Microsatellite markers for *Fluminicola spp.*

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Objective

The purpose of this research is to design, test and optimize microsatellite primers for pebblesnails. These primers can be used to study population structure and manage these relatively unknown species for biodiversity and conservation purposes.

Background

Fluminicola is a genus of freshwater snails found in western United States, they are commonly known as the pebblesnail (Figure 1) (Hershler et al., 2017). There are currently 25 recognized species. They exist in the Pacific Coast Watershed, from northern California to southern British Columbia. There are several petitions to enter pebblesnails into the endangered species list, however many species have never been formally described and/or studied.

Microsatellites are made up of short tandem repeats ranging in length from 2-6 base pairs. It is typically repeated 5-50 times. Microsatellites are useful genetic markers because they tend to be highly polymorphic. The development of microsatellite primers can be used for studies of population structure and conservation genetics of these relatively unknown pebblesnails.

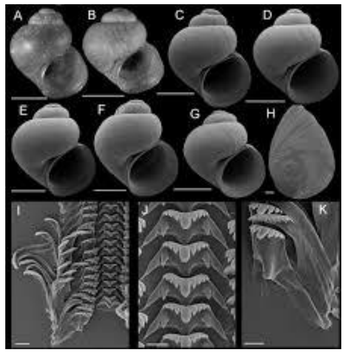

Figure 1. This is an example of pebblesnail, *Fluminola modoci*. A-G shows shell variation, H shows the operculum, and I-K is the radula (Hershler et al., 2017).

Acknowledgements

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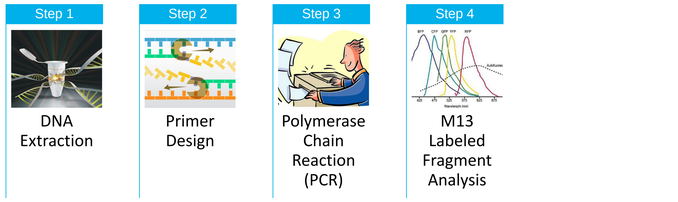
Works Cited

Boutin-Ganache, I., Raposo, M., Raymond, M., and Deschepper, C.F. (2001) M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. Biotechniques 31: 24-28.

Hershler, R., Liu, H-P., and Hubbart, N. (2017) Two new species of *Fluminicola* (Caenogastropoda, Lithoglyphidae) from southwest Oregon, USA, and a range extension for *F. multifarius*. ZooKeys 679: 1-20.

Liu, H-P., and Hershler, R. (2008) Microsatellite markers for the threatened Bliss Rapids snail (*Taylorconcha serpenticola*) and cross-amplification in its congener, *T. insperata*. Molecular Ecology Resources 8: 418–420.

Methods and Materials



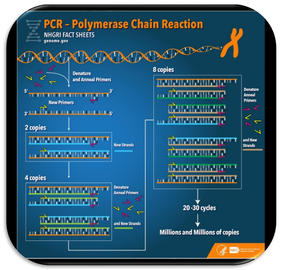


Figure 2: Detailed image of how polymerase chain reaction works

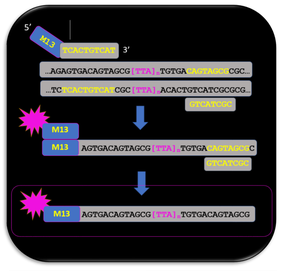


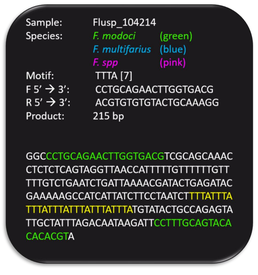
Figure 3: Depicts how fluorescent labeled DNA fragments are created.

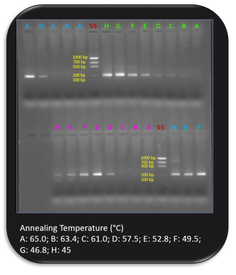
- DNA samples were extracted from 3 species of pebblesnails *Fluminicola modoci, Fluminicola multifarius,* and *Fluminicola spp.*
- Primer design: primers are 18-24 base pairs long. The primers were designed based on the desired DNA fragment size, appropriate GC percent, and avoiding hairpin structure.
- Primers were tested and optimized using polymerase chain reactions (PCR) with temperature gradients (Figure 2).
- 1.5% agarose gel was used to visualize the PCR products
- For successfully amplified microsatellite locus, PCRs were performed with M13 primer labeled with fluorescent molecule, M13 tailed forward primer, and reverse primer to generate fluorescent labeled DNA fragment (Figure 3) (Boutin – Ganche et at 2001).
- PCR products were run on the genetic analyzer ABI 3500.

Results

- 156 pair of primers were designed.
- 108 microsatellites loci were tested, including 36 di-, tri-, and tetranucleotide repeats. Only 9 loci (8%) were successfully amplified.
- 88 microsatellite loci were retested. 32 loci were successfully amplified (Figure 4) (36%). 8 out of 32 dinucleotide (25%), 7 out of 29 trinucleotide (24%), 17 out of 27 tetranucleotide (63%) repeats were successfully amplified.
- 26 forward primers were tailed with M13 sequences, of those 5 were dinucleotide, 7 were trinucleotide, and 14 were tetranucleotide repeats.
- Fluorescently labeled DNA fragment were shown in figures 5 and 6.

Figure 4: (Left) PCR product for three species of *Fluminicola* were visualized using 1.5% agarose gel. The PCR products were run using a temperature gradient ranging from 45 – 65C. Bands can be seen at ~215 bp, representing the expected PCR Product. **(Right)** Primer design details for gel.





Discussion

- **Primer Optimization:** The original test results showed less than a 8% success rate of microsatellite loci. After retesting there was a 36% success rate.
- **Microsatellites:** The successful rate of the tetranucleotide loci was 63% which was significantly higher than di- and trinucleotide loci, 25% and 24% respectively.
- **M13 primers:** Instead of fluorescently labeling forward primer, M13 labeled primers were used. It can reduce the cost of microsatellite development. In addition, samples can be ran in multiplex.

Future Work

- Completion of M13 tailed primer amplification testing.
- Continue data collection using fluorescent M13 fragment analysis.
- Determine polymorphism for each microsatellite loci.
- Use microsatellite loci for species identification and examination of population structure.

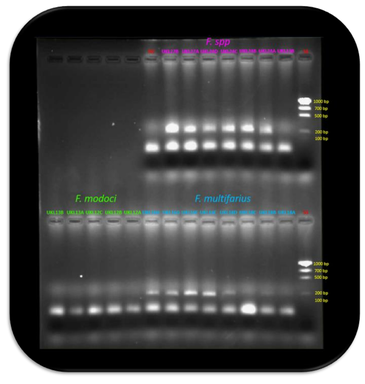


Figure 5 (M13 Tailed Primers Results): 1.5% agarose gel showing amplification results using M13 tailed primers for 20 individuals.

Lane 6: Negative control; Lanes 7 – 13: *F. modoci,* Lanes 10 – 14 *F. spp*, Lanes 16 – 23 *F. multifarious,* Lanes 14 and 28: Size standard.

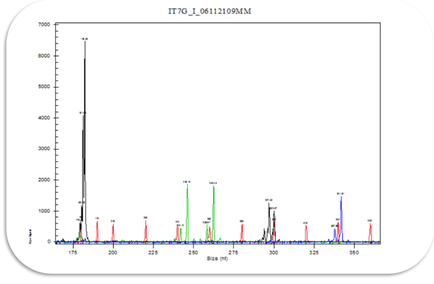


Figure 6 (M13 Fragment Analysis): Multiplex showing genotype analysis of several loci. Blue: FAM; Green: VIC, Black: NED

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