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Molecular phylogenetics of sculpins of the subfamily Oligocottinae (Cottidae)

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ABSTRACT

The sculpin subfamily Oligocottinae includes 18–20 species of nearshore benthic fishes with a diverse array of reproductive strategies. As a first step toward understanding the evolution of that diversity, we conducted a phylogenetic study based on DNA sequences from eight genomic regions from 31 sculpin species aimed at testing monophyly and relationships of the Oligocottinae. Representatives from the perciform families Agonidae, Cottidae, Hemitripteridae, Hexagrammidae, Psychrolutidae, and Rhamphocottidae served as outgroups. The sequence data were analyzed in maximum likelihood and Bayesian phylogenetic inference frameworks. Results of these analyses show that a systematic revision of the group is warranted. The genus *Clinocottus* is a polyphyletic assemblage of three distinct lineages, which should be indicated by resurrection of the subgenera *Blennicottus*, *Clinocottus*, and *Oxycottus*; *Leiocottus hirundo* is more closely related to *Clinocottus analis* than *C. analis* is related to any other member of *Clinocottus*; the composition of the tribe Oligocottini should be revised to include only the genera *Oligocottus*, *Clinocottus*, and *Orthonopias*; and the genus *Sigmistes* should be removed from the subfamily Oligocottinae.

1. Introduction

The subfamily Oligocottinae Hubbs 1926 comprises 18–20 species of nearshore sculpins (family Psychrolutidae Günther 1861) that range along the north Pacific coast from the Baja Peninsula in Mexico to the Kuril Islands in Russia (Hubbs, 1926b; Taranets, 1941; Masuda and Muzik, 1992; Mecklenburg et al., 2002; Smith and Busby, 2014). This group includes many intertidal species and is remarkable for the diversity of coloration and reproductive specializations found among its constituent taxa. The subfamily was first delineated to include the members of the currently accepted genera *Oligocottus* Girard 1856, *Clinocottus* Gill 1861, and *Sigmistes* Rutter 1898 (in Jordan and Evermann, 1898) (Hubbs, 1926b). It was later expanded with the inclusion of the species currently assigned to the genera *Artedius* Girard 1856 and *Orthonopias* Starks and Mann 1911 (Taranets, 1941). Though never explicitly stated by Hubbs (1926b) or Taranets (1941), *Oligocottus* is considered the type genus of Oligocottinae and has priority. These early studies united the group using morphological characters that can be found throughout Cottidae (i.e., reduction in scales, reduced number of preopercular spines, three soft pelvic rays, "upper pharyngeal" teeth; Greeley, 1899; Hubbs, 1926b; Taranets, 1941). Subsequent systematic research allied the genera *Phallocottus* Schultz 1938 (Howe and Richardson, 1978), *Ruscarius* Jordan and Starks 1895 (Bolin, 1944, 1947), and *Leiocottus* Girard 1856 (Bolin, 1944, 1947) with various oligocottine genera. Since the major revisions of Bolin (1944), the only taxonomic change within the subfamily was the split of *Artedius* (*sensu* Bolin, 1944; 7 spp.) into *Artedius* (5 spp.) and a resurrected *Ruscarius* (2 spp.; Begle, 1989). Other studies note the close relationship of oligocottine sculpins but offer limited evidence (i.e., reduction in scales, reduction in preopercular spines) to support the group (Bolin, 1947; Howe and Richardson, 1978). Similarly, studies that apply cladistic methods to oligocottine phylogenetics have yielded only a few putative synapomorphies (see Begle, 1989; Strauss, 1993).

The phylogeny of Oligocottinae was recently revisited using evidence from DNA sequences from mitochondrial (cyt b and NADH1) and nuclear (S7 intron 1) gene regions (Ramon and Knope, 2008; Knope, 2013). Results of those two studies differ from Bolin (1944, 1947) in rejecting the monophyly of *Clinocottus*, and supporting the validity of *Ruscarius*. Notably, results of those studies

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differ from Bolin (1947) and from each other in the placement of *Orthonopias triacis* and *Clinocottus acuticeps*. Smith and Busby (2014) constructed an aggregate dataset of all previously published morphological and molecular data pertaining to cottoids in order to resolve the higher-level relationships of the group. Their results show that many family-level revisions are in order, but show clear support for a monophyletic grouping of all the oligocottine genera included in their study. With regard to oligocottine phylogeny, the results of Smith and Busby (2014) generally agree with those of Bolin (1944, 1947) Ramon and Knope (2008), and Knope (2013). One notable disagreement between the phylogeny proposed by Smith and Busby (2014) and that proposed by Bolin (1944, 1947) is the placement of the genus *Orthonopias*, where, like the molecular-based studies of Ramon and Knope (2008) and Knope (2013), Smith and Busby (2014) show *Orthonopias* nested within the *Oligocottus–Clinocottus* clade, rather than sister to *Artedius*, as was suggested by Bolin (1944, 1947). Given the broad scale of Smith and Busby (2014), it does little to further resolve oligocottine systematics, and does not provide any new synapomorphies for the group.

None of these purely molecular or the combined morphology and molecular studies included samples of the oligocottine genus *Sigmistes*. In fact, *Sigmistes* has been included in only three phylogenetic studies since its description (Hubbs, 1926b; Taranets, 1941; Howe and Richardson, 1978), none of which used explicit phylogenetic methods to infer relationships.

Given the lack of morphological synapomorphies to support the monophyly of Oligocottinae and the inconsistent results found in recent phylogenetic studies of the subfamily, we assembled and analyzed an extensive DNA sequence dataset from a broad sample of oligocottine and possible outgroup taxa. The objectives of this study were to: (1) test the monophyly of the subfamily Oligocottinae and each of its constituent genera, (2) test the phylogenetic placement of the oligocottine genus *Sigmistes*, and (3) develop a stable phylogenetic hypothesis for the oligocottine sculpins. Sequence data for this study were derived from seven nuclear genome regions and one mitochondrial genome segment.

2. Materials and methods

2.1. Taxon sampling

Specimens representing all species of the genera: *Oligocottus*, *Clinocottus*, *Sigmistes*, *Artedius*, *Phallocottus*, *Leiocottus*, and *Orthonopias* were assembled from field and museum collections (Table 1). This taxonomic sample includes all species that have been included directly or indirectly within Oligocottinae with the exception of *Ruscarius creaseri* (Hubbs, 1926a) and *R. meanyi* Jordan and Starks 1895. Samples from these two species were not available for this study. Nineteen other cottoid species were included in the taxon sample to allow tests of the monophyly of Oligocottinae. These species were chosen based on phylogenetic relationships hypothesized in previous studies (i.e., Bolin, 1947; Yabe, 1985; Smith and Wheeler, 2004; Knope, 2013), and the choice in outgroup taxa is consistent with the most recent phylogenetic hypothesis of cottoids (i.e., Smith and Busby, 2014). The outgroup species represent the genera: *Blepsias* Cuvier 1829, *Chitonotus* Lockington 1879, *Enophrys* Swainson 1839, *Hemilepidotus* Cuvier 1829, *Hexagrammos* Tilesius 1810, *Hemitripterus* Cuvier 1829, *Icelinus* Jordan 1885, *Icelus* Krøyer 1845, *Leptocottus* Girard 1854, *Myoxocephalus* Tilesius 1811, *Percis* Scopoli 1777, *Podothecus* Gill 1861, *Radulinus* Gilbert 1890, *Rhamphocottus* Günther 1874, and *Triglops* Reinhardt 1830.

Sculpins were collected from nearshore and intertidal habitats from 38 localities across Alaska, British Columbia, Washington, and Oregon (Table 1). Collections were made with dip nets in intertidal habitats at low tide from the shore, and in sub-tidal habitats by SCUBA diving. Voucher specimen and tissue samples were archived in fish collections at University of Alaska Museum (UAM) and the University of Washington (UWFC). In addition to targeted collections, specimens and/or tissue samples were provided by the Alaska Sea Life Center, Mayumi Arimitsu (United States Geological Survey), Milton Love (University of California, Santa Barbara), Marina Ramon (University of Southern California), Scripps Institution of Oceanography, University of Washington Fish Collection and the University of Kansas. In total, 119 individuals representing 37 species of cottoids were examined in this study.

2.2. DNA sequence determinations

Total genomic DNA was extracted from fin and muscle tissue with reagents and protocols from the DNEasy Blood and Tissue Kit (Qiagen Corp.). Targeted polymerase chain reactions (PCR) was used to amplify DNA fragments from the following eight loci (Table 2): one mitochondrial protein-coding locus (Cytochrome c oxidase, COI), two nuclear introns [exon-primed intron crossing (EPIC) locus 1777E10 and EPIC locus 4174E20] and five protein-coding nuclear loci [early growth response protein 1 (EGR1); mixed-lineage leukemia (MLL); patched domain-containing protein 1 (ptchd1); Rhodopsin; and Sushi, von Willebrand factor type A, and pentraxin domain-containing 1 (SVEP)]. Standard reagent concentrations (1× Buffer, 0.8 mM dNTP, 1–2 mM Mg++, 0.4 μM F/R primer, 0.025 U/μl Taq polymerase, and 1 μl of DNA template of variable concentration per 25 μl reaction) were used in all reactions. With the exception of SVEP, thermal cycler profiles for each reaction were adapted from published amplification conditions for each locus (see Table 2), with minor adjustments to annealing temperature and/or extension time. A nested PCR strategy was used to generate amplicons of ptchd1 and SVEP suitable for sequence determination. For SVEP, novel primers were designed for the nested reaction (see Table 2) and, for this second reaction, the thermal cycler conditions were as follows: initial denaturation at 94 °C for 90 s (s); 40 cycles of 94 °C denaturation for 30 s, 65 °C annealing for 30 s, 72 °C extension for 45 s; and final extension at 72 °C for 4 min.

Amplicons were purified and sequenced in both directions by Sanger sequencing at the University of Washington High-Throughput Genomics Unit. Sequences were trimmed, visually checked for quality, and forward and reverse complementary reads were assembled into contiguous sequences using CodonCode Aligner Software (CodonCode Corp.) Multiple sequence alignments (MSAs) for each locus were generated in ClustalW (Larkin et al., 2007). MSAs were trimmed to eliminate missing data sites at 5′ and 3′ regions. The reading frame for protein-coding MSAs was identified using Se–Al (Rambaut, 2002). MSAs for all loci were concatenated using Mesquite (Maddison and Maddison, 2011). To measure divergence, *p*-distances were calculated for the combined MSA dataset as well as for each locus individually in PAUP* (Swofford, 2003).

2.3. Phylogenetic inference

To assess the possible effects of analysis-specific inference artifacts, multiple phylogenetic approaches were used, each in several configurations. Phylogenetic relationships were estimated by analyses of the concatenated dataset using Maximum Likelihood (ML) and Bayesian (B) optimality criteria. Discrepancies and similarities between the results of different analyses were used to evaluate the degree of confidence and expected stability of inferred relationships.

Table 1
Summary of sampled taxa and source of specimens. Museums abbreviations: KU = University of Kansas; SIO = Scripps Institution of Oceanography; UAM = University of Alaska Museum; UW = Burke Museum at the University of Washington. Region abbreviations: AI = Aleutian Islands, USA; AK = Alaska excluding the Aleutian Islands; BC = British Columbia; CA = California; OR = Oregon; WA = Washington.

Taxon	*n*	Catalog number	Region	Collection locality
Ingroup				
Artedius corallinus	1	SIO:Fishes:01-124	CA	San Diego
Artedius fenestralis	3	UAM:Fishes:6252	AK	Kodiak Island
		UAM:Fishes:6159	AK	Kasitsna Bay
		UAM:Fishes:6167	AK	Kasitsna Bay
Artedius harringtoni	6	UAM:Fishes:6189	WA	Bremerton
		UAM:Fishes:6186	WA	Bremerton
		UAM:Fishes:6163	AK	Kasitsna Bay
		UAM:Fishes:6155	AK	Kasitsna Bay
		UAM:Fishes:6158	AK	Kasitsna Bay
		UAM:Fishes:4702	CA	Monterey Bay
Artedius lateralis	5	UAM:Fishes:6254	AK	Kodiak Island
		UAM:Fishes:2951	AK	Sitka
		UAM:Fishes:2962	AK	Sitka
		UAM:Fishes:2976	OR	Newport
		UAM:Fishes:2976	OR	Newport
Artedius notospilotus	1	SIO:Fishes:04-2	CA	San Diego
Clinocottus acuticeps	9	UAM:Fishes:6260	AK	Kodiak Island
		UAM:Fishes:6164	AK	Jakolof Bay
		UAM:Fishes:6179	BC	Tofino
		UAM:Fishes:2947	AK	Sitka
		UAM:Fishes:2947	AK	Sitka
		UAM:Fishes:2973	OR	Newport
		UAM:Fishes:2973	OR	Newport
		UAM:Fishes:47693	AI	Attu
		UAM:Fishes:47693	AI	Attu
Clinocottus analis	5	UAM:Fishes:4699	CA	Monterey Bay
		SIO:Fishes:06-42	CA	Cambria
		N/A	CA	Gaviota
		N/A	CA	Gaviota
		N/A	CA	Gaviota
Clinocottus embryum	8	UAM:Fishes:6154	AK	Kasitsna Bay
		UAM:Fishes:6165	AK	Kasitsna Bay
		UAM:Fishes:6154	AK	Kasitsna Bay
		UAM:Fishes:4695	AK	Kodiak Island
		UAM:Fishes:2948	AK	Sitka
		UAM:Fishes:2974	OR	Newport
		UAM:Fishes:47694	AI	Attu
		UAM:Fishes:47694	AI	Attu
Clinocottus globiceps	6	UAM:Fishes:6180	BC	Tofino
		UAM:Fishes:6180	BC	Tofino
		UAM:Fishes:6182	BC	Uculet
		UAM:Fishes:2942	AK	Sitka
		UAM:Fishes:2968	WA	Neah Bay
		UAM:Fishes:2975	OR	Newport
Clinocottus recalvus	3	N/A	CA	Vandenberg Air Force Base
		N/A	CA	Vandenberg Air Force Base
		N/A	CA	Vandenberg Air Force Base
Oligocottus maculosus	7	UAM:Fishes:4698	AK	Prince William Sound
		UAM:Fishes:6259	AK	Kodiak Island
		UAM:Fishes:6188	WA	Bremerton
		UAM:Fishes:6178	BC	Port Hardy
		UAM:Fishes:6166	AK	Kasitsna Bay
		UAM:Fishes:6181	BC	Tofino
		UAM:Fishes:6154	AK	Middleton Island
Oligocottus rimensis	5	UAM:Fishes:2955	AK	Sitka
		UAM:Fishes:2945	AK	Sitka
		UAM:Fishes:2964	AK	Sitka
		UAM:Fishes:2964	AK	Sitka
Oligocottus rubellio	2	N/A	CA	Big Sur
		N/A	CA	Big Sur
Oligocottus snyderi	9	UAM:Fishes:4700	CA	Monterey Bay
		UAM:Fishes:2946	AK	Sitka
		UAM:Fishes:2946	AK	Sitka
		UAM:Fishes:4683	BC	Uculet
		UAM:Fishes:4683	BC	Uculet

Table 1 (*continued*)

Taxon	*n*	Catalog number	Region	Collection locality
		UAM:Fishes:2972	WA	Seiku
		UAM:Fishes:2972	WA	Seiku
		UAM:Fishes:2978	OR	Newport
		UAM:Fishes:2979	OR	Newport
Orthonopias triacis	4	UAM:Fishes:4701	CA	Monterey Bay
		SIO:Fishes:03-166	CA	Carmel
		N/A	CA	Monterey
		N/A	CA	Monterey
Phallocottus obtusus	2	UAM:Fishes:4697	AI	Adak
		UAM:Fishes:4697	AI	Adak
Sigmistes caulias	6	UAM:Fishes:47726	AI	Adak
		UAM:Fishes:47684	AI	Adak
		UAM:Fishes:47715	AI	Tanaga
		UAM:Fishes:47715	AI	Tanaga
		UAM:Fishes:47705	AI	Amchitka
		UAM:Fishes:47706	AI	Amchitka
Sigmistes smithi	4	UAM:Fishes:47712	AI	Ogliuga
		UAM:Fishes:47727	AI	Adak
		UAM:Fishes:47727	AI	Adak
		UAM:Fishes:47727	AI	Adak
Outgroup				
Blepsias cirrhosus	2	UAM:Fishes:2941	AK	Alaska Sea Life Center
		UAM:Fishes:2941	AK	Alaska Sea Life Center
Chitonotus pugetensis	5	UW:Fishes:151078	WA	Puget Sound
		UW:Fishes:151079	WA	Puget Sound
		UW:Fishes:47298	WA	Puget Sound
		UW:Fishes:47675	WA	Myrtle Edwards Park
		UW:Fishes:47676	WA	Myrtle Edwards Park
Enophrys bison	2	UAM:Fishes:6255	AK	Kodiak Island
		UAM:Fishes:6186	WA	Bremerton
Enophrys lucasi	3	UAM:Fishes:6160	AK	Kasitsna Bay
		UAM:Fishes:6160	AK	Kasitsna Bay
		UAM:Fishes:6160	AK	Kasitsna Bay
Hemilepidotus hemilepidotus	1	UAM:Fishes:6177	BC	Smith Sound
Hemilepidotus jordani	1	UAM:Fishes:2938	AK	Alaska Sea Life Center
Hemitripterus bolini	1	UAM:Fishes:2936	AK	Alaska Sea Life Center
Hexagrammos lagocephalus	2	UAM:Fishes:6256	AK	Kodiak Island
		UAM:Fishes:2937	AK	Alaska Sea Life Center
Icelinus filamentosus	1	KU:Fishes:28049	CA	Southern California
Icelus spiniger	2	UAM:Fishes:4703	AK	UNK.
		UAM:Fishes:4703	AK	UNK.
Leiocottus hirundo	2	SIO:Fishes:08-60	CA	San Clemente
		N/A	CA	Los Angeles County
Leptocottus armatus	2	UAM:Fishes:6174	BC	Rivers Inlet
		UAM:Fishes:6174	BC	Rivers Inlet
Myoxocephalus jaok	1	UAM:Fishes:6246	AK	Kodiak Island
Myoxocephalus polyacanthocephalus	3	UAM:Fishes:6257	AK	Kodiak Island
		UAM:Fishes:6257	AK	Kodiak Island
		UAM:Fishes:6168	AK	Kasitsna Bay
Percis japonicus	1	UAM:Fishes:2935	AK	Alaska Sea Life Center
Podothecus veterus	1	UW:Fishes:125588	AK	Bering Sea
Radulinus taylori	1	UAM:Fishes:6191	WA	Bremerton
Rhamphocottus richardsonii	1	UAM:Fishes:2940	AK	Alaska Sea Life Center
Triglops scepticus	1	UAM:Fishes:4704	AK	UNK.

Maximum likelihood analysis of the concatenated dataset was conducted with RaxML v. 7.3.0 (Stamatakis, 2006) using the rapid bootstrapping algorithm (Stamatakis et al., 2008). The dataset was partitioned by locus (e.g., COI, EGR1, etc.) and the General Time Reversible (GTR) model of molecular evolution with a four-category gamma distribution of rate variation and invariable sites was applied to each data partition. A bootstrap analysis with 5000 iterations was performed to assess the strength of different components of the phylogenetic inference.

For Bayesian analyses, the best fitting model of molecular evolution for each locus was identified using the Akaike information criterion (AIC; Akaike, 1973; Posada and Buckley, 2004) with the model comparison routines implemented in MrModeltest (Nylander, 2004). Bayesian analysis of the concatenated dataset

Table 2
Loci examined, primer sequences and annealing temperatures. Primer sources are: 1 = Betancur et al., 2013, 2 = Campbell et al., 2013, 3 = Chen et al., 2003, 4 = Chen et al., 2008, 5 = Chen et al., 2013, 6 = Li et al., 2007, 7 = Li et al., 2010, 8 = This study, 9 = Ward et al., 2005.

Locus	Primer name	Primer sequence 5′–3′	Amplicon length (bp)	Annealing temperature (°C)	Source
COI	FISH_F1	TCAACCAACCACAAAGACATTGGCAC	655	52	9
	FISH_R1	TAGACTTCTGGGTGGCCAAAGAATCA		52	9
EPIC 1777E4	1777E4F	AGGAGYTGGTGAACCAGAGCAAAGC	300	58	7
	1777E4R	AGATCRGCCTGAATSAGCCAGTT		58	7
EPIC 4174E20	4174E20F	CTYTCGCTGGCTTTGTCTCAAATCA	350	58	7
	4174E20R	CTTTTACCATCKCCACTRAAATCCAC		58	7
EGR1	EGR1 290F	TMTCTTACACAGGCCGYTTCAC	828	55	4
	EGR1 1118R	CTTCTTGTCCTTCTGCCGYAGRT		55	5
MLL	MLL 1459F	TCCCAGACTCARGTTTCCAG	711	55	2
	MLL 2170R	CTCTGCTGAAKGAGAGTAGTKGG		55	2
ptchd1	ptr458F	AGAATGGATWACCAACACYTACG	990	55	6
	ptr1248R	TAAGGCACAGGATTGAGATGCT		55	6
	ptr463F	GGATAACCAACACYTACGTCAA	779	62	6
	ptr1242R	ACAGGATTGAGATGCTGTCCA		62	6
Rhodopsin	RH 193F	CNTATGAATAYCCTCAGTACTACC	846	55	3
	RH 1039R	TGCTTGTTCATGCAGATGTAGA		55	3
SVEP1	SVEP1 7960F	CCTCCNCAYATYGAYTTTGGDGAMTA	929	50	1
	SVEP1 8889R	TTCAGGWARCCRTGRCTRATRTCCTC		50	1
	SVEP 8058F	TCACATTCRTAGCTCACCTTGCTGTTGAAGCCRAACT	652	65	8
	SVEP 8710R	AGCCCCACCAGGTTRGCGTGYCAGGAG		65	8

was conducted in MrBayes v. 3.2.0 (Ronquist et al., 2012). The dataset was partitioned by locus and each partition was assigned the best-fitting model structure as determined in MrModeltest. Character state frequencies, substitution rates, gamma shape parameter, and proportion of invariable sites were unlinked across partitions. A 50% consensus tree was generated from six independent runs of 20 million generations, sampled every 5000 generations, with the first 25% discarded as burn-in.

The phylogeny was tested for the presence of destabilizing "rogue" taxa using RogueNaRok (Aberer et al., 2013). Any individuals that failed to find consistent placement among pseudo-replications were flagged as problematic (Aberer et al., 2013), removed from the alignment, and phylogeny re-inferred from the reduced dataset.

To test for contamination or other identification errors, phylogenies were produced for each locus individually to ensure that each specimen of a given species grouped with its conspecifics.

2.4. Alternative character coding and data permutations

To evaluate the effect of alternative partition and character coding schemes, alternative configurations of the maximum likelihood and Bayesian analyses were performed. These alternative coding/partitioning schemes included: (1) treating the dataset as a single partition, (2) partitioning by gene and codon position [noted as Gene (1_N, 2_N, 3_N)], (3) partitioning by gene with the third codon position sites coded as only purines/pyrimidines [noted as Gene ($1_N2_N3_{RY}$)], and (4) partition by gene with deletion of the third codon position sites [noted as Gene (1_N2_N)]. The latter two analysis configurations were used to identify the possible role of substitution saturation of the third codon position sites on inferred topologies. The optimal partitioning scheme was also identified using PartitionFinder (Lanfear et al., 2012) under the Bayesian information criterion (BIC). For this analysis, sites from each protein-coding region were first partitioned by codon position (cp) so that, for example, COI was broken into three sets: COI _cp1, COI _cp2, and COI _cp3. Here again discrepancies and similarities between the results of the analyses were used to evaluate the confidence of relationships inferred and in particular to identify strong historical patterns in the dataset.

3. Results

3.1. Sequences

All the sequences determined in this study are available on Genbank under accession numbers KP826911–KP827632 (Supplementary Table 1). Table 3 lists lengths for each of the locus-specific alignments generated. The concatenated MSA spans a total of 4695 aligned nucleotide sites, of which 1037 are variable and 368 are parsimony-informative. Amplification success was generally high, with ample coverage across loci for each species (Supplementary Table 1). Additionally, the phylogenetic trees produced for each individual locus show no evidence of contamination or misidentification/mislabeling of specimens. The loci with the greatest degrees of divergence were the mitochondrial protein-coding region, COI; the two nuclear introns, EPIC loci 1777E4 and 4174E20; and the nuclear protein-coding region, SVEP1 (Table 3). GC composition for each locus across the full dataset and for the ingroup taxa is reported in Table 3. For each locus, the following models of molecular evolution represent the best fit to the observed patterns of variation based on the AIC: EPIC locus 1777E4 and SVEP best fit the General Time Reversible (GTR) model (Tavaré, 1986) with among site rate variation (ASRV); COI, ptchd1, and Rhodopsin best fit the GTR model with ASRV and invariable sites; EPIC locus 4174E20 and MLL best fit the Hasegawa–Kishino–Yano (HKY; Hasegawa et al., 1985) model with ASRV; and EGR1 best fit the HKY model with ASRV and invariable sites. These models were applied to the appropriate partitions in the Bayesian analyses of the concatenated dataset.

A PartitionFinder analysis showed that the optimal partitioning scheme consists of ten partitions, with the third codon position of each protein-coding region often as a distinct partition (Table 4). This partitioning scheme was used for an additional Bayesian analysis of the dataset, run for five million generations but with otherwise identical parameters to the initial Bayesian analysis.

3.2. Phylogeny

Likelihood and Bayesian inferred tree topologies were largely congruent. Fig. 1 shows the 50% majority-rule consensus tree

Table 3
Alignment lengths, maximum observed proportion of nucleotide differences between sequences, GC composition, and best fit substitution models for the eight loci examined and for the overall dataset.

Locus	Trimmed alignment length (bp)	Group	Max uncorrected distance (*p*-distance)	GC (%)	Model best fit (AIC)
EPIC1777E4	263	All	12.01	51.07	GTR
		Ingroup	7.26	51.64	
EPIC4174E20	283	All	9.99	40.45	HKY + G
		Ingroup	5.73	40.27	
COI	651	All	20.74	50.91	GTR + G
		Ingroup	20.12	51.70	
EGR1	783	All	5.24	58.51	HKY + G + I
		Ingroup	3.19	58.34	
MLL	693	All	6.88	54.64	HKY + G
		Ingroup	4.64	54.59	
ptchd1	678	All	6.94	53.35	GTR + G
		Ingroup	4.58	53.89	
Rhodopsin	738	All	8.41	62.57	GTR + G
		Ingroup	5.01	63.22	
SVEP1	606	All	11.08	56.87	GTR
		Ingroup	7.59	57.28	
All Loci	4695	All	19.85	54.75	
		Ingroup	15.25	55.26	

produced by the Bayesian analysis, rooted with the hexagrammid species *Hexagrammos lagocephalus*. Hexagrammidae lies just outside the cottoid superfamily (Shinohara, 1994; Betancur et al., 2013; Smith and Busby, 2014) and was therefore chosen as the most appropriate root for the tree. The Bayesian posterior probabilities (BPP) and ML bootstrap values (MLB) associated with each node. The only difference between the Bayesian and ML topologies is the placement of *Phallocottus obtusus*, which is placed as sister to *Sigmistes smithi* in the Bayesian inference and as sister to a monophyletic *Sigmistes* under maximum likelihood, but in both analyses indices of support for the conflicting relationships are weak (<0.55 BPP and <50% MLB). Both analyses show strong support for a clade consisting of the members of the oligocottine genera *Clinocottus*, *Orthonopias*, *Artedius*, *Oligocottus*, and the genus *Leiocottus* (clade A in Fig. 1). Neither inference places within that clade the oligocottine genus *Sigmistes* or the genus *Phallocottus*. Rather, these two genera are allied to the genus *Icelus* with strong and unanimous support across both analyses (1.00 BPP and 100% MLB).

Table 4
Best fit partitions and models. Best partitions of molecular dataset and corresponding best fitting models were calculated in PartitionFinder (Lanfear et al., 2012) using the Bayesian information criterion. Protein-coding regions were subdivided by codon position site (cp; i.e., COI was subdivided into COI_cp1, CPlcp2, and COI_cp3). K80 = Kimura 2-parameter (Kimura, 1980), F81 = Felsenstein, 1981 (Felsenstein, 1981), HKY = Hasegawa, Kishino, and Yano (Hasegawa et al., 1985), GTR = Generalized Time-Reversible (Tavaré, 1986), I = invariable Sites, G = among site rate variation.

Partition	Best model	Loci
1	K80 + I + G	EPIC1777E4, EPIC4174E20, MLL_cp1, SVEP_cp1 SVEP_cp2
2	GTR + G	COI_cp1
3	GTR + I + G	COI_cp2, Rhodopsin_cp2, ptchd1_cp2
4	GTR + G	COI_cp3
5	F81 + I	EGR1_cp1, EGR1_cp2, MLL_cp2, ptchd1_cp1
6	GTR + G	EGR1_cp3, ptchd1_cp3
7	K80 + I	MLL_cp3
8	K80 + I	Rhodopsin_cp1
9	HKY + G	Rhodopsin_cp3
10	HKY + G	SVEP_cp3

Within clade A, there are two primary lineages: *Clinocottus acuticeps* + the genus *Artedius* (clade B in Fig. 1), and a group consisting of all the remaining species of clade A (clade C in Fig. 1). Within clade B, *A. corallinus* is sister to *A. lateralis* with unanimous support; *A. notospilotus* is sister to *A. fenestralis* with high support (1.00 BPP and 98% MLB); these two groups are most closely related to one another; and form a clade that is sister to *A. harringtoni*. This clade comprises the genus *Artedius* and is well supported (1.00 BPP and 98% MLB). *Artedius* and *Clinocottus acuticeps* are placed as sister taxa but the relationship is weakly supported (0.58 BPP and <50% MLB). Within the *Artedius* clade, the sister relationship of the *A. fenestralis* + *A. notospilotus* clade with the *A. corallinus* + *A. lateralis* clade is only moderately supported (0.65 BPP and 78% MLB; Fig. 1).

Within clade C, there are two primary lineages: *L. hirundo* + *C. analis*, which was supported unanimously (clade D in Fig. 1), and a weakly supported clade containing all remaining taxa (0.64 BPP and 53% MLB; clade E in Fig. 1). Clade E is split into two well-supported groups: the genus *Oligocottus* and a clade containing *Orthonopias triacis*, *C. recalvus*, *C. globiceps*, and *C. embryum* (clade F in Fig. 1). Within clade F, all relationships are resolved with high support indices (Fig. 1): *C. recalvus* is sister to *C. globiceps*; this group is sister to *C. embryum*; and this larger group (clade G in Fig. 1) is sister to *O. triacis*. Within *Oligocottus*, all relationships are resolved with high support indices (Fig. 1): *O. rubellio* is most closely related to *O. snyderi*; this group is sister to *O. maculosus*; and this larger group (clade H in Fig. 1), is sister to *O. rimensis*.

RogueNaRok testing suggested the removal of five individuals from the alignment. These individuals suffered from low amplification success (<50% of loci) and this is a likely reason that they were flagged. However, these rogue individuals were from species represented by multiple individuals, as a result no species was excluded from the alignment after pruning. Inter-species relationships before pruning and after pruning were unchanged. Pruning had minor changes in some support values (i.e., <10%).

All alternative coding and partitioning schemes produced phylogenies with similar topologies to those produced by the initial BI and ML analyses. The two coding schemes that produced the greatest deviations were those that affected third codon position sites by either coding them as RY or deleting them. RY coding of third codon position sites affected the tree topology by changing the relationship of *C. acuticeps* from a weakly supported sister-group of *Artedius* to a weakly supported (i.e., <60% MLB) sister of clade C, collapsing the sister relationship of *Oligocottus* and clade F, and by collapsing the sister relationship of *O. triacis* with clade G. Additionally, under this coding scheme the analyses results showed strong support (94% bootstrap) for the clade that contains *A. corallinus*, *A. lateralis*, *A. notospilotus*, and *A. fenestralis*, compared to the low to moderate support (0.650 BPP and 78% MLB) for this clade in the initial BI and ML phylogenies (Fig. 1). Deleting third codon position sites produced similar results with the following notable differences: a weakly supported (0.540 BPP) sister-group relationship of *O. triacis* and clade G; collapse of the sister-group relationship between *O. rimensis* and clade H to produce a polytomy between *O. rimensis*, *O. maculosus*, and the *O. rubellio* + *O. snyderi* clade; moderate support for a sister-group relationship between *Oligocottus* and clade G; collapse of the relationship between *A. lateralis* and *A. corallinus*, nesting *A. corallinus* within *A. lateralis*; collapse of the *A. notospilotus* + *A. fenestralis* clade; and strong support (99% MLB) for the clade containing *A. lateralis*, *A. corallinus*, *A. notospilotus*, and *A. fenestralis*. It should be noted that the phylogeny produced after complete deletion of third codon position sites was the only instance where monophyly of clade H was not supported.

Fig. 2 shows a phylogeny depicting the most stable and well-supported relationships based on a synthesis of the results described above. The purpose of this phylogeny is to highlight

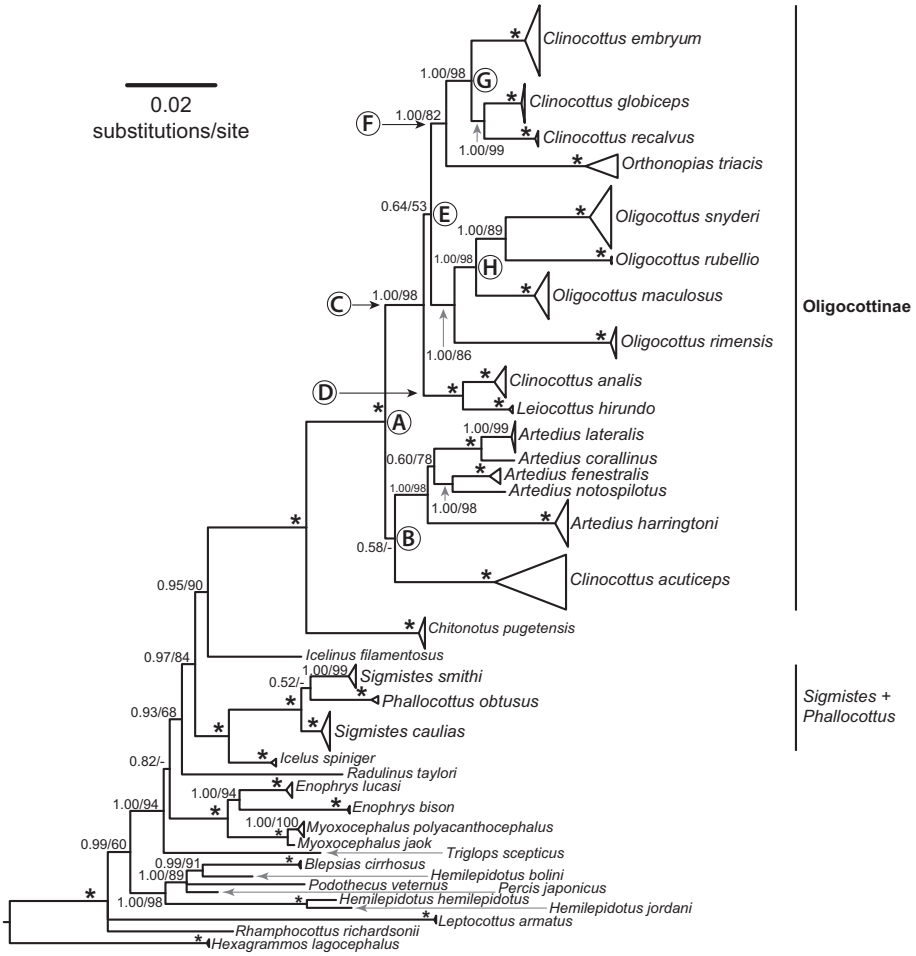

Fig. 1. Phylogeny of oligocottine relationships based on Bayesian analysis of the concatenated dataset. Bayesian and ML support values are indicated at each node left and right of '/', respectively. Asterisks indicate nodes with 1.0 posterior probability and 100% ML bootstrap support. Dash in place of support value indicates less than 50% support. Clades consisting of multiple conspecific individuals are collapsed with the size of the collapsed clade proportional to the number of individuals examined.

clades supported by a strong phylogenetic signal in the sequence data as evidenced by the consistent appearance and strong support of a given clade in multiple analyses and permutations of the data. Relationships were collapsed if the support values were low in the initial ML and BI analyses (i.e., >0.65 BPP and >65% MLB) and in those using alternative phylogenetic approaches (i.e., clade E, clade B, and the relationship of *Phallocottus obtusus*, *Sigmistes caulias*, and *S. smithi*). The sister-group relationship of the *Artedius fenestralis* + *A. notospilotus* clade with the *A. lateralis* + *A. corallinus* clade was only moderately supported in the initial analyses (Fig. 1), however, a clade containing these four species was well supported in the phylogenies produced by alternative coding of the third codon position (94–99% support values), and has been proposed by some previous studies (i.e., Bolin, 1944, 1947; Ramon and Knope, 2008), though others (i.e., Begle, 1989; Knope, 2013) came to different conclusions. Several clades were supported by the primary and all alternative analyses: *Sigmistes* + *Phallocottus*, together allied with *Icelus spiniger*; clade A, *Artedius*, clade C, clade G, clade D; the sister-group relationship of *O. snyderi* with *O. rubellio*; and the monophyly of *A. lateralis* + *A. corallinus*.

4. Discussion

4.1. Monophyly of the Oligocottinae

Since the subfamily Oligocottinae was first delineated, its monophyly has been examined in a modest number of studies (i.e., Strauss, 1993; Ramon and Knope, 2008; Knope, 2013). Although these studies did not examine all genera assigned to the Oligocottinae, they support a close relationship between *Oligocottus*, *Clinocottus*, *Orthonopias*, and *Artedius*, including the monotypic genus *Leiocottus* in this group, in agreement with earlier work (e.g., Bolin, 1944, 1947). Broader systematic studies of cottoid relationships (i.e., Yabe, 1985; Jackson, 2003; Smith and Wheeler, 2004; Smith and Busby, 2014) have examined only single representatives of some of the oligocottine genera, which, aside from *Artedius* (see Begle, 1989), had themselves not been systematically tested for monophyly until recently (i.e., Ramon and Knope, 2008; Knope, 2013).

The evidence presented here provides strong support for a monophyletic group consisting of all the species that make up the genera: *Clinocottus*, *Oligocottus*, *Artedius*, *Leiocottus*, and *Orthonopias* (Clade A in Fig. 1). This grouping was present and strongly supported in the analyses of every permutation of the data and all methods of phylogenetic inference (Fig. 2). This grouping is in agreement with early evolutionary hypotheses (i.e., Bolin, 1944, 1947), the morphology-based cladistic analysis of the genera (Strauss, 1993), and recent molecular-based analyses of many of the oligocottine species (Ramon and Knope, 2008; Knope, 2013). Significantly, no analyses conducted in this study placed the oligocottine genus *Sigmistes* in clade A. Instead, every analysis allied *Sigmistes* with the monotypic genus *Phallocottus*, and this clade was allied with *Icelus spiniger* with very high support values (Fig. 2). This finding contradicts previous work, which had

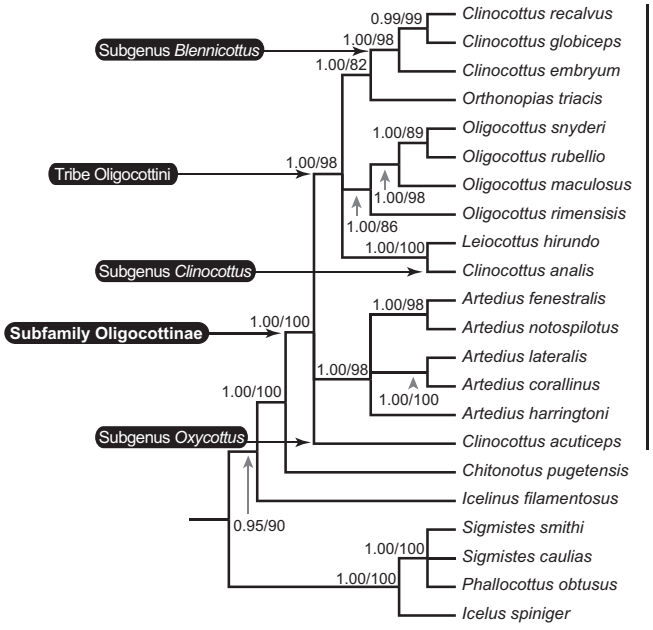

Fig. 2. Phylogeny of Oligocottinae including only stable and strongly supported relationships. Bayesian and ML support values are indicated at each node left and right of '/', respectively. Dash in place of support value indicates less than 50% support. Clades with taxonomic labels referred to in the text are indicated with black bubbles and an arrow pointing to the base of the clade.

unanimously allied *Sigmistes* with *Clinocottus* (Hubbs, 1926b; Taranets, 1941; Howe and Richardson, 1978). However, it should be noted that the present study represents the first analysis of the phylogenetic placement of either *Sigmistes* or *Phallocottus* using formalized methods of phylogenetic inference.

The morphological traits that have been proposed to delineate Oligocottinae (e.g., soft pelvic rays, simple preopercular spines; see Hubbs, 1926b; Taranets, 1941) are found throughout the cottoid suborder (see descriptions in Bolin, 1944). Studies that have analyzed the morphology of oligocottine species in a cladistic framework have either failed to resolve their relationship (e.g., Jackson, 2003) or been so limited in taxon-sampling that the results are difficult to interpret in either a broad phylogenetic sense or as being generally applicable to all oligocottines (Washington, 1986; Begle, 1989; Strauss, 1993). The most recent and thorough analysis of higher cottoid relationships (Smith and Busby, 2014) supports a monophyletic grouping of *Oligocottus*, *Orthonopias*, *Clinocottus*, and *Artedius*, but is unable to provide any new synapomorphies for the group, given the broad scope of the study and instead describes the traits shared by oligocottine genera as homoplastic synapomorphies. Given the results presented here and the absence of relevant contradictory evidence in the literature, we propose to delineate the Oligocottinae to include only the members of clade A, thus the genus *Sigmistes* should removed from the subfamily (Table 5).

Rather than an affinity with any oligocottine genera, our data show a close relationship between *Sigmistes* and the genus *Icelus*. Given this close relationship, future work may show that *Sigmistes* would be best placed in a modified form of the subfamily *Icelinae* (*sensu* Taranets, 1941). The results of Knope (2013) and Smith and Busby (2014) each unite some of the putative iceline genera, though their results show that there is clear need for further investigation into the constituency and validity of the subfamily Icelinae, especially as it relates to the subfamily Pseudoblenniinae (*sensu* Taranets, 1941).

4.2. Inter-generic relationships

Outside of Oligocottinae, as defined above, lies the *Sigmistes* + *Phallocottus* clade. These genera were grouped together and as a sister-group to *Icelus spiniger* in every analysis conducted in this study. Within the *Sigmistes* + *Phallocottus* clade, however, there was great discrepancy on the placement of *Phallocottus*; some analyses placed it as sister to *Sigmistes* while others nested it within *Sigmistes* as sister to *S. smithi*. Given the lack of clear consensus and the low support values for either of the two placements of *Phallocottus*, the relationship of the constituent species of the two genera could not be confidently resolved and we present the clade as an unresolved polytomy (Fig. 2). Regardless, the three species in the clade together form a stable and well-supported monophyletic group.

Within Oligocottinae, there was stable and strong support for division of the subfamily into three primary lineages: the genus *Artedius*, clade C (hereafter referred to as the tribe Oligocottini *sensu* Hubbs, 1926b, modified from Taranets, 1941, see Fig. 2), and *Clinocottus acuticeps*. Relationships between these three lineages vary between analyses. Thus, given the available evidence, these three lines are best arranged in an unresolved trichotomy at the base of the oligocottine clade.

Within the tribe Oligocottini there are three unambiguously supported groups: the genus *Oligocottus*, clade D (discussed below in Section 4.3.2), and clade F. Clade F contains clade G, the latter corresponding to the subgenus *Blennicottus* Gill 1861 *sensu* Bolin

Table 5
Proposed revisions to taxonomy and classification of species and higher-order groups examined in this study.

Current taxonomy	Proposed revision
Species taxonomy	Species taxonomy
Artedius corallinus	*Artedius corallinus*
Artedius fenestralis	*Artedius fenestralis*
Artedius harringtoni	*Artedius harringtoni*
Artedius lateralis	*Artedius lateralis*
Artedius notospilotus	*Artedius notospilotus*
Clinocottus acuticeps	*Clinocottus (Oxycottus) acuticeps*
Clinocottus analis	*Clinocottus (Clinocottus) analis*
Clinocottus embryum	*Clinocottus (Blennicottus) globiceps*
Clinocottus globiceps	*Clinocottus (Blennicottus) embryum*
Clinocottus recalvus	*Clinocottus (Blennicottus) recalvus*
Leiocottus hirundo	*Leiocottus hirundo*
Oligocottus maculosus	*Oligocottus maculosus*
Oligocottus rimensis	*Oligocottus rimensis*
Oligocottus rubellio	*Oligocottus rubellio*
Oligocottus snyderi	*Oligocottus snyderi*
Sigmistes caulias	*Sigmistes caulias*
Sigmistes smithi	*Sigmistes smithi*
Tribe Oligocottini (*sensu* Taranets, 1941)	Tribe Oligocottini
Clinocottus	*Clinocottus*
Oligocottus	*Leiocottus*
Sigmistes	*Oligocottus*
	Orthonopias
Tribe Artediini (*sensu* Taranets, 1941)	Tribe Artediini = genus *Artedius*
Artedius	
Orthonopias	
Subfamily Oligocottinae (*sensu* Taranets, 1941)	Subfamily Oligocottinae
Artedius	*Artedius*
Clinocottus	*Clinocottus*
Oligocottus	*Leiocottus*
Orthonopias	*Oligocottus*
Sigmistes	*Orthonopias*

(1944), and *O. triacis* (Fig. 2). These two groups share very little apparent morphological similarity aside from an anteriorly placed vent (Bolin, 1944). *Orthonopias triacis* is perhaps the most confounding of any oligocottine taxa. Previous morphological studies have allied this species with *Artedius* (i.e., Taranets, 1941; Bolin, 1944, 1947) or placed it completely outside of Oligocottinae (i.e., Begle, 1989). Indeed, *O. triacis* possesses an unusual mixture of primitive and derived traits (*sensu* Bolin, 1944, 1947) compared to other oligocottine species. For example, *O. triacis* possesses four distinct preopercular spines while in all other oligocottine sculpins only the uppermost preopercular spine is distinct and the lower three are either reduced to small nubs or are completely obscure. *Orthonopias triacis* is also the only oligocottine species to have both an advanced anus and an *Artedius*-type dorsal scale band. Additionally, *O. triacis* has a unique morphology of the pelvic fins that is sexually dimorphic (see Bolin, 1944, 1947).

The unique suite of morphological characters found in *Orthonopias triacis* has perhaps contributed to the lack of consensus among attempts to infer its phylogenetic placement using comparative morphology (i.e., Taranets, 1941; Bolin, 1944, 1947; Begle, 1989; Jackson, 2003). There is, however, strong support on the molecular level for the phylogenetic placement of *O. triacis* within the tribe Oligocottini, and a strong association between *O. triacis* and the subgenus *Blennicottus* (Fig. 1). Therefore, given the support of a close relationship between *O. triacis* and *Blennicottus* found in this study, we conclude that the physical differences (notably, presence of scales and number of preopercular spines) are autapomorphies of the species and not indicative of its affinities elsewhere. Further study may provide insight into the apparent discrepancy between genotype and phenotype in this group.

4.3. Intra-generic relationships

Our results provide further support for the monophyly of *Artedius* and moderate support for the intra-generic relationships suggested in previous morphological (i.e., Bolin, 1947) and molecular (i.e., Ramon and Knope, 2008) studies (Figs. 1 and 2). In contrast, *Clinocottus* as currently defined does not represent a natural group, but rather an assemblage of three distinct and distantly related lineages (Figs. 1 and 2). The first includes only *C. acuticeps* and its phylogenetic placement is at the base of the oligocottine tree as part of a polytomy with *Artedius* and Oligocottini. We recommend that given the clear evolutionary distinction of this species, it should be recognized as a distinct subgenus, *Clinocottus (Oxycottus) acuticeps* (sensu Bolin, 1944, 1947; see Fig. 2 and Table 5).

The second group within *Clinocottus* is the subgenus *Blennicottus*. This group contains *C. recalvus*, *C. globiceps*, and *C. embryum*. Morphologically, these species are united with each other and differentiated from other members of *Clinocottus* by the "deep" and "heavy" caudal peduncle and a comb of cirri at each anterior pore of the lateral line (Bolin, 1944). *Clinocottus globiceps* and *C. recalvus* have been closely allied throughout their taxonomic history, and were in fact believed to be subspecies for a time (see Greeley, 1899). It comes as no surprise then that within the subgenus *Blennicottus*, *C. globiceps* and *C. recalvus* are most closely related, with *C. embryum* as sister to their clade. This grouping and structure are shown with high support in all permutations and analyses of our dataset with the exception of analyses that exclude third codon positions, where the overall grouping remains with high support but relationships between the three species is unresolved. This result is in agreement with those reported in recent DNA-based studies (Ramon and Knope, 2008; Knope, 2013). A larval-character based study by Washington (1986) and the follow up study by Strauss (1993), incorporating Washington's character matrix with that of Begle (1989), placed *C. embryum* in a separate clade from *C. recalvus* and *C. globiceps*. However, those results were not generated using explicitly phylogenetic analyses or suffered from methodological flaws (as described above). Given the strong support in our analyses, coupled with the support of other DNA-based studies, the existence of candidate synapomorphies for the group, and the relatively few characters used in the only dissenting studies (i.e., Washington, 1986 and Strauss, 1993), we conclude that the subgenus *Blennicottus* (*sensu* Bolin, 1944) is a valid taxonomic grouping, distinct from other members of *Clinocottus*.

The third and final lineage of species of *Clinocottus* is the *C. analis* lineage. Bolin (1944, 1947) places *C. analis* in its own subgenus (subgenus *Clinocottus*) and our results show clearly that *C. analis* is distinct from its congeners and we support the recognition of this subgenus (Fig. 2 and Table 5). Every analysis configuration conducted in this study shows a highly supported clade formed by *C. analis* + *Leiocottus hirundo*. This relationship was also shown in other DNA-based phylogenies (i.e., Ramon and Knope, 2008 and Knope, 2013). No cladistic, morphology-based study has examined both *C. analis* and *L. hirundo*. However, Bolin (1944, 1947) considered *L. hirundo* to be allied to the genus *Clinocottus* and pointed to differences in the attachment of the gill membrane as the morphological feature warranting generic distinction. Side-by-side examination of the two species shows clear differences in the side profile of the head and fins, especially in the first two spines of the spinous dorsal fin, which in *L. hirundo* are elongated and quite robust (see descriptions in Bolin, 1944), however, none of these apparent differences have been described and tested cladistically. Shared characters between the two genera include the "structure of the preopercular spine," "advanced anus," and "blunt" genital papilla (Bolin, 1947). The latter two features are some of the most notable distinguishing features of *Clinocottus*, as described in Bolin (1944). We therefore question the generic distinction of *Leiocottus* and *Clinocottus*. *Leiocottus* has priority of nomenclature and it is our recommendation that if future researchers choose to update the nomenclature to better fit our best understanding of the evolutionary relationships of these species, the three subgenera outlined herein that currently make up the genus *Clinocottus* should each be given generic distinction, and the then monotypic genus *Clinocottus* should be synonymized with *Leiocottus*. If these recommendations were followed, the affected species would be renamed as follows: *Oxycottus acuticeps*, *Blennicottus globiceps*, *B. recalvus*, *B. embryum*, *Leiocottus hirundo*, and *L. analis* (see Fig. 2 and Table 5).

The species of *Oligocottus* form a well-supported clade in the phylogenies produced by the primary ML and BI phylogenies (Fig. 1), as well as the phylogeny produced by coding the third codon position sites as R/Y. *Oligocottus rimensis* is morphologically distinct from the rest of *Oligocottus* in that its body is almost completely covered in prickles, and the upper preopercular spine is simple, but is united with other members of the genus by the general modification of the anterior anal fin rays and the placement of the vent with respect to the anal fin (see Bolin, 1944, 1947). The rest of the members of *Oligocottus* (*O. maculosus*, *O. snyderi*, and *O. rubellio)* make up clade H, (subgenus *Oligocottus* in Bolin, 1944, 1947) and were well supported in our analyses and united by several morphological similarities: complete loss of all scales but those on the lateral line, bifurcation or trifurcation of the upper preopercular spine, and a simple, elongated genital papilla in males (see Bolin, 1944, 1947). Within this group, *O. snyderi* and *O. rubellio* are most closely related, and their sister-relationship was recovered in all analyses. The close relationship of these two species is supported morphologically by the modification of the anterior-most anal fin ray into an elongated, prehensile organ (used by *O. snyderi* to grasp females during copulation; Morris, 1956), and by the abundance and distribution of multifid/palmate cirri across their head and body (Bolin, 1944, 1947). We conclude that

Oligocottus, in its present form, forms a monophyletic, well-defined, stably and strongly supported clade.

The monotypic *Phallocottus* is known only from a few locations in the Aleutian archipelago. This genus has received little scientific study and, prior to the present study, the phylogenetic placement of *Phallocottus* had never been examined. The initial description of *P. obtusus* asserted that it was "most closely related to the Oligocottinae of Hubbs, 1926," based on comparisons of several morphological features (e.g., the arched lateral line) with those found in the oligocottine (*sensu* Hubbs, 1926b) genus *Sigmistes* (Schultz, 1938). *Phallocottus obtusus* is described as distinct from its close relatives (i.e., *Sigmistes* spp.) based primarily on its rounded preopercular spines, lack of palatine teeth, and obscured nasal spines (Schultz, 1938). The only other mention of *Phallocottus* in an evolutionary context is found in an unpublished study of meristic characteristics of NE Pacific sculpins, which agreed with the conclusions of Schultz (1938) that *Phallocottus* was most closely related to *Sigmistes* (Howe and Richardson, 1978).

All of the results of the present study show strong support for a clade containing *P. obtusus* and both species of *Sigmistes*. However, because the results do not resolve the relationship of these three species, we consider an unresolved 'soft' polytomy consisting of *P. obtusus*, *S. smithi*, and *S. caulias* as the best phylogenetic estimate available at present. The morphological features uniting *Phallocottus* and *Sigmistes* are a lack of scales and a strong arch in the lateral line above the pectoral fins (see Jordan and Evermann, 1898; Schultz, 1938; Howe and Richardson, 1978). Given the consistent and overwhelming support of a monophyletic relationship of the members of these two genera in all of our analyses, combined with the morphological similarities and historical affinities, we conclude that *Phallocottus* forms a monophyletic group with the members of the genus *Sigmistes*.

Sigmistes was allied with the modern genus *Clinocottus* in the earliest systematic classifications of Oligocottinae (Hubbs, 1926b; Taranets, 1941). Prior to that, *Sigmistes* was allied to *C. acuticeps* and *C. embryum* (Jordan and Evermann, 1898). Indeed, there are several morphological similarities between *Sigmistes* and members of *Clinocottus* (e.g., a lack of scales, advanced anus, enlarged genital papilla in males). However, none of those similarities has been tested in a formal systematic analysis. In fact, the only other mention of the evolutionary relationships of *Sigmistes* was in an unpublished study of meristic traits, which suggested a close relationship between *Sigmistes* and *Phallocottus*, and that the two genera together are most closely related to the "*Oligocottus–Clinocottus* group," especially *Clinocottus* (Howe and Richardson, 1978), but gives no indication of the evidence supporting such a grouping.

The sequence data presented here overwhelmingly support a close relationship between *Sigmistes* and *Phallocottus* but ally them to *Icelus spiniger* rather than *Clinocottus*. Furthermore, our results never place the *Sigmistes* + *Phallocottus* clade within, or even sister to the Oligocottinae, as defined in this study.

The relationship between *Sigmistes* and *Phallocottus*, or rather, the validity of *Phallocottus*, is unclear. As was discussed above, the relationship of *S. smithi*, *S. caulias*, and *P. obtusus* is inconsistent and weakly supported across our various analyses, making a confident resolution impossible. Rather, we place the three species in a soft polytomy.

As in the case of *Phallocottus*, the present study represents the first test of the classification and phylogenetic placement of *Sigmistes*. Given the strong support of a close relationship between *Sigmistes* and *Phallocottus*, the morphological similarities between the two genera (see above discussion of *Phallocottus*), we conclude that *Phallocottus* and *Sigmistes* form a stably and well-supported monophyletic group. Additionally, we conclude that *Sigmistes* should be removed from Oligocottinae (Fig. 2 and Table 5).

5. Conclusions

The membership of the subfamily Oligocottinae should be revised to include only the genera *Oligocottus*, *Clinocottus*, *Orthonopias*, *Leiocottus*, and *Artedius* (Table 5). The genus *Sigmistes* should not be grouped in this subfamily, despite the superficial morphological similarities it shares with some members of the oligocottine genus *Clinocottus*. Rather, *Sigmistes* and the genus *Phallocottus* form a monophyletic clade, closely related to the genus *Icelus*.

Artedius and *Oligocottus* are both consistently supported as monophyletic genera within Oligocottinae. The genus *Clinocottus* is polyphyletic as currently defined as it groups three distinct lineages that have closer affinities to other oligocottine groups than they do to one another. The three independent lines of *Clinocottus* are: the subgenus *Blennicottus*, the subgenus *Oxycottus*, and the subgenus *Clinocottus*. The monotypic genus *Leiocottus* is most closely related to *Clinocottus analis*, and the distinction of these two genera carries no benefit of morphological clarity, especially given the morphological diversity within the currently defined *Clinocottus* (see descriptions in Bolin, 1944). Taxonomic revision of *Clinocottus* is clearly in order.

Orthonopias triacis is not closely related to *Artedius*, as previously proposed. Rather, it represents a morphologically distinct lineage with strong molecular support for a close relationship with the subgenus *Blennicottus*.

The groups receiving consistent and strong support in this study represent three evolutionary lines within Oligocottinae: the genus *Artedius*, the tribe Oligocottini, and *Clinocottus acuticeps* (Table 5). The Oligocottini contains three distinct groups in an unresolved polytomy (Fig. 2).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2015.03.006.

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