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Cottus schitsuumsh, a new species of sculpin (Scorpaeniformes: Cottidae) in the Columbia River basin, Idaho-Montana, USA

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Abstract

Fishes of the genus *Cottus* have long been taxonomically challenging because of morphological similarities among species and their tendency to hybridize, and a number of undescribed species may remain in this genus. We used a combination of genetic and morphological methods to delineate and describe *Cottus schitsuumsh*, Cedar Sculpin, a new species, from the upper Columbia River basin, Idaho-Montana, USA. Although historically confused with the Shorthead Sculpin (*C. confusus*), the genetic distance between *C. schitsuumsh* and *C. confusus* (4.84–6.29%) suggests these species are distant relatives. Moreover, the two species can be differentiated on the basis of lateral-line pores on the caudal peduncle, head width, and interpelvic width. *Cottus schitsuumsh* is also distinct from all other *Cottus* in this region in having a single small, skin-covered, preopercular spine. Haplotypes of mtDNA cytochrome oxidase *c* subunit 1 of *C. schitsuumsh* differed from all other members of the genus at three positions, had interspecific genetic distances typical for congeneric fishes (1.61–2.74% to nearest neighbors), and were monophyletic in maximum-likelihood trees. Microsatellite analyses confirmed these taxonomic groupings for species potentially sympatric with *C. schitsuumsh* and that fish used in morphological comparisons were unlikely to be introgressed. Its irregular distribution, in the Spokane River basin in Idaho and portions of the Clark Fork River basin in Montana, may have resulted from human-assisted translocation.

Key words: Cedar Sculpin, COI, Couer d'Alene River, DNA barcode, microsatellite, Shorthead Sculpin, St. Joe River

Introduction

North American freshwater fishes of the genus *Cottus* (Actinopterygii; Scorpaeniformes; Cottidae), commonly known as sculpins, have long been a taxonomic challenge (McAllister & Lindsey 1961; Kinziger *et al.* 2005). Sculpins are among the most difficult freshwater fishes to identify based on morphological characteristics (Jenkins & Burkhead 1994; Wydoski & Whitney 2003), a difficulty compounded by occasional hybridization between sympatric species (Strauss 1986; Markle & Hill 2000; Nolte *et al.* 2005) and geographic variation in phenotypically diagnostic characters within individual species (Maughan 1978; McPhail 2007). The limited movement and home ranges (< 250 m) exhibited by many fluvial sculpins (Petty & Grossman 2007; Hudy & Shiflet 2009) should favor speciation at relatively small geographic scales, yet the tendency has been to assign populations across broad geographical ranges to single species (e.g., *C. bairdii* in eastern and western North America; Jenkins & Burkhead 1994; Markle & Hill 2000). Recent morphological and genetic evaluations of sculpins in portions of the eastern U.S. have delineated several species of *Cottus* once thought to be part of more broadly distributed taxa (Neely *et al.* 2007; Kinziger & Wood 2010; Adams *et al.* 2013). Thus, it seems likely that diversity in *Cottus* elsewhere in North America has been underestimated.

One such region is the upper Columbia River basin in western North America. There is little consensus on the number and distribution of *Cottus* species in this basin, despite the efforts of several authors (McAllister & Lindsey 1961; Bailey & Bond 1963; Maughan 1978; Neely 2003; McPhail 2007). Particularly problematic has been delimiting the range of the Shorthead Sculpin, *C. confusus*. This species was described as occupying waters across

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the Columbia River basin (Bailey & Bond 1963), a range later expanded to coastal rivers in Washington (Wydoski & Whitney 2003). In the upper Columbia River basin, this species was believed to be distributed throughout the tributaries and upper main-stems of the Salmon and Clearwater Rivers in Idaho, with disjunct populations in the St. Joe River basin (and adjacent Coeur d'Alene River basin; Wallace & Zaroban 2013) in northern Idaho and the North and Middle Fork Flathead River basins in Montana (Bailey & Bond 1963). Presently, the sculpins of the upper Flathead River once thought to be *C. confusus* are recognized as belonging to a separate but as yet undescribed species (Neely 2003; McPhail 2007; COSEWIC 2010) on the basis of genetic and morphological differences. Recently, a broad-scale genetic inventory of fishes in the upper Columbia River basin provided evidence that some sculpins in tributaries of the Spokane River basin, which includes the St. Joe and Coeur d'Alene Rivers, constituted yet another species (Young *et al.* 2013). That observation led us to conduct further sampling and to search for morphological differences that would refute or confirm that hypothesis.

In this paper, we describe a new species of sculpin, *Cottus schitsuumsh,* Cedar Sculpin, from portions of northern Idaho and western Montana on the basis of meristic, morphological, and morphometric characteristics, diagnostic nucleotides in mtDNA sequences, genetic distances, reciprocal monophyly in phylogenetic trees, and a geographically restricted range.

Material and methods

We examined whole specimens of *Cottus* (n = 324) from 69 locations in northern Idaho, eastern Washington, and western Montana (Fig. 1). These sites included presumed locations of the new species and all neighboring *Cottus* species from the upper Columbia River basin, and were chosen based on the geographic distribution of haplotype groups (Young *et al.* 2013) and recognized species (Simpson & Wallace 1982; Holton & Johnson 2003; Wydoski & Whitney 2003). Samples were collected as part of this study or provided by the Orma J. Smith Museum (College of Idaho, Caldwell; CIDA), the Burke Museum (University of Washington, Seattle; UW), the Salmon-Challis National Forest, or the Washington Department of Fish and Wildlife.

We conducted meristic counts following Robins and Miller (1957) and McAllister (1964), and most morphological assessments and morphometric measurements following Bailey and Bond (1963). An exception was that head length was measured ventrally from the anterior edge of the head to the point where the gill membranes attached to the isthmus. Meristic counts were made for both sides of paired structures. Asymmetry was rare, so we report right-side counts following Maughan (1974), except for the number of palatine tooth rows which were described for both sides. We also noted lateral-line pore presence on either side of the caudal peduncle and measured the distance between the proximal pelvic-fin rays (interpelvic distance). Measurements were made to the nearest 0.1 mm with calipers. To evaluate phenotypic differences between the new species and others, we compared minimum, maximum, and modal counts of meristic characters. We used ANCOVA, with standard length (SL) as the covariate, to compare most morphometric measures among species. For mouth width, we used head width as the covariate. We used Scheffé multiple-comparison tests for pairwise comparisons among species. Specimens of the new species from the Spokane and Clark Fork River basins were pooled because pairwise comparisons between the two populations were not significant for any morphometric measure. The specimens we examined were fixed in a 10% formalin solution or in 70 or 95% ethanol, and stored in 70% ethanol. Although different preservation techniques sometimes influence morphometry, our comparisons of specimens fixed with different methods did not reveal morphometric differences (data not shown).

Tissue samples (pelvic fin clips) were taken from a subset of the whole-body specimens fixed in alcohol (n = 63) for genetic analysis. These were supplemented with tissue samples (n = 378) collected from *Cottus* in western Montana and northern and southeastern Idaho (Young *et al.* 2013; M. Young, B. Gamett, and E. Keeley, unpubl. data). We extracted DNA from samples using the DNeasy Blood and Tissue kit (Qiagen Inc., Hilden, Germany) following the manufacturer's instructions for tissue. To delineate and identify potential species (Hebert *et al.* 2003; April *et al.* 2011), we amplified the cytochrome *c* oxidase 1 (COI) region of mitochondrial DNA (mtDNA) using primers *FF2d* and *FR1d* (Ivanova *et al.* 2007). Reaction volumes of 50 µl contained 50–100 ng DNA, 1× reaction buffer (Life Technologies, CA, USA), 2.5 mM $MgCl_2$, 200 µM each dNTP, 1 µM each primer, 1 U Taq polymerase (Life Technologies). The PCR program was 94 °C/5 min, [94 °C /1 min, 55 °C /1 min, 72 °C/1 min 30s] × 34 cycles, 72 °C /5 min. The quality and quantity of template DNA were determined by 1.6% agarose gel electrophoresis. PCR products were purified using ExoSap-IT (Affymetrix-USB Corporation, OH, USA) according to manufacturer's instructions. We used the Big Dye kit and the 3700 DNA Analyzer (ABI; High Throughput Genomics Unit, WA, USA) to obtain DNA sequences, which were viewed and aligned with Sequencher (Gene Codes Corporation, MI, USA).

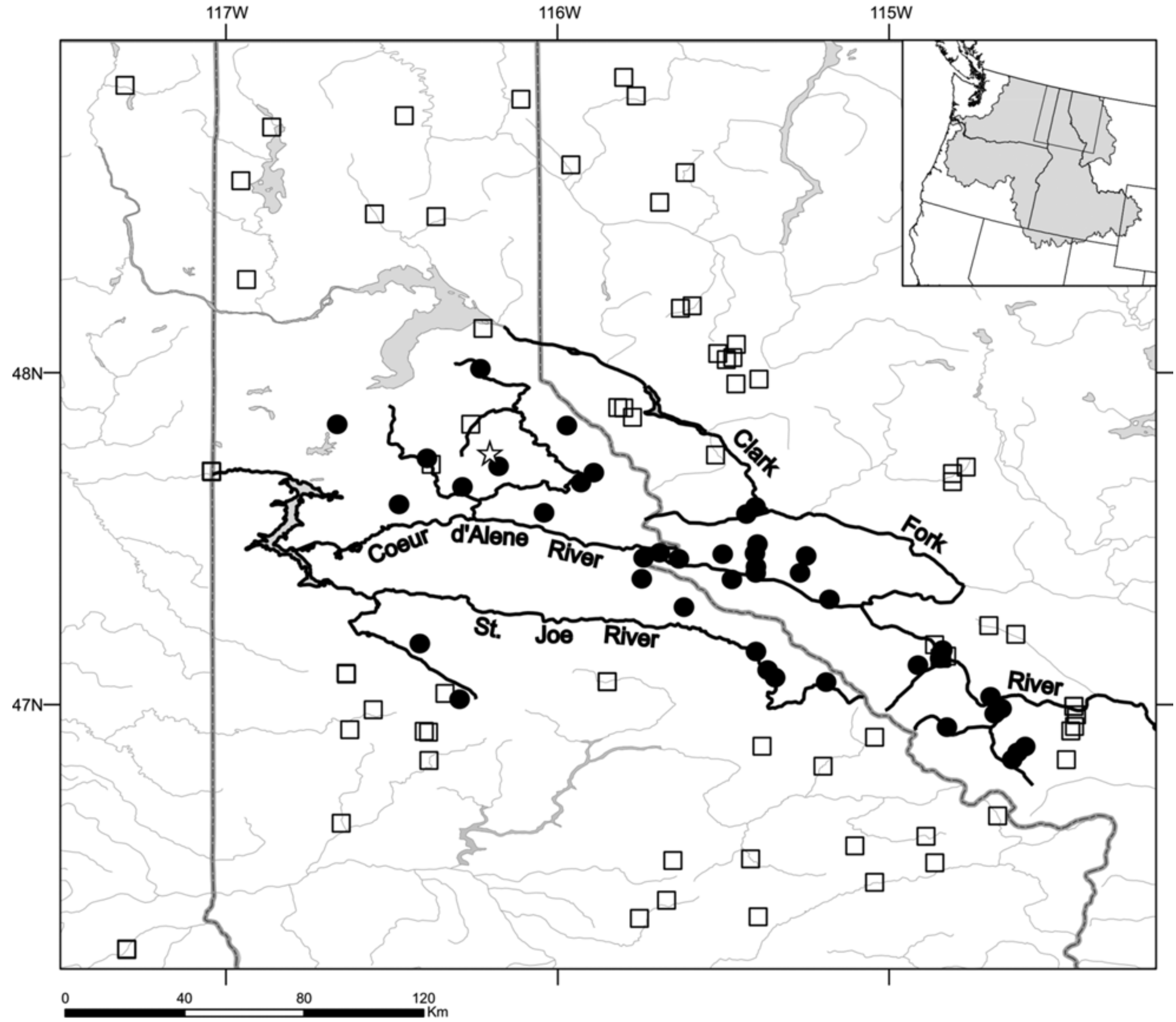

FIGURE 1. Distribution of specimens of *Cottus schitsuumsh* (filled circles; type location, unfilled star) examined within the upper Spokane River (the Couer d'Alene and St. Joe rivers) in Idaho and the Clark Fork River in Montana. Locations of specimens of other *Cottus* species examined from adjacent basins are depicted (unfilled squares); others beyond this area are noted in the text. Inset: Columbia River basin in the United States.

We used diagnostic nucleotide characters (DeSalle *et al.* 2005), genetic distances, and tree-based phylogenies derived from COI sequences to assist with species diagnosis. We analyzed two sets of sequences representing 33 recognized species (including the species described herein): 1) the 104 distinct haplotypes among all accessions (*n* = 283) of COI sequences for *Cottus* in GenBank (accessed 5 May 2013); and 2) 44 COI haplotypes of *Cottus* collected as part of a comprehensive inventory of streams in the upper Columbia and Missouri River basins (Young *et al.* 2013) or detected among specimens examined for this study. Sequences of a number of North American species are not currently available, but all represent taxa from eastern North America or the *Cottopsis* clade (Kinziger *et al.* 2005) that are unlikely to be confused with the species we examined. Diagnostic nucleotides consisted of pure and conditional characters. Pure diagnostic characters were those nucleotide-position combinations unique to all haplotypes of the new species globally or in North America. Conditional diagnostic characters were those nucleotide-position combinations that were present in one of the haplotypes of the new species but were not observed in any other species (Wong *et al.* 2009). We used MEGA 5.1 (Tamura *et al.* 2011) to construct an outgroup-rooted neighbor-joining phylogenetic tree of all *Cottus* haplotypes based on uncorrected *p*-distances (rather than Kimura 2-parameter distances; Collins *et al.* 2012; Srivathsan & Meier 2012). We used *Leptocottus armatus* as the outgroup (Kinziger *et al.* 2005). We built the tree to display genetic distances among taxa and to assess whether the new species was reciprocally monophyletic from all recognized species available in GenBank.

To provide evidence that hybridized individuals of the new species were not included in morphological or mtDNA analyses, we analyzed microsatellites of the new species and potentially sympatric taxa. This included morphologically examined specimens of the new species (n = 28), as well as tissue samples from representatives from the rest of its putative range (n = 24). Potentially sympatric species were represented by tissues and whole-body specimens of *C. rhotheus* (n = 14) and *C. cognatus* (n = 65) from northern Idaho and western Montana. We analyzed 11 microsatellite loci used in previous studies on sculpin (Englbrecht *et al.* 1999; Fiumera *et al.* 2002; Nolte *et al.* 2005): *Cott100*, *Cott113*, *Cott130*, *Cott255*, *Cott687*, *CottES10*, *CottES19*, *Cba42*, *Cgo33*, *Cgo310*, and *Cgo1114*. The reaction volume (10 µl) contained 1.0µL DNA, 1x reaction buffer (Life Technologies), 2.0 mM $MgCl_2$, 200µM of each dNTP, 1µM reverse primer, 1µM dye-labeled forward primer, 1.5 mg/ml BSA, and 1U *Taq* polymerase (Life Technologies). The PCR profile was 94°C/5 min, [94°C/1 min, 55°C/1 min, 72°C/30s] x 36 cycles). The resultant products were visualized on a LI-COR DNA analyzer (LI-COR Biotechnology). We inspected principal coordinate analysis (PCoA) plots generated using GenAlEx (Peakall and Smouse 2006) to gauge whether introgressed specimens were present.

Results

Genetic evidence supports recognition of a sculpin from tributaries of the Spokane River basin in Idaho, as well as in the middle Clark Fork River basin in Montana, as a distinct taxon (cf. Young *et al.* 2013). Based on a sample of 91 individuals, this new species was represented by three COI haplotypes throughout its range: haplotype 1 in the Coeur d'Alene River basin in Idaho and the middle Clark Fork River basin in Montana, haplotype 2 in most of the St. Joe River basin in Idaho, and haplotype 3 in the St. Maries River basin (tributary to the St. Joe River) in Idaho (Fig. 2). The maximum intraspecific genetic distance among haplotypes of this species (0.65%) was less than the minimum interspecific distances to its three nearest neighbors (*C. cognatus* (ID-MT), 1.61–2.26%; *C. cf. bairdii* (MT), 1.77–2.74%; and *C. caeruleomentum*, 1.77–2.74%). These interspecific distances are typical of minimum differences among congeneric fishes (Ward 2009). The genetic distance between the new species and *C. confusus* (mean, 5.52%, range 4.84–6.29%) and their positions on the neighbor-joining tree imply that these constitute separate species that are not sister to one another. The new species was reciprocally monophyletic with respect to all other sculpin taxa, despite evidence for substantial paraphyly and polyphyly in the phylogenetic tree for many other species. This indicates ambiguity in the taxonomy of *Cottus*, large numbers of misidentified species, or the influence of introgression (Kinziger *et al.* 2005; April *et al.* 2011; Young *et al*. 2013), none of which altered the distinctiveness of the new species. Furthermore, six nucleotide positions were globally or continentally diagnostic for the new species (Table 1).

Microsatellite data distinguished between the new species and its two potentially sympatric congeners (*C. rhotheus* and *C. cognatus*), with no evidence of intermediate haplotypes suggestive of introgression. The first two axes of the PCoA scatterplot accounted for 70% of the variation in the data (axis 1, 53.25%; axis 2, 16.76%) and produced three well-separated groups (Fig. 3).

***Cottus schitsuumsh*, new species**
Cedar Sculpin

Holotype. UW 151670, male, 91 mm SL, haplotype group 1 (JX311895), East Fork Steamboat Creek, a tributary of the Coeur d'Alene River at West Fork Road (USFS Road 409) (47.71821°N, 116.20010°W), Shoshone County, Idaho, 29 September 2011.

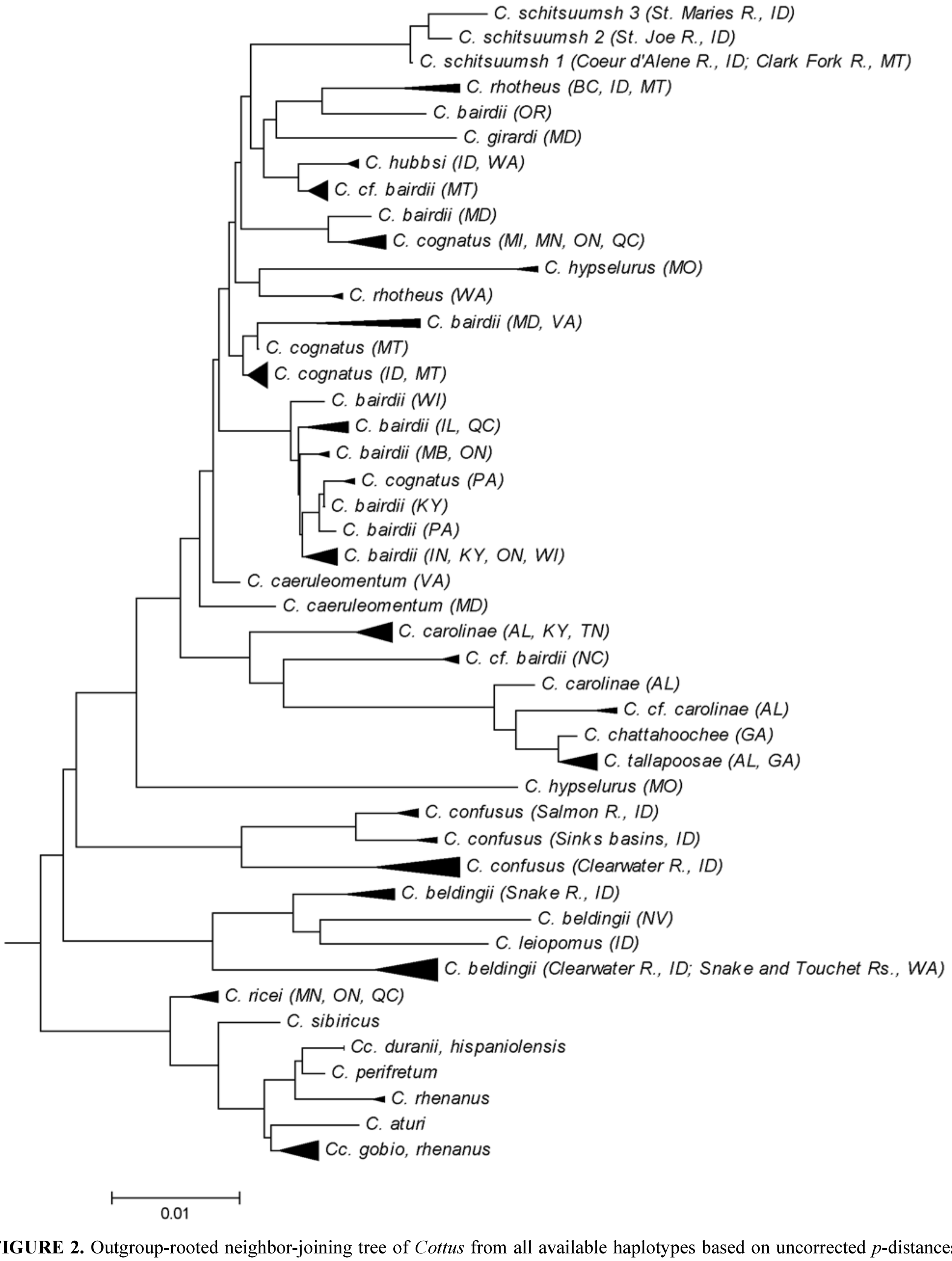

FIGURE 2. Outgroup-rooted neighbor-joining tree of *Cottus* from all available haplotypes based on uncorrected *p*-distances for cytochrome *c* subunit 1 sequences. State and province abbreviations are given for North American samples, and river basins are noted for *C. beldingii*, *C. confusus*, and *C. schitsuumsh* in Idaho and Montana. *Cottus poecilopus*, *C. reinii*, those in the *Cottopsis* clade (Kinziger *et al.* 2005), and the outgroup *Leptocottus armatus* are not shown.

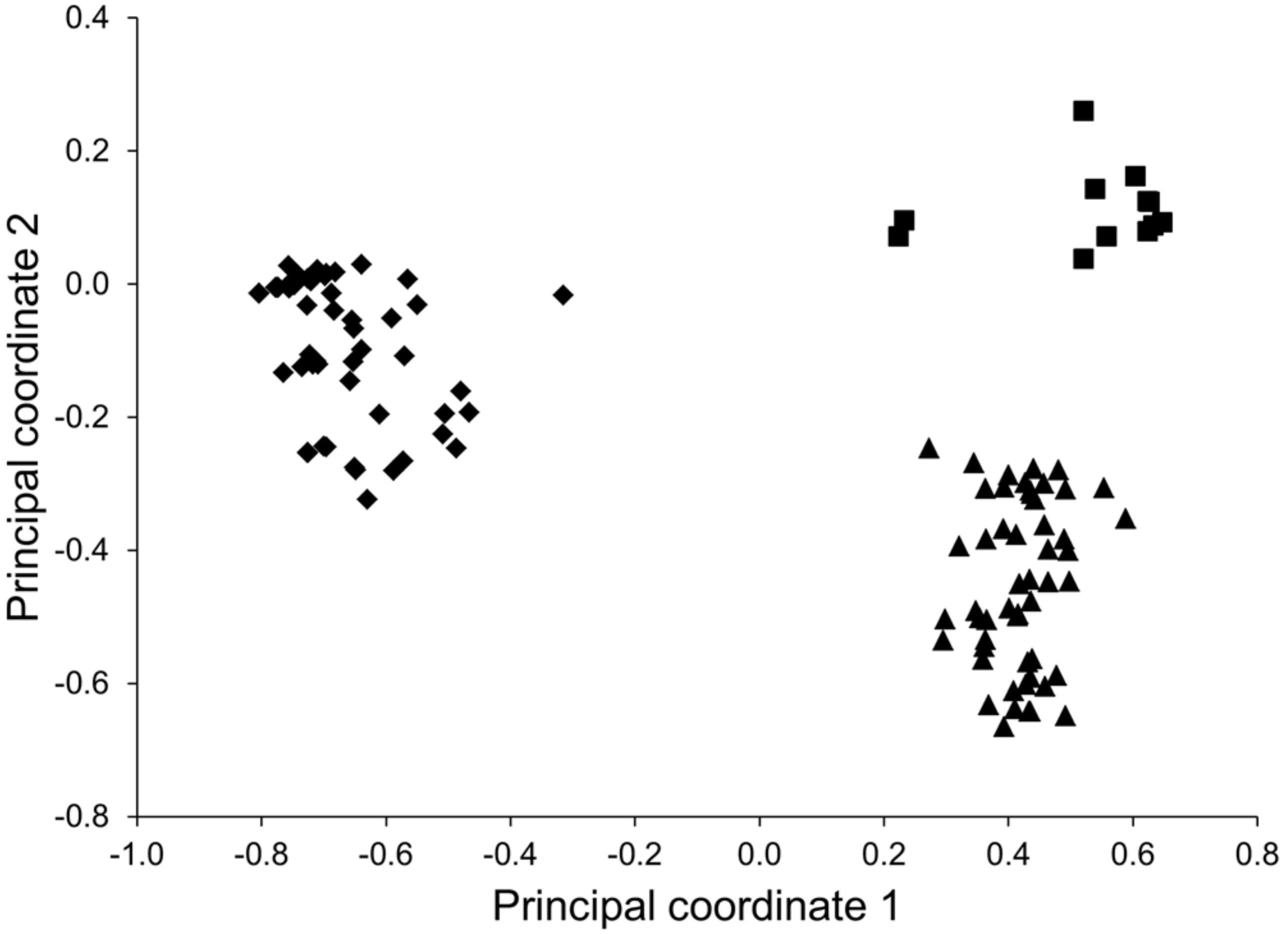

FIGURE 3. Plot of the first two principal coordinates based on 11 microsatellite loci for *Cottus schitsuumsh* (triangles) and potentially sympatric *C. cognatus* (diamonds) and *C. rhotheus* (squares).

Paratypes. UW 151671, 2 males and 1 female, 57–73 mm SL, haplotype group 1 (JX311896), CIDA 101,703, 2 males and 1 female, 72–99 mm SL, collected with the holotype.

Additional materials (nontypes, examined morphologically and genetically). Idaho, Shoshone County: (5, 66–81 mm SL, haplotype group 2, JX311919) Bird Creek (47.27235°N, 115.60885°W), 29 September 2011; (5, 68–80 SL, haplotype group 1, JX311898) Leiberg Creek (47.72100°N, 116.38335°W), 6 September 2011; (5, 64–88 mm SL, haplotype group 2, JX311921) Red Ives Creek (47.06016°N, 115.33448°W), 29 September 2011; (5, 69–96 mm SL, haplotype group 1, JX311902) West Fork Steamboat Creek (47.71718°N, 116.20380°W), 7 September 2011. Tissue samples (and extracted DNA) are archived at the Wildlife Genetics Laboratory, Rocky Mountain Research Station, Missoula, MT.

Additional materials (nontypes, examined morphologically). Idaho, Benewah County: (2, 50–70 mm SL, CIDA68425) Renfro Creek (47.16278°N, 116.40462°W), 11 July 1996. **Idaho, Shoshone County**: (5, 69–84 mm SL) Beaver Creek (47.08260°N, 115.35738°W), 29 September 2011; (1, 62 mm SL, CIDA69113) Buckskin Creek (47.99148°N, 116.22247°W), 24 July 1997; (2, 71–86 mm SL, CIDA68475) Eagle Creek (47.64813°N, 115.91920°W), 23 August 1996; (5, 71–83 mm SL) East Fork Steamboat (47.71821°N, 116.20010°W), 29 September 2011; (5, 72–79 mm SL) Leiberg Creek (47.72100°N, 116.38335°W), 6 September 2011; (10, 44–95 mm SL) Steamboat Creek (47.69685°N, 116.167867°W), 18 October 2011; (4, 70–97 mm SL) West Fork Steamboat Creek (47.71718°N, 116.2038°W), 18 October 2011; (1, 73 mm SL, UW 152969) West Fork Steamboat Creek (47.71718°N, 116.2038°W), 7 September 2011. **Montana, Mineral County**: (5, 48–62 mm SL) Fish Creek (46.951°N, 114.67359°W), 12 October 2011; (5, 50–76 mm SL) Fish Creek (46.96693°N, 114.65292°W), 12 October 2011; (5, 45–67 mm SL) St. Regis River (47.43131°N, 115.74357°W), 15 October 2011; (5, 48–86 mm SL) Trout Creek (47.09768°N, 114.90475°W), 12 October 2011; (5, 51–67 mm SL) Trout Creek (47.13611°N, 114.85036°W), 12 October 2011; (5, 51–91 mm SL) Trout Creek (47.14311°N, 114.83298°W), 12 October 2011;

(6, 49–83 mm SL) Twomile Creek (47.295958°N, 115.171311°W), 12 October 2011. **Montana, Sanders County**: (8, 53–77 mm SL) Prospect Creek (47.57452°N, 115.39381°W), 15 October 2011.

Additional materials (nontypes, examined genetically). Haplotype group 1. **Idaho, Kootenai County**: (2, JX282597) 4th of July Creek (47.58208°N, 116.46721°W); (2, JX311897) Hayden Creek (47.82378°N, 116.65348°W). **Idaho, Shoshone County**: (2, JX311893) Bumblebee Creek (47.63522°N, 116.27645°W); (2, JX311899) Moon Creek (47.55719°N, 116.03062°W); (2, JX311900) Rampike Creek (47.81965°N, 115.96189°W); (2, JX311901) West Fork Eagle Creek (47.67772°N, 115.88087°W). **Montana, Mineral County**: (2, JX311903) Big Creek (47.37580°N, 115.39283°W). (1, JX311904) Burdette Creek (46.81331°N, 114.62097°W). (1, JX311905) Burdette Creek (46.83436°N, 114.60488°W). (2, JX311907) East Fork Packer Creek (47.43260°N, 115.49248°W). (2, JX311908) Savenac Creek (47.39438°N, 115.39210°W). (1, JX311909) Savenac Creek (47.43467°N, 115.39532°W). (1, JX311910) Savenac Creek (47.46251°N, 115.38812°W). (2, JX311911) St. Regis River (47.41834°N, 115.62450°W). (1, JX311912) St. Regis River (47.43131°N, 115.74357°W). (1, JX311913) St. Regis River (47.43675°N, 115.68220°W). (2, JX311914) Straight Creek (46.91092°N, 114.81761°W). (2, JX311915) Twelvemile Creek (47.37603°N, 115.25982°W). (1, JX311915) Twelvemile Creek (47.42681°N, 115.24120°W). **Montana, Sanders County**: (2, JX311917) Wilkes Creek (47.55313°N, 115.42111°W). Haplotype group 2. **Idaho, Shoshone County**: (2, JX282598) Beaver Creek (47.08314°N, 115.35790°W). (2, JX311918) Bird Creek (47.27229°N 115.60893°W). (2, JX311920) North Fork St. Joe River (47.35768°N, 115.73658°W). (2, JX311922) Simmons Creek (47.13888°N, 115.39229°W). (2, JX311923) St. Joe River (47.04652°N, 115.18094°W). Haplotype group 3. **Idaho, Shoshone County**: (2, JX282599) Bechtel Creek (46.99509°N, 116.28479°W). Tissue samples (and extracted DNA) are archived at the Wildlife Genetics Laboratory, Rocky Mountain Research Station, Missoula, MT. See Young *et al.* (2013; table S1) for additional materials used in this analysis.

Morphological diagnosis. *Cottus schitsuumsh* is diagnosed from all other *Cottus* in the upper Columbia River basin by the lack of a second preopercular spine, palatine tooth patches smaller and separate from the vomerine tooth patch, and axillary prickling.

Cottus schitsuumsh is distinguished from *C. cognatus*, *C. confusus*, *C. hubbsi*, and *C. rhotheus* by the lack of a second preopercular spine. *Cottus schitsuumsh* is distinguished from *C. cf. bairdii* and *C. beldingii* by the presence of axillary prickling. In addition, *C. schitsuumsh* has palatine tooth patches smaller than the vomerine tooth patch and the patches are separated. *Cottus cf. bairdii* has palatine tooth patches equal to or larger in size than the vomerine tooth patch. *Cottus beldingii* generally lacks palatine teeth.

Molecular diagnosis. Haplotypes of *C. schitsuumsh* differed from those of all other members of the genus *Cottus* (represented in GenBank or in this study) at three positions (Table 1). Additionally, they differed from all other North American *Cottus* haplotypes at an additional position. Nucleotides at two other positions were diagnostic for individual haplotypes.

TABLE 1. Diagnostic characters for *Cottus schitsuumsh* in the mtDNA cytochrome *c* oxidase subunit 1 (COI) region.

Position[1]	Nucleotide	Diagnostic for[2]
5577	T	*C. schitsuumsh* haplotype 3
5664	G	*C. schitsuumsh* in North America
5685	T	*C. schitsuumsh* haplotype 2
5868	T	*C. schitsuumsh*
6081	T	*C. schitsuumsh*
6120	T	*C. schitsuumsh*

[1]Based on *C. reinii* mitome (GenBank accession AP004442; Miya *et al.* 2003).

[2]Relative to all *Cottus* haplotypes in GenBank (n = 104 from 283 accessions) and from *Cottus* collected throughout the upper Columbia and Missouri River basins (Young *et al.* 2013) and in this study (n = 44).

Description. *Cottus schitsuumsh* is a moderately sized species of *Cottus* rarely exceeding 90 mm SL with anal fin rays 14 (rarely 13 or 15), the final posterior anal-fin ray commonly branched; dorsal-fin spines 8 (rarely 7 or 9); dorsal-fin rays 17–20 (usually 18–19) with the posterior dorsal-fin ray branched; pectoral-fin rays 13–14 (usually 14); and pelvic-fin rays 4 (rarely 3) and of similar length. There are 2 medial-chin pores, 10–12 preoperculomandibular pores, and 2 postmaxillary pores (rarely 0). Dorsal fins are generally not connected but occasionally have a slight connection at the base. Prickling is consistent and restricted to a trapezoidal area, widest in the axillary region and narrowest along the lateral line under the beginning of the second dorsal fin. Palatine teeth are present in two or fewer rows and sometimes on only one side. The palatine tooth patches are smaller than and separated from the vomerine tooth patch and generally equal if present on both sides. The lateral line is incomplete, ending below the latter half of the second dorsal fin. One preopercular spine was observed among all specimens. Preopercular spines are greatly reduced and skin-covered. The mouth width/head width fraction ranges from ½ to 2/3.

Morphometrics of the holotype (91 mm SL, male) and 3 paratypes (57–73 mm SL, 2 males and 1 female) as a percentage of SL (mean and range): caudal peduncle depth (8.8, 8.1–10.6); caudal peduncle length (13.9, 12.3–17.4); head length (19.7, 16.8–23.4); head width (26.8, 21.6–34.3); lateral line length (59.5, 51.3–70.6); snout length (9.0, 6.5–17.4); mouth width (14.2, 12.9–15.9); interpelvic width (3.5, 2.3–4.6); longest pectoral ray (24.3, 21.2–33.7), longest pelvic ray (18.5, 15.1–24.4).

Mottling of *C. schitsuumsh* is variable and consists of at least two forms. One has black-brown mottling on a brown-olive background mainly above the lateral line that stops under the soft dorsal fin (Fig. 4), and weak saddling is occasionally present (Fig. 5). The pectoral fins have faint black-brown contours running vertically. The other form lacks mottling or spotting over the body and the background color can be light brown, rust orange, or yellow. Both types have a dark blotchy marking near the base of the caudal rays and the first dorsal fin is generally dorsally darkened. Both forms are sympatric in the upper Spokane River basin, Idaho, but the mottled form has only been observed in Montana.

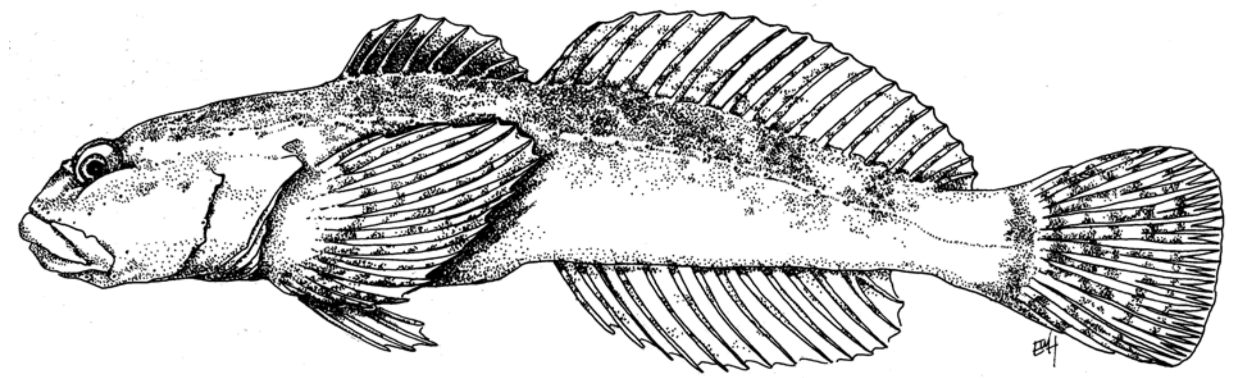

FIGURE 4. Illustration of the holotype of *Cottus schitsuumsh.*

Comparisons. *Cottus schitsuumsh* tended to have more anal fin rays (mode, 14 rays) than the other species (modes, 12 or 13 rays) among our samples (Table 2) and relative to other sculpins in this region (Wydoski and Whitney 2003). Pelvic ray counts are similar among the observed *Cottus* species, but *C. cognatus* commonly had a greatly reduced fourth pelvic-fin ray (cf. Wydoski & Whitney 2003). *Cottus rhotheus* has more pectoral rays than *C. schitsuumsh*. Counts of medial-chin pores did not differ among species, although *C. schitsuumsh* tended to have more preoperculomandibular pores than other species and more postmaxillary pores than *C. cf. bairdii*, *C. beldingii*, and *C. cognatus* (Table 3). *Cottus schitsuumsh* can be further distinguished from *C. cognatus* and *C. beldingii* by the presence of palatine teeth (Table 4). The palatine tooth patch of *C. schitsuumsh* consists of two or fewer rows, may occur on only one side, and is about ½ the size of the vomerine tooth patch. The palatine tooth patch is similar to or larger than the vomerine tooth patch in *C. rhotheus, C. hubbsi*, and *C. cf. bairdii*. *Cottus schitsuumsh* also differs from *C. hubbsi* and *C. rhotheus* by having an incomplete lateral line.

Originally confused with *C. confusus* (Bailey & Bond 1963), *C. schitsuumsh* can be distinguished from it by having only one preopercular spine that is small (length less than half the eye diameter) and skin-covered (Fig. 6). Most specimens of *C. schitsuumsh* also lack lateral-line pores on the caudal peduncle (Fig. 7). Pores along the lateral line of *C. confusus*, usually end beneath the latter half of the second dorsal fin and reappear at the end of the caudal peduncle near the origin of the caudal rays.

TABLE 2. Range (mode) for counts of *Cottus* fin spines and rays examined in this study. Samples sizes are noted after each sampling location or species.

Species	Dorsal spines	Dorsal rays	Anal rays	Pelvic rays	Pectoral rays
Spokane River basin (*n* = 64)	7–9 (8)	17–19 (18)	13–15 (14)	3–4 (4)	12–14 (14)
Clark Fork River basin (*n* = 44)	7–9 (8)	17–19 (18)	12–14 (14)	3–4 (4)	12–15 (14)
C. beldingii (*n* = 39)	7–8 (7)	16–18 (17)	11–13 (12)	3–4 (4)	13–15 (14)
C. cf. bairdii (*n* = 10)	6–8 (8)	16–19 (17)	12–14 (13)	4	13–15 (14)
C. cognatus (*n* = 73)	7–9 (8)	15–19 (18)	10–13 (12)	3–4 (4)[1]	12–14 (13)
C. confusus (*n* = 49)	7–9 (8)	15–19 (17)	10–14 (13)	4	11–15 (13)
C. hubbsi (*n* = 19)	7–8 (8)	15–18 (17)	12–14 (13)	4	13–16 (15)
C. rhotheus (*n* = 26)	7–9 (8)	15–18 (16)	11–14 (12)	4	15–17 (16)

[1]Fourth ray in the pelvic fin of *C. cognatus* reduced (ca. ½ of other rays; cf. Wydoski & Whitney 2003).

TABLE 3. Range (mode) of pore counts for *Cottus* examined in this study.

Species	Medial chin pores	Preoperculomandibular pores	Postmaxillary pores
Spokane River basin (*n* = 64)	(2)	10–12 (12)	0–2 (2)
Clark Fork River basin (*n* = 44)	(2)	10–12 (12)	0–2 (2)
C. beldingii (*n* = 39)	(2)	10–12 (11)	(2)
C. cf. bairdii (*n* = 10)	(2)	10–11 (11)	0–2 (1)[1]
C. cognatus (*n* = 73)	(2)	10–12 (10)	0–1 (0)
C. confusus (*n* = 49)	(2)	10–12 (11)	1–2 (2)
C. hubbsi (*n* = 19)	(2)	10–12 (11)	1–2 (2)
C. rhotheus (*n* = 26)	(2)	10–12 (11)	1–2 (2)

[1]Postmaxillary pores for *C. cf. bairdii* were consistently in a lower position compared to that of other species.

TABLE 4. Additional meristic and morphological characters of *Cottus* examined in this study.

Species	Preopercular spines	Prickling	Palatine teeth	Lateral line pores on caudal peduncle
Spokane River basin (*n* = 64)	1	Consistent and restricted to a trapezoidal area, widest in the axillary region and narrowest along the lateral line	1–2 rows on 1–2 sides	No (59), yes (5)
Clark Fork River basin (*n* = 44)	1	Consistent and restricted to a trapezoidal area, widest in the axillary region and narrowest along the lateral line	1–2 rows on 1–2 sides	No (40), yes (4)
C. beldingii (*n* = 39)	1–2 (1)	Absent	Absent	No
C. cf. bairdii (*n* = 10)	1–2 (2)	Generally absent; some prickles (1–7) can be present axial to the pectoral fin	≥ 2 rows on both sides	No
C. cognatus (*n* = 73)	2–3 (2)	Generally absent; occasionally small patches in the axillary region	Absent	No
C. confusus (*n* = 49)	2–3 (2)	Consistent and restricted to a trapezoidal area, widest in the axillary region and narrowest along the lateral line	1–2 rows on 1–2 sides	No (3), yes (46)
C. hubbsi (*n* = 19)	3	Large patch under pectoral fins, scattered elsewhere	≥ 2 rows on both sides	Yes
C. rhotheus (*n* = 26)	3–4 (3)	Covers most of the body	≥ 2 rows on both sides	Yes

FIGURE 5. Dorsal, lateral, and ventral views of a 73 mm SL male *Cottus schitsuumsh* (UW 152969) from West Fork Steamboat Creek (47.71718°N, 116.2038°W), Shoshone County, Idaho, 7 September 2011. A pelvic fin was removed for genetic sequencing. Photographs by Zachary Randall.

Most morphometric features separated *C. schitsuumsh* from one or more of the *Cottus* species. Caudal-peduncle depth, head length, mouth width, and interpelvic width statistically differed more often between *C. schitsuumsh* and the other species than did other measures (Table 5). Frequency distributions of these features emphasized these differences (Tables 6, 7). With respect to *C. confusus*, *C. schitsuumsh* differed by having a shorter lateral line, wider mouth, longer snout, and narrower interpelvic width.

TABLE 5. Mean values for 11 morphometric characteristics among *Cottus* examined in this study. Morphometric values were standardized by dividing by standard length, except mouth width was standardized by head width. An asterisk denotes a significant pairwise difference (ANCOVA, Scheffé test; $P < 0.05$) between the pooled sample of *C. schitsuumsh* and another species.

Species	Caudal peduncle depth	Caudal peduncle length	Head length	Head width	Lateral line length	Snout length
Spokane River basin (*n* = 64)	0.086	0.144	0.191	0.264	0.582	0.086
Clark Fork River basin (*n* = 44)	0.088	0.144	0.187	0.265	0.584	0.086
C. beldingii (*n* = 39)	0.097*	0.134*	0.211*	0.273	0.620*	0.092
C. cf. bairdii (*n* = 10)	0.077*	0.131	0.208*	0.301*	0.599	0.084
C. cognatus (*n* = 73)	0.078*	0.165*	0.221*	0.257	0.565	0.076*
C. confusus (*n* = 49)	0.084	0.157	0.202	0.265	0.667*	0.078*
C. hubbsi (*n* = 19)	0.081*	0.131	0.245*	0.299*	0.711*	0.095
C. rhotheus (*n* = 26)	0.062*	0.134	0.213*	0.304*	0.678*	0.109*

continued.

Species	Mouth width	Interpelvic width	Longest pectoral ray	Longest pelvic ray	Longest dorsal ray
Spokane River basin (*n* = 64)	0.608	0.034	0.338	0.180	0.124
Clark Fork River basin (*n* = 44)	0.605	0.036	0.337	0.179	0.123
C. beldingii (*n* = 39)	0.509*	0.042*	0.282*	0.167*	0.116
C. cf. bairdii (*n* = 10)	0.522*	0.043*	0.325	0.191	0.106
C. cognatus (*n* = 73)	0.505*	0.044*	0.287*	0.198*	0.110*
C. confusus (*n* = 49)	0.527*	0.039*	0.314	0.178	0.117
C. hubbsi (*n* = 19)	0.541*	0.045*	0.302	0.217*	0.126
C. rhotheus (*n* = 26)	0.599	0.038	0.284*	0.185	0.129

TABLE 6. Frequency distribution of head length divided by standard length (%) for *Cottus* examined in this study.

	Standardized head length										
Species	12	14	16	18	20	22	24	26	28	30	32
Spokane River basin (*n* = 64)	1		4	21	33	5					
Clark Fork River basin (*n* = 44)		3	8	15	10	4	4				
C. beldingii (*n* = 39)			1	9	10	12	4	1	2		
C. cf. bairdii (*n* = 10)				2	2	6					
C. cognatus (*n* = 73)	1		7	9	11	17	13	7	4	2	2
C. confusus (*n* = 49)	2	1	5	7	10	16	6	2			
C. hubbsi (*n* = 19)					2	3	6	6	2		
C. rhotheus (*n* = 26)			2	3	5	11	2	3			

Distribution and ecology. *Cottus schitsuumsh* is distributed in all streams sampled in the upper Coeur d'Alene and St. Joe River drainages of the Spokane River basin above Post Falls near Post Falls, Idaho, and sporadically in the middle Clark Fork River basin between Fish Creek and Prospect Creek, approximately between Tarkio and Thompson Falls, Montana (Fig. 1). This species is common to abundant in cool to cold tributaries with cobble and gravel bottoms. *Cottus schitsuumsh* is sympatric with Westslope Cutthroat Trout, *Oncorhynchus clarkii lewisi,* and Bull Trout, *Salvelinus confluentus,* throughout its distribution, with *C. rhotheus* in Idaho, and with *C. cognatus* in Montana. No effort was undertaken to find this species in Lake Coeur d'Alene or in the river main stems, but haplotype differences between fish from the Coeur d'Alene and St. Joe River basins imply that lineages in these basins are isolated and diverging.

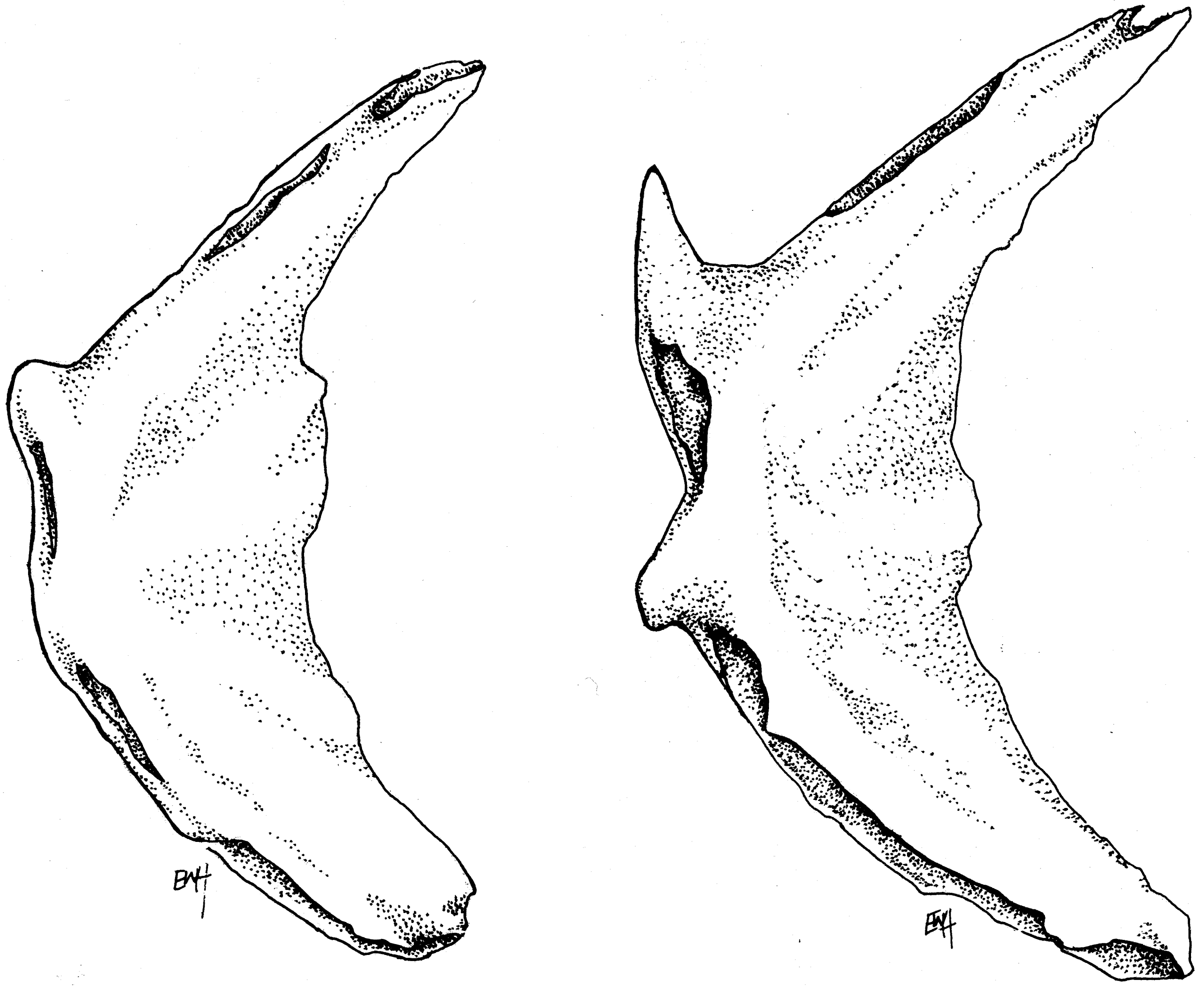

FIGURE 6. Preopercular bone of *Cottus schitsuumsh* (left) and of *Cottus confusus* (right).

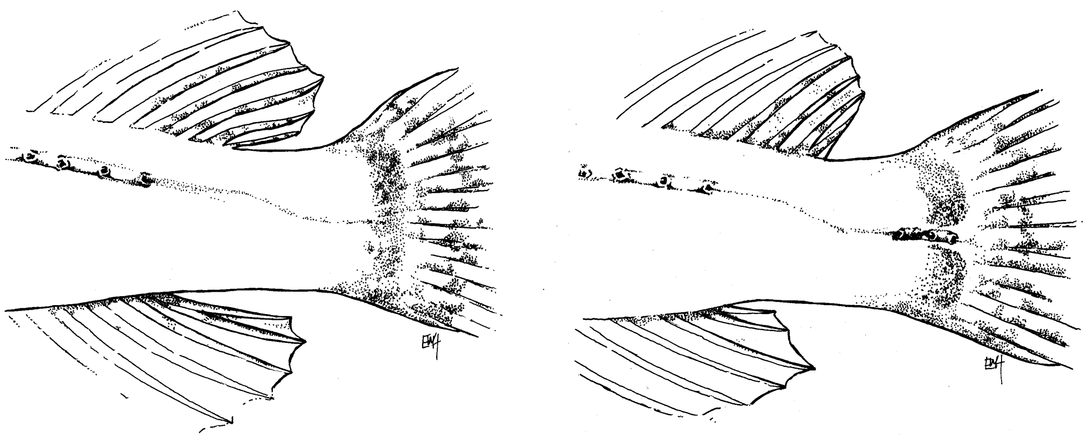

FIGURE 7. Lateral pores absent on the caudal peduncle on *Cottus schitsuumsh* (left) and present on *Cottus confusus* (right).

The distribution in Idaho is consistent with that of other terrestrial and aquatic fauna e.g., Idaho Giant Salamander, *Plethodon idahoensis*, that persisted immediately south of the extent of Cordilleran glaciation in the Northern Rocky Mountain Refugium (Carstens *et al.* 2005; Shafer *et al.* 2010). The disjunct distribution in the Clark Fork River basin in western Montana is puzzling (although known for some time; Hendricks 1997). There are no known peri- or post-glacial hydrological connections between these basins because all mountain divides are more than 150 m above the glacial Lake Missoula highstand (Smith 2006). Because sculpins were a common and legal baitfish in Montana until this century, it is plausible that populations in western Montana resulted from human-assisted translocation. Regardless of their means of arrival, the Montana populations have haplotypes similar to those in the Coeur d'Alene River basin and probably originated from that watershed.

TABLE 7. Frequency distribution of interpelvic width divided by standard length (%) for *Cottus* examined in this study.

	Standardized interpelvic width											
Species	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0
Spokane River basin (*n* = 64)	2	1	5	18	16	19	2		1			
Clark Fork River basin (*n* = 44)		2	1	10	15	12	2			2		
C. beldingii (*n* = 39)				2	7	12	11	4	1		2	
C. cf. bairdii (*n* = 10)						4	4	2				
C. cognatus (*n* = 73)		1		2	7	19	19	15	7	2		1
C. confusus (*n* = 49)				5	15	16	11	1	1			
C. hubbsi (*n* = 19)					4	3	6	2	2	1	1	
C. rhotheus (*n* = 26)			4	2	6	8	5	1				

Etymology. The word schitsu'umsh (pronounced s-CHEET-sue-umsh) is Americanist phonetic notation of the spoken word used by the first peoples who inhabited portions of northern Idaho and western Montana, now recognized as the Coeur d'Alene Tribe. In modern usage it means "those who were found here" and refers to the people of this tribe. The current distribution of C. *schitsuumsh* overlaps with most of the historical homeland of this tribe. The common name refers to a common riparian tree in this area, Western Redcedar, *Thuja plicata*.

Remarks. Although originally assigned to *C. confusus*, a broad-scale genetic biodiversity inventory of fishes in the northern Rocky Mountains (Young *et al.* 2013) indicated, based on sequence differences among two mtDNA regions, that *C. schitsuumsh* was not closely related to that species. In the present study, the more geographically comprehensive sample of fishes subjected to genetic analysis coupled with the morphological assessment confirmed that interpretation. In a broader context, however, these findings support previous assertions that the taxonomy of sculpins in North America is far from resolved (Neely 2003; Kinziger *et al.* 2005; April *et al.* 2011). Paraphyly and polyphyly among a number of taxa are evident in phylogenetic trees based on COI (Fig. 2). Although partly attributable to a lack of resolution resulting from the limited number of nucleotides in this region (Pollock *et al.* 2002), the results are similar to those observed for other mtDNA regions that included additional taxa and more nucleotides (Kinziger *et al.* 2005; Young *et al.* 2013). In addition, the large genetic differences between clades within other species of *Cottus* in the western U.S., such as *C. beldingii* and *C. confusus*, suggest that additional taxa remain to be described (also see April *et al.* 2011).

The ambiguous morphological distinctions among sculpin species (Jenkins & Burkhead 1994; McPhail 2007) argue for the use of genetic information for species discovery and delineation (Baldwin & Weigt 2012). Moreover, some of the variation in diagnostic traits within species ascribed to geography may instead reflect the existence of cryptic taxa (Bickford *et al.* 2007). As has been suggested for other groups of species, an integrative taxonomy relying on morphology and genes will be necessary to understand the details of the systematic relations and boundaries of individual taxa among members of the genus *Cottus*.

Comparative material for morphological and genetic analyses (GenBank accession numbers for COI haplotypes). *Cottus beldingii*: (4, 45–54 mm SL, JX311884) North Fork Asotin Creek, Asotin County, WA (46.25856°N, 117.29962°W), 9 August 2011. *Cottus cognatus*: (5, 65–81 mm SL, JX311885) Marten Creek, Sanders County, MT (47.89233°N 115.82088°W), 20 September 2011; (5, 44–74 mm SL, JX311886, JX311887) Skalkaho Creek, Ravalli County, MT (46.18128°N, 114.07984°W), 8 January 2012. *Cottus cf. bairdii* (Rocky Mountain Sculpin): (5, 60–91 mm SL, JX311882) Elk Creek, Missoula County, MT (46.92707°N, 113.43934°W), 23 August 2011. *Cottus confusus*: (5, 77–89 mm SL, JX311888) Little North Fork Clearwater River, Shoshone County, ID (47.06579°N, 115.85287°W), 19 September 2011; (2, 74–89 mm SL, pending) Pole Creek, Blaine County, ID (43.90974°N, 114.76370°W), 7 November 2012; (2, 59–68 mm SL, pending) Salmon River, Blaine County, ID (43.88382°N, 114.76370°W), 7 November 2012; (2, 67–90 mm SL, pending) Smiley Creek, Blaine County, ID (43.89650°N, 114.79853°W), 7 November 2012. *Cottus hubbsi*: (1, 112 mm SL, JX311892) Dragoon

Creek, Spokane County, WA (47.88619°N 117.38400°W), 16 August 2011. *Cottus rhotheus*: (1, 71 mm SL, JX311890) Dragoon Creek, Spokane County, WA (47.88619°N 117.38400°W), 16 August 2011; (5, 59–84 mm SL, JX311891) Spokane River, Spokane County, WA (47.66060°N, 117.43559°W), 12 September 2011.

Comparative material for morphological analyses. *Cottus beldingii*: (2, 56–57 mm SL, CIDA68169) Big Bear Creek, Latah County, ID (46.63927°N, 116.65417°W), 1 July 1995; (4, 57–68 mm SL, CIDA68443) Clear Creek, Clearwater County, ID (46.04655°N, 115.79120°W) 18 July 1996; (2, 57–58 mm SL, CIDA68110) East Fork Potlatch River, Clearwater County, ID (46.82873°N, 116.38967°W), 25 July 1969; (2, 52–60 mm SL, CIDA68369) Glade Creek, Idaho County, ID (46.22338°N, 115.52972°W), 18 July 1996; (2, 51–58 mm SL, CIDA68309) Lochsa River, Idaho County, ID (46.46113°N, 115.04775°W), 15 August 1969; (4, 39–64 mm SL, CIDA68192) Middle Fork Clearwater River, Idaho County, ID (46.14052°N, 115.69819°W), 23July 1969; (1, 46 mm SL) North Fork Asotin Creek, Asotin County, WA (44.25856°N, 117.29962°W), 9 August 2011; (4, 49–63 mm SL, CIDA68389) North Fork Clearwater River, Clearwater County, ID (46.81125°N, 115.20330°W), 20 August 1969; (4, 40–64 mm SL, CIDA81824) South Fork Clearwater River, Idaho County, ID (46.03107°N, 115.97420°W), 20 August 2003; (2, 48–55 mm SL) Swauk Creek, Kittatas County, WA (47.17556°N, 120.72944°W), 9 June 1996; (6, 42–75 mm SL) Tucannon River, Columbia County, WA (46.21694°N, 117.71111°W), 1 August 1993; (2, 35–68 mm SL, CIDA69562) Weitas Creek, Clearwater County, ID (46.53143°N, 115.42108°W), 4 August 1998. *Cottus cf. bairdii* (Rocky Mountain Sculpin): (5, 84–99 mm SL) Elk Creek, Missoula County, MT (46.92707°N, 113.43934°W), 23 August 2011. *Cottus cognatus*: (10, 32–81 mm SL) Blodgett Creek, Ravalli County, MT (46.28756°N, 114.16837°W), 3 August 2012; (2, 51–54 mm SL, CIDA70563) Burton Creek, Boundary County, ID (48.772227°N, 116.46445°W), 26 July 2001; (2, 31–40 mm SL, CIDA69961) Kalispell Creek, Bonner County, ID (48.57615°N, 116.95607°W), 18 July 2000; (10, 59–88 mm SL) Overwhich Creek, Ravalli County, MT (45.66498°N, 114.28005°W), 21 August 2012; (3, 50–56 mm SL, CIDA68609) Pack River, Bonner County, ID (48.47588°N, 116.55390°W), 12 October 1972; (5, 48–71 mm SL) Petty Creek, Mineral County, MT (46.91728°N, 114.45675°W), 12 October 2011; (5, 52–82 mm SL) Petty Creek, Mineral County, MT (46.99196°N, 114.44762°W), 12 October 2011; (5, 48–76 mm SL) Petty Creek, Mineral County, MT (46.93116°N, 114.44568°W), 12 October 2011; (2, 48–53 mm SL) Skalkaho Creek, Ravalli County, MT (46.18128°N, 114.07984°W), 8 January 2012; (10, 66-77 mm SL) Sleeping Child Creek, Ravalli County, MT (46.11015°N, 114.00677°W), 10 August 2012; (3, 32–81 mm SL) Sullivan Creek, Pend Oreille County, WA (48.86333°N, 117.30500°W), 20 August 1996; (6, 42–71 mm SL) Tonata Creek, Ferry County, WA (48.83308°N, 118.80858°W), 1 September 1996. *Cottus confusus:* (3, 69–77 mm SL) Alpine Creek, Blaine County, ID (43.89740°N, 114.91070°W), 15 August 2011; (1, 47 mm SL) American River, Yakima County, WA (46.92611°N, 121.36389°W), 1 September 1993; (3, 35–70 mm SL) Entiat River, Chelan County, WA (47.92528°N, 120.51167°W), 1 October 1996; (2, 31–56 mm SL) Frenchman Creek, Blaine County, ID (43.87108°N, 114.77263°W), 1 August 1998; (3, 54–64 mm SL) Lost River, Okanogan County, WA (48.65556°N, 120.50444°W), 31 August 1999; (3, 52–77 mm SL, CIDA69441) Pole Creek, Blaine County, ID (43.91063°N, 114.74935°W), 26 August 1998; (3, 47–70 mm SL, CIDA69831) Salmon River, Blaine County, ID (43.83368°N, 114.75885°W), 26 July 1990; (5, 32–60 mm SL, CIDA70993) Salmon River, Blaine County, ID (43.85745°N, 114.75448°W), 3 August 2003; (3, 54–74 mm SL) Salmon River, Blaine County, ID (43.88382°N, 114.76370°W), 7 November 2012; (7, 33–78 mm SL, CIDA69443) Smiley Creek, Blaine County, ID (43.82638°N, 114.82743°W), 26 July 1998; (3, 33–78 mm SL) Smiley Creek, Blaine County, ID (43.89650°N, 114.79853°W), 7 November 2012; (2, 50 mm SL, CIDA69503) Vat Creek, Blaine County, ID (43.97637°N, 114.84807°W), 8 August 1998. *Cottus hubbsi*: (4, 57–68 mm SL) Chewuch River, Okanogan County, WA (48.56472°N, 120.17667°W), 31 August 1999; (2, 44–53 mm SL, CIDA68934) Siegel Creek, Idaho County, ID (45.78730°N, 115.36688°W), 11 July 1997; (5, 35–73 mm SL) Similkameen River, Okanogan County, WA (48.95667°N, 119.64583°W), 21 July 1998; (3, 53–63 mm SL, CIDA69699) Skull Creek, Clearwater County, ID (46.87165°N, 115.38710°W), 9 September 1999; (3, 67–103 mm SL, CIDA68494) Selway River, Idaho County, ID (45.73492°N, 114.75885°W), 12 July 1970; (1, 68 mm SL) West Fork Sanpoil River, Okanogan County, WA (48.51417°N, 118.88889°W), 23 August 1999. *Cottus rhotheus*: (5, 67–79 mm SL) Ramskull Creek, Benewah County, ID (47.08918°N, 116.63813°W), 11 August 2011; (5, 63–94 mm SL) Spokane River, Spokane County, WA (47.66060°N, 117.43559°W), 12 September 2011; (10, 41–90 mm SL, JX311891) Spokane River, Spokane County, WA (47.69878°N, 117.04451°W), 12 September 2011.

Comparative material for genetic analyses from GenBank. *Cottus aleuticus*: (5) EU523991, EU523992, HQ010070, JN024987, JN024988. *Cottus asper*: (2) EU523994, JQ354065. *Cottus aturi*: (1) EF416966. *Cottus* *bairdii*: (20) EF416967, EU522459, EU523998, EU524490, EU524496, EU524506, HQ557187, HQ557189, JN024992, JN024996, JN024997, JN024999, JN025004, JN025006, JN025010, JN025011, JN025012, JN025013, JN025022, JN025023. *Cottus beldingii*: (5) HQ557196, HQ579024, JN025027, JN025029, JN025034. *Cottus caeruleomentum*: (2) JN025040, JN025041. *Cottus carolinae*: (8) JN025046, JN025047, JN025051, JN025052, JN025053, JN025058, JN025060, JN025064. *Cottus cf. bairdii*: JN025069, JN025070, JN025071. *Cottus cf. carolinae*: (2) JN025072, JN025073. *Cottus chattahoochee*: (1) JN025077. *Cottus cognatus*: (7) EU523999, EU524511, EU524517, JN025085, JN025090, JN025091, JN025099. *Cottus duranii*: (1) EF416968. *Cottus girardi*: (1) HQ557143. *Cottus gobio*: (6) EF416970, EF416971, EF416973, HQ960512, HQ960869, HQ960870. *Cottus gulosus*: (2) JN025102, JN025103. *Cottus hispaniolensis*: (1) EF416977. *Cottus hubbsi*: (1) JN025104. *Cottus hypselurus*: (3) JN025105, JN025106, JN025110. *Cottus klamathensis*: (1) JN025112. *Cottus leiopomus*: (1) HQ971431. *Cottus perifretum*: (1) EF416978. *Cottus perplexus*: (3) EF416984, JN025117, JN025120. *Cottus pitensis*: (1) JN025122. *Cottus poecilopus*: (5) HQ536339, HQ960874, HQ960878, HQ961093, JN025128. *Cottus reinii*: (1) AP004442. *Cottus rhenanus*: (3) EF416985, EF416986, EF416988. *Cottus rhotheus*: (3) EU524000, HQ579026, JN025131. *Cottus ricei*: (4) EU522462, EU524521, HQ557327, JN025134. *Cottus sibiricus*: (1) EF416990. *Cottus tallapoosae*: (6) JN025137, JN025140, JN025143, JN025144, JN025145, JN025146. *Cottus tenuis*: (2) HQ579025, JN025147. *Leptocottus armatus*: (1) FJ164712.

Comparative material for genetic analyses from regional sampling (with GenBank accession numbers, if available). *Cottus beldingii*: (5, JX282576–JX282580, 2 pending) Feather Creek, Clearwater County, ID (46.91599°N, 116.40383°W), Potlatch River, Clearwater County, ID (46.91376°N, 116.39121°W); Goddard Creek, Idaho County, ID (46.10088°N, 115.55723°W); Musselshell Creek, Clearwater County, ID (46.35239°N, 115.75575°W); Lolo Creek, Clearwater County, ID (46.40720°N, 115.67415°W); Portneuf River, Bannock County, ID (42.70837°N, 112.21222°W); Pass Creek, Custer County, ID (43.93533°N, 113.44703°W). *Cottus cf. bairdii*: (5, JX282572–JX282575, JX311883, 2 pending) Alice Creek, Lewis and Clark County, MT (47.10702°N, 112.4767°W); Colts Creek, Flathead County, MT (48.99259°N, 114.49092°W); Trout Creek, Lewis and Clark County, MT (46.90852°N, 112.38711°W); Taylor Fork Gallatin River, Gallatin County, MT (45.04594°N, 111.37768°W); Highwood Creek, Choteau County, MT (47.47503°N, 110.61261°W); Thayer Creek, Beaverhead County, MT (45.20349°N, 113.29354°W); Mussigbrod Creek, Beaverhead County, MT (45.76858°N, 113.58538°W). *Cottus cognatus*: (8, JX282581–JX282588) Beaver Creek, Missoula County, MT (47.38776°N, 113.66057°W); Little Thompson River, Sanders County, MT (47.69347°N, 114.81318°W); Lake Creek, Lincoln County, MT (48.03698°N, 115.49420°W); Kraft Creek, Missoula County, MT (47.45570°N, 113.75697°W); Twelvemile, Mineral County, MT (47.37603°N, 115.25982°W); Lower Twin Creek, Flathead County, MT (47.99185°N, 113.56328°W); Overwhich Creek, Ravalli County, MT (45.66399°N, 114.15168°W). *Cottus confusus*: (7, JX282589–JX282594, JX311889, 9 pending) Crooked Fork Creek, Clearwater County, ID (46.66158°N, 114.67718°W); Fishing Creek, Clearwater County, ID (46.51996°N, 114.86661°W); Cayuse Creek, Clearwater County, ID (46.59984°N, 114.89314°W); French Creek, Clearwater County, ID (46.52659°N, 115.65508°W); South Fork Red River, Idaho County, ID (45.67204°N, 115.36070°W); Newsome Creek, Idaho County, ID (45.91054°N, 115.62920°W); Baldy Creek, Idaho County, ID (45.91278°N, 115.64021°W); Fallert Springs, Butte County, ID (43.91625°N, 112.12339°W); Wildhorse Creek, Custer County, ID (43.85320°N, 114.08758°W); Pass Creek, Clark County, ID (44.13444°N, 112.99285°W); Big Gulch, Custer County, ID (44.34647°N, 113.52006°W); Eighteenmile Creek, Lemhi County, ID (44.53546°N, 113.24102°W); Loon Creek, Custer County, ID (44.80694°N, 114.81070°W); Salmon River, Blaine County, ID (43.88382°N, 114.76370°W); Pole Creek, Blaine County, ID (43.90974°N, 114.75629°W); Smiley Creek, Blaine County, ID (43.89650°N, 114.79853°W). *Cottus hubbsi*: (1, pending) South Fork Rock Creek, Power County, ID (42.50190°N, 112.82991°W). *Cottus rhotheus*: (2, JX282595–JX282596) East Fork Emerald Creek, Latah County, ID (47.02486°N, 116.32903°W); Little Sand Creek, Latah County, ID (46.92103°N, 116.62829°W).

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