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The phylogeny of marine sculpins of the genus *Icelinus* with comments on the evolution and biogeography of the Pseudoblenninae

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Abstract

The marine sculpins (Psychrolutidae) are a diverse percomorph family with notable morphological variation and repeated biogeographic patterns within the group. The psychrolutid genus *Icelinus* is unusual because it is one of the few near-shore members of the family that exhibits a trans-Pacific distribution; it has two species in the western Pacific and nine species in the eastern Pacific. Furthermore, the placement of *Icelinus* has been more inconsistent across molecular and morphological analyses than many genera. Previous phylogenetic studies have hypothesized sister taxa to *Icelinus* ranging from *Antipodocottus, Chitonotus*, and *Stlengis,* to a mixed clade of psychrolutids. The varied placements across these studies may be due to limited taxon sampling within *Icelinus*, and previous authors have never included western Pacific species of *Icelinus* in their analyses. This study tests the monophyly of the genus, examines the relationships between eastern and western Pacific species of *Icelinus,* and explores the relationships of *Icelinus* within Psychrolutidae. Our results show that the traditional grouping of *Icelinus* is polyphyletic. The eastern Pacific species of *Icelinus* are restricted to a clade sister to *Furcina* and *Antipodocottus*. The western Pacific species of *Icelinus* are recovered sister to the genus *Stlengis*. Given the polyphyly of *Icelinus*, the sister-group pairing of western Pacific species of *Icelinus* and *Stlengis*, as well as morphological similarity between the two groups, we recommend treating the western Pacific species of *Icelinus* as members of the genus *Stlengis.* With this taxonomic change, species in the genus *Icelinus* are now limited to the eastern Pacific, ranging from Alaska to Mexico.

Key words: Psychrolutidae, *Icelinus*, *Antipodocottus*, Sculpins

Background

The limits and relationships of the cottoid families and subfamilies have been thoroughly examined since the revisionary work by Jordan (1896) and Jordan and Evermann (1898). Several of the subsequent investigations have laid the groundwork for the modern phylogeny of cottoids. Specifically, Taranets (1941) revised the limits of cottoids by dividing the group into 12 families and 13 cottid subfamilies. Further, Bolin (1947; Figure 1) and Yabe (1985; Figure 1) built off of previous work and refined the limits and relationships of cottoid genera using morphological variation. Recently, studies by Knope (2013) and Smith and Busby (2014) have built upon these foundational morphological works and revised the limits and relationships of cottoids, specifically the marine sculpins, using molecular or a combination of morphological and molecular data.

The marine sculpins are members of a diverse percomorph family (Psychrolutidae, 64 genera, 214 species) with notable biogeographic distributions and morphological variation within the group (Knope, 2013; Smith and Busby, 2014; Eschmeyer *et al*. 2016). Psychrolutids (*sensu* Smith and Busby, 2014, and used hereafter) are predominantly found in the North Pacific Ocean, but the psychrolutines can be found in deep water throughout all oceans. These fishes exhibit morphological variation that includes, but is not limited to, the loss of pelvic-fin rays, the loss of body scales, and the development of an external intromittent organ. Among psychrolutids, the subfamily Pseudoblenninae is notable because of its comparative phylogenetic stability, while the larger Cottoidea has undergone substantive revision (e.g., Yabe, 1985; Knope, 2013; Smith and Busby, 2014; Figure 1).

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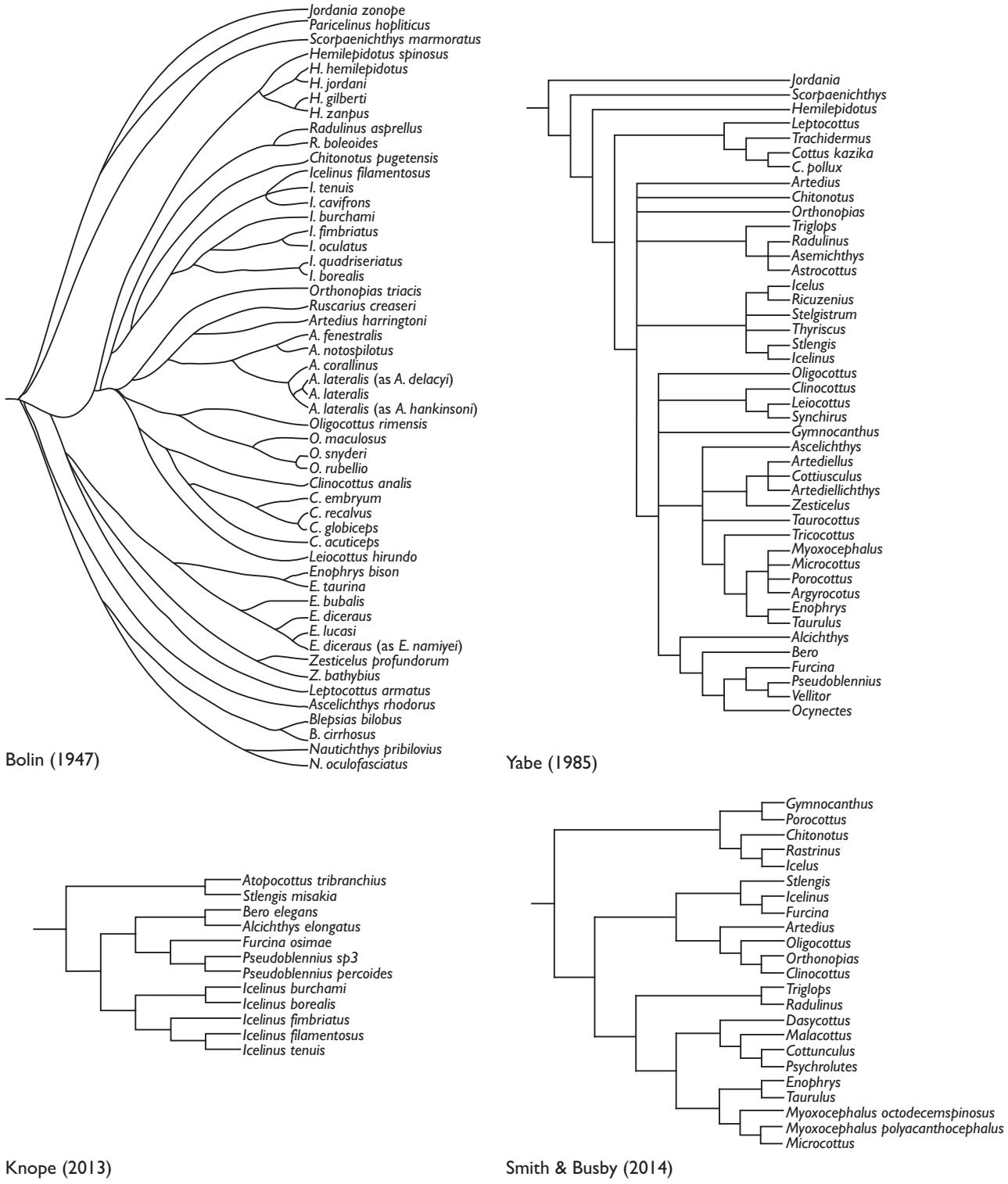

FIGURE 1. Historical placement and previous phylogenetic hypotheses for *Icelinus* from Bolin (1947), Yabe (1985), Knope (2013), and Smith & Busby (2014).

As described by Jordan (1896), Pseudoblenninae was characterized by the presence of vomerine and palatine teeth, scaleless bodies, and males possessing a non-retractile intromittent organ. In addition to the characters recognized by Jordan, Taranets (1941) diagnosed the Pseudoblenninae by the absence of dorsal head spines or ridges protruding through skin, pelvic fins with one spine and two soft rays, bony plates on lateral line, when present, that are weakly developed, and numerous other characters. The Pseudoblenninae was later studied by Watanabe (1960) who used morphological data to refine the subfamily to include eight genera: *Alcichthys,*

Argyrocottus, Bero, Crossias, Furcina, Ocynectes, Pseudoblennius, and *Vellitor* (hereafter "traditional" Pseudoblenninae). Following these revisionary works, the subfamily has generally been recovered in morphological (Yabe, 1985; Figure 1), molecular (Knope, 2013; Figure 1), and combined analyses (Smith and Busby, 2014; Figure 1). Recent studies (Knope, 2013 and studies cited within; Figure 1) have mostly recovered the traditional Pseudoblenninae with modest changes to the group, including the recovery of the genus *Icelinus* within or sister to the Pseudoblenninae (Knope, 2013; Smith and Busby, 2014). In light of this placement, it is noteworthy that Taranets (1941) originally placed *Icelinus* in Icelinae, which was defined by the presence of bony plates along the lateral line and base of the dorsal fin. Yabe (1981) noted problems with the monophyly of the Icelinae, so the potential addition of *Icelinus* to Taranets' (1941) otherwise resilient clade demands further investigation.

Icelinus was described by Jordan (1885: 898) for *Artedius quadriseriatus* due to its distinctive bands of ctenoid scales running below the dorsal fins (Figure 2), preopercular armature, and "distinct body form." Bolin (1944) recognized eight species, all distributed in the northern and eastern Pacific Ocean, that he classified into four subgenera: *Tarandichthys*, including *Icelinus cavifrons, I. filamentosus*, and *I. tenuis*; *Medicelinus*, including *I. burchami*; *Penicelinus*, including *I. fimbriatus* and *I. oculatus*; and *Icelinus*, including *I. borealis* and *I. quadriseriatus*. Following Bolin's revision, one additional eastern Pacific species (*I. limbaughi* Rosenblatt and Smith, 2004) has been described. These nine species of *Icelinus* will be treated as the "eastern Pacific" species of *Icelinus* hereafter. Furthermore, two western Pacific species (*I. japonicus*, Yabe *et al.* [1980]; *I. pietschi,* Yabe *et al.* [2001]; "western Pacific" species of *Icelinus* hereafter) have also been described. With the addition of the western Pacific species of *Icelinus*, the genus exhibits an atypical distribution compared to most other cottoids, particularly psychrolutids. Only five other psychrolutid genera distributed in the North Pacific Ocean have been found to inhabit both the eastern and western regions (*Dasycottus, Gilbertidia, Malacocottus, Psychrolutes,* and *Zesticelus*). Among these psychrolutids, *Icelinus* is the only near-shore group to exhibit this pattern.

With the recovery of *Icelinus* near or among the Pseudoblenninae in molecular studies, the description of three new species that cannot be placed in the existing subgeneric classification, and the unusual trans-Pacific distribution of the genus, a comprehensive study is warranted to explore the limits and relationships of *Icelinus*. The aim of this study is to test the phylogenetic placement, intrarelationships, and biogeography of the psychrolutid genus *Icelinus*.

Materials and methods

The taxon sampling for this study encompasses 19 taxa, including all 11 described species of *Icelinus*. To test the monophyly of *Icelinus* and its relationships to the larger Psychrolutidae, five additional psychrolutid genera were included in the analyses: *Antipodocottus*, *Artedius*, *Chitonotus, Furcina, Icelus, Radulinus,* and *Stlengis*. These outgroup taxa were chosen based on previous hypotheses that suggested that the taxa are closely allied to the clade (Bolin, 1944; Nelson, 1985; Yabe, 1985; Knope, 2013; Smith and Busby, 2014). Analyses were rooted with *Leptocottus*, a member of the predominantly freshwater Cottidae, which has been recovered as the sister group to the Psychrolutidae (Smith and Busby, 2014).

This study combined molecular and morphological data to improve resolution, add more heritable information, and allow for the inclusion of species that can only be represented by morphological or molecular data (Wiley *et al*., 2011; McMahan *et al*., 2013; Davis, 2015). The dataset consisted of 3,814 molecular and morphological characters (Tables 1 and 2). Of these 3,814 characters, 24 were soft and hard tissue morphological characters from the following sources: Taranets (1941), Bolin (1947), Yabe (1985), and Jackson (2003). The morphological dataset is 98% complete at the individual character level.

Some specimens were cleared and double stained for bone and cartilage following the methods of Pothoff (1984). These specimens were dissected and documented via digital photography with a Nikon SMZ18 microscope under normal as well as epifluorescent lighting. One specimen of *Icelinus quadriseriatus* was prepared for scanning electron microcopy (SEM) in order to examine scalation. The specimen was dehydrated in an ascending ethanol series, critical-point dried in CO_2, mounted on stubs with silver paint (following Webb, 1989), and viewed using a Hitachi model S5-7 scanning electron microscope. Examined vouchers use institutional acronyms recommended by Sabaj Pérez (2016).

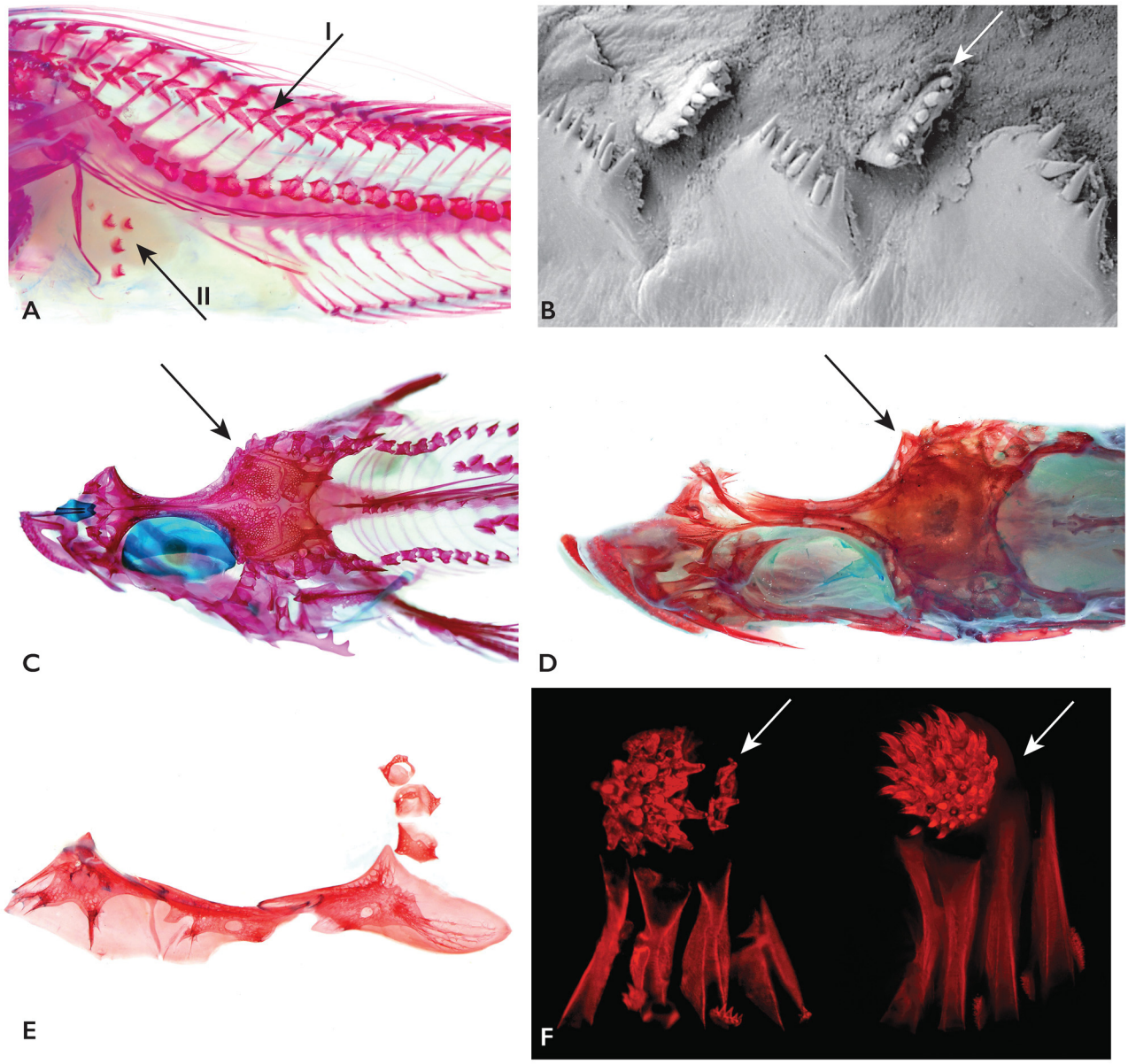

FIGURE 2. Notable morphological variation coded in this study: A) Presence of dorsal scale band (Character 12_1)—*Icelinus filamentosus* (SIO 83-82), arrow (I). Presence of pectoral axillary scales (Character 10_1), arrow (II). B) Scanning electron micrograph of dorsal scale band (Character 20_1)—*Icelinus quadriseriatus* (SIO 02-19). Arrow indicates 'toothed' nature of dorsal scales. C) Absence of frontal spine (Character 15_0)—*Icelinus quadriseriatus* (SIO 02-19), arrow. D) Presence of frontal spine (Character 15_1)—*Icelinus fimbriatus* (SIO 94-130), arrow. E) Infraorbitals (Character 1_0)—*Icelinus filamentosus* (SIO 83-82), dissected. F) Second pharyngobranchial tooth plate presence (Character 3_1)—*Cottus bairdii* (KU 15228), dissected, left. Second pharyngobranchial tooth plate absence (Character 3_0)—*Icelinus filamentosus* (SIO 83-82), dissected, right.

In addition to morphological data, 3,790 aligned nucleotides were analyzed from two mitochondrial and three nuclear loci; 12S-tRNA-Val-16S fragment, COI, ENC1, TMO-4c4, and ZIC1 (Table 1). Tissue samples were preserved in 95% ethanol prior to extraction of DNA. Tissues for three taxa, *Antipodocottus galatheae, Icelinus japonicus,* and *I. pietschi*, were not available for sequencing due to species rarity and lack of recent collection. For novel sequences, genomic DNA was extracted from muscle tissue using a DNeasy Tissue Extraction Kit (Qiagen, Valencia, CA). The polymerase chain reaction (PCR) was used to amplify all gene fragments. Double-stranded amplifications were performed in a 25 µL volume containing one Ready-To-Go PCR bead (GE Healthcare, Piscataway, NJ), 1.25 µL of each primer (10 pmol), and 2–5 µL of undiluted DNA extract. All primers and primer sources are listed in Table 3. Amplifications for all novel DNA fragments were carried out in 36 cycles using the

TABLE 1. Molecular vouchers with GenBank accession numbers.

Taxon	Molecular Voucher	12S	tRNA-Val-16S	COI	TMO-4c4	ENC1	ZIC1
Cottidae (Root)							
Leptocottus armatus	FMNH Uncat. – Bodega Bay	KM057968	AY539537	JQ354163	AY539435	KX353740	KX353727
Psychrolutidae							
Artedius fenestralis	AMNH Uncat. – Friday Harbor	KM057943	KJ010593	JQ353989	AY539428	KX353741	KX353728
Chitonotus pugetensis	SIO 02-19	KX353700	KM057853	KX353713	KM058001	KX353742	KX353729
Icelus spiniger	KU 2365	KX353708	KX353712	KX353716	KX353726	KX353752	KX353738
Radulinus asprellus	KU 2410	KM057983	AY539028	KF918897	AY539437	KX353753	KC831262
Stlengis japonicus	Unavailable	Unavailable	Unavailable	Unavailable	Unavailable	Unavailable	Unavailable
Stlengis misakia	FMNH Uncat. – Pet Trade	KM057989	KM057876	KX353717	KM058022	KX353754	KX353739
Stlengis pietschi	Unavailable	Unavailable	Unavailable	Unavailable	Unavailable	Unavailable	Unavailable
Pseudoblenninae							
Antipodocottus galatheae	Unavailable	Unavailable	Unavailable	Unavailable	Unavailable	Unavailable	Unavailable
Furcina osimae	FMNH Uncat. – Pet Trade	KM057958	KM058037	KX353714	KM058008	KX353743	KX353730
Icelinus borealis	AMNH Uncat. – Friday Harbor	KX353701	EF458346	JQ354140	KX353718	KX353744	KX353731
Icelinus burchami	SIO 97-132	KX353702	AY835647	EU403065	KX353719	KX353745	KX353732
Icelinus cavifrons	KU 518	KX353703	AY835648	KF929992	KX353720	KX353746	KX353733
Icelinus filamentosus	SIO 97-184	KM057965	AY539023	JQ354141	AY539433	KX230217	KC831205
Icelinus fimbriatus	KU 509	KX353704	KX353709	JQ354143	KX353721	KX353747	Unavailable
Icelinus limbaughi	LACM 56818.001	KX353705	KX353710	KX353715	KX353722	KX353748	KX353734
Icelinus oculatus	SIO 99-94	KX353706	AY835650	EU403068	KX353723	KX353749	KX353735
Icelinus quadriseriatus	SIO 02-19	Unavailable	AY835651	GU440356	KX353724	KX353750	KX353736
Icelinus tenuis	UW 152151	KX3537067	KX353711	GU440357	KX353725	KX353751	KX353737

TABLE 2. Matrix of phenotypic characters analyzed in the current study (characters 1–24). Characters that are inapplicable are indicated by '-'.

	111111111122222
	123456789012345678901234
Leptocottus armatus	001001000-1---00000--001
Antipodocottus galatheae	0001?1000-1---0000011000
Artedius fenestralis	001000000001110100111100
Chitonotus pugetensis	001010111010011111--110
Furcina osimae	000111000-0---00000--1?0
Icelinus borealis	000110100011000100000-010
Icelinus burchami	000110100011110001111000
Icelinus cavifrons	000110110101110000010-110
Icelinus filamentosus	000110111101110100010-110
Icelinus fimbriatus	000110100011101110111110
Icelinus limbaughi	000110100001110000000-100
Icelinus oculatus	000110100011100111111010
Icelinus quadriseriatus	000110100001100100000-010
Icelinus tenuis	000110111101111001100-010
Icelus spiniger	000000000111001000110110
Radulinus asprellus	111000100100--101110-000
Stlengis japonicus	00?1?0?000110001011111?0
Stlengis misakia	000100000011100001111000
Stlengis pietschi	00?1?0?000110101011100

following temperature profile: initial denaturation for 6 min at 94°C, denaturation for 60 s at 94°C, annealing for 60 s at 46–53°C (see Table 3 for core annealing temperature for each locus), and extension for 75 s at 72°C, with an additional terminal extension at 72°C for 6 min. Sequencing of PCR products was done either on an ABI 3730 at the Field Museum of Natural History (FMNH; Chicago, IL) or submitted to Beckman Coulter Genomics (Danvers, MA) for sequencing. For DNA products that were sequenced at FMNH, amplification products were cleaned, desalinated, and concentrated using AMPure (Agencourt Biosciences, Beverly, MA). Purified PCR products were then amplified as templates for sequencing using the amplification primers listed in Table 3 and a Prism Dye Terminator Reaction Kit Version 1.1 (Applied Biosystems, Foster City, CA). The second amplification products were then cleaned and desalinated using cleanSEQ (Agencourt Biosciences). All sequence contigs were built using Geneious 8.1.5 (Biomatters, Auckland, New Zealand) using DNA sequences from the complementary heavy and light strands. Sequences were edited in Geneious 8.1.5 (Kearse *et al.*, 2012) and assembled into FASTA files. A total of 37 previously published DNA sequences were used in this study from the following sources: Smith and Wheeler (2004), April *et al.* (2011), Betancur-R. *et al.* (2013), Smith and Busby (2014), Smith *et al.* (2016), Bentley and Wiley (unpublished), and Park *et al.* (unpublished). These sequences were combined with 55 novel DNA sequences for the analyses. For taxa with molecular data, the molecular matrix is 98% complete at the amplicon level and 94% complete at the individual base-pair level. One gene region was unable to be collected for *Icelinus fimbriatus* (ZIC1) and *I. quadriseriatus* (12S). Sequences analyzed in this study are listed in Table 1 in conjunction with GenBank accession numbers corresponding to the sequenced loci. The novel sequences were submitted to GenBank (accession numbers: KX353700–KX353754).

Four separate phylogenetic analyses were conducted in this study and analyzed in a maximum-likelihood framework: morphology-only or "morphological" analysis, DNA sequence data-only or "molecular" analysis, an analysis composed of a both morphological and molecular dataset or "combined" analysis, and an analysis

composed of all morphological and molecular data for species that had any DNA sequence data (i.e., excluding *Antipodocottus galatheae, Icelinus japonicus,* and *I. pietschi*) or "support" analysis. For the analyses that included molecular data, each of the five loci were individually aligned in MUSCLE (Edgar, 2004) using default values. The maximum-likelihood molecular dataset was broken into 13 partitions: one partition designated for the mitochondrial (12S, tRNA-Val, and 16S) fragment and 12 partitions designated for the three codon positions in each of the four protein coding genes: mitochondrial (COI) and nuclear (ENC1, TMO-4c4, and ZIC1). In the combined and morphology-only analyses, one partition was designated for the morphological dataset (Table 2). The optimal nucleotide substitution model for each molecular partition was determined empirically (Table 3) by comparing different models under an Akaike information criterion (AIC) as executed in jModelTest (Guindon and Gascuel, 2003; Darriba *et al.*, 2012). The maximum likelihood analyses were conducted in GARLI v2.01 (Zwickl, 2006), and the tree with the maximum likelihood score from 100 independent analyses was selected as the preferred hypothesis. A nonparametric maximum-likelihood bootstrap analysis was conducted for 500 random pseudoreplicates to assess nodal support in the support analysis. We recognize two levels of nodal support: 70% bootstrap support represents a moderately supported node or clade, and 95% bootstrap support represents a well-supported node or clade.

TABLE 3. PCR Primers, substitution models, and annealing temperatures for each amplicon analyzed in the current study.

Primer Name (Source)—Substitution model(s)	Primer Sequence	Primary Annealing Temperature (°C)
12S (Tang, 2001)—whole amplicon: GTR+I+G		
Phe2-L	5'-AAAGCATAACACTGAAGATGTTAAGATG-3'	47
12Sb-H	5'-AGGAGGGTGACGGGCGGTGTGT-3'	47
tRNA-Val-16S (Titus, 1992; Feller and Hedges, 1998)—whole amplicon: GTR+I+G		
12SL13-L	5'-TTAGAAGAGGCAAGTCGTAACATGGTA-3'	48
TitusI-H	5'-GGTGGCTGCTTTTAGGCC-3'	48
COI (Folmer *et al.*, 1994)—1st Pos.: TIM3+I; 2nd Pos.: TVM+I; 3rd Pos.: GTR+G		
LCO1490	5'-GGTCAACAAATCATAAAGATATTGG-3'	48
HCO2198	5'-TAAACTTCAGGGTGACCA- AAAAATCA-3'	48
TMO-4c4 (Streelman and Karl, 1997)—1st Pos.: HKY+I; 2nd Pos.: F81+I; 3rd Pos.: HKY+I		
TMO-f1	5'-CCTCCGGCCTTCCTAAAACCTCTC-3'	51
TMO-r1	5'-CATCGTGCTCCTGGGTGACAAAGT-3'	51
ENC1 (Li *et al.*, 2007)—1st Pos.: TIM2; 2nd Pos.: HKY; 3rd Pos.: K81uf+I+G		
ENC1_F85	5'-GACATGCTGGAGTTTCAGGA-3'	56
ENC1_R982	5'-ACTTGTTRGCMACTGGGTCAAA-3'	56
ZIC1 (Li *et al.*, 2007)—1st Pos.: K81uf+I + G; 2nd Pos.: F81; 3rd Pos.: K81uf+I		
ZIC1_F9	5'-GGACGCAGGACCGCARTAYC-3'	58
ZIC1_R967	5'-CTGTGTGTGTCCTTTTGTGRATYTT-3'	58

Results

The combined likelihood analysis (molecular and morphological data) resulted in a single optimal tree (Figure 3). Most nodes recovered in the support analysis were moderately to well supported with 11 nodes (85%) being supported by a bootstrap value ≥70% and five nodes (38%) being supported by a bootstrap value ≥95%. The combined analysis resulted in a polyphyletic *Icelinus*. Western Pacific species (*Icelinus japonicus* and *I. pietschi*) were recovered in a polytomy with *Stlengis misakia.* Eastern Pacific species of *Icelinus* were recovered as a clade most closely related to a clade consisting of *Furcina osimae* and *Antipodocottus galatheae*. Two additional

analyses, morphological and molecular, were conducted. Each of the additional analyses also resulted in a single optimal tree. The molecular analysis was completely congruent with the combined analysis with the exception of the three taxa that were not included due to lack of molecular data (*Antipodocottus galatheae*, *Icelinus japonicus*, and *I. pietschi*). The morphological analysis recovered a non-monophyletic *Icelinus* and *Radulinus asprellus, Furcina osimae,* and *Chitonotus pugentensis* were recovered within a clade of eastern Pacific species of *Icelinus.* Further, *Artedius fenestralis* and *Icelus spiniger* were recovered within a clade of western Pacific species of *Icelinus*.

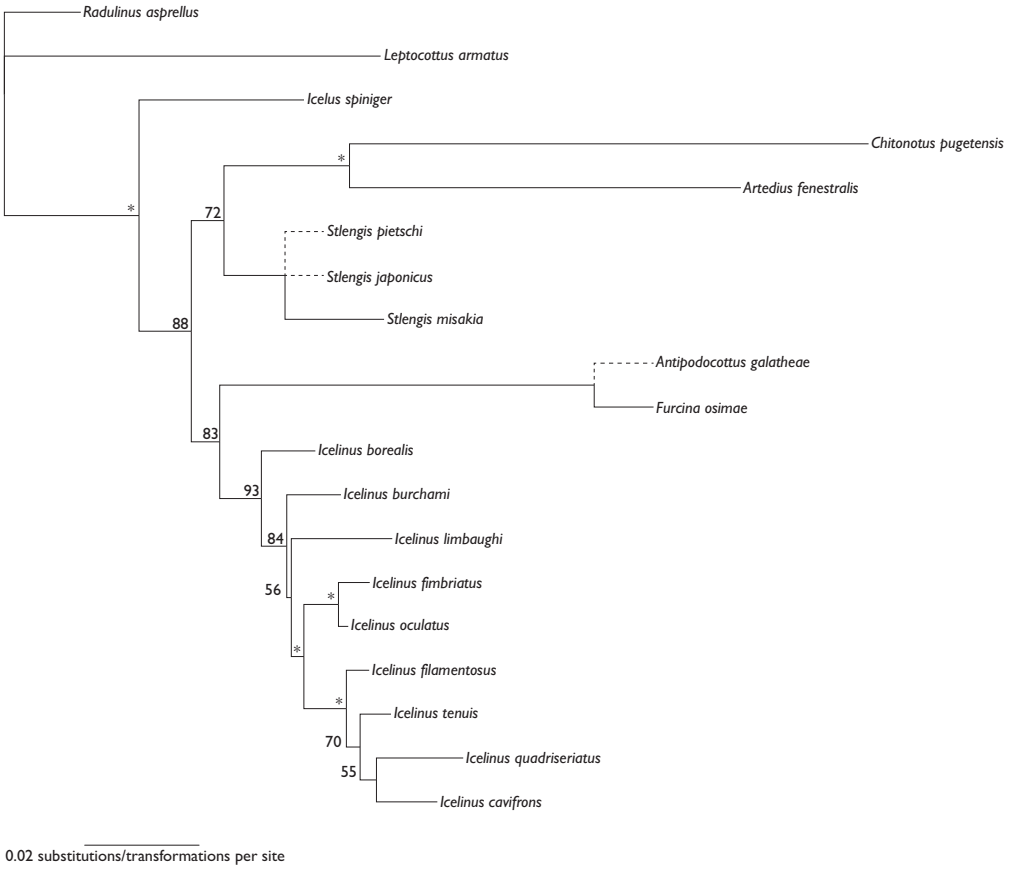

FIGURE 3. Optimal phylogeny from partitioned likelihood analysis of *Icelinus* and allies. Data set comprised of 24 soft and hard tissue characters and 3,790 molecular characters. Numbers above branches represent bootstrap resampling percentages (500 pseudoreplicates) of analyses of species with molecular and morphological data from the support analysis (>50%). Hatched bars indicate placement of species based on combined morphological and molecular analyses of all taxa, but that were excluded from the support analysis due to the lack sequence data. Nodes with resampling percentages ≥95% were marked with an "*".

Discussion

Our combined analysis recovers a polyphyletic *Icelinus.* One clade consists of only eastern Pacific species of *Icelinus*. Another clade consists of western Pacific species of *Icelinus*. Notably, the clade of *Furcina* and *Antipodocottus* is recovered as the sister to the eastern Pacific, and name-bearing, clade of *Icelinus*. This finding supports previous inferences that *Furcina* is sister to eastern Pacific species of *Icelinus* (Smith and Busby, 2014: figure 3). However, the recovery of an independent clade of western Pacific species of *Icelinus* is a novel hypothesis. Our finding that western Pacific species of *Icelinus* are sister to *Stlengis misakia* supports previous inferences (Yabe, 1985) that have recovered the genus *Icelinus* as sister to the genus *Stlengis* (despite that inference being made using eastern Pacific species of *Icelinus*). It is also worth noting that in both species descriptions of the western Pacific species of *Icelinus* and other studies on *Icelinus*, the authors were unable to place the new species

into Bolin's (1936) subgeneric classification due to discrepancies in the diagnostic morphological characters. (Yabe *et al*., 1981, 2001; Peden, 1984). Our hypothesis supports these describing authors' hesitation to group western Pacific species with eastern Pacific species of *Icelinus*, as they are not a monophyletic grouping.

As the eastern and western Pacific species of *Icelinus* do not form a monophyletic group, taxonomic changes are required to recognize that western Pacific species are separate and distinct from the eastern Pacific clade. We recommend the recognition of the former western Pacific species of *Icelinus* as members of the genus *Stlengis*: *S. japonicus* n. comb., and *S. piestchi* n. comb. It should be noted that molecular data were not available for all members of *Stlengis*. Given the rarity of the species in tissue collections, it was not possible to obtain molecular data for all three included species. The resulting clade of eastern Pacific *Icelinus* (*Icelinus* hereafter) is diagnosed by the presence of a double row of ctenoid scales between the dorsal fin and the lateral line that have a characteristic alternating toothed pattern (Figure 2).

Given these phylogenetic and taxonomic revisions, it was important to identify the sister group of *Icelinus* and determine whether the genus might be better classified within the Pseudoblenninae. Although *Icelinus* was not included within the traditional Pseudoblenninae (Taranets, 1941; Watanabe, 1960), it is notable that the genus was recovered as the sister group to this subfamily as first shown by Knope (2013). No molecular analyses, to date, have included *Velitor,* so its interrelationships have not been investigated with molecular data. Other recent analyses (Smith and Busby, 2014) on psychrolutids have also recovered *Icelinus* sister to the traditional Pseudoblenninae.

We recovered *Icelinus* sister to a clade of *Furcina*, a member of the traditional Pseudoblenninae, and *Antipodocottus*, a genus that has never been formally classified into any cottid or psychrolutid subfamily. Our finding corroborates the hypothesis of Knope (2013) and Smith and Busby (2014) that *Icelinus* is sister to the Pseudoblenninae. This result suggests that *Icelinus* should best be treated as a member of the subfamily Pseudoblenninae (Figure 3) rather than its own independent subfamily. Bolin (1952) and Nelson (1985) suggested that the southern hemisphere genus *Antipodocottus* was closely related to the north Pacific *Icelinus* based on their morphological examination. Despite this assertion, *Antipodocottus* has never been formally included in an explicit phylogenetic analysis, most likely due to the rarity of specimens. Based on our finding of *Furcina* and *Antipodocottus* sister to *Icelinus,* we recommend the following revised composition of the Pseudoblenninae: *Alcichthys, Antipodocottus, Argyrocottus, Bero, Crossias, Furcina, Icelinus, Ocynectes, Pseudoblennius,* and *Vellitor*. The current study and Yabe (1985) suggest that *Stlengis* should not be included in the Pseudoblenninae; however, a re-analysis of Yabe's (1985) matrix by Smith and Wheeler (2004: figure 4a) and Knope's (2013) phylogeny suggest that *Stlengis* and possibly *Atopocottus* might also belong in the Pseudoblenninae. Additional molecular and morphological work is needed to resolve the placement of these genera, the possible placement of these genera within Pseudoblenninae, and the subfamilial classification of psychrolutids generally.

Material examined

Comparative material examined, included the following ("cs" indicates cleared and stained material, "etoh" indicates alcohol preserved specimens that were examined whole): *Artedius fenestralis* SIO 63-1068, 4, etoh. *Chitonotus pugetensis* SIO H51-32, 17, 15 etoh, 2 cs. *Cottus bairdii* KU 15228, 14, 9 etoh, 5 cs. *Furcina osimae* HUMZ 40980, 1, cs. *Icelinus australis* USNM 41917, 1 (syntype), etoh. *Icelinus borealis* AMNH 2638, 32, etoh, CAS 102292, 1 (paralectotype), etoh, CAS 105045, 1 (syntype of *Icelinus strabo*), etoh, SIO 63-595, 2, etoh, SIO 76-299, 5, etoh, SIO 76-300, 3, cs, SIO 77-12, 2, etoh, USNM 53037, 6 (paralectotypes), etoh. *Icelinus burchami* SIO 97-123, 1, etoh, SIO 97-130, 2, etoh, SIO 97-132, 2, etoh, SIO 97-133, 1, cs, SIO 97-135, 2, etoh, USNM 57822, 1 (holotype), etoh, USNM 75812, 1 (holotype of *Icelinus fuscescens*), etoh. *Icelinus cavifrons* CAS 128111, 1 (syntype), etoh, SIO 48-217, 1, etoh, SIO 48-30, 3, etoh, SIO H48-306, 1, cs, SIO H51-260, 1, etoh, SIO 52-102, 6, etoh, SIO 62-381, 1, etoh, SIO 62-631, 1, etoh, SIO 76-300, 3, cs, USNM 44405, 2 (syntypes), etoh. *Icelinus filamentosus* CAS 100118, 1 (syntype), etoh, SIO 51-252-55A, 1, etoh, SIO 83-64, 3, etoh, SIO 83-68, 1, etoh, SIO 83-69, 3, etoh, SIO 83-82, 1 etoh, 4 cs, SIO 98-24, 1, etoh, USNM 44407, 1 (syntype), etoh. *Icelinus fimbriatus* SIO 94-130, 2, 1 etoh, 1 cs, SIO 97-130, 1, etoh, USNM 43087, 1 (syntype), etoh. *Icelinus limbaughi* LACM 56817.001, 1, etoh, SIO 51-253, 2 (paratypes), etoh, SIO 54-112, 18 (paratypes), etoh, SIO 62-628, 1 (holotype), etoh, SIO 62-673, 1 (paratype), cs. *Icelinus oculatus* CAS 051404, 1, etoh, CAS 100080, 1 (holotype), etoh, CAS

102559, 1, etoh, SIO 97-59, 2, 1 etoh, 1 cs, SIO 97-126, 1, etoh. *Icelinus quadriseriatus* SIO 60-468-55A, 8, etoh, SIO 60-471, 14, etoh, SIO 84-91, 2, cs, SIO 85-139, 5, etoh, USNM 23503, 2 (syntypes) etoh. *Icelinus tenuis* CAS 128110, 1 (syntype), etoh, SIO 66-4-55A, 2, etoh, SIO 83-15, 3, etoh, SIO 83-86, 1 etoh, 3 cs, SIO 85-58, 1, etoh, USNM 43086, 1 (syntype) etoh. *Icelus spiniger* SIO 76-299, 20, 19 etoh, 1 cs. *Leptocottus armatus* SIO 45-120a, 20, 19 etoh, 1 cs. *Radulinus asprellus* SIO 88-125, 5, 1 etoh, 4 cs. *Stlengis japonicus* HUMZ 77562, 1 (paratype), etoh. *Stlengis misakia* SIO 98-103, 2, 1 etoh, 1 cs. *Stlengis pietschi* HUMZ 151944, 1 (paratype), etoh. Whole specimens of *Antipodocottus galatheae* were unavailable but were coded based on Nelson (1985).

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APPENDIX 1.

Characters examined in the phylogenetic analysis. Data for characters 1–6 were taken from Yabe (1985) and expanded upon with data from the following sources: Begle, 1989; Bolin, 1944; Jackson, 2003; and Nelson, 1985. Data for characters 7–10 and 12–14 were taken from Bolin (1936) and expanded upon with data from the following sources: Begle, 1989; Bolin, 1944; Jackson, 2003; and Nelson, 1985. Data for characters 23–24 were taken from Jackson (2003) and expanded upon with data from the following sources: Bolin, 1944; Begle, 1989; Nelson, 1985; and Yabe, 1985.

1. Number of infraorbitals—shown in Fig. 3 (based in part on Yabe [1985] character 1):
   (1_0) = 5
   (1_1) = 4
2. Palatine teeth (based in part on Yabe [1985] character 16):
   (2_0) = Present
   (2_1) = Absent
3. Second pharyngobranchial tooth plate—shown in Fig. 3 (based in part on Yabe [1985] character 20):
   (3_0) = Absent
   (3_1) = Present
4. Number of soft rays in pelvic fin (based in part on Yabe [1985] character 31):
   (4_0) = 3 soft rays
   (4_1) = 2 soft rays
5. Anterior pterygiophore insertion (based in part on Yabe [1985] character 32):
   (5_0) = Second interneural space
   (5_1) = First interneural space
6. Characteristics of body scales (based in part on Yabe [1985] character 45):
   (6_0) = Ctenoid scales throughout
   (6_1) = Scales limited to dorsal & LL bands
7. Stegural:
   (7_0) = Absent
   (7_1) = Present
8. Elongate filamentous spine in dorsal fin—males (based in part on Bolin [1936]):
   (8_0) = Absent
   (8_1) = Present
9. Elongate filamentous spine in dorsal fin—females (based in part on Bolin [1936]):
   (9_0) = Absent
   (9_1) = Present
10. Pectoral axillary scales—shown in Fig. 3 (based in part on Bolin [1936]):
   (10_0) = Absent
   (10_1) = Present
11. Ornamentation of preopercular spine:
   (11_0) = Not antlered

(11_1) = Antlered

12. Dorsal scale band above lateral line—shown in Fig. 3 (based in part on Bolin [1936]):
    (12_0) = Absent
    (12_1) = Present
13. Dorsal scale band origin (based in part on Bolin [1936]):
    (13_0) = Origin at first dorsal element
    (13_1) = Origin posteriorly displaced
14. Dorsal scale band termination (based in part on Bolin [1936]):
    (14_0) = On caudal peduncle
    (14_1) = Not reaching caudal peduncle
15. Spines on frontal—shown in Fig. 3:
    (15_0) = Absent
    (15_1) = Present
16. Cirri on base of nasal:
    (16_0) = Absent
    (16_1) = Present
17. Penis noticeably enlarged:
    (17_0) = Absent
    (17_1) = Present
18. Spination on lateral line scales:
    (18_0) = Absent
    (18_1) = Present, lateral line scales have spines, teeth, or nodules on posterior margin
19. Canal at mandibular symphysis:
    (19_0) = 1 pore for both canals
    (19_1) = 1 pore per canal (two pores)
20. Spination on dorsal scales—shown in Fig. 3:
    (20_0) = Absent
    (20_1) = Present, dorsal scales have 1 or more spines or ctenii
21. Characteristics of dorsal scale spination:
    (21_0) = 1 large spine per scale
    (21_1) = Many small spines or ctenii per scale
22. Overall body physiognomy:
    (22_0) = Flattened
    (22_1) = Notably humped
23. Parietal extrascapular spine (based in part on Jackson [2003] character 14):
    (23_0) = Absent
    (23_1) = Present
24. Branchiostegal membrane connection to each other in relation to isthmus (based in part on Jackson [2003] character 59):
    (24_0) = Free
    (24_1) = Connected