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Host-race specificity in the endemic pygmy mistletoe *Korthalsella salicornioides* (Viscaceae)

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In

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**ABSTRACT**

*Korthalsella* Tiegh. is a genus of stem hemiparasites in the family Viscaceae, represented in New Zealand by three endemic species: *K. clavata*, *K. lindsayi*, and *K. salicornioides*. The most host-specific is *K. salicornioides* as it parasitizes two main host genera *Leptospermum* (Myrtaceae) and *Kunzea* (Myrtaceae), while the other two species are considered generalists parasitizing a wider range of host species. *K. salicornioides* is naturally uncommon and sparse, although it can be locally abundant on occasion. Mistletoe populations are at risk primarily due to habitat destruction and subsequent loss of hosts. Cross-infection experiments in *K. salicornioides* provided some insight into the presence of putative host races, as better mistletoe seedling establishment success rates were apparent when the maternal and recipient hosts were the same. However, because previous molecular sequence data (nuclear internal transcribed spacers and chloroplast *trnQ-rps16*) for *K. salicornioides* were not informative about specific host-races, more rapidly evolving molecular markers might be expected to detect host races.

In this study, next generation sequencing was used to develop novel microsatellite markers for *Korthalsella*. Eleven markers were reliably amplifiable and the most polymorphic for *K. salicornioides* were used to genotype 272 *K. salicornioides* individuals from 16 populations. Across all populations few alleles were identified, and within-population assessment of genetic variation indicated that many populations have low levels of genetic diversity and high proportions of homozygotes. Despite the presence of few alleles, a high degree of genetic differentiation between most populations was detected and was found to reflect host species and geography.

The findings of this study that *Korthalsella salicornioides* populations have low levels of genetic variation but host-specific races, has important conservation implications. The main conservational focus should be maintaining and increasing host *Leptospermum* and *Kunzea*
populations. The spread of mistletoe seed on hosts within or between populations may also increase the chances of continued survival. However, it is imperative that genetic material comes from the same host species, and consideration should also be given to the geographic area, especially in the Wairarapa. This study provides insights into the population structure within and between the different host populations and suggests several interesting areas of future study.
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ABBREVIATIONS

\%P  - percentage of polymorphic loci
A  - number of alleles
As  - allele size
AT  - total number of alleles
AFLP  - Amplified Fragment Length Polymorphism
AMOVA  - analysis of molecular variance
BLAST  - basic local alignment search tool
bp  - base pairs
cpDNA  - chloroplast DNA
CASS  - cheaply amplified size standard
CTAB  - hexa-decetylammomium bromide
DNA  - deoxyribonucleic acid
dNTP  - deoxyribonucleotide triphosphate
DOC  - Department of Conservation (New Zealand)
ER  - Ecological Region
FIS  - component of Wright’s (1921) fixation index, used to define within population structure by calculating the average observed heterozygosity of an individual relative to the expected heterozygosity of individuals in the population it belongs to
FST  - component of Wright’s (1921) fixation index, used to define between population structure by comparing the expected heterozygosity of individuals within a subpopulation to the total expected heterozygosity of individuals across all populations
HE  - expected heterozygosity
HO  - observed heterozygosity
IBD  - Isolation by Distance
ITS  - internal transcribed spacer
LnP(D)  - mean posterior probability
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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<tbody>
<tr>
<td>MA</td>
<td>Million years</td>
</tr>
<tr>
<td>MPN</td>
<td>Dame Ella Campbell Herbarium</td>
</tr>
<tr>
<td>mtDNA</td>
<td>Mitochondrial DNA</td>
</tr>
<tr>
<td>N</td>
<td>Number of individuals</td>
</tr>
<tr>
<td>N_A</td>
<td>Number of alleles</td>
</tr>
<tr>
<td>N_e</td>
<td>Effective number of alleles</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>RAPD</td>
<td>Randomly Amplified Polymorphic DNA</td>
</tr>
<tr>
<td>SNP</td>
<td>Single Nucleotide Polymorphism</td>
</tr>
<tr>
<td>Tm</td>
<td>Melting temperature</td>
</tr>
<tr>
<td>VNTR</td>
<td>Variable Number of Tandem Repeat loci</td>
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