Molecular Phylogenetics and Evolution 66 (2013) 341–349

Contents lists available at SciVerse ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Phylogenetics of the marine sculpins (Teleostei: Cottidae) of the North American Pacific Coast

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A R T I C L E I N F O

Article history:
Received 7 June 2012
Revised 2 October 2012
Accepted 8 October 2012
Available online 23 October 2012

Keywords:
Allopatry
Intertidal
Radiation
Subtidal
Speciation
Sympatry

A B S T R A C T

With 92 species along the North American Pacific Coast, marine sculpins represent the most species-rich radiation of fishes in this region. I used the mitochondrial cytochrome *b* gene and the nuclear ribosomal S7 intron for 99 species (76 North American, 19 Asian, and four North Atlantic) to produce the most complete phylogenetic hypothesis yet generated for this assemblage. Maximum likelihood and Bayesian analyses produced highly similar tree topologies. While many previously proposed groupings based on morphology are recovered, the molecular data suggest that a number of genera are para- or polyphyletic. However, this analysis supports the monophyly of one large clade that is found exclusively along the North American Pacific Coast (*Chitonotous–Ruscarius–Artedius–Orthonopius–Clinocottus–Leiocottus–Oligocottus*). Some sibling species have disjunct ranges, suggesting allopatric speciation. However, many other sibling species have largely overlapping ranges, and repeated habitat shifts appear to have facilitated diversification.

1. Introduction

The process of species formation in the marine environment is poorly understood (Palumbi, 1994; Burford and Bernardi, 2008; Miglietta et al., 2011). Typically, marine species have large population sizes, the potential for long-distance dispersal, and large geographic ranges, and these characteristics can inhibit genetic differentiation and species formation (Palumbi, 1994; Miglietta et al., 2011). However, many marine radiations are species-rich (e.g., Eastman and McCune, 2000; Hyde and Vetter, 2007) and it remains unclear why some lineages diversify greatly while others do not. One such lineage that is exceptionally species-rich is the marine fishes in the northern Pacific Ocean commonly known as sculpins. With 92 species found along the North American Pacific Coast, marine sculpins represent the most diverse radiation of fishes in this region (Howe and Richardson, 1978; Love, 2011).

There has been nearly 125 years of attempts to reconstruct the evolutionary history of the sculpins of the North American Pacific Coast (Gill, 1889; Hubbs, 1926; Taranets, 1941; Bolin, 1947) and current ideas about the systematics of this group are based primarily on morphology (Bolin, 1944, 1947). Using an overall similarity (phenetic) approach, Bolin (1947) was the first to offer a branching diagram for this assemblage. However, Bolin limited his sampling to the 50 species found in California waters alone, which are now recognized to be both a polypheletic and a paraphyletic assemblage. More recently, Yabe (1985) and Jackson (2003) used internal and external morphological characters, respectively, to address taxonomic issues among the superfamily Cottoidea and the family Cottidae. Further, Ramon and Knope (2008) used molecular markers to address the phylogenetic relationships of 27 species, with a focus on the putative subfamily Oligocottinae (family Cottidae) and found both congruencies and differences between their molecular and previous morphological assessments of relationships. Despite this long history of work, most generic and specific relationships within the family Cottidae remain unresolved.

The Cottidae is the largest family of sculpins with about 70 genera and 275 species worldwide, found primarily in boreal and cold-temperate regions (Mecklenburg et al., 2002; Nelson, 2006), and the family is considered to be of recent Oligocene or Miocene origin (MacFarlane, 1923; David, 1943; Berg, 1947). Sculpins in the family Cottidae are called cottids (*sensu stricto*) to avoid confusion with other sculpins in the superfamily Cottoidea. Cottids are generally small, intertidal and subtidal benthic marine fishes, although about 75 species are found in freshwater (Nelson, 2006). Marine cottids are generally restricted to the near shore and continental shelf, but display great habitat diversity and can be found on deep reefs and soft sediments, shallow reefs and soft sediments, in kelp forests, the intertidal, estuarine, and freshwater environments (Miller and Lea, 1972; Ramon and Knope, 2008; Love, 2011). While sculpins are found in all oceans of the world except the Indian Ocean (four species occur in the Southern Hemisphere), the north Pacific has been identified as their center of diversity and hypothesized to also be the center of origin for the family (Eschmeyer et al., 1983;

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http://dx.doi.org/10.1016/j.ympev.2012.10.008

Watanabe, 1969; Yabe, 1985). They are generally not commercially important, but a few species in the genera *Hemilepidotus* and *Scorpaenichthys* are important in sport fisheries.

Like other scorpaeniform ("mail-cheeked") fishes, cottids have a suborbital stay which is a backwards extension of the third circumorbital bone (part of the lateral head/cheek skeleton, below the eye socket) across the cheek to the pre-operculum. Yabe (1985) defined cottids as a monophyletic group having one autapomorphy (presence of the lateral process of the hyomandibular) and a unique combination of nine morphological synapomorphies. Cottids are proposed to differ from sailfin sculpins (Hemitripteridae) and grunt sculpins (Rhamphocottidae) in that they are not densely covered with prickles and from most fathead sculpins (Psychrolutidae) by not being extremely tadpole-shaped and lacking loose skin over a gelatinous layer (Mecklenburg et al., 2002). However the monophyly of the Cottidae remains contentious (Crow et al., 2004; Smith and Wheeler, 2004). Cottids exhibit wide variation in color and in distribution of skin pigments and in some genera there is considerable intraspecific morphological variability (including between the sexes), as well as individual ontogenetic shifts (Bolin, 1944; Miller and Lea, 1972; Mecklenburg et al., 2002), making morphological assessments of phylogenetic relationships difficult.

The primary objectives of this study were to: (i) reconstruct a phylogenetic hypothesis for the sculpins of the North American Pacific Coast (NAPC), (ii) determine at which hierarchical level the NAPC radiation is monophyletic; and (iii) determine if the molecular phylogenetic hypothesis is consistent with previous morphological hypotheses. To this end, I generated a molecular phylogeny based on the mitochondrial cytochrome *b* gene and the intron of nuclear ribosomal protein S7 for 99 species and compared the resultant phylogenetic relationships with that of previous studies.

2. Materials and methods

2.1. Specimen collection

Of the 99 species examined, 80 are represented by new collections and 19 are from a previous study (Ramon and Knope, 2008). A total of 130 new sculpins were collected in the field, from aquaria at the Alaska Sea Life Center, or donated from the University of Washington Burke Ichthyology Museum, the University of Helsinki Natural History Museum, or the Maizuru Fisheries Research Station (Table 1). Intertidal specimens were collected by dipnet at low tides and deepwater specimens were collected by bottom-trawl on the NOAA vessels F/V Alaska Knight, F/V Vesteraalen, F/V Dominator, F/V Sea Storm, and F/V Ocean Explorer.

2.2. DNA extraction, amplification, sequencing, and alignment

Whole genomic DNA was extracted from fin clippings stored in 95% ethanol. Tissues were digested and DNA extracted using standard DNEASY protocol (Qiagen Corp.). Samples were PCR amplified for mitochondrial cytochrome *b* (GLUDG-L: 5′-TGACTTGAARAACCAYCGTTG-3′ and CB3-H: 5′-GGCAAATAGGAARTATCATTC-3′ from Palumbi, 1996) and the first intron of the nuclear S7 ribosomal protein (custom designed primers S72F: 5′-TCTCAAGGCTCGGATACGTT-3′ and S74R: 5′-TACTGAACATGGCCGTTGTG-3′). PCR amplifications were performed in a 25 μl volume with 10–12.5 μl MyTaq™ Red Mix (Bioline Corp.), 1 μl (10 μM) each primer, and the remaining volume H_2O. PCR thermalcycling was performed using the following protocol for cyt *b*: 1 min initial denaturation at 95 °C, followed by 35 cycles of 95 °C for 30 s, 50 °C for 30 s, and 72 °C for 45s. PCR thermalcycling was performed using the following protocol for S7: 1 min initial denaturation at 95 °C, followed by 30 cycles of 95 °C for 15 s, 50 °C for 15 s, and 72 °C for 20 s. PCR products were visualized in 1% sodium borate agarose gels and enzymatically cleaned with 2 μl of Exo-Sap-It (USB Corp.). Double stranded PCR products were sequenced directly with the same primers used for the PCR amplifications by ELIM Biopharmaceuticals, Inc.

Raw forward and reverse sequences were assembled and edited in Sequencher 4.8 (Gene Codes, Corp). The mitochondrial cyt *b* locus exhibited no insertions or deletions (indels), as it is protein-coding. The nuclear ribosomal S7 exhibited some indels, which were included in the analysis, but were coded as gaps. Double peaks in the nuclear sequences, reflecting heterozygous positions, were coded with IUPAC degeneracy codes and treated as polymorphisms. When multiple sequences for a single species were available, few or no nucleotide differences were found among individuals. Therefore, for the phylogenetic analyses I used sequences from the individual with the most complete coverage. All alignments were created in MUSCLE (Edgar, 2004) and were unambiguous. The matrix analyzed was over 97% complete. Genes not sequenced for given individuals were coded as missing. All previous sequences from Ramon and Knope (2008) were downloaded from GenBank (accession numbers EF521313–EF521387).

2.3. Model selection

Models of evolution were determined using jModelTest 0.1.1 (Posada, 2008) for each gene independently and for both loci combined. Akaike Information Criterion (AIC) was used to discriminate among 88 progressively more complex models of nucleotide evolution. The models chosen for each of the datasets are as follows: the cyt *b* data set best fit the general time reversible model with invariable sites and rate variation among sites included (GTR + I + G), the S7 data best fit the Hasegawa, Kishino, Yano 85 model (Hasegawa et al., 1985) with rate variation among sites included (HKY + G) and the combined data set best fit the transversion model with rate variation among sites included (TVM + G; Posada, 2008).

2.4. Phylogenetic analyses

All trees were outgroup rooted with *Stellerina xyosterna* in the family Agonidae, which has been identified as the most appropriate outgroup by previous studies (Crow et al., 2004; Smith and Wheeler, 2004; Ramon and Knope, 2008). In addition, other putative outgroup species were tested (e.g. *Apeltes quadracus* [Gasterosteidae], *Lycodes diapterus* [Zoarcidae], *Ophiodon elongatus* [Hexagrammidae]) and doing so made no difference in terms of the tree topology of the ingroup, so *Stellerina xyosterna* was utilized as the outgroup as it is outside the family Cottidae, but within the suborder Cottoidei, whereas the other taxa are not (Nelson, 2006). In addition, representatives of three other cottoid families (Rhamphocottidae, Hemitripteridae, and Psychrolutidae; Table 1) were included in this analysis to further evaluate the monophyly of Cottidae. Among-taxa base composition differences and overall base composition bias values were calculated according to Irwin et al. (1991). Phylogenetic relationships were assessed by maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) methods. Both loci were examined individually and in a concatenated analysis in all three phylogenetic reconstruction approaches. In general, individual analyses were less resolved and had lower statistical support (not shown) than the combined loci analyses. The resulting topologies for shared species were compared with Bolin's (1947) morphological tree based on overall similarity, using the

Table 1
Summary of taxa sampled, distribution, and collection numbers. Distributions are abbreviated as follows: Arctic (Arc), north Atlantic (NAtl), north Pacific (NP), northwestern Pacific (NWP), eastern Pacific (EP), eastern central Pacific (ECP), and northeastern Pacific (NEP). Distributions are based on range data given in Miller and Lea (1972), Howe and Richardson (1978), Mecklenburg et al. (2002), Nakobo (2002), Lamb and Edgell (2010), and Love (2011). Collection numbers begin with two-letter code for location of voucher specimen (FK = Maizuru Fisheries Research Station, Kyoto University; SU = Stanford University, Dept. of Biology; UH = University of Helsinki, Natural History Museum; UW = University of Washington, Burke Ichthyology Museum; N/A = Not applicable, as sequence data is from GenBank).

Family	Genus	Species	Distribution	Collection number
Cottidae	*Alcichthys*	*elongatus*	NWP	FK13118
Cottidae	*Archaulus*	*biseriatus*	NP	UW11997
Cottidae	*Artediellus*	*fuscimentus*	NWP	FK131111
Cottidae	*Artediellus*	*pacificus*	NP	UW150025
Cottidae	*Artediellus*	*scaber*	NAtl	UW150030
Cottidae	*Artedius*	*corallinus*	EP	N/A
Cottidae	*Artedius*	*fenestralis*	EP	N/A
Cottidae	*Artedius*	*harringtoni*	EP	N/A
Cottidae	*Artedius*	*lateralis*	NP	SUKN010
Cottidae	*Artedius*	*notospilotus*	EP	N/A
Cottidae	*Atopocottus*	*tribranchius*	NWP	FK132895
Cottidae	*Bero*	*elegans*	NWP	FK130488
Hemitripteridae	*Blepsias*	*bilobus*	NP	SUKN014
Hemitripteridae	*Blepsias*	*cirrhosus*	NP	SUKN008
Cottidae	*Chitonotus*	*pugetensis*	EP	SUKN057
Cottidae	*Clinocottus*	*acuticeps*	EP	SUKN015
Cottidae	*Clinocottus*	*analis*	EP (ECP)	N/A
Cottidae	*Clinocottus*	*embryum*	EP	SUKNO25
Cottidae	*Clinocottus*	*globiceps*	EP	N/A
Cottidae	*Clinocottus*	*recalvus*	EP	N/A
Cottidae	*Cottiusculus*	*gonez*	NWP	FK132213
Cottidae	*Cottus*	*amblystomopsis*	Asia	UW29174
Cottidae	*Cottus*	*cognatus*	N. America	UH114
Cottidae	*Cottus*	*kazika*	Japan	FK132173
Cottidae	*Cottus*	*pollux* (*mid-size egg*)	Japan	SUKN130
Cottidae	*Dasycottus*	*setiger*	NP	SUKN0013
Cottidae	*Enophrys*	*bison*	EP	N/A
Cottidae	*Enophrys*	*diceraus*	NP	UW150180
Cottidae	*Enophrys*	*lucasi*	NP	UW112174
Cottidae	*Enophrys*	*taurina*	EP (ECP)	SUKN058
Cottidae	*Furcina*	*osimae*	NWP	FK131882
Cottidae	*Gymnocanthus*	*galeatus*	NP	UW 44189
Cottidae	*Gymnocanthus*	*pistilliger*	NP	UW150257
Cottidae	*Gymnocanthus*	*tricuspis*	Arc & NAtl	UW150277
Cottidae	*Hemilepidotus*	*gilberti*	NP	UW49723
Cottidae	*Hemilepidotus*	*hemilepidotus*	NP	SUKN001
Cottidae	*Hemilepidotus*	*jordani*	NP	SUKN002
Cottidae	*Hemilepidotus*	*papilio*	Arc & NP	UW 49428
Cottidae	*Hemilepidotus*	*spinosus*	EP	SUKN018
Cottidae	*Hemilepidotus*	*zapus*	NP	UW111999
Cottidae	*Hemitripterus*	*bolini*	NP	SUKN017
Cottidae	*Icelinus*	*borealis*	EP	N/A
Cottidae	*Icelinus*	*burchami*	EP	SUNK059
Cottidae	*Icelinus*	*filamentosus*	EP	UW49116
Cottidae	*Icelinus*	*fimbriatus*	EP	SUKN038
Cottidae	*Icelinus*	*tenuis*	EP	UW117021
Cottidae	*Icelus*	*euryops*	NP	UW117205
Cottidae	*Icelus*	*ochotensis*	NWP	FK132551
Cottidae	*Icelus*	*spatula*	Arc & NAtl	UW150028
Cottidae	*Icelus*	*toyamensis*	NWP	FK132550
Cottidae	*Icelus*	*uncinalis*	NEP	UW117176
Cottidae	*Icelus*	*canaliculatus*	NP	UW112091
Cottidae	*Icelus*	*spiniger*	NP	UW115870
Cottidae	*Jordania*	*zonope*	EP	N/A
Cottidae	*Leiocottus*	*hirundo*	EP (ECP)	N/A
Cottidae	*Leptocottus*	*armatus*	EP	SUKN016
Cottidae	*Megalocottus*	*platycephalus*	NP	UW150203
Cottidae	*Microcottus*	*sellaris*	NP	UW150203
Cottidae	*Myoxocephalus*	*brandtii*	NWP	FK130458
Cottidae	*Myoxocephalus*	*cf. scorpioides*	NP	UH112
Cottidae	*Myoxocephalus*	*jaok*	NP	UW150274
Cottidae	*Myoxocephalus*	*polyacanthocephalus*	NP	UW 47635
Cottidae	*Myoxocephalus*	*scorpius*	Arc and NAtl	SUKN039
Cottidae	*Myoxocephalus*	*stelleri*	NWP	FK130458
Cottidae	*Myoxocephalus*	*verrucosus*	NP	UW150284
Hemitripteridae	*Nautichthys*	*oculofasciatus*	EP	SUKN003
Hemitripteridae	*Nautichthys*	*pribilovius*	NP	UW117335
Cottidae	*Oligocottus*	*maculosus*	NP	SUKN023
Cottidae	*Oligocottus*	*rimensis*	EP	N/A
Cottidae	*Oligocottus*	*rubellio*	EP	N/A

(*continued on next page*)

Table 1 (*continued*)

Family	Genus	Species	Distribution	Collection number
Cottidae	*Oligocottus*	*snyderi*	EP	N/A
Cottidae	*Orthonopias*	*triacis*	EP (ECP)	N/A
Cottidae	*Porocottus*	*allisi*	NWP	UW 47873
Cottidae	*Porocottus*	*camtschaticus*	NWP	UW 44501
Cottidae	*Pseudoblennius*	*percoides*	NWP	FK131881
Cottidae	*Pseudoblennius*	sp.3 *sensu Nakabo*	NWP	FK132480
Psychrolutidae	*Psychrolutes*	*phrictus*	NP	SUKN079
Cottidae	*Radulinus*	*asprellus*	EP	SUKN037
Cottidae	*Rastrinus*	*scutiger*	NEP	UW117240
Rhamphocottidae	*Rhamphocottus*	*richardsonii*	NP	SUKN011
Cottidae	*Ricuzenius*	*pinetorum*	NWP	FK131167
Cottidae	*Ruscarius*	*creaseri*	EP (ECP)	N/A
Cottidae	*Ruscarius*	*meanyi*	EP	N/A
Cottidae	*Scorpaenichthys*	*marmoratus*	EP	N/A
Cottidae	*Stellerina*	*xyosterna*	EP	N/A
Cottidae	*Stlengis*	*misakia*	NWP	FK132495
Cottidae	*Synchirus*	*gilli*	EP	UW49430
Cottidae	*Trichocottus*	*brashnikovi*	NP	UW150262
Cottidae	*Triglops*	*forficatus*	NP	UW49483
Cottidae	*Triglops*	*macellus*	EP	UW110461
Cottidae	*Triglops*	*metopias*	NP	UW49475
Cottidae	*Triglops*	*murrayi*	NAtl	UW111785
Cottidae	*Triglops*	*nybelini*	NAtl	UW111786
Cottidae	*Triglops*	*pingelii*	Arc, NP, NAtl	UW49659
Cottidae	*Triglops*	*quadricornis*	Arc and NAtl	UW150271
Cottidae	*Triglops*	*scepticus*	NP	UW111989
Cottidae	*Triglops*	*xenostethus*	NP	UW117184
Cottidae	*Zesticelus*	*profundorum*	NP	UW115868

Shimodaira and Hasegawa test (hereafter referred to as S–H test; Shimodaira and Hasegawa, 1999) as implemented in PAUP* version 4.0b10 (Swofford, 2002).

2.4.1. Parsimony analysis

The total number of characters, the number of variable characters, and the number of parsimony informative characters were calculated in PAUP*. Trees were outgroup rooted and full heuristic searches were performed for the individual and combined loci matrices and the maximum number of trees retained was set to 2000. Gaps were treated as missing data, starting trees were obtained via stepwise addition, branch swapping was performed by tree-bisection–reconnection, and the optimality criterion set to ACCTRAN (which puts the character change as close to the root of the phylogeny as possible). Consensus trees were evaluated by 50% majority-rule and the confidence index (CI) and retention index (RI) were calculated on this tree. Statistical confidence in nodes was assessed by bootstrapping with 1000 pseudoreplicates and "FAST" stepwise-addition (Felsenstein, 1985).

2.4.2. Maximum likelihood and Bayesian analyses

ML analyses were conducted with PhyML (Guindon and Gascuel, 2003). The appropriate models of sequence evolution, as detected by jModelTest, were used to find the best ML tree and statistical support values for nodes were obtained by approximate likelihood ratio tests (aLRT). BI analyses were conducted with MrBayes 3.1 (Huelsenbeck and Ronquist, 2001) as implemented within Geneious (Drummond et al., 2009). Again, the appropriate models of nucleotide evolution as determined by jModelTest were implemented. Four chains were run for one million generations each, with one chain heated at a setting of 0.2. The first 100,000 generations were discarded as burn-in and trees were sampled every 200 generations. A consensus tree was constructed and statistical confidence in nodes was evaluated by Bayesian posterior probabilities.

3. Results

3.1. Nucleotide composition and DNA sequences

Approximately 1270 bp were sequenced for almost all 130 individuals. The fragment lengths used for phylogenetic analysis for cyt *b* = 800 bp and S7 = 470 bp. The mtDNA cyt *b* locus showed a positive C bias and an anti-G bias ($A = 0.2489$, $C = 0.4009$, $G = 0.1258$, $T = 0.2245$) but the nuclear ribosomal S7 locus did not ($A = 0.2074$, $C = 0.2457$, $G = 0.2546$, $T = 0.2923$). However, for both loci, chi-square analyses showed no difference in base frequency across taxa ($p > 0.95$ in both cases).

3.2. Maximum parsimony

Maximum parsimony analysis of the cyt *b* gene resulted in 355 parsimony informative sites and 53 equally parsimonious trees of length 4070 steps (not shown). The S7 locus resulted in 188 parsimony informative sites and reached the maximum of 2000 equally parsimonious trees of length 910 (not shown). The combined data analysis from both loci yielded 543 parsimony informative characters and also reached the maximum of 2000 equally parsimonious trees of 4851 steps. The 50% majority-rule consensus tree (not shown) resulted in a tree length of 6435 steps (CI = 0.177, RI = 0.371).

3.3. Maximum likelihood and Bayesian inference

The BI and ML tree topologies were nearly identical to each other, therefore only the BI tree is shown with Bayesian posterior probabilities and aLRT support plotted on each node (Fig. 1). Generally, statistical confidence in nodes was high for both basal and derived nodes, but varied considerably (Fig. 1). For shared species, the molecular ML phylogenetic hypothesis proposed here is statistically significantly different from that based on morphology (S–H test $P < 0.05$; Bolin, 1947). For cyt *b* the GTR + G + I model was selected as the best-fit with Gamma shape parameter = 0.716 and

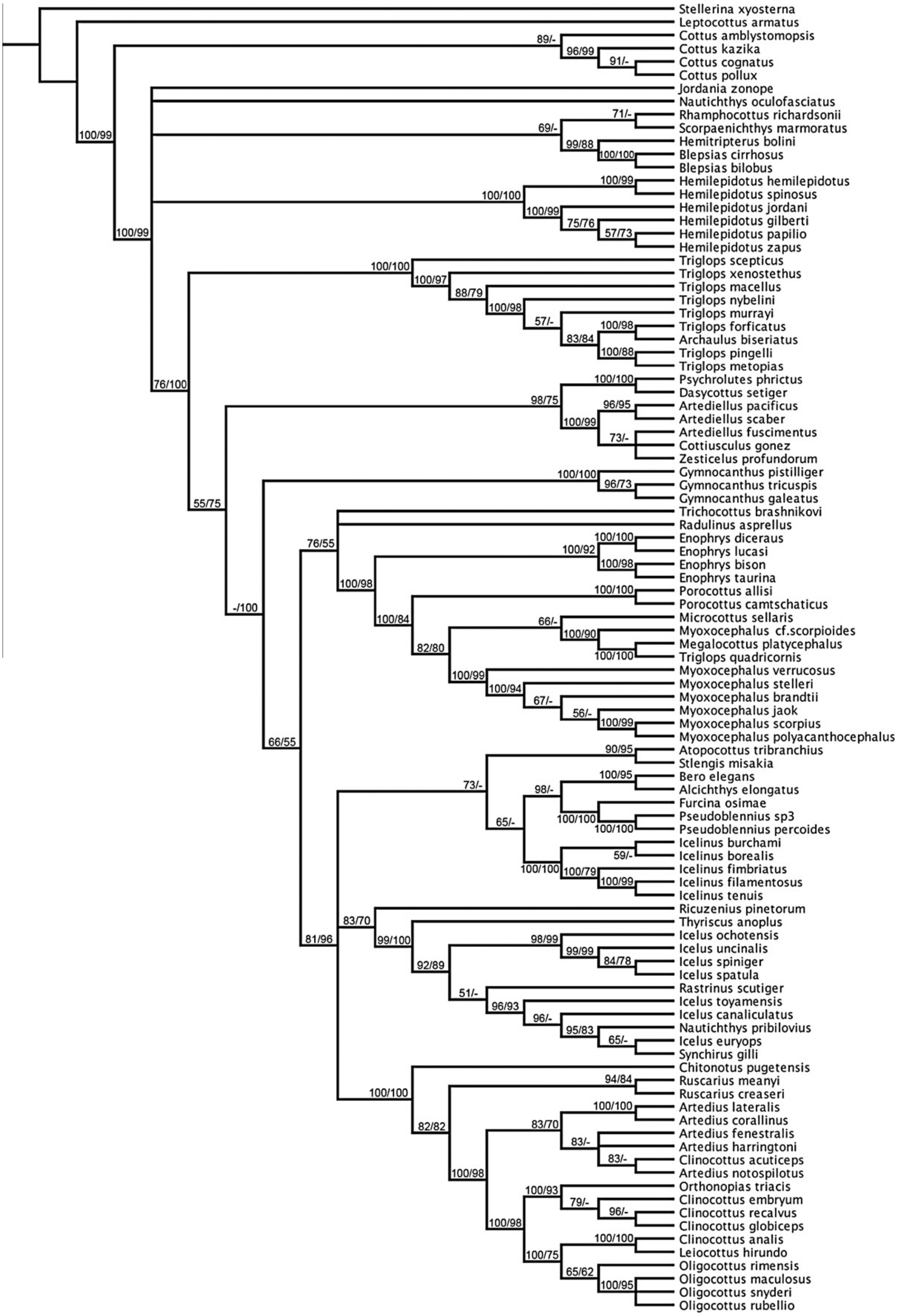

Fig. 1. Molecular phylogenetic hypothesis. Bayesian phylogenetic reconstruction using the mitochondrial gene cyt *b* and the nuclear ribosomal S7 intron. Statistical support values are either above or below each node with Bayesian posterior probabilities to the left and maximum likelihood aLRT values to the right. Dashed lines indicate less than 50% support.

proportion of invariant sites = 0.489 and the resultant ML tree length $-\ln L = 8299.29$. For S7 the TIMef + I + G model was selected as the best-fit with Gamma shape parameter = 0.859 and proportion of invariant sites = 0.000 and the resultant ML tree length $-\ln L = 4822.85$. For both loci combined the TVM + G model was selected as the best-fit with Gamma shape parameter = 0.313 and proportion of invariant sites = 0.000 and the resultant ML tree length $-\ln L = 23460.41$. The ML substitution rate matrix for cyt *b* was AC = 0.8518, AT = 0.7871, CT = 5.6744, AG = 7.8251, CG = 0.5392, and GT = 1.0000. The ML substitution rate matrix for S7 was AC = 1.6804, AT = 1.0000, CT = 2.2493, AG = 1.6804, CG = 0.9727, and GT = 1.0000. For both loci combined, the ML substitution rate matrix was AC = 0.8539, AT = 0.7715, CT = 4.0213, AG = 4.0213, CG = 0.4488, and GT = 1.0000.

4. Discussion

4.1. A phylogenetic hypothesis for North American Pacific Coast sculpins

With the inclusion of 76 species from the NAPC and 99 species in total, this study nearly doubles the taxonomic coverage of previous work (Bolin, 1947) with few unresolved nodes and generally high statistical support (Fig. 1).

4.2. Monophyly of North American Pacific Coast radiation?

The NAPC sculpin assemblage is not monophyletic as a number of genera also include exclusively Northwestern Pacific species and/or Arctic and North Atlantic species (Table 1). For example, *Icelus ochotensis* and *I. toyamensis* are only found in the Northwestern Pacific but are nested within the otherwise NAPC genus *Icelus* (Fig. 1). Similarly, *Myoxocephalus brandtii* and *M. stelleri* are only found in the Northwestern Pacific but again are nested within the otherwise NAPC genus *Myoxocephalus* (Fig. 1). However, it has not previously been addressed if there is a monophyletic assemblage nested within the NAPC assemblage. All members of the *Chitonotous–Ruscarius–Artedius–Orthonopius–Clinocottus–Leiocottus–Oligocottus* clade (Fig. 1) are strictly found along the NAPC (predominately in the Eastern Pacific; Lamb and Edgell, 2010; Miller and Lea, 1972; Mecklenburg et al., 2002) and since many representative species of almost every closely related clade are included in this analysis, this clade likely constitutes a monophyletic assemblage.

4.3. Molecular vs. morphological hypotheses

For species represented in both this study and Bolin (1947), the phylogenetic hypotheses are statistically significantly different from each other (S–H test $P < 0.05$). This work also suggests that the family Cottidae is not monophyletic, as representatives of three other cottoid families (Rhamphocottidae, Hemitripteridae, and Psychrolutidae; Table 1) included in this study nest within the Cottidae (Fig. 1). However, formerly these families were included in the Cottidae (Taranets, 1941; Bolin 1944,1947). The Rhamphocottidae is comprised of a single species, the grunt sculpin (*Rhamphocottus richardsonii*). Some taxonomists classify grunt sculpins in the Cottidae, but Washington et al. (1984) and Yabe (1985) classified them as a primitive sister family to the other members of the superfamily Cottoidea. The Hemitripteridae, or sailfin sculpins, are comprised of eight marine species that all have tall first dorsal fins. Again, these species were formerly considered members of the Cottidae, but Washington et al. (1984) and Yabe (1985) classified them as the sister group to the Agonidae (poachers). Lastly, the Psychrolutidae (fathead, soft, or blob sculpins), includes about 30 species of loose-skinned, demersal marine sculpins that differ from other members of the Cottoidea by the presence of well-developed bony arches (which may bear spines) over the lateral line system of the head (Nelson, 1982). Taranets (1941) defined many subfamilies of cottids that are largely considered no longer reliable due to the body of evidence that now points to too many exceptions, and most researchers consider it premature to define subfamilies (Bolin, 1947; Mecklenburg et al., 2002).

4.3.1. Intrageneric relationships

I focus here on the evolutionary relationships within genera recovered in this phylogenetic analysis and how those proposed relationships compare to previous assessments. All genera found along the NAPC with two or more species included in this analysis are discussed below in alphabetical order.

4.3.1.1. Artedius. Bolin (1947) defined *Artedius* by its large head, normal pelvic fins, and unadvanced anus. However, as Begle (1989) points out, these are not synapomorphic characters for the group and are symplesiomorphic characters for the family. However, Begle (1989) found six morphological synapomorphies that support *Artedius*. Bolin (1947) was the first to offer a branching diagram for the *Artedius* species and Howe and Richardson (1978) agreed with Bolin that *A. corallinus* and *A. lateralis* appear to be closely related and that *A. fenestralis* and *A. notospilotus* share some characteristics suggesting affinity, but not to the extent as the above species pair. This work supports the conclusion that *A. corallinus* and *A. lateralis* are more closely related to each other than either is to the other *Artedius* species. However, this work places *A. fenestralis* and *A. harringtoni* in a polytomy with the split to *A. notospilotus* and *Clinocottus acuticeps* (Fig. 1), suggesting the genus as currently described is not monophyletic and may include *C. acuticeps*, but this warrants further investigation.

4.3.1.2. Blepsias. This genus is currently classified in the cottoid family Hemitripteridae along with the genera *Nautichthys* and *Hemitripterus* based on the presence of numerous prickles on the head and body, a knobby front-parietal ridge, broad plate-like epurals, and the absence of the basihyal bone (Yabe, 1985; Mecklenburg et al., 2002). There are only two currently recognized species in the genus *Blepsias* and they form a monophyletic pair with high statistical confidence (Fig. 1). However, they do not group with the *Nautichthys* species or with *Hemitripterus bolini* in this analysis (Fig. 1).

4.3.1.3. Clinocottus. Bolin (1947) defined *Clinocottus* based on the more anterior position of the anus between the anal fin and pelvic fins, the heavy and blunt penis, and the unmodified anal fin in both sexes. Howe and Richardson (1978) concurred with Bolin (1947) that *Clinocottus globiceps* and *Clinocottus recalvus* appear quite closely related and that *Clinocottus embryum* appears more closely related to these two species than to either *Clinocottus analis* or *Clinocottus acuticeps*. However, Howe and Richardson noted that the interrelationships of the species within the genus are in need of study. This study does not support the monophyly of *Clinocottus* in any of the analyses performed (Fig. 1) and is in agreement with Ramon and Knope (2008) that *Clinocottus* is not a natural group.

In this study, *C. acuticeps* appears to be the most distantly related to any of the other nominal species of *Clinocottus*. *C. analis* consistently groups with *Leiocottus hirundo* and basal to the *Oligocottus* clade (Fig. 1). All previous morphological investigators have concluded that *C. recalvus* and *C. globiceps* are sister species, with *C. embryum* the closest relative to this pair and this study supports that conclusion. This work also supports the conclusion by Howe and Richardson (1978) and Ramon and Knope (2008) that *C. acuticeps* and *C. analis* do not appear closely related to each other or to the other three species of *Clinocottus,* suggesting *C. acuticeps* and *C. analis* should be removed from the genus.

The genus *Clinocottus* was originally described as three separate genera (*Oxycottus, Blennicottus,* and *Clinocottus*) and Bolin (1947) subsumed them into *Clinocottus*. Bolin demoted the generic designations to subgeneric designations based on the following three lines of evidence: (1) progressive loss of preopercular spines, scales, and last gill slit, (2) head change from pointed to hemispherical, and (3) enlargement and elaboration of the penis. However, *C. analis* and *C. acuticeps* retain the primitive pointed head structure, unlike the other three *Clinocottus* species that have very large rounded heads (Bolin, 1944) and form a monophyletic group in this analysis (Fig. 1). In addition, Bolin (1947) was making an argument for the retention of the genus as a valid systematic category to counter the trend of systematic splitting that was common at the time, which may explain why *Clinocottus* is not a natural group.

4.3.1.4. Cottus. The freshwater sculpins in the genus *Cottus* have radiated throughout Northern Hemisphere freshwater habitats. The phylogenetic relationships among *Cottus* and related taxa were evaluated with molecular markers by Yokoyama and Goto (2005) and Kinziger et al. (2005). Yokoyama and Goto (2005) suggested that the common ancestor of freshwater sculpins was a euryhaline species, similar to *Leptocottus*, which is primarily marine but often enters lower reaches of rivers and streams. Kinziger et al. (2005) recognized the lack of monophyly for *Cottus* with respect to the Lake Baikal sculpins and the genus *Leptocottus.* Both Yokoyama and Goto (2005) and Kinziger et al. (2005) showed that *Cottus* is both polyphyletic and paraphyletic and suggest that the genus is in need of revision. While freshwater sculpins were not the focus of this study, the four species included form a monophyletic group with *Leptocottus* basal to this group (Fig. 1).

4.3.1.5. Enophrys. The genus and the relationships of the six recognized species are currently based on the morphometric analysis of Sandercock and Wilimovsky (1968). The *Enophrys* species are exclusively marine with plates along the lateral line, prickly scales below the lateral line in some species, a very long, sharp upper preopercular spine, and sharp nasal spines (Sandercock and Wilimovsky, 1968). This study includes only four of the six *Enophrys* species, limiting the ability to infer evolutionary relationships within the genus. However, it is important to note that the four species of *Enophrys* included in this study form a monophyletic group in all analyses performed (Fig. 1). Quast and Hall (1972) questioned the validity of *Enophrys lucasi* as a species distinct from *E. diceraus*. This analysis places the two as sister species with high statistical confidence (Fig. 1) and the two species are 2.7% divergent at cyt *b* and 3.4% divergent at S7, perhaps suggesting speciation or incipient speciation.

4.3.1.6. Gymnocanthus. Wilson (1973) reviewed the six species of the genus *Gymnocanthus* that inhabit the North Pacific, Arctic, and North Atlantic. Wilson described the genus as characterized by edentulous palatines and prevomer, granulations on the nape, scales restricted to axillary prickles, and an elongate, multi-cusped pre-opercular spine. The three species included in this study form a monophyletic cluster with high statistical support (Fig. 1), but since only half of the species in the genus are represented, I do not discuss their relationships further.

4.3.1.7. Hemilepidotus. Peden (1978) revised the systematics of *Hemilepidotus* and there are six currently recognized species in the genus. They all have three bands of scales: a dorsal band, a lateral band, and a ventral band and are generally similar in appearance (Bolin, 1947). All six species form a monophyletic clade in this analysis with very high statistical support (Fig. 1).

4.3.1.8. Icelus. The most recent revision of the genus *Icelus* and the very closely related *Rastrinus* were based on an osteological assessment by Nelson (1984). The monotypic *Rastrinus scutiger* is so closely related to *Icelus* that some researchers classify it in that genus (Mecklenburg et al., 2002). *Icelus* has one row of large, spiny plate-like scales below the dorsal fins, spiny tubular lateral line scales, scales on the pectoral axil and on the upper portion of the eye, and a nuchal spine or protuberance (Mecklenburg et al., 2002). As only seven of the broadly distributed twenty species of the genus are included here, I do not discuss relationships within the genus. However, I note that four species cluster in a monophyletic clade and the remaining three species cluster with *Rastrinus scutiger, Nautichthys pribilovius,* and *Synchirus gilli* in a sister group (Fig. 1).

4.3.1.9. Icelinus. Bolin (1936) described eight species in the genus, but recent discoveries place 11 species in the genus (Peden, 1984; Yabe et al., 1980; Yabe et al., 2001; Rosenblatt and Smith, 2004). The genus is diagnosed as including an antler-like fourth (dorsal-most) preopercular spine, a pelvic fin with one spine and two soft rays, and two rows of ctenoid scales extending along the base of the dorsal fins (Bolin, 1936, 1944). All five species included in this analysis form a monophyletic group with high statistical confidence (Fig. 1).

4.3.1.10. Myoxocephalus. Neylov (1979) described the genus as having a long, straight upper pre-opercular spine, an opening behind the last gill arch, a lower jaw that does not extend beyond the upper, and absence of cirri from the cheeks, jaws, and lateral line. Of the twenty species in the genus, six were included in this study and from a monophyletic clade with high statistical support (Fig. 1), except for one specimen identified as *M. cf. scorpiodes* which groups with a sister clade and basal to *Megalocottus platycephalus* and *Triglops quadricornis*, and could represent a previously undescribed species.

4.3.1.11. Nautichthys. There are three species of sailfin sculpins in the genus *Nautichthys* (Hemitripteridae) differentiated from other members of the putative family Hemitripteridae by branched rays in the caudal fin and lack of cirri on the lower jaw (Mecklenburg et al., 2002). The two species included in this study (*N. oculofasciatus* and *N. pribilovius*) appear distantly related to one another (Fig. 1), with *N. oculofasciatus* in a basal polytomy with *Jordania zonope* and *N. pribilovius* nested within the clade primarily comprised of *Icelus* species (Fig. 1).

4.3.1.12. Oligocottus. The genus *Oligocottus* contains four species originally identified as sharing common ancestry by Hubbs (1926). Bolin (1947) concurred with this conclusion, presenting the affinities of the four species and diagnosed the genus *Oligocottus* by the location of the anus directly in front of anal fin origin, the shape of the penis as a long, slender, simple cone, and the modification of the anterior anal rays in males. Bolin suggested that *O. rubellio* and *O. snyderi* are the most specialized and the most closely related. *O. maculosus* was considered less specialized and less closely related to both of the aforementioned species. Bolin stated that *O. rimensis* is the most primitive and equally related to the other three species. Howe and Richardson (1978) agreed with the designation of *Oligocottus* as a monophyletic genus, but did not suggest any further affinities. This analysis confirms the monophyly of *Oligocottus* and supports the conclusion that *O. rimensis* is the basal species in the group, but places the other three species into a polytomy (Fig. 1).

4.3.1.13. Porocottus. Neylov (1976, 1979) classified *Porocottus* and *Microcottus* by the upper preopercular spine being abruptly curved upward and by the lack of slit or pore behind the fourth gill arch. Neylov distinguished *Porocottus* from *Microcottus* by the postocular and occipital cirri being usually multifid. There are currently ten species classified in *Porocottus* and the two species of *Porocottus* in this study group together with high statistical support (Fig. 1), but without the inclusion of the other species in the genus they are not necessarily sister species.

4.3.1.14. Pseudoblennius. There are currently six described species in the genus *Pseudoblennius* and an additional three undescribed species (Nakobo, 2002; Sado et al., 2005). All species are restricted to the Northwestern Pacific. The two species included in this study (*P. percoides* and *P.* sp. 3 *sensu* Nakobo, 2002) form a monophyletic grouping with high statistical support (Fig. 1).

4.3.1.15. Ruscarius. Begle (1989) proposed that the former *Artedius creaseri* and *Artedius meanyi* are the sister clade to the monotypic *Chitonotus*. In fact, Begle found no synapomorphies that unite *A. creaseri* and *A. meanyi* with the other *Artedius* species. However, these two species share seven synapomorphic morphological characters with each other, prompting Begle to resurrect the genus *Ruscarius* for these two species. In addition to Begle's study, Washington (1986), Strauss (1993), and Ramon and Knope (2008) have found evidence that these two species do not belong with the other *Artedius* species and concurred that these species should be placed back in their original genus *Ruscarius*. This study further supports that conclusion with strong statistical support (Fig. 1).

4.3.1.16. Triglops. Pietsch (1994) revised the classification of *Triglops* and concluded that there are nine valid species of *Triglops*. Pietsch defined the genus based on the following characters: a small head, a narrow, elongate body, a slender caudal peduncle, a long anal fin containing 18–32 rays, pelvic fins with a single spine and three soft rays, branchiostegal membranes united on the ventral midline but lying free from the isthmus, and the scales below the lateral line modified to form discrete rows of tiny serrated plates that lie in close-set, oblique dermal folds. Eight of the nine *Triglops* species included here form a monophyletic clade with high statistical support (Fig. 1), however *T. quadricornis* does not group with the other species. Neylov (1979) changed *Myoxocephalus quadricornis* to *Triglops quadricornis* and this work suggests that this species does not belong in either genus (Fig. 1).

4.4. Why is the North American Pacific Coast sculpin radiation so speciose?

Many marine organisms have the potential for long distance dispersal with long-lived and planktonic eggs and/or larvae, but recent work shows this is not always the case (e.g., Bernardi and Bucciarelli, 1999; Swearer et al., 1999; Bernardi and Talley, 2000; Bernardi, 2005; Cowen et al., 2006; Shanks and Shearman, 2009). Sculpins are known to display high site fidelity as adults (Yoshiyama et al., 1986; Yoshiyama et al., 1992; Knope and Tice, unpubl. data) and are not broadcast spawners, so gene flow is likely to be mediated only through the larval stage (Budd, 1940; Hubbs, 1966; Swank, 1979; Ramon, 2007). Judging from the widespread distribution of species, sculpin larvae can likely disperse great distances. However, along the NAPC, the larvae of intertidal species are generally only found near shore in plankton tows (Marliave, 1986) and population genetic surveys have found low to moderate levels of gene flow among populations (Swank, 1979; Waples, 1987; Ramon, 2007; Knope, unpubl. data). So it may be that sculpin larvae can disperse enough to colonize novel ranges and habitats, but have limited ongoing gene flow facilitating speciation by diversifying selection (Simpson, 1953; Schluter, 2000). Further, colonization of new habitats is hypothesized to open up new ecological opportunities and thus promote lineage diversification (Simpson, 1953; Schluter, 2000). Sculpins can be found in almost every aquatic habitat type and it appears that repeated habitat shifts have facilitated their diversification. For example, Ramon and Knope (2008) and Mandic et al. (2009) found a clear trend in the *Chitonotous–Ruscarius–Artedius–Orthonopius–Clinocottus–Leiocottus–Oligocottus* clade that subtidal sculpins in this clade are basal-rooted species, while the most recently derived species are primarily found in the intertidal. In addition to repeated habitat shifts, it appears that allopatric speciation may have been the mode of speciation for a number of sculpin taxa (Ramon and Knope, 2008). For example, *R. meanyi* has a northern distribution ranging from the Gulf of Alaska to northern California and *R. creaseri* has a southern distribution ranging from central California to central Baja California (Miller and Lea, 1972). This biogeographic pattern suggests the possibility that allopatric speciation has occurred in the somewhat recent geologic past, but the possibility that sympatric speciation was followed by subsequent range separation cannot be discounted (Losos and Glor, 2003).

5. Summary

In many respects, this work supports the phylogenetic relationships proposed by previous authors working with morphological characters. However, this phylogeny differs in a number of respects from those of previous investigators. Other molecular markers could be useful in resolving the discrepancies. In addition, the phylogenetic relationships may become more clearly resolved by reexamining the morphological characters that Bolin investigated using modern phylogenetic methods that can distinguish shared ancestral from shared derived characters. Future research should aim towards complete taxonomic sampling of all approximately 275 cottid species and related cottoid families to fully resolve the evolutionary history of this successful radiation of fishes. In addition, further identification of drivers of speciation in this radiation may provide insights into what causes some lineages to diversify greatly, while others do not.

Acknowledgments

I thank: Giacomo Bernardi and two anonymous reviewers for improvements to the manuscript; Tadashi Fukami for support and advice; Jilian Chapman, David Christie, Robert Foy, Erica Fruh, Stewart Grant, Michael Hardman, Richard Hocking, Shama Hinard, Yoshiaki Kai, Dan Kamikawa, Aimee Keller, Andrew Kinziger, Heather Leba, Ty Linderoth, Katherine Maslenikov, Mifuyu Nakajima, Carmen Olito, James Orr, Sheamus Riley, Gento Shinohara, and David Tallmon for assistance with obtaining samples; Nicole Bitler, Pasha Feinberg, Olivia Isaac, Christine Kyauk, and Alexandra Ritchie for assistance with lab work; Greg Cailliet, Frank Cipriano, Liz Hadly, Rodolfo Dirzo, Ralph Larson, Robert Lea, Milton Love, Jonathan Payne, Marina Ramon, Greg Spicer, and Ward Watt for advice; and Melinda Belisle, Lis Castillo Nelis, Tadashi Fukami, Jimmy O'Donnell, and Marina Oster for comments. Financial support was provided by Stanford University, a Stanford University VPUE SCORE grant, and a Dr. Earl H. Myers and Ethel M. Myers Oceanographic and Marine Biology Trust grant.

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