DNA UNVEILS NEW FRESHWATER FISH SPECIES IN CALIFORNIA

Peter B. Moyle and Matthew A. Campbell

No doubt you have watched a crime show where DNA analysis reveals the identity of a victim or criminal. Or, you have read accounts of how Neanderthal genes are part of our DNA. It is still astonishing to think that such uses of DNA did not exist until the Human Genome Project, finished about 20 years ago at the cost of millions of dollars. Even more astonishing is that low-cost methods of examining the genome of any animal or plant are now available. Specifically, the genomes of fishes can be examined to determine evolutionary relationships among species and to identify new "cryptic" species of fishes that otherwise are hard to identify. This means that ancient fish biologists (like Moyle) can team up with geneticists steeped in new methodologies (like Campbell) to explore fish genomes. We can identify "new" (to us) species and confirm (or deny) species identified by standard methods, such as counting scales and fin rays.

Moyle's first venture into the genomic world, with postdoc Jason Baumsteiger as his guide, was to explore the genome of California Roach *Hesperoleucus symmetricus*, a small fish endemic to much of central and coastal California. They found that the single species recognized when they started was actually five species (Baumsteiger et al. 2019). In this article, we summarize our findings that the Riffle Sculpin *Cottus gulosus* is also multiple species based on analysis of the genome (genomics) but supported by other genetic, distributional, and meristic studies (Moyle and Campbell 2022).

Freshwater sculpins as a family (Cottidae, 42+ recognized species) are good subjects for genomic analysis because the species are naturally hard to tell apart, being small (usually less than 80 mm in length), with no scales, and with habits and color patterns that keep them camouflaged. Most species are indicators of high-water quality, inhabiting cool, clear streams and lakes throughout the northern hemisphere. Their frequent preference for permanent headwaters leads to isolation and formation of new species, some with ironically hilarious scientific names such as *Cottus perplexus* and *C. confusus*. They are typically abundant and important parts of the ecosystems they inhabit, coexisting with diverse trout and salmon species, as well as other endemic fishes.

Reprinted with permission, with minor edits, from California WaterBlog. January 29, 2023. See https://californiawaterblog.com/ for this and many more interesting articles about water and fishes, including many native species.

Peter Moyle is an Emeritus Professor and Associate Director of the Center for Watershed Sciences, UC Davis; Matthew Campbell is a Research Scientist in the Genomic Variation Laboratory, UC Davis.

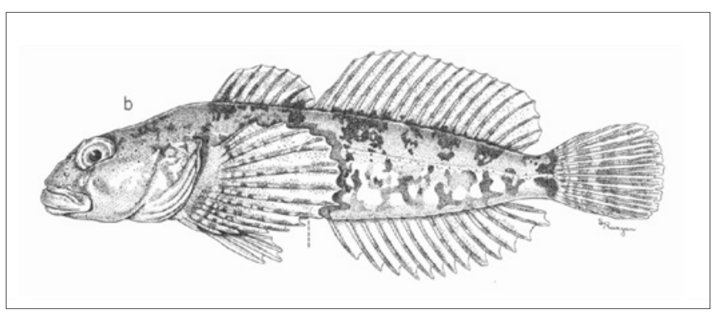
The Riffle Sculpin species "complex" we discuss here consists of the following three species and four subspecies:

Cottus pitensis: Pit Sculpin Bailey and Bond 1963
Cottus gulosus: Inland Riffle Sculpin (Girard 1854)
C. g. gulosus: San Joaquin Riffle Sculpin (Girard 1854), nominate subspecies
C. g. wintu: Sacramento Riffle Sculpin, Moyle and Campbell 2022, new subspecies
Cottus ohlone: Coastal Riffle Sculpin Moyle and Campbell 2022, new species
C. o. ohlone: Ohlone Riffle Sculpin Moyle and Campbell 2022, new subspecies
C. o. pomo: Pomo Riffle Sculpin Moyle and Campbell 2022, new subspecies.

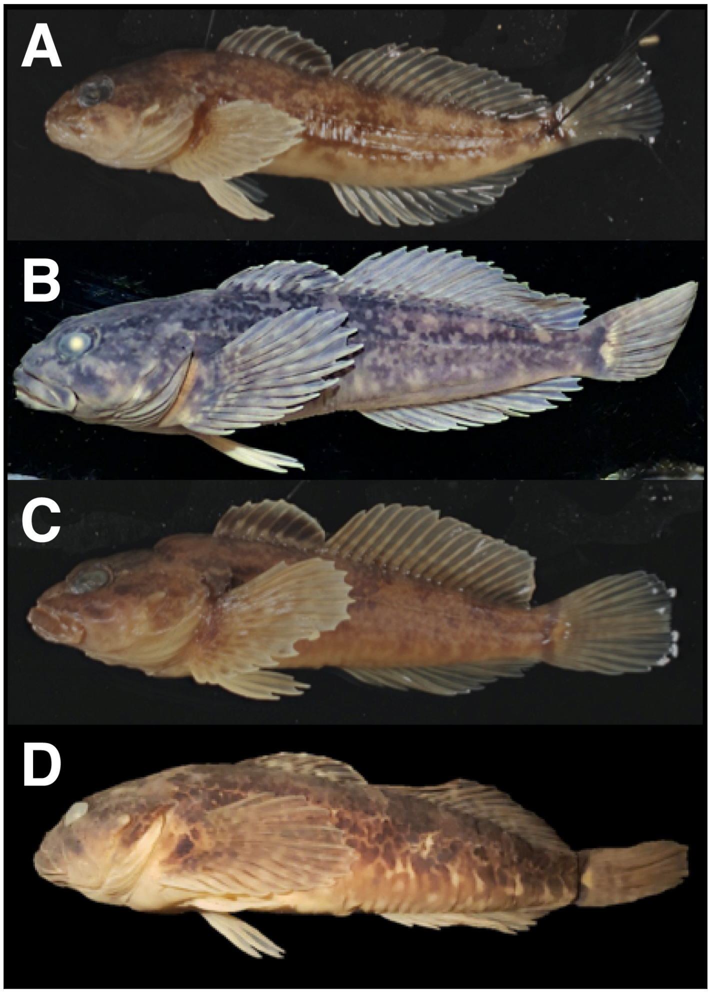
The **Pit Sculpin** was described as a distinct species in 1963 using conventional taxonomic techniques, but its distinguishing features were minor, indicating its close relationship to the Inland Riffle Sculpin. Our genomic study showed that it did indeed merit continued recognition as a separate species. This is the only sculpin species in the Pit River watershed of northeastern California and the tributaries to Goose Lake in Oregon.

The **Inland Riffle Sculpin** was described in 1854 by pioneering ichthyologist Charles Girard. His description was brief and confusing and was applied to all Riffle Sculpins in California (including the Pit Sculpin). Our genomic study showed that Girard's sculpins in the Pit, Sacramento, and San Joaquin rivers and their tributaries, as well as in San Francisco Bay tributaries and the Russian River, were distinct from each other. Girard's description seems to have been mainly based on fish from the San Joaquin River, so *C. gulosus* was retained as the scientific name of the Inland Riffle Sculpin.

Our genomic analysis indicated that the Inland Riffle Sculpin contained two distinct evolutionary lineages that we designated as subspecies because the genetic differences were less than we found between species-level lineages in our data set. Yet the differences



Pit Sculpin, from Bailey and Bond (1963).



Four species/subspecies of riffle sculpin endemic to California. A. San Joaquin Riffle Sculpin, B. Sacramento Riffle Sculpin, C. Ohlone Riffle Sculpin, D. Pomo Riffle Sculpin. (Photos by Irene Englis)

are substantial and correspond to the major river basins, so we recognized the **San Joaquin Riffle Sculpin** *C. g. gulosus* and the **Sacramento Riffle Sculpin** *C. g. wintu*. One outcome of our genomics study was finding that the Sacramento Riffle Sculpin is a hybrid lineage of ancient origin, with a nuclear genome largely of the Inland Riffle Sculpin lineage but with maternally-inherited mitochondrial DNA of the Pit Sculpin type. Surveying only mitochondrial DNA with barcoding approaches would be misleading in this case and is an argument to apply genomic approaches when possible.

Baumsteiger et al. (2012, 2014), in part by using mitochondrial DNA, found that the sculpins in San Francisco Bay drainages were quite different genetically from the inland sculpin populations. This finding is what prompted our study using the more complete genetic picture provided by genomics, which examines the entire genome. Our study led to the designation of coastal and SF Bay populations as a new species **Coastal Riffle Sculpin** *C. ohlone*, with two subspecies, **Ohlone Riffle Sculpin** *C. ohlone ohlone* and **Pomo Riffle Sculpin** *C. o. pomo*. The two subspecies were named to honor the native peoples that lived in the watersheds they occupied, coexisting with the fishes for thousands of years.

Today, the Ohlone Riffle Sculpin lives mostly in the headwater streams of the Guadalupe River, which drains the Santa Clara Valley. These streams flow through and are highly altered by urban areas of San Jose. They also are found in a few small streams that flow directly into the Bay (e.g., Coyote Creek). The Pomo Riffle Sculpin is present in the upper Russian River watershed, above the mouth of Mark West Creek. Their range includes the East Fork Russian River, as well as tributaries to northern San Francisco Bay: Napa River, Petaluma River, Sonoma Creek, and smaller tributaries. These SF Bay streams had connections in the past to the Russian River, via the shifting headwaters of Sonoma Creek. For both subspecies the exact distribution needs to be clarified, as does the status of each isolated population.

Our finding of "new" species and subspecies of sculpin is an example of how genomics can be used to identify cryptic species in the California fish fauna. The five sculpin lineages we have identified cannot, for the most part, be told apart using non-genetic techniques. Furthermore, the use of mitochondrial barcoding techniques would also not have captured the entire picture of sculpin diversity in California. These discoveries increase our appreciation of the uniqueness of California fish fauna, where over 80% of the species are endemic to the state or shared with parts of watersheds in Oregon or Nevada (Moyle 2002, Leidy and Moyle 2022). If these special species are going to be around for future generations to admire, including the species and subspecies of Riffle Sculpin, a way must be found to systematically protect aquatic habitats statewide while surveying for cryptic diversity. There are other cryptic species waiting to be discovered!

Further Reading

Bailey, R.M., and C.E. Bond. 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. https://deepblue.lib.umich.edu/handle/2027.42/57070

Baumsteiger, J., A.P. Kinziger, and A. Aguilar. 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: 940–52. https://doi.org/10.1016/j.ympev.2012.08.015

Baumsteiger, J., A.P. Kinziger, S.B. Reid., and A. Aguilar. 2014. Complex phylogeography and historical hybridization between sister taxa of freshwater sculpin (*Cottus*). Molecular Ecology 23: 2602–2618. https://doi.org/10.1111/mec.12758

Baumsteiger, J., and P.B. Moyle. 2019. A reappraisal of the California Roach/Hitch (Cypriniformes, Cyprinidae, *Hesperoleucus/Lavinia*) species complex. Zootaxa 4543 (2): 2221–240.

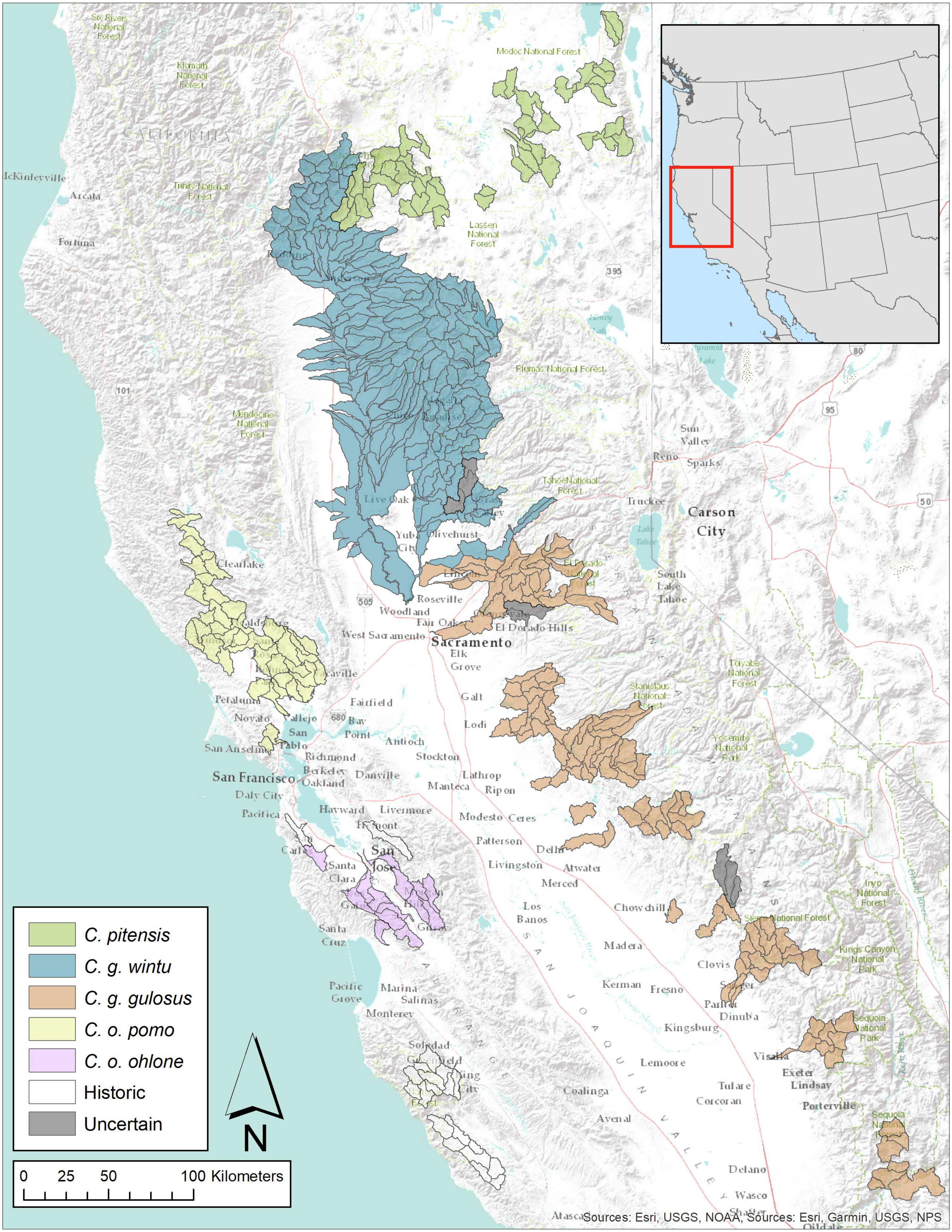
Leidy, R.A., and P.B. Moyle. 2021. Keeping up with the status of freshwater fishes: a California (USA) perspective. Conservation Science and Practice 3(8): e474. https://doi.org/10.1111/csp2.474.

Moyle, P.B. 2002. Inland Fishes of California. Revised and Expanded. University of California Press, Berkeley, 517 pp.

Moyle, P. B., and M.A. Campbell. 2022. Cryptic species of freshwater sculpin (Cottidae, *Cottus*) in California, USA. Zootaxa 5154 (5): 501–527. https://doi.org/10.11646/zootaxa.5154.5.1

Moyle, P.B., J.V.E. Katz, and R.M. Quiñones. 2011. Rapid decline of California's native inland fishes: a status assessment. Biological Conservation 144: 2414–2423. https://doi.org/10.1016/j.biocon.2011.06.002

Opposite: Map of current distribution of Riffle Sculpin species/subspecies. Note the fragmentation of distributions, which is the result of habitat alteration by people. From Moyle and Campbell 2022. Map by CWS staff.


C. pitensis
C. g. wintu
C. g. gulosus
C. o. pomo
C. o. ohlone
Historic
Uncertain
N
0 25 50 100 Kilometers
Sources: Esri, USGS, NOAA, Sources: Esri, Garmin, USGS, NPS
Sacramento
San Francisco
Carson City
Reno
Fresno
Modoc National Forest
Lassen National Forest
Plumas National Forest
Mendocino National Forest
Yosemite National Park
Sierra National Forest
Sequoia National Park