

Pallid Sturgeon Genetics for MRRP and PRRIP

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Genetics for MRRP Program

- **Population Augmentation**

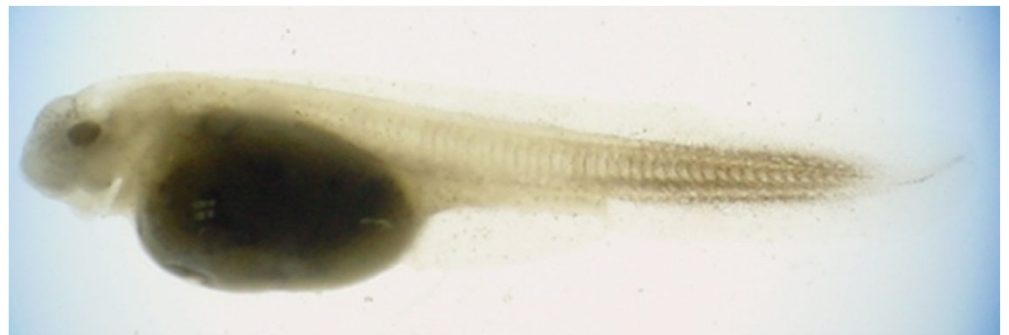
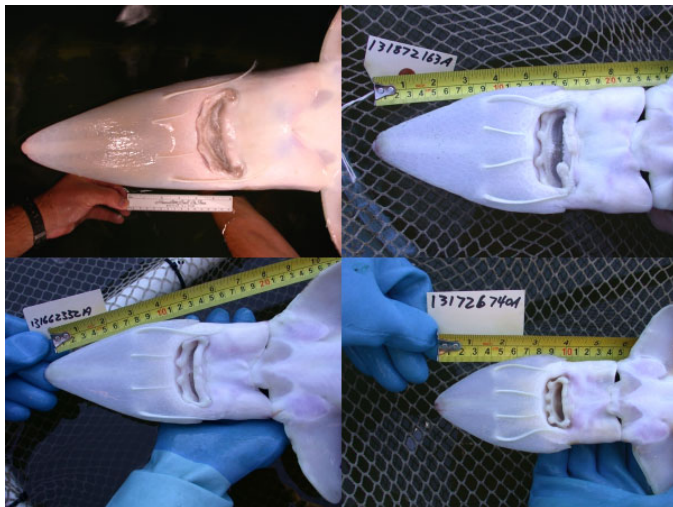
- Avoid using hybrid or back-cross pallid sturgeon for broodstock.
- Use only wild pallid sturgeon.

- **Natural Reproduction**

- Identify sturgeon embryos and larvae
 - Where and when are pallid sturgeon spawning successfully in the Missouri River?
 - Platte River?
- Effective population size (N_e)
 - Recovery criteria based on an N_e of 500 in each management unit.
 - Can be determined using large numbers of independent (unlinked) SNP markers.

Hybridization in Pallid Sturgeon

- Threat to continued existence of pallid sturgeon.
 - Stocking hybrids is detrimental.
- Difficult to discriminate between hybrids and backcrosses.
 - Especially problematic for early life history stages.



We Need Better Sturgeon Genetic ID

- Current ID based on 19 microsatellite markers
- Modeling in Jordan et al. (2019)*
 - 2% of pure Pallid Sturgeon identified as hybrids.
 - 9% of F_1 x Pallid back-crosses identified as pure Pallid Sturgeon.
 - 52% of F_1 x Shovelnose back-crosses identified as pure Shovelnose Sturgeon.
- We lack certainty about species ID of broodstock.
- We don't know what fraction of wild fish are hybrids.

*Jordan, G., E. J. Heist, B. R. Kuhajda, G. R. Moyer, P. Hartfield and M. Piteo (2019). "Morphological Identification Over-Estimates The Number Of Pallid Sturgeon (*Scaphirhynchus albus*) In The Lower Mississippi River Due To Extensive Introgressive Hybridization." Transactions of the American Fisheries Society **148**: 1004-1023.

Case study #1

- Pallid Broodstock #6C00111938
- Gravid female currently at Gavins Point Fish Hatchery.
- Borderline microsatellite assignment = 0.976
 - Type I error – Stock hybrid offspring
 - Type II error – Lose female's demographic and genetic contribution

Case study #2

- Larval Sturgeon 20-07947
- Collected from lower MO River in 2020
 - Marginal microsatellite assignment ($p = 0.946$)
 - Pure pallid sturgeon or possibly a hybrid?

Single Nucleotide Polymorphisms (SNPs)

Pallid ...AGCGCGTACACG**T**GTCTAGAGCAT...

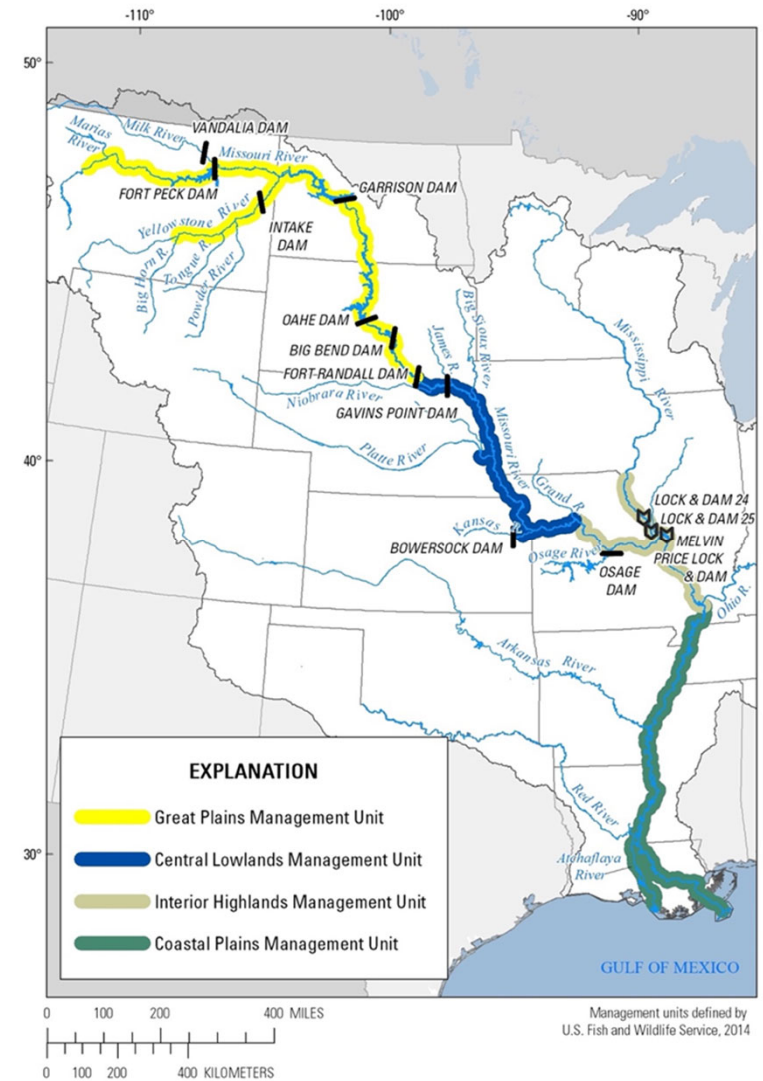
Shovelnose ...AGCGCGTACACG**A**GTCTAGAGCAT...



- Discovered by sequencing
- Efficient to score

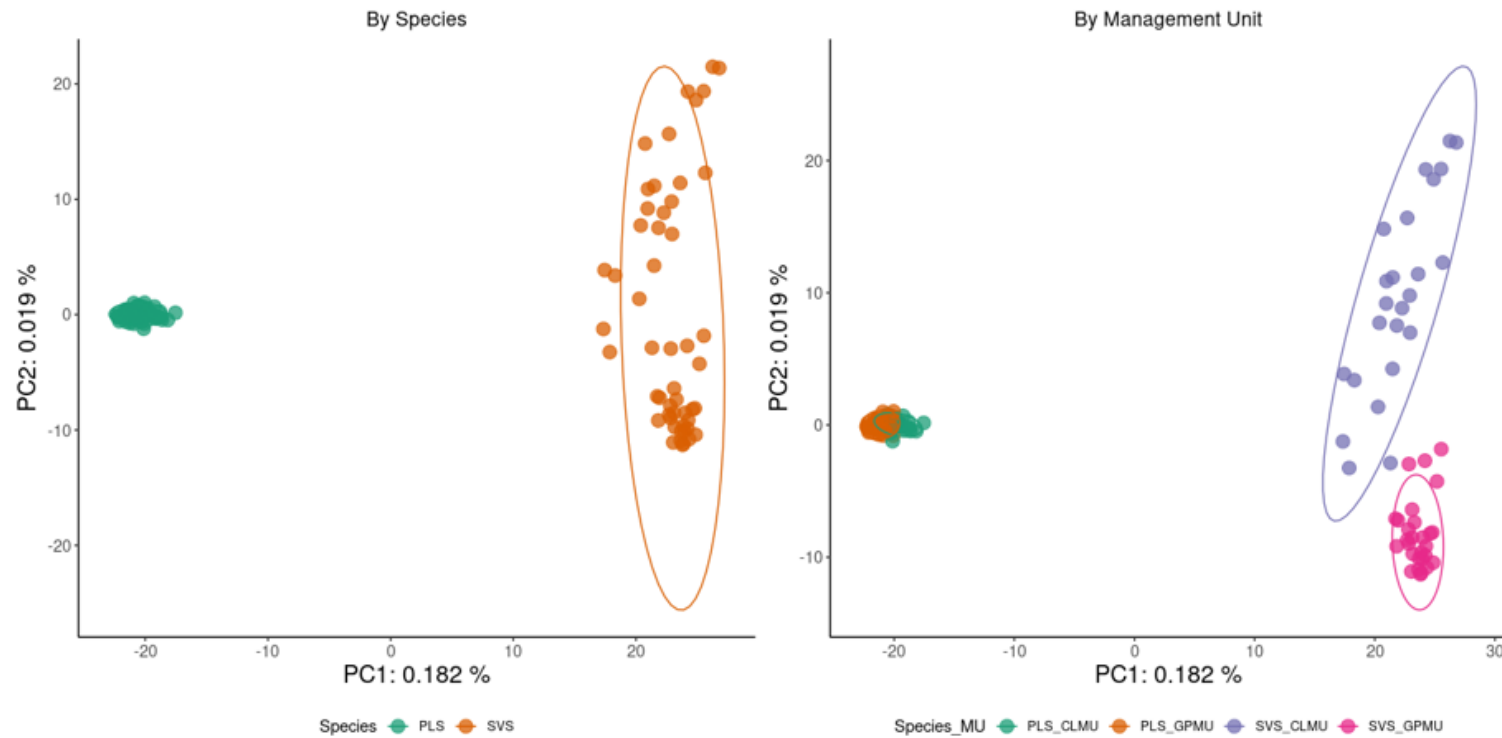
Wild Sturgeon SNP Study

- Examine SNP frequency differences between species and management units
 - 57 Pallid Sturgeon
 - 26 CLMU
 - 31 GPMU
 - 52 Shovelnose Sturgeon
 - 24 CLMU
 - 28 GPMU
- 11,140 SNP loci
 - > 500-fold increase over current 19 markers
 - Most extensive examination of genetic differentiation within *Scaphirhynchus*



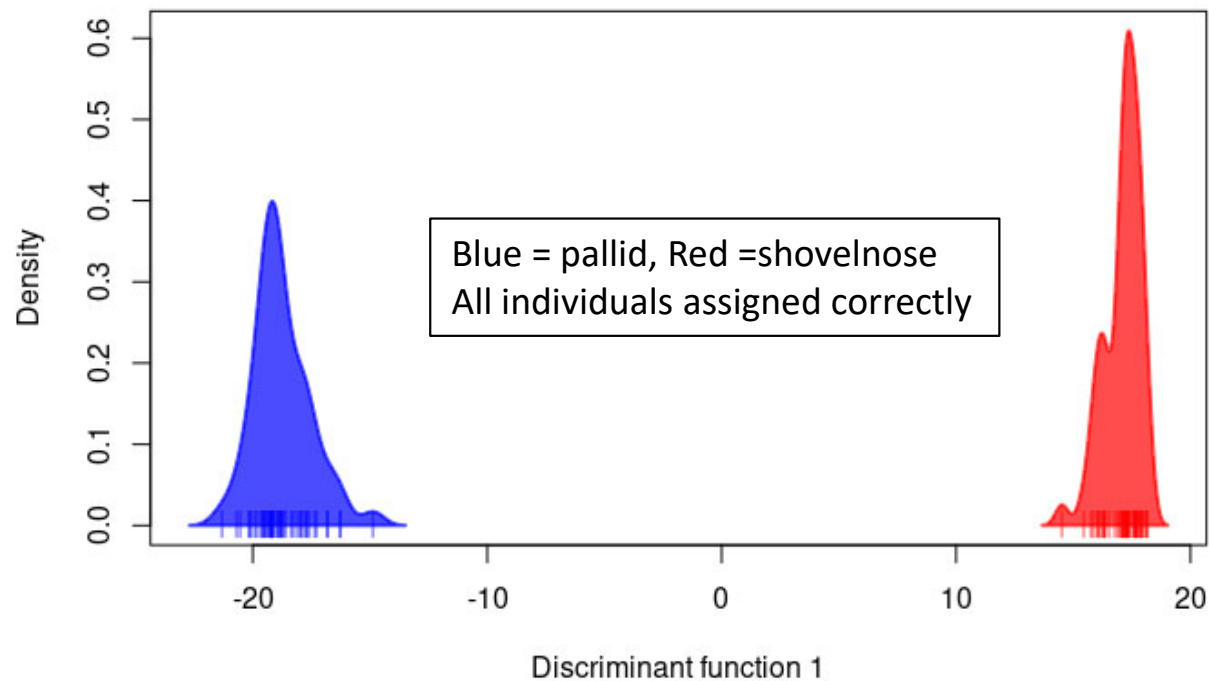
Principle Component Analysis

11,140 SNPs



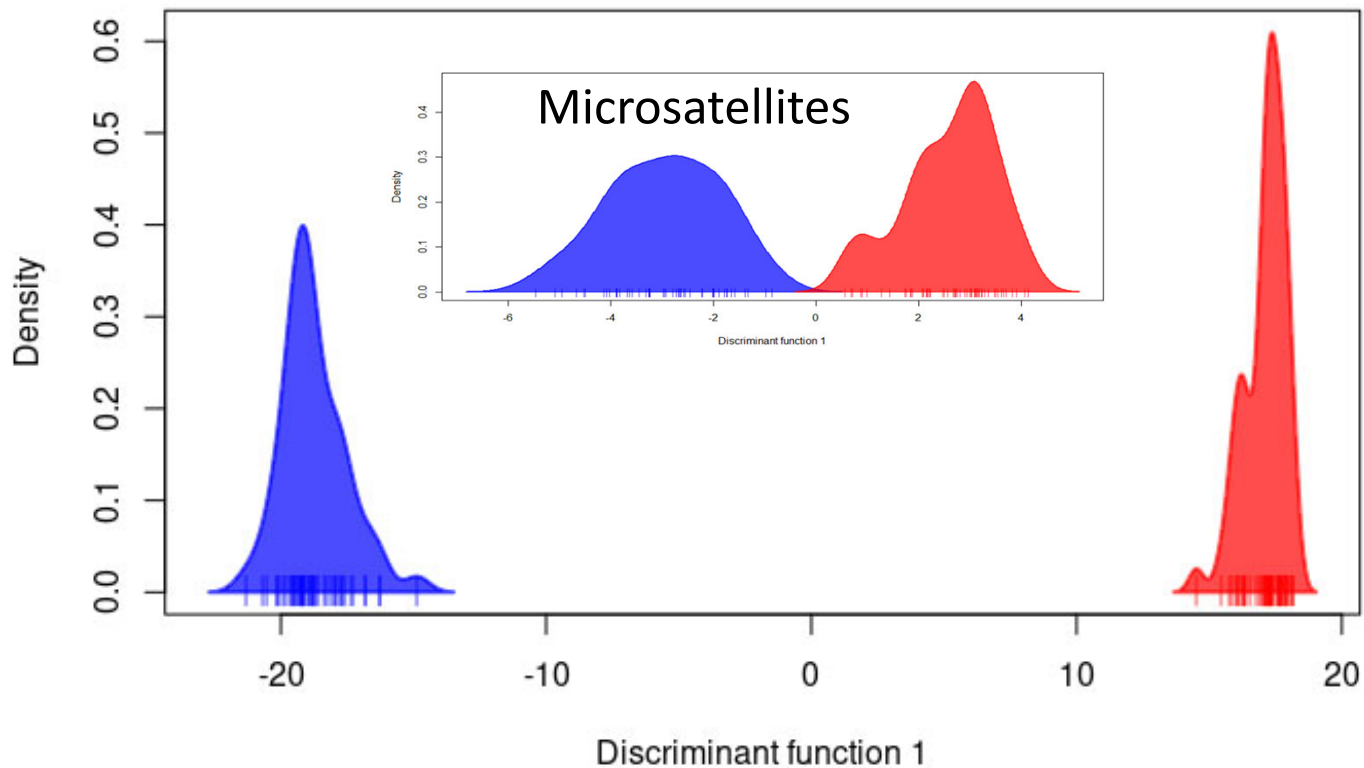
- Pallid and Shovelnose Sturgeon Genetically Distinct (separate species).
- None of the fish utilized appear to be hybrids.
- Some signal associated with management units.

Discriminant Analysis using Principal Components

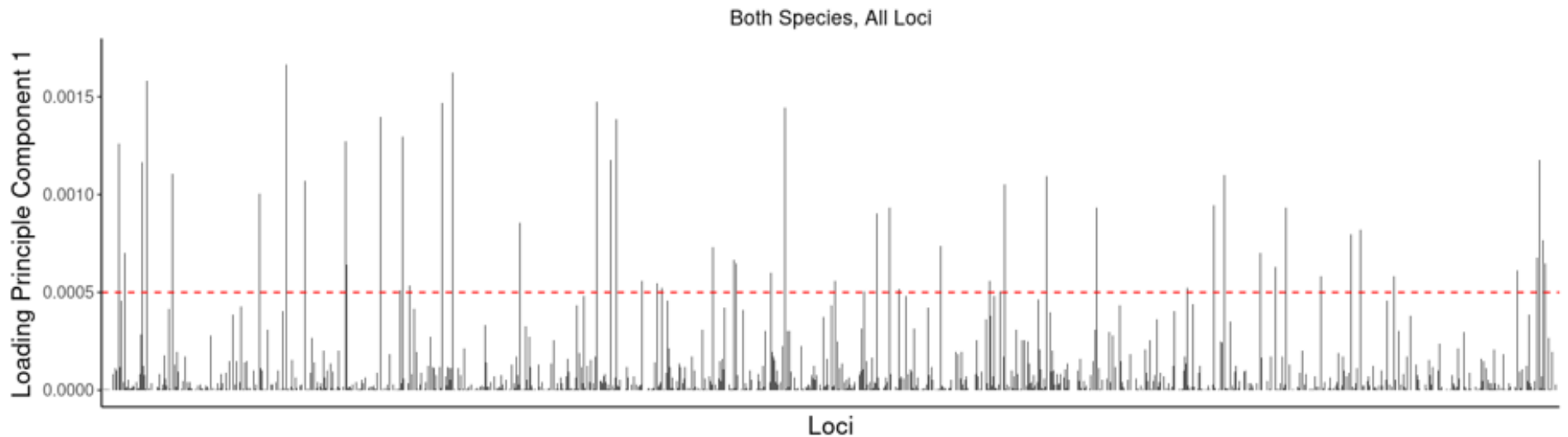


Genetic groups were 100% concordant with field identification.

SNPs are much better at species discrimination!

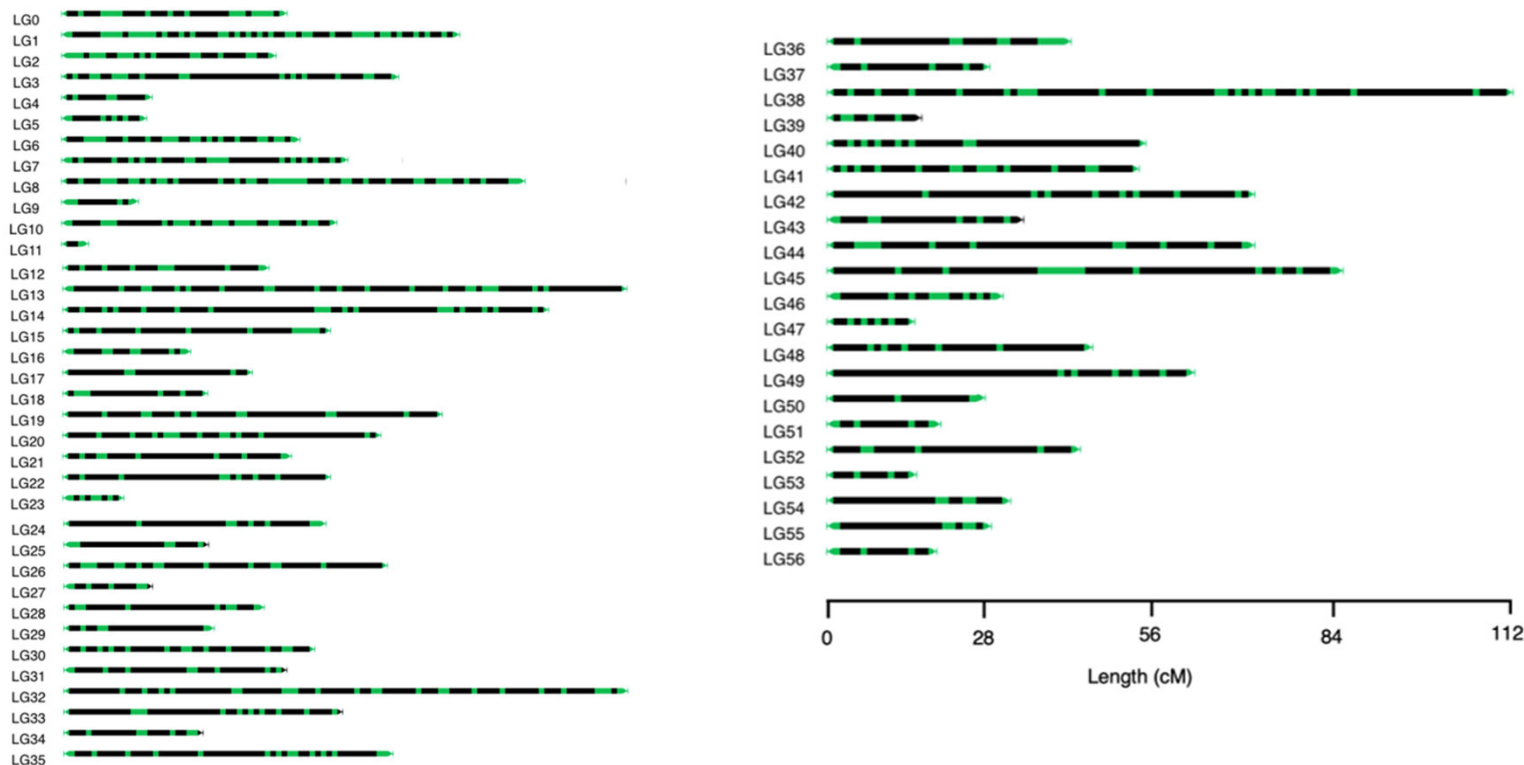


Microsatellites grouped one Shovelnose with the Pallid Sturgeon.



- Some loci provide more signal than others.
- Loci can be individually extracted and assayed.
- New “tool” will be an efficient means of genotyping several hundred of the most powerful SNPs.

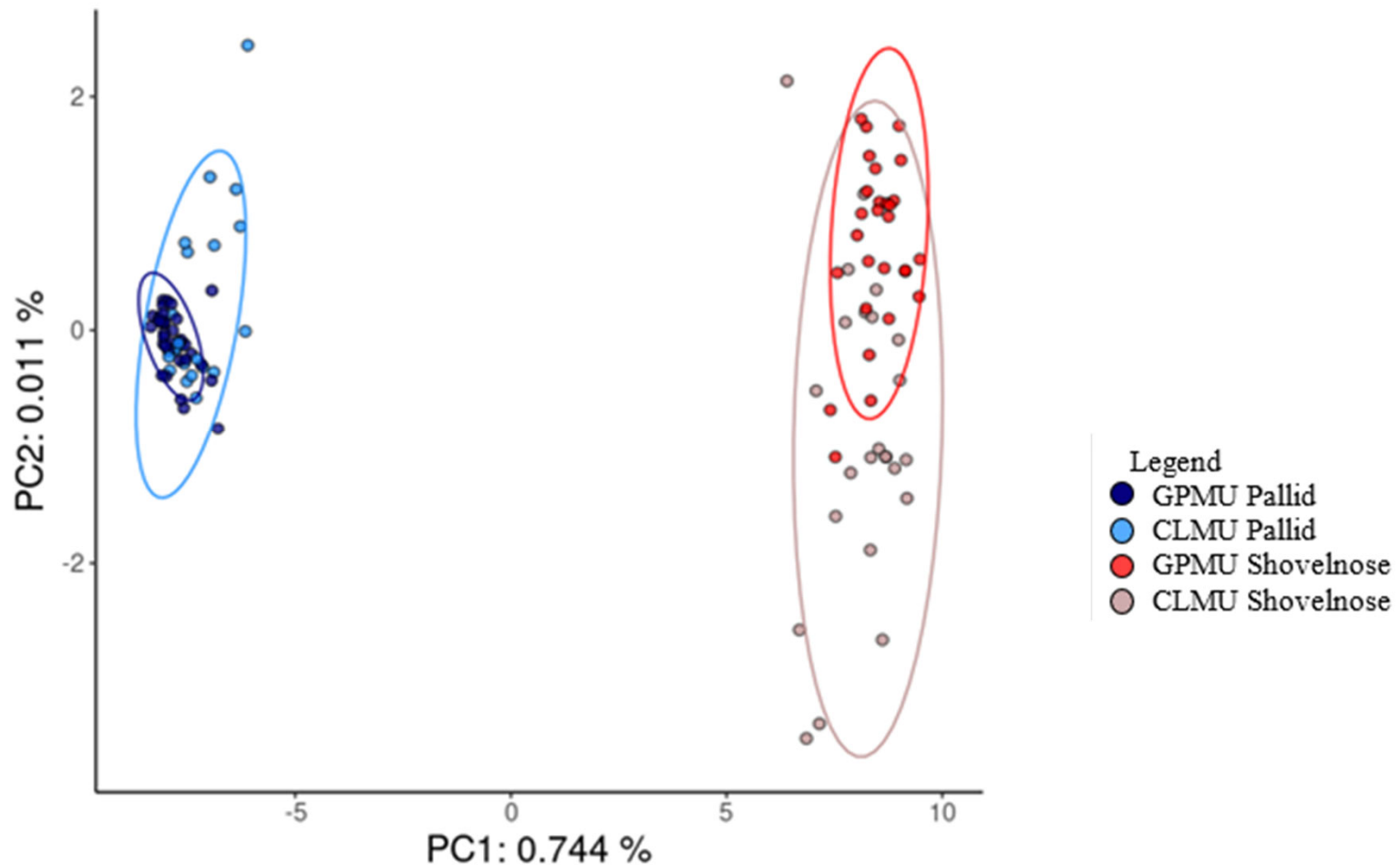
Scaphirhynchus linkage map

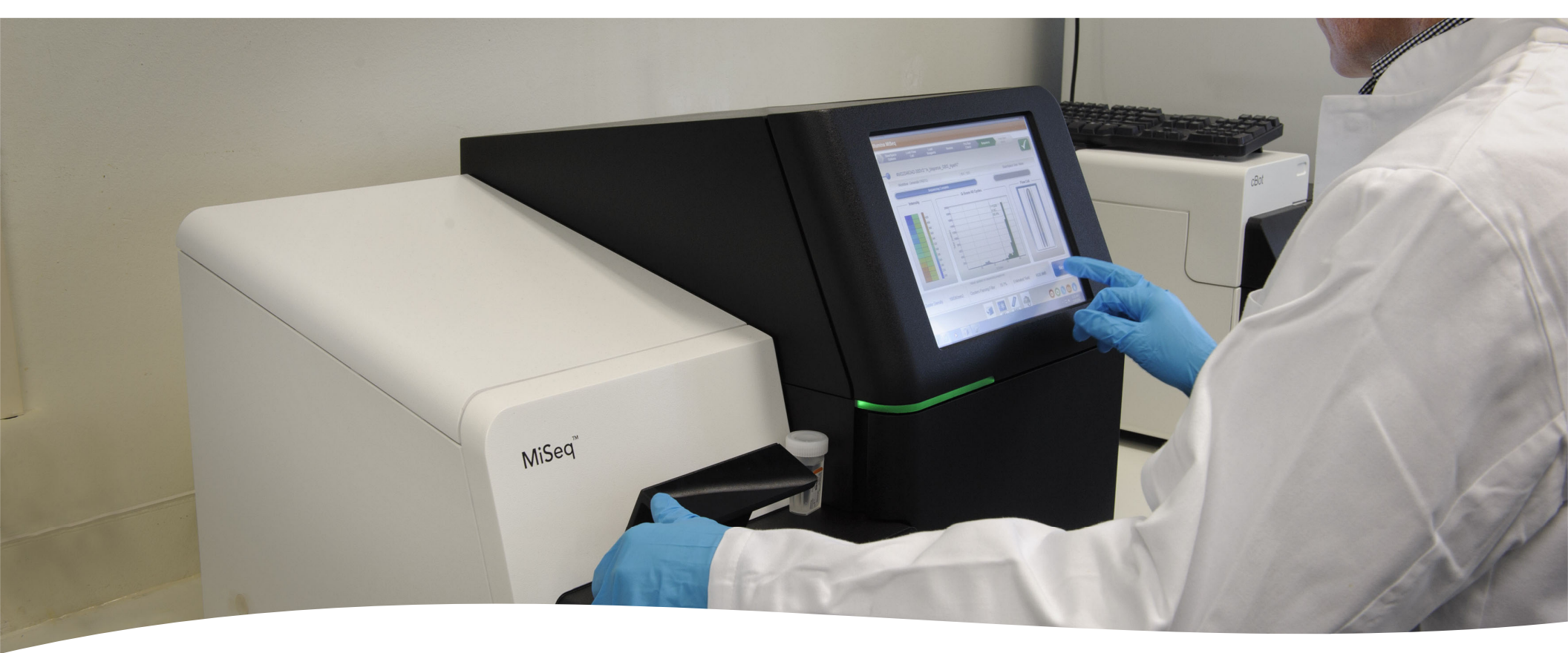


Unlinked markers provide more powerful estimate of hybrid status and population genetic measures including effective population size.

Principle Component Analysis

200 most informative SNPs





Illumina MiSeq

- High Throughput DNA sequencer.
- Can genotype hundreds of individuals at hundreds of loci in a single run.
- Recently acquired with funding from PRRIP.



Next Steps



- Genotyping by Thousands (GT-Seq) for larger study
 - Genotype 4000 MO River sturgeon at 200 loci over the next 4 years.
 - Genotype every age-0 sturgeon and adult pallid sturgeon collected in Platte River.
 - What is the role of Platte River in pallid sturgeon recovery?
 - Estimate N_e
- Funding through PRRIP (Platte River) and Army Corps of Engineers (MO River).

Summary

- Powerful SNP markers were developed for discriminating between pallid, shovelnose and hybrid sturgeon and for estimating effective population size.
- GT-Seq will provide unrivalled resolution for several conservation goals (augmentation, monitoring etc.) in the Missouri and Platte rivers.

