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MORPHOMETRIC ANALYSIS OF TWO ENIGMATIC SCULPIN SPECIES, *COTTUS GULOSUS* AND *COTTUS PERPLEXUS* (SCORPAENIFORMES: COTTIDAE)

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ABSTRACT—The freshwater sculpin species *Cottus gulosus* and *Cottus perplexus* present a challenge to biologists because of their overlapping sympatric distributions, morphological similarity, and historical inconsistency regarding species limits and ranges. We applied linear morphometric analyses in conjunction with meristic and discrete character counts to 1) assess the validity of previously cited diagnostic characters, 2) test multivariate combinations of characters to elucidate novel diagnostic characters useful in distinguishing the 2 species, and 3) apply these diagnostic characters to identify previously unidentified specimens obtained from Washington streams, where the 2 species occur in sympatry. Our analysis of 270 *Cottus* specimens encompassing the range of both species uncovered little support for the diagnostic utility of previously cited characters, although significant differences between mean measurements for each group were detected in the proportion of individuals with palatine teeth and a median chin pore, as well as in mean mouth-width to body-width ratio. Additionally, we were unable to detect linear transformations of continuous measurements that could be used to reliably identify individuals of these 2 species. These results prevented us from confidently identifying the unknown Washington specimens and illustrate that multi-locus phylogenetic studies are needed to assess species boundaries for these morphologically similar fishes.

Key words: California, identification, morphometrics, Oregon, Reticulate Sculpin, Riffle Sculpin, taxonomy, Washington

Sculpins of the genus *Cottus* are an important component of the freshwater ichthyofauna of the Pacific Northwest. There are currently 19 valid species native to Washington, Oregon, and British Columbia. Most species are restricted to freshwater habitats, but others exhibit varying degrees of salinity tolerance and a few can be found in brackish environments (Moyle 2002; Wydoski and Whitney 2003; McPhail 2007). *Cottus* species are generally benthic and can be found in a variety of habitats including wetlands, lakes, large low-gradient rivers, and small coldwater mountain streams (Wydoski and Whitney 2003).

Sculpins are often widely distributed and highly variable morphologically (Moyle 2002). These 2 factors have contributed to a significant degree of confusion surrounding their taxono-

my. Sculpins are among the most notoriously difficult North American freshwater fishes to positively identify to species. Recent studies using DNA sequence data to distinguish lineages have begun to shed light on the problem (Kinziger and others 2005; Baumsteiger and others 2012, 2014; Young and others 2013), but there is still a high degree of uncertainty surrounding species boundaries in many taxa and in many regions throughout western North America.

One particularly vexing taxonomic challenge concerns the distinction between the Riffle Sculpin (*Cottus gulosus*) and the Reticulate Sculpin (*Cottus perplexus*), 2 species that are sympatric in western Oregon and Washington streams. Although both species have some salinity tolerance (Bond 1963) their range is

restricted to freshwater environments spanning the West Coast of the United States (Fig. 1). The type localities for these species are located at the northern and southern edges of their distributions: *C. perplexus* in the Skookumchuck River near Chehalis, Washington (Gilbert and Evermann 1894) and *C. gulosus* in San Mateo Creek, California (Girard 1854). *Cottus gulosus* has a disjunct distribution, with southern populations in the Sacramento-San Joaquin and coastal systems of California, and northern populations in western Oregon and Washington. *Cottus perplexus* is also found in western Washington streams, but its range does not extend south beyond the Rogue River in Oregon (Bond 1973a; Moyle 2002; Wydoski and Whitney 2003). In some areas of sympatry, one species dominates a particular stretch of stream, whereas in other areas they are equally abundant (Bond 1963).

Reliably distinguishing between these 2 species has been particularly problematic in areas of sympatry in northern Oregon and western Washington. Snyder (1907) failed to separate the 2 species and suggested that specimens of *C. gulosus* from the Sacramento River were indistinguishable from specimens of *C. perplexus* from the Columbia River. Schultz (1930), working with specimens identified as *C. perplexus* and *C. gulosus* from western Washington, also failed to find consistent morphological distinctions between the species and synonymized the species into *C. gulosus*. In their broader revision of western *Cottus* species, Robins and Miller (1957) once again recognized *C. perplexus* as a distinct species, stating that most records of *C. gulosus* from Washington and Oregon were in fact *C. perplexus*, not *C. gulosus* as they defined the species. Based on an examination of types, they concluded that *C. perplexus* could be distinguished from *C. gulosus* in having a more robust body form, strongly marbled anal and dorsal fins, and joined dorsal fins. However, in reestablishing the validity of *C. perplexus*, Robins and Miller (1957) never explicitly stated that *C. gulosus* could be found in northern Oregon or Washington.

Continuing extensive work on western *Cottus*, Bond (1963) also stated that many specimens of *C. perplexus* had been misidentified as *C. gulosus*, likely due to the temporary synonymy of the two. Bond (1963) concluded that *C. perplexus* is more widely distributed in Oregon than *C. gulosus*, which is absent from the Willamette

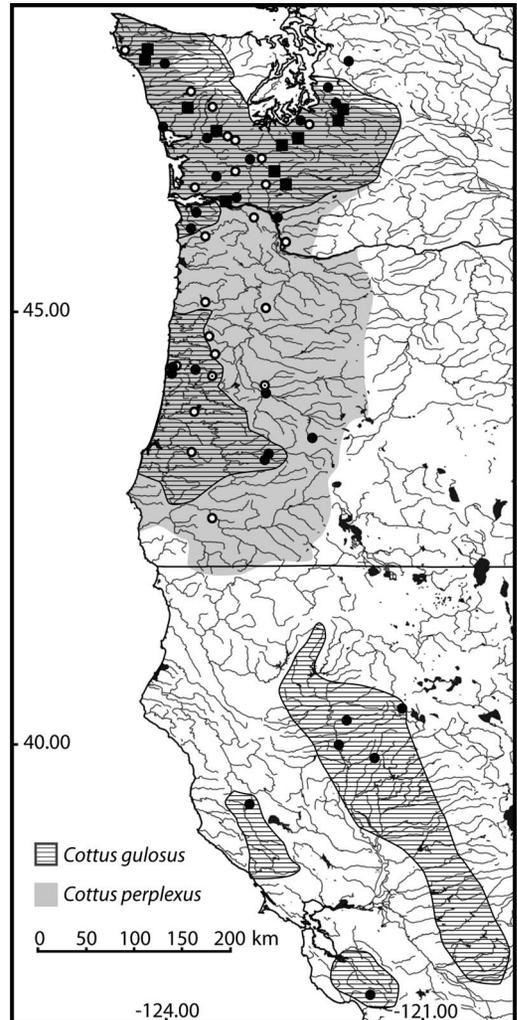


FIGURE 1. Range of *Cottus gulosus* and *Cottus perplexus*. Localities for specimens used in this studies indicated by white circles, *Cottus gulosus*; black circles, *Cottus perplexus*; black squares, unidentified *Cottus* spp. (either *C. perplexus* or *C. gulosus*). Compiled from Bond (1963), Lee and others (1980), Moyle (2002), Wydoski and Whitney (2003), and Baumsteiger and others (2012, 2014).

River system altogether. Characters that have been proposed as useful for distinguishing between the 2 species are listed in Table 1.

Although many characters show significant overlap between species, one that is often considered the most reliable diagnostic is the absence or weak development of palatine teeth in *C. perplexus* and the presence of palatine teeth in *C. gulosus* (Bailey and Bond 1963; Bond 1973a

TABLE 1. Characters that have been cited as diagnostic in distinguishing *Cottus perplexus* from *Cottus gulosus* by previous authors.

Character	<i>Cottus perplexus</i>	<i>Cottus gulosus</i>
Palatine teeth ^{a,c,d,e}	Absent	Present
Dorsal fins ^{b,c,d}	Usually conjoined, most often broadly so	Conjoined or separate
Vertical fins ^b	Strongly marbled	Not marbled
Percent of specimens with complete lateral line (specimens >50 mm) ^{c,d}	36%	23%
Preopercular spines ^{c,d}	2 to 3; usually 2 or 2+	3 or 3+; usually 3
Head length (%SL), extremes in parentheses ^{c,d}	(23) 28–33 (38)	(30) 31–36 (39)
Maxilla extending to ^{c,d}	Anterior part of eye	Posterior part of eye
Mouth width ^{c,d,e}	Less than body width behind pectorals	Equal to or exceeds body width behind pectorals
Pigmentation ^{c,d}	Variable, usually with vermiculations and small blotches that do not have high contrast with background	Usually with large irregular dark blotches on a lighter background
Median chin pores ^e	2 in 20% of specimens	2 in 50% of specimens
Snout shape ^e	Head triangular, more pointed snout	More rounded snout

^a Gilbert and Evermann (1892)

^b Robins and Miller (1957)

^c Bailey and Bond (1963)

^d Bond (1973b)

^e Wydoski and Whitney (2003)

and b; Wydoski and Whitney 2003). In their original description of *Cottus perplexus*, Gilbert and Evermann (1894) stated that *C. perplexus* lacks palatine teeth, as is the case for the holotype. However, the subtopotypes are a mix of specimens with and without palatine teeth (Robins and Miller 1957). There is no mention of palatine teeth in the description of *C. gulosus* (Girard 1854). Potentially an informative character, palatine teeth are difficult to find on a live specimen in the field. Even confirming their presence in a preserved specimen often requires the aid of a microscope, and in many cases the jaws are either intentionally or unintentionally damaged in the process.

Biologists working in streams and rivers in western Washington and Oregon regularly encounter sculpins that are either *C. perplexus* or *C. gulosus*, but positive identification is difficult due to the lack of reliable morphological distinction combined with the taxonomic uncertainty surrounding these 2 species (Tabor and others 2007). This problem is particularly relevant in western Washington, where relatively little work on these species has been done. In order to further elucidate the distinction between these 2 species and establish reliable morphological diagnostic characters, we as-

sessed the utility of previously proposed morphological traits and explored novel characters by performing morphometric analyses on a combination of previously identified and unidentified museum specimens.

METHODS

We conducted all statistical analyses in the R statistical software program, using the packages “reshape2,” “ggplot2,” “ape,” “MASS,” “candisc,” and “car” (Venables and Ripley 2002; Paradis and others 2004; Wickham 2007, 2009; Fox and Weisberg 2011; Friendly and Fox 2015; R Core Team 2015).

Material Examined

We sampled a total of 270 fluid-preserved museum specimens comprising the geographic range of both *Cottus gulosus* and *Cottus perplexus* (Fig. 1; Appendix). When possible, at least 5 individuals of each species were measured from each major drainage where the species is known to occur. Specimens were obtained from the Oregon State Ichthyology Collection (OS), the Fish Collection of the Burke Museum at the University of Washington (UW), the Natural History Museum of Los Angeles County

(LACM), the California Academy of Sciences (CAS), the Smithsonian National Museum of Natural History (USNM), and the Pacific Lutheran University Natural History Collection (PLUNHC). All specimens had been previously identified as either *Cottus gulosus* or *Cottus perplexus* except those from PLUNHC, which were designated as *Cottus* spp. at the time of collection, with the intent of identifying them based on emergent diagnostic characters. Four specimens from 3 different localities in the Willamette River system were previously identified as *C. gulosus*, although Bond (1963) suggested that the species is absent from this system. We treated them as *C. gulosus* in all analyses.

For all analyses, specimens were grouped in 1 of 3 ways. First, we used the prior species identification (henceforth referred to as prior ID) to determine whether the current identification, assuming accuracy, corresponded with multivariate character differences. Second, we used presence of palatine teeth, a character claimed to be diagnostic by several sources (Bailey and Bond 1963; Bond 1963, 1973a and b; Wydoski and Whitney 2003), to assess morphological distinctness without assuming accuracy in species identification. Third, we analyzed sampled specimens by drainage, as the species have incompletely overlapping ranges (Reimers and Bond 1967; Bond 1973a). For all analyses other than prior ID, we disregarded prior ID to examine differences solely based on the grouping variable in question.

Meristic and Discrete Character Data Collection and Analysis

We took counts for 5 meristic characters following the guidelines set by Robins and Miller (1957): 1st dorsal fin rays (1DR), 2nd dorsal fin rays (2DR), pectoral fin rays (PCR), pelvic fin rays (PVR), and anal fin rays (ANR). Additionally, we assessed the presence of palatine teeth, the connectivity of the dorsal fin, and the configuration of preoperculo-mandibular pores as described by Robins and Miller (1957): specifically, whether an individual had a median chin pore ("10-1-10" configuration) or not ("11-11" configuration). We observed and recorded these traits based on their previously reported utility in distinguishing members of *Cottus gulosus* from *Cottus perplexus* (Robins and Miller

TABLE 2. Summary statistics of meristics taken from all previously identified *Cottus* specimens. Values are represented as follows: Median (Min-Max); Mode.

	<i>C. gulosus</i>	<i>C. perplexus</i>
<i>n</i>	106	90
Dorsal spines	8 (7–9); 8	8 (7–9); 8
Dorsal rays	18 (15–21); 18	18 (15–20); 18
Pectoral rays	15 (14–16); 15	15 (13–16); 15
Pelvic rays	4 (4–4); 4	4 (4–4); 4
Anal rays	15 (12–18); 15	15 (13–18); 15
Proportion with palatine teeth	0.69	0.10
Proportion with median chin pore	0.34	0.15

1957; Bond 1973a; Wydoski and Whitney 2002; Table 1). The amount of variation in the connectivity of the dorsal fin was not easily determined on a categorical scale and was frequently damaged in the preserved specimens, but was nonetheless recorded as "separate," "nearly separate," or "continuous." However, the variation in this character is essentially continuous and therefore placing the variation into discrete categories was problematic. Thus, these results are not reported. Where applicable, we recorded measurements from the right side of the specimen unless pertinent structures were damaged. We tabulated median, mode, and range for each variable, as well as the percentage of individuals with palatine teeth and a median chin pore (Table 2). Differences between species for the proportion of individuals with palatine teeth and those with a median chin pore were tested using Wilson's (1927) test of equal proportions.

Morphometric Data Collection

We measured 27 morphometric distances based on 37 landmarks on 256 specimens (Fig. 2). All measurements were collected to the nearest 0.01 millimeter (mm) using Mitutoyo ABSOLUTE Digimatic calipers. Prior to collecting data, each measurement was taken 5 times on 5 different specimens. Measurements with greater than 5% standard deviation from the mean were measured again 5 more times until error was reduced to less than 5%. All measurements, except those for USNM 45387 (the holotype for *Cottus perplexus*) were performed by DR to minimize error associated with differences in character coding.

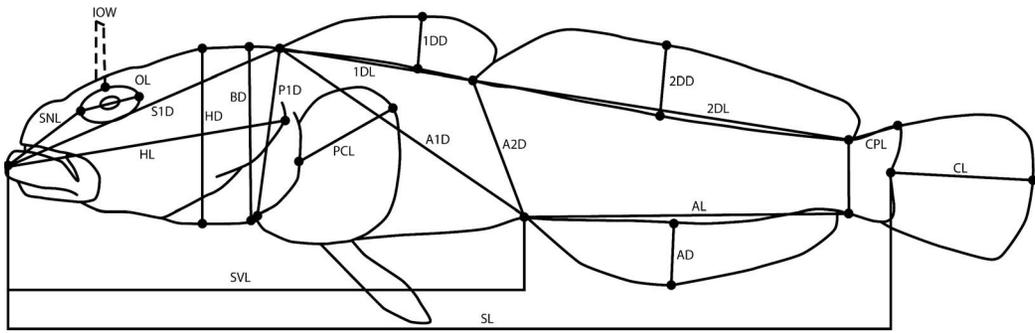
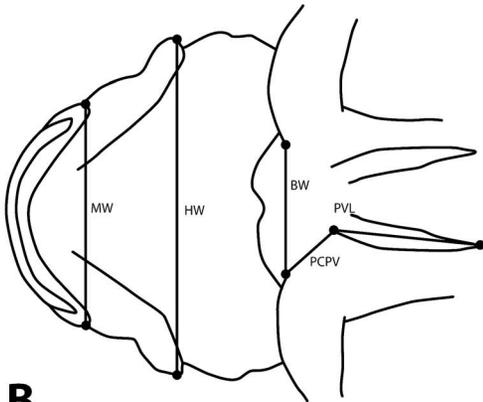
**A****B**

FIGURE 2. Landmarks and morphometric distances measured. (A) Lateral view: standard length from snout to end of last vertebra [SL]; snout-vent length [SVL]; length of 1st dorsal fin [1DL]; depth of 1st dorsal fin at 4th fin ray [1DD]; length of 2nd dorsal fin [2DL]; depth of 2nd dorsal fin at 7th fin ray [2DD]; length of pectoral fin [PFL]; length of anal fin [AL]; depth of anal fin at 5th ray [AD]; length of caudal fin [CL]; length of caudal peduncle from 2nd dorsal fin insertion to dorsal insertion of caudal fin [CPL]; depth of caudal peduncle at minimum [CPD]; length of snout to 1st dorsal fin origin [S1D]; length of 1st dorsal fin origin to lower insertion of pectoral fin [P1D]; length of 1st dorsal fin origin to anal fin origin [A1D]; length of 2nd dorsal fin origin to anal fin origin [A2D]; head length from snout to posteriormost point of operculum [HL]; depth of head at maximum [HD]; length of snout from anteriormost point to anteriormost point of right orbit [SNL]; length of orbit at maximum [OL]; and interorbital width at minimum [IOW]. (B) ventral view: mouth width [MW]; width of head at maximum [HW]; body width between origins of pectoral fins [BW]; length of pelvic fin origin to lower insertion of pectoral fin [PCPV]; and length of pelvic fin [PVL].

Univariate Analytical Methods for Morphometric Data

To test for differences between specimens based on prior ID, as well as between groups based on presence or absence of palatine teeth, we used Welch 2-sample, 2-sided *t*-tests on an allometry-corrected data set of mouth-width to body-width ratio (MBR), a character that has been cited as diagnostic (Wydoski and Whitney 2003). Potential allometric effects were removed by regressing MBR against standard length (SL)

and extracting the residuals of the linear fit of these data (rMBR). This method was performed for a data set including all individuals previously identified as *Cottus gulosus* or *Cottus perplexus* (for prior ID) and all individuals with complete measurement data greater than 45 mm SL (for palatine tooth condition). We also examined whether individuals with the combination of palatine teeth and no median chin pore had significantly different mean rMBR than individuals with other combinations of these traits.

TABLE 3. Results of tests of equal proportions. Null hypothesis: the character in question is equally proportional across species (prior ID).

Test of equal proportions	df	Min 95% CI	Max 95% CI	X ² -Value	p-value
Palatine teeth by species	1	-0.6940	-0.4774	68.46	<2.2×10 ⁻¹⁶
Median chin pore by species	1	0.06961	0.3050	8.917	2.826×10 ⁻³

Multivariate Analytical Methods for Morphometric Data

Individuals with missing measurement data were excluded from multivariate analyses. The multivariate analysis data set comprised a total of 256 individuals. A subset of these data excluding head measurements was analyzed using principle components analysis (PCA). In all analyses other than PCA grouped by prior ID, individuals smaller than 45 mm SL were removed to minimize potential variation due to ontogenetic factors.

We then performed hierarchical cluster analyses on head and body measurements for all individuals greater than 45 mm SL. To minimize potential allometric effects, head and body distances were linearly regressed against standard length and the residuals of these distances from the best-fit line were extracted. Euclidean distances between individuals were calculated in multivariate space and grouped using Ward's (1963) method, an agglomerative algorithm which creates clusters based on minimizing the added variance about the centroid of a group of Euclidean distances.

We also performed canonical discriminant analyses on the principal component scores with data partitioned by prior ID and palatine tooth condition. This analysis constructs the linear combination of predictor variables that maximizes the variance explained between or among grouping variables, which are then defined as canonical axes. The 1st canonical axis is the rescaled combination of variables that maximizes the variance between the groups, the 2nd canonical axis is the combination that explains the 2nd-largest variance that is uncorrelated with the previous axis in this transformed space, and so on until the number of canonical variables equals the number of input variables minus 1 or the number of grouping variables minus 1, whichever is smaller.

Finally, we performed a quadratic discriminant analysis, a variation of Fisher's (1936) linear discriminant analysis that does not assume equal

variance of input variables, on the PCA data using prior ID as the grouping variable. This analysis attempts to classify individuals into their designated group using canonical variables and returns a proportion of correct assignment of individuals based on these canonical variables; the higher the proportion of correct assignment, the greater power the input variables have for predicting group membership.

RESULTS

Meristic Comparisons

Analysis of meristic data showed no significant differences between species among any of the fin ray counts (prior ID) (Table 2). Although we found variation among individuals in all fin ray counts other than pelvic, this variation was partitioned within, rather than between, species (Table 2). However, significant differences were detected between species for the proportion of individuals with palatine teeth ($X^2 = 68.46$, $p < 0.001$, $n = 195$, $df = 1$, but see discussion) and for the proportion of individuals with a median chin pore ($X^2 = 8.92$, $p < 0.003$, $n = 196$, $df = 1$) (Table 3).

Univariate Results

Two-sided *t*-tests found significant differences in mean rMBR based on prior species identification (*t*-test; $t = 4.45$, $P < 0.001$, $n = 188$, $df = 173.14$), but they were only marginally significant when grouped by condition of palatine teeth (*t*-test; $t = -1.91$, $P = 0.058$, $n = 268$, $df = 228.92$) (Fig. 3). Additionally, we detected significantly different mean rMBR between individuals with the combination of palatine teeth and no median chin pore compared to all other combinations of these two traits grouped together ($t = 2.23$, $df = 81.94$, $p = 0.028$). These results roughly coincide with the identification guidelines of Wydoski and Whitney (2003), who concluded that the mouth width is typically greater than the body width in *Cottus gulosus* (the inverse is reported for *Cottus perplexus*).

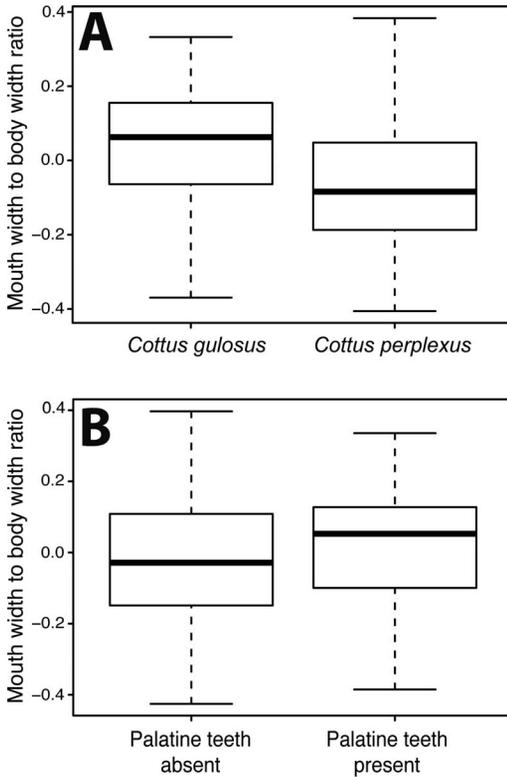


FIGURE 3. Box plots of mouth-width to body-width ratios (rMBR). (A) Specimens grouped by prior species identification (prior ID) (t -test; $t = 4.45$, $p < 0.001$, $n = 188$, $df = 173.14$). (B) Specimens grouped by condition of palatine teeth (t -test; $t = -1.91$, $p = 0.058$, $n = 268$, $df = 228.92$).

However, the distributions of ratios based on these grouping criteria substantially overlap.

Multivariate Results

In all specimen-partitioning schemes, relative contributions of each measurement to each component axis were similar. The 1st principal component (PC1), variation due to size, explained a large majority of variation (range of proportion of variance explained: 0.74–0.85). Slenderness versus stoutness of the caudal peduncle contributed most strongly to variation in PC2 (range of proportion of variance explained: 0.037–0.046). PC3 varied most in terms of the depth of the dorsal fins and the distance between the pectoral and pelvic fins (range of variance explained: 0.025–0.041). The overwhelming proportion of variation described

based on size suggests that these data have low effective dimensionality, that is, that the amount of variation captured by our measurements can be explained using comparatively few component axes and that variation in shape among specimens is low compared to body size.

Across all partitions, clear clustering according to prior ID or “diagnostic” characters was not evident. However, we did notice patterns according to diagnostic characters, irrespective of the lack of clustering. The unidentified *Cottus* spp. from Washington occupy the same distribution across PC1, PC2, and PC3 as *Cottus gulosus* and *Cottus perplexus*, arguing against the geographic distinctiveness of Washington populations (Fig. 4A, 4B). Individuals without palatine teeth occupy a subset of the variation of individuals with palatine teeth along PC1 and PC2. Very slight differentiation occurs along these axes, suggesting that specimens of *Cottus* with palatine teeth tend to be slightly larger, with stouter caudal peduncles, than those without palatine teeth (Fig. 4C). The distinctiveness of these characters is not extreme enough to be diagnostic, as the distribution of values overlaps considerably. When grouped by drainage, several individuals from the Sacramento–San Joaquin system occupy areas of morphospace that differentiate them from all other *Cottus* individuals measured (Fig. 4D).

A similar lack of concordance is exhibited in cluster analyses of the allometry-corrected head and body measurements. Group admixture occurred regardless of whether the grouping variable was prior ID, palatine tooth condition, or drainage (Fig. 5). These cluster analyses provide evidence that these grouping variables do not correspond with morphological differences.

Canonical variate analyses create slight separation between prior ID groups when individuals < 45 mm SL are excluded, with the variables contributing to SL weighted strongest (PC1 and PC19, Fig. 6A). The species-wise difference in mean score is statistically significant (t -test: $t = 8.29$, $P < 0.001$, $n = 113$, $df = 109.76$). Canonical discriminant analysis of palatine teeth condition with individuals < 45 mm SL excluded showed very slight separation based on SL, SID, and slenderness of caudal peduncle, which are greater in magnitude among individuals with palatine teeth compared to those without (PC1, 2, 18, and 19, Fig. 6B), with means that were also

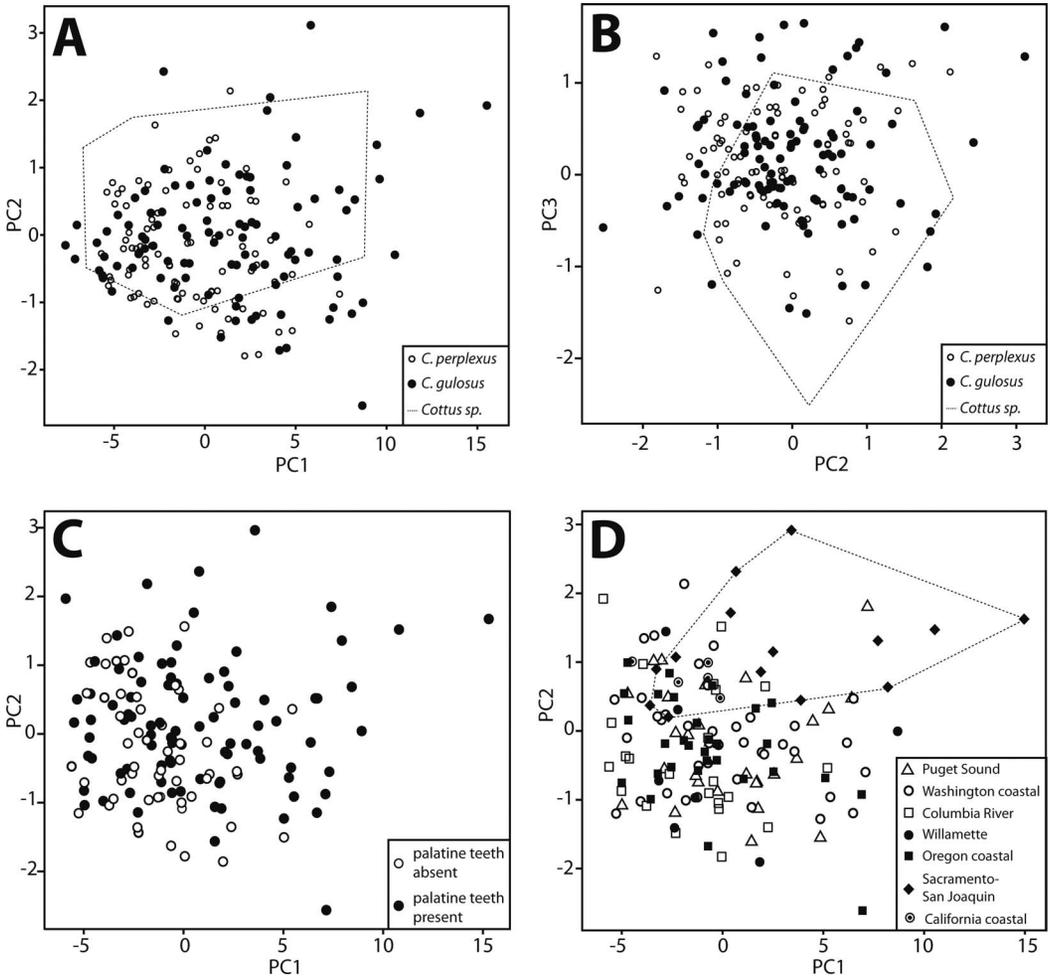


FIGURE 4. Scatterplots of body measurement principal component (PC) scores under different groupings of specimens. (A) PC 1 and 2 with individuals labeled according to prior species identification (prior ID), with extremes of the *Cottus* spp. group represented by a dashed convex hull; (B) PC 2 and 3 with individuals labeled according to prior ID, with extremes of the *Cottus* spp. group represented by a dashed convex hull; (C) PC 1 and 2 of individuals >45 mm SL, with individuals labeled according to palatine tooth condition; (D) PC 1 and 2 of individuals >45 mm SL, with individuals labeled according to the drainage in which they were collected. Sacramento-San Joaquin individuals sampled are emphasized using a dashed convex hull, for clarity.

significantly different (t -test: $t = 8.28, P < 0.001, n = 144, df = 128.83$). Despite this, the distribution of individuals across categories still overlaps considerably, meaning the categories are still undiagnosable based on these differences. Finally, only 66% of prior ID individuals were correctly identified to species using all measurement data as predictor variables in quadratic discriminant analyses, arguing against the utility of our measurements for matching current species designations.

DISCUSSION

Our goals were threefold: (1) assess the utility of previously cited diagnostic characters for these 2 species as they are currently defined; (2) determine whether multivariate statistical analyses uncover novel diagnostic-character combinations that coincide with current species definitions, previously cited diagnostic characters, or major drainages; and (3) apply these new characters, if found, to identifying the unidentified *Cottus* specimens (*Cottus* sp.) from western

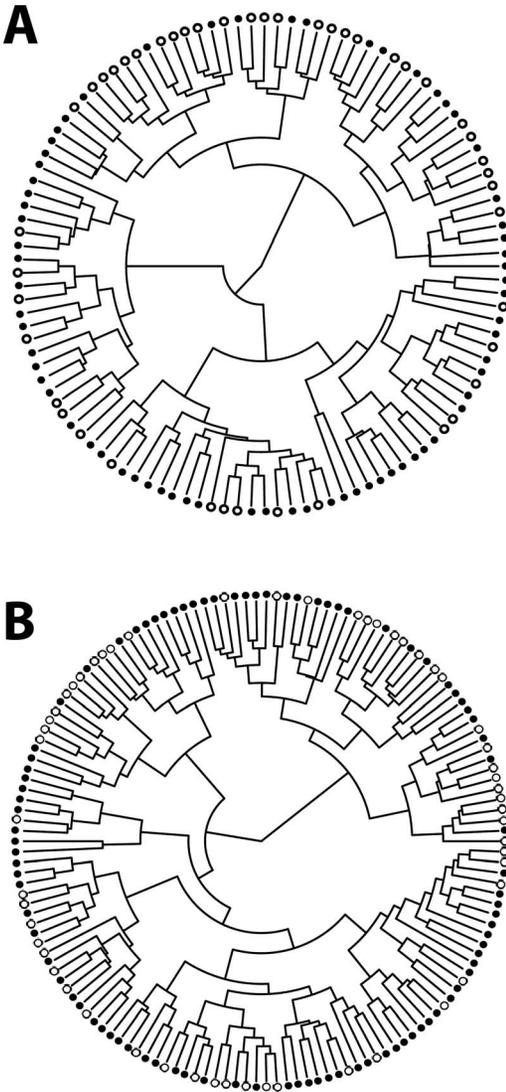


FIGURE 5. Dendrograms of hierarchical cluster analyses of allometry-corrected head and body measurement data. Differences between individuals are measured as the multivariate euclidean distance between measurements for each individual. Individuals are clustered based on Ward's (1963) algorithm, a hierarchical agglomerative algorithm that minimizes the addition of variance to each cluster. (A) Clustering based on prior species identification (prior ID). Black circles: *Cottus gulosus*; white circles: *Cottus perplexus*; (B) Clustering based on palatine teeth condition. Black circles: palatine teeth present; white circles: palatine teeth absent.

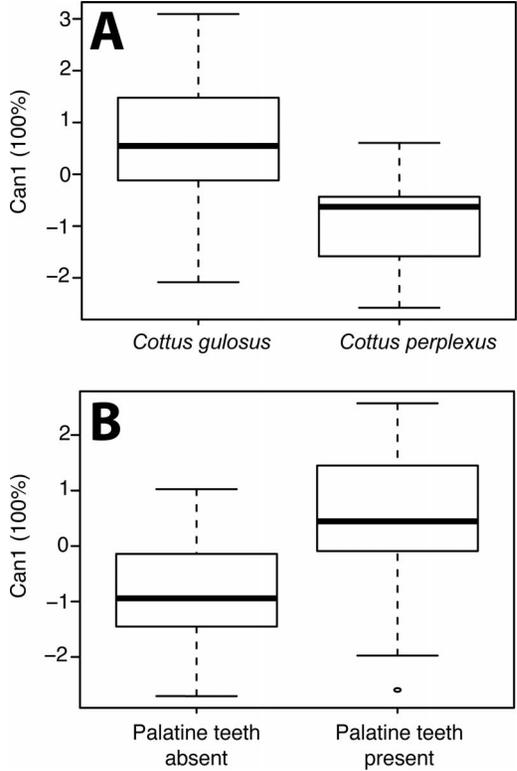


FIGURE 6. Boxplots illustrating distributions of canonical scores. (A) Specimens grouped by prior species identification (prior ID). PC 1 and 19 are most strongly correlated with the canonical scores (canonical correlations: 0.573 and -0.445, respectively), and the means between groups are significantly different (t -test: $t = 8.29$, $p < 0.001$, $n = 113$, $df = 109.76$); (B) Specimens grouped by palatine teeth condition. PC 1, 2, 19, and 18 are most strongly correlated with the canonical scores (canonical correlations: 0.472, 0.389, 0.371, and -0.327 respectively), with means which were also significantly different (t -test: $t = 8.28$, $p < 0.001$, $n = 144$, $df = 128.83$).

Washington collected in localities where the species occur in sympatry.

Our assessment of diagnosability using previously cited morphological characters found that the proportion of individuals with a median chin pore and palatine teeth differed significantly between species ($P < 0.003$ and $P < 0.001$, respectively); however, these are differences of proportion and do not represent simple diagnostic 'present' versus 'absent' conditions. Neither species was found to have fixed differences for either character. Furthermore, the specimens we obtained from OS were likely assigned to

species based solely on whether they possessed palatine teeth, given that all specimens identified as *Cottus gulosus* had palatine teeth and all *Cottus perplexus* specimens lacked palatine teeth. This includes the 4 individuals identified as *C. gulosus* from the Willamette system, a drainage in which Bond (1963) suggested *C. gulosus* was absent. Designating species based solely on a single character introduces circularity in the analysis when the character in question is not demonstrated to be autapomorphic. In this case, the designation of species identity based solely on palatine teeth elevates the potential for Type I error in a test of equal proportions, that is, false recovery of a significant relationship when none exists.

Similarly, we found significant differences between species in mean rMBR when specimens were grouped by prior ID or palatine tooth condition (Fig. 3). Like the discrete characters we examined, species overlap in their rMBR such that any one individual could not reliably be identified based on this character alone. Apart from size variation, caudal peduncle shape varied most strongly in our multivariate analyses. Wydoski and Whitney (2003) suggest that *C. gulosus* has a deeper caudal peduncle, but the slight separation along PC2 for our Prior ID data set suggests that *C. gulosus* have more slender (longer, shallower) caudal peduncles, and thus higher PC2 scores, than does *C. perplexus*. This is particularly the case for individuals collected in the Sacramento–San Joaquin drainage system (Fig. 4D).

Given the limited definitive diagnostic utility of any individual previously described character, we attempted to uncover character suites or linear combinations of characters that would prove useful in reliably diagnosing these species. Slight separation occurred along PC1 with respect to prior ID in terms of gross body size (PC1), and this measure was weighted most strongly in canonical discriminant analyses grouped by prior ID (Fig. 4A, Fig. 6A). Palatine tooth categories exhibit slight separation under canonical discriminant analysis driven primarily by component axes in which caudal peduncle length and width are strongly weighted. Again, however, none of the variables analyzed create diagnosable clusters, although we found significant differences in mean rMBR between the 2 categories for each grouping variable. Furthermore, we found no evidence for geographic

distinctiveness based on drainage, and our cluster analyses do not exhibit any groupwise clustering. Even examining multivariate differences between species transformed to maximize between-group variance failed to produce a reliable diagnostic tool, as evidenced by the poor performance of the quadratic discriminant function to accurately assign individuals to the correct species. Taken together with the results from our previous goal, we fail to identify reliable diagnostic characters of any sort to confirm existing species boundaries or delineate new ones.

Because of our inability to find morphological characters that allow discrete diagnosis of these 2 species and the poor performance of a quadratic discriminant function in categorizing previously identified *Cottus* specimens, the identity of the *Cottus* spp. obtained from Washington drainages where the 2 species are supposedly sympatric remains uncertain. Although more morphological characters could be studied to elucidate potentially uncaptured differences between these 2 *Cottus* species, we assert that a comprehensive, population-level, molecular phylogenetic study is warranted to validate the species boundaries of *Cottus gulosus* and *Cottus perplexus* in the absence of diagnostic morphological characters. In the meantime, biologists examining *Cottus gulosus* and *Cottus perplexus* from Washington should consider palatine tooth condition, MBR, and median chin pore counts in making identifications, with the caveat that a species designation based on morphology for any one individual will be tenuous given lack of clear diagnosability.

Recent phylogeographic studies in northern and central California have begun to unravel the tangled web of species relationships among *C. gulosus* and other sympatric *Cottus* species, recovering evidence for both ancient and recent hybridization as well as distinct genetic signatures in populations otherwise identified as *C. gulosus* (Baumsteiger and others 2012, 2014; Baumsteiger and Aguilar 2014). We have found evidence that may corroborate these findings in our multivariate analyses, specifically the clustering of *C. gulosus* from the Sacramento–San Joaquin system (Fig. 4D). Comprehensive phylogenetic studies incorporating *C. gulosus* and *C. perplexus* will determine whether introgression, hybridization, and cryptic speciation characterize the remainder of the range of *C. gulosus*.

Currently, we cannot reconstruct *C. gulosus* and *C. perplexus* as distinct, diagnosable species. However, because we did detect significant differences among several of our measurements, we conclude that at least 2 species are likely present, and that species boundaries and morphometric characters must be reevaluated in light of molecular genetic data. One potential reason behind this broad morphological overlap is recent divergence between *C. gulosus* and *C. perplexus*, allowing relatively little time for the 2 species to diverge morphologically. An ideal scenario is one in which a multi-locus phylogenetic study of these species is conducted, followed by *post hoc* analysis of morphometric data to identify morphological signatures that correspond with the molecular phylogeny.

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LITERATURE CITED

- BAILEY RM, BOND CE. 1963. Four new species of freshwater sculpins, genus *Cottus*, from western North America. *Occasional Papers of the Museum of Zoology, University of Michigan* 634:1–27.
- BAUMSTEIGER J, AGUILAR A. 2014. Impact of dams on distribution, population structure, and hybridization of two species of California freshwater sculpin (*Cottus*). *Conservation Genetics* 15:729–742.
- BAUMSTEIGER J, KINZIGER AP, AGUILAR A. 2012. Life history and biogeographic diversification of an endemic western North American freshwater fish clade using a comparative species tree approach. *Molecular Phylogenetics and Evolution* 65:940–952.
- BAUMSTEIGER J, KINZIGER AP, REID SB, AGUILAR A. 2014. Complex phylogeography and historical hybridization between sister taxa of freshwater sculpin (*Cottus*). *Molecular Ecology* 23:2602–2618.
- BOND CE. 1963. Distribution and ecology of freshwater sculpins, genus *Cottus*, in Oregon [dissertation]. Ann Arbor, MI: University of Michigan. 186 pp.
- BOND CE. 1973a. Occurrence of the Reticulate Sculpin, *Cottus perplexus*, in California, with distributional notes on *Cottus gulosus* in Oregon and Washington. *California Fish and Game* 59:93–94.
- BOND CE. 1973b. Keys to Oregon freshwater fishes. *Technical Bulletin of Oregon State University* 58:1–48.
- FISHER RA. 1936. The use of multiple measurements in taxonomic problems. *Annals of Eugenics* 7:179–188.
- FOX J, WEISBERG S. 2011. An R companion to applied regression (2nd edition). Thousand Oaks, CA: Sage. <http://socserv.socsci.mcmaster.ca/jfox/Books/Companion>.
- FRIENDLY M, FOX J. 2015. Candisc: visualizing generalized canonical discriminant and canonical correlation analysis. R package version 0.6-7. <http://CRAN.R-project.org/package=candisc>
- GILBERT CH, EVERMANN, BW. 1894. A report upon investigations in the Columbia River Basin, with descriptions of four new species of fishes. *Bulletin of the United States Fish Commission* 14:169–207.
- GIRARD C. 1854. Descriptions of new fishes, collected by Dr. A. L. Heermann, naturalist attached to the survey of the Pacific railroad route, under Lieut. R. S. Williamson, U.S.A. *Proceedings of the Academy of Natural Sciences of Philadelphia* 7:1854–1855.
- KINZIGER AP, WOOD RM, NEELY DA. 2005. Molecular systematics of the genus *Cottus* (Scorpaeniformes: Cottidae). *Copeia* 2005:303–311.
- LEE DS, GILBERT CR, HOCUTT CH, JENKINS RE, McALLISTER DE, STAUFFER JR (editors). 1980. Atlas of North American freshwater fishes. Raleigh, NC: North Carolina State Museum of Natural History. 867 p.
- McPHAIL JD. 2007. The Freshwater Fishes of British Columbia. Edmonton, Alberta: University of Alberta Press. 620 p.
- MOYLE PB. 2002. Inland Fishes of California. Berkeley, CA: University of California Press. 507 p.
- PARADIS E, CLAUDE J, STRIMMER K. 2004. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* 20:289–290.
- R CORE TEAM. 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>
- REIMERS PE, BOND CE. 1967. Distribution of fishes in tributaries of the lower Columbia River. *Copeia* 1967:541–550.
- ROBINS CR, MILLER RR. 1957. Classification, variation, and distribution of the sculpins, genus *Cottus*, inhabiting Pacific slope waters, in California and Oregon, with a key to the species. *California Fish and Game* 43:213–233.
- SCHULTZ LP. 1930. Notes on the species of *Cottus* in western Washington. *Copeia* 1930:14–15.

- SNYDER JO. 1907. The fishes of the coastal streams of Oregon and northern California. *Bulletin of the Bureau of Fisheries* 27:153–189.
- TABOR RA, FRESH KL, PAIGE DK, WARNER EJ, PETERS RJ. 2007. Distribution and habitat use of cottids in the Lake Washington Basin. *American Fisheries Society Symposium* 53:135–150.
- VENABLES WN, RIPLEY BD. 2002. *Modern applied statistics with S*, 4th Ed. New York, NY: Springer.
- WARD JH JR. 1963. Hierarchical grouping to optimize an objective function. *Journal of the American Statistical Association* 58:236–244.
- WICKHAM H. 2007. Reshaping data with the reshape package. *Journal of Statistical Software* 21:1–20.
- WICKHAM H. 2009. *Ggplot2: elegant graphics for data analysis*. New York, NY: Springer-Verlag.
- WILSON EB. 1927. Probable inference, the law of succession, and statistical inference. *Journal of the American Statistical Association* 22:209–212.
- WYDOSKI RS, WHITNEY RR. 2003. *Inland Fishes of Washington*. Seattle, WA: University of Washington Press. 322 p.
- YOUNG MK, MCKELVEY KS, PILGRIM KL, SCHWARTZ MK. 2013. DNA barcoding at riverscape scales: assessing biodiversity among fishes of the genus *Cottus* (Teleostei) in northern Rocky Mountain streams. *Molecular Ecology Resources* 13:583–595.

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APPENDIX. Voucher number, drainage, locality and number of individuals sampled from each locality for study.

Species	Voucher number	Drainage	Locality	<i>n</i>
<i>Cottus gulosus</i>	UW 019808	Puget Sound	Skykomish River, WA	1
<i>Cottus gulosus</i>	UW 111077	Puget Sound	May Creek, WA	5
<i>Cottus gulosus</i>	UW 020065	Puget Sound	Soos Creek, WA	5
<i>Cottus gulosus</i>	UW 020471	Puget Sound	Newaukum Creek, WA	1
<i>Cottus gulosus</i>	PLUNHM F0247	Puget Sound	Clover Creek, WA	1
<i>Cottus gulosus</i>	UW 018233	Washington coastal streams	Conner Creek, WA	5
<i>Cottus gulosus</i>	PLUNHM F0477	Washington coastal streams	Hoh River, WA	1
<i>Cottus gulosus</i>	UW 019782	Washington coastal streams	Chehalis River, WA	4
<i>Cottus gulosus</i>	UW 112373	Washington coastal streams	Deep Creek, WA	9
<i>Cottus gulosus</i>	UW 112354	Washington coastal streams	Halfway Creek, WA	5
<i>Cottus gulosus</i>	OS 010544	Columbia River	Klaskanine River, WA	5
<i>Cottus gulosus</i>	UW 029618	Columbia River	Columbian River, WA	3
<i>Cottus gulosus</i>	UW 000941	Columbia River	Kalama River, WA	5
<i>Cottus gulosus</i>	OS 016098	Willamette River	Mohawk River, OR	2
<i>Cottus gulosus</i>	OS 010047	Willamette River	Pudding Creek, OR	1
<i>Cottus gulosus</i>	OS 016213	Willamette River	Willamette River, OR	1
<i>Cottus gulosus</i>	OS 005846	Oregon coastal streams	Necanicum River, OR	5
<i>Cottus gulosus</i>	OS 009350	Oregon coastal streams	Tenmile Creek, OR	4
<i>Cottus gulosus</i>	OS 016491	Oregon coastal streams	Camp Creek, OR	4
<i>Cottus gulosus</i>	OS 000355	Oregon coastal streams	Swamp Creek, OR	4
<i>Cottus gulosus</i>	OS 000835	Oregon coastal streams	Yachats River, OR	1
<i>Cottus gulosus</i>	OS 009039	Oregon coastal streams	Smith River, OR	5
<i>Cottus gulosus</i>	OS 000584	Oregon coastal streams	Little River, OR	2
<i>Cottus gulosus</i>	OS 005843	Oregon coastal streams	Umpqua River, OR	3
<i>Cottus gulosus</i>	CAS 023556	Oregon coastal streams	Cold Creek, OR	1
<i>Cottus gulosus</i>	LACM 024646	Sacramento/San Joaquin	Deer Creek, CA	5
<i>Cottus gulosus</i>	LACM 024801	Sacramento/San Joaquin	Antelope Creek, CA	4
<i>Cottus gulosus</i>	LACM 024822	Sacramento/San Joaquin	Butte Creek, CA	1
<i>Cottus gulosus</i>	LACM 045413-1	Sacramento/San Joaquin	Domingo Creek, CA	5
<i>Cottus gulosus</i>	CAS 022868	California Coastal Streams	Kaweah River, CA	3
<i>Cottus gulosus</i>	LACM 025000	California Coastal Streams	Little Arthur Creek, CA	5
<i>Cottus perplexus</i>	UW 111169	Puget Sound	Clover Creek, WA	1
<i>Cottus perplexus</i>	UW 041224	Washington coastal streams	Coal Creek, WA	5
<i>Cottus perplexus</i>	UW 029612	Washington coastal streams	Ziegler Creek, WA	2
<i>Cottus perplexus</i>	UW 020337	Washington coastal streams	Satsop River, WA	2
<i>Cottus perplexus</i>	UW 111056	Washington coastal streams	Delezene Creek, WA	5
<i>Cottus perplexus</i>	UW 111083	Washington coastal streams	Porter Creek, WA	5
<i>Cottus perplexus</i>	UW 112369	Washington coastal streams	Stowe Creek, WA	1

APPENDIX. Continued.

Species	Voucher number	Drainage	Locality	<i>n</i>
<i>Cottus perplexus</i>	USNM 45387	Washington coastal streams	Skookumchuck River, WA	1
<i>Cottus perplexus</i>	UW 041238	Washington coastal streams	Naselle River, WA	2
<i>Cottus perplexus</i>	UW 020344	Columbia River	Lacamas Creek, WA	5
<i>Cottus perplexus</i>	OS 016506	Columbia River	Clatskanie River, WA	4
<i>Cottus perplexus</i>	UW 111130	Columbia River	Packard Creek, WA	5
<i>Cottus perplexus</i>	OS 016085	Willamette River	Mohawk River, OR	5
<i>Cottus perplexus</i>	OS 018451	Willamette River	Little Pudding River, OR	3
<i>Cottus perplexus</i>	OS 009744	Oregon coastal streams	Nehalem River, OR	5
<i>Cottus perplexus</i>	OS 000394	Oregon coastal streams	Trask River, OR	4
<i>Cottus perplexus</i>	OS 009046	Oregon coastal streams	Coos River, OR	2
<i>Cottus perplexus</i>	OS 006171	Oregon coastal streams	Yaquina River, OR	5
<i>Cottus perplexus</i>	OS 000354	Oregon coastal streams	Siuslaw River, OR	5
<i>Cottus perplexus</i>	OS 016128	Oregon coastal streams	Alea River, OR	5
<i>Cottus perplexus</i>	OS 005329	Oregon coastal streams	Beamer Creek, OR	5
<i>Cottus perplexus</i>	OS 009047	Oregon coastal streams	Vincent Creek, OR	3
<i>Cottus perplexus</i>	OS 009995	Oregon coastal streams	Rogue River, OR	5
<i>Cottus perplexus</i>	OS 017095	Oregon coastal streams	Rogue River, OR	5
<i>Cottus</i> spp.	PLUNHM F0705	Puget Sound	Green River, WA	9
<i>Cottus</i> spp.	PLUNHM F0710	Puget Sound	Prairie River, WA	1
<i>Cottus</i> spp.	PLUNHM F0724	Puget Sound	Nisqually River, WA	5
<i>Cottus</i> spp.	PLUNHM F0726	Puget Sound	Nisqually River, WA	5
<i>Cottus</i> spp.	PLUNHM F0687	Puget Sound	Deschutes River, WA	3
<i>Cottus</i> spp.	PLUNHM F0673	Washington coastal streams	Bogachiel River, WA	6
<i>Cottus</i> spp.	PLUNHM F0669	Washington coastal streams	Calawah River, WA	4
<i>Cottus</i> spp.	PLUNHM F0667	Washington coastal streams	Humptulips River, WA	3
<i>Cottus</i> spp.	PLUNHM F0677	Washington coastal streams	Satsop River, WA	3
<i>Cottus</i> spp.	PLUNHM F0713	Washington coastal streams	Newaukum River, WA	5
<i>Cottus</i> spp.	PLUNHM F0714	Washington coastal streams	Newaukum River, WA	5
<i>Cottus</i> spp.	PLUNHM F0718	Columbia River	Salmon Creek, WA	3
<i>Cottus</i> spp.	PLUNHM F0682	Columbia River	Elochoman River, WA	22