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A transcriptome resource for the copepod *Calanus glacialis* across a range of culture temperatures

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ABSTRACT

The copepod *Calanus glacialis* plays a key role in the Arctic pelagic ecosystem. Despite its ecological importance and ongoing climate changes, limited knowledge at the genomic level has hindered the understanding of the molecular processes underlying environmental stress responses and ecological adaptation. Transcriptome data was generated from an experiment with *C. glacialis* copepodite (CV) subjected to five different temperatures. We obtained a total of 512,352 high-quality 454 pyrosequencing reads, which were assembled into 55,562 contigs distributed in 128 KEGG pathways. Functional analysis revealed numerous genes related to diverse biological functions and processes, including members of all major conserved signaling pathways. Comparative analysis of acclimated individuals to experimental temperatures has provided information about gene variations observed in several pathways (e.g. genes involved in energy, lipid and amino acid metabolism were shown to be down-regulated with increasing temperatures). These mRNA sequence resources will facilitate further studies on genomics and physiology-driven molecular processes in *C. glacialis* and related species.

1. Introduction

Climate change is dramatically affecting Arctic ecosystems, causing changes in oceanic circulation, sea ice loss and temperature increases that may alter marine community structure (e.g., demographic traits, spatial range, biological interactions) and ecosystem function (Post et al., 2009; Slagstad et al., 2011). The calanoid copepode *Calanus glacialis* plays a major role in the trophodynamics of Arctic pelagic ecosystems and is the dominant species of the genus in the northern Barents Sea (Tande, 1991). Warming of the Arctic is predicted to induce a possible replacement of *C. glacialis* by its boreal sibling *Calanus finmarchicus* (Reygondeau and Beaugrand, 2011; Weydmann et al., 2014a). Consequently, it is essential to understand how climate change might affect the biogeography and population dynamics of *C. glacialis*, and to predict the response and adaptability of the species to environmental fluctuations (Wassmann et al., 2011). In an effort to provide comprehensive genomic resources for *C. glacialis* and a baseline for future physiological studies, we have used Roche 454 pyrosequencing technology to characterize the temperature responsive transcriptome of this species.

2. Methods and analysis

2.1. Sample collection and temperature experiment

Mesozooplankton samples were collected in the Barents Sea, NE of the Hopen Island (77° 08.6′N 28° 11.0′E; average water temperature −0.6 °C), with vertical tows using a WP-2 net (0.25 m-2 opening; 0.2 mm mesh size; with a large non-filtrating cod end) in June 2009. Sixty *C. glacialis* copepodites at the 5th stage (CV) were gently picked and randomly assigned to 6 groups of 10 individuals. One of these, representing natural conditions (NAT), was immediately frozen in liquid nitrogen and stored at −80 °C. The other 5 groups were placed in flasks (200 ml) filled with filtered seawater and placed in a laboratory cooler (type CHL 1 B) at 0 °C. After 36 h of incubation all but one of the groups were transferred to a second cooler at 2.5 °C. This process was repeated with 2.5 °C increments every 36 h. At the end of the experiment (204 h) the individuals incubated at 0 °C (T0), 2.5 °C (T2.5), 5 °C (T5), 7.5 °C (T7.5) and 10 °C (T10) were flash frozen in liquid nitrogen and stored at −80 °C. See Supplementary methods for RNA preparation, cDNA synthesis and pyrosequencing.

2.2. Bioinformatic analysis

Sequence quality-filtering, assembly and annotation were performed essentially as described in Martins et al. (2013). An overview

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Table 1
Summary of 454 sequencing, assembly and BLASTx annotation.

Source	No of raw reads	Assembled quality-filtered reads (% of total)	No. of contigs	Assembled reads with BLASTx[b] matches (% of total)
Total	721,973	512,352 (71.0)	55,562[a]	324,538 (67.7)
NAT	181,118	134,983 (74.5)	14,957	85,963 (65.1)
T0	169,830	121,663 (71.6)	16,297	80,183 (67.6)
T2.5	59,914	43,080 (71.9)	9307	27,551 (65.3)
T5	62,862	43,639(69.4)	8860	27,061 (63.2)
T7.5	111,683	76,266 (68.3)	12,661	47,231 (63.0)
T10	136,566	92,691 (67.9)	13,466	56,549 (61.9)

[a] Median length (N50)—620.
[b] E-value ≤ $1e^{-6}$.

of the sequencing and assembly results is shown in Table 1. A total of 512,352 quality-filtered reads were pooled and assembled using MIRA (v. 3.0; Chevreux et al., 2004) into 55,562 contiguous sequences (contigs) and 12,369 singletons. 41% of the assembled contigs with significant BLASTx homology (NCBI nr database, E-value ≤ $1e^{-6}$) were annotated against KEGG pathway and Pfam protein databases (Kanehisa and Goto, 2000; Finn et al., 2014). A total of 2733 KEGG terms were identified, mapping to 128 KEGG pathways (22,424 contigs). Annotation against the Pfam database identified 1691 terms (16,998 contigs). Highly represented domains, as determined by the total number of reads (>1000) mapping to the domain, were associated with cytoskeletal-related proteins and essential cell functions including energy production (glyceraldehyde 3-phosphate dehydrogenase, ATP synthase, and NADH dehydrogenase), metabolite transport (mitochondrial carrier, sugar transport and lipocalin), fatty acid biosynthesis (fatty acid desaturase), lipid catabolism (Acyl-coA dehydrogenase), cell differentiation (Ras family), protein synthesis (ribosomal genes), and signal transduction and transcription regulation (protein kinases, protein tyrosine kinases, WD40). Additionally, numerous abundant transcripts were involved in the cellular stress response; redox, antioxidant reactions and stress-related processes (cytochrome P450, glutathione S-transferase, NADH ubiquitone, thioredoxin and heat shock proteins—HSP70, HSP90, HSP40). Several potential homologues belonging to the major conserved animal signaling pathways were also identified (e.g. Wnt, Notch, Hedgehog, TGF-, JAK-STAT and MAPK; Pires-da Silva and Sommer, 2003). Overall response to temperature of metabolic and regulatory pathways (*R* statistics using IDEG6, significant threshold of 0.05, corrected for multiple tests using the False Discovery Rate, FDR < 0.1; Romualdi et al., 2003; Stekel et al., 2000) showed different regulation mechanisms and a patchwork of up- and down-regulated steps in some KEGG pathways was observed (Table 2). Furthermore, we tested a subset of simple sequence repeat (SSR) types and nine polymorphic microsatellites were suitable for population genetic studies as described in Weydmann et al., 2014b. In conclusion, we performed de novo transcriptome sequencing of *C. glacialis* incubated at increasing temperatures representing realistic warming scenarios. This pyrosequencing effort provides clues to the identification of genes potentially involved in temperature responses and generates essential molecular tools that will be useful in further genetic and genomic studies of this species.

2.3. Data deposition

The 454 sequence reads of *C. glacialis* were submitted to NCBI Short Read Archive (SRA) under the accession number SRP053198. The assembled transcriptome data were deposited in the European Nucleotide Archive (accession numbers HACJ01000001–HACJ01054344).

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Table 2
Selected list of KEGG biochemical mappings for *C. glacialis* transcriptome data and functional annotation of potential up- and down-regulated genes showing significant differential expression in the temperature experiment.

KEGG pathway	Pathway ID	No of annotated enzymes	Stress regulation[a]
Metabolism			
Glycolysis/gluconeogenesis	00010	18	↓ EC:3.1.3.11, EC:5.1.3.3
Citric acid cycle	00020	21	↑ EC:1.2.4.2, EC:1.1.1.37
Pentose phosphate pathway	00030	14	↓ EC:2.2.1.1, EC:2.7.6.1, EC:3.1.3.11
Oxidative phosphorylation	00190	24	↓ EC:1.6.5.3 U: EC:1.9.3.1, EC:3.6.3.14
Fatty acid elongation	00062	8	↓ EC:2.3.1.199
Fatty acid degradation	00071	14	↓ EC:1.3.8.8, EC:1.3.8.9 ↑ EC:5.3.3.8
Glycerolipid metabolism	00561	12	↓ EC 2.3.1.20
Glyceropholipid metabolism	00564	23	↓ EC:3.1.3.4, EC:2.3.1.23, EC:3.1.1.5
Biosynthesis of unsaturated fatty acids	01040	7	↑ EC:2.3.1.199, EC:1.1.1.100
Purine metabolism	00230	40	↓ EC:2.7.6.1, EC:1.7.3.3 ↑EC:2.7.4.6
Cysteine and methionine metabolism	00270	18	↑ EC:3.1.3.77, EC:1.13.11.20, EC:1.1.1.37
Arginine and proline metabolism	00330	26	↑ EC:1.5.-.-, EC:1.2.1.88
Glutathione metabolism	00480	19	↓ EC:1.1.1.42, EC:4.1.1.17
Genetic information processing			
Ribosome	03010	75	↓ RP-S20e, RP-S24e, RP-S2e ↑ RP-L18e, RP-L22e, RP-L24e, RP-L29e
RNA transport	03013	29	↑ SMT3, EIF4E, PABPC
Proteosome	03050	9	↑ PSMD11, PSMA6, PSMA2, PSMA5
Cellular processes			
Peroxisome	04146	35	↓: EC:1.17.1.4

[a] Down (↓)/up- (↑) regulated genes with temperature increase; *p*-value < 0.05, FDR < 0.1; contigs with more than 20 reads and $\log_2$ (fold change) > 1.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.margen.2015.03.014.

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