National Doctoral Course in Sustainable Development and Climate Change IUSS Pavia - Cycle XXXIX

Transposable elements response to heat stress in *Trematomus bernacchii*

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Introduction

Climate change rates have exceeded historical levels, with oceans experiencing faster and intense temperature changes. Antarctic ecosystems are especially vulnerable to this phenomenon. Fishes of this region, such as those of the Nototheniidae family, are highly adapted to extreme cold environments. *Trematomus bernacchii*, an Antartic fish belonging to this genre, is considered a key species to study environmental stress. Nowadays, a consistent number of papers has highlighted the crucial role of transposable elements (TEs) in stress responses. These genomic elements can have an impact on the gene expression, in particular when the specimens are exposed to stress conditions. In this study, we investigated the transcriptional responses of TEs in two tissues (liver and gills) of *T. bernacchii* exposed to experimental warming (+1°C and +3°C). We performed RNA extraction, RNA-Seq, and bioinformatic analyses to assess the expressions levels of TEs and genes known to be involved in TE control in these specimens exposed to thermal stress.

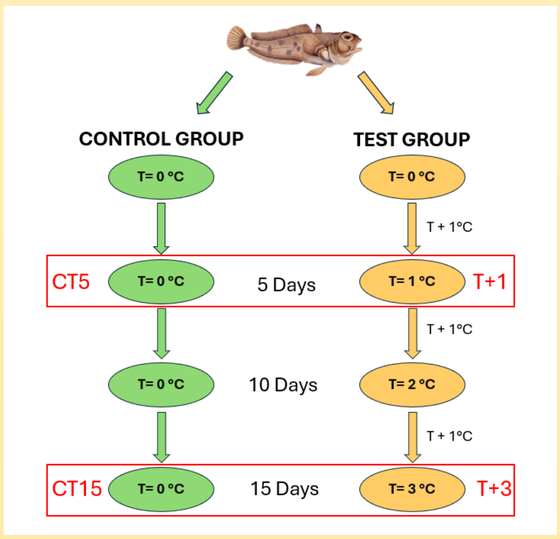

Figure 1. Scheme of the experimental design.
Specimens of *T. bernacchii* were collected and divided into two tanks. The tank of the control group was set at a constant temperature of 0°C for fifteen days. The experimental group was initially kept at a 0°C for one day, followed by a gradual increase of +1°C within 24 hours and then maintained for four days. Then, the temperature was increased by a further degree and maintained for other four days, and finally this was repeated until +3°C was reached over fifteen days. Liver and gills tissue samples (three biological replicates) were collected on the fifth and fifteenth days for two control conditions (CT5 and CT15) and two experimental groups (T+1 and T+3). Afterwards RNA extractions were carried out followed by RNA sequencing.

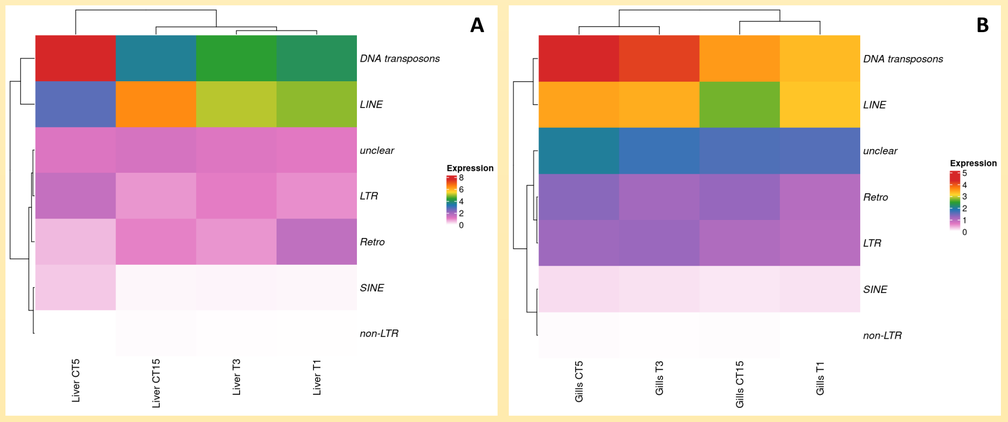

Figure 2. Heatmaps showing the expression levels of major TEs types in liver (A) and gill (B) tissues across different experimental conditions in *T. bernacchii*.
The heatmap illustrates the expression levels of various TE classes (*DNA transposons, LINE, unclear, Retro, LTR, SINE,* and *non-LTR*) across the experimental conditions and time points (*CT5, CT15, T1* and *T3*). The color gradient reflects the intensity of expression, ranging from red, indicating high expression levels, through yellow and blue for moderate levels, to light pink and white, which denote low to negligible expression.

The analyses were performed on the assembly of each condition and allowed to assess the expression level of different TE types. In this figure samples are hierarchically clustered based on expression profile similarities. In liver tissue we observed a strong expression of DNA transposons and LINEs, with remarkable variations across the different conditions. In gills, TE expression appears more homogeneous overall, although DNA transposons and LINEs remain to be highly expressed compared to other categories. SINEs and non-LTR elements exhibited low expression levels in both tissues in all conditions. In the liver, TE transcriptional profile of the two exposed groups (T1 and T3) were more closely, whereas in gills the tested conditions displayed greater divergence. Overall, these data indicated that the thermal stress had an effect on the TE transcriptional activities.

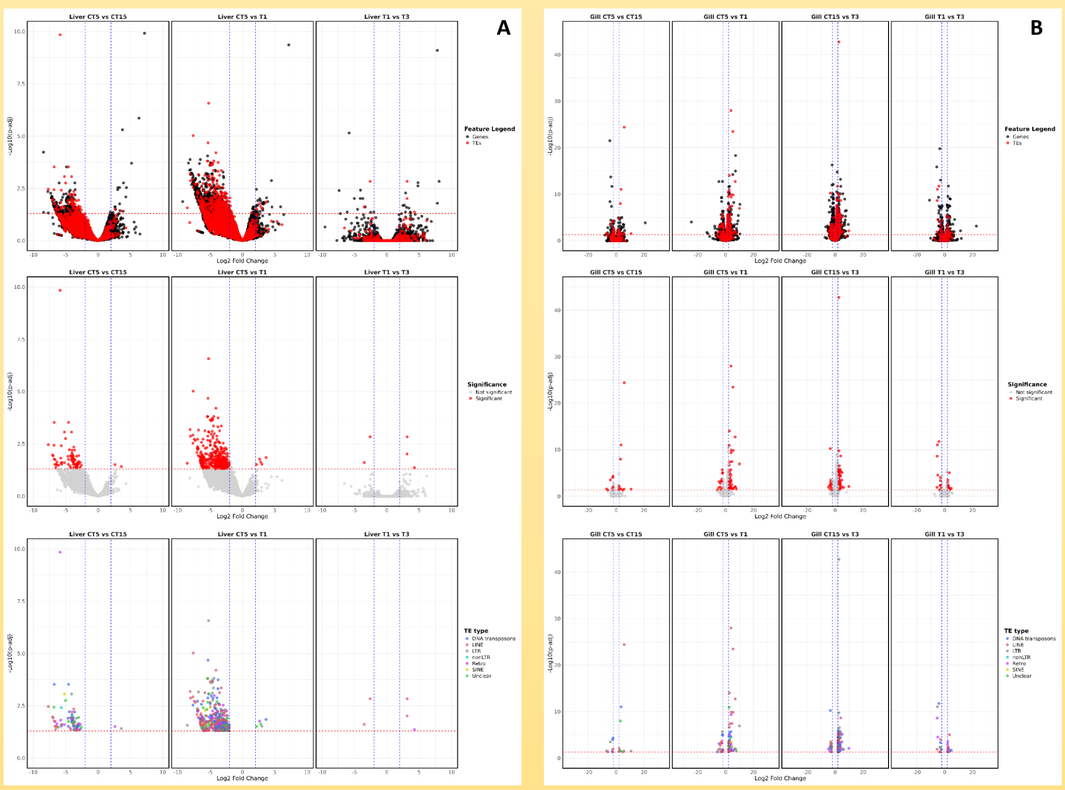

Figure 3. Volcano plot of differentially expressed genes and TEs of *T. bernacchii*. A. Volcano plots related to liver comparisons (*CT5 vs CT15, CT5 vs T1* and *T1 vs T3*). **B.** Volcano plots related to gills comparisons (*CT5 vs CT15, CT5 vs T1, CT15 vs T3* and *T1 vs T3*). These differential expression analyses were performed using TETRANSCRIPTS.
In the first row of each panel, black dots represent genes, while red dots indicate TEs. In the second row, significantly differentially expressed TEs are highlighted in red, while non-significant TEs are shown in grey. In the third row, the differentially expressed TEs (DETEs) are colored according to their TE types. For each graphs the X-axis represents the Log2 fold change, indicating the magnitude of expression change between conditions, while the Y-axis shows the -log10 adjusted p-value (padj), reflecting the statistical significance.
Overall, these analyses revealed distinct patterns of TE modulation in liver and gills. The comparison performed for the liver showed a major number of DETEs compared to gills. In liver (panel A), the comparison CT5 *vs* T1 showed a higher number of DETEs compared to the other comparison. No significant changes were observed between CT15 and T3. Moreover, 365 differentially expressed TEs (DETEs) were identified in the CT5 *vs* T1 comparison, with a strong prevalence of downregulation (360/365), mainly involving LINE retrotransposons, DNA transposons, and LTR retrotransposons. In gills (panel B) a low number of DETEs were detected compared to liver. It was observed a predominant upregulation both in CT5 *vs* T1 (68 upregulated DETES of 86) and CT15 *vs* T3 (122 upregulated DETEs of 138). These upregulated DETEs mainly belong to LINE, LTR, and DNA transposons for both the comparisons. Meanwhile, from the analyses of CT5 *vs* CT15 and T1 *vs* T3, emerged minimal DETE changes.
Overall, exposure to different temperatures significantly impacted TE transcriptional activity, with liver and gills displaying distinct TE regulation patterns, highlighting tissue-specific responses in the thermal adaptation of these fish.

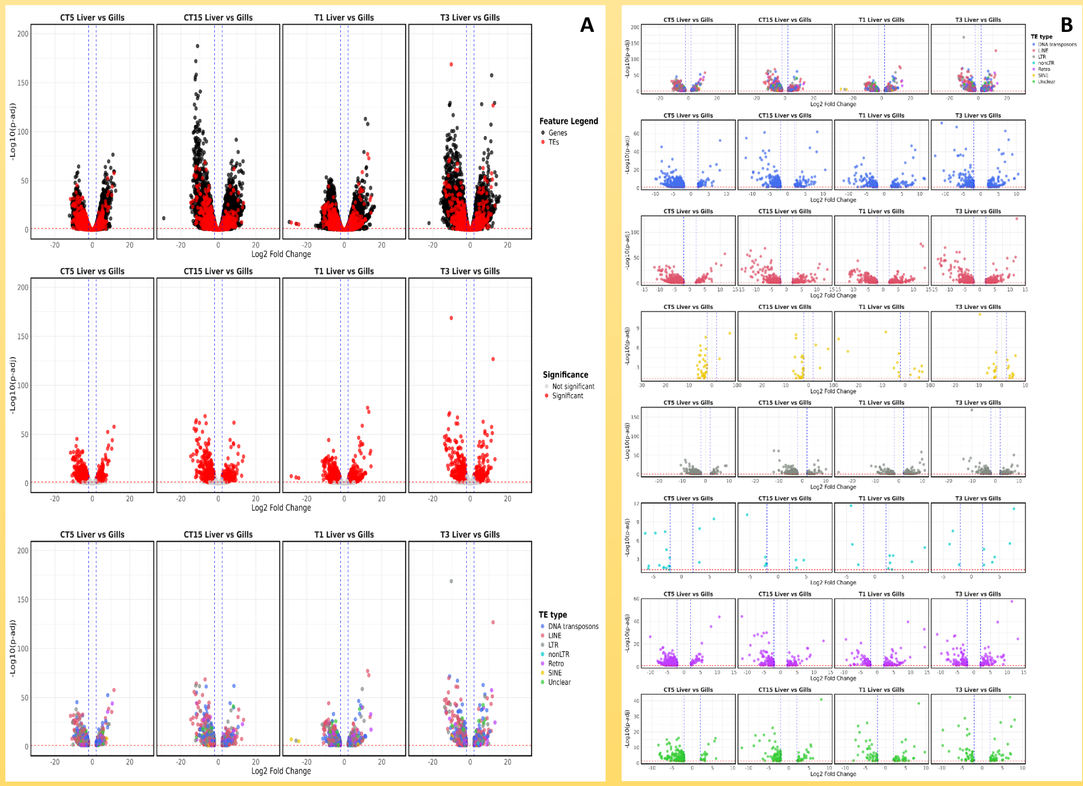

Figure 3. Volcano plot of differentially expressed genes and TEs comparing liver and gills of *T. bernacchii*. A. Volcano plots of genes and TEs. **B.** Volcano plots of the different TE types.
In the first row of the panel A, black dots represent genes, while red dots indicate TEs. In the second row, significantly differentially expressed TEs are highlighted in red, while non-significant TEs are shown in gray. In the third row, the differentially expressed TEs (DETEs) are colored according to their TE class. For each graph, the X-axis represents the Log2 fold change, indicating the magnitude of expression change between conditions, while the Y-axis shows the -log10 adjusted p-value (padj), reflecting statistical significance.
In the panel B the first row is identical to the third row of panel A. The subsequent rows represent the DETEs for each specific TE type.
The volcano plots illustrate a marked difference in the transcriptional response of genes and TEs between liver and gill tissues under the four experimental conditions (CT5, CT15, T1, and T3). Throughout these comparisons a substantial number of DETEs were observed in particular of LINE retrotransposons, followed by DNA transposons and LTR retrotransposons. The right panel, which breaks down the response by TE type, indicates that certain classes are more responsive to experimental conditions, suggesting functional specialization or differential regulatory mechanisms within the TE repertoire. A considerable number of TEs shows significant differential expression, suggesting that TEs might play a more dynamic role in response to heat stress. In the comparisons between control conditions (CT5 and CT15) downregulated DETEs were predominant, on the contrast comparisons between temperature treatments showed a more balanced distribution between up- and downregulated elements. These data suggest that the two tissues are transcriptional active in a different way during heat exposure.

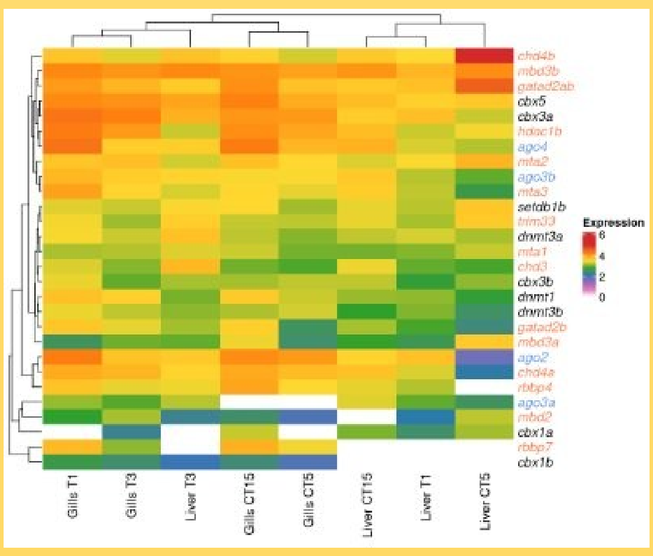

Figure 3. . Heatmap showing the expression profiles of gene involved in silencing mechanism across the different experimental conditions in liver and gill tissues of *T. bernacchii*.
The heatmap shows gene expression profiles in different tissues (gills and liver) under various conditions (time points T1, T3, CT15, and CT5). The color gradient from blue to red represents increasing expression levels. The genes considered are of members of the Argonaute gene subfamily (*ago1*, *ago2*, *ago3*, and *ago4*), genes encoding proteins involved in heterochromatin formation (*cbx5*, *cbx1a*, *cbx1b*, *cbx3a*, *cbx3b*, *dnmt1*, *dnmt3Aa*, and *dnmt3Ab*) and genes of the NuRD complex (*chd3*, setdb1a, *setdb1b*, *chd4a*, *hdac1*, *hdac3*, *mbd2*, *mbd3a*, *mbd3b*, *mta1*, *mta2*, *mta3*, *gatad2Ab*, *gatad2b*, *rbbp4*, *rbbp7*, *FiNZ-ZNF family*, *krab-like ZNF family*, *trim33*).
One of the most notable findings is the clear tissue-specific response observed. The gill and liver samples form distinct clusters, with the exception of Liver T3, which clusters with gills. This pattern indicates that the transcriptional regulation of TE-silencing genes significantly differs between the two tissues, suggesting that each may utilize unique regulatory mechanisms to cope with heat stress. Notably, no transcripts corresponding to *ago1* were detected in either liver or gill samples across all conditions. In contrast, transcripts for *ago2, ago3a, ago3b*, and *ago4* were consistently expressed in both control and experimental samples, with all showing increased expression in the T1 condition compared to CT15.
Some genes, such as ***ago2***, ***mbd3a***, and ***gata4***, exhibit reduced expression in gills under stress conditions (T1 and T3), while others, including ***cbx3a*** and ***hdac1b***, display upregulation. These expression changes may indicate a compensatory activation of alternative pathways to maintain genome integrity when standard silencing mechanisms are disrupted.
Crucially, core epigenetic regulators, including ***dnmt3a***, ***dnmt3b***, and several members of the Argonaute family, display variable expression patterns depending on both temperature and tissue type. This dynamic modulation suggests that *T. bernacchii* actively adapts its genome surveillance mechanisms in response to thermal stress, potentially as a strategy to prevent the activation of TEs.
In summary, the results underlight the complexity and fine-tuning of the TE regulatory system in *T. bernacchii*, reflecting the species molecular adaptability to environmental changes. The distinct temperature-dependent regulation highlights the fish capacity to maintain genome stability despite varying thermal conditions, revealing its adaptive flexibility at the molecular level.

Conclusion -

Our study demonstrates that thermal stress significantly affects TE transcription in *T. bernacchii*, with distinct patterns observed between liver and gill tissues. Liver samples show marked downregulation of LINE, DNA transposons, and LTR retrotransposons, while gill samples predominantly exhibit upregulation, indicating tissue-specific regulatory responses. Core epigenetic regulators also show differential expression, suggesting that both temperature and tissue type influence TE silencing mechanisms. These findings highlight the adaptive flexibility of *T. bernacchii* in responding to environmental changes.