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 the ability to live in intertidal habitats, particularly in tide pools, is likely a primitive state for Oligocottinae, with a single species that has secondarily come to occupy only subtidal habitats. Contrary to previous hypotheses, maximum size and presence of scales do not show a statistically significant correlation with depth. However, the maximum size for all species is generally small (250mm or less) and all show a reduction in scales, as would be expected for an intertidal group. Also contrary to previous hypotheses, 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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2 **intertidal sculpin subfamily Oligocottinae (Pisces: Cottoidea)**

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17   **Abstract**
18   While intertidal habitats are often productive, species-rich environments, they are also harsh and
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 the ability to live in intertidal habitats, particularly in tide pools, is likely
29   a primitive state for Oligocottinae, with a single species that has secondarily come to occupy
30   only subtidal habitats. Contrary to previous hypotheses, maximum size and presence of scales do
31   not show a statistically significant correlation with depth. However, the maximum size for all
32   species is generally small (250mm or less) and all show a reduction in scales, as would be
33   expected for an intertidal group. Also contrary to previous hypotheses, we show that copulation
34   and associated characters are the ancestral condition in Oligocottinae, with copulation most
35   likely being lost in a single lineage within the genus *Artedius*. Lastly, we show that body shape
36   appears to be constrained among species with broader depth ranges, but lineages that occupy
37   only a narrow range of intertidal habitats display novel body shapes, and this may be associated
38   with habitat partitioning, particularly as it relates to the degree of wave exposure.
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41   **Introduction**
42   Intertidal habitats are often highly-productive, species rich environments (Leigh et al., 1987).
43   Yet, intertidal areas are also one of the harshest marine environments, often subject to rapidly
44   changing physical conditions such as wave action, temperature, and current, as well as factors
45   that affect homeostasis of resident organisms, such as pH and dissolved oxygen (Davenport &
46   Woolmington, 1981; Bridges, 1993; Martin, Lawson & Engebretson, 1996). Fishes living in
47   these areas often display common physical characteristics such as small size (Gibson, 1982) and
48   a reduction of scales (e.g., intertidal members of Blenniidae, Gobiesocidae, Pholidae, see
49   Chotkowski, Buth & Prochazka, 1999; Knope & Scales, 2013), presumably as means of coping
50   with the unique set of challenges presented by intertidal habitats. Likewise, the body shapes of
51   intertidal fishes appear constrained to take on one of only a few stereotypical shapes, such as
52   elongate and eel-like (e.g., Pholidae), cylindrical and tapered (e.g., Cottoidea), or dorso-ventrally
53   compressed (e.g., Gobiesocidae; for full descriptions see Horn, 1999).
54   In contrast to the somewhat predictable morphological characteristics of intertidal fishes,
55   the reproductive biology of these species is diverse and does not show apparent patterns between
56   intertidal and subtidal taxa (reviewed in DeMartini, 1999 and Coleman, 1999). However, our

57 understanding of many of these morphological and reproductive patterns in intertidal fishes is
58 based only on qualitative assessments. Body shape, for instance, has never been quantitatively
59 described and compared among or between any group(s) of intertidal fishes. A quantitative
60 approach may shed additional light on the patterns and processes of adaptation to intertidal
61 habitats in fishes. A phylogenetic comparative approach is one way to better understand the
62 relationship of habitat, morphological, and reproductive characters in intertidal fishes, and the
63 marine sculpin (family Psychrolutidae *sensu* Smith & Busby, 2014) subfamily Oligocottinae is a
64 relatively well-studied group and excellent candidate in which to do so.
65   The 16 species that make up Oligocottinae are found in a variety of shallow nearshore
66 habitats across the Pacific coast of North America (Hubbs, 1926; Taranetz, 1941; Ramon &
67 Knope, 2008; Buser & López, 2015). The members of this subfamily occupy a range of subtidal
68 and intertidal habitats, with varying degrees of intertidal occupation across species (Bolin, 1944;
69 Lamb & Edgell, 1986; Mecklenburg, Mecklenburg & Thorsteinson, 2002). Likewise,
70 oligocottines display a broad range of reproductive strategies ranging from copulation and
71 internal insemination to spawning and external mixing of gametes (Petersen et al., 2005; Abe &
72 Munehara, 2009).
73   Recent studies have suggested that the diversification of Oligocottinae is associated with
74 a shift in habitat within the group (Ramon & Knope, 2008; Knope & Scales, 2013). Subtidal
75 habitats are believed to be the ancestral condition of the subfamily and the putative shift from
76 subtidal to intertidal habitats is thought to have been followed by relatively rapid diversification
77 in the intertidal lineage. The shift in habitat is associated with adaptive morphological changes in
78 the intertidal group, which include smaller body size and fewer scales in intertidal species when
79 compared to their deeper-dwelling relatives (Knope & Scales, 2013). Critically, however, this
80 transition is thought to have occurred within the subfamily, such that the group contains subtidal
81 taxa, intertidal taxa, and "transitional" taxa, with the intertidal taxa being the most species rich
82 (Ramon & Knope, 2008).
83   At the heart of these results, however, is an unanswered question, namely: how does one
84 categorize the habitat type (e.g., "intertidal") of each species? Intertidal habitats comprise a range
85 of depths which change on daily, seasonal, and yearly cycles. Categorizing these habitats and
86 ascribing them to a fish, which is free to move across and occupy all habitat types with every
87 flooding tide, presents many potential pitfalls (this conundrum is reviewed in Gibson &
88 Yoshiyama, 1999). The ways in which fishes use these habitats ranges from intertidal residents
89 to intertidal transients (Breder, 1948; Gibson, 1969; Thomson & Lehner, 1976; Potts, 1980) and
90 this continuum only further complicates the qualitative categorization of these fishes. Given
91 these uncertainties, and the potential for arbitrary categorizations to impact the results of
92 comparative analyses, it could be useful to take a different approach.
93   Many species venture into intertidal habitats during high tide but do not remain during
94 low tides ("intertidal transients"). Conversely, some species remain in intertidal habitats
95 throughout the tidal cycle. These "intertidal residents" are often found in special habitats during
96 low tides, such as in tide pools or under exposed rocks, and use a suite of behavioral and

97 physiological adaptations to cope with the challenging conditions that they present (Martin 1996,
98 Gibson and Yoshiyama 1999, Mandic et al. 2009, Martin and Bridges 1999, Evans et al. 1999).
99 The number of prerequisite adaptations needed to survive in tide pool habitats suggests that
100 species that regularly utilize them possess at least the capacity to function as intertidal residents.
101   Small size (i.e., length) and a reduction of scales have been reported for many resident
102 intertidal species and these characters show an adaptive shift between subtidal and intertidal
103 oligocottine sculpins (Knope & Scales, 2013). While reproductive characters are not known to
104 correspond to intertidal vs subtidal habitats (Coleman, 1999; DeMartini, 1999), the relationship
105 between depth and reproductive characters has yet to be formally tested. Reproductive traits are
106 very diverse in sculpins, particularly regarding copulation and parental care (Abe & Munehara,
107 2009). While copulation is difficult to observe directly, characters that are putatively associated
108 with this trait, such as the presence of an enlarged genital papilla, and spermatozoon
109 morphology, are more readily observable. Parental care is also difficult to observe in many
110 species, but has important evolutionary implications.
111   In this study, we forego categorization of habitat and instead use known depth ranges for
112 each species to test whether the host of morphological and reproductive traits putatively linked to
113 species in intertidal habitats in fact correlate with depth. To do so, we construct a phylogenetic
114 hypothesis of the subfamily Oligocottinae using previously published molecular sequence data
115 and use ancestral state reconstruction and phylogenetic comparative methods to test the
116 relationship between depth range and morphological, reproductive, and body shape characters in
117 the group.
118
119

120 **Materials & Methods**
121 ***Phylogenetic framework***
122 We constructed a phylogenetic framework using previously reported DNA sequences from all
123 oligocottine species (sample size per species: 1-9 individuals, median 5) and several outgroups
124 from the cottoid families (*sensu* Smith & Busby, 2014): Agonidae (n = 6 spp.), Cottidae (n = 1
125 sp.), Hexagrammidae (n = 1 spp.), Psychrolutidae (n = 11 spp.), and Rhamphocottidae (n = 1
126 sp.). These outgroup taxa are consistent with the most recent phylogenetic hypotheses of broader
127 cottoid relationships (Knope, 2013; Smith & Busby, 2014). This dataset is accessible on
128 Genbank (accession numbers KP826911–KP827632, see Supplementary Table 1) and contains
129 sequence data from eight molecular loci: one mitochondrial protein-coding locus (Cytochrome c
130 oxidase, COI), two nuclear introns [exon-primed intron crossing (EPIC) locus 1777E10 and
131 EPIC locus 4174E20] and five protein-coding nuclear loci [early growth response protein 1
132 (EGR1); mixed-lineage leukemia (MLL); patched domain-containing protein 1 (ptchd1);
133 Rhodopsin; and Sushi, von Willebrand factor type A, and pentraxin domain-containing 1
134 (SVEP). Multiple sequence alignments (MSAs) for each locus were generated in ClustalW
135 (Larkin et al., 2007). Alignments were visually inspected, trimmed, and concatenated in
136 Mesquite v3.2 (Maddison & Maddison, 2016). The best fitting model of molecular evolution for

137 each locus was identified using the Akaike information criterion (Akaike, 1973; Posada &
138 Buckley, 2004), with the model comparison routines implemented in MrModeltest v2 (Nylander,
139 2004).
140   The molecular dataset contains multiple representatives for each species, so we estimated
141 a species tree using the multispecies coalescent model (Heled & Drummond, 2010) in BEAST
142 v1.8.2 (Drummond et al., 2012). A species set was defined, based on the results of recent
143 phylogenetic hypotheses (Knope, 2013; Smith & Busby, 2014), for the superfamily Cottoidea,
144 which contains all taxa included in the dataset except *Hexagrammos lagocephalus*, which was
145 treated as an outgroup. The species of each sampled individual in the dataset was assigned as a
146 discrete trait. These species assignments were tested and validated in (Buser & López, 2015). For
147 each locus, the model of molecular evolution yielding the lowest AIC value (as calculated in
148 MrModeltest) was applied. The rate of molecular evolution was modeled as an uncorrelated
149 lognormal relaxed clock (Drummond et al., 2006) and was unlinked across all loci. All tree
150 models share a birth-death speciation tree prior with a piecewise linear and constant root
151 population size model and a UPGMA starting tree. Four independent analyses were run for 200
152 million generations each and were sampled every 20,000 generations. MCMC logs were
153 visualized using Tracer v1.6 (Rambaut et al., 2014) to determine convergence and an appropriate
154 number of generations to discard as burn-in. Burn-in was removed and trees combined using
155 LogCombiner v1.7.5 (Drummond & Rambaut, 2007). The phylogeny was pruned in the R
156 statistical environment (R Development Core Team 2015) using the "extract.clade" function
157 from the package "ape" (Paradis, Claude & Strimmer, 2004) to only include members of the
158 subfamily Oligocottinae plus the outgroup taxon *Chitonotus pugetensis*. An R script,
159 "LitorallyAdaptiveScript.R," detailing these commands and all other operations performed in R,
160 along with all pertinent data (phylogeny, data matrix, etc.) is available in the Supplementary
161 Materials in the folder "LitorallyAdaptive_PeerJ_Rfolder."
162
163 ***Character coding***
164 To understand the relationship between a species' depth preferences and its size, reproductive
165 habits, or scale patterns, we coded the following characters from previous studies and, where
166 possible, verified our findings by examining museum specimens and/or collection data
167 (summarized in Table 1):
168
169 1.   **Depth range (Continuous).** Collection data for all specimens of each species of
170 Oligocottinae and the outgroup taxon *C. pugetensis* were collated from museum records from the
171 following natural history collections: University of Alaska Museum (UAM), University of
172 British Columbia Beaty Biodiversity Museum (UBCBBM), University of Washington Burke
173 Museum Fish Collection (UW), Oregon State University Fish Collection (OS), California
174 Academy of Sciences (CAS), Natural History Museum of Los Angeles County (LACM),
175 University of Michigan Museum of Zoology (UMMZ) and Scripps Institute of Oceanography
176 Marine Vertebrates Collections (SIO). These records were accessed through institution-specific

177   (UW, UBCBBM, CAS) or the multi-institutional database interfaces (all others) VertNet.org,
178   Arctos.Database.Museum, and FishNet2.org (see Supplementary Table 2 for all museum records
179   analyzed). For each species, we extracted collection depth data from all museum holdings of
180   adult specimens for which it had been recorded. Some collection depths are recorded as a range,
181   in these cases, we used the maximum depth in the range. Where the collection depth and/or
182   locality is described as "tide pool," "intertidal," etc., we assigned a collection depth of 0 m. To
183   lessen the effects of outliers, we selected a depth range (i.e., minimum depth and maximum
184   depth) for each species that includes 95% of museum collection depths (illustrated in Figure 1).
185   For the purposes of this study, we will refer to this depth range as the range where each species is
186   "commonly" collected. To verify these depth ranges, maximum and minimum depth records for
187   each species were cataloged and cross-examined from multiple sources (Bolin, 1944; Miller &
188   Lea, 1972; Eschmeyer, Herald & Hammann, 1983; Mecklenburg, Mecklenburg & Thorsteinson,
189   2002; see Supplementary Table 3). Where these previously published depth maxima and minima
190   disagree, we chose the median value for each. Many of these ranges include only imprecise
191   descriptions such as "tide pools" and "intertidal areas." In these cases, we assigned a minimum
192   depth value of 0 m and a maximum depth value of 2 m. We used a phylogenetic paired t-test
193   (Lindenfors, Revell & Nunn, 2010) to compare the maximum and minimum depth for each
194   species using the museum records vs. the descriptions published in the literature using the
195   "phyl.pairedttest" function in the R package "phytools" (Revell, 2012; see "Character coding-
196   Depth Range" section in "LitorallyAdaptiveScript.R" in Supplementary Folder
197   "LitorallyAdaptive_PeerJ_Rfolder").
198   2.   **Tide pool occupancy (Presence, absence).** We noted which taxa were explicitly
199   collected from tide pools in museum collection data, in previously published depth ranges, and in
200   primary literature.
201   3.   **Length (Continuous).** Maximum recorded length of each species was cataloged and
202   cross-examined from multiple sources (Bolin, 1944; Miller & Lea, 1972; Eschmeyer, Herald &
203   Hammann, 1983; Mecklenburg, Mecklenburg & Thorsteinson, 2002; Knope & Scales, 2013).
204   Where sources disagreed, we used the median value.
205   4.   **Squamation (Presence, absence).** For the purposes of this study, squamation is defined
206   as any dermal ossification outside of the lateralis system. This includes scales, prickles, and
207   scutes. The evolution of scale types in sculpins is poorly understood, but what is known suggests
208   that the modified scales found in Oligocottinae may each represent an equal number of
209   evolutionary steps away from the ancestral ctenoid scale type, with the latter not represented in
210   any extant cottoid (Jackson, 2003). We therefore feel that in the context of this study it is
211   unjustifiable to discriminate between scale types in oligocottines until further study indicates
212   otherwise. Presence of squamation was coded from descriptions in the literature (Bolin, 1944;
213   Begle, 1989; Mecklenburg, Mecklenburg & Thorsteinson, 2002; Jackson, 2003).
214   5.   **Enlarged genital papilla (Presence, absence).** This character was coded directly from
215   descriptions in the literature (Bolin, 1944; Mecklenburg, Mecklenburg & Thorsteinson, 2002).

216 6. **Spermatozoon morphology (Oval, intermediate, slender).** Character states were
217 adapted from descriptions in the literature (Hann, 1930; Petersen et al., 2005; Koya et al., 2011).
218 Slender sperm morphology is associated with internal insemination in many groups of fishes
219 (Mattei, 1991). Petersen et al. (2005) confirmed this observation in Oligocottinae by
220 demonstrating that spermatozoa with a slender-type morphology are active only in seawater that
221 has been diluted to approximate the osmolality of ovarian fluid in these sculpins, while
222 spermatozoa with oval-type morphology are active in both dilute and full-strength seawater. This
223 suggests that slender-type spermatozoon morphology is indicative of obligate insemination, but
224 oval-type morphology indicates the capacity for external mixing of gametes (i.e., spawning).
225 7. **Copulation (Presence, absence).** For the purposes of this study, copulation is defined as
226 the transfer of sperm from a male into the ovary of a female. The presence of copulation, where
227 known, was determined from descriptions found in the literature (Bolin, 1941; Morris, 1952,
228 1956; Hubbs, 1966; Misitano, 1980; Petersen et al., 2005; Abe & Munehara, 2009).
229 8. **Parental care (Presence, absence).** For the purposes of this study, egg guarding by one
230 or both parents is considered parental care. The presence of parental care, where known, was
231 determined from a review of behavioral descriptions from previous literature (Morris, 1952;
232 Hubbs, 1966; Petersen et al., 2005; Abe & Munehara, 2009).
233
234 *Character mapping and ancestral state reconstruction*
235 To visualize the inferred evolutionary history of characters, we performed ancestral state
236 reconstruction (ASR) of each character examined in this study. For discrete characters, we used
237 maximum likelihood (ML) with a Markov k-state 1 parameter (Mk1) model of evolution (Lewis,
238 2001), implemented in Mesquite. The evolutionary history of continuous characters was inferred
239 using ML in the R statistical environment with functions from the package "phytools" (Revell,
240 2012; see "Character mapping and ancestral state reconstruction" section in
241 "LitorallyAdaptiveScript.R" in Supplementary Folder "LitorallyAdaptive_PeerJ_Rfolder").
242
243 *Body shape analysis*
244 Qualitative assessment of body shape in intertidal fishes has not only shown differences in the
245 shape of some intertidal species compared to their subtidal relatives (e.g., *Hypsoblennius* spp.;
246 Thomson & Lehner, 1976), but also that many groups of intertidal fishes (including intertidal
247 sculpins) have converged on a small number of stereotypical body shapes (reviewed in Horn,
248 1999). While thought provoking, these observations have yet to be tested using quantitative
249 methods. We used landmark-based geometric morphometrics to describe and compare the body
250 shape of each species in this study and test for correlation between body shape and the depth at
251 which each species occurs. Body shape data were collected from digital photographs of the
252 lateral view of museum specimens of each species. Photography followed the phototank method
253 of Sabaj Pérez (2009). To minimize the likelihood of introducing variation due to photographic
254 artifacts (i.e., image distortion), lighting, distance to the subject, focal length, camera angle, and
255 camera settings (e.g., aperture) were kept constant. We photographed 115 specimens,

256   representing all 16 species in Oligocottinae plus the outgroup taxon *C. pugetensis*. Sample size
257   per species ranges from 2 to 15 individuals, median six (Table 2). To capture overall head and
258   body shape, landmarks were adapted from those described in previous studies of sculpin body
259   shape (Strauss & Bookstein, 1982; Strauss & Fuiman, 1985). Preliminary analysis revealed a
260   high frequency of distended stomachs and upturned caudal peduncles, presumably from prior
261   feeding and preservation (respectively), so landmarks that appeared to be influenced by these
262   variables were not included. Thirteen landmarks were ultimately used in this study (Fig. 2). We
263   used tps-Dig2.2 (Rohlf, 2007) to locate the landmarks on each specimen from the digital
264   photographs. To compare body shape across the group, landmark configurations were Procrustes
265   superimposed using MorphoJ v1.06 (Klingenberg, 2011). The aligned landmark coordinates
266   were used to calculate a covariance matrix on which we performed a principal component
267   analysis (PCA) in MorphoJ. The number of significant principal component axes was calculated
268   using the broken stick method (Frontier, 1976; Jackson, 1993; Legendre & Legendre, 2012),
269   implemented with the "screeplot.cca" function in the R package "vegan" (Oksanen et al., 2017).
270   The significant principal component axes were used to interpret overall shape variation and
271   visualize the distribution of species in body shape morphospace. To visually check for evidence
272   of morphological convergence or divergence, we projected phylogenetic relatedness into the
273   principal component morphospaces and inferred states of each significant PC axis for each
274   ancestral node (i.e., phylomorphospace analysis; Sidlauskas, 2008) using the
275   "phylomorphospace" function in the R package "phytools" (Revell, 2012; see "Body shape
276   analysis" section in "LitorallyAdaptiveScript.R" in Supplementary Folder
277   "LitorallyAdaptive_PeerJ_Rfolder").
278
279   ***Depth correlation analysis***
280   For both museum records and previously published depth ranges, preliminary results indicated
281   that, while there is considerable variability in the maximum collection depth of each species in
282   Oligocottinae, all species share a minimum recorded depth of zero meters. Given this
283   invariability in minimum depth, we chose to use only maximum depth as our depth variable for
284   regression analysis. We used phylogenetic generalized least squares regression (PGLS)
285   implemented using the "gls" function in the R package "nlme" (Pinheiro et al., 2015) to test for a
286   linear correlation between depth and each of the putatively associated characters examined in
287   this study: presence of scales and maximum length. We also tested for linear correlation between
288   depth and the presence of a genital papilla, which is the only reproductive character in this study
289   for which states are known for all oligocottine species. To account for potential variability in trait
290   evolution (e.g., Brownian motion, selection, etc.), we tested three alternate single-parameter
291   correlation structures supplied in the R package "ape" (Paradis, Claude & Strimmer, 2004) in
292   each of our regression models: a Brownian motion model with correlation due to phylogenetic
293   relatedness represented by Pagel's lambda (Pagel, 1994, 1999), which we estimated using ML; a
294   Brownian motion model with the rate of evolution (accelerated or decelerated) estimated using
295   ML; and a single optimum (i.e., stabilizing selection) Ornstein-Uhlenbeck (OU) model
296   (Felsenstein, 1988; Hansen, 1997) with the strength of attraction towards the optimum

297   represented by alpha and estimated using ML. The best fitting model for each regression was
298   determined by comparing AIC values.
299   One of our hypotheses is that shallow-dwelling species show convergent morphology
300   differing from that of their subtidal sister taxa. To test for convergent or divergent evolution of
301   body shape, we used a stepwise model-fitting approach, "surface," that detects shifts and
302   convergence in phenotypic optima (Ingram & Mahler, 2013). In this approach, each optimum
303   contributes a parameter to an OU process of evolution. The "surface" method finds the
304   maximum-likelihood estimate of the number and location of phenotypic optima under the OU
305   model and collapses similar phenotypic optima together if it improves the AIC score (Ingram &
306   Mahler, 2013). Phenotypic convergence is indicated when independent lineages share a common
307   optimum. These analyses were conducted in the R environment using functions from the package
308   "surface" (Ingram & Mahler, 2013).
309   To visualize only the aspects of body shape that covary with depth, a partial least squares
310   analysis (PLS) was conducted on a matrix of depth variables (minimum depth and maximum
311   depth) and Procrustes-aligned shape variables (Rohlf & Corti, 2000). This analysis was
312   conducted in MorphoJ and in R using functions from the package "geomorph v2.0" (Adams &
313   Otárola-Castillo, 2013). In both cases, the significance of the covariance was tested using a
314   permutation test with 10,000 iterations (see "Correlation with depth" section in
315   "LitorallyAdaptiveScript.R" in Supplementary Folder "LitorallyAdaptive_PeerJ_Rfolder").
316
317
318   **Results**
319   ***Character mapping and ancestral state reconstruction***
320   The trimmed, concatenated MSA dataset spans 4695 aligned nucleotide sites, containing 1037
321   variable sites. The topology of the Bayesian maximum clade credibility (MCC) phylogeny
322   produced herein is identical to the topology of the MCC phylogeny reported in Buser & López
323   (2015), with similar levels of support for each clade (Figure 3). As noted in Buser & López
324   (2015), this topology is similar to that of other molecular-based phylogenetic inferences of
325   Oligocottinae (i.e., Ramon & Knope, 2008; Knope, 2013), but has substantially higher support
326   values (i.e., Bayesian posterior probability) for many of the inferred relationships. We will use
327   the classification and taxonomy suggested by Buser & López (2015) for discussion of the
328   interrelationships of oligocottine sculpins.
329   The outgroup taxon, *C. pugetensis*, rarely (if ever) occurs in intertidal areas (Fig. 1, Table
330   1, Supplementary Tables 3 & 4). However, apart from *L. hirundo*, all the constituent species of
331   Oligocottinae are regularly found in intertidal habitats and both museum records and published
332   depth ranges include tide pools in the common collection depth or depth range data for all
333   oligocottine species but *L. hirundo* (Fig. 1, Table 1, Supplementary Tables 3 & 4). There is also
334   explicit discussion of tide pool and intertidal occupancy for all oligocottine species except *L.*
335   *hirundo* in the primary literature (Supplementary Table 3). However, while the occupation of
336   intertidal and subtidal habitats is often portrayed as an either/or scenario, there is considerable

337   variation in the maximum depth at which each species occurs (Fig. 1, Table 1). Generally

338   though, all oligocottine species occur at relatively shallow depths: none is commonly collected at

339   depths greater than 55 m, most (12/16 spp.) are not commonly collected below 25m (though

340   there is some discrepancy between the museum collection data and the published depth ranges

341   for *A. corallinus* and *A. fenestralis*), and four (published ranges) to seven (museum depth data)

342   species are common only in very shallow (i.e., 2 m depth or less) habitats (Table 1). There is

343   considerable disagreement between the museum collection data and the published depth range

344   for *A. notospilotus*, *C. acuticeps*, *C. analis*, and *L. hirundo*. In each case, published depth ranges

345   indicate a maximum depth that is > 10 m deeper than the depths where these species have been

346   commonly collected in museum holdings (Supplementary Tables 3 and 4). However, the depth

347   ranges are otherwise largely congruent, and the differences between the two datasets are not

348   statistically significant (phylogenetic paired t-test p-value > 0.89). All remaining analyses show

349   identical outcomes when using either the common museum collection data or the previously

350   published depth range data for each species. Given the congruence of the datasets, the

351   indistinguishable outcome of using one over the other, and the more verifiable nature of the

352   museum collection records, we present the results of the remaining analyses using only the

353   common museum collection depth range of each species.

354   Predictably, the ASR of minimum depth shows that the most recent common ancestor

355   (MRCA) of Oligocottinae likely occurred in shallow habitats (ML estimate: 1 m; 95%

356   confidence interval: 0 m, 2 m). Ancestral state reconstruction of tide pool occupancy shows that

357   with extremely high proportional likelihood (0.9988) the MRCA of Oligocottinae occurred in

358   tide pools. In fact, even the MRCA of the *Leiocottus* lineage was likely (0.9215 proportional

359   likelihood) capable of living in tide pools (Supplementary Figure 1). Thus, the absence of tide

360   pool occupation in *L. hirundo* likely represents a derived state. The ASR of maximum depth

361   suggests that the MRCA of Oligocottinae occurred down to only moderate depths (ML estimate:

362   23 m; 95% confidence interval: 2 m, 44 m; see Fig. 4) and suggests that the habitation of only

363   very shallow-water habitats (maximum depth = 2 m or less) seen in members of *Oligocottus*

364   *maculosus*, *O. rimensis*, and *O. snyderi* and in all members of the subgenus *Clinocottus*

365   (*Blennicottus*) represents a derived state (see Table 1, Fig. 4). However, given the uncertainty of

366   the ML estimates of maximum depth at each node (Fig. 4), and the uncertain phylogenetic

367   relationships of *Blennicottus*, *Leiocottus*, and *Oligocottus* lineages (Fig. 1), it is not possible to

368   claim with confidence the number of transitions that may have occurred within the subfamily.

369   Maximum length shows no obvious relationship with depth in Oligocottinae (illustrated

370   in Fig. 4). All species (including the outgroup) are relatively small (none longer than 250mm),

371   most (12/16 spp.) do not grow longer than 150mm, and seven species do not grow longer than

372   100mm (Table 1). The ASR of maximum length suggests that the MRCA of Oligocottinae was

373   small (132mm, 95% confidence interval: 83mm, 182mm), but like maximum depth, the

374   uncertainty of the ML estimates at each node precludes additional inference into the

375   diversification of this trait (Supplementary Figure 2).

376   Squamation is common among members of Oligocottinae but is completely absent in two
377 clades: the subgenus *Clinocottus* (*Blennicottus*), and the clade in *Oligocottus* made up of *O.*
378 *maculosus*, *O. snyderi*, and *O. rubellio* (Table 1, Supplementary Figure 3). Predictably, the ASR
379 shows that the presence of squamation is the most likely state for the MRCA of Oligocottinae
380 (proportional likelihood: 0.81), and that the absence of scales represents an independent loss of
381 the trait in the MRCA of each of the above clades (Supplementary Figure 3). It should be noted
382 that we inferred the evolution of squamation using an Mk1 model, which assumes that all
383 potential changes in state are equally probable (Lewis, 2001). Given the lack of rigorous study of
384 scale evolution in sculpins, specifying a more complex model is not warranted. However, it is
385 our opinion that re-acquisition of squamation is an extremely unlikely evolutionary scenario in
386 oligocottine sculpins (i.e., less probable than the loss of squamation) and thus the proportional
387 likelihood of the presence of scales for the ancestral nodes in Oligocottinae should be taken as a
388 conservative estimate.
389   An enlarged genital papilla is found in all but five species of Oligocottine sculpins (Fig.
390 5). The ASR shows with high proportional likelihood (>0.98) that this character was present at
391 all ancestral nodes except those within the clade composed of the members of the genus
392 *Artedius*. Within *Artedius*, an enlarged genital papilla was likely lost in the MRCA of the clade
393 containing *A. corallinus*, *A. fenestralis*, *A. lateralis*, and *A. notospilotus* (Fig. 5). An independent
394 loss of the enlarged genital papilla occurred in the oligocottinin species *Orthonopias triacis*.
395   The distribution of spermatozoon morphology follows a pattern similar to that of the
396 enlarged genital papilla. Outside of the clade composed of members of the genus *Artedius*,
397 slender-type spermatozoa are present in all Oligocottine sculpins and, with high proportional
398 likelihood (>0.90), this is the state at all ancestral nodes (Fig. 5). Within *Artedius*, an oval-type
399 spermatozoon likely evolved in the MRCA of the clade containing *A. corallinus*, *A. fenestralis*,
400 *A. lateralis*, and *A. notospilotus*. *Artedius harringtoni* possess an intermediate spermatozoon
401 morphology and is the only oligocottine to do so (Fig. 5). With one notable exception, this shows
402 that all species with a slender-type spermatozoon morphology (which in other species is known
403 to function only in ovarian fluid) also possess an enlarged genital papilla, which is presumably
404 used in copulation. The exception to this observation is the species *Orthonopias triacis*, which
405 does not possess an enlarged genital papilla, but does possess a slender-type sperm morphology.
406   Though not known for all species in Oligocottinae, the distribution of copulatory
407 behavior closely follows that of spermatozoon morphology, where copulating species possess
408 either slender or intermediate-type spermatozoon morphology, and non-copulating species
409 possess only oval-type spermatozoon morphology (illustrated in Fig. 5; see also Supplementary
410 Figure 4). The ASR of this character shows that, with high proportional likelihood (>0.95),
411 presence of copulation is the likely state for all ancestral nodes outside of the clade composed of
412 the members of the genus *Artedius*. Within *Artedius*, copulation was likely lost in the MRCA of
413 the clade containing *A. corallinus*, *A. fenestralis*, *A. lateralis*, and *A. notospilotus*. This finding
414 reinforces the observations of Mattei (1991) and Petersen et al. (2005) who each show that
415 slender-type sperm morphology is indicative of copulating species. Once again, *Orthonopias*

416   *triacis* presents a noteworthy case as there is evidence of copulation for the species and the
417   species possesses a slender-type spermatozoon morphology, yet the species lacks an enlarged
418   genital papilla or other known intromittent organ (Fig. 5).
419   The presence or absence of parental care has been described in less than half of all
420   oligocottine species (6/16 spp.), but follows a similar pattern to those seen in other reproductive
421   characters in the group, where members of the genus *Artedius* tend to differ from all other
422   species. In this case, parental care is observed only in members of *Artedius* (Fig. 6). The ASR
423   shows that parental care was likely present in the MRCA of *Artedius*, while a lack of parental
424   care is the most likely state for the MRCA of the tribe Oligocottini. However, given the
425   substantial amount of missing data for this trait, the ASR is subject to change with the addition of
426   new observations.
427
428   ***Body shape analysis***
429   Observed body shape variation was captured by two significant principal components, which
430   cumulatively describe 70% of the total variance. We used an outline of a specimen of
431   *Clinocottus analis* to visualize shape change represented by each PC axis in MorphoJ (Fig. 7).
432   Principal component (PC) 1 (52% of total observed variance) describes antero-posterior
433   elongation/compression of the head and mouth as well as the relative size of the eye. Principal
434   component 2 (19% of total observed variance) captures dorso-ventral elongation/compression of
435   the body, the shape of the eye, and the slope of the snout.
436   There is no clear evidence of a consistent relationship between the minimum depth or
437   maximum depth of a species and its morphology, nor evidence of morphological convergence
438   among shallow or deep-ranging species (Fig. 8). Species with deeper ranges appear to be
439   constrained to a common morphospace, while species that inhabit only shallow depth ranges
440   (e.g., *Oligocottus* spp., *Clinocottus* (*Blennicottus*) spp.) appear to occupy novel and distinct areas
441   of morphospace (Fig. 8). This observation is supported by the results of the "surface" analysis,
442   which inferred three optima for body shape in the morphospace described by the significant PC
443   axes: one for members of the genus *Oligocottus* plus *Orthonopias triacis*, one for members of the
444   subgenus *Clinocottus* (*Blennicottus*), and a third that is occupied by all other oligocottines
445   (Supplementary Figure 5). However, there is no clear pattern in terms of the direction of the
446   divergence in morphospace of these taxa. Interestingly, two of the optima are occupied almost
447   entirely by taxa that are found exclusively in shallow water (i.e., *Clinocottus* (*Blennicottus*) and
448   all but one species of *Oligocottus*), while the remaining optimum is made up almost exclusively
449   of deeper-ranging taxa (the exception being *C.* (*O.*) *acuticeps*. Here again *Orthonopias triaci*s is
450   remarkable in that it is a deeper-ranging species, but appears to be drawn to the phenotypic
451   optimum occupied otherwise exclusively by the genus *Oligocottus*.
452
453   ***Depth correlation analysis***
454   No morphological, reproductive, and body shape variables examined in this study show a
455   significant correlation with maximum depth. The phylogenetic generalized least squares

456 regression showed no significant linear correlation between depth and the putative covariates
457 examined herein: maximum length, presence of scales, or presence of an enlarged genital papilla.
458 Likewise, the results of the PLS analysis were identical in MorphoJ and in R, and failed to show
459 a statistically significant correlation between body shape and depth range (p-value > 0.05).
460 However, the analysis did reveal a few interesting observations: species that are restricted
461 entirely to shallow water tend to display greater morphological diversity than those that range
462 into deeper water, but generally have smaller eyes, smaller mouths, terminal placement of the
463 mouth, and more robust bodies, while deeper-ranging species tend to have larger eyes, larger
464 mouths, subterminal placement of the mouth, and dorso-ventrally compressed bodies (Fig. 9).
465
466

467 **Discussion**
468 The results of our study show support for some previous hypotheses of the general evolution of
469 intertidal fishes in that, excepting *L. hirundo*, all oligocottines regularly occur in tide pools, and
470 all show small bodies and few scales. However, our results do not support previous hypotheses
471 of the diversification of the subfamily Oligocottinae as it relates to depth, nor do we support
472 previous hypotheses regarding the evolution of reproductive modes in oligocottines or, to a
473 certain extent, sculpins in general. In some cases, our conclusions directly oppose those made by
474 previous authors. We discuss these results and some plausible explanations for our congruous
475 and incongruous conclusions below.
476

477 *Squamation, length, and depth*
478 The subfamily Oligocottinae should be thought of as a clade of intertidal-occurring fishes and the
479 ability to live in intertidal depths and specialized intertidal habitats such as tide pools is likely the
480 ancestral state of the group. This finding does not support the hypothesis that there is differential
481 diversification of intertidal vs. subtidal oligocottine groups (e.g., Ramon & Knope, 2008; Knope
482 & Scales, 2013), as we conclude that virtually all oligocottines reside with some frequency in
483 intertidal habitats. However, this ability to live in tide pools does not preclude residency in other
484 habitat types within the same species, as many of the extant and ancestral species are capable of
485 living in a variety of depths in addition to the intertidal ones. Thus, the diversification of
486 Oligocottinae should not be characterized as occurring between intertidal and subtidal habitats,
487 but rather occurring within a habitat range that includes both. This may explain the general lack
488 of correlation between depth the other characters examined in this study.
489 Small maximum size and a reduction in scales have been noted as common features of
490 intertidal fishes by previous authors (Gibson, 1982; Knope & Scales, 2013), and while we found
491 no evidence to support these hypotheses within Oligocottinae, oligocottines as a whole may in
492 fact offer support. All oligocottines are small (none longer than 250 mm), and all show a
493 reduction in scales when compared to a "typical" scaled member of Cottales, such as *Oxylebius*
494 *pictus* (see Jackson, 2003). Outside of the lateral line, the most heavily scaled oligocottines
495 possess only modified scales in a narrow band on the body along each side of the dorsal fins and

496   on the dorsal surfaces of the head and caudal peduncle (e.g., *Orthonopias triacis*; see
497   descriptions in Bolin, 1944; Jackson, 2003). Others possess highly reduced scales in the form of
498   prickles (e.g., *Clinocottus (Clinocottus) analis*; see description in Bolin, 1944). It is presumed
499   that the primary reason scales are often reduced in intertidal fishes is to promote cutaneous
500   respiration, which is dependent on well vascularized skin that is free from obstructions (Feder &
501   Burggren, 1985; Martin & Bridges, 1999). Within Oligocottinae, it may simply be the case that
502   the highly-reduced scales seen in the group do not cover enough surface area to interfere with
503   cutaneous respiration in a meaningful way. Or perhaps that cutaneous respiration is restricted to
504   only certain areas, such as the head (as seen in *Coryphoblennius galerita*, see Zander, 1972), or
505   buccal chamber (reviewed in Bridges, 1993). This may explain the presence of scales within the
506   group, and the fact that even the most heavily scaled members of Oligocottinae occur intertidally
507   and in tide pools (Fig. 2). Interestingly, the one oligocottine species that does not occur
508   intertidally (*L. hirundo*) possess only a few highly reduced scales in the form of a scattered patch
509   of prickles located just posterior to the base of the pectoral fins (see Jackson, 2003). We interpret
510   this as evidence that a reduction in scales in an ancestral condition for *Leiocottus*, and possibly
511   Oligocottinae as a whole.
512   The evolutionary history of maximum size, depth range, and the presence of squamation
513   all support the idea that the subfamily Oligocottinae is a primitively intertidal group. All species
514   in this subfamily display conditions of these traits that are common in intertidal taxa, and all but
515   one species are commonly found intertidally and explicitly in tide pools. We thus conclude the
516   opposite of previous studies and suggest that rather than containing an intertidal radiation
517   (Ramon & Knope, 2008; Knope & Scales, 2013), the subfamily Oligocottinae itself represents an
518   intertidal radiation.
519
520   ***Body shape and depth***
521   While the body shape of deeper-ranging species is relatively conserved, groups that occupy only
522   intertidal areas appear to be exploring novel areas of morphospace (Figure 8, Supplementary
523   Figure 5). This is most pronounced in members *Oligocottus* and the subgenus *Clinocottus*
524   (*Blennicottus*), where the two groups each occupy a morphological optimum that is distinct from
525   each other and from all but one other oligocottine (i.e., the enigmatic *O. triacis*). The
526   morphological distinctiveness of *Clinocottus* (*Blennicottus*) is readily apparent, even to the
527   casual observer, with antero-posteriorly compressed heads and highly robust bodies and fins (see
528   illustrations in Bolin, 1944). The constituent species of this subgenus occur most abundantly in
529   steep, rocky habitats with high wave exposure and are often the only oligocottine species found
530   in these areas (T. Buser pers. obs.; Green, 1971; Yoshiyama, 1981; Yoshiyama, Sassaman &
531   Lea, 1986; Mgaya, 1992, see also descriptions in Eschmeyer, Herald & Hammann, 1983; Lamb
532   & Edgell, 1986; Mecklenburg, Mecklenburg & Thorsteinson, 2002). The blunt heads and short,
533   stocky bodies of these species are also seen in other intertidal fishes occupying similarly
534   exposed, rocky habitats and may reflect a common evolutionary response to the physical
535   demands of living in such areas (Kotrschal, 1988, 1989; Thomson, Findley & Kerstitch, 2010,

536   reviewed in Kotrschal, 1999). If the diverse morphologies seen among intertidal specialist clades
537   are reflective of their respective habitat partitions, it may also be the case that the constrained
538   morphologies seen in deeper-ranging taxa reflect a kind of stabilizing selection of generalist
539   traits that are optimal for occupying a comparatively wide variety of habitats. The relatively high
540   diversity of morphotypes seen in shallow vs deep-ranging species may mask morphological traits
541   that are in fact associated with depth, as is suggested by the results of our PSL analysis (Fig. 9).
542   However, the lack of statistical significance of this trend warrants caution on interpretation of
543   this finding until the question can be revisited with additional taxa.
544
545   ***Reproduction***
546   While reproductive characters do not show any correlation with depth range in Oligocottinae, the
547   evolution of these traits in the subfamily may offer new insight into the evolution of reproductive
548   modes in cottoids. Copulation in oligocottines is associated with an enlarged genital papilla and a
549   slender-type spermatozoon morphology. These traits are broadly distributed in Oligocottinae and
550   are likely the ancestral state of the subfamily (Fig. 5). Importantly, the absence of copulation and
551   associated traits in most members of the genus *Artedius* represents a loss and is thus a derived
552   state. This finding runs counter to previous hypotheses of the evolution of reproductive modes in
553   sculpins, which interpreted the seemingly scattered distribution of copulation in cottoids as
554   indicative of parallel or convergent evolution of copulation from non-copulating ancestors (Abe
555   & Munehara, 2009; Muñoz, 2010). Under this paradigm, Petersen et al. (2005) suggests that the
556   ability of the oval-type sperm morphology (uniquely capable of functioning well in seawater and
557   ovarian fluid, seen in non-copulating members of *Artedius*) to function in ovarian fluid
558   represents a derived condition and concludes that the presence of this trait in most members of
559   *Artedius* represents an evolutionary step *towards* copulation in the group. We conclude the
560   opposite of Petersen et al. (2005), and suggest that rather than the sperm's ability to function in
561   ovarian fluid, it is in fact the sperm's ability to function in seawater that is a derived state and
562   this, along with the loss of an intromittent organ, represents an evolutionary step *away* from
563   copulation within *Artedius*.
564   A reduction or loss of the enlarged genital papilla is seen in other oligocottines as well.
565   Critically, however, these species maintain a slender-type spermatozoon morphology and, where
566   known, copulation. For example, while most oligocottines possess genital papillae that are quite
567   large and robust, males in the genus *Oligocottus* possess papillae that are uniquely small, gracile,
568   and thread-like (Supplementary Figure 6). Also unique to the males of this genus are
569   modifications of the anterior portion of the anal fin (Supplementary Figure 7) which, where
570   known, is used for grasping females during copulation (*O. snyderi*; Morris, 1956). It is possible
571   that the added security and stability during copulation provided by the prehensile anal fin rays
572   has rendered the large genital papilla seen in other oligocottines redundant. The other example of
573   a reduction in the size of the male genital papilla is seen in *Orthonopias triacis*, where males lack
574   an intromittent organ altogether, yet also possess slender-type sperm morphology and are known
575   to copulate. Males of this species possess enlarged pelvic fins that face inwardly "palm to palm,"

576 and project postero-ventrally from a "pedunculated" base (Bolin, 1944). Perhaps these highly
577 modified, sexually dimorphic pelvic fins are used in a grasping manner that, like in *Oligocottus*,
578 is used to aid in copulation and has eliminated the need of a large, robust male genital papilla.
579 Copulation without the use of an intromittent organ is seen in at least one other member of
580 Cottales, the sea raven (*Hemitripterus villosus*). In this species, males are not known to possess
581 any putative grasping organs. Instead, the female everts her genital tract, which is covered in
582 mucus, and the male ejaculates onto it, whereby the sperm become entrained in the mucus and
583 enter the female when she inverts her genital tract (Munehara, 1996).
584   Our results show that while the presence of an enlarged male genital papilla is a likely
585 indicator of copulation, the absence of an intromittent organ does not necessarily indicate the
586 absence of copulation. Furthermore, our results show that non-copulating species may evolve
587 from copulating ancestors. Given the widespread distribution of copulation and/or an enlarged
588 genital papillae within Cottoidea (Abe & Munehara, 2009; Muñoz, 2010), we suggest that
589 copulation and associated traits may have evolved much earlier in cottoids than has been
590 previously estimated. Perhaps the seemingly disparate distribution of copulation in cottoids is not
591 due to many independent evolutions of copulation, but rather to a single early evolution of
592 copulation and multiple subsequent losses of the trait. Given the suite of complex physiological
593 and behavioral traits associated with copulation in sculpins (e.g., internal gamete association
594 with delayed fertilization, see Munehara, Takano & Koya, 1989, 1991; Munehara et al., 1997;
595 Petersen et al., 2005), the independent loss of copulation by certain lineages would, in our
596 opinion, be a far simpler explanation for the modern distribution of the trait than would the
597 independent evolution of copulation and all associated characters.
598
599 ***Parental care***
600 Like other reproductive traits, the distribution of parental care in Oligocottinae does not appear
601 to be related to the distribution of depth ranges. Rather, only members of the genus *Artedius*
602 display parental care. Strong phylogenetic signal of parental care has been reported for other
603 groups as well (reviewed in Coleman, 1999), but this does not provide a satisfying explanation
604 for why *Artedius* differs from all other oligocottines in this trait. It is interesting to note that
605 parental care shows an almost inverse distribution to oval-type sperm morphology (i.e., obligate
606 copulation; see Figs. 5 and 6), but the limited sample size and degree of missing data for parental
607 care make this a tenuous connection. Many other non-copulating sculpins also display parental
608 care (e.g., *Hemitripterus* spp., *Enophrys bison*, *Myoxocephalus* spp., *Cottus* spp.), but this trait is
609 also seen in some copulating species, including *Artedius harringtoni* (Abe & Munehara, 2009).
610 Clearly, more research is needed to better understand the evolution of parental care in cottoids,
611 and its relationship with other aspects of their complex reproductive biology.
612
613 **Conclusions**
614 Considering the depth ranges rather than previously published habitat categorizations of
615 oligocottine sculpins reveals substantial overlap of almost all species in intertidal habitats. This

616   new understanding of the group agrees with our findings that all oligocottine sculpins are
617   relatively small and bear relatively few scales, two common attributes of intertidal fishes. This
618   finding also helps to explain why the maximum depth of the common depth range does not
619   correlate with most of the characters examined in this study, as we would expect them to vary
620   with depth only if we are comparing intertidal fishes with subtidal fishes, and for the most part
621   we are not. While body shape does not significantly correlate with the maximum common
622   collection depth, the body shape of most species with broader depth ranges appear constrained to
623   what we interpret as a generalist morphology, while most groups that inhabit a narrow, wholly-
624   intertidal depth range appear to have unique body shapes, perhaps suited to their specialized
625   habitat partitions. Likewise, we find no evidence of an association between maximum depth and
626   reproductive characters, but we do find that the evolution of these characters has likely
627   proceeded from a primitive condition of obligate copulation using an intromittent organ to a
628   derived state of spawning and/or the loss of an intromittent organ. This sequence is the opposite
629   direction of that inferred by previous authors, but is clearly supported by the distribution of
630   reproductive traits across our phylogenetic hypothesis of the group.
631
632

641
642

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Figure 1

Histogram of all recorded collection depth from museum records of each species of the sculpin subfamily Oligocottinae and the outgroup taxon *Chitonotus Pugetensis*.

The x-axis represents bins of collection depth in meters. The first bin "0," contains only collection depths that were recorded as 0 meters or where the habitat or collection depth is described as "tide pool," "intertidal," or the like. The median collection depth is indicated with a red arrow above the x-axis. The range of collection depths that contains 95% of all museum collection depth records is indicated with a black double-sided arrow below x-axis. Where the 95% range extends beyond the 35 m depth bin, the arrow points to the right, indicating the "More" depth bin. All museum data analyzed herein are included in Supplementary Table 2. Precise values of minimum, maximum, 95% depth ranges, mean, and sample size for the museum depth records of each species are given in Supplementary Table 4.
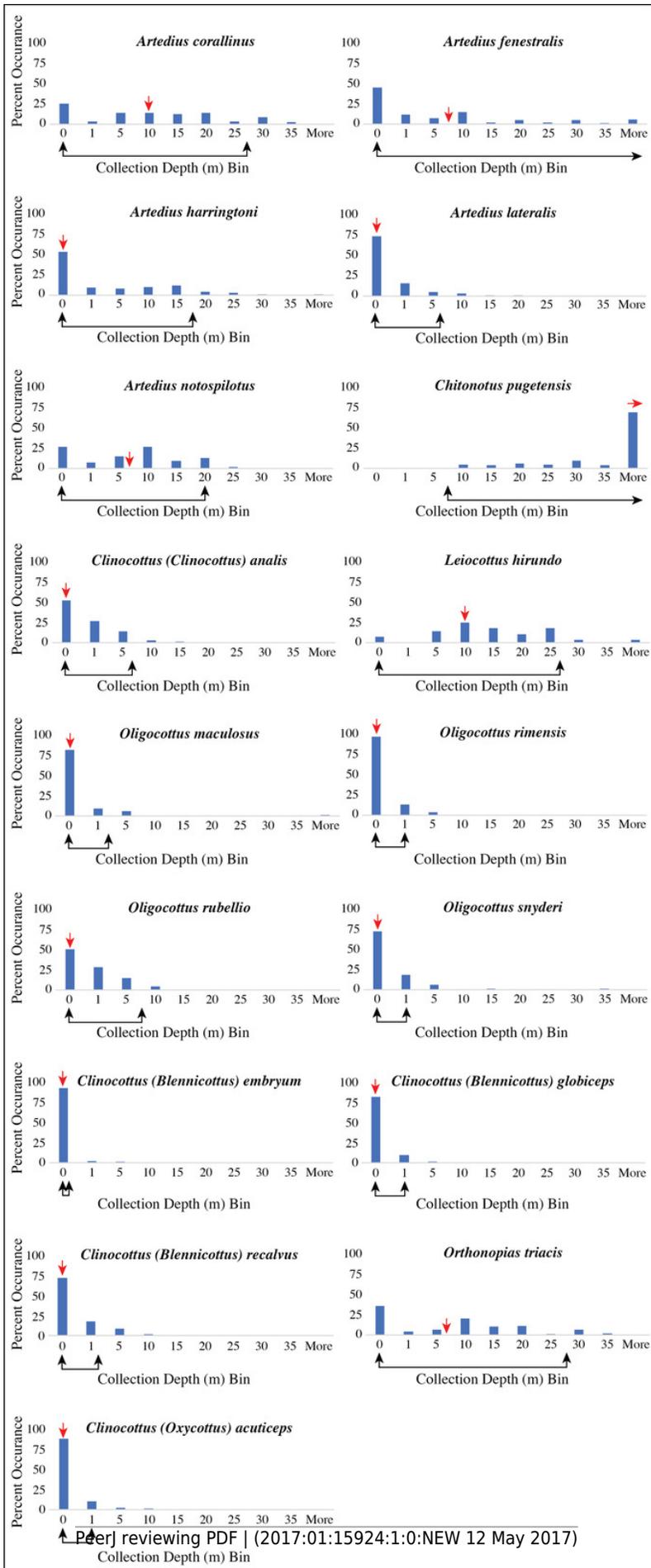
Figure 2

Lateral photograph of *Clinocottus analis* (OSIC 6710, 75.9mm SL) showing thirteen homologous landmarks used to capture overall body shape in oligocottine sculpins.

Landmark descriptions: 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.
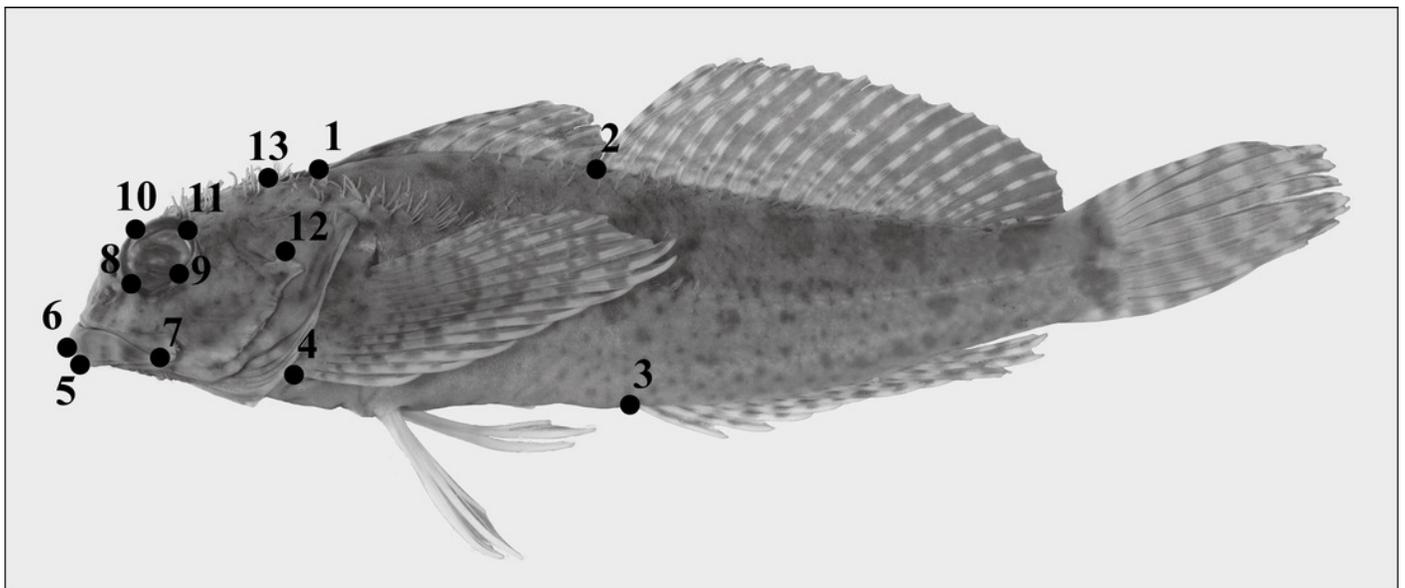
Figure 3

Phylogenetic hypothesis of the subfamily Oligocottinae.

Phylogeny is the maximum clade credibility tree from Bayesian phylogenetic inference conducted using four independent runs of 200 million generations each using the molecular dataset published in Buser and López (2015). Bayesian posterior probability scores are indicated at each node. Probabilities less than 0.50 are not displayed. The subfamily Oligocottinae, along with pertinent clades therein are labeled with arrows following the taxonomy suggested in Buser and López (2015).
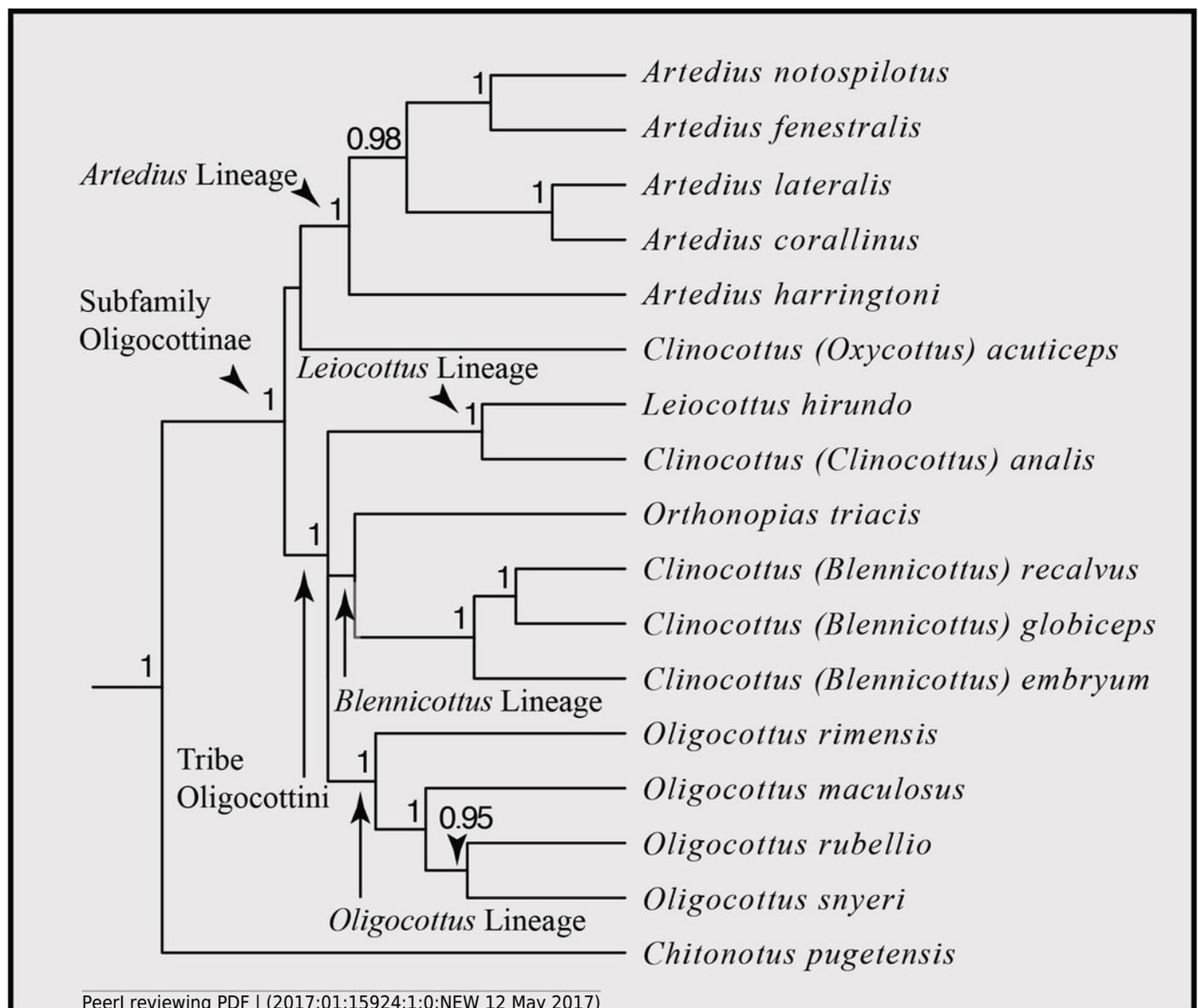
Figure 4

Inferred evolutionary history of maximum depth and maximum size of oligocottine species.

A phenogram showing the inferred evolutionary history of maximum depth is indicated on the left panel. Phylogenetic relationships are represented by white edges and bifurcation points represent inferred speciation events. Phylogenetic topology is from Bayesian MCC tree shown in Figure 3. Relative time is indicated on the horizontal axis and depth in meters indicated on the vertical axis. The tips and nodes of the phylogeny are positioned on the vertical axis to reflect the maximum depth or inferred maximum depth (respectively) of each taxon. Ancestral states for each node were inferred using maximum likelihood and 95% confidence intervals for each state are represented with blue lines. 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.*
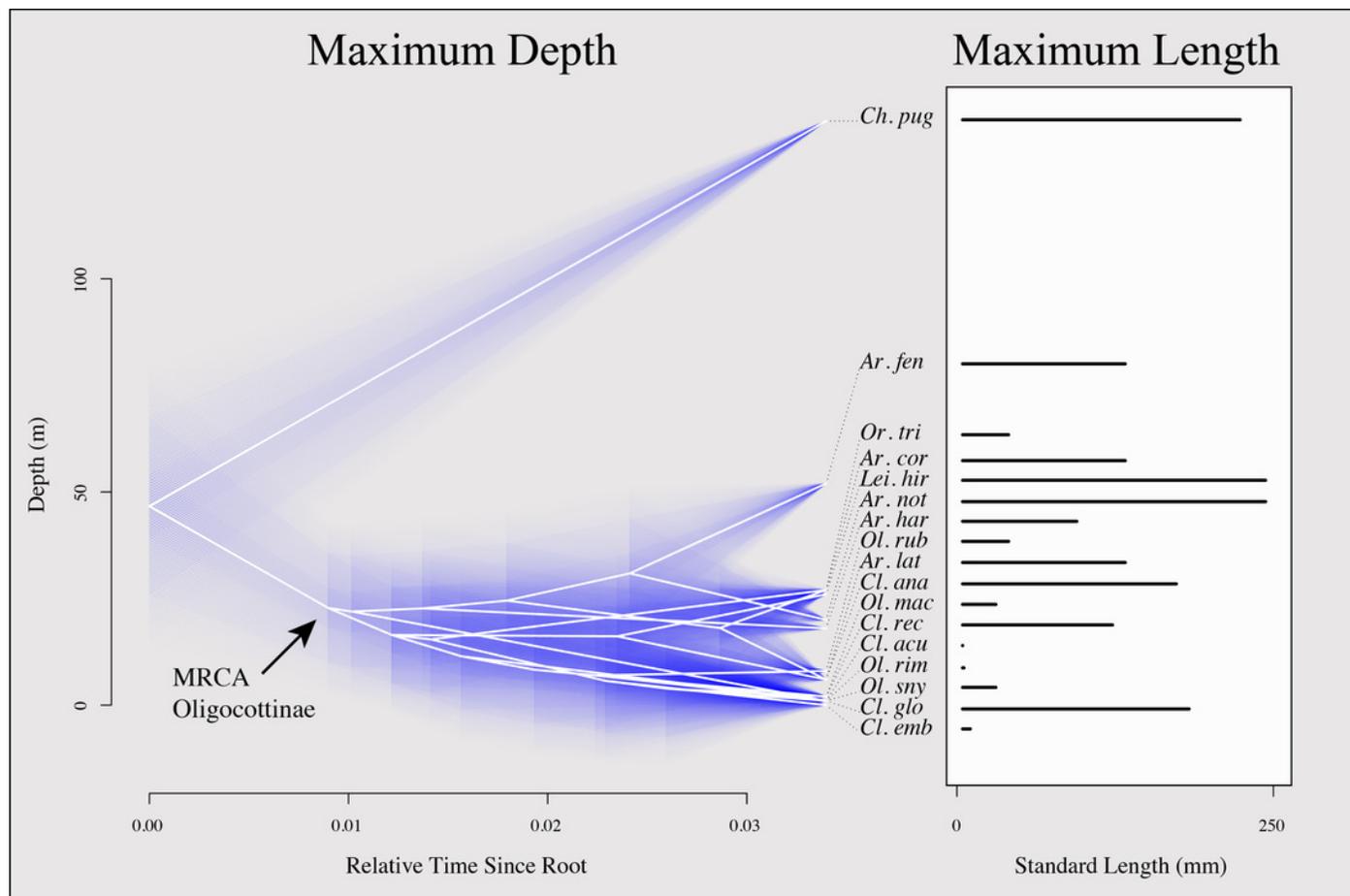
Figure 5

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 for the ancestor of a given clade is depicted with a pie chart at each respective node. This scheme is also depicted on the branches between each 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 4.
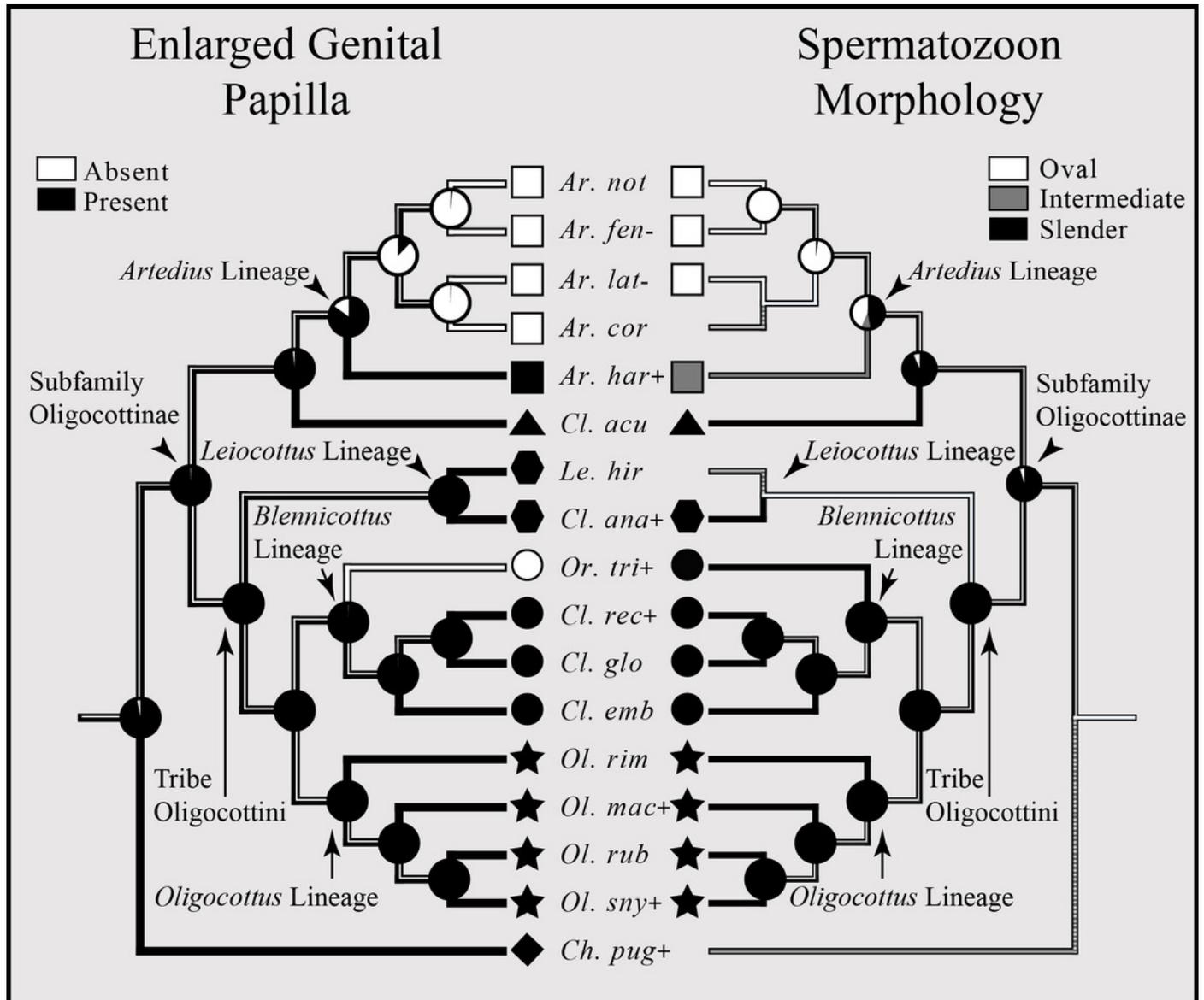
Figure 6

Bayesian MCC phylogeny of Oligocottinae with distribution and inferred evolutionary history of parental care.

The proportional likelihood of each character 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 4. Absence of a symbol or pie at a tip or node (respectively) indicates an unknown state. Species abbreviations in bold indicate a slender-type spermatozoon morphology present in that species.

Parental Care

□ Absent
■ Present

Artedius Lineage

Subfamily Oligocottinae

Leiocottus Lineage

Blennicottus Lineage

Tribe Oligocottini

Oligocottus Lineage

Ar. not
Ar. fen
Ar. lat
Ar. cor
Ar. har
Cl. acu
Le. hir
Cl. ana
Or. tri
Cl. rec
Cl. glo
Cl. emb
Ol. rim
Ol. mac
Ol. rub
Ol. sny
Ch. pug
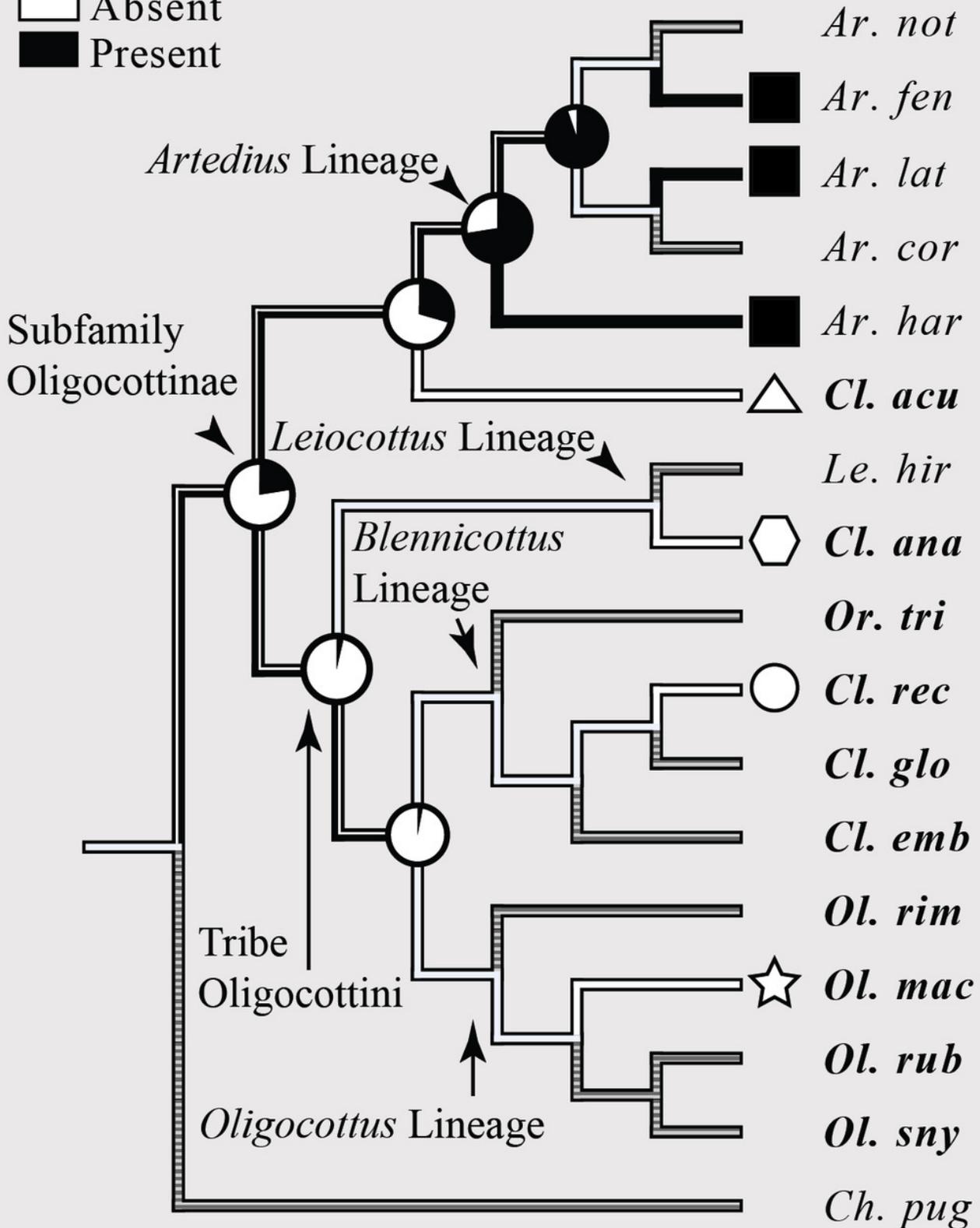
Figure 7

Body shape change in oligocottine sculpins represented by each of the two 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 2. 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.
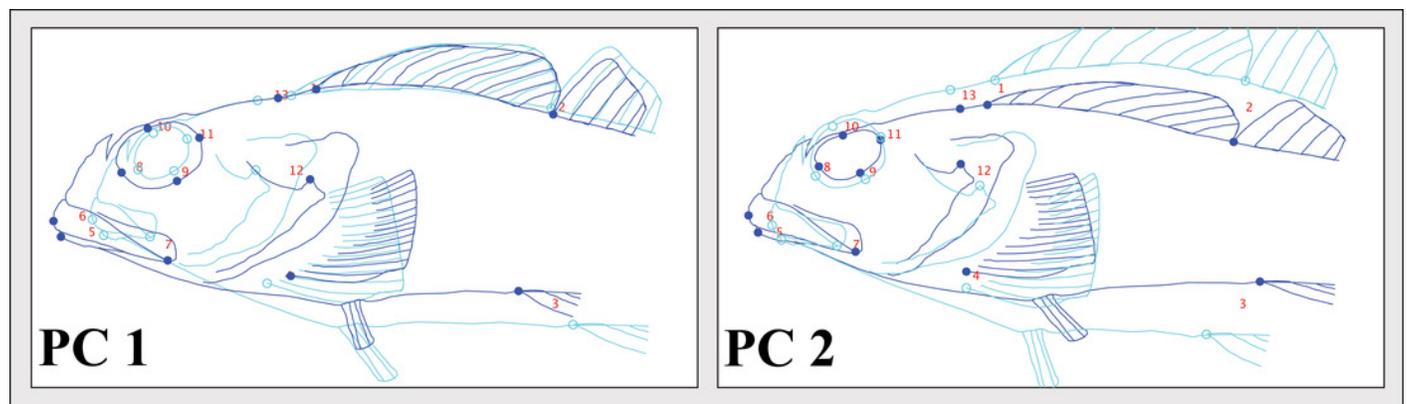
Figure 8

Phylomorphospace of the two significant 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 5, 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. The depth data values are provided in Table 1.
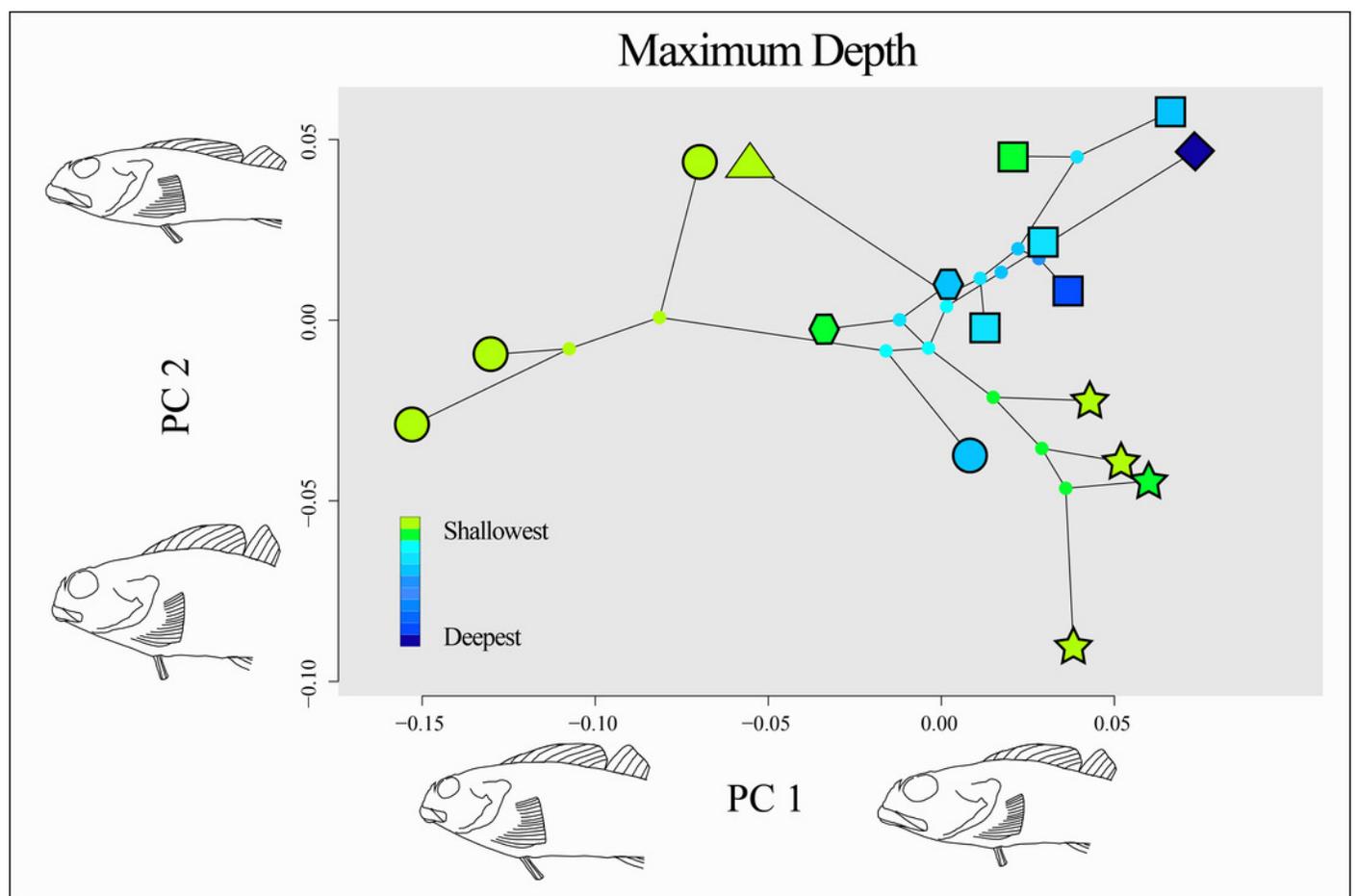
Figure 9

Body shape change associated with change in depth range.

Body shape is represented by the average shape variables (Procrustes-aligned landmark coordinates, illustrated in Figure 2) for each species. Depth range is represented by minimum and maximum collection depth from museum specimens of each species (see Table 1). 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 2. 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 depth range is indicated on the vertical axis. Symbols are indicative of the phylogenetic lineage of each species, as in Figure 5.
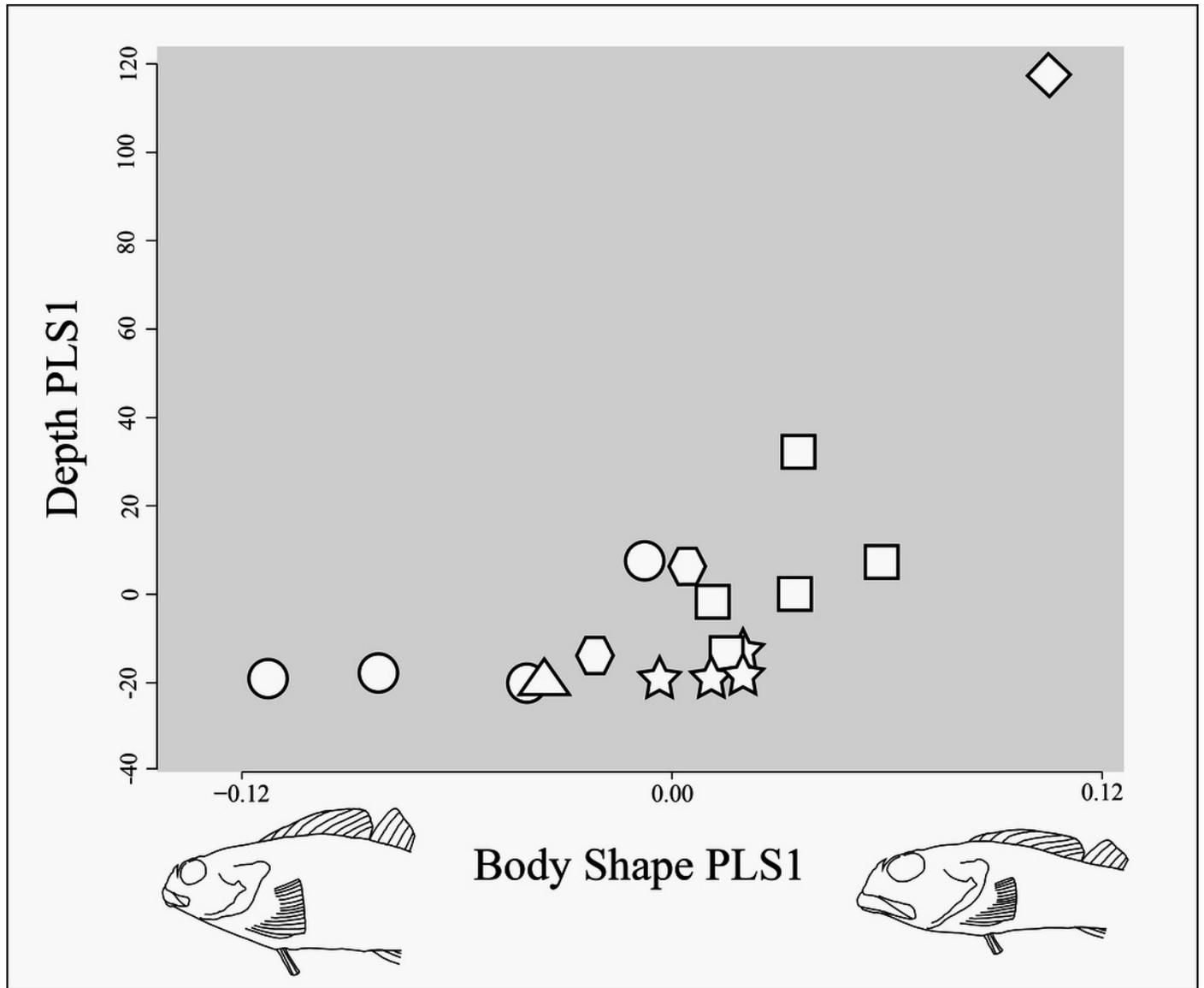
Table 1(on next page)

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). *Minimum and maximum depth are taken from the depth range that contains 95% of museum collection depths for each species. See Methods Supplementary Table 4.

Species	Minimum depth (m)*	Maximum depth (m)*	Tide pool occupancy	Maximum length (mm)	Squamation	Enlarged genital papilla	Spermatozoon morphology	Copulation	Parental care
Artedius corallinus	0	27	Present	140[3,12,13]	Present[3]	Absent[3]	?	?	?
Artedius fenestralis	0	52	Present	140[3,10,12,13]	Present[3]	Absent[3,10]	Oval[9]	Absent[9]	Present[9]
Artedius harringtoni	0	18	Present	102[3,10,12,13]	Present[3]	Absent[3,10]	Intermediate[4,9]	Present[9]	Present[9]
Artedius lateralis	0	6	Present	140[3,10,12,13]	Present[3,11]	Absent[3,10]	Oval[4,9,15]	Absent[9]	Present[9]
Artedius notospilotus	0	20	Present	250[3,12,13]	Present[3]	Absent[3]	Oval[4]	?	?
Chitonotus pugetensis	7	137	Absent	230[3,10,12,13]	Present[3,11]	Present[3,10]	?	Present[1,6]	?
Clinocottus (Oxycottus) acuticeps	0	1	Present	64[3,10,12,13]	Absent[3]	Present[3,10]	Slender[4]	?	Absent[14]
Clinocottus (Clinocottus) analis	0	6	Present	180[3,12,13]	Present[3]	Present[3]	Slender[4]	Present[1,5]	Absent[1,5]
Clinocottus (Blennicottus) embryum	0	0	Present	70[3,10,12,13]	Absent[3]	Present[3,10]	Slender[4]	?	?
Clinocottus (Blennicottus) globiceps	0	1	Present	190[3,10,12,13]	Absent[3]	Present[3,10]	Slender[4]	?	?
Clinocottus (Blennicottus) recalvus	0	2	Present	130[3,12,13]	Absent[3]	Present[3]	Slender[4]	Present[1,7]	Absent[1,7]
Leiocottus hirundo	0	26	Absent	250[3,12,13]	Present[11]	Present[3]	?	?	?
Oligocottus maculosus	0	2	Present	90[3,10,12,13]	Absent[3,11]	Present[3,10]	Slender[4,15]	Present[1]	Absent[1]
Oligocottus rimensis	0	1	Present	65[3,10,12,13]	Present[3]	Present[3,10]	Slender[4]	?	?

Oligocottus rubellio	0	8	Present	100[3,12,13]	Absent[3]	Present[3]	Slender[4]	?	?
Oligocottus snyderi	0	1	Present	90[3,10,12,13]	Absent[3]	Present[3,10]	Slender[4,15]	Present[1,8]	?
Orthonopias triacis	0	27	Present	100[3,12,13]	Present[3,11]	Absent[3]	Slender[4,15]	Present[1,2]	?

1

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Table 2(on next page)

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

Note: many museum lots contain multiple individuals.

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

1