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GENETIC STRUCTURE OF
PLEUROBRANCHAEA MACULATA IN
NEW ZEALAND

A thesis presented in partial fulfilment of the
requirements for the degree of **Doctor of Philosophy (PhD)** in Genetics

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ABSTRACT

AIMS

The grey side-gilled sea slug (*Pleurobranchaea maculata*), which is native to the western and south Pacific, is known to contain high concentrations of tetrodotoxin (TTX). *P. maculata* populations around New Zealand exhibit individual, spatial and temporal differences in TTX concentration, but the origin of TTX in *P. maculata* is not fully understood. The main goal of my PhD project was to examine the genetic structure and demographic history of *P. maculata* populations from different regions in New Zealand and to clarify whether there is a correlation between variability in TTX concentrations and genetic structure.

METHODS

A sample of 146 *P. maculata* individuals were collected from three populations from the north-eastern North Island (Ti Point-TP, Auckland-AKL and Tauranga-TR), one population from the southern North Island (Wellington-WL) and one population from the northern South Island (Nelson-NL). TP, AKL and TR were designated the “Northern cluster”, whereas the WL and NL population were labelled as the “Southern cluster” due to the relative geographical locations of these clusters. Twelve nuclear microsatellite markers that were developed based on shotgun sequence were obtained from the genome of *P. maculata*. The markers were used to analyse the genetic structure of *P. maculata* populations. The mitochondrial cytochrome c oxidase subunit I (1153 bp) and cytochrome b (1060 bp) genes were also partially sequenced in *P. maculata* individuals.

RESULTS AND MAIN CONCLUSIONS

The microsatellite data reveal high genetic diversity and lead to the rejection of the hypothesis of panmixia: populations from the Northern cluster are highly connected but significantly differentiated from the Southern cluster. A weak differentiation was also observed between the WL and NL populations. The two populations correlate with regional variations in TTX concentrations: the Northern cluster populations contain highly toxic individuals, whereas the Southern cluster (WL and NL) populations

harbour either slightly toxic or non-toxic populations. The disjunction between the Northern and Southern clusters can be explained by biogeographical barriers specific to New Zealand but also with a stepping stone model. The geographical gap between the sampling locations made it impossible to draw firm conclusions as to the origin of the disjunction. The mtDNA sequence data reveal high haplotype diversity, low nucleotide diversity and a star-shaped haplotype network. These data can be explained by a population expansion dating back to the Pleistocene era. All the sampling locations are significantly differentiated from each other according to mtDNA data. Given that microsatellite and mitochondrial sequences evolve at different rates, incomplete linkage sorting is expected to be completed for mtDNA before, which should be reflected in a more pronounced structure for mtDNA markers where members of the populations have diverged recently. Although this may explain the geographical conflict between the microsatellite and mtDNA data, it is necessary to consider the possibility that the discordance between microsatellite markers and mtDNA may be in part attributable to the relatively small sample size.

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