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

MATTHEW G. GIRARD ${ }^{1,2} \&$ W. LEO SMITH ${ }^{1,3}$<br>${ }^{1}$ Biodiversity Institute and Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS 66045, USA. E-mail: mgirard@ku.edu ${ }^{2}$ leosmith@ku.edu ${ }^{3}$


#### 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).


Knope (2013)


Yabe (1985)


Smith \& Busby (2014)

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 $\mathrm{CO}_{2}$, 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 101), arrow (II). B) Scanning electron micrograph of dorsal scale band (Character 201) -Icelinus quadriseriatus (SIO 02-19). Arrow indicates 'toothed' nature of dorsal scales. C) Absence of frontal spine (Character 150)—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 8382), 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 $25 \mu \mathrm{~L}$ volume containing one Ready-To-Go PCR bead (GE Healthcare, Piscataway, NJ), $1.25 \mu \mathrm{~L}$ of each primer ( 10 pmol ), and $2-5 \mu \mathrm{~L}$ 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 temuis | 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 |
| :--- | :--- |
| Leptocottus armatus | $001001000-1---00000--001$ |
| Antipodocottus galatheae | $0001 ? 1000-1---0000011000$ |
| Artedius fenestralis | 001000000001110100111100 |
| Chitonotus pugetensis | $0010101110110011111--110$ |
| Furcina osimae | $000111000-0---00000--1 ? 0$ |
| Icelinus borealis | $00011010001100010000-010$ |
| Icelinus burchami | 000110100011110001111000 |
| Icelinus cavifrons | $00011011010111000010-110$ |
| Icelinus filamentosus | $00011011110111010010-110$ |
| Icelinus fimbriatus | 000110100011101110111110 |
| Icelinus limbaughi | $00011010000111000000-100$ |
| Icelinus oculatus | 00011010001110011111010 |
| Icelinus quadriseriatus | $00011010000110010000-010$ |
| Icelinus tenuis | $00011011110111100110-010$ |
| Icelus spiniger | 00000000111001000110110 |
| Radulinus asprellus | $111000100100--101110-000$ |
| Stlengis japonicus | $00 ? 1 ? 0 ? 00011000101111 ? 0$ |
| Stlengis misakia | 00010000001110000111000 |
| Stlengis pietschi | $00 ? 1 ? 0 ? 00011010101011100$ |

following temperature profile: initial denaturation for 6 min at $94^{\circ} \mathrm{C}$, denaturation for 60 s at $94^{\circ} \mathrm{C}$, annealing for 60 s at $46-53^{\circ} \mathrm{C}$ (see Table 3 for core annealing temperature for each locus), and extension for 75 s at $72^{\circ} \mathrm{C}$, with an additional terminal extension at $72^{\circ} \mathrm{C}$ 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 ( $\mathrm{ENC1}, \mathrm{TMO}-4 \mathrm{c} 4$, and $\mathrm{ZIC1}$ ). 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 (Zwick1, 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 wellsupported 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 $\left({ }^{\circ} \mathrm{C}\right)$ |
| :---: | :---: | :---: |
| 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-fl | 5'-CCTCCGGCCTTCCTAAAACCTCTC-3' | 51 |
| TMO-r 1 | 5'-CATCGTGCTCCTGGGTGACAAAGT-3' | 51 |
| ENC1 (Li et al., 2007)—1st Pos.: TIM2; 2nd Pos.: HKY; 3rd Pos.: K81uf $+\mathrm{I}+\mathrm{G}$ |  |  |
| 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 $\geq 70 \%$ and five nodes ( $38 \%$ ) being supported by a bootstrap value $\geq 95 \%$. 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.

0.02 substitutions/transformations per site

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 $\geq 95 \%$ 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):
$\left(1_{0}\right)=5$
$\left(1_{1}\right)=4$
2. Palatine teeth (based in part on Yabe [1985] character 16):
$\left(2_{0}\right)=$ Present
$\left(2_{1}\right)=$ Absent
3. Second pharyngobranchial tooth plate-shown in Fig. 3 (based in part on Yabe [1985] character 20):

$$
\left(3_{0}\right)=\text { Absent }
$$

$\left(3_{1}\right)=$ Present
4. Number of soft rays in pelvic fin (based in part on Yabe [1985] character 31):
$\left(4_{0}\right)=3$ soft rays
$(4)=2$ soft rays
5. Anterior pterygiophore insertion (based in part on Yabe [1985] character 32):
$\left(5_{0}\right)=$ Second interneural space
$\left(5_{1}\right)=$ First interneural space
6. Characteristics of body scales (based in part on Yabe [1985] character 45):
$\left(6_{0}\right)=$ Ctenoid scales throughout
$\left(6_{1}\right)=$ Scales limited to dorsal \& LL bands
7. Stegural:
$\left(7_{0}\right)=$ Absent
$\left(7_{1}\right)=$ Present
8. Elongate filamentous spine in dorsal fin-males (based in part on Bolin [1936]):
$\left(8_{0}\right)=$ Absent
$\left(8_{1}\right)=$ Present
9. Elongate filamentous spine in dorsal fin-females (based in part on Bolin [1936]):
$\left(9_{0}\right)=$ Absent
$(9)=$ Present
10. Pectoral axillary scales—shown in Fig. 3 (based in part on Bolin [1936]):
$\left(10_{0}\right)=$ Absent
$\left(10_{1}\right)=$ Present
11. Ornamentation of preopercular spine: $\left(11_{0}\right)=$ Not antlered
$\left(11_{1}\right)=$ Antlered
12. Dorsal scale band above lateral line-shown in Fig. 3 (based in part on Bolin [1936]):
$\left(12{ }_{0}\right)=$ Absent
$\left(12_{1}\right)=$ Present
13. Dorsal scale band origin (based in part on Bolin [1936]):
$\left(13_{0}\right)=$ Origin at first dorsal element
$\left(13_{1}\right)=$ Origin posteriorly displaced
14. Dorsal scale band termination (based in part on Bolin [1936]):
$\left(14_{0}\right)=$ On caudal peduncle
$\left(14_{1}\right)=$ Not reaching caudal peduncle
15. Spines on frontal-shown in Fig. 3:
$\left(15_{0}\right)=$ Absent
$\left(15_{1}\right)=$ Present
16. Cirri on base of nasal:
$\left(16_{0}\right)=$ Absent
$\left(16_{1}\right)=$ Present
17. Penis noticeably enlarged:
$\left(17_{0}\right)=$ Absent
$\left(17_{1}\right)=$ Present
18. Spination on lateral line scales:
$\left(18{ }_{0}\right)=$ Absent
$\left(18_{1}\right)=$ Present, lateral line scales have spines, teeth, or nodules on posterior margin
19. Canal at mandibular symphysis:
$\left(19_{0}\right)=1$ pore for both canals
$\left(19_{1}\right)=1$ pore per canal (two pores)
20. Spination on dorsal scales-shown in Fig. 3:
$\left(20_{0}\right)=$ Absent
$\left(20_{1}\right)=$ Present, dorsal scales have 1 or more spines or ctenii
21. Characteristics of dorsal scale spination:
$\left(21_{0}\right)=1$ large spine per scale
$\left(21_{1}\right)=$ Many small spines or ctenii per scale
22. Overall body physiognomy:
$\left(22_{0}\right)=$ Flattened
$\left(22_{1}\right)=$ Notably humped
23. Parietal extrascapular spine (based in part on Jackson [2003] character 14):
$\left(23_{0}\right)=$ Absent
$\left(23_{1}\right)=$ Present
24. Branchiostegal membrane connection to each other in relation to isthmus (based in part on Jackson [2003] character 59):
$\left(24_{0}\right)=$ Free
(24) $=$ Connected

