A highly contiguous nuclear genome assembly of the mandarinfish *Synchiropus splendidus* (Syngnathiformes: Callionymidae)

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Abstract

The fish order Syngnathiformes has been referred to as a collection of misfit fishes, comprising commercially important fish such as red mullets as well as the highly diverse seahorses, pipefishes, and seadragons—the well-known family Syngnathidae, with their unique adaptations including male pregnancy. Another ornate member of this order is the species mandarinfish. No less than two types of chromatophores have been discovered in the spectacularly colored mandarinfish: the cyanophore (producing blue color) and the dichromatic cyano-erythrophore (producing blue and red). The phylogenetic position of mandarinfish in Syngnathiformes, and their promise of additional genetic discoveries beyond the chromatophores, made mandarinfish an appealing target for whole-genome sequencing. We used linked sequences to create synthetic long reads, producing a highly contiguous genome assembly for the mandarinfish. The genome assembly comprises 483 Mbp (longest scaffold 29 Mbp), has an N50 of 12 Mbp, and an L50 of 14 scaffolds. The assembly completeness is also high, with 92.6% complete, 4.4% fragmented, and 2.9% missing out of 4584 BUSCO genes found in ray-finned fishes. Outside the family Syngnathidae, the mandarinfish represents one of the most contiguous syngnathiform genome assemblies to date. The mandarinfish genomic resource will likely serve as a high-quality outgroup to syngnathid fish, and furthermore for research on the genomic underpinnings of the evolution of novel pigmentation.

Keywords: mandarin dragonet; barcoded linked-reads; synthetic long-reads; 10x Genomics; Illumina sequencing; syngnathids

Introduction

The mandarinfish *Synchiropus splendidus* (Herre 1927), also known as a mandarin dragonet, is an intensely colored west Pacific species (Figure 1) that is popular in aquarium trade, even though captive breeding is difficult and most specimens therefore are wild-caught (Sadovy et al. 2001). Aside from the commercial interest in the species, the evolution of such vibrant coloration has captured the eye and attention of biologists. Studies of mandarinfish coloration have been the source for the discovery of two types of chromatophores. While blue colors in animals are generally structural, the mandarinfish has cyano-erythrophores producing a strong blue color (Goda and Fujii 1995). More recently a dichromatic chromatophore producing blue and red was described in mandarinfish (Goda et al. 2010).

The phylogenetic and taxonomic placement of the dragonet families Draconettidae and Callionymidae—to which the mandarinfish belongs—has long been subject for debate. Morphology-based assessment has traditionally placed dragonets with a phylogenetic affinity to clingfishes (within order Gobiesociformes; e.g., Springer and Johnson 2004) or independently comprising the order Callionymiformes (e.g., Nelson et al. 2016). Early molecular studies based on a few, predominantly mitochondrial sequence markers also resulted in wildly different phylogenetic placements of dragonets (Chen et al. 2003; Smith and Wheeler 2006). However, molecular studies of larger genetic material have demonstrated that dragonets form a monophyletic clade with the morphologically distant families Syngnathidae, Solenostomidae, Aulostomidae, Fistulariidae, Centriscidae, Dactylopteridae, Mullidae, and Pegasidae, albeit with varying internal arrangements (Kawahara et al. 2008; Betancur-R et al. 2013, 2017; Near et al. 2013; Sanciangco et al. 2016; Longo et al. 2017; Alfaro et al. 2018; Hughes et al. 2018).

The clade composed of the aforementioned families makes up order Syngnathiformes (sensu Betancur-R et al. 2017, Hughes et al. 2018), in which genome assemblies have been available for suborders Syngnathoidei, Dactylopteroidae, and Mulloidae (sensu Betancur-R et al. 2017; cf. Figure 2), and the first genome assembly for suborder Callionymoidei was only recently published (Winter et al. 2020). Whereas several high-quality assemblies within family Syngnathidae, i.e., seahorses, pipefishes, and seadragons have been published in the last few years (Lin et al. 2016; Small et al. 2016; Vertebrate Genomes Project 2019; Roth et al. 2020; Zhang et al. 2020; Li et al. 2021), nonsyngnathid syngnathiform species...
been collected close to the Kapal and Kahyangan Islands north of Java, Indonesia, at approximately 6°02'10"S 106°44'05"E, through a commercial aquarium fish trader (Blue Zoo Aquatics, Gardena, CA, USA). Upon receipt of the fish on March 20, 2019, we euthanized it using 0.0168% tricaine methanesulfonate (MS-222), immediately dissected it, and flash froze tissues separately in liquid nitrogen, followed by storage at –80°C. All vertebrate handling and euthanization followed approved, IACUC-regulated protocols (University of Oregon #17-05).

We extracted high molecular weight DNA using a prototype Nanohind Tissue Big DNA Kit (Circulomics, Baltimore, MD, USA), modified as follows: We mechanically homogenized 15 mg liver tissue within a tissueTUBE TT1 (Covaris, Woburn, MA, USA) by chilling the tissue for ~7 s in liquid nitrogen, then immediately crushing it with a pre-chilled steel hammer and anvil. The homogenate was then used for extraction according to the manufacturer’s instructions, with elution in 100 μl elution buffer EB. The DNA was quantified using a Qubit dsDNA BR Assay on a Qubit 2.0 Fluorometer (Thermo Fisher, Waltham, MA, USA), purity was checked on a Nanodrop 2000 (Thermo Fisher, Waltham, MA, USA), and DNA size distribution was determined with a HS Large Fragment 50Kb Kit on a Fragment Analyzer (Agilent, Santa Clara, CA, USA).

We ran 3.5 μg genomic DNA on a BluePippin (Sage Science, Beverly, MA, USA), collecting fragments ≥45 Kbp, which were used to construct a whole-genome linked-reads library with a Chromium Genome Reagent Kit (10x Genomics, Pleasanton, CA, USA) following the manufacturer’s instructions. The synthetic long-read library was checked with an HS NGS Fragment Kit (1–6000 bp) on a Fragment Analyzer (Agilent, Santa Clara, CA, USA), and then loaded on half a lane on a HiSeq 4000 (Illumina, San Diego, CA, USA) in the University of Oregon Genomics & Cell Characterization Core Facility (GC3F) which produced 152 million 150-bp, paired-end reads.

 Genome assembly and assessment

In order to assess the raw data and make a preliminary estimations of the genome size, level of heterozygosity, and read error rate, we trimmed all reads with Trimmomatic v. 0.36 (Bolger et al. 2014) using arguments LEADING: 3 TRAILING: 3 SLIDINGWINDOW: 4:15 MINLEN: 36. We also ran kmer analyses at kmer sizes 19–31 bp with Jellyfish v. 1.1.11 (Marçais and Kingsford 2011) on the remaining 125,818,427 read pairs. We then used the generated histo files for genome profiling with Genomescope v. 1 (Vurture et al. 2017) at kmer size = 19, 25, and 31 bp.

For the assembly, we followed the recommendations from 10x Genomics and used raw (nontrimmed) reads as input to Supernova v. 2.0.1 (Weisenfeld et al. 2017) because the assembly pipeline addresses trimming needs and no advantage has been demonstrated by trimming reads ahead of assembly (10x Genomics 2019). We ran the genome assembly with all 152,197,312 raw read pairs on a single core of Talapas, University of Oregon’s high performance computing environment, with 28 CPU and 114 Mb RAM for 47 h. The result of this assembly was two pseudohaplotypes. Supernova is known to produce duplicate scaffolds (Ozerov et al. 2020), and among the 10,652 scaffolds (referred to as contigs by 10x Genomics), 1441 were identified as duplicates and therefore removed with seqkit rmdup (Zou et al. 2016). One scaffold was removed because it consisted of Ns only. We also detected seven instances where Supernova failed to remove one of two Illumina adaptor sequences at the end of a scaffold (one instance) or within scaffolds between true sequence and runs of Ns (six instances). While contamination of adaptor sequence usually indicates a risk of mis-assembly, because reads

**Materials and methods**

Sample acquisition, tissue collection, DNA extraction, and sequencing

We purchased an adult male mandarinfish *S. splendidus*, that had been collected close to the Kapal and Kahyangan Islands north of...
may have been mistakenly aligned specifically based on matching adaptor sequence, this risk should be low based on the location of those adaptor sequence remnants and the DNA molecule-specific barcoding.

We evaluated the de-duplicated pseudohaplotype 1 of the assembly using Quast v. 4.6.0 (Gurevich et al. 2013), and assessed assembly completeness by searching for near-universal single-copy orthologous genes in the ray-finned fish (actinopterygii_odb9) and vertebrate (vertebrata_odb10) ortholog sets with BUSCO v. 3.0.2 (Waterhouse et al. 2018).

**Repeat content analyses**

We ran RepeatModeler v. 10.11 (Smit and Hubley 2014) using the NCBI engine and combined the custom repeat library with publicly available fish repeats (RepeatMasker queryRepeatDatabase.pl –species Teleostei), which we used with RepeatMasker v. 4.0.9 (Smit et al. 2014) with databases Dfam_3.0, RepBase-20170127 and arguments -nomat -xsmall.

**Results and discussion**

Using the prototype Nanobind Tissue Big DNA Kit, the yield was 11.69 μg DNA extracted from 15 mg liver tissue, with absorbance ratios at 260/230 nm of 2.13, and at 260/280 nm 1.83. The DNA produced was of high molecular weight, with a mode size of 50,933 bp. The size distribution contained 51.6% of the DNA among fragments ≥40 Kbp, 37.6%, ≥50 Kbp, 22.5% ≥40 Kbp, and 9.8% ≥75 Kbp, which allowed for size selection of ≥45 Kbp.

**Genome assembly and repeat content**

The preliminary kmer analyses with Genomescope estimated a genome size of 481–492 Mbp a heterozygosity level of 1.20%–1.31% (Table 1). The final assembly had an effective coverage of 59× (raw coverage of 81×), was highly contiguous, and comprised 483 Mbp, with N50 > 12 Mbp and L50 of 14 scaffolds (Table 2).

The assembly completeness was relatively high as supported by the finding that only 2.9% of 4584 Actinopterygi preassembly completeness by searching for near-universal single-copy orthologous genes in the ray-finned fish (actinopterygii_odb9) and vertebrate (vertebrata_odb10) ortholog sets with BUSCO v. 3.0.2 (Waterhouse et al. 2018).

**Table 1** Estimations of nuclear genome size and heterozygosity for the mandarinfish Synchiropus splendidus, based on kmer analyses in Genomescope of 126 million trimmed read pairs

| Kmer length | Genome size (Mbp) | Heterozygosity (%) |
|-------------|------------------|-------------------|
| 19          | 481              | 1.31              |
| 25          | 488              | 1.26              |
| 31          | 492              | 1.20              |

not recovered and 4.4% were fragmented. The remaining 92.6% of BUSCO genes were complete in our assembly, with similar figures for the Vertebrata gene set (Table 3). Total repeat content of the genome was 17.6%, comprising predominantly DNA elements (5.87%), LINEs (2.47%), and simple repeats (1.39%; Table 4). These data are comparable to other syngnathiform assemblies (Lin et al. 2016, 2017; Small et al. 2016, Roth et al. 2020, Zhang et al. 2020), where total repeat content (albeit not calculated identically between studies) ranges between 11.5% and 68.5% of the assembly size. The repeat content largely determines assembly size (linear regression: b = 7.52, t19 = 5.21, r² = 0.63, P < 0.0001). A phylogenetic signal of repeat content exists because species of the subfamily Serpaecephalidae (Syngnathoidei: Syngnathidae; n = 4 assemblies) have a higher ratio of repeat:nonrepeat content (1.00–2.17) compared to species within the other syngnathid subfamily Syngnathinae (0.18–0.49; n = 7), other families within Syngnathoidei (Centriscidae and Fistulariidae; 0.13–0.30; n = 3), and representatives of suborders Mulloidei (0.20; n = 1), Dactylopteriodae (0.32; n = 1), and Callinymoidei (0.21–0.37; n = 2; this study).

Since the input DNA for the synthetic long-read library was primarily long fragments (≥45 Kbp), the 16,430 bp mitochondrion (Song et al. 2014) was not assembled. The absence of the mtDNA genome was confirmed by BLAST for cytochrome b, COI, ND1, and ND4, which resulted in short (27–236 bp) best hits at low similarity (pairwise identity 75%–93%, e-values at 10⁻¹–10⁻⁶⁰).

**Synthetic long-read sequencing**

The development of high-throughput sequencing continues to be rapid, with decreasing user costs (Goodwin et al. 2016). Long-read
Table 2 Summary statistics for the mandarinfish Synchiropus splendidus nuclear genome assembly fSynSpl_1.0

| Assembly statistic | fSynSpl_1.0 |
|-------------------|-------------|
| Number of scaffolds | 9,210 |
| Total length (Mbp) | 482.93 |
| Largest scaffold (Mbp) | 29.25 |
| N50 (Mbp) | 12.19 |
| N75 (Mbp) | 5.85 |
| L50 | 14 |
| L75 | 27 |
| GC content (%) | 43.79 |
| Number of Ns per Kbp | 36.21 |

Table 3 BUSCO assessment of the mandarinfish Synchiropus splendidus nuclear genome assembly (fSynSpl_1.0) completeness through searching for single-copy orthologs from the Vertebrata and Actinopterygii datasets

| BUSCOs | Vertebrata | Actinopterygii |
|--------|------------|----------------|
| Complete | 3,074 (91.7%) | 4,247 (92.6%) |
| single copy | 3,045 (90.8%) | 4,127 (90.0%) |
| duplicated | 29 (0.9%) | 120 (2.6%) |
| Fragmented | 145 (4.3%) | 202 (4.4%) |
| Missing | 135 (4.0%) | 135 (2.9%) |
| Total | 3,354 | 4,584 |

Table 4 Repeat contents of the mandarinfish Synchiropus splendidus nuclear genome assembly (fSynSpl_1.0), determined with RepeatMasker, using a custom assembly-specific repeat library and publicly available repeats in Teleostei

| Repeat type | N elements | Repeat length (bp) | Proportion of assembly (%) |
|-------------|------------|--------------------|---------------------------|
| SINEs | 3,898 | 76,558,225 | 15.85 |
| ALU1s | 0 | 376,741 | 0.08 |
| MIRs | 180 | 4,098 | 0.00 |
| LINEs | 57,871 | 11,932,669 | 2.47 |
| LINE1 | 2,822 | 772,252 | 0.16 |
| LINE2 | 5,219 | 884,904 | 0.18 |
| L3/CR1 | 0 | 0 | 0.00 |
| LTR | 22,877 | 5,559,049 | 1.15 |
| ERVL | 139 | 200,533 | 0.04 |
| ERVL-MaLRs | 0 | 0 | 0.00 |
| ERV classI | 1,630 | 501,062 | 0.10 |
| ERV classII | 110 | 41,130 | 0.01 |
| DNA elements | 151,460 | 28,367,333 | 5.87 |
| hAT-Charlie | 30,384 | 5,308,847 | 1.0 |
| TcMar-Tigger | 10,929 | 3,438,056 | 0.71 |
| Unclassified | 199,626 | 30,322,433 | 6.28 |
| Satellites | 1,161 | 179,831 | 0.04 |
| Simple repeats | 142,303 | 6,729,931 | 1.39 |
| Low complexity | 16,696 | 849,897 | 0.18 |
camouflage through elaborate appendages (e.g., Ahnesjö and Craig 2011; Lin et al. 2016; Small et al. 2016; Li et al. 2021). Similar to family Syngnathidae, other families within suborder Syngnathoidei display elongated snouts (Solenostomidae, Fistulariidae, Aulostomidae, and Centriscidae; Figure 2) and body plans (Solenostomidae, Fistulariidae, and Aulostomidae). The mandarinfish will therefore also represent a beautiful (and useful) outgroup to suborder Syngnathoidei.

Data availability

The data underlying this article can be accessed with accession number JAFFPX000000000 from the GenBank Assembly Database at https://www.ncbi.nlm.nih.gov/assembly, and with accession number SRR12233697 from the GenBank Sequence Read Archive at https://www.ncbi.nlm.nih.gov/sra, both connected to accession number PRJNA646594 in the GenBank BioProject Database at https://www.ncbi.nlm.nih.gov/bioproject.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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