HAPLOTYPE DIVERSITY IN PILBARUS MILLSI, A WIDESPREAD GROUNDWATER SPECIES OF AMPHIPOD FROM THE PILBARA, WESTERN AUSTRALIA

[1]T. FINSTON, [1]M. JOHNSON, [2]W. HUMPHREYS, [3]S. EBERHARD and [3]S. HALSE.

1. School of Animal Biology M092, The University of Western Australia, 35 Stirling Hwy., Crawley, WA 6009, Australia (tfinston@cyllene.uwa.edu.au).

2. Western Australian Museum, Locked Bag 49, Welshpool DC, Western Australia 6986, Australia. (bill.humphreys@museum.wa.gov.au).

3. Department of Conservation and Land Management, P.O. Box 51, Wanneroo, WA6946. Australia. (StefanE@calm.wa.gov.au), (stuarth@calm.wa.gov.au).

ABSTRACT

The amphipod Pilbarus millsi, has a widespread distribution in northwestern Western Australia. It is unclear whether ongoing gene flow or historical processes account for this pattern. Analysis of mtDNA sequence divergence between samples from ten catchments showed no evidence of contemporary gene flow. Instead, amphipods from each catchment had unique and highly distinct haplotypes, indicating that the distribution is ancient. Phylogenetic analysis revealed clades containing haplotypes from only a single catchment, suggesting past fragmentation events.

KEYWORDS : AMPHIPODS, CO1, MTDNA, PHYLOGEOGRAPHY, STYGOFAUNA.

1. INTRODUCTION

Twenty-six species of stygobitic amphipods have been described from the Pilbara, an arid region in the northwest of Western Australia (Bradbury and Williams, 1997; Bradbury, 2000). Many are known from single bores. One species, *Pilbarus millsi*, is widespread, occurring in multiple catchments throughout the Pilbara. However, its distribution appears to be limited to areas containing calcrete formations (Finston and Johnson, 2004).The present study examined specimens of *P. millsi* from ten catchments, using a partial sequence of the mitochondrial COI gene, to test hypotheses about the origin and maintenance of a widespread species in a highly subdivided habitat. The species may be widespread due to its dispersal capabilities, perhaps linked to its occasional occupation of surface waters and flooding events associated with the area. Alternately, historical processes

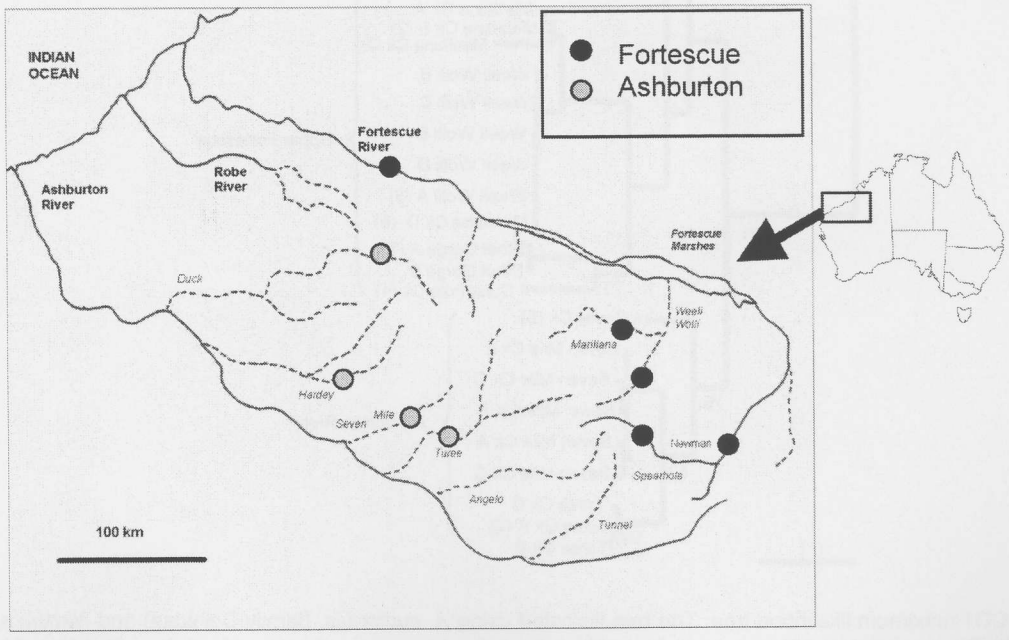

Figure 1 : Map of the Pilbara, Western Australia, showing sample sites within the Ashburton and Fortescue River basins.

may have involved a past fragmentation event of a once widespread ancestor, perhaps linked to the onset of aridity in Australia.

2. METHODS

Specimens of *P. millsi* were collected from ten creek catchments located within two major river basins, the Ashburton, and the Fortescue (Fig. 1). With some exceptions, between five and ten individuals per catchment were sequenced. Whole genomic DNA was extracted from specimens in 50µl of a proteinase K extraction buffer for between 10 and 20 hours. A 710 base pair (bp) fragment of the 3' end of the cytochrome oxidase subunit 1 (COI) gene was amplified, using the primers LCO1490 (forward, 5' GGT CAA CAA ATC ATA AAG ATA TTG G 3') and HCO2198 (reverse, 5' TAA ACT TCA GGG TGA CCA AAA AAT CA 3'; Folmer *et al.*, 1994). The 25 µl PCR reactions used 0.2mM dNTPs, 4.0mM $MgCl_2$, 1x buffer, 12.5 pmoles of each primer, 1 unit Taq, and 2.5 µl template. Sequences were cleaned using the UltraClean™ PCR Clean-up DNA purification kit (MoBio Laboratories, Inc.) prior to sequencing. The sequencing reaction was carried out using the BigDye V3 Ready Reaction Mix (ABI Prism) and the products were sequenced using both primers on an ABI 373 automated sequencer (Applied Biosystems). Sequences were aligned and edited by eye with GeneDoc, version 2.6.002, using default settings. Relationships among the sequences were analysed using PHYLIP, version 3.57c. Pair-wise nucleotide sequence divergence was calculated using Kimura's two-parameter distance model in the DNADIST module. A maximum likelihood phylogeny was produced using the module DNAML on 100 multiple data sets (generated in SEQBOOT).

3. RESULTS

The number of haplotypes detected varied among catchments, ranging from a single haplotype at Caves Creek, Roy Hill and Spearhole Creek, to six at Weeli Wolli Creek. Importantly, haplotypes were not shared among catchments. Sequence divergence between haplotypes within the same catchment did not exceed 2.3%. Within the Ashburton, sequence divergence ranged from 8.9 to 13.1% between catchments and within the Fortescue, divergence ranged from 16.4 to 26.5% between catchments. Sequence divergence between haplotypes in different basins ranged from 20.1 to 26.8%. The phylogenetic analysis showed that genetic structure within *P. millsi* reflected hydrological structure. Each mtDNA lineage contained haplotypes from only a single catchment (Fig. 2). While the use of a molecular clock is fraught with assumptions that are often violated, it can give a rough estimate of the time frame associated with phylogenetic events. Using an estimate of the evolutionary rate of the COI gene of approximately 2% divergence per million years, haplotypes from the Ashburton and Fortescue River basins last shared a common ancestor between 10 and 13 mya.

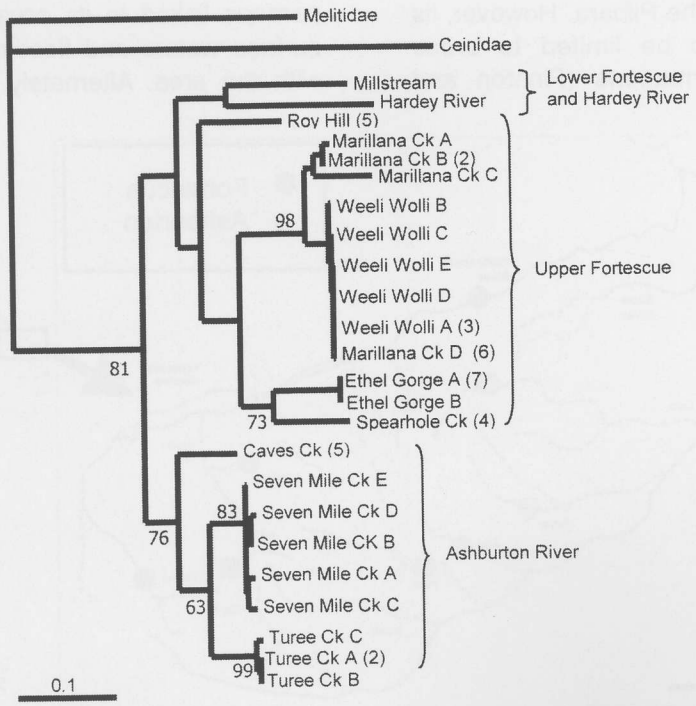

Figure 2 : CO1 maximum likelihood tree. The tree is rooted using *A. subtenuis*, (family Ceinidae) and *Nedsia sp.* (family Melitidae). Confidence levels > 50%, assessed by 100 replicate bootstraps are shown on branches. Number of individuals represented by each haplotype is shown for n>1.

4. DISCUSSION

Genetic structure within *P. millsi* reflected the hydrological structure of the region. There was no apparent gene flow between catchments, and each lineage corresponded to individual creek catchments. Strong geographical associations of haplotypes may be evidence of a past fragmentation event (Templeton, 1998). This pattern of diversity suggests that the current distribution of *P. millsi* is not due to gene flow, but supports the hypothesis that a historical fragmentation event isolated populations of the species within catchments and basins. One explanation is that a surface ancestor sought refuge in subterranean waters when the climate became more arid during the Miocene (Humphreys, 2001). In support of this hypothesis, the molecular clock estimate places divergence between the two basins at 10 – 13 my, corresponding to the late Miocene or early Pliocene. *Pilbarus millsi* may comprise multiple morphologically cryptic species, or may be an example of morphological stasis in a genetically diverse, widespread species.

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