Title

De novo assembly and annotation of the eastern fence lizard (*Sceloporus undulatus*) transcriptome

Authors

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Abstract

Background: The eastern fence lizard (*Sceloporus undulatus*) has been a model species for ecological and evolutionary research. Genomic and transcriptomic resources for this species would promote investigation of genetic mechanisms that underpin plastic responses to environmental stress, such as climate warming. Moreover, such resources would aid comparative studies of complex traits at the molecular level, such as the transition from oviparous to viviparous reproduction, which happened at least four times within *Sceloporus.*

Findings: A *de novo* transcriptome assembly for *Sceloporus undulatus*, Sund_v1.0, was generated using over 179 million Illumina reads obtained from three tissues (whole brain, skeletal muscle, and embryo) as well as previously reported liver sequences. The Sund_v1.0 assembly had an average contig length of 782 nucleotides and an E90N50 statistic of 2,550 nucleotides. Comparing *S. undulatus* transcripts with the benchmarking universal single-copy orthologs (BUSCO) for tetrapod species yielded 97.2% gene representation. A total of 13,422 protein-coding orthologs were identified in comparison to the genome of the green anole lizard, *Anolis carolinensis*, which is the closest related species with genomic data available.

Conclusions: The multi-tissue transcriptome of *S. undulatus* is the first for a member of the family Phrynosomatidae, offering an important resource to advance studies of adaptation in this species and genomic research in reptiles.

Keywords: *Sceloporus undulatus*, eastern fence lizard, Phrynosomatidae, RNA-Seq, transcriptome, assembly, annotation.

Data description

Context

Eastern fence lizards belong to a clade, the *Sceloporus undulatus* complex, which spans much of the United States and northern Mexico [1]. Because these lizards occupy a wide range of habitats and environmental conditions, *S. undulatus* has been a good model for studies of organismal ecology [2–4], population dynamics [5,6], and local adaptation [7–9]. In particular, embryos of oviparous *S. undulatus* are subjected to oscillations in nest temperature that are known to affect development [10–13], which could potentially be compensated for by egg-laying behavior in adult females [14,15]. Embryos of this species have a threshold for thermal tolerance at high temperatures and are thus susceptible to potential warming due to climate change [11,16]. Other species in the genus *Sceloporus* evolved either prolonged or complete retention of eggs in response to cold environments. In fact, viviparity has evolved in association with cooler climates at least four times within *Sceloporus* and another two times in the Phrynosomatidae [17], along with numerous physiological and morphological adaptations expected to accompany this convergent trait. Specifically, a congeneric species (*S. jarrovi*) displays specialized features in the placenta, although relying mostly on yolk nutrients during development (lecithotrophy) [18,19]. A comparative study of gene expression among *Sceloporus* species that

67 differ in parity mode (oviparous vs. viviparous) would allow testing for
68 convergence with the pregnant-specific gene expression profile described for
69 viviparous lizard species from family Scincidae, whose development depend
70 mostly on nutrients from the mother (matrotrophy) [20]. To begin to identify the
71 genes for molecular studies of these processes, we have sequenced and
72 annotated a *de novo* multi-tissue transcriptome for *Sceloporus undulatus*.
73

74 ***Methods***

75 *a) Sampling*

76 Gravid females of *Sceloporus undulatus* were collected in Edgefield County,
77 South Carolina (33.7°N, 82.0°W) and transported to Arizona State University.
78 These animals were maintained under conditions described in previous
79 publications [21,22], which were approved by the Institutional Animal Care and
80 Use Committee (Protocol #14-1338R). Approximately two days after laying eggs,
81 each lizard was euthanized by injecting sodium pentobarbital into the coelomic
82 cavity. The whole brain and skeletal muscle samples were removed and placed
83 in RNA-lysis buffer (mirVana miRNA Isolation Kit, Ambion) and flash-frozen.
84 Additionally, three early-stage embryos from each clutch were dissected, pooled
85 together, and homogenized in RNA-lysis buffer using the same protocol.

86 *b) Sequencing*

87 Total RNA was isolated from three tissue samples (whole brain, skeletal muscle
88 and embryos) from each individual using the mirVana miRNA Isolation Kit

89 (Ambion) protocol. Samples were checked for quality on a 2100 Bioanalyzer
90 (Agilent). One sample from each tissue was selected for RNA-Seq based on the
91 highest RIN, with a cutoff of 8.0. For each selected sample, 3 µg of total RNA
92 was sent to the University of Arizona Genetics Core (Tucson, AZ) for library
93 preparation and with TruSeq v3 chemistry for a standard insert size. RNA
94 samples were multiplexed and sequenced using an Illumina HiSEq 2000 to
95 generate 100-bp paired-end reads. Publicly available raw Illumina RNA-Seq
96 reads from *S. undulatus* liver [23] were added to our dataset. After removing
97 adaptors, raw reads from the four tissues were evaluated using FastQC
98 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/, v-0.11.5) and
99 trimmed using Trimmomatic (v-0.32, [24]), filtering for quality score (≥Q20) and
100 using HEADCROP:9 to minimize nucleotide bias. This procedure yielded
101 179,374,469 quality-filtered reads. **Table 1** summarizes read-pair counts from
102 whole brain, skeletal muscle, whole embryos, and liver.

103 *c) Assembly and annotation*

104 All trimmed reads were pooled and assembled *de novo* using Trinity (v-2.2.0,
105 default k-mer size of 25 [25]), which is an efficient transcriptome assembly
106 method for non-model species without a reference genome available [26]. The
107 most comprehensive transcriptome, obtained using reads from four tissues,
108 consists of 547,370 contigs with an average length of 781.5 nucleotides (**Table**
109 **2**)—shorter than other assemblies because of the range of contig sizes that
110 varied among datasets (1, 3 and 4 tissues; **Table S1, Fig. S1**). The N50 of the
111 most highly expressed transcripts that represent 90% of the total normalized

112 expression data (E90N50) was highest in the assembly based on four tissues,
113 hereafter referred to as Sund_v1.0 (**Table 2**). A subset of contigs containing the
114 longest open reading frames (ORFs), representing 123,323 transcripts, was
115 extracted from the Sund_v1.0 assembly using TransDecoder (v-3.0.0,
116 http://transdecoder.github.io) with homology searches against the databases
117 UniProtKB/SwissProt [27] and PFAM [28]. The transcriptome obtained was
118 annotated using Trinotate (v-3.0, http://trinotate.github.io), which involved
119 searching against multiple databases (as UniProtKB/SwissProt, PFAM, signalP,
120 GO) to identify sequence homology and protein domains, as well as to predict
121 signaling peptides. **Table 3** summarizes the annotation results.

122

123 ***Data validation and quality control***

124 Trimmed reads were aligned back to the assembled contigs using Bowtie2 (v-
125 2.2.6 [29]). From the 176,086,787 reads that aligned, 97% represented proper
126 pairs (**Table S2**), indicating good read representation in the Sund_v1.0
127 assembly. To assess quality and completeness of the assemblies, we first
128 compared the Sund_v1.0 transcripts with the BUSCO profile for Tetrapoda
129 (BUSCO v-2.0 [30]), which has BLAST+ (v-2.2.31 [31]) and HMMER (v-3.1b2
130 [32]) as dependencies. This procedure revealed that the Sund_v1.0 assembly
131 captured 97.1% of the expected orthologs, a result comparable to the 97.8%
132 obtained for *Anolis carolinensis* transcriptome using 14 tissues [33] (**Table 4**).
133 Next, nucleotide sequences of Sund_v1.0 transcripts with the longest ORFs were

134 compared to the protein set of *Anolis carolinensis* (AnoCar2.0, Ensembl) using
135 BLASTX (evalue=1e-20, max_target_seqs=1). This comparison showed that
136 11,223 transcripts of *S. undulatus* have nearly full-length (>80%) alignment
137 coverage with *A. carolinensis* proteins (**Table S3**). Predicted proteins of *S.*
138 *undulatus* were also used to identify 13,422 one-to-one orthologs with proteins of
139 *A. carolinensis* through reciprocal BLAST (evalue=1e-6, max_target_seqs=1).

140

141 **Availability of supporting data**

142 Novel RNA-Seq data for *Sceloporus undulatus* samples are available under the
143 NCBI accession identifiers listed in Table 1, and are associated with BioProject
144 PRJNA371829. RNA-Seq data for the liver sample [23] were downloaded from
145 NCBI from BioProject PRJNA183121, Run SRR629640. Datasets referring to the
146 assembled and annotated transcriptome are available for download at Dryad.

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List of abbreviations

BLAST: Basic local alignment search tool; BUSCO: Benchmarking Universal Single-Copy Orthologs; GO: Gene Ontology; National Center for Biotechnology Information NCBI; ORFs: open reading frames; RIN: RNA integrity number.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

MBG performed transcript assemblies and bioinformatics analyses; JJR performed transcript assemblies and bioinformatics analyses; MAT, OL, MJA and KK conceived the study; MAT and KK supervised bioinformatics analyses; MJA and OL provided samples. MBG drafted the manuscript, with edits from MT, KK, and MJA. All authors read and approved the final version.

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267
268
269

270 **Figures and tables**

271

272 **Table 1** Number of pairs and accessions numbers for *Sceloporus undulatus*

273 sequence reads.

Tissue	Number of read pairs	Accession numbers
	This study	
Whole Brain	51,537,265	SAMN06312741
Embryo	49,112,293	SAMN06312742
Skeletal muscle	42,922,488	SAMN06312743
	McGaugh et al., 2015	
Liver	35,802,423	SRR629640
Total	179,374,469	_

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Table 2 Statistics for the *de novo* assembly of *Sceloporus undulatus* transcriptome (Sund_v1.0).

Assembly	**1 tissue** [23]	**3 tissues**	**4 tissues (Sund_v1.0)**
Total of Trinity transcript contigs	158,323	492,249	547,370
Total of Trinity 'genes'	138,031	422,687	467,658
GC%	43.8	42.9	42.8
Contig N50 (bp)	1,720	1,648	1,438
Contig E90N50 (bp)	2,254	2,640	2,550
Average contig length (bp)	833.0	822.4	781.5
Transcripts with the longest	86,630	212,172	217,756
ORFs	(54.7%)	(43.1%)	(39.8%)

277

278 The different assemblies used data from a previous study (1 tissue, liver [23]),
279 data from this study (3 tissues:: whole brain, skeletal muscle, embryos), and the
280 two datasets combined (4 tissues, or Sund_v1.0).

281

282

283 **Table 3** Annotation summary of *Sceloporus undulatus de novo* transcriptome

284 assembly (Sund_v1.0).

Annotation of the Sund_v1.0 assembly	
Annotated genes	467,658
Annotated transcript isoforms	547,370
Annotated isoforms/gene	1.17
Transcripts with Swiss-Prot annotation	(71,944)
Transcripts with PFAM annotation	51,018 (46,432)
Transcripts with KEGG annotation	65,694 (21,520)
Transcripts with GO annotation	73,936 (66,554)

285

286 Unique annotation numbers are indicated by parentheses.

287 **Table 4** BUSCO results for the transcriptomes of *Sceloporus undulatus* and

288 *Anolis carolinensis*.

	Sceloporus undulatus			***Anolis carolinensis***
	1 tissue	**3 tissues**	**4 tissues (Sund_v1.0)**	**14 tissues**
Complete genes	72.5%	91.7%	92.3%	96.7%
Duplicated genes	25%	43.8%	43.9%	37.9%
Fragmented genes	9.2%	4.8%	4.8%	1.1%
Missing genes	18.3%	3.5%	2.9%	2.2%
Reference	McGaugh et al, 2015 [23]	This study	This study	Eckalbar et al, 2013 [33]

289

290 *For S. undulatus*, the Sund_v1.0 assembly includes 4 tissues, specifically 3

291 tissues from this study (whole brain, skeletal muscle and embryos) and 1

292 previously reported tissue (liver [23]). For *A. carolinensis*, transcriptomes

293 included adrenal gland, brain, dewlap skin, embryos, and pooled samples, heart,

294 liver, lung, original tail, ovary, regenerating tail tip, regenerating tail base, and

295 skeletal muscle [18].

296

297 **Supporting information – Tables**

298

299 **Table S1** Contig length statistics for *Sceloporus undulatus de novo* assemblies.

	1 tissue	**3 tissues**	**4 tissues**
Minimum length	201.0	201.0	201.0
1st Quartile	266.0	266.0	266.0
Median	382.0	377.0	375.0
Mean	829.9	822.4	781.0
3rd Quartile	808.0	732.0	711.0
Maximum length	16,776.0	30,410.0	30,258.0

300

301 The Sund_v1.0 assembly includes 4 tissues, specifically 3 tissues sequenced in
302 this study (whole brain, skeletal muscle and embryos) and 1 previously reported
303 tissue (liver [23]).

304

305 **Table S2** Reads mapped to *Sceloporus undulatus de novo* Sund_v1.0 assembly.

Read classification	**Counts**	**Percentage of mapped reads**
Proper pairing	170,981,981	97.10%
Left read only	3,778,790	2.15%
Right read only	1,015,874	0.58%
Improper pairing	310,142	0.18%

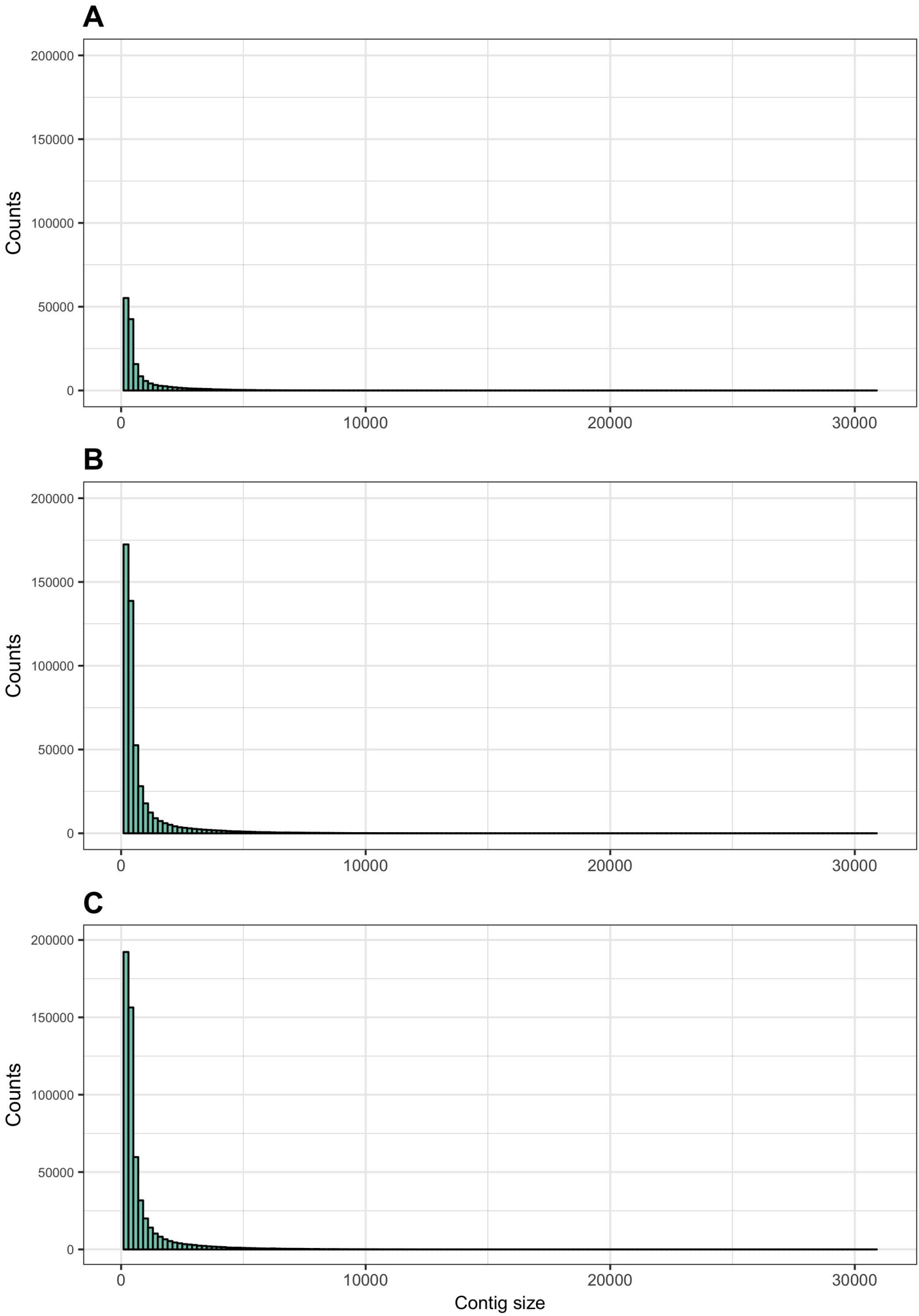
306 **Table S3** Representation of full-length reconstructed protein-coding genes in
307 *Sceloporus undulatus de novo* Sund_v1.0 transcriptome assembly, using the
308 protein set of *Anolis carolinensis* (AnoCar2.0, Ensembl) as a reference.

Alignment coverage	Counts	Cumulative counts
100%	9,874	9,874
90%	1,349	11,223
80%	799	12,022
70%	757	12,779
60%	725	13,504
50%	577	14,081
40%	463	14,544
30%	455	14,999
20%	358	15,357
10%	97	15,454

309

310 **Supporting information – Figure**

311 **Figure S1** Contig sizes for different *Sceloporus undulatus* assemblies.
312 Assemblies used (**A**) the previously published single tissue transcriptome (liver
313 [23]), (**B**) transcriptomes from the 3 tissues sequenced in this study (brain,
314 skeletal muscle and embryos), and (**C**) the combined data set of 4 tissues ([23]
315 and this study).


A
B
C
Counts
200000
150000
100000
50000
0
0
10000
20000
30000
Contig size