Journal of Heredity, 2023, **114**, 81–87
https://doi.org/10.1093/jhered/esac056
Advance access publication 12 October 2022
Genome Resources

American Genetic Association

OXFORD

Genome Resources

A draft reference genome of the Vernal Pool Fairy Shrimp, *Branchinecta lynchi*

Shannon Rose Kieran Blair[1], Andrea Schreier[1], Merly Escalona[2], Amanda J. Finger[1], Shannon E.K. Joslin[3], Ruta Sahasrabudhe[4], Mohan P.A. Marimuthu[4], Oanh Nguyen[4], Noravit Chumchim[4], Emily Reister Morris[5], Hayley Mangelson[5], Joshua Hull[6]

[1]Genomic Variation Laboratory, Department of Animal Science, University of California, Davis, Davis, CA, United States,
[2]Department of Biomolecular Engineering, University of California, Santa Cruz, Santa Cruz, CA, United States,
[3]U.S. National Park Service, Yosemite National Park, El Portal, CA, United States,
[4]DNA Technologies and Expression Analysis Cores, UC Davis Genome Center, University of California, Davis, Davis, CA, United States,
[5]Phase Genomics, Seattle, WA, United States,
[6]U.S. Fish and Wildlife Service, Sacramento Fish and Wildlife Office, Sacramento, CA, United States

Address correspondence to S.R.K. Blair at the address above, or e-mail: sckieran@ucdavis.edu.

Corresponding Editor: Rachel Meyer

Abstract

We present the reference genome of the Vernal Pool Fairy Shrimp *Branchinecta lynchi*. This branchiopod crustacean is endemic to California's freshwater ephemeral ponds. It faces enormous habitat loss and fragmentation as urbanization and agriculture have fundamentally changed the vernal pool landscape over the past 3 centuries. The assembled genome consists of 22 chromosome-length scaffolds that account for 96.85% of the total sequence. One hundred and ninety-five unscaffolded contigs comprise the rest of the genome's 575.6 Mb length. The genome is substantially complete with a BUSCO score of 90.0%. There is no immediately identifiable sex chromosome, typical for this class of organism. This new resource will permit researchers to better understand the adaptive capacity of this imperiled species, as well as answer lingering questions about anostracan physiology, sex determination, and development.

Key words: Anostraca, branchiopods, California Conservation Genomics Project, CCGP, conservation genomics, crustaceans

Introduction

The Vernal Pool Fairy Shrimp *Branchinecta lynchi* (Eng *et al.* 1990) (phylum: Crustacea, order: Branchiopoda, class: Anostraca, family: Branchinectidae) is an ephemeral wetlands specialist endemic to California and southern Oregon. It is a key member of California's vernal pool ecosystem. Vernal pools in California are important, imperiled habitats which support native plant diversity. They also provide food and habitat for many of the millions of waterfowl that utilize these pools as winter habitat (Wilson 2010). Vernal pool habitat in California has been lost, degraded and fragmented due to anthropogenic factors. These factors, which include land conversion, urban development, and large-scale water projects, have resulted in an estimated 95% loss of vernal pool habitat since Spanish settlement (King 1998). Over the past 30 yr, federal and state conservation efforts attempted to recover and conserve vernal pools and their inhabitants across California. *B. lynchi* was Federally listed as threatened under the Endangered Species Act in 1994 (United States Fish and Wildlife Service 1994).

B. lynchi faces increasing pressure from habitat loss, habitat fragmentation, and loss of dispersal vectors. Recent studies have attempted to identify genetic structuring in the disjunct populations of *B. lynchi* using mitochondrial DNA (Aguilar 2011; Deiner *et al.* 2017) and RAD sequencing (Kieran and Finger 2020). Despite this, much is still unknown about the species, including sex determination, chromosome structure and even genome size. *B. lynchi* was chosen as a California Conservation Genetics Project (CCGP) (Shaffer *et al.* 2022) species in order to facilitate future adaptation and dispersal research, to identify the distribution of genomic variation across the landscape and determine how historical habitat loss has impacted contemporary genomic variation.

This genomic resource will provide tools for researchers to better understand how *B. lynchi* moves across the landscape, colonizes new habitats, and adapts to local conditions, which will in turn help conservationists and managers better prepare this species against a future that contains 50 million Californians, less rain, and longer drought periods (Ullrich *et al.* 2018).

Received August 16, 2022; Accepted October 10, 2022

Methods

Biological materials

Specimens were collected on 5 March 2021, under Federal 10(A)1(a) collection permit TE-28101C-0. Specimens were collected via dip-net from private conservation lands in Placer County, CA (38.884802, −121.452608) and were transferred live to the lab, where they were immediately frozen in liquid nitrogen and stored at −80 °C until extraction (Fig. 1).

Nucleic acid library preparation

High molecular weight (HMW) genomic DNA (gDNA) extraction and nucleic acid library preparation were carried out by the University of California Davis DNA Technologies Core (Davis, CA). A whole-body sample from a male and a female *B. lynchi* was homogenized in 500 µl of homogenization buffer (10 mM Tris–HCl, pH 8.0 and 25 mM EDTA) using TissueRuptor II (Qiagen, Hilden, Germany; Cat. # 9002755). 500 µl of lysis buffer (10 mM Tris, 25 mM EDTA, 200 mM NaCl, and 1% SDS) and proteinase K (100 µg/ml) were added to the homogenate and incubated overnight at room temperature followed by RNAse A (20 µg/ml) treatment at 37 °C for 30 min. The lysate was cleaned with equal volumes of phenol/chloroform using phase-lock gels (Quantabio, Beverley, MA; Cat. # 2302830) and the DNA was precipitated by adding 0.4× volume of 5 M ammonium acetate and 3× volume of ice-cold ethanol. The DNA pellet was washed twice with 70% ethanol and resuspended in an elution buffer (10 mM Tris, pH 8.0). The purity of the DNA was accessed using NanoDrop spectrophotometer (260/280 and 260/230 ratios) and the integrity of the HMW gDNA was verified on a Femto pulse system (Agilent Technologies, Santa Clara, CA).

DNA sequencing and genome assembly

The HiFi SMRTbell libraries were constructed using the SMRTbell Express Template Prep Kit v2.0 (Pacific Biosciences—PacBio, Menlo Park, CA, Cat. #100-938-900) according to the manufacturer's instructions. HMW gDNA was sheared to a target DNA size distribution between 12 and 20 kb. For library preparation input, the sheared gDNA was concentrated using 1.8× of AMPure PB beads (PacBio, Cat. #100-265-900) for the removal of single-strand overhangs at 37 °C for 15 min, followed by further enzymatic steps of DNA damage repair at 37 °C for 30 min, end repair and A-tailing at 20 °C for 10 min and 65 °C for 30 min, and ligation of overhang adapter v3 at 20 °C for 60 min. The SMRTbell libraries were purified and concentrated with 0.8× AMPure PB beads for size selection with 40% diluted AMPure PB beads to remove short SMRTbell templates, <3 kb. The 15 to 17 kb average HiFi SMRTbell libraries were sequenced on 8M SMRT cells (1 per library), Sequel II sequencing chemistry 2.0, and 30-h movies each at UC Davis DNA Technologies Core (Davis, CA) on a PacBio Sequel II sequencer.

Initial contig assembly

PacBio HiFi Reads were assembled into contigs using the PacBio "ipa" software program version 1.3.1 with default parameters. Each specimen (male and female) was assembled separately, and each assembly was evaluated for quality and completeness. Because the male assembly was slightly larger and more complete, further sequencing and assembly were carried out for the male assembly only.

Proximo Hi-C sequencing and scaffolded assembly

Chromatin conformation capture data were generated using a Phase Genomics (Seattle, WA) Proximo Hi-C 4.0 Kit, which is a commercially available version of the Hi-C protocol (Lieberman-Aiden *et al.* 2009). Following the manufacturer's instructions for the kit, intact cells were crosslinked using a formaldehyde solution, digested using the DPNII, DDE1, HINF, and MSEI restriction enzymes, end repaired with biotinylated nucleotides, and proximity ligated to create chimeric molecules composed of fragments from different regions of the genome that were physically proximal in vivo, but

Fig. 1. (A) A microscope image of *Branchinecta lynchi* females at 10×. (B) A large vernal pool being dip-net in Solano County, CA. Photos courtesy Shannon Kieran Blair.

not necessarily genomically proximal. Continuing with the manufacturer's protocol, molecules were pulled down with streptavidin beads and processed into an Illumina-compatible sequencing library. Sequencing was performed on an Illumina NovaSeq (San Diego, CA).

Reads were aligned to the draft assembly also following the Phase Genomics manufacturer recommendations. Briefly, reads were aligned using BWA-MEM (Li and Durbin 2010) with the -5SP and -t 8 options specified, and all other options default. SAMBLASTER (Faust and Hall 2014) was used to flag PCR duplicates, which were later excluded from analysis. Alignments were then filtered with samtools (Li *et al.* 2009) using the -F 2304 filtering flag to remove non-primary and secondary alignments. Putative misjoined contigs were broken using Juicebox (Rao *et al.* 2014; Durand *et al.* 2016) based on the Hi-C alignments.

Phase Genomics' Proximo Hi-C genome scaffolding platform was used to create chromosome-scale scaffolds from the corrected assembly as described in Bickhart *et al.* (2017). As in the LACHESIS method (Burton *et al.* 2013), this process computes a contact frequency matrix from the aligned Hi-C read pairs, normalized by the number of restriction enzyme cut sites on each contig, and constructs scaffolds in such a way as to optimize expected contact frequency and other statistical patterns in Hi-C data. Approximately 40,000 separate Proximo runs were performed to optimize the number of scaffolds and scaffold construction in order to make the scaffolds as concordant with the observed Hi-C data as possible. Finally, Juicebox was again used to correct scaffolding errors.

Assembly metrics and validation

The assembly completeness was estimated by running BUSCO (Waterhouse *et al.* 2018) version 5.2.2 in genome mode using the arthropoda_odb10 database. Assembly statistics were calculated using genometools (Gremme *et al.* 2013) version 1.5.9 and QUAST (Mikheenko *et al.* 2018) version 5.0.2. Further quality assessment was carried out following the frameshift pipeline described in Korlach *et al.* (2017).

Initial sex determination analysis

Two methods were used to investigate a potential sex-determining chromosome in *B. lynchi*. First, PacBio HiFi sequences from the female *B. lynchi* specimen described earlier in this manuscript were aligned to the male reference assembly using minimap2 version 2.16 (Li 2021) using the -asm20 option. Alignments were filtered male HiFi reads were aligned identically. Next, per-base coverage was calculated using bedtools version 2.27 (Quinlan and Hall 2010) separately for the male and female specimens. The ratio of male/female coverage for each scaffold and contig was calculated separately. Scaffolds were considered potential sex chromosomes if the ratio of male/female coverage exceeded 1.96× the median ratio (1.45). Next, association tests were undertaken using RAD sequencing data previously collected and sequenced from 47 *B. lynchi* individuals (34 males and 13 females) collected from a single vernal pool complex in Merced, CA (Kieran and Finger 2020). These RAD sequencing data were aligned to the reference genome using bwa-mem and filtered using samtools. The angsd (Korneliussen *et al.* 2014) software kit version 9.34

Table 1. Assembly pipeline and software used.

Assembly	Software	Version
K-mer counting	Jellyfish	2.2.6
Estimation of genome size and heterozygosity	GenomeScope	2
De novo assembly (contigging)	Ipa	1.3.1
Long read, genome–genome alignment	Minimap2	2.22
Scaffolding		
Hi-C mapping	Phase Genomics Proximo Hi-C pipeline https://phasegenomics.github.io/2019/09/19/hic-alignment-and-qc.html	Commit 5f9d55ea3162f8d21988f486b5d012f0800abdc4
Hi-C scaffolding	Juicebox	2
Hi-C contact map generation		
Short-read alignment	BWA-MEM	0.7.17-r1188
SAM/BAM processing	SAMBLASTER	1.11
SAM/BAM filtering	samtools	0.3.0
Matrix generation and balancing	Phase Genomics Proximo Hi-C pipeline	Commit 5f9d55ea3162f8d21988f486b5d012f0800abdc4
Benchmarking		
Basic assembly stats	QUAST	5.0.2
	GenomeTools	1.5.9
Assembly completeness	BUSCO	5.2.2
	Merqury	1.3
	Blobtoolkit	3.1.6

Software citations are listed in the text.

was used to run an association test with sex as the tested phenotype. Genes were predicted ab initio by Augustus v3.04 (Stanke *et al.* 2006) trained on the *Daphnia magna* annotated gene set (accession number GCF_003990815.1) retrieved from NCBI March 2022. To explore the contigs, we used Augustus to predict genes on each contig and used Blast2Go version 6.0.3 to query the sequence of each predicted protein against the NCBI nucleotide database with an *e*-value threshold of 1*e*−5 as a cutoff for potential matches (Table 1).

Results

Genome assembly

The Phase Genomics Proximo Hi-C Illumina Novaseq sequencing generated a total of 90,119,568 PE150 read pairs. Initial analysis in Juicebox introduced a total of 9 breaks in 9 contigs. The assembled genome (see Table 2) is 575,641,406 bp (575.6 Mb) in length. It comprises 22 chromosome-length scaffolds (96.85% of all sequence length) and 195 unscaffolded contigs. Because these long scaffolds have not been officially assigned to chromosomes, NCBI reports 217 "scaffolds" (all scaffolds and unscaffolded contigs) and 1,304 "contigs" (all scaffolds and unscaffolded contigs after spanning 1,087 gaps). The N50 is 23,437,814 bp. The scaffold L50 is 10. The longest scaffold is 36,901,223 bp. The BUSCO score for this genome is 90.0% (89.2% complete and unduplicated, 0.8% complete and duplicated, 4.0% fragmented, and 6.0% missing, n = 1,013) (Fig. 2).

Sex chromosome determination

Analysis of male/female chromosome coverage revealed no potential scaffolds corresponding to an X or Z chromosome (Fig. 3). Association testing of RAD-seq-derived SNPs from 47 individuals in a single population revealed no potential SNPs associated with sex. There were 6 unscaffolded contigs totaling 1,189,164 bases found in the male sequence with no coverage at all in the female sequence. Because we recovered more overall sequence from the male than the female, this is not necessarily indicative of a contig or scaffold related to sex

Table 2. Sequencing and assembly statistics, and accession numbers.

BioProjects and vouchers	CCGP NCBI Bio-project	PRJNA720569 http://www.ncbi.nlm.nih.gov/bioproject/PRJNA720569
	Branchinecta lynchi NCBI Bio-project	PRJNA811230 https://www.ncbi.nlm.nih.gov/bioproject/PRJNA811230
	NCBI Bio-sample	SAMN26264359 https://www.ncbi.nlm.nih.gov/biosample/SAMN26264343
Genome sequence	PacBio HiFi long-read runs (male)	1 PACBIO_SMRT (Sequel II) runs: 1M spots, 10.9G bases, 2.6 Gb downloads
	Phase Genomics Proximo Hi-C sequencing	1 Illumina NovaSeq 6000 run: 90.1M spots, 27G bases, 11.4 Gb downloads
	PacBio HiFi NCBI SRA Accession	SRX15225444 https://www.ncbi.nlm.nih.gov/sra/SRX15225444
	Proximo HiC Illumina NCBI SRA Accession	SRX15225445 https://www.ncbi.nlm.nih.gov/sra/SRX15225445
Genome assembly	HiFi read coverage	18.8×
	Number of contigs	1,304
	Contig N50 (bp)	902,028
	Longest scaffold	36,901,223
	Number of scaffolds	217
	Scaffolds assigned to chromosomes	22
	Scaffold N50 (bp)	23,437,814
	Size of final assembly (bp)	575,641,406
	Gaps per Gbp	1,889
	NCBI Genome Assembly Accession	GCA_023053575.1 https://www.ncbi.nlm.nih.gov/assembly/GCA_023053575.1
Assembly Quality	Assembly Quality identifier[a]	6.c.Q57
	Base pair QV (Merqury)	57.7
	Indel QV (frameshift analysis)	50.1
	k-mer completeness	77.9%
	BUSCO completeness (C:S:D:F:M)	90.0%:89.2%:0.8%:4.0%:6.0%

[a]We follow the quality metric nomenclature established by (Rhie *et al.* 2021) with the genome quality code $x{\cdot}y{\cdot}Q$, where x = log10[contig NG50]; y = log10[scaffold NG50], "c" denotes "complete" telomere-to-telomere continuity; Q = Phred base accuracy QV (quality value).

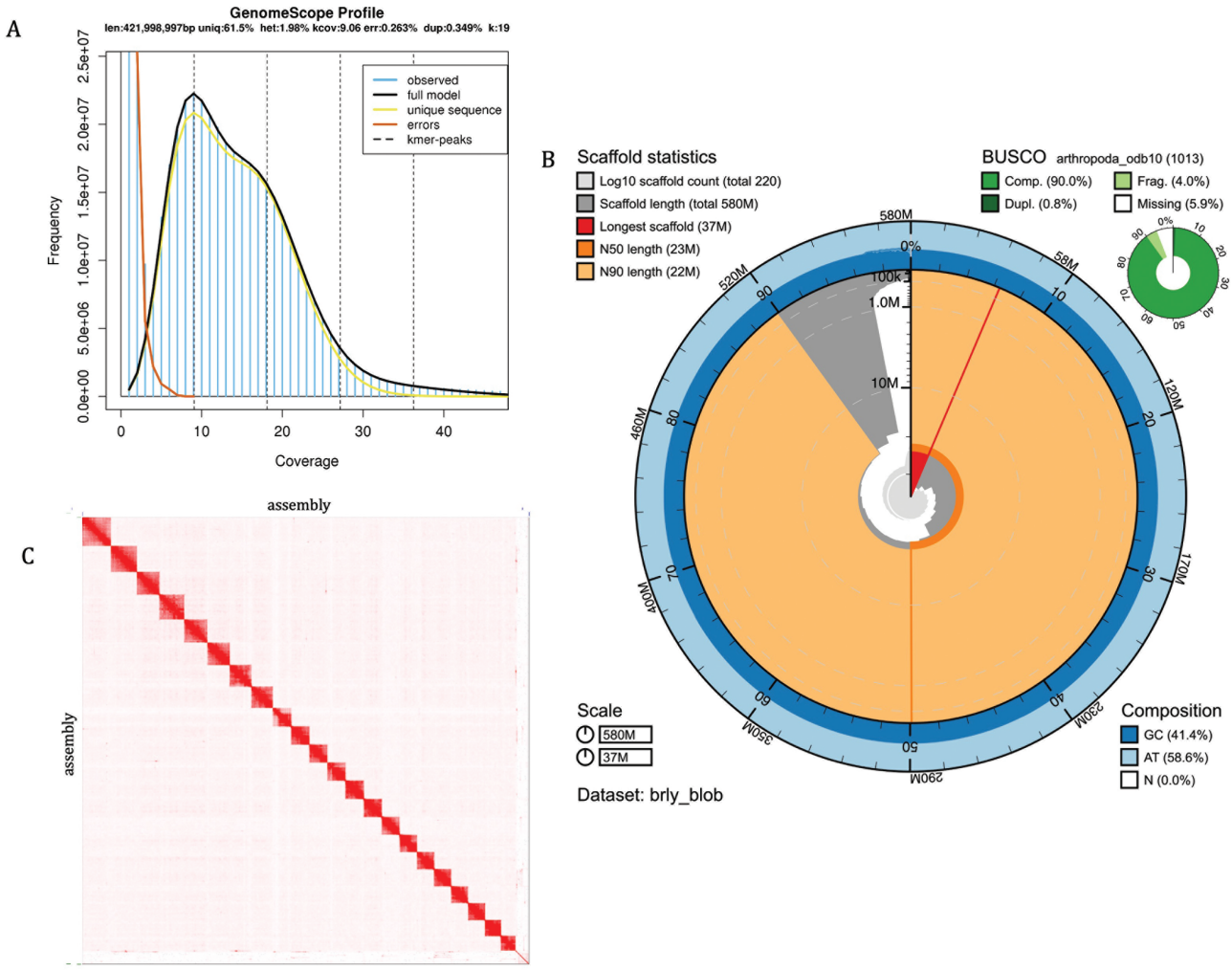

Fig. 2. (A) K-mer spectra produced by genoscope. (B) BlobToolKit snail plot showing N50 metrics for *Branchinecta lynchi* assembly and BUSCO scores for the Arthopoda set of orthologs. (C) Contact map of the final assembly. This map visualizes the high percentage of sequence contained in scaffolds, suggesting high contiguity of the assembly.

determination, but we investigated each contig for genomic content anyway. Of 123 predicted genes, only 29 returned positive BLAST hits using Blast2Go. Of these 29, 23 were *B. lynchi* microsatellite sequences. Three of remaining 6 sequences were uncharacterized. Of the 3 characterized hits 2 were for a trehalase gene and 1 was for frizzled receptor 8a (see Table 3).

Discussion

The genome, consisting of 22 chromosome-length scaffolds that comprise 96.85% of the 575.6 Mb total sequence, fits into the size range of other sequenced branchiopods, which range from 80 to 850 Mb (de Vos *et al.* 2021). Although no sex chromosome could be easily identified by coverage analysis or association testing, this is not surprising. Few crustaceans have sex chromosomes, and many branchiopods show complex sexual development patterns including androdioecy, variable hermaphrodism, and gynandromorphism. In the branchiopod clam shrimp *Eulimnadia texana*, the sex-determining region takes up nearly half the genome (Baldwin-Brown *et al.* 2018). RAD sequencing is a form of reduced-representation sequencing that produces sparse markers. We recommend whole genome resequencing and differential expression analysis on multiple male/female specimens be used to identify more definitely the method of sex determination in this species. The 2 characterized genes we found on unscaffolded contigs missing from the female assembly (but present in the male assembly) did not suggest either contig was specifically a sex-determining region.

This genome will provide direct resources for California conservationists as they attempt to protect and recover the endangered, endemic Vernal Pool Fairy Shrimp. Exploring adaptation and differentiation on a genome scale will help managers determine how and where to translocate or introduce new populations. The ability to perform large-scale ecological genomics studies may allow researchers to finally understand the key hydrological and biochemical variables that are key to ensuring new populations of the species survive, as well as the genetic basis and plasticity of the response to environmental change.

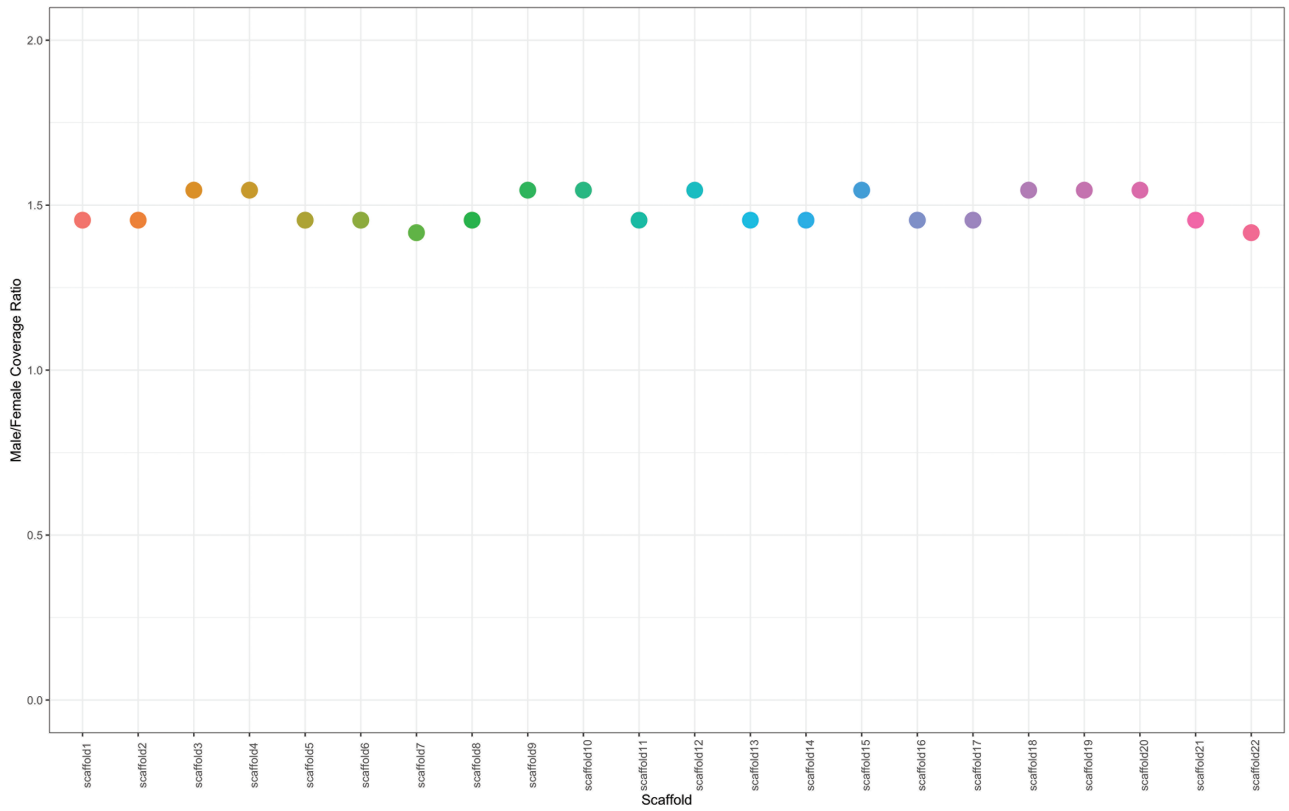

Fig. 3. Male/female coverage ratio of each of the 22 chromosome-length scaffolds for the *B. lynchi* assembly. No scaffold had high enough coverage differential to be considered a likely sex chromosome. Genome-wide median coverage ratio was 1.45.

Table 3. Characterization of predicted genes on scaffolds with no coverage in the female sequence.

Contig	Length	Predicted genes	Successful BLAST hits	Gene descriptions
ctg.000028F_ fragment_2_debris	50,665	4	2	Microsatellite (2)
ctg.000028F_ fragment_3	152,102	15	6	Microsatellite (4), frizzled receptor 8a (1), uncharacterized (1)
ctg.000467F	605,364	57	11	Microsatellite (11)
ctg.001020F	162,245	17	4	Microsatellite (4)
ctg.001305F	194,778	29	6	Microsatellite (2), trehalase (2), uncharacterized (2)
ctg.002381F	24,010	1	0	NA

Values in parentheses after gene descriptions reflect the number of BLAST hits matching that description for each contig.

Funding

This work was funded by the U.S. Bureau of Reclamation [Grant #R20AP00037]. This work was supported by the California Conservation Genomics Project, with funding provided to the University of California by the State of California, State Budget Act of 2019 [UC Award ID RSI-19-690224].

Acknowledgments

PacBio Sequel II library prep and sequencing were carried out at the DNA Technologies and Expression Analysis Cores at the UC Davis Genome Center, supported by NIH Shared Instrumentation Grant 1S10OD010786-01. We thank the staff at the UC Davis DNA Technologies and Expression Analysis Cores for their diligence and dedication to generating high-quality sequence data. We wish to acknowledge Westervelt Ecological Services, especially Matt Gause, Charlotte Marks, and Tara Collins, for their timely assistance in accessing Westervelt properties to collect specimens for this project.

Data availability

Data generated for this study are available under NCBI BioProject PRJNA720569. Raw sequencing data for sample BRLY_1 (NCBI BioSample SAMN26264359 are deposited in the NCBI Short Read Archive (SRA) under SRX15225444 to SRX15225445.

References

Aguilar A. Weak phylogeographic structure in the endemic western North American fairy shrimp *Branchinecta lynchi* (Eng, Belk and Erickson 1990). *Aquat Sci*. 2011;73(1):15–20.

Baldwin-Brown JG, Weeks SC, Long AD. A new standard for crustacean genomes: the highly contiguous, annotated genome assembly of the clam shrimp *Eulimnadia texana* reveals HOX gene order and identifies the sex chromosome. *Genome Biol Evol*. 2018;10(1):143–156.

Bickhart DM, Rosen BD, Koren S, Sayre BL, Hastie AR, Chan S, Lee J, Lam ET, Liachko I, Sullivan ST, et al. Single-molecule sequencing and chromatin conformation capture enable de novo reference assembly of the domestic goat genome. *Nat Genet*. 2017;49(4):2503–2505.

Burton JN, Adey A, Patwardhan RP, Qiu R, Kitzman JO, Shendure J. Chromosome-scale scaffolding of de novo genome assemblies based on chromatin interactions. *Nat Biotechnol*. 2013;31(12):1119–1125.

de Vos S, Rombauts S, Coussement L, Dermauw W, Vuylsteke M, Sorgeloos P, Clegg JS, Nambu Z, van Nieuwerburgh F, Norouzitallab P, et al. The genome of the extremophile *Artemia* provides insight into strategies to cope with extreme environments. *BMC Genomics*. 2021;22(1):1–26.

Deiner K, Hull JM, May B. Range-wide phylogeographic structure of the vernal pool fairy shrimp (*Branchinecta lynchi*). *PLoS One*. 2017;12(5):e0176266.

Durand NC, Robinson JT, Shamim MS, Machol I, Mesirov JP, Lander ES, Aiden EL. Juicebox provides a visualization system for Hi-C contact maps with unlimited zoom. *Cell Syst*. 2016;3(1):99–101.

Eng LL, Belk D, Eriksen CH. Californian Anostraca: distribution, habitat, and status. *J Crustac Biol*. 1990;10(2):247–277.

Faust GG, Hall IM. SAMBLASTER: fast duplicate marking and structural variant read extraction. *Bioinformatics*. 2014;30(17):2503–2505.

Gremme G, Steinbiss S, Kurtz S. GenomeTools: a comprehensive software library for efficient processing of structured genome annotations. *IEEE/ACM Trans Comput Biol Bioinf*. 2013;10(3):645–656.

Kieran SRC, Finger AJ. *Final report for Cesu R15AC00525: comparative population genetics across vernal pool branchiopod species reveals incongruous patterns of geographic structuring, genetic differentiation*. 2020. Prepared for the United States Fish and Wildlife Service. Sacramento, CA. 76 pp.

King J. Loss of diversity as a consequence of habitat destruction in California vernal pools. In: Witham CW, Bauder ET, Belk D, Ferren WR Jr., and Ornduff R. (eds.) *Ecology, conservation, and management of vernal pool ecosystems*. 1998. p. 119–123. https://vernalpools.ucmerced.edu/sites/vernalpools.ucmerced.edu/files/page/documents/2.5_loss_of_diversity_as_a_consequence_of_habitat_destruction_in_california_vernal_pools_by_jamie_l._king__0.pdf. Sacramento, CA: California Native Plant Society.

Korlach J, Gedman G, Kingan SB, Chin CS, Howard JT, Audet JN, Cantin L, Jarvis ED. De novo PacBio long-read and phased avian genome assemblies correct and add to reference genes generated with intermediate and short reads. *GigaScience*. 2017;6(10):1–16.

Korneliussen TS, Albrechtsen A, Nielsen R. ANGSD: analysis of next generation sequencing data. *BMC Bioinformatics*. 2014;15(1):356.

Li H. New strategies to improve minimap2 alignment accuracy. *Bioinformatics*. 2021;37(23):4572–4574.

Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics*. 2010;26(5):589–595.

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R. The Sequence Alignment/Map (SAM) format and SAMtools 1000 Genome Project data processing subgroup. *Bioinformatics*. 2009;25(16):2078–2079.

Lieberman-Aiden E, van Berkum NL, Williams L, Imakaev M, Ragoczy T, Telling A, Amit I, Lajoie BR, Sabo PJ, Dorschner MO, et al. Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science*. 2009;326(5950):289–293.

Mikheenko A, Prjibelski A, Saveliev V, Antipov D, Gurevich A. Versatile genome assembly evaluation with QUAST-LG. *Bioinformatics*. 2018;34(13):i142–i150.

Quinlan AR, Hall IM. BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics*. 2010;26(6):841–842.

Rao SSP, Huntley MH, Durand NC, Stamenova EK, Bochkov ID, Robinson JT, Sanborn AL, Machol I, Omer AD, Lander ES, et al. A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell*. 2014;159(7):1665–1680.

Rhie A, McCarthy SA, Fedrigo O, Damas J, Formenti G, Koren S, Uliano-Silva M, Chow W, Fungtammasan A, Kim J. Towards complete and error-free genome assemblies of all vertebrate species. *Nature*. 2021;592(7856):737–746.

Shaffer HB, Toffelmier E, Corbett-Detig RB, Escalona M, Erickson B, Fiedler P, Gold M, Harrigan RJ, Hodges S, Luckau TK, et al. Landscape genomics to enable conservation actions: the California Conservation Genomics Project. *J Hered*. 2022; esac020.

Stanke M, Keller O, Gunduz I, Hayes A, Waack S, Morgenstern B. AUGUSTUS: ab initio prediction of alternative transcripts. *Nucleic Acids Res*. 2006;34(Web Server issue):W435–W439.

Ullrich PA, Xu Z, Rhoades AM, Dettinger MD, Mount JF, Jones AD, Vahmani P. California's drought of the future: a midcentury recreation of the exceptional conditions of 2012–2017. *Earth's Future*. 2018;6(11):1568–1587.

United States Fish and Wildlife Service. Endangered and threatened wildlife and plants; determination of endangered status for the conservancy fairy shrimp, longhorn fairy shrimp, and the vernal pool tadpole shrimp; and threatened status for the vernal pool fairy shrimp. *Fed Reg*. 1994;59(180):48136.

Waterhouse RM, Seppey M, Simao FA, Manni M, Ioannidis P, Klioutchnikov G, Kriventseva E, Zdobnov EM. BUSCO applications from quality assessments to gene prediction and phylogenomics. *Mol Biol Evol*. 2018;35(3):543–548.

Wilson RM. *Seeking refuge: birds and landscapes of the Pacific flyway*. University of Washington Press; 2010. https://books.google.com/books/about/Seeking_Refuge.html?id=e8j5zdiCdSgC. Accessed June 10, 2022.