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Impacts of Stress Caused by Copper Sulfate (CuSO4) on the Genome of the Tambaqui (*Colossoma macropomum*): Quantification of *Rex1* and Heterochromatic Profile

Hallana Cristina Menezes da Silva[1]
https://orcid.org/0000-0002-8998-0855

Leila Braga Ribeiro[2]
https://orcid.org/0000-0003-2529-9806

Adolfo José da Mota[3]
https://orcid.org/0000-0002-9745-1128

Eliana Feldberg[4]
https://orcid.org/0000-0002-2962-4541

Daniele Aparecida Matoso[5]
https://orcid.org/0000-0001-7346-6352

[1]Instituto Nacional de Pesquisas da Amazônia, Programa de Pós-Graduação em Genética, Conservação e Biologia Evolutiva. Manaus, Amazonas, Brasil; [2]Universidade Federal de Roraima, Centro de Ciências da Saúde, Faculdade de Medicina, Boa Vista, Roraima, Brasil; [3]Universidade Federal do Amazonas, Instituto de Ciências Biológicas, Laboratório de Biodregadação, Manaus, Amazonas, Brasil; [4]Instituto Nacional de Pesquisas da Amazônia, Laboratório de Genética Animal, Manaus, Amazonas, Brasil; [5]Universidade Federal do Amazonas, Instituto de Ciências Biológicas, Departamento de Genética, Laboratório de Biotecnologia e Citogenômica Animal, Manaus, Amazonas, Brasil.

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*Correspondence: hallana19@gmail.com; Tel.: +55-42-988918138; (H.C.M.S.).

HIGHLIGHTS

- Retrotransposable elements can respond to environmental stress.
- Cooper sulfate can activate retrotransposon *Rex1* within 72h of exposure.
- *Rex1* copy number is higher in exposed animals.
- The heterochromatin profile was different in exposed animals.

Abstract: The transposable elements are known by their ability to move and integrate into the genome of the host organism. They are classified in retrotransposons class I, which has, as intermediate of the transposition, the RNA and retrotransposons class II, which is composed of the DNA transposons, whose DNA migrates directly or is copied and inserted into the genome. The retrotransposable element *Rex*1 is a non-LTR retrotransposon found in several types of organisms. Many studies indicate that the *Rex* retrotransposons have the capacity to respond to environmental stress. The results found in this study corroborate the hypothesis that this retrotransposon possesses a response to environmental stress, since the chromosomal mapping, obtained through FISH, showed a higher number of markings on animals that were submitted to stress in 48h by the copper sulfate action. Furthermore, we can identify an increase in heterochromatic regions in the chromosomes. In the absolute quantification by Real-Time PCR, we found

the largest numbers of *Rex*1 copies in the samples that were submitted to environmental stress by copper sulfate.

Keywords: transposable elements; Real-Time PCR; Copper sulfate.

INTRODUCTION

In Brazil, the tambaqui (*Colossoma macropomum*) is the fish species that accounts about 20% of the production of all freshwater aquaculture operations [1]. The fish farmers often use copper sulfate ($CuSO_4$) to control the proliferation of organic material and parasites in the fish ponds. This substance represents a stressor and potential toxin for captive fish [2,3] in particular by causing physiological alterations [4].

Studies of Amazonian fishes have revealed that the *Rex* retrotransposable elements have a response mechanism for stressful conditions, derived either from the physical-chemical parameters of the water or from the pollution of the aquatic environment [5,6]. The *Rex*1 retrotransposon was described by Volff and coauthors (2000) and has been identified in some fish species with an ample distribution in the genome of these animals, where it plays an important role in the evolutionary dynamics of this group [7,8]. The insertion of TEs within distinct regions of the host genome typically triggers an adaptive cellular response in the genome with the TEs interference in the modulation of the gene expression regulation. This confers an adaptive capacity on the organism due to its genetic variability [9], although this process may also generate genomic instability through insertion in euchromatic regions. However, the TEs may also be kept inactive by the silencing mediated by heterochromatinization. Several studies have shown that heterochromatinization is a highly effective mechanism to block the action of a range of different transposable elements, which guarantees cellular homeostasis [10,11].

In the present study, tambaqui specimens were exposed to a sublethal concentration (30% of the LC_{50}-96h) of copper sulfate ($CuSO_4$), diluted in the water, to verify whether exposure to this compound results in the activation of the *Rex*1 retroelement and whether the genome deactivates it through the formation of heterochromatin.

MATERIAL AND METHODS

Experimental design

All procedures followed the norms recommended by the Committee for Ethics in Animal Experimentation of the Federal University of Amazonas, in Manaus (process number 011/2018 – CEUA UFAM).

Sixty juvenile tambaquis acquired from a fish farming and measuring approximately 15 centimeters in length each were acclimatized for 14 days in the annex of the Applied Evolution Laboratory, Block M, Federal University of Amazonas. The animals were placed in six 240-liter polyethylene tanks, where each tank received 10 animals. The water and oxygen systems were kept open and the fish were fed twice a day with commercial feed (36% protein). The water temperature was maintained at 27°C (+/- 2°C). After the acclimatization period, three tanks were then randomly assigned to each of the two different experimental groups: treatment, and control. The treatment group received 30% of the $LC_{50\text{-}96h}$ dose of copper sulfate ($CuSO_4$), as described for the tambaqui (CL_{50}-96h = 0.380 mg/L), 30% CL_{50}-96h = 0.114 mg/L [12] (Figure 1), while the control group had no copper sulfate being added. The water system was then closed in both tanks.

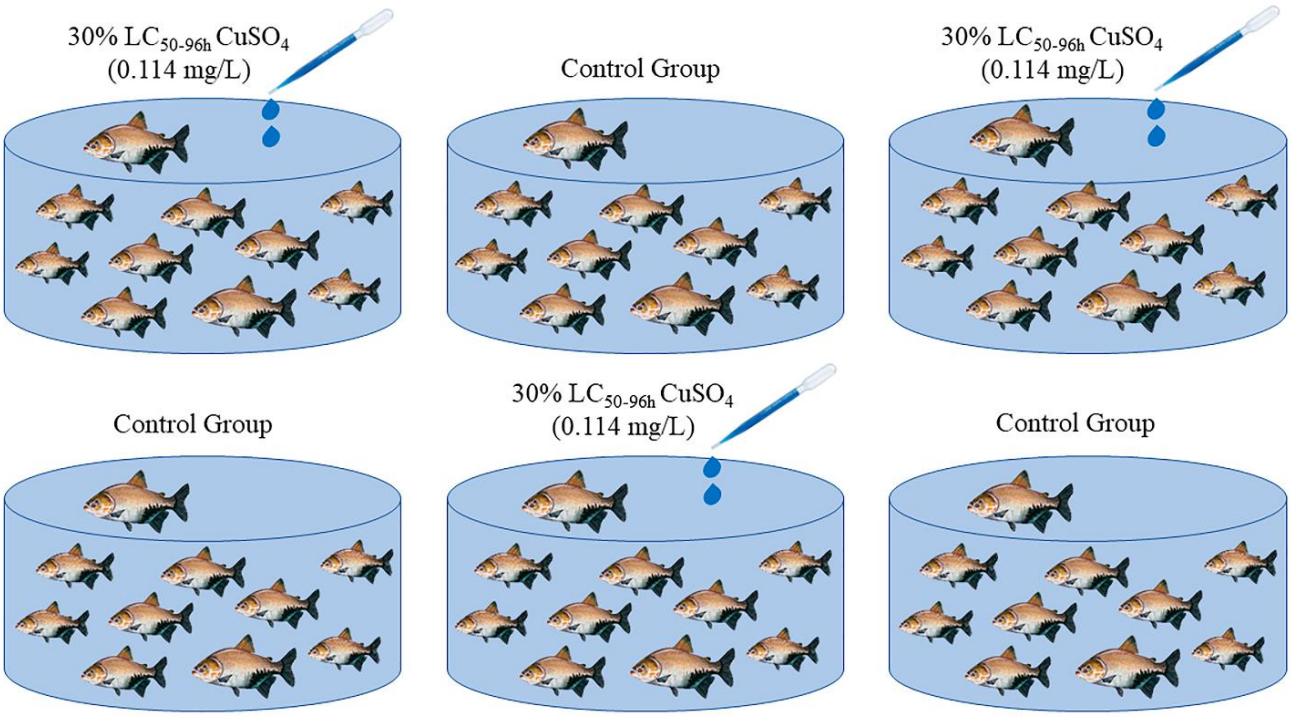

Figure 1. Experimental design scheme.

The material was collected at two different times: 48 hours of CuSO4 exposure and 72 hours. Each experimental time had its respective control group. After 48 h, five animals from each tank were anesthetized with freezing water and then euthanized. Another five specimens from each group were collected after 72 h. Kidney tissue was extracted from each specimen to obtain cell suspensions in order to analyze the heterochromatin, and muscle tissue samples were taken for the absolute quantification of the *Rex*1 by Real-Time PCR.

C-banding

Mitotic chromosomes were obtained using the Gold and coauthors (1990) [13] protocol. The slides were air-dried and then stained with 5% Giemsa for 10 minutes before observation under light microscopy. The C-banding technique, used to detect heterochromatic regions, was applied following the protocol described by Sumner (1972) [14], with some modifications. The slides were treated with 0.2 M HCl for 2 minutes and then washed in distilled water before being air-dried. The slides were then treated with 5% Barium hydroxide (BaOH) at 42ºC for 2 minutes and 20 seconds and immersed in 2xSSC saline solution at 60ºC for 20 minutes before being stained with Propidium Iodide.

Sequencing of the *Rex*1 retrotransposable element

The DNA was extracted using the Sambrook and coauthors (1989) [15] protocol. The *Rex*1 was amplified by PCR using the Taq DNA Polymerase kit (Invitrogen™ by Thermo) and the primers described by Volff and coauthors (2000) [7]. The amplicons were cloned in pGEM-T Easy (Promega) and sequenced with the Big Dye Terminator Cycle Sequencing, v. 3.1 kit (Applied Biosystems by Thermo Scientific). The sequences were analyzed in SeqManPro (DNASTAR v.12). Similarities with other *Rex*1 sequences were verified using the nr database of the BLAST program.

The *Rex*1 sequence obtained in the present study was deposited at the European Bioinformatics Institute (EMBL-EBI) under identification code PRJEB28635 (http://www.ebi.ac.uk/ena/data/view/PRJEB28635). The primers used for the absolute quantification by Real-Time PCR were designed based on this sequence, using Primer Quest Tool (IDT DNA). The efficiency of the primers was determined based on the maker's recommendations and the reaction conditions were adjusted for a final volume of 10 µL containing 0.2 pmol/µL of each primer.

The absolute quantification was performed in a StepOne system (Applied Biosystems by Thermo Scientific) using the SYBR Green Master Mix kit (Applied Biosystems by Thermo Scientific). The plasmid containing the sequence was linearized using the *Nco*I restriction enzyme to determine the concentration

curve. The pDNA was quantified by fluorescence using the Qubit® dsDNA HS (High Sensitivity) Assay kit (Invitrogen by Thermo Scientific) and the mass of the sample was transformed into a number of copies using the equation:

$$\frac{quantification\ average\ (\mu g).\ Avogrado\ number\ (6{,}022.1023^{23})}{dalton\ numbers\ (656{,}6.10^{9})\ .\ amplicon\ size\ (115bp)}$$

The standard curve was drawn up based on four reference points of the number of copies ($7.6423.10^{10}$, $7.6423.10^{9}$, $1.5285.10^{9}$, and $3.0571.10^{8}$ copies). All reactions were run in triplicate and contained 5 ng of the genomic DNA. The number of copies of each sample was obtained by interpolating the Ct data of the samples with those from the standard curve.

Statistical analysis

The Kolmogorov-Smirnov test was used to verify the normal distribution of the absolute values determined by the Real-Time PCR, and Student's *t* test was used to analyze the significance of the differences between the two experimental treatments results. Both tests were run in the R software (The R Project for Statistical Computing-The R Foundation).

RESULTS

Heterochromatic profile and FISH

The heterochromatic profiles of the tambaqui specimens from the different treatment groups (control, 48 hours, and 72 hours) showed considerable differences in the distribution of the heterochromatic blocks (Figure 2). When we compared the three treatments, we found conspicuous signals in the samples of the fish exposed to the heavy metal, in particular in the samples exposed for 72 hours (Figure 2). Pericentromeric heterochromatic signals were detected in almost all chromosome pairs in the control samples. In the samples exposed to copper sulfate for 48h, pericentromeric signals were observed in several pairs, an interstitial signal in one pair, bitelomeric markings, signals on the short arms of two pairs, and practically in the whole chromosome of the other two pairs (Figura 2b). In the 72-hour exposure group, heterochromatin signals were observed on the short arms of six pairs, with an interstitial signal in one pair, bitelomeric signals on one pair, and chromosomes almost totally marked, in the two pairs, in addition to one of the homologs of the one pair (Figure 2c).

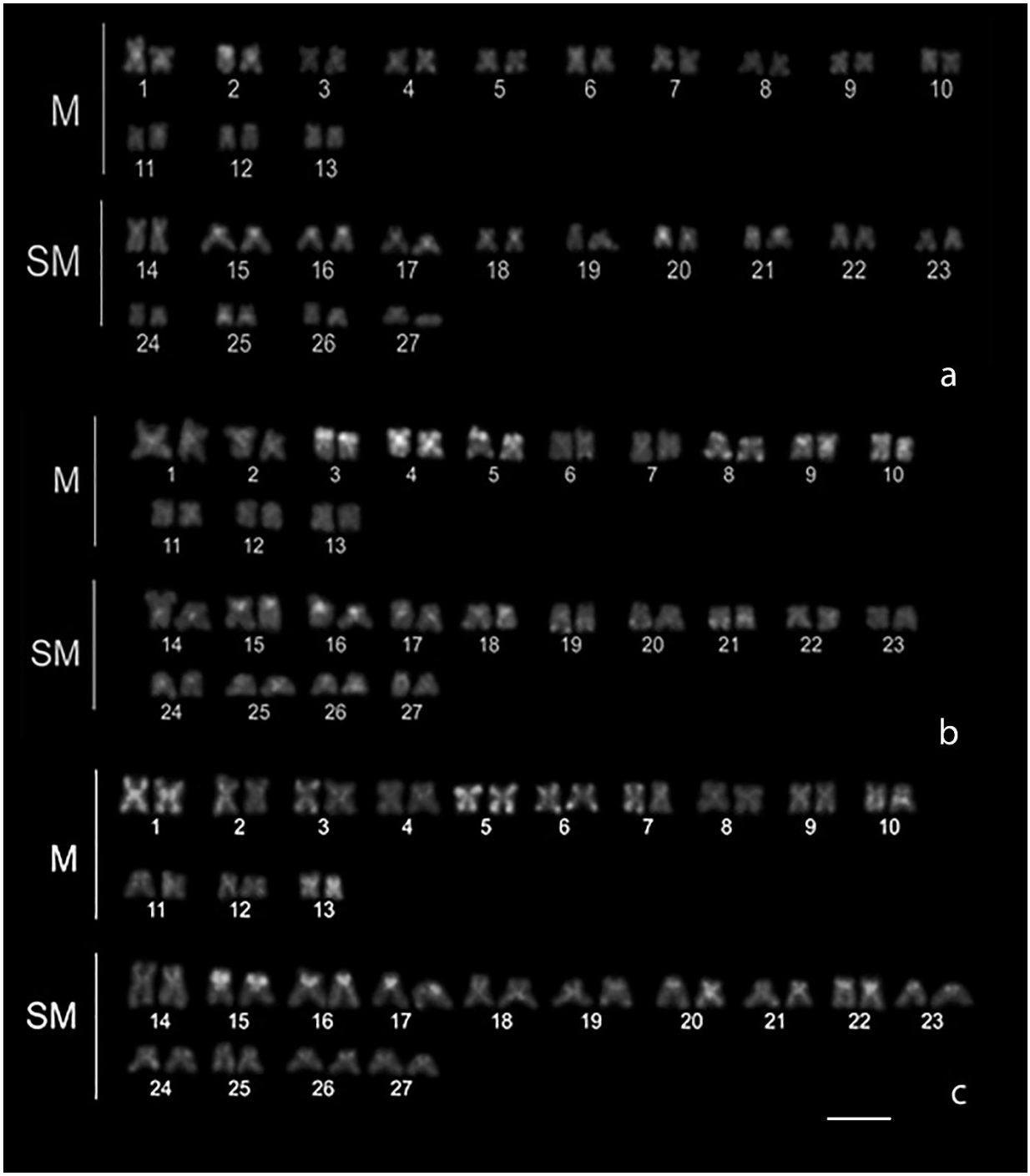

Figure 2. Heterochromatin distribution in *Colossoma macropomum* specimens. a) C-banding in control. The arrows show two marked chromosomes in the pericentromeric region. b) C-banding in 48h condition, the arrows show chromosomes almost completely stained. The arrowhead shows bitelomeric signals. c) C-banding in 72h condition. The arrow tail shows chromosomes almost completely stained and the arrowhead shows short arms staining.

As there was no cell division induction in the analyzed fish, the analysis of metaphases good enough for the FISH technique has become limited. Therefore, in this technique, only the samples that were submitted to 48 hours of $CuSO_4$ exposure were analyzed. In the case of the FISH of the *Rex*1 retroelement, the animals exposed to $CuSO_4$ for 48h presented signals dispersed throughout several different chromosomes when compared to the control group (Figure 3a), including arms entirely marked (Figure 3b).

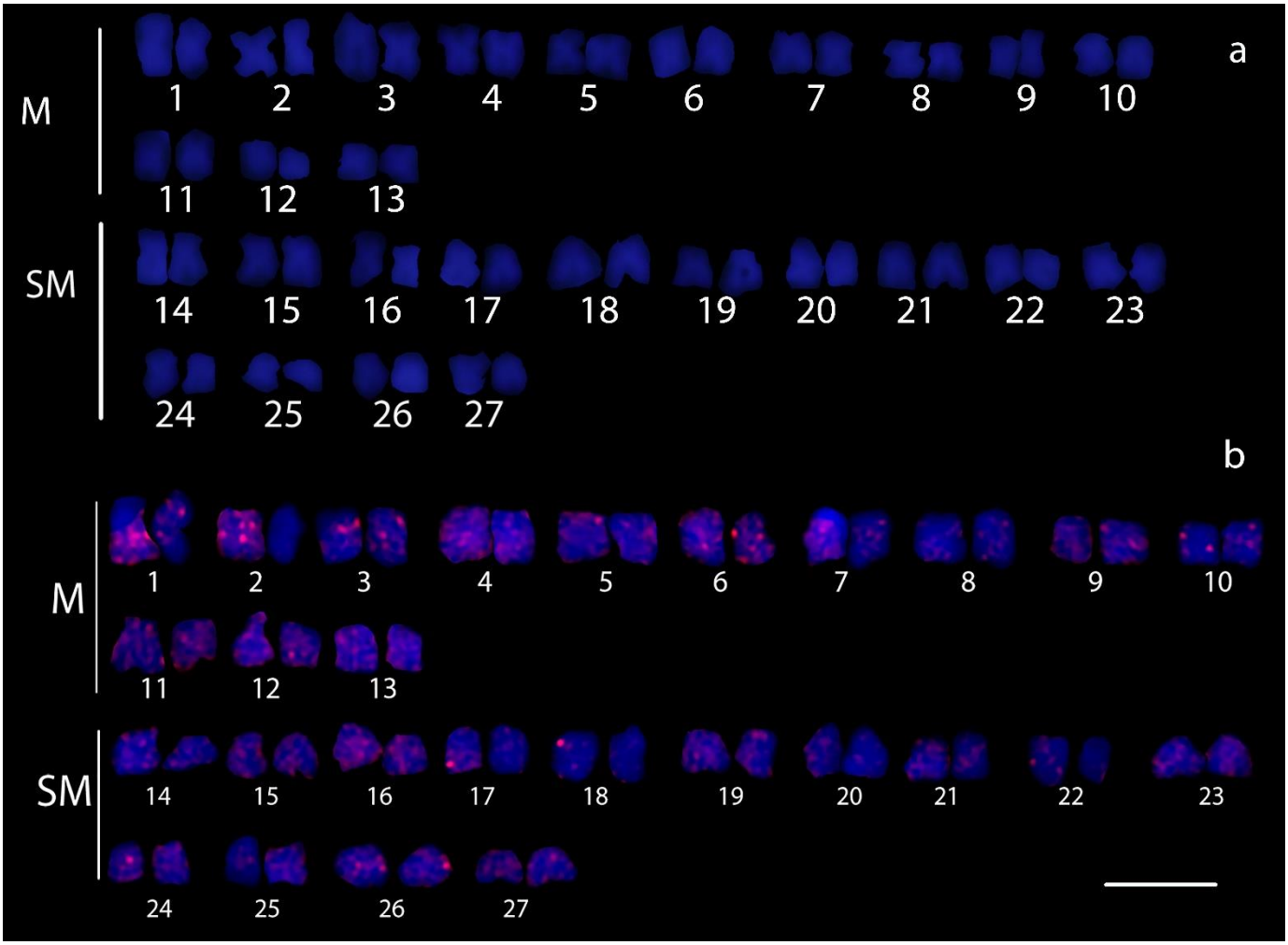

Figure 3. a) FISH of the *Rex1* in the control group; b) FISH of the *Rex1* of the samples submitted to 48 hours of CuSO4 exposure.

Absolute quantification of the *Rex*1 retroelement

The quantitative analysis by Real-Time PCR was designed to evaluate the impact of $CuSO_4$ exposure on the tambaqui genome, *i.e.*, monitor the potential increase in the *Rex*1 retroelement number of copies found in the genome when compared to the control group. Table 1 shows the difference between the *Rex1* number of copies found in the tambaqui genome, both in control and experimental samples.

Table1. Quantification, in copy number, of *Rex1* in the tambaqui genome (Colossoma macropoum), exposed (experimental group) and not exposed (control group) to $CuSO_4$.

Sample	Condition		Number of copies in the genome
T01	Experimental Group (50% LC50-96h = 0.114mg/L)	48h	68,982,950
T07			40,963,005
T08			36,548,895
T10			44,240,384
T12			52,543,675
T19	Control Group (not exposed)		26,596,265
T20			28,205,556
T21			26,074,322
T32	Experimental Group (50% LC50-96h = 0.114mg/L)	72h	52,241,898
T37			58,792,352
T38			397,912,585
T40			110,914,519
T53	Control Group (not exposed)		29,784,025
T55			41,720,309
T56			45,666,232

In the first 48 hours of exposure, there was an increase in the number of copies, when compared to the control group, although the difference was not statistically significant based on the value of *t* (Figure 4A). However, *t* was significant when the samples exposed for 72 hours were compared with the respective control (Figure 4B). The Kolmogorov-Smirnov test confirmed with confidence that the data are normally distributed (P=0,6347).

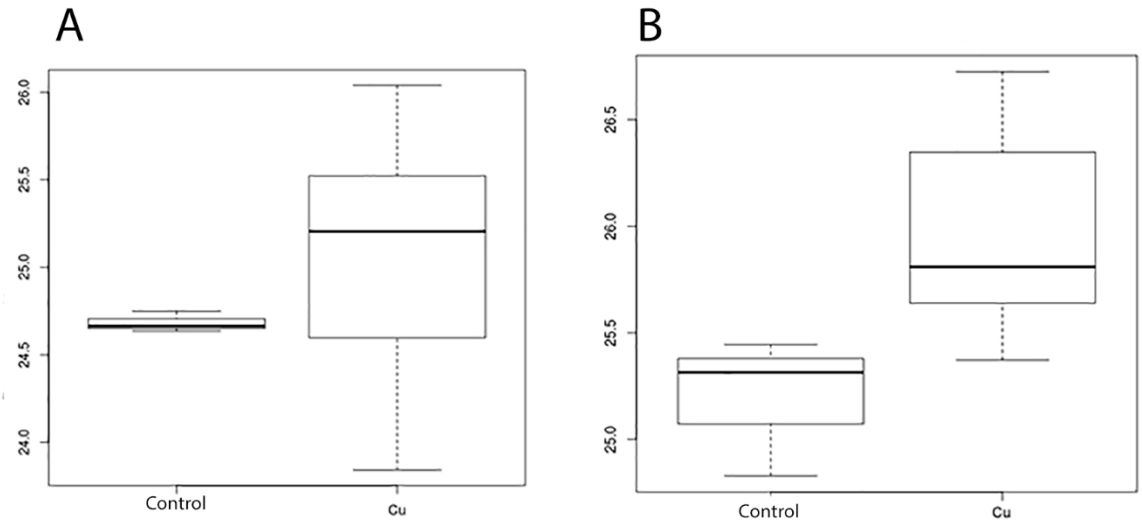

Figure 4. A) Graph of samples exposed for 48 hours to $CuSO_4$, showing overlapping values of the averages between the experimental group and the control group. The numbers of copies are presented on a logarithmic scale, on the axis of the ordered (y). B) Graph of samples exposed for 72 hours to $CuSO_4$. The differences observed between the averages of the control and experimental groups are statistically significant. The experimental group presents a significantly higher number of *Rex1* retroelement copies in the samples. The numbers of copies are presented on a logarithmic scale, on the axis of the ordered (y).

DISCUSSION

Transposable elements (TEs) are the most abundant components in the organism's genome, corresponding to about 50% of it. In some organisms such as maize, the coding regions are just small islands floating in a sea of retrotransposons [16]. Due to the stochastic nature of the activity [17], many elements show fluctuations related to responses to environmental stress and its effects at individual and genomic levels. This variation may refer to the type of organism response, the type of stress, or the type of regulation to which the TE is subjected. In this context, retrotransposons present the widest variation range seen so far [18]. For this balance, dynamic protection systems such as chromosomal heterochromatinization are known to repress the activity of these mobile sequences, thus, although they are abundant in the genome, the transposable elements remain silenced most of the time [19, 20]. According to Trojer and Reinberg (2007) [21], heterochromatinization in response to environmental changes is a process dynamically controlled by epigenetic changes, which provides a quick and efficient way to flex the cellular tolerance level when facing environmental stress [22].

The environment adaptation is probably responsible for linking *Rex1* activation and heterochromatinization. If $CuSO_4$ exposure induces an increase in *Rex1* activity, an increase in heterochromatinization is also expected as consequence. In this study, this mechanism was consistently observed as an increase in the number of heterochromatic blocks was effectively verified in individuals kept for 48 and 72 hours in contact with the solution, when compared to animals in the control condition (Figure 2). This dispersion of heterochromatin may be an epigenetic response to the activation of the retrotransposable elements, in this case to the retroelement *Rex1*, when the organism was exposed to the stressor. In microorganisms several studies relate the TEs activity with environmental adaptation, making clear its relationship with adaptive phenotypic change. Studies such as Stoebel and coauthors (2009), Sun and coauthors (2009), Chou and coauthors (2009), and Gaffé and coauthors (2011) [23-26], used microorganisms such as *Escherichia coli*, *Candida tropicalis*, *Candida albicans*, *Sacharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Methyloacterium extorquens* when facing stress, and concluded that there was a direct relationship between stress conditions and TE activation. Thus, to quickly adapt to environmental changes or to protect itself from exposure to toxic agents, epigenetic alterations have to alter the conformation of chromatin, initially allowing the expression of TEs.

However, responding to the risk that random insertions bring to genomic stability, regions with TE abundance are again silenced by heterochromatinization. Whitelaw and Martins (2001) [17] observed that in mammals, analyzing isogenic mouse strains, it was found morphological phenotypic variation related to the action of retrotransposons. This action was related to the stochastic activity of retroelements, producing subtle phenotypic variations in genetically identical individuals. According to the authors, due to the differential gene expression of retrotransposons in somatic cells, individuals can be considered epigenetic mosaics and this inheritance does not follow a Mendelian pattern. Several studies have already demonstrated the emergence of epigenetic marks and consequently the heterochromatin appearance in a variety of organisms when exposed to stressor agents [27-29].

Hereupon, analyzing rodents' brains exposed to a moderate acute stress for only an hour, Hunter and coauthors (2013) [10] were able to observe a regionally selective increase in the epigenetic marks of H3K9me3 in the hippocampus, which is directly related to heterochromatin appearance. These marks persisted for 24 hours and after 7 days of stress, they began to disappear and were absent after 3 weeks. The authors claim that it remains unclear whether this characterizes some learning form or a stressor agent adaptation. Consistent with this mechanism, in the present approach we observed that the retrotransposable element *Rex1* from tambaqui was activated by the stressor agent copper sulfate during the experiment course. This was confirmed by the observation of a *Rex1* copies increase in the genome of animals that were exposed to the compound for 48 and 72 hours when compared to the control condition. At the exposure time of 72 hours, this increase in the number of copies was significantly greater than in the control condition (Figure 3).

In the Amazon, several studies have been described reporting the possible adaptive responses and environmental changes related to TEs in fish. Ribeiro (2013) [30] performed the chromosomal mapping of *Rex1*, *Rex3*, and *Rex6* in tambaqui specimens acclimated to three different temperatures: 30ºC, 28ºC, 22-23ºC. As a result, it was found that at the lowest temperature the animals showed more markings when compared to the other specimens. This result corroborated the hypothesis that these retrotransposons have a response to environmental stress by temperature change. Silva and coauthors (2016) [6] carried out a study with specimens of *Hoplosternum litoralle*, known as tamoatá, where through chromosomal mapping they could analyze the *Rex3* markings in specimens collected in environments with polluted water and environments with unpolluted water, observing that the levels of *Rex3* markings in the polluted water environments were greater than in the unpolluted. The first work with differential expression analysis was carried out by Barbosa and coauthors (2014) [5] using the retrotransposable element *Rex6*. In this study, it was found that in the gills, liver, and muscle tissues of tambaqui (*Colossoma macropomum*) there was greater expression of *Rex6* in specimens from clear water when compared with specimens from black water. The authors associated this difference in expression with the water physicochemical differences. These studies in Amazonian fish support the idea of previous studies which presented that Rex retroelements have a response to environmental changes. In addition to rDNAomics, Silva and coauthors 2019 [31] analyzed the constitutive heterochromatin of fish species in polluted and unpolluted water environments. As a result, it was observed that fish from polluted water had greater markings on the chromosomes referring to heterochromatin, indicating that these fish used this mechanism in an attempt to adapt the genome to this environmental condition. Silva and coauthors 2020 [32] also mapped the retrotransposables *Rex1*, *Rex3*, and *Rex6* from the same environments and found that fish from polluted water had higher chromosomal markings of these retroelements, with *Rex1* and *Rex3* showing more differences between the markings while *Rex6* showed fewer differences.

Regarding the results obtained on the heterochromatin patterns found in our work, they support the evidence that there was an adaptive response of the fish to the environmental stress caused by copper sulfate as a consequence of retrotransposable elements activation with their subsequent inactivation by the heterochromatin assembly. This heterochromatin molecular characterization as well as the assembly mechanisms, scattering, containment, and its maintenance is outside the scope of the discussion in this article. We can, however, conclude that the heterochromatin observed in the control group in a pericentromeric position must be constitutive and related to the functional kinetochores assembly in DNA regions that are centromeres satellites, whose primary function is to ensure correct chromosomal segregation during cell division [33]. For the assembled heterochromatin, in samples exposed to copper sulfate, we believe that the change in this pattern is related to the necessity of the fish to activate or silence genes that are responsible for the cell function maintenance under stress, since gene silencing mediated by repetitive DNA sequences heterochromatinization is well established in the literature, and there are also reports that the formation of heterochromatin is required for gene activation [34].

The real consequences of the *Rex1* element transport and insertion in the genome of these specimens is still an open question that should be addressed in future approaches that aim to understand the functioning

of adaptive genetic and epigenetic mechanisms required by the organism to face different environmental challenges. However, the role of TEs in important events of gene expression control and the genome is undeniable, favoring processes such as speciation, genetic variability, phenotypic plasticity, and genomic malleability in species. The heterochromatinization process is also essential for the genomic stability maintenance and cell machinery balance, in humans it has medical relevance as it is involved in the regulation of processes such as viral dormancy, obesity, premature aging, and metabolism changes [33]. Future work will be carried out in order to identify these changes at the molecular level, along with the expression of genes that may be candidates for the animal stress response.

In the present study, we applied an integrated approach between cytogenomics and absolute quantification through Real-Time PCR, combining controlled environmental change and adaptive responses in Amazonian aquatic organisms. This approach provided the acquisition of greater knowledge about the tambaqui functional genomic organization, proving to be fundamental to supporting applied genetics studies.

CONCLUSION

The Real-Time PCR technique showed an increase in the retrotransposable element *Rex1* number of copies when compared to the control group, in the two sampling times used: 48 and 72 hours. Absolute qPCR showed a significant increase in the number of *Rex1* copies in the tambaqui genome exposed to $CuSO_4$ for 72 hours when compared to the control group. The FISH technique showed an increase in the *Rex1* number of markings in the group submitted to $CuSO_4$ for 48h when compared to the control group. C-banding heterochromatin analysis showed that there was an increase in heterochromatic regions in the tambaqui genome when exposed to $CuSO_4$, probably showing a gene silencing process of *Rex1* retrotransposable elements. Therefore, we conclude that the heavy metal copper sulfate showed to be toxic at the molecular level through the activation of *Rex1* in the tambaqui genome.

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Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

1. Ministry of Fisheries and Aquaculture (MPA). Fisheries and Aquaculture Statistical Bulletin. Brasília, Ministry of Fisheries and Aquaculture. 2011;160.
2. Beaumont MW, Butler PJ, Taylor EW. Exposure of brown trout, *Salmo trutta*, to a sub-lethal concentration of copper in soft acidic water: Effects upon muscle metabolism and membrane potential. Aquat Toxicol. 2000;51:259–72.
3. Novelli-Filho JLVB, Novelli ELB, Manzano MA, Lopes AM, Cataneo AC, Barbosa LL, et al. Effect of alpha-tocopherol on superoxide radical and toxicity of cadmium exposure. Int J Environ Health Res. 2000;10:125–34.
4. Nriagu JO. A global assessment of natural sources of atmospheric trace metals. Nature. 1989;338,47–9.
5. Barbosa CM, Mareco EA, Silva MDP, Martins C, Alves-Costa FA. Differential expression of a retrotransposable element, Rex6, in *Colossoma macropomum* fish from different Amazonian environments. Mob Genet Elements. 2014;4:e30003.
6. Silva FA, Schneider CH, Feldberg E, Baccaro FB, Carvalho NDM, Gross MC. Genomic Organization Under Different Environmental Conditions: Hoplosternum Littorale as a Model. Zebrafish. 2016;13(3):197-206.
7. Volff JN, Körting C, Schartl M. Multiple lineages of the non-LTR retrotransposon Rex1 with varying success in invading fish genomes. Mol Biol Evol. 2000;17:1673–84.
8. Volff JN, Körting C, Froschauer A, Sweeney K, Schartl M. Non-LTR Retrotransposons Encoding a Restriction Enzyme-Like Endonuclease in Vertebrates. J Mol Evol. 2001;52:351–60.
9. Kazazian HH. Mobile elements: drivers of genome evolution. Science. 2004; 303:1626–32.
10. Hunter RG, McEwen BS, Pfaff DW. Environmental stress and transposon transcription in the mammalian brian. Mobile Genetics Elements. 2013;3:e24555.
11. Wang J, Reddy BD, Jia S. Rapid epigenetic adaptation to uncontrolled heterochromatin spreading. eLife. 2015:1–17.
12. Figueiredo RO, Silva KYS, Oliveira CPF, Duarte RM, Silva MNP, Val AL. Cooper and lead toxicity for Matrinxã (*Brycon amazonicus*, Spix e Agassiz, 1829). 2014.
13. Gold JR, Li YC, Shipley NS, Powers PK. Improved methods for working with fish chromosomes with a review of metaphase chromosome banding. Journal of Fish Biology. 1990;37:563-75.
14. Sumner AT. A simple technique for demonstrating centrometic heterochromatin. Experimental Cell Research. 1972; 75, 304-6.

15. Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning a Laboratory Manual. Cold Springs Harbor Laboratory Press, Cold Springs Harbor, NY. 1989.
16. Mita P, Boeke JD. How retrotransposons shape genome regulation. Genet Dev. 2016;37:90-100.
17. Whitelaw E, Martin DIK. Retrotransposons as epigenetic mediators of phenotypic variation in mammals. Nat. Genet. 2001:361–5.
18. Capy P, Gasperi G, Biémont C, Bazin C. Stress and transposable elements: co-evolution or useful parasites. Heredity. 2000; 85,101-6.
19. Okamoto H, Hirohiko H. Silencing of transposable elements in plants. Trends Plant Sci. 2001; 6(11),527-34.
20. Mansour A. Epigenetic activation of genomic retrotransposons. J Cell Mol Biol. 2007; 6(2), 99-107.
21. Trojer P, Reinberg D. Facultative Heterochromatin: Is There a Distinctive Molecular Signature? Mol Cell. 2007; 28:1–13.
22. Wang J, Jia ST, Jia S. New Insights into the Regulation of Heterochromatin. Trends Genet. 2016;32(5):284–94.
23. Stoebel DM, Hokamp K, Last MS, Dorman CJ. Compensatory evolution of gene regulation in response to stress by *Escherichia coli* lacking RpoS. Plos Genet. 2009;5(10):e1000671/1-9.
24. Sun J, Yu EY, Yang Y, Confer LA, Sun SH, Wan K, et al. Stn1-Ten1 is an Rpa2-Rpa3-like complex at telomers. Genes Dev. 2009;23(24):2900-14.
25. Chou HH, Berthet J, Marx CJ. Fast growth increases the selective advatage of a mutation arising recurrently during evolution inder metal limitation. Plos Genet. 2009; 5(9).
26. Gaffé J, McKenzie C, Maharjan RP, Coursange E, Ferenci T, Schneider D. Insertion Sequence-driven evolution of *Escherichia coli* in Chemostats. J Mol Evol. 2011;72:398-412.
27. Ayoub N, Goldshmidt I, Cohen A. Position effect variegation at the mating-type locus of fission yeast: A cis-acting element inhibits covariegated expression of genes in the silent and expressed domains. Genetics. 1999;152(2):495–508.
28. Hunter RG, McCarthy KJ, Milne TA, Pfaff DW, McEwen BS. Regulation of hippocampal H3 histone methylation by acute and chronic stress. PNAS. 2009:20912–7.
29. Andres F, Coupland G. The genetic basis of flowering responses to seasonal cues. Nat Rev Genet. 2012;13,627–39.
30. Ribeiro LB. Chromosomal physical mapping of repetitive elements in *Colossoma macropomum* (Characiformes, Serrasalmidae) associated with fish farming. Doctoral Thesis, National Institute for Research in the Amazon. 2013.
31. Silva FA, Feldberg E, Carvalho NDM, Rangel SMH, Schneider CH, Carvalho-Zilse GA, et al. Effects os environmental pollution on the rDNAomics of Amazonian fish. Environ Pollut. 2019; 252:180-7.
32. Silva FA, Guimarães EMC, Carvalho NDM, Ferreira AMV, Schneider CH, Carvalho-Zilse GA, et al. Transposable DNA elements in Amazonian fish: from genome enlargement to genetic adaptation to stressful environments. Cytogenet Genome Res. 2020;160(3):148-55.
33. Allshire RC, Madhani HD. Ten principles of heterochromatin formation and function. Mol Cell Biol. 2017;19(4):229–44.
34. Grewal SIS, Jia S. Heterochromatin revisited. Nat Rev Genet. 2007;8,35–46.