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Multi-Scale Phylogenomics of Gadiformes With Emphasis on Hakes (Merluccius, Merlucciidae)

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MULTI-SCALE PHYLOGENOMICS OF GADIFORMES WITH EMPHASIS ON HAKES (*MERLUCCIUS*, MERLUCCIIDAE)

A Dissertation

Presented to

The Faculty of the School of Marine Science

The College of William and Mary in Virginia

In Partial Fulfillment

of the Requirements for the Degree of

Doctor of Philosophy

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by

Adela Y. Roa-Varón

August 2018

APPROVAL PAGE

This Multi-scale Phylogenomics of Gadiformes with Emphasis on Hakes (*Merluccius*, Merlucciidae)

is submitted in partial fulfillment of

the requirements for the degree of

Doctor of Philosophy

Adela Y. Roa-Varón

Approved by the Committee, August, 2018

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Bruce Collette, Ph.D. Washington, DC. USA

This Ph.D. is dedicated to

Naikoa & Itziar

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ABSTRACT PAGE

Gadiformes include some of the most important commercially harvested fishes in the world (e.g. cods, hakes, and grenadiers). Currently, different authors recognize anywhere between 11 and 14 families, approximately 84 genera, and over 600 species. The monophyly of the order has been supported by both morphological and molecular data, yet the relationships among families and subfamilies remain poorly understood and interpreting phylogenetic patterns to date has been difficult. My dissertation research on multi-scale phylogenetics of Gadiformes with emphasis on hakes (*Merluccius*, Merlucciidae) has three primary objectives: (1) to improve the understanding of the phylogenetic relationships among families of Gadiformes (Teleostei); (2) to analyze the phylogenetic relationships within the family Merlucciidae (*Merluccius*) and (3) to explore the evolution of the caudal skeleton using molecular, morphological and ecological data. In chapter two, a gene capture approach was used, targeting coding DNA sequences (CDS) from single-copy protein-coding genes to study the higher-level relationships (i.e., above the genus level) of Gadiformes. Matrices of 14,208 loci (~2.8 M bp) were generated from a total of 57 species representing all recognized Gadiformes families and subfamilies. Species tree and concatenated maximum likelihood analyses resolved a highly congruent and well-supported phylogeny at both shallow and deep levels that contributes towards stabilizing higher-level Gadiformes classification. In accordance with these results a revised classification of the group is provided in chapter two.

In Chapter three the systematics, evolution, and taxonomy of a particularly problematic family, the hakes (*Merluccius*, Merlucciidae), using genomic data from 13,771 loci and 74 taxa were reassessed. This study resolved the controversy surrounding the taxonomic extent of Merlucciidae and the phylogenetic placement of the genera historically included within the family, based on complete taxonomic sampling at the family and subfamily levels among gadiforms. Additionally, the phylogenetic analysis confirmed an early separation of two lineages of *Merluccius*, the Old World and the New World clades. The Old World clade includes five well defined species and the New World clade includes three species and two complexes of species suggesting the presence of only one species in the eastern Pacific, as well as, one species in the Southern Ocean (Patagonia – New Zealand).

Chapter four provided for the first time a description of the caudal skeleton for all the families among Gadiformes and explores the character evolution of 11 characters (morphological and ecological) mapped on a taxonomically robust phylogenetic hypothesis proposed in chapter two. The ancestral state reconstruction analyses suggest that the ancestral condition among gadiforms had a caudal fin and a pelagic origin. Its loss arose at least two times within Gadiformes resulting in two main phenotypes - the tailed and the tailless fishes, neither of which form a monophyletic grouping.

This study is the most comprehensive phylogenomic study of Gadiformes to date. This dissertation used a novel molecular technique coupled with morphological and ecological data that resulted in a better understanding of the evolution of commercially and ecological valuable fishes, which is necessary for effective fisheries management and the preservation of reproductive and genetic diversity. Further steps, including morphological data of extant and extinct taxa, is essential to fully understand macroevolutionary patterns and processes in phenotypic evolution and lineage diversification of gadiforms.

MULTI-SCALE PHYLOGENOMICS OF GADIFORMES WITH EMPHASIS ON HAKES (*MERLUCCIUS*, MERLUCCIIDAE)

CHAPTER 1

Introduction

The order Gadiformes – the cods, hakes, grenadiers, and their allies – includes some of the most commercially important fishes in the world, and collectively forms about 18% of all marine fishes caught commercially (FAO 2004). Included in this order is the Atlantic cod, *Gadus morhua*, which supported a massive fishery in the north Atlantic that crashed in the mid-1990s; the species was red listed by several conservation groups but it has yet to recover (e.g. HELCOM 2013). Gadiformes inhabit cool waters in every ocean of the world (Nelson 2006), although they exploit a broad range of primarily marine habitats. In high latitudes, they occur throughout the water column, from deep-sea benthic habitats to near-shore coastal waters. In tropical seas, they are found mainly in deep waters. There is only one exclusively freshwater species, the Holarctic burbot (*Lota lota*). Despite their great commercial and ecological importance, knowledge of the evolutionary relationships of Gadiformes is poor.

Gadiformes have been characterized as a monophyletic group within the supra-ordinal taxon Paracanthopterygii (Greenwood et al. 1966). The authors included in this superorder the lophiiforms, ophidiiforms (grouped together with gadiforms), zoarciforms, gobiesociforms, batrachoidiforms, percopsiforms, and gadiforms fishes. In 1969, Rosen and Patterson added the Polymixiiformes, as a possible sister group to all other paracanthopterygians. Twenty years later, the same authors redefined the group and inferred interrelationships among the orders Percopsiformes, Ophidiiformes, Batrachoidiformes, Lophiiformes, and Gadiformes (Patterson & Rosen 1989). They suggested that Gadiformes are a monophyletic group and proposed a group Pediculati, composed by Batrachoidiformes and Lophiiformes, as a sister group of Gadiformes. However, they stated that "few tenuous characters define the Paracanthopterygii" and that it is possible that Percopsiformes and Ophidiiformes are non-monophyletic. Lauder & Liem (1983) proposed Percopsiformes as the sister group of Gadiformes, and included also

Batrachoidiformes, Lophiiformes, and Gobiesociformes within Paracanthopterygii. Markle (1989) state that Batrachoidiformes should be the sister group of Gadiformes and Nolf & Steurbaut (1989) proposed Ophidiiformes based on otolith morphology.

Molecular analyses of higher teleosts have shown the Paracanthopterygii as a polyphyletic group. For example, Wiley et al. (2000) found Zeidae to be the sister group of Gadiformes. In addition, Ophidiformes and Batrachoidiformes, two of the other members of paracanthopterygians were recovered as polyphyletic groupings within Percomorpha. Miya et al. (2001, 2003, 2005) also found strong evidence indicating that Gadiformes and zeioids are sister taxa in a mitogenomic exploration of higher teleostean phylogenies focused on the phylogenetic position of Batrachoidiformes. Some members of the Paracanthopterygii (Ophidiiformes, Lophiiformes, and Batrachoidiformes) were recovered as highly advanced percomorphs. Based on their findings, Miya et al. (2003) suggested that the name "Paracanthopterygii" should be restricted to a clade comprising (((gadiforms + zeioids) percopsiforms) polymixiiforms)) and two years later the same authors suggest ((gadiforms + zeioids) + (percopsiforms + polymixiiforms)) (Miya et al. 2005). Holcroft (2004), while testing alternative hypotheses of the sister group of Tetraodontiforms using the nuclear RAG1 gene (1497 bp) found strong support (97%) for the clade (Gadiformes + Percopsiformes) included inside a clade weakly supported ((Gadiformes + Percopsiformes) Zeioids)). In 2007, Miya et al. recovered the lampriform *Stylephorus chordatus* as sister to the Gadiformes and it was subsequently placed in a new order, Stylephoriformes. Later studies corroborated the sister relationship between Gadiformes and *Stylephorus* (Betancur et al. 2013; Grande et al. 2013; Near et al. 2013).

Within the order different authors recognize between 11 and 14 families, about 75 genera, and more than 500 species (Roa-Varón & Ortí 2009: table 1, fig. 1). Two classifications

of Gadiformes have been published recently based on morphology (Endo 2002) and molecular data (Roa-Varón & Ortí 2009), although many issues remain unresolved. Up to six morphological characters have been identified supporting Gadiformes although none are unambiguous or unreversed (Cohen 1984, Patterson & Rosen 1989, Murray & Wilson 1999, Endo 2002, Wiley & Johnson 2010). Roa-Varón & Ortí (2009) analyzed one nuclear and two mitochondrial DNA loci from 117 taxa representing virtually all families and subfamilies in the order. The monophyly of the order and most families was supported, although relationships among families were poorly resolved. Analysis of more taxa and more extensive molecular and morphological data sampling will undoubtedly help to clarify the relationships among these important fishes, as it has for other groups of organisms (e.g., Rokas et al. 2003, Prasad et al. 2008).

The present study seeks to expand on past studies of Gadiformes by combining greater taxonomic sampling with more extensive molecular character sampling, and by incorporating next generation sequencing (NGS) approaches to molecular data acquisition and analysis, with the overall goal of improving our understanding of the evolution of the order. Using the resulting phylogenetic hypothesis of Gadiformes as a framework, the systematics, evolution, and taxonomy of a particularly problematic family, the hakes (*Merluccius*, family Merlucciidae), will be reassessed. Despite the extreme commercial importance of hakes, the taxonomic composition of the genus *Merluccius*, with 16 species considered valid, is not well understood. The putative species of this genus are morphologically similar and have overlapping geographic ranges, and different species are often captured together, hindering effective management of this group. Some merluccids are currently overexploited and are on IUCN's Red List (e.g., European hake *M. merluccius*).

Molecular studies of Merlucciidae have been based on allozymes (Roldan et al. 1999), mtDNA (Quinteiro et al. 2000), both (Grant & Leslie 2001), or mtDNA and nDNA data (Campo et al. 2007, Roa-Varón & Ortí 2009). These studies and previous biogeographic analyses (Kabata & Ho 1981, Inada 1981, Ho 1990) identified an Old World (eastern Atlantic) clade and a New World (western Atlantic, eastern Pacific) clade. However, in the most taxonomically extensive study only a single outgroup was used and the basal nodes were poorly supported (Campo et al. 2007). Additionally, a revised date for the closure of the Isthmus of Panama (15 ma vs 4-3 ma; Coates & Stallard 2013) calls for a re-evaluation of the biogeographic history of putative Atlantic-Pacific species pairs. Vicariance, dispersal, and hybridization all have been invoked to explain the evolution of *Merluccius*. No comprehensive phylogeny, including morphological and molecular data from all species, has yet been conducted, limiting conclusions.

Many aspects of hake biology and management are hindered by the confusing taxonomy of the group and poor understanding of their population structure. Most importantly, this has led to mixed-species in landings data, making species-specific stock assessment and conservation difficult. In order to provide critical information for the conservation biology and management of these fishes, this study seeks to address the unsettled phylogenetic position of the genera that have been included historically within Merlucciidae (e.g., the taxonomic extent of the family) and the phylogenetic relationships among species of *Merluccius* using a more comprehensive phylogenetic data set, both in terms of taxon sampling and nucleotide data, and by using Next Generation Sequencing approaches to molecular data acquisition and analysis.

Chapter four builds on chapter two by using the phylogenetic hypothesis proposed to explore the evolution of the caudal skeleton of the order. The caudal skeleton of Gadiformes is a complex structure that exhibits substantial diversity among the major subgroups of the order,

which range from tailless fishes (e.g., macrouroids) to those with externally symmetrical caudal fins (e.g. cod-like fishes). This morphological diversity is mirrored by the exceptional ecological diversity of Gadiformes, which are distributed from the Arctic to Antarctic oceans, and occupy deep-sea to shallow marine waters, with a single fully freshwater species. Many fish use the tail fin as the main propulsive and steering device (Videler 1993) and its shape is usually adapted to the type of swimming required to optimize survival and fitness in different habitats (Webb 1984, Pavlov and Kasumyan 2002). Correlating morphological, ecological and molecular data for understanding systematic relationships can provide valuable insight into the evolution of gadiforms.

Thus, gadiforms are a very promising group to investigate a variety of questions ranging from the systematics of conflicting taxa at different levels, to the implications of key innovations to adapt from pelagic to hadalpelagic habitats in the speciation process. Nevertheless, the inference of species boundaries and phylogenetic relationships is fundamental for any systematic, ecological or evolutionary study and consequently, a robust phylogenetic framework of the order is a necessary backbone to explore any of the aforementioned questions.

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GREEK-STYLE BAKED COD RECIPE WITH LEMON AND GARLIC

@themediterraneandish

INGREDIENTS
1.5 lb Cod fillet pieces (4-6 pieces)
5 garlic cloves, peeled and minced
1/4 cup chopped fresh parsley leaves

Lemon Juice Mixture

5 tbsp fresh lemon juice
5 tbsp Private Reserve extra virgin olive oil
2 tbsp melted butter

For Coating

1/3 cup all-purpose flour
1 tsp ground coriander
3/4 tsp sweet Spanish paprika
3/4 tsp ground cumin
3/4 tsp salt
1/2 tsp black pepper

PREPARATION

1. Preheat oven to 400 degrees F.
2. Mix lemon juice, olive oil, and melted butter in a shallow bowl. Set aside
3. In another shallow bowl, mix all-purpose flour, spices, salt and pepper. Set next to the lemon juice mixture.
4. Pat fish fillet dry. Dip fish in the lemon juice mixture then dip in the flour mixture. Shake off excess flour.
5. Heat 2 tbsp olive oil in a cast iron skillet over medium-high heat (watch the oil to be sure it is sizzling but not smoking). Add fish and sear on each side to give it some color, but do not fully cook (about a couple minutes on each side) Remove from heat.
6. To the remaining lemon juice mixture, add the minced garlic and mix. Drizzle all over the fish fillets.
7. Bake in the heated oven for until it begins to flake easily with a fork (10 minutes should do it but begin checking earlier). Remove from heat and sprinkle chopped parsley.
8. Serving suggestions: Serve immediately with Jasmine or Basmatic rice and a Mediterranean chickpea salad or a traditional Greek salad.

Prep Time: 10 mins Cook Time: 12 mins Yield: 4

CHAPTER 2

Phylogenomic Insights into the Systematic Interrelationships of Gadiform Fishes (Paracanthopterygii: Gadiformes)

2.1. Introduction

Phylogenetic hypotheses are essential for understanding all aspects of evolutionary biology, including the spatiotemporal patterns of diversification and phenotypic evolution. High-throughput sequencing (HTS) now allows the acquisition of hundreds or even thousands of loci at once. Such advances in phylogenomics have promise to improve phylogenetic reconstruction across a wide range of divergence times and taxa (e.g., Streicher & Wiens 2017, Campbell et al. 2017, Branstetter et al. 2017, Harrington et al. 2016, Gilbert et al. 2015, O'Hara et al. 2014, Faircloth et al. 2013, Li et al. 2013, Hughes et al 2018). A number of studies have identified protein-coding exons in transcriptome sequences and designed probes to capture hundreds or thousands of exons to address population genetic and phylogenetic questions (e.g., Bragg et al. 2016, Teasdale et al. 2016, Huggall et al. 2015, Ilves & Lopez-Fernandez 2014, Li et al. 2013, Song et al. 2017). These advances in genome-scale dataset production and phylogenetic inference represent a unique opportunity to improve our knowledge of the systematics of gadiforms fishes. Gadiformes are a complex, morphologically and ecologically diverse order, which despite their commercial importance and previous morphological and molecular studies still lacks a well-resolved phylogeny.

Gadiformes (cods, hakes, grenadiers, and their relatives) are inhabitants of cool water distributed in all oceans; they inhabit all portions of the water column at high latitudes but are primarily found in deeper layers of tropical seas. Anywhere between 11 and 14 families, 84 genera, and about 613 species are recognized (Svetovidov 1948, Nolf and Steurbaut 1989, Markle 1989, Howes 1989, Endo 2002, Roa-Varón and Ortí 2009, Nelson et al. 2016, Betancur-R et al. 2017). Only the burbot (*Lota lota*) and some populations of the Atlantic tomcod

(*Microgadus tomcod*) are confined to freshwater (Nelson et al. 2016). Gadiformes includes some of the most important commercial fishes in the world (Alaskan pollock, Atlantic cod, Blue whiting, Pacific cod, hake, etc.), accounting for 20.6% of the world's marine fish catch (FAO 2016). The status of some stocks assessed according to FAO (2016) is worrisome. For example, the Alaskan pollock (*Theragra chalcogramma*) is considered fully fished in the North Pacific, the Atlantic cod (*Gadus morhua*) is overfished in the Northwest Atlantic, but fully fished to overfished in the Northeast Atlantic and all hake (*Merluccius* spp. stocks are considered overfished.

The order Gadiformes has been characterized as a monophyletic group within Paracanthopterygii (Greenwood et al. 1966, Gosline 1971, Cohen 1984), although well-defined morphological synapomorphies supporting its monophyly have yet to be established (e.g. Patterson & Rosen 1989, Murray and Wilson 1999, Endo 2002). In their classification of teleostean fishes, Greenwood et al. (1966) included five suborders within Gadiformes: (1) Muraenolepoidei; (2) Gadoidei; (3) Ophidioidei; (4) Zoarcoidei; and (5) Macrouroidei. The modern conceptualization of Gadiformes (i.e., limited to Greenwood et al.'s 1966 Muraenolepoidei, Gadoidei, and Macrouroidei) is a consensus of previous classifications proposed by Nelson (1976). Since that time, there have been several attempts to resolve the relationships of Gadiformes, based primarily on morphological data. The most emblematic effort was in January 1986, when a workshop on Gadiformes systematics (WOGADS) was held at the Natural History Museum of Los Angeles County. None of the participants questioned the monophyly of gadiforms and only three authors attempted broad analyses of the phylogeny of the order: Howes based on cranial myology and arthrology, Markle based on adult morphology,

and Nolf and Steurbaut based on otoliths (Fig. 2.1). These studies produced largely conflicting results, and no consensus regarding the phylogeny and classification emerged (Cohen, 1989). Endo (2002) published the most recent and extensive morphologically based classification of Gadiformes (Fig. 2.1). The synapomorphies of Gadiformes *sensu* Endo (2002) include the presence of a scapular foramen between the scapula and the coracoid, the presence of a single hyomandibular condyle, saccular otoliths characterized by a pince-nez-shape and many taxa with a central collicular crest above the ostium-caudal junction of the crista inferior, levator arcus palatini lying laterally on abductor mandibular, and the absence of a basihyal. Endo (2002) proposed that Gadiformes should be classified into three suborders: Melanonoidei, Macrouroidei and Gadoidei. Melanonoidei was supported by four synapomorphies (Howes 1993: supraoccipital excluded from the margin of the foramen magnum, cranial neuromast pattern and innervation, brain position and morphology, and enlarged pterosphenoid contacting lateral line). Macrouroidei was supported by two synapomorphies (transverse median process of the pelvic girdle and absence of a caudal skeleton) and Gadoidei by only one synapomorphy (presence of X and Y bones in the caudal skeleton). However, Endo (2002) noted that the analysis of more taxa was still necessary and studies of molecular evolution should be developed to clarify the phylogeny of the group.

Several genetic studies have focused on the systematic relationships of subgroups (i.e., families or the phylogenetic affinities of specific taxa) of Gadiformes (e.g., Carr et al., 1999; Roldan et al., 1999; Quintero and Mendez, 2000; Grant and Leslie, 2001; Møller et al., 2002; Teletchea et al., 2006). Roa-Varón & Ortí (2009) published the first molecular phylogeny for Gadiformes as a whole (Fig. 2.1). The study was based on one nuclear and two mitochondrial

DNA (mtDNA) genes from 117 taxa, including all of the recognized families and subfamilies (except the monotypic family Lyconidae). The monophyly of the order and most families was supported although relationships among them were poorly resolved. This study resolved the position of 12 families within Gadiformes in three suborders, Muraenolepidoidei, Macrouroidei, and Gadoidei. Other studies, while not directly addressing the phylogeny of Gadiformes, presented results based on substantial yet incomplete taxon sampling for this order. Betancur-R et al (2017) used 20 protein coding gene sequences for 42 taxa (10 families) and Hughes et al (2018) used 1105 protein coding genes for 26 taxa (10 families) (Fig. 2.1). Recently, Malmstrøm et al. (2016) used low-coverage genome sequencing and assemblies of 66 novel teleostean genomes to demonstrate how the major histocompatibility complex (MHC I and II) gene composition in teleostean fishes has influenced diversification rates in the vertebrate linage (Fig. 2.1). After filtering, the data set included 567 exons including 111 genes (71,418 bp) for 28 gadiform species (11 families). They reported that the loss of MHCII is shared by all Gadiformes, and this loss occurred approximately 105 Ma (million years ago), accompanied by a highly variable MHCI copy number. Despite the large molecular data set, their conclusion of firmly establishing the relationships of gadiform fishes is questionable due to the poor taxonomic sampling, which included only seven of the thirteen families (following Nelson et al., 2016 classification).

There is also a lack of consensus between morphological and molecular data over the precise identity of the Gadiformes sister group. According to morphological studies, the sister group for Gadiformes could be Pediculati, a group comprised of Batrachoidiformes and Lophiiformes (Patterson and Rosen 1989). Lauder and Liem (1983) proposed Percopsiformes as the sister group of Gadiformes, while Markle (1989) proposed Batrachoidiformes and Nolf &

Steurbaut (1989) state that Ophidiiformes could be its sister group based on otolith morphology. Dispute over the taxonomic extent of Paracanthopterygii has increased with analyses of whole mitochondrial genome sequences (Miya et al, 2001, 2003, 2005). These analyses recovered Zeidae as the sister group of Gadiformes and suggested that the name "Paracanthoptherygii" should be retained for a clade comprising Gadiformes, Zeidae, Percopsiformes, and Polymixiiformes. A later study based on the entire mitochondrial genome and a single nuclear gene (RAG1) suggested that the monotypic genus *Stylephorus*, which has been historically included within Lampridiformes, is the sister group of all Gadiformes (Miya et al. 2007). The sister-group relationship between Gadiformes and *Stylephorus* has been corroborated by several molecular studies (Near et al. 2013, Malstrøm et al. 2016, Betancur-R et al. 2017, Hughes et al. 2018), but is poorly supported based on morphological data (Grande et al. 2013). A comprehensive morphological revision of both fossil and extant specimens within the Gadiformes, with reconsideration of character homologies under a phylogenetic framework, is needed to provide a framework for further genetic and evolutionary study of this commercially-important group. As a first step in better understanding the phylogeny of Gadiformes, additional molecular data needs to be analyzed to complement past and future morphological studies.

In this study, the interrelationships of Gadiformes were analyzed using phylogenomic data, species-tree and concatenation methods, with the overall goal of improving our understanding of the evolution of the group. Protein coding DNA sequence data (CDS) from 14,208 loci and a taxonomic dataset including 50 gadiform species were obtained representing all the families and subfamilies. The goals of this study are to confirm or establish: (1) the monophyly of Gadiformes, (2) the status of the major lineages of Gadiformes and (3) the identity and phylogenetic relationships of the order at the family and subfamily levels.

2.2. Materials and Methods

2.2.1. Species Sampling and Molecular Techniques

2.2.1.1. Taxon Sampling

Fifty-seven species including 50 species of Gadiformes representing all the currently recognized families and subfamilies within the order were sampled. Representatives of seven additional orders were sampled for outgroup comparison: Lampridiformes (*Regalecus glesne*, *Desmodema polystictum*); Ophidiiformes (*Brotula brotula*); Percopsiformes (*Percopsis transmontana*); Polymixiiformes (*Polymixia japonica*); Stylephoriformes (*Stylephorus chordatus*) and Zeiformes (*Zenopsis conchifera*). This study generated genomic data for 42 species. From all remaining species, loci of interest were extracted from published genomic data (Malstrom et al. 2016) and analyzed following a modified pipeline for harvesting loci from genomes (Faircloth 2016).

2.2.1.2. Gene capture and probe design

The genome sequences of *Anguilla japonica*, *Danio rerio*, *Gadus morhua*, *Gasterosteus aculeatus*, *Lepisosteus oculatus*, *Oreochromis niloticus*, *Orizias laticeps* and *Tetraodon nigrovirilis*, were compared using the EvolMarkers tool pipeline (Li et al. 2012) to identify single-copy, conserved, protein coding sequences (CDS). A total of 14,217 exons shared by the eight-model species were retained by this search. Baits for target capture enrichment (Li et al 2013) were designed for these loci based on the sequences of the Atlantic cod (*G. morhua*). Bait sequences 120 bp in length were tiled to obtain 2X coverage of each targeted locus (60 bp overlap between baits). Biotinylated RNA probes of bait sequences were synthesized by Arbor Bioscience (formerly MYcroarray, Ann Arbor, Michigan).

2.2.1.3. Library preparation, gene capture and sequencing

Genomic DNA was quantified using a Qubit 2.0 fluorometer (Life Technologies Corporation, California, USA) and a sample of 0.5-3 ug was sheared to *c*. 500 bp using acoustic ultra-sonication on a Covaris E220 Focused-Ultrasonicator (Covaris, Inc., Massachusets, USA). Illumina sequencing libraries (Meyer & Kircher 2010) were then prepared for each sample using the "with-bead" method following Li et al. (2013), using adaptors labeled with unique 8 bp indices for identification. RNA baits were hybridized twice to individual libraries to increase the number of captured loci as suggested by Li et al. (2013). After target capture, the enriched individual libraries were pooled in equimolar ratios for paired-end sequencing on an Illumina HiSeq 2500 (Illumina, Inc, San Diego, CA). (Figure 2a)

2.2.1.4. Data assembly, orthology testing and alignment

The raw sequence data were demultiplexed according to the custom 8 bp indices for each sample. Adapters and low-quality reads (Phred score less than 20) were removed using the 'cutadapt' and 'FastQC' functions available in the wrapper script Trim Galore! (v0.4.4; Krueger 2012). PCR amplification duplicates were removed, and then the reads were parsed into different files according to their similarity representing each targeted sequence of the query (*G. morhua*) using a custom Perl script (S2, Supplementary material). Then, *de novo* assembly was performed using Trinity (Grabherr et al. 2011). Multiple overlapping contigs derived from the same targeted region were merged in Geneious vR10 (Kearse et al. 2012). The Smith-Waterman algorithm (Smith & Waterman 1981) was used to find the best matched sequence by comparing the query targeted sequence (*G. morhua*) with contigs derived from the *de novo* assembly. Finally, putatively orthologous genes were chosen by aligning the best matching contigs to the reference

genome of *G. morhua* using BLAST+ (v. 2.4.0; Camacho et al. 2009). If the alignment returned a hit outside of the targeted region, the contig was discarded. The final output included two files, the coding regions without flanking regions, and the intronic sequences. The sequences without flanking regions were used for the downstream analysis. Each individual locus was translated into amino acids (AA), and then the AA and DNA data were aligned using the auto option in MAFFT (v.7.221; Katoh & Standley 2013). (Simplified pipeline in Fig. 2b).

2.2.2. Phylogenomic Analyses

Concatenated DNA and amino acid datasets were assembled with FASconCAT-G (Kück & Longo 2014). Phylogenetic reconstruction analyses were conducted on various matrices using the maximum likelihood (ML) criterion: (i) original supermatrix with 57 species (DNA57), (ii) a reduced supermatrix with 54 species (DNA54) and (iii) three **s**elected **o**ptimal **s**ubsets (SOS-1, SOS-2 and SOS-3) from DNA54 generated with MARE v0.1.2-rc (**MA**trix **RE**duction, Meyer et al. 2011). Maximum likelihood analyses were conducted using RAxML-NG v0.2.0 BETA (DOI:10.5281/zenodo.492245) with a GTRGAMMA substitution model for the nucleotides datasets. The BLOSUM62 substitution model was applied to the amino-acid dataset. The ML tree searches for each dataset were conducted using 20 distinct random starting trees. One-hundred non-parametric bootstrap replicates were conducted in RAxML-NG to assess nodal support. Additionally, maximum likelihood analysis was conducted to the SOS-1 matrix partitioned by codon position and implementing the best fit model in as selected by IQ-TREE (v1.6 Kalyaanamoorthy et al. 2016). Non-parametric bootstrap -slow bootstrap (Felsenstein 1985) was calculated by running 100 replicates. Individual loci were also analyzed in RAxML with 1000 ultrafast bootstrap replicates (Hoang et al. 2017). Species-tree analyses were carried

out with ASTRAL 4.10.2 (Mirabab et al. 2015) with the individual RAxML locus "gene" trees as input trees. Pairwise uncorrected *p*-distances (proportion of nucleotide sites at which two sequences being compared are different) under were generated with MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets (Kumar et al. 2015) to compare genetic divergence among and within families, although they represent only a rough view of sequence divergence (Nei and Kumar, 2000).

2.2.2.1. Long Branch Attraction

Non-phylogenetic signal can result in long branch attraction (LBA), an artifact in which two or more lineages group together irrespective of their true relationships because they have much longer branches than the other taxa included in the analysis (e.g., Felsenstein 1978, Philippe 1998). The outgroup taxa are a common source of long branches, as they that may attract fast-evolving species of the ingroup through LBA, and as a result, the rapidly evolving taxa emerge too deeply in the tree (Whelan et al. 2015, Philippe et al. 2011). the potential source of LBA artifact due to outgroup was addressed by removing the most distant taxa: Lampridiformes (*Regalecus glesne*, *Desmodema polystictum*) and Ophidiiformes (*Brotula brotula*); but still including the current members of the Paracanthopterygii, for a total taxonomic sampling of 54 species.

2.2.2.2. Coverage and Information Content

The software MARE v0.1.2-rc (**MA**trix **RE**duction, Meyer et al. 2011) was used to assess the information content of loci. MARE combines potential signal of the loci with data coverage to generate **S**elected **O**ptimal **S**ubsets (SOS) of taxa and loci. The relative

informativeness of each single locus in the supermatrix is calculated based on weighted geometry quartet mapping (Nieselt-Struwe and von Haeseler 2001). Each locus received a value of informativeness between 0.0 and 1.0, reflecting the relative number of resolved quartet trees. Then a matrix indicating absence (0) or presence (1) was transformed into a matrix of potential information content of each loci and taxon by multiplying the availability (0/1) with scores of informativeness. The relative information content of each gene was calculated as the average value over all taxa including missing taxa; while the total average information content of the supermatrix was calculated as the sum of relative information content of all loci in relation with the number of taxa. Then MARE uses a hill climbing algorithm to reduce supermatrices into SOS of loci and taxa (see equations in MARE v0.1.2-rc manual). The optimality function of matrix reduction takes into account α as a scaling factor (default =3) and λ as the size ratio between the reduced matrix and the original matrix (Misof et al. 2013). . Three SOS matrices were built by adjusting the α value: α=1, α=3 and α=5 (α1 more relaxed and α5 stricter; S3, Supplementary Material) in order to search for the highest information content while excluding as few loci as possible. The -c option followed by a list of all taxa was included in the settings in order to retain all 54 species.

2.2.2.3. Data Partitioning and Model Selection

Best-fit partitioning schemes were estimated using Partition Finder v2 (Lanfear et al. 2016, 2014) and BLOSUM62 and GTRGAMMA evolutionary models were set for the AA54 and the DNA datasets (DNA54, SOS-1, SOS2 and SOS-3, Fig. 2.2). Parameters were set as follow: ‘rcluster-max’ to 1000 and ‘rcluster-percent’ to 10 for the relaxed clustering algorithm.

Because these data are protein coding, the DNA SOS-1 data set was also analyzed by codon position for the whole alignment and ran IQTREE (v1.6 Kalyaanamoorthyet al. 2017) to find the best-fit model for each partition using two strategies: (1) models in common between IQ-TREE and RAxML-NG (JC, K80, F81, HKY, TPM2, TPM3, TIM3, TVM, SYM, GTR) and (2) models available in IQ-TREE v1.6 (JC, F81, K80, HKY, TN, K81, K81u, TPM2, TPM2u, TPM3, TPM3u, TIM, TIMe, TIM2, TIM2e, TPM3, TVM, TVMe, SYM, GTR). Then, 100 slow standard nonparametric bootstrap (Felsenstein 1985) replicates were run for both strategies in IQ-TREE (v. 1.6 Nguyen, et al. 2015) (Fig. 2.2).

2.2.2.4. Saturation

Saturation bias can produce inconsistent phylogenetic results and phylogenomic data sets may be especially vulnerable to this source of bias (e.g., Breinholt et al. 2017, Philippe 2011, Dávalos & Perkins, 2009). To examine the extent of saturation for the nucleotide datasets (DNA54, SOS-1, SOS-2 and SOS-3) the degen v1.4. Perl script (Zwick et al. 2012) was used to exclude synonymous signal that can contribute to saturation (Fig. 2.2).

2.3. Results

2.3.1. Gene Capture Data Collection

The number of sequences generated, accession numbers, raw reads, contigs and values to assess the quality of the assembled loci, can be found in the electronic Supplementary Material, Table 2.1. Fifty species of gadiforms were included representing all recognized families and subfamilies, and seven outgroups. Enrichment and assembly recovered 14,210 loci with percentage of capture efficiency by family ranging from 12.3 to 76.0 % (except for

Bregmacerotidae, which registered the lowest capture 4.8%). The DNA57 and DNA54 matrices comprised 14,210 single copy loci and 2,874,858 and 2,868,792 base pairs, with 35.6 % and 33.7 % median missing data, respectively. Three DNA supermatrices were generated from the DNA54 dataset using MARE v0.1.2-rc by adjusting the alpha variable: α1: SOS-1 (8,478 loci; 1,821,891 sites), α 3: SOS-2 (5,172 loci; 1,088,988 sites) and α 5: SOS-3 (3,738 loci; 744,399 sites). The AA data set included 14,210 loci and 956,264 sites. (Table 2.1).

2.3.2. Maximum likelihood analysis

A ML phylogeny including 57 taxa was inferred using a single GTRGAMMA model across the alignment for the DNA57 data matrix, resulting in a well-resolved and well-supported topology with few exceptions (Fig. 2.3). Early diverging relationships are highly supported, as are the nodes defining families and subfamilies. A fully-congruent topology was resolved for DNA54 (Fig. 2.4 a) and for the SOS-1 (Fig. 2.4b) with slightly higher bootstrap values. Six main lineages were supported among gadiforms in the concatenated ML topology. The first one included (((Macrouridae , Steindachneriidae) , ((Macruronidae , Lyconidae) , Bathygadidae))) and Moridae as the sister group. The second clade consisted of (((Melanonidae , Muraenolepididae) , Euclichthyidae) , Trachyrincidae)) and Merlucciidae is the sister group of (Clade 1 + Clade 2). A separate lineage representing Ranicipitidae was found as the sister group of all of the previous clades. A fifth clade included all the gadoids (((Gadidae , Lotidae) , Gaidropsaridae) , Phycidae))). Finally, Bregmacerotidae was found as the sister group of all other Gadiformes (Fig. 2.3).

Overall, the ML trees estimated for the SOS-2 and SOS-3 (Fig. 2.4 c, d respectively) had lower bootstrap support at many nodes compared with the topologies from the DNA54 and SOS-

1 alignments. The lower support values across the two trees could be related to the decreased information content of the matrices compared with the DNA54 and SOS-1 (Fig. 2.4 a, b). ML topologies were largely consistent with those obtained from the ML analysis of the DNA57 and DNA54 and SOS-1 and differed only in one clade: ((Melanonidae , Euclichthyidae) , Trachyrincidae) sister to (Muraenolepididae , Merlucciidae) instead of (((Muraenolepididae , Melanonidae) , Euclichthyidae) , Trachyrincidae) and Merlucciidae as their sister group.

The values of *p*-distances revealed a pattern of increased nucleotide diversity at three levels: (1) within families, (2) among families of Gadiformes, and (3) among orders. Scores ranges of average *p*-distances of the three categories were (1) 0.017 – 0.065, (2) 0.024 – 0.102 and (3) 0.106 – 0.184, respectively. The family Bregmacerotidae showed the highest distances values (0.113 – 0.148) while families including only one genus the lowest values (e.g. Macruronidae $p = 0.017$, Merlucciidae $p = 0.020$, Melanonidae $p = 0.025$).

2.3.2.1. Saturation and base composition bias

Saturation is a factor that obscures phylogenetic signal (e.g., Breinholt et al. 2017, Borowiec et al. 2016, Dávalos et al. 2008), and base compositional bias also is a known source of systematic error in phylogenetic analyses of genome-scale data sets (Nabholz et al. 2011, Jeffroy et al. 2006, Phillips et al. 2004). The topology from the DNA54 differed from the DNA54 + degen and had lower bootstrap support at many nodes after removing synonymous signal with degen v1.4. In contrast, after removing saturation, SOS-1 recovered exactly the same topology as DNA54 and SOS-1.

2.3.2.2. The placement of Bregmacerotidae

Bregmacerotidae was placed as the earliest branching lineage of all gadiforms (on a long branch and with strong support) in both DNA and amino acid data analyses. Bregmacerotids have been difficult to amplify with PCR and have been shown in previous studies to constitute long branches (see Roa-Varón & Ortí 2009; Malstrøm et al. 2016, Supl. fig. 1; Grande et al. 2013, fig.1; Hughes et al. 2018). Over a long evolutionary separation, bregmacerotids have accumulated multiple substitutions occurring at the same position and there is insufficient phylogenetic signal despite using genomic data (e.g., homoplasy). The potential source of LBA artifact was addressed by: (a) reducing the outgroup taxa from seven to four taxa -- multiple outgroups may have attracted fast-evolving bregmacerotids species to spuriously make them diverge earlier on the tree; (b) removing saturation due to accelerated substitution rates causing homoplasy in the genomic dataset with degen v1.4. Perl script (Zwick et al. 2012); (c) reducing missing data (ML analysis using only the loci found in *Bregmaceros* spp. - 145 loci and 83,025 sites); (d) using partitions by codon position and applying the most realistic model of sequence evolution (Fig. 2.5a). Another potential way to solve or reduce LBA is increasing the taxonomic sampling within the family to detect convergences and reversions more easily. Loci from *Bregmaceros cantori* were harvested from Malstrøm et al. (2016), but a very low number of the loci were recovered (0.2 % capture efficiency). In all the analyses, Bregmacerotidae was found again as the sister group of all gadiforms on a long branch. Additionally, the topology recovered in the reduced data set including only loci found in Bregmacerotidae changed dramatically and reduced bootstrap support at many nodes, suggesting significant loss of phylogenetic signal.

2.3.3. Amino Acid Dataset

The concatenated amino acid alignment consisted of 956,264 sites and 107 subsets resulted from PF2 using the BLOSUM62 model. ML analysis generated phylogenetic trees in which the majority of nodes were well supported, with some exceptions. Topological conflict between the amino acid and DNA ML trees was observed at only two nodes -- the placement of Ranicipitidae within the gadoids clade with low support (bs = 83%) and Merlucciidae as the sister group of all gadiforms except Bregmacerotidae, with moderate support (bs=92%).

2.3.4. Gene tree species tree

The consensus topology of the SOS-1 matrix retrieved the monophyly of all the families (excepting lotids) and similar relationships among them. The few exceptions were the unsupported placement of (Moridae + ((Melanonidae + Trachyrincidae) + Euclichthyidae))) and Muraenolepididae in a separate clade more related to merlucciids (Fig. 2.5b).

2.4. Discussion

Taxonomic classification of the Gadiformes has been controversial due to partial taxonomic sampling and to the discordance among character types - musculature, early life stages, osteology, fossil data, and DNA (Malstrøm et al. 2016, Roa-Varón & Ortí 2009, Endo 2002, Howes 1989, Markle 1989, Nolf & Steurbaut 1989 among others). This study is the first order-wide phylogenomic analysis of the Gadiformes including representatives of all families and subfamilies. The utility of a gene capture approach-targeting coding DNA sequences (CDS) from single-copy protein-coding genes - for assessing the relationships of fishes at multiple evolutionary depths was also demonstrated. Using matrices of 14,208 and 8,478 loci, a well-

supported molecular phylogeny of the order is proposed that seeks to stabilize the higher-level Gadiformes classification (Fig. 2.6). Six lineages were recovered with strong support, and while there were some differences between topologies recovered by the DNA and the amino acid datasets, they agree on a number of fundamental points resolving unresolved relationships among gadiform lineages.

Clade 1

This study recovered the family Macrouridae including only macrourids as proposed by Roa-Varon & Ortí (2009). The family has about 364 species within 29 genera (Nelson et al. 2016) and includes more than half of all gadiform species. Traditionally, the family has included four subfamilies - Macrourinae, Bathygadinae, Trachyrincinae and Macrouroidinae (Marshall 1965, Cohen 1984, Nolf & Steurbaut 1989, Iwamoto 1989, Cohen et al. 1990, Endo 2002). However, the interrelationships among them have been often contentious. For example, Howes (1989) suggested that the family was a paraphyletic assemblage among gadiforms, but he could not resolve the polytomy with steindachnerids, melanonids and other gadoids (Howes 1990) or with steindachnnerids and morids (Howes 1993). Additionally, Howes (1991) could not determine the phylogenetic placement of trachyrincids and bathygadids among basal gadoids. On the other hand, Okamura (1989) suggested a close relationship among macrourids, trachyrincids, macrourines, bathygadines and euclichthyids. The present study indicates once again that the family is paraphyletic (Fig. 2.6). The uncorrected average *p*-distance between Macrouridae and Bathygadidae is $p = 0.035$, Macrouridae and Trachyrincidae is $p = 0.041$, similar to average *p*-distances among different families in the order (e.g. Melanonidae - Lotidae $p = 0.040$, Moridae – Macrouridae $p = 0.032$) - the placement of the other three "subfamilies" will be discussed in more detail below.

A close relationship between macrourids and the monotypic family Steindachneriidae was strongly supported (bs = 100; Fig. 2.6). This finding, however, contradicts some previously published phylogenies based on molecular (Malstrøm et al. 2016, Hi et al. 2016, Roa-Varón & Ortí 2009) and morphological hypotheses (Endo 2002, Cohen et al. 1990, Okamura 1989), which support a sister relationship between Macrouridae and Bathygadidae. In contrast, Jordan and Evermann (1898) included *Steindachneria* within macrourids. Howes (1990, 1991, 1993) considered it an unresolved basal gadiform, while Fahay (1989) based on ontogenetic and osteological data, proposed the *Steindachneria* as the sister group of macrourids. This study supports the conclusions of Fahay (1989), in which *Steindachneria* may be the primitive sister group of the Macrouridae, evolving before the development of a special benthopelagic juvenile stage of macrourids. The close relationship of *Steindachneria* with merlucciids as proposed by some others (Parr 1946, Norman 1966, Marshall 1966, Nelson 1976, 1984, Cohen 1984, Cohen et al. 1990 and Inada 1989) is rejected with the present findings (Merlucciidae – Steindachneriidae $p = 0.094$).

Within this clade, the dataset also reveals a close relationship among (Macruronidae, Lyconidae), and Bathygadidae (Fig. 2.6). Macruronidae has been placed as an independent family composed of *Macruronus* and *Lyconus* (Markle 1989, Howes 1991 a,b and Endo 2002) or as a subfamily within Merlucciidae (Inada 1989). Based on mtDNA (regions of COI and cyt*b*), von der Heyden and Matthee (2008) proposed Steindachneridae and Macruronidae as independent families, and resurrected Lyconidae - a poorly known family with potentially two genera *Lyconus*, with two species and the monotypic genus *Lyconodus argenteus* (potentially extinct). The family was proposed by Günther (1887) based on the description of *L. pinnatus*, a small specimen collected in the middle of the South Atlantic; twenty years later Holt & Byrne

(1906) described *Lyconus brachycolus* from the eastern North Atlantic. This study found that Macruronidae and Lyconidae are not related with Merlucciidae, but closely related to Macrouridae and Moridae. Molecular distance among Macruronidae – Merlucciidae and Lyconidae – Merlucciidae ($p = 0.070$ for both of them) are larger than the genetic distances between Macrouridae and Lyconidae ($p = 0.045$) and Moridae and Lyconidae ($p = 0.044$). Additionally, the distance between Macruronidae-Lyconidae ($p = 0.047$) corroborated the resurrection of the family Lyconidae proposed by von der Heyden and Matthew (2008) and for the first time, this study sheds light about the phylogenetic placement of the family Lyconidae due to the complete taxonomic sampling at family and subfamily level among gadiforms used in this study (Fig. 2.6).

The family Moridae was recovered as the sister group of the five previously discussed families (Fig. 2.6). Moridae is the second largest family among gadiforms, comprising about 108 species in about 18 genera. Morids have been considered as being sister lineage to *Euclichthys* (Markle 1989), bathygadids (Howes 1989), or representing an offshoot among basal gadoids (Howes 1990, 1991a). Morids remained as an unresolved polychotomy with euclichthyids, macrourids, bathygadines, trachyrincids, macrouroids, and melanonids in the Nolf & Steurbaut (1989) study based on otolith morphology. The relationships among all families within this clade were strongly supported and fully resolved.

Clade 2

Within the second clade, (((Muraenolepididae, Melanonidae) , Euclichthyidae) , Trachyrincidae) was resolved based on DNA data analyses (Fig. 2.6). In contrast, the amino acid dataset recovered Euclichthyidae as the sister group of Melanonidae, and Muraenolepididae

closely related to them; Trachyrincidae remained as the sister group of all of them. All these families represent phylogenetically enigmatic families. Muraenolepididae includes two genera (*Muraenolepis* with eight species and the monotypic genus *Notomuraenobathys microcephalus*) and has been considered the earliest offshoot of the gadiforms (Roa-Varón & Ortí 2009, Cohen 1984) or belonging to advanced gadoids (Endo 2002; Markle 1989; Howes 1991a, b, 1989; Siebert 1990).

Melanonidae is another puzzling family that includes one genus *Melanonus* and two species (*M. gracilis* and *M. zugmayeri*). The genus was first included as a subfamily within Gadidae by Goode & Bean (1896) and subsequently as a genus within Gadidae (Jordan 1923), Moridae (Svetovidov 1948), and Morinae within gadids (Norman 1966). Since the study of Marshall and Cohen (1973), it has been recognized as a distinct family, although its phylogenetic position is controversial. It has been proposed as the sister group of all Gadiformes (Howes 1993, Ends 2002), the sister group of all gadiforms except ranicipitids (Markle 1989), closely related to Moridae (Schwarzhans 1980, 1984; Cohen 1984; Fahay & Markle 1984), the sister group of steindachnerids (Howes 1989), or as an unresolved polychotomy with euclichthyids, bathygadines, macrourines, macrouroidines, trachyrincids and morids (Nolf & Streurbaut 1989). Howes (1990, 1991a, b) also found them in a polychotomy but with Bathygadinae and Steindachneriinae within gadoids. Based on molecular data it has been found to be closely related to Merlucciidae and Euclichthyidae (Roa-Varón and Ortí, 2009) or within a clade including merlucciids, muraenolepids, trachyrincids, morids, bathygadids and macrourids (Malstrøm et al. 2016). This study found Melanonidae within the clade (Melanonidae, Muraenolepididae), Euclichthyidae, Trachyrincidae). These results are partially in agreement with Nolf & Staurbaut (1989) and Siebert (1990).

Euclichthyidae is represented by a single species, *Euclichthys euclichthys*, from South Australia and New Zealand. It was included within the Moridae, but removed by Svetovidov (1969), who suggested some similarities with Macrouridae. Cohen (1984) proposed the family because it could not be placed in any recognized family within gadiforms. This study recovered the family as the sister group of Melanonidae and Muraenolepididae.

The family Trachyrincidae includes two subfamilies, Trachyrincinae (two genera - *Trachyrincus* with six species and monotypic *Idiolophorhynchus andriashevi*) and Macrouroidinae with two monotypic genera (*Macrouroides inflaticeps* and *Squalogadus modificatus*). The two subfamilies have been ranked at the family level, Macrouroididae (Okamura 1970 a,b, 1989) and Trachyrincidae (Okamura 1989, Howes 1988, 1989), as subfamilies within Macrouridae (Marshall 1965, 1973; Cohen 1984; Nolf & Steurbaut 1989; Iwamoto 1989; Cohen et al. 1990; Endo 2002), or subfamilies within Trachyrincidae (Nelson et al. 2016, Roa-Varón & Ortí 2009). The molecular distance of Trachyrincidae compared with other families such us Moridae ($p = 0.025$), Melanonidae ($p = 0.030$), Lotidae ($p = 0.038$) is smaller than the distance with the family Macrouridae ($p = 0.041$) in which was formerly included, corroborating its status at family level.

The relationships among the families included in clade one and two, were anticipated in part by several authors who included some or all these families within “Macrouroidei”. For example, Cohen (1984) suggested that Macrouroidei should be composed by Euclichthyidae and Macrouridae. Markle (1989) proposed a close relationship among Macrouridae + Steindachneriidae and the two of them having split off from a common linage with morids and *Euclichthys*. Nolf & Steurbaut (1989) proposed euclichthyids, bathygadids, macrouroidines, macrourids, melanonids and morids.

Clade 3

This clade recovered the family Merlucciidae (including only the genus *Merluccius*) with strong support as the sister group of the two clades discussed previously (Fig. 2.6). In contrast, when the nucleotide dataset was translated into amino acid dataset, Merlucciidae was recovered as the sister group of all other sampled gadiforms except Bregmacerotidae; in this topology, support values were reduced for several clades (S4, Supplementary Material). The phylogenetic signals were weakened, suggesting that fast-evolving sites and the third-codon position have useful information for inferring the phylogenetic placement of the family among gadiforms. The phylogenetic position and composition of merlucciids has long been controversial as some authors suggested they could be placed within Gadidae (Kaup 1858, Regan 1903, Cadenat 1937) or as a subfamily within the Gadidae, along with Lotinae and Gadinae (Svetovidov 1948, Dunn & Matarese 1984). In contrast, other authors (based on morphological or molecular data) recognized merluccids as a separate family including: a) only *Merluccius* (Gill 1884, Jordan and Evermann 1898, Jordan 1923, Belloc 1928, Norman 1937, Endo 2002, von der Heyden & Matthew 2008, Roa-Varón & Ortí 2009); b) *Merluccius* and *Macruronus* (Norman 1937); c) *Merluccius, Macruronus*, *Lyconus*, *Steindachneriia* and potentially *Lyconodes* (Marshall 1966, Lloris eta l. 2005); d) *Merluccius*, *Macruronus*, *Lyconus* and the fossil genera *Rhinocephalus* and *Paleogadus* (Marshall and Cohen 1973); and e) only *Merluccius*, *Macruronus* and *Lyconus* *(*Fahay & Markle 1984, Lloris et al. 2005). The present results support the original hypothesis proposed by Gill (1884).

Clade 4

The family Ranicipitidae, represented by *Raniceps raninus,* was resolved as an independent lineage with strong support (bs = 100; Fig. 2.6). The family was proposed by Gill (1890) and has been regarded as a subfamily within Gadidae (Berg 1940), as a member of lotines (Svetovidov 1948, Nolf & Steurbaut 1989), as a member of phycines (Dunn & Matarese 1984, Fahay & Markle 1984, Cohen et al. 1990), ranked at family level within Gadoidei (Endo 2002, Roa-Varón & Ortí 2009), or proposed as a separated suborder (Markle 1989). Its ranking at family level is supported in the present study by the molecular distance found among families ranging from $p = 0.049$ (Ranicipitidae - Moridae) to $p = 0.078$ (Ranicipitidae – Gaidropsaridae).

Clade 5

This clade includes one of the most-studied groups among fishes, which despite of their commercial importance and previous morphological and molecular studies, still lacks a resolved phylogeny. The topology of (Phycinae, (Gaidropsarinae , (Lotinae, Gadinae))) was strongly supported throughout all of the analyses (Figs. 6). Svetovidov (1948) considered 22 genera (including *Merluccius*) within the family Gadidae and organized them in three subfamilies: Lotinae, Merlucciinae and Gadinae (including taxa now assigned to gadines, lotines, gaidropsarines, phycines and ranicipitids). Gaemers (1976), based on otolith characters, proposed five subfamilies (Gadinae, Lotinae, Gaidropsarinae, Phycinae and Merlucciinae). Cohen (1984) ranked some of these subfamilies at family level: Gadidae, Lotidae and Phycidae (phycines, gaidropsarines and ranicipitids). Endo (2002) included within Gadidae, four subfamilies (Gadinae, Lotinae, Gaidropsarinae and Phycinae) and merlucciids and ranicipitids at family level within “Gadoidei”. Teletchea et al. (2006), based on analyses of two mitochondrial loci and 30

morphological characters from the literature, proposed a new provisional classification of the suborder Gadoidei containing two families, Merlucciidae (one genus) and the Gadidae (21 genera) distributed into four subfamilies: Gadinae (12 genera), Lotinae (three genera), Gaidropsarinae (three genera), and Phycinae (three genera). Interestingly, the authors included *Raniceps raninus* (which was not included in their molecular analyses) within Phycinae, based on two morphological characters (moderate number of rays in dorsal and anal fins). The authors noted that the characters were continuous and did not show clear-cut states between species and therefore further analyses, both molecular and morphological, were needed in order to clarify the phylogenetic position of ranicipitids. The present results agreed with Howes' (1990, 1991a,b) hypothesis ranking these taxa at family level (Gadidae - Phycidae $p = 0.053$; Gadidae - Gaidropsaridae $p = 0.050$; Lotidae $p = 0.024$). Phycidae comprises two genera (*Phycis* and *Urophycis*), Gaidropsaridae includes *Ciliata*, *Enchelyopus* (not included in this study) and *Gaidropsaurus*. The monophyly of Lotidae (*Brosme*, *Lota* and *Molva*) was recovered with strong support for the first time based on molecular data (see Betancur et al. 2017, Malstrøm et al. 2016) and Gadidae represented by 10 of the 12 genera currently known. However, morphological data of these two families could provide further evidence given the relatively low genetic distance between both of them.

Ranicipitidae and Merlucciidae were ranked at family level representing independent lineages (see Clades 3 and 4). These results shed light onto one of the most contentious relationships among gadiforms.

Clade 6

Bregmacerotids represent one of the most challenging families within gadiforms in terms of its taxonomy and phylogenetic placement. The family comprises one genus (*Bregmaceros*) and about 16 nominal species (Nelson et al. 2016). It has been included within gadoids by many authors (Markle 1989, Svetovidov 1948, Gill 1872), ranked at suborder level Cohen (1984), as an unresolved trichotomy with muraenolepids and the other gadiforms (Nolf & Steurbaut 1989), as the sister group of Muraenolepididae (Markle 1989, Endo 2002), or sister group of the higher gadoids excluding macruronids (Howes 1990, 1991a). Based on molecular data Roa-Varón & Ortí (2009) recovered bregmacerotids on a long branch closely related to macrourids using mitochondrial DNA data, and to Ranicipitidae in the combined (mtDNA and nDNA) dataset (Fig. 2.1). Malstrøm et al. (2016), recovered bregmacerotids as the sister group of all other sampled gadiforms (on a long branch and with strong support, Fig. 1 Supplemental Material). The present study also found bregmacerotids sister to the remaining gadiform taxa, despite the efforts to break the long branches by: (a) reducing the outgroup taxa; (b) removing saturation; (c) reducing missing data; (d) using partitions by codon position and applying the most realistic model of sequence evolution. The placement of the family Bregmacerotidae as the earliest branching family of gadiforms has not been postulated before based on morphology, and therefore it should be viewed as provisional until additional morphological data are considered from bregmacerotids and other gadiforms.

2.5. Conclusions

This study is the most comprehensive phylogenomic study of Gadiformes to date. In accordance with these results, and to contribute to the state of gadiforms higher level

systematics, a revised classification of the group is provided including 17 families (Fig. 2.6). The monophyly of all the families and subfamilies among gadiforms was resolved for first time with strong support, and the large amount of congruence across analyses was reassuring. Few differences were noted among the DNA, the amino acid and the species phylogenetic trees, and those ambiguities were generally poorly supported. Gene capture in conjunction with next generation sequencing has much promise as an approach to phylogenomics. It can be used to generate sequence information for thousands of loci that vary substantially in their rates of evolution and allow the use of bioinformatic workflows that take advantage of protein and reading-frame information. The baits used in this study had a lot of variation in coverage of different loci, and strongly suggests that future studies should work to improve the efficiency of the baits prior to initiation of a large-scale study. Future efforts will focus on refining the bait set to mask over-sequenced regions and even out read coverage across loci and increasing the taxonomic sampling. Despite these limitations, these molecular data provided insights into the phylogeny of the Gadiformes. An extended analysis, including morphological data of extant and fossils taxa, is needed, in order to reach conclusions about the evolution of these fishes.

Table 2.1 Alignment and Matrices Summary

Datasets	# Taxa	# Loci	# Partitions	Global Evol. Model	Evol. Model by Codon Position
DNA57	57	14,210		GTR+G	
DNA54	54	14,210	11,068	GTR+G	
DNA 54 + degen	54	14,210	NA	GTR+G	
DNA SOS-1	54	8,479	6,445	GTR+G	RAxML P1:GTR+R4; P2 & P3:GTR+R6 IQ-TREE P1: GTR+R5, P2: GTR+R4, P3: GTR+R6
DNA SOS-1 + degen	54	8,479	NA	GTR+G	
DNA SOS-2	54	5,173	3,575	GTR+G	
DNA SOS-3	54	3,739	2,190	GTR+G	
AA54	54	14,210	107	BLOSUM62	

Table 2.2 Sampling and Sequencing Information of the Taxa Used in this Study

Origin	Order	Family	*Species*	Tissue	Voucher / Donation	# Loci captured	% Cap. Effic.
a	Gadiformes	Macrouridae	*Coryphaenoides subserrulatus*	Gill arch - 1	P. McMillan	1931	13.6
a	Gadiformes	Euclichthyidae	*Euclichthys polynemus*	Gill arch - 1	P. McMillan	2394	16.8
a	Gadiformes	Muraenolepidida	*Muraenolepis sp.*	Gill arch - 1	P. McMillan	1982	13.9
a	Ophidiiformes	Ophidiidae	*Brotula brotula*	Gill arch - 2	T. Iwamaoto		
a	Gadiformes	Macrouridae	*Coelorinchus geronimo*	Gill arch	CAS 223169	1713	12.0
a	Gadiformes	Bathygadidae	*Bathygadus melanobranchus*	Gill arch	CAS 224389	8575	60.3
a	Gadiformes	Moridae	*Lepidion capensis*	Gill arch - 2	T. Iwamaoto	2160	15.2
a	Gadiformes	Melanonidae	*Melanonus gracilis*	Gill arch - 2	T. Iwamaoto	2921	20.5
a	Gadiformes	Trachyrincidae	*Squalogadus modificatus*	Gill arch - 2	T. Iwamaoto	7485	52.6
a	Gadiformes	Moridae	*Gadella imberbis*	Muscle	TS 950 (KU) - MCZ 138027	9495	66.8
a	Gadiformes	Phycidae	*Urophycis regia*	Muscle	TS 1000 (KU) - KU 26953	2707	19.0
a	Gadiformes	Bregmacerotidae	*Bregmaceros cantori*	Muscle	TS 5132 (KU) - KU 30244	519	3.7
a	Gadiformes	Gadidae	*Microgadus tomcod*	Muscle	TS 5884 (KU) - KU 34104	13374	94.1
a	Gadiformes	Bathygadidae	*Gadomus colleti*	Muscle - 3	E. Hiromitsu	5186	36.5
a	Gadiformes	Gaidropsaridae	*Ciliata mustela*	Muscle	ZMUC 373657	2685	18.9
a	Gadiformes	Ranicipitidae	*Raniceps raninus*	Muscle	ZMUC 375239	4637	32.6
a	Gadiformes	Trachyrincidae	*Trachyrincus murrayi*	Muscle	ZMUC 375204	3432	24.1
a	Percopsiforme	Percopsidae	*Percopsis transmontana*	Muscle	TS 1891 - KU 29775	2346	16.5
a	Gadiformes	Steindachneriidae	*Steindachneria argentea*	Gill arch - 4	S. W. Ross	3138	22.1
a	Gadiformes	Moridae	*Physiculus fulvus*	Gill arch - 4	S. W. Ross	6746	47.5
a	Gadiformes	Lotidae	*Brosme brosme*	Muscle	TS 8722 (KU) - MCZ 168090	2618	18.4
a	Gadiformes	Gaidropsaridae	*Gaidropsarus ensis*	Muscle	TS 5924 (KU) - MCZ 163253	11563	81.3
a	Stylephoriform	Stylephoridae	*Stylephorus chordatus*	Muscle	TS 8138 (KU) - MCZ 165920	2257	15.9
a	Polymixiiform	Polymixiidae	*Polymixia japonica*	Muscle	TS 258 (KU) - KU 21392	2437	17.1
a	Zeiformes	Zeidae	*Zenopsis conchifera*	Muscle	TS 2929 (KU) - MCZ 155779	817	5.7
a	Gadiformes	Lyconidae	*Lyconus brachycolus*	DNA aliquot - 5	S. von der Heyden	2325	16.4
a	Gadiformes	Lyconidae	*Lyconus pinnatus*	DNA aliquot - 5	S. von der Heyden	2883	20.3
a	Gadiformes	Merlucciidae	*Merluccius merluccius*	Gill arch - 6	R. Banon	5400	38.0
a	Gadiformes	Phycidae	*Phycis blennoides*	Gill arch - 6	R. Banon	8645	60.8
a	Gadiformes	Trachyrincidae	*Trachyrincus scabrus*	Gill arch - 6	R. Banon	4388	30.9
a	Gadiformes	Moridae	*Mora moro*	Gill arch - 6	R. Banon	4374	30.8
a	Gadiformes	Merlucciidae	*Merluccius paradoxus*	Gill arch	USNM 440552	8049	56.6
a	Gadiformes	Macruronidae	*Macruronus cf. capensis*	Gill arch - 7	R. Leslie	1625	11.4
a	Gadiformes	Merlucciidae	*Merluccius hubbsi*	DNA aliquot - 8	UNMdP 1569	8075	56.8
a	Gadiformes	Bregmacerotidae	*Bregmaceros houdei*	Muscle	TCWCID 15940.02	847	6.0
a	Gadiformes	Merlucciidae	*Merluccius australis*	Gill arch - 1	P. McMillan	6740	47.4
a	Gadiformes	Macruronidae	*Macruronus magellanicus*	Gill arch - 9		6371	44.8
a	Gadiformes	Macruronidae	*Macruronus magellanicus*	Gill arch - 9	L.M. Adasme	4585	32.3
a	Gadiformes	Merlucciidae	*Merluccius angustimanus*	DNA aliquot	USNM 422438	6362	44.7
a	Gadiformes	Merlucciidae	*Merluccius polli*	Gill arch	CAS 223407	5782	40.7
a	Gadiformes	Gadidae	*Gadus morhua*		Genome	14208	100.0
b	Gadiformes	Gadidae	*Arctogadus glacialis*	Fin	Malstrøm et al. 2016	10497	73.8
b	Gadiformes	Lotidae	*Molva molva*	Thymus	Malstrøm et al. 2016	8166	57.4
b	Gadiformes	Lotidae	*Lota lota*	Muscle	Malstrøm et al. 2016	7474	52.6
b	Gadiformes	Melanonidae	*Melanonus zugmayeri*	Muscle	ZSCM 32519	3527	24.8
b	Gadiformes	Macrouridae	*Macrourus berglax*	Muscle	Malstrøm et al. 2016	1651	11.6
b	Gadiformes	Macrouridae	*Malacocephalus occidentalis*	Muscle	CFM 117884	1699	12.0
b	Gadiformes	Gadidae	*Boreogadus saida*	Fin	Malstrøm et al. 2016	10769	75.7
b	Gadiformes	Muraenolepidida	*Muraenolepis marmoratus*	Muscle	Malstrøm et al. 2016	2146	15.1
b	Gadiformes	Moridae	*Laemonema laureysi*	Muscle	ZSCM 32710	3309	23.3
b	Gadiformes	Gadidae	*Trisopterus minutus*	Spleen	Malstrøm et al. 2016	7490	52.7
b	Gadiformes	Gadidae	*Pollachius virens*	Spleen	Malstrøm et al. 2016	10762	75.7
b	Gadiformes	Gadidae	*Melanogrammus aeglefinus*	Spleen	Malstrøm et al. 2016	10855	76.4
b	Gadiformes	Gadidae	*Merlangius merlangus*	Thymus	Malstrøm et al. 2016	10709	75.3
b	Gadiformes	Gadidae	*Gadus chalcogrammus*	Fin	Malstrøm et al. 2016	11371	80.0
b	Gadiformes	Gadidae	*Gadiculus argenteus*	Spleen	Malstrøm et al. 2016	8038	56.5

Data origin
a This study
b Malstrøm et al. 2016

Institutional abbreviations
CAS voucher from California Academy of Sciences
CFM vouchers from Chicago Field Museum collection
KU voucher and tissue sample (TS) from Biodiversity Institute & Natural History Museum, The University of Kansas
MZC Museum of Comparative Zoology, Harvard University
TCWC Texas Cooperative Wildlife Collection, The Natural History Collection at Texas A&M University
UNDMP Universidad Nacional de Mar del Plata
ZMUC voucher from Zoological Museum University of Copenhagen collection
ZSCM numbers are vouchers from Zoological State Collection Munich

Tissues samples donated by
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3 - Endo Hiromitsu (Kochi University, Kochi, Japan)
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7 - Rob Leslie (Department of Agriculture, Forestry and Fisheries -DAFF. Cape Town, South Africa)
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9 - Luis Marcos Adasme Martinez (Instituto de Fomento pesquero - IFOP. Valparaiso, Chile)

Table 2.3 Uncorrected *p*-Distances Among and Within Families of Gadiformes

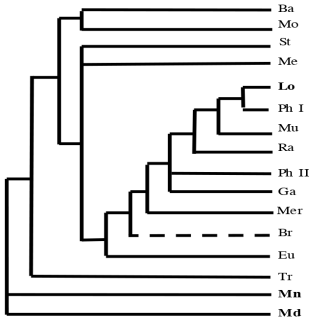
Family	Gadidae	Lotidae	Melanonidae	Macrouridae	Muraenolepididae	Moridae	Merlucciidae	Euclichthyidae	Bathygadidae	Trachyrincidae	Phycidae
Gadidae	0.039										
Lotidae	0.024	0.035									
Melanonidae	0.049	0.040	0.025								
Macrouridae	0.053	0.050	0.044	0.052							
Muraenolepididae	0.062	0.060	0.055	0.059	0.026						
Moridae	0.043	0.032	0.026	0.032	0.045	0.054					
Merlucciidae	0.067	0.054	0.046	0.053	0.063	0.041	0.020				
Euclichthyidae	0.073	0.060	0.050	0.061	0.074	0.049	0.072	NA			
Bathygadidae	0.056	0.042	0.034	0.035	0.057	0.029	0.054	0.058	0.042		
Trachyrincidae	0.053	0.038	0.030	0.041	0.050	0.025	0.049	0.053	0.034	0.035	
Phycidae	0.053	0.040	0.050	0.050	0.065	0.045	0.070	0.077	0.058	0.055	0.037
Bregmacerotidae	0.128	0.133	0.133	0.113	0.141	0.122	0.139	0.161	0.134	0.138	0.134
Gaidropsaridae	0.050	0.042	0.051	0.047	0.063	0.047	0.070	0.080	0.062	0.058	0.057
Ranicipitidae	0.070	0.055	0.056	0.065	0.073	0.049	0.072	0.076	0.060	0.053	0.072
Percopsiformes	0.137	0.134	0.135	0.121	0.140	0.127	0.148	0.164	0.139	0.142	0.147
Steindachneriidae	0.086	0.081	0.075	0.064	0.090	0.068	0.094	0.102	0.074	0.080	0.089
Stylephoriformes	0.119	0.112	0.118	0.110	0.129	0.112	0.136	0.153	0.126	0.124	0.140
Polymixiiformes	0.131	0.127	0.128	0.117	0.135	0.122	0.143	0.152	0.133	0.134	0.140
Zeiformes	0.125	0.124	0.119	0.106	0.130	0.117	0.132	0.150	0.126	0.127	0.133
Lyconidae	0.064	0.054	0.047	0.045	0.069	0.044	0.070	0.074	0.042	0.051	0.072
Macruronidae	0.066	0.056	0.050	0.046	0.069	0.044	0.070	0.075	0.044	0.052	0.069

Dark grey: *p* -uncorrected distance within family

Family	Bregmacerotidae	Gaidropsaridae	Ranicipitidae	Percopsiformes	Steindachneriidae	Stylephoriformes	Polymixiiformes	Zeiformes	Lyconidae	Macruronidae
Gadidae										
Lotidae										
Melanonidae										
Macrouridae										
Muraenolepididae										
Moridae										
Merlucciidae										
Euclichthyidae										
Bathygadidae										
Trachyrincidae										
Phycidae										
Bregmacerotidae	0.030									
Gaidropsaridae	0.121	0.065								
Ranicipitidae	0.157	0.078	NA							
Percopsiformes	0.180	0.136	0.162	NA						
Steindachneriidae	0.153	0.091	0.102	0.162	NA					
Stylephoriformes	0.184	0.132	0.147	0.163	0.158	NA				
Polymixiiformes	0.175	0.134	0.156	0.124	0.160	0.150	NA			
Zeiformes	0.178	0.126	0.146	0.153	0.155	0.170	0.142	NA		
Lyconidae	0.149	0.074	0.073	0.157	0.088	0.147	0.147	0.140	0.023	
Macruronidae	0.148	0.072	0.077	0.152	0.085	0.142	0.146	0.138	0.047	0.017

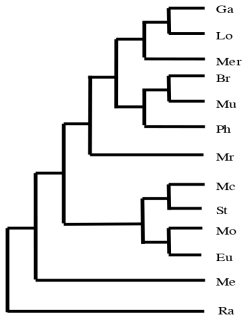
Dark grey: *p* -uncorrected distance within family

Howes 1989



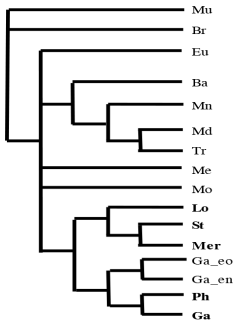
Muscles & Joints
(18 characters)

Markle 1989



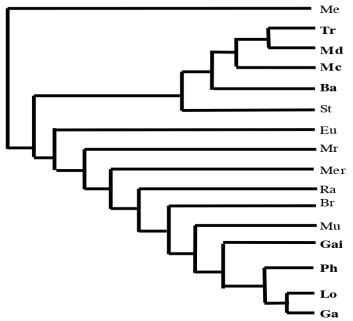
Adult Morphology
(40 characters)

Nolf & Steurbaut 1989



Otoliths

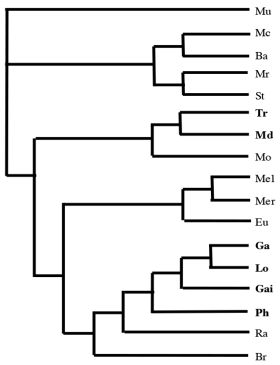
Endo 2002



Osteology and Myology
(23 characters)

Gadiformes

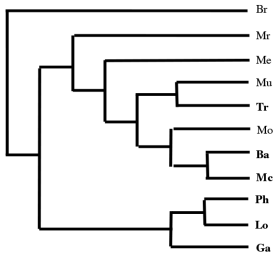
Roa-Varón & Ortí 2009



117 species
46 genera
12/13 Families
3 genes (2,740 bp)

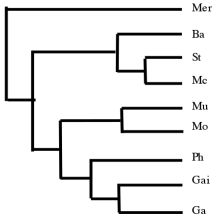
Studies Across Teleosts

Malström et al. 2016



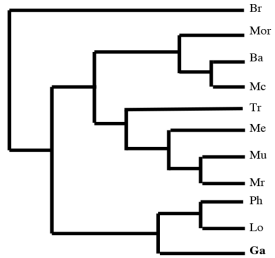
27 Species
22 genera
7/13 Families
111 genes (71,418 bp)

Betancur-R et al. 2017



42 Species
27 genera
10/13 Families
21 genes (20,874 bp)

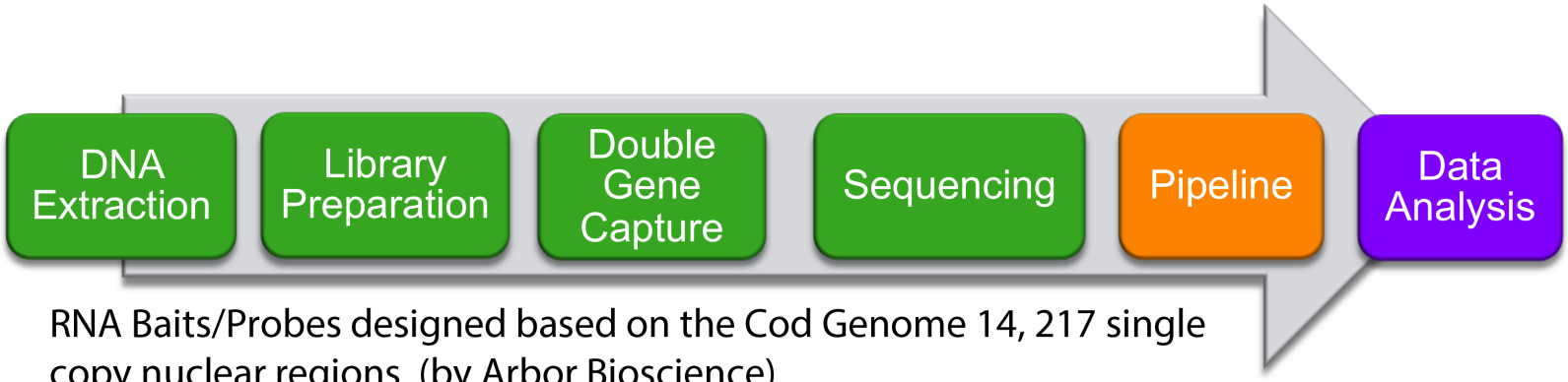
Hughes et al. 2018



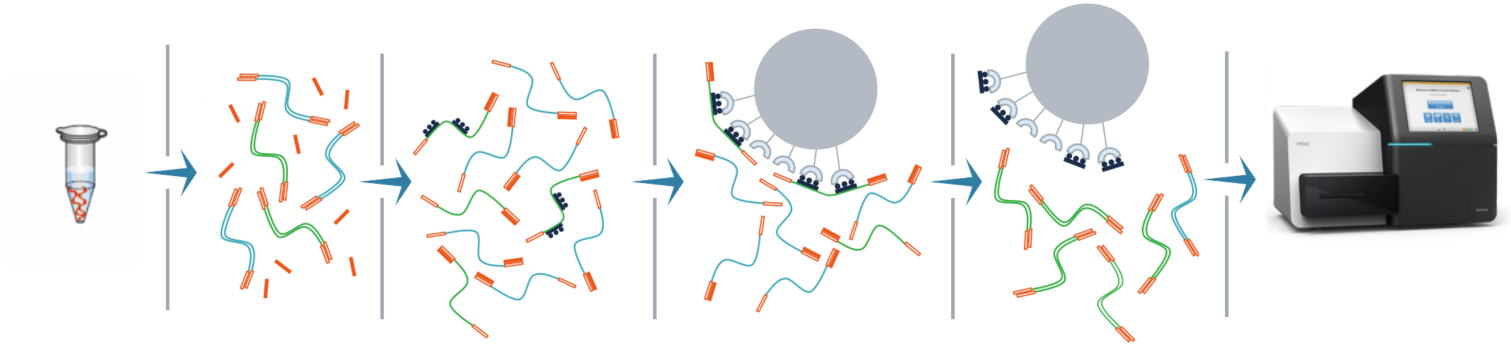
27 Species
22 genera
7/13 Families
1,105 loci (555,288 bp)

Figure 2.1 Classifications of Gadiformes Based on Morphological (top row) and Molecular Data (bottom row) data. Abbreviations correspond to: Ba: Bathygadidae (**Ba**: Bathygadinae); Bre: Bregmacerotidae; Eu: Euclichthyidae; Ga: Gadidae (**Ga**: Gadinae); Gai: Gaidropsarinae; Lo: Lotidae (**Lo**: Lotinae); Mc: Macrouridae; Md: Macrouroidinae; Mn: Macrourinae; Mr: Macruronidae; Mo: Moridae; Me: Melanonidae; Mer: Merlucciidae (**Mer**: Merlucciidae); Mu: Muraenolepididae; Ph I and II: Phycidae (**Ph**: Phycinae); Ra: Ranicipitidae; St: Steindachneriidae (**St**: Steindachneriinae); Tr: Trachyrincidae (**Tr**: Trachyrincinae). **All bolded abbreviations represent subfamily ranking provided by the authors.**

Protein-Coding Genes Capture Approach



RNA Baits/Probes designed based on the Cod Genome 14, 217 single copy nuclear regions (by Arbor Bioscience)



Quantify & Shear DNA (Qubit & Covaris)

Library

Hybridize targets to capture probes

add streptavidin beads to bin probes

Capture targets with magnet; Wash away off-target sequences

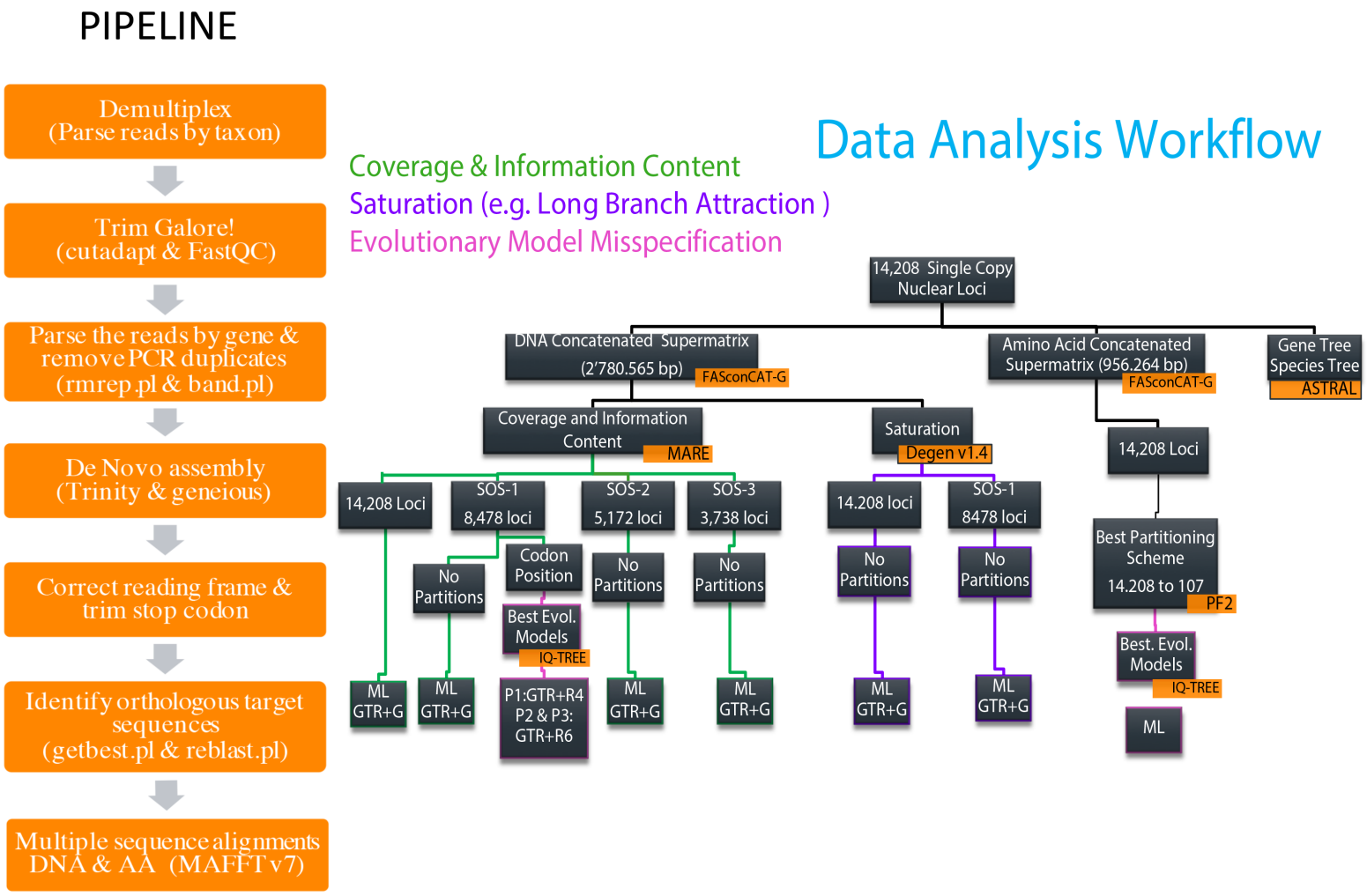


Figure 2.2 Protein gene capture approach; b) Pipeline simplified and c) Data analysis workflow

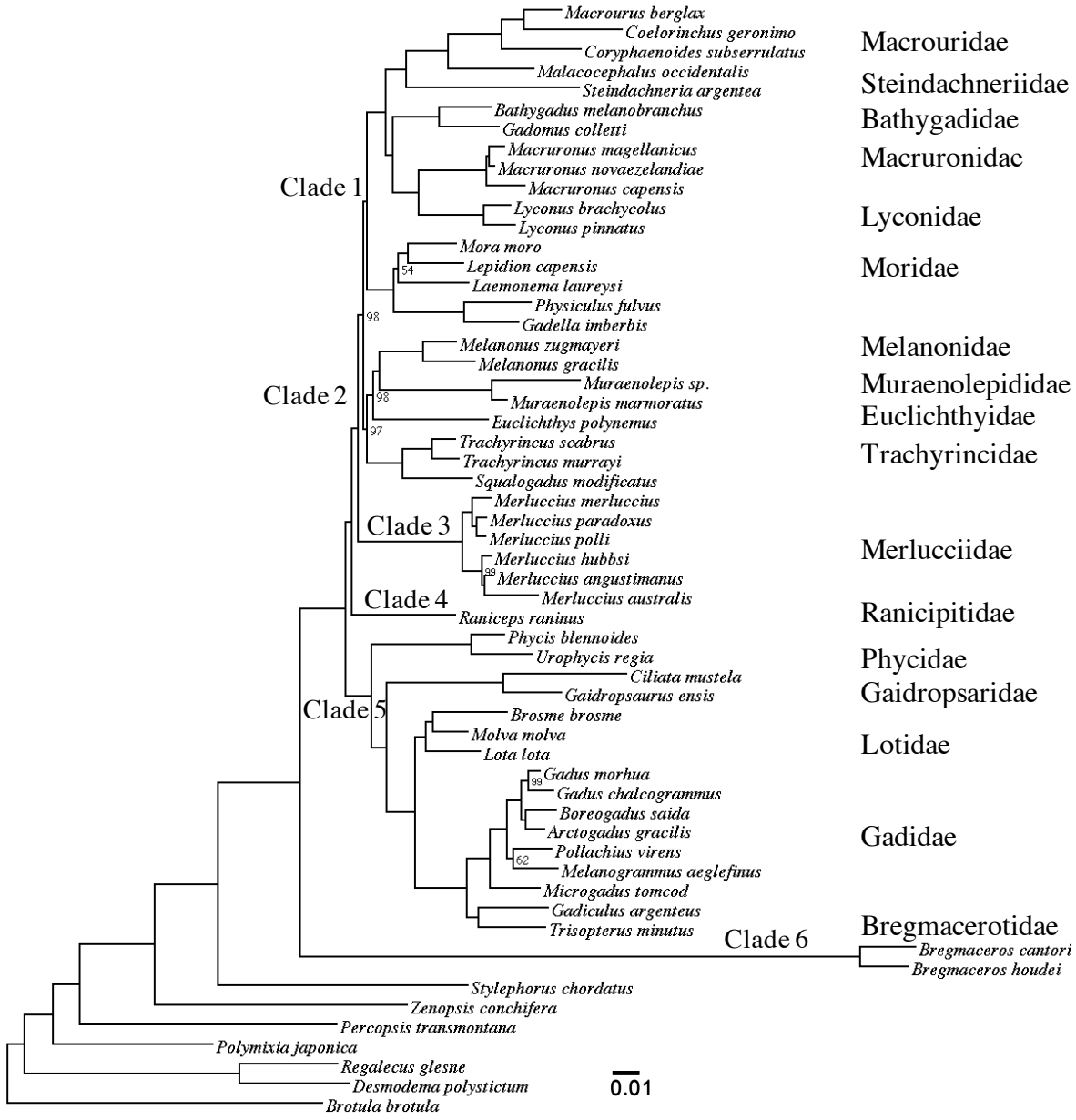


Figure 2.3 Relationships of Gadiformes inferred from ML analysis of 14,210 loci (2,874.858 sites) including 57 species and GTRGAMMA substitution model. All nodes had 100 % bootstrap values except when noted.

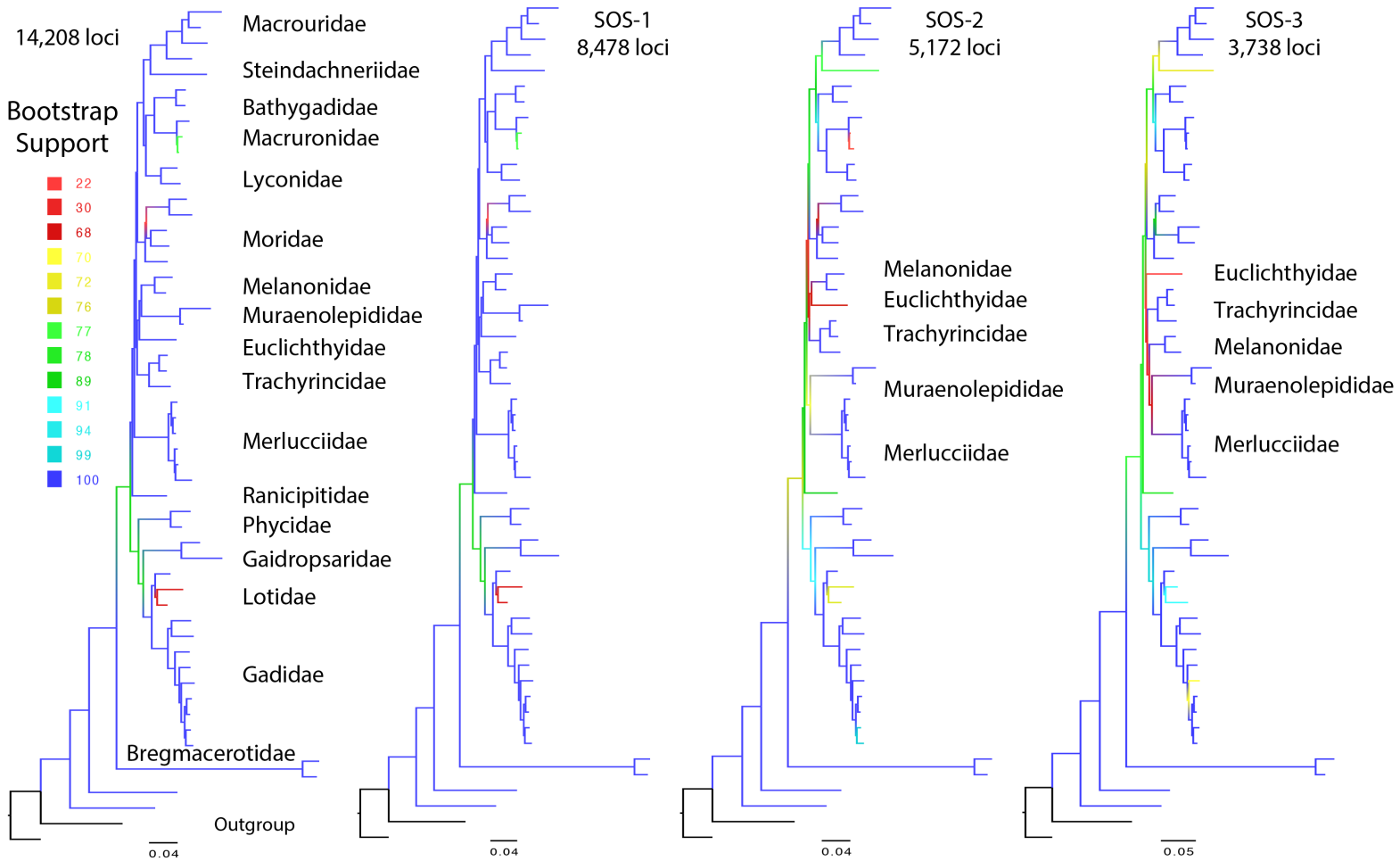


Figure 2.4 Data coverage and loci information content: a) DNA54 (11,068 loci), b) SOS-1 (8,478 loci), c) SOS-2 (5,172 loci), d) SOS-3 (5,172 loci). Color gradient correspond to bootstrap support values.

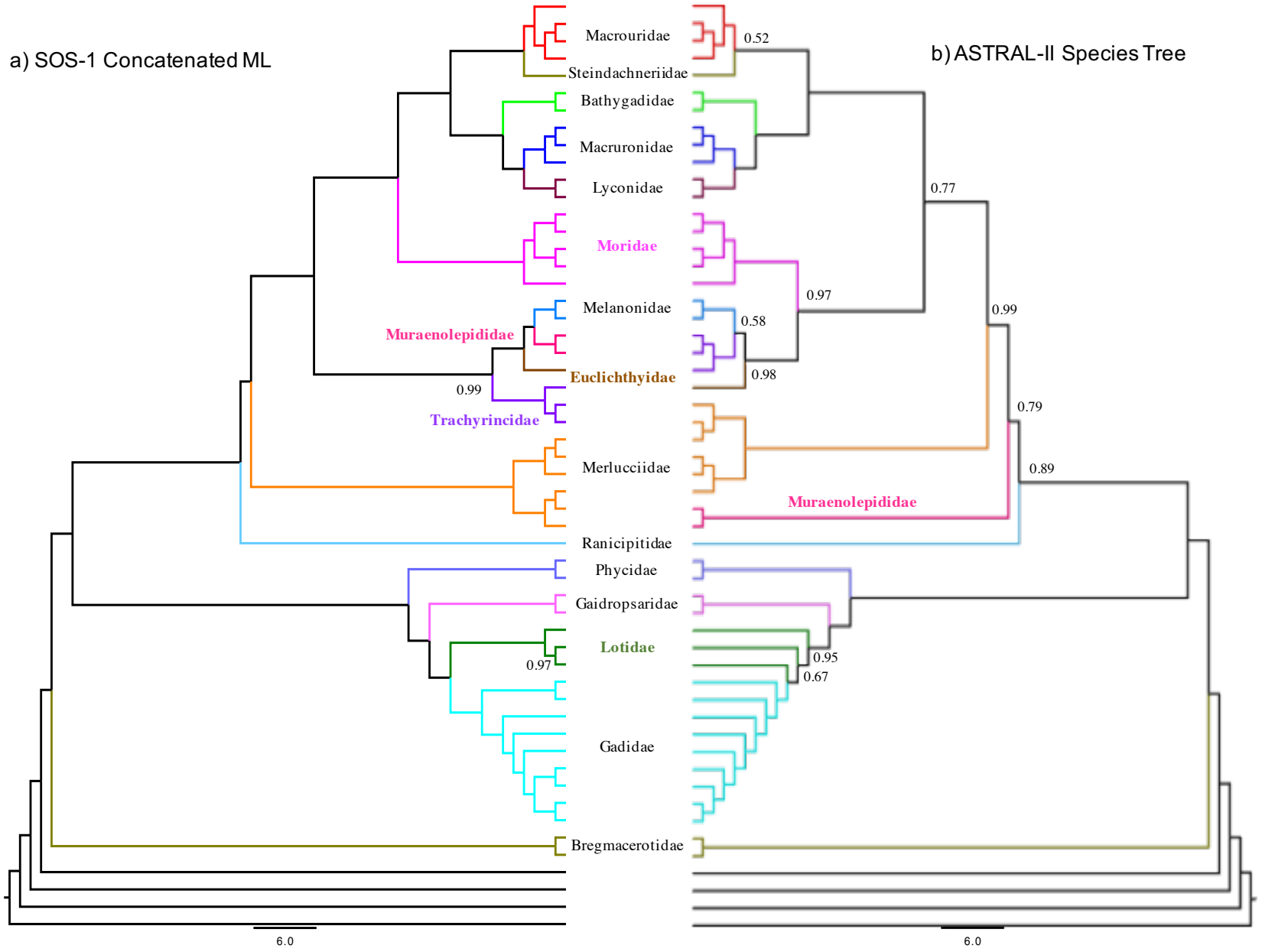

a) SOS-1 Concatenated ML
b) ASTRAL-II Species Tree
Macrouridae
Steindachneriidae
Bathygadidae
Macruronidae
Lyconidae
Moridae
Melanonidae
Muraenolepididae
Euclichthyidae
Trachyrincidae
Merlucciidae
Ranicipitidae
Phycidae
Gaidropsaridae
Lotidae
Gadidae
Bregmacerotidae
0.52
0.77
0.97
0.58
0.98
0.99
0.99
0.79
0.89
Muraenolepididae
0.97
0.95
0.67
6.0
6.0

Figure 2.5 Relationships of Gadiformes inferred from a) ML analysis of 8, 478 loci (1,821,891 sites) including 54 species and substitution model by codon position (P1: GTR+R4; P2 & P3: GTR+R6). b) Species-tree analyses carried out with ASTRAL-II. Number on nodes indicate 100 % bootstrap values except when noted.

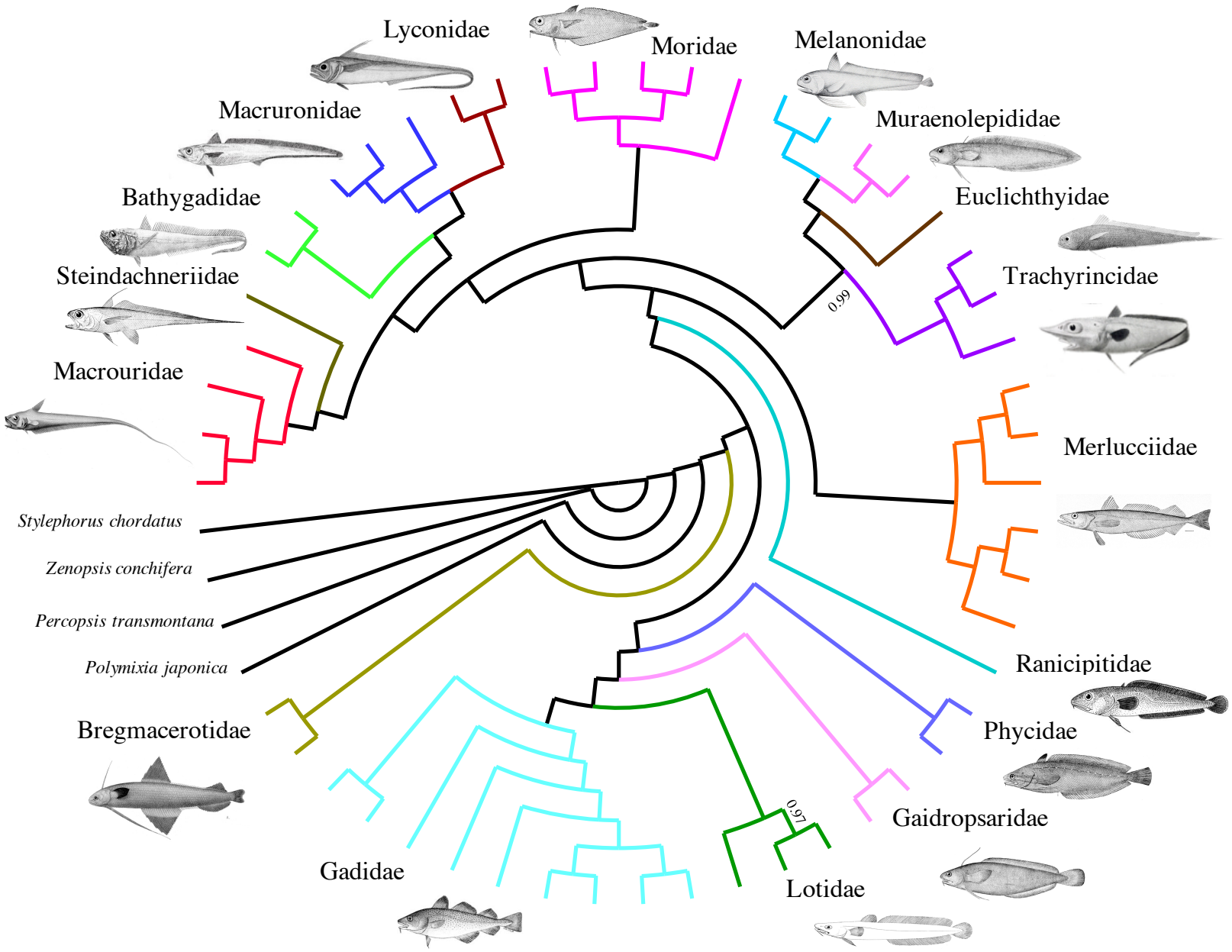

Lyconidae
Moridae
Melanonidae
Macruronidae
Muraenolepididae
Bathygadidae
Euclichthyidae
Steindachneriidae
Trachyrincidae
0.99
Macrouridae
Merlucciidae
Stylephorus chordatus
Zenopsis conchifera
Percopsis transmontana
Polymixia japonica
Ranicipitidae
Bregmacerotidae
Phycidae
0.97
Gaidropsaridae
Gadidae
Lotidae

Figure 2.6 Maximum likelihood tree and preferred phylogenomic hypothesis of Gadiformes inferred from 8, 478 loci (1,821,891 sites) and substitution model by codon position (P1: GTR+R4; P2 & P3: GTR+R6).

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POLLOCK WITH CLAMS AND CHORIZO

(by Anna Hansen)

INGREDIENTS

4 pollock or cod fillets, each weighing 150g
100g of cooking chorizo, spicy, diced
30 clams, fresh, scrubbed
10 cherry tomatoes, halved
125ml of white wine
1 handful of parsley, chopped
1 knob of butter, large
50ml of olive oil

PREPARATION

1. In a large pan with a tight-fitting lid (it needs to be large enough to hold the fish and all other ingredients), heat some olive oil over a moderate heat. Add the chorizo and fry until the fat begins to run out.

2. Move the chorizo to the side of the pan to create some room. Increase the heat and add the pollock, skin-side down, and fry for 1-2 minutes.

3. Add the clams, cherry tomatoes and white wine, cover with the lid and reduce to a medium heat once again. Cook for 2-3 minutes, be careful not to overcook the pollock.

4. Transfer the pollock along with any open clams to plates. Increase the heat, add the parsley and butter and simmer, shaking the pan occasionally.

Prep Time: 30 mins Cook Time: 15 mins Yield: 4

CHAPTER 3

Phylogeny of Merlucciidae (Gadiformes: Paracanthopterygii)

Based on Genome-Wide Molecular Data

3.1 Introduction

Hakes (*Merluccius*, Merlucciidae) are among the most important commercially harvested fishes in the world (e.g., Alheit and Pitcher 1985, Arancibia 2015, FAO 2016), and most species are currently considered to be over-exploited (FAO 2014). They are ecologically important in continental shelf ecosystems as predators and preys (Bolles and Begg 2000, Garrison and Link 2000, Perez-Perez et al. 2012). Merluciids are demersal fishes that have a high dispersal capacity (Inada 1981) and are widely distributed in the Atlantic and Pacific oceans and the waters around New Zealand; there are also isolated records of *Merluccius productus* (Ayres 1855) from the coast of Japan and the Indian Ocean (e.g., Lloris et al. 2005, Endo and Kitagawa 2006. Fig. 3.1). Despite their ecological importance, a significant commercial interest from at least the medieval times, and a long history of taxonomic study (Lopez 2000), the understanding of the systematic relationships among hake species remains poor.

Belon (1553) was the first to describe what is currently known as *Merluccius merluccius*, as Marlutiu vulgari. "Marlutiu" makes reference to Maris lucium (Mar: sea; lutiu: pike) and was the origin of the name *Merluccius*. It was not until Linnaeus (1758), that the first hake species (Marlutiu vulgaris) was described following the binomial system of nomenclature as *Gadus merluccius* and included within Gadidae. The genus *Merluccius* was described by Rafinesque (1810), based on *M. smiridus* (a junior synonym of *Merluccius merluccius)* using the type species of Marlutio vulgari. Adams (1864) separated *Merluccius* from Gadidae and proposed the family Merlucciidae and Gill (1884: 172-173) defined the characteristics of the family:

Gadoidea with a moderate caudal region coniform behind and with caudal rays procurrent forwards, the anus submedian, moderate suborbital bones, terminal mouth, subjugular ventral fins, dorsal double, a short anterior and long anal corresponding to the second dorsal; ribs wide, approximated, and channeled before with inflected sides, and paired excavated fontal bones with divergent crests continuous from the forked occipital crest.

The genus *Merluccius* has been included as a member of Gadidae (Kaup 1858, Günter 1862, 1887, Regan 1903, Cadenat 1937, Svetovidov 1937), or within the family Merlucciidae (e.g., Jordan and Evermann 1898, Jordan 1923, Belloc 1928, Norman 1937, Endo 2002, Nelson et al. 2016), sometimes as a subfamily Merlucciinae (Svetovidov 1948, Lloris et al. 2005). The fossil genus †*Palaeogadus* was included within Merlucciinae by Nikol'skii (1954). Norman (1937) included *Macruronus* in the family based on the attachment of the first vertebra to the skull and having separate frontal bones with ridges diverging from the occipital crest. Norman (1966) was the first to include both *Macruronus* and *Steindachneria* in the family Merlucciidae and placed them within the subfamily Macruroninae. Marshall (1966) expanded the family by including *Lyconus* (Lyconidae; Günther 1887), *Lyconodes* (Gilchrist 1922) along with *Merluccius*, *Macruronus,* and *Steindachneria argentea*. Marshall and Cohen (1973) removed *Steindachneria angentea* from the Merlucciidae to establish a monotypic family Steindachneridae, based on the position of the anus, having the urogenital pores widely separated and the presence of a light organ; within Merlucciidae they included the genera *Merluccius*, *Macruronus*, *Lyconus* and the fossil genera †*Paleogadus* and †*Rhinocephalus*. However, different authors continued to recognize different taxonomic concepts for the family. For example, Inada (1981, 1989) included four genera

(*Merluccius, Macruronus, Lyconus* and *Steindachneria*) and 19 species within two subfamilies (Merlucciinae and Steindachneriinae). Fahay and Markle (1984) recognized Steindachneriidae and Merlucciidae, but Dunn and Matarese (1984) included *Merluccius* in its own subfamily within Gadidae. Nelson (1984) proposed three subfamilies within Merlucciidae (Merlucciinae, Macruroninae, and Steindachneriinae). Endo (2002) recovered the Merlucciidae (*Merluccius*) and Macruronidae (*Macruronus* and *Lyconus*) within Gadoidei and Steindachneriidae within Macrouroidei. Lloris et al. (2005) recognized two subfamilies within Merlucciidae: (i) Merlucciinae (including only *Merluccius*) and (ii) Macruroninae (including *Macruronus, Lyconus* and *Lyconodes*).

As Inada (1989: 197) pointed out, within "gadoids, the merlucciids in particular seem to be composed of problem genera." Indeed, in addition to *Merluccius*, the other four genera historically included within Merlucciidae (*Lyconus*, *Lyconodes*, *Macruronus* and *Steindachneria*) also have a complex taxonomic history. Günther (1887) described *Lyconus pinnatus* based on a single specimen that was collected in the South Atlantic and proposed for it the family Lyconidae. Holt and Byrne (1906) described *L. brachycolus* based on another single specimen from the northeastern Atlantic. A second monotypic genus, *Lyconodes,* was described by Gilchrist (1922) from a single specimen (presumably lost, Lloris et al. 2005) and differentiated it from *Lyconus* by the absence of anterior caniniform teeth, elongate anterior dorsal-fin rays, and gill membranes fused to the isthmus. Howes (1991) reexamined the type specimens of both species of *Lyconus* (noting their poor condition) and suggested that *L. pinnatus* may be a juvenile specimen of *Macruronus,* as it differs only in having fewer teeth on the jaws, while *L. brachycolus* differs in having a single dorsal fin and the origin of the pelvic is more anterior. Howes (1991) recognized *L. brachycolus* as *Cynogadus* due to their caniniform teeth and for its similarities with the

macrourid genus *Cynomacrurus*, a subgenus of *Macruronus* and placed them within Macruronidae. Endo (2002) recognized *L, brachycolus* as *M. brachycolus* within Macruronidae and recovered macruronids and merlucciids as paraphyletic assemblages based on morphological data. Lyconidae was resurrected by von der Heyden and Matthee (2008) based on analysis of mitochondrial Cytochrome c oxidase I (COI) and Cytochrome b (Cyt *b*). However, the incomplete taxonomic sampling in their study limited the phylogenic placement of the family among gadiforms. Roa-Varón & Ortí (2009) presented the results of an analysis of one nuclear and two mitochondrial DNA (mtDNA) loci from 117 taxa, including all of the then-recognized families in gadiforms except the monotypic family Lyconidae. These authors recovered Merlucciidae, composed only of the genus *Merluccius,* within Gadoidei, with taxa assigned to Macruronidae and Steindachneriidae within Macrouroidei. However, their study was not able to test the phylogenetic relationship of Lyconidae within the Gadiformes due to the lack samples for *Lyconus* spp.

Macruronus has been placed as a subfamily (Macruroninae) or just as a genus within Macrouridae (e.g., Goode and Bean 1896, Regan 1903, Jordan 1923, Berg 1940). It has been also recognized as a subfamily Macruroninae within Merluciidae (e.g., Norman 1966, Nelson 1984) or at family level (Merlucciidae) including *Macruronus* and *Lyconus* (Markle 1989; Howes 1990, 1991a, 1991b; Endo 2002). *Steindachneria argentea* also has been regarded as a member of macrourids within Macruroninae (along with *Macruronus,* Regan 1903) or it its own subfamily (Steindachneriinae, Parr 1946); within the subfamilies Macruroninae (e.g., Norman 1966, Marshall 1966, Nelson 1976) or Steindachneriinae within merluccids (Nelson 1984; Cohen 1984, 1990; Inada 1981) or ranked at family level (Marshall and Cohen 1973, Fahay and Markle 1984, Markle 1989, Okamura 1989, Howes 1988, 1989, 1990, 1991a, 1991b, 1993, Nelson 1994). Several authors have considered the species as the sister group of macrourids (Markle 1989, Fahay

1989, Endo 2002) or Melanonidae (Howes 1989). In spite of these attempts to clarify the extent of Merlucciidae, consensus has not been reached (Endo 2002, von der Heyden and Matthee 2008, Roa-Varón and Ortí 2009).

Another level of disagreement is at the species level within *Merluccius*. In recent years there has been much splitting and lumping of taxa based on limited sampling and limited data on morphological and genetic variability, thereby increasing the confusion regarding the alpha taxonomy of the genus. Currently, the number of the species ranges from 12 (Eschmeyer et al. 2018) to 16 (Froese and Pauly 2018) depending on the author (Table 3.1). Identifying *Merluccius* species into species or subspecies is challenging due to similarities in their external morphology and ontogenetic changes – divergence is lessened and even disappears with growth in some species (e.g., Ginsburg 1954, Inada, 1981). Identification is further complicated by characters of considerable importance used in the keys that are difficult to use in practice. For example, scale characters (e.g., number of the scales in the lateral line, presence or absence of scales on lachrymal, scales on lower part of cheek and preopercular) often are broken even in nicely preserved specimens (Ginsburg 1954, Lozano-Cabo 1965). Further, ontogenetic variation has not been evaluated making intra and interspecific variability, if any, difficult to evaluate (Ginsburg 1954). The number of gill rakers, including tubercles (short rakers with the diameter of their bases greater than their height), is inconsistent among keys (Ginsburg 1954). The tubercles on the lower limb tend to merge, particularly in large individuals, or the change from the gill rakers to tubercles is gradual, thereby masking the individual variability in their number; including the tubercles represent another problem for separating species (Ginsburg 1954).

Many species in the genus *Merluccius* are sympatric, although species with overlapping geographic ranges may be found at different depths, these may have moderate overlap in their

depth profiles (e.g., *M. paradoxus* Franca 1960, *M. capensis* Castelnau 1861, and *M. polli* Cadenat 1950 are all found off Angola and partially overlapping in deep areas between 200-400 m). As a further complication, there are a number of potentially invalid species (e.g., *M. hernandezi* Mathews 1985 is possibly a synonym of *M. angustimanus* Garman 1899 or both could be synonyms of *M. productus*; Silva-Segundo et al. 2011) and the monophyly of several species with subspecies has yet to be satisfactorily established (e.g., *M. gayi peruanus* more closely resembles *M. angustimanus* than *M. gayi gayi*).

There have been few molecular studies focusing on species relationships within Merlucciidae. Roldan et al., (1999; based on allozyme data), Quintero and Mendez (2000; based on mitochondrial DNA), Grant and Leslie (2001; based on new and previous allozyme data and previous mitochondrial DNA data) and von der Heyden and Matthee (2008; based on COI and Cyt *b*) analyzed the systematic relationships of the family and recovered well-supported Old World (Euro-Africa) and New World clades (West Atlantic and East Pacific). However, no study included all currently recognized species. Grant and Leslie (2001) suggested that the genetic distance between these two primary clades corresponds to 10-15 million years of separation. This timing corresponds to the expansion of the North Atlantic Basin through the separation of North American and European plates (Van Andel 1976) and a gradual drop in high-latitude ocean temperatures, thereby preventing dispersal routes across the North Atlantic. Vicariance, dispersal, and hybridization all have been invoked to explain the evolution and distribution of *Merluccius* (e.g., Inada 1981, Ho 1982, Grant and Leslie 2001).

No comprehensive phylogenetic analysis that includes representatives of all the families among Gadiformes and all species of *Merluccius* has yet been conducted. Many aspects of the biology and management of Merlucciidae are hindered by the confusing taxonomy of the group.

Most importantly, this has led to mixed species in landings data, making stock assessment and conservation difficult. As a first step to improving the understanding of the phylogeny and taxonomy of Merlucciidae, this study aims to address the unsettled phylogenetic position of the genera that have been included historically within Merlucciidae (e.g., the taxonomic extent of the family) and the phylogenetic relationships among species of *Merluccius* using a more comprehensive phylogenetic data set, both in terms of taxon sampling and nucleotide data, and by using Next Generation Sequencing approaches to molecular data acquisition and analysis.

3.2 Materials and Methods

3.2.1. Species Sampling and Molecular Techniques

Two datasets were generated in order to assess (i) the phylogenetic placement of the genera historically included within Merlucciidae (SOS-54, Ch. 1) and (ii) the relationships among species of *Merluccius* (SOS-74). The first dataset included 54 taxa representing all recognized Gadiformes families and subfamilies (8,478 loci; see Ch. 1, Table 3.1). The second data set included 74 taxa (65 individuals of *Merluccius* from all potential species included in the genus and the outgroup species; Table 2). The outgroup taxa included nine representatives of gadiform families (*Bathygadus melanobranchus, Ciliata mustela, Euclichthys euclinichthys, Gadus morhua, Lyconus pinnatus, Macruronus magellanicus, Melanonus gracilis, Raniceps raninus, Steindachneria argentea*). Specimens of *Merluccius* were identified to species following Lloris et al. (2003) and Inada (1981) (Table 2). Vouchered museum specimens are from collections at United States National Museum of Natural History, Smithsonian Institution (**USNM**), California Academy of Sciences (**CAS**), Florida Museum of Natural History (**FLMNH**), National History Museum of Los Angeles County (**LACM**), Muséum National

d'Histoire Naturelle (**MNHN**), Makuriwa Museo de Historia Natural Marina de Colombia (**MHNMC**), Museo de Historia Natural, Universidad Ricardo Palma (**MHN-URP**).

3.2.1.1. Gene capture and probe design

The genome sequences of *Anguilla japonica*, *Danio rerio*, *Gadus morhua*, *Gasterosteus aculeatus*, *Lepisosteus oculatus*, *Oreochromis niloticus*, *Orizias laticeps* and *Tetraodon nigrovirilis*, were compared using the EvolMarkers tool pipeline (Li et al. 2012) to identify single-copy, conserved, protein coding sequences (CDS). A total of 14,217 exons shared by the eight-model species were retained by this search. Baits for target capture enrichment (Li et al 2013) were designed for these loci based on the sequences of the Atlantic cod (*Gadus morhua*). Bait sequences 120 bp in length were tiled to obtain 2X coverage of each targeted locus (60 bp overlap between baits). Biotinylated RNA probes of bait sequences were synthesized by Arbor Bioscience (formerly MYcroarray, Ann Arbor, Michigan).

3.2.1.2. Library preparation, gene capture and sequencing

Genomic DNA was quantified using a Qubit 2.0 fluorometer (Life Technologies Corporation, California, USA) and a sample of 0.5-3 ug was sheared to *c*. 500 bp using acoustic ultra-sonication on a Covaris E220 Focused-Ultrasonicator (Covaris, Inc., Massachusets, USA). Illumina sequencing libraries (Meyer & Kircher 2010) were then prepared for each sample using the "with-bead" method following Li et al. (2013), using adaptors labeled with unique 8 bp indices for identification. RNA baits were hybridized twice to individual libraries to increase the number of captured loci as suggested by Li et al. (2013). After target capture, the enriched individual libraries were pooled in equimolar ratios for sequencing on an Illumina HiSeq 2500

(Illumina, Inc, San Diego, CA). (Figure 2A; detailed laboratory protocol in S1, Supplementary Material)

3.2.1.3. Data assembly, orthology testing and alignment

The raw sequence data were demultiplexed according to the custom 8 bp indices for each sample. Adapters and low-quality reads (Phred score less than 20) were removed using the 'cutadapt' and 'FastQC' functions available in the wrapper script Trim Galore! (v0.4.4; Krueger 2012). PCR amplification duplicates were removed, and then the reads were parsed into different files according to their similarity representing each targeted sequence of the query (*G. morhua*) using a custom Perl script (S2, Supplementary material). Then, *de novo* assembly was performed using Trinity (Grabherr et al. 2011). Multiple overlapping contigs derived from the same targeted region were merged in Geneious vR10 (Kearse et al. 2012). The Smith-Waterman algorithm (Smith & Waterman 1981) was used to find the best matched sequence by comparing the query targeted sequence (*G. morhua*) with contigs derived from the *de novo* assembly. Finally, putatively orthologous genes were chosen by aligning the best matching contigs to the reference genome of *G. morhua* using BLAST+ (v. 2.4.0; Camacho et al. 2009). If the alignment returned a hit outside of the targeted region, the contig was discarded. The final output included two files, the coding regions without flanking regions, and the intronic sequences. The sequences without flanking regions were used for the downstream analysis. Each individual locus was translated into amino acids (AA), and then the AA and DNA data were aligned using the auto option in MAFFT (v.7.221; Katoh & Standley 2013). (Figure 2B; pipeline and scripts available in S2, Supplementary Material).

3.2.2. Phylogenomic Analyses

Concatenated DNA datasets (SOS-54 and SOS-74) were reconstructed with FASconCAT-G (Kück & Longo 2014). After assessing the coverage and the information content of loci using the software MARE v0.1.2-rc (**MA**trix **RE**duction, Meyer et al. 2011, see Ch. 1 for detailed description) two supermatrices were generated: SOS-54 (from14,210 to 8,478 from loci) and SOS-74 (from 13,771 to 8,243 loci), with percentage of capture efficiency by species ranging from 10.8 to 99.6 % (Table 2). Maximum likelihood (ML) analyses were conducted using RAxML-NG v0.2.0 BETA (DOI:10.5281/zenodo.492245) with a GTRGAMMA substitution model by codon position. The ML tree searches for each dataset were conducted using 20 distinct random starting trees. One-hundred non-parametric bootstrap replicates were conducted in RAxML-NG to assess nodal support. Pairwise uncorrected *p*-distances (proportion of nucleotide sites at which two sequences being compared are different) under were generated with MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets (Kumar et al. 2015) to compare genetic divergence among genera and within and among species, although they represent only a rough view of sequence divergence (Nei and Kumar, 2000).

3.3. Results

3.3.1. Maximum likelihood analysis

The ML analysis to infer the taxonomic extent of the family Merlucciidae included 54 taxa representing all the families and subfamilies among gadiforms. The topology inferred using GTRGAMMA model by codon position resulted in a well-resolved and well-supported topology (bs > 97%). At least four main lineages were recovered among gadiforms, the first one included (((Macrouridae , Steindachneriidae) , ((Macruronidae , Lyconidae) , Bathygadidae))) and

Moridae as the sister group. The second clade consisted of (((Melanonidae , Muraenolepididae) , Euclichthyidae) , Trachyrincidae)) with the sister group Merlucciidae. A separate lineage representing Ranicipitidae was found as the sister group of all of the previous clades. A fourth clade included all the gadoids (((Gadidae , Lotidae) , Gaidropsaridae) , Phycidae))). Finally, Bregmacerotidae was recovered as the sister group of all other Gadiformes (Ch. 1).

A ML analysis to test if there were reciprocally monophyletic clades among species of *Merluccius* species included 74 taxa representing all the current species described in the genus. The gene tree topology (Fig. 3.2) recovered two well-defined and supported lineages (bs = 100%): one including the eastern Atlantic species (i.e., taxa found along the coasts of Europe and Africa) and a second clade comprising western Atlantic and eastern Pacific species (Fig. 3.2, 3.3). The eastern Atlantic lineage was well resolved (bs = 100%) and was formed by two major clades (Fig. 3.2). The first of these subclades included *M. merluccius* (Linnaeus 1758) and its sister group *M. senegalensis* Cadenat 1950 + *M. capensis*. The second subclade was formed by *M. paradoxus*, which is found along the west coast of equatorial Africa, and *M. polli*, which is present along the continental shelf along southern and south-western Africa.

The New World lineage recovered *M. bilinearis* (from the western North Atlantic) as the earliest branching species (bs = 100%), followed by *M. albidus* (Mitchill 1818) (western Atlantic, bs = 100%) and *M. hubbsi* Marini 1933 (western South Atlantic, bs = 60%) (Fig. 3.3). *Merluccius hubbsi* is the sister group of a weakly supported clade (bs = 59%) involving two complexes of species: The first of these complexes includes the eastern Pacific species (*M. productus*, *M. hernandenzi*, *M. angustimanus* and *M. gayi gayi* (Guichenot 1848) and *M. gayi peruanus* Ginsburg 1954; bs = 60%) and the second includes *M. australis* (Hutton 1872) and *M.*

polylepis, both from southern South America and New Zealand (bs = 100%). The divergences within each of these clades are very low and weakly supported.

The values of *p*-distances revealed a pattern of increased nucleotide diversity at three levels: (1) within species, (2) among species of *Merluccius*, and (3) among genera. Scores ranges of average *p*-distances of the three categories were (1) 0.004 – 0.025, (2) 0.008 – 0.822 (3) and (4) , respectively.

3.4. Discussion

2.4.1. Taxonomic Extent of the Family Merlucciidae

The extent of the family Merlucciidae has been debated, ranging from including only one genus (*Merluccius*; Howes 1991) to five genera (*Merluccius*, *Macruronus, Lyconus, Lyconodes* and *Steindachneria*; Cohen et al. 1990) with two (Merluccinae and Steindachneriinae; van der Laan 2014) or three (Merlucciinae, Macruroninae and Steindachneriinae; Marshall 1966, Cohen 1984, Nelson 1984) subfamilies. The present study recovered the family Merlucciidae including only the genus *Merluccius* with strong support (bs = 100%). This corroborates the morphological concept of the family according to Howes (1991a) and Endo (2002), who suggested morphological synapomorphies for the family include enlarged vertebral parapophyses, medial prootic shelves forming a pseudo-posterior myodome, and the presence of an intermuscular process on the hyomandibula.

Among the most enigmatic and poorly known genera within Gadiformes are *Lyconus* and *Lyconodes,* because they are rarely captured. *Lyconus* includes two species, *L. brachycolus* with few reports from the northwestern, northeastern and southeastern Atlantic (Cohen et al. 1990) and *L. pinnatus* with isolated captures from southern Atlantic, Madagascar shelf, southern

Australia and east of New Zealand (Cohen et al. 1990). The other genus, is *Lyconodes,* is monotypic (*L. argenteus*) and known from only one a single specimen that has been lost (Lloris et al. 2005). The family was resurrected by von der Heyden and Matthee (2008), but its phylogenetic placement remained elusive. The current study included samples of both *Lyconus* species and they were recovered as a monophyletic group with strong support (bs = 100%), corroborating the resurrection of the family. Macruronidae was recovered as a monophyletic group with strong support (bs =100%) as the sister group of Lyconidae (BS=100%). These two families were previously placed as *incertae sedis* by Marshall (1973) or closely related but phylogenetically separated from *Merluccius* by Howes (1991b). Lyconidae and Macruronidae share some morphological characters such as the anal and the urogenital opening are close together and anterior to the anal fin, and the pelvic fins are posterior to the pectoral fins, but differ in other characters, such as in the presence of two dorsal fins and elements of the caudal skeleton elements in Macrunonidae (Lyconidae has a single dorsal and the caudal fin is essentially absent). These two families are not closely related to Merlucciidae, but rather together are the sister group to Bathygadidae ((Macruronidae + Lyconidae) + Bathygadidae). Inada (1981) provided the following morphological characters supporting this relationship: (i) the foramen of the trigeminofacilis nerve is absent; (ii) the upper window on the suspensorium is closed; (iii) the caudal fin tapers and is confluent with the dorsal and anal fins, (iv) and the presences of few epipelurals. This group in turn is the sister-group of Macrouridae + Steindachneriidae (Ch. 2: Fig. 2.6). These findings support the results of Marshall and Cohen (1973), who removed *Steindachneria angentea* from the Merlucciidae to establish a monotypic Steindachneridae based on the presence of a unique light organ, the position of the anus between the pelvic fins, and the urogenital opening close to the anal fin. The sister relationship among

Macrouridae and Steindachneriidae is supported by the vertically developed anterior portion of the parasphenoid also present in Bathygadidae while the development in *Merluccius*, *Macruronus* and *Lyconus* is horizontally (Okamura 1970, Inada 1989).

3.4.2. Phylogenetic relationships among species of *Merluccius*

This study recovered two clades representing the Old World clade and the New World clades of *Merluccius*, which corroborates previous molecular studies (e.g., Campo et al. 2007, Grant and Leslie 2001, Quintero et al. 2000, Roldan 1999). Two hypotheses have been proposed for the origin and dispersal of *Merluccius*: (i) hakes originated in the north-eastern Atlantic-Arctic and dispersed along the west coast of Europe and along the east coast of North America as a result of the expansion of the North Atlantic Basin and the gradual drop in high-latitude ocean temperatures (e.g., Svetovidov 1948, Inada 1981, Kabata and Ho 1981, Fedotov and Bannikov 1989, Grant and Leslie 2001, Fig. 3.5a). (ii) hakes originated in the western North Atlantic (Szidat 1961; Ho, 1974, 1990; Roldan et al. 1999) and *M. albidus* was proposed as the earliest branching of all the species of *Merluccius* (Ho 1990). The results obtained in this study are consistent with a north-eastern Atlantic-Arctic origin for the genus *Merluccius*, which diverged into two major *Merluccius* lineages (the Old and the New World). *Merluccius albidus* was found nested within the New World clade and the earliest branch within the clade was *M. bilinearis*. The oldest known fossils of merluccids (*Paleogadus sinangulatus*) from the Selandian of Denmark, is about 61 Ma. also supports the north-eastern Atlantic-Arctic origin for the genus *Merluccius* when an inland sea connected to a temperate Arctic Ocean, covered mostly of central Europe and separated Europe from Asia.

Within the Old World clade, *M. merluccius* and *M. senegalensis* have been suggested to be sister species (e.g., Campo et al. 2007, Quinteiro et al. 2000) whereas in other studies, *M. senegalensis* and *M. capensis* are recovered as sister taxa (Grant and Leslie 2001, Roldan et al. 1999). In this study, the Old World clade was fully resolved (bs = 100%) and two lineages were found, one comprising *M. polli* and *M. paradoxus* and the other comprising the clade *M. senegalensis* + *M. capensis*, with *M. merluccius* as its sister taxa (Fig. 3.2).

In the coastal waters of southern Africa *M. capensis* (the Shallow-water Cape Hake) and *M. paradoxus* (the Deep-water Cape Hake) have overlapping distributions along the coasts of Namibia and South Africa, but as their common names suggest, the two species inhabit different depths (e.g., Botha et al. 1985, von der Heyden et al. 2007). They are commercially and ecologically important and have been recently subject of several studies, as potential hybridization was suspected due to their sympatric distribution. Millares et al. (2014) sampled 296 individuals of Cape hakes and used eight microsatellite loci, mitochondrial (COI) and nuclear (5S rDNA) molecular markers. Hybridization and introgression was found between both species with a north-south gradient and the highest proportion in the North Benguela off the Namibian coast. However, von der Heyden et al. (2016) analyzed 1,137 Cape hake collected across Southern Africa between 2012-2013. This study used nine microsatellite loci and mtDNA control region sequences to assess the validity of the hybridization proposed by Millares et al. (2014). Their results suggested that the reported hybridization was a result of homoplasy, and therefore the two species are distinct. Their study highlighted the importance of conducting a priori simulation studies for avoiding bias of the hybrids identification associated with the number of loci, variability and models used in the analyses. von der Heyden et al. (2016) drew attention to the relevance of the analytical methods used in population genetics, especially when

the species are commercially exploited, because accurate data is needed for adequate management and conservation strategies. The stock assessment, however, is still performed for *Merluccius paradoxus* and *M. capensis* combined (Kirchner et al. 2012; Paterson and Kainge 2014). Therefore, there is uncertainty surrounding the fishing mortality as well as the reporting measures because of the non-segregation of these two species; According to the Ministry of Fisheries and Marine Resources report a recently implemented management plan anticipates future stock assessments to be separated by species (Esau 2014). The current study corroborates the absence of a sister-species relationships between *Merluccius paradoxus* and *M. capensis* and supported the hypothesis proposed by Grant & Leslie (2001) and Campo et al. (2007, 2009) in which these species colonized the southeastern Atlantic in two independent events (Fig. 3.4 c,d).

Merluccius senegalensis has an overlapping distribution with *M. merluccius* in the north, up to Cape Blanc and with the *M. polli* in the south, from Cape Barbas to Cape Roxo, (FAO 2012, Lloris et al 2005). *Merluccius merluccius* and *M. senegalensis* are commercially important while *M. polli* is of little commercial interest, but likely still is taken in the fishery (Lloris et al. 2005). *Merluccius merluccius* is distributed over the Northeast Atlantic shelf, from Norway and Iceland to Mauritania (Lloris et al. 2005). Two different stock units have been recognized since the late 1970s: (i) the northern stock distributed throughout the Kattegat, the Skagerrak, the North Sea, the English Channel, to the west of Scotland and Ireland and into the Bay of Biscay and the (ii) southern stock along the Spanish and Portuguese coasts. Cape Breton canyon is considered to be a geographical boundary limiting exchanges between the two stocks (ICES, 2013). Recent molecular studies have suggested genetic connectivity among Atlantic grounds as a result of migration from the Celtic Sea towards its adjacent Atlantic grounds, which could imply that the spawning biomass of the northern *M. merluccius* plays a crucial role in ensuring

the sustainability of southern fishing grounds (Pita et al. 2010, 2013). *Merluccius merluccius* is listed as Vulnerable and there is evidence supporting that this species is over-exploited and that over-fishing is a major threat to the populations (Di et al. 2011)

This study included three samples, one from the Mediterranean (southern stock) and two from the Bay of Biscay (northern stock). The species was recovered as monophyletic and sister to the clade including *M. senegalensis* and *M. polli*. The sister relationships among *M. senegalensis* and *M. polli* support allopatric speciation over parapatric speciation, in which individuals of *M. capensis* migrated northward across the equator to establish the West African population of *M. senegalensis* (Roldan et al. 1999, Grant and Leslie 2001, Fig.3.3 a,c).

In the New World clade, *Merluccius bilinearis* (Mitchill 1814) was recovered as the earliest branch (bs = 100%), followed by the *M. albidus* (western Atlantic, bs = 100%). These species are sympatric and morphologically similar. *Merluccius bilinearis* is distributed on the continental shelf from Canada south along the U.S. to southeast Florida between 0-914 m, but is most abundant on sandy continental shelf between 50-300 m (Carpenter, K.E. 2015, Lloris et al. 2000, Cohen *et al*. 1990), whereas *M. albidus* is distributed from Nova Scotia south along the U.S. coast, throughout the Gulf of Mexico and along the Central and South American coast from Mexico to French Guiana and some areas in the Caribbean between 60 and 1170 m, although it is most commonly found between 160-640 m (McEachran et al. 2018, Cohen *et al*. 1990). Ginsburg (1954) described *M. magnoculus* from the Gulf of Mexico (GoM), differing from *M. albidus* by a longer head and shorter pectoral and pelvic fins. However, Karnella (1973) and Inada (1981) considered it to be a synonym of *M. abidus*. Lloris et al. (2005) proposed that *M. albidus* from the GoM formed a valid subspecies (*M. abidus magnoculus*) due to the variability in the number of vertebrae, fin rays, and gill rakers. *Merluccius bilinearis* accounts for one-half of the total European consumption of hake

(Alheit & Pitcher 1995), but *M. albidus* is often sold in European markets as *M. bilinearis* (Garcia-Vazquez et al. 2009, 2012; Helser and Alade 2012). There is a pressing need to better diagnose western Atlantic hakes to avoid inclusion of *M. albidus* in landings statistics (Garcia-Vazquez et al. 2009). However, the geographic overlap these two species and high levels of bidirectional introgressive hybridization make it difficult to understand the species boundaries (Machado-Schiaffino et al. 2010). This study recovered a monophyletic *M. albidus* (bs = 100%) (Fig. 3.1, 3.2). Geographic structure was revealed, with the two specimens from the GoM grouping together and the other three specimens from the southeast Atlantic recovered as more closely related to each other. However, this ML analysis performed represents only a first step in testing for reciprocal monophyletic clades, and further analyses are required to infer species delimitation among these populations and species.

The monophyly of the Argentinean or common hake, *Merluccius hubbsi* was weakly supported (bs = 60%) in this analysis and sister to a clade involving two species complexes (the Patagonia – New Zealand complex and the eastern Pacific complex) with strong support (bs = 100%). *Merluccius hubbsi* inhabits the shelf and upper continental slope from southern Brazil to southern Argentina with four stocks: (i) from 21°S to 29°S, in Southeastern Brazil, (ii) from 29°S to 41°S, shared by Brazil, Uruguay and Argentina, (iii) from 41°S to 54°S in Southern Argentina (Patagonian stock), and (iiii) Falkland Islands/ Malvinas stock (Bezzi et al. 1997, Vaz-dos-Santos et al. 2009, Arkhipkin et al. 2015). The species is the main target of the Uruguayan and Argentinean fleets, and since 2001 has been targeted by Brazilian trawlers (Perez et al. 2003; Vaz-dos-Santos & Rossi-Wongtschowski 2005). *Merluccius hubbsi* is over-exploited, and both their total and spawning biomass have crashed (Cordo 2006), and it was listed as threatened species (Vaz-dos- Santos et al. 2010). More recent evaluation has showed the stock of the

Patagonian shelf to be overexploited, while the Falkland/Malvinas Islands is only moderately exploited (Arkhipkin et al. 2012). High recruitment levels have been recorded, although it appears that most fish do not survive to become older spawners (Santos and Villarino 2015) and the impacts of the fishery on the ecosystem are poorly known (CeDePesca 2015).

Lloris & Matallanas (2003) described *M. patagonicus* Lloris & Matallanas 2003 (Synonym of *Merluccius hubbsi* Marini 1933; Díaz de Astarloa et al. 2011) from the Argentine Sea and discussed its relationships to *M. hubbsi* and *M. australis*. These three species are sympatric and are difficult to distinguish in commercial catches (Lloris et al. 2005). According to the original description, *M. patagonicus* is characterized by the presence of scales on the lower part of the check and preopercle, and their absence on the lower part of the interopercle (Lloris & Matallanas 2003). Díaz de Astarloa et al. (2011), however, considered *M. hubbsi* and *M. patagonicus* to be conspecific based on examination of six paratypes and 209 specimens of *M. hubssi* and the holoytype and three paratypes of *M. patagonicus*. Using external (meristic and morphometric data) and internal (the shape of the hyomandibula, urohyal and sagittal otolith) morphology, these authors found no difference between the two species and therefore the two species was synonymized. No specimens with the putative characteristics of *M. patagonicus* were available for this study.

This study included specimens of *M. polylepis* from Chile and Argentina and specimens of *M. australis* from New Zealand. No clear support for monophyly of either species was found, despite their allopatry (bs = 30%). Ginsburg (1954) described *M. polylepis* from Chile-Argentina, but Inada (1981) synonymized *M. polylepis* with *M. australis* for New Zealand-Australia and southern Chile-Argentina, based on morphological data. According to molecular data, using allozyme data considered initially two species, *M. australis* from New Zealand-Australia and M.

polylepis southern Chile-Argentina, but Grant and Leslie (2001) found low levels of genetic divergence, leading to the suggestion of a single species and further subspecific recognition. Matallanas & Lloris (2006) described *M. tasmanicus* Matallanas & Lloris 2006 (Synonym of *Merluccius australis* (Hutton 1872); Deli Antoni et al. 2015) from New Zealand and redescribed *M. australis*. However, *M. tasmanicus* was described based on the holotype of *M. polylepis* (Ginsburg 1954), and therefore the new species was automatically a junior synonym of *M. polylepis*. I alerted Drs. Collette and Eschmeyer about this issue in 2013, and the online Catalog of Fishes was corrected in 2014. Subsequently, Deli Antoni et al. (2015) suggested that *M. tasmanicus* is a junior synonym of *M. australis* based on morphological and DNA-based barcoding. However, following the ICZN (1999), the species is a junior synonym of the first described species, in this case *M. polylepis* (W. N. Eschmeyer, pers. comm. 2014). The taxonomic status for *M. polylepis* is confusing because it was synonymized with *M. australis* and two geographic populations are recognized: one in New Zealand and the other in waters of southern South America (Inada 1981).

Currently, in fisheries *M. polylepis* is recognized as a population of *M. australis* and at species level (Eschmeyer et al. 2018). *Merluccius polylepis* and *M. australis* support important industrial and artisanal fisheries in Chile, Argentina and New Zealand. *Merluccius polylepis* is found from the Chilean coast down to the southern tip of the continent and it is managed and assessed by Chile (Lloris et al. 2005). The fishery is overfished and overfishing is occurring (IFOP 2017). On the other hand, *Merluccius australis* has three biological stocks in New Zealand waters (Chatham Rise, Sub-Antarctic, and West coast of South Island), but no ecosystem-based management has been implemented in New Zealand (Horn 2015). The distribution, growth, reproduction, recruitment success and feeding are well understood, but there are uncertainties

concerning the stock structure and natural mortality rate (Horn 2015). There are several other species with disjunct distributions along southern Australia-New Zealand and Chile-Argentina, for example, the blue grenadier, *Macruronus novazelandiae* and the Patagonian grenadier *M. magellanicus*. The lack of genetic isolation and larval and adult morphological differentiation has led to the suggestion that these species are synonyms (Olavarria et al. 2006, Lloris et al. 2005). A comparable lack of genetic isolation and similar disjunctive distributions across the Southern Hemisphere has been observed in *Micromesistius australis*, two subspecies (i) *M. australis australis* off Chile, southern Argentina, and the Falkland Islands and (ii) *M. australis pallidus* from sub Antarctic waters off New Zealand are recognized based on morphological data (Inada & Nakamura 1975) and molecular data (Ryan et al. 2002). Therefore, it will not be surprising if this complex represents only one species, *M. australis*, after the implementation of species delimitation analyses.

The eastern Pacific complex of *Merluccius* includes *M. productus, M. hernandezi*, *M. angustimanus*, *M. gayi gayi* and *M. g. peruanus*. The Pacific hake, *M. productus* is the target of one of the most important commercial fisheries along North America's west coast (Ressler et al. 2007, Hamel et al. 2015). The species is widely distributed along the Pacific west coast of North America from Baja California to southeastern Alaska (Dark et al. 1980, Saunders and McFarlane 1997, Wilson et al. 2000, Ressler et al. 2007). The distribution of the migrating coastal stock varies from about lat. 25°N to lat. 55°N, moving north in summer and south in winter (Ressler et al. 2007). High levels of environmental variability (e.g., reduction of the influence of the upwelling systems) in the equatorial Pacific region induced by the ENSO (El Niño Southern Oscillation) and LNSO (La Niña Southern Oscillation) affects its distribution and abundance within the California Current Large Marine Ecosystem (CCLME) (Benson and Trites 2002, King 2005, Ressler et al.

2007). At least three biologically and genetically distinct stocks are recognized along the coast of North America: (i) Puget Sound (ii), Strait of Georgia and (iii) Baja California (e.g., Iwamoto et al. 2004, King et al. 2012, Hamel et al. 2015). The Baja California stock is known as the dwarf species and its status is controversial. Vrooman and Paloma (1976) suggested that it could be a different species distinct from *M. productus*, while Inada (1981) recognized it as a population of *M. angustimanus*. Silva-Segundo et al. (2011) suggested that *M. productus*, *M. hernandezi* and *M. angustimanus*, all are a single species and proposed *M. productus* as the only species present along the North American and northern Central American coast. Ichthyoplankton surveys have not able to locate larvae of *M. angustimanus* from the coast of off California and Baja California and only larval *M. productus* has been found in these regions (Ahlstrom and Counts 1955, Ahlstrom 1969, Ambrose 1996, Smith and Moser 2003). Silva-Segundo et al. (2011) analyzed 461 individuals covering the range of distribution of *M. productus* and *M. angustimanus* using traditional meristic and morphometric characters applied to identify the species (e.g., the number of anal fin rays of the first and second dorsal; gill rakers number; the number of vertebrae, diameter of eye orbit, length of the pectoral fin, pre-orbital length). These authors were unable to distinguish species of hake since most characteristics overlapped and suggested the presence of three contiguous and overlapping groups in three geographic areas (northern, southern and eastern group) that could result in response to ocean-climate variation within the distributional range. High levels of haplotype diversity but low nucleotide diversity was found analyzing molecular data (Cyt *b*, COI, and 16S rDNA), which suggest a high degree of gene flow and supports the hypothesis of a single hake species for this region, with two population units (one population from Washington to Costa Rica and the other in the upper Gulf of California) with some degree of gene flow between them. The authors suggested a single taxonomic entity (*M. productus*) with a minor degree of

morphological and genetic intra-specific variation. Based on a study at population level to determine the magnitude of gene flow among the Pacific populations, Silva-Segundo et al. (2011) anticipated a future reclassification renaming all hake in the eastern Pacific, probably as *M. gayi* (Guichenot 1848), according to the principle of priority (ICZN 1999).

Two subspecies of *Merluccius gayi* were proposed by Ginsburg (1954): *M. gayi-gayi* distributed on the continental shelf and slope off Chile and *M. gayi-peruanus* from off Ecuador, and the Galápagos Islands to Peru (García Domínguez et al. 2014, Lloris et al. 2005). Leiblie-Diaz (1979) and (Inada 1981) suggested the slight morphological difference between these two subspecies is due to latitudinal gradients along the west coast of South America and they are better recognized as a single species. The habitat of these two subspecies is influenced by the interaction of three water masses (the equatorial subsurface waters, ESSW flowing southward along the Peru-Chile undercurrent; The Antarctic intermediate water, AAIW and subantarctic water, SAW), poleward circulation of the Peru-Chile undercurrent, and stronger wind-driven upwelling events during summer time (e.g., Strub et al. 1998, Vidal et al. 2012). There is little geographic separation between the subspecies as a result of latitudinal shifts in distribution associated with climate-ocean oscillations such as ENSO events (Espino et al. 1995, Guevara-Carrasco and Lleonart 2008). During el Niño conditions for a wider distribution of hake is generated (from Chilean water waters when the southern limit of the *M. gayi-peruanus* may be shifted to c. 18°), while during La Niña events have the opposite effects (Guillén et al. 1985, Espino et al. 1995, Guevara-Carrasco and Lleonart 2008, Vidal et al. 2012). *Merluccius gayi-peruanus* more closely resembles *M. angustimanus* than *M. gayi-gayi* in terms of some meristic characters, such as number of gill rakers, vertebrae and all fin rays (Lloris et al. 2005). In 1965, Berry (unpublished, cited by Inada 1981) suggested that only two species occur in the eastern Pacific from Alaska to southern Chile: *M.*

polylepis from southern Chile, and *M. gayi* (including *M. productus*, *M. angustimanus*, *M. gayi-peruanus,* and *M. gayi-gayi*). According to Berry, the morphological characters used to identify the additional species were environmentally induced variations related to its geographic range. Moreover, Ho (1990) using a cladistic analysis of Inada's (1981) osteological data was not able to resolve the relationships among the eastern Pacific hakes (*M. gayi*, *M. angustimanus* and *M. productus*) and the two western North Atlantic species (*M. albidus* and *M. bilinearis*) due to the high level of homoplasy.

For first time in any molecular based phylogenic analysis of the family, the present study included specimens of *M. angustimanus* from Costa Rica and Colombia (three for each location) and three specimens identified tentatively as *M. hernandezi* from Mexico (pers. comm. Dr. Hector Espinosa - UNAM). Additionally, the sampling included one specimen from *M. productus* off Point Loma (San Diego, California); four specimens of *M. gayi-peruanus* from the fishing ground of the Santa Rosa District, Chiclayo, Peru and three specimens of *M. gayi-gayi* from Talcahuano, Bío-Bío Region in Chile. Two weakly supported clades were recovered, the first one including *M. productus*, *M. cf. hernandezi*, *M. angustimanus*, *M. gayi-peruanus* and *M. gayi-gayi* (bs = 58 %). The second clade contains *M. angustimanus*, *M. gayi-peruanus* and *M. gayi-gayi* (bs = 29 %). The preliminary results from this study did not recover the monophyly for any of the species in the eastern Pacific. Similarly, phylogenetic clades did not match geographic origin of the samples. These results support the presence of only one species in the eastern-Pacific from Alaska to southern Chile (*M. gayi*, including *M. productus, M. angustimanus*, *M. gayi-peruanus*, and *M. gayi-gayi*) and *M. australis* from southern Chile. However, further species delimitation analyses integrating molecular and morphological data, as well as more detailed genetic analyses using SNP data, are necessary to assess the extent of genetic isolation

among putative species and to determine the magnitude of gene flow among Pacific populations. This type of analyses could provide further resolution of the taxonomy of hake in the eastern Pacific.

3.4.3. Biogeography

Three hypotheses have been proposed for the origin and dispersal of *Merluccius*. The first one suggested that hakes originated in the north-eastern Atlantic-Arctic and dispersed along the west coast of Europe and along the east coast of North America as a result of the expansion of the North Atlantic Basin and the gradual drop in high-latitude ocean temperatures (e.g., Svetovidov 1948, Inada 1981, Kabata and Ho 1981, Fedotov and Bannikov 1989, Grant and Leslie 2001, Fig. 3.6a). The second hypothesis proposed by Szidat (1961) based on parasites distributions considered *Merluccius* to have originated in the North Pacific; however, the parasites' taxonomy was rejected and therefore his zoogeographical hypothesis. The third hypothesis implied that hakes originated in the western North Atlantic (Ho 1974, 1990; Roldan et al. 1999; Fig. 3.5b) and *M. albidus* was proposed as the earliest branching of all the *Merluccius* species (Ho 1990). The results obtained in this study are consistent with a northeastern Atlantic-Arctic origin for the genus and earliest known fossils of merluccids in the Middle and Upper Oligocene in Europe when an inland sea connected to a temperate Arctic Ocean, covered mostly of central Europe and separated Europe from Asia (e.g., Inada 1981, Fedotov and Bannikov 1989, Grant and Leslie 2001).

The two lineages recovered in the Old World indicates two independent diversification events from an ancestral *Merluccius*. These two lineages are known as the "paradoxus" (*M. paradoxus* + *M. polli*) and the "capensis" (*M. Merluccius* (*M. senegalensis* + *M. capensis*) lineages (Grant and Leslie 2001) The ancestral *Merluccius* migrated into southern Africa and

dispersed back across the equator to establish the population of *M. polli,* first in southern Africa and then in west Africa (Grant and Leslie 2001, Fig. 3.6b). In the second diversification event individuals of *M. capensis* moved northward across the equator to establish the West African populations of *M. senegalensis* (Grant and Leslie 2001, Fig. 3.6c). The results of this study corroborate the absence of a sister-species relationship between *M. paradoxus* and *M. capensis* and supported the hypothesis proposed by Grant & Leslie (2001, Fig. 3.4c) and Campo et al. (2007, 2009; Fig. 3.4d) in which these species colonized the southeastern Atlantic in two independent events.

Two hypotheses for the origin and dispersal of *Merluccius* in the New World have been proposed, and the phylogenetic position of *M. hubbsi* plays a key role in both. These hypotheses have in common that hakes originated in the Atlantic and entered to the Pacific through the Panama seaway, but they disagree in whether *M. hubbsi* arose from an eastern South Pacific stock around Cape Horn (Szidat 1955, Inada 1991, Grant and Leslie 2001; Fig. 3.5a, 3.6e) or from a North Atlantic stock (Kabata and Ho 1981, Ho 1990; Fig. 3.5b). According to Inada (1981) *Merluccius* migrated from the western Atlantic once during the Pliocene over the submerged Panamanian Isthmus and discarded the migration to the South Atlantic along the coast of Brazil because the barrier effect of the Amazon River. However, prior to the reversal of the drainage pattern of the Amazon River from the western and northwestern direction to an eastern direction between 11.8 and 11.3 Ma ago (Middle to Late Miocene), the salinity was not as low as it reached in the late Pliocene (Hoorn 1993, Hoorn et al. 1995, Caputo and Amaral 2016) and it would have not represented a barrier to a southern migration along the coast of Brazil. On the other hand, the rise of the Panama isthmus separated the Eastern Pacific from the Caribbean Sea gradually over a period of 12 million years (My) process and finally closing

completely in the Pleistocene, three to four My ago (Coates and Stallard 2013, O'Dea et al. 2016). The formation of the Isthmus of Panama has been associated with tectonic vicariance as a result to changes in current flow, salinity, temperature and primary production in the Atlantic and eastern Pacific and potential dispersal path-ways (e.g., Van Andel, 1976, Lessios 2008, Cowman and Bellwood 2013, Thacker 2016).

Finding *M. hubbsi* as the earliest branch to a clade formed by the two complexes of species with strong support (bs = 100 %), does not falsify the hypothesis of a North Atlantic ancestor that migrated southward along South America. Grant and Leslie (2001) suggested dispersal and allopatric isolation as an important mechanism explaining the distribution in hakes and for other temperate marine fishes. Additionally, a dispersal event associated with the drop in the surface temperatures as a result of the closure of the Panama Seaway, which allowed the cool waters of the California Current to reach Central America and northwestern South America, promoted a shift from tropical to temperate species in the Chilean marine fauna (Duque-Cano 1990, Lindberg 1991).

The timing of the dispersal from South America to New Zealand for the second complex of the species including *M. polylepis* and *M. australis* is under debate. Inada (1981; Fig. 3.5a) proposed a South African origin for the Australian lineage, whereas Kabata and Ho (1981), Ho (1990) and Grant and Leslie (2001) suggested an Argentinean origin (Figs. 5b, 6e). The current study supports the later hypothesis of a recent dispersal from South America to New Zealand when a cold Southern Ocean was fully developed (Grant and Leslie 2001). However, the lack of resolution of the internal relationships in the Patagonia – New Zealand complex and the eastern Pacific complex in this study leads to uncertainty about the potential origin and dispersal of *Merluccius* in the Pacific.

3.5. Conclusions

This study is the first attempt to resolve the controversy surrounding the taxonomic extent of Merlucciidae and the phylogenetic placement of the genera historically included within the family, based on complete taxonomic sampling at the family and subfamily levels among gadiforms. Merlucciidae includes only the genus *Merluccius* and the other three genera are not related with *Merluccius*. *Lyconus* is best regarded as being in a monogeneric Lyconidae and *Macruronus* within a monogeneric Macruronidae, with both families being closely related to each other and sister to Bathygadidae. *Steindachneria argentea* is placed in its own family, Steindachneriidae, and recovered as the sister group of Macrouridae. The monophyletic clade including (((Macrouridae , Steindachneriidae) , ((Macruronidae , Lyconidae) , Bathygadidae))) was strongly supported at all nodes.

This phylogenetic analysis confirmed an early separation of two lineages of *Merluccius*, the Old World and the New World clades as suggested by other authors (e.g., Inada 1981, Kabata and Ho 1981, Lombarte and Castellon 1991, Roldan et al. 1999, Quintero et al. 2000, Grant and Leslie 2001, Campo et al. 2007). This study also corroborated a deep separation of the Old World into two clades, one including (*M. paradoxus* + *M. polli*) and another comprising (*M. merluccius* + (*M. senegalensis* + *M. capensis*)). Recovering *M. paradoxus* and *M. capensis* in different clades supports the dispersal of North Atlantic taxa along the west coast of Africa and explains their sympatric distribution.

The ML results provided intriguing but limited information about the species delimitation for some species in the New World clade. *Merluccius bilinearis* is the earlies branch among the clade which agrees with previous studies (e.g., Roldan et al. 1999, Quintero et al. 2000, Grant and Leslie 2001, Campo et al. 2007, 2009; Fig. 3.4a-d). *Merluccius albidus* is the sister taxa of

the rest of hakes and included two clades, one from the Atlantic and the other one from the Gulf of Mexico, suggesting that samples from the Gulf of Mexico could represent two species or a geographical population as it has been described in the past (e.g., Ginsburg 1954, Lloris et al. 2005 respectively). However, until more detailed species delimitation analyses are performed no conclusions challenging the species status can be made.

Merluccius hubbsi was recovered as a weakly supported monophyletic clade but strongly supported as sister to a well-supported clade involving two species complexes (the Patagonia – New Zealand complex and the eastern Pacific complex). The ML topology suggests a North Atlantic ancestor for the Pacific species, although the relationships of these complexes of species need to be clarified in order to make a more solid inference.

The results presented here based on molecular data suggest the presence of only one species in the eastern Pacific, as well as, one species in the Southern Ocean (Patagonia – New Zealand). Further analyses to test clinal morphological variation will be performed using principal component analyses (PCA), and the correlation of each principal component to latitude and longitude will be explored using multiple regression analysis. Morphological data will be derived from the vouchers of the specimens used in the molecular analyses as possible and at least 25 specimens per species/population along the distribution range. All specimens are deposited at the Nunnally Ichthyology Collection (VIMS; 258 lots, *M. albidus, M. bilinearis* and *M. productus*) and at the Smithsonian Institution (NMNH; 649 lots, including all the putative *Merluccius* species). Both collection of the morphological data and molecular analyses using genome-wide SNP data are currently ongoing. These analyses will help to: (i) identify putative species boundaries by implementing non-coalescent and coalescent-based species delimitation methods such as BPP (Yang & Rannala 2010), IBPP (Solis-Lemus et al. 2014) and BFD* (Leache et al. 2014) and (ii)

assess the extent of genetic isolation among putative species by estimating spatio-temporal gene flow with fastsimcoal2 (Excoffier et al. 2013) and ABBA-BABA (Martin et al. 2015). In order to better understand the tempo and mode of speciation in *Merluccius*, a fossil calibrated phylogeny will provide estimate times of separation between Atlantic and Pacific sister species independent from the emergence of the Isthmus of Panama.

Defining species is essential for the fundamental understanding of biodiversity and its conservation. In the case of *Merluccius,* one of the most heavily exploited fishes worldwide, their taxonomic relationships and species delimitation is an essential basis for the conservation, development and management of their fishery across boundaries between nations. Historically, morphological analyses have been used for taxonomic studies. Combining these types of analyses with genetic studies provides a more robust foundation to make taxonomic decisions that could have a positive impact in fisheries management so that the fisheries they support remain biologically productive and economically valuable.

Table 3.1 Taxonomic classification of *Merluccius* species

Species	Inada (1981)	Lloris et al (2003) & Matallanas & Lloris (2006)	Eschmeyer (2018)	Froese & Pauly (20178	Synonyms (synonymized by)	Types
Merluccius albidus					*M. magnoculus* (Karnella 1973)	**H =** No known **; N** = USNM 31630
Merluccius angustimanus						**H =** No known **; S** = MCZ 28612-15 (6); USNM 57873 (1), 74575 (2), 120424
Merluccius australis		*M. australis-australis & M. australis polylepis*				**L** = BMNH 1872.4.26.8. ; **PL** = BMNH 1905.11.30.38 (1)
Merluccius bilinearis						**H =** No known ; **N** = USNM 39935
Merluccius capensis						**H** = No known ;
Merluccius gayi gayi						**H** = MNHN B-1280
Merluccius gayi peruanus	*M. gayi*	*M. gayi-gayi & M. gayi-peruanus*	*M. gayi*			**H =** USNM 77727 **; P** = USNM 77525 (5), 77539 (3), 128180-81 (1, 1)
Merluccius hernandezi					*M. angustimanus* (Lloris et al. 2003, Eschmeyer et al. 2018).	**P** = SIO 74-70
Merluccius hubbsi						**H =** UMMZ/MACN (presumed lost) ; **P** = BMNH 1935.8.29.14-15 [ex USNM 77291] (2); UMMZ 95461 (1); USNM 43388 (15, disintegrated), 77291(40), 77292 (1)
Merluccius magnoculus					*M. albidus* (Inada in Cohen et al. 1990)	**P** = USNM 144303 (3), 157758-63 (2, 5, 5, 4, 2, 10)
Merluccius merluccius		*M. merluccius-merluccius, M. merluccius-smiridus*			*M. lanatus* (Linnaeus 1758)	**H** = BMNH 1853.11.12.113
					Trachinoides moroccanus (Linnaeus 1758)	**H** = MM 1349 (now at AMNH, presumed lost)
					Onus riali (Linnaeus 1758)	**H** = No known
					M. sinuatus (Linnaeus 1758)	**H** = No known
					M. smiridus (Linnaeus 1758)	**H** = No known
					M. vulgaris (Linnaeus 1758)	**H** = No known
Merluccius paradoxus						**H** = No known
Merluccius patagonicus					*M. hubbsi* (Diaz de Astarloa et al. 2011)	**P** = CMIMA-CSIC IIPB 501-504/2001 (4, 1 dissected)
Merluccius polli		*M. polli-polli & M. polli-cadenati*			*M. cadenati* (Inada 1981)	**H** = No known
Merluccius polylepis					*M. australis* (Inada in Cohen et al. 1990)	**P** = USNM 157765-66 (1, 2)
Merluccius productus					*Homalopomus trowbridgii* (Ayres 1855)	**S** = USNM 529 (1) ; USNM 285 (1)
Merluccius senegalensis						**H** = No known
Merluccius tasmanicus					*M. australis* (Deli Antoni et al. 2015) - Description warranted	**H** = NMNZ P.5566 **; P** = MOVI 27490 (1), 27491 (1); NMNZ P.3963 (1)

H = Holotype; **P** = paratype; **N** = Neotype; **S** = Syntypes; **L** = Lectotype; **PL** = Paralectotype
Geographic Distrubution, depth range and types from Froese and Pauly (2018) and IUCN (2018)
Colors in cells - white: Not valid by the author(s) or the species was described after the publication, dark grey (accepted by the author(s).

Table 3.2 Sampling and sequencing information of the taxa used in this study

Family	Species	Tissue	Voucher	Sequenced	# Loci captured	% Capt. Effic.
Euclichthyidae	*Euclichthys polynemus*	Gill arch - 1		SHOU	2394	16.8
Merlucciidae	*Merluccius capensis*	Gill arch - 2	CAS 224386	HML	6698	47.1
Merlucciidae	*Merluccius capensis*	Gill arch - 2	CAS 224387	HML	5527	38.9
Merlucciidae	*Merluccius capensis*	Gill arch - 2	CAS 224387	HML	4266	30.0
Merlucciidae	*Merluccius polli*	Gill arch - 2	CAS 223407	HML	5782	40.7
Merlucciidae	*Merluccius polli*	Gill arch - 2	CAS 224779	SHOU	2785	19.6
Merlucciidae	*Merluccius polli*	Gill arch - 2	CAS 224779	HML	5074	35.7
Melanonidae	*Merluccius polli*	Gill arch - 2	CAS 223133	SHOU	3404	23.9
Bathygadidae	*Bathygadus melanobranchus*	Gill arch - 2	CAS 224389	SHOU	8575	60.3
Melanonidae	*Melanonus gracilis*	Gill arch - 2		SHOU	2921	20.5
Gadidae	*Microgadus tomcod*		KU 5884	SHOU	13374	94.1
Merlucciidae	*Merluccius hubbsi*	Muscle - 14		HML	1533	10.8
Merlucciidae	*Merluccius polylepis*	Muscle - 14		HML	5405	38.0
Merlucciidae	*Merluccius polylepis*	Muscle - 14		HML	4361	30.7
Merlucciidae	*Merluccius gayi-gayi*	Muscle - 14		SHOU	2155	15.2
Merlucciidae	*Merluccius gayi-gayi*	Muscle - 14		HML	9477	66.7
Merlucciidae	*Merluccius gayi-gayi*	Muscle - 14		HML	4874	34.3
Gadidae	*Ciliata mustela*	Muscle	ZMUC 373657	SHOU	2685	18.9
Merlucciidae	*Merluccius albidus*	Gill arch - 4	NCMNS- 73191 / Ross167	SHOU	5144	36.2
Merlucciidae	*Merluccius albidus*	Gill arch - 4	Ross235	SHOU	4193	29.5
Merlucciidae	*Merluccius paradoxus*	Muscle - 5	1-11740 - von der Heyden	HML	8026	56.5
Merlucciidae	*Merluccius paradoxus*	Muscle - 5	1-11733 - von der Heyden	HML	6279	44.2
Merlucciidae	*Merluccius merluccius*	Muscle - 15		SHOU	2546	17.9
Merlucciidae	*Merluccius merluccius*	Gill arch - 6		HML	5400	38.0
Merlucciidae	*Merluccius merluccius*			HML	4956	34.9
Merlucciidae	*Merluccius polli*	Gill arch - 2	TI-185	HML	5102	35.9
Merlucciidae	*Merluccius polli*	Gill arch - 2	TI-191	HML	7246	51.0
Merlucciidae	*Merluccius senegalensis*	Gill arch - 2	TI-192	HML	6370	44.8
Merlucciidae	*Merluccius capensis*	Muscle - 7		SHOU	3923	27.6
Merlucciidae	*Merluccius capensis*	Muscle - 7		SHOU	5181	36.4

Family	Species	Tissue	Voucher	Sequenced	# Loci captured	% Capt. Effic.
Merlucciidae	*Merluccius capensis*	Muscle - 7		SHOU	3126	22.0
Merlucciidae	*Merluccius paradoxus*	Gill arch - 7	USNM 440552	HML	8049	56.6
Merlucciidae	*Merluccius paradoxus*	Gill arch - 7		HML	8528	60.0
Merlucciidae	*Merluccius australis*	Muscle - 8	UNMDP 108	SHOU	1944	13.7
Merlucciidae	*Merluccius australis*	Muscle - 8	UNMDP 109	SHOU	3015	21.2
Merlucciidae	*Merluccius australis*	Muscle - 8	UNMDP 111	SHOU	2574	18.1
Merlucciidae	*Merluccius hubbsi*	Muscle - 8	UNMDP 1565	SHOU	4431	31.2
Merlucciidae	*Merluccius hubbsi*	Muscle - 8	UNMDP 1567	SHOU	3544	24.9
Merlucciidae	*Merluccius hubbsi*	DNA aliquot - 8	UNMDP 1569	HML	8075	56.8
Merlucciidae	*Merluccius angustimanus*	Muscle - 10	NMNH 102451499	HML	6362	44.7
Merlucciidae	*Merluccius angustimanus*	DNA aliquot - 8	USNM 102451482	SHOU	2211	15.6
Merlucciidae	*Merluccius angustimanus*	Muscle - 10	NMNH 102451482	HML	5183	36.5
Merlucciidae	*Merluccius australis*	Gill arch - 1	PMc-TAN_1412_Sta67_T1	SHOU	3490	24.5
Merlucciidae	*Merluccius australis*	Gill arch - 1	PMc-TAN_1412_Sta74_T1	SHOU	3218	22.6
Merlucciidae	*Merluccius australis*	Gill arch - 1	PMc-TAN_1412_Sta76_T1	HML	6740	47.4
Merlucciidae	*Merluccius australis*	Gill arch - 1	PMc-TAN_1412_Sta78_T1	SHOU	2226	15.7
Merlucciidae	*Merluccius albidus*	Muscle - 13	VIMS TS-2015-266	SHOU	3255	22.9
Merlucciidae	*Merluccius productus*	Muscle	SIO-12-14	SHOU	3062	21.5
Merlucciidae	*Merluccius albidus*	Muscle	CNPE-IBUNAM 16503	SHOU	1841	13.0
Merlucciidae	*M.* cf. *hernandezi*	Muscle		SHOU	3043	21.4
Merlucciidae	*M.* cf. *hernandezi*	Muscle		SHOU	4056	28.5
Merlucciidae	*M.* cf. *hernandezi*	Muscle		SHOU	1763	12.4
Merlucciidae	*Merluccius albidus*	Muscle	TS2005-0074 / FLMNH 180335	SHOU	3648	25.7
Merlucciidae	*Merluccius gayi-peruanus*	Muscle - 12	MURP00002516	HML	7282	51.2
Merlucciidae	*Merluccius gayi-peruanus*	Muscle - 12	MURP00002516	HML	5228	36.8
Merlucciidae	*Merluccius gayi-peruanus*	Muscle - 12	MURP00002517	HML	4762	33.5
Merlucciidae	*Merluccius gayi-peruanus*	Muscle - 12	MURP00002518	HML	4784	33.6
Merlucciidae	*Merluccius bilinearis*	Muscle - 13	VIMS TS -2013-58 / VIMS 34594	SHOU	5261	37.0
Merlucciidae	*Merluccius bilinearis*	Muscle - 13	VIMS TS -2013-64 / VIMS 34609	SHOU	5672	39.9
Merlucciidae	*Merluccius albidus*	Muscle - 13	VIMS TS -2013-76 / VIMS 34624	SHOU	4313	30.4
Merlucciidae	*Merluccius bilinearis*	Muscle - 13	VIMS TS -2013-79 / VIMS 34696	SHOU	5120	36.0

Family	Species	Tissue	Voucher	Sequenced	# Loci captured	% Capt. Effic.
Merlucciidae	*Merluccius bilinearis*	Muscle - 13	VIMS TS -2013-93 / VIMS 34637	SHOU	5395	38.0
Merlucciidae	*Merluccius bilinearis*	Muscle - 13	VIMS TS -2013-96 / VIMS 34612	SHOU	4857	34.2
Merlucciidae	*Merluccius bilinearis*	Muscle - 13	VIMS TS -2013-178	SHOU	6008	42.3
Merlucciidae	*Merluccius angustimanus*	Gill arch - 11	INV-TEJ1675	SHOU	2074	14.6
Merlucciidae	*Merluccius angustimanus*	Gill arch - 11	INV-TEJ1676	SHOU	2422	17.0
Merlucciidae	*Merluccius angustimanus*	Gill arch - 11	INV-TEJ1677	SHOU	3135	22.1
Merlucciidae	*Merluccius polylepis*	Muscle - 9		HML	2962	20.8
Merlucciidae	*Merluccius polylepis*	Muscle - 9		HML	5167	36.3
Merlucciidae	*Merluccius polylepis*	Muscle - 9		HML	5115	36.0
Merlucciidae	*Merluccius polylepis*	Muscle - 9		HML	5217	36.7
Merlucciidae	*Merluccius polylepis*	Muscle - 9		HML	4798	33.7
Gadidae	*Gadus morhua*			SHOU	13771	96.9

CAS voucher from California Academy of Sciences
CFM vouchers from Chicago Field Museum collection
CNPE-IBUNAM Colección Nacional de Peces del Instituto de Biología, UNAM
FLMNH Florida Museum of Natural History
KU voucher and tissue sample (TS) from Biodiversity Institute & Natural History Museum, The University of Kansas
MZC Museum of Comparative Zoology, Harvard University
TCWC Texas Cooperative Wildlife Collection, The Natural History Collection at Texas A&M University
UNDMP Universidad Nacional de Mar del Plata
ZMUC voucher from Zoological Museum University of Copenhagen collection
ZSCM numbers are vouchers from Zoological State Collection Munich

Tissues samples donated by:
1- Peter McMillan (National Institute of Water and Atmospheric Reseach - NIWA, Willington, NZ)
2 - Tomio Iwamoto (California Academy of Sciences, San Francisco, CA. USA)
3 - Endo Hiromitsu (Kochi University, Kochi, Japan)
4 - Steve Ross (Center for Marine Science, University of North Carollina Wilmington. Wilmington, NC. USA)
5 - Sophie von der Heyden (Stellenbosch University, Stellenbosch, South Africa)
6 - Rafael Banon (Consejo Superior de Investigaciones Cientificas - CSIC. Madrid, Spain)
7 - Rob Leslie (Department of Agriculture, Forestry and Fisheries -DAFF. Cape Town, South Africa)
8 - Juan Manuel Diaz de Astarloa (Universidad Nacional de Mar del Plata - UNMdP, Buenos Aires, Argentina)
9 - Luis Marcos Adasme Martinez (Instituto de Fomento pesquero - IFOP. Valparaiso, Chile)
10 - Carole Baldwin (National Museum of Natural History - Washington DC, USA)
11 - Mario Rueda, Marisol Santos-Acevedo (Programa de Valoracion y Aprovechamiento Pesquero and MHNMC - INVEMAR. Santa Marta, Colombia)
12 - Francis Paola Castro, Museo de Historia Natural "Vera Alleman Haeghebaert". Lima, Peru Universidad Ricardo Palma
13 - Jakub Kircun (Woods Hole Laboratory - WHL and National Oceanic Atmospheric Administration - NOAA)
14 - Guillermo Ortí (George Washington University. Washington, DC. USA
15 - Peter Warth (Institut f€ur Spezielle Zoologie undEvolutionsbiologie mit PhyletischemMuseum, Friedrich-Schiller-Universität Jena,Germany)
15 - Hector Espinosa (Colección Nacional de Peces - CNP, Universidad Nacional Autónoma de México, México)

Table 3.3 Uncorrected *p*-Distances Among species of *Merluccius* and representatives of the families Bathygadidae, Euclichthyidae and Gadidae.

Species	*M. polylepis*	*M. angustimanus*	*M. gayi-peruanus*	*M. albidus*	*M. hernandezi*	*M.productus*	*M. australis*	*M. hubbsi*	*M. paradoxus*
M. polylepis	0.007								
M. angustimanus	0.012	0.013							
M. gayi-peruanus	0.008	0.008	0.004						
M. albidus	0.014	0.016	0.013	0.016					
M. hernandezi	0.014	0.013	0.010	0.018	0.014				
M.productus	0.014	0.015	0.011	0.020	0.016	n/c			
M. australis	0.016	0.022	0.016	0.023	0.022	0.021	0.025		
M. hubbsi	0.009	0.011	0.008	0.013	0.012	0.013	0.017	0.007	
M. paradoxus	0.019	0.021	0.018	0.024	0.022	0.024	0.026	0.019	0.005
M. capensis	0.025	0.028	0.024	0.029	0.029	0.031	0.034	0.025	0.017
M. senegalensis	0.021	0.022	0.019	0.025	0.023	0.025	0.028	0.020	0.012
M. polli	0.021	0.023	0.020	0.025	0.024	0.026	0.029	0.020	0.010
M. merluccius	0.021	0.024	0.020	0.025	0.024	0.026	0.029	0.021	0.013
M. gayi-gayi	0.014	0.015	0.009	0.019	0.017	0.018	0.022	0.013	0.023
Bathygadus	0.082	0.082	0.082	0.082	0.083	0.084	0.086	0.080	0.082
Euclichthys	0.081	0.083	0.080	0.081	0.084	0.087	0.088	0.079	0.081
M. bilinearis	0.016	0.017	0.015	0.020	0.018	0.019	0.024	0.015	0.024
Gadus morhua	0.098	0.099	0.098	0.096	0.099	0.099	0.102	0.097	0.098

Dark grey: p-uncorrected distance within genus

Species	*M. capensis*	*M. senegalensis*	*M. polli*	*M. merluccius*	*M. gayi-gayi*	*Bathygadus*	*Euclichthys*	*M. bilinearis*	*Gadus morhua*
M. polylepis									
M. angustimanus									
M. gayi-peruanus									
M. albidus									
M. hernandezi									
M.productus									
M. australis									
M. hubbsi									
M. paradoxus									
M. capensis	0.015								
M. senegalensis	0.011	NA							
M. polli	0.018	0.013	0.008						
M. merluccius	0.014	0.009	0.014	0.008					
M. gayi-gayi	0.031	0.024	0.026	0.026	0.017				
Bathygadus	0.087	0.083	0.082	0.081	0.087	NA			
Euclichthys	0.088	0.081	0.080	0.080	0.088	0.081	NA		
M. bilinearis	0.030	0.025	0.024	0.025	0.020	0.082	0.082	0.010	
Gadus morhua	0.102	0.099	0.099	0.098	0.102	0.103	0.099	0.096	NA

Dark grey: p-uncorrected distance within genus

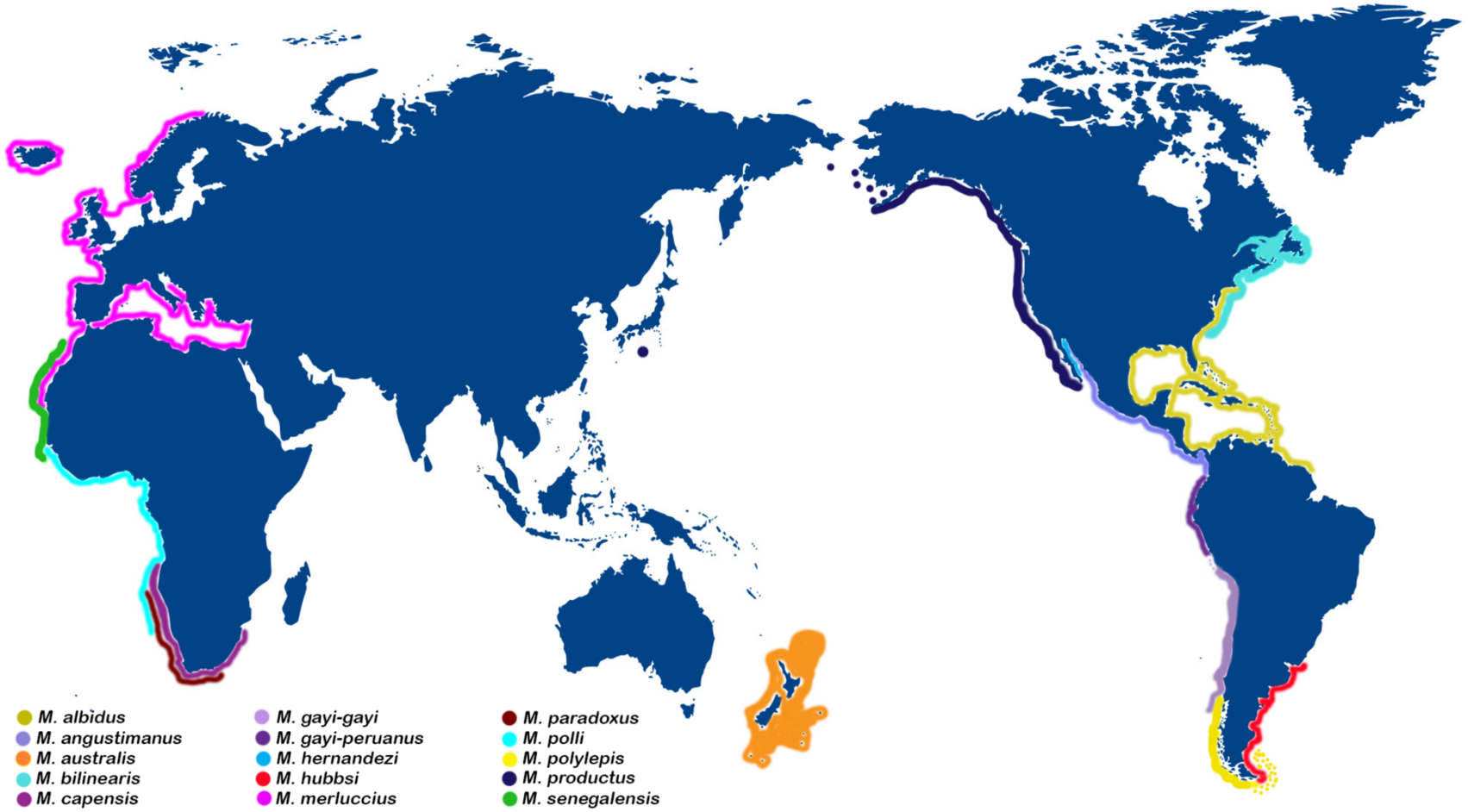

Figure 3.1. Geographical distribution of the genus *Merluccius*.

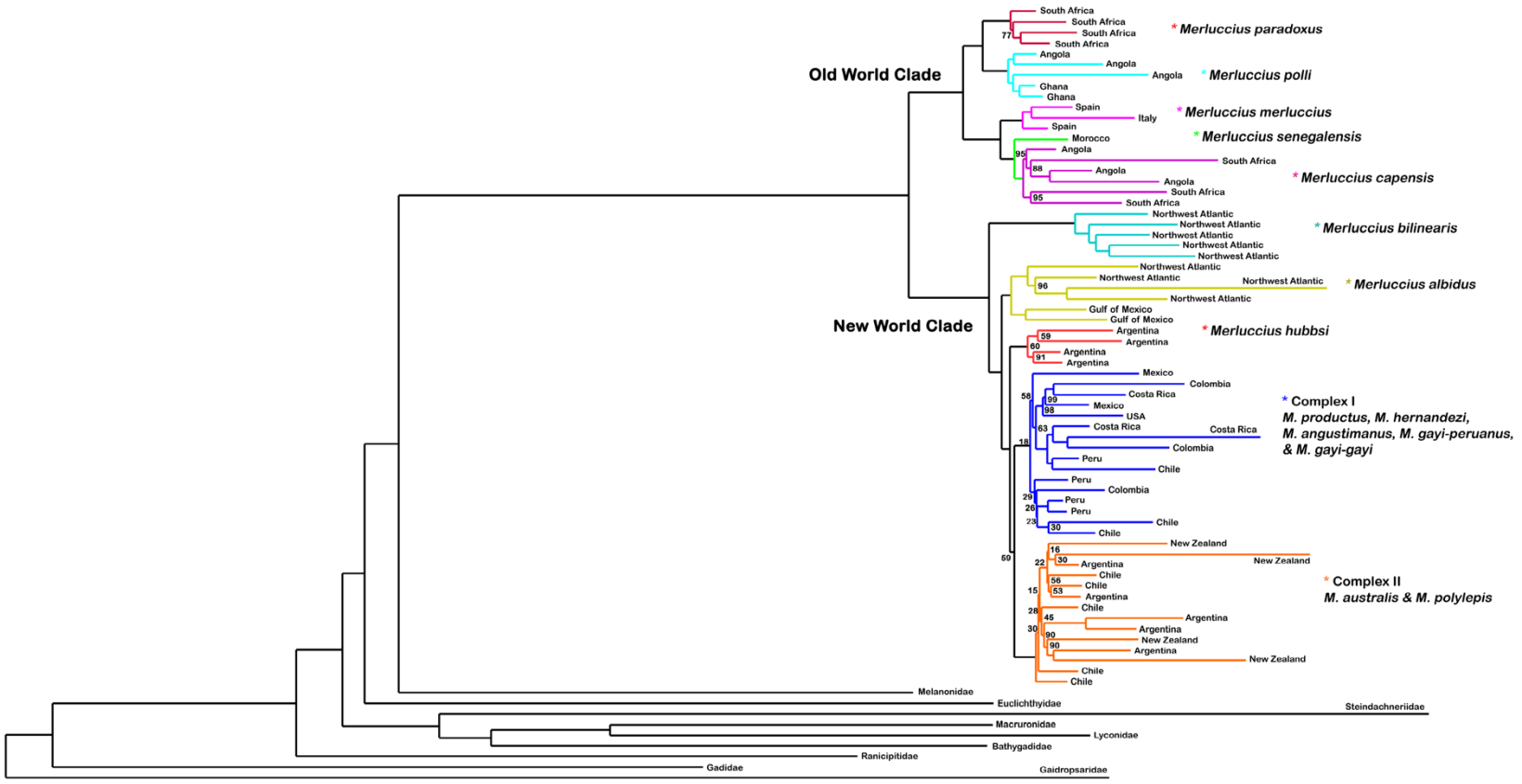

Figure 3.2 Relationships of the family Merlucciidae inferred from ML analysis of 8,243 loci including 74 taxa and GTRGAMMA substitution model by codon position. Number at nodes indicate bootstrap values; 100 %except when noted.

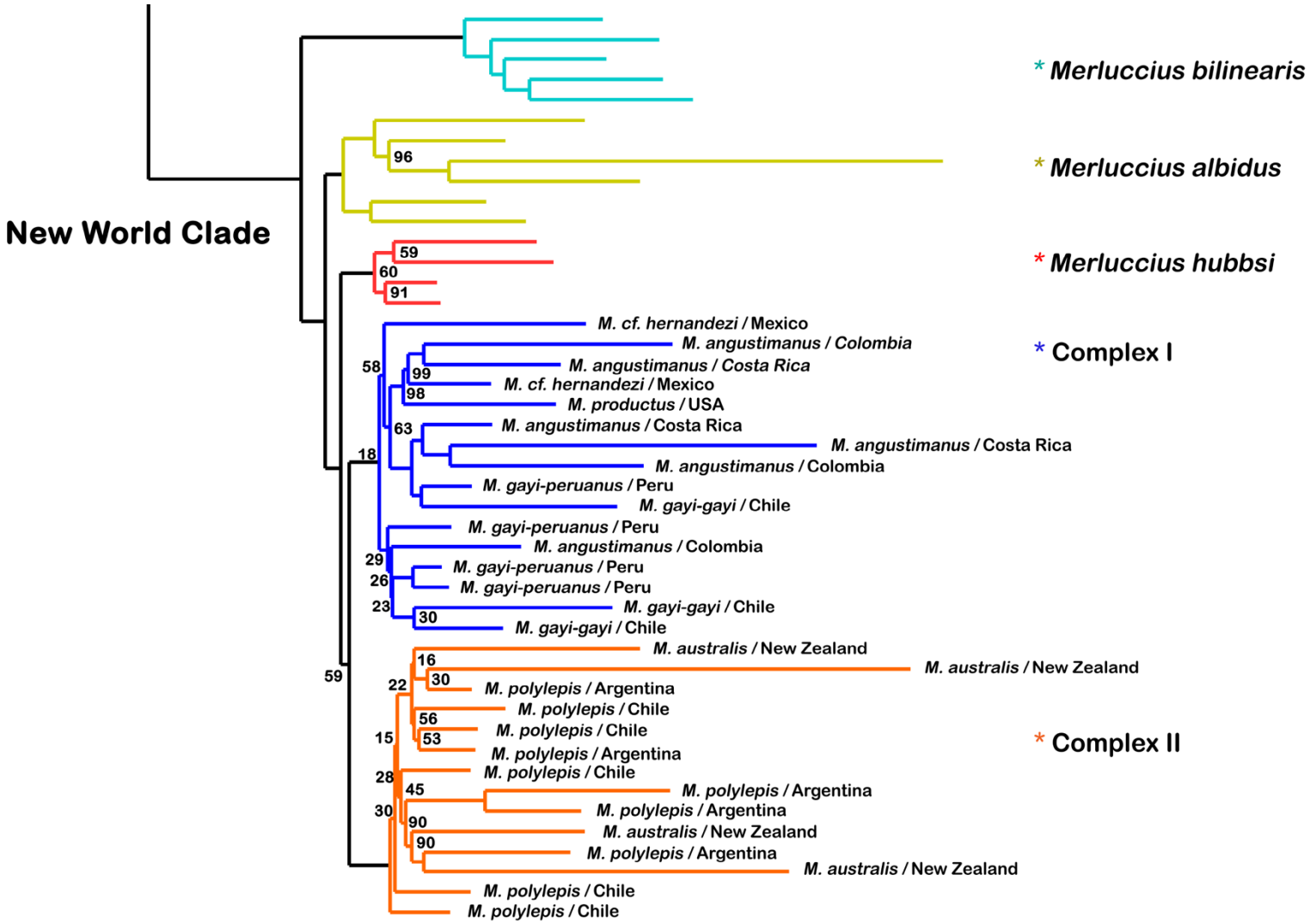

Figure 3.3 Relationships of the New World species. Number on nodes indicate bootstrap values; 100 % except when noted.

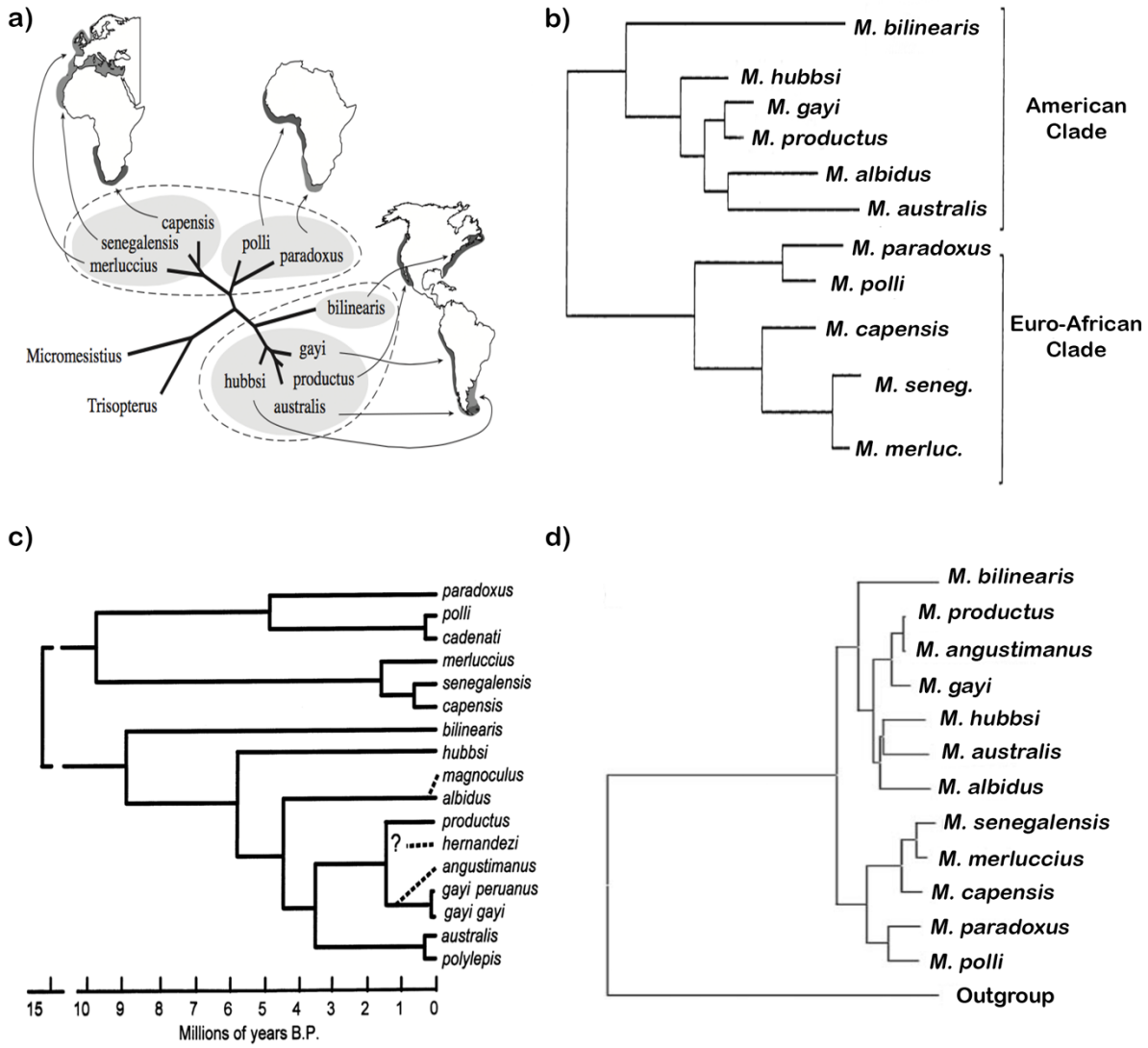

Figure 3.4 Phylogenetic Hypotheses proposed by a) Roldan et al. 1999; b) Quinteiro et al. 2000; c) Grant and Leslie 2001; d) Campo et al. 2007, 2009. Figures Modified from original references.

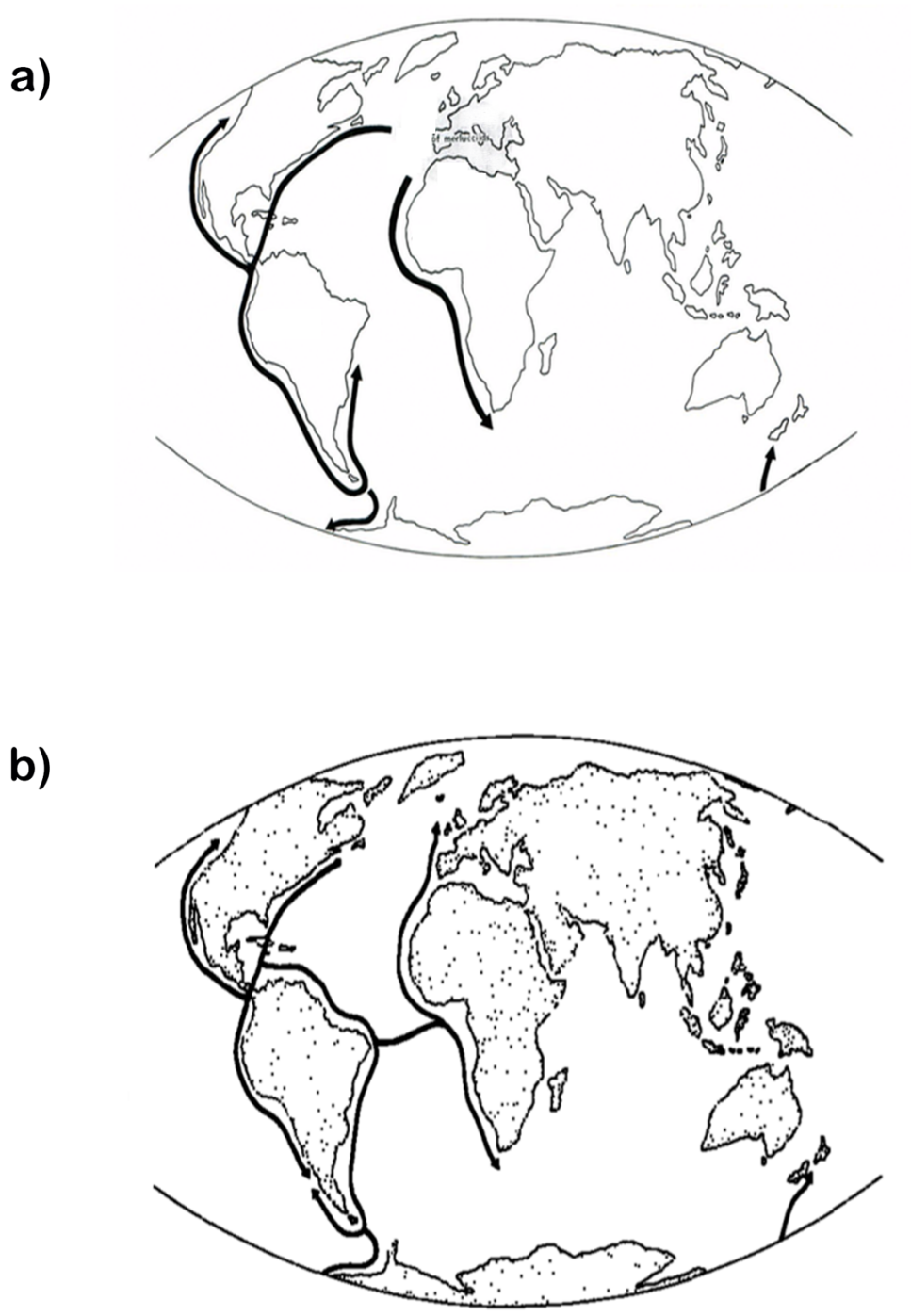

Figure 3.5 Biogeographic hypotheses of the origin and dispersal of *Merluccius* species by: a) Inada 1981 and b) Ho (1990).

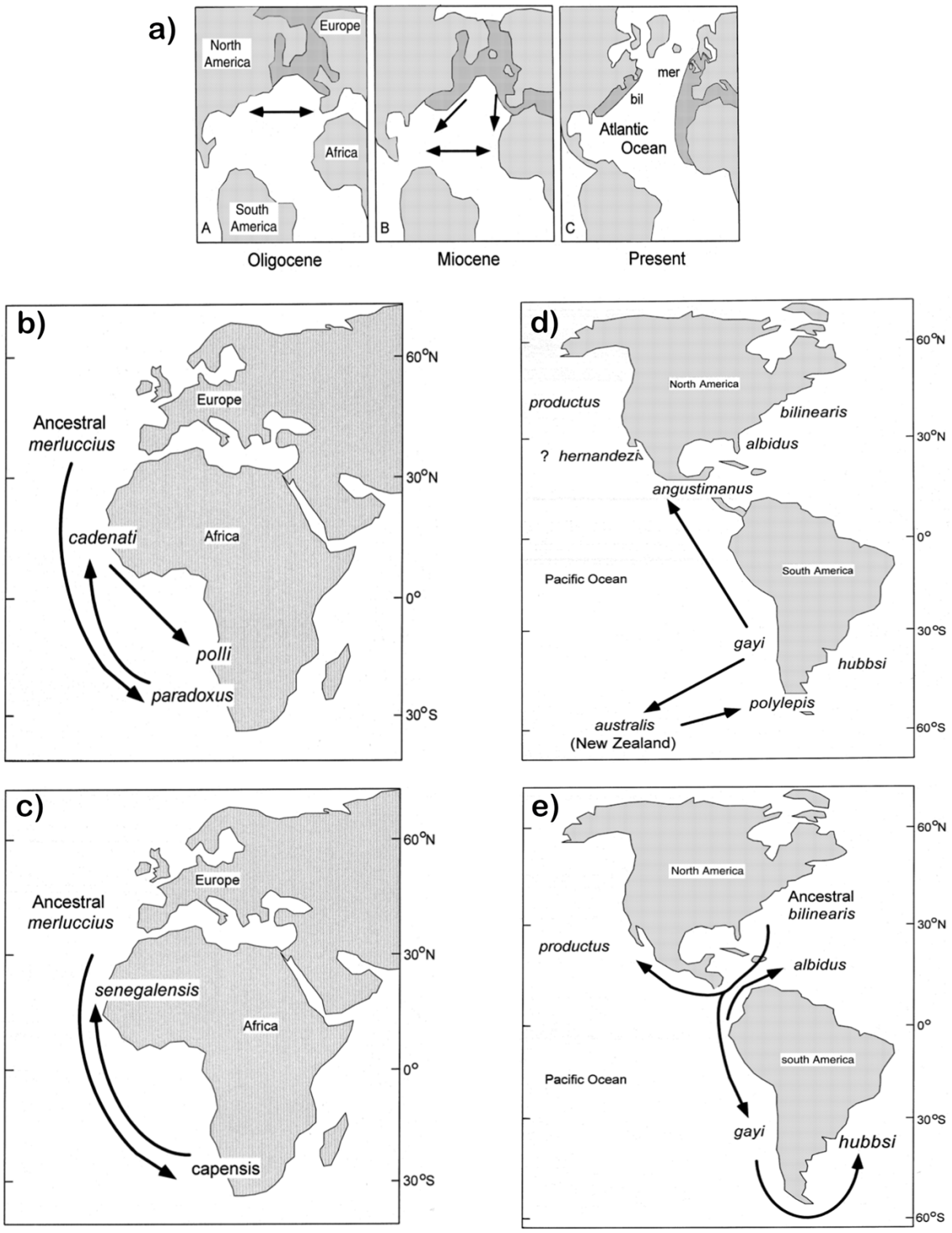
a)
North America
Europe
Africa
South America
A
B
C
mer
bil
Atlantic Ocean
Oligocene
Miocene
Present
b)
Europe
Ancestral merluccius
cadenati
Africa
polli
paradoxus
60°N
30°N
0°
30°S
c)
Europe
Ancestral merluccius
senegalensis
Africa
capensis
60°N
30°N
0°
30°S
d)
North America
productus
bilinearis
albidus
? hernandezi
angustimanus
Pacific Ocean
South America
gayi
hubbsi
polylepis
australis
(New Zealand)
60°N
30°N
0°
30°S
60°S
e)
North America
Ancestral bilinearis
productus
albidus
Pacific Ocean
south America
gayi
hubbsi
60°N
30°N
0°
30°S
60°S

Figure 3.6 Biogeographic hypotheses of the origin and dispersal of *Merluccius* species by Grant and Leslie (2001): a) Initial separation between the Old and New World *Merluccius* species; b) Geographical dispersal of the "paradoxus" lineage; c) Geographical dispersal of the "capensis" lineage; d) Origin of the *Merluccius* eastern Pacific species; e) Origin of *M. hubbsi* from an ancestral Pacific hake by dispersal around Cape Horn and origin of *M. albidus* as a consequence of the emergence of the Isthmus of Panama.

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AMATXU'S BASQUE HAKE FILLETS WITH CLAMS IN SALSA VERDE

INGREDIENTS (Serves 4)

https://www.spain-recipes.com/hake-clams 1

24 Manila or small littleneck clams
1 tablespoon coarse sea salt
4 cups water
1/3 cup olive oil
2 cloves garlic, finely minced
1/2 teaspoon hot red pepper flakes (optional)
1 tablespoon all-purpose flour
1 !/2 teaspoons salt
2 tablespoons chopped fresh flat-leaf parsley
1/2 cup dry white wine
2 pounds hake fillet, cut into 16 pieces
Salt
4 white asparagus, freshly cooked or canned, halved crosswise
2 hard-boiled eggs, peeled and quartered lengthwise, for garnish
Chopped fresh flat-leaf parsley for garnish

PREPARATION

1. Scrub the clams under cold running water, discarding any that fail to close to the touch. In a large bowl, combine the clams, coarse salt, and water to cover and let stand for at least 30 minutes or up to 2 hours so that the clams release any sand trapped in their shells. Drain.
2. In a large saucepan, combine the clams with the 4 cups water and bring to a boil over medium-high heat. Cover and cook for about 5 minutes, or until they open. As the clams cook, uncover the pan occasionally and stir with a wooden spoon to encourage them all to open at about the same time. Drain the clams, reserving the cooking liquid. Discard any clams that have not opened.
3.In a large cazuela, heat the olive oil over high heat. Add the garlic and red pepper flakes, if using, and fry, stirring often, for 1 to 2 minutes, or until the garlic begins to turn golden. Sprinkle the flour over the garlic and stir with a wooden spoon until the mixture is well blended. Add 3 cups of the reserving cooking liquid and the salt, parsley and wine. Decrease the heat to medium and boil, stirring occasionally, for 5 minutes, or until the sauce thickens slightly. Add more cooking liquid if you prefer a thinner sauce. Rotate the cazuela in circular motions over the burner to mix all the ingredients, and boil gently for 2 minutes, or until the sauce is blended and looks whitish green.
4.Sprinkle the hake pieces with the salt and place in a single layer in the sauce. Cook, turning once, for 2 minutes on each side, or until opaque at the center when tested with a knife tip. Add the clams and asparagus, shake the pan gently to prevent sticking, and simmer for 2 more minutes so heat all the ingredients through.
Garnish with the egg wedges and sprinkle with the parsley. Serve immediately.

Prep Time: 30 mins Cook Time: 15 mins Yield: 4

CHAPTER 4

Evolution of the Caudal Skeleton of Gadiformes (Teleostei: Paracanthopterygii)

4.1 Introduction

The caudal skeleton of Gadiformes (e.g. cods, hakes, and grenadiers) is a complex structure that exhibits substantial diversity among the major subgroups of the order, which range from taillless fishes (e.g., macrouroids) to those with externally symmetrical caudal fins (e.g. cod-like fishes). This morphological diversity is mirrored by the exceptional ecological diversity of Gadiformes, which are distributed from the Arctic to Antarctic oceans, and occupy deep-sea to shallow marine waters, with a single fully freshwater species. Many fishes use the caudal fin as the main propulsive and steering device (Videler 1993) and its shape is usually adapted to the type of swimming required to optimize survival and fitness in different habitats (Webb 1984, Pavlov and Kasumyan 2002). Correlating morphological, ecological and phylogenetic can provide valuable insight into the evolution of gadiforms.

Gadiformes are widely regarded as a monophyletic group within Paracanthopterygii (Cohen 1984, Gosline 1971, Greenwood et al. 1966) although no well-defined morphological synapomorphies supporting its monophyly have yet been established (Patterson & Rosen 1989, Murray and Wilson 1999). Currently, different authors recognize between 11 and 17 families, approximately 84 genera, and over 600 species within the Gadiformes (e.g., Endo 2002, Roa-Varón and Ortí 2009, Nelson et al. 2016, Betancur-R et al. 2017). Further, the composition of Paracanthopterygii has changed substantially since Greenwood et al. (1966) first named it (e.g. Patterson and Rosen 1989, Wiley and Johnson 2010, Betancur-R et al. 2017). As currently recognized, Paracanthopterygii comprises Gadiformes, Percopsiformes, Polymixiiformes, Stylephoriformes, and Zeiformes (e.g., Miya et al. 2007, Grande et al. 2013, Betancur-R et al., 2017). Within Paracanthopterygii (in both the traditional usage, *sensu* Rosen and Patterson, 1969, and in its modern reconstitution) there is a lack of consensus between morphological and

molecular data regarding the sister group of Gadiformes. Morphological studies havesuggest that the sister group of Gadiformes is the Pediculati (= Batrachoidiformes + Lophiiformes; Patterson and Rosen 1989), Percopsiformes (Lauder and Liem 1983), Batrachoidiformes (Markle 1989), or Ophidiiformes (Nolf & Steurbaut 1989). The most recent molecular hypothesis suggests that Gadiformes and Stylephoriformes are sister-groups (Miya et al. 2007). This has been corroborated by several molecular studies (Near et al. 2013, Malstrøm et al. 2016, Betancur-R et al. 2017) but is poorly supported based on morphological data (Grande et al. 2013). In chapter 1, a highly congruent and well-supported phylogeny at both shallow and deep levels that contributes towards stabilizing higher-level Gadiformes classification was proposed. This phylogeny provides a framework for interpreting the evolution of the caudal skeleton of Gadiformes.

4.1.1 A Brief History of the Study of the Caudal Skeleton of Gadiformes

Among the earliest studies to describe the caudal skeleton of gadiforms was that of Agassiz (1877), who described the developing caudal skeleton of a larval *Gadus morhua* (20 mm). Based on the structure of the caudal skeleton, Cunningham (1897) suggested that Gadidae were descended from ganoid fishes with a diphycercal tail. Boulenger (1902) and Regan (1903) followed Cunningham (1897) and concluded that the condition found in Gadidae resulted from the formation of a novel caudal skeleton from direct ancestors that have lost the tail (e.g. macrouroids). However, Whitehouse (1910) found an upwardly directed urostyle along the dorsal edge of the posteriormost hypurals in *Gadus minutus* (= *Trisopterus minutus*), which became in later stages reduced or almost completely lost.

Based on a comparative study of the structure and development of the caudal-fin skeleton, Barrington (1937) suggested that the caudal skeleton of *Gadus morhua* was derived from a less-specialized state, such as that of *Pleuronectes*. Barrington (1937) also noted that the caudal skeleton of *Gadus* contains elements from all three median fins (i.e., dorsal, anal and caudal fins), and referred to it using the term "pseudocaudal", which was originally proposed by Goodrich (1909). The pseudocaudal concept has been advanced in several subsequent studies of Gadiformes (e.g., Marshall and Cohen, 1973; Cohen, 1984), but was not accepted by Ahlstrom (pers. comm. in Cohen 1984) due to the lack of pterygiophores associated with the procurrent rays. Barrington (1937) also proposed that the two hypurals of *Gadus* were fused hypurals that can be homologized with the more numerous elements of other fishes. However, Gosline (1961) suggested that these elements cannot be homologized with those of other teleosts and that the unusual and unique nature of the caudal fin of gadiforms resulted from sequential fusion of elements. Rosen and Patterson (1969) suggested fusion of centra as a process resulting in a free parhypural, but vertebral loss as the process that produced free neural and haemal spines (see below in discussion of the X and Y bones). In contrast, Markle (1989) proposed that the ancestral first preural centrum was lost in gadiforms, leaving a free parhypural.

Several studies have focused on the ontogeny of the skeleton of Gadidae (e.g. Markle 1982, Dunn and Matarese, 1984, Fahay and Markle 1984), some of which have included description of the development of the caudal skeleton (e.g. Boulenger 1902, Totton 1914, Barrington 1937, Matarese et al. 1981). A reduction in the number of elements in the caudal skeleton has been regarded as an evolutionary loss of independent elements (Gosline 1997), whether through actual loss or apparent loss through so-called "phylogenetic fusion" (see Nelson 1969; Hilton 2003). For example, the fusion of the dorsal hypurals with the second ural centrum

has characterized the Paracanthopterygii according to several authors (Gosline 1963, Monod 1968, Rosen and Patterson 1969, Marshall and Cohen 1973). However, Matarese et al. (1981) were unable to corroborate the fusion of hypurals during ontogeny (e.g., based on study of *Microgadus proximus*). The rattail or gephyrocercal tail (*sensu* Goodrich 1909) present in macrouroids has been thought to be formed by elements of the dorsal and anal fins. However, in *Coelorhynchus hubbsi*, Okamura (1970: fig. 58a, b) described a posterior series of elongated centra lacking neural and haemal spines. The two most posterior vertebrae support five ventral elements, which were homologized to the hypurals of other teleostean fishes. Okamura perhaps followed Monod (1968: figs. 571-576), who interpreted the caudal skeleton of *Gaidropsaurus mediterraneus*, *Phycis phycis*, *Merluccius Merluccius* and *Polachius virens* to contain two free ural centra and described the parhypural as the first hypural and the second hypural as the first hypural; this confusion has been continued in several studies (e.g., Dunn and Matarese 1984).

Over half of the known species of the gadiforms lack a caudal skeleton. When present, the caudal skeleton ranges from a reduced caudal skeleton (e.g. gadids) to a more caudal complex (e.g. morids, melanonids), widely regarded as representing the plesiomorphic condition for the order (e.g. Markle 1989, Patterson and Rosen 1989, Cohen 1984). All tailed-gadiforms have two epurals. In contrast, presence of X and Y bones, the number of hypurals, and the number of caudal-fin rays are variable across the order. The presence of X and Y bones (Monod 1968), also called accessory bones (Rosen and Patterson 1969), in the caudal skeleton has been regarded as a synapomorphy for the order (Markle 1982, Patterson and Rosen 1989), although they are homoplastically absent in several gadiform taxa (considered to be a derived condition; Markle 1982, Cohen 1984). X and Y bones are located anterior to the neural (ns) and haemal (hs) spines of the second preural vertebra (pu2), and two hypotheses have been proposed for their

evolution. According to the "vertebral subtraction" hypothesis proposed by Rosen and Patterson (1969), the X and Y bones represent neural and haemal spines for which arches and centra never develop. In contrast, Fahay and Markle (1984: fig. 144) proposed the "continuous caudal" hypothesis, in which the X and Y bones and spines associated with pu1 are homologues to dorsal and anal-fin pterygiophores.

In this chapter, I describe the structure of the caudal skeleton of Gadiformes and discuss the evolution of this morphological complex within this group of fishes based on the phylogenomic hypothesis proposed in Chapter One. The objectives of this study are (i) to review the osteology of the caudal skeleton among gadiform families based on x-rays and cleared and stained specimens of juvenile and adult specimens, as well as information from the literature, (ii) to explore character evolution of the caudal skeleton based on a taxonomically robust phylogenetic hypothesis

4.2 Materials and Methods

One-hundred thirty-four specimens were examined that represent most currently recognized gadiform families and subfamilies (sensu Chapter 1, S1) and 35 of the 84 genera within the order; specimens examined are listed in S1. All body lengths are reported as standard (SL) or total length (TL); preanal-fin lengths (PAL) are provided in cases in which the tail is broken or absent (e.g. in macrouroids). Preserved specimens were cleared and double stained following protocols adapted from Dingerkus & Uhler (1977) and Taylor & Van Dyke (1985) and/or radiographed with a PXS5-927EA MicroFocus 90kV X-Ray Source and a Paxscan 4030E Digital X-Ray Receptor (settings: 40 kv and uA 180) at the Museum Support Center, Smithsonian Institution. Cleared and stained specimens were examined with binocular dissecting

microscopes and images were obtained using a Zeiss Discovery V12 stereomicroscope with Zeiss Axiocam camera attached and rendered as Z-stacked images using AxioVision software to increase depth of field. Images were adjusted for contrast and color balance, and backgrounds were cleaned using Adobe Photoshop CS6.

4.2.1 Anatomical Terminology

Few ontogenetic data on the caudal skeleton of Gadiformes exist (see Matarese et al. 1981). Although there is no direct evidence of ontogenetic fusion of elements among caudal elements I have chosen to follow the terminology in the literature, which assumes: (i) fusion of the hypurals such that the single anteroventral hypural element represents hy 1+2; separate anterior hypural represents hy 1 and hy 2; one posterodorsal hypural element represents fused hy 3-5, separate posterodorsal hypurals represent separate hy 3, 4, 5; and the presence of two posterodorsal hypural elements can either represent hy 3-4 and hy 5 or hy 3 and hy 4-5; (ii) and two compound elements (pu1+u1 and the second ural centrum, u2, the latter of which may be fused with one or more of the upper hypurals). No statement of primary homology with the hypurals of other teleosts is implied or intended, however. Additionally information clarifying other characters/assumptions: (i) an autogenous ural centrum 2 is posterior to pu1+u1 and supports some or all hypural elements; (ii) two hypurals are typically associated with ural centrum 1 and the more posterior hypurals with ural centrum 2; (iii) the number of hypural elements varies from one to six and this variation may be the result of fusion or loss; (iv) parahypural is associated with preural centrum 1 and represents the posteriormost element through which the blood vessels pass before bifurcating (in many gadiformes, the arch portion of the parhypural is lost, and the element is not supported directly by the centrum); (v) epurals are median elements that are

anterodorsal to the uroneural(s) and the ural centra, and are thought to be homologous to neural spines that have lost their connection to a neural arch of the ural or preural centra (Monod 1968, Schultze and Arratia 1989); (vi) the X and Y bones are median elements that are positioned between the neural and the haemal spines (respectively) of preural centra 2 and 3. Additionally, caudal-fin rays in gadiforms have been reported as: (1) primary rays (those articulating with dorsal hypurals) and secondary rays (articulating with ventral hypurals, parhypural, epurals, X and Y bones and neural and haemal spines including the rays between them) (e.g. Fahay & Markle 1984: table 76); (2) number of rays supported by the upper hypurals and lower hypurals (e.g. Markle 1982: table 5); or (3) listed as branched and unbranched (e.g. Fahay & Markle 1984: table 76). In this study, only the total number of caudal-fin rays is reported due to the inconsistency among studies and the small number of specimens examined per taxon (i.e., natural variation could not be assessed based on samples observed here).

4.2.2. Anatomical Abbreviation*s*

ep: epurals; hy: hypurals; hyp: hypurapophysis; phy: parhypural; pu, preural centrum; ns: neural spine; hs: haemal spine; nspu: neural spine associated with pu; hspu: haemal spine associated with pu; pu1+u1: compound vertebrae preural 1 and ural 1; u2: ural two; un1: uroneural 1; un2: uroneural 2; pmr: proximal middle radial; ds: distal radial; fr: fin rays. PAL: preanal length; SL: standard length; TL: total length. Anterior is to the left in all figures.

4.2.3. Institutional Abbreviations

CSIRO, Australian National Fish Collection, Hobart, Australia; **CAS**, California Academy of Sciences, San Francisco, USA; **MCZ**, Museum of Comparative Zoology, Harvard University,

Cambridge, MA, USA; **SAIAB**, South African Institute for Aquatic Biodiversity, Grahamstown, South Africa; **FLMNH**, Florida Museum of Natural History, Gainesville, USA; **USNM**, National Museum of Natural History, Smithsonian Institution, Washington D.C., USA; **VIMS**, Nunnally Ichthyology Collection, Virginia Institute of Marine Science, Gloucester Point, USA.

4.2.4. Ancestral State Reconstruction

Ancestral character states were reconstructed for eleven characters: 1) Caudal fin (presence/absence), 2) number of ventral hypural elements, 3) number of dorsal hypural elements, 4) X bone (presence/absence), 5) Y bone (presence/absence); 6) number of uroneurals and 7) number of epurals, 8) tail caudal fin propulsion, 9) number of dorsal fins, 10) number of anal fins and 11) biozones (Table 4.1). Each taxon was scored based on information extracted from direct examination of specimens or published literature. The data matrix and the description of the characters used for the analysis are presented in Table 4 and the scripts in the Supplementary S2 and S3. The ASR package in Mesquite (v3.31; Maddison & Maddison 2015) was used to visualize the character distribution on the topology and reconstruct the ancestral states that minimize the character change (i.e., parsimony). Traits were mapped onto the phylogeny generated from the partitioned by codon position maximum likelihood analysis in Chapter 2 (Fig 2.6).

4.3. Results

4.3.1 Caudal skeleton of gadiform fishes

In this section, I describe the caudal-fin anatomy of extant gadiform taxa. The taxonomy, distribution, and general habitat and ecology of each family is briefly discussed. Each family

(sensu the phylogenetic hypothesis of Chapter 1) is described and arranged in alphabetical order. The outgroup includes species representing each order, beyond Gadiformes, currently included within Paracanthopterygii sensu Betancur et al. 2013 (i.e., Stylephoriformes, Zeiformes, Percopsiformes, and Polymixiiformes).

Bathygadidae

Bathygadidae includes two genera (*Bathygadus* and *Gadomus*) and about 26 species (Nelson et al. 2016). They are distributed worldwide in tropical and subtropical waters from about 200 m to more than 2,700 m (*B. favosus* recorded at 2,745 m, but mainly within the depth range 650 – 1,350 m; Priede 2017, Froese and Pauly 2018). Members of this family have two dorsal fins (the second of which has rays that are longer than those of the anal fin), a rounded snout, the anus positioned next to the origin of the anal fin, and no luminous organ (Cohen et al. 1990). Six specimens of both genera, representing four species, were examined in this study (Fig. 4.1, Table 4.1)

An externally discernible caudal fin is absent in the family and the long dorsal and anal fins meet at the tip of a tapering tail (Fig. 4.1A). Internally, there are several elongate posterior vertebrae with neural and haemal spines. Most of the specimens examined in this study have lost the tail due to damage and the "pseudocaudal fin" is composed of an incomplete centrum. According to Howes and Crimmen (1990), bathygadids do not have a true caudal skeleton and the caudal rays could be derived from dorsal and/or anal-fin rays surrounding the posteriormost centrum. These authors found the last centrum always to be incomplete, ending in a flat cartilaginous plate supporting two to five rays and with neural and haemal spines that have variable shapes (e.g. elongated, lamellate). Okamura (1970) presumed that the cartilaginous plate

is generated from a cartilage between centra after the tip of the tail was broken. The specimens examined in this study showed both conditions: having a cartilaginous plate with regenerated fin rays (Fig. 4.1B, C) or with the posterior portion missing and only a portion of the centrum remaining (Fig. 4.1D).

Bregmacerotidae

One genus, *Bregmaceros*, and 14 species are recognized within the family (Nelson et al. 2016). They are small pelagic fishes (less than 12 cm SL) found inshore and on the continental shelf in tropical and subtropical waters from the surface to depths of approximately 2,000 m (Priede 2017, Froese and Pauly 2018). The family is characterized by having the first dorsal fin with a single long ray positioned on top of the head, second dorsal- and anal fins with well-developed anterior and posterior lobes, pelvic fins that are jugular and extend beyond the anterior lobe of anal fin, a well-developed caudal fin, and having the lateral line extend along dorsal margin of body (Cohen et al. 1990). Seven specimens belonging to at least four species were examined in this study (some specimens could be identified only to genus, Fig. 4.2, Table 4.1).

The caudal fin has 27-36 total fin rays (see also Fahay and Markle 1984, Swidnicki 1991). The caudal-fin rays are supported by six or seven vertebrae, the compound vertebrae, pu1+u1, and u2 (Fig. 4.2). The last centrum (u2) shows no clear upward turning in some specimens (e.g., Fig. 4.2B), while in others it is a dorsally angled splinter that was variably separate from (Fig. 2A) or fused to (Fig. 4.2D) the dorsal hypural plate. The dorsal hypural plate is composed of the fused hypurals 3-5 supporting seven to nine branched fin rays, whereas the ventral plate contains the fused hypurals 1 and 2 supporting a single fin ray (Fig. 4.2, Table 4.1). The two epurals, the ventral hypural plate, and the parhypural each support a single branched

ray. X and Y bones are present and each supports a single procurrent fin ray, while the neural and haemal spines of preural vertebrae 4-7 or 8 may support 1-3 fin rays. The X and Y bones, epurals, and parhypural have either a flattened and wide shape (Fig. 4.2A-B, D- F) or are thin and more elongate (Fig. 4.2C). The presence of a uroneural in *Bregmaceros* has been suggested by several authors (Swidnicki 1991: fig. 18A; Markle 1982: fig. 7B; see Fig. 4.2F). Other studies do not report the presence of this element (Fujita 1990: fig. 138; see Fig. 4.2E). Several specimens examined in this study show an ossification in the dorsal part of u2 that could be confused with the uroneural (e.g., Fig. 4.2A, D). However, after close inspection of the cleared and stained specimens it was determined that this was better interpreted as the dorsally directed portion of u2 at its point of fusion with the dorsal hypurals.

Euclichthyidae

There is only one species in this family, *Euclichthys polynemus,* and it is only found in the southwest Pacific (off New Zealand and around Australia). It is a bathydemersal fish found mainly between 250 - 920 m (Nelson et al. 2016, Froese and Pauly 2018). Four specimens were examined in this study (Fig.4.3, Table 4.1)

The caudal fin of *E. polynemus* has 33 - 41 total rays (this study). The specimen with 33 fin rays was examined in this study (Fig. 4.3A) and no fin rays were associated with the hypurals. The low number of total rays relative to other specimens (36 - 41), therefore, is likely due to damage. The caudal-fin rays are supported by ten vertebrae, pu1+u1, and u2. There is only one dorsal hypural plate (hy3 + hy4 + hy5) and one ventral hypural plate (hy1 + hy2) (Fig. 4.3). Anterior to the dorsal hypural plate there are two epurals that lie dorsal to pu1+u1, and a nspu2. There is inconsistency in the literature regarding the presence or absence of X and Y bones in *E.*

polynemus. According to Paulin (1983: fig. 5c) the X bone is present and the Y bone is absent; Cohen (1984:263) reported only one of the bones in each of two specimens. In contrast, Patterson and Rosen (1989: fig. 6) found both elements, while Markle (1989: figs. 16-17) found a small cartilaginous "X bone" and several cartilaginous "Y bones". The specimens examined in this study lack both X and Y bones (Figs. 3A-C) and no cartilaginous elements were found in any of the specimens that could be interpreted as X bones (e.g., Markle 1989). However, one specimen (Fig. 4.3D) has three structures that are similar to those reported by Markle (1989) as "cartilaginous Y bones". I interpret these to be rudimentary posterior anal-fin pterygiophores. Anterior to the ventral hypural plate is the parhypural with its base adjacent to the haemal spine of p1+u1, and haemal spines of the more anterior vertebrae. The epurals and parhypural have a flattened, wide shape (Fig. 4.3).

Gadidae

Gadidae includes some of the most valuable commercial species of fishes (e.g. Cod, Haddock, Pollock and Whiting). They inhabit circumpolar and temperate waters, mainly of the northern hemisphere. Most species are demersal or benthopelagic and several species are recorded in deeper waters to about 1,000 m (e.g., *Arctogadus glacilis*, *Gadiculus argenteus, Micromesistius australis, Theragra chalcogramma*) and the record is retained by *M. poutassou* at 3,000 m (Nelson et al. 2016, Priede 2017, Froese and Pauly 2018). The family is characterized by the presence of three dorsal fins and two anal fins, X and Y bones being absent, and the eggs lack an oil globule (Markle 1982, Cohen 1984, Nelson et al. 2016). Seven specimens representing six of the 12 genera currently recognized in the family were examined (Fig. 4.4, Table 4.1).

The gadid caudal fin has 35-70 total fin rays (Fahay and Markle 1984). The caudal-fin rays are supported by 13 to 16 vertebrae, pu1+u1, and u2. There is one ventral hypural plate (hy1 + hy2) and one dorsal hypural plate (hy3 + hy4 + hy5) (Fig. 4.4). Anterior to the dorsal hypural plate there are two epurals that are positioned dorsal to pu2, a neural spine arising from the second pre-ural centrum (nspu2). Anterior to the ventral hypural plate is the parhypural, which has its base adjacent to the haemal spine of pu2, and neural and haemal spines of more anterior vertebrae. Double spines (a double neural, a double haemal spine, or both) on pu2 have been reported for *Melanogrammus aeglefinus* and *Gadus morhua* (Markle 1982: fig. 8e, f). This phenomenon was observed on pu2, but also on preceding centra from pu4 to pu10 in five of the six genera examined in this study (Fig. 4.4B-F).

Gaidropsaridae

The family includes three genera (*Gaidropsaurus*, *Ciliata* and *Enchelyopus)* with 18 species (Nelson et al. 2016, Priede 2017). They are found in the northern Atlantic and in the oceans of the southern hemisphere. They typically inhabit shallow waters, although one species (*Gaidropsaurus argentatus*) is found in deep-waters down to 2,260 m (Priede 2017). The family is characterized by (i) the presence of three almost continuous dorsal fins (the first one consists in only a single unsegmented ray, the second consist of several unsegmented rays within a groove, and the third one has segmented rays and runs most of the body length) and (ii) two to four barbels on the snout and one at the tip of the lower jaw (Nelson et al. 2016). Six specimens representing the three genera and four species were examined (Fig. 4.5, Table 4.1).

The caudal fin comprises 29-46 total caudal-fin rays (see also Fahay and Markle 1984). The caudal-fin rays are supported by six to seven regular vertebrae, pu1+u1, and u2. The u2

shows no clear upward turning in most specimens (only the specimen in Fig. 4.5A has a splinter turning upwards and which is separated from the dorsal hypural plate). The dorsal hypural plate (hy3 + hy4 + hy5) has five caudal-fin rays attached. The ventral hypural plate (hy1 + hy2) originates on the ventral side of the preceding compound vertebra (pu1+u1) and two caudal-fin rays are attached to it (Fig. 4.5A-C). Dorsal to pu1+u1 there are two epurals that are broadened distally; each supports a single fin ray. An X bone is present between nspu2 and nspu3. It is only slightly distally broadened and supports a caudal-fin ray. Between the ventral hypural plate and hspu2 the autogenous parhypural is located. Anterior to hspu2 is the Y bone, and haemal spines of the preceding centra. The more anterior haemal and neural spines (nspu2-nspu7, hspu2-hspu7) are full, distally broadened spines that support caudal-fin rays.

Lotidae

Lotidae includes three genera, two of which are monotypic (*Brosme brosme*, *Lota lota*); *Molva* includes three species (Nelson et al. 2016). *Lota lota* is the only member of the family that lives in freshwater and has circumarctic distribution. *Brosme brosme* and the three species of *Molva* are found in the North Atlantic from shallow waters to 1,000 m (Froese and Pauly 2018). All species are demersal and three of them (*Brosme brosme*, *M. dypterygia* and *M. molva*) are commercially exploited (Priede 2017). Members of the family have one or two dorsal fins, a chin barbel, a rounded caudal fin, and eggs with an oil gobule. The X and Y bones can be present or absent (Fahay and Markle 1984, Nelson et al. 2016). Seven specimens representing all genera and three of the currently recognized five species were examined in the present study (Fig. 4.6, Table 4.1).

The caudal fin has 42-53 total caudal rays (Fahay and Markle 1984) that are supported by 11-14 vertebrae, pu1+u1, and u2 (Fahay and Markle 1984). There is one ventral hypural plate (hy1 + hy2) and one dorsal (hy3+hy4+hy5) hypural plate (Fig. 4.6A-E). Anterior to the dorsal hypural plate there are two epurals, which are dorsal to pu1+u1, and a neural spine arising from pu2. Anterior to the ventral hypural plate is the parhypural, which has its base adjacent to the haemal spine of pu2 (Fig. 4.6). Double neural and haemal spines were observed on pu2 in *B. brosme* and *L. lota* (Fig. 4.6A, B). Within Lotidae X and Y bones are present only in *M. molva* (Fig. 4.6D, E).

Lyconidae

The family Lyconidae was recently resurrected by von der Heyden and Matthee (2008) but remains poorly known. It contains two genera *Lyconus* (with two species, *L. brachycolus* and *L. pinnatus*) and the potentially extinct *Lyconodus argenteus* (Lloris and Matellanas 2005). All species are known from only a few specimens with depth range of 150 - 997 m (*L. brachycolus*) and 150 - 700 m (*L. pinnatus*); juveniles are found from 150-700 m whereas adults are found in the continental shelf and seamounts (Cohen et al. 1990, Lloris and Matallanas 2005). Ten specimens of *Lyconus* spp. were examined in this study (Fig. 4.7, Table 4.1)

The family does not have a distinct caudal fin and as in other tailless gadiforms the caudal region terminates posteriorly with the confluence of the dorsal and anal fins. Marshall (1966) reported a specimen collected in the Indian Ocean with a caudal fin similar to *Macruronus* (see below), although no specimen number or illustration was presented. Despite of the rarity of specimens, a specimen in excellent condition was found for this study (*Lyconus* sp., LACM 115919; Fig. 4.7A, B). All specimens examined except one had the distal portion torn off

and only a portion of a centrum remaining. This specimen has two fin rays supported by the posteriormost centrum, although there is no evidence of any other elements associated with the caudal skeleton (Fig. 4.7B). No cartilaginous plate was observed in any of the specimens with broken tail. In one specimen there are three neural spines and three haemal spines followed by several distorted vertebrae (Fig. 4.7C).

Macrouridae

This is the most species-rich family within Gadiformes and includes 29 genera and about 364 species. They are among the most abundant of the deep-sea fishes (Nelson et al. 2016, Froese and Pauly 2018) and are distributed worldwide except in the high Arctic Ocean. Almost all the species are bottom-dwelling (benthopelagic and bathydemersal) found from the upper slope (~ 200 m) to the hadal trenches (*Coryphaenoides yaquinae,* 6008-7012 m; Linley et al. 2016). Members of the family are known as rattails because of their slender bodies that tapers to a very thin tail. The family has two dorsal fins (the second dorsal has fin rays that are smaller than those of the anal fin) and a terminal to subterminal snout; a luminous organ located in the middle of the abdomen is present in most species (Cohen et al. 1990). Twelve specimens including six genera and nine species were examined in this study (Fig. 4.8, Table 4.1)

Externally macrouroids do not have a distinct caudal fin and its tail appears to be surrounded by rays from both the dorsal and anal fins. The internal caudal skeleton of macrouroids (formerly including Bathygadinae, Macrouroidinae and Trachyrincinae) was described based on *Coelorhynchus hubbsi* Matsubara 1936 by Okamura (1970: fig. 58; Fig. 4.8B-C). According to this author the caudal fin skeleton consists of elongate centra; the four most posterior of which do not have neural or haemal spines. Additionally, the last vertebra was

described supporting three “interneurals” or “interhaemals”, which I interpret as hypural elements (= hy 4, 5, 6), whereas the penultimate vertebra was described supporting an “interneural” dorsally and two “interhaemals” ventrally (hy 1 and hy 2-3) (Fig. 4.8B). The tail was either broken (Fig. 4.8D) or a cartilaginous plate was observed (Fig. 4.8E) on all specimens examined in this study. Therefore, it was not possible to corroborate the existence of the bones forming the caudal skeleton as described by Okamura (1970).

Macruronidae

The family includes only the genus *Macruronus* and four species (*M. capensis*, *M. maderensis*, *M. magellanicus* and *M. novazelandiae*) until a series of recent reviews that rendered the genus monotypic (*M. novezelandiae*). According to Lloris et al. (2003), *M. magellanicus* is a junior synonym of *M. novaezelandiae* based on morphological data. Their findings were corroborated by Olavarría et al. (2006) using DNA barcoding (cytochrome c oxidase subunit I gene, COI). Leslie et al. (2018) examined the taxonomic status of *M. capensis* using molecular (COI) and morphological data and concluded that *M. capensis* is also a junior synonym of *M. novaezelandiae*. Maul (1951), described *M. maderensis* from eight juvenile specimens recovered from the stomach of *Alepisaurus ferox* and its status is uncertain (Inada 1990; Howes 1991; Lloris et al. 2016). This species is benthopelagic and inhabits the cold temperate regions of the Southern Hemisphere and southeastern Atlantic (South Africa) with depth range of 30 - 1,000 m (Froese and Pauly 2018). In South America, Australia and New Zealand waters, *M. novaezelandiae* supports an important commercial fishery (Inada 1990, Lloris et al. 2003, Leslie et al. 2018). Six specimens, including representatives formerly identified as *M. magellanicus* and *M. novazelandiae*, were examined (Fig. 4.9, Table 4.1)

The dorsal, anal, and caudal fins are confluent. The caudal fin comprises 10-12 total caudal-fin rays (Fahay and Markle 1984). All of the specimens examined in this study have only 10 total caudal-fin rays that were supported by pu2, pu1+u1 and u2. The u2 shows no clear dorsal turning in any specimen. Two dorsal hypurals supporting one or two caudal-fin rays each were observed (Fig. 4.9A, B). The ventral hypural plate (hy1 + hy2) originates on the ventral side of pu1+u1 and has two caudal-fin rays attached to it (Fig. 4.9A, B). Dorsal to pu1+u1 there are two epurals that are distally broadened; each supports a caudal-fin ray. Between the ventral hypural plate and hspu2 there is an autogenous parhypural. The haemal and neural spines of the caudal region are long, thin, and pointed. Intercalated between adjacent neural and haemal spines are the pterygiophores are located. The proximal-middle radials are situated between the haemal and neural spines, the posterior portion has a triangular shape where the dorsal and anal fin rays articulate on small-round distal radials (Fig. 4.9A, B).

Melanonidae

Melanonidae comprises two species in a single genus (*Melanonus gracilis* and *M. zugmayeri*). *Melanonus gracilis* has a circumpolar distribution in the Southern Hemisphere whereas *M. zugmayeri* is found in tropical and temperate waters of the Atlantic, Indian and Pacific Oceans (Nelson et al. 2016, Froese and Pauly 2018). Both species are bathypelagic with the deepest records at 3,613 m for *M. gracilis* (Miller 1993) and 3,000 m for *M. zugmayeri*. Five specimens representing both species were examined in the present study (Fig. 4.10, Table 4.1).

The caudal fin comprises 55-60 total caudal rays (Fahay and Markle 1984) that are supported by 12 vertebrae, pu1+u1, and u2. The last centrum (u2) shows no clear upward turning in any specimen. Three dorsal hypurals support a total of six caudal-fin rays (Fig. 4.10 A, B).

Two ventral hypurals (hy1, hy2), which contact pu1+u1 proximally, support a total of three caudal-fin rays (Fig. 4.10). Dorsal to pu1+u1 there are two epurals that are broadened distally; each supports a single caudal-fin ray. The nspu2 and hspu2 are similarly broadened distally (Fig. 4.10). The autogenous parhypural is positioned between the ventral hypural plate and hspu2. Double spines (either a double neural or a double haemal spine or both) were observed on pu2. The more anterior of the double nspu2 and nspu3 has a similar shape to that of more anterior neural spines (Fig. 4.10). In contrast, the double haemal spine is a fork of the broadened hspu2. The more anterior haemal and neural spines are long, thin and pointed, with each supporting a single caudal-fin ray.

Merlucciidae

The family Merlucciidae includes only the genus *Merlucccius* and the number of the species ranges from 12 (Eschmeyer et al. 2018) to 16 (Froese and Pauly 2018) depending on the author. They are demersal fishes that have a high dispersal capacity (Inada 1981) and are widely distributed in the Atlantic and Pacific Oceans and the waters around New Zealand; there are isolated records of *Merluccius productus* (Ayres 1855) from the coast of Japan and the Indian Ocean (Lloris et al. 2005, Endo and Kitagawa 2006). Merlucciids occur in continental shelf waters around 50 m depth to 1,000 m or more. They are sometimes found off at bottom or in midwater (Cohen et al. 1990, Priede 2017). Three species were examined in the present study (Fig. 4.11, Table 4.1).

The caudal fin comprises 34-43 total caudal rays (Fahay and Markle 1984). The caudal-fin rays are supported by nine to ten vertebrae, pu1+u1, and u2. The u2 shows no clear upward turning in any specimen examined in this study. The dorsal hypural plate (hy3 + hy4 + hy5)

supports six caudal-fin rays. The ventral hypural plate (hy1 + hy2) contacts the ventral side of pu1+u1 and supports three caudal-fin rays (Fig. 4.11). Dorsal to pu1+u1 there are two epurals that are distally broadened; each supports a single caudal-fin ray. An X bone is present between nspu2 and nspu3, and it is only slightly distally broadened; it supports a single caudal-fin ray. Between the ventral hypural plate and hspu2 the autogenous parhypural is located. Anterior to hspu2 is the Y bone, and haemal spines on more anterior vertebrae (Fig. 4.11).

Moridae

There are approximately 110 morid species in 18 genera are currently recognized (Priede 2017). These fishes are benthopelagic to pelagic fishes ranging from shallow coastal areas to deep waters below 2,500 m with circumglobal distribution. The family is characterized by the presence of an otophysic connection, four or five hypurals, X and Y bones, a first neural spine joined to the supraoccipital crest, and distinctively shaped sagittal otoliths that have a deeply channeled bifurcate cauda (Svetovidov 1948, Fitch and Barker 1972, Cohen 1984, Paulin 1989, Schwarzhans et al. 2017). Ten specimens including seven genera and eight species were examined in this study (Fig. 4.12, Table 4.1)

The morid caudal fin has a total of 20-40 rays. The caudal-fin rays are supported by four to nine vertebrae, pu1+u1 and u2. There are two ventral hypurals (hy1 and hy2) which contact pu1+u1 proximally (Fig 12). Although these are separate distally, they are fused at their base. Three dorsal hypurals (hy3, hy4, hy5) are fused with u2 and diverge posteriorly (Fig. 4.12). Anterior to the dorsal hypurals are two epurals that lie dorsal to pu1+u1. More anteriorly there is the nspu2, the X bone, and the neural spines of the more anterior vertebrae. Anterior to the

ventral hypurals is the parhypural, the base of which is typically in contact with the pu2. Anterior to the hspu2 is the Y bone and more anterior haemal spines.

In this study, *Mora mora, Lepidion eques,* and *Halargyreus johnsoni* (Fig. 4.12A-C) correspond to the above description, which is consistent with what has been reported for the family (e.g. Fujita 1990: fig.137, Paulin 1983: figs. 4, 5D). However, variation in other taxa related to the fusion of several hypurals was observed. For example, *Gadella imberbis* (Fig. 4.12D) has hy1 and hy2 fused distally and separate proximally. In *Physiculus fulvus* (Fig. 4.12E) ventral hypurals are either separated (Fig. 4.12E) or completely fused (Fig. 4.12F). Both individuals of *G. imberbis* that were examined have the three dorsal hypurals fused (Fig. 4.12D, G), while *P. fulvus* has hy3 separated from hy4 + hy5 (Fig. 4.12E) or all three dorsal hypurals fused (Fig. 4.12F). However, Fujita (1990: fig. 138, reproduced here as Fig. 4.12H) illustrated all three dorsal hypurals separated in *P. jordani* by. Further, *G. imberbis* has a double nspu2 (which is common in gadoids) and a thin splinter-like element that could be the X bone. In one of the specimens of *G. imberbis* examined (Fig. 4.12G), the nspu2 is followed by the nspu3 and next to it (between npu3 and nspu4), a thin splinter was observed that could be interpreted as the X bone. If this element is the X bone, it is placed in a unique position among gadiforms (typically found between the nspu2 and nspu3). Paulin (1983: fig. 4, reproduced here as Fig. 4.12H) suggested the presence of an uroneural in Moridae, although none of the specimens examined here have an uroneural, corroborating the findings of most studies (e.g., Fahay and Markle 1984, Fujita, 1990).

Muraenolepididae

Muraenolepididae includes two genera, *Muraenolepis* with seven species and the monotypic *Notomuraenobathys microcephalus*. *Muraenolepis microps* Lönnberg 1905 was synonymized with *M. marmorata* by Balushkin & Prirodina (2010) and *M. microcephalus* Norman 1937 was placed in its own genus, *Notomuraenobathys*, based on a greater number of vertebrae, differences in the cephalic sensory canals and body proportions (Balushkin and Prirodina 2010). They are bathydemersal or benthopelagic fishes found in the Southern Hemisphere at 30 – 2,010 m (*Muraenolepis*) and 1976 - 3040 m (*N. microcephalus*) (Cohen et al. 1990, Froese and Pauly 2018). The family is characterized by (i) two dorsal fins, the first one with only one or two rays, and the second dorsal fin runs most of the body length (ii) a caudal fin connected with the dorsal and anal fin, (iii) one anal fin, and (iv) a chin barbel that is always present (Cohen et al. 1990, Nelson et al. 2016). Eight specimens of *Muraenolepis* representing three species were examined in the present study (Fig.4.13, Table 4.1).

The dorsal, anal, and caudal fins are confluent. The caudal fin comprises eight to 11 total caudal-fin rays supported by pu2, pu1+u1 and u2. There is not clear turning in u2 show in any specimen. The dorsal hypural plate (hy 3 + hy4 +hy5) supports four caudal-fin rays (Fig. 4.13). The ventral hypural plate (hy1 + hy2) originates on the ventral side of pu1+u1 and has two caudal-fin rays attached to it (Fig. 4.13). There are two epurals dorsal to pu1+u1 that are distally broadened; each supports a caudal-fin ray. Between the ventral hypural plate and hspu2 there is an autogenous parhypural. X and Y bones are absent (Fig. 4.13). The haemal and neural spines of the caudal region are short, thin, and pointed. Double neural and haemal spines were observed on pu2 (Fig. 4.13A). The pterygiophores are intercalated between adjacent neural and haemal spines. The proximal-middle radials are situated between the haemal and neural spines, they are

thin and the posterior portion has a triangular shape where the dorsal and anal fin rays articulate on small-round distal radials (Fig. 4.13).

Phycidae

The family Phycidae includes two genera (*Phycis* and *Urophycis)* with 11 species (Nelson et al. 2016). They are benthopelagic fishes found in the outer continental shelves and slopes in the eastern and western Atlantic Ocean (Froese and Pauly 2018). Some species are non-migratory while in other species juveniles spend part of their time in estuaries (Froese and Pauly 2018). The family is characterized by (i) the presence of two dorsal fins and one anal fin, neither of which is connected with the caudal fin and (ii) pelvic fin with two elongate rays (Nelson et al. 2016). Seven specimens representing both genera and four species were examined (Fig. 4.14, Table 4.1).

The caudal fin comprises 28-39 total caudal rays (see also Fahay and Markle 1984). The caudal-fin rays are supported by four to six regular vertebrae, pu1+u1, and u2. There is not clear upward turning of u2 in any of the specimens examined (Fig. 4.14). The dorsal hypural plate (hy3 + hy4 + hy5) has six caudal-fin rays attached. The ventral hypural plate (hy1 + hy2) originates on the ventral side of the preceding compound vertebra (pu1+u1) and three caudal fin rays are attached to it (Fig. 4.14). Dorsal to pu1+u1 there are two epurals that are broadened distally; each supports a single fin ray. An X bone is present between nspu2 and nspu3. It is distally broadened and supports a caudal fin ray. Between the ventral hypural plate and hspu2 the autogenous parhypural is located. Anterior to hspu2 is the Y bone, and haemal spines of the preceding centra (Fig. 4.14).

Ranicipitidae

The family includes only *Raniceps raninus,* which lives in the coastal waters (from zero to 100 m, but usually between 10 to 20 m depth) of the northeastern Atlantic Ocean from Trondheim on the Norwegian coast to the Bay of Biscay, as well as around the British Isles (Nelson et al. 2016, Froese and Pauly 2018). Four specimens were examined in this study (Fig. 4.15, Table 4.1).

The caudal fin has 33-36 total rays. The caudal-fin rays are supported by six preural vertebrae, pu1+u1, and u2. There are two ventral hypurals (hy1 and hy2) that are separate distally and fused at their base; they are supported by pu1+u1 (Fig. 4.15). The dorsal hypural plate (hy3, hy4 + hy5) diverges posteriorly from u2 in some specimens (Fig. 4.15). In two specimens, hy3 and hy4+5 appear to be either partially (Fig. 4.15A) or nearly completely separate (Fig. 4.15C). In the other two specimens, the fusion between hy3 and hy4+5 is limited to the proximal portion of the element and the distal part is completely fused (hy3+hy4+hy5; Fig. 4.15B, D). Fusion among the six hypural elements (counting phy as hy1) resulting in three distinct elements in Ranicipitidae was proposed as a character of the family by Dunn and Matarese (1984) based on ontogenetic data. Anterior to the dorsal hypurals there are two epurals dorsal to pu1+u1, a neural spine arises from the second pre-ural centrum (nspu2), and the X bone. Anterior to hy1 and hy2 is the parhypural, which is positioned adjacent to hspu2. Borden et al. (2013; CAS 225749) reported that the parhypural is attached to the ventral hypural elements. However, all specimens examined in this study have the parhypural well separate from the ventral hypural elements. Between the haemal spines of pu2 and pu3 is the Y bone. The neural and haemal spines of pu6 and pu7 support caudal-fin rays. In all four specimens examined in this study, hspu2 does not appear to be fused to pu2 (Fig. 4.15).

Steindachneriidae

Steindachneriidae includes only *Steindachneria argentea*. It is a bathydemersal species found in the outer shelf and slopes (400-500 m) of the Western Atlantic Ocean from Florida and the Gulf of Mexico through the northeastern corner of South America (Cohen et al. 1990, Nelson et al. 2016). The family is characterized by (i) the presence of the anus between the pelvic fins and separated from the urogenital pore, which is anterior to the anal fin, (ii) an externally discernible caudal fin is absent and the long dorsal and anal fins meet at the tip of a tapering tail, (iii) light organ present in the ventral part of the body and the head (Priede 2017). Five specimens were examined in this study (Fig. 4.16, Table 4.1).

According to Marshall (1966: fig. 1), the posteriormost centum supports a long fin ray with a broad base. Borden et al. (2013: fig. 7d), however, interpreted the species to have two cartilaginous hypurals, identifying them as such because they articulate with a centrum that was interpreted as representing u2. These authors further documented that each plate supports two filamentous fin rays. Most of the specimens examined in this study had the tail broken (16A), only one cleared and stained specimen revealed the presence of caudal skeleton elements (Fig. 4.16B). The posteriormost vertebrae, which is only represented by its centrum, supports two posterior cartilaginous elements, of which one is directed posteriorly along the axis of the vertebra, and the second extends posteroventrally from the posterior tip of the centrum; each of these elements supports a single fin ray (Fig. 4.16C). These observations support Borden et al.'s interpretation, and I tentatively identify these elements as two hypural elements (hy1-2 and hy3-5, Fig. 4.16C). The proximal-middle radials are situated between the haemal and neural spines, they are thin and the posterior portion has a triangular shape where the dorsal and anal fin rays articulate on small-round distal radials (Fig. 4.16B).

Trachyrincidae

Trachyrinchidae includes two subfamilies, Trachyrincinae with two genera (*Trachyrinchus* with six species and the monotypic *Idiolophorhynchus andriashevi*), and Macrouroidinae with two monotypic genera (*Macrouroides inflaticeps* and *Squalogadus modificatus*) (Nelson et al. 2016). *Trachyrinchus* species are found in the northern and southern hemispheres between 450-1400 m, whereas *I. andriashevi* is found off New Zealand and West and South Australia between 1190 - 2350 m (Nelson et al. 2016, Froese and Pauly 2018). All species are characterized by a pointed snout and ventral mouth and differ in the presence of a chin barbel (*Trachyrincus* spp.) versus lacking a chin barbel (*I. andriashevi*). The two species of the subfamily Macrouroidinae (*M. inflaticeps* and *S. modificatus*) are found circumglobally associated with continental slopes in tropical to temperate waters (747 - 4000 and 600 - 1740 m respectively) (Sazonov and Iwamoto 1992, Hoese et al. 2006). Both species have a large head, ventral mouth, small eyes and long dorsal and anal fins that meet at the tip of a tapering tail. They differ in the presence of a small pelvic fin with five rays in *S. modificatus*; the pelvic fin is absent in *M. inflaticeps* (Cohen et al. 1990). Twelve specimens, including one genus of each subfamily and five species in total, were examined for this study (Fig. 4.17, Table 4.1).

Marshall (1973: 501) reported the presence of a modified caudal skeleton in two specimens of *T. scabrus* (formerly *Trachyrincus trachyrincus*). This author found a caudal fin with seven or eight caudal-fin rays, the last partial centrum supporting two hypural plates, the penultimate centrum supporting one hypural plate, and the presence of X and Y bones. Howes (1989, fig. 6) was not able to corroborate the presence of X and Y bones, but he reported two hypural plates; the dorsal plate supporting two fin rays and the ventral plate supporting a single fin ray, two potential epurals and a total of eight caudal fin rays. These findings were supported

by Borden et al. (2013, fig. 7C) although they were not able to corroborate the presence of the X and Y bones. All specimens examined in the current study, ranging from perfect condition (whole external tail present) to damaged, are tailless (caudal region terminates posteriorly with the confluence of the dorsal and anal fins (Fig. 4.17). A specimen identified as *T. villegai* (USNM 129943) has a tail with at least six vertebral centra with small neural and/or haemal spines (Fig. 4.17 B). Two specimens of *T. murrayi* (Fig. 4.17 C, E) have a partial last centrum surrounded by rays from both the dorsal and anal fins. A specimen of *T. scabrus* has the last centrum distorted and bearing several fin rays; the next anterior vertebra has shorter neural and haemal spines (Fig. 4.17E). The haemal and neural spines of the caudal region are long, thin, and pointed. The pterygiophores are deeply intercalated between adjacent neural and haemal spines. They are thin and the posterior portion has a triangular shape where the dorsal and anal fin rays articulate on small-round distal radials (Fig. 4.17B-G). Three neural and haemal spines on one vertebra were observed (Fig. 4.17E). It was not possible to corroborate the presence or absence of a caudal fin in Macrouroidinae due to the lack of specimens with a complete tail (Fig. 4.17F).

4.3.2 Caudal skeleton of non-gadiform Paracanthopterygii

Stylephoriformes

Stylephorus chordatus is the sole member of the monotypic order Stylephoriformes. It has been historically included within Lampridiformes (e.g., Lampridae, Lophotidae, Trachipteridae, Regalecidae) and is now considered the sister group of Gadiformes (Miya et al. 2007). The sister-group relationship between Gadiformes and *Stylephorus* has been corroborated by several molecular studies (Near et al. 2013, Malstrøm et al. 2016, Betancur-R et al. 2017), but is poorly supported based on morphological data (Grande et al. 2013). It is found in all oceans

and is an abyssal species, occurring at depths of during the day 600-800 m and 300-600 m at night (Nelson et al. 2016, Priede 2107, Froese and Pauly 2018). The species is characterized by (i) a ribbonlike body, (ii) vertical head-up posture in the water column, (iii) tubular eyes, (iv) protrusible tubular mouth, (v) a highly modified skull and (vi) two ventral caudal-fin rays elongated, which may extend more than twice the standard length (Pietsch 1978, Priede 2017). Three specimens were examined in this study (Fig. 4.18, Table 4.1).

The caudal fin of *Stylephorus* has two elongate fin rays and five to seven unmodified rays for a total of eight to nine caudal-fin rays (Regan 1924, Pietsch 1978). The caudal-fin rays are supported by pu1+u1, and u2. The last centrum (u2) shows a clear upward turning in the cleared and stained specimen and is fused to the dorsal hypural plates (Fig. 4.18B). There are three hypural elements, the ventral plate (hy1+hy2) is supported by pu1+u1 and the dorsal hypural plates (hy3, hy4+hy5) by u2. The ventral hypural plate and hy3 each support an elongate caudal-fin ray. These two fin rays are tightly attached to one another by lateral-line scales (Pietsch 1978: fig. 8; pers. obs. Fig. 4.18C). The posteriormost hypural plate (hy4+hy5) has been hypothesized to be a fusion of hy3 +hy4 + hy5, which diverge posteriorly from u2 (Pietsch 1978: fig. 8, Borden et al. 2013: fig. 7E; Fig. 4.18D). Because pu1+u1 supports the parhypural and the anteriormost two hypurals in more basal teleosts, where there is only one hypural element (as in *Stylephorus*), it is presumed to be the product of fusion between the hy1 and hy2. Anterior to hy1 is the parhypural, which is positioned between pu1+ u1 and hspu2 (Fig. 4.18B, C). The neural and haemal spines are thin and short. Double neural spines were observed on pu2 (Fig. 4.18B, C)

Zeiformes

Zeiformes contains six families with about 16 genera and 33 species (Tyler et al. 2003, Nelson et al. 2016, Grande et al. 2018). They are mostly deep-sea fishes found on the upper slope or at mesopelagic depths (200-1000 m), usually associated submarine summits, ridges, and canyons (Nelson et al. 2016, Priede 2017). The order is characterized by (i) body deep and laterally-flattened, (ii) protrusible jaws, (iii) large eyes (Nelson et al. 2016, Priede 2017). The same species (*Zenopsis conchifer*) used in the phylogenomic analyses was examined in this study (Fig. 4.19A-B, Table 4.1).

The species examined has 15 caudal-fin rays. Fusion between preural centrum 1, ural centrum 1, and ural centrum 2 to form a single terminal centrum is hypothesized in all zeiforms (pu1+u1+u2; Tyler et al. 2003). There are two hypural plates. The anteriormost is formed by the proximal fusion between hy1 + hy2 and hy3 + hy4, and each of these subplates supports five caudal-fin rays (Fig 19B). The posterior hypural element (hy5) is autogenous and intercalated between hy3 + hy4 and ep. Anterior to hy5 is a single epural that lies dorsal to pu1+u1+u2 and is similar in shape and size to hy5. Anterior to hspu2 is the Y bone; it does not support a caudal-fin ray. The more anterior neural and haemal spines are relatively short and thick (Fig. 4.19B).

Variation in the degree of fusion and number of elements has been reported for the family Zeidae, among species and at intraspecific level (e.g., Monod 1968, Rosen and Patterson 1969, Tyler et al. 2003, Borden et al. 2013). For example, the number of epurals varies from one (e.g., *Zeus faber*) to two (e.g., *Parazen pacificus*). The degree of fusion between the hypurals 1-4 ranges from completely fused at the base to form a single compound element (pu1+u1+u2+hy1-4), with the hypurals fused proximally but separate distally (e.g., *Zenopsis conchifer*; Fig. 4.19B), diverging from the base to the end in two large plates (pu1+u1+u2+hy1-2 and hy3-4;

Parazen pacificus; Borden et al. 2003: fig. 4.10C), or fused at the base with pu1+u1+u2, but separated into hy1-2, hy3 and hy4 (*Xenolepidichthys dalgleishi*; Borden et al. 2003: fig. 4.10D). Hypural five can be fused with pu1+u1+u2+hy1-5 (e.g., *Zeus faber*; Borden et al. 2003: fig. 10A), can articulate with the last centrum (*Zenopsis conchifer*; Fig. 4.19B) or can be autogenous (*X. dalgleishi*; Borden et al. 2003: fig. 10D). Rosen (1984: fig. 31) identified hy5 as one of three epurals. Intraspecific variation was noted among different studies. For example, in *Xenolepidichthys dalgleishi* the degree of fusion between the hypurals 1-4 ranges from fused at the base with pu1+u1+u2, but separated into hy1-2, hy3 and hy4 (Borden et al. 2003: fig. 10D, USNM 320016, SL not provided) to completely fused at the base to form a single compound element (pu1+u1+u2+hy1-4) (Tyler et al. 2013: fig. 69, CAS 38406, 75 mm SL).

Percopsiformes

The order Percopsiformes includes three families: Percopsidae with a single genus *Percopsis* and two species*;* Aphredoderidae with only one species, *Aphredoderus sayanus*; and Amblyopsidae with five genera and six species (Nelson et al. 2016). They inhabit freshwater habitats in North America (Nelson et al. 2016). The monophyly of the order has been questioned, with amblyopsids suggested to be closer to anacanthines or to gobiods (e.g. Murray and Wilson 1999), however molecular data supported their monophyly (e.g. Dillman et al. 2011), which is consistent with most morphological studies (e.g. Springer and Johnson 2004). The same species used in the phylogenomic analyses (*Percopsis transmontana*) was examined in this study (Fig. 4.19 C-D)

The specimen examined has 40 total caudal-fin rays supported by four preural vertebrae, fused hypurals 3+4 and u2. *Percopsis* has six hypurals in total of which four hypurals are fused

into two plates. The ventral hypural plate (hy1+ hy2) lies below the diastema and is supported by pu1+u1 (Fig. 4.19D). The dorsal hypural elements are interpreted as hy3+4, hy5 and hy6, the latter two are autogenous elements that lie dorsal to u2. A uroneural is present and located between hy6 and the most posterior epural. The ventral (hy1+hy2) and dorsal hypural plate (hy3+4) each support six caudal-fin rays; hy5 supports two and hy6 one. The uroneural supports two caudal-fin rays. There are two long, slender epurals that lie anterior to the dorsal hypurals; each epural supports two caudal-fin rays. The parhypural is anterior to hy1 + hy2 and is positioned adjacent to the hspu2. It has a flattened and wide shape (Fig. 4.19D). Both the nspu2 and hspu2 are long, and have bony outgrowths (flanges) that almost contact the adjacent hspu and nspu forming a strong bony caudal peduncle. The primary variation among elements of the caudal skeleton across percopsiforms includes the presence of four hypural elements in percopsids (Fig. 4.19D), three in aphredoderids (Borden et al. 2013: fig. 5a) and one in amblyopsids (Borden et al. 2013: fig. 6). The parhypural can articulate with (e.g., *Percopsis*) or be positioned below pu1+u1 (amblyopsids, *Aphredoderus*) (Tyler et al. 2003, Borden et al. 2013).

Polymixiiformes

The order Polymixiiformes includes one genus, *Polymixia* with 10 species (Nelson et al. 2016). All are bathydemersal species distributed in tropical and subtropical portions of the western Indian and western and eastern Atlantic Oceans (Nelson et al. 2016). They are found in the upper slopes of continental margins associated with oceanic islands and seamounts in semi-hard and soft bottoms between 20 and 800 m (Kotlyar 1993, Mundy 2005, Priede 2017). The genus is characterized by the presence of a pair of hyoid barbels, body moderately elongate and

compressed and the retention of three sets of intermuscular bones (Patterson & Johnson, 1995). Eight specimens representing two species were examined in the present study directly (Fig. 4.19E, F, Table 4.1).

The caudal fins of the examined species have 28-29 caudal fin rays. The caudal-fin rays are supported by pu1+u1, and u2. There are two autogenous ventral hypurals (hy1 and hy2) that contact pu1+u1 proximally (Fig 19F). There are four dorsal hypurals (hy3, hy4, hy5, hy6) that are supported by u2 (Fig. 4.19F). Two uroneurals are present (Fig. 4.19F). Anterior to the un1 are three short, thin epurals that are positioned dorsal to pu1+u1. The neural and haemal spines of pu2 and nspu3 have similar broadened outgrowths like *Percopsis transmontana*; the neural spines of the more anterior vertebrae are shorter. Anterior to the ventral hypurals is the parhypural, the base of which is typically in contact with the pu1+u1 (Fig. 4.19F).

4.3.3 Ancestral State Reconstruction

The results of the Ancestral State Reconstruction (ASR) of the eleven characters are shown in Figures 4.20-4.25 (see also Table 4.1, Supplementary Material S2). Within Paracanthopterygii, the total number of hypurals account for six (Polymixiiformes and Percopsiformes), five (Zeiformes, Stylephoriformes and Gadiformes) or none (gadiforms tailless families). Two separate ventral hypural elements are present in Polymixiiformes (Fig. 4.20) and some Gadiformes (Fig. 4.21), others have only a single ventral hypural element, presumably fused out of two hypurals (Percopsiformes, Zeiformes, Stylephoriformes and some Gadiformes families). The number of dorsal hypural elements is highly variable within the Paracanthopterygii, i.e., four hypural elements in the outgroups Polymixiiformes, three hypural elements in Percopsiformes and some Gadiformes, two hypural elements in Zeiformes and some

Gadiformes, one hypural element in Stylephoriformes, and zero in the tailless gadiform fishes (Fig. 4.22). The Y bone is sometimes present in Zeiformes (Border et al. 2013), and the earliest group to branch among Gadiformes (Bregmacerotidae) has both X and Y bones present (This study, Ch. 2.1, Fig. 2.6); these bones were lost at least three times in Gadiformes, and their presence is variable in the family Lotidae (Fig. 4.23). Two uroneurals are present in Polymixiiformes and Percopsiformes and lost in Zeiformes, Stylephoriformes and Gadiformes (Fig. 4.24). Three epurals are present in Polymixiiformes, two in Percopsiformes, Zeiformes and the tailed families in gadiforms, and are absent in Stylephoriformes and tailless gadiforms' families (Fig. 4.25).

Within Gadiformes there are two main phenotypes the tailed and the tailless fishes, neither of which form a monophyletic grouping. Among the tailed Gadiformes, five configurations of caudal skeleton elements were found. (I) Euclichthyidae, Gadidae and Muraenolepididae lack X and Y bones and have one ventral (hy1+hy2) and one dorsal (hy3+hy4+hy5) hypural element. (II) Bregmacerotidae, Gaidropsaridae, Lotidae, Merlucciidae, and Phycidae have X and Y bones and one ventral and one dorsal hypural element. (III) Moridae and Ranicipitidae have X and Y bones, two ventral hypurals and two or three dorsal hypural elements. (IV) Macruronidae and Moridae have one ventral hypural element and two dorsal hypural elements (hy3, hy4+hy5 or hy3+hy4, hy5), the two families only differ in that the Macruronidae do not have X and Y bones. (V) Moridae and Melanonidae have two ventral and three hypural elements, Melanonidae lack X and Y bones (Figs. 21-23). The family Moridae displays multiple conditions in the number of both ventral and dorsal hypural elements and this variation is reflected in their presence in the last three groupings.

The caudal skeleton was lost at least two times within Gadiformes. The tailless gadiforms include the families Bathygadidae, Lyconidae Macrouridae, Steindachneriidae, and Trachyrincidae (Fig. 4.20). Steindachneriidae is included within this group due to the extreme reduction of the caudal skeleton elements and their similarity in body shape with the tailless families (e.g. large eyes, slender body that tapers into a very thin caudal region) and living and feeding on the bottom below 200 m (Fig. 4.16).

4.4. Discussion

In this study the caudal skeleton of all Gadiform families was investigated and the evolution of this complex structure was examined in a phylogenetic framework. The most informative and variable caudal skeleton elements among gadiforms are the X and Y bones and the number of ventral and dorsal hypural elements, therewith the discussion will focus on those.

The presence of X and Y bones has been regarded and argued as a possible synapomorphy for the order, and their absence as a derived character due to secondary loss (e.g. Markle 1982, Cohen 1884) or as a primitive condition (e.g. Endo 2002). Patterson and Rosen (1989) pointed out the character evolution depends on prior knowledge of gadiform phylogeny and therefore the earliest branch plays a pivotal role. Muraenolepididae (e.g. Svetovidov 1948, Cohen 1984) and Melanonidae (e.g. Howes 1993, Endo 2002) were proposed as the earliest branches among Gadiformes. However, Muraenolepididae has the dorsal and anal fins confluent with the caudal fin and the X and Y bones cannot be identified. Melanonidae, on the other, hand lacks X and Y bones. Therefore, the diagnostic of these bones has remained ambiguous until the present study (Ch. 2, Fig. 2.6) that found Bregmacerotidae as the earliest branch among Gadiformes and consequently supports the presence of X and Y bones as a synapomorphy for the

order. The X and Y bones are present in Bregmacerotidae, Gaidropsaridae, Merlucciidae, Moridae, Phycidae, Ranicipitidae. Their presence is variable in Lotidae and they are absent in Gadidae, Melanonidae, Muraenolepididae and the tailless families (Markle 1989). This study did not observe the X and Y bones in the monotypic family Euclichthyidae, but other authors have reported one of the elements (Paulin 1983, Cohen 1984,) or both of them (Markle 1989, Patterson and Rosen1989) as present.

The evolutionary trend is that hypural elements fuse in Teleost (e.g. Gosline 1961, Rosen and Patterson 1969, Marshall and Cohen 1973, Markle 1989). In Gadiformes, the presence of five separate hypural elements (Melanonidae, Moridae and Ranicipitidae), has been proposed as a plesiomorphic condition and fusion of some of the elements (Bregmacerotidae, Euclichthyidae, Gadidae, gaidropsaridae, Lotidae, Macruronidae, Muraenolepididae and Phycidae) has been regarded as the derived condition (Svetovidov 1948, Greenwood et al. 1986, Rosen and Patterson 1969; Fig 21). Gadiformes fossil record is rich with otoliths, but few non-otolith fossils exist that could be useful to interpret the caudal skeleton (Kriwet and Hecht 2008). The oldest known fossil gadoid is † *Protocodus* from the early Paleocene of Greenland has a typical morid form and five hypurals elements (Rosen and Patterson 1969, Cohen 1984). On the other hand, †*Palaeogadus* and †*Bregmaceros albyi* have two hypural plates (Fedotov and Bannikov 1989), implying that hypurals fusion is a derived character (Cohen 1984). Ontogenetic fusion of elements can be verified with early life history stages, however, insufficient ontogenetic data on the caudal skeleton of Gadiformes is available (e.g. Matarese et al. 1981, Border et al. 2013) and so far, there is no direct evidence of ontogenetic fusion of elements in the caudal skeleton. In this study two hypural plates have been found as the primitive character (Bregmacerotidae) within the Gadiformes.

The ancestral state reconstruction suggests that the MRCA of gadiform fishes had a caudal skeleton and was pelagic (Fig. 4.20). The order evolved into different lineages with a predominantly benthopelagic form, which is reflected in a more elongate body with increased number of vertebrae, dorsal and anal rays (Marshall and Cohen 1973). One evolutionary trend includes those families with the caudal skeleton consolidated into two enlarged hypural plates (hy1+hy2 and hy3+hy4+hy5). This form of caudal skeleton may play an important role in locomotion. For example, families such as Gadidae and Merlucciidae have a wide range of habitats, from inshore to the continental shelf habitats (Cohen et al. 1990, Froese and Pauly 2018). They are some of the most highly mobile species among Gadiformes, capable of moving between shallow and deep habitats on diel (e.g. Pacific cod, *Gadus macrocephalus*) or seasonal timeframes (e.g. silver hake, *Merluccius bilinearis*). In contrast, other families with limited dispersal ranges in searching for food (Løkkeborg et al. 2000) such as Lotidae and Gaidropsaridae have a more elongate body shape designed for slow cruising (Koslow 1996).

Moridae and Ranicipitidae have the caudal skeleton with more elements among gadiforms including five hypurals, the X and Y bones, and the other elements present in the tailed families (e.g. epurals, parahypural). These two families are at the two ends of taxonomic, morphological, and ecological spectra. Morids are the second most diverse family among Gadiformes (with approximately 110 species), are benthopelagic to pelagic fishes, globally distributed, and found in shallow coastal areas to below 2,500 m (Cohen et al. 1990). In contrast, the monotypic *Raniceps raninus* is restricted to the northeastern Atlantic and lives in coastal waters at shallow depths, from 20 to 100 m (generally from 10 to 20 meters) on rocky bottoms and undertakes only limited local movements (Cohen et al. 1990). Morids have two or three dorsal fins and one anal fin that, in some genera, has a deep notch and appears as two (e.g.

Halargyreus, Mora, Lepidion). *Raniceps raninus* has two dorsal fins, but the first one is poorly developed consisting of three short rays; the second dorsal and the anal are long based. The disparity between these two families offers small opportunity for conjecture.

Moridae and Melanonidae share the presence of five hypural elements, but melanonids lack X and Y bones. Melanonids are deep-living pelagic fishes consisting of only two species, one with circumpolar distribution in the Southern Hemisphere (150 – 3,650 m) and the other is found in tropical and temperate waters of the Atlantic, Indian and Pacific oceans (1,000 – 3,000 m). While morids are benthopelagic to pelagic, melanonids are pelagic, being neither close to the bottom nor near the shore. These two families are externally very similar, but internally melanonids lack an otophysic (inner ear to swim bladder) connection correlated with increased hearing sensitivity (Deng et al. 2011). Such a connection evolved only in morids among Gadiformes. The other pelagic family within Gadiformes is Bregmacerotidae and they also lack the otophysic connection. They are considered the most anatomically aberrant among cod-like fishes (Marshall and Cohen 1973). The caudal fin is well developed in Bregmacerotidae and they are, together with Merlucciidae, considered the most active swimmers within the gadiforms (Marshall and Cohen 1973).

Based on the ASR analyses the caudal fin has been lost at least twice in Gadiformes (Macrouridae, Bathygadidae, Trachyrincidae and Lyconidae). Three of these families (Macrouridae, Bathygadidae, Trachyrincidae) were traditionally recognized by several authors as subfamilies within Macrouridae (Regan 1903, Marshall 1965, Marshall and Cohen 1973; Okamura 1970 a,b; Iwamoto 1989) and Lyconidae was placed *incertae sedis* until resolved by my analysis in Chapter 1. The traditionally constituted Macrouridae (i.e., including Bathygadinae, Macrourinae, Macrouroidinae and Trachyrincinae) was defined among other

characters by the extreme reduction or loss of the caudal skeleton and a tapering tail. However, the family Macrouridae including only macrouroids was strongly supported (bs = 100%) based on molecular data; the other three subfamilies Bathygadinae, Macrouroidinae and Trachyrincinae were placed in two independent families (Bathygadidae and Trachyrincidae, which includes Macrouroidinae and Trachyrincinae (Roa-Varón & Ortí 2009 and this study). Most of the species among these families are benthopelagic fishes recorded from depths of about 200 to 7,000 m (Priede 2017). The families without a tail have large heads with large mouths and eyes, and a body attenuated posteriorly to very thin tail. This morphology is the source of the common name "rattail," and the name of the largest family, Macrouridae comes from the Greek *makros* meaning "great" and *oura* meaning "tail" (McKee and Compton 2014). The first dorsal fin is small, high, and pointed; the second dorsal fin runs along the rest of the back and merges with the tail and the long anal fin. Many deep living fishes such as the rattails, halosaurs, notacanthids, saccopharyngids, and ophidiiforms among others share this character (e.g. the tapering body form and merging fins) as well as a well-developed lateral line system, numerous chemoreceptors located on the head and lips, and chemosensory chin barbels (Gordon 2001). The phenotypic convergence could be due to the environmental and similar niche exploitation, swimming and foraging strategies despite significant geographical distances and taxonomic dissimilarity (Grundler et al. 2014, Bridge et al. 2016). Additionally, changes in gradients of key environmental variables (e.g. light, pressure, and food availability) restrict some species to different depth ranges (Bridge et al. 2016). For example, Gadidae decreases in dominance with depth whilst macrouroids become more dominant in terms of biomass on the lower slope and rise making them of great ecological importance (Merrett and Haedrich 1997).

There are two dominant types of swimming modes among Gadiformes, anguilliform and sub-carangiform. In anguilliform locomotion, the whole body participates in large amplitude undulations (Sfakiotakis et al. 1999). Many anguilliform swimmers are capable of backward as well as forward swimming by altering the propagation direction of the propulsive wave (pers. obser. Okeanos Explorer). It uses less energy, which is useful in deep-water environments where food is less available than in shallow waters (Phleger 1971). Deep-sea fishes that live near the bottom usually feed on bottom-dwelling prey and a slow cruising swimming mode along the bottom, with the head down and tail up assists in feeding and predator sensing (Drazen and Sutton 2017). The sub-carangiform mode is characteristic of the cod-like Gadiformes, which have a stiffer body, making up for higher swimming speed with reduced maneuverability (Sfakiotakis et al. 1999). The tailless families are all anguilliform swimmers (e.g. Macrouridae, Bathygadidae) (Fig. 4.26). But also, some families with caudal skeleton such as Melanonidae and Macruronidae have an anguilliform swimming type (Fig. 4.26). Both families have evolutionary trends in opposite directions, Melanonidae has two species in a single widespread genus with bathypelagic mode of life recorded from 150 to 3,613 m. In contrast, Macruronidae inhabits the cold temperate Southern Hemisphere and southeastern Atlantic (South Africa) with depth range of 30 - 1,000 m. Both families have long dorsal and anal fins, and the caudal skeleton is smaller in size indicating a minor propulsive role compared with Gadidae in which the caudal skeleton is large and plays a more important role in locomotion.

Of the 613 recognized species in Gadiformes, about 402 of them have lost the caudal skeleton. Most of these (c. 90%) are in the family Macrouridae. The rattail-shaped fish have been most successful in colonizing the deep ocean by fine-tuning anatomy, swimming mode and feeding behavior. Their elongate bodies allow for more sensory cells (neuromasts) along the

length of the body in the lateral line sensory canal and the cephalic sensory canals. The sensory cells are used to identify and locate sources of sound with more precision (Haedrich 1997). Within Gadiformes, this increased precision could be useful in detecting the movements of potential predators or prey, or those emitted by the drumming muscles of potential mates (Haedrich 1997, Marshall, 1971, Marshall 1965).

Based on morphology, biology and distribution, Andriyashev (1953) suggested that fishes had colonized deep-water habitats at two different times: (i) ancient deep-water forms, which had their primary evolution and radiation there and exhibit bizarre structural adaptations to deep-sea life; and (ii) secondary deep-water forms, which underwent their primary evolution and radiation on the shallow continental shelve and do not display a marked morphological adaptation to the deep-sea environment. Andriyashev (1953) included macrouroids and morids, along with other neoteleosts, elopomorphs and clupeiforms within the "ancient deep-water" forms, and gadoids within the secondary deep-water forms. However, the ASR analyses suggest a diversification from shallow waters to the deep sea for gadiform fishes. The results of this study support the "deep-allopatry" hypothesis proposed by White (1988), in which lower abyssal benthic species are more derived than their sister groups restricted to mesopelagic and upper slope benthic habitats. White (1988) also predicted that derived deep-sea taxa had originated from the late Cretaceous or early Tertiary. The fossil record of gadiforms (particularly macrouroids), which extends to the Paleocene, also supports a shallow origin in the shelf environments and adaptations to deep-water habitats early in their evolution (Kriwet and Hecht 2008, Nolf and Steurbaut 1989). However, the apparent lack of macrouroids in shallow water deposits might represent a sampling artifact, because most fossils have been recovered from neritic deposits up to now, which are more accessible and as a result more sampled (Kriwet and Hecht 2008).

4.5. Conclusions

This study provided for the first time a description of the caudal skeleton for all the families among Gadiformes and explores the character evolution of 11 characters mapped on a taxonomically robust phylogenetic hypothesis proposed in chapter two based on genomic data.

In Bregmacerotidae, the most primitive member of the Gadiformes, the X and Y bones are present confirming them as a synapomorphy for the order and its absence is due to a secondary loss as suggested by several authors (Markle 1982, Cohen 1984).

The family Moridae is highly polymorphic in the number of hypural elements, individual variation can be found within some species. Caudal skeletons composed of two hypural plates are a synapomorphy of the gadoids (Gadidae, Gaidropsaridae, Lotidae and Phycidae).

The loss of the caudal fin has been proven successful and reflected by the diversification into the deep sea by fine-tuning anatomy, swimming mode and feeding behavior.

The caudal skeleton of the genera *Lyconus* and *Trachyrincus* were erroneously defined having a reduced caudal skeleton by several authors (e.g. Howes 1989, Borden et al. 2013). The specimens revised in this study showed a tailless condition for both genera.

The proximal-middle radials have a unique morphology in tailless families and Muraenolepididae. They are situated between the haemal and neural spines and the posterior portion has a triangular shape where the dorsal and anal fin rays articulate on small-round distal radials.

The ASR analyses suggest that the ancestral condition among gadiforms had a caudal fin and a pelagic origin. Its loss arose at least two times within Gadiformes resulting in two main phenotypes - the tailed and the tailless fishes, neither of which form a monophyletic grouping.

Among the tailed Gadiformes, five configurations of caudal skeleton elements were found depending on the presence or absence of the X and Y bones and the fusion of hypurals elements.

The ASR was useful for the reconstruction of the traits to study the evolutionary relationships among Gadiformes. An important next step is the calibration of the phylogeny of Gadiformes with fossil data since they provide data that are closer to the ancestors being reconstructed and will most likely improve the analysis, especially when rates of character change vary through time.

Table 4.1. The coding and scoring for the characters used in this study

Characters	Character States
1. Tail	0 Absent 1 Present
2. Number of ventral hypural elements	0 Absent 1 one element (hy1+hy2) 2 two elements (hy1, hy2)
3. Number of dorsal hypural elements	0 absent 1 one element (hy3+hy4+hy5) 2 two elements (hy3, hy4+hy5 or hy3+hy4, hy5) 3 three elements (hy3, hy4, hy5) 4 three elements (hy1+2, hy3+4, hy5) 5 three elements (hy3+hy4, hy5, hy6) 6 four elements (hy3, hy4, hy5, hy6)
4. X bone	0 Absent 1 Present
5. Y bone	0 Absent 1 Present
6. Uroneural	0 Absent 1 one 2 two
7. Number of epurals	0 Absent 1 one 2 two 3 three
8. Caudal fin propulsion	1 Anguilliform 2 Sub-carangiform
9. Number of dorsal fins	0 one 1 two 2 three 3 two or three
10. Number of anal fins	0 one 1 two 2 one or two
11. Biozones	0 Freshwater 1 Epipelagic 2 Epipelagic to Mesopelagic 3 Mesopelagic 4 Blackish, Epipelagic, Mesopelagic, Bathypelagic 5 Epipelagic to abyssalpelagic 6 Mesopelagic to abyssalpelagic 7 Mesopelagic to hadalpelagic.

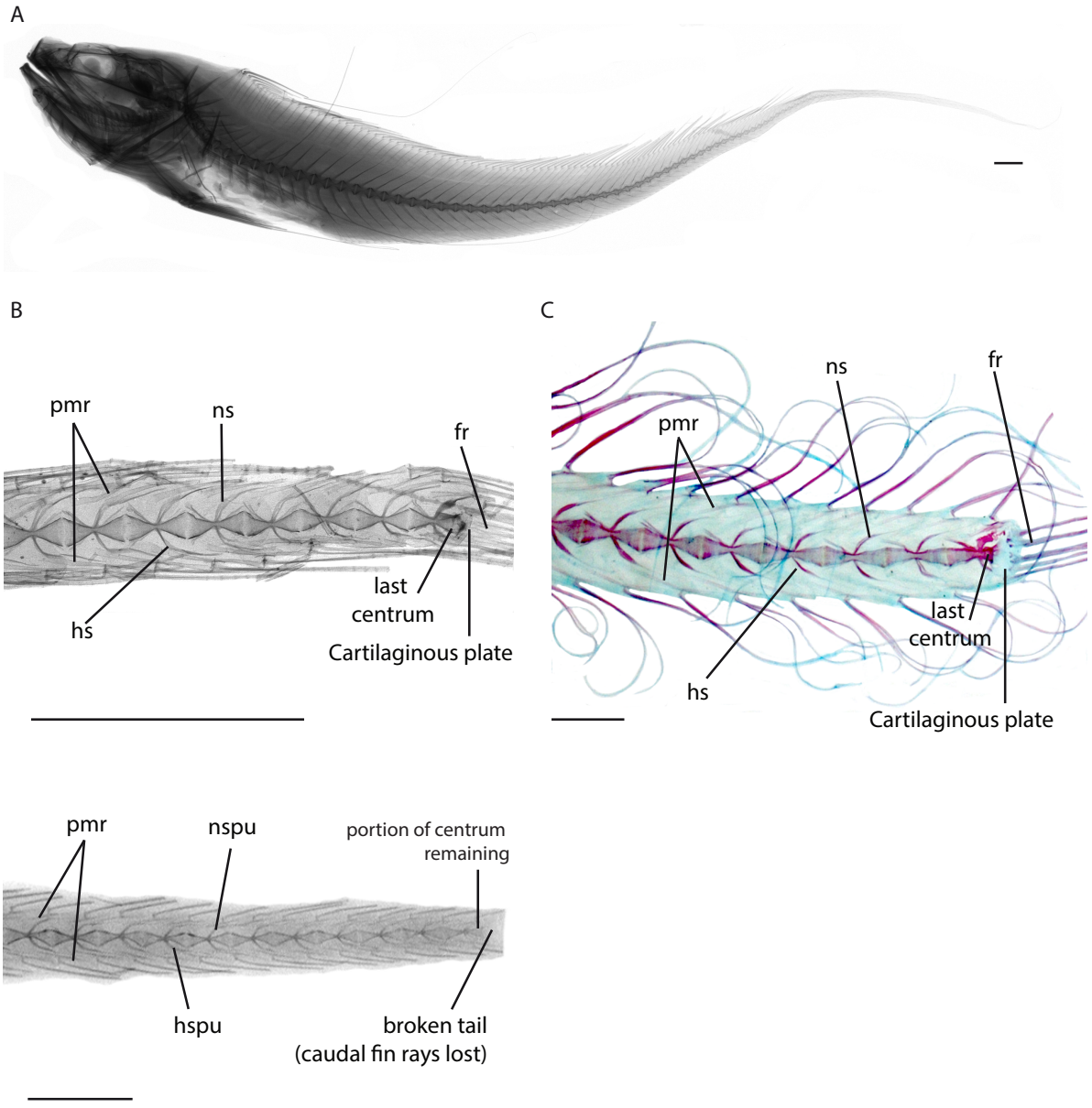


Figure 4.1 Caudal skeleton of the family Bathygadidae: A) USNM 1482 *Gadomus multifilis* lateral view. B) USNM 192639 *Gadomus* sp. (360 mm TL). C) UF 125711 *Gadomus arcuatus* (178mm TL). D) USNM 135351 *Bathygadus garretti*. Scale bars equal 1.0 mm.

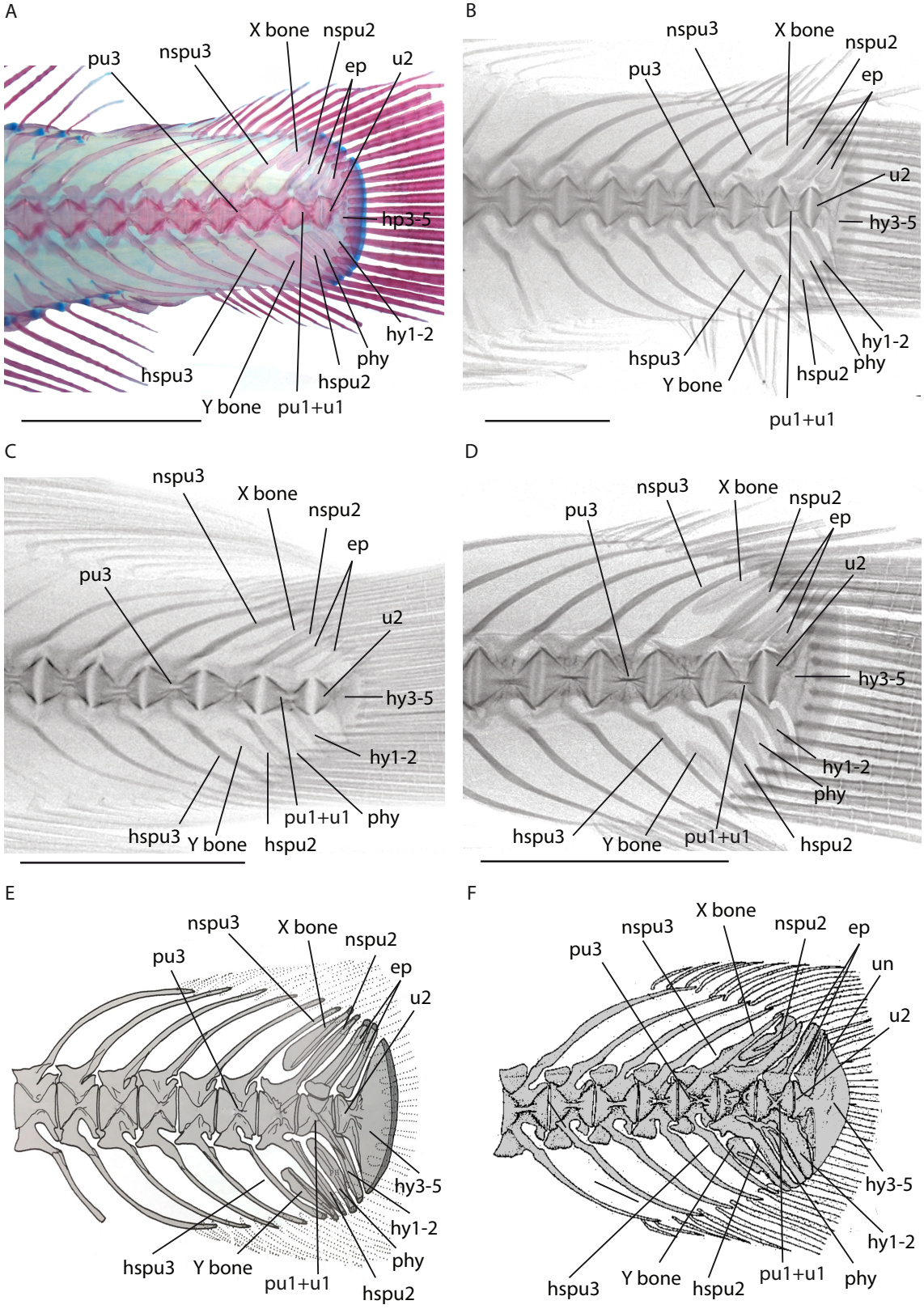

A
pu3
nspu3
X bone
nspu2
ep
u2
hp3-5
hy1-2
phy
hspu3
hspu2
Y bone
pu1+u1
B
nspu3
X bone
nspu2
pu3
ep
u2
hy3-5
hy1-2
hspu3
Y bone
hspu2
phy
pu1+u1
C
nspu3
X bone
nspu2
ep
pu3
u2
hy3-5
hy1-2
hspu3
Y bone
pu1+u1
phy
hspu2
D
nspu3
X bone
nspu2
pu3
ep
u2
hy3-5
hy1-2
phy
hspu3
Y bone
pu1+u1
hspu2
E
nspu3
X bone
nspu2
pu3
ep
u2
hy3-5
hy1-2
phy
hspu3
Y bone
pu1+u1
hspu2
F
X bone
nspu3
nspu2
ep
pu3
un
u2
hy3-5
hy1-2
hspu3
Y bone
hspu2
pu1+u1
phy

Figure 4.2 Caudal skeleton of the family Bregmacerotidae: A) USNM 441818 *Bregmaceros japonicus* (5.1 mm SL). B) USNM 441716 *Bregmaceros* sp. (8.0 mm SL). C) USNM 399939 *Bregmaceros* sp. (8.5 mm SL). D) USNM 309305 *Bregmaceros* sp. (7.3 mm SL). E) *Bregmaceros japonicus* (modified from Fujita 1990, fig. 138). F) SEFC 28625/2 *Bregmaceros houdi* (Świdnicki 1991). Scale bars equal 0.2 mm. Scale bars equal 1.0 mm.

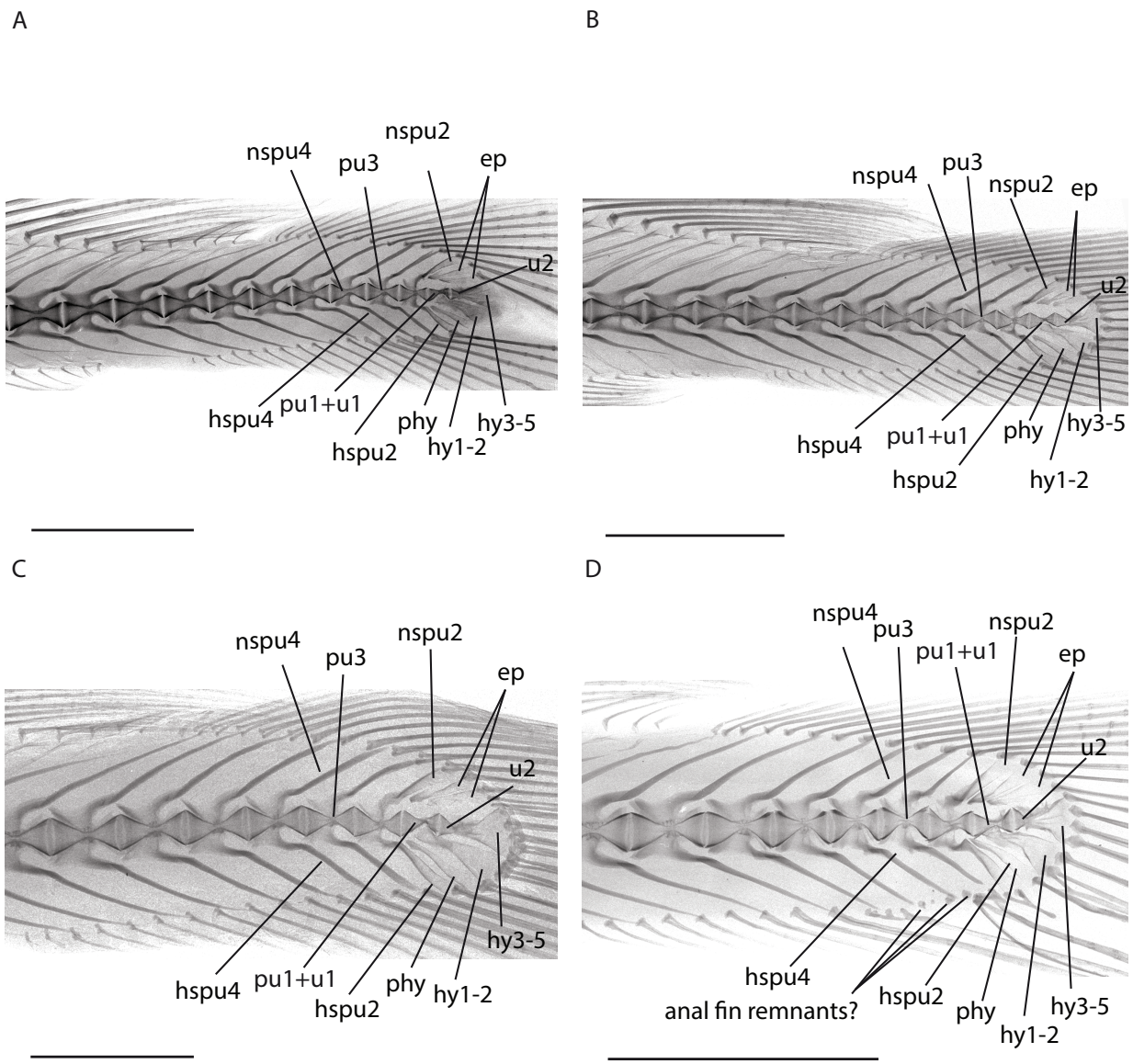


Figure 4.3 Caudal skeleton of the family Euclichthyidae: A) CAS 213338 *Euclichthys polynemus* 1. B) CAS 213338 *Euclichthys polynemus* 2. C) CAS 213338 *Euclichthys polynemus* 3. D) USNM 222071 *Euclichthys polynemus* (245 mm SL). Scale bars equal 1.0 mm.

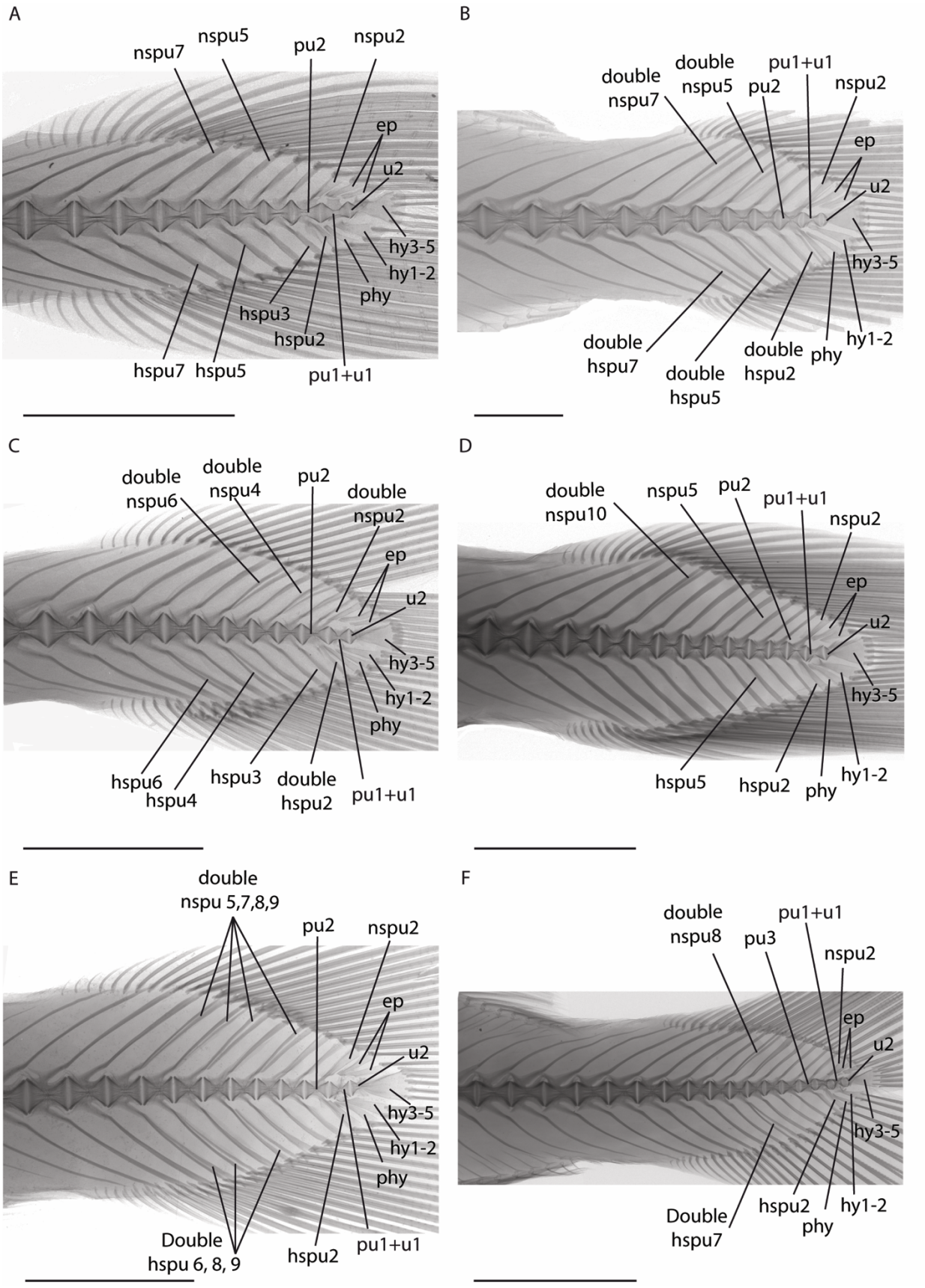

A
nspu7
nspu5
pu2
nspu2
ep
u2
hy3-5
hy1-2
phy
hspu3
hspu2
hspu7
hspu5
pu1+u1
B
double nspu7
double nspu5
pu1+u1
pu2
nspu2
ep
u2
hy3-5
hy1-2
phy
double hspu7
double hspu5
double hspu2
C
double nspu6
double nspu4
pu2
double nspu2
ep
u2
hy3-5
hy1-2
phy
hspu6
hspu4
hspu3
double hspu2
pu1+u1
D
double nspu10
nspu5
pu2
pu1+u1
nspu2
ep
u2
hy3-5
hspu5
hspu2
phy
hy1-2
E
double nspu 5,7,8,9
pu2
nspu2
ep
u2
hy3-5
hy1-2
phy
Double hspu 6, 8, 9
hspu2
pu1+u1
F
double nspu8
pu3
pu1+u1
nspu2
ep
u2
hy3-5
Double hspu7
hspu2
phy
hy1-2

Figure 4.4 Caudal skeleton of the family Gadidae: A) USNM 211611 *Boreogadus saida* (123 mm SL). B) USNM 141781 *Gadiculus argenteus* fish 3 (105 mm SL). C) USNM 441718 *Gadus morhua* (165 mm SL), D) USNM 396626 *Melanogrammus aeglefinus* (300 mm SL). E) USNM 017382 *Merlangius-merlangus* (278 mm SL). F) USNM 103639 *Microgadus proximus* (265 mm SL). Scale bars equal 1.0 mm.

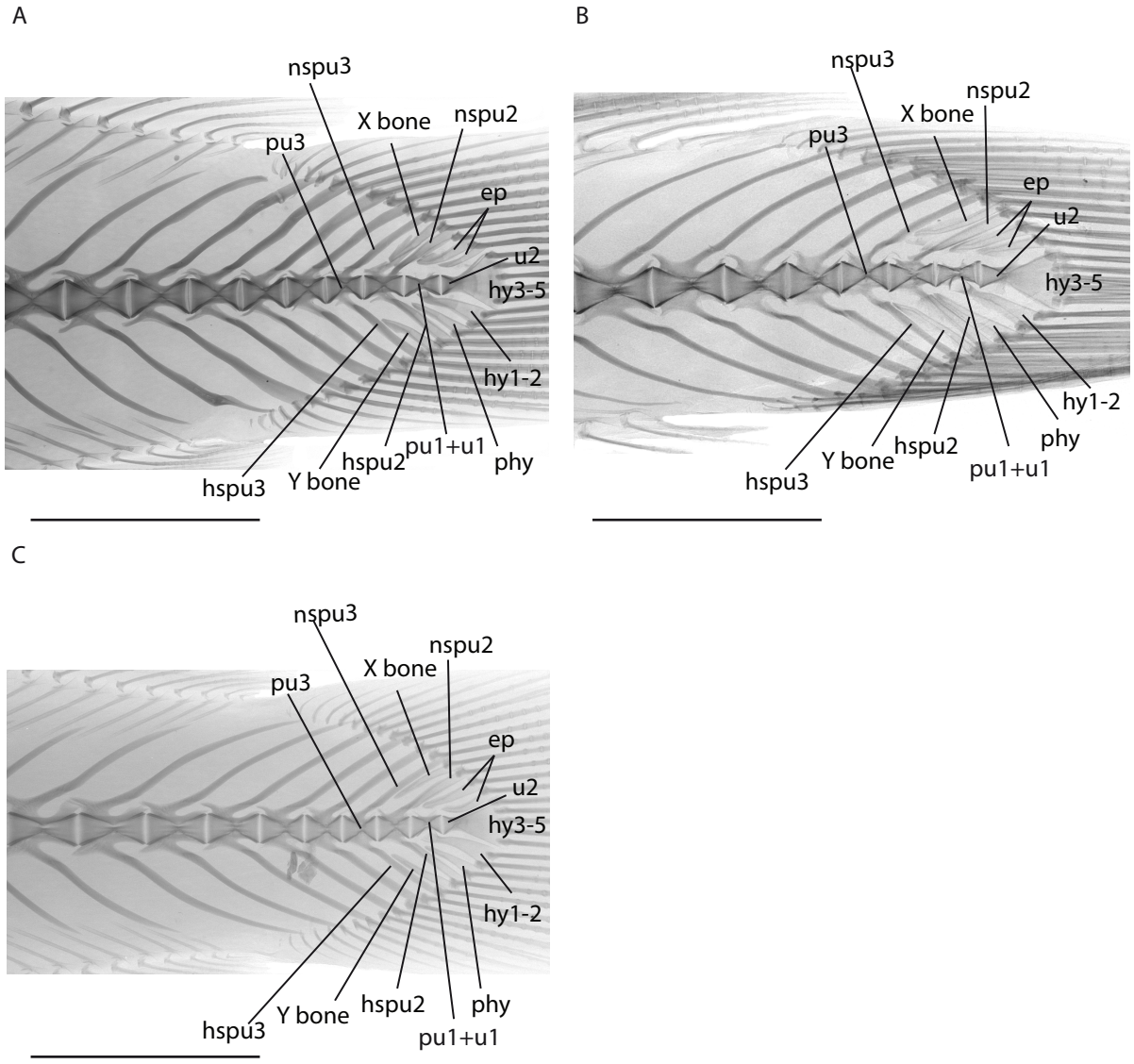


Figure 4.5 Caudal skeleton of the family Gaidropsaridae: A) USNM 216711 *Ciliata mustela*. B) USNM 217843 *Enchelyopus cimbrius*. C) USNM 394678 *Gaidropsaurus novazelandiae* (165 mm SL). Scale bars equal 1.0 mm.

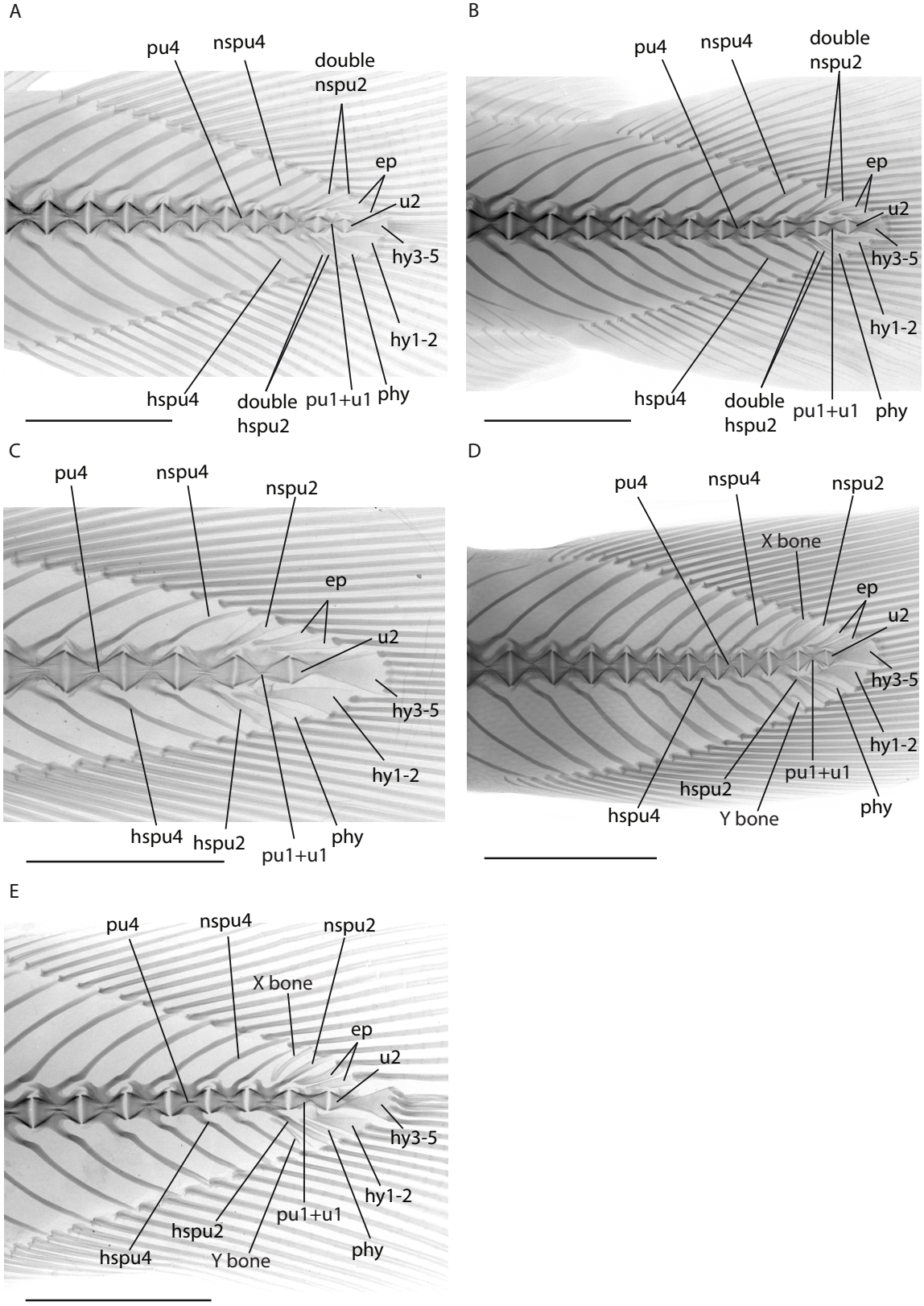

A
pu4
nspu4
double nspu2
ep
u2
hy3-5
hy1-2
hspu4
double hspu2
pu1+u1
phy
B
pu4
nspu4
double nspu2
ep
u2
hy3-5
hy1-2
hspu4
double hspu2
pu1+u1
phy
C
pu4
nspu4
nspu2
ep
u2
hy3-5
hy1-2
hspu4
hspu2
pu1+u1
phy
D
pu4
nspu4
nspu2
X bone
ep
u2
hy3-5
hy1-2
hspu2
pu1+u1
hspu4
Y bone
phy
E
pu4
nspu4
nspu2
X bone
ep
u2
hy3-5
hy1-2
pu1+u1
hspu2
hspu4
Y bone
phy

Figure 4.6 Caudal skeleton of the family Lotidae: A) USNM 211621 *Brosme brosme* (193 mm SL). B) USNM 441778 Lota lota. C) USNM 211613 *Molva dyterygia* (335 mm SL). D) USNM 10050 *Molva molva* (360 mm SL). E) USNM 28563 *Molva vulgaris*. Scale bars equal 1.0 mm.

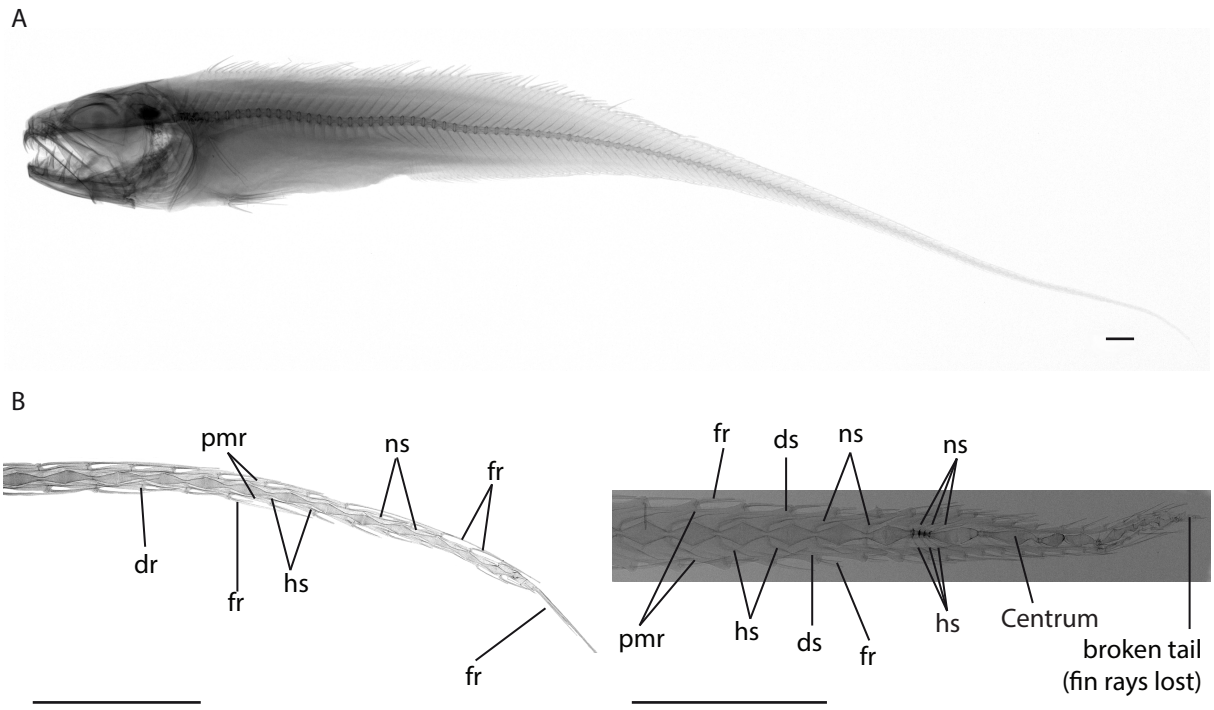


Figure 4.7 Caudal skeleton of the family Lyconidae: A-B) LACM 11591-9 *Lyconus* sp. C) SIO73 336 *Lyconus* sp. Scale bars equal 1.0 mm.

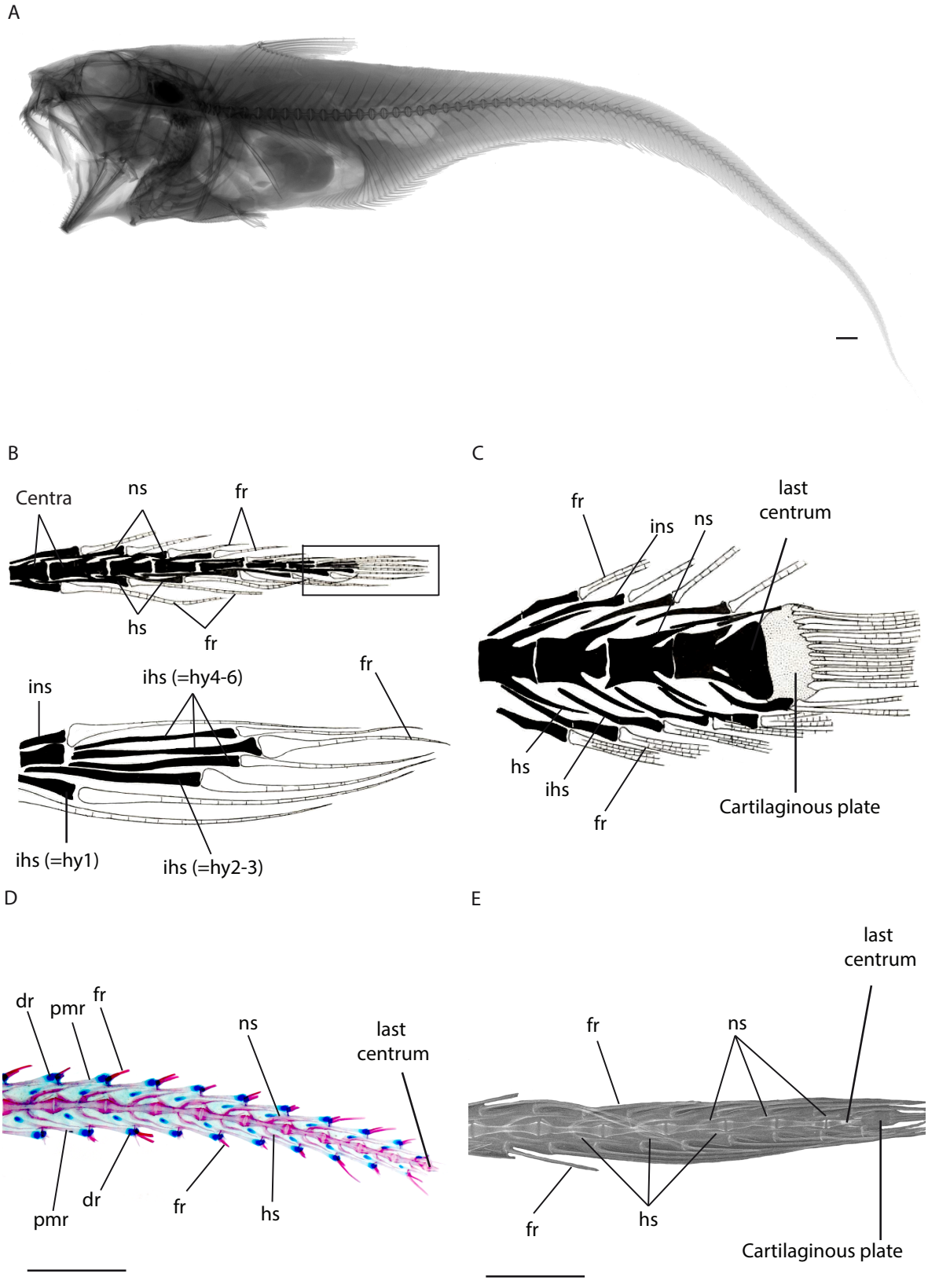

A
B
Centra
ns
fr
hs
fr
ins
ihs (=hy4-6)
fr
ihs (=hy1)
ihs (=hy2-3)
C
fr
ins
ns
last centrum
hs
ihs
fr
Cartilaginous plate
D
dr
pmr
fr
ns
last centrum
pmr
dr
fr
hs
E
last centrum
fr
ns
fr
hs
Cartilaginous plate

Figure 4.8 Caudal skeleton of the family Macrouridae: A) USNM 156901 *Malacocephalus occidentalis* (333 mm TL). B-C) Okamura 1970: fig. 58. D) USNM 441779 *Macrourus holotrachys* (198 mm TL / 64 mm PAL). E) USNM 303751 *Coryphaenoides serrulatus* fish 3 (456 mm TL / 125 mm PAL). Scale bars equal 1.0 mm.

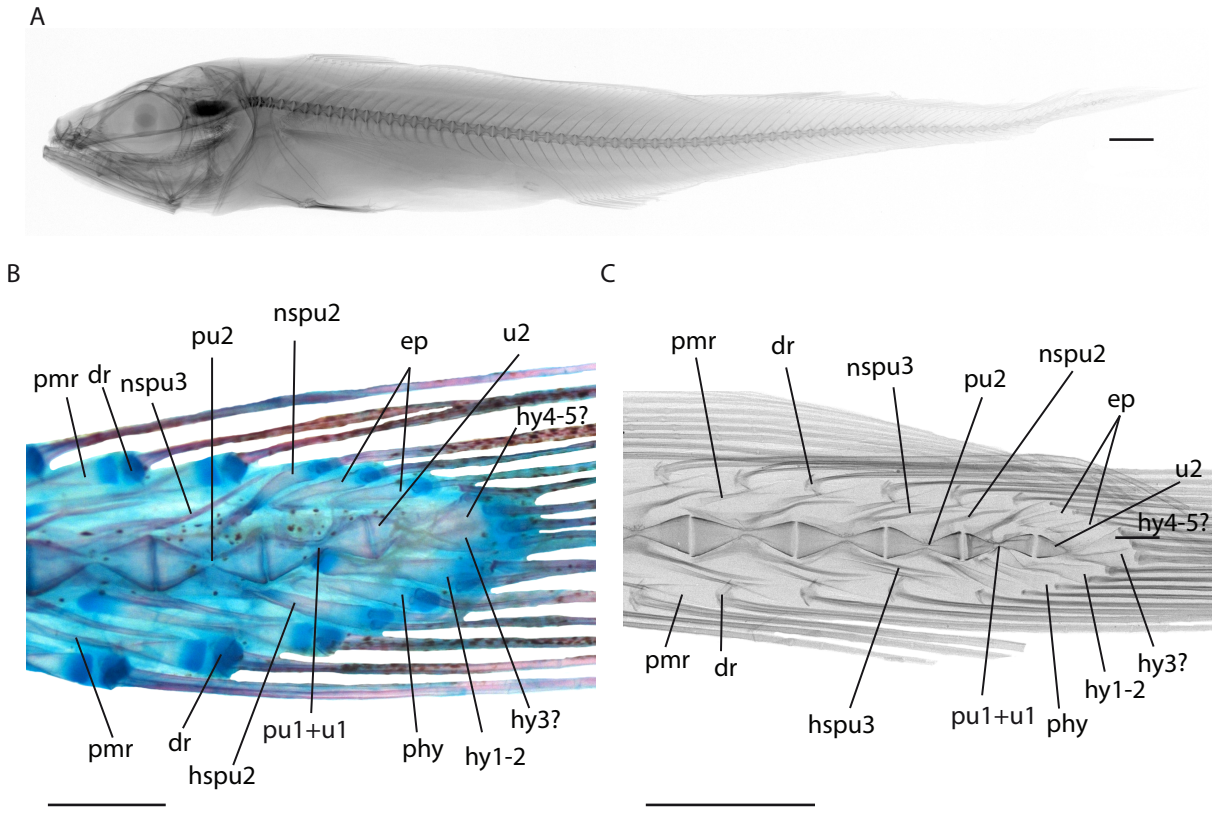


Figure 4.9 Caudal skeleton of the family Macruronidae: A) USNM 103773 *Macruronus magellanicus* (188 mm TL). B. Australian Museum 1-25-229-001 *Macruronus novaezelandiae* (240 mm TL / 105 mm PAL). C) USNM 176587 *Macruronus* sp. (470 mm SL). Scale bars equal 1.0 mm.

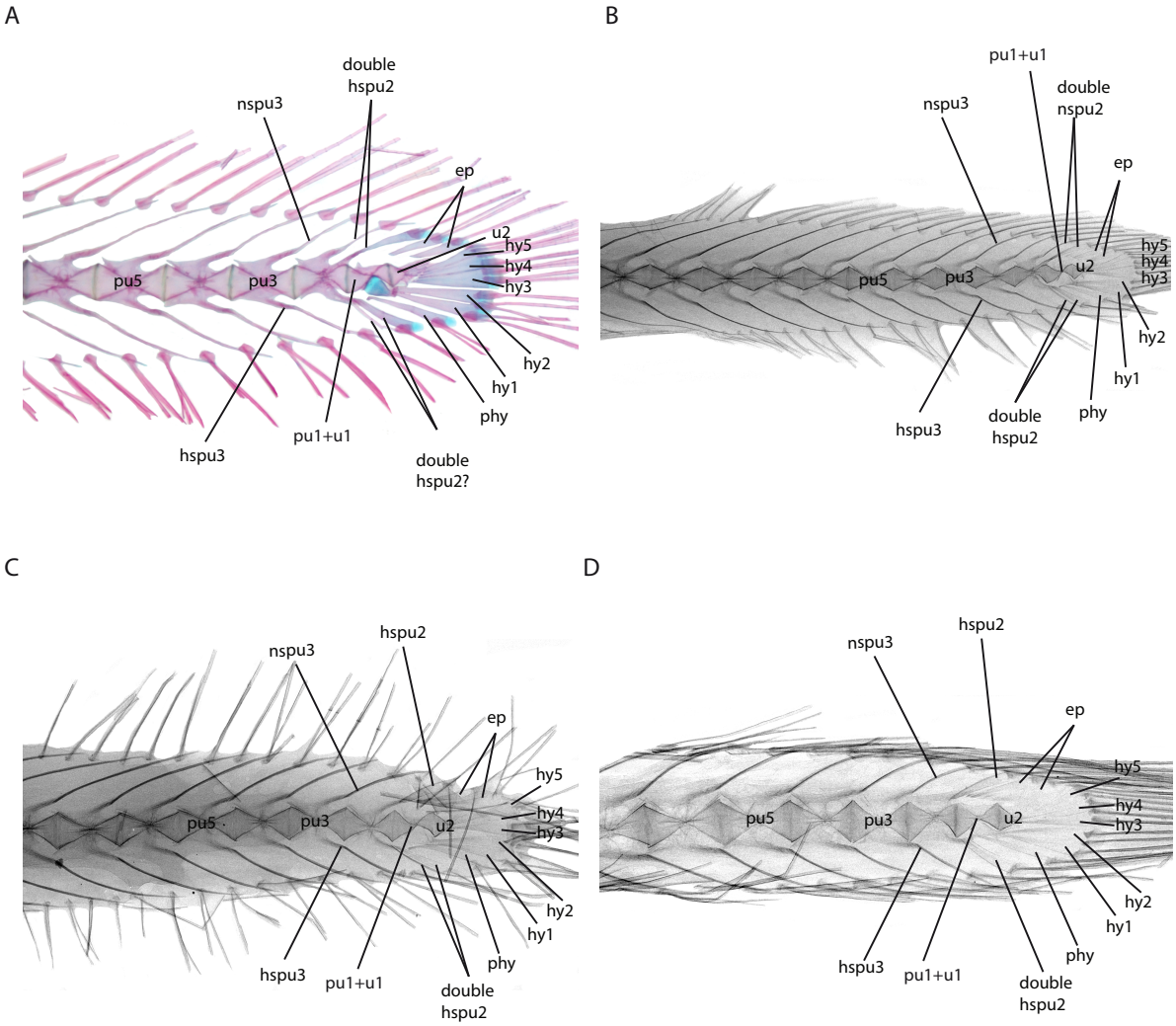


Figure 4.10 Caudal skeleton of the family Melanonidae: A) USNM 441715 *Melanonus* sp. Fish 1 (215mm SL). B) USNM 441715 *Melanonus* sp. Fish 2. C) USNM 441814 *Melanonus zugmayeri*. D) CAS 243394 *Melanonus zugmayeri*. Scale bars equal 1.0 mm.

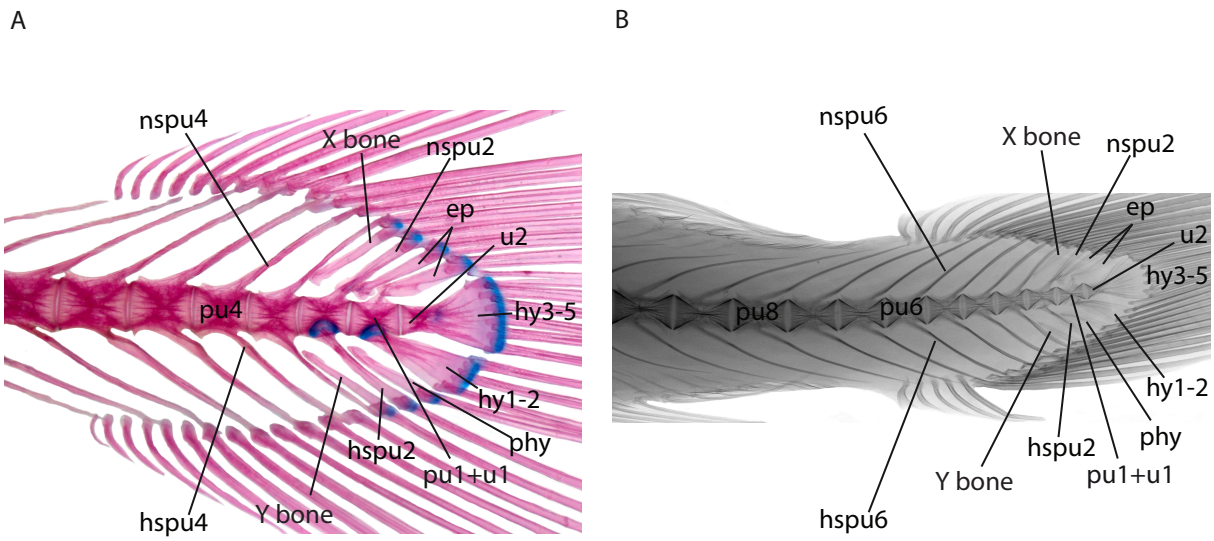
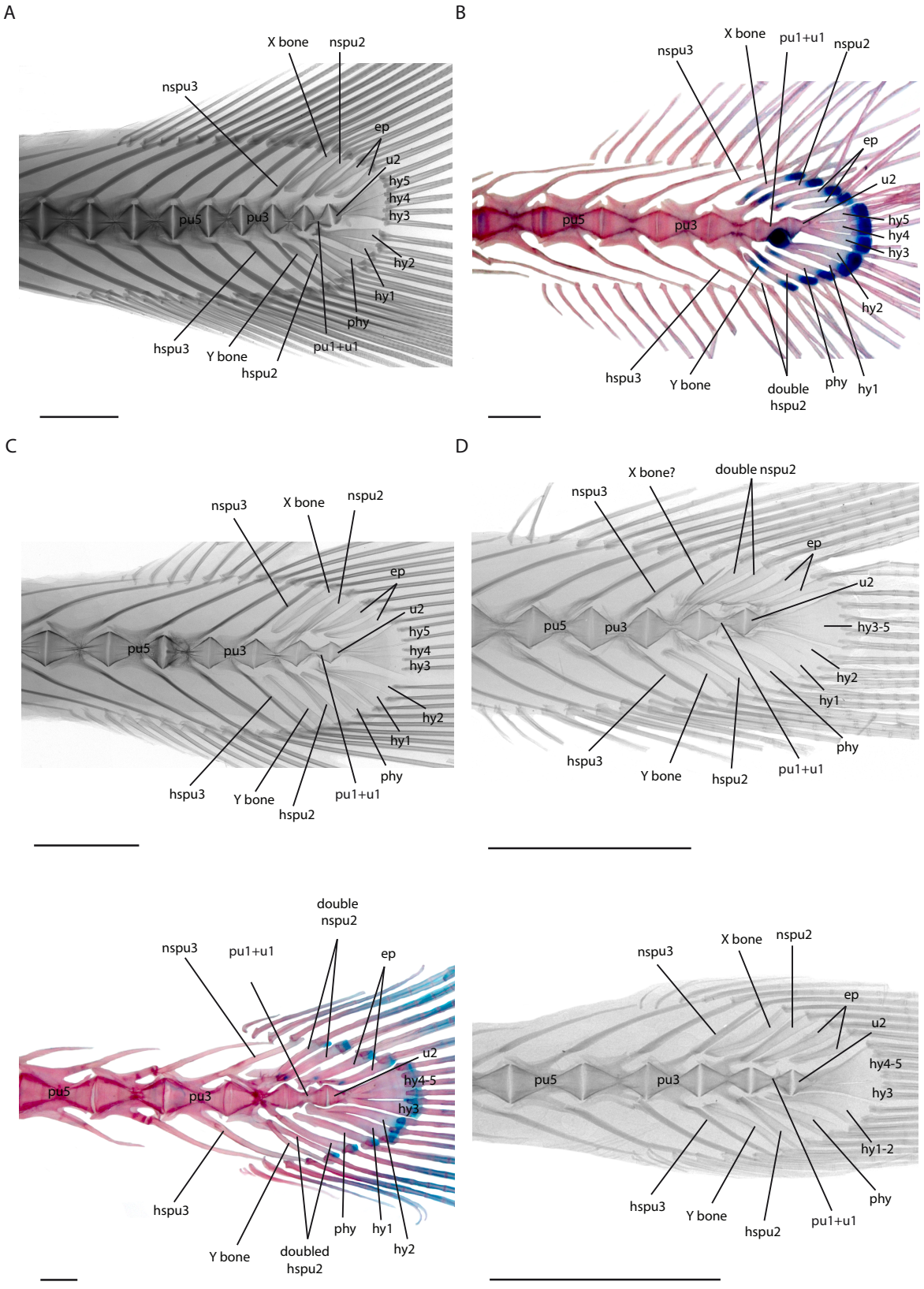


Figure 4.11 Caudal skeleton of the family Merlucciidae: SIO 00157 *Merluccius productus* fish 2. B) USNM 302413 *Merluccius bilinearis*. Scale bars equal 1.0 mm.


A
X bone
nspu2
nspu3
ep
u2
hy5
hy4
hy3
pu5
pu3
hy2
hy1
phy
hspu3
Y bone
pu1+u1
hspu2
B
nspu3
X bone
pu1+u1
nspu2
ep
u2
pu5
pu3
hy5
hy4
hy3
hy2
hspu3
Y bone
double
hspu2
phy
hy1
C
nspu3
X bone
nspu2
ep
u2
pu5
pu3
hy5
hy4
hy3
hy2
hy1
hspu3
Y bone
hspu2
pu1+u1
phy
D
X bone?
double nspu2
nspu3
ep
u2
pu5
pu3
hy3-5
hy2
hy1
phy
hspu3
Y bone
hspu2
pu1+u1
double
nspu2
nspu3
pu1+u1
ep
u2
hy4-5
pu5
pu3
hy3
hspu3
phy
hy1
hy2
Y bone
doubled
hspu2
nspu3
X bone
nspu2
ep
u2
hy4-5
pu5
pu3
hy3
hy1-2
phy
hspu3
Y bone
hspu2
pu1+u1

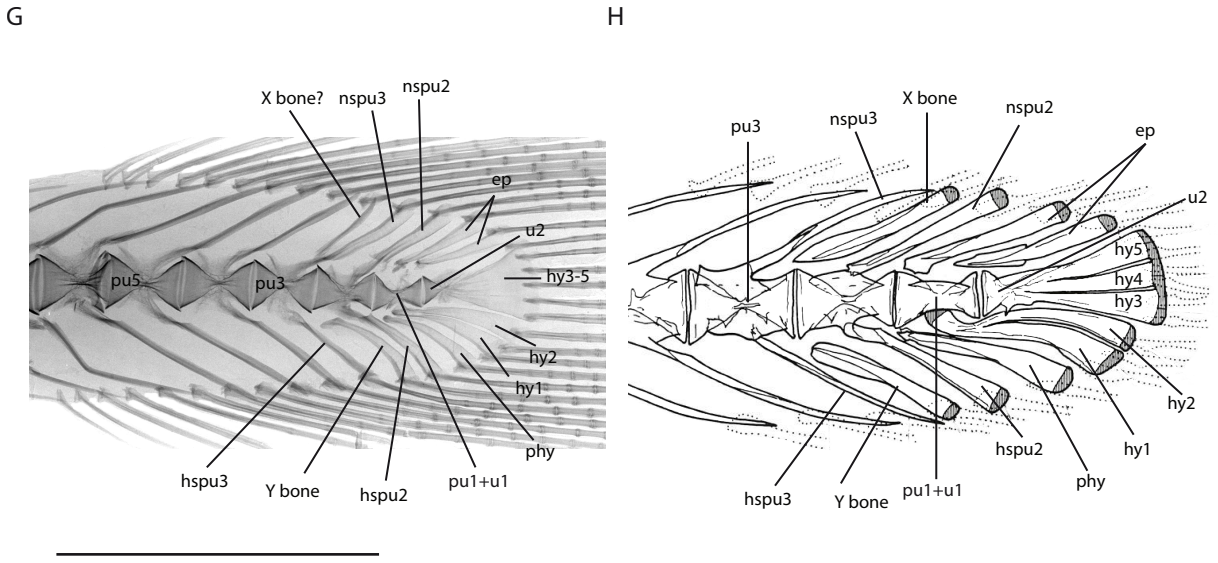


Figure 4.12 Caudal skeleton of the family Moridae: A) USNM 320679 *Mora mora* (345 mm SL). B) USNM 211787 *Lepidiom eques*. C) USNM 214030 *Halargyreus johnsoni* (310 mm SL). D) USNM 273286 *Gadella imberbis* fish 1 (195 mm SL). E) USNM 232481 *Physiculus fulvus*. F) USNM 266304 *Physiculus fulvus*. G) USNM 273286 *Gadella imberbis* 2 (195 mm SL). H) *Physiculus jordani* (Fujita 1990, fig. 138). Scale bars equal 1.0 mm.

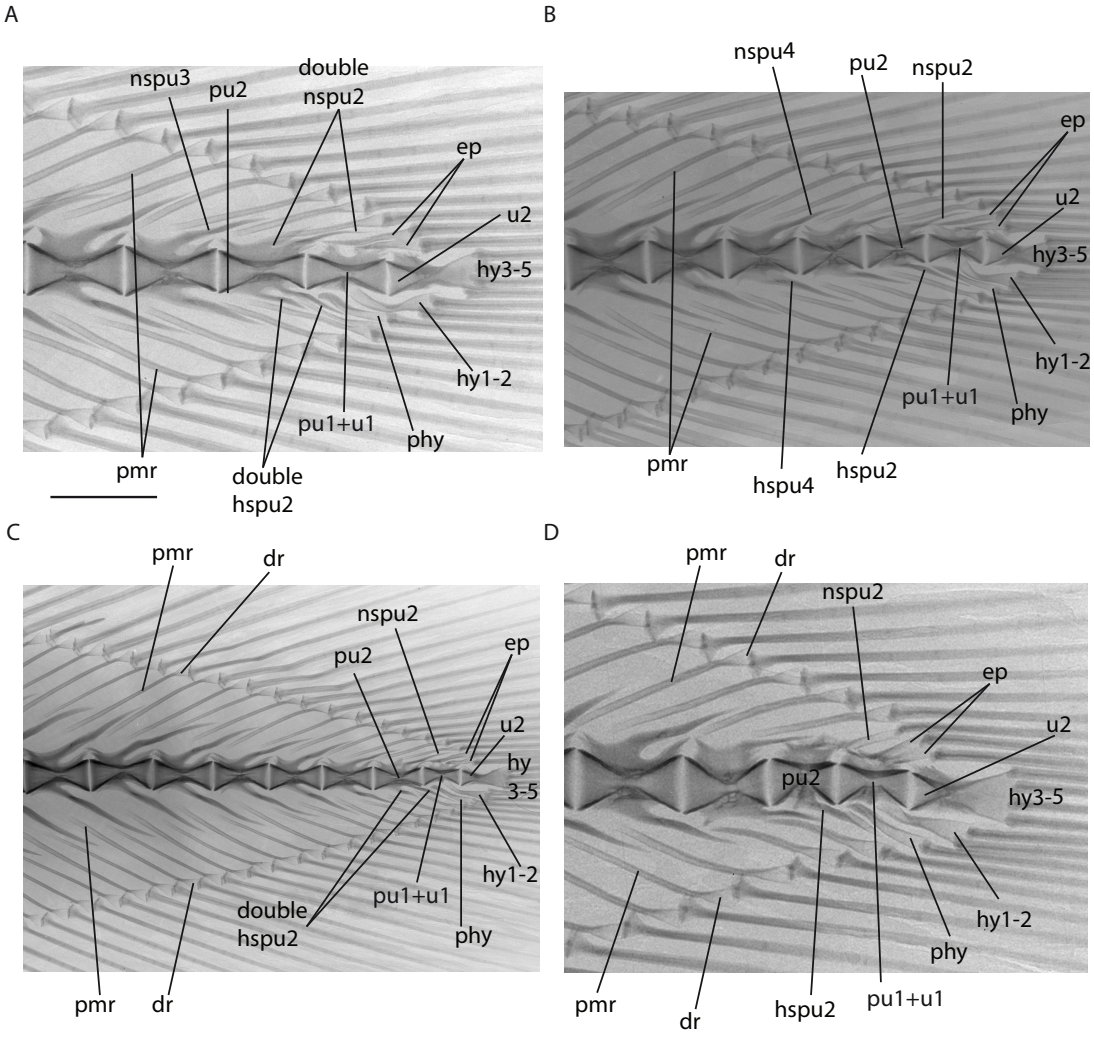


Figure 4.13 Caudal skeleton of the family Muraenolepididae: A) USNM 397108-282 *Muraenolepis microps*. B) USNM 397108-182 *Muraenolepis microps*. C) USNM 397108-369 *Muraenolepis microps*. D) USNM 380028 *Muraenolepis orangiensis*. Scale bars equal 1.0 mm.

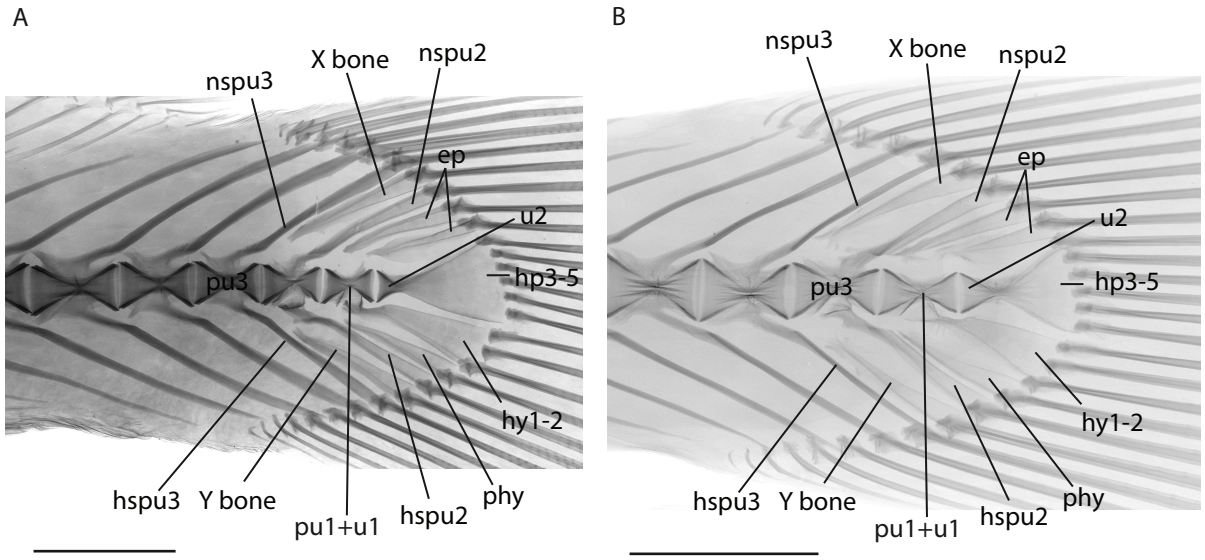


Figure 4.14 Caudal skeleton of the family Phycidae: A) USNM 017371 *Phycis blennoides* (420 mm SL); B) USNM 396321 *Urophycis regia* (230 mm SL). Scale bars equal 1.0 mm.

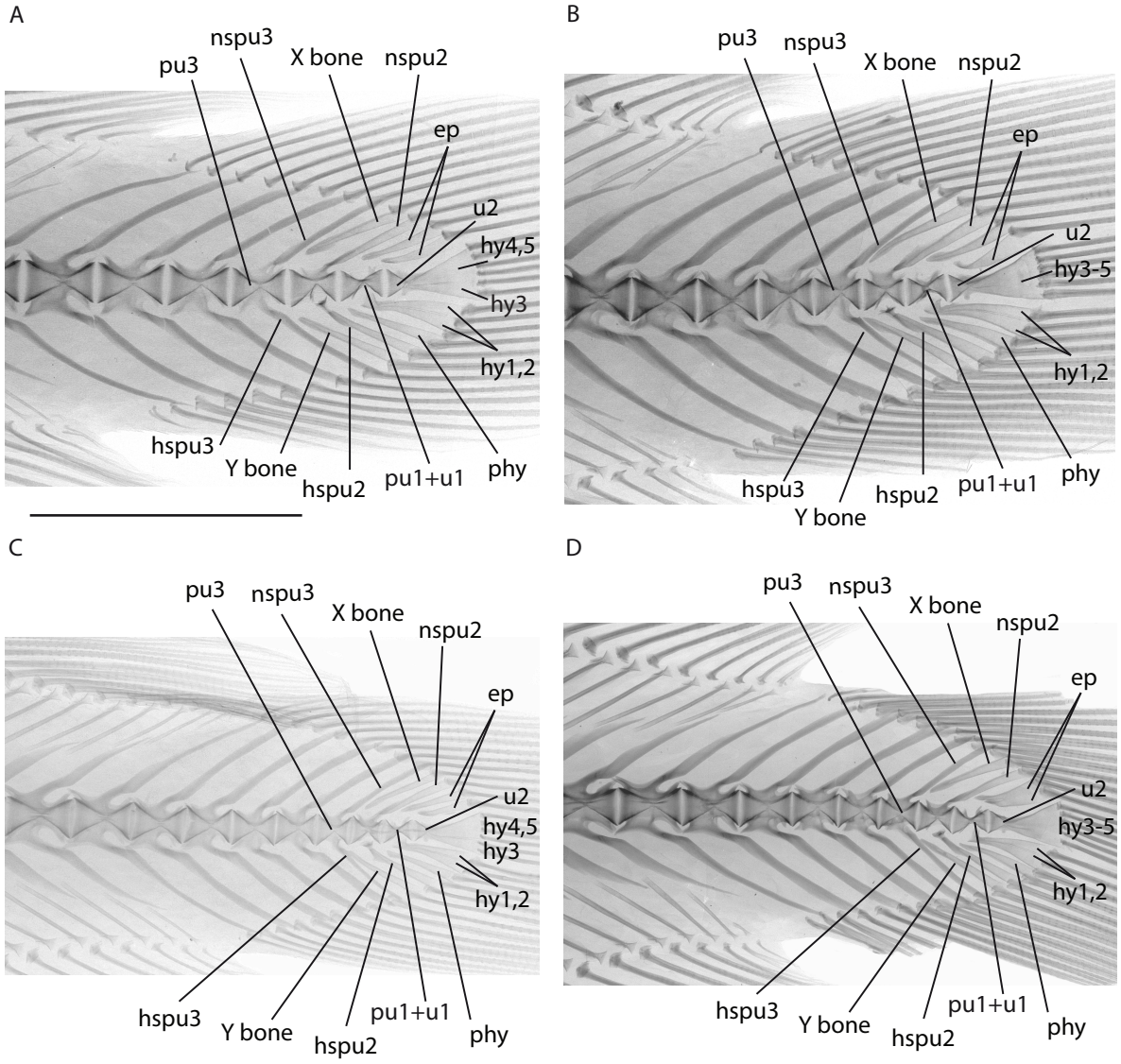


Figure 4.15 Caudal skeleton of the family Ranicipitidae: *Raniceps raninus* A) USNM 010056; B) USNM 017367; C) USNM 23045; D) USNM 044512. Scale bars equal 1.0 mm.

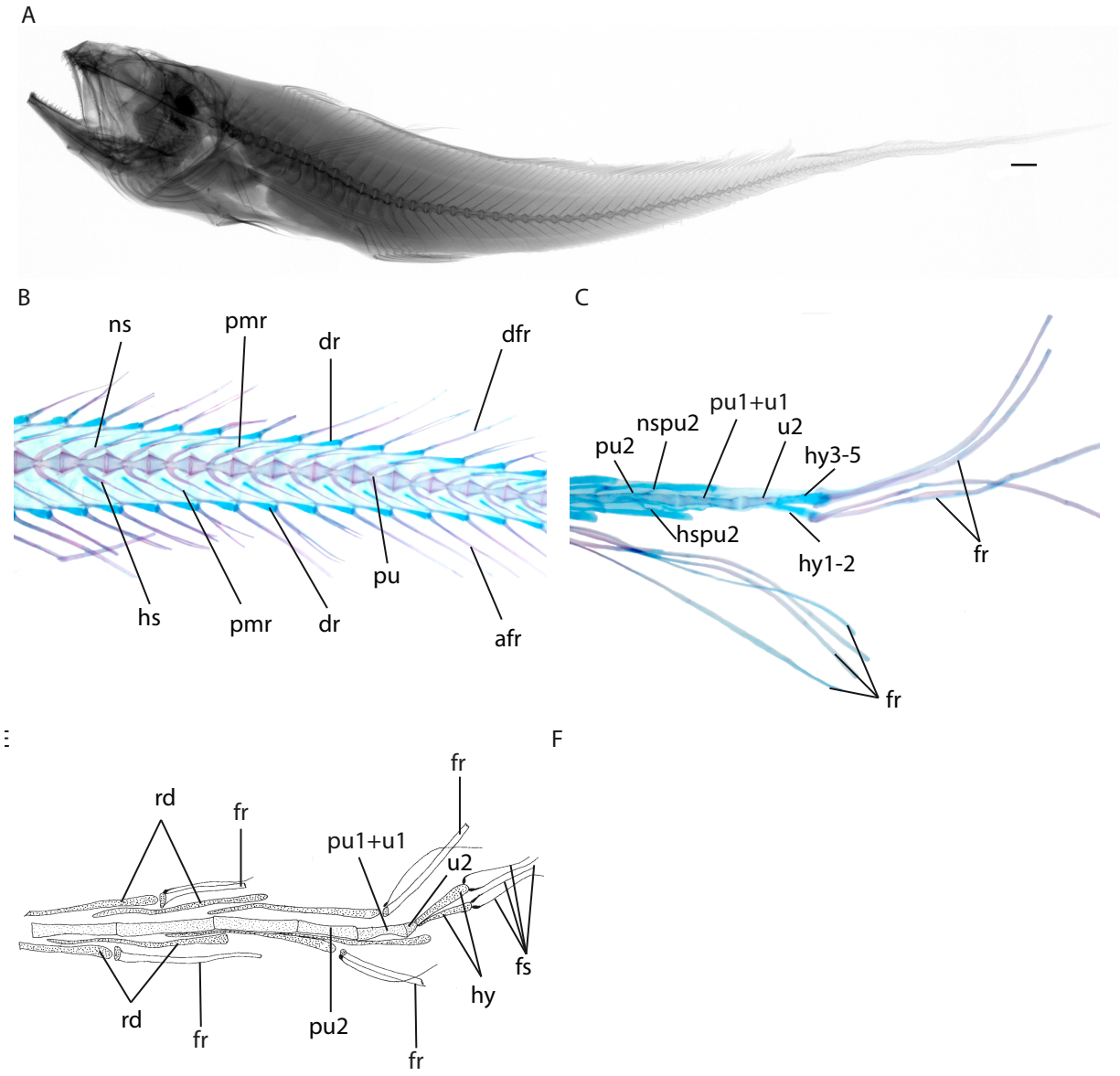


Figure 4.16 Caudal skeleton of the family Steindachneriidae: *Steindachneria argentea* A) USNM 427842 Lateral view (340 mm TL / 110 mm PAL); B-C) USNM 441817; D) FMNH 67856 (Borden et al. 2013: fig. 7D (143.1 SL mm). Scale bars equal 1.0 mm.

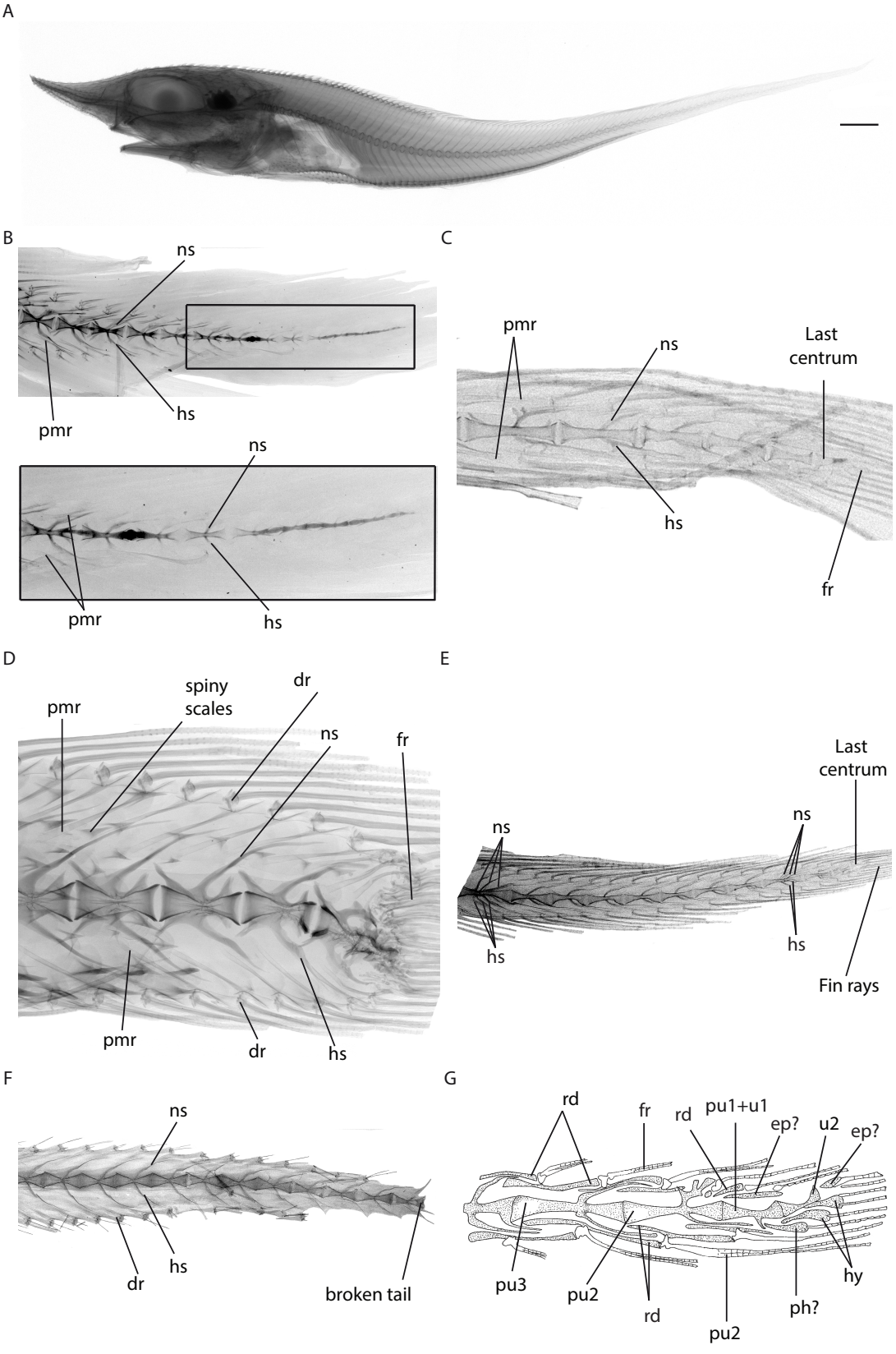

A
B
ns
hs
pmr
ns
pmr
hs
C
pmr
ns
Last
centrum
hs
fr
D
pmr
spiny
scales
dr
ns
fr
pmr
dr
hs
E
Last
centrum
ns
ns
hs
hs
Fin rays
F
ns
hs
dr
broken tail
G
rd
fr
rd
pu1+u1
ep?
u2
ep?
pu3
pu2
rd
pu2
ph?
hy

Figure 4.17 Caudal skeleton of the family Trachyrincidae: A) USNM 212159 *Trachyrincus trachyrincus*. B) USNM 149923 *Trachyrincus villegai*. C) CAS 53215 *Trachyrincus murrayi*. D) CAS 223414 *Trachyrincus scabrus*. E) CAS 53215 *Trachyrincus murrayi*. F) CAS 98154 *Squalogadus modificatus*. G) *Trachyrincus trachyrincus* (Howes 1989, fig. 6). Scale bars equal 1.0 mm.

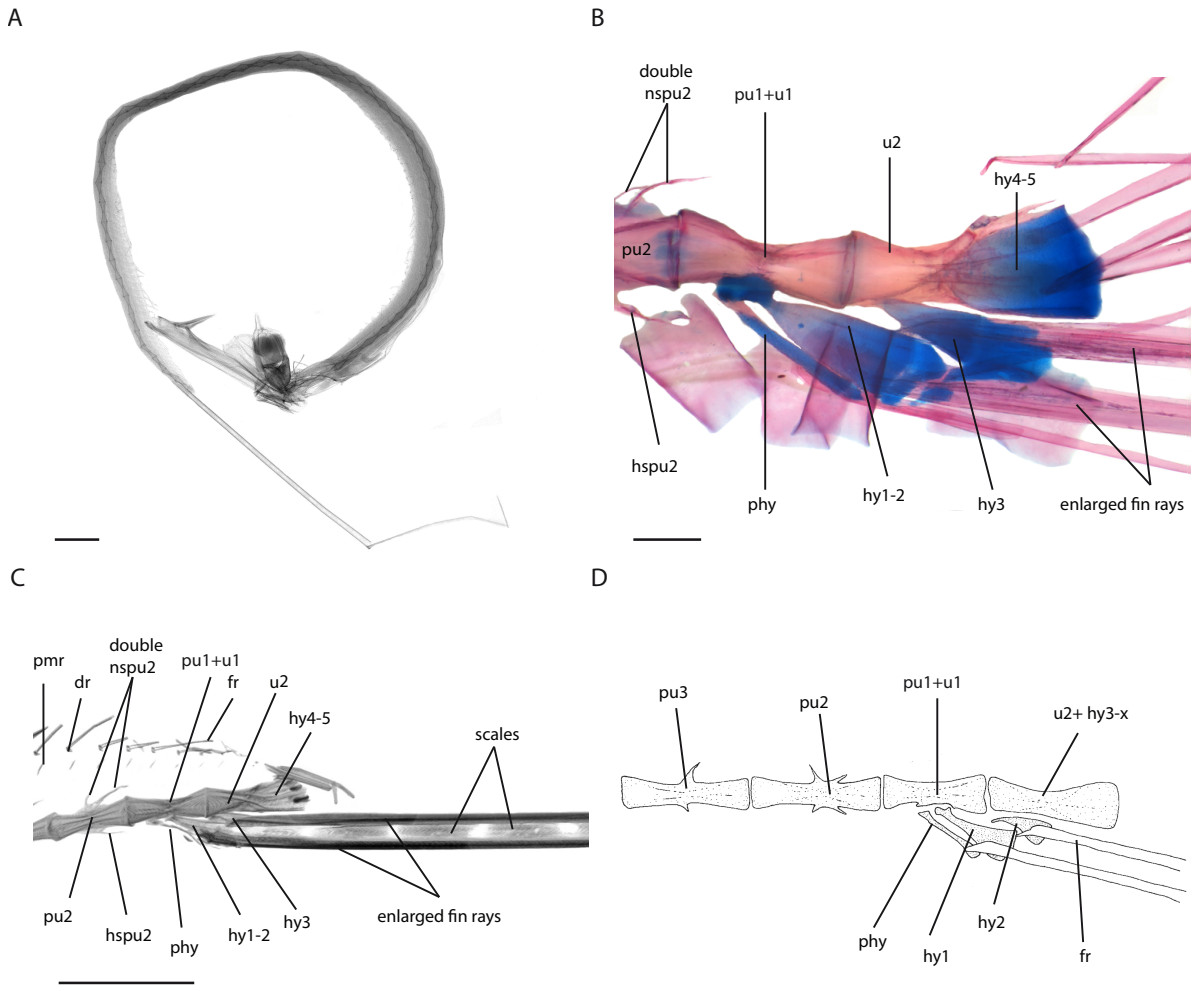


Figure 4.18 Caudal skeleton of the outgroup. Part I: Stylephoriformes, *Stylephorus chordatus* A) MCZ 138107 Lateral view (160 mm TL), B) Cleared and stained image was provided by Dave Johnson (NMNH); C) CT-scan image was available through the Museum of Comparative Zoology, Harvard University - MCZ 138107 (160 mm TL) Scale bars equal 1.0 mm.

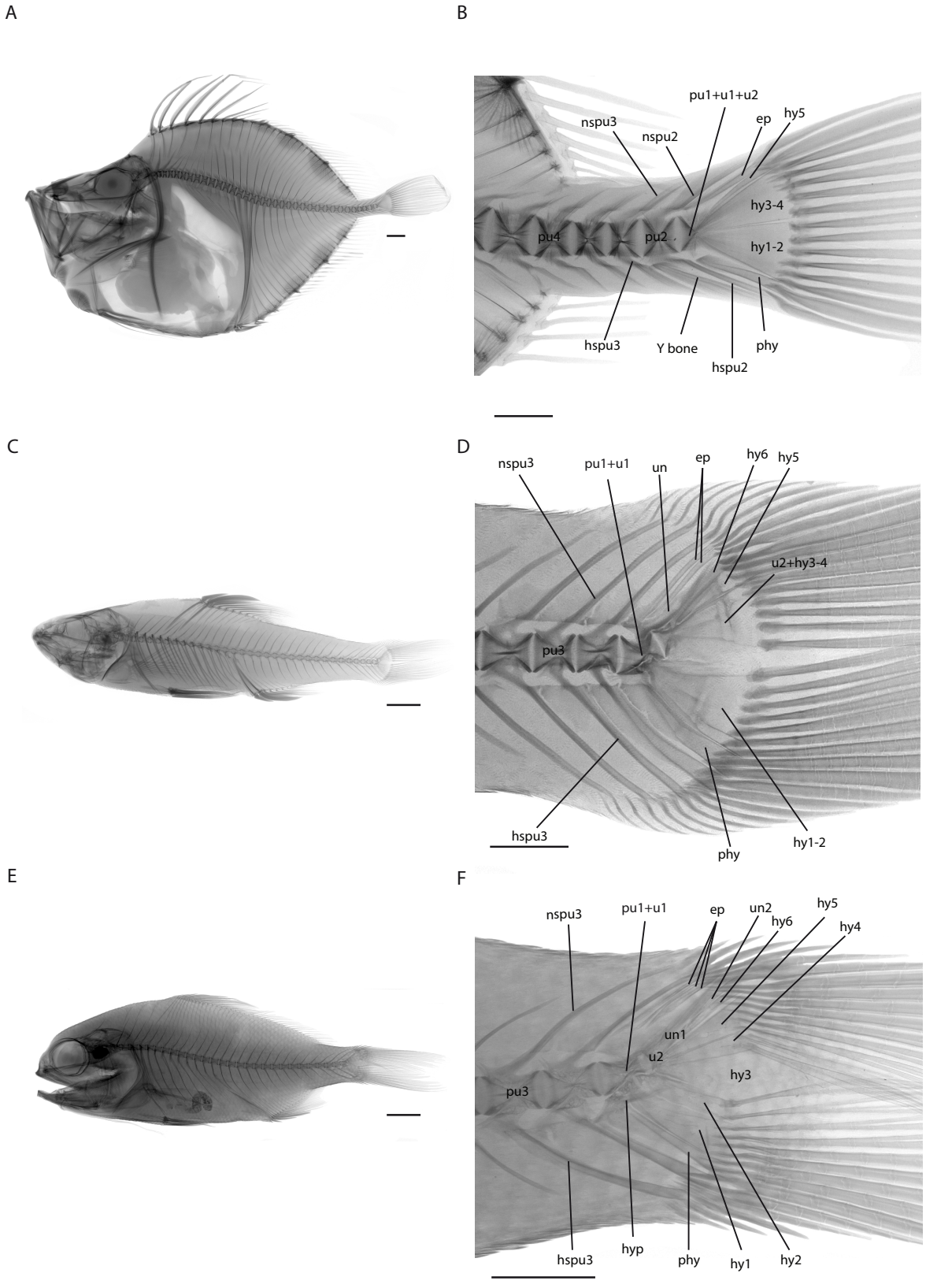

A
B
pu1+u1+u2
nspu3
nspu2
ep
hy5
hy3-4
pu4
pu2
hy1-2
hspu3
Y bone
phy
hspu2
C
D
nspu3
pu1+u1
un
ep
hy6
hy5
u2+hy3-4
pu3
hspu3
phy
hy1-2
E
F
nspu3
pu1+u1
ep
un2
hy6
hy5
hy4
un1
u2
hy3
pu3
hspu3
hyp
phy
hy1
hy2

Figure 4.19 Caudal skeleton of the outgroups. Part II: Zeiformes, Zeidae, USNM 302363 *Zenopsis conchifer* (160 mm SL): A) Lateral view and B) Caudal skeleton Scale bars equal 1.0 mm. Percopsiformes, Percopsidae, USNM 304445 *Percopsis transmontana* (80 mm SL): C) Lateral view and D) Caudal skeleton. Scale bars equal 0.5 mm. Polymixiiformes, Polymixiidae, USNM 383299 *Polymixia japonica* (120 mm SL): E) Lateral view and F) Caudal skeleton. Scale bars equal 1.0 mm.

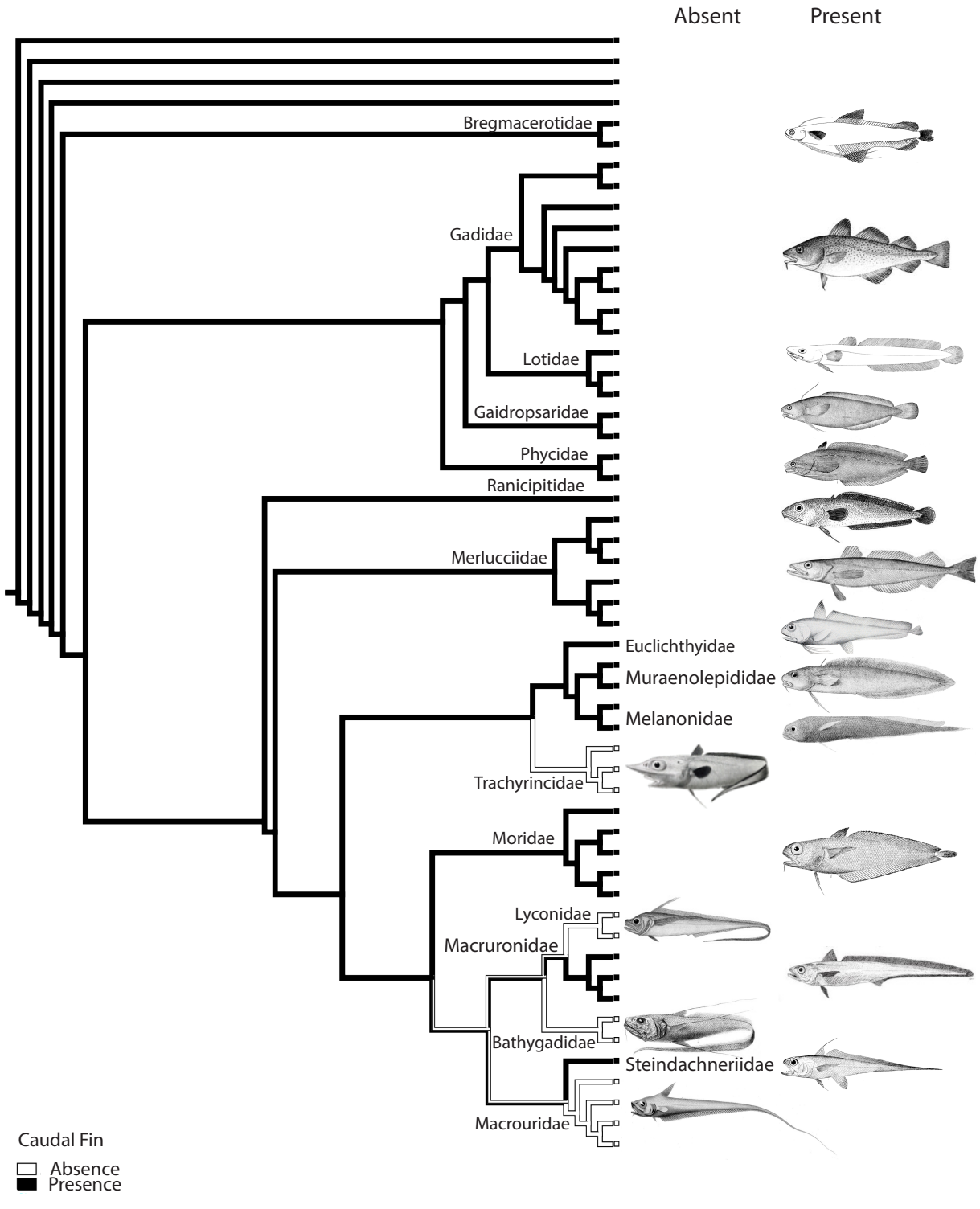


Figure 4.20 Ancestral State Reconstruction – Tail

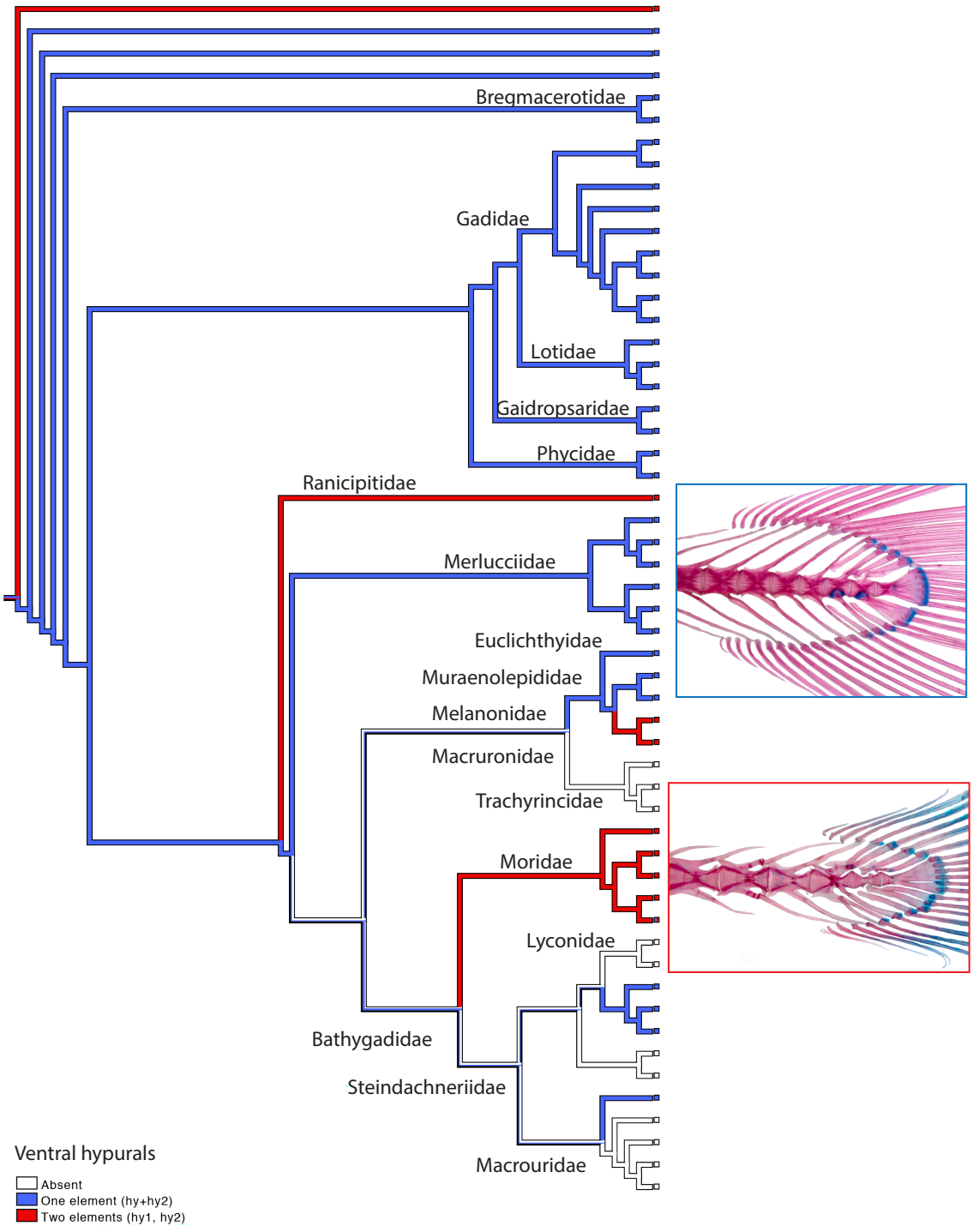


Figure 4.21 Ancestral State Reconstruction – Ventral hypurals elements

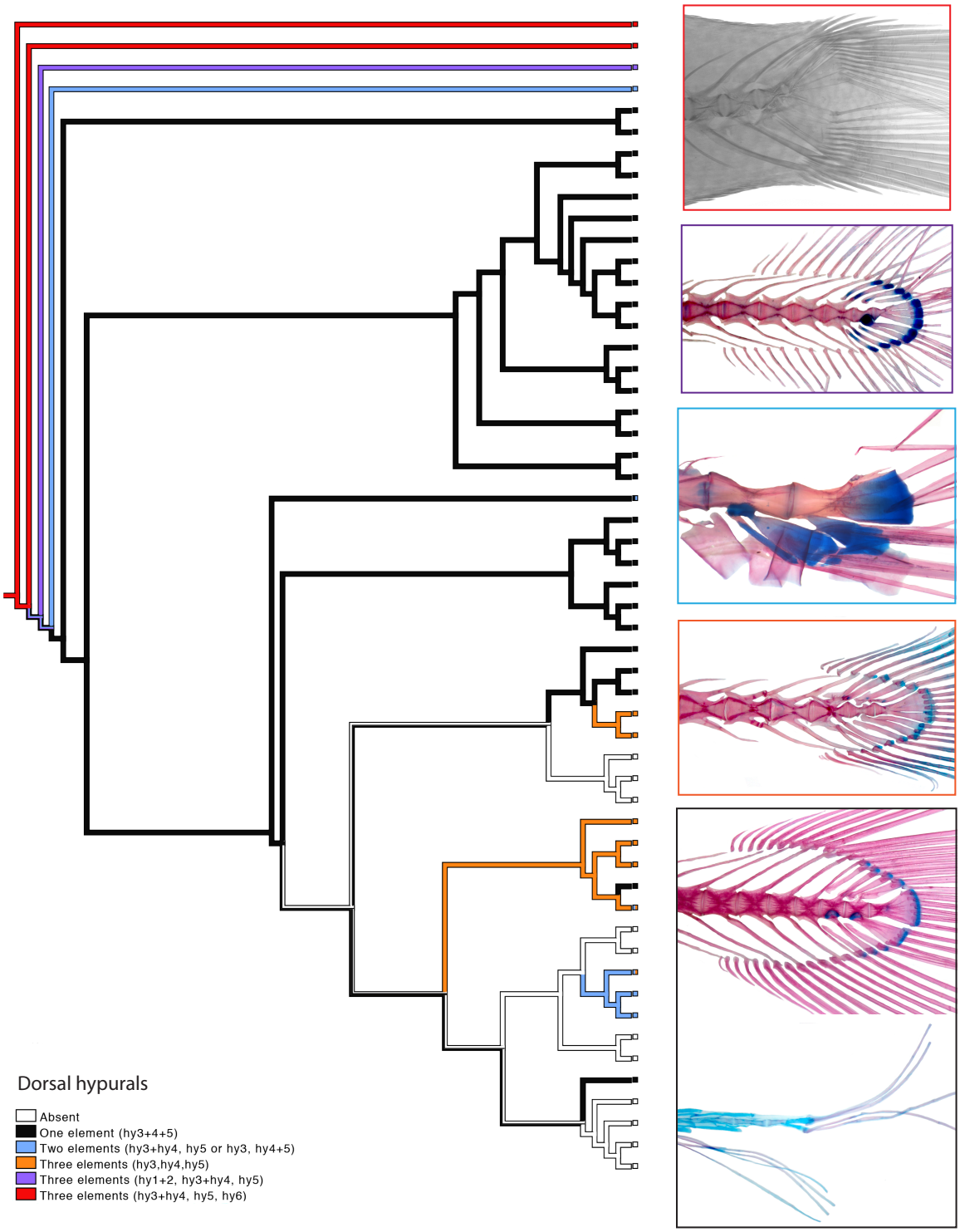


Figure 4.21 Ancestral State Reconstruction – Dorsal hypurals elements

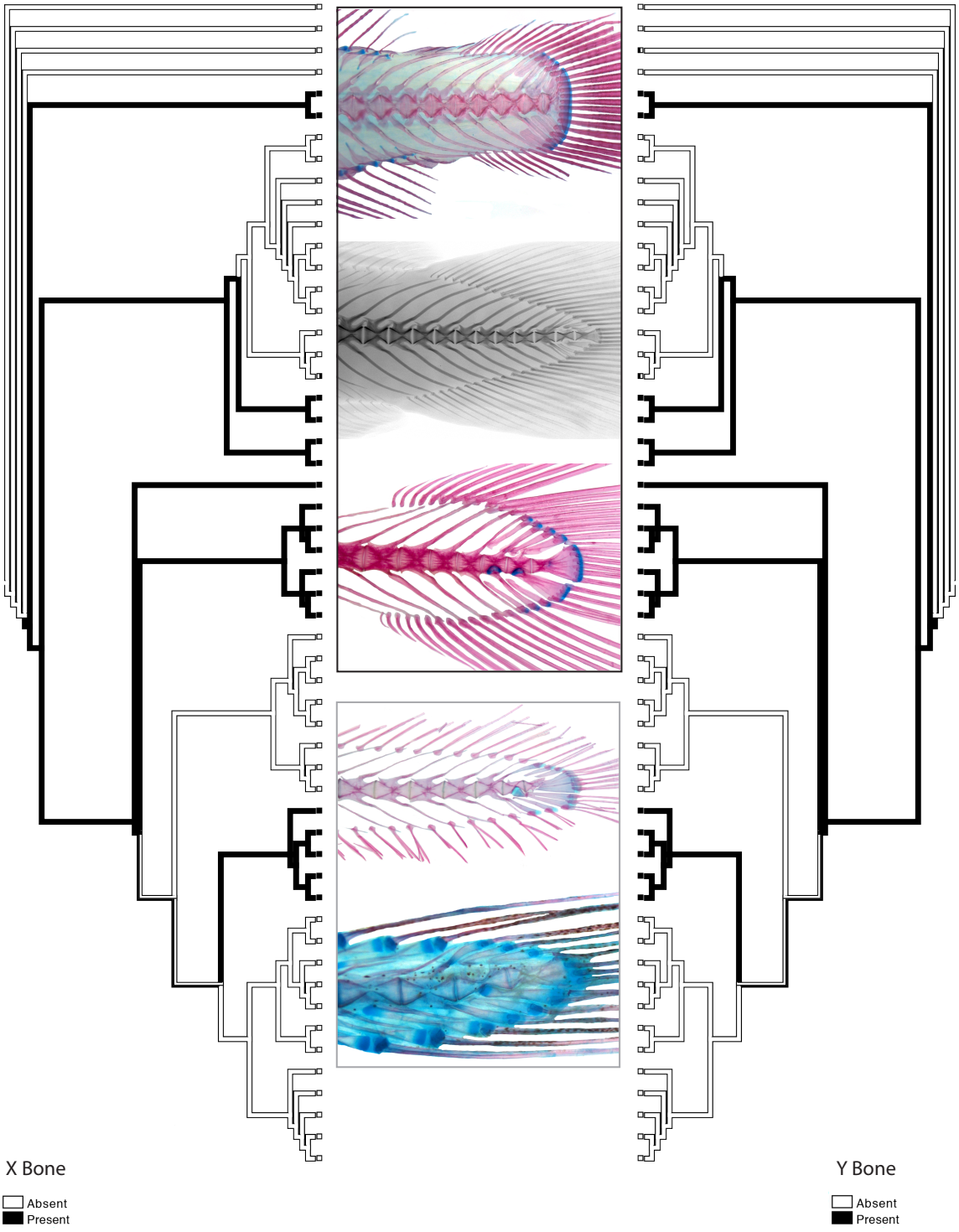


Figure 4.22 Ancestral State Reconstruction – X and Y Bones

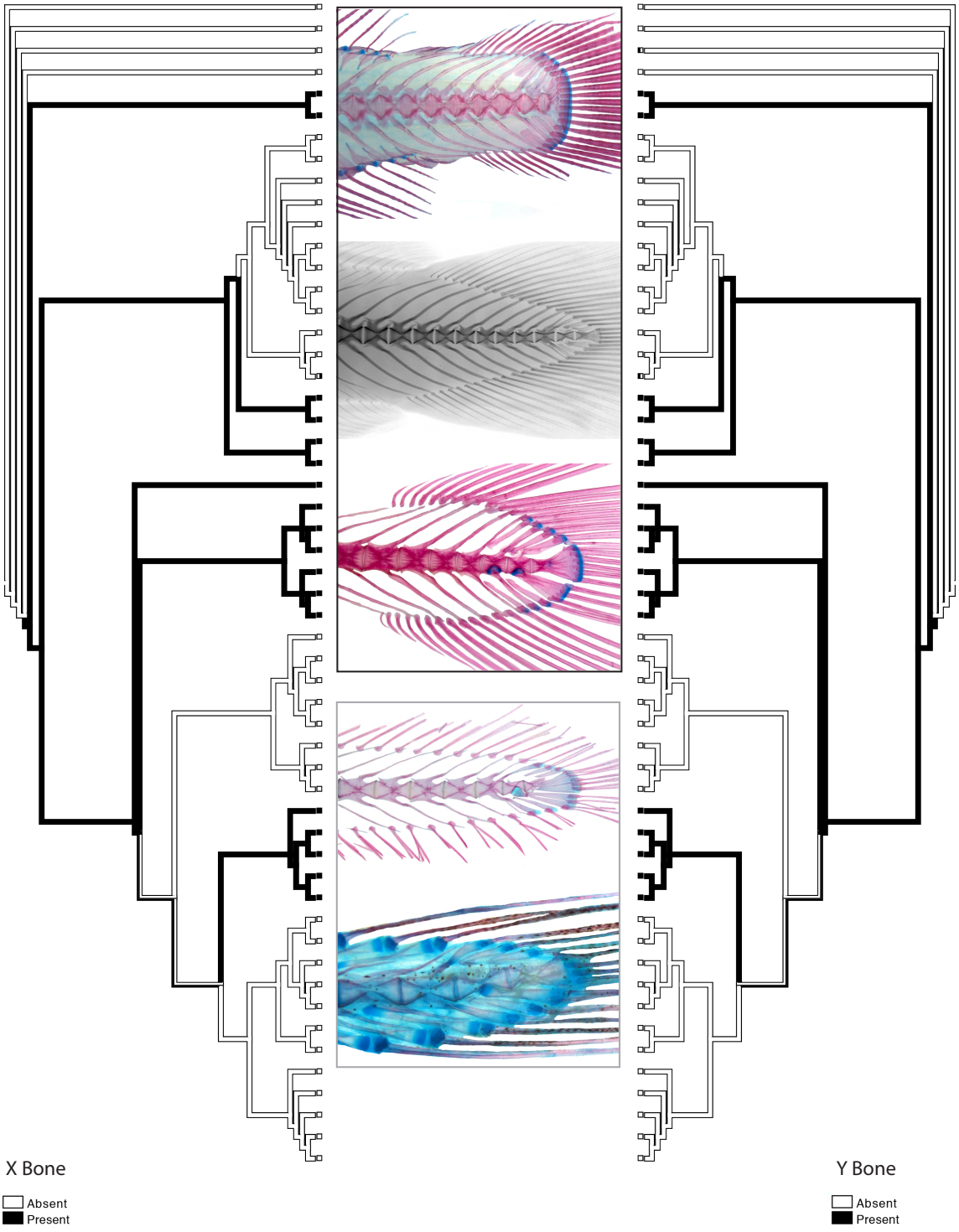


Figure 4.23 Ancestral State Reconstruction – Uroneural

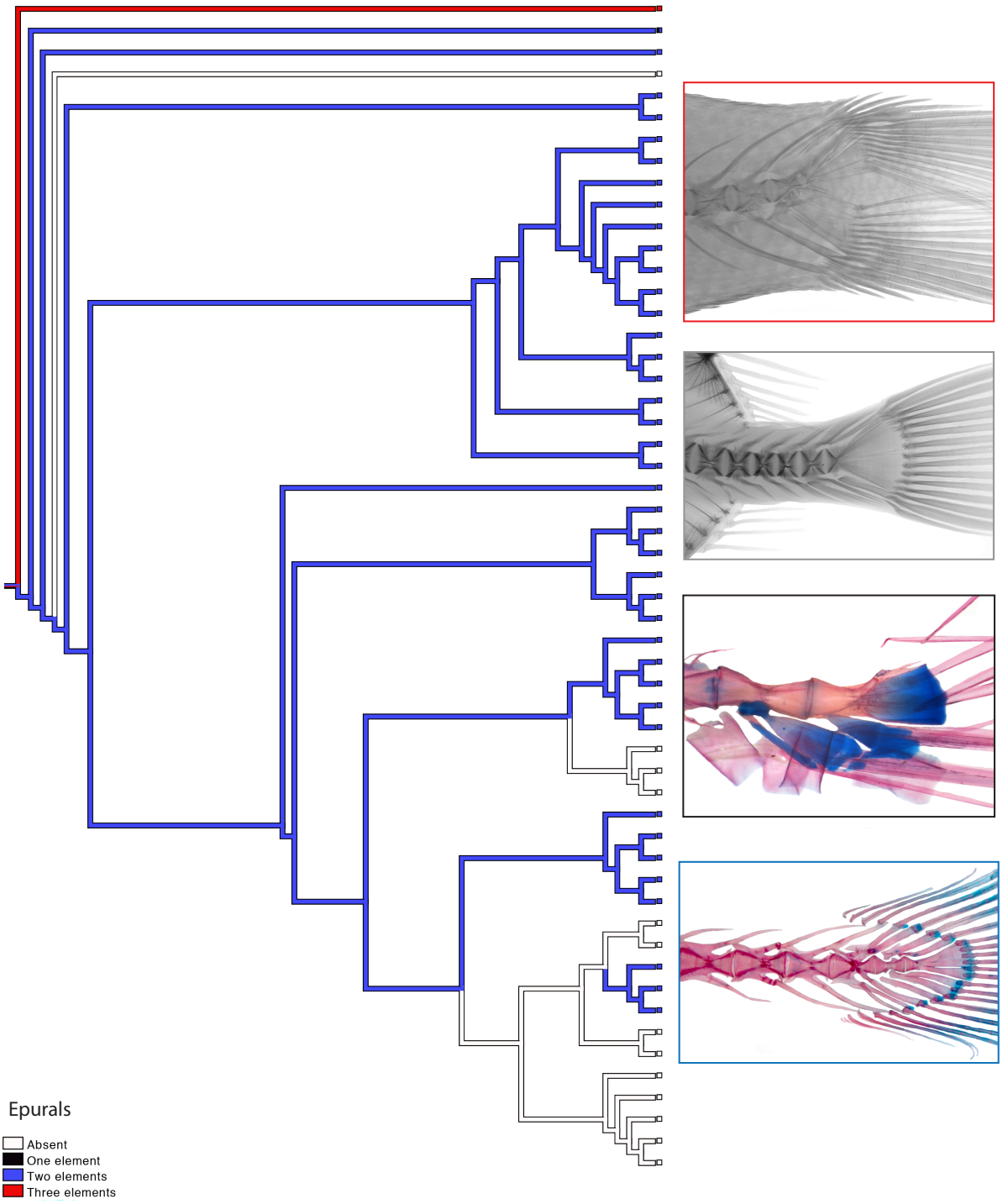


Figure 4.24 Ancestral State Reconstruction – Epurals

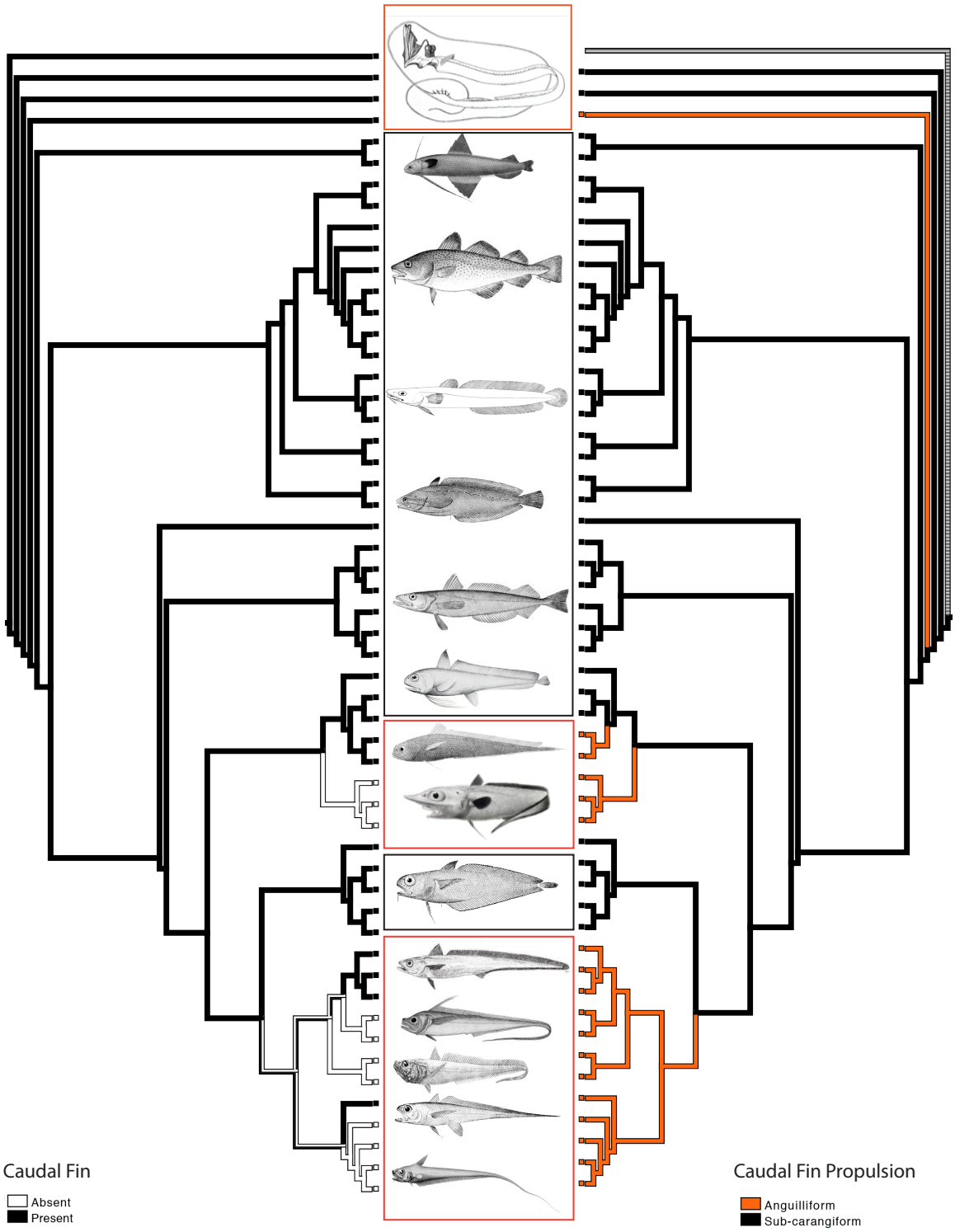


Figure 4.25 Ancestral State Reconstruction – Caudal fin propulsion

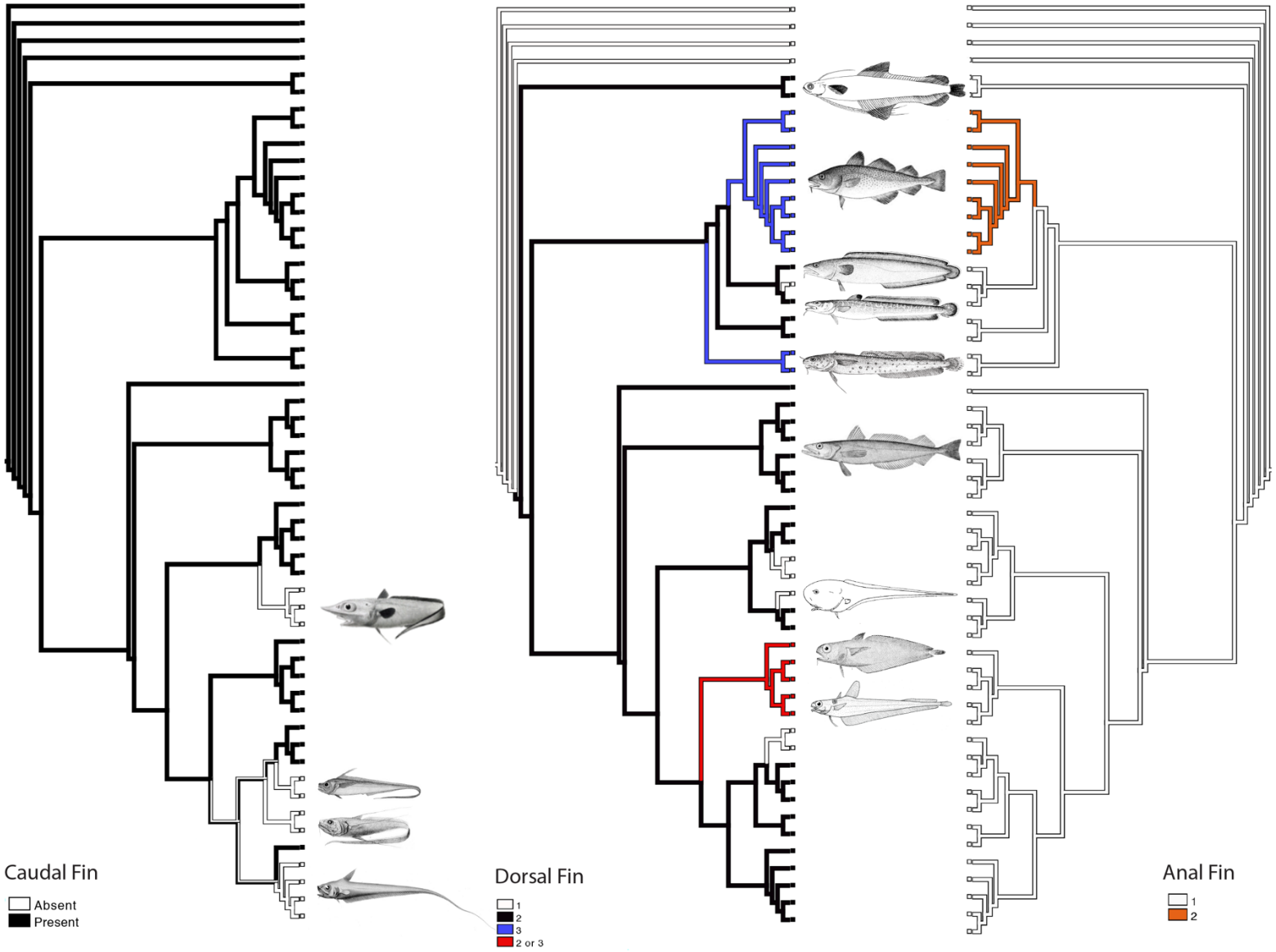


Figure 4.26 Ancestral State Reconstruction – Number of dorsal and anal fins

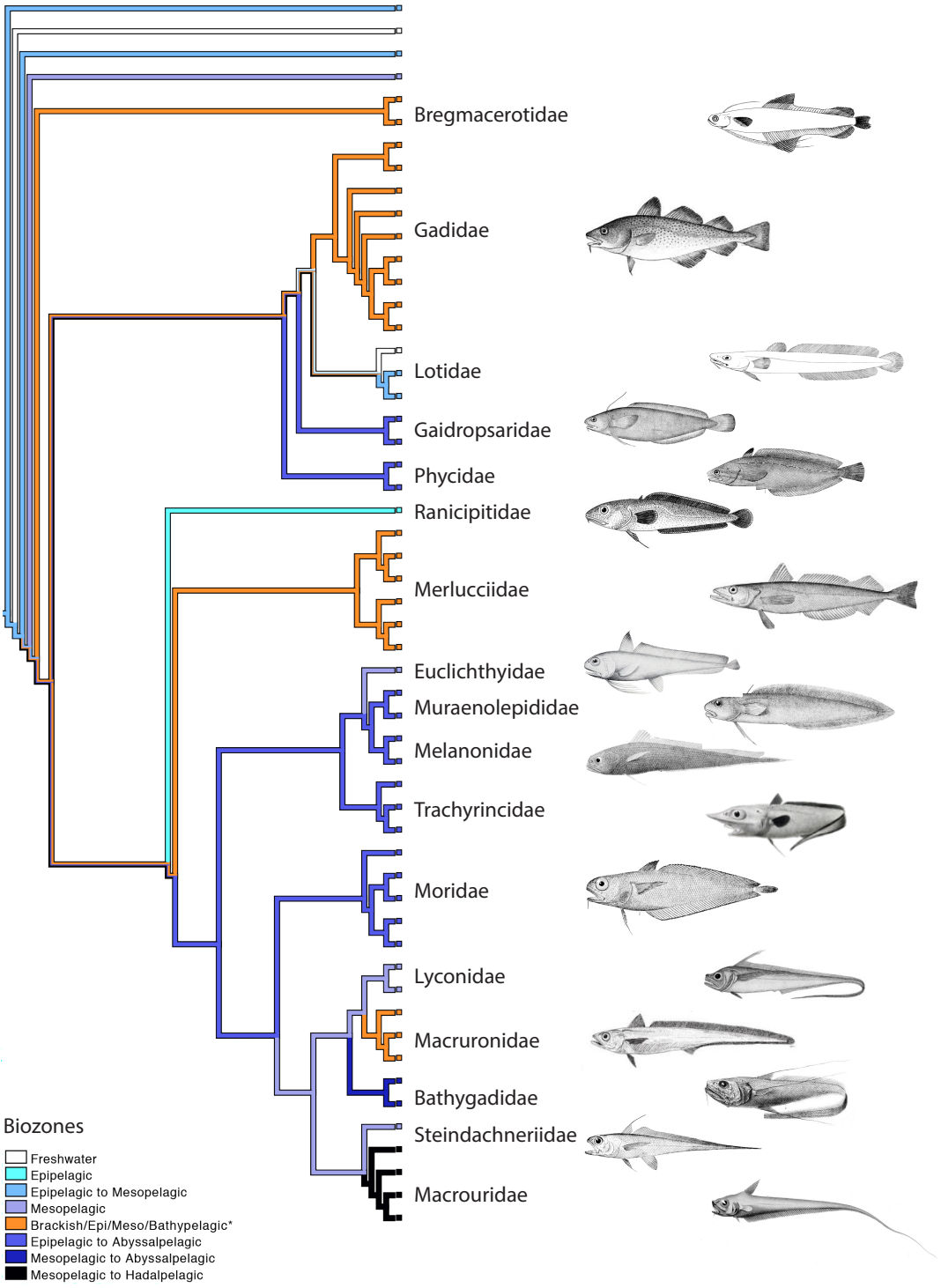


Figure 4.27 Ancestral State Reconstruction – Biozones

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TURMERIC AND COCONUT WHITHIN CURRY

(Christine Manfield)

INGREDIENTS

Credit: Jeremy Simons

4 garlic cloves, chopped
4 small green chillies, chopped
1 tbs finely chopped ginger
2 tsp finely chopped fresh turmeric
2 tbs sunfower oil
1 onion, finely chopped
2 tsp each ground turmeric and coriander
1 tsp ground cumin
1/4 tsp ground cloves
6 green cardamom pods, cracked
12 curry leaves, plus extra deep-fried leaves to serve
400ml can coconut milk
1 cup (250ml) fish stock
600g whiting fillets (skin on), cut into 4cm pieces
Juice of 1 lime
Steamed basmati rice and coriander leaves, to serve

PREPARATION

1 Using a mortar and pestle, pound the garlic, chilli, ginger and turmeric to a paste.

2 Heat oil in a deep frypan over medium heat. Cook onion, stirring, for 3-4 minutes until softened. Add chilli paste and cook, stirring, for 3-4 minutes until fragrant. Add spices and curry leaves, and cook, stirring, for a further 2 minutes. Add coconut milk and stock, then bring to a simmer.

3 Cook, stirring occasionally, for 10 minutes or until slightly reduced. Add whithin and cook for 4 minutes or until just cooked. Remove from heat. Season with lime juice and salt.
4 Serve curry with rice, topped with coriander and deep-fried curry leaves.

Prep Time: 20 mins Cook Time: 35 mins Yield: 4

VITA

Adela Roa-Varón was born in Bogotá, Colombia. She graduated with Magna Cum Laude from Universidad Nacional de Colombia where she earned his B.A. in Biology, with a major in Icthyology in 2001. Her dissertation on the Characterization of the demersal fish community of the Colombian Caribbean slope (300- 500 m) and some zoogeographic resulted in the publication of four scientific papers. After graduation Adela gained experience as a research associate at Makuriwa Museo de Historia Natural Marina de Colombia (INVEMAR), working in the evaluation of the ecological impacts caused by the construction of Urrá I hydroelectric dam on the fish community of the Sinú River, Colombia. Then, she pursued her M.S. in Biological Sciences from the University of Nebraska-Lincoln working on the molecular approaches to study relationships among families of Gadiformes fishes (cods, haddocks, rattail and their allies). Prior to entering the graduate program at VIMS, Adela worked as a research technician at UNC-Wilmington in taxonomy, biodiversity, ecology, and population dynamics of deep-sea fishes and then as a molecular lab technician working on the Euteleost Tree of Life (EToL) project at The George Washington University. In her doctoral research she continued working on improving the understanding of the evolutionary relationships among gadiform fishes using genomics and morphology. Adela has received many awards and grants, including two National Science Foundations awards, the East Asia and Pacific Summer Institute Fellowship (EAPSI) and the Doctoral Dissertation Improvement Grant (DDIG), the Lakeside Foundation Grant from the California Academy of Sciences and a summer fellowship from the Museum of Comparative Zoology at Harvard University and a predoctoral fellowship from the National Museum of Natural History, Smithsonian Institution. Adela has 10 first or co-authored peer-reviewed publications and has given many professional presentations in the U.S. and abroad. In addition to her research conference presentations and publications, Adela served as the VIMS International Student Representative and as a member of the W&M International Student Advisory Board. Her advisor was Dr. Eric J. Hilton, Department of Fisheries Science.