Phylogenetic analyses of ITS and *rbcL* DNA sequences for sixteen genera of Australian and New Zealand Brassicaceae result in the expansion of the tribe Microlepidieae

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Abstract Sequence data from the internal transcribed spacer (ITS) nrDNA and *rbcL* cpDNA regions were used to determine relationships of genera of Brassicaceae from Australia and New Zealand (NZ) that were previously unassigned to a tribe. Maximum likelihood analysis of 71 ITS sequences identified a monophyletic clade of Australian genera, including *Carinavalva* and *Microlepidium* that had previously been assigned to the tribe Microlepidieae. *Pachycladon* is not supported as monophyletic, comprising a clade of the NZ species and another clade of the Tasmanian *P. radicatum*. These two *Pachycladon* clades form a polytomy with the Australian clade. Maximum likelihood analysis of the *rbcL* region generally supports the ITS analysis with the Australian genera forming a monophyletic clade with *Pachycladon*. *Arabidella* is polyphyletic in the *rbcL* phylogeny as *A. eremigena* is member of the Australian clade but *A. trisecta* is placed in a sister clade that comprises mainly genera of tribe Camelineae. As a result of these phylogenetic analyses the tribe Microlepidieae is expanded and now includes 16 genera and 56 species endemic to Australia and New Zealand. Genera included in the Microlepidieae are *Arabidella*, *Ballantinia*, *Blennodia*, *Carinavalva*, *Cuphonotus*, *Drabastrum*, *Geococcus*, *Harmsiodoxa*, *Irenepharsus*, *Menkea*, *Microlepidium*, *Pachycladon*, *Pachymitus*, *Phlegmatospermum*, *Scambopus* and *Stenopetalum*. Whole-genome duplication has previously been shown to have occurred in the ancestry of *Arabidella*, *Ballantinia*, *Pachycladon* and *Stenopetalum* and is likely to be a defining feature of the tribe Microlepidieae. Future research needs to investigate circumscription of the Australian genera as there is a predominance of closely related monotypic genera in the Microlepidieae. With resolution of the tribal placement of these Australian and New Zealand genera, ca. 94% (302) of the 321 genera in the family have been assigned to a tribe.

Keywords Australian flora; Brassicaceae; Microlepidieae; New Zealand flora; tribal classification

Supplementary Material The alignment files are available in the Supplementary Data section of the online version of this article (http://www.ingentaconnect.com/content/iapt/tax).

■ INTRODUCTION

In recent years there has been considerable advancement of the phylogenetic and evolutionary understanding of New Zealand (NZ) and Australian species of Brassicaceae. Notable among these studies has been the phylogenetic relationships of NZ Brassicaceae (Mitchell & Heenan, 2000; Heenan & al., 2002), placement of the NZ endemic *Notothlaspi* Hook. f. in the tribe Notothlaspideae and the Australian genera *Carinavalva* Ising and *Microlepidium* F. Muell. in the tribe Microlepidieae (Warwick & al., 2010), demonstration of a hybrid origin in Australian and NZ *Lepidium* L. (Mummenhoff & al., 2004; Dierschke & al., 2009), gene expression in *Pachycladon* Hook. f. (Voelckel & al., 2008, 2010; Sooda & al., 2011), and allopolyploidy and chromosome evolution in *Arabidella* O.E. Schulz, *Ballantinia* O.E. Schulz, *Stenopetalum* R. Br. ex DC. and *Pachycladon* (Joly & al., 2009; Mandáková & al., 2010a, b; Zhao & al., 2010).

Pachycladon has been central to much of this research (Yogeeswaran & al., 2011), and from this several issues have been identified that remain unresolved for taxa in Australia and NZ. For example: Which genera are part of the allopolyploid lineage that includes *Arabidella*, *Ballantinia*, *Stenopetalum* and *Pachycladon*? Are the two whole-genome duplication (WGD) events identified in the Australian species and *Pachycladon* (Mandáková & al., 2010a, b) independent, or was it a single event with a different tempo of diploidization? What is the relationship of *Pachycladon* to the Australian genera? To what tribe(s) should Australian and NZ genera be assigned? These issues are consistent with two of the four major tasks that have been defined in Brassicaceae systematics, taxonomy and evolution (Koch & Al-Shehbaz, 2009): (1) achieving a new infrafamiliar classification system based on phylogenetically circumscribed new tribes, and (2) recognition and assignment of monophyletic genera. There are two major impediments to resolving these issues for Australian and NZ taxa. Firstly, no DNA sequence data is available for eight Australian genera (*Cuphonotus* Ising, *Drabastrum* (F. Muell.) O.E. Schulz, *Harmsiodoxa* O.E. Schulz, *Irenepharsus* Hewson, *Menkea* Lehm., *Pachymitus* O.E. Schulz, *Phlegmatospermum* O.E. Schulz and *Scambopus* O.E. Schulz) that comprise 21 species and, secondly, no phylogenetic analyses of all genera that have already been sequenced have been undertaken.

As currently delimited, the Brassicaceae comprise 49 tribes, 321 genera, and 3660 species (Al-Shehbaz, 2012). Prior to this synopsis 34 genera and 90 species remained to be assigned to tribes (I. Al-Shehbaz, pers. comm. in 2011). The genera that were not assigned to any tribes included *Pachycladon* from NZ and Tasmania and 13 genera from Australia (*Arabidella*, *Ballantinia*, *Blennodia* R. Br., *Cuphonotus*, *Drabastrum*, *Geococcus* J.L. Drumm. ex Harv., *Harmsiodoxa*, *Irenepharsus*, *Menkea*, *Pachymitus*, *Phlegmatospermum*, *Scambopus*, *Stenopetalum*). The most recent treatment of the Australian genera was by Hewson (1982), and for the tribal placement of the genera this follows the now outdated treatment of Schulz (1936), with the exception of the more recently described genera. The Australian genera were placed by Hewson (1982) in the tribes Hesperideae, Lepidieae, Sisymbrieae and Stenopetaleae nom. inval.

In recent years molecular data has contributed significantly to the tribal subdivision of the Brassicaceae. However, due to variable taxonomic sampling in the phylogenetic analyses the classifications have not always been definitive or stable, and this has particularly affected the Australian and NZ genera. *Pachycladon*, for example, was placed in the tribe Camelineae (Al-Shehbaz & al., 2006) and was considered to be a “New Zealand clade” by Couvreur & al. (2010). Among the Australian genera, *Arabidella* was tentatively included in Cardamineae (Al-Shehbaz & al., 2006), *Stenopetalum* was provisionally assigned to Camelineae (Al-Shehbaz & al., 2006), *Blennodia* was placed in Camelineae (Warwick & al., 2007), *Carinavalva*, *Geococcus* and *Stenopetalum* were treated as an “Australian Clade” by Couvreur & al. (2010), and *Microlepidium* and *Carinavalva* were placed in the newly described tribe Microlepdieae by Warwick & al. (2010), having been traditionally placed in the tribe Lepidieae (Schulz, 1936). Furthermore, the Australian *Irenepharsus magicus* and the NZ *Pachycladon wallii* were sister species and referred to as the “ex-Camelineae II” clade by German & al. (2009). The tribe Camelineae is where the Australian and NZ genera have mostly been assigned, but recent studies have demonstrated that this tribe is heterogeneous and consequently segregate tribes have been recognised (Bailey & al., 2006; German & al., 2009; Koch & Al-Shehbaz, 2009; Warwick & al., 2010).

The objectives of this study are (1) to estimate the phylogeny for the endemic Australian genera and *Pachycladon* in relation to other Brassicaceae; (2) to test the potential monophyly of the Australian genera and *Pachycladon*; and (3) to provide a tribal classification for the Australian and NZ genera. The internal transcribed spacers and the 5.8S gene of nuclear ribosomal DNA (ITS) and the large subunit of the ribulose-1,5-bisphosphate carboxylase/oxygenase chloroplast DNA gene (*rbcL*) were sequenced for eight genera whose phylogenetic relationships had not previously been studied. ITS sequence data was used as this has been the region of choice for many Brassicaceae studies including those from NZ (e.g., Koch & al., 1999; Mitchell & Heenan, 2000; Heenan & al., 2002; O’Kane & Al-Shehbaz, 2003; Bailey & al., 2006; Warwick & al., 2007) and the chloroplast *rbcL* gene was selected since a large dataset exists.

■ MATERIALS AND METHODS

Plant material. — This study included 15 new ITS sequences representing genera from Australia that have not previously been included in phylogenetic analyses of ITS data. This includes each species of the monotypic *Drabastrum*, *Pachymitus* and *Scambopus*, and one species from *Arabidella* (10 spp.), *Cuphonotus* (2 spp.), *Harmsiodoxa* (3 spp.), *Irenepharsus* (3 spp.), *Menkea* (6 spp.) and *Phlegmatospermum* (4 spp.). A sequence was also obtained for *Blennodia canescens*, even though the second species in this genus (*B. pterosperma*) had been sequenced. In addition, ITS sequences representing five Australian genera were obtained from GenBank (*Blennodia pterosperma*, *Carinavalva glauca*, *Geococcus pusillus*, *Microlepidium pilosulum*, *Stenopetalum decipiens*), along with sequences of nine species of *Pachycladon* and 43 of other Brassicaceae. Multiple accessions were sequenced for *Drabastrum alpestre*, *Irenepharsus magicus*, *Pachymitus cardaminoides* and *Phlegmatospermum eremaeum*, but only one sequence was used in the maximum likelihood (ML) analyses. Likewise, several ITS clones were available for *Pachycladon* (McBreen & Heenan, 2006), but for each species only one was used in the ML analysis.

For *rbcL* we obtained new sequences representing nine genera from Australia that have not previously been included in phylogenetic analyses of *rbcL* data. These include *Blennodia*, *Cuphonotus*, *Drabastrum*, *Harmsiodoxa*, *Irenepharsus*, *Menkea*, *Pachymitus*, *Phlegmatospermum* and *Scambopus*. A sequence was obtained for *Arabidella trisecta*, even though another species in this genus (*Arabidella eremigena*) had been sequenced. From Genbank we acquired *rbcL* sequences of the Australian *Arabidella eremigena*, *Ballantinia antipoda*, *Stenopetalum nutans* and *Stenopetalum velutinum*, along with six *Pachycladon* sequences and 41 of other Brassicaceae.

In constructing the ITS and *rbcL* datasets care was taken to ensure there was good representation of genera from Brassicaceae lineage 1 where *Pachycladon* and *Stenopetalum* were placed in the analyses of Beilstein & al. (2010: fig. S2), and also the large clade in Warwick & al. (2010: fig. 1) that included *Microlepidium* and *Carinavalva* of the then newly recognised tribe Microlepidieae. Collection information, vouchers or literature citations, and GenBank accession numbers are listed in the Appendix.

DNA sequencing and analysis. — DNA extraction was performed using the iNtRON DNA Plant Kit (iNtRON Biotechnology, Sungnam, Kyungki-Do, Korea) following the manufacturer’s instructions. Extractions were additionally purified using a phenol-chloroform extraction and DNA concentrations determined with Nanodrop. DNA samples were then amplified and sequenced for (1) the two internal transcribed spacers (ITS1, ITS2) that flank the 5.8S nuclear ribosomal DNA region and (2) the chloroplast *rbcL* region. The touchdown PCR procedure involved initial denaturation of 4 min at 94°C, 10 cycles of 30 s 94°C, 30 s 56°C (−1°C per cycle), 1 min 72°, and 30 cycles of 30 s 94°C, 30 s 46°C, 1 min 72°C, and a final elongation step for 10 min at 72°C. The ITS primers used were ITS5-GGAAGTAAAAGTCGTAACAAGG (White & al., 1990), ITS4-TCCTCCGCTTATTGATATGC (White & al., 1990), FFB-TATGCTTAAATTCAGGGGGT (Mitchell & Heenan, 2000), and 18D-CACACCGCCCGTCGCTCCTACCGATTG (Mitchell & Heenan, 2000). The *rbcL* primers used were a_f-ATGTCACCACAAACAGAGACTAAAGC (Kress & Erickson, 2007), 724r-TCGCATGTACCTGCAGTAGC (Fay & al., 1997), rbcL-5′-GGCCGTCGACATGTCACCACAAACAGARACTAAAGC (Olmstead & al., 1992), and 1351R-TTCACAAGCTGCGGCTAGTTCAGGACTCCA (S. Wagstaff, pers. comm.).

For ITS and *rbcL* the new sequences were aligned with those obtained from GenBank (Appendix) using ClustalW as implemented in MEGA 5 (Tamura & al., 2011). Multiple alignment parameters were set to 12 for gap opening penalty and 0.1 for gap extension penalty. Alignments were confirmed manually using sequential pairwise comparisons. Insertion/deletion events were inferred where necessary. *Aethionema* is well-established as the sister to all other Brassicaceae (Galloway & al., 1998; Koch & al., 2001, 2007; Hall & al., 2002; Mathews & McBreen, 2008) and was therefore used to root the ITS and *rbcL* analyses. Phylogenetic ML analyses were undertaken using RAxML v.7.0.4 (Stamatakis, 2006) and FigTree v.1.3.1 was used to view and save trees in graphic format. Branch support was estimated using 1000 bootstrap replicates.

■ RESULTS

ITS sequence data. — The data matrix contained 71 taxa and the exclusion of ambiguous sites resulted in a total of 648 characters, 375 of which were variable and 291

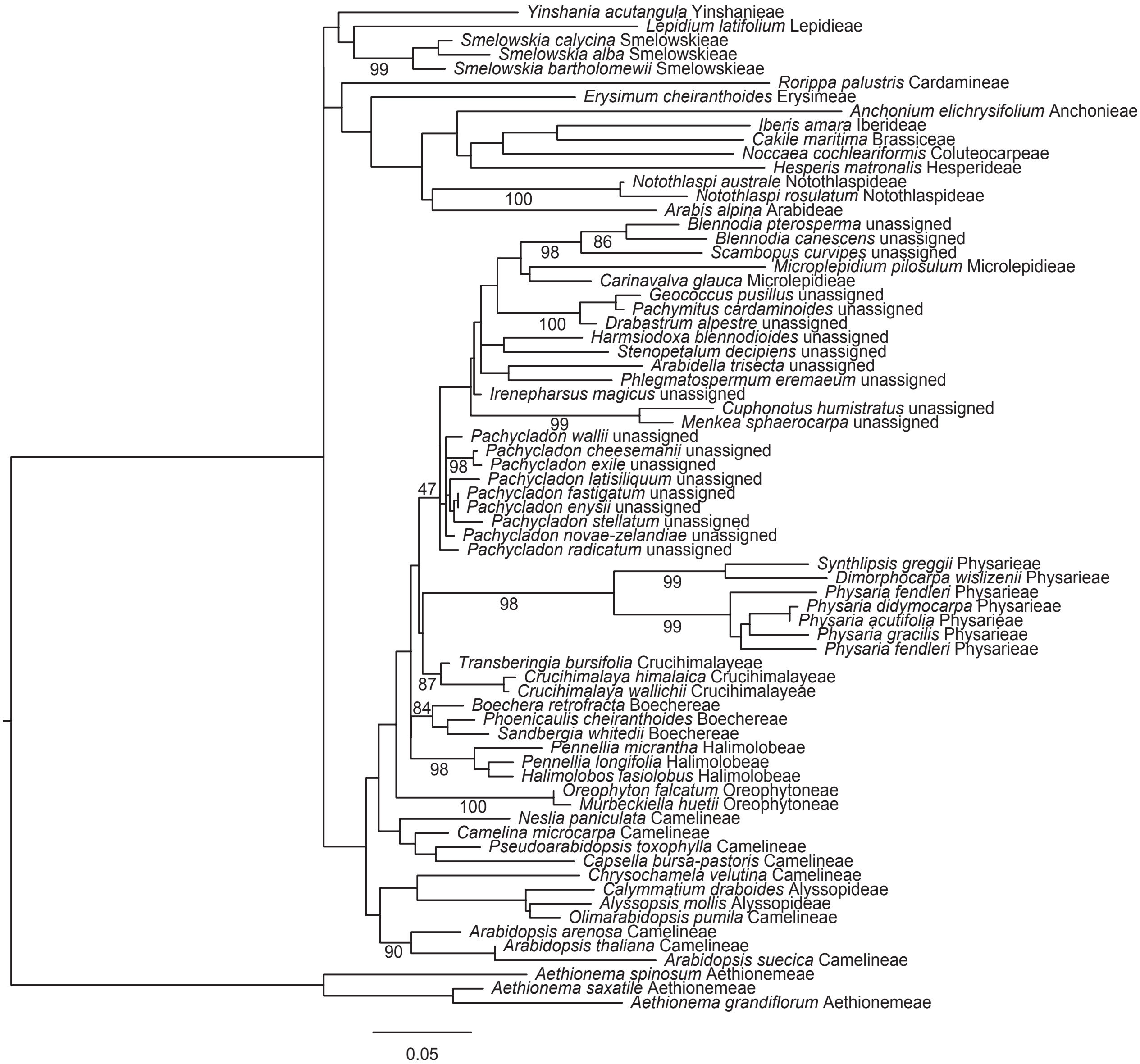

Fig. 1. Maximum likelihood phylogeny of ITS showing the position of sequences from the Australian genera and *Pachycladon* that are unassigned to a tribe in relation to other Brassicaceae taxa. Bootstrap values are shown below the branches.

parsimony-informative. Model selection as implemented in MEGA v.5.05 (Tamura & al., 2011) showed the general time reversible (GTR) model (GTR gamma+invariant) to be the best model with −Ln likelihood score = 9421.602. The GTR model accounts for variable base frequencies, variable transversions, and equal transitions. Base frequencies were A = 0.219, C = 0.284, G = 0.271, and T = 0.227. The substitution rate matrix determined by RAxML was R(a) [A-C] = 0.834542, R(b) [A-G] = 1.806899, R(c) [A-T] = 1.739994, R(d) [C-G] = 0.204375, R(e) [C-T] = 2.807188, and R(f) [G-T] = 1.0. The gamma distribution shape parameter was 0.431624. Maximum likelihood analysis revealed a single tree, −Ln likelihood score = 9821.306528 (Fig. 1).

A monophyletic Australian clade was retrieved that includes the genera *Arabidella*, *Blennodia*, *Carinavalva*, *Cuphonotus*, *Drabastrum*, *Geococcus*, *Harmsiodoxa*, *Irenepharsus*, *Menkea*, *Microlepidium*, *Pachymitus*, *Phlegmatospermum*, *Scambopus*, and *Stenopetalum* (Fig. 1). *Pachycladon* is not supported as a monophyletic genus, comprising a clade of NZ species and a clade of the single Tasmanian species (*P. radicatum*). The two *Pachycladon* clades form a polytomy with the clade comprising the 14 Australian genera. Sister to the clade of Australian and NZ genera is a weakly supported clade that includes the tribes Crucihimalayeae and Physarieae.

***rbcL* sequence data. —** The matrix contained 61 taxa and the exclusion of ambiguous sites resulted in a total of 1342 characters, 213 of which were variable and 99 parsimony-informative. Incomplete sequences were obtained for *Cuphonotus humistratus* and *Arabidella trisecta* despite using different primers and several attempts to resolve this issue. Model selection as implemented in MEGA v.5.05 (Tamura & al., 2011) showed the GTR model (GTR gamma+invariant) to be the best model with −Ln likelihood score = 4318.583879. Base frequencies were A = 0.271, C = 0.20, G = 0.246, and T = 0.283. The substitution rate matrix determined by RAxML was R(a) [A-C] = 0.804399, R(b) [A-G] = 1.992619, R(c) [A-T] = 0.289044, R(d) [C-G] = 0.398757, R(e) [C-T] = 2.768927, and R(f) [G-T] = 1.0. The gamma distribution shape parameter was 0.690131. Maximum likelihood analysis revealed a single tree with a −Ln likelihood score = 4316.383091 (Fig. 2).

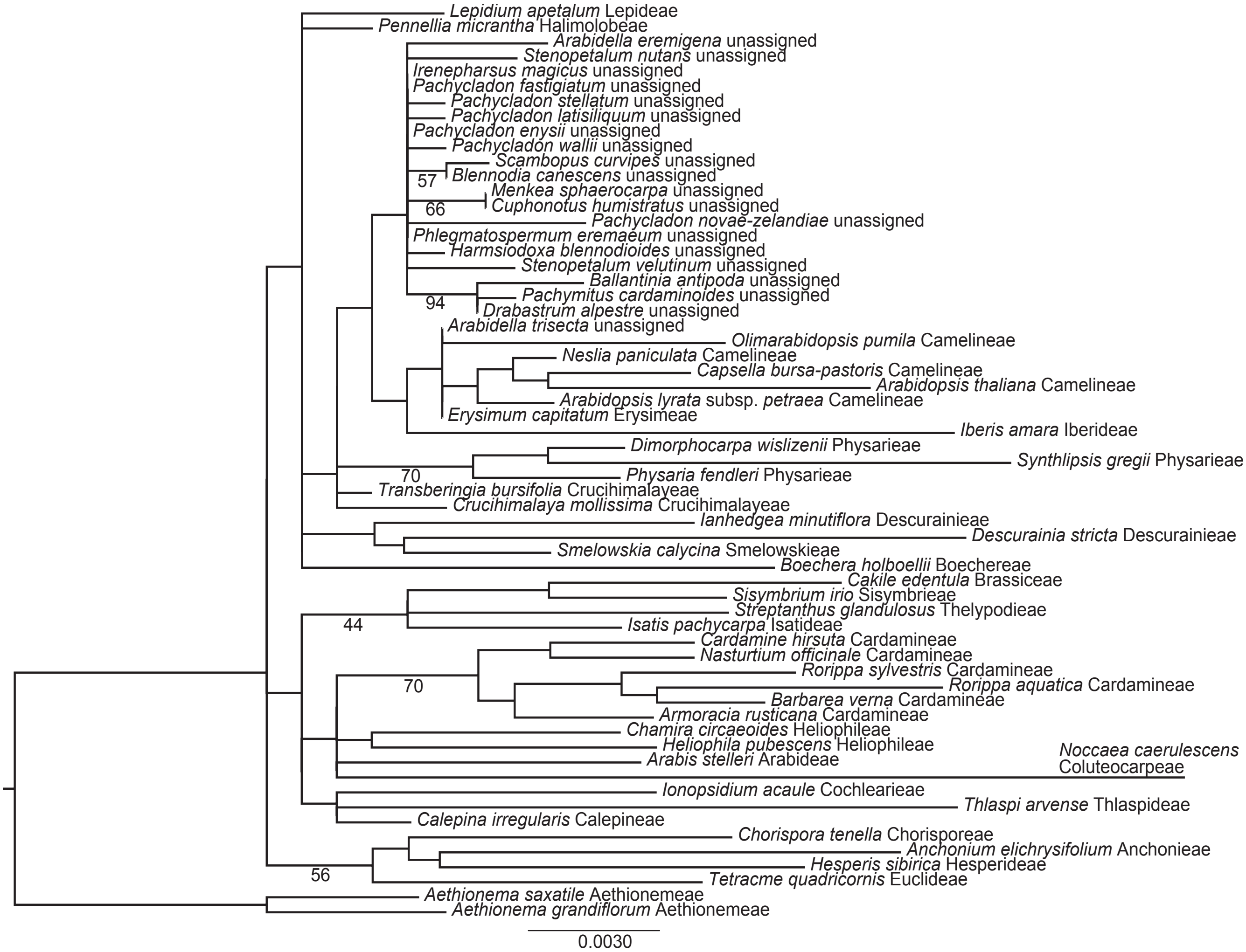

Fig. 2. Maximum likelihood phylogeny of *rbcL* showing the position of sequences from the Australian genera and *Pachycladon* that are unassigned to a tribe in relation to other Brassicaceae taxa. Bootstrap values are shown below the branches.

A monophyletic clade was retrieved that comprises *Pachycladon* and 12 Australian genera (*Arabidella*, *Ballantinia*, *Blennodia*, *Cuphonotus*, *Drabastrum*, *Harmsiodoxa*, *Irenepharsus*, *Menkea*, *Pachymitus*, *Phlegmatospermum*, *Scambopus*, *Stenopetalum*). Relationships are poorly resolved among these genera with only three monophyletic clades retrieved. These include the genera *Ballantinia*, *Drabastrum* and *Pachymitus*; *Menkea* and *Cuphonotus*; and *Blennodia* and *Scambopus*. *Pachycladon* is not monophyletic. *Arabidella* is polyphyletic as *A. eremigena* is part of the clade of Australian genera but *A. trisecta* is placed in a sister clade that comprises genera of the tribes Camelineae and Erysimeae.

The Australian genera included in the ITS and *rbcL* analyses were not exactly the same. *Carinavalva*, *Geococcus* and *Microlepidium* were not included in the *rbcL* analysis, but were shown to be part of the Australian clade in the ITS analysis. Likewise, *Ballantinia* was not included in the ITS analysis, but is shown with *rbcL* data to be part of the Australian and NZ clade.

■ DISCUSSION

Tribal classification. — Tribal classifications of the Brassicaceae, primarily founded on morphology and embryology, were proposed by Candolle (1821), Hayek (1911), and Schulz (1924, 1936), but these have now been superseded by molecular-based tribal classifications (e.g., Al-Shehbaz & al., 2006; German & Al-Shehbaz, 2008, 2010; Warwick & al., 2010). The analyses of DNA sequence data presented here are a further contribution to the effort to construct phylogenetically circumscribed tribes (Koch & Al-Shehbaz, 2009) and are especially important for determining the relationships of currently unassigned Australian genera and *Pachycladon*.

In the ITS ML phylogeny the 14 Australian genera form a monophyletic clade, although as discussed below *Arabidella* is polyphyletic in the *rbcL* analysis. Based on the tree topology and tribe relationships depicted in the ITS phylogeny, that are mostly well-supported in the *rbcL* phylogeny, and following the criteria of tribal recognition being based on monophyletic clades as adopted in other recent studies (e.g., Warwick & al., 2010), the most pragmatic solution to the assignment to a tribe of these Australian genera and *Pachycladon* is to place all genera in the tribe Microlepidieae. Consideration was given to restricting this tribe to only the Australian genera, but this clade is very poorly supported (21% bootstrap) and it would leave *Pachycladon* without adequate support to be recognised as a monogeneric tribe. Bootstrap support in the ITS phylogeny for the *Pachycladon*/Australian genera clade is low at 47%, and so importance was also placed on these genera being a geographically distinct Australian and New Zealand clade.

In the ITS phylogeny support for the clade comprising the tribes Crucihimalayeae and Physarieae as sister to the Microlepdieae is very weak (10% bootstrap), and so the sister clade to the Microlepidieae has not been confidently identified (Fig. 1). These tribes also have unresolved relationships in the *rbcL* phylogeny (Fig. 2). *Crucihimalaya* Al-Shehbaz & al. has previously been shown to be a close relative of *Pachycladon* (e.g., Heenan & al., 2002; Joly & al., 2009; Beilstein & al., 2010). In the *rbcL* phylogeny the sister clade to the Microlepidieae includes genera of the Camelineae and Erysimeae as well as *Arabidella trisecta*. Based on the ITS and *rbcL* ML trees, recognition of the expanded tribe Microlepidieae retains the tribes Physarieae and Crucihimalayeae as monophyletic and as circumscribed by O'Kane & Al-Shehbaz (2003) and Warwick & al. (2010), respectively. Basal relationships among the tribes Crucihimalayeae, Physarieae and Microlepidieae (as "ex-Camelineae II") were also unresolved in the ITS phylogeny of German & al. (2009), as these three clades formed a polytomy. These three tribes may represent more-or-less simulataneous radiations in Asia, North America and Australia/New Zealand, respectively, and therefore the polytomy could be difficult to resolve.

Arabidella is polyphyletic (see below) in the *rbcL* analysis and is therefore difficult to assign to one particular tribe using these data. Based on the ITS phylogeny where *A. trisecta* is placed with the other Australian genera, and the *rbcL* phylogeny where *A. eremigena* is placed with the Australian genera, the genus *Arabidella* is assigned to the Microlepidieae. Comparative chromosome painting (CCP) data provided by Mandáková & al. (2010b: 2280) also demonstrates that *A. eremigena* (and *Blennodia canescens*) should be regarded as belonging to the Australian clade as it has a unique chromosome reshuffling of the ancestral chromosome AK8-like homeologue also shared by *Stenopetalum nutans*, *S. lineare* R. Br. ex DC. and *Ballantinia antipoda*. However, further sampling of *Arabidella* species and additional genes and phylogenetic analyses are required to resolve the conflict between *A. trisecta* in the ITS and *rbcL* phylogenies.

Generic circumscription. — The ITS and *rbcL* ML phylogenetic hypotheses retrieved here provide a new framework for reevaluating the relationships and generic circumscriptions of the Australian genera, although detailed analyses of this are not undertaken here. Three generic complexes are retrieved in both the ITS and *rbcL* analyses that require further taxonomic study to confirm whether the genera are distinct or if consideration should be given to merging them. Firstly, *Menkea* and *Cuphonotus* are well-supported as sister genera with high bootstrap values for both ITS (99%) and *rbcL* (66%). These two genera share several characters including pinnate leaves, they are glabrous or with simple hairs, the median nectary glands are absent, the petals are white, pink or yellow, and the fruit is a silicula (Hewson, 1982). Secondly, in the ITS analysis the three monotypic genera *Drabastrum*, *Geococcus* and *Pachymitus* are strongly supported as a monophyletic clade with high bootstrap values (100% bootstrap). In the *rbcL* analysis *Ballantinia* (also monotypic), *Drabastrum*, and *Pachymitus* are strongly supported (94% bootstrap) as a monophyletic clade. These four genera share branched hairs and pinnate leaves, but *Ballantinia*, *Geococcus* and *Pachymitus* are annuals with white flowers and *Drabastrum* is a perennial undershrub with white or lavender flowers (Hewson, 1982). These genera also differ in fruit type (siliqua or silicula) and hairiness (glabrous or hairy). Thirdly, the bitypic *Blennodia* and the monotypic *Scambopus* are sister genera with high bootstrap support in ITS (98%) and *rbcL* (57%) data. These genera are erect annuals up to 50 cm tall, with pinnate leaves, stellate or branched hairs, and the fruit is a densely hairy siliqua (Hewson, 1982).

The ITS and *rbcL* ML phylogenies have not confirmed the monophyly of *Pachycladon*, which is in contrast to previous phylogenetic studies (Mitchell & Heenan, 2000; Heenan & al., 2002; Heenan & Mitchell, 2003; Joly & al., 2009, notwithstanding its allopolyploid origin) and the identification of *Pachycladon* as a "New Zealand clade" by Couvreur & al. (2010). *Pachycladon radicatum* was not included in the monophyletic clade of NZ *Pachycladon* species, and is the only Australian representative of the genus. It is considered to have diverged relatively early from the New Zealand *Pachycladon* species (Heenan & Mitchell, 2003). However, *P. radicatum* does share several features with *P. wallii*, including being glabrous, perennial, having a semi-woody caudex, spreading branches, oblong to oval and usually lobed or toothed leaves, more-or-less slender lateral inflorescences, and winged seeds. *Pachycladon*, including *P. radicatum*, is a morphologically discrete genus and should remain as circumscribed by Heenan & al. (2002).

The most difficult generic problem to deal with is *Arabidella*, as in the *rbcL* phylogeny this genus is polyphyletic. *Arabidella eremigena* occurs in a clade comprising *Pachycladon* and the other Australian genera, and *A. trisecta* is in a clade with genera of Camelineae and Erysimeae. The situation is further complicated by the ITS phylogeny, as in this *A. trisecta* is part of the large Australian clade. *Arabidella* has two distinct species groups (Wege & Lepschi, 2007), and the type species of *Arabidella* is the shrubby *A. trisecta* (Hewson, 1982; Al-Shehbaz, 2012). Three species (*A. trisecta*, *A. filifolia* (F. Muell.) E.A. Shaw, *A. glaucescens* E.A. Shaw) are shrubby, with most or all leaves 2- to multi-pinnatisect, and their inflorescences elongate at or after flowering (Wege & Lepschi, 2007). In contrast, four species (*A. nasturtium* (F. Muell.) E.A. Shaw, *A. procumbens* (Tate) E.A. Shaw, *A. eremigena*, *A. chrysodema* Lepschi & Wege) are annuals with lyrate-pinnatisect to 3-pinnatisect leaves and a dense inflorescence (Wege & Lepschi, 2007). In reference to *A. nasturtium*, Shaw (1965: 196) commented: "It is most closely related to *A. trisecta* …. It is also closely related to *A. eremigena* and forms a link between *A. trisecta*, *A. filifolia* and *A. glaucescens* on the one hand, and *A. eremigena* and *A. procumbens* on the other. Were it not for the existence of *A. nasturtium* it would seem better to place these two latter species in a genus distinct from *Arabidella*." If it was necessary to split *Arabidella* into two genera corresponding to the annual and shrubby clades, as implied by the *rbcL* phylogeny and the morphological characters, there are two generic names available for the annual species. Schulz (1924) provided the names *Lemphoria* O.E. Schulz for *L. procumbens* (Tate) O.E. Schulz and *Micromystria* O.E. Schulz for *M. nasturtium* (F. Muell.) O.E. Schulz and *M. eremigena* (F. Muell.) O.E. Schulz.

Resolution of this unexpected problem with *Arabidella* is beyond the scope of this paper, but future phylogenetic studies will require sequencing of all species of *Arabidella* along with analyses of additional genes. Indeed, to provide a more thorough phylogenetic framework for all studies of generic circumscription discussed here for the Microlepidieae, future phylogenetic studies will require that all species of the relevant genera are included and that more rapidly evolving genes are used for the phylogenetic analyses.

Attributes of the Microlepidieae clade. — The clade of Australian genera and *Pachycladon* retrieved here corresponding to the expanded tribe Microlepidieae features well-documented allopolyploidy (Joly & al., 2009; Mandáková & al., 2010b; Zhao & al., 2010) and major genome reshuffling (Mandáková & al., 2010a, b). In both of the CCP studies, chromosome reorganisation accompanying the reduction of chromosome number was mediated by inversions, translocations and centromere inactivation/loss, but there are significant differences between *Pachycladon* (Mandáková & al., 2010a) and *Ballantinia antipoda*, *Stenopetalum nutans* and *S. lineare* (Mandáková & al., 2010b). The Australian species have low chromosome numbers ($n = 4–6$), exhibit species-specific complex mosaics of duplicated ancestral genomic blocks reshuffled by numerous chromosome rearrangements, and the hybridization event is estimated to have occurred 5.9 (3.7–8.7) million years ago. In contrast, *Pachycladon* allopolyploids experienced less extensive diploidization than the Australian group and chromosome stasis prevails as these species share $n = 10$, have identical karyotype structure, and the hybridisation event occurred more recently at 1.61 ± 0.63 million years ago. The differences in genome structure between *Pachycladon* and the Australian endemic genera are also likely to be correlated with differences in traits such as life cycle and habitat. For example, *Pachycladon* is primarily alpine and has a long-lived perennial growth habit (Heenan & Mitchell, 2003), whereas the Australian species studied are annuals and occur in low rainfall, arid to semi-arid, dry hills and plains (Hewson, 1982). The cytogenetic and phylogenetic differences described here require more detailed analyses in regard to the generation time hypothesis and differences in annual and perennial habit (e.g., Soria-Hernanz & al., 2008). In this context it will be interesting to compare genome structure of *Drabastrum* and *Pachycladon* since these genera are both perennials, and to compare *Drabastrum* with its annual Australian relatives.

An attribute of allopolyploidy is that each of the parental taxa will be represented in the genome of the hybrid, and therefore the phylogenetic relationships of each genome can be retrieved. Analyses of five single-copy nuclear genes show that *Pachycladon* species have two copies of each gene representing two highly diverged evolutionary lineages (Joly & al., 2009). Further analysis of chalcone synthase (*CHS*) by Mandáková & al. (2010b) for *Pachycladon* and the Australian genera *Arabidella*, *Ballantinia* and *Stenopetalum* also retrieved two separate phylogenetic lineages for these genera. They demonstrated that genome "a1" is polyphyletic as two separate clades corresponding to *Pachycladon* and the Australian genera are placed within a larger clade that also includes *Boechera* Á. Löve & D. Löve, *Capsella* Medik., *Crucihimalaya*, and *Transberingia* Al-Shehbaz & O'Kane. The second lineage (genome "a2") was monophyletic for *Pachycladon* and the Australian genera, but does not have any obvious close relative. Zhao & al. (2010) provided better resolution of relationships for genome "a2" of Mandáková & al. (2010b), and showed that this lineage of *Pachycladon* is closely related to the Asian tribe Smelowskieae. Beilstein & al. (2010) provided further support for this relationship as phylogenetic analyses of phytochrome A retrieved two *Pachycladon* lineages, one sister to *Crucihimalaya* and the other sister to *Smelowskia* C.A. Mey. (tribe Smelowskieae). Additional sequencing is now required of other Australian genera placed in the Microlepidieae to determine if they are allopolyploid, and if so what the phylogenetic relationships of the two parental genomes are.

The close relationship between the tribes Physarieae and Microlepidieae as retrieved in the ITS ML analysis presented here and by German & al. (2009) requires further study. The Physarieae includes seven genera and 133 species (Al-Shehbaz, 2012), and *Physaria* Rchb. is characterized by a diversity of chromosome numbers ($2n$ = 8–140; Warwick & Al-Shehbaz, 2006) and variation in genome size (Lysak & Lexer, 2006; Lysak & al., 2009). These observations lead Lysak & al. (2009) to suggest that in *Physaria* there may have been one or more polyploidization events followed by a series of chromosome rearrangements leading to chromosome number reduction. The tribe Physarieae will be included in future CCP studies, as its phylogenetic position as a possible sister tribe to Microlepidieae is of considerable relevance to the evolution of WGD in this large Australian-New Zealand-North American tribal complex.

Two species of Crucihimalayeae, *Transberingia bursifolia* and *Crucihimalaya wallichii*, have also been studied by CCP and in both cases the analysis showed that their chromosome complements are almost completely collinear with the Ancestral Crucifer Karyotype (Mandáková & al., 2010b: suppl. material). There was no evidence in the Crucihimalayeae of the WGD event that characterises the Microplepidieae. This may indicate that among these three closely related tribes the genome evolution of Microlepidieae and Physarieae was impacted by WGD, whereas there is no evidence for a WGD event in the ancestry of the Crucihimalayeae.

■ NOMENCLATURE

Tribe Microlepidieae Al-Shehbaz, Warwick, Mummenhoff & M.A. Koch in Pl. Syst. Evol. 285: 228. 2010 – Type: *Microlepidium* F. Muell. in Linnaea 25: 371. 1852.

As originally circumscribed this tribe comprised two Australian genera and three species, *Microlepidium alatum* (J.M. Black) E. Shaw, *M. pilosulum* F. Muell., and *Carinavalva glauca* Ising (Warwick & al., 2010). The tribe Microlepidieae is expanded here to also include an additional 14 genera. Additional nomenclatural information for these genera is provided by Al-Shehbaz (2012).

Genera included

Arabidella (F. Muell.) O.E. Schulz in Engler, Pflanzenr. IV. 105 (Heft 86): 177. 1924.

Seven species: *A. chrysodema* Lepschi & Wege, *A. eremigena* (F. Muell.) E.A. Shaw, *A. filifolia* (F. Muell.) E.A. Shaw, *A. glaucescens* E.A. Shaw, *A. nasturtium* (F. Muell.) E.A.Shaw, *A. procumbens* (Tate) E.A. Shaw, *A. trisecta* (F. Muell.) O.E. Schulz.

Ballantinia Hook. f. ex E.A. Shaw in Contr. Gray Herb. 205: 160. 1974.

One species: *B. antipoda* (F. Muell.) E.A. Shaw.

Blennodia R. Br. in Sturt, Narr. Exped. C. Australia 2: 67. 1849.

Two species: *B. canescens* R. Br., *B. pterosperma* (J.M. Black) J.M. Black.

Carinavalva Ising, Trans. Roy. Soc. South Australia 78: 114. 1955.

One species: *C. glauca* Ising.

Cuphonotus O.E. Schulz in Bot. Jahrb. Syst. 66: 92. 1933.

Two species: *C. andraeanus* (F. Muell.) E.A. Shaw, *C. humistratus* (F. Muell.) O.E. Schulz.

Drabastrum (F. Muell.) O.E. Schulz in Engler, Pflanzenr. IV. 105 (Heft 86): 257. 1924.

One species: *D. alpestre* (F. Muell.) O.E. Schulz.

Geococcus J. Drumm. ex Harv. in Hooker's J. Bot. Kew Gard. Misc. 7: 52. 1855.

One species: *G. pusillus* J. Drumm. ex Harv.

Harmsiodoxa O.E. Schulz in Engler, Pflanzenr. IV. 105 (Heft 86): 260. 1924.

Three species: *H. blennodioides* O.E. Schulz, *H. brevipes* (F. Muell.) O.E. Schulz, *H. puberula* E. Shaw.

Irenepharsus Hewson in George (ed.), Fl. Australia 8: 391. 1982.

Three species: *I. magicus* Hewson, *I. phasmatodes* Hewson, *I. trypherus* Hewson.

Menkea Lehm. in Del. Sem. Hort. Hamburg.: 8. 1843.

Six species: *M. australis* Lehm., *M. crassa* E.A. Shaw, *M. draboides* Hook. f. ex Benth., *M. lutea* E.A. Shaw, *M. sphaerocarpa* F. Muell., *M. villosula* (F. Muell. & Tate) J.M. Black.

Microlepidium F. Muell. in Linnaea 25: 371. 1853.

Two species: *M. alatum* (J.M. Black) E.A. Shaw, *M. fenestratum* Rupr., *M. pilosulum* F. Muell.

Pachycladon Hook. f., Handb. N. Zeal. Fl. 2: 724. 1867.

Eleven species: *P. cheesemanii* Heenan & A.D. Mitch., *P. crenatus* Philipson, *P. enysii* (Cheeseman) Heenan & A.D. Mitch., *P. exile* (Heenan) Heenan & A.D. Mitch., *P. fasciarium* Heenan, *P. fastigiatum* (Hook. f.) Heenan & A.D. Mitch., *P. latisiliquum* (Cheeseman) Heenan & A.D. Mitch., *P. novaezelandiae* (Hook. f.) Hook. f., *P. radicatum* (Hook. f.) Heenan & A.D. Mitch., *P. stellatum* (Allan) Heenan & A.D. Mitch., *P. wallii* (Carse) Heenan & A.D. Mitch.

Pachymitus O.E. Schulz in Engler, Pflanzenr. IV. 105 (Heft 86): 266. 1924.

One species: *P. cardaminoides* (F. Muell.) O.E. Schulz.

Phlegmatospermum O.E. Schulz in Bot. Jahrb. Syst. 66: 93. 1933.

Four species: *P. cochlearinum* (F. Muell.) O.E. Schulz, *P. drummondii* (Benth.) O.E. Schulz, *P. eremaeum* E. Shaw, *P. richardsii* (F. Muell.) E.A. Shaw.

Scambopus O.E. Schulz in Engler, Pflanzenr. IV. 105 (Heft 86): 259. 1924.

One species: *Scambopus curvipes* (F. Muell.) O.E. Schulz.

Stenopetalum R. Br. ex DC., Syst. Nat. 2: 513. 1821.

Ten species: *S. anfractum* E.A. Shaw, *S. decipiens* E.A. Shaw, *S. filifolium* Benth., *S. lineare* R. Br. ex DC., *S. nutans* F. Muell., *S. robustum* Endl., *S. salicola* Keighery, *S. saxatile* Keighery, *S. sphaerocarpum* F. Muell., *S. velutinum* F. Muell.

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Appendix. GenBank accession numbers for the sequences used in the phylogenetic analyses. For the new sequences studied here the following information is given: Taxon name — tribe; voucher information; ITS GenBank accession number, *rbcL* GenBank accession number. New GenBank sequences are marked with an asterisk (*) and a dash (–) indicates missing sequences.

Aethionema grandiflorum Boiss. & Hohen. — Aethionemeae; DQ249867, AY167983. ***Aethionema saxatile*** (L.) W.T. Aiton — Aethionemeae; GQ284853, AY483262. ***Aethionema spinosum*** (Boiss.) N. Busch. — Aethionemeae; GQ424545, –. ***Alyssopsis mollis*** (Jacq.) O.E. Schulz — Alyssopideae; GQ497845, –. ***Anchonium elichrysifolium*** (DC.) Boiss. — Anchonieae; DQ357515, FN594834. ***Arabidella eremigena*** (F. Muell.) E.A. Shaw — Unassigned; –, FN597049. ***Arabidella trisecta*** (F. Muell.) O.E. Schulz — Unassigned; Australia, Queensland, *N.N. Donner 6113*, 5 Aug 1978 (CHR 488253); *JX630158, *JX630168. ***Arabidopsis arenosa*** (L.) Lawalrée — Camelineae; U52187, –. ***Arabidopsis lyrata* subsp. *petraea*** (L.) O'Kane & Al-Shehbaz — Camelineae; –, AY174652. ***Arabidopsis suecica*** (Fr.) Norrl. ex O.E. Schulz — Camelineae; ASU52185, –. ***Arabidopsis thaliana*** (L.) Heynh. — Camelineae; DQ528813, ATU91966. ***Arabis alpina*** L. — Arabideae; AF137559, –. ***Arabis stelleri*** DC. — Arabideae; –, D88903. ***Armoracia rusticana*** G. Gaertn., B. Mey. & Scherb. — Cardamineae; –, AF020323. ***Ballantinia antipoda*** (F. Muell.) E.A. Shaw — Unassigned; –, FN597048. ***Barbarea verna*** (Mill.) Asch. — Cardamineae; –, HM849808. ***Blennodia canescens*** R. Br. — Unassigned; Australia, South Australia, *R.H. Kuchel 555*, 2 Aug 1963 (CHR 153232);*JX630159, *JX630159. ***Blennodia pterosperma*** J.M. Black — Unassigned; DQ357519, –. ***Boechera holboellii*** (Hornem.) Á. Löve & D. Löve — Boechereae; –, FN594845. ***Boechera retrofracta*** (Graham) Á. Löve & D. Löve — Boechereae; AF183105, –. ***Cakile edentula*** (Bigelow) Hook. — Brassiceae; –, HM849832. ***Cakile maritima*** Scop. — Brassiceae; AY722494, –. ***Calepina irregularis*** (Asso) Thell. — Calepineae; –, HE616642.***Calymmatium draboides*** (Korsh.) O.E. Schulz — Alyssopideae; GQ497854, –. ***Camelina microcarpa*** Andrz. ex DC. — Camelineae; AF137574, –. ***Capsella bursa-pastoris*** (L.) Medik. — Camelineae; DQ310530, FN594844. ***Cardamine hirsuta*** L. — Cardamineae; –, HM849847. ***Carinavalva glauca*** Ising — Microlepidieae; GQ424527, –. ***Chamira circaeoides*** (L.f) Zahlbr. — Heliophileae; –, AM234932. ***Chorispora tenella*** (L.) DC. — Chorisporeae; –, FN594833. ***Chrysochamela velutina*** Boiss. — Camelineae; DQ249856, –. ***Crucihimalaya himalaica*** (Edgew.) Al-Shehbaz, O'Kane & R.A. Price — Crucihimalayeae; AY662283, –. ***Crucihimalaya mollissima*** (C.A. Mey.) Al-Shehbaz, O'Kane & R.A. Price — Crucihimalayeae; –, FN594843. ***Crucihimalaya wallichii*** (Hook. f. & Thomson) Al-Shehbaz, O'Kane & R.A. Price — Crucihimalayeae; DQ399114, –. ***Cuphonotus humistratus*** (F. Muell.) O.E. Schulz — Unassigned; Australia, Western Australia, *B.H. Smith 620*, 22 Aug 1985 (MEL 679795); *JX630160, *JX630170. ***Descurainia stricta*** Reiche — Descurainieae; –, FN594838. ***Dimorphocarpa wislizenii*** (Engelm.) Rollins — Physarieae; AF137593, FN594839. ***Drabastrum alpestre*** (F. Muell.) O.E. Schulz — Unassigned; Australia, Victoria, *J.A. Jeanes 1852*, 15 Jan 2008 (MEL 2296588); *JX630161, *JX630176. ***Erysimum capitatum*** (Douglas ex Hook.) Greene — Erysimeae; –, AY167980. ***Erysimum cheiranthoides*** L. — Erysimeae; DQ005989, –. ***Geococcus pusillus*** J. Drumm. ex Harv. — Unassigned; GQ424571, –. ***Halimolobos lasiolobus*** (Link) O.E. Schulz — Halimolobeae; AF307647, –. ***Harmsiodoxa blennodioides*** O.E. Schulz — Unassigned; Australia, New South Wales, *J.G. West 4994*, 24 Sep 1985 (CHR 486935); *JX630162, *JX630172. ***Heliophila pubescens*** Burch. — Heliophileae; –, AM234933. ***Hesperis matronalis*** L. — Hesperideae; DQ357547, –. ***Hesperis sibirica*** L. — Hesperideae; –, FN594835. ***Ianhedgea minutiflora*** (Hook. f. & Thomson) Al-Shehbaz & O'Kane — Descurainieae; –, FN594825. ***Iberis amara*** L. — Iberideae; AJ440311, FN594828. ***Ionopsidium acaule*** (Desf.) DC. Ex Rchb. — Cochlearieae; –, FN594827. ***Irenepharsus magicus*** Hewson — Unassigned; Australia, Victoria, *J. Turner 1116*, 4 Dec 2004 (MEL 2278721); *JX630163, *JX630175. ***Isatis pachycarpa*** Rech.f., Aellen & Esfand. — Isatideae; –, FN594830. ***Lepidium apetalum*** Willd. — Lepidieae; –, GQ436651. ***Lepidium latifolium*** L. — Lepidieae; AJ582447 and AJ582521, –. ***Menkea sphaerocarpa*** F. Muell — Unassigned; Australia, Western Australia, *B.J. Conn*

Appendix. Continued.

1972, 5 Sep 1985 (MEL 712563); *JX630164, *JX630171. ***Microlepidium pilosulum*** F. Muell. — Microlepidieae; GQ497869, –.***Murbeckiella huetii*** (Boiss.) Rothm. — Oreophytoneae; GQ424546, –. ***Nasturtium officinale*** W.T. Aiton — Cardamineae; –, HM850197. ***Neslia paniculata*** (L.) Desv. — Camelineae; DQ310524, DQ310541. ***Noccaea caerulescens*** (J. Presl & C. Presl) F.K. Mey. — Coluteocarpeae; –, FN594826. ***Noccaea cochleariformis*** (DC.) A. Löve & D. Löve — Coluteocarpeae; DQ249838, –. ***Notothlaspi australe*** Hook. f. — Notothlaspideae; AF100689, –. ***Notothlaspi rosulatum*** Hook. f. — Notothlaspideae; AF100690, –. ***Olimarabidopsis pumila*** (Stephan) Al-Shehbaz, O'Kane & R.A. Price — Camelineae; DQ310528, DQ310543. ***Oreophyton falcatum*** (Hochst ex A. Rich) O.E. Schulz — Oreophytoneae; GQ424549, –. ***Pachycladon cheesemanii*** Heenan & A.D. Mitch. — Unassigned; EF015686, –. ***Pachycladon enysii*** (Cheeseman) Heenan & A.D. Mitch. — Unassigned; EF015678, EF015668. ***Pachycladon exile*** (Heenan) Heenan & A.D. Mitch. — Unassigned; EF015676, –. ***Pachycladon fastigiatum*** (Hook. f.) Heenan & A.D. Mitch. — Unassigned; EF015669, EF015666. ***Pachycladon latisiliquum*** (Cheeseman) Heenan & A.D. Mitch. — Unassigned; EF015694, EF015665. ***Pachycladon novae-zelandiae*** (Hook. f.) Hook. f. — Unassigned; EF015677, FN594852. ***Pachycladon radicatum*** (Hook. f.) Heenan & A.D. Mitch. — Unassigned; EF015692, –. ***Pachycladon stellatum*** (Allan) Heenan & A.D. Mitch. — Unassigned; EF015689, EF015664. ***Pachycladon wallii*** (Carse) Heenan & A.D. Mitch. — Unassigned; EF015681, EF015663. ***Pachymitus cardaminoides*** (F. Muell.) O.E. Schulz — Unassigned; Australia, Victoria, *I.R.K. Sluiter 04–34*, 18 Sep 2003 (MEL 2231915); *JX630165, *JX630174. ***Pennellia longifolia*** (Benth.) Rollins — Halimolobeae; AF307627, –. ***Pennellia micrantha*** (A. Gray) Nieuwl. — Halimolobeae; F307629, FN594847. ***Phlegmatospermum eremaeum*** E. Shaw — Unassigned; Australia, Victoria, *J.H. Browne 626*, 27 Aug 1990 (MEL 1589968); *JX630166, *JX630169. ***Phoenicaulis cheiranthoides*** Nutt. — Boechereae; GQ497874, –. ***Physaria acutifolia*** Rydb. — Physarieae; AF137582, –. ***Physaria didymocarpa*** (Hook.) A. Gray — Physarieae; AF137583, –. ***Physaria fendleri*** (A. Gray) O'Kane & Al-Shehbaz — Physarieae; AF055198, FN594840. ***Physaria gracilis*** (Hook.) O'Kane & Al-Shehbaz — Physarieae; DQ310523, –. ***Physaria pruinosa*** (Greene) O'Kane & Al-Shehbaz — Physarieae; AF137584, –. ***Pseudoarabidopsis toxophylla*** (M. Bieb.) Al-Shehbaz, O'Kane & R.A. Price — Camelineae; AF137558, –. ***Rorippa aquatica*** (Eaton) E.J. Palmer & Steyerm. — Cardamineae; –, AF020326. ***Rorippa sylvestris*** (L.) Besser — Cardamineae; –, AF020328. ***Rorippa palustris*** (L.) Besser — Cardamineae; X98639, –. ***Sandbergia whitedii*** (Piper) Greene — Boechereae; AJ628295 and AJ628296, –. ***Scambopus curvipes*** (F. Muell.) O.E. Schulz — Unassigned; Australia, South Australia, *D.J. Duval 1087 & T.S. Te*, 11 Aug 2008 (AD 225630); *JX630167, *JX630177. ***Sisymbrium irio*** L. — Sisymbrieae; –, AY167982. ***Smelowskia alba*** (Pall.) Regel — Smelowskieae; AY230562, –. ***Smelowskia bartholomewii*** (Al-Shehbaz) Al-Shehbaz & Warwick — Smelowskieae; AY230609, –. ***Smelowskia calycina*** (Stephan ex Willd.) C.A. Mey. — Smelowskieae; AY230588, FN594837. ***Stenopetalum decipiens*** E.A. Shaw — Unassigned; GQ424564, –. ***Stenopetalum nutans*** F. Muell. — Unassigned; –, FN594848. ***Stenopetalum velutinum*** F. Muell. — Unassigned; –, FN594850. ***Streptanthus glandulosus*** Hook. — Thelypodieae; –, FN594831. ***Synthlipsis greggii*** A. Gray — Physarieae; AF137590, FN594841. ***Tetracme quadricornis*** (Steph.) Bunge — Euclidieae; –, FN594832. ***Thlaspi arvense*** L. — Thlaspideae; –, FN594829. ***Transberingia bursifolia*** (DC.) Al-Shehbaz & O'Kane — Crucihimalayeae; DQ399110, FN598779. ***Yinshania acutangula*** (O.E. Schulz) Al-Shehbaz, G. Yang, L.L. Lu & T.Y. Cheo — Yinshanieae; AH007969, –.