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Molecular support for marine sculpin (Cottidae; Oligocottinae) diversification during the transition from the subtidal to intertidal habitat in the Northeastern Pacific Ocean

Marina L. Ramon [a,*], Matthew L. Knope [b,1]

[a] University of California Santa Cruz, Department of Ecology and Evolutionary Biology, Long Marine Laboratory, 100 Shaffer Road COH 247, Santa Cruz, CA 95064, USA
[b] San Francisco State University, Department of Biology, 1600 Holloway Avenue, San Francisco, CA 94132, USA

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Abstract

Sculpins in the genera *Ruscarius*, *Artedius*, *Clinocottus*, and *Oligocottus* are common intertidal and subtidal benthic fishes of the Northeastern Pacific Ocean. While there has been a long history of attempts to reconstruct the evolutionary relationships within this clade, studies have largely resulted in conflicting conclusions. Current ideas regarding the limits of species and genera in this subfamily (Oligocottinae) and their branching order are based primarily on morphology [Bolin, R.L., 1944. A Review of the Marine Cottid Fishes of California. Natural History Museum of Stanford University, Stanford University, California; Bolin, R.L., 1947. The Evolution of the Marine Cottidae of California with a Discussion of the Genus as a Systematic Category. Stanford University, California]. The primary objectives of this study are: (a) to determine if the phylogenetic relationships inferred from DNA characters are concordant with those inferred from morphological characters and (b) to determine if a habitat shift from the subtidal to the intertidal environment resulted in the diversification of the group. Cytochrome *b* and Nicotinamide Adenine Dinucleotide Dehydrogenase subunit one mitochondrial gene fragments and one nuclear intron (S7 ribosomal protein) were sequenced in order to infer the phylogenetic relationships within this subfamily. Maximum likelihood and Bayesian algorithms were employed to reconstruct phylogenetic trees. We found that several genera in this clade are not monophyletic and that there is a clear phylogenetic signal indicating that a habitat shift from the subtidal to the intertidal habitat has resulted in the diversification of the Oligocottinae.

Keywords: Cottidae; Phylogeny; mtDNA; Nuclear DNA; Sculpins; Intertidal

1. Introduction

The origin of marine intertidal communities has long been a perplexing question to natural historians. Approximately 150 years ago, Charles Darwin asked what causes some lineages to diversify more than others. More recently, evolutionary ecologists have begun to explore the particular mechanisms that may have facilitated marine intertidal radiations (Appelbaum et al., 2002; Burton et al., 2006; Marko, 2005). In a review of the systematics of intertidal fishes, Chotkowski et al. (1999) concluded that the ichthyofaunal assemblage is a result of repeated invasions by subtidal species and subsequent speciation events. While it is clear that intertidal fish assemblages have evolved from multiple independent lineages, how these radiations have evolved remains an unanswered question.

Intertidal fish are found in several superorders throughout the world (including Euteleostei, Paracanthopterygii, and Acanthopterygii). Within the Acanthopterygii, the percomorph orders Scorpaeniformes, and Perciformes contain the greatest number of intertidal species. However, neither

* Corresponding author. Fax: +1 831 459 3383.
E-mail address: ramon@darwin.ucsc.edu (M.L. Ramon).
[1] Present address: University of Hawaii, Manoa, Department of Zoology, Edmondson 256, Honolulu, HI 96822, USA.

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of these orders are comprised exclusively of intertidal species (Chotkowski et al., 1999; Nelson, 2006). Within the order Scorpaeniformes, sculpins in the family Cottidae provide an excellent opportunity to examine whether a habitat shift into the intertidal has lead to speciation. In particular, we chose to examine the subfamily Oligocottinae, which is considered to be a monophyletic group comprised of 16 species. Within this subfamily some species are found only in the intertidal, others only in the subtidal, and some in both habitats. Hence, this is an ideal system for a case study exploring whether a habitat shift can lead to diversification.

1.1. Background on Northeast Pacific sculpins

Worldwide, sculpins in the family Cottidae include approximately 275 species found primarily in boreal and cold-temperate regions (Nelson, 2006). Cottids (*sensu stricto*) are generally small, intertidal and subtidal benthic fishes that exhibit extensive diversity in their reproductive biology, feeding ecology, and habitat choice (Bolin, 1944; Normark et al., 1991; Petersen et al., 2005; Yaakub et al., 2006; Yoshiyama et al., 1986). The family Cottidae is generally considered to be of recent (Oligocene or Miocene) North Pacific origin (Berg, 1947; Macfarlane, 1923) but is not well represented in the fossil record. Although sculpins are found in all oceans of the world except the Indian Ocean, the Northern Pacific has been identified as the center of origin and diversity for the family (Eschmeyer, 1983; Watanabe, 1969; Yabe, 1985). With over 40 currently recognized genera and 90 species distributed between Baja California and the Aleutian Islands, the Cottidae represent one of the most species-rich taxa with approximately 10% of the known species in the ichthyofauna of this region (Bolin, 1944; Howe and Richardson, 1978).

While there has been a long history of attempts to reconstruct the phylogenetic relationships of the Northeastern Pacific cottids (Bolin, 1947; Gill, 1889), many relationships remain largely unresolved. Current ideas about the evolutionary history and definitions of genera in this group of cottids are based primarily on Bolin (1944, 1947). Using a phenetic approach, Bolin (1947) was the first to offer a branching diagram for the Northeastern Pacific cottids. There has since been little formal re-investigation of Bolin's proposed phylogeny. Yabe (1985) used 60 osteologic and myologic characters to construct a hand-rendered cladistic phylogeny of 61 genera of the superfamily Cottoidea. In addition, Jackson (2003) used 100 plus morphological characters to address the phylogenetic relationships within the Cottoidea. Although these phylogenies helped to resolve cottoid/cottid arguments, most generic and specific relationships within the Cottidae remain largely unresolved.

The primary objectives of this study are: (a) to determine if the phylogenetic relationships inferred from DNA characters are concordant with those inferred from morphological characters and (b) to determine if a habitat shift from the subtidal to the intertidal environment caused the diversification of the group. To evaluate these questions, we generated a molecular phylogeny based on two mitochondrial genes (cytochrome *b* and Nicotinamide Adenine Dinucleotide Dehydrogenase subunit one) and a nuclear intron (ribosomal protein S7) for 27 recognized taxa.

2. Materials and methods

2.1. Collection, amplification, and sequencing

Samples of sculpins were collected from several locations in Baja California, California, Oregon, Washington, and Alaska (Table 1). Whenever possible, several individuals were collected from different locations to assess geographical intraspecific genetic variation. Intertidal specimens were collected in the field by dipnet at low tides. Nearshore subtidal specimens were collected by dipnet or pole speared by divers using SCUBA. Deepwater specimens were collected by trawl on the R/V *David Starr Jordan*. Whole genomic DNA was extracted from muscle tissue or fin clippings that had been stored in 95% ethanol. Tissues were digested overnight at 55 °C in 500 μl salt extraction buffer (400 mM NaCl, 10 mM Tris, 2 mM EDTA, 1% SDS, and 20 μg/ml proteinase K). DNA was purified by standard chloroform extractions and isopropanol precipitation (Sambrook et al., 1989). Samples were processed using a standard PCR method to amplify mitochondrial cytochrome *b* (GluDG-L and CB3H from Palumbi, 1991), Nicotinamide Adenine Dinucleotide Dehydrogenase subunit one (L3827, L4500, H5191 modified from Sorenson et al., 1999) and the first nuclear intron S7 ribosomal protein (S7RPEX1F and S7RPEX2R, Chow and Hazama, 1998). PCR amplifications were performed in a 25 μl volume with 1 U AmpliTaq DNA Polymerase (Perkin-Elmer), the manufacturer's buffer, 2.5 mM $MgCl_2$, 0.25 mM each dNTP, and 1 μM each primer. PCR products were gel purified in 1% agarose, excised from the gel, and then recovered using a QIAquick Gel Extraction Kit (QIAGEN). Double-stranded PCR products were sequenced directly with the same primers used for the PCR amplifications using BigDye Chemistry (Applied Biosystems). The sequencing reaction products were cleaned of unincorporated dyes and salts by isoproponal precipitation and then run on an ABI3100 DNA Sequencer (Applied Biosystems). Sequences of the two DNA strands were aligned and reconciled in Sequence Navigator (Applied Biosystems). All sequences have been deposited in GenBank (Accession Nos. EF521313–EF521387).

2.2. Phylogenetic analysis of mtDNA and nuclear data

Alignments of mitochondrial gene regions were unambiguous and were aligned by eye using the program Se–Al (Rambaut, 1996). The nuclear gene was aligned using Clustal X with the default settings. Double peaks in nuclear

Table 1
Taxa represented in the phylogenetic analysis, abbreviation, corresponding sampling locations, number of individuals sampled, and number of bases analyzed per species for each of the three genes used

Taxon	Abbreviation	Locations[a]	Total Sampled	Cyt *b*[b]	ND1[c]	S7[d]
Rhamphocottus richardsonii	RHRI	CA	1	393	0	468
Stellerina xyosterna	STXY	CA	1	393	507	486
Scorpaenichthys marmoratus	SCMA	CA	3	731	507	387
Jordania zonope	JOZO	CA	1	752	507	0
Hemilepidotus hemilepidotus	HEHE	CA	2	810	488	486
Leptocottus armatus	LEAR	CA	1	810	507	138
Enophrys bison	ENBI	CA	2	810	507	0
Orthonopias triacis	ORTR	CA	4	810	507	486
Icelinus borealis	ICBO	AK	1	810	484	171
Chitonotus pugetensis	CHPU	CA	1	810	507	486
Ruscarius meanyi	RUME	CA	1	754	507	141
Ruscarius creaseri	RUCR	CA	2	810	507	384
Artedius corallinus	ARCO	CA	1	810	495	308
Artedius lateralis	ARLA	CA	4	810	507	486
Artedius fenestralis	ARFE	AK	4	810	507	486
Artedius harringtoni	ARHA	AK	3	810	507	486
Artedius notospilotus	ARNO	CA	1	408	507	0
Oligocottus snyderi	OLSN	CA, OR	5	810	507	486
Oligocottus maculosus	OLMA	OR	2	810	507	486
Oligocottus rimensis	OLRI	CA	2	810	507	486
Oligocottus rubellio	OLRU	CA	3	810	498	486
Clinocottus analis	CLAN	CA, MEX	8	810	495	486
Clinocottus acuticeps	CLAC	CA, OR	4	810	507	486
Clinocottus embryum	CLEM	AK, WA	3	810	494	486
Clinocottus globiceps	CLGL	CA, OR	5	810	507	486
Clinocottus recalvus	CLRE	CA	3	810	507	486
Leiocottus hirundo	LEHI	CA	1	810	507	486

[a] CA, California; AK, Alaska; OR, Oregon; WA, Washington, MEX, Baja California, Mexico.
[b] Cyt *b*, cytochrome *b*.
[c] ND1, nicotinamide adenine dinucleotide dehydrogenase subunit one.
[d] S7, S7 ribosomal protein.

gene sequences, reflecting heterozygous positions, were coded with IUPAC degeneracy codes and were treated as polymorphisms. Missing or incomplete data were treated as gaps (TreeBASE Accession No. S1924). All trees were rooted with *Rhamphocottus richardsonii* (Family: Rhamphocottidae) except for the Nicotinamide Adenine Dinucleotide Dehydrogenase subunit one gene tree (NADH1), which was rooted with *Stellerina xyosterna* in the family Agonidae (Crow et al., 2004; Nelson, 2006; Smith and Wheeler, 2004). Phylogenetic relationships were assessed by maximum likelihood (ML) and Bayesian inference (BI) methods.

For the ML analysis, the computer program Modeltest 3.1 (Posada and Crandall, 1998) was used to determine the appropriate model of sequence evolution for each gene independently and for all genes combined. Modeltest uses a set of hierarchical likelihood ratio tests to discriminate among 56 progressively more complex models of nucleotide evolution. The models chosen for each of the datasets are as follows: all genes combined and the cytochrome *b* (Cyt *b*) dataset alone best fit the transversional model with invariable sites and rate variation among sites included (TVM + I + G), the NADH1 data best fit the general time-reversible model (Lanave et al., 1984; Rodriguez et al., 1990) with invariable sites and rate variation among sites included (GTR + I + G) and the S7 data best fit the Hasegawa, Kishino, Yano 85 model (Hasegawa et al., 1985) with rate variation among sites included (HKY + G). The appropriate models of sequence evolution were used to find the best ML tree using a heuristic tree search with 10 random, stepwise additions and TBR branch swapping. Bootstrap support values were obtained with 1000 pseudoreplicates and "FAST" stepwise addition in PAUP* version 4.0b10 (Swofford, 2002).

MrModeltest 2.1 (Nylander, 2004) was used to determine the optimal model of nucleotide evolution to implement in MrBayes 2.1 (Huelsenbeck and Ronquist, 2001) and statistical confidence in nodes were evaluated by Bayesian posterior probabilities. The model invoked for the Bayesian analysis with the data combined was the general time reversible with invariable sites and rate variation among sites included (GTR + I + G). When the data was partitioned by gene, the models of best fit were the GTR + I + G for Cyt *b* and NADH1 and the HKY + G for S7. Starting trees were chosen by random selection. Stationarity of tree likelihood, sampled every 100 cycles, was achieved after 40,000 (combined dataset) and 30,000 (data partitioned by gene) generations and all sampled trees preceding stationarity were discarded.

2.3. Testing alternative phylogenetic hypotheses

The phylogenetic hypothesis based on morphological characters (Bolin, 1947) was compared to our combined ML topology. In addition, Bolin's unresolved polytomy for the *Artedius–Clinocottus–Oligocottus* (A–C–O) split was resolved for this test by grouping *Oligocottus* as the sister clade to *Artedius* and alternatively as the sister clade to *Clinocottus*. Both of these alternative trees were compared to the best maximum likelihood tree for the molecular data. In addition we tested the monophyly of *Clinocottus*, *Oligocottus*, *Artedius*, and *Ruscarius*. Since Bolin's phylogeny did not include *R. richardsonii, S. xyosterna*, and *Ruscarius meanyi* they were removed from the molecular tree for the test. The alternative phylogenetic hypotheses were used as topological constraints for ML analysis with identical parameters as the unconstrained analysis. The resulting topologies were compared with the unconstrained ML tree using the Shimodaira and Hasegawa test (hereafter referred to as S–H test; Shimodaira and Hasegawa, 1999) as implemented in PAUP*.

2.4. Habitat mapping

To evaluate the evolution of habitat shifts we mapped habitat affinities onto our BI tree using MacClade 4.08 (Maddison and Maddison, 2005). Habitat affinities were determined using data from Miller and Lea (1972), Fish Base (http://www.fishbase.org/search.php), and personal observations.

3. Results

3.1. Sequences

Approximately 1803 bp were sequenced for 69 individuals representing 27 species of cottids. The fragment lengths used for phylogenetic analysis for each of the genes are as follows: Cyt $b = 810$ bp, NADH1 = 507 bp, and S7 = 486 bp, resulting in a total of 486 bp of nuclear and 1317 bp of mitochondrial DNA (Table 1). Mitochondrial loci exhibited no insertions or deletions (indels). The nuclear gene contained 13 indels, which were each coded as single evolutionary changes. We analyzed the data with and without indels, and we included them in the final analysis because excluding them made no difference (S–H test $p < 0.05$). Few or no nucleotide differences were found for several individuals within each species, therefore, for the phylogenetic analysis we used a consensus sequence per species.

Due to PCR amplification difficulties S7 was not sequenced for *Enophrys bison*, *Jordania zonope*, and *Artedius notospilotus*. Sequencing attempts were unsuccessful in *R. richardsonii* for NADH1 and Karen D. Crow provided the S7 sequence. Of a total 810 bp fragment for cytochrome *b*, only 408 bp were successfully sequenced for *A. notospilotus*, and 393 bp for *R. richardsonii* (GenBank Accession No. AY582105) and *S. xyosterna* (GenBank Accession No. AY582108).

Plots of transitions and transversions versus genetic distances for independent loci indicated that saturation was not reached for any of the genes used (not shown). Observed transitions to transversion ratios ranged from 0.97 to 2.935.

3.2. Tree topologies

Both of the mitochondrial gene trees were similar, but not identical, and few nodes had more than 50% bootstrap support (Fig. 1A and B). The nuclear ribosomal S7 gene recovered even less support for many of the tree nodes (Fig. 1C). Because each of the genes analyzed independently did not show strong bootstrap support for many nodes and a S–H test indicated that neither tree was significantly different, we combined all of the data. The combined data analysis of the 1803 bp characters from the three genes yielded 635 parsimony informative characters. The ML ($-\ln L = 16362.155$) and BI tree topologies were identical to each other, therefore only the Bayesian tree is shown (Fig. 1).

Our combined analysis tree was compared to Bolin's tree (Fig. 2) and the alternative hypotheses of monophyly for *Oligocottus*, *Clinocottus*, *Artedius*, and *Ruscarius*. In all cases, our tree was found to be the significantly shorter (S–H test $p < 0.05$). Several of the major differences between Bolin's morphological tree and our tree are that in the molecular tree *Clinocottus* is not monophyletic and *Orthonopias triacis* is subsumed within *Oligocottus* as opposed to being a sister taxon to the *Artedius–Clinocottus–Oligocottus* (A–C–O) clade. Our tree also supports the hypothesis that *Ruscarius* is sister and basal to the A–C–O clade and not incorporated within *Artedius*.

3.3. Habitat mapping

The mapping of habitat affinity onto our BI combined data tree resulted in a clear trend that shows that subtidal sculpins in this clade are ancestral species, while the most derived species are primarily found in the intertidal. However, among the ancestral species, *Hemilepidotus hemilepidotus* can be found in both intertidal and subtidal habitats and *Leptocottus armatus* can be found in the intertidal and freshwater habitats (Fig. 1).

4. Discussion

Our work supports the ideas of several others who have suggested that intertidal fauna have arisen through invasions from the subtidal zone (for review see Chotkowski et al., 1999; Johnson and Baarli, 1999). In addition, the monophyly of several genera based on morphological assessments are not supported by the molecular phylogeny we constructed from mitochondrial and nuclear loci (Fig. 1).

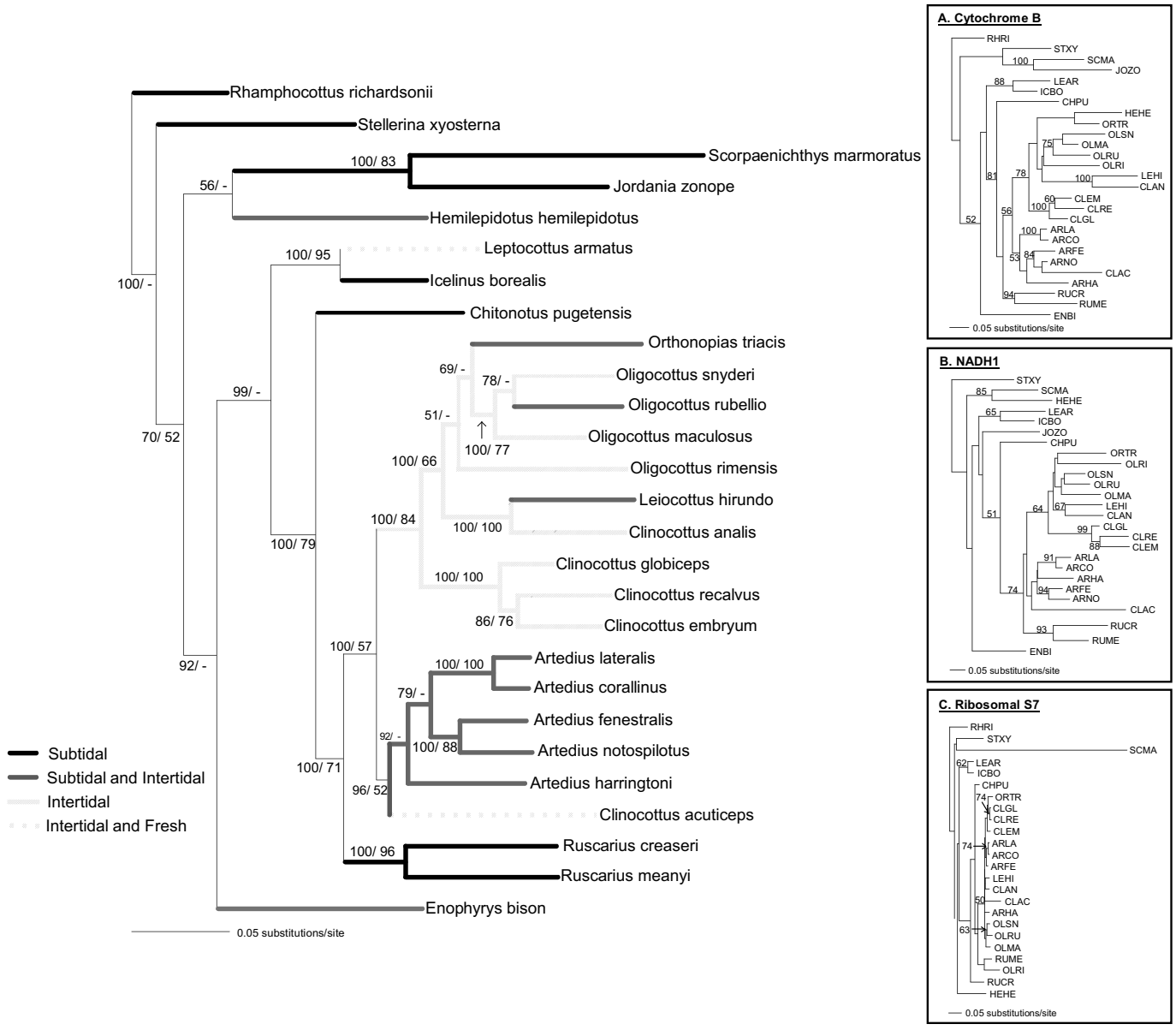

Fig. 1. Bayesian reconstruction of several genera of sculpins using two mitochondrial genes (Cyt *b*, NADH1) and one nuclear gene (S7). Bootstrap values are either above or below each node (BI and ML, respectively). Dashed lines mean that there was less than 50% bootstrap support. Side panels (A–C) are ML tree topologies with bootstrap support values shown for clades with more than 50% support. Habitat affinities have been mapped onto the tree and are depicted with different line patterns.

4.1. Morphological versus molecular phylogeny

In addition to Bolin's analysis, several phylogenetic hypotheses have been reconstructed based on morphological traits and little consensus has been reached on the relationships of some species, while others have received consistent support. We compared our molecular phylogeny with Bolin's morphological tree and found many similarities and several discrepancies between the two (Fig. 2). It is important to note that the molecular phylogeny supports the monophyly of the Oligocottinae (A–C–O) if the *Ruscarius* species are removed.

The genus *Artedius* has had several revisions in the last two decades and our results support the most recent revisions. Both Washington (1986) and Begle (1989) suggested that *Artedius creaseri* and *Artedius meanyi* do not share a unique common ancestor with the other *Artedius* species. These two species share several synapomorphic morphological characters with each other and none with the other *Artedius* species. This prompted Begle (1989) to resurrect the genus *Ruscarius* (Jordan and Starks, 1895) for these two species. Our data strongly support the monophyly of *Ruscarius creaseri* and *R. meanyi* for both the combined dataset and each of the mitochondrial markers (Fig. 1). Therefore we concur with both authors that *Ruscarius* should not be included in the same genus with *Artedius*. Further, we support the name change from *A. creaseri* and *A. meanyi* to their original designa-

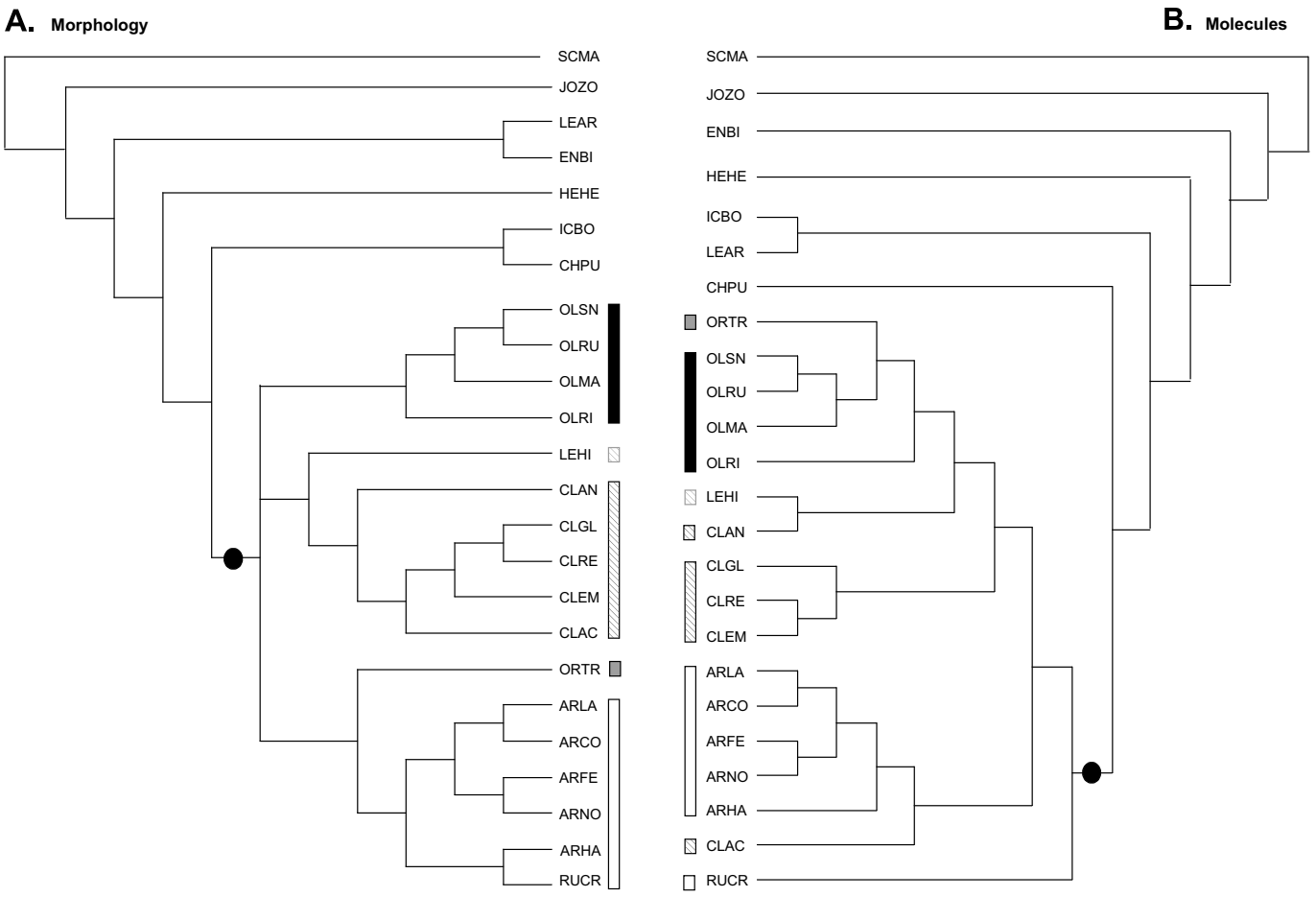

Fig. 2. (A) Reconstruction of Bolin (1947) morphological phylogeny. (B) Molecular phylogeny, combined analysis of three genes, using a maximum likelihood approach. Limits of genera, as defined by Bolin (1947) are coded by boxes with different shading patterns (see Table 1 for taxon names).

tion of *R. creaseri* and *R. meanyi* (Jordan and Starks, 1895).

In addition, another difference between the morphological and molecular trees is that the genus *Clinocottus* is polyphyletic in the molecular tree (Figs. 1 and 2). This genus was originally described as three separate genera (*Oxycottus*, *Blennicottus*, and *Clinocottus*) and Bolin later subsumed them all into *Clinocottus*. While others (Begle, 1989; Strauss, 1993) were not certain of their relationships and suggested that more work was needed. In contrast, Swank (1988) using phenetic and cladistic analyses based on allozyme evidence, provided support for Bolin's assessment of the evolution of the *Clinocottus* genus. However, our work consistently supports the conclusion by Howe and Richardson (1978) that *C. acuticeps* and *C. analis* do not appear closely related to each other or to the other three species of *Clinocottus*. In our phylogeny, *C. analis* is the sister taxon to the monotypic *Leiocottus hirundo* and *C. acuticeps* is immediately basal to *Artedius*.

Another important discrepancy between the two trees is the monophyly of *Oligocottus* (Fig. 2). In our molecular phylogeny, the monotypic *O. triacis* falls within *Oligocottus*, however it is important to note that statistical support for this node is weak and this relationship should be considered tenuous awaiting further investigation. Several authors (Begle, 1989; Howe and Richardson, 1978) have been uncertain about where *O. triacis* should fall in the phylogeny. Bolin (1947) and Howe and Richardson (1978) believed that it was probably related to *Artedius* based on scales, cirri, preopercular spines, gills, and gill membranes and the structure and pattern of the teeth. Yet, it is important to note that many of these characters appear to be highly plastic and convergent within the Cottidae (see Horn, 1999). It is perhaps the use of these types of characters that have fueled the historical systematic confusion within the family.

Our results also suggest that several genera are not monophyletic, but this is perhaps not surprising given their contentious systematic history. For example ichthyologists have long disagreed about the systematic position of *C. analis* and *O. triacis*. Additionally, Crow et al. (2004) suggest that *Scorpaenichthys marmoratus* might not belong within the family Cottidae based on a molecular phylogenetic assessment, but more data are needed to resolve this important question. On the other hand, there are a number of relationships that are supported by both previous morphological phylogenies and our molecular phylogeny. For example, *Clinocottus globiceps*, *Clinocottus recalvus*, and

Clinocottus embryum are consistently a monophyletic group in all assessments, which is supported by their extremely similar morphologies. Additionally, after the removal of the *Ruscarius* species, the remaining five species in the genus *Artedius* are supported as a monophyletic group. Further, our molecular phylogeny strongly corroborates the monophyly of *Ruscarius*. Although our phylogeny does not include all cottid species, we believe that the A–C–O clade is sister to *Ruscarius* and that the A–C–O are more derived when compared to the other cottid species, based on a mitochondrial gene rearrangement found only in the Oligocottinae subfamily (A–C–O) including *L. hirundo* and *O. triacus* (Ramon and Sorenson, unpublished, Fig. 1).

4.2. Subtidal origin

Mapping habitat affinities onto a phylogeny can provide a fuller understanding of the evolutionary patterns within a clade (Ahn and Ashe, 2004; Bargelloni et al., 2000; Ruber et al., 2003; Williams and Reid, 2004). When we mapped habitat affinities onto our molecular phylogeny, a very clear trend appeared where ancestral species are found primarily in the subtidal and derived species are primarily intertidal (Fig. 1). All species basal to the A–C–O clade are subtidal species, with the exceptions of *L. armatus* (intertidal and freshwater), *H. hemilepidotus* (subtidal and intertidal) and *E. bison* (subtidal and intertidal). While we did not sample all species in the branch considered to be immediately basal to the Oligocottinae, the entire clade is a deep-water subtidal branch (Bolin, 1947).

Within the A–C–O, the *Artedius* species can be found both in the intertidal and subtidal. However all of the *Oligocottus* and *Clinocottus* species are intertidal with the exceptions of two species (*Oligocottus rubellio* and *C. aculiceps*) found in the intertidal as well as in other habitats (subtidal and freshwater). Martin (1991) and others (Wright and Raymond, 1978; Yoshiyama and Cech, 1994) have found that several resident intertidal species (*Oligocottus maculosus*, *Oligocottus snyderi*, *C. analis*, *C. globiceps*, and *C. recalvus*) have specific physiological (air-breathing) and behavioral traits not present in the ancestral subtidal species (*J. zonope, Icelinus borealis and Chitonotus pugetensis*), which further supports our hypothesis of a subtidal to intertidal invasion. However, Martin (1996) is the only study that has tested for air-breathing ability using depth as an ecological gradient in fish. Clearly, more studies like this are needed to conclude that air-breathing ability is an adaptation to the intertidal and not the result of an ancestral trait in subtidal species. We are, of course, not the first to suggest that subtidal species invaded the intertidal, however our phylogeny is the first that we are aware of to clearly support the hypothesis that subtidal sculpin species are ancestral to intertidal sculpin species.

Lastly, there is evidence from two sister species of *Artedius* (*A. lateralis* and *A. corallinus*) of depth stratification. *A. lateralis* is primarily found in the intertidal and *A. corallinus* in the subtidal. This depth stratification suggests the possibility of ecological speciation initiating the cladogenic split, however, the possibility of vicariant speciation followed by displacement of depth ranges after their ranges expanded cannot be discounted. In contrast, two species pairs (*R. creaseri–R. meanyi* and *A. fenestralis–A. notospilotus*) have largely non-overlapping ranges. *R. meanyi* and *A. fenestralis* have northern distributions and *R. creaseri* and *A. notospilotus* have southern distributions (Miller and Lea, 1972). This biogeographic pattern suggests the possibility that allopatric speciation has occurred in the somewhat recent geologic past, or alternatively, that sympatric speciation was followed by subsequent range separation. However, caution is warranted when using modern geographic range data to infer historical modes of speciation (Losos and Glor, 2003).

4.3. Comments on systematics and future work needed

While we had hoped that molecular markers might give us a clearer understanding of the relationships within the Oligocottinae subfamily, they have in many instances complicated the story (Fig. 2). In those cases where this work is congruent with morphological phylogenies, we can be reasonably confident of the proposed relationships. In those cases where the molecular and morphological trees are incongruent, the proposed relationships based on either method should be considered tenuous and further investigation is necessary to resolve these differences. In addition, the phylogenetic relationships may become more clearly resolved if the morphological characters investigated in the past by several authors (Bolin, 1947; Jackson, 2003; Yabe, 1985) are reexamined to create a data matrix that could be used to polarize the characters and synapomorphies could be distinguished from symplesiomorphies. We are not advocating a revision of generic names based solely on molecular markers and instead hope that our results bring attention to which phylogenetic relationships are well supported by both molecular and morphological approaches, and which require further attention. Specifically, with the addition of new molecular markers and morphological characters, questions regarding the timing of speciation, adaptations to the intertidal, and convergent evolution can be further explored.

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References

Ahn, K.J., Ashe, J.S., 2004. Phylogeny of the Myllaenini and related taxa (Coleoptera: Staphylinidae: Aleocharinae). Cladistics Int. J. Willi Hennig Soc. 20, 123–138.

Appelbaum, L., Achituv, Y., Mokady, O., 2002. Speciation and the establishment of zonation in an intertidal barnacle: specific settlement vs. selection. Mol. Ecol. 11, 1731–1737.

Bargelloni, L., Marcato, S., Zane, L., Patarnello, T., 2000. Mitochondrial phylogeny of notothenioids: a molecular approach to antarctic fish evolution and biogeography. Syst. Biol. 49, 114–129.

Begle, D.P., 1989. Phylogenetic analysis of the Cottid genus Artedius (Teleostei, Scorpaeniformes). Copeia, 642–652.

Berg, L.S., 1947. Classification of Fishes Both Recent and Fossil. J.W. Edwards, Ann Arbor, Mich.

Bolin, R.L., 1944. A Review of the Marine Cottid Fishes of California. Natural History Museum of Stanford University, Stanford University, California.

Bolin, R.L., 1947. The Evolution of the Marine Cottidae of California with a Discussion of the Genus as a Systematic Category. Stanford University, California.

Burton, R.S., Ellison, C.K., Harrison, J.S., 2006. The sorry state of F-2 hybrids: consequences of rapid mitochondrial DNA evolution in allopatric populations. Am. Nat. 168, S14–S24.

Chotkowski, M.A., Buth, D.G., Prochazka, K., 1999. Systematics of intertidal fishes. In: Horn, M.H., Martin, K.L.M., Chotkowski, M.A. (Eds.), Intertidal Fishes: Life in Two Worlds. Academic Press, San Diego, California, pp. 297–331.

Chow, S., Hazama, K., 1998. Universal PCR primers for S7 ribosomal protein gene introns in fish. Mol. Ecol. 7, 1255.

Crow, K.D., Kanamoto, Z., Bernardi, G., 2004. Molecular phylogeny of the hexagrammid fishes using a multi-locus approach. Mol. Phylogen. Evol. 32, 986–997.

Eschmeyer, W.N.H.E.S., 1983. A Field Guide to Pacific Coast Fishes: North America. Houghton Mifflin, Boston, Mass.

Gill, T., 1889. On the classification of the mail-cheeked fishes. In: Proceedings of the United States National Museum, pp. 567–592.

Hasegawa, M., Kishino, H., Yano, T.A., 1985. Dating of the human ape splitting by a molecular clock of mitochondrial-DNA. J. Mol. Evol. 22, 160–174.

Horn, M.H., 1999. Convergent evolution and community convergence: research potential using intertidal fishes. In: Horn, M.H., Martin, K.L.M., Chotkowski, M.A. (Eds.), Intertidal Fishes: Life in Two Worlds. Academic Press, San Diego, California, pp. 356–372.

Howe, K.M., Richardson, S.L., 1978. Taxonomic review and meristic variation in marine sculpins (Osteichthyes: Cottidae) of the northeast Pacific Ocean. Nat. Mar. Fisheries.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Jackson, K.L., 2003. Contributions to the Systematics of Cottoid Fishes (Teleostei: Scorpaeniformes). Dept. of Biological Sciences,University of Alberta, pp. 13, 181 leaves 128 cm.

Johnson, M.E., Baarli, B.G., 1999. Diversification of rocky-shore biotas through geologic time. Geobios 32, 257–273.

Jordan, D.S., Starks, E.C., 1895. The Fishes of Puget Sound. Cal. Leland Stanford Junior University, Hopkins Laboratory of Biology, Contributions, No. III, Palo Alto.

Lanave, C., Preparata, G., Saccone, C., Serio, G., 1984. A new method for calculating evolutionary substitution rates. J. Mol. Evol. 20, 86–93.

Losos, J.B., Glor, R.E., 2003. Phylogenetic comparative methods and the geography of speciation. Trends Ecol. Evol. 18, 220–227.

Macfarlane, J.M., 1923. The Evolution and Distribution of Fishes. The Macmillan Company, New York.

Maddison, D.R., Maddison, W.P., 2005. MacClade 4: Analysis of Phylogeny and Character Evolution. Sinauer Associates, Sunderland, Massachusetts.

Marko, P.B., 2005. An intraspecific comparative analysis of character divergence between sympatric species. Evolution 59, 554–564.

Martin, K.L.M., 1991. Facultative aerial respiration in an intertidal sculpin, *Clinocottus analis* (Scorpaeniformes, Cottidae). Physiol. Zool. 64, 1341–1355.

Martin, K.L.M., 1996. An ecological gradient in air-breathing ability among marine cottid fishes. Physiol. Zool. 69, 1096–1113.

Miller, D.J., Lea, R.N., 1972. Guide to the Coastal Marine Fishes of California. California Department of Fish and Game Fish Bulletin, Sacramento.

Nelson, J.S., 2006. Fishes of the World. John Wiley & Sons, Inc., New Jersey.

Normark, B.B., McCune, A.R., Harrison, R.G., 1991. Phylogenetic relationships of Neopterygian fishes, inferred from mitochondrial DNA sequences. Mol. Biol. Evol. 8, 819–834.

Nylander, J.A.A., 2004. MrModeltest v2. Program Distributed by the Author, Evolutionary Biology Centre, Uppsala University.

Palumbi, S.R., 1991. The Simple Fool's Guide to PCR. Dept. of Zoology and Kewalo Marine Laboratory, University of Hawaii, Honolulu, HI.

Petersen, C.W., Mazzoldi, C., Zarrella, K.A., Hale, R.E., 2005. Fertilization mode, sperm characteristics, mate choice and parental care patterns in *Artedius* spp. (Cottidae). J. Fish Biol. 67, 239.

Posada, D., Crandall, K.A., 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Rambaut, A., 1996. Se–Al: Sequence Alignment Editor. Available from: <http://evolve.zoo.ox.ac.uk/>.

Rodriguez, F., Oliver, J.L., Marin, A., Medina, J.R., 1990. The general stochastic-model of nucleotide substitution. J. Theor. Biol. 142, 485–501.

Ruber, L., Van Tassell, J.L., Zardoya, R., 2003. Rapid speciation and ecological divergence in the American seven-spined gobies (Gobiidae, Gobiosomatini) inferred from a molecular phylogeny. Evolution 57, 1584.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning a Laboratory Manual. Cold Spring Harbor Laboratory Press, New York.

Shimodaira, H., Hasegawa, M., 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol. Biol. Evol. 16, 1114.

Smith, W.L., Wheeler, W.C., 2004. Polyphyly of the mail-cheeked fishes (Teleostei: Scorpaeniformes): evidence from mitochondrial and nuclear sequence data. Mol. Phylogen. Evol. 32, 627–646.

Sorenson, M.D., Ast, J.C., Dimcheff, D.E., Yuri, T., Mindell, D.P., 1999. Primers for a PCR-based approach to mitochondrial genome sequencing in birds and other vertebrates. Mol. Phylogen. Evol. 12, 105–114.

Strauss, R.E., 1993. Relationships among the Cottid Genera Artedius, Clinocottus, and Oligocottus (Teleostei, Scorpaeniformes). Copeia, 518–522.

Swank, S.E., 1988. Biochemical systematics of the genus Clinocottus Cottidae. Bull. S. Cal. Acad. of Sci. 87, 57–66.

Swofford, D.L., 2002. PAUP* Phylogenetic Analysis using Parsimony (* and other methods). Sinauer, Sunderland, Massachusetts.

Washington, B.B., 1986. Systematic relationships and ontogeny of the sculpins *Artedius, Clinocottus*, and *Oligocottus* (Cottidae: Scorpaeniformes). Proc. Calif. Acad. Sci. 44, 157–224.

Watanabe, M., 1969. Fauna Japonica: Cottidae (pisces). Tokyo News Service, Tokyo.

Williams, S.T., Reid, D.G., 2004. Speciation and diversity on tropical rocky shores: a global phylogeny of snails of the genus *Echinolittorina*. Evolution 58, 2227–2251.

Wright, W.G., Raymond, J.A., 1978. Air-breathing in a California sculpin. J. Exp. Zool. 203, 171–176.

Yaakub, S.M., Bellwood, D.R., van Herwerden, L., Walsh, F.A., 2006. Hybridization in coral reef fishes: introgression and bi-directional gene exchange in Thalassoma (family Labridae). Mol. Phylogen. Evol. 40, 84–100.

Yabe, M., 1985. Comparative Osteology and Myology of the Superfamily Cottoidea (Pisces: Scorpaeniformes), and its Phylogenetic Classification. Faculty of Fisheries, Hokkaido University, Hakodate, Japan.

Yoshiyama, R.M., Cech, J.J., 1994. Aerial respiration by rocky intertidal fishes of California and Oregon. Copeia, 153–158.

Yoshiyama, R.M., Sassaman, C., Lea, R.N., 1986. Rocky intertidal fish communities of California—temporal and spatial variation. Environ. Biol. Fishes 17, 23–40.