Science of the Total Environment 817 (2022) 152749

Contents lists available at ScienceDirect

Science of the Total Environment

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

How to thrive in unstable environments: Gene expression profile of a riparian earthworm under abiotic stress

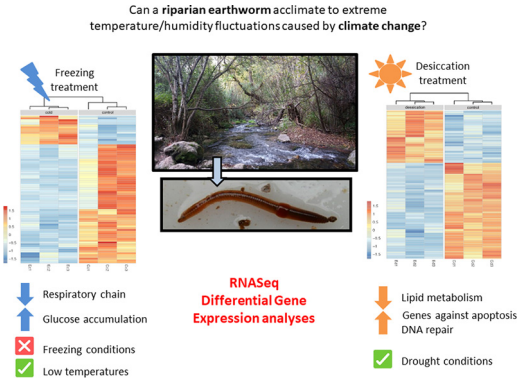
Irene de Sosa [a,*], Aída Verdes [b], Natasha Tilikj [a], Daniel F. Marchán [c], Rosario Planelló [d], Óscar Herrero [d], Ana Almodóvar [a], Darío Díaz Cosín [a], Marta Novo [a]

[a] Biodiversity, Ecology and Evolution Department, Faculty of Biology, Complutense University of Madrid, C/José Antonio Nováis 12, 28040 Madrid, Spain
[b] Department of Biodiversity and Evolutionary Biology, Museo Nacional de Ciencias Naturales, Consejo Superior de Investigaciones Científicas, C/Jose Gutiérrez Abascal 2, 28006 Madrid, Spain
[c] CEFE, UMR 5175, CNRS–Univ Montpellier–Univ Paul–Valéry–EPHE–SupAgro Montpellier–INRA–IRD, Montpellier, France
[d] Biology and Environmental Toxicology Group, Faculty of Science, Universidad Nacional de Educación a Distancia (UNED), Campus UNED Las Rozas, Avda. Esparta s/n, 28232, Las Rozas de Madrid, Madrid, Spain

HIGHLIGHTS

- RNA seq analyses were performed in earthworms under cold and desiccation stress.
- *E. tetraedra* adults can acclimate to low temperatures but do not tolerate freezing.
- Genes against apoptosis and DNA repair were upregulated in low humidity.
- Lipid metabolism and transport were downregulated under desiccation conditions.
- No common responses were found between cold and desiccation treatments.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 9 November 2021
Received in revised form 17 December 2021
Accepted 24 December 2021
Available online 3 January 2022

Editor: Jay Gan

Keywords:
Climate change
Cold
Desiccation
Eiseniella tetraedra
Transcriptomics

ABSTRACT

Nowadays, extreme weather events caused by climate change are becoming more frequent. This leads to the occurrence of extreme habitats to which species must adapt. This challenge becomes crucial for species living in unstable environments, such as the riparian earthworm *Eiseniella tetraedra*. Its cosmopolitan distribution exposes it to various environmental changes, such as freezing in subarctic regions or droughts in Mediterranean areas. Transcriptional changes under cold and desiccation conditions could therefore shed light on the adaptive mechanisms of this species. An experiment was performed for each condition. In the cold experiment, the temperature was lowered to −14 °C ± 2 °C (compared to 8 °C for control samples), and in the desiccation treatment, humidity was lowered from 60% to 15%. Comparisons of gene expression levels between earthworms under freezing conditions and control earthworms revealed a total of 84 differentially expressed genes and comparisons between the desiccation experiment and the control yielded 163 differentially expressed genes. However, no common responses were found between the two treatments. The results suggest that *E. tetraedra* can acclimate to low temperatures due to the upregulation of genes involved in glucose accumulation. However, downregulation of the respiratory chain suggests that this earthworm does not tolerate freezing conditions. Under desiccation conditions, genes involved in cell protection from apoptosis and DNA repair were upregulated. In contrast, lipid metabolism was downregulated, presumably to conserve resources by reducing the rate at which they are consumed.

* Corresponding author.
E-mail address: iscarrasco@ucm.es (I. de Sosa).

http://dx.doi.org/10.1016/j.scitotenv.2021.152749

1. Introduction

Recent climate change has impacted a wide range of organisms worldwide, responding at population and species levels with changes in phenology, physiology, and range shifts, as well as changes in community and ecosystem structure and dynamics (Walther et al., 2002; Brown et al., 2016). Projected climate changes are likely to significantly affect the spatial extent, distribution, and function of wetlands (IPCC, 1992). Changes in precipitation will alter water availability, river flows and affect ecosystem productivity. Populations of aquatic organisms are sensitive to the effects of floods and droughts (Dawson et al., 2003). In assessing recent climate change and that projected for the coming decades, the 3rd IPCC report on climate change (Houghton et al., 2001) notes that increasing summer drought over most continental mid-latitude areas and associated drought risk was likely in the 20th century and is to continue in the 21st century (Dubrovsky et al., 2009). Moreover, the importance of winter conditions is often overlooked, especially in temperate ecology (Kreyling, 2010). A single extreme cold event can offset all distributional adjustments to the general warming trend (Jalili et al., 2010). These extreme weather events have increased in recent years due to climate change. This is creating new extreme habitats for species, which need to develop adaptive mechanisms for their survival.

Earthworms are probably the most important animals of the soil biota in terms of soil formation and maintenance of soil structure and fertility. Their activities are important for maintaining soil fertility in a variety of ways in forest, grassland and agroecosystems (Edwards, 2004). The local species richness and abundance of earthworm communities typically peak at mid-latitudes, and their global distribution is mostly determined by climatic variables and habitat cover rather than soil properties Phillips et al. (2020), suggesting that climate and habitat changes can have serious impacts on earthworm communities and the functions they provide.

Soil moisture and temperature are the main factors limiting earthworm survival, growth and reproduction (Lee, 1985). Below a critical soil moisture level, there is an adverse osmotic effect (desiccation) on earthworms (Grant, 1955). This critical moisture level depends on the physical properties of the soil and it is species-specific, as desiccation tolerance varies among earthworm species (Grant, 1955; Buckman and Brady, 1969; Lee, 1985). Moderately low soil moisture decreases aerobic metabolism (Diehl and Williams, 1992), fertility (Reinecke and Venter, 1987) and whole organism growth rate (Viljoen and Reinecke, 1989), whereas high soil moisture lowers fertility and growth rate (Reinecke and Venter, 1987). Levels beyond the critical point for desiccation, interfere mainly with the respiratory system (Williams and Diehl, 1992) due to both the difference in oxygen diffusion rate in water compared to air (Cameron, 1986) and the effects of soil moisture on cutaneous oxygen uptake (Lee, 1985).

Temperature also affects earthworm physiology and ecology (Lee, 1985), with upper thermal limits for survival varying among species, ranging from 25 to 33 °C (Wolf, 1938; Miles, 1963). When temperatures are low, earthworms employ two main strategies: (1) freeze avoidance either by migration or physiological adaptation, such as the synthesis of cryoprotectants to avoid the formation of ice within the animal; and (2) allowing the formation of extracellular ice (Mazur, 1963; Holmstrup and Zachariassen, 1996). Glucose loading appears to be essential for freezing tolerance in earthworms, but other factors may also be involved (Berman and Leirikh, 1985; Holmstrup and Petersen, 1997; Holmstrup et al., 1999). According to Holmstrup (2014) there seems to be a common response between cold tolerance and desiccation tolerance in soil invertebrates, such as initiating same modifications in membrane composition.

Eiseniella tetraedra (Savigny, 1826) is a parthenogenetic earthworm (Casellato, 1987) with a cosmopolitan distribution (Blakemore, 2006), being found in a variety of climates, such as mediterranean or subarctic, and thus coping with a wide range of climatic conditions. It is a semi-aquatic earthworm (Omodeo and Rota, 1991) and inhabits both stable habitats, such as rivers or streams, and unstable waters, which may be frozen or dried up depending on the season. The abundance of *E. tetraedra* populations can reach 1000 ind/m^2 (Malevich, 1956) and it occurs mainly in soils with mull humus, under leaf litter or moss in the upper 2 cm of the soil profile (Terhivuo et al., 1994). In the Russian habitats studied by Barne and Striganova (2005), adults predominated in the populations, accounting for 70–75% of the total abundance. The proportion of juveniles and subadults ranged from 10 to 16%. This earthworm can be adapted to laboratory cultures with relatively high survival and reproduction rates under certain temperature and moisture conditions, 17–18 °C and 90% humidity (Barne and Striganova, 2005).

RNA-Seq is a powerful high-throughput sequencing method that provides rapid and comprehensive gene expression data (Chen et al., 2017; Jin et al., 2017). It is an effective way to identify a range of novel protein-coding and non-coding genes/transcripts of organisms of interest (Roberts et al., 2011; Pauli et al., 2012; Chettoor et al., 2014). Additionally, RNA-Seq technology can detect gene transcription, characterize gene expression, and discover novel genes and biomarkers at the aggregate level (Diao et al., 2019). Advances in high-throughput sequencing technology along with relevant bioinformatics tools and pipelines have allowed us to study the transcriptome profile of non-model species (Ekblom and Galindo, 2011). Unfortunately, whole transcriptomic studies for earthworms are rare. However, Paul et al. (2018) examined differential gene expression under freezing and warm conditions in *Dendrobaena octaedra* (Savigny, 1826), identifying numerous genes involved in the response to each abiotic stressor, but very few shared between the two conditions.

The aim of this study was to explore i) the transcriptional changes in the riparian earthworm *E. tetraedra* during freezing and ii) during desiccation conditions. Also, iii) to investigate possible common responses to both abiotic stressors.

2. Material and methods

2.1. Experimental animals and laboratory conditions

In February 2018 we collected by manual sorting juveniles and adults of *Eiseniella tetraedra* in the surroundings of Fuente de las Hondillas, in Guadarrama, Madrid, Spain (40°41′50.79″N, 4° 8′22.75″ O). In order to establish a culture of *E. tetraedra* in the laboratory we prepared boxes with dry soil from the collection site and distilled water up to 60% humidity. We stored the earthworms in this medium at 8 °C for 1 year and 4 months, changing soil every month approximately. We tried other culture methods, such as the one followed by Barne and Striganova (2005) or those described in Supplementary Table 1 but earthworms died.

2.2. Freezing experiment

Eight mature earthworms from the laboratory culture were weighted and ranged between 0.13 g and 0.28 g. Eight petri dishes were prepared with 30 g of soil and distilled water at 60% of humidity. One earthworm was placed on each dish and they were introduced in a freezer at −14 °C ± 2 °C. The reaction of earthworms was checked every 10 min for the first 2 h and every 20 min afterwards for less manipulation by touching them with tweezers for a further 3 h. Then, earthworms were returned to the initial control conditions and two of them died the next day. Final conditions for the RNA-Seq experiment were based on the results from this preliminary trial (Supplementary Table 2) and earthworms were exposed to freezing conditions for 1 h (the first time point at which all of the earthworms were less reactive). For the RNA-Seq experiment six petri dishes were prepared with 30 g of soil and distilled water (60% moisture). Six mature earthworms from laboratory culture were weighted and ranged between 0.08 g and 0.22 g. One earthworm was introduced per petri dish; three of them were placed at 8 °C (control) and three of them were introduced in the freezer at −14 °C ± 2 °C (cold). After 1 h all individuals were flash frozen in dry ice and stored at −80 °C (Fig. 1).

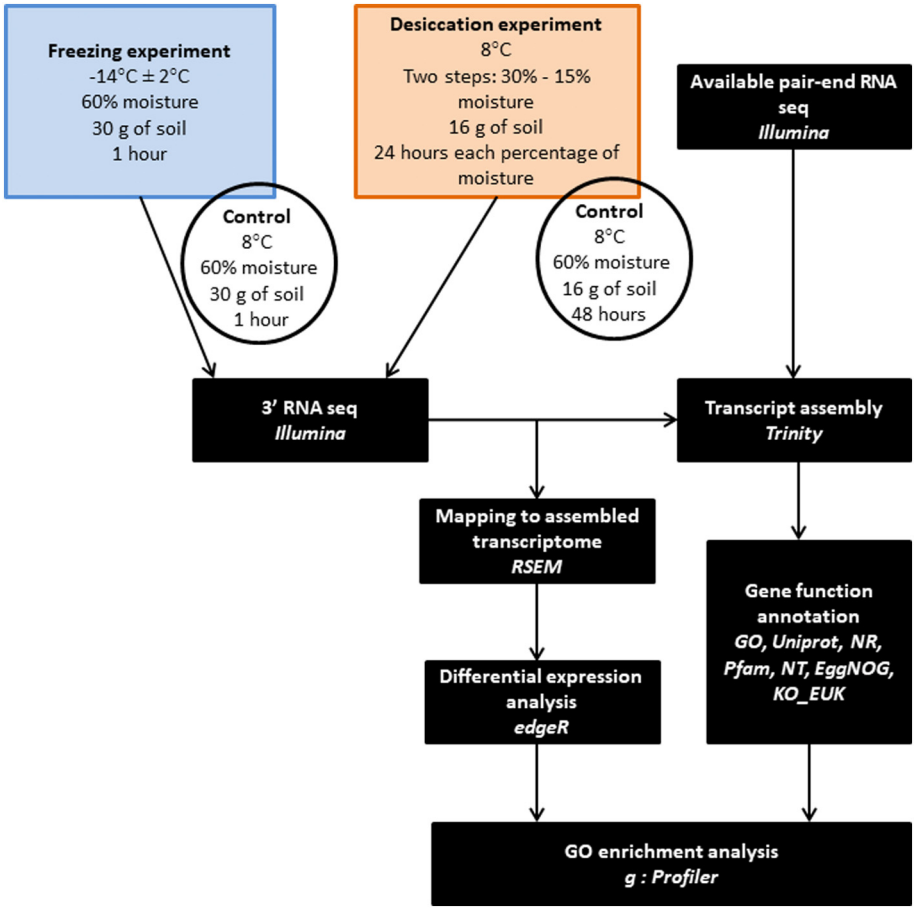


Fig. 1. Schematic representation of the experimental design and data analysis workflow performed for desiccation and cold conditions in the earthworm *Eiseniella tetraedra*.

2.3. Desiccation experiment

Similar to the freezing experiment, a preliminary experiment was conducted to understand the limiting desiccation conditions for earthworms (Supplementary Table 3). For this, we performed a two-step experiment for lowering the humidity in a more realistic way. Eight petri dishes were prepared with 16 g of soil and distilled water at 30% moisture. One earthworm (weight range 0.13–0.27 g) was placed per plate and kept at 8 °C for 24 h. Then, we placed the same earthworms in new petri dishes with soil at 15% moisture and kept them at 8 °C for 24 h. After this preliminary experiment, the worms were really affected, but they recovered after they were put in the control conditions again. Therefore, the conditions for the RNA-Seq experiment were exactly the same as in this preliminary experiment. Six petri dishes were prepared with 16 g of soil. Six mature earthworms from the laboratory culture were weighted and ranged in weight from 0.16 g to 0.39 g. One earthworm was introduced per petri dish; three of them at 30% moisture (desiccation) and three at 60% moisture (control) and kept at 8 °C for 24 h. To ensure that all individuals had the same manipulation, the six earthworms were placed in new petri dishes; three of them (desiccation) at 15% humidity and three (control) at 60% humidity and kept at 8 °C. After 24 h, all individuals were flash frozen in dry ice and stored at −80 °C (Fig. 1).

2.4. RNA extraction and species verification

The frozen tissue from six earthworms per experiment was powdered with a mortar and pestle and 50 mg were used for RNA and DNA extractions. Powdered tissue was homogenized in 1.5 ml of Trizol (Invitrogen) and RNA was extracted according to the manufacturer's protocol. Subsequently, samples were treated with RNAse-free DNAse (Roche) for 90 min and organic extraction was performed using phenol-chloroform-isoamyl alcohol and Phase Lock Gel Light tubes (5Prime). RNA integrity was verified using Agilent 2100 Bioanalyzer and quality and concentration of RNA was estimated with Nanodrop. For DNA extraction, the organic phases left from the RNA extractions were used and DNA was precipitated with ethanol.

A fragment of cytochrome *c* oxidase subunit I (COI) was amplified using primer sequences and polymerase chain reactions (PCR) following Pop et al. (2003). All PCRs were specific and resolved via 1% agarose gel electrophoresis; they were visualised with GelRed stain (Biotium). All products were purified using ExoSAP-IT reagent (ThermoFisher Scientific). PCR products were sequenced by Macrogen Spain Inc. and species identity was verified through blasting with previous sequences from De Sosa et al. (2017).

2.5. Transcriptome sequencing

Once verified the identity of the animals for which RNA was extracted, three samples per treatment were further processed for RNA-seq. 3′RNAseq libraries were prepared from ~500 ng of total RNA per sample using the Lexogen QuantSeq 3′ mRNA-Seq Library Prep Kit FWD for Illumina (https://www.lexogen.com/quantseq-3mrna-sequencing/). The libraries were quantified on a Molecular Devices Spectra Max M2 plate reader (with the intercalating dye QuantiFluor) and pooled accordingly for maximum evenness. The pool was quantified by digital PCR and sequenced on one lane of an Illumina NextSeq500 sequencer, single-end 1x86bp, and de-multiplexed based upon six base i7 indices using Illumina bcl2fastq2 software (version 2.18; Illumina, Inc., San Diego, CA). Illumina adapters,

poly-A tails, and poly-G stretches of at least 10 bases in length were removed from the de-multiplexed fastq files using the BBDuk program in the package BBMap (https://sourceforge.net/projects/bbmap/), keeping reads at least 18 bases in length after trimming. Poly-G stretches result from sequencing past the ends of short fragments (G = no signal).

2.6. Establishing a reference transcriptome

Trinity v2.8.4 (Grabherr et al., 2011) was used for establishing two different reference transcriptomes. Firstly, a transcriptome was assembled with default options using available 101 bp paired-end reads from an ongoing work (Planelló and Herrero unpublished, ENA reference number PRJEB49685 (ERP134202)). These reads were sequenced from an individual of *E. tetraedra* collected in North Western Spain. A second transcriptome was generated by combining the mentioned paired-end reads with 3´RNAseq reads from this experiment in order to check whether we could improve mapping. We normalised reads according to Trinity protocol (insilico_read_normalization.pl) and then combined 3´RNAseq reads with R1 from the paired-end set and proceeded with the assembly including the R2. We then assessed both assemblies with BUSCO using metazoa_odb10 database (Seppey et al., 2019) and performed some mapping trials with Bowtie 1 and 2 (Langmead et al., 2009; Langmead and Salzberg, 2012). We found that the combined transcriptome included too much duplication, which provoked a dispersion of the reads in the mapping and therefore lower mapping rates (398,361 contigs, 91% complete BUSCOS, 16% complete and single, 75% complete and duplicate). The transcriptome assembled only with the pair-end reads on the other hand showed lower duplication rates and higher mapping (143,339 contigs, 91% complete BUSCOS, 87% complete and single, 4% complete and duplicate). For this reason, we decided to continue the analyses with the transcriptome assembled only with the paired-end reads.

2.7. Quantitative transcriptomic analysis

A differential gene expression analysis was performed with the Trinity module (Grabherr et al., 2011) which integrates Bowtie 1.3.0 (Langmead et al., 2009) to align input reads from each individual library against the reference transcriptome, RSEM (Li and Dewey, 2011) to estimate transcript abundance using the alignments, and edgeR (Robinson et al., 2010) to perform pairwise comparisons of expression levels and extract Differentially Expressed Genes (DEGs). edgeR was run with a *p*-value cut off of 0.001, a false discovery rate <0.05 and min abs(log2(a/b)) change of 1 (therefore, minimally, 2-fold change). Principal component analyses (PCAs) were performed and plotted with the same package to visualize variation of expression levels among samples and treatments (Supplementary Figs. 1 and 2).

Enrichment analyses were performed in g: Profiler (Reimand et al., 2019) using the detected differentially expressed transcripts (FDR < 0.05) for each experiment, specifically for upregulated and downregulated genes subsets separately, using the complete transcriptome of *E. tetraedra* as reference. GO terms with adjusted *P* value <0.05 were considered significantly enriched.

3. Results

3.1. Sequencing output

Reads were submitted to the European Nucleotide Archive (ENA) under study number PRJEB46360 (Samples ERS6655476-ERS6655487). For each sample, 2–40 million clean single-ended reads were used for analyses (Supplementary Table 4). Between 45 and 65% of the reads from each sample mapped against the reference transcriptome, and were therefore informative for subsequent quantitative analyses.

3.2. Differential gene expression under freezing conditions

Hierarchical clustering analysis of the differentially expressed transcripts revealed differences in gene expression between control earthworms and those under freezing conditions (Fig. 2A). A complete list of DEGs for this experiment is shown in Supplementary File 2. Comparisons of gene expression levels between earthworms under freezing conditions and control earthworms generated a total of 84 DEGs. Of those, 17 DEGs were upregulated, while 67 were downregulated and a total of 21 could be functionally annotated (Table 1). Among the downregulated DEGs, seven genes related to the respiratory chain were expressed: chains 4, 5, and 6 of NADH-ubiquinone oxidoreductase, cytochrome *c* oxidase (subunits 1 and 2), cytochrome *b*, and ATP synthase subunit a. Moreover, glutamyl aminopeptidase and C-type lectin domain family 4 member D were also found downregulated. In contrast, the genes encoding giant extracellular hemoglobin linker 2 chain, uridine-cytidine kinase-like 1, GPI-linked NAD (P)(+)-arginine ADP -ribosyltransferase 1-like (GLP1R) and lectin, were upregulated. To improve our knowledge of the biological functions represented by DEGs, a GO enrichment analysis was performed (Fig. 2B). Among the downregulated DEGs, nine GO terms belonging to the Biological Processes (BP) category were enriched. Transcriptional changes under freezing conditions were characterized in *E. tetraedra* by downregulation of aerobic respiration (GO:0009060), including several steps of this process, such as oxidative phosphorylation (GO:0006119) which is an ATP biosynthetic process (GO:0006754), proton transmembrane transport (GO:1902600) and mitochondrial electron transport, cytochrome *c* to oxygen (GO:0006123). In addition, purine biosynthesis processes were downregulated (GO:0009206 and GO:0009145). In contrast, pyrimidine ribonucleotide and nucleotide salvage (GO:0010138 and GO:0032262) and pyrimidine biosynthesis and metabolism processes such as the pyrimidine ribonucleoside triphosphate biosynthesis process (GO:0009209) and the pyrimidine ribonucleoside monophosphate metabolism process (GO:0009173) were upregulated. As for the molecular function category, uridine kinase activity (GO:0004849) and nucleoside kinase activity (GO:0019206) were upregulated. As expected, most of the GO terms belonging to the category of molecular functions were consistent with the downregulation of aerobic cellular respiration. Thus, detected that genes associated with seven aerobic cellular respiration-related GO terms were downregulated, such as proton transmembrane transporter activity (GO:0015078), cytochrome *c* oxidase activity (GO:0004129), and electron transfer activity (GO:0009055), among others (Fig. 2B). Genes implied in copper ion binding (GO:0005507) were also downregulated.

3.3. Differential gene expression under desiccation conditions

Hierarchical clustering analysis of the differentially expressed transcripts revealed clearly differences in gene expression between control and earthworms under desiccation conditions (Fig. 3A). Comparison of genes between earthworms under desiccation conditions and control earthworms yielded a total of 163 differentially expressed genes (DEGs), with 58 upregulated and 105 downregulated, of which we could functionally annotate 43 (Table 2). For a complete list of DEGs see Supplementary File 2. Several genes were found upregulated under desiccation conditions, including genes encoding enzymes involved in protein modification, such as UDP-*N*-acetylglucosamine-peptide-*N*-acetylglucosaminyltransferase (110 kDa subunit), which glycosylates a large and diverse number of proteins, and peptidyl-prolyl cis-trans isomerase B. In addition, maternal embryonic leucine zipper kinase, which is involved in various processes such as cell cycle regulation, stem cell self-renewal, apoptosis, and splicing regulation, and the protein CREBRF homolog, a transcriptional regulator that acts in the TORC1 pathway to regulate energy homeostasis and promote survival during nutrient deprivation, were also upregulated. In contrast, several genes involved in protein transport and degradation were downregulated, including sorting nexin-27 and UBX domain-containing protein. Apoptosis regulators such as serine/threonine protein kinase 17A were also found downregulated. To improve our knowledge of the biological functions represented by DEGs, a GO enrichment analysis was performed (Fig. 3B). We only found enriched GO terms belonging to the Molecular Function (MF) among the downregulated DEGs. Enriched GO terms were related to dehydrogenase activities such as testosterone dehydrogenase (NAD +) activity

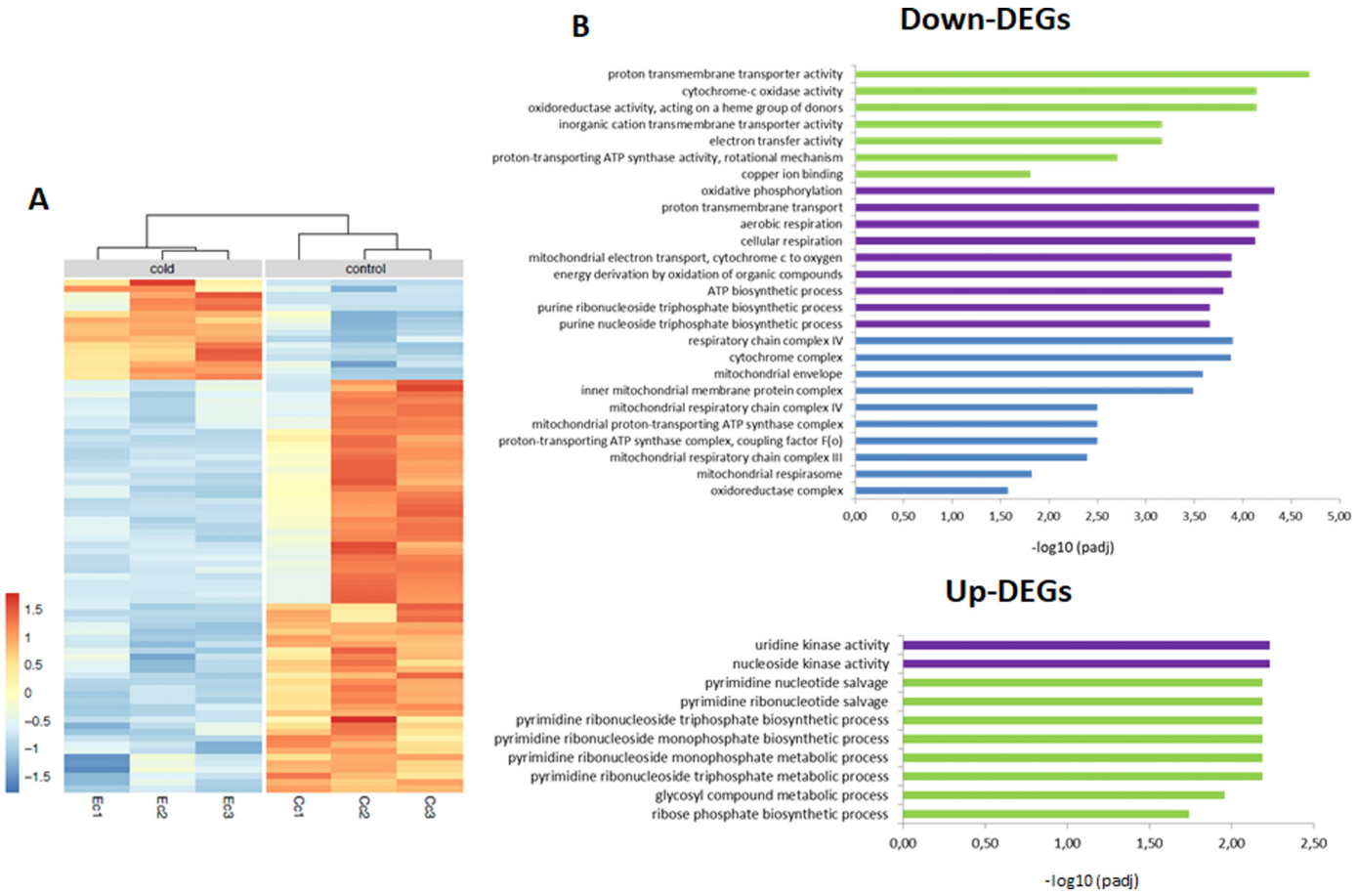


Fig. 2. A: Hierarchically-clustered heatmap of all differentially expressed genes under freezing conditions in the earthworm *Eiseniella tetraedra*. Red color indicates high expression values and blue color indicates low expression values. B: Results of GO enrichment analysis performed with differentially expressed genes (DEGs). Terms in the Molecular Function (MF) category are shown in green, Biological Processes (BP) in purple, and Cellular Components (CC) in blue. All shown GO terms were significantly enriched (adjusted *p* value <0.05). For a complete list of results of GO enrichment analysis see Supplementary File 3. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(GO:0047035) and alcohol dehydrogenase [NAD (P)+] activity (GO:0018455). Also fucosyltransferase activity (GO:0008417), such as galactoside 2-alpha-L-fucosyltransferase activity (GO:0008107). In addition, GO terms such as protein folding chaperone (GO:0044183) and long chain fatty acid transporter activity (GO:0005324) appeared enriched. Isomerase activity (GO:0016853) and racemase and epimerase activity (GO:0016854) were also enriched among the downregulated DEGs.

4. Discussion

A complex machinery of gene expression and biochemical adaptive responses is induced by thermal stress in most organisms (Lindquist, 1986; Fujita, 1999). This response enables organisms to survive and adapt to thermal stress and it is a fundamental requirement for species exposed to temperature fluctuations, such as *Eiseniella tetraedra*. Its cosmopolitan distribution implies survival in a wide range of climatic conditions. Cold and desiccation have many similar effects at the cellular level in other invertebrates, such as dehydration and osmotic stress (Sinclair et al., 2013; Paul et al., 2018). However, we found no common responses of both treatments in *E. tetraedra*. Further experiments will unveil whether those common responses exist.

4.1. Cold tolerance

Even though cold shock response has been studied for several decades in a number of different organisms, we have only recently begun to understand the molecular mechanisms that control the adaptation to cold stress. Remarkably, all organisms, from prokaryotes to plants to higher eukaryotes, respond to cold shock in comparatively similar ways (Al-Fageeh and Smales, 2006).

Post-translational modifications of cellular proteins by ADP-ribosylation have been associated with a number of cellular processes (Fabrizio et al., 2015). The *ART1* gene, which encodes the protein GPI-linked NAD (P)(+)-arginine ADP-ribosyltransferase 1-like, was found to be upregulated in our freezing experiments. It has ADP -ribosyltransferase activity towards the glucagon-like peptide-1 receptor (GLP1R), which is involved in the control of blood glucose levels. In mammals, GLP1R has the ability to induce insulin secretion in response to high glucose levels (Gutniak et al., 1992). In general, ADP-ribosyltransferases inhibit the function of the target protein (Álvarez, 2004). Since the accumulation of glucose can slow down the freezing process and even reduce the amount of ice formed (Holmstrup, 2003), it seems reasonable that *ART1* is upregulated to inhibit the function of GLP1R and thus accumulate glucose to cope with freezing temperatures.

Earthworms have cutaneous respiration; they perform gas exchange through the skin. Therefore, air dissolves on the mucus of their skin, so they must remain moist to breathe. The skin of *E. tetraedra* was nearly frozen during the freezing experiment, causing it to lose much of its moisture and making gas exchange more difficult. The giant extracellular hemoglobin of annelids has been studied in detail because of its exceptional oxygen transport properties. Interestingly, expression of the gene for giant extracellular hemoglobin of *Eisenia fetida* Eisen, 1874 is induced in the regenerating tissue (Bhambri et al., 2018). It is possible that the upregulation of the gene encoding the linker 2 chain of extracellular giant hemoglobin contributes to

Table 1
Annotated differentially expressed genes (DEGs) for the cold experiment in the earthworm *Eiseniella tetraedra*. Positive log fold change values (logFC) represent upregulated DEGs (in dark blue), while negatives represent downregulated DEGs (in light blue).

Gene	Protein	logFC
	GPI-linked NAD(P)(+)--arginine ADP-ribosyltransferase	
ART1	1-like	3,66501667
LEC	Lectin	2,71902448
UCKL1	Uridine-cytidine kinase-like 1	7,82968242
N/A	Giant extracellular hemoglobin linker 2 chain	2,53081466
ENPEP	Glutamyl aminopeptidase	−5,18492199
Clec4d	C-type lectin domain family 4 member D	−3,47079202
COI	Cytochrome c oxidase subunit 1	−7,47972063
MT-CYB	Cytochrome b	−7,15222671
ND5	NADH-ubiquinone oxidoreductase chain 5	−7,93382089
ND6	NADH-ubiquinone oxidoreductase chain 6	−7,4433782
ATP6	ATP synthase subunit a	−6,40678916
N/A	Vasotocin-neurophysin VT 1	−4,4569175
N/A	Genome polyprotein	−4,80785628
	Retrovirus-related Pol polyprotein from type-1	
N/A	retrotransposable element R2	−5,99953981
Ttn	Titin	−7,90297656
COII	Cytochrome c oxidase subunit 2	−6,25792581
ND4	NADH-ubiquinone oxidoreductase chain 4	−7,08596604

the enhancement of respiration in *E. tetraedra* at low temperatures due to its oxygen carrier activity. Also, it may be involved in tissue repair due to its destruction by freezing.

Uridine kinase activity (GO:0004849) is involved in the pyrimidine salvage pathway (GO:0032262), and both molecular functions have been upregulated. In the cell, nucleotides are produced either by the de novo pathway or by the salvage pathway. Uridine kinase phosphorylates uridine to uridine monophosphate using ATP as a phosphate donor (Kashuba et al., 2002). It has been suggested that de novo synthesized UTP is preferentially used for the production of UDP-sugars and phospholipids, whereas UTP produced via the salvage pathway is used for RNA synthesis (Anderson and Parkinson, 1997). There seems to be general agreement in the literature that increased RNA and protein synthesis may be part of the resistance mechanism of plants to low temperatures (Sarhan and D'aoust, 1975). Ribosomal RNA has been shown to be the RNA class most responsive to the physiological stimuli that causes acclimation (Khan et al., 1968; Brown, 1972; Gusta and Weiser, 1972). This is consistent with the upregulation of pyrimidine ribonucleotide salvage (GO:0010138) found in the freezing experiment.

The C-type lectin (CTL) protein family has been extensively studied in both vertebrates and invertebrates (Wei et al., 2010). CTL is Ca^{2+} dependent, and all members of the CTL family of invertebrates have one or more carbohydrate recognition domains (CRD) (Li et al., 2020a). CTLs from *Bombyx mori* Linnaeus, 1758 play an important role in the activation of immune signaling pathways (Zhu et al., 2016). They are also associated with tissue regeneration (Gao et al., 2017). In addition, it has been demonstrated that there is some relationship between herring antifreeze protein (AFP) type II and the carbohydrate binding site of C-type lectin CRDs. The ice binding site of AFP corresponds to the CRD binding site of CTL, showing that the carbohydrate binding site of C-type lectin evolved into an ice binding site (Ewart and Fletcher, 1993; Ewart et al., 1998). Thus, CTL has a potential function in low temperature tolerance and cold resistance. In samples of *E. tetraedra* subjected to cold treatment, the gene *CLEC4D* encoding C-type lectin domain family 4 member D was downregulated, similarly to the transcription of CTL mRNA in the liver of *Oreochromis niloticus* Linnaeus, 1758 under cold stress, which is significantly decreased (Yang et al., 2016). However, the expression level of CTLs in rainbow trout was hardly affected by cold stress (Rebl et al., 2012) and in *Venerupis* (*Ruditapes*) *philippinarum* (Adams and Reeve, 1850) low-temperature stress promotes the synthesis of CTLs in the hepatopancreas, which could explain the upregulation of the *LEC* gene in *E. tetraedra* that may be involved in protection of eggs and embryos against microorganisms.

From our results, we can conclude that the respiratory chain of *E. tetraedra* is clearly affected under cold conditions. This mechanism appeared also downregulated under other stress conditions such as different light intensities (Li et al., 2020b) or anoxia (Cai and Storey, 1996). Moreover, several biological processes and molecular functions involved in the respiratory chain were enriched among the downregulated DEGs in our results, such as oxidative phosphorylation (GO:0006119), cytochrome *c* oxidase activity (GO:0004129) or cellular respiration (GO:0045333). Nevertheless, some studies have shown the importance of energy in maintaining metabolic homeostasis at low temperatures for poikilotherms. For

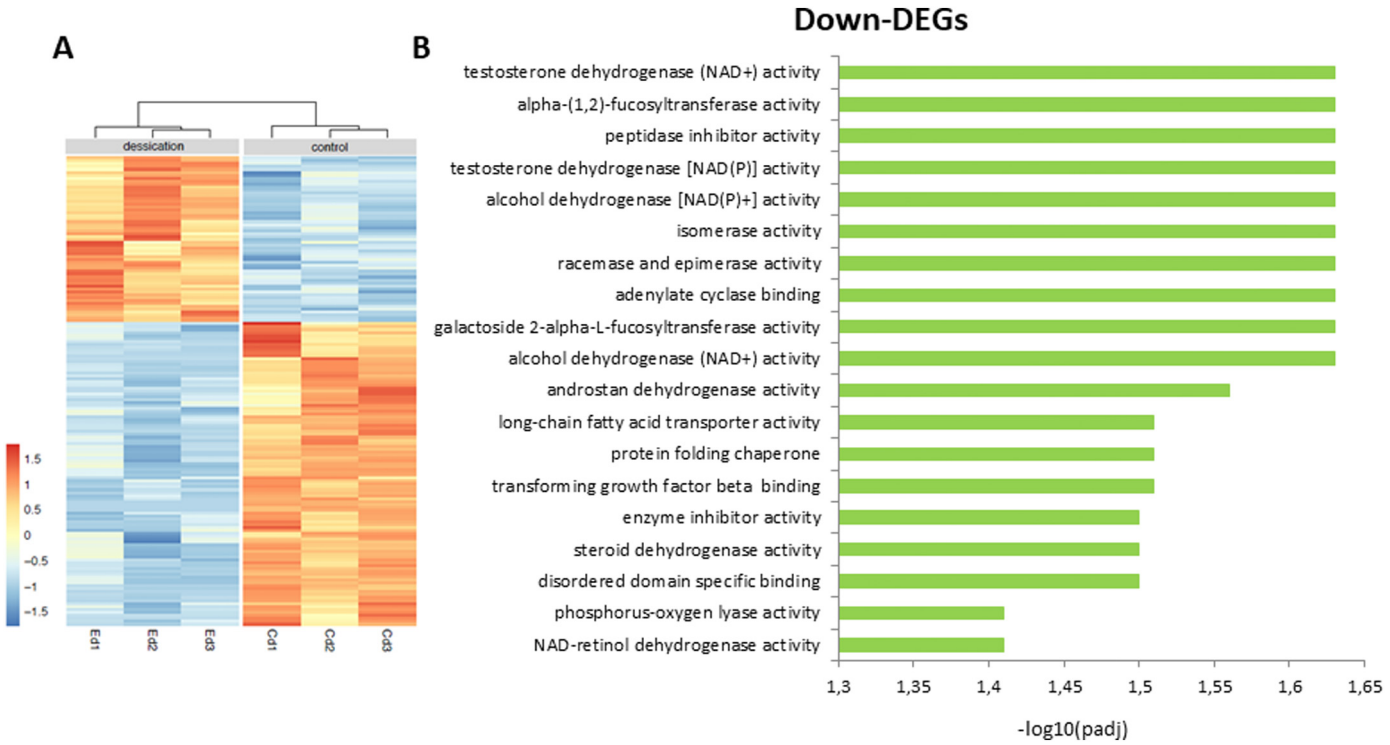


Fig. 3. A: Hierarchically-clustered heatmap of all differentially expressed genes under desiccation conditions in *Eiseniella tetraedra* earthworms. Red color indicates high expression values and blue color indicates low expression values. B: Enriched GO terms obtained from downregulated differentially expressed genes (DEGs) in dessication samples. Terms corresponding to the molecular function (MF) category are shown in green. All shown GO terms were significantly enriched (adjusted p value <0.05). No terms for Biological Process (BP) or Cellular Component (CC) were enriched. No enriched GO terms were obtained from upregulated DEGs. For a complete list of results of GO enrichment analysis see Supplementary File 3. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

example, animals with a high capacity for ATP production under cold conditions also show good tolerance to cold stress (Wang et al., 2014; Lu et al., 2017). Thus, the downregulation of a variety of genes involved in the respiratory chain suggests that *E. tetraedra* may not be a good candidate to sustain low temperature conditions. However, it is a cosmopolitan species present in even subarctic climates. It is possible that the upregulation of *ART1*, which presumably leads to glucose accumulation, counteracts the lack of energy from ATP and allows *E. tetraedra* to live in low temperature zones, but not in freezing conditions. This may explain why Terhivuo and Saura (1997) reported the yearly disappearance of *E. tetraedra* caused by habitat freezing in the Aland Archipelago (Baltic Sea). However, some cocoons of *E. tetraedra* are able to survive at −28 °C (personal obs). Whether the cocoons may be the frost-resistant form of this species needs to be investigated in further studies.

4.2. Desiccation tolerance

Lack or scarcity of water is a big stressor for organisms. A loss of more than 20–50% of their water content is fatal to most higher plants (Kranner et al., 2002) and most animals die when they lose more than 15–20% of their body water (Barrett, 1991). Seasonal variation in earthworm habitats is a natural phenomenon. As a semi-aquatic species (Omodeo and Rota, 1991), *E. tetraedra* is highly dependent on the availability of water. Therefore, it is believed to be extremely susceptible to drought stress.

Maternal embryonic leucine zipper kinase (MELK), encoded by the gene also known as *MELK*, is a serine/threonine kinase involved in various cellular processes such as cell division (Nakano et al., 2005; Cordes et al., 2006; Niesler et al., 2007; Le Page et al., 2011; Chien et al., 2013), transcription (Seong et al., 2002), pre-mRNA splicing (Vulsteke et al., 2004), DNA repair (Bensimon et al., 2011) and apoptosis (Lin et al., 2007; Jung et al., 2008). In addition, *MELK* has been implicated in DNA damage response pathways (Hurov et al., 2010; Bensimon et al., 2011). Thus, elevated MELK protein levels increase resistance to DNA-damaging events (Choi and Ku, 2011). In severe cases, abiotic stress can trigger apoptosis or programmed cell death (Wang and Kaufman, 2012) and DNA damage response pathways (Nisa et al., 2019). Serine threonine protein kinase 17A, encoded by the gene *STK17A*, appeared downregulated in our results. It belongs to a member of the death associated protein kinase family, and acts as a positive regulator of apoptosis (Sanjo et al., 1998; Mao et al., 2011). Therefore, upregulation of *MELK* and downregulation of *STK17A* in *E. tetraedra* during desiccation conditions could protect the animal from apoptosis and DNA damage.

Peptidyl-prolyl cis-trans isomerases (PPiase) are a group of chaperones found in all organisms. They are involved in many biochemical processes such as signal transduction, protein folding and development. Their occurrence in plants is comparable to that of heat shock proteins (Schiene-Fischer and Yu, 2001). As in the desiccation treatment of *E. tetraedra*, PPiases were up-regulated under drought stress in rice (Wang and Kaufman, 2012) and under heat conditions in the springtail *Folsomia candida* Willem, 1902 (Nota et al., 2010). Moreover, they are involved in the anhydrobiosis process of the nematode *Panagrolaimus superbus* Fuchs, 1930. In contrast, in tardigrades subjected to desiccation stress, the concentration of another chaperone family, namely Hsp70, was reduced, whereas elevated levels were detected after rehydration. This suggests that the role of Hsp70 may be related to repair processes after desiccation rather than biochemical stabilization in the dry state (Jönsson and Schill, 2007). Consequently, this could be the reason why the GO: 0044183, which belongs to the protein folding chaperones, appeared downregulated in *E. tetraedra*.

The gene *REPTOR*, which encodes the protein CREBRF homolog, plays a key role in regulating energy homeostasis and promotes survival during nutrient deprivation (Tiebe et al., 2015). It maintains the organism's metabolism by activating the expression of target genes of the stress response, including genes involved in glycogenesis and triglyceride biosynthesis. Therefore, the upregulation of this gene under the drought conditions in our experiment seems to be reasonable, as it maintains homeostasis and

covers the lack of nutrients caused by the absence of moss or plant roots in the petri dishes, which *E. tetraedra* also eats (personal obs).

The UBX domain-containing protein family is evolutionarily conserved and is present in several model organisms (Hartmann-Petersen et al., 2003; Schuberth et al., 2004; Yamauchi et al., 2007; Lee et al., 2010) where it is involved in oxidative stress and osmotic stress response (Zhang et al., 2017). Two other domains are found in the UBX domain: ubiquitin-associated (UBA) and ubiquitin-associated (UAS) (Zhang et al., 2017). The UAS domain has been shown to interact with long-chain unsaturated fatty acids (Kim et al., 2013). Like UBX, the transporter activity for long-chain fatty acids (GO:0005324) and several GO terms related to dehydrogenase activities (such as GO:0047035, GO:0018455 and GO:0047044) were also downregulated in our desiccation experiment. The gene *DHRS9*, which is involved in all these dehydrogenase activities, is also related to lipid metabolism.

Table 2
Annotated differentially expressed genes (DEGs) for the desiccation experiment in the earthworm *Eiseniella tetraedra*. Positive log fold change values (logFC) represent upregulated DEGs (in dark orange), while negatives represent downregulated DEGs (in light orange).

Gene	Protein	logFC
melk	Maternal embryonic leucine zipper kinase	2,42595792
Ogt	UDP-N-acetulglucosamine peptide N-acetylglusaminyltransferase 110 kDa subunit	2,32822656
DIO3	Thyrosine 5-deiodinase	2,57000343
RNF11	RING finger protein 11	2,17304219
Ppib	Peptidyl-prolyl cis-trans isomerase B	3,03414854
REPTOR	Protein CREBRF homolog	1,95570455
gag-pol	Gag-Pol polyprotein	1,81829307
SEC61A2	Protein transport protein Sec61 subunit alpha isoform 2	−2,99656266
vps25	Vacuolar protein-sorting-associated protein 25	−9,57780365
Mtorc2	Mitochondrial amodoxime reducing component 2	−5,35907783
UGT2A3	UDP-glucoronosyltransferase 2A3	−3,15670948
Igf2bp1	insuline-like growth factor 2 mRNA-binding protein 1	−5,79504615
BSG	Basigin	−2,79364374
adcy5	Adenylate cyclase type 5	−5,26211640
STK17A	Serine/threonine-proteine kinase 17A	−5,00263744
N/A	Soma ferritin	−2,15117738
N/A	Parvalbumin alpha	−4,78357177
SNX27	Sorting nexin-27	−4,11397031
KIDINS220	Kinase D-interacting substrate of 220 kDa	−8,94636595
clec4a	C-type lectin domain family 4 member A	−3,22700454
Rmdn1	regulator of microtubule dynamics protein 1	−4,52941819
Plod3	Multifunctional procollagen lysine hydroxylase and glycosyltransferase LH3	−3,58851204
TM1	Tropomyosin	−2,28803424
CD109	CD109 antigen	−3,49888030
dld	Delta-like protein D	−4,69949756
N/A	Extracellular globin-1	−4,20395984
DHRS9	Dehydrogenase/reductase SDR family member 9	−2,91725090
TMEM26	Transmembrane protein 26	−4,25613240
FUT1	Galactoside alpha-(1,2)-fucosyltransferase 1	−3,93638172
AMY2	Pancreatic alpha-amylase	−5,54200867
chmp3	Charged multivesicular body protein 3	−2,51423075
ABCD4	Lysosomal cobalamin transporter ABCD4	−7,77174965
N/A	C-type lectin mannose-binding isoform	−2,80057574
SLC1A2	Excitatory amino acid transporter 2	−2,27316235
DAZAP2	DAZ-associated protein 2	−2,12466453
Mocs2	Molybdopterin synthase catalytic subunit	−2,48858001
Ubxn4	UBX domain-containing protein 4	−7,62518905
FASTKD3	FAST kinase domain-containing protein 3, mitochondrial	−3,35739905
RAB14	Ras-related protein Rab-14	−1,78479830
CREB3L2	Cyclic AMP-responsive element-binding protein 3-like protein 2	−2,73883959
SPRYD3	SPRY domain-containing protein 3	−2,37690372
PI16	Peptidase inhibitor 16	−2,75902813
FKBP8	Peptidyl-prolyl cis-trans isomerase FKBP8	−2,57298054

The *FUT1* gene is also involved in the fatty acid biosynthesis pathway. It encodes galactoside-2-alpha-L-fucosyltransferase, which mediates the attachment of L-fucose to the terminal β-D-galactose residues of the glycan via an α1,2 linkage (Kim et al., 2020). Fucosylation is a biological process that is widely distributed in vertebrates, invertebrates, plants, bacteria, and fungi (Li et al., 2018). It plays an important role in molecular functions such as cell adhesion and immune regulation (Kim et al., 2020). Fucosylated carbohydrates are involved in the regulation of several cellular functions such as cell trafficking, immune cell development, and interaction with gut microbes (Aplin and Jones, 2012; Goto et al., 2016). Galactoside 2-alpha-L-fucosyltransferase is also needed for the glycosylation of N-glycans, O-glycans, and glycosphingolipids (Taniguchi and Yoshida, 2017). *FUT1* appeared to be upregulated under water stress in plants (Maheswary et al., 2016), whereas it appeared downregulated in our desiccation experiments. Survival under stress conditions can be maximized by two physiological mechanisms: increasing the storage of resources (energy or water) aimed for consumption during stress, or conserving resources by reducing the rate at which they are consumed (Marron et al., 2003). Experiments with *Drosophila melanogaster* Meigen, 1830 show that selection for stress resistance in the laboratory can lead to a reduction in metabolic rate (Hoffmann and Parsons, 1993). Lipids provide more than twice as much energy per gram as carbohydrates (Withers, 1992), so they would be a suitable fuel for starvation resistance and explain the downregulation of lipid metabolism and transport in *E. tetraedra*.

5. Conclusions

In this study, we identified 84 differentially expressed genes in *Eiseniella tetraedra* under cold conditions and 163 in the desiccation experiment. Some mechanisms such as dehydration and osmotic stress have been

reported as common at low temperatures and low humidity, but in this case we did not detect common responses between the two experiments.

Despite its cosmopolitan distribution, even in subarctic zones, *E. tetraedra* did not tolerate freezing conditions as demonstrated by the down-regulation of the respiratory chain. However, upregulation of genes related to glucose accumulation suggests acclimation to low temperatures. We hypothesized that cocoons are the frost-resistant forms of this species, but this needs to be confirmed in future experiments. Earthworms perform gas exchange through their skin, and under cold conditions their skin remains nearly frozen, making it difficult for them to breathe. The upregulation of extracellular giant hemoglobin could be helpful in this process due to its exceptional oxygen transport properties. In contrast, under desiccation conditions, genes that protect earthworms from apoptosis and DNA damage were upregulated. Lipid metabolism, however, appeared to be downregulated, presumably due to resource accumulation in case the adverse conditions persisted for a prolonged period of time.

CRediT authorship contribution statement

Conceptualization: I. de Sosa, D. F. Marchán, A. Almodóvar, D. Díaz Cosín, M. Novo.

Methodology: I. de Sosa, D. Díaz Cosín, M. Novo.

Software: A. Verdes, M. Novo.

Validation: I. de Sosa, A. Verdes, M. Novo.

Formal analysis: I. de Sosa, A. Verdes, N. Tilikj, M. Novo.

Investigation: I. de Sosa, D. Díaz Cosín, M. Novo.

Resources: I. de Sosa, A. Verdes, O. Herrero, R. Planelló, A. Almodóvar, D. Díaz Cosín, M. Novo.

Data curation: I. de Sosa, A. Verdes, O. Herrero, R. Planelló, M. Novo.

Writing – Original Draft: I. de Sosa, A. Verdes, M. Novo.

Writing – Review & Editing: I. de Sosa, A. Verdes, D. F. Marchán, N. Tilikj, O. Herrero, R. Planelló, A. Almodóvar, D. Díaz Cosín, M. Novo.

Visualization: I. de Sosa, A. Verdes, N. Tilikj, M. Novo.

Supervision: M. Novo.

Project administration: M. Novo.

Funding acquisition: O. Herrero, R. Planelló, D. Díaz Cosín, M. Novo.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are grateful to the team of the Soil Zoology Group of Complutense University of Madrid (UCM) for laboratory and field support. IS was supported by a Predoctoral Fellowship grant by Universidad Complutense de Madrid, Spain. AV was funded by the European Union's Horizon 2020 research and innovation program through a Marie Sklodowska-Curie individual fellowship (841576). MN was supported by Ramón y Cajal Fellowship (RYC2018-024654-I) and this study was funded by Grant PGC2018-094112-A-I00, both from MCIN/AEI/10.13039/501100011033 and by "ESF: Investing in your future" and "ERDF: A way of making Europe" respectively.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.152749.

References

Al-Fageeh, M.B., Smales, C.M., 2006. Control and regulation of the cellular responses to cold shock: the responses in yeast and mammalian systems. Biochem. J. 397, 247–259. https://doi.org/10.1042/BJ20060166.

Álvarez, A.H., 2004. Mono-adp-ribosilación: implicación en la fisiología de los organismos. REB 23, 149–156.

Anderson, C.M., Parkinson, F.E., 1997. Potential signalling roles for UTP and UDP: sources, regulation and release of uracil nucleotides. Trends Pharmacol. Sci. 18, 387–392. https://doi.org/10.1016/S0165-6147(97)90667-2.

Aplin, J.D., Jones, C.J., 2012. Fucose, placental evolution and the glycocode. Glycobiology 22, 470–478. https://doi.org/10.1093/glycob/cwr156.

Barne, A.Z., Striganova, B.R., 2005. Evaluation of production parameters of earthworms eiseniella tetraedra sav. In a laboratory culture. Biol. Bull. 32, 264–267. https://doi.org/10.1007/s10525-005-0100-8.

Barrett, J., 1991. Water and fertilizer movement in greenhouse subirrigation systems. Greenhouse Manager 10, 89–90.

Bensimon, A., Aebersold, R., Shiloh, Y., 2011. Beyond ATM: the protein kinase landscape of the DNA damage response. FEBS Lett. 585, 1625–1639. https://doi.org/10.1016/j.febslet.2011.05.013.

Berman, D.I., Leirikh, A.N., 1985. The ability of earthworm Eisenia-nordenskioldi (Eisen) (Lumbricidae, Oligochaeta) to resist minus temperatures. Dokl. Akad. Nauk SSSR 285, 1258–1261.

Bhambri, A., Dhaunta, N., Patel, S.S., Hardikar, M., Bhatt, A., Srikakulam, N., Pillai, B., 2018. Large scale changes in the transcriptome of Eisenia fetida during regeneration. PLoS One 13, e0204234. https://doi.org/10.1371/journal.pone.0204234.

Blakemore, R.J., 2006. Cosmopolitan Earthworms-an Eco-taxonomic Guide to the Peregrine Species of the World. 2nd ed. VermEcology, Australia.

Brown, N.G., 1972. Changes in ribosomal patterns and a related membrane fraction during induction of cold hardiness in mimosa epicotyl tissues. Plant Cell Physiol. 13, 345–351.

Brown, C.J., O'Connor, M.I., Poloczanska, E.S., Schoeman, D.S., Buckley, L.B., Burrows, M.T., Richardson, A.J., 2016. Ecological and methodological drivers of species' distribution and phenology responses to climate change. Glob. Chang. Biol. 22, 1548–1560. https://doi.org/10.1111/gcb.13184.

Buckman, H.O., Brady, N.C., 1969. The nature and properties of soils. The MacMillan Company, London, UK.

Cai, Q., Storey, K.B., 1996. Anoxia-induced gene expression in turtle heart: upregulation of mitochondrial genes for NADH-ubiquinone oxidoreductase subunit 5 and cytochrome c oxidase subunit 1. Eur. J. Biochem. 241, 83–92. https://doi.org/10.1111/j.1432-1033.1996.0083t.x.

Cameron, J., 1986. Appendix 2-Solubility of O_2 and CO_2 at different temperatures and salinities. Princ. of Physiol. Meas. Academic Press, Inc, Orlando, Florida, USA, pp. 254–259.

Casellato, S., 1987. On polyploidy in Oligochaetes with particular reference to Lumbricids. On Earthworms. Selected symposia and monographs UZI, Modena, pp. 75–87.

Chen, L., Sun, F., Yang, X., Jin, Y., Shi, M., Wang, L., Wang, Q., 2017. Correlation between RNA-seq and microarrays results using TCGA data. Gene 628, 200–204. https://doi.org/10.1016/j.gene.2017.07.056.

Chettoor, A.M., Givan, S.A., Cole, R.A., Coker, C.T., Unger-Wallace, E., Vejlupkova, Z., Evans, M.M., 2014. Discovery of novel transcripts and gametophytic functions via RNA-seq analysis of maize gametophytic transcriptomes. Genome Boil. 15, 1–23. https://doi.org/10.1186/s13059-014-0414-2.

Chien, S.C., Brinkmann, E.M., Teuliere, J., Garriga, G., 2013. Caenorhabditis elegans PIG-1/MELK acts in a conserved PAR-4/LKB1 polarity pathway to promote asymmetric neuroblast divisions. Genetics 193, 897–909. https://doi.org/10.1534/genetics.112.148106.

Choi, S., Ku, J.L., 2011. Resistance of colorectal cancer cells to radiation and 5-FU is associated with MELK expression. Biochem. Biophys. Res. Commun. 412, 207–213. https://doi.org/10.1016/j.bbrc.2011.07.060.

Cordes, S., Frank, C.A., Garriga, G., 2006. The C. elegans MELK ortholog PIG-1 regulates cell size asymmetry and daughter cell fate in asymmetric neuroblast divisions. Development 133, 2747–2756. https://doi.org/10.1242/dev.02447.

Dawson, T.P., Berry, P.M., Kampa, E., 2003. Climate change impacts on freshwater wetland habitats. J. Nat. Conserv. 11, 25–30. https://doi.org/10.1078/1617-1381-00031.

De Sosa, I., Marchan, D.F., Novo, M., Almodovar, A., Díaz Cosín, D.J., 2017. Bless this phylogeographic mess–Comparative study of eiseniella tetraedra (Annelida, Oligochaeta) between an Atlantic area and a continental Mediterranean area in Spain. Eur. J. Soil Biol. 78, 50–56. https://doi.org/10.1016/j.ejsobi.2016.11.006.

Diao, P., Song, Y., Ge, H., Wu, Y., Li, J., Zhang, W., Cheng, J., 2019. Identification of 4-lncRNA prognostic signature in head and neck squamous cell carcinoma. J. Cell. Biochem. 120, 10010–10020. https://doi.org/10.1002/jcb.28284.

Diehl, W.J., Williams, D.L., 1992. Interactive effects of soil moisture and food on growth and aerobic metabolism in Eisenia fetida (Oligochaeta). Comp. Biochem. Physiol. A 1021, 179–184. https://doi.org/10.1016/0300-9629(92)90031-K.

Dubrovsky, M., Svoboda, M.D., Trnka, M., Hayes, M.J., Wilhite, D.A., Zalud, Z., Hlavinka, P., 2009. Application of relative drought indices in assessing climate-change impacts on drought conditions in Czechia. Theor. Appl. Climatol. 96, 155–171. https://doi.org/10.1007/s00704-008-0020-x.

Edwards, C.A., 2004. The importance of earthworms as key representatives of the soil fauna. Earthworm Ecol. 2, 3–11.

Ekblom, R., Galindo, J., 2011. Applications of next generation sequencing in molecular ecology of non-model organisms. Heredity 107, 1–15. https://doi.org/10.1038/hdy.2010.152.

Ewart, K.V., Fletcher, G.L., 1993. Herring antifreeze protein: primary structure and evidence for a C-type lectin evolutionary origin. Mol. Mar. Biol. Biotechnol. 2, 20–27.

Ewart, K.V., Li, Z., Yang, D.S., Fletcher, G.L., Hew, C.L., 1998. The ice-binding site of Atlantic herring antifreeze protein corresponds to the carbohydrate-binding site of C-type lectins. Biochemistry 37, 4080–4085. https://doi.org/10.1021/bi972503w.

Fabrizio, G., Di Paola, S., Stilla, A., Giannotta, M., Ruggiero, C., Menzel, S., Di Girolamo, M., 2015. ARTC1-mediated ADP-ribosylation of GRP78/BiP: a new player in endoplasmic-reticulum stress responses. Cell. Mol. Life Sci. 72, 1209–1225. https://doi.org/10.1007/s00018-014-1745-6.

Fujita, J., 1999. Cold shock response in mammalian cells. J. Mol. Microbiol. Biotechnol. 1, 243–255.
Gao, L., Han, Y., Deng, H., Hu, W., Zhen, H., Li, N., Pang, Q., 2017. The role of a novel C-type lectin-like protein from planarian in innate immunity and regeneration. Dev. Comp. Immunol. 67, 413–426. https://doi.org/10.1016/j.dci.2016.08.010.
Goto, Y., Uematsu, S., Kiyono, H., 2016. Epithelial glycosylation in gut homeostasis and inflammation. Nat. Immunol. 17, 1244–1251. https://doi.org/10.1038/ni.3587.
Grabherr, M.G., Haas, B.J., Yassour, M., Levin, J.Z., Thompson, D.A., Amit, I., Regev, A., 2011. Trinity: reconstructing a full-length transcriptome without a genome from RNA-seq data. Nat. Biotechnol. 29, 644–652. https://doi.org/10.1038/nbt.1883.
Grant, W.C., 1955. Studies on moisture relationships in earthworms. Ecology 36, 400–407. https://doi.org/10.2307/1929574.
Gusta, L.V., Weiser, C.J., 1972. Nucleic acid and protein changes in relation to cold acclimation and freezing injury of Korean boxwood leaves. Plant Physiol. 49, 91–96. https://doi.org/10.1104/pp.49.1.91.
Gutniak, M., Orskov, C., Holst, J.J., Ahren, B.B., Efendic, S., 1992. Antidiabetogenic effect of glucagon-like peptide-1(7–36) amide in normal subjects and patients with mellitus. N. Engl. J. Med. 326, 1316–1322. https://doi.org/10.1056/NEJM199205143262003.
Hartmann-Petersen, R., Semple, C.A., Ponting, C.P., Hendil, K.B., Gordon, C., 2003. UBA domain containing proteins in fission yeast. Int. J. Biochem. Cell Biol. 35, 629–636. https://doi.org/10.1016/S1357-2725(02)00393-X.
Hoffmann, A.A., Parsons, P.A., 1993. Direct and correlated responses to selection for desiccation resistance: a comparison of Drosophila melanogaster and D. simulans. J. Evol. Biol. 6, 643–657. https://doi.org/10.1046/j.1420-9101.1993.6050643.x.
Holmstrup, M., 2003. Overwintering adaptations in earthworms: the 7th international symposium on earthworm ecology· Cardiff· Wales· 2002. Pedobiologia 47, 504–510. https://doi.org/10.1078/0031-4056-00220.
Holmstrup, M., 2014. The ins and outs of water dynamics in cold tolerant soil invertebrates. J. Therm. Boil. 45, 117–123. https://doi.org/10.1016/j.jtherbio.2014.09.001.
Holmstrup, M., Petersen, B.F., 1997. Freeze-tolerance in the subarctic earthworm Eisenia nordenskioeldi (Eisen). Cryo-Letters 18, 153–156.
Holmstrup, M., Zachariassen, K.E., 1996. Physiology of cold hardiness in earthworms. Comp. Biochem. Physiol. A 115, 91–101. https://doi.org/10.1016/0300-9629(96)00010-2.
Holmstrup, M., Costanzo, J.P., Lee Jr., R.E., 1999. Cryoprotective and osmotic responses to cold acclimation and freezing in freeze-tolerant and freeze-intolerant earthworms. Comp. Biochem. Physiol. B. 169, 207–214. https://doi.org/10.1007/s003600050213.
Houghton, J.T., Ding, Y., Griggs, D.J., Noguer, M., van der Linden, P.J., Dai, X., Maskell, K., Johnson, C.A., 2001. Climate change. The Scientific Basis. Cambridge University Press, Cambridge, UK.
Hurov, K.E., Cotta-Ramusino, C., Elledge, S.J., 2010. A genetic screen identifies the triple T complex required for DNA damage signaling and ATM and ATR stability. Genes Dev. 24, 1939–1950. https://doi.org/10.1101/gad.1934210.
Intergovernmental Panel on Climate Change (IPCC), 1992. Climate Change 1992. The Suplementary Report to the IPCC Scientific Assessment. Cambridge University Press, Cambridge, UK.
Jalili, A., Jamzad, Z., Thompson, K., Araghi, M.K., Ashrafi, S., Hasaninejad, M., Parvaneh, K., 2010. Climate change, unpredictable cold waves and possible brakes on plant migration. Glob. Ecol. Biogeogr. 19, 642–648. https://doi.org/10.1111/j.1466-8238.2010.00553.x.
Jin, H., Wan, Y.W., Liu, Z., 2017. Comprehensive evaluation of RNA-seq quantification methods for linearity. BMC Bioinformatics 18, 51–59. https://doi.org/10.1186/s12859-017-1526-y.
Jönsson, K.I., Schill, R.O., 2007. Induction of Hsp70 by desiccation, ionising radiation and heat-shock in the eutardigrade Richtersius coronifer. Comp. Biochem. Physiol. BBiochem. Mol. Biol. 146, 456–460. https://doi.org/10.1016/j.cbpb.2006.10.111.
Jung, H., Seong, H.A., Ha, H., 2008. Murine protein serine/threonine kinase 38 activates apoptosis signal-regulating kinase 1 via Thr838 phosphorylation. J. Biol. Chem. 283, 34541–34553. https://doi.org/10.1074/jbc.M807219200.
Kashuba, E., Kashuba, V., Sandalova, T., Klein, G., Szekely, L., 2002. Epstein-Barr virus encoded nuclear protein EBNA-3 binds a novel human uridine kinase/uracil phosphoribosyltransferase. BMC Cell. Boil. 3, 1–12. https://doi.org/10.1038/sj.onc.1203501.
Khan, A.A., Heit, C.E., Lippolo, P.C., 1968. Increase in nucleic acid synthesizing capacity during cold treatment of dormant pear embryos. Biochem. Biophys. Res. Commun. 33, 391–396. https://doi.org/10.1016/0006-291X(68)90583-4.
Kim, H., Zhang, H., Meng, D., Russell, G., Lee, J.N., Ye, J., 2013. UAS domain of Ubxd8 and FAF1 polymerizes upon interaction with long-chain unsaturated fatty acids. J. Lipid Res. 54, 2144–2152. https://doi.org/10.1194/jlr.M037218.
Kim, K.W., Ryu, J.S., Ko, J.H., Kim, J.Y., Kim, H.J., Lee, H.J., Oh, J.Y., 2020. FUT1 deficiency elicits immune dysregulation and corneal opacity in steady state and under stress. Cell Death Dis. 11, 1–11. https://doi.org/10.1038/s41419-020-2489-x.
Kranner, I., Beckett, R.P., Wornik, S., Zorn, M., Pfeifhofer, H.W., 2002. Revival of a resurrection plant correlates with its antioxidant status. Plant J. 31, 13–24. https://doi.org/10.1046/j.1365-313X.2002.01329.x.
Kreyling, J., 2010. Winter climate change: a critical factor for temperate vegetation performance. Ecology 91, 1939–1948. https://doi.org/10.1890/09-1160.1.
Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with bowtie 2. Nat. Methods 9, 357–359. https://doi.org/10.1038/nmeth.1923.
Langmead, B., Trapnell, C., Pop, M., Salzberg, S.L., 2009. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol. 10, 1–10. https://doi.org/10.1186/gb-2009-10-3-r25.
Le Page, Y., Chartrain, I., Badouel, C., Tassan, J.P., 2011. A functional analysis of MELK in cell division reveals a transition in the mode of cytokinesis during xenopus development. J. Cell Sci. 124, 958–968. https://doi.org/10.1242/jcs.069567.
Lee, K.E., 1985. Earthworms: their ecology and relationships with soils and land use. Academic Press Inc.
Lee, J.N., Kim, H., Yao, H., Chen, Y., Weng, K., Ye, J., 2010. Identification of Ubxd8 protein as a sensor for unsaturated fatty acids and regulator of triglyceride synthesis. Proc. Natl. Acad. Sci. 107, 21424–21429. https://doi.org/10.1073/pnas.1011859107.
Li, B., Dewey, C.N., 2011. RSEM: accurate transcript quantification from RNA-seq data with or without a reference genome. BMC Bioinformatics 12, 1–16. https://doi.org/10.1186/1471-2105-12-323.
Li, J., Hsu, H.C., Mountz, J.D., Allen, J.G., 2018. Unmasking fucosylation: from cell adhesion to immune system regulation and diseases. Cell Chem. Biol. 25, 499–512. https://doi.org/10.1016/j.chembiol.2018.02.005.
Li, D., Nie, H., Jahan, K., Yan, X., 2020. Expression analyses of C-type lectins (CTLs) in Manila clam under cold stress provide insights for its potential function in cold resistance of Ruditapes philippinarum. Comp. Biochem. Physiol. C: Toxicol. Pharmacol. 230, 108708. https://doi.org/10.1016/j.cbpc.2020.108708.
Li, N., Zhou, J., Wang, H., Mu, C., Shi, C., Liu, L., Wang, C., 2020. Transcriptome analysis of genes and pathways associated with metabolism in Scylla paramamosain under different light intensities during indoor overwintering. BMC Genomics 21, 1–15. https://doi.org/10.1186/s12864-020-07190-w.
Lin, M.L., Park, J.H., Nishidate, T., Nakamura, Y., Katagiri, T., 2007. Involvement of maternal embryonic leucine zipper kinase (MELK) in mammary carcinogenesis through interaction with Bcl-G, a pro-apoptotic member of the Bcl-2 family. Breast Cancer Res. 9, 1–13. https://doi.org/10.1186/bcr1650.
Lindquist, S., 1986. The heat-shock response. Annu. Rev. Biochem. 55, 1151–1191. https://doi.org/10.1146/annurev.bi.55.070186.005443.
Lu, X., Zhou, X., Cao, Y., Zhou, M., McNeil, D., Liang, S., Yang, C., 2017. RNA-seq analysis of cold and drought responsive transcriptomes of Zea mays ssp. mexicana L. Front. Plant Sci. 8, 136. https://doi.org/10.3389/fpls.2017.00136.
Malevich, I.I., 1956. Oligohetes of the Moscow region. Uch. Zap. MGPI im. VP Potemkina 61, 403–437.
Mao, P., Hever, M.P., Niemaszyk, L.M., Haghkerdar, J.M., Yanco, E.G., Desai, D., Spinella, M.J., 2011. Serine/threonine kinase 17A is a novel p53 target gene and modulator of cisplatin toxicity and reactive oxygen species in testicular cancer cells. J. Biol. Chem. 286, 19381–19391. https://doi.org/10.1074/jbc.M111.218040.
Marron, M.T., Markow, T.A., Kain, K.J., Gibbs, A.G., 2003. Effects of starvation and desiccation on energy metabolism in desert and Mesic drosophila. J. Insect Physiol. 49, 261–270. https://doi.org/10.1016/S0022-1910(02)00287-1.
Mazur, P., 1963. Kinetics of water loss from cells at subzero temperatures and the likelihood of intracellular freezing. J. Gen. Physiol. 47, 347–369. https://doi.org/10.1085/jgp.47.2.347.
Miles, H.B., 1963. Heat-death temperature in allolobophora terrestris (Sav.) forma longa (Ude) and Eisenia foetida (Sav.). Nature 199, 826. https://doi.org/10.1038/199826a0.
Nakano, I., Paucar, A.A., Bajpai, R., Dougherty, J.D., Zewail, A., Kelly, T.K., Kornblum, H.I., 2005. Maternal embryonic leucine zipper kinase (MELK) regulates multipotent neural progenitor proliferation. J. Cell Biol. 170, 413–427. https://doi.org/10.1083/jcb.200412115.
Niesler, C.U., Myburgh, K.H., Moore, F., 2007. The changing AMPK expression profile in differentiating mouse skeletal muscle myoblast cells helps confer increasing resistance to apoptosis. Exp. Physiol. 92, 207–217. https://doi.org/10.1113/expphysiol.2006.034736.
Nisa, M.U., Huang, Y., Benhamed, M., Raynaud, C., 2019. The plant DNA damage response: signaling pathways leading to growth inhibition and putative role in response to stress conditions. Front. Plant Sci. 10, 653. https://doi.org/10.3389/fpls.2019.00653.
Nota, B., Van Straalen, N.M., Ylstra, B., Roelofs, D., 2010. Gene expression microarray analysis of heat stress in the soil invertebrate Folsomia candida. Insect Mol. Biol. 19, 315–322. https://doi.org/10.1111/j.1365-2583.2009.00990.x.
Omodeo, P., Rota, E., 1991. Earthworms of Turkey.II. Ital. J. Zool. 58, 171–181. https://doi.org/10.1080/11250009109355749.
Paul, S., Heckmann, L.H., Sørensen, J.G., Holmstrup, M., Arumugaperumal, A., Sivasubramaniam, S., 2018. Transcriptome sequencing, de novo assembly and annotation of the freeze tolerant earthworm, Dendrobaena octaedra. Gene Rep. 13, 180–191. https://doi.org/10.1016/j.genrep.2018.10.010.
Pauli, A., Valen, E., Lin, M.F., Garber, M., Vastenhouw, N.L., Levin, J.Z., Schier, A.F., 2012. Systematic identification of long noncoding RNAs expressed during zebrafish embryogenesis. Genome Res. 22, 577–591. https://doi.org/10.1101/gr.133009.111.
Phillips, H.R.P., Guerra, C.A., Bartz, M.L.C., Briones, J.I., Brown, G., Crowther, T.W., Eisenhauer, N., 2020. Global distribution of earthworm diversity (vol 366, pg 480, 2019). Science 369. https://doi.org/10.1126/science.abd9834.
Pop, A.A., Wink, M., Pop, V.V., 2003. Use of 18S, 16S rDNA and cytochrome c oxidase sequences in earthworm taxonomy (Oligochaeta, Lumbricidae): the 7th international symposium on earthworm ecology· Cardiff· Wales· 2002. Pedobiologia 47, 428–433. https://doi.org/10.1078/0031-4056-00208.
Rebl, A., Verleih, M., Korytář, T., Kühn, C., Wimmers, K., Köllner, B., Goldammer, T., 2012. Identification of differentially expressed protective genes in liver of two rainbow trout strains. Vet. Immunol. Immunopathol. 145, 305–315. https://doi.org/10.1016/j.vetimm.2011.11.023.
Reimand, J., Isserlin, R., Voisin, V., Kucera, M., Tannus-Lopes, C., Rostamianfar, A., Bader, G.D., 2019. Pathway enrichment analysis and visualization of omics data using g: profiler, GSEA, cytoscape and EnrichmentMap. Nat. Protoc. 14, 482–517. https://doi.org/10.1038/s41596-018-0103-9.
Reinecke, A.J., Venter, J.M., 1987. Moisture preferences, growth and reproduction of the compost worm Eisenia fetida (Oligochaeta). Biol. Fert. Soils 3, 135–141. https://doi.org/10.1007/BF00260595.
Roberts, A., Pimentel, H., Trapnell, C., Pachter, L., 2011. Identification of novel transcripts in annotated genomes using RNA-seq. Bioinformatics 27, 2325–2329. https://doi.org/10.1093/bioinformatics/btr355.
Robinson, D.M., McCarthy, D.J., Smyth, G.K., 2010. edgeR: a bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26 (1), 139–140. https://doi.org/10.1093/bioinformatics/btp616.

Sanjo, H., Kawai, T., Akira, S., 1998. DRAKs, novel serine/threonine kinases related to death-associated protein kinase that trigger apoptosis. J. Biol. Chem. 273, 29066–29071. https://doi.org/10.1074/jbc.273.44.29066.

Sarhan, F., D'aoust, M.J., 1975. RNA synthesis in spring and winter wheat during cold acclimation. Physiol. Plant. 35, 62–65. https://doi.org/10.1111/j.1399-3054.1975.tb03868.x.

Schiene-Fischer, C., Yu, C., 2001. Receptor accessory folding helper enzymes: the functional role of peptidyl prolyl cis/trans isomerases. FEBS Lett. 495, 1–6. https://doi.org/10.1016/S0014-5793(01)02326-2.

Schuberth, C., Richly, H., Rumpf, S., Buchberger, A., 2004. Shp1 and Ubx2 are adaptors of Cdc48 involved in ubiquitin-dependent protein degradation. EMBO Rep. 5, 818–824. https://doi.org/10.1038/sj.embor.7400203.

Seong, H.A., Gil, M., Kim, K.T., Kim, S.J., Ha, H., 2002. Phosphorylation of a novel zinc-finger-like protein, ZPR9, by murine protein serine/threonine kinase 38 (MPK38). Biochem. J. 361, 597–604. https://doi.org/10.1042/bj3610597.

Seppey, M., Manni, M., Zdobnov, E.M., 2019. BUSCO: assessing genome assembly and annotation completeness. In: Kollmar, M. (Ed.), Gene Prediction. Methods in Molecular Biology. 1962. Humana, New York, NY. https://doi.org/10.1007/978-1-4939-9173-0_14.

Sinclair, B.J., Ferguson, L.V., Salehipour-Shirazi, G., MacMillan, H.A., 2013. Cross-tolerance and cross-talk in the cold: relating low temperatures to desiccation and immune stress in insects. Integr. Comp. Biol. 53, 545–556. https://doi.org/10.1093/icb/ict004.

Taniguchi, M., Yoshida, H., 2017. TFE3, HSP47, and CREB3 pathways of the mammalian Golgi stress response. Cell Struct. Funct. e16023. https://doi.org/10.1247/csf.16023.

Terhivuo, J., Saura, A., 1997. Island biogeography of north european parthenogenetic lumbricidae: I. Clone pool affinities and morphometric differentiation of Åland populations. Ecography 20, 185–196. https://doi.org/10.1111/j.1600-0587.1997.tb00361.x.

Terhivuo, J., Saura, A., Hongell, K., 1994. Genetic and morphological variation in the parthenogenetic earthworm Eiseniella tetraedra (Sav.)(Oligochaeta: Lumbricidae) from South Finland and North Norway. Pedobiologia (Jena) 38, 81–96.

Tiebe, M., Lutz, M., De La Garza, A., Buechling, T., Boutros, M., Teleman, A.A., 2015. REPTOR and REPTOR-BP regulate organismal metabolism and transcription downstream of TORC1. Dev. Cell 33, 272–284. https://doi.org/10.1016/j.devcel.2015.03.013.

Viljoen, S.A., Reinecke, A.J., 1989. Moisture and growth, maturation and cocoon production of Eudrilus eugeniae (Oligochaeta). Rev. Ecol. Biol. Sol. 26, 291–303.

Vulsteke, V., Beullens, M., Boudrez, A., Keppens, S., Van Eynde, A., Rider, M.H., Bollen, M., 2004. Inhibition of spliceosome assembly by the cell cycle-regulated protein kinase MELK and involvement of splicing factor NIPP1. J. Biol. Chem. 279, 8642–8647. https://doi.org/10.1074/jbc.M311466200.

Walther, G.R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J., Bairlein, F., 2002. Ecological responses to recent climate change. Nature 416, 389–395. https://doi.org/10.1038/416389a.

Wang, S., Kaufman, R.J., 2012. The impact of the unfolded protein response on human disease. J. Cell Biol. 197, 857–867. https://doi.org/10.1083/jcb.201110131.

Wang, Q., Tan, X., Jiao, S., You, F., Zhang, P.J., 2014. Analyzing cold tolerance mechanism in transgenic zebrafish (Danio rerio). PloS one 9, e102492. https://doi.org/10.1371/journal.pone.0102492.

Wei, J., Xu, D., Zhou, J., Cui, H., Yan, Y., Ouyang, Z., Qin, Q., 2010. Molecular cloning, characterization and expression analysis of a C-type lectin (Ec-CTL) in orange-spotted grouper Epinephelus coioides. Fish Shellfish Immunol. 28, 178–186. https://doi.org/10.1016/j.fsi.2009.10.020.

Williams, D.L., Diehl, W.J., 1992. Interactive effects of soil moisture and food on glycolytic metabolism in Eisenia fetida (Oligochaeta). Comp. Biochem. and Physiol. B. 102, 911–917. https://doi.org/10.1016/0305-0491(92)90101-V.

Withers, P.C., 1992. Comparative animal physiology. Saunders College Pub, Philadelphia.

Wolf, A.V., 1938. Notes on the effect of heat in lumbricus terrestris L. Ecology 19, 346–348. https://doi.org/10.2307/1929655.

Yamauchi, S., Sasagawa, Y., Ogura, T., Yamanaka, K., 2007. Differential expression pattern of UBX family genes in Caenorhabditis elegans. Biochem. Biophys. Res. Commun. 358, 545–552. https://doi.org/10.1016/j.bbrc.2007.04.163.

Yang, C., Jiang, M., Wu, F., Yu, L., Tian, J., Liu, W., Wen, H., 2016. Identification of a C-type lectin from tilapia (Oreochromis niloticus) and its functional characterization under low-temperature stress. Fish Shellfish Immunol. 58, 631–640. https://doi.org/10.1016/j.fsi.2016.10.004.

Zhang, M., Yu, Q., Liu, Z., Liang, C., Zhang, B., Li, M., 2017. UBX domain-containing proteins are involved in lipid homeostasis and stress responses in Pichia pastoris. Int. J. Biochem. Cell Biol. 90, 136–144. https://doi.org/10.1016/j.biocel.2017.08.006.

Zhu, Y.T., Zhang, X., Wang, S.C., Li, W.W., Wang, Q., 2016. Antimicrobial functions of EsLecH, a C-type lectin, via JNK pathway in the chinese mitten crab, Eriocheir sinensis. Dev. Comp. Immunol. 61, 225–235. https://doi.org/10.1016/j.dci.2016.04.007.