Biogeography of endemic New Zealand *Isoëtes*

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Introduction

Isoëtes are spore-bearing vascular plants comprising approximately 200 species world-wide (Hoot & Taylor 2001), and are included in the plant division Pteridophyta, which includes both ferns and fern allies.

Their conservative and simple anatomy means that taxonomic classification of *Isoëtes* based on morphology is problematic (Rydin & Wikström 2002). However morphological characteristics commonly used to differentiate species of *Isoëtes*, include megaspore ornamentation (Marsden 1976) and cytology (Marsden 1976; Taylor et al. 1985; Hoot & Taylor 2001).

Isoëtes in New Zealand has been described as two species, *I. kirkii* A. Braun and *I. alpinus* T. Kirk (Allan 1961). However, unpublished work by Marsden (1979) described three varieties within one species, *I. kirkii* (*I. kirkii* var. *kirkii, I. kirkii* var. *alpina* (T. Kirk) Marsden & Chinnock, *I. kirkii* var. *flabellata*). Cytology for one population of each of those three varieties of *Isoëtes* established a diploid chromosome number of 2n = 22 (Marsden 1979).

More recently, D. Britton (University of Guelph, Canada) and D. Brunton (D. Brunton Consulting Services, Canada) investigated New Zealand *Isoëtes* using herbarium specimens, spore morphology, and cytology on fresh material supplied by NIWA from 11 lakes. They suggest New Zealand *Isoëtes* is best viewed as a single species with three morphologically similar subspecies, which exhibit reasonably distinctive geographical, cytological and ecological characteristics. Two of these subspecies were diploid and sexual: *Isoëtes kirkii* (s.str) found at low elevation lakes, mostly on the North Island and currently a rare and declining population; and *Isoëtes kirkii* ssp. *alpina* found in higher elevation lakes on South Island and relatively common. Some entities on both Islands were difficult to assign to one or the other taxon. More difficult to interpret was the fact that several populations in Central North Island lakes were tetraploid, without microspores and apparently apomictic. These demonstrated virtually identical megaspore morphology, apart from a larger spore size, to that of the subspecies *Isoëtes kirkii* ssp. *alpina*.

Isoëtes forming a dense turf with the occasional plant of *Potamogeton cheesemanii*

Objective

The objective of this research project was to assess genetic variation among New Zealand *Isoëtes*: to determine if the genetic data support any of the morphological hypothesis, and to determine what levels of variation reside between and within a wide geographic sample of populations.

Methods

Plant sampling and cultivation

- *Isoëtes* were sampled from 20 lakes throughout New Zealand (5 in the North Island and 15 in the South Island. (Figure 1)).
- In four large lakes (Rotoiti, Taupo, Wanaka and Te Anau) plants were sampled at multiple sites, and in the remaining lakes plants were sampled based on accessibility.
- Following collection plants were cultivated at Ruakura (NIWA, Hamilton). *Isoëtes* plants from Lake Omapere (Northland) were already in cultivation.

DNA extraction and PCR

- CTAB or the DNEasy plant mini kit (Qiagen) was used for all extractions.
- PCR primers for sequenced DNA regions were ITS (using ITS4 and ITS5 primers designed for higher plants) and the trnL intron (Taberlet et al 1991). RAPD primers were OPP3, OPP4, OPP8, OPP9, OPP16, OPP17, OPP18, and OPP19 (by Operon Technologies). All PCR was performed under standard conditions (Hofstra and Gemmill 1999).
- DNA sequences were aligned and edited using Sequencer 3, and parsimony analyses employed heuristic searches and were conducted using PAUP.
- UPGMA analyses were conducted for the RAPD (band presence/absence) data using Nei's (1972) genetic distance, with the software program TFPGA (Tools for Population Genetic Analysis, Version 1.3, Miller 1997).

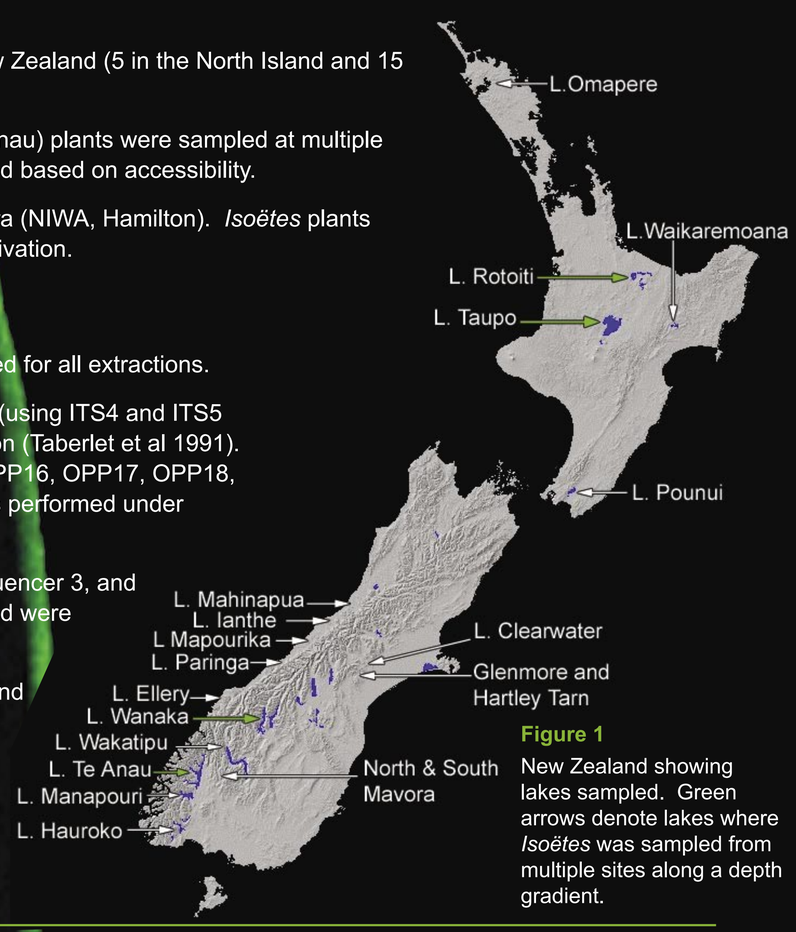
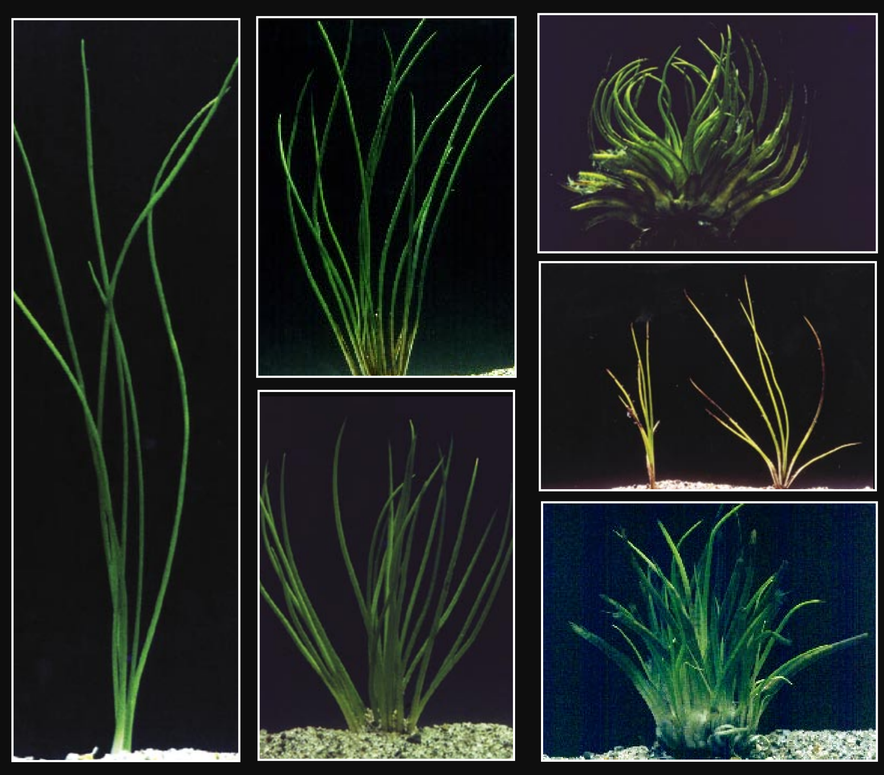


Figure 1
New Zealand showing lakes sampled. Green arrows denote lakes where *Isoëtes* was sampled from multiple sites along a depth gradient.



This collection of plants illustrates some of the morphological variation that is seen in *Isoëtes*, including plants from Lake Omapere (middle right).

Results and Discussion

Sequence data

- Amplification of the ITS region (ITS-1, ITS-2 and the intervening 5.8s gene) resulted in PCR products of ca. 800 base pairs. The trnL amplification products were ca 600 base pairs long.
- ITS and cpDNA spacers lack resolution (Figure 2) between the New Zealand *Isoëtes* samples and indeed those from Tasmania (Hofstra and Gemmill 1999).

RAPDs

- The 8 primers used generated 92 polymorphic bands, and showed distinct differences in band presence/absence between plants from different lake populations (Figure 3).
- RAPDs reveal biogeographic differences between *Isoëtes* plants and suggest taxa are distinct (Figure 4). For example, the largest genetic distance was between the flabellate *I. kirkii* historically from Lake Omapere in Northland (North Island), and all other populations which were grouped into two clusters one with the remaining North Island lakes (with two South Island exceptions), and the other the South Island lakes.
- The remaining North Island lakes include the *I. kirkii* plants that are known tetraploids (e.g. Lakes Taupo and Rotoiti) whilst the cytology of the other plants in this cluster have not yet been investigated. Interestingly *I. kirkii* has been described from the South Island before (Marsden 1979).
- The South Island lakes include plants that have previously been described as *I. alpinus*. Amongst the South Island lakes genetic distance is closely related to geographic distance for the smaller lakes in the northern West Coast group (eg Lakes Mahinapua, Ianthe and Mapourika) for the central lakes North and South Mavora, and Hartley and Glenmore Tarns, and the two southern most lakes (Hauroko and Manapouri).

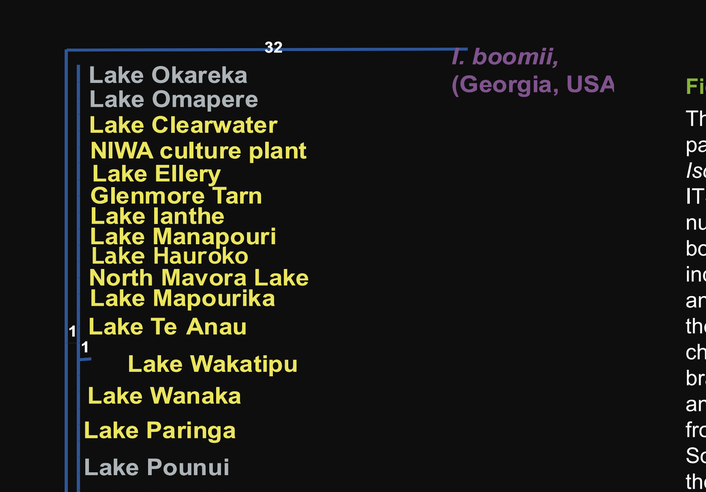


Figure 2
This is one of the equally parsimonious trees for *Isoëtes* based on the ITS sequence data. The number and band on the bottom right hand corner indicates the branch length and is proportional to the amount of character change along each branch. Samples in grey and yellow denote those from the North Island and South Island respectively, those in purple were sequenced in an earlier study (Hofstra and Gemmill 1999).

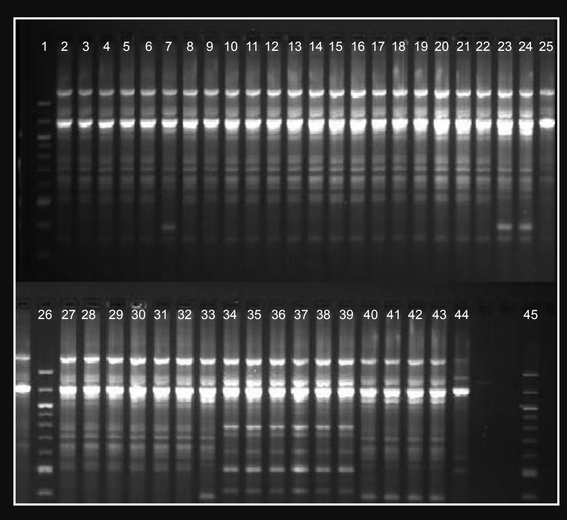


Figure 3
Photograph of a RAPD gel, showing population differences for a single primer. Lanes 1, 26 and 45 are DNA (ladder) markers. Lanes 7, 23 and 24, compared with other samples in the top row, are examples of within population (lake) differences. Lanes 27 to 33, 34 to 39 and 40 to 44 show differences between three different populations.

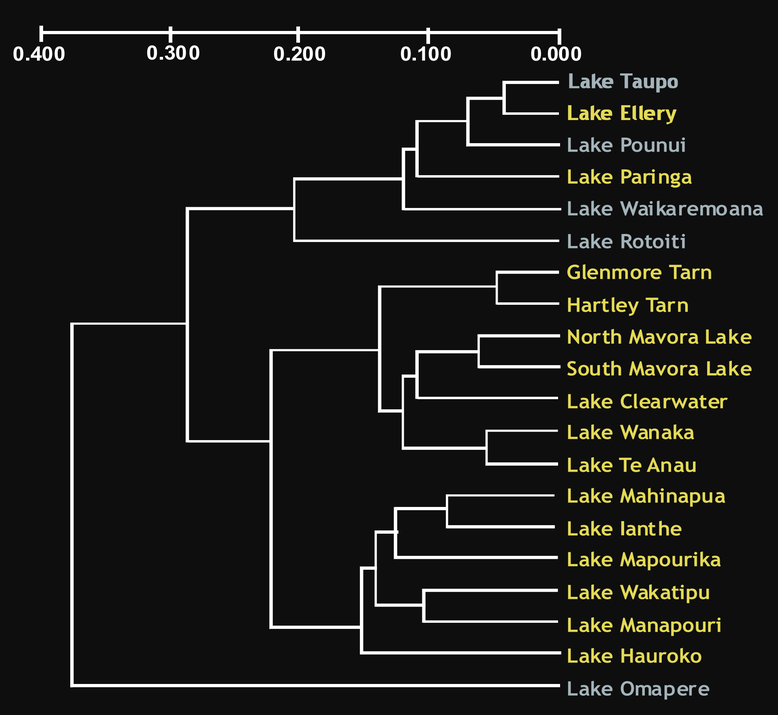


Figure 4
UPGMA cluster diagram generated from *Isoëtes* RAPD data using Nei's (1972) genetic distance. Samples in grey and yellow denote those from the North Island and South Island respectively.

Conclusions

- This study provides genetic evidence in support of unpublished morphological and cytological investigations (ie there are diverse and distinct entities of *Isoëtes* such as the flabellate *I. kirkii* and the tetraploid North Island *Isoëtes*, and the Southern *Isoëtes*).
- These findings have significant implications for New Zealand biodiversity because some populations of these endemic plants are under threat (and already extinct from the Lower Waikato Region).
- Future directions include using other DNA regions for sequencing, such as the Lfy intron and ETS to attempt to resolve the relationship between New Zealand and Tasmanian *Isoëtes*, and at the population level using ISSR markers to corroborate our RAPD findings.

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