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# Recent, small beginnings: genetic analysis suggests *Catostomus rimiculus* (Klamath smallscale sucker) in the Smith River, California, are introduced

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## Abstract

Identification of introduced species can be important to understanding ecological systems and meeting conservation and management goals, but the process can be surprisingly challenging. The Klamath smallscale sucker *Catostomus rimiculus* seems likely to be native to the Smith River because the drainage separates two basins believed to be within the fish's native range, the Rogue and Klamath rivers. Further, *C. rimiculus* is broadly distributed in the Smith River, and the indigenous Dee-ni' People of the Smith River have a unique word for sucker. Nonetheless, a historical survey of fishes that described *C. rimiculus* from the Rogue and Klamath rivers did not include *C. rimiculus* among the fishes of the Smith River. To determine whether the genetic structure of the Smith River *C. rimiculus* reflects expectations for a native sucker population, the authors of this study examined variation in microsatellite and mitochondrial genetic markers from the Smith River and surrounding drainages. The genetic analyses revealed a pattern consistent with extreme founder effects in Smith River *C. rimiculus*, as would be expected from a single introduction of six or fewer effective individuals. The sharing of a high-frequency haplotype between the Smith River and Klamath River that is not detected in the Rogue River suggests the Klamath River as the likely source for the introduction. The findings highlight that local-scale introductions can be easily overlooked because the newly established populations can appear to be parts of contiguous natural distributions.

**KEYWORDS**
catostomidae, DNA, introduced species, Klamath smallscale sucker, microsatellites, mitochondrial

## 1 | INTRODUCTION

Identification of introduced species is a necessary step in enhancing the understanding of their colonization pathways, probability of establishment and ecological effects. In some cases, determining whether a species is native or introduced can be surprisingly difficult (*e.g.*, Scott *et al.*, 2009). These cases most often occur when the distribution of populations of interest abut areas almost certain to be within the native range of a species. Drainages adjacent to the native ranges of freshwater fishes may frequently present favourable introduction opportunities for those fishes because they are likely to offer suitable habitat. The relative ease of local introductions makes it reasonable to hypothesize that they are more common than intercontinental or across-continent introductions. Like longer-distance introductions, local-scale transfers have the potential to harm native fauna. For example, Sacramento pikeminnow (*Ptychocheilus grandis*) in the Eel River of northwestern California have been introduced from an adjacent drainage (Kinziger

  

*et al*., 2014) to the detriment of native sculpins (White & Harvey, 2001).

The objective of this study was to evaluate the status (*e.g*., native or introduced) of the Klamath smallscale sucker (*Catostomus rimiculus*) in the Smith River of northern California, U.S.A. A survey of fishes in the late 1890s encountered *C. rimiculus* in the Rogue River of southern Oregon and the Klamath River of southern Oregon and northern California, but did not include any catostomids in the species list for the Smith River, a coastal river that bisects the Rogue and Klamath drainages (Snyder, 1908) (Figure 1). Monroe *et al*. (1975) recognized the occurrence of catostomids in the Smith River in a resource agency report. White and Harvey (1999) were apparently the first authors to note in the scientific literature the occurrence of *C. rimiculus* in the Smith River; they assumed it was a native species. *C. rimiculus* represents the only obligate freshwater dispersant fish known from the Smith River.

The absence of catostomids from the Smith River in historical records suggests that the current population is the result of a recent introduction. Nonetheless, it is possible that *C. rimiculus* was present but undetected in historical field surveys (*e.g*., MacKenzie *et al*., 2003). Three additional observations support the hypothesis that *C. rimiculus* is native to the Smith River: (a) the Smith River is not an outlier population but instead geographically sandwiched between the Rogue and Klamath rivers, (b) *C. rimiculus* are currently broadly distributed in the Smith River and (c) the Smith-River dialect of the language of the indigenous Dee-ni' People includes a possibly unique word for sucker (da'-srch'e). Nonetheless, on the last point, indigenous people of the Smith River had frequent contact with people occupying the Rogue River drainage, and available documentation of the local language includes words for non-native taxa.

The authors conducted a genetic analysis to evaluate the status of *C. rimiculus* in the Smith River, California. They examined microsatellite and mitochondrial DNA from *C. rimiculus* collected from the Smith, Rogue and Klamath rivers. If *C. rimiculus* has been introduced, the authors would expect to observe founder effects (*e.g*., reductions in genetic diversity and shifts in allele/haplotype frequency; *e.g*., Dlugosch & Parker, 2008), whereas if the species is native, they would expect genetic structuring to be consistent with patterns from the other populations in the species range.

## 2 | MATERIALS AND METHODS

### 2.1 | Microsatellite genotyping and analysis

A total of 274 *C. rimiculus* were genotyped at 15 microsatellite loci described by Tranah *et al*. (2001), including 190 individuals from the Smith River, 37 from the Klamath River and 47 from the Rogue River (Supporting Information Table S1). Genomic DNA was extracted from fin tissue using chelex methods (Walsh *et al*., 1991). PCR volumes and thermocycling conditions followed Tranah *et al*., 2001 (Supporting Information Table S2). Microsatellite allele sizes were determined using the Beckman Coulter CEQ 8000 Genetic Analysis System. Allele sizes were determined from visual inspection of electropherograms.

Tests for conformance to Hardy–Weinberg expectations were conducted for each locus in each population using the Markov chain Monte Carlo approximation of Fisher's exact test implemented in the software GENEPOP version 4.0.10 (Raymond & Rousset, 1995; Rousset, 2008). Critical values were corrected for multiple tests using the Bonferroni method (Rice, 1989). The software Genotype Viewer (Steven Kalinowski, Unpubl., available at: http://www.montana.edu/kalinowski/software/genotypeviewer.html) was used to calculate Hardy–Weinberg expected heterozygosity and allele frequency differentiation between population pairs ($F_{ST}$). Uncorrected allelic richness,

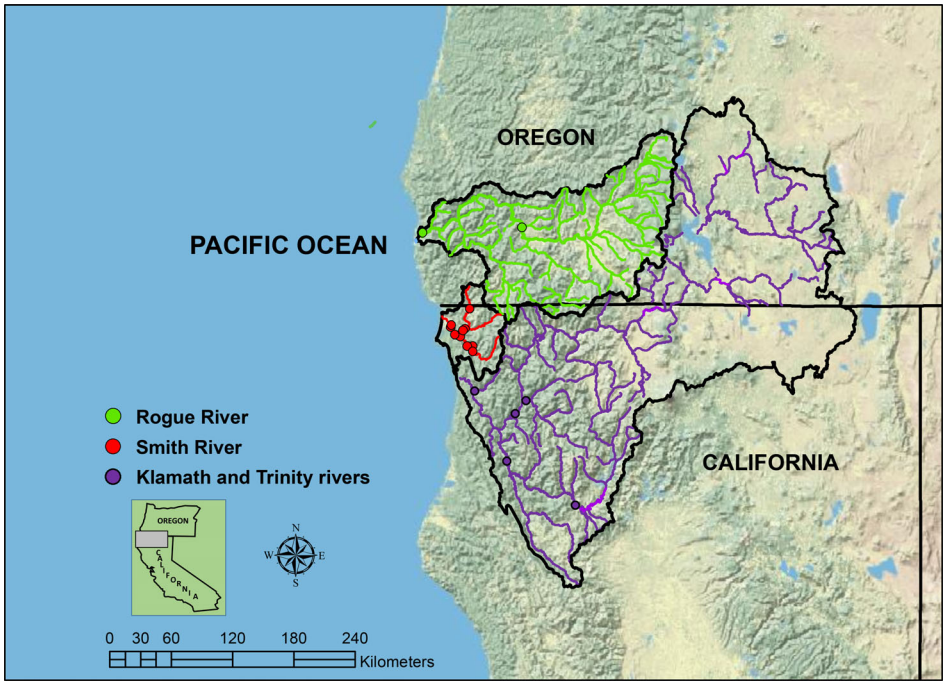


**FIGURE 1** The geographic distribution of *Catostomus rimiculus* in northern California and southern Oregon. Collection locations used for microsatellite and mitochondrial DNA analysis (circles) from the Smith River (red), Klamath River (purple) and Rogue River (green)

corrected allelic richness and corrected private allelic richness were estimated using the software HP-RARE 1.0 (Kalinowski, 2005). Corrected allelic richness estimates were equalized to a sample size of 62 genes using rarefaction. To generate graphical descriptions of allele frequency divergence between populations, the authors conducted discriminant analysis of principal components (DAPC) (Jombart *et al*., 2010) using the adegenet package (Jombart, 2008; Jombart & Ahmed, 2011) for the statistical software R (R Development Core Team, 2019).

Because the analysis of this study supported the introduced status for Smith River *C. rimiculus*, the authors used the coalescent-based maximum likelihood methods implemented in the software COALIT and NFCONE (Anderson & Slatkin, 2007) to estimate the effective founder number. Given no information on the demographic history of the population, the authors explored a range of reasonable demographic parameter values and evaluated their effects on the estimate of founder numbers. As indicated by the analysis, the Klamath River was designated as the source population. The number of generations between the introduction and the field collections was set to 5, 10 and 20. This range brackets the uncertainty in the introduction date, which presumably occurred sometime between Snyder's (1908) observation of the absence of *C. rimiculus* from the Smith River and the first record (Monroe *et al*., 1975). Thus, given a generation time of *c*. 5–7 years for *C. rimiculus* (Moyle, 2002), the authors of this study explored founding from *c*. 25 to 140 years before the field collections (2013–2017). They assumed carrying capacities for *C. rimiculus* in the Smith River of 5000 and 16,000, derived from their personal observations. They explored intrinsic rates of increase of 0.25 and 0.50. These values seemed reasonable given that Miller *et al*. (2013) estimated an intrinsic rate of increase of 0.25 for a *Catostomus commersoni* (white sucker) population, whereas fecundity estimates for *C. rimiculus* (15,300–20,000 eggs per female, for fish measuring 35–38 cm standard length) suggest that a rapid increase in abundance is possible (Moyle, 2002). The authors generated estimates of founder number for all 12 combinations of generations since founding (5, 10 and 20), carrying capacity (5000 and 16,000) and intrinsic rate of increase (0.25 and 0.50).

## 2.2 | Mitochondrial DNA sequencing and analysis

The authors compiled mitochondrial DNA cytochrome *b* sequence data from a total of 124 individuals, combining 34 individuals from GenBank with an additional 90 individuals sequenced for this study (Figure 1; Supporting Information Tables S1 and S3). They analysed 25 individuals from the Smith River, 27 individuals from the Rogue River and 60 individuals from the Klamath River. They also included one individual each of upper Klamath River endemic sucker species, *Deltistes luxatus*, *Chasmistes brevirostris* and *Catostomus snyderi*, because they may hybridize with *C. rimiculus* (Markle *et al*., 2005; Tranah & May, 2006). Finally, nine *Catostomus occidentalis* sequences were included because the geographic range of this species abuts the southern edge of *C. rimiculus* geographic range. The *C. occidentalis* included seven

**TABLE 1** Microsatellite and mitochondrial DNA diversity in *Catostomus rimiculus* from northern California and southern Oregon

| | | | Microsatellite DNA diversity | | | | Mitochondrial DNA diversity | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Population | Site | N | Private allelic richness | Rarefied allelic richness | Uncorrected allelic richness | Expected heterozygosity | N | Number of segregating sites | Number of haplotypes | Haplotype diversity | Average number of differences | Nucleotide diversity |
| Smith River | SM | 190 | 0.56 | 4.41 | 6.20 | 0.61 | 25 | 6 | 6 | 0.42667 | 0.89333 | 0.00087 |
| Klamath River | KL | 37 | 5.84 | 19.27 | 20.13 | 0.92 | 60 | 18 | 15 | 0.75254 | 2.03616 | 0.02216 |
| Rogue River | RO | 47 | 3.53 | 16.68 | 18.07 | 0.90 | 27 | 23 | 13 | 0.85185 | 3.99430 | 0.00393 |

sequences from GenBank (Supporting Information Table S3) and two sequences generated herein (Supporting Information Table S1).

Mitochondrial DNA was extracted from fin tissue using chelex methods (Walsh *et al*., 1991). The authors sequenced a 1023 base-pair fragment of the mitochondrial DNA gene cytochrome *b* gene using the PCR primers, thermocycling conditions and sequencing primers described by Kettratad and Markle (2010). Amplified PCR products were Sanger sequenced at MCLAB (San Francisco, California). DNA sequences were aligned in MEGAX (Kumar *et al*., 2018) using MUSCLE (Edgar, 2004). The number of segregating sites, number of haplotypes, haplotype diversity, average number of nucleotide differences within sites, nucleotide diversity and the pair-wise number of nucleotide differences between sites were calculated using the software DNASP v6 (Rozas *et al*., 2017). The relationships among haplotypes were evaluated and depicted by constructing a statistical parsimony network using PopART v1.7 (Clement *et al*., 2002; Leigh & Bryant, 2015).

**TABLE 2** Maximum likelihood estimates (MLE) of the effective number of *Catostomus rimiculus* individuals founding the Smith River and 95% support limits for all 12 combinations of generations since founding (T), carrying capacity (NK) and intrinsic rate of increase (R)

| MLE | Support limits | T | NK | R |
|---|---|---|---|---|
| 4.06 | 3.51–4.63 | 5 | 5000 | 0.25 |
| 4.07 | 3.51–4.63 | 5 | 16,000 | 0.25 |
| 4.77 | 4.2–5.54 | 10 | 5000 | 0.25 |
| 4.77 | 4.2–5.53 | 10 | 16,000 | 0.25 |
| 5.07 | 4.4–5.86 | 20 | 5000 | 0.25 |
| 5.07 | 4.39–5.83 | 20 | 16,000 | 0.25 |
| 3.03 | 2.76–3.31 | 5 | 5000 | 0.5 |
| 3.03 | 2.76–3.31 | 5 | 16,000 | 0.5 |
| 3.12 | 2.84–3.48 | 10 | 5000 | 0.5 |
| 3.12 | 2.83–3.47 | 10 | 16,000 | 0.5 |
| 3.14 | 2.85–3.51 | 20 | 5000 | 0.5 |
| 3.13 | 2.84–3.50 | 20 | 16,000 | 0.5 |

## 3 | RESULTS

### 3.1 | Microsatellite

The 15 microsatellite loci were highly polymorphic, containing a total of 374 alleles (mean 24.9 alleles per locus; range 16–35). Of a total of 45 tests (15 loci and 3 populations), three loci in the Rogue River did not conform to Hardy–Weinberg proportions, following Bonferroni correction for multiple tests (critical value = 0.0011). The authors suspect that these departures resulted from tissue degradation. The

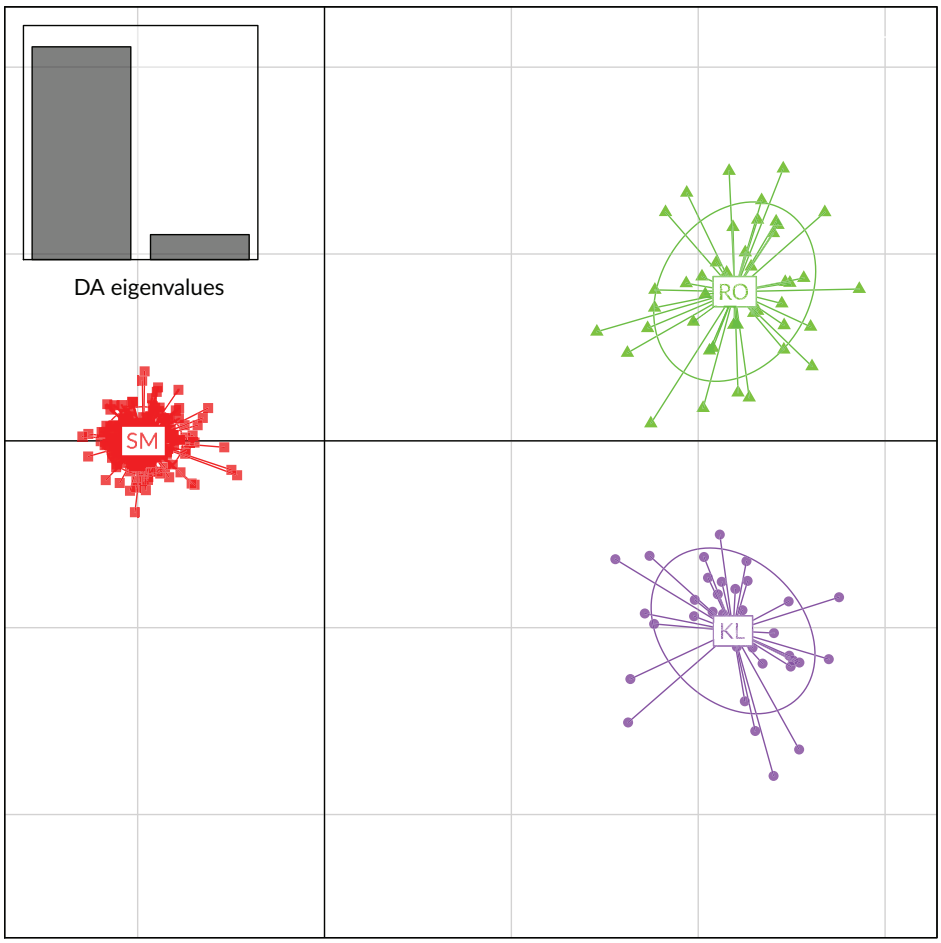


**FIGURE 2** Scatterplot from discriminant analysis of principal components analysis of *Catostomus rimiculus* microsatellite genotypes. The populations, Smith River (SM, squares, red), Klamath River (KL, circles, purple) and Rogue River (RO, triangles, green), are labelled inside their 95% inertia ellipses. Dots represent individuals. The inset indicates the eigenvalues of the first two principal components

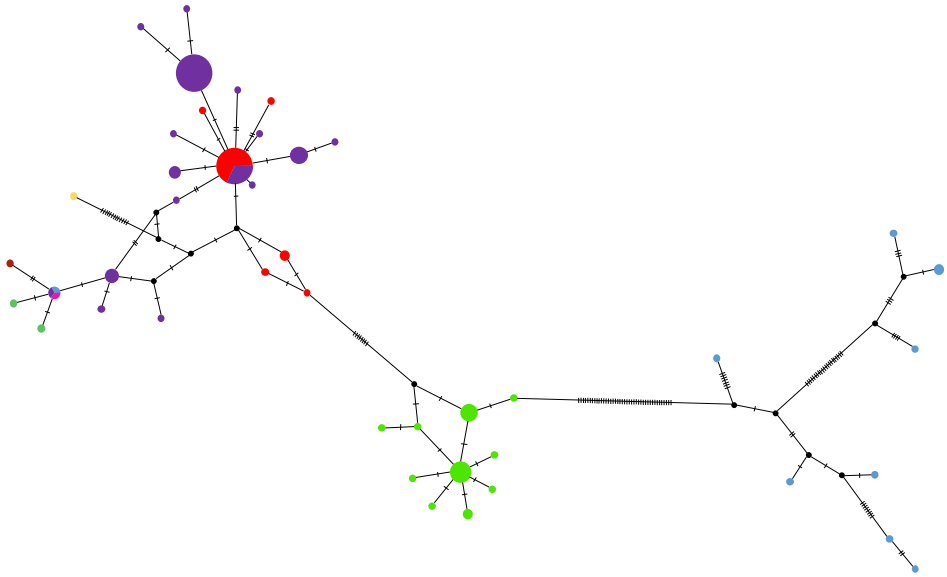

**FIGURE 3** Statistical parsimony network for *Catostomus rimiculus* constructed using mitochondrial DNA haplotypes. Each hatch along a branch indicates a single nucleotide substitution and the dots are inferred substitutions. The circle diameters are proportional to haplotype frequency. ◌, Ten Samples, One Sample; ●, Smith River; ●, Rogue River; ●, Klamath/Trinity River; ●, *Deltistes luxatus*; ●, *Catostomus brevirostris*; ●, *Catostomus snyderi*; ●, *Catostomus occidentalis*

**TABLE 3** Examples of introductions established by few founders

| Species | Introduction | Founder number | Citation |
|---|---|---|---|
| Speckled dace, (*Rhinichthys osculus*) | Trinity River to Van Duzen River, California | 10 EF | Kinziger *et al*., 2011 |
| Lake trout (*Salvelinus namaycush*) | Flathead Lake to Swan Lake, Montana | 2 EF | Kalinowski *et al*., 2010 |
| Sacramento pikeminnow (*Ptychocheilus grandis*) | Sacramento River to Eel River, California | 3–4 EF | Kinziger *et al*., 2014 |
| Bluegill sunfish (*Lepomis macrochirus*) | Iowa, U.S.A., to Japan | 15 EF | Kawamura *et al*., 2006 |
| Leon Springs pupfish (*Cyprinodon bovinus*) | Roughly equal number of males and females founded in hatchery mesocosms and left undisturbed | 80 C | Edds and Echelle (1989) |
| Comanche Springs pupfish (*Cyprinodon elegans*) | Roughly equal number of males and females founded in hatchery mesocosms and left undisturbed | 40 C | Edds and Echelle (1989) |
| Pecos gambusia (*Gambusia nobilis*) | Roughly equal number of males and females founded in hatchery mesocosms and left undisturbed | 30–40 C | Edds and Echelle (1989) |
| Northern pike (*Esox lucius*) | Illegal introduction into Lake Davis, California, from an unknown source | 7–12 EF | Aguilar *et al*. (2005) |
| Fountain darter (*Etheostoma fonticola*) | Thought to be extirpated from Comal River, reintroduced in the early 1970s using 457 individuals from the San Marcos River. | 457 C<br>49 EF | Olsen *et al*. (2016) |
| Klamath smallscale sucker (*Catostomus rimiculus*) | Klamath River to Smith River, California | <6 EF | This study |

*Note*. C: census counts from field observations; EF: effective founder number estimated from genetic analysis.

Rogue River collection (OS 15913) dates to 1993, and the DNA was analysed more than 20 years later. The Rogue River collection also exhibited the highest proportion of missing genotypes (the number of missing genotypes divided by the total number of genotypes possible): 0.087 for the Rogue River, 0.041 for the Klamath River and 0.016 for the Smith River.

The Smith River *C. rimiculus* exhibited particularly low genetic diversity, including only one-quarter of the rarified allelic richness in both the Klamath and Rogue river collections (4.41 *vs*. 19.27 and 16.68, respectively), and a 30% reduction in expected heterozygosity in comparison to the Klamath and Rogue rivers (0.62 *vs*. 0.90 and 0.92, respectively; Table 1). Estimates of pair-wise genetic differentiation indicated that the Smith River *C. rimiculus* were more divergent from the Klamath ($F_{ST}$ 0.1801) and the Rogue rivers ($F_{ST}$ 0.1934) than the Klamath and Rogue were from each other ($F_{ST}$ 0.0260). In the DAPC, 90.1% of the total genetic variation was captured by the first 69 principal components of principal components analysis, and these were used as input to discriminant analysis (DA). The eigenvalues resulting from DA indicated that the first axis captured the majority of genetic structure (89.5%) and separated the Smith River from the Klamath and

Rogue rivers (Figure 2). The second axis captured less genetic structure (10.5%) and separated the Klamath River from the Rogue River.

The estimated number of founders establishing the Smith River *C. rimiculus* population ranged between 3 and 5 (2.76–5.86), depending upon the parameter inputs (Table 2). Additional analyses using an intrinsic rate of increase >1 and generation time <5 resulted in errors, indicating the improbability of these values given the data.

### 3.2 | Mitochondrial

The final cytochrome *b* alignment included 118 polymorphic sites, 80 parsimony informative sites, 38 singleton variable sites and 42 different haplotypes. The Smith River *C. rimiculus* contained strikingly less mitochondrial DNA diversity for all metrics in comparison to the Klamath and Rogue rivers (Table 1). The 25 individuals from the Smith River yielded six haplotypes, including one haplotype that occurred at high frequency in the Smith River (0.76) and the Klamath River (0.15) but was not detected in the Rogue River. The remaining five haplotypes from the Smith River were not shared with the other locations; all occurred at low frequency (0.04–0.08) (Figure 3).

Consistent with previous research, *C. rimiculus* appears to share haplotypes with upper Klamath River endemic sucker species (Dowling *et al*., 2016; Kettratad, 2008; Kettratad & Markle, 2010). Nonetheless, unlike previous studies, the authors of this study found that *C. rimiculus* from both the Klamath River and Rogue River, not just the Klamath River, share mtDNA lineages with upper Klamath River species (Figure 3).

## 4 | DISCUSSION

The patterns of genetic structuring resolved in this study for *C. rimiculus* in the Smith River correspond with expectations for a species introduction. The authors of this study resolved extreme reductions in microsatellite and mitochondrial diversity in the Smith River in comparison to the Klamath and Rogue rivers. In addition, the Smith River population exhibited substantial allele frequency divergence in comparison to the Klamath and Rogue river populations, in contrast to the lesser divergence between the other two populations. The combined reduction in genetic diversity and shifts in allele frequency are consistent with a severe reduction in population size (Allendorf, 1986; Dlugosch & Parker, 2008; Spencer *et al*., 2000) that the authors of this study hypothesize occurred during the introduction of *C. rimiculus* to the Smith River. This conclusion is consistent with the historical survey data that failed to detect catostomids in the Smith River (Snyder, 1908).

One part of the results of this study appears to conflict with the hypothesis that Smith River *C. rimiculus* are introduced: the occurrence of private microsatellite alleles and mtDNA haplotypes in Smith River *C. rimiculus*. Nonetheless, the numbers of individuals and locations examined in this study were almost certainly insufficient to fully inventory genetic variation in the likely source areas (*e.g*., Klamath and Rogue rivers). When sampling is insufficient and imbalanced (*e.g*., see Table 1), it is not surprising to find unique variation in introduced populations (Hänfling *et al*., 2002; Johnson *et al*., 2011; Kelly *et al*., 2006; Kinziger *et al*., 2019; Marques *et al*., 2016; Vidal *et al*., 2010). The possibility remains that the results of this study reflect a bottleneck event rather than a founder effect associated with an introduction. Nonetheless, it seems unlikely that even the most significant disturbances in the Smith River in the recent past, most probably historic floods in the 1950s and 1960s, would have reduced *C. rimiculus* numbers to the extent required to explain the genetic patterns that were observed (*e.g*., reductions in genetic diversity and shifts in allele frequency).

The results of this study show that *C. rimiculus* from the Klamath River most likely founded the Smith River population. The sharing of a high frequency mitochondrial DNA haplotype between the Smith River and Klamath River and the divergence of Smith/Klamath haplotypes from those in the Rogue River (Figure 3) both support this assertion. To a lesser extent, the microsatellite results also suggest a Klamath River source for the Smith River population, given lower genetic differentiation between the Smith and Klamath rivers ($F_{ST}$ 0.1896) than between the Smith and Rogue rivers ($F_{ST}$ 0.2017). The absence of any evidence for admixture from multiple founding sources (Figure 2) combined with the large reductions in genetic diversity suggests that a single transfer established the introduced population, rather than multiple introductions from the same or different sources (*e.g*., Roman & Darling, 2007).

The authors estimated that six or fewer individuals founded the introduced Smith River *C. rimiculus* population. This finding adds to a large number of examples from natural and experimental settings that illustrate the potential for few individuals to establish new populations (Table 3). Nonetheless, it should be noted that the frequency of successful invasion by a small number of founders is difficult to assess because many introduced populations that fail to become established probably remain undocumented. Elucidating the mechanisms that enable populations with limited genetic diversity to flourish represents a critical unknown in invasion genetics (Dlugosch *et al*., 2015; Estoup *et al*., 2016).

This likely example of introduction from an adjacent drainage joins three others involving obligate freshwater fishes in northwestern California (Kinziger *et al*., 2011, 2014, 2019). Combined with the fact that all four of these examples and many others offer evidence of successful establishment of populations by very few founders, single vicinal introduction events appear to deserve consideration in efforts to limit invasive species. Although vicinal introductions may enhance the overall probability of persistence for the introduced species, they can also have negative consequences for native taxa (White & Harvey, 2001).

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## AUTHOR CONTRIBUTIONS

J.L.W., R.J.N. and B.C.H. performed field collections. R.J.N. conducted the molecular lab processing and A.P.K. conducted the data analysis. A.P.K. wrote the first version of the manuscript, which was edited primarily by B.C.H.

## ETHICAL STATEMENT

This research followed guidelines for use of fishes in research co-published by the American Society of Ichthyologists and Herpetologists, the American Fisheries Society and the American Institute of Fisheries Research Biologists.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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