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Evolution of Life-History Characteristics in Gadoidei

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EVOLUTION OF LIFE-HISTORY CHARACTERISTICS IN GADOIDEI

A THESIS SUBMITTED TO

THE FACULTY OF THE GRADUATE SCHOOL

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PROGRAM IN BIOLOGY

BY

JOSHUA HITTIE

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Life-history characteristics (e.g., age and growth) have been used extensively to understand the temporal population dynamics of fish species, but less so within a phylogenetic framework. This study investigates life-history characteristics within the suborder Gadoidei (order: Gadiformes) and to test the extent of phylogenetic signal for those characteristics. To accomplish this, a phylogeny of Gadoidei was first constructed based on both mitochondrial and nuclear genes. Within this phylogenetic framework, life-history traits, including growth rate, age at maturity, and longevity, as well as ecological data, such as water depth and diet type, were mapped to the phylogeny using parsimony analysis to examine the extent of phylogenetic signal. A phylomorphospace was constructed to estimate an ancestral body plan for gadoid fishes, to examine possible convergences and divergences among the target groups, and whether the morphological features relate to the life-history aspect of the study. Lastly, life-history characteristics were mapped onto the phylomorphospace to compare body shape and life-history data within a comprehensive phylogenetic framework. The results of both the parsimony and morphometric analyses show support for the hypothesis that shared ancestry plays a role in the evolution of life-history traits.

CHAPTER 1

INTRODUCTION

The order Gadiformes is a diverse group of fishes within the superorder

Paracanthopterygii (Fig. 1). The overall placement of Gadiformes within Paracanthopterygii is

well supported (Endo, 2002; Roa-Varón and Ortí, 2009; Borden et al., 2013; Grande et al., 2013;

Nelson et al., 2016). The taxon Paracanthopterygii includes the orders Polymixiiformes

(beardfishes), Percopsiformes (trout-perches), Gadiformes (cods and hakes), Zeiformes (dories),

and Stylephoriformes (tube-eyes) (Nelson et al., 2016). Although once controversial, both

morphological and molecular studies support the sister-group relationship between Gadiformes

and *Stylephorus*, the only genus within the order Stylephoriformes (Miya et al., 2007; Grande et

al., 2013). They in turn are sister to Zeiformes (Miya et al., 2007; Grande et al., 2013; Betancur-

R et al., 2013; Grande et al., 2018, Hughes et al., 2018; Ghezelayagh et al., 2021; Figs. 1 and 2).
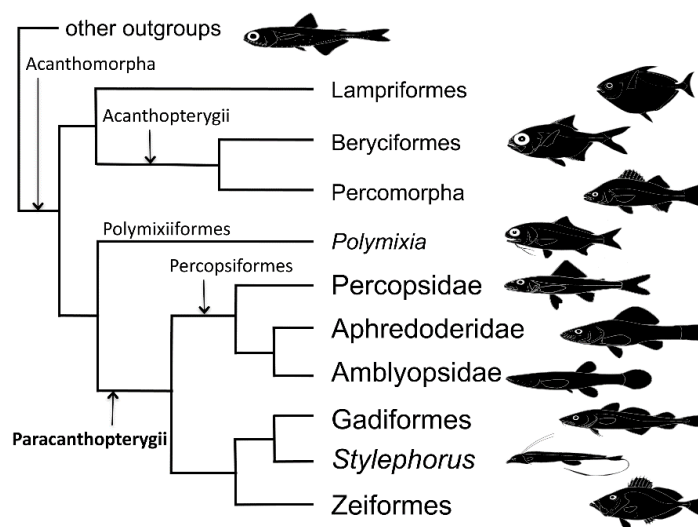
Figure 1. Overview of Paracanthopterygii showing the relationships of Gadiformes within the superorder, from Borden et al. (2013).
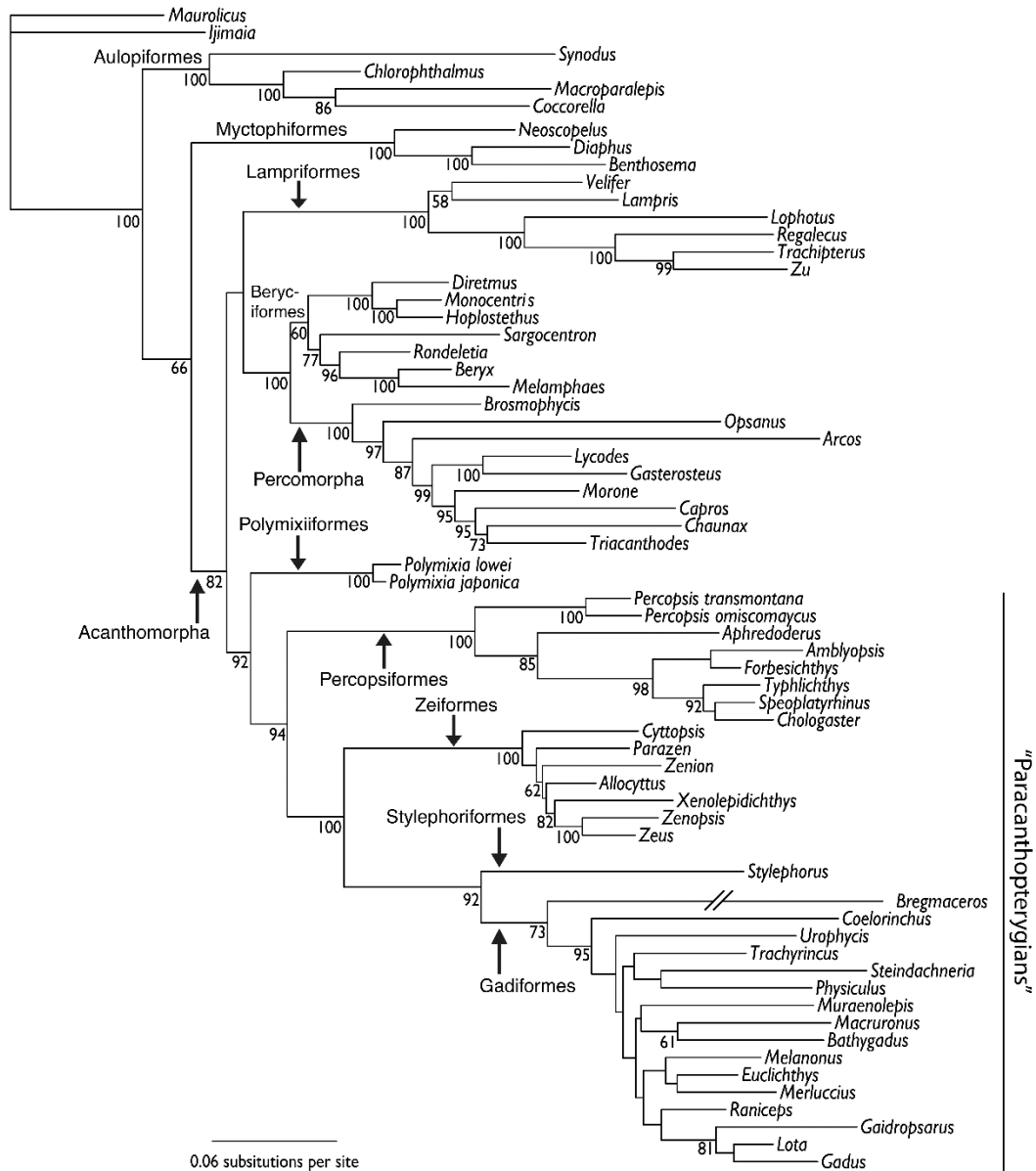
Figure 2. Phylogeny of Paracanthopterygii and related groups of Teleostei, from Grande et al. (2013).

Despite the placement of the order within Paracanthopterygii being well supported, the arrangement of lineages within Gadiformes is still contentious. The overview of Gadiformes presented in the study of Nelson et al. (2016) notes this lack of consensus, and as such did not resolve the major families of Gadiformes as monophyletic. Nelson et al. (2016) included three

suborders within Gadiformes: Melanoidei (consisting only of the family Melanonidae), Macrouroidei (consisting of the families Steindachneriidae, Bathygadidae, Macrouridae, and Trachyrincidae), and Gadoidei (consisting of the families Euclichthyidae, Macruronidae, Merluciidae, Ranicipitidae, Muraenolepididae, and Gadidae). Endo (2002) published the first comprehensive study of gadiform interrelationships, which was based exclusively on morphological data, followed by the molecular-based gadiform phylogeny created by Roa-Varón and Ortí (2009) followed. Endo (2002) and Roa-Varón and Ortí (2009) are two comparably comprehensive phylogenies of Gadiformes, but they differ substantially in their inter-gadiform relationships. Endo (2002) included the suborder Melanonoidei (which consists only of the family Melanonidae) as a basal sister clade to a Macrouroidei + Gadoidei clade. Roa-Varón and Ortí (2009), however, suggested the superorder Muraenolepidoidei is the basal gadiform group. More recently, based on molecular results, both Grande et al. (2013) and Roa-Verón et al. (2021) agree that the genus *Bregmaceros* is the most basal gadiform, Roa-Verón et al. (2021) placing it within the suborder Bregmacerotoidei.

This study focuses on a suborder of Gadiformes called the Gadoidei, which includes important gadid fishes such as the genus *Gadus* (a genus of particular commercial importance) and the only freshwater species of Gadiformes, *Lota lota*. The taxonomic composition of Gadoidei has varied greatly among published phylogenies. Endo (2002) recovered a suborder that included nearly every family within Gadiformes, excluding the family Melanonidae and the suborder Macrouroidei. Roa-Varón and Ortí (2009) recovered a similar suborder of almost equal size, and they divided it into two main clades: the clade ((Trachyrincinae + Macrouroidinae) + ((Merlucciidae + Melanonidae) Euclichthyidae)), and the clade Gadidae made up of the subfamilies Gadinae, Lotinae, Gaidropsarinae, and Phycinae. More recently, the Roa-Varón et al.

(2021) phylogeny restricted Gadoidei to four families: Phycidae, Gaidropsaridae, Lotidae, and Gadidae. The family names used in this thesis are based on this most recent phylogeny.

Apart from the taxonomic composition within Gadoidei, the relationship and placement of particular lineages also varies in different publications. The lotid fishes have been placed as a monophyletic subfamily Lotinae, sister to a subfamily Gadinae and placed within Gadidae (Endo, 2002; Teletchea et al., 2006), or as non-monophyletic within Gadidae (Roa-Varón and Ortí, 2009), or as a monophyletic family Lotidae, which is sister to Gadidae (Roa-Varón et al., 2021). Gaidropsarid and phycid fishes have been placed as sister subfamilies (Gaidropsarinae + Phycinae) within Gadidae as sister to a (Lotinae + Gadinae) clade (Endo, 2002), as sister to Gadinae with the (Gadinae + (Gaidropsarinae + Phycinae)) clade, as sister to a subfamily Lotinae (Teletchea et al., 2006), as non-sister clades (Roa-Varón and Ortí, 2009), or as non-sister families (Roa-Varón et al., 2021).

The several gadiform phylogenies proposed previously (e.g., Roa-Varón and Ortí, 2009; Endo, 2002; Nelson et al., 2016; Teletchea et al., 2006), however, produced a lack of consensus as to which lineages belong within Gadoidei and the relationships within. As such, the first goal of this thesis was to construct molecular phylogenetic trees to determine the relationships among lineages within Gadoidei as well as to provide a phylogenetic foundation for further life history and morphometric studies. Secondarily, the constructed gene trees and combined phylogeny were compared with phylogenetic studies such as that of Roa-Varón et al. (2021).

CHAPTER 2

PHYLOGENY

The first step of this study was to construct a phylogeny of the Gadoidei based on

molecular data. Five gene sequences were used: 12S, 16S, cytochrome b, and COI for the

mitochondrial genes, and RAG-1, a nuclear gene. The genes chosen are established markers for

molecular systematics of fishes (Roa-Varón and Ortí, 2009).

The 12S (mitochondrially encoded 12S ribosomal RNA) gene is a part of the small

subunit of the mitochondrial ribosome. The gene encodes a protein responsible for regulating

metabolic homeostasis and insulin sensitivity. The 12S gene, along with the 16s (mitochondrially

encoded 16S ribosomal RNA) gene, are involved in the translation of messenger RNA into

mitochondrial proteins. Both genes are considered conserved and evolve more slowly than other

mitochondrial genes such as COI and cytochrome b, providing a counter to more rapidly

evolving mitochondrial genes such as COI (Cawthorn et al., 2012). Within fishes, the rate of

evolution for 12S and 16S is about 0.23% (percent substitutions per position per million years;

Gomes, 1999).

COI, or cytochrome c oxidase I, is a mitochondrial gene for an enzyme involved in the

electron transport chain of mitochondrial oxidative phosphorylation, involved in catalyzing the

reduction of oxygen to water (Denis, 1986). The subunits perform identical or very similar

functions across multiple groups of vertebrates and the genes themselves are considered highly

conserved due to the final protein product being necessary for basic functionality of the electron

transport chain. However, the third codon position shows faster levels of mutation, while the functional first and second positions remain highly conserved (Ward et al., 2005). COI is particularly useful for resolving lower-level relationships between taxa due to its role in coding for the electron transport protein *cox1*, limiting any potential mutations to the third codon position, which would not disrupt the functioning of the protein itself. Within different genera of fishes, the conservation of COI is as high as 85% (flathead *Platycephalus*, *Neoplatycephalus*, *Cymbacephalus*) and upwards of 99% conserved within the tuna genus *Thunnus* (Ward et al., 2005). COI mutations in the third codon position occur at a rapid pace, causing enough differences between species in a way that those species can be differentiated. The mutations are most likely to occur in the third position of the codon which does not interfere with protein function. Because overall functionality is so highly maintained, COI has a low rate of overall amino acid change within the mitochondrial genome (Hebert et al., 2003). COI has substitution rates between 0.06% to 3.3% per million years within some marine fishes (Horne and Herwerden, 2013), although the rate can vary between taxonomic groups.

The Cytochrome b gene (MT-CYB) codes for a protein that is one of 11 (depending on species) components of the complex III structure responsible for mediating electron transfer to cytochrome c (Beattie et al., 1994). Like COI, it is highly conserved due to its importance in general cellular functionality. Within different groups of vertebrates, it is suggested that the differences in amino acid replacement rates within cytochrome b are due to differences in DNA substitution rates; additionally, there is little evidence that the types of amino acid changes differ significantly between groups (such as sharks and mammals; Martin and Palumbi, 1993). Overall, evidence points toward the patterns of evolution for cytochrome b being relatively constant throughout vertebrate evolutionary history. Cytochrome b has been established as a useful

marker for fish species identification, specifically within Gadidae and Merlucciidae (Pepe et al., 2004), and when used in conjunction with other genes for phylogenetic analysis, can yield well-resolved phylogenetic trees (Farias et al., 2001).

RAG-1 is one part of a pair of highly conserved recombination-activating genes, involved in the rearrangement and recombination of genes that encode immunoglobulin and T-cell receptor molecules. RAG-1 is one of the key mediators of the somatic gene rearrangement process (known as V(D)J recombination), which is responsible for the diversity of antigen receptors in the adaptive immune system of jawed vertebrates. It is suggested that RAG-1, and its paralog RAG-2, were present in a common ancestor of deuterostomes and their role in the adaptive immune system was co-opted later during early jawed vertebrate evolution (Fugmann et al., 2006). Within sharks it was shown that upwards of 94% of the translated protein sequence is identical, with 63% and 64% shared between chicken and human translated protein sequences, and 64% and 63% of the shark DNA sequence shared with chicken and human DNA sequences (Bernstein et al., 1996), further indicating RAG-1's high degree of functionality and conservation. Within sister lineages, the rates of molecular evolution of RAG-1 are strongly conserved and are relatively slow, though the exact rate depends on the size of the overall genome. Additionally, it is suggested that these mutation rates are evolving as lineages split and may coincide with speciation events (Sclavi and Herrick, 2013), making them particularly useful in a phylogenetic context.

Methods

Several gadiform phylogenies and classifications (Endo, 2002; Bakke et al., 2005; Teletchea et al., 2006; Roa-Varón and Ortí, 2009; Nelson et al., 2016) were used as initial starting points to determine which taxa should be included in this study for construction of

individual gene trees and the subsequent combined molecular gene phylogeny. The family

Merlucciidae (hakes) was used as an outgroup relative to Gadoidei, but still within Gadiformes

as a whole. The gene trees were rooted using *Polymixia japonica* and *Percopsis transmontana* as

representatives of non-gadiform Paracanthopterygii.

For the constructed gene trees, 22 taxa (17 gadoid species, three merluccid species, and

two non-gadiform species as the outgroup) were chosen. The species used in the present study

are a different from those used by Roa-Varón et al. (2021); the species of Gadoidei used herein

are a robust representation of the key target clades, focused on species for which both life-history

data and gene sequence information was available. Gene sequences used for the individual gene

trees and the combined gene tree were obtained from the GenBank molecular database (Table 1).

Sequence data for all taxa were retrieved from GenBank as FASTA files and input into

the Geneious software (version 9.1.5) for sequence alignment and editing. The alignment in

Geneious was done using pairwise global alignment, based on the algorithm developed by Feng

and Doolittle (1987). The evolutionary models for the selected genes were determined using

jModeltest v2.1.1 (Darriba et al., 2012; Guindon and Gascuel, 2003). The model for all included

genes was GTR+G.

Maximum likelihood (ML) analyses were performed using Garli version 2.0 (Zwickl,

2006) with 1000 bootstrap replicates. Bayesian inference (BI) analyses were conducted using

MrBayes v3.2.6 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck 2003) with 10

million generations and a burn-in of 25%. The Bayesian-based phylogenies were selected as

preferred over the maximum likelihood trees due to the overall greater resolution of their gene

trees and combined gene tree.

A gene tree was first constructed for each individual gene using both ML and BI methods, and then the sequence data for all genes were combined into one dataset and run using the same ML and BI analyses to create a combined gene tree. The combined datasets were also analyzed using gene-by-gene jackknifing to evaluate each individual gene's contribution to the phylogeny.

Taxon	12S	16S	COI	CytB	RAG1
Gadus morhua	NC_002081.1	NC_002081.1	KP975728.1	M57662.1	FJ215242.1
Gadus ogac	FJ215031.1	FJ215140.1	-	DQ174047.1	-
Gadus macrocephalus	LC021203.1	KR779486.1	JF952739.1	AB078152.1	FJ215241.1
Microgadus proximus	FJ215062.1	FJ215170.1	GU440406.1	DQ174067.1	FJ215274.1
Microgadus tomcod	FJ215063.1	FJ215171.1	KF930129.1	KP644319.1	-
Lota lota	NC_004379.1	DQ174053.1	HQ961089.1	AJ517496.1	FJ215254.1
Molva molva	FJ215065.1	FJ215173.1	KP975793.1	AJ517494.1	FJ215275.1
Urophycis tenuis	FJ215092.1	FJ215199.1	KF930528.1	DQ174086.1	FJ215302.1
Urophycis regia	FJ215091.1	FJ215200.1	KC016028.1	-	FJ215301.1
Urophycis floridana	FJ215094.1	FJ215198.1	KF930526.1	-	FJ215300.1
Urophycis chuss	FJ215093.1	FJ215197.1	KF930524.1	-	FJ215299.1
Phycis chesteri	FJ215077.1	FJ215184.1	KC015811.1	DQ174075.1	FJ215287.1
Gaidropsarus ensis	FJ215034.1	FJ215143.1	KF929907.1	DQ174049.1	FJ215244.1
Gaidropsarus argentatus	FJ215033.1	FJ215142.1	KF929906.1	MZ234305.1	FJ215243.1
Gaidropsarus vulgaris	KC980977.1	KJ128775.1	KJ204889.1	DQ174051.1	-
Enchelyopus cimbrius	FJ215025.1	FJ215134.1	KJ204849.1	AJ517498.1	FJ215237.1
Ciliata mustela	FJ215012.1	FJ215121.1	KJ204807.1	DQ174039.1	FJ215225.1
Merluccius australis	FJ215060.1	FJ215162.1	KM255106.1	AB248670.1	FJ215266.1
Merluccius bilinearis	FJ215056.1	FJ215165.1	KF930127.1	DQ174060.1	FJ215267.1
Merluccius capensis	FJ215059.1	FJ215166.1	JF493884.1	EF456009.1	FJ215268.1
Percopsis transmontana	NC_003168.1	NC_003168.1	KF930253.1	AP002928.1	AY308766.1
Polymixia japonica	NC_002648.1	NC_002648.1	KF930291.1	AB034826.1	AY308765.1

Table 1. List of individual genes and GenBank ascension numbers used for each gene tree and combined gene tree.

Individual Gene Tree Results

12S Gene Tree

The Bayesian inference conditions used to construct the 12S gene tree resulted in generally more resolution at the genus level than the family level, excluding the three *Urophycis* species. The 12S tree (Fig. 3) placed Phycidae as the most basal family within the included gadoid taxa, with a posterior probability values support value of 1.00, which indicates high support for that arrangement within this tree. This placement is in line with that of Roa-Varón et al. (2021), who placed Phycidae as the most basal clade of their Gadoidei (which includes Gadidae, Lotidae, Phycidae, and Gaidropsaridae), with Merluccioidei (outgroup to Gadoidei) being a separate sister suborder. The present study recovered a monophyletic Phycidae, with *Phycis* sister to *Urophycis* (support value of 1). Data for 12S were unable to resolve the relationships among three *Urophycis* species (*U. tenuis, U. chuss,* and *U. regia*), although this trichotomous clade is sister to *U. floridana*. Lotidae were not recovered as monophyletic. Instead, *Molva molva* was recovered as sister (with support value of 0.8004) to a polychotomy consisting of *Lota lota,* Gadidae*,* and (*Gaidropsaridae + Merluccioidei*) with a support value of 0.9116 (Fig. 3).

Gadidae in this analysis included only *Gadus* and *Microgadus*. Within Gadidae, *Microgadus* (containing *M. proximus* sister to *M. tomcod*, with a support value of 0.9428) is placed sister to *Gadus* with a support value of 1.00. Within *Gadus*, *G. macrocephalus* and *G. ogac* form a clade with a support value of 0.9565, whereas *G. morhua* is sister to the previous genera with a support value of 0.9942 (Fig. 3).

Within Gaidropsaridae, the species of *Gaidropsarus* are united and placed as sister to *Ciliata mustela + Enchelyopus cimbrius*, with a support value for the family being 1.00. Within

Merluccioidei, *Merluccius capensis* and *Merluccius bilinearis* are placed as sister genera with a

support value 0.6351, with *Merluccius australis* then placed sister to those two genera with a

support value of 1.00 (Fig. 3).
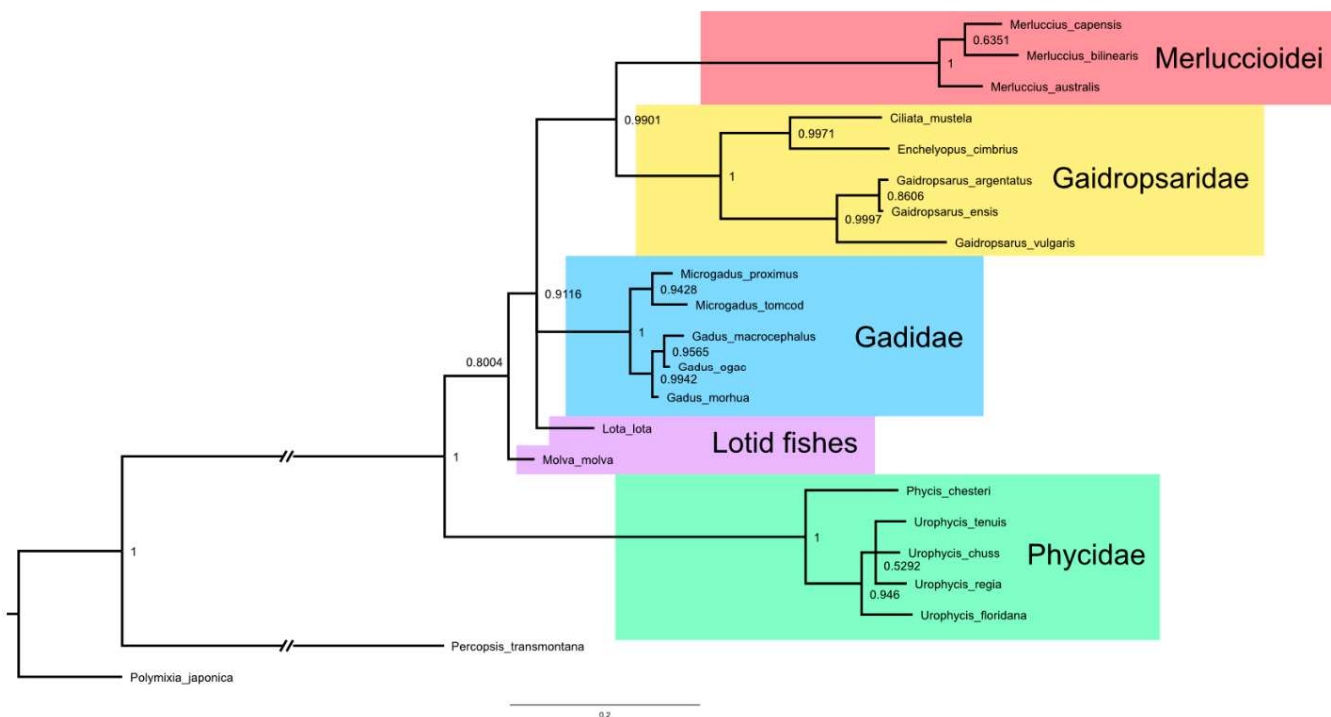
Figure 3. Bayesian Inference (BI) consensus tree based on sequence data for the non-coding mitochondrial 12S gene. Support values at the nodes are posterior probability values. Diagonal lines indicate truncation. Colors indicate gadiform families (or suborder in the case of Merluccioidei) as recognized by Roa-Varón et al. (2021).

16S Gene Tree

The 16S tree (Fig. 4) placed Phycidae as the most basal family within the included

gadiform taxa, with a support value of 1.00. This placement is congruent with that of the 12S

gene tree. However, with 16S, the suborder Merluccioidei was recovered as a sister clade to

Gaidropsaridae + Gadidae with a support value of 0.7504. This placement is contrary to Roa-

Varón et al. (2021) who placed Phycidae as the most basal clade of the suborder Gadoidei, with

Merluccioidei being a separate sister suborder. In this 16S study, Lotidae are not recovered as monophyletic but instead, both lotid taxa (*Lota lota* and *Molva molva*) were included within a larger Gadidae family as opposed to their conventional sister group relationship (*Molva molva* was placed with a support value of 0.9805, and *Lota lota* with a support value of 0.9273). Within the gadoid families, the included genera resolved as expected. The Bayesian inference conditions used to construct the 16S gene tree resulted in more resolution at the genus level than family level. The result for 16S shares the same genera placement as 12S in Gaidropsaridae (all genera with support values of 1.00, though the support value for the family itself is 0.9925) and with Gadidae of *M. proximus* and *M. tomcod* having a support value of 0.9907, while within *Gadus*, *G. macrocephalus* and *G. ogac* have a support value of 0.5521, and those two plus *G. morhua* have a support value of 0.9989.

Figure 4. Bayesian Inference (BI) 16S consensus tree using sequence data for the non-coding mitochondrial 16S gene. Support values at the nodes are posterior probability values. Diagonal lines indicate truncation. Colors indicate gadiform families (or suborder in the case of Merluccioidei) as recognized by Roa-Varón et al. (2021).

COI Gene Tree

The COI gene tree (Fig. 5) resolved Merluccioidei as sister to all of Gadoidei (with a support value of 1), agreeing with Roa-Varón et al. (2021), but unlike the 12S and 16S gene trees. Within Gadoidei, Phycidae were recovered as sister to Gaidropsaridae (with a support value of 1), as opposed to its placement in Roa-Varón et al. (2021), where it was placed as the most basal clade of the suborder Gadoidei. Lotidae are resolved as monophyletic and sister to Gadidae + (Phycidae + Gaidropsaridae) with a support value of 1. Within Gadidae, resolution is as expected, agreeing with 12S and 16S. However, within Gaidropsaridae, *Ciliata* and

*Enchelyopus* are not placed as sister genera, contrary to their placement by 12S and 16S.

Figure 5. Bayesian Inference (BI) COI consensus tree using sequence data for the protein coding coenzyme I gene. Support values at the nodes are posterior probability values. Diagonal lines indicate truncation. Colors indicate gadiform families (or suborder in the case of Merluccioidei) as recognized by Roa-Varón et al. (2021).

Cytochrome b Gene Tree

The placement of taxa on the cytochrome b phylogeny (Fig. 6) is the same as in the COI gene tree. The suborder Merluccioidei is placed sister to Gadoidei with a support value of 1 (this includes the polytomies and unexpected placement of *Gaidropsarus argentatus*). Phycidae are sister to Gaidropsaridae with a support value of 1. Lotidae are placed as the most basal within Gadoidei, with a support value of 1. Within families, genera follow the same placement as in COI. *Microgadus* is sister to *Gadus* with a support value of 1. Within Gaidropsaridae, the genera *Ciliata* and *Enchelyopus* are not placed as sister genera, whereas they were sisters in 12S and 16S results.
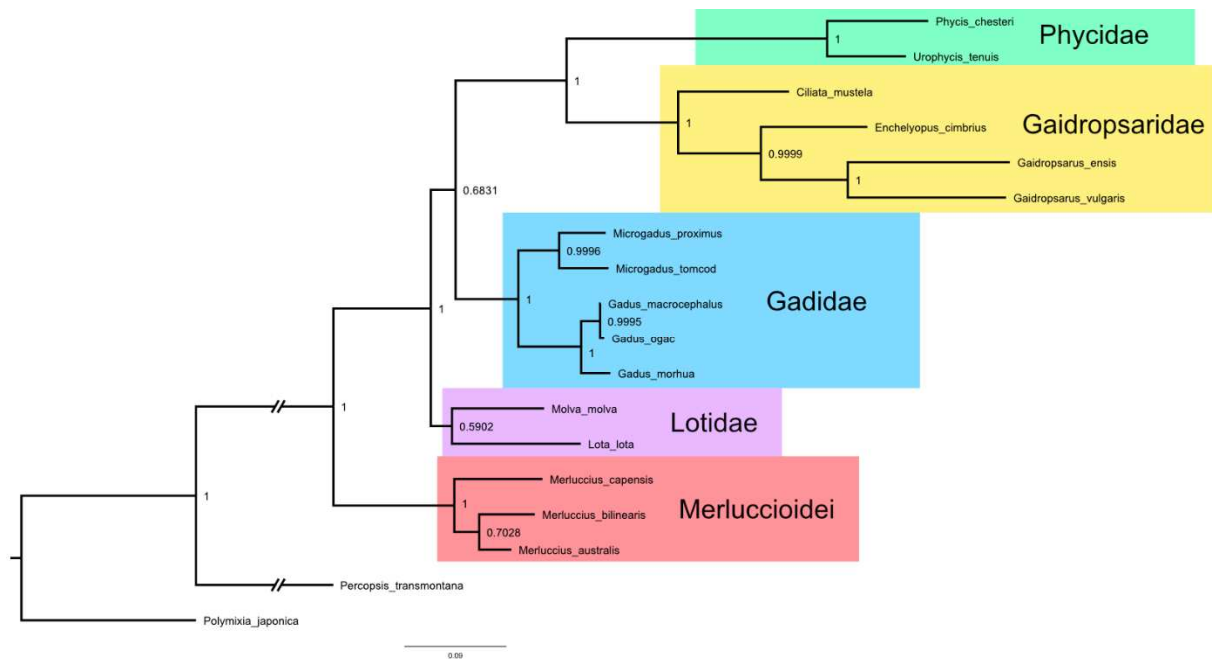
Figure 6. Bayesian Inference (BI) Cytochrome b consensus tree using sequence data for the protein coding cytochrome b gene. Support values at the nodes are posterior probability values. Diagonal lines indicate truncation. Colors indicate gadiform families (or suborder in the case of Merluccioidei) as recognized by Roa-Varón et al., (2021).

RAG-1 Gene Tree

In the RAG-1 gene tree (Fig. 7), Merluccioidei are placed as sister to the Gadoidei with a support value of 1.00. Phycidae are placed as the most basal family within Gadoidei, as in Roa-Varón et al. (2021), with a support value of 0.9211. Gaidropsaridae follow with a support value of 0.9849. Lotidae, once again, are not recovered as monophyletic, with lotids (*Molva molva* and *Lota lota*) being placed within Gadidae. Despite the families being largely resolved, RAG-1 was unable to fully resolve all genera within the families. Within Phycidae, a polytomy is present containing three branches (*Phycis chesteri + Urophycis tenuis* with a support value of 0.6001, *Urophycis floridana + Urophycis regia* with a support value of 0.9936, and *Urophycis chuss*). Within Gaidropsaridae, the genera *Ciliata* and *Enchelyopus* form a polytomy with the *Gaidropsarus* clade.
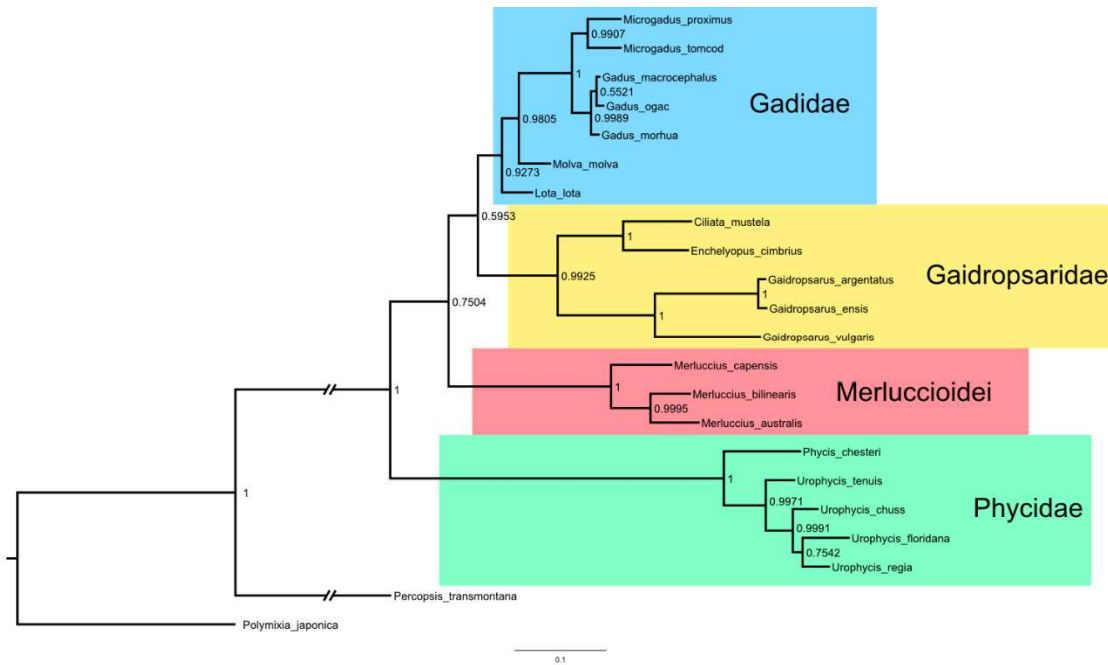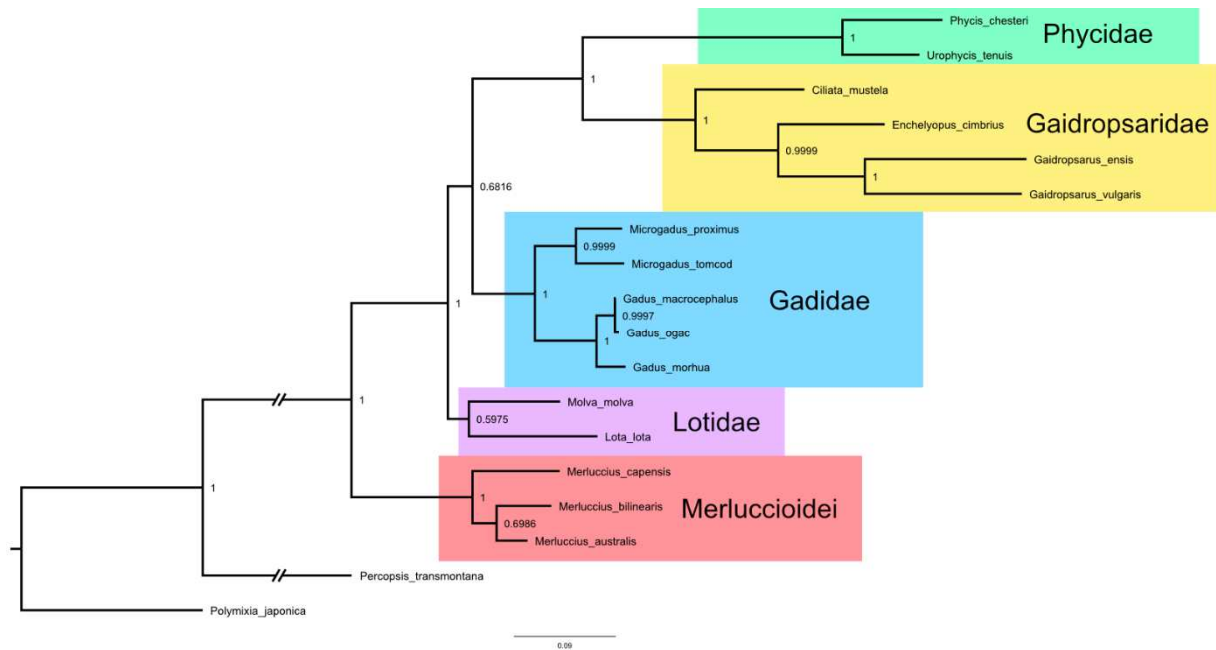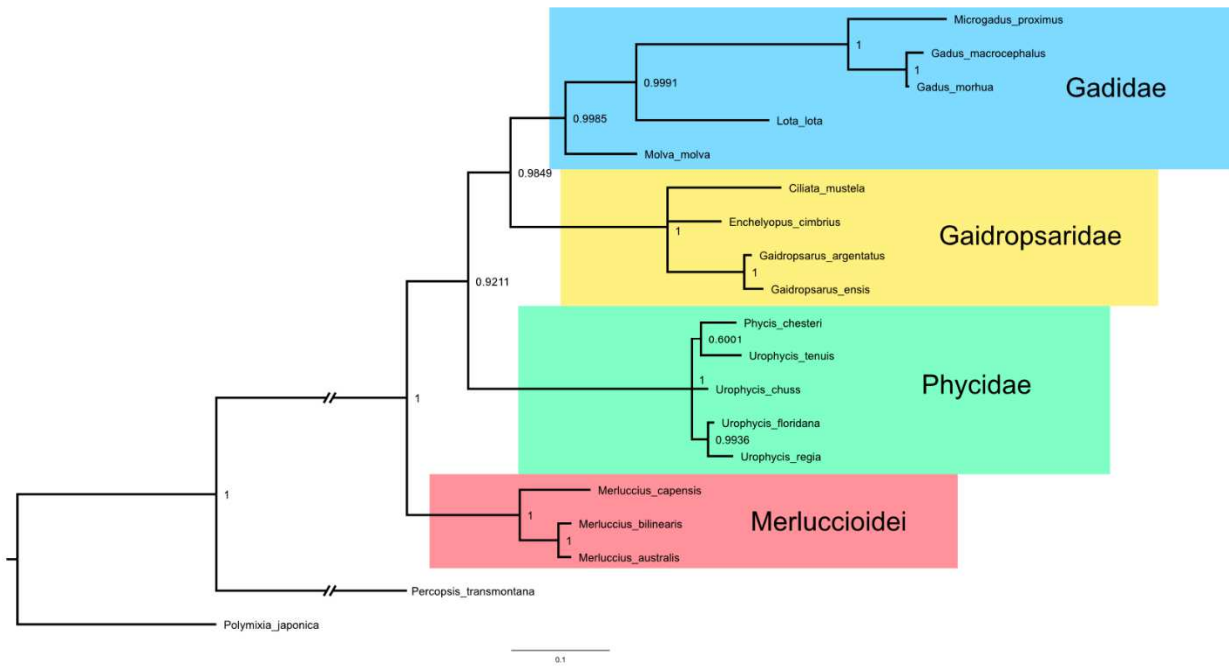
Figure 7. Bayesian Inference (BI) consensus tree using sequence data for the nuclear RAG-1 gene. Support values at the nodes are posterior probability values. Diagonal lines indicate truncation. Colors indicate gadiform families (or suborder in the case of Merluccioidei) as recognized by Roa-Varón et al. (2021).

Combined Gene Tree Results

Based on the results of the gene-by-gene jackknifing, the final combined gene tree used all five individual genes. In the combined gene tree (Fig. 8), Merluccioidei (*M. capensis, M. bilinearis,* and *M. australis*) is placed as the outgroup to Gadoidei, with a support value of 1.00. Phycidae, consisting of *Phycis chesteri, Urophycis tenuis, Urophycis chuss, Urophycis floridana,* and *Urophycis regia,* form a monophyletic group, with a support value of 0.9791. *Ciliata mustela* and *Enchelyopus cimbrius* are sister taxa with a support value of 0.9893 and are in turn sister to the three *Gaidropsarus* species (*G. argentatus, G. ensis,* and *G. vulgaris*), with a support value of 0.9986. Phycidae form a monophyletic group with Gaidropsaridae, with a support value of 0.9985.

For the gadids, the genera *Microgadus* and *Gadus* form a monophyletic group, with a support value of 1.00. The lotids (*Lota lota* and *Molva molva*) do not form a monophyletic group; instead, *Lota lota* is placed as sister to the gadid clade with a support value of 0.8348. The lotids and gadids together form a monophyletic group with a support value of 0.9992. The combined gene tree follows the lotid-gadid placement recovered by Roa-Varón and Ortí (2009), but not that of Roa-Varón et al. (2021); in the combined tree, the gadid clade, which includes the genera *Gadus* and its closest relatives, is sister to *Lota lota*, but not to an entire lotid clade, instead placing *Lota lota* and *Molva molva* as the second most and the single most basal species within the gadid clade, respectively. This result indicates that the two "lotid" genera could be included within the family Gadidae, perhaps as separate subfamilies.
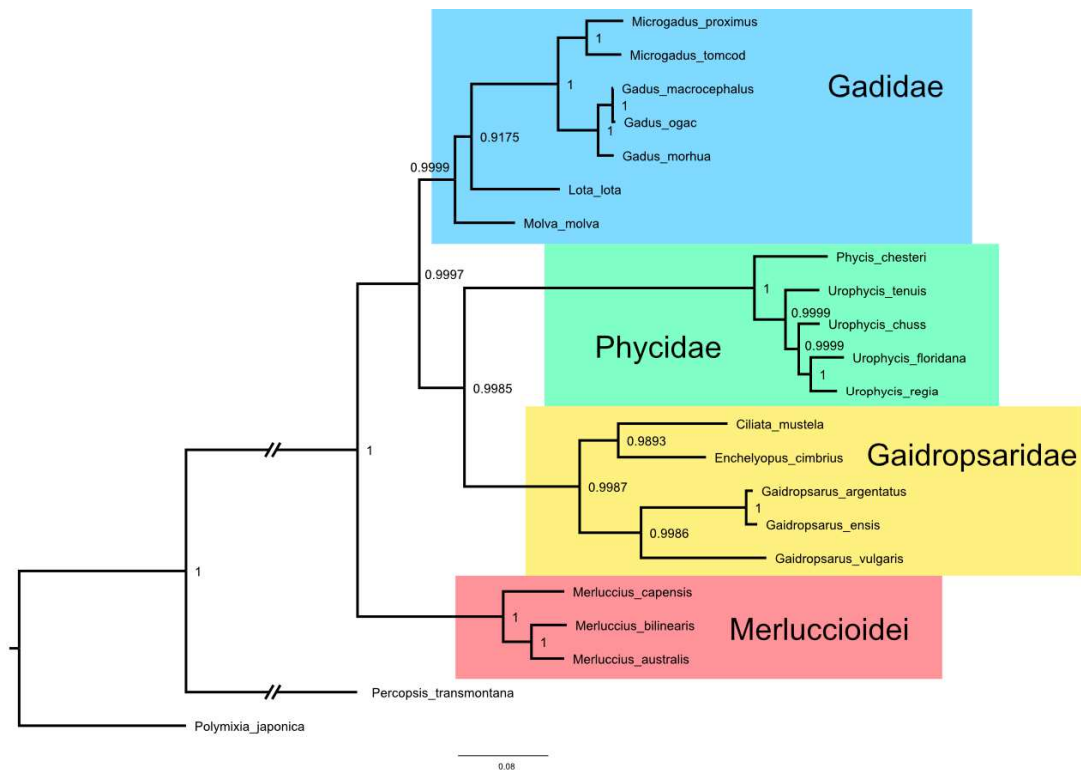
Figure 8. Combined Bayesian Inference (BI) consensus gene tree. Support values at the nodes are posterior probability values. Diagonal lines indicate truncation. Colors indicate gadiform families (or suborder in the case of Merluccioidei) as recognized by Roa-Varón et al. (2021).

Combined Gene Tree Discussion

Roa-Varón and Ortí (2009) placed *Gaidropsarus ensis, Gaidropsarus argentatus, Enchelyopus cimbrius,* and *Ciliata mustela* as sister to the gadid clade. These two sister clades were then sister to a *Urophycis + Phycis* clade, all of which were designated as the family Gadidae. However, in this study, in the combined gene tree, the clade *Urophycis + Phycis* is sister to the clade of *Gaidropsarus ensis, Gaidropsarus argentatus, Enchelyopus cimbrius,* and *Ciliata mustela*; this resembles the placement of the groups found in Endo (2002), which was a morphology-based phylogeny. Endo (2002), however, defined the groups as Gaidropsarinae and Phycinae: subfamilies within Gadidae. In both the Roa-Varón and Ortí (2009) phylogeny and this study's combined gene tree, a monophyletic lotid clade was not recovered, though *Lota lota* is consistently placed as closely related to the gadid clade.

Comparing the Roa-Varón (2021) phylogeny to the constructed combined gene tree has different results. It should be noted that it was not appropriate simply to use the phylogeny of Roa-Varón and Ortí (2009), because of its weaker taxon sampling for the study group, nor that of Roa-Varón et al. (2021), because of the lack of sufficient life-history data for their chosen taxon sample.

In the Roa-Varón (2021) phylogeny, *Molva molva* and *Lota lota* are placed in a monophyletic family Lotidae, which is sister to the Gadidae. The placement of Gaidropsaridae and Phycidae mirrors the Roa-Varón (2009) placement of the clade of *Gaidropsarus ensis, Gaidropsarus argentatus, Enchelyopus cimbrius* (not analyzed by Roa-Varón et al. 2021)*,* and *Ciliata mustela,* together with the clade of Urophycis + Phycis, but as separate families rather than being within the family Gadidae.

The placement of phycids and gaidropsarids differs among gadiform phylogenies. The two groups are either placed as sister clades, which are in turn sister to a gadid group (as in the combined gene tree, in Endo (2002), and in the COI and cytochrome b gene trees), or the gaidropsarid clade is placed as sister to the gadid clade with the phycid clade then being sister to the [gaidropsarid + gadid] clade, as in Roa-Varón and Ortí (2009), Roa-Varón (2021), and the RAG-1 gene tree. The main point of contention lies in the placement of the phycid fishes, and whether they are sister to just the gaidropsarids, or sister to a combined [gaidropsarid + gadid] clade. Based on the combined gene tree, there is support for the family Phycidae being sister to Gaidropsaridae, with the combined clade then being sister to Gadidae.

Based on the combined gene tree, there is also support for placing the lotid genera within Gadidae. Within Gadidae, there is a clear gadid clade or Gadinae subfamily, with the possibility of recognizing subfamilies Lotinae and Molvinae as well.

An interesting note is that the COI and cytochrome b gene trees placed *Lota lota* and *Molva molva* into a monophyletic lotid clade. There is support for a monophyletic lotid clade based on previous phylogenies (Endo, 2002; Roa-Varón et al., 2021); this is potentially strengthened when paired with the above COI and cytochrome b placement. Despite this, there are several important differences between the individual gene trees and the combined gene tree that highlight the importance of including multiple genes. Chief among these differences is the correct placement of the suborder Merluccioidei as the outgroup to Gadoidei. (Note that in all constructed trees, both *Polymixia japonica* and *Percopsis transmontana* were placed as the non-gadiform paracanthopterygian outgroups, and all trees were rooted to *Polymixia*.)

CHAPTER 3

LIFE HISTORIES OF GADOIDEI

Life-history characteristics are traits that affect an organism's survival, reproduction, and success in their given environment, and thus in many ways help to define it. Life-history characteristics include an organism's longevity, age and size at sexual maturity, and fecundity. These characteristics can show large amounts of variation across different species and environments (Flatt and Heyland, 2011). Life-history characteristics are often explored under the context of trade-offs and constraints, such as when an increase in investment in one trait results in a decrease in investment in another life-history characteristic (Flatt and Heyland, 2011).

Life-history research also considers life-history characteristics as tied to phenotypic plasticity, where one genotype can produce multiple phenotypes when under different environmental selective pressures (Stearns, 1992). However, the role of shared ancestry in the evolution of life-history characteristics cannot be dismissed.

Life-history characteristics have been used extensively to understand the temporal population dynamics of fish species (Ribeiro et al., 2008; Devine et al., 2012), but less so within a phylogenetic framework. Investigating life histories within the context of a phylogeny can help understand how important a role shared ancestry has played in the evolution of life-history traits within the suborder Gadoidei.

Water temperature is known to affect the growth of teleost fishes, with fishes in colder waters tending to grow more slowly and reach maturity at later ages, including Gadiformes (Wootton, 1998; Handeland et al., 2008; Lorenzo et al., 2011). Cold-water species such as *Gadus*

morhua are specialized to grow more optimally in those colder temperatures (Pörtner et al., 2008).

Gadoidei are typically found in temperate or colder regions, being found usually in deeper and thus cooler waters; they are considered stenothermal fishes that tolerate a relatively narrow range of temperatures, typically from 0°C to 15°C (Hardewig et al., 2004). All species within Gadoidei are marine, except for *Lota lota*, which is found exclusively in cold, deep lakes and rivers (e.g., the North American Great Lakes; Cohen et al., 1990). Some species of Gadoidei (*Microgadus proximus* and *Microgadus tomcod*) can tolerate brackish environments, but are primarily marine fishes (Riede, 2004). Most gadoids are demersal benthopelagic fishes (Cohen et al., 1990), spending most of their time just above the sea floor (or riverbed/lakebed in the case of *Lota lota*).

Methods

For this study, life-history data for gadoid fishes were collected (Table 2), from available literature. Cohen et al. (1990), Scott and Scott (1988), and Muus and Nielson (1999) were key references. The characteristics chosen for examination reflect those that affect a species' survivability and reproduction, including longevity (years), age of female sexual maturity (days), age of male sexual maturity (days), standard adult length (cm), and fecundity (millions of eggs produced). Fecundity is recorded using absolute fecundity, which measures eggs produced during a breeding season and is estimated using number of eggs in a subsample times gonad weight divided by weight of the subsample (Kosior et al., 2001). However, a large proportion of gadiform fishes, including some Gadoidei, remain understudied as they are unlikely to be sampled using typical collection methods (Alcorn and Stone, 2012), and as such some species-specific data are unavailable. In addition to the life-history characteristics, ecological data were

also collected from available literature. The ecological data used herein are typical depths (m) at

which they were found and general diet.

Species	Longevity (yrs.)	Female Age at Maturity (days)	Male Age at Maturity (days)	Adult Length (cm)	Fecundity (millions of eggs produced)	Depth (m)	Diet
Microgadus proximus	?	?	?	38.1 cm	?	25-120 m	Crustaceans
Microgadus tomcod	?	?	?	30.5 cm	?	0-69 m	Crustaceans
Gadus macrocephalus	25 yrs.	1514	1514	85 cm	1.0-5.7	100-400 m	Fishes
Gadus ogac	20 yrs.	1277	1277	77 cm	0.5-15.0	0-200 m	Fishes
Gadus morhua	25 yrs.	790	778	100 cm	2.5-9.0	0-600 m	Fishes
Lota lota	20 yrs.	1460	1095	40 cm	1.3-5.0	0-700 m	Fishes
Molva molva	25 yrs.	?	?	106 cm	20.0-60.0	100-400 m	Fishes
Phycis chesteri	?	?	?	42 cm	1.3	360-800 m	Crustaceans
Urophycis tenuis	23 yds	351	327	70 cm	1.0-15	100-247 m	Crustaceans
Urophycis chuss	?	?	?	66 cm	?	110-130 m	Crustaceans
Urophycis floridana	?	?	?	25 cm	?	0-400 m	Crustaceans
Urophycis regia	?	?	?	17 cm	?	110-185 m	Crustaceans
Cilita mustela	3 yrs.	365	365	17 cm	0.009-0.030	17-22 m	Crustaceans
Enchelyopus cimbrius	9 yds	1095	1095	30 cm	0.005-0.045	20-25 m	Crustaceans
Gaidropsarus argentatus	?	?	?	35 cm	?	150-2260 m	Crustaceans
Gaidropsarus ensis	?	?	?	40 cm	?	0-2000 m	Crustaceans
Gaidropsarus vulgaris	?	?	?	25 cm	?	20-120 m	Crustaceans
Merluccius capensis	16 yrs.	1790	1425	50 cm	?	50-1000 m	Fishes
Merluccius bilinearis	12 yds	602	602	37 cm	0.7	55-914 m	Fishes
Merluccius australis	30 yrs.	2859	2615	80 cm	?	28-1000 m	Fishes

Table 2. Collected Life-History Data. Question mark indicates no available data.

Life-history and ecological data were then mapped onto the topology of the combined

gene tree (Fig. 8) using the Trace Character History analysis of the Mesquite program (Maddison

and Maddison, 2021). The ancestral character states were reconstructed using parsimony

analysis, which minimizes the number of evolutionary state changes for each character, taking

into account missing data when possible.

Results

Longevity

Gadoid longevity (Fig. 9) was measured in years and represents the maximum recorded

lifespan for the species. Within Gadoidei, a lifespan of 20–25 years was the most common

recorded result. Longevity values were separated into four states based on the collected data: less

than 10 years, 10–19 years, 20–29 years, and 30+ years. Not all taxa had recorded longevity data.

Based on the parsimony analysis, the predicted ancestral state for Gadoidei is 20–29 years.

Within the family Gadidae, the overall lifespan is roughly the same, being 20 to 25 years

total (Muus and Dahlström, 1974; Cohen et al., 1990; Muus and Nielson, 1999; Munk, 2001),

which falls within the 20–29 years state. Longevity data for *Microgadus* were not available.

Based on the parsimony analysis, their longevity is predicted to be the same state (20–29 years)

as that of the rest of Gadidae. This prediction is also supported by the close phylogenetic

relationship of the genus to the other Gadidae.

Among Phycidae, the only recorded longevity data available were for *Urophycis tenuis* at

23 years (Beverton and Holt, 1959). The analysis predicts that the most parsimonious state for

the family is 20–29 years.

Within Gaidropsaridae, longevity data were only available for *Ciliata mustela* and

Enchelyopus cimbrius, at 3 and 9 years, respectively (Cohen et al., 1990). The ancestral state for

Gaidropsaridae is ambiguous, being either the short lifespan state, or the 20–29 years state.

The longevity of specific species of Merluccioidei as reported is varied, although

Merluccius capensis and *Merluccius bilinearis* are similar (16 years and 12 years, respectively;

Cohen et al., 1990) with *Merluccius australis* being more of an outlier at 30 years (Annala,

1994).
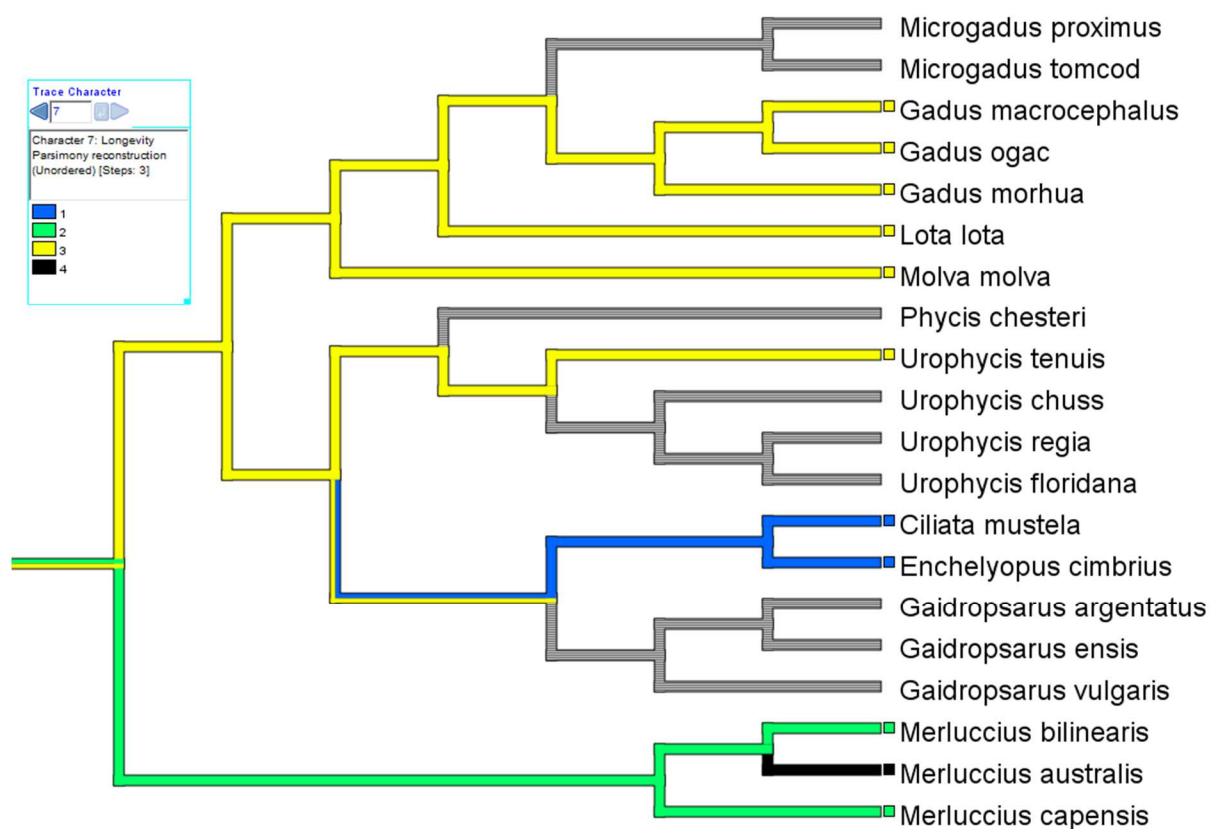
Figure 9. Combined gene tree (see Fig. 8) with data for maximum longevity (years) based on parsimony analysis using Mesquite. Colors indicate lifespan ranges with 1. blue = <10 years; 2. green = 10–19 years; 3. yellow = 20–29 years; and 4. black = 30+ years. Grey lines indicate no available data for those species. Mixed colors indicate ambiguous ancestral states.

Longevity appears to show phylogenetic signal within Gadidae when compared to Merluccioidei. Given the lack of available data for Phycidae and Gaidropsaridae and ambiguity of the reconstructed ancestral states, it is more difficult to draw a conclusion for those lineages.

Female and male age at maturity

Age at maturity for both females and males of Gadoidei (Fig. 10) was measured in days. Age at maturity is the age at which the species can reproduce. Both female and male ages followed the same pattern, with the specific ages of some species differing between female and male. However, the difference between female and male maturity data values is small. The values of age at maturity were separated into three states based on the collected data; 0-500 days, 501-1000 days, and 1000+ days to reach maturity. Based on the parsimony analysis, the predicted ancestral state for age at maturity for Gadoidei is over 1000 days.

Within Gadidae, most species (both male and female) matured at ages over 1000 days or approximately three years (Cohen et al., 1990; Fryhof and Kottelat, 2007), the exception being *Gadus morhua*, which matures at 790 days in females and 778 days in males (Ajiad et al., 1999). Maturity data were not available for *Microgadus* or *Molva molva*. Based on the analysis, the most parsimonious state for both the genus *Microgadus* and for *Molva molva* would be reaching maturity at over 1000 days for both females and males.

Within Phycidae, the only available maturity data were for *Urophycis tenuis*, with females maturing at 351 days and males at 327 (O'Brien et al., 1993). This is noticeably lower than the ages found within Gadidae, being closer to one year than three years. The most parsimonious ancestral state for Phycidae is ambiguous, being either 0 to 500 days or over 1000 days, because of older ages of maturity within its sister group.

Within Gaidropsaridae, the only available age data were for *Ciliata mustela*, which matures at 365 days for both male and females (Cohen et al., 1990), and *Enchelyopus cimbrius*, which matures at 1095 days for both males and females (Cohen et al., 1990). Based on the parsimony analysis, the ancestral state for Gaidropsaridae is reaching maturity at over 1000 days.

The age at maturity in Merluccioidei showed a high degree of variation among the three species and between sexes. *Merluccius capensis* matures at 1790 days in females and 1425 days in males (Botha, 1980). *Merluccius bilinearis* matures at 602 days in both females and males (Myers et al., 1995). *Merluccius australis* matures at 2859 days in females and 2615 days in males (Myers et al., 1995). The predicted ancestral state for Merluccioidei is reaching maturity at over 1000 days. *Merluccius australis* exhibits a far later maturity than Gadoidei, taking roughly seven years to reach maturity. The species is also an outlier within longevity, with a recorded lifespan of 30 years. The longer lifespan may account for the later maturity.
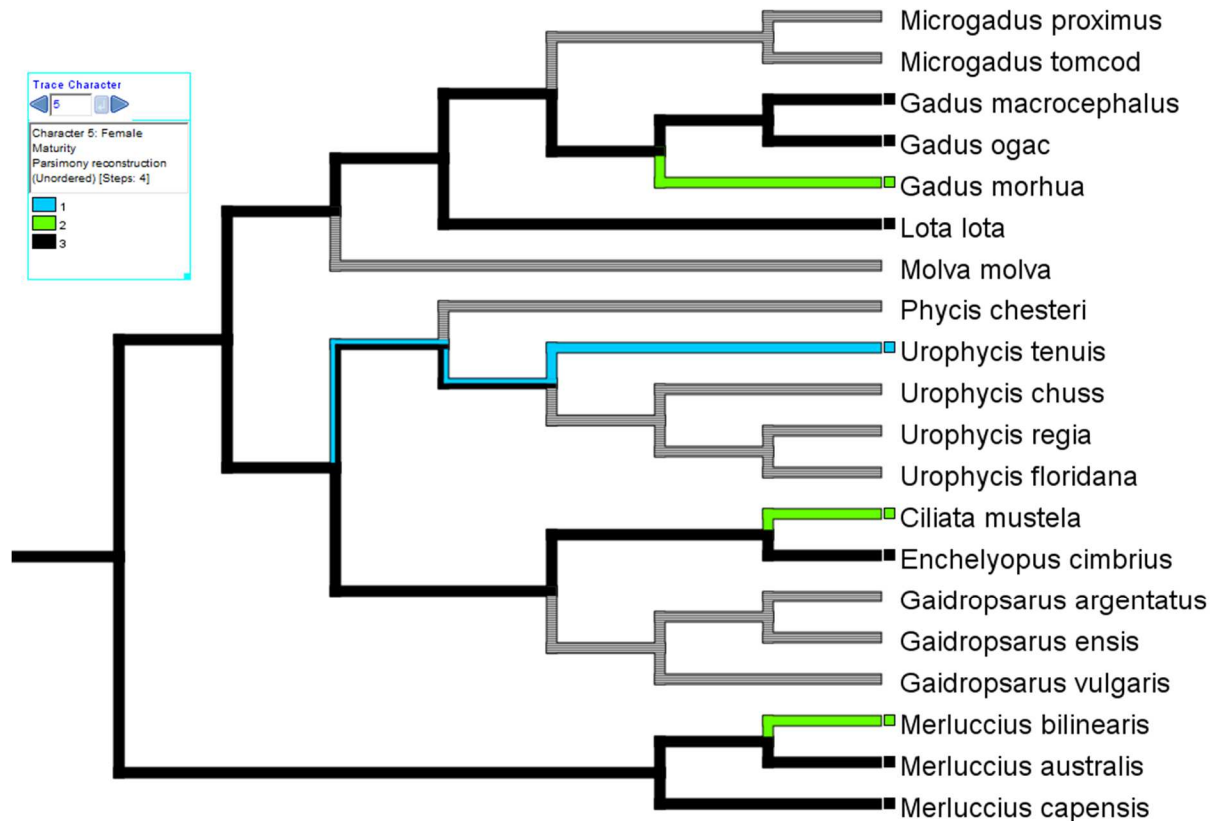
Figure 10. Combined gene tree with age at maturity (days) data based on parsimony analysis in Mesquite. Both male and female data produced the same result. Colors indicate lifespan ranges: 1. blue = 0–500 days; 2. green = 501–1000 days; and 3. black = 1000+ days. Grey lines indicate no available data for those species. Mixed colors indicate ambiguous ancestral states.

The degree of phylogenetic signal present within age at maturity is unclear, in part due to lack of data. The difference in maturity between *Gadus morhua* and the rest of the gadid species is discussed further in the study.

Adult length

Adult standard length of gadoids (Fig. 11) measures a species' modal standard length, recorded in centimeters. The length values were separated into four states based on the collected

data; 0–25 cm, 26–50 cm, 51–75 cm, and greater than 75 cm. Based on the parsimony analysis, the predicted ancestral state for Gadoidei is a standard length of 26–50 cm.

Within Gadidae, the genus *Gadus* and the species *Molva molva* are recorded as the largest. *Gadus morhua* is the largest within its genus, at 100 cm (Cohen et al., 1990). *Gadus macrocephalus* is typically 85 cm (Cohen et al., 1990), and *Gadus ogac* is the smallest of the genus, typically 77 cm in length (Cohen et al., 1990). Both species of *Microgadus* are typically much smaller than *Gadus* spp. *Microgadus proximus* is recorded at 38 cm and *M. tomcod* at 30 cm (Lamb and Edgell, 1986; Cohen et al., 1990). *Lota lota* is typically 40 cm in length (Cohen et al., 1990), and *Molva molva* typically 100 cm (Cohen et al., 1990). Based on the parsimony analysis, the ancestral state for Gadidae is 26–50 cm. Despite the large size of some gadids, the smaller predicted ancestral size is influenced by the smaller sizes reached in closely related clades.

Within Phycidae, a wide range of sizes was recorded. Both *Urophycis regia* and *Urophycis floridana* are found at lengths less than 25 cm (Cohen et al., 1990). *Urophycis tenuis* and *Urophycis chuss* are recorded at lengths of 70 cm and 66 cm respectively (Cohen et al., 1990), which is much larger than the other species within the genus. The predicted ancestral state for the *Urophycis regia + Urophycis floridana* clade is 0–25 cm, suggesting some phylogenetic signal for that state within that clade specifically. *Phycis chesteri* is typically recorded at 42 cm (Coad and Reist, 2004). The predicted ancestral state for the family Phycidae is 21–50 cm in length.

Within Gaidropsaridae, *Ciliata mustela* at 17 cm (Cohen et al., 1990) is a notable outlier. *Enchelyopus cimbrius* is typically 30 cm in length (Cohen et al., 1990). *Gaidropsarus vulgaris* is commonly 25 cm in length (Bauchot, 1987), *Gaidropsarus argentatus* is 35 cm in length (Muus

and Nielson, 1999), and *G. ensis* is 40 cm in standard length (Lein and Scott, 1966). The predicted ancestral state for the genus is unambiguously 21–50 cm, suggesting phylogenetic signal for that variable.

Within Merluccioidei, *Merluccius australis* is recorded as the largest, at 80 cm (Cohen et al., 1990). *Merluccius capensis* and *Merluccius bilinearis* are both smaller at 50 cm and 37 cm, respectively (Cohen et al., 1990).
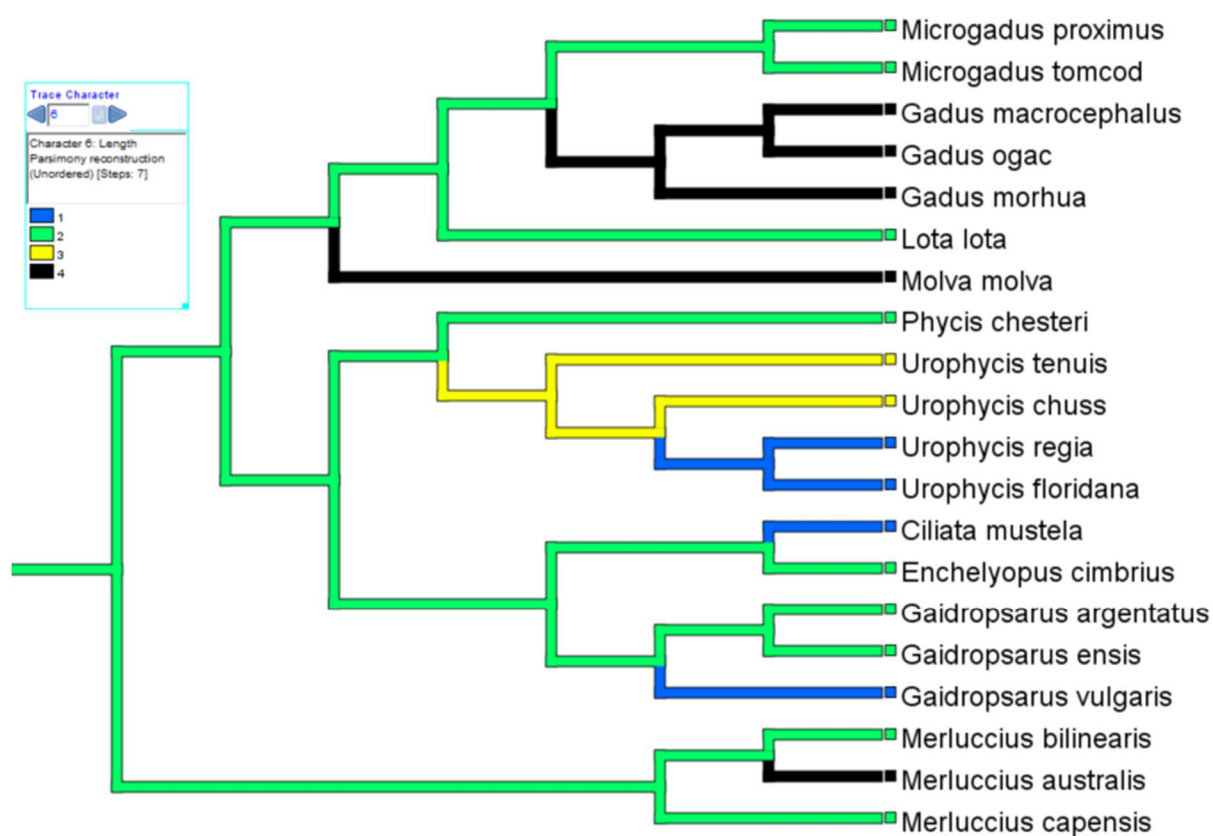
Figure 11. Combined gene tree with modal adult standard length (cm) data based on the parsimony analysis of Mesquite. Colors indicate length ranges: 1. blue = 0–25 cm; 2. green = 26–50 cm; 3. yellow = 51–75 cm; and 4. black = greater than 75 cm.

Fecundity

Gadoid fecundity (Fig. 12) is recorded as millions of eggs produced during a spawning season (absolute fecundity). Overall, the gadoids showed a large range of fecundity, both within species and across lineages. Fecundity values were separated into three states based on the collected data; less than 0.5 million eggs produced, 0.5-20 million eggs produced, and greater than 20 million eggs produced. Based on parsimony analysis, the predicted ancestral state for Gadoidei was the production of 0.5 to 20 million eggs.

Within Gadidae, data were not available for all species, including *Microgadus proximus* and *Microgadus tomcod*. For species with available data, all except *Molva molva* fall within the range of 0.5 to 20 million eggs produced (Cohen et al., 1990). *Molva molva* was the only recorded species to produce over 20 million eggs in a season, with records ranging from 20 to 60 million eggs produced (Cohen et al., 1990). *Lota lota* exhibited a smaller range that was also at the lower end of that for Gadidae, producing between 1.3 and 5.0 million eggs at a time (Cohen et al., 1990). Within the genus *Gadus*, *G. macrocephalus, G. ogac*, and *G. morhua* produce 1.5–7 million eggs, 0.5–15 million eggs, and 2.5–9 million eggs, respectively (Cohen et al., 1990). Species in *Gadus* exhibit both larger numbers of eggs, as well as a larger range in number of eggs produced.

Within Phycidae, *Phycis chesteri* (1.3 million eggs) and *Urophycis tenuis* (1.0 to 15 million eggs) were the only species with data available (Cohen et al., 1990). Based on the parsimony analysis, the predicted ancestral state for the family is within the 0.5–20 million eggs produced range.

Within Gaidropsaridae, the only species with available data were *Ciliata mustela*, with 9,000 to 30,000 eggs produced, and *Enchelyopus cimbrius* with 5,000 to 45,000 eggs produced

(Cohen et al., 1990). Both species produce significantly fewer eggs than any other gadoid fishes. The ancestral state of the family is ambiguous. However, based on the parsimony analysis, the substantially lower number of eggs produced by *Ciliata mustela* and *Enchelyopus cimbrius*, and the two species' close phylogenetic relationship, there could well be phylogenetic signal for this character state within the clade.

Within Merluccioidei, *Merluccius bilinearis* is recorded as producing 0.7 million eggs at a time (Mertz and Myers, 1996).

Within Gadidae there appears to be phylogenetic signal for fecundity, as most species are found to produce a similar range of eggs, *Molva molva* being the one known exception within the family.
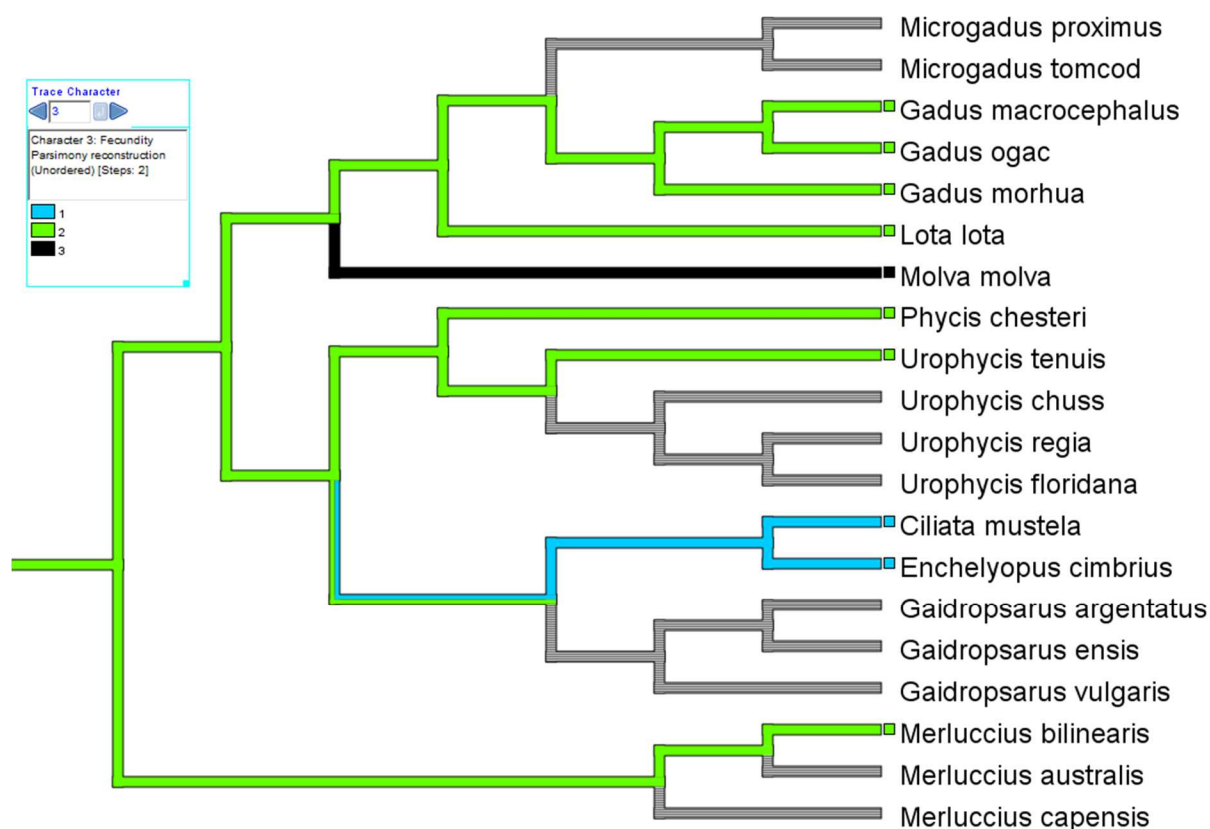
Figure 12. Combined gene tree with data for absolute fecundity (millions of eggs) based on parsimony analysis in Mesquite. Colors indicate fecundity ranges: 1. blue = <0.5 million eggs; 2. green = 0.5–20 million eggs; and 3. black = >20 million eggs produced. Grey lines indicate no available data for those species. Mixed colors indicate ambiguous ancestral states.

Water depth

Water depth (Fig. 13) at which each species was typically found was recorded in meters and assigned to one specific water depth category: epipelagic (0–200 m), mesopelagic (200–1000 m), or bathypelagic (1000–4000 m). The deepest range at which a species was typically found served as the threshold for water depth, e.g., a fish that ranged from 0 to 500 meters was considered mesopelagic, despite also being found within epipelagic waters. Based on parsimony analysis, the predicted ancestral depth range for Gadoidei is mesopelagic (200–1000 m).

Species within Gadidae are typically found within the mesopelagic range. However, *Gadus ogac* has been found only in the epipelagic range (Fedorov, 2003). *Gadus morhua* and *Gadus macrocephalus* have depth ranges that extend into mesopelagic depths, but they can be found within the epipelagic ranges of 0–600 m and 100–400 m respectively (Cohen et al., 1990; Fedorov, 2003). Both *Microgadus proximus* and *Microgadus tomcod* are found only within the epipelagic ranges of 25–120 m and 0–69 m respectively (Cohen et al., 1990). As with most species of *Gadus*, *Lota lota* occurs at 0–700 m (Scott and Crossman, 1973) and *Molva molva* at 100–400 m (Muus and Nielson, 1999); thus, both extend through the epipelagic depths and into the mesopelagic range.

Species within Phycidae are mostly found only within the epipelagic range. However, *Phycis chesteri* (360–800 m) is found only within the mesopelagic range (Cohen et al., 1990), and *Urophycis floridana* (0–400 m) extends its range from epipelagic into mesopelagic depths (Cohen et al., 1990). *Phycis chesteri* might be unique within the family for its restricted depth range, as it has yet to be recorded in the epipelagic range. Similarly, *Urophycis tenuis, Urophycis regia,* and *Urophycis chuss*, the exclusively epipelagic species, have yet to be recorded at depths below 200 meters.

Species within Gaidropsaridae are found largely within the epipelagic range. *Ciliata mustela* and *Enchelyopus cimbrius* have the tightest ranges within Gadoidei, being found at 17–22 m and 20–50 m respectively (Cohen et al., 1990). *Gaidropsarus argentatus* and *Gaidropsarus ensis* have the largest range within Gadoidei, at 150–2260 m and 0–2000 m, respectively; these are the only species within the suborder to extend into bathypelagic depths (Coad and Reist, 2004). *Gaidropsarus vulgaris* is the only species within that genus found only within the epipelagic range (Muus and Nielson, 1999). The bathypelagic range found in the *Gaidropsarus*

argentatus + *Gaidropsarus ensis* clade may be indicative of phylogenetic signal; however, it is

possible that *Gaidropsarus vulgaris* has merely yet to be recorded from depths below 200 meters

and is in fact found within the bathypelagic range. The predicted ancestral state for Phycidae +

Gaidropsaridae is ambiguous.

The species within Merluccioidei show a similarly large range in the depths at which they

are found, extending from epipelagic to the boundary of mesopelagic depths (Bianchi et al.,

1999; Stevenson, 2004; Lloris et al., 2005).

Figure 13. Combined gene tree with data for depth (meters) based on the parsimony analysis of
Mesquite. Colors indicate depth ranges: 1. blue = epipelagic (0–200 m); 2. green = mesopelagic
(200–1000 m); 3. black = bathypelagic (1000–4000 m). Mixed colors indicate ambiguous
ancestral states.

Based on the parsimony analysis, there is support for phylogenetic signal within the depth data. Within Gadidae, *Microgadus* appears to be a clear divergence away from the predicted ancestral state for Gadidae. *Gadus ogac* appears to be an outlier within Gadidae. Though the predicted ancestral state is ambiguous for Phycidae + Gaidropsaridae, the predominance of epipelagic species might show support for phylogenetic signal.

Diet

The adult diets of the gadoid fishes were generalized to two main groups; those that feed primarily on other fishes (piscivores) and those that feed primarily on crustaceans (crustacivores). All taxa fell within one of the two categories (Fig. 14). Based on the parsimony analysis, the predicted ancestral state for Gadoidei is piscivory.

 Within Gadidae, there are two distinct groups: *Microgadus*, which feeds primarily on crustaceans (Scott and Scott, 1988), and *Gadus, Lota lota,* and *Molva molva*, all of which feed on fishes as adults (Cohen et al., 1990; Etnier and Starnes, 1993; Muus and Nielson, 1999). Both Phycidae and Gaidropsaridae feed primarily on crustaceans (Scott and Scott, 1988; Cohen et al., 1990; Frimodt, 1995). The species within Merluccioidei feed primarily on fishes (Cohen et al., 1990).

There appears to be a strong phylogenetic signal for diet. The ancestor of Gaidropsaridae + Phycidae appears to have diverged from the predicted ancestral state of piscivory and become more specialized to feed on crustaceans. *Microgadus* also appears to have evolved to eat crustaceans, differing from the predicted ancestral state for Gadidae of piscivory, independently from the similar shift in the Gaidropsaridae + Phycidae clade.
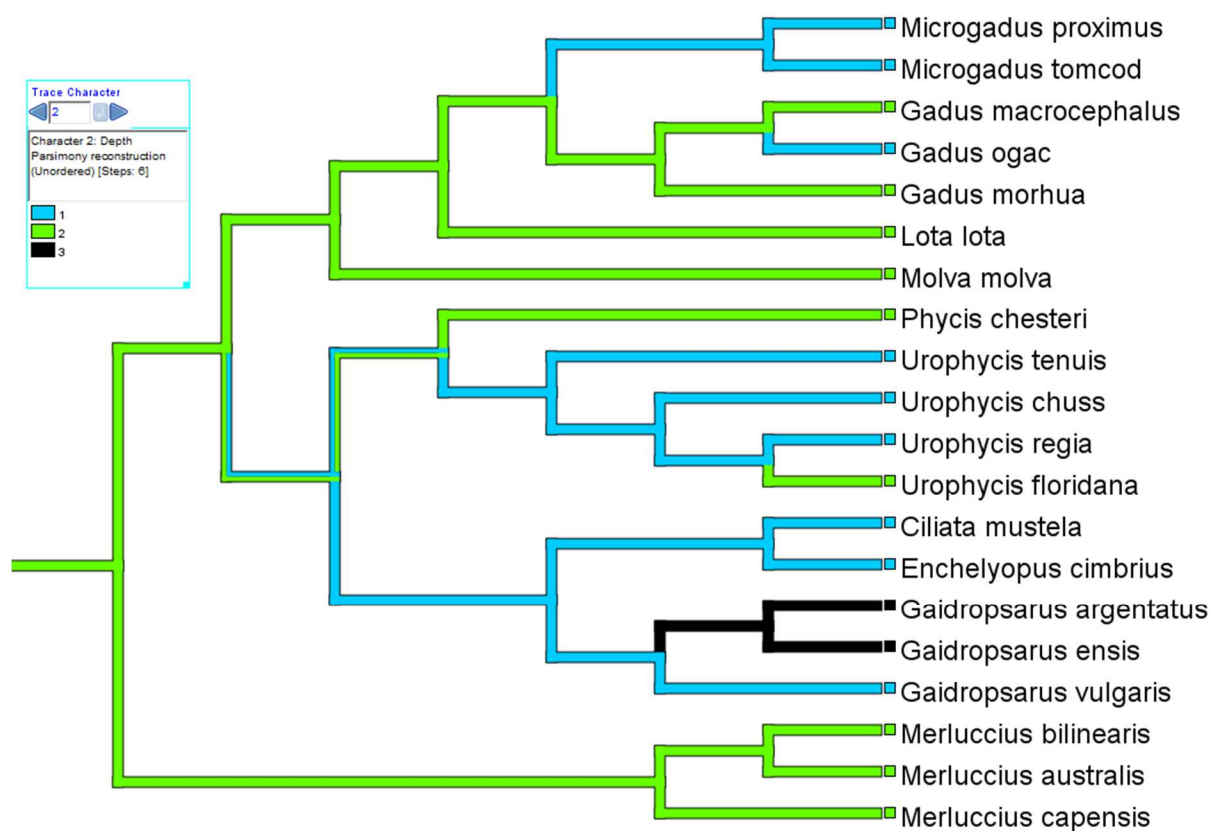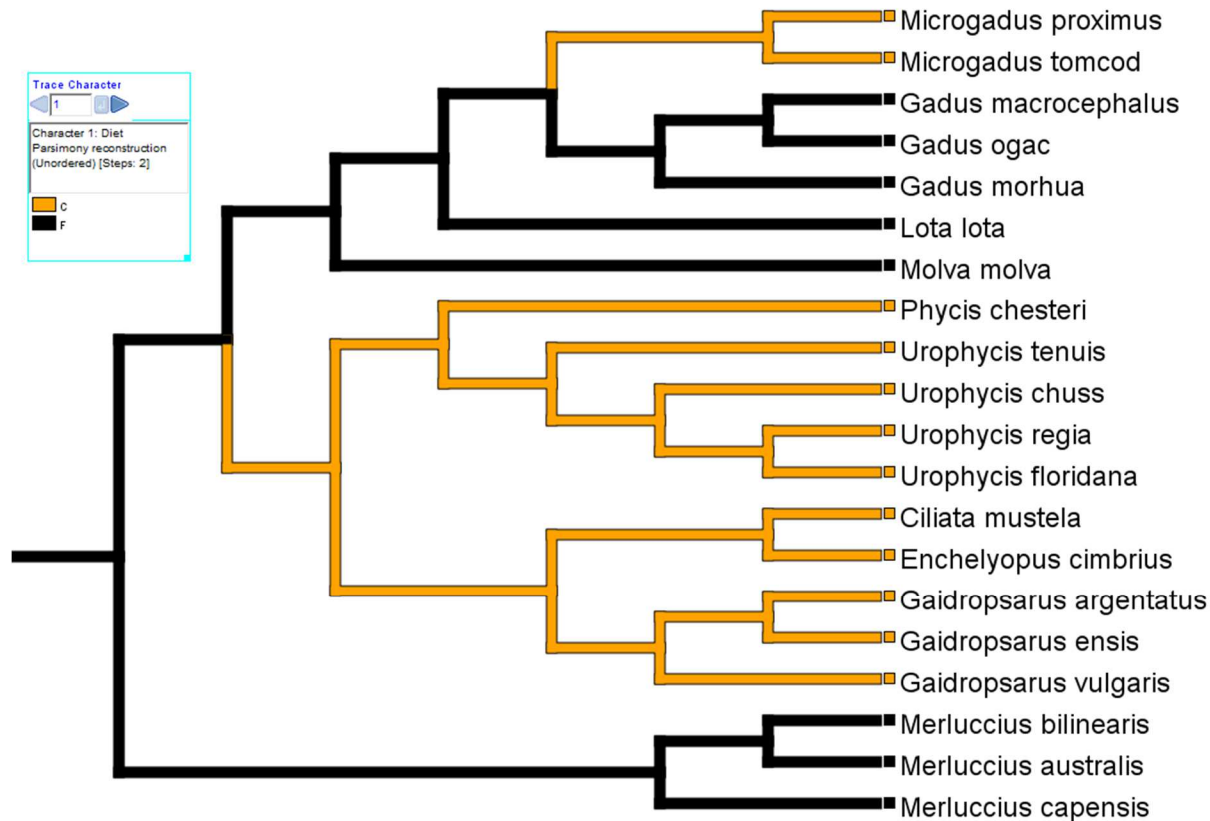
Figure 14. Combined gene tree with data for diet based on the parsimony analysis of Mesquite. Colors indicate diet type: C. orange = mainly crustacivorous; F. black = mainly piscivorous.

Discussion

There are few well-preserved fossil gadiforms (an exception is *Rhinocephalus* from the Lower Eocene of the London Clay). The fossil record of Gadoidei is, instead, primarily known from otoliths (Rosen and Patterson, 1969; Nolf and Steurbaut, 1989; Kriwet and Hecht, 2008). The earliest fossil gadiform otoliths come from the Paleocene (66–56 mya; Nolf and Steurbaut, 1989). Based on the available fossil records for Gadoidei, both Phycidae and Gaidropsaridae can be traced back to the Oligocene (Nolf and Steurbaut, 1989), whereas the genus *Gadus* is found

only as far back as the Pliocene (5.4–2.4 mya), making it a very recent lineage within Gadoidei (Nolf and Steurbaut, 1989).

Over the course of their evolutionary history, Gadiformes have increased in size, as seen in Eocene fossils of *Merluccius*, which was about 15 cm, estimated from otolith size, compared to more recent Pliocene and modern *Merluccius*, which exceed 50 cm (Nolf and Steurbaut, 1989).

Based on such fossil records, gadoid fishes likely had their geographic origins in the North Atlantic and North Sea Basin continental shelf areas, which likely had a depth of approximately 250 meters at the time, before the group experienced a rapid diversification (Kriwet and Hecht, 2008). This estimated depth is within the predicted ancestral depth of Gadoidei, being mesopelagic (200–1000 m) (Fig. 13). This agreement of the fossil data with the estimated ancestral state based on phylogeny and extant life-history data further supports the results found within this study.

Analysis of fossil otoliths is uncommon and can be difficult due to changes caused to the structure during fossilization (e.g., Woydack and Morales-Nin, 2001), but it has produced valuable information on life-history characteristics including, age, growth, and maturity (Woydack and Morales-Nin, 2001; Schwarzhans et al., 2016). Further research and analysis of fossil otoliths could provide more specific information on the evolution of life-history characteristics within Gadoidei.

Some life-history characteristics show stronger phylogenetic signal than others within Gadoidei. Longevity, size, and fecundity, as well as depth and diet, appear to show phylogenetic signal based on parsimony analysis. Age at maturity is more ambiguous due to lack of data for many species, though within Gadidae, there is less ambiguity. The main outlier is *Gadus*

morhua, which has seen a recorded reduction in age at maturity, linked to both climate change (Perry et al., 2005) and over exploitation of fishing stocks (Jørgensen, 1988). These factors likely account for the lower age at maturity recorded for *Gadus morhua*.

A trait with some of the strongest evidence for phylogenetic signal in Gadoidei is diet, with clear delineation of diet type among lineages (Fig. 14), and few changes of diet type during the evolution of the group. Parsimony analysis suggests only two changes of predominant diet during the evolution of Gadoidei, both from predominantly fish-eating to predominantly crustacean-eating, once in the ancestor of *Microgadus* and the other in the ancestry of Phycidae + Gaidropsaridae. The shift in diet might be correlated with a reduction in body size, as the crustacivores do tend to exhibit generally smaller body sizes than the piscivores; however, there are some exceptions, such as *Urophycis chuss* and *Urophycis tenuis*, which are recorded at sizes comparable to most gadid piscivores, despite being primarily crustacivores.

Lota lota is notable among Gadiformes and Gadoidei for being the sole freshwater species. Despite living in a different habitat (freshwater vs marine), it still exhibits similar life-history character states to those of the other Gadidae, namely similar longevity, size, and fecundity. *Lota lota* also shares those characteristics with the predicted ancestral states of Gadoidei itself. *Lota lota* is also found in colder waters typical of Gadoidei, ranging from 4°C to 18°C (Baensch and Riehl, 1999), although for *Lota* they are fresh waters.

Given that Gadoidei are hypothesized to have evolved in colder waters, the initial ancestral set of life-history characters likely evolved in response to that environment and have since been passed down to the extant Gadoidei. Divergences away from the predicted ancestral states, such as the epipelagic-only *Urophycis* species, could be a result of incomplete records. However, the difference present in the fecundity of *Ciliata mustela* + *Enchelyopus cimbrius*,

compared to the predicted ancestral state for Gadoidei, appears to be more substantial,

representing a clearer phylogenetic divergence of that clade. The lack of data for *Gaidropsarus*

means that we do not know whether the switch happened in the ancestor of *Ciliata +*

Enchelyopus or whether it occurred sooner, in the ancestor of all Gaidropsaridae.

CHAPTER 4

MULTIVARIATE MORPHOMETRIC ANALYSIS

Gadoid fishes vary greatly in size and body shape (Nelson et al., 2016). There are three

major body forms found within Gadoidei: the phycid and gaidropsarid body form (Fig. 15A)

characterized by a tapered body profile and long posterior dorsal fin and anal fin, the lotid body

form (Fig. 15B) characterized by a long, comparatively shallower body profile with a uniform

depth, a relatively small and pointed head, and a long posterior dorsal fin and anal fin, and the

gadid body form (Fig. 15C) characterized by a relatively deep body, pointed head, rounded

caudal fin, and an extra dorsal and anal fin. The gadid body form is the only type in the

gadiforms to have three separate dorsal fins and two separate anal fins. A fourth body form (Fig.

15D) is found within the outgroup Merluccioidei; it has a more pointed head, forked caudal fin,

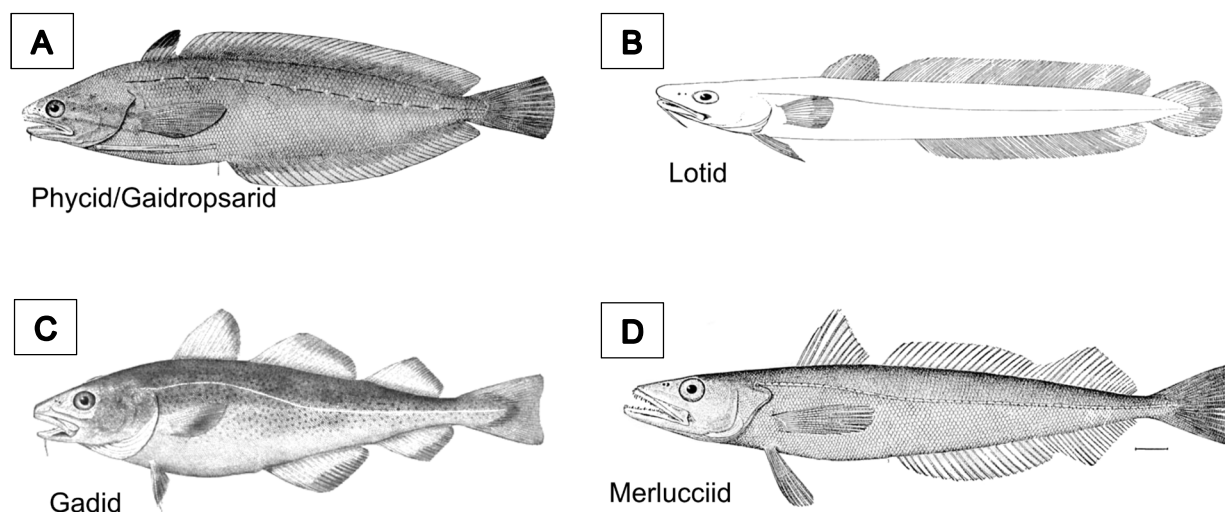and continuous second dorsal and anal fins, the posterior of each having a unique "peak".

Fig 15. Typical body forms of Gadoidei based on the gadoid morphospace analysis (see below).
Illustrations are from Wikimedia Commons.

This chapter will examine gadoid body shape using 2-D multivariate geometric morphometrics. By way of introduction, a morphospace is a graphical representation of the form, shape, and structure of an organism based on landmarked morphological characteristics. The axes of a morphospace, derived from a principal components analysis, correspond to specific morphological features and shape changes away from an average shape. A data point, representing a taxon, sits within the morphospace, and based on its position, different conclusions about that taxon's shape can be made. The principal components analysis helps to identify patterns within complex and interconnected data by transforming high-dimensional data into low-dimensional data, while retaining most of the information contained in the dataset.

The morphospace framework can also be combined with ecological methods. One such study used a landmark shape analysis and morphospace to compare the diversity of two fish assemblages: one of an artificial reef, the other a natural formation (Recasens et al., 2006). In both assemblages, the same pattern of clustering of taxa in the morphospace was present, indicating that both communities had similar species compositions in similar relative abundances occupying the same three niches: benthic fishes, epibenthic species, and nektonic fishes. A geometric morphometric analysis utilizing a morphospace captures shape information that mere linear body measurements cannot. The shape information can then be used to interpret morphological variability and its links to different characteristics. For example, in fishes, body shape is linked to characteristics such as locomotion efficiency and habitat use (Recasens et al., 2006).

There is a strong link between life-history traits (e.g., age and growth), ecological occupancy, and morphospace clustering (Farré et al., 2016). The morphospace shows what morphological characteristics distinguish individual groups. Based on the morphological

characteristics, a better understanding of the ecological niches of that cluster and its individual taxa can be reached. For example, particular head or mouth shapes can suggest functional feeding groups. In addition to morphological traits, other life-history characteristics can be mapped onto the morphospace. Mapping a characteristic such as water depth (which may act as an evolutionary driver; Gaither et al., 2016) onto the morphospace adds more context to the potential evolutionary drivers of the order. Then, mapping the phylogeny onto the morphospace to form a phylomorphospace clarifies the evolutionary history of the various characteristics, and reveals morphological convergences and divergences among the various groups of fishes. Another use of a phylomorphospace is to reconstruct a hypothetical ancestral gadiform body shape.

Methods

Twenty images were included in this study (Table 4), consisting of each species of gadoid and three species of merlucciids. Images were assembled from museum image databases and new photographs of preserved specimens; these were supplemented with high quality images from scientific databases, including World Register of Marine Species (WoRMS), International Union for Conservation of Nature (IUCN; Nielsen et al., 2014), and National Oceanic and Atmospheric Administration (NOAA). *Polymixia japonica* and *Percopsis transmontana* were excluded from the morphometric analysis because they fall too far outside the scope of the body forms of the ingroup. All images were of adult fishes. Based partly on previously established landmarks (Farré et al., 2016; Grande et al., 2018), thirty-five landmarks (Table 3) were chosen to capture the body shape.

LM	Placement	LM	Placement
1	Anterior tip of premaxilla	19	Distal tip from the posterior insertion of the anal fin when the fin is in position of maximum extension or distal tip of anal fin 2 when the fin is in position of maximum extension when two anal fins are present
2	Posterior tip of maxilla	20	Posterior insertion of the anal fin
3	Anterior tip dentary	21	Ventral insertion of the caudal fin
4	Anterior margin of the maximum eye width	22	Distal tip of the ventral lobe of the caudal fin when the fin is in position of maximum extension
5	Posterior margin of the maximum eye width	23	Posterior margin of the caudal fin between dorsal and ventral lobes
6	Dorsal margin in the end of the head	24	Distal tip of the dorsal lobe of the caudal fin when the fin is in position of maximum extension
7	Posterior margin in the end of the head	25	Dorsal insertion of the caudal fin
8	Ventral margin in the end of the head	26	Posterior insertion of most posterior dorsal fin
9	Central point in the baseline of the pectoral fin	27	Distal tip from the posterior insertion of the most posterior dorsal fin when the fin is in position of maximum extension or distal tip of posterior dorsal fin 2 when the fin is in position of maximum extension when two posterior dorsal fins are present
10	Posterior tip of the pectoral fin when the fin is in position of maximum extension	28	Center of the dorsal margin of the posterior dorsal fin or anterior insertion of posterior dorsal fin 2 when two posterior dorsal fins are present
11	Ventral margin of the pectoral fin when the fin in position of maximum extension	29	Center of the dorsal margin of the posterior dorsal fin or posterior insertion of posterior dorsal fin 1 when two posterior dorsal fins are present
12	Anterior insertion of the pelvic fin	30	Distal tip from the most anterior insertion of the posterior dorsal fin when the fin is in position of maximum extension or distal tip of posterior dorsal fin 1 when the fin is in position of maximum extension when two posterior dorsal fins are present
13	Distal tip of the pelvic fin when the fin is in position of maximum extension	31	Anterior insertion of the posterior dorsal fin
14	Posterior insertion of the pelvic fin	32	Posterior insertion of the anterior dorsal fin
15	Anterior insertion of the anal fin	33	Distal tip of the anterior dorsal fin when the fin is in position of maximum extension
16	Distal tip from the anterior insertion of the anal fin when the fin is in position of maximum extension or distal tip of anal fin 1 when the fin is in position of maximum extension when two anal fins are present	34	Anterior insertion of the anterior dorsal fin
17	Center of the ventral margin of the anal fin or posterior insertion of anterior anal fin when two anal fins are present	35	Dorsal margin of the head above the center of the eye
18	Center of the ventral margin of the anal fin or anterior insertion of posterior anal fin when two anal fins are present		

Table 3. List of landmarks (LM) and body placement collected for geometric morphometric analysis based on previously established landmarks (Farré et al., 2016; Grande et al., 2018).

Images were landmarked with ImageJ version 2.0.0 (Rasband, 2016) using the Point Picker plugin (Thévenaz, 2016). Morphometric analysis was performed in MorphoJ version 1.06d (Klingenberg, 2011). A Procrustes fit aligned by principal axes was applied to the landmarks to remove the effects of size, translation, and rotation from the analysis, and a covariance matrix was generated from the Procrustes coordinates. The covariance matrix was then subjected to the principal components analysis (PCA) to create a morphospace.

The combined-gene phylogeny was then mapped onto the PCA morphospace using MorphoJ, to create a phylomorphospace.

Taxa	Reference	Taxa	Reference
Gadus morhua	USNM 272799	*Urophycis chuss*	USNM 395748
Gadus ogac	WoRMS	*Phycis chesteri*	USNM 21840
Gadus macrocephalus	USNM 404987	*Gaidropsarus ensis*	NHMD P374966
Microgadus proximus	KU 6825	*Gaidropsarus argentatus*	NHMD P374988
Microgadus tomcod	USNM 73480	*Gaidropsarus vulgaris*	IUCN
Lota lota	UAMZ F4981.2	*Enchelyopus cimbrius*	USNM 21721
Molva molva	WoRMS	*Ciliata mustela*	WoRMS
Urophycis tenuis	USNM 21029	*Merluccius australis*	MNHN-IC-1884-0838
Urophycis regia	USNM 20923	*Merluccius bilinearis*	USNM 21016
Urophycis floridana	NOAA	*Merluccius capensis*	MNHN-IC-2006-1099

Table 4. Table of museum specimens and databases used to collect specimen images for morphometric analysis.

Results

Principal components analysis of Procrustes coordinates from 35 landmarks (e.g., Fig. 16) of 17 species of Gadoidei and three species of Merluccioidei produced 20 principal components, of which the first four principal components explain a cumulative 79.22% of the total variance in shape changes (PC1 50.73%, PC2 15.41%, PC3 7.61%, and PC4 5.47%).

PC1

PC1 (Fig. 16) corresponds to a difference in body shape from the average form, with slightly deeper bodies, longer heads, larger anterior dorsal fin, and a shift toward the more pointed double posterior dorsal fin arrangement seen in *Gadus* and *Microgadus*, and a shift toward a more pointed double anal fin arrangement seen in *Gadus* and *Microgadus*. Low values of PC1 correspond to species with a single, shorter (in height), and long posterior dorsal fin and anal fin, along with more slender bodies, while high scores of PC1 correspond to species with posterior dorsal and anal fin arrangements closer to those of *Gadus*, as well as deeper bodies.

The change in dorsal fin shape is the most drastic body change away from the average form seen in PC1. *Gadus*, *Microgadus*, and Merluccioidei all show a similar shift toward a double posterior dorsal fin and double anal fin arrangement, except that *Gadus* and *Microgadus* exhibit two fully separate posterior dorsal fins, along with two separate anal fins. The species of *Merluccius* have a single, continuous posterior dorsal fin and a single, continuous anal fin, both of which exhibit "peaks" due to elongated fin rays closer to the caudal region, as seen in the typical merlucciid body form in Figure 14D. The shape change of the posterior dorsal fin and anal fin of *Merluccius* is more explained by PC2 than PC1.

Figure 16. PC1 wireframe diagram showing the average body form in red, and the change in the direction of the principal component in blue.

PC2

PC2 (Fig. 17) corresponds to a change in the posterior dorsal fin and the anal fin toward a more posteriorly peaked, posterior dorsal fin and similarly shaped anal fin as seen in the species of *Merluccius*, and a lengthening and forking of the caudal fin. Low values of PC2 correspond to species with posterior dorsal fins and anal fins of uniform height, and more rounded caudal fins, as in *Lota lota*, while high values correspond to species with more forked caudal fins and a posteriorly peaked posterior dorsal fin and anal fin, like those found in *Merluccius australis*.

Figure 17. PC2 wireframe diagram showing the average body form in red, and the change in the direction of the principal component in blue.

PC3

PC3 (Fig. 18) corresponds to changes in body depth, length of pectoral fin, size of anterior dorsal fin, and length of pelvic fin. Low values of PC3 correspond to species with deeper bodies, longer pectoral fins, smaller anterior dorsal fins, and longer, more narrow pelvic fins, as seen in *Urophycis floridana*. High values of PC3 correspond to species with narrower bodies, wider pectoral fins, larger anterior dorsal fins, and shorter pelvic fins, as in *Ciliata mustela*.
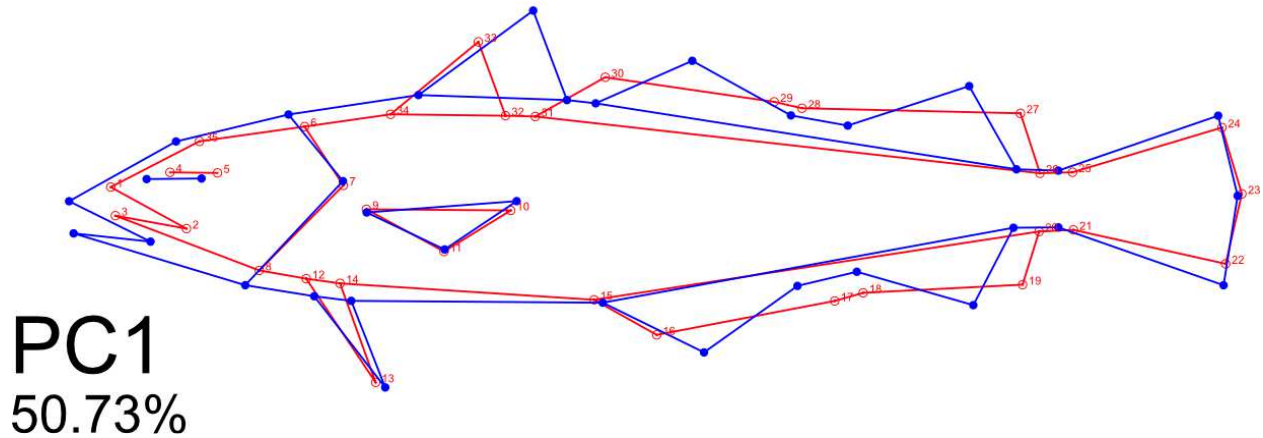

Figure 18. PC3 wireframe diagram showing the average body form in red, and the change in the direction of the principal component in blue.

PC4

PC4 (Fig. 19) corresponds to a change in the depth of the body, a shortening of the head, and a lengthening of the pelvic fin. Low values of PC4 correspond to species with longer, narrower heads and bodies, and shorter pelvic fins, as in *Lota lota*. High values of PC4 correspond to species with shorter heads, deeper bodies, and longer pelvic fins, as found in *Urophycis chuss*.
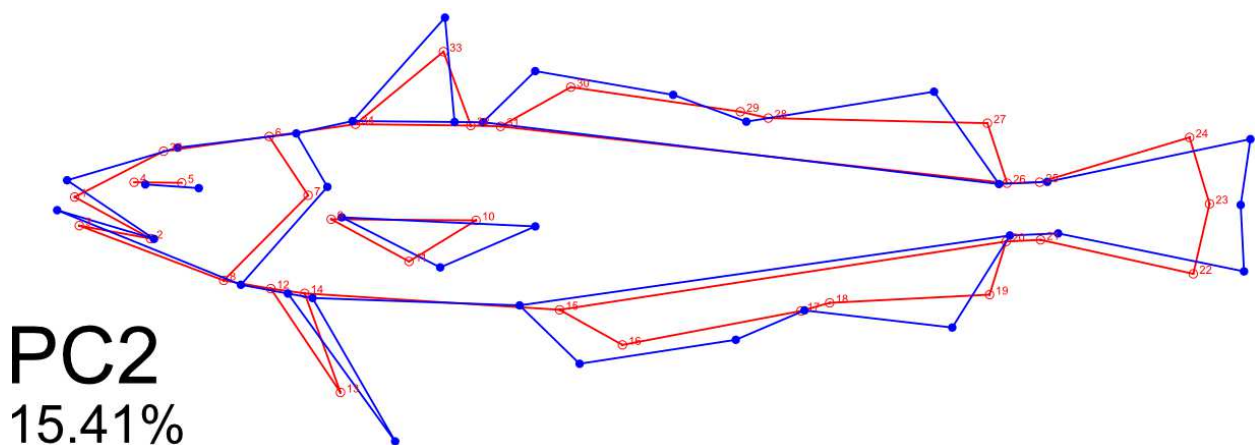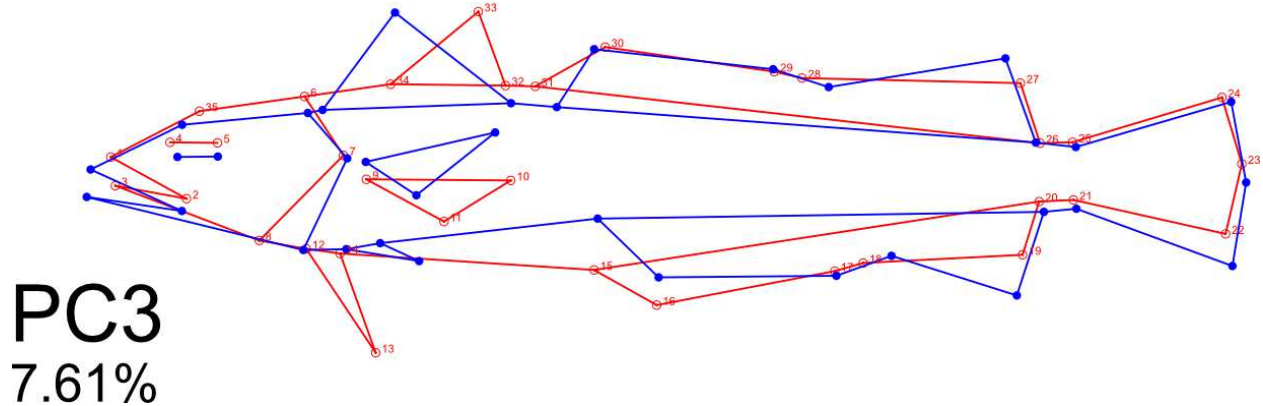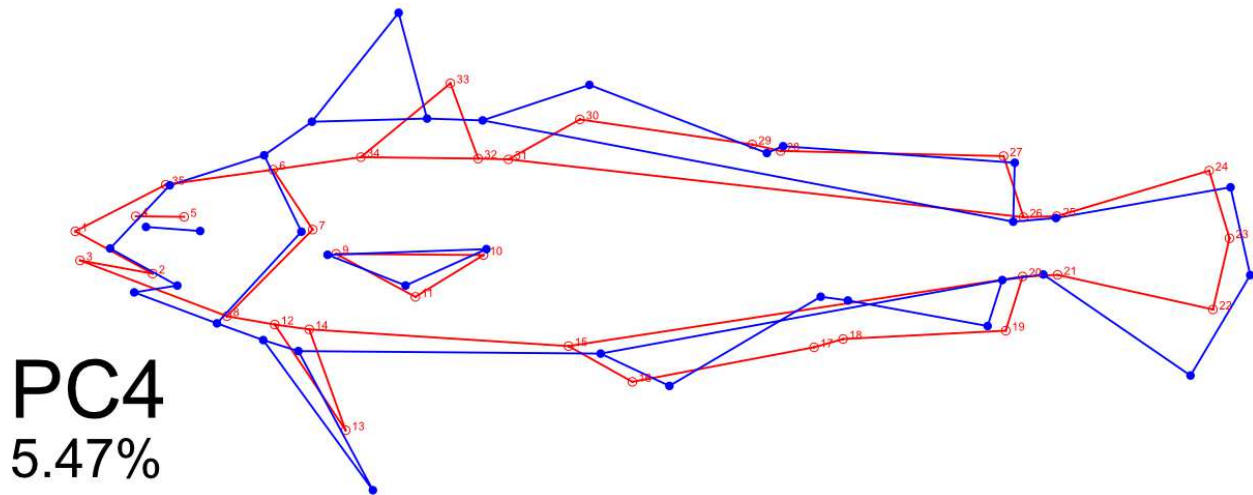
Figure 19. PC4 wireframe diagram showing the average body form in red, and the change in the direction of the principal component in blue.

Scatterplots of PC1 vs PC2 and PC3 vs PC4

The scatterplot of PC1 vs PC2 (Fig. 20) shows four clusters: a primary cluster for Gadidae (made up of *Gadus* and *Microgadus*), a secondary cluster for Gadidae (made up of *Lota lota* and *Molva molva*, a Phycidae + Gaidropsaridae cluster, and a Merluccioidei cluster. In the scatterplot of PC1 vs PC2, the shape changes of the posterior dorsal fins and the anal fin account for the most drastic variation, followed by body shape.

The two gadid clusters are largely distinguished by dorsal and anal fin shape and arrangement. The cluster made up of *Gadus* and *Microgadus* contains the only two genera within Gadoidei to have three fully separate dorsal fins, and two fully separate anal fins, as well as deeper bodies. The secondary gadid cluster of *Lota lota* and *Molva molva* is characterized by the more typical gadoid dorsal and anal fin shapes and arrangement, and a more narrow body that remains relatively the same depth for most of the length of the fish.

There is overlap of the two families within the Phycidae + Gaidropsaridae cluster. Both families have similar dorsal and anal fin shapes and arrangements, possessing a single, long, posterior dorsal fin, and a single, long anal fin. The two families also have similar body shapes in terms of body depth. The clustering of the two families close together indicates a strong similarity in body shape between the two families.

The cluster for Merluccioidei is mostly characterized by its forked caudal fin and by its unique posterior dorsal fin and anal fin shapes, both of which exhibit a peak toward the posterior end of the fin, approaching the caudal region.
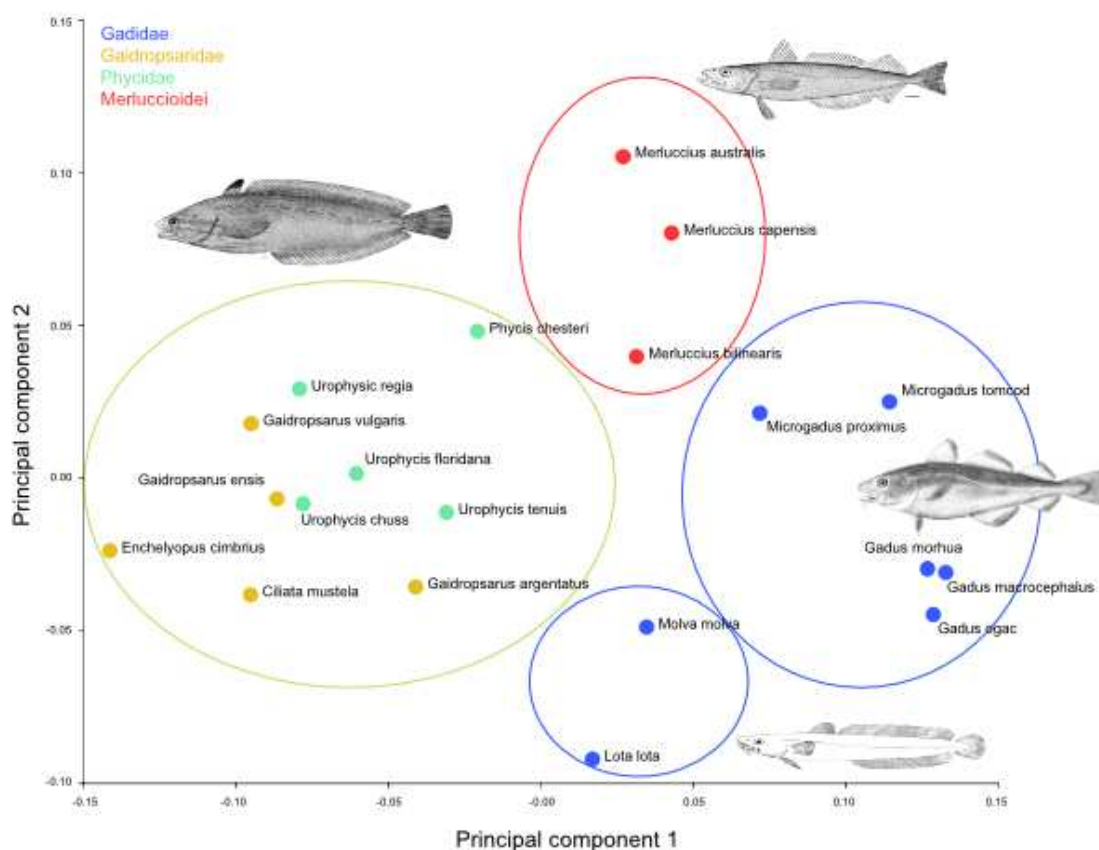
Figure 20. Scatterplot of PC1 vs PC2, which together explain a total 66.14% of variation in shape changes. Colors indicate family (or suborder for Merluccioidei). Circles indicate clusters. Illustrations denote generalized body form present in the associated cluster. Images are from Wikimedia Commons.

The scatterplot of PC3 vs PC4 (Fig. 21A) does not show clear clustering, a difference from the scatterplot for PC1 vs PC2. The shape changes of the pelvic and pectoral fins and shape change of the head present in PC3 and PC4 account for far less variation than PC1 or PC2. *Lota lota* appears to be an outlier in the scatterplot for PC3 vs PC4. This is likely due to the shape of its caudal fin, which has a larger upper half than lower half. Even when *Lota lota* is removed from the scatterplot, PC3 vs PC4 still does not show clear clustering (Fig. 21B), indicating that PC3 vs PC4 is not producing variations in shape that can be attributed to the different families (and merluccioid suborder).
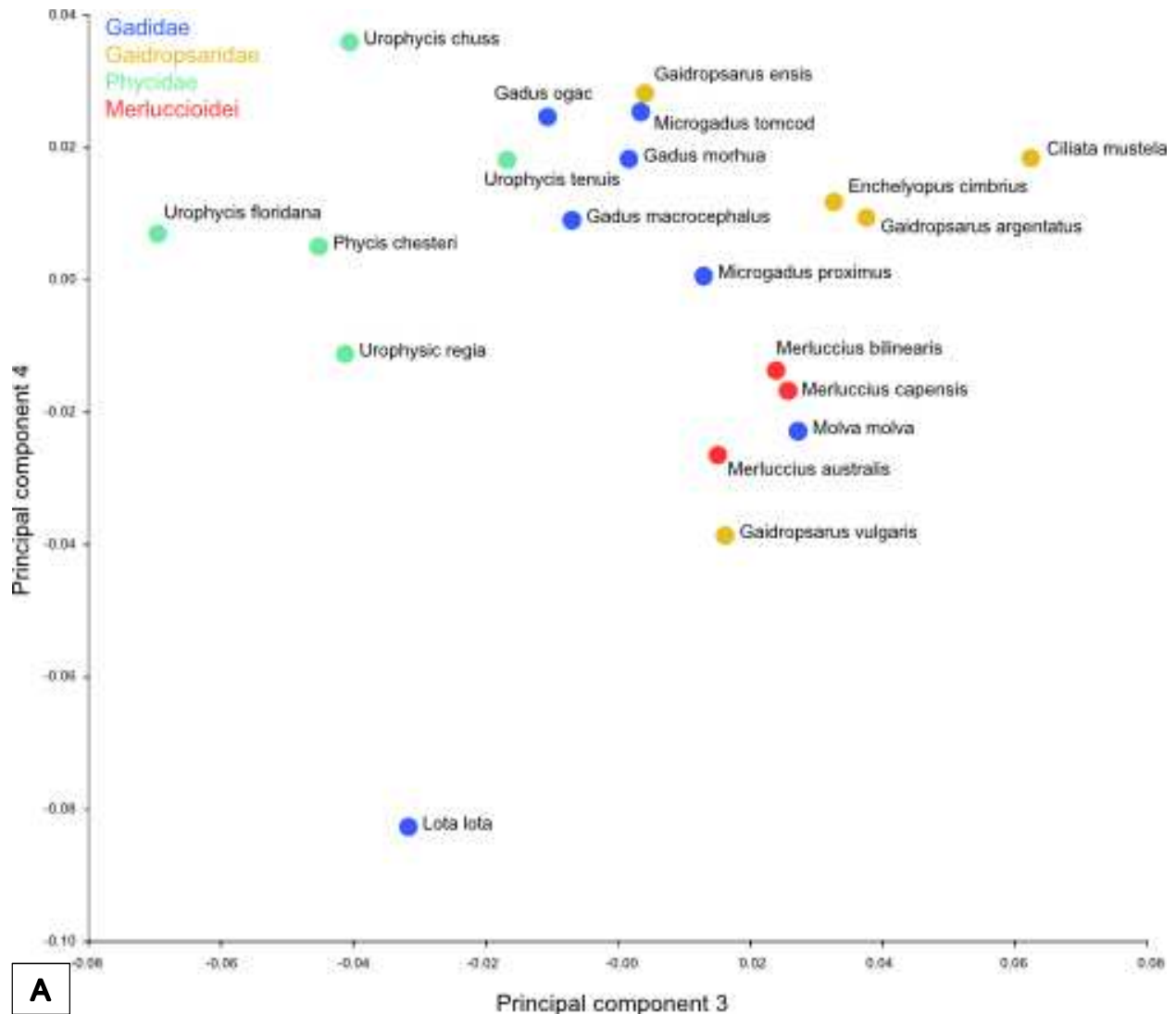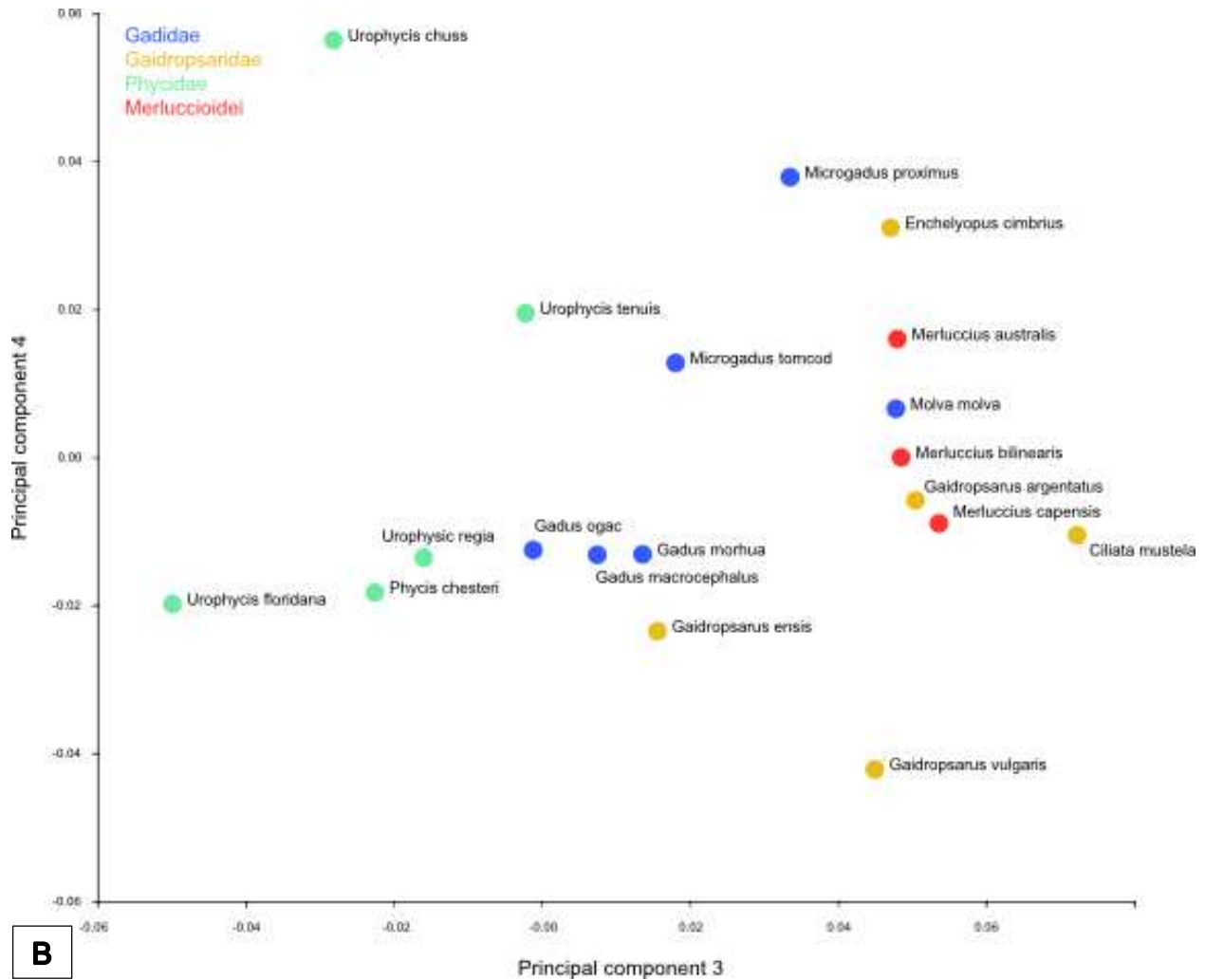
Figure 21. Scatterplot for PC3 vs PC4 with (A, previous page) and without (B) *Lota lota*, explaining a total 13.08% of variation in shape changes. Colors indicate family (or suborder for Merluccioidei).

Phylomorphospace analysis

The phylomorphospace analysis (Fig. 22) indicates that the ancestral body form was

close to the average body form for Gadoidei seen in the principal components, but with a

transition in the lengths of the fin rays in the posterior dorsal fin and anal fin (however, it would

not have two separate posterior dorsal fins and two separate anal fins, like those of the primary

cluster of Gadidae). The ancestor also had a relatively shallow body, not as uniformly slender as the body of *Lota lota*, but intermediate between the bodies of the Phycidae + Gaidropsaridae cluster and those of the Merluccioidei cluster.

The large overlap of body shape present in the Phycidae + Gaidropsaridae cluster is unsurprising given that the two families are sister to each other. The primary cluster of Gadidae that contains *Gadus* and *Microgadus* represents the largest divergence away from the ancestral body shape, primarily due to changes in the dorsal and anal fins. *Gadus* and *Microgadus* are also the most divergent genera within Gadoidei based on the phylogeny in the combined gene tree, as also supported by fossil evidence, the two genera first appearing in the fossil record in the Pliocene (5.4-2.4 mya) making them among the most recently evolved genera within Gadidae (Nolf and Steurbaut, 1989). The development of a second posterior dorsal fin and a second anal fin apparently happened after the ancestor of the two genera diverged from the ancestral gadid.

The shape change of the posterior dorsal fin and anal fin seen in Merluccioidei seems to parallel changes seen in *Gadus* and *Microgadus*, though the posterior dorsal fin and anal fin of the merlucciids remains a single fin. However, the gadid and merluccioid changes occur in different parts of the tree. Therefore, the change in shape of the merluccioid fins seems to be independent of the change in the Gadinae to completely separate fins.

Figure 22. Phylomorphospace diagram of Gadoidei showing variation in the morphospace defined by PC1 and PC2, with the combined gene tree phylogeny of Figure 8 mapped onto the morphospace. Colors indicate the families (or suborder in the case of Merluccioidei) as used in the combined gene tree. The open yellow circle indicates the root of the phylogeny. Illustrations denote generalized body form present in the associated cluster. Images are from Wikimedia Commons.

Phylomorphospace and Water Depth

Results of the parsimony analysis of water-depth states (Fig. 13) mapped onto the phylomorphospace indicate which body form is found at each depth (Fig. 23). Within the primary Gadidae cluster, *Gadus* and *Microgadus* have similar body forms, but are recorded at different depths. Based on its position within the phylomorphospace, *Gadus ogac* would be

expected to be found within the mesopelagic range, though it is recorded as being epipelagic.

Microgadus is epipelagic while *Gadus morhua* and *Gadus macrocephalus* are mesopelagic.

Figure 23. Phylomorphospace diagram showing variation in the morphospace defined by PC1 and PC2, with the combined gene tree phylogeny of Figure 8 mapped onto the morphospace and colored by depth states from Figure 13. The open yellow circle indicates the root of the phylogeny. Illustrations denote generalized body form present in the associated cluster. Images are from Wikimedia Commons.

Microgadus occupies a slightly separate cluster away from *Gadus* and has a generally more narrow body than *Gadus*, which tends to be deeper bodied. Within the Phycidae + Gaidropsaridae cluster, most species are epipelagic.

Phylomorphospace and Diet

Results of the parsimony analysis of diet states (Fig. 14) mapped onto the

phylomorphospace indicate which body form is associated with which diet (Fig. 24). All

members of the Phycidae + Gaidropsaridae body-form cluster feed primarily on crustaceans.

These two families are sister groups, so these results favor one origin of crustacean feeding for

the ancestor of these two families. *Microgadus,* although belonging to the gadid clade, also feeds

on crustaceans. All other gadoids are piscivorous. This points to a shift in diet from the ancestral

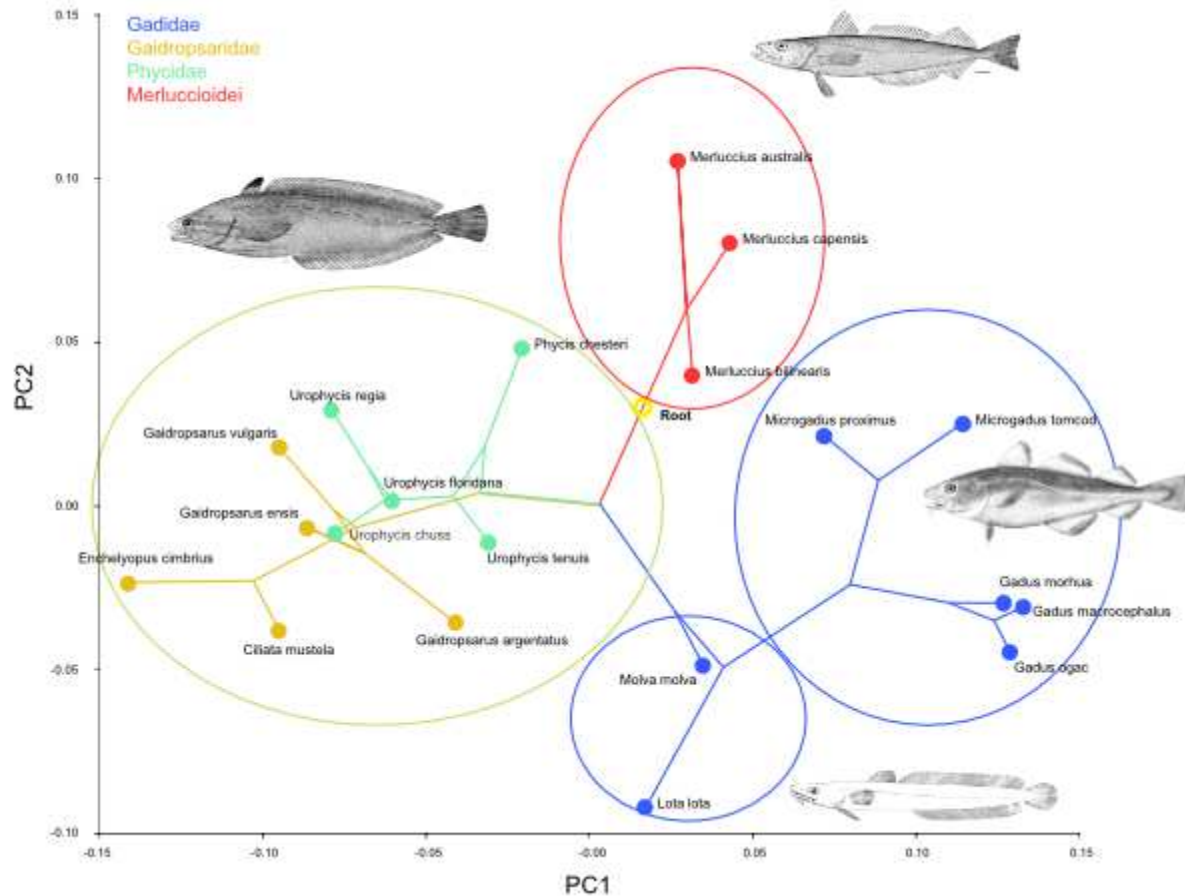state of piscivory seen in most gadids to the crustacean-eating *Microgadus.*
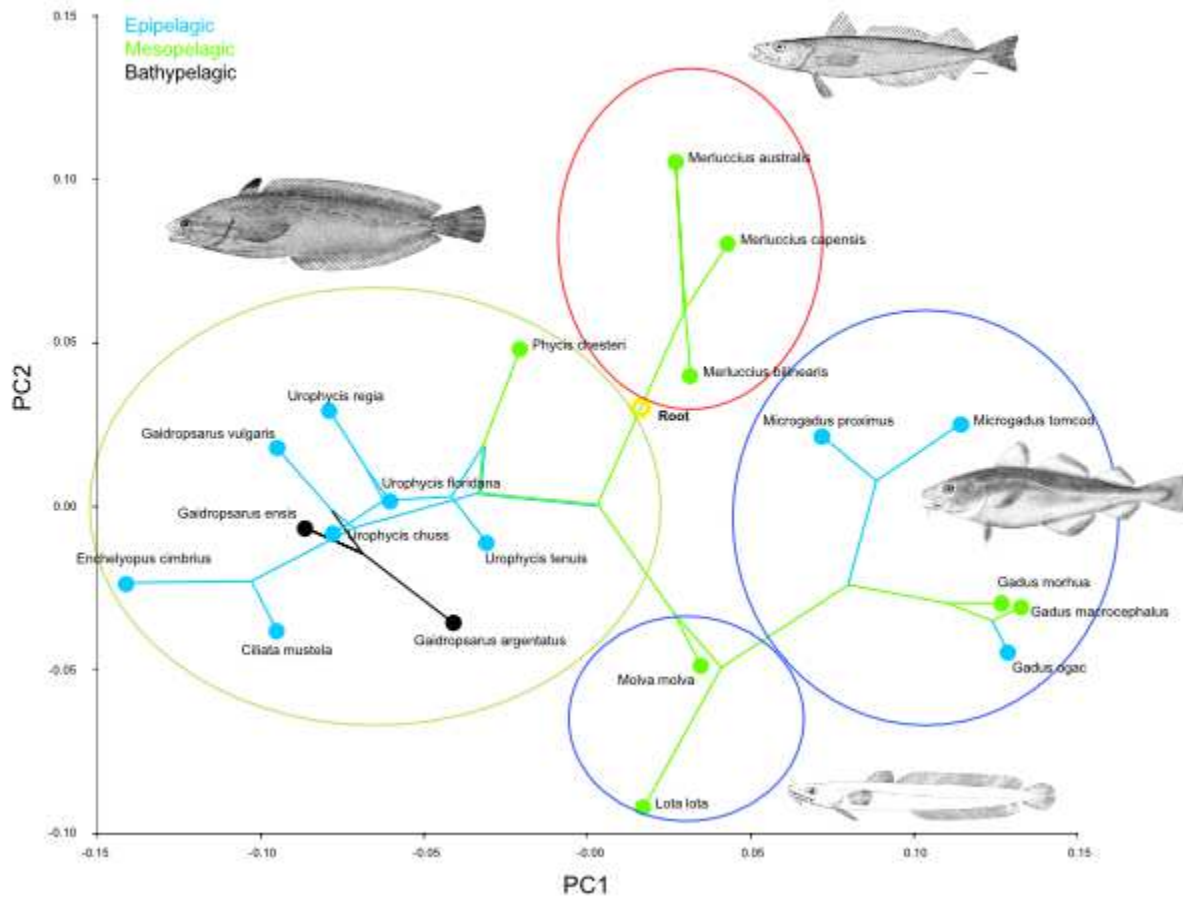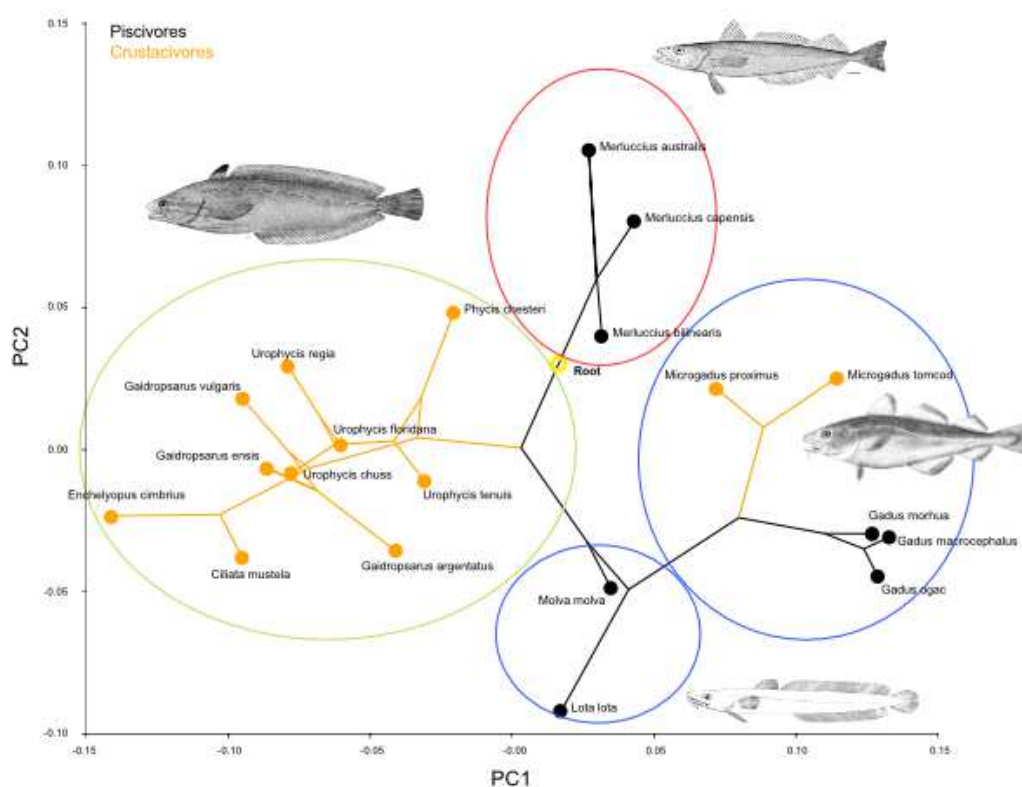

Figure 24. Phylomorphospace diagram showing variation in the morphospace defined by PC1 and PC2, with the combined gene-tree phylogeny of Figure 8 mapped onto the morphospace and colored by diet type from Figure 14. The open yellow circle indicates the root of the phylogeny. Illustrations denote generalized body form present in the associated cluster. Images are from Wikimedia Commons.

Discussion

Body Form and Depth

There appears to be some connection between body depth and water depth, with deeper bodied families such as Phycidae, Gaidropsaridae, and some species of Gadidae being found within the epipelagic range. The Phycidae + Gaidropsaridae lineage and morphospace cluster are particularly strongly associated with the epipelagic depth range. However, most species of Gadidae are found within the mesopelagic range, with *Gadus ogac* and both species of *Microgadus* being found in the epipelagic range.

Within Gadidae, the larger-sized species (*Gadus*, *Molva molva*, and *Lota lota*, ranging from 40 to 100 cm) are also the mesopelagic species, which might suggest that body size is also connected to depth range. *Gadus ogac* remains an outlier within *Gadus* for its epipelagic range. Additionally, both *Urophycis chuss* and *Urophycis tenuis* are of sizes (66–70 cm) comparable to those of the larger species of Gadidae, indicating that size alone is not the only factor associated with water depth.

The type of environment a species is found in is another likely variable that affects body form. Species with elongate and narrow body forms and typical dorsal and anal fin arrangements (a single posterior dorsal fin and a single anal fin), such as *Lota lota* and *Molva molva*, are known to shelter under rocks or within crevices (Etnier and Starnes, 1993; Frimodt, 1995), which suggests that the combination of elongate and narrow body form and typical dorsal and anal fin arrangements might be more advantageous in that environment. Both Gaidropsaridae and Phycidae are typically found on muddy bottoms (Svetovidov, 1986; Cohen et al., 1990), and are not noted to shelter under rocks. Deeper-bodied species such as Gaidropsaridae and Phycidae might not be able to fit as easily into those confined environments.

Body Form and Diet

Fish size is considered one of the largest explanatory variables for feeding habits of demersal fishes, including multiple species of Gadoidei (Jaworski and Ragnarsson, 2006). Jaworski and Ragnarsson (2006) also found that species below 30 cm in length tended to feed primarily on crustaceans and other invertebrates. This trend aligns largely with the results in this study, in which species closer to and below 30 cm in length feed primarily on crustaceans. However, there are some partial exceptions in *Urophycis chuss* and *Urophycis tenuis*, which are recorded above 30 cm in length but remain mainly crustacivorous, and *Merluccius bilinearis*, which is recorded as only 37 cm in length but is a piscivore. Within Gadidae, the smaller size of *Microgadus* likely is a reason or a result for its shift in diet toward crustacivory.

Hypothesis for the Origin of Multiple Median Fins in Gadidae

Within ray-finned fishes, there is a well-known evolutionary trend for different shifts in the size and relative positions of median (anal, caudal, dorsal) fins (Larouche et al., 2018). The evolvability of the median fins has been attributed to the existence of different evolutionary modules (Larouche et al., 2018), with these modules being susceptible to duplication, dissociation, divergence, and co-option (Mabee et al., 2002; Larouche et al., 2017). The presence of the second posterior dorsal fin and second anal fin seen in *Microgadus* and *Gadus* is entirely unique to that lineage within Gadidae. All other fishes within Gadiformes have two dorsal fins, with the anterior dorsal fin typically being much shorter than the posterior dorsal fin, and a single anal fin that is almost as long as the posterior dorsal fin. The unique dorsal fin and anal fin arrangement seen in the gadid body form represents a divergent trait.

The second posterior dorsal fin and second anal fin appear early during development (Patchell et al., 1987; Auditore et al., 1994). Dorsal and anal fins have been suggested to share

the same developmental module (Mabee et al., 2002). The appearance of the second posterior dorsal fin and second anal fin might plausibly be a result of a module duplication event that occurred within the ancestor of *Gadus* and *Microgadus* (Larouche et al., 2018).

Merluccioidei superficially appear to have a similar dorsal and anal fin arrangement to that of gadids, but their posterior dorsal fin and anal fin are continuous and not fully separated like those of the gadids. Based on developmental series (Olivar et al., 1988; Palomera et al., 2005) the posterior "peaks" of the merlucciid posterior dorsal fin and anal fin arise much later in development than the separate second posterior dorsal fin and second anal fin found in gadids. Thus, these merlucciid traits are less likely to be explained by co-option of fundamental developmental modules.

CHAPTER 5

DISCUSSION

This research supports the existence of phylogenetic signal within life-history characteristics based both on the parsimony analysis for reconstruction of ancestral life-history characteristics and the phylomorphospace analysis. A strong phylogenetic signal means that the existence of a particular trait in a species owes much to inheritance of the trait from its ancestors, and that particular life-history traits typically characterize clades of multiple species. Depth and diet showed strong phylogenetic signal among gadoids. Additionally, there was corroboration from fossil records for the reconstructed ancestral depth state of Gadoidei being the mesopelagic range (200–400 meters), as the water depth of early fossil gadoids has been estimated at 250 meters (Kriwet and Hecht, 2008).

Depth has also been implicated as a driver for evolution among abyssal (below 4000 m) fishes (Gaither et al., 2016). Most abyssal species are thought to have originated at bathyal depths (1000–2000 m; Etter and Rex, 1990; Etter et al., 2005). For example, Gaither et al. (2016) conducted a phylogenetic study of *Coryphaenoides* (rattails), a genus belonging to the family Macrouridae within Gadiformes, and recovered an abyssal-specific clade within the genus.

Adaptation to the abyssal depths was found to happen only once within the genus, which suggests that the abyssal lineage only diversified after adapting to the deep waters (Gaither et al., 2016). This shows support for a strong phylogenetic signal for the depth trait, similar to the support found within the depth parsimony analysis of the present study. The *Coryphaenoides* conclusion also helps support the reconstructed ancestral depth state for Gadoidei being within

the mesopelagic range, as deeper waters were shown to only be colonized later in the evolutionary history of *Coryphaenoides* (Gaither et al., 2016).

Diet also showed strong phylogenetic signal, with distinct clades of piscivores and divergent crustacivores in the parsimony analysis, and patterns of body shape within the phylomorphospace. Further morphological and morphometric analysis of the mouths of both groups may provide additional information as to unique adaptations to the different diet groups found within the major piscivore and crustacivore clades. The effect of body shape on preferred prey type might also be examined, given that the major piscivore clades were Merluccioidei and Gadidae (excluding *Microgadus*). The two groups have different body forms, particularly the dorsal and anal fin arrangements, and the forked caudal fin found in *Merluccius*. The differing fin shapes might provide locomotive advantages when hunting faster piscine prey. The median (dorsal, anal, and caudal) fins play an important role in generating locomotor force during swimming, accelerating, and maneuvering (Lauder et al., 2002). The dorsal fin, in particular, has been known to be involved in steady swimming, turning, and braking as well as thrust generation (Lauder et al., 2002). The change in median fin arrangements of the gadid and merlucciid body forms might similarly confer a mechanical advantage. However, this mechanical benefit would presumably not be shared by *Lota lota* and *Molva molva*, which are still piscivores, but have median fin forms much closer to those found in the Phycidae + Gaidropsaridae crustacivore group. That *Microgadus* shares the unique median fin shapes of *Gadus*, yet is a crustacivore, is likely partially explained by the smaller body size of that genus than of its relatives in *Gadus*, as smaller-sized species of Gadoidei tend to feed on crustaceans and other invertebrates (Jaworski and Ragnarsson, 2006).

Fossil otoliths of *Trisopterus* (a genus belonging to Gadidae) were analyzed to compare the recovered life-history data of the fossil species to the life-history data of extant species. It was found that the age structure of fossil species corresponds to data for extant species (Woydack and Morales-Nin, 2001). The species studied showed the highest increase in otolith growth and therefore somatic growth during their first year of life, a pattern that corresponds to the fast growth and maturation recorded in living species of *Trisopterus minutus* (Woydack and Morales-Nin, 2001). However, the otoliths indicated that the fossil species were likely overall smaller in size than living species (Woydack and Morales-Nin, 2001), which corresponds to the evolutionary trend of increasing size also seen within fossil merlucciids compared to living species (Nolf and Steurbaut, 1989).

The data obtained from fossil otoliths shows further support for a level of phylogenetic signal in life-history characteristics, particularly growth and maturity (Woydack and Morales-Nin, 2001). Size is less clearly supported given the range of variables that can affect the state, such as water temperature (Wootton, 1998; Handeland et al., 2008), and the evolutionary trend toward larger size as seen in different lineages within Gadiformes (Nolf and Steurbaut, 1989; Woydack and Morales-Nin, 2001).

Further data collection of life-history characteristics of Gadoidei, as well as further fossil otolith analysis, will likely continue to untangle some of the less-clear cases of phylogenetic signal in life-history characteristics.

Not only was the parsimony analysis of life-history characteristics useful for reconstructing ancestral states, but the phylomorphospace analysis provides an estimation of ancestral morphological traits linking body form with life history. The phylomorphospace indicates that the ancestral body form of Gadoidei + Merluccioidei was close to the average body

form for Gadoidei, but with a transition in the lengths of the fin rays in the posterior dorsal fin

and anal fin, and with a relatively shallow body, not as uniformly slender as the body of *Lota*

lota, but intermediate between the bodies of the Phycidae + Gaidropsaridae cluster and those of

the Merluccioidei cluster.

More recent phylogenetic analyses (Grande et al., 2013; Roa-Verón et al., 2021) recover

Bregmaceros as the most basal gadiform, and as such it might be predicted to resemble the

ancestral body form. The general body form of *Bregmaceros* does somewhat resemble the

predicted form, particularly with respect to its body shape and depth, but the genus has its own

unique posterior dorsal and anal fin shapes (Fig. 25). Further analysis of fossil record of

Bregmaceros, of which there are skeletal remains (Gaudant, 2005; Argyriou, 2022), might

provide more information as to whether the unique posterior dorsal fin and anal fin shapes are

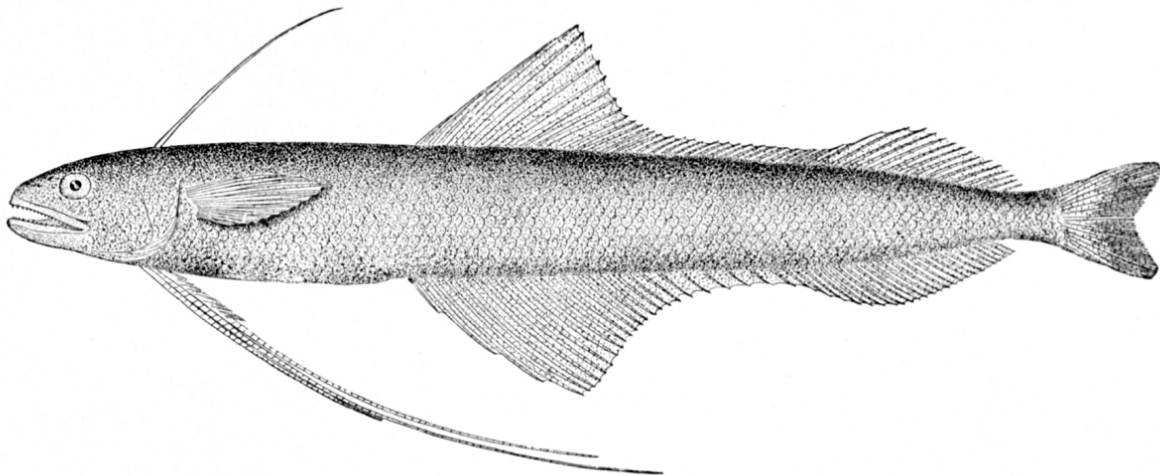characteristics ancestral to the genus, or more recent developments.

Figure 25. Illustration of *Bregmaceros* demonstrating its body form and the unique shapes of its posterior dorsal fin and anal fin. Image from Wikimedia Commons.

The Effects of Overfishing and Climate Change on Life-History Traits

Multiple fish stocks have seen a decrease in abundance since the 1970s (Engelhard et al., 2014), with *Gadus morhua* showing one of the most dramatic decreases, one that has yet to return to historic abundances (Hislop 1996; Brander, 2010). The overexploitation of fishing stocks has been shown to cause a decrease in age at maturity due to increased mortality of adults, particularly among cod and merlucciid hakes (Jørgensen, 1988; King and McFarlane, 2006; Brander, 2010). While overfishing is blamed for the dramatic collapse of multiple stocks, there has been a decrease in fishing mortality over the past ten years across fifty different stocks, indicating some success of more recent changes in fish stock management (Brander, 2010).

While the effects of overexploitation of fishing stocks have had a significant impact on the life-history characteristics of Gadoidei, the effects of climate change add another layer to the discussion. Ongoing climate change is predicted to affect organisms at all of their life stages, in their environment, and through changes to the composition of their food sources (Pörtner et al., 2008). Based on climate models, the North Sea area is predicted to see a decrease in cold winters and an increase in hot summers (Pörtner et al., 2008), with mean annual sea-surface temperatures of the North Sea predicted to increase by 1.0 to 2.5º C by 2050, and 1.5 to 4.0º C by 2080 (Perry et al., 2005). With Gadoidei being notable stenothermal species, they will be greatly affected by increases in water temperature. Climate change has already been linked to changes in multiple life-history characteristics of species within Gadoidei. *Gadus morhua* (Atlantic cod) is the most evident example, which has seen shifts in size, maturity, and fecundity (Pörtner et al., 2008; Brander, 2010; Perry et al., 2005).

Temperature influences the rates of spawning events per season and offspring recruitment success of *Gadus morhua*, with Atlantic Cod found in the North Sea seeing a decrease of

spawning events and recruitment success with an increasing temperature; these might be attributed to spawning behavior being linked to a temperature preference, or to temperature sensitivity of the eggs and larvae (Pörtner et al., 2008). In stenothermal boreal species such as cod, larger body size is shown to enhance thermal sensitivity, meaning that larger bodied fishes have a more restricted optimal growth range, growing less optimally at higher temperatures than smaller bodied fishes (Pörtner et al., 2008; Brander, 2010). Smaller bodied fish are more likely to tolerate warmer water temperatures and are more likely to reach maturity faster and at smaller sizes (Pörtner et al., 2008); these data suggest that if species such as *Gadus morhua* are to inhabit warmer water bodies, there will likely be an overall reduction in size and individuals will reach maturity at a younger age.

Multiple species of fishes, including cod, have seen a northward shift in their distributions in response to warming waters (Pörtner et al., 2008; Nye et al., 2011; Perry et al., 2005), with nearly two-thirds of North Sea species shifting northwards (Perry et al., 2005), indicating a limited ability to adapt to the changing temperatures present in their historic ranges (Pörtner et al., 2008). Species that have shifted their distributions are reported to mature faster and at smaller sizes compared to species that have not shifted their distributions, which include multiple species within Gadoidei and the whole of Gadiformes (Perry et al., 2005).

The existence not only of shifts in geographic range but also of changes in maturity and size suggests that the range shifts by themselves have not been sufficient to mitigate the effects of warming waters. Species that shift their distribution are also likely to face competition from other species, as well as changes and reductions in their prey species, which are also changing their distributions due to climate change (Pörtner et al., 2008); this has already been suggested as another factor in the decrease in the abundance of *Gadus morhua* (Beaugrand et al., 2003).

Optimal life-history characteristics and water temperature are tightly linked within cold-water adapted gadoid fishes (Wootton, 1998; Handeland et al., 2008; Pörtner et al., 2008; Lorenzo et al., 2011). Other stenothermal fishes that are specialized to cold climates, such as Antarctic notothenioids, are shown to have less phenotypic plasticity, which might limit the extent to which they can adapt to increasing water temperatures (Patarnello et al., 2011). While some populations of cod have shifted their distributions northward, there might be an upper limit, due to evolutionary constraints, to the extent to which they can successfully respond to increasing temperatures.

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VITA

Joshua Hittie graduated from Loyola University Chicago, in May 2015 with a B.S. in Biology, concentration in Ecology, and a minor in Biostatistics. As an undergraduate he studied the early development of caudal fin-rays in Northern Pike (*Esox lucius*) to determine stages of ossification in Dr. Terry Grande's lab. This early research introduced him to fish development and evolution, paving the way for an increased interest in fish phylogenetics, as well as providing the opportunity to present his undergraduate research at the 2014 Joint Meeting of Ichthyologists and Herpetologists.

Upon completing his undergraduate degree, Mr. Hittie continued pursuing research in Dr. Grande's lab as he began pursuing his Master of Science at Loyola University Chicago, focusing on the phylogenetics and life history of gadiform fishes. He was able to present his early graduate research at the 2016 Joint Meeting of Ichthyologists and Herpetologists. During his time at Loyola, he also assisted in Dr. Grande's Comparative Anatomy lab course, as well as teaching as the Instructor of Record for the General Biology I and II lab courses. It was through both his work in Comparative Anatomy and General Biology that he discovered his love of teaching that continues to this day.