Modes of Speciation in Subterranean Diving Beetles from a Single Calcrete Aquifer in Central Western Australia

Paroster macrosturtensis (Dytiscidae)

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This thesis is dedicated to my children

Nikita and Jack

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Calcrete aquifers from the Yilgarn region in central Western Australia's arid zone contain a highly diverse range of obligate groundwater invertebrate species (stygofauna), with many endemic to single calcretes. Phylogenetic studies on the diving beetles from the Yilgarn calcretes suggest a scenario of invasion of the subterranean environment by several surface dwelling ancestors during aridification of the region since the Miocene. This system is ideal for examining modes of speciation within a closed calcrete body and in particular the relative roles of allopatric, parapatric and sympatric speciation in the generation of diving beetle (Dytiscidae) species diversity. Previous phylogenetic analyses have identified 13 independent cases of sympatric sister species pairs of large and small diving beetles in separate calcretes, suggesting the potential for their speciation in sympatry as a result of ecological niche partitioning. A single calcrete at Sturt Meadows contains a sympatric sister triplet of large and small diving beetles (*Paroster macrosturtensis, P. mesosturtensis, P microsturtensis*), and can be accessed by an extensive grid of mineral exploration bores (3.5km^2, 115 bores), allowing intensive sampling for population genetic studies and biodiversity assessment. Comparative phylogeographic analyses by others on these *Paroster* beetle species found no evidence for long term allopatric processes operating within the calcrete, although any fragmentation event that could have led to the evolution of the three beetle species may not have persisted post-speciation, and thus would not been seen in their gene genealogies.

The aim of this study was to investigate the possibility that the three beetle species at Sturt Meadows may have evolved by sympatric speciation due to trophic niche partitioning. Two main approaches were used to achieve this aim. First, whether the different beetle species have different trophic niches was determined and, second, whether micro-allopatric processes, such as fragmentation events, may have led to the evolution of the three beetle species was investigated. To detect evidence for such fragmentation events, a comparative phylogeography of chiltoniid amphipods in the Sturt Meadows calcrete was undertaken.

A DNA barcoding framework was established for the macro-invertebrates in the Sturt Meadows calcrete, in order to obtain sequence information on potential prey groups for the diving beetles. A 623-bp fragment of the mitochondrial cytochrome *c* oxidase 1 (*COI*) gene was amplified from stygofauna plus terrestrial organisms that were found in the calcrete. Phylogenetic analyses revealed the existence of 12 divergent monophyletic groups of haplotypes, including three unrelated lineages of chiltoniid amphipod that are

morphologically cryptic. Allozyme analyses on the amphipods showed them to be three species that can be separated at multiple allozyme loci.

Spatial variation in genetic diversity was assessed for the chiltoniid amphipods, enabling a comparative phylogeography of the three species across the bore field. There was no common pattern in the gene trees of the amphipod species, so no evidence for three isolation events in the diversification of the diving beetles at this site. However, high haplotype diversity coupled with low nucleotide diversity, plus signatures of population expansion and isolation by distance in two out of three species, match previous findings for diving beetles at the same site and indicate the potential for micro-allopatric evolutionary processes to have operated within the calcrete. Isolation of populations in pockets of favourable habitat (refugia) within the calcrete followed by expansion events, are proposed as the most likely generator of population genetic diversity, and are thought to be related to water level changes in the aquifer.

Trophic niche partitioning in the sister triplet of large and small *Paroster* diving beetles and their larvae was investigated by molecular amplification of small fragments of the *COI* gene identified by the barcode. Amphipods (Chiltoniidae) and copepods (Harpacticoida, Cyclopoidea) were chosen for the analysis as they were the most abundant potential prey items in the calcrete. There was not complete trophic partitioning in the adult beetles, with all three species feeding on amphipods and copepods. As the trophic analysis was molecular, differences in size of the prey were not tested for. There was some evidence for preferential feeding on particular prey species by the adult beetle species, however, small sample sizes precluded making comparisons between their larvae. It is thought that in the impoverished environment of the aquifer, the adult beetles are scavengers and opportunistic feeders, as well as active predators. Stable isotope analysis confirmed that the three diving beetle species are feeding on similar food items, and indicated a separate source of carbon and nitrogen to the tree roots as the basis of the food web of the calcrete.

In summary, there was no evidence for complete trophic niche partitioning in the adult diving beetles of different sizes that could have led to their speciation in sympatry. Any further investigation of trophic differences needs to concentrate on preferential feeding in the adults, and the trophic niche of the beetle larvae which are active predators. The potential for micro-allopatry in the diversification of the different sized diving beetles at Sturt Meadows has been identified, through congruence in current population genetic patterns for the amphipods and the diving beetles. Stygofauna in the calcrete have high genetic diversity, which is thought to be the result of historical water level fluctuations leading to frequent population bottlenecks, followed by range expansion after aquifer recharge. Identification of

at least 12 macro-invertebrate species in a single calcrete increases the total number of stygobytic and troglobytic species discovered in the Yilgarn, and has enabled estimates to be made of possible numbers of subterranean species in the region still to be discovered.

The presence of large and small dytiscid beetles in multiple calcretes that are sympatric sister species still points to some common ecological niche differentiation within the calcrete environment. It is hypothesized that there could be depth partitioning in the different sized diving beetles related to their oxygen requirements. The population genetic and ecological data generated at Sturt Meadows provide a baseline for the Yilgarn calcretes. Extending such a study to examine spatial and ecological differentiation in sympatric sister species of subterranean diving beetles across multiple calcretes, would be a powerful approach in the investigation of modes of speciation.

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