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Project Title:	Revisiting old friends: using molecular data to characterise the diversity of a lichen genus in New Zealand
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Executive Summary

This project aims to determine which species of the lichen genus *Parmotrema* are found in New Zealand, describe any new species and identify if any species are threatened with extinction.

The project has two phases. Phase 1 uses molecular data from DNA sequences to compare specimens collected from New Zealand with data from the rest of the world. Phase 2 uses more traditional approaches (morphology, anatomy and chemistry) to expand on this and generate a consensus scheme of species for New Zealand. When a confirmed species list is available, distributional and ecological data will be used to update the threat classification information.

Phase 1 is partly completed and indicates that the species we have examined have the same DNA sequence as those with the same name from overseas. One exciting result is that one species has significant genetic variation, which suggests that cryptic species may exist.

It is too early in the project for significant conclusions. The research continues into 2013 without further funding being required from the Unitec Research Committee at this stage.

Background

The currency or unit of measurement for conservation management is the species. Accurate knowledge of what species exist in a country and their distribution and ecology is essential before conservation measures can be taken. Lichens are not as well known as many other groups of organisms in New Zealand, with perhaps 85% of lichen species catalogued and named (Galloway 2007). A National Threat Classification Panel met and rated the New Zealand lichen flora in 2009, but nearly half could not be rated and were designated as being "Data Deficient" (de Lange et al (2012)). A number of lichen genera have been comprehensively studied in New Zealand, and these were easily assigned threat classifications. Examples include *Sticta* (Galloway 1997), *Pseudocyphellaria* (Galloway 1988) and *Ramalina* (Blanchon et al 1996). This research aims to add the genus *Parmotrema* to the list of well-studied genera. *Parmotrema* is a widespread and commonly collected genus in New Zealand. It is often collected by members of the public, students and for biodiversity and environmental impact studies (usually measuring impacts of air pollution from coal-fired power stations), but individual species are commonly misidentified, undermining the accuracy of these studies and frustrating collectors. This study will result in the production of a corrected species list for *Parmotrema* and a freely-available, interactive online identification key using Lucid software (www.lucidcentral.com). Most lichen generic revisions have traditionally been done using morphology, anatomy and chemistry. Modern revisions have moved into using molecular data – particularly DNA sequencing as standard (Lumbsch & Leavitt 2011). Some research has been done on *Parmotrema* using molecular data. Blanco et al (2005) revised the generic limits of *Parmotrema* for the world, but did not include New Zealand specimens. Adding New Zealand sequence data will enhance the biogeographic knowledge of the genus worldwide. This project aims to study and classify the New Zealand species, fit them into the international classification scheme for the genus and make this information available to end users via publication in refereed journals, and

online on the Manaaki Whenua Landcare Research website (Flora of New Zealand series) and Lucid keys.

Aims and Objectives

1. What species of the lichen genus *Parmotrema* are found in New Zealand?
2. Are there any new, undescribed species of the lichen genus *Parmotrema* found in New Zealand?
3. Are any species of *Parmotrema* threatened?

Methodology

Phase 1: Molecular study (2012-2013)

A modern lichen taxonomic revision of a genus requires multiple samples from different geographic locations of each species being studied. Fresh material from around New Zealand of each species and suspected species in *Parmotrema* has been collected for DNA extraction and production of molecular data (material less than 1 year old is best for DNA work). The chief investigator collected specimens from Auckland, Northland and Waikato (day trips), and specimens were obtained from East Cape, some northern offshore islands and Otago (as different species are found there), supplemented with collections from colleagues in universities elsewhere. DNA has been extracted from selected specimens (5+ per species). DNA amplification (PCR) is performed on DNA samples using fungal primers for the ITS and IGS spacers. PCR products are purified and sent to Massey University, Palmerston North for sequencing (Unitec does not have sequencing facilities). Sequences are being aligned and analysed (including foreign sequences supplied by Pradeep Divakar) using Geneious software. These techniques have recently been successfully used by part of this research team at Unitec and routinely by Thorsten Lumbsch and Pradeep Divakar. It is likely that 150+ specimens will need to be sequenced.

Phase 2: Morphology, anatomy and chemistry (2013-2014)

The same lichen specimens have been preserved as voucher specimens in the Unitec Herbarium. These can then be used for morphological, anatomical and chemical studies (using thin layer chromatography, a technique routinely used by the chief investigator at Unitec).

An extensive range of specimens are preserved at the Herbarium of the Auckland Institute and Museum, and can be studied there. Further material can be loaned from other herbaria around New Zealand. It will be necessary to examine several thousand dried herbarium specimens. An extensive source of vital literature has been offered by Dr David Galloway (Landcare Research).

Molecular, morphological and chemical data will be combined to produce a robust idea of the diversity within *Parmotrema* in New Zealand and how this compares with other international studies. It is likely that some species will have name changes and it may be necessary to name new species for New Zealand. Once the names are known, it will be possible to decide if any species are threatened, using Department of Conservation guidelines.

Important morphological, anatomical and chemical characters will be identified and entered into Lucid Builder software. Photographs will be taken over the course of the study and also entered into the program. The Department of Natural Sciences is already planning to purchase Lucid.

We asked for funding for Phase 1, but some aspects of Phase 2 were done at the same time. Phase 2 is not likely to require significant extra funding – mainly time (thin layer chromatography is already set up and being performed at Unitec).

Outcomes/findings

We have collected or otherwise obtained around 180 specimens of *Parmotrema* from around the country. Of these, around 50 have had DNA extracted and stored. Of these, we have generated 48 DNA sequences from 6 species, and have completed DNA extraction for three species (we aim to have 5 samples for each species across a geographic spread).

So far, our DNA sequence data is in agreement with published data from other parts of the world, confirming the identity of the species we have examined. (Objective 1)

Excitingly, we are finding some genetic variation in one of our species, which may suggest the presence of cryptic species. (Objective 2).

We still need to collect/obtain more samples from around the country, and colleagues at the Department of Conservation and University of Otago are assisting with this. We have the kits we need to extract the DNA, so can continue the research this year. After a concerted effort in the laboratory we will send more samples to Massey University for DNA sequencing. The Faculty of Social and Health Science Research Committee has given us further funding to complete this.

We intend to complete Phase 1 later in 2013.

Conclusions

None yet

Implications

None yet

Recommendations (optional)

None yet

Publications and dissemination

None yet

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