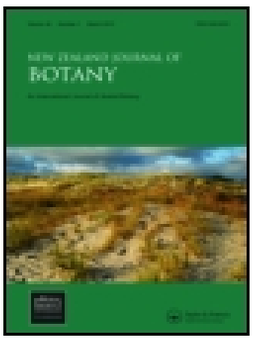

New Zealand Journal of Botany

ISSN: 0028-825X (Print) 1175-8643 (Online) Journal homepage: http://www.tandfonline.com/loi/tnzb20

Natural variation and conservation of Lepidium sisymbrioides Hook, f. and L. solandri Kirk (Brassicaceae) in South Island, New Zealand, based on morphological and DNA sequence data

P. B. Heenan , A. D. Mitchell , P. A. McLenachan , P. J. Lockhart & P. J. de Lange

To cite this article: P. B. Heenan , A. D. Mitchell , P. A. McLenachan , P. J. Lockhart & P. J. de Lange (2007) Natural variation and conservation of Lepidium sisymbrioides Hook, f. and L. solandri Kirk (Brassicaceae) in South Island, New Zealand, based on morphological and DNA sequence data, New Zealand Journal of Botany, 45:1, 237-264, DOI: 10.1080/00288250709509712

To link to this article: http://dx.doi.org/10.1080/00288250709509712

New Zealand Journal of Botany, 2007, Vol. 45: 237–264
0028–825X/07/4501–0237

Natural variation and conservation of *Lepidium sisymbrioides* Hook. f. and *L. solandri* Kirk (Brassicaceae) in South Island, New Zealand, based on morphological and DNA sequence data

P. B. HEENAN
Allan Herbarium
Landcare Research
PO Box 40
Lincoln 7640, New Zealand

A. D. MITCHELL
Christchurch School of Medicine & Health Sciences
PO Box 4345
Christchurch 8140, New Zealand

P. A. McLENACHAN

P. J. LOCKHART
Allan Wilson Centre for Molecular Ecology and Evolution
Institute of Molecular BioSciences
Massey University
Private Bag 11222
Palmerston North 4442, New Zealand

P. J. de LANGE
Terrestrial Conservation Unit
Department of Conservation
Private Bag 68908
Newton
Auckland 1145, New Zealand

Abstract The South Island, New Zealand, endemics *Lepidium sisymbrioides* and *L. solandri* are accepted at species rank. *L. solandri* Kirk is reinstated and includes *L. matau* Petrie in synonymy, and *L. kawarau* is reduced to synonymy of *L. sisymbrioides*. Twenty-nine floral and vegetative morphological characters were measured and used for analyses of character variation and principal components analysis. Leaf characters proved to be the most useful in distinguishing between *L. sisymbrioides* and *L. solandri*, with 12 of the 15 leaf characters having significantly different means. Only 7 of the 14 floral characters included in the study have significantly different means. In comparison to *L. solandri*, *L. sisymbrioides* has longer and narrower terminal and primary pinnae and cauline leaves, more secondary pinnae and cauline leaf lobes, less hairy sepals and ovaries, narrower ovaries, and shorter filaments. *L. sisymbrioides* occurs on rock outcrops in the upper Manuherikia, Waitaki, and Kawarau river valleys. *L. solandri* grows mainly on alluvium and outwash gravels in semi-arid parts of inland Canterbury and Central Otago.

DNA sequence data from ITS, ETS, and *trn*L-*trn*F markers were used to examine phylogenetic patterns in *L. sisymbrioides* and *L. solandri*, and the related species *L. kirkii*, *L. naufragorum*, and *L. tenuicaule*. There is substantial variation in these markers in *L. sisymbrioides* and *L. solandri*, and this is considered to be due to introgression from other species of *Lepidium*, such as *L. kirkii* and *L. tenuicaule*. Much of the variation in the ETS sequences occurs in samples of *L. sisymbrioides* and *L. solandri* from the Manuherikia River valley, and this can be attributed to geneflow with the sympatric *L. kirkii*.

Lepidium sisymbrioides and *L. solandri* are considered to be "Acutely Threatened, Nationally Endangered" using the New Zealand threatened plant classification criteria. In this assessment both species are considered to be data poor. Further field survey for new populations is required.

Keywords Brassicaceae; *Lepidium*; *L. sisymbrioides*; *L. solandri*; *L. kawarau*; *L. matau*; *L. sisymbrioides* subsp. *matau*; *L. sisymbrioides* subsp. *kawarau*; *L. sisymbrioides* subsp. *sisymbrioides*; ITS; ETS; *trn*L-*trn*F; conservation; introgression; New Zealand flora

INTRODUCTION

Lepidium in New Zealand includes 8 indigenous species and 13 naturalised species (Webb et al. 1988; Garnock-Jones & Norton 1995; Al-Shehbaz et al.

B06021; Online publication date 29 March 2007
Received 2 June 2006; accepted 17 November 2006

2002; *Coronopus didymus*, *C. squamatus*, and *Cardaria draba* are placed in *Lepidium*). One species, the South Island endemic *L. sisymbrioides*, is unusual in the Brassicaceae in being the only species of the family to be dioecious. Despite the distinct breeding system, the taxonomy of the *L. sisymbrioides* complex has proven particularly difficult to resolve; this is reflected by a number of different taxonomic classifications and uses of taxonomic rank.

Four of the taxa involved in the *L. sisymbrioides* complex were first described at the rank of species, these being *L. sisymbrioides* (Hooker 1864), *L. solandri* (Kirk 1882), *L. kawarau* (Petrie 1885), and *L. matau* (Petrie 1887). Kirk (1899) and Cheeseman (1906) accepted three of these species (*L. kawarau*, *L. matau*, and *L. sisymbrioides*), with both authors relegating *L. solandri* into synonymy of *L. sisymbrioides*. Kirk (1899) was the first to use an infraspecific rank (*L. kawarau* var. *dubium*). This was followed by the complex infraspecific classification system of Thellung (1906), who accepted only *L. sisymbrioides* at species rank but recognised three subspecies (subspp. *solandri*, *matau*, and *kawarau*). For each of these subspecies Thellung also published combinations at the rank of variety: *L. sisymbrioides* subsp. *matau* var. *lobulatum*, *L. sisymbrioides* subsp. *kawarau* var. *dubium*, and *L. sisymbrioides* subsp. *solandri* var. *typicum* and var. *ovatum*.

Thellung's (1906) treatment was not accepted by Cheeseman (1925), who continued to recognise *L. kawarau*, *L. matau*, and *L. sisymbrioides*. Indeed, Cheeseman (1925) commented, "I cannot at all agree with Dr. Thellung in combining the three into one species." Cheeseman (1925) continued to recognise one infraspecific taxon, *L. kawarau* var. *dubium* Kirk. Allan (1961) followed Cheeseman (1925) in accepting three species, only referring to the infraspecific taxa and their protologues in the notes for each species entry. Allan (1961) appears not to have been convinced that the taxonomy of the dioecious taxa was adequately resolved as he commented, "Further field and cultural work is necessary to determine the status of the forms of the dioec. spp. The occurrence of intermediate forms suggests hybridism may occur." Earlier, Cockayne & Allan (1934) had listed the hybrid combination *L. matau* × *L. kawarau*, observing, "we have seen these species growing together in one locality, and intermediates were present." The most recent taxonomic treatment has been provided by Garnock-Jones (in Webb et al. 1988) who followed Thellung (1906) in accepting three subspecies, but did not recognise any taxa at the rank of variety.

As part of a study on the ecology of *L. sisymbrioides*, Allen (1998) examined variation of several leaf and floral characters. He commented, "The taxonomic characters listed by Allan (1961) were generally adequate to distinguish between plants of the three subspecies, although there was a closer similarity between *L. s.* subsp. *sisymbrioides* and *L. s.* subsp. *matau* than between either of these and *L. s.* subsp. *kawarau*." However, Allen (1998) cast some doubt on the taxonomy by suggesting, "The possibility that [*L. s.* subsp. *sisymbrioides* and *L. s.* subsp. *matau*] are geographic variants of a single species should be further explored." Furthermore, examination of Allen's (1998) text and figures (e.g., fig. 6, 7) indicates considerable overlap among subspecies and populations for some characters.

The preceding account of the taxonomic history of the *L. sisymbrioides* complex shows that the taxonomy of the group has been unstable for nearly 150 years, particularly in regard to the number of taxa recognised and the application of different taxonomic ranks. It is notable, however, that some nomenclatural stability was provided for over 60 years by the consecutive flora treatments of Kirk (1899), Cheeseman (1906, 1925), and Allan (1961), who all accepted at species rank *L. kawarau*, *L. matau*, and *L. sisymbrioides*.

A biological feature of the *L. sisymbrioides* complex is that the different subspecies are uncommon, as they usually occur naturally as small and disjunct populations between North Canterbury and Central Otago in eastern South Island. This pattern of abundance and distribution has led to the different taxa being listed as rare and threatened plants (e.g., *L. kawarau* and *L. matau*; Given 1976). The most recent inventory of threatened plants (de Lange et al. 2004) lists the three currently recognised *L. sisymbrioides* subspecies (Webb et al. 1988): *L. sisymbrioides* subsp. *matau* (Acutely Threatened, Nationally Critical), *L. sisymbrioides* subsp. *kawarau* (Acutely Threatened, Nationally Endangered), and *L. sisymbrioides* subsp. *sisymbrioides* (Chronically Threatened, Gradual Decline). Furthermore, such is the concern for the conservation of the three subspecies of *L. sisymbrioides* that a recovery and management strategy and work plan to halt their decline has been proposed (Allen 2000).

Due to the variable taxonomic and nomenclatural history and the significant conservation issues surrounding the *L. sisymbrioides* complex, the Department of Conservation approached PBH in 2003 to undertake a taxonomic revision of the group. The purpose of this revision is to assess morphological

variation throughout the geographic range of *L. sisymbrioides* using common garden experiments and to use molecular techniques to provide an independent data set for comparison with the morphology. The results of these studies are presented here.

MATERIALS AND METHODS

Plant material

Field studies and collections of the *L. sisymbrioides* complex have been made throughout the South Island by PBH. Plant material was collected from populations representing the range of *L. sisymbrioides* sens. lat. (Table 1), with particular emphasis given to populations from Otago since the majority of the morphological and taxonomic diversity occurs there. Seeds were obtained from additional natural populations but these did not germinate, and some other populations comprised so few plants that live plants could not be removed or they did not set seed.

Plants representing 10 populations were successfully grown from seed or from whole plant transplants. Plants were cultivated under similar conditions in a glasshouse at the experimental nursery, Landcare Research, Lincoln. This approach minimises the effect of environmentally induced variation, and plants were allowed to establish and mature in an unheated glasshouse for one season before measurements were taken.

Samples for the DNA study were taken from 21 populations, usually by removing several leaves from individual plants. Vouchers for the DNA and morphological studies are deposited in CHR (Tables 1 and 2).

To provide consistency throughout the paper the names *L. solandri* and *L. sisymbrioides* are used in the experimental, result, and taxonomic sections. *Lepidium solandri* includes plants previously assigned to *L. sisymbrioides* subsp. *matau* (Alexandra area) and *L. sisymbrioides* subsp. *sisymbrioides* (throughout Canterbury and Otago). *Lepidium sisymbrioides* includes *L. sisymbrioides* subsp. *kawarau* (Kawarau Gorge). Populations previously assigned to *L. sisymbrioides* subsp. *kawarau* from Manuherikia Gorge/Falls Dam, as well as similar plants from populations in the Waitaki River valley, are referred to as "*L. sisymbrioides* subsp. *kawarau* II". For the morphological analyses plants were a priori assigned to the three subspecies following Webb et al. (1988), and to "*L. sisymbrioides* subsp. *kawarau* II". This approach enabled the existing taxomony to be critically tested as well as providing the opportunity for any new groups to be identified.

Morphological characters and analyses

For each of the cultivated plants of *L. sisymbrioides* sens. lat. that were cultivated at Lincoln, 29 vegetative and floral characters were scored. To obtain the mean of each of the floral and vegetative characters for each plant, three measurements were made. Exploratory data analysis was carried out for all characters to check for errors, outliers, and normal distribution; all characters were approximately normal and no transformations were required. Furthermore, box plots and scatter graphs were also used to explore patterns of character variation. A principal components analysis (PCA) was undertaken of these data. A correlation matrix scaled to have unit variance was used for the PCA because the variables are in different units of measurement. For the PCA, the occurrence of dioecy in the *L. sisymbrioides* complex meant that the entire data set could not be analysed at once since the floral morphology of the male and female plants differed. Therefore, separate analyses were undertaken to test for groups or patterns in the data. These analyses included:

a. Leaf characters for all samples. Plants were also analysed by sex, to assess whether variation in leaf characters is sex linked.
b. Male plants: analyses of leaf characters only; floral characters only; leaf and floral characters combined.
c. Female plants: analyses of leaf characters only; floral characters only; leaf and floral characters combined.
d. Leaf and floral characters combined for characters where the means were different at 95% confidence level in the one-way analysis of variance: male plants; female plants.

One-way analysis of variance (ANOVA) was undertaken for each character to test the ability of the measurement data to discriminate between the taxonomic groups determined by the PCA. Box plots were used to examine length to width ratios of leaf pinnae and cauline leaves for populations in the Manuherikia River valley that had previously been referred to *L. sisymbrioides* subsp. *sisymbrioides* and *L. sisymbrioides* subsp. *matau*; these characters were used, for example, by Webb et al. (1988) to distinguish these two subspecies. Data for these analyses were taken from the herbarium specimens of plants collected in the wild. For each plant measurements were taken from three cauline leaves from the middle

Table 1 Collection details for *Lepidium* samples used for the morphological and molecular studies.

Name	Code	Population	NZMS 260 grid reference	Morphology vouchers
L. sisymbrioides	k-MG	gorge, upper Manuherikia River, Otago	H41/638855	-
	k-Fd a	Falls Dam, upper Manuherikia River, Otago	H40/663895	-
	k-FD b	Falls Dam, upper Manuherikia River, Otago	H41/652882	CHR 573547; CHR 573555
	k-HT	Hakataramea Road, Waitaki River, Otago	I40/134035	-
	k-GR	Gards Road, Waitaki River, Otago	I40/948172	CHR 573559; CHR 573548
	k-OT	Otematata, Waitaki River, Otago	H40/85-19-	-
	k-CR	Chards Road, Kawarau River, Otago	F41/866694	CHR 573554; CHR 573553
	k-NB	Nevis Bluff, Kawarau River, Otago	F41/946669	CHR 573546; CHR 573550
	k-SC	Slapjack Creek, Kawarau River, Otago	F41/993614	CHR 573551; CHR 573557
	k-SC-M	Slapjack Creek, Kawarau River, Otago	-	-
L. kirkii	*L. kirkii*	Galloway, near Alexandra, Otago	G42/338497	-
L. naufragorum	*L. nau*	Open Bay Islands, Westland	F37/791970	-
	L. nau a	Cape Foulwind, Westland	K29/817370	-
	L. nau b	Seal I., Punakaiki, Westland	K30/748071	-
L. solandri	s-CH	Castle Hill, Canterbury	K34/056756	-
	s-SS	Sawdon Stream, Tekapo, Canterbury	I38/087785	CHR 573560
	s-TW	Ohau River, Twizel, Canterbury	H38/779538	-
	s-PF	Pisa Flats, Clutha River, Otago	G41/159792	CHR 573558; CHR 573583
	s-PF-M	Pisa Flats, Clutha River, Otago	-	-
	s-LV	Little Valley, Alexandra, Otago	G42/368439	CHR 573543; CHR 573544
	s-Wa, s-Wb, s-Wc, s-Wd	property of K. & R. Wardle, Alexandra, Otago	G42/322489	-
	s-GW	Galloway, Alexandra, Otago	G42/337497	-
	s-GW-M	Galloway, Alexandra, Otago	-	-
	s-Sa, s-Sb	Springvale, Manuherikia River valley, Otago	G42/297506	CHR 573552; CHR 573545
	s-CC	Chatto Creek, Manuherikia River valley, Otago	G42/355575	-
	s-MP	Michael Peak Station, Manuherikia River valley, Otago	H41/604777	-
	s-P	Patearoa, Taieri River, Otago	H42/716394	CHR 573549; CHR 573556
	s-ES	Eden Creek, Taieri River valley, Otago	H42/782532	-
L. tenuicaule	*L. ten*	Shag Point, Otago	J43/398233	-
	L. ten a	Watson's Beach, Otago	I45/898455	-
	L. ten b	Taieri Beach, Otago	I45/927513	-
	L. ten c	Waipapa Point, Southland	F47/918864	-

of the inflorescence and three pinnae from the middle of the leaf. Each box plot depicts the median (central line in each box), 25% and 75% quartiles (upper and lower limits of each box), the maximum point within 1.5 times the interquartile range from the quartiles (indicated by whiskers), and outliers (points greater than 1.5 times from the quartiles, indicated by lines outside the whiskers). All analyses were made using S-Plus (Statistical Sciences 1998).

Table 2 Herbarium vouchers and GenBank accessions for samples included in the DNA study.

Species	Code	ITS GenBank number	ETS GenBank number	*trn*L-*trn*F GenBank number	Herbarium voucher or collection number
L. sisymbrioides	k-OT	DQ997565	DQ989376	DQ997054	CHR 573572
	k-HT	DQ997570	DQ989377	-	CHR 573568
	k-GR	DQ997560	DQ989378	DQ997064	CHR 569975
	k-MG	DQ997564	DQ989379	DQ997063	No voucher
	k-FDa	DQ997559	DQ989380	-	No voucher
	k-FDb	DQ997569	DQ989381	DQ997061	CHR 573579
	k-SC	DQ997568	DQ989382	DQ997065	CHR 569976
	k-SC-M	AJ582419; AJ582476	-	AY015894; AY015978	KM 667
	k-NB	DQ997561	DQ989383	DQ997067	CHR 569978
	k-CR	DQ997562	DQ989384	DQ997068	CHR 569977
L. kirkii	*L. kirkii*	EF109738; EF109739	-	-	KM 1413
	L. kirkii	-	DQ989385	DQ997075	CHR 586002
L. naufragorum	*nau*	AJ582422; AJ582479	-	AY015872; AY015958	KM 669
	nau	-	DQ989386	-	CHR 586005
	*nau*_a	-	DQ989387	-	CHR 586004
	*nau*_b	-	DQ989388	-	CHR 586003
L. solandri	s-CH	DQ997553	DQ989389	DQ997060	CHR 569936
	s-SS	DQ997555	DQ989390	DQ997070	CHR 569982
	s-TW	DQ997566	DQ989391	DQ997056	CHR 573596
	s-PF	DQ997557	DQ989392	DQ997073	CHR 569979
	s-PF-M	AJ582420; AJ582477	-	AY015896; AY015980	KM 665
	s-LV	DQ997554	DQ989393	DQ997069	CHR 569980
	s-GW	-	DQ989394	DQ997066	CHR 569972
	s-GW-M	AJ582418; AJ582475	-	AY015895; AY015979	KM 666
	s-Sa	-	DQ989395	DQ997071	CHR 569981
	s-Sb	-	DQ989396	DQ997055	CHR 573573
	s-CC	-	DQ989397	DQ997062	No voucher
	s-ES	DQ997558	DQ989398	DQ997074	CHR 569974
	s-MP	DQ997571	DQ989399	-	CHR 573584
	s-P	DQ997563	DQ989400	DQ997053	CHR 573575
	s-Wa	DQ997556	DQ989401	DQ997072	CHR 569971
	s-Wb	DQ997567	DQ989402	DQ997059	No voucher
	s-Wc	-	-	DQ997057	No voucher
	s-Wd	-	DQ989403	DQ997058	No voucher
L. tenuicaule	*ten*	AJ582421; AJ582478	-	AY015899; AY015982	KM 664
	ten	-	DQ989404	-	CHR 586006
	*ten*_a	-	DQ989405	-	CHR 586008
	*ten*_b	-	DQ989406	-	CHR 586009
	*ten*_c	-	DQ989407	-	CHR 586007

The 29 characters scored for the study are described below:

Lamina length (mm). Measured from the lowest primary pinna to the apex of the terminal pinnae.

Petiole length (mm). Measured from the lowest primary pinna to the base of the petiole.

Ratio of petiole length to total leaf length. Scored as a percentage.

Leaf hair type. Linear; linear and dolabriform; dolabriform; glabrous.

Leaf colour. Green (0); green-brown (1); brown (2).

Leaf terminal pinnae width (mm). Measured at the widest point.

Leaf terminal pinnae length (mm). Measured from the distal primary pinna to the apex of the terminal pinna.

Leaf primary pinnae number. Primary pinnae counted.

Leaf primary pinnae length (mm). Three primary pinnae were selected in the middle third of the lamina and measured from the central part of the lamina to the apex of the pinna.

Leaf primary pinnae width (mm). Measured at the widest point.

Leaf secondary pinnae number. Number of secondary pinnae (including lobes and serrations) on each primary pinna.

Central part of lamina width (mm). Measured at the middle of the lamina.

Inflorescence diameter (mm). Measured at the base of the inflorescence.

Inflorescence branch number. Number of lateral inflorescence branches.

Inflorescence length (mm).Measured from the base to the apex.

Inflorescence rachis angle. Measured in degrees from the base.

Cauline leaf length (mm). Three most distal cauline leaves measured from their base to their apex.

Cauline leaf width (mm). Measured at the widest point.

Cauline leaf lobe number. Primary pinnae (including lobes and serrations) counted.

Petal length (mm).

Sepal hair number. Number of hairs on the abaxial surface.

Sepal width (mm). Measured at the widest point.

Style length (mm).

Ovary length (mm).

Ovary width (mm).

Ovary hair number. Number of hairs on one face of the ovary.

Nectary colour. Green (0); green and red (1); red (2).

Filament number.

Filament length (mm).

DNA sequence data

Plant specimens

Plant material used for the molecular study included specimens of *L. sisymbrioides* sens. lat. that were recently collected in the field (Tables 1 and 2). We also included published sequences of *L. sisymbrioides* sens. lat. from the studies of Mummenhoff et al. (2001, 2004). Herbarium vouchers for accessions are deposited in CHR (Table 2). Vouchers were not available for some of the collections since the wild populations comprised too few plants to secure a suitable herbarium specimen. Sequences of *L. kirkii*, *L. naufragorum*, and *L. tenuicaule* were also included, since ITS sequence data have shown they form a monophyletic group with *L. sisymbrioides* sens. lat. (Mitchell & Heenan 2000; Mummenhoff et al. 2004). In this study we have used the *nr*DNA Internal Transcribed Spacer (ITS) and External Transcribed Spacer (ETS) and the *cp*DNA *trn*L-*trn*F markers for phylogenetic reconstruction. These markers were sequenced for most of the accessions included in this study (Table 2).

DNA extraction, PCR, and sequencing

Genomic DNA was extracted from the specimens of *Lepidium* listed in Table 2 using a modification of the CTAB method (Doyle & Doyle 1987). The total amplification reaction volumes were 25 µl. Each PCR reaction mixture contained 16.6 µl sterile water, 2.5 µl 10× PCR reaction buffer (without $MgCl_2$), 2.0 µl $MgCl_2$, 0.65 µl of dNTPs (8 m*M*), 1.0 µl of each primer (10 µ*M*), 0.25 µl *Taq* polymerase (5 U/µl), and, typically, 1.0 µl template DNA (unquantified). Oligonucleotide primers were ITS-28CC and ITS-18d (Wagstaff & Garnock-Jones 1998) and ETS-18S and ETS-9 (Wright et al. 2001). Temperature and cycling conditions for both ETS and ITS amplifications were identical as follows: one 94°C denaturation cycle for 2 min, followed by 10 cycles in which the annealing temperature was reduced by 1°C in each cycle, of 94°C denaturation for 30 s, primer annealing at 65°C–56°C for 30 s, and elongation at 72°C for 1 min, followed by 38 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 1 min, and finally an elongation at 72°C for 5 min; in the *trn*L-*trn*F PCR the primer annealing step (50°C for 1 min) was changed to 55°C for 1 min. PCR products were purified using

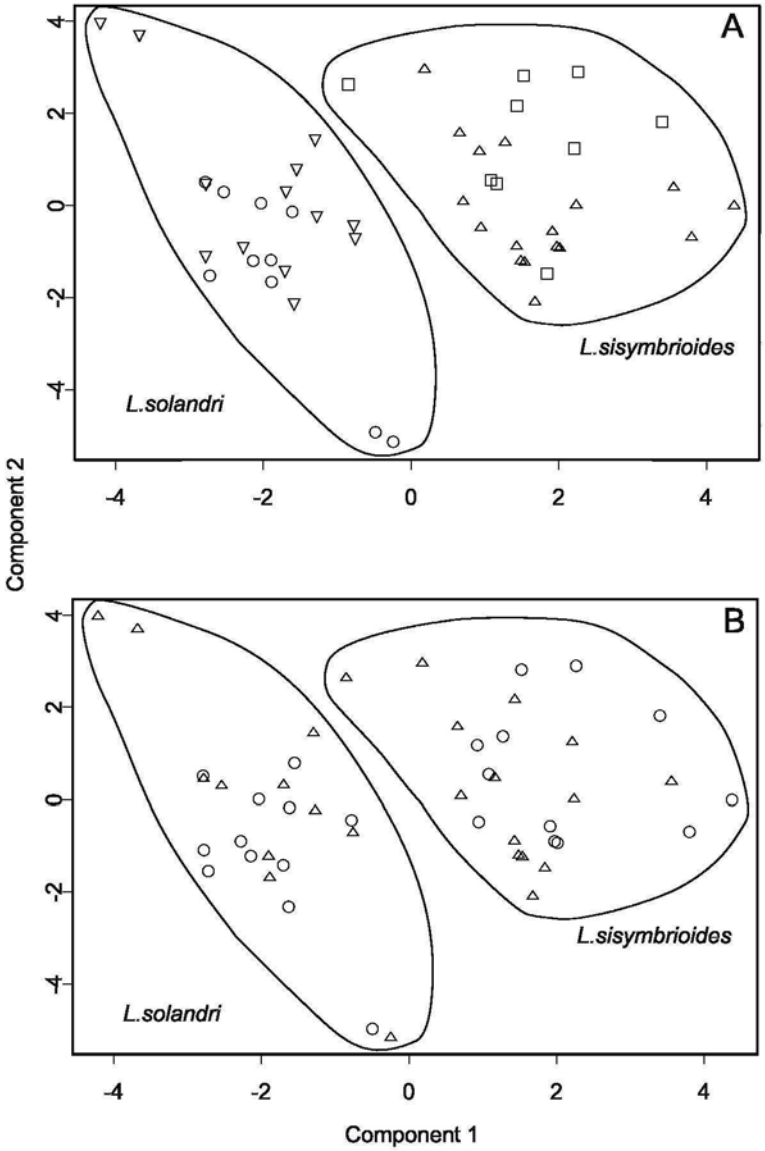

Fig. 1 Principal components analysis of leaf characters only for male and female plants. Component 1 explained 29.5% of the variation and component 2 explained 22.5%. **A**, *L. sisymbrioides* (△, as subsp. *kawarau*; □, as subsp. *kawarau* II); *L. solandri* (○, as *L. sisymbrioides* subsp. *sisymbrioides*; ▽, as *L. sisymbrioides* subsp. *matau*); **B**, distribution of male (○) and female (△) plants for *L. sisymbrioides* and *L. solandri*.

Perfectprep PCR cleanup kits (Eppendorf). Each sample was sequenced in the sense and antisense direction. Sequencing reactions were performed with the same primers as the PCR amplifications using the 3.1 ABI Prism™ Big Dye Terminator Sequencing Kit (Applied Biosystems, Scoresby, Vic.) by the Allan Wilson Centre Genome Service, Massey University, Albany. Sequences obtained in this study have been assigned GenBank accession numbers (Table 2).

Data analysis

Sequence alignment was initially performed using ClustalX vers. 1.81 (Thompson et al. 1997). Multiple alignment parameters were set to 12 for gap opening penalty and 6.0 for gap extension penalty. Sequence alignments were subsequently improved manually using BioEdit (Hall 1999). Splits Tree 4, V 4.2 (Huson & Bryant 2006; http://www-ab.informatik.uni-tuebingen.de/software/jsplits/welcome.html) was used, retaining all characters, to convert sequence

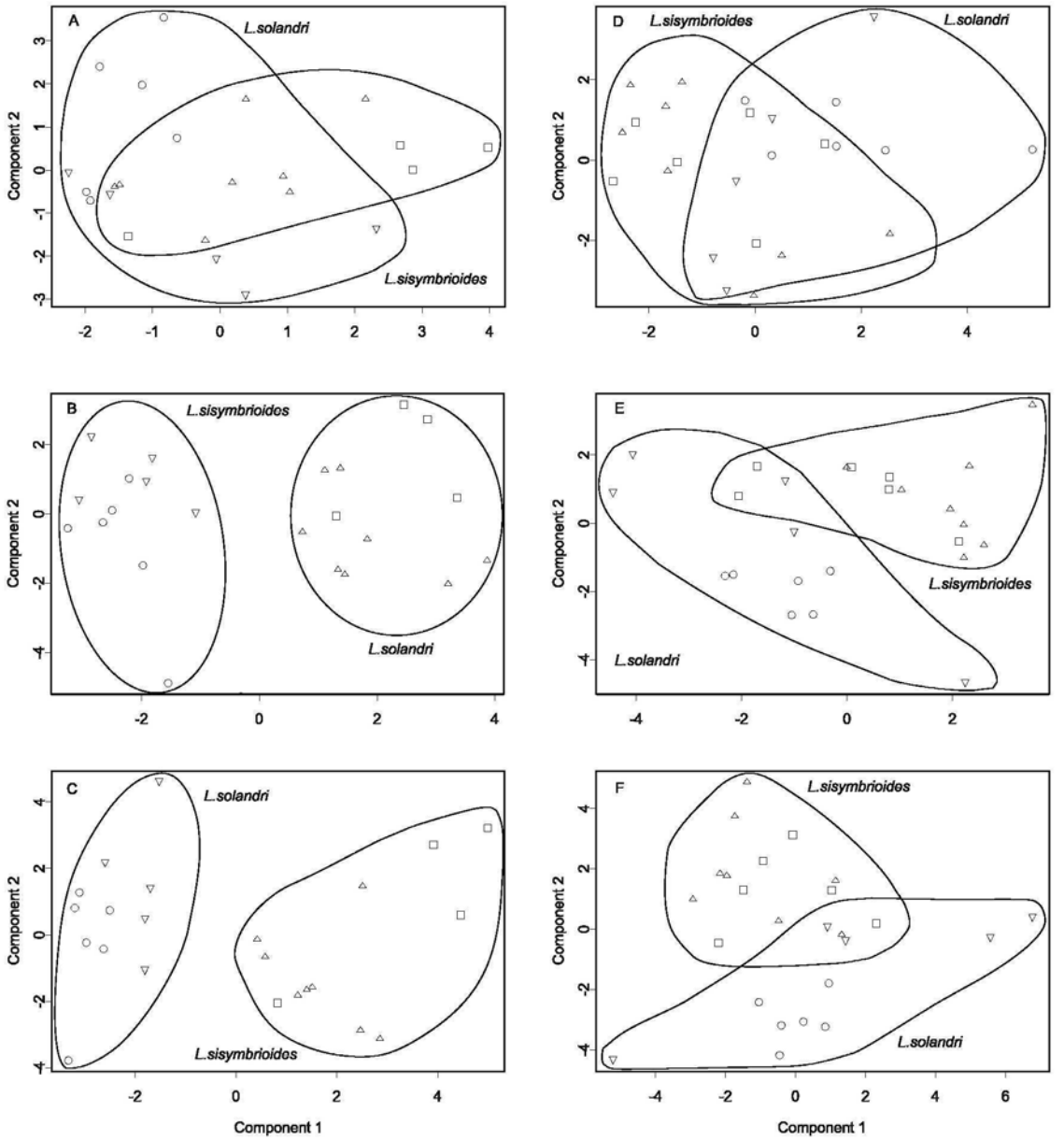

Fig. 2 Principal components analysis for *L. sisymbrioides* (△, as subsp. *kawarau*; □, as subsp. *kawarau* II) and *L. solandri* (○, as *L. sisymbrioides* subsp. *sisymbrioides*; ▽, as *L. sisymbrioides* subsp. *matau*). **A**, Male flowers only (component 1 explained 30.8% of the variation and component 2 explained 21.5%); **B**, male leaves only (component 1, 35.9%; component 2, 19.9%); **C**, male leaves and flowers combined (component 1, 27.6%; component 2, 16.9%); **D**, female flowers only (component 1, 24.6%; component 2, 20.6%); **E**, female leaves only (component 1, 28.8%; component 2, 21.7%); **F**, female leaves and flowers combined (component 1, 35.9%; component 2, 19.9%).

data to uncorrected-P distances and construct networks using NeighborNet. A total of 5000 bootstrap iterations were performed.

RESULTS AND DISCUSSION

Morphological characters

The PCA of all leaf characters distinguished two discrete groups (Fig. 1A). One group included all samples a priori assigned to *L. sisymbrioides* subsp. *matau* and *L. sisymbrioides* subsp. *sisymbrioides* (hereafter referred to as *L. solandri* group). The second group comprised all samples a priori assigned to *L. sisymbrioides* subsp. *kawarau* and *L. sisymbrioides* subsp. *kawarau* II (hereafter referred to as *L. sisymbrioides* group). Within the *L. sisymbrioides* and *L. solandri* groups, the two taxonomic subgroups overlapped, implying that variation in leaf characters is continuous. The outliers in *L. solandri* (Fig. 1A) are conspicuous and considered to be the result of inadequate sampling of a threatened species that is known from few populations with a small number of variable individuals; they do not warrant recognition as additional taxonomic groups. Male and female plants were scattered across the *L. sisymbrioides* and *L. solandri* groups and did not show any pattern or bias in leaf morphological characters that could be sex linked (Fig. 1B).

The PCA of male plants only did not retrieve any groups when male flower characters only were used (Fig. 2A). However, in the analyses of leaf characters only (Fig. 2B) and leaf and flower characters combined (Fig. 2C) two discrete groups were distinguished. These two groups correspond to the *L. sisymbrioides* and *L. solandri* groups obtained in the analysis of all leaf characters (Fig. 1A). The three PCAs of only female plants did not retrieve any discrete groups (Fig. 2D–F), although in the leaf characters only (Fig. 2E) and leaf and flower characters combined (Fig. 2F) two groups with a small amount of overlap were recognised; these two groups correspond to the *L. sisymbrioides* and *L. solandri* groups of the other PCAs (Fig. 1, 2A–C). The PCA of the leaf and floral characters with significantly different means in the ANOVA (Table 3) continued to distinguish two groups (*L. sisymbrioides* and *L. solandri*) in the male plants (result not presented, similar to Fig. 2C); for the female plants two groups (*L. sisymbrioides* and *L. solandri*) were retrieved with only a slight overlap, which improved resolution in comparison to the analyses of all of the characters for the female plants (result not presented; similar to Fig. 2F).

Leaf characters proved to be the most useful in distinguishing between the *L. sisymbrioides* and *L. solandri* groups, with 12 of the 15 leaf characters having significantly different means (Table 3). Variation in leaf characters between *L. sisymbrioides* and *L. solandri* is illustrated in Fig. 3–5. The most obvious differences include *L. sisymbrioides* having longer and narrower terminal and primary pinnae and cauline leaves, and more secondary pinnae and cauline leaf lobes.

Of the 15 floral characters included in the study 8 have significantly different means (Table 3). Important flower characters include *L. sisymbrioides* typically having less hairy sepals and ovaries, narrower ovaries, and shorter filaments. There is often variation within these characters with, for example, some plants having wider and more hairy ovaries (Fig. 4). *L. sisymbrioides* shows variation in the presence and absence of petals, with all plants examined from the Waitaki River valley having petals (Fig. 6). Plants from Falls Dam and the Kawarau River usually lacked petals, although one plant from Chards Road (Kawarau River) had two petals per flower. *L. solandri* also usually lacks petals, although one plant from Springvale had 1–2 small petals per flower.

Plants from the Manuherikia and Taieri river valleys previously assigned to *L. sisymbrioides* subsp. *sisymbrioides* and *L. sisymbrioides* subsp. *matau* (herein treated as *L. solandri*) show variation in the length-to-width ratio of the cauline leaves and leaflet pinnae (Fig. 7). A clinal pattern of variation is particularly striking for the cauline leaf length-to-width ratio. For example, plants from the lower Manuherikia River valley (e.g., Alexandra, Galloway, and Springvale) that have previously been assigned to *L. sisymbrioides* subsp. *matau* have broad cauline leaves relative to their length, which gives a low length-to-width ratio. In contrast, plants from the central Manuherikia River valley (e.g., Michael Peak Station) and upper Taieri River valley (e.g., Eden Creek, Eweburn, and Gimmerburn) that have previously been assigned to *L. sisymbrioides* subsp. *sisymbrioides* have narrower cauline leaves relative to their length and this gives a high length-to-width ratio. These two characters cannot, therefore, reliably distinguish *L. sisymbrioides* subsp. *matau* from *L. sisymbrioides* subsp. *sisymbrioides* (cf. Webb et al. 1988). Box plot analyses of characters by geographic area or population and scatter graphs of different combinations of characters did not identify

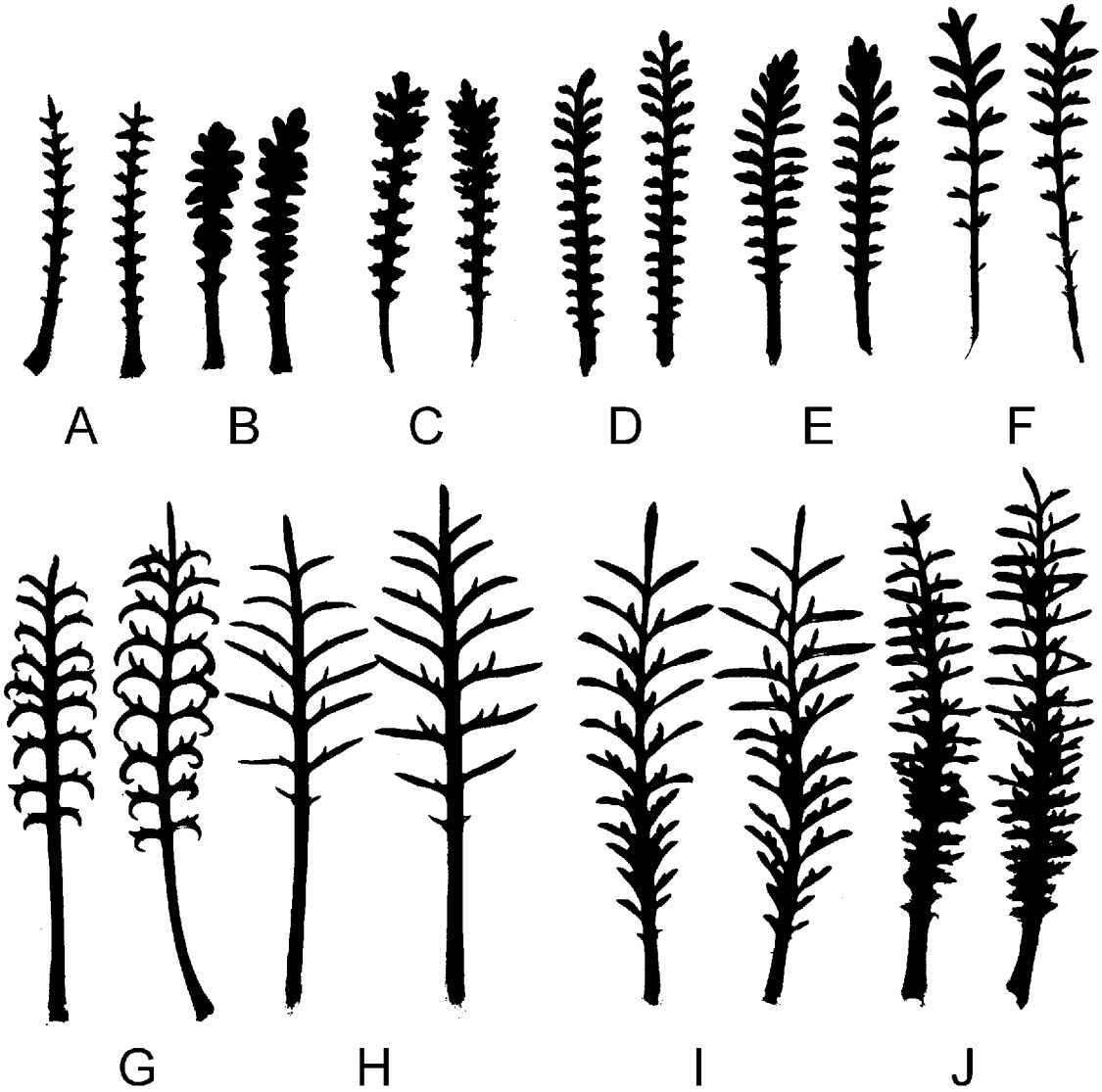

Fig. 3 Leaves of *L. solandri* (A–F) and *L. sisymbrioides* (G–J). **A**, Sawdon Stream, Tekapo; **B**, Springvale, Manuherikia River valley; **C**, Little Valley, Alexandra; **D**, Pisa Flats, Clutha River valley; **E**, Patearoa, upper Taieri River; **F**, Castle Hill; **G**, Nevis Bluff, Kawarau River; **H**, Slapjack Creek, Kawarau River; **I**, Falls Dam, upper Manuherikia River; **J**, Gards Road, Waitaki River valley.

any other geographic or clinal patterns of variation (results not presented).

DNA sequence data

trn*L*-trn*F*

The aligned *trn*L-*trn*F data set contained 28 samples and 902 characters; 15 of the characters were variable, and 9 were potentially parsimony-informative. *L. naufragorum* and *L. tenuicaule* share similar sequences, differing only at positions 790 and 866 (Table 4). One sample of *L. sisymbrioides* from Falls Dam (upper Manuherikia River valley; Fig. 8, 9) has an identical sequence type to *L. tenuicaule*. *L. kirkii* and most of the samples of *L. sisymbrioides* and *L. solandri* have the same basic sequence type (Table 4), although 10 samples do differ. One sample of *L. sisymbrioides* (Gards Road) and three of *L. solandri*

(Galloway, Twizel, and Sawdon) share bases with *L. naufragorum* and *L. tenuicaule*. Six other samples of *L. solandri* and one of *L. sisymbrioides* also have a single transition (Table 4).

The Splitstree graph of the *trn*L-*trn*F sequence data illustrates the patterns of sequence variation described above (Fig. 9). Notably, the *L. sisymbrioides* sample from Falls Dam groups with *L. tenuicaule*, and there is a strong network linking samples of *L. sisymbrioides* from Gards Road and *L. solandri* from Twizel and Sawdon to both *L. tenuicaule* and the core group of samples representing *L. sisymbrioides* and *L. solandri*.

ITS

The aligned ITS data set contained 31 samples and 648 characters; 15 of the characters were variable and five were potentially parsimony-informative. Of the 15 variable characters 13 occurred in the *L. sisymbrioides* complex (Table 4). *L. naufragorum*, *L. tenuicaule*, and *L. kirkii* all share the same ITS sequence (Table 4). In contrast, *L. sisymbrioides* and *L. solandri* show considerable variation with three main sequence types: mixed, ctttc-gttgcaa (type I), and ctcc--gttgctg (type II) (Table 4). Six samples comprised mixed sequences and these occur only in *L. solandri* from near Alexandra (Fig. 10); these were excluded from the Splitstree analysis. Type I is relatively uniform and occurs in samples assigned to both *L. sisymbrioides* and *L. solandri*; one sample from Hakataramea does show variation in two base positions (Table 4). Type I is the most widespread sequence type and occurs near Alexandra and at localities outside of the Manuherikia River valley (Fig. 10). Type II includes samples of *L. sisymbrioides* and *L. solandri*, is most similar to the *L. kirkii*

Table 3 Leaf and flower measurement data from the cultivated plants included in morphometric study. One-way ANOVA significant at 95% (*) and 99% (**) confidence limits.

	L. solandri		*L. sisymbrioides*		ANOVA	
Character	Range	Mean	Range	Mean	*F*	*Pr (F)*
Lamina length (mm)	27.00–89.66	69.73 ± 15.14	72.66–169.33	99.88 ± 23.45	27.61	0.0**
Petiole length (mm)	9.66–33.00	20.38 ± 6.14	8.33–44.66	27.67 ± 10.45	8.56	0.0**
Petiole length/leaf length ratio	12.00–47.33	22.79 ± 7.95	6.33–35.33	21.60 ± 8.52	0.25	0.61
Leaf hair type	0.00–1.00	0.27 ± 0.44	0.00–3.00	1.26 ± 1.04	17.99	0.0**
Leaf colour	0.00–2.00	0.82 ± 0.65	0.00–1.00	0.38 ± 0.49	7.23	0.0**
Leaf terminal pinnae width (mm)	1.06–3.86	2.39 ± 0.73	1.03–2.60	1.73 ± 0.37	16.33	0.0**
Leaf terminal pinnae length (mm)	3.20–13.60	5.45 ± 2.11	9.43–24.33	14.35 ± 4.20	84.14	0.0**
Leaf primary pinnae number	14.66–30.33	22.76 ± 3.84	12.66–42.33	24.29 ± 8.14	0.67	0.41
Leaf primary pinnae length (mm)	3.40–10.26	6.84 ± 1.88	8.53–24.33	15.40 ± 4.18	81.28	0.0**
Leaf primary pinnae width (mm)	0.80–3.53	1.87 ± 0.57	0.96–2.40	1.57 ± 0.31	5.61	0.02*
Leaf secondary pinnae number	0.33–4.00	1.94 ± 0.90	2.00–4.66	3.00 ± 0.83	18.09	0.0**
Central lamina width (mm)	0.83–4.76	2.32 ± 0.82	1.10–2.93	2.12 ± 0.42	1.16	0.28
Cauline leaf length (mm)	5.30–18.60	10.22 ± 2.82	9.96–22.06	14.97 ± 3.23	29.55	0.0**
Cauline leaf width (mm)	1.46–8.70	3.97 ± 1.46	1.93–5.16	3.30 ± 0.90	3.79	0.05*
Cauline leaf pinnae number	0.00–1.00	0.05 ± 0.21	0.00–6.33	1.58 ± 1.78	16.59	0.0**
Inflorescence diameter (mm)	0.88–3.70	2.03 ± 0.69	1.80–4.65	2.76 ± 0.63	14.40	0.0**
Inflorescence branch number	0.00–12.00	6.44 ± 2.82	4.00–10.50	7.69 ± 1.83	3.44	0.06
Inflorescence length (mm)	7.46–160.00	93.15 ± 46.94	9.60–130.00	35.41 ± 39.88	21.67	0.0**
Inflorescence angle	7.50–90.00	57.02 ± 26.79	40.00–90.00	67.94 ± 14.87	3.20	0.08
Petal length: males (mm)	1.3–1.5	1.4 ± 0.10	1.5–2.2	1.85 ± 0.21	11.92	0.0**
Petal length: females (mm)	0.8–1.1	0.96 ± 0.15	1.2–1.5	1.36 ± 0.10	32.55	0.0**
Sepal hair number	2.00–33.00	14.56 ± 9.42	1.00–19.00	7.97 ± 5.39	9.16	0.0**
Sepal width (mm)	0.73–1.43	1.06 ± 0.19	0.70–1.53	1.09 ± 0.20	0.44	0.51
Nectary colour	0.00–1.66	0.62 ± 0.64	0.00–1.00	0.41 ± 0.49	1.64	0.2
Style length (mm); female only	0.10–0.43	0.26 ± 0.08	0.10–1.10	0.24 ± 0.14	0.37	0.54
Ovary length (mm); female only	0.90–2.40	1.70 ± 0.40	1.00–2.70	1.69 ± 0.33	0.001	0.99
Ovary width (mm); female only	1.10–1.80	1.44 ± 0.22	0.80–1.90	1.24 ± 0.24	11.76	0.0**
Ovary hair number; female only	0.0–61.00	24.84 ± 22.38	0.00–53.00	14.16 ± 14.85	5.93	0.01**
Filament number; male only	4.00–6.00	4.75 ± 0.84	4.00–6.00	5.12 ± 0.92	3.04	0.08
Filament length (mm); male only	1.50–2.80	2.07 ± 0.29	1.60–3.00	2.24 ± 0.32	5.5	0.02*

Table 4 Alignment and sequence differences for species included in the study. Shaded caps in *L. sisymbrioides* and *L. solandri* are either a substitution from a putative parent (*L. kirkii*, *L. naufragorum*, or *L. tenuicaule*) or a mixed sequence representative of both putative parent sequences. Unshaded caps are a substitution or a mixed sequence that is not representative of both putative parent sequences. W = a/t; Y = c/t; K = g/t; R = g/a; S = g/c; M = a/c.

	ITS 0011122223446 8912436788091 7037796137348	ETS 0011111234444445 4512389470244591 3347630754928632	*trnL-trnF* 1255677788 8336599968 2319505666
L. naufragorum	ctCC-CgttgcTG	acaSgcctgACggggg	AaAcgcAGtg
L. naufragorum a		acaggcctgACggggg	
L. naufragorum b		acaggcctgACggggg	
L. tenuicaule	ctCC-CgttgcTG	acaggcctgACggggg	AaAcgAAGCg
L. tenuicaule a		acaggcctgACggggg	
L. tenuicaule b		acaggcctgACggggg	
L. tenuicaule c		aSaggcctgACggggg	
L. kirkii	ctCC-CgttgcTG	acaggcctgcCAgAgg	caccgccatg
L. solandri			
Twizel s-TW	ctttc-gttgcaa	acaggccAgctggggg	AaAcgccatA
Sawdon s-SS	ctttc-gttgcaa	acaggccWgctggggg	AaAcgccatA
Castle Hill s-CH	ctttc-gttgcaa	acaggccWgctggggg	cCccgccatg
Pisa Flats s-PF	ctttc-gttgcaa	acaggcctgctggggg	caccgccatg
Pisa Flats s-PF-M	ctttc-gttgcaa		caccgccatg
Little Valley s-LV	ctttc-gttgcaa	acaggcctKcCAgAgS	caccgccatg
Wardles s-Wa	ctttc-gttgcaa	acaggcctgcYgggggg	caccTccatg
Wardles s-Wb	ctttc-gttgcaa	acaggcctgctgggggg	caccTccatg
Wardles s-Wc	mixed	mixed	caccgccatg
Wardles s-Wd	mixed	acaggcctgcYRRRgg	caccgccatg
Galloway s-GW	mixed	acaggcctgctgggggg	caccgccatg
Springvale s-Sa	mixed	acaggcctgcYRgRgg	caccgccatg
Springvale s-Sb	mixed	acaggcctgcYRgRgg	caccgccatg
Chatto Creek s-CC	mixed	acKSgYctgcCAgAgg	cacTgccatg
Michael Peak s-MP	ctCC-CgttgcTG	aSagRcYtgMCggggg	
Galloway s-GW-M	ctCC--gttgcTG		caccgccGtg
Eden Creek s-ES	ctCC--gttgcTG	acaggcctgcCAgAgg	caccgccatg
Patearoa s-P	ctCC--gttgcTG	acaggcctgcCAgAgg	caccgccatg
L. sisymbrioides			
Manuherikia k-MG	ctCC-CRttgcTG	acaggcTtgcCAgRgg	cacTgccatg
Falls Dam k-FDa	SKCC-CRKKRcTG	acaggcTtgACggggg	
Falls Dam k-FDb	ctCC-CAttgcTG	acaggcYtgACgggSg	AaAcgAAGCg
Otematata k-OT	ctCC--gttgcTG	acaggcctgcCAgAgg	caccgccatg
Gards Road k-GR	ctCC-CAttgcTG	acaggccAgctggggg	AaAcgccatA
Hakataramea k-HT	ctttc-gttRYaa	acaggccAgctggggg	
Slapjack k-SC	ctttc-gttgcaa	Tcaggcctgctggggg	caccgccatg
Slapjack k-SC-M	ctttc-gttgcaa		caccgccatg
Nevis Bluff k-NB	ctttc-gttgcaa	acaggcctgctggggg	caccgccatg
Chards Road k-CR	ctttc-gttgcaa	acaggcctgctggggg	caccgccatg

sequence type, is particularly variable, and includes samples only from the Manuherikia River valley or the Waitaki River valley (Fig. 10).

The Splitstree graph of the ITS sequence data shows two distinct groups (Fig. 11). One of these groups comprises *L. naufragorum*, *L. tenuicaule*, and *L. kirkii*, and samples of *L. sisymbrioides* and *L. solandri* from the type II group. The *L. sisymbrioides* samples came from Falls Dam, Manuherikia River gorge, Otematata, and Gards Road, and those of *L. solandri* came from Patearoa, Michael Peak Station, Galloway, and Eden Creek. The second group includes samples of *L. sisymbrioides* and *L. solandri* with ITS type I. The sample of *L. sisymbrioides* from

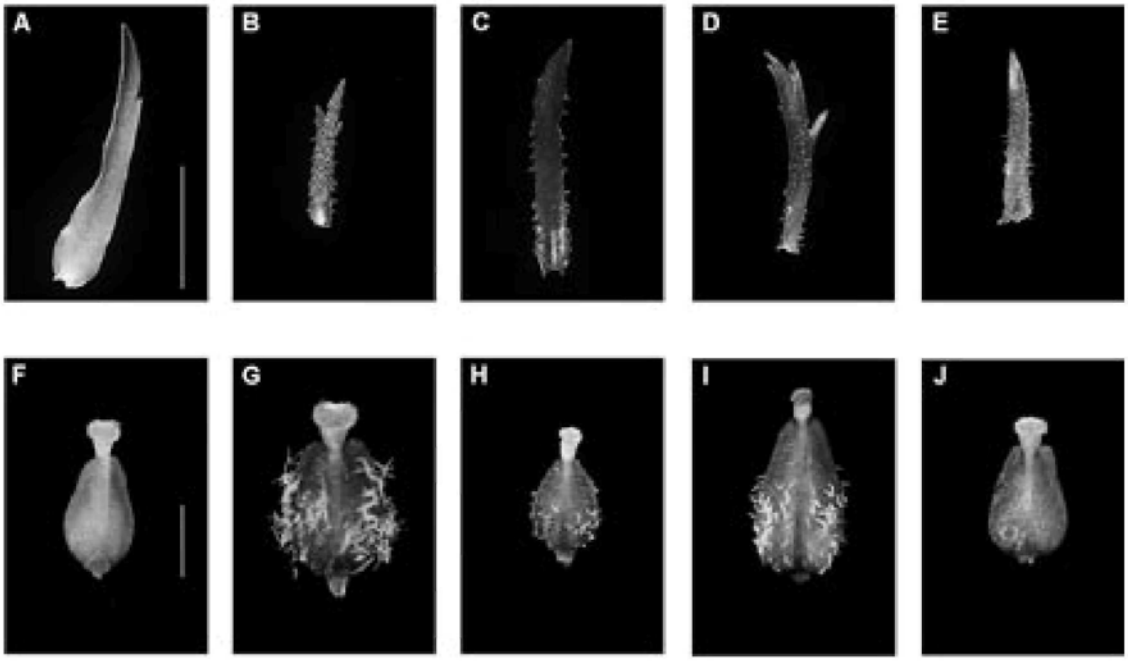

Fig. 4 *Lepidium sisymbrioides* cauline leaves (A–E) and ovaries (F–J). **A, F**, Slapjack Creek, Kawarau River; **B, G**, Chards Road, Kawarau River; **C, H**, Gards Road, Waitaki River valley; **D, E, I**, Falls Dam, upper Manuherikia River; **J**, Nevis Bluff, Kawarau River. Scale bars: A–E = 10 mm; F–J = 1 mm.

Slapjack Creek included from GenBank (k-SC-M) differs from the other *L. sisymbrioides* samples (including another from Slapjack Creek) by six substitution and three insertion/deletion events; this maybe due to sequencing or editing error.

ETS

The aligned ETS data set contained 28 samples and 565 characters; 8 of the characters were variable, and 6 were potentially parsimony-informative. One sample included mixed sequences and it was therefore excluded from the Splitstree analysis. *L. tenuicaule* and *L. naufragorum* share the same sequence type with the only variation being a single heteroplasmy in one sample of each species (Table 4). *L. kirkii* differs from *L. naufragorum* and *L. tenuicaule* at three nucleotide positions (Table 4). *L. sisymbrioides* and *L. solandri* are very variable and comprise three main sequence types (X, Y, and Z). Type X occurs in *L. sisymbrioides* and *L. solandri*, is relatively uniform, and is the most widespread, occurring near Alexandra and at localities outside of the Manuherikia River valley (Fig. 12). Some samples of type X have one base substitution, but this has no obvious affinity to either the *L. kirkii* or *L. naufragorum*/*L. tenuicaule* sequence types. Type Y represents the signature of *L. kirkii* and occurs in 10 of the *L. sisymbrioides* and *L. solandri* samples, with 7 of these from the Manuherikia River valley, 2 from the upper Taieri River valley, and 1 from the Waitaki River valley (Otematata) (Fig. 12; Table 4). In *L. sisymbrioides* and *L. solandri* the *L. kirkii* signature is particularly well shown by the direct substitution or mixed sequences of bases 430, 443, and 457 (Table 4). Type Z represents the signature of *L. tenuicaule*/*L. naufragorum* and this occurs in one sample of *L. solandri* from Michael Peak Station and two of *L. sisymbrioides* from Falls Dam, both sites in the upper Manuherikia River valley (Fig. 12). Nucleotide position 404 shows this signature in *L. sisymbrioides* and *L. kawarau* by either direct substitution or a mixed sequence. One sample from near Alexandra (Wardles 5) has a mixed sequence at position 430 and this could represent *L. kirkii*, *L. tenuicaule*, or *L. naufragorum*.

The Splitstree graph of the ETS sequence data shows *L. solandri* from Michael Peak Station and the two *L. sisymbrioides* samples from Falls Dam

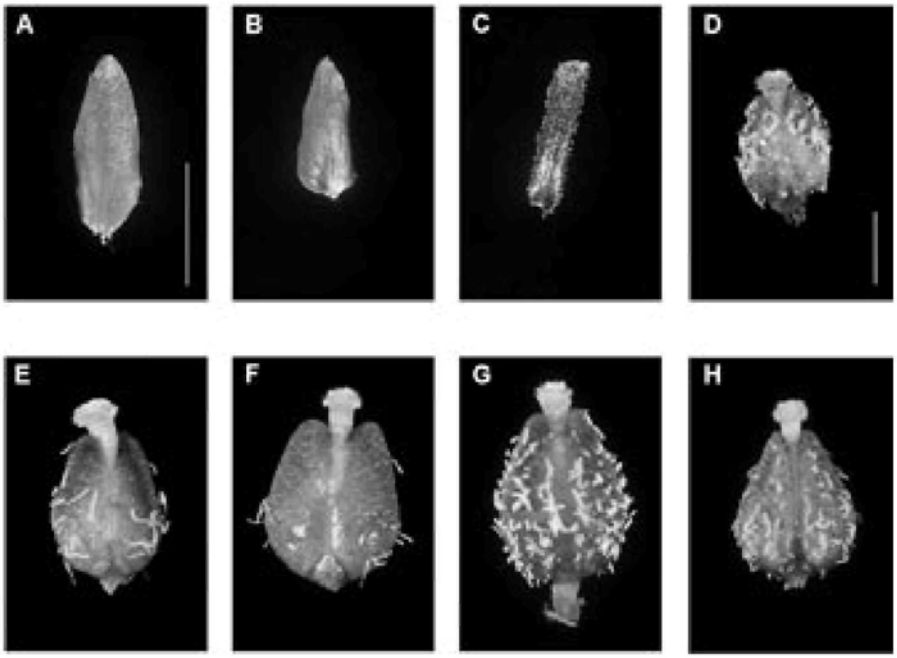

Fig. 5 *Lepidium solandri* cauline leaves (A–C) and ovaries(D–H). **A**, **E**, Little Valley, Alexandra; **B**, **F**, Springvale, Manuherikia River valley; **C**, **G**, Patearoa, upper Taieri River; **D**, Pisa Flats, Clutha River valley; **H**, Sawdon Stream, Tekapo. Scale bars: A–C = 10 mm; D–H = 1 mm.

associated with *L. tenuicaule*/*L. naufragorum* by a strong network (Fig. 13). The nine samples of *L. sisymbrioides* and *L. solandri* that show the *L. kirkii* signature (type Y) all associate with *L. kirkii* and have a well-developed network linking them to the more typical *L. sisymbrioides*/*L. solandri* sequence type.

Synthesis of DNA sequence data

DNA sequence data presented here for *L. sisymbrioides* and *L. solandri* is particularly variable in the Manuherikia River valley, with virtually all of the samples from that area showing mixed sequences, substitutions, or heteroplasmic sites (Table 4). A lesser number of specimens sampled from the Waitaki River valley (e.g., Gards Road and Otematata) show a similar pattern. In most of the samples of *L. sisymbrioides* and *L. solandri* with variable ITS and ETS sequences, the substitutions and heteroplasmic sites are considered to be the result of introgression from *L. kirkii* and *L. tenuicaule*. One specimen of *L. sisymbrioides* from the upper Manuherikia River valley (Falls Dam 2) shows this pattern for all three molecular markers, as it has the ITS and ETS sequence type of either *L. naufragorum* or *L. tenuicaule* and the *trn*L-*trn*F sequence type of *L. tenuicaule*. Two other samples of *L. sisymbrioides* and *L. solandri* from the upper Manuherikia River valley (Falls Dam and Michael Peak Station) also have the *L. tenuicaule* or *L. naufragorum* ETS signature (Fig. 12).

Neither *L. tenuicaule* nor *L. naufragorum* is known to occur in the vicinity of the upper Manuherikia River valley. *L. naufragorum* occurs in coastal sites in Westland (Garnock-Jones & Norton 1995), and is unlikely to have occurred inland in the upper Manuherikia River valley. *L. tenuicaule* occurs in coastal habitats in Wellington, Otago, and Southland, and is currently found on the Otago coast south of Oamaru (Webb et al. 1988). A number of other plants from the Otago coast (e.g., *Selliera radicans* and *Apium prostratum*) occur at inland sites in the upper Taieri River, and it is therefore possible that *L. tenuicaule* once occurred, and perhaps still does occur, in the area.

In regard to *L. kirkii*, nine samples of *L. sisymbrioides* and *L. solandri* from the Manuherikia River valley have ETS substitutions that are strongly

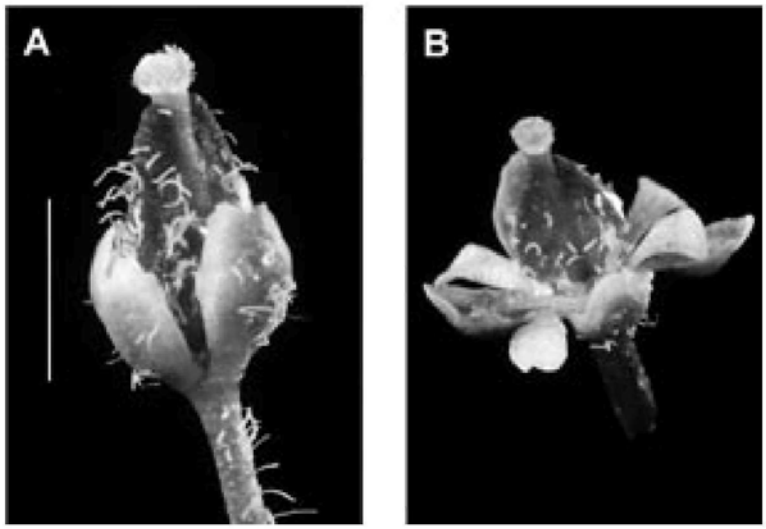

Fig. 6 *Lepidium sisymbrioides* female flowers. **A**, flower without petals (Chards Road, Kawarau River valley); **B**, flower with petals (Gards Road, Waitaki River valley). Scale bar = 2 mm, for both flowers.

indicative of introgression from *L. kirkii* (Table 4; Fig. 13). *L. kirkii* is a Central Otago endemic occurring on saline patches, and although now a threatened species it is likely to have once been more common on saline habitats that occurred throughout the Manuherikia River valley. Therefore, the widespread occurrence of the *L. kirkii* sequence type in samples of both *L. sisymbrioides* and *L. solandri* should not be surprising. The latter two species are dioecious and often occur in small and sparsely distributed populations, and female plants could therefore be likely to cross with other species of *Lepidium* that occur in close geographic proximity. Indeed, many of the extant populations of *L. sisymbrioides* occur in close proximity to saline patches. One sample of *L. sisymbrioides* from Otematata also showed evidence of introgression from *L. kirkii* in the ETS sequence data. The Otematata population of *L. sisymbrioides* is adjacent to a saline patch, and although *L. kirkii* is not currently known from the area, we infer from these data that it once grew there.

The majority of the other samples of *L. sisymbrioides* and *L. solandri* that occur at localities other than the Manuherikia River and Waitaki River valleys are relatively uniform and exhibit little, if any, nucleotide variation for the three DNA markers studied here. These more uniform ITS, ETS, and *trn*L-*trn*F sequence types are considered to represent the typical sequence type for *L. sisymbrioides* and *L. solandri*. For example, the ITS, ETS, and *trn*L-*trn*F sequences of *L. sisymbrioides* from Chards Road and *L. solandri* from Pisa Flats are considered typical of both these species.

Conclusions

In this study we used the vegetative and floral morphology of plants of known wild origin to identify taxonomic groups in the *L. sisymbrioides* complex; as a result of these studies we distinguished two species (*L. sisymbrioides* and *L. solandri*). These two species are closely related as is indicated by the DNA sequence data and their general morphological similarity and breeding system (e.g., both are dioecious). The phenetic analyses presented here highlight the difficulty of retrieving taxonomic groups from a species complex that comprises few, small, and disjunct populations that are naturally variable. These individual phenetic analyses do not unequivocally support the recognition of two species. Nevertheless, when all of the data presented are integrated and collectively considered, two species can be recognised. In particular, the character dimensions given in Table 3 are suitable for the identification of the two species from wild and cultivated plants; statistical support for many of these floral and vegetative characters is significant at 95% and 99% confidence limits. The range of leaf dimensions and variation in floral characters for cultivated plants is very similar to the range of variation found on naturally occurring wild-collected plants and were generally found to be reliable in distinguishing the two species. Care needs to be taken in identifying small plants of wild-collected specimens of *L. sisymbrioides* and *L. solandri* since the leaf characters have become contracted in size and are therefore difficult to discern. For both cultivated and wild-collected plants, their ultimate size and vigour is variable and determined by features

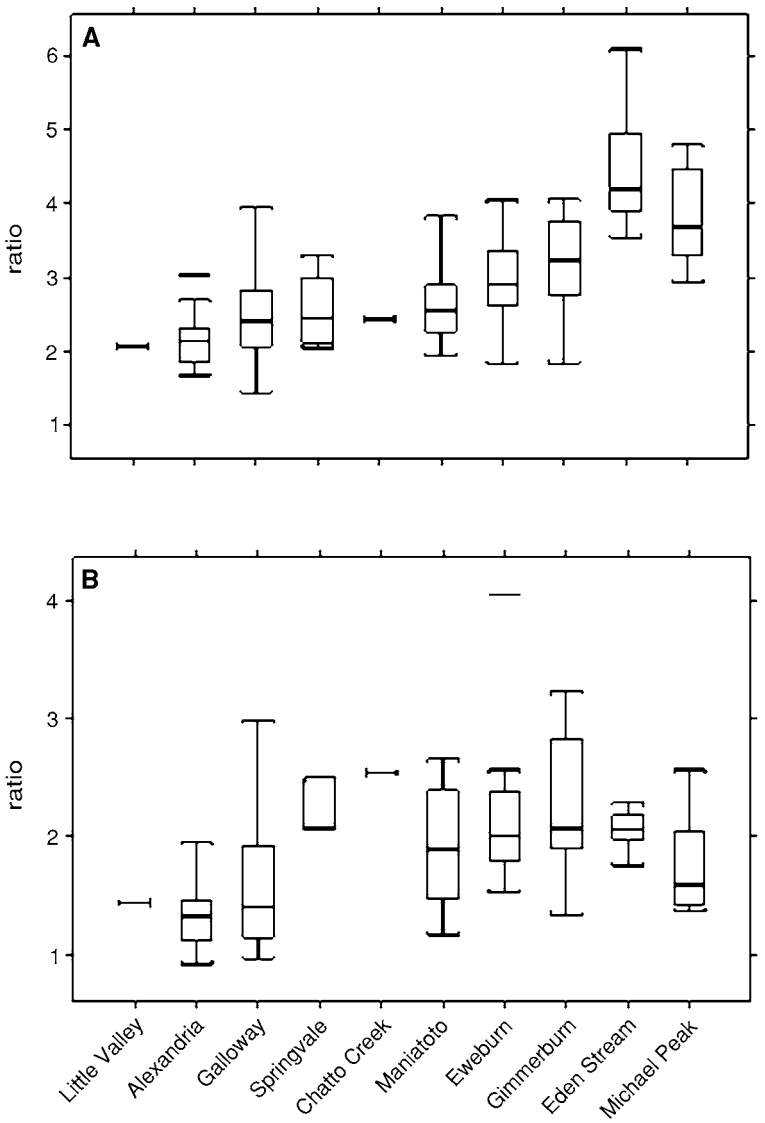

Fig. 7 *Lepidium solandri* variation in cauline bract (**A**) and leaf pinnae (**B**) length to width ratios for 10 populations in the Manuherikia and upper Taieri river valleys. Populations in geographical order from the lower Manuherikia River valley (e.g., Little Valley) to upper Manuherikia River valley (e.g., Michael Peak).

at the site at which the plant grows. Soil moisture and soil fertility are considered the most important determinants of plant size and vigour.

As indicated in the Methods (Morphological characters and analyses), in the experimental parts of this paper we tested the most recently accepted taxonomic classification (sensu Webb et al. 1988) by a priori assigning collections used in this study to one of the three named *L. sisymbrioides* subspecies or "*L. sisymbrioides* subsp. *kawarau* II" (see Fig. 1, 2). In addition to testing the subspecies classification used by Webb et al. (1988), the PCA and other analyses were critically examined for other groups. The identification of groups in, for example, the PCA analyses is a subjective process and it is possible that other discrete groups could be defined by arbitrarily drawing circles around any number of individuals (e.g., *L. solandri* outliers, see Results and Discussion). This is a similistic approach, and while attempting to be objective it does not consider other biological attributes such as distribution patterns and the occurrence of small and disjunct populations.

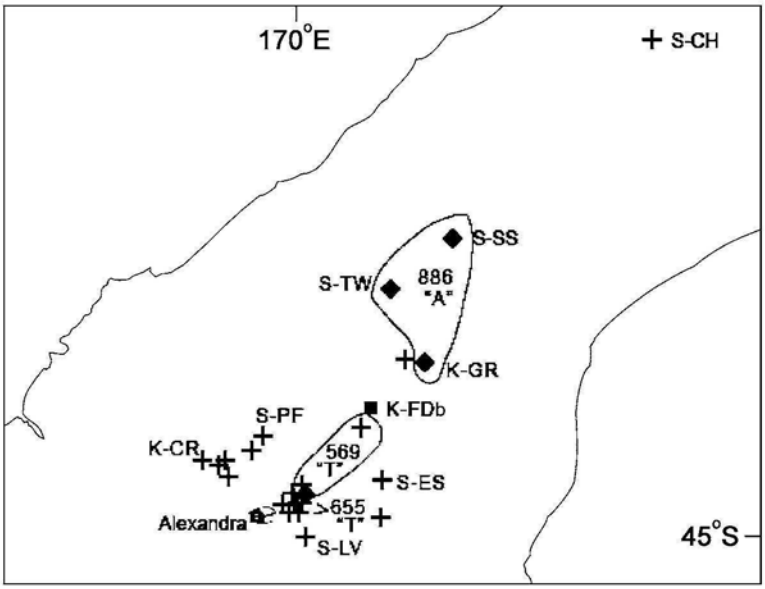

Fig. 8 Distribution map (central South Island) of *trn*L-*trn*F sequence types for *L. sisymbrioides* and *L. solandri*. +, common sequence type shared by *L. sisymbrioides* and *L. solandri*; ■, *L. tenuicaule* signature; ◆, *L. naufragorum* or *L. tenuicaule* signature. Base substitutions shown for some samples. See Table 1 for definitions of abbreviations.

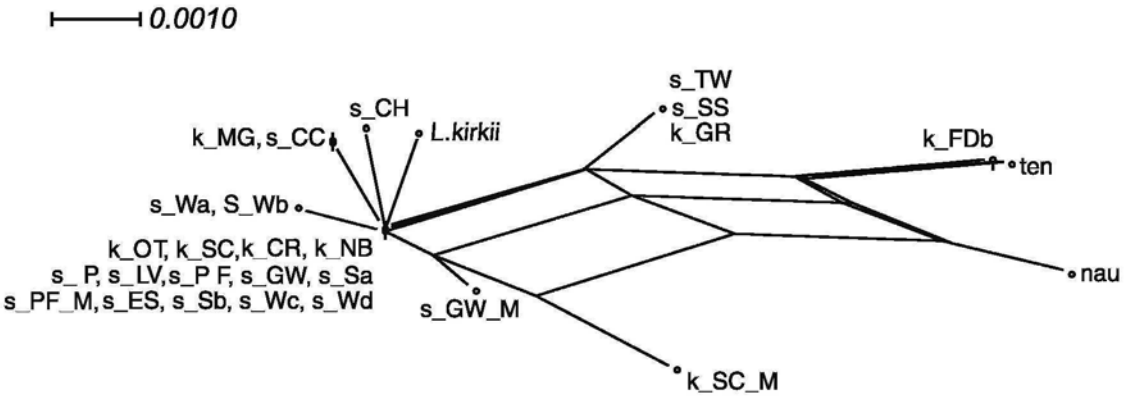

Fig. 9 Splitstree graph for *trn*L-*trn*F sequence data. See Table 1 for definitions of abbreviations.

Furthermore, it does not allow weighting of particular characters that may be of greater or lesser taxonomic significance.

The decision to recognise two species is a subjective decision that is based on amalgamation and consensus of a number of different attributes. These include patterns of variation of morphological characters (Fig. 1–6), the taxonomic usefulness of individual characters (Fig. 7; Table 3), geographic distribution patterns (Fig. 8, 10, 12, 14), and previous taxonomic classifications. The analyses of morphological variation presented in Table 3, for example, highlight those characters that are significant at 95% and 99% confidence levels whereas other characters may vary naturally and be of limited taxonomic value (e.g., Fig. 3, 7). Geographic distribution is considered important when populations of similar plants cluster together irrespective of whether the populations are in close geographic proximity or distant from each other (e.g., Fig. 14). Consideration of previous classifications is necessary as these also reflect patterns of character variation and subsequent taxonomic interpretation; previously recognised taxa may be shown to be indistinguishable from each

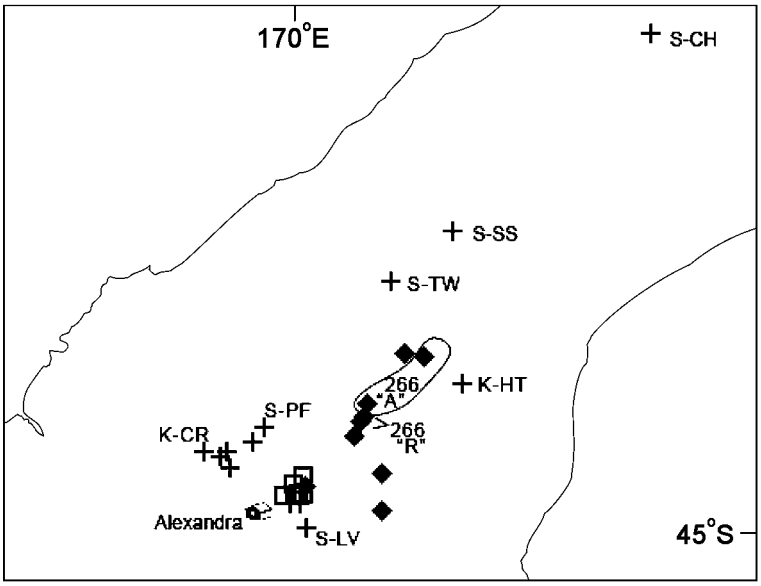

Fig. 10 Distribution map (central South Island) of ITS sequence types for *L. sisymbrioides* and *L. solandri*. +, common sequence type shared by *L. sisymbrioides* and *L. solandri*; ◆, *L. kirkii*, *L. naufragorum*, or *L. tenuicaule* signature; □, mixed sequences. Base substitutions shown for some samples. See Table 1 for definitions of abbreviations.

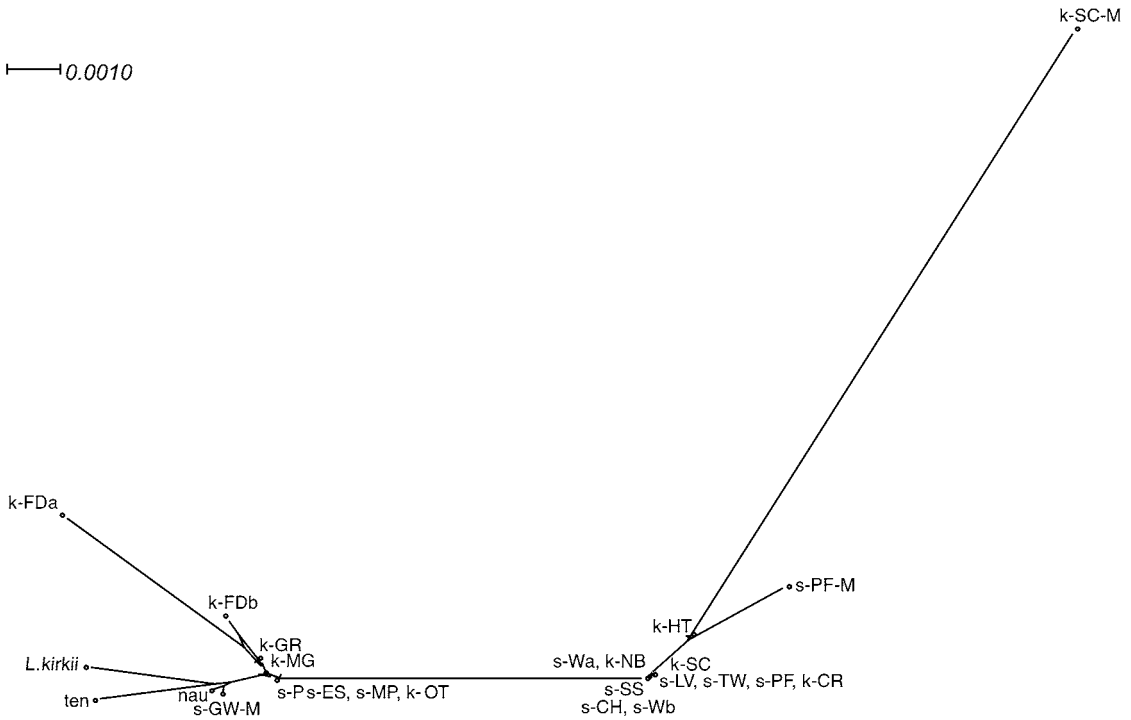

Fig. 11 Splitstree graph for ITS sequence data. See Table 1 for definitions of abbreviations.

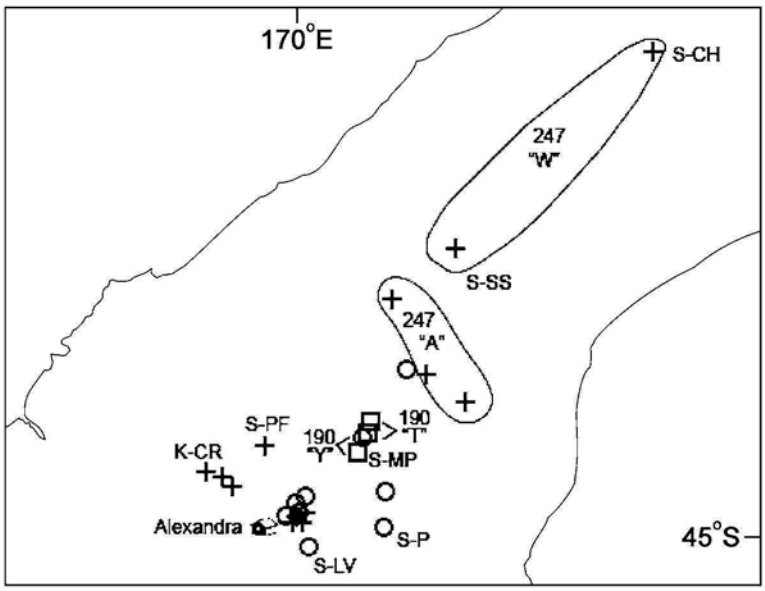

Fig. 12 Distribution map (central South Island) of ETS sequence types for *L. sisymbrioides* and *L. solandri*. +, common sequence type shared by *L. sisymbrioides* and *L. solandri*; ◆, *L. kirkii* or *L. tenuicaule* signature; ○, *L. kirkii* signature; □, *L. tenuicaule* signature. Base substitutions shown for some samples. See Table 1 for definitions of abbreviations.

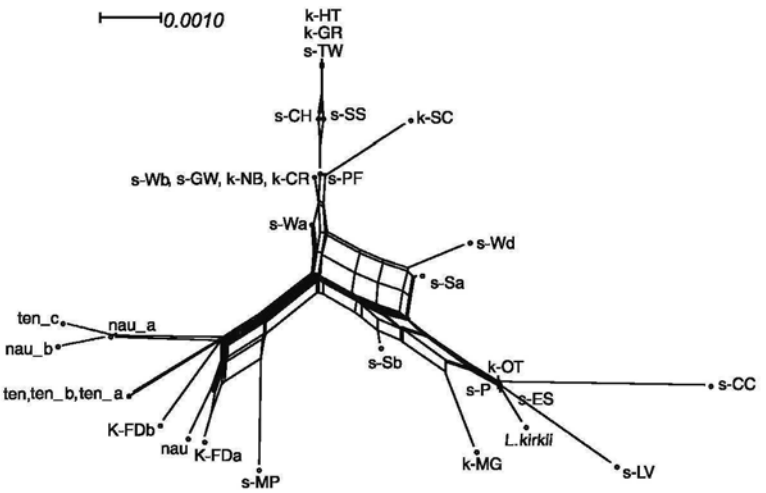

Fig. 13 Splitstree graph for ETS sequence data. See Table 1 for definitions of abbreviations.

other and/or more similar to each other than to other taxa (e.g., subsp. *matau* and subsp. *sisymbrioides* clustering together; Fig. 1, 2).

Species concept

As outlined in the above discussion the species concept adopted here emphasises discontinuities in quantitative vegetative and floral morphological characters; these morphological discontinuities also have a geographic distribution pattern in that they comprise discrete and disjunct populations. The intention of this study, therefore, is to recognise populations at species rank that constitute shared morphological characters. The rank of species rather than subspecies is appropriate since *L. sisymbrioides* and *L. solandri* comprise populations that are morphologically distinct in a number of vegetative and floral characters and they are sympatric (although at a finer scale the populations of the two species are disjunct and do not overlap; Fig. 14). Morphological and distributional differences are taken as evidence that refutes a hypothesis of conspecificity.

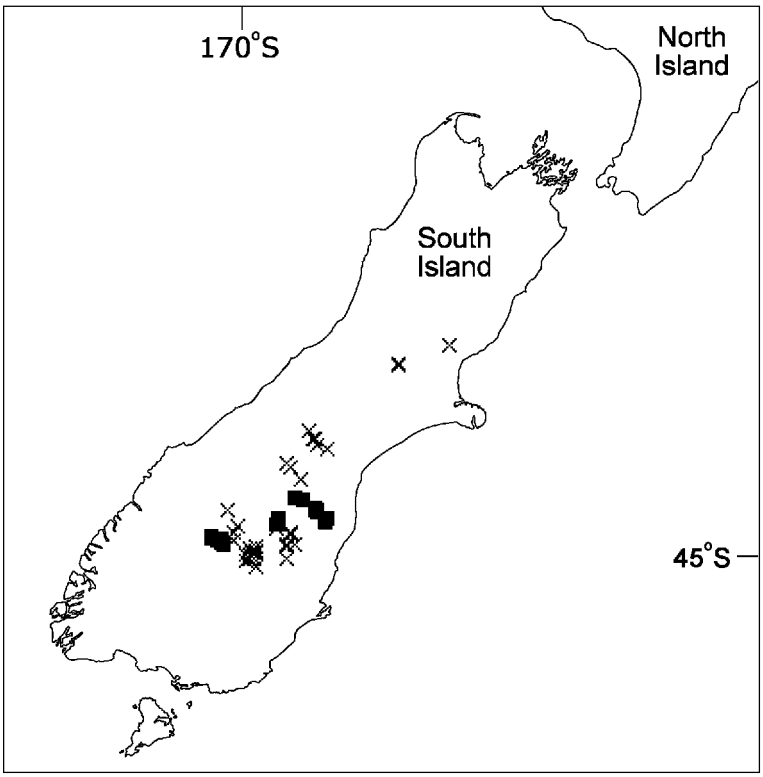

Fig. 14 Distribution. ■, *L. sisymbrioides*; ×, *L. solandri*.

Nomenclature

In the morphological studies presented here two species are recognised in the *L. sisymbrioides* complex. As a result of the typification undertaken of the names published in the *L. sisymbrioides* complex it is necessary to correct the application of these names. Therefore, *L. sisymbrioides* is applied to plants treated by Webb et al. (1988) as *L. sisymbrioides* subsp. *kawarau* (including *L. sisymbrioides* subsp. *kawarau* II of this study), and *L. solandri* is reinstated for plants previously known as *L. sisymbrioides* subsp. *sisymbrioides* and *L. sisymbrioides* subsp. *matau*. A formal taxonomic and nomenclatural treatment for these is provided below.

TAXONOMY

Lepidium solandri Kirk, *Trans. & Proc. New Zealand Inst. 14*, 380–381 (1882)

≡ *Lepidium sisymbrioides* subsp. *solandri* (Kirk) Thell., *Die Gatt.* Lepidium, 313 (1906).

TYPE COLLECTION: "South Island: limestone rocks, Broken River Basin, Canterbury—*J. D. Enys* and *T. Kirk*."

LECTOTYPE (here designated): Limestone rocks, Castle Hill basin, Canterbury, 2300 feet, *T. Kirk*, 18 Jan 1876, WELT 28575.

NOTES: There are several sheets in the Kirk Herbarium from the Castle Hill/Broken River area but not one of these gives the collector as J. D. Enys, and no sheet is labelled as being from the "Broken River Basin". Only two sheets are dated (18 January 1876) prior to the publication of *L. solandri*. One of these sheets (WELT 28575) has the specific locality of "Limestone rocks, Castle Hill Basin", and this is chosen as the lectotype since the label information and plant specimens best represent the protologue.

= *Lepidium matau* Petrie, *Trans. & Proc. New Zealand Inst. 19*, 323–324 (1887).

≡ *Lepidium sisymbrioides* subsp. *matau* (Petrie) Thell., *Die Gatt.* Lepidium, 312–313 (1906).

TYPE COLLECTION: "Alexandra South."

LECTOTYPE (here designated): Alexandra S., Central Otago, *D. Petrie*, WELT 28621.

NOTES: The lectotype is selected from the Petrie Herbarium and is consistent with the protologue, but it is undated. Another specimen in the Cockayne Herbarium is labelled "L. matau n. sp. Alexandra

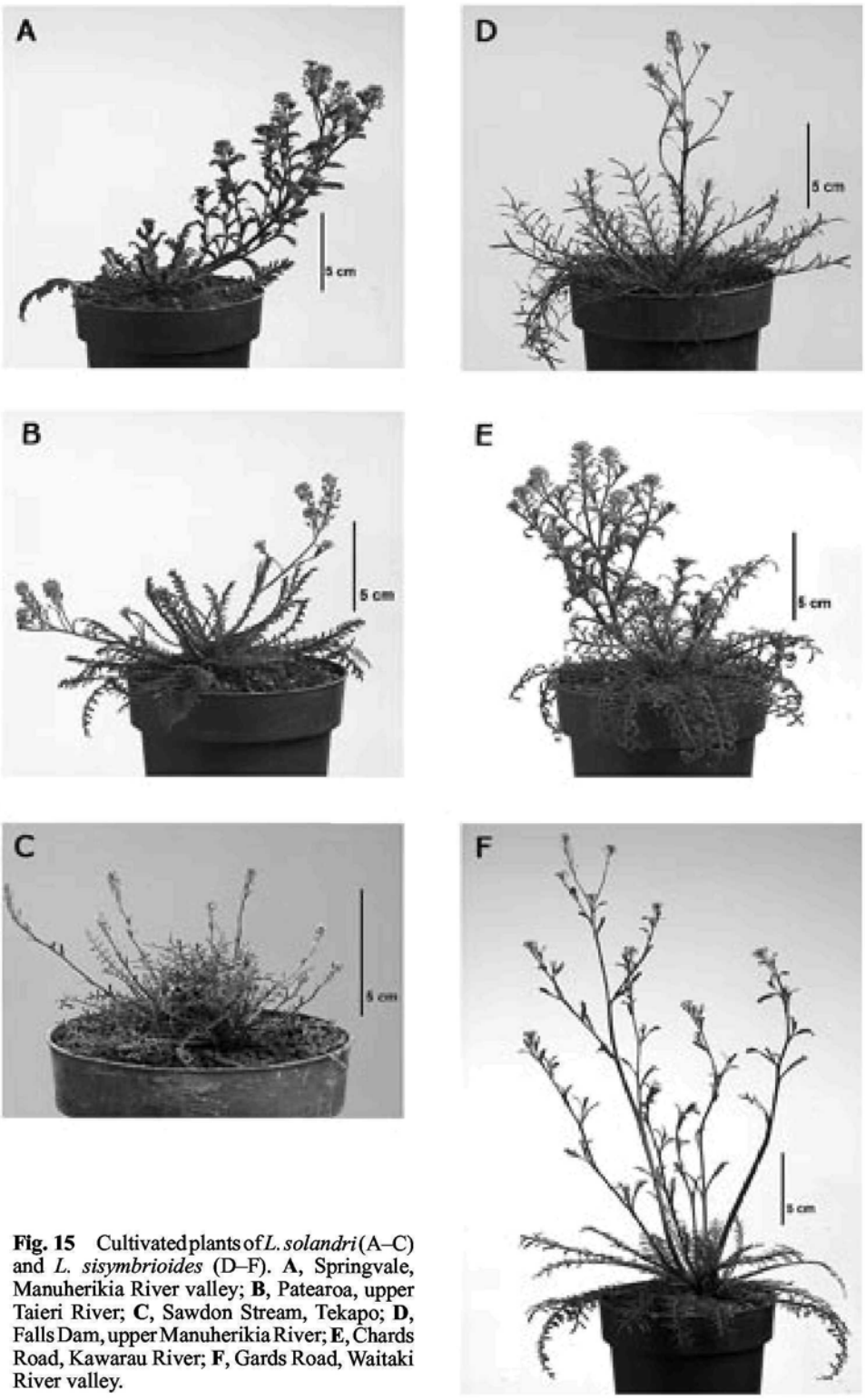

Fig. 15 Cultivated plants of *L. solandri* (A–C) and *L. sisymbrioides* (D–F). **A**, Springvale, Manuherikia River valley; **B**, Patearoa, upper Taieri River; **C**, Sawdon Stream, Tekapo; **D**, Falls Dam, upper Manuherikia River; **E**, Chards Road, Kawarau River; **F**, Gards Road, Waitaki River valley.

S. Coll. D. Petrie 4 Dec 1885". The 10 plants on this sheet are probably syntype collections and, notably, were collected prior to the publication of the name in 1887.

= *Lepidium sisymbrioides* subsp. *matau* var. *lobulatum* Thell., *Die Gatt.* Lepidium, 314 (1906).

TYPE COLLECTION: "Neuseeland: South Isl.: Otago, Maniototo Plain, 1500′, Petrie ("*L. Matau* var.") — Univ. Zürich."

HOLOTYPE: Maniototo Plain, 1500 ft, Herb. *D. Petrie*, Z.

DESCRIPTION (Fig. 15): Perennial dioecious herb, with up to 24 compact, leafy rosettes. Rootstock deep rooted, up to 28 mm diam. near crown; stems spreading to erect, up to 60 mm long, 10.0 mm wide. Basal and lower stem leaves persistent, pinnatifid, pinnate, to bipinnatifid, narrow-oblong to oblong, up to 100 mm long, green, green-brown, or brown, central part of lamina 0.7–6.2 mm wide; pinnae in 14–32 pairs, linear, obovate or broadly oblong, with up to 5 secondary pinnae, terminal pinnae 3.0–16.0 × 1.0–4.9 mm, lateral pinnae 2.6–11.3 × 0.8–3.9 mm. Middle stem leaves similar, often becoming shallowly pinnatifid, serrate, or entire. Cauline leaves 2.5–19.8 × 1.2–9.8 mm, with up to 3 serrations or small lobes, or entire. Inflorescences terminal, 1.5–16.0 cm long, 0.8–3.7 mm diam. at base, usually spreading to ascending, with up to 12 lateral branches, glabrous to sparsely hairy; pedicels 2.5–6.5 mm long, 0.2–0.35 mm wide, slightly recurved, adaxial surface glabrous to moderately hairy, abaxial surface glabrous to rarely sparsely hairy. Flowers up to 4 mm wide. Sepals 0.7–1.3 × 0.7–1.6 mm, green to maroon, sparsely to moderately hairy, rarely glabrous, margins scarious, apex obtuse. Petals usually absent, rarely present and then clawed, white, limb obovate, apex emarginate; males: 1.3–1.5 mm long; females: 0.8–1.1 mm long. Female flowers: ovary 1.0–2.4 × 1.1–1.8 mm, usually orbicular to rhomboid, sometimes ovate, sparsely to moderately hairy, rarely glabrous; style up to 0.1–0.4 mm long; stigma 0.3–0.4 mm wide; 3–7 staminodes, 0.8–1.4 mm long, rarely with malformed anthers to 0.3 mm long. Male flowers: 4–6 stamens, 1.5–2.8 mm long, white; anthers 0.3–0.6 mm long, white or maroon; ovary rudimentary, 0.2–1.1 × 0.3–1.3 mm. Nectaries 0.25–0.5 mm long, green, green-red, to red, oblong. Siliques 3.1–5.0 × 2.3–3.8 mm, usually orbicular to rhomboid, sometimes ovate, suture usually maroon, apex emarginate to retuse, style base often persistent. Seed usually obovate, rarely obovate-oblong, straighter along one margin, compressed but with broad rounded margins, 1.7–2.5 mm long, not winged; both surfaces with a distinct groove from hilum at base towards apex, and the seed folded around it; apex broad and rounded; base cuneate or slightly rounded. Testa dull, orange or orange-brown to dark henna, with a fine reticulum of very thick-walled cells. FL Sep–Dec; FR Sep–Mar.

REPRESENTATIVE SPECIMENS: CANTERBURY: Waipara Gorge, *W. B. B*[*rockie*], 18 Oct 1938, CHR 222224; Porter's River, *L. M. Cranwell & L. B. Moore*, 4 Jan 1931, AK 100097; Broken River, *D. Petrie*, Jan 1893, WELT 28605; Castle Hill Basin, *T. Kirk*, 18 Jan 1876, WELT 28576; Castle Hill, *P. J. de Lange 3484 & G. M. Crowcroft*, 23 Dec 1994, AK 235163; Lake Tekapo, *T. F. C*[*heeseman*], Jan 1883, AK 4494; Castle Hill, *M. J. A. Simpson*, 8 Feb 1956, CHR 92966; Lake Tekapo, *H. H. Allan*, Jan 1936, CHR 17538; Burkes Pass, *A. J. Healy 64/512*, 18 Dec 1965, CHR 152231; Black Forest flats, *H. H. Allan*, 20 Oct 1943, CHR 59487; Twizel, *P. B. Heenan*, 11 Nov 2004, CHR 572565; Twizel, *P. B. Heenan*, 22 Dec 2004, CHR 572828; Ohau River, *B. H. Macmillan, P. C. Douglas & D. L. Beetham*, 14 Dec 1976, CHR 384111; Edwards Stream, Tekapo, *P. B. Heenan*, 20 Jan 2004, CHR 569982. OTAGO: Pisa Flat, *G. Rogers*, 20 Mar 1999, CHR 541370; Wanaka, *J. Buchanan*, WELT 28579; Cromwell, *J. E. Holloway*, OTA 1528; Eweburn, *B. C. A*[*ston*], Jan 1898, WELT 28620; Maniototo Plain, *H. J. Matthews*, 1 Nov 1897, WELT 28617; Ranfurly, *D. Petrie*, 18 Dec 1910, CHR 5399; Alexandra, *D. Petrie*, 4 Dec 1885, CHR 329233; Alexandra, *D. Petrie*, Dec 1893, WELT 28612; Galloway, *A. J. Healy 73/170*, 13 Nov 1973, CHR 234750; Gimmerburn, *D. Petrie*, 20 Dec 1910, CHR 5397; Taieri Lake, Maniototo, *J. Barkla*, 6 Nov 2000, CHR 573232; Halls Bridge, Taieri River, Maniototo, *J. Barkla*, 6 Nov 2000, CHR 573231; Duffy's Lane, Taieri River, Maniototo, *J. Barkla*, 6 Nov 2000, CHR 573248; Michael Peak Station, Manuherikia River valley, *B. P. J. Molloy*, 15 Jan 2003, CHR 573584; Galloway, *J. E. Holloway*, OTA 1527; Galloway, *P. B. Heenan, M. Thorsen, T. Murdoch & A. Temple*, 11 Nov 2004, CHR 573561; Eden Creek, Maniototo, *J. Barkla*, 6 Nov 2000, CHR 573233; Eden Creek, Ranfurly, *P. B. Heenan, M. Thorsen & T. Murdoch*, 10 Nov 2004, CHR 573566; Chatto Creek, Manuherikia River valley, *P. B. Heenan, M. Thorsen, T. Murdoch & A. Temple*, 11 Nov 2004, CHR 573573; Patearoa, *T. Partridge & J. Child*, 19 Dec 1976, OTA 36566; Patearoa, *B. P. J. Molloy*, 15 Jan 2003, CHR 573582.

DISTRIBUTION AND HABITATS: *Lepidium solandri* occurs in dry and semi-arid inland parts of Canterbury and Otago (Fig. 14). Its northern limit is the Waipara Gorge (North Canterbury), scattered populations occur in the Mackenzie Basin (particularly in the vicinity of lakes Tekapo and Ohau), and in Central Otago it is found in the catchments of the upper Clutha, Manuherikia, and upper Taieri rivers.

Lepidium solandri occurs in a variety of mainly dry and stony habitats. These include limestone talus and debris (e.g., Castle Hill), greywacke (e.g., Mackenzie Basin) and schist (e.g., Pisa Flats) alluvial river terraces and floodplains, and schist ridges and hill slopes (e.g., Patearoa). At one site near Springvale (lower Manuherikia River valley), *L. solandri* occurs on severely eroded silts and clays. Allen (1998, 2000) provides further discussion of the habitats of *L. solandri* (as *L. sisymbrioides*).

Invalid name

Lepidium sisymbrioides subsp. *solandri* var. *typicum* Thell., *Die Gatt.* Lepidium, 312 (1906).

TYPE COLLECTION: "Neuseeland: South Isl.: Canterbury, Limestone debris, Irelissick Basin, Southern Alps, 2500′, 1893, L. Cockayne — Herb. Berlin, Petersbg.; ibidem, Broken River, Jatle Land, 2400′, 1893, Cockayne — Univ. Zürich."

NOTES: Infraspecific names with final epithets such as *typicum* purporting to indicate the taxon containing the type of the name of the next higher taxon are not validly published (Greuter et al. 2000; Article 24.3). Specimens have not been located in Zürich and Petersburg, and those at Berlin were probably destroyed in the fire at the Botanical Museum in 1943 (R. Vogt pers. comm., 2006).

Lepidium sisymbrioides Hook.f., *Handb. N. Zeal. fl.*, 14 (1864)

TYPE COLLECTION: "Middle Island: Dry Grass flats, Lake Ohau, alt. 2000 ft., *Haast*; Otago, grassy plains, Waitaki valley, *Hector & Buchanan*".

LECTOTYPE (here designated; Fig. 16): on dry grassy flats, 2000′, Alps, Lake Ohau, *Haast*, 1863, K.

≡ *Nasturtium sisymbrioides* (Hook.f.) Kuntze, *Revisio Generum Plantarum I*, 937 (1891).

NOTES: Both the Haast and Hector & Buchanan collections have flowers with petals, which is in agreement with Hooker's (1864) description. The Haast specimens are selected as lectotype since they are consistent with the protologue, also include immature fruit, and are in good condition.

= *Lepidium kawarau* Petrie, *Trans. & Proc. New Zealand Inst. 17*, 270 (1885).

TYPE COLLECTION: "Kawarau River, near Victoria Bridge, Cromwell; "Earthquakes," near Duntroon."

LECTOTYPE: Victoria Bridge, Kawarau, Otago, *D. Petrie*, WELT 28587.

≡ *Lepidium sisymbrioides* subsp. *kawarau* (Petrie) Thell., *Die Gatt.* Lepidium, 314 (1906).

= *Lepidium kawarau* var. *dubium* Kirk, *Stud. fl. New Zealand*, 36 (1899).

≡ *Lepidium sisymbrioides* subsp. *kawarau* var. *dubium* (Kirk) Thell., *Die Gatt.* Lepidium, 314 (1906).

TYPE COLLECTION: "Otago: Earthquakes near Duntroon, *Petrie*!"

LECTOTYPE: The Earthquakes, near Duntroon, Waitaki R., *D. Petrie*, WELT 28589.

= *Lepidium sisymbrioides* subsp. *solandri* var. *ovatum* Thell., *Die Gatt.* Lepidium, 313 (1906).

TYPE COLLECTION: "Neuseeland: South Island: East Coast of Otago & Kurm Otago, Petrie — Univ. Zürich."

LECTOTYPE: East Coast of Otago, Herbarium *D. Petrie*, Z.

DESCRIPTION (Fig. 15): Perennial dioecious herb, with up to 15 compact, leafy rosettes. Rootstock deep rooted, up to 20 mm diam. near crown; stems spreading to erect, up to 25 mm long and 6.0 mm wide. Basal and lower stem leaves persistent, pinnatifid, pinnate, to bipinnatifid, narrow-oblong to oblong, up to 120(–190) mm long, green or green-brown, central part of lamina 1.0–3.4 mm wide; pinnae in 6–25 pairs, linear to lanceolate, usually recurved, with 1–6 secondary pinnae, terminal pinnae 7.8–30.0 × 0.9–2.9 mm, lateral pinnae 8.0–28.9 × 0.8–2.7 mm. Middle stem leaves similar, often becoming shallowly pinnatifid, serrate, or entire. Cauline leaves 8.3–25.6 × 1.6–6.2 mm, with up to 8 narrow or small lobes, or entire. Inflorescences terminal, 2–40 cm long, 1.0–5.6 mm diam. at base, usually ascending or erect, sometimes spreading, with up to 12 lateral branches, glabrous to sparsely hairy; pedicels 2.7–6.4 mm long, 0.25–0.35 mm wide, slightly recurved, adaxial surface glabrous to moderately hairy, abaxial surface glabrous. Flowers up to 4 mm wide. Sepals 0.7–1.0 × 0.6–1.6 mm, green to maroon, glabrous to sparsely hairy, sometimes moderately hairy, margins scarious, apex obtuse. Petals present or absent, when present spreading and clawed, white, limb obovate, apex obtuse to emarginate; males: 1.5–2.2 mm long;

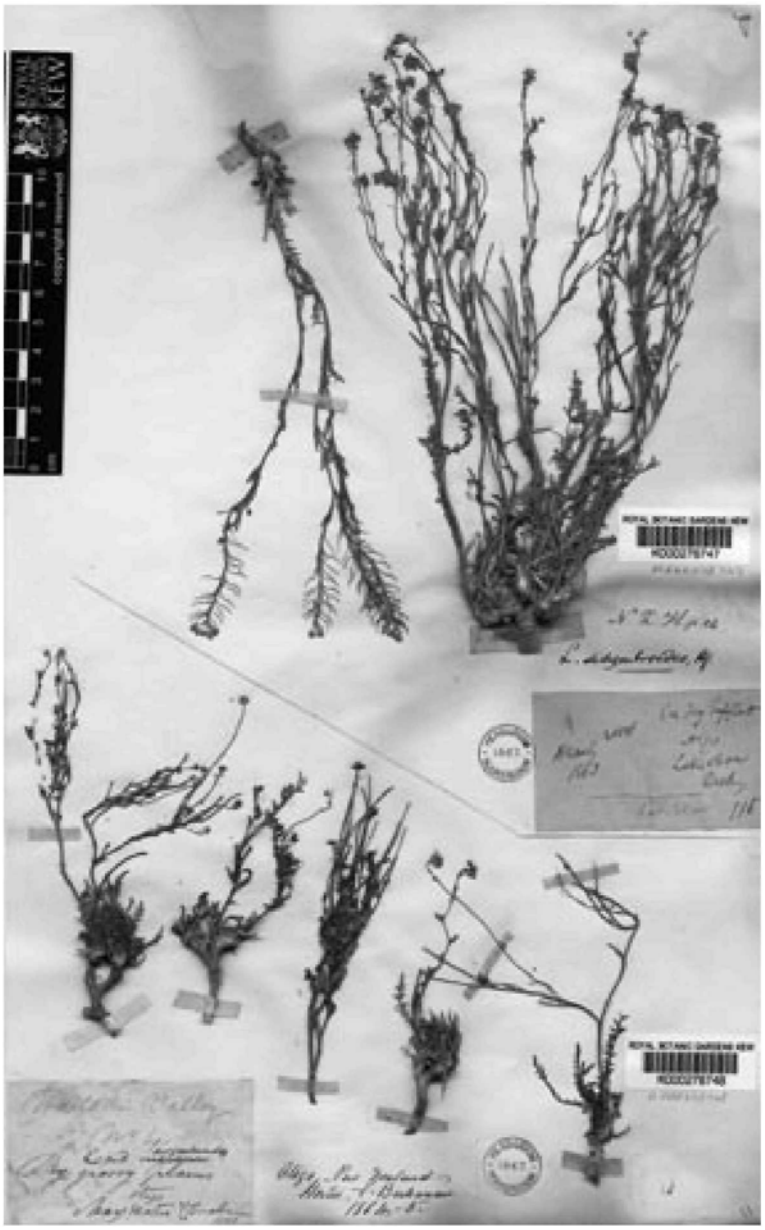

Fig. 16 *Lepidium sisymbrioides* lectotype. The top two specimens collected by Haast are designated as the lectotype.

females 1.2–1.5 mm long. Female flowers: ovary 1.0–2.7 × 0.8–1.9 mm, ovate, orbicular, to rhomboid, glabrous to sparsely hairy, sometimes moderately hairy; style 0.1–1.1 mm long; stigma 0.2–0.4 mm wide; 4–7 staminodes, 0.6–1.4 mm long. Male flowers: 4–6 stamens, 1.6–3.0 mm long, white; anthers 0.3–0.4 mm long, white or maroon; ovary rudimentary, 0.2–0.9 × 0.3–0.9 mm. Nectaries 0.2–0.6 mm long, oblong, green to green-red. Siliques 3.5–5.0 × 1.9–4.6 mm, usually ovate to rhomboid, sometimes orbicular, suture usually maroon, apex emarginate to retuse, style base often persistent. Seed usually obovate, rarely obovate-oblong, straighter along one margin, compressed but with broad rounded margins, 1.5–2.5 mm long, not winged; both surfaces with a distinct groove from hilum at base towards apex, and the seed folded around it; apex broad and rounded; base cuneate or slightly rounded.

Testa dull, orange or orange-brown to dark henna, with a fine reticulum of very thick walled cells. FL Nov–Jan; FR Dec–Jan.

REPRESENTATIVE SPECIMENS: OTAGO: Waitaki R., *L. Cockayne*, 25 Nov 1919, AK 100098; Kurow, Waitaki River, *A. Wall*, Jan 1931, CHR 329232; Kurow village, *Herb L. Cockayne*, WELT 28570; near Kurow, *B. Patrick*, 18 Nov 1988, CHR 461172; Kurow, *W. G. Rutherford*, Mar 1897, WELT 28622; Kurow, *Herb. L. Cockayne*, WELT 28570; Kurow, *J. E. Holloway*, OTA 1529; Duntroon, Otago, *D. Petrie*, AK 4487; Gards Road, Kurow, *P. J. de Lange*, 10 Apr 1995, AK 222322; Gards Road, Waitaki River valley, *P. B. Heenan & M. Thorsen*, 10 Nov 2004, CHR 573577; Otematata, *P. A. Williams*, Nov 1976, CHR 284621; Otematata, Waitaki River valley, *P. B. Heenan & M. Thorsen*, 10 Nov 2004, CHR 573567; Hakataramea, Waitaki River valley, *P. B. Heenan & M. Thorsen*, 10 Nov 2004, CHR 573568; Earthquakes, Waitaki R., *D. Petrie*, WELT 28590; Falls Dam, upper Manuherikia River, *G. Rogers*, 18 Nov 2004, CHR 573580; Falls Dam, *J. Barkla*, 7 Nov 2001, CHR 573270; Falls Dam, upper Manuherikia River, *P. B. Heenan, M. Thorsen & T. Murdoch*, 10 Nov 2004, CHR 573574; Kawarau River, *D. Petrie*, Nov 1890, AK 4486; Nevis Bluff, *Petrie*, 24 Nov, WELT 28596; Nevis Bluff, Kawarau River valley, *P. B. Heenan & A. Temple*, 11 Nov 2004, CHR 573571; Gibbston, Kawarau R., *D. Petrie*, WELT 28591; near Gibbston, Kawarau River, *D. Petrie*, WELT 28593; near Gibbston, Kawarau River, *D. Petrie*, WELT 28592; Victoria Bridge, Kawarau River, *D. Petrie*, WELT 28588; Chard Rd, Kawarau gorge, *P. N. Johnson*, 6 Dec 1983, CHR 415715; Chard Road, Kawarau River valley, *P. B. Heenan & A. Temple*, 11 Nov 2004, CHR 573569; Slapjack Creek, Kawarau valley, *A. Robertson*, 1 Dec 1983, CHR 415716.

DISTRIBUTION AND HABITATS: *Lepidium sisymbrioides* occurs in three distinct geographic areas in semi-arid or low rainfall parts of Central Otago, North Otago, and South Canterbury (Fig. 14). These three areas are the Kawarau River, upper Manuherikia River, and Waitaki River valley.

Lepidium sisymbrioides occurs in a range of mainly dry and rock outcrop habitats. These include outcrops of limestone (e.g., Gards Road, Waitaki River valley), greywacke (Falls Dam, upper Manuherikia River), and schist (Kawarau River). In the Waitaki River valley *L. sisymbrioides* also occurs on alluvial river terraces and floodplains. Allen (1998, 2000) provides further discussion of the habitats of *L. sisymbrioides*.

CONSERVATION

With the recognition of *L. sisymbrioides* and *L. solandri* at species rank, and their altered circumscriptions, their conservation status needs revision. Although it is now difficult to be sure of the exact distribution and abundance of *L. sisymbrioides* and *L. solandri* prior to human-induced disturbance to their habitat, it is likely that *L. sisymbrioides* and *L. solandri* were once more common and widespread, and the implication of this is that these two species are now greatly reduced in distribution, number of populations, and abundance of plants. Currently, *L. sisymbrioides* and *L. solandri* are known from sparse and fragmented populations, with *L. solandri* occupying a wide geographic area and *L. sisymbrioides* being more restricted (Fig. 14). In both species, populations now normally comprise small (< 30 individuals) to moderate (c. 100 individuals) numbers of plants, and these usually occupy only a few square metres of habitat. There are very few large (> 100 individuals) populations known for either species, and of these none is considered secure. All the populations that we have seen are at serious risk due to degradation of habitat from agricultural practices, invasion by weeds (e.g., *Echium vulgare*, *Hieracium* spp., and *Thymus vulgaris*), browsing animals (especially rabbits, hares, and sheep), and stochastic events.

L. sisymbrioides

Lepidium sisymbrioides occurs in three main geographic areas: the Kawarau River valley, upper Manuherikia River valley, and Waitaki River valley (Fig. 14). In the Kawarau, there are five known populations. The first of these, Nevis Bluff, was formerly the largest population known and was long regarded as a "safe" stronghold for the species. It now has five known plants, the rest having been destroyed following a major collapse of the unstable schist rock forming the bluff. At the other Kawarau River sites there are 38 plants known from Chards Road, 10 from Slapjack Creek, 4 from Mt Rosa, and 1 from the Swift Burn. At Chards Road there is some field evidence to suggest that plants are being displaced by wild thyme (*Thymus vulgaris*). This is an issue that urgently needs further research. In the upper Manuherikia River, *L. sisymbrioides* is known from only about 110 plants at 6 sites in the

immediate vicinity of Falls Dam. *L. sisymbrioides* is most common in the Waitaki River valley, and the populations from there sampled in this study have about 20 (Hakataramea), 200 (Gards Road), and probably over 300 (Otematata) plants.

It is highly likely that *L. sisymbrioides* will be found at other sites in the Kawarau, Waitaki, and upper Manuherikia river valleys, and it is important that further survey for the species is undertaken. Herbarium specimens from the Waitaki River valley indicate it was once more widespread and common there, occurring on outwash gravels and alluvium.

Based on these data *L. sisymbrioides* qualifies under the New Zealand Threat Classification System (Molloy et al. 2002) as "Acutely Threatened/Nationally Endangered" (Assessment Nationally Endangered A) because there are c. 700 individuals known. While these are scattered over several populations, only one of these has c. 300 mature plants. Overall the trend for this species has been toward extinction, and we estimate that over 30% of the species' populations have been lost over the last 100 years and that the potential for loss exceeding 30% in the next 10 years is extremely high. None of the Waitaki Valley populations occurs on protected land and they are all vulnerable to changes in land-use management. For example, the limestone bluff population at Gards Road remains vulnerable to possible future mining for limestone for agricultural purposes. We assign *L. sisymbrioides* the qualifier "DP" (Data Poor) because field observations suggest recruitment failure at most sites, but at this stage we have insufficient data to confirm any particular trend.

Beyond the species' threat assessment it is clear that all three geographic areas (Kawarau, Waitaki, and Manuherikia) in which *L. sisymbrioides* occurs require urgent protection. This study has shown that plants from the three areas have distinctive molecular and morphological characters that need to be preserved to maintain intact what remains of the full diversity of this species. For example, the Kawarau River populations are characterised by green leaves (brown or green-brown in other populations) and their ITS, ETS, and *trn*L-*trn*F data show no evidence of introgression or gene flow with *L. kirkii*, *L. naufragorum*, or *L. tenuicaule*. The few plants in the Kawarau River populations are, therefore, morphologically distinct and genetically important for conservation. The Falls Dam populations comprise few plants and although their DNA sequences indicate introgression from *L. tenuicaule* they are geographically distinct and, therefore, important populations. The Waitaki River valley populations are the largest known and are morphologically distinct in the flowers consistently having petals. Thus, they too are very significant for conservation.

Although the conservation assessment criteria can be applied only to plants that have a formal taxonomic rank (see Molloy et al. 2002), it is clear that the Kawarau River populations with only 58 plants qualify as "Acutely Threatened/National Critical"; the same conservation assessment applies to the Falls Dam populations. Therefore, it is imperative that urgent steps are taken to prevent the further loss of these populations.

L. solandri

Lepidium solandri, although more widespread than *L. sisymbrioides*, is also under severe threat. As circumscribed here this species occurs in three main geographic areas: the Manuherikia River/upper Taieri River, Clutha River (Pisa Flat), and Mackenzie Basin/central Canterbury. Seven populations from the Manuherikia River valley and upper Taieri River show DNA evidence of extensive introgression with *L. kirkii* and *L. tenuicaule*/*L. naufragorum*. Morphological variation in the Manuherikia and upper Taieri river valleys is considerable and some of these populations comprise the most robust plants of *L. solandri*; the cauline and rosette leaves are particularly broad and stout. Populations sizes range from as few as 3–4 plants (e.g., Chatto Creek) to about 100 plants (e.g., Springvale).

Only one population of about 200 plants is known from the upper Clutha River (Pisa Flat); this is a geographically isolated population of *L. solandri* and also shows no evidence of introgression with *L. kirkii* or *L. tenuicaule*. Part of the Pisa Flat population occurs in the Mahaka Katia Scientific Reserve. The populations of *L. solandri* from the Mackenzie Basin and Castle Hill have unique ETS and *trn*L-*trn*F sequences, and they also represent the central and northern geographic range of *L. solandri*.

Threats to *L. solandri* are myriad. As a species of lowland to montane distribution, predominantly occurring in short-tussock grassland communities, generally overlying fertile substrates, populations have been lost to conversion of these habitats for agricultural purposes (e.g., sheep and mixed animal grazing). Where this has not happened, the species remains at risk through the spread of weeds, particularly the hawkweeds (*Hieracium* spp.), which can smother and kill plants and prevent recruitment. Many of the Otago populations are uniquely at risk from the conversion of land from sheep farming to viticulture, as further land is found suitable for the

production of high-yielding grapes. *L. solandri* is also extremely vulnerable to browsing from rabbits, which not only browse the foliage but will on occasion dig out entire plants. In many sites only scattered individuals are known, and in a dioecious species this presents unique reproductive problems. At some sites only single plants have been recorded, while at others we suspect that all of the plants present may have originated from a single past seeding event. The largest population known at Castle Hill is also probably the most secure because it is partially within a Nature Reserve, and the portion of the population outside of the reserve is likely to remain intact because of local land-use practices. However, here the spread of *Hieracium pilosella* and *H. lepidulum* is of major concern and anecdotal reports suggest that *L. solandri* has been declining from key areas over the last 15–20 years (D. A. Norton pers. comm.).

We have no exact figures on the number of mature plants left in the wild, but observations made for this study in Otago and Canterbury suggest it is likely to be less than 1000 individuals scattered over about 12–15 populations from Castle Hill in the north to the Manuherikia River Valley and Pisa Flats in the south. Herbarium specimens indicate a considerable range contraction that equates to an effective loss of this species from over 60% of its former range in the last 100 years. As noted, threats are myriad and continue at most sites unabated. We suggest that *L. solandri* might be better assessed as "Acutely Threatened/Nationally Endangered" (Molloy et al. 2002). This assessment needs qualification with the qualifier "DP" (Data Poor) because an exact assessment of the numbers of mature wild plants is unknown.

Conclusions

The molecular, morphological, and distributional information presented here highlights the urgent need for conservation management of *L. sisymbrioides* and *L. solandri*. All of the populations sampled for this study have a unique combination of attributes, including their geographic location, number of plants, habitat disturbance, DNA sequence variation, and morphological variation. Therefore, as noted by Allen (2000), all populations of *L. sisymbrioides* and *L. solandri* are highly significant for conservation purposes.

Allen (2000) provided a recovery plan for the conservation management of the three *L. sisymbrioides* subspecies, treated here as *L. sisymbrioides* and *L. solandri*. He concluded that management in the wild is the only feasible option for their long-term survival, and that this should be supplemented with some management in cultivation. The recovery plan proposed by Allen (2000) is detailed and should be consulted for further information, but he emphasised four recovery strategy objectives. These are increased public awareness; precise information on distribution, abundance, and threats; research; and in situ conservation management. To ensure the long-term survival of *L. sisymbrioides* and *L. solandri* it is essential that the recovery plan proposed by Allen (2000) continues to be actively implemented.

A successful translocation of *L. solandri* has been undertaken by the Department of Conservation in Central Otago (J. Barkla pers. comm.). Plants grown from seed sourced from Patearoa, upper Taieri River, were planted at Aldinga Creek, Alexandra, on 12 Nov 2003. In Jan 2006, at least 66 plants were still alive. Other translocations of *L. sisymbrioides* and *L. solandri* should be undertaken to establish new populations in secure and unmodified habitats.

ACKNOWLEDGMENTS

Funds for this research were provided to PBH by the Department of Conservation (contract 3736) and the Foundation for Research, Science and Technology. We thank John Barkla, Joy Comrie, Phil Knightbridge, Brian Molloy, Trudy Murdoch, Brian Rance, Geoff Rogers, Alan Temple, Mike Thorsen, and Kate Wardle for providing information on the distribution of *L. sisymbrioides* and *L. solandri* and/or collecting plants for the project; Juliet Wege, Australian Botanical Liason Officer at Kew, for examining the type specimens of *L. sisymbrioides*; Anouk Wanrooy for assistance in preparing the figures; David Purcell for cultivating the plants at the Landcare Research experimental nursery; and John Barkla, David Glenny, David Norton, Phil Garnock-Jones, and Christine Bezar for their comments on the draft manuscript.

REFERENCES

Allan HH 1961. Flora of New Zealand. Vol. I. Wellington, Government Printer.

Allen RB 1998. Germination, growth, reproduction, and population structure of three subspecies of *Lepidium sisymbrioides* (Brassicaceae) with respect to taxon rarity. New Zealand Journal of Botany 36: 439–452.

Allen RB 2000. Inland *Lepidium* recovery plan 2000–2010. Threatened Species Recovery Plan 32. Wellington, Department of Conservation.

Al-Shehbaz IA, Mummenhoff K, Appel O 2002. *Cardaria*, *Coronopus*, and *Stroganowia* are united with *Lepidium* (Brassicaceae). Novon 12: 5–11.

Cheeseman TF 1906. Manual of the New Zealand flora. Wellington, Government Printer.

Cheeseman TF 1925. Manual of the New Zealand flora. 2nd ed. Wellington, Government Printer.

Cockayne L, Allan HH 1934. An annotated list of groups of wild hybrids in the New Zealand flora. Annals of Botany 48: 1–55.

de Lange PJ, Norton D, Heenan PB, Courtney SP, Molloy BPJ, Ogle CC, Rance BD, Johnson PN, Hitchmough R 2004. Threatened and uncommon plants of New Zealand. New Zealand Journal of Botany 42: 45–76.

Doyle JJ, Doyle JL 1987. A rapid isolation procedure from small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11–15.

Garnock-Jones PJ, Norton DA 1995. *Lepidium naufragorum* (Brassicaceae), a new species from Westland, and notes on other coastal species of *Lepidium*. New Zealand Journal of Botany 33: 43–51.

Given DR 1976. A register of rare and endangered indigenous plants in New Zealand. New Zealand Journal of Botany 14: 135–149.

Greuter W, McNeill J, Barrie FR, Burdet H-M, Demoulin V, Filgueiras TS, Nicolson DH, Silva PC, Skog JE, Trehane P, Turland NJ, Hawksworth DL 2000. International Code of Botanical Nomenclature (St Louis code). Regnum Vegetabile 138.

Hall TA 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Hooker JD 1864. Handbook of the New Zealand flora, Part 1. London, Reeve.

Huson DH, Bryant D 2006. Application of phylogenetic networks in evolutionary studies. Molecular Biology and Evolution 23: 254–267.

Kirk T 1882. A revision of the New Zealand Lepidia, with descriptions of new species. Transactions and Proceedings of the New Zealand Institute 14: 379–382.

Kirk T 1899. The students' flora of New Zealand and outlying islands. Wellington, Government Printer.

Mitchell AD, Heenan PB 2000. Systematic relationships of New Zealand endemic Brassicaceae inferred from rDNA sequence data. Systematic Botany 25: 98–105.

Molloy J, Bell B, Clout M, de Lange P, Gibbs G, Given D, Norton D, Smith N, Stephens T 2002. Classifying species according to threat of extinction – a system for New Zealand. Wellington, Department of Conservation.

Mummenhoff K, Brueggemann H, Bowman JL 2001. Chloroplast DNA phylogeny and biogeography of *Lepidium* (Brassicaceae). American Journal of Botany 88: 2051–2063.

Mummenhoff K, Linder P, Friesen N, Bowman JL, Lee J, Franzke A 2004. Molecular evidence for bicontinental hybridogenous genomic constitution in *Lepidium* sensu stricto (Brassicaceae) species from Australia and New Zealand. American Journal of Botany 91: 254–261.

Petrie D 1885. Descriptions of new species of native plants. Transactions and Proceedings of the New Zealand Institute 17: 269–271.

Petrie D 1887. Descriptions of new native plants. Transactions and Proceedings of the New Zealand Institute 19: 323–324.

Statistical Sciences 1998. S-Plus for Windows user's manual, Version 4.5. Release 2. Seattle, Statistical Sciences.

Thellung A 1906. Die Gattung *Lepidium* (L.) R.Br. Eine Monographische Studie. Neue Denkschriften der Schweizerischen Naturforschenden Gesellschaft 41: 1–304.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG 1997. The ClustalX Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 24: 4876–4882.

Wagstaff SJ, Garnock-Jones PJ 1998. Evolution and biogeography of the *Hebe* complex (Scrophulariaceae) inferred from ITS sequences. New Zealand Journal of Botany 36: 425–437.

Webb CJ, Sykes WR, Garnock-Jones PJ 1988. Flora of New Zealand. Vol. IV. Christchurch, Botany Division DSIR.

Wright SD, Young CG, Keeling DJ, Dawson JW, Gardner RC 2001. Stepping stones to Hawaii: a transequatorial dispersal pathway for *Metrosideros* (Myrtaceae) inferred from nrDNA (ITS+ETS). Journal of Biogeography 28: 769–774.