Phylogeny of an undescribed *Helobdella* leech species found in Montezuma Well, Arizona

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Abstract

Montezuma Well (the Well), a collapsed travertine well located in the Verde Valley of Central Arizona, has been isolated from other aquatic systems for over 11,000 years. Due to high levels of dissolved CO_2, the Well lacks aquatic vertebrate species, which has allowed invertebrates to fill the niches normally occupied by fishes. Four leech species, including one endemic species and another currently described as *Helobdella stagnalis*, a common fresh water leech, now inhabit the Well. To determine whether the *H. stagnalis* population is in fact endemic sequenced the cytochome c oxidase subunit I (COI) and nicotinamide adenine dinucleotide dehydrogenase subunit I (NDI) mtDNA gene sequences and compared these to two other Arizona populations of *Helobdella stagnalis* and other leech sequences collected from GenBank. Our results suggest that the Well leech may be an undescribed *Helobdella* species.

Introduction

Montezuma Well (The Well) is located 72 km south of Flagstaff in the Verde Valley of Northern Arizona. It is thermally constant (19-24˚C) and continuously replenished by two vents located at the bottom. It is 0.76 ha in diameter and approximately 15m deep. Most of the shoreline is steep and falls straight into the open water, but in the northeast corner there is a shallow region called the “swallet”. The water drains through this region and empties into both Wet Beaver Creek and the Sinagua Canal System. The water within the Well has unique water chemistry, it contains a high levels of arsenic (>100µg/L) and high levels dissolved CO_2 (>300mg/L).

There are four known species of leech that inhabit the Well one of which is an endemic predator of the pelagic zone (*Motobdella montezuma*). The other three are currently described as *Helobdella triserialis, Helobdella elongate,* and *Helobdella stagnalis* all are found in the shallow waters of the swallet. Beresic-Perrins (2010) began studying *H. stagnalis* in 2007 and found that their brood size, parental behavior, and life history differ from other populations of *H. stagnalis*. We investigated, through the use of phylogeny, if this population is an endemic species to the Well. Both the COI and NDI mitochondrial gene regions were chosen due to their rapid mutation rates (Apakupakul et al., 1999). These regions were used in collaboration to eliminate any bias that one may have, this “total evidence” approach aims reduce the chances of using inaccurate characteristics in the creation of the phylogeny (Light and Siddall 1999).

Basic Research

Undescribed *Helobdella* species from Montezuma Well

Methods

Leeches were collected from the swallet in Montezuma Well and our ingroup samples were collected from the Rio Del Flag ponds at the Rio de Flag Waste Water Facility outflow, and Oak Creek, all located in Northern Arizona. These samples were extracted, purified, amplified and sequenced using Siddall & Borda methods (2001). Sequences of other *Helobdella* species as well as *Haementeria* species were collected from Genebank. *Haementeria* was chosen as the outgroup taxa based on evidence suggesting it is the most closely related genera to *Helobdella* (Siddall & Borda 2001).

Caudal suckers and side slices were used for DNA extraction to minimize contamination from the gastrointestinal tract. We used QIAGEN DNeasy Tissue kit for DNA extraction and purification. We visualized and quantified the extracted DNA using gel-electrophoresis and Nano-Drop. We then amplified two mitochondrial gene regions, cytochrome c oxidase subunit I (COI) and nicotinamide adenine dinucleotide dehydrogenase subunit I (NDI), using Siddall’s 2001 PCR method. We used the COI region primers LCO1490 5’-GGTCAACAAA-TCATAAAGATATTGG-3’ and HCO2198 5’-TAAACTTCAGGGTGACCAAAAAATCA-3’ and the NDI region primers LND300 5’-TGGCAGAGTAGTGCATTAGG-3’ and HND1932 5’-CCTCAGCAAAATCAAATGG-3’. The samples were first heated to 94˚C for 5 min, they then went through 15 cycles of 94˚C for 45 sec, 46˚C for 45 sec, and 72˚C for 45 sec, followed by 25 cycles of 94˚C for 20 sec, 45˚C for 20 sec and 72˚C for 30 sec and finally 72˚C for 6 min. All products were purified through the use of the QIAquick PCR Purification Protocol.

Products were mixed with 4µL BigDye, 2µL of 1µM primer and 5µL of DNA template. This was sent through 40 cycles of 96˚C for 10 sec, 50˚C for 10 sec and 60˚C for 4 min. The products were then purified to remove primers and dyes and sequenced in both directions. Finally they were electrophoresed in an ABI Prism 3730 sequencer. Sequences were then aligned in DNASTAR Lasergene 8 and analyzed in PAUP* 4.0.

Results

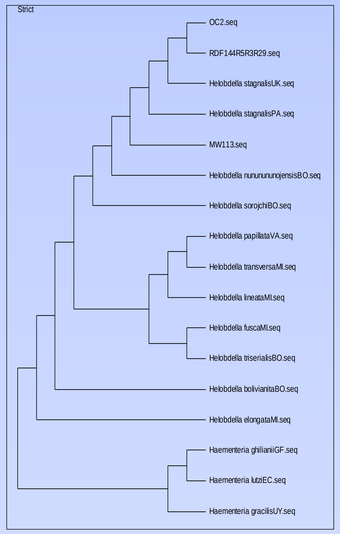

Figure 1: Most parsimonious tree created from COI region of mtDNA. This tree has a length of 869. MW: Montezuma Well, OC: Oak Creek, RDF: Rio de Flag

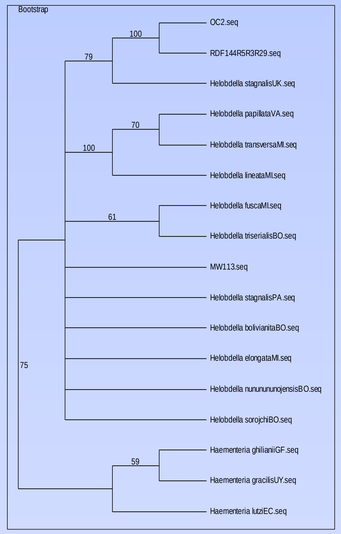

Figure 2: Bootstrap analysis of COI region of mtDNA. Numbers above branches indicate bootstrap values. MW: Montezuma Well, OC: Oak Creek, RDF: Rio de Flag

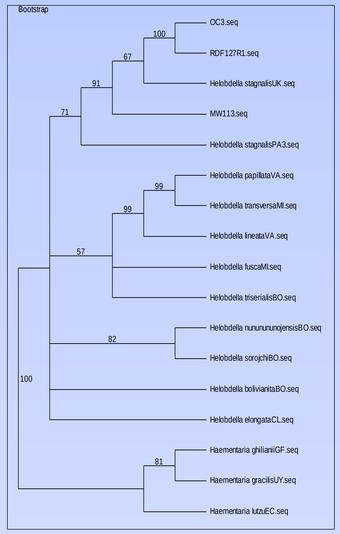

Figure 3: Most parsimonious tree created from NDI region of mtDNA. Numbers above branches indicate bootstrap values. This tree has a length of 872. MW: Montezuma Well, OC: Oak Creek, RDF: Rio de Flag

Both the COI (figures 1 and 2) and NDI (Figure 3) regions produced a single most parsimonious tree with CI values of 0.432 and 0.440 respectively and RI values of 0.426 and 0.475 respectively. Bootstrap analysis of the COI region resulted in loss of resolution of many relationships including those associated with the population from Montezuma Well. The bootstrap analysis of the NDI region however, supported our hypothesis that the population of leeches in Montezuma Well is an undescribed endemic species.

The “swallet” in the Northeast corner of Monetzuma Well

Conclusion

The phylogeny of both the COI and NDI regions of the mtDNA suggest this is a new species, which was expected due to its isolation for the past 11,000 years, however we cannot be certain based solely on the two phylogenies produced by these regions. Although both regions produced similar trees a few differences were present, by combining these trees a more accurate tree would be produced. Future analysis is needed to determine why the phylogeny of the COI region lost resolution after performing a bootstrap analysis. Investigation into both morphology and behavior of this population is also needed to increase certainty that this is in fact a new endemic species.

References

Siddall, M.E., & Borda E. 2001, DNVA, 32, 23

Light, J.E., & Siddall M.E. 1999, ASP, 85, 815

Apakupakul K., Siddall M.E., Burreson E.M., 1999, MPE, 12, 350

Beresic-Perrins, R.K., 2010, NAU thesis.

Acknowledgments

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