Selection and Screening of aquatic taxa for proposed dryland refugium project.

Final Report

Jane Hughes

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Aim: The broad aim of this project was to provide preliminary data for the dryland river refugia project.

Context:

Two key elements of the dryland river refugia project were:

1. the need to understand the pattern and extent of dispersal of aquatic organisms (how do they move within and between refugia, how far do they move and how quickly could they recolonise disturbed systems? This is of particular relevance to understanding how flow regulation and/r changes to water movement on floodplains affect refugia.
2. identification of particular catchments or subcatchments which contain unusually high levels of endemism among aquatic biota. Are there particular catchments of subcatchments with particularly high levels of biodiversity?

Analysis of the genetic structure of populations is one of the ,major tools that will be used in addressing these questions. These will be used as 'markers' for dispersal studies, as well as direct measures of endemism and biodiversity.

Preliminary data collection was required to determine:

1. the range of aquatic species to be examined
2. the spatial scale for sampling
3. the kinds of genetic markers to be used.

The specific objectives for the scoping study were:

1. to identify potential study sites for research proposed under the dryland refugia project
2. to determine the species of most interest to the project (e.g. species with apparently different life-histories and dispersal abilities that occur in sufficient numbers to be able to be sampled from each of a number of major river systems
3. for a subset of these species, to determine whether allozyme and/or mitochondrial DNA can be reliably used as markers. This was to include an initial screening to ensure assess the levels of variation between major drainages. For species of interest that showed limited variation microsatellite loci would need to be developed.

Methods

Sampling

Samples were collected from rivers in a transect across southern Queensland and NT, from the Condamine River in the east to the Diamintina in the west. Samples were collected for a number of fish species (Table 1), one turtle, two crustaceans (*Macrobrachium* and *Cherax*), two bivalve molluscs and a gastropod mollusc. For *Macrobrachium*, 100 individuals were collected from each site, as this species is the subject of Ben Cook's Honours project. For the other species, smaller numbers were collected for screening purposes. With the exception of *Macrobrachium,* sampling on a small scale was not sufficient , so at this stage we have examined mostly variation within and among drainages, rather than concentrating on the within-stream variation that will follow in the main study.

Laboratory methods

Allozymes

For all species where we had samples of more than 10 individuals from two or more sites, we screened 25 allozyme loci for variation. We used cellulose acetate electrophoresis (Titan III plates, Helena Laboratories) and followed standard methods similar to those described in Richardson et al (1986) and our previous work (e.g. Hughes et al 1995). We recorded a locus as variable (polymorphic) if variation was recorded either within or between sites.

Mitochondrial DNA

Mitochondrial DNA (chelex extraction) was extracted for all species for which we had two or more individuals from two or more sites. Polymerase chain reaction (PCR) was then used to amplify specific fragments of the mitochondrial genome. We chose genes which had been shown to vary intraspecifically in other species of the group (e.g. ATPase genes for the fish, CO1 for the molluscs and crustaceans. PCR products for each individual; were sequenced in an ABI automatic sequencing machine and sequences compared among individuals of a species using BIOEDIT. The levels of variation in mitochondrial DNA for each species are shown in Table 1. Neighbour-joining trees were constructed in MEGA for each species to examine divergence within and among drainages.

Microsatellite Loci

For the turtle, for which we already knew that variation at allozyme and mtDNA was negligible, a microsatellite library was made and 15 positive clones were sequenced. From these sequences, microsatellite loci were identified, primers designed and the loci were trialled for variation within and between populations, by running on a GELSCAN DNA analyser.

Results

Table 1 shows the species for which sufficient samples were collected. It includes a summary of the number of individuals analysed for mtDNA for each site, the gene analysed, the number of different haplotypes identified and the number of variable sites. The final column gives the number of polymorphic loci identified of the 25 allozyme loci that were screened. We were able to find some variable allozyme loci for all species that were screened with the exception of the crayfish (*Cherax*). There was also no variation among *Cherax* individuals in the CO1 gene (mtDNA) that we have used extensively in other crustaceans in our lab. Figures 1-7 give the neighbour joining trees showing relationships among the individuals that were sequenced for each species. These data are preliminary and more samples and more sophisticated analysis would be required before definite conclusions can be drawn. However some obvious trends can be seen.

For *Hypseleotris*, we could recognise three distinct species. This was on the basis of allozymes and mtDNA (Figure 6). Each species occurred sympatrically with at least one other species at one or more sites. The allozyme data clearly showed that no interbreeding occurred between them (fixed differences at three or more loci). The neighbour joining tree also shows that each species is monophyletic. Note that Species 1 occurs in the Cooper and Murray-Darling systems, Species 2 was only found in the Cooper and Species 3 was only found in the Murray-Darling. Further sampling and morphological analysis is required . *Neosiluris* was only collected from the Cooper drainage and Figure 3 shows there are probably two species. Limited variation was observed in one of these.

For *Ambassis* , *Retropinna* and *Melanotaenia*, there are obvious differences among drainages. Although samples are limited, these data suggest that the populations within a drainage are monophyletic. This clearly suggests no movement between drainages and that there has been no movement in recent historical times. In contrast, the very limited data for *Nematolosa* suggests a very different pattern, with shared haplotypes between the Cooper and Murray-Darling drainages. More data are needed, but these suggest that

this species moves between these drainages, (or has done in the recent past), possibly during flood times.
The snail *Notopala* also shows monophyly within drainages, again indicating no recent dispersal.
Variation within drainages was greatest for the snail and the fish . This suggests that these two species would be most useful to examine dispersal on a smaller spatial scale.
Screening of turtle microsatellite loci is not complete, but we have identified six loci which show variation within and between populations. The number of alleles per locus varies from 2 to 7.

Conclusions

The following species should be included in the main study, on the basis of genetic variation identified and the ease of sampling:
Crustaceans: *Macrobrachium.*
Molluscs: *Notopala*
Fish*: Melanotaenia, Hypseleotris, Nematolosa, Retropinna*
Turtle:
The spatial scale suggested for further sampling would be to sample extensively across the broad range, as in this study, but to include more small scale sampling within drainages and even within the same tributary., especially for the species showing within drainage variation.

References

Hughes, J.M. S.E. Bunn, D. Kingston and D.A. Hurwood, 1995. Genetic differentiation and dispersal among populations of *Paratya australiensis* (Atyidae) in rainforest streams in south-east Queensland. J. North. American Benthological Society 14,158-173.
Richardson, B.J., P.R. Baverstock and M. Adams, 1986. Allozyme Electrophoresis: A Handbook for Animal Systematics and Population Studies.Academic Press, Sydney.

Table 1. Species analysed for mitochondrial DNA and allozyme variation.

Species	# sites	#/ site*	# sequences	Fragment (size)	# haplotypes	# variable sites	# polymorphic allozyme loci
Pisces							
Ambassis	4	3	10	ATP6,8 (507)	2	28	NA
Nematolosa	4	3	10	ATP6,8 (480)	2	3	6
Neosiluris	4	1-3	8	ATP6,8 (498)	3	46	NA
Melanotaenia	4	2-3	11	ATP6,8 (450)	7	10	7
Retropinna	4	6	22	ATP6,8 (405)	10	21	NA
Hypseleotris	8	2-9	26	ATP6,8 (498)	16	not yet calculated	8
Crustacean							
Macrobrachium	1	5	5	CO1 (500)	3	3	9
Chare	2	2-4	6	COI (500)	1	0	0
Mollusc							
Notopala	6	3-4	20	CO1 (333)	14	57	5
Velesunio	2	1	2	CO1 (402)	2	40	5

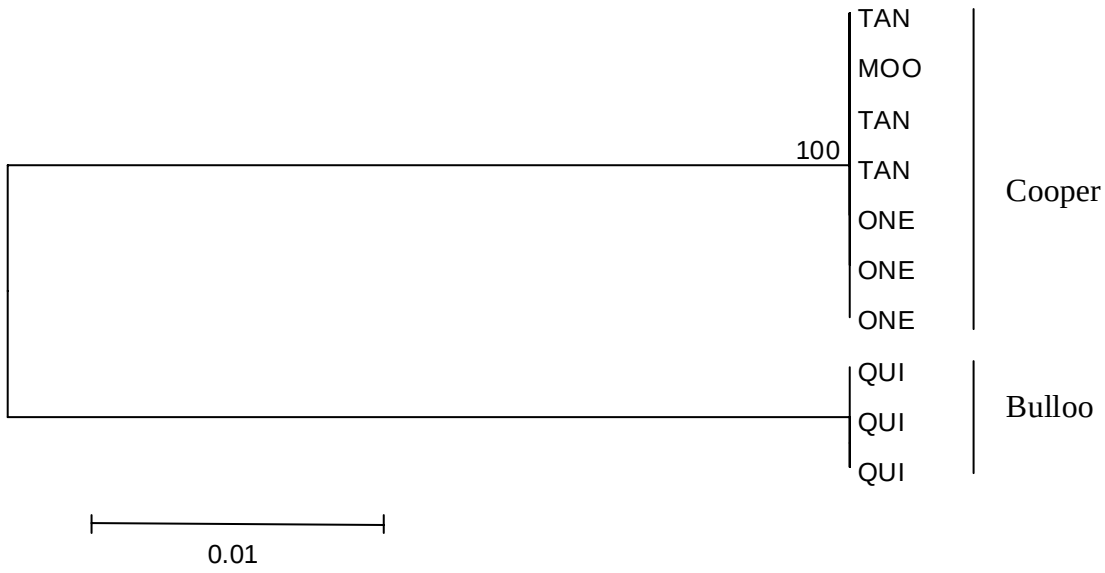

Figure 1. Neighbour-Joining tree of Kimura's two parameter genetic distance for *Ambassis*.

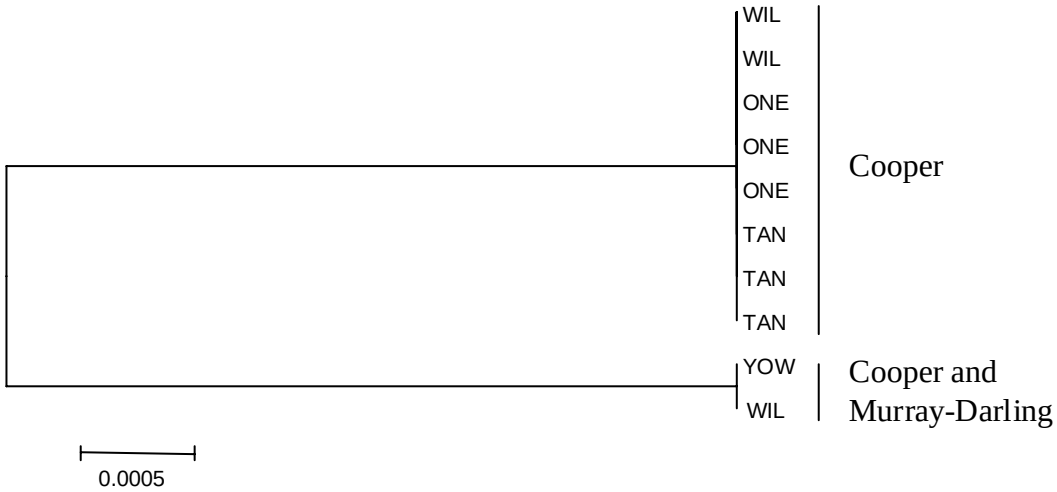

Figure 2. Neighbour-Joining tree of Kimura's two parameter's two parameter genetic distance for *Nematolosa*.

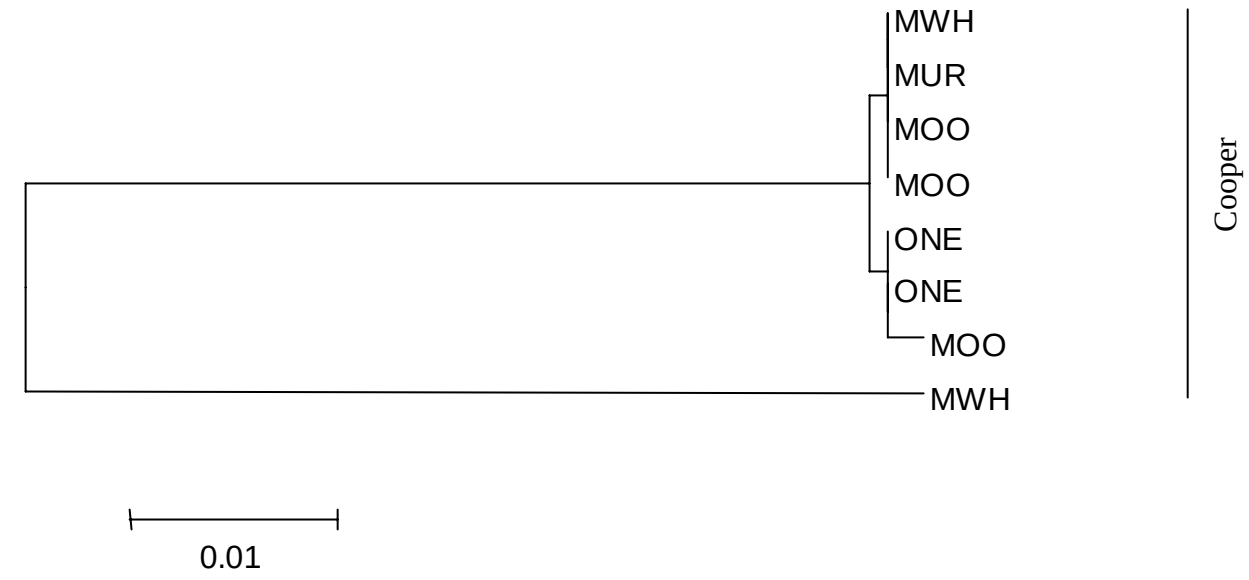

Figure 3. Neighbour-Joining tree of Kimura's two parameter genetic distance of *Neosiluris*.

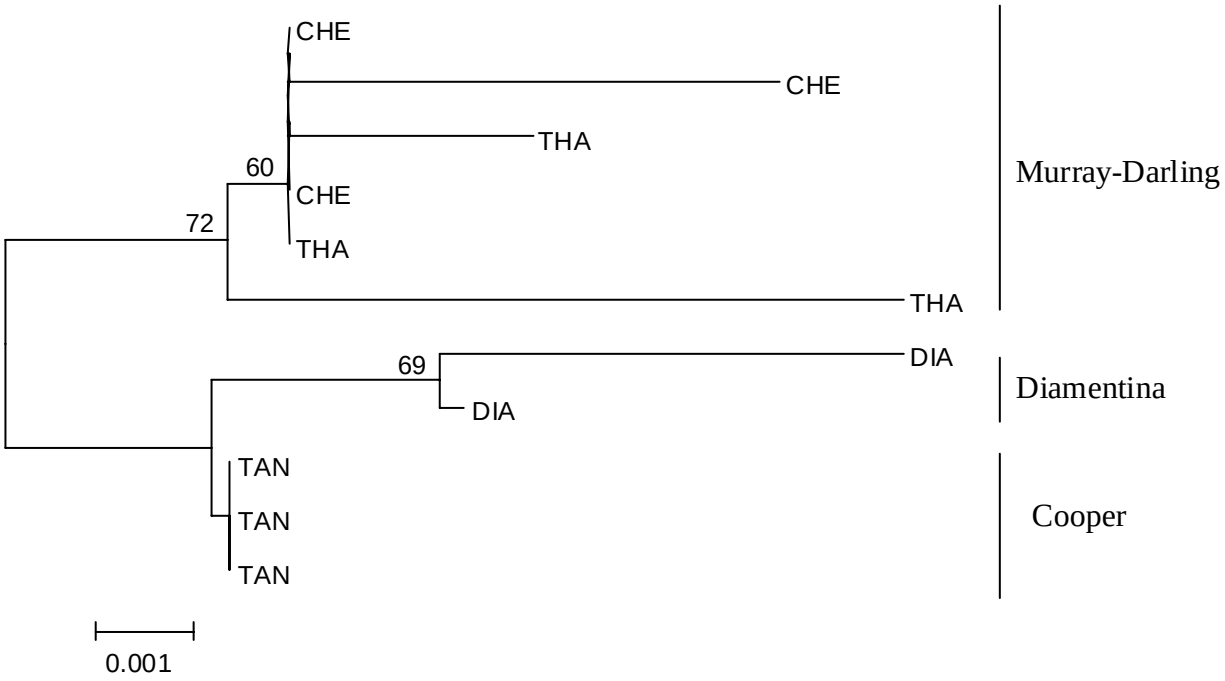

Figure 4. Neighbour-Joining tree of Kimura's two parameter two parameter's genetic distance for *Melanotaenia*.

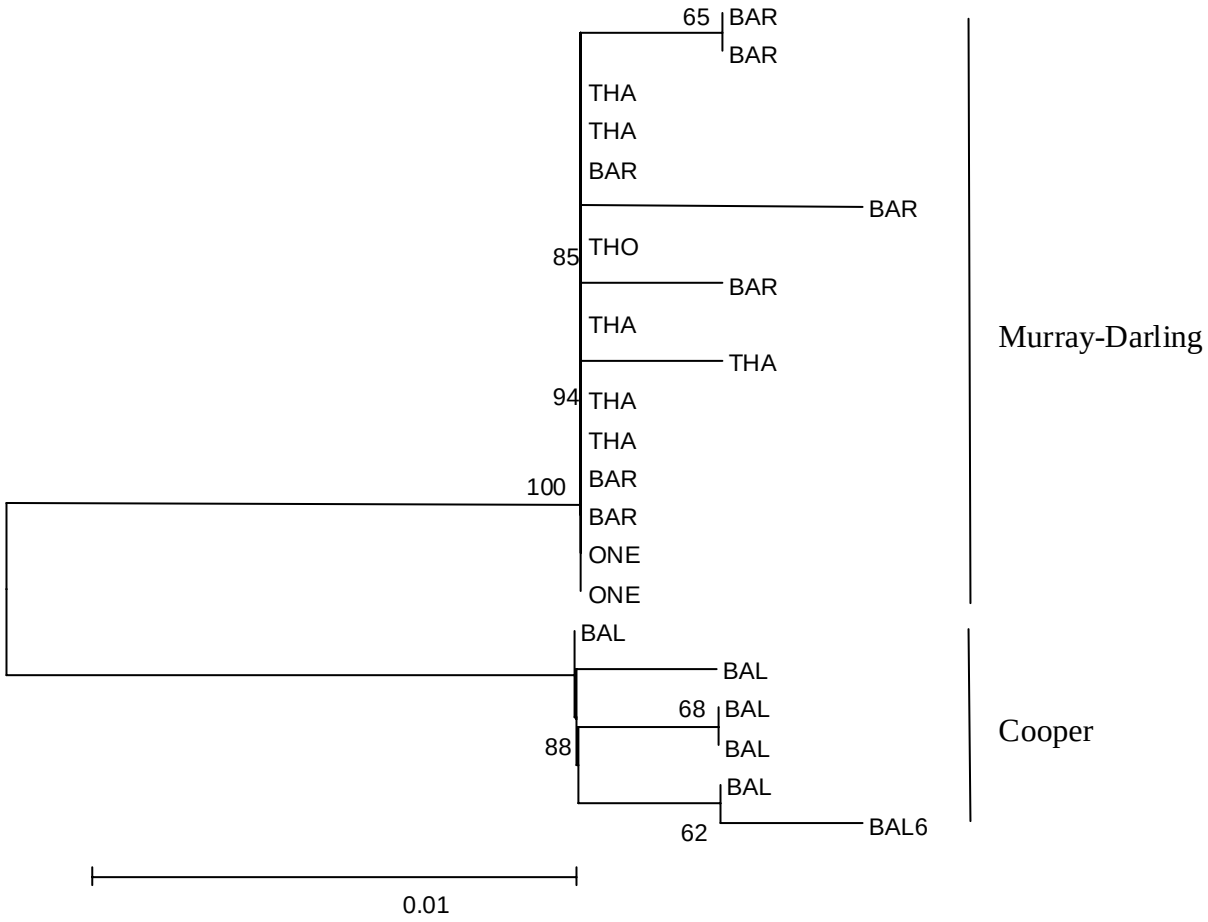

Figure 5. Neighbour-Joining tree of Kimura's two parameter genetic distance for *Retropinna*.

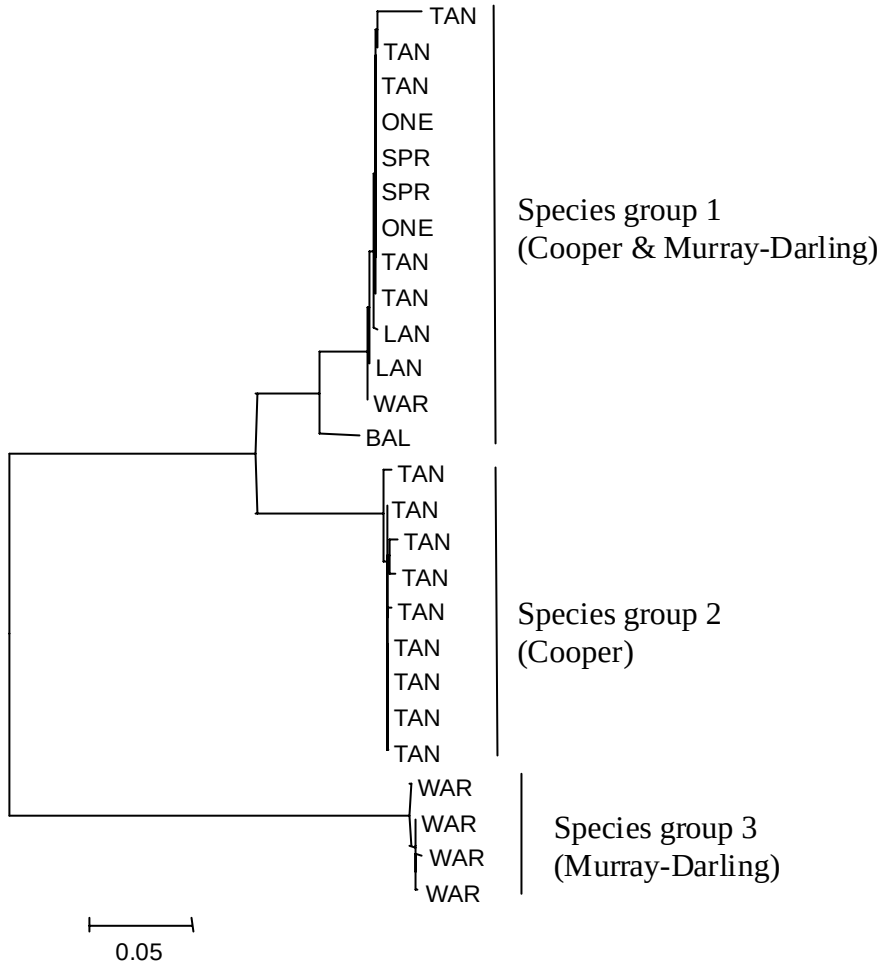

Figure 6. Neighbour-Joining tree of Kimura's two parameter genetic distance for *Hypseleotris* spp.

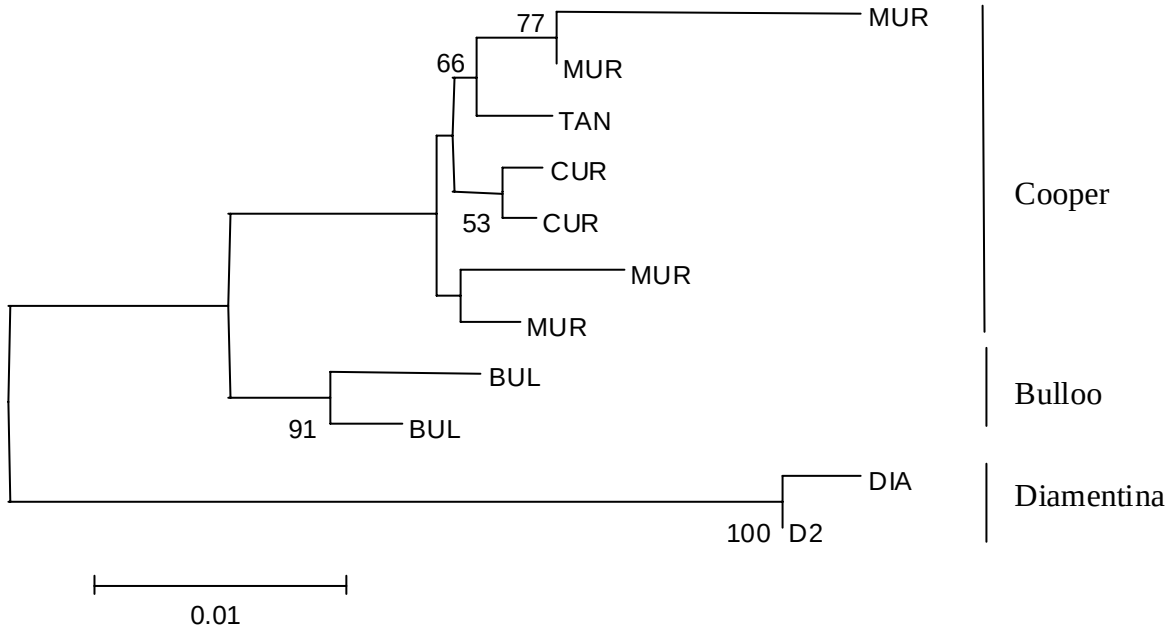

Figure 7. Neighbour-Joining tree of Kimura's two parameter genetic distance for unique mtDNA haplotypes of *Notopala*.