De novo genome assembly of the marine teleost, bluefin trevally (Caranx melampygus)

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Abstract

The bluefin trevally, Caranx melampygus, also known as the bluefin kingfish or bluefin jack, is known for its remarkable, bright-blue fins. This marine teleost is a widely prized sportfish, but few resources have been devoted to the genomics and conservation of this species because it is not targeted by large-scale commercial fisheries. Population declines from recreational and artisanal overfishing have been observed in Hawai‘i, USA, resulting in both an interest in aquaculture and concerns about the long-term conservation of this species. Most research to-date has been performed in Hawai‘i, raising questions about the status of bluefin trevally populations across its Indo-Pacific range. Genomic resources allow for expanded research on stock status, genetic diversity, and population demography. We present a high quality, 711 Mb nuclear genome assembly of a Hawaiian bluefin trevally from noisy long-reads with a contig NG50 of 1.2 Mb and longest contig length of 8.9 Mb. As measured by single-copy orthologs, the assembly was 95% complete, and the genome is comprised of 16.9% repetitive elements. The assembly was annotated with 33.1 K protein-coding genes, 71.4% of which were assigned putative functions, using RNA-seq data from eight tissues from the same individual. This is the first whole-genome assembly published for the carangoid genus Caranx. Using this assembled genome, a multiple sequentially Markovian coalescent model was implemented to assess population demography. Estimates of effective population size suggest population expansion has occurred since the Late Pleistocene. This genome will be a valuable resource for comparative phylogenomic studies of carangoid fishes and will help elucidate demographic history and delineate stock structure for bluefin trevally populations throughout the Indo-Pacific.

Keywords: bluefin trevally; ‘Omilu; Carangidae; de novo genome assembly; MSMC

Introduction

The bluefin trevally (Caranx melampygus; Cuvier 1833) is a marine teleost fish (Carangiformes: Carangoidei) inhabiting coastal environments throughout the tropical and subtropical Indo-Pacific (Figure 1). C. melampygus is a top predator on coral and rocky reef ecosystems, reaching up to 117 cm in length and feeding predominantly on shallow-water fishes and invertebrates (Sudekum et al. 1991; Meyer et al. 2001). In the Northwestern Hawaiian Islands, for example, bluefin trevallys consume an estimated 11,000 metric tons of prey per year, confirming their role as important predators in this region (Sudekum et al. 1991). C. melampygus is also targeted by small-scale and recreational fisheries in Hawai‘i, where it is known by its Native Hawaiian name, ‘omilu (Meyer et al. 2001). In recent decades, the C. melampygus population in Hawai‘i has been impacted by overharvesting and habitat destruction (Friedlander and Dalzell 2004). For this reason, there has been significant interest in Hawai‘i in captive breeding for aquaculture (Moriwake et al. 2001; Zhao and Lu 2006). Because the bulk of research on the bluefin trevally has been conducted in Hawai‘i, observations of population declines raise concerns for populations in other parts of its range, where abundance and biomass estimates remain unknown.

Recent genomic evidence suggests C. melampygus comprises a unique population in Hawai‘i compared with several localities sampled across the Indo-Pacific (Glass et al. 2021), and an analysis of complete mitochondrial genomes suggests individuals from Guam are also genetically distinct (Genomic Resources Development Consortium et al. 2014). Given population declines and evidence of unique stock structure in Hawai‘i, whole-genome data for C. melampygus would provide unprecedented value for inferring demographic history, estimating effective population size, and testing for selection and local adaptation. Juvenile and adult individuals frequently utilize estuarine habitats, for example, and have a strong tolerance for freshwater in coastal locations where estuaries are present (Blaber and Cyrus 1983). Studying the evolution and physiology of C. melampygus in a genomic context is valuable to the broader scientific and reef fish community, especially given interest in the genomic mechanisms of adaptation of marine and anadromous fishes to freshwater (Kültz 2015). Furthermore, whole-genome data provide baseline biological
C. melampygus 2016). Here, we present an annotated which diverged from comparator phylogenomic analysis. development and expand the genomic resources of carangoid fishes Ozaki and Araki 2017; Araki et al. (Linnaeus 1758; Zhang ovatus (Linnaeus 1758; Koepfli 2015). Data are available for only 7 out of read DNA sequencing. The heart tissue was also used for long- Sample acquisition and sequencing

One C. melampygus individual was captured in 3–9 m of water <1 km off the coast of O‘ahu (near Kaneohe, Hawai‘i, USA: 21° 26’ 45.3” N 157° 48’ 07.5” W) in April 2018. The specimen was caught using a Shimano (Sakai, Osaka, Japan) ocean rod outfitted with a Daiwa (Cypress, CA, USA) Saligma 6500 reel and a white feather jig. Brain, eye, fin, gill, heart, kidney, liver, and muscle tissue samples were collected immediately upon capture, flash-frozen in liquid nitrogen, and packaged in dry ice for transportation to Brigham Young University (BYU; Provo, UT, USA) for storage at −80°C until sequencing. All tissue samples were used for short-read RNA sequencing. The heart tissue was also used for long-read DNA sequencing.

High-molecular weight (HMW) DNA was extracted and prepared for long-read sequencing following the protocol “Procedure & Checklist—Preparing >30 kb SMRTbell Libraries Using Megaruptor Shearing and BluePippin Size-Selection for PacBio RS II and Sequel Systems.” Briefly, HMW DNA was extracted using a wide-bore pipette tip and quality was assessed using an Agilent (Santa Clara, CA, USA; https://www.agilent.com) Fragment Analyzer and Sage Science (Beverly, Massachusetts, USA; https://sage-science.com) Pippin Pulse. DNA quantitation was performed at each step using a Beckman Coulter (Brea, CA, USA; https://www.beckman.com) Qubit v3.0 Fluorometer. No shearing, for example, with a Diagenode (Seraing, Belgium; https://www.diagenode.com) Megaruptor, was performed because the DNA was already sufficiently fragmented (average size of 25 Kb). After exonuclease removal and damage and end repairs, adapter ligation was performed using a Pacific Biosciences (PacBio; Menlo Park, CA, USA; https://www.pacb.com) SMRTbell Library Kit. Purification was performed using BC AMPure PB beads, and the resulting DNA was size selected for fragments >20 Kb with the Sage Science BluePippin. Continuous long-read (CLR) sequencing was performed on ten SMRT cells for a 10-h movie on the PacBio Sequel at the BYU DNA Sequencing Center (DNASC; https://dnasc.byu.edu), a PacBio Certified Service Provider.

RNA was extracted using Invitrogen (part of Thermo Fisher Scientific, Carlsbad, CA, USA; https://www.thermofisher.com) TRizol Reagent (Catalog Numbers 15596018 and 15596026), as described in the User Guide (Document Part Number 15596026.PPS; Publication Number MAN0001271, Revision B.0). The quality and quantity of the RNA were assessed using an Agilent Fragment Analyzer with their RNA standard sensitivity kit (Part Number DNF-471-0500), and 4 μg of RNA was prepared for sequencing with Roche (Basel, Switzerland; https://sequencing.roche.com) KAPA Stranded RNA-Seq kit, following recommended protocols as described in the Technical Data Sheet titled “KAPA Stranded mRNA-Seq Kit Illumina platform” with product code KR0960, version 3.15 (this is now outdated, and newer versions can be found on Roche’s website at https://sequencing.roche.com/en-us/products-solutions/by-category/library-preparation/rna-library-preparation/kapa-stranded-rna-seq-kits/ordering.html). The average insert size of the library was 300 bp. Paired-end RNA sequencing was performed in Rapid Run mode for 250 cycles with the eight samples across two lanes on the Illumina (San Diego, CA, USA; https://www.illumina.com) Hi-Seq 2500 at the DNASC.

Sequence assembly

The PacBio CLR reads were self-corrected and assembled with Canu v1.6 (Koren et al. 2017). Repeat characterization was performed with RepeatMasker v4.1.2-p1 (Smit et al. 2021) using Dfam v3.3 (Storer et al. 2021) and the RepBase RepeatMasker Library (2018). Assembly continuity statistics, for example, N50 and area under the NG-curve (auNG; Li 2020), were calculated with calcN50 downloaded April 2020 (https://github.com/ih3/calN50) and a custom Python (https://www.python.org) script. The genome size provided to Canu and used for the computation of assembly statistics was based on values recorded in the Animal Genome Size Database (Gregory 2018). A C-value was not listed in the database for C. melampygus; we used 0.8 (782.4 Mb) as an upper limit based on recorded genome size values for other Caranx species.

The transcriptome was assembled from Illumina RNA-seq reads from all eight tissues (i.e., brain, eye, fin, gill, heart, kidney, liver, and muscle). The RNA-seq reads were not corrected, but the quality was assessed using fastqc (Babraham Bioinformatics

Figure 1 Bluefin trevally (C. melampygus) adult and juvenile. Quantitative morphological data for this illustration of C. melampygus were obtained primarily from (Heemstra et al. 2021). These were then evaluated by the artist who selected specific values for details such as number of lateral line scutes (32), number of rays (23), and spines (8) in the dorsal fin, and number of rays (19) and spines (2) in the anal fin. Each of these was portrayed in the illustration to be near the middle of the ranges reported.
Computational genome annotation

The MAKER v3.01.02-beta (Holt and Yandell 2011) pipeline was used to annotate the genome assembly. Generally speaking, annotation proceeded according to the process described in the most recent Maker Wiki tutorial (Holt and Yandell 2018). A custom repeat library was created using RepeatModeler v1.0.11 (Smit and Hubley 2008). The transcriptome assembly, genome assembly, and proteins from UniProtKB Swiss-prot (Boutet et al. 2007; The Uniprot Consortium 2019) were used as input to MAKER to create initial annotations. Gene models based on these annotations were used to train the following ab initio gene predictors: AUGUSTUS v3.3.2 (Stanke and Waack 2003; Stanke et al. 2006) and SNAP downloaded 3 June 2019 (Korf 2004). AUGUSTUS was trained using BUSCO (Simão et al. 2015) as a wrapper; SNAP was trained without a wrapper. GeneMark-ES v4.38 (Lomsadze et al. 2005, 2014; Brünó et al. 2020) was also trained, though necessarily without the initial models from MAKER. These models were all provided to MAKER for a second round of structural annotation. The gene models based on those annotations were filtered with gFACs v1.1.1 (Caballero and Wegrzyn 2019) and again provided to AUGUSTUS and SNAP. As GeneMark-ES does not accept initial gene models, it had no need to be run again. The gene models from the ab initio gene predictors were again provided to MAKER for a third and final round of annotation. Functional annotations were added using MAKER accessory scripts, the BLAST+ Suite v2.9.0 (Altschul et al. 1990; Camacho et al. 2009), and InterProScan v5.45-80.0 (Jones et al. 2014; Mitchell et al. 2019).

Demographic history

We inferred the historical demography of C. melampygus and its close relative, the giant trevally (Caranx ignobilis), by implementing the multiple sequentially Markovian coalescent (MSMC) model (Schiffels and Durbin 2014) to generate estimates of effective population size \( N_e \) over time. MSMC estimates the rate of coalescent events between two alleles at each locus along an unphased, single diploid genome. We used the self-corrected PacBio reads mapped against the assembly, filtered for contigs > 500 Kb, and applied additional cutoffs to ensure sufficient sequencing depth and quality using MSMC-tools downloaded October 8, 2020 (https://github.com/stschiff/msmc-tools; Mather et al. 2020; Schiffels and Wang 2020). We used a draft de novo genome for C. ignobilis (Glass et al. 2021). We ran MSMC v1.1.0 using the following time patterning parameters to estimate 20-time intervals and one free coalescent rate parameter: “12 + 16*1 + 12”. We then generated 1000 bootstrap estimates using a simulated dataset that randomly pulled, with replacement, 500 Kb long segments and arranged them into 52 segments per “chromosome”. We generated 30 simulated “chromosomes” to construct artificial 780 Mb long genomes, reflecting the estimated size of the C. melampygus genome, to determine confidence intervals around \( N_e \) estimates. We used the same MSMC parameters for C. ignobilis, except that the 30 simulated “chromosomes” contained 42 segments of length 500 Kb to construct 630 Mb long genomes to reflect the estimated size of the C. ignobilis genome (Hardie and Hebert 2004; Gregory 2018). After running MSMC, we converted population sizes and times into number of individuals and years, respectively, using a per site per generation mutation rate \( \mu = 3.7 \times 10^{-8} \) from another marine teleost species (Liu et al. 2016). For C. melampygus, we used a generation time of four, based on the average age of sexual maturity of C. melampygus (two) multiplied by two (Nadachowska-Brzyska et al. 2016; Mather et al. 2020). For C. ignobilis, we used a generation time of six, given an average age of three for sexual maturity in this species. The scripts to perform this analysis are available on GitHub (https://github.com/pickettbd/msmc-slurmPipeLine) with supporting documentation.

Results and discussion

Sequencing

CLR sequencing (PacBio) generated 4.45 M reads with a total of 52.67 Gb, which is \(~67\times\) physical coverage of the genome. The mean and N50 read lengths were 11,834.768 and 19,264 base pairs (bp), respectively. The longest read was 116,429 bp. The read length distribution is plotted in Figure 2. A summary of the results for the sequencing run is available in Table 1. This genome represents the first for the Caranx genus and ranks among the highest quality genomes available for Carangoidea in terms of N50 (Zhang et al. 2019).

RNA-seq from the eight tissues (i.e., brain, eye, fin, gill, heart, kidney, liver, and muscle) generated 257.47 M pairs of reads totaling 114.61 Gb. Across all eight tissues, the mean and N50 read lengths were 222.6 and 249 bp, respectively. The combined results from all eight tissues are represented in Table 1, whereas the results from each tissue are made available in Table 2.

PacBio CLR error correction

The self-correction strategy reduced the number of reads from 4.45 to 1.77 M and the total number of bases from 52.67 to 29.6 Gb for an approximate physical coverage of 37.8×. The mean and N50 read lengths were changed from 11,835 and 19,264 to 16,769 and 25,074 bp, respectively. The longest read was 78,163 bp. The distribution of read lengths can be viewed in Figure 2.

Genome assembly

The initial assembly from Canu was comprised of 3.6 K contigs with a total assembly size of 711 Mb. The mean contig length, N50, NG50, and maximum contig length were 198.8 Kb, 1.5 Mb, and 8.9 Mb, respectively. The L50 was 120, and the LG50 was 147. The auNG was 1.93 M. Table 3 summarizes the assembly continuity statistics, and the auNG is visualized in Figure 3.

The assembly completeness, as assessed with single-copy orthologs, was also evaluated (Table 3). The final set of contigs had 3,480 complete single-copy orthologs (95.6% of 3,640 from the ODB10 Actinopterygii set). Of these 99.3% (3,248) were present...
in the assembly only once, and 6.7% (232) were present more than once. Twenty-one (0.6%) and 139 (3.8%) single-copy orthologs were fragmented in and missing from the assembly, respectively. Approximately 16.9% of the genome was comprised of repetitive elements (Table 4), which is similar to other Carangoid genomes, for example, Pseudocaranx georgianus at 12.8% (Ruigrok et al. 2021) and T. ovatus at 20.3% (Zhang et al. 2019).

Transcriptome assembly and computational genome annotation

The transcriptome assembly generated by Trinity was comprised of 680 K sequences with a mean sequence length of 1,171 bp. The N50 and L50 were 2.4 Kb and 419 K, respectively. Of the 3640 single-copy orthologs in the ODB10 Actinopterygii set, 93.3% (3,399) were complete; 33.8% (1,148) of which were present only once in the transcript set. 112 (3.1%) single-copy orthologs were fragmented in the transcript set, 129 (3.6%) were missing. Computational structural and functional annotation using the transcriptome assembly and the MAKER pipeline yielded 33.1 K protein-coding genes, 71.4% of which were assigned putative functions. Of these, 20.9 and 19.9 K have annotated 5' and 3' UTRs, respectively. 2.1 K tRNA genes were also identified. A BUSCO analysis of the annotated genes (as extracted from the assembly with an extra 1 Kb from the ends of each gene) yielded 22.4% (814/3640 of Actinopterygii genes from OrthoDB v10) complete single-copy orthologs. Of the total, 20.1% were present only once, and 2.3% were duplicated. Twelve percent were fragmented, and 65.5% were duplicated. Twelve percent were fragmented, and 65.5% were duplicated. Twelve percent were fragmented, and 65.5% were duplicated.
The increase in $N_e$ was greater for *C. melampygus* than *C. ignobilis*. Our observations corroborate a previous demographic analysis of both species from Hawai‘i using mitochondrial loci, which also recovered evidence of population expansion compared with *C. ignobilis* (Santos et al. 2011). Other demographic components of wild populations (e.g., population structure, nonrandom mating, selection) are also known to affect estimates of coalescent rates (Mazet et al. 2016). For example, decreases in sea level have been linked to the isolation of marine populations (Norris and Hull 2012; Cacciapaglia et al. 2021), which would lead to demographic changes such as population structure and nonrandom mating. Sea levels decreased globally from the beginning of the Upper Pleistocene (~129 kya) until the last glacial maximum (~19–26 kya), with several fluctuations in-between caused by glacial-interglacial cycles (Grant et al. 2014). Moreover, ocean circulation patterns were weaker during glacial periods (Rahmstorf 2002), which would limit connectivity between populations of marine fishes such as *C. melampygus* and *C. ignobilis* that disperse primarily via pelagic larval drifting.

Recent evidence suggests *C. melampygus* and *C. ignobilis* individuals are a genetically unique population in Hawai‘i (Glass et al. 2021). During the last glacial maximum, exposed limestone bridges linked the Hawaiian Islands of Maui, Lāna‘i, and Moloka‘i and supported reef habitats which became drowned after sea levels began rising (Grigg et al. 2002). These limestone reef features may have created increased habitat availability in Hawai‘i during periods of glaciation and supported population expansion. Notably, these species are large-bodied and associated with coastal habitats, including rock and coral reefs, but are not reef-obligate. Overall, some reef fishes exhibit evidence of dramatic declines in population size during glaciation periods (Gaither et al. 2009), whereas others exhibit evidence of population expansion similar to what is reported here for *C. melampygus* (Delrieu-Trottin et al. 2017). An analysis of demographic history for *C. melampygus* individuals from the widespread, Indo-West Pacific population, and individuals of *C. ignobilis* from other identified populations (Glass et al. 2021) would allow us to compare population expansion and contractions over time and assess how sea level changes may have affected *C. melampygus* and *C. ignobilis* differently across the Indo-Pacific.

## Conclusion

The assembled genome of *C. melampygus* represents the first whole-genome assembly and annotation for the genus *Caranx* and second, after the Atlantic Horse Mackerel (*Trachurus trachurus*; Vertebrate Genomes Project 2020), in the clade Carangini, the most speciose subclade of Carangoidea. The high quality of this reference genome builds on previous carangoid whole-genome datasets and is important for delineating stock structure and demographic history of *C. melampygus*, especially given evidence of a unique genetic lineage in Hawai‘i. The bluefin trevally genome is also a valuable resource for comparative phylogenomic studies of carangoid fishes.

## Data availability

Raw reads have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under BioProject PRJNA670455. The genome assembly and annotations are associated with the same BioProject and can be found in GenBank under accession JAFELL0100000. The genome assembly as reported by RepeatMasker (Smit et al. 2021) using the Dfam v3.3 (Storer et al. 2021) and RepBase RepeatMasker v20181026 (Jurka 1998; Bao et al. 2015) repeat libraries.

## Supplementary material

Supplementary material is available at G3 online.

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## Conflicts of interest

None declared.
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