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Data article

European sea bass (*Dicentrarchus labrax*) skin and scale transcriptomes

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Abstract

Fish skin and their appendages, the mineralized scales, are important organs for protection and homeostasis, but little is known about their specific transcript or protein repertoire. This study used RNA-seq to generate transcriptome data for skin and scales in the European sea bass (*Dicentrarchus labrax*), an important species for fisheries and aquaculture. RNA was extracted from the pectoral skin and from scales collected above the midline of immature one-year old sea bass. More than $20x10^6$ reads were obtained for each tissue, using RNA-seq Illumina technology. *De novo* assembly resulted in 31,842 transcripts (of 500 base pairs or greater) for skin and 20,423 transcripts for scale. This dataset provides a useful resource for both aquaculture and fish conservation studies and for research into the physiology and molecular biology of fish skin and scales.

Keywords: RNA-seq, teleost fish, fish scale, integument

1- **Introduction**

The skin is a multifunctional organ and an important innate immune barrier that is essential for organism survival across the vertebrates. It is a mechanical and immune barrier, it is used for sensing and inter-individual communication and it also appears to be a site of hormone action and production (Nejati et al. 2013). Teleost fish skin has a similar organization to that of mammals and is composed of the outer epidermis and underlying dermis and hypodermis. Key specializations of fish skin in the majority of species include: i) an outer non-keratinized epithelial cell layer that is overlaid by a protective layer of mucous and ii) mineralized scales that emerge from scale pockets in the dermis (reviewed by Power 2014). In addition, fish skin and scales play an important role in osmotic and mineral homeostasis (Guerreiro et al. 2007) but the physiology and the underlying molecular basis of their response to different environmental stimuli remains largely unexplored.

The European sea bass (*Dicentrarchus labrax*) is a marine teleost of great importance in European marine fisheries and aquaculture (FAO 2005) and it is also a representative of the species rich order, the Perciformes, which includes many other commercially important aquaculture species. The sea bass genome (Tine et al. 2014) and the transcriptomes of several tissues (Louro et al. 2010; Louro et al. 2014) have been fully sequenced, mainly by Sanger sequencing of expressed sequence tag (ESTs) libraries or by 454 pyrosequencing. However, until now there are few available transcripts for the skin and scale tissues.

In the context of a larger project aiming to characterize the impacts and mechanisms of action of estrogenic compounds in fish skin and scale (Pinto et al. 2009, 20014, 2016; Ibarz et al. 2013), and in an effort to increase the transcriptomic resources, we have used next-generation sequencing to obtain the skin and scale transcriptomes from sea bass.

2- Data description

2.1- Sea bass sampling and library construction

Sea bass larvae (weighing less than 1g) were obtained from Station Expérimentale d'Aquaculture Ifremer (Palavas-les-flot, France). These were maintained in Ramalhete Marine Station (CCMAR, Faro, Portugal) in 500 L flow-through seawater tanks, continuously supplied with natural sea water from the lagoon system "Ria Formosa", at an average salinity of 35.7 PSU (practical salinity units) and natural temperature and photoperiod, and were fed with commercial dry pellets. On the day of sampling, carried out in January 2014 at a water temperature of 14.6 ºC and 10L:14D (light-dark) photoperiod, tissues from six immature (one year-old) sea bass (18.8 ± 0.3 cm; 75.8 ± 1.1g) were sampled, from a mixed sexes population. Fish were anesthetized in 2-phenoxyethanol (Sigma-Aldrich, diluted 1:5,000 in seawater), washed with clean seawater, measured and weighed. Skin samples (approximately 1 × 0.5 cm) were collected from the pectoral region (Figure 1), which contains a low number of scales, using a scalpel and carefully removing adherent muscle. Individual scales (10/fish) were plucked with forceps from below the dorsal fin and above the midline, in approximately the same position from all fish. Tissues were immediately frozen in liquid nitrogen and stored at -80ºC. Manipulation of animals was performed in compliance with international and national ethics guidelines for animal care and experimentation, under a "Group-I" license from the Portuguese Government Central Veterinary service to CCMAR and conducted by a certified investigator (DMP).

Total RNA was extracted from frozen tissues using an automated Maxwell 16 Instrument and a Maxwell 16 SEV total RNA purification kit (Promega, UK) after mechanical disruption using an Ultra Turrax homogenizer (IKA, Germany) with the dispersing element S25N-8G for skin (soft tissue) and S25N-8G-ST for scales (fibrous tissue). Total RNAs of each individual fish were pooled per tissue (0.5µg/fish for skin and 1µg/fish for scales, n=6 fish for each tissue) and one RNA-seq library was prepared for each tissue. The combined RNA pools for each tissue were treated with a DNA-free kit (Ambion, UK) using an optional rigorous treatment, consisting of two sequential 30 min treatments

with double the normal amount recommended of TURBO DNAse (4-6 U). RNA concentration and integrity were assessed using an Agilent 2100 Bioanalyser (Agilent Technologies, USA), which confirmed that both samples had a RIN (RNA integrity number) above 8. Library preparation was conducted at Shanghai Ocean University, China using a TruSeq mRNA library prep kit (Illumina, USA) and 0.5ug per library (skin or scale) of the pooled RNA for each tissue. The skin and scale cDNA libraries were subjected to 100 bp paired-end (PE) sequencing using an Illumina HiSeq 1500. MIxS descriptors of the project, samples and sequencing are presented in Table 1.

2.2- Assembly and annotation

Sequencing produced 20.7 M paired-end reads (100 bp) for sea bass pectoral skin and 20.1 M for scales. These were examined for quality (mean bp quality of 35) and adapters were trimmed by fastqc (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and cutadapt (http://journal.embnet.org/index.php/embnetjournal/article/view/200). The remaining sequences were assembled with SOAPdenovo v.2.04 (Luo et al. 2012), using default genome style parameters with a kmer value of 47, resulting in 31,842 and 20,423 transcripts of 500bp or greater for skin and scale respectively. Mean transcript coverage was 17.23 for skin and 11.63 for scales. The two assembled transcriptomes were compared to annotated genes in the sea bass genome (June 2012 draft assembly of dicLab v1.0c, annotation of July 2013, accessed at http://seabass.mpipz.de/ on January 2015), using stand-alone Blast with an E-value threshold of $1 \times 10e^{-10}$. This resulted in 88% of the skin and 89% of the scale transcriptomes being annotated with sea bass genes, while only 12% (for skin) and 11% (for scales) remained unannotated, and these transcripts may represent novel, unannotated genes in the sea bass genome. Table 2 summarizes the assembly and annotation statistics for this study.

A comparison between the assembled transcripts for each tissue was carried out using a stringent stand-alone Blast (with E-value threshold set at 1 × 10e-50) and 16,989 skin transcripts matched 17,150 scale transcripts. This result reveals a high similarity between the two transcriptomes, with the common transcripts comprising 53% or 84% of the skin or scale transcriptomes, respectively.

The skin-scale structure existing in sea bass and in most teleost fish consists of a multilayer of mineralized and partially mineralized tissue composing the elasmoid scale, with its anterior region inserted into the scale pocket in the dermis and its protruding posterior region covered by the epidermis (Pinto et al., 2009; Sire and Akimenko, 2004). When scales are sampled or when scales are lost in the wild or in captivity, part of the epidermal layer and the superficial dermis may also be collected or lost together with the mineralized portion of the scale; scale loss is then followed by rapid re-epithelisation and scale regeneration (Guerreiro et al., 2013; Vieira et al., 2011). Thus, the interconnected nature of the skin and scale tissues can explain the high similarity found between their transcriptomes. Nevertheless, skin and scale specific transcripts were identified, which may represent the activity of specific cell types, such as for example the scale forming osteoblasts or the scale resorbing osteoblasts (Pinto et al., 2014) that dynamically maintain the mineral homeostasis in fish scales, in common with what happens in other bony tissues.

2.3- Data deposition

The obtained raw RNA-seq data were deposited to the NCBI Sequence Read Archive (SRA) (http://www.ncbi.nlm.nih.gov/sra/) under project number SRP071200 (accession numbers SRX1615511 for skin and SRX1615510 for scale). Assembled contigs have been deposited in GenBank as Transcriptome Shotgun Assembly (https://www.ncbi.nlm.nih.gov/genbank/tsa/) projects GFJW00000000 (skin) and GFJX00000000 (scale).

These resources are of great interest for future studies directed at better understanding the physiology of fish skin and scales and their molecular responses to different environmental and internal stimulus, hormones or endocrine disruptors. They are also of great value in understanding the impact of aquaculture conditions on the skin and scale in relation to animal health. The present skin and scale transcriptomes add to previously available fish skin transcriptomes (e.g. Jiang et al., 2014; Malachowicz et al., 2017) and provide, to our knowledge, the first available transcriptome from fish scales.

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Table1

MIxS descriptors of the study (Yilmaz et al. 2011)

Item	Description
Submitted_to_insdc	Yes (SRA)
Investigation_type	Eukaryote
Project_name	Transcriptome of European sea bass (*Dicentrarchus labrax*) skin and scale
Experimental_factor	Skin (EFO_0000962); Scales (EFO:0000960)
Geo_loc_name	Portugal:Algarve:Faro
Lat_lon	37° 1' 0" N / 7° 56' 0" W
Depth	<1.5m (500L sea water tanks)
Alt_elev	0 m
Collection_date	2014-01-14T14:30+00:00
Collected_by	Patricia Pinto
Env_biome	Sea water (ENVO:00002149)
Env_feature	Lagoon (ENVO:00000038)
Env_material	sea water(ENVO:00002149)
Env_package	Water
Temp	14.6ºC
Salinity	35.7 PSU (practical salinity units)
Sequencing method	Illumina HiSeq
Assembly method	Soapdenovo

Table 2

Assembly and annotation statistics of the study

	Skin	Scale
Sequencing and assembly		
Raw reads	20,694,759	20,122,512
Number of contigs (≥ 500bp)	31,842	20,423
Mean contig length	1,070 bp	976 bp
Maximum contig length	11,920 bp	5,939 bp
Annotation		
Annotated genes in genome	28,163 (88%)	18,225 (89%)
Non-annotated	3,679 (12%)	2,198 (11%)

Figure 1

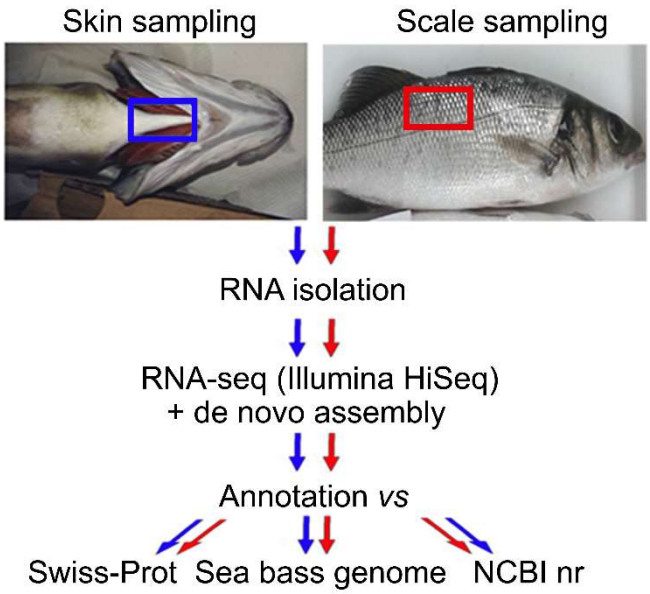

Figure1- Workflow of the study.

The areas sampled of sea bass pectoral skin and dorsal scales are represented by rectangles and technical details are given for the sequencing, assembly and annotation of their transcriptomes.