Notes

The First Record of the Large-scale Loach *Paramisgurnus dabryanus* (Cobitidae) in the United States

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Abstract

Exotic species have been implicated as a major threat to native freshwater fish communities in the Unites States. The San Francisco Estuary watershed has been recognized as one of the most invaded systems where exotics often dominate the fish community. On October 6, 2014, the U.S. Fish and Wildlife Service detected a previously unknown exotic fish in a disconnected pool immediately upstream from the Chowchilla Bifurcation Structure in the San Joaquin River, a major tributary of the San Francisco Estuary. The fish was initially identified as an Oriental Weatherfish *Misgurnus anguillicaudatus* using external morphological characteristics. We conducted additional fish sampling near the Chowchilla Bifurcation Structure in November 2014 and collected a total of six additional specimens in disconnected pool habitats. Unexpectedly, genetic and meristic techniques revealed that these specimens were Large-scale Loach *Paramisgurnus dabryanus*. To our knowledge this is the first confirmed occurrence of Large-scale Loach in the United States and the suspected pathway of introduction is release from aquaria. Very little is known about the population in the San Joaquin River. We recommend further evaluation of the ecology, distribution, and abundance of Large-scale Loach to better understand their potential impact on the fish communities of the San Joaquin River and the likelihood of establishment throughout the United States.

Key Words: exotic fish, United States, San Joaquin River, Large-scale Loach, *Paramisgurnus dabryanus*, ecological risk, Oriental Weatherfish, misidentification

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Introduction

The introduction of exotic (i.e., nonnative) fish species is a topic of concern throughout North America, where approximately 46% of the described native freshwater fish taxa are considered imperiled (Jelks et al. 2008). The decline of native fish species is largely attributed to habitat degradation coupled with the introduction of exotic fish species (Richter et al. 1997; Jelks et al. 2008). In general, exotic species can negatively impact native fish through multiple mechanisms including habitat alteration, competition, predation, hybridization, and pathogen transfer (Douglas et al. 1994; Mooney and Cleland 2001; Dunham et al. 2004). The impacts of exotic fish on a fish assemblage is often mediated at local and landscape scales by the suitability of habitat, magnitude and extent of introduction, structure of the existing assemblage, and the implementation of management or control actions (Courtenay and Robins 1989; Moyle and Light 1996; Marchetti et al. 2004). Although the United States has a multitude of regulations to prevent the introduction and establishment of exotic fishes (Lodge et al. 2006), more than 138 exotic species have been introduced and become established in the United States through development of gamefish or baitfish stocks, disposal of aquarium fish, release of baitfish or escape from aquaculture facilities (Nico and Fuller 1999; Pimentel et al. 2000; Fuller 2003).

One group of fishes with great invasive potential is the family Cobitidae, which includes approximately 177 freshwater loach species from 26 genera (Nelson 2006; Kottelat 2012). The most widely introduced species, the Oriental Weatherfish *Misgurnus anguillicaudatus,* has become established in several areas in the United States including southern California, Florida, Hawaii, Idaho, Illinois, Michigan, New York, Oregon, and Washington (Courtenay et al. 1987; Tabor et al. 2001; Simon et al. 2006) and has been largely introduced by aquaculture escapes or releases by aquarists or fisherman (Courtenay and Stauffer 1990; Chang et al. 2009). The potential negative impacts of invasive loaches such as the Oriental Weatherfish on native fishes include predation of eggs or larvae, increased competition for macroinvertebrate or algal prey, introduction of foreign parasites, and the elevation of ammonia, nitrate, and turbidity levels within the environment (Logan et al. 1996; Keller and Lake 2007; Lintermans et al. 2007).

On October 6, 2014, the U.S. Fish and Wildlife Service collected a previously unknown exotic loach in a disconnected pool during a fish assemblage survey on the San Joaquin River immediately upstream from the Chowchilla Bifurcation Structure in Madera County, California. The specimen was sacrificed, measured, photographed, and discarded. Following Moyle (2002) and Nelson (2006), the specimen was later identified as an Oriental Weatherfish using the morphological characteristics visible in the photographs (e.g., veliform body shape, subterminal mouth, five barbels present on each side of the jaw; Crystal Castle, personal communication, U.S. Fish and Wildlife Service). The detection of Oriental Weatherfish in the San Joaquin River would constitute a range expansion of the species within California. However, misidentification often occurs among cobitids when relying solely on external morphological characteristics (Bohlen et al. 2005). Further, Oriental Weatherfish are known to hybridize with Large-scale Loach *Paramisgurnus dabryanus* and the hybrids exhibit morphological similarities to that of the Oriental Weatherfish (You et al. 2009). As a result, the true identity of the exotic loach was unknown. The objectives of this study were to collect additional loach specimens near the Chowchilla Bifurcation Structure in the San Joaquin River and validate the species identification using meristics and genetics.

Methods

Study area

The San Joaquin River basin has a Mediterranean-montane climate (i.e., wet-cool winters and dry-hot summers; Null and Viers 2013) where natural flows are largely generated during the spring from snowmelt runoff. In general, the San Joaquin River downstream of Millerton Lake (river kilometer [rkm] 430.5 of the San Joaquin River measuring from its confluence with the Sacramento River) is subjected to artificial flow regimes, agricultural and municipal contaminants, excessive groundwater pumping, channel confinement and surface water diversion and export (Galloway and Riley 1999; Traum et al. 2014). As a result, the San Joaquin River is currently dominated by losing or effluent reaches where flows become intermittent and habitat becomes disconnected between Gravelly Ford (rkm 370.0) and the Merced River confluence (rkm 187.6) during non-flood conditions with the exception of where irrigation water from the federal Central Valley Project is conveyed. We sampled for the exotic loach in all disconnected pools occurring within 1 km of the Chowchilla Bifurcation Structure (rkm 347.8; Figure 1).

Sample methods

A total of four disconnected pools were sampled on November 12, 2014 by U.S. Fish and Wildlife Service and California Department of Fish and Wildlife staff. Prior to sampling the pools, we collected dominant substrate and water quality data along the shoreline of all pools but one. A YSI 85 or YSI PRO 2030 meter was used to measure water temperature to the nearest 0.1˚C, specific conductance to the nearest 0.1 μS/cm and dissolved oxygen to the nearest 0.1 mg/L. Turbidity was measured using a HACH 2100Q turbidity meter to the nearest 0.1 Nephelometric Turbidity Unit (NTU). Dominant substrate type was determined visually and was broadly classified based on particle size diameter as mud (< 0.5 mm) or sand (0.5–5.0 mm). Pool size was also broadly classified as either small or large. Small pools were, on average, shallow (i.e., < 2 m in depth) and less than 15-m wide or long. Conversely, large pools were, on average, deep (i.e., at least 2 m in depth) and greater than or equal to 15-m wide or long.

We sampled fish in each disconnected pool during daylight hours using two Smith-Root LR 24 pulsed DC backpack electrofishers operating between 0.1 and 0.2 amperes. Fishes were generally sampled using single-pass electrofishing throughout the wadeable portions of each pool starting at one end of the disconnected pool and moving to the opposite end of the pool. In large pools, only the shoreline habitats were sampled. The total shock time was recorded for each disconnected pool. Fish were additionally sampled in two of the pools using a 15.0-m x 1.2-m beach seine with 3-mm square Delta mesh in combination with the backpack electrofishers. In these pools, the beach seine was deployed at one end of each disconnected pool following behind the electrofishers to collect stunned fish that could not be detected visually. In large pools, a block net with 3-mm square Delta mesh was used to partition the pool into smaller units to allow for more effective sampling given the gear and methods used.

We identified all fish in the field that were ≥ 25-mm total length (TL) to species following Moyle (2002). All loach-like fishes collected were measured for TL, euthanized, preserved in a 95% ethanol solution, and retained for genetic and meristic analyses. Preserved specimens were sent to the University of California–Davis for DNA barcoding and the Natural History Museum of Los Angeles County Section of Ichthyology for radiograph-based identification.

Genetic analysis

We extracted DNA from dried barbel clips from the loach specimens using a PureGene DNA extraction kit. The Barcode of Life BOLD System repository (BoL; http://www.boldsystems.org/) was used to identify appropriate universal primers to amplify and sequence the cytochrome *c* oxidase I (COI) gene in teleost species. We used PCR primers VF2_t1 and FR1d_t1 and sequencing primers M13F and M13R for DNA barcoding (Table 1; Messing 1983, Ivanova et al. 2007, Geiger et al. 2014). The PCR amplifications were performed using the thermal profile described in Geiger et al. (2014). Sanger sequencing was outsourced to QuintaraBio in Richmond, California. Poor quality ends were trimmed from the COI sequences in the program Sequencher leaving high quality sequences ranging in length from 662–689 base pairs. Preliminary species identification was made based on sequence similarity between the unknown samples and species-specific sequences in the BoL and NCBI Nucleotide Database (NCBI).

Next, we downloaded COI sequences from BoL and NCBI for the closest genetic matches to the unknown specimens, the Large-scale Loach (N = 5) and Fine-scale Loach *Misgurnus mizolepsis* (N = 3), along with two commonly cultured loach species, the Oriental Weatherfish (N = 16) and European Weatherfish *Misgurnus fossilis* (N = 13). We trimmed the COI sequences from all 41 individuals to 607 bp and aligned them using the Clustal W approach with default settings in the program MEGA7 (Kumar et al. 2016). We created five separate groups, one for each species and one for the unknowns, and calculated the between group mean pairwise distances in MEGA7 using all nucleotide substitutions (transitions and transversions, coding and non-coding), assuming uniform evolution among lineages and sites, and using a gap treatment of complete deletion. A thousand bootstrap replicates were performed to estimate variance.

Meristic analysis

A subset of preserved specimens were cataloged, photographed, and radiographed at the Natural History Museum of Los Angeles County, Section of Ichthyology (LACM; Sabaj 2016). Meristics were counted on each specimen including dorsal, anal, pectoral, and pelvic fin rays, vertebrae, and barbels. We also assessed fin morphology, the presence of spots on the body or peduncle, and the presence and type of adipose crests on the caudal peduncle. Thereafter, each specimen was compared to Oriental Weatherfish and identified following Kottelat (2001), Kottelat and Freyhof (2007), Kottelat (2012), and Froese and Pauly (2015).

Results

The disconnected pools sampled (n=4) represented a narrow range of habitat conditions. In general, all of the pools were all dominated by mud substrate and contained non-flowing (0 m/s), cool (16.2–18.5°C), well oxygenated (8.4–9.8 mg/L), and clear (7.3 NTU) water with little conductance (39.3–47.7 µS/cm). On average, each disconnected pool was sampled using the backpack electrofishers for 35.2 minutes (range = 21.9 to 49.5). A total of 417 fish were detected during the study, which represented a total of 14 known fish species (Table 2). One Sacramento Sucker *Catostomus occidentalis* represented the only native fish detected.

A total of six loach specimens (93 to 131 mm TL) were captured in two of the four disconnected pools in the study area (Table 2). Five loach specimens were collected from the pool immediately upstream of the Chowchilla Bifurcation Structure where the original loach was captured on October 6, 2014. One loach specimen was collected from the disconnected pool approximately 1-km upstream of the Chowchilla Bifurcation Structure.

Genetic identification of loach

All unknown loaches exhibited an identical haplotype at 607 base pairs of COI sequence. Although an identification of Oriental Weatherfish was anticipated, our preliminary analysis of sequence similarity found that the closest matches were the Large-scale Loach (BoL and NCBI;

99% similarity) and Fine-scale Loach (NCBI; 99% similarity), a taxon likely conspecific to Large-scale Loach (see Discussion). Our analysis of pairwise distance between groups revealed that the unknown specimens were most genetically similar to Large-scale Loach (Table 3). Interestingly, the unknowns and Large-scale Loach samples were nearly equally divergent from the Fine-scale Loach (0.062 vs. 0.063; Table 3). Greater sequence divergence was observed in comparisons involving Oriental Weatherfish and European Weatherfish (Table 3).

Meristic identification of loach

We selected one specimen to send to the Natural History Museum of Los Angeles County for meristic identification. The other specimens, in poor physical condition, were retained for additional genetic analysis. The specimen was identified as Large-scale Loach (catalog number LACM 58237-1). The radiograph revealed the meristic 8 dorsal rays, 7 anal rays, 11–12 pectoral rays, about 8 pelvic rays and 46 vertebrae (Figure 2). Physical characteristics included a pair of rostral, maxillary, and mandibular barbels, with two pairs of barbel-like mental lobes on the lower lip. Scales covered the body of the specimen. The tail was not forked, somewhat pointed, and the pectoral fin was longer than the head, with the second ray thickened. A small pouch was present on the inside of each pectoral fin but did not contain lamina circularis. The caudal peduncle had adipose crests that extended to the dorsal and anal fins, and there were spots on the side of the body but not a distinct spot on the upper caudal base. These characters are consistent with Large-scale Loach or Fine-scale Loach (Kottelat 2001; Kottelat 2012; Froese and Pauly 2015). However, the specimen was identified as a Large-scale Loach following Kottelat and Freyhof (2007) based on the presence of four barbel-like mental lobes on the lower lip, the absence of a midlateral stripe from the eye to the caudal base, the absence of a narrow stripe from the opercle to the pelvic origin, and the presence of high adipose crests on the caudal peduncle. An Oriental Weatherfish (catalog number LACM 36986-2) was radiographed for comparative purposes and was found to have only 10 pectoral rays and 45 vertebrae, a pectoral fin that was shorter than the head (although this may be due to it being a female), a rounded tail, and a dense black spot on the upper caudal base.

Discussion

A total of six loach specimens were captured in the San Joaquin River near the Chowchilla Bifurcation Structure. All were identified as Large-scale Loach using genetic techniques and a meristic analysis of one specimen corroborated the genetic results. Although our initial queries of BoL and NCBI revealed strong genetic similarity between our specimens and both Large-scale and Fine-scale Loach, the subsequent pairwise distance analysis supported identification of the unknowns as Large-scale Loach. Genetic similarity between Large-scale and Fine-scale Loach was not unexpected given the findings of phylogenetic studies of the family Cobitidae. In two recent studies using both nuclear (RAG-1) and mitochondrial (cytochrome b) gene sequence data, Large-scale and Fine-scale Loach resolved into a single lineage with low genetic divergence within it, supporting the hypothesis posed by several authors that the two taxa are very genetically similar (Perdices et al. 2016) or conspecific (Vasil'eva 2001; Kottelat 2012; Perdices et al. 2012). Morphological similarity of some Fine-scale Loach specimens to Oriental Weatherfish have led to recommendations that those taxa are conspecific (Kottelat 2012), but this is not supported by genetic data (Perdices et al. 2012, 2016, this study). Our results are the first to confirm the occurrence of Large-scale Loach in the United States following a review of the literature and the U.S. Geological Survey Nonindigenous Aquatic Species Database (U.S. Geological Survey 2017). The specimens are currently retained by the University of California–Davis (catalog numbers WFB 3240a through WFB 3240d), California Academy of Sciences (catalog number CAS 243916), and Los Angeles Natural History Museum (catalog number LACM 58237-1) as vouchers for future study.

The native distribution of Large-scale Loach includes the Yangtze River basin in China and the inland waters of Taiwan (Kottelat 2012) where water temperatures can range from 10–30°C (Wang and Li 2005; Zhang et al. 2012). They are found in lentic and lotic habitats containing muddy substrate, low gradient, slack water and high turbidity (Gao et al. 2010; Sato et al. 2011; Huang et al. 2013). Additionally, Large-scale Loach can occur in irrigation or diversion canals, ponds, and rice fields (Kanou et al. 2007; Greshishchev et al. 2015) and are able to breathe air in oxygen depleted waters and burrow into mud to avoid desiccation or thermal extremes (Wang and Li 2005; Zhang et al. 2012). These habitat preferences and characteristics make the Large-scale Loach suited to the San Joaquin River with its labyrinth of agricultural diversion canals and managed waterfowl habitat, muddy substrate and thermal extremes (Galloway and Riley 1999; Traum et al. 2014).

We speculate that the introduction to the San Joaquin River may have been the result of one or more aquarium releases within 1-km of the Chowchilla Bifurcation Structure or further upstream when the river was connected. The Large-scale Loach is considered to have nutritional, medicinal and ornamental value outside of the species' native distribution (Freyhof and Korte 2005; Wang and Li 2005; Kanou et al. 2007) and the species is commonly exported from eastern Asia for the aquarium or aquaculture trade (Freyhof and Korte 2005; Kanou et al. 2007; Chu et al. 2012). It has been introduced to Europe and Japan by means of aquaculture escape and aquarium release (Kanou et al. 2007; Tang et al. 2008). In the United States, cobitids are commonly sold as aquarium fish in retail stores (Courtenay and Stauffer 1990; Rixon et al. 2005) including those within the San Francisco Estuary watershed (Chang et al. 2009), providing a source of introduction.

The ecological impact of the Large-scale Loach population in the San Joaquin River is unknown. The species has high reproductive potential and can become sexually mature at one or two years of age when fish range in length from 100 to 200 mm (Chu et al. 2012; Zhang et al. 2012). The Large-scale Loach is an omnivorous benthic feeder and its prey consists of zooplankton, macroinvertebrates, and algae (Wang and Li 2005; Kanou et al. 2007). High population densities of Large-scale Loach could potentially alter the invertebrate and algal community within invaded waters. Further, Large-scale Loach could potentially interfere with other benthic feeders (Mills et al. 2004; Hazelton and Grossman 2009), although interference competition has not been documented between Large-scale Loach and other species possessing diet overlap within Japan (Kanou et al. 2007). Additionally, the introduction of Large-scale Loach has the potential to introduce foreign fish parasites. Introductions of Oriental Weatherfish have resulted in the transport and introductions of pathogens including tapeworms and nematodes (Sohn et al. 1993; Ernst and Dove 1998; Lintermans et al. 2007), which could affect both fish and humans.

For all these reasons, we believe the establishment of Large-scale Loach in the San Joaquin River could influence the aquatic ecosystems and the success of large-scale river restoration projects being implemented in the basin including the San Joaquin River Restoration Program. Therefore, we recommend further research to assess their level of establishment in the San Francisco Estuary watershed. Additional field sampling is needed throughout the San Joaquin River downstream of Friant Dam including the San Francisco Estuary, adjacent diversion canals, and managed waterfowl habitat to document the distribution and abundance of the species. This information can be used to facilitate an assessment of impact, guide further research, and inform natural resource management decisions concerning the implementation of appropriate management actions (Courtenay and Robins 1989; Lodge et al. 2006). Although the ecological impacts of introduced Large-scale Loach are not well understood, bans on the import of Large-scale Loach and eradication strategies may be warranted to help prevent establishment and further introductions within the United States.

Supplemental Material

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Data S1. All fish capture and habitat data collected from the San Joaquin River, California near the Chowchilla Bifurcation Structure on November 12, 2014 is contained in the xls file titled Data_S1. (37 KB).
Found at DOI: http://dx.doi.org/10.3996//012017-JFWM-008.S1 (90 KB CSV).

Reference S1. Galloway DL, Riley FS. 1999. San Joaquin Valley, California—largest human alteration of the Earth’s surface. Pages 23–34 in Galloway DL, Jones DR, Ingebritsen SE, editors. Land Subsidence in the United States. U.S. Geological Survey Circular 1182. Found at DOI: http://dx.doi.org/10.3996//012017-JFWM-008.S2 (5471 KB PDF); also found at DOI: https://pubs.usgs.gov/circ/1999/1182/report.pdf (5,340 KB PDF).

Reference S2. Traum JA, Phillips SP, Bennett GL, Zamora C, and Metzger LF. 2014. Documentation of a groundwater flow model (SJRRPGW) for the San Joaquin River Restoration Program Study Area, California. U.S. Geological Survey Scientific Investigations Report 2014–5148. Found at DOI: http://dx.doi.org/10.3996//012017-JFWM-008.S3 (16985 KB PDF); Found at DOI: https://pubs.usgs.gov/sir/2014/5148/pdf/sir2014-5148.pdf (16,500 KB PDF).

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Table and Figure Captions

Table 1. Cytochrome *c* oxidase I (COI) primer sequences used for DNA barcoding to identify the unknown loach specimens collected in the San Joaquin River, California near the Chowchilla Bifurcation Structure on November 12, 2014.

Table 2. Fish species observed and numbers captured in each disconnected pool sampled within the San Joaquin River, California near the Chowchilla Bifurcation Structure on November 12, 2014.

Table 3. Pairwise mean sequence distances between Large-scale Loach *Paramisgurnus dabryanus* (LSL), Chinese fine-scale Loach *Misgurnus mizolepsis* (FSL), Oriental Weatherfish *Misgurnus anguillicaudatus* (OW), European Weatherfish *Misgurnus fossilis* (EW), and unknown loach samples (UNK) collected from the San Joaquin River, California near the Chowchilla Bifurcation Structure on November 12, 2014. Pairwise distance values are below the diagonal and standard errors are above the diagonal.

Figure 1. The location of the Chowchilla Bifurcation Structure where sampling occurred to collect unknown loach specimens in the San Joaquin River, California on November 12, 2014.

Figure 2. A photograph (A) and radiograph (B) of a Large-scale Loach *Paramisgurnus dabryanus* captured (112 mm TL; LACM 58237-1) in a disconnected pool upstream of the Chowchilla Bifurcation Structure in the San Joaquin River, California on November 12, 2014.

Table 1. Cytochrome c oxidase I (COI) primer sequences used for DNA barcoding to identify the unknown loach specimens collected in the San Joaquin River, California near the Chowchilla Bifurcation Structure on November 12, 2014.

Primer name	Sequence	Reference
VF2_t1	TGTAAAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC	Ivanova et al. (2007)
FR1d_t1	CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAYCARAA	Ivanova et al. (2007)
M13F	GTAAAACGACGGCCAGT	Geiger et al. (2014)
M13R	CAGGAAACAGCTATGAC	Messing et al. (1983)

Table 2. Fish species observed and numbers captured in each disconnected pool sampled within the San Joaquin River, November 12, 2014.

Species	Pool in river immediately upstream of the Chowchilla Bifurcation Structure *	Pool in river 1 rkm upstream of the Chowchilla Bifurcation Structure
Goldfish, *Carassius auratus*	0	1
Common Carp, *Cyprinus carpio*	16	0
Golden Shiner, *Notemigonus crysoleucas*	3	0
Sacramento Sucker, *Catostomus occidentalis*	1	0
Western Mosquitofish, *Gambusia affinis*	0	7
Redear Sunfish, *Lepomis microlophus*	14	2
Green Sunfish, *L. cyanellus*	17	36
Bluegill, *L. macrochirus*	40	3
Spotted Bass, *Micropterus punctulatus*	38	15
Largemouth Bass, *M. salmoides*	16	11
White Catfish, *Ameiurus catus*	0	0
Yellow Bullhead, *A. natalis*	0	0
Channel Catfish, *Ictalurus punctatus*	4	0
Bigscale Logperch, *Percina macrolepida*	14	8
Large-scale Loach, *Paramisgurnus dabryanus*	5	1

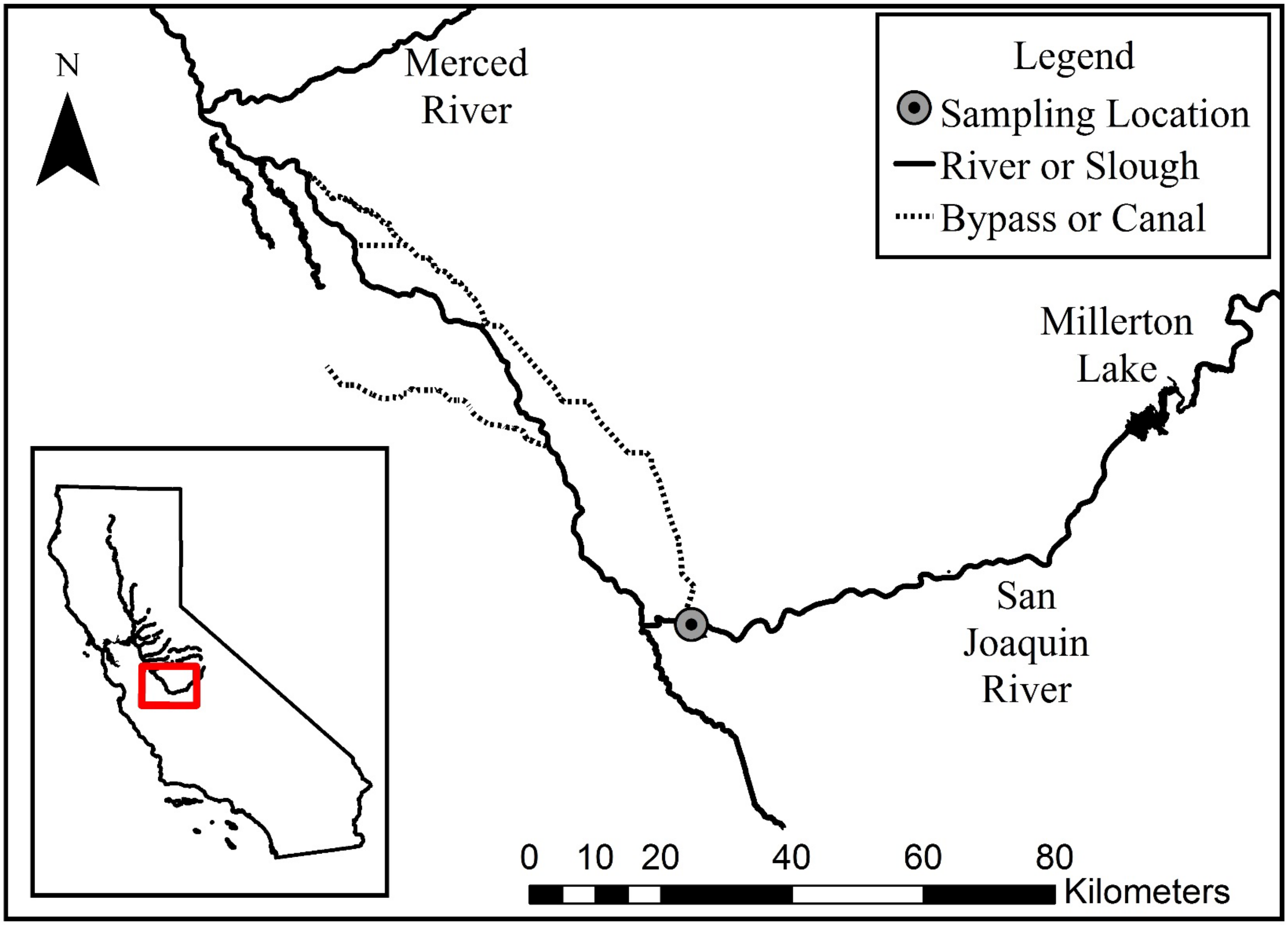
* Sampling location where the original loach was detected on October 6, 2014.

California near the Chowchilla Bifurcation Structure on

Pool in river immediately downstream of the Chowchilla Bifurcation Structure	Pool in bypass immediately downstream of the Chowchilla Bifurcation Structure
0	0
4	0
3	0
0	0
0	9
2	0
25	61
23	14
2	1
1	0
2	0
1	0
16	0
1	0
0	0

Table 3. Pairwise mean sequence distances between Large-scale Loach *Paramisgurnus dabryanus* (LSL), Chinese fine-scale Loach *Misgurnus mizolepsis* (FSL), Oriental Weatherfish *Misgurnus anguillicaudatus* (OW), European Weatherfish *Misgurnus fossilis* (EW), and unknown loach samples (UNK) collected from the San Joaquin River, California near the Chowchilla Bifurcation Structure on November 12, 2014. Pairwise distance values are below the diagonal and standard errors are above the diagonal.

	LSL	FSL	OW	EW	UNK
LSL		0.005	0.011	0.013	0.004
FSL	0.063		0.010	0.012	0.006
OW	0.144	0.132		0.013	0.012
EW	0.150	0.151	0.155		0.014
UNK	0.022	0.062	0.149	0.155	



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