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The complete mitochondrial genome of *Achatinella mustelina* (Gastropoda: Pulmonata: Stylommatophora)

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ABSTRACT

In this study, we report the complete mitochondrial genome sequence of *Achatinella mustelina*, an endangered Hawaiian tree snail. The mitogenome is 16 323 bp in length and has a base composition of A (34.7%), T (42.6%), C (12.7%) and G (10.0%). Similar to other Pulmonates, it contains 13 protein-coding genes, 2 rRNA genes and 22 tRNA genes. To our knowledge, this is the first mitochondrial genome sequenced within the Achatinelloidea superfamily, which contains a high number of endangered species. As such, this mitogenome will be useful in conservation genetics studies.

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The genus *Achatinella*, endemic to the island of O'ahu, included at least 41 species of Hawaiian tree snails (Pilsbry & Cooke 1912–1914). Habitat loss, predation by introduced species and over-harvesting by collectors led to the extinction of at least 30 species of *Achatinella*, and resulted in the declaration of all remaining species in the genus as Endangered (Hadfield & Mountain 1980; U.S.A Fish and Wildlife Service 1981; Hadfield 1986). Of these, *Achatinella mustelina* is the most abundant, with at least 2000 individuals remaining in the wild.

We sequenced the complete mitochondrial genome of *A. mustelina* (GenBank accession number KU525108). Small tissue samples were collected from 15 populations across the range (15–40 individuals per population), using non-lethal methods, and preserved in 100% ethanol until DNA extraction (Thacker & Hadfield 2000). DNA was individually extracted from tissue samples using a DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) according to the manufacturer's protocol. Extracted DNA was quantified using the Biotium AccuClear Ultra High Sensitivity dsDNA quantitation kit, Hayward, CA with seven standards. Equal quantities of DNA from each individual within a population were pooled to a total of 1μg. From these pools, libraries were prepared for genome scanning using the ezRAD protocol (Toonen et al. 2013) version 2.0 (http://ingridknapp.weebly.com/current-research.html). Samples were digested with the frequent cutter restriction enzyme DpnII from New England Biolabs®, Ipswich, MA and prepared for sequencing on the Illumina® MiSeq using the Kapa Biosystems Hyper Prep kit, Wilmington, MA. All samples were amplified to generate 1 μg of adapter-ligated DNA, then validated and quantified to ensure equal pooling on the MiSeq flow cell, using a Bioanalyzer and qPCR. Quality control checks and sequencing were performed by the Hawaii Institute of Marine Biology Genetics Core Facility.

Reads were cleaned, and then assembled using an iterative method within Geneious 6.0, Newark, NJ. Ends were trimmed to remove barcode sequences, adapters, primers and low-quality regions (quality score <20). We obtained 69 178 116 sequences (mean ± S.D. = 4 494 912 ± 1 818 900 per population).

Reads were initially mapped to the mitogenome of *Albinuria coerulea* (Hatzoglou et al. 1995). The alignment of mapped sequences was inspected, and a consensus sequence was generated. This consensus sequence was used as a reference for the next iteration, in which all ~69 million sequences were mapped against the consensus sequence achieved in the previous round of alignment. This process was repeated until the complete mitochondrial genome was obtained. In total, 30 695 reads mapped to the complete mitochondrial genome, with coverage ranging from 10× to 4409× per site (256 ± 50). Annotation of mitochondrial elements was carried out with DOGMA (Wyman et al. 2004) and MITOS (Bernt et al. 2013).

The *Achatinella mustelina* mitogenome is similar to those of other Pulmonates (Figure 1), with 13 protein-coding genes, 2 rRNA genes and 22 tRNA genes. The total length is

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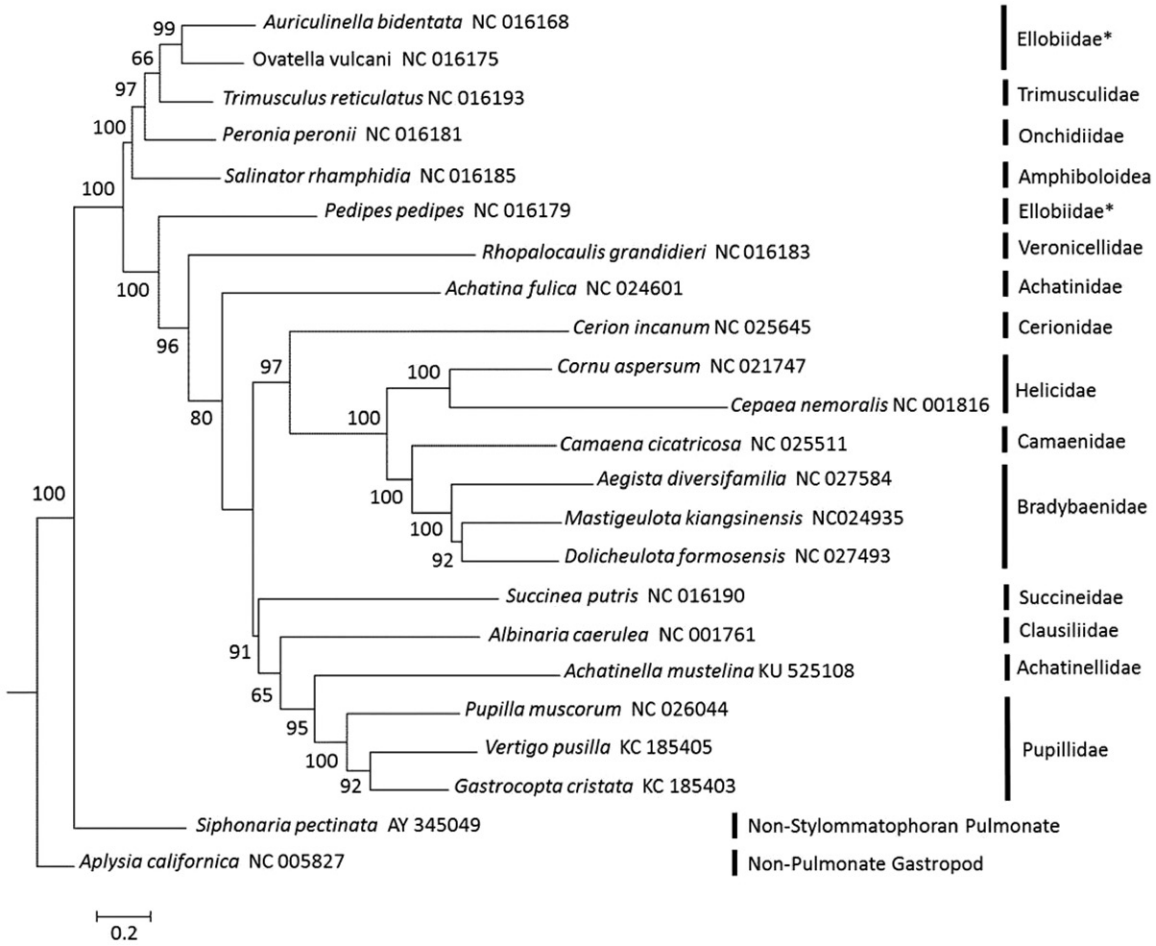

Figure 1. Placement of *Achatinella mustelina* among Stylommatophoran snails. Alignments, model tests and maximum likelihood analyses were performed using MEGA version 6.2 (Tamura et al. 2013). The 13 protein-coding mitochondrial gene sequences were individually translated into amino acid sequences, and then aligned using ClustalW in MEGA version 6.2 (Tamura et al. 2013). Default settings were used with the following exception: the multiple alignment parameters were changed to a gap opening penalty of 3.0; and the gap extension penalty was set to 1.8. The amino acid substitution model was found to be LG + G + I + F using the Akaike Information Criterion (AIC). Maximum likelihood analysis of the amino acid sequences was run using the identified model, with bootstrap support values based on 1000 replicates. The resulting tree shows similar relationships to previous studies, though species in one family (Ellobiidae) did not group together (Hatzoglou et al. 1995; Grande et al. 2004; Knudsen et al. 2006; White et al. 2011; Yamazaki et al. 1997; Gaitán-Espitia et al. 2013; Gonzalez et al. 2014 (unpublished); He et al. 2014; Wang et al. 2014; Huang et al. 2015; Marquardt et al. 2015 (unpublished); Deng et al. 2016).

16 323 bp, slightly larger than other Pulmonates (White et al. 2011). The base composition of the genome is: A (34.7%), T (42.6%), C (12.7%) and G (10.0%). This is the first mitochondrial genome sequenced within the Achatinelloidea superfamily.

Collection sites

Samples were collected from the following locations (decimal degrees): 21.413406, -158.099816; 21.438595, -158.097806; 21.467780, -158.103860; 21.476095, -158.125562; 21.488028, -158.138127; 21.498169, -158.143955; 21.505067, -158.127069; 21.510198, -158134197; 21.514961, -158153266; 21.525394, -158.170789; 21.496982, -158.172874; 21.511052, -158.190455; 21.514602, -158.201006; 21.537079, -158.199123; 21.538785, -158.198855.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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