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Short Note

Head Kidney Transcriptome Analysis and Characterization for the Sub-Antarctic Notothenioid Fish *Eleginops maclovinus*

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Abstract: This study describes de novo transcriptome sequencing and annotation analyses for the head kidney of the sub-Antarctic notothenioid fish *Eleginops maclovinus*, a sister group of the Antarctic notothenioid fish clade. Moreover, *E. maclovinus* is one of the most eurythermal and euryhaline representatives of the Notothenioidei suborder. RNA-seq data were generated by the 454 GS Junior system, resulting in 11,207 contigs that were then assembled by the Genomic Workbench CLC software. The transcriptome was annotated by BLASTing each sequence against the universal, non-redundant NCBI database (National Center for Biotechnology Information) using the AUSTRAL-omics computer cluster. A significant number of transcripts related to innate and adaptive immunity were found in the sequences, which could be used as references in future immunological studies in *E. maclovinus*.

Keywords: *Eleginops maclovinus*; transcriptome; notothenioid; immunity; head kidney

1. Introduction

Eleginops maclovinus (Valenciennes 1830) is a notothenioid (Perciformes) endemic to the temperate and sub-Antarctic waters of South America. This fish is the only representative of the Eleginopsidae (Osteichthyes) family within the Notothenioidei suborder. Regarding phylogeny, the Eleginopsidae, Pseudaphritidae, and Bovichtidae families emerged before a severe decrease in water temperature approximately 24 million years ago [1]. Therefore, these families are known as non-Antarctic Notothenioidei that diverged early from the main notothenioid lineage. These families also never established on the Antarctic shelf [2], either due to a lack of crucial biochemical and physiological adaptations to cold (e.g., antifreeze glycoproteins), the lack of dispersal potential (e.g., small eggs and larval stages with reduced mobility), or the dependence on estuarine environments that are absent in the Antarctic [3]. Meanwhile, the Antarctic fish lack a common cellular defense mechanism called the heat shock response (HSR) by molecular chaperones [4], developing a cardiovascular system with

modifications that involve large-bore blood vessels, large blood volumes; and large hearts with high content of mitochondria [5].

Genetic analyses of Antarctic fish are possible due to an availability of cDNA sequence libraries for various species, including *Dissostichus mawsoni* (Nototheniidae) [6], *Harpagifer antarcticus* (Harpagiferidae) [7], *Chionodraco hamatus* (Channichthyidae) [8], *Trematomus bernacchii* [9,10], *Pagothenia borchgrevinki* [11], *Chaenocephalus aceratus* (Channichthyidae), *Notothenia coriiceps*, and *Pleuragramma antarcticum* (Nototheniidae) [12,13]. To date, relatively little sequence information is available for *E. maclovinus* [2], despite the physiological characteristics that consider it an eurythermic, euryhaline and stenobatic species [14], with a hermaphrodite type of reproduction (unique among the notothenioids) [15] and with a relevant participation in the transmission of diseases that affect aquaculture as the Piscirickettsiosis [16]. This report describes the de novo assembly and annotation of the *E. maclovinus* head kidney transcriptome, providing a new source of genetic information that can be used in future studies dealing with the immunological response of notothenioids.

2. Results

Functional annotation against the Gene Ontology database revealed transcripts involved in cellular components, molecular functions, and biological processes (Figure 1). Among the biological processes, several transcripts were found with functions in the innate and adaptive immune response of *E. maclovinus*, representing a significant advance in respect with the genomic data available for this species (Table 1). The high prevalence of immune-related transcripts in the *E. maclovinus* head kidney is in line with previous reports from *T. bernacchii* [10]. The head kidney has been reported a primary lymphoid organs in the bony fishes that functions as lymphocyte precursor tissue responsible for the generation as well as maturation of B cells, and the generation of T-cell progenitor cells that migrate to the thymus throughout life [17]. The head kidney in fish also has endocrine functions, including cortisol synthesis by interrenal cells and catecholamine synthesis by chromaffin cells. Therefore, this tissue presents functions analogous to the mammalian adrenal gland [18].

Table 1. List of annotated Gene Ontology (GO) biological processes relevant to the innate and/or adaptive immune systems, detailing the number of corresponding annotated contigs in the *Eleginops maclovinus* transcriptome.

GO Category	Description
GO:0002376	H-2 class ii histocompatibility antigen gamma chain-like
GO:0045087	Tyrosine-protein kinase fgr isoform ×1
GO:0002755	Ubiquitin-40s ribosomal protein s27a
GO:0045087	Af280815_1calmodulin partial
GO:0006955	Major histocompatibility class i receptor
GO:0006955	Tumor necrosis factor ligand superfamily member 13b
GO:0006054	Macrophage colony-stimulating factor 1 receptor 2-like
GO:0006055	Ectodysplasin splice variant-8 partial
GO:0002474	Mhc class i alpha partial
GO:0001916	Mhc class i antigen
GO:0006955	Invariant chain-like protein 14-1
GO:0002504	Mhc class ii antigen beta chain
GO:0006355	Paired amphipathic helix protein sin3a
GO:0002376	G-protein coupled receptor 183-like
GO:0045087	Actin-related protein 3
GO:0045087	Cell division control protein 42 homolog
GO:0006954	Toll-like receptor 1
GO:0045087	Catenin beta-1-like
GO:0006954	Toll-like receptor 8

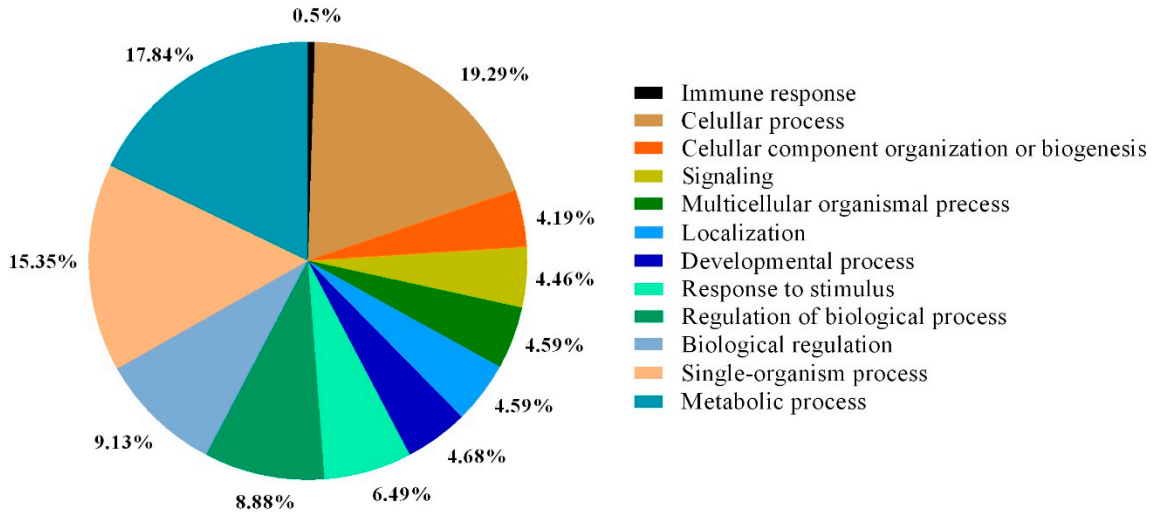

Figure 1. Functional Gene Ontology annotation of the *Eleginops maclovinus* head kidney transcriptome revealed transcripts involved in biological processes.

3. Materials and Methods

3.1. Animal Specimens

Eleginops maclovinus specimens were collected with nets from the wild and transferred to the Calfuco Coastal Laboratory (Faculty of Science, Universidad Austral de Chile, Valdivia, Chile). Fish were acclimated for four weeks to seawater (32 psu, 1085 mOsm kg^{-1}) in 500 L, flow-through system ponds at a stocking density of 3.1 kg m^{-3} and under natural photoperiod and temperature (12 ± 0.5 °C) conditions, per methodology described by Vargas-Chacoff et al. [19]. Fish were fed using commercial Nutreco Defense 100 dry pellets (Skretting, Stavanger, Norway) containing 48% protein, 22% fat, 13.5% carbohydrates, 8% humidity, and 8.5% ash. Fish were fed once daily at a ratio of 1% body weight. All experiments complied with animal use guidelines established by the National Commission for Scientific and Technological Research of Chile (CONICYT) and the Universidad Austral de Chile.

3.2. RNA Isolation, Library Construction, and Sequencing

Fish were anaesthetized with a lethal dose of 2-fenoxietanol (1 mL L^{-1}), weighed, measured, and dissected for sample collection. The head kidney was aseptically removed and stored at −80 °C. All steps involving massive cDNA sequencing were performed using a GS Junior Titanium Series System (Roche, Basel, Switzerland) at the AUSTRAL-omics Laboratory (Universidad Austral of Chile) following the manufacturer's protocols. In detail, total RNA was extracted from the head kidney with the NucleoSpin® RNA kit (Macherey-Nagel, Dueren, Germany). Total RNA quantity and quality were evaluated spectrophotometrically (A260) using the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Waltham, MA, USA) and Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA), respectively.

Head kidney mRNA was isolated with the PolyATtract kit® mRNA Isolation System III (Promega, Madison, WI, USA), and mRNA concentration was determined using the Quant-iT™ RiboGreen® RNA Assay kit. The cDNA library was constructed following the "Rapid cDNA library preparation" protocol recommended by Roche. This protocol begins with a minimal amount of mRNA (300 ng). Then, mRNA samples were fragmented with ZnCl to an optimal range of 200 to 1200 base pairs before the subsequent synthesis of double-stranded cDNA (Synthesis System kit, Roche) and removal of small fragments. The library was quantified by fluorescence, obtaining an approximate concentration of 6.4^8 molecules DNA/µL. The Roche-recommended protocol advised preparing a working dilution of 1×10^7 DNA molecules DNA/µL to ensure successful emulsion PCR. Therefore, the working

solution was prepared using 7.3 µL of library molecules in 492.7 µL of TE buffer. This solution (10 µL) was then used for emulsion PCR following the protocol outlined in the *emPCR Amplification Method Manual—Lib-L, GS Junior Titanium Series* (ed. March 2012), which resulted in 10% bead recovery. The amplified cDNA for each microsphere was pyrosequenced in a 454 GS Junior System (Roche). The raw data were processed from a series of individual images taken for each well of the sequenced plate. The data were extracted and then quantified and normalized with control DNA microspheres. The results generated by Roche 454 sequencing are show in Table 2.

Table 2. Roche 454 sequencing results for the head kidney transcriptome of *Eleginops maclovinus*.

A. Number of Obtained Readings	
Raw Wells	291,469
Key Pass Wells	281,128
Passed Filter Wells	208,809
Total Bases	95,309,200
B. Sizes of the Obtained Sequences	
Length Average	456
Longest Read Length	1173
Shortest Read Length	40
Median Read Length	500
Mode Read Length	542

3.3. De Novo Transcriptome Assembly, Identification of Protein Coding Region, and Annotation

Regarding bioinformatics analysis, sequences were filtered using the prinseq-lite.pl software [20], with different cut-off parameters at both ends of each sequence. The cut-off values are given by adapters or for low quality existing at the ends of the readings. Furthermore, average quality filters were applied to discard reads that did not meet minimum threshold cut-off values. De novo transcriptome assembly was performed using the CLC Genomic Workbench software 7.0.3 and the transcriptome was annotated by BLASTing each sequence against the universal, non-redundant NCBI database (National Center for Biotechnology Information) using the AUSTRAL-omics computer cluster. This step consequently allowed for functional annotation against the Gene Ontology database using the Blast2GO software [21]. Annotation was performed for transcripts assembled by the Genomic Workbench CLC software, as well as for unassembled reads to identify low-abundance transcripts that could only be sequenced once. The results of bioinformatics analyses are show in Table 3. Raw sequencing data have been deposited in the NCBI SRA database (Sequence Read Archive) with the accession ID SRP129851.

Table 3. Bioinformatics analysis results for the head kidney transcriptome of *Eleginops maclovinus*.

	Assembled Transcripts	**Unassembled Reads**
Number of transcripts	5088	6119
Base pairs	2,398,375	1,780,104
Annotated with "nr"	2000	1602
Not annotated with "nr"	3088	4517

4. Conclusions

The obtained *E. maclovinus* transcriptome represents a significant advancement in the genomic data available for this species. Papetti et al. [2] assessed the GenBank information for *E. maclovinus*, finding 125 nucleotide entries (71 mitochondrial, 54 nuclear, with 10 microsatellite markers among these), 124 protein entries (78 mitochondrial, 46 nuclear), and 38 popsets (phylogenetic studies). The sequences reported by the present study provide a new source of genetic information that can be

used in future studies dealing with immunological response of *E. maclovinus* and other notothenioids Antarctic at a comparative level.

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Conflicts of Interest: The authors declare that they have no competing interests

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