Animal Reproduction Science xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Sex-related gonadal gene expression differences in the Russian sturgeon (*Acipenser gueldenstaedtii*) grown in stable aquaculture conditions

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ARTICLE INFO

Keywords:
Acipenser
Gonad
Ovary
Testes
Transcriptome

ABSTRACT

The Russian sturgeon (*Acipenser gueldenstaedtii*) is a primitive freshwater fish and a source of caviar. In the present study, the gonadal transcriptomes of male and female Russian sturgeons grown in stable aquaculture conditions were analyzed. RNA sequencing of whole-gonad transcriptomes from pools of 4-year old fish (five females, four males), resulted in the identification of 28,170 unique transcripts. Of these, 16,191 could be annotated by similarity to gene sequences from other species. There were 392 transcripts that showed differential abundance by a factor of 20-fold or more between the sexes; 272 of these were annotated; of these, 175 and 97 were in greater abundance in ovaries and testes, respectively. Functional annotation and clustering of the genes with differential abundances of mRNA allowed for identification of several clusters. Thus, a group of transcriptional regulators and factors involved in cell division, especially septins, were in greater abundance in the ovaries; while a different set of transcription factors (including *sox6* and *sox30*) and a group of protein kinases were in greater abundance in the testes. The transcript abundances of nine highly abundant candidate transcripts, as well as of two additional genes previously known to be involved in reproduction, *cyp19* (*p450* aromatase) and *foxl2*, were assessed in the individual samples by qRT-PCR. Of these, five (including *cyp19* and *foxl2*) were in greater abundance in ovaries, while the abundance of *ighm1* mRNA was greater in testes. Phylogenetic analysis based on the *k1c18* keratin gene placed the sturgeon sequence nearest those of other primitive fish species, supporting the ancient origin of the sturgeon. In conclusion, this study details transcriptome differences between male and female sturgeon and identifies key genes that may contribute to sexual determination and differentiation.

1. Introduction

Acipenseridae (common name Sturgeon) are a primitive freshwater fish family of 27 species. With over 200 million years of existence of the sturgeon, these fish represent an important stage in vertebrate evolution (Birstein et al., 1997). In some mechanisms of hormonal control of growth and reproduction, these fish are more similar to mammals than to Osteichthyes fish (Yom Din et al., 2008), and are thus of interest in the study of the evolution of reproduction mechanisms in other vertebrates. The Russian sturgeon (*Acipenser gueldenstaedtii*) is of great economical value as a source of caviar. The over-fishing, pollution and habitat destruction of the

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https://doi.org/10.1016/j.anireprosci.2018.11.013
Received 16 August 2018; Received in revised form 6 November 2018; Accepted 30 November 2018

Russian sturgeon have dramatically decreased the Russian sturgeon populations in its natural habitats (Black Sea, Azov Sea and Caspian Sea), as well as significantly increased caviar value. This accelerated the development of Russian sturgeon aquaculture for caviar production, not only proximal to the natural habitats of the Russian sturgeon but also in other places such as Israel (Hurvitz et al., 2007; Degani et al., 2017).

In their natural habitat (Black Sea), Russian sturgeons reach sexual maturity at 8–13 years of age for males and 10–16 years for females (Hochleithner and Gessner, 1999). With aquaculture conditions, however, sturgeons usually mature at an earlier age: 3–4 years for males and 6–8 years for females (Doroshov et al., 1997; Hurvitz et al., 2005, 2007; Degani et al., 2017). Aspects of hormonal control of reproduction and growth of the Russian surgeon grown in northern Israel (Dan Fish Farm) have adapted to stable water temperatures (18 ± 6 °C all year round), indicating that culturing environments affect sturgeon physiology (Hurvitz et al., 2007; Degani et al., 2017). Considerable attention has been paid to oogenesis (caviar development) due to the economic value of the eggs. Oogenesis in the Russian sturgeon is controlled by FSH and LH and involves signaling by GH, IGF-1 and IGF-1R (Hurvitz et al., 2005, 2007; Yom Din et al., 2008, 2016; Degani et al., 2017).

Sex determination and sexual differentiation of fish are important for aquaculture (reviewed by (Tzchori et al., 2004a; Brykov, 2014)). These can be affected by genetics, environment or both (Kushnirov and Degani, 1991; Devlin and Nagahama, 2002). In the sturgeon, sex identification based on external morphological variation among males and females is difficult; therefore, there have been efforts to establish different methods for sex identification (Vecsei et al., 2003); e.g. ultrasonics (Wildhaber et al., 2005) or endoscopy (Hurvitz et al., 2007). Some Acipenseriformes are tetraploid species with 250 ± 8 chromosomes (Fontana, 1994). Although the sex chromosomes have not been identified, genetic sex determination suggests that in the sturgeon the ZZ genotype leads to development of males and the ZW, of females (Eenennaam and Doroshov, 1998; Van Eenennaam et al., 1999).

Transcriptome studies of sexual variation of gene expression have been conducted in the Russian sturgeon (Hagihara et al., 2014; Chen et al., 2016) and in other sturgeon species (Jin et al., 2015; Wang et al., 2017; Burcea et al., 2018; Chen et al., 2018). Thus, examination of the gonadal transcriptome in male and female fish at three developmental stages (1, 3 and 5 years old) (Chen et al., 2016) suggested that expression of the *foxl2* gene is responsible for ovary development, and *Sox9* gene for testis development. Hagihara et al. (2014) focused on differentially expressed genes in undifferentiated gonads of juvenile fish. Thus, increased abundances of *foxl2, hsd17b1* and *cyp19a1a* mRNA suggested differentiation into females, while greater transcript numbers of *gsdf* indicated differentiation into males. Transcriptomic studies of sex determination and differentiation were also conducted in other sturgeon species, e.g. *A. schrenckii* (Jin et al., 2015), *A. ruthenus* (Wang et al., 2017; Chen et al., 2018) and *A. stellatus* (Burcea et al., 2018). Although these studies identified additional candidate genes, the findings generally led to the conclusion that there are greater abundances of *dmrt1* mRNA in males and of *foxl2* and *cyp19a1* mRNA in females. Some of these findings were independently confirmed by qRT-PCR (Fajkowska et al., 2016).

It is well-known that the sexual differentiation of some fish responds to environmental factors (Kushnirov and Degani, 1991; Degani and Kushnirov, 1992; Devlin and Nagahama, 2002). Previous studies (e.g. (Chen et al., 2016) were conducted in environmental conditions different from those at the Dan Fish farm in north Israel, where stable temperatures (18 ± 6 °C throughout the year) (Hurvitz et al., 2007) may affect sexual differentiation. Thus, under these conditions 14% of the individuals with intersex gonads (combined testis and ovary-like tissues) were identified among a population of 5000 fish that originated from the same lot of eggs that had been pre-selected as females (Jackson et al., 2006). The specific effects of constant aquaculture conditions on the expression of genes involved in sexual differentiation is still unknown. In the present study, we examined the gonadal transcriptome variation (by RNA-seq) and determined the abundances of transcripts (by qRT-PCR) of nine genes that are differentially expressed between the sexes of Russian sturgeon fish maintained in aquaculture conditions including a stable water temperature.

2. Materials and methods

2.1. Fish and sampling procedure

Russian sturgeon fish (*A. gueldenstaedtii*) maintained and bred at the Kibbutz Dan Fishery in Israel, were used in this study. Investigations were conducted in accordance with the International Guiding Principles for Biomedical Research Involving Animals as promulgated by the Society for the Study of Reproduction. The fish were developed and maintained at Dan Fish Farms, (Upper Galilee, Israel; 31°30′N, 34°45′E) and were managed in aquaculture conditions, as previously described (Hurvitz et al., 2007; Levavi-Sivan et al., 2017). Briefly, fish were maintained in 250 m^3 concrete ponds at a water temperature of 18 ± 6 °C. The oxygen concentration was maintained at 6 ppm using an oxygenic machine. Ammonia and nitrite were maintained at concentrations of less than 0.5 ppm. For the study of gene expression in the gonads, 4-year-old males and females (4–6 kg) were anaesthetized with 0.03% tricaine methane sulfonate (MS222, Sigma-Aldrich), and their fork length and body weight were quantified. Gonad samples were removed by endoscopy from males and females as described previously (Hurvitz et al., 2007) and frozen in 1.5 ml tubes with RNALater (Ambion) at −25 °C until further analysis. In the relatively constant conditions at the Dan Fish Farms, the 4-year old females are at the pre-vitellogenic stage which immediately precedes and is essential for the production of caviar (Hurvitz et al., 2007), while males are in full spermatogenesis (Hurvitz et al., 2007) as observed using routine endoscopy and macroscopic examination and histology procedures that are conducted annually at the Dan Fish Farm.

2.2. RNA extraction

Samples of ovaries from five females and testes from four males were removed from RNALater (Thermo-Fisher Scientific) and

homogenized using a TissueRuptor (Qiagen). Total RNA was extracted from each sample with TRI Reagent (Sigma) using the manufacturer's protocol. The concentration and integrity of RNA were examined using a Thermo-Fisher Scientific NanoDrop 8000 Spectrophotometer and an Agilent 2100 Bioanalyzer. All RNA samples had OD260/280 ≥ 1.8 and RNA integrity number (RIN) ≥7. Equal amounts of the RNA samples from each group were then pooled together for cDNA synthesis and sequencing.

2.3. Library construction, Illumina sequencing and transcriptome assembly

The RNA-Seq library preparation and sequencing were conducted at the Genomics Center of the Silberman Institute of Life Sciences, Hebrew University of Jerusalem. The cDNA libraries were prepared with ~2.5 μg of total RNA using the NEBNext Ultra RNA library prep kit (New England Biolabs). The libraries were sequenced with one lane on an Illumina NextSeq 500 instrument with 75-bp single-end reads. The raw read files have been deposited in the NCBI Sequence Read Archive (SRA) with the accession SRP157973. Adaptor-only reads and low-quality reads were filtered using the Illumina BaseSpace environment; and the files were then examined using FastQC software (www.bioinformatics.babraham.ac.uk/projects/fastqc/). Cleaned reads were used for *de novo* assembly with Trinity software (Grabherr et al., 2011; Haas et al., 2013) with Kallisto transcript abundance estimation (Bray et al., 2016) and Bowtie alignment (Langmead et al., 2009).

2.4. Functional annotation

The assembly of RNA-Seq contigs was used for open reading frame extraction by TransDecoder (Haas et al., 2013) and a similarity search was conducted as follows: BLASTX and BLASTP programs (Altschul et al., 1990) with E value cutoffs of default and 1e-5, respectively, against the SwissProt/UniProt protein database (Bateman et al., 2017); and HMMSCAN program against the PFAM domain database (Finn et al., 2016). Trinotate software (Haas et al., 2013) was used to integrate the hits into a single SQLITE database and generate an annotation report.

2.5. Gene expression and differentially expressed genes

Trinity software was used with the edgeR (Robinson et al., 2010) method to identify differentially expressed genes between two libraries. The dispersion value was set at 0.2. DAVID online tools (Huang et al., 2008, 2009) were used to find clusters of annotated genes with similar functions in the differentially regulated gene list. Specifically, the Functional Annotation Clustering tool was used with default parameters. Gene annotations were limited to one species to prevent duplicate results; a mammalian species (*Bos Taurus*) was chosen based on the quality of transcript annotation.

2.6. Phylogenetic analysis

For phylogenetic analysis, the *k1c18* keratin gene was chosen as its sequence was of sufficient conservation for abundant BLASTX hits but there also was enough variation to allow the construction of a detailed tree. BLASTX (at the NCBI website) was used to obtain the 100 most precise matches to the predicted *A. gueldenstaedtii k1c18* transcript. The multiple alignments were generated using the MUSCLE multiple alignment software, version 3.8.31 (Edgar, 2004) and FastTreeMP tree-building software (Price et al., 2009). The resulting tree file was visualized on https://itol.embl.de, and the dataset underwent trimming to remove 15 closest-neighbor sequences if these came from the same species (assumed to be duplicate entries). The multiple alignment and tree-building was then repeated with the remaining 85 sequences to obtain the final tree. A scheme of the procedural and bioinformatics pipeline is shown in Fig. 1A.

2.7. Quantitative RT-PCR

To validate the differential abundance of transcript for candidate genes based on RNA-seq results as well as prior knowledge, reverse transcription, primer design and quantitative PCR using SYBR Green chemistry and DNA primers were performed. Primer sequences are provided in Supplementary Information. All primers were tested for efficiency (by serial dilutions) and specificity (by melting peak analysis). The RT was performed on an Applied Biosystems ABI-9600 with reagents from New England Biolabs. The qPCR was performed in technical quadruplicates on an Applied Biosystems ABI-7900HT Sequence Detection System equipped with a 384-well block. Data were analyzed using SDS 2.3 software (Applied Biosystems) and Microsoft Excel. Relative quantification and the ΔCq method were used. Results were normalized to the transcript abundance median of all measured genes per sample.

2.8. Statistics

The significance of differential transcript abundance in the RNA-seq results was estimated by the edgeR differential expression pipeline (Robinson et al., 2010; McCarthy et al., 2012). Significance of differential transcript abundances as measured by qRT-PCR was estimated by a two-tailed Student's t-test. Differences were considered statistically significant at $P < 0.05$.

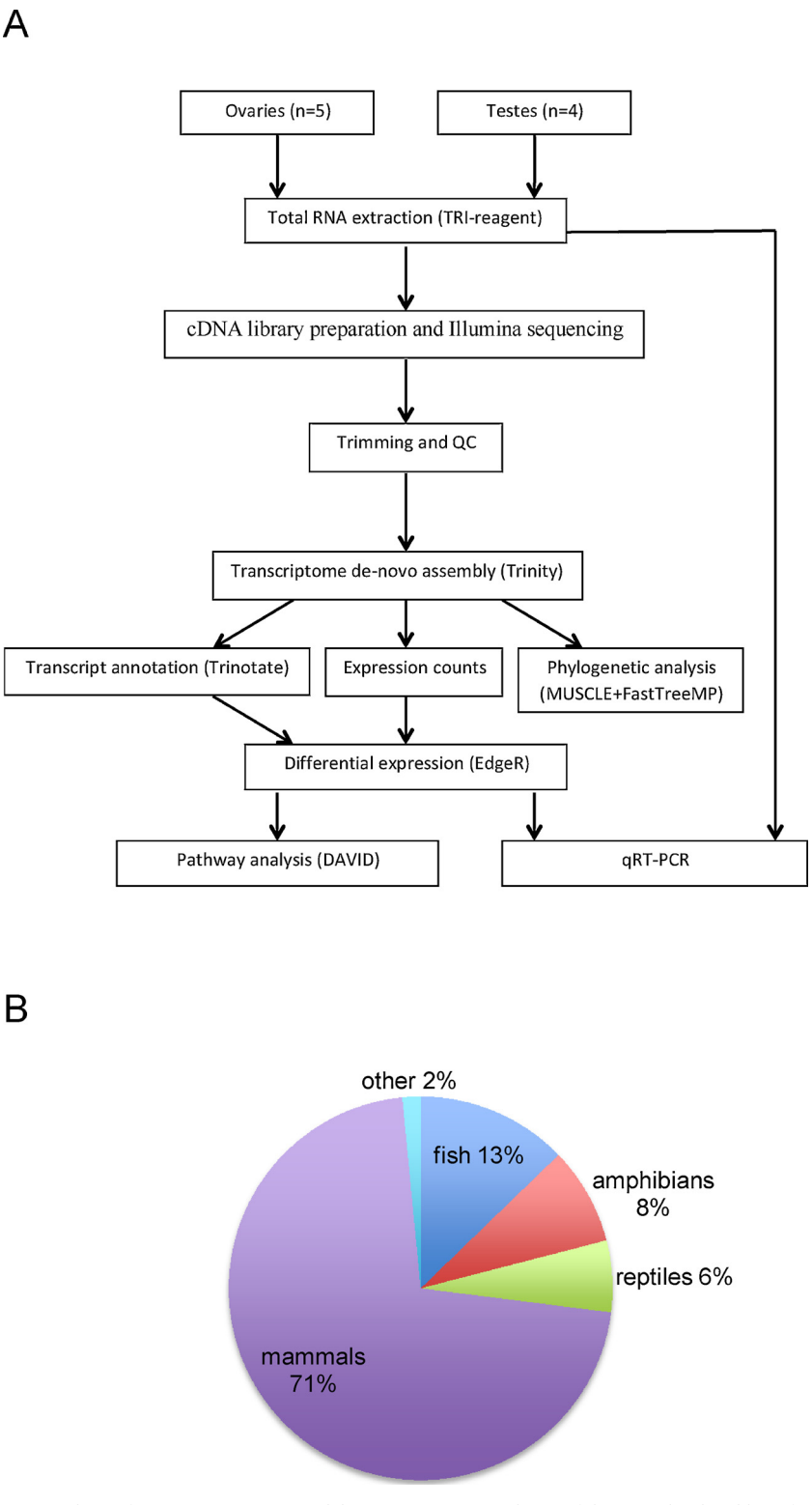


Fig. 1. Overview of RNA-seq analysis of Russian sturgeon gonadal transcriptome; A. Scheme of the procedural and bioinformatics pipeline used for the study. B. Summarized distribution of classes among transcript sequences used to annotate sturgeon gonadal transcripts identified by RNA-seq. Detailed annotation statistics appear in Supplementary Information.

3. Results

3.1. RNA-seq

Two cDNA libraries were prepared from pooled gonad RNAs of five females and four males. A total of 69.7 M and 39.1 M sequencing reads, respectively, were obtained from the two libraries after trimming and QC. The combination of both pools served to assemble the transcriptome, while the sex-specific pools were compared to determine differential abundance of specific transcripts. Of the 28,170 unique transcripts identified, 16,191 could be annotated by similarity to genes in other species. The homologous sequences were predominantly from mammalian (71%), fish (13%), amphibian (8%) and reptilian origin (6%), with all other sequences accounting for less than 2% (Fig. 1B). There were 392 transcripts that were in differential abundance by a factor of 20-fold or more between the sexes; 272 of these were annotated of which 175 and 97 were upregulated 20X fold or more in the ovaries and testes, respectively. A subset of 60 annotated genes with the greatest relatively abundance (based on RNA-seq reads from either ovaries or testes) that were X20-fold or more in abundance in ovaries as compared with testes or vice versa is presented in Table 1.

Additional genes previously described as involved in sexual differentiation of fish that were identified in the Russian sturgeon gonadal transcriptome included *sox3, sox9*, and *star*. Of these, the *sox9* transcript was 5.6X fold greater in testes compared to ovaries; *star* transcript abundance did not differ significantly between ovaries and testes; and *sox3* transcript abundance could not be quantified in both sexes. Other genes previously described as involved in sexual differentiation of fish could not be identified in samples in the present study. These included *ar, dmrt1* and *cyp17a1*. The annotation report and the list of transcripts in differential abundance are included in Supplementary Information.

Functional annotation and clustering of the genes with transcripts in differential abundance were identified in several clusters. Thus, a group of transcriptional regulators and factors involved in cell division, and specifically *septins 6, 7* and *9*, were in greater relative abundance in ovaries; while a different set of transcription factors (including *sox6* and *sox30*) and a group of protein kinases were in greater relative abundance in testes. The highest-score functional annotation clusters of transcripts in relatively greater abundance are shown in Fig. 2 for ovaries (Fig. 2A) and testes (Fig. 2B), with respective enrichment scores of 2.1 and 1.78. Detailed DAVID outputs containing additional clusters appear in Supplementary information.

3.2. qRT-PCR for genes of interest

To validate several of the genes for which there was differential transcript abundance as identified by RNA-seq in individual samples, qRT-PCR measurements were conducted on nine transcripts that were in greatest relative abundance and in differential abundances between the pooled ovaries and testes samples. Additionally, the relative abundances of transcripts of two genes previously known to be involved in reproduction, *cyp19* (*p450* aromatase) and *foxl2*, were measured by qRT-PCR in the individual samples. Of these, the transcripts for five (including *cyp19* and *foxl2*) were in greater relative abundance in ovaries, while the relative abundance of *ighm1* transcripts was greater in the testes (Fig. 3). For other candidate genes, such as *sf3b3*, there was a trend for differential relative transcript abundance, however, there was not a statistical significance due to variation between the samples.

3.3. Phylogenetics

For phylogenetic analysis, multiple alignment was performed using the predicted sturgeon *k1c18* keratin gene and the top 100 BLASTX hits. The resulting phylogenetic tree indicated the relative proximity of the sturgeon sequence to that of other primitive fish species, namely the spotted gar (*Lepistosteus oculatus*), gray bichir (*Polypterus senegalus*), coelacanth (*Latimeria chalumnae*) and marbled lungfish (*Protopterus aethiopicus*), as well as another sturgeon species, the Siberian sturgeon (*Acipenser baerii*) (Fig. 4). Furthermore, the Russian sturgeon was located on the same phylogenetic branch with amphibian, reptilian and even avian sequences, while there was separation from the teleosts, which contributed most of the aligned sequences (Fig. 4).

4. Discussion

The great economic value of caviar and as a result the development of sturgeon aquaculture farms where the value of females is greater than that of males, increase the importance of knowledge about mechanisms of sex determination and differentiation in the sturgeon (Devlin and Nagahama, 2002; Hurvitz et al., 2007). This knowledge may be applied to develop monosex populations, as was successfully done with other commercial fish (Cnaani and Levavi-Sivan, 2009) and in prawns (*Macrobrachium rosenbergii*) (Levy et al., 2017) but has not, as far as we know, been done in the sturgeon.

The findings of our gonadal gene expression analysis in male and female Russian sturgeons maintained in specific aquaculture conditions are overall consistent with prior studies in sturgeon species (Burcea et al., 2018; Chen et al., 2016). Thus, in agreement with a previous study (Chen et al., 2016), more genes were expressed in ovaries than in testes, suggesting that more genes may be involved in female differentiation. However, the present study identified additional specific genes for which there was differential transcript abundance in the gonads and which are likely involved in oogenesis and spermatogenesis. While the functions of many genes are still unknown, the observed greater abundance of septin transcripts in ovaries is intriguing, as septins have important and still poorly understood functions in cell division and maintenance of cell polarity (Kinoshita, 2006; Mostowy and Cossart, 2012; Neubauer and Zieger, 2017). In mammals, the function of septins has been linked to male fertility (Shen et al., 2017). Results in the present study suggest that during early vertebrate evolution, these proteins were also instrumental in female reproductive

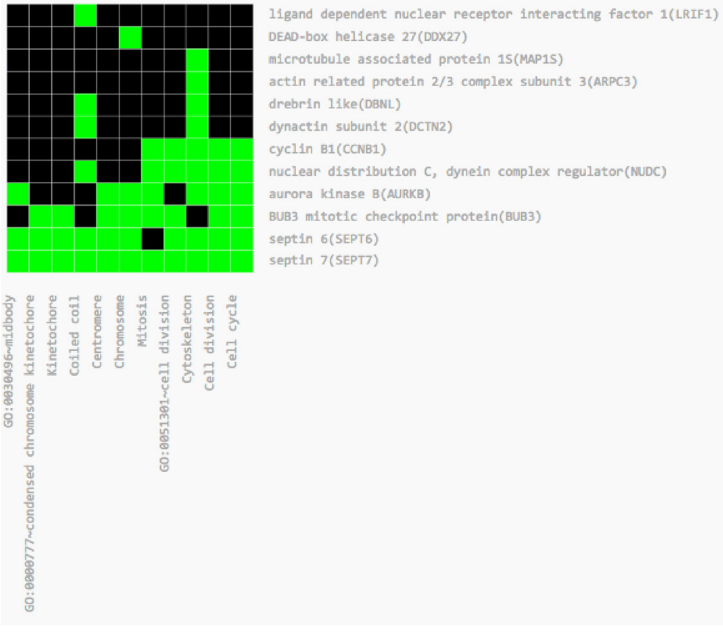
Table 1
A subset of 60 annotated genes with the greatest transcript abundance (based on RNA-seq reads from either ovaries or testes) that were X20-fold or more in transcript abundance in ovaries compared with testes (top of list) or vice versa (bottom of list). A full list of genes for which there was differential transcript abundance appears in Supplementary Information.

Gene name	Ovary norm reads	Testes norm reads	ovary/testes ratio
zp2	4796.9	4.4	1085.8
zp1	10828.7	23.5	460.7
cpne1	2586.7	11.6	222.3
rbps2	999.1	4.6	218.4
zp4	403.7	2.9	137.6
socs4	224.8	1.6	136.3
aurkb	402.6	4.0	100.7
rbps2	307.8	3.1	99.1
cdca7	5897.5	59.8	98.7
cpeb1	537.0	5.5	98.2
lis1a	1988.8	20.5	96.9
pyc	260.7	2.8	92.3
npl1a	2109.7	26.0	81.0
eomes	898.4	11.2	80.2
ccnb1	301.4	4.0	76.1
tisb	351.7	4.8	73.2
po5f1	773.8	12.9	59.8
b4	452.3	9.5	47.6
trefoil domain	5171.6	126.6	40.8
pmel	292.0	10.8	26.9
kctd5	226.3	8.5	26.7
tbr1	272.3	11.4	23.8
rm45	926.4	44.4	20.9
auraa	478.0	23.0	20.8
sf3b3	142.2	3064.8	0.046
atp6	327.1	7135.1	0.046
mark2	124.9	2836.1	0.044
ca194	13.0	314.3	0.041
hsf5	10.8	289.8	0.037
hspb1	17.3	467.3	0.037
hspb1	14.9	405.3	0.037
md19b	19.4	545.2	0.036
hes1	31.3	908.8	0.034
tisb	16.2	493.2	0.033
ca194	11.9	401.8	0.030
egr1	9.6	338.6	0.028
atp6	561.0	21507.2	0.026
nu5m	6.9	265.1	0.026
hes4	7.7	296.9	0.026
nlrc3	11.1	437.5	0.025
tar1	33.8	1544.7	0.022
cox1	55.5	2665.5	0.021
nupr2	12.6	681.2	0.018
sik2	4.1	232.6	0.018
aldoa	4.7	282.6	0.017
rtxe	4.2	257.8	0.016
tba3	31.7	1956.4	0.016
mast2	3.1	224.6	0.014
rl29	2.9	226.8	0.013
cox3	1195.2	106239.0	0.011
rtbs	3.5	333.6	0.011
pol	2.5	263.5	0.009
odf3a	1.7	230.6	0.007
ttll3	2.6	436.4	0.006
tba1	72.6	12674.9	0.006
t53i2	1.3	307.7	0.004
ighm	2.5	2150.2	0.001
sox30	0.9	974.2	0.001
tba	38.3	54335.9	0.001

development. This hypothesis warrants further investigation.

In the present study, there was a greater abundance of *cyp19* and *foxl2* transcripts in ovaries which is consistent with findings from other studies of the sturgeon (Hagihara et al., 2014; Chen et al., 2016; Wang et al., 2017; Burcea et al., 2018) and also in other fish species (Tzchori et al., 2004a, 2004b; Ezagouri et al., 2008; Zhang et al., 2017). *Ighm1* showed a greater transcript abundance in the testis compared to the ovary as previously described in other fish (Bengtén et al., 2006). These results are consistent with the

A



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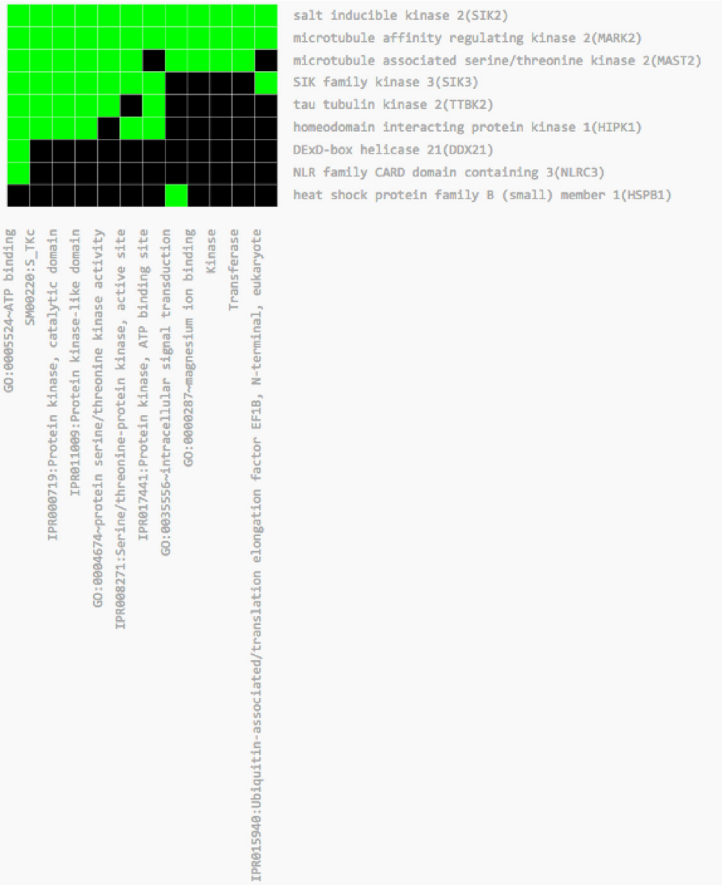

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Fig. 2. Highest-score functional annotation clusters of transcripts in greater abundance in Russian sturgeon gonads. (A) ovaries; (B) testes. Clusters are based on RNA-seq results and DAVID software output (Huang et al., 2008, 2009). The 2 clusters had enrichment scores of 2.1 and 1.78, respectively. Detailed DAVID outputs appear in Supplementary Information.

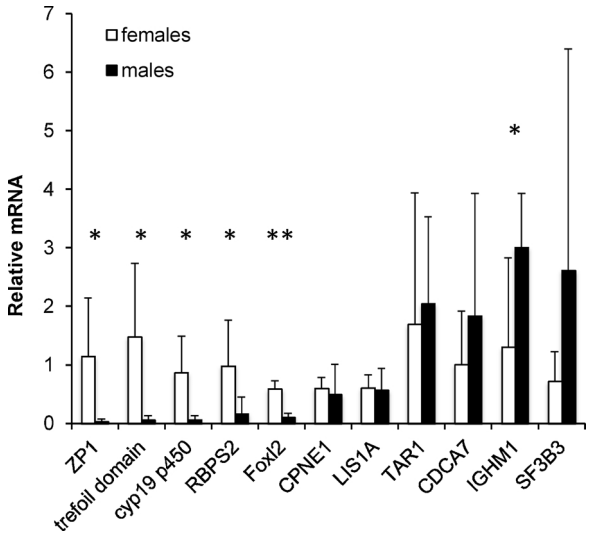

Fig. 3. Relative mean mRNA abundance (based on qRT-PCR) of transcripts annotated to the listed genes, in gonadal samples from five female and four male 4-year old sturgeons. The genes are sorted according to mean female/male signal ratio. *: $P < 0.05$; **: $P < 0.001$ (t-test). Bars, stdev.

hypothesis that the genes involved in sex differentiation also control oogenesis and spermatogenesis in mature fish (Degani, 2014, p. 3; Vizziano-Cantonnet et al., 2016).

Both the genetics and environment affect gonad development and gametogenesis in fish (Devlin and Nagahama, 2002; Hurvitz et al., 2007). Thus, in the European eel (*Anguilla anguilla*) sex differentiation is affected by population density (Kushnirov and Degani, 1991) and involves environmental-hormonal interactions (Degani, 2016). The differences between the results of the present and other studies in the same species (Burcea et al., 2018; Chen et al., 2016) may result from such environmental effects on gene expression. Specifically, at the Dan fish farm the Russian sturgeon are maintained in specific aquaculture conditions including stable water temperatures throughout the year, which markedly differs from the natural habitats of the Russian sturgeon. Technical differences between bioinformatic pipelines and parameters used, however, are also a likely contributor to the inconsistent results; especially because there is no existing reference and transcripts have to be annotated based on domain prediction and partial sequence homology. This could explain why other genes previously described as involved in sexual differentiation of fish could not be identified in the present study (e.g., *ar, dmrt1* and *cyp17a1*).

Phylogenetic analysis based on the *k1c18* keratin gene indicated that the sturgeon is genetically more similar to other primitive/ancient species of fish and land vertebrates then to teleosts, supporting the ancient origin of the *Acipenser* genus. The lack of sequences from other sturgeon species in this phylogenetic tree stems from the currently incomplete annotation of their genomes. Density of annotation also partially accounts for the fact that by far the most sequences used to annotate sturgeon transcripts originated in mammalian species rather than fish. However, greater similarity of sturgeon with mammalian gene sequences than with Osteichthyes sequence was previously observed for specific genes; e.g., in the cloning of Russian sturgeon growth hormone and insulin-like growth factor 1 (Yom Din et al., 2008), and the cloning of *fshβ, lhβ* and glycoprotein α subunits (Hurvitz et al., 2005; Yom-Din et al., 2016). Taken together, these findings suggest that understanding the mechanisms of sexual differentiation in the Russian sturgeon is not only of commercial value, but may lead to a greater understanding of the evolution of reproductive mechanisms that exist in higher vertebrates.

The present study was limited by the small sample size and as a result of the use of pooling at the RNA-seq stage, both of which could have resulted in the lack of detection of candidate genes. Additionally, the present study focused on an endpoint in the sexual differentiation of the sturgeon, while some genes important to this process are likely to be transiently up- or down-regulated at particular developmental stages and thus were not detected with the present analysis. Future studies would thus benefit from quantifying the relative abundance of gene transcripts at different stages of sexual differentiation in the sturgeon. We are conducting further studies to establish genetic sex markers based on transcriptomic as well as genomic characterization of the Russian sturgeon.

Funding

This study was funded by an internal grant from MIGAL.

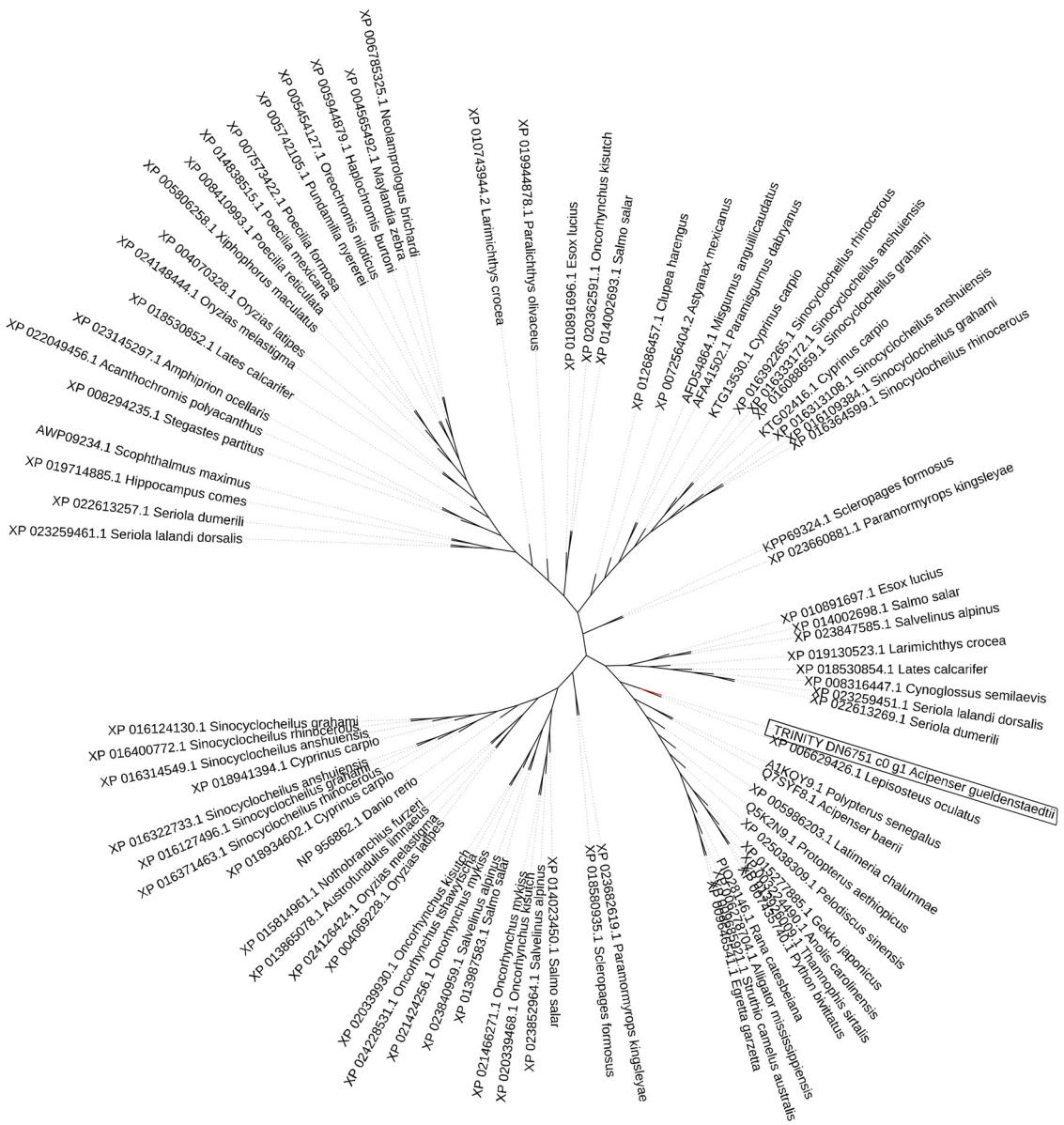

Fig. 4. Phylogenetic trees showing the relationship between the sturgeon gonadal transcript sequence annotated as the keratin-encoding gene *k1c18* and the most similar sequences in other species identified by BLASTX against the non-redundant nucleotide database. The alignments were generated using MUSCLE software and the tree was constructed using FastTreeMP. The top 85 alignments are shown. The branch representing the input sturgeon sequence is indicated by a box.

Declaration of interest

AH has commercial interest in sturgeon aquaculture at the Kibbutz Dan Fishery, Israel.

Acknowledgements

The authors would like to thank Hila Yehuda (MIGAL) for expert technical assistance.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.anireprosci.2018.11.013.

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