

The male-type mitochondrial genome of the freshwater mussel *Potamilus streckersoni* Smith, Johnson, Inoue, Doyle, & Randklev, 2019 (Bivalvia: Unionidae)

Raquel Mejia-Trujillo  and Chase H. Smith 

Department of Integrative Biology, University of Texas, Austin, TX, USA

ABSTRACT

The global decline of freshwater mussels emphasizes the need to establish genetic resources to better understand their biology, including a unique mitochondrial biology known as doubly uniparental inheritance. In this study, we present the complete male-type (M-type) mitochondrial genome of the freshwater mussel, *Potamilus streckersoni* Smith, Johnson, Inoue, Doyle, & Randklev, 2019. The M-type mtDNA is approximately 16 kilobases and contains 22 tRNAs, two rRNAs, and 14 protein-coding genes, including a male-specific open reading frame. Read coverage revealed that M-type mtDNA was more abundant than female-type mtDNA in male gonadal tissue, with respect to a non-spawning male individual. Novel mitogenomes were resolved within previously described sex-specific monophyletic clades across the subfamily Ambleminae. The availability of high-quality nuclear and mitochondrial genomic data for *P. streckersoni* makes it a model for future research into the potential role of mtDNA in sex determination or sexual development in freshwater mussels.

ARTICLE HISTORY

Received 18 August 2022
Accepted 6 October 2022

KEYWORDS

Doubly uniparental inheritance;
Unionida; Ambleminae

Introduction

Freshwater mussels (Bivalvia: Unionidae) are aquatic mollusks concentrated in North America (Graf and Cummings 2021), with approximately 300 species in the United States and Canada (Williams et al. 2017). Unfortunately, systemic declines have led the group to be considered one of the most imperiled in North America (Strayer et al. 2004; Haag and Williams 2014), which is problematic considering freshwater mussels perform essential functional roles in freshwater ecosystems (Vaughn and Hakenkamp 2001). Recent declines have invoked a need for additional research (Ferreira-Rodríguez et al. 2019), which includes the establishment of genetic resources to facilitate the research of freshwater mussel biology.

Several bivalve lineages, including freshwater mussels, possess a unique mitochondrial inheritance pattern known as doubly uniparental inheritance (DUI), which involves the transmission of sex-specific mtDNAs (Hoeh et al. 1991; Breton et al. 2007; Zouros 2013). Females are homoplasmic for a female-type (F-type) mtDNA that is transmitted to all offspring (Zouros 2013), similar to organisms with strictly maternal mitochondrial inheritance. In males, however, male-type (M-type) mtDNA is transmitted from fathers only to their sons. In freshwater mussels, F- and M-type mtDNAs are highly distinctive, differing by more than 50% of their amino acid sequences (Breton et al. 2007). Freshwater mussel mtDNAs also contain haplotype-specific open reading frames

(ORFs) that have no homology to known genes. A female ORF (F-ORF) has been detected in F-type mtDNA, while a male ORF (M-ORF) has been detected in M-type mtDNA (Breton et al. 2011). Given this, an association between nuclear gene products and mtDNA ORFs is speculated to play a role in sex determination (Breton et al. 2022), but the lack of genetic resources has hindered research into these topics (e.g. Renaut et al. 2018; Capt et al. 2019).

Potamilus streckersoni Smith, Johnson, Inoue, Doyle, & Randklev, 2019 (Figure 1) is one of the few freshwater mussel species with a high-quality *de novo* nuclear genome assembly (Smith 2021). In this study, we assemble the M-type mtDNA of *P. streckersoni*, which completes the availability of genetic resources for the species. With reference F-type mtDNA, M-type mtDNA, and nuclear genome assemblies available, *P. streckersoni* represents a model for investigating the biological function of DUI and mitonuclear ecology in freshwater mussels.

Methods

One adult male *P. streckersoni* was collected from the type locality in the Brazos River drainage (Latitude: 30.86586° N, Longitude: 96.69575° W) under Scientific Research Permit SPR-0717-156 issued by the Texas Parks and Wildlife Department. The specimen was deposited in the Joseph Britton Freshwater Mussel Collection (Charles Randklev;

CONTACT Chase H. Smith  chase.smith@austin.utexas.edu  Department of Integrative Biology, University of Texas, Austin, TX, USA

© 2022 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Figure 1. *Potamilus streckersoni* holotype (UF439497) designated by Smith et al. (2019).

Charles.Randklev@ag.tamu.edu) under voucher number 10001.6. DNA was extracted from gonadal tissue using the Qiagen PureGene Kit (Hilden, Germany) with the manufacturer's recommended protocol. High-molecular weight DNA was ensured by visualizing the isolation on a 1% agarose gel stained with GelGreen[®] nucleic acid stain (Biotium, Hayward, CA). DNA quantity and quality was assessed using a Qubit[™] fluorometer and a NanoDrop[™] One (ThermoFisher Scientific, Waltham, MA), respectively. A genomic DNA library was constructed with the NEBNext Ultra[™] II Library Prep Kit (Ipswich, MA) using standard protocols. The resulting library was sequenced using 100-bp single end reads on an Illumina NovaSeq 6000 (San Diego, CA).

TRIM GALORE! v 0.6.7 (Krueger et al. 2021) was used to trim adaptor sequences and filter reads of less than 30 bases. F- and M-type mtDNAs were *de novo* assembled from the trimmed reads using MitoFinder v 1.4 (Allio et al. 2020). Gene limits for the M-type mtDNA were constructed using MITOS2 (Donath et al. 2019) and manually curated when needed. The M-ORF was manually annotated using Geneious v 2022.1.1 (Kearse et al. 2012). Final M-type tRNA gene limits were assessed using ARWEN v 1.2 (Laslett and Canbäck 2008) and manually curated when needed.

We investigated start codon variation by compiling start codons of all available Ambleminae mtDNAs in 14 PCGs, including the haplotype-specific ORFs when available. Considering both F- and M-type mtDNAs are present in gonadal tissue, we also compared relative read depth of each type. We mapped trimmed reads to the final assemblies using bwa-mem2 v 2.2.1 (Vasimuddin et al. 2019) and calculated average read depth using SAMtools v 1.15.1 (Li et al. 2009).

Annotations for 13 PCGs (excluding haplotype-specific ORFs) were extracted from 15 F- and five M-type mtDNAs (Figure 2) from NCBI and the novel *P. streckersoni* F- and M-type assemblies. PCGs were aligned using MACSE v 2 (Ranwez et al. 2011), and the resulting alignments were trimmed using trimAl v 1.4 (Capella-Gutierrez et al. 2009) with a gap threshold of 50%. Phylogenetic inference was performed on an amino acid alignment of 13 PCGs using IQ-TREE V 2.1.4 (Minh et al. 2020). ModelFinder (Kalyaanamoorthy et al. 2017) was used to select the best invertebrate mitochondrial model of amino acid evolution and 1000 ultrafast bootstrap replicates were used to assess nodal support (Hoang et al. 2018).

Results and discussion

Illumina sequencing generated approximately 70 million 100-bp single end reads. We were able to assemble both F- and M-type mtDNAs from a single male gonadal sample (GenBank accession nos. ON855351 and ON881148). The F-type assembly was 16,152 bp and similar, but smaller in size, than the previously published *P. streckersoni* F-type assembly that reported 16,293 bases (GenBank accession no. MW413895; Smith 2021). The M-type assembly was 16,482 bases and similar in size to the closely related species *Potamilus alatus* (Say, 1817), which was reported to be 16,560 bases (GenBank accession no. KU559010; Wen et al. 2017). Our annotations were consistent with annotations from available Ambleminae mitogenomes (Table 1). Each of the haplotype assemblies consisted of 22 tRNAs, two rRNAs, and 14 PCGs, which includes a haplotype-specific ORF (Figure 3). Both haplotypes utilize alternative start codons consistent with the invertebrate mitochondrial genetic code. Additionally, there are haplotype-specific start codon biases across *COX1*, *COX3*, *ND2*, *ND4*, and the ORFs (Table 1).

We investigated the relative amount of F- and M-type mtDNA in gonadal tissue using read depth, which showed a ratio of ~1.5 M-type to F-type mtDNA (F-type mean coverage = 146, M-type mean coverage = 231). Relative read depth was similar across each mtDNA, other than a peak in the putative F-type mtDNA control region, which is likely due to an unassembled repetitive region (Figure 3). However, this ratio may vary based on sampling timing given M-type mtDNA is localized in sperm. The adult male specimen used in this study was collected in November, and given spermatogenesis occurs in the closely related species *P. alatus* in July–August (Haggerty and Garner 2000), the specimen was likely collected outside of spawning season. Male specimens collected during active spermatogenesis may exhibit increased dosage of M mtDNA in gonadal tissues. Resources presented in this study will allow for the development of assays (e.g. qPCR and ddPCR) to assess relative ratios of F and M type mtDNAs, which may be useful for future research in the phenology and mitochondrial physiology of *P. streckersoni*.

Similar to previous studies, our phylogenetic reconstruction resolved F- and M-type mitogenomes as monophyletic, while also generally resolves tribal relationships across

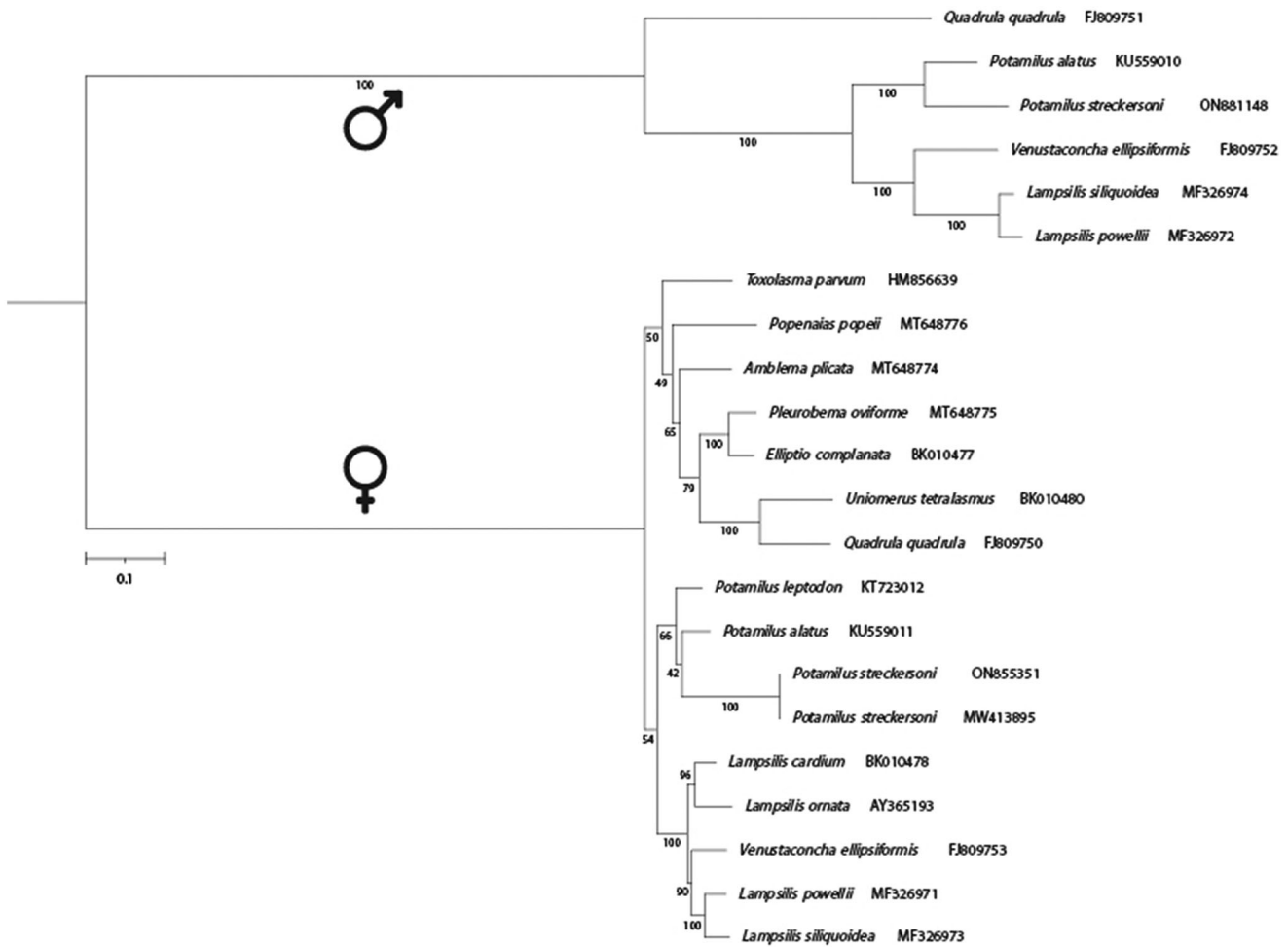


Figure 2. A multi-gene phylogenetic reconstruction of translated female- and male-type mitochondrial genes involved in oxidative phosphorylation. Associated GenBank accessions are included adjacent to each species in the subfamily Ambleminae. Ultrafast bootstrap support is referenced on nodes, where values greater than or equal to 95 represent strong support.

Table 1. Start codons based on the invertebrate mitochondrial translation code for annotated genes in female- (F) and male-type (M) mitogenomes examined in this study.

Taxon	Accession	Type	ATP6	ATP8	COX1	COX2	COX3	CYTB	ND1	ND2	ND3	ND4	ND4L	ND5	ND6	ORF
<i>Amblema plicata</i>	MT648774	HO	M	V	L	M	M	I	I	M	M	I	V	M	I	-
<i>Elliptio complanata</i>	BK010477	HO	M	-	L	M	M	I	I	M	M	I	M	M	I	-
<i>Lampsilis cardium</i>	BK010478	HO	M	M	L	M	M	I	I	M	M	I	M	M	I	-
<i>Lampsilis ornata</i>	AY365193	HO	M	M	L	M	M	I	I	M	M	I	V	M	I	-
<i>Lampsilis siliquoidea</i>	MF326973	HO	M	M	L	M	M	I	I	M	M	I	V	M	I	L
<i>Pleurobema oviforme</i>	MT648775	HO	M	V	L	M	M	I	I	M	M	I	M	M	I	-
<i>Popenaias popeii</i>	MT648776	HO	M	V	L	M	M	I	I	M	M	I	V	M	I	-
<i>Potamilus alatus</i>	KU559011	HO	M	V	I	M	M	I	I	M	M	I	V	M	M	I
<i>Potamilus leptodon</i>	KT723012	HO	M	V	L	M	M	I	I	M	M	I	M	I	I	-
<i>Potamilus streckersoni</i>	MW413895	HO	M	V	I	M	M	I	I	M	M	I	V	V	I	I
<i>Potamilus streckersoni</i>	ON855351	HO	M	V	I	M	M	I	I	M	M	I	V	V	I	I
<i>Quadrula quadrula</i>	FJ809750	HO	M	M	L	M	M	I	M	M	M	I	V	M	M	-
<i>Toxolasma parvum</i>	HM856639	HO	M	V	L	M	M	M	I	M	M	I	V	M	I	I
<i>Unio merus tetralasmus</i>	BK010480	HO	M	V	L	V	M	I	I	M	M	I	I	M	V	-
<i>Venustaconcha ellipsiformis</i>	FJ809753	HO	M	V	L	M	M	I	I	M	M	I	V	L	I	-
<i>Lampsilis powellii</i>	MF326972	HO	M	V	V	M	I	V	I	I	I	M	L	M	M	V
<i>Lampsilis siliquoidea</i>	MF326974	HO	M	V	V	M	I	V	M	I	M	M	L	M	M	V
<i>Potamilus alatus</i>	KU559010	HO	M	M	M	M	I	M	I	I	M	M	M	M	M	M
<i>Potamilus streckersoni</i>	ON881148	HO	M	M	M	M	I	M	M	I	M	M	M	M	M	M
<i>Quadrula quadrula</i>	FJ809751	HO	M	V	V	M	I	M	M	I	I	M	M	M	M	-
<i>Venustaconcha ellipsiformis</i>	FJ809752	HO	M	V	V	M	I	I	M	I	M	M	L	M	M	-

GenBank accession numbers and type are provided for each mitogenome. Dashes indicate a gene that was not annotated on the assembly.

^aHermaphroditic mtDNA.

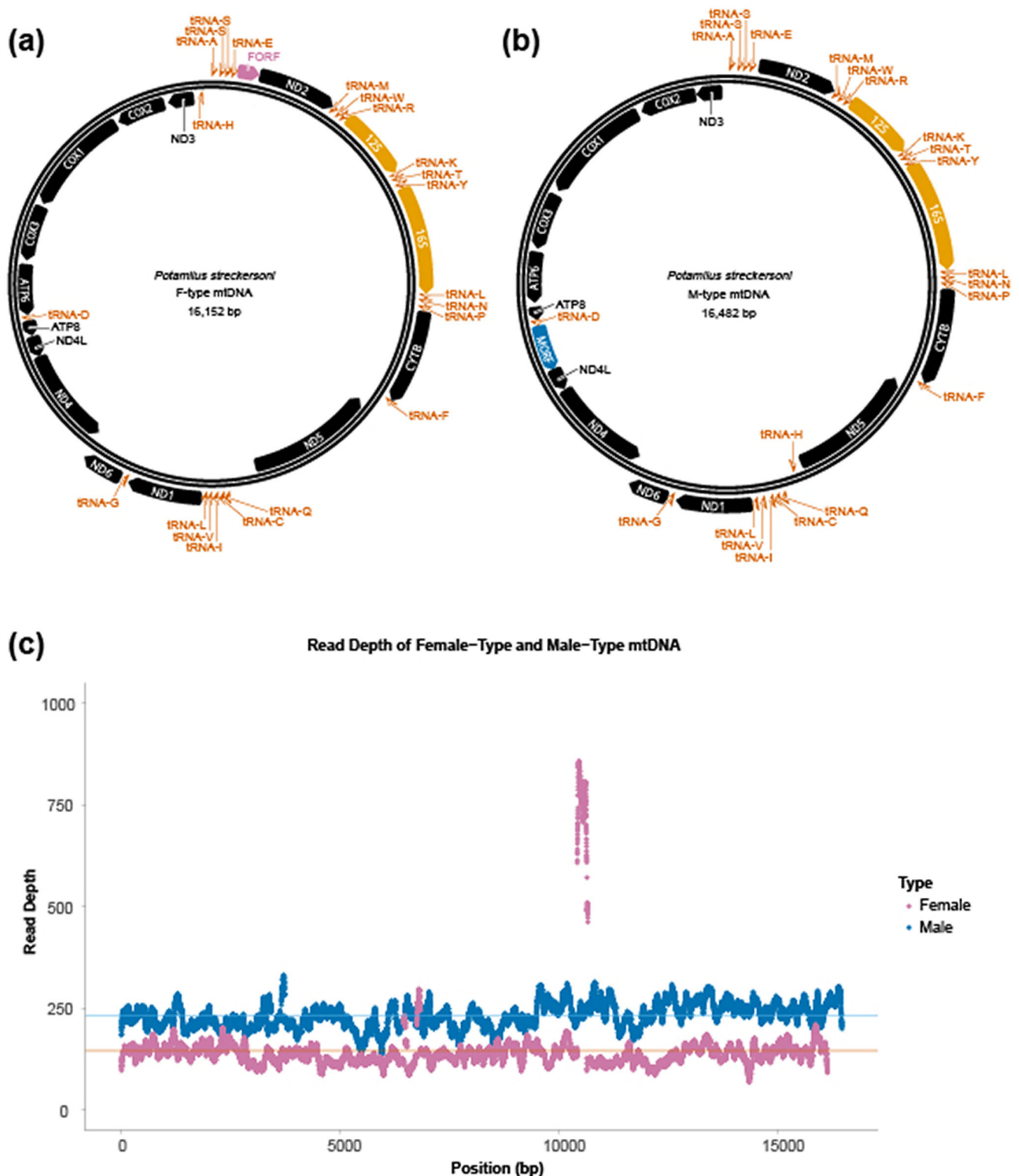


Figure 3. Female- (F-type) (a) and male-type (M-type) (b) mitogenome assemblies for *P. streckersoni*. Coloration of annotated features is as follows: protein-coding oxidative phosphorylation genes are represented in black, tRNAs are represented in dark orange, rRNAs are represented in light orange, and haplotype-specific open reading frames (ORFs) are represented in pink (F-ORF) and blue (M-ORF). Per base read depth statistics for F- and M-type mtDNA (c). Each blue (M-type) and pink (F-type) point represents coverage at a specific site. Lines represent the average value across each mtDNA.

Ambleminae (Figure 2; Huang et al. 2019; Pfeiffer et al. 2019). *Potamilus* was resolved as monophyletic in both the F- and M-type lineages, with *P. streckersoni* sister to *P. alatus* (Figure 2).

Author contributions

RM-T – conceptualization, methodology, formal analysis, investigation, writing – original draft, visualization, and

funding acquisition. CHS – conceptualization, methodology, formal analysis, resources, data curation, writing – review and editing, supervision, and funding acquisition.

Disclosure statement

The authors report there are no competing interests to declare.

Funding

This work was supported by the University of Texas at Austin Stengl-Wyer Endowment (InSTnCT REU – Raquel Mejia-Trujillo; Scholars Program – Chase Smith).

ORCID

Raquel Mejia-Trujillo  <http://orcid.org/0000-0002-2581-5674>

Chase H. Smith  <http://orcid.org/0000-0002-1499-0311>

Data availability statement

Mitogenome sequence data generated in this study are available on GenBank of NCBI at <https://www.ncbi.nlm.nih.gov> under the accession numbers ON855351 and ON881148. The associated BioProject and SRA numbers are PRJNA851076 and SRR19754570, respectively.

References

- Allio R, Schomaker-Bastos A, Romiguier J, Prosdocimi F, Nabholz B, Delsuc F. 2020. MitoFinder: efficient automated large-scale extraction of mitogenomic data in target enrichment phylogenomics. *Mol Ecol Resour.* 20(4):892–905.
- Breton S, Beaupré HD, Stewart DT, Hoeh WR, Blier PU. 2007. The unusual system of doubly uniparental inheritance of mtDNA: isn't one enough? *Trends Genet.* 23(9):465–474.
- Breton S, Stewart DT, Brémaud J, Havird JC, Smith CH, Hoeh WR. 2022. Did doubly uniparental inheritance (DUI) of mtDNA originate as a cytoplasmic male sterility (CMS) system? *BioEssays.* 44(4):2100283.
- Breton S, Stewart DT, Shepardson S, Trdan RJ, Bogan AE, Chapman EG, Ruminas AJ, Piontkivska H, Hoeh WR. 2011. Novel protein genes in animal mtDNA: a new sex determination system in freshwater mussels (Bivalvia: Unionoida)? *Mol Biol Evol.* 28(5):1645–1659.
- Capella-Gutierrez S, Silla-Martinez JM, Gabaldon T. 2009. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics.* 25(15):1972–1973.
- Capt C, Renaut S, Stewart DT, Johnson NA, Breton S. 2019. Putative mitochondrial sex determination in the Bivalvia: insights from a hybrid transcriptome assembly in freshwater mussels. *Front Genet.* 10:840.
- Donath A, Jühling F, Al-Arab M, Bernhart SH, Reinhardt F, Stadler PF, Middendorf M, Bernt M. 2019. Improved annotation of protein-coding genes boundaries in metazoan mitochondrial genomes. *Nucleic Acids Res.* 47(20):10543–10552.
- Ferreira-Rodríguez N, Akiyama YB, Aksenova OV, Araujo R, Christopher Barnhart M, Bespalaya YV, Bogan AE, Bolotov IN, Budha PB, Clavijo C, et al. 2019. Research priorities for freshwater mussel conservation assessment. *Biol Conserv.* 231:77–87.
- Graf DL, Cummings KS. 2021. A “big data” approach to global freshwater mussel diversity (Bivalvia: Unionoida), with an updated checklist of genera and species. *J Mollusc Stud.* 87(2):1–36.
- Haag WR, Williams JD. 2014. Biodiversity on the brink: an assessment of conservation strategies for North American freshwater mussels. *Hydrobiologia.* 735(1):45–60.
- Haggerty TM, Garner JT. 2000. Seasonal timing of gametogenesis, spawning, brooding and glochidia discharge in *Potamilus alatus* (Bivalvia: Unionidae) in the Wheeler Reservoir, Tennessee River, Alabama, USA. *Inverteb Reprod Dev.* 38(1):35–41.
- Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS. 2018. UFBoot2: improving the ultrafast bootstrap approximation. *Mol Biol Evol.* 35(2):518–522.
- Hoeh W, Blakley K, Brown W. 1991. Heteroplasmy suggests limited biparental inheritance of *Mytilus* mitochondrial DNA. *Science.* 251(5000):1488–1490.
- Huang X-C, Su J-H, Ouyang J-X, Ouyang S, Zhou C-H, Wu X-P. 2019. Towards a global phylogeny of freshwater mussels (Bivalvia: Unionida): species delimitation of Chinese taxa, mitochondrial phylogenomics, and diversification patterns. *Mol Phylogenet Evol.* 130:45–59.
- Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermin LS. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat Methods.* 14(6):587–589.
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, et al. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics.* 28(12):1647–1649.
- Krueger F, James F, Ewels P, Afyounian E, Schuster-Boeckler B. 2021. FelixKrueger/TrimGalore: v0.6.7 – DOI via Zenodo (0.6.7). Zenodo.
- Laslett D, Canbäck B. 2008. ARWEN: a program to detect tRNA genes in metazoan mitochondrial nucleotide sequences. *Bioinformatics.* 24(2):172–175.
- Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, 1000 Genome Project Data Processing Subgroup. 2009. The Sequence Alignment/Map format and SAMtools. *Bioinformatics.* 25(16):2078–2079.
- Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R. 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol Biol Evol.* 37(5):1530–1534.
- Pfeiffer JM, Breinholt JW, Page LM. 2019. Unioverse: phylogenomic resources for reconstructing the evolution of freshwater mussels (Unionoida). *Mol Phylogenet Evol.* 137:114–126.
- Ranwez V, Harispe S, Delsuc F, Douzery EJP. 2011. MACSE: Multiple Alignment of Coding SEquences accounting for frameshifts and stop codons. *PLOS One.* 6(9):e22594.
- Renaut S, Guerra D, Hoeh WR, Stewart DT, Bogan AE, Ghiselli F, Milani L, Passamonti M, Breton S. 2018. Genome survey of the freshwater mussel *Venustaconcha ellipsiformis* (Bivalvia: Unionida) using a hybrid *de novo* assembly approach. *Genome Biol Evol.* 10(7):1637–1646.
- Smith CH. 2021. A high-quality reference genome for a parasitic bivalve with doubly uniparental inheritance (Bivalvia: Unionida). *Genome Biol Evol.* 13(3):evab029.
- Smith CH, Johnson NA, Inoue K, Doyle RD, Randklev CR. 2019. Integrative taxonomy reveals a new species of freshwater mussel, *Potamilus streckeri* sp. nov. (Bivalvia: Unionidae): implications for conservation and management. *Syst Biodivers.* 17(4):331–348.
- Strayer DL, Downing JA, Haag WR, King TL, Layzer JB, Newton TJ, Nichols SJ. 2004. Changing perspectives on pearly mussels, North America's most imperiled animals. *BioScience.* 54(5):429.
- Vasimuddin M, Misra S, Li H, Aluru S. 2019. Efficient architecture-aware acceleration of BWA-MEM for multicore systems. 2019 IEEE International Parallel and Distributed Processing Symposium.
- Vaughn CC, Hakenkamp CC. 2001. The functional role of burrowing bivalves in freshwater ecosystems. *Freshw Biol.* 46(11):1431–1446.
- Wen HB, Cao ZM, Hua D, Xu P, Ma XY, Jin W, Yuan XH, Gu RB. 2017. The complete maternally and paternally inherited mitochondrial genomes of a freshwater mussel *Potamilus alatus* (Bivalvia: Unionidae). *PLOS One.* 12(1):e0169749.
- Williams JD, Bogan AE, Butler RS, Cummings KS, Garner JT, Harris JL, Johnson NA, Watters GT. 2017. A revised list of the freshwater mussels (Mollusca: Bivalvia: Unionida) of the United States and Canada. *Freshw Mollusk Biol Conserv.* 20(2):33.
- Zouros E. 2013. Biparental inheritance through uniparental transmission: the doubly uniparental inheritance (DUI) of mitochondrial DNA. *Evol Biol.* 40(1):1–31.