic sampling. In future molecular studies, every effort should be made to sample each oegopsid family as exhaustively as possible. This would require a concomitant increase in the number of characters analyzed, such that multiple genes in tandem with longer stretches of individual genes must be utilized. In the wake of rapid improvements in DNA sequencing and computer technology, the situation is not hopeless. For example, since the inception of the research described in this dissertation, the rate of increase at which DNA sequence data was gathered and analyzed due to technological improvements alone increased by at least a factor of four. Although there is a significant lag period in the technology used in big budget research such as the human genome project versus small budget esoteric projects such as the phylogeny of coleoid cephalopods, consider what might be accomplished given a 10 year lag period. The rate of data accumulation for the human genome project currently averages 100,000 bases per laboratory per day in the most productive laboratories (Pennisi, 1998), approximately the same amount of data used to produce this dissertation!

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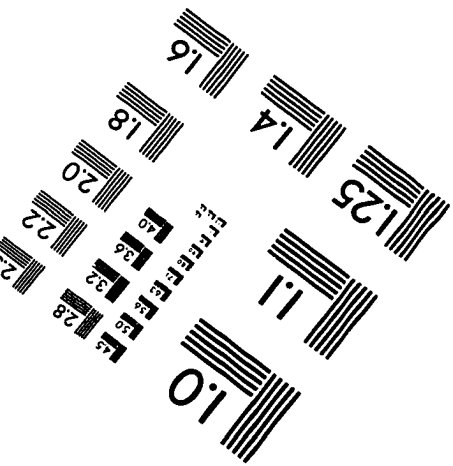
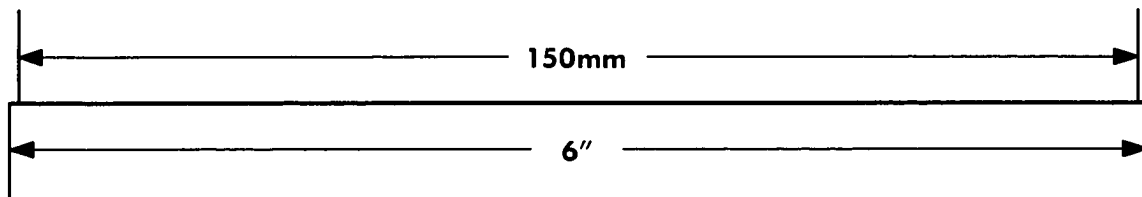
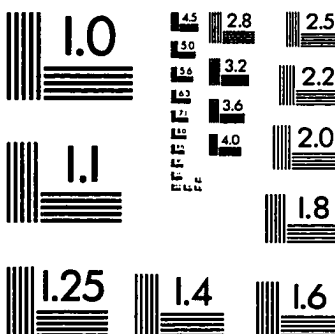
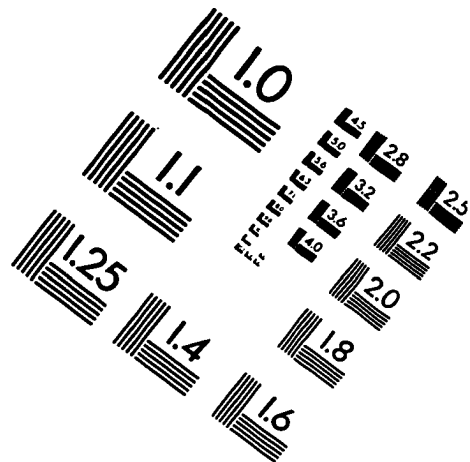
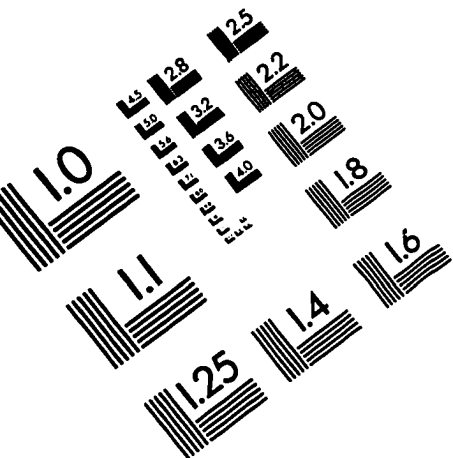
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