

Systematic Relationships of New Zealand Endemic Brassicaceae Inferred from nrDNA ITS Sequence Data

ANTHONY D. MITCHELL

Ecology and Entomology Group, Soil, Plant and Ecological Sciences, Lincoln University, PO Box 84, Lincoln, New Zealand

PETER B. HEENAN

Landcare Research, PO Box 69, Lincoln, New Zealand

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ABSTRACT. Phylogenetic relationships of New Zealand species of Brassicaceae belonging to the South Island endemic *Ischnocarpus*, *Iti*, *Notothlaspi*, and *Pachycladon*, and the indigenous *Cardamine*, *Cheesemania*, *Lepidium*, and *Rorippa* were studied using nuclear ribosomal internal transcribed spacer sequences. Results support a monophyletic *Notothlaspi* and suggest that species of this genus are not closely related to *Thlaspi*, the genus to which Hooker provisionally assigned *N. australe*. The cosmopolitan genus *Cardamine* is paraphyletic by the inclusion of the monotypic genus *Iti*. The association of *Iti* with New Zealand *Cardamine* is of particular significance, as the relationships of this monotypic genus have historically proven elusive. *Cheesemania* species, *Ischnocarpus novae-zelandiae*, and *Pachycladon novae-zelandiae* form a closely related monophyletic group. The species of *Lepidium* form a monophyletic group, but the New Zealand species are not monophyletic indicating two possible dispersal events to New Zealand. Additionally, the tribes Arabidieae, Lepidieae, and Sisymbrieae are polyphyletic. This study highlights the difficulty of using traditional characters such as fruit type and cotyledon arrangement to define the tribes of the Brassicaceae.

The New Zealand Brassicaceae are notable for their high degree of generic endemism, and the few species in each genus. Several endemic genera occur in the mountains of the South Island, including *Ischnocarpus* O.E.Schulz, *Notothlaspi* Hook.f., and *Pachycladon* Hook.f., with two species each (Allan 1961; Molloy et al. 1999), and the monotypic *Iti* Garn.-Jones et P.N.Johnson (Garnock-Jones and Johnson 1987). *Cheesemania* O.E.Schulz has five species in the South Island mountains (Heenan and Garnock-Jones 1999), and a single species is endemic to Tasmania (Curtis and Morris 1993). The cosmopolitan *Cardamine* L. is well represented in New Zealand with about 20–25 species, most of which are unnamed and occur in the South Island mountains (P. B. Heenan unpubl. data). *Lepidium* L. and *Rorippa* Scop. are also cosmopolitan. *Lepidium* in New Zealand includes five coastal and two inland South Island species (Webb et al. 1988; Garnock-Jones and Norton 1995). *Rorippa* is represented in New Zealand by two species (Webb et al. 1988).

The taxonomic history of the New Zealand Brassicaceae is complex and, like other groups of New Zealand plants (e.g., Apiaceae, Mitchell et al. 1998), reflects the influence of Northern Hemisphere taxonomists (Garnock-Jones 1991). Most of the New Zealand species were initially placed in well-

known or common Northern Hemisphere genera, but as better quality and more plant material became available, these species were placed in new and often exclusively New Zealand genera. Species now assigned to *Pachycladon* were originally placed in *Braya* (Hooker 1864, 1867), *Ischnocarpus* in *Sisymbrium* L. (Hooker 1864; Schulz 1924), *Notothlaspi* in *Thlaspi* L. (Hooker 1855, 1864), and *Cheesemania* in *Arabis* L., *Cardamine*, and *Nasturtium* R.Br (Hooker 1855, 1864; Cheeseman 1925; Schulz 1929). The most recent assessments of the New Zealand genera have been provided by Garnock-Jones and Johnson (1987) where the relationships of *Iti* were considered, and in a study of seed anatomy and morphology (Garnock-Jones 1991). Seed characters indicate a possible relationship between *Ischnocarpus* and *Pachycladon*, whereas the seeds of *Cheesemania*, *Notothlaspi*, and *Iti* are more distinctive (Garnock-Jones 1991). Apart from these studies the group has been neglected.

The tribal classification of the Brassicaceae is also problematic because the characters traditionally used at this rank are few in number and may not support natural groups (Vaughan and Whitehouse 1971; Hedge 1976; Al-Shehbaz 1988). Traditionally important characters include the orientation of the radicle in the embryo, the number of rows of seeds

in each locule of the silique, flower color, trichome types, and features of the nectaries. Currently, *Pachycladon*, *Ischnocarpus*, and *Iti* are placed in the tribe Sisymbrieae, *Cheesemanina* and *Cardamine* in the tribe Arabideae, and *Lepidium* and *Notothlaspi* in the Lepidieae (Schulz 1924; Garnock-Jones and Johnson 1987; Webb et al. 1988). The presence of incumbent or accumbent cotyledons has been a particularly important character for tribal classification. However, this character can be polymorphic within a species, as has been shown for *Cheesemanina enysii* (Cheeseman) O.E.Schulz, *C. fastigiata* (Hook.f.) O.E.Schulz, *C. latesiliqua* (Cheeseman) O.E.Schulz, and *C. stellata* (Allan) Heenan et Garn.-Jones, where both incumbent and accumbent cotyledons can be found (Garnock-Jones 1991; Heenan and Garnock-Jones 1999).

The New Zealand Brassicaceae have not previously been studied using phylogenetic methods; although the named genera include species of similar growth habit and morphology, their affinities are poorly understood. This study poses two questions: (1) What are the relationships among the New Zealand genera and species? (2) Can monophyletic groups be circumscribed that include these taxa? To resolve these questions, phylogenetic analysis of nucleotide sequences of the internal transcribed spacers (ITS) of nuclear ribosomal DNA was employed.

MATERIALS AND METHODS

Plant Materials. The study group consisted of representatives of all New Zealand genera of Brassicaceae, including four species of *Cardamine* (New Zealand species are currently under revision by P. B. Heenan), five of *Lepidium*, four of *Cheesemanina*, two of *Ischnocarpus*, two of *Pachycladon*, one of *Rorippa*, two species and one possible undescribed species of *Notothlaspi*, and the monotypic *Iti* (Table 1). This sampling included four out of the five endemic species of *Cheesemanina*, five out of seven endemic species of *Lepidium*, and one each of the two species of *Pachycladon* and *Rorippa*. Brassicaceae from outside of New Zealand, and from the tribes to which the New Zealand taxa belong, have been included to provide a broader context for relationships of the New Zealand genera. The taxa included in this study are listed in Table 1 with details of their provenance, herbarium voucher (CHR), and GenBank accession number. Twenty-three New Zealand species were sequenced and an additional 20 sequences were obtained from GenBank.

DNA Analysis. Total DNA was extracted from fresh leaf tissue or dried leaves stored in silica gel, although herbarium material was also used as a source of DNA for analysis (Table 1). Total DNA was extracted using a modification of the CTAB method (Doyle and Doyle 1987). Initially 0.5 μ l of the total DNA extract (diluted 1:500) was used as a template for the amplification of double-stranded DNA (dsDNA) in a thermocycler. Excess primers and salts were removed by precipitation with isopropanol in the presence of 2.5 M NH_4Ac followed by a 70% EtOH wash. Both the forward and reverse strands of the ITS-1, 5.8S, and ITS-2 regions of nuclear encoded ribosomal DNA were sequenced according to the methods of Baldwin (1992). Primers used were ITS4 and ITS5 (White et al. 1990) as well as FFB (5'-TATGCTTAAATTCAGGGGGT-3') and 18D (5'-CACACCGCCCGTCGCTCCTACCGATTG-3'). The purified PCR fragments were sequenced using the ABI BigDye cycle sequencing kit and run on an ABI 373 automated sequencer.

Sequences were aligned using Clustal W vers. 1.6 (Thompson and Gibson 1996), then confirmed manually using sequential pairwise comparisons. The aligned data used in this analysis are available from the first author upon request. Gaps were created to ensure positional homology in instances where an insertion or deletion event may have occurred, and gaps were coded as missing data. The 5.8S region was not available in some of the sequences obtained from GenBank; these were coded as missing data. The 'Filter Taxa' option was used in MacClade vers. 3.1 (Maddison and Maddison 1992), where taxa were considered redundant even if states were not identical, when the resolution of missing or uncertain data made them potentially identical.

Phylogenetic Analyses. Analyses were conducted using PAUP vers. 3.1.1 (Swofford 1993) and the Maximum Likelihood program DNAML, from PHYLIP vers. 3.5c (Felsenstein 1993). Data were analysed as unordered characters using the heuristic search and the random addition sequence (10 replicates) options in PAUP. TBR branch swapping, COLLAPSE and MULPARS options were in effect. No characters were excluded, all characters were equally weighted, and branch lengths of 0 were collapsed to polytomies. A strict consensus tree of equally parsimonious trees (Sokal and Rohlf 1981) was constructed to assess the information that was common all most parsimonious trees. Bootstrap values (Felsenstein 1985) were calculated from 500 replicate parsimony analyses, using the simple addition sequence of taxa and the heuristic search op-

TABLE 1. List of taxa used for the study. CHR = Landcare Research Herbarium (Lincoln, New Zealand).

Taxon	Tribe	Origin	Source (including vouchers)	GenBank number
<i>Arabidopsis arenosa</i> (L.) Lawalrée	Sisymbrieae	Europe	G. Sheridan	U52188
<i>Arabidopsis thaliana</i> (L.) Heynh.	Sisymbrieae	Europe	O'Kane et al. 1996	U43225
<i>Arabis caucasia</i> Willd.	Arabideae	S. Europe	G. Sheridan	X98629
<i>Arabis scabra</i> All.	Arabideae	S. Europe	G. Sheridan	X98630
<i>Barbarea verna</i> (Mill.) Asch.	Arabideae	Europe	G. Sheridan	X98631
<i>Barbarea vulgaris</i> R.Br.	Arabideae	Eurasia, N.W. Africa	G. Sheridan	X98632
<i>Brassica oleracea</i> L.	Brassicaceae	Europe	Francisco-Ortega et al. in press	AF03994 / AF040087
<i>Camelina microcarpa</i> Andr. ex DC.	Sisymbrieae	Europe	Francisco-Ortega et al. in press	AF039999 / AF040042
<i>Cardamine amara</i> L.	Arabideae	Europe	G. Sheridan	X98633
<i>Cardamine</i> aff. <i>bilobata</i> Kirk	Arabideae	New Zealand	CHR 513362	AF100677
<i>Cardamine</i> aff. <i>corymbosa</i> Hook.f.	Arabideae	New Zealand	CHR 515295	AF100678
<i>Cardamine flexuosa</i> With.	Arabideae	Europe	G. Sheridan	X98634
<i>Cardamine hirsuta</i> L.	Arabideae	Europe	G. Sheridan	X98635
<i>Cardamine</i> sp. (a)	Arabideae	New Zealand	CHR 515296	none
<i>Cardamine</i> sp. (b)	Arabideae	New Zealand	CHR 515294	none
<i>Cheesemannia erysiti</i> (Cheeseman) O.E. Schulz	Arabideae	New Zealand	CHR 514938	AF100679
<i>Cheesemannia fastigiata</i> (Hook.f.) O.E. Schulz	Arabideae	New Zealand	CHR 514939	AF100680
<i>Cheesemannia fastigiata</i> (Hook.f.) O.E. Schulz	Arabideae	New Zealand	CHR 514934	none
<i>Cheesemannia stellata</i> (Allan) Heenan et Garn- Jones	Arabideae	New Zealand	CHR 517801	none
<i>Cheesemannia wallii</i> (Carse) Allan	Arabideae	New Zealand	CHR 514940	AF100681
<i>Eruca sativa</i> Miller	Brassicaceae	Europe, Africa, Asia	Francisco-Ortega et al. in press	AF039996 / AF040039
<i>Erysimum vitruvianii</i> Zaw.	Hesperideae	Europe	Francisco-Ortega et al. in press	AF040000 / AF040043
<i>Hirschfeldia incana</i> (L.) Lagr.-Foss.	Brassicaceae	Europe, Asia	Francisco-Ortega et al. in press	AF039989 / AF040032
<i>Ischnocarpus novae-zelandiae</i> (Hook.f.) O.E. Schulz	Sisymbrieae	New Zealand	CHR 514941	AF100682
<i>Ischnocarpus exilis</i> Heenan	Sisymbrieae	New Zealand	CHR 514942	none
<i>Isti lacustris</i> Garn.-Jones et P.N. Johnson	Sisymbrieae	New Zealand	CHR 514945	AF100683
<i>Lepidium banksii</i> Kirk	Lepidieae	New Zealand	CHR 515311	AF100684
<i>Lepidium flexicaule</i> Kirk	Lepidieae	New Zealand	CHR 515312	AF100685
<i>Lepidium naufragorum</i> Garn.-Jones et D.A. Nor- ton	Lepidieae	New Zealand	CHR 515313	AF100686
<i>Lepidium oleraceum</i> Sparrm.	Lepidieae	New Zealand	CHR 515314	AF100687
<i>Lepidium sativum</i> L.	Lepidieae	Egypt, W. Asia	Mummenhoff et al. 1997	none

TABLE 1. Continued.

Taxon	Tribe	Origin	Source (including vouchers)	GenBank number
<i>Lepidium sisymbrioides</i> subsp. <i>kaararau</i> (Pettie) Thell.	Lepidieae	New Zealand	CHR 515315	AF100688
<i>Lepidium virginicum</i> L.	Lepidieae	N. America	Mummenhoff et al. 1997	none
<i>Notothlaspi australe</i> (Hook.f.) Hook.f.	Lepidieae	New Zealand	CHR 515310	AF100689
<i>Notothlaspi rosulatum</i> Hook.f.	Lepidieae	New Zealand	CHR 514159	AF100690
<i>Notothlaspi</i> sp. (a)	Lepidieae	New Zealand	CHR 514943	AF100691
<i>Pachycladon novae-zelandiae</i> (Hook.f.) Hook.f.	Sisymbrieae	New Zealand	CHR 514944	AF100692
<i>Pachycladon</i> aff. <i>novae-zelandiae</i> (Hook.f.) Hook.f.	Sisymbrieae	New Zealand	CHR 517802	none
<i>Rorippa ditricata</i> (Hook.f.) Gam-Jones et Jonsell	Arabideae	New Zealand	CHR 190851	AF100693
<i>Rorippa heterophylla</i> (Blume) Williams	Arabideae	Asia, America	G. Sheridan	X98638
<i>Rorippa palustris</i> (L.) Besser	Arabideae	Europe, New Zealand	G. Sheridan	X98639
<i>Sisymbrium altissimum</i> L.	Sisymbrieae	Asia, Europe	Francisco-Ortega et al. in press	AF039997/AF040043
<i>Thlaspi arvense</i> L.	Lepidieae	Europe	Mummenhoff et al. 1997	none

tion in PAUP. The data set was jumbled for Maximum Likelihood analysis, which was carried out with a transition:transversion ratio of 1.3, estimated from the most parsimonious trees using MacClade. Taxa from the tribe Brassiceae were designated as the root for trees, as this tribe is considered one of the most natural in the Brassicaceae (Avetisian 1976, 1983; Al-Shehbaz 1984). Three parsimony analyses were conducted using topological constraints enforcing the monophyly of species from tribes Arabideae, Sisymbrieae, and Lepidieae respectively, and tree lengths were compared with those obtained from unconstrained analysis.

RESULTS

Taxa merged as a result of applying the FILTER TAXA option in MacClade were: (1) *Cardamine* aff. *corymbosa* + *Cardamine* sp. (a) + *Cardamine* sp. (b); (2) *Cheesemania fastigiata* + *Cheesemania stellata* + *Cheesemania* aff. *fastigiata*, and (3) *Ischnocarpus novae-zelandiae* + *Ischnocarpus exilis*, and (4) *Pachycladon novae-zelandiae* + *Pachycladon* aff. *novae-zelandiae*. These merged taxa were eliminated from the analysis and are represented in Fig. 1 by *Cardamine* aff. *corymbosa*, *Cheesemania fastigiata*, *Ischnocarpus novae-zelandiae*, and *Pachycladon novae-zelandiae*, respectively. The resulting matrix used for analysis contained a total of 37 taxa, representing five Brassicaceae tribes (Table 1). A total of 661 nucleotide positions were included in the analysis. The nucleotide composition was 22.4% A, 28.3% C, 26.8% G, 22.5% T, with 355–385 unambiguous transitions and 313–343 unambiguous transversions, calculated in MacClade using the RESOLVE POLYTOMY option. The PAUP analysis yielded 13 equally parsimonious trees, each 698 steps long, with a consistency index (CI) of 0.619 and a rescaled consistency index (RC) of 0.481. Of the 661 characters included in the analysis, 196 were phylogenetically informative. The log likelihood index (ln) = -4675.04.

The New Zealand genera occur in four major monophyletic groups (Fig. 1). The first includes *Arabidopsis* species, *Camelina microcarpa*, *Cheesemania* species, *Ischnocarpus novae-zelandiae*, *Pachycladon novae-zelandiae*, and *Erysimum witmannii*; the second comprises all *Lepidium* species sampled, the third includes *Barbarea* species, *Cardamine* species, *Iti lacustris*, and the *Rorippa* species; and the fourth includes *Notothlaspi*. Bootstrap analysis indicates that the monophyly of these groups is well supported. Except for the resolved relationships of *Cheesemania*

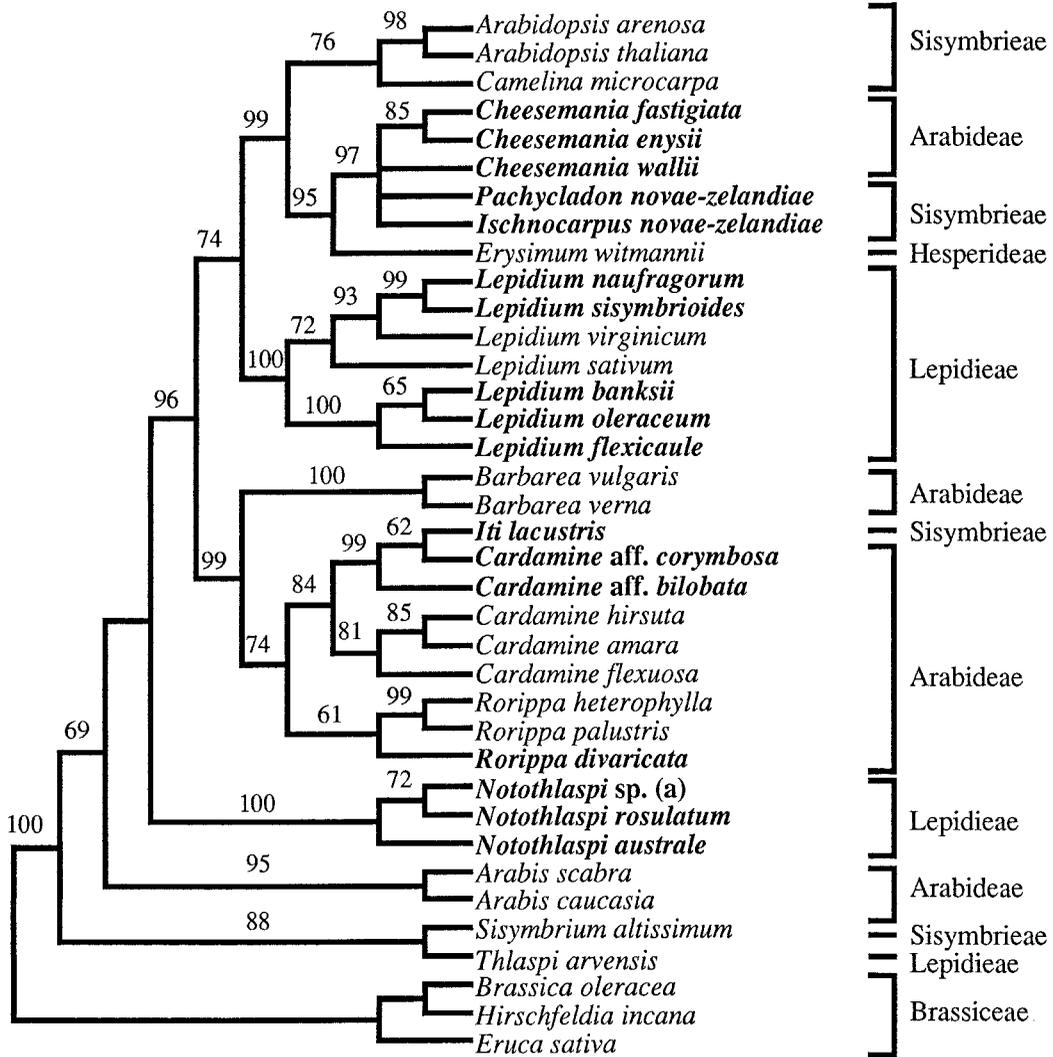


FIG. 1. Strict consensus of 13 equally parsimonious trees constructed from ITS sequence data, of 698 steps, with a consistency index (CI) = 0.619 and rescaled consistency index (RC) = 0.481. Species endemic to New Zealand are highlighted.

species, *Ischnocarpus novae-zelandiae* and *Pachycladon novae-zelandiae* in the maximum likelihood tree, which collapsed in the strict consensus parsimony tree, the topologies are identical (Fig. 1).

Trees 75 steps longer than the most parsimonious trees were found when topological constraints were imposed that placed species from the tribe Sisymbrieae together. Similarly, when species from the Arabideae were constrained to monophyly, the resulting trees were 59 steps longer. When species from the Lepidieae were constrained, trees were 19 steps longer.

DISCUSSION

Phylogenetic analyses of the DNA data provide significant new insights into relationships among New Zealand genera of the Brassicaceae, which were found to occur in five separate clades. These include: (1) *Notothlaspi*; (2) *Lepidium*; (3) *Cardamine* + *Iti*; (4) *Rorippa*; (5) *Cheesemanina* + *Ischnocarpus* + *Pachycladon*.

Notothlaspi, *Lepidium*, and the Asian/European *Thlaspi* share angustiseptate silicles and have traditionally been placed in the tribe Lepidieae (Schulz

1936). However, our results (Fig. 1) do not support monophyly of the Lepidieae, with genera from this tribe occurring in distinct clades composed of other Brassicaceae tribes. New Zealand endemic species of *Lepidium* constitute two different but related lineages, from which it may be inferred that there have been at least two dispersals and colonizations of *Lepidium* to New Zealand. *Lepidium virginicum* and *L. sativum* (found in the Northern Hemisphere) are inferred to have diverged prior to the New Zealand endemic *Lepidium sisymbrioides* and *L. naufragorum*. The sister group to these species includes the other New Zealand endemics *L. flexicaule*, *L. banksii*, and *L. oleraceum*. The three species of *Notothlaspi* form a monophyletic group with no close affinities to other Lepidieae (Fig. 1). In particular, our results suggest *Notothlaspi* is not closely related to *Thlaspi*, the genus to which Hooker provisionally assigned the species *N. australe* when it was first described (Hooker 1855, "Thlaspi? Australe"). *Notothlaspi* has been placed in its own subtribe, the *Notothlaspidinae* (Schulz 1936).

The cosmopolitan genus *Cardamine* was found to be paraphyletic by the inclusion of the monotypic genus *Iti* (Fig. 1). However, New Zealand *Cardamine* and *Iti* form a monophyletic group, which is sister group to other species of *Cardamine*, and are closely related to the Australian *C. lilacina* (Franzke et al. 1998). The association of *Iti* with New Zealand *Cardamine* is of particular significance as the relationships of this monotypic genus have proven elusive (Garnock-Jones and Johnson 1987; Garnock-Jones 1991; Garnock-Jones and Breitwieser 1998). *Iti* has small flowers, narrow usually simple leaves, sparse indumentum, barely dehiscent fruits, and embryos with elongated cotyledons (Garnock-Jones and Johnson 1987). These characters could be regarded as adaptations to its lake-shore habitat, since some of these characters it shares with unrelated species that occupy similar habitats in New Zealand (Wagstaff and Garnock-Jones 1998). Characters in *Iti* that could indicate relationships with other New Zealand Brassicaceae, particularly with *Cardamine*, may have been lost, e.g., *Iti* lacks recoiling valves and explosive fruit dehiscence, which have been considered synapomorphies for *Cardamine* (Garnock-Jones and Breitwieser 1998). However, the possibility of a relationship between *Iti* and *Cardamine* has been previously suggested (Garnock-Jones and Breitwieser 1998), and the chromosome numbers for both *Iti* and New Zealand *Cardamine* are $2n = 48$ (Pritchard 1957; Garnock-Jones and Johnson 1987).

Iti has incumbent cotyledons and an oblong sil-

icle and was, therefore, placed in the *Sisymbrieae* (following Schulz 1936 and Janchen 1942), and compared with *Ischnocarpus* and *Pachycladon* (Garnock-Jones and Johnson 1987). However, the placement of *Iti* with other taxa from the *Sisymbrieae* was not supported in this analysis (Fig. 1), and its incumbent cotyledons appear to be a derived condition within the group that included representatives from the tribe *Arabideae*, which otherwise has accumbent cotyledons. The *Sisymbrieae*, as circumscribed using traditional characters, are therefore polyphyletic.

The monophyly of *Cheesemanina*, *Ischnocarpus*, and *Pachycladon* (Fig. 1) conflicts with hypotheses of relationships proposed by Schulz (1924, 1929, 1936). The presence of accumbent cotyledons is traditionally used to define the *Arabideae*, while incumbent cotyledons are used to define the *Sisymbrieae* (Schulz 1936; Al-Shehbaz 1984). *Cheesemanina* is presently classified in the *Arabideae*, with species previously assigned to *Arabis*, *Cardamine*, and *Nasturtium* (Schulz 1929, 1936). However, *Cheesemanina* has polymorphic cotyledons that may be incumbent, accumbent, obliquely accumbent, and/or obliquely incumbent (Garnock-Jones 1991; Heenan and Garnock-Jones 1999). Polymorphism of the cotyledon in *Cheesemanina* is derived since the closely related *Ischnocarpus* and *Pachycladon*, and the more distantly related *Erysimum*, *Arabidopsis*, and *Camelina*, all possess incumbent cotyledons.

Ischnocarpus and *Pachycladon* historically have been placed in different subtribes of the *Sisymbrieae* (Schulz 1924, 1936). However, *Ischnocarpus* and *Pachycladon* share several characters that may support a close relationship, some of which occur elsewhere in the Brassicaceae. These include chromosome numbers of $2n = 20$ (Dawson 1995; M. I. Dawson, unpubl. data), branched or dendritic hairs on the leaves and inflorescences, introrse anthers, a papillate stigma, inflorescences with lateral branches, and septa with a prominent midvein. *Cheesemanina* is most similar to *Pachycladon*, sharing a stout caudex and seeds in two rows in each locule. The inflorescences of *Cheesemanina* are either lateral (*C. wallii*) and/or terminal (other *Cheesemanina* species), the pod valves are generally flat, and the anthers are usually at a similar height to the stigma at anthesis. Exceptions are the siliques of *C. wallii* being slightly keeled, and the anthers in *C. latesiliqua* held well beyond the stigma.

The seeds of *Ischnocarpus* and *Pachycladon* are similar in sharing a single reticulation formed of epidermal cells, large rounded mucilage bodies vir-

tually filling the epidermal cells, thin-walled palisade which does not collapse, a well-defined aleurone layer, and imbricate radicles (Garnock-Jones 1991). *Pachycladon* has larger seeds and more numerous areoles than *Ischnocarpus*. The seeds of New Zealand species of *Cheesemania* differ from these two genera in being winged (Garnock-Jones 1991). Based on seed anatomy, it is difficult to infer a relationship between *Ischnocarpus/Pachycladon* and *Cheesemania*.

Additional support for the relationship between *Ischnocarpus* and *Pachycladon* is provided by artificial intergeneric F₁ and F₂ hybrids between the two genera (Heenan 1999), and the issue of merging *Ischnocarpus* and *Pachycladon* as members of the same genus is under consideration. The ITS data support a close relationship of these two genera with *Cheesemania*. However, before any generic changes are made, it is necessary to identify characters that adequately circumscribe the expanded generic concept. In addition, the possible inclusion of some of the endemic Australian Brassicaceae in this clade needs to be considered before generic boundaries are redrawn.

The New Zealand endemic *Rorippa divaricata* (Garnock-Jones and Jonsell 1988) formed a monophyletic clade with the other species of *Rorippa* (Fig. 1). *Rorippa divaricata* fits within the current circumscription of *Rorippa* (Al-Shehbaz and Price 1998), but is part of a group of taxa from Australasia, New Guinea, and the west Pacific with white petals, linear siliques, and large reticulate seeds that has been recognized at the sectional rank as *Nasturtium* sect. *Ceriosperma* (Schulz 1933). It has been suggested that this section could be raised to generic rank (Garnock-Jones 1978), and their distinct testa morphology and anatomy might provide some support for this view (P. J. Garnock-Jones and B. Jonsell pers. comm.). The phylogenetic relationships within *Rorippa* presented here indicate that additional species should be examined to establish whether sect. *Ceriosperma* is sister to *Rorippa* s.s.

Erysimum witmannii, the only representative of the Hesperideae included in this study, was found to be sister to species traditionally classified in the Sisymbrieae and Arabideae (Schulz 1936) (Fig. 1). However, the relationships of the Hesperideae historically have been problematic (Al-Shehbaz 1984), with Janchen (1942) merging it with the Matthioleae, and Avetisian (1976, 1983) including it in the Sisymbrieae. Our results are congruent with the classification proposed by Avetisian, but this should be tested by the inclusion of additional species representative of the Hesperideae.

Based only on this phylogenetic study of ITS data it could be argued that *Iti* should be included in *Cardamine*, and perhaps that *Cheesemania*, *Ischnocarpus*, and *Pachycladon* be placed in a single genus. However, a general lack of agreement between molecular data and morphological characters makes taxonomic interpretation difficult, and further work is needed prior to making any taxonomic changes. In particular, Australian genera, whose affinities are also poorly known, should be included in future work. This study has highlighted the difficulty of using fruit type and cotyledon arrangement for defining tribes of the Brassicaceae.

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