

Littorally adaptive? Testing the link between habitat, morphology, and reproduction in the intertidal sculpin subfamily Oligocottinae (Pisces: Cottoidea)

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While intertidal habitats are often productive, species-rich environments, they are also harsh and highly dynamic. Organisms that live in these habitats must possess morphological and physiological adaptations that enable them to do so. Intertidal fishes are generally small, often lack scales, and the diverse families represented in intertidal habitats often show convergence into a few general body shapes. However, few studies have quantified the relationship between phenotypes and intertidal living. Likewise, the diversity of reproductive traits and parental care in intertidal fishes has yet to be compared quantitatively with habitat. We examine the relationship of these characters in the sculpin subfamily Oligocottinae using a phylogenetic hypothesis, geometric morphometrics, and phylogenetic comparative methods to provide the first formal test of associations between fish phenotypes and reproductive characters with intertidal habitats. We show that Oligocottinae is a primitively and overwhelmingly intertidal group, with a single species that has secondarily come to occupy only subtidal habitats. Maximum size is the only character to show a statistically significant correlation with depth, but the maximum for all species is generally small (250mm or less) and all species show a reduction in scales, as would be expected for an intertidal group. Also contrary to previous studies, we show that copulation and associated characters are the ancestral condition in Oligocottinae, with copulation most likely being lost in a single lineage within the genus *Artedius*. Lastly, we show that body shape appears to be constrained among species with broader depth ranges, but lineages that occupy only a narrow range of intertidal habitats display novel body shapes, and this may be associated with habitat partitioning, particularly as it relates to the degree of wave exposure.

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17 Abstract

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19 highly dynamic. Organisms that live in these habitats must possess morphological and
20 physiological adaptations that enable them to do so. Intertidal fishes are generally small, often
21 lack scales, and the diverse families represented in intertidal habitats often show convergence
22 into a few general body shapes. However, few studies have quantified the relationship between
23 phenotypes and intertidal living. Likewise, the diversity of reproductive traits and parental care
24 in intertidal fishes has yet to be compared quantitatively with habitat. We examine the
25 relationship of these characters in the sculpin subfamily Oligocottinae using a phylogenetic
26 hypothesis, geometric morphometrics, and phylogenetic comparative methods to provide the first
27 formal test of associations between fish phenotypes and reproductive characters with intertidal
28 habitats. We show that Oligocottinae is a primitively and overwhelmingly intertidal group, with
29 a single species that has secondarily come to occupy only subtidal habitats. Maximum size is the
30 only character to show a statistically significant correlation with depth, but the maximum for all
31 species is generally small (250mm or less) and all species show a reduction in scales, as would
32 be expected for an intertidal group. Also contrary to previous studies, we show that copulation
33 and associated characters are the ancestral condition in Oligocottinae, with copulation most
34 likely being lost in a single lineage within the genus *Artedius*. Lastly, we show that body shape
35 appears to be constrained among species with broader depth ranges, but lineages that occupy
36 only a narrow range of intertidal habitats display novel body shapes, and this may be associated
37 with habitat partitioning, particularly as it relates to the degree of wave exposure.

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40 Introduction

41 Intertidal habitats are often highly-productive, species rich environments (Leigh et al., 1987).
42 Yet, intertidal areas are also one of the harshest marine environments, often subject to rapidly
43 changing physical conditions such as wave action, temperature, and current, as well as factors
44 that affect homeostasis of resident organisms, such as pH and dissolved oxygen (Davenport &
45 Woolmington, 1981; Bridges, 1993; Martin, Lawson & Engebretson, 1996). Fishes living in
46 these areas often display common physical characteristics such as small size (Gibson, 1982) and
47 a reduction of scales (e.g., intertidal members of Blenniidae, Gobioidae, Pholidae, see
48 Chotkowski, Buth & Prochazka, 1999; Knope & Scales, 2013), presumably as means of coping
49 with the unique set of challenges presented by intertidal habitats. Likewise, the body shapes of
50 intertidal fishes appear constrained to take on one of only a few stereotypical shapes, such as
51 elongate and eel-like (e.g., Pholidae), cylindrical and tapered (e.g., Cottoidea), or dorso-ventrally
52 compressed (e.g., Gobioidae; for full descriptions see Horn, 1999).

53 In contrast to the somewhat predictable morphological characteristics of intertidal fishes,
54 the reproductive biology of these species is diverse and does not show apparent patterns between
55 intertidal and subtidal taxa (reviewed in DeMartini, 1999 and Coleman, 1999). However, our
56 understanding of many of these morphological and reproductive patterns in intertidal fishes is

57 based only on qualitative assessments. Body shape, for instance, has never been quantitatively
58 described and compared among or between any group(s) of intertidal fishes. A quantitative
59 approach may shed additional light on the patterns and processes of adaptation to intertidal
60 habitats in fishes. A phylogenetic comparative approach is one way to better understand the
61 relationship of habitat, morphological, and reproductive characters in intertidal fishes, and the
62 marine sculpin (family Psychrolutidae *sensu* Smith & Busby, 2014) subfamily Oligocottinae is a
63 relatively well-studied group and excellent candidate in which to do so.

64 The 16 species that make up Oligocottinae are found in a variety of shallow nearshore
65 habitats across the Pacific coast of North America (Hubbs, 1926; Taranetz, 1941; Ramon &
66 Knope, 2008; Buser & López, 2015). The members of this subfamily occupy a range of subtidal
67 and intertidal habitats, with varying degrees of intertidal occupation across species (Bolin, 1944;
68 Lamb & Edgell, 1986; Mecklenburg, Mecklenburg & Thorsteinson, 2002). Likewise,
69 oligocottines display a broad range of reproductive strategies ranging from copulation and
70 internal insemination to spawning and external mixing of gametes (Petersen et al., 2005; Abe &
71 Munehara, 2009).

72 Recent studies have suggested that the diversification of Oligocottinae is associated with
73 a shift in habitat within the group (Ramon & Knope, 2008; Knope & Scales, 2013). Subtidal
74 habitats are believed to be the ancestral condition of the subfamily and the putative shift from
75 subtidal to intertidal habitats is thought to have been followed by relatively rapid diversification
76 in the intertidal lineage. The shift in habitat is associated with adaptive morphological changes in
77 the intertidal group, which include smaller body size and fewer scales in intertidal species when
78 compared to their deeper-dwelling relatives (Knope & Scales, 2013). Critically, however, this
79 transition is thought to have occurred within the subfamily, such that the group contains subtidal
80 taxa, intertidal taxa, and “transitional” taxa, with the intertidal taxa being the most species rich
81 (Ramon & Knope, 2008).

82 At the heart of these results, however, is an unanswered question, namely: how does one
83 categorize the habitat type (e.g., “intertidal”) of each species? Intertidal habitats comprise a range
84 of depths which change on daily, seasonal, and yearly cycles. Categorizing these habitats and
85 ascribing them to a fish, which is free to move across and occupy all habitat types with every
86 flooding tide, presents many potential pitfalls (this conundrum is reviewed in Gibson &
87 Yoshiyama, 1999). The ways in which fishes use these habitats ranges from intertidal residents
88 to intertidal transients (Breder, 1948; Gibson, 1969; Thomson & Lehner, 1976; Potts, 1980) and
89 this continuum only further muddies the waters. Given these uncertainties, and the potential for
90 arbitrary categorizations to impact the results of comparative analyses, it could be useful to take
91 a different approach.

92 In this study, we forego categorization of habitat and instead use known depth ranges for
93 each species to test whether the host of morphological and reproductive traits putatively linked to
94 species in intertidal habitats in fact correlate with depth. To do so, we construct a phylogenetic
95 hypothesis of the subfamily Oligocottinae using previously published molecular sequence data
96 and use ancestral state reconstruction and phylogenetic comparative methods to test the

97 relationship between depth range and morphological, reproductive, and body shape characters in
98 the group.

99
100

101 **Materials & Methods**

102 ***Phylogenetic framework***

103 We constructed a phylogenetic framework using previously reported DNA sequences from all
104 oligocottine species and several outgroups (see Buser & López, 2015 Supplementary Table 1 for
105 list of species, sample size, molecular loci, and Genbank IDs). Multiple sequence alignments
106 (MSAs) for each locus were generated in ClustalW (Larkin et al., 2007). Alignments were
107 visually inspected, trimmed, and concatenated in Mesquite v3.2 (Maddison & Maddison, 2016).
108 The best fitting model of molecular evolution for each locus was identified using the Akaike
109 information criterion (Akaike, 1973; Posada & Buckley, 2004), with the model comparison
110 routines implemented in MrModeltest v2 (Nylander, 2004).

111 The molecular dataset contains multiple representatives for each species, so we estimated
112 a species tree using the multispecies coalescent model (Heled & Drummond, 2010) in BEAST
113 v1.8.2 (Drummond et al., 2012). A species set was defined, based on the results of recent
114 phylogenetic hypotheses (Knape, 2013; Smith & Busby, 2014), for the superfamily Cottoidea,
115 which contains all taxa included in the dataset except *Hexagrammos lagocephalus*, which was
116 treated as an outgroup. The species of each sampled individual in the dataset was assigned as a
117 discrete trait. These species assignments were tested and validated in (Buser & López, 2015). For
118 each locus, the model of molecular evolution yielding the lowest AIC value (as calculated in
119 MrModeltest) was applied. The rate of molecular evolution was modeled as an uncorrelated
120 lognormal relaxed clock (Drummond et al., 2006) and was unlinked across all loci. All tree
121 models share a birth-death speciation tree prior with a piecewise linear and constant root
122 population size model and a UPGMA starting tree. Four independent analyses were run for 200
123 million generations each and were sampled every 20,000 generations. MCMC logs were
124 visualized using Tracer v1.6 (Rambaut et al., 2014) to determine convergence and an appropriate
125 number of generations to discard as burn-in. Burn-in was removed and trees combined using
126 LogCombiner v1.7.5 (Drummond & Rambaut, 2007). The phylogeny was pruned using
127 Mesquite to only include members of the subfamily Oligocottinae plus the outgroup taxon
128 *Chitonotus pugetensis*.

129

130 ***Character coding***

131 Small size (i.e., length) and a reduction of scales have been reported for many intertidal species
132 and these characters show an adaptive shift between subtidal and intertidal oligocottine sculpins
133 (Knape & Scales, 2013). While reproductive characters are not known to correspond to intertidal
134 vs subtidal habitats (Coleman, 1999; DeMartini, 1999), the relationship between depth and
135 reproductive characters has yet to be formally tested. Reproductive traits are very diverse in
136 sculpins, particularly regarding copulation and parental care (Abe & Munehara, 2009). While

137 copulation is difficult to observe directly, characters that are putatively associated with this trait,
138 such as the presence of an enlarged genital papilla, and spermatozoon morphology, are more
139 readily observable. Parental care is also difficult to observe in many species, but has important
140 evolutionary implications. To understand the relationship between a species depth preferences
141 and its size, reproductive habits, or scale patterns, we coded the following characters from
142 previous studies and, where possible, verified our findings by examining museum specimens
143 and/or collection data:

144

145 1. **Depth range (Continuous).** Maximum and minimum depth records for each species
146 were cataloged and cross-examined from multiple sources (Bolin, 1944; Miller & Lea, 1972;
147 Eschmeyer, Herald & Hammann, 1983; Mecklenburg, Mecklenburg & Thorsteinson, 2002). The
148 records for museum lots of each species were obtained from various institutions (accessed
149 through the Fishnet2 Portal, www.fishnet2.org, 2016-06-22) and examined to verify the reported
150 depth ranges (Supplementary Tables 1-8).

151 2. **Length (Continuous).** Maximum recorded length of each species was cataloged and
152 cross-examined from multiple sources (Bolin, 1944; Miller & Lea, 1972; Eschmeyer, Herald &
153 Hammann, 1983; Mecklenburg, Mecklenburg & Thorsteinson, 2002; Knope & Scales, 2013).

154 3. **Squamation (Presence, absence).** For the purposes of this study, squamation is defined
155 as any dermal ossification outside of the lateralis system. This includes scales, prickles, and
156 scutes. The evolution of scale types in sculpins is poorly understood, but what is known suggests
157 that the modified scales found in Oligocottinae may each represent an equal number of
158 evolutionary steps away from the ancestral ctenoid scale type, with the latter not represented in
159 any extant cottoid (Jackson, 2003). We therefore feel that in the context of this study it is
160 unjustifiable to discriminate between scale types in oligocottines until further study indicates
161 otherwise. Presence of squamation was coded from descriptions in the literature (Bolin, 1944;
162 Begle, 1989; Mecklenburg, Mecklenburg & Thorsteinson, 2002; Jackson, 2003).

163 4. **Enlarged genital papilla (Presence, absence).** This character was coded directly from
164 descriptions in the literature (Bolin, 1944; Mecklenburg, Mecklenburg & Thorsteinson, 2002).

165 5. **Spermatozoon morphology (Oval, intermediate, slender).** Character states were
166 adapted from descriptions in the literature (Hann, 1930; Petersen et al., 2005; Koya et al., 2011).
167 Slender sperm morphology is associated with internal insemination in many groups of fishes
168 (Mattei, 1991). (Petersen et al., 2005) confirmed this observation in Oligocottinae by
169 demonstrating that spermatozoa with a slender-type morphology are active only in seawater that
170 has been diluted to approximate the osmolality of ovarian fluid in these sculpins, while
171 spermatozoa with oval-type morphology are active in both dilute and full-strength seawater. This
172 suggests that slender-type spermatozoon morphology is indicative of obligate insemination, but
173 oval-type morphology indicates the capacity for external mixing of gametes (i.e., spawning).

174 6. **Copulation (Presence, absence).** For the purposes of this study, copulation is defined as
175 the transfer of sperm from a male into the ovary of a female. The presence of copulation, where

176 known, was determined from descriptions found in the literature (Bolin, 1941; Morris, 1952,
177 1956; Hubbs, 1966; Misitano, 1980; Petersen et al., 2005; Abe & Munehara, 2009).

178 7. **Parental care (Presence, absence).** For the purposes of this study, egg guarding by one
179 or both parents is considered parental care. The presence of parental care, where known, was
180 determined from a review of behavioral descriptions from previous literature (Morris, 1952;
181 Hubbs, 1966; Petersen et al., 2005; Abe & Munehara, 2009).

182

183 *Character mapping and ancestral state reconstruction*

184 To visualize the inferred evolutionary history of characters, we performed ancestral state
185 reconstruction (ASR) of each character examined in this study. For discrete characters, we used
186 maximum likelihood (ML) with a Markov k-state 1 parameter (Mk1) model of evolution (Lewis,
187 2001), implemented in Mesquite. The evolutionary history of continuous characters was inferred
188 using ML in the R statistical environment (R Development Core Team 2015) with functions
189 from the package “phytools” (Revell, 2012).

190

191 *Body shape analysis*

192 Qualitative assessment of body shape in intertidal fishes has not only shown differences in the
193 shape of some intertidal species compared to their subtidal relatives (e.g., *Hypsoblennius* spp.;
194 Thomson & Lehner, 1976), but also that many groups of intertidal fishes (including intertidal
195 sculpins) have converged on a small number of stereotypical body shapes (reviewed in Horn,
196 1999). While thought provoking, these observations have yet to be tested using quantitative
197 methods. We used landmark-based geometric morphometrics to describe and compare the body
198 shape of each species in this study and test for correlation between body shape and the depth at
199 which each species occurs. Body shape data were collected from digital photographs of the
200 lateral view of museum specimens of each species. Photography followed the phototank method
201 of (Sabaj Pérez, 2009). To minimize the likelihood of introducing variation due to photographic
202 artifacts (i.e., image distortion), lighting, distance to the subject, focal length, camera angle, and
203 camera settings (e.g., aperture) were kept constant. We photographed 115 specimens,
204 representing all 16 species in Oligocottinae plus the outgroup taxon *Chitonotus pugetensis*.
205 Sample size per species ranges from 2 to 15 individuals, median six (Table 1). To capture overall
206 head and body shape, landmarks were adapted from those described in previous studies of
207 sculpin body shape (Strauss & Bookstein, 1982; Strauss & Fuiman, 1985). Preliminary analysis
208 revealed a high frequency of distended stomachs and upturned caudal peduncles, presumably
209 from prior feeding and preservation (respectively), so landmarks that appeared to be influenced
210 by these variables were not included. Thirteen landmarks were ultimately used in this study (Fig.
211 1). We used tps-Dig2.2 (Rohlf, 2007) to locate the landmarks on each specimen from the digital
212 photographs. To compare body shape across the group, landmark configurations were Procrustes
213 superimposed using MorphoJ v1.06 (Klingenberg, 2011). The aligned landmark coordinates
214 were used to calculate a covariance matrix on which we performed a principal component
215 analysis (PCA) in MorphoJ. The number of significant principal component axes was calculated

216 using the “testdim” function in the R package “ade4” (Dray & Dufour, 2007). The significant
217 principal component axes were used to interpret overall shape variation and visualize the
218 distribution of species in body shape morphospace. To visually check for evidence of
219 morphological convergence or divergence, we projected phylogenetic relatedness into the
220 principal component morphospaces and inferred states of each significant PC axis for each
221 ancestral node (i.e., phylomorphospace analysis; (Sidlauskas, 2008) using the
222 “phylomorphospace” function in the R package “phytools” (Revell, 2012)

223

224 ***Correlation with depth***

225 Preliminary results indicated that, while there is considerable variability in the maximum
226 recorded depth of each species in Oligocottinae, all but one species (*Leiocottus hirundo*) share a
227 minimum recorded depth of zero meters (i.e., intertidal). We therefore chose a single variable
228 (maximum depth) to represent depth and used phylogenetic generalized least squares regression
229 (PGLS) implemented using the “gls” function in the R package “nlme” (Pinheiro et al., 2015) to
230 test for a linear correlation between depth and each of the putatively associated characters
231 examined in this study. The relationship between depth and body shape was also tested using
232 PGLS, with body shape represented by the significant PC axes. To account for potential
233 variability in trait evolution (e.g., Brownian motion, selection, etc.), we tested three alternate
234 single-parameter correlation structures supplied in the R package “ape” (Paradis, Claude &
235 Strimmer, 2004) in each of our regression models: a Brownian motion model with correlation
236 due to phylogenetic relatedness represented by Pagel’s lambda (Pagel, 1994, 1999), which we
237 estimated using ML; a Brownian motion model with the rate of evolution (accelerated or
238 decelerated) estimated using ML; and a single optimum (i.e., stabilizing selection) Ornstein-
239 Uhlenbeck (OU) model (Felsenstein, 1988; Hansen, 1997) with the strength of attraction towards
240 the optimum represented by alpha and estimated using ML. The best fitting model for each
241 regression was determined by comparing AIC values.

242 One of our hypotheses is that shallow-dwelling species show convergent morphology
243 differing from that of their subtidal sister taxa. To test for convergent or divergent evolution of
244 body shape, we used a stepwise model-fitting approach, “surface,” that detects shifts and
245 convergence in phenotypic optima (Ingram & Mahler, 2013). In this approach, each optimum
246 contributes a parameter to an OU process of evolution. The “surface” method finds the
247 maximum-likelihood estimate of the number and location of phenotypic optima under the OU
248 model and collapses similar adaptive optima together if it improves the AIC score (Ingram &
249 Mahler, 2013). Phenotypic convergence is indicated when independent lineages share a common
250 optimum. These analyses were conducted in the R environment using functions from the package
251 “surface” (Ingram & Mahler, 2013).

252 To visualize only the aspects of body shape that covary with depth, a partial least squares
253 analysis (PLS) was conducted on a matrix of depth variables (minimum depth and maximum
254 depth) and Procrustes-aligned shape variables (Rohlf & Corti, 2000). This analysis was
255 conducted in MorphoJ and in R using functions from the package “geomorph v2.0” (Adams &

256 Otárola-Castillo, 2013). In both cases, the significance of the covariance was tested using a
257 permutation test with 10,000 iterations.

258

259

260 **Results**

261 ***Character mapping and ancestral state reconstruction***

262 The topology of the MCC phylogeny produced herein is identical to the topology of the MCC
263 phylogeny reported in Buser & López (2015), with similar levels of support for each clade
264 (Supplementary Figure 1). We will use the classification and taxonomy suggested by Buser &
265 López (2015) for discussion of the interrelationships of oligocottine sculpins.

266 The outgroup taxon, *C. pugetensis*, rarely (if ever) occurs in intertidal areas. However,
267 apart from *Leiocottus hirundo* (minimum depth: 5m), all the constituent species of Oligocottinae
268 are found in intertidal habitats and have been collected from tide pools explicitly (Table 2).
269 There is considerable variation in the maximum depth at which each species occurs, but all
270 oligocottine species occur at relatively shallow depths: none has been collected at depths greater
271 than 55 m, most (12/16 spp.) have never been collected below 25m, and five species occur
272 exclusively in intertidal habitats (Table 2). Predictably, the ASR of minimum depth shows that
273 the most recent common ancestor (MRCA) of Oligocottinae likely occurred in intertidal habitats
274 (ML estimate: 1 m; 95% confidence interval: 0m, 2m), and the subtidal distribution of *L. hirundo*
275 likely represents a derived state. The ASR of maximum depth suggests that the MRCA of
276 Oligocottinae occurred down to only moderate depths (ML estimate: 24m; 95% confidence
277 interval: 11m, 37m) and suggests that the habitation of only intertidal habitats represents a
278 derived state. This intertidal specialization likely evolved independently in two groups: once in
279 the ancestor of the subgenus *Clinocottus* (*Blennicottus*) and once or perhaps twice in the genus
280 *Oligocottus*. Unfortunately, the uncertainty of the ML estimates at each node precludes further
281 inference of the diversification of this trait (Fig. 2).

282 Maximum length shows a similar pattern to depth in Oligocottinae. All species (including
283 the outgroup) are relatively small (none longer than 250mm), most (12/16 spp.) do not grow
284 longer than 150mm, and seven species do not grow longer than 100mm (Table 2). The ASR of
285 maximum length suggests that the MRCA of Oligocottinae was small (130mm, 95% confidence
286 interval: 80mm, 180mm), but like maximum depth, the uncertainty of the ML estimates at each
287 node precludes additional inference into the diversification of this trait.

288 Squamation is common among members of Oligocottinae but is completely absent in
289 only two clades: the subgenus *Clinocottus* (*Blennicottus*), and the clade in *Oligocottus* made up
290 of *O. maculosus*, *O. snyderi*, and *O. rubellio* (Table 2). Predictably, the ASR shows that the
291 presence of squamation is the most likely state for the MRCA of Oligocottinae (proportional
292 likelihood: 0.81), and that the absence of scales represents an independent loss of the trait in the
293 MRCA of each of the above clades (illustrated in Fig. 2; Supplementary Figure 2). It should be
294 noted that we inferred the evolution of squamation using an Mk1 model, which assumes that all
295 potential changes in state are equally probable (Lewis, 2001). Given the lack of rigorous study of

296 scale evolution in sculpins, specifying a more complex model is not warranted. However, it is
297 our opinion that re-acquisition of squamation is an extremely unlikely evolutionary scenario in
298 oligocottine sculpins (i.e., less probable than the loss of squamation) and thus the proportional
299 likelihood of the presence of scales for the ancestral nodes in Oligocottinae should be taken as a
300 conservative estimate.

301 An enlarged genital papilla is found in all but five species of Oligocottine sculpins (Fig.
302 3). The ASR shows with high proportional likelihood (>0.98) that this character was present at
303 all ancestral nodes except those within the clade composed of the members of the genus
304 *Artedius*. Within *Artedius*, an enlarged genital papilla was likely lost in the MRCA of the clade
305 containing *A. corallinus*, *A. fenestralis*, *A. lateralis*, and *A. notospilotus* (Fig. 3). An independent
306 loss of the enlarged genital papilla occurred in the oligocottine species *Orthonopias triacis*.

307 The distribution of spermatozoon morphology follows a pattern similar to that of the
308 enlarged genital papilla. Outside of the clade composed of members of the genus *Artedius*,
309 slender-type spermatozoa are present in all Oligocottine sculpins and, with high proportional
310 likelihood (>0.90), is the state at all ancestral nodes (Fig. 3). Within *Artedius*, an oval-type
311 spermatozoon likely evolved in the MRCA of the clade containing *A. corallinus*, *A. fenestralis*,
312 *A. lateralis*, and *A. notospilotus*. *Artedius harringtoni* possess an intermediate spermatozoon
313 morphology and is the only oligocottine to do so (Fig. 3). With one notable exception, this shows
314 that all species with a slender-type spermatozoon morphology (which in other species is known
315 to function only in ovarian fluid) also possess an enlarged genital papilla, which is presumably
316 used in copulation. The exception to this observation is the species *Orthonopias triacis*, which
317 does not possess an enlarged genital papilla, but does possess a slender-type sperm morphology.

318 Though not known for all species in Oligocottinae, the distribution of copulatory
319 behavior closely follows that of spermatozoon morphology, where copulating species possess
320 either slender or intermediate-type spermatozoon morphology, and non-copulating species
321 possess only oval-type spermatozoon morphology (Fig. 3). The ASR of this character shows that,
322 with high proportional likelihood (>0.95), presence of copulation is the likely state for all
323 ancestral nodes outside of the clade composed of the members of the genus *Artedius*. Within
324 *Artedius*, copulation was likely lost in the MRCA of the clade containing *A. corallinus*, *A.*
325 *fenestralis*, *A. lateralis*, and *A. notospilotus*. These finding reinforces the observations of Mattei
326 (1991) and Petersen et al. (2005) who each show that slender-type sperm morphology is
327 indicative of copulating species. Once again, *Orthonopias triacis* presents a noteworthy case as
328 there is evidence of copulation for the species and the species possesses a slender-type
329 spermatozoon morphology, yet the species lacks an enlarged genital papilla or other known
330 intromittent organ (Fig. 3).

331 The presence or absence of parental care has been described in less than half of all
332 oligocottine species (6/16 spp.), but follows a similar pattern to those seen in other reproductive
333 characters in the group, where members of the genus *Artedius* tend to differ from all other
334 species. In this case, parental care is observed only in members of *Artedius* (Fig. 4). The ASR
335 shows that parental care was likely present in the MRCA of *Artedius*, while a lack of parental

336 care is the most likely state for the MRCA of the tribe Oligocottini. However, given the
337 substantial amount of missing data for this trait, the ASR is subject to change with the addition of
338 new observations.

339

340 ***Body shape analysis***

341 Observed body shape variation was captured by four significant principal components, which
342 cumulatively describe 85% of the total variance. We used an outline of a specimen of
343 *Clinocottus analis* to visualize shape change represented by each PC axis in MorphoJ (Fig. 5).
344 Principal component (PC) 1 (52% of total observed variance) describes antero-posterior
345 elongation/compression of the head and mouth as well as the relative size of the eye. Principal
346 component 2 (19% of total observed variance) captures dorso-ventral elongation/compression of
347 the body, the shape of the eye, and the slope of the snout. Principal component 3 (9% of total
348 observed variance) captures the size and orientation of the mouth, the size and relative position
349 of the eye, and the relative position of the uppermost preopercular spine. Principal component 4
350 (6% of total observed variance) captures the relative length of the snout and dorso-ventral
351 elongation/compression of the head.

352 There is no clear evidence of a consistent relationship between the minimum depth or
353 maximum depth of a species and its morphology, nor evidence of morphological convergence
354 among shallow or deep-ranging species (Fig. 6). Species with deeper ranges appear to be
355 constrained to a common morphospace, while species that inhabit only shallow depth ranges
356 (e.g., *Oligocottus* spp., *Clinocottus (Blennicottus)* spp.) appear to occupy novel and distinct areas
357 of morphospace (Fig. 6). This observation is supported by the results of the “surface” analysis,
358 which inferred three adaptive optima for body shape in the morphospace described by PC 1 and
359 PC 2 (cumulatively 71% of total observed variance): one for each of the two intertidal specialist
360 lineages, and a third that is occupied by all other oligocottines (Supplementary Figure 3).
361 However, there is no clear pattern in terms of the direction of the divergence in morphospace of
362 these shallow (i.e. purely intertidal) specialists from their respective deeper-ranging sister taxa.

363

364 ***Correlation with depth***

365 Phylogenetic generalized least squares regression shows that maximum length has a slightly
366 significant (p -value < 0.05), positive linear correlation with maximum depth (coefficient:
367 0.136656, standard error: 0.058674). For this relationship, the best fitting model of evolution is a
368 Brownian motion model with Pagel’s lambda estimated at a value of 1, indicating strong
369 phylogenetic signal. No other morphological, reproductive, and body shape variables examined
370 in this study show a significant correlation with maximum depth.

371 The results of the PLS analysis were identical in MorphoJ and in R, and, like the other
372 analyses, failed to show a statistically significant correlation between body shape and depth
373 range (p -value > 0.05). However, the analysis did reveal a few interesting observations: species
374 that are restricted entirely to shallow water tend to display greater morphological diversity than
375 those that range into deeper water, but generally have smaller eyes, smaller mouths, terminal

376 placement of the mouth, and more robust bodies, while deeper-ranging species tend to have
377 larger eyes, larger mouths, subterminal placement of the mouth, and dorso-ventrally compressed
378 bodies (Fig. 7).

379

380

381 **Discussion**

382 The results of our study show support for some previous hypotheses of the general evolution of
383 intertidal fishes. We support the purported relationship between body size and habitat depth, as
384 well as some general trends in the body shape of intertidal fishes compared to their deeper-
385 dwelling relatives. However, our results do not support previous hypotheses of the diversification
386 of the subfamily Oligocottinae as it relates to depth, nor do we support previous hypotheses
387 regarding the evolution of reproductive modes in oligocottines or, to a certain extent, sculpins in
388 general. In some cases, our conclusions directly oppose those made by previous authors. We
389 discuss these results and some plausible explanations for our congruous and incongruous
390 conclusions below.

391

392 ***Squamation, length, and depth***

393 The subfamily Oligocottinae should be thought of as a clade of intertidal-occurring fishes and the
394 ability to live in intertidal depths is likely the ancestral state of the group. This finding does not
395 support the hypothesis that there is differential diversification of intertidal vs. subtidal
396 oligocottine groups (e.g., Ramon & Knope, 2008; Knope & Scales, 2013), as we conclude that
397 virtually all oligocottines occur with some frequency in intertidal habitats. Thus, the
398 diversification of Oligocottinae should not be characterized as occurring between intertidal and
399 subtidal habitats, but rather occurring primarily within both habitats simultaneously. This may
400 explain the general lack of correlation between depth the other characters examined in this study,
401 as well as the relatively small maximum size observed in all oligocottine species (none longer
402 than 250mm).

403 Small maximum size and a reduction in scales have been noted as common features of
404 intertidal fishes by previous authors (Gibson, 1982; Knope & Scales, 2013), and our results
405 support the former. While we found no evidence to support the latter, it should be noted that all
406 oligocottine sculpins show a reduction in scales when compared to a “typical” scaled member of
407 Cottales, such as *Oxylebius pictus* (see Jackson, 2003). Outside of the lateral line, the most
408 heavily scaled oligocottines possess only modified scales in a narrow band on the body along
409 each side of the dorsal fins and on the dorsal surfaces of the head and caudal peduncle (e.g.,
410 *Orthonopias triacis*; see descriptions in Bolin, 1944; Jackson, 2003). Others possess highly
411 reduced scales in the form of prickles (e.g., *Clinocottus (Clinocottus) analis*; see description in
412 (Bolin, 1944). It is presumed that the primary reason scales are often reduced in intertidal fishes
413 is to promote cutaneous respiration, which is dependent on well vascularized skin that is free
414 from obstructions (Feder & Burggren, 1985; Martin & Bridges, 1999). Within Oligocottinae, it
415 may simply be the case that the highly reduced scales seen in the group do not cover enough

416 surface area to interfere with cutaneous respiration in a meaningful way. Or perhaps that
417 cutaneous respiration is restricted to only certain areas, such as the head (as seen in
418 *Coryphoblennius galerita*, see Zander, 1972), or buccal chamber (reviewed in Bridges, 1993).
419 This may explain the presence of scales within the group, and the fact that even the most heavily
420 scaled members of Oligocottinae occur intertidally and in tide pools (Fig. 2). Interestingly, the
421 one oligocottine species that does not occur intertidally (*L. hirundo*) possess only a few highly
422 reduced scales in the form of a scattered patch of prickles located just posterior to the base of the
423 pectoral fins (see Jackson, 2003). We interpret this as evidence that a reduction in scales in an
424 ancestral condition for *Leiocottus*, and possibly Oligocottinae as a whole.

425 The evolutionary history of maximum size, depth range, and the presence of squamation
426 all support the idea that the subfamily Oligocottinae is a primitively intertidal group. All species
427 in this subfamily display conditions of these traits that are common in intertidal taxa, and all but
428 one species are found intertidally and explicitly in tide pools. We thus conclude the opposite of
429 previous studies and suggest that rather than containing an intertidal radiation (Ramon & Knope,
430 2008; Knope & Scales, 2013), the subfamily Oligocottinae itself represents an intertidal
431 radiation.

432

433 ***Body shape and depth***

434 While the body shape of deeper-ranging species is relatively conserved, groups that occupy only
435 intertidal areas appear to be exploring novel areas of morphospace. This is most pronounced in
436 members *Oligocottus* and the subgenus *Clinocottus* (*Blennicottus*), where the two groups each
437 occupy a morphological optimum that is distinct from each other and from all other
438 oligocottines. The morphological distinctiveness of *Clinocottus* (*Blennicottus*) is readily
439 apparent, even to the casual observer (see illustrations in Bolin, 1944). The constituent species of
440 this subgenus occur most abundantly in steep, rocky habitats with high wave exposure and are
441 often the only oligocottine species found in these areas (T. Buser pers. obs.; Green, 1971;
442 Yoshiyama, 1981; Yoshiyama, Sassaman & Lea, 1986; Mgaya, 1992, see also descriptions in
443 Eschmeyer, Herald & Hammann, 1983; Lamb & Edgell, 1986; Mecklenburg, Mecklenburg &
444 Thorsteinson, 2002). The blunt heads and short, stocky bodies of these species are also seen in
445 other intertidal fishes occupying similarly exposed, rocky habitats and may reflect a common
446 evolutionary response to the physical demands of living in such areas (Kotrschal, 1988, 1989;
447 Thomson, Findley & Kerstitch, 2010, reviewed in Kotrschal, 1999). If the diverse morphologies
448 seen among intertidal specialist clades are reflective of their respective habitat partitions, it may
449 also be the case that the constrained morphologies seen in deeper-ranging taxa reflect a kind of
450 stabilizing selection of generalist traits that are optimal for occupying a comparatively wide
451 variety of habitats. The relatively high diversity of morphotypes seen in shallow vs deep-ranging
452 species may mask morphological traits that are in fact associated with depth, as is suggested by
453 the results of our PSL analysis (Fig. 7). However, the lack of statistical significance of this trend
454 warrants caution on interpretation of this finding until the question can be revisited with
455 additional taxa.

456

457 **Reproduction**

458 While reproductive characters do not show any correlation with depth range in Oligocottinae, the
459 evolution of these traits in the subfamily may offer new insight into the evolution of reproductive
460 modes in cottoids. Copulation in oligocottines is associated with an enlarged genital papilla and a
461 slender-type spermatozoon morphology. These traits are broadly distributed in Oligocottinae and
462 are likely the ancestral state of the subfamily (Fig. 3). Importantly, the absence of copulation and
463 associated traits in most members of the genus *Artedius* represents a loss and is thus a derived
464 state. This finding runs counter to previous hypotheses of the evolution of reproductive modes in
465 sculpins, which interpreted the seemingly scattered distribution of copulation in cottoids as
466 indicative of parallel or convergent evolution of copulation from non-copulating ancestors (Abe
467 & Munehara, 2009; Muñoz, 2010). Under this paradigm, (Petersen et al., 2005) suggests that the
468 ability of the oval-type sperm morphology (uniquely capable of functioning well in seawater and
469 ovarian fluid, seen in non-copulating members of *Artedius*) to function in ovarian fluid
470 represents a derived condition and concludes that the presence of this trait in most members of
471 *Artedius* represents an evolutionary step *towards* copulation in the group. We conclude the
472 opposite of (Petersen et al., 2005), and suggest that rather than the sperm's ability to function in
473 ovarian fluid, it is in fact the sperm's ability to function in seawater that is a derived state and
474 this, along with the loss of an intromittent organ, represents an evolutionary step *away* from
475 copulation within *Artedius*.

476 A reduction or loss of the enlarged genital papilla is seen in other oligocottines as well.
477 Critically, however, these species maintain a slender-type spermatozoon morphology and, where
478 known, copulation. For example, while most oligocottines possess genital papillae that are quite
479 large and robust, males in the genus *Oligocottus* possess papillae that are uniquely small, gracile,
480 and thread-like (Supplementary Figure 4). Also unique to the males of this genus are
481 modifications of the anterior portion of the anal fin (Supplementary Figure 5) which, were
482 known, is used for grasping females during copulation (*O. snyderi*, Morris, 1956). It is possible
483 that the added security and stability during copulation provided by the prehensile anal fin rays
484 has rendered the large genital papilla seen in other oligocottines redundant. The other example of
485 a reduction in the size of the male genital papilla is seen in *Orthonopias triacis*, where males lack
486 an intromittent organ altogether, yet also possess slender-type sperm morphology and are known
487 to copulate. Males of this species possess enlarged pelvic fins that face inwardly “palm to palm,”
488 and project postero-ventrally from a “pedunculated” base (Bolin, 1944). Perhaps these highly
489 modified, sexually dimorphic pelvic fins are used in a grasping manner that, like in *Oligocottus*,
490 is used to aid in copulation and has eliminated the need of a large, robust male genital papilla.
491 Copulation without the use of an intromittent organ is seen in at least one other member of
492 Cottales, the sea raven (*Hemitripterus villosus*). In this species, males are not known to possess
493 any putative grasping organs. Instead, the female everts her genital tract, which is covered in
494 mucus, and the male ejaculates onto it, whereby the sperm become entrained in the mucus and
495 enter the female when she inverts her genital tract (Munehara, 1996).

496 Our results show that while the presence of an enlarged male genital papilla is a likely
497 indicator of copulation, the absence of an intromittent organ does not necessarily indicate the
498 absence of copulation. Furthermore, our results show that non-copulating species may evolve
499 from copulating ancestors. Given the widespread distribution of copulation and/or an enlarged
500 genital papillae within Cottoidea (Abe & Munehara, 2009; Muñoz, 2010), we suggest that
501 copulation and associated traits may have evolved much earlier in cottoids than has been
502 previously estimated. Perhaps the seemingly disparate distribution of copulation in cottoids is not
503 due to many independent evolutions of copulation, but rather to a single early evolution of
504 copulation and multiple subsequent losses of the trait. Given the suite of complex physiological
505 and behavioral traits associated with copulation in sculpins (e.g., internal gamete association
506 with delayed fertilization, see Munehara, Takano & Koya, 1989, 1991; Munehara et al., 1997;
507 Petersen et al., 2005), the independent loss of copulation by certain lineages would, in our
508 opinion, be a far simpler explanation for the modern distribution of the trait than would the
509 independent evolution of copulation and all associated characters.

510

511 ***Parental care***

512 Like other reproductive traits, the distribution of parental care in Oligocottinae does not appear
513 to be related to the distribution of depth ranges. Rather, only members of the genus *Artedius*
514 display parental care. Strong phylogenetic signal of parental care has been reported for other
515 groups as well (reviewed in Coleman, 1999), but this does not provide a satisfying explanation
516 for why *Artedius* differs from all other oligocottines in this trait. It is interesting to note that
517 parental care shows an almost inverse distribution to oval-type sperm morphology (i.e., obligate
518 copulation; see Figs. 3 and 4), but the limited sample size and degree of missing data for parental
519 care make this a tenuous connection. Many other non-copulating sculpins also display parental
520 care (e.g., *Hemitripterus* spp., *Enophrys bison*, *Myoxocephalus* spp., *Cottus* spp.), but this trait is
521 also seen in some copulating species, including *Artedius harringtoni* (Abe & Munehara, 2009).
522 Clearly, more research is needed to better understand the evolution of parental care in cottoids,
523 and its relationship with other aspects of their complex reproductive biology.

524

525 **Conclusions**

526 Considering the depth ranges rather than previously published habitat categorizations of
527 oligocottine sculpins reveals substantial overlap of almost all species in intertidal habitats. This
528 new understanding of the group agrees with our findings that all oligocottine sculpins are
529 relatively small and bear relatively few scales, two common attributes of intertidal fishes. This
530 finding also helps to explain why maximum recorded depth does not correlate with most of the
531 characters examined in this study, as we would expect them to vary with depth only if we are
532 comparing intertidal fishes with subtidal fishes, and for the most part we are not. While body
533 shape does not significantly correlate with depth, the body shape of species with broader depth
534 ranges appear constrained to what we interpret as a generalist morphology, while groups that
535 inhabit a narrow, wholly-intertidal depth range appear to have unique body shapes, perhaps

536 suited to their specialized habitat partitions. Likewise, we find no evidence of an association
537 between depth and reproductive characters, but we do find that the evolution of these characters
538 has likely proceeded from a primitive condition of obligate copulation using an intromittent
539 organ to a derived state of spawning and/or the loss of an intromittent organ. This sequence is the
540 opposite direction of that inferred by previous authors, but is clearly supported by the
541 distribution of reproductive traits across our phylogenetic hypothesis of the group.

542

543

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Figure 1

Homologous landmarks used in this study.

Lateral photograph of *Clinocottus analis* (OSIC 6710, 75.9mm SL) showing thirteen homologous landmarks used to capture overall body shape in oligocottine sculpins: 1) insertion of spinous dorsal fin, 2) insertion of soft dorsal fin, 3) insertion of anal fin, 4) ventral-most ray of the pectoral fin, 5) anterior-most tip of dentary 6) anterior-most tip of premaxilla 7) posterior-most tip of maxilla, 8) anteroventral-most point of orbit, 9) anteroventral-most point of orbit 10) anterodorsal-most point of orbit, 11) posteroventral-most point of orbit 10) anterodorsal-most point of orbit, 12) dorsal tip of dorsal-most preopercular spine, 13) insertion of epaxial musculature onto neurocranium.

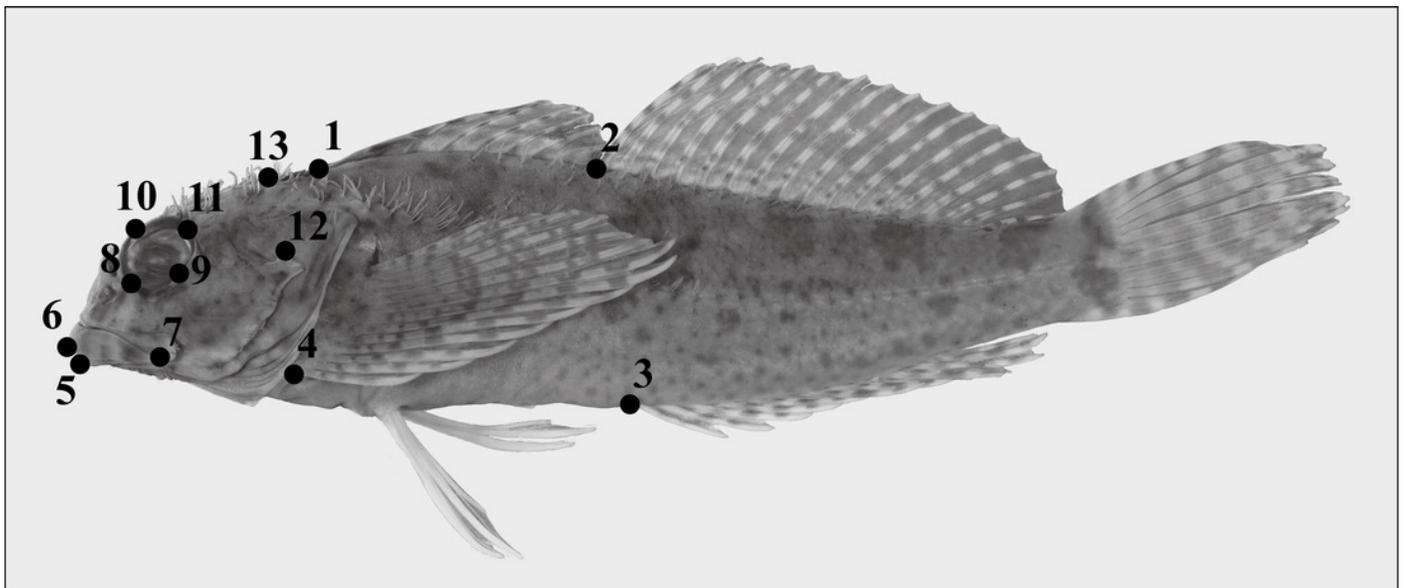


Figure 2

Distribution of maximum depth, maximum size, and squamation in Oligocottinae.

Bayesian MCC phylogeny of Oligocottinae with maximum depth, maximum size, and presence of squamation of extant species. The inferred evolutionary history of maximum depth is indicated on the left panel with relative time indicated on the horizontal axis and depth in meters indicated on the vertical axis. Ancestral states for each node were inferred using maximum likelihood and 95% confidence intervals for each state are represented with blue lines. White lines on the phylogeny indicate species and inferred ancestors that possess squamation, black lines indicate species and inferred ancestors that have lost squamation outside of the lateral line. Species names are abbreviated as follows: *Artedius corallinus* = *Ar. cor.*, *A. fenestralis* = *Ar. fen*, *A. harringtoni* = *Ar. har.*, *A. lateralis* = *Ar. lat.*, *A. notospilotus* = *Ar. not.*, *Chitonotus pugetensis* = *Ch. pug.*, *Clinocottus (Oxycottus) acuticeps* = *Cl. acu.*, *Clinocottus (Clinocottus) analis* = *Cl. ana.*, *Clinocottus (Blennicottus) embryum* = *Cl. emb.*, *C. (B.) globiceps* = *Cl. glo.*, *C. (B.) recalvus* = *Cl. rec.*, *Leiocottus hirundo* = *Li. hir.*, *Oligocottus maculosus* = *Ol. mac.*, *O. rimensis* = *Ol. rim.*, *O. rubellio* = *Ol. rub.*, *O. snyderi* = *Ol. sny.*, *Orthonopias triacis* = *Or. tri.*

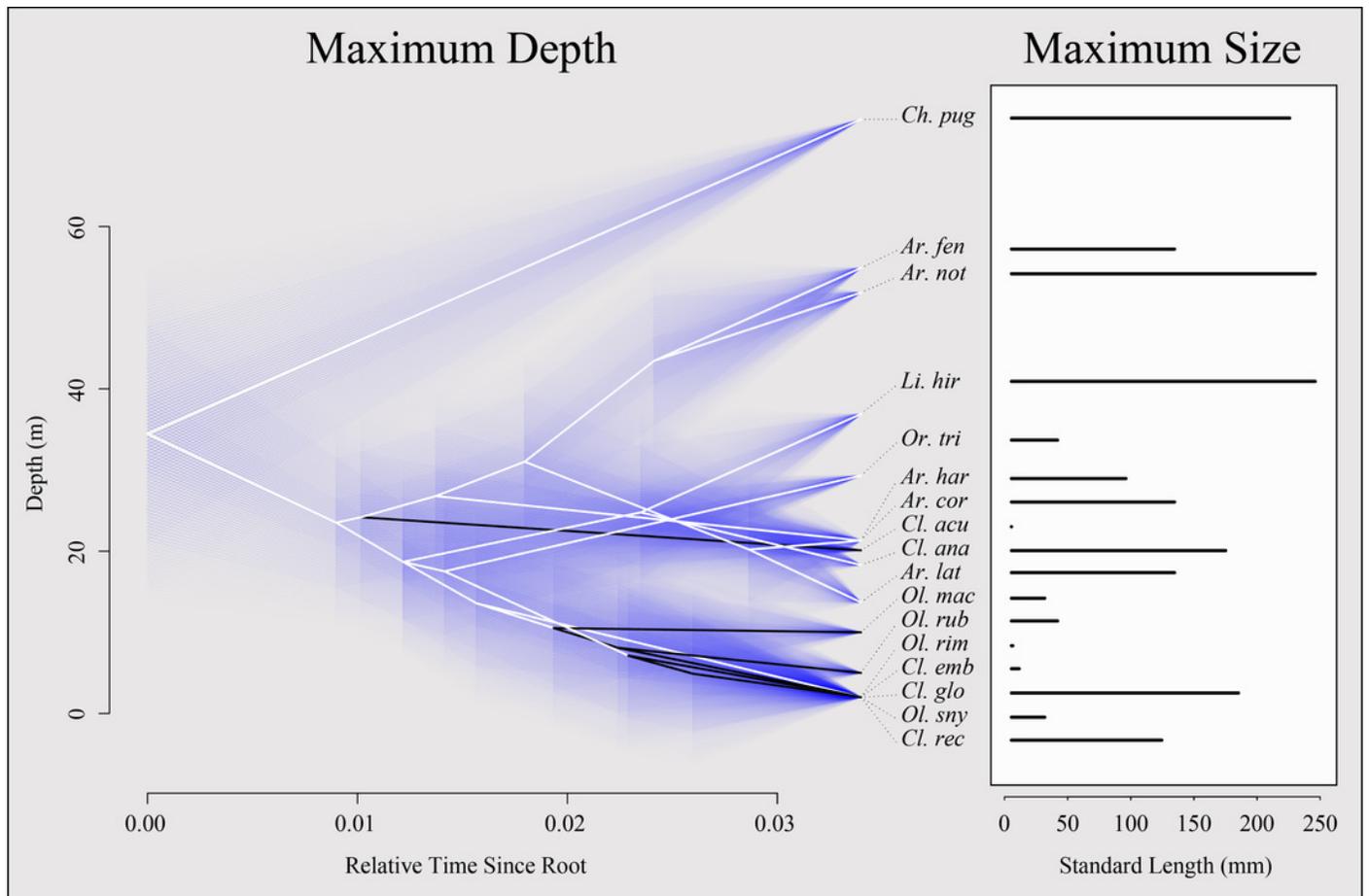


Figure 3

Inferred evolutionary history of enlarged genital papilla and spermatozoon, and distribution of copulation in Oligocottinae.

Bayesian MCC phylogeny of Oligocottinae with inferred evolutionary histories of the presence of an enlarged genital papilla and spermatozoon shape. Where known, the presence of copulation in extant species is indicated by a “+” symbol following the abbreviated species name. Absence of copulation is indicated by a “-” symbol. Unknown states are indicated by the absence of a symbol. For the ancestral state reconstructions of the presence of an enlarged genital papilla and spermatozoon morphology, the proportional likelihood of each character state for the ancestor of a given clade is depicted with a pie chart at each respective node. Symbols at the tips of each phylogeny are indicative of the phylogenetic lineage of each species. Absence of a symbol at a tip indicates an unknown state. Squares represent the *Artedius* lineage, a triangle represents the lineage composed solely of the species *Clinocottus (Oxycottus) acuticeps*, hexagons represent the *Leiocottus* lineage, circles represent the *Blennicottus* lineage, stars represent the *Oligocottus* lineage, and a diamond represents a lineage composed solely of the species *Chitonotus pugetensis*. Species names are abbreviated as in Figure 2.

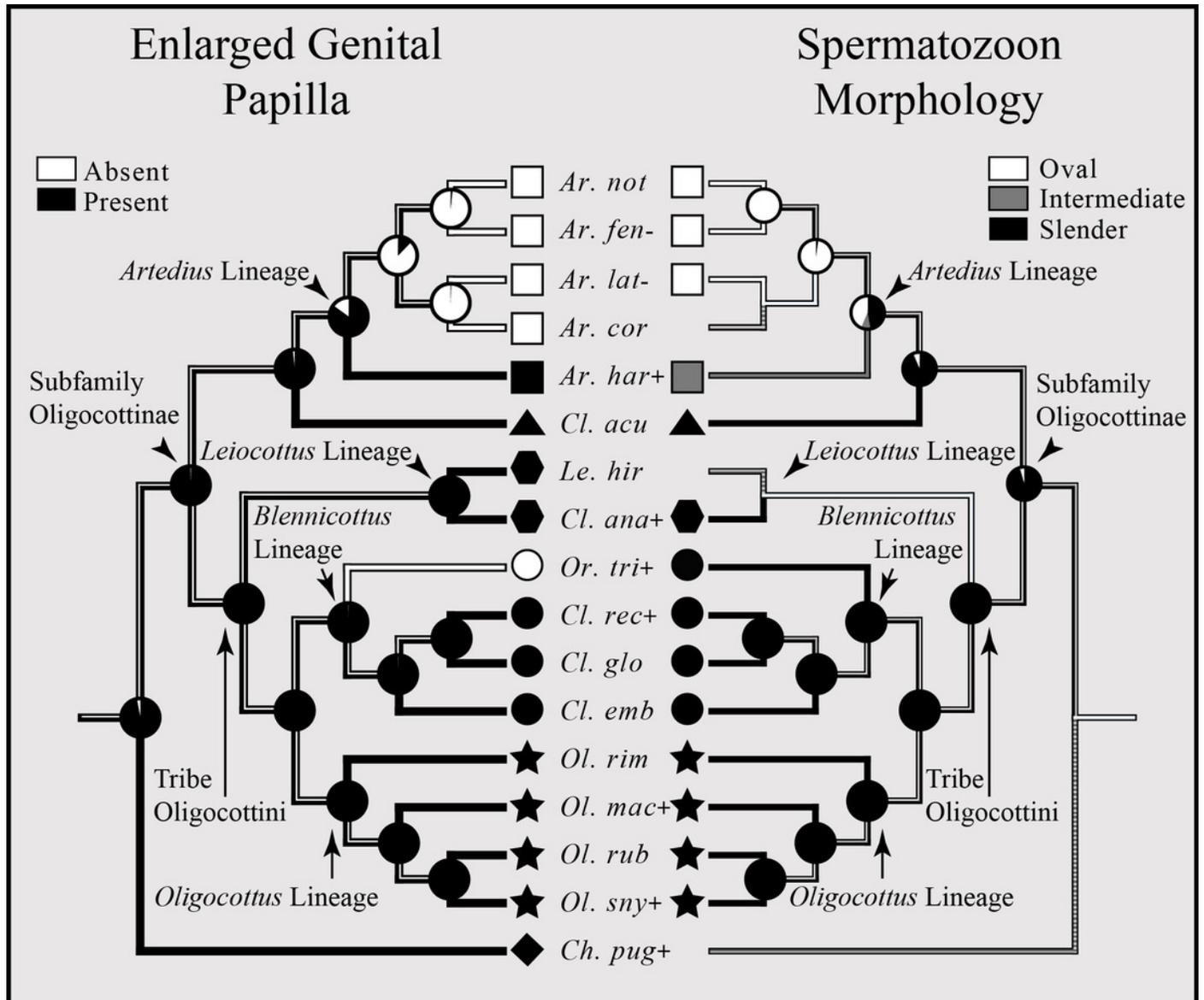


Figure 4

Inferred evolutionary history of parental care in Oligocottinae.

Bayesian MCC phylogeny of Oligocottinae with distribution and inferred evolutionary history of parental care. The proportional likelihood of each character state for the ancestor of a given clade is depicted with a pie chart at each respective node. Symbols at the tips of the phylogeny are indicative of the phylogenetic lineage of each species, as in Figure 3. Absence of a symbol at a tip indicates an unknown state. Species abbreviations in bold indicate a slender-type spermatozoon morphology present in that species.

Parental Care

□ Absent
 ■ Present

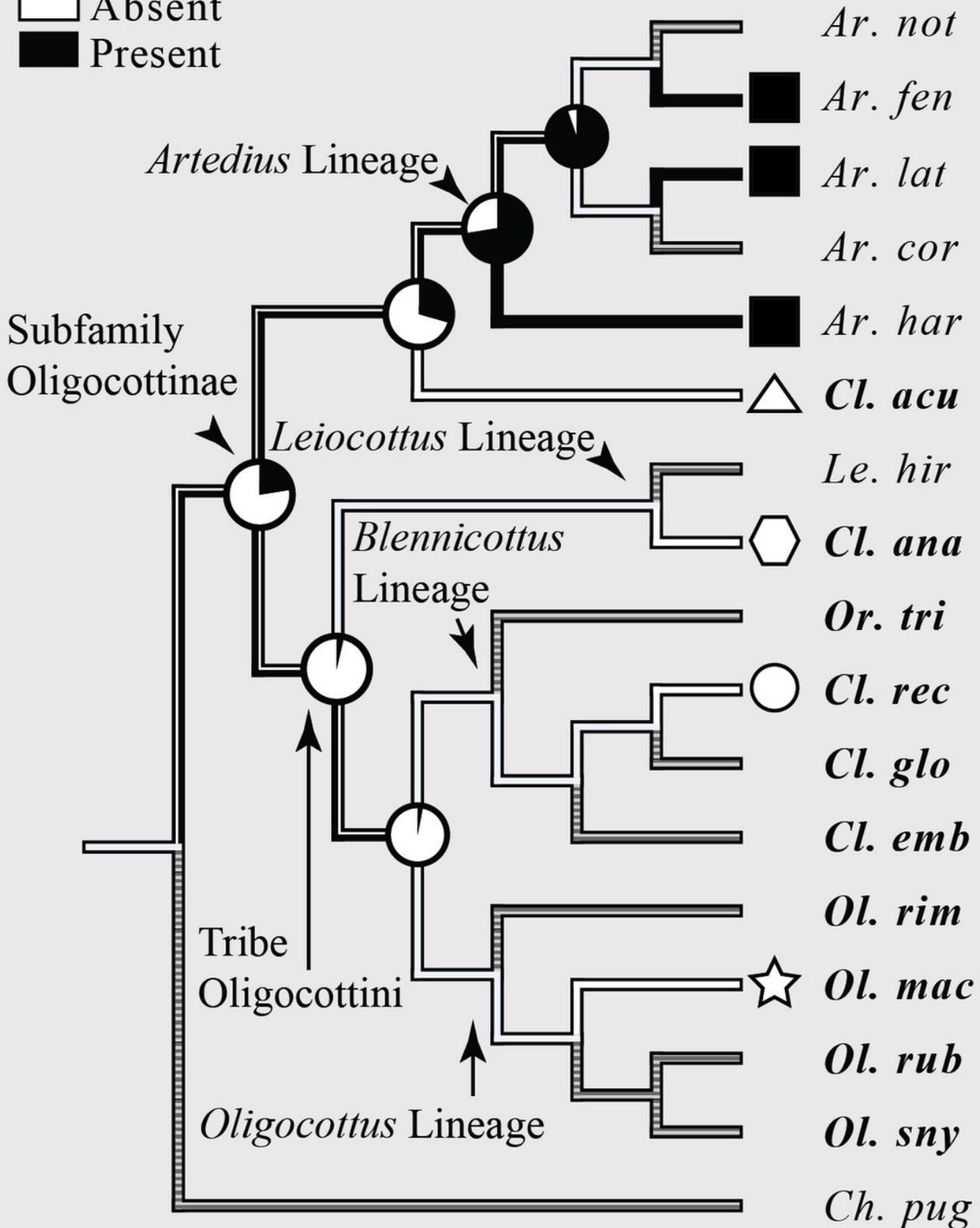


Figure 5

Body shape change in oligocottine sculpins represented by each of the four significant principal component axes.

Landmark locations are indicated by circles and are numbered as in Figure 1. Outline sketched from the lateral photograph of *Clinocottus analis* (OSIC 6710, 75.9mm SL) in Figure 1. Light blue and open circles shows displacement of landmarks and interpolated warping of the outline at a value of -1 on each PC axis. Dark blue and closed circles show displacement of landmarks and interpolated warping of the outline at a value of +1 on each PC axis.

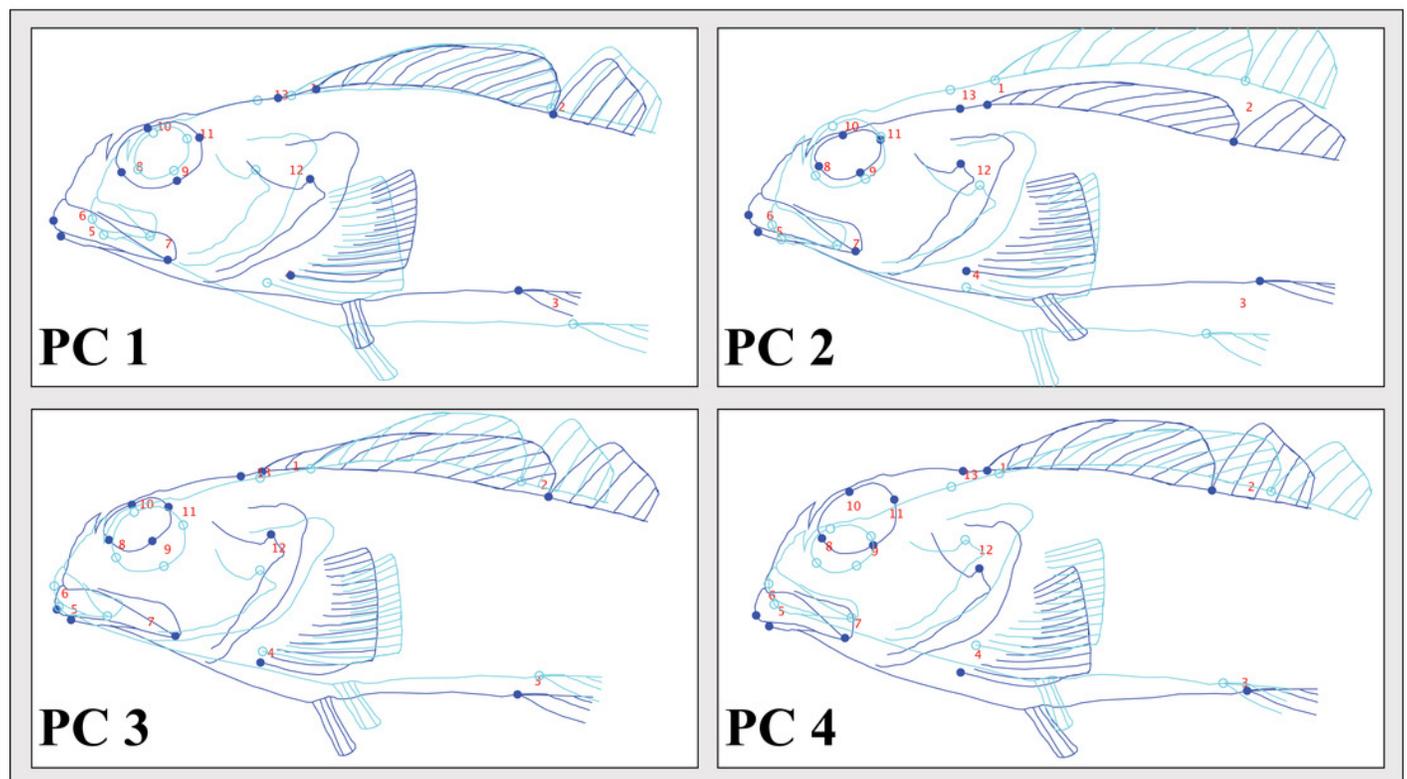


Figure 6

Phylomorphospace of the first two principal components of body shape in the subfamily Oligocottinae.

Outlined symbols at the tips of each phylogeny are indicative of the phylogenetic lineage of each species as in Figure 3, ancestral nodes are indicated by small circles that are not outlined. Colors of each tip and internal node are indicative of the maximum depth of the species or the maximum likelihood (ML) estimate of the ancestral state of maximum depth, respectively. The shape change described by each PC axis is as shown as in Figure 5.

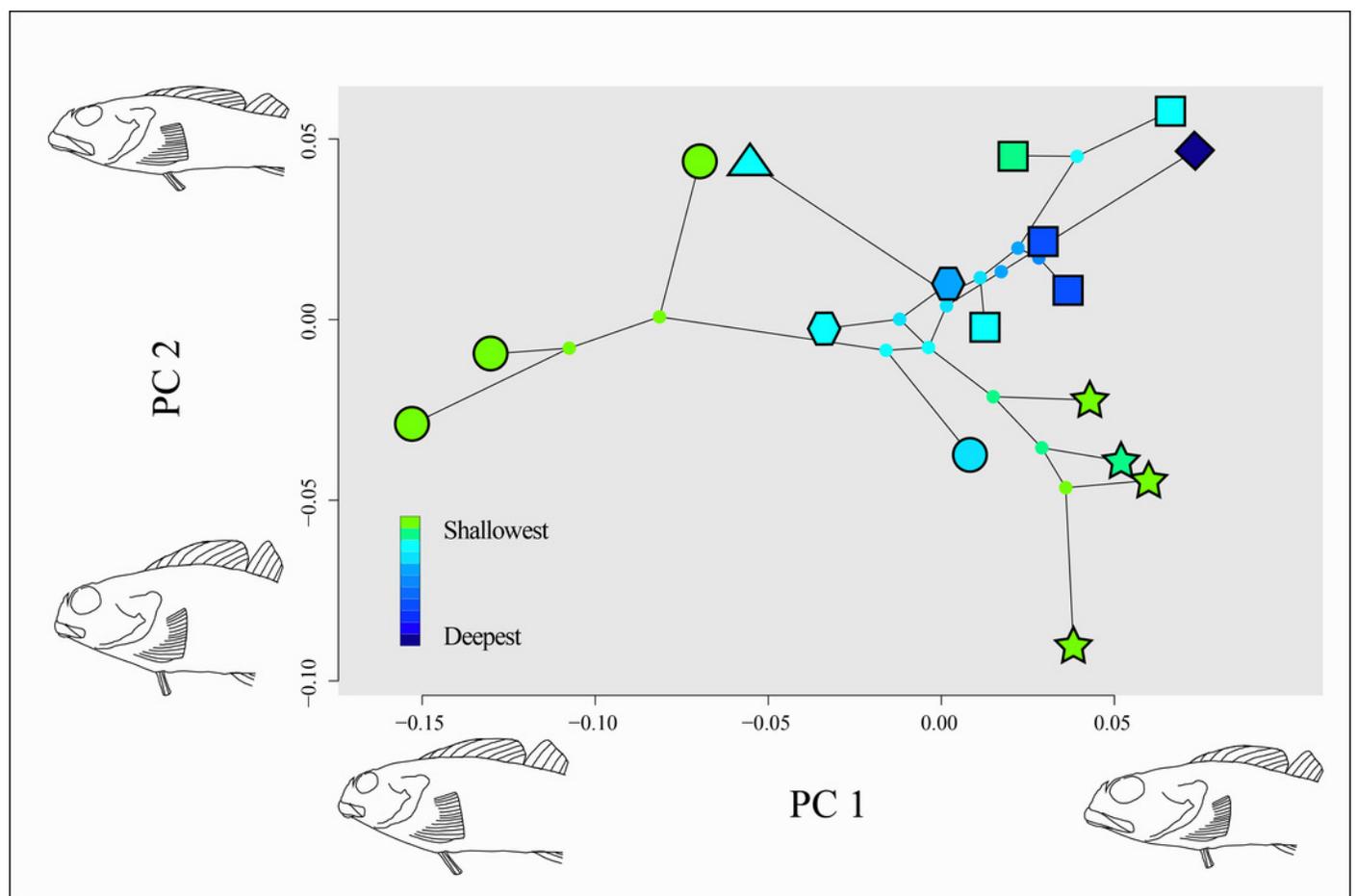


Figure 7

Body shape change correlated with change in maximum depth.

Partial least squares (PLS) axis 1 of body shape is indicated on the horizontal axis with body shape change described by the axis shown through a warped outline sketched from the lateral photograph of *Clinocottus analis* (OSIC 6710, 75.9mm SL) in Figure 1. The outline on the negative side of the axis shows body shape associated with a value of -1 on PLS axis 1, the outline on the positive side of the axis shows body shape associated with a value of +1 on PLS axis 1. The PLS axis 1 of maximum depth is indicated on the vertical axis. Symbols are indicative of the phylogenetic lineage of each species, as in Figure 3.

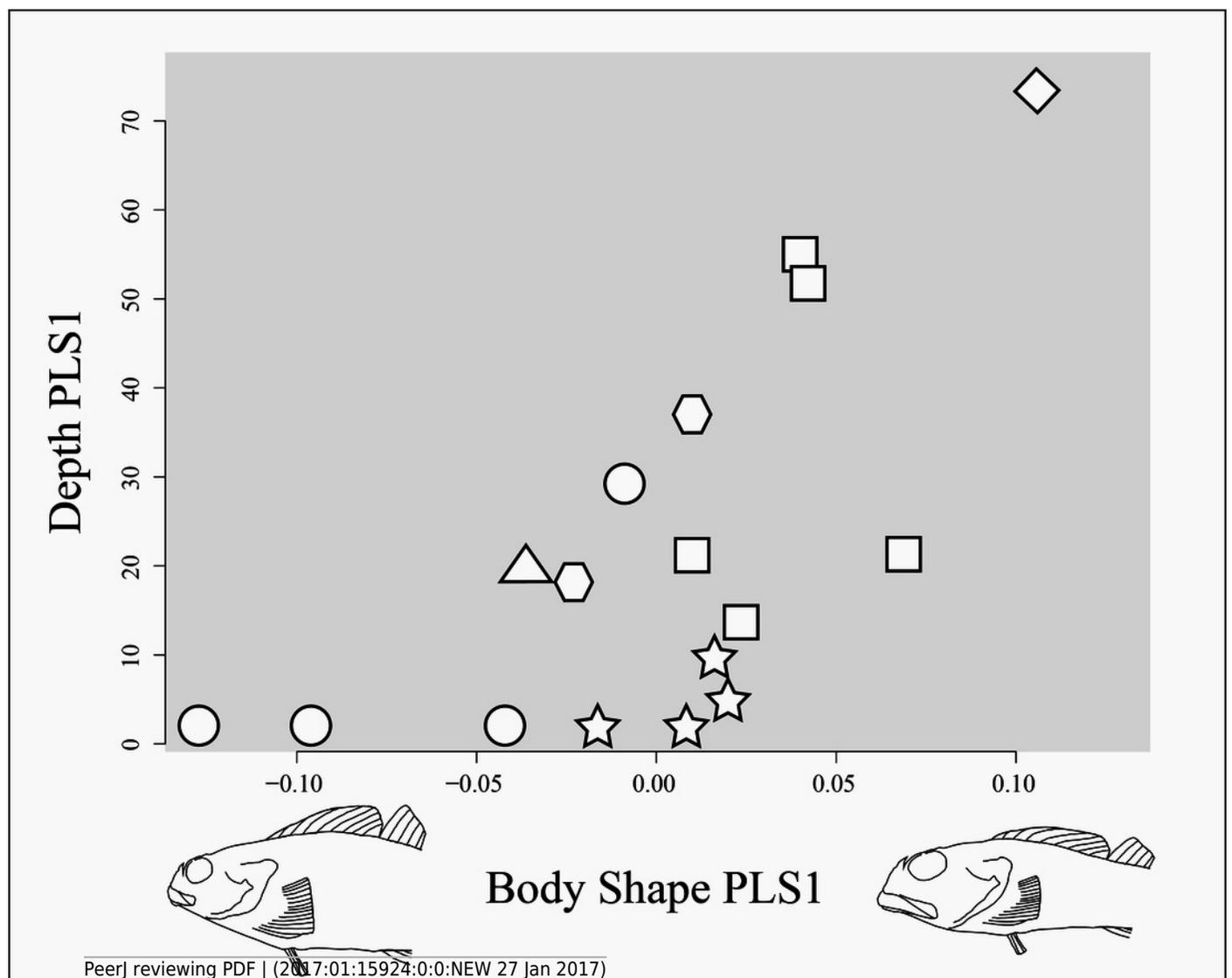


Table 1 (on next page)

Photographed specimens for geometric morphometrics.

Sample size (n) and museum lot number (Museum ID) of specimens examined for each species that was photographed for landmarking and body shape analysis.

Taxon	n	Museum ID
<i>Arteidius corallinus</i>	8	OSIC 08140, SIO 457-34-55, SIO 057-34-55, SIO H51-34-55C
<i>Arteidius fenestralis</i>	9	OSIC 05879, OSIC 09206, UW 000587, UW 017420, UW 118839
<i>Arteidius harringtoni</i>	15	OSIC 04533, OSIC 07471, OSIC 11055, UW 001011, UW 027119, OSIC 07474
<i>Arteidius lateralis</i>	10	OSIC 03175, OSIC 03178
<i>Arteidius notospilotus</i>	2	OSIC 02995, OSIC 07523
<i>Chitonotus pugetensis</i>	4	OSIC 05269, OSIC 07016
<i>Clinocottus acuticeps</i>	7	OSIC 06539, UAM 047689, UAM 047713
<i>Clinocottus analis</i>	5	OSIC 06707, OSIC 06710, OSIC 08136
<i>Clinocottus embryum</i>	6	OSIC 03009, OSIC 07071, UAM 47704
<i>Clinocottus globiceps</i>	7	OSIC 00272, OSIC 00275, OSIC 06600
<i>Clinocottus recalvus</i>	5	OSIC 08134
<i>Leiocottus hirundo</i>	9	OSIC 08132, SIO 059-307-55D, SIO 071-62-55
<i>Oligocottus maculosus</i>	8	OSIC 06628, OSIC 06663, OSIC 07467
<i>Oligocottus rimensis</i>	6	SIO 67-151
<i>Oligocottus rubellio</i>	4	OSIC 08133
<i>Oligocottus snyderi</i>	4	OSIC 06541, OSIC 06668
<i>Orthonopias triacis</i>	6	OSIC 08137

Table 2(on next page)

Matrix of characters examined and character states for each species

Numbered references for each state are indicated in superscript and are as follows: 1: Abe and Munehara (2009), 2: Bolin (1941), 3: Bolin (1944), 4: Hann (1930) , 5: Hubbs (1966) , 6: Misitano (1980) , 7: Morris (1952) , 8: Morris (1956) , 9: Petersen et al. (2005) , 10: Mecklenburg et al. (2002) , 11: Jackson, (2003) , 12: Miller and Lea (1972) , 13: Eschmeyer et al. (1983) , 14: Marliave (1981) , 15: Koya et al. (2011) . *References for minimum depth and maximum depth are located in Supplementary Table 8.

Species	Minimum Depth (m)*	Maximum Depth (m)*	Maximum Length (mm)	Squamation	Enlarged genital papilla	Spermatozoon morphology	Copulation	Parental care
<i>Arteidius corallinus</i>	0	21	140 ^{3,12,13}	Present ³	Absent ³	?	?	?
<i>Arteidius fenestralis</i>	0	55	140 ^{3,10,12,13}	Present ³	Absent ^{3,10}	Oval ⁹	Absent ⁹	Present ⁹
<i>Arteidius harringtoni</i>	0	21	102 ^{3,10,12,13}	Present ³	Absent ^{3,10}	Intermediate ^{4,9}	Present ⁹	Present ⁹
<i>Arteidius lateralis</i>	0	14	140 ^{3,10,12,13}	Present ^{3,11}	Absent ^{3,10}	Oval ^{4,9,15}	Absent ⁹	Present ⁹
<i>Arteidius notospilotus</i>	0	52	250 ^{3,12,13}	Present ³	Absent ³	Oval ⁴	?	?
<i>Chitonotus pugetensis</i>	8	73	230 ^{3,10,12,13}	Present ^{3,11}	Present ^{3,10}	?	Present ^{1,6}	?
<i>Clinocottus acuticeps</i>	0	20	64 ^{3,10,12,13}	Absent ³	Present ^{3,10}	Slender ⁴	?	Absent ¹⁴
<i>Clinocottus analis</i>	0	18	180 ^{3,12,13}	Present ³	Present ³	Slender ⁴	Present ^{1,5}	Absent ^{1,5}
<i>Clinocottus embryum</i>	0	2	70 ^{3,10,12,13}	Absent ³	Present ^{3,10}	Slender ⁴	?	?
<i>Clinocottus globiceps</i>	0	2	190 ^{3,10,12,13}	Absent ³	Present ^{3,10}	Slender ⁴	?	?
<i>Clinocottus recalvus</i>	0	2	130 ^{3,12,13}	Absent ³	Present ³	Slender ⁴	Present ^{1,7}	Absent ^{1,7}
<i>Leiocottus hirundo</i>	5	37	250 ^{3,12,13}	Present ¹¹	Present ³	?	?	?
<i>Oligocottus maculosus</i>	0	10	90 ^{3,10,12,13}	Absent ^{3,11}	Present ^{3,10}	Slender ^{4,15}	Present ¹	Absent ¹
<i>Oligocottus rimensis</i>	0	2	65 ^{3,10,12,13}	Present ³	Present ^{3,10}	Slender ⁴	?	?
<i>Oligocottus rubellio</i>	0	5	100 ^{3,12,13}	Absent ³	Present ³	Slender ⁴	?	?
<i>Oligocottus snyderi</i>	0	2	90 ^{3,10,12,13}	Absent ³	Present ^{3,10}	Slender ^{4,15}	Present ^{1,8}	?