Convergence in Diet and Morphology in Marine and Freshwater Cottoid Fishes

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Abstract

Habitat transitions provide opportunities for drastic changes in ecology, morphology, and behavior of organisms. The goal of this study is to determine whether the numerous evolutionary transitions from marine to freshwaters have altered the pattern and pace of morphological and lineage diversification within the sculpins (Cottoidea). The broad global distribution and wide-ranging ecology of sculpins make them an ideal study system in which to analyze marine invasions in northern latitudes. The sheer diversity of sculpins in isolated systems like Lake Baikal has led some to suggest these fishes (particularly *Cottus*) underwent an adaptive radiation upon their invasion of freshwaters in north Asia and Europe. Marine sculpins appear to be more diverse than freshwater sculpins, and while cottoids show signs of explosive radiation early in their evolutionary history, our study shows that unequal patterns of clade disparity among these lineages has led to constant rates of morphological and lineage diversification. Feeding morphology traits are highly conserved in cottoids, with both marine and freshwater species displaying similar morphologies despite widely-varying diets. While convergence in feeding morphology and dietary ecology is widespread in freshwater and marine cottoids, some specialist taxa, including planktivores and piscivores, show notable departures from the ancestral sculpin body plan. These include planktivores like *Comephorus dybowskii*, as well as piscivores *Hemitripterus bolini* and *Myoxocephalus polyacanthocephalus*. *C. dybowskii*'s unique feeding morphology, including a high posterior mechanical advantage and long dentary bones, may be further explained by its habitation of the pelagic environment.

Introduction

Marine to freshwater transition events occur rarely (Gray, 1988; Lee and Bell, 1999; Vermeij and Dudley, 2000; Vermeij and Wesselingh, 2002), likely due to physiological and ecological barriers associated with changing environmental conditions, including lower salinity (Lee and Bell, 1999; Lovejoy et al, 2006; Bloom and Lovejoy 2011, 2012; Heger et al, 2009) and competition from primary freshwater fish lineages (Vermeij and Wesselingh, 2002). Despite these challenges, many fish lineages have successfully invaded freshwater habitats, including terapontids of Australia (Davis et al, 2012) and anchovies, stingrays, needlefish, and drums in South America and Asia (Bloom and Lovejoy et al, 2006; Bloom and Lovejoy, 2012). Habitat shifts by marine derived lineages (MDLs) are often accompanied by changes in diet and morphology, sometimes evident as a burst in ecomorphological diversification. This signal of ecological and morphological diversification may result from ecological opportunity presented by new habitat and unoccupied niches, requirements for an adaptive evolution (Schluter, 2000, Yoder et al, 2010).

Although most studies of adaptive radiation in fishes have been in temperate regions (Ingram 2011, 2015, Near et al, 2003), most research on MDLs has been concentrated in the neotropics (Bloom et al 2013, Bloom & Lovejoy 2017, Davis and Betancur-R 2017), with temperate and boreal areas remaining mostly unexplored. Much debate surrounds continental radiations and whether they exhibit the requirements of an adaptive radiation (Harmon et al, 2010; Arbour and Lopez-Fernandez, 2013), but recent glacial advance and retreat in North America and Asia provide ideal conditions for habitat transitions and ecological opportunity for fishes (Schluter 2000). Fishes exhibiting recent evolutionary and ecological divergence allow for comparison with related lineages who diverged much earlier when analyzing timelines for radiations.

One such group are the cottoids (sculpins), benthic fishes found globally in freshwaters and nearshore marine habitats throughout Holarctic regions (Kinzinger et al, 2005). Previous studies have made morphological and phylogenetic comparisons of marine cottoids transitioning between subtidal to intertidal habitats (Ramon and Knope, 2008), but despite large and frequent invasions of freshwater habitats, the evolution of these cottoids across the marine to freshwater barrier has remained largely unexplored, (but see Goto 2015).

The northern hemisphere contains more than 60 freshwater species of sculpins (Kinzinger et al, 2005), across six geographical localities: Europe, Siberia, East Asia, Western North America, Eastern North America, and Lake Baikal (Goto, 2015). These freshwater sculpins are represented by distinct invasions in all regions; the radiation of East Asian cottoids occurred during the Pliocene-Pleistocene, while European, Siberian, and North American cottoids are older, diversifying during the late Miocene or early Pliocene (Yokoyama and Goto, 2005). The timeline of freshwater invasion varies widely across the cottid family, and each locality shows large separation in evolutionary divergence (Goto et al, 2015).

The greatest diversity of freshwater sculpins is in Lake Baikal, containing 33 species across three families (Goto et al, 2015), but molecular data suggests that Baikal sculpins are relatively young, beginning divergence in the Pliocene or early Pleistocene

(Kirilchik and Slobodyanuk, 1997; Hunt et al, 1997; Sherbakov, 1999; Kontula et al, 2003), suggesting an adaptive radiation. Baikal sculpins fit within the genus Cottus and form a single lineage but display vastly different ecological and physiological characteristics (Yokoyama and Goto 2005; Kinziger et al 2005; Goto et al 2015).

Sculpins can occupy a variety of habitats, ranging from intertidal and deep water marine environments to freshwater streams, rivers, and lakes, but are demersal fishes in general. This trend carries over to Lake Baikal's sculpins, but some of these fishes also exhibit a secondary microhabitat diversification to live in the pelagic water column (Goto et al 2015; Kontula et al 2003), a rare trait among cottoids. While much progress has been made involving the phylogeny of cottidae (Kinzinger et al 2005; Goto et al 2015), little research has been done that addresses potential links between habitat shifts and ecology across the evolutionary history of cottid lineages. Those that have examined ecomorphological diversification (Norton 1995; Riffel and Schrieber 1998; Goto et al 2015) have not addressed the diverse morphology of these Baikal sculpins in comparison to sister taxa and the rest of the cottoids.

How have marine to freshwater transitions affected evolutionary changes in the diet, morphology, and lineage diversification of sculpins? This study used micro-computed tomography to measure ecomorphological traits in marine and freshwater sculpins and analyzed how these traits evolved across lineages using a phylogenetic comparative context. We expect that freshwater sculpins will show greater morphological diversity than their marine relatives, due to adaptation to new niches and diets in freshwater habitats. Our goals were threefold: we examined 1) how diet and morphology have changed following marine-freshwater transitions, 2) whether freshwater cottids possess greater diversity than marine species, resulting from increased ecological opportunity in freshwater, and 3) whether new adaptive optima arise in freshwater habitats, underlying morphological differences among dietary groups. Answering these questions will indicate whether cottoids have exhibited adaptive evolution as a result of marine to freshwater transitions.

Methods

Specimen collection and micro-computed tomography scanning

Our study system includes 63 species (including outgroup species), including 15% of Lake Baikal species diversity and a complimentary sampling of related marine sculpins. Specimens were obtained from various museum institutions, including: Burke Museum (Seattle, WA), Oregon State University (Corvallis, OR), University of Washington- Friday Harbor Laboratories (Friday Harbor, WA), National Museum of Natural History (Washington, D.C.), University of Michigan- Museum of Zoology (Ann Arbor, MI), California Academy of Sciences (San Francisco, CA), Cornell University Museum of Vertebrates (Ithaca, NY), and the Virginia Institute of Marine Science (Gloucester Point, VA). All data are freely available via the ScanAllFishes project database on Open Science Network and MorphoSource (morphosource.org).

Specimens were micro-computed tomography (µCT) scanned using the Bruker 1173 Skyscan (Micro Photonics Inc., Allentown, PA) at Friday Harbor Laboratories' Karel Liem Memorial Bio-Imaging Facility. Prior to µCT scanning, specimens were photographed with an individual radio-opaque label and scale bar and wrapped in ethanol-soaked cheesecloth to prevent drying. These wrapped specimens were packed as a group into 3D-printed PVA plastic tubes, and then wrapped with plastic wrap to limit moisture loss and protect the scanner. Scans were typically run at 65 kV and 123 uA, with a voxel size spanning 17.4-50.0 um, and reconstructed as stacks of .bmp images. Specimens were digitally isolated using Dataviewer software (2012-15 Bruker microCT, Dataviewer Version 1.5.24 64-bit) and converted to .dcm format using DicomConverter software (2007-17 Bruker microCT, DicomCT Version 2.5).

Morphological Measurements

Morphological measurements were quantified using the line tool in the 3D-MPR function in Horos software (The Horos Project, Horos v2.0.1). Ecological speciation as proposed for adaptive radiations requires that traits must show adaptive, or ecologically-relevant function (Schluter, 2000). Thus, we measured traits demonstrated to be tied to feeding performance in other fish functional morphology literature (Davis et al 2012, 2013, 2014; Anderson et al., 2009).

Our measurements included: a) anterior mechanical advantage from the ratio of in-lever (muscle insertion to jaw joint) to anterior out-lever (bite point to jaw joint) to indicate the leverage (force) of the jaw, b) posterior mechanical advantage from the ratio of in-lever to posterior out-lever (last dentition to jaw joint), c) jaw aspect ratio (upper and lower) and symphyseal height to indicate the jaw’s ability to resist bending, d) occlusal offset, obtained by measuring the line tangent from the jaw joint to the plane of the tooth row, to determine angular rotation, e) tooth aspect ratio to indicate biting pressure, f) ascending process length and premaxilla length to indicate jaw protrusion, which is a movement important in suction feeding (Cook, 1996), and g) dorsal dentary length to indicate speed. Ecological traits, such as diet, habitat, and microhabitat of each fish were compiled from fishbase.org and other literature (see Table 2).

Phylogeny

We used a newly revised phylogenetic tree of cottoids developed by Thaddaeus Buser of Oregon State University. The families Cottocomephoridae, Agonidae, Jordaniidae, Psychrolutidae, Ramphocottidae, Scorpaenichthyidae, and Hexagrammidae served as outgroups. This tree is constructed with both mitochondrial and nuclear loci, allowing us to capture both young and old divergences among lineages. This phylogeny was 'time-calibrated,' meaning the dates of certain divergences have been calibrated with fossil and geological data, namely *Eocottus* fossil, which is 33-38 mya and serves as the ancestor to our ingroup, excluding *Hexagrammos decagrammos*.

Statistical Analyses

To generate morphospaces, we used phylogenetically-explicit principle component analysis (phyPCA), which reduces the dimensionality of our multivariate trait dataset and corrects for phylogenetic relatedness (phytools package; Revell, 2012). Before ordination, using PCA, we accounted for body size bias in our variables by regressing each measurement against specimen standard length, and taking the residual of that regression as our size-corrected variables. Our cottid phylogeny was read into R using the ape (APE) and phytools (Revell) packages and the tree was trimmed to include only the species in our dataset using Mesquite (Maddison and Maddison, 2017).

We used lineage-through-time (LTT) plots to determine how cottoid lineages have accumulated through time. This provides an indication of whether these species underwent adaptive evolution, as we would expect there to be a burst in new lineage generation early in the history of Cottoidea, followed by an exponential decline as niche spaces are filled to capacity. Disparity through time (DTT) plots provide an additional, perhaps more indicative, means of diagnosing an adaptive radiation by measuring how trait disparification proceeds along with lineage accumulation and how this disparity is divided among lineages. DTT simulations compare our observed diversification measures (PC1, PC2) against a Brownian motion null model, which follows a random-walk process for explaining trait diversification under little ecological selection. We might expect a negative overall clade disparity at the beginning of the cottoid radiation if adaptive radiation is occurring, while positive clade disparity suggests that niche partitioning proceeds randomly. We generated SURFACE models, following Ingram and Mahler (2013), to fit adaptive peaks to the phylogenetic tree, allowing us to determine whether adaptive optima and/or convergence in morphological configurations have occurred in cottoid evolution using the SURFACE package in R (Ingram and Mahler, 2013). This method fits adaptive peaks to the phylogeny by running both a “forward” and “backward” run to eliminate poorly supported peaks, with an AIC threshold of -2.

Results

Functional morphospace of cottoid feeding

The first 3 axes of phyPCA represent 61.2% of the variance in the data (PC1- 32.2%, PC2 15.8%, PC3- 13.2%). Species that loaded positively on the PC1 axis had longer dentary bones, longer upper jaws, and greater upper symphyseal height. This suggests greater jaw speed and more protrusible jaws.Those that loaded negatively on the PC1 axis had a higher anterior mechanical advantage, a taller upper jaw, and a greater occlusal offset. This is characteristic of species with a large gape, robust jaw skeleton, and a bite in which teeth occlude on top of each other. Species that loaded positively on the PC2 axis displayed longer in-levers. No characteristics loaded strongly negative on the PC2 axis. Species that loaded positively on PC3 showed a longer posterior out-lever (ventral dentary process) and greater posterior mechanical advantage, suggesting greater levels of speed and force toward the back of the jaw, while those that loaded negatively on PC3 displayed a higher lower jaw aspect ratio and greater lower jaw width and tooth height, showing increased jaw robustness. Results of PC1, PC2, and PC3 variance for each trait are shown in Table 1.

Convex hulls of freshwater and saltwater fishes broadly overlap to form a shared morphospace, suggesting a general lack of morphological partitioning between fishes in the two habitats (Figure 1a). This overlapping region suggests strong convergence of morphological traits throughout cottoid evolution (Fig. 3). Fig. 1(b) shows greater morphological diversity in saltwater species than freshwater. Omnivores, general invertivores, and insectivores show overlap in the same region of the morphospace. Those that loaded more positively on PC1 tended to be planktivores and piscivores (Fig.

2a). Both piscivores and planktivores exhibit low mechanical advantage and long dentary bones relative to other taxa, traits characteristic of fishes which require greater levels of jaw closing or opening speed (Anderson et al 2013). Figure 2 (b) showed similar morphological disparity among all dietary guilds but accounts for only 29% of variance.

Patterns of Morphological & Lineage Diversification

Our DTT plots (Fig. 3) showed negative clade disparity early in cottoid evolution, from 33-38 mya to 22.7-26.1 mya, consistent with sub-clades equally partitioning overall morphological diversity. This pattern is consistent until approximately 23-20 mya, when clade disparity shifts to positive, exceeding the simulated random expectation from the Brownian model. Younger clade diversification following this period is evidenced by a continued positive trend in disparity. At 11.5-10 mya, cottoids showed a steep drop in clade disparity, suggesting that some clades were diversifying further than others; i.e. that overall clade disparity was not partitioned equally among all lineages. This pattern suggests increased functional convergence in feeding morphology among lineages. LTT plots (Fig. 4) show constant (log-linear) accumulation of lineages through time, with no sudden bursts in diversity or apparent slowdowns in accumulation. These results do not support an adaptive radiation, which is characterized by an explosive ‘burst’ of lineage and morphological diversification, followed by a gradual decline in these diversification patterns as niches are filled (Schluter 2000).

Six novel adaptive peaks (in addition to the ancestral optima at the base of the cottoid tree) were estimated from repeated SURFACE iterations (Figure 6 shows trail 5 of 10). The clade composed of *Psychrolutes phrictus* and *Dasycottus setiger* were indicated as occupying a novel adaptive optima, which also showed convergence with peak 4, *Hemitripterus bolini* (Fig. 5a). *Stellarina xyosterna*, an agonid poacher, was the sole occupant of peak 5. Peak 2 was made up of the clade containing *Porocottus camschaticus, Myoxocephalus polyacanthocephalus*, *Microcottus sellaris,* and *Enophrys bison*, mostly large piscivorous species. Finally, peak 6 represented the only freshwater lineage, containing *Comephorus dybowskii* (Fig. 5a). Peak 6 (represented in bright green) also shows the most disparity from the other peaks (Fig. 5a and 5b).

Discussion

Dietary and morphological changes marked by functional equifinality

Cottoid evolutionary history is characterized by conservative patterns of morphological diversification despite marked changes in both habitat and diet. Strong convergence in phylomorphospace between marine invertivores and insectivorous freshwater cottids suggests that little overall morphological change was needed in order to take advantage of insect prey. Interestingly, though sculpins showed little adaptive modifications for insectivory, most other MDLs showed marked morphological change to occupy this niche (Kolmann et al, 2016).

Despite overwhelming functional overlap between most dietary guilds, those few taxa which do show stark departures in functional morphology from other sculpins also demonstrate unique dietary habits. Some piscivores and planktivores possess jaw morphologies which are optimized for greater levels of speed, i.e. faster opening and closing of jaws (Hulsey et al., 2005), characterized by having comparably low mechanical advantage and long snouts relative to invertivorous sculpins (Fig. 2a).

Perhaps the most remarkable example of these trends is *Comephorus dybowskii*, a peculiar pelagic species. *C. dybowskii* is a morphological outlier in our dataset and as a filter feeder of macro-zooplankton in Lake Baikal (Sideleva 1996), is quite distinctive relative to other planktivorous, semi-pelagic sculpins like *Psychrolutes*. The large convex hull encompassing the few planktivorous sculpins shown in Fig. 2(a) suggests that many ecomorphological configurations can be successful as planktivores, and their morphology may ultimately depend on the type of plankton (Friedman et al., 2016). Two of three specialized piscivorous species loaded highly positive on the PC1 and PC2 axes, suggesting that sculpins who specialize in fish require a different set of morphological traits, including longer lower jaw bones and low mechanical advantage for increased speed, as well as a greater distance from joint to muscle attachment area. This has been supported in other studies, including those on sunfishes, which has also shown that piscivory may serve as a constraint on adaptive evolution due to a strict set of requirements for ambush predatory strategies (Coller et al 2009).

Species Diversity and Adaptive Evolution

The low species diversity seen in freshwater cottoids (namely the European *Cottus* radiation) suggests that marine to freshwater transitions have not resulted in adaptive radiation. Timing of freshwater invasions occurred well after the initial burst in disparity early on in cottoid evolution (Fig. 4), and lineage accumulation has been constant following these transitions (Fig. 5). Estimates of early cottoid lineage diversification within this study aligns with those of others on sculpins (Kirilchik and Slobodyanuk 1997, Hunt et al 1997, Sherbakov 1999, Kontula et al, 2003, Yokoyama and

Goto 2005, Goto et 2015), and sculpin diversification falls within the timeframe of diversification seen in many other groups of fishes, including notothenioids (Colombo et al., 2015) and cyprinids (Hollingsworth et al., 2013). These events also occurred at approximately the same period in which many tropical fishes invaded freshwater (Bloom and Lovejoy 2017). Similar timeframes for invasions of freshwater both at higher latitudes and in tropical species demonstrates how climate fluctuations and sea-level change can structure biodiversity at a global scale, with replicated marine-freshwater transitions occurring simultaneously across continents and drastically different faunal assemblages.

Speciation of European and Siberian cottoids may have resulted from shrinkage of the Paratethys Sea in the late Miocene (Ramstein et al, 1997), while later diversification of Baikal sculpins may have occurred following isolation of Lake Baikal due to lower water levels and glaciation events during the Pliocene-Pliestocene (Williams et al 1997, Koskinen et al 2002, Khlystov et al 2008), though much is still unknown about the timing of Baikal sculpin radiation (but see Sideleva 2003). Research on Baikal grayling (Thymallinae) suggests that their invasion and speciation within Lake Baikal resulted from its isolation from the Lena Basin and the formation of the Angara River (Koskinen et al 2002), which could also be a possible explanation for Baikal sculpin speciation. Diversification of Baikal crustaceans, such as candonids, in the late Miocene may have paved the way for larger predators such as sculpin and grayling to diversify (Karanovic and Sitnikova 2017).

Adaptive Peaks in Freshwater

Lack of novel adaptive peaks in most freshwater sculpin suggests either that the ancestral sculpin body plan is particularly adaptable or that the niches presented by freshwater systems are not marked departures from marine niches, as most sculpins in our dataset occupied an ancient, broad adaptive plateau (Fig. 6). The sole freshwater adaptive peak is occupied by the pelagic sculpin, *Comephorus dybowskii*, a notable outlier relative to most other cottoids due to its planktivorous feeding habits and pelagic microhabitat association. Its traits of high posterior mechanical advantage and a long dentary may suggest a large gape for filtering macrozooplankton. The lack of adaptive peaks throughout the rest of the cottids further supports the conclusion that most freshwater species underwent little morphological change, despite numerous marine to freshwater transitions and dietary shifts.

Conclusions

The evolution and feeding morphology of sculpins is indicative of sustained diversification of both feeding morphology and lineage accumulation, as morphological traits have been highly conserved throughout their species diversification despite a wide array of diets. The dietary shift to insects following marine to freshwater transitions did not create new adaptive peaks, but some piscivores and planktivores within Cottoidea show very distinct morphological configurations relative to predominantly benthic invertivorous species, suggesting that although morphological conservatism is widespread in cottoids, trophic specialization for capturing elusive prey has driven morphological evolution away from ancestral body plans only in select lineages.

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Variable	PC1	PC2	PC3
Head Length	0.527	.0802	-0.398
Anterior MA	-0.814	.420	-0.101
Posterior MA	.304	-0.176	0.449
Tooth Aspect Ratio	.291	-.0618	-0.329
Lower Jaw Aspect Ratio	-0.583	-0.176	-0.582
Upper Jaw Width	-0.0361	0.529	0.307
Anterior Out-Lever	0.846	0.148	0.326
Post Out-Lever	0.053	0.263	0.581
In-Lever	-0.312	.772	-0.136
Tooth Height	0.334	0.441	-0.632
Tooth Width	0.139	0.492	-0.358
Lower Jaw Height	0.769	0.249	-0.123
Lower Jaw Width	0.168	0.611	-0.536
Lower Symphseal Height	0.463	0.581	0.339
Upper Symphseal Height	0.747	0.164	0.230
Upper Jaw Height	-.737	0.239	0.0368
Upper Jaw Aspect Ratio	-.682	0.0844	-0.0988
Ascending Process Length	0.108	0.567	-0.400
Upper Jaw Length	0.678	0.156	0.351
Occlusal Offset	-0.782	0.230	0.351
Dentary Length	0.934	0.0906	0.106

Table 1. Statistics resulting from phylogenetically explicit principle component.t analysis on size corrected data of jaw feeding morphology.

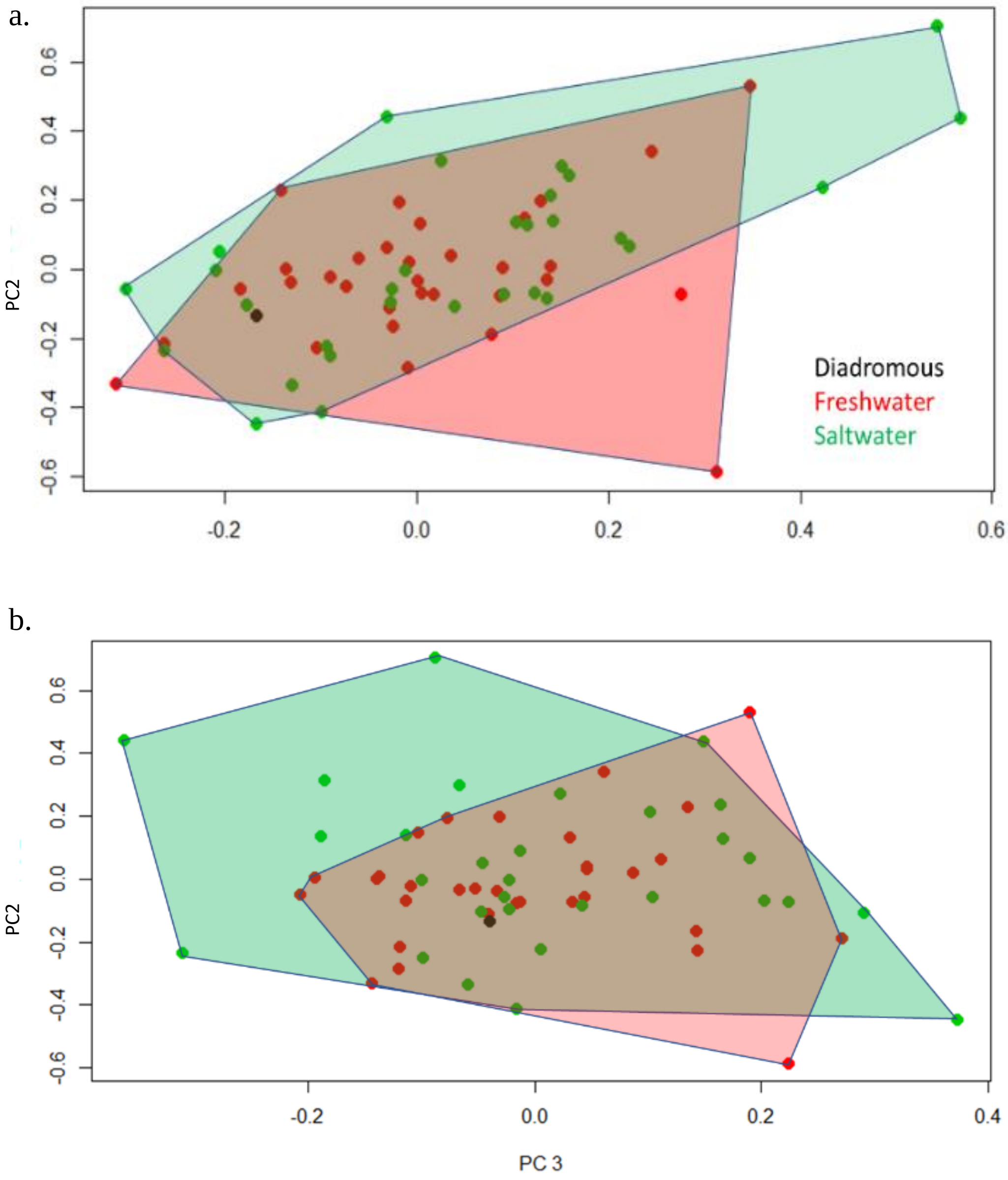

Figure 1. Scores of phylogenetically-explicit principal component analysis (phyPCA) for sculpin feeding morphology. Points represent each species, convex hulls represent a particular group. Red convex hulls shows space occupied by freshwater species, green denotes saltwater species. a) phyPCA of PC1 and PC2. b) phyPCA of PC2 and PC3.

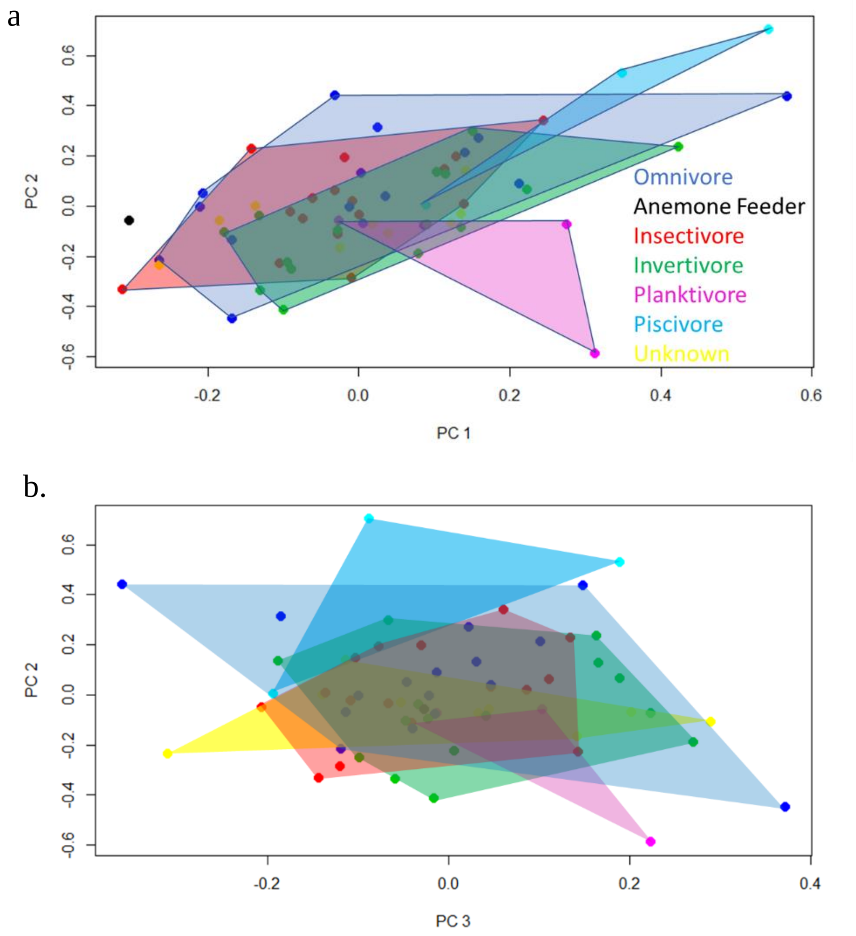

Figure 2. Scores of phylogenetically-explicit principal component analysis (phyPCA) for sculpin feeding morphology. Points represent each species, convex hulls represent a particular feeding guild. a) phyPCA of PC1 and PC2. b) phyPCA of PC2 and PC3.

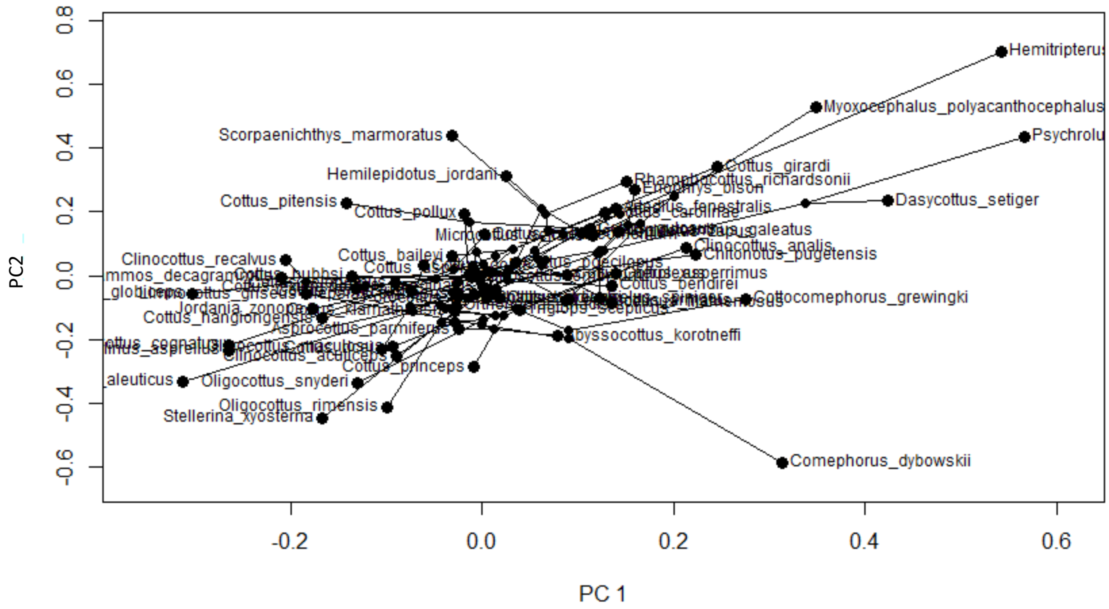

Figure 3. Phylomorphospace plot showing the phylogenetic relationships among sister taxa overlaying morphospace to show levels of convergence in morphological traits between species and clades.

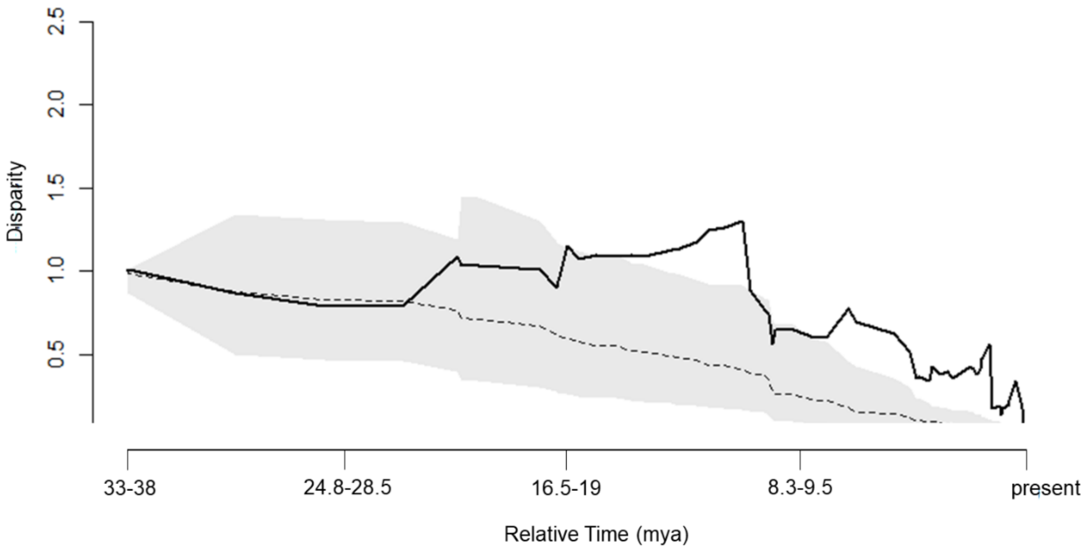

Figure 4. Disparity-through-time for jaw morphology. The dashed line represents the simulated median disparity based on repeated Brownian motion and the gray region represents range of disparity. The solid line represents relative clade disparity of cottoids through time, in millions of years.

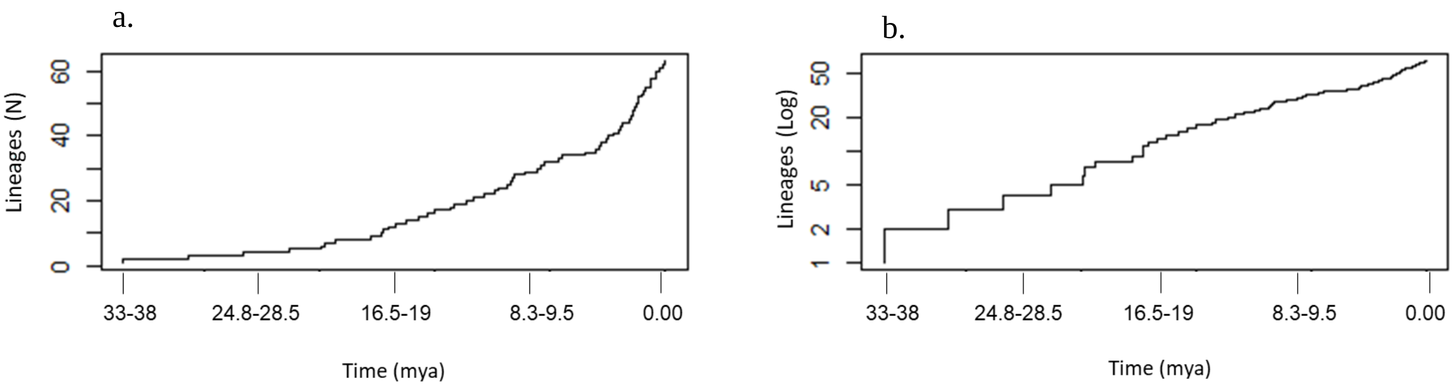

Figure 5. Lineage-through-time for Cottoidea. a) Shows number of lineages accumulated over time (in millions of years). b) Show log number of lineages accumulated over time (in millions of years).

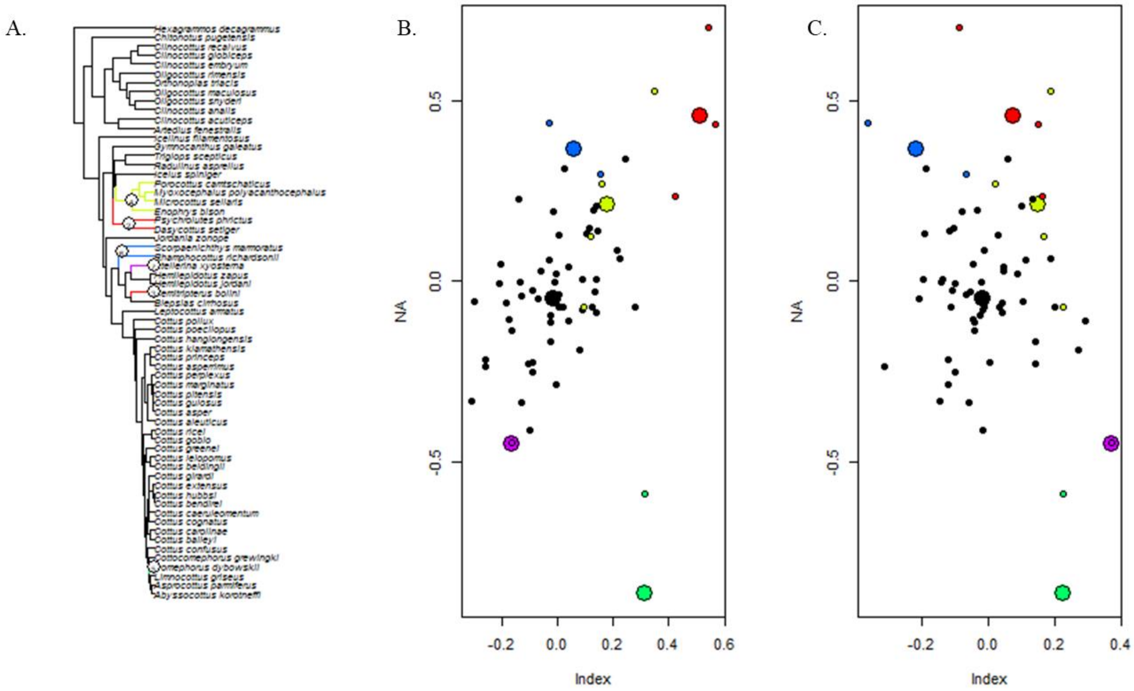

Figure 6. Adaptive optima for Cottoidea. a) Shows six novel adaptive peaks that form throughout evolution. b) Shows disparity of traits using PC1 and PC2. c) Shows disparity of traits using PC2 and PC3.

Species	Diet Data	Source
Clinocottus_acuticeps	Invertivore	Fishbase.org
Clinocottus_recalvus	Omnivore	Based off sister group
Clinocottus_globiceps	Anemone Feeder	Augustine and Muller-Parker 1998
Clinocottus_embryum	Omnivore	Based off sister group
Clinocottus_analis	Omnivore	Boyle and Horn 2006
Oligocottus_snyderi	Invertivore	Fishbase.org
Oligocottus_maculosus	Invertivore	Grossman 1986
Oligocottus_rimensis	Invertivore	Grossman 1986
Abyssocottus_korotneffi	Invertivore	Sideleva 1996
Asprocottus_parmiferus	Unknown	NA
Limnocottus_griseus	Unknown	NA
Comephorus_dybowskii	Planktivore	Sideleva 1996
Cottus_aleuticus	Insectivore	Fishbase.org
Cottus_asper	Insectivore	Fishbase.org
Cottus_asperrimus	Insectivore	Daniels and Moyle 1978
Cottus_baileyi	Insectivore	McGinley et al 2013
Cottus_bairdii	Insectivore	Fishbase.org
Cottus_beldingii	Omnivore	Ebert and Summerfelt 1969
Cottus_bendirei	Unknown	NA
Cottus_caeruleomentum	Omnivore	Based off sister group
Cottus_carolinae	Insectivore	Fishbase.org
Cottus_cognatus	Omnivore	Fishbase.org
Cottus_confusus	Insectivore	Johnson et al 1983
Cottus_extensus	Invertivore	Neverman and Wurtsbaugh 1994
Cottus_girardi	Insectivore	Based off sister group
Cottus_gobio	Insectivore	Fishbase.org
Cottus_greenei	Unknown	NA
Cottus_gulosus	Insectivore	Baltz et al 1982
Cottus_hangiongensis	Omnivore	Based off sister group
Cottus_hubbsi	Unknown	Based off sister group
Cottus_klamathensis	Insectivore	Based off sister group
Cottus_leiopomus	Insectivore	Merkley and Griffith 1993
Cottus_marginatus	Insectivore	Based off sister group
Cottus_perplexus	Piscivore	Phillips and Claire 1966
Cottus_pitensis	Insectivore	Li and Moyle 1976
Cottus_poecilopus	Omnivore	Fishbase.org
Cottus_pollux	Insectivore	Natsumeda et al 2012
Cottus_princeps	Insectivore	Based off sister group

Cottus_rhotheus	Insectivore	Fishbase.org
Cottus_ricei	Insectivore	Fishbase.org
Leptocottus_armatus	Omnivore	Fishbase.org
Cottocomephorus_grewingki	Planktivore	Fishbase.org
Blepsias_cirrhosus	Planktivore	Cross et al 1980
Hemilepidotus_jordani	Omnivore	Spies et al 2013
Hemilepidotus_zapus	Invertivore	Fishbase.org
Hemitripterus_bolini	Piscivore	Tenbrink and Hutchinson 2009
Stellerina_xyosterna	Omnivore	Eschmeyer et al 1983
Jordania_zonope	Invertivore	Norton 1991
Chitonotus_pugetensis	Invertivore	Fishbase.org
Enophrys_bison	Omnivore	Fishbase.org
Gymnocanthus_galeatus	Unknown	Coad and Reist 2004
Icelinus_filamentosus	Invertivore	Fishbase.org
Icelus_spiniger	Unknown	NA
Microcottus_sellaris	Invertivore	Eschmeyer et al 1983
Myoxocephalus_polyacanthocephalus	Piscivore	Fishbase.org
Porocottus_camtschaticus	Invertivore	Saveliey and Kolpakov 2016
Radulinus_asprellus	Unknown	NA
Triglops_scepticus	Unknown	NA
Artedius_fenestralis	Omnivore	Fishbase.org
Orthonopias_triacis	Invertivore	Snook 1997
Dasycottus_setiger	Planktivore	Jewett et al 1989
Psychrolutes_phrictus	Planktivore	Fishbase.org
Rhamphocottus_richardsonii	Invertivore	Fishbase.org
Scorpaenichthys_marmoratus	Omnivore	Fishbase.org
Hexagrammos_decagrammus	Omnivore	Fishbase.org

Table 2. Diet information and sources for all species included in dataset. Those without diet information available were based off sister groups when possible or listed as "unknown."