GENOME SEQUENCING

The Complete Genome Sequence of *Protonotaria citrea,* the Prothonotary Warbler

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Biodiversity Genomes

The Prothonotary Warbler (*Protonotaria citrea*) is a nearctic-neotropical migratory songbird that breeds in forested swamps and riparian areas in the eastern-central United States and southern Ontario. It is the sole eastern North American wood-warbler that nests in cavities, the only species in the genus *Protonotaria*, and is one of the few hole-nesting hosts of the Brown-headed Cowbird (*Molothrus ater*), an obligate brood parasite. We present the whole genome sequence of this wood-warbler species. Illumina sequencing was performed on a genetic sample from a single female individual. The reads were assembled using a de novo method followed by a finishing step. The raw and assembled data is publicly available via Genbank: Sequence Read Archive (SRR19579445) and Assembly (JAOYNP000000000).

Introduction

The Prothonotary Warbler (*Protonotaria citrea*), from the family Parulidae (New World Warblers), is the only species in the genus *Protonotaria* and measures 13 cm in length, weighs 12.5 g, and has an average wingspan of 22 cm. The plumage is sexually dichromatic: adult males have a bright orange-yellow head whereas females and immatures are duller and have a yellowish-green head. Both sexes have an olive-coloured back with blue-grey wings and tail, yellow underparts, a relatively long pointed bill, and black legs (Dunne 2006).

The Prothonotary Warbler is a nearctic-neotropical migratory songbird that breeds in bottomland forests and riparian areas of temperate regions of east-central North America from southern Ontario to the Gulf Coast and east to the southern and central Atlantic coast (Petit 2020). It consumes a mix of aquatic and terrestrial invertebrate prey (Dodson, Moy, and Bulluck 2016; Petit 2020). It is the only cavity nesting wood-warbler in eastern North America and readily adapts to using human-made structures such as nest boxes, making it accessible for studies of its breeding ecology. Its global population has declined over the last four decades; though populations in some USA regions are stable (Sauer et al. 2019), it is listed as endangered in Canada (Petit 2020). In its wintering range, >90% of the global population of prothonotary warblers is thought to migrate to a relatively small region in northern Columbia, that is roughly 20% of the size of their breeding range in the eastern USA and Canada (Tonra et al. 2019). The species is parasitized by the brown-headed cowbird (*Molothrus ater*) throughout much of its range, serving as one of the

few cavity nesting hosts of this generalist obligate brood parasite. Conspecific brood parasitism has also been documented in this species (Tucker et al. 2016). Recent evidence demonstrated the susceptibility of the Prothonotary Warbler's breeding phenology to climate change, with the date of the first laid egg advancing earlier into the spring with increasing spring temperatures (Hoover and Schelsky 2020).

Methods

We collected brachial blood from a 2 year old adult breeding female at the Cache River watershed, in southern Illinois, USA, in spring 2021 and stored it in 95% ethanol at room temperature. DNA extraction was performed in fall 2021 using the Qiagen DNAeasy genomic extraction kit following the standard protocol for tissues. A paired-end sequencing library was constructed using the Illumina TruSeq kit according to the manufacturer's instructions. The library was sequenced on an Illumina Hi-Seq platform in paired-end, 2 × 150 bp format. The resulting fastq files were trimmed of adapter/primer sequence and low-quality regions with Trimmomatic v0.33 (Bolger, Lohse, and Usadel 2014). The trimmed sequence was assembled by SPAdes v2.5 (Bankevich et al. 2012) followed by a finishing step using Zanfona (Kieras, O'Neill, and Pirro 2021).

Results

The genome assembly yielded a total sequence length of 1,048,280,936 bp over 23,559 scaffolds, and an N50 of 71.442 MB.

Data availability

Raw and assembled data is publicly available via GenBank.

RAW GENOME DATA

https://www.ncbi.nlm.nih.gov/sra/?term=SRR19579445

ASSEMBLED GENOME

https://www.ncbi.nlm.nih.gov/nuccore/JAOYNP000000000

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Biodiversity Genomes 2

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Biodiversity Genomes 3