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ORIGINAL ARTICLE

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Multispeed genome diploidization and diversification after an ancient allopolyploidization

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Abstract

Hybridization and genome doubling (allopolyploidy) have led to evolutionary novelties as well as to the origin of new clades and species. Despite the importance of allopolyploidization, the dynamics of postpolyploid diploidization (PPD) at the genome level has been only sparsely studied. The Microlepidieae (MICR) is a crucifer tribe of 17 genera and c. 56 species endemic to Australia and New Zealand. Our phylogenetic and cytogenomic analyses revealed that MICR originated via an intertribal hybridization between ancestors of Crucihimalayeae (n = 8; maternal genome) and Smelowskieae (n = 7; paternal genome), both native to the Northern Hemisphere. The reconstructed ancestral allopolyploid genome (n = 15) originated probably in northeastern Asia or western North America during the Late Miocene (c. 10.6–7 million years ago) and reached the Australian mainland via long-distance dispersal. In Australia, the allotetraploid genome diverged into at least three main subclades exhibiting different levels of PPD and diversity: 1.25-fold descending dysploidy (DD) of n = 15 → n = 12 (autopolyploidy → 24) in perennial *Arabidella* (3 species), 1.5-fold DD of n = 15 → n = 10 in the perennial *Pachycladon* (11 spp.) and 2.1–3.75-fold DD of n = 15 → n = 7–4 in the largely annual crown-group genera (42 spp. in 15 genera). These results are among the first to demonstrate multispeed genome evolution in taxa descending from a common allopolyploid ancestor. It is suggested that clade-specific PPD can operate at different rates and efficacies and can be tentatively linked to life histories and the extent of taxonomic diversity.

KEYWORDS
Brassicaceae, comparative genomics, distant hybridization, long-distance dispersal, phylogeography, whole-genome duplication

1 | INTRODUCTION

Species, genera or whole clades occurring outside diversity centres of their respective lineages have always attracted the attention of biologists. The nature and mechanisms of these long-distance dispersal (LDD) events were analysed in a large set of land plants, particularly from reconstructed gene phylogenies, and have been reviewed on several occasions (e.g., Baldwin & Wagner, 2010; Blattner, 2006; Gillespie et al., 2012; Givnish et al., 2016; Nathan et al., 2008; Schaefer, Heibl, & Renner, 2009). Colonizations of other continents, new islands or habitats (ecological islands, Hughes & Atchison, 2015) were often followed by adaptive radiations and speciation events. In plants, colonization and diversification events are frequently associated with hybridization and polyploidy (e.g., Baldwin & Wagner, 2010; te Beest et al., 2012; Linder & Barker, 2014; Mummenhoff et al., 2004). Polyploidization (whole-genome duplication, WGD) can either precede a successful colonization event (e.g., Barrier, Baldwin, Robichaux, & Purugganan, 1999; Vijverberg, Mes, & Bachmann,

1999), or dispersal(s) of one or more species into new geographic regions and/or habitats may trigger hybridization and be the origin of new polyploid genomes and lineages (e.g., Ainouche, Baumel, Salmon, & Yannic, 2004; Mummenhoff et al., 2004; Paterson et al., 2012; Soltis et al., 2004).

Whereas the link between hybridization and genome doubling (predominantly allopolyploidization), on the one hand, and LDDs, colonization and adaptive radiations, on the other, has been corroborated repeatedly (for a review, see te Beest et al., 2012; Linder & Barker, 2014), we have only a very limited knowledge of what genomic changes accompany diversification of polyploid founders into new species and clades. In a broad sense, it might be envisaged that populations of the allopolyploid founder would gradually become reproductively isolated and differentiate into new species. This could be followed by autopolyploidization or interspecies hybridization and be the origin of new auto/allopolyploid genomes. In parallel, polyploid genomes are exposed to postpolyploid diploidization (PPD, Dodsworth, Chase, & Leitch, 2016) through intra- and inter-subgenome (homeologous) recombination, resulting in chromosomal rearrangements and genome-wide reorganization, including aneuploidy and descending dysploidy (i.e., reduction in chromosome number). We know that except for gross chromosomal changes (e.g., Chester et al., 2012; Geiser, Mandáková, Arrigo, Lysak, & Parisod, 2016; Mandáková, Gloss, Whiteman, & Lysak, 2016; Murat et al., 2014), PPD includes a range of diverse processes, such as genome downsizing (Zenil-Ferguson, Ponciano, & Burleigh, 2016 and references therein), epigenetic reprogramming, modulation of gene expression (e.g., Chelaifa, Monnier, & Ainouche, 2010; Flagel, Udall, Nettleton, & Wendel, 2008) and genome-specific fractionation, including biased gene retention/loss (e.g., Conant, Birchler, & Pires, 2014; Freeling, 2009; Garsmeur et al., 2013).

As PPD is an understudied topic (Dodsworth et al., 2016), the functional links between diploidization and adaptive radiations and speciation have only recently been unveiled. On the whole-genome level, it remains elusive whether diploidization is largely incidental to speciation or whether it can promote postpolyploid radiations. The fact that postpolyploid diversification in several angiosperm lineages occurred million of years after the respective WGDs (Schranz, Mohammadin, & Edger, 2012) points to an important dependency between PPD and increased rates of net diversification in palaeo- and mesopolyploid clades (Dodsworth et al., 2016; Hohmann, Wolf, Lysak, & Koch, 2015; Tank et al., 2015).

The Australian flora consists of some 20,000 to 21,000 angiosperm species, 90% of which are endemic (Chapman, 2009). Transoceanic dispersals played a major role in the origin of the present-day Australian flora (see Crisp & Cook, 2013), and several documented LDDs from other landmasses to Australia and New Zealand were associated with hybridization and polyploidization (e.g., Dierschke, Mandáková, Lysak, & Mummenhoff, 2009; Mummenhoff et al., 2004; Prebble, Cupido, Meudt, & Garnock-Jones, 2011; Vijverberg et al., 1999). However, except for chromosome counts, ploidy levels and parental species inferred from reconstructed molecular phylogenies, none of the immigrant lineages were analysed for detailed genome structures in the context of postcolonization diversification.

The tribe Microlepidieae (named MICR thereafter) includes 17 genera and c. 56 species endemic to Australia (16 genera) and New Zealand (*Pachycladon*; Heenan, Goeke, Houliston, & Lysak, 2012; Warwick, Mummenhoff, Sauder, Koch, & Al-Shehbaz, 2010; see Table S1 for the complete species list). The genus *Pachycladon* comprises ten species endemic to the South Island of New Zealand and one to Tasmania (Heenan & Mitchell, 2003; Heenan et al., 2012). However, most tribe members (i.e., 16 genera and 45 spp.) occur on the Australian mainland, with only two species co-occurring in Tasmania. Within Australasia, the diversity of MICR is comparable with another crucifer tribe, Lepidieae, harbouring some 36 and 19 *Lepidium* species endemic to Australia and New Zealand, respectively (Hewson, 1981; de Lange, Heenan, Houliston, Rolfe, & Mitchell, 2013).

Compared to many Eurasian crucifer clades, the 16 Australian genera and the New Zealand *Pachycladon* were neglected and their phylogenetic position was unknown until recently. The first representative phylogenetic analysis of *Pachycladon* revealed its origin through an intertribal hybridization (Joly, Heenan, & Lockhart, 2009). Later, the allopolyploid origin of *Pachycladon* was confirmed by comparative chromosome painting (CCP) analysis (Mandáková, Heenan, & Lysak, 2010), revealing that probably all 11 *Pachycladon* species (n = 10 chromosomes) possess the same genome structure resulting from a merger of two identical or very similar parental genomes with eight chromosomes (known as the Ancestral Crucifer Karyotype—ACK; Schranz, Lysak, & Mitchell-Olds, 2006). The origin of the ten *Pachycladon* chromosomes was reconstructed as PPD of the ancestral mesotetraploid genome (n = ~16) accompanied by descending dysploidy (~16 → 10). These data also showed that both progenitor genomes belonged to the supertribe Lineage I (Franzke, Lysak, Al-Shehbaz, Koch, & Mummenhoff, 2011; Clade A sensu Huang et al., 2016), and not to Lineages I and II (Franzke et al., 2011; Clade B sensu Huang et al., 2016), as suggested by Joly et al. (2009).

A later detailed phylo-cytogenetic study of three Australian MICR species revealed their allopolyploid origin concealed by extensive PPD, including up to fourfold descending dysploidy (Mandáková, Joly, Krzywinski, Mummenhoff, & Lysak, 2010). As for *Pachycladon*, CCP data suggested that the three diploid Australian MICR species (n = 4–6) have descended from a mesotetraploid genome (n = ~16) merging two ACK-like genomes. This and other phylogenetic studies (Heenan et al., 2012; Zhao, Liu, Tan, & Wang, 2010) confirmed that the parental genomes of *Pachycladon* and Australian genera have to be searched for among the Northern Hemisphere tribes of Lineage I/Clade A, but failed to narrow down the putative parental genomes.

All these studies raise a number of questions on the origin of the New Zealand and Australian MICR taxa and its spatiotemporal context. For instance, did both MICR subclades originate through a single or two different hybridization events? Which of the two alternative scenarios better explains the strikingly different extent of genome diploidization between the New Zealand *Pachycladon* and the Australian genera? Did the allopolyploidization(s) take place in

Australasia or in the Northern Hemisphere followed by LDD of the ancestor(s) to Australia/New Zealand? Are there any causal relationships between diploidization patterns and the phylotaxonomic diversity of the 16 Australian MICR genera? To settle these issues, we embarked on a comprehensive phylogenetic analysis with the aim of inferring the most probable parental genomes of the MICR clade, and to gain insight into phylogenetic relationships within the tribe. Complementary to the phylogenetics, we carried out CCP analysis of nine different MICR genera and several putative parental genomes to identify possible parent-specific genomic signatures and to obtain new information on the role of the PPD process in diversification of a monophyletic mesopolyploid clade.

2 | MATERIALS AND METHODS

2.1 | Plant material

Plants analysed cytogenetically were provided as seeds by institutions or individuals and cultivated under standard conditions (21/18°C, 16/8 h of light/dark) in growth chambers. The same plants were used for phylogenetic analyses in addition to the donated silica-dried leaf samples. The origins of all plant materials are listed in Table S2.

2.2 | DNA extraction, PCR amplification and cloning

Genomic DNA was extracted from either fresh or silica-dried leaves using a modified CTAB method (Doyle & Doyle, 1987). Two nuclear genes (chalcone synthase—*CHS* and phytochrome A—*PHYA*) and one chloroplast marker (NADH dehydrogenase subunit F—*ndhF*) were selected for phylogenetic analyses. Markers and PCR conditions were taken from published studies by Koch, Haubold, and Mitchell-Olds (2000) for *CHS*, Beilstein, Al-Shehbaz, Mathews, and Kellogg (2008) for *PHYA* and Beilstein, Al-Shehbaz, and Kellogg (2006) for *ndhF*. PCR products of nuclear genes were extracted from agarose gels using a QIAEX II Gel Extraction Kit (Qiagen) and cloned using the pGEM Teasy vector system (Promega). Subsequently, 5–10 clones per accession were sequenced (Macrogen) to identify expected homeologous copies (orthologs/paralogs) in MICR species. Clone sequences that shared >99% similarity with each other were assumed to come from a single gene copy, and only one representative clone was retained for the subsequent analyses. Purified amplicons of *ndhF* gene were sequenced directly. The DNA sequences for *CHS* (KY212711–KY212741, KY228937, and MF287798–MF287799), *PHYA* (KY228895–KY228936 and MF287796–MF287797) and *ndhF* (KY172921–KY172945) were deposited to the GenBank database.

2.3 | Phylogenetic analysis

A megablast nucleotide search in the GenBank database using *A. thaliana* sequences as a query (*CHS*—AF112086, *PHYA*—HM138769 and *ndhF*—HM120263) was performed to identify available Brassicaceae sequences. Based on earlier phylogenetic analyses (e.g., Beilstein, Nagalingum, Clements, Manchester, & Mathews, 2010; Heenan et al., 2012; Mandáková, Joly, et al., 2010; Zhao et al., 2010), *CHS* and *PHYA* sequences, representing 13 of the 15 tribes belonging to Lineage I/Clade A according to Al-Shehbaz (2012), were analysed (Table S3). The maternal phylogeny was based on *ndhF* sequences for 12 Lineage I tribes (Table S3). DNA sequences were aligned using MAFFT 7.017 (Katoh & Standley, 2013), implemented in GENEIOUS v8.1.8 (http://www.geneious.com, Kearse et al., 2012). UTR and introns were removed from final alignments as they were too variable. Multiple alignments of *CHS*, *PHYA* and *ndhF* genes can be found in the Dryad Digital Repository, https://doi.org/10.5061/dryad.q39k3. The substitution model for each gene was selected according to the Akaike Information Criterion (AIC) in JMODELTEST v2.1.7 software (Darriba, Taboada, Doallo, & Posada, 2012). The evolutionary models with the highest score were HKY+I+G for *CHS*, TVM+I+G for *PHYA* and GTR+I+G for *ndhF*. The Bayesian analysis of nuclear genes was conducted using MRBAYES v3.2.3 (Ronquist & Huelsenbeck, 2003). Two independent Markov chain Monte Carlo (MCMC) analyses were run for 5×10^6 generations, sampling trees every 1,000 generations and discarding 25% of samples as burn-in. Distributions of posterior probabilities obtained from MRBAYES were evaluated by TRACER v1.6 (http://tree.bio.ed.ac.uk/software/tracer/).

2.4 | Molecular dating

Divergence time dating using the *ndhF* gene was performed in BEAST v2.3.2 (Bouckaert et al., 2014). Two secondary calibration points were used based on Hohmann et al. (2015): (i) split of *Aethionema* from the Brassicaceae crown-group—32.4 mya (±4 my) and (ii) split of *A. thaliana* from the rest of *Arabidopsis* species—5.97 mya (±2 my). The relaxed clock log normal approach with birth–death model was used. Two independent MCMC runs were carried out for 100 million generations, sampling trees every 5,000 generations. The first 10% of trees were discarded as burn-in. The resulting trees from both runs were combined in LogCombiner, and the maximum clade credibility (MCC) tree was generated by TREEANNOTATOR. Distributions of posterior probabilities were evaluated by TRACER v1.6. The MCC tree was visualized in FIGTREE v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

2.5 | Detection of diversification rate shifts

To identify putative shifts in net diversification rate in the MICR clade, a BAMM analysis (Rabosky, 2014) was performed on the *ndhF* MCC tree. For this analysis, the MCC tree was pruned so that only the MICR clade and related tribes were retained. Incomplete taxon sampling was accounted for using estimates of sampling fraction for the MICR clade and for each of the tribes (indicated in Table S4). Two rounds of BAMM analysis were conducted: one with only the MICR clade taxa and the other with the MICR clade plus the other

Brassicaceae tribes in Figure 1c. BAMM (version 2.5.0) was run for 10 million generations on four MC3 chains with a sampling frequency of every 100 steps and with 25% of the initial samples discarded as burn-in (using default priors). The trace was summarized and visualized using the R package BAMMTOOLS (version 2.1.6). All the evolutionary and statistical analyses (as described in this and subsequent section) were performed using R (R Core Team 2017).

2.6 | Inference of trait-dependent diversification

Using the BiSSE (Maddison, Midford, & Otto, 2007) framework, we tested whether diversification rates in the MICR clade are dependent on the life history state of the subclades. Briefly, the BiSSE model describes the rates of speciation and extinction of lineages under two states, for example, annuality vs. perenniality (see Table S1), as well as the rates of transition between the two states along a given phylogeny. We estimated the six parameters of the BiSSE model using the following MCMC procedure. One hundred trees were randomly selected from the set of Beast *ndhF* phylogenies; all species not belonging to the MICR clade were pruned from each tree. For each tree, we applied the MCMC procedure as implemented in the R package DIVERSITREE (version 0.9-8; FitzJohn, 2012), using exponential priors on all the parameters and accounting for incomplete taxon sampling using an estimated global sampling fraction of ~45% (Table S4). For the first tree, starting parameters were determined using a state-independent birth–death model, while for all other trees the parameters sampled at the last generation of the preceding tree were used as the starting point. For the first tree, a MCMC chain was run for 2,000 generations (with 1,000 discarded as burn-in) with a sampling frequency of every 10 steps; for the remaining 99 trees, a MCMC chain was run for 1,500 generations (with 500 discarded as burn-in). The MCMC samples from the 100 trees were pooled together to form a posterior distribution for the rates of speciation and extinction of annual and perennial lineages. In particular, we computed the posterior probability that the net diversification rate (i.e., speciation rate minus extinction rate) of the annual lineages in the MICR clade is higher than that of the perennial lineages. We interpreted a high posterior probability (i.e., >0.90) to be evidence for higher diversification rates in annual versus perennial lineages.

2.7 | Chromosome preparation

For chromosome preparations, entire young inflorescences were collected in ethanol/acetic acid (3:1) fixative, fixed overnight and stored in 70% ethanol at −20°C until analysis. Mitotic and meiotic (pachytene) chromosome preparations were prepared as described by Mandáková and Lysak (2016a). Chromosome preparations were treated with 100 μg/ml RNase in 2× sodium saline citrate (SSC; 20× SSC: 3 M sodium chloride, 300 mM trisodium citrate, pH 7.0) for 60 min and with 0.1 mg/ml pepsin in 0.01 M HCl at 37°C for 5 min, then postfixed in 4% formaldehyde in distilled water and dehydrated in an ethanol series (70%, 90% and 100%, 2 min each).

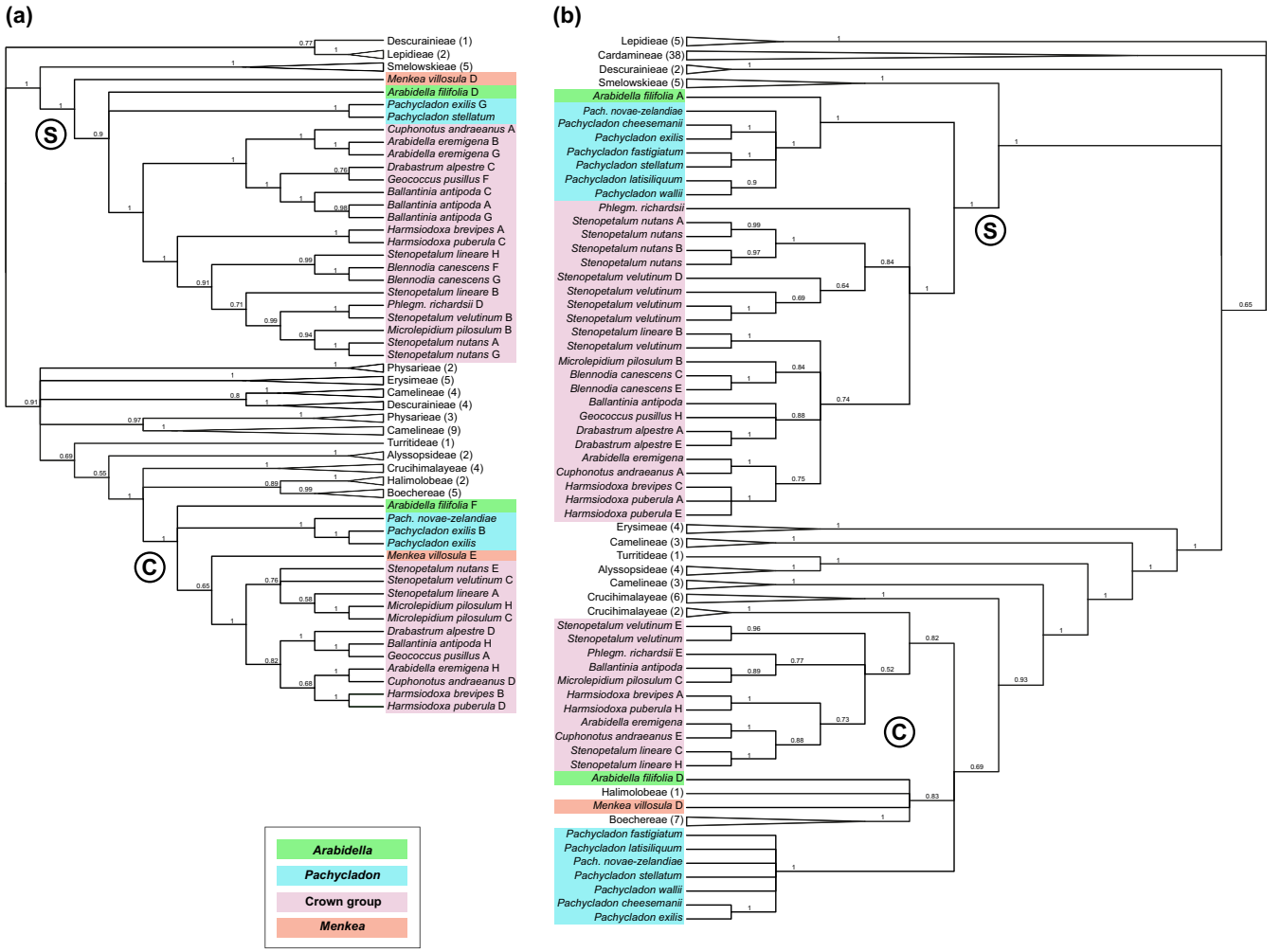
2.8 | DNA probes for comparative chromosome painting

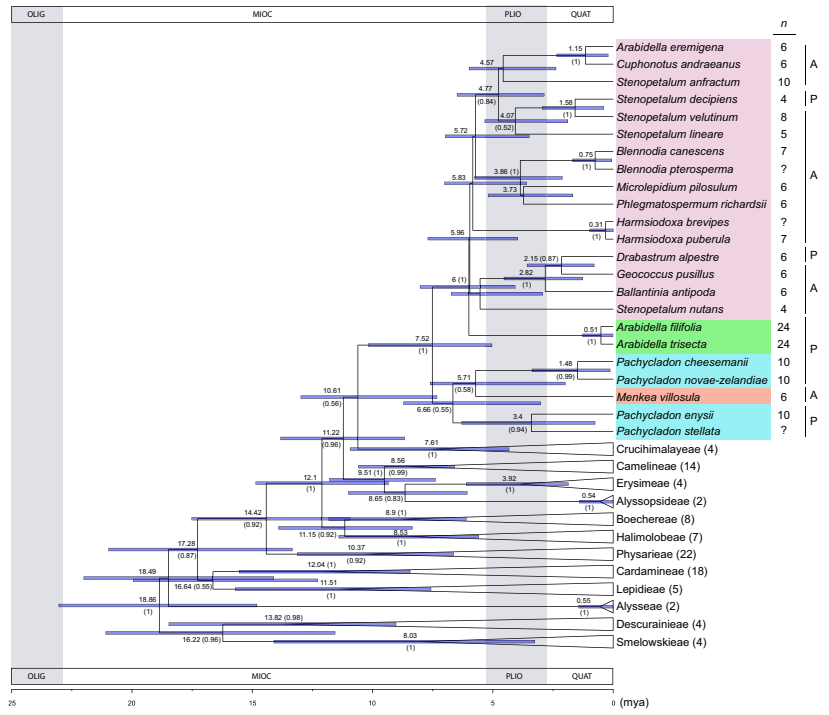
For CCP, on average every third *Arabidopsis thaliana* BAC clone was used to establish contigs corresponding to the 22 genomic blocks (GBs) of the Ancestral Crucifer Karyotype (ACK, Lysak, Mandáková, & Schranz, 2016; Schranz et al., 2006). For BAC contigs used for CCP, see Mandáková and Lysak (2008); for borders of individual GBs, see the ideogram in Figure 2b. To determine and characterize species-specific inversions and splits of GBs, after initial CCP experiments, some BAC contigs were split into smaller subcontigs and differentially labelled. *A. thaliana* BAC clone T15P10 (AF167571) bearing 45S rRNA gene repeats was used for in situ localization of 45S rDNA, and *A. thaliana* clone pCT 4.2 (M65137), corresponding to a 500-bp 5S rRNA repeat, was used for localization of the 5S rDNA loci. All DNA probes were labelled with biotin-dUTP, digoxigenin-dUTP or Cy3-dUTP by nick translation as described by Mandáková and Lysak (2016b).

2.9 | CCP

Selected labelled DNA probes were pooled together, ethanol-precipitated, dissolved in 20 μl of 50% formamide, 10% dextran sulphate in 2× SSC and pipetted onto microscopic slides. The slides were heated at 80°C for 2 min and incubated at 37°C overnight. Posthybridization washing was performed in 20% formamide in 2× SSC at 42°C. Hybridized probes were visualized either as the direct fluorescence of Cy3-dUTP or through fluorescently labelled antibodies against biotin-dUTP and digoxigenin-dUTP following Mandáková and Lysak (2016b). Chromosomes were counterstained with 4′,6-diamidino-2-phenylindole (DAPI, 2 μg/ml) in Vectashield antifade. Fluorescence signals were analysed and photographed using a Zeiss Axioimager epifluorescence microscope and a CoolCube camera (MetaSystems). Individual images were merged and processed using

FIGURE 1 Reconstructed origin and relationships of Microlepidieae. Fifty per cent majority-rule consensus tree of the Bayesian inference of nuclear genes *PHYA* (a) and *CHS* (b). Two paralogous copies of MICR accessions are marked as C (for Crucihimalayeae) and S (for Smelowskieae). The other Lineage I/Clade A tribes are collapsed and the number of accessions analysed is given in parentheses. (c) *ndhF*-based chronogram (maternal phylogeny) of Brassicaceae Lineage I/Clade A tribes. Divergence times (million years ago) are displayed with 95% confidence intervals. Node posterior probabilities (PP) are indicated in parentheses; PP lower than 0.5 are not shown. Shown are also chromosome numbers and life histories of the analysed MICR species. A: annual; P: perennial; OLIG: Oligocene; MIOC: Miocene; PLIO: Pliocene; and QUAT: Quaternary. Tribal classification follows Al-Shehbaz (2012); chromosome numbers correspond to Table S1. Plant drawings are used with permission of the Museum of New Zealand, Te Papa Tongarewa, Wellington (*P. latisiliqua*), the Board of the Botanic Gardens and State Herbarium, Adelaide (*A. trisecta*), and the Royal Society of South Australia, Adelaide


(a)
Descurainieae (1)
Lepidieae (2)
Smelowskieae (5)
Menkea villosula D
Arabidella filifolia D
Pachycladon exilis G
Pachycladon stellatum
Cuphonotus andraeanus A
Arabidella eremigena B
Arabidella eremigena G
Drabastrum alpestre C
Geococcus pusillus F
Ballantinia antipoda C
Ballantinia antipoda A
Ballantinia antipoda G
Harmsiodoxa brevipes A
Harmsiodoxa puberula C
Stenopetalum lineare H
Blennodia canescens F
Blennodia canescens G
Stenopetalum lineare B
Phlegm. richardsii D
Stenopetalum velutinum B
Microlepidium pilosulum B
Stenopetalum nutans A
Stenopetalum nutans G
Physarieae (2)
Erysimeae (5)
Camelineae (4)
Descurainieae (4)
Physarieae (3)
Camelineae (9)
Turritideae (1)
Alyssopsideae (2)
Crucihimalayeae (4)
Halimolobeae (2)
Boechereae (5)
Arabidella filifolia F
Pach. novae-zelandiae
Pachycladon exilis B
Pachycladon exilis
Menkea villosula E
Stenopetalum nutans E
Stenopetalum velutinum C
Stenopetalum lineare A
Microlepidium pilosulum H
Microlepidium pilosulum C
Drabastrum alpestre D
Ballantinia antipoda H
Geococcus pusillus A
Arabidella eremigena H
Cuphonotus andraeanus D
Harmsiodoxa brevipes B
Harmsiodoxa puberula D
S
C
(b)
Lepidieae (5)
Cardamineae (38)
Descurainieae (2)
Smelowskieae (5)
Arabidella filifolia A
Pach. novae-zelandiae
Pachycladon cheesemanii
Pachycladon exilis
Pachycladon fastigiatum
Pachycladon stellatum
Pachycladon latisiliquum
Pachycladon wallii
Phlegm. richardsii
Stenopetalum nutans A
Stenopetalum nutans
Stenopetalum nutans B
Stenopetalum nutans
Stenopetalum velutinum D
Stenopetalum velutinum
Stenopetalum velutinum
Stenopetalum velutinum
Stenopetalum lineare B
Stenopetalum velutinum
Microlepidium pilosulum B
Blennodia canescens C
Blennodia canescens E
Ballantinia antipoda
Geococcus pusillus H
Drabastrum alpestre A
Drabastrum alpestre E
Arabidella eremigena
Cuphonotus andraeanus A
Harmsiodoxa brevipes C
Harmsiodoxa puberula A
Harmsiodoxa puberula E
Erysimeae (4)
Camelineae (3)
Turritideae (1)
Alyssopsideae (4)
Camelineae (3)
Crucihimalayeae (6)
Crucihimalayeae (2)
Stenopetalum velutinum E
Stenopetalum velutinum
Phlegm. richardsii E
Ballantinia antipoda
Microlepidium pilosulum C
Harmsiodoxa brevipes A
Harmsiodoxa puberula H
Arabidella eremigena
Cuphonotus andraeanus E
Stenopetalum lineare C
Stenopetalum lineare H
Arabidella filifolia D
Halimolobeae (1)
Menkea villosula D
Boechereae (7)
Pachycladon fastigiatum
Pachycladon latisiliquum
Pach. novae-zelandiae
Pachycladon stellatum
Pachycladon wallii
Pachycladon cheesemanii
Pachycladon exilis
S
C
Arabidella
Pachycladon
Crown group
Menkea


(c)
OLIG
MIOC
PLIO
QUAT
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Stenopetalum decipiens 4 P
Stenopetalum velutinum 8
Stenopetalum lineare 5
Blennodia canescens 7
Blennodia pterosperma ? A
Microlepidium pilosulum 6
Phlegmatospermum richardsii 6
Harmsiodoxa brevipes ?
Harmsiodoxa puberula 7
Drabastrum alpestre 6 P
Geococcus pusillus 6
Ballantinia antipoda 6 A
Stenopetalum nutans 4
Arabidella filifolia 24
Arabidella trisecta 24 P
Pachycladon cheesemanii 10
Pachycladon novae-zelandiae 10
Menkea villosula 6 A
Pachycladon enysii 10 P
Pachycladon stellata ?
Crucihimalayeae (4)
Camelineae (14)
Erysimeae (4)
Alyssopsideae (2)
Boechereae (8)
Halimolobeae (7)
Physarieae (22)
Cardamineae (18)
Lepidieae (5)
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Descurainieae (4)
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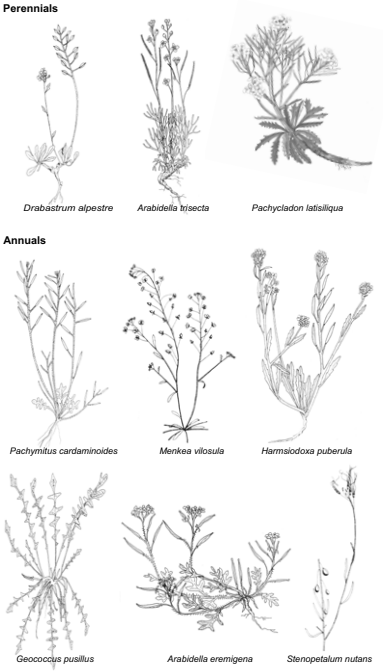
25
20
15
10
5
0 (mya)


Perennials
Drabastrum alpestre
Arabidella trisecta
Pachycladon latisiliqua
Annuals
Pachymitus cardaminoides
Menkea vilosula
Harmsiodoxa puberula
Geococcus pusillus
Arabidella eremigena
Stenopetalum nutans

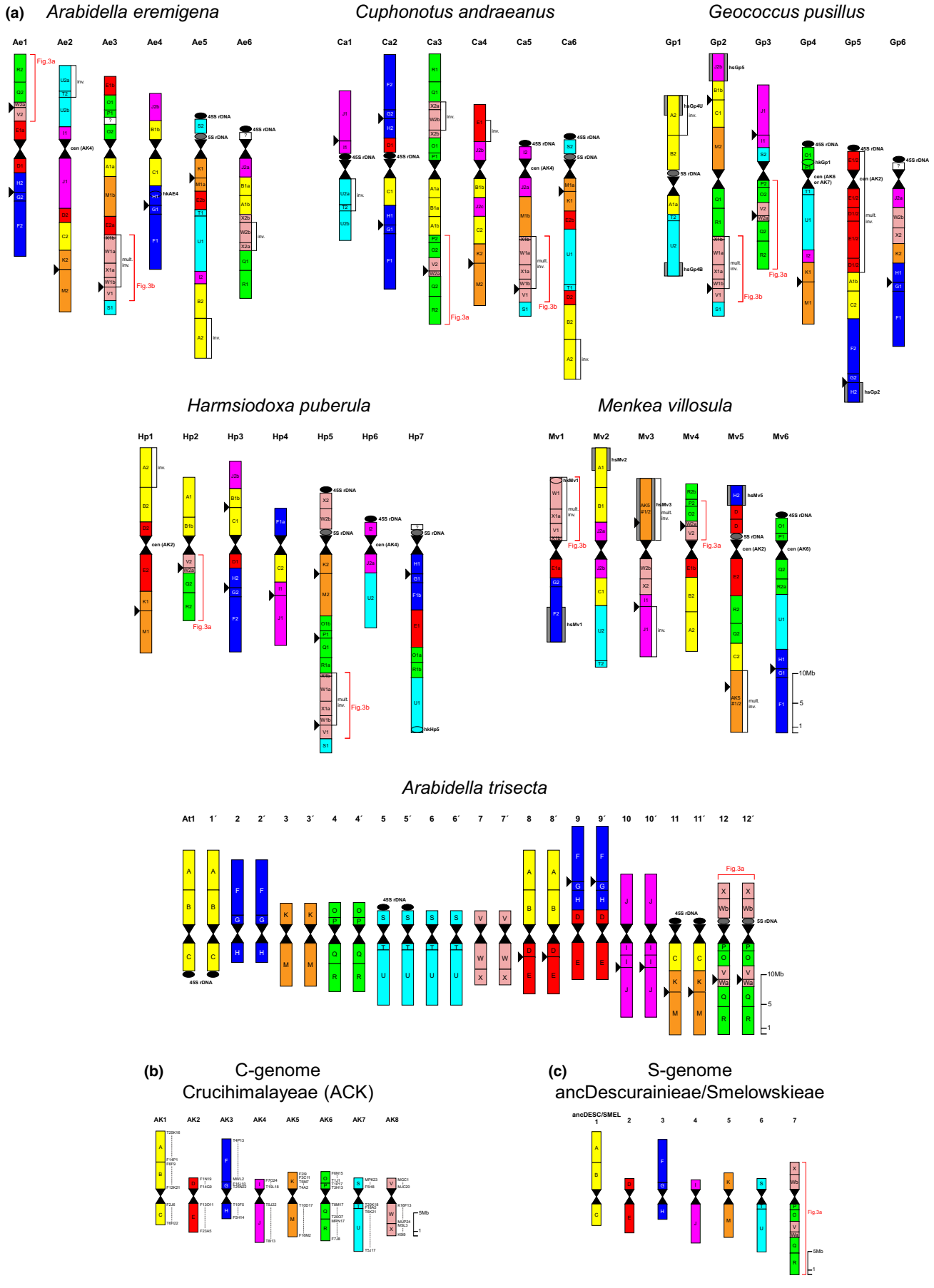

(a)
Arabidella eremigena
Cuphonotus andraeanus
Geococcus pusillus
Harmsiodoxa puberula
Menkea villosula
Arabidella trisecta
(b)
C-genome
Crucihimalayeae (ACK)
(c)
S-genome
ancDescurainieae/Smelowskieae
45S rDNA
5S rDNA
cen (AK4)
cen (AK2)
cen (AK6 or AK7)
cen (AK6)
hkAE4
hsGp1
hsGp2
hsGp4U
hsGp4B
hsGp5
hsMv1
hsMv2
hsMv3
hsMv5
hkHp5
Fig.3a
Fig.3b
10Mb
5
1
5Mb

FIGURE 2 Comparative cytogenomic maps of the six analysed Microlepidieae species and two putative parental genomes. (a) Six comparative maps based on CCP analyses. Black arrowheads point to the inferred sites of lost ancestral centromeres. Inv.: inversion; hk: heterochromatic knob; hs: heterochromatic segment. (b) Maternal Crucihimalayeae-like genome structurally resembling Ancestral Crucifer Karyotype (ACK). BAC clones of *Arabidopsis thaliana* defining painting probes used for each of the 22 genomic blocks (A-X) are shown. (c) Paternal ancDESC/SMEL genome—ancestral karyotype of Descurainieae and Smelowskieae. In all ideograms, colour coding and capital letters correspond to the eight chromosomes and 22 genomic blocks (A-X) of ACK, respectively. Block splits into two or three parts are labelled as "a," "b," and "c." All ideograms are drawn to scale, whereby Mb units refer to the *A. thaliana* genome sequence at The Arabidopsis Information Resource (TAIR, http://www.arabidopsis.org)

PHOTOSHOP CS software (Adobe Systems). Pachytene chromosomes were straightened using the "straighten-curved-objects" plugin in the IMAGE J software (Kocsis, Trus, Steer, Bisher, & Steven, 1991).

3 | RESULTS

3.1 | *CHS* and *PHYA* phylogenies point to an allopolyploid origin of the MICR clade and reveal its parental genomes among tribes of Clade A

As all previous studies on the phylogenetic placement of the MICR were inconclusive and/or suffered from limited taxon sampling (Heenan et al., 2012; Huang et al., 2016; Joly et al., 2009; Mandáková, Joly, et al., 2010; Warwick et al., 2010; Zhao et al., 2010), we carried out phylogenetic analysis using two nuclear genes—*CHS* (chalcone synthase) and *PHYA* (phytochrome A). The aligned data matrix of DNA sequences comprised 1,041 characters in 227 taxa for *CHS* and 1,705 characters in 273 taxa for *PHYA*. PCR amplification and subsequent cloning of both nuclear genes usually resulted in retrieving two or more paralogous copies for the MICR species. At least two paralogous copies of *PHYA* were identified in 14 of 19 MICR species analysed, and similar results were obtained for *CHS* gene with two paralogues identified in 17 of 22 MICR species analysed. Higher numbers (i.e., three or more) of paralogous gene copies indicated that the MICR species has undergone two subsequent polyploidizations (e.g., *Stenopetalum velutinum*, Mandáková, Joly, et al., 2010). In both gene trees, gene paralogues of the MICR species formed two clades assigned as C- and S-clades, respectively (Figure 1a,b). In the *PHYA* phylogeny (Figure 1a), the C-clade clustered with the representatives of Boechereae (BOEC), Crucihimalayeae (CRUC) and Halimolobeae (HALI), whereas the S-clade was highly supported (posterior probability, PP 1.0) as sister to Smelowskieae (SMEL). Within the C-clade, *Arabidella filifolia*, *Pachycladon* and the MICR crown-group including *Menkea* were retrieved as three subclades. Within the S-clade, *A. filifolia*, *Pachycladon* and the crown-group formed a polytomic clade sister to *Menkea*. In the *CHS* tree (Figure 1b), the C-clade was retrieved as sister to CRUC but polyphyletic due to BOEC and HALI embedded within the clade. The monophyletic S-clade was recovered as sister to SMEL (PP 1.0), with two subclades—*A. filifolia* + *Pachycladon* and the MICR crown-group (S-clade paralogous copy of *CHS* was not retrieved for *Menkea*). In both nuclear gene trees, *Arabidella* was polyphyletic—the perennial *A. filifolia* formed own subclade outside the MICR crown-group, whereas the annual *A. eremigena* was embedded in the crown-group and sister to *Cuphonotus*.

The recovery of two paralogous genomic copies of *CHS* and *PHYA* in most MICR species corroborated the allotetraploid origin of the tribe. SMEL were pinpointed as the ancestor of the S-clade subgenome, whereas tribes BOEC/CRUC/HALI were identified as the candidate parental donor of the C-clade subgenome (Figure 1a, b).

3.2 | Time-calibrated *ndhF* phylogeny suggests Crucihimalayeae as the maternal ancestor of MICR and dates the hybridization event between 10 and 7 mya

To further investigate the allopolyploid origin of MICR inferred from nuclear phylogenies and to date the purported intertribal hybridization event, we have analysed the maternally inherited *ndh*F (NADH dehydrogenase subunit F) gene in 201 Brassicaceae taxa, including 24 MICR species. The aligned data matrix of DNA sequences comprised 1,969 characters. Within the *ndh*F tree, MICR genera were recovered as a monophyletic clade sister to tribe CRUC (Figure 1c). Thus, *ndh*F data further narrowed down CRUC, although with lower support (PP 0.56), as the most probable maternal donor of the C-clade subgenome of MICR taxa. The well-supported MICR clade (PP 1.0) consisted of two subclades formed by *A. filifolia* and *A. trisecta* together with the crown-group genera and by *Pachycladon* species with *Menkea* nested among them. The peculiar position of *Menkea* breaking the monophyly of *Pachycladon* was not recovered in the nuclear gene phylogenies (Figure 1a,b). *Arabidella* and *Stenopetalum* were recovered as polyphyletic; *Ballantinia*, *Drabastrum* and *Geococcus* formed a monophyletic subclade. The polyphyletic character of *Arabidella* retrieved in all three gene phylogenies is in full agreement with conclusions of Heenan et al. (2012) and reflects the existence of two species groups (cf. Shaw, 1965; Wege & Lepschi, 2007): one of three perennial shrubby species (*Arabidella* sensu stricto) and a second one containing four annual herbaceous species.

We used the *ndh*F phylogeny as the basis for divergence time estimation analyses. To date the origin of MICR and closely related tribes, two secondary calibration points were used (Hohmann et al., 2015): 32.42 million years ago (mya) for the split between the Aethionemeae and the Brassicaceae crown-group and 5.97 mya for the split between *A. thaliana* and the remaining *Arabidopsis* species. According to our analysis, the initial diversification of Lineage I/

Clade A tribes occurred during the Early Miocene (approx. 19 mya) and the most recent common ancestor (MRCA) of CRUC and SMEL was dated to 18.66 mya [95% highest posterior density interval (HPD) = 23.04–14.81]. The MRCA of tribes CRUC and MICR was dated to 10.61 mya (95% HPD = 12.98–7.32)—the Late Miocene (Tortonian). The divergence of (*Pachycladon* + *Menkea*) from the clade comprising other MICR genera was estimated at 7.52 mya (95% HPD = 10.17–5.04) and that between *Arabidella filifolia*/*trisecta* and the crown-group Australian MICR at 6 mya (95% HPD = 8.03–4.07). These divergence time estimates date the origin of the CRUC × SMEL hybrid between 10.61 and 7.52 mya to the Late Miocene (the Tortonian–Messinian transition).

3.3 | Cytogenomic analysis of ten species reveals the mesotetraploid origin of the MICR clade

Karyotypes of nine species with two diploid-like chromosome numbers (n = 6 and 7) and that of *Arabidella trisecta* with a polyploid-like number (n = 24) were investigated by CCP using chromosome-specific BAC contigs of *Arabidopsis thaliana*. The CCP approach reveals the extent of shared chromosomal collinearity between an analysed species, the *A. thaliana* genome as well as other extant and hypothetical crucifer genomes. By selecting the 10 species, we largely covered the phylogeographic and life form diversity of Australian MICR genera (Figure 1 and Table S3). Except for *Arabidella trisecta* ($2n$ = 48) and *Menkea villosula* ($2n$ = 12), the following species from the MICR crown-group were analysed: *A. eremigena* ($2n$ = 12), *Blennodia canescens* ($2n$ = 14), *Cuphonotus andraeanus* ($2n$ = 12), *Drabastrum alpestre* ($2n$ = 12), *Geococcus pusillus* ($2n$ = 12), *Harmsiodoxa puberula* ($2n$ = 14), *Microlepidium pilosulum* ($2n$ = 12) and *Phlegmatospermum richardsii* ($2n$ = 12). Based on CCP and comparisons with ACK, detailed comparative cytogenetic maps were constructed for six MICR species (Figure 2a) and draft maps for another four species (*B. canescens*, *D. alpestre*, *Mi. pilosulum* and *P. richardsii*). In the latter four species, higher levels of chromosome heterochromatinization and corresponding clumping of pachytene bivalents precluded construction of detailed cytogenetic maps. Results of comparative cytogenetic analyses of MICR species are detailed in Appendix S1.

Fluorescently labelled chromosome-specific BAC contigs from *Arabidopsis thaliana*, representing eight ancestral chromosomes (AK1 to AK8) and 22 (A–X) ancestral genomic blocks (GBs) of ACK (Figure 2b; Lysak et al., 2016; Schranz et al., 2006), were used to paint pachytene complements of the 10 MICR species. 99% of painting probes unambiguously identified two homeologous regions within the nine haploid chromosome complements and four homeologous regions in the haploid complement of *A. trisecta* (see Figure 3 for examples of CCP and Appendix S1).

As these BAC contigs decorated unique chromosomal regions in diploid *Arabidopsis* species (e.g., Lysak, Fransz, Ali, & Schubert, 2001), two genomic copies in MICR genomes were interpreted as evidence of allotetraploidization (cf. Mandáková, Heenan, et al., 2010; Mandáková, Joly, et al., 2010), whereas four homeologous copies in *A. trisecta* indicated an additional duplication of the allotetraploid genome (neo-octoploidy).

3.4 | Chromosomal rearrangements corroborate Smelowskieae as the putative paternal genome of MICR

Three species of two tribes, namely SMEL (*Smelowskia altaica*, $2n$ = 12, Figure S1) and Descurainieae (DESC, *Descurainia tanacetifolia*, $2n$ = 14, Figure 2c and Figure S1; and *Hornungia alpina*, $2n$ = 12, Lysak et al., 2006), were investigated by CCP as the candidate paternal S-genome. DESC were repeatedly found to be closely related to SMEL (Figure 1b,c; Beilstein et al., 2010; Couvreur et al., 2010; Mandáková, Joly, et al., 2010: figure S4; Zhao et al., 2010). In the purported paternal SMEL-like genome, represented here by *S. altaica* (n = 6), three modern chromosomes (Sa1, Sa2 and Sa3) have retained the ancestral structure of AK1, AK4 and AK7, whereas the other three chromosomes originated by shuffling of the remaining five AK chromosomes (Figure S1). Our parsimonious reconstruction unequivocally showed that one of the three translocation ("fusion") chromosomes was formed by an end-to-end translocation (EET) between chromosomes AK6 and AK8, accompanied by centromere inactivation and a pericentric inversion later on (Figure S1). In DESC (*D. tanacetifolia*, n = 7), six of the seven chromosomes resemble the ancestral AK chromosomes (Figure S1). The seventh chromosome (Dt7) was formed through EET between chromosomes AK6 and AK8, and later reshuffled by a pericentric inversion (Figure 3a and Figure S1). The same translocation chromosome AK6/8 was also identified in another member of DESC—*H. alpina* (Lysak et al., 2006). Based on the shared unique structure of chromosome AK6/8 and the close phylogenetic affinity between DESC and SMEL, we inferred an ancestral ancDESC/SMEL genome (Figure 2c) resembling the extant DESC karyotype with n = 7 (Figure S1). The ancDESC/SMEL genome originated from the more ancestral ACK (n = 8) via descending dysploidy (Figure 3a) and after the DESC-SMEL split was further reshuffled to form the modern genomes of SMEL with n = 6 (Figure S1).

In the ancDESC/SMEL genome, the AK6/8 chromosome comprises two unique GB associations, namely X#2-Wb#2 on the upper arm and P#2-O#2-V#2-Wa#2-Q#2-R#2 on the bottom arm (Figure 2c and 3a). Comparison of these structures with genomes of MICR species revealed that the entire AK6/8 chromosome corresponds to two chromosomes in *A. trisecta* (At12 and At12') and chromosome Pc6 in *Pachycladon* (Mandáková, Heenan, et al., 2010), and it is also present within chromosome Ba2 in *Ballantinia antipoda* (Mandáková, Joly, et al., 2010). In all the analysed crown-group species, the bottom-arm association (P#2-O#2-V#2-Wa#2-Q#2-R#2) was always identified within the S-subgenome (Figure 2a, Mandáková, Joly, et al., 2010). Altogether, the unique ancDESC/SMEL-specific cytogenetic signature identified in all MICR species supports the monophyletic origin of the clade and identifies the ancDESC/SMEL-like subgenome as the paternal genome of the allopolyploid ancestor of MICR.

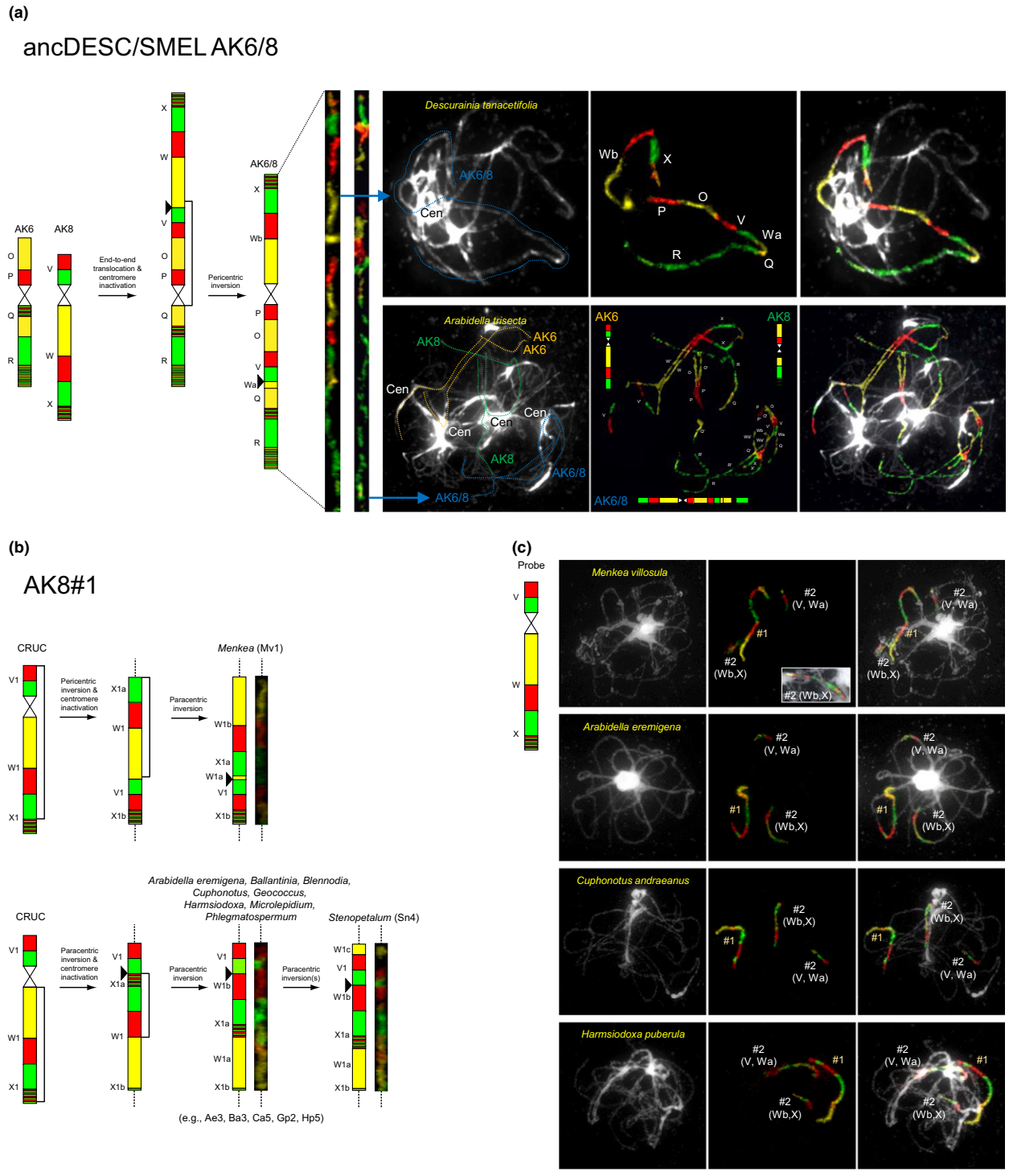


FIGURE 3 Parental- and subclade-specific chromosomal rearrangements identified by CCP in Microlepidieae species. (a) Parsimoniously reconstructed origin of chromosome AK6/8 and its CCP-based identification within pachytene complements of *Descurainia tanacetifolia* (representing the paternal ancDESC/SMEL genome, see Figure 2c) and *Arabidella trisecta*. Note that in the diploid genome of *D. tanacetifolia*, the AK6/8 probe identified only one bivalent, whereas in the mesoautopolyploid genome of *A. trisecta* the probe decorated one AK6/8 quadrivalent contributed by the paternal ancDESC/SMEL subgenome, and AK6 and AK8 quadrivalents of the maternal Crucihimalayeae-like subgenome. (b) Rearrangements of maternal-subgenome chromosome AK8#1 specific for the *Menkea* and crown-group subclade, respectively. (c) In situ localization of ancestral chromosome AK8 by CCP with differentially labelled *A. thaliana* BAC contigs on pachytene complements of crown-group species. The probe identified AK8#1 homeologue of the maternal subgenome and AK8#2 homeologue of the paternal subgenome later split between two different chromosomes (see Figure 2a). Capital letters refer to 22 genomic blocks (A-X) of ACK (Lysak et al., 2016). Black arrowheads point to the sites of lost ancestral centromeres. Three colours correspond to fluorochromes used to label BAC contig DNA (green—Alexa 488, yellow—Cy3 and red—Texas Red). Chromosomes were counterstained by DAPI and captured as black-and-white images

3.5 | Phylogenomic and geographic patterns pinpoint Crucihimalayeae as the putative maternal genome of MICR

Based on our phylogenetic analyses, we investigated the genome structure in selected species of Lineage I/Clade A tribes inferred to be putative maternal C-genome donor (Figure 1c). Five species of three tribes were analysed by CCP previously or within the present study: BOEC (*Boechera stricta*, 2*n* = 14; Mandáková, Schranz, Sharbel, de Jong, & Lysak, 2015), CRUC (*Crucihimalaya tibetica*, Figure 2b and Figure S1; *C. wallichii* and *Transberingia bursifolia*, Mandáková, Joly, et al., 2010; all 2*n* = 16) and HALI (*Pennelia micrantha*, 2*n* = 16, Figure S1).

The uniform base number of *n* = 7 (BrassiBase, Kiefer et al., 2014) and BOEC-specific rearrangements (Mandáková et al., 2015), absent in MICR genomes, make the North American BOEC an unlikely parental candidate. All the analysed CRUC and HALI species possess ACK-like genomes (*n* = 8) with no tribe- or MICR-specific chromosomal rearrangements (Figure S1 and Mandáková, Joly, et al., 2010). HALI species are restricted to Central and South America (Al-Shehbaz, 2012), with minimal distribution and no ecological overlaps with the paternal genome donor (SMEL) in the southwestern US states (Al-Shehbaz et al., 2010). In contrast, as CRUC overlap geographically with SMEL (Al-Shehbaz et al., 2010), and our *ndhF* tree (Figure 1c) and other published phylogenies (see above) pointed to a close relationship between CRUC and MICR, Crucihimalayeae were the most probable maternal progenitor of MICR.

3.6 | Ancestral allopolyploid CRUC × SMEL genome had 15 chromosome pairs

Combining the phylogenetic and cytogenomic evidence, we conclude that the MICR clade originated via an intertribal hybridization between ancestors of CRUC (maternal C-genome, *n* = 8) and SMEL (paternal S-genome, *n* = 7), and propose that the allotetraploid genome had 15 chromosome pairs (*n* = 15; Figure 4). The least diploidized genome of *A. trisecta*, with 67% of homeologues resembling the 15 ancestral chromosomes (Figure 2a), corroborates this conclusion.

3.7 | Subgenome-specific fractionation

In genomes of *Menkea* and the crown-group species analysed by CCP, one homeologous copy (#1) was, on average, longer and, in some instances, brighter than the other genomic copy (#2) (Figures 2 and 3). As the ancDESC/SMEL-specific rearrangement allowed us to assign the majority of GBs to either of the two parental subgenomes, on average shorter and weaker painting signals were found to belong to the paternal S-genome. Thus, the paternal subgenome has most likely undergone a more extensive and faster genome fractionation than the maternal C-genome. The biased chromosomal fractionation was not observed in the less diploidized genomes of *A. trisecta* (Figure 3a) and *Pachycladon* (Mandáková, Heenan, et al., 2010).

The subgenome-specific chromosomal fractionation was corroborated by sequence variability between maternal (C-genome) and paternal (S-genome) paralogous copies for *CHS* and *PHYA* genes for the crown-group species and *Menkea*. For *CHS* and *PHYA*, the average pairwise similarities of orthologous gene sequences were 95.5% (93.8–97.7%) and 96.7% (95.7–98.7%) for C-copy and 94.2% (89.3–97.9%) and 96.2% (93.0–98.9%) for S-copy. For *CHS*, the multiple nucleotide alignment of orthologous sequences possessed 83.7% of identical sites for the C-copy and 78.3% for the S-copy. Similarly, for *PHYA*, the multiple alignment possessed 86.2% of identical sites for the C-copy and 80.6% for the S-copy. Thus, S-genome orthologues, consistently exhibiting a broader sequence diversity, indicated slightly faster mutation rates of the paternal subgenome, presumably reflecting its more extensive fractionation at the chromosomal level.

3.8 | No diversification rate shifts could be detected among the MICR subclades

Establishing different levels of PPD for the three MICR groups (i.e., perennial *Arabidella* spp., *Pachycladon* and crown-group genera including *Menkea*), we conducted a BAMM analysis to test for statistically supported diversification rate shifts among the MICR subclades. This analysis did not detect statistically significant support for diversification rate shifts being correlated with the multispeed structural diploidization in MICR.

3.9 | Weak correlation between diversification and life forms in MICR

A BiSSE analysis was performed to test whether annual lineages diversify at different rates than perennial lineages in the MICR clade. The median net diversification rate of the annual lineages was higher than that of the perennial ones (0.38 vs. 0.21; Figure S2). This result is consistent with the annual MICR taxa undergoing more rapid diversification than their perennial relatives. Yet, the posterior probability that the diversification rate of annual lineages is higher than that of perennial ones was ~0.65, indicating that the association between life form and diversification in the MICR clade could not be statistically supported. Given such a small tree, it is quite possible that the failure to detect a correlation, if any, could be attributed to lack of data or insufficient signal.

4 | DISCUSSION

4.1 | MICR originated via hybridization between ancestors of Crucihimalayeae and Smelowskieae

The allopolyploid origin of MICR via an intertribal hybridization was previously proposed by Joly et al. (2009), Mandáková et al. (2010), Mandáková et al. (2010), Zhao et al. (2010) and Heenan et al. (2012). Whereas CRUC (formerly included within the broadly defined Camelineae, CAME) were purported to be a possible parental genome of MICR (Joly et al., 2009; Mandáková, Joly, et al., 2010; Zhao

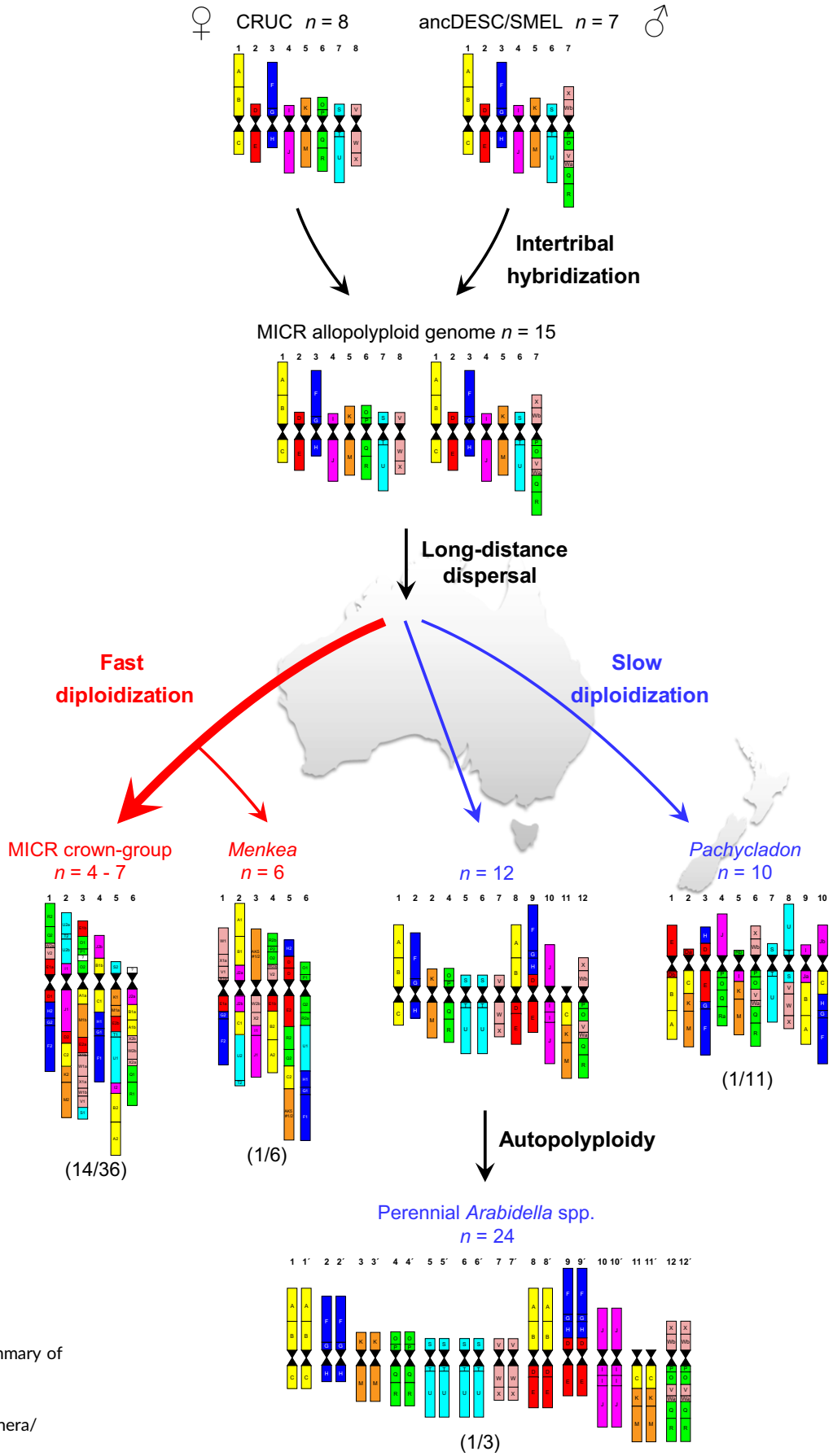


FIGURE 4 A phylogenomic summary of the origin and evolution of the Microlepidieae clade. Numbers in parentheses refer to number of genera/species

et al., 2010), the identity of the second parent was more elusive (cf. Heenan et al., 2012; Joly et al., 2009; Mandáková, Joly et al. 2010, Mandáková, Heenan et al. 2010). Joly et al. (2009) argued for an allopolyploid origin of *Pachycladon* by an intertribal hybridization between tribes of Lineage I (A genomic copy) and Lineage II (B copy for *Brassica*/Brassiceae). In a family-wide *CHS* phylogeny, Zhao et al. (2010) identified one parental genome of *Pachycladon* as being closely related to tribes BOEC, CRUC and HALI, whereas the second one clustered together with SMEL. Similarly, Beilstein et al. (2010: figure S2 and S3) showed *Pachycladon* to be sister to CRUC in the *ndh*F tree and to SMEL in the *PHYA* phylogeny. Here, based on phylogenetic reconstructions and cytogenomic analyses, we elucidated the origin of the mesotetraploid ancestor of Microlepidieae ($n = 15$) through an intertribal hybridization between ancestral genomes of tribes CRUC ($n = 8$) and SMEL ($n = 7$).

4.2 | Parental genomes could have co-occurred in the Northern Hemisphere

As the present-day distribution of the two parental clades is restricted to the Northern Hemisphere (mainly to Central and northeastern Asia and western North America) following the Brassicaceae radiation in Eurasia (Franzke, German, Al-Shehbaz, & Mummenhoff, 2009), the intertribal hybridization event must have occurred in the Northern Hemisphere. Tribe CRUC contains 13 species mostly distributed in the high mountains of Central and southwestern Asia; *Transberingia bursifolia* has a bicontinental distribution in the Russian Far East and northern and western North America (Al-Shehbaz, 2012; Al-Shehbaz et al., 2010). The closest extant relatives of the paternal ancDESC/SMEL genome have to be looked for among representatives of closely related tribes DESC, SMEL (e.g., Beilstein et al., 2010; Couvreur et al., 2010; Mandáková, Joly, et al., 2010: figure S4; Zhao et al., 2010 and this study) and the monogeneric Yinshanieae (YINS) as shown recently (Chen, Deng, Yue, Al-Shehbaz, & Sun, 2016). Tribe DESC comprise 6 genera and 45 species, dominated by 35 *Descurainia* species being particularly common in North and South America (Al-Shehbaz, 2012). Monospecific genera *Ianhedgea* and *Robeschia* are distributed in Central and southwestern Asia, and the Middle East, respectively. *Hornungia* (4 spp.) is centred in the mountains of Europe, while two other genera, *Trichotolinum* and *Tropidocarpum*, are confined to California, Central and South America (Al-Shehbaz, 2012; Al-Shehbaz, Beilstein, & Kellogg, 2006). Tribe SMEL contains 25 *Smelowskia* species distributed in Central to northeastern Asia and through the northwestern and western part of North America (Al-Shehbaz & Warwick 2006; Al-Shehbaz et al., 2010; Carlsen, Elven, & Brochmann, 2010). The YINS, at least four species endemic to southwestern and northern China, closely related to DESC and SMEL (Chen et al., 2016), represent mostly $n = 6$ genomes (Zhang, 2003) derived from the ancDESC/SMEL genome as in SMEL.

The tribes DESC, SMEL and YINS diverged from an ACK-like ancestor ($n = 8$) most likely in Central Eurasia, and the first two spread further east to North America via the Bering land bridge (Carlsen et al., 2010; Warwick, Al-Shehbaz, Sauder, Murray, & Mummenhoff, 2004). The extant amphiberingian distribution of *T. bursifolia* is probably the result of a single dispersal event from the Asian to the North American Beringia and further inland. The Bering land bridge served as an important migration route from Asia to the American continent in the Late Tertiary (Wen, Ickert-Bond, Nie, & Li, 2010) and several Brassicaceae groups are purported to have spread from Asia to North America (Carlsen et al., 2010; German & Koch, 2017; Koch & Al-Shehbaz, 2002; Mummenhoff, Brüggemann, & Bowman, 2001; Warwick et al., 2004).

CRUC, SMEL and YINS harbour mostly, although not exclusively, high-mountain and (sub)arctic species that may have co-occurred in the past and some still occur today in Central, North and northeastern Asia (*Crucihimalaya mollissima* and *S. calycina* in the Altai Mts., *T. bursifolia* and *Smelowskia* spp.; D. A. German, personal communication) and western North America (*T. bursifolia* and *S. porsildii* and *S. parryoides*: Alaska and Yukon, *T. bursifolia* and *S. americana*: the Rocky Mts.). From tribe DESC, *Ianhedgea minutiflora* and *H. procumbens* may co-occur with the Asian *Crucihimalaya* species, and a number of *Descurainia* species may co-occur with *T. bursifolia* subsp. *virgata* in North America. Considering the current distribution of CRUC and SMEL and the estimated age of the hybridization (10 to 7 mya), it seems conceivable to propose that the hybridization between ancestors of CRUC and SMEL has taken place in northeastern Asia or American Beringia.

4.3 | Long-distance dispersal to Australasia

The allotetraploid ancestor of MICR was transported to Australasia via a LDD. As spreading of small Brassicaceae seeds by sea currents is not likely, extreme meteorological events (Nathan et al., 2008; Gillespie et al. 2012) or dispersals by migratory birds are more feasible options (Mummenhoff & Franzke, 2007; Gillespie et al. 2012; Viana, Santamaría, & Figuerola, 2016). There are two main migration flyways connecting Asia and western North America with Australasia—the East Asian-Australasian (EAA) and the Central Pacific flyways (e.g., Bamford, Watkins, Bancroft, Tischler, & Wahl, 2008; Gillespie et al. 2012). The EAA flyway is predominately used by shorebirds to migrate from Australia and New Zealand to reach their breeding grounds in southern and eastern Asia, up to the Russian Far East and Alaska (Bamford et al., 2008). The present-day flyways suggest that long-distance bird migrations could have existed already million of years ago and that potentially mucilaginous seeds of the allopolyploid founder plant could adhere to a bird or have survived in its gut (Mummenhoff & Franzke, 2007). The origin of endemic Australian and New Zealand *Lepidium* species through hybridization between North American and African progenitor species (Dierschke et al., 2009; Mummenhoff & Franzke, 2007; Mummenhoff et al., 2004), as well as the dispersal of the North American *Microseris* to Australia and New Zealand (Vijverberg et al., 1999), was attributed to bird dispersals. The asymmetric present-day diversity of MICR in Australia (16 genera) and New Zealand (one genus) favours the Australian mainland as the primary landing site of the trans-oceanic dispersal, followed by a later dispersal of the *Pachycladon* ancestor to

New Zealand through migratory birds (Williams, Gummer, Powlesland, Robertson, & Taylor, 2006) or prevailing strong westerly winds (Sanmartin, Wanntorp, & Winkworth, 2007).

4.4 | Independent PPDs potentially facilitated the occupation of new habitats

The highest diversity of the MICR clade is reached in Southeast Australia with several taxa expanding or being endemic to the Eremean biome, and some species also occur in the Southwest Australian Floristic Region (Figure S3). Except for three shrubby *Arabidella* species (Shaw, 1965), three perennial *Stenopetalum* taxa (Keighery, 2002) and the high-mountain perennial *D. alpestre* (Shaw, 1965), most Australian MICR species are selfing annuals of semi-arid shrublands (Table S1). This prevailing life history suggests that similar to the Australian chenopods (Kadereit, Gotzek, & Freitag, 2005) and *Lepidium* species (Mummenhoff et al., 2004), diversification of the MICR crown-group was probably associated with the expansion of semi-arid ecosystems (Semi-arid Cradle Hypothesis, Hopper, 2009). The Mid-Miocene (15–14 mya) was marked by continuing aridification and cooling of Australia (Byrne et al., 2008; Crisp & Cook, 2013) and the next, Late Pliocene (c. 4–2 mya) aridification was key to the formation of the central Australian desert (Crisp & Cook, 2013; Martin, 2006). The Late Miocene origin of the MICR ancestor and colonization of the Australian mainland followed by diploidization and cladogenesis (Figures 1 and 4) coincide well with the onset of aridification and the origin of seasonally dry habitats. However, the absence of significant pollen fossils of Brassicaceae species in Australia and New Zealand older than the earliest Pleistocene (Gelasian, 2.58–1.80 mya; M. Macphail and D. Mildenhall, personal communication) could imply that most speciation events within the Australian crown-group might have occurred during or after the Pliocene aridification, that is, between 3 and 1 mya. In New Zealand, Alpine habitats in the South Island started to form at about 1.9 mya and most Alpine radiations occurred after 0.95 mya (Heenan & McGlone, 2013). The Pleistocene (0.8–1.9 mya) radiation of *Pachycladon* (Joly et al., 2009) is also supported by narrow endemism (Heenan, Mitchell, & Koch, 2002) and structural genome uniformity of *Pachycladon* species (Mandáková, Heenan, et al., 2010). As the divergence of *Pachycladon* from its closest relatives was estimated to occur 3.5–1 mya (Heenan et al., 2002), 2.77 mya (Hohmann et al., 2015), (2.48-) 1.61 (-0.83) mya (Joly et al., 2009) and even (10.17-) 7.52 (-5.04) mya (this study), it is conceivable that the Pleistocene radiation was preceded by PPD ($n = 15 \rightarrow n = 10$) and a relatively long "lag phase."

4.5 | MICR genera originated via a shared WGD but exhibit a multispeed postpolyploid diploidization

Whereas the species of the crown-group and *Menkea* subclades show an advanced level of PPD due to numerous chromosomal rearrangements and 2- to 3.75-fold reduction in the ancestral chromosome number, in the perennial *Arabidella* and *Pachycladon* species, reshuffling of the ancestral duplicated genome was much less extensive due to only a 1.25- and 1.5-fold descending dysploidy, respectively. In addition, the cytological differentiation of two parental genomes, as revealed by CCP in the crown-group Australian genera and *Menkea* (Mandáková, Joly, et al., 2010 and this study), was not observed in *A. trisecta* and *Pachycladon* (see above and Mandáková, Heenan, et al., 2010). These contrasting genomic features differentiating the crown-group genera and *Pachycladon* were initially interpreted as two, subsequent WGD events involving the same or closely related parental genomes (Mandáková, Joly, et al., 2010; Mandáková, Heenan, et al., 2010). However, this should be revisited in the light of the present data. Newly identified tribe-specific cytogenetic signatures and the consistently retrieved monophyly of MICR collectively suggest a single origin for all the Australian and New Zealand subclades through a shared allotetraploidization event (Figure 4).

However, if the MICR clade originated as a single WGD event, how can the surprisingly diverse levels of genome diploidization be explained? Our data convincingly point to a multispeed tempo of structural postpolyploid diploidization (Figure 4). The slowest tempo of PPD can be inferred for the perennial *Arabidella* genomes with both parental subgenomes being largely conserved. The ancestral *Pachycladon* genome experienced a more progressed PPD than *Arabidella* (Mandáková, Heenan, et al., 2010) and both subclades stand in stark contrast to the extensive PPD of the *Menkea* and crown-group subclades. Our recent transcriptomic analyses of *Pachycladon* and *Stenopetalum* (the latter representing the crown-group subclade) pointed to a shared mesotetraploid origin and suggested that the *Pachycladon* genomes were evolving ~21% slower than that of *Stenopetalum* (Mandáková, Zheng, Barker, & Lysak, 2017). Similarly, comparison of paralogous gene pairs among grass genomes showed the slowest nucleotide substitution in the ancestor-like rice genome and up to 48% faster in other grasses with more reshuffled genomes (Wang et al., 2015). This is in accord with findings that palaeopolyploid genomes of relatively slow-evolving taxa (such as grapevine *Vitis vinifera*) are less rearranged compared to faster-evolving ones (Murat et al., 2015). As noted by Stebbins (1950), annuals exhibit descending dysploidy to a greater extent than perennials, probably due to selection for reduced recombination in annuals. It was suggested that perennials exhibit slower nucleotide substitution rates than annuals (Luo et al., 2015; Soria-Hernanz, Fiz-Palacios, Braverman, & Hamilton, 2008; Yue et al., 2010). In MICR, the two basal subclades showing the least degree of PPD, that is, the shrubby *Arabidella* species and *Pachycladon*, are perennials. In contrast, *Menkea* and the species-rich crown-group genera are characterized by annual life histories (except for *D. alpestre* and three *Stenopetalum* taxa in which the perennial habit most likely evolved secondarily). Although we did not find statistically significant association between life form and diversification rates in MICR, the median net diversification rate of the annual MICR subclades was higher than that of the perennial subclades (Figure S2). In the light of these findings, we propose that PPDs and diversification rates were on average faster in annual subclades than in perennial ones. As montane adaptive radiations are

generally linked to the perennial life cycle (Hughes & Atchison, 2015; Karl & Koch, 2013), the divergence of the *Pachycladon* ancestor into the extant 11 species was possible only due to its perenniality (Heenan & McGlone, 2013; Heenan & Mitchell, 2003). The three shrubby *Arabidella* species (*A. filifolia*, *A. glaucescens* and *A. trisecta*) represent genomes adapted to arid conditions where the perennial growth habit might be a successful alternative (e.g., Huang, Wang, & Chen, 2011) to short-lived annuals and ephemerals prevailing among the highly diploidized genomes of the MICR crown-group. The slower diploidization rates can be associated with selection against diploidizing chromosomal rearrangements in genomes well adapted to extreme environments.

4.6 | Underlying mechanisms of PPDs and descending dysploidies

Differences in karyotype structure among the MICR subclades and species suggest that the diploidizing descending dysploidies were independent and resulted in subclade- and species-specific patterns (Figures 2a and 4, Tables S5 and S6; and Mandáková, Joly, et al., 2010; Mandáková, Heenan, et al., 2010). Considering chromosome number of the inferred ancestral mesotetraploid genome ($n = 15$) and lower chromosome counts in the extant MICR taxa ($n = 4$–7, 10, and inferred $n = 12$ for *Arabidella*), it becomes obvious that end-to-end translocations (i.e., "chromosome fusions," Lysak, 2014) were the exclusive (*Arabidella*) or dominating mechanism of independent descending dysploidies across MICR. EET events can be inferred from the high number of retained ancestral whole-chromosome GB associations (from two in *Menkea* to seven in *Harmsiodoxa* and up to 13 in *A. trisecta*) and eliminated centromeres within collinear homeologous chromosomal regions (20%, 33.3% and 37.5% of the ancestral centromeres were eliminated in *A. trisecta*, *Pachycladon* and the crown-group including *Menkea*, respectively). The comparable number of EETs/inactive centromeres in *Pachycladon* and the crown-group genomes including *Menkea* point to a twofold conclusion. First, EETs must have been the initial mechanism of independent descending dysploidies in all MICR subclades, and second, the more extensive descending dysploidies in *Menkea* and across the MICR crown-group occurred after the EET phase and were probably mediated by a pericentric inversion–reciprocal translocation mechanism (Lysak et al., 2006; Schubert & Lysak, 2011).

In the MICR crown-group species and *Menkea*, 60% of inactive centromeres (=EET events) occurred within the maternal C-subgenome, compared to 40% within the paternal S-subgenome. This may suggest that whereas the C-subgenome chromosomes were preferentially involved in diploidizing EETs, the S-subgenome chromosomes were reshuffled by additional (post-EET) rearrangements and presumably followed by biased subgenome-specific fractionation on a sequence level. This is reflected by a more extensive and faster fractionation of the paternal S-genome chromosomes as revealed by CCP (Figure 3c). This observation is, for instance, in agreement with a biased loss of paternally derived repetitive DNA sequences in the allotetraploid *Nicotiana tabacum* (Renny-Byfield et al., 2011). The subgenome-specific CCP hybridization patterns were also observed in previously analysed Australian MICR species (Mandáková, Joly, et al., 2010) and other Brassicaceae mesopolyploids (Geiser et al., 2016; Haudry et al., 2013; Lysak, Koch, Pecinka, & Schubert, 2005; Mandáková et al., 2017). The absence of the biased chromosomal fractionation in less diploidized genomes of *Arabidella* (Figure 3a) and *Pachycladon* (Mandáková, Heenan, et al., 2010) would suggest that the original parental genomes did not differ in the level of their fractionation, and biased fractionation was rather associated with or triggered by major structural rearrangements mediating PPDs.

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DATA ACCESSIBILITY

Multiple alignments of *CHS*, *PHYA* and *ndhF* genes: Dryad, https://doi.org/10.5061/dryad.q39k3.

AUTHOR CONTRIBUTIONS

M.A.L. and T.M. designed the study. T.M, M.P. and K.H. performed the laboratory work. T.M., M.P., S.H.Z. and I.M. analysed the data. M.A.L. wrote the manuscript with contribution of T.M., M.P., S.H.Z. and I.M.

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