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# Phylogeny, biogeography and character evolution in the tribe Desmodieae (Fabaceae: Papilionoideae), with special emphasis on the New Caledonian endemic genera

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## A B S T R A C T

The nearly cosmopolitan tribe Desmodieae (Fabaceae) includes many important genera for medicine and forage. However, the phylogenetic relationships among the infratribal groups circumscribed using morphological traits are still poorly known. In this study, we used chloroplast (*rbcL*, *psbA-trnH*) and nuclear (ITS-1) DNA sequences to investigate the molecular phylogeny and historical biogeography of Desmodieae, and infer ancestral states for several vegetative and reproductive traits. Three groups, corresponding to the Desmodium, Lespedeza, and Phylloidium groups *sensu* Ohashi were retrieved in the phylogenetic analyses. Conflicts in the topologies inferred from the chloroplast and nuclear datasets were detected. For instance, the Lespedeza clade was sister to the groups Phylloidium + Desmodium based on chloroplast DNA, but nested within the Desmodium group based on ITS-1. Moreover, the New Caledonian endemic genera *Arthroclianthus* and *Nephrodesmus* were not monophyletic but together formed a clade, which also included *Hanslia* and *Ohwia* based on chloroplast DNA. The hypothetical common ancestor of Desmodieae was dated to the Middle Oligocene (ca. 28.3 Ma) and was likely an Asian shrub or tree producing indehiscent loments. Several colonization events towards Oceania, America, and Africa occurred (all less than ca. 17.5 Ma), most probably through long distance dispersal. The fruits of Desmodieae repeatedly evolved from indehiscence to dehiscence. We also showed that indehiscent loments allow for more variability in the number of seeds per fruit than indehiscent legumes. Modularity seems here to allow variability in the number of ovules produced in a single ovary.

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## 1. Introduction

The tribe Desmodieae (Benth.) Hutchinson is an Old World tribe of the legume subfamily Papilionoideae. It includes many important genera used for medicine and forage. The tribe Desmodieae comprises 32 genera and ca. 530 species that mostly inhabit tropical, subtropical, and warm-temperate regions, but also extend into the cool-temperate and sub-boreal regions of Eastern Asia and North America (Ohashi, 2005; Ohashi and Ohashi, 2012a, 2012b). Two genera, *Arthroclianthus* Baill. and *Nephrodesmus* Schindl., are endemic to New Caledonia. The Desmodieae are commonly erect and trifoliolate herbs, shrubs or rarely trees, with fruits either composed of a single article (a legume) or of several segments (a loment; Ohashi et al., 1981) (Fig. 1). The loment consists of a single carpel that disarticulates into single-seeded

segments when ripe (Spjut, 1994). Fruit characters are of high taxonomical value in Desmodieae and in Papilionoideae as a whole (e.g. segmentation, dehiscence, size, pubescence, isthmus margin in loments; Kirkbride et al., 2003).

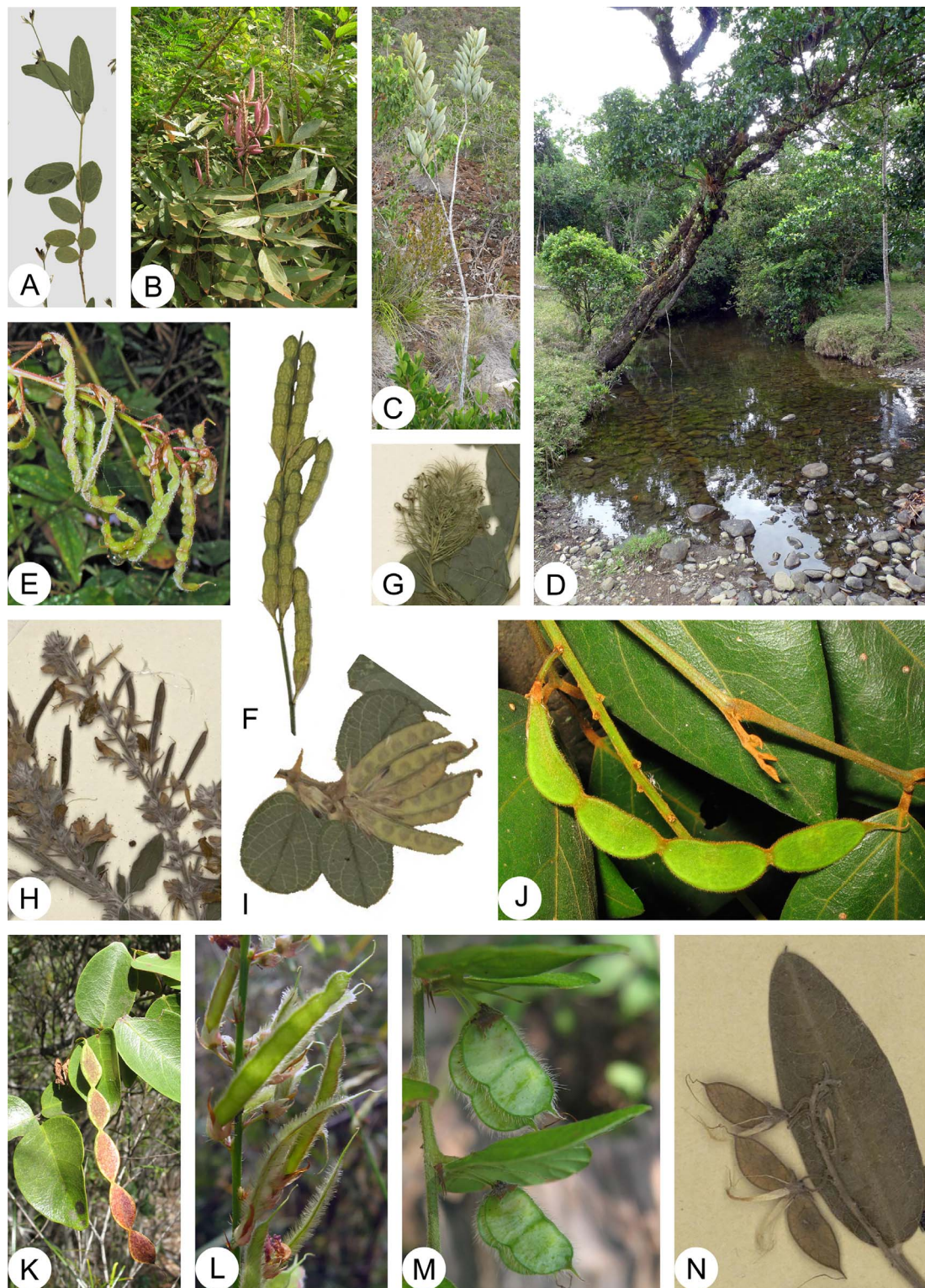
The Desmodieae have been once considered similar to tribe Phaseoleae (Polhill, 1981). They were subsequently shown to be a monophyletic group included within Phaseoleae *sensu lato*, and closely related to subtribe Kennediinae (Doyle and Doyle, 1993; Bruneau et al., 1995; Kajita et al., 1996; Doyle et al., 1997; Wojciechowski et al., 2004; Egan et al., 2016). A supertree using chloroplast DNA data (cpDNA; *rbcL*, *matK*, *trnL* intron; Lewis et al., 2005) placed the Desmodieae in the Millettoid/Phaseoloid clade, the single clade of the Papilionoideae subfamily characterized by a macromorphological apomorphy, pseudoracemose inflorescences (Wojciechowski et al., 2004). The

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**Fig. 1.** Diversity of habits and fruit types in the tribe Desmodieae. (A) Herb: *Alysicarpus ovalifolius* (Desmodium group; one individual from the specimen *Labat 3952*, Paris Herbarium (P), barcode P00577613), (B) Subshrub: *Urvia crinita* (Desmodium group; credit: Sovanmoly Hul), (C) Shrub: *Nephrodesmus sericeus* (Desmodium group; credit: Jean-Noël Labat, *Labat 3913* (P), P00609010), (D) Tree: *Arthroclianthus angustifolius* (Phylloidium group; credit: J.N. Labat), (E) Indehiscent loment: *Desmodium intortum* (Desmodium group; credit: J.N. Labat, *Labat 3797* (P), P00527423), (F) Indehiscent loment: *Alysicarpus ovalifolius* (Desmodium group; fruits from the specimen *Labat 3952* (P), P00758171), (G) Dehiscent legume: *Mecopus nidulans* (Desmodium group; fruits from the specimen *Cheng, David & Leti CL721* (P), P00625251), (H) Dehiscent legume: *Pseudarthria hookeri* (Desmodium group; fruits from the specimen *Jongkind 2462* (P), P03496356), (I) Dehiscent legume: *Melliniella micrantha* (Desmodium group; fruits from the specimen *Raynal-Roques 22514* (P), P03091983), (J) Indehiscent loment: *Nephrodesmus francii* (Phylloidium group; credit: J.N. Labat, *Labat 3932* (P), P00609037), (K) Indehiscent loment: *Arthroclianthus deplanchei* (Phylloidium group; credit: J.N. Labat, *Labat 3915* (P), P00609013), (L) Indehiscent loment: *Tadehagi triquetrum* (Phylloidium group; credit: S. Hul), (M) Indehiscent loment: *Phylloidium pulchellum* (Phylloidium group; credit: S. Hul), (N) Indehiscent legume: *Campylotropis polyantha* (Lespedeza group; fruits from the specimen *Soulié 3968* (P), P03089856).

**Table 1**  
Generic composition, sampling ratio, and geographic distribution of the infratribal groups of the tribe Desmodieae (from: Ohashi, 2005, Ohashi and Ohashi, 2012a, 2012b; Huang et al., 2010).

Infratribal groups of Desmodieae	Genera	Sampling ratio	Distributional range
<i>Desmodiinae subtribe</i>			
Desmodium Group (11 genera studied/17 genera in total)	<i>Alysicarpus</i> <i>Christia</i> <i>Codariocalyx</i> <i>Desmodium</i> <i>Desmodiastrum</i> <i>Eliotis</i> <i>Hegnera</i> <i>Hylodesmum</i> <i>Leptodesmia</i> <i>Mecopus</i> <i>Melliniella</i> <i>Monarthrocarpus</i> <i>Ototropis</i> <i>Pseudarthria</i> <i>Pycnospora</i>	2/25-30 3/ca. 10 1/2 5/ca. 260 0/4 0/2 1/1 3/14 1/3 1/3 1/2 0/1 0/13 1/3-4 0/1	Africa, Asia (China, India, Japan, Malaysia, South-East Asia), Oceania (Australia) Asia (China, India, Malaysia, South-East Asia), Oceania (Australia) Asia (China, India, Malaysia, South-East Asia, Sri Lanka, Taiwan) Africa (Madagascar), America, Asia, Oceania Asia (India, Malaysia) Asia (India, South-East Asia, Sri Lanka) Asia (Malaysia, South-East Asia) Africa, America (East North America), Asia (East Asia, India through China) Africa (Madagascar), Asia (India) Asia (India, Malaysia, South China, South-East Asia) Africa (Central and West Africa) Asia (East Malaysia), Oceania (Papua New Guinea) Asia, Oceania Africa, Asia (India, Malaysia) Africa (East Africa, Somalia), Asia (East and South-East Asia, India), Oceania (Australia) Asia (Malaysia, South China, South-East Asia) Africa, Asia (India, Malaysia, South China, South-East Asia, Taiwan)
Phyllodium Group (11/12)	<i>Trifidacanthus</i> <i>Uraria</i> <i>Akschindium</i> <i>Apylloidium</i> <i>Arthroclianthus</i> <i>Dendrobium</i> <i>Droognansia</i> <i>Hanslia</i> <i>Nephrodesmus</i> <i>Ohwia</i> <i>Ougenia</i> <i>Phyllodium</i> <i>Tadehagi</i> <i>Verdesmum</i>	0/1 3/ca. 20 1/1 1/7 10/ca. 30 2/18 2/ca. 5 1/2 4/5 1/2 1/1 3/8 1/ca. 6 0/1	Asia (South-East Asia) Asia (India, Malaysia, South China, South-East Asia, Sri Lanka), Oceania (Australia, Papua New Guinea) Oceania (endemic to New Caledonia) Asia (India to Japan, Malaysia, South-East Asia), Oceania (Australia) Africa (Central to South Central Africa) Oceania (Australia, Papua New Guinea, Vanuatu), Asia (Malaysia) Oceania (endemic to New Caledonia) Asia (China to Japan, India, Malaysia, South-East Asia) Asia (India and West Nepal) Asia (East and South-East Asia, India), Oceania (North Australia) Asia (India to South-East Asia, China, Malaysia), Oceania (North Australia) Oceania (Papua New Guinea)
<i>Lespedezinae subtribe</i>	<i>Campyloropsis</i> <i>Kummerowia</i> <i>Lespedeza</i>	5/ca. 37 1/2 2/ ca. 35	Asia (India to South-East Asia, China) Asia (East Asia) Asia (China, East Asia, India, Malaysia), America (North America)

**Table 2**

List and characteristics of the species/samples included in the study, and GenBank accession numbers. Within each group, species names are ordered according to the position of the relative accession in the chloroplast phylogenetic tree. Group: D, Desmodium group; L, Lespedeza group; P, Phyllodium; O: outgroup. Sequences generated for the first time for particular species are marked with an asterisk. The genera that were sequenced for the first time (*Leptodesmia* and *Ougeinia*) are also marked with an asterisk. Voucher information includes the Paris Herbarium barcode (followed by 'SI' for silica-dried samples), the geographic origin, name of the collector, and collection number. Distribution: (a) Asia, (b) Oceania, (c) Africa, (d) America, (ab) Asia and Oceania; (ac) Asia and Africa; (abc) Asia and Oceania and Africa. Habit: (1) Herb; (2) Shrub; (3) Shrub or tree; (4) Herb and subshrub; (5) Subshrub and 'shrub or tree'. Fruit: (1) Indehiscent loment; (2) dehiscent legume; (3) Indehiscent legume. Size of the fruit and number of seeds per fruit: (1) small fruit with up to 3 seeds; (2) average-sized fruit with up to 6 seeds; (3) long fruit with up to 7 seeds and more. References used for coding the character states are the following: [Ohashi \(1973\)](#); [Kirikbride et al. \(2003\)](#) and [Lewis et al. \(2005\)](#).

Taxa	Group	P barcode	Origin and voucher	GenBank accession number	Distribution	Habit	Fruit	Seed number
Desmodieae				<i>rbcL</i> <i>psbA-trnH</i> <i>ITS-1</i>				
<i>Alysicarpus bupleurifolius</i> (L.) DC.	D	P03114294	Thailand, A.F.G. Kerr 16207	KY702606	ab	1	1	2
<i>Alysicarpus ovalifolius</i> (Schum.) J. Léonard	D	P00577613	Mauritania, J.-N. Labat et al. 3952	KY702607	ac	1	1	3
<i>Christia conwalliana</i> (Schindl.) H. Ohashi	D	P03089594	Vietnam, E. Poilane 12517	KY702608*	a	1	1	2
<i>Christia obcordata</i> (Poir.) Bakh. f. ex Meenwen	D	P03089572	Thailand, T. Sørensen et al. 2493	KY702609*	ab	1	1	2
<i>Christia vesperitilonis</i> (L. f.) Bakh. f. ex Meeuwen	D	P00633105	Cambodia, Cheng et al. Cl.683	KY702610	a	1	1	2
<i>Codariocalyx groides</i> (Roxb. ex Link) X.Y. Zhu	D	P00695519	Cambodia, M. Newman et al. 2233	KY702611*	ab	5	3	3
<i>Desmodium adscendens</i> (Sw.) DC.	D	P03623122	New Caledonia, M. Baumann-Bodenheim & A. Guillaumin 7945	KY702612*	abc	4	1	2
<i>Desmodium heterocarpum</i> (L.) DC.	D	P00695889	Cambodia, M. Newman et al. 2139	KY702613	abc	4	1	3
<i>Desmodium heterophyllum</i> (Willd.) DC.	D	P03623154	New Caledonia, M. Baumann-Bodenheim & A. Guillaumin 12157	KY702614*	abc	4	1	2
<i>Desmodium intortum</i> (Mill.) Urb.	D	P00527423	Comoros, J.-N. Labat et al. 3797	KY702615	d	4	1	3
<i>Desmodium velutinum</i> (Willd.) DC.	D	P00181647	Mayotte, M. Pignal 1294	KY702616*	ac	5	1	3
<i>Hegnora obcordata</i> (Miq.) Schindl.	D	P00096071	Thailand, C.F. van Beusekom et al. 4135	KY702617	a	2	1	2
<i>Hyolodessum oldhamii</i> (Oliv.) H. Ohashi & R.R. Mill	D	P02937755	Japan, G. Murata 18538	KY702618*	a	1	1	1
<i>Hyolodessum podocarpum</i> (DC.) H. Ohashi & R.R. Mill	D	P02939195	Korea, V. Komarov 960	KY702619	a	1	1	1
<i>Hyolodessum repandum</i> (Vahl) H. Ohashi & R.R. Mill	D	P02938380	Laos, E. Poilane 28355	KY702620*	abc	2	1	2
<i>Leptodesmia congesta</i> Baker*	D	P00533699	Madagascar, J.-N. Labat et al. 3655	KY702621*	ac	4	3	1
<i>Mecopus nidulans</i> Benn.	D	P00623521	Cambodia, K.C. Cheng et al. Cl.721	KY702622	a	1	3	1
<i>Melliniella micrantha</i> Harms	D	P03091983	Cambodia, K.C. Cheng et al. Cl.721	KY702623*	c	1	3	3
<i>Pseudarthria hookeri</i> Wight & Arn.	D	P03496356	Mali, A. Reynal-Roques 22514	KY702624	c	1	3	3
<i>Uraria cinnata</i> (L.) Desv. ex DC.	D	P00626168	Ghana, C.C.H. Jongkind 2462	KY702625	c	4	3	3
<i>Uraria lagopodioides</i> (L.) DC.	D	P03103654	Cambodia, C. Long et al. Cl.314	KY702626	a	2	1	2
<i>Uraria rufescens</i> (DC.) Schindl.	D	P02756238	China, Hong Kong, J.P.W. Woo & T.K. Woo 647	KY702626	ab	1	1	1
<i>Campylotropis bonii</i> Schindl.	L	P03089256	Thailand, C.F. van Beusekom & C. Charoenpol 1987	KY702628*	a	2	1	3
<i>Campylotropis delavayi</i> (Franch.) Schindl.	L	P03089355	Thailand, K. Larsen et al. 42555	KY702628*	a	3	2	1
<i>Campylotropis macrocarpa</i> (Bunge) Rehder	L	-	China, G. Forrest 16948	KY702629*	a	3	2	1
<i>Campylotropis polyantha</i> (Franch.) Schindl.	L	P03089856	Cultivated, MNHN Garden, Paris	KY702630	a	3	2	1
<i>Campylotropis striata</i> (Thunb.) Schindl.	L	P03089792	China, Tibet, J.A. Soutilé 3968	KY702631*	a	3	2	1
<i>Lespedeza juncea</i> (L. f.) Pers.	L	P02751576	China, W.P. Fang 3504	KY702632*	a	3	2	1
<i>Lespedeza simulata</i> Mack. & Bush	L	P03093515	Japan, M. Toghshi 589	KY702633	a	1	2	1
<i>Akschindium godefroyanum</i> (Kuntze) H. Ohashi	P	P00093355	Japan, H. Ohashi s.n.	KY702634*	a	4	2	1
<i>Aphyllodium biarticulatum</i> (L.) Gagnep.	P	P00096063	USA, D. Demaree 36231	KY702635*	d	4	2	1
<i>Arthroclianthus balansae</i> Schindl.	P	P02296319	Thailand, K. Larsen et al. 31817	KY702636*	a	3	1	2
<i>Arthroclianthus sanguineus</i> Baill.	P	P02296364	Cambodia, Poré-Maspéro 242	KY702640*	ab	2	1	1
<i>Arthroclianthus macrobaryosus</i> Hochr.	P	P02296319	New Caledonia, J.M. Veillon 6127	KY702641*	b	3	1	3
<i>Arthroclianthus obovatus</i> Hochr.	P	P02296364	New Caledonia, G.L. Webster 18371	KY702642*	b	3	1	3
<i>Arthroclianthus deplanchei</i> Hochr.	P	P02296557	New Caledonia, V. Hequet 3627	KY702643*	b	3	1	3
<i>Arthroclianthus angustifolius</i> Hochr.	P	P02296383	New Caledonia, T. Jaffré 3291	KY702644*	b	3	1	3
<i>Arthroclianthus leratii</i> Schindl.	P	P02296411	New Caledonia, H.S. Mackee 12283	KY702603*	b	3	1	3
<i>Arthroclianthus cuneatus</i> Schindl.	P	P02296353	New Caledonia, A.J. Le Rat 454	KY702601*	b	3	1	3
<i>Arthroclianthus andersonii</i> Schindl.	P	P02296282	New Caledonia, E.-I. Franc 2500	KY702602*	b	3	1	3
<i>Arthroclianthus maximus</i> Schindl.	P	P00749540 (SI)	New Caledonia, G.D. McPherson 6511	KY702600*	b	3	1	3
<i>Dendrobium lanceolatum</i> (Dunn) Schindl.	P	P00606856	New Caledonia, J.-N. Labat et al. 4029	KY702605*	b	3	1	3
<i>Dendrobium triangulare</i> (Retz.) Schindl.	P	P00626170	Cambodia, C. Long & K.C. Cheng Cl.024	KY702645	a	3	1	2
<i>Droognansia montana</i> Jacq.-Fél.	P	P03092486	Cambodia, H. Long et al. Cl.1316	KY702646	a	3	1	2
<i>Droognansia pteropus</i> (Baker) De Wild.	P	P03092470	Guinea, H. Jacques-Félix 1959	KY702647*	c	3	1	3
<i>Hanslia ormocarpoides</i> (DC.) H. Ohashi	P	P00591396	Burundi, M. Reekmans 6409	KY702648*	c	5	1	2
			Vanuatu, M. Pignal & F. Brunois 2771	KY702649*	ab	3	1	2

(continued on next page)

Table 2 (continued)

Taxa	Group	P barcode	Origin and voucher	Genbank accession number		Disribution	Habit	Fruit	Seed number
				<i>rbcl</i>	<i>psbA-trnH</i> ITS-1				
<b>Desmodieae</b>									
<i>Nephrodesmus francii</i> (Harms) Schindl.	P	P00609041 (Sil)	New Caledonia, J.-N. Labat et al. 3933	KY702650*	MF084275*	b	5	1	2
<i>Nephrodesmus ferrugineus</i> Daniker	P	P02296269 (Sil)	New Caledonia, J. Munzinger et al. 3011	KY702651*	MF084276*	b	3	1	2
<i>Nephrodesmus sericeus</i> Schindl.	P	P00609010 (Sil)	New Caledonia, J.-N. Labat et al. 3913	KY702652*	MF084277*	b	3	1	1
<i>Nephrodesmus parvifolius</i> Schindl.	P	P00454765 (Sil)	New Caledonia, J.-N. Labat et al. 3503	KY702638*	MF084263*	b	3	1	1
<i>Ohwia caudata</i> (Thunb.) H. Ohashi	P	P03993618	Japan, H. Kanai 666	KY702653*	MF084278*	a	5	1	3
<i>Ougeinia dalbergioides</i> Benth.*	P	P02396263	India, J.D. Hooker s.n.	KY702654*	MF084279*	a	3	1	2
<i>Ougeinia dalbergioides</i> Benth.*	P	P02396265	India, R. Strachey & J.E. Winterbottom 2	KY702655*	MF084280	a	3	1	2
<i>Phyllodium elegans</i> (Lour.) Desv.	P	P00625262	Cambodia, K.C. Cheng et al. CL712	KY702656*	MF084281	a	3	1	2
<i>Phyllodium longipes</i> (Craib) Schindl.	P	P00606870	Cambodia, C. Long & K.C. Cheng CL037	KY702657*	MF084282*	a	3	1	2
<i>Phyllodium pulchellum</i> (L.) Desv.	P	P00625078	Cambodia, K.C. Cheng et al. CL807	KY702658*	MF084283	ab	3	1	1
<i>Tadehagi triquetrum</i> (L.) H. Ohashi	P	P00695517	Cambodia, M. Newman et al. 2111	KY702659	MF084284	ab	5	1	3
<b>Phaseoleae</b>									
<i>Phaseolus vulgaris</i> L.	O	-	-	NC_009259	NC_009259	d	1	3	3
<i>Vigna radiata</i> (L.) R. Wiltzek	O	-	-	NC_013843	NC_013843	a	1	3	3
<b>Millerieae</b>									
<i>Fordia cauliflora</i> Hensl.	O	P02942273	China, Hong-Kong, K.Y. Chan 108	KY702636	MF084261	a	3	3	1
<i>Muelleria frutescens</i> (Aubl.) Standl.	O	P03088013	Colombia, H.P. Fuchs & L. Zanella 21740	KY702637	MF084262*	d	3	1	1

phylogenies published by the Legume Phylogeny Working Group (LPWG, 2013, 2017) showed that the Desmodieae tribe is included within the Indigoferoid/Millettioid clade, together with Phaseoleae *sensu lato*, Psoraleae, Abreae, and Indigoferae. Within the phaseoloid Fabaceae, The Desmodieae tribe was strongly supported as monophyletic (for seven of the eight cpDNA markers used; Stefanović et al., 2009), and was sister to *Mucuna* Adans.

Based on morphological traits, Ohashi et al. (1981) defined three subtribes within the Desmodieae: the Bryinae, the Desmodiinae, and the Lespedezinae. Subsequent molecular analyses placed the Bryinae within the *Pterocarpus* clade of the Dalbergieae *sensu lato* (Bailey et al., 1997; Doyle et al., 2000; Lavin et al., 2001). Also, the genera *Phyllacium* Benn. and *Neocolletia* Hemsl. were moved from the subtribe Lespedezinae to the tribe Phaseoleae based on morphological, palynological, and molecular evidences (Bailey et al., 1997; Doyle et al., 2000; Kajita et al., 2001). Two subtribes are now recognized within the Desmodieae: the Desmodiinae and Lespedezinae (Ohashi, 2005). The subtribe Desmodiinae shows a higher generic diversity in tropical South and South-East Asia (Dy Phon et al., 1994), while the centres of diversity of subtribe Lespedezinae are in temperate East Asia (Huang et al., 2010) and North America (Isely, 1998). The tribe was further circumscribed into three groups based on an analysis of the chloroplast gene *rbcl* (Kajita et al., 2001; Ohashi, 2005): the Lespedeza group (three genera) corresponding to the Lespedezinae subtribe, and the Phyllodium (12 genera) and Desmodium (17 genera) groups, together corresponding to the Desmodiinae subtribe (Table 1).

The Lespedezinae subtribe has been extensively studied during the last decade. Phylogenetic analyses based on cpDNA regions (Kajita et al., 2001; Stefanović et al., 2009; Nemoto et al., 2010) and nuclear ribosomal ITS (Han et al., 2010) strongly supported the monophyly of the Lespedezinae subtribe, including the three genera *Lespedeza* Michx., *Campylotropis* Bunge, and *Kummerowia* Schindl. *Campylotropis* appeared as the sister group of *Kummerowia* and *Lespedeza*. Asia was inferred as the ancestral distribution of the subtribe (Han et al., 2010; Nemoto et al., 2010), and the disjunct distribution of *Lespedeza*, with eastern Asian and eastern North American species, would be the result of migration through the Bering land bridge during the Miocene (Xu et al., 2012). Later diversification and introgression may have yielded the present species diversity (Xu et al., 2012).

Our aim was to provide a robust phylogeny of the tribe Desmodieae using combined chloroplast (*rbcl*, *psbA-trnH*) and nuclear (ITS-1) sequence data with a large genus sampling including the previously unanalysed genera *Hanslia* Schindl. and *Leptodesmia* (Benth) Benth. We also examined ancestral distribution areas and ancestral states for the habit, fruit type and number of seeds per fruit. Special emphasis was placed on the New Caledonian endemic genera *Arthroclianthus* and *Nephrodesmus*, for which a substantial sampling was available.

Our results were used to: (1) verify the monophyly of the subtribe Lespedezinae; (2) assess the monophyly of the subtribe Desmodiinae and of each of the two groups defined by Ohashi (2005) within the Desmodiinae; (3) interpret ancestral distribution areas and ancestral states for the habit, fruit type and number of seeds per fruit; (4) specify the phylogenetic relationships of the two endemic New Caledonian genera *Arthroclianthus* and *Nephrodesmus* within Desmodiinae; and (5) date their origin in New Caledonia.

## 2. Materials and methods

### 2.1. Taxonomic sampling

The sampling included 25 genera out of the 32 recognized in the tribe (Ohashi, 2005; generic sampling ratio = 78%; Table 1). We investigated a total of 58 species of Desmodieae and four outgroup species listed in Table 2. The outgroup species were chosen based on Queiroz et al. (2015): the genera *Vigna* Savi and *Phaseolus* L. belong to the Phaseoleinae, while *Muelleria* L.f. and *Fordia* Hemsl. belong to the

core Millettieae. Leaf material was either silica-dried in the field, in New Caledonia (for some species of *Arthroclianthus* and *Nephrodesmus*), or sampled from herbarium specimens preserved at the Paris Herbarium (Muséum national d'Histoire naturelle; code P). Taxa sampled, voucher information, and GenBank accession numbers are listed in Table 2.

## 2.2. DNA sequencing

We analyzed the nuclear ribosomal ITS-1 and two chloroplast regions (*rbcL* and *psbA-trnH*), because these have been used successfully for phylogenetic studies of closely related plants (Shaw et al., 2005), and also for phylogenetic studies within Fabaceae and especially within Papilionoideae (Käss and Wink, 1997; Hu et al., 2002; Han et al., 2010; Nemoto et al., 2010). The chloroplast gene *rbcL* has been especially used to circumscribe the groups and genera within the Desmodieae tribe (Kajita et al., 2001).

Herbarium or silica-dried leaf material was grounded into powder and total genomic DNA was extracted using the Qiagen DNeasy Plant Mini Kit (Valencia, California, USA). PCR amplifications of the two cpDNA markers (*rbcL*, *psbA-trnH*) and of the nuclear marker ITS-1 were prepared in 20 µl including 1 µl of non diluted genomic DNA, 2 µl of 10x *Taq* buffer (with 1.5 µM of MgCl<sub>2</sub>), 1 µl of dimethyl sulfoxide 2% (DMSO), 1 µl of bovine serum albumine (BSA), 0.8 µl of dNTPs (6.6 mM), 0.32 µl of each forward and reverse primers, 0.6 U *Taq* polymerase (Qiagen), and sterile distilled water.

The following primers were used: *rbcL1F* (5'-ATGTCACCACAAACAGAAAC-3') and *rbcL724R* (5'-TCGCATGTACTGCAGTAGC-3') for *rbcL* (Fay et al., 1997); *psbA* (5'-GTTATGCATGAACGTAATGCTC-3') and *trnH<sup>GUG</sup>* (5'-CGCGCATGGTGGATTACAATCC-3') for *psbA-trnH<sup>GUG</sup>* (Sang et al., 1997; Tate and Simpson, 2003); ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS2 (5'-GCTGCGTCTTCATCGATGC-3') for the nuclear marker ITS-1 (White et al., 1990).

The PCR programs consisted of 35 cycles of 1 min of denaturation at 94 °C, 1 min annealing at 53 °C for *rbcL*, 56 °C for *psbA-trnH* and 58 °C for ITS-1, 2 min extension at 72 °C and 10 min final extension at 72 °C. PCR amplifications were performed in a Thermal cycler Biometra. The resulting PCR products were then checked on a 1% agarose gel with ethidium bromide. The PCR products were sequenced using Sanger sequencing on a 3730xl DNA Analyzer at Genoscope (Centre National de Séquençage, Evry, France).

Forward and reverse sequences were visually edited and assembled using Sequencher v4.9 (Gene Codes Corp., Ann Arbor, Michigan, USA). All newly generated sequences were deposited in GenBank (Table 2).

## 2.3. Phylogenetic analyses

Sequences were automatically aligned in MUSCLE v3.6 (Edgar, 2004) before the alignment was manually revised in BioEdit v.7.2.5 (Hall, 1999). Indels were coded using the simple coding method of Simmons and Ochoterena (2000) in SeqState (Müller, 2005). Both Bayesian inferences (BI) and Maximum Likelihood (ML) analyses were used to estimate phylogenetic relationships. Bayesian inferences were performed using MrBayes v.3.2.5 (Ronquist et al., 2011). For each region, the most adequate model of nucleotide substitution was identified under the Akaike information criterion in MrModelTest v.2.3 (Nylander, 2004): GTR + I + G for ITS-1 and *rbcL*, and GTR + G for *psbA-trnH*. For indels, we used the restriction site (binary) model with the option lset coding = variable. Two independent but parallel analyses were conducted using flat priors, starting from random trees and consisting of four chains each. The analyses were run for 5 million generations, sampling every 100 generations and with a 10% burnin. For comparison, the analyses were also run for 30 million generations, sampling every 1000 generations and with a 25% burnin. Analysis of output parameters, in Tracer v.1.6 (Rambaut et al., 2014), confirmed the convergence of chains and adequate burnin length. Post-burnin

trees were pooled and 50% majority-rule consensus trees were computed with posterior probability (PP) estimates for all nodes. We ran separate analyses for each region, checked that there was no conflict between the topologies based on the two cpDNA regions, and concatenated the *rbcL* and *psbA-trnH* sequences to infer a single topology based on the combined cpDNA dataset. Comparison of the combined cpDNA tree with the nuclear ITS-1 tree, however, revealed supported contradictions (with PP > 0.8 and bootstrap support (BS) > 70%); both datasets were hence not combined. The ML analyses were performed in raxmlGUI 1.5.1 (Silvestro and Michalak, 2012; Stamatakis, 2014), using the same partitions and models of nucleotide evolution as for the BI. We performed 1000 rapid bootstrap replicates and searched for the best-scoring ML tree.

## 2.4. Molecular dating

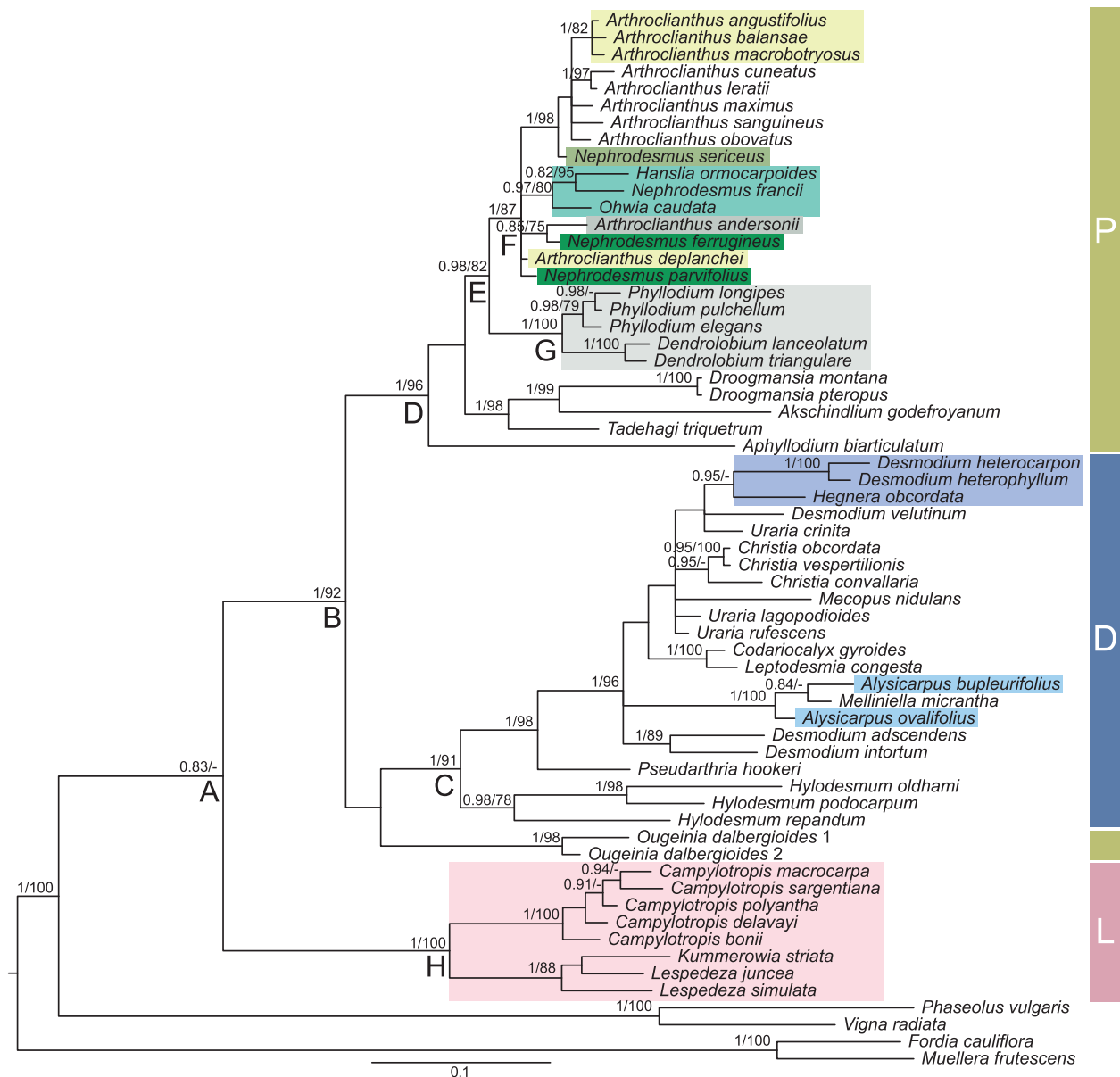
We implemented an uncorrelated lognormal relaxed clock approach in BEAST v.1.8.1 (Drummond et al., 2012), based on the combined cpDNA dataset. The prior for the ucl.d.mean parameter was uniform between 0 and 10E100, with an initial value of 1. A Birth-Death process was employed as tree prior, and other parameters were left to default values. Node calibration relied on one fossil and one secondary calibration point. A fossil of a *Campylotropis* species most similar to the extant *C. macrocarpa* (Bunge) Rehder was dated to 5.3 Ma (Guo and Zhou, 1992; Xu et al., 2012). We therefore modelled the stem node of *C. macrocarpa* by a uniform prior between 5.3 and 53.0 Ma. In addition, the crown node of tribe Desmodieae was dated to ca. 27.0 Ma by Simon et al. (2009). Therefore, we modelled the age of the tribe by a normal distribution with a mean of 27.0 and a standard deviation of 2.0 Ma. Two Markov Chain Monte Carlo (MCMC) analyses were run for 50 million generations, and sampled every 2000 generations. Tracer v.1.6 was used to confirm convergence among chains and adequate effective sample sizes (ESS > 200). Both chains were combined using Log-Combiner 1.4.8, after discarding the first 10% generations as burnin. Trees were summarized in a maximum clade credibility tree obtained in TreeAnnotator 1.4.8, and visualized in FigTree 1.1.2 (available at <<http://tree.bio.ed.ac.uk/software/figtree/>>).

## 2.5. Biogeography and character evolution

The distribution areas and character states of the extant species were coded according to Ohashi (1973), Kirkbride et al. (2003) and Lewis et al. (2005). We used a Dispersal-Extinction-Cladogenesis model (DEC; Ree and Smith, 2008) implemented in RASP v.3.2 (Yu et al., 2015) to infer ancestral biogeographical areas. Distribution ranges of extant taxa were coded as combinations of four geographic areas: (a) Asia; (b) Oceania; (c) Africa; (d) America. The condensed tree was the maximum clade credibility tree estimated in BEAST, and the maximum number of ancestral areas was not constrained.

To infer the ancestral states for the morphological traits, we used maximum likelihood as implemented in Mesquite v.3.11 (Maddison and Maddison, 2016). Analyses were carried out on the Bayesian cpDNA tree (with 61 tips, after collapsing the branches leading to the two accessions of *Ougeinia dalbergioides* Benth.), taking into account branch lengths and using the Markov k-state one-parameter model, which is a generalization of the Jukes-Cantor model (Lewis, 2001) and assumes a single rate for all transitions between character states.

The habit was coded using five states (including two combined states), namely: (1) Herb (Fig. 1A); (2) Subshrub (Fig. 1B); (3) Shrub (Fig. 1C) or tree (Fig. 1D); (4) Herb and subshrub; (5) Subshrub and shrub or tree. We defined three categories of fruit types: (1) Indehiscent loment (Fig. 1E-F, J-M); (2) Dehiscent legume (Fig. 1G-I); (3) Indehiscent legume (Fig. 1N). The size of the fruit characterized by the number of seeds in the fruit was coded using three states: (1) small fruit with up to 3 seeds; (2) average-sized fruit with up to 6 seeds; (3) long fruit with up to 7 seeds and more. For the species producing loment,



**Fig. 2.** Phylogenetic tree of the tribe Desmodieae obtained with Bayesian inference from a 62-accession and 1444-position long alignment of concatenated *psbA-trnH* and *rbcL* chloroplast sequences. Posterior probability values > 0.8 and bootstrap supports > 70% are indicated. Nodes that are discussed in the text are noted A to H. The Phyllodium (P), Desmodium (D), and Lespedeza (L) groups are indicated. Lineages belonging to the P, D, and L groups that are incongruently placed in the nuclear tree of Fig. 3 are highlighted with shades of green, blue, and pink, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

this character corresponds to the number of articles. For this last character, personal observations (CS) on herbarium specimens were included in the matrix in order to decrease the number of missing data. The matrix of four characters coded for 61 species (including four outgroup species) is presented in Table 2.

For comparison, we used parsimony in Mesquite to infer the ancestral states for the phenotypic traits (habit, fruit type, and number of seeds in the fruit).

### 3. Results

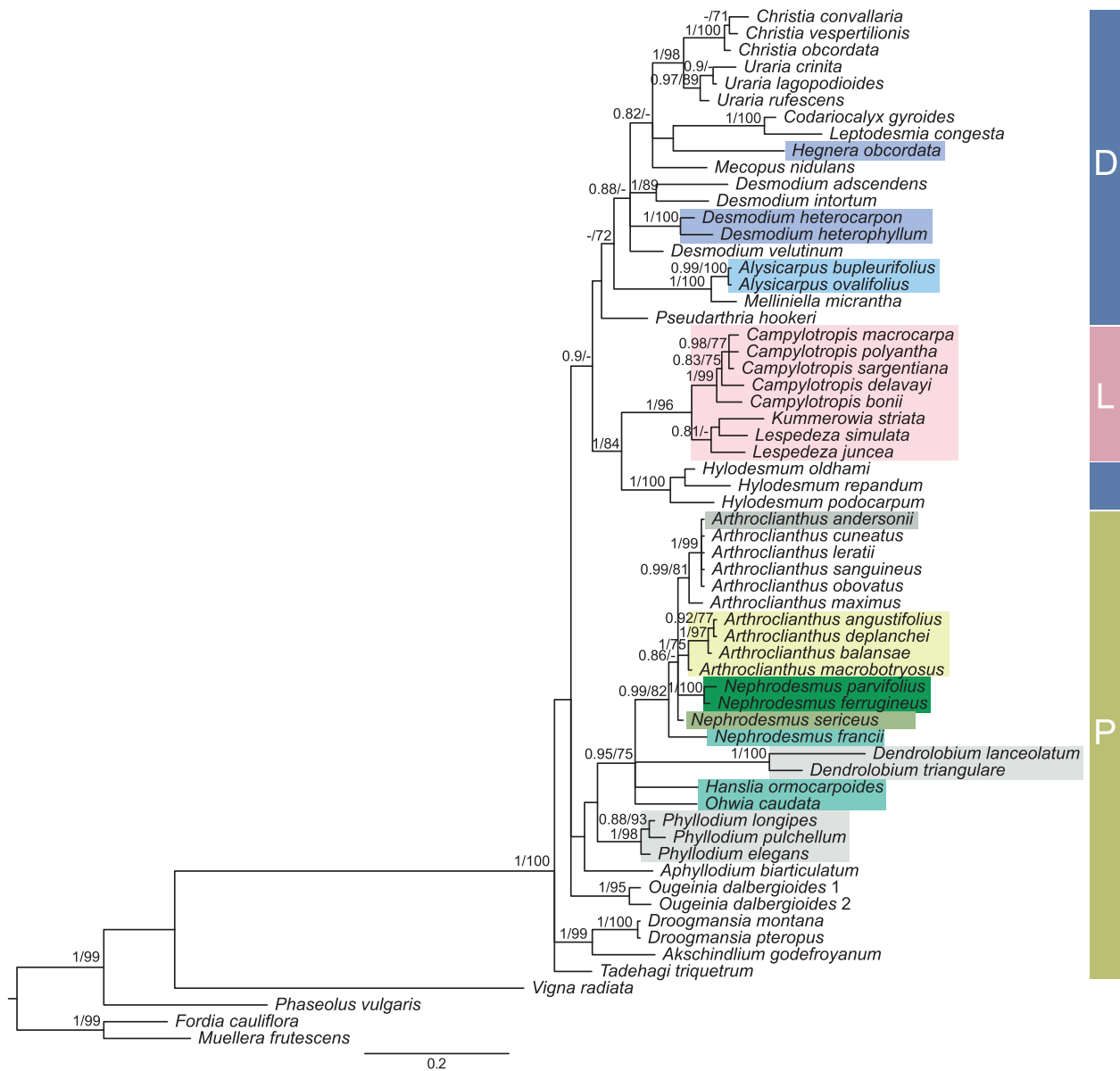
#### 3.1. Phylogenetic relationships

In total, our analyses included 62 species and 180 DNA sequences were newly generated. The ITS-1 alignment was 381-position long and included 100 coded indels, whereas the combined cpDNA alignment was 1444-position long (676 for *rbcL* and 768 for *psbA-trnH*) with 117

indels coded. Both BI analyses (5 and 30 million generations) gave identical tree topologies and nearly identical support values (results from the second run are not shown). Topologies of trees obtained from the BI and ML analyses were similar, only differing for groups with low support values. We hence showed the BS values on the tree topology obtained with the BI (Figs. 2 and 3).

Based on the BI using the combined cpDNA dataset, we showed that the tribe Desmodieae is monophyletic (although moderately supported, PP = 0.83; Fig. 2), but there was a trichotomy including the two groups of Desmodieae and the Phaseoleae tribe based on ML. Within the tribe, three clades were identified. First, the clade whose crown node is noted D (PP = 1, BS = 96%; Fig. 2) partly corresponded to the Phyllodium group and included the genera *Arthroclianthus* Baill., *Nephrodesmus* Schindl., *Hanslia* Schindl., *Ohwia* H. Ohashi, *Phyllodium* Desv., *Dendrolobium* (Wight & Arn.) Benth., *Droogmansia* De Wild., *Akschindlium* H. Ohashi, *Tadehagi* H. Ohashi, and *Aphyllodium* (DC.) Gagnep. Second, the clade whose crown node is noted C (PP = 1, BS = 91%; Fig. 2)





**Fig. 3.** Phylogenetic tree of the tribe Desmodieae obtained with Bayesian inference from a 62-accession and 381-position long alignment of ITS-1 nuclear sequences. Posterior probability values  $> 0.8$  and bootstrap supports  $> 70\%$  are indicated. The Phyllodium (P), Desmodium (D), and Lespedeza (L) groups are indicated. Lineages belonging to the P, D, and L groups that are incongruently placed in the chloroplast tree of Fig. 2 are highlighted with shades of green, blue, and pink, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

corresponded to the Desmodium group and was composed of *Desmodium* Desv., *Hegnere* Schindl., *Uria* Desv., *Christia* Moench, *Mecopus* Benn., *Codariocalyx* Hassk., *Leptodesmia* (Benth.) Benth., *Alysicarpus* Neck. ex Desv., *Melliniella* Harms, *Pseudarthria* Wight & Arn., and *Hylodesmum* H. Ohashi & R.R. Mill. The genus *Ougeinia* Benth. in Miquel (belonging to the Phyllodium group) was sister to the Desmodium group but with very low support (PP = 0.52, BS = 36%; Fig. 2). Third, the group whose crown node is noted H (PP = 1, BS = 100%; Fig. 2) corresponded to the Lespedeza group. It was the earliest diverging of the tribe and included the genera *Campylotropis*, *Kummerowia*, and *Lespedeza*. Clade D was sister to the Desmodium group + *Ougeinia*, all together being sister to the Lespedeza group. In our sampling, twelve genera were represented by several species, and four of them were non-monophyletic: *Arthroclianthus*, *Nephrodesmus*, *Desmodium*, and *Alysicarpus* (*Uria* and *Lespedeza* were also possibly paraphyletic, but the associated support values were low; PP = 0.76, BS = 20% and PP = 0.77, BS = 68%, respectively). In particular, *Arthroclianthus* and *Nephrodesmus* species were intermingled in the phylogeny, and formed

a highly supported clade with *Hanslia* and *Ohwia* (PP = 1, BS = 87%).

Based on ITS-1, the monophyly of the tribe Desmodieae was strongly supported (PP = 1, BS = 100%; Fig. 3). The Phyllodium group was composed of four lineages, two of which included in the basal trichotomy of the tribe: one consisted of *Tadehagi* and the other of *Akschindium* and *Droogmansia* (PP = 1, BS = 99%; Fig. 3). The third lineage was the genus *Ougeinia*, and the fourth clustered most genera of the group with PP = 0.76 and BS = 51%. *Ougeinia*, the main part of the Phyllodium group and all the remaining genera of Desmodieae formed an unresolved and poorly supported clade (PP = 0.64, BS = 32%). The Desmodium group was paraphyletic since the genus *Hylodesmum* was sister to the Lespedeza group (PP = 1, BS = 84%) and *Hylodesmum* + the Lespedeza group was sister to the remaining genera of the Desmodium group (PP = 0.90, BS = 57%). Last, the Lespedeza group was retrieved with high support (PP = 1, BS = 96%).

Considering the three groups Phyllodium, Desmodium, and Lespedeza, there was one supported conflict between the cpDNA and ITS-1 topologies involving the position of the Lespedeza group. It was

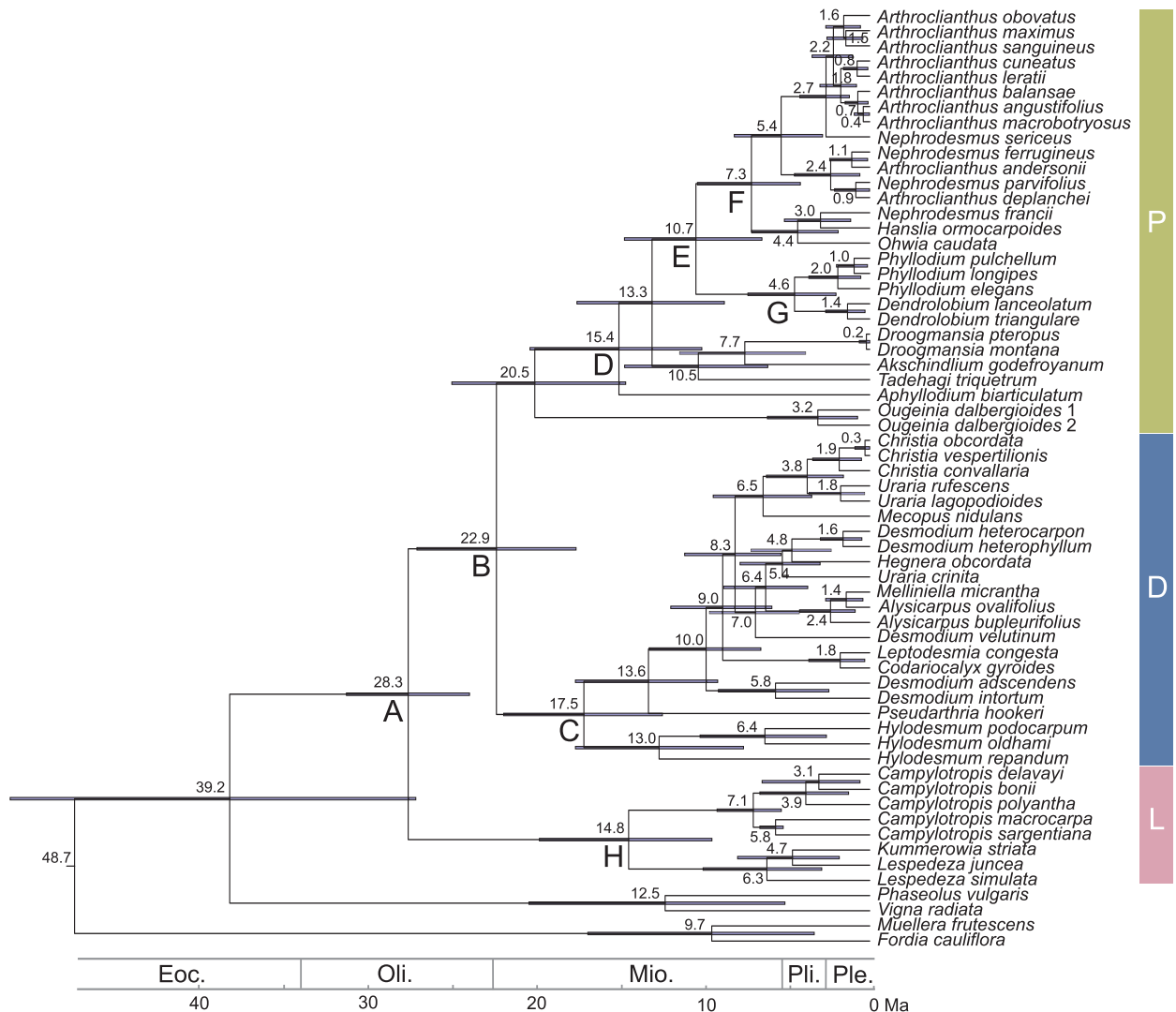


Fig. 4. Chronogram of the tribe Desmodieae obtained under a Bayesian relaxed clock with log-normally distributed rates (cpDNA matrix of 62 accessions, 1444 positions). Bars around node ages indicate the 95% highest posterior density intervals. Nodes that are discussed in the text are noted A to H. The Phyllodium (P), Desmodium (D), and Lespedeza (L) groups are indicated.

retrieved as basal to the tribe and sister group to the groups Phyllodium + Desmodium based on cpDNA, but nested within the Desmodium group and most closely related to genus *Hylodesmum* based on ITS-1. Within each group, the relationships were similar based on cpDNA and ITS-1, albeit with a few exceptions: (i) in the cpDNA phylogeny, *Phyllodium* and *Dendrolobium* were grouped (PP = 1, BS = 100%) and *Phyllodium* + *Dendrolobium* was sister to *Arthroclianthus* + *Nephrodesmus* + *Hanslia* + *Ohwia* with PP = 0.98 and BS = 82% whereas in the ITS-1 phylogeny, *Dendrolobium* was included in the *Arthroclianthus* + *Nephrodesmus* + *Hanslia* + *Ohwia* + *Dendrolobium* clade with PP = 0.95 and BS = 75%. (ii) *Arthroclianthus* and *Nephrodesmus* formed a supported clade based on ITS-1 (PP = 0.99, BS = 82%), whereas they were grouped with *Hanslia* and *Ohwia* based on cpDNA (PP = 1, BS = 87%) because of the position of *Nephrodesmus francii* (Harms) Schindl. clustering with *Hanslia* and *Ohwia* (PP = 0.97, BS = 80%). Also, the respective non-monophyly of *Arthroclianthus* and *Nephrodesmus* relied on a moderate support value in the ITS-1 phylogeny (PP = 0.86, BS = 63%) whereas it was more supported in the cpDNA phylogeny (since *N. sericeus* Schindl. was sister to several *Arthroclianthus* species with PP = 1 and BS = 98%). Last, the relationships among *Arthroclianthus* and *Nephrodesmus* samples differed between the cpDNA and ITS-1 topologies, e.g., *A. angustifolius*

Hochr., *A. balansae* Schindl., and *A. macrobotryosus* Hochr. were grouped with PP = 1 and BS = 82% based on the cpDNA, whereas *A. deplanchei* Hochr. appeared more closely related to *A. angustifolius* and *A. balansae* than *A. macrobotryosus* based on ITS-1. (iii) *Alysicarpus* was monophyletic based on ITS-1 (PP = 0.99, BS = 100%) whereas it was paraphyletic based on cpDNA, but it must be noted that this paraphyly relied on a moderate support value in the cpDNA topology (PP = 0.84, BS = 55%). (iv) *Hegnere* and *Desmodium heterocarpon* (L.) DC. + *D. heterophyllum* (Willd.) DC. were grouped based on cpDNA (PP = 0.95, BS = 51%), but not based on ITS-1.

### 3.2. Molecular dating

Our molecular estimations suggest that the tribe Desmodieae started to diversify 28.3 Ma (95% CI 24.5–32.1 Ma; Fig. 4, crown node A), and that the Phyllodium, Desmodium, and Lespedeza groups started to diversify 20.5 Ma (15.0–25.6 Ma), 17.5 Ma (12.7–22.4 Ma; crown node C), and 14.8 Ma (9.7–20.2 Ma; crown node H), respectively. The clade comprising *Arthroclianthus*, *Nephrodesmus*, *Ohwia* and *Hanslia* (crown node F) diverged from *Phyllodium* and *Dendrolobium* (crown node G) 10.7 Ma (6.6–15.0 Ma) and started to diversify 7.3 Ma (4.3–10.6 Ma).

Geographical regions

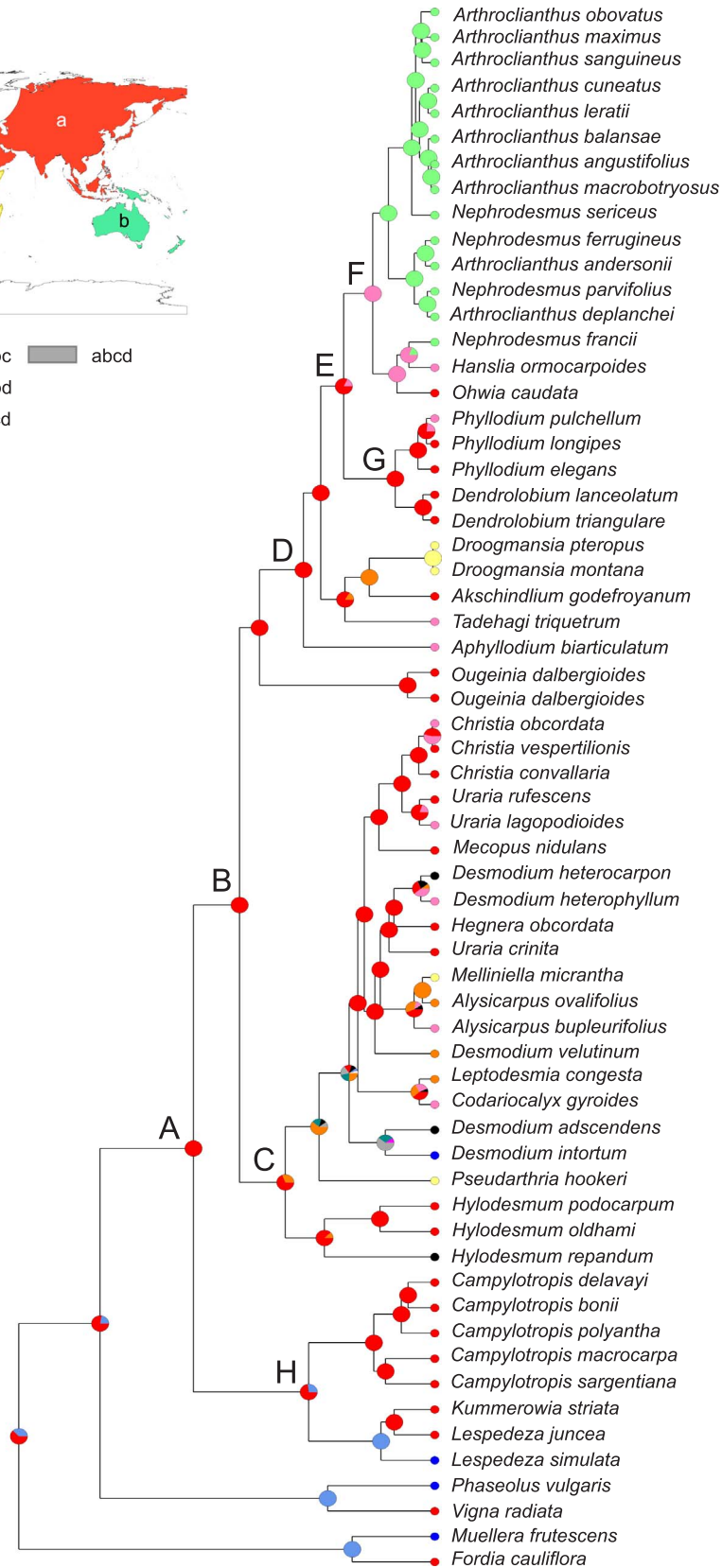
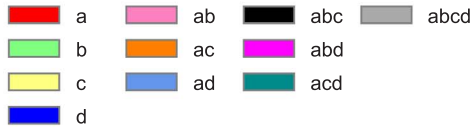
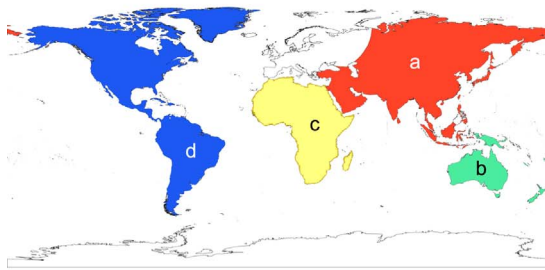
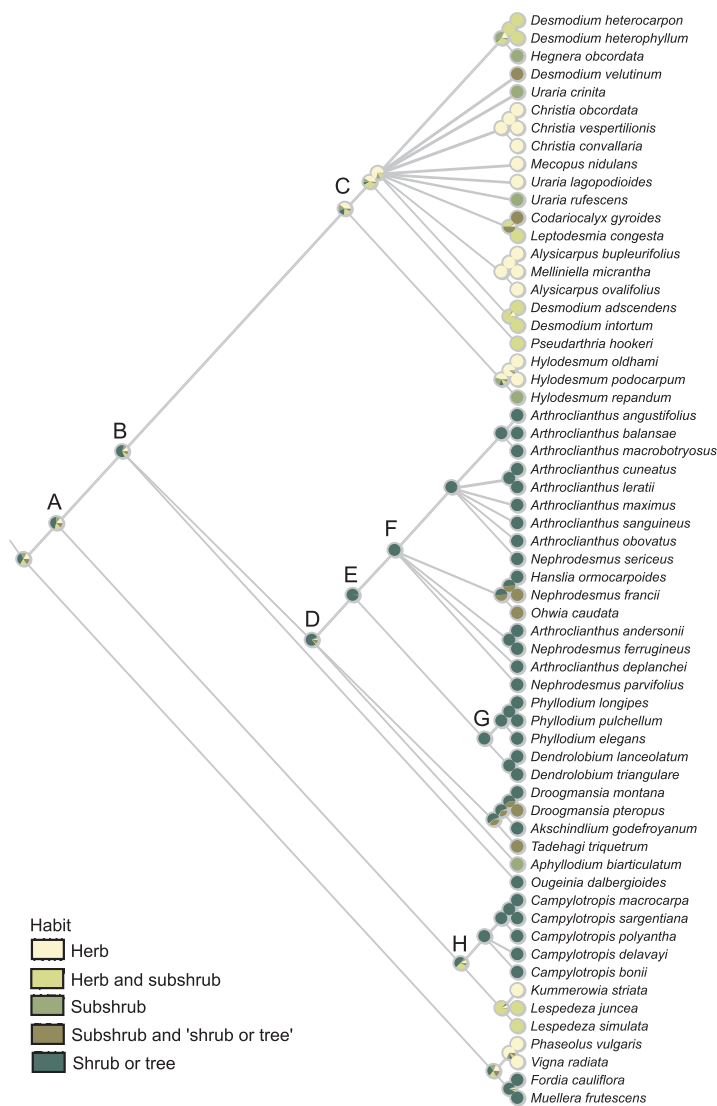


Fig. 5. Evolution of the distribution area of the tribe Desmodieae inferred under the DEC model using RASP v.3.2 on the chloroplast tree obtained with Bayesian inference. Nodes that are discussed in the text are noted A to H. The colored bars refer to the Phyllodium (green), Desmodium (blue), and Lespedeza (pink) groups. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



**Fig. 6.** Evolution of habit in the tribe Desmodieae inferred under maximum likelihood optimization on the 61-accession tree obtained with Bayesian inference. Nodes that are discussed in the text are noted A to H. The colored bars refer to the Phyllodium (green), Desmodium (blue), and Lespedeza (pink) groups. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

### 3.3. Ancestral distribution areas

The hypothetical ancestor of Desmodieae (crown node A) and of each of the Desmodium (crown node C), Phyllodium excluding *Ougeinia* (crown node D), and Lespedeza (crown node H) groups most likely occurred in Asia (with probabilities of 1, 0.69, 1, and 0.73, respectively; Fig. 5). The Lespedeza clade hosts a single colonization event, from Asia to America, which occurred later than 14.8 Ma and led to the *Kummerowia* and *Lespedeza* lineage. In the Phyllodium group, Oceania was probably colonized between ca. 10.7 (node E) and 7.3 Ma (node F), since the most likely ancestral areas were Asia at node E (probability of 0.82) and Asia + Oceania at node F (1), giving rise to the *Arthroclianthus*, *Nephrodesmus*, *Ohwia* and *Hanslia* lineage. Oceania was colonized three additional times, later than 15.4, 10.5, and 1.0 Ma in the lineages leading to *Aphyllodium biarticulatum* (L.) Gagnep., *Tadehagi triquetrum* (L.) H. Ohashi, and *Phyllodium pulchellum* (L.) Desv., respectively.

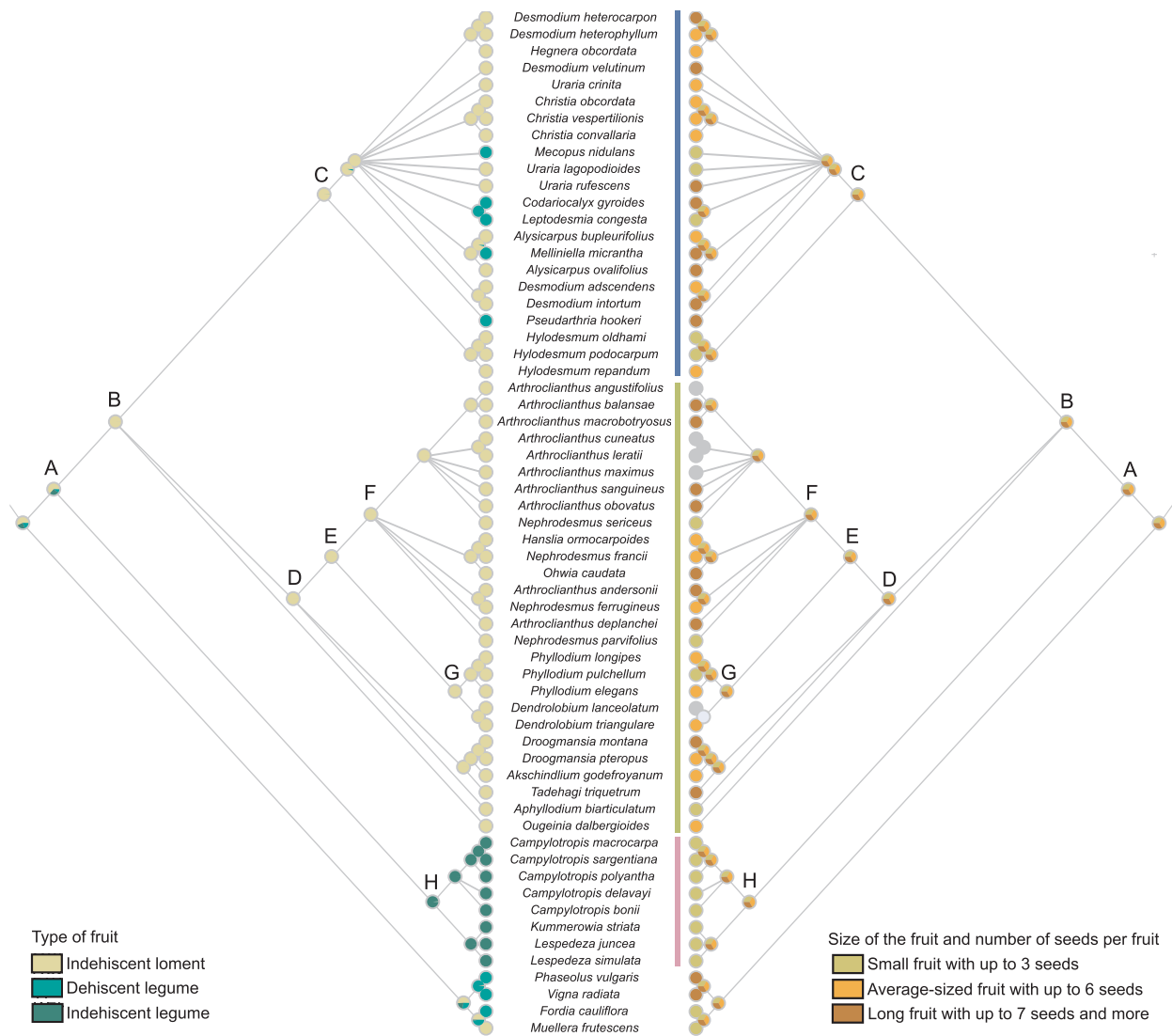
The group Phyllodium also hosts a single colonization from Asia to Africa between 10.5 and 7.7 Ma. Within the Desmodium group, the low resolution did not allow inferring a detailed biogeographic scenario, but the results suggested that America was colonized once, giving rise to the *Desmodium intortum* (Mill.) Urb. and *D. adscendens* (Sw.) DC. lineage, and that Oceania and Africa were colonized several times.

### 3.4. Ancestral states for the habit, fruit type and number of seeds per fruit

The hypothetical ancestor of Desmodieae (crown node A; Fig. 6) was most likely a shrub or a tree (probability of 0.52). In the Lespedeza group (crown node H), the most probable ancestral state was the 'shrub or tree' state (0.51). All *Campylotropis* retained this ancestral state, while plant size decreased in the genus *Lespedeza*, and even more in its sister genus *Kummerowia* (herb).

In the Phyllodium group excluding *Ougeinia* (crown node D), starting from a most likely 'shrub or tree' state (0.84), a few and slight decreases in plant size (from 'shrub or tree' to 'subshrub or 'shrub or tree') were inferred in the lineages leading to *N. francii* and *Ohwia caudata* (Thunb.) H. Ohashi, to *Droogmansia pteropus* (Baker) De Wild. and *Tadehagi triquetrum* (L.) H. Ohashi, and to *Aphyllodium biarticulatum* (L.) Gagnep. The ancestor of the Desmodium group (crown node C) was most likely herbaceous (0.49). Several transitions to higher plant sizes (subshrub) occurred in this group. Among these transitions, the state 'subshrub or 'shrub or tree' evolved at least once and perhaps twice, in the lineage(s) leading to *Desmodium velutinum* (Willd.) DC. and *Codariocalyx gyroides* (Roxb.) Hassk. Ancestral state reconstructions using the parsimony criterion provided similar results (result not shown).

The hypothetical ancestor of Desmodieae (crown node A; Fig. 7) most likely produced indehiscent loments (probability of 0.64; although



**Fig. 7.** Evolution of fruit type and number of seeds per fruit in the tribe Desmodieae inferred under maximum likelihood optimization on the 61-accession chloroplast tree obtained with Bayesian Inference. Nodes that are discussed in the text are noted A to H. The colored bars refer to the Phyllodium (green), Desmodium (blue), and Lespedeza (pink) groups. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

this state is ambiguous when using parsimony (result not shown)). Lineages with dehiscent legumes evolved at least three times, exclusively in the Desmodium group. The production of indehiscent legumes is a synapomorphy of the Lespedeza group (Fig. 7).

The ancestral state for the number of seeds in the fruit could neither be inferred unequivocally in the groups Desmodium, Phyllodium excluding *Ougeinia* (crown node D), and Lespedeza, nor in the tribe Desmodieae as a whole (Fig. 7). The extant lineages included in the Lespedeza group produce indehiscent legumes with up to three seeds.

## 4. Discussion

### 4.1. Phylogenetic relationships within Desmodieae

The monophyly of the tribe Desmodieae was shown in previous studies (Bruneau et al., 1995; Kajita et al., 1996; Doyle et al., 1997; Wojciechowski et al., 2004; Egan et al., 2016; LPWG, 2017), and our analysis confirmed this result based on an unprecedented sampling. Except for the uncertain phylogenetic placement of the genus *Ougeinia*, the main clades identified on the cpDNA tree correspond to the three groups previously circumscribed based on both morphological and molecular characters in the tribe, i.e. Desmodium, Lespedeza, and

Phyllodium. In addition, we found that the genus *Kummerowia* is nested within the genus *Lespedeza* (Figs. 2 and 3), in contrast with the results of Ohashi and Nemoto (2014).

Our sampling included two accessions of *O. dalbergioides* Benth. in order to discard the hypothesis that the uncertain placement of *Ougeinia* was due to a problem when sequencing the cpDNA markers on the first accession. This monospecific genus was not included in the sampling of LPWG (2017). Regarding the tribe Desmodieae, our results were similar to those shown by LPWG (2017), although the latter study was based on a different taxonomic sampling and on a single chloroplast marker (*matK*). However, in our study, *T. triquetrum*, *Akschindlium godefroyanum*, and *Droogmansia* formed a clade together with *Aphyllodium* and the clade gathering the remaining accessions of the Phyllodium group (crown node E), whereas *T. triquetrum* was sister to all the other members of the Phyllodium group (including *T. pseudotriquetrum* (DC.) H. Ohashi) in LPWG (2017).

Our dense genus sampling allowed showing several statistically supported conflicts between the chloroplast and nuclear topologies. Similarly, strong differences in the evolutionary history of different cellular compartments have been reported in other plant groups (e.g. Nauheimer et al., 2012; Kramina et al., 2016). Such conflicts can result from reticulate evolution, due to interspecific hybridization.

Hybridization events in Desmodieae have already been reported in a few studies (e.g. Raveill, 2006; Han et al., 2010; Xu et al., 2017) and hybrid forms (in respect to taxonomically important traits such as fruit form and leaf shape) have been detected when studying herbarium specimens (C. Sarthou, pers. obs.). Other causes of incongruent chloroplast and nuclear tree topologies are incomplete lineage sorting, and particular phylogeographic structure (Rieseberg et al., 1996; Naciri and Linder, 2015). Whether these factors apply to Desmodieae is still hypothetical, and further investigations are needed.

The reconstruction of ancestral states for distribution areas and morphological traits relied on the chloroplast phylogenetic tree of Desmodieae, as the topology was mostly in accordance with the circumscription of infratribal groups as proposed by Ohashi (2005) based on morphological traits, contrary to what was observed on the nuclear tree topology, where the Lespedeza group was nested within the Desmodium group.

#### 4.2. Biogeography and life history trait evolution in Desmodieae

The hypothetical common ancestor of Desmodieae most likely occurred in Asia and dates back to the Middle Oligocene, ca. 28.3 Ma. While the ancestral lineages leading to the three major clades likely remained in Asia, several colonization events towards Oceania, America, and Africa occurred later on, within the last 17.5 Ma. They were most probably mediated through long distance dispersal (LDD) events since landmasses had then already diverged for a long time (McLoughlin, 2001), excluding the potential influence of vicariance. Long distance dispersal has been shown to be a common dispersal process in Fabaceae (Lavin et al., 2004), and it is probable that the seeds drifted through ocean currents such as in the tribe Fabeae (Schaefer et al., 2012) and *Mucuna* (Moura et al., 2016). Nevertheless, the two colonization events from Asia to America in the Desmodium group and in the Lespedeza group occurred less than 10.0 and 14.8 Ma, respectively. This was before the Bering Strait got submerged (Brigham-Grette, 2001) and dispersal could therefore have been mediated through a land bridge.

Two contrasted trends for the evolution of the habit were identified. In the Phyllodium group excluding *Ougeinia*, the habit tends to decrease in size, while it increases in some lineages of the Desmodium group. Both colonization events to America (giving rise to *L. simulata* in the Lespedeza group and to *D. intortum* in the Desmodium group) occurred in lineages presenting herbaceous or shrub habit. The single colonization event of Africa found in the Phyllodium group led to two *Droogmansia* species that differ by their habit. While *Droogmansia montana* Jacq.-Fél. retained the likely ancestral state (trees and shrubs), *D. pteropus* evolved towards lower life forms (trees, shrubs, but also subshrubs). This may be explained by niche competition between sister lineages colonizing the same area.

The type of fruit is a taxonomically useful character to distinguish the species from the Lespedeza group from the other Desmodieae species, as indehiscent legumes are only found in the Lespedeza group. Within the Desmodium group, lineages with dehiscent legumes evolved once or several times from ancestors with indehiscent loments. Furthermore, in the tribe as a whole, only transitions from indehiscence to dehiscence were observed. This may indicate that reduced fruit modularity and dehiscing fruits have been selected during the evolution of Desmodieae, probably because of lower morphogenetic costs and better dispersal abilities.

Indehiscent loments allow for more variability in the number of seeds per fruit than indehiscent legumes (Fig. 7). Evolving from segmented fruits (loments, the ancestral state for Desmodieae) to fruits with a single segment (a synapomorphy of the Lespedeza group) may increase structural constraints during fruit development for ovaries bearing a high number of ovules. This may explain why the Lespedeza lineage consists exclusively of species producing fruits with less than three ovules only. Modularity seems here to allow variability in the

number of ovules produced in a single ovary. The dehiscent legume fruit type is apomorphic in the Desmodium group. In this group, when both the degree of fruit segmentation (loment to legume) and the mode of dehiscence (indehiscent to dehiscent) change, the number of seeds is not necessarily low. The mode of dehiscence might hence add a degree of freedom to carpel development in lineages where species producing legumes evolved from ancestors producing loments.

When comparing the evolution of habit and number of seeds per fruit, it seems that more seeds are produced per fruit when plant size is high (except in the *Melliniella* + *Alysicarpus* clade). Seed production may thus be linked to the age of the individual (positively correlated with plant height in the case of trees and shrubs) or to adaptations leading to more effective dispersal (Norghauer and Newbery, 2015).

#### 4.3. The New Caledonian endemic genera *Arthroclianthus* and *Nephrodesmus*

Although the genera *Arthroclianthus* and *Nephrodesmus* were circumscribed using morphological characters (but see Ohashi et al. (1981), who noted that *Arthroclianthus* is scarcely distinct from *Nephrodesmus*), they were not respectively monophyletic based on our molecular data. A taxonomic revision of both genera is hence much needed.

*Arthroclianthus* and *Nephrodesmus* either formed a clade, or were grouped together with *H. ormocarpoides* (sister to *N. francii*) and *O. caudata* based on the nuclear and chloroplast datasets, respectively. Oceania was likely colonized between 10.7 and 7.3 Ma by the lineage leading to the common ancestor of *Arthroclianthus*, *Nephrodesmus*, *Hanslia*, and *Ohwia* (node E in Fig. 5), and the colonization of New Caledonia necessarily occurred either at this time, or later on. This is long after the period of total oceanic submersion of the island shown by geological evidence, which ended ca. 37 Ma (Pelletier, 2007; Nattier et al., 2017), and colonization by an Asian lineage was most likely due to LDD. Such dispersal from Asia to New Caledonia was already shown in other plant groups, e.g., *Piper* L. (Piperaceae; Smith et al., 2008) and *Diospyros* L. (Ebenaceae; Turner et al., 2013).

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