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## Data Article

# Transcriptome of *Xenopus andrei*, an octoploid frog, during embryonic development



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## ABSTRACT

Although polyploidy occurs throughout the fish and amphibian lineages, the *Xenopus* genus exhibits a high incidence of polyploidy, with 25 out of the 26 known species being polyploid. However, transcriptomic information is currently available for only one of these species, the tetraploid *Xenopus laevis*. *Xenopus andrei*, an octoploid species within the *Xenopus* genus, offers an opportunity for assessing a novel polyploid transcriptome during vertebrate development. RNA-Seq data was generated at nine different developmental stages ranging from unfertilized eggs through swimming tadpole stages and raw FASTQ files were deposited in the NCBI SRA database (accession number SRP134281). Additionally, RNA-seq data from all nine stages were pooled to create a *de novo* assembly of the transcriptome using Trinity and has been deposited in the NCBI GEO database (accession number GSE111639). To our knowledge, this represents the first published assembly of an octoploid vertebrate transcriptome. In total, 849 Mb were assembled, which led to the identification of 1,650,048 transcripts in the assembly with a contig N50 of 630 bases. This RNA-Seq and transcriptome data will be valuable for comparing polyploid transcriptomes across *Xenopus* species, as well as understanding evolutionary implications of whole-genome duplication and polyploidy in vertebrates.

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## Specifications Table

<b>Subject area</b>	Biology
<b>More specific subject area</b>	Developmental Biology and Transcriptomics
<b>Type of data</b>	Transcriptome assembly, raw sequences
<b>How data was acquired</b>	High-throughput sequencing carried out by Oklahoma Medical Research Facility (OMRF) Clinical Genomics Center using an Illumina HiSeq. 3000.
<b>Data format</b>	Analyzed, raw
<b>Experimental factors</b>	One pair of adult frogs was mated to produce embryos sampled in groups of five individual embryos at nine different developmental time points.
<b>Experimental features</b>	Total RNA was extracted from samples of five pooled embryos. Library preparation and sequencing was performed by OMRF. Analysis was carried out in-house.
<b>Data source location</b>	Adult <i>X. andrei</i> were purchased from Xenopus Express Inc. (Brooksville, FL, USA).
<b>Data accessibility</b>	Raw FASTQ files were deposited on NCBI SRA database with accession number SRP134281. Transcriptome fasta file was deposited on NCBI GEO database with accession number GSE111639. All data can be accessed at the following link <a href="https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE111639">https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE111639</a> .

## Value of the data

- To our knowledge, this is the first published *de novo* transcriptome assembly of an octoploid vertebrate species.
- *Xenopus andrei* presents a novel model system for studying transcriptional regulation of an octoploid genome during vertebrate development.
- Comparisons between *X. tropicalis*, *X. laevis*, and *X. andrei* transcriptomes will provide insight into how multiple genome duplications have affected gene expression.

## 1. Data

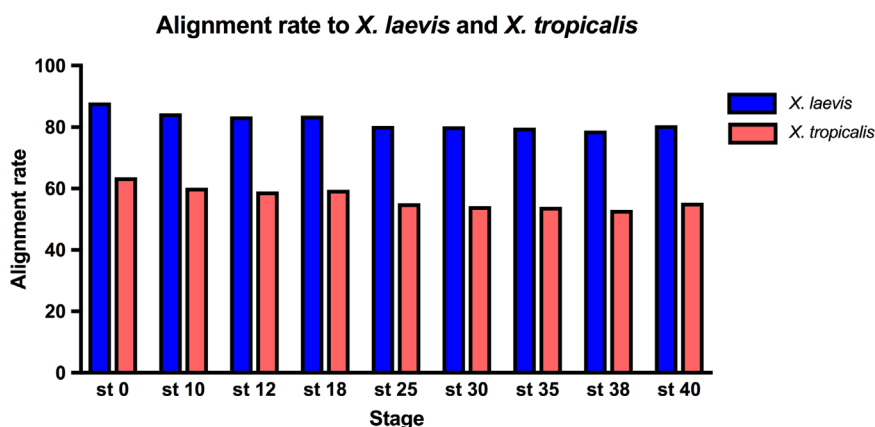
Although comparatively less prevalent than in plants, polyploidy in animals occurs throughout the amphibian and fish lineages as well as in mammalian tissues including heart, placenta, and in pathogenic conditions such as wound healing and cancer [1,2]. The publication of the completed tetraploid *Xenopus laevis* genome [3] as well as associated transcriptome data has presented the possibility of studying comparative gene expression in polyploid vertebrate animals. *Xenopus andrei* is an octoploid ( $2n = 72$ ) species [4] and provides the unique opportunity to compare transcriptomes among a range of ploidy levels in related frogs. We generated RNA-seq data at nine developmental time points and produced a draft assembly of the transcriptome. The data presented here can be accessed at SRA: SRP134281 for raw FASTQ files of all sequencing, and GEO: GSE111639 for assembly of the transcriptome.

Using Trinity [5], a *de novo* assembly of the transcriptome was produced by combining all of the sequence data generated from the nine samples in this experiment. Statistics of the assembly are summarized in Table 1.

A filtered transcriptome assembly was also generated using a minimum transcript per million (TPM) threshold of 2 to filter out lowly expressed transcripts in the initial *de novo* assembly. This is

**Table 1**Statistics of *X. andrei* transcriptome assembly.

Number of reads used in assembly	341,149,792
Number assembled bases	849,005,380
Number of assembled genes	1,023,069
Number of assembled transcripts	1,650,048
Assembly GC percent	41.15
Contig N50	631
Contig Ex90N50	1215
Average contig length	514.53



**Fig. 1.** Overall alignment rate to *X. laevis* and *X. tropicalis*. *X. andrei* RNA-Seq reads at each of the indicated stages were aligned to *X. laevis* and *X. tropicalis* reference genomes.

because many assembled transcripts lacked read support, meaning that paired-end reads did not concordantly align to the transcriptome. This has been observed by others [6,7] when using Trinity to assemble polyploid transcriptomes. The filtered assembly consists of 149,471 transcripts and 100,936 genes, representing 9.06% and 9.86% of the transcripts and genes in the initial *de novo* assembly, respectively. Data including TPM abundance measures, genes from the transcriptome assembly, and the filtered transcriptome assembly are available as supplementary files. While the tetraploid *Xenopus laevis* genome has approximately 46,000 genes [3], our filtered assembly contains approximately twice as many genes in octoploid *Xenopus andrei*.

To provide additional comparative data, *X. laevis* v9.2 and *X. tropicalis* v9.1 reference genomes were downloaded from Xenbase.org [8]. At each developmental stage, RNA-seq reads were aligned to both reference genomes. *X. andrei* reads align to the *X. laevis* reference on average at 82% and to the *X. tropicalis* reference on average at 57% (Fig. 1).

## 2. Experimental design, materials and methods

### 2.1. Animal usage

All animal care and use was approved by the College of William and Mary Institutional Animal Care and Use Committee (IACUC). Mating was induced by subcutaneous injection of human chorionic gonadotropin (hCG) as described by Sive et al. [9], with the female receiving 150 units and the male 100 units. Embryos were collected and dejellied in basic 2% cysteine for five minutes. After dejelling, embryos were washed three times in  $0.1 \times$  Marc's Modified Ringers (MMR) with 50  $\mu$ g/ml gentamicin. Embryos were then reared in  $0.1 \times$  MMR + gentamicin at room temperature (22 °C) until the

desired stage was reached. All staging was based on equivalent Nieuwkoop and Faber [10] *Xenopus laevis* stages.

## 2.2. Sample preparation and RNA extraction

At stages 0 (unfertilized egg), 10 (early gastrula), 12 (gastrula), 18 (neurula), 25 (tailbud), 30 (hatching), 35 (swimming tadpole), 38 (swimming tadpole), and 40 (swimming tadpole), groups of five embryos were flash frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ . RNA was extracted by homogenizing embryos in 600  $\mu\text{l}$  TRI reagent (Ambion) then dividing the homogenate equally between two tubes. Extraction was then carried out using 60  $\mu\text{l}$  of chloroform. This partially separated mixture was spun down in a Phase Lock Gel Heavy tube (QuantaBio) and RNA was then purified using the MagMAX™-96 for Microarrays (Thermo Fisher Scientific) kit according to the manufacturer's instructions. RNA quality and yield was assessed by spectrophotometry (NanoDrop<sup>®</sup>) and agarose gel electrophoresis.

## 2.3. RNA-seq

Total RNA samples were shipped to OMRF where libraries were generated using the Illumina TruSeq Stranded rRNA depletion Library Prep kit. Library quality was confirmed with Kapa qPCR and Agilent TapeStation prior to paired-end sequencing with an Illumina HiSeq 3000.

## 2.4. Transcriptome analysis and de novo assembly

Raw reads were first analyzed by FastQC [11] for per base sequence quality. Reads were then aligned using Hisat2 with relaxed parameters [12] to the *Xenopus laevis* reference genome, and alignment files were used to infer read strandedness using RSeQC (Version 2.6.4) [13]. Trimmomatic (Version 0.36) [14] was used to trim Illumina TruSeq paired-end adaptors and bases with phred quality scores less than 5. *De novo* transcriptome assembly was performed using Trinity (Version 2.4.0) [5] with all reads from the nine samples run on a high performance computing cluster with settings to normalize read coverage to  $50\times$  and minimum of 2 k-mers to be assembled by the Inchworm module. Raw reads were then mapped back to the *de novo* assembled transcriptome using Bowtie2 (Version 2.3.4.1) [15] with default parameters to assess assembly quality and RNA-Seq read representation (Supplementary Fig. 1). Transcript isoforms were collapsed into 'genes' to construct a genome-like reference using the Trinity SuperTranscripts module. Transcript abundance quantification was performed with Salmon (Version 0.8.2) [16] and transcripts were then filtered using a minimum TPM threshold of 2 using the Trinity transcript filtering module. Detailed commands, scripts, and parameters used in this pipeline are available in the supplementary files.

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## Transparency document. Supplementary material

Transparency data associated with this article can be found in the online version at <https://doi.org/10.1016/j.dib.2018.05.017>.

## Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <https://doi.org/10.1016/j.dib.2018.05.017>.

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