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Genome Resources

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A chromosome-level genome assembly of a vernal pool specialist amphibian, the Western Spadefoot, *Spea hammondii*

Ben Thompsky[1,2,*], Eric Beraut[3], Robert D. Cooper[1,2,4], Merly Escalona[5,6], Robert E. Espinoza[7], Robert N. Fisher[8], Courtney Miller[1,2], Oanh Nguyen[9], Samuel Sacco[3], Ruta Sahasrabudhe[9], William E. Seligmann[3], Erin Tofflemier[1,2], Ian J. Wang[10,11], H. Bradley Shaffer[1,2]

[1]Department of Ecology & Evolutionary Biology, University of California, Los Angeles, Los Angeles, CA, United States, [2]La Kretz Center for California Conservation Science, Institute of the Environment and Sustainability, University of California, Los Angeles, Los Angeles, CA, United States, [3]Department of Ecology and Evolutionary Biology, University of California, Santa Cruz, Santa Cruz, CA, United States, [4]Department of Fish and Wildlife Conservation, Virginia Polytechnic Institute and State University, Blacksburg, VA, United States, [5]Institute for Systems Genomics, University of Connecticut, Storrs, CT, United States, [6]Department of Biomolecular Engineering, University of California, Santa Cruz, Santa Cruz, CA, United States, [7]Department of Biology, California State University, Northridge, Northridge, CA, United States, [8]U.S. Geological Survey, San Diego, CA, United States, [9]DNA Technologies and Expression Analysis Core Laboratory, Genome Center, University of California-Davis, Davis, CA, United States, [10]Department of Environmental Science, Policy, and Management, University of California, Berkeley, CA, United States, [11]Museum of Vertebrate Zoology, University of California, Berkeley, CA, United States

*Corresponding author. Department of Ecology and Evolutionary Biology 610 Charles E. Young Drive East Box 957239 University of California, Los Angeles Los Angeles, CA 90095-7239, USA. Email: benthompsky@g.ucla.edu

Abstract

We assembled and annotated a chromosome-level genome for the Western Spadefoot, *Spea hammondii* (Anura, Scaphiopodidae) representing one of only three amphibians included in the California Conservation Genomics Project (CCGP). *Spea hammondii* is a vernal pool breeding anuran native to California and northwestern Baja California which has undergone both range contractions and local extirpations across its distribution, primarily due to habitat loss and degradation and drought. The species is recognized by the state of California as a Species of Special Concern and is proposed for listing under the United States Endangered Species Act. Using the established CCGP pipeline, this *S. hammondii* genome was produced using Pacific Biosciences HiFi long-reads and Omni-C proximity ligation, resulting in a de novo genome assembly 1.14 Gb in length, distributed across 479 scaffolds (scaffold N50 = 120.8 Mb; largest scaffold = 183.6 Mb) with a BUSCO completeness score of 90.9% using a conserved tetrapod ortholog set. Our assembly shows high base accuracy (quality value [QV] = 63.7) and low frameshift error in coding regions (QV 50.42). Annotation of this genome yielded 20,434 genes with a BUSCO completeness score of 94.7%. This genome assembly, in combination with range-wide resequencing data from CCGP, will facilitate statewide population genomic assessments to delineate conservation units, quantify inbreeding and genomic load, and test for adaptive variation associated with vernal pool hydrology and drought tolerance, all of which are important considerations in the proposed federal listing.

Key words: California Conservation Genomics Project, conservation genomics, genome assembly, endangered species, Scaphiopodidae

Introduction

The Western Spadefoot, *Spea hammondii*, is a species of medium-sized fossorial anuran that was historically widespread in the low-elevation, non-desert regions of central and southern California and northern Baja California, Mexico (Fig. 1A). The species often occurs in discrete and isolated populations tied to small seasonal wetlands, including human-constructed livestock ponds. Adult *S. hammondii* are primarily found in grassland, chaparral and oak woodland habitats with loose soil conducive to burrowing (Morey 2005; Hansen and Shedd 2025). During the non-rainy season, post-metamorphic juveniles and adults live underground in self-constructed burrows, preferring sand and silt over clay soils (Baumberger 2019). They emerge from burrows during rainy nights, generally in the winter and spring. *Spea hammondii* typically breed between January and June (Dodd 2013). Breeding is wholly dependent on the presence of ephemeral water bodies, rendering the species exceptionally vulnerable to drought and climatic changes that lower or change the frequency of precipitation (Thomson et al. 2016).

Larval development is extremely rapid and has been the focus of substantial research on the genus *Spea*. In short-hydroperiod vernal pools, *S. hammondii* exhibit rapid development, with smaller sizes at metamorphosis (Denver 1998). *Spea* larvae also show remarkable life history adaptations and phenotypic plasticity, with the potential to develop into

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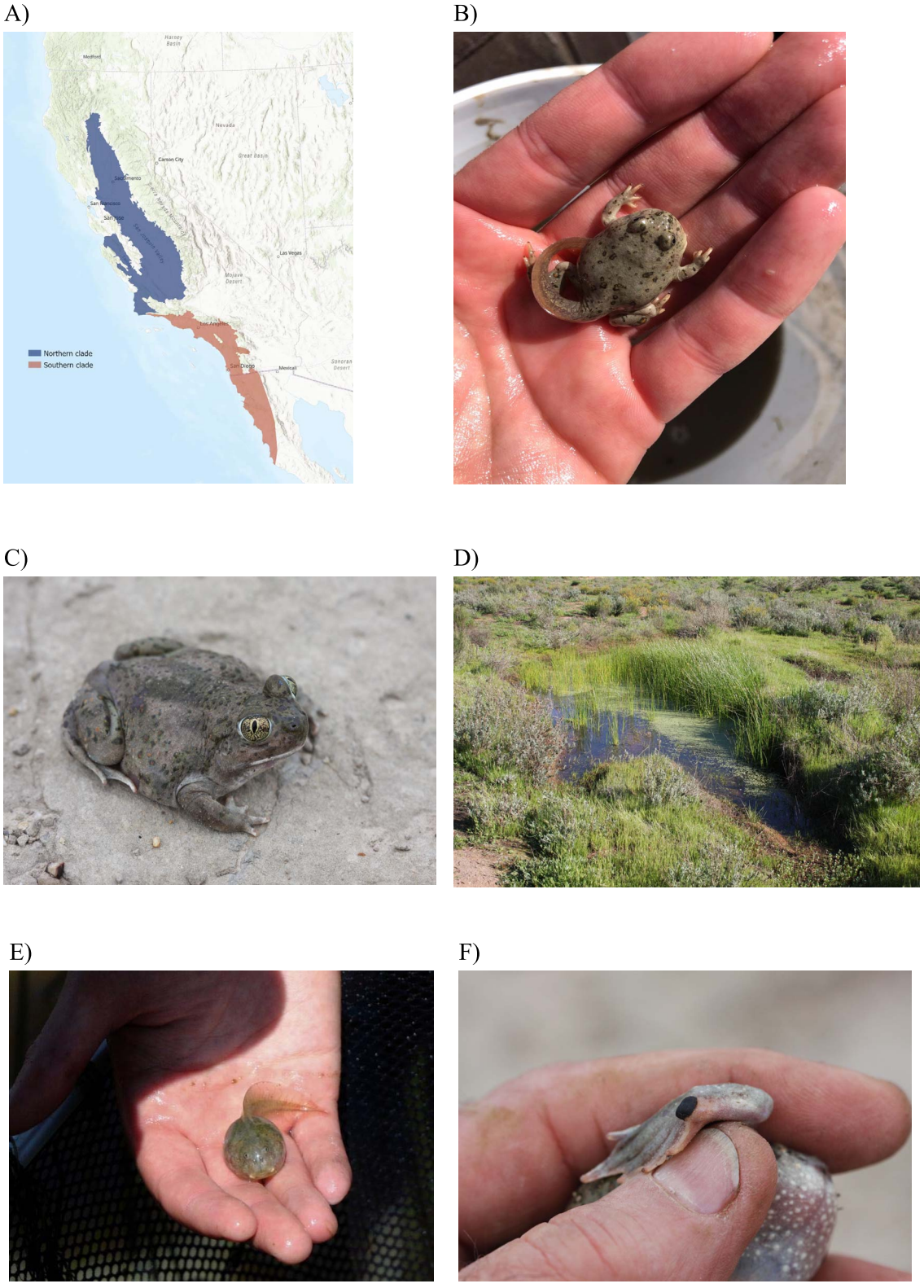

Fig. 1. (A) Range map of *Spea hammondii* showing the two genetically separate subpopulations north and south of the Transverse Ranges (Kristi Cripe [CDFW], map). (B) Mid-metamorphosis spadefoot tadpole. Animals at this stage have well-developed spades on their rear feet and are capable of completing metamorphosis on land (Robert D. Cooper, image). (C) Adult Western spadefoot from northern Santa Barbara County, California (H. Bradley Shaffer, image). (D) Vernal pool used by *S. hammondii* for breeding (San Diego County, California, H. Bradley Shaffer, image). (E) *S. hammondii* tadpole, displaying their relatively large size (Nora Papian/USFWS, Public Domain, https://www.fws.gov/media/spadefoot-toad-tadpole). (F) Close-up of the keratinized spade on the rear foot of an adult *S. hammondii*. Spadefoots use this spade to dig burrows in loose sandy soil (H. Bradley Shaffer, image).

cannibalistic carnivorous tadpoles with increased keratinization of the jaw in response to prey density (Levis et al. 2018). Although this "cannibal morph" phenotype is better documented in other species in the genus (Levis et al. 2015), at least one published observation from northwestern Baja California has documented cannibalistic behaviors in metamorphosing *S. hammondii* (Alvarez et al. 2024).

The taxonomic history of the genus *Spea,* including *S. hammondii,* has been a source of confusion at multiple levels. Long placed in the family Pelobatidae, current taxonomy recognizes the New World spadefoots as members of Scaphiopodidae, a small clade of seven species and two genera (*Spea* and *Scaphiopus*) (see Frost 2025 for a detailed taxonomic history). Scaphiopodids, along with the Old World families Pelodytidae, Megophryidae, and Pelobatidae, form a clade that is the sister group to Neobatrachia, a large clade that includes well more than 90% of living anurans (Pyron and Wiens 2011). Within *S. hammondii*, recent genetic analyses (Neal et al. 2018) have identified two species-level lineages that are divided into allopatric northern and southern groups by the Transverse Ranges, a mid-elevation mountain range that often forms a biogeographic boundary between northern and southern California biotic elements (Rissler et al. 2006). Neal et al. (2018) suggested that each clade should be managed as a distinct conservation unit, based on both genetic data and species distribution modeling. Although a single range-wide entity is currently recognized by California as a Species of Special Concern (Thomson et al. 2016) the northern and southern clades are each currently under consideration for listing under the United States Endangered Species Act (USFWS 2023).

This genome release is part of the California Conservation Genomics Project (CCGP), which provides genomic resources and their analyses to inform conservation actions at a statewide scale (Fiedler et al. 2022; Shaffer et al. 2022; Toffelmier et al. 2022). This chromosome-level genome, the third available for the genus (see also *Spea multiplicata* (Seidl et al. 2019), GenBank accession GCA_009364415.1, and *Spea bombifrons*, GenBank accession GCF_027358695.1) provides a key resource to help address outstanding questions in the systematics and conservation of the genus *Spea* and *S. hammondii*. As part of the CCGP, this genome will enable range-wide population-genomic analyses to delineate conservation units, quantify inbreeding and genomic load, and map adaptive variation associated with hydroperiod, temperature, and drought (Shaffer et al. 2022). This information is of particular importance for *S. hammondii*, as it exists in increasingly disjointed and potentially inbred populations. A reference genome is a vital resource necessary to develop cost-effective marker panels for genetically informed reintroductions, translocations, and long-term monitoring across California's fragmented seasonal-wetland landscapes. Finally, this genome adds to a growing set of California vernal pool specialist species, including the freshwater crustaceans *Branchinecta lynchi* (Blair et al. 2023a), *Branchinecta lindahli* (Blair et al. 2023b), and *Lepidurus packardi* (Blair et al. 2022), and the vernal pool plants *Neostapfia colusana* (Pennington et al. 2025) and *Tuctoria greenei* (Toews et al. 2025), that will allow cross-taxon analyses of the genomic health of California's rich, endemic, and extremely endangered vernal pool ecosystems.

Methods

Biological materials

We sequenced an adult *S. hammondii* (HBS 135690) from the southern clade/distinct population segment (Neal et al. 2018) that was collected as a tadpole from a drying ephemeral puddle (a tire track) on a dirt trail in Los Angeles County (coordinates: 34.4680837, -118.5479482), reared in captivity for approximately 2.5 yrs in the California State University Northridge vivarium, and euthanized on 19 August 2020. Liver, thigh muscle, tongue, and heart tissues were harvested and flash-frozen in liquid nitrogen within 2 min of euthanasia, and the remaining carcass was formalin-fixed for later museum deposition.

High molecular weight genomic DNA isolation

High molecular weight genomic DNA (HMW gDNA) was isolated from a liver sample. Flash frozen liver tissue (20 mg) was homogenized using a mortar and a pestle in liquid nitrogen. Homogenized tissue was lysed with 2 ml of lysis buffer containing 100 mM NaCl, 10 mM Tris–HCL-pH 8.0, 25 mM EDTA, 0.5% SDS, and 100 μg/ml proteinase K overnight at room temperature. Lysate was treated with 20 μg/ml RNase at 37 °C for 30 min and cleaned with equal volumes of phenol/chloroform using phase-lock gels (Quantabio, Beverly, MA, USA; Cat # 2302830). DNA was precipitated by adding 0.4X volume of 5M ammonium acetate and 3X volume of ice-cold ethanol. The resulting DNA pellet was washed twice with 70% ethanol and resuspended in an elution buffer (10 mM Tris, pH 8.0). Purity of DNA was assessed with a spectrophotometer (NanoDrop ND-1000, Thermo Fisher Scientific, Waltham, MA, USA) where 260/280 ratios of 1.88 and 260/230 of 2.14 were observed. Total DNA yield of 13.3 μg was obtained as quantified using Qbit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). DNA fragment size was quantified using a Femto pulse system (Agilent Technologies, Santa Clara, CA, USA); 44% of the DNA was in fragments > 100 kilobases (kb).

HiFi library preparation

The HiFi SMRTbell library was constructed using the SMRTbell Express Template Prep Kit v2.0 (Pacific Biosciences—PacBio, Menlo Park, CA, USA; Cat. #100-938-900) according to the manufacturer's instructions. HMW gDNA was sheared to a target DNA size distribution between 15 to 20 kb using Diagenode's Megaruptor 3 system (Diagenode, Belgium; Cat. B06010003). The sheared gDNA was concentrated using 0.45X 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, ligation of overhang adapters v3 at 20 °C for 60 min and 65 °C for 10 min to inactivate the ligase, then nuclease treated at 37 °C for 1 h. The SMRTbell library was purified and concentrated with 0.45X AMPure PB beads for size selection using the BluePippin/PippinHT system (Sage Science, Beverly, MA, USA; Cat #BLF7510/HPE7510) to collect fragments greater than 7 to 9 kb. The 15 to 20 kb average HiFi SMRTbell library was sequenced at the UC Davis DNA Technologies Core (Davis, CA, USA) using two 8M SMRT cells (PacBio, Cat #101-389-001), Sequel II sequencing chemistry 2.0, and 30-h movies each on a PacBio Sequel IIe sequencer.

Omni-C library preparation

The Omni-C library was prepared using the Dovetail Omni-C Kit (Dovetail Genomics, Scotts Valley, CA, USA) according to the manufacturer's protocol with slight modifications. First, liver tissue was thoroughly ground with a mortar and pestle while cooled with liquid nitrogen. Subsequently, chromatin was fixed in place in the nucleus. The suspended chromatin solution was then passed through 100 and 40 μm cell strainers to remove large debris. Fixed chromatin was digested under various conditions of DNase I until a suitable fragment length distribution of DNA molecules was obtained. Chromatin ends were repaired and ligated to a biotinylated bridge adapter followed by proximity ligation of adapter-containing ends. After proximity ligation, crosslinks were reversed and the DNA was purified from proteins. Purified DNA was treated to remove biotin that was not internal to ligated fragments. An NGS library was generated using an NEB Ultra II DNA Library Prep kit (New England Biolabs, Ipswich, MA, USA) with an Illumina compatible y-adaptor. Biotin-containing fragments were then captured using streptavidin beads. The post-capture product was split into two replicates prior to PCR enrichment to preserve library complexity with each replicate receiving unique dual indices. The library was sequenced at Vincent J. Coates Genomics Sequencing Lab (Berkeley, CA, USA) on an Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA) to generate approximately 100 million 2 × 150 bp read pairs per giga base pair (Gb) of genome size.

Transcriptome RNA extraction, library preparation, and sequencing

Total RNA from three tissues (tongue, muscle, heart) was extracted using a Qiagen RNeasy Mini Kit (Qiagen, Netherlands), according to the manufacturer's protocol. RNA libraries were then prepared using the KAPA mRNA HyperPrep Kit (Roche, Switzerland) according to the manufacturer's protocol. Libraries were sequenced with 100 bp reads on an Illumina NovaSeq 6000 platform (Illumina), to generate approximately 50M reads per library at the Technology Center for Genomics and Bioinformatics (UCLA, CA, USA) on an Illumina NovaSeq X.

Nuclear genome assembly

We assembled the genome of a *S. hammondii* individual following the CCGP assembly pipeline Version 5.0, as outlined in Table 1, where we list the tools and non-default parameters used in the assembly process. We removed the remnants adapter sequences from the PacBio HiFi dataset using HiFiAdapterFilt (Sim et al. 2022) and generated an initial diploid phased assembly using HiFiasm (Cheng et al. 2021) in HiC mode with the filtered PacBio HiFi reads and the Omni-C short-reads, a process that generates two assemblies, one per haplotype. We then aligned the Omni-C data to each assembly following the Arima Genomics Mapping Pipeline (https://github.com/ArimaGenomics/mapping_pipeline) and scaffolded them independently with SALSA (Ghurye et al. 2017, 2019.

The assemblies for both haplotypes were manually curated by iteratively generating and analyzing their corresponding Omni-C contact maps. To generate the contact maps, we aligned the Omni-C data with BWA-MEM (Li 2013), identified ligation junctions, and generated Omni-C pairs (Lee et al. 2022) using pairtools (Abdennur et al. 2024). Then, we generated multi-resolution Omni-C matrices with cooler (Abdennur and Mirny 2020) and balanced them with hicExplorer (Ramírez et al. 2018). We used HiGlass (Kerpedjiev et al. 2018) and the PretextSuite (ttps://github.com/wtsi-hpag/PretextView; https://github.com/wtsi-hpag/PretextMap; https://github.com/wtsi-hpag/PretextSnapshot) to visualize the contact maps. We identified misassemblies and misjoins in these contact maps and modified the assemblies using the Rapid Curation pipeline from the Wellcome Trust Sanger Institute, Genome Reference Informatics Team (https://gitlab.com/wtsi-grit/rapid-curation). Some of the remaining gaps (joins generated during scaffolding and/or curation) were closed using the PacBio HiFi reads and YAGCloser (https://github.com/merlyescalona/yagcloser). We checked for contamination using the BlobToolKit Framework (Challis et al. 2020).

Mitochondrial genome

We assembled the mitochondrial genome of *S. hammondii* from the PacBio HiFi reads using the reference-guided pipeline MitoHiFi (Allio et al. 2020; Uliano-Silva et al. 2023). The mitochondrial sequence of a *Pelobates fuscus* (NCBI:NC_051953.1) was used as the starting sequence. After completion of the nuclear genome, we searched for matches of the resulting mitochondrial assembly sequence in the nuclear genome assembly using BLAST+ (Camacho et al. 2009) and filtered out contigs and scaffolds from the nuclear genome with a percentage of sequence identity > 99% and size smaller than the mitochondrial assembly sequence.

Genome quality assessment and size estimation

We generated k-mer counts from the PacBio HiFi reads using meryl (https://github.com/marbl/meryl). The k-mer counts were then used in GenomeScope2.0 (Ranallo-Benavidez et al. 2020) to estimate genome features including genome size, heterozygosity, and repeat content. To obtain general contiguity metrics, we ran QUAST (Gurevich et al. 2013). To evaluate genome quality and functional completeness, we used BUSCO (Manni et al. 2021) with the Tetrapoda ortholog database (tetrapoda_odb10) which contains 5,310 genes. Assessment of base level accuracy (quality value [QV]) and k-mer completeness were performed using the previously generated meryl database and merqury (Rhie et al. 2020). We further estimated genome assembly accuracy via BUSCO gene set frameshift analysis using the pipeline described in Korlach et al. (2017). Measurements of the size of the phased blocks were based on the size of the contigs generated by HiFiasm on HiC mode. We follow the quality metric nomenclature established by Rhie et al. (2021), with the genome quality code x.y.P.Q.C, where, x = log10[contig NG50]; y = log10[scaffold NG50]; P = log10 [phased block NG50]; Q = Phred base accuracy QV; and C = % genome represented by the first "n" scaffolds, following a karyotype of $2n = 26$ for this species (Wasserman 1970). Quality metrics for the notation were calculated on the assembly for the haplotype one assembly.

Genome annotation

We annotated the genome assembly using the NCBI Eukaryotic Genome Annotation Pipeline v0.4.1-alpha (hereafter, "EGAPx") which is published in the NCBI RefSeq database (O'Leary et al. 2016) and accessible through the NCBI GitHub page ("ncbi/egapx"). Annotation features were identified by aligning transcripts and proteins from related taxa in the RefSeq database using BLAST (Camacho et al. 2009).

Table 1. Assembly pipeline and software used.

Assembly step	Software and any non-default options	Version	Reference
Initial assembly			
Filtering PacBio HiFi adapters	HiFiAdapterFilt	Commit 64d1c7b	Sim et al. 2022
K-mer counting	Meryl (k = 21)	1	https://github.com/marbl/meryl
Estimation of genome size and heterozygosity	GenomeScope	2	Ranallo-Benavidez et al. 2020
De novo *assembly (contiging)*	HiFiasm (Hi-C Mode, –primary, output hic.hap1.p_ctg, hic.hap2.p_ctg)	0.16.1-r375	Cheng et al. 2021
Scaffolding			
Omni-C data alignment	*Arima Genomics Mapping Pipeline*	Commit 2e74ea4	https://github.com/ArimaGenomics/mapping_pipeline
Arima Genomics Mapping Pipeline (AGMP)	BWA-MEM	0.7.17-r1188	Li 2013
	samtools	1.11	Danecek et al. 2021
	filter_five_end.pl (AGMP)	Commit 2e74ea4	https://github.com/ArimaGenomics/mapping_pipeline
	two_read_bam_combiner.pl ((AGMP))	Commit 2e74ea4	https://github.com/ArimaGenomics/mapping_pipeline
	picard	2.27.5	https://broadinstitute.github.io/picard/
Omni-C Scaffolding	SALSA (-DNASE, -i 20, -p yes)	2	Ghurye et al. 2017, Ghurye et al. 2019
Omni-C Contact map generation			
Short-read alignment	BWA-MEM (-5SP)	0.7.17-r1188	Li 2013
SAM/BAM processing	samtools	1.11	Danecek et al. 2021
SAM/BAM filtering	pairtools	0.3.0	Abdennur et al. 2024
Pairs indexing	pairix	0.3.7	Lee et al. 2022
Matrix generation	cooler	0.8.10	Abdennur and Mirny 2020
Matrix balancing	hicExplorer (hicCorrectmatrix correct –filterThreshold -2 4)	3.6	Ramírez et al. 2018
Contact map visualization	HiGlass	2.1.11	Kerpedjiev et al. 2018
	PretextMap	0.1.4	https://github.com/wtsi-hpag/PretextView
	PretextView	0.1.5	https://github.com/wtsi-hpag/PretextMap
	PretextSnapshot	0.0.3	https://github.com/wtsi-hpag/PretextSnapshot
Manual curation tools	Rapid curation pipeline (Wellcome Trust Sanger Institute, Genome Reference Informatics Team)	Commit 7acf220c	https://gitlab.com/wtsi-grit/rapid-curation
Genome quality assessment			
Basic assembly metrics	QUAST (–est-ref-size)	5.0.2	Gurevich et al. 2013
Assembly completeness	BUSCO (-m geno, -l tetrapoda)	5.0.0	Manni et al. 2021
	Merqury		Rhie et al. 2020
Contamination screening			
Local alignment tool	BLAST+ (-db nt, -outfmt '6 qseqid staxids bitscore std' , -max_target_seqs 1, -max_hsps 1, -evalue 1e-25)	2.15	Camacho et al. 2009
General contamination screening	BlobToolKit (HiFi coverage, BUSCO = tetrapoda, NCBI Taxa ID = 228 670)	2.3.3	Challis et al. 2020
Mitochondrial assembly			
Mitochondrial genome assembly	MitoHiFi (-r, -p 90, -o 1) Reference: *Pelobates fuscus* (NCBI:NC_051953.1)	2.2	Uliano-Silva et al. 2023
MitoHiFi: Mitochondrial genome annotation	MitoFinder (MitoHiFi pipeline parameters)	1.4	Allio et al. 2020

Novel, species-specific RNA-Seq reads were also aligned to the assembly using the alignment software STAR (Dobin et al. 2013). Additional features are predicted using HMM-based gene models using the NCBI Gnomon software. We evaluated the quality and completeness of our annotation by comparing the longest protein for each annotated coding gene to tetrapods (odb10) using BUSCO v5.7.1 (Manni et al. 2021). We report the number of annotation features and the BUSCO results in Table 2.

Results

Sequencing data

The Omni-C library generated 289.96 million read pairs, and the PacBio HiFi library generated 4.65 million reads. The PacBio HiFi sequences yielded ~56X genome coverage and had an N50 read length of 18,307 bp; a minimum read length of 1 bp; a mean read length of 18,118 bp; and a maximum read length of 65,001 bp (see Supplementary Fig. S1 for read length distribution). Based

Table 2. Sequencing and assembly statistics and accession numbers.

Bio Projects & Vouchers	CCGP NCBI BioProject		PRJNA720569			
	Genera NCBI BioProject		PRJNA765864			
	Species NCBI BioProject		PRJNA937275			
	NCBI BioSample		Assembly: SAMN33386125 Transcriptome: SAMN40863797, SAMN40863796, SAMN40863795			
	Specimen identification		HBS 135690			
	NCBI Genome accessions		**Haplotype one**		**Haplotype two**	
	Assembly accession		JARDYI000000000		JARDYJ000000000	
	Genome sequences		GCA_029215705.1		GCA_029215755.1	
Genome sequence	PacBio HiFi reads	Run	1 PACBIO_SMRT (Sequel II) run: 2.9M spots, 48.5G bases, 35.2 Gb			
		Accession	SRR25482847			
	Omni-C Illumina reads	Run	2 ILLUMINA (Illumina NovaSeq 6000) runs: 101.3M spots, 30.6 G bases, 10.4 Gb			
		Accession	SRR25482845-46			
Genome assembly quality metrics	Assembly identifier (quality code[a])		aSpeHam1 (7.8.P7.Q64.C90)			
	HiFi read coverage[b]		44.26X			
			Haplotype one		**Haplotype two**	
	Number of contigs		677		541	
	Contig N50 (bp)		11,561,794		14,335,781	
	Contig NG50[b]		12,894,986		15,063,856	
	Longest contigs		61,120,978		46,956,032	
	Number of scaffolds		479		351	
	Scaffold N50		120,773,319		120,842,655	
	Scaffold NG50[b]		120,773,319		120,842,655	
	Largest scaffold		183,606,164		188,005,797	
	Size of final assembly		1,142,014,214		1,155,539,324	
	Phased block NG50[b]		12,894,986		15,063,856	
	Gaps per Gbp (#gaps)		173(198)		164(190)	
	Indel QV (frame shift)		50.42043639		50.31166	
	Base pair QV		64.3477		64.3477	
			Full assembly = 64.0148			
	k-mer completeness		95.968		96.1908	
			Full assembly = 99.1337			
	BUSCO	**C**	**S**	**D**	**F**	**M**
	completeness H1[c]	90.90%	89.90%	1.00%	2.30%	6.80%
	(tetrapoda) $n = 5310$ H2[c]	91.50%	90.50%	1.00%	2.30%	6.20%
	Organelles	1 complete mitochondrial sequence			CM055131	
Genome annotation quality metrics			**Count of features**			
	Genes		20,434			
	mRNA		24,883			
	lncRNA		887			
	CDSs		24,883			
	BUSCO completeness	**C**	**S**	**D**	**F**	**M**
	(tetrapoda_odb10) $n = 5310$	94.70%	93.20%	1.50%	0.70%	4.60%

[a]Assembly quality code x.y.P.Q.C derived notation, from (Rhie et al. 2021). x = log10[contig NG50]; y = log10[scaffold NG50]; P = log10 [phased block NG50]; Q = Phred base accuracy QV; C = % genome represented by the first "*n*" scaffolds, following a known karyotype for *S. hammondii* of $2n = 26$ (Wasserman 1970). Quality code for all the assembly denoted by haplotype one assembly (aSpeHap1.0.hap1). [b]Read coverage and NGx statistics have been calculated based on the estimated genome size of 1.09 Gb. [c](H1) Haplotype one and (H2) haplotype two assembly values.

on the PacBio HiFi data, GenomeScope 2.0 estimated a genome size of 1.09 Gb, a 0.164% sequencing error rate, and 0.355% heterozygosity. The k-mer spectrum shows a unimodal distribution with a major coverage peak at ~43-fold coverage (Fig. 2A). Sequencing of mRNA libraries for SAMN40863797, SAMN40863796, and SAMN40863795 yielded 28M, 28M, 32M paired end reads, respectively.

Nuclear genome assembly

The final genome assembly (aSpeHam1) consists of two phased haplotypes. Both assemblies are similar in size, but not equal to the estimated genome size from GenomeScope2.0, as has been observed in other taxa (e.g. Pflug et al. 2020).

The haplotype one assembly (aSpeHam1.0.hap1) consists of 479 scaffolds spanning 1.14 Gb with a contig N50 of 11.56 Mb, a scaffold N50 of 120.77 Mb, the largest contig size of 61.12 Mb, and the largest scaffold size of 183.6 Mb. The haplotype two assembly (aSpeHam1.0.hap2) consists of 351 scaffolds spanning 1.15 Gb with a contig N50 of 14.33 Mb, a scaffold N50 of 120.84 Mb, the largest contig size of 46.95 Mb, and the largest scaffold size of 188 Mb. The haplotype one assembly has a BUSCO completeness score for the Tetrapoda gene set of 90.9%, a base pair QV of 63.7, a k-mer completeness of 95.96%, and a frameshift indel QV of 50.42. The haplotype two assembly has a BUSCO completeness score for the Tetrapoda gene set of 91.5%, a

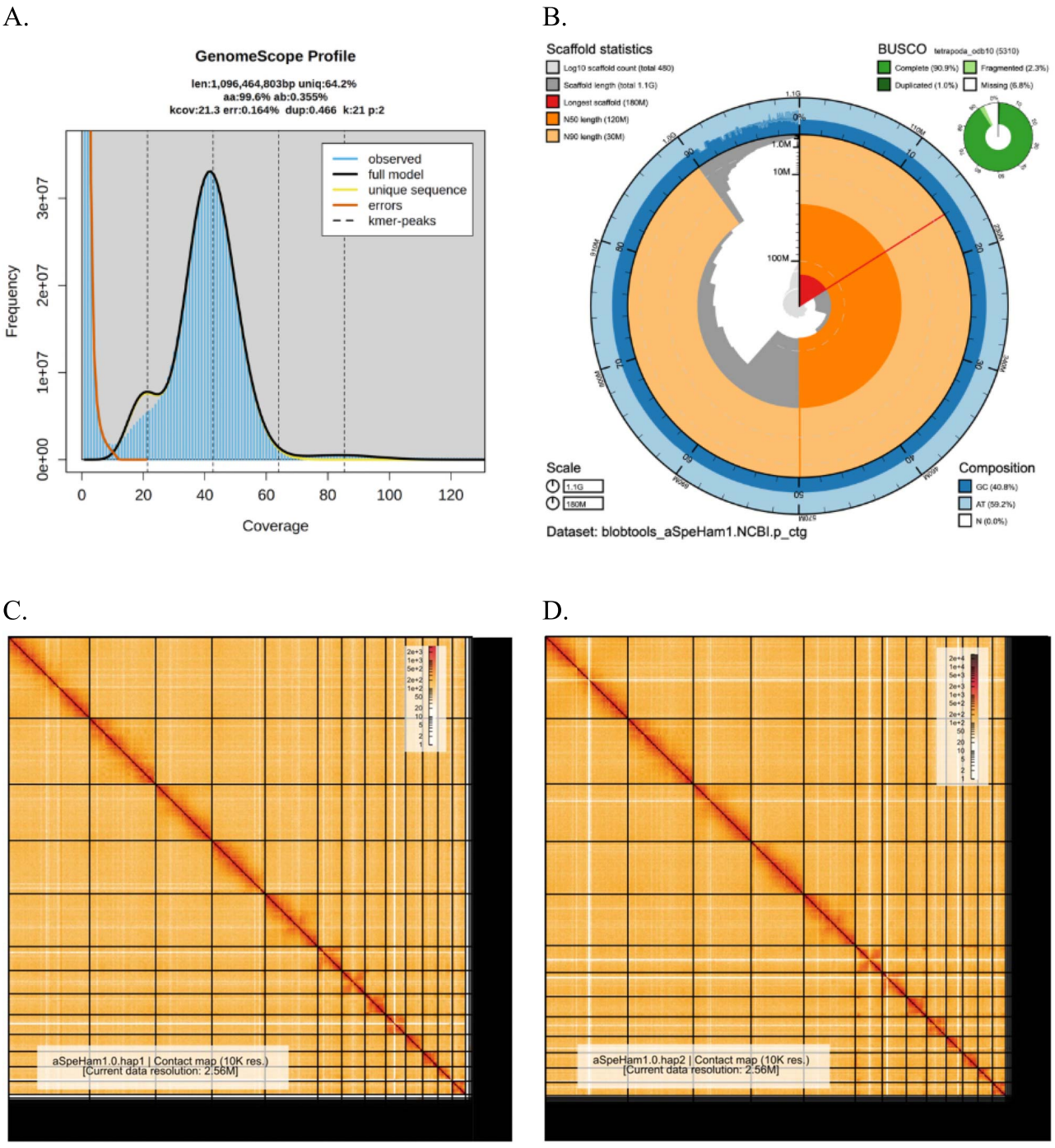

Fig. 2. Visual overview of assembly metrics. (A) K-mer spectrum output generated from PacBio HiFi data without adapters using GenomeScope2.0. K-mers covered at lower coverage and low frequency correspond to differences between haplotypes, whereas the higher coverage and high frequency k-mers correspond to the similarities between haplotypes. (B) BlobToolkit Snail plot showing a graphical representation of the quality metrics presented in Table 2 for the *Spea hammondii* primary assembly (haplotype one; GenBank accession no. GCA_029215705.1). The plot circle represents the full size of the assembly. From the inside-out, the central plot covers length related metrics. The red line represents the size of the longest scaffold; all other scaffolds are arranged in size-order moving clockwise around the plot and drawn in gray starting from the outside of the central plot. Dark and light orange arcs indicate the scaffold N50 and scaffold N90 values. The central light gray spiral shows the cumulative scaffold count with a white line deliniating each order of magnitude. White regions in this area reflect the proportion of Ns in the assembly. The dark vs. light blue area around it shows mean, maximum, and minimum GC vs.AT content at 0.1% intervals (Challis et al. 2020). For BUSCO summary data, the definitions are "complete and single copy" (Comp.) or "complete and duplicated" (Dupl.). (C and D) Omni-C Contact maps for the haplotype 1 (2C) and haplotype 2 (2D) assemblies generated with PretextSnapshot. Omni-C contact maps translate proximity of genomic regions in 3-D space to contiguous linear organization. Each line in the contact map corresponds to sequencing data supporting the linkage (or join) between two of such regions. Scaffolds are separated by black lines, and higher density corresponds to high levels of fragmentation.

base pair QV of 64.35, a k-mer completeness of 96.1%, and a frameshift indel QV of 50.31.

During manual curation, we made 217 joins (103 on haplotype one and 114 on haplotype two) and 28 breaks (12 on haplotype one and 16 on haplotype two) based on the Omni-C contact map signal. We closed 20 gaps (9 on haplotype one and 11 on haplotype two). We filtered out two contigs corresponding to mitochondrial contamination. No further contigs were removed. The Omni-C contact maps show highly contiguous assemblies supporting the genome organization of 13 chromosomes per haplotype (Fig. 2C and D). Classic cytogenetic work reports a diploid number of $2n = 26$ for *S. hammondii*, historically treated as *Scaphiopus hammondii* (Wasserman 1970). By sorting our haploid assembly scaffolds by length, we found that the largest 13 contain 90.87% of the total genome length. We believe the remaining sequences represent repeat-dense, unresolved regions. Assembly statistics are reported in Table 2 and represented graphically in Fig. 2B. We have deposited the genome assembly on NCBI GenBank (See Table 2 and Data Availability for details).

Mitochondrial genome assembly

We assembled a mitochondrial genome for *S. hammondii* with MitoHiFi. The final mitochondrial sequence has a size of 16,682 bp, with a base composition of A = 29.85%, C = 27.89%, G = 15.2%, T = 27.07%, and consists of 2 rRNAs, 22 unique transfer RNAs, and 13 protein-coding genes.

Genome annotation

Our final genome annotation for *S. hammondii* included 20,434 genes, with a Tetrapoda BUSCO completeness of 94.70%. A list of annotation statistics and BUSCO score breakdowns are reported in Table 2.

Discussion

As of the publication date of this manuscript, there are 101 chromosome-level genome assemblies for anurans in the NCBI database representing 51 species. With this publication, this list now includes what we consider a chromosome-level reference genome for the Western Spadefoot, *S. hammondii*. Assembly quality is reflected in core metrics (scaffold N50 = 120.8 Mb) with the largest scaffolds reaching 183.6 to 188.0 Mb, and completeness (BUSCO = 90.9% against a conserved tetrapod ortholog set).

Amphibian genomes are remarkably variable in size, with many anurans showing clade-specific genome expansions or contractions. Compared with other anurans, *Spea* genomes are extremely compact. Of the 495 anuran genomes reported in the Animal Genome Size Database (https://www.genomesize.com), scaphiopodids are almost always among the smallest 10 species; their reported c-values (average = 1.42, $n = 17$ observations) are much smaller than anurans generally (average = 4.47, $n = 495$). Zeng et al. (2014) analyzed the genome size distribution within pelobatoid frogs (a group including *Spea* and related families) and found a strong statistical association between larval development time and genome size. However, the genome size statistics reported by Zeng et al. (2014) are also derived from c-values in the Animal Genome Size Database rather than assembled genomes, and these two estimates can vary. Our assembly spans 1.142 Gb, and our haploid genome size estimate is 1.09 Gb, which aligns with the other two available *Spea* genomes but is on the low end of previously published c-values for *S. hammondii* (c-values of 1.27, 1.34, 1.55, and 2.34; Gregory 2026). We interpret this discrepancy as reflecting a modest underrepresentation of repetitive sequence in our current assembly and/or variability in older Feulgen c-value estimates. Regardless, as additional annotated genomes of anurans are produced, future studies should explore whether the developmental time/genome size correlation persists across pelobatoids and anurans more generally.

This *S. hammondii* genome also offers a comparative framework for studying phenotypic plasticity in spadefoots. Studies in congeners (e.g. *S. multiplicata*) found that rapid development and resource polyphenism involve shifts in lipid and cholesterol metabolism and peroxisome biology and have identified candidate loci involved in plasticity (Isdaner et al. 2023), while population genomic analyses have identified candidate loci involved in plasticity (Levis et al. 2020). Our reference genome is a key resource supporting future mapping of these candidate pathways and their regulatory neighborhoods in *S. hammondii*, as well as tests for selection on these regions along hydroperiod gradients.

This genome directly supports the conservation goals of the CCGP. Range-wide genome resequencing built on this coordinate system will enable delineation of conservation units across the patchy distribution for this species (including the genetic division north and south of the Transverse Ranges identified by Neal et al. (2018) based on RADseq data), estimation of relatedness, runs of homozygosity, and the quantification of genomic load on a population basis. Landscape genetics analyses can identify loci linked to hydroperiod, desiccation tolerance, developmental rate, and temperature sensitivity. If inbreeding is detected, and we suspect that this will be the case, this information can be used to plan reintroductions and assisted gene flow to potentially minimize the negative effects of inbreeding while preventing the proliferation of harmful alleles and the degradation of natural diversity.

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Author contributions

Ben Thompsky (Writing—original draft, Writing—review & editing), Eric Beraut (Resources, Writing—original draft), Robert D. Cooper (Methodology), Merly Escalona (Resources, Software, Writing—review & editing), Robert E. Espinoza (Methodology, Resources), Robert N. Fisher (Resources), Courtney Miller (Data curation, Project administration, Writing—review & editing), Oanh Nguyen (Resources), Samuel Sacco (Resources), Ruta Sahasrabudhe (Resources), William Seligmann (Resources), Erin Toffelmier (Project administration, Supervision, Writing—review & editing), Ian J. Wang (Conceptualization, Writing—review & editing), and H. Bradley Shaffer (Conceptualization, Supervision, Visualization, Writing—original draft, Writing—review & editing)

Supplementary material

Supplementary material is available at *Journal of Heredity* online.

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Data availability

Data generated for this study are available under NCBI BioProject PRJNA937275. Raw sequencing data for sample HBS135690 (NCBI BioSample SAMN33386125) are deposited in the NCBI Short Read Archive (SRA) under SRR25482847 for PacBio HiFi sequencing data, and SRR25482845-46 for the Omni-C Illumina sequencing data. GenBank accessions for both primary and alternate assemblies are GCA_029215705.1 and GCA_029215755.1; and for genome sequences JARDYI000000000 and JARDYJ000000000. The GenBank genome assembly for the mitochondrial genome is CM055131. Data generated for the annotation in this study are available under NCBI BioProject PRJNA1013362. Raw RNA sequencing data (NCBI BioSamples SAMN40863797, SAMN40863796 and SAMN40863795) are deposited in the NCBI SRA [SRR29346342, SRR29346343, SRR29346344], respectively). Assembly scripts and other data for the analyses presented can be found at the following GitHub repository: www.github.com/ccgproject/ccgp_assembly

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