Mummenhoff, K., Linder, P., Friesen, N., Bowman, J.L. Lee, J.-Y. & Franzke, A. (2006). African species of *Lepidium* (Brassicaceae) contributed via hybridization to the origin of Australian/New Zealand species. In: S.A. Ghazanfar & H.J. Beentje (eds), Taxonomy and ecology of African plants, their conservation and sustainable use, pp. 291–308. Royal Botanic Gardens, Kew.

AFRICAN SPECIES OF *LEPIDIUM* (BRASSICACEAE) CONTRIBUTED VIA HYBRIDIZATION TO THE ORIGIN OF AUSTRALIAN/NEW ZEALAND SPECIES

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Abstract

Lepidium sensu stricto (Brassicaceae) (± 150 species) is distributed world-wide with endemic species on every continent. It is represented in Australia and New Zealand by 19 and 7 native species respectively. In the present study we used a nuclear ribosomal ITS phylogeny in comparison with a *cp*DNA phylogeny to unravel the origin of Australian/New Zealand species. The *cp*DNA data indicate a Californian origin of *Lepidium* species from Australia/New Zealand. With respect to the Australian/New Zealand taxa, strongly conflicting signals between the *cp*- and nuclear DNA phylogenetic analysis clearly indicated hybridogenous genomic constitution of Australian *Lepidium* s.s. species: all 18 studied Australian/New Zealand *Lepidium* s.s. species examined shared a Californian *cp*DNA type. While eleven Australian/New Zealand taxa appeared to harbor a Californian ITS type, a group of seven species shared a South African ITS type. This pattern is most likely explained by two trans-oceanic dispersals of *Lepidium* from California and Africa to Australia/New Zealand and subsequent hybridization followed by homogenization of the ribosomal DNA either to the Californian or South African ITS type in the two different lineages. Calibration of our molecular trees indicates a Pliocene/Pleistocene origin of *Lepidium* in Australia/New Zealand. Low levels of *cp*DNA- and ITS sequence divergence and unresolved topologies within Australian/New Zealand species suggest a rapid and recent radiation of *Lepidium* after the hybridization event. This coincides with dramatic climatic changes in that geological epoch shaping the composition of the vegetation.

Résumé

Des espèces africaines de *Lepidium* (Brassicaceae) ont contribué, par hybridation, à l'origine d'espèces australiennes/néo-zélandaises. Le genre *Lepidium* sensu stricto (Brassicaceae) (± 150 espèces) est distribué à travers le monde et est constitué d'espèces endémiques sur chaque continent. Il est représenté en Australie et en Nouvelle-Zélande par 19 et 7 espèces natives, respectivement. Dans le cadre de la

présente étude, nous avons utilisé une phylogénie basée sur l'ITS de l'ADN ribosomique (non codant) et nous l'avons comparée à une phylogénie du *cp*DNA afin de trouver l'origine des espèces australiennes/néo-zélandaises. En ce qui concerne les taxons australiens/néo-zélandais, des signaux fortement conflictuels sont apparus entre les analyses du *cp*- et de l'ADN nucléaire. Ils prouvaient clairement la constitution génomique hybridogène des espèces de *Lepidium* s.s. australiens. Les 18 espèces étudiées de *Lepidium* s.s. australiens/néo-zélandais avaient en commun un *cp*DNA de type californien, 11 taxons australiens/néo-zélandais avaient un type d'ITS californien et un groupe de sept espèces montrait un type d'ITS sud-africain. Cette situation peut vraisemblablement être expliquée par deux dispersions transocéaniques de *Lepidium* à partir de la Californie et de l'Afrique vers l'Australie/Nouvelle Zélande, suivies par une hybridation et par l'homogénéisation de l'ADN ribosomique en deux lignées, l'une avec un type d'ITS californien, l'autre sud-africain. Le calibrage de nos arbres moléculaires indique une origine Pliocène/Pleistocène de *Lepidium* en Australie/Nouvelle Zélande. Une faible divergence des séquences *cp*DNA et ITS et des topologies non résolues entre les espèces australiennes/néo-zélandaises suggèrent une radiation rapide et récente de *Lepidium* après l'événement d'hybridation. Celui-ci coïncide avec les changements climatiques drastiques de cette époque géologique qui ont conditionné la composition de la végétation.

Key words: biogeography, Brassicaceae, hybridization, *Lepidium*, long-distance dispersal

1 Introduction

Lepidium L. is one of the largest genera in the Brassicaceae, consisting of ± 175 species worldwide. Recent molecular studies of *Lepidium* phylogeny utilizing the nuclear *r*DNA internal transcribed spacer (ITS), noncoding *cp*DNA and single copy nuclear DNA sequences (an intron of PISTILLATA, PI), respectively, clarified some relationships within the genus (Bowman *et al.*, 1999; Mummenhoff *et al.*, 2001; Lee *et al.*, 2002). However, an important unanswered question concerns the evolutionary history, i.e., the biogeographic context and time of origin, of Australian/New Zealand *Lepidium* s.s. species (for a definition of *Lepidium* s.s. see Mummenhoff *et al.*, 2001). Based on morphological differences among native Australian taxa, H. Hewson (personal communication, CSIRO, Canberra) suggested independent introductions of *Lepidium* in Australia from South America, South Africa, and Southeast Asia.

In the present study we clarify the evolutionary history of *Lepidium* s.s. in Australia/New Zealand by comparing noncoding *cp*DNA and ITS sequences from 18 Australian/New Zealand species along with 38 species from the other continents. This represents the whole spectrum of variation in *Lepidium* s.s. Molecular footprints in Australian/New Zealand *Lepidium* revealed that these species arose through hybridization with Californian and South African *Lepidium* species being involved.

2 Materials and methods

2.1 Taxon sampling

Fifty-six taxa were chosen to represent the whole spectrum of variation in the *Lepidium* s.s lineage and cover all major geographic distributions areas: Europe/Asia, Africa, North and South America, and Australia/New Zealand. Australia and New

Zealand harbor 19 and nine native taxa, respectively; our sampling of ten Australian and eight New Zealand taxa comprises representatives of all taxonomic entities and thus represents the full range of variation of *Lepidium* s.s. in Australia and New Zealand. As an outgroup, species of section *Lepia* s.l., i.e., *L. campestre* and *L. hirtum* subsp. *hirtum*, were used. In previous analyses section *Lepia* appeared as sister to *Lepidium* s.s. (Bowman *et al.*, 1999, Mummenhoff *et al.*, 2001). GenBank accession numbers and collection data are given in Table 1.

2.2 Molecular methods

Methods for DNA extraction, PCR and direct sequencing of ITS and noncoding *cp*DNA (*trn*T/*trn*L spacer, *trn*L intron, *trn*L/*trn*F spacer) are given elsewhere (Bowman *et al.*, 1999, Mummenhoff *et al.*, 2001). To study the parental ITS units in presumed hybridogenous Australian *Lepidium*, the PCR (Polymerase Chain Reaction) products of selected Australian/New Zealand polyploids (*L. fasciculatum*, *L. pseudohyssopifolium, L. pseudotasmanicum, L. muelleri-ferdinandi, L. oleraceum*, *L. banksii*) were cloned and 2–8 clones were sequenced. The same primers as for the PCR amplification were used for sequencing. Compilation of the information obtained from direct sequencing and from clone sequencing reveals one nucleotide polymorphism in *L. oleraceum* and *L. fasciculatum*, respectively, two polymorphic sites in *L. pseudohyssopifolium*, and three in *L. muelleri-ferdinandi*. Thus, we treated these nucleotide sites as polymorphic character states in the ITS data set used for subsequent phylogenetic analyses. DNA sequences were aligned by hand. Regions of ambiguous alignment were eliminated.

2.3 Phylogenetic analysis

Parsimony analysis of ITS and *cp*DNA assumed unordered and unweighted character states (i.e., Fitch parsimony) and used the heuristic search strategy in PAUP (version 4.0b10; Swofford, 2000) with TBR (tree bisection-reconnection) branch swapping, and 100 random taxon additions. Up to 10,000 trees were kept from each random addition sequence replicate. Bootstrap support values were obtained from 100 replicates by using a heuristic search and simple addition.

2.4 Sequence divergence and relative rate test

Pairwise distance divergences were calculated in PAUP under Kimura's two parameter model (K2P) by using the pairwise-deletion option for gaps and ambiguous data. Rate heterogeneity among lineages in the *cp*DNA and ITS trees was examined by using a tree-wide likelihood ratio (LR) test (Modeltest 3.04; Posada & Crandall, 1998). This test compares the log likelihoods (determined in 4.0b10) of constrained and unconstrained hypotheses (constrained: DNA substitution rates are equal among lineages; unconstrained: rates are allowed to vary among lineages) under the most adequate substitution model that best fits the data (Huelsenbeck & Rannala, 1997).

3 Results

3.1 *cp*DNA- and ITS sequence variation

Details on the characterization of noncoding *cp*DNA regions (*trn*T/*trn*L spacer, *trn*L intron, *trn*L/*trn*F spacer) are given in Mummenhoff *et al.* (2001). After eliminating regions with ambiguous alignment, 1435 positions were available for phylogenetic analysis. Of the 220 variable characters, 89 were potentially parsimony informative.

TABLE 1. Collection data and GenBank accession numbers of *Lepidium* species studied.

Species	Origin	Provenance/Source [a]/Collector	GenBank accession number				
			*trn*T/*trn*L spacer [b]	*trn*L *intron*	*trn*L/*trn*F spacer [c]	ITS1	ITS2
L. africanum (Burm.f.) DC.	Africa	South Africa, Cape Province, Williston District/PRE, 95056-102/ G. Germishuizen	AY015703 AY015704	AY015833	AY015921	AJ582441	AJ582498
L. alluaudii Maire	Africa	Morocco, near Tazenakh/ ETSIA, 418-1483-68	AY015705 AY015706	AY015834	AY015922	AJ582436	AJ582493
L. apetalum Willd.	Asia	China, Beijing/ B.G. Beijing, China, s.n.	AY015823	AY015835	AY015923	AJ582466	AJ582514
L. arbuscula Hillebr.	Hawaii	Hawaii, Oahu , Makua Valley, Ohikilolo Ridge/National Tropical B.G. Lawai, Kauaii, Hawaii, 945176/ S. P. Pearlman	AY015707 AY015708	AY015836	AY015924	AJ582451	AJ582517
L. armoracia Fisch. & Mey.	Africa	Arabian Republic Yemen, Shibam/ K, MWC 2307/A. G. Miller	AY015709 AY015710	AY015837	AY015925	AJ582454	AJ582502
L. aschersonii Thell.	Australia	Australia, Victoria, Lake Omeo/ La Trobe Univ., Australia, s.n./ N. H. Scarlett	AY015711 AY015712	AY015838	AY015926	AJ582426	AJ582483
L. aucheri Boiss.	Asia	Jordania, Wadi Araba/Orient Herbarium, FU Berlin, Germany, s.n./ H. U. Baierle & C. Prime	AY015713 AY015714	AY015839	AY015927	AJ582443	AJ582525
L. austrinum Small	North America	USA, Texas, Hidalgo Co./Tucker Herbarium, Univ. of California, Davis, 17451/L. H. Shinners	AY015715 AY015716	AY015840	AY015928	AJ582467	AJ582515
L. banksii Kirk	New Zealand	New Zealand, Abel Tasman National Park, Totaranui/P. de Lange, Dept. Conservation, Auckland Conservancy, New Zealand, 942179	AY015717 AY015718	AY015841	AY015929	AJ582433	AJ582490
L. bidentatum Montin	Hawaii	Hawaii, Kauaii, Haupu/National Tropical B.G. Lawai, Kauaii, Hawaii, 905054/S. P. Pearlman	AY015719 AY015720	AY015842	AY015930	AJ582468	AJ582516

TABLE 1. continued

Species	Origin	Provenance/Source [a]/Collector	GenBank accession number				
			*trn*T/*trn*L spacer [b]	*trn*L *intron*	*trn*L/*trn*F spacer [c]	ITS1	ITS2
L. bipinnatifidum Desv.	South America	Bolivia, La Paz , railway station/ Univ. Osnabrück, Germany, 451/ K. Mummenhoff & H. Brüggemann	AY015721 AY015722	AY015843	AY015931	AJ582446	AJ582522
L. bonariense L.	South America	Chile, Prov. Atacama, Dept. Valle Iorquera/CETYBO, 3078/O. Zöllner	AY015723 AY015724	AY015844	AY015932	AJ582458	AJ582506
L. campestre (L.) R.Br.	Europe	France, Meurthe-et-Moselle, Villers-les-Nancy/B.G. Nancy, France, s.n.	AY015725 AY015726	AY015845	AY015906 AY015907	AJ582412	AJ582469
L. capense Thunb.	Africa	South Africa, Cape Province/ PRE, 95056/49/H. C. Taylor	AY015727 AY015728	AY015846	AY015933	AJ582452	AJ582500
L. desertorum Eckl. & Zeyh.	Africa	South Africa, Cape Province/ PRE, 95056/68/M. B. Bayer	AY015729 AY015730	AY015847	AY015934	AJ582453	AJ582501
L. desvauxii Thell.	Australia	Australia, Victoria, Townsend/ La Trobe Univ., Australia , s.n./ N. H. Scarlett	AY015731 AY015732	AY015848	AY015935	AJ582429	AJ582486
L. dictyotum A.Gray	North America	USA, California, San Luis Obispo Co./ Tucker Herbarium, Univ. of California, Davis, 32057/E. C. Twisselmann	AY015733 AY015734	AY015849	AY015936	AJ582415	AJ582472
L. divaricatum Ait.	Africa	South Africa, Cape Province, Williston District/PRE, 95056/134/ P. Germishuizen	Sequence data not available	AJ582565	AJ582566	AJ582437	AJ582494
L. fasciculatum Thell.	Australia	Australia, Victoria, river road near Lake Walla Walla/La Trobe Univ., Australia, s.n./J. H. Browne	AJ582562	AJ582563	AJ582564	AJ582428	AJ582485
L. ferganense Korsh.	Asia	Russia, Moscow/B.G. Moscow, Russia, s.n.	AY015737 AY015738	AY015851	AY015938	AJ582449	AJ582519
L. flavum Torr.	North America	USA, Nevada, Churchill Co., Slate Mountain/MO/A. Tiehm & P. Lott, 4011	AY015739 AY015740	AY015852	AY015908 AY015909	AJ582444	AJ582524

TABLE 1. continued

Species	Origin	Provenance/Source [a]/Collector	GenBank accession number				
			*trn*T/*trn*L spacer [b]	*trn*L *intron*	*trn*L/*trn*F spacer [c]	ITS1	ITS2
L. flexicaule Kirk	New Zealand	New Zealand/Auckland B. G., cultivated plants, 950769	AY015741 AY015742	AY015853	AY015939	AJ582430	AJ582487
L. fremontii S.Wats.	North America	USA, California, Joshua Tree Desert/ Univ. Osnabrück, Germany/ H. Hurka, s.n.	AY015815	AY015854	AY015940	AJ582456	AJ582504
L. hirtum (L.) Sm. ssp. *hirtum*	Europe	France, Dept. Aude, Montagne de Tauch/B.G. Univ. Liege, Belgium, 85-3863	AY015819	AY015858	AY015944	AJ582413	AJ582470
L. hyssopifolium Desv.	Australia	Australia, Victoria, Beveridge/ La Trobe Univ., Australia, 70-296-940/ N. H. Scarlett	AY015743 AY015744	AY015861	AY015947	AJ582435	AJ582492
L. lasiocarpum Nutt.	North America	USA, California, Joshua Tree Desert/ ETSIA, 430-1738-69	AY015745 AY015746	AY015862	AY015948	AJ582455	AJ582503
L. latifolium L.	Europe	Germany, Leipzig, garbage dump/ B.G. Leipzig, Germany/P. Gutte	AY015747 AY015748	AY015863	AY015949	AJ582447	AJ582521
L. latipes Hook.	North America	USA, California, Solano Co./Tucker Herbarium, Univ. of California, Davis, 37209/J. M. Tucker	AY015749 AY015750	AY015864	AY015950	AJ582416	AJ582473
L. lyratum L.	Asia	Iran, mountains near Abadeh/ ETSIA, 433-3758-75	AY015755 AY015756	AY015867	AY015953	AJ582448	AJ582520
L. meyenii Walpers	South America	Peru, Dept. Junin, Huayre/ M. Hermann, Intern. Potato Center, Quito, Ecuador, JTA-106/J. Anco	AY015757 AY015758	AY015868	AY015954	AJ582445	AJ582523
L. montanum Nutt.	North America	USA, Arizona, Shonto/B.G. Univ. Liege, Belgium, s.n.	AY015759 AY015760	AY015869	AY015955	AJ582457	AJ582505
L. muelleriferdinandi Thell.	Australia	Australia, New South Wales, Menindee Lakes/La Trobe Univ., Australia, s.n./J. H. Browne	AY015761 AY015762	AY015870	AY015956	AJ582427	AJ582484

TABLE 1. continued

Species	Origin	Provenance/Source [a]/Collector	GenBank accession number				
			*trn*T/*trn*L spacer [b]	*trn*L *intron*	*trn*L/*trn*F spacer [c]	ITS1	ITS2
L. myriocarpum Sond.	Africa	South Africa, Cape Province, Vaalbos National Park/PRE, 95056/ 53/P. C. Zietsman	AY015763 AY015764	AY015871	AY015957	AJ582442	AJ582499
L. naufragorum Garnock-Jones & D.A.Norton	New Zealand	New Zealand/P. de Lange, cultivated plants, Dept. Conserv., Auckland Conservancy, New Zealand, 950771	AY015765 AY015766	AY015872	AY015958	AJ582422	AJ582479
L. nitidum Nutt.	North America	USA, California , Table Mountains/ Univ. Osnabrück, Germany, 338/ H. Hurka	AY015767 AY015768	AY015873	AY015959	AJ582414	AJ582471
L. oblongum Small	North America	Cultivated plants/B.G. Copenhagen, Denmark, s.n.	AY015769 AY015770	AY015874	AY015960	AJ582462	AJ582510
L. oleraceum Sparrm.	New Zealand	New Zealand, Port Waikato, Ngatutura Point/Dept. Conserv., Auckland Conservancy, New Zealand, 941265/P. de Lange	AY015771 AY015772	AY015875	AY015961	AJ582434	AJ582491
L. oxycarpum Torrey & A.Gray	North America	USA, California, Merced Co./Tucker Herbarium, Univ. of California, Davis, 115743/C. A. & L. P. Janeway	AY015773 AY015774	AY015876	AY015962	AJ582417	AJ582474
L. oxytrichum Sprague	Australia	Australia, Northern Territorium/ National B.G. Canberra, Australia, s.n.	AY015775 AY015776	AY015877	AY015963	AJ582424	AJ582481
L. papillosum F.Muell.	Australia	Australia, Victoria, Red Cliffs, Bottle Bend River/T.H. Browne, Australia, s.n.	AY015777 AY015778	AY015878	AY015964	AJ582425	AJ582482
L. pinnatifidum Ledeb.	Asia	USA, California, Yolo Co./Tucker Herbarium, Univ. of California, Davis, 96441/A. M. Shapiro	AY015787 AY015788	AY015883	AY015968	AJ582464	AJ582512
L. pinnatum Thunb.	Africa	South Africa, Cape Province, Drinkriver farm/PRE, 95056/45/ K. A. Dahlstrand	AY015827	AY015884	AY015969	AJ582439	AJ582496

TABLE 1. continued

Species	Origin	Provenance/Source [a]/Collector	GenBank accession number				
			*trn*T/*trn*L spacer [b]	*trn*L *intron*	*trn*L/*trn*F spacer [c]	ITS1	ITS2
L. pseudohyssopifolium Hewson	Australia	Australia, Victoria, Mitre Rock, near Mt. Arapiles/La Trobe Univ., Australia, s.n./N. H. Scarlett	AY015789 AY015790	AY015885	AY015970	AJ582431	AJ582488
L. pseudopapillosum Thell.	Australia	Australia, Victoria, Kamarooka Forest/ La Trobe Univ., Australia, s.n./ N. H. Scarlett *et al.*	AY015791 AY015792	AY015886	AY015971	AJ582423	AJ582480
L. pseudotasmanicum Thell.	Australia	Australia, Victoria, George National Park/La Trobe Univ., Australia s.n./ N. H. Scarlett *et al.*	AY015826	AY015887	AY015972	AJ582432	AJ582489
L. quitense Turcz.	South America	Ecuador, Prov. Tungurakua, road from Pillaro to Ambato/MO, 3792042/ C. E. & M. Ceron	AY015793 AY015794	AY015888	AY015973	AJ582463	AJ582511
L. ruderale L.	Europe	Germany, Borgholzberg near Oldenburg/B.G. Oldenburg, Germany, s.n.	AY015795 AY015796	AY015890	AY015975	AJ582465	AJ582513
L. sativum L.	Europe	Denmark, Jersie/B.G. Copenhagen, Denmark, s.n.	AY015828	AY015891	AY015912 AY015913	AJ582459	AJ582507
L. schinzii Thell.	Africa	South Africa, Orange Free State Excelsior, Korannaberg/ PRE, 95056/6/J. du Perez	AY015797 AY015798	AY015892	AY015976	AJ582440	AJ582497
L. serra H.Mann	Hawaii	Hawaii, Kauai, Kalalau valley near Puu O Kila/National Tropical B.G. Lawai, Hawaii, Kauai, 915398/ S.P. Pearlman	AY015799 AY015800	AY015893	AY015977	AJ582450	AJ582518
L. sisymbrioides ssp. *kawarau* (Petrie) Thell.	New Zealand	New Zealand, Central Otago, Slapjack Creek/P. de Lange, Dept. Conserv., Auckland Conservancy, New Zealand, 950766/R. B. Allen	AY015801 AY015802	AY015894	AY015978	AJ582419	AJ582476

TABLE 1. continued

Species	Origin	Provenance/Source [a]/Collector	GenBank accession number				
			*trn*T/*trn*L spacer [b]	*trn*L *intron*	*trn*L/*trn*F spacer [c]	ITS1	ITS2
L. sisymbrioides ssp. *matau* (Petrie) Thell.	New Zealand	New Zealand, Central Otago, Galloway/P. de Lange, Dept. Conserv., Auckland Conservancy, New Zealand , 950767/R. B. Allen	AY015803 AY015804	AY015895	AY015979	AJ582418	AJ582475
L. sisymbrioides Hook.f. ssp. *sisymbrioides*	New Zealand	New Zealand, Central Otago, Pisa Flats/P. de Lange, Dept. Conserv., Auckland Conservancy, New Zealand , 950768/R. B. Allen	AY015805 AY015806	AY015896	AY015980	AJ582420	AJ582477
L. spinescens DC.	Asia	Israel, Upper Galilee/B.G. Univ. Tel Aviv, Israel, s.n.	AY015807 AY015808	AY015897	AY015981	AJ582461	AJ582509
L. spinosum Ard.	Asia, Europe	Turkey, Central Anatolia, near Gaziantep/ETSIA, 436-6229-83	AY015824	AY015898	AY015914 AY015915	AJ582460	AJ582508
L. tenuicaule Kirk	New Zealand	New Zealand, Kakanui, Shag Point/ P. de Lange, Dept. Conserv., Auckland Conservancy, New Zealand, 950191/P. de Lange	AY015809 AY015810	AY015899	AY015982	AJ582421	AJ582478
L. trifurcum (Sond.) Marais	Africa	South Africa, Cape Province, SW slopes of Pakhuispiek, cedarberg/ PRE, 95056-111/H. C. Taylor	AY015811 AY015812	AY015900	AY015983	AJ582438	AJ582495
L. virginicum L.	North America	Mexico, Carrizal Chico, riverbed/ Univ. of Osnabrück, Germany/ R. Stöckmann & K. Bosbach, s.n.	AY015813 AY015814	AY015902	AY015984	AF283496	AF283497

[a] ETSIA = Escuela Tecnica Superior de Ingenieros Agronomos, Madrid, Spain; B.G. = Botanical Garden; CETYBO = Centro de Estudios Farmacologicos y Botanicos, Buenos Aires, Argentina; DAV = Davis Herbarium, Deptartment of Botany, University of California, Davis, USA; K = Herbarium, Royal Botanic Gardens, Kew, UK; MO = Herbarium, Missouri Botanical Garden, St. Louis, USA; PRE = National Herbarium, Botanical Research Institute, Pretoria, South Africa.

[b] Species with two GenBank accession numbers refer to sequences starting from the 5′ and 3′ end, respectively. In these species presence of poly-A/T tracts caused premature termination of sequencing reactions.

[c] Species with two GenBank accession numbers refer to sequences starting from the 5′ and 3′ end, respectively. For these species overlapping sequencing could not be achieved due to extreme length of spacer regions.

	ITS1 ITS2 1111111112233344 6788001344677593693 3 8347896568024179540 2
Californian taxa, clade C	
dic (4x), lat (4x), nit (4x), oxyc	CATTAGCCCGTCATCGG–TA
Australian/New Zealand taxa, clade C	
nau (4x), oxyt (3x), pap,	
sis (3 ssp., 4x), ten	CATTACCCCGTCATCGG–TA
fasc (4x)	CATTATCCCGTCATCGG–TA
pseup	CGTTACCCCGTCATCGG–TA
asch	CATTATCCCGTCATCGG–TW
muel (6x)	CATTRCCCCGTCATCGG–TA
Australian/New Zealand taxa, clade A	
ban (4x), des, fle (4x),	
hys (4x), ole (4x), pseut (4x)	TTCGGCGTGCAACCTA–TCT
pseuh (4x)	TTCGGCGTGCAACCYA–TCT
South African taxa, clade A	
afr (2x), dese, div, myr,	
pin, schi, tri	TTCGGCGTGCAACCTA–TCT
cap	TTCGGCGTGCAACCAA–TCT

FIG. 1. Composition of the ITS sequences of Californian, Australian/New Zealand and South African *Lepidium* s.s. species. This data matrix contains only those positions of a complete alignment (not shown) that distinguish Californian from South African *Lepidium* taxa. Californian and South African species are extant members of two different lineages suggested to have been involved in the hybridogenous origin of Australian/New Zealand *Lepidium*. Clade A and clade C refer to the ITS tree in Fig. 2. Site numbers are those of the complete alignment. R = A and G; W = A and T; Y = C and T. Taxon abbreviations; Californian taxa: dic = *L. dictyotum*; lat = *L. latipes*; nit = *L. nitidum*; oxyc = *L. oxycarpum*; Australian/New Zealand clade C taxa: nau = *L. naufragorum*; oxyt = *L. oxytrichum*; pap = *L. papillosum*; sis (3 ssp.) = *L. sisymbrioides* ssp. *kawarau*, ssp. *sisymbrioides*, ssp. *matau*; ten = *L. tenuicaule*; fasc = *L. fasciculatum*; pseup = *L. pseudopapillosum*; asch = *L. aschersonii*; muel = *L. muelleri-ferdinandi*; Australian/New Zealand clade A taxa: ban = *L. banksii*; des = *L. desvauxii*; fle = *L. flexicaule*; hys = *L. hyssopifolium*; ole = *L. oleraceaum*; pseut = *L. pseudotasmanicum*; pseuh = *L. pseudohyssopifolium*; South African taxa: afr = *L. africanum*; dese = *L. desertorum*; div = *L. divaricatum*; myr = *L. myriocarpum*; pin = *L. pinnatum*; schi = *L. schinzii*; tri = *L. trifurcum*; cap = *L. capense*. Ploidy levels (2x–6x) are based on own chromosome counts.

Averaged over the three regions K2P pairwise sequence divergence among the *Lepidium* s.s. taxa varied between 0 and 2.5% and within Australian/New Zealand taxa between 0 and 1.5%.

The ITS regions were sequenced for 56 species and 34 clones of selected Australian *Lepidium* species. The alignment generated a matrix of 456 characters, of which 87 are potentially informative in parsimony analysis. K2P pairwise sequence divergence among the *Lepidium* s.s. taxa ranged between 0 and 7.9%, and within Australian/New Zealand taxa between 0 and 1.5%.

3.2 ITS sequence polymorphism in Australian/New Zealand *Lepidium* and presumed progenitors

Eighteen Australian *Lepidium* s.s. taxa were analyzed by direct sequencing. Known polyploids (*L. fasciculatum*, *L. pseudohyssopifolium*, *L. pseudotasmanicum*, *L. muelleri-ferdinandi*, *L. oleraceum*, *L. banksii;* ploidy level see Fig.1) were also analyzed by cloning amplified ITS sequences. Three species (*L. aschersonii*, *L. muelleri-ferdinandii*, *L. pseudohyssopifolium*) show additivity at one nucleotide position that is variable between the Californian (clade C) and South African (clade A) species group (Fig. 1, sites 108, 297, 432). This indicates that the Australian/New Zealand species may have originated by hybridization. With respect to those sites that are variable between the California and South Africa species group (Fig. 1) the Australian species of clade C and A show exactly those character states of the Californian (clade C) or South African (clade A) species, respectively. However, at site 109 most Australian/New Zealand taxa including those of clade C (related to Californian species) are characterized by the nucleotide found in the South African species (clade A).

3.4 Phylogenetic position of Australian/New Zealand *Lepidium*

We analyzed noncoding *cp*DNA regions and nuclear ITS regions from 56 *Lepidium* s.s. taxa of all major geographic distributions to unravel the origin of Australian/New Zealand *Lepidium* in this lineage. Comparison of the phylogenetic trees (strict consensus of most parsimonious trees) generated from the two data sets is illustrated in Fig. 2. Several nodes are reasonably well supported (>70% bootstrap values), while other nodes are less so, due to low numbers of nucleotide substitutions. This is not surprising in view of a Pliocene/Pleistocene origin of *Lepidium* s.s. (Mummenhoff *et al.*, 2001). In the current study of *Lepidium* s.s. both phylogenies agree in grouping geographically related species, but there are strongly conflicting signals between the *cp*DNA and ITS topologies (Fig. 2). In the *cp*DNA strict consensus tree all Australian/New Zealand species are grouped into a monophyletic assemblage arising from a polytomy. However, in 60% of all maximally parsimonious trees (clade B, *cp*DNA, Fig. 2) four coastal Californian species are found as sister to the Australian/New Zealand clade. Within clade B species relationships are not well resolved due to lack of characters. However, within this clade two distinct groups may be recognized composed of New Zealand species, i.e., *L. sisymbrioides* with three subspecies (100% bootstrap support) and *L. naufragorum, L. flexicaule, L. banksii,* and *L. oleraceum* (53% bootstrap support), respectively. In the ITS tree Australian species are distributed among two different lineages. One group shares common ancestry with the same four Californian species mentioned above (clade C). The remaining species are nested within an unresolved clade A along with South African species and one East African species.

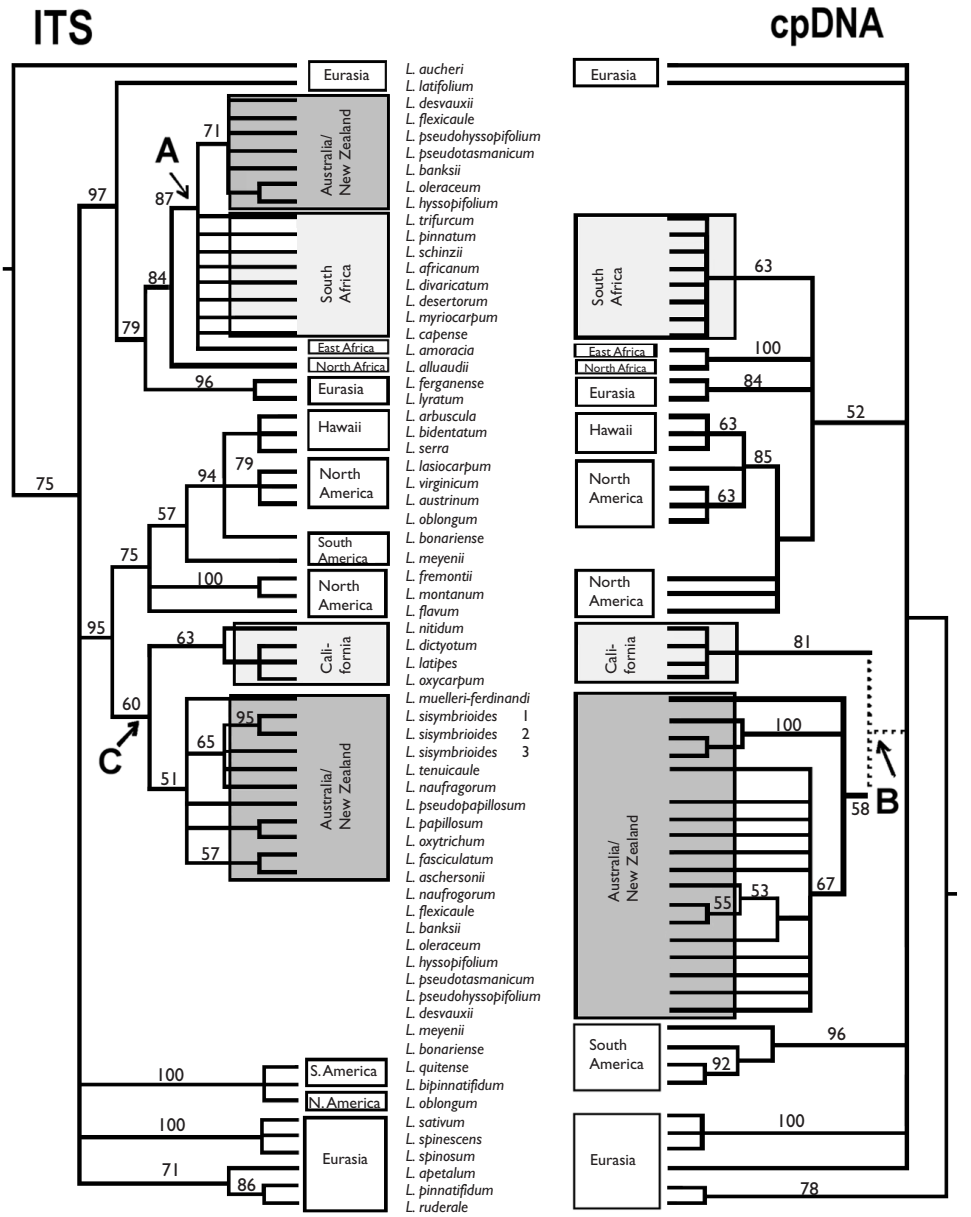

FIG. 2. Comparison of nuclear and chloroplast phylogeny of Australian/New Zealand *Lepidium* s.s. species and continental relatives based on sequence analysis of ribosomal ITS and three noncoding *cp*DNA regions (*trn*T/*trn*L spacer, *trn*L intron, *trn*L/*trn*F spacer), respectively. Shown is the strict consensus each of 1369 most parsimonious ITS and 990 001 *cp*DNA trees with 202 and 275 steps and a consistency index (autapomorpies excluded) of 0.80 and 0.71, respectively. Dashed line in the *cp*DNA tree indicates a branch present in 60% of the minimum length trees. Arrows indicate clades involving *Lepidium* species of South Africa, Australia/New Zealand (clade A), and California, Australia/New Zealand (clade C), and Australia/New Zealand solely (clade B). Bootstrap values >50% are given above branches. *Lepidium sisymbrioides* 1 through 3 refer to subspecies *sisymbrioides*, *kawarau*, and *matau*. Trees were rooted by outgroup comparison.

3.5 Relative-rate-test and molecular clock calibration

Rate heterogeneity among lineages in the *cp*DNA and ITS trees, respectively, was examined by using a tree-wide likelihood ratio (LR) test (Modeltest 3.04; Posada & Crandall, 1998). Rate constancy across lineages throughout the ITS and *cp*DNA tree was rejected. So we followed the recommendations given in Sanderson (1998) to perform localized LR tests on subclades of the phylogenies. As we have focussed in the present study on the origin of Australian/New Zealand *Lepidium* species we tested for rate constancy within those clades containing Australian/New Zealand species (Fig. 2: ITS tree, clade A, C; *cp*DNA tree, clade B). Substitution rate constancy was rejected for clade B in the *cp*DNA tree, but rate constancy within clades A and C of the ITS phylogeny cannot be rejected at the $\alpha = 0.01$ level.

We used fossil data (Mai, 1995) of *Rorippa* (Brassicaceae) to calibrate mutation rates in *Lepidium*. *Rorippa* was found sister to *Cardamine* in recent phylogenetic analyses (Franzke *et al.*, 1998; Mitchell and Heenan, 2000). Assuming a correct dating for the *Rorippa* fossil, a minimum of K2P sequence divergences of 1.8% (*trn*T/L spacer, *trn*L intron) and 4.4% (ITS), respectively, observed between *Rorippa* and *Cardamine* taxa analyzed (Franzke *et al.*, 1998), might then correspond to 2.5–5 million years ago (mya), as a rough estimate for divergence time between *Rorippa* and *Cardamine*. Thus, 1% sequence divergence corresponds to 0.6–1.1 my (million years) for the ITS regions and 1.3–2.8 my for the *cp*DNA. Our calibrated ITS rates are in the same order of magnitude as recently published ITS rates, e.g., *Gossypium*: ca 1% = 1 my (Wendel *et al.*, 1995b), *Gentianella* 1% = 0.6–1.1 my (Hagen & Kadereit 2001), *Soldanella* 1% = 0.6–1.3 my (Zhang *et al.*, 2001), *Robinsonia* 1% = 0.6 my (Sang *et al.*, 1995b), and are thus reasonable.

Sequence divergence among the Australian/New Zealand species ranged from 0% to 1.2% (0.5% mean) for the *trn*T/L spacer and *trn*L intron. ITS divergence ranged from 0.2 to 3.4% (1.2% mean) and from 0 to 1.1% (0.5% mean) among the Australian/New Zealand species of lineage A and C (Fig. 2), respectively. By using the ITS substitution rate calibrated with *Cardamine*/*Rorippa* (see above), we roughly estimated an age of 0.7–1.3 my and 0.3–0.55 my for the Australian/New Zealand species of lineage C and A, respectively. As we had to reject a tree-wide constant rate of ITS, the deviating divergence values of these two lineages might result from rate heterogeneity and reticulation between basal lineages. Although noncoding *cp*DNA evolution (even within the Australia/New Zealand clade) was not clocklike, the estimated age for the Australian/New Zealand lineage ranges from 0.6 to 1.4 my. Our time estimates based on ITS and *cp*DNA data are in the same order of magnitude indicating a Quaternary origin of Australian/New Zealand *Lepidium* species.

4 Discussion

4.1 Reticulate evolution in *Lepidium* s.s.

Lepidium s.s. includes species from all continents. Most of the species are characterized by reduced flowers, an autogamous breeding system and polyploidy, all these being typical features of colonizing plants (Al-Shehbaz 1986, Bowman *et al.*, 1999). Our recent phylogenetic analysis of the *PISTILLATA* (PI) intron, a member of the MADS-box gene family involved in stamen and petal specification, indicates that many species have originated from alloploidization (Lee *et al.*, 2002). The present ITS- and *cp*DNA data reinforces this hypothesis. Strongly conflicting signals between the different genome phylogenies are easily observed throughout the trees. Although

incongruence among gene trees can result from a variety of factors, including sampling error, evolutionary rate heterogeneity, and phylogenetic sorting, the most common source of such phylogenetic discordance in plants appeared to reticulation events (Rieseberg *et al.*, 1996; Barrier *et al.*, 1999). Although reticulate evolution seems to have played an important role in the phylogeny of *Lepidium* s.s. world-wide, the current study focuses on the origin of Australian/New Zealand *Lepidium* s.s. species.

4.2 Two nuclear genomes in Australian/New Zealand *Lepidium*

Judged from the *cp*DNA phylogeny all Australian/New Zealand species harbor a *cp*DNA type most closely related to that of Californian species, and they are grouped into Clade B in 60% of maximally parsimonious trees (Fig. 2). The *cp*DNA mean sequence divergence values of 0.48% between species from California and Australia/New Zealand compared to the 0.92% sequence divergence observed between Australian/New Zealand species of clade B and South American species, i.e., *L. bonariense*, *L. meyenii*, and *L. quitense*, also supports this close relationship between Californian and Australian/New Zealand species. However, the ITS data provides strong evidence that some Australian/New Zealand *Lepidium* species contain the nuclear genome of the Californian species (clade C), while others have the nuclear genome of the South African lineage (clade A). The predominance of a distinct ITS type within each Australian lineage (clade C and A), respectively, may be explained by rapid bidirectional concerted evolution, following ancient hybridization between species from California and South Africa. The *cp*DNA data indicates that species of a Californian lineage represented by e.g., *L. dictyotum* was the most probable maternal parent of the Australian/New Zealand species, both groups sharing a similar *cp*-genome. The ITS data confirms this Californian lineage to be among the parental taxa but indicates that South African species (e.g., *L. africanum* etc.) were also involved. The hybridogenous genomic constitution of Australian/New Zealand *Lepidium* species can also be observed directly in ITS sequences. Nucleotide additivity at three nucleotide sites (Fig. 1) in the ITS regions Australian/New Zealand *Lepidium* s.s. species indicates that reticulate evolution has occurred as has been demonstrated in *Paeonia* (Sang *et al.*, 1995a) and that parental taxa of both continents were involved. The Australian/New Zealand species of clade C and A show with one exception exactly those character states of the Californian or South African species, respectively (Fig. 1). Thus, concerted evolution via gene conversion or unequal crossing-over is apparently operating. The observation of (nearly) homogenous ITS sequences (Fig. 1) indicates (nearly) complete uni- or bidirectional homogenization or transition stages in the homogenization process of the ITS region in the alloploids, respectively (Wendel *et al.*, 1995a, Campbell *et al.*, 1997).

Based on PI intron sequences(Lee *et al.*, 2002) demonstrated that many polyploid taxa harbored two or more phylogenetically distinct sequences, confirming a hybrid nature of Australian/New Zealand taxa.

4.3 Bicontinental hybrid origin of Australian/New Zealand *Lepidium* s.s.

We suggest that Australian *Lepidium* s.s. species are hybrids derived from a cross between colonizing species of the Californian and South African lineages C and A, respectively. Uniform/similar *cp*DNA and ITS sequences within the lineages containing Australian/New Zealand *Lepidium* taxa indicate that this ancient hybridization scenario predated the rapid speciation within these lineages. These speciation events probably also include later hybridization events as evidenced from grouping of species in the *cp*DNA tree (i.e., *L. naufragorum*, *L. banksii*, *L. flexicaule*, *L. oleraceum*) that are

distributed among the two different clades A and C in the ITS tree (Fig. 2). The question whether polyploid Australian/New Zealand *Lepidium* are homoploid or alloploid hybrids cannot be answered from our data. The very few South African and Californian species from which chromosome numbers are known are *L. africanum* (2n = 2x = 16) and *L. dictyotum*, *L. latipes*, *L. nitidum*, (all 2n = 4x = 32), respectively. We generally used herbarium specimens in the current study and thus the ploidy level of Californian and South African *Lepidium* could not be analyzed. Until this point is clarified, one may suggest that the parental species were perhaps polyploid as polyploidy may have facilitated the establishment of the colonizing taxa from California and South Africa prior to hybridization. This is an attractive hypothesis because polyploidy may confer various characteristics appropriate to colonizing organisms including self-compatibility and buffering the effects of selfing (i.e., inbreeding depression), better vigor, and broader ecological tolerance (Barrier *et al.*, 1999; Miller & Venables, 2000). Indeed, based on PI intron sequences, *L. nitidum* is suggested to be an allopolyploid (Lee *et al.*, 2002).

An attractive hypothesis is that the ancient hybridization event occurred in Australia. This implies one dispersal from South Africa and California, respectively. Dispersal from California to South Africa and subsequent hybridization with an endemic taxon (or vice versa) followed by transoceanic migration of the hybrid (that retained the two different parental ITS copies) to Australia is equally parsimonious. But this scenario may be less likely because to our knowledge there are no sea birds migrating from California to South Africa (or vice versa) potentially having transported seeds. Whereas well documented sea bird migration pathways between coastal California and Australia/New Zealand (Lincoln *et al.*, 1998) are compatible with the former colonization scenario.

The origin of *Lepidium* and of the Brassicaceae as a whole presumably occurred in an area encompassing the Mediterranean and the Irano-Turanian territory, a region extremely diverse ecologically, altitudinally, and geologically (Thellung, 1906; Mummenhoff *et al.*, 2001). Fossil data, easy dispersible mucilaginous seeds, widespread autogamy and polyploidy, and low levels of *cp*DNA divergence between species from different continents or islands suggest a rapid radiation of *Lepidium* by long distance dispersal in the Pliocene/Pleistocene (Mummenhoff *et al.*, 2001). As a consequence of climatic changes in this geological epoch, arid/semiarid regions were established, providing favorable conditions for the radiation of *Lepidium* worldwide. South Africa was reached by the "arid corridor", a belt of dry country that stretched from the Horn of Africa to Namibia (Hedge, 1976; Jürgens, 1997), and immigration of *Lepidium* into North and subsequently South America in Quaternary times is compatible with our estimates of divergence times, based on *cp*DNA sequence data (Mummenhoff *et al.,* 2001).

There are several genera centred in southern Africa with a few species in Australia, e.g., *Bulbine* (Asphodeliaceae), *Wurmbea* (Colchicaceae), *Caesia* (Hemerocallidaceae), *Spiloxene* (Hypoxidaceae), and *Dietes* (Iridaceae). In a phylogenetic analysis of *Pelargonium* (Geraniaceae) the close relationship between the South African and Australian species is caused by long distance dispersal to Australia, probably as recent as the late Pliocene (Bakker *et al.,* 1998). In the other cases mentioned above the possibility of recent dispersal across the Indian Ocean has not been confirmed, but it seems unlikely that the distributions date to the Jurassic, which is when the continents were last physically connected via Antarctica.

Our data provide evidence that Australian/New Zealand *Lepidium* are polyploid descendants of two different continental ancestors, one probably immigrating from California, and the other from South Africa. Long distance dispersal of colonizing

species from California and South Africa to Australia/New Zealand seems unlikely given that these areas are currently separated by more than 10,000 km, respectively. Carlquist (1983) however, demonstrated intercontinental dispersal (California to Chile) of mucilaginous *Lepidium* seeds adhering to birds (Mummenhoff *et al.*, 1992; Norton *et al.*, 1997) and sea bird migration pathways between coastal California and Australia/New Zealand and between South Africa and Australia/New Zealand (Lincoln *et al.*, 1998) are compatible with the proposed colonization scenario.

4.4 Time of origin

Calibration of our molecular trees by using *Rorippa* fossil data yield ages of approximately 0.7–1.3 my and 0.3–0.55 my for the Australian/New Zealand species of clade C and A, respectively. These age differences might slightly modify our hypothesis that the hybridization of more or less concurrently arriving Californian and South African ancestors predates the radiation of Australian/New Zealand species. Different estimates of time of origin of Australian/New Zealand representatives of clades A and C, respectively might indicate that the diversification of Californian ancestors in Australia predates a chloroplast transfer into a later arriving South African colonizing species. The evidence against this scenario are the additive sequences in *L. aschersonii* and *L. muelleri-ferdinandi* (Fig. 1). Of course, another possibility is that the A-clade nucleotides in these two species were acquired later by secondary hybridization between clades A and C in Australia and are not relicts of an original hybridization. This would be more consistent with the dating analyses. However this discrepancy in time estimations may simply be explained by the observed rate heterogeneity between these two lineages and across the whole ITS tree. The low levels of *cp*DNA and ITS sequence divergence and unresolved topologies within Australian/New Zealand species (due to low numbers of nucleotide substitutions) suggest a rapid and recent radiation of *Lepidium* after the hybridization event. This coincides with dramatic climatic fluctuations of the Quaternary when a cooling climate and the formation of a more mountainous landscape in New Zealand, and a drying trend in Australia may have created novel habitats and thus highly invasible terrain (Markgraf *et al.*, 1995; McGlone *et al.*, 2001). This could have provided the necessary ecological space into which *Lepidium* could have radiated.

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