

Abschlussbericht

Anschubprojekt:

**„Novel mechanisms of P-dependent energy transductions in an animal
extremophile“**

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* z.B. Beiträge auf Konferenzen, Publikationen (mit Status), Drittmittelanträge (mit Status), auch Ziele (wie Publikationen), welche im (Verlängerungs)antrag angegeben wurden!

1. Zusammenfassung und Schlussfolgerung

Hypoxia is a common stressor in the ocean oxygen minimum zones (OMZ) and is currently enhanced by the global climate change and anthropogenic input of nutrients into the ocean. Most marine metazoans cannot tolerate persistent hypoxia as reflected in mass mortalities and major decreases in biodiversity when the ambient oxygen concentration drops below 2 mg/L. However, a recent study of the OMZ off the Namibian coast led to a discovery of a unique bivalve species, *Lembulus bicuspidatus*, that thrives at extremely low oxygen concentrations (<0.7 mg/L) where it forms abundant populations with high biomass. This ecological success of *L. bicuspidatus* at the OMZ margins implies existence of unique physiological and molecular mechanisms that regulate ATP production and P-dependent energy fluxes under extremely hypoxic conditions. We have sequenced the genome of *L. bicuspidatus* collected at the OMZ margin and obtained sequence information covering ~82% of the *Lembulus* genome. Over 35,000 genes were identified and annotated, and based on these data, specific primers were designed to investigate the transcriptomic response of *L. bicuspidatus* to different oxygen conditions. The ongoing bioinformatics analysis is expected to generate the complete assembly of the mitochondrial genome and a partial (~82%) assembly of the nuclear genome of *L. bicuspidatus*, followed with annotation, phylogenetic and functional analysis. These data will be used as a basis of at least three peer-reviewed publications and a DFG proposal submitted in 2020-2021.

2. Einleitung und Ziele des Projektes

Phosphorus (P) is a key element involved in energy transduction (due to its role in formation of ATP), cell signaling, and regulation of metabolic pathways. Aerobic organisms have high rates of P turnover due to the key role of adenosine triphosphate (ATP) as the universal energy currency of an organism. The P turnover rate and P-dependent energy fluxes in the organism are highly sensitive to environmental stressors, particularly oxygen deficiency (hypoxia). Hypoxia is a common stressor in the ocean oxygen minimum zones (OMZ) and is currently enhanced by the global climate change and anthropogenic input of nutrients into the ocean. Most marine metazoans cannot tolerate persistent hypoxia as reflected in mass mortalities and major decreases in biodiversity when the ambient oxygen concentration drops below 2 mg/L. However, a recent study (led by Dr. Zettler) of the OMZ off the Namibian coast led to a discovery of a unique bivalve species, *Lembulus bicuspidatus*, that thrives at extremely low oxygen concentrations (<0.7 mg/L) where it forms abundant populations with high biomass. **This ecological success of *L. bicuspidatus* at the OMZ margins implies existence of unique physiological and molecular mechanisms that regulate ATP production and P-dependent energy fluxes under extremely hypoxic conditions.** The proposed project uses discovery-oriented approach to identify such potentially adaptive mechanisms in hypoxia-adapted bivalves. For this, we have isolated genomic DNA from frozen tissues of *L. bicuspidatus* and submitted it to the next-generation sequencing (NGS). The ultimate goal of this proposal is to assemble and annotate mitochondrial and nuclear genome of *L. bicuspidatus* and compare it with the published genomes of bivalves not adapted to OMZ margin conditions (the blue mussels *Mytilus galloprovincialis* and the Pacific oyster *Crassostrea virginica*). The study will provide the first genomic resource for the OMZ-adapted macrofauna species and will test the hypothesis that adaptations to the OMZ margin zones is associated with the stressome expansion involving duplications of the genes involved in energy metabolism and stress protection. This investigation will lay foundation for the future research proposal to investigate physiological

and biochemical adaptations of *L. bicuspidatus* to the life at the OMZ margins. Due to the difficulties of conducting physiological studies on live mussels, the experimental studies on the OMZ margin animals require approaches can test functional characteristics on preserved specimens. One such promising approach involves investigations of the transcriptomic and proteomic changes in response to oxygen fluctuations (which will be the part of the proposed project to be submitted to the DFG in 2019), and genome sequence of *L. bicuspidatus* is an essential prerequisite for such studies. Therefore, our pilot study will serve to: 1) generate the novel data about the genome-based metabolic adaptations of *L. bicuspidatus* to the extreme conditions of the OMZ margin; 2) develop the genomic tools for a future externally funded project to study novel mechanisms of P-dependent energy transductions in an animal extremophile from the OMZ margin zone.

3. Material und Methoden

We have isolated and purified the total DNA from *L. bicuspidatus* obtained during a recent expedition to Namibia by Dr. Zettler. Due to the requirements of the NGS, high purity and integrity of DNA was required to generate sequencing library. Because of the small size of *L. bicuspidatus* and the problems with maintaining genomic DNA integrity in frozen samples during transport from Namibia to Germany, the isolation and purification of DNA had to be repeated several times from a total of 12 animals to obtain the necessary DNA quality for sequencing. The purified DNA has been submitted for the NGS to Macrogen Inc. that conducted DNA library preparation, HiSeq de novo sequencing with PacBio gap closing and draft genome assembly.

The NGS services were delayed due to the technical issues at Macrogen Inc., and we received the sequence data only in October 2019. Approximately 2,350,000,000+ paired-end reads that passed 90% quality cut-off were generated, assembled into ~62,500+ contigs spanning ~82% of its (estimated) 1,55 Gb genome, with N50 of about 27,000 bp. Genome assembly was performed with wtdbg2.

4. Ergebnisse

Identification of putative protein-coding genes

Assembled *Lembulus* contigs were searched for matches to putative protein-coding genes using available predicted proteins from genome assemblies of *Crassostrea virginica* (3.0; https://www.ncbi.nlm.nih.gov/assembly/GCF_002022765.2/), *C. gigas* (https://www.ncbi.nlm.nih.gov/assembly/GCF_000297895.1/) and *Mytilus galloprovincialis* (https://www.ncbi.nlm.nih.gov/assembly/GCA_001676915.1/). TBLASTN search with a relaxed cut-off value of 0.001 was used to ensure that even distantly homologous sequences are identified. Because *C. virginica* has the best annotated assembly of the three taxa, we have used *virginica* genes as references for our analyses. Of 60,213 predicted *C. virginica* proteins, 47,149 had homologs with *C. gigas* (BLASTP with E-value cut-off of 0.001), and 47,244 had matches to *Lembulus* sequences. Homologs of 37,717 *C. virginica* sequences were shared among all three genomes (shared-3 subset), and additional 9527 sequences were shared only between *C. virginica* and *Lembulus*. Notably, of the former (shared-3) subset, 11,309 *C. virginica* genes are annotated as “uncharacterized”, while the other 26,408 genes had at least some functional characterization available. Of these characterized genes, 21,505 passed the stricter cut-off E-value of 10^{-10} , suggesting that these are likely homologous. Of these sequences, 195 were metabolic and stress-related genes of interest

used for further functional analyses. Currently, a PhD student (Katherine Amorim) working under the supervision of Dr. Zettler, co-supervised by Dr. Sokolova is performing the RT-qPCR studies to determine the effects of different oxygen levels on expression of key metabolic and stress protection-related genes in *Lembulus* using the gene-specific primers designed based on the newly identified *Lembulus* gene sequences. This work will be a part of her PhD thesis and is expected to result in a peer-reviewed publication.

Final genome assembly of *Lembulus*

The work is currently in progress to improve the complete genome assembly of *Lembulus decuspidatus* based on the generated sequence reads. Dr. Piontkivska, as a bioinformatics expert, is the lead on this part of the project. Once the final genome draft is obtained, Dr. Piontkivska and Dr. Sokolova will collaborate to produce the functional annotation and GO classification of the genes in *Lembulus* genome. The assemble genome and separate contigs will be submitted to the open-access NCBI database and will serve as a potential resource for other scientists interested in physiology, molecular ecology and evolution of *Lembulus*.

5. Diskussion

The current project has successfully generated a draft genome of a hypoxia-tolerant marine bivalve, *Lembulus bicuspidatus*, with unique ecology and potentially unique physiology. Over 35,000 of *Lembulus* genes were identified and annotated based on the homology with other bivalves. Based on these data, we designed specific primers that a PhD student currently uses to assess the transcriptomic response to different oxygen levels in this species. Due to the massive amount of the generated sequence data, the data analysis is still ongoing and is expected to generate significant contributions to the functional genomics of this species over the next two years.

6. weitere Leistungen und Ziele aus dem Projekt

The project is expected to generate at least three peer-reviewed publications with the provisional titles: 1) “Mitochondrial genome of a hypoxia-tolerant bivalve *Lembulus bicuspidatus* from the oxygen minimum zone of the Northern Benguela upwelling system”; 2) “Genome assembly of a hypoxia-tolerant bivalve *Lembulus bicuspidatus* from the oxygen minimum zone of the Northern Benguela upwelling system”; 3) “Effects of hypoxia-reoxygenation stress on metabolic and stress gene expression of a hypoxia-tolerant bivalve *Lembulus bicuspidatus* from the oxygen minimum zone of the Northern Benguela upwelling system”. Manuscripts 1 and 3 are expected to be submitted in 2020, and manuscript 2 (requiring lengthy bioinformatics analysis) is planned for 2021.

The preliminary data generated in the current project will serve as a basis for a DFG research proposal entitled “Evolution of the bivalve mitochondria in low oxygen environments”. We have requested a Mare Balticum travel fellowship for Prof. Piontkivska to visit the University of Rostock in spring 2020 to work on the joint proposal development. The proposal will aim to analyze mitochondrial genomes of 160+ species of mollusks that broadly differ in their tolerance to hypoxia, identify the potential targets for selection that may contribute to high hypoxia tolerance in marine bivalves, and test the functional consequences of the putative adaptive changes by measuring activities of the relevant mitochondrial enzymes from selected species with different degree of mitochondrial tolerance. Mitochondrially-encoded proteins are fundamental players of the oxidative phosphorylation

machinery, with genes encoding multiple protein subunits of complex I (CI, NADH:ubiquinone oxidoreductase) and complex IV (CIV, cytochrome c oxidase) being shared across mitochondrial genomes. Using publicly available mitochondrial genomes, we will reconstruct phylogenetic trees of individual and concatenated subunits to determine the distribution of hypoxia tolerance phenotype(s) across broad groups of bivalves. Using the genome reads generated in the current pilot project, we will assemble a novel, previously unknown, mitochondrial genome of a representative *Lembulus* species, which is characterized by high hypoxia tolerance. Predicted mitochondrial genes of *Lembulus* will be contrasted with genes from other species to determine molecular evolutionary patterns, including signature of positive selection that can be associated with the degree of hypoxia tolerance across bivalves, to enable subsequent testing of their physiological relevance.

7. Danksagung

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Anhang

List of the selected annotated sequences of *Lembulus bicuspidatis* generated in this project.