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Genetic Identification and Population Characteristics of Deep-Sea Cephalopod Species in the Gulf of Mexico and Northwestern Atlantic Ocean

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by

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ABSTRACT

Nearly all deep-sea cephalopod life history studies have been completed by examination of specimens collected in the wild. Much of this work is like piecing together a puzzle; knowledge of the life history of many species remains fragmented and hence, taxonomically and phylogenetically confused. Molecular approaches and sequencing technologies are powerful tools for deciphering wild-type cephalopod life history and population dynamics. Use of molecular markers offers additional certainty for identifying specimens damaged during deep-sea collections and can elucidate often cryptic, intra- and interspecific diversity. The research presented in this study assessed broad genetic patterns of biodiversity in deep-sea cephalopods from the Gulf of Mexico and northwestern Atlantic Ocean. This study has two key objectives: [1] to examine intraspecies variation among regionally disjunct subpopulations, comparing collections separated by the Florida Peninsula, and [2] to examine intraspecies variation within deep-sea cephalopods in the Gulf of Mexico. Through Sanger sequencing marker genes COI, 16S rRNA, and 28S rRNA, this study has generated a genetic baseline characterization of deep-sea cephalopods in the Gulf of Mexico, assessed intraspecies genetic variation, and linked morphological identification with DNA barcodes, testing morphological hypotheses of species identification and naming. Results of investigating intraspecies variation within regionally disjunct subpopulations reveal there is no regional distinction between the Gulf of Mexico subpopulations of *Vampyroteuthis infernalis*, *Pyroteuthis margaritifera*, and *Cranchia scabra*, and the Bear Seamount subpopulations in the northwestern Atlantic Ocean. Results of

investigating intraspecies variation within the Gulf of Mexico displayed potential for cryptic species, novel sequence records, and large expansions to sequence records for species known to inhabit the Gulf of Mexico. Analysis of intraspecies variation within the Gulf of Mexico facilitated identification of damaged specimens used for this study, but also revealed GenBank database issues of misidentified records, and outdated nomenclature in accession records. Because cephalopods play a central role in most oceanic ecosystems, characteristics like a short average life span and a rapid growth rate mean that cephalopod populations have the potential to serve as an invaluable reflection of ecosystem change.

CHAPTER ONE: Introduction

1.1 Cephalopoda: Decapodiformes and Octopodiformes

The group referred to as cephalopods, class Cephalopoda (Cuvier, 1797), belongs to the phylum Mollusca (Linnaeus, 1758). Molluscs are soft-bodied invertebrates with these defining characteristics: specialized tissue known as the mantle, a feeding structure made up of a band of teeth known as radula, and a modified foot used for locomotion (Pechenik, 2010). The body plan of Cephalopoda is a merged head and foot; arms, tentacles, and funnel are all modifications of the foot. Mature cephalopod size has an enormous range, from smaller than 1 cm (Jackson, 1990) to around 20 m in total length (Roper & Jereb, 2010). While cephalopods have high growth rates, on average most live for a period of a few months to a few years (Judkins, 2009). The life-history strategy of high growth rates, short lifespans, and life-history adaptability, allows cephalopods to take advantage of rapidly changing environmental conditions: extreme climate change and anthropogenic influences like overfishing, pollution, etc. (Doubleday et al., 2016).

Cephalopods are strictly marine organisms, inhabiting both benthic and pelagic zones, found in all the world's oceans except the Black Sea (Jereb & Roper, 2010). The vertical distribution of cephalopods is extensive and highly variable, from the shallow intertidal to depths of over 5,000 m (Judkins, 2009). It is common for cephalopods to undergo diel migration, occurring deeper in the water column during the day (400 m to 1000 m) and ascending to the euphotic zone for the night (Judkins, 2009; Roper & Young, 1975). The class Cephalopoda

currently consists of around 700 extant recognized species (Judkins et al., 2016b; Uribe & Zardoya, 2017).

The subclass Coleoidea comprises almost all cephalopod species within the extant superorders: Decapodiformes (Leach, 1817) and Octopodiformes (Berthold & Engeser, 1987) (Allcock et al., 2015). Squids and cuttlefishes belong to the superorder Decapodiformes. All squids have the following defining characteristics distinguishing them from other cephalopods: a torpedo-shaped body with a pair of external fins situated on the mantle, internal chitinous gladius, buccal crown, multifunctional funnel, ten appendages surrounding the mouth, eight arms with two or four series of suckers with chitinous rings (transformed into hooks in some), and two elongate tentacles with tentacular clubs composed of series of suckers and/or hooks (Jereb & Roper, 2010). The octopods and vampire squid, *Vampyroteuthis infernalis* (Chun, 1903), belong to the superorder Octopodiformes (Berthold & Engeser, 1987). Defining characteristics of this cephalopod superorder include: a moderately spherical body with a mantle, multifunctional funnel, lack of a buccal crown, eight arms surrounding the mouth, lack of a pair of tentacles, and one or two series of symmetrically rounded suckers lacking chitinous rings on the arms (Jereb, et al., 2016).

1.2 Molecular Systematics

Systematics is the study of the diversity of and evolutionary relationships among life forms (Wiley & Lieberman, 2011). The field of systematics requires comprehensive understanding of organismal diversity to identify, name, describe, classify, and determine evolutionary relationships (Wiley & Lieberman, 2011). Systematics research remains vital to a comprehensive understanding of marine flora and fauna; it is fundamental to all biology. The

rate of discovery of new marine species indicates that there is still an enormous number of undiscovered and/or unidentified marine species (Bucklin et al., 2011). For fisheries to manage target stocks and the ecosystems in which they occur, we must understand how ecosystems work, this requires a foundation of knowledge of individual species that make up each ecosystem (Ward et al., 2016).

Traditional systematics relies on morphological characters to determine classification and phylogeny of taxa. Because morphological characters are rooted in the organism's genetic make-up, molecular characters are thought to be more direct and accurate (Patwardhan et al., 2014). However, molecular characters can still suffer from aspects of subjectivity due to difficulties with polymorphisms and base determination in DNA/RNA sequencing (Patwardhan et al., 2014).

Molecular characters are proxies of genetic traits evolved from common ancestral genes (Patwardhan et al., 2014). Because phylogeny seeks to 'map out' the decent, lineages, and potential divergence of taxa from common ancestors, it is logical to examine molecular characters to classify taxa. Advantages of using molecular characters include: a larger data set that is easier to obtain than examination of specimens at various life stages and no sampling bias involved, helping to correct gaps in species' life histories. Molecular characters can also aid in distinguishing specimens damaged during collection, and elucidating often cryptic, intra- and interspecific diversity. In terms of phylogeny, molecular characters aid in yielding clearer phylogenetic trees. Because every taxon is the result of an evolutionary process, its evolutionary history must be determined to understand and express the taxon in biological terms (Patwardhan et al., 2014).

A more comprehensive, robust approach to systematics is combining data of morphological characters and molecular characters to determine the phylogeny and classification

of taxa. This includes a combination of: phenotypic information gained from expressed genes including both internal and external morphology, genotypic information obtained from the genetic material, and phylogenetic information of a taxon's phylogeny when homologues of DNA, RNA or protein sequences are compared (Patwardhan et al., 2014).

Comparing homologous genes from different organisms will reveal the amount of similarity (relation), or dissimilarity, allowing one to infer population connectivity and branching of a phylogenetic tree (Patwardhan et al., 2014). The larger the dissimilarity in homologous genes from different organisms, the further the organisms are separated from each other in the evolutionary timescale (Patwardhan et al., 2014). Because genetic molecules can mutate at different rates, phylogenetic information from a single marker gene or protein sequence only correlates to the evolutionary time scale of that particular gene (Strugnell & Lindgren, 2007). Hence, the use of a singular molecular marker can cause a bias, where different genes in an organism can show differing rates of evolution or differing evolutionary histories (Strugnell & Lindgren, 2007; Patwardhan et al., 2014).

1.3 Cephalopod Genetics

In 1983 the first cephalopod DNA sequence was published, followed by the first molecular paper on cephalopods in 1994 (Allcock et al., 2015). Bonnaud et al. (1994) performed a phylogenetic study which ultimately found no support for higher-level cephalopod relationships (Allcock et al., 2015). A few years later, Bonnaud et al. (1997) followed-up with a molecular study on various cephalopod species using a mitochondrial 16S rRNA marker, which supported higher-level relationship hypotheses of prior morphological systematics studies (Lindgren et al., 2004). While the Bonnaud et al. (1997) study supported the morphological

understanding of high-level cephalopod relationships, a more comprehensive study was soon to follow, by Carlini and Graves (1999). The Carlini and Graves (1999) study investigated the utility of cytochrome c oxidase subunit I (COI) marker gene for determining higher-level relationships of 48 cephalopod species, confirming some morphological data, but unable to resolve the phylogeny of the vampire squid and many Decapodiformes' interfamilial relationships (Lindgren et al., 2004).

Molecular methods have improved with time, in particular, universal primers like those targeting the 658-base pair region of the COI gene have been employed across a broad spectrum of metazoans successfully (Bucklin et al., 2011; Allcock et al., 2015). The universal COI primers have been widely used as a new tool to quickly and reliably identify known cephalopod species and aid in elucidation of cryptic species (Dai et al., 2012). Strugnell & Lindgren, (2007) noted potential pitfalls utilizing only one universal barcode in cephalopods: slow rates of evolution and gene duplication in some taxa (Sanchez et al., 2016). Then, Lindgren (2010) performed a comprehensive study to further investigate relationships within Oegopsida, as well as investigate higher-level relationships within Decapodiformes, through five molecular markers (18S rRNA, 28S rRNA, Histone H3, 16S rRNA, COI).

COI has been sequenced and used as a barcode for most known coleoid species (e.g. Dai et al. 2012, Allcock et al. 2015). Barcodes (COI sequences) and other molecular markers are entered into an open-access genetic sequence database (e.g. GenBank: www.ncbi.nlm.nih.gov/genbank/, or BOLD (www.barcodinglife.org) as reference for future researchers. Queries can be run through Basic Local Alignment Search Tool (BLAST: blast.ncbi.nlm.nih.gov) to find pairwise comparisons between nucleotide sequences and infer functional and evolutionary relationships between sequences (Altschul et al., 1990). One

difficulty with open-access genetic sequence databases like GenBank is they are built by direct submissions from individual laboratories, leaving room for morphological misidentifications which lead to incorrect reference sequences and potential misrepresentations of marker genes due to variable laboratory methods. Incorrect annotations in the GenBank database can be propagated by assigning species names to new accession records solely based on sequence similarity without morphological identity confirmation; this leads to a decline in database quality.

Other important contributions to cephalopod genetics include studies that have focused on genomics (Allcock et al., 2015). The first, complete cephalopod mitochondrial genome sequenced was a combined effort by Sasuga et al. (1999), followed by Tomita et al. (2002), of the commercially important squid: *Heterololigo bleekeri* (Allcock et al., 2015). Since then, several cephalopod species' complete mitochondrial genomes have been added to GenBank (Allcock et al., 2015). Most recently, Uribe and Zardoya (2017) and Strugnell et al. (2017) have utilized complete mitochondrial genomes to reconstruct the basal phylogenies of Cephalopoda, and the Ram's Horn Squid, respectively. Uribe and Zardoya (2017) harnessed the utility of all 39 currently published complete mitochondrial genomes and concatenating available partial mitochondrial genomes for genera that lack complete mitochondrial genomes to infer evolution of gene rearrangements and estimated dates of pivotal cladogenetic events within Cephalopoda.

With Next Generation Sequencing (NGS) and bioinformatics on the forefront, a wave of evolutionary and developmental studies focusing on transcriptomics, epigenetics, whole genome sequencing, and population genetics are in motion. Lindgren and Anderson (2017) just published a study which examined the utility of coleoid transcriptome data for deriving phylogenetic relationships. Cephalopods have been shown to be unique in RNA editing (Liscovitch-Brauer et

al., 2017), showcasing an unparalleled epigenetic source of phenotypic plasticity. Liscovitch-Brauer et al. (2017) highlight the cephalopod's, specifically coleoids, tremendous capacity to edit RNA with high frequency, unlike in mammals where RNA editing is both infrequent and usually limited to non-coding RNA. One of the newest technologies employed for cephalopod genetics from primarily one lab (Cheng, 2015) is double digest Restriction Site Associated DNA sequencing (ddRADseq), which is a method for sampling the genomes of many wild-type individuals in a population using NGS. The dissertation of Cheng (2015) focused on ddRADseq to resolve issues of species identity and to delineate spatial patterns of population connectivity in commercially important species of squids.

In addition, a new effort by the Cephalopod Sequencing Consortium (CephSeq) with a NGS approach is focused on sequencing entire genomes of select cephalopod species: *Octopus vulgaris*, *Octopus bimaculoides*, *Hapalochlaena maculosa*, *Sepia officinalis*, *Doryteuthis pealeii*, *Euprymna scolopes*, *Idiosepius sp.*, *Architeuthis dux*, and *Nautilus sp.* (Albertin et al., 2012). A little over two years ago, a breakthrough in cephalopod genetics made history. For the first time, CephSeq successfully sequenced the entire genome of a cephalopod, *Octopus bimaculoides* (Albertin et al., 2015). This enormous advance in cephalopod genetics serves as both a vehicle into a new realm of evolutionary and developmental questions, and as a stark contrast to the nominal, existing baseline of genetic data for cephalopods. While there have been vast improvements in cephalopod genetics, the cephalopod tree is not yet fully resolved (Allcock et al., 2015).

1.4 Overview of Thesis

Nearly all deep-sea cephalopod life history studies have been completed by examination of specimens collected in the wild. Much of this work is like piecing together a puzzle; knowledge of the life history of many species remains fragmented and hence, taxonomically and phylogenetically confused. Modern molecular approaches and sequencing technologies are powerful tools for deciphering wild-type cephalopod life histories and population dynamics (e.g. Dai et al., 2012; Vecchione et al., 2015; Judkins et al., 2016a). Use of molecular markers offers additional certainty for identifying specimens damaged during deep-sea collections and can elucidate often cryptic, intra- and interspecific diversity. This study examines the genetic diversity of deep-sea cephalopods in the Gulf of Mexico, with a look into population connectivity outside of the Gulf of Mexico. This study has two key objectives:

1. To examine intraspecies variation among regionally disjunct subpopulations, comparing collections separated by the Florida Peninsula (northern Gulf of Mexico and northwestern Atlantic Ocean).
2. To examine intraspecies variation within deep-sea cephalopods in the northern Gulf of Mexico, testing each specimen's identity assigned by morphology with molecular methods.

It is imperative to understand the genetic exchange of cephalopods to determine if demographic independence exists among populations and subsequently, assess their susceptibility to impact and recovery after disturbance. Collecting baseline information about deep-sea, genetic biodiversity is vital to improving ocean health and vitality.

CHAPTER TWO: Genetic Identification and Population Characteristics of Deep-Sea Cephalopod Species in the Gulf of Mexico and Northwestern Atlantic Ocean

2.1 Introduction

2.1.1. Gulf of Mexico

The Gulf of Mexico basin is roughly 1,500 km in diameter, with an average depth of approximately 1,615 m (Rivas et al., 2005), comprised of four distinct areas: 38% shallow and intertidal areas (< 20 m deep), 22% continental shelf (< 180 m), 20% continental slope (180 - 3,000 m), and 20% abyssal areas deeper than 3,000 m (Gore, 1992; Moretzsohn et al., 2010). The deep waters of the Gulf of Mexico (> 1,000 m), reflect the characteristics of North Atlantic Deep Water (NADW) (Nowlin et al., 2001): cold and oxygen-rich (Rivas et al., 2005).

Water enters the Gulf of Mexico from the Caribbean through the Yucatan Channel, feeding the Loop Current as it sheds warm-core eddies, and exits through the Florida Straits as the main contribution to the Gulf Stream (Rivas et al., 2005; Moretzsohn et al., 2010). The Gulf Stream moves north, through the Bahamas and east coast of Florida, as a poleward transfer of heat and salt (Rivas et al., 2005; Judkins, 2009). Because the Florida Sill of the Florida Straits is at a depth of 800 m, deep water exchange in the Gulf of Mexico must take place through the Yucatan Sill (2040 m) of the Yucatan Channel, as well as mixing and diffusion with upper layers (Rivas et al., 2005). Based on net transport measured over the Yucatan Sill (~0.32 Sv), Rivas et al. (2005) suggested an extremely brief residence time of the Gulf of Mexico's deep waters: approximately 250 years.

In the northern Gulf of Mexico on April 20, 2010, a mechanical failure occurred on the Deepwater Horizon drilling unit, releasing 4.9 million of barrels of crude oil (Ramseur, 2011), resulting in the unprecedented use of 2.1 million gallons of dispersants (Allen et al., 2012; Love et al., 2015). Within the scientific community prior knowledge on how deep-sea ecosystems and habitats would react to crude oil and dispersant exposure, as well as how to approach a recovery of those ecosystems and habitats, was minimal (Love et al., 2015). After the Deepwater Horizon oil spill in 2010, new research programs were initiated in the Gulf of Mexico deep water environment through the Gulf of Mexico Research Initiative (GoMRI). The Deep Pelagic Nekton Dynamics of the Gulf of Mexico (DEEPEND) program, funded by GoMRI, is focused on generating a comprehensive evaluation of extant deep-pelagic communities to inform the quantification of deep-pelagic susceptibility to impacts and restoration after disturbance between 0-1500 m in depth (Sutton et al., 2015).

Even before the Deepwater Horizon oil spill, knowledge of deep-sea cephalopod species in the Gulf of Mexico was limited with two comprehensive studies conducted by Voss in 1956 and Judkins in 2009. The most recent compilation of the cephalopods in the Gulf of Mexico is that of Judkins' (2009) monograph and Vecchione's (2002) *The Living Marine Resources of the Western Central Atlantic* FAO Cephalopod Species Identification Guide. To date, roughly 109 species of cephalopods have been identified in the western central Atlantic, including the Caribbean Sea and the Gulf of Mexico, with 89 species found specifically in the Gulf of Mexico (Judkins, 2009). While records of cephalopods in the Gulf of Mexico originate with Lesueur's (1821) publication, most cephalopod studies have included this region in broader studies (e.g. Vecchione, 2002) or examined fisheries-related species based on limited geographic area (e.g. Avila-Poveda et al., 2009; Sales et al., 2014). The types of cephalopods listed in the Gulf of

Mexico include squids, octopods, and the vampire squid (Voss, 1956; 1962; Roper, 1964; Roper et al., 1969; Lipka, 1975; Passarella, 1990; Salcedo-Vargas, 1991; Vecchione, 2002; Judkins et al., 2010).

In Judkins' (2009) comprehensive study of cephalopods in the Gulf of Mexico and Broad Caribbean, the eastern coast of Florida exhibited the highest species richness (*n*=32). It was suggested the large species diversity might be due to increased nutrient mixing in the Gulf Stream, patterns of current transport in the region (the convergence of the Florida current and the North Equatorial current), and the presence of both shallow and deep water habitats existing along the eastern coast of Florida (Judkins, 2009). It was also noted that the Florida Straits, only 80 km wide and 800 m deep, is an important barrier to consider in the dispersal of Gulf of Mexico biota (Judkins, 2009). The study recommended DNA analysis to gain a better understanding of this complex cephalopod assemblage in the Gulf of Mexico region (Judkins, 2009).

2.1.2 Bear Seamount

Bear Seamount (39º55'N 67º30'W) is an undersea mountain formed by volcanic activity, located in the northwestern Atlantic Ocean inside the US Exclusive Economic Zone, southeast of Georges Bank (Moore et al., 2003). While the top of Bear Seamount is flat, around 1,100 m below the sea surface, the seamount rises out of the continental slope at depths of 2,000 m to 3,000 m. In 1954 the Woods Hole Oceanographic Institution (WHOI) discovered, named, and mapped Bear Seamount (Moore et al., 2003). Recently, President Obama established the first Marine National Monument in the Atlantic Ocean, protecting Bear Seamount and its neighboring chain of seamounts and canyons: the New England Seamounts (NES), which form the longest seamount chain in the North Atlantic.

A chain of more than 30 major extinct volcanic peaks make up the NES (Moore et al., 2003), of which Bear Seamount is the most inshore. Two major currents intersect with the NES chain: the Gulf Stream, which flows to the north-east, and the north Atlantic Deep Western Boundary under Current (DWBC), a cold-water current which flows deep, south-west along the continental slope (Hamilton et al., 1996; Moore et al., 2003). The cold, dense, Antarctic Bottom Water (AABW) also flows around the eastern NES bases. As with other seamounts the NES are considered to be biological hotspots supporting species diversity (Moore et al., 2004). The steep slopes and shapes of seamounts cause nutrient upwelling as well as alterations in the flow of currents nearby (Vastano & Warren, 1976; Hogg et al., 1986). The alteration of nearby, deep-water currents influences the recruitment of seamount flora and fauna (Moore et al., 2003).

Flowing from the Labrador Sea in the northern Atlantic, the Deep Western Boundary under Current crosscuts the westernmost seamount of the NES chain on the continental slope: Bear Seamount and nearby seamounts (Moore et al., 2004). The Deep Western Boundary under Current is a potential dispersal route of exotic northern species brought southwards to Bear Seamount (Moore et al., 2004). The rest of the NES chain is not subjected to as cold water temperatures from the Deep Western Boundary Current as at Bear Seamount, hence, species richness and species diversity associated with Bear Seamount is high (Moore et al., 2004). Another dispersal route into Bear Seamount is from the Gulf Stream and the warm-core eddies from the Gulf Stream (Markle et al., 1980; Harold & Clark, 1990; Moore et al., 2004). Both benthic and pelagic cephalopod species found in the deep waters of the Caribbean and Gulf of Mexico regions, have been found in the vicinity of Bear Seamount (Moore et al., 2004; Shea et al., 2017). Moore et al. (2004) recommended population-genetics studies to better understand genetic drift and establishment of populations at Bear Seamount from various dispersal routes;

suggesting that new populations of species colonized at Bear Seamount from distant original source populations.

2.1.3 Molecular Markers

It is important to note, not all genes are fit to be taxonomic markers, and not all molecular markers are appropriate for phylogenetic analyses of a given taxon. Potential molecular markers must be evaluated on their ability to retrieve full-fledged phylogenetic relationships within clades of similar evolutionary timescales (Patwardhan et al., 2014). Molecular markers that achieve this, serve as genetic records and archives, which can then be compared amongst related organisms to yield evolutionary histories of the genes, and therefore, the phylogenetic relationships of the organisms (Patwardhan et al., 2014).

Gene sequences can be categorized by genome; nuclear or mitochondrial, and by function: protein-coding, noncoding, or structural RNA (Springer et al., 2001). For higher taxonomic levels, conserved molecular markers are necessary to examine basal phylogenetic relationships. With closely related taxa, a gene with a high substitution rate is necessary to allow for enough mutations to accumulate over a small evolutionary time period (Strugnell & Lindgren, 2007; Patwardhan et al., 2014). The ribosomal RNA (rRNA) genes serve a critical role, helping to assemble amino acids, the protein building blocks, into proteins (Patwardhan et al., 2014). Commonly, mitochondrial ribosomal RNA genes have a higher rate of nucleotide substitution than the nucleotide substitution rate of nuclear ribosomal RNA genes (Springer et al., 2001). In addition, mitochondrial DNA (mtDNA) is inherited from mother to child as a single, haploid linkage unit, which yields a smaller genetically effective population size compared with the nuclear ribosomal DNA (rRNA) (W. S. Moore, 1995; Patwardhan et al., 2014). Therefore, with a smaller genetically effective population size, there will be faster genetic

drift, and hence, a faster evolution of mitochondrial genes compared with nuclear genes (Moore, 1995). This will lead to a higher likelihood that the mitochondrial gene tree accurately reflects the species tree for closely spaced speciation events compared with nuclear gene trees (Moore, 1995; Patwardhan et al., 2014). In order to analyze population connectivity and various phylogenetic relationships within clades of similar evolutionary timescales, it is helpful to utilize both nuclear genes for distinguishing more distantly related taxa and mitochondrial genes to distinguish closely related taxa. Ideal marker genes with varying degrees of sequence conservation were selected for this study (Hwang & Kim, 1999; Aguilera-Munoz et al., 2008; Lindgren, 2010; Allcock et al., 2015): two mitochondrial genes (cytochrome c oxidase subunit I (COI) and 16S rRNA) and one nuclear gene (28S rRNA).

Nuclear rRNA genes encode rRNAs and have differing rates of evolution among coding regions and spacer regions (Hwang & Kim, 1999; Patwardhan et al., 2014). Nuclear rRNA coding regions evolve slower than nuclear rRNA spacer regions due to non-coding (i.e. non-functional) nucleotide substitutions occurring in spacer regions. While nucleotide substitutions in nuclear rRNA spacer regions do not cause harmful effects on organisms, the effects on rRNA coding regions can cause problems with ribosome construction or protein synthesis (Hwang & Kim, 1999). Consequently, rRNA coding regions are more conserved relative to the spacer regions in nuclear rRNA (Hwang & Kim, 1999). Due to rRNA coding regions' and nuclear rRNA spacer regions' differing rates of evolution, a more conserved coding region (28S rRNA, D3 region) was selected to help address objective 1, serving as a standard confirmation for clear, delineation of each putative species from regionally disjunct subpopulations, comparing collections separated by the Florida Peninsula (Gulf of Mexico and northwestern Atlantic Ocean).

The mitochondrial genome evolves faster than the nuclear genome (Hwang & Kim, 1999; Springer et al., 2001; Patwardhan et al., 2014). As a result, mitochondrial protein coding genes are often employed to examine phylogeny among lower taxonomic levels (i.e. genera and species). Because mtDNA is faster evolving than nuclear rRNA, more conserved regions of mtDNA ensure an appropriate evolutionary scale for the scope of this study. The mitochondrial 16S rRNA gene is the most conserved region among the mitochondrial genes, and COI is the most conserved among the three cytochrome oxidase coding genes (Hwang & Kim, 1999). In addition, both 16S rRNA and COI are conserved enough to have standard universal primers. The mitochondrial protein coding genes 16S rRNA and COI were therefore selected to examine intraspecies variation within deep-sea cephalopods in the Gulf of Mexico.

2.2 Materials and Methods

2.2.1 Sample Sites and Collections

Deep-sea cephalopod specimens were collected at various stations in the northern Gulf of Mexico over a three-year period through the DEEPEND Program (2015-2017), as well as near Bear Seamount in the northwestern Atlantic Ocean (39^{o}55'N 67^{o}30'W) through NOAA's Deepwater Biodiversity Project (2014) (Figure 2.1).

Figure 2.1. Map of research regions
Specimens were collected from two regionally disjunct basins, separated by the Florida Peninsula: the northern Gulf of Mexico and northwestern Atlantic Ocean. DEEPEND sampling stations were taken from within the red box. The star symbol indicates the location of Bear Seamount, sampling stations encircled the seamount itself.

Gulf of Mexico specimens were caught by a 10 m^2 mouth area Multiple Opening/Closing Net and Environmental Sensing System (MOC-10) rigged with six 3 mm mesh nets (Sutton et al., 2015). Sampling at each station was conducted twice, with one deployment at solar noon (1000 h-1600 h) and one at midnight (2200 h-0400 h) (Sutton et al., 2015). The first net (Net 0) was towed from the surface to 1500 m, Net 1 from 1500 m to 1200 m, Net 2 from 1200 m to 1000 m, Net 3 from 1000 to 600 m, Net 4 from 600 to 200 m, and Net 5 from 200 m back to the surface (Sutton et al., 2015). This was the same depth sampling scheme used for the NOAA NRDA Offshore Nekton Sampling and Analysis Program. Bear Seamount specimens were caught using a Superior midwater Trawl rigged with deep-water floats and White Nets doors

(standard tom weights and spectra bridles) and a 4-Seam Trawl (non-standard, bottom trawl) rigged with deep-water floats and rock-hopper sweep used with Perfect Doors (Moore et al., 2003; 2004; Shea et al., 2017). Both measured environmental parameters: conductivity, temperature, and depth during the tows.

To address objective 1, three focal deep-sea cephalopod species with high relative abundance and distribution including both the Gulf of Mexico and Bear Seamount were collected: *Vampyroteuthis infernalis*, *Pyroteuthis margaritifera*, and *Cranchia scabra*. To address objective 2, only deep-sea cephalopod specimens collected through the DEEPEND program in the Gulf of Mexico were utilized. Tissue samples were collected at sea. DEEPEND tissue samples were directly frozen at -20^{o}C in RNALater, while tissue samples from Bear Seamount were directly frozen at -20^{o}C in 95% ethanol. Respective vouchers were fixed in 10% formalin, and later transferred to 50% isopropyl alcohol. In total, 215 individual specimens were sampled for genetic data from DEEPEND. From 215 specimens, 78 of the three focal species were combined with an additional 89 individuals sampled from Bear Seamount for objective 1 population connectivity analyses.

2.2.2 Morphological Identification

Morphological analyses were conducted in two stages to verify each specimen's identity: initial specimen identification was completed at sea by a designated researcher (Dr. M. Vecchione or Dr. H. Judkins) during field collection, a second round of morphological analyses occurred at H. Judkins lab to verify all specimen identifications. Cephalopods were identified to species when possible. The dorsal mantle length (DML) was measured on all intact specimens for reference. Morphological analyses were conducted with the use of taxonomic descriptions in: Vecchione (2002), Jereb and Roper (2010), the Tree of Life Web Project

(http://tolweb.org/tree/), morphometrics (measurements of size and external shape), and meristics (counts of structures such as suckers and hooks). Appendix A includes the species, abundance, and selected organisms that were utilized for this study.

2.2.3 DNA Extraction, PCR Conditions and Sequencing

Tissue samples were cut individually in sterile conditions for sequencing. DNA was extracted from a 12-24 mg portion (when available) of each tissue sample using the Qiagen DNeasy Blood and Tissue Kit (qiagen.com/dna-preparation/dneasy-blood-and-tissue-kit), following the manufacturer's protocol. Polymerase chain reaction (PCR) was then performed to amplify the DNA locus of interest: COI (658 bp), 16S rRNA (~520 bp), and 28S rRNA (~590 bp). The universal primers used in this study are listed in Table 2.1 and sourced from (Folmer et al., 1994), (Xiong & Kocher, 1991), and (Whiting et al., 1997) for COI, 16S rRNA, and 28S rRNA, respectively.

Table 2.1 List of primers
A list of primers, by locus, used in this study.

Locus	Forward Primer	Reverse Primer
COI	LCO1490 (5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′)	HCO2198 (5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′)
16S rRNA	16Sa (5′-CGC CTG TTT ATC AAA AAC AT-3′)	16Sb (5′-CTC CGG TTT GAA CTC AGA TCA-3′)
28S rRNA (D3 region)	28Sa (5′-GAC CCG TCT TGA AAC ACG GA-3′)	28Sb (5′-TCG GAA GGA ACC AGC TAC-3′)

PCR reactions contained the following in a 25-µl final reaction volume: 12.5 µl GoTaq® DNA Polymerase, 1 µl forward primer (i.e. LCO1490, 16Sa, or 28Sa), 1 µl reverse primer (i.e. HCO2198, 16Sb, or 28Sb), 8.5 µl of sterile distilled water, and 2 µl of diluted template DNA.

Template DNA was diluted to a 1:10 ratio for all reactions as DNA was highly concentrated in extracted samples; there were some cases where template DNA had to be diluted to 1:100 and 1:1000 for successful amplification.

Lindgren (2010) thermocycling protocols were followed for PCR amplification of COI, 16S rRNA, and 28S rRNA (Table 2.2).

Table 2.2 List of thermocycling protocols
A list of thermocycling protocols by locus used in this study.

Locus	Initial Denaturation	Denaturation	Annealing	Extension	Final Extension	Hold
COI and 16S rRNA	95°C, 2 min	94°C, 1 min	48°C, 1 min	72°C, 1 min	72°C, 7 min	4°C, ∞
28S rRNA (D3 region)	95°C, 2 min	94°C, 1 min	40°C, 1 min	72°C, 1 min	72°C, 7 min	4°C, ∞
	1 Cycle	35 Cycles			1 Cycle	

For some of the more difficult taxa/loci, individual gene PCR products were cloned using the CloneJET PCR Cloning Kit (Thermo Scientific) and commercially sequenced using vector primers, following the same protocol used in Judkins et al. (2016a). All PCR products were visualized with gel electrophoresis, run at a voltage of 120v for 60 min in a 1.5% agarose gel stained with ethidium bromide.

Unpurified PCR products were sent for commercial clean-up and bidirectional Sanger sequencing (Sanger et al., 1977) at GeneWiz sequencing facility (www.genewiz.com). The resulting forward and reverse sequences were assembled and edited manually in Geneious (www.geneious.com). Any assembled sequences under a minimum average quality score of 85 were not used in this study. The assembled sequences that passed quality check were imported into the publically available sequence alignment software: Molecular Evolutionary Genetics Analysis, or MEGA7 (megasoftware.net) (Kumar et al., 2016), to trim primers and align sequences.

2.2.4 Analyses of Sequence Data

A total of 549 sequences (238 COI, 216 16S rRNA, and 95 28S rRNA) were included in this analysis of sequence data using MEGA7 (Kumar et al., 2016). Appendix B includes the sequence labels and associated vouchers for future reference. To address objective 1, sequences of the three focal species (*Vampyroteuthis infernalis*, *Pyroteuthis margaritifera*, and *Cranchia scabra*) from the Gulf of Mexico and Bear Seamount were grouped into individual datasets for each gene: COI, 16S rRNA, and 28S rRNA. To address objective 2, sequences generated exclusively from the Gulf of Mexico were compiled into individual datasets based on Family for each gene: COI and 16S rRNA. Sequences for each dataset were aligned using the ClustalW algorithm (Thompson et al., 1994), with alignment quality checks performed manually. Additionally, the COI alignments were checked for the occurrence of nuclear copies of mitochondrial genes (referred to as: numts or pseudogenes) that have been translocated to the nuclear genome. The COI alignments were checked for pseudogenes by looking at the translated protein sequences using the invertebrate mitochondrial code '5' for the presence of premature stop codons and indels (Strugnell & Lindgren, 2007; Allcock et al., 2015).

The phylogenetic trees were also constructed in this study using MEGA7 (Kumar et al., 2016). Molecular phylogenetic analyses were performed by the Maximum Likelihood method based on the Tamura-Nei model (Tamura & Nei, 1993) and bootstrap analyses were carried out using 1000 replicates. Using MEGA7, initial tree(s) for the heuristic search were obtained by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood approach, and then selecting the phylogenetic tree with highest log likelihood value (Kumar et al., 2016). The trees were drawn to scale, with branch lengths representing the number of nucleotide substitutions per site and the percentage of trees in

which the associated taxa clustered together shown next to the branches as bootstrap support (Kumar et al., 2016).

The Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990) and GenBank sequence database (www.ncbi.nlm.nih.gov/genbank/) were used to check the new sequences by comparing to available nucleotide sequences in GenBank and identify which database sequences match the specimens at a threshold of 98% or higher sequence identity (Lindgren, 2010; Dai et al., 2012). Relevant GenBank accession records were added to each alignment and phylogenetic tree for reference and comparison purposes.

2.3 Results

2.3.1 Intraspecies Variation within Regionally Disjunct Subpopulations

One hundred and ten COI amplicons were successfully recovered from specimens in this study, with only one specimen failing to amplify with the COI primers used. An additional 101 16S rRNA amplicons and 95 28S rRNA amplicons were successfully recovered in this study. Phylogenetic trees of COI, 16S rRNA, and 28S rRNA are shown in Figures 2.2, 2.3, and 2.4., with Gulf of Mexico specimens indicated by blue symbols and Bear Seamount specimens indicated by black symbols. While the trees resolved by Maximum Likelihood methods were not identical, the recovered putative species of all three trees were the same. Each species is clearly and definitively clustered, with agreement among all genetic loci. The three trees show that species form monophyletic clusters in agreement with their current, accepted systematic resolutions. Each cluster shares between 98-100% pairwise identity, with high bootstrap support. Between the clusters, there are substantial differences in nucleotide sequences, with less than 90% pairwise identity.

In each phylogenetic tree, the Gulf of Mexico and Bear Seamount sequences from each species display no region-specific grouping. These results show that there is no genetic differentiation between the Gulf of Mexico and Bear Seamount subpopulations of *Vampyroteuthis infernalis*, *Pyroteuthis margaritifera*, and *Cranchia scabra*. The genetic resolution of COI, 16S rRNA, and 28S rRNA at the species level for these focal taxa is explicit. It is interesting to note the differences in nucleotide substitution rate between COI, with a slightly higher substitution rate between species (around four changes per 100 nucleotides), and 16S rRNA (around three changes per 100 nucleotides). Slightly more intraspecies genetic variability is seen in the COI tree than in the 16S rRNA tree. As expected (Hwang & Kim, 1999), the nucleotide substitution rate of the nuclear 28S rRNA gene is the slowest (with around two changes per 100 nucleotides), showing the poorest intraspecies resolution, but clear confirmation of species delineations.

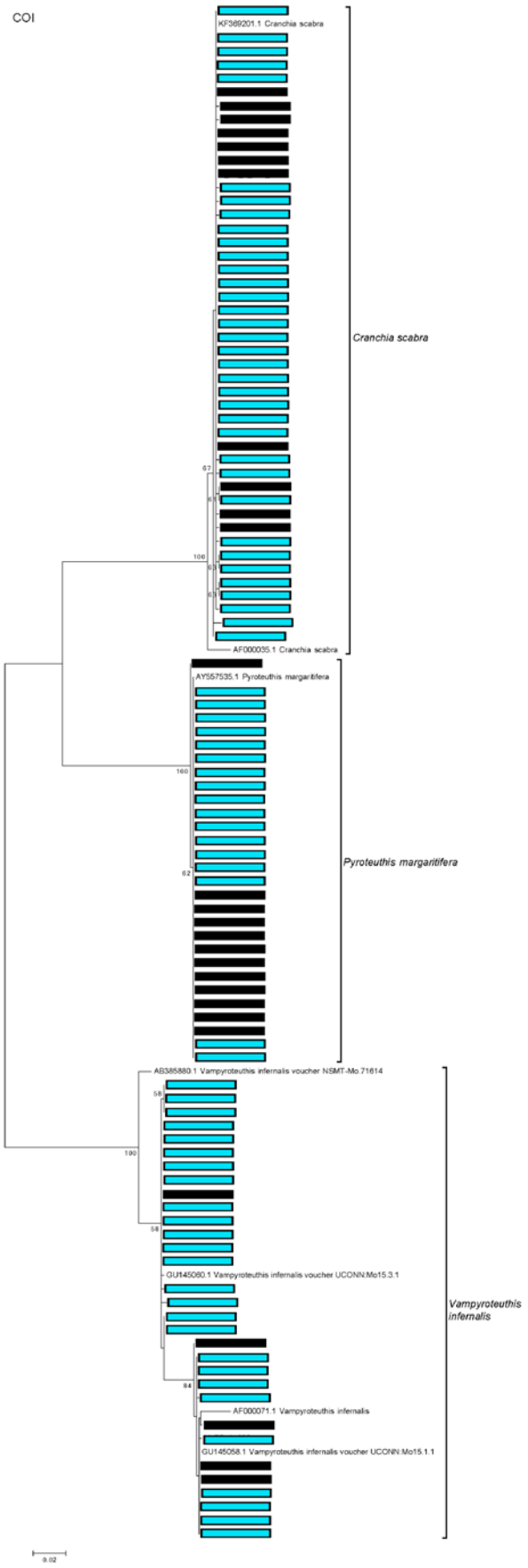

Figure 2.2 COI phylogenetic tree

COI Maximum Likelihood phylogenetic tree with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Blue symbols indicate specimens collected from the Gulf of Mexico, while black symbols indicate specimens collected from Bear Seamount. Relevant GenBank accession records were added for reference.

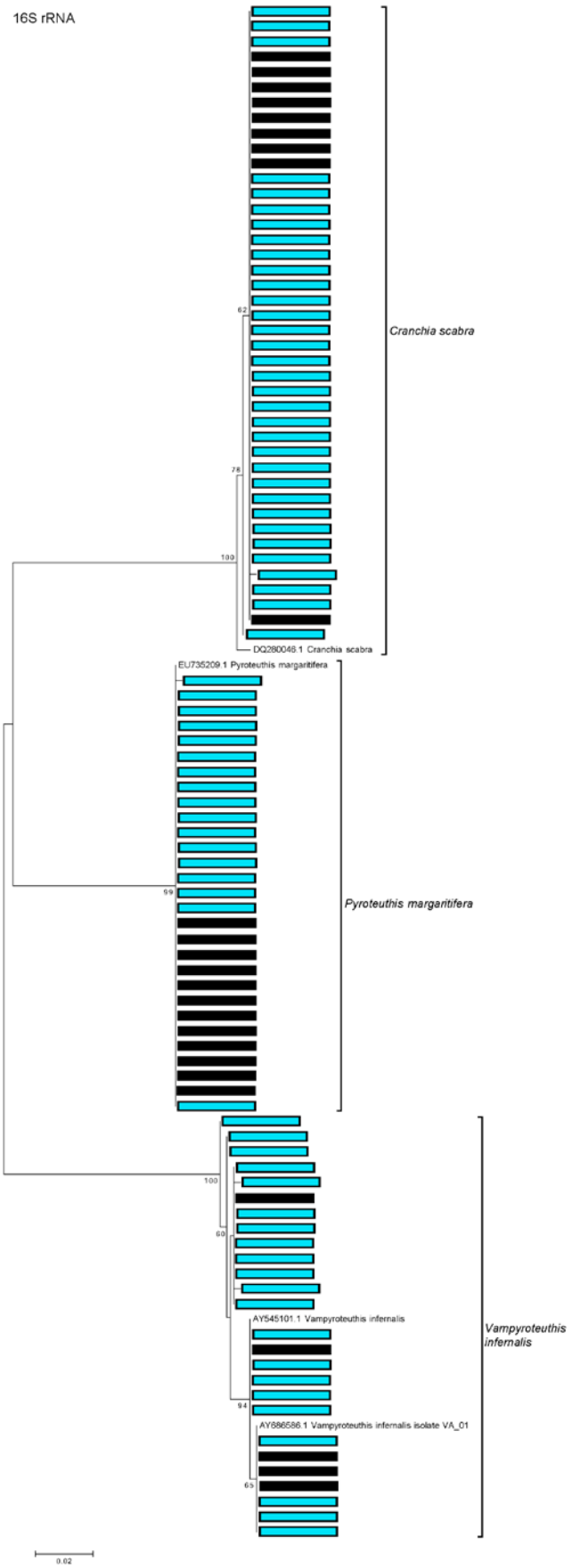

Figure 2.3 16S rRNA phylogenetic tree

16S rRNA Maximum Likelihood phylogenetic tree with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Blue symbols indicate specimens collected from the Gulf of Mexico, while black symbols indicate specimens collected from Bear Seamount. Relevant GenBank accession records were added for reference.

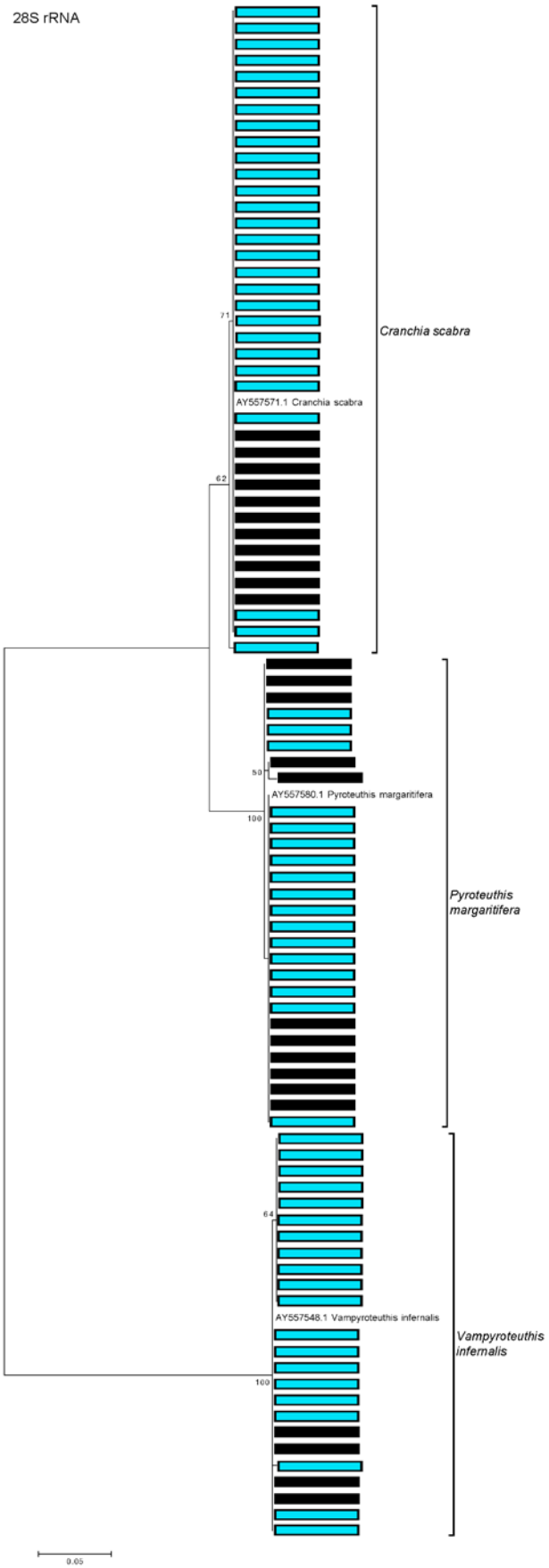

Figure 2.4 28S rRNA phylogenetic tree

28S rRNA Maximum Likelihood phylogenetic tree with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Blue symbols indicate specimens collected from the Gulf of Mexico, while black symbols indicate specimens collected from Bear Seamount. Relevant GenBank accession records were added for reference.

2.3.2 Intraspecies Variation within the Gulf of Mexico

Objective 2 focused on deep-sea cephalopod intraspecies genetic diversity within the Gulf of Mexico. This study's approach was a combined effort of morphological and molecular systematics, testing morphological hypotheses of species identification and naming through genetics. It is important to note the current accepted delimitation of a cephalopod species is at a threshold of 98% or higher sequence identity for COI and 16S rRNA (Lindgren, 2010; Dai et al., 2012). Dai et al. (2012) generated average species delimitation genetic distances for coleoids in the study, with average COI intraspecific distances around 0.2% and interspecific distances of 17.1%, while that of average 16S rRNA intraspecific distances were around 0.1% and interspecific distances of 7.5%. Specimen identities in this study were first assigned names through morphology and separately identified through sequencing, run as a double-blind test. Phylogenetic trees were generated to compare species delineation methods and evaluate conflicting results. Trees of COI and 16S rRNA sequences were grouped by taxonomic Family shown in Figures 2.5-2.36, with sequences from the Gulf of Mexico generated from this study highlighted in yellow.

Ancistrocheiridae and Enoploteuthidae

Ancistrocheiridae and Enoploteuthidae were combined for reference into two trees shown in Figures 2.5 and 2.6. Among these families, all assemblages of conspecific individuals were clustered clearly among both loci. Each genus resolved into a monophyletic clade with high bootstrap support. The COI and 16S rRNA sequences for *Abraliopsis atlantica* and *Abralia redfieldi* will be the first records contributed to GenBank.

Bolitaenidae

Bolitaenidae represented by two species belonging to two genera were analyzed. In total, 62 sequences from the Gulf of Mexico contributed to the Bolitaenidae trees shown in Figures 2.7 and 2.8. The phylogenetic trees show two distinct clades, *Japetella diaphana* and *Bolitaena pygmaea*, sharing 92% and 95% pairwise identity with moderate bootstrap values in the COI and 16S rRNA trees, respectively. GenBank sequences annotated as *Japetella diaphana* cluster within both clades.

Brachioteuthidae and Neoteuthidae

The Brachioteuthidae and Neoteuthidae trees were combined for reference, shown in Figures 2.7 and 2.8. One sequence represents Brachioteuthidae from the Gulf of Mexico, and two sequences represent Neoteuthidae from the Gulf of Mexico. The recovered putative species for Gulf of Mexico specimens of both genetic loci are the same with high bootstrap support. The *Narrowteuthis nesisi* clade shares 86% and 94% pairwise identity with the *Architeuthis dux* clade in the COI and 16S rRNA trees, respectively. The *Brachioteuthis sp.* sequence from the Gulf of Mexico shares 85% and 93% pairwise identity with the *Brachioteuthis beanii* clade in the COI and 16S rRNA trees, respectively. The *Brachioteuthis sp.* from the Gulf of Mexico highest match when run through a BLAST search is 93% identity score with *Brachioteuthis beanii* (EU735201.1) 16S rRNA. The *Narrowteuthis nesisi* highest match when run through a BLAST search is 94% identity score with *Architeuthis dux* (AY37769.1) 16S rRNA. The two COI and two 16S rRNA sequences will be the first genetic records of the monotypic genus *Narrowteuthis* contributed to GenBank.

Chiroteuthidae

Chiroteuthidae represented by four species belonging to three genera were analyzed. Eight COI sequences and eight 16S rRNA sequences from the Gulf of Mexico contributed to this analysis. The COI and 16S rRNA Chiroteuthidae trees are shown in Figures 2.11 and 2.12. All four species clustered clearly with 100% bootstrap support. Each cluster shares between 98-100% pairwise identity. A GenBank sequence annotated as *Chiroteuthis veranyi* (GU145077.1) clusters within the *Planctoteuthis levimana* clade of the COI tree.

Cranchiidae

Cranchiidae represented by eight species belonging to seven genera were analyzed. In total, 103 sequences from the Gulf of Mexico contributed to the Cranchiidae trees shown in Figures 2.13 and 2.14. Cranchiidae species clearly and definitively clustered with high bootstrap support in the COI and 16S rRNA trees. *Cranchia scabra*, *Galiteuthis armata*, and *Leachia atlantica* all form monophyletic clusters in agreement with their current, accepted phylogenetic relationships. *Teuthowenia megalops* from the Gulf of Mexico ('PP_' Figures 2.13 and 2.14) did not group with the GenBank sequences annotated as *Teuthowenia megalops* in the COI and 16S rRNA trees, sharing only 84% and 94% pairwise identity, respectively. Within each tree cluster there is 98-100% shared pairwise identity. The genetic loci COI and 16S rRNA show two distinct clades of *Helicocranchia* Gulf of Mexico sequences: *Helicocranchia pfefferi* and *Helicocranchia sp. A*, with only 88% and 96% shared pairwise identity between the two clades in the COI and 16S rRNA trees, respectively.

Cycloteuthidae

Two species, *Cycloteuthis sirventi* and *Discoteuthis discus*, belonging to Cycloteuthidae were analyzed. Twenty sequences from the Gulf of Mexico contributed to the Cycloteuthidae trees shown in Figures 2.15 and 2.16. *Cycloteuthis sirventi* clustered clearly sharing 98-99% pairwise identity with 100% bootstrap support in the COI and 16S rRNA trees, respectively. One unidentified 'unid' Cycloteuthidae Gulf of Mexico specimen does not group with the *Cycloteuthis sirventi* clade or the *Discoteuthis discus* clade ('E_' Figures 2.15 and 2.16). The *Discoteuthis discus* sequences from the Gulf of Mexico did not form a monophyletic clade, only sharing 86% and 96% pairwise identity with the other *Discoteuthis discus* Gulf of Mexico sequence ('G_' Figures 2.15 and 2.16) in the COI and 16S rRNA trees, respectively. Notably, the *Discoteuthis discus* sequences from the Gulf of Mexico only share 84% pairwise identity with the GenBank sequence annotated as *Discoteuthis discus* in the COI tree.

Histioteuthidae

Histioteuthidae represented by two species belonging to two genera were analyzed. Four sequences from the Gulf of Mexico contributed to the Histioteuthidae trees shown in Figures 2.17 and 2.18. *Stigmatoteuthis arcturi* clustered clearly sharing 99% pairwise identity, with 100% bootstrap support. *Histioteuthis corona* did not group with the GenBank 16S rRNA sequence annotated as *Histioteuthis corona* (EU735211.1), the two sequences only share 94% pairwise identity.

Joubiniteuthidae

The single species from this monotypic family was represented by two specimens, with four new sequences from the Gulf of Mexico contributing to the genetic records of *Joubiniteuthis portieri* shown in Figures 2.19 and 2.20. All of the *Joubiniteuthis portieri* sequences formed one monophyletic cluster with 98-99% shared pairwise identity.

Lycoteuthidae

Lycoteuthidae represented by one species, *Selenoteuthis scintillans*, was analyzed. Four sequences from the Gulf of Mexico contributed to the Lycoteuthidae trees shown in Figures 2.21 and 2.22. While all *Selenoteuthis scintillans* sequences clustered clearly, there is low bootstrap support. The *Selenoteuthis scintillans* cluster shares 99% pairwise identity in both trees, and less than 86% and 93% pairwise identity to *Lycoteuthis lorigera* with COI and 16S rRNA, respectively.

Mastigoteuthidae

Mastigoteuthidae consisting of three species belonging to three genera were analyzed. Fifteen COI sequences and 15 16S rRNA sequences from the Gulf of Mexico contributed to the Mastigoteuthidae trees shown in Figures 2.23 and 2.24. All assemblages of conspecific individuals clustered clearly with 100% bootstrap support. Within each cluster there is 98-100% shared pairwise identity. Each genus resolved into a monophyletic clade.

Octopoteuthidae

Two species, *Octopoteuthis megaptera* and *Taningia danae*, belonging to Octopoteuthidae were analyzed. Ten sequences from the Gulf of Mexico contributed to the Octopoteuthidae trees shown in Figures 2.25 and 2.26. The *Octopoteuthis* sequences did not form a monophyletic clade. *Octopoteuthis* sequences from the Gulf of Mexico only share 88% and 96% pairwise identity with the clade of GenBank sequences annotated as *Octopoteuthis* in the COI (EU735358.1, EU735402.1, GU812407.2) and 16S rRNA (EU35266.1, EU735258.1, GU812406.1) trees, respectively. A BLAST search of the Gulf of Mexico COI and 16S rRNA *Octopoteuthis megaptera* sequences matched GenBank sequences annotated as *Octopoteuthis nielseni*, with 93% (AF000055.1) and 99% (AY616983.1) shared pairwise identity.

Ommastrephidae

Sixteen sequences from the Gulf of Mexico contributed to the Ommastrephidae trees shown in Figures 2.27 and 2.28. *Ornithoteuthis antillarum* and *Sthenoteuthis pteropus*, clustered clearly, with 98-99% shared pairwise identity in each cluster and 100% bootstrap support. There are three unidentified 'unid' Ommastrephidae Gulf of Mexico specimens that discretely cluster with 100% bootstrap support. These specimens are not available for morphological verification post-analysis.

Onychoteuthidae

Two species, *Onychoteuthis cf. banksii* and *Onychoteuthis compacta*, belonging to Onychoteuthidae were analyzed. Eight sequences from the Gulf of Mexico contributed to the Onychoteuthidae trees shown in Figures 2.29 and 2.30. Both trees are not well resolved with

moderate bootstrap support. *Onychoteuthis cf. banksii* and *Onychoteuthis compacta* sequences did not form monophyletic clades. There is also an unidentified 'unid' Onychoteuthidae Gulf of Mexico specimen that does not group with any existing GenBank onychoteuthid sequences ('D_' Figures 2.29 and 2.30).

Pyroteuthidae

Pyroteuthidae represented by two species belonging to two genera were analyzed. In total, 48 sequences from the Gulf of Mexico contributed to the Pyroteuthidae trees shown in Figures 2.31 and 2.32. While the resolved trees are not identical, the two major clades recovered on both trees are the same. Both groups are discretely clustered with high bootstrap support. *Pyroteuthis margaritifera* forms a monophyletic clade, while the various species of *Pterygioteuthis* do not cluster distinctively. The second highly supported clade, *Pterygioteuthis gemmata*, is placed in both trees among GenBank sequences annotated as *Pterygioteuthis*, however, the *Pterygioteuthis gemmata* Gulf of Mexico sequences do not match any *Pterygioteuthis* species when run through a BLAST search. The clade's highest shared pairwise identity is with *Pterygioteuthis giardi* (GU145065.1) at 89% in the COI tree, and with *Pterygioteuthis microlampas* (EU735253.1) at 94% in the 16S rRNA sequences.

Sepiolidae

Ten sequences of one species, *Heteroteuthis dagamensis*, representing Sepiolidae were analyzed. Four COI sequences and six 16S rRNA sequences were recovered from the Gulf of Mexico. The COI and 16S rRNA Sepiolidae trees are shown in Figures 2.33 and 2.34. *Heteroteuthis dagamensis* species are clearly and definitively clustered, with agreement among

trees and 100% bootstrap support. The two species clusters, *Heteroteuthis dagamensis* and *Heteroteuthis hawaiiensis*, share 95% and 98% pairwise identity, in the COI and 16S rRNA trees, respectively.

Vampyroteuthidae

The single species, *Vampyroteuthis infernalis,* from this monotypic family was represented by 26 COI sequences and 20 16S rRNA sequences from the Gulf of Mexico shown in Figures 2.35 and 2.36. The trees show two distinct clades with a variety of intraspecific distances among the *Vampyroteuthis infernalis* sequences. The two clades share 98-99% pairwise identity with moderate bootstrap values.

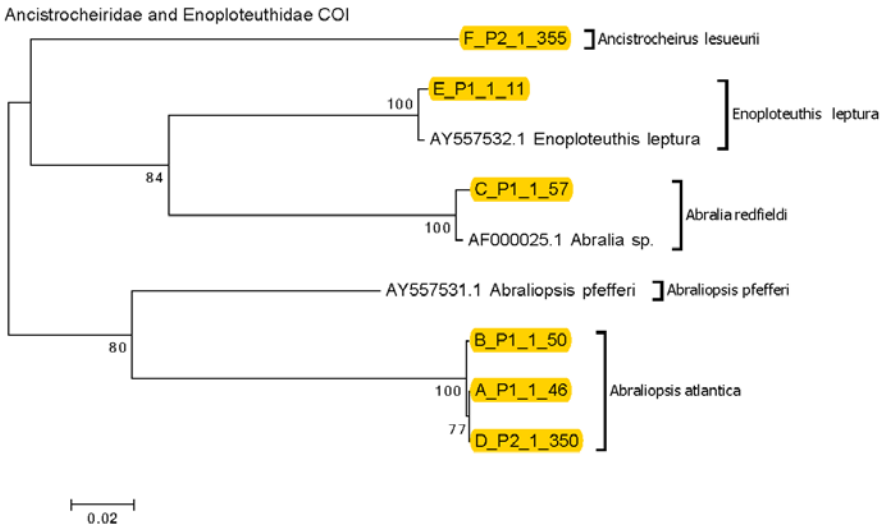

Figure 2.5 Ancistrocheiridae and Enoploteuthidae COI phylogenetic tree

COI Maximum Likelihood phylogenetic tree for species of Ancistrocheiridae and Enoploteuthidae with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Highlighted sequence labels indicate sequences from the Gulf of Mexico. The starting letter of highlighted sequence labels designates the same specimen in both trees (i.e. A_ in the COI tree corresponds with A_ in the 16S rRNA tree). Relevant GenBank accession records were added for reference.

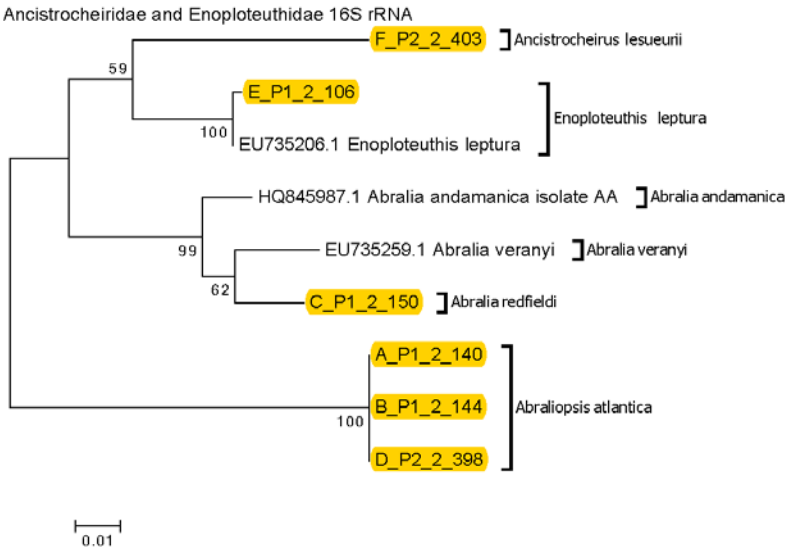

Figure 2.6 Ancistrocheiridae and Enoploteuthidae 16S rRNA phylogenetic tree

16S rRNA Maximum Likelihood phylogenetic tree for species of Ancistrocheiridae and Enoploteuthidae with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Highlighted sequence labels indicate sequences from the Gulf of Mexico. The starting letter of highlighted sequence labels designates the same specimen in both trees (i.e. A_ in the COI tree corresponds with A_ in the 16S rRNA tree). Relevant GenBank accession records were added for reference.

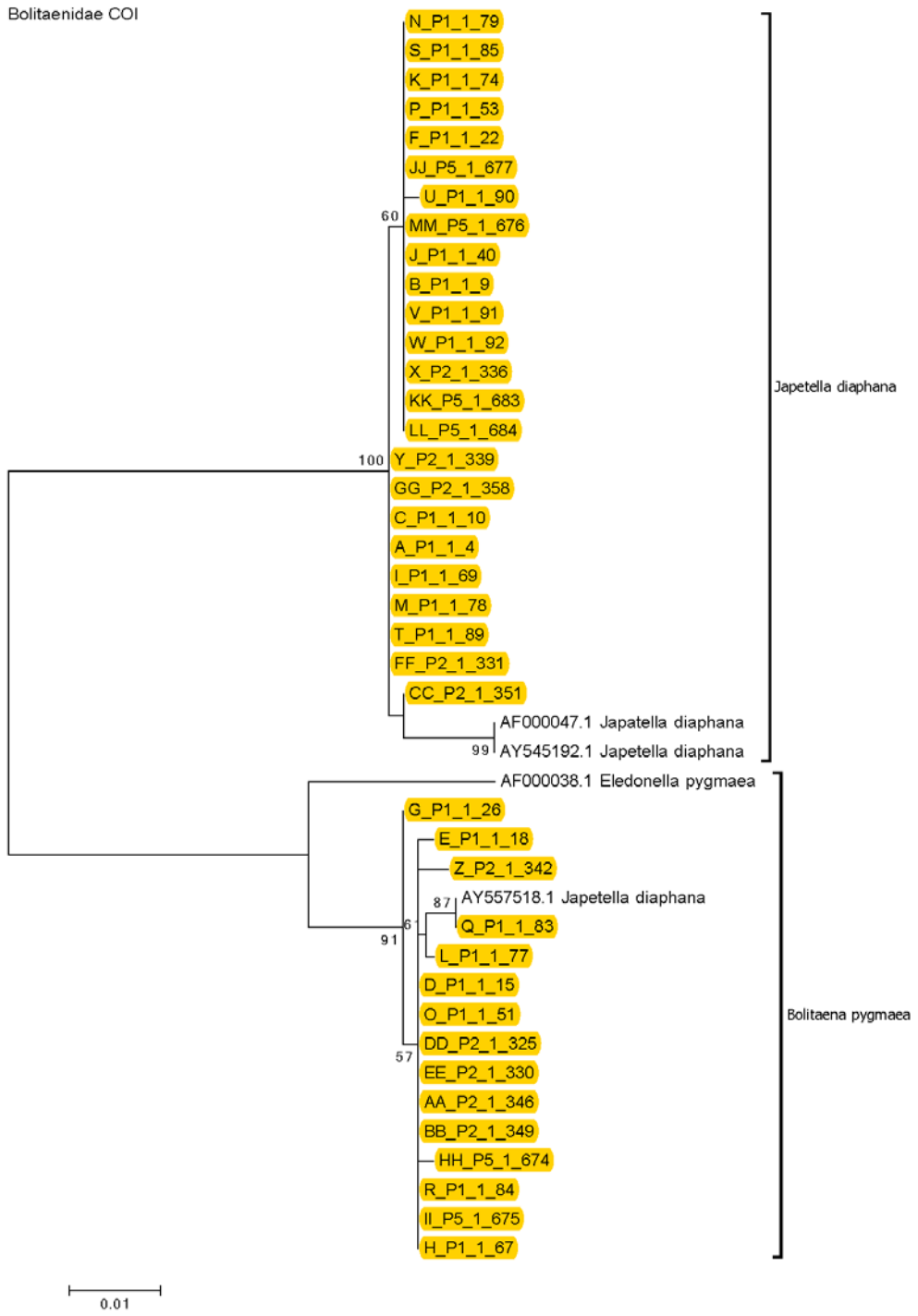

Figure 2.7 Bolitaenidae COI phylogenetic tree

COI Maximum Likelihood phylogenetic tree for species of Bolitaenidae with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Highlighted sequence labels indicate sequences from the Gulf of Mexico. The starting letter of highlighted sequence labels designates the same specimen in both trees (i.e. A_ in the COI tree corresponds with A_ in the 16S rRNA tree). Relevant GenBank accession records were added for reference.

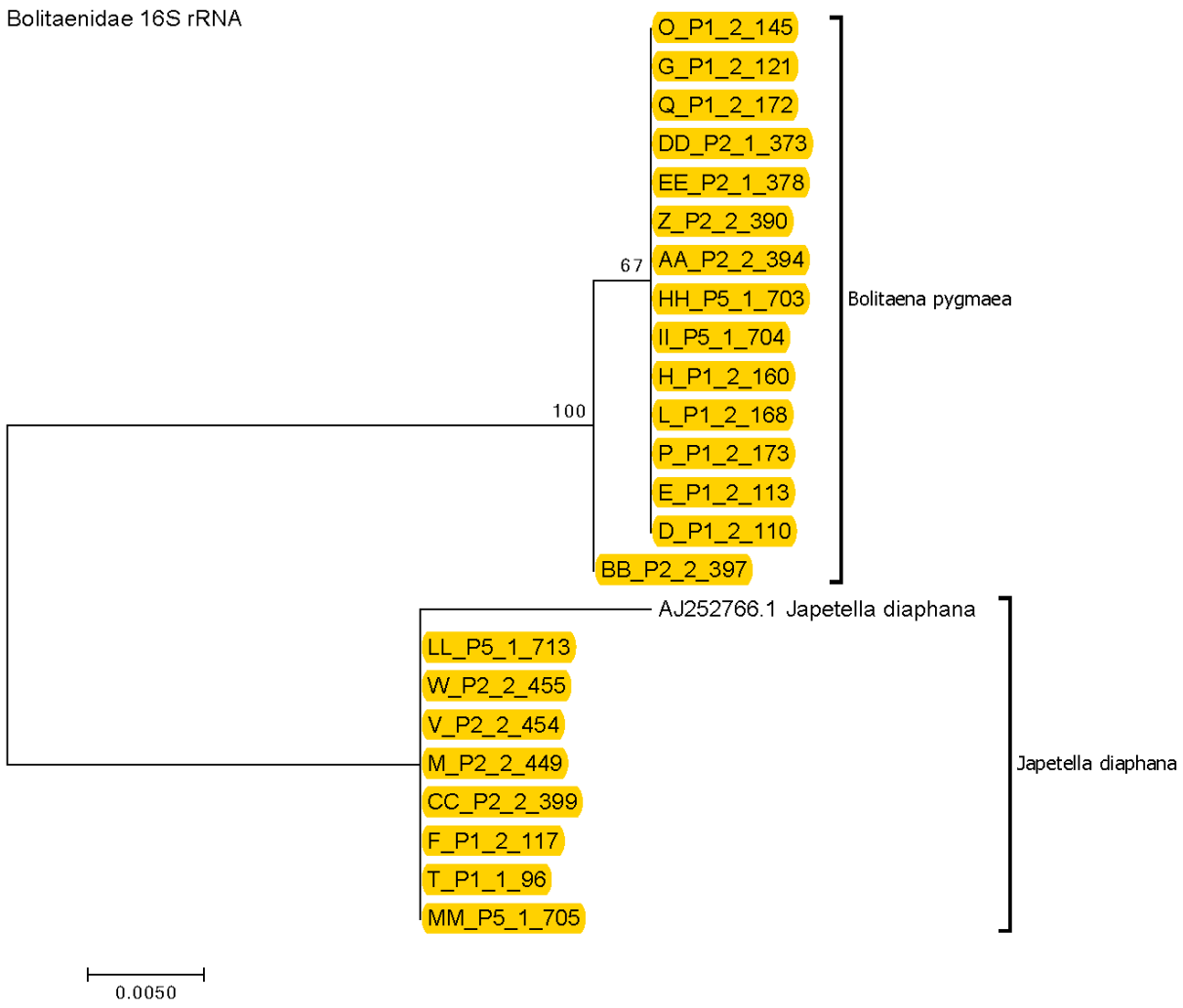

Figure 2.8 Bolitaenidae 16S rRNA phylogenetic tree

16S rRNA Maximum Likelihood phylogenetic tree for species of Bolitaenidae with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Highlighted sequence labels indicate sequences from the Gulf of Mexico. The starting letter of highlighted sequence labels designates the same specimen in both trees (i.e. A_ in the COI tree corresponds with A_ in the 16S rRNA tree). Relevant GenBank accession records were added for reference.

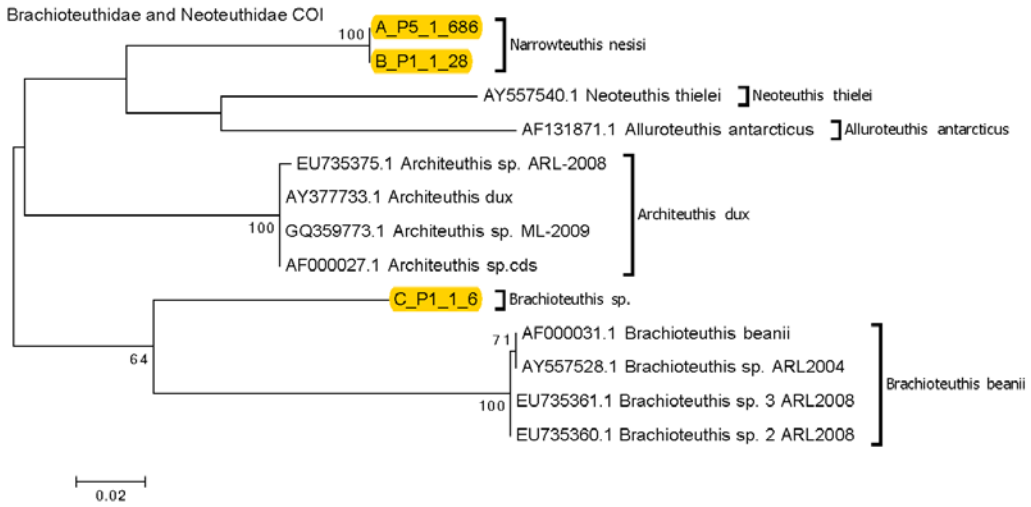

Figure 2.9 Brachioteuthidae and Neoteuthidae COI phylogenetic tree

COI Maximum Likelihood phylogenetic tree for species of Brachioteuthidae and Neoteuthidae with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Highlighted sequence labels indicate sequences from the Gulf of Mexico. The starting letter of highlighted sequence labels designates the same specimen in both trees (i.e. A_ in the COI tree corresponds with A_ in the 16S rRNA tree). Relevant GenBank accession records were added for reference.

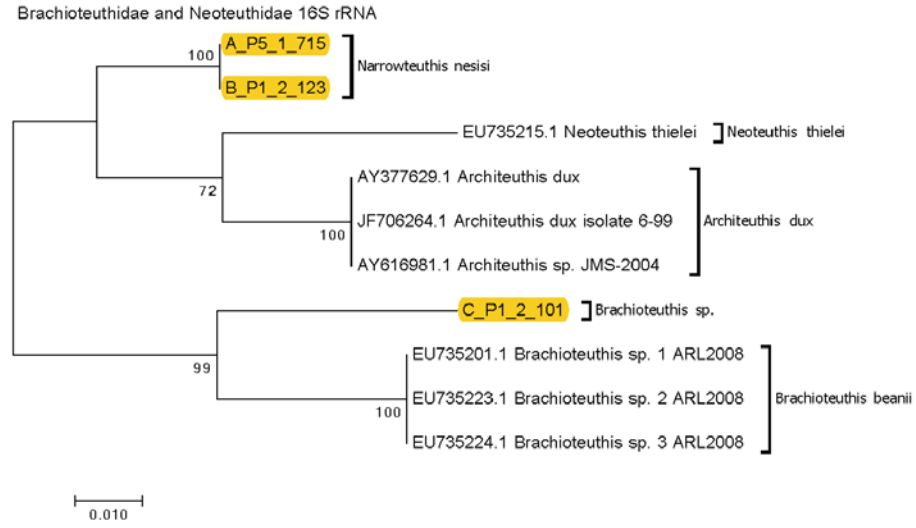

Figure 2.10 Brachioteuthidae and Neoteuthidae 16S rRNA phylogenetic tree

16S rRNA Maximum Likelihood phylogenetic tree for species of Brachioteuthidae and Neoteuthidae with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Highlighted sequence labels indicate sequences from the Gulf of Mexico. The starting letter of highlighted sequence labels designates the same specimen in both trees (i.e. A_ in the COI tree corresponds with A_ in the 16S rRNA tree). Relevant GenBank accession records were added for reference.

Figure 2.11 Chiroteuthidae COI phylogenetic tree

COI Maximum Likelihood phylogenetic tree for species of Chiroteuthidae with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Highlighted sequence labels indicate sequences from the Gulf of Mexico. The starting letter of highlighted sequence labels designates the same specimen in both trees (i.e. A_ in the COI tree corresponds with A_ in the 16S rRNA tree). Relevant GenBank accession records were added for reference.

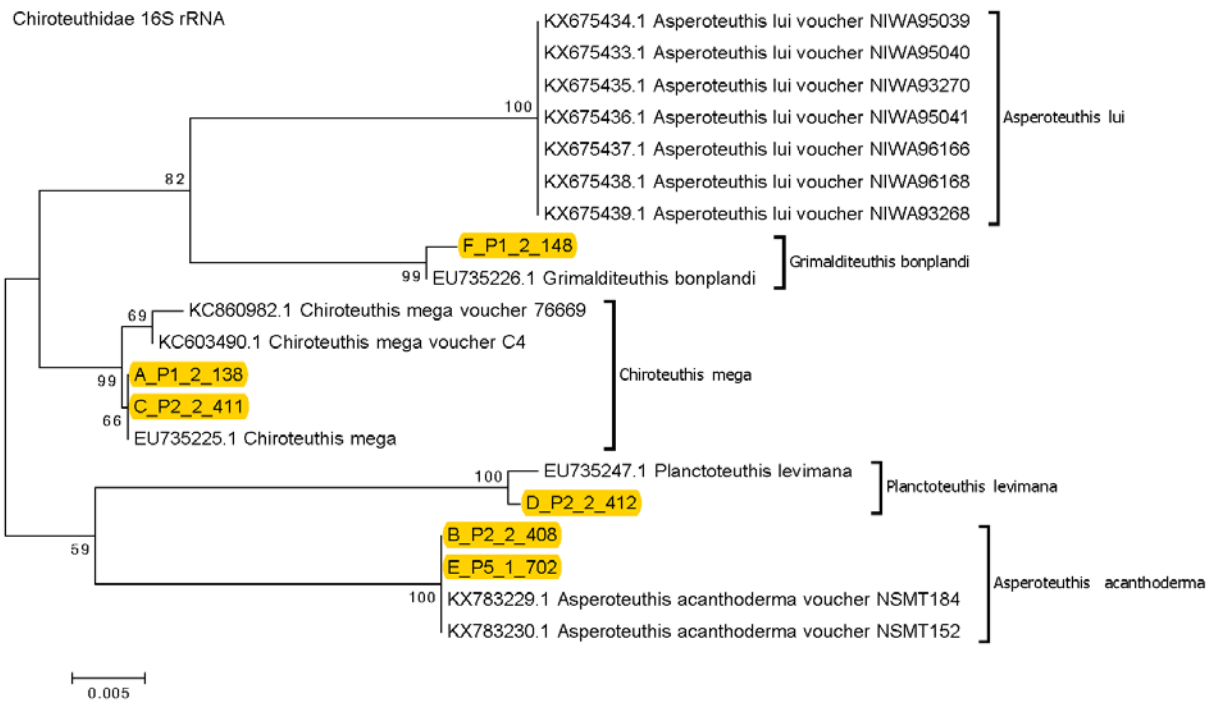

Figure 2.12 Chiroteuthidae 16S rRNA phylogenetic tree

16S rRNA Maximum Likelihood phylogenetic tree for species of Chiroteuthidae with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Highlighted sequence labels indicate sequences from the Gulf of Mexico. The starting letter of highlighted sequence labels designates the same specimen in both trees (i.e. A_ in the COI tree corresponds with A_ in the 16S rRNA tree). Relevant GenBank accession records were added for reference.

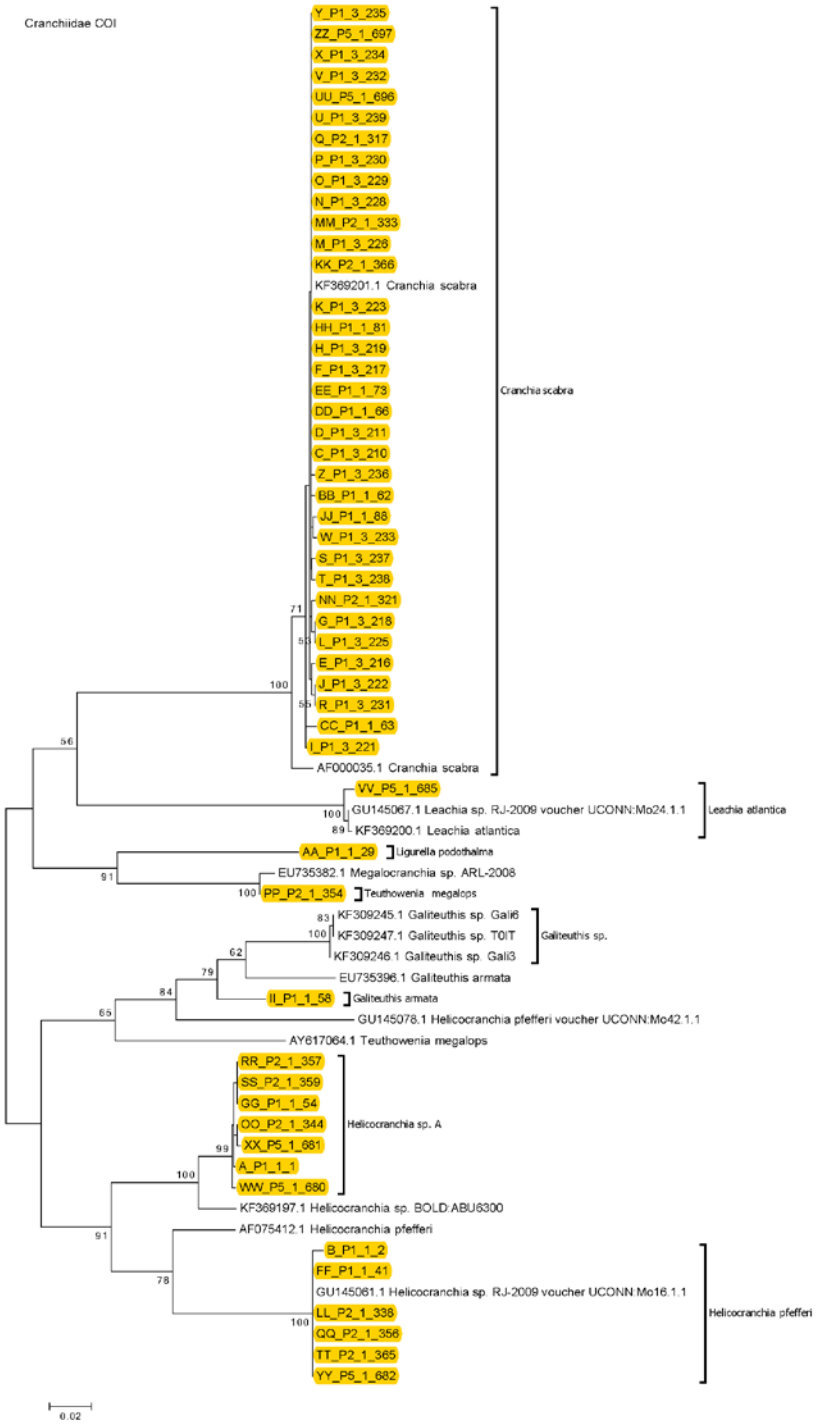

Figure 2.13 Cranchiidae COI phylogenetic tree

COI Maximum Likelihood phylogenetic tree for species of Cranchiidae with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Highlighted sequence labels indicate sequences from the Gulf of Mexico. The starting letter of highlighted sequence labels designates the same specimen in both trees (i.e. A_ in the COI tree corresponds with A_ in the 16S rRNA tree). Relevant GenBank accession records were added for reference.

Figure 2.14 Cranchiidae 16S rRNA phylogenetic tree

16S rRNA Maximum Likelihood phylogenetic tree for species of Cranchiidae with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Highlighted sequence labels indicate sequences from the Gulf of Mexico. The starting letter of highlighted sequence labels designates the same specimen in both trees (i.e. A_ in the COI tree corresponds with A_ in the 16S rRNA tree). Relevant GenBank accession records were added for reference.

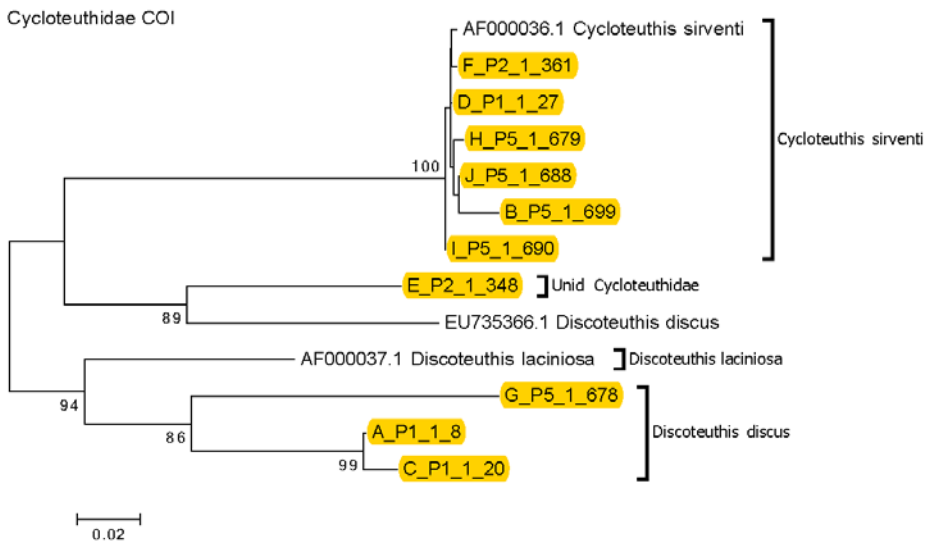

Figure 2.15 Cycloteuthidae COI phylogenetic tree

COI Maximum Likelihood phylogenetic tree for species of Cycloteuthidae with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Highlighted sequence labels indicate sequences from the Gulf of Mexico. The starting letter of highlighted sequence labels designates the same specimen in both trees (i.e. A_ in the COI tree corresponds with A_ in the 16S rRNA tree). Relevant GenBank accession records were added for reference.

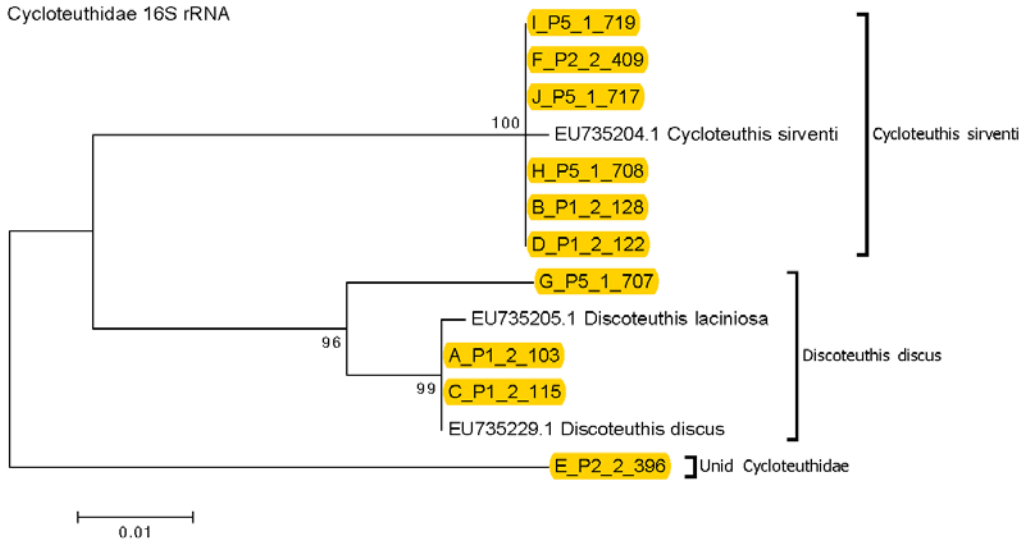

Figure 2.16 Cycloteuthidae 16S rRNA phylogenetic tree

16S rRNA Maximum Likelihood phylogenetic tree for species of Cycloteuthidae with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Highlighted sequence labels indicate sequences from the Gulf of Mexico. The starting letter of highlighted sequence labels designates the same specimen in both trees (i.e. A_ in the COI tree corresponds with A_ in the 16S rRNA tree). Relevant GenBank accession records were added for reference.

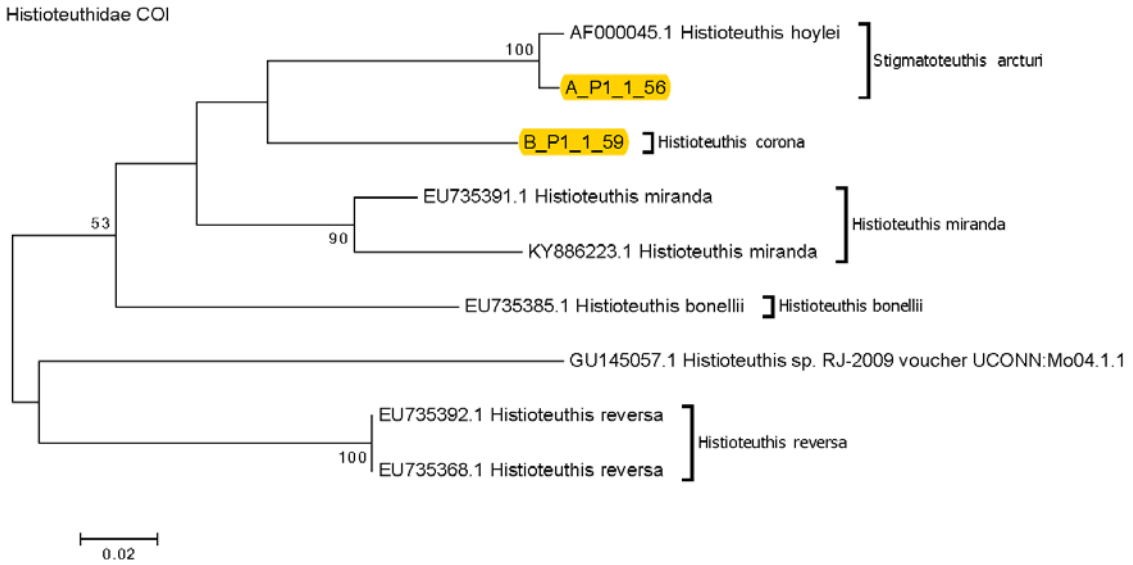

Figure 2.17 Histioteuthidae COI phylogenetic tree

COI Maximum Likelihood phylogenetic tree for species of Histioteuthidae with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Highlighted sequence labels indicate sequences from the Gulf of Mexico. The starting letter of highlighted sequence labels designates the same specimen in both trees (i.e. A_ in the COI tree corresponds with A_ in the 16S rRNA tree). Relevant GenBank accession records were added for reference.

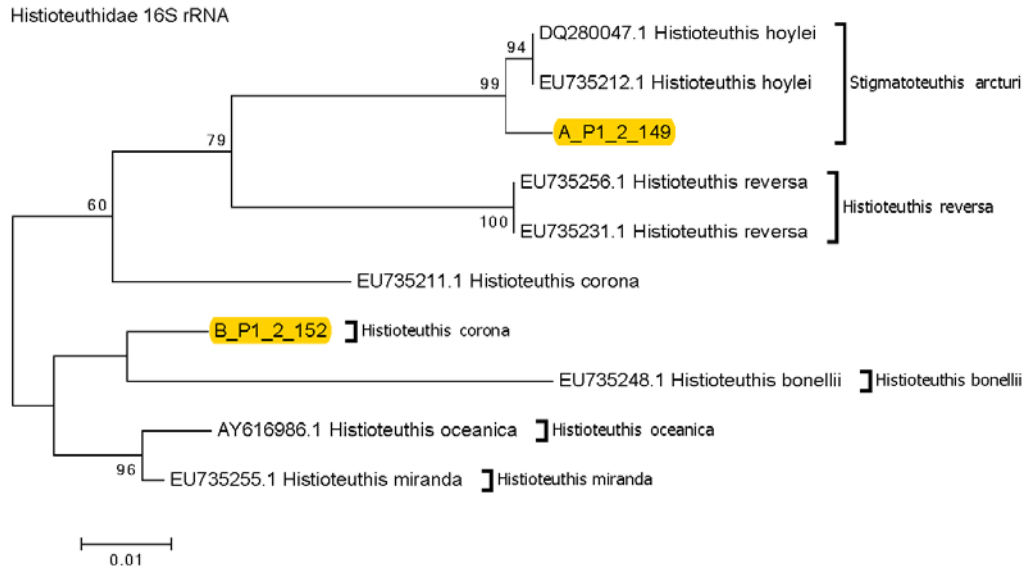

Figure 2.18 Histioteuthidae 16S rRNA phylogenetic tree

16S rRNA Maximum Likelihood phylogenetic tree for species of Histioteuthidae with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Highlighted sequence labels indicate sequences from the Gulf of Mexico. The starting letter of highlighted sequence labels designates the same specimen in both trees (i.e. A_ in the COI tree corresponds with A_ in the 16S rRNA tree). Relevant GenBank accession records were added for reference.

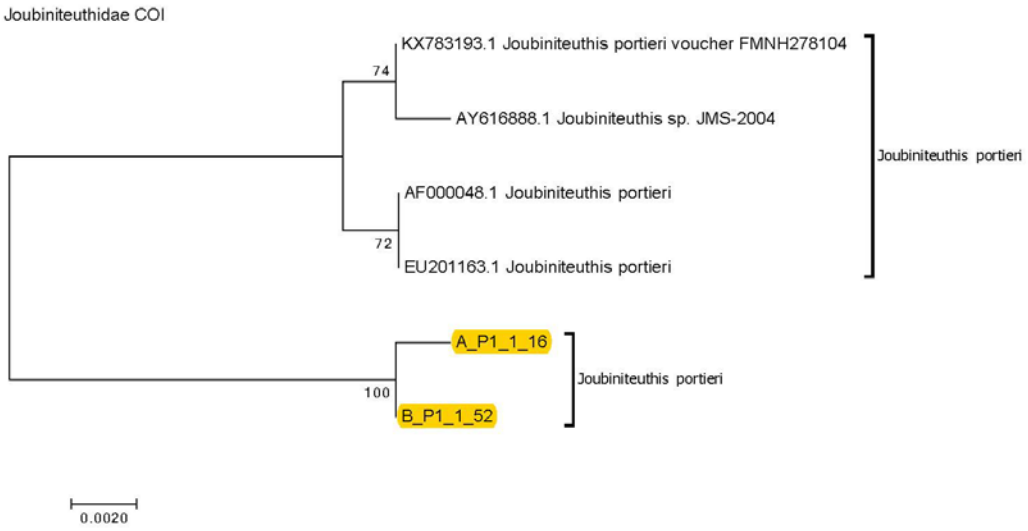

Figure 2.19 Joubiniteuthidae COI phylogenetic tree

COI Maximum Likelihood phylogenetic tree for species of Joubiniteuthidae with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Highlighted sequence labels indicate sequences from the Gulf of Mexico. The starting letter of highlighted sequence labels designates the same specimen in both trees (i.e. A_ in the COI tree corresponds with A_ in the 16S rRNA tree). Relevant GenBank accession records were added for reference.

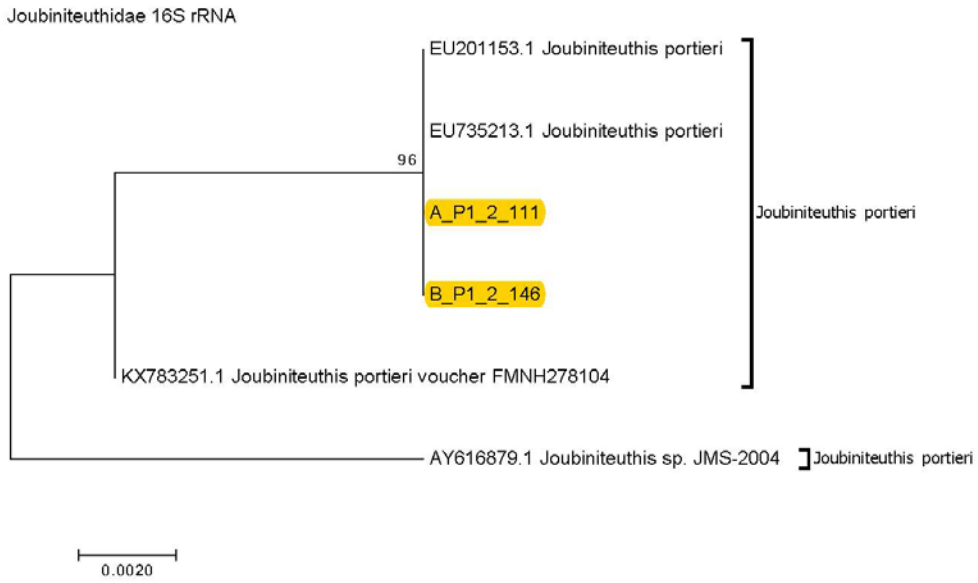

Figure 2.20 Joubiniteuthidae 16S rRNA phylogenetic tree

16S rRNA Maximum Likelihood phylogenetic tree for species of Joubiniteuthidae with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Highlighted sequence labels indicate sequences from the Gulf of Mexico. The starting letter of highlighted sequence labels designates the same specimen in both trees (i.e. A_ in the COI tree corresponds with A_ in the 16S rRNA tree). Relevant GenBank accession records were added for reference.

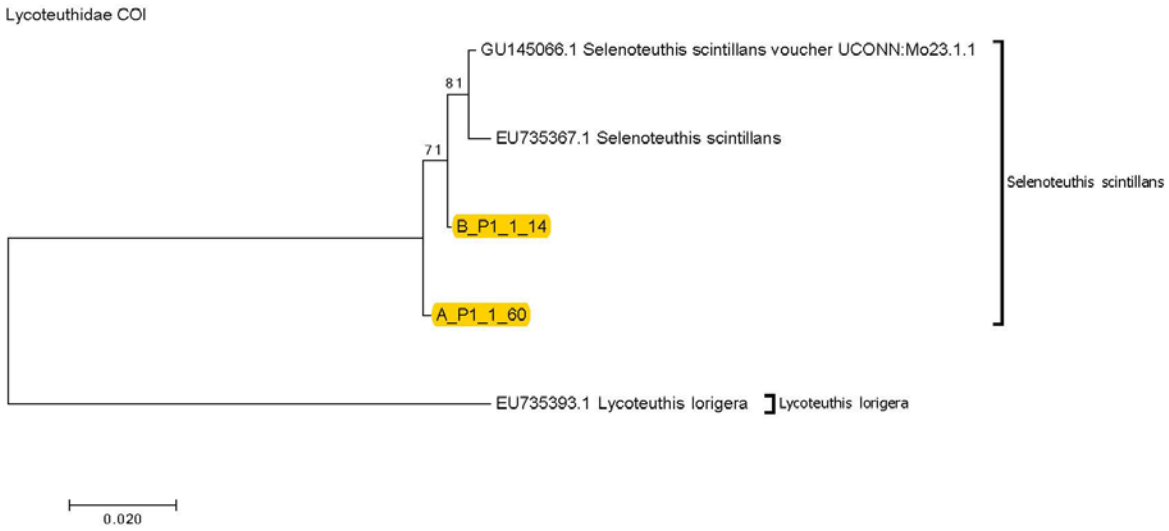

Figure 2.21 Lycoteuthidae COI phylogenetic tree

COI Maximum Likelihood phylogenetic tree for species of Lycoteuthidae with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Highlighted sequence labels indicate sequences from the Gulf of Mexico. The starting letter of highlighted sequence labels designates the same specimen in both trees (i.e. A_ in the COI tree corresponds with A_ in the 16S rRNA tree). Relevant GenBank accession records were added for reference.

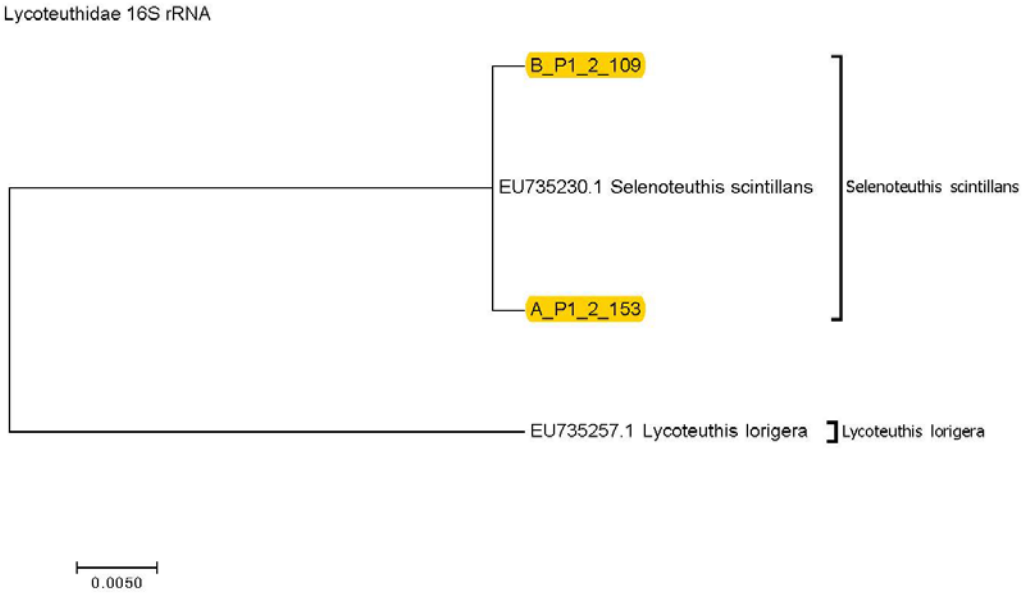

Figure 2.22 Lycoteuthidae 16S rRNA phylogenetic tree

16S rRNA Maximum Likelihood phylogenetic tree for species of Lycoteuthidae with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Highlighted sequence labels indicate sequences from the Gulf of Mexico. The starting letter of highlighted sequence labels designates the same specimen in both trees (i.e. A_ in the COI tree corresponds with A_ in the 16S rRNA tree). Relevant GenBank accession records were added for reference.

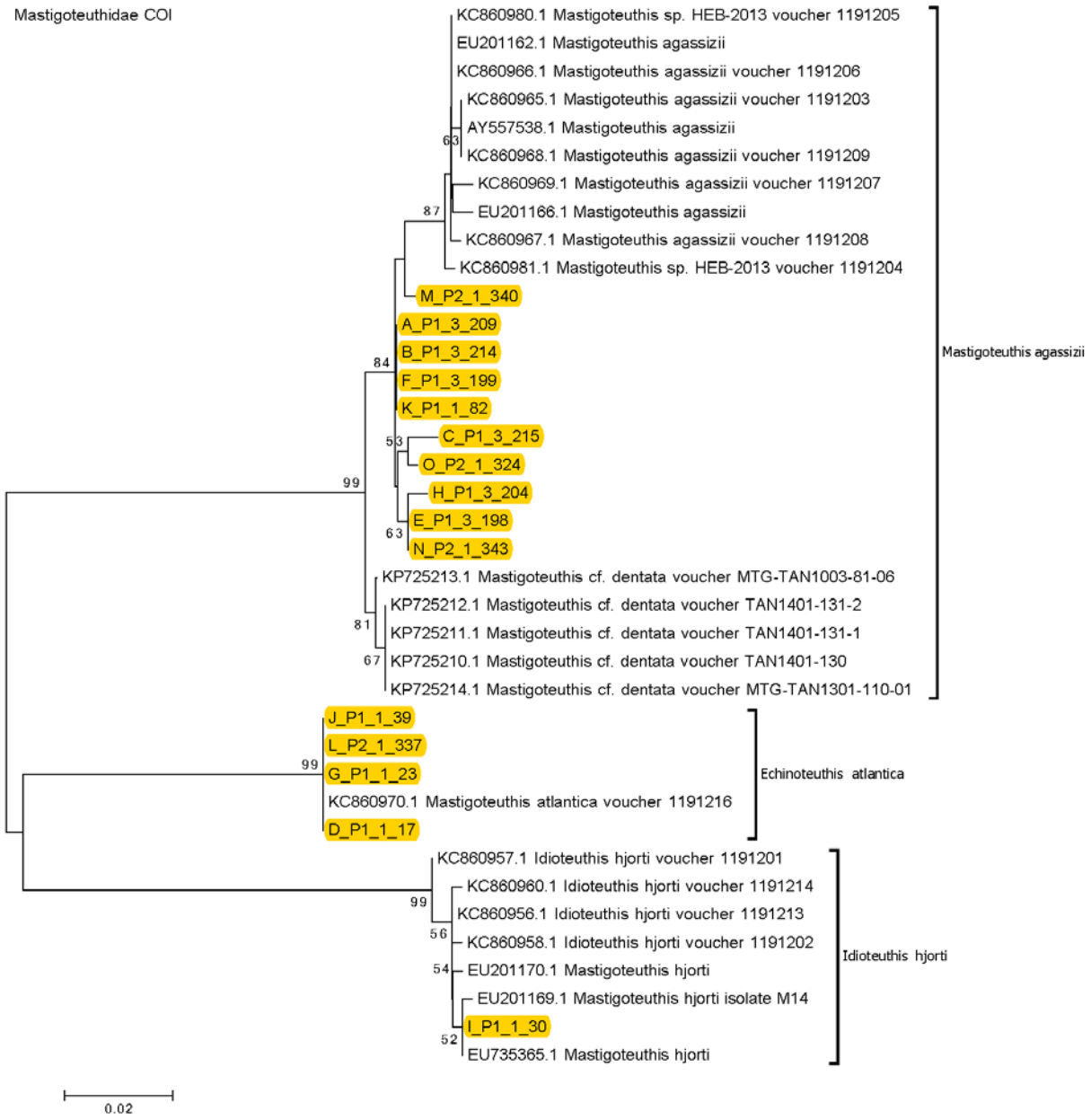

Figure 2.23 Mastigoteuthidae COI phylogenetic tree

COI Maximum Likelihood phylogenetic tree for species of Mastigoteuthidae with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Highlighted sequence labels indicate sequences from the Gulf of Mexico. The starting letter of highlighted sequence labels designates the same specimen in both trees (i.e. A_ in the COI tree corresponds with A_ in the 16S rRNA tree). Relevant GenBank accession records were added for reference.

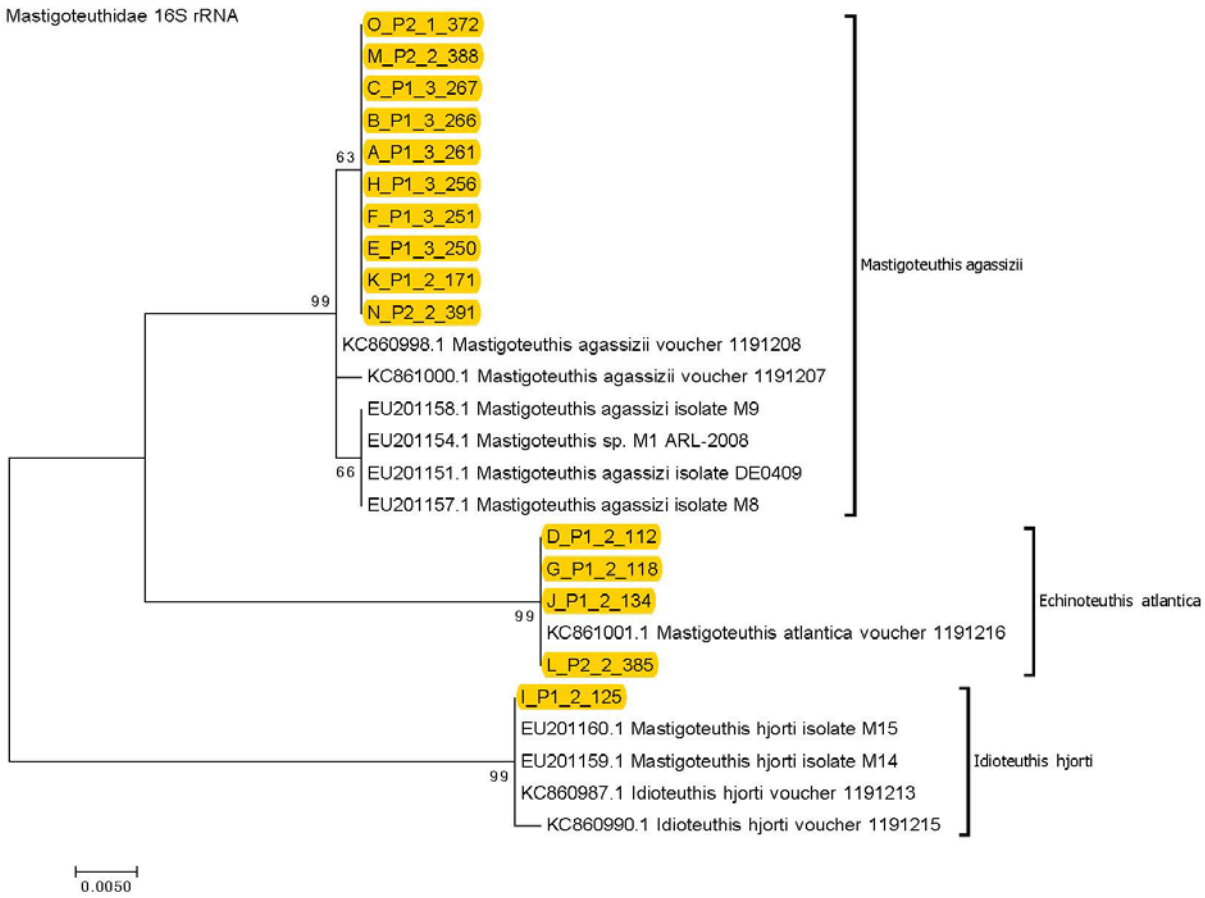

Figure 2.24 Mastigoteuthidae 16S rRNA phylogenetic tree

16S rRNA Maximum Likelihood phylogenetic tree for species of Mastigoteuthidae with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Highlighted sequence labels indicate sequences from the Gulf of Mexico. The starting letter of highlighted sequence labels designates the same specimen in both trees (i.e. A_ in the COI tree corresponds with A_ in the 16S rRNA tree). Relevant GenBank accession records were added for reference.

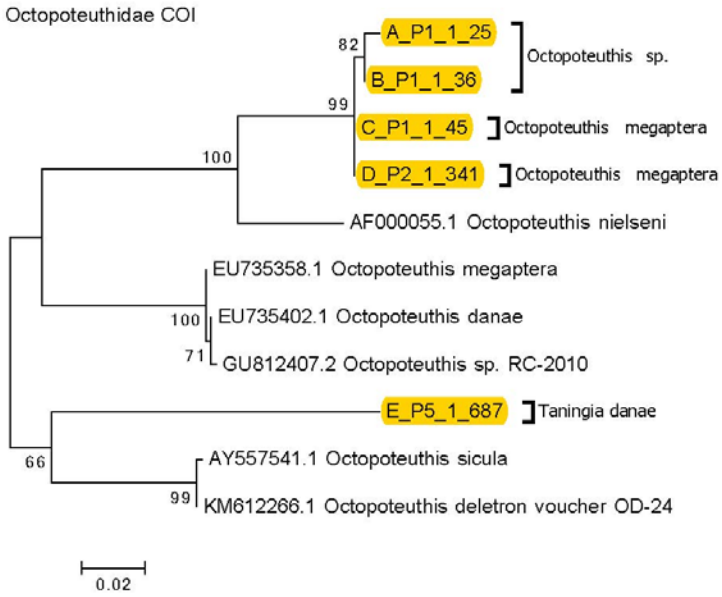

Figure 2.25 Octopoteuthidae COI phylogenetic tree

COI Maximum Likelihood phylogenetic tree for species of Octopoteuthidae with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Highlighted sequence labels indicate sequences from the Gulf of Mexico. The starting letter of highlighted sequence labels designates the same specimen in both trees (i.e. A_ in the COI tree corresponds with A_ in the 16S rRNA tree). Relevant GenBank accession records were added for reference.

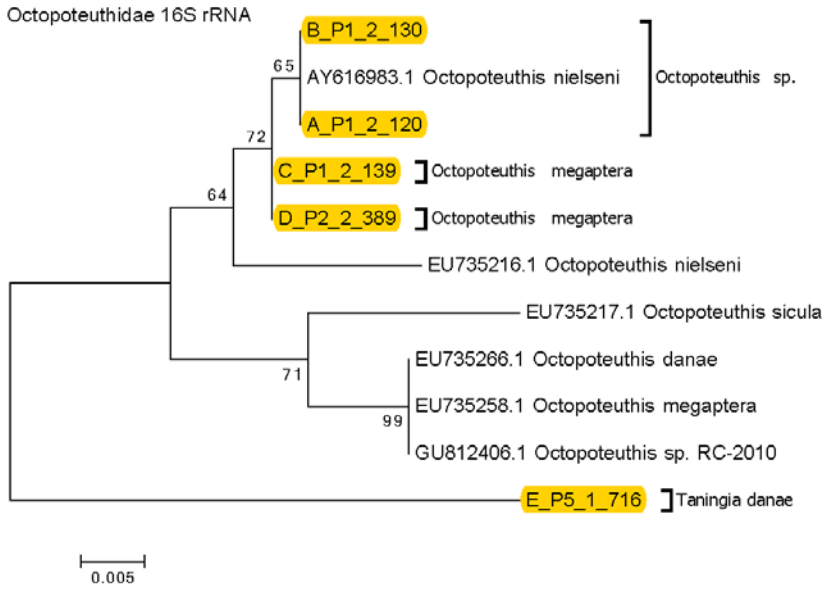

Figure 2.26 Octopoteuthidae 16S rRNA phylogenetic tree

16S rRNA Maximum Likelihood phylogenetic tree for species of Octopoteuthidae with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Highlighted sequence labels indicate sequences from the Gulf of Mexico. The starting letter of highlighted sequence labels designates the same specimen in both trees (i.e. A_ in the COI tree corresponds with A_ in the 16S rRNA tree). Relevant GenBank accession records were added for reference.

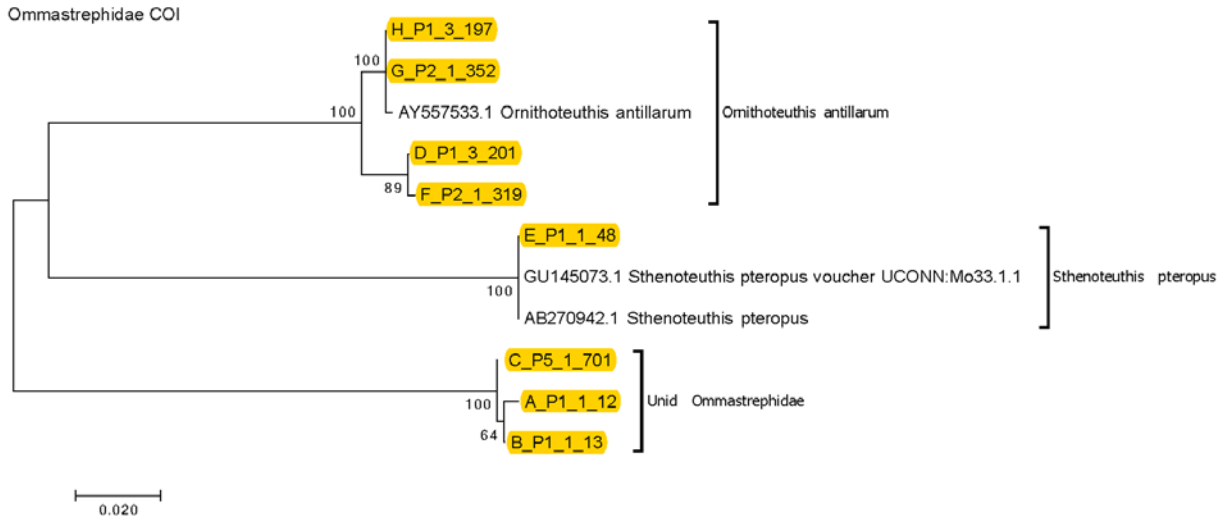

Figure 2.27 Ommastrephidae COI phylogenetic tree

COI Maximum Likelihood phylogenetic tree for species of Ommastrephidae with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Highlighted sequence labels indicate sequences from the Gulf of Mexico. The starting letter of highlighted sequence labels designates the same specimen in both trees (i.e. A_ in the COI tree corresponds with A_ in the 16S rRNA tree). Relevant GenBank accession records were added for reference.

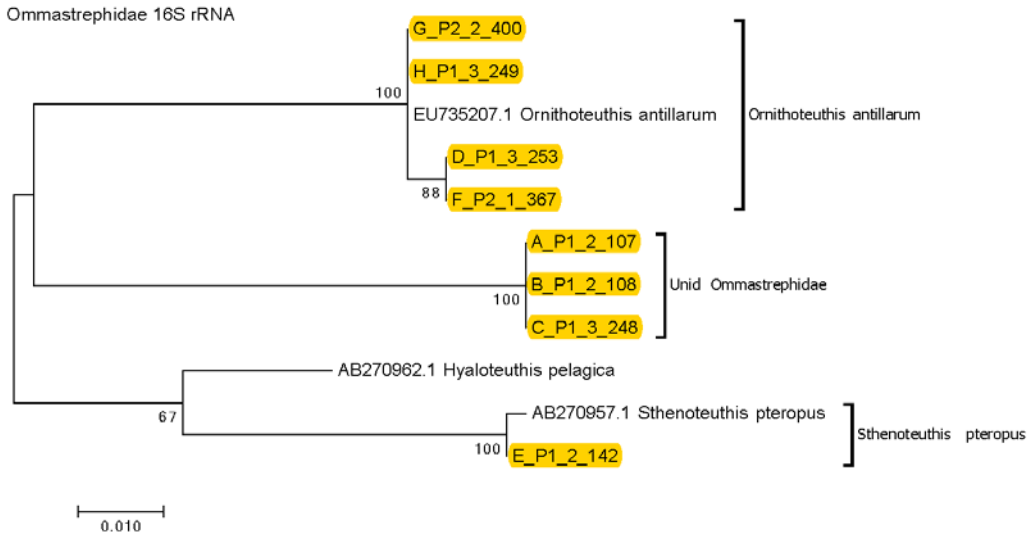

Figure 2.28 Ommastrephidae 16S rRNA phylogenetic tree

16S rRNA Maximum Likelihood phylogenetic tree for species of Ommastrephidae with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Highlighted sequence labels indicate sequences from the Gulf of Mexico. The starting letter of highlighted sequence labels designates the same specimen in both trees (i.e. A_ in the COI tree corresponds with A_ in the 16S rRNA tree). Relevant GenBank accession records were added for reference.

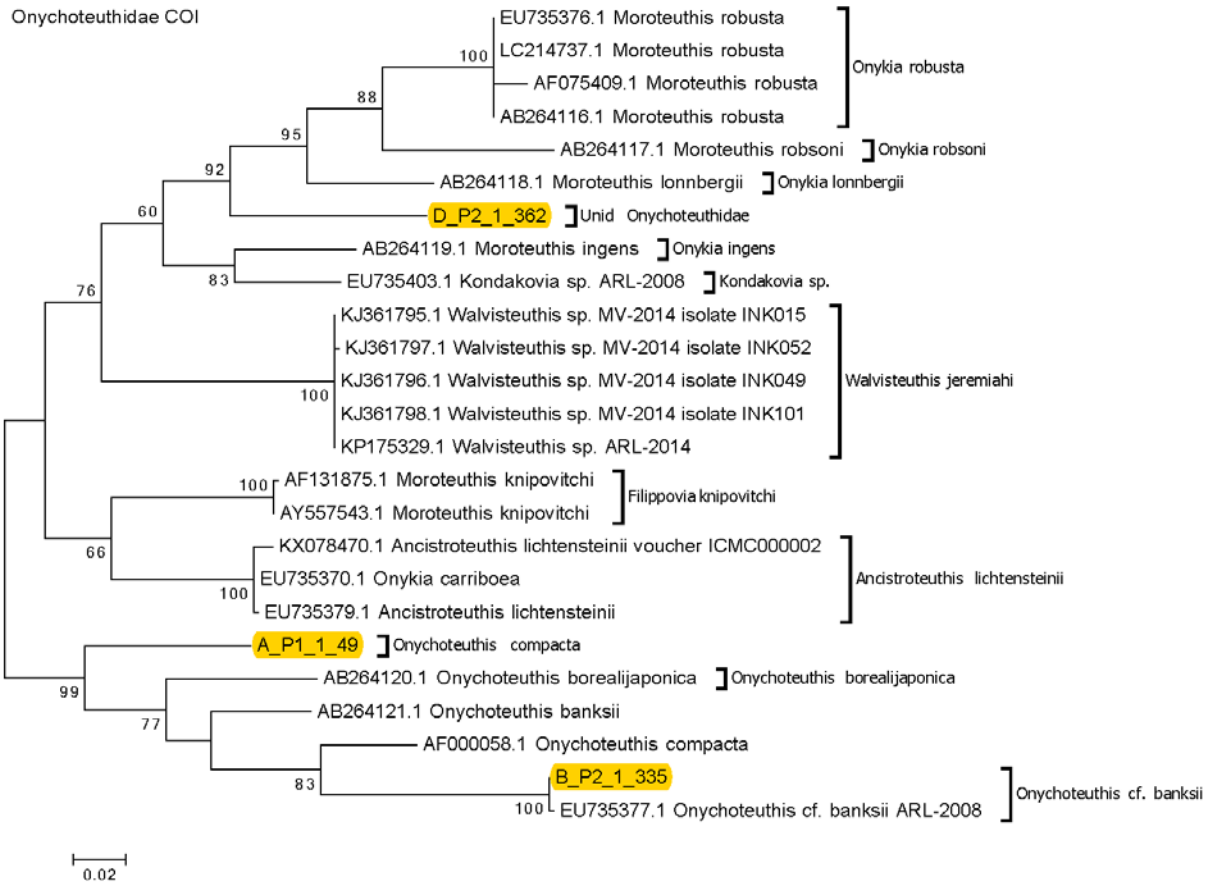

Figure 2.29 Onychoteuthidae COI phylogenetic tree

COI Maximum Likelihood phylogenetic tree for species of Onychoteuthidae with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Highlighted sequence labels indicate sequences from the Gulf of Mexico. The starting letter of highlighted sequence labels designates the same specimen in both trees (i.e. A_ in the COI tree corresponds with A_ in the 16S rRNA tree). Relevant GenBank accession records were added for reference.

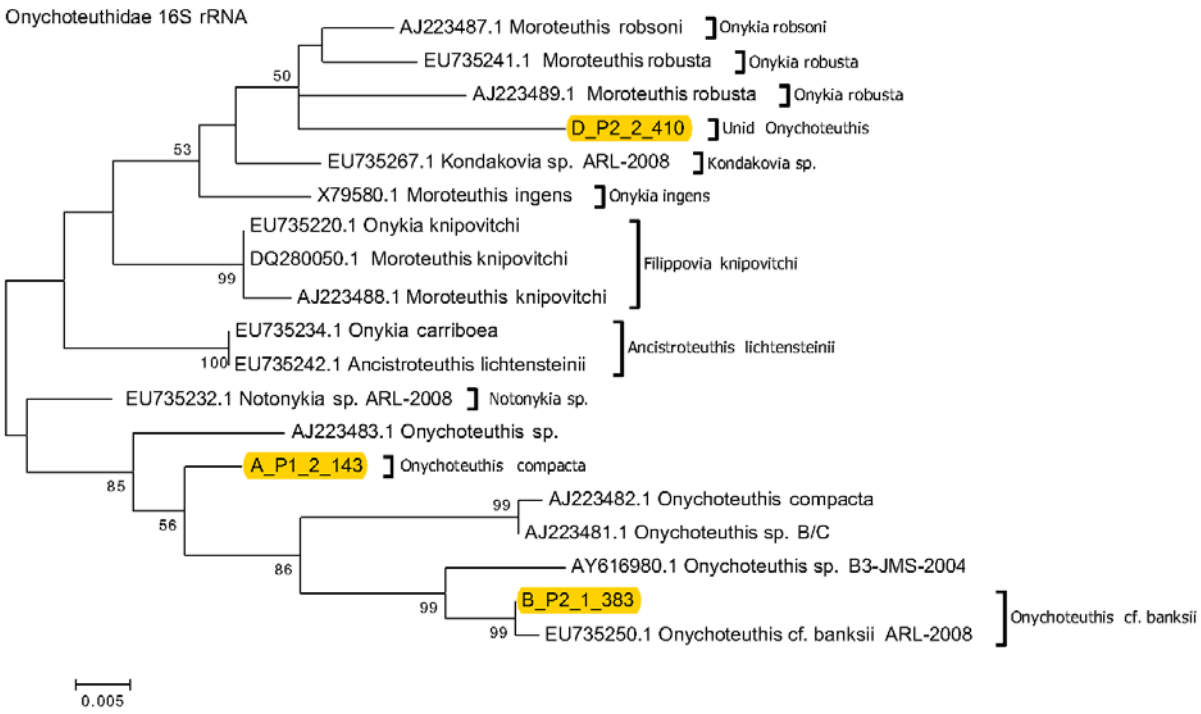

Figure 2.30 Onychoteuthidae 16S rRNA phylogenetic tree

16S rRNA Maximum Likelihood phylogenetic tree for species of Onychoteuthidae with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Highlighted sequence labels indicate sequences from the Gulf of Mexico. The starting letter of highlighted sequence labels designates the same specimen in both trees (i.e. A_ in the COI tree corresponds with A_ in the 16S rRNA tree). Relevant GenBank accession records were added for reference.

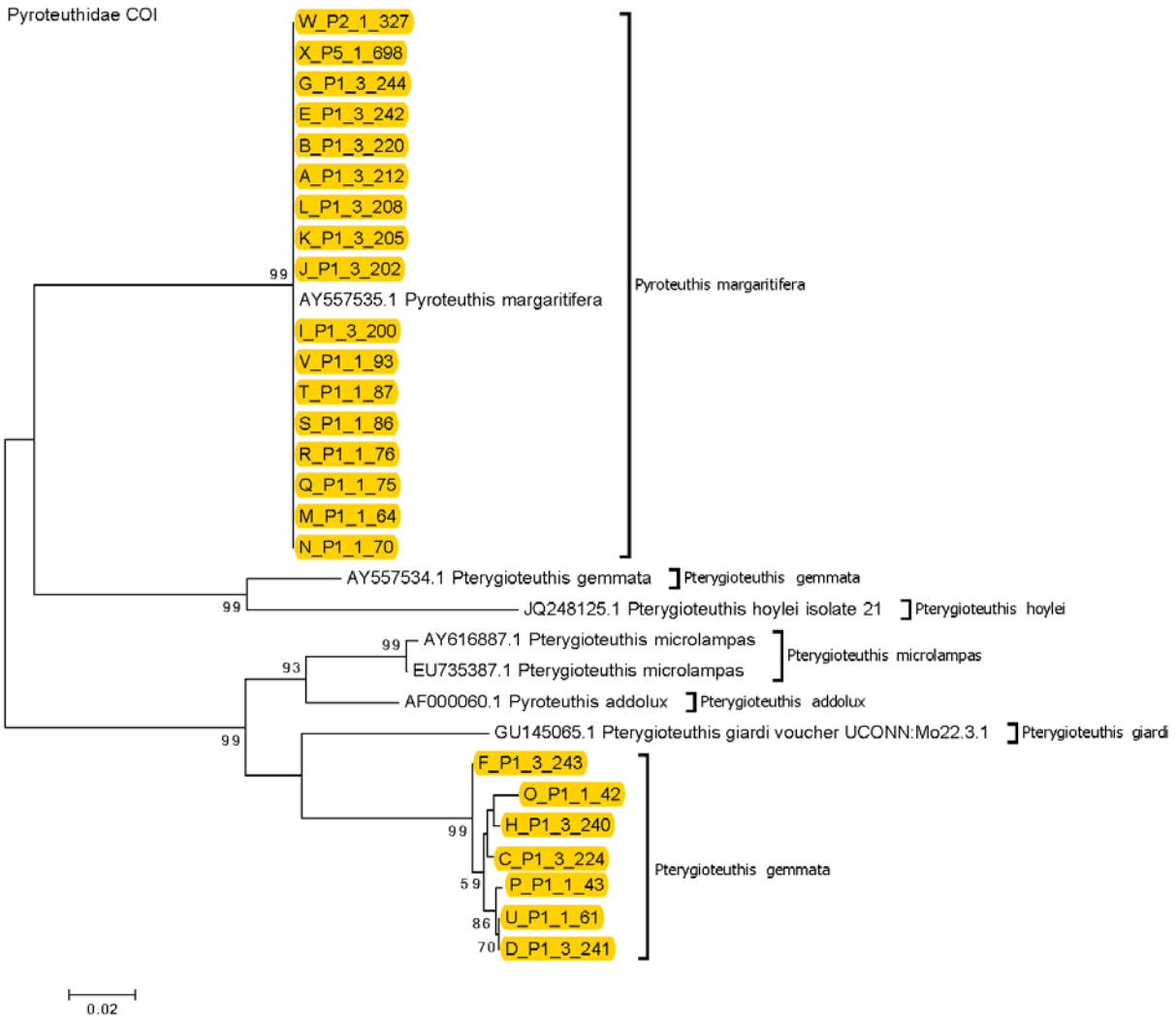

Figure 2.31 Pyroteuthidae COI phylogenetic tree

COI Maximum Likelihood phylogenetic tree for species of Pyroteuthidae with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Highlighted sequence labels indicate sequences from the Gulf of Mexico. The starting letter of highlighted sequence labels designates the same specimen in both trees (i.e. A_ in the COI tree corresponds with A_ in the 16S rRNA tree). Relevant GenBank accession records were added for reference.

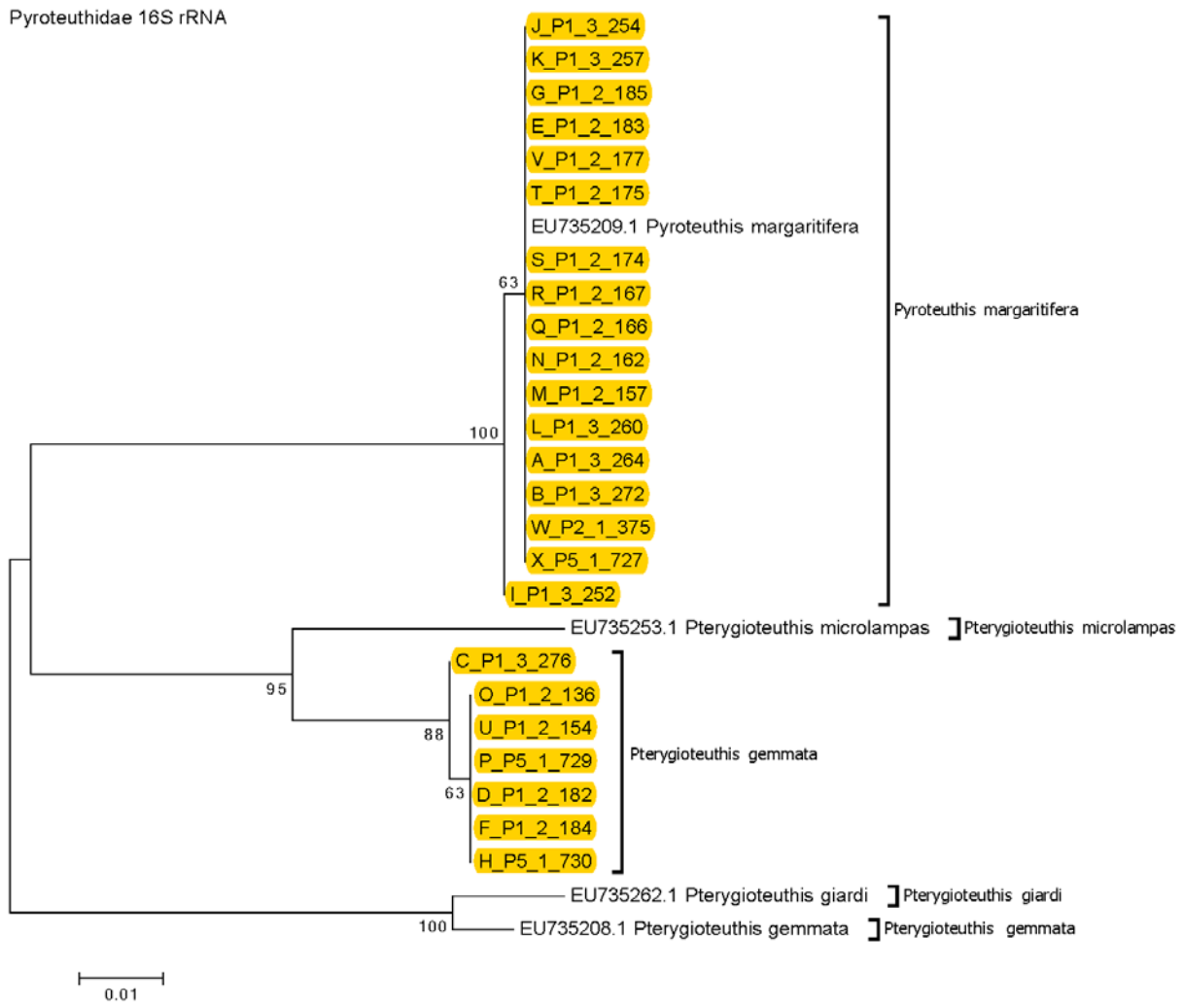

Figure 2.32 Pyroteuthidae 16S rRNA phylogenetic tree

16S rRNA Maximum Likelihood phylogenetic tree for species of Pyroteuthidae with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Highlighted sequence labels indicate sequences from the Gulf of Mexico. The starting letter of highlighted sequence labels designates the same specimen in both trees (i.e. A_ in the COI tree corresponds with A_ in the 16S rRNA tree). Relevant GenBank accession records were added for reference.

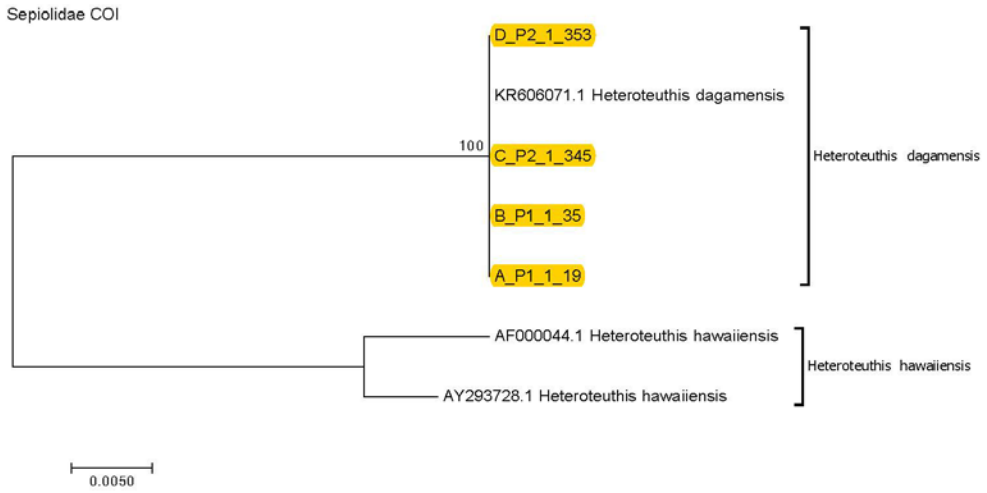

Figure 2.33 Sepiolidae COI phylogenetic tree

COI Maximum Likelihood phylogenetic tree for species of Sepiolidae with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Highlighted sequence labels indicate sequences from the Gulf of Mexico. The starting letter of highlighted sequence labels designates the same specimen in both trees (i.e. A_ in the COI tree corresponds with A_ in the 16S rRNA tree). Relevant GenBank accession records were added for reference.

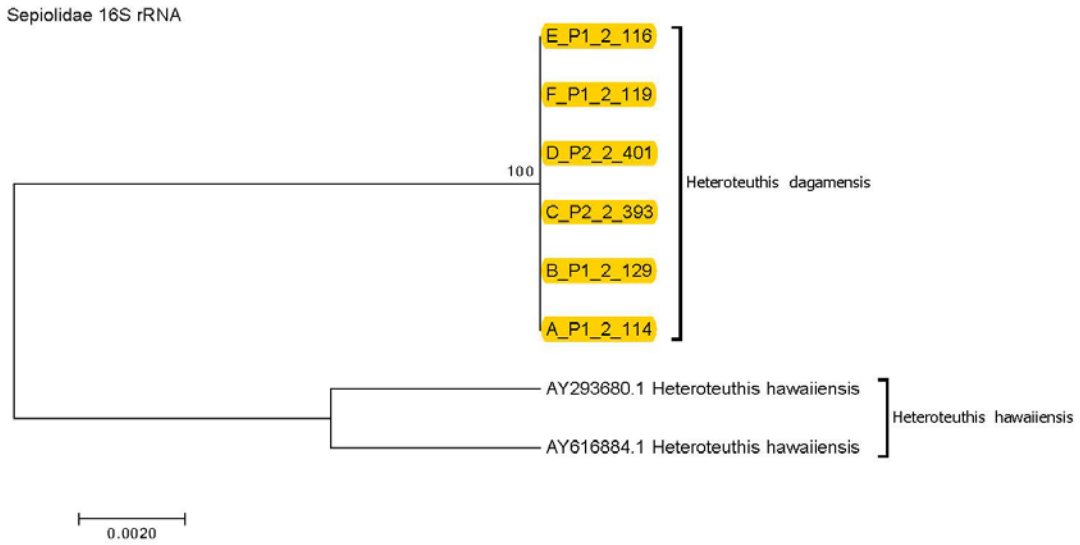

Figure 2.34 Sepiolidae 16S rRNA phylogenetic tree

16S rRNA Maximum Likelihood phylogenetic tree for species of Sepiolidae with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Highlighted sequence labels indicate sequences from the Gulf of Mexico. The starting letter of highlighted sequence labels designates the same specimen in both trees (i.e. A_ in the COI tree corresponds with A_ in the 16S rRNA tree). Relevant GenBank accession records were added for reference.

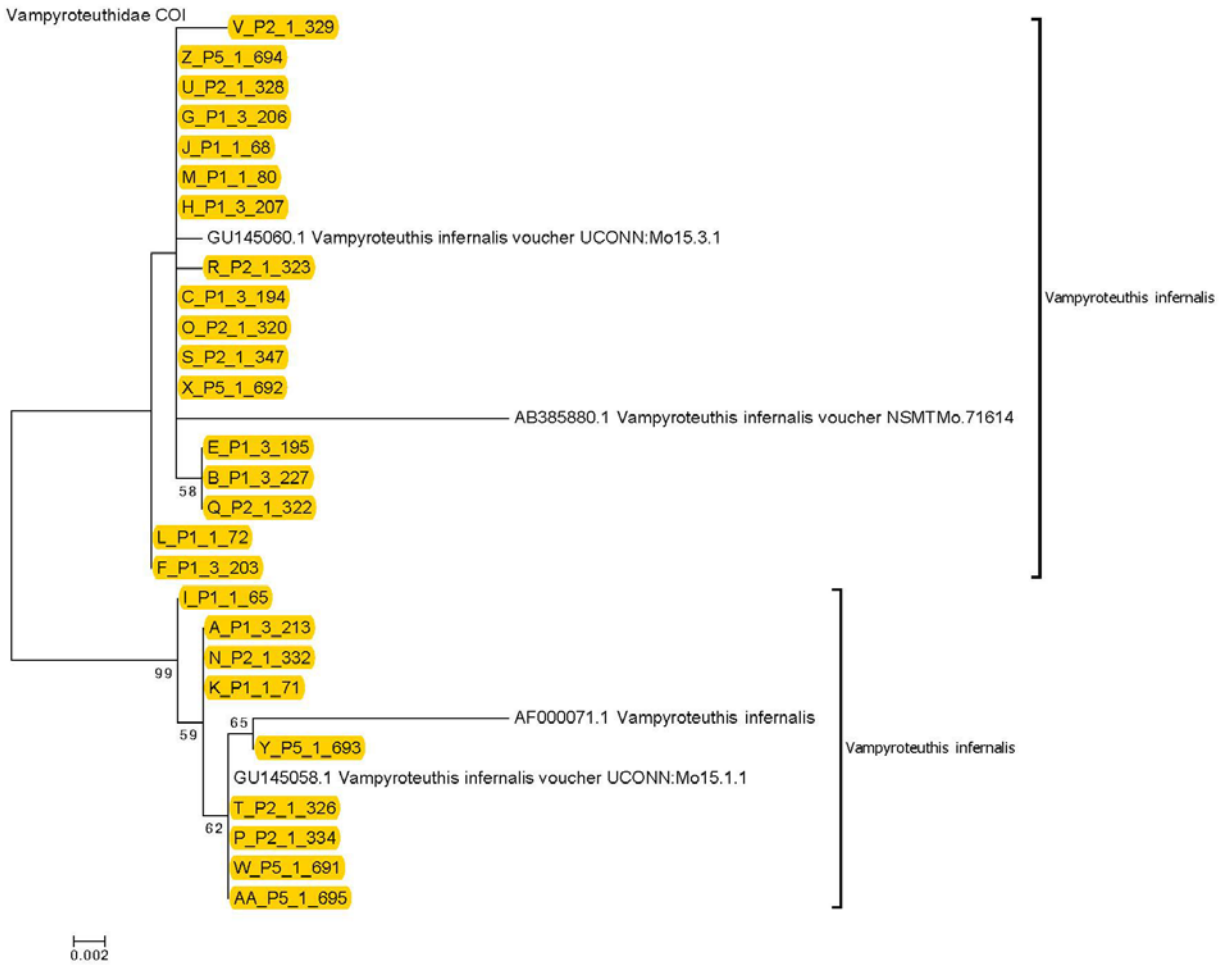

Figure 2.35 Vampyroteuthidae COI phylogenetic tree

COI Maximum Likelihood phylogenetic tree for species of Vampyroteuthidae with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Highlighted sequence labels indicate sequences from the Gulf of Mexico. The starting letter of highlighted sequence labels designates the same specimen in both trees (i.e. A_ in the COI tree corresponds with A_ in the 16S rRNA tree). Relevant GenBank accession records were added for reference.

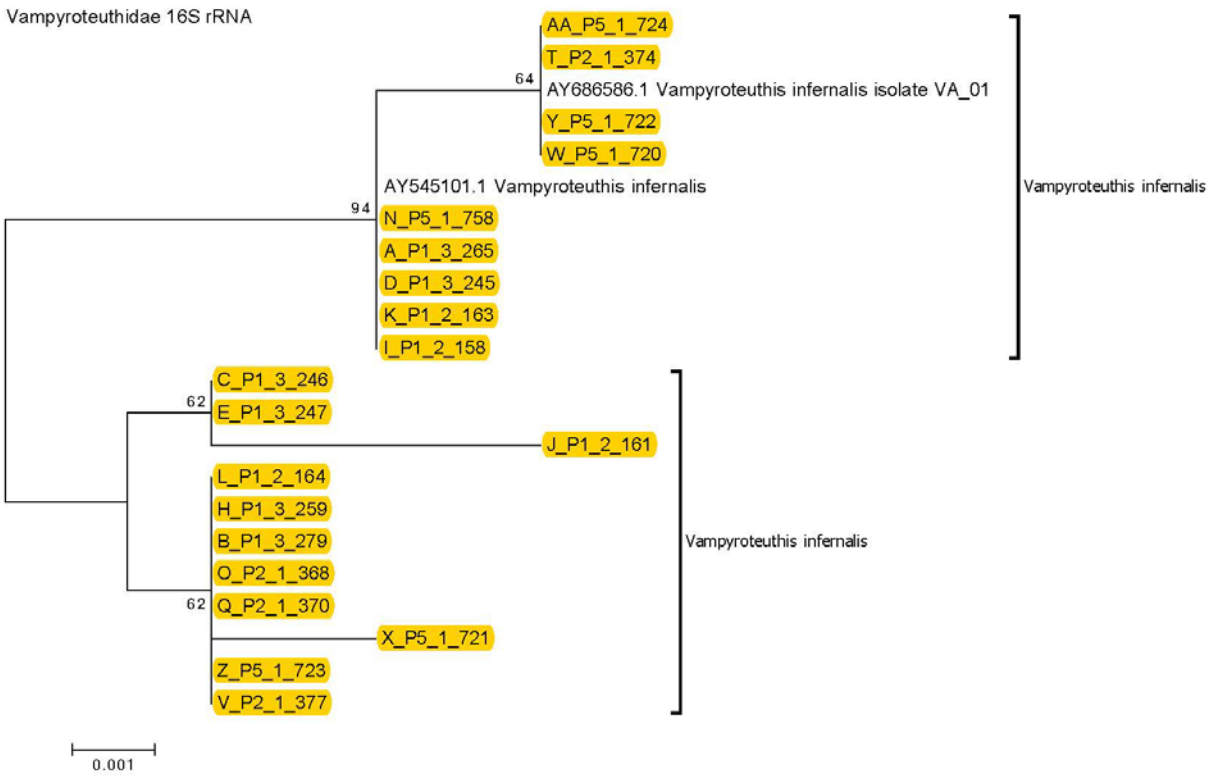

Figure 2.36 Vampyroteuthidae 16S rRNA phylogenetic tree

16S rRNA Maximum Likelihood phylogenetic tree for species of Vampyroteuthidae with bootstrap test of 1000 replicates. Branches with <50% bootstrap support were collapsed. Highlighted sequence labels indicate sequences from the Gulf of Mexico. The starting letter of highlighted sequence labels designates the same specimen in both trees (i.e. A_ in the COI tree corresponds with A_ in the 16S rRNA tree). Relevant GenBank accession records were added for reference.

2.4 Discussion

2.4.1 Intraspecies Variation within Regionally Disjunct Subpopulations

Objective 1 examined subpopulations of the Gulf of Mexico and Bear Seamount separated by the Florida Peninsula, with the Florida Straits acting as a potential barrier to dispersal of deep Gulf of Mexico biota. Dispersal through the Florida Straits would likely occur for *Pyroteuthis margaritifera,* which has a short average life span but is a known diel vertical migrator (Roper & Young, 1975), and *Cranchia scabra* which has juveniles and paralarvae in the epipelagic to mesopelagic while adults descend to the mesopelagic to bathypelagic zones (Clarke & Lu, 1975). *Vampyroteuthis infernalis* is not known to be a vertical migrator, with a peak distribution in the bathypelagic between 600 and 1500 m in the Gulf of Mexico (Judkins et al., in prep). This would make dispersal through the 800 m deep Florida Straits for *Vampyroteuthis infernalis* possible as well.

The lenses of COI, 16S rRNA, and 28S rRNA loci revealed there is no regional distinction between the Gulf of Mexico subpopulations of *Vampyroteuthis infernalis*, *Pyroteuthis margaritifera,* and *Cranchia scabra* and the Bear Seamount subpopulations in the northwestern Atlantic Ocean. The resulting trees reflect enough gene flow occurring within each taxon that there are no apparent regional genetic differences. It was necessary to employ a multi-loci approach to elucidate and corroborate the lack of intraspecies variation across regionally disjunct subpopulations. Although the 28S rRNA tree was the most conservative with the least resolution, it recovered each species clearly and in agreement with the two mitochondrial trees. 28S rRNA served as a standard confirmation for the delineation of each putative species. COI was the most informative of the chosen marker genes and likely has enough characteristic single nucleotide polymorphisms (SNPs) that could be used in molecular systematics studies to delineate

cephalopod subpopulations (Dai et al., 2012).

This finding is consistent with a recent review paper discussing the population genetics of benthic invertebrate species across the deep-sea (Taylor & Roterman, 2017). A general outcome of deep-sea population connectivity assessments discussed by Taylor and Roterman (2017) is wide-spread horizontal connectivity at the regional and oceanic scale. While the pattern of deep-sea population connectivity lends itself to high horizontal connectivity, there seems to be less vertical connectivity across steep environmental gradients (Taylor & Roterman, 2017). A relevant, recent paper by Shea et al. (2017) discussed cephalopod assemblages over a 15-year study conducted at Bear Seamount. Shea et al. (2017) explained that while seamounts were once considered to host mostly endemic species (Wilson & Kaufmann, 1987), Bear Seamount is now known to have cephalopod assemblages similar to the neighboring continental slope and to support many cosmopolitan cephalopod species (Vecchione, 2001). The Shea et al. (2017) findings, as well as this study's findings, further support the hypothesis that Bear Seamount is an intermediary in oceanic-scale dispersal (Wilson & Kaufmann, 1987). The ecological importance of this study is tied into genetic diversity. While it is an advantage for these deep-sea cephalopod populations to have such a geographically expansive gene pool to replenish populations after disruptions and isolated environmental disturbances, the trade-off seems to be reduced genetic diversity, at least for the three genetic loci examined in this study. With reduced genetic diversity among species populations, those species are more vulnerable to widespread critical events and reduced in their ability to recover from damage.

Another interesting point this finding supports relates to the recent discovery of extensive cephalopod RNA editing (Liscovitch-Brauer et al., 2017). Liscovitch-Brauer et al. (2017) suggest that slowed-down genome evolution is a trade-off for higher transcriptomic plasticity. It

was found that mutations and SNPs in cephalopod coding sequences are reduced to conserve the genome, slowing down the rate of conventional, genome evolution (Liscovitch-Brauer et al., 2017). The shared genetic identity across regionally disjunct subpopulations seen in this study supports the Liscovitch-Brauer et al. (2017) idea of slow genome evolution.

After having examined intraspecies relationships within a population through specific Sanger-based sequencing molecular markers, a further assessment through Next Generation Sequencing (NGS) will be used for higher resolution. The NGS method: double digest Restriction site-Associated DNA sequencing (ddRADseq) (Peterson et al., 2012) genotypes single nucleotide polymorphisms (SNPs). Double digest Restriction site-Associated DNA sequencing allows for the discovery on the order of thousands of SNP markers in comparison with Sanger sequencing (Peterson et al., 2012). The abundance of markers generated through ddRADseq allows for much finer-scale analysis of population level questions (i.e. selection, diversity, connectivity, etc.) (Peterson et al., 2012). Both NGS and Sanger-based sequencing data will be compared for divisions and delineations at the subpopulation level in an upcoming publication (Sosnowski et al., in prep).

2.4.2 Intraspecies Variation within the Gulf of Mexico

Objective 2 is the first comprehensive genetics study of deep-sea cephalopods in the Gulf of Mexico. This study examined intraspecies variation within deep-sea cephalopods in the Gulf of Mexico, testing each specimen's identity assigned by morphology with molecular methods. Currently, morphological identification is used to verify molecular taxonomic identities of cephalopods, but with the increase in sampling and sequencing efforts (e.g. Moore et al., 2003; Sutton et al., 2015; Judkins et al., 2016b; Shea et al., 2017) molecular identification has become a necessary and universal tool in biological studies. As such, objective 2 tested the application of

molecular systematics related to morphological identities and is discussed by each taxonomic family below.

Ancistrocheiridae and Enoploteuthidae

The two *Ancistrocheirus lesueurii* sequences and all ten enoploteuthid sequences will provide new genetic records of these species from the Gulf of Mexico. Six sequences for *Abraliopsis atlantica* and two sequences for *Abralia redfieldi* will be the first records contributed to GenBank for those enoploteuthid species. This analysis helped pinpoint and correct a misidentification originally identified as *Abraliopsis atlantica* to *Enoploteuthis leptura* ('E_' in Figures 2.5 and 2.6).

Bathyteuthidae

Four specimens from the family Bathyteuthidae, representing *Bathyteuthis abyssicola* and a new species *Bathyteuthis sp. A*, were originally included in this study. All amplicons failed to amplify with the COI and 16S rRNA primers used. Ongoing processing is underway and it should be noted that a separate description for the morphology and genetics of this family is being written by H. Judkins et al..

Bolitaenidae

Thirty nine COI sequences and 23 16S rRNA sequences representing Bolitaenidae provide additional information on this group in the Gulf of Mexico. COI amplicons were more successfully recovered than 16S rRNA, many individuals failed to amplify with the 16S rRNA primers used. Further sequencing of 16S rRNA for this family is recommended to better

understand the inconsistencies in successful amplicon recovery. The two clades, *Japetella diaphana* and *Bolitaena pygmaea,* are clearly and definitively clustered sharing only 92% and 95% pairwise identity in the COI and 16S rRNA trees, respectively. Both trees support the current accepted phylogenetic relationships of Bolitaenidae having two monotypic, genera (Thore, 1949; Hochberg et al., 1992). These phylogenetic trees helped highlight potential misidentifications of GenBank sequences annotated as *Japetella diaphana* which cluster within both clades, as well as potential misidentifications made of specimens sequenced from the Gulf of Mexico. This is unsurprising as the morphological characters separating bolitaenids are subtle: the size of the eyes (larger in *Japetella*) and the distance between the eyes (larger in *Bolitaena*) (Thore, 1949; Hochberg et al., 1992; Vecchione, 2002). This is a great example of where genetics serves as a powerful elucidation tool when morphology falls short. Within this study, the confusion between misidentified specimens was resolved by designating a sequence as *Japetella diaphana,* whose voucher confidently keys out to *Japetella diaphana*, and do the same for *Bolitaena pygmaea*.

Brachioteuthidae and Neoteuthidae

The four *Narrowteuthis nesisi* sequences will be the first COI and 16S rRNA records contributed to GenBank. The two sequences representing *Brachioteuthidae* will provide new information on species in the Gulf of Mexico. Both genetic loci reveal the *Narrowteuthis nesisi* sequences form a monophyletic cluster in agreement with the current, accepted systematic resolution as a monotypic taxon (Young & Vecchione, 2005). COI and 16S rRNA sequencing also revealed the *Brachioteuthis sp.* Gulf of Mexico specimen as potential cryptic species, sharing only 85% and 93% pairwise identity, respectively, with GenBank *Brachioteuthis beanii*

sequences. This will require further collection of material, genetic sequencing, and analysis of the Brachioteuthidae family to resolve the *Brachioteuthis sp.* Gulf of Mexico specimen's identity, as it is clearly not *Brachioteuthis beanii*.

Chiroteuthidae

All four sequenced species (16 sequences in total) representing Chiroteuthidae provide new information on the group in the Gulf of Mexico. The sequences for *Chiroteuthis mega* and *Grimalditeuthis bonplandi* will be the first contributions to GenBank from the Gulf of Mexico (Braid et al., 2016). This genetic analysis of chiroteuthids helped resolve a *Chiroteuthis sp*. specimen to *Chiroteuthis mega* ('C_' in Figures 2.9 and 2.10), as it was too damaged to morphologically identify to species. The COI tree helped bring to light a potential misidentification in GenBank of a conspecific individual, *Chiroteuthis veranyi* (GU145077.1), which grouped with the *Planctoteuthis levimana* clade.

Cranchiidae

All 103 sequences representing Cranchiidae provide new information on the species in the Gulf of Mexico. The COI and 16S rRNA sequences of *Ligurella podothalma* and *Galiteuthis armata* will be novel records contributed to GenBank. The 16S rRNA sequence of *Bathothauma lyromma* will also be the first record contributed to GenBank. *Bathothauma lyromma* failed to amplify with the COI primers used. It is interesting to note that *Cranchia scabra* shows very low intraspecies diversity, for both loci *Cranchia scabra* sequences share 99-100% pairwise identity. These analyses brought to light a discrepancy between *Teuthowenia megalops* from the Gulf of Mexico ('PP_' Figures 2.13 and 2.14) and GenBank sequences annotated as *Teuthowenia*

megalops (AY617064.1, AY616984.1) in the COI and 16S rRNA trees, with only 84% and 94% shared pairwise identity, respectively. The *Teuthowenia megalops* from the Gulf of Mexico highest match when run through a BLAST search is 99% identity score with *Megalocranchia sp.* COI (EU735382.1). These trees demonstrate that *Teuthowenia megalops* needs further investigation to resolve the observed intraspecies variations and discrepancies. Both trees' clustering of conspecific individuals into two clades of *Helicocranchia*, *Helicocranchia pfefferi* and *Helicocranchia sp. A*, support the findings of Judkins et al. (2016b), which suggested a new species, *Helicocranchia sp. A*, and possible additional undescribed species of Taoniinae. These genetic loci brought to light two unique clades that would have otherwise been morphologically grouped as *Helicocranchia pfefferi*. The COI tree also highlighted a potential misidentification of a GenBank sequence annotated as *Helicocranchia pfefferi* (GU145078.1). The Gulf of Mexico *Helicocranchia pfefferi* sequences only share 91% and 97% pairwise identity with GenBank sequences annotated as *Helicocranchia pfefferi* (AF075412.1, AF110099.2) in the COI and 16S rRNA trees, respectively. Because both clades are clearly clustered and do not share much similarity to existing GenBank sequences, H. Judkins will be furthering analyses to resolve the *Helicocranchia* clades and describe the new species *Helicocranchia sp. A.*

Cycloteuthidae

Ten COI sequences and ten 16S rRNA sequences representing Cycloteuthidae provide additional information on the group in the Gulf of Mexico. While *Cycloteuthis sirventi* is well resolved, *Discoteuthis discus* remains unresolved in both COI and 16S rRNA trees. There is high bootstrap support for each node in both trees. This analysis helped correct a misidentification originally identified as *Discoteuthis discus* to *Cycloteuthis sirventi* ('H_' in Figures 2.15 and

2.16), highlighted a potential misidentification of a GenBank sequence annotated as *Discoteuthis laciniosa* (EU735205.1), and brought to light an unresolved unid Cycloteuthidae Gulf of Mexico specimen too damaged to morphologically identify to species. These trees demonstrate that *Discoteuthis discus* needs further investigation to resolve the observed intraspecies variations and discrepancies. As noted by Young and Roper (1969), there seems to be potential for four species under the genus *Discoteuthis*. It is unsurprising there are misidentifications as cycloteuthid specimens are typically collected in poor condition, making morphological identifications difficult. With the unresolved *Discoteuthis discus* sequences, there is an opportunity for further genetic analysis of the family Cycloteuthidae.

Histioteuthidae

All four sequences representing Histioteuthidae provide new information on two species in the Gulf of Mexico. The *Histioteuthis corona* sequence will be the first COI record contributed to GenBank. While *Histioteuthis corona* did not resolve into an assemblage of conspecific individuals in the 16S rRNA tree, it was noted by Voss (1969) and Voss et al. (1998) that there are several subspecies under *Histioteuthis* species (Young & Vecchione, 2013). These trees helped bring to light a need for further genetic sequencing and analysis of the Histioteuthidae family to better understand species and potential subspecies complexes. The Histioteuthidae COI and 16S rRNA analyses highlighted an important nomenclature-related issue of updating taxa synonyms in GenBank. GenBank sequences annotated as *Histioteuthis hoylei* are currently accepted as *Stigmatoteuthis arcturi.*

Joubiniteuthidae

The four *Joubiniteuthis portieri* sequences will provide a new genetic record of this species from the Gulf of Mexico. The high, shared pairwise identities of all *Joubiniteuthis portieri* sequences supports the family's current, accepted systematic resolution as monospecific. This analysis helped resolve a GenBank sequence currently annotated as *Joubiniteuthis sp.* (AY616888.1 COI and AY616879.1 16S rRNA) to species.

Lycoteuthidae

The contribution of four *Selenoteuthis scintillans* sequences from the Gulf of Mexico will double the number of existing genetic records in GenBank. The low bootstrap values reflected in both trees is most likely due to a small sample size. This genetic analysis of lycoteuthids helped correct a misidentification originally identified as *Lycoteuthis lorigera* to *Selenoteuthis scintillans* ('B_' in Figures 2.21 and 2.22).

Mastigoteuthidae

Thirty sequences representing Mastigoteuthidae provide additional information on the group in the Gulf of Mexico and double the existing genetic records of the three sequenced species in GenBank. Both trees agree with the 'Maximum-likelihood consensus tree for eight species of Mastigoteuthidae' in the Braid et al. (2013) review of the family Mastigoteuthidae. Braid et al. (2013) noted mastigoteuthids are typically recovered as badly damaged specimens, making it difficult to identify to species. This genetic analysis helped resolve three *Mastigoteuthis sp*. specimens to *Mastigoteuthis agassizii* ('K_', 'M_', and 'N_' in Figures 2.23 and 2.24), as they were too damaged to morphologically identify to species. The

Mastigoteuthidae COI and 16S rRNA analyses highlighted an important nomenclature-related issue of updating taxa synonyms in GenBank. The current accepted Mastigoteuthidae genera are *Echinoteuthis* and *Idioteuthis*, as well, *Mastigoteuthis cf. dentata* is now considered *Mastigoteuthis agassizii*.

Octopoteuthidae

All ten sequences representing Octopoteuthidae provide new information on two species in the Gulf of Mexico. These trees demonstrate that *Octopoteuthis* species included in the Octopoteuthidae analyses need further investigation to resolve the observed intraspecies variations and discrepancies. It seems likely that there are misidentifications in GenBank. With the resultant Octopoteuthidae trees, a determinate answer cannot be drawn. This highlights the difficulties of morphological identification and an opportunity for further genetic analysis to resolve morphological problems.

Ommastrephidae

All 16 sequences belonging to Ommastrephidae will contribute new genetic information from the Gulf of Mexico. The non-verifiable unid Ommastrephidae Gulf of Mexico specimens in these analyses highlight the need for further genetic sequencing to resolve the unid Ommastrephidae identity. This genetic analysis of helped resolve a *Ommastrephidae sp*. specimen to *Ornithoteuthis antillarum* (‘G_’ in Figures 2.27 and 2.28), as it was too damaged to morphologically identify to species.

Onychoteuthidae

Four COI sequences and four 16S rRNA sequences representing Onychoteuthidae provide additional information on the group in the Gulf of Mexico. This analysis helped place the unverifiable unid Onychoteuthidae specimen with GenBank sequences annotated as *Onykia*, sharing 89% and 96% pairwise identity in the COI and 16S rRNA trees, respectively. These trees demonstrate that species included in the Onychoteuthidae analyses need further genetic sequencing to resolve the observed intraspecies variations and discrepancies. It seems likely there are potential misidentifications in GenBank. These analyses highlight an opportunity for further genetic analysis for the family Onychoteuthidae, as the family was recently resolved through morphological systematics by Bolstad (2008).

Pyroteuthidae

All 48 sequences representing Pyroteuthidae provide new information on two species in the Gulf of Mexico. These COI and 16S rRNA sequences helped bring to light a discrete clade, *Pterygioteuthis gemmata*, that would have otherwise been marked as misidentifications. Because the clade is clearly clustered and does not share much similarity to GenBank sequences annotated as *Pterygioteuthis gemmata*, it will require further genetic sequencing and analysis to resolve the *Pterygioteuthis* species. There is the potential for misidentifications in GenBank confusing the true identities of *Pterygioteuthis* species. It is interesting to note that *Pyroteuthis margaritifera* shows very low intraspecies diversity; *Pyroteuthis margaritifera* sequences share 99-100% pairwise identity, while the *Pterygioteuthis gemmata* clade shows some intraspecific diversity.

Sepiolidae

The six 16S rRNA *Heteroteuthis dagamensis* sequences will be the first 16S rRNA records contributed to GenBank. 16S rRNA amplicons were more successfully recovered than COI, two individuals failed to amplify with the COI primers used. The problem of successful COI amplicon recovery and the resulting trees support the recent report of *Heteroteuthis dagamensis* in the Gulf of Mexico by Judkins et al. (2016). This analysis helped confirm two damaged specimen identifications to species ('C_' and 'D_' in Figures 2.33 and 2.34). It is interesting to note the high shared pairwise identity (98%) between *Heteroteuthis dagamensis* and *Heteroteuthis hawaiiensis* in the 16S rRNA tree. 16S rRNA has shown to be a slower evolving gene in comparison with COI. As such, the 16S rRNA high shared pairwise identity speaks to the evolutionary history of this gene and might suggest *Heteroteuthis dagamensis* is a more recently evolved species.

Vampyroteuthidae

All 46 sequences representing Vampyroteuthidae will contribute new genetic information from the Gulf of Mexico. COI amplicons were more successfully recovered than 16S rRNA, six individuals failed to amplify with the 16S rRNA primers used. While there are two clades supported and identical between genetic loci, the high shared pairwise identity between clades is in agreement with the family's current accepted systematic resolution as a monotypic taxon (Yokobori et al., 2007). The COI and 16S rRNA sequences demonstrate a variety of intraspecific distances among the *Vampyroteuthis infernalis* sequences, indicating various nucleotide substitutions occurred. Although the number of nucleotide substitutions are not enough to reach

the accepted threshold of different species (Strugnell & Lindgren, 2007; Dai et al., 2012), this might suggest there are several subspecies under *Vampyroteuthis*.

2.5 Conclusion

The research presented in this study assessed broad genetic patterns of biodiversity in deep-sea cephalopods from the Gulf of Mexico and northwestern Atlantic Ocean. As the first comprehensive phylogenetic assessment of deep-sea cephalopods in the Gulf of Mexico, this research expanded our overall understanding of cephalopod genetics. The research in this study was also the first to compare population connectivity of deep-sea cephalopods in the Gulf of Mexico with other subpopulations outside of the basin. Before the Deepwater Horizon oil spill, knowledge of deep-sea cephalopod species in the Gulf of Mexico was limited with two comprehensive studies conducted by Judkins in 2009 and Voss in 1956. The most recent compilation of the cephalopods in the Gulf of Mexico is that of Judkins' (2009) monograph and Vecchione's (2002) *The Living Marine Resources of the Western Central Atlantic* FAO Cephalopod Species Identification Guide.

In an effort to examine intraspecies variation of deep-sea cephalopods, specimens from the Gulf of Mexico and in the northwestern Atlantic Ocean were sampled using molecular markers and Sanger-based sequencing. Results of investigating intraspecies variation within regionally disjunct subpopulations reveal there is no regional distinction between the Gulf of Mexico subpopulations of *Vampyroteuthis infernalis*, *Pyroteuthis margaritifera*, and *Cranchia scabra* and the Bear Seamount subpopulations in the northwestern Atlantic for the three genetic loci examined in this study. The resulting trees reflect enough gene flow occurring among each taxon that there are no apparent regional genetic differences.

Results of investigating intraspecies variation within the Gulf of Mexico displayed potential for cryptic species in Brachioteuthidae, Cranchiidae, and Pyroteuthidae, novel sequence records with two molecular markers for Brachioteuthidae, Chiroteuthidae, Cranchiidae, Neoteuthidae, and Sepiolidae, and large expansions to sequence records for species known to inhabit the Gulf of Mexico. Analysis of intraspecies variation within the Gulf of Mexico facilitated identification of damaged specimens used for this study, but also revealed database issues of misidentified records, and outdated nomenclature in accession records. The multi-loci sequencing approach used in this study to investigate complex cephalopod assemblages improved accuracy by having loci with differing rates of evolution to corroborate and support species delimitations and intraspecific diversity. This in turn, strengthened the system of determining population connectivity and phylogenetic relationships of cephalopods greatly. Future deep-sea cephalopod biodiversity studies should remain technique-driven and designed to improve accuracy, staying at the forefront of modeling patterns of connectivity and genetic diversity. The currently underway NGS and Sanger sequencing methods comparison paper aims to fulfil this initiative by producing a higher-resolution picture of subpopulation gene flow of deep-sea cephalopods (Sosnowski et al., in prep). Because cephalopods play a central role in most oceanic ecosystems, characteristics like a short average life span and a rapid growth rate mean that cephalopod populations have the potential to serve as an invaluable reflection of ecosystem change. For ecosystems to be understood and managed, a foundation of knowledge of the species and populations that make up each ecosystem is required, and must continue to be explored.

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APPENDICIES

Appendix A. List of species abundances

Abundances (*n*) of cephalopods collected during all DEEPEND cruises 2015-2017 and those utilized during this study.

Superorder	Species	*n* (all cruises)	*n* (current study)
	unid Cephalopod	3	0
Octopodiformes	*Argonauta argo*	3	0
Octopodiformes	*Argonautidae*	1	0
Octopodiformes	*Bolitaena pygmaea*	59	15
Octopodiformes	*Bolitaenidae*	4	0
Octopodiformes	*Haliphron atlanticus*	1	0
Octopodiformes	*Japetella diaphana*	83	24
Octopodiformes	*Macrotritopus defilippi*	4	0
Octopodiformes	*unid Octopod*	1	0
Octopodiformes	*Vampyroteuthis infernalis*	49	26
Decapodiformes	*Abralia redfieldi*	10	1
Decapodiformes	*Abraliopsis atlantica*	14	3
Decapodiformes	*Ancistrocheirus lesueurii*	2	1
Decapodiformes	*Asperoteuthis acanthoderma*	2	2
Decapodiformes	*Bathothauma lyromma*	2	1
Decapodiformes	*Bathyteuthis abyssicola*	1	0
Decapodiformes	*Bathyteuthis sp.*	4	0
Decapodiformes	*Bathyteuthis sp. A*	6	0
Decapodiformes	*Brachioteuthis sp.*	4	1
Decapodiformes	*Chiroteuthidae*	4	0
Decapodiformes	*Chiroteuthis mega*	7	2
Decapodiformes	*Cranchia scabra*	72	35
Decapodiformes	*Cranchiidae*	1	0
Decapodiformes	*Cycloteuthidae*	3	1
Decapodiformes	*Cycloteuthis sirventi*	16	6
Decapodiformes	*Discoteuthis discus*	5	3
Decapodiformes	*Echinoteuthis atlantica*	5	4

Superorder	Species	*n* (all cruises)	*n* (current study)
Decapodiformes	*Egea inermis*	1	0
Decapodiformes	*Enoploteuthidae*	1	0
Decapodiformes	*Enoploteuthis anapsis*	1	0
Decapodiformes	*Enoploteuthis leptura*	1	1
Decapodiformes	*Enoploteuthis sp.*	1	0
Decapodiformes	*Galiteuthis armata*	4	1
Decapodiformes	*Grimalditeuthis bonplandi*	8	1
Decapodiformes	*Helicocranchia papilata*	1	0
Decapodiformes	*Helicocranchia pfefferi*	15	6
Decapodiformes	*Helicocranchia sp. A*	9	7
Decapodiformes	*Helicocranchia sp.*	3	0
Decapodiformes	*Heteroteuthis dagamensis*	8	6
Decapodiformes	*Heteroteuthis sp.*	1	0
Decapodiformes	*Histioteuthidae*	1	0
Decapodiformes	*Histioteuthis corona*	13	1
Decapodiformes	*Histioteuthis sp.*	1	0
Decapodiformes	*Idioteuthis hjorti*	2	1
Decapodiformes	*Joubiniteuthis portieri*	8	6
Decapodiformes	*Leachia atlantica*	4	1
Decapodiformes	*Liguriella podophthalma*	1	1
Decapodiformes	*Lycoteuthidae*	1	1
Decapodiformes	*Lycoteuthis lorigera*	2	0
Decapodiformes	*Mastigoteuthis agassizii*	18	10
Decapodiformes	*Mastigoteuthis sp.*	4	0
Decapodiformes	*Megalocranchia fisheri*	1	0
Decapodiformes	*Megalocranchia sp.*	1	0
Decapodiformes	*Narrowteuthis nesisi*	2	2
Decapodiformes	*Neoteuthidae*	1	0
Decapodiformes	*Neoteuthis nesisi*	1	0
Decapodiformes	*Neoteuthis thielei*	1	0
Decapodiformes	*Octopoteuthis cf. myoptera*	1	0
Decapodiformes	*Octopoteuthis megaptera*	2	2
Decapodiformes	*Octopoteuthis sp.*	8	2
Decapodiformes	*Ommastrephidae*	3	3
Decapodiformes	*Onychoteuthidae*	2	1

Superorder	Species	*n* (all cruises)	*n* (current study)
Decapodiformes	*Onychoteuthis banksii*	2	0
Decapodiformes	*Onychoteuthis cf. banksii*	3	1
Decapodiformes	*Onychoteuthis compacta*	1	1
Decapodiformes	*Onychoteuthis sp.*	1	0
Decapodiformes	*Ornithoteuthis antillarum*	4	4
Decapodiformes	*Planctoteuthis levimana*	1	1
Decapodiformes	*Pterygioteuthis gemmata*	41	7
Decapodiformes	*Pterygioteuthis giardi*	45	0
Decapodiformes	*Pterygioteuthis sp.*	23	0
Decapodiformes	*Pyroteuthidae*	2	0
Decapodiformes	*Pyroteuthis margaritifera*	28	17
Decapodiformes	*Sandalops melancholicus*	12	0
Decapodiformes	*Selenoteuthis scintillans*	14	2
Decapodiformes	*Spirula spirula*	1	0
Decapodiformes	*Sthenoteuthis pteropus*	3	1
Decapodiformes	*Stigmatoteuthis arcturi*	35	1
Decapodiformes	*Taningia danae*	3	1
Decapodiformes	*Taoniinae*	9	0
Decapodiformes	*Taonius pavo*	1	0
Decapodiformes	*Teuthowenia megalops*	1	1
Decapodiformes	*unid Oegopsid*	1	0
Decapodiformes	*unid Squid*	4	0
Decapodiformes	*Walvisteuthis jeremiahi*	4	0
Decapodiformes	*Walvisteuthis sp.*	2	0

Appendix B. List of sequence labels

A list of sequence labels used in the phylogenetic trees of this study, along with the corresponding DEEPEND jar labels, and species name. The DEEPEND jar labels will be submitted to GenBank as each Accession Number's Sequence ID and Specimen Voucher for future reference.

Sequence Label	Jar Label	Species
P1_1_1	DP20895	*Helicocranchia sp. A*
P1_1_10	DP0025X	*Japetella diaphana*
P1_1_11	DP0026X	*Enoploteuthis leptura*
P1_1_14	DP0027X	*Selenoteuthis scintillans*
P1_1_15	DP0760	*Bolitaena pygmaea*
P1_1_16	DP0759	*Joubiniteuthis portieri*
P1_1_17	DP0757	*Echinoteuthis atlantica*
P1_1_18	DP0752	*Bolitaena pygmaea*
P1_1_19	DP0746	*Heteroteuthis dagamensis*
P1_1_2	DP0001X	*Helicocranchia pfefferi*
P1_1_20	DP0754	*Discoteuthis discus*
P1_1_22	DP0029X	*Japetella diaphana*
P1_1_23	DP2114	*Echinoteuthis atlantica*
P1_1_25	DP2064	*Octopoteuthis sp.*
P1_1_26	DP0030X	*Bolitaena pygmaea*
P1_1_27	DP0466	*Cycloteuthis sirventi*
P1_1_28	DP0748	*Narrowteuthis nesisi*
P1_1_29	DP0747	*Liguriella podophthalma*
P1_1_30	DP2065	*Idioteuthis hjorti*
P1_1_35	DP0751	*Heteroteuthis dagamensis*
P1_1_36	DP0495	*Octopoteuthis sp.*
P1_1_39	DP2063	*Echinoteuthis atlantica*
P1_1_4	DP0012	*Japetella diaphana*
P1_1_40	DP0771	*Japetella diaphana*
P1_1_41	DP0812	*Helicocranchia pfefferi*
P1_1_42	DP0034X	*Pterygioteuthis gemmata*
P1_1_43	DP0787	*Pterygioteuthis gemmata*
P1_1_44	DP0800	*Chiroteuthis mega*
P1_1_45	DP0802	*Octopoteuthis megaptera*
P1_1_46	DP0035X	*Abraliopsis atlantica*
P1_1_48	DP0038X	*Sthenoteuthis pteropus*

Sequence Label	Jar Label	Species
P1_1_49	DP0826	*Onychoteuthis compacta*
P1_1_50	DP0039X	*Abraliopsis atlantica*
P1_1_51	DP0808	*Bolitaena pygmaea*
P1_1_52	DP0739	*Joubiniteuthis portieri*
P1_1_53	DP0040X	*Japetella diaphana*
P1_1_54	DP0790	*Helicocranchia sp. A*
P1_1_55	DP0740	*Grimalditeuthis bonplandi*
P1_1_56	DP0803	*Stigmatoteuthis arcturi*
P1_1_57	DP0041X	*Abralia redfieldi*
P1_1_58	DP2118	*Galiteuthis armata*
P1_1_59	DP0809	*Histioteuthis corona*
P1_1_6	DP0049	*Brachioteuthis sp.*
P1_1_60	DP0042X	*Selenoteuthis scintillans*
P1_1_61	DP0798	*Pterygioteuthis gemmata*
P1_1_62	DP0831	*Cranchia scabra*
P1_1_63	DP0768	*Cranchia scabra*
P1_1_64	DP0783	*Pyroteuthis margaritifera*
P1_1_65	DP1994	*Vampyroteuthis infernalis*
P1_1_66	DP0011X	*Cranchia scabra*
P1_1_67	DP2025	*Bolitaena pygmaea*
P1_1_68	DP2087	*Vampyroteuthis infernalis*
P1_1_69	DP0032X	*Japetella diaphana*
P1_1_70	DP0012X	*Pyroteuthis margaritifera*
P1_1_71	DP0013X	*Vampyroteuthis infernalis*
P1_1_72	DP0014X	*Vampyroteuthis infernalis*
P1_1_73	DP0792	*Cranchia scabra*
P1_1_74	DP0033X	*Japetella diaphana*
P1_1_75	DP2084	*Pyroteuthis margaritifera*
P1_1_76	DP0015X	*Pyroteuthis margaritifera*
P1_1_77	DP0036X	*Bolitaena pygmaea*
P1_1_78	DP0037X	*Japetella diaphana*
P1_1_79	DP0782	*Japetella diaphana*
P1_1_8	DP2021	*Discoteuthis discus*
P1_1_80	DP0016X	*Vampyroteuthis infernalis*
P1_1_81	DP0736	*Cranchia scabra*

Sequence Label	Jar Label	Species
P1_1_82	DP0815	*Mastigoteuthis agassizii*
P1_1_83	DP0020X	*Bolitaena pygmaea*
P1_1_84	DP0021X	*Bolitaena pygmaea*
P1_1_85	DP0022X	*Japetella diaphana*
P1_1_86	DP1993	*Pyroteuthis margaritifera*
P1_1_87	DP0017X	*Pyroteuthis margaritifera*
P1_1_88	DP0018X	*Cranchia scabra*
P1_1_89	DP0829	*Japetella diaphana*
P1_1_9	DP0766	*Japetella diaphana*
P1_1_90	DP0023X	*Japetella diaphana*
P1_1_91	DP0024X	*Japetella diaphana*
P1_1_92	DP0024X	*Japetella diaphana*
P1_1_93	DP0019X	*Pyroteuthis margaritifera*
P1_1_96	DP0829	*Japetella diaphana*
P1_2_101	DP0049	*Brachioteuthis sp.*
P1_2_103	DP2021	*Discoteuthis discus*
P1_2_106	DP0026X	*Enoploteuthis leptura*
P1_2_109	DP0027X	*Selenoteuthis scintillans*
P1_2_110	DP0760	*Bolitaena pygmaea*
P1_2_111	DP0759	*Joubiniteuthis portieri*
P1_2_112	DP0757	*Echinoteuthis atlantica*
P1_2_113	DP0752	*Bolitaena pygmaea*
P1_2_114	DP0746	*Heteroteuthis dagamensis*
P1_2_115	DP0754	*Discoteuthis discus*
P1_2_116	DP0028X	*Heteroteuthis dagamensis*
P1_2_117	DP0029X	*Japetella diaphana*
P1_2_118	DP2114	*Echinoteuthis atlantica*
P1_2_119	DP2023	*Heteroteuthis dagamensis*
P1_2_120	DP2064	*Octopoteuthis sp.*
P1_2_121	DP0030X	*Bolitaena pygmaea*
P1_2_122	DP0466	*Cycloteuthis sirventi*
P1_2_123	DP0748	*Narrowteuthis nesisi*
P1_2_124	DP0747	*Liguriella podophthalma*
P1_2_125	DP2065	*Idioteuthis hjorti*
P1_2_126	DP1963	*Bathothauma lyromma*

Sequence Label	Jar Label	Species
P1_2_128	DP0765	*Cycloteuthis sirventi*
P1_2_129	DP0751	*Heteroteuthis dagamensis*
P1_2_130	DP0495	*Octopoteuthis sp.*
P1_2_134	DP2063	*Echinoteuthis atlantica*
P1_2_135	DP0812	*Helicocranchia pfefferi*
P1_2_136	DP0034X	*Pterygioteuthis gemmata*
P1_2_138	DP0800	*Chiroteuthis mega*
P1_2_139	DP0802	*Octopoteuthis megaptera*
P1_2_140	DP0035X	*Abraliopsis atlantica*
P1_2_142	DP0038X	*Sthenoteuthis pteropus*
P1_2_143	DP0826	*Onychoteuthis compacta*
P1_2_144	DP0039X	*Abraliopsis atlantica*
P1_2_145	DP0808	*Bolitaena pygmaea*
P1_2_146	DP0739	*Joubiniteuthis portieri*
P1_2_147	DP0790	*Helicocranchia sp. A*
P1_2_148	DP0740	*Grimalditeuthis bonplandi*
P1_2_149	DP0803	*Stigmatoteuthis arcturi*
P1_2_150	DP0041X	*Abralia redfieldi*
P1_2_151	DP2118	*Galiteuthis armata*
P1_2_152	DP0809	*Histioteuthis corona*
P1_2_153	DP0042X	*Selenoteuthis scintillans*
P1_2_154	DP0798	*Pterygioteuthis gemmata*
P1_2_155	DP0831	*Cranchia scabra*
P1_2_156	DP0768	*Cranchia scabra*
P1_2_157	DP0783	*Pyroteuthis margaritifera*
P1_2_158	DP1994	*Vampyroteuthis infernalis*
P1_2_159	DP0011X	*Cranchia scabra*
P1_2_160	DP2025	*Bolitaena pygmaea*
P1_2_161	DP2087	*Vampyroteuthis infernalis*
P1_2_162	DP0012X	*Pyroteuthis margaritifera*
P1_2_163	DP0013X	*Vampyroteuthis infernalis*
P1_2_164	DP0014X	*Vampyroteuthis infernalis*
P1_2_165	DP0792	*Cranchia scabra*
P1_2_166	DP2084	*Pyroteuthis margaritifera*
P1_2_167	DP0015X	*Pyroteuthis margaritifera*

Sequence Label	Jar Label	Species
P1_2_168	DP0036X	*Japetella diaphana*
P1_2_170	DP0736	*Cranchia scabra*
P1_2_171	DP0815	*Mastigoteuthis agassizii*
P1_2_172	DP0020X	*Bolitaena pygmaea*
P1_2_173	DP0021X	*Japetella diaphana*
P1_2_174	DP1993	*Pyroteuthis margaritifera*
P1_2_175	DP0017X	*Pyroteuthis margaritifera*
P1_2_176	DP0018X	*Cranchia scabra*
P1_2_177	DP0019X	*Pyroteuthis margaritifera*
P1_2_178	DP0064X	*Cranchia scabra*
P1_2_179	DP0065X	*Cranchia scabra*
P1_2_180	DP0066X	*Cranchia scabra*
P1_2_182	DP0068X	*Pterygioteuthis gemmata*
P1_2_183	DP0069X	*Pyroteuthis margaritifera*
P1_2_184	DP0070X	*Pterygioteuthis gemmata*
P1_2_185	DP0071X	*Pyroteuthis margaritifera*
P1_2_98	DP0001X	*Helicocranchia pfefferi*
P1_3_194	DP0744	*Vampyroteuthis infernalis*
P1_3_195	DP0048X	*Vampyroteuthis infernalis*
P1_3_197	DP0755	*Ornithoteuthis antillarum*
P1_3_198	DP0756	*Mastigoteuthis agassizii*
P1_3_199	DP0046X	*Mastigoteuthis agassizii*
P1_3_200	DP0045X	*Pyroteuthis margaritifera*
P1_3_201	DP0430	*Ornithoteuthis antillarum*
P1_3_202	DP0441	*Pyroteuthis margaritifera*
P1_3_203	DP0742	*Vampyroteuthis infernalis*
P1_3_204	DP0741	*Mastigoteuthis agassizii*
P1_3_205	DP0750	*Pyroteuthis margaritifera*
P1_3_206	DP0743	*Vampyroteuthis infernalis*
P1_3_207	DP2120	*Vampyroteuthis infernalis*
P1_3_208	DP0044X	*Pyroteuthis margaritifera*
P1_3_209	DP2093	*Mastigoteuthis agassizii*
P1_3_210	DP0051X	*Cranchia scabra*
P1_3_211	DP0052X	*Cranchia scabra*
P1_3_212	DP0053X	*Pyroteuthis margaritifera*

Sequence Label	Jar Label	Species
P1_3_213	DP0016	*Vampyroteuthis infernalis*
P1_3_214	DP0018	*Mastigoteuthis agassizii*
P1_3_215	DP2028	*Mastigoteuthis agassizii*
P1_3_216	DP0002X	*Cranchia scabra*
P1_3_217	DP0003X	*Cranchia scabra*
P1_3_218	DP0004X	*Cranchia scabra*
P1_3_219	DP0005X	*Cranchia scabra*
P1_3_220	DP0054X	*Pyroteuthis margaritifera*
P1_3_221	DP0006X	*Cranchia scabra*
P1_3_222	DP0007X	*Cranchia scabra*
P1_3_223	DP0008X	*Cranchia scabra*
P1_3_224	DP0009X	*Pterygioteuthis gemmata*
P1_3_225	DP0010X	*Cranchia scabra*
P1_3_226	DP0053	*Cranchia scabra*
P1_3_227	DP0055X	*Vampyroteuthis infernalis*
P1_3_228	DP0056X	*Cranchia scabra*
P1_3_229	DP0058X	*Cranchia scabra*
P1_3_230	DP0059X	*Cranchia scabra*
P1_3_231	DP0031	*Cranchia scabra*
P1_3_232	DP0060X	*Cranchia scabra*
P1_3_233	DP0061X	*Cranchia scabra*
P1_3_234	DP0062X	*Cranchia scabra*
P1_3_235	DP0072X	*Cranchia scabra*
P1_3_236	DP0063X	*Cranchia scabra*
P1_3_237	DP0064X	*Cranchia scabra*
P1_3_238	DP0065X	*Cranchia scabra*
P1_3_239	DP0066X	*Cranchia scabra*
P1_3_240	DP0067X	*Pterygioteuthis gemmata*
P1_3_241	DP0068X	*Pterygioteuthis gemmata*
P1_3_242	DP0069X	*Pyroteuthis margaritifera*
P1_3_243	DP0070X	*Pterygioteuthis gemmata*
P1_3_244	DP0071X	*Pyroteuthis margaritifera*
P1_3_245	DP0049X	*Vampyroteuthis infernalis*
P1_3_246	DP0744	*Vampyroteuthis infernalis*
P1_3_247	DP0048X	*Vampyroteuthis infernalis*

Sequence Label	Jar Label	Species
P1_3_249	DP0755	*Ornithoteuthis antillarum*
P1_3_250	DP0756	*Mastigoteuthis agassizii*
P1_3_251	DP0046X	*Mastigoteuthis agassizii*
P1_3_252	DP0045X	*Pyroteuthis margaritifera*
P1_3_253	DP0430	*Ornithoteuthis antillarum*
P1_3_254	DP0441	*Pyroteuthis margaritifera*
P1_3_256	DP0741	*Mastigoteuthis agassizii*
P1_3_257	DP0750	*Pyroteuthis margaritifera*
P1_3_259	DP2120	*Vampyroteuthis infernalis*
P1_3_260	DP0044X	*Pyroteuthis margaritifera*
P1_3_261	DP2093	*Mastigoteuthis agassizii*
P1_3_262	DP0051X	*Cranchia scabra*
P1_3_263	DP0052X	*Cranchia scabra*
P1_3_264	DP0053X	*Pyroteuthis margaritifera*
P1_3_265	DP0016	*Vampyroteuthis infernalis*
P1_3_266	DP0018	*Mastigoteuthis agassizii*
P1_3_267	DP2028	*Mastigoteuthis agassizii*
P1_3_268	DP0002X	*Cranchia scabra*
P1_3_269	DP0003X	*Cranchia scabra*
P1_3_270	DP0004X	*Cranchia scabra*
P1_3_271	DP0005X	*Cranchia scabra*
P1_3_272	DP0054X	*Pyroteuthis margaritifera*
P1_3_273	DP0006X	*Cranchia scabra*
P1_3_274	DP0007X	*Cranchia scabra*
P1_3_275	DP0008X	*Cranchia scabra*
P1_3_276	DP0009X	*Pterygioteuthis gemmata*
P1_3_277	DP0010X	*Cranchia scabra*
P1_3_278	DP0053	*Cranchia scabra*
P1_3_279	DP0055X	*Vampyroteuthis infernalis*
P1_3_280	DP0056X	*Cranchia scabra*
P1_3_281	DP0058X	*Cranchia scabra*
P1_3_283	DP0031	*Cranchia scabra*
P1_3_284	DP0060X	*Cranchia scabra*
P1_3_286	DP0062X	*Cranchia scabra*
P1_3_287	DP0072X	*Cranchia scabra*

Sequence Label	Jar Label	Species
P1_3_288	DP0063X	*Cranchia scabra*
P2_1_289	P14-069	*Cranchia scabra*
P2_1_290	P14-070	*Cranchia scabra*
P2_1_291	P14-140	*Cranchia scabra*
P2_1_292	P14-143	*Cranchia scabra*
P2_1_293	P14-144	*Cranchia scabra*
P2_1_294	P14-146	*Cranchia scabra*
P2_1_295	P14-150	*Cranchia scabra*
P2_1_296	P14-152	*Cranchia scabra*
P2_1_297	P14-295	*Cranchia scabra*
P2_1_298	P14-298	*Cranchia scabra*
P2_1_299	P14-299	*Cranchia scabra*
P2_1_300	P14-074	*Pyroteuthis margaritifera*
P2_1_301	P14-075	*Pyroteuthis margaritifera*
P2_1_302	P14-137	*Pyroteuthis margaritifera*
P2_1_303	P14-138	*Pyroteuthis margaritifera*
P2_1_304	P14-151	*Pyroteuthis margaritifera*
P2_1_305	P14-276	*Pyroteuthis margaritifera*
P2_1_306	P14-278	*Pyroteuthis margaritifera*
P2_1_307	P14-280	*Pyroteuthis margaritifera*
P2_1_308	P14-282	*Pyroteuthis margaritifera*
P2_1_309	P14-287	*Pyroteuthis margaritifera*
P2_1_310	P14-288	*Pyroteuthis margaritifera*
P2_1_311	P14-289	*Pyroteuthis margaritifera*
P2_1_312	P14-071	*Vampyroteuthis infernalis*
P2_1_313	P14-141	*Vampyroteuthis infernalis*
P2_1_314	P14-275	*Vampyroteuthis infernalis*
P2_1_315	P14-286	*Vampyroteuthis infernalis*
P2_1_316	P14-296	*Vampyroteuthis infernalis*
P2_1_317	DP0057X	*Cranchia scabra*
P2_1_319	DP9033	*Ornithoteuthis antillarum*
P2_1_320	DP9044	*Vampyroteuthis infernalis*
P2_1_321	DP9060	*Cranchia scabra*
P2_1_322	DP9071	*Vampyroteuthis infernalis*
P2_1_323	DP9078	*Vampyroteuthis infernalis*

Sequence Label	Jar Label	Species
P2_1_324	DP9131	*Mastigoteuthis agassizii*
P2_1_325	DP9139	*Bolitaena pygmaea*
P2_1_326	DP9167	*Vampyroteuthis infernalis*
P2_1_327	DP9181	*Pyroteuthis margaritifera*
P2_1_328	DP9184	*Vampyroteuthis infernalis*
P2_1_329	DP9194	*Vampyroteuthis infernalis*
P2_1_330	DP9199	*Bolitaena pygmaea*
P2_1_331	DP9211	*Japetella diaphana*
P2_1_332	DP9035	*Vampyroteuthis infernalis*
P2_1_333	DP9052	*Cranchia scabra*
P2_1_334	DP9064	*Vampyroteuthis infernalis*
P2_1_335	DP9000	*Onychoteuthis cf. banksii*
P2_1_336	DP9028	*Japetella diaphana*
P2_1_337	DP9027	*Echinoteuthis atlantica*
P2_1_338	DP9039	*Helicocranchia pfefferi*
P2_1_339	DP9042	*Japetella diaphana*
P2_1_340	DP9055	*Mastigoteuthis agassizii*
P2_1_341	DP9061	*Octopoteuthis megaptera*
P2_1_342	DP9067	*Bolitaena pygmaea*
P2_1_343	DP9065	*Mastigoteuthis agassizii*
P2_1_344	DP9080	*Helicocranchia sp. A*
P2_1_345	DP9082	*Heteroteuthis dagamensis*
P2_1_346	DP9088	*Bolitaena pygmaea*
P2_1_347	DP9143	*Vampyroteuthis infernalis*
P2_1_349	DP2083	*Bolitaena pygmaea*
P2_1_350	DP9109	*Abraliopsis atlantica*
P2_1_351	DP9108	*Japetella diaphana*
P2_1_352	DP9110	*Ornithoteuthis antillarum*
P2_1_353	DP9123	*Heteroteuthis dagamensis*
P2_1_354	DP9153	*Teuthowenia megalops*
P2_1_355	DP9168	*Ancistrocheirus lesueurii*
P2_1_356	DP9178	*Helicocranchia pfefferi*
P2_1_357	DP9202	*Helicocranchia sp. A*
P2_1_358	DP9212	*Japetella diaphana*
P2_1_359	DP9218	*Helicocranchia sp. A*

Sequence Label	Jar Label	Species
P2_1_360	DP9225	*Asperoteuthis acanthoderma*
P2_1_361	DP9230	*Cycloteuthis sirventi*
P2_1_363	DP9245	*Chiroteuthis mega*
P2_1_364	DP9248	*Planctoteuthis levimana*
P2_1_365	DP9262	*Helicocranchia pfefferi*
P2_1_366	DP9021	*Cranchia scabra*
P2_1_367	DP9033	*Ornithoteuthis antillarum*
P2_1_368	DP9044	*Vampyroteuthis infernalis*
P2_1_369	DP9060	*Cranchia scabra*
P2_1_370	DP9071	*Vampyroteuthis infernalis*
P2_1_372	DP9131	*Mastigoteuthis agassizii*
P2_1_373	DP9139	*Bolitaena pygmaea*
P2_1_374	DP9167	*Vampyroteuthis infernalis*
P2_1_375	DP9181	*Pyroteuthis margaritifera*
P2_1_377	DP9194	*Vampyroteuthis infernalis*
P2_1_378	DP9199	*Bolitaena pygmaea*
P2_1_381	DP9052	*Cranchia scabra*
P2_1_383	DP9000	*Onychoteuthis cf. banksii*
P2_2_385	DP9027	*Echinoteuthis atlantica*
P2_2_386	DP9039	*Helicocranchia pfefferi*
P2_2_388	DP9055	*Mastigoteuthis agassizii*
P2_2_389	DP9061	*Octopoteuthis megaptera*
P2_2_390	DP9067	*Bolitaena pygmaea*
P2_2_391	DP9065	*Mastigoteuthis agassizii*
P2_2_392	DP9080	*Helicocranchia sp. A*
P2_2_393	DP9082	*Heteroteuthis dagamensis*
P2_2_394	DP9088	*Bolitaena pygmaea*
P2_2_397	DP2083	*Bolitaena pygmaea*
P2_2_398	DP9109	*Abraliopsis atlantica*
P2_2_399	DP9108	*Japetella diaphana*
P2_2_400	DP9110	*Ornithoteuthis antillarum*
P2_2_401	DP9123	*Heteroteuthis dagamensis*
P2_2_402	DP9153	*Teuthowenia megalops*
P2_2_403	DP9168	*Ancistrocheirus lesueurii*
P2_2_404	DP9178	*Helicocranchia pfefferi*

Sequence Label	Jar Label	Species
P2_2_405	DP9202	*Helicocranchia sp. A*
P2_2_407	DP9218	*Helicocranchia sp. A*
P2_2_408	DP9225	*Asperoteuthis acanthoderma*
P2_2_409	DP9230	*Cycloteuthis sirventi*
P2_2_411	DP9245	*Chiroteuthis mega*
P2_2_412	DP9248	*Planctoteuthis levimana*
P2_2_413	DP9262	*Helicocranchia pfefferi*
P2_2_414	DP9021	*Cranchia scabra*
P2_2_415	P14-069	*Cranchia scabra*
P2_2_416	P14-070	*Cranchia scabra*
P2_2_417	P14-140	*Cranchia scabra*
P2_2_418	P14-143	*Cranchia scabra*
P2_2_420	P14-146	*Cranchia scabra*
P2_2_422	P14-152	*Cranchia scabra*
P2_2_424	P14-298	*Cranchia scabra*
P2_2_425	P14-299	*Cranchia scabra*
P2_2_426	P14-074	*Pyroteuthis margaritifera*
P2_2_427	P14-075	*Pyroteuthis margaritifera*
P2_2_428	P14-137	*Pyroteuthis margaritifera*
P2_2_429	P14-138	*Pyroteuthis margaritifera*
P2_2_430	P14-151	*Pyroteuthis margaritifera*
P2_2_431	P14-276	*Pyroteuthis margaritifera*
P2_2_432	P14-278	*Pyroteuthis margaritifera*
P2_2_433	P14-280	*Pyroteuthis margaritifera*
P2_2_434	P14-282	*Pyroteuthis margaritifera*
P2_2_435	P14-287	*Pyroteuthis margaritifera*
P2_2_436	P14-288	*Pyroteuthis margaritifera*
P2_2_437	P14-289	*Pyroteuthis margaritifera*
P2_2_438	P14-071	*Vampyroteuthis infernalis*
P2_2_439	P14-141	*Vampyroteuthis infernalis*
P2_2_440	P14-275	*Vampyroteuthis infernalis*
P2_2_441	P14-286	*Vampyroteuthis infernalis*
P2_2_442	P14-296	*Vampyroteuthis infernalis*
P2_2_444	DP0057X	*Cranchia scabra*
P2_2_449	DP0037X	*Japetella diaphana*

Sequence Label	Jar Label	Species
P2_2_454	DP0024X	*Japetella diaphana*
P2_2_455	DP0024X	*Japetella diaphana*
P3_1_481	DP0768	*Cranchia scabra*
P3_1_482	DP0783	*Pyroteuthis margaritifera*
P3_1_483	DP1994	*Vampyroteuthis infernalis*
P3_1_484	DP0011X	*Cranchia scabra*
P3_1_485	DP2087	*Vampyroteuthis infernalis*
P3_1_486	DP0012X	*Pyroteuthis margaritifera*
P3_1_487	DP0013X	*Vampyroteuthis infernalis*
P3_1_488	DP0014X	*Vampyroteuthis infernalis*
P3_1_489	DP0792	*Cranchia scabra*
P3_1_490	DP2084	*Pyroteuthis margaritifera*
P3_1_491	DP0015X	*Pyroteuthis margaritifera*
P3_1_492	DP0736	*Cranchia scabra*
P3_1_493	DP1993	*Pyroteuthis margaritifera*
P3_1_494	DP0017X	*Pyroteuthis margaritifera*
P3_1_495	DP0018X	*Cranchia scabra*
P3_1_496	DP0019X	*Pyroteuthis margaritifera*
P3_1_497	DP0049X	*Vampyroteuthis infernalis*
P3_1_498	DP0744	*Vampyroteuthis infernalis*
P3_1_499	DP0048X	*Vampyroteuthis infernalis*
P3_1_500	DP0045X	*Pyroteuthis margaritifera*
P3_1_501	DP0441	*Vampyroteuthis infernalis*
P3_1_502	DP0750	*Pyroteuthis margaritifera*
P3_1_504	DP0044X	*Pyroteuthis margaritifera*
P3_1_506	DP9044	*Cranchia scabra*
P3_1_507	DP9071	*Vampyroteuthis infernalis*
P3_1_508	DP9078	*Vampyroteuthis infernalis*
P3_1_510	DP9181	*Pyroteuthis margaritifera*
P3_1_513	DP9035	*Vampyroteuthis infernalis*
P3_1_514	DP9052	*Cranchia scabra*
P3_1_515	DP9064	*Vampyroteuthis infernalis*
P3_1_516	DP9143	*Vampyroteuthis infernalis*
P3_1_517	DP9021	*Cranchia scabra*
P3_1_518	DP0051X	*Cranchia scabra*

Sequence Label	Jar Label	Species
P3_1_519	DP0052X	*Cranchia scabra*
P3_1_520	DP0053X	*Pyroteuthis margaritifera*
P3_1_521	DP0016	*Vampyroteuthis infernalis*
P3_1_522	DP0002X	*Cranchia scabra*
P3_1_523	DP0003X	*Cranchia scabra*
P3_1_524	DP0004X	*Cranchia scabra*
P3_1_525	DP0005X	*Cranchia scabra*
P3_1_526	DP0054X	*Pyroteuthis margaritifera*
P3_1_527	DP0006X	*Cranchia scabra*
P3_1_529	DP0009X	*Pterygioteuthis sp.*
P3_1_530	DP0010X	*Cranchia scabra*
P3_1_531	DP0053	*Cranchia scabra*
P3_1_532	DP0055X	*Vampyroteuthis infernalis*
P3_1_533	DP0056X	*Cranchia scabra*
P3_1_535	DP0058X	*Cranchia scabra*
P3_1_536	DP0059X	*Cranchia scabra*
P3_1_537	DP0031	*Cranchia scabra*
P3_1_538	DP0060X	*Cranchia scabra*
P3_1_539	DP0061X	*Cranchia scabra*
P3_1_540	DP0062X	*Cranchia scabra*
P3_1_541	DP0063X	*Cranchia scabra*
P3_1_542	DP0065X	*Cranchia scabra*
P3_1_543	DP0066X	*Cranchia scabra*
P3_1_544	DP0067X	*Pterygioteuthis sp.*
P3_1_545	DP0068X	*Pterygioteuthis sp.*
P3_1_546	DP0069X	*Pyroteuthis margaritifera*
P3_1_547	DP0070X	*Pterygioteuthis sp.*
P3_1_548	DP0071X	*Pyroteuthis margaritifera*
P3_1_549	P14-069	*Cranchia scabra*
P3_1_550	P14-070	*Cranchia scabra*
P3_1_551	P14-140	*Cranchia scabra*
P3_1_552	P14-143	*Cranchia scabra*
P3_1_553	P14-144	*Cranchia scabra*
P3_1_554	P14-146	*Cranchia scabra*
P3_1_555	P14-150	*Cranchia scabra*

Sequence Label	Jar Label	Species
P3_1_556	P14-152	*Cranchia scabra*
P3_1_557	P14-295	*Cranchia scabra*
P3_1_558	P14-298	*Cranchia scabra*
P3_1_559	P14-299	*Cranchia scabra*
P3_1_560	P14-074	*Pyroteuthis margaritifera*
P3_1_561	P14-075	*Pyroteuthis margaritifera*
P3_1_562	P14-137	*Pyroteuthis margaritifera*
P3_1_563	P14-138	*Pyroteuthis margaritifera*
P3_1_564	P14-151	*Pyroteuthis margaritifera*
P3_1_565	P14-276	*Pyroteuthis margaritifera*
P3_1_567	P14-280	*Pyroteuthis margaritifera*
P3_1_568	P14-282	*Pyroteuthis margaritifera*
P3_1_569	P14-287	*Pyroteuthis margaritifera*
P3_1_570	P14-288	*Pyroteuthis margaritifera*
P3_1_571	P14-289	*Pyroteuthis margaritifera*
P3_1_573	P14-141	*Vampyroteuthis infernalis*
P3_1_574	P14-275	*Vampyroteuthis infernalis*
P3_1_575	P14-286	*Vampyroteuthis infernalis*
P3_1_576	P14-296	*Vampyroteuthis infernalis*
P5_1_673	DP2746	*Asperoteuthis acanthoderma*
P5_1_674	DP2637	*Bolitaena pygmaea*
P5_1_675	DP2657	*Bolitaena pygmaea*
P5_1_676	DP2737	*Japetella diaphana*
P5_1_677	DP2662	*Japetella diaphana*
P5_1_678	DP2703	*Discoteuthis discus*
P5_1_679	DP1991	*Cycloteuthis sirventi*
P5_1_680	DP2713	*Helicocranchia sp. A*
P5_1_681	DP2727	*Helicocranchia sp. A*
P5_1_682	DP0073X	*Helicocranchia pfefferi*
P5_1_683	DP2685	*Japetella diaphana*
P5_1_684	DP2103	*Japetella diaphana*
P5_1_685	DP2683	*Leachia atlantica*
P5_1_686	DP1966	*Narrowteuthis nesisi*
P5_1_687	DP2097	*Taningia danae*
P5_1_688	DP2640	*Cycloteuthis sirventi*

Sequence Label	Jar Label	Species
P5_1_690	DP0043X	*Cycloteuthis sirventi*
P5_1_691	DP2099	*Vampyroteuthis infernalis*
P5_1_692	DP1962	*Vampyroteuthis infernalis*
P5_1_693	DP2122	*Vampyroteuthis infernalis*
P5_1_694	DP2098	*Vampyroteuthis infernalis*
P5_1_695	DP2700	*Vampyroteuthis infernalis*
P5_1_696	DP2678	*Cranchia scabra*
P5_1_697	DP1964	*Cranchia scabra*
P5_1_698	DP0074X	*Pyroteuthis margaritifera*
P5_1_699	DP0765	*Cycloteuthis sirventi*
P5_1_702	DP2746	*Asperoteuthis acanthoderma*
P5_1_703	DP2637	*Bolitaena pygmaea*
P5_1_704	DP2657	*Bolitaena pygmaea*
P5_1_705	DP2737	*Japetella diaphana*
P5_1_707	DP2703	*Discoteuthis discus*
P5_1_708	DP1991	*Cycloteuthis sirventi*
P5_1_709	DP2713	*Helicocranchia sp. A*
P5_1_710	DP2727	*Helicocranchia sp. A*
P5_1_711	DP0073X	*Helicocranchia pfefferi*
P5_1_713	DP2103	*Japetella diaphana*
P5_1_714	DP2683	*Leachia atlantica*
P5_1_715	DP1966	*Narrowteuthis nesisi*
P5_1_716	DP2097	*Taningia danae*
P5_1_717	DP2640	*Cycloteuthis sirventi*
P5_1_719	DP0043X	*Cycloteuthis sirventi*
P5_1_720	DP2099	*Vampyroteuthis infernalis*
P5_1_721	DP1962	*Vampyroteuthis infernalis*
P5_1_722	DP2122	*Vampyroteuthis infernalis*
P5_1_723	DP2098	*Vampyroteuthis infernalis*
P5_1_724	DP2700	*Vampyroteuthis infernalis*
P5_1_725	DP2678	*Cranchia scabra*
P5_1_726	DP1964	*Cranchia scabra*
P5_1_727	DP0074X	*Pyroteuthis margaritifera*
P5_1_728	DP20895	*Helicocranchia sp. A*
P5_1_729	DP0787	*Pterygioteuthis gemmata*

Sequence Label	Jar Label	Species
P5_1_730	DP0067X	*Pterygioteuthis gemmata*
P5_1_731	DP2099	*Vampyroteuthis infernalis*
P5_1_733	DP2122	*Vampyroteuthis infernalis*
P5_1_734	DP2098	*Vampyroteuthis infernalis*
P5_1_735	DP2700	*Vampyroteuthis infernalis*
P5_1_736	DP2678	*Cranchia scabra*
P5_1_737	DP1964	*Cranchia scabra*
P5_1_738	DP2747	*Pyroteuthis margaritifera*
P5_1_743	DP9194	*Vampyroteuthis infernalis*
P5_1_748	P14-295	*Cranchia scabra*
P5_1_758	DP9035	*Vampyroteuthis infernalis*