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## A Molecular Phylogeny of the Echeneoidea (Perciformes: Carangoidei) and an Investigation of Population Structuring Within the Echeneidae

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A MOLECULAR PHYLOGENY OF THE ECHENEIOIDEA  
(PERCIFORMES: CARANGOIDEI)  
AND  
AN INVESTIGATION OF POPULATION STRUCTURING  
WITHIN THE ECHENEIDAE

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A Thesis  
Presented to  
The Faculty of the School of Marine Science  
The College of William and Mary

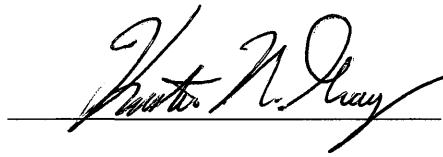
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Of the Requirements for the Degree of  
Master of Science

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by  
Kurtis N. Gray  
2005

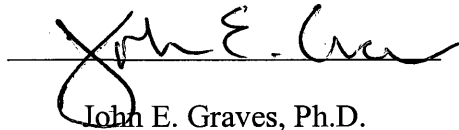
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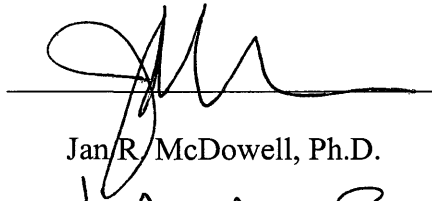


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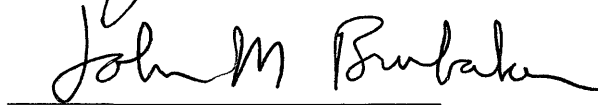
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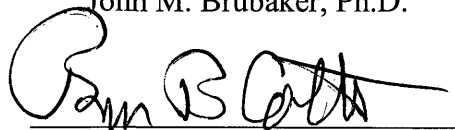
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## TABLE OF CONTENTS

ACKNOWLEDGMENTS .....	v
LIST OF TABLES .....	vii
LIST OF FIGURES .....	ix
LIST OF APPENDICES .....	xii
ABSTRACT .....	xiv
GENERAL INTRODUCTION .....	2
Definitions .....	2
An Introduction to the Echeneoidea .....	4
Objectives .....	6
CHAPTER ONE. A MOLECULAR PHYLOGENY OF THE SUPERFAMILY ECHENEOIDEA (PERCIFORMES: CARANGOIDEI) INFERRED FROM MITOCHONDRIAL 12S rRNA, 16S rRNA, ND2, AND NUCLEAR ITS-1 GENE REGION SEQUENCE ANALYSES .....	8
INTRODUCTION .....	8
Taxonomic Classification .....	9
Utility of Molecular Markers .....	12
Genome Comparisons .....	13
Molecular Marker Selection .....	15
Objectives .....	16
MATERIALS AND METHODS .....	17
Sample Collection .....	17
DNA Extraction and PCR Amplification .....	18
DNA Sequencing and Sequence Analyses .....	21
Data Set Partitioning .....	23
Phylogenetic Analyses .....	24
RESULTS .....	26
Sequence Variation .....	26
Partition Homogeneity Analyses .....	30
Phylogenetic Relationships – Combined .....	30
Phylogenetic Relationships - Individual Gene Regions .....	32
DISCUSSION .....	35



Echeneoid Phylogenetics. . . . .	35
Unresolved Relationships. . . . .	37
Discrepancies within <i>Echeneis</i> . . . . .	38
Importance of Multiple Genes. . . . .	40

CHAPTER TWO. AN INVESTIGATION OF POPULATION STRUCTURING  
 WITHIN THE FAMILY ECHENEIDAE (PERCIFORMES: CARANGOIDEI)  
 INFERRED FROM MITOCHONDRIAL CONTROL REGION DNA

SEQUENCE ANALYSES. . . . .	63
INTRODUCTION. . . . .	63
Host-Symbiont Behavior. . . . .	64
Utility of Molecular Markers . . . . .	67
Objectives. . . . .	69
MATERIALS AND METHODS. . . . .	71
Sample Collection . . . . .	71
DNA Extraction and PCR Amplification . . . . .	72
DNA Sequencing and Sequence Analyses . . . . .	73
Population Genetic Analyses. . . . .	74
RESULTS. . . . .	77
Sequence Variation. . . . .	77
Genetic Diversity. . . . .	78
Clade Distribution . . . . .	78
Phylogeography and Population Structuring. . . . .	80
Neutrality and Population Demography . . . . .	81
Cladogenesis and Putative Recolonization. . . . .	82
DISCUSSION . . . . .	83
Sequence Variation . . . . .	83
Genetic Diversity . . . . .	84
Clade Distribution. . . . .	85
Phylogeography and Population Structuring. . . . .	88
Cladogenesis and Putative Recolonization. . . . .	92
Neutrality and Population Demography. . . . .	93
Host-symbiont Phylogeography. . . . .	94
Sampling Concerns . . . . .	98
CONCLUSIONS. . . . .	101
APPENDICES . . . . .	123
LITERATURE CITED . . . . .	161
VITA . . . . .	176

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## LIST OF TABLES

Table	Page
1. PCR and cycle sequencing primer sequences. . . . .	43
2. Nucleotide sequence variation within mitochondrial 12S rRNA, 16S rRNA and ND2 gene regions, and the nuclear ITS-1 region in the Echeneoidea. . . . .	44
3. Summary statistics of parsimony and likelihood analyses. . . . .	45
4. Echeneid host-symbiont occurrence records. . . . .	103
5. Mitochondrial DNA diversity estimates within collections of marlinsucker . . .	104
6. Matrix of corrected average nucleotide sequence divergence ( $\delta$ ) and associated p-values among collections of marlinsucker. . . . .	105
7. Distribution of Clade I and Clade II mitochondrial haplotypes among seven geographic sampling locations. . . . .	106
8. Matrix of pairwise $\Phi_{st}$ values and associated p-values among collections of marlinsucker. . . . .	107
9. Analyses of molecular variance (AMOVA) among collections of marlinsucker. . . . .	109
10. Matrix of pairwise $\Phi_{st}$ values and associated p-values among Atlantic collections of marlinsucker. Haplotypes binned by istiophorid host. . . . .	111

11. Analyses of molecular variance (AMOVA) among Atlantic collections of marlinsucker. Haplotypes binned by istiophorid host. . . . .	112
12. Neutrality and mismatch distribution statistics estimated within collections of marlinsucker. . . . .	113
13. Divergence and recolonization estimates inferred from coalescence and nucleotide divergence based analyses. . . . .	114

## LIST OF FIGURES

Figure	Page
1. Incongruent hypotheses of the taxonomic relationships within the Echeneoidea. . . . .	46
2. Locations of PCR primers used to amplify DNA sequences from the mitochondrial 12S rRNA, 16S rRNA, and ND2 gene regions and nuclear ITS-1 gene region. . . . .	47
3. Nucleotide substitution patterns observed in pairwise comparisons of complete 12S rRNA gene region sequences. . . . .	48
4. Nucleotide substitution patterns observed in pairwise comparisons of partial 16S rRNA gene region sequences. . . . .	49
5. Nucleotide substitution patterns observed in pairwise comparisons of complete ND2 gene region sequences. . . . .	50
6. Nucleotide substitution patterns observed in pairwise comparisons of complete ITS-1 gene region sequences. . . . .	52
7. Phylogenetic estimates of the relationships within the Echeneoidea, based upon analyses of 3213 bp of concatenated mitochondrial 12S rRNA, 16S rRNA, and ND2 gene region sequence data. . . . .	53
8. Phylogenetic estimates of the relationships within the Echeneoidea, based upon analyses of 4223bp of concatenated mitochondrial 12S rRNA,	

16S rRNA, ND2, and nuclear ITS-1 gene region sequence data. . . . .	56
9. Phylogenetic estimates of the relationships within the Echeneoidea, based upon analyses of 916bp of 12S rRNA gene region sequence data. . . . .	59
10. Phylogenetic estimates of the relationships within the Echeneoidea, based upon analyses of 1599bp of 16S rRNA gene region sequence data. . . . .	60
11. Phylogenetic estimates of the relationships within the Echeneoidea, based upon analyses of 698bp of ND2 gene region sequence data. . . . .	61
12. Phylogenetic estimates of the relationships within the Echeneoidea, based upon analyses of 1010bp of ITS-1 gene region sequence data. . . . .	62
13. Patterns of host association between marlinsucker, <i>R. osteochir</i> and istiophorid billfishes, N = 580 records. . . . .	115
14. Geographic sampling locations . . . . .	116
15. Sequence variation across 971 aligned base pairs of the complete mitochondrial control region. . . . .	117
16. UPGMA trees of complete mitochondrial control region DNA sequences with annotated geographic distribution patterns. . . . .	118
17. Graphical representation of the distribution of Clade I and Clade II mitochondrial haplotypes among seven geographic sampling locations. . . . .	119
18. UPGMA trees of complete mitochondrial control region DNA sequences with annotated host association patterns. . . . .	120

19. Mismatch distributions observed in pairwise comparisons of complete  
mitochondrial control region DNA sequences. . . . .121



## LIST OF APPENDICES

Appendix	Page
1. Sample collection and voucher information. . . . .	124
2. Pairwise nucleotide sequence divergence estimates between echeneoid specimens based upon complete mitochondrial 12S rRNA DNA sequence data. . . . .	126
3. Pairwise nucleotide sequence divergence estimates between echeneoid specimens based upon partial mitochondrial 16S rRNA DNA sequence data. .	127
4. Pairwise nucleotide sequence divergence estimates between echeneoid specimens based upon complete mitochondrial ND2 DNA sequence data. . . .	128
5. Pairwise nucleotide sequence divergence estimates between echeneoid specimens based upon complete nuclear ITS-1 DNA sequence data. . . . .	129
6. Pairwise nucleotide sequence divergence estimates between specimens of marlinsucker, <i>R. osteochir</i> , based upon complete mitochondrial control region DNA sequence data. . . . .	130
7. Aligned mitochondrial 12S rRNA DNA sequence data collected from echeneoid specimens. . . . .	139
8. Aligned mitochondrial 16S rRNA DNA sequence data collected from echeneoid specimens. . . . .	141

9. Aligned mitochondrial ND2 DNA sequence data collected from echeneoid specimens. . . . .145

10. Aligned nuclear ITS-1 DNA sequence data collected from echeneoid specimens. . . . .148

11. Aligned mitochondrial control region DNA sequence data collected from marlinsucker specimens. . . . .151

## ABSTRACT

The Echeneoidea comprise three families of cosmopolitan tropical/subtropical marine fishes: the Echeneidae (remoras), Coryphaenidae (dolphin) and Rachycentridae (cobia). The present study addresses specific aspects of the systematic relationships within this superfamily using molecular evidence. Two separate projects were undertaken to address both interspecific and intraspecific relationships within the Echeneoidea. In chapter one of this thesis, complete nucleotide sequences from the mitochondrial 12S rRNA, 16S rRNA, protein-coding ND2, and nuclear ITS-1 gene regions were used to reconstruct the phylogenetic history of these fishes. Parsimony, likelihood and Bayesian analyses of combined data sets resolved trees of similar topology. Congruent with evolutionary hypotheses based upon larval morphology, a monophyletic Rachycentridae + Coryphaenidae was resolved with high support. Within a monophyletic Echeneidae, the subfamilies Echeneiinae and Remorinae were monophyletic. In agreement with recent morphological analyses, the genus *Remora* was paraphyletic based upon the position of *Remora albescens*. Consistent resolution within the Remorinae using parsimony, likelihood and Bayesian inference was not achieved with the markers used in this study.

In chapter two of this thesis, nucleotide sequences from the hypervariable mitochondrial control region were used to investigate phylogeographic structuring in the marlinsucker, *Remora osteochir*. Complete DNA sequences were isolated from 71 individuals collected from seven geographically distant sample collections (Atlantic, n=5, Pacific n=2). Analyses of molecular variance (AMOVA) and  $\Phi_{st}$  analyses resolved significant levels of population structuring among Atlantic and Pacific Ocean samples, whereas negligible levels of population structuring were resolved within both Atlantic and Pacific Ocean samples. Cluster analysis of haplotypes based upon inter-haplotype divergence resolved two distinct evolutionary lineages, one composed of Atlantic-only haplotypes, and one composed of both Atlantic and Pacific haplotypes. Observations are consistent with vicariant isolation of Atlantic and Pacific marlinsucker, followed by geologically recent secondary colonization of the Atlantic from the Indian Ocean. Congruence between patterns of genetic differentiation between marlinsucker and their istiophorid hosts, particularly blue marlin, *Makaira nigricans*, and sailfish, *Istiophorus platypterus*, highlights the possibility that symbiont phylogeography is ultimately governed by host dispersal. Alternatively, these findings indicate that these pelagic fishes have been influenced by similar vicariant events and processes in the distant past.

A MOLECULAR PHYLOGENY OF THE ECHENEIOIDEA  
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## GENERAL INTRODUCTION

### Definitions

Systematics is a broad discipline within the field of biology that aims to identify, describe and organize biological diversity. Traditionally, systematics has been divided into two areas of specialization: the field of phylogenetics (Greek: *phylon* = race, *genetic* = birth), which focuses on describing interspecific relationships, and population biology, which focuses on intraspecific relationships. Both areas of specialization aim to describe how living (or once living) organisms relate evolutionarily and ascertain how past and present processes have influenced evolution and speciation. For this reason, it is not surprising that systematics crosses into the fields of paleontology, comparative morphology, behavioral ecology, physiology, and most recently, molecular biology. Molecular systematics attempts to resolve evolutionary relationships through analyses of genetic variation. In the case of phylogenetics, evolutionary hypotheses at the organismal level are generated on the basis of gene phylogenies. Phylogenetic trees graphically represent hypothesized evolutionary relationships among extant and extinct life forms, based upon the degree of genetic similarity or dissimilarity. Population genetics aims to understand how the forces of mutation, migration, selection and genetic drift affect genetic variation (Hartl and Clark, 1997). Moreover, population genetics attempts to describe how

events and processes in the past and present affect evolutionary change (i.e. adaptation and speciation).

Systematic ichthyology aims to identify and describe fish biodiversity. Using available morphological, behavioral and molecular evidence, systematic ichthyologists construct evolutionary hypotheses to explain the relationships within and among the diverse array of fishes that exist today. With the aid of fossil evidence, this information can be framed against the backdrop of marine and freshwater fish lineages that became extinct tens or hundreds of millions of years ago. Systematic ichthyology spans the spectrum from purely academic investigations (e.g. identifying and cataloging fish biodiversity), to theoretic interpretations (e.g. understanding past processes and events that have given rise to the diversity of fishes seen today), to more applied studies (e.g. identification and conservation of threatened or endangered species). Phylogenetics within the field of systematic ichthyology has traditionally been based upon detailed investigation of morphological characters at all developmental stages (egg, larvae, juvenile and adult). Analyses of physiology and behavior have also been used to supplement hypotheses based upon these characters. Molecular phylogenetics is a relatively new field that has facilitated the reanalysis of existing hypotheses and the generation of novel theories to explain the evolutionary relationships between extinct and extant fishes. Population genetics involves quantification and analysis of present day genetic variation in target fish species. Variation can be interpreted in light of past processes, such as glaciation, changing ocean circulation patterns, seaway closures (e.g. closure of the Tethys Sea), or present day activities, such as overfishing and species introductions (both exotic and

rehabilitation-related). This study bridges the gap between phylogenetics and population biology. Molecular markers are used to answer systematic questions at the family-level down to the intrapopulation-level in the superfamily Echeneoidea.

### An Introduction to the Echeneoidea

The superfamily Echeneoidea includes the Echeneidae (remoras), Coryphaenidae (dolphins) and Rachycentridae (cobia). These three families comprise 11 extant marine teleostean species found in tropical and subtropical waters worldwide. The family Echeneidae contains four genera, with eight recognized species that inhabit open-ocean, coastal and reef environments. The members of this family bear a transversely laminated cephalic disc that is used to attach to a diverse group of hosts, and includes sharks, bony fishes, marine mammals, as well as inanimate objects (e.g. buoys). Possible benefits of this unique symbiotic behavior include protection from potential predators, access to food resources (ecto-parasites, food scraps), increased reproductive chances, and free transportation (lower energy expenditure) (O'Toole, 2002). Feeding strategies vary from species to species and between different developmental stages within a species. An assortment of marine invertebrates (amphipods, copepods, mollusks, cephalopods) and juvenile fishes form the majority of their diet (Strasburg, 1959; Cressey and Lachner, 1970). Overall, very little is known about the life history of these fishes. Physical description (both as larvae and adults), diet composition and host association patterns are the dominant focus of existing literature. The remoras are traditionally divided into two subfamilies (Lachner, 1981), the Echeneiinae and the Remorinae. In addition to a number of

morphological characters, members of these subfamilies differ in habitat preference and behavior. The Echeneiinae are most frequently found near coral reefs and exhibit only generalized host-association patterns: they do not appear to specialize, and are often seen free swimming. In contrast, the Remorinae are most frequently found in oceanic environments and exhibit a higher degree of host-specificity: they are moderate to highly specific in host choice and are rarely seen free-swimming (O'Toole, 2002).

The family Coryphaenidae consists of one genus with two cosmopolitan species found in oceanic and coastal waters: the common dolphin, *Coryphaena hippurus* (Linnaeus, 1758), and the pompano dolphin, *Coryphaena equiselis* (Linnaeus, 1758). The common dolphin (and to a lesser degree, the pompano dolphin) are prized commercial and recreational fishes worldwide (reviewed in Palko et al. 1982). Coryphaenids are commonly found in association with flotsam and sargassum, although not exclusively. They are highly migratory, and are known to spawn multiple times during the year (potentially year round in the tropics). Eggs and larvae are planktonic. These fishes are generalist feeders and have been reported to consume an assortment of pelagic fishes including flying fishes, juvenile tunas, mackerels and billfishes, as well as cephalopods and other marine invertebrates. Dolphin exhibit an extremely rapid growth rate and short lifespan ( $\leq 4$  years).

The monotypic family Rachycentridae consists of a single cosmopolitan species, the cobia, *Rachycentron canadum* (Linnaeus, 1766), that inhabits open-ocean, coastal and estuarine waters, with the exception of the central and eastern Pacific Ocean. Cobia are a valuable recreational fishing resource in the United States;



Australia, Africa and parts of the Caribbean (reviewed in Shaffer and Nakamura, 1989). Rachycentrids are frequently found in the proximity of stationary and free-floating structure (mangroves, pilings, submerged wrecks, reefs and buoys) and are known to associate with large marine vertebrates (bony fishes, sharks, rays, and turtles). These migratory pelagic fishes spawn in large aggregations, where they release pelagic eggs. They are voracious feeders, and are known to consume an array of crustaceans, cephalopods and demersal fishes. Cobia exhibit rapid growth rates and a moderate lifespan ( $\leq 15$  years).

### Objectives

In this thesis, I present the results of an investigation into a fairly focused area of ichthyology. Using molecular evidence, I address specific aspects of the systematic relationships within the superfamily Echeneoidea (remoras, dolphin, and cobia). To further our understanding of the evolutionary biology of these fishes, the genetic basis of both interspecific and intraspecific relationships was examined. In chapter one, molecular evidence was used to clarify taxonomic ambiguities within this superfamily. DNA sequence data collected from the mitochondrial and nuclear genome were used to test the contradictory phylogenetic hypotheses of Johnson (1984, 1993) and O'Toole (2002). In chapter two, molecular evidence was used to test the hypothesis that population genetic structure exists within the Echeneidae. Genetic variation among and within populations of marlinsucker, *Remora osteochir* (Cuvier, 1829), sampled from seven locations spread throughout their geographic range, was used to test for geographic homogeneity. Collectively, this information

builds upon existing knowledge in the areas of systematics, ecology and population biology of this unique group of marine fishes.

CHAPTER ONE. A MOLECULAR PHYLOGENY OF THE SUPERFAMILY  
ECHENEOIDEA (PERCIFORMES: CARANGOIDEI) INFERRED FROM  
MITOCHONDRIAL 12S rRNA, 16S rRNA, ND2, AND NUCLEAR ITS-1  
GENE REGION SEQUENCE ANALYSES.

## INTRODUCTION

### Taxonomic Classification

The superfamily Echeneoidea belongs to the most speciose group of extant vertebrates. Over 25,000 species of marine and freshwater fishes are currently recognized. The most dominant group are the jawed fishes (gnathostomes), which include the Osteichthyes (bony fishes, ca. 24,000 spp.), and the Chondrichthyes (cartilaginous fishes, ca. 850 spp.). The Agnatha includes approximately 85 species of jawless fishes. Within the Osteichthyes, the Echeneoidea fall within the order Perciformes (perch-like fishes), the largest of the orders within the Actinopterygii (ray-finned fishes). Of the 148 families that define the order Perciformes, the Echeneoidea comprise three: the Echeidae, Coryphaenidae and Rachycentridae (Nelson, 1994; Helfman et al. 2000).

On the basis of prenasal canal ossification and scale structure, the Echeneoidea has been grouped with the Carangidae (jacks, pompanos) and the Nematistiidae (roosterfish; Freihof, 1978). Collectively these five families define the suborder Carangoidei (Johnson, 1984). The carangids and echeneoids form a monophyletic group based upon three features: these fishes lack the bony stay posterior to the ultimate dorsal and anal pterygiophores, possess two prenasal canal units, and bear a lamellar expansion of the coracoid. Synapomorphies that unite the Rachycentridae, Coryphaenidae, and the Echeidae include the absence of predorsal

bones, an anterior shift of the first dorsal pterygiophore, presence of several anal pterygiophores anterior to the first haemal spine, loss of the beryciform foramen in the ceratohyal, tubular ossifications surrounding both prenasal canal units and elongate larvae with late dorsal fin completion (Johnson, 1984).

Placement of the Echeneoidea within the Carangoidei is uncontested. Despite a number of morphological investigations (Freihofner, 1978; Johnson, 1984, 1993; Ditty and Shaw, 1992; Ditty et al. 1993; O'Toole, 2002), the phylogenetic relationships of the species within the Echeneoidea remain unresolved. Based upon larval characters relating to neurocranial development, head spination, mandibular structure and epithelial cell composition, Johnson (1984) hypothesized a rachycentrid-coryphaenid sister group relationship (Figure 1). This hypothesis was supported by the work of Ditty and Shaw (1992) and Ditty et al. (1993), in an examination of larval development in cobia and dolphin, respectively. O'Toole (2002) recently published a phylogeny based on 138 putatively informative osteological characters that is inconsistent with this hypothesis (Figure 1). Specifically, in O'Toole's phylogeny, the Coryphaenidae were placed as a sister group to the Rachycentridae-Echeneidae clade. In addition, the phylogeny did not support the subfamilies Echeneiinae and Remorinae, or the monophyly of the genus *Remora*. O'Toole (2002) supports his phylogeny with behavioral characters concerning the development of the symbiotic "hitchhiking" association behavior and the degree of host specialization within the Echeneidae. O'Toole describes a progression from general schooling behavior to close association with floating objects (demonstrated by the coryphaenids), which progresses further to following behavior (exhibited by

*Rachycentron*), and finally, direct host attachment via a modified dorsal fin (demonstrated by the echeneids). Within the Echeneidae, O'Toole suggests a progression from coral-reef associating generalist symbiont (*Phtheichthys*, *Echeneis*) to pelagic generalist (*R. brachyptera*, Lowe 1839) to pelagic specialist (*R. remora*, Linnaeus 1758) and finally, pelagic obligate (*R. osteochir*, *R. australis*, Bennet 1840, and *R. albescens*, Temminck and Schlegel 1850).

Family level relationships with the Echeneoidea have also been addressed using molecular evidence. In an analysis of alpha-level taxonomy within the Carangidae, Reed et al. (2002) hypothesized a coryphaenid-rachycentrid sister-group relationship. Analyses of mitochondrial cytochrome *b* nucleotide sequences resolved *C. hippurus* and *R. canadum* as a monophyletic outgroup to the Carangidae using parsimony, likelihood and Bayesian inference methods. Placement of the lone echeneid examined was problematic. *E. naucrates* (Linnaeus 1758) was alternately resolved within and as an outgroup-to the carangids studied, depending on optimality criterion used. Preliminary molecular evidence from Miya and Nishida (pers. comm.) supports the alternate hypothesis of a rachycentrid-echeneid sister group relationship. This relationship was hypothesized based upon analyses of whole mitochondrial genome sequences from 336 species of ray-finned fishes (Actinopterygii). The level of support for this finding, however, was unclear. Detailed species-level analyses were not conducted, as these goals were not within the scope of their study. To date, a comprehensive molecular investigation into the taxonomic relationships within the Echeneoidea has not been performed.

### Utility of Molecular Markers

Analyses of molecular markers provide a means to infer evolutionary history. DNA sequence analysis is one method whereby the genetic basis of both interspecific and intraspecific relationships can be addressed (Avice, 1994). Comparative analyses of DNA polymorphisms can provide insight into the genealogical relationships among taxa. Phylogenetic hypotheses generated using DNA sequence data can be used to supplement and/or evaluate hypotheses based upon morphological, physiological or behavior characters. Beginning in the late 1970s this methodology was in its infancy. The dominant molecular systematic methods of the time included allozyme electrophoresis and restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA (mtDNA) (Hillis et al. 1996; Avice, 1994). With the advent of modern molecular techniques including the polymerase chain reaction (PCR), the development of high fidelity enzymes, and advances in DNA amplification and analysis equipment, genetic analyses have become more streamlined, allowing large scale, rapid comparisons of genetic information. In the field of molecular systematics, it is now common to address taxonomic questions using multiple genes (both mitochondrial and nuclear-encoded), or even entire mitochondrial genomes (Inoue et al. 2001) in order to generate robust evolutionary hypotheses. This methodology has been successfully used to address ambiguities within the Actinopterygii at a number of taxonomic levels (Chen et al. 2003; Thacker, 2003; Zardoya and Doadrio, 1999; Inoue et al. 2001).

## Genome Comparisons

The mitochondrial genome is a closed-circular DNA molecule that is contained within the mitochondria, organelles within living cells that are involved in cellular respiration. This genome is a unique resource that can be exploited to address phylogenetic ambiguities such as those within the Echinozoa (reviewed in Avise, 1994; Hillis et al. 1996; Avise, 2001; Hallerman, 2003; Tsaousis et al. 2005). The mitochondrial genome is haploid, maternally inherited, and rarely exhibits recombination (crossing over). The mitochondrial genome is relatively small (14,000 – 42,000 base pairs in length) and encodes a diverse array of genes (13 proteins, 22 transfer RNAs, 2 ribosomal RNAs), as well as a hypervariable non-coding region. By size, mtDNA makes up approximately 0.0005% of the total genetic information vertebrates possess, but is present in such high copy number ( $\sim 10^2$ - $10^3$  per cell; Robin and Wong, 1988), that the relative abundance compared with nuclear DNA is on the order of 0.1%.

The mitochondrial genome is more responsive to evolutionary change than the nuclear genome. Because mtDNA is haploid and maternally inherited, the effective population size of the genome is approximately one-quarter that of nuclear DNA (Moore, 1995; Hallerman, 2003). As such, genetic divergence tends to accumulate more rapidly, assuming an equal rate of mutation, selection, drift and gene flow (Hallerman, 2003). The mitochondrial genome is considered a single genetic unit (Saccone, 1999; Avise, 1994; Stepien and Kocher, 1997), although the tRNAs, rRNAs and proteins for which it encodes are under different selection regimes. For this reason, an unequal rate of sequence evolution is noted across different gene



regions. The tRNA, 12S and 16S rRNA regions evolve at a substantially slower rate than the mitochondrial genome as a whole (2.0%; Brown, 1979), and have been estimated to evolve at a rate between 0.34 and 0.45% per million years (Pesole et al. 1999). Protein-encoding regions evolve at a moderate rate, and display a significant range in substitution rates between gene families (0.29-0.66% per million years, Zardoya and Meyer, 1996; 0.37-2.82% per million years, Zhang and Gu, 1998; 0.54-1.44% per million years; Ho et al. 2005; 1.0-2.5%; McMillan and Palumbi, 1996). The non-coding control region exhibits the highest rate of sequence evolution, and has been estimated to evolve at a rate 3 to 5 times that of the mitochondrial genome as a whole (Brown, 1993; Avise, 2001). In teleosts, substitution rates within hypervariable regions of the control region have been estimated to evolve at a rate approaching 38% per million years (McMillan and Palumbi, 1996).

The nuclear genome differs from the mitochondrial genome by a number of characteristics. In eukaryotes, organisms with true nuclei, the nuclear DNA (nDNA) is located within the nucleus, and is encoded on a number of separate chromosomes. In humans, the nuclear genome size is on the order of  $3 \times 10^9$  base pairs, which represents greater than 99.99% of the total genetic information an organism may possess, and encodes nearly 80,000 genes including tRNAs, rRNAs, and a diverse array of proteins and enzymes (Makalowski, 2001). In contrast with mtDNA, the nuclear genome is diploid, biparentally inherited, exhibits recombination (i.e. crossing over occurs), and contains multiple intervening DNA sequences (e.g. introns) (Avise, 1994; Hallerman, 2003). Furthermore, the nuclear genome is less sensitive to introgression, the spread of genes from one species to another as a result

of hybridization (Ballard and Whitlock, 2004). Overall, nDNA is considered to evolve at a slower rate than the mtDNA, although a range in sequence evolution rate is found between different regions (as found in mtDNA). To resolve ambiguities at different taxonomic levels, a diverse array of genes should be studied to provide a reasonable hypothesis of evolutionary history.

### Molecular Marker Selection

To generate an accurate hypothesis of evolutionary history using molecular evidence, it is wise to contrast taxonomic hypotheses generated using both mitochondrial and nuclear encoded genes. Agreement between nuclear and mitochondrial based phylogenies is ultimately desired. Given the genome differences noted above, agreement lends support to the hypothesized taxonomic relationships. Because taxonomic ambiguities exist at the family, genus and species levels within the Echineidae, genetic variation was evaluated using four gene regions (three mitochondrial and one nuclear) that exhibit differing rates of sequence evolution. Variation was examined in the relatively slowly evolving mitochondrial 12S and 16S ribosomal RNA (rRNA) gene regions, the moderately rapidly evolving protein-coding NADH-dehydrogenase subunit 2 (ND2) gene region, and the rapidly evolving nuclear-encoded internal transcribed spacer subunit 1 (ITS-1) gene region. These gene regions have been used successfully to estimate phylogenetic relationships in a number of taxa (Mattern, 2004; Thacker, 2004; Westneat and Alfaro, 2004; Broughton and Gold, 2002; Domanico et al. 1997). As a comparative index of support, these data were analyzed using three different inference methods (optimality

criteria): maximum parsimony (Felsenstein, 1983), maximum likelihood (Felsenstein, 1981), and Bayesian inference (Ronquist and Huelsenbeck, 2003).

### Objectives

In this study, complete nucleotide sequences from the mitochondrial 12S rRNA, 16S rRNA, protein-coding ND2, and nuclear ITS-1 gene regions were collected from extant members of the suborder Carangoidei. Gene-based phylogenies were generated using maximum parsimony, maximum likelihood and Bayesian inference methodologies. These phylogenies were used to test existing taxonomic hypotheses based upon larval morphology (Johnson, 1984,1993) and both adult osteology and behavior characters (O'Toole, 2002). Specifically, molecular evidence was used to address the following:

1. The monophyly of the superfamily Echeneoidea.
2. Family level sister-group relationships within the Echeneoidea.
3. The monophyly of the Echeneidae.
4. The monophyly of the subfamilies Echeneiinae and Remorinae.
5. The monophyly of the genus *Remora*.
6. Species-level relationships within the Echeneidae.

## MATERIALS AND METHODS

### Sample Collection

To address taxonomic ambiguities within the superfamily Echeneoidea, representatives from six families of marine fishes (families Pomatomidae, Nematistiidae, Carangidae, Coryphaenidae, Rachycentridae and Echeneidae) were collected. A single non-carangoid species, *Pomatomus saltatrix* (Linnaeus 1766) and two non-echeneoid, carangoid species, *Nematistius pectoralis* (Gill 1862) and *Carangoides armatus* (Rüppell, 1830), were used to root the phylogenetic comparisons performed. With the exception of *C. armatus*, all samples were procured from coastal and off-shore collections in the Atlantic and Pacific oceans. Molecular data from *C. armatus*, Genbank accession number AP004444, supplemented the outgroup taxa data set.

Collections were made between August, 2002 and July, 2005 using a host of academic, federal, commercial and recreational fishing resources. Samples were caught by hook and line individually or in association with their pelagic hosts (e.g. billfish, sharks, rays, dolphin, buoys). Upon capture, whole specimens were placed on ice or immediately frozen to prevent tissue breakdown. Tissue samples were stored in either DMSO tissue storage buffer (0.25 M disodium ethylenediamine-tetraacetic acid (EDTA), 20% dimethyl sulfoxide (DMSO), saturated sodium chloride (NaCl), pH 8.0) or 95% ethanol. Samples were identified using the keys of Lachner (1984)

and Collette (2003), photographed and processed in the VIMS Fisheries Genetics Laboratory. Voucher specimens are held at the Virginia Institute of Marine Science (VIMS), the National Museum of Natural History (USNM) and the Scripps Institution of Oceanography (SIO). Pertinent collection and voucher informationa are noted in Appendix 1.

#### DNA Extraction and PCR Amplification

Following the methods of Sambrook et al. (2001), total genomic DNA was extracted from 0.03-0.10g skeletal and/or heart muscle. Tissue was digested at 37°C over night with 15µl proteinase K (25mg/ml), 15µl RNase (10mg/ml), 60µl 10% sodium dodecyl sulfate (SDS) and 500µl isolation buffer (50mM EDTA, 50mM Tris, 150mM NaCl, ph 8.0). Genomic DNA was isolated through a series of washes with equilibrated phenol, phenol/chloroform/isoamyl alcohol (25:24:1) and chloroform/isoamyl alcohol (24:1). DNA was precipitated using an equal volume of isopropanol and 0.04x volume 5M NaCl and pelleted by high-speed centrifugation. DNA was washed with 70% EtOH to remove salts, lyophilized to remove trace EtOH and resuspended in 0.1X TE buffer, pH 8.0. Complete double-stranded nucleotide sequences from the mitochondrial 12S, 16S and ND2 gene regions and nuclear ITS-1 region were amplified following standard polymerase chain reaction (PCR) methodology using *Taq* PCR Core reagents (Qiagen Corp. Valencia, CA). Multiple primer sets were utilized to amplify gene sequences across all echeneoid samples (Table 1, Figure 2). Universal mitochondrial PCR primers were designed based upon consensus identity of published primer sequences (Palumbi, 1996; Broughton and

Gold, 2000) with gene sequences from carp, *Cyprinus carpio* (Sorenson et al. 1999), little tunny, *Euthynnus alletteratus* (AB099716) and sailfish, *Istiophorus platypterus* (McDowell, 2002). Superfamily-specific internal 16S rRNA primers were designed based upon consensus identity of echeneoid DNA sequences. Nuclear ITS amplifications were performed using primers designed by Johnson (2003). Primer locations are depicted graphically in Figure 2. Each 25 $\mu$ l PCR reaction contained the following: approximately 5-25ng purified gDNA, 2.5 $\mu$ l 10X PCR reaction buffer (Tris·Cl, KCl, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 15 mM MgCl<sub>2</sub>; pH 8.7), 0.5 $\mu$ l 10mM dNTP mix (dATP, dCTP, dGTP, dTTP, 10mM each), 0.125 $\mu$ l *Taq* DNA polymerase @ 5 units/ $\mu$ l, 0.5 $\mu$ l bovine serum albumin (BSA) @ 10mg/ml, 10pmoles of each primer. Negative (no DNA) control reactions were set up alongside experimental reaction mixtures to confirm that contamination via extraneous DNA did not occur. PCR amplification conditions consisted of an initial denaturation of 4 minutes at 94°C, followed by 35 cycles of 1 minute at 94°C, 1 minute at 50°C, and 1.5 minutes at 72°C, followed by a final extension of 5 minutes at 72°C (with minor exceptions). Alternate cycling conditions were utilized when the above conditions were unsuccessful, and consisted of a “touchdown” cycle defined by an initial denaturation of 4 minutes at 94°C, followed by 45 cycles of 1 minute at 94°C, 1 minute at 56°C (decreasing 2°C every 5 cycles), and 1.5 minutes at 72°C, followed by a final extension of 5 minutes at 72°C. All PCR amplifications were performed using an MJ Research PTC-200 thermocycler (Watertown, MA). Products were electrophoresed through an agarose gel matrix, stained with ethidium bromide and visualized using an ultraviolet-light transilluminator.

PCR products were either purified or cloned into a plasmid vector prior to DNA sequencing. Amplicon purification was performed via column filtration by using QIAquick PCR Purification reagents (Qiagen Corp., Valencia, CA), or by using EXOSAP (USB Scientific, Cleveland, OH) following manufacturer's specifications. Nuclear ITS-1 amplicons (and mitochondrial fragments that could not be successfully sequenced directly) were cloned using the TOPO-TA plasmid cloning system (Invitrogen Corp., San Diego, CA). Briefly, fresh PCR product was ligated into the TOPO 2.1 plasmid vector and transformed into competent TOP10 *Escherichia coli* bacterial cells. *E. coli* cells were grown overnight on nutrient rich Luria-Bertani (LB) agar plates containing ampicillin (@ 50ug/ml) and 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside (X-gal; 40µl @40mg/ml). Recombinant plasmids (white colonies) were selected for subsequent analysis and grown up overnight in 3ml Luria-Bertani liquid media containing ampicillin (@ 50ug/ml). Cloned fragments were isolated and purified using QIAprep Spin Miniprep reagents (Qiagen Corp., Valencia, CA) following the manufacturer's specifications. Recombinant plasmids were confirmed by *EcoRI* restriction endonuclease digestion. Purified plasmids were digested for a period of at least two hours @ 37°C, electrophoresed through an agarose gel matrix, stained with ethidium bromide and visualized with a UV transilluminator. Concentration of purified products was measured using a Dynaquant 200 fluorometer (Hofer, Inc. San Francisco, CA) or Biomate-3 UV spectrophotometer (Thermo Spectronic, Rochester, NY) prior to sequencing.

### DNA Sequencing and Sequence Analyses

Purified PCR products and recombinant plasmids were sequenced in forward and reverse directions following dideoxynucleotide chain termination sequencing methodology developed by Sanger et al. (1977). Samples were sequenced using either BigDye Terminator v3.1 Cycle Sequencing (Applied Biosystems, Warrington, UK) or Thermo Sequenase cycle sequencing reagents (Amersham Biosciences, Piscataway, NJ) with minor modifications of the manufacturer's recommendations. ABI sequencing reactions were composed of 10-50 ng template DNA, 0.25 $\mu$ l sequencing primer, 0.25 $\mu$ l BigDye master mix, 1 $\mu$ l 5x reaction mix and milli-q water to a final volume of 5 $\mu$ l. Cycle sequencing conditions consisted of an initial denaturation of 1 minute at 96°C, followed by 25 cycles of 10s at 96°C, 5s at 50°C, and 4 minutes at 60°C. Primers used for cycle sequencing were identical to primers used in original PCR amplification reactions. Thermo Sequenase reactions were composed of 25-50 ng template DNA, 1.5  $\mu$ l infrared-labeled (IR700 or IR800) M13F or M13R sequencing primer @ 1.0pmol/ $\mu$ l, and milli-q water to a final volume of 17 $\mu$ l. Master reaction mixtures were divided equally into four 0.2 $\mu$ l reaction tubes, mixed with 1 $\mu$ l of appropriate dideoxynucleotide terminator mix (ddA, ddC, ddG or ddT) and overlain with one drop of silicon oil to prevent condensation during cycling. Cycle sequencing conditions consisted of an initial denaturation of 5 minutes at 95°C, followed by 30 cycles of 30s at 92°C, 30s at 52°C, and 30s at 70°C (with minor exceptions).

Products amplified with the BigDye reagents were electrophoresed using an ABI 3100 or ABI 3130 DNA sequencer equipped with either a 50cm or 80cm



capillary loaded with POP7 or POP4 gel matrix, respectively. Results were analyzed using Sequencing Analysis v. 5.1.1 software (Applied Biosystems, Warrington, UK). Products amplified with the Thermo Sequenase reagents were electrophoresed on a Li-Cor Global IR2 System slab-gel DNA sequencer through a 3.7% polyacrylamide gel matrix. Results were analyzed using E-seq v2.0 software (Li-Cor Biosciences, Lincoln, NE). Standard chromatogram format (SCF) curves were exported for subsequent analyses. Consensus sequences from multiple SCF sequence files were created using Sequencher 3.0 (Gene Codes Corp., Ann Arbor, MI). Preliminary alignments of consensus sequences were generated using the Clustal W algorithm in MacVector 7.2 (Accelrys Inc., San Diego CA) using default parameters (with minor exceptions). 12S and 16S rRNA alignments were adjusted by eye using secondary structure models of Orti et al. (1996), Burk et al. (2002), and Wang and Lee (2002) following the methods of Kjer (1995). Ambiguous (unalignable) regions were excluded from further analyses to prevent loss of phylogenetic signal. Putative stem (paired) and loop/bulge (unpaired) regions were located in both data sets. Base pair complementarity in stem regions was confirmed by eye. Prior to alignment, all ND2 sequences were translated to ensure a single, continuous open reading frame from start to stop. Adjustment of aligned ND2 consensus sequences was not necessary, as each sequence encoded a protein of the exact same length. The alignment of the ITS-1 region was performed on the basis of conserved sequence motifs using default pairwise gap opening and extension penalties. Flanking transfer RNA (tRNA) locations within each mitochondrial alignment were located using tRNAscan SE (Lowe and Eddy, 1997). 18S and 5.8S gene regions were located in the nuclear ITS-1

alignment with the aid of ITS region sequences from *Auxis rochei*, Genbank accession AB193747.

Pairwise comparisons of all taxa were performed using PAUP\* v.4.0b4 (Swofford, 1999). Sequence features including nucleotide composition, site variability and relative contribution by transitions (Ts) and transversions (Tv) were estimated. Homogeneity of base composition was investigated using a chi-square ( $X^2$ ) test ( $\alpha = 0.05$ ). To infer sequence saturation, transitions and transversions were plotted against uncorrected sequence divergence (p-distance), a measure of the genetic distance between two DNA sequences. Ts and Tv vs. p-distance plots were generated for stem and loop partitions in the 12S and 16S data sets, for the first, second and third positions in the protein-coding ND2 alignment and for the ITS-1 data set overall. Saturation (multiple substitutions at the same nucleotide position), which tends to obscure true phylogenetic signal, is inferred when an asymptotic pattern is found. Exclusion of potentially homoplasious data partitions or implementation of an alternate weighting scheme are two ways to resolve this issue.

#### Data Set Partitioning

To generate a robust phylogenetic hypothesis, it is common to combine DNA sequence alignments from different gene regions into one large, concatenated data set. Prior to analysis, however, one should test for congruence between data partitions. To determine the validity of using a combined data set, a partition homogeneity test (Farris et al. 1995) was executed in PAUP\* to infer congruence between 12S, 16S, ND2 and ITS-1 data partitions. Incongruence length differences

(ILDs) were explored with a heuristic search of 1000 replicates and 10 random sequence additions to assess dataset combinability. A significance estimate (p-value) less than 0.05 indicates potential incongruence between data partitions.

### Phylogenetic Analyses

DNA sequence alignments from 12S, 16S, ND2 and ITS-1 gene regions were analyzed separately and as two concatenated data sets (combined mitochondrial gene regions; mitochondrial plus nuclear gene regions) using three different inference methods: maximum parsimony, maximum likelihood, and Bayesian inference. Parsimony analyses were conducted using a heuristic search algorithm in PAUP\* with tree-bisection-reconnection (TBR) branch swapping. Nonparametric bootstrapping (Felsenstein, 1985) was used to measure robustness of clade support. A total of 1000 bootstrap pseudoreplicates with 1000 random sequence addition replicates was performed for all data sets. Characters were unordered and equally weighted. Gaps were considered missing information. Likelihood analyses were performed using a heuristic search algorithm in PAUP\* with base frequency, substitution rate and site variation parameters estimated using Modeltest 3.06 (Posada and Crandall, 1998). Each data set was examined using a hierarchical likelihood ratio test of 56 models of character evolution. Parameters calculated using the Akaike Information Criterion (AIC) were implemented in a heuristic likelihood search with TBR branch swapping. Robustness of clade support was measured using 100 bootstrap pseudoreplicates with 10 random addition sequence replicates. Bayesian analyses were performed using MrBayes 3.1.1 (Ronquist and Huelsenbeck, 2003).

For each individual gene region data set, two metropolis coupled markov chain monte carlo (MCMCMC) analyses were run for one million generations each, sampling every 1000 generations. Concatenated data sets were run for a total of four million generations sampling every 1000 generations. The GTR + I + G (general time reversible with a proportion of invariant sites and among site rate heterogeneity (gamma) parameter) model of character change was used in all analyses. The number of generations required to reach stationarity was determined by plotting the log likelihood (-ln) score against generation number. Stationarity was assumed when -ln, tree length, GTR rate, stationary nucleotide frequency, gamma shape, and proportion of invariant site parameters reached a stable level (asymptote). In all cases, stationarity was reached after approximately 2000 generations. Data collected prior to this point were excluded in subsequent analyses to account for "burn in". Posterior probabilities were calculated as a measure of clade support with PAUP\* using a 50% majority rule consensus. In all cases, phylogenetic trees were rooted using genetic data from *P. saltatrix*, *N. pectoralis* and *C. armatus* (AP004444).

## RESULTS

### Sequence Variation

Complete 12S rRNA, ND2 and ITS-1, and nearly complete 16S rRNA gene region sequences were isolated from all taxa, with the exception of *C. armatus*, which was downloaded from Genbank (AP004444). Complete 12S rRNA DNA sequences ranged between 946 and 960 bp upon removal of tRNA<sup>Phe</sup> and tRNA<sup>Val</sup>. Alignment was relatively straightforward following the proposed secondary structure models of Wang and Lee (2002). Two ambiguously aligned regions of length 20bp and 49bp were excluded from subsequent analyses, and were located in putative loop regions near the 5' and 3' end of the alignment, respectively. Nine-hundred and sixteen aligned bases were included in the final alignment, of which 269 (29.4%) were variable, and 179 (19.5%) were parsimony informative (Table 2). Putative paired (stem) regions contained 464 nucleotide positions, of which 58 were parsimony informative. Putative unpaired (loop, bulge) regions contained 452 nucleotide positions, of which 121 were parsimony informative. Negligible base composition bias was noted in the alignment. An elevated transition/transversion ratio (1.9 overall) was estimated across the data set, a finding common among mitochondrial genes (Saccone et al. 1999). No evidence of saturation was noted in paired regions (Figure 3). A minor amount of transitional saturation was noted in unpaired regions, although not enough to warrant data exclusion or the use of an alternate character weighting

scheme. Nucleotide sequence divergence between ingroup (i.e. echeneneoid) and outgroup (i.e. non-echeneoid; *P. saltatrix*, *N. pectoralis*, *C armatus*) taxa ranged from 12.3-18.9% with a mean of 15.6% (SD = 0.045, n = 55). Nucleotide sequence divergence within ingroup taxa ranged from 0.02-16.0% with a mean of 10.1% (SD = 0.019, n = 33). The lowest divergence values (0.02%) were found between *E. naucrates* and *E. neucratoides* (Appendix 2).

Nearly complete 16S rRNA gene region sequences were collected from all taxa. Sequences analyzed ranged between 1613 and 1660 bp upon removal of tRNA<sup>Val</sup> and tRNA<sup>Leu</sup> fragments. Alignment was relatively straightforward following the proposed secondary structure models of Orti et al. (1996) and Burk et al. (2002). Two ambiguous regions of length 46bp and 44bp were excluded from subsequent analyses, and were located in putative loop regions in the central and 3' end of the alignment, respectively. A total of 1599 aligned bases was included in the final alignment, of which 589 (36.8%) were variable, and 426 (26.6%) were parsimony informative (Table 2). Putative paired (stem) regions contained 718 nucleotide positions, of which 121 were parsimony informative. Putative unpaired (loop, bulge) regions contained 881 nucleotide positions, of which 305 were parsimony informative. Slight anti-G bias was noticed in the final data set. A moderately elevated transition/transversion ratio (1.3 overall) was estimated across the data set. No evidence of saturation was noted in paired regions (Figure 4). As was found in the 12S rRNA data set, a minor amount of transitional saturation was noted in unpaired regions, although not enough to warrant data exclusion or the use of an alternate weighting scheme. Nucleotide sequence divergence between ingroup and outgroup

taxa ranged from 17.1-22.6% with a mean of 19.1% (SD = 0.014, n = 55 ).

Nucleotide sequence divergence within ingroup taxa ranged from 0.02-20.1% with a mean of 13.2% (SD = 0.056, n = 33). As in the 12S data set, the lowest divergence (0.02%) was found between *E. naucrates* and *E. neucratoides* (Appendix 3).

ND2 gene region sequences from all taxa totaled 1047bp in length. As there were no amino acid insertions or deletions in the data set, adjustment of the alignment was not necessary. Of the 1047 aligned bases 603 (57.6%) were variable, and 518 (49.5%) were parsimony informative (Table 2). As characteristic of protein coding genes, the greatest amount of variation was found at the third position, followed by the first, then second position (Nei, 1987). One hundred and seventy-nine first position sites, 87 second position sites and 337 third position sites were variable. While no evidence of saturation was found at first or second positions, severe transition saturation was noted in the third codon position (Figure 5). As a conservative measure, third position data were excluded from further analyses to reduce the possibility of misinterpreting true phylogenetic signal due to multiple substitutions at the same position (homoplasy). Of the remaining 698 aligned bases, 266 (38.1%) were variable and 196 (28.1%) were parsimony informative. Slight A-C bias was noticed in the first position, whereas relatively strong C-T bias was noted in the second position. An elevated transition/transversion ratio was noted in both first and second positions (2.5 and 2.3, respectively). Based upon first and second position data alone, nucleotide sequence divergence between ingroup and outgroup taxa ranged from 12.9-21.3% with a mean of 16.9% (SD = 0.025, n = 33). Nucleotide sequence divergence within ingroup taxa ranged from 0.14-20.6% with a mean of

12.5% (SD =0.067, n = 55 ). Lowest divergence values were found between *E. naucrates* and *E. neucratooides* (Appendix 4).

ITS gene sequences were isolated from all taxa, with the exception of *C. armatus*. Nuclear data from this species was not available to combine with the data set (as was the case with the mitochondrial data). Two or three clones from each ITS-1 gene region were assayed to account for allelic variation. In all cases, only minor differences (base changes, insertions, deletions) existed between variants within an individual. The clone with the highest sequence quality (i.e. cleanest sequence, fewest ambiguities) was chosen for phylogenetic analysis. The final ITS alignment included complete ITS-1 region sequences which ranged between 416 and 725 bp, and partial flanking 18S and 5.8S region sequences which totaled 68 and 72bp, respectively. A total of 1010 aligned bases was included in the final alignment, of which 572 (56.6%) were variable, and 350 (34.7%) were parsimony informative (Table 2). Strong C-G bias was noticed across the alignment. Base composition was skewed towards C and G (30.3% and 33.6%, respectively). A chi-square test used to test base composition homogeneity demonstrated highly significant heterogeneity ( $X^2 = 58.8$ ,  $p < 0.01$ ,  $df = 36$ ). An equal number of transition and transversion mutations were noted (Ts/Tv ratio = 0.99). A minor amount of transitional saturation was found, although not enough to warrant data exclusion or the use of an alternate weighting scheme (Figure 6). Nucleotide sequence divergence between ingroup and outgroup taxa ranged from 28.1-33.8% with a mean of 31.0% (SD = 0.015, n = 22 ). Nucleotide sequence divergence within ingroup taxa ranged from 0.6-32.6% with a mean of 21.5% (SD =



0.087,  $n = 55$ ). Lowest divergence values were found between *E. naucrates* and *E. neucratoides* (Appendix 5).

### Partition Homogeneity Analyses

Two concatenated gene region alignments were assembled and tested for data set congruence: a mitochondrial only and mitochondrial plus nuclear (M + N) gene region alignment. Results of the partition homogeneity test indicated congruence between data partitions in the mitochondrial only alignment ( $p = 0.362$ ). On the contrary, incongruence was found between data partitions in the M + N alignment ( $p = 0.001$ ). ILD tests of data congruence have been shown to be inaccurate under certain conditions (Cunningham, 1997; Yoder et al. 2001). Furthermore, it has been argued that the combination of potentially incongruent (i.e. heterogeneous) data sets may increase phylogenetic accuracy (reviewed in Barker and Lutzoni, 2002). For this reason, despite the indication of potential incongruence within the M + N alignment, both data sets were analyzed using maximum parsimony, maximum likelihood and Bayesian inference methods. In essence, I took both an aggressive (i.e. “the more data the better”) and a conservative (“incongruence may lead to phylogenetic inaccuracy”) stance. Data set characteristics of each concatenated alignment are noted in Table 2 and Table 3.

### Phylogenetic Relationships – Combined

Parsimony (MP) analyses of the mitochondrial-only combined data set resulted in a single most parsimonious tree of length 2458 (Consistency Index (CI) =

0.6452; Retention Index (RI) = 0.6220; Rescaled Consistency Index (RC) = 0.4014 ; Table 3). Tree topology is shown in Figure 7. All nodes were supported by moderate (60-85%) to strong (>85%) bootstrap support and moderate (70-90%) to strong (>90%) posterior probabilities. The subfamilies Echeneiinae and Remorinae were both monophyletic within a monophyletic Echeneidae. Rachycentridae + Coryphaenidae formed a monophyletic sister group to the Echeneidae. A moderate level of support (67%) was estimated for a Echeneoidea + *C. armatus* clade. Within the Remorinae, *R. australis* was placed at the most basal position, whereas a *R. remora* + *R. brachyptera* clade occupied the most derived position. Likelihood (ML) analyses produced a tree of somewhat different topology (-ln = 15270.2; Figure 7). Differences include the placement *C. armatus* and the relationships within the Remorinae. In this case, *N. pectoralis* and *C. armatus* form a monophyletic outgroup to the Echeneidae. Within the Remorinae clade, a polytomy of *R. albescens* + *R. remora* + *R. australis* + a weakly supported *R. osteochir* + *R. brachyptera* clade was resolved. Bayesian (BN) analyses resulted in a 95% credible set of 3996 trees. A 50% majority rule consensus of these trees was generated in PAUP\*. Topology resembled that of the parsimony tree, except that *N. pectoralis* and *C. armatus* form a monophyletic outgroup to the Echeneidae, as was found in the likelihood tree. Posterior probabilities of clade support were strong (>90%) at all nodes except at the node containing *R. albescens* + *R. remora* + *R. osteochir* + *R. brachyptera* (86%) and the most derived node within the Remorinae containing *R. osteochir* and *R. brachyptera* (82%). As found in the parsimony tree, *R. australis* was the most basal member within the Remorinae.

Analyses of the M + N data set yielded congruent tree topologies to those generated by the mitochondrial only alignment (Figure 8). Using all three inference methods, a monophyletic Echeneoidea was resolved with high bootstrap support (100% MP; 100% ML) and high posterior probability (100% BN). Rachycentridae + Coryphaenidae were resolved in all cases. Within a monophyletic Echeneidae, subfamilies Echeneiinae and Remorinae were both monophyletic. In all trees, the Echeneoidea + *C. armatus* were resolved with a moderate to strong level of bootstrap support (67% MP; 84% ML) and high posterior probability (100% BN; Figure 8). Parsimony analyses of these data resulted in a single most parsimonious tree of length 3500 (CI = 0.6452; RI = 0.6220; RC = 0.4526; Table 3). Tree topology was an exact match of that found with the mitochondrial only data set, with slightly different bootstrap support values. Relationships within the Remorinae differed between trees generated using the three different inference methods. In all cases, however, *R. australis* was found at the most basal position and *R. brachyptera* within a clade at the most derived position. In all trees, *R. albescens* was alternately placed at intermediary positions with the Remorinae.

#### Phylogenetic Relationships – Individual Gene Regions

Individual gene phylogenies differed somewhat depending on the gene region examined and inference method used. 12S rRNA phylogenies agreed upon the monophyly of the Echeneiinae within a monophyletic, but polytomic Echeneidae clade (Figure 9). A monophyletic Rachycentridae + Coryphaenidae clade was resolved in all trees, with low to moderate bootstrap (63% MP; 53% ML), but high

posterior probability (99% BN) support. Relationships between ingroup + outgroup taxa and within outgroup taxa differed between inference method. Parsimony analyses resolved a weakly supported (54%) Pomatomidae + Rachycentridae + Coryphaenidae clade, whereas Bayesian analyses grouped Pomatomidae with the monophyletic Echeneidae with moderate support (76%). Likelihood placed Pomatomidae in at the root of the phylogeny.

16S phylogenies closely resembled the results of the combined data analyses, with a few minor differences. A Rachycentridae + Coryphaenidae grouping was resolved with strong support (100% MP; 100% ML; 100% BN) in all cases (Figure 10). Subfamilies Echeneiinae and Remorinae were both monophyletic within a monophyletic Echeneidae. Parsimony analyses produced a topology identical to the one found using the M + N data set, except *C. armatus* was not grouped with the monophyletic Echeneoidea clade. Likelihood analyses resolved a Pomatomidae + Echeneoidea clade, and an undefined relationship with the Echeneiinae. Unlike either of the combined data sets, likelihood resolved a clear relationship within the Remorinae: *R. albescens* at the basal position, and a *R. osteochir* + *R. brachyptera* clade at the most derived. Results of the Bayesian analyses were identical to those seen in the combined (mitochondrial only) data set, with the exception of the arrangement of species with the Remorinae.

Analyses of 698 bases of the ND2 gene yielded three trees of differing topology (Figure 11). In all case, however, the Echeneoidea were monophyletic. In addition, a monophyletic Rachycentridae + Coryphaenidae clade was grouped sister to a monophyletic Echeneidae. Topologies within the Echeneidae differed

significantly among inference methods. A monophyletic, polytomic and polyphyletic Remorinae was resolved using parsimony, likelihood and Bayesian inference, respectively. A monophyletic Echeneiinae was resolved in all topologies.

Phylogenies based upon ITS data were in agreement with most of the hypotheses generated using combined data (Figure 12). Each inference method resolved a monophyletic Echeneoidea. A monophyletic Rachycentridae + Coryphaenidae clade was resolved, sister to a monophyletic Echeneidae. The family Echeneidae was defined by monophyletic subfamilies Echeneiinae and Remorinae. Relationships within the Remorinae differed among inference methods. Parsimony analyses placed *R. australis* in the basal position, and a clade containing *R. brachyptera* and *R. osteochir* at the most derived. Likelihood analyses produced a polytomic arrangement. Bayesian analyses placed *R. brachyptera* in the basal position and a clade containing *R. australis* and *R. remora* at the most derived position.

## DISCUSSION

### Echeneoid Phylogenetics

This study represents the first comprehensive molecular investigation into the taxonomic relationships within the superfamily Echeneoidea. Hypotheses of the evolutionary relationships within the Echeneoidea were based upon analyses of both concatenated mitochondrial DNA sequences and concatenated mitochondrial + nuclear DNA sequences. The hypotheses generated were largely consistent between mitochondrial-only and mitochondrial + nuclear gene phylogenies, despite potential incongruence between mitochondrial and nuclear data sets. Overall, the phylogenetic hypotheses generated using three different optimality criterion (parsimony, likelihood, Bayesian) were largely congruent. Nodal support at the superfamily, family and subfamily levels was very high, as measured by nonparametric bootstrapping and posterior probability calculations.

The taxonomic relationships presented here, observed in both concatenated mitochondrial-only and mitochondrial + nuclear phylogenies, corroborate the family-level morphology-based hypotheses of Johnson (1984, 1993) and molecular hypotheses of Reed et al. (2002). These results contradict the morphology and behavior-based hypotheses of O'Toole (2002), and the molecular hypotheses of Miya and Nishida (pers. comm.). Furthermore, the present results disagree with specific

aspects of alpha level taxonomy theorized by O'Toole (2002). Below are the relevant findings, in reference to the six objectives outlined in the first section of this chapter:

1. In agreement with the work of Johnson (1984, 1993) and O'Toole (2002), the Echeneoidea were resolved as a monophyletic group. The Echeneidae, Coryphaenidae and Rachycentridae were resolved together with strong support in all cases.
2. The families Rachycentridae and Coryphaenidae form a monophyletic group. This hypothesis agrees with Johnson (1984, 1993), who cites a number of synapomorphies relating to neurocranial development, head spination, mandibular structure and epithelial cell composition in support of this relationship. This phylogeny also agrees with the hypotheses of Reed et al. (2002) and the work of Ditty (1993) and Ditty and Shaw (1992).
3. In agreement with the work of Lachner (1981) and O'Toole (2002), the Echeneidae form a monophyletic group.
4. Subfamilies Echeneiinae and Remorinae were both monophyletic. These data contradict the subfamily-level hypotheses of O'Toole (2002), who found the subfamily Echeneiinae to be polyphyletic based upon analyses of 138 putatively informative osteological characters. In his phylogeny, the Remorinae + *E. naucrates* + *E. neucratoides* form a monophyletic group. For this reason, O'Toole (2002) recommended the elimination of subfamilial designations. Results of this study contradict O'Toole's (2002) hypotheses and validate their designation.

5. The genus *Remora* is paraphyletic based upon the position of the monotypic genus *Remorina*. These results agree with O'Toole's (2002) findings. To amend this situation, O'Toole (2002) recommended that *Remorina albescens* be subsumed under the genus *Remora*, which yielded a five-member monophyletic genus. Results of this study support this recommendation.
6. The final objective, a clarification of the species-level relationships within the Echeneidae, was not fully achieved using the genetic information surveyed. This goal was not realized due to poor resolution within the Remorinae and potential discrepancies with the genus *Echeneis* (both discussed below).

#### Unresolved Relationships

Two nodes were unresolved in the analysis of combined data: the node defining the outgroups *N. pectoralis* and *C. armatus* and the node defining the Remorinae. *Carangoides armatus* was alternately grouped with either a monophyletic Echeneoidea or *N. pectoralis*. Freihofner (1978) united the five families Nematistiidae, Carangidae, Echeneidae, Coryphaenidae and Rachycentridae based upon two synapomorphies: an extension of the nasal canal surrounded by tubular ossifications and cycloid scales. Johnson (1984) and Smith-Vaniz (1984) further clarified the relationships with additional larval characters. The Carangidae were grouped with the Echeneoidea based upon three characters: the lack of a bony stay posterior to the ultimate dorsal and anal pterygiophores, presence of two prenasal canal units, and a



lamellar expansion of the coracoid. Parsimony, likelihood and Bayesian phylogenies of the combined (M +N) data set agree upon the placement of *C. armatus* as a sister-taxon to the monophyletic echeneoid clade with a moderate (67%) to high (100%) level of support. Parsimony analyses of the combined (mitochondrial only) data set agrees with this relationship. On the contrary, likelihood and Bayesian analyses of these data resolve a monophyletic *N. pectoralis* + *C. armatus* clade. The phylogenetic relationships among these taxa were undefined by O'Toole (2002). In light of the previous, uncontested work of Johnson and Smith-Vaniz, it seems highly likely that the grouping of (Nematistiidae + (Carangidae + Echeneoidea)) is the most phylogenetically accurate.

The second unresolved aspect of the proposed echeneoid phylogeny involves the relationships within the Remorinae. Likelihood analyses of both combined data sets produced an unresolved relationship among the five member taxa. Parsimony and Bayesian analyses of both combined data sets (M only, M+N) agree upon the placement of *R. australis* at the most basal position and *R. brachyptera* at the most derived position along the branch. The relative positions of the remaining taxa varied among data sets and inference methods used. As such, the relationships within this subfamily are still unresolved. To address this issue, future work should involve sampling a gene region that exhibits a higher rate of sequence evolution (e.g. the mitochondrial control region).

#### Discrepancies within *Echeneis*

The level of genetic divergences between sister-species within the genus

*Echeneis* is surprisingly low. Pairwise sequence divergence of 0.21% (12S rRNA), 0.25% (16S rRNA), 0.14% (ND2), and 0.66% (ITS) were estimated between the sharksucker (*E. naucrates*), and whitefin sharksucker (*E. neucratoides*) specimens. Cursory analysis of these data suggests that these two specimens are in fact, the same species, as this level of divergence is comparable to that found between individuals of the same species. For example, intraspecific sequence divergence between individuals of *R. brachyptera* exhibited the following ranges: 12S (0.32–1.16%); 16S (0.18–1.07%); ND2 (0.43–1.00%) (data not shown).

Two reasons could account for the observed levels of divergence between *Echeneis* species. First, a sample could have been misidentified. Alternatively, these two putative sister species could, in fact, comprise a species that simply demonstrates a wider range in character states and alternate color morphs than other echeneids. Debate currently exists as to whether *Echeneis* is monotypic (Collette, pers. comm.). *E. neucratoides* exhibits a restricted geographic distribution (western Atlantic only), whereas *E. naucrates* is found worldwide. These two species are nearly identical in external appearance and many of the putative characters used to differentiate the two species overlap. Only one putative sample of *E. neucratoides* was collected during this investigation. The specimen was identified by two separate researchers (K. Gray, B.B. Collette) using the keys of Lachner (1984) and Collette (2003), following standard identification procedures (including x-ray analysis of skeletal elements). On the basis of dorsal and anal fin coloration alone (as the other identifiable features fell within the range of both species), this specimen was identified as *E. neucratoides*. Although tree topology is unlikely to change, even if divergence values between these

sister species were to increase to a level comparable to that seen between other remora species, these results highlight the need for inclusion of additional individuals to properly address this discrepancy. Moreover, multiple individuals of each member of the superfamily should be included, to ensure consistent and accurate phylogenetic interpretation.

### Importance of Multiple Genes

In a molecular phylogeny of the Gasterosteidae, Mattern (2004) stressed the importance of using multiple gene regions to identify the “true” organismal phylogeny. In an analysis of stickleback taxonomy using mitochondrial 12S rRNA, 16S rRNA, cytochrome *b*, ATPase 6, and control region DNA sequences, Mattern (2004) resolved significant topological differences among different gene trees. This is surprising given that all of these gene regions are linked on the same mitochondrial gene. One would expect the gene trees of different regions of the same gene to be more congruent (i.e. more so than if multiple nuclear and mitochondria gene regions were compared). Nonetheless, in combined analyses of all molecular data, Mattern’s (2004) phylogeny very closely resembled that of a morphologic and behavioral based study. She noted that if the scope of gene sampling had been more limited, her phylogenetic interpretation might have led to a vastly different hypothesis of the relationships among these fishes. Above all, this highlights the need for a diverse genetic sampling regimen, preferably including numerous independent mitochondrial and nuclear encoded genes, in order to infer accurate evolutionary relationships.

The results of the present study lend support to Mattern's observations to a degree. The individual gene phylogenies varied somewhat among gene regions sampled and inference methods used. Overall, each gene tree resolved most of the major nodes (e.g. monophyletic Echeneoidea, Rachycentridae + Coryphaenidae, Echeneidae and Echeneiinae clades), although the relationships between *Remora* and *Remorina* were almost consistently unresolved. A polytomic Remorinae was resolved using parsimony, likelihood and Bayesian analyses of 12S rRNA and ND2 data. On the contrary, the relationships within the Remorinae were resolved with 16S rRNA and ITS-1 data, although differing hypotheses were generated.

The observed range in phylogenetic performance among the gene regions is most likely a product of the amount of phylogenetically informative data each data set contained. The 12S rRNA and ND2 data sets contained 179 and 196 parsimony informative sites, respectively (after exclusion of third position ND2 data), whereas the 16S rRNA and ITS-1 data sets contained 426 and 350 parsimony informative sites, respectively. Furthermore, due to differences in evolutionary rate, each gene region comparison resolved different levels of interspecific divergence. Interspecific divergence estimates were lowest in 12S rRNA and ND2 data sets (after exclusion of third position data), and ranged from 0.21 to 18.9%, and 0.14 to 21.3%, respectively. The greater levels of interspecific sequence divergence estimated using 16S rRNA and ITS-1 data sets (0.25-22.6%, and 0.66-33.8%, respectively), combined with a greater amount of parsimony informative data overall, likely provided for a clearer interpretation of the interspecific relationships within the Echeneoidea.

Analyses of concatenated DNA sequence data facilitated the generation of robust gene phylogenies that effectively addressed the objectives of this study. Over 3200 bp of mitochondrial sequence data and over 4200 bp of combined nuclear and mitochondrial sequence data were effectively utilized to address family, genus and species-level ambiguities within the superfamily Echeneoidea. Although minor differences exist among concatenated and individual gene phylogenies, most of the major nodes were defined in the phylogenies generated. As such, the taxonomic hypotheses presented here appear well supported and potentially represent the “true” echeneoid phylogeny.

Table 1. PCR and cycle sequencing primer sequences used to amplify complete mitochondrial 12S rRNA, 16S rRNA, ND2 and nuclear ITS-1 DNA sequences in the Echeneoidea.

Gene Region	Primer	Sequence	Reference	Notes
<u>PCR Amplification</u>				
12S rRNA	Phe-5M13F	5' aaagcataacactgaagatgt 3' *	d	5' M13F tail
	Phe-5.1F	5' aaagcrtaacactgaagatgt 3'	d	-
	16S-3M13R	5' accagctatmacyaggttcg 3' *	d	5' M13R tail
	16S-3.1R	5' accagctatsacyaggttcg 3'	d	-
	12SA-L (12SA-5')	5' aaactgggattagatacccactat 3'	a	
	16SA-H (16SA-3')	5' atgttttgataaacaggcg 3'	a	
16S rRNA	Val-5M13F	5' gcawagcatytcmttacacyg 3' *	d	5' M13F tail
	Val-5.1F	5' gcrtyticccttacacygagaagtc 3'	d	-
	Leu-3M13R	5' rytgggragaggayttgaacc 3' *	d	5' M13R tail
	Leu-3.1R	5' rytgggagaggayttgaacc 3'	d	-
	16S-1AM13F	5' agttartcaaarggggkacagc 3' *	d	5' M13F tail
	16S-1BM13R	5' caartgattacgctacctthgc 3' *	d	5' M13R tail
ND2	ND2B-LM13F	5' taagcttlygggccatcac 3' *	b	5' M13F tail
	ND2B-L	5' taagcttlygggccatcac 3'	b	-
	ND2B-HM13R	5' crttaggrctttgaaggc 3' *	b	5' M13R tail
	ND2B-H	5' crttaggrctttgaaggc 3'	b	-
ITS-1	X18SF	5' cttgactatctagaggaagt 3'	c	
	X28SR	5' atatgcttaaattcagcggg 3'	c	
	5.8SR1	5' attcacattagttctgcagcta 3'	c	
	5.8SR2	5' atgatcatcgacmyttcgaacgcac 3'	c	
<u>Cycle Sequencing</u>				
	M13F	5' cagcaggttgtaaaacgac 3'	-	
	M13R	5' ggataacaattcacacagg 3'	-	

References: <sup>a</sup> Modified from Palumbi, S.R. 1996

<sup>b</sup> Modified from Broughton and Gold, 2000

<sup>c</sup> K. Johnson, thesis

<sup>d</sup> This Study

Table 2. Nucleotide sequence variation within mitochondrial 12S rRNA, 16S rRNA and ND2 gene regions, and the nuclear ITS-1 region in the Echeneoidea.



c

Region	Sequence length (bp)	No. variable sites	No. pars. inform. sites	Base frequencies				Base freq. homogeneity	Ts/Tv ratio
				A	C	G	T		
<u>12S rRNA</u>									
Stems	464	97 (20.9%)	58 (12.5%)	0.22308	0.27405	0.28536	0.21751	1.00000	4.50565
Loops	452	172 (38.1%)	121 (26.8%)	0.39363	0.21225	0.16290	0.23123	0.99993	2.07875
Overall	916	269 (29.4%)	179 (19.5%)	0.31029	0.24656	0.21786	0.22529	0.99737	1.91144
<u>16S rRNA</u>									
Stems	718	186 (25.9%)	121 (16.9%)	0.23096	0.27049	0.27687	0.22168	1.00000	3.37622
Loops	881	403 (45.7%)	305 (34.6%)	0.43750	0.19053	0.14084	0.23113	0.30220	1.08000
Overall	1599	589 (36.8%)	426 (26.6%)	0.34278	0.22893	0.19813	0.23016	0.44166	1.31495
<u>ND2</u>									
1st Position	349	179 (51.3%)	140 (40.1%)	0.30311	0.30270	0.18707	0.20712	0.81868	2.50632
2nd Position	349	87 (24.9%)	56 (16.0%)	0.15923	0.33402	0.10684	0.39992	1.00000	2.33229
3rd Position	349	337 (96.6%)	322 (92.3%)	0.35510	0.32788	0.07307	0.24396	0.00000	1.41637
Overall	1047 (698) <sup>a</sup>	266 (38.1%)	196 (28.1%)	0.27248	0.32153	0.12232	0.28367	0.98521	1.74747
<u>ITS-1</u>									
Overall	1010	572 (56.6%)	350 (34.7%)	0.17147	0.30322	0.33609	0.18922	0.00946	0.99449
<u>Combined</u>									
Mitochondrial Only	3213	1124 (35.0%)	801 (24.9%)	0.30767	0.25212	0.19718	0.24302	0.13738	1.88121
Overall (Mito + Nuclear)	4223	1685 (39.9%)	1164 (27.6%)	0.28467	0.26091	0.22063	0.23379	0.00091	1.46769

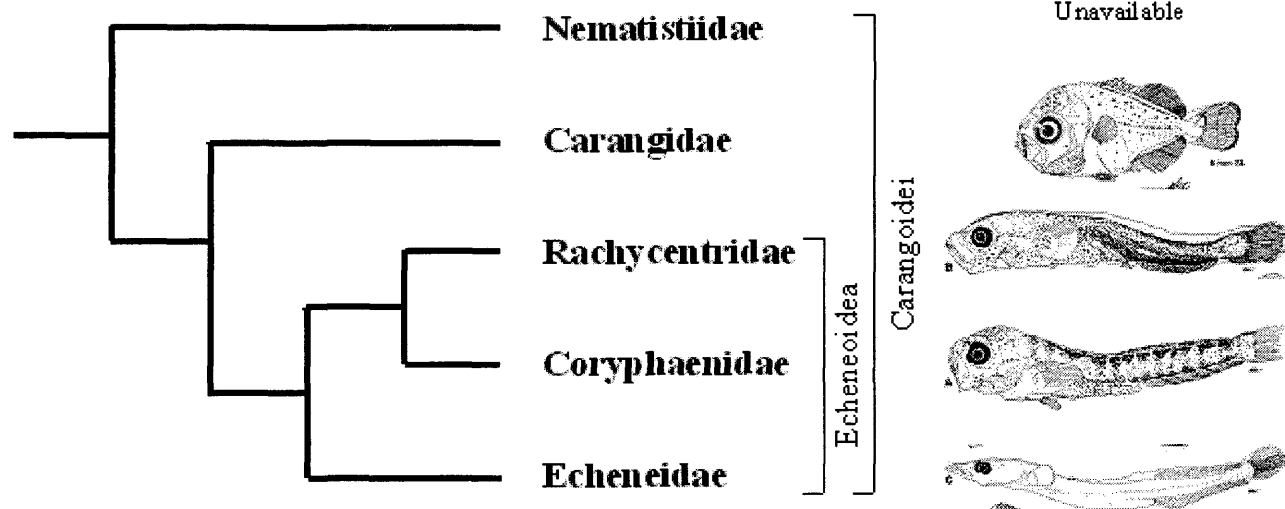
<sup>a</sup> Third position excluded

Table 3. Summary statistics of parsimony and likelihood analyses of 12S rRNA, 16S rRNA, ND2 and ITS-1 DNA sequences in the Echeneoidea. Abbreviations: consistency index (CI); retention index (RI); rescaled consistency index (RC); homoplasy index (HI); log-likelihood (-ln).

Region	Maximum Parsimony					Maximum Likelihood			
	Tree length	CI	RI	RC	HI	Evol. model	Prop. Invar. Sites (I)	Alpha shape parameter (G)	-ln
<u>12S rRNA</u>									
Overall	611	0.6007	0.4969	0.2985	0.3993	GTR+I+G	0.5481	0.7593	3850.5
<u>16S rRNA</u>									
Overall	1334	0.6499	0.6311	0.4102	0.3501	GTR+I+G	0.3121	0.4836	7884.9
ND2									
1 <sup>st</sup> + 2 <sup>nd</sup> Positions Only	772	0.6010	0.5000	0.3347	0.3990	TIM+I+G	0.3822	0.6807	3404.4
ITS-1									
Overall	1041	0.8204	0.7700	0.6317	0.1796	GTR+I+G	0.2675	4.0054	5442.2
Combined									
Mitochondrial Only	2458	0.6452	0.6220	0.4014	0.3548	GTR+I+G	0.4311	0.6863	15270.2
Overall (Mito + Nuclear)	3500	0.6906	0.6554	0.4526	0.3094	GTR+I+G	0.4329	1.1719	30186.4

Figure 1. Incongruent hypotheses of the taxonomic relationships within the Echeneoidea based upon (A) larval morphology (Johnson, 1984,1993; Smith-Vaniz, 1984) and (B) adult osteology and behavioral characters (O'Toole, 2002). Diagram credit: Johnson, G.D. 1984; O'Toole, B. 2002; LARVALBASE: <http://www.larvalbase.org>, 2005.

A



B

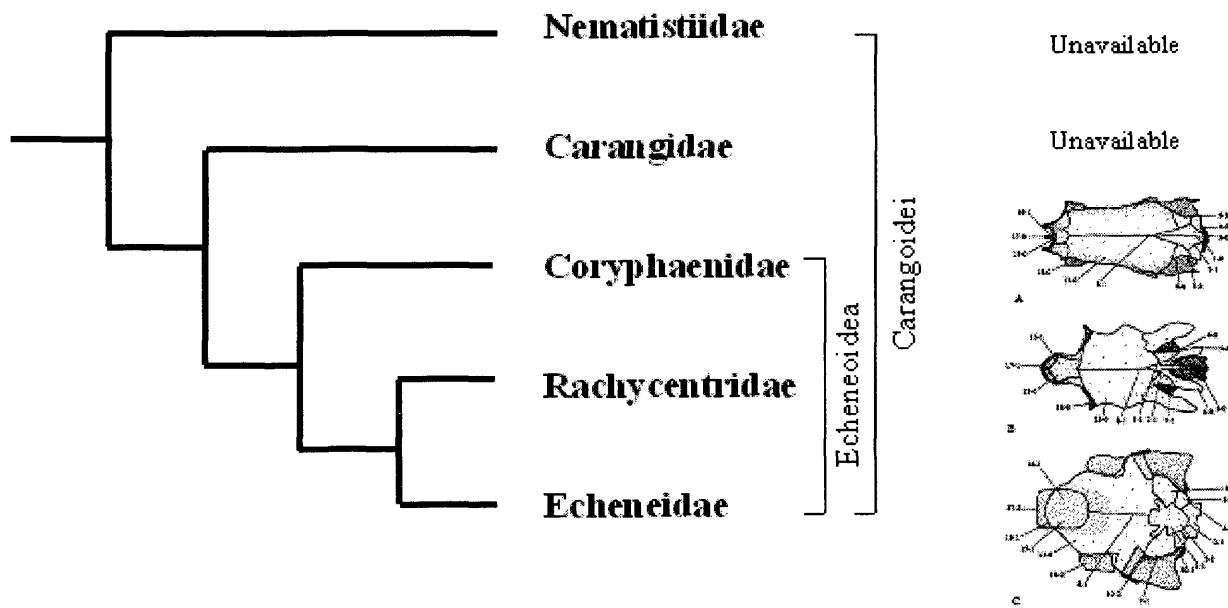
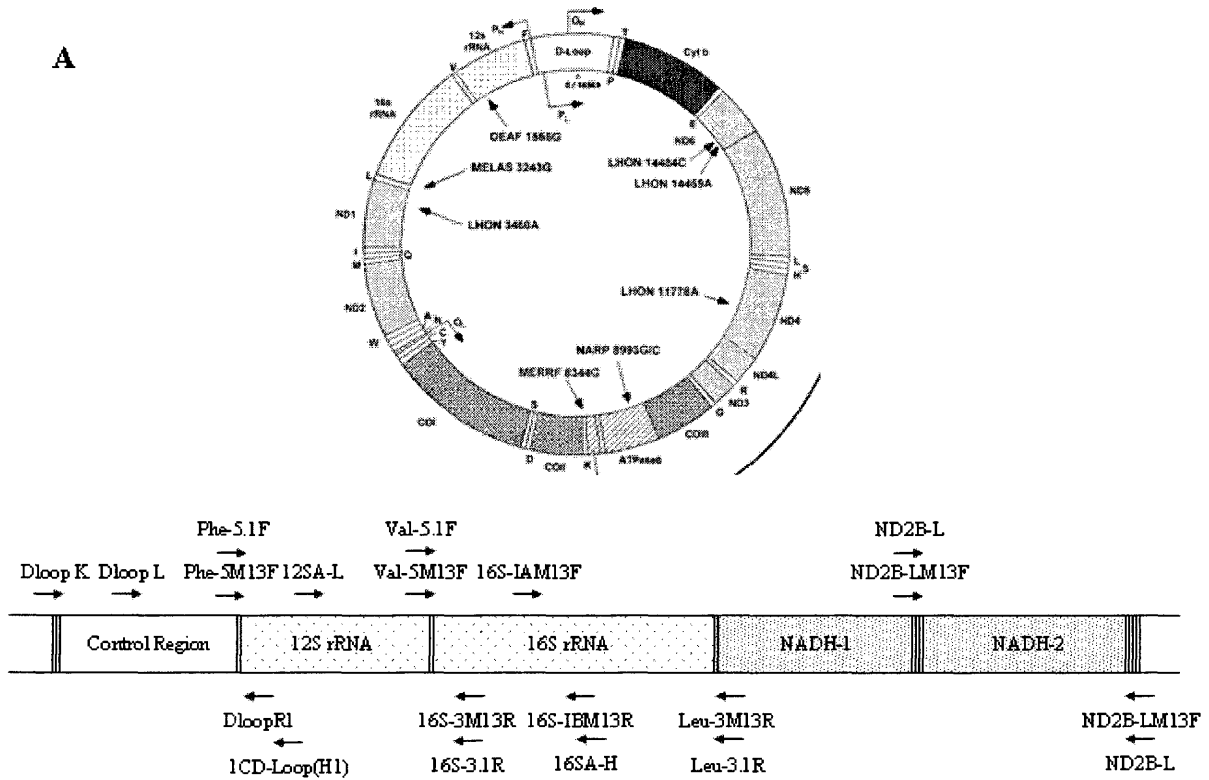


Figure 2. Locations of PCR primers used to amplify DNA sequences from (A) the mitochondrial 12S rRNA, 16S rRNA, and ND2 gene regions and (B) the nuclear ITS-1 gene region in the Echinoidea. Mitochondrial genome diagram credit: MITOMAP: A Human Mitochondrial Genome Database. <http://www.mitomap.org>, 2005.

A



B

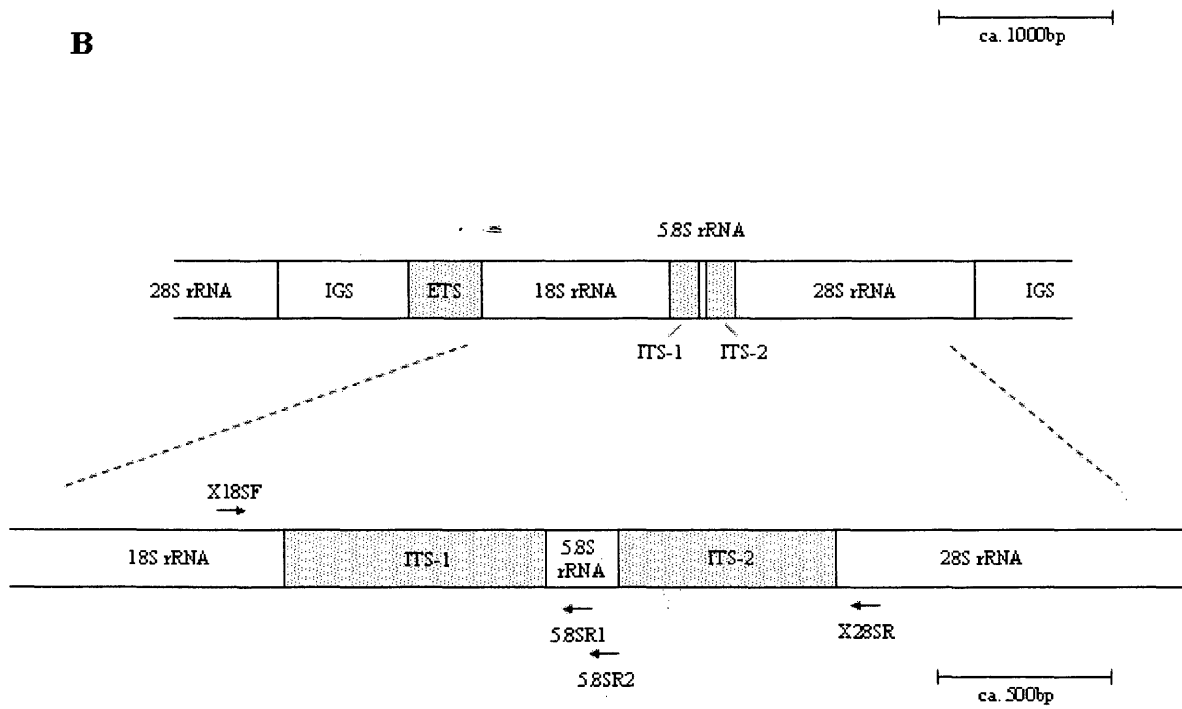


Figure 3. Nucleotide substitution patterns observed in pairwise comparisons of complete 12S rRNA gene region sequences in the Echeuoidea. Transitions (diamonds) and transversions (triangles) observed (A) overall and (B) within putative paired and unpaired regions were plotted against pairwise uncorrected sequence divergence (p-distance) to explore sequence saturation.



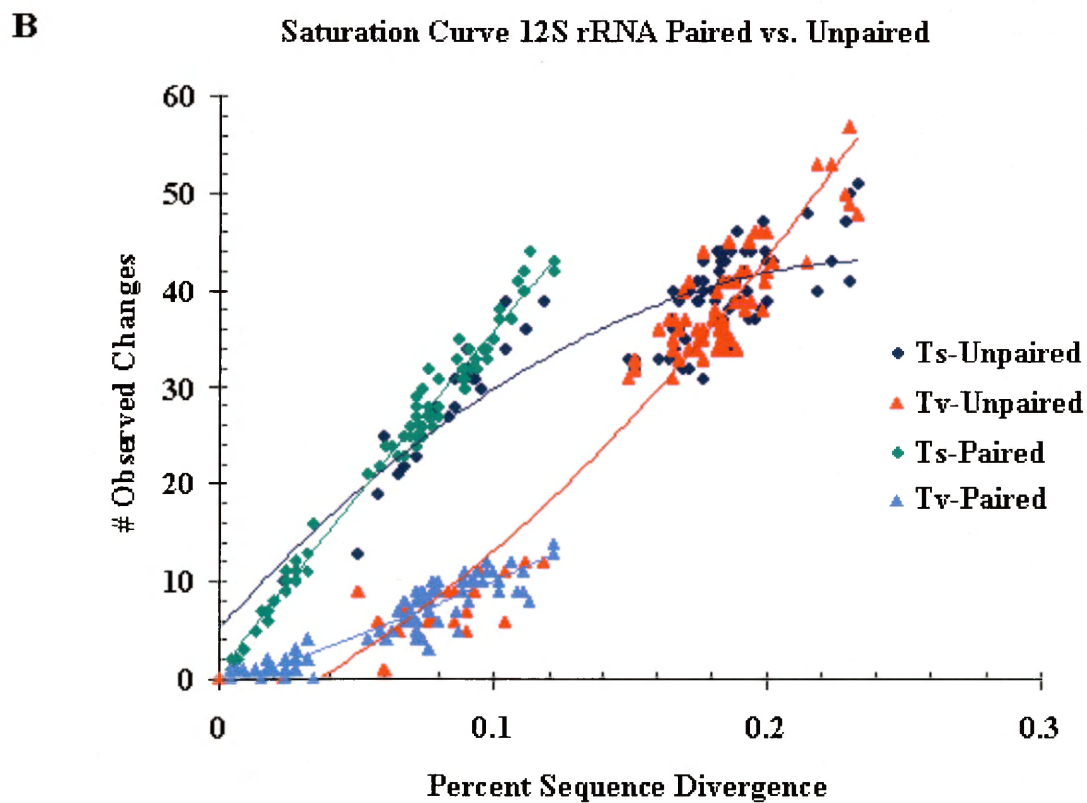
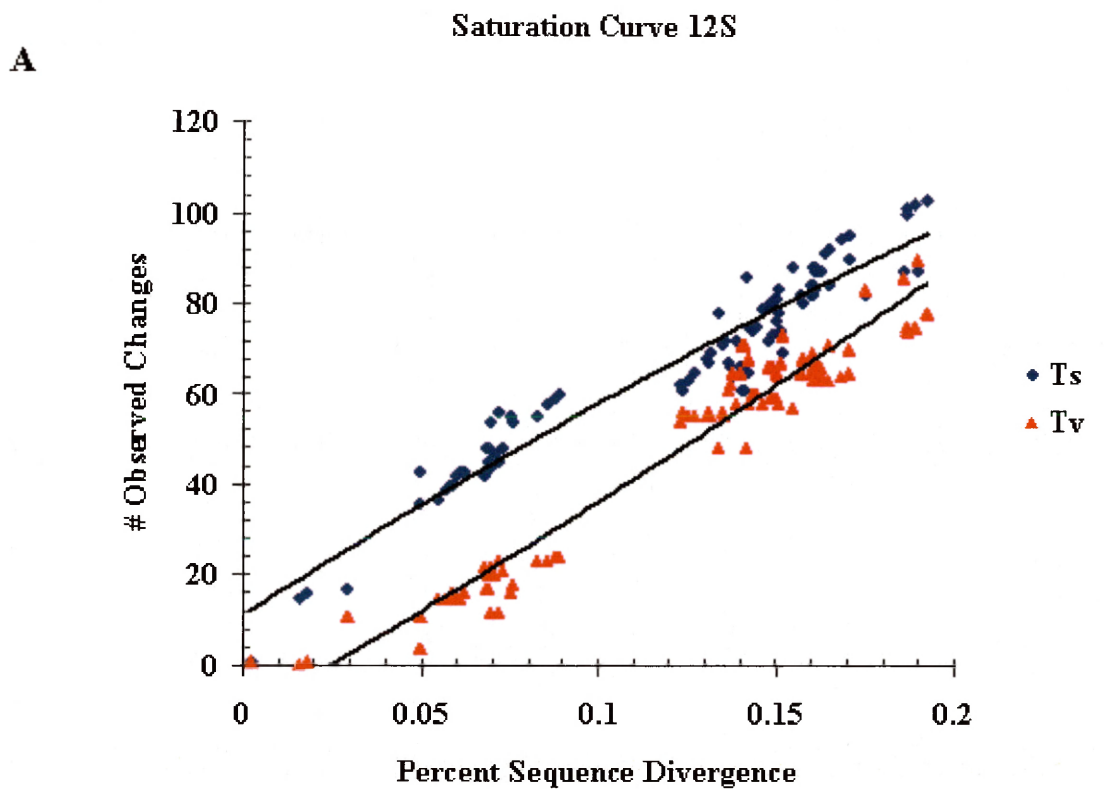
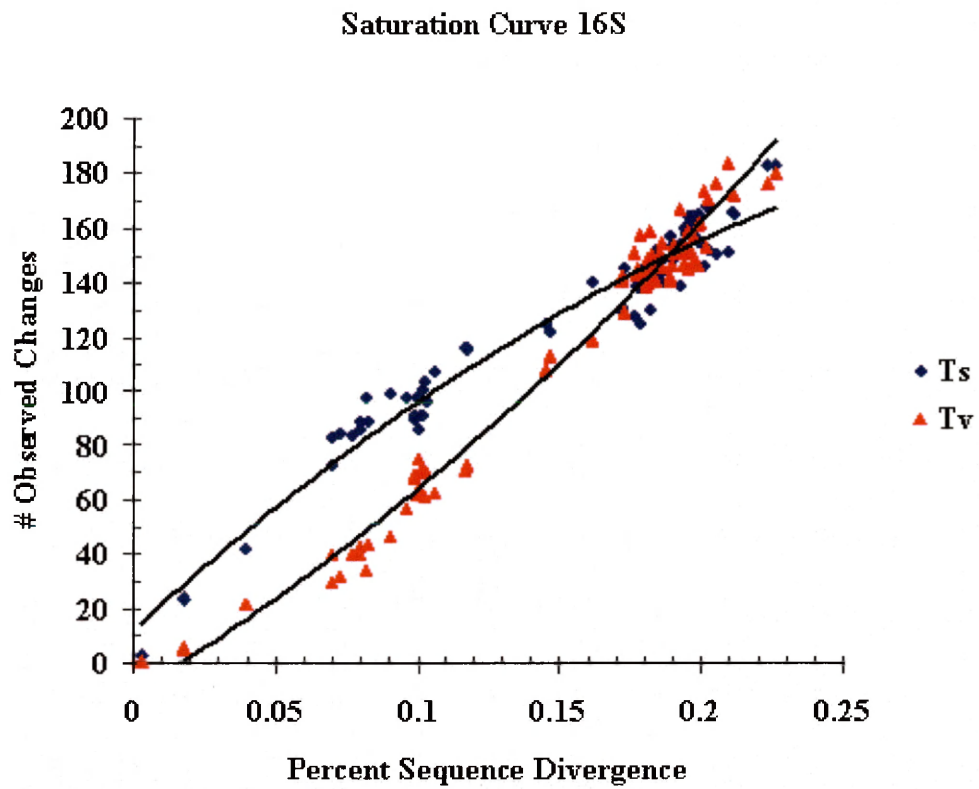


Figure 4. Nucleotide substitution patterns observed in pairwise comparisons of partial 16S rRNA gene region sequences in the Echeoidea. Transitions (diamonds) and transversions (triangles) observed (A) overall and (B) within putative paired and unpaired regions were plotted against pairwise uncorrected sequence divergence (p-distance) to explore sequence saturation.

A



B

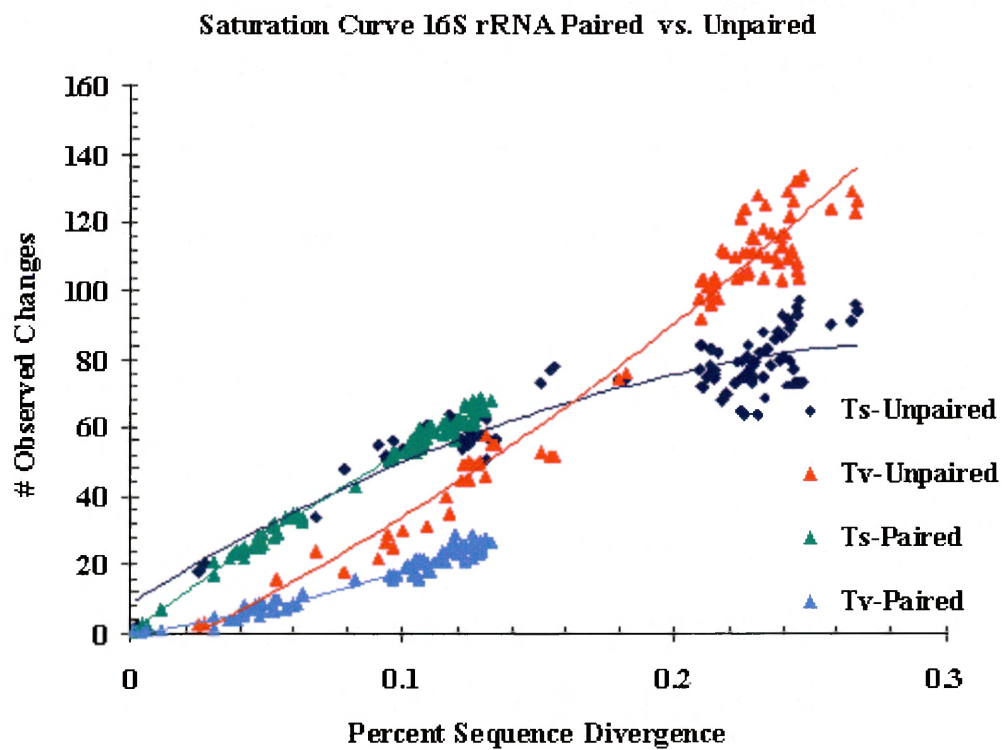


Figure 5. Nucleotide substitution patterns observed in pairwise comparisons of complete ND2 gene region sequences in the Echeneoidea. Transitions (diamonds) and transversions (triangles) observed (A) overall and within (B) first (C) second, and (D) third codon positions, were plotted against pairwise uncorrected sequence divergence (p-distance) to explore sequence saturation.

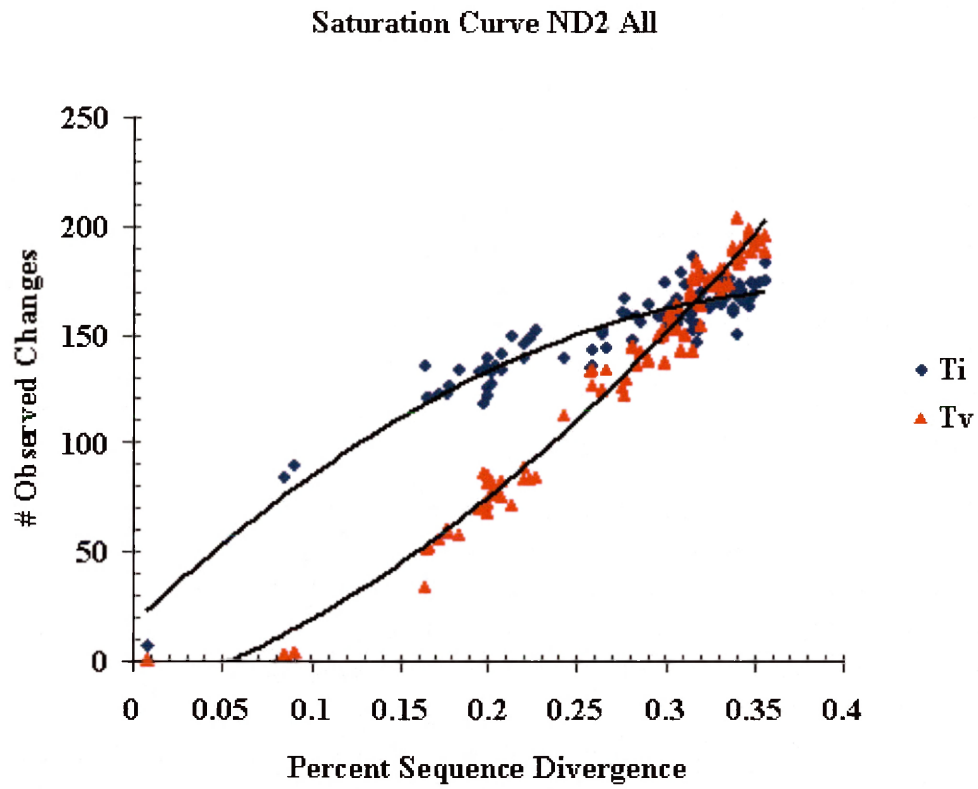
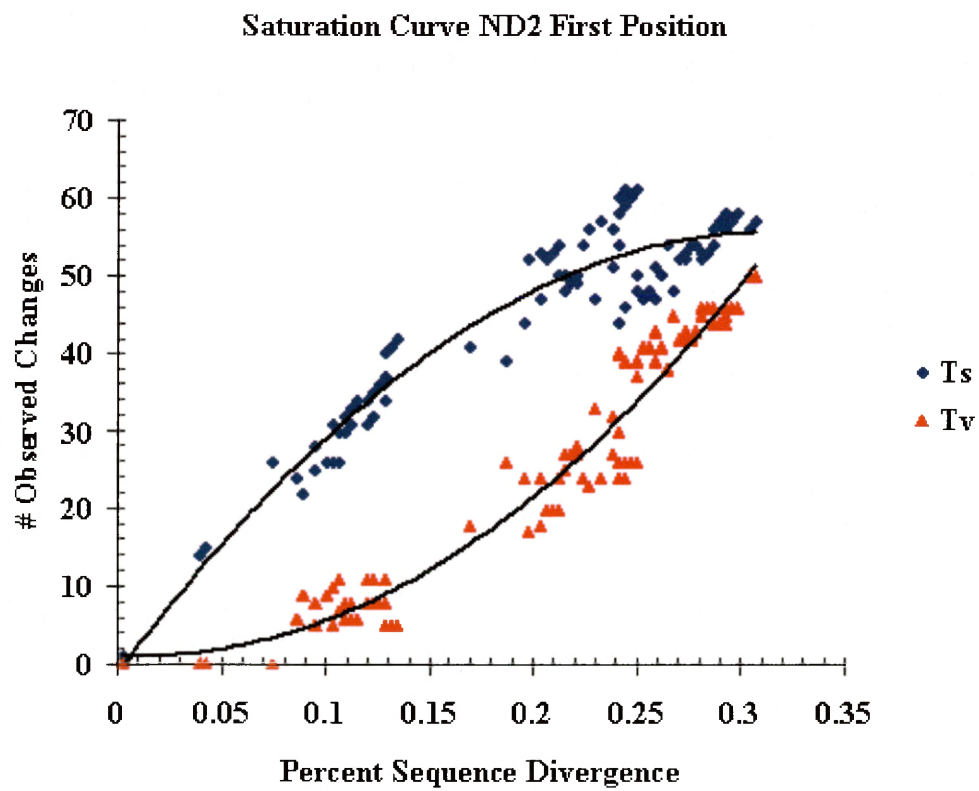
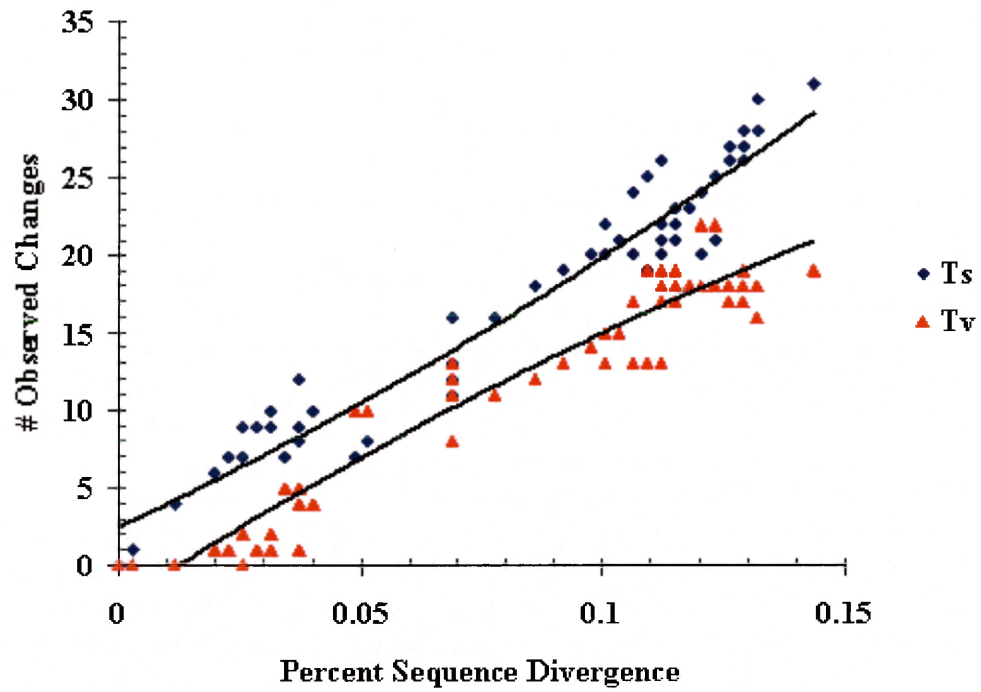
**A****B**

Figure 5. (continued).

C

Saturation Curve ND2 Second Position



D

Saturation Curve ND2 Third Position

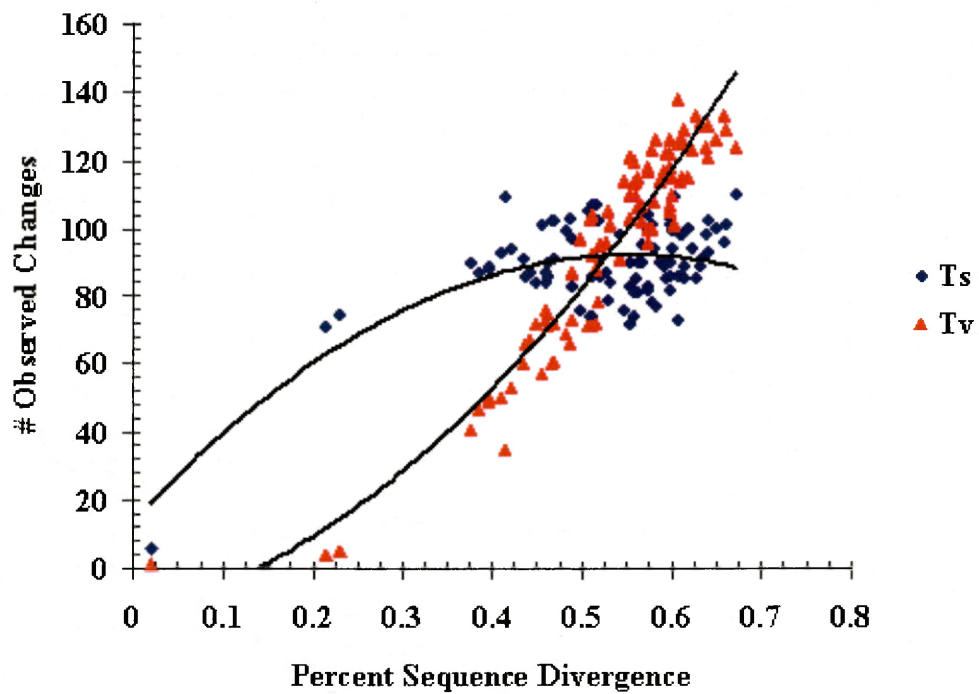


Figure 6. Nucleotide substitution patterns observed in pairwise comparisons of complete ITS-1 gene region sequences in the Echeoidea. Transitions (diamonds) and transversions (triangles) observed were plotted against pairwise uncorrected sequence divergence (p-distance) to explore sequence saturation.



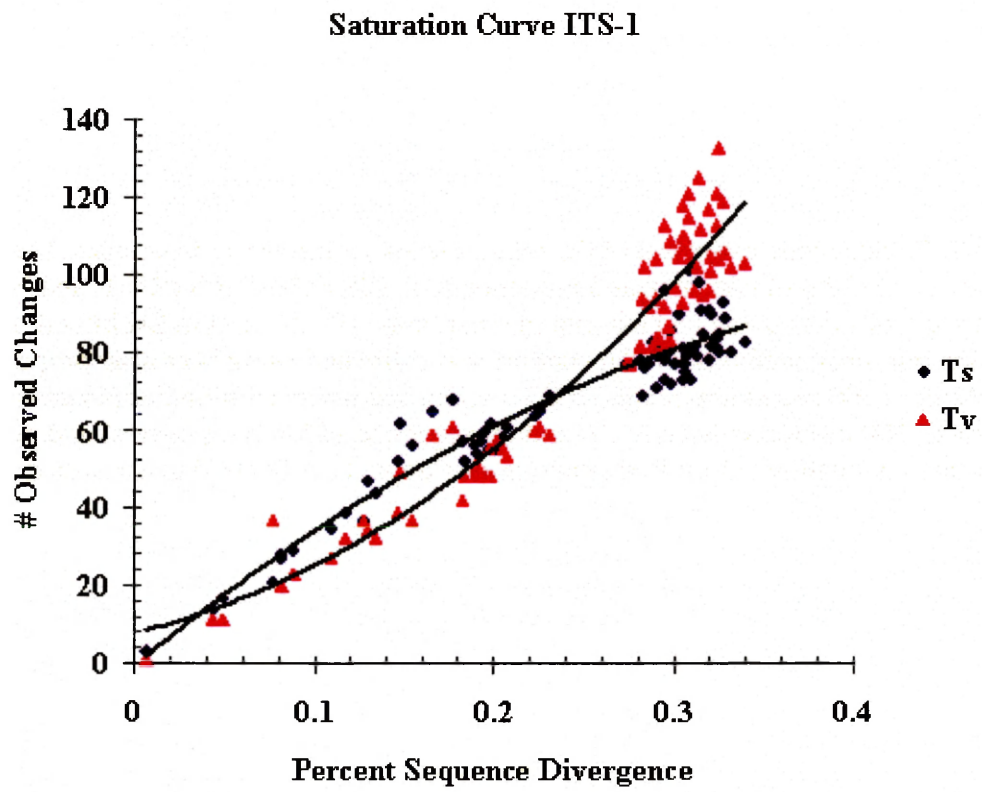


Figure 7. Phylogenetic estimates of the relationships within the Echeneoidea, based upon analyses of 3213 bp of concatenated mitochondrial 12S rRNA, 16S rRNA, and ND2 gene region sequence data using (A) maximum parsimony, (B) maximum likelihood, and (C) Bayesian inference methods. Nodal support was estimated using bootstrap proportions (parsimony: 1000 pseudoreplicates, 1000 random sequence additional replicates; likelihood: 100 pseudoreplicates, 10 random sequence addition replicates) and posterior probability calculations (4 million generations sampled every 1000 generations).

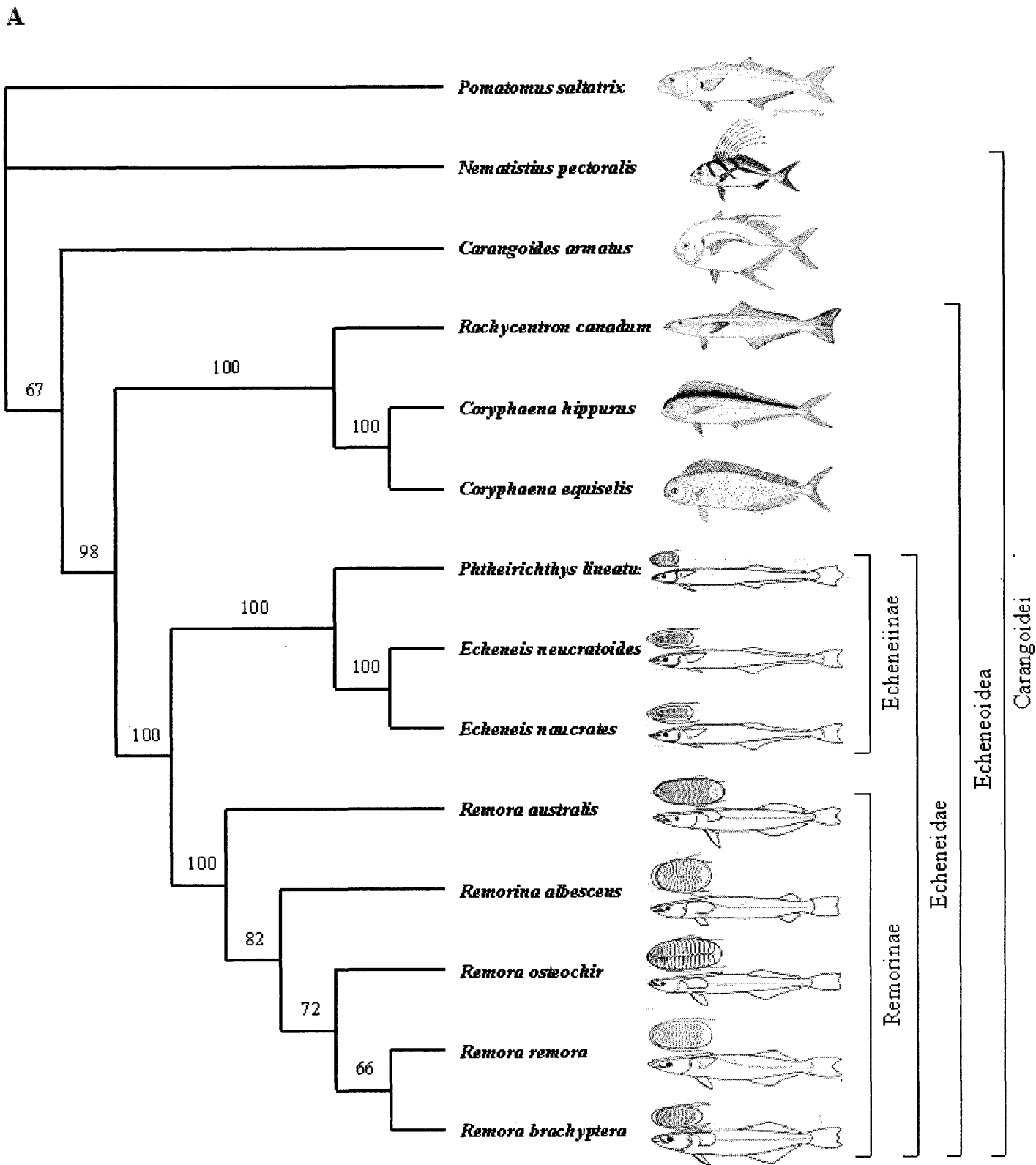


Figure 7. (continued).

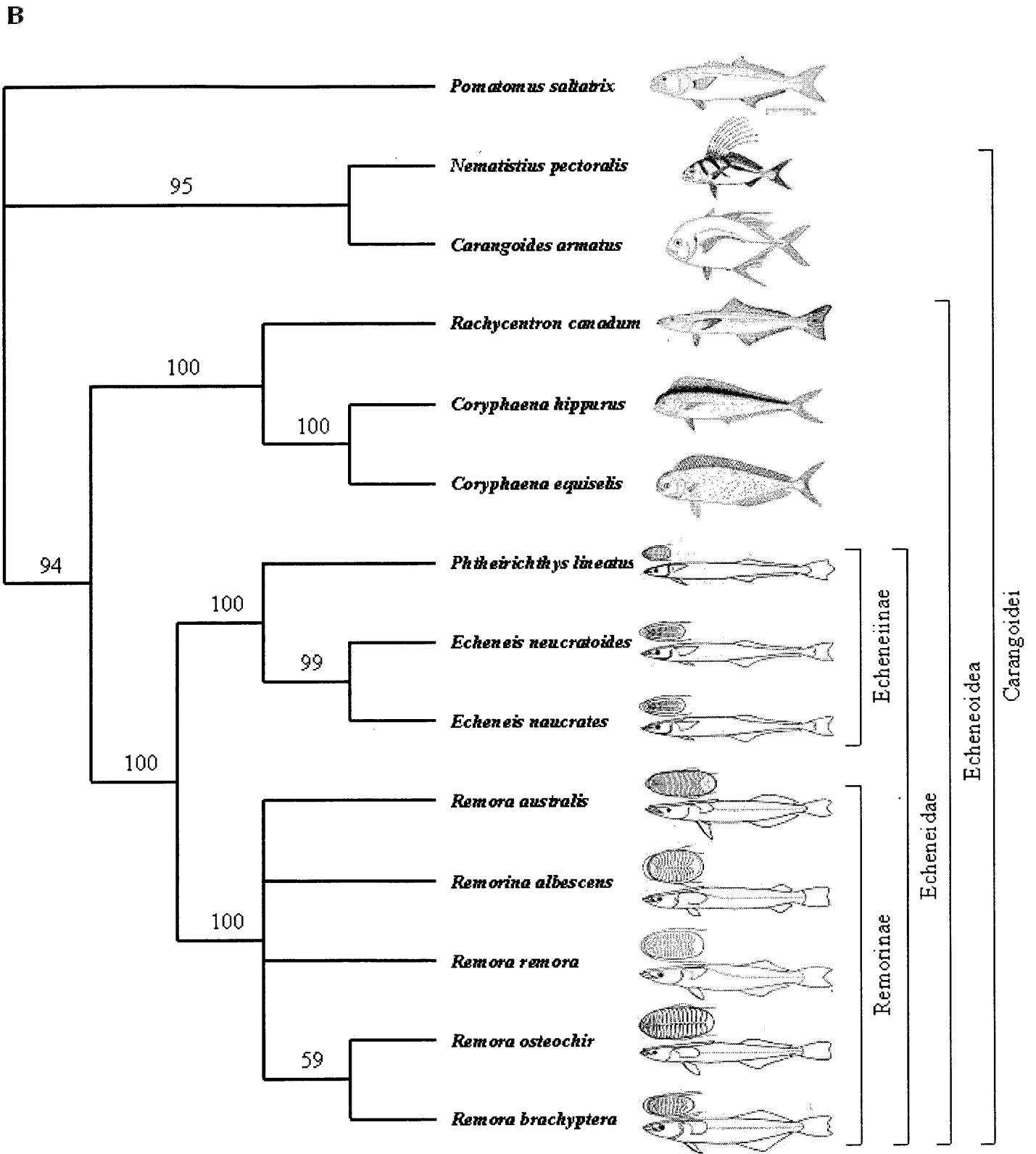


Figure 7. (continued).

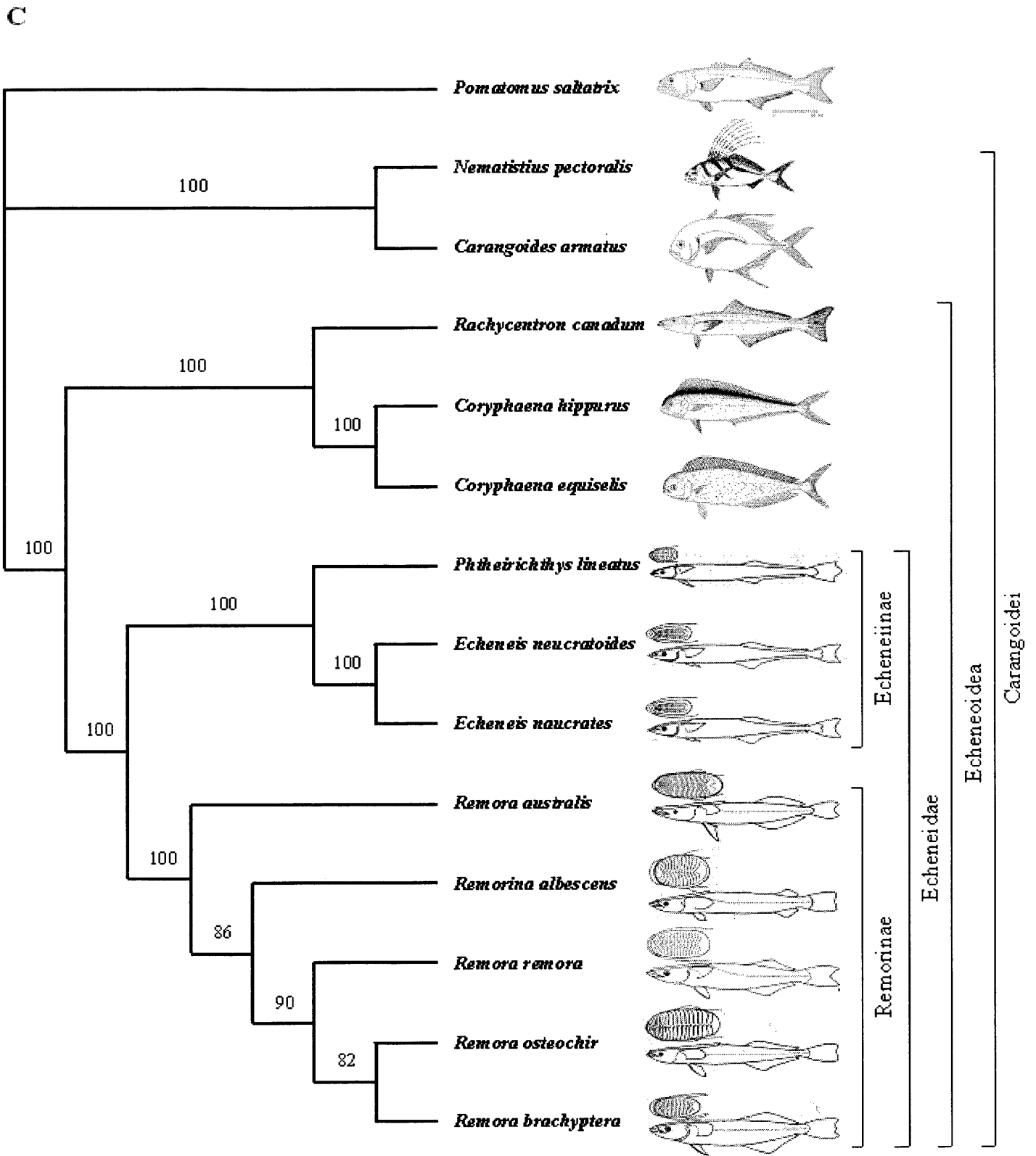


Figure 8. Phylogenetic estimates of the relationships within the EcheNeoidea, based upon analyses of 4223bp of concatenated mitochondrial 12S rRNA, 16S rRNA, ND2, and nuclear ITS-1 gene region sequence data using (A) maximum parsimony, (B) maximum likelihood, and (C) Bayesian inference methods. Nodal support was estimated using bootstrap proportions (parsimony: 1000 pseudoreplicates, 1000 random sequence additional replicates; likelihood: 100 pseudoreplicates, 10 random sequence addition replicates) and posterior probability calculations (4 million generations sampled every 1000 generations). Diagram credit: FISHBASE: <http://www.fishbase.org>, 2005.



A

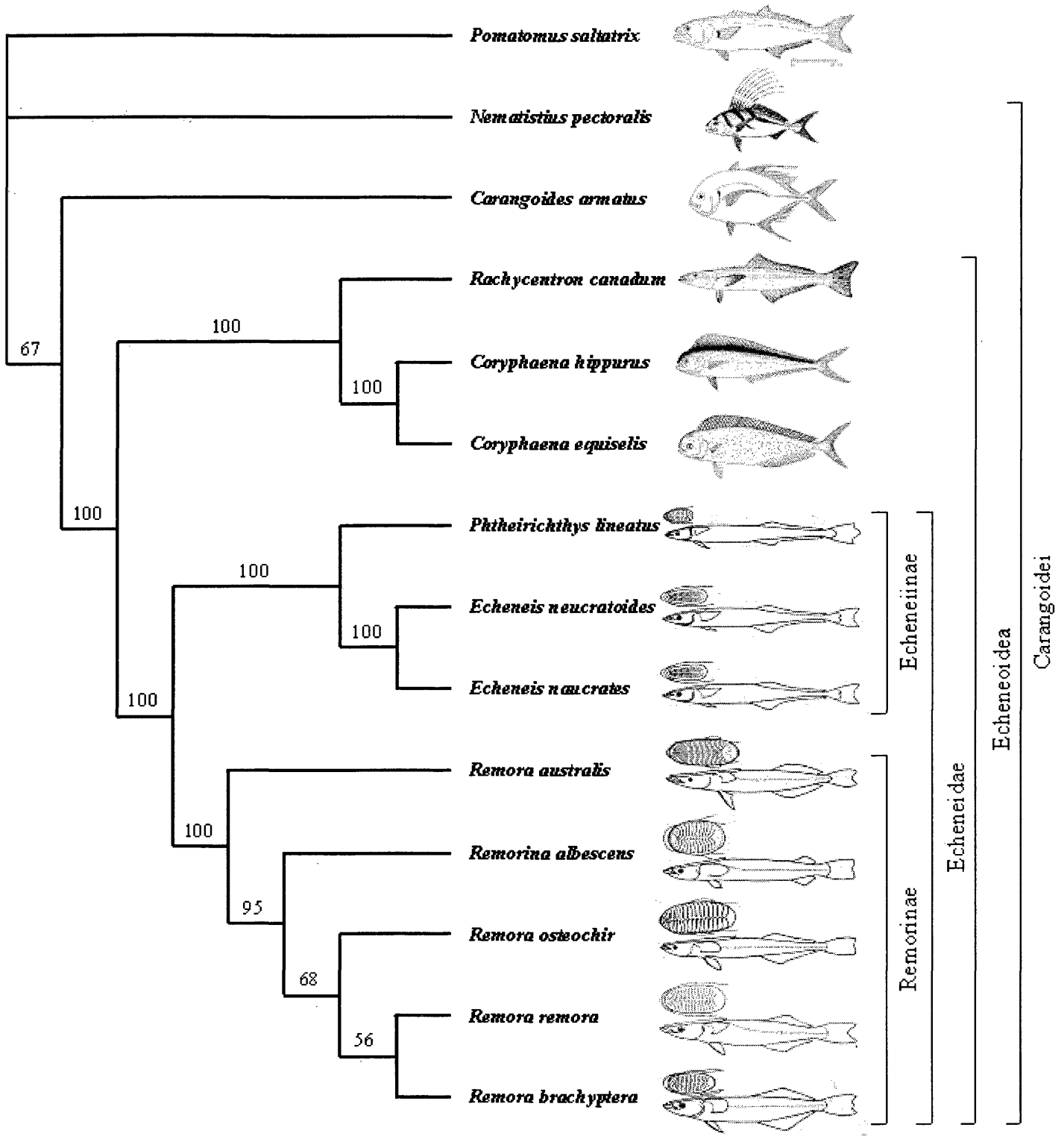


Figure 8. (continued).

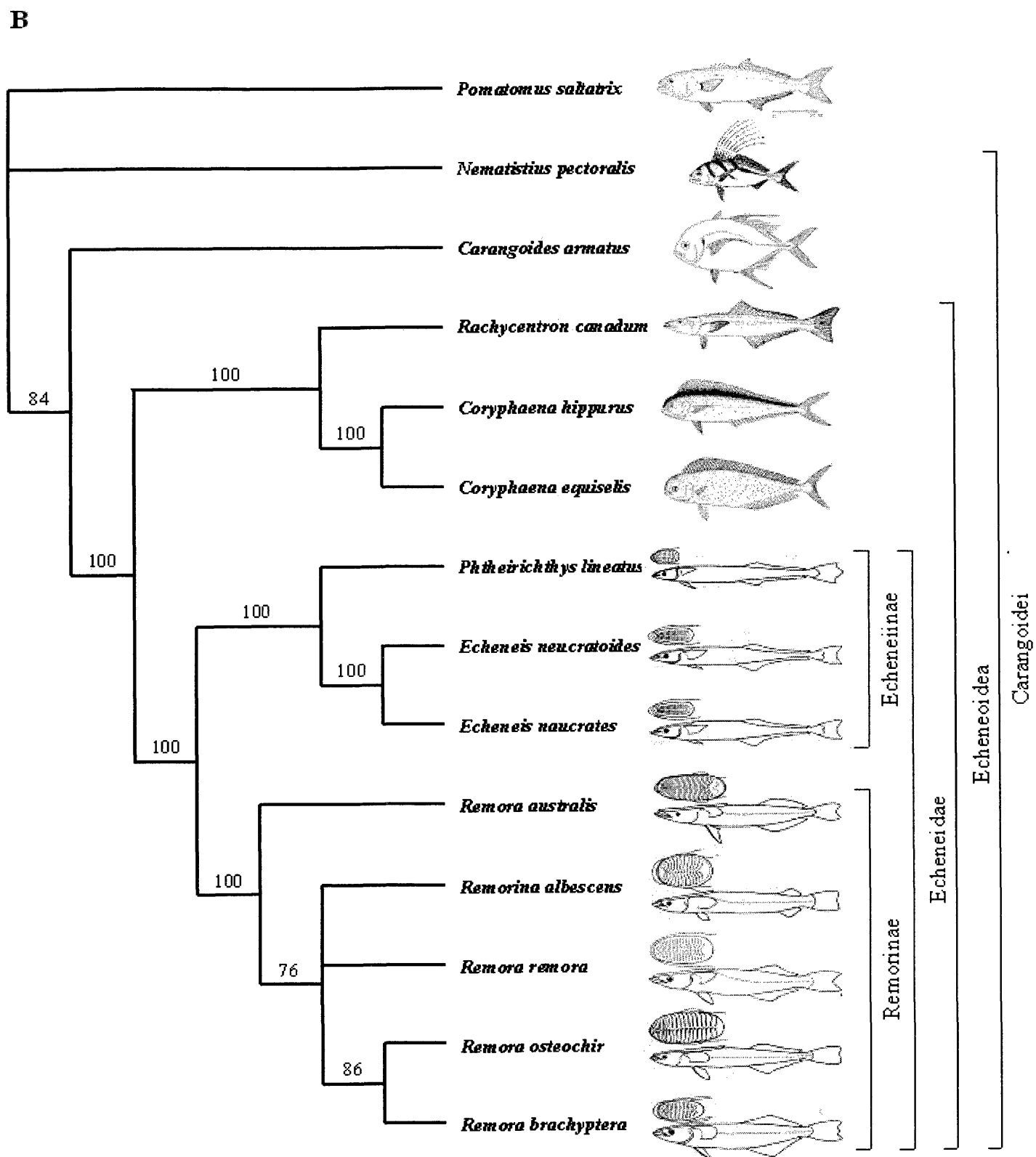


Figure 8. (continued).

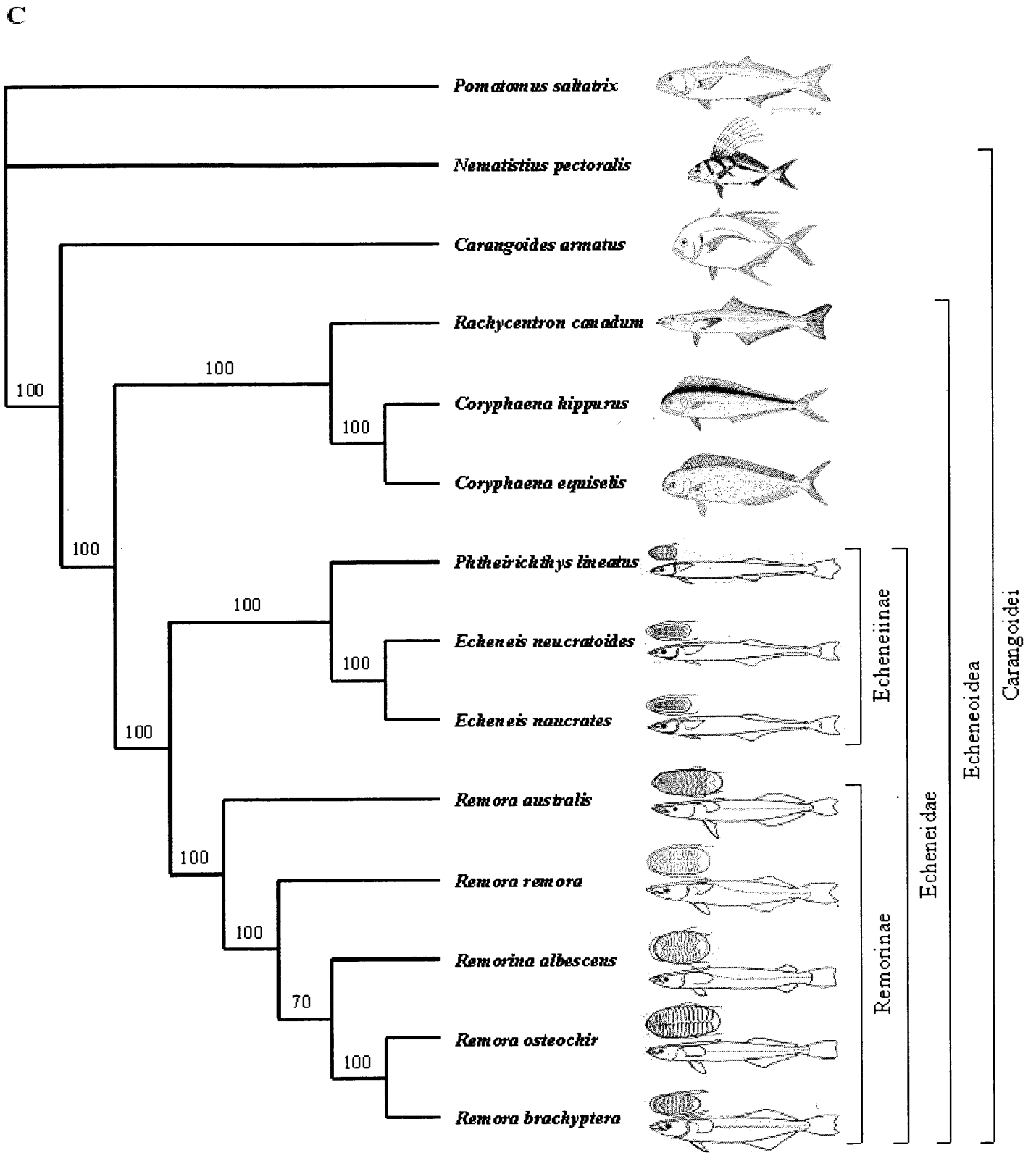
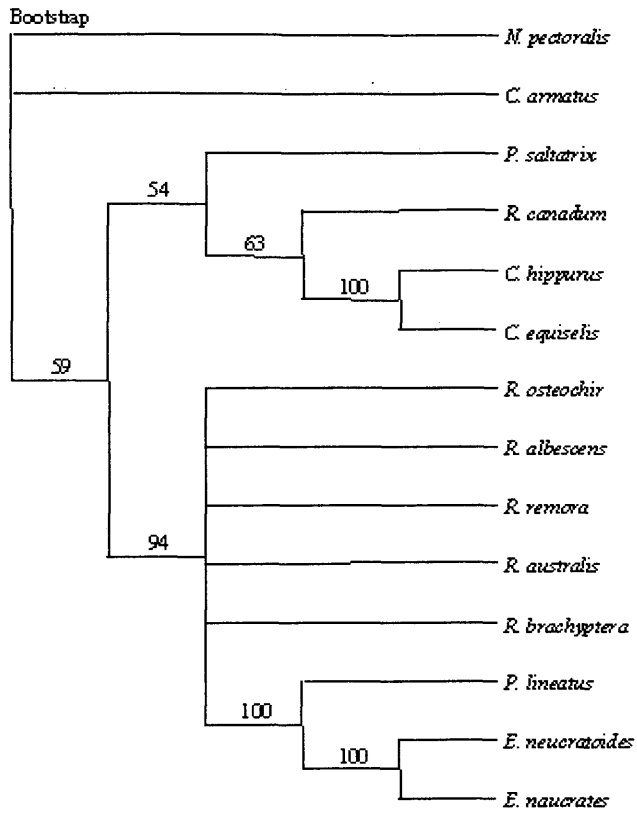
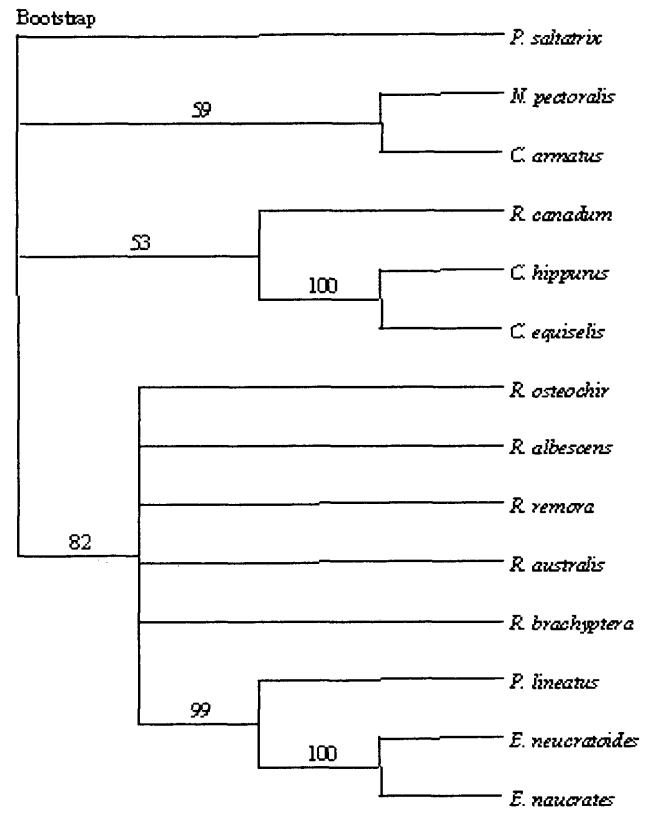


Figure 9. Phylogenetic estimates of the relationships within the Echeneoidea, based upon analyses of 916bp of 12S rRNA gene region sequence data using (A) maximum parsimony, (B) maximum likelihood, and (C) Bayesian inference methods. Nodal support was estimated using bootstrap proportions (parsimony: 1000 pseudoreplicates, 1000 random sequence additional replicates; likelihood: 100 pseudoreplicates, 10 random sequence addition replicates) and posterior probability calculations (2 million generations sampled every 1000 generations).

A



B



C

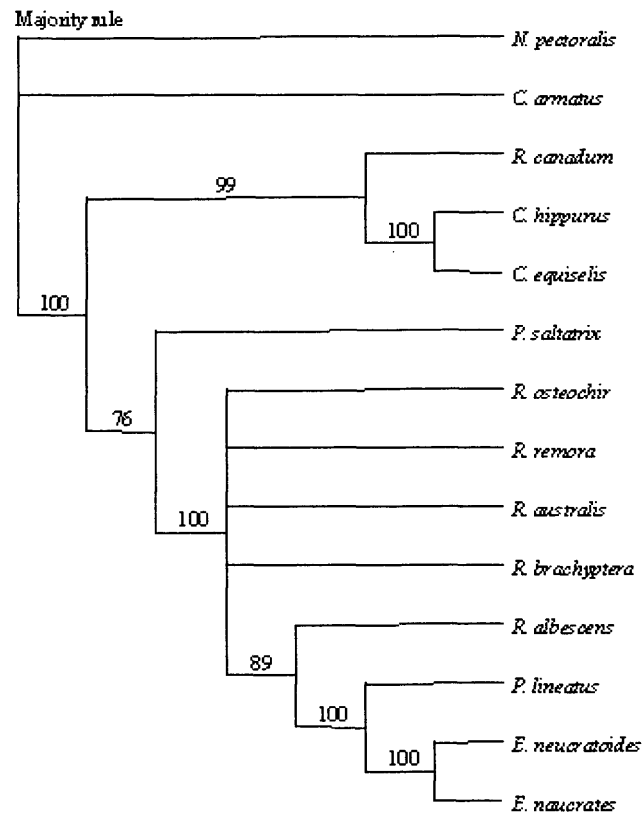


Figure 10. Phylogenetic estimates of the relationships within the Echeneoidea, based upon analyses of 1599bp of 16S rRNA gene region sequence data using (A) maximum parsimony, (B) maximum likelihood, and (C) Bayesian inference methods. Nodal support was estimated using bootstrap proportions (parsimony: 1000 pseudoreplicates, 1000 random sequence additional replicates; likelihood: 100 pseudoreplicates, 10 random sequence addition replicates) and posterior probability calculations (2 million generations sampled every 1000 generations).



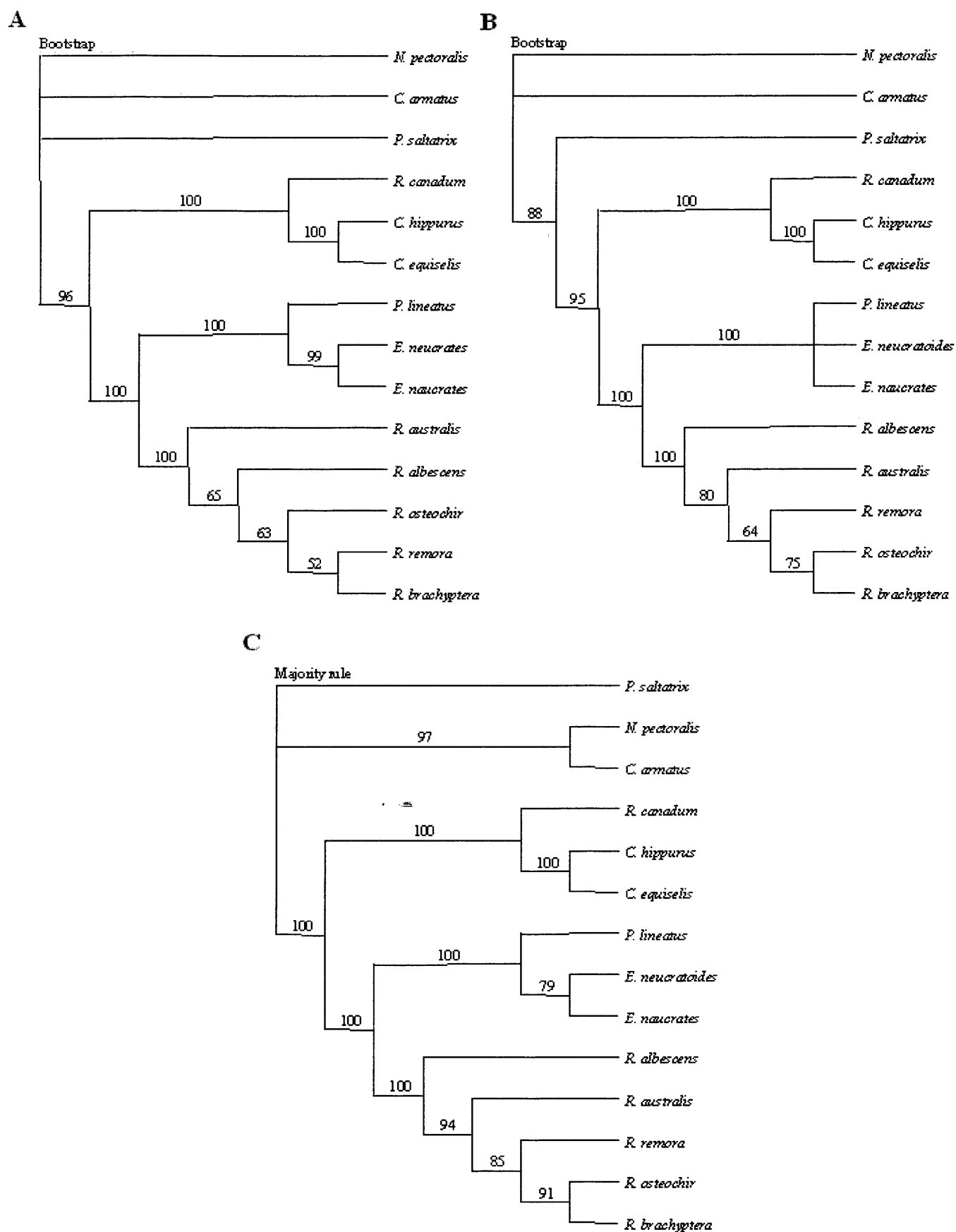
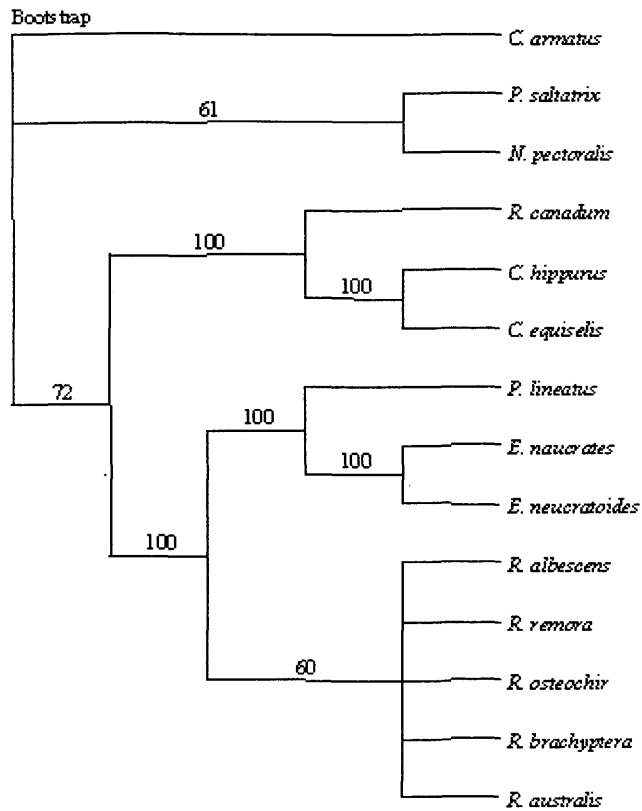
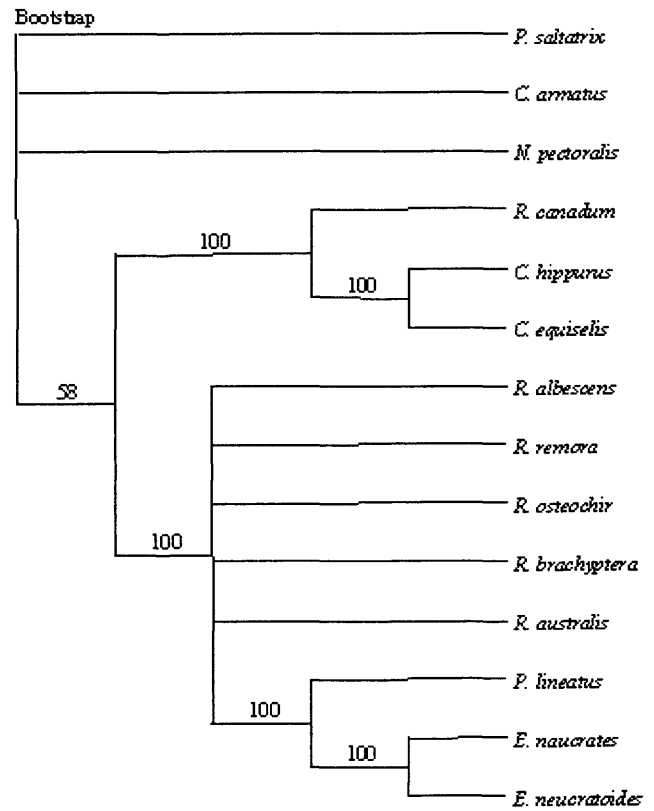


Figure 11. Phylogenetic estimates of the relationships within the Echeneoidea, based upon analyses of 698bp of ND2 gene region sequence data using (A) maximum parsimony, (B) maximum likelihood, and (C) Bayesian inference methods. Nodal support was estimated using bootstrap proportions (parsimony: 1000 pseudoreplicates, 1000 random sequence additional replicates; likelihood: 100 pseudoreplicates, 10 random sequence addition replicates) and posterior probability calculations (2 million generations sampled every 1000 generations).

A



B



C

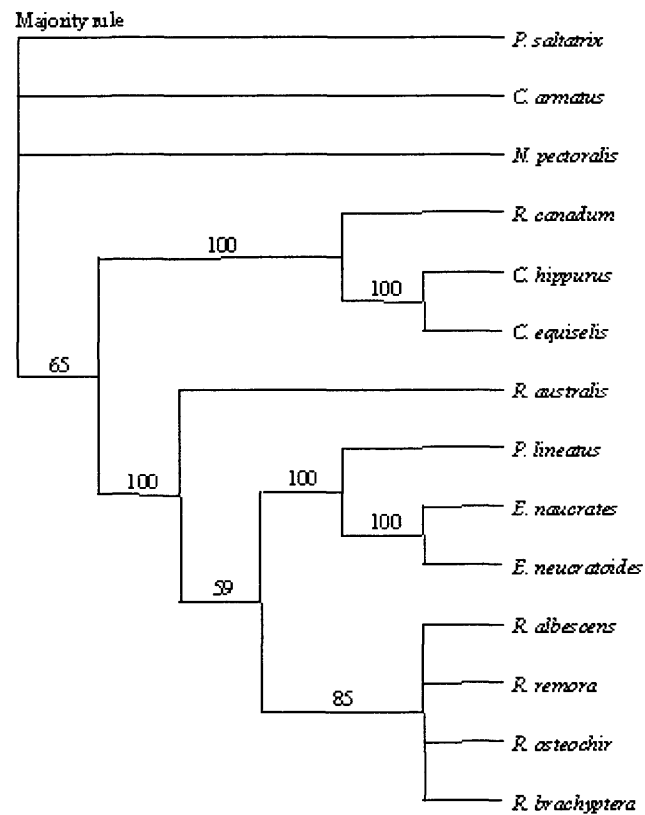
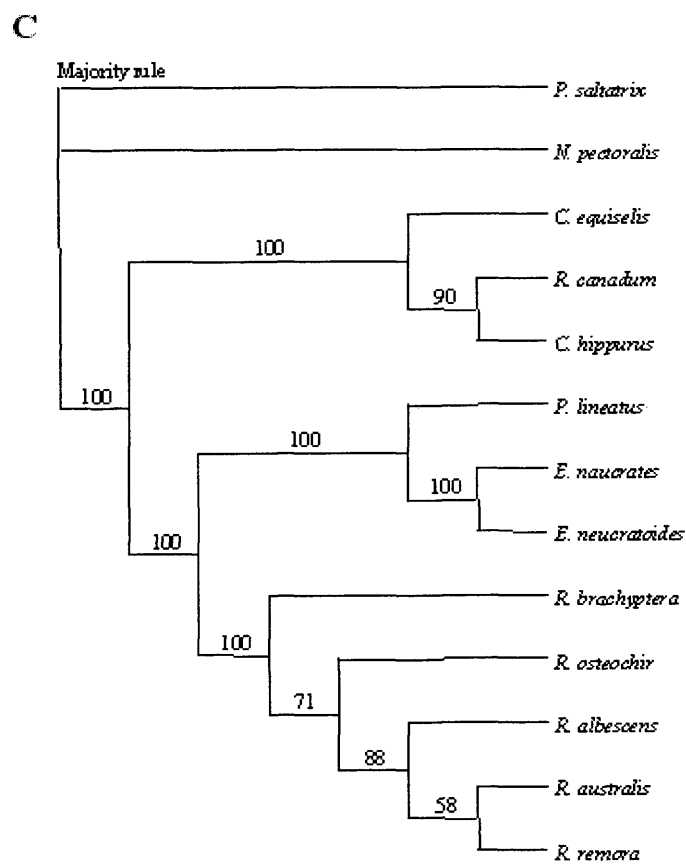
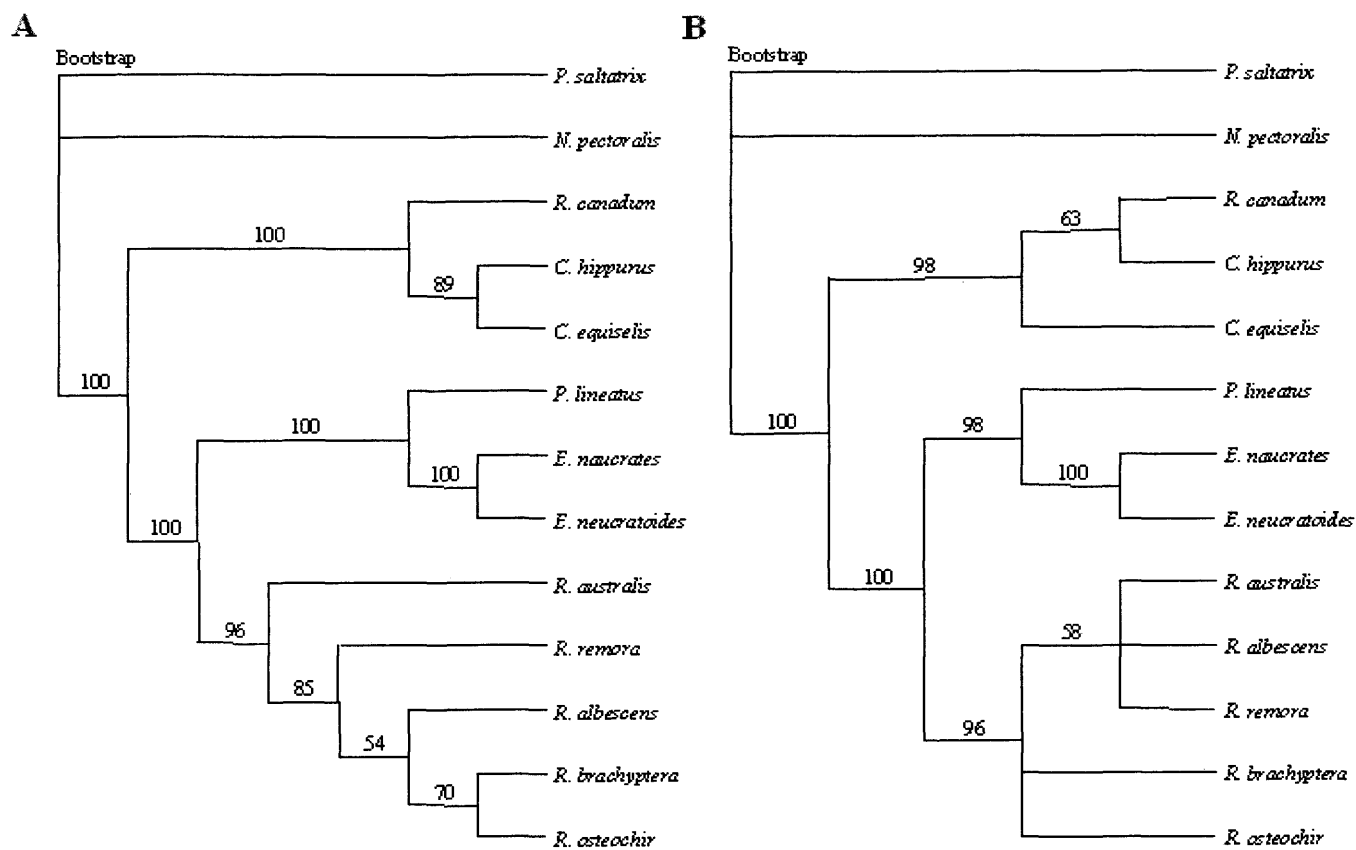


Figure 12. Phylogenetic estimates of the relationships within the Echeneoidea, based upon analyses of 1010bp of ITS-1 gene region sequence data using (A) maximum parsimony, (B) maximum likelihood, and (C) Bayesian inference methods. Nodal support was estimated using bootstrap proportions (parsimony: 1000 pseudoreplicates, 1000 random sequence additional replicates; likelihood: 100 pseudoreplicates, 10 random sequence addition replicates) and posterior probability calculations (2 million generations sampled every 1000 generations).



CHAPTER TWO. AN INVESTIGATION OF POPULATION STRUCTURING  
WITHIN THE FAMILY ECHENEIDAE (PERCIFORMES: CARANGOIDEI)  
INFERRED FROM MITOCHONDRIAL CONTROL REGION  
DNA SEQUENCE ANALYSES.

## INTRODUCTION

### Host-Symbiont Behavior

The Echeneidae (remoras) exhibit unique symbiotic interactions with a diverse group of marine organisms. Facilitated by a modified dorsal fin in the form of a transversely laminated cephalic suction disc, these fishes readily attach to an array of hosts, including bony fishes, sharks, rays, marine mammals and reptiles. Most echeneids attach to the external surface of their hosts, although some species are known to enter the gill cavities (Cressey and Lachner, 1970). Possible benefits of this behavior include protection from predators, access to food resources (e.g. ectoparasites, food scraps), increased reproductive chances, and free transportation (i.e. lower energy expenditure; O'Toole, 2002). It has been shown that the remoras exhibit commensal, mutualistic and potentially parasitic interactions with their marine hosts (Strasburg, 1959; Strasburg, 1962; Strasburg, 1967; Cressey and Lachner, 1970; O'Toole, 2002). Assuming negligible ill effects to the host, the remora-host association is considered commensal on the basis of the factors noted above. The work of Strasburg (1959, 1967) and Cressey and Lachner (1970) demonstrated that this association is often mutualistic given the relative contribution of parasitic copepods (specifically family Caligidae) to the remora's diet. The degree of dependence upon host parasites differs by species and level of development, often changing through ontogeny (Cressey and Lachner, 1970). The presence of

unidentified fish scales in the stomachs of juvenile slender suckerfish, *Phtheichthys lineatus* (Gray, unpublished data), indicates the potential for parasitism. Scale eating behavior (lepidophagy) is common to a number of fish taxa, including cichlids of the genus *Perissodus* (Hori, 1993), Asiatic glassfishes, *Chanda nama* (Grubh and Winemiller, 2004), and cleaner wrasse mimics, the fang tooth blennies of the genus *Plagiotremus* (Moland and Jones, 2004). Pronounced recurved teeth present during the juvenile stages of *P. lineatus* may function as scale removal implements.

Independent of the type of symbiotic interaction, it is evident that host-association is an important behavioral element in the life history of the remoras. The relative importance of host-association, and the degree of host-reliance during development from larvae through adult are topics of great interest. The degree to which host ecology might affect the ecology of the symbiont is completely unknown. This last question is particularly intriguing in the light of the work of Strasburg (1959), Cressey and Lachner (1970), and others who have demonstrated host specificity in this family. In his summary of available host/remora occurrence records published between 1959 and 1996, O'Toole (2002) reported that remoras exhibit a considerable range in both the degree of host specificity and free-swimming behavior (Table 4). Specifically, the members of the subfamily Echeiinae demonstrate generalized host association patterns and elevated free-swimming behavior, particularly as adults. In contrast, the Remorinae exhibit moderate to highly specific host association patterns, and low to moderate levels of free-swimming behavior. Collectively, these characteristics indicate that host ecology may directly influence the ecology of the symbiont. Specifically, structuring within remora populations



(particularly the Remorinae) could be greatly affected, or driven by distribution and movement patterns of their hosts. Moreover, the distribution of remoras that exhibit a high degree of host specificity and depressed free-swimming behavior has the potential to mirror that of their host.

The marlinsucker, *Remora osteochir* (Cuvier, 1829) is an ideal candidate that can be used to contrast host and symbiont population patterns. This “pelagic specialist” (as defined by O’Toole), preferentially associates with istiophorid billfishes (Cressey and Lachner, 1970; O’Toole, 2002). Of 495 host occurrence records, 483 (97.6%) involved istiophorid billfishes (O’Toole, 2002; Table 4; Figure 13). The remaining 2.4% of host association records involve swordfish, *Xiphias gladius* (Linnaeus, 1758), shortfin mako shark, *Isurus oxyrinchus* (Rafinesque, 1810), wahoo, *Acanthocybium solandri* (Cuvier, 1832), dolphin, (*Coryphaena* sp.), ocean sunfish, *Mola mola* (Linnaeus, 1758), and fishing gear (bait, buoys).

Population genetic structure in istiophorid billfishes has been noted between, and in some cases within ocean basins (reviewed in Graves and McDowell, 2003). Based upon studies utilizing allozymes, mtDNA restriction fragment length polymorphism (RFLP) patterns, mtDNA, anonymous single copy nuclear DNA (ascnDNA) sequences, and nuclear microsatellites, it has been demonstrated that both blue marlin (*Makaira nigricans*, Lacepède 1802), and sailfish (*Istiophorus platypterus*, Shaw 1792) exhibit significant levels of genetic partitioning between Atlantic and Pacific oceans. In both blue marlin and sailfish, two distinct evolutionary lineages have been described: one lineage that is restricted to the Atlantic, and a second “ubiquitous” lineage composed of both Atlantic and Pacific individuals.

Significant heterogeneity has also been noted among sailfish collections within the Indo-Pacific (McDowell, 2002). Based upon analyses of both allozymes and mtDNA RFLP patterns (Graves and McDowell, 1994; Graves and McDowell, 2003) and mitochondrial DNA sequences and nuclear microsatellites (McDowell and Graves, unpublished) highly significant levels of population genetic structure within the Indo-Pacific have been inferred in striped marlin (*Tetrapterus audax*, Philippi 1887).

Given their highly specific host-association pattern and depressed level of free-swimming behavior, it is plausible that *R. osteochir* exhibit similar patterns of geographic partitioning to that of their istiophorid hosts. To date, symbioses-dependent structuring has not been explored in these (or any other) marine fishes. To address this intriguing evolutionary question, samples of *R. osteochir* were collected from seven sampling locations spread throughout Atlantic and Pacific oceans. Genetic variability was assayed to infer phylogeographic patterns within this species.

#### Utility of Molecular Markers

Analyses of molecular markers provide a means to infer evolutionary history (Avice, 1994). Using these markers, intraspecific genetic relationships can be evaluated in a geographic context to determine if population structure exists. In light of the forces that influence genetic variation (mutation, migration, selection and genetic drift), these observations can be related to events and processes in the past, as well as the present, to infer the factors likely influencing evolutionary change. In this study, DNA sequence analyses of the hypervariable mitochondrial control region were used to infer marlinsucker phylogeography.

The mitochondrial genome is an ideal candidate for population-level analyses for a number of reasons (reviewed in Avise, 1994; Stepien and Kocher, 1997; Avise, 2001; Hallerman, 2003). The mitochondrial genome is haploid, maternally inherited and does not undergo recombination (crossing over). A single identical mitochondrial genotype (haplotype) is found throughout the organism, except in the rare case of paternal leakage (heteroplasmy) (Magoulas and Zouros, 1993; Avise, 2001). The mitochondrial genome experiences a higher rate of nucleotide substitution than nuclear DNA and has been estimated to approach a rate 5-10 times that of nDNA (Brown et al. 1979; Saccone et al. 1999; Avise, 2001). Furthermore, it has been demonstrated that the non-coding control region, which contains the transcription and replication control elements for the genome, evolves 3-5 times faster than the mitochondrial genome as a whole (Brown, 1993; Avise, 2001). Because the mitochondrial genome is haploid and uniparentally inherited, it has been argued that the mtDNA exhibits an effective population size one-quarter that of nuclear DNA. As such, genetic divergence between isolated populations tends to accumulate more rapidly, assuming an equal rate of mutation, selection, drift and gene flow (Hallerman, 2003). Although debate exists as to the frequency of mitochondrial recombination (Tsaousis et al. 2005), it is sufficiently rare that mtDNA can be used to effectively evaluate historical events and processes. In sum, the mitochondrial genome is quite sensitive to evolutionary pressure and can be exploited to detect population genetic substructure.

Analyses of mitochondrial DNA (mtDNA) sequence variation have been successfully used to address phylogeographic questions in a number of fish taxa.

These include studies pertinent to stock management that have focused on pelagic marine fishes such as swordfish, *X. gladius* (Reeb et al. 2000; Alvarado-Bremer et al. 2005), blue marlin, *M. nigricans* (Buonaccorsi et al. 1999), black marlin, *Makaira indica* (Faltermann, 1999), sailfish, *I. platypterus* (Graves and McDowell, 1995; McDowell, 2002), white marlin, *Tetraodon albidus* (Graves and McDowell, 2001; Graves and McDowell, 2003), striped marlin, *T. audax* (Graves and McDowell, 1994; Graves and McDowell, 2003; McDowell and Graves, unpublished), bigeye tuna, *Thunnus obesus* (Chow et al. 2000; Durand et al. 2005; Martinez et al. in press), bluefin tuna, *Thunnus thynnus* (Carlsson et al. 2004; Alvarado-Bremer et al. 2005), albacore tuna, *Thunnus alalunga* (Vinas et al. 2004), and wahoo, *A. solandri* (Garber et al. 2004).

### Objectives

This study builds upon our understanding of the intraspecific evolutionary relationships within the Echeneidae. Using the marlinsucker, *R. osteochir*, as a case study organism, genetic relationships among geographically distant collections were elucidated. To test the null hypothesis that a single, panmictic population of *R. osteochir* is distributed through Atlantic and Indo-Pacific oceans, DNA sequence analyses of the hypervariable mitochondrial-encoded control region were performed. Intraspecific variation within and among samples collected from geographically distant locations within Atlantic and Pacific oceans was used to infer population structure within this species. The results of this work help to better understand the ecology of *R. osteochir* and the Echeneidae as a whole. A comparison of the

population genetic patterns between host and symbiont provide insight into the factors potentially affecting remora phylogeography.

## MATERIALS AND METHODS

### Sample Collection

Between August, 2002 and April, 2005, over 100 samples of marlinsucker were collected. In all cases, samples were secured by pelagic longline or recreational capture of their istiophorid hosts. Numerous academic, federal, commercial and recreational fishing resources were exploited to sample from seven broad geographic provinces within the marlinsucker's geographic range (five Atlantic and two Pacific; Figure 14). In this study, 71 specimens were used to test for geographic homogeneity. Sampling locations include the following: the western North Atlantic (WNA; n = 6) the Gulf of Mexico/Caribbean Sea (GOM; n = 15), the western Equatorial Atlantic (WEA; n = 13), the eastern Equatorial Atlantic (EEA; n = 12) , the central North Atlantic (CNA; n = 5), the western South Pacific (WPAC) n = 8, and the eastern Equatorial Pacific (EPAC; n = 12). Specific collection information is noted in Appendix 1. Due to logistic constraints, it was not feasible to sample throughout the entire marlinsucker range (e.g. Indian Ocean), although care was made to sample in as wide a range of locations as possible.

Upon capture, whole specimens were placed on ice or immediately frozen to prevent tissue degradation. In most cases, tissue samples were taken at capture and stored in either DMSO tissue storage buffer (0.25 M disodium ethylenediamine-tetraacetic acid (EDTA), 20% dimethyl sulfoxide (DMSO), saturated sodium chloride

(NaCl), pH 8.0) or 95% ethanol. Samples were identified, photographed and processed in the VIMS Fisheries Genetics Laboratory. Voucher specimens were deposited at the Virginia Institute of Marine Science (VIMS) and the National Museum of Natural History (USNM; Appendix 1).

#### DNA Extraction and PCR Amplification

Following the methods of Sambrook et al. (2001), total genomic DNA was extracted from 0.03-0.10g skeletal and/or heart muscle. Tissue was digested at 37°C over night with 15µl proteinase K (25mg/ml), 15µl RNase (10mg/ml), 60µl 10% sodium dodecyl sulfate (SDS) and 500µl isolation buffer (50mM EDTA, 50mM Tris, 150mM NaCl, pH 8.0). Genomic DNA was isolated through a series of washes with equilibrated phenol, phenol/isoamyl alcohol/chloroform (25:1:24) and isoamyl alcohol/chloroform (1:24). DNA was precipitated using an equal volume of isopropanol and 0.04x volume 5M NaCl and pelleted by high-speed centrifugation. DNA was washed with 70% EtOH to remove salts, lyophilized to remove trace EtOH and resuspended in 0.1X TE buffer, pH 8.0. Complete double-stranded nucleotide sequences from the hypervariable mitochondrial control region were amplified following standard polymerase chain reaction (PCR) methodology using *Taq* PCR Core reagents (Qiagen Corp. Valencia, CA) with published universal PCR primers DloopK (5' AGCTCAGCGCCAGAGCGC CGGTC TTGTAAA 3'; Lee et al. 1995), DloopL (5' AGTAAGAGCCCACCATCAGT 3'; Lee et al. 1995), 1CD-Loop(H1) (5' TTGGGTTTCTCGTATGACCG 3'; Cronin et al. 1993) and echeneoid specific primer DloopR1 (5' GCRGATACTTGCATGTCTAART 3'; this study). Primer

locations are depicted graphically in Figure 2. Each 25 $\mu$ l PCR reaction contained the following: approximately 5-25ng purified gDNA, 2.5 $\mu$ l 10X PCR reaction buffer (Tris·Cl, KCl, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 15 mM MgCl<sub>2</sub>; pH 8.7), 0.5 $\mu$ l 10mM dNTP mix (dATP, dCTP, dGTP, dTTP, 10mM each), 0.125 $\mu$ l *Taq* DNA polymerase @ 5 units/ $\mu$ l, 0.5 $\mu$ l bovine serum albumin (BSA) @ 10mg/ml, 10pmoles of each primer. Negative (no DNA) control reactions were set up alongside experimental reaction mixtures to confirm that contamination via extraneous DNA did not occur. PCR amplification conditions consisted of an initial denaturation of 4 minutes at 94°C, followed by 35 cycles of 1 minute at 94°C, 1 minute at 50°C, and 1.5 minutes at 72°C, followed by a final extension of 5 minutes at 72°C (with minor exceptions). All PCR amplifications were performed using an MJ Research PTC-200 thermocycler (Watertown, MA). Products were visualized using an ultraviolet-light transilluminator following electrophoresis through an ethidium bromide stained agarose gel matrix. PCR products were purified by column filtration (QIAquick PCR Purification, Qiagen Corp., Valencia, CA), or by using EXOSAP (USB Scientific, Cleveland, OH) prior to DNA sequencing.

#### DNA Sequencing and Sequence Analyses

Purified PCR products were sequenced in forward and reverse directions following dideoxynucleotide chain termination sequencing methodology developed by Sanger et al. (1977). Samples were sequenced using BigDye Terminator v3.1 Cycle Sequencing reagents (Applied Biosystems, Warrington, UK) with minor modifications of the manufacturer's recommendations. Sequencing reactions were



composed of 10-50ng template DNA, 0.25µl sequencing primer, 0.25µl BigDye master mix, 1µl 5x reaction mix and milli-q water to a final volume of 5µl. Cycle sequencing conditions consisted of an initial denaturation of 1 minute at 96°C, followed by 25 cycles of 10s at 96°C, 5s at 50°C, and 4 minutes at 60°C. Primers used for cycle sequencing were identical to primers used in original PCR amplification reactions. Amplification products were electrophoresed using an ABI 3100 or ABI 3130 DNA sequencer equipped with either a 50cm or 80cm capillary loaded with POP7 or POP4 gel matrix, respectively. Results were analyzed using Sequencing Analysis v. 5.1.1 software (Applied Biosystems, Warrington, UK). Standard chromatogram format (SCF) curves were exported for subsequent analyses. Consensus sequences from multiple SCF sequence files were created using Sequencher 3.0 (Gene Codes Corp., Ann Arbor, MI). Preliminary alignments of consensus sequences were generated using the Clustal W algorithm in MacVector 7.2 (Accelrys Inc., San Diego CA), with default parameters. Minor adjustments were made to align consensus sequences on the basis of conserved sequence motifs. Sequence characteristics including base composition and number of substitutions (as well as relative contribution by transitions, transversions, indels) were calculated in Arlequin v 2.0.4 (Schneider et al. 2000). A different haplotype designation was given to each unique DNA sequence.

### Population Genetic Analyses

An estimate of molecular diversity within each collection was calculated using Arlequin. This includes haplotype diversity ( $h$ ), which represents the probability of

encountering a unique haplotype on repeated draws from the same sample collection, and nucleotide sequence diversity ( $\pi$ ), which represents the average number of differences per site between two sequences sampled from the same collection (Nei, 1987). Divergence between populations was estimated using pairwise nucleotide sequence divergence ( $\delta$ ), which represents the average number of differences per site between two sequences sampled from separate collections. Nucleotide sequence divergence values were used to generate an unweighted pair group method with arithmetic mean (UPGMA) (Sneath and Snokal, 1973) tree to visualize phylogeographic patterns within this species. The control region sequence of the slender suckerfish, *P. lineatus*, was used to root this analysis. Sequence divergence values were used to evaluate geographic structuring of molecular variance. Hierarchical analyses of molecular variance (AMOVA) was employed to partition variation between ocean basins, among collections within ocean basins, and among individuals within collections. Population pairwise  $\Phi_{st}$  values (Excoffier et al. 2002), which are analogous to Wright's F-statistics (Wright, 1978), were calculated and used as a proxy of gene flow. All analyses were performed using the Tamura-Nei model of character evolution. For AMOVA and  $\Phi_{st}$  calculations, the probability of significance was assessed using 10000 permutations of the data. Neutrality tests were performed to infer violation from the neutral theory of molecular evolution (Kimura, 1983). Mismatch distributions (Harpending et al. 1994) were generated using the graphing function in Microsoft Excel using absolute pairwise differences between haplotypes. Neutrality statistics including  $\tau$ ,  $\theta_0$ ,  $\theta_1$ , Harpending's raggedness index (Harpending et

al. 1993), Tajima's D (Tajima, 1989), and Fu's  $F_s$  (Fu, 1997) were estimated in Arlequin using 1000 replicates.

Interclade divergence estimates were used to estimate time since separation and putative secondary colonization of Atlantic marlinsucker using two different methods: a simple back-calculation based upon nucleotide sequence divergence ( $\delta$ ) and an estimated rate of mutation ( $\mu$ ), and a more complex formula based upon coalescence theory. The first formula estimates the time since divergence (T) using the equation  $T = \delta/2\mu$ . The second formula estimates of the amount of time since divergence from a common ancestor (coalescence time, t) using the formula  $t = \tau/2\nu$ , where  $\nu = m\mu$ . Estimates of  $\tau$  were calculated with Arlequin using 1000 replicates. Mutation rate per haplotype ( $\nu$ ) was defined by the product of  $m$ , the total aligned sequence length, and  $\mu$ , the estimated mutation rate (Harpending et al. 1993). A mutation rate of 3.6% per million years, which was estimated using control region variation in amphi-Panamic geminate pairs of snook (Donaldson and Wilson, 1999), was implemented in all analyses. This mutation rate estimate falls between the commonly cited whole mtDNA mutation rate of 2% per million years (Brown et al. 1979), and an empirical estimate based upon the transition (Ts):transversion (Tv) ratio of the hypervariable 5' end of the control region (McMillian and Palumbi, 1997). The empirical estimate was based on a Ts/Tv ratio of 4.3 observed in a 300bp region encompassing the hypervariable region in the marlinsucker, which corresponds to a "moderate" mutation rate (~5% per million years).

## RESULTS

### Sequence Variation

Complete mitochondrial control region sequences were collected from 71 individuals of *R. osteochir*, and ranged from 952 to 959 base pairs (bp) in length. A final alignment of 971 bp exhibited 223 polymorphic sites (23%), which included 160 transitions, 59 transversions and 32 indels. Sequence variability was unequally distributed across the control region. The 5' (tRNA<sup>Pro</sup>) region demonstrated the highest degree of polymorphism, although additional highly variable segments were spread throughout the gene region, interspersed between segments that exhibited high sequence conservation (Figure 15). Transition:transversion ratio across the entire control region was estimated at 2.8. Average nucleotide composition was biased towards adenine and thymine. Relative contribution of each nucleotide was approximately 32.9% A, 20.8% C, 13.8% G, and 32.5% T. Seventy-one unique haplotypes were observed in the 71 samples examined. The absolute number of differences between haplotypes ranged from 2 to 62 (mean = 34.34, SE = 0.26) and nucleotide sequence divergence between haplotypes ranged from 0.21 to 6.71% (mean = 3.49%, SE = 0.02; Table 5, Appendix 6).

### Genetic Diversity

High levels of haplotype ( $h$ ) and nucleotide sequence diversity ( $B$ ) were estimated at each of the seven sampling locations (Table 5). Given that 71 unique haplotypes were found, haplotype diversity was unity at all locations. Nucleotide sequence diversity, which better captures the amount of genetic variation at each location, ranged from 1.95 to 3.76% (overall mean = 3.67%, SE = 0.0003; Table 5). The lowest level of sequence diversity was found in the CNA collection, which coincidentally had the smallest sample size ( $n = 5$ ). The mean number of pairwise differences between individuals collected in this region was also low (18.6) compared to the other sampling locations (mean = 26.3). The highest level of diversity was found in the GOM sample, in which the mean number of pairwise differences between haplotypes was 36.1. Within the Atlantic, corrected nucleotide sequence divergence between sample locations ranged from zero to 0.82% (GOM:CNA; Table 6). In the Pacific, average corrected nucleotide sequence divergence between samples collected in eastern and western sampling locations measured 0.58%. Between Atlantic and Pacific collections, the lowest corrected nucleotide sequence divergence was found between CNA and WPAC (0.00%), and the highest level of divergence was found between GOM and WPAC (0.89%; Table 6).

### Clade Distribution

Evidence of structuring was noted in the UPGMA tree generated from pairwise nucleotide sequence divergence values (Figure 16). Although tree topology could not resolve a clear relationship between sampling location and genetic

relatedness, two distinct lineages were resolved: one lineage (Clade I) was composed of only Atlantic specimens, whereas the other lineage (Clade II) was composed of both Atlantic and Pacific samples. Clade I included 24 haplotypes from four Atlantic sampling locations (no Clade I haplotypes were found at the CNA sampling location). Clade II included 47 haplotypes represented in all seven sampling locations (27 Atlantic and 20 Pacific; Table 7). Relative contribution of Clade I and Clade II haplotypes at each sampling location is depicted graphically in Figure 17. When all haplotypes were considered, roughly equal estimates of nucleotide sequence diversity were calculated for Clades I and II (2.46 and 2.44%, respectively; Table 5). Between clades, average corrected nucleotide sequence divergence was 2.30% (4.75% uncorrected), and a  $\Phi_{st}$  of 0.485 ( $p < 0.0001$ ) was estimated.

Based upon clade organization, three subgroups were defined to further evaluate the level of differentiation between haplotypes: Atlantic-I (Atlantic samples found in Clade I), Atlantic-II (Atlantic haplotypes found in Clade II) and Pacific. Corrected nucleotide sequence divergence between Atlantic-II:Pacific groups was estimated at 0.11%, whereas divergence between both Atlantic-I:Atlantic-II and Atlantic-I:Pacific pairs was nearly twenty times as great (2.31 and 2.35%, respectively; Table 7). Within the Atlantic, neither Atlantic-I nor Atlantic-II haplotypes demonstrated significant genetic differentiation by sampling location (Table 8).  $\Phi_{st}$  values between Atlantic-I haplotypes binned by sampling location ranged from  $-0.0704$  to  $0.0346$  ( $p \gg 0.05$ ).  $\Phi_{st}$  values between Atlantic-II haplotypes binned by sampling location ranged from  $-0.1262$  to  $0.0605$  ( $p \gg 0.05$ ).

AMOVA analyses resolved 100% of the variance within sampling locations (Table 9).

### Phylogeography and Population Structuring

Analyses of molecular variance demonstrated significant genetic heterogeneity between Atlantic and Pacific collections (Table 9). When haplotypes were binned into Atlantic and Pacific groups, a highly significant proportion (14.88%) of the variance was calculated between ocean basins ( $p = 0.0001$ ). When haplotypes were binned into collections nested within ocean basins (Atlantic  $n=5$ , Pacific  $n=2$ ), 14.66% of the variance was estimated between ocean basins ( $p < 0.0001$ ). A minor amount of the variance (0.64%,  $p < 0.001$ ) was accounted for by differences between collections within ocean basins. The majority of the variance (84.7%) was partitioned among individuals within collections.

Population pairwise  $\Phi_{st}$  analyses resolved significant levels of population differentiation between both the Atlantic and Pacific samples and one sub-region pair within the Atlantic (Table 8). Elevated  $\Phi_{st}$  values (0.1137-0.2105,  $p < 0.05$ ) were estimated between all Atlantic and Pacific collections, with the exception of CNA:WPAC and CNA:EPAC pairs. Both CNA-Pacific pairwise  $\Phi_{st}$  values were negative and non-significant ( $p > 0.5409$ ). Pairwise  $\Phi_{st}$  values among Atlantic sampling locations ranged from -0.0550 to 0.1707. All values were non-significant, except between CNA and GOM, where a  $\Phi_{st}$  of 0.1707 ( $p = 0.0243$ ) was estimated. No evidence of structure was noted between eastern and western Pacific collections ( $\Phi_{st} = -0.0245$ ,  $p = 0.7998$ ).

No evidence of structuring in the Atlantic was resolved when marlinsucker samples were binned by istiophorid host (Figure 18). Since only one blue marlin and one swordfish-marlinsucker pair were observed, only three host classes were specified (white marlin, WHM; sailfish, SAI; and spearfish, SPR). Population pairwise  $\Phi_{st}$  values in all pairwise comparisons were negative and non-significant (Table 10). Analyses of molecular variance (AMOVA) resolved 100% of the variance within collections (Table 11).

#### Neutrality and Population Demography

Significant differences were observed in the mismatch distributions estimated from pairwise comparisons of marlinsucker haplotypes. Overall, a bimodal mismatch distribution was resolved when Atlantic and Pacific haplotypes were binned together (Figure 19). When binned by clade, Clade I haplotypes demonstrated a ragged, multimodal distribution, whereas Clade II haplotypes exhibited a broad unimodal distribution. Both Atlantic-I and Pacific haplotypes demonstrated broad multimodal mismatch distributions, whereas Atlantic-II haplotypes exhibited a nearly unimodal distribution. Estimates of  $\tau$  differed significantly by haplotype binning strategy: when binned by ocean basin, clade, and clade within ocean basin, the lowest values of  $\tau$  were estimated in Pacific, Clade II and Atlantic-II subgroups, respectively. Large differences between  $\theta_0$  and  $\theta_1$  were noted in all combinations of marlinsucker haplotypes. Both Harpending's raggedness index and Tajima's D estimates were non-significant ( $p > 0.08$ ) for all binned haplotype classes. Fu's  $F_s$  values were negative (-6.2212 to -24.1513) and highly significant in all cases ( $p < 0.008$ ; Table 12).



### Cladogenesis and Putative Recolonization

Using a mutation rate of 3.6% per million years (Donaldson and Wilson, 1999), cladogenesis was estimated to occur between 0.33 and 0.46 million years ago using Atlantic-I and Pacific  $\tau$  estimates ( $\tau = 31.891$  and  $23.204$ , respectively). Based upon corrected nucleotide sequence divergence ( $\delta$ ) between Atlantic-I and Pacific haplotypes (0.02353), cladogenesis occurred approximately 0.33 million years ago (Table 13). Putative recolonization of the Atlantic was estimated to occur 0.22 million years ago using the  $\tau$  value estimated from Atlantic-II haplotypes. In contrast, recolonization was estimated to occur 16 000 thousand years ago using corrected nucleotide sequence divergence ( $\delta$ ) estimated between Atlantic-II and Pacific haplotypes (0.00114).

## DISCUSSION

### Sequence Variation

The mitochondrial control region of the marlinsucker, *R. osteochir*, displayed sequence characteristics similar to those examined in other fish taxa. The size of the non-coding region (952 to 959bp) is slightly larger than that noted in swordfish (835bp; Rosel and Block, 1996), bluefin tuna (820-860bp; Carlsson et al. 2004), wahoo (889-894bp; Garber et al. 2005) and sailfish (839-855bp; McDowell, 2002), but falls within the range noted in Percidae (888-1223bp; Faber and Stepien, 1997). Consistent with the observations of Saccone et al. (1999), average nucleotide composition was biased towards adenine and thymine, which together accounted for 65.4% of the total nucleotide usage. As with the mtDNA of other vertebrates (reviewed in Meyer, 1995), there was a transitional bias. A transition:transversion ratio of 2.8 was estimated over the entire control region, which is consistent with observations in brook charr, *Salvelinus fontinalis* (2.3; Bernatchez and Danzmann, 1993) and red drum, *Sciaenops ocellatus* (3.4; Seyoum et al. 1999). Nucleotide sequence variability was not equally distributed across the entire control region. The region of highest variability was located in the 5' (tRNA<sup>Pro</sup>) region, which is congruent with observations in swordfish, *Xiphias gladius* (Reeb et al. 2000; Rosel and Block, 1996), white sturgeon, *Acipenser transmontanus* (Brown et al. 1993), wahoo, *A. solandri* (Garber et al. 2005), and ninespine stickleback of the genus

*Pungitius* (Takahashi and Goto, 2001), Overall, the level of nucleotide polymorphism (23%) across the entire marlinsucker control region is comparable to that seen in partial control region sequences analyzed from red snapper (22%; Garber et al. 2004), and red drum (22%; Seyoum et al. 1998) and slightly lower than the degree of polymorphism observed in swordfish (30%; Alvarado-Bremer et al. 1995) and albacore tuna (35%; Vinas et al. 2004). Both the level of polymorphism and the magnitude of divergence noted between haplotypes indicate that the control region contains sufficient genetic variation to infer population heterogeneity in this species.

### Genetic Diversity

All sampling locations displayed extremely high levels of haplotype diversity and moderate levels of nucleotide diversity. Haplotype diversity estimates of unity at all locations were unexpected, although not unrealistic for the control region. Analyses of control region sequences from other cosmopolitan pelagic fishes including swordfish (Alvarado-Bremer et al. 1996; Rosel and Block, 1996), bluefin tuna (Carlsson et al. 2004; Alvarado-Bremer et al. 2005), albacore tuna (Vinas et al. 2004), and wahoo (Garber et al. 2005), have also revealed high levels of haplotype diversity ( $h = 0.99$ ). Population-wide nucleotide diversity estimates in marlinsucker (1.95 to 3.76% at seven sampling locations, 3.67% overall), are also consistent with observations in other species, including swordfish (3.45%; Rosel and Block, 1996), bluefin tuna (1.5%; Carlsson et al. 2004; 4.3%; Alvarado-Bremer, 2005), albacore tuna (5.4%; Vinas et al. 2004), and wahoo (5.3%; Garber et al. 2004).

On the basis of elevated haplotype and mean nucleotide sequence diversities across all sample collections, (with the exception of the CNA collection, which shows a moderate level of nucleotide diversity) it is unlikely that either Atlantic or Pacific populations were recently colonized. High  $h$  and high  $\pi$  patterns are indicative of a long time interval since the split from a common ancestral haplotype (Hallerman, 2003). A recent colonization event is usually characterized by large number of haplotypes (high  $h$ ) that differ from each other by only a few base pairs (low  $\pi$ ) and often exhibit a star shaped haplotype phylogeny (Slatkin and Hudson, 1991; Avise, 2001). Observations from this study do not follow these patterns, namely the high estimates of both  $h$  and  $\pi$ , large absolute number of differences between haplotypes (range = 2 – 62, mean = 34.34), and the distribution of haplotypes in the UPGMA phylogenetic tree.

### Clade Distribution

The most intriguing result of the present study was the resolution of two distinct marlinsucker mtDNA lineages: Clade I composed of samples restricted to the Atlantic, and Clade II composed of both Atlantic and Pacific samples. These observations are congruent with observations in Atlantic populations of blue marlin (Graves and McDowell, 1995; Buonaccorsi, 2001), sailfish (Graves and McDowell, 1995; McDowell, 2002), bigeye tuna (Chow et al. 2000; Durand et al. 2005; Martinez et al. 2005) and swordfish (Alvarado-Bremer et al. 1996; Rosel and Block, 1996; Alvarado-Bremer et al. 2005). Within the Mediterranean, this pattern has also been demonstrated in albacore tuna (Vinas et al. 2004). In this study, nearly equal numbers

of Clade I and Clade II haplotypes were resolved overall (24 Clade I, 27 Clade II), although a homogeneous distribution of haplotypes by sampling location was not observed. Within the Atlantic, the relative contribution of Clade II haplotypes at each sampling location ranged from 40% to 100%, although no significant geographic pattern to the haplotype distribution was found.

Potential explanations for the presence of two mitochondrial lineages in highly vagile, cosmopolitan fishes such as swordfish, billfish and tunas have been offered by a number of authors. Theories used to explain this pattern include mitochondrial introgression (Manchado et al. in press) and stochastic extinction of haplotypes (Vinas et al. 2004). The most frequently used explanation in fishes (and perhaps the most logical explanation for the patterns seen in marlinsucker), involves vicariant isolation during the Pleistocene (Graves and McDowell, 1995; Chow et al. 2000; Buonaccorsi et al. 2001; Durand et al. 2004; Alvarado-Bremer et al. 2005; Martinez et al. in press), secondary contact of populations that have evolved in allopatry, followed by subsequent isolation.

Three vicariant events that occurred during the last 20 million years have been postulated to be responsible for the isolation between tropical and subtropical marine fishes of the Atlantic and Pacific: the closure of the Tethys Seaway (15-20mya), the rise of the Isthmus of Panama (3.1-3.5mya) and the development of the Benguela upwelling system off South Africa (2.0-2.5mya) (Bermingham et al. 1997; Bowen et al. 2001; Rocha et al. 2005). Recent studies have shown that the Benguela upwelling is somewhat permeable, and that contact between Atlantic and Indian Ocean fauna has occurred since its inception (Graves and McDowell, 1995, 2003; Vermeij and

Rosenberg, 1993; Chow et al. 2000; Buonaccorsi et al. 2001; McDowell, 2002; Peeters et al., 2004; Durand et al. 2005; Martinez et al. 2005). Secondary contact involving unidirectional migration across the Benguela upwelling barrier has been used to explain the presence of two separate mtDNA lineages in a number of pelagic fishes in the Atlantic (Chow et al. 2000; Buonaccorsi et al. 2001; McDowell, 2002; Graves and McDowell, 2003; Durand et al. 2005; Martinez et al., in press).

Periods of secondary contact likely occurred during warm interglacial periods, when tropical/subtropical marine habitat was less constricted and the cold Benguela upwelling barrier was somewhat more permeable (Bowen et al. 2001; Graves and McDowell, 2003; Rocha et al. 2005). Westward traveling warm-water eddies that develop at the mixing zone between the Benguela current of the South Atlantic and the Agulhas current of the western Indian Ocean likely provided a vector for eggs, larvae, juvenile and adult fishes to be introduced into the South Atlantic (Bowen et al. 2001; Peeters et al. 2004; Rocha et al. 2005). During the last 2 million years, glacial-interglacial cycles have occurred on the order of every 100,000 years (with multiple transient heating/cooling events in between), suggestive of numerous potential inter-ocean contact periods between isolated populations of pelagic fishes of the Atlantic and Pacific (Fennel, 1999; Petit et al. 1999; Peeters et al. 2005; Rocha et al. 2005).

Results of the present study support the theory that Atlantic marlinsucker were isolated from their Pacific conspecifics, and subsequently brought back into contact in the relatively recent geological past. Furthermore, these results support the hypothesis that the Atlantic population was secondarily colonized by Indo-Pacific individuals, although it is unclear when these event(s) occurred. This hypothesis is supported by

several lines of evidence. Overall, the level of genetic diversity estimated in the Atlantic samples ( $\pi = 0.0346$ ) was substantially more than that estimated in the Pacific samples ( $\pi = 0.0273$ ). Two significantly different mitochondrial lineages (Clades I and II) were resolved in the Atlantic ( $\Phi_{st} = 0.485$ ,  $p < 0.0001$ ), only one of which was found in the Pacific. The level of divergence between collections of Atlantic-II (putative Atlantic recolonists) and Pacific haplotypes ( $\delta = 0.11\%$ ,  $p = 0.0055$ ;  $\Phi_{st} = 0.044$ ,  $p = 0.0041$ ) was significantly lower than that measured between collections of Atlantic-I and Pacific haplotypes ( $\delta = 2.35\%$ ,  $p < 0.0001$ ;  $\Phi_{st} = 0.472$ ,  $p < 0.0001$ ). Furthermore, the level of divergence between collections of Atlantic-II and Pacific haplotypes was significantly more than the level of divergence resolved between Pacific sampling locations ( $\delta = -0.01\%$ ,  $p = 0.7262$ ;  $\Phi_{st} = -0.0245$ ,  $p = 0.7998$ ). Analyses of Atlantic-II haplotypes resolved a nearly unimodal mismatch distribution curve, whereas broad multimodal mismatch distributions were resolved in analyses of both Atlantic-I and Pacific haplotypes.

#### Phylogeography and Population Structuring

Patterns of genetic differentiation between samples of marlinsucker collected from seven locations in the Atlantic and Pacific indicate that marlinsucker exhibit significant levels of population structuring between ocean basins, but negligible geographic heterogeneity within ocean basins. Both population pairwise fixation indices and hierarchical analyses of variance indicate a significant level of genetic differentiation between marlinsucker collections from different ocean basins.  $\Phi_{st}$  estimates between Atlantic and Pacific samples resolved a "moderate" to "strong"

level of fixation (as defined by Wright, 1978) between all sampling locations ( $\Phi_{st} = 0.1137-0.2105$ ,  $p < 0.05$ ), with the exception of pairwise comparisons involving CNA. These results are congruent with AMOVA analyses, which estimate a highly significant portion of the total genetic variance partitioned between collections from different ocean basins. When haplotypes were binned by ocean basin (two collections), 14.88% of the total variance was partitioned between ocean basins. When haplotypes were binned by sampling location within ocean basins (seven collections), 14.66% of the total variance was partitioned between ocean basins. These results are consistent with observations in several other cosmopolitan pelagic fishes (Graves and McDowell, 1995; Alvarado-Bremer et al. 1996; Rosel and Block, 1996; Chow et al. 2000; Buonaccorsi et al. 2001; McDowell, 2002; Durand et al. 2005; Alvarado-Bremer et al. 2005; Martinez et al., in press). On the basis of DNA sequence variability in a 300bp fragment of the control region, Rosel and Block (1996) estimated a  $\Phi_{st}$  of 0.153 between Atlantic and Pacific samples and AMOVA analyses partitioned 15.6% of the total variance between samples. Using RFLP analysis of whole molecule mitochondrial DNA, McDowell (2002) demonstrated significant levels of genetic partitioning between sailfish collected from different ocean basins. When haplotypes were binned by ocean basin, 32.53% of the total variance was partitioned between samples from different ocean basins, corresponding to an  $\Phi_{st}$  estimate of 0.325. In agreement with the work of Graves and McDowell (1995), Alvarado-Bremer et al. (1996), Rosel and Block, (1996), Chow et al. (2000), Buonaccorsi et al. (2001), McDowell (2002), Durand et al. (2005), Alvarado-Bremer et al. (2005), Martinez et al. (in press), the levels of interocean divergence estimated



between Atlantic and Pacific marlinsucker samples suggest a significant barrier to gene flow between ocean basins.

No evidence of geographic structuring was found in marlinsucker collections within the Pacific Ocean. The  $\Phi_{st}$  estimate between eastern and western Pacific collections was negative and non-significant ( $p = 0.7998$ ), suggesting basin-wide genetic homogeneity. These results parallel observations in blue marlin, where negligible levels of structuring were resolved within the Pacific using cytochrome *b* DNA sequencing (Finnerty and Block, 1992), allozyme and anonymous single copy nuclear DNA (ascnDNA; Buonaccorsi et al. 1999) and whole mtDNA restriction fragment length polymorphism (RFLP) analyses (Buonaccorsi et al. 2001). In contrast, the results of the present study differ from the observations made in a number of other pelagic fishes with which marlinsucker associate. Significant levels of population structuring within the Pacific Ocean have been described in striped marlin (Graves and McDowell, 1995; Graves and McDowell, 2003; McDowell and Graves, unpublished), sailfish (McDowell, 2002), and swordfish (Reeb et al. 2000). Although the results of the present study suggest that a significant amount of gene flow occurs between distant sampling locations, further sampling is required to evaluate these observations with moderate statistical power. In this study, samples were collected from only two sampling locations (eastern Australia and southwestern Panama) and totaled 8 and 12 individuals, respectively. A broader geographic sampling regimen with increased sample sizes will be required to reevaluate the levels of differentiation measured.

The interpretation of the genetic relationships among geographically distant collections in the Atlantic was less clear than that of the Pacific. When Atlantic marlinsucker haplotypes were binned by sampling location, a highly significant amount of genetic differentiation was observed between CNA and GOM marlinsucker collections ( $\Phi_{st} = 0.1707$ ,  $p = 0.0243$ ), which is indicative of restricted gene flow. In contrast,  $\Phi_{st}$  estimates in all other intraocean comparisons were not significant ( $p > 0.0762$ ), implying negligible structuring between sampling locations. The observed divergence between the CNA and GOM collections is most likely a product of the small sample size in the CNA collection ( $n = 5$ ). As such, the interpretation of these data could be flawed due to type 1 error. The CNA sample consisted exclusively of Clade II haplotypes, whereas the relative proportion of Clade II haplotypes at all other Atlantic sampling locations ranged from 40 to 58%. Due to the low sampling effort in this location, it is possible that the local genetic diversity was not adequately sampled.

To more effectively evaluate intraocean substructuring, AMOVA and  $\Phi_{st}$  analyses were performed by separately binning Atlantic-I and Atlantic-II haplotypes by sampling location. Results of these tests indicated a lack of intraocean structure:  $\Phi_{st}$  values between all Atlantic sampling locations were not significant for either Clade I or Clade II samples; 100% of the variance was partitioned within collections for both Clade I and Clade II haplotypes. These observations do not reject the hypothesis of panmixia, and thus indicate that a significant amount of gene flow exists between Atlantic sampling locations.

### Cladogenesis and Putative Recolonization

Estimates of cladogenesis and putative recolonization of the Atlantic by Indo-Pacific marlinsucker are similar to estimates in other pelagic fishes exhibiting this intriguing two-clade mtDNA pattern (Buonaccorsi et al. 2001; McDowell, 2002; Graves and McDowell, 2003; Alvarado-Bremer et al. 2005; Martinez et al. in press). In the present study, cladogenesis was estimated to have occurred between 0.33 and 0.46 million years ago. These values are roughly consistent with divergence time estimates between Atlantic and Pacific stocks of blue marlin (0.6 million years; Graves and McDowell, 1995; Buonaccorsi et al. 2001.), sailfish (0.37-0.66 million years; McDowell, 2002), bigeye tuna (0.32-0.43 million year; Martinez et al., in press) and swordfish (0.7 – 3.0 million years; Alvarado-Bremer et al. 2005). Results suggest that contact between Atlantic and Pacific marlinsucker persisted long after the rise of the Isthmus of Panama and the development of the Benguela upwelling system. Based upon  $\tau$  estimates from Atlantic-II haplotypes and the level of divergence measured between Atlantic-II and Pacific haplotypes (0.11%,  $p = 0.0055$ ), the putative recolonization of the Atlantic by Indo-Pacific marlinsucker could have occurred between 16 000 and 220 000 years before present. Although coalescent and divergence-based estimates disagree upon a definitive point in time when recolonization most likely occurred, these results suggest that Atlantic and Pacific marlinsucker stocks were separated for a period of at least 100 000 years prior to secondary contact. Furthermore, the most recent contact between Atlantic and Pacific stocks could have occurred near the end of the Pleistocene. It should be noted, however, that the conversion of genetic distances into geologic time should be

approached with caution. Mutation rate heterogeneity has been described at the nucleotide position, gene region, gene and organismal level (reviewed in Hillis et al. 1996; Avise, 1994) making this methodology somewhat contentious. A more conservative approach would be to use relative, rather than absolute distance. As such, it would appear that secondary colonization occurred in the “relatively recent” geological past. A low, but highly significant level of fixation ( $\Phi_{st} = 0.0442$ ,  $p = 0.0041$ ) between Atlantic-II and Pacific samples suggests that sufficient time has passed for genetic divergence to develop between these samples. As such, it appears that contemporary gene flow between Atlantic and Pacific marlinsucker is not occurring.

#### Neutrality and Population Demography

Distinct signatures of past population expansion were noted in mismatch distribution analyses of marlinsucker control region haplotypes. In the present study, long-term population stability was inferred in Pacific and Atlantic Clade I samples, based upon broad, multimodal mismatch distributions. In contrast, Atlantic Clade II samples (putative Atlantic Ocean recolonists) demonstrated a roughly unimodal distribution, suggestive of rapid population growth. Harpending’s index, which estimates the probability of deviation from a rapid population expansion model, was insignificant in all haplotype comparisons. Fu’s  $F_s$ , which can be used to evaluate population growth (or alternatively selection), was significant in all binned haplotype analyses. Taken together, these data indicate that both Atlantic and Pacific populations of marlinsucker experienced rapid population growth in the past. A

significant amount of time may have passed, however, for the ancient population growth signature to be lost in the mismatch distribution analyses of Atlantic-I and Pacific haplotypes. These observations are consistent with the hypothesis that Indo-Pacific marlinsucker colonized and subsequently proliferated in the Atlantic Ocean in the recent geological past.

#### Host-symbiont Phylogeography

The intimate association between marlinsucker and the fishes of the family Istiophoridae has important implications to the ecology of *R. osteochir*. Barriers to genetic homogenization that commonly affect free living (i.e. not host-associating) marine fishes, are likely circumvented by attaching to istiophorids. Based upon the highly migratory nature of their hosts, marlinsucker are not likely to be isolated by distance, which represents a reduction in gene flow due to increased geographic distance between individuals (Avice, 2001). Tagging data demonstrate that a number of istiophorids (e.g. blue marlin, black marlin; Ortiz et al. 2003) are capable of long-distance migration, including cross-ocean excursions. These observations support the hypothesis that barriers to dispersion, such as the eastern Pacific barrier, may not be significant obstacles to the marlinsucker. Localized spawning and recruitment, which promotes genetic isolation, also does not appear to be a controlling factor. Ripe male and female marlinsucker pairs have been observed cohabitating on the same istiophorid host (Cressey and Lachner, 1970; Collette, pers. obs.; Gray, pers. obs.), suggesting the potential for repeated spawning events between cohabitating fishes, and a wide distribution area for their progeny given the highly migratory nature of

their hosts. In this study, structuring was not revealed in the Atlantic when marlinsucker were binned by istiophorid host. This suggests that while marlinsucker selectively associate with istiophorid billfishes generally, they are not selective of a particular species of billfish, at least in the Atlantic. The combination of these factors provides a strong homogenization force that potentially reduces (or prevents) intraocean population structuring in the marlinsucker.

Given the stochastic nature of dispersal in cosmopolitan marine fishes (Graves, 1998), it seems plausible that at a maximum, the levels of population structure observed in marlinsucker should reflect the level of structure of the least structured host. Patterns of interocean genetic differentiation observed in marlinsucker are strikingly similar to patterns observed in blue marlin and sailfish (Graves and McDowell, 1995; Buonaccorsi et al. 2001; McDowell, 2002; Graves and McDowell, 2003), and include significant levels of genetic divergence and the presence of two mitochondrial lineages in the Atlantic Ocean. Consistent with observations in white marlin (Graves and McDowell, 2001), sailfish (McDowell, 2002) and blue marlin (Buonaccorsi et al. 2001), no evidence of intraocean structuring was resolved among Atlantic collections of marlinsucker. Consistent with observations in black marlin (Faltermann, 1999) and blue marlin (Buonaccorsi et al. 1999; Buonaccorsi et al. 2001), and in contrast to observations in sailfish (McDowell, 2002) and striped marlin (McDowell and Graves, unpublished), no evidence of population structuring was resolved among collections in the Pacific. Overall, the patterns of genetic differentiation in marlinsucker most closely resemble the patterns

seen in blue marlin, the istiophorid species that exhibits the least amount of intraocean structure.

The nature of the two-clade mtDNA pattern in marlinsucker is intriguing in light of similar observations made in blue marlin and sailfish (Graves and McDowell, 1995; Buonaccorsi, 2001; McDowell, 2002). Vicariant isolation during the Pleistocene, followed by secondary contact and subsequent isolation has been used to explain the two-clade pattern found in Atlantic collections of these two species. Congruence between the mtDNA patterns observed in marlinsucker, blue marlin and sailfish suggests that marlinsucker were either secondarily introduced into the Atlantic in association with one (or more) of these species, or colonization occurred in parallel, independent of host association. Given that these two fishes account for nearly one-third of the present day host association records, it is plausible that marlinsucker were associated with blue marlin or sailfish when the putative recolonization occurred. Ultimately, it is impossible to discern which of these two alternative explanations is correct. Three independent colonizations (e.g. marlinsucker, blue marlin, and sailfish) could have occurred in the same time period, and the genetic signature would still appear the same.

Intraspecific and interspecific relationships within white marlin and striped marlin, which collectively account for 36.4% of marlinsucker host association records, have been recently evaluated by McDowell and Graves (unpublished). Based upon analyses of control region mtDNA, McDowell and Graves (unpublished) found that the magnitude of divergence between these two species is similar to the level of divergence observed between mtDNA clades in blue marlin and sailfish. Furthermore,

it appears that these two species diverged during the late Pleistocene, likely when mtDNA clades in blue marlin and sailfish diverged (McDowell, pers. comm.) The lack of significantly different mtDNA lineages within either white marlin or striped marlin suggests that secondary contact between these two species has not occurred (contrary to blue marlin and sailfish). As such, it appears that if secondary contact in marlinsucker was host-dependent, it is unlikely that white marlin and striped marlin were the vectors. Likewise, given the lack of significantly different mtDNA lineages within each of the three Atlantic spearfish species, the longbill (*Tetrapterus pfluegeri*), Mediterranean (*Tetrapterus belone*), and roundscale (*Tetrapterus georgei*), and the one Indo-Pacific species, the shortbill (*Tetrapterus angustirostris*), it is unlikely that spearfish acted as vectors (McDowell, pers. comm.). Furthermore, given that black marlin are mainly restricted to the Pacific, it is unlikely that this species contributed to the patterns of interocean genetic differentiation in marlinsucker.

Results of the present study demonstrate clear similarities between host and symbiont phylogeography. It is unclear, however, whether the congruent phylogeographic patterns in marlinsucker are a direct result of host ecology or parallel evolution that occurred in response to past vicariant events. On one hand, the congruent patterns of genetic differentiation between these species highlight the possible effect that tight linkage between host and symbiont may have upon the geographical distribution of the symbiont. As such, these patterns potentially demonstrate how host natural history may directly affect the evolutionary trajectory of the symbiont. Morphological evidence supports this theory: the marlinsucker is engineered for host association, not free swimming behavior. The disc in



marlinsucker may command up to 40% of the total length and may extend beyond the maximum body width, implying that this species is well equipped to remain attached to highly mobile pelagic hosts such as the istiophorids. Furthermore, given their general body design, marlinsucker are more suited for burst swimming, rather than the continuous, high-speed swimming behavior exhibited by their hosts.

Alternatively, since correlation does not denote causation, the congruent phylogeographic patterns between host and symbiont may simply indicate parallels between two divergent groups of pelagic fishes that have been influenced by similar events and processes in the distant past. Most likely however, remora phylogeography is ultimately a product of both of these factors. The patterns of genetic differentiation observed in marlinsucker are likely a result of both host-dependent effects (e.g. habitat preference, migration patterns) as well as parallel evolutionary forces (e.g. vicariant isolation, selection, genetic drift) affecting both the host and symbiont.

### Sampling Concerns

The interpretation of these results requires critical examination in light of two relevant concerns: specimen and gene region sampling. In the present study, genetic variation was surveyed in 71 marlinsucker specimens collected from seven different sampling locations (two Pacific, five Atlantic). Between 5 and 15 samples were analyzed from each sampling location. Within the Atlantic, geographic sampling range was primarily concentrated in the North Atlantic ( $\sim 39^{\circ}\text{N}$  to  $\sim 9^{\circ}\text{S}$  and  $\sim 87^{\circ}\text{W}$  to  $\sim 21^{\circ}\text{W}$ ). Within the Pacific, a broader geographic range was sampled ( $\sim 8^{\circ}\text{N}$  to  $\sim 32^{\circ}\text{S}$  and  $\sim 153^{\circ}\text{E}$  to  $\sim 79^{\circ}\text{W}$ ), although only two sampling locations were included in

this study. From the level of genetic variability assayed in these 71 individuals, interpretations were made concerning present day geographic structuring and the past events that may have shaped these observations. While the results were statistically significant, it could be argued that both sample size and breadth of geographic coverage are too low to accurately infer geographic structuring. Overall, statistical power was low, especially in the intra-ocean comparisons. However, low sample sizes are more likely to reveal a lack of structure, rather than the presence of structure. As such, the inferences regarding structure are likely valid, although the underlying phylogeographic signal may not be fully explored. The results of this study must be considered preliminary until both a broader sampling range and large number of samples per location are included.

The second area of concern pertains to the gene region sampled.

Phylogeographic patterns were inferred from DNA sequence variation in the mitochondrial control region. Because the mitochondrial genome is maternally inherited, the observations made here may not fully describe the evolutionary history of the marlinsucker population. Phylogeographic analyses inferred from mtDNA variation capture only the female component of the broader population demography. If males and females exhibit similar life history traits, it is possible that mtDNA variation may be used to accurately describe the evolutionary history of the species. However, if life history differences exist between sexes (such as the case of male or female philopatry), inferences made from mitochondrial genealogies will not accurately represent the "true" evolutionary history of the species. Although no evidence of sex biased dispersal in marlinsucker has been documented to date, the

phylogeographic interpretations made in this study must be viewed in light of the inherent limitations of this molecular marker.

To most effectively infer marlinsucker phylogeography, genetic variation should be assayed using multiple unlinked (mitochondrial and nuclear) molecular markers. Inclusion of additional molecular markers, such as single nucleotide polymorphisms (SNPs), rapid amplification of polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), and microsatellites would provide a means to evaluate the mitochondrial-based hypotheses generated in this study. Additional markers would afford a more accurate measure of geographic distribution of genetic variation in marlinsucker and lend greater statistical support to phylogeographic hypotheses generated.

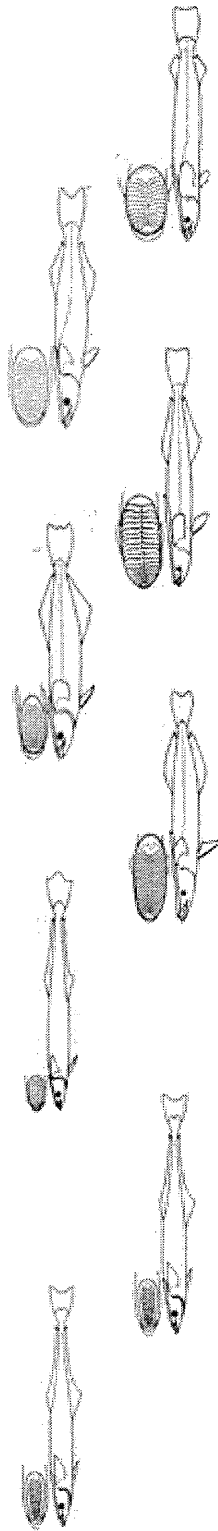
## CONCLUSIONS

The present study represents the first comprehensive molecular investigation into the interspecific taxonomic relationships within the Echeneoidea. Results of this study agree with the family-level hypotheses of Johnson (1984,1993), and specific aspects of the alpha taxonomy described by O'Toole (2002). In agreement with both authors, the Echeneoidea were resolved as a monophyletic group. Consistent with the classification based upon larval characters (and in contrast with that based on osteology and behavior), a monophyletic Rachycentridae + Coryphaenidae was resolved with high support. In agreement with the work of O'Toole (2002), the Echeneidae form a monophyletic group. In contrast, however, the subfamilies Echeneiinae and Remorinae were each monophyletic, indicating that both subfamily designations are valid. As described by O'Toole (2002), the genus *Remora* was paraphyletic based on the position of the monotypic genus *Remorina*, suggesting that taxonomic revision is necessary. Overall, the relationships within the Remorinae were not well resolved in this study. Future work should include nuclear or mitochondrial loci that exhibit a higher degree of sequence variability to more accurately infer the taxonomic relationships within the Remorinae. Furthermore, analyses of multiple specimens of each species would lend greater support to the hypotheses generated.

Phylogeographic structuring in the marlinsucker, *R. osteochir*, was evaluated using nucleotide sequence data from the hypervariable mitochondrial control region.

Based upon the geographic distribution of genetic variation, the null hypothesis that marlinsucker constitute a single, global panmictic population was confidently rejected. Significant levels of population structuring were resolved among marlinsucker collections from the Atlantic and Pacific oceans. In contrast, there was no evidence of structuring among collections within ocean basins. Based upon inter-haplotype divergence, two evolutionary lineages were described, one composed of Atlantic-only individuals, the other composed of both Atlantic and Pacific individuals. The presence of two marlinsucker mtDNA lineages in the Atlantic is consistent with a period of vicariant isolation during the Pleistocene, secondary contact involving unidirectional migration from the Indian to Atlantic Ocean, followed by a subsequent period of isolation. Patterns of genetic differentiation in marlinsucker are congruent with patterns observed in their istiophorid hosts (particularly blue marlin, *M. nigricans*, and sailfish, *I. platypterus*). These findings highlight the possibility that marlinsucker phylogeography is ultimately governed by host dispersal. Alternatively, these observations suggest that these pelagic fishes have been influenced by similar vicariant events and processes in the distant past. Future work should aim to augment the existing marlinsucker collection by broadening geographic sampling coverage and intensifying sampling at each collection location. Additional molecular markers, particularly nuclear (i.e. microsatellites) should be used to evaluate the observed levels of genetic differentiation.

Table 4. Echineid host-symbiont occurrence records compiled from literature sources.  
Data modified from O'Toole (2002) and augmented with samples from this study.  
Diagram credit: FISHBASE: <http://www.fishbase.org>, 2005.



Host	<i>Echeneis</i>		<i>Pitheirichthys</i>		<i>Remora</i>		<i>Remora</i>		<i>Remora</i>		<i>Remora</i>	
	<i>naucratis</i> sharksucker	<i>naucratoides</i> whitefin shark sucker	<i>lineatus</i> slender suckerfish	<i>australis</i> whalesucker	<i>brachyptera</i> spearfish remora	<i>osteoclar</i> madlinesucker	<i>remora</i> common remora	<i>remora</i> common remora	<i>albescens</i> white suckerfish	<i>albescens</i> white suckerfish	<i>albescens</i> white suckerfish	<i>albescens</i> white suckerfish
Istiophorid billfishes	1	0	0	0	184	564	20	0	0	0	0	0
<i>X. gladius</i>	0	1	0	0	300	8	3	0	0	0	0	0
Sharks	26	5	3	0	198	1	890	4	0	0	0	4
Rays	3	0	0	0	1	0	3	27	0	0	0	27
Cetaceans	2	2	0	43	0	0	0	0	0	0	0	0
Reef fishes	32	0	15	0	2	0	0	0	0	0	0	0
Pelagic fishes	3	1	0	0	7	4	6	0	0	0	0	0
Other (i.e. bait, gear)	2	0	19	0	1	2	15	0	0	0	0	0
Unknown	5	0	0	0	6	1	1	0	0	0	0	0
Total	74	9	37	43	699	580	938	31	0	0	0	31

Subfamily Echeneinae

Subfamily Remorinae

Table 5. Mitochondrial DNA diversity estimates within collections of marlinsucker, *R. osteochir*, based upon complete mitochondrial control region sequence data.



Genetic Diversity Indices of *R. osteocirca* subpopulations

Locality	No. of Samples	No. of Haplotypes	Haplotype diversity ( $h$ ) +/- SE	Nucleotide diversity ( $\pi$ ) +/- SE	Mean No. of Pairwise Differences +/- SE
<b>By Ocean Basin</b>					
Atlantic	51	51	1.00	0.0346 +/- 0.0005	33.4449 +/- 0.4150
Pacific	20	20	1.00	0.0273 +/- 0.0010	26.2574 +/- 0.8720
<b>By Sampling Location</b>					
Western Pacific (WPAC)	8	8	1.00	0.0244 +/- 0.0026	23.3430 +/- 2.1769
Eastern Pacific (EPAC)	12	12	1.00	0.0297 +/- 0.0019	28.5012 +/- 1.6532
Central North Atlantic (CNA)	5	5	1.00	0.0195 +/- 0.0039	18.6392 +/- 3.1627
Western North Atlantic (WNA)	6	6	1.00	0.0358 +/- 0.0054	34.2698 +/- 4.5137
Gulf of Mexico (GOM)	15	15	1.00	0.0376 +/- 0.0019	36.0587 +/- 1.6239
Eastern Equatorial Atlantic (EEA)	12	12	1.00	0.0359 +/- 0.0023	34.5391 +/- 1.9948
Western Equatorial Atlantic (WEA)	13	13	1.00	0.0332 +/- 0.0020	31.8297 +/- 1.6836
<b>By Clade</b>					
Clade I	24	24	1.00	0.0246 +/- 0.0008	23.6058 +/- 0.6475
Clade II	47	47	1.00	0.0244 +/- 0.0004	23.6273 +/- 0.3217
<b>By Intra-clade Grouping</b>					
Atlantic-I	24	24	1.00	0.0246 +/- 0.0008	23.6058 +/- 0.6475
Atlantic-II	27	27	1.00	0.0213 +/- 0.0011	20.5329 +/- 0.4996
Pacific	20	20	1.00	0.0273 +/- 0.0010	26.2574 +/- 0.8720
Overall	71	71	1.00	0.0367 +/- 0.0003	26.2574 +/- 0.2998

Table 6. Matrix of corrected average nucleotide sequence divergence ( $\delta$ ) and associated p-values among collections of marlinsucker, *R. osteochir* based upon complete mitochondrial control region sequence data. Haplotypes were binned by (A) sampling location, and (B) ocean basin, clade, and clade within ocean basin.

**A**Population Pairwise Divergence ( $\delta$ )

	CNA	WEA	EEA	GOM	WNA	EPAC	WPAC
CNA		0.05634	0.07673	0.02059	0.15673	0.29604	0.58317
WEA	0.00663		0.57842	0.95743	0.81356	0.00198	0.00485
EEA	0.00393	-0.00066		0.47277	0.65525	0.00238	0.00851
GOM	<b>0.00817</b>	-0.00138	-0.00030		0.63248	0.00178	0.00168
WNA	0.00364	-0.00189	-0.00120	-0.00110		0.01762	0.01950
EPAC	0.00039	<b>0.00693</b>	<b>0.00489</b>	<b>0.007312</b>	<b>0.00378</b>		0.72624
WPAC	-0.00044	<b>0.00821</b>	<b>0.00495</b>	<b>0.008942</b>	<b>0.00528</b>	-0.00054	

Above Diagonal: P-values. Tests significant &lt; 0.05

Below Diagonal: Divergence values

**B**Population Pairwise Divergence ( $\delta$ )

	Divergence	P-value
Atlantic/Pacific	<b>0.00581</b>	0.00010
Clade I/ Clade II	<b>0.02300</b>	0.00000
Atlantic-I/Pacific	<b>0.02353</b>	0.00000
Atlantic-II/Pacific	<b>0.00114</b>	0.00554
Atlantic-I/Atlantic-II	<b>0.02310</b>	0.00000

Tests significant &lt; 0.05

Table 7. Distribution of marlinsucker, *R. osteochir*, Clade I and Clade II mitochondrial haplotypes among seven geographic sampling locations.

## Distribution of Haplotypes

Locality	Clade I	Clade II	Total
Central North Atlantic (CNA)	0	5	5
Western North Atlantic (WNA)	3	3	6
Gulf of Mexico (GOM)	9	6	15
Western Equatorial Atlantic (WEA)	7	6	13
Eastern Equatorial Atlantic (EEA)	5	7	12
Atlantic Subtotal	24	27	51
Western Pacific (WPAC)	0	8	8
Eastern Pacific (EPAC)	0	12	12
Pacific Subtotal	0	20	20
Total	24	47	71

Table 8. Matrix of pairwise  $\Phi_{st}$  values and associated p-values among collections of marlinsucker, *R. osteochir*, based upon complete mitochondrial control region sequence data. Haplotypes were binned by (A) sampling location, (B) ocean basin, clade, and clade within ocean basin, and (C, D) both sampling location and clade within ocean basin.

**A**Population Pairwise  $\Phi_{st}$  values: Atlantic and Pacific Subpopulations

Locality	CNA	WEA	EEA	GOM	WNA	EPAC	WPAC
CNA		0.0762	0.1014	0.0243	0.1632	0.5409	0.6520
WEA	0.1613		0.5760	0.9655	0.7871	0.0020	0.0051
EEA	0.0842	-0.0191		0.4786	0.6678	0.0024	0.0105
GOM	<b>0.1707</b>	-0.0410	-0.0085		0.6755	0.0019	0.0019
WNA	0.1048	-0.0550	-0.0348	-0.0331		0.0162	0.0169
EPAC	-0.0091	<b>0.1800</b>	<b>0.1298</b>	<b>0.1753</b>	<b>0.1137</b>		0.7998
WPAC	-0.0292	<b>0.2105</b>	<b>0.1300</b>	<b>0.2048</b>	<b>0.1599</b>	-0.0245	

Above Diagonal: P-values. Tests significant &lt; 0.05

Below Diagonal:  $\Phi_{st}$  values**B**Population Pairwise  $\Phi_{st}$  values:

	$\Phi_{st}$	P-value
Atlantic/Pacific	<b>0.14880</b>	0.0001
Clade I/Clade II	<b>0.48450</b>	< 0.0000
Atlantic-I/Pacific	<b>0.47022</b>	< 0.0001
Atlantic-II/Pacific	<b>0.04420</b>	0.0041
Atlantic-I/Atlantic-II	<b>0.50051</b>	< 0.0001

Tests significant &lt; 0.05

Table 8. (continued).



**C**Population Pairwise  $\Phi_{st}$  values: Atlantic Clade I haplotypes

Locality	WEA	EEA	GOM	WNA
WEA		0.4157	0.6449	0.3905
EEA	-0.0195		0.7314	0.8602
GOM	-0.0385	-0.0545		0.2910
WNA	-0.0027	-0.0704	0.0346	

Above Diagonal: P-values. Tests significant &lt; 0.05

Below Diagonal:  $\Phi_{st}$  values**D**Population Pairwise  $\Phi_{st}$  values: Atlantic Clade II haplotypes

Locality	CNA	WEA	EEA	GOM	WNA
CNA		0.8088	0.3864	0.7948	0.9092
WEA	-0.0777		0.4603	0.5142	0.6951
EEA	0.0069	-0.0001		0.0549	0.2827
GOM	-0.0549	-0.0144	0.0605		0.8656
WNA	-0.1262	-0.0707	0.0226	-0.0847	

Above Diagonal: P-values. Tests significant &lt; 0.05

Below Diagonal:  $\Phi_{st}$  values

Table 9. Analyses of molecular variance (AMOVA) among collections of marlinsucker, *R. osteochir* based upon complete mitochondrial control region sequence data. Haplotypes were binned by (A) ocean basin (B) sampling location, (C) clade within ocean basin, and (D, E) both sampling location and clade within ocean basin.

**A**

Analysis of Molecular Variance (AMOVA): Separated into two subpopulations: Pacific, Atlantic

Source of Variation	df	Sum of Squares	Variance Components	Percentage of Variation	P-value	Fixation Indices
Among populations	1	95.24	2.76438	14.88	0.0001	FST : 0.14880
Within populations	69	1091.091	15.81291	85.12		
Total	70	1186.331	18.57728			

**B**

Analysis of Molecular Variance (AMOVA): Separated into seven subpopulations: two Pacific, five Atlantic

Source of Variation	df	Sum of Squares	Variance Components	Percentage of Variation	P-value	Fixation Indices
Among groups	1	95.24	2.72281	14.66	< 0.0001	FCT : 0.14662
Among populations within groups	5	84.438	0.11857	0.64	0.0008	FSC : 0.00748
Within populations	64	1006.653	15.72895	84.7	0.0018	FST : 0.14662
Total	70	1186.331	18.57034			

**C**

Analysis of Molecular Variance (AMOVA): Separated into three subpopulations: Pacific, Atlantic-I, Atlantic-II

Source of Variation	df	Sum of Squares	Variance Components	Percentage of Variation	P-value	Fixation Indices
Among populations	2	391.68	7.82902	39.66	< 0.0001	FST : 0.39656
Within populations	68	810.094	11.90305	60.34		
Total	70	1201.775	19.74218			

Table 9. (continued).

**D**Analysis of Molecular Variance (AMOVA): Clade I haplotypes separated into four Atlantic subpopulations

Source of Variation	df	Sum of Squares	Variance Components	Percentage of Variation	P-value	Fixation Indices
Among populations	3	30.575	-0.324	-2.760	0.6745	-0.02763
Within populations	20	240.892	12.045	102.760		
Total	23	271.467	11.721			

**E**Analysis of Molecular Variance (AMOVA): Clade II haplotypes separated into five Atlantic subpopulations

Source of Variation	df	Sum of Squares	Variance Components	Percentage of Variation	P-value	Fixation Indices
Among populations	4	38.198	-0.159	-1.56	0.6445	-0.01557
Within populations	22	228.729	10.397	101.56		
Total	26	266.927	10.237			

Table 10. Matrix of pairwise  $\Phi_{st}$  values and associated p-values among Atlantic collections of marlinsucker, *R. osteochir*, based upon complete mitochondrial control region sequence data. Haplotypes were binned by istiophorid host: white marlin (WHM); sailfish (SAI); spearfish (SPR).

Population Pairwise  $\Phi_{st}$  values: Host Analyses

Host	WHM	SAI	SPR
WHM		0.6818	0.9505
SAI	-0.01657		0.7703
SPR	-0.04258	-0.02369	

Above Diagonal: P-values. Tests significant < 0.05

Below Diagonal:  $\Phi_{st}$  values

Table 11. Analyses of molecular variance (AMOVA) among collections of marlinsucker, *R. osteochir* based upon complete mitochondrial control region sequence data when haplotypes were binned by isitiophorid host.



Analysis of Molecular Variance (AMOVA): Atlantic haplotypes binned by host association

Source of Variation	df	Sum of Squares	Variance Components	Percentage of Variation	P-value	Fixation Indices
Among populations	2	21.248	-0.418	-2.500	0.9053	F <sub>ST</sub> : -0.0250
Within populations	45	770.580	17.124	102.500		
Total	47	791.828	16.706			

Table 12. Neutrality and mismatch distribution statistics estimated within collections of marlinsucker, *R. osteochir*, based upon complete mitochondrial control region sequence data.

Mismatch Distribution Parameters

	No. of Samples	Mismatch		$\tau$	$\theta_0$	$\theta_1$	Raggedness Index		Tajima's D		F <sub>st</sub> F <sub>s</sub>	
		Mean	Variance				Statistic	P-value	Statistic	P-value	Statistic	P-value
<u>By Ocean Basin</u>												
Atlantic	51	34.109	183.144	44.561	0.033	74.879	0.0018	0.994	-0.550	0.311	<b>-24.151</b>	0.000
Pacific	20	26.989	77.185	23.204	7.014	162.920	0.0069	0.906	-1.012	0.169	<b>-6.221</b>	0.008
<u>By Clade</u>												
Clade I	24	24.225	99.753	31.891	0.004	58.837	0.0076	0.857	-0.697	0.262	<b>-9.313</b>	0.002
Clade II	47	24.168	64.564	18.503	7.656	282.812	0.0026	0.682	-1.381	0.080	<b>-24.152</b>	0.001
<u>By Intra-clade Grouping</u>												
Atlantic-I	24	24.225	99.753	31.891	0.004	58.837	0.0076	0.835	-0.697	0.262	<b>-9.313</b>	0.003
Atlantic-II	27	21.162	60.799	15.485	8.944	159.922	0.0067	0.645	-1.331	0.092	<b>-12.813</b>	0.000
Pacific	20	26.989	77.185	23.204	7.014	162.920	0.0069	0.906	-1.012	0.169	<b>-6.221</b>	0.013
All	71	34.336	174.396	17.729	27.725	770.625	0.0008	0.9750	-0.810	0.223	<b>-24.094</b>	0.000

Table 13. Divergence and recolonization estimates in marlinsucker, *R. osteochir* inferred from coalescence and nucleotide divergence based analyses. Calculations assume a mutation rate of 3.6% per million years after Donaldson and Wilson (1993).

## Estimate of time since divergence/recolonization

Coalescence Theory			$\delta$ Based Theory		
Location	$\tau$	$\tau$ age estimate	Regional Pair	Corrected Divergence ( $\delta$ )	$\delta$ age estimate
Atlantic-I	31.891	456159	Atlantic-I/Pacific	0.02353	326778
Pacific	23.204	331903	Atlantic-II/Pacific	0.00114	15841
Atlantic-II	15.485	221493			

Figure 13. Patterns of host association between marlinsucker, *R. osteochir* and istiophorid billfishes, N = 580 records. Diagram credit: FISHBASE: <http://www.fishbase.org>, 2005.

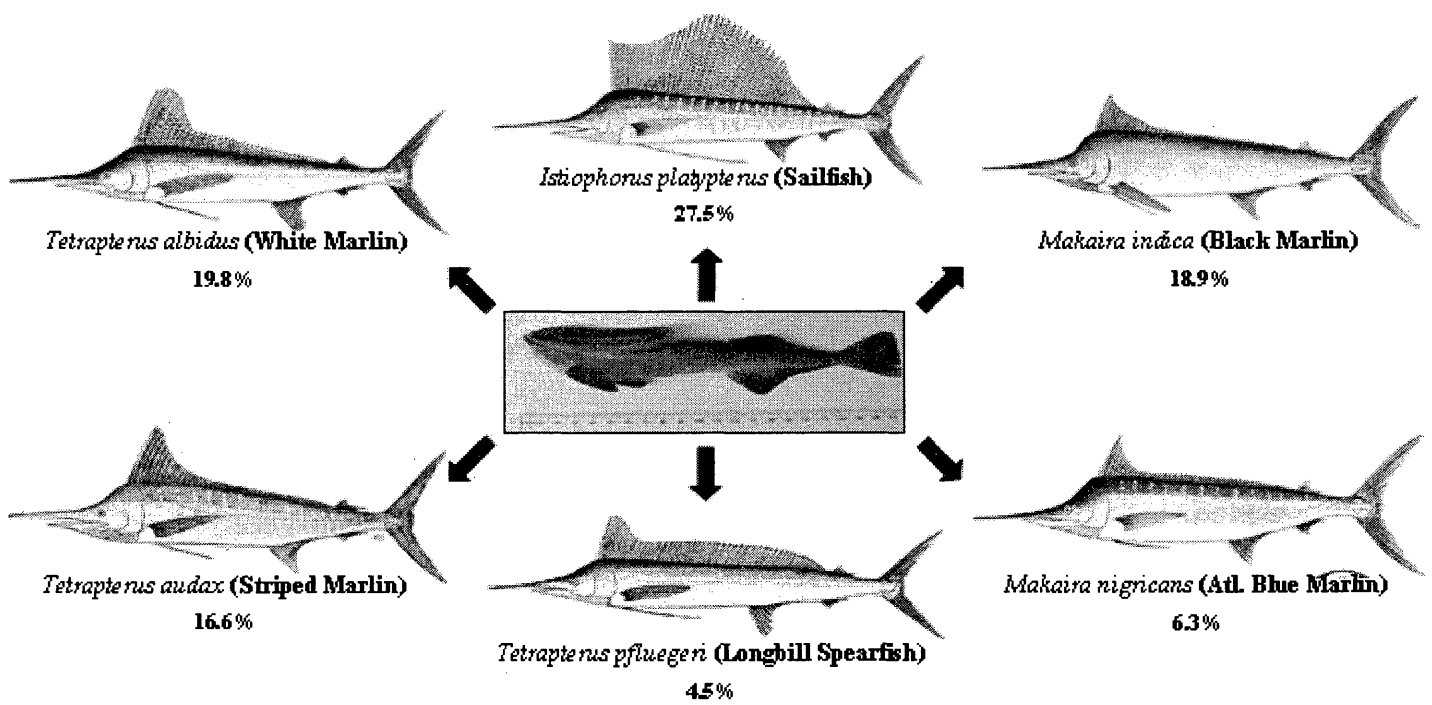


Figure 14. Marlinsucker, *R. osteochir*, sampling locations in the western North Atlantic (WNA), central North Atlantic (CNA), Gulf of Mexico (GOM), western equatorial Atlantic (WEA), eastern equatorial Atlantic (EEA), western Pacific (WPAC), and eastern Pacific (EPAC).



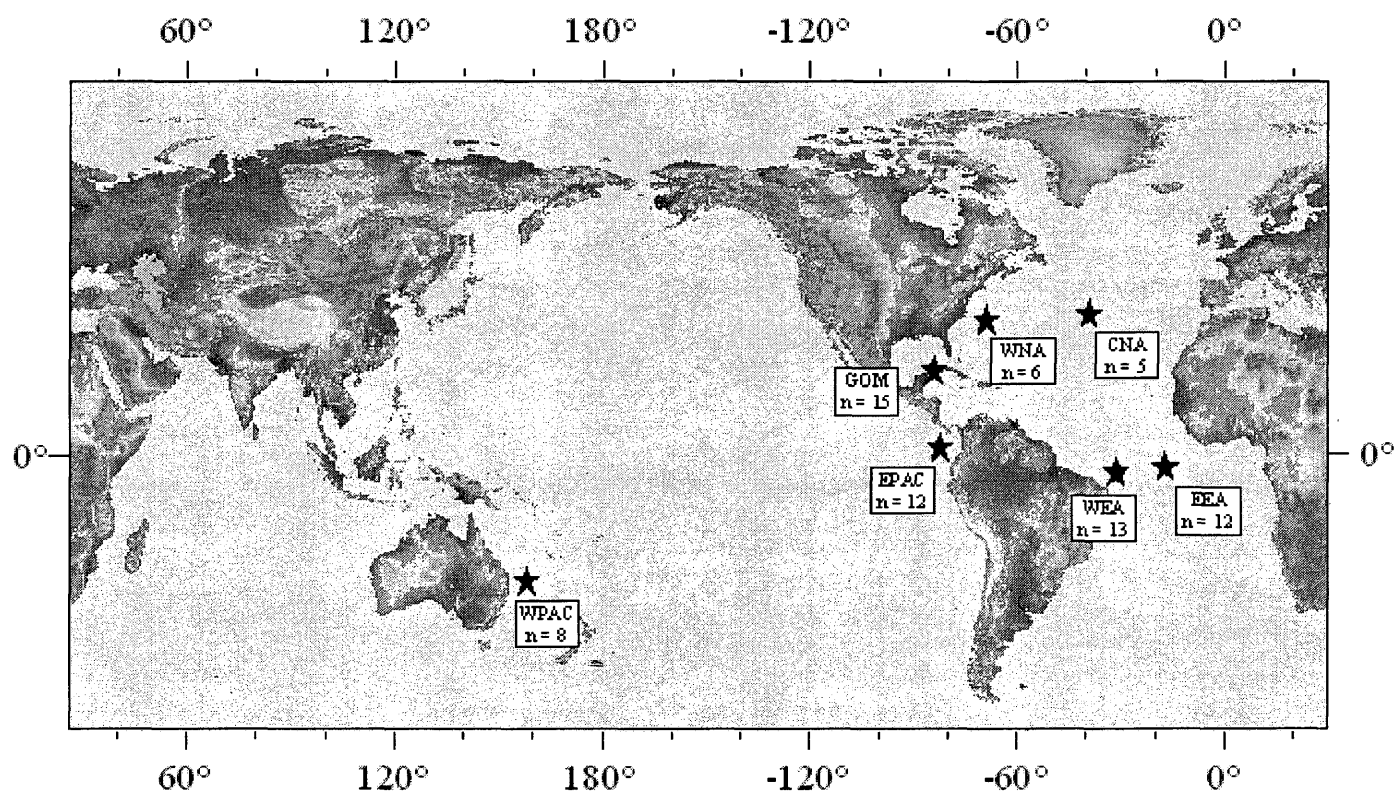


Figure 15. Sequence variation across 971 aligned base pairs of the complete mitochondrial control region in marlinsucker, *R. osteochir*.

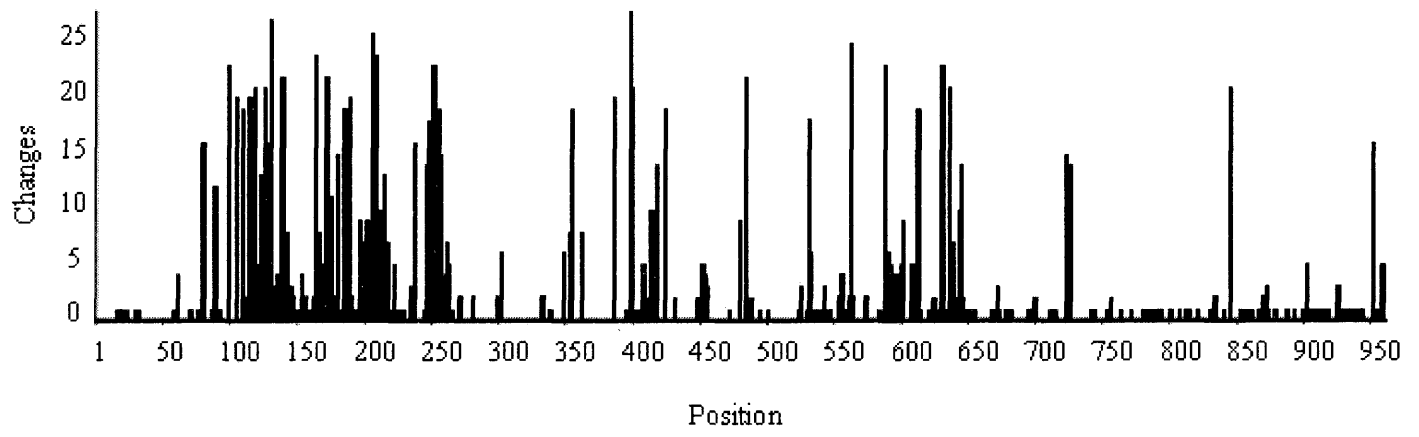


Figure 16. UPGMA trees of complete mitochondrial control region DNA sequences amplified from marlinsucker, *R. osteochir*. (A) Phylogenetic tree rooted with slender suckerfish, *P. lineatus*. (B) Unrooted phylogenetic tree with geographic sampling location information: western North Atlantic (WNA); central North Atlantic (CNA); Gulf of Mexico (GOM); western equatorial Atlantic (WEA); eastern equatorial Atlantic (EEA); western Pacific (WPAC); eastern Pacific (EPAC). Dark and light boxes indicate presence or absence each haplotype in Atlantic and Pacific Oceans. Trees derived from pairwise nucleotide sequence divergence estimated using the Tamura-Nei model of character evolution.

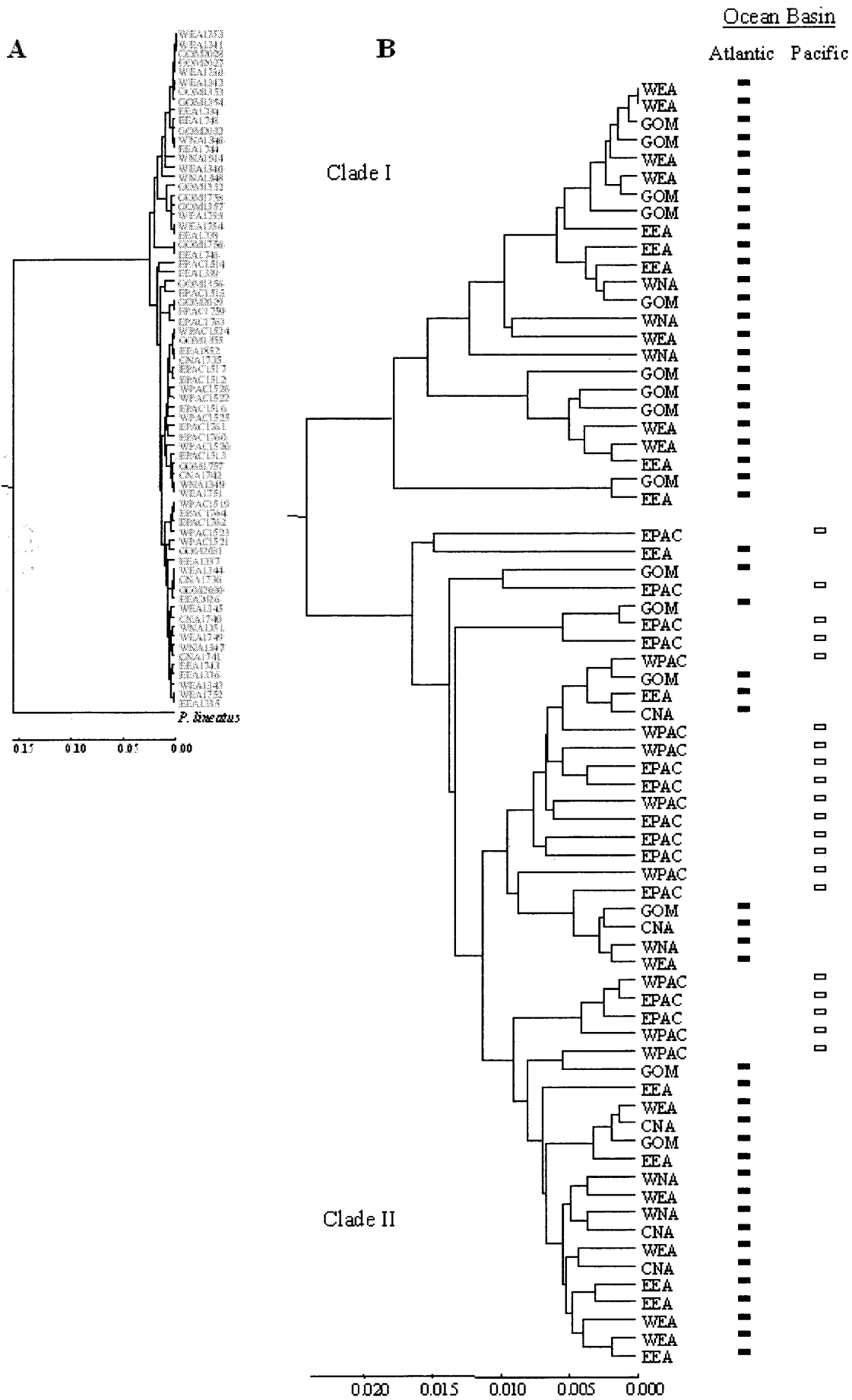


Figure 17. Graphical representation of the distribution of marlinsucker, *R. osteochir*, Clade I and Clade II mitochondrial haplotypes among seven geographic sampling locations: western North Atlantic (WNA); central North Atlantic (CNA); Gulf of Mexico/Caribbean Sea (GOM); western equatorial Atlantic (WEA); eastern equatorial Atlantic (EEA); western Pacific (WPAC); eastern Pacific (EPAC)

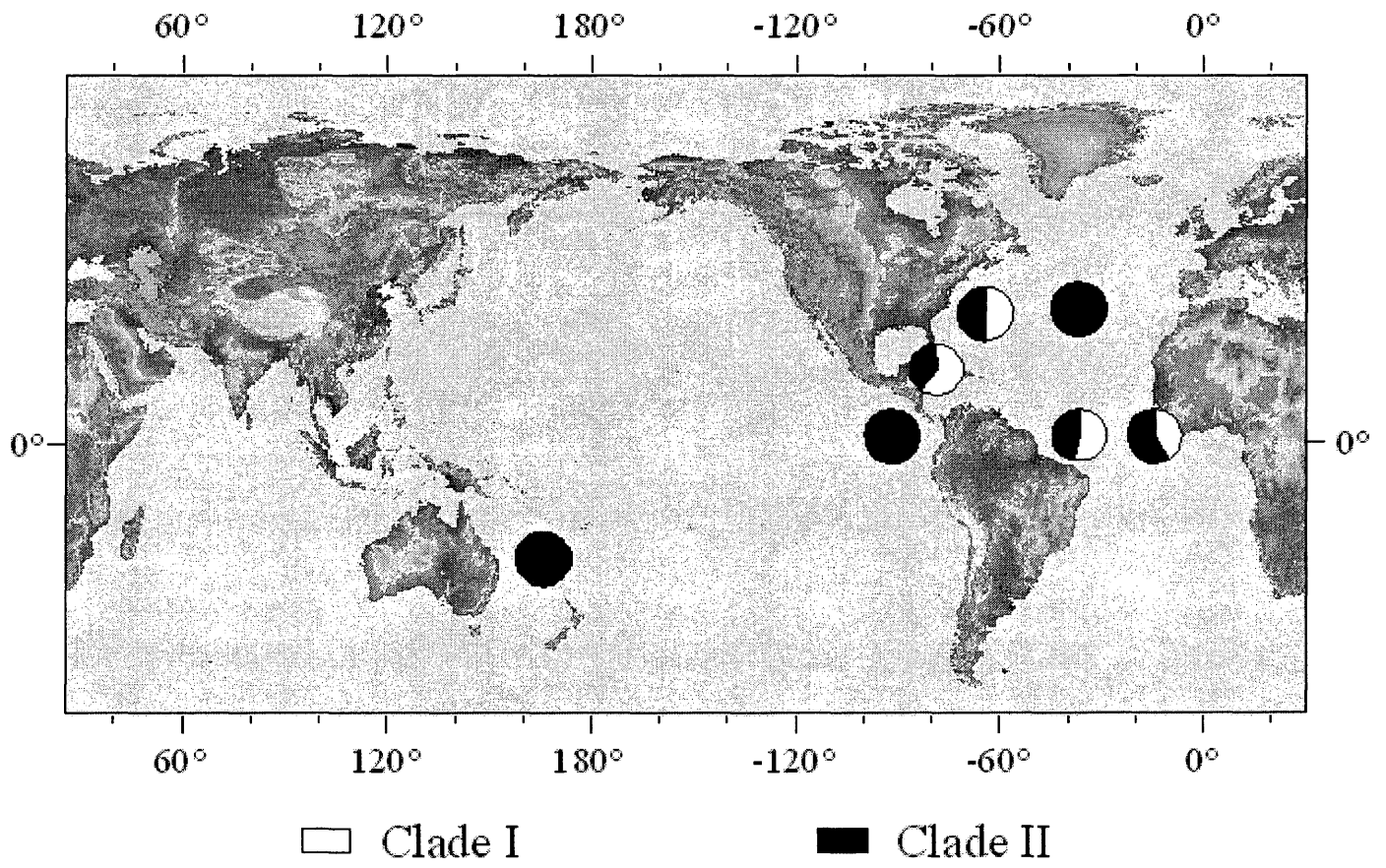


Figure 18. UPGMA trees of complete mitochondrial control region DNA sequences amplified from marlinsucker, *R. osteochir*. Unrooted phylogenetic tree demonstrating host association. Black boxes indicate the host from which the marlinsucker individual was collected: white marlin (WHM); spearfish (SPR); striped marlin (STR); sailfish (SAI); blue marlin (BUM); swordfish (SWO); unknown (UNK). Trees derived from pairwise nucleotide sequence divergence estimated using the Tamura-Nei model of character evolution.



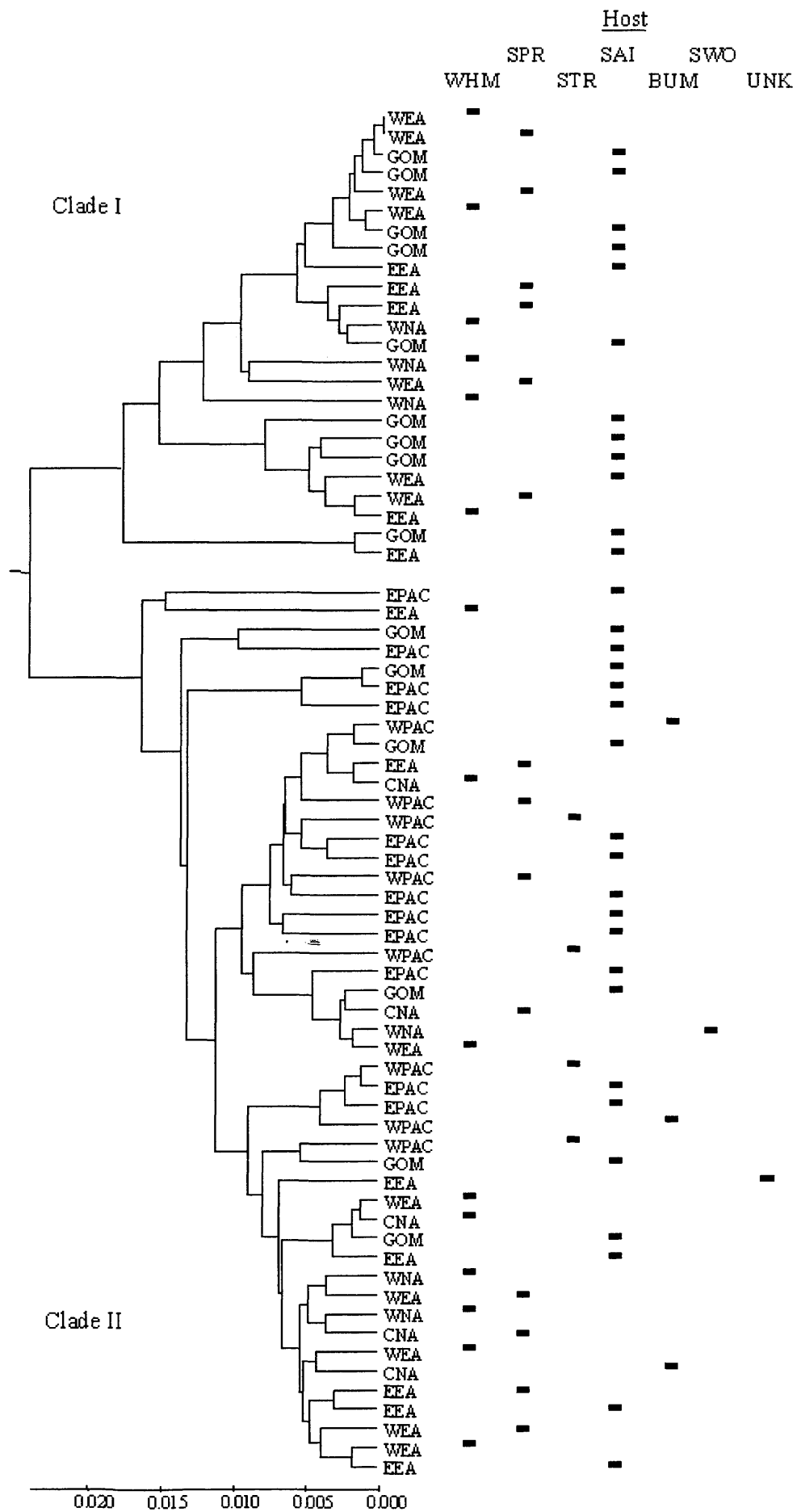


Figure 19. Mismatch distributions observed in pairwise comparisons of complete mitochondrial control region DNA sequences amplified from marlinsucker, *R. osteochir*. Pairwise comparisons were performed using (A) the entire data set; n = 71, (B) Clade I haplotypes; n = 24, (C) Clade II haplotypes; n = 47, (D) Atlantic Clade I haplotypes; n = 24, (E) Atlantic Clade II haplotypes; n = 27, and Pacific haplotypes; n = 20.

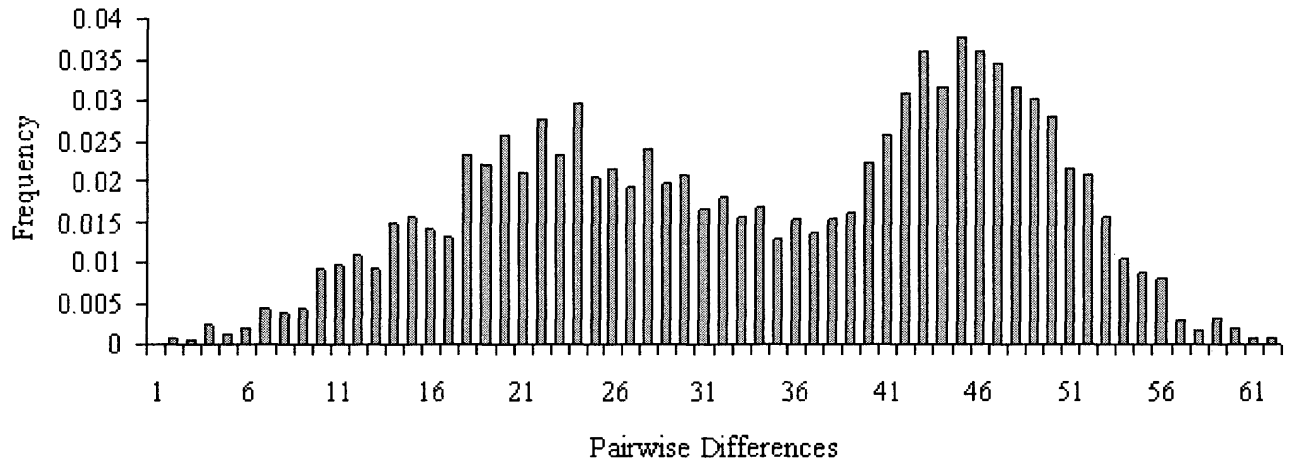
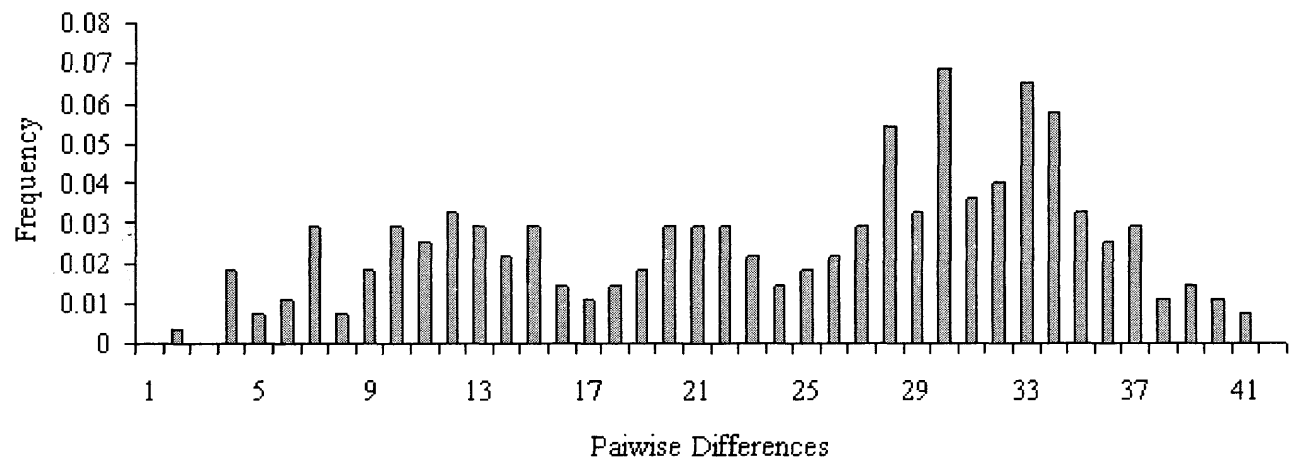
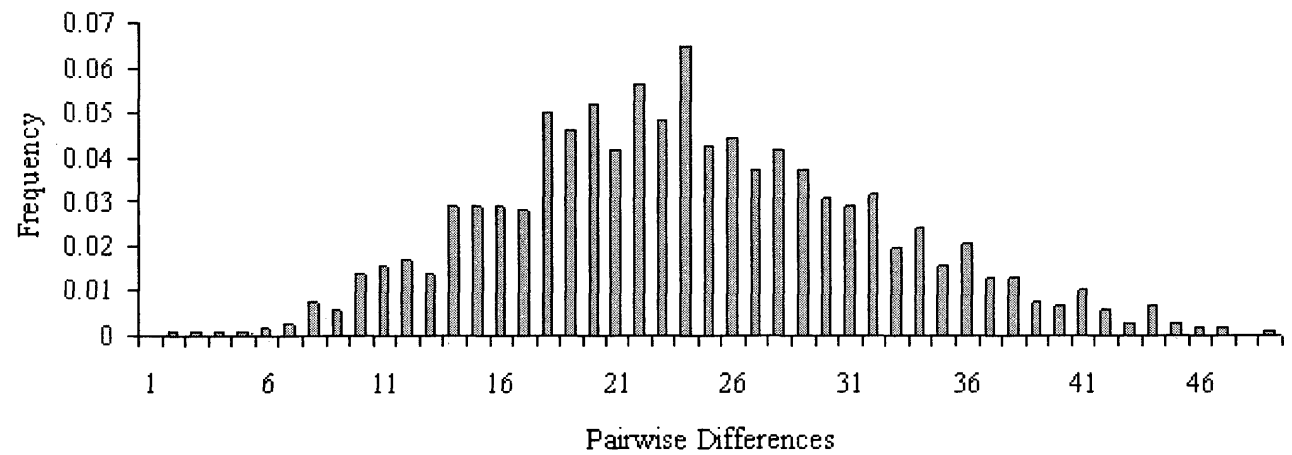
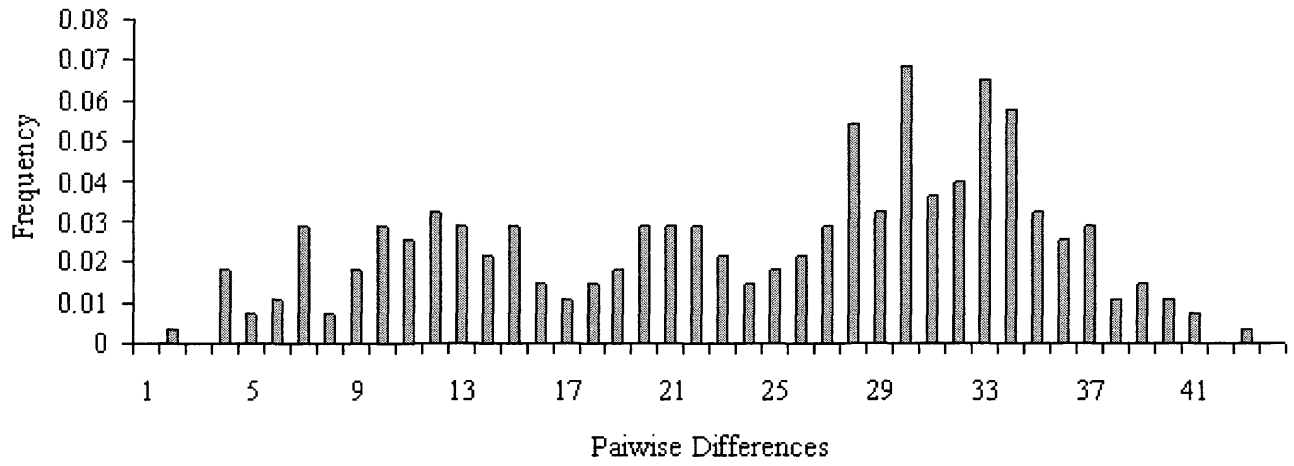
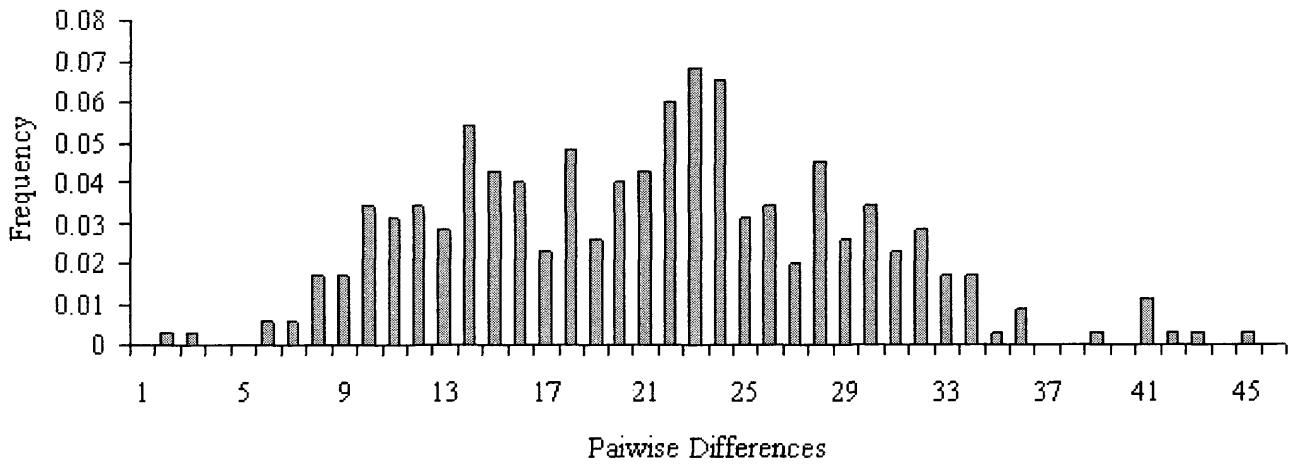
**All Samples****A****B****Clade I****C****Clade II**

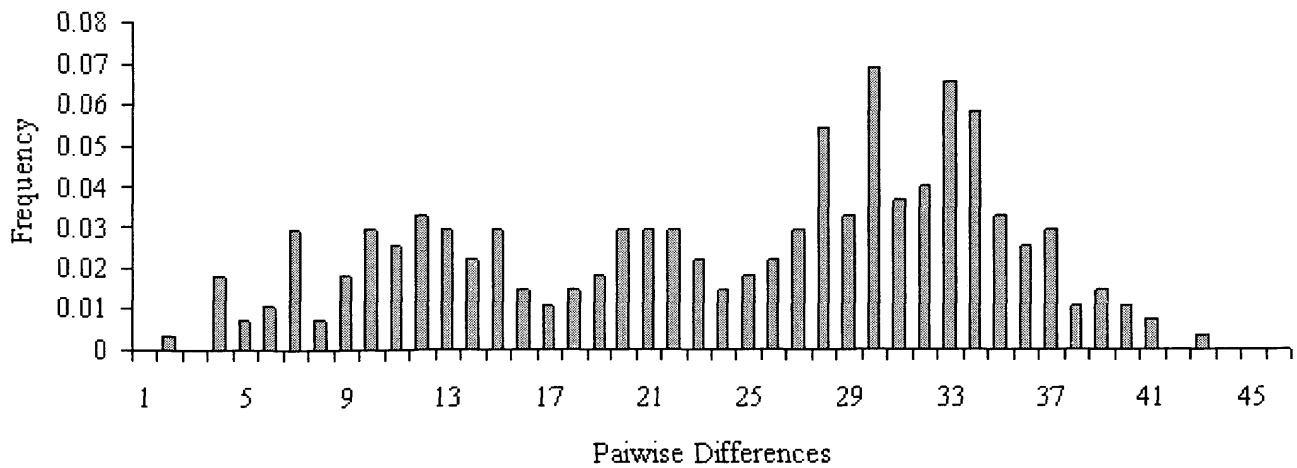
Figure 19. (continued).

**D**

Atlantic-II

**E**

Pacific

**F**

APPENDICES

Appendix 1. Sample collection and voucher information.

Collection Date	Species	Tissue ID	Latitude	Longitude	Geographic Location / Notes	Host	Voucher ID
2/10/04	<i>P. sabatini</i>	Atl. Pom. 1	N/A	N/A	Chesapeake Bay, VA	N/A	
2/28/05	<i>N. pectoralis</i>	Pac. Nem. 1	~8° N	~78° 30' W	Pinas Bay, Panama	N/A	
	<i>C. armatus</i>				Genbank Accession # AP004444	N/A	
	<i>R. canadum</i>	Atl. Rach. 1			Jim Franks cobiatissue, sample #7	N/A	
3/24/04	<i>C. hippocampus</i>	Atl. Cor. 1			Bayside Seafood, Hayes, VA	N/A	
8/02	<i>C. equiseetus</i>	Atl. Cor. 4			N. Atlantic - Shoyo Maru Leg 2	N/A	SMB036
5/13/04	<i>P. lineatus</i>	Pac. Rem. 4	~18° 06' N	~158° 34' W	Hawaii, SW of Big Island	baifish	
2/27/05	<i>E. naustrates</i>	Pac. Rem. 28	~32° 45' S	~152° 30' E	Port Stephens, Australia	<i>T. audax</i>	
8/8/03	<i>E. neucratoides</i>	Atl. Rem. 7	~39° 31' N	~69° 32' W	MAB- Hudson Canyon	<i>C. hippocampus</i>	
9/2/03	<i>R. remora</i>	Atl. Rem. 1	~39° 23' N	~71° 11' W	MAB- Hudson Canyon	<i>X. gladius</i>	
9/11/02	<i>R. brachyptera</i>	SIM105	~8° 8' S	~24° 24' W	Eq. S. Atlantic - Shoyo Maru Leg 3	<i>I. platypterus</i>	NMFS 0546
9/4/03	<i>R. osteochir</i>	Atl. Rem. 3	~39° 32' N	~71° 9' W	MAB- Hudson Canyon	<i>T. albidus</i>	
6/1/05	<i>R. australis</i>	SIO-0532	~19.5° N	~156.5° W	Kona, Hawaii	neuston tow	SIO-0532
2/27/05	<i>R. albescens</i>	Pac. Rem. 37	~32° 45' S	~152° 30' E	Port Stephens, Australia	<i>G. curvier</i>	



Appendix 1. (continued).

Ocean Basin	Collection Date	Sample Number	Tissue ID	Latitude	Longitude	Geographic Location	Host	Voucher ID
Atlantic	8/9/02	CNA1735	SMB004	36° 46' N	46° 10' W	Shoyo Maru Leg 2 - N. Atlantic	<i>T. albidus</i>	-
	8/9/02	CNA1736	SMB005	36° 46' N	46° 10' W	Shoyo Maru Leg 2 - N. Atlantic	<i>T. albidus</i>	-
	8/16/02	CNA1740	SMB013	31° 50' N	27° 53' W	Shoyo Maru Leg 2 - N. Atlantic	<i>M. nigricans</i>	-
	8/17/02	CNA1741	SMB017	30° 24' N	28° 42' W	Shoyo Maru Leg 2 - N. Atlantic	<i>T. pfluegeri</i>	-
	8/17/02	CNA1742	SMB018	30° 24' N	28° 42' W	Shoyo Maru Leg 2 - N. Atlantic	<i>T. pfluegeri</i>	-
	8/9/03	WNA1347	Atl. Rem. 8	39° 53' N	69° 38' W	MAB - Hudson Canyon	<i>T. albidus</i>	-
	8/9/03	WNA1348	Atl. Rem. 9	39° 53' N	69° 50' W	MAB - Hudson Canyon	<i>T. albidus</i>	-
	8/18/03	WNA1349	Atl. Rem. 10	39° 07' N	72° 37' W	MAB - Hudson Canyon	<i>X. scadius</i>	-
	9/4/03	WNA1346	Atl. Rem. 3	39° 53' N	71° 15' W	MAB - Hudson Canyon	<i>T. albidus</i>	-
	8/11/04	WNA1351	Atl. Rem. 44			Eastern Shore, NC	<i>T. albidus</i>	-
	8/11/04	WNA1914	Atl. Rem. 43			Eastern Shore, NC	<i>T. albidus</i>	-
	3/20/04	GOM1756	Atl. Rem. 57	~24° N	~82° W	Gulf of Mexico/Fl Straights	<i>I. platypterus</i>	-
	3/20/04	GOM1757	Atl. Rem. 58	~24° N	~82° W	Gulf of Mexico/Fl Straights	<i>I. platypterus</i>	-
	3/20/04	GOM1758	Atl. Rem. 59	~24° N	~82° W	Gulf of Mexico/Fl Straights	<i>I. platypterus</i>	-
	5/8/04-5/6/04	GOMB52	Atl. Rem. 33	~21° N	~87° W	Elk Mujeres, Mexico	<i>I. platypterus</i>	-
	5/8/04-5/6/04	GOMB53	Atl. Rem. 34	~21° N	~87° W	Elk Mujeres, Mexico	<i>I. platypterus</i>	-
	5/8/04-5/6/04	GOMB54	Atl. Rem. 35	~21° N	~87° W	Elk Mujeres, Mexico	<i>I. platypterus</i>	-
	5/8/04-5/6/04	GOMB55	Atl. Rem. 36	~21° N	~87° W	Elk Mujeres, Mexico	<i>I. platypterus</i>	-
	5/8/04-5/6/04	GOMB56	Atl. Rem. 46	~21° N	~87° W	Elk Mujeres, Mexico	<i>I. platypterus</i>	-
	5/8/04-5/6/04	GOMB57	Atl. Rem. 47	~21° N	~87° W	Elk Mujeres, Mexico	<i>I. platypterus</i>	-
	5/8/04-5/6/04	GOM2027	Atl. Rem. 48	~21° N	~87° W	Elk Mujeres, Mexico	<i>I. platypterus</i>	-
	5/8/04-5/6/04	GOM2028	Atl. Rem. 49	~21° N	~87° W	Elk Mujeres, Mexico	<i>I. platypterus</i>	-
	5/8/04-5/6/04	GOM2029	Atl. Rem. 50	~21° N	~87° W	Elk Mujeres, Mexico	<i>I. platypterus</i>	-
	5/8/04-5/6/04	GOM2030	Atl. Rem. 51	~21° N	~87° W	Elk Mujeres, Mexico	<i>I. platypterus</i>	-
	5/8/04-5/6/04	GOM2031	Atl. Rem. 52	~21° N	~87° W	Elk Mujeres, Mexico	<i>I. platypterus</i>	-
	5/8/04-5/6/04	GOM2032	Atl. Rem. 53	~21° N	~87° W	Elk Mujeres, Mexico	<i>I. platypterus</i>	-
	9/30/02	WEA1340	L48-1-1	8° 22' S	29° 39' W	Shoyo Maru Leg 4 - Eq. S. Atlantic	<i>M. nigricans</i>	-
	9/30/02	WEA1341	L48-1-2	8° 22' S	29° 39' W	Shoyo Maru Leg 4 - Eq. S. Atlantic	<i>M. nigricans</i>	-
	10/1/02	WEA1342	L48-16-1	8° 21' S	29° 40' W	Shoyo Maru Leg 4 - Eq. S. Atlantic	<i>T. pfluegeri</i>	-
	10/1/02	WEA1343	L48-16-2	8° 21' S	29° 40' W	Shoyo Maru Leg 4 - Eq. S. Atlantic	<i>T. pfluegeri</i>	-
	10/1/02	WEA1749	L48-4	8° 21' S	29° 40' W	Shoyo Maru Leg 4 - Eq. S. Atlantic	<i>T. pfluegeri</i>	-
	10/1/02	WEA1750	L48-13-1(L48-13)	8° 21' S	29° 40' W	Shoyo Maru Leg 4 - Eq. S. Atlantic	<i>T. albidus</i>	-
	10/1/02	WEA1751	L48-13-2(L48-13-1)	8° 21' S	29° 40' W	Shoyo Maru Leg 4 - Eq. S. Atlantic	<i>T. albidus</i>	-
	10/1/02	WEA1752	L48-17-1(L48-17)	8° 21' S	29° 40' W	Shoyo Maru Leg 4 - Eq. S. Atlantic	<i>T. albidus</i>	-
	10/1/02	WEA1753	L48-17-2(L48-17-1)	8° 21' S	29° 40' W	Shoyo Maru Leg 4 - Eq. S. Atlantic	<i>T. albidus</i>	-
	10/3/02	WEA1344	L50-8-1	9° 05' S	29° 42' W	Shoyo Maru Leg 4 - Eq. S. Atlantic	<i>T. albidus</i>	-
	10/3/02	WEA1345	L50-8-2	9° 05' S	29° 42' W	Shoyo Maru Leg 4 - Eq. S. Atlantic	<i>T. albidus</i>	-
10/8/02	WEA1754	L52-8	3° 30' N	40° 30' W	Shoyo Maru Leg 4 - Eq. N. Atlantic	<i>T. pfluegeri</i>	-	
10/9/02	WEA1755	L53-1	3° 44' N	40° 29' W	Shoyo Maru Leg 4 - Eq. N. Atlantic	<i>I. platypterus</i>	-	
9/1/02	EEA1334	SM032	4° 36' N	22° 18' W	Shoyo Maru Leg 3 - Eq. N. Atlantic	<i>I. platypterus</i>	NMFS 0506	
9/1/02	EEA1335	SM033	4° 36' N	22° 18' W	Shoyo Maru Leg 3 - Eq. N. Atlantic	<i>I. platypterus</i>	NMFS 0507	
9/7/02	EEA1336	SM074	7° 36' S	21° 6' W	Shoyo Maru Leg 3 - Eq. S. Atlantic	<i>I. platypterus</i>	NMFS 0517	
9/7/02	EEA1337	SM077	7° 36' S	21° 6' W	Shoyo Maru Leg 3 - Eq. S. Atlantic	N/A	NMFS 0520	
9/7/02	EEA1338	SM078	7° 36' S	21° 6' W	Shoyo Maru Leg 3 - Eq. S. Atlantic	<i>T. albidus</i>	NMFS 0521	
9/7/02	EEA1339	SM079	7° 36' S	21° 6' W	Shoyo Maru Leg 3 - Eq. S. Atlantic	<i>T. albidus</i>	NMFS 0522	
9/8/02	EEA1743	SM083	7° 27' S	21° 11' W	Shoyo Maru Leg 3 - Eq. S. Atlantic	<i>T. pfluegeri</i>	NMFS 0528	
9/9/02	EEA1744	SM087	7° 30' S	21° 15' W	Shoyo Maru Leg 3 - Eq. S. Atlantic	<i>T. pfluegeri</i>	NMFS 0531	
9/13/02	EEA1746	SM130	7° 28' S	22° 8' W	Shoyo Maru Leg 3 - Eq. S. Atlantic	<i>I. platypterus</i>	NMFS 0555	
9/18/02	EEA1748	SM189	8° 12' S	21° 36' W	Shoyo Maru Leg 3 - Eq. S. Atlantic	<i>T. pfluegeri</i>	NMFS 0577	
9/14/02	EEA1852	SM143	7° 36' S	21° 0' W	Shoyo Maru Leg 3 - Eq. S. Atlantic	<i>T. pfluegeri</i>	NMFS 0560	
9/11/02	EEA2026	SM108	8° 8' S	24° 24' W	Shoyo Maru Leg 3 - Eq. S. Atlantic	<i>I. platypterus</i>	NMFS 0548	
Pacific	N/A	WPAC1525	Pac. Rem. 35	~26° 40' S	~153° 20' E	Malookaba, Australia	<i>T. onus inostris</i>	-
	N/A	WPAC1526	Pac. Rem. 36	~26° 40' S	~153° 20' E	Malookaba, Australia	<i>T. onus inostris</i>	-
	2/1/04	WPAC1523	Pac. Rem. 33	~32° 45' S	~152° 30' E	Port Stephens, Australia	<i>M. nigricans</i>	-
	2/1/04	WPAC1524	Pac. Rem. 34	~32° 45' S	~152° 30' E	Port Stephens, Australia	<i>M. nigricans</i>	-
	2/27/05	WPAC1519	Pac. Rem. 29	~32° 45' S	~152° 30' E	Port Stephens, Australia	<i>T. audax</i>	-
	2/27/05	WPAC1520	Pac. Rem. 30	~32° 45' S	~152° 30' E	Port Stephens, Australia	<i>T. audax</i>	-
	3/6/05	WPAC1521	Pac. Rem. 31	~32° 45' S	~152° 30' E	Port Stephens, Australia	<i>T. audax</i>	-
	3/6/05	WPAC1522	Pac. Rem. 32	~32° 45' S	~152° 30' E	Port Stephens, Australia	<i>T. audax</i>	-
	1/05	EPAC1512	Pac. Rem. 22	~8° N	~78° 30' W	Pinas Bay, Panama	<i>I. platypterus</i>	-
	1/05	EPAC1513	Pac. Rem. 23	~8° N	~78° 30' W	Pinas Bay, Panama	<i>I. platypterus</i>	-
	1/05	EPAC1514	Pac. Rem. 24	~8° N	~78° 30' W	Pinas Bay, Panama	<i>I. platypterus</i>	-
	1/05	EPAC1515	Pac. Rem. 25	~8° N	~78° 30' W	Pinas Bay, Panama	<i>I. platypterus</i>	-
	1/05	EPAC1516	Pac. Rem. 26	~8° N	~78° 30' W	Pinas Bay, Panama	<i>I. platypterus</i>	-
	1/05	EPAC1517	Pac. Rem. 27	~8° N	~78° 30' W	Pinas Bay, Panama	<i>I. platypterus</i>	-
	2/2005-4/2005	EPAC1759	Pac. Rem. 41	~8° N	~78° 30' W	Pinas Bay, Panama	N/A	-
	2/2005-4/2005	EPAC1760	Pac. Rem. 42	~8° N	~78° 30' W	Pinas Bay, Panama	N/A	-
	2/2005-4/2005	EPAC1761	Pac. Rem. 43	~8° N	~78° 30' W	Pinas Bay, Panama	N/A	-
	2/2005-4/2005	EPAC1762	Pac. Rem. 44	~8° N	~78° 30' W	Pinas Bay, Panama	N/A	-
	2/2005-4/2005	EPAC1763	Pac. Rem. 45	~8° N	~78° 30' W	Pinas Bay, Panama	N/A	-
2/2005-4/2005	EPAC1764	Pac. Rem. 46	~8° N	~78° 30' W	Pinas Bay, Panama	N/A	-	
Outgroup	5/13/2004	<i>P. lineatus</i>	Pac. Rem. 4	18° 06' N	158° 34' W	Hawaii, SW of Big Island	baiffish	-

Appendix 2. Pairwise nucleotide sequence divergence estimates between echeneoid specimens based upon complete mitochondrial 12S rRNA DNA sequence data.

## Pairwise uncorrected 'p-distances' among the Ethnoscioidea

	<i>M. pectoralis</i>	<i>C. ornatus</i>	<i>R. ostensifer</i>	<i>R. albescens</i>	<i>P. brevis</i>	<i>E. neurocrabridae</i>	<i>P. subarctic</i>	<i>R. remora</i>	<i>E. maculipes</i>	<i>C. hispidus</i>	<i>R. australis</i>	<i>R. brachyptera</i>	<i>C. equisetis</i>	<i>R. canadum</i>
<i>M. pectoralis</i>														
<i>C. ornatus</i>	0.1276													
<i>R. ostensifer</i>	0.1268	0.1010												
<i>R. albescens</i>	0.1380	0.1179	0.0561											
<i>P. brevis</i>	0.1335	0.1078	0.0471	0.0561										
<i>E. neurocrabridae</i>	0.1368	0.1124	0.0482	0.0572	0.0135									
<i>P. subarctic</i>	0.1684	0.1572	0.1385	0.1462	0.1361	0.1372								
<i>R. remora</i>	0.1302	0.1033	0.0382	0.0561	0.0448	0.0437	0.1327							
<i>E. maculipes</i>	0.1380	0.1146	0.0504	0.0594	0.0157	0.0023	0.1384	0.0460						
<i>C. hispidus</i>	0.1680	0.1252	0.1172	0.1319	0.1284	0.1273	0.1679	0.1217	0.1284					
<i>R. australis</i>	0.1302	0.1033	0.0382	0.0527	0.0493	0.0516	0.1418	0.0370	0.0538	0.1136				
<i>R. brachyptera</i>	0.1368	0.1145	0.0584	0.0673	0.0684	0.0718	0.1507	0.0383	0.0740	0.1307	0.0583			
<i>C. equisetis</i>	0.1680	0.1307	0.1240	0.1420	0.1341	0.1330	0.1735	0.1239	0.1341	0.0280	0.1180	0.1329		
<i>R. canadum</i>	0.1605	0.1264	0.1218	0.1342	0.1264	0.1275	0.1340	0.1308	0.1287	0.1171	0.1184	0.1309	0.1239	

Appendix 3. Pairwise nucleotide sequence divergence estimates between echeneoid specimens based upon partial mitochondrial 16S rRNA DNA sequence data.

## Paires uncorrected 'b-dissorté' au orghe Ethnoscopia

	<i>M. pectoralis</i>	<i>C. armatus</i>	<i>R. canadum</i>	<i>R. ostrochir</i>	<i>R. albescens</i>	<i>P. brentus</i>	<i>E. neocentoides</i>	<i>P. solitari</i>	<i>R. remora</i>	<i>E. macrotus</i>	<i>C. hippurus</i>	<i>R. australis</i>	<i>R. brachyptera</i>	<i>C. equiseis</i>
<i>M. pectoralis</i>														
<i>C. armatus</i>	0.1513													
<i>R. canadum</i>	0.1985	0.1717												
<i>R. ostrochir</i>	0.1904	0.1690	0.1837											
<i>R. albescens</i>	0.1934	0.1686	0.1729	0.0614										
<i>P. brentus</i>	0.1800	0.1620	0.1642	0.0979	0.0919									
<i>E. neocentoides</i>	0.1869	0.1619	0.1635	0.0939	0.0880	0.0166								
<i>P. solitari</i>	0.1701	0.1605	0.1760	0.1777	0.1710	0.1716	0.1715							
<i>R. remora</i>	0.1908	0.1789	0.1803	0.0615	0.0703	0.0918	0.0910	0.1774						
<i>E. macrotus</i>	0.1862	0.1612	0.1622	0.0946	0.0873	0.0160	0.0019	0.1701	0.0898					
<i>C. hippurus</i>	0.2023	0.1798	0.1290	0.1770	0.1814	0.1696	0.1714	0.1888	0.1793	0.1701				
<i>R. australis</i>	0.1895	0.1731	0.1685	0.0680	0.0705	0.0895	0.0908	0.1713	0.0660	0.0895	0.1785			
<i>R. brachyptera</i>	0.1966	0.1735	0.1829	0.0732	0.0879	0.1077	0.1082	0.1743	0.0731	0.1059	0.1831	0.0802		
<i>C. equiseis</i>	0.2026	0.1748	0.1265	0.1789	0.1788	0.1693	0.1705	0.1847	0.1842	0.1681	0.0841	0.1819	0.1903	

Appendix 4. Pairwise nucleotide sequence divergence estimates between echeoid specimens based upon complete mitochondrial ND2 DNA sequence data.

Pairwise uncorrected 'p-distance' among the Echinoidika

	<i>P. solitaria</i>	<i>C. ornatus</i>	<i>C. hippocampus</i>	<i>C. equisetis</i>	<i>R. albescens</i>	<i>E. neauratus</i>	<i>R. remora</i>	<i>R. canadum</i>	<i>R. ostreolar</i>	<i>E. neauratoides</i>	<i>R. brachyptera</i>	<i>N. peratravis</i>	<i>P. lineatus</i>	<i>R. auratus</i>
<i>P. solitaria</i>	-													
<i>C. ornatus</i>	0.1390	-												
<i>C. hippocampus</i>	0.2135	0.1991	-											
<i>C. equisetis</i>	0.2135	0.1977	0.0387	-										
<i>R. albescens</i>	0.1691	0.1332	0.1963	0.2034	-									
<i>E. neauratus</i>	0.1820	0.1318	0.2006	0.2034	0.0788	-								
<i>R. remora</i>	0.1848	0.1361	0.1894	0.1920	0.0573	0.0731	-							
<i>R. canadum</i>	0.2049	0.1994	0.1605	0.1705	0.1963	0.1848	0.1877	-						
<i>R. ostreolar</i>	0.1705	0.1433	0.2049	0.2034	0.0645	0.0716	0.0530	0.1891	-					
<i>E. neauratoides</i>	0.1805	0.1304	0.2020	0.2049	0.0774	0.0014	0.0745	0.1863	0.0731	-				
<i>R. brachyptera</i>	0.1805	0.1461	0.2063	0.2063	0.0716	0.0817	0.0645	0.1920	0.0745	0.0881	-			
<i>N. peratravis</i>	0.1504	0.1189	0.1920	0.1920	0.1390	0.1648	0.1547	0.1905	0.1519	0.1633	0.1734	-		
<i>P. lineatus</i>	0.1791	0.1289	0.1977	0.1977	0.0759	0.0301	0.0673	0.1891	0.0702	0.0815	0.0745	0.1590	-	
<i>R. auratus</i>	0.1719	0.1318	0.1920	0.1963	0.0659	0.0639	0.0602	0.1864	0.0888	0.0673	0.0716	0.1504	0.0702	-



Appendix 5. Pairwise nucleotide sequence divergence estimates between echeneoid specimens based upon complete nuclear ITS-1 DNA sequence data.

Pairwise uncorrected 'p-distance' among IS-1 DNA sequences in the Eubacteriia

	<i>P. salinarum</i>	<i>N. perströmii</i>	<i>R. canadum</i>	<i>C. hippocampus</i>	<i>C. equi</i>	<i>P. hansenii</i>	<i>E. neaurum</i>	<i>E. neaurum</i>	<i>E. neaurum</i>	<i>R. australis</i>	<i>R. adhaerens</i>	<i>R. brassilyperna</i>	<i>R. ostreolitor</i>	<i>R. remora</i>
<i>P. salinarum</i>	0.1770													
<i>N. perströmii</i>	0.3143	0.3071												
<i>R. canadum</i>	0.3075	0.3132	0.1477											
<i>C. hippocampus</i>	0.2827	0.2938	0.1657	0.0772										
<i>C. equi</i>	0.3187	0.2997	0.2965	0.2747	0.2727									
<i>P. hansenii</i>	0.3307	0.3200	0.3102	0.2809	0.2809	0.0438								
<i>E. neaurum</i>	0.3383	0.3240	0.3156	0.2869	0.2863	0.0492	0.0066							
<i>E. neaurum</i>	0.3121	0.3225	0.3232	0.3044	0.3052	0.2009	0.2045	0.2072						
<i>R. australis</i>	0.3269	0.3257	0.3264	0.3179	0.3020	0.2243	0.2224	0.2200	0.1461					
<i>R. adhaerens</i>	0.2938	0.3060	0.3196	0.2895	0.2854	0.1818	0.1880	0.1919	0.1340	0.1544				
<i>R. brassilyperna</i>	0.2940	0.2810	0.3042	0.2871	0.2800	0.1832	0.1903	0.1885	0.1096	0.1273	0.0817			
<i>R. ostreolitor</i>	0.3088	0.3099	0.3046	0.2867	0.2890	0.1964	0.1948	0.1980	0.1176	0.1293	0.0881	0.0815		

Appendix 6. Pairwise nucleotide sequence divergence estimates between specimens of marlinsucker, *R. osteochir*, based upon complete mitochondrial control region DNA sequence data.

	EPAC1763	WEA1751	CNA1740	CNA1741	CNA1742	CNA1735	EEA1334	EEA1335	EEA1336	EEA1337	EEA1338	EEA1339
EPAC1763	-											
WEA1751	0.0325	-										
CNA1740	0.0247	0.0194	-									
CNA1741	0.0224	0.0239	0.0127	-								
CNA1742	0.0370	0.0063	0.0192	0.0238	-							
CNA1735	0.0303	0.0181	0.0182	0.0204	0.0203	-						
EEA1334	0.0452	0.0508	0.0417	0.0405	0.0485	0.0487	-					
EEA1335	0.0280	0.0271	0.0116	0.0116	0.0269	0.0215	0.0416	-				
EEA1336	0.0236	0.0291	0.0160	0.0138	0.0291	0.0214	0.0441	0.0127	-			
EEA1337	0.0336	0.0303	0.0192	0.0148	0.0238	0.0247	0.0475	0.0159	0.0160	-		
EEA1338	0.0496	0.0555	0.0462	0.0449	0.0531	0.0532	0.0292	0.0484	0.0439	0.0473	-	
EEA1339	0.0382	0.0427	0.0314	0.0325	0.0427	0.0314	0.0428	0.0336	0.0291	0.0325	0.0496	-
EEA1743	0.0269	0.0281	0.0149	0.0106	0.0280	0.0204	0.0429	0.0116	0.0074	0.0127	0.0450	0.0280
EEA1744	0.0464	0.0521	0.0406	0.0416	0.0475	0.0452	0.0161	0.0405	0.0452	0.0463	0.0314	0.0440
EEA1746	0.0495	0.0506	0.0438	0.0439	0.0483	0.0495	0.0348	0.0471	0.0473	0.0506	0.0347	0.0392
EEA1748	0.0487	0.0545	0.0429	0.0440	0.0498	0.0476	0.0204	0.0406	0.0442	0.0441	0.0304	0.0441
EEA1852	0.0347	0.0203	0.0225	0.0247	0.0247	0.0063	0.0487	0.0259	0.0236	0.0291	0.0532	0.0337
EEA2026	0.0325	0.0292	0.0203	0.0138	0.0314	0.0259	0.0486	0.0192	0.0214	0.0203	0.0508	0.0404
EPAC1512	0.0269	0.0203	0.0160	0.0203	0.0203	0.0149	0.0440	0.0214	0.0236	0.0258	0.0462	0.0371
EPAC1513	0.0386	0.0091	0.0237	0.0317	0.0117	0.0250	0.0600	0.0346	0.0345	0.0360	0.0672	0.0414
EPAC1514	0.0453	0.0489	0.0407	0.0406	0.0443	0.0466	0.0477	0.0395	0.0407	0.0407	0.0443	0.0395
EPAC1515	0.0346	0.0338	0.0246	0.0224	0.0369	0.0347	0.0496	0.0235	0.0301	0.0312	0.0517	0.0449
CNA1736	0.0269	0.0238	0.0171	0.0106	0.0280	0.0226	0.0441	0.0160	0.0182	0.0171	0.0439	0.0371
EPAC1516	0.0325	0.0203	0.0139	0.0181	0.0203	0.0149	0.0308	0.0193	0.0192	0.0203	0.0530	0.0338
EPAC1517	0.0239	0.0160	0.0139	0.0182	0.0160	0.0106	0.0463	0.0193	0.0214	0.0225	0.0486	0.0339
EPAC1759	0.0127	0.0302	0.0225	0.0224	0.0347	0.0258	0.0428	0.0236	0.0214	0.0291	0.0450	0.0382
EPAC1760	0.0239	0.0160	0.0205	0.0226	0.0181	0.0171	0.0510	0.0238	0.0259	0.0270	0.0309	0.0405
EPAC1761	0.0303	0.0203	0.0182	0.0204	0.0203	0.0149	0.0463	0.0237	0.0259	0.0269	0.0485	0.0405
EPAC1762	0.0281	0.0316	0.0182	0.0225	0.0338	0.0281	0.0322	0.0214	0.0258	0.0247	0.0544	0.0417
EPAC1764	0.0269	0.0305	0.0193	0.0214	0.0305	0.0270	0.0498	0.0203	0.0247	0.0236	0.0520	0.0394
GOMI352	0.0531	0.0601	0.0520	0.0530	0.0601	0.0543	0.0381	0.0519	0.0474	0.0555	0.0203	0.0556
GOMI353	0.0429	0.0510	0.0395	0.0382	0.0487	0.0464	0.0128	0.0384	0.0418	0.0429	0.0259	0.0429
GOMI354	0.0476	0.0532	0.0440	0.0428	0.0486	0.0510	0.0106	0.0440	0.0464	0.0451	0.0303	0.0427
GOMI355	0.0239	0.0138	0.0161	0.0161	0.0181	0.0085	0.0463	0.0172	0.0193	0.0204	0.0309	0.0337
GOMI356	0.0337	0.0325	0.0237	0.0192	0.0337	0.0270	0.0465	0.0203	0.0248	0.0259	0.0510	0.0417
GOMI357	0.0507	0.0589	0.0474	0.0484	0.0542	0.0544	0.0359	0.0497	0.0428	0.0485	0.0117	0.0484

Appendix 6. (continued).

	EEA1743	EEA1744	EEA1746	EEA1748	EEA1852	EEA2026	EPAC1512	EPAC1513	EPAC1514	EPAC1515	CNA1736	EPAC1516
EEA1743												
EEA1744	0.0417											
EEA1746	0.0484	0.0336										
EEA1748	0.0442	0.0085	0.0348									
EEA1852	0.0248	0.0475	0.0495	0.0499								
EEA2026	0.0181	0.0474	0.0517	0.0498	0.0281							
EPAC1512	0.0203	0.0429	0.0449	0.0475	0.0192	0.0281						
EPAC1513	0.0332	0.0601	0.0626	0.0615	0.0263	0.0415	0.0276					
EPAC1514	0.0407	0.0466	0.0440	0.0478	0.0489	0.0488	0.0396	0.0480				
EPAC1515	0.0268	0.0484	0.0539	0.0508	0.0382	0.0301	0.0313	0.0470	0.0486			
CNA1736	0.0149	0.0429	0.0449	0.0453	0.0247	0.0074	0.0247	0.0387	0.0407	0.0268		
EPAC1516	0.0182	0.0497	0.0517	0.0521	0.0192	0.0236	0.0192	0.0277	0.0465	0.0323	0.0203	
EPAC1517	0.0204	0.0429	0.0438	0.0453	0.0149	0.0237	0.0063	0.0250	0.0397	0.0292	0.0204	0.0149
EPAC1739	0.0225	0.0394	0.0448	0.0417	0.0303	0.0302	0.0247	0.0386	0.0453	0.0346	0.0247	0.0302
EPAC1760	0.0248	0.0475	0.0484	0.0499	0.0215	0.0282	0.0193	0.0250	0.0409	0.0325	0.0226	0.0193
EPAC1761	0.0204	0.0429	0.0484	0.0476	0.0193	0.0259	0.0171	0.0291	0.0466	0.0325	0.0226	0.0192
EPAC1762	0.0226	0.0487	0.0542	0.0488	0.0304	0.0303	0.0239	0.0347	0.0466	0.0347	0.0270	0.0292
EPAC1764	0.0214	0.0464	0.0495	0.0464	0.0293	0.0292	0.0226	0.0347	0.0409	0.0324	0.0239	0.0281
GOM1352	0.0532	0.0416	0.0448	0.0382	0.0543	0.0613	0.0496	0.0629	0.0464	0.0647	0.0543	0.0588
GOM1353	0.0429	0.0139	0.0348	0.0161	0.0464	0.0463	0.0464	0.0557	0.0408	0.0485	0.0418	0.0486
GOM1354	0.0475	0.0138	0.0325	0.0160	0.0310	0.0309	0.0474	0.0586	0.0453	0.0519	0.0464	0.0532
GOM1355	0.0182	0.0452	0.0495	0.0476	0.0106	0.0193	0.0127	0.0236	0.0419	0.0325	0.0160	0.0128
GOM1356	0.0193	0.0453	0.0543	0.0477	0.0292	0.0269	0.0236	0.0388	0.0477	0.0192	0.0237	0.0291
GOM1357	0.0439	0.0370	0.0403	0.0337	0.0567	0.0542	0.0473	0.0672	0.0500	0.0529	0.0473	0.0542

Appendix 6. (continued).

	EPAC1517	EPAC1759	EPAC1760	EPAC1761	EPAC1762	EPAC1764	GOM1352	GOM1353	GOM1354	GOM1355	GOM1356	GOM1357
EPAC1517	-											
EPAC1759	0.0236	-										
EPAC1760	0.0128	0.0259	-									
EPAC1761	0.0128	0.0259	0.0149	-								
EPAC1762	0.0237	0.0237	0.0260	0.0259	-							
EPAC1764	0.0205	0.0225	0.0249	0.0248	0.0053	-						
GOM1352	0.0520	0.0484	0.0602	0.0578	0.0380	0.0556	-					
GOM1353	0.0441	0.0406	0.0463	0.0441	0.0476	0.0476	0.0537	-				
GOM1354	0.0463	0.0451	0.0510	0.0487	0.0522	0.0498	0.0382	0.0106	-			
GOM1355	0.0085	0.0236	0.0128	0.0149	0.0215	0.0204	0.0567	0.0441	0.0487	-		
GOM1356	0.0237	0.0337	0.0293	0.0292	0.0315	0.0292	0.0616	0.0477	0.0512	0.0226	-	
GOM1357	0.0497	0.0461	0.0543	0.0497	0.0556	0.0532	0.0237	0.0337	0.0339	0.0567	0.0546	-



Appendix 6. (continued).

	EPAC1763	WEA1751	CNA1740	CNA1741	CNA1742	CNA1735	EEA1334	EEA1335	EEA1336	EEA1337	EEA1338	EEA1339
GOM2031	0.0291	0.0291	0.0203	0.0181	0.0302	0.0258	0.0382	0.0214	0.0236	0.0247	0.0450	0.0358
GOM1757	0.0337	0.0053	0.0205	0.0270	0.0074	0.0182	0.0522	0.0282	0.0303	0.0292	0.0345	0.0440
GOM1758	0.0496	0.0531	0.0439	0.0449	0.0484	0.0309	0.0325	0.0461	0.0440	0.0474	0.0106	0.0496
GOM2027	0.0394	0.0474	0.0371	0.0347	0.0451	0.0429	0.0117	0.0359	0.0383	0.0393	0.0247	0.0393
GOM2028	0.0440	0.0522	0.0406	0.0393	0.0498	0.0476	0.0138	0.0405	0.0429	0.0440	0.0292	0.0439
GOM2029	0.0138	0.0302	0.0247	0.0247	0.0347	0.0303	0.0405	0.0280	0.0280	0.0336	0.0427	0.0430
GOM1756	0.0495	0.0507	0.0438	0.0460	0.0484	0.0496	0.0326	0.0449	0.0451	0.0484	0.0326	0.0370
GOM2030	0.0313	0.0302	0.0192	0.0127	0.0324	0.0269	0.0451	0.0181	0.0303	0.0214	0.0495	0.0392
WEA1340	0.0522	0.0579	0.0509	0.0497	0.0553	0.0537	0.0281	0.0309	0.0309	0.0343	0.0370	0.0321
WEA1341	0.0440	0.0521	0.0406	0.0393	0.0498	0.0475	0.0138	0.0405	0.0429	0.0440	0.0292	0.0440
GOM2032	0.0496	0.0554	0.0438	0.0449	0.0507	0.0485	0.0214	0.0438	0.0440	0.0461	0.0347	0.0450
WEA1342	0.0428	0.0533	0.0395	0.0405	0.0509	0.0487	0.0149	0.0394	0.0440	0.0451	0.0281	0.0451
WEA1343	0.0236	0.0248	0.0117	0.0116	0.0247	0.0193	0.0395	0.0106	0.0127	0.0160	0.0439	0.0314
WEA1344	0.0291	0.0236	0.0149	0.0106	0.0238	0.0204	0.0440	0.0160	0.0181	0.0171	0.0462	0.0348
WEA1345	0.0280	0.0269	0.0116	0.0138	0.0268	0.0214	0.0405	0.0170	0.0192	0.0203	0.0426	0.0347
WEA1749	0.0280	0.0239	0.0160	0.0116	0.0238	0.0237	0.0417	0.0127	0.0149	0.0138	0.0438	0.0314
WEA1750	0.0486	0.0568	0.0428	0.0439	0.0521	0.0522	0.0127	0.0451	0.0475	0.0485	0.0314	0.0473
WEA1752	0.0247	0.0237	0.0106	0.0084	0.0236	0.0182	0.0405	0.0032	0.0095	0.0127	0.0473	0.0303
WEA1753	0.0440	0.0521	0.0405	0.0393	0.0486	0.0475	0.0127	0.0405	0.0428	0.0439	0.0280	0.0439
WNA1914	0.0454	0.0486	0.0408	0.0406	0.0463	0.0441	0.0226	0.0395	0.0420	0.0476	0.0293	0.0453
WPAC1519	0.0324	0.0360	0.0203	0.0246	0.0339	0.0325	0.0320	0.0214	0.0280	0.0268	0.0366	0.0415
WPAC1520	0.0404	0.0170	0.0237	0.0238	0.0192	0.0204	0.0367	0.0248	0.0236	0.0269	0.0602	0.0427
WEA1754	0.0520	0.0578	0.0485	0.0472	0.0534	0.0532	0.0303	0.0308	0.0463	0.0497	0.0063	0.0496
WEA1755	0.0495	0.0530	0.0438	0.0448	0.0507	0.0308	0.0303	0.0461	0.0439	0.0472	0.0085	0.0472
WNA1346	0.0440	0.0498	0.0383	0.0393	0.0451	0.0429	0.0160	0.0382	0.0429	0.0440	0.0282	0.0417
WNA1347	0.0279	0.0291	0.0159	0.0137	0.0268	0.0257	0.0437	0.0148	0.0171	0.0203	0.0482	0.0380
WNA1348	0.0487	0.0498	0.0428	0.0486	0.0474	0.0499	0.0248	0.0451	0.0475	0.0486	0.0314	0.0463
WNA1349	0.0335	0.0053	0.0203	0.0269	0.0095	0.0192	0.0543	0.0280	0.0302	0.0291	0.0389	0.0438
WNA1351	0.0247	0.0249	0.0149	0.0106	0.0270	0.0249	0.0406	0.0160	0.0181	0.0192	0.0427	0.0325
WPAC1521	0.0247	0.0247	0.0192	0.0149	0.0280	0.0225	0.0439	0.0303	0.0225	0.0236	0.0461	0.0325
WPAC1522	0.0280	0.0181	0.0181	0.0225	0.0181	0.0149	0.0463	0.0236	0.0193	0.0247	0.0463	0.0382
WPAC1523	0.0251	0.0320	0.0185	0.0207	0.0331	0.0263	0.0484	0.0195	0.0229	0.0251	0.0517	0.0355
WPAC1524	0.0238	0.0182	0.0182	0.0160	0.0203	0.0106	0.0441	0.0172	0.0214	0.0225	0.0309	0.0339
WPAC1525	0.0360	0.0248	0.0248	0.0225	0.0237	0.0085	0.0476	0.0259	0.0259	0.0270	0.0498	0.0383
WPAC1526	0.0303	0.0171	0.0149	0.0182	0.0170	0.0117	0.0463	0.0204	0.0192	0.0214	0.0497	0.0348
P. lineatus	0.3352	0.3319	0.3175	0.3209	0.3320	0.3286	0.3316	0.3197	0.3229	0.3286	0.3311	0.3270

Appendix 6. (continued).

	EEA1743	EEA1744	EEA1746	EEA1748	EEA1832	EEA2026	EPAC1512	EPAC1513	EPAC1514	EPAC1515	CNA1736	EPAC1516
GOM2031	0.0225	0.0394	0.0437	0.0417	0.0303	0.0258	0.0225	0.0338	0.0395	0.0192	0.0225	0.0238
GOM1757	0.0293	0.0511	0.0495	0.0535	0.0236	0.0327	0.0214	0.0130	0.0502	0.0370	0.0292	0.0214
GOM1758	0.0451	0.0337	0.0370	0.0304	0.0532	0.0531	0.0462	0.0599	0.0466	0.0494	0.0462	0.0530
GOM2027	0.0394	0.0128	0.0314	0.0150	0.0428	0.0427	0.0428	0.0528	0.0396	0.0449	0.0383	0.0450
GOM2028	0.0440	0.0128	0.0359	0.0150	0.0475	0.0451	0.0475	0.0571	0.0419	0.0496	0.0429	0.0497
GOM2029	0.0292	0.0372	0.0426	0.0418	0.0325	0.0302	0.0247	0.0427	0.0453	0.0357	0.0247	0.0325
GOM1756	0.0461	0.0315	0.0042	0.0303	0.0496	0.0519	0.0450	0.0613	0.0441	0.0540	0.0450	0.0518
GOM2030	0.0170	0.0440	0.0305	0.0463	0.0291	0.0116	0.0269	0.0359	0.0475	0.0290	0.0084	0.0246
WEA1340	0.0498	0.0204	0.0437	0.0226	0.0557	0.0579	0.0532	0.0615	0.0512	0.0589	0.0533	0.0578
WEA1341	0.0440	0.0128	0.0359	0.0150	0.0475	0.0474	0.0475	0.0558	0.0431	0.0496	0.0429	0.0497
GOM2032	0.0450	0.0117	0.0369	0.0117	0.0485	0.0507	0.0484	0.0571	0.0523	0.0540	0.0462	0.0530
WEA1342	0.0452	0.0161	0.0370	0.0183	0.0487	0.0486	0.0487	0.0585	0.0431	0.0508	0.0441	0.0509
WEA1343	0.0117	0.0383	0.0426	0.0407	0.0237	0.0192	0.0192	0.0318	0.0385	0.0279	0.0160	0.0149
WEA1344	0.0149	0.0429	0.0449	0.0453	0.0225	0.0074	0.0225	0.0359	0.0407	0.0246	0.0021	0.0181
WEA1345	0.0160	0.0393	0.0414	0.0417	0.0258	0.0214	0.0214	0.0304	0.0452	0.0279	0.0181	0.0192
WEA1749	0.0117	0.0382	0.0426	0.0383	0.0259	0.0170	0.0236	0.0318	0.0395	0.0279	0.0138	0.0215
WEA1750	0.0486	0.0128	0.0349	0.0150	0.0498	0.0497	0.0498	0.0615	0.0466	0.0530	0.0475	0.0543
WEA1752	0.0085	0.0394	0.0437	0.0395	0.0225	0.0159	0.0181	0.0304	0.0384	0.0246	0.0127	0.0160
WEA1753	0.0440	0.0117	0.0348	0.0139	0.0474	0.0474	0.0463	0.0571	0.0431	0.0485	0.0428	0.0496
WNA1914	0.0431	0.0215	0.0381	0.0215	0.0441	0.0464	0.0440	0.0572	0.0489	0.0473	0.0420	0.0486
WPAC1519	0.0247	0.0485	0.0517	0.0486	0.0348	0.0324	0.0302	0.0361	0.0441	0.0388	0.0291	0.0313
WPAC1520	0.0204	0.0557	0.0576	0.0580	0.0247	0.0314	0.0247	0.0249	0.0512	0.0357	0.0280	0.0214
WEA1754	0.0450	0.0337	0.0369	0.0349	0.0532	0.0531	0.0462	0.0658	0.0466	0.0541	0.0462	0.0542
WEA1755	0.0450	0.0315	0.0325	0.0281	0.0307	0.0307	0.0438	0.0642	0.0466	0.0494	0.0438	0.0530
WNA1346	0.0418	0.0042	0.0314	0.0063	0.0452	0.0451	0.0429	0.0572	0.0443	0.0461	0.0406	0.0474
WNA1347	0.0160	0.0426	0.0481	0.0427	0.0301	0.0213	0.0214	0.0304	0.0428	0.0267	0.0181	0.0203
WNA1348	0.0487	0.0237	0.0281	0.0238	0.0475	0.0545	0.0463	0.0602	0.0443	0.0531	0.0475	0.0520
WNA1349	0.0291	0.0555	0.0540	0.0579	0.0235	0.0325	0.0203	0.0130	0.0522	0.0368	0.0291	0.0213
WNA1351	0.0149	0.0417	0.0426	0.0441	0.0270	0.0181	0.0304	0.0292	0.0384	0.0268	0.0149	0.0204
WPAC1521	0.0192	0.0428	0.0425	0.0452	0.0269	0.0225	0.0214	0.0331	0.0406	0.0181	0.0192	0.0247
WPAC1522	0.0182	0.0452	0.0462	0.0487	0.0192	0.0280	0.0106	0.0276	0.0456	0.0335	0.0247	0.0149
WPAC1523	0.0229	0.0448	0.0457	0.0426	0.0275	0.0286	0.0263	0.0332	0.0425	0.0364	0.0252	0.0274
WPAC1524	0.0204	0.0453	0.0519	0.0477	0.0149	0.0238	0.0149	0.0250	0.0419	0.0325	0.0204	0.0149
WPAC1525	0.0226	0.0441	0.0331	0.0465	0.0127	0.0281	0.0203	0.0305	0.0503	0.0381	0.0248	0.0214
WPAC1526	0.0193	0.0452	0.0449	0.0452	0.0160	0.0214	0.0149	0.0250	0.0443	0.0302	0.0203	0.0138
P. lineatus	0.3229	0.3262	0.3294	0.3303	0.3305	0.3242	0.3247	0.3342	0.3260	0.3299	0.3214	0.3210

Appendix 6. (continued).

	EPAC1517	EPAC1758	EPAC1760	EPAC1761	EPAC1762	EPAC1764	GOM1352	GOM1353	GOM1354	GOM1355	GOM1356	GOM1357
GOM2031	0.0225	0.0291	0.0259	0.0281	0.0325	0.0303	0.0554	0.0394	0.0405	0.0237	0.0193	0.0461
GOM1757	0.0171	0.0314	0.0171	0.0215	0.0327	0.0316	0.0590	0.0500	0.0545	0.0171	0.0337	0.0555
GOM1758	0.0486	0.0427	0.0309	0.0462	0.0521	0.0497	0.0225	0.0281	0.0325	0.0532	0.0510	0.0095
GOM2027	0.0406	0.0370	0.0429	0.0406	0.0452	0.0440	0.0525	0.0032	0.0095	0.0406	0.0441	0.0303
GOM2028	0.0452	0.0417	0.0476	0.0453	0.0487	0.0487	0.0570	0.0053	0.0117	0.0452	0.0488	0.0348
GOM2029	0.0237	0.0063	0.0281	0.0303	0.0281	0.0248	0.0484	0.0406	0.0428	0.0236	0.0349	0.0484
GOM1756	0.0438	0.0426	0.0485	0.0485	0.0520	0.0473	0.0403	0.0326	0.0303	0.0496	0.0544	0.0358
GOM2030	0.0247	0.0290	0.0292	0.0269	0.0291	0.0260	0.0601	0.0451	0.0475	0.0203	0.0258	0.0530
WEA1340	0.0309	0.0474	0.0557	0.0533	0.0557	0.0533	0.0438	0.0237	0.0237	0.0533	0.0539	0.0427
WEA1341	0.0452	0.0417	0.0476	0.0452	0.0476	0.0476	0.0370	0.0032	0.0117	0.0452	0.0488	0.0348
GOM2032	0.0462	0.0426	0.0308	0.0485	0.0496	0.0473	0.0425	0.0171	0.0170	0.0485	0.0532	0.0380
WEA1342	0.0464	0.0405	0.0487	0.0441	0.0476	0.0476	0.0359	0.0021	0.0128	0.0464	0.0500	0.0359
WEA1343	0.0171	0.0214	0.0193	0.0215	0.0193	0.0182	0.0473	0.0372	0.0418	0.0150	0.0226	0.0474
WEA1344	0.0182	0.0269	0.0248	0.0204	0.0270	0.0259	0.0544	0.0418	0.0464	0.0160	0.0237	0.0473
WEA1345	0.0192	0.0258	0.0259	0.0214	0.0214	0.0225	0.0495	0.0382	0.0428	0.0193	0.0247	0.0427
WEA1749	0.0215	0.0236	0.0260	0.0259	0.0214	0.0204	0.0520	0.0371	0.0416	0.0172	0.0225	0.0473
WEA1750	0.0475	0.0462	0.0322	0.0498	0.0510	0.0487	0.0392	0.0095	0.0106	0.0498	0.0522	0.0370
WEA1752	0.0160	0.0203	0.0204	0.0226	0.0203	0.0192	0.0508	0.0085	0.0428	0.0139	0.0192	0.0485
WEA1753	0.0440	0.0416	0.0475	0.0452	0.0487	0.0475	0.0358	0.0032	0.0106	0.0452	0.0476	0.0336
WNA1914	0.0440	0.0406	0.0464	0.0441	0.0300	0.0464	0.0382	0.0205	0.0248	0.0441	0.0467	0.0349
WPAC1519	0.0281	0.0280	0.0281	0.0280	0.0084	0.0074	0.0589	0.0474	0.0520	0.0238	0.0339	0.0566
WPAC1520	0.0225	0.0336	0.0303	0.0204	0.0360	0.0349	0.0648	0.0569	0.0615	0.0204	0.0303	0.0589
WEA1754	0.0309	0.0473	0.0332	0.0485	0.0545	0.0321	0.0225	0.0303	0.0348	0.0532	0.0510	0.0160
WEA1755	0.0462	0.0426	0.0309	0.0462	0.0320	0.0496	0.0225	0.0239	0.0302	0.0508	0.0309	0.0138
WNA1346	0.0406	0.0394	0.0452	0.0429	0.0464	0.0441	0.0393	0.0117	0.0117	0.0429	0.0454	0.0348
WNA1347	0.0193	0.0235	0.0258	0.0258	0.0246	0.0214	0.0528	0.0438	0.0460	0.0214	0.0257	0.0482
WNA1348	0.0452	0.0463	0.0452	0.0476	0.0534	0.0511	0.0404	0.0249	0.0204	0.0499	0.0523	0.0348
WNA1349	0.0170	0.0313	0.0170	0.0214	0.0325	0.0314	0.0636	0.0544	0.0543	0.0170	0.0336	0.0600
WNA1351	0.0183	0.0247	0.0251	0.0249	0.0226	0.0215	0.0509	0.0383	0.0429	0.0183	0.0237	0.0485
WPAC1521	0.0193	0.0247	0.0248	0.0248	0.0269	0.0225	0.0566	0.0429	0.0462	0.0204	0.0160	0.0473
WPAC1522	0.0184	0.0280	0.0193	0.0149	0.0281	0.0248	0.0498	0.0487	0.0510	0.0127	0.0239	0.0451
WPAC1523	0.0241	0.0207	0.0287	0.0263	0.0097	0.0086	0.0543	0.0437	0.0483	0.0207	0.0309	0.0530
WPAC1524	0.0106	0.0258	0.0150	0.0171	0.0237	0.0226	0.0544	0.0419	0.0464	0.0042	0.0225	0.0544
WPAC1525	0.0182	0.0314	0.0193	0.0171	0.0326	0.0304	0.0591	0.0465	0.0499	0.0149	0.0292	0.0533
WPAC1526	0.0117	0.0259	0.0160	0.0128	0.0293	0.0282	0.0554	0.0441	0.0463	0.0117	0.0281	0.0508
P. lineate	0.3196	0.3317	0.3263	0.3250	0.3316	0.3316	0.3368	0.3372	0.3338	0.3267	0.3234	0.3370

Appendix 6. (continued).

	GOM1757	GOM1758	GOM1757	GOM1758	GOM2027	GOM2028	GOM2028	GOM2028	GOM1756	GOM2030	WEA1340	WEA1341	GOM2032	WEA1342
GOM2031														
GOM1757	0.0303													
GOM1758	0.0427	0.0497												
GOM2027	0.0359	0.0464	0.0270											
GOM2028	0.0405	0.0512	0.0314	0.0042										
GOM2029	0.0302	0.0337	0.0450	0.0371	0.0417									
GOM1756	0.0438	0.0496	0.0326	0.0292	0.0337	0.0426								
GOM2030	0.0224	0.0336	0.0518	0.0415	0.0462	0.0291	0.0506							
WEA1340	0.0497	0.0568	0.0416	0.0226	0.0451	0.0438	0.0438	0.0544						
WEA1341	0.0405	0.0512	0.0314	0.0042	0.0417	0.0417	0.0337	0.0462	0.0204					
GOM2032	0.0426	0.0544	0.0347	0.0170	0.0160	0.0427	0.0324	0.0472	0.0238					
WEA1342	0.0417	0.0523	0.0303	0.0053	0.0074	0.0405	0.0348	0.0474	0.0239	0.0160				
WEA1343	0.0214	0.0259	0.0462	0.0337	0.0383	0.0236	0.0427	0.0181	0.0463	0.0383	0.0193		0.0395	
WEA1344	0.0203	0.0270	0.0462	0.0382	0.0429	0.0269	0.0450	0.0084	0.0533	0.0429	0.0461		0.0440	
WEA1345	0.0235	0.0259	0.0404	0.0359	0.0382	0.0258	0.0414	0.0202	0.0496	0.0393	0.0426		0.0405	
WEA1749	0.0236	0.0271	0.0438	0.0336	0.0382	0.0258	0.0404	0.0139	0.0462	0.0382	0.0392		0.0394	
WEA1750	0.0416	0.0558	0.0337	0.0085	0.0063	0.0439	0.0327	0.0485	0.0225	0.0085	0.0160		0.0117	
WEA1752	0.0181	0.0248	0.0450	0.0348	0.0384	0.0247	0.0415	0.0148	0.0497	0.0394	0.0404		0.0405	
WEA1753	0.0384	0.0511	0.0303	0.0042	0.0042	0.0405	0.0326	0.0461	0.0214	0.0021	0.0021	0.0149	0.0053	
WNA1914	0.0405	0.0475	0.0315	0.0193	0.0193	0.0384	0.0382	0.0452	0.0204	0.0171	0.0247	0.0247	0.0226	
WPAC1519	0.0347	0.0371	0.0542	0.0450	0.0474	0.0325	0.0495	0.0312	0.0590	0.0485	0.0495	0.0495	0.0474	
WPAC1520	0.0291	0.0181	0.0366	0.0332	0.0381	0.0404	0.0553	0.0324	0.0630	0.0580	0.0589	0.0589	0.0592	
WEA1754	0.0473	0.0568	0.0149	0.0292	0.0337	0.0450	0.0348	0.0519	0.0416	0.0337	0.0392	0.0392	0.0326	
WEA1755	0.0426	0.0521	0.0106	0.0247	0.0292	0.0427	0.0281	0.0494	0.0392	0.0292	0.0324	0.0324	0.0281	
WNA1346	0.0371	0.0488	0.0315	0.0106	0.0107	0.0371	0.0292	0.0417	0.0204	0.0106	0.0095	0.0095	0.0139	
WNA1347	0.0224	0.0302	0.0459	0.0403	0.0438	0.0257	0.0459	0.0202	0.0529	0.0449	0.0436	0.0436	0.0461	
WNA1348	0.0417	0.0510	0.0314	0.0237	0.0237	0.0440	0.0270	0.0510	0.0239	0.0237	0.0269	0.0269	0.0271	
WNA1349	0.0301	0.0085	0.0365	0.0308	0.0355	0.0335	0.0541	0.0345	0.0613	0.0555	0.0588	0.0588	0.0567	
WNA1351	0.0247	0.0283	0.0473	0.0348	0.0384	0.0247	0.0427	0.0170	0.0475	0.0394	0.0450	0.0450	0.0406	
WPAC1521	0.0117	0.0258	0.0438	0.0393	0.0440	0.0258	0.0426	0.0213	0.0509	0.0440	0.0461	0.0461	0.0451	
WPAC1522	0.0269	0.0192	0.0486	0.0451	0.0498	0.0280	0.0462	0.0291	0.0532	0.0498	0.0508	0.0508	0.0510	
WPAC1523	0.0285	0.0332	0.0494	0.0412	0.0436	0.0252	0.0434	0.0273	0.0530	0.0448	0.0447	0.0447	0.0436	
WPAC1524	0.0259	0.0193	0.0309	0.0383	0.0430	0.0280	0.0521	0.0247	0.0510	0.0430	0.0509	0.0509	0.0441	
WPAC1525	0.0293	0.0248	0.0498	0.0429	0.0476	0.0349	0.0532	0.0291	0.0522	0.0476	0.0474	0.0474	0.0488	
WPAC1526	0.0236	0.0182	0.0473	0.0405	0.0429	0.0303	0.0450	0.0247	0.0556	0.0452	0.0484	0.0484	0.0463	
P. lineare	0.3313	0.3329	0.3402	0.3365	0.3248	0.3280	0.3296	0.3299	0.3405	0.3353	0.3392	0.3392	0.3338	



Appendix 6. (continued).

	WEA1343	WEA1344	WEA1345	WEA1749	WEA1750	WEA1752	WEA1753	WNA1914	WPAC1519	WPAC1520	WEA1754	WEA1755
WEA1343												
WEA1344	0.0160											
WEA1345	0.0127	0.0138										
WEA1749	0.0149	0.0149										
WEA1750	0.0428	0.0474	0.0439	0.0428								
WEA1752	0.0074	0.0127	0.0138	0.0095	0.0439							
WEA1753	0.0382	0.0428	0.0393	0.0382	0.0074	0.0393						
WNA1914	0.0374	0.0419	0.0395	0.0396	0.0215	0.0384	0.0182					
WPAC1519	0.0192	0.0291	0.0171	0.0286	0.0309	0.0203	0.0485					
WPAC1520	0.0259	0.0258	0.0291	0.0270	0.0627	0.0214	0.0580	0.0510	0.0381			
WEA1754	0.0462	0.0485	0.0449	0.0462	0.0359	0.0497	0.0325	0.0315	0.0566	0.0602		
WEA1755	0.0461	0.0438	0.0403	0.0415	0.0314	0.0449	0.0280	0.0281	0.0541	0.0565	0.0127	
WNA1346	0.0360	0.0406	0.0371	0.0360	0.0106	0.0371	0.0096	0.0193	0.0462	0.0536	0.0337	0.0292
WNA1347	0.0170	0.0181	0.0149	0.0170	0.0472	0.0116	0.0437	0.0415	0.0203	0.029	0.0494	0.0439
WNA1348	0.0429	0.0476	0.0440	0.0452	0.0215	0.0440	0.0226	0.0260	0.0532	0.0536	0.0337	0.0292
WNA1349	0.0258	0.0269	0.0279	0.0291	0.0402	0.0247	0.0555	0.0520	0.0369	0.0181	0.0612	0.0564
WNA1351	0.0160	0.0149	0.0181	0.0095	0.0440	0.0127	0.0394	0.0407	0.0247	0.0304	0.0428	0.0427
WPAC1521	0.0203	0.0170	0.0203	0.0181	0.0474	0.0170	0.0429	0.0417	0.0291	0.028	0.0484	0.0437
WPAC1522	0.0171	0.0225	0.0214	0.0258	0.0321	0.0203	0.0486	0.0464	0.0325	0.0214	0.0463	0.0462
WPAC1523	0.0185	0.0252	0.0206	0.0196	0.0471	0.0184	0.0447	0.0474	0.0107	0.0354	0.0518	0.0493
WPAC1524	0.0172	0.0204	0.0215	0.0216	0.0475	0.0161	0.0429	0.0453	0.0281	0.0226	0.0533	0.0509
WPAC1525	0.0237	0.0248	0.0258	0.0259	0.0510	0.0226	0.0464	0.0430	0.0348	0.0247	0.0498	0.0497
WPAC1526	0.0182	0.0181	0.0203	0.0225	0.0474	0.0171	0.0451	0.0440	0.0314	0.0192	0.052	0.0473
P. lineatus	0.3229	0.3195	0.3304	0.3250	0.3351	0.3214	0.3330	0.3313	0.3356	0.3279	0.3289	0.3331

Appendix 6. (continued).



Appendix 7. Aligned mitochondrial 12S rRNA DNA sequence data collected from echeneoid specimens analyzed in the present study.

10 20 30 40 50 60 70 80 90 100  
*P. saltatrix* CAAAGGCTTGGTCTGACTTTTCTATCAGCTCTAGCTAGATTACACATGCAAGTATCCGGCCCTGTGAGATGCCCTGACAGTTTCTGCA--TGAA  
*N. pectoralis* CAAAGGCTTGGTCTGACTTTACTGTCCGGCTTTAGCTAGACTTACACATGCAAGTATCCGGCCCTGTGAGATGCCCTATAGT--TCCTGTT--TGGG  
*C. armatus* CAAAGGCTTGGTCTGACTTTACTGTCCGGCTTTAGCTAGACTTACACATGCAAGTATCCGGCCCTGTGAGATGCCCTATAGT--TCCTGTT--TGGG  
*R. canadum* CAAAGGCTTGGTCTGACTTTACTGTCCGGCTTTAGCTAGACTTACACATGCAAGTATCCGGCCCTGTGAGATGCCCTATAGT--TCCTGTT--TGGG  
*C. hippurus* CAAAGGCTTGGTCTGACTTTACTGTCCGGCTTTAGCTAGACTTACACATGCAAGTATCCGGCCCTGTGAGATGCCCTATAGT--TCCTGTT--TGGG  
*C. sequisilis* CAAAGGCTTGGTCTGACTTTACTGTCCGGCTTTAGCTAGACTTACACATGCAAGTATCCGGCCCTGTGAGATGCCCTATAGT--TCCTGTT--TGGG  
*P. lineatus* CAAAGGCTTGGTCTGACTTTACTGTCCGGCTTTAGCTAGACTTACACATGCAAGTATCCGGCCCTGTGAGATGCCCTATAGT--TCCTGTT--TGGG  
*E. naucrates* CAAAGGCTTGGTCTGACTTTACTGTCCGGCTTTAGCTAGACTTACACATGCAAGTATCCGGCCCTGTGAGATGCCCTATAGT--TCCTGTT--TGGG  
*E. neucratoides* CAAAGGCTTGGTCTGACTTTACTGTCCGGCTTTAGCTAGACTTACACATGCAAGTATCCGGCCCTGTGAGATGCCCTATAGT--TCCTGTT--TGGG  
*R. australis* CAAAGGCTTGGTCTGACTTTACTGTCCGGCTTTAGCTAGACTTACACATGCAAGTATCCGGCCCTGTGAGATGCCCTATAGT--TCCTGTT--TGGG  
*R. albescens* CAAAGGCTTGGTCTGACTTTACTGTCCGGCTTTAGCTAGACTTACACATGCAAGTATCCGGCCCTGTGAGATGCCCTATAGT--TCCTGTT--TGGG  
*R. remora* CAAAGGCTTGGTCTGACTTTACTGTCCGGCTTTAGCTAGACTTACACATGCAAGTATCCGGCCCTGTGAGATGCCCTATAGT--TCCTGTT--TGGG  
*R. ostteochir* CAAAGGCTTGGTCTGACTTTACTGTCCGGCTTTAGCTAGACTTACACATGCAAGTATCCGGCCCTGTGAGATGCCCTATAGT--TCCTGTT--TGGG  
*R. brachyptera* CAAAGGCTTGGTCTGACTTTACTGTCCGGCTTTAGCTAGACTTACACATGCAAGTATCCGGCCCTGTGAGATGCCCTATAGT--TCCTGTT--TGGG

110 120 130 140 150 160 170 180 190 200  
*P. saltatrix* AACAGGAACTGGTATCAGGCACCCCTA-----AATTTTTGCCACGACGCTTGCTCTTAGCCACCCCTCAAGGGARCCAGCAGTGATAAATTTA  
*N. pectoralis* AATAGGAGCTGGTATCAGGCACACCTATTTGATTTTGGGCCACTACACCTTGCT--CAGCCACACCCCAAGGGATTCAGCAGTGATAAATTTA  
*C. armatus* AACAGGAGCCGGTATCAGGCACACAC-----ATAGCTTAGCCACACACCTTGCT--TAGCCACACCCCAAGGGARCCAGCAGTGATAAATTTA  
*R. canadum* AACAGGAGCCGGTATCAGGCACACCCA-----ACTAATAGCCCATGACGCTTGCT--TAGCCACACCCCTCAAGGGARCCAGCAGTGATAAATTTA  
*C. hippurus* GATAGGAGCTGGTATCAGGCACACAG-----CTAGCCACACACCTTGCT--TAGCCACACCCCAAGGGARCCAGCAGTGATAAATTTA  
*C. sequisilis* GATAGGAGCTGGTATCAGGCACACAAA-----TTAGCCACACACCTTGCT--TAGCCACACCCCAAGGGARCCAGCAGTGATAAATTTA  
*P. lineatus* GATAGGAGCTGGTATCAGGCACACAC-----TCTGTAGCCATACACCTTGCT--TAGCCACACCCCAAGGGARCCAGCAGTGATAAATTTA  
*E. naucrates* GATAGGAGCTGGTATCAGGCACACAC-----TCTGTAGCCATACACCTTGCT--TAGCCACACCCCAAGGGARCCAGCAGTGATAAATTTA  
*E. neucratoides* GATAGGAGCTGGTATCAGGCACACAC-----TCTGTAGCCATACACCTTGCT--TAGCCACACCCCAAGGGARCCAGCAGTGATAAATTTA  
*R. australis* GATAGGAGCTGGTATCAGGCACACTTA-----CTTAGTACCCACACACCTTGCT--TAGCCACACCCCAAGGGARCCAGCAGTGATAAATTTA  
*R. albescens* GATAGGAGCTGGTATCAGGCACACAA-----TAT--GTAGCCCATGACACCTTGCT--TAGCCACACCCCAAGGGARCCAGCAGTGATAAATTTA  
*R. remora* GATAGGAGCTGGTATCAGGCACACTTA-----ACCGTAGCCACACACCTTGCT--TAGCCACACCCCAAGGGARCCAGCAGTGATAAATTTA  
*R. ostteochir* GATAGGAGCTGGTATCAGGCACACTTA-----ACAGTAGCCACACACCTTGCT--TAGCCACACCCCAAGGGARCCAGCAGTGATAAATTTA  
*R. brachyptera* GATAGGAGCTGGTATCAGGCACACTTA-----TTTAGTAGCCACACACCTTGCT--TAGCCACACCCCAAGGGARCCAGCAGTGATAAATTTA

210 220 230 240 250 260 270 280 290 300  
*P. saltatrix* AGCTATAGTGAARACTTGACTTAGTTAAGCTAATTAGGGCCGGTAAACTCGTGCCAGCCACCCGGTTATACGAGAGGCCAAGTTGACAGATACC  
*N. pectoralis* AGCCATAGTGAARACTTGACTTAGTTAAGCTAAG--AGGCGCGTAAACTCGTGCCAGCCACCCGGTTATACGAGAGGCCAAGTTGACAGACACCG  
*C. armatus* AGCCATAGTGAARACTTGACTTAGTTAAGCTAAG--AGGCGCGTAAACTCGTGCCAGCCACCCGGTTATACGAGAGGCCAAGTTGACAGACACCG  
*R. canadum* AGCTATAGTGAARACTTGACTTAGTTAAGCTAAG--AGGCGCGTAAACTCGTGCCAGCCACCCGGTTATACGAGAGGCCAAGTTGACAGACATCG  
*C. hippurus* AGCCATAGTGAARACTTGACTTAGTTAAGCTAATTAGGGTTGGTAAATTCGTGCCAGCCACCCGGTTAGACGARTGACCCAGTTGACAGARTACG  
*C. sequisilis* AGCTATAGTGAARACTTGACTTAGTTAAGCTAAG--AGGCGCGTAAACTCGTGCCAGCCACCCGGTTATACGAGAGGCCAAGTTGACAGACACCG  
*P. lineatus* AGCCATAGTGAARACTTGACTTAGTTAAGCTAAG--AGGCGCGTAAACTCGTGCCAGCCACCCGGTTATACGAGAGGCCAAGTTGACAGACACCG  
*E. naucrates* AGCCATAGTGAARACTTGACTTAGTTAAGCTAAG--AGGCGCGTAAACTCGTGCCAGCCACCCGGTTATACGAGAGGCCAAGTTGACAGARTACG  
*E. neucratoides* AGCCATAGTGAARACTTGACTTAGTTAAGCTAAG--AGGCGCGTAAACTCGTGCCAGCCACCCGGTTATACGAGAGGCCAAGTTGACAGARTACG  
*R. australis* AGCCATAGTGAARACTTGACTTAGTTAAGCTAAG--AGGCGCGTAAACTCGTGCCAGCCACCCGGTTATACGAGAGGCCAAGTTGACAGAAACCG  
*R. albescens* AGCCATAGTGAARACTTGACTTAGTTAAGCTAAG--AGGCGCGTAAACTCGTGCCAGCCACCCGGTTATACGAGAGGCCAAGTTGACAGACACCG  
*R. remora* AGCCATAGTGAARACTTGACTTAGTTAAGCTAAG--AGGCGCGTAAACTCGTGCCAGCCACCCGGTTATACGAGAGGCCAAGTTGACAGARTACG  
*R. ostteochir* AGCCATAGTGAARACTTGACTTAGTTAAGCTAAG--AGGCGCGTAAACTCGTGCCAGCCACCCGGTTATACGAGAGGCCAAGTTGACAGACACCG  
*R. brachyptera* AGCCATAGTGAARACTTGACTTAGTTAAGCTAAG--AGGCGCGTAAACTCGTGCCAGCCACCCGGTTATACGAGAGGCCAAGTTGACAGACACCG

310 320 330 340 350 360 370 380 390 400  
*P. saltatrix* GCGTAAAGCTGGTTAAGGAAAGCCTGAARACTAAGCCGATACCTTCAGAGCAGTTATACGATCCGAAACAC--GAGGCCCTACCAGAAAGTGGCTT  
*N. pectoralis* GCGTAAAGCTGGTTAAGGAAACAGAAACTAAGCCGACCTTCAGGACTGTTATAGTGTCTGAAGGTAA--GAGGCCCTACCAGAAAGTGGCTT  
*C. armatus* GCGTAAAGCTGGTTAAGGAAATACATAACTAAGCCGACACCTTCACAGCTGTTATACGCTTCCGAGGCGAT--GACCCAGACTACGAAAGTGGCTT  
*R. canadum* GCGTAAAGCTGGTTAAGGAAATTTA--ACTAAGCCGACACCTTCAGGACTGTTATACGATCCGAAAGCAG--GAGGCCCTACCAGAAAGTGGCTT  
*C. hippurus* GCGTAAAGCTGGTTAAGGAAATTTA--ACTAAGCCGACACCTTCAGGACTGTTATACGCTTATGAG--ACTGAGGCACACTACGAAAGTGGCTT  
*C. sequisilis* GCGTAAAGCTGGTTAAGGAAATTTA--ACTAAGCCGACACCTTCAGGACTGTTATACGCTTATGAG--ACTGAGGCACACTACGAAAGTGGCTT  
*P. lineatus* GCGTAAAGCTGGTTAAGGAAATTTA--ACTAAGCCGACACCTTCAGGACTGTTATACGCTTATGAG--ACTGAGGCACACTACGAAAGTGGCTT  
*E. naucrates* GCGTAAAGCTGGTTAAGGAAATTTA--ACTAAGCCGACACCTTCAGGACTGTTATACGCTTATGAG--ACTGAGGCACACTACGAAAGTGGCTT  
*E. neucratoides* GCGTAAAGCTGGTTAAGGAAATTTA--ACTAAGCCGACACCTTCAGGACTGTTATACGCTTATGAG--ACTGAGGCACACTACGAAAGTGGCTT  
*R. australis* GCGTAAAGCTGGTTAAGGAAATTTA--ACTAAGCCGACACCTTCAGGACTGTTATACGCTTATGAG--ACTGAGGCACACTACGAAAGTGGCTT  
*R. albescens* GCGTAAAGCTGGTTAAGGAAATTTA--ACTAAGCCGACACCTTCAGGACTGTTATACGCTTATGAG--ACTGAGGCACACTACGAAAGTGGCTT  
*R. remora* GCGTAAAGCTGGTTAAGGAAATTTA--ACTAAGCCGACACCTTCAGGACTGTTATACGCTTATGAG--ACTGAGGCACACTACGAAAGTGGCTT  
*R. ostteochir* GCGTAAAGCTGGTTAAGGAAATTTA--ACTAAGCCGACACCTTCAGGACTGTTATACGCTTATGAG--ACTGAGGCACACTACGAAAGTGGCTT  
*R. brachyptera* GCGTAAAGCTGGTTAAGGAAATTTA--ACTAAGCCGACACCTTCAGGACTGTTATACGCTTATGAG--ACTGAGGCACACTACGAAAGTGGCTT

410 420 430 440 450 460 470 480 490 500  
*P. saltatrix* TACCATTCTGACCCACGAAAGCTATGATACAACTGGGATTAGATACCCACTATGCTTAGCCGTAAACATTGATAGATAGTACATCCCATCTATCC  
*N. pectoralis* TACATTACCTGACCCACGAAAGCTATGATACAACTGGGATTAGATACCCACTATGCTTAGCCGTAAACATTGATAGTATGCTATACACACCT--ACATATCC  
*C. armatus* TACATTACCTGACCCACGAAAGCTATGATACAACTGGGATTAGATACCCACTATGCTTAGCCGTAAACATTGATAGTATGCTATACACACCT--ACATATCC  
*R. canadum* TATGACTCTGACCCACGAAAGCTATGATACAACTGGGATTAGATACCCACTATGCTTAGCCGTAAACATTGATAGTATGCTATACACACCT--ACATATCC  
*C. hippurus* TAAACACCTGAAACCCAGAAAGCTAAGAAACAACTGGGATTAGATACCCACTATGCTTAGCCGTAAACATTGATAGTATGCTATACACACCT--ACATATCC  
*C. sequisilis* TAAATCACCTGAAACCCAGAAAGCTAAGAAACAACTGGGATTAGATACCCACTATGCTTAGCCGTAAACATTGATAGTATGCTATACACACCT--ACATATCC  
*P. lineatus* TATAAARCTGAAACCCAGAAAGCTAAGAAACAACTGGGATTAGATACCCACTATGCTTAGCCGTAAACATTGATAGTATGCTATACACACCT--ACATATCC  
*E. naucrates* TATAAARCTGAAACCCAGAAAGCTAAGAAACAACTGGGATTAGATACCCACTATGCTTAGCCGTAAACATTGATAGTATGCTATACACACCT--ACATATCC  
*E. neucratoides* TATAAARCTGAAACCCAGAAAGCTAAGAAACAACTGGGATTAGATACCCACTATGCTTAGCCGTAAACATTGATAGTATGCTATACACACCT--ACATATCC  
*R. australis* TATAAARCTGAAACCCAGAAAGCTAAGAAACAACTGGGATTAGATACCCACTATGCTTAGCCGTAAACATTGATAGTATGCTATACACACCT--ACATATCC  
*R. albescens* TATAAARCTGAAACCCAGAAAGCTAAGAAACAACTGGGATTAGATACCCACTATGCTTAGCCGTAAACATTGATAGTATGCTATACACACCT--ACATATCC  
*R. remora* TACAAARCTGACTCCACGAAAGCTAAGAAACAACTGGGATTAGATACCCACTATGCTTAGCCGTAAACATTGATAGTATGCTATACACACCT--ACATATCC  
*R. ostteochir* TATAAARCTGAAACCCAGAAAGCTAAGAAACAACTGGGATTAGATACCCACTATGCTTAGCCGTAAACATTGATAGTATGCTATACACACCT--ACATATCC  
*R. brachyptera* TACAAARCTGAAACCCAGAAAGCTAAGAAACAACTGGGATTAGATACCCACTATGCTTAGCCGTAAACATTGATAGTATGCTATACACACCT--ACATATCC

Appendix 7. (continued).

510 520 530 540 550 560 570 580 590 600  
*P.\_saltatrix* GCCGGGGTACTACGAGCACCAGCTTGAARCCCAAGGACTTGGCGGTACTTTAGATCCCCCTAGAGGAGCTGTCTGTARCCGATACCCCGCTTTAAC  
*N.\_pectoralis* GCCCGGGATTACGAGCAGCTAGTTTGAARCCCAAGGACTTGGCGGTGCTTAACTTCCACCTAGAGGAGCTGTCTATARCCGATACCCCGCTTTAAC  
*C.\_armatus* GCCTGGGATTACGAGCAGCTAGTTTGAARCCCAAGGACTTGGCGGTGCTTAACTTCCACCTAGAGGAGCTGTCTATARCCGATACCCCGCTTTAAC  
*R.\_canadum* GCCTGGGATTACGAGCAGCTAGTTTGAARCCCAAGGACTTGGCGGTGCTTAACTTCCACCTAGAGGAGCTGTCTATARCCGATACCCCGCTTTAAC  
*C.\_hippurus* GCCTGGGATTACGAGCAGCTAGTTTGAARCCCAAGGACTTGGCGGTGCTTAACTTCCACCTAGAGGAGCTGTCTATARCCGATACCCCGCTTTAAC  
*C.\_equisetis* GCCTGGGATTACGAGCAGCTAGTTTGAARCCCAAGGACTTGGCGGTGCTTAACTTCCACCTAGAGGAGCTGTCTATARCCGATACCCCGCTTTAAC  
*P.\_lineatus* GCCCGGGATTACGAGCAGCTAGTTTGAARCCCAAGGACTTGGCGGTGCTTAACTTCCACCTAGAGGAGCTGTCTATARCCGATACCCCGCTTTAAC  
*E.\_naucratus* GCCCGGGATTACGAGCAGCTAGTTTGAARCCCAAGGACTTGGCGGTGCTTAACTTCCACCTAGAGGAGCTGTCTATARCCGATACCCCGCTTTAAC  
*E.\_neucratoides* GCCCGGGATTACGAGCAGCTAGTTTGAARCCCAAGGACTTGGCGGTGCTTAACTTCCACCTAGAGGAGCTGTCTATARCCGATACCCCGCTTTAAC  
*R.\_australis* GCCTGGGATTACGAGCAGCTAGTTTGAARCCCAAGGACTTGGCGGTGCTTAACTTCCACCTAGAGGAGCTGTCTATARCCGATACCCCGCTTTAAC  
*R.\_albescens* GCCTGGGATTACGAGCAGCTAGTTTGAARCCCAAGGACTTGGCGGTGCTTAACTTCCACCTAGAGGAGCTGTCTATARCCGATACCCCGCTTTAAC  
*R.\_remora* GCCCGGGATTACGAGCAGCTAGTTTGAARCCCAAGGACTTGGCGGTGCTTAACTTCCACCTAGAGGAGCTGTCTATARCCGATACCCCGCTTTAAC  
*R.\_osteoichir* GCCCGGGATTACGAGCAGCTAGTTTGAARCCCAAGGACTTGGCGGTGCTTAACTTCCACCTAGAGGAGCTGTCTATARCCGATACCCCGCTTTAAC  
*R.\_brachyptera* GCCCGGGATTACGAGCAGCTAGTTTGAARCCCAAGGACTTGGCGGTGCTTAACTTCCACCTAGAGGAGCTGTCTATARCCGATACCCCGCTTTAAC

610 620 630 640 650 660 670 680 690 700  
*P.\_saltatrix* CTCACCCCTCCCTCTCCTC--TGTCTATATACCAGCCGCTGTAAGCTTACCCTGTGAGGG-TCTARTAGTTAGCAAAATTTGGCACAGCCGAGACGTCAG  
*N.\_pectoralis* CTCACCCCTCTTGTTCCTCCACCGCTATATACCAGCCGCTGTCAGCTTACCCTGTGAGGG-CCAAATAGTGTAGCAGGATTAGCATATCCAGACGTCAG  
*C.\_armatus* CTCACCCCTCTAGTTTAACTACCAGCCGCTATATACCAGCCGCTGTCAGCTTACCCTGTGAGGG-CCTARTAGTGTAGCAGGATTTGGCATAGCCCAAAACGTCAG  
*R.\_canadum* CTCACCTTTTCTGTATTT-CAGCCTATATACCAGCCGCTGTCAGCTTACCCTGTGAGGG-ATTAGTAGTGTAGCAGGATTTGGCAATGCCAGTACGTCAG  
*C.\_hippurus* CTCACCTCTCTCGCAATAT-CAGCCTATATACCAGCCGCTGTCAGCTTACCCTGTGAGGG-ACTARAGTGTAGCAGGATTTGGTACARCCCAATACGTCAG  
*C.\_equisetis* CTCACCTCTCTTGCACAT-CAGCCTATATACCAGCCGCTGTCAGCTTACCCTGTGAGGG-ACTARTAGTGTAGCAGGATTTGGTAAARCCCAATACGTCAG  
*P.\_lineatus* CTCACCCCTCCCTTGTTTTTT-CCGCCTATATACCAGCCGCTGTCAGCTTACCCTGTGAGGG-TCTARTAGTGTAGCAGGATTTGGTAAARCCCAAGACGTCAG  
*E.\_naucratus* CTCACCCCTCCCTTGTTTTTT-CCGCCTATATACCAGCCGCTGTCAGCTTACCCTGTGAGGG-TCTARTAGTGTAGCAGGATTTGGTAAARCCCAAGACGTCAG  
*E.\_neucratoides* CTCACCCCTCCCTTGTTTTTT-CCGCCTATATACCAGCCGCTGTCAGCTTACCCTGTGAGGG-TCTARTAGTGTAGCAGGATTTGGTAAARCCCAAGACGTCAG  
*R.\_australis* CTCACCCCTCCCTTGTTTTTT-CCGCCTATATACCAGCCGCTGTCAGCTTACCCTGTGAGGG-TTARTAGTGTAGCAGGATTTGGTAAARCCCAAGACGTCAG  
*R.\_albescens* CTCACCCCTCCCTTGTTTTTT-CCGCCTATATACCAGCCGCTGTCAGCTTACCCTGTGAGGG-TTARTAGTGTAGCAGGATTTGGTAAARCCCAAGACGTCAG  
*R.\_remora* CTCACCCCTCCCTTGTTTTTT-CCGCCTATATACCAGCCGCTGTCAGCTTACCCTGTGAGGG-CCTARTAGTGTAGCAGGATTTGGTAAARCCCAAGACGTCAG  
*R.\_osteoichir* CTCACCCCTCCCTTGTTTTTT-CCGCCTATATACCAGCCGCTGTCAGCTTACCCTGTGAGGG-CCTARTAGTGTAGCAGGATTTGGTAAARCCCAAGACGTCAG  
*R.\_brachyptera* CTCACCCCTCCCTTGTTTTTT-CCGCCTATATACCAGCCGCTGTCAGCTTACCCTGTGAGGG-CTARTAGTGTAGCAGGATTTGGTAAARCCCAAGACGTCAG

710 720 730 740 750 760 770 780 790 800  
*P.\_saltatrix* GTCGAGGTTAGTCATATGGGAGGGGAGGACARTGGG-CTACATTCGCTARTGA--AGCGAAC-ACGAA-TGGTGCCTGAAARACTGTGCGCTCTGAGGG  
*N.\_pectoralis* GTCGAGGTTAGTCATATGGGAGGGGAGGAGAA--ATGGG-CTACATTCGCTARTGA-TAGCGAAAC-ACGAA-TGACACGTTGAAAR-CATAG-TATTGAGGG  
*C.\_armatus* GTCGAGGTTAGTCATATGGGAGGGGAGGAGAA--ATGGG-CTACATTCGCTARTGA-TAGCGAAAC-ACGAA-TGACACGTTGAAAR-CATAG-AGCTGAGGG  
*R.\_canadum* GTCGAGGTTAGTCATATGGGAGGGGAGGAG--ATGGG-CTACATTCGCTARTGA-TAGTGAAT-ACGAA-TARTACAAATTTGAAAR--CATGT-ATCTGAGGG  
*C.\_hippurus* GTCGAGGTTAGTCATATGGGAGGGGAGGAG--ATGGG-CTACATTCGCTARTGA-TAGTGAAT-ACGAA-TAGTATATTGAAAR--CATAT-ACTTGAAGG  
*C.\_equisetis* GTCGAGGTTAGTCATATGGGAGGGGAGGAG--ATGGG-CTACATTCGCTARTGA-TAGTGAAT-ACGAA-TAGTATATTGAAAR--CATAT-ACTTGAAGG  
*P.\_lineatus* GTCGAGGTTAGTCATATGGGAGGGGAGGAG--ATGGG-CTACATTCGCTARTGA-TAGTGAAT-ACGAA-TAGTATATTGAAAR--CATAT-ACTTGAAGG  
*E.\_naucratus* GTCGAGGTTAGTCATATGGGAGGGGAGGAG--ATGGG-CTACATTCGCTARTGA-TAGTGAAT-ACGAA-TAGTATATTGAAAR--TATAC-TTCTGAGGG  
*E.\_neucratoides* GTCGAGGTTAGTCATATGGGAGGGGAGGAG--ATGGG-CTACATTCGCTARTGA-TAGTGAAT-ACGAA-TAGTATATTGAAAR--TATAC-TTCTGAGGG  
*R.\_australis* GTCGAGGTTAGTCATATGGGAGGGGAGGAG--ATGGG-CTACATTCGCTARTGA-TAGTGAAT-ACGAA-TAGTATATTGAAAR--TATAT-CCTGAGGG  
*R.\_albescens* GTCGAGGTTAGTCATATGGGAGGGGAGGAG--ATGGG-CTACATTCGCTARTGA-TAGTGAAT-ACGAA-TAGTATATTGAAAR--TATAT-CCTGAGGG  
*R.\_remora* GTCGAGGTTAGTCATATGGGAGGGGAGGAG--ATGGG-CTACATTCGCTARTGA-TAGTGAAT-ACGAA-TAGTATATTGAAAR--TATAC-TCTGAGGG  
*R.\_osteoichir* GTCGAGGTTAGTCATATGGGAGGGGAGGAG--ATGGG-CTACATTCGCTARTGA-TAGTGAAT-ACGAA-TAGTATATTGAAAR--TATAC-CCTGAGGG  
*R.\_brachyptera* GTCGAGGTTAGTCATATGGGAGGGGAGGAG--ATGGG-CTACATTCGCTARTGA-TAGTGAAT-ACGAA-TAGTATATTGAAAR--TATAT-TTCTGAGGG

810 820 830 840 850 860 870 880 890 900  
*P.\_saltatrix* TGGATTTAGCAGTAGTGGAAATAGAGAGTCCCACTGAGGCGGCTCTAAGTGGCGCACACACCCGCCGTCACTCTCCCAAGCCGAGGCCAGATTT  
*N.\_pectoralis* TGGATTTAGCAGTAGCAGGAGGACAGAGTGCCTACTGARTTCCGGCTATTAGCAGCGCACATACCCGCCGTCACTCTCCCAAGCCGAGGCCAGATTT  
*C.\_armatus* TGGATTTAGCAGTAGCAGGAGGAGGAGGAGTGTCCGCTAGAGCCGCTCTTAAAGCGCGCACACACCCGCCGTCACTCTCCCAAGCCGAGGCCAGATTT  
*R.\_canadum* TGGATTTAGCAGTAGGTTGGAATAGAGTGTCCCACTGAGGCGGCTCTTAAAGCGGTGCACATACCCGCCGTCACTCTCCCAAGCCGAGGCCAGATTT  
*C.\_hippurus* TGGATTTAGCAGTAGCAGGAGGAGGAGGAGTGTCTGCTGAGGCGGCTCTTAAAGCGGTGCACATACCCGCCGTCACTCTCCCAAGCCGAGGCCAGATTT  
*C.\_equisetis* TGGATTTAGCAGTAGCAGGAGGAGGAGGAGTGTCTGCTGAGGCGGCTCTTAAAGCGGTGCACATACCCGCCGTCACTCTCCCAAGCCGAGGCCAGATTT  
*P.\_lineatus* TGGATTTAGCAGTAGTGGGAAATAGAGAGTCCCTCTGAAATGGCTCTAAGGCGCGCACACACCCGCCGTCACTCTCCCAAGCCGAGGCCAGATTT  
*E.\_naucratus* TGGATTTAGCAGTAGTGGGAAATAGAGAGTCCCTCTGAAAR-TGGCTCTAAGGCGCGCACACACCCGCCGTCACTCTCCCAAGCCGAGGCCAGATTT  
*E.\_neucratoides* TGGATTTAGCAGTAGTGGGAAATAGAGAGTCCCTCTGAAAR-TGGCTCTAAGGCGCGCACACACCCGCCGTCACTCTCCCAAGCCGAGGCCAGATTT  
*R.\_australis* TGGATTTAGCAGTAGTGGGAAATAGAGAGTGTCCCACTGAAAR-TGGCTCTAAGGCGCGCACACACCCGCCGTCACTCTCCCAAGCCGAGGCCAGATTT  
*R.\_albescens* TGGATTTAGCAGTAGTGGGAAATAGAGAGTGTCCCACTGAAAR-TGGCTCTAAGGCGCGCACACACCCGCCGTCACTCTCCCAAGCCGAGGCCAGATTT  
*R.\_remora* TGGATTTAGCAGTAGTGGGAAATAGAGAGTGTCCCACTGAAAR-TGGCTCTAAGGCGCGCACACACCCGCCGTCACTCTCCCAAGCCGAGGCCAGATTT  
*R.\_osteoichir* TGGATTTAGCAGTAGTGGGAAATAGAGAGTGTCCCACTGAAAR-TGGCTCTAAGGCGCGCACACACCCGCCGTCACTCTCCCAAGCCGAGGCCAGATTT  
*R.\_brachyptera* TGGATTTAGCAGTAGTGGGAAATAGAGAGTGTCCCACTGAAAR-TGGCTCTAAGGCGCGCACACACCCGCCGTCACTCTCCCAAGCCGAGGCCAGATTT

910 920 930 940 950 960 970 980  
*P.\_saltatrix* ACATTARCCGCTA-----AACC-TGA-AGGGGAGGACAGTCTGTRACATGGTAAAGCGTACCAGGAGGTGCGCTTGGAAATATAT  
*N.\_pectoralis* AT-TCAAGCCCTC-----TACAGTAGCGA--AGGTGAGGAAAGTCTGTRACATGGTAAAGTGTACCAGGAGGTGACTTGGCT-AC--  
*C.\_armatus* TTCTTAAACCTA--AACACCCGCA--AGGAGGAGGAAAGTCTGTRACATGGTAAAGCGTACCAGGAGGTGCGCTTGGTAAAC--  
*R.\_canadum* TAACTAARCCACATATTTTTATGCT--AGGAGAGGAGGAGTCTGTRACATGGTAAAGCGTACCAGGAGGTGCGCTTGGAA- TAATC-  
*C.\_hippurus* TTTCTAARCTAC--TTATAAAGCT--AGGAGAGGAGGAGTCTGTRACATGGTAAAGCGTACCAGGAGGTGCGCTTGGAA- TAATC-  
*C.\_equisetis* TTTCTAARCTAC--TTATAAAGCT--AGGAGAGGAGGAGTCTGTRACATGGTAAAGCGTACCAGGAGGTGCGCTTGGAA- TAATC-  
*P.\_lineatus* TAACTAARCTAT--ACCACCCGCTAC--AGGAGAGGAGGAGTCTGTRACATGGTAAAGCGTACCAGGAGGTGCGCTTGGAAATAC--  
*E.\_naucratus* TAACTAARCTAT--ACCACCCGCTAC--AGGAGAGGAGGAGTCTGTRACATGGTAAAGCGTACCAGGAGGTGCGCTTGGAAATAC--  
*E.\_neucratoides* TAACTAARCTAT--ACCACCCGCTAC--AGGAGAGGAGGAGTCTGTRACATGGTAAAGCGTACCAGGAGGTGCGCTTGGAAATAC--  
*R.\_australis* TAACTAARAAAT--ATCACCCTGCTT--AGGAGAGGAGGAGTCTGTRACATGGTAAAGCGTACCAGGAGGTGCGCTTGGAAATAC--  
*R.\_albescens* TTATTTAAAGT--GCCCCCGCTAC--AGGAGAGGAGGAGTCTGTRACATGGTAAAGCGTACCAGGAGGTGCGCTTGGAAATAC--  
*R.\_remora* TAACTAARAGTAC--ATCACCCTGCTC--AGGAGAGGAGGAGTCTGTRACATGGTAAAGCGTACCAGGAGGTGCGCTTGGAAATAC--  
*R.\_osteoichir* TAACTAARAAAT--ATTGACCCGCTAT--AGGAGAGGAGGAGTCTGTRACATGGTAAAGCGTACCAGGAGGTGCGCTTGGAAATAC--  
*R.\_brachyptera* TAACTAARATAT--ATTARCCGCTAT--AGGAGAGGAGGAGTCTGTRACATGGTAAAGCGTACCAGGAGGTGCGCTTGGAAATAC--



Appendix 8. Aligned mitochondrial 16S rRNA DNA sequence data collected from echeneoid specimens analyzed in the present study.

10 20 30 40 50 60 70 80 90 100  
*P. saltatrix* -CGCCACAGCTAGCCACCC--CCACAAACACACATTCACACTTAAATACCCACATACAC-ACCTCCCCACTTAAATTAATCATCTCCDC-CC  
*N. pectoralis* -TGCTACTAGTAGCCCCCCCAACAAAACACACACCCCAATTAATTAACCC-CAATAC-ACAAACCCACATTAACCAACATTATTC-CTC  
*C. armatus* -TGCTATTAGTAGCCCCCTC--ACTAARCTTACACAGTACCATGAT-ARCC-CAARAGC-ACTAARCTTATTCARACAAACATTTTCTCC  
*R. canadum* -CGCCAAATAGCTAGCCCTAC--CCACACCCACACATTCACACTTAAATACCCCTTAAATACACCCACAAACAC--CAATCAATCATTTTATTTCC  
*C. hippurus* CAGCTAATTAGTAGCCCTACCT--ACACACCCATCAA-ATACCTTACTACCCCTTAAATACACTAARAAATATT--CACTAARACCATTCATCTTCC  
*C. lequisei* TAGCTAATTAGTAGCCCTACCT--ACACACCCATCAA-ATACCTTACTACCCCTTAAATACACTAARAAATATT--TAACTAARACCATTCATTTCC  
*P. lineatus* -AGCCACCCAGCTAGCCCTAAT--ACTAARCTTACACACCCAAATTAATTAARACCCCTAATTAATTAATTAAR-ACCTA--TAAATAAACATTTTCCA-CCC  
*E. naucrates* -AGCCACCCAGCTAGCCCTAAT--ACTAARCTTACACACCCAAATTAATTAARACCCCTAATTAATTAATTAAR-ACCTA--TAAATAAACATTTTCCA-CCC  
*E. neucratoides* -AGCCACCCAGCTAGCCCTAAT--ACTAARCTTACACACCCAAATTAATTAARACCCCTAATTAATTAATTAAR-ACCTA--TAAATAAACATTTTCCA-CCC  
*R. australis* -AGCCACCCAGCTAGCCCTAAT--ACTAARCTTACACACCCAAATTAATTAARACCCCTAATTAATTAATTAAR-ACCTA--TAAATAAACATTTTCCA-CCC  
*R. albescens* -AGCCACCCAGCTAGCCCTAAT--ACTAARCTTACACACCCAAATTAATTAARACCCCTAATTAATTAATTAAR-ACCTA--TAAATAAACATTTTCCA-CCC  
*R. remora* -AGCCACCCAGCTAGCCCTAAT--ACTAARCTTACACACCCAAATTAATTAARACCCCTAATTAATTAATTAAR-ACCTA--TAAATAAACATTTTCCA-CCC  
*R. osteochir* -AGCCACCCAGCTAGCCCTAAT--ACTAARCTTACACACCCAAATTAATTAARACCCCTAATTAATTAATTAAR-ACCTA--TAAATAAACATTTTCCA-CCC  
*R. brachyptera* -CGCCATCAGCTAGCCCTAAT--ACTAARCTTACACACCCAAATTAATTAARACCCCTAATTAATTAATTAAR-ACCTA--TAAATAAACATTTTCCA-CCC

110 120 130 140 150 160 170 180 190 200  
*P. saltatrix* TC--AGTATGGCGACAGAAAGGACCTATTGGAGCAGTAGAAA-AGTACCCTAGGGAAAGCTGAAGAGAGATGAATTAACCCAGTG-AGGCTAAR  
*N. pectoralis* TCTAGTATAGCCGATAGAAAGAGGCTCGTGGAGCAGTAGAAA-AGTACCCTAGGGAAAGCTGAAGAGAGATGAATTAACCCAGTG-AGGCTAAR  
*C. armatus* T--AGTATGGCGACAGAAAGGACCTTCGGGCGCAGTAGAAA-AGTACCCTAGGGAAAGCTGAAGAGAGATGAATTAACCCAGTG-AGGCTAAR  
*R. canadum* CC--AGTATAGCCGATAGAAAGGAACTTATAGAGCTACAGAAA-AGTACCCTAGGGAAAGCTGAAGAGAGATGAATTAACCCAGTG-AGGCTAAR  
*C. hippurus* T--AGTATAGCCGATAGAAAGGAACTAARAGGACACAGATATCAGTACCCTAGGGAAAGCTGAAGAGAGATGAATTAACCCAGTG-AGGCTAAR  
*C. lequisei* T--AGTATAGCCGATAGAAAGGAACTAARAGGACACAGATATCAGTACCCTAGGGAAAGCTGAAGAGAGATGAATTAACCCAGTG-AGGCTAAR  
*P. lineatus* TT--AGTATAGGAGATAGAAAGGAAATATAGGAGCAGTAGAAA-AGTACCCTAGGGAAAGCTGAAGAGAGATGAATTAACCCAGTG-AGGCTAAR  
*E. naucrates* TT--AGTATAGGAGATAGAAAGGAAATATAGGAGCAGTAGAAA-AGTACCCTAGGGAAAGCTGAAGAGAGATGAATTAACCCAGTG-AGGCTAAR  
*E. neucratoides* TT--AGTATAGGAGATAGAAAGGAAATATAGGAGCAGTAGAAA-AGTACCCTAGGGAAAGCTGAAGAGAGATGAATTAACCCAGTG-AGGCTAAR  
*R. australis* C--AGTACAGCCGATAGAAAGGAACTAATGGAGCAGTAGAAA-AGTACCCTAGGGAAAGCTGAAGAGAGATGAATTAACCCAGTG-AGGCTAAR  
*R. albescens* TT--AGTATAGCCGATAGAAAGGAACTATGGAGCAGTAGAAA-AGTACCCTAGGGAAAGCTGAAGAGAGATGAATTAACCCAGTG-AGGCTAAR  
*R. remora* CC--AGTATAGCCGATAGAAAGGAACTATGGAGCAGTAGAAA-AGTACCCTAGGGAAAGCTGAAGAGAGATGAATTAACCCAGTG-AGGCTAAR  
*R. osteochir* TT--AGTATAGCCGATAGAAAGGAACTA--CGAGCAGTAGAAA-AGTACCCTAGGGAAAGCTGAAGAGAGATGAATTAACCCAGTG-AGGCTAAR  
*R. brachyptera* C--AGTATAGGCTAGAAAGGAAATATGGAGCAGTAGAAA-AGTACCCTAGGGAAAGCTGAAGAGAGATGAATTAACCCAGTG-AGGCTAAR

210 220 230 240 250 260 270 280 290 300  
*P. saltatrix* AAGCAGAGATTTTACCTCGTACCTTTTGCATCATGATTTAGCTAGTATTACCCAGCAAGAGGACCTTTAGTTTGGGACCCCGAAGCTAC-GTAGGCTA  
*N. pectoralis* AAGCAGAGATTTTACCTCGTACCTTTTGCATCATGATTTAGCTAGTATTACCCAGCAAGAGGACCTTTAGTTTGGGACCCCGAAGCTAC-GTAGGCTA  
*C. armatus* AAGCAGAGATTTTCCCTCGTACCTTTTGCATCATGATTTAGCCAGTAC-CTTCAGCAAGAGGAGCTTTAGTTTGGGACCCCGAAGCTAC-GTAGGCTA  
*R. canadum* AAGCAGAGATTTTCCCTCGTACCTTTTGCATCATGATTTAGCCAGTAC-CTTCAGCAAGAGGAGCTTTAGTTTGGGACCCCGAAGCTAC-GTAGGCTA  
*C. hippurus* AAGCAGAGATTTCCCTCGTACCTTTTGCATCATGATTTAGCCAGTAC-CTTCAGCAAGAGGAGCTTTAGTTTGGGACCCCGAAGCTAC-GTAGGCTA  
*C. lequisei* AAGCAGAGATTTCCCTCGTACCTTTTGCATCATGATTTAGCCAGTAC-CTTCAGCAAGAGGAGCTTTAGTTTGGGACCCCGAAGCTAC-GTAGGCTA  
*P. lineatus* AAGCAGAGATATACCTCGTACCTTTTGCATCATGATTTAGCCAGTAA-ACCTAAGCAAGAGGACCTTTAGTTTGGGACCCCGAAGCTAC-GTAGGCTA  
*E. naucrates* AAGCAGAGATATACCTCGTACCTTTTGCATCATGATTTAGCCAGTAA-ACCTAAGCAAGAGGACCTTTAGTTTGGGACCCCGAAGCTAC-GTAGGCTA  
*E. neucratoides* AAGCAGAGATATACCTCGTACCTTTTGCATCATGATTTAGCCAGTAA-ACCTAAGCAAGAGGACCTTTAGTTTGGGACCCCGAAGCTAC-GTAGGCTA  
*R. australis* AAGCAGAGCTATATCCCTCGTACCTTTTGCATCATGATTTAGCTAGTAA-ACCTAAGCAAGAGGACCTTTAGTTTGGGACCCCGAAGCTAC-GTAGGCTA  
*R. albescens* AAGCAGAGCTTATACCTCGTACCTTTTGCATCATGATTTAGCTAGTAA-ACCTAAGCAAGAGGACCTTTAGTTTGGGACCCCGAAGCTAC-GTAGGCTA  
*R. remora* AAGCAGAGTTTATACCTCGTACCTTTTGCATCATGATTTAGCTAGTAA-ACCTAAGCAAGAGGACCTTTAGTTTGGGACCCCGAAGCTAC-GTAGGCTA  
*R. osteochir* AAGCAGAGTTTATACCTCGTACCTTTTGCATCATGATTTAGCTAGTAA-ATTTAAGCAAGAGGACCTTTAGTTTGGGACCCCGAAGCTAC-GTAGGCTA  
*R. brachyptera* AAGCAGAGTTTATACCTCGTACCTTTTGCATCATGATTTAGCTAGTAA-ACCTAAGCAAGAGGACCTTTAGTTTGGGACCCCGAAGCTAC-GTAGGCTA

310 320 330 340 350 360 370 380 390 400  
*P. saltatrix* CTCACAGACGCCCTATCAATAGGCAACCCGCTCTCTGTGGCAAAAGAGTGGGAGAGGCTTCAGTAGAGGTGACAGACCTACCGAACCTAGTTATAGCT  
*N. pectoralis* CTCACAGACGCCCTATCAATAGGCAACCCGCTCTCTGTGGCAAAAGAGTGGGAGAGGCTTCAGTAGAGGTGACAGACCTATCGAACCTAGTTATAGCT  
*C. armatus* CTCACAGACGCCCTATTATAGGCAACCCGCTCTCTGTGGCAAAAGAGTGGGAGAGGCTTCAGTAGAGGTGACAGACCTATCGAACCTAGTTATAGCT  
*R. canadum* CTCACAGACGCCCTATT-ACAGGCAACCCGCTCTCTGTGGCAAAAGAGTGGGAGAGGCTTCAGTAGAGGTGACAGACCTATCGAACCTAGTTATAGCT  
*C. hippurus* CTCACAGACGCCCTATT-ATAGGCAACCCGCTCTCTGTGGCAAAAGAGTGGGAGAGGCTTCAGTAGAGGTGACAGACCTATCGAACCTAGTTATAGCT  
*C. lequisei* CTCACAGACGCCCTATT-ATAGGCAACCCGCTCTCTGTGGCAAAAGAGTGGGAGAGGCTTCAGTAGAGGTGACAGACCTATCGAACCTAGTTATAGCT  
*P. lineatus* CTCACAGACGCCCTATT-ATAGGCAACCCGCTCTCTGTGGCAAAAGAGTGGGAGAGGCTTCAGTAGAGGTGACAGACCTACCGAACCTAGTTATAGCT  
*E. naucrates* CTCACAGACGCCCTATT-ATAGGCAACCCGCTCTCTGTGGCAAAAGAGTGGGAGAGGCTTCAGTAGAGGTGACAGACCTACCGAACCTAGTTATAGCT  
*E. neucratoides* CTCACAGACGCCCTATT-ATAGGCAACCCGCTCTCTGTGGCAAAAGAGTGGGAGAGGCTTCAGTAGAGGTGACAGACCTACCGAACCTAGTTATAGCT  
*R. australis* CTCACAGACGCCCTATT-ATAGGCAACCCGCTCTCTGTGGCAAAAGAGTGGGAGAGGCTTCAGTAGAGGTGACAGACCTACCGAACCTAGTTATAGCT  
*R. albescens* CTCACAGACGCCCTATT-ATAGGCAACCCGCTCTCTGTGGCAAAAGAGTGGGAGAGGCTTCAGTAGAGGTGACAGACCTACCGAACCTAGTTATAGCT  
*R. remora* CTCACAGACGCCCTATT-ATAGGCAACCCGCTCTCTGTGGCAAAAGAGTGGGAGAGGCTTCAGTAGAGGTGACAGACCTACCGAACCTAGTTATAGCT  
*R. osteochir* CTCACAGACGCCCTATT-ATAGGCAACCCGCTCTCTGTGGCAAAAGAGTGGGAGAGGCTTCAGTAGAGGTGACAGACCTACCGAACCTAGTTATAGCT  
*R. brachyptera* CTCACAGACGCCCTATT-ATAGGCAACCCGCTCTCTGTGGCAAAAGAGTGGGAGAGGCTTCAGTAGAGGTGACAGACCTACCGAACCTAGTTATAGCT

410 420 430 440 450 460 470 480 490 500  
*P. saltatrix* GGTTCCTGAGAGTGGATAGAGTTCAGCCCTTTGGG-CTTCTCTTTCAC-----TACAAATTTATCTTATTACTGAGATTCAGAGAGGCCCA  
*N. pectoralis* GGTTCCTGAGAGTGGATAGAGTTCAGCCCTTTGGG-CTTCTCTTTCAC-----TACAAATTTATCTTATTACTGAGATTCAGAGAGGCCCA  
*C. armatus* GGTTCCTGAGAGTGGATAGAGTTCAGCCCTTTGGG-CTTCTCTTTCAT-----ACTAATCTAATACCCAGCATAT-----AAGAGACTGT  
*R. canadum* GGTTCCTGAGAGTGGATAGAGTTCAGCCCTTTGGG-CTTCTCTTTCAT-----TACAAATTTATCTTATTACTGAGATTCAGAGAGGCCCA  
*C. hippurus* GGTTCCTGAGAGTGGATAGAGTTCAGCCCTTTGGG-CTTCTCTTTCACCTA-----TTGTACACCAACCAACTGAT-----TTAGAGACTGT  
*C. lequisei* GGTTCCTGAGAGTGGATAGAGTTCAGCCCTTTGGG-CTTCTCTTTCACCTA-----TTGTACACCAACCAACTGAT-----TTAGAGACTGT  
*P. lineatus* GGTTCCTGAGAGTGGATAGAGTTCAGCCCTTTGGG-CTTCTCTTTCACCTA-----TATT-TACTTCTTAAATGATCA-----CAGAGATTAT  
*E. naucrates* GGTTCCTGAGAGTGGATAGAGTTCAGCCCTTTGGG-CTTCTCTTTCACCTA-----TATT-TACTTCTTAAATGATCA-----CAGAGATTAT  
*E. neucratoides* GGTTCCTGAGAGTGGATAGAGTTCAGCCCTTTGGG-CTTCTCTTTCACCTA-----TATT-TACTTCTTAAATGATCA-----CAGAGATTAT  
*R. australis* GGTTCCTGAGAGTGGATAGAGTTCAGCCCTTTGGG-CTTCTCTTTCACCTA-----TATT-TACTTCTTAAATGATCA-----CAGAGATTAT  
*R. albescens* GGTTCCTGAGAGTGGATAGAGTTCAGCCCTTTGGG-CTTCTCTTTCACCTA-----TATT-TACTTCTTAAATGATCA-----CAGAGATTAT  
*R. remora* GGTTCCTGAGAGTGGATAGAGTTCAGCCCTTTGGG-CTTCTCTTTCACCTA-----TATT-TACTTCTTAAATGATCA-----CAGAGATTAT  
*R. osteochir* GGTTCCTGAGAGTGGATAGAGTTCAGCCCTTTGGG-CTTCTCTTTCACCTA-----TATT-TACTTCTTAAATGATCA-----CAGAGATTAT  
*R. brachyptera* GGTTCCTGAGAGTGGATAGAGTTCAGCCCTTTGGG-CTTCTCTTTCACCTA-----TATT-TACTTCTTAAATGATCA-----CAGAGATTAT

Appendix 8. (continued).

510 520 530 540 550 560 570 580 590 600  
*P. saltatrix* TGGAGTTAGTCAARAGGGGACAGCCCTTTGATATAGACACACACTTTTCAGAGGGGTAAGGATCATARTAACTAAGGCAACATCGCCCTGGTGGGC  
*N. pectoralis* GAGAGTTAGTCAARAGGGGACAGCCCTTTGATATAGACACACACTTTTCAGAGGGGTAAGGATCATARTTT-TCRAGGCA-ACAGTTTGGTGGGC  
*C. armatus* AAGAGTTAGTCAARAGGGGACAGCCCTTTGATATAGACACACACTTTTCAGAGGGGTAAGGATCATARTTT-TCRAGGCA-ACAGTTTGGTGGGC  
*R. canadum* GAGAGTTAGTCAARAGGGGACAGCTCCTTTGATATAGACACACACTTTTARAGGGGTAAGGATCATATA-TTTTARAGGTAA-ARTGTTCTAGTGGGC  
*C. hippurus* GAGAGTTAGTCAARAGGGGACAGCTCCTTTGATATAGACACACACTTTTARACTGGGTAAGGATCATATA-TTATTTARAGGTAA-ARATCTCAGTGGGC  
*C. sequiselis* AAGAGTTAGTCAARAGGGGACAGCTCCTTTGATATAGACACACACTTTTARACTGGGTAAGGATCATATA-GTARTTARAGGTAA-ARATCTCAGTGGGC  
*P. lineatus* AAGAGTTAGTCAARAGGGGACAGCCCTTTGATATAGACACACACTTTTARAGGGGTAAGGATCATATA-ATCTAARAGGTAA-ARTGTTCTAGTGGGC  
*E. naucrates* AAGAGTTAGTCAARAGGGGACAGCCCTTTGATATAGACACACACTTTTARAGGGGTAAGGATCATATA-ATCTAARAGGTAA-ARTGTTCTAGTGGGC  
*E. neucratoides* AAGAGTTAGTCAARAGGGGACAGCCCTTTGATATAGACACACACTTTTARAGGGGTAAGGATCATATA-ATCTAARAGGTAA-ARTGTTCTAGTGGGC  
*R. australis* AAGAGTTAGTCAARAGGGGACAGCCCTTTGATATAGACACACACTTTTARAGGGGTAAGGATCATATA-ATCTAARAGGTAA-ARTGTTCTAGTGGGC  
*R. albescens* AAGAGTTAGTCAARAGGGGACAGCCCTTTGATATAGACACACACTTTTARAGGGGTAAGGATCATATA-TTATARAGGTAA-ARTGTTCTAGTGGGC  
*R. remora* AAGAGTTAGTCAARAGGGGACAGCCCTTTGATATAGACACACACTTTTARAGGGGTAAGGATCATATA-CTTTARAGGTAA-ARTGTTCTAGTGGGC  
*R. osteochir* AAGAGTTAGTCAARAGGGGACAGCCCTTTGATATAGACACACTTTTARAGGGGTAAGGATCATATA-ATTTARAGGTAA-ARTGTTCTAGTGGGC  
*R. brachyptera* AAGAGTTAGTCAARAGGGGACAGCCCTTTGATATAGACACACTTTGACRAGCGGGTAAGGATCATG-TTTTARAGGTAA-ARTGTTCTAGTGGGC

610 620 630 640 650 660 670 680 690 700  
*P. saltatrix* CTAAAGCAGCCACCCCTTACAGAAAGCGTTACAGCTCAGCATTATACCTTARCCCATATATTTAGATAAACAATCCACACCCCTACACTT-ATCAGG  
*N. pectoralis* CTAAAGCAGCCACCCCTTACAGAAAGCGTTACAGCTCAGCATTATACCTTARCCCATATATTTAGATAAACAATCCACACCCCTACACTT-ATCAGG  
*C. armatus* CTAAAGCAGCCACCCCTTACAGAAAGCGTTACAGCTCAGCATTATACCTTARCCCATATATTTAGATAAACAATCCACACCCCTACACTT-ATCAGG  
*R. canadum* CTAAAGCAGCCACCCCTTACAGAAAGCGTTACAGCTCAGCATTATACCTTARCCCATATATTTAGATAAACAATCCACACCCCTACACTT-ATCAGG  
*C. hippurus* CTAAAGCAGCCACCCCTTACAGAAAGCGTTACAGCTCAGCATTATACCTTARCCCATATATTTAGATAAACAATCCACACCCCTACACTT-ATCAGG  
*C. sequiselis* CTAAAGCAGCCACCCCTTACAGAAAGCGTTACAGCTCAGCATTATACCTTARCCCATATATTTAGATAAACAATCCACACCCCTACACTT-ATCAGG  
*P. lineatus* CTAAAGCAGCCACCCCTTACAGAAAGCGTTACAGCTCAGCATTATACCTTARCCCATATATTTAGATAAACAATCCACACCCCTACACTT-ATCAGG  
*E. naucrates* CTAAAGCAGCCACCCCTTACAGAAAGCGTTACAGCTCAGCATTATACCTTARCCCATATATTTAGATAAACAATCCACACCCCTACACTT-ATCAGG  
*E. neucratoides* CTAAAGCAGCCACCCCTTACAGAAAGCGTTACAGCTCAGCATTATACCTTARCCCATATATTTAGATAAACAATCCACACCCCTACACTT-ATCAGG  
*R. australis* CTAAAGCAGCCACCCCTTACAGAAAGCGTTACAGCTCAGCATTATACCTTARCCCATATATTTAGATAAACAATCCACACCCCTACACTT-ATCAGG  
*R. albescens* CTAAAGCAGCCACCCCTTACAGAAAGCGTTACAGCTCAGCATTATACCTTARCCCATATATTTAGATAAACAATCCACACCCCTACACTT-ATCAGG  
*R. remora* CTAAAGCAGCCACCCCTTACAGAAAGCGTTACAGCTCAGCATTATACCTTARCCCATATATTTAGATAAACAATCCACACCCCTACACTT-ATCAGG  
*R. osteochir* CTAAAGCAGCCACCCCTTACAGAAAGCGTTACAGCTCAGCATTATACCTTARCCCATATATTTAGATAAACAATCCACACCCCTACACTT-ATCAGG  
*R. brachyptera* CTAAAGCAGCCACCCCTTACAGAAAGCGTTACAGCTCAGCATTATACCTTARCCCATATATTTAGATAAACAATCCACACCCCTACACTT-ATCAGG

710 720 730 740 750 760 770 780 790 800  
*P. saltatrix* CCGTCTCATGCAARCATGAGAGTGCACATGCTAATATGAGTAAATAGAGAGCACC--TGCCCTCTCCTTGCCACAGTGTARTTGGAGCGACCC-CCA  
*N. pectoralis* CCGTCTCATGCAARCATGAGAGTGCACATGCTAATATGAGTAAATAGAGAGCACCCTCTCTCCACAGCAGTGTARTTGGAGCGGACCCACCA  
*C. armatus* CCGTCTCATGCAARCATGAGAGTGCACATGCTAATATGAGTAAATAGAGAGCACC--TGCCCTCTCCTTGCCACAGTGTARTTGGAGCGGACCCACCA  
*R. canadum* CCGTCTCATGCAARCATGAGAGTGCACATGCTAATATGAGTAAATAGAGAGCACC--TGCCCTCTCCTTGCCACAGTGTARTTGGAGCGGACCCACCA  
*C. hippurus* CCGTCTCATGCAARCATGAGAGTGCACATGCTAATATGAGTAAATAGAGAGCACC--TGCCCTCTCCTTGCCACAGTGTARTTGGAGCGGACCCACCA  
*C. sequiselis* CCGTCTCATGCAARCATGAGAGTGCACATGCTAATATGAGTAAATAGAGAGCACC--TGCCCTCTCCTTGCCACAGTGTARTTGGAGCGGACCCACCA  
*P. lineatus* CCGTCTCATGCAARCATGAGAGTGCACATGCTAATATGAGTAAATAGAGAGCACC--TGCCCTCTCCTTGCCACAGTGTARTTGGAGCGGACCCACCA  
*E. naucrates* CCGTCTCATGCAARCATGAGAGTGCACATGCTAATATGAGTAAATAGAGAGCACC--TGCCCTCTCCTTGCCACAGTGTARTTGGAGCGGACCCACCA  
*E. neucratoides* CCGTCTCATGCAARCATGAGAGTGCACATGCTAATATGAGTAAATAGAGAGCACC--TGCCCTCTCCTTGCCACAGTGTARTTGGAGCGGACCCACCA  
*R. australis* CCGTCTCATGCAARCATGAGAGTGCACATGCTAATATGAGTAAATAGAGAGCACC--TGCCCTCTCCTTGCCACAGTGTARTTGGAGCGGACCCACCA  
*R. albescens* CCGTCTCATGCAARCATGAGAGTGCACATGCTAATATGAGTAAATAGAGAGCACC--TGCCCTCTCCTTGCCACAGTGTARTTGGAGCGGACCCACCA  
*R. remora* CCGTCTCATGCAARCATGAGAGTGCACATGCTAATATGAGTAAATAGAGAGCACC--TGCCCTCTCCTTGCCACAGTGTARTTGGAGCGGACCCACCA  
*R. osteochir* CCGTCTCATGCAARCATGAGAGTGCACATGCTAATATGAGTAAATAGAGAGCACC--TGCCCTCTCCTTGCCACAGTGTARTTGGAGCGGACCCACCA  
*R. brachyptera* CCGTCTCATGCAARCATGAGAGTGCACATGCTAATATGAGTAAATAGAGAGCACC--TGCCCTCTCCTTGCCACAGTGTARTTGGAGCGGACCCACCA

810 820 830 840 850 860 870 880 890 900  
*P. saltatrix* CCGAGACTTACCGTCCCAACCAAGAGGGTARTGACACACAAATT--ACCA-ACCAGAAACCCACCAACAAACACCCGTTAATCCACACTGGTGTG  
*N. pectoralis* CCGAGACTTACCGTCCCAACCAAGAGGGTARTGACACACAAATT--ACCA-ACCAGAAACCCACCAACAAACACCCGTTAATCCACACTGGTGTG  
*C. armatus* CCGAGACTTACCGTCCCAACCAAGAGGGTARTGACACACAAATT--ACCA-ACCAGAAACCCACCAACAAACACCCGTTAATCCACACTGGTGTG  
*R. canadum* CCGAGACTTACCGTCCCAACCAAGAGGGTARTGACACACAAATT--ACCA-ACCAGAAACCCACCAACAAACACCCGTTAATCCACACTGGTGTG  
*C. hippurus* CCGAGACTTACCGTCCCAACCAAGAGGGTARTGACACACAAATT--ACCA-ACCAGAAACCCACCAACAAACACCCGTTAATCCACACTGGTGTG  
*C. sequiselis* CCGAGACTTACCGTCCCAACCAAGAGGGTARTGACACACAAATT--ACCA-ACCAGAAACCCACCAACAAACACCCGTTAATCCACACTGGTGTG  
*P. lineatus* CCGAGACTTACCGTCCCAACCAAGAGGGTARTGACACACAAATT--ACCA-ACCAGAAACCCACCAACAAACACCCGTTAATCCACACTGGTGTG  
*E. naucrates* CCGAGACTTACCGTCCCAACCAAGAGGGTARTGACACACAAATT--ACCA-ACCAGAAACCCACCAACAAACACCCGTTAATCCACACTGGTGTG  
*E. neucratoides* CCGAGACTTACCGTCCCAACCAAGAGGGTARTGACACACAAATT--ACCA-ACCAGAAACCCACCAACAAACACCCGTTAATCCACACTGGTGTG  
*R. australis* CCGAGACTTACCGTCCCAACCAAGAGGGTARTGACACACAAATT--ACCA-ACCAGAAACCCACCAACAAACACCCGTTAATCCACACTGGTGTG  
*R. albescens* CCGAGACTTACCGTCCCAACCAAGAGGGTARTGACACACAAATT--ACCA-ACCAGAAACCCACCAACAAACACCCGTTAATCCACACTGGTGTG  
*R. remora* CCGAGACTTACCGTCCCAACCAAGAGGGTARTGACACACAAATT--ACCA-ACCAGAAACCCACCAACAAACACCCGTTAATCCACACTGGTGTG  
*R. osteochir* CCGAGACTTACCGTCCCAACCAAGAGGGTARTGACACACAAATT--ACCA-ACCAGAAACCCACCAACAAACACCCGTTAATCCACACTGGTGTG  
*R. brachyptera* CCGAGACTTACCGTCCCAACCAAGAGGGTARTGACACACAAATT--ACCA-ACCAGAAACCCACCAACAAACACCCGTTAATCCACACTGGTGTG

910 920 930 940 950 960 970 980 990 1000  
*P. saltatrix* CC--CTTAGGAAAGACTAARAGAAATGAGAGGAACTCGGCARACAC--RAGCCTCGCCTGTTTACCAAAAACATCGCCTCTTGCAAAA-TAAAGT  
*N. pectoralis* CC--CTTAGGAAAGACTAARAGAAATGAGAGGAACTCGGCARACACATA--RAGCCTCGCCTGTTTACCAAAAACATCGCCTCTTGCAAAA-TAAAGT  
*C. armatus* CC--TACTAGGAAAGACTAARAGAAATGAGAGGAACTCGGCARACACATA--RAGCCTCGCCTGTTTACCAAAAACATCGCCTCTTGCAAAA-TAAAGT  
*R. canadum* CA--TTTAGGAAAGACTAARAGAAATGAGAGGAACTCGGCARACAC--GAT--RAGCCTCGCCTGTTTACCAAAAACATCGCCTCTTGCAAAA-TTAA-GA  
*C. hippurus* CTCTTTTAGGAAAGACTAARAGAAATGAGAGGAACTCGGCARAC--AC--RAGCCTCGCCTGTTTACCAAAAACATCGCCTCTTGCAAAA-TAAAGT  
*C. sequiselis* CCCTTTTAGGAAAGACTAARAGAAATGAGAGGAACTCGGCARAC--AC--RAGCCTCGCCTGTTTACCAAAAACATCGCCTCTTGCAAAA-TAAAGT  
*P. lineatus* CA--TACAGGAAAGACTAARAGAAATGAGAGGAACTCGGCARACACAT--RAGCCTCGCCTGTTTACCAAAAACATCGCCTCTTGCAAAA-TAAAGT  
*E. naucrates* CA--TACAGGAAAGACTAARAGAAATGAGAGGAACTCGGCARACACAT--RAGCCTCGCCTGTTTACCAAAAACATCGCCTCTTGCAAAA-TAAAGT  
*E. neucratoides* CA--TACAGGAAAGACTAARAGAAATGAGAGGAACTCGGCARACACAT--RAGCCTCGCCTGTTTACCAAAAACATCGCCTCTTGCAAAA-TAAAGT  
*R. australis* CA--CCCAGGAAAGACTAARAGAAATGAGAGGAACTCGGCARACAC--RAGCCTCGCCTGTTTACCAAAAACATCGCCTCTTGCAAAA-TAAAGT  
*R. albescens* CA--CTCAGGAAAGACTAARAGAAATGAGAGGAACTCGGCARACACAT--RAGCCTCGCCTGTTTACCAAAAACATCGCCTCTTGCAAAA-TAAAGT  
*R. remora* CA--CCCAGGAAAGACTAARAGAAATGAGAGGAACTCGGCARACACAT--RAGCCTCGCCTGTTTACCAAAAACATCGCCTCTTGCAAAA-TAAAGT  
*R. osteochir* CA--CTCAGGAAAGACTAARAGAAATGAGAGGAACTCGGCARACACAT--RAGCCTCGCCTGTTTACCAAAAACATCGCCTCTTGCAAAA-TAAAGT  
*R. brachyptera* CA--CCCAGGAAAGACTAARAGAAATGAGAGGAACTCGGCARACACATA--RAGCCTCGCCTGTTTACCAAAAACATCGCCTCTTGCAAAA-TAAAGT

Appendix 8. (continued).

1010 1020 1030 1040 1050 1060 1070 1080 1090 1100  
*P.\_saltatrix* ATAGAGGTCCACCTGCCAGTGACTATA-GGTTACACGGCCGGTATTTTACCGTGCAAGGTAGCGTAACTTGTCTTTAATGGGACCCG  
*N.\_pectoralis* -TAAGAGGTCCCTGCCCTGCCTATGATA-ATGATTACACGGCCGGTATTTTACCGTGCGAAGGTAGCGTAACTTGTCTTTAATGAAGACCCG  
*C.\_armatus* ATAGAGGTCCCGCTGCCAGTGACATATGGTTACACGGCCGGTATTTTACCGTGCAAGGTAGCGTAACTTGTCTTTAATGAAGACCCG  
*R.\_canadum* ATAGAGGTCTTACCTGCCAGTGACAA--CTCGTTTACCGCCGGTATCTTACCGTGCAAGGTAGCGTAACTTGTCTTTAATGAAGACCCG  
*C.\_hippurus* ATAGAGGTCCACCTGCCCGTGACAA--CCATTTTACCGCCGGTATCTTACCGTGCGAAGGTAGCGTAACTTGTCTTTAATGGGACTTG  
*C.\_equiselis* ATAGAGGTCCACCTGCCCGTGACAA--CCATTTTACCGCCGGTATCTTACCGTGCGAAGGTAGCGTAACTTGTCTTTAATGGGACTTG  
*P.\_lineatus* ATAGAGGTCCCGCTGCCAGTGACAA-CTAGTTTACCGCCGGTATTTTACCGTGCTAAGGTAGCGTAACTTGTCTTTAATGAAGACCCG  
*E.\_naucratus* ATAGAGGTCCCGCTGCCAGTGACAA-CTAGTTTACCGCCGGTATTTTACCGTGCTAAGGTAGCGTAACTTGTCTTTAATGAAGACCCG  
*E.\_neucratoides* ATAGAGGTCCCGCTGCCAGTGACAA-CTAGTTTACCGCCGGTATTTTACCGTGCTAAGGTAGCGTAACTTGTCTTTAATGAAGACCCG  
*R.\_australis* ATAGAGGTCCCGCTGCCAGTGACAA-CTAGTTTACCGCCGGTATTTTACCGTGCTAAGGTAGCGTAACTTGTCTTTAATGAAGACCCG  
*R.\_albescens* ATAGAGGTCCCGCTGCCAGTGACAA-CTAGTTTACCGCCGGTATTTTACCGTGCTAAGGTAGCGTAACTTGTCTTTAATGAAGACCCG  
*R.\_remora* ATAGAGGTCCCGCTGCCAGTGACAA-CTAGTTTACCGCCGGTATTTTACCGTGCTAAGGTAGCGTAACTTGTCTTTAATGAAGACCCG  
*R.\_osteoichir* ATAGAGGTCCCGCTGCCAGTGACAA-CTAGTTTACCGCCGGTATTTTACCGTGCTAAGGTAGCGTAACTTGTCTTTAATGAAGACCCG  
*R.\_brachyptera* ATAGAGGTCCCGCTGCCAGTGACAA-CTAGTTTACCGCCGGTATTTTACCGTGCTAAGGTAGCGTAACTTGTCTTTAATGAAGACCCG

1110 1120 1130 1140 1150 1160 1170 1180 1190 1200  
*P.\_saltatrix* TATGAATGGCACACGAGGGCTTACTGTCTCCTTTTTCCGGTACGTGAATTTGATCTCCCGTGCAGAGCGGGGATAGACCATAGACGAGAGACC  
*N.\_pectoralis* TATGAATGGCACACGAGGGCTTACTGTCTCCTTTTTTACTGCAATGAATTTGATCTCCCGTGCAGAGCGGGGATAGACCATAGACGAGAGACC  
*C.\_armatus* TATGAATGGCACACGAGGGCTTACTGTCTCCTCTCCAGTCAATGAATTTGATCTCCCGTGCAGAGCGGGGATTTACATAGACGAGAGACC  
*R.\_canadum* TATGAATGGTATACGAGGGCTTACTGTCTCCTCTCCAGTCAATGAATTTGATCTCCCGTGCAGAGCGGGGATTTACATAGACGAGAGACC  
*C.\_hippurus* TATGAATGGTATACGAGGGCTTACTGTCTCCTCTCTGTCATGAATTTGATCTCCCGTGCAGAGCGGGGATTTACTCATAGACGAGAGACC  
*C.\_equiselis* TATGAATGGTATACGAGGGCTTACTGTCTCCTCTCTGTCATGAATTTGATCTCCCGTGCAGAGCGGGGATTTACTCATAGACGAGAGACC  
*P.\_lineatus* TATGAATGGTATACGAGGGCTTACTGTCTCCTCTCTGTCATGAATTTGATCTCCCGTGCAGAGCGGGGATTTACTCATAGACGAGAGACC  
*E.\_naucratus* TATGAATGGTATACGAGGGCTTACTGTCTCCTCTCTGTCATGAATTTGATCTCCCGTGCAGAGCGGGGATTTACTCATAGACGAGAGACC  
*E.\_neucratoides* TATGAATGGTATACGAGGGCTTACTGTCTCCTCTCTGTCATGAATTTGATCTCCCGTGCAGAGCGGGGATTTACTCATAGACGAGAGACC  
*R.\_australis* TATGAATGGTATACGAGGGCTTACTGTCTCCTCTCTGTCATGAATTTGATCTCCCGTGCAGAGCGGGGATTTACTCATAGACGAGAGACC  
*R.\_albescens* TATGAATGGTATACGAGGGCTTACTGTCTCCTCTCTGTCATGAATTTGATCTCCCGTGCAGAGCGGGGATTTACTCATAGACGAGAGACC  
*R.\_remora* TATGAATGGTATACGAGGGCTTACTGTCTCCTCTCTGTCATGAATTTGATCTCCCGTGCAGAGCGGGGATTTACTCATAGACGAGAGACC  
*R.\_osteoichir* TATGAATGGTATACGAGGGCTTACTGTCTCCTCTCTGTCATGAATTTGATCTCCCGTGCAGAGCGGGGATTTACTCATAGACGAGAGACC  
*R.\_brachyptera* TATGAATGGTATACGAGGGCTTACTGTCTCCTCTCTGTCATGAATTTGATCTCCCGTGCAGAGCGGGGATTTACTCATAGACGAGAGACC

1210 1220 1230 1240 1250 1260 1270 1280 1290 1300  
*P.\_saltatrix* CTATGGAGCTTAAAGACACAGGGCATATCATGTTAAACACCC--AAGATAAAGGACTGACTGAGTGAATTA---TGCCCTATGTCTTTGGTTGGGGCG  
*N.\_pectoralis* CTATGGAGCTTAAAGACACAGGGCATATCATGTTAAACACCC--AAGATAAAGGACTGACTGAGTGAATTA---TGCCCTATGTCTTTGGTTGGGGCG  
*C.\_armatus* CTATGGAGCTTAAAGACACAGGGCATATGTTAAACACCCCTGACTGAAATTTGATCTCCCGTGCAGAGCGGGGATTTACTCATAGACGAGAGACC  
*R.\_canadum* CTATGGAGCTTAAAGACTAAGGTAGACTAGATTAAATTACTTCAACACAGGACTAAATCATCAC--CACCTACCCCTATATCTTCGGTTGGGGCG  
*C.\_hippurus* CTATGGAGCTTAAAGACTAAGGTAGACTAGATTAAATTACTTCAACACAGGACTAAATCATCAC--CACCTACCCCTATATCTTCGGTTGGGGCG  
*C.\_equiselis* CTATGGAGCTTAAAGACTAAGGTAGACTAGATTAAATTACTTCAACACAGGACTAAATCATCAC--CACCTACCCCTATATCTTCGGTTGGGGCG  
*P.\_lineatus* CTATGGAGCTTAAAGACTAAGGTAGACTAGATTAAATTACTTCAACACAGGACTAAATCATCAC--CACCTACCCCTATATCTTCGGTTGGGGCG  
*E.\_naucratus* CTATGGAGCTTAAAGACTAAGGTAGACTAGATTAAATTACTTCAACACAGGACTAAATCATCAC--CACCTACCCCTATATCTTCGGTTGGGGCG  
*E.\_neucratoides* CTATGGAGCTTAAAGACTAAGGTAGACTAGATTAAATTACTTCAACACAGGACTAAATCATCAC--CACCTACCCCTATATCTTCGGTTGGGGCG  
*R.\_australis* CTATGGAGCTTAAAGACTAAGGTAGACTAGATTAAATTACTTCAACACAGGACTAAATCATCAC--CACCTACCCCTATATCTTCGGTTGGGGCG  
*R.\_albescens* CTATGGAGCTTAAAGACTAAGGTAGACTAGATTAAATTACTTCAACACAGGACTAAATCATCAC--CACCTACCCCTATATCTTCGGTTGGGGCG  
*R.\_remora* CTATGGAGCTTAAAGACTAAGGTAGACTAGATTAAATTACTTCAACACAGGACTAAATCATCAC--CACCTACCCCTATATCTTCGGTTGGGGCG  
*R.\_osteoichir* CTATGGAGCTTAAAGACTAAGGTAGACTAGATTAAATTACTTCAACACAGGACTAAATCATCAC--CACCTACCCCTATATCTTCGGTTGGGGCG  
*R.\_brachyptera* CTATGGAGCTTAAAGACTAAGGTAGACTAGATTAAATTACTTCAACACAGGACTAAATCATCAC--CACCTACCCCTATATCTTCGGTTGGGGCG

1310 1320 1330 1340 1350 1360 1370 1380 1390 1400  
*P.\_saltatrix* ACCACGGGAGAACAAARACCCACCGGACTGGGG--TACATCA-----TATCTTACACCAAGAGGCT  
*N.\_pectoralis* ACCCTGGG-GAAACAAARACCCCGTGGGACTGGGAGCAATGATTTACCCGGATTCCTTATCTACCATCTCTCTCCCAATTA-AGAGCT  
*C.\_armatus* ACCATGGG-GAAGCAAAARACCCCATGGGACTAGGG-----GACACCC--ACTATCTCCCCCTCCCAACAGCA-AGAGTT  
*R.\_canadum* ACAATGGG-GAA-CAAAATCCCCATGCGGACGGGAGCAAA-----ACCTGAATATCACTTTCACATCAACTTCTCCCAATTAAGAGTG  
*C.\_hippurus* ACAATGGG-GAAATAAAATCCCCATGCGGACGGGAAATTT-----TATCTAAGAAATTTCTTACCATAAGACTACTCC-CAATTAAGAGTG  
*C.\_equiselis* ACAATGGG-GAAATAAAATCCCCATGCGGACGGGAAATTT-----TATTAAGAAATTTCTTACCATAAGACTACTCC-CAATTAAGAGTG  
*P.\_lineatus* ACCATGGG-GAAATATAAAARACCCACGTTGGGACTAGAGAAC-----TA-ACCCATATCAA-----ACCTCTAAARATATAGAGCC  
*E.\_naucratus* ACCATGGG-GAAATATAAAARACCCACGTTGGGACTAGAGAAC-----CA-ACCCATATCAA-----CCCTCTAAARATATAGAGCC  
*E.\_neucratoides* ACCATGGG-GAAATATAAAARACCCACGTTGGGACTAGAGAAC-----TA-ACCCATATCAA-----CCCTCTAAARATATAGAGCC  
*R.\_australis* ACCATGGG-GAAATATAAAARACCCACGTTGGGACTAGAGAAC-----TAGACCCCTAATGA-----TCCTCTTAARATATAGAGCC  
*R.\_albescens* ACCATGGG-GAAATATAAAARACCCACGTTGGGACTAGAGAAC-----TAATCCCTTACAG-----CTCTCCCAACAGCACAGAGCC  
*R.\_remora* ACCATGGG-GAAATATAAAARACCCACGTTGGGACTAGAGAAC-----TAGACCCCTAATGA-----ACCTCTCAAAATATAGAGCC  
*R.\_osteoichir* ACCATGGG-GAAATATAAAARACCCACGTTGGGACTAGAGAAC-----TCCTCCCTCAACAA-----CCCTCTCAAAACACAGAGCC  
*R.\_brachyptera* ACCATGGG-GAAGTACAAARACCCACGTTGGGACTAGAGAAC-----TCCTTCCCTTAATA-----CTCTCTCAAAACACAGAGTT

1410 1420 1430 1440 1450 1460 1470 1480 1490 1500  
*P.\_saltatrix* GCACCTCTAGTATACAGAAATTTCTGACCAAAATGATCCGGCAAC-GCCGATCAACGGACCAAGTTACCCTAGGGATACAGCGCAATCCCTTTTAGA  
*N.\_pectoralis* GCACCTCTAGTATACAGAA-CCTCTGACCTACCATGATCCGGCAAA-CGCCATCAACGGACCAAGTTACCCTAGGGATACAGCGCAATCCCTTTTAGA  
*C.\_armatus* ACACCTCTAGTATACAGAA-CCTCTGACCTATATGATCCGGCTTCTGCGGATCAACGGACCAAGTTACCCTAGGGATACAGCGCAATCCCTTTTAGA  
*R.\_canadum* ACACCTCTAGTATACAGTA-CCTCTGACCAAAATGATCCGGCAAT-GCCGATCAACGGACCAAGTTACCCTAGGGATACAGCGCAATCCCTTTTAGA  
*C.\_hippurus* ACACCTCTAGTATACAGTA-TTTCTGACCAAAATGATCCGGCAAT-TTCCGATTAACGGACCAAGTTACCCTAGGGATACAGCGCAATCCCTTTTAGA  
*C.\_equiselis* ACACCTCTAGTATACAGTA-TTTCTGACCAAAATGATCCGGCAAT-TTCCGATTAACGGACCAAGTTACCCTAGGGATACAGCGCAATCCCTTTTAGA  
*P.\_lineatus* ACACCTCTAATTAACAGAA-CCTCTGACCAAAATGATCCGGCAAT-GCCGATCAACGGACCAAGTTACCCTAGGGATACAGCGCAATCCCTTTTAGA  
*E.\_naucratus* ACACCTCCAATTAACAGAA-CCTCTGACCAAAATGATCCGGCAAT-GCCGATCAACGGACCAAGTTACCCTAGGGATACAGCGCAATCCCTTTTAGA  
*E.\_neucratoides* ACACCTCCAATTAACAGAA-CCTCTGACCAAAATGATCCGGCAAT-GCCGATCAACGGACCAAGTTACCCTAGGGATACAGCGCAATCCCTTTTAGA  
*R.\_australis* ACACCTCTAATTAACAGAA-CCTCTGACCAAAATGATCCGGCAAT-GCCGATCAACGGACCAAGTTACCCTAGGGATACAGCGCAATCCCTTTTAGA  
*R.\_albescens* ACACCTCTAATTAACAGAA-CCTCTGACCAAAATGATCCGGCAAT-GCCGATCAACGGACCAAGTTACCCTAGGGATACAGCGCAATCCCTTTTAGA  
*R.\_remora* ACACCTCTAATTAACAGAA-TCTCTGACCAAAATGATCCGGCAAT-GCCGATCAACGGACCAAGTTACCCTAGGGATACAGCGCAATCCCTTTTAGA  
*R.\_osteoichir* ACACCTCTAATTAACAGAA-TTTCTGACCAAAATGATCCGGCAAT-GCCGATCAACGGACCAAGTTACCCTAGGGATACAGCGCAATCCCTTTTAGA  
*R.\_brachyptera* ACACCTCTAATTAACAGAA-TTTCTGACCAAAATGATCCGGCAAT-GCCGATCAACGGACCAAGTTACCCTAGGGATACAGCGCAATCCCTTTTAGA

Appendix 8. (continued).

	1510	1520	1530	1540	1550	1560	1570	1580	1590	1600
<i>P._saltatrix</i>	GCCCTTATCAGCAGGGGGTTACGACCTCGATGTTGGATCAGGACATCCTATGGTGCACCCGCTACTGAGGGTTCGTTTGTTCACCGATTAAAGTCCT									
<i>N._pectoralis</i>	GCCCATATCGACAGGGGGTTACGACCTCGATGTTGGATCAGGACATCCTATGGTGCACCCGCTATTAAAGGGTTCGTTTGTTCACCGATTAAAGTCCT									
<i>C._armatus</i>	GCCCATATCGACAGGGGGTTACGACCTCGATGTTGGATCAGGACATCCTATGGTGCACCCGCTATTAAAGGGTTCGTTTGTTCACCGATTAAAGTCCT									
<i>R._canadum</i>	GCCCGCATCAGCAGGGGGTTACGACCTCGATGTTGGATCAGGACATCCTATGGTGCACCCGCTATTAAAGGGTTCGTTTGTTCACCGATTAAAGTCCT									
<i>C._hippurus</i>	GCCACATCAGCAGGGGGTTACGACCTCGATGTTGGATCAGGACATCCTATGGTGCACCCGCTATTAAAGGGTTCGTTTGTTCACCGATTAAAGTCCT									
<i>C._lewisii</i>	GCCACATCAGCAGGGGGTTACGACCTCGATGTTGGATCAGGACATCCTATGGTGCACCCGCTATTAAAGGGTTCGTTTGTTCACCGATTAAAGTCCT									
<i>P._lineatus</i>	GCCCTTATCAGCAGGGGGTTACGACCTCGATGTTGGATCAGGACATCCTATGGTGCACCCGCTATTAAAGGGTTCGTTTGTTCACCGATTAAAGTCCT									
<i>E._naucratus</i>	GCCCTTATCAGCAGGGGGTTACGACCTCGATGTTGGATCAGGACATCCTATGGTGCACCCGCTATTAAAGGGTTCGTTTGTTCACCGATTAAAGTCCT									
<i>E._neucratoides</i>	GCCCTTATCAGCAGGGGGTTACGACCTCGATGTTGGATCAGGACATCCTATGGTGCACCCGCTATTAAAGGGTTCGTTTGTTCACCGATTAAAGTCCT									
<i>R._australis</i>	GCCCTTATCAGCAGGGGGTTACGACCTCGATGTTGGATCAGGACATCCTATGGTGCACCCGCTATTAAAGGGTTCGTTTGTTCACCGATTAAAGTCCT									
<i>R._albescens</i>	GCCCTTATCAGCAGGGGGTTACGACCTCGATGTTGGATCAGGACATCCTATGGTGCACCCGCTATTAAAGGGTTCGTTTGTTCACCGATTAAAGTCCT									
<i>R._remora</i>	GCCCTTATCAGCAGGGGGTTACGACCTCGATGTTGGATCAGGACATCCTATGGTGCACCCGCTATTAAAGGGTTCGTTTGTTCACCGATTAAAGTCCT									
<i>R._osteochir</i>	GCCCTTATCAGCAGGGGGTTACGACCTCGATGTTGGATCAGGACATCCTATGGTGCACCCGCTATTAAAGGGTTCGTTTGTTCACCGATTAAAGTCCT									
<i>R._brachyptera</i>	GCCCTTATCAGCAGGGGGTTACGACCTCGATGTTGGATCAGGACATCCTATGGTGCACCCGCTATTAAAGGGTTCGTTTGTTCACCGATTAAAGTCCT									

	1610	1620	1630	1640	1650	1660	1670	1680
<i>P._saltatrix</i>	ACGTGATCTGAGTTCAGACCCGGAGTATCCAGGTCAGTTTCTATCTATGACAT-GTTCCTTTCTAGTACGAAAGGACCCGAAAGAGAGAG							
<i>N._pectoralis</i>	ACGTGATCTGAGTTCAGACCCGGAGTATCCAGGTCAGTTTCTATCTATGAAATGATCTTTTCTAGTACGAAAGGACCCGAAAGAGAGAG							
<i>C._armatus</i>	ACGTGATCTGAGTTCAGACCCGGAGTATCCAGGTCAGTTTCTATCTATGAAAC-ATCTTTTCTAGTACGAAAGGACCCGAAAGAGAGAG							
<i>R._canadum</i>	ACGTGATCTGAGTTCAGACCCGGAGTATCCAGGTCAGTTTCTATCTATGTAAT-RAACTTTTCTAGTACGAAAGGACCCGAAAGAGAGAG							
<i>C._hippurus</i>	ACGTGATCTGAGTTCAGACCCGGAGTATCCAGGTCAGTTTCTATCTATGTAAT-ATTCTTTTCTAGTACGAAAGGACCCGAAAGAGAGAG							
<i>C._lewisii</i>	ACGTGATCTGAGTTCAGACCCGGAGTATCCAGGTCAGTTTCTATCTATGTAAT-ATTCTTTTCTAGTACGAAAG-ACCGAAAGAGAGAG							
<i>P._lineatus</i>	ACGTGATCTGAGTTCAGACCCGGAGTATCCAGGTCAGTTTCTATCTATGTAAT-RAACTTTTCTAGTACGAAAGGACCCGAAAGAGAGAG							
<i>E._naucratus</i>	ACGTGATCTGAGTTCAGACCCGGAGTATCCAGGTCAGTTTCTATCTATGTAAT-RAACTTTTCTAGTACGAAAGGACCCGAAAGAGAGAG							
<i>E._neucratoides</i>	ACGTGATCTGAGTTCAGACCCGGAGTATCCAGGTCAGTTTCTATCTATGTAAT-RAACTTTTCTAGTACGAAAGGACCCGAAAGAGAGAG							
<i>R._australis</i>	ACGTGATCTGAGTTCAGACCCGGAGTATCCAGGTCAGTTTCTATCTATGTAAT-RAACTTTTCTAGTACGAAAGGACCCGAAAGAGAGAG							
<i>R._albescens</i>	ACGTGATCTGAGTTCAGACCCGGAGTATCCAGGTCAGTTTCTATCTATGTAAT-RAACTTTTCTAGTACGAAAGGACCCGAAAGAGAGAG							
<i>R._remora</i>	ACGTGATCTGAGTTCAGACCCGGAGTATCCAGGTCAGTTTCTATCTATGTAAT-RAACTTTTCTAGTACGAAAGGACCCGAAAGAGAGAG							
<i>R._osteochir</i>	ACGTGATCTGAGTTCAGACCCGGAGTATCCAGGTCAGTTTCTATCTATGTAAT-RAACTTTTCTAGTACGAAAGGACCCGAAAGAGAGAG							
<i>R._brachyptera</i>	ACGTGATCTGAGTTCAGACCCGGAGTATCCAGGTCAGTTTCTATCTATGTAAT-RAACTTTTCTAGTACGAAAGGACCCGAAAGAGAGAG							



Appendix 9. Aligned mitochondrial ND2 DNA sequence data collected from echeneoid specimens analyzed in the present study.

10 20 30 40 50 60 70 80 90 100  
 P.\_saltatrix ATGAGCCCTTATATCTTAGCCGCGCTCTATTAGCCCTAGGCTTGGGAGCAGACGTTACATTGCGARAGCTCTCACTGACTACTTGCATGATAGGACTAG  
 N.\_pectoralis ATGAAACCCCTTATATCTTAGCTACCCCTGCTATTAGCCCTAGGCTTAGGGACCACTACCACCTTTATAGCTCCCACTGACTACTCGCCGATAGGACTCG  
 C.\_armatus ATGAAACCCCTTATATCTTAGCTACCCCTGCTATTAGCCCTAGGCTTAGGGACCACTACCACCTTTATAGCTCCCACTGACTACTCGCCGATAGGACTCG  
 R.\_canadum ATGAGCCCTTACATCTTAGCCATCTTCTTAATAGCTTAGGCTTAGGACTAGGACTACCACATTTGCGAGCTCACACTGACTACTCGCCGATAGGACTCG  
 C.\_hippurus ATGAAACCCCTTATATCTTAGCCATCTTCTATTAGCTTAGGCTTAGGACTTAGGACTACTACCACATTTGCTAGCTCACACTGACTACTTGCATGATAGGACTCG  
 C.\_sequiselis ATGAAACCCCTTATATCTTAGCTACCCCTGCTATTAGCTTAGGCTTAGGACTACTATTACATTTGCTAGCTCGCACTGACTACTTGCATGATAGGACTCG  
 P.\_lineatus ATGAAACCCCTTATCTTAACCATTTACTCTTTGCCCTTGGCCCTAGGACTACCACATTTACATTTATAGCTCACACTGATTTATTTGCTTGAATAGGCTTAG  
 E.\_naucrates ATGAAACCCCTTATCTTAACCATTTACTCTTTGCCCTTGGCCCTAGGACTACCACATTTACATTTATAGCTCACACTGATTTATTTGCTTGAATAGGCTTAG  
 E.\_neucratoides ATGAAACCCCTTATCTTAACCATTTACTCTTTGCCCTTGGCCCTAGGACTACCACATTTACATTTATAGCTCACACTGATTTATTTGCTTGAATAGGCTTAG  
 R.\_australis ATGAAACCCCTTATCTTAACCATTTACTCTTTGCCCTTGGCCCTAGGACTACCACATTTACATTTATAGCTCACACTGATTTATTTGCTTGAATAGGCTTAG  
 R.\_albescens ATGAAACCCCTTATCTTAACCATTTACTCTTTGCCCTTGGCCCTAGGACTACCACATTTACATTTATAGCTCACACTGATTTATTTGCTTGAATAGGCTTAG  
 R.\_remora ATGAAACCCCTTATCTTAACCATTTACTCTTTGCCCTTGGCCCTAGGACTACCACATTTACATTTATAGCTCACACTGATTTATTTGCTTGAATAGGCTTAG  
 R.\_osteoichir ATGAAACCCCTTATCTTAACCATTTACTCTTTGCCCTTGGCCCTAGGACTACCACATTTACATTTATAGCTCACACTGATTTATTTGCTTGAATAGGCTTAG  
 R.\_brachyptera ATGAAACCCCTTATCTTAACCATTTACTCTTTGCCCTTGGCCCTAGGACTACCACATTTACATTTATAGCTCACACTGATTTATTTGCTTGAATAGGACTTAG

110 120 130 140 150 160 170 180 190 200  
 P.\_saltatrix AARATTAATACCCCTAGCCATTCTTCCACTAATGGCACAARACCCACCCTCGAGCAGTAGAGCAACACCACCAATACTTCTCACTCAGGGCCACCGCAGC  
 N.\_pectoralis AARATTAATACCCCTAGCCATTCTTCCACTAATGGCACAARACCCACCCTCGAGCAGTAGAGCAACACCACCAATACTTCTCACTCAGGGCCACCGCAGC  
 C.\_armatus AARATTAATACCCCTAGCCATTCTTCCACTAATGGCACAARACCCACCCTCGAGCAGTAGAGCAACACCACCAATACTTCTCACTCAGGGCCACCGCAGC  
 R.\_canadum AARATTAATACCCCTAGCCATTCTTCCACTAATGGCACAARACCCACCCTCGAGCAGTAGAGCAACACCACCAATACTTCTCACTCAGGGCCACCGCAGC  
 C.\_hippurus AARATTAATACCCCTAGCCATTCTTCCACTAATGGCACAARACCCACCCTCGAGCAGTAGAGCAACACCACCAATACTTCTCACTCAGGGCCACCGCAGC  
 C.\_sequiselis AARATTAATACCCCTAGCCATTCTTCCACTAATGGCACAARACCCACCCTCGAGCAGTAGAGCAACACCACCAATACTTCTCACTCAGGGCCACCGCAGC  
 P.\_lineatus AARATTAATACCCCTAGCCATTCTTCCACTAATGGCACAARACCCACCCTCGAGCAGTAGAGCAACACCACCAATACTTCTCACTCAGGGCCACCGCAGC  
 E.\_naucrates AARATTAATACCCCTAGCCATTCTTCCACTAATGGCACAARACCCACCCTCGAGCAGTAGAGCAACACCACCAATACTTCTCACTCAGGGCCACCGCAGC  
 E.\_neucratoides AARATTAATACCCCTAGCCATTCTTCCACTAATGGCACAARACCCACCCTCGAGCAGTAGAGCAACACCACCAATACTTCTCACTCAGGGCCACCGCAGC  
 R.\_australis AARATTAATACCCCTAGCCATTCTTCCACTAATGGCACAARACCCACCCTCGAGCAGTAGAGCAACACCACCAATACTTCTCACTCAGGGCCACCGCAGC  
 R.\_albescens AARATTAATACCCCTAGCCATTCTTCCACTAATGGCACAARACCCACCCTCGAGCAGTAGAGCAACACCACCAATACTTCTCACTCAGGGCCACCGCAGC  
 R.\_remora AARATTAATACCCCTAGCCATTCTTCCACTAATGGCACAARACCCACCCTCGAGCAGTAGAGCAACACCACCAATACTTCTCACTCAGGGCCACCGCAGC  
 R.\_osteoichir AARATTAATACCCCTAGCCATTCTTCCACTAATGGCACAARACCCACCCTCGAGCAGTAGAGCAACACCACCAATACTTCTCACTCAGGGCCACCGCAGC  
 R.\_brachyptera AARATTAATACCCCTAGCCATTCTTCCACTAATGGCACAARACCCACCCTCGAGCAGTAGAGCAACACCACCAATACTTCTCACTCAGGGCCACCGCAGC

210 220 230 240 250 260 270 280 290 300  
 P.\_saltatrix TGCCATTAATATTTGCGAGCACCACCGCTTGACTTACCAGCAATGAGCATTGACAAATACCCACCCTCTCCAACTACAAATGATCATTTTAA  
 N.\_pectoralis TGCCATTAATATTTGCGAGCACCACCGCTTGACTTACCAGCAATGAGCATTGACAAATACCCACCCTCTCCAACTACAAATGATCATTTTAA  
 C.\_armatus TGCCATTAATATTTGCGAGCACCACCGCTTGACTTACCAGCAATGAGCATTGACAAATACCCACCCTCTCCAACTACAAATGATCATTTTAA  
 R.\_canadum TGCCATTAATATTTGCGAGCACCACCGCTTGACTTACCAGCAATGAGCATTGACAAATACCCACCCTCTCCAACTACAAATGATCATTTTAA  
 C.\_hippurus TGCCATTAATATTTGCGAGCACCACCGCTTGACTTACCAGCAATGAGCATTGACAAATACCCACCCTCTCCAACTACAAATGATCATTTTAA  
 C.\_sequiselis TGCCATTAATATTTGCGAGCACCACCGCTTGACTTACCAGCAATGAGCATTGACAAATACCCACCCTCTCCAACTACAAATGATCATTTTAA  
 P.\_lineatus TGCCATTAATATTTGCGAGCACCACCGCTTGACTTACCAGCAATGAGCATTGACAAATACCCACCCTCTCCAACTACAAATGATCATTTTAA  
 E.\_naucrates TGCCATTAATATTTGCGAGCACCACCGCTTGACTTACCAGCAATGAGCATTGACAAATACCCACCCTCTCCAACTACAAATGATCATTTTAA  
 E.\_neucratoides TGCCATTAATATTTGCGAGCACCACCGCTTGACTTACCAGCAATGAGCATTGACAAATACCCACCCTCTCCAACTACAAATGATCATTTTAA  
 R.\_australis TGCCATTAATATTTGCGAGCACCACCGCTTGACTTACCAGCAATGAGCATTGACAAATACCCACCCTCTCCAACTACAAATGATCATTTTAA  
 R.\_albescens TGCCATTAATATTTGCGAGCACCACCGCTTGACTTACCAGCAATGAGCATTGACAAATACCCACCCTCTCCAACTACAAATGATCATTTTAA  
 R.\_remora TGCCATTAATATTTGCGAGCACCACCGCTTGACTTACCAGCAATGAGCATTGACAAATACCCACCCTCTCCAACTACAAATGATCATTTTAA  
 R.\_osteoichir TGCCATTAATATTTGCGAGCACCACCGCTTGACTTACCAGCAATGAGCATTGACAAATACCCACCCTCTCCAACTACAAATGATCATTTTAA  
 R.\_brachyptera TGCCATTAATATTTGCGAGCACCACCGCTTGACTTACCAGCAATGAGCATTGACAAATACCCACCCTCTCCAACTACAAATGATCATTTTAA

310 320 330 340 350 360 370 380 390 400  
 P.\_saltatrix GCGCTGCGCTAAAAGTGGCTAGCCCTAGCCCTGACTTACCGGCTGACTACCTGAGACTCCAGGCTTAGACTCTCACTACGGGACTTATCTTATCCACTTGC  
 N.\_pectoralis GCGCTGCGCTAAAAGTGGCTAGCCCTAGCCCTGACTTACCGGCTGACTACCTGAGACTCCAGGCTTAGACTCTCACTACGGGACTTATCTTATCCACTTGC  
 C.\_armatus GCGCTGCGCTAAAAGTGGCTAGCCCTAGCCCTGACTTACCGGCTGACTACCTGAGACTCCAGGCTTAGACTCTCACTACGGGACTTATCTTATCCACTTGC  
 R.\_canadum GCGCTGCGCTAAAAGTGGCTAGCCCTAGCCCTGACTTACCGGCTGACTACCTGAGACTCCAGGCTTAGACTCTCACTACGGGACTTATCTTATCCACTTGC  
 C.\_hippurus GCGCTGCGCTAAAAGTGGCTAGCCCTAGCCCTGACTTACCGGCTGACTACCTGAGACTCCAGGCTTAGACTCTCACTACGGGACTTATCTTATCCACTTGC  
 C.\_sequiselis GCGCTGCGCTAAAAGTGGCTAGCCCTAGCCCTGACTTACCGGCTGACTACCTGAGACTCCAGGCTTAGACTCTCACTACGGGACTTATCTTATCCACTTGC  
 P.\_lineatus GCGCTGCGCTAAAAGTGGCTAGCCCTAGCCCTGACTTACCGGCTGACTACCTGAGACTCCAGGCTTAGACTCTCACTACGGGACTTATCTTATCCACTTGC  
 E.\_naucrates GCGCTGCGCTAAAAGTGGCTAGCCCTAGCCCTGACTTACCGGCTGACTACCTGAGACTCCAGGCTTAGACTCTCACTACGGGACTTATCTTATCCACTTGC  
 E.\_neucratoides GCGCTGCGCTAAAAGTGGCTAGCCCTAGCCCTGACTTACCGGCTGACTACCTGAGACTCCAGGCTTAGACTCTCACTACGGGACTTATCTTATCCACTTGC  
 R.\_australis GCGCTGCGCTAAAAGTGGCTAGCCCTAGCCCTGACTTACCGGCTGACTACCTGAGACTCCAGGCTTAGACTCTCACTACGGGACTTATCTTATCCACTTGC  
 R.\_albescens GCGCTGCGCTAAAAGTGGCTAGCCCTAGCCCTGACTTACCGGCTGACTACCTGAGACTCCAGGCTTAGACTCTCACTACGGGACTTATCTTATCCACTTGC  
 R.\_remora GCGCTGCGCTAAAAGTGGCTAGCCCTAGCCCTGACTTACCGGCTGACTACCTGAGACTCCAGGCTTAGACTCTCACTACGGGACTTATCTTATCCACTTGC  
 R.\_osteoichir GCGCTGCGCTAAAAGTGGCTAGCCCTAGCCCTGACTTACCGGCTGACTACCTGAGACTCCAGGCTTAGACTCTCACTACGGGACTTATCTTATCCACTTGC  
 R.\_brachyptera GCGCTGCGCTAAAAGTGGCTAGCCCTAGCCCTGACTTACCGGCTGACTACCTGAGACTCCAGGCTTAGACTCTCACTACGGGACTTATCTTATCCACTTGC

410 420 430 440 450 460 470 480 490 500  
 P.\_saltatrix AAAAFACTGCCCCATTGCCCCACTATTACAAATCACCCAGCAARACCCAAATATCTTATGCTTTAGGCCTAATATCTACACTCATCGGTGGCTGGGG  
 N.\_pectoralis AAAAFACTGCCCCATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTG  
 C.\_armatus AAAAFACTGCCCCATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTG  
 R.\_canadum AAAAFACTGCCCCATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTG  
 C.\_hippurus AAAAFACTGCCCCATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTG  
 C.\_sequiselis AAAAFACTGCCCCATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTG  
 P.\_lineatus AAAAFACTGCCCCATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTG  
 E.\_naucrates AAAAFACTGCCCCATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTG  
 E.\_neucratoides AAAAFACTGCCCCATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTG  
 R.\_australis AAAAFACTGCCCCATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTG  
 R.\_albescens AAAAFACTGCCCCATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTG  
 R.\_remora AAAAFACTGCCCCATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTG  
 R.\_osteoichir AAAAFACTGCCCCATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTG  
 R.\_brachyptera AAAAFACTGCCCCATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTGCCCCACTATTG

Appendix 9. (continued).

510 520 530 540 550 560 570 580 590 600  
*P. saltatrix* TGGACTTAATCAGACCAACTACGAAAAATCCTCGCCTACTCTTCTATTGACACCTCGGGTGAATGATTTTAGTCCTACRATTCTCCCATCCCTGACA  
*N. pectoralis* CGGCTTAARCCAACTCAACTGCGAAAAATCCTGCGTACTCCTCAATTGCCCCTTAGGGTGAATAGCCTTAGTCATTCAATTTTTCCCATCCCTCGCC  
*C. armatus* GGGGCTAARCCAACTCAACTACGAAAAATCCTCGCCTACTCCTCAATTGCCCCTTAGGGTGAATAGCCTTAGTCATTCAATTTTTCCCATCCCTCGCC  
*R. canadum* CGGACTGAACCAACCAACTACGTAARATCCTAGCCTACTCCTCCATTTGCCACTTAGGGTGAATAGCCTTAGTCATTCAATTTTTCCCATCCCTCGCC  
*C. hippurus* AGGCTTGAACTCAARCCCAACTGCGGAAAGTCTTGCATACCTCAATCTCCTACTAGGGTGAATAGCCTTAGTCATTCAATTTTTCCCATCCCTCGCC  
*C. equiselis* GGGCTTAARCCAACTCAACTACGAAAGTCTAGCATATCCTCAATCTCCCATCGGGTGAATAGCCTTAGTCATTCAATTTTTCCCATCCCTCGCC  
*P. lineatus* AGGCTTAARCCAACTCAACTACGAAAAATCCTCGTACTCATCAATTGACACCTTGGTGAATAGCCCTAATCCTCCATTGACCCCTCCCTAACC  
*E. naucrates* AGGCTTAARCCAACTCAACTACGAAAAATCCTCGTACTCATCAATTGACACCTTGGTGAATAGCCCTAATCCTCCATTGACCCCTCCCTAACC  
*E. neucratoides* AGGCTTAARCCAACTCAACTACGAAAAATCCTCGTACTCATCAATTGACACCTTGGTGAATAGCCCTAATCCTCCATTGACCCCTCCCTAACC  
*R. australis* GGGCTTAARCCAACTCAACTACGAAAAATCCTGCGTACTCATCTATTGACACCTCGGGTGAATAGCCTTAGTCATTCAATTTTTCCCATCCCTCGACT  
*R. albescens* AGGCTTAARCCAACTCAACTACGAAAAATCCTGCGTACTCATCAATTGACACCTTGGTGAATAGCCTTAGTCATTCAATTTTTCCCATCCCTCGACT  
*R. remora* GGGCTTAARCCAACTCAACTACGAAAAATCCTGCGTACTCATCTATTGACACCTTGGTGAATAGCCTTAGTCATTCAATTTTTCCCATCCCTCGACT  
*R. osteochir* CGGCTTAARCCAACTCAACTACGAAAGATCTTGCCTACTCATCCATGACACCTCGGGTGAATAGCCTTAGTCATTCAATTTTTCCCATCCCTCGACT  
*R. brachyptera* CGGCTTAARCCAACTCAACTACGAAAAATCCTGCGTACTCATCAATTGACACCTTGGTGAATAGCCTTAGTCATTCAATTTTTCCCATCCCTCGACT

610 620 630 640 650 660 670 680 690 700  
*P. saltatrix* CTACTCACCCTTCTCACCTATTTTGTATGACATTTTCARACTTTCTGGTATTTAARCTCAATAAGCAACAAATATCAACACACTTGCACCTCCTGGG  
*N. pectoralis* ATCTTACTTTTGCATATATATTTATATACACTTCCATCACTTCCCTGTATTTAARCTGACAAATCAACAAATGTCAACTCCCTAGCTACCTCCTGAA  
*C. armatus* TTATTARCCCTTCTACTACTTCAATATACCTTTTCARACTTCCCTGTATTTAARCTCAATAAGCAACAAATATCAACACACTTGCACCTCCTGGG  
*R. canadum* ATAAATACCCCTTGAAATTTACATCGCCATACACTTTCARACTTCCCTGTATTTAARCTGACAAAGCAACAAATATCAACACACTTGCACCTCCTGAA  
*C. hippurus* ATGATACACTAGTTATTTACATCATTATACACTCATCTGTTTATCTTAACTGACAAAGCAACAAATATCAACACACTTGCACCTCCTGAA  
*C. equiselis* ATGATACACTCATCTTTATATGCTGATGACACTCATCTGTTTATCTTAACTGACAAAGTAAACCAAGTAACTGCTGACTACTCCTGAA  
*P. lineatus* CTGCTTGCCTATTARCTACCTTATCATGACTTTCTCATCTTCTAGTGTAAARCTCAATAAGCAACAAATATCAACACACTTGCACCTCCTGAA  
*E. naucrates* CTACTTGCCTATTARCTATCTTATATACCCCTCTCATCTTCTAGTGTAAARCTCAATAAGCAACAAATATCAACACACTTGCACCTCCTGAA  
*E. neucratoides* CTACTTGCCTATTARCTATCTTATATACCCCTCTCATCTTCTAGTGTAAARCTCAATAAGCAACAAATATCAACACACTTGCACCTCCTGAA  
*R. australis* ATACTTGCCTTAACTTATCTTATCATACCTTCTCAACTTCCCTGTATTTAARCTCAATAAGCAACAAATATCAACACACTTGCACCTCCTGAA  
*R. albescens* CTCTGCTTGTAACTTATCTCATCATACCTTCTCAACTTCCCTGTATTTAARCTCAATAAGCAACAAATATCAACACACTTGCACCTCCTGAA  
*R. remora* ATCTGCTTGTAACTTATCTCATCATACCTTCTCAACTTCCCTGTATTTAARCTCAATAAGCAACAAATATCAACACACTTGCACCTCCTGAA  
*R. osteochir* CTACTTGCCTCCTAATCTTATCATACCTTCTCAACTTCCCTGTATTTAARCTCAATAAGCAACAAATATCAACACACTTGCACCTCCTGAA  
*R. brachyptera* CTCTTGCCTGTTAACTTACCTTATATACACTTTCARACTTCCCTGTGTTAARCTCAATAAGCAACAAATATCAACACACTTGCACCTCCTGAA

710 720 730 740 750 760 770 780 790 800  
*P. saltatrix* CAAAAGCCCTGCGCTTATATCCCTTACCCCTTATCTCCTCTCACTAGGAGGCCCTCCCCCGCTAACAGGCTTCAATGCCAAATGACTAATCTGCA  
*N. pectoralis* CAAAARACCCCGCCCTTACATCCCTTACCCCTTATCTACTCTCATAGGAGGCCCTCCCCCGCTTACAGGCTTCAATGCCAAATGACTAATCTGCA  
*C. armatus* CTAARACCCCGGTTATACCTCCCTGCGCCCTGATTTTACTTCCCTGAGGAGGCCCTCCCCCGCTTACAGGCTTCAATGCCAAATGACTAATCTGCA  
*R. canadum* CAAATACCTATTTTAGCAGCATTACGCCCTTAGTGTTACTGTCCTAGGAGGCCCTCCCCCGCTTACAGGCTTCTCCCTAATGATTTATCTCCA  
*C. hippurus* TTAARACCCCACTTACATACCTTATGCCCCCTTAGTTTATCTCATAGGAGGCCCTCCCCCGCTTACAGGCTTCAATGCCAAATGACTAATCTGCA  
*C. equiselis* TGAARACCCCTACGCTTACCTTATACCTTTGCTGTTCTCATAGGAGGCCCTCCCCCGCTTACAGGCTTCAATGCCAAATGACTAATCTGCA  
*P. lineatus* CAAAARACCCCTTATACCCACTTACACCTTGTCTTATATCATAGGAGGCCCTCCCCCGCTTACAGGCTTATACCAAAATGGTTATTTTACA  
*E. naucrates* CTAARACCCCACTTATACCCACTTACACCTTACTCTTACTATCACTAGGAGGCCCTCCCTCCTTACAGGATTTATACCAAAATGGTTATTTTACA  
*E. neucratoides* CTAARACCCCACTTATACCCACTTACACCTTACTCTTACTATCACTAGGAGGCCCTCCCTCCTTACAGGATTTATACCAAAATGGTTATTTTACA  
*R. australis* CAAAARACCCCTTATTTACTGCTTACCCCTTCTTCTTACTCCTGCGGCCCTCCCTCCTTACAGGATTTATACCAAAATGGTTATTTTACA  
*R. albescens* CAAAARACCCCTTATTTACTGCTTACCCCTTCTTCTTACTCCTGCGGCCCTCCCTCCTTACAGGATTTATACCAAAATGGTTATTTTACA  
*R. remora* CAAAARACCCCTTATTTACTGCTTACCCCTTCTTCTTACTCCTGCGGCCCTCCCTCCTTACAGGATTTATACCAAAATGGTTATTTTACA  
*R. osteochir* CAAAARACCCCTTATTTACTGCTTACCCCTTCTTCTTACTCCTGCGGCCCTCCCCCGCTTACAGGATTTATACCAAAATGGTTATTTTACA  
*R. brachyptera* CAAAARACCCCTTATTTACTGCTTACCCCTTCTTCTTACTCCTGCGGCCCTCCCCCGCTTACAGGATTTATACCAAAATGGTTATTTTACA

810 820 830 840 850 860 870 880 890 900  
*P. saltatrix* AGAATTAARACAGAGACTCGCCCTTAGCCACTTAGCCGCTTACAGGCCCTTCTAGCCCTTACTTCTACCTACGATTTGCTTACGCATAGCC  
*N. pectoralis* AGAATTAARACAGAGACTCGCCCTTAGCCACTTAGCCGCTTACAGGCCCTTCTAGCCCTTACTTCTACCTACGATTTGCTTACGCATAGCC  
*C. armatus* AGAGCTCACTAARACAGACTCTCCAGTACTAGCCACTTAGCCGCTTACAGGCCCTTACTAAGCCCTACTTACTAGCCCTACTGCTTCCGCTCCTAGCARTAGCC  
*R. canadum* TGAACTAARACAGAAATCTACTTTTATAGCCCTTATAGCGTTTACAGGCCCTTACTAGCCCTTACTTCTAGCTACGACTAGCATATGCTATACCA  
*C. hippurus* TGAACTAARACAGAAATCTACTTTTATAGCCCTTATAGCGTTTACAGGCCCTTACTAAGCCCTACTTACTAGCCCTACTGCTTCTAGCTTGTACTTTAGCTACGACTATCTAGCCTGACCA  
*C. equiselis* TGAGCTCACTAARACAGACTTCCGCCCCCTAGCCACTTACTGACAGCCTACTGCCCCCTTACTAGCCCTTACTTCTAGCTTGTACTTTAGCTACGACTATCTAGCCTGACCA  
*P. lineatus* AGAGCTTACAAAGCAAACTCCCACTTATGGCAAGCTTACCAGCCTACTGCCCCCTTACTAGCCCTTACTTCTAGCTTGTACTTTATCTCCGCTCCTACTCCATACCC  
*E. naucrates* AGAGCTTACAAAGCAAACTCCCACTTATAGCAACATTTACTGCACTACTGCCCCCTTACTAGCCCTTACTTCTAGCTTGTACTTTATCTCCGCTCCTACTCCATAGCC  
*E. neucratoides* AGAGCTTACAAAGCAAACTCCCACTTATAGCAACATTTGCTGCACTACTGCCCCCTTACTAGCCCTTACTTCTAGCTTGTACTTTATCTCCGCTCCTACTCTATGACC  
*R. australis* AGAATTAARACAGAGACTCCCCCTCATTTGCAACACTACTGCCCCCTACTGCACTACTTACTAGCCCTTACTTCTAGCTTGTACTTTTACTCCGCTTCTACTCCATAGCC  
*R. albescens* AGAGCTTACAAAGCAAACTCCCCCTCATTTGCAACACTACTGCCCCCTACTGCACTACTTACTAGCCCTTACTTCTAGCTTGTACTTTTACTCCGCTTCTACTCCATAGCC  
*R. remora* AGAATTAARACAGAAATCTTTCCGCTTAGCAACATTTACCAGCCTTACCAGCCTTCTAGCCCTTACTTCTAGCTTGTACTTTTACTCCGCTTCTACTCCATAGCC  
*R. osteochir* AGAATTAARACAGAGACTCCCTTACTAGCGAATTTACTGCCCCCTTACTGCTTCTACTGCTTCTACTAGCCCTGATTTTACTCCGCTTCTACTCCATAGCC  
*R. brachyptera* AGAGCTTACAAAGCAAACTCCCCCTTAGCCACTTACTGCACTACTGCCCCCTTACTAGCCCTTACTTCTAGCTTGTACTTTTACTCCGCTTCTACTCCATAGCC

910 920 930 940 950 960 970 980 990 1000  
*P. saltatrix* CTAACCATATCCCAATACCTCCCTGGTATCACTCATGAGCTTCCATCCACACACAGCTTCTCTGGGCGGTGTCGCTGGGCTACCTTA  
*N. pectoralis* CTAACCATATCCCAATACCTCAGCGCAACACTCTCCGCGCTTCCCTACTCTCAACTTCCCTCCCACTAGCCATTTCAACCAACAGCTCAACTCT  
*C. armatus* CTAACCATATTTCCAAACACCTAATAGGAGACAGCCCTGAGCCTTCCATACCCCTCACTTAACTCCCACTAGCCATCTCAACTCTGCTACTATTC  
*R. canadum* CTAACCTCCTTCCCAACACCCACAGGTTGACACCTTGGAGATTTCCCTTAACTCAACACTCACTCCCAATCGCCATTTATCATGGTCTCAATCT  
*C. hippurus* CTAACCTCCTTCCCAATACTATTACTAGCAGCCTACTGAGGCTTCTTAACTCAACAGCCTTCTTCCCTGTCATATTTATAGTGGGCAACTTC  
*C. equiselis* CTTACGCTTTCCCTAATACTGTGACCCAGCAGGCCCTACTGCGGATTTCTGCTTAACTCAACAGCCTTCTTCCCTTTCAGTGTATAGTGGGCACTATCT  
*P. lineatus* CTCACCATGTTCCCAATATCTAATGGTACACCCCTGAGGATTTCTTAACTCAACAGCCTTCTTAACTCAACAGCCTTCCCTCCTAGCCCTCAGATCTGATAGCCATTG  
*E. naucrates* CTTACCATATTTCCAAATACCTAATAGGACTACTCCATGAGGCTTCTTAACTCAACAGCCTTCCCTCCTAGCCCTCAGATCTGATAGCCATTG  
*E. neucratoides* CTCACATATTTCCAAATATCTAATGGGCAACCTCCATGAGCCTTCTTAACTCAACAGCCTTCCCTCCTAGCCCTCAGATCTGATAGCCATTG  
*R. australis* CTCACCATGTTCCCAATACCTAATGGGCAACCTGCCCCCTGAGGCTTCTTAACTCAACAGCCTTCCCTCCTAGCCCTCAGATCTGATAGCCATTG  
*R. albescens* CTCACCTCCTTCCCAATACCTAATGGGCAACCTGCCCCCTGAGGCTTCTTAACTCAACAGCCTTCCCTCCTAGCCCTCAGATCTGATAGCCATTG  
*R. remora* CTCACATGTTTCCAAACACTTAGCTGGCAGCAGCCCTGAGGCTTCTTAACTCAACAGCCTTCCCTCCTAGCCCTCAGATCTGATAGCCATTG  
*R. osteochir* CTCACCATGTTCCCAACACTTAACTGGGCAACCTGAGGCTTCTTAACTCAACAGCCTTCCCTCCTAGCCCTCAGATCTGATAGCCATTG  
*R. brachyptera* CTTACTATGTTCCCAATATTTGAGCGGACTTCCCGGAGCCTTCTTAACTCAACAGCCTTCCCTCCTAGCCCTCAGATCTGATAGCCATTG

Appendix 9. (continued).

	1010	1020	1030	1040
<i>P._saltatrix</i>	GCCTCCTACCCCTACCCCAGCCATTACGGCCCTTTTGACCCCTCTAA			
<i>N._pectoralis</i>	CCCTACTACCCCTACCCCTGCCGACGCTCCCTCCTCGCCCTCTAA			
<i>C._armatus</i>	TCCTTCTCCCTTACCCCTGCTATCACAGCCTTACTTACCCCTAG			
<i>R._canadum</i>	CACCTCTACCAATCGCTCCGACTCTGATGACCCCTCTCCATTCTTGA			
<i>C._hippurus</i>	TACTCCTCCTGTTACACCAGCCATCGTTGCCCTTTTCTATCTCTAG			
<i>C._equiselis</i>	TTCTTCTCCCTATCACACCAGCCATCGTTGCCCTTTTCTACCTCTAG			
<i>P._lineatus</i>	CCCTACTCCCCCTTGCCCCGCTATAATAGCCCTCCTTACCCCTTAA			
<i>E._hauchrates</i>	CCCTACTCCCCCTTGCCCCGCTATAATAGCACTCCTTACCCCTTAA			
<i>E._neuchrotoides</i>	CCCTACTCCCCCTTGCCCCGCTATAATAGCACTCCTTACCCCTTAA			
<i>R._australis</i>	TATTGCTTCCTCTAGCACCCGCTGTAATAGCCCTATTATCCCCATAA			
<i>R._albescens</i>	TGCTCCTTCCCTTACCCCGCTGCATAGCACTCTTGAGCCCTTAA			
<i>R._remora</i>	TTCTACTCCCCCTGCCCCGCTGCATAGCCCTTTTAGCCCTTAA			
<i>R._osteocheir</i>	TACTTCTCCCGCTAGCCCCGCTGCCATAGCCCTTTAGGCCCTTAA			
<i>R._brachyptera</i>	TACTCCTCCCCCTGGCTCCCGCGTAAATAGCCCTCTTGACCCGTTAA			

Appendix 10. Aligned nuclear ITS-1 DNA sequence data collected from echeneoid specimens analyzed in the present study.

	10	20	30	40	50	60	70	80	90	100
<i>P._saltatrix</i>	ATTACATTAGTTC	CGCAGCTAGCT	GCGTTCTT	CATCGACGCAC	GAGCCGAGT	GATCCACC	GCTAAGAGTT	GTGCACAC	CTTTTT	TTTTGTT
<i>N._pectoralis</i>	ATTACATTAGTTC	CGCAGCTAGCT	GCGTTCTT	CATCGACGCAC	GAGCCGAGT	GATCCACC	GCTAAGAGTT	GTGCACAC	CTTTTT	TTTTGTT
<i>R._canadum</i>	ATTACATTAGTTC	CGCAGCTAGCT	GCGTTCTT	CATCGACGCAC	GAGCCGAGT	GATCCACC	GCTAAGAGTT	GTGCACAC	CTTTTT	TTTTGTT
<i>C._hippurus</i>	ATTACATTAGTTC	CGCAGCTAGCT	GCGTTCTT	CATCGACGCAC	GAGCCGAGT	GATCCACC	GCTAAGAGTT	GTGCACAC	CTTTTT	TTTTGTT
<i>C._equisetis</i>	ATTACATTAGTTC	CGCAGCTAGCT	GCGTTCTT	CATCGACGCAC	GAGCCGAGT	GATCCACC	GCTAAGAGTT	GTGCACAC	CTTTTT	TTTTGTT
<i>P._lineatus</i>	ATTACATTAGTTC	CGCAGCTAGCT	GCGTTCTT	CATCGACGCAC	GAGCCGAGT	GATCCACC	GCTAAGAGTT	GTGCACAC	CTTTTT	TTTTGTT
<i>E._naucratus</i>	ATTACATTAGTTC	CGCAGCTAGCT	GCGTTCTT	CATCGACGCAC	GAGCCGAGT	GATCCACC	GCTAAGAGTT	GTGCACAC	CTTTTT	TTTTGTT
<i>E._neucratoides</i>	ATTACATTAGTTC	CGCAGCTAGCT	GCGTTCTT	CATCGACGCAC	GAGCCGAGT	GATCCACC	GCTAAGAGTT	GTGCACAC	CTTTTT	TTTTGTT
<i>R._australis</i>	ATTACATTAGTTC	CGCAGCTAGCT	GCGTTCTT	CATCGACGCAC	GAGCCGAGT	GATCCACC	GCTAAGAGTT	GTGCACAC	CTTTTT	TTTTGTT
<i>R._albescens</i>	ATTACATTAGTTC	CGCAGCTAGCT	GCGTTCTT	CATCGACGCAC	GAGCCGAGT	GATCCACC	GCTAAGAGTT	GTGCACAC	CTTTTT	TTTTGTT
<i>R._remora</i>	ATTACATTAGTTC	CGCAGCTAGCT	GCGTTCTT	CATCGACGCAC	GAGCCGAGT	GATCCACC	GCTAAGAGTT	GTGCACAC	CTTTTT	TTTTGTT
<i>R._osteoichir</i>	ATTACATTAGTTC	CGCAGCTAGCT	GCGTTCTT	CATCGACGCAC	GAGCCGAGT	GATCCACC	GCTAAGAGTT	GTGCACAC	CTTTTT	TTTTGTT
<i>R._brachyptera</i>	ATTACATTAGTTC	CGCAGCTAGCT	GCGTTCTT	CATCGACGCAC	GAGCCGAGT	GATCCACC	GCTAAGAGTT	GTGCACAC	CTTTTT	TTTTGTT

	110	120	130	140	150	160	170	180	190	200
<i>P._saltatrix</i>	AGCTTTCTCCGG	TTTTTACAC	CGAARAGGG	GTTCG	CAAAAGT	TCCAA	AGACAR	ACGGG	TTTTG	CGAR
<i>N._pectoralis</i>	TCCATTTTTC	TTTTCC	GGGAA	AAAAA	CAAGAG	GGCCAC	AGTTC	CGAGAC	AGGART	TGG
<i>R._canadum</i>	TTGGT	TTCC	GGCCAAA	A	GAA	AA	G	GTT	CT	GGAG
<i>C._hippurus</i>	TACGCT	TTCT	GGCCAA	CTTC	AA	CGA	GA	G	TTT	CC
<i>C._equisetis</i>	GTTTCGTCG	TTCC	GGCCAA	AGTC	A	CGA	GA	G	TTT	CC
<i>P._lineatus</i>	CATCCGTTTC	AGTTC	GGCCAA	ACCT		A	GAT	TGTG	C	TTT
<i>E._naucratus</i>	TCCGTTTC	GGTTC	GGCCAA	ACCT		A	GA	G	G	TTT
<i>E._neucratoides</i>	TCCGTTTC	GGTTC	GGCCAA	ACCT		A	GA	G	G	TTT
<i>R._australis</i>	T	CTTT	TCCC	GGCCAA	GCCTT	TTCTTC	CGAG	GTGTT	TGG	TTT
<i>R._albescens</i>	T	C	TTT	TCCC	GGCCAA	GCCTT	CGAG	GTGTT	TGG	TTT
<i>R._remora</i>	T	C	TTT	TCCC	GGCCAA	GCCTT	CGAG	GTGTT	TGG	TTT
<i>R._osteoichir</i>	T	CTTT	TCCC	GGCCAA	GCCTT	TTCTTC	CGAG	GTGTT	TGG	TTT
<i>R._brachyptera</i>	T	CTTT	TCCC	GGCCAA	GCCTT	TTCTTC	CGAG	GTGTT	TGG	TTT

	210	220	230	240	250	260	270	280	290	300
<i>P._saltatrix</i>	A	CGAAC	CCGGC	GGCGCT	CCCGCT	CGCC	CCG	CCG	GGG	GGG
<i>N._pectoralis</i>	A	CGAAC	CCGGC	GGCGCT	CCCGCT	CGCC	CCG	CCG	GGG	GGG
<i>R._canadum</i>	A	CGAAC	CCGGC	GGCGCT	CCCGCT	CGCC	CCG	CCG	GGG	GGG
<i>C._hippurus</i>	A	CGAAC	CCGGC	GGCGCT	CCCGCT	CGCC	CCG	CCG	GGG	GGG
<i>C._equisetis</i>	A	CGAAC	CCGGC	GGCGCT	CCCGCT	CGCC	CCG	CCG	GGG	GGG
<i>P._lineatus</i>	A	CGAAC	CCGGC	GGCGCT	CCCGCT	CGCC	CCG	CCG	GGG	GGG
<i>E._naucratus</i>	A	CGAAC	CCGGC	GGCGCT	CCCGCT	CGCC	CCG	CCG	GGG	GGG
<i>E._neucratoides</i>	A	CGAAC	CCGGC	GGCGCT	CCCGCT	CGCC	CCG	CCG	GGG	GGG
<i>R._australis</i>	A	CGAAC	CCGGC	GGCGCT	CCCGCT	CGCC	CCG	CCG	GGG	GGG
<i>R._albescens</i>	A	CGAAC	CCGGC	GGCGCT	CCCGCT	CGCC	CCG	CCG	GGG	GGG
<i>R._remora</i>	A	CGAAC	CCGGC	GGCGCT	CCCGCT	CGCC	CCG	CCG	GGG	GGG
<i>R._osteoichir</i>	A	CGAAC	CCGGC	GGCGCT	CCCGCT	CGCC	CCG	CCG	GGG	GGG
<i>R._brachyptera</i>	A	CGAAC	CCGGC	GGCGCT	CCCGCT	CGCC	CCG	CCG	GGG	GGG

	310	320	330	340	350	360	370	380	390	400
<i>P._saltatrix</i>	CCCCG	CCTCC	CGA	GGAG	GGAG	GGAG	GGAG	GGAG	GGAG	GGAG
<i>N._pectoralis</i>	CCCCG	CCTCC	CGA	GGAG	GGAG	GGAG	GGAG	GGAG	GGAG	GGAG
<i>R._canadum</i>	CCCCG	CCTCC	CGA	GGAG	GGAG	GGAG	GGAG	GGAG	GGAG	GGAG
<i>C._hippurus</i>	CCCCG	CCTCC	CGA	GGAG	GGAG	GGAG	GGAG	GGAG	GGAG	GGAG
<i>C._equisetis</i>	CCCCG	CCTCC	CGA	GGAG	GGAG	GGAG	GGAG	GGAG	GGAG	GGAG
<i>P._lineatus</i>	CCCCG	CCTCC	CGA	GGAG	GGAG	GGAG	GGAG	GGAG	GGAG	GGAG
<i>E._naucratus</i>	CCCCG	CCTCC	CGA	GGAG	GGAG	GGAG	GGAG	GGAG	GGAG	GGAG
<i>E._neucratoides</i>	CCCCG	CCTCC	CGA	GGAG	GGAG	GGAG	GGAG	GGAG	GGAG	GGAG
<i>R._australis</i>	CCCCG	CCTCC	CGA	GGAG	GGAG	GGAG	GGAG	GGAG	GGAG	GGAG
<i>R._albescens</i>	CCCCG	CCTCC	CGA	GGAG	GGAG	GGAG	GGAG	GGAG	GGAG	GGAG
<i>R._remora</i>	CCCCG	CCTCC	CGA	GGAG	GGAG	GGAG	GGAG	GGAG	GGAG	GGAG
<i>R._osteoichir</i>	CCCCG	CCTCC	CGA	GGAG	GGAG	GGAG	GGAG	GGAG	GGAG	GGAG
<i>R._brachyptera</i>	CCCCG	CCTCC	CGA	GGAG	GGAG	GGAG	GGAG	GGAG	GGAG	GGAG

	410	420	430	440	450	460	470	480	490	500
<i>P._saltatrix</i>	TCCGA	TTG	G	CGG	TCC	AGGC	CCGG	CG	CAC	CGG
<i>N._pectoralis</i>	TCCGA	TTG	G	CGG	TCC	AGGC	CCGG	CG	CAC	CGG
<i>R._canadum</i>	TCCGA	TTG	G	CGG	TCC	AGGC	CCGG	CG	CAC	CGG
<i>C._hippurus</i>	TCCGA	TTG	G	CGG	TCC	AGGC	CCGG	CG	CAC	CGG
<i>C._equisetis</i>	TCCGA	TTG	G	CGG	TCC	AGGC	CCGG	CG	CAC	CGG
<i>P._lineatus</i>	TCCGA	TTG	G	CGG	TCC	AGGC	CCGG	CG	CAC	CGG
<i>E._naucratus</i>	TCCGA	TTG	G	CGG	TCC	AGGC	CCGG	CG	CAC	CGG
<i>E._neucratoides</i>	TCCGA	TTG	G	CGG	TCC	AGGC	CCGG	CG	CAC	CGG
<i>R._australis</i>	TCCGA	TTG	G	CGG	TCC	AGGC	CCGG	CG	CAC	CGG
<i>R._albescens</i>	TCCGA	TTG	G	CGG	TCC	AGGC	CCGG	CG	CAC	CGG
<i>R._remora</i>	TCCGA	TTG	G	CGG	TCC	AGGC	CCGG	CG	CAC	CGG
<i>R._osteoichir</i>	TCCGA	TTG	G	CGG	TCC	AGGC	CCGG	CG	CAC	CGG
<i>R._brachyptera</i>	TCCGA	TTG	G	CGG	TCC	AGGC	CCGG	CG	CAC	CGG



Appendix 10. (continued).



Appendix 10. (continued).

1010  
P.\_saltatrix GATAGTCAG  
N.\_pectoralis GATAGTCAG  
R.\_canadum GATAGTCAG  
C.\_hippurus GATAGTCAG  
C.\_equisetis GATAGTCAG  
P.\_lineatus GATAGTCAG  
E.\_naucratus GATAGTCAG  
E.\_neucratoides GATAGTCAG  
R.\_australis GATAGTCAG  
R.\_albescens GATAGTCAG  
R.\_remora GATAGTCAG  
R.\_ostechir GATAGTCAG  
R.\_brachyptera GATAGTCAG

Appendix 11. Aligned mitochondrial control region DNA sequence data collected from marlinsucker specimens analyzed in the present study.



Appendix 11. (continued).





Appendix 11. (continued).



Appendix 11. (continued).



Appendix 11. (continued).



Appendix 11. (continued).





Appendix 11. (continued).



Appendix 11. (continued).



Appendix 11. (continued).



Appendix 11. (continued).





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## VITA

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