

## Mitochondrial DNA Part B Resources

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# Complete mitochondrial genomes of the freshwater mussels *Amblema plicata* (Say, 1817), *Pleurobema oviforme* (Conrad, 1834), and *Popenaias popeii* (Lea, 1857) (Bivalvia: Unionidae: Ambleminae)

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## Complete mitochondrial genomes of the freshwater mussels *Amblema plicata* (Say, 1817), *Pleurobema oviforme* (Conrad, 1834), and *Popenaias popeii* (Lea, 1857) (Bivalvia: Unionidae: Ambleminae)

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### ABSTRACT

Freshwater mussels are a critically imperiled group of mollusks that play key ecological roles and provide important services to humans. The Ambleminae is the only subfamily of these mussels, endemic to North America. Complete mitogenomes have only been sequenced for two of five tribes of the subfamily. *Pleurobema oviforme*, *Amblema plicata*, and *Popenaias popeii* each belong to tribes Pleurobemini, Amblemini, and Popenaidini, respectively, and have not had published mitogenomes. Thus, this study aims to present the complete mitogenomes for these species, to provide a phylogeny of the Ambleminae and confirm the gene arrangements with representation from each of its tribes. The newly sequenced mitogenomes range from 15,852 to 15,993 nucleotides, are composed of 13 PCGs, 22 tRNAs, and two rRNAs and all share the same (UF1) gene order.

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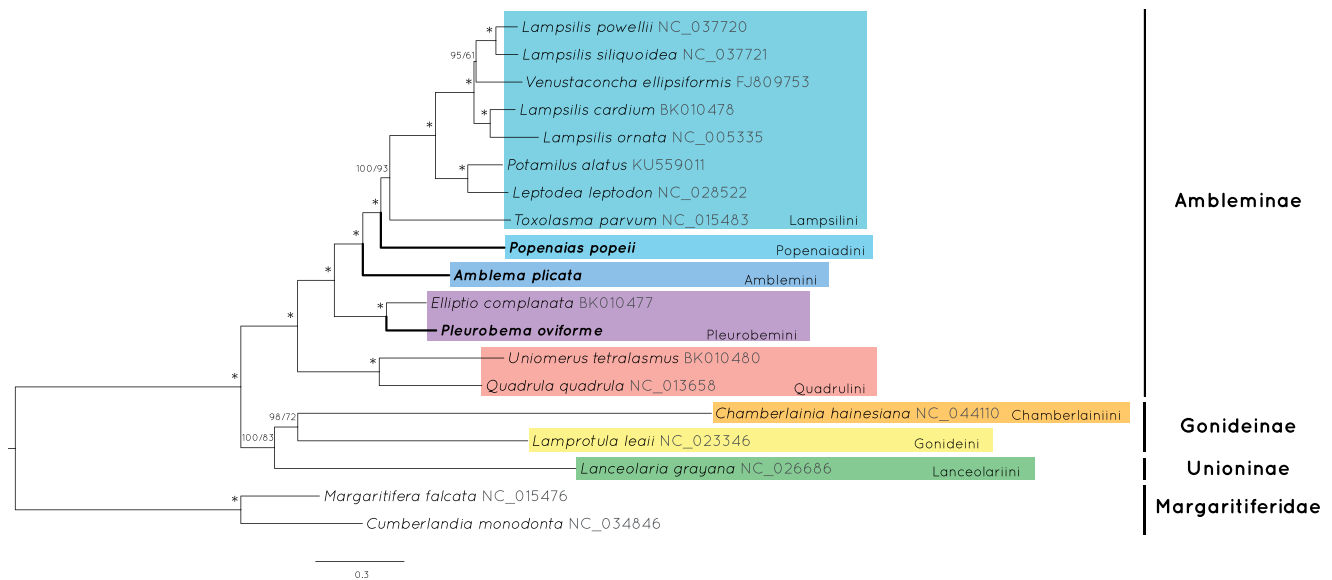
*Amblema plicata*;  
*Pleurobema oviforme*;  
*Popenaias popeii*;  
Ambleminae; mitogenome  
phylogenetics

Freshwater mussels (Bivalvia: Unionida) are important members of aquatic ecosystems and are among the most threatened faunistic groups worldwide. Both North and Central America are among the regions with higher freshwater mussel diversity (Lopes-Lima et al. 2018). However, these taxa have suffered a dramatic decline and a high number of extinctions in these regions (Haag and Williams 2014), emphasizing the need for understanding their evolutionary relationships to guide future conservation measures (Levine et al. 2012). To date, three subfamilies are recognized within the Unionidae in the North and Central America: Gonideinae Ortmann (1916), Unioninae Rafinesque (1820), and Ambleminae Rafinesque (1820). The Ambleminae is the only subfamily endemic in this region. Several studies have been published to characterize the phylogenetic relationships within the Ambleminae in North America, although genera that occur in Central America were rarely included. The most recent classification separates the amblemines into five tribes: Lampsilini, Popenaidini, Amblemini, Pleurobemini, and Quadrulini (Pfeiffer et al. 2019). Complete mitogenomes are only available for two of these tribes, Lampsilini with eight,

Quadrulini with two, Pleurobemini with only one partial mitogenome and Popenaidini and Amblemini with no available complete mitogenome. Freshwater mussels present different gene orders that might be useful for phylogenetic inference. Within Unionidae, the gene order UF1 is shared among all members of the Ambleminae and Unioninae subfamilies, while UF2 and UF3 occur in members of Gonideinae (Lopes-Lima et al. 2017).

Here, we present the complete mitogenomes of *Amblema plicata* (Say, 1817), *Pleurobema oviforme* (Conrad, 1834), and *Popenaias popeii* (Lea, 1857) and their evolutionary relationships. The latter two have a high conservation concern being considered as threatened by the IUCN Red List (<https://www.iucnredlist.org/>).

*Pleurobema oviforme* was collected from the Little Tennessee River, Tennessee 35.325820 longitude 83.523930 latitude and deposited in North Carolina Museum of Natural Sciences USA (voucher: NCSM30708). *Popenaias popeii* was collected in Rio Grande River, Texas 99.49890 longitude, 27.49921 latitude, and deposited in the Great Lakes Center Invertebrate Collection (BSGLC) (SUNY Buffalo State College,



**Figure 1.** Bayesian Inference phylogenetic tree of the concatenated protein coding and rRNA genes of the available mitogenomes of Ambleminae and two outgroup taxa. Values in branches represent the Bayesian posterior probabilities percentage obtained by MrBayes and the bootstrap support values from IQ-Tree. Values above 95% for both are replaced with an \*.

Buffalo, NY, USA) (voucher: 067TS). *Amblema plicata* was collected in the San Marcos River, TX, 29.588390 latitude 97.586110 longitude, deposited in the BSGLC (voucher: 061TS). The collection in Texas was carried out with an appropriate Scientific Research Permit SPR-0503-300 issued by the Texas Parks and Wildlife Department. Genomic DNA was extracted using standard protocols and thereafter sequenced using MiSeq Illumina runs. Resulting reads were trimmed in Trimmomatic (Bolger et al. 2014). Each mitogenome was assembled using MITObim (Hahn et al. 2013), and annotated in MITOS (Bernt et al. 2013). Additional sequences of all Ambleminae species were downloaded from GenBank together with two margaritiferid species as outgroup. Each individual protein-coding gene and rRNA gene were aligned and trimmed using GUIDANCE2 (Sela et al. 2015) with the MAFFT algorithm (Katoh and Standley 2013). The resulting trimmed alignments of the individual genes were concatenated. The phylogenetic relationships were inferred by maximum likelihood (ML) using IQTree version 1.6.10 (Nguyen et al. 2015) with 20,000 ultrafast-bootstraps and Bayesian inference (BI) using MrBayes version 3.2.7a (Ronquist et al. 2012) with two independent runs of  $10^7$  generations and a sampling frequency from every 1,000 trees. The best partition models were selected under the software PartitionFinder version 2.2.2.1 (Lanfear et al. 2016) for both IQ-Tree and MrBayes. These were run on XSEDE through the CIPRES Science Gateway (Miller et al. 2010). Complete mitogenomes of *A. plicata*, *P. oviforme*, and *P. popeii* were submitted to GenBank: MT648774, MT648775, and MT648776. Each contained 15,946, 15,852, and 15,993 bps length, respectively, and are composed by 13 PCGs, 2 rRNAs, and 22 tRNAs.

The resulting ML and BI trees share the same topology (Figure 1), rooted with the two margaritiferid species. An initial split occurs between the Ambleminae and Gonideinae + Unioninae. The Ambleminae is further separated between Quadrilini and the rest of the tribes. The new mitogenome of *P. oviforme* clusters with *E. complanata* in a well-

supported clade. Both *A. plicata* (Amblemini) and *P. popeii* (Popenaiadini) represent the first available mitogenomes within their tribes and therefore do not show any close phylogenetic relationship. This tree confirms the same phylogeny of the most recent tribal classification within Ambleminae (Pfeiffer et al. 2019). Finally, all Ambleminae mitogenomes (including the newly sequenced) share the same gene order, i.e. UF1.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

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## Data availability statement

The data produced in this study are available in GenBank of NCBI at <https://www.ncbi.nlm.nih.gov>, reference numbers MT648774–MT648776 or from the corresponding author.

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