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PhD thesis title: The genus Paramacrobiotus (Tardigrada): integrative taxonomy, biogeography and effects of stress factors on the selected species.

Paramacrobiotus is one of the genera of the phylum Tardigrada (commonly referred as water bears). This genus was erected more than a decade ago. Previously all representatives of this genus were included in the *richtersi-areolatus* complex within the genus *Macrobiotus*, but with the help of molecular and phylogenetic analysis, this new genus was identified and established.

As the title of thesis suggests, the goal of this dissertation was the overall study of the genus *Paramacrobiotus*, including: incorporating integrative taxonomy in new species descriptions, working out the distribution patterns of parthenogenetic *Pramacrobiotus* species, testing if the 'everything is everywhere' hypothesis is true for them, testing the influence of different types of stressors on the species *Pam. experimentalis*, assessing biogeography, distribution, microbiome, reproduction, feeding behaviour, life history, *Wolbachia* endosymbiont identification and cryptobiotic abilities of the species from the genus *Pramacrobiotus* and providing a new diagnostic key for the genus using morphological and morphometric characters of adults and eggs.

Using an integrative taxonomy approach (classical morphology and morphometry, as well as, genotypic using DNA barcodes and phylogenetic tree), a new species: *Pam. gadabouti* was described from a moss sample collected in Ribeiro Frio, Madeira. Furthermore, the mode of reproduction of this species was studied experimentally, which corroborates the interrelatedness between wide distribution and parthenogenesis.

In the subsequent paper constituting the doctoral dissertation, two parthenogenetic species of the *Paramacrobiotus*, i.e., *Pam. gadabouti* and *Pam. fairbanksi* were analysed to study the distribution, as well as genetic variability which showed that the 'everything is everywhere' hypothesis is true for, at least, some tardigrade species. Environmental niche modelling performed using MaxEnt supports the wide distribution of these two parthenogenetic species.

In the next publication, survivability of the *Pam. experimentalis* was tested at various concentrations (0.25%, 0.50% and 1.00%) of magnesium perchlorates (in range with the concentration present in Martian regolith) for two different time points (24h and 72h). In experiments where specimens were exposed to 24h time period, 33.3%, 16.7% and 0% were

active in 0.25%, 0.50% and 1.00% solutions, respectively. However, more than 75% returned to activity after transferring them to the culture medium (93.3%, 76.7% and 86.7% of specimens exposed to 0.25%, 0.50% and 1.00% solutions, respectively). In experiments where specimens were exposed to 72h time period, 30.0%, 26.7% and 0% were active in 0.25%, 0.50% and 1.00% solutions, respectively and after transferring them to the culture medium, 83.3%, 86.7% and 10.0% of specimens exposed to 0.25%, 0.50% and 1.00% solutions, respectively, returned to activity.

In the following paper constituting the doctoral dissertation, changes in ultrastructure for both active animals and desiccated tuns, as well as levels of heat shock proteins (Hsp27, Hsp60 and Hsp70) in active animals of *Pam. experimentalis* were studied when exposed at higher temperatures (35 °C, 37 °C, 40 °C, and 42 °C) for 5 hours, compared to optimal temperature (20 °C). Isolated storage cells from the control group persisted in the body cavity among internal organs in an amoeboid or spherical shape. Small, but visible changes were observed in specimens exposed to 35°C in the form of alterations in the mitochondria. Significant ultrastructural changes were observed in storage cells of specimens exposed to 37 °C. There were multiple deteriorating mitochondria with the loss of its cristae and there was a presence of autophagic structures. The level of changes increased at 40°C, with the irregular and shrunken shape of storage cells, deteriorated cell organelles and mitochondria with a higher number of autophagosomes and autolysosomes. Most drastic changes were observed at 42 °C, with full degeneration of cells and organelles showing signs of necrosis, making even cell identification difficult. However, when exposed to higher temperatures, tuns exhibited absolutely no differences from the control group. Out of five temperatures tested, the three deemed most important were selected (20 °C – optimum temperature, 35 °C – the highest temperature from which the return to activity was observed and 42 °C – where full necrosis was observed) and used for quantification of heat shock proteins (Hsp27, Hsp60 and Hsp70) in active specimens. All of them showed significant upregulation with the increase of the temperature.

In the last paper, all available information on the genus *Paramacrobiotus* were summarized. Thus, in summary, the genus *Paramacrobiotus* currently includes 45 species (including *Pam. gadabouti* added as a part of the present thesis). The species of this genus can be found everywhere throughout the globe, supporting the statement that the genus is cosmopolitan. Both dioecious and unisexual species are present in the genus, with both long and short lifespan. The species in this genus are generally carnivorous with their food preference, including certain rotifers, nematodes and juvenile tardigrades. However, it was

reported that they could also feed on cyanobacteria, algae, and fungi. The species generally have a good affinity for cryptobiosis, which is why multiple studies involving anhydrobiosis have been performed to date using specimens of various species of the *Paramacrobiotus*.