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Intraspecific and interspecific genetic and phylogenetic relationships in the genus *Populus* based on AFLP markers

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Abstract Although *Populus* has become the model genus for molecular genetics and genomics research on forest trees, genetic and phylogenetic relationships within this genus have not yet been comprehensively studied at the molecular level. By using 151 AFLP® (AFLP® is a registered trademark of Keygene) markers, 178 accessions belonging to 25 poplar species and three interspecific hybrids were analyzed, using three accessions belonging to two willow species as outgroups. The genetic and phylogenetic relationships were generally consistent with the known taxonomy, although notable exceptions were observed. A dendrogram as well as a single most parsimonious tree, ordered the *Populus* sections from the oldest *Leuce* to the latest *Aigeiros*, a pattern consistent with their known evolutionary relationships. A close relationship between *Populus deltoides* of the *Aigeiros* section and species of the *Tacamahaca* section was observed and, with the exception of *Populus wilsonii*, between the species of the *Leucoides*, *Tacamahaca*, and *Aigeiros* sections. *Populus nigra* was clearly separated from its consectional *P. deltoides*, and should be classified separately from *P. deltoides*. The AFLP profiles pointed out to the lack of divergence between some species and revealed that some accessions corresponded with interspecific hybrids. This molecular study provides useful information about genetic relationships among several *Populus* species and, together with morphological descriptions and crossability, it may help review and update systematic classification within the *Populus* genus.

Keywords AFLP fingerprinting · *Populus* · Genetic and phylogenetic relationships · Molecular systematics · Evolution

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Introduction

Populus has become the model of choice for molecular genetics and genomics research on forest trees, mainly because of its fast growth, easy vegetative propagation, amenability to genetic transformation by *Agrobacterium tumefaciens*, and its small genome size. These features have resulted in the development of a range of tools, such as microarrays, activation and gene trap libraries, and genetic maps (Hertzberg et al. 2001; Wullschleger et al. 2002; Bhalerao et al. 2003; Cervera et al. 2004; Boerjan, 2005), and in the genome sequencing (http://genome.jgi-psf.org/Poptr1/Poptr1.home.html) that will aid understanding the specific biology of woody plants.

The genus *Populus* (of which 27 species are listed in Table 1) is a member of the Salicaceae family and is subdivided into six sections (Rehder 1947; Dickmann and Stuart 1983; Eckenwalder 1996). There are approximately 30 species that are widely distributed,

Table 1 *Populus* species and hybrids analyzed

Section	Species	Common name	Accessions per species[a]
Turanga Bge.	*P. euphratica* Oliv.	Euphrates poplar	1 (1/1)
Leucoides Spach.	*P. ciliata* Wall.	Himalayan poplar	5 (1/1)
	P. lasiocarpa Oliv.	Chinese necklace poplar	7 (4/4)
	P. violascens Dode		1 (1/1)
	P. wilsonii Schneid.	Wilson poplar	1 (1/1)
Leuce Duby			
Sub-section *Albidae*	*P. alba* L.	White poplar	7 (7/7)
Sub-section *Trepidae*	*P. davidiana* Schneid.	Korean poplar	1 (1/1)
	P. sieboldii Miq.	Japanese aspen	1 (0/0)
	P. tremula L.	European aspen	5 (5/5)
	P. tremuloides Michx.	Trembling aspen	3 (1/1)
Tacamahaca Spach.	*P. angustifolia* James	Narrowleaf cottonwood	2 (2/2)
	P. balsamifera L.	Balsam poplar	6 (5/5)
	P. candicans Ait.	Balm-of-Gilead	2 (2/3)
	P. cathayana Rehd.		3 (1/1)
	P. koreana Rehd.	Korean poplar	6 (2/2)
	P. laurifolia Ledeb.	Laurel poplar	6 (1/1)
	P. maximowiczii Henry	Japanese poplar	15 (11/11)
	P. simonii Carr.	Simon poplar	11 (9/9)
	P. suaveolens Fisch.		5 (1/1)
	P. szechuanica Schneid	Toghrak poplar	4 (2/3)
	P. yunnanensis Dode		5 (3/3)
	P. trichocarpa Torr. & Gray	Black cottonwood	12 (9/9)
	P. tristis Fisch.	Himalayan balsam poplar	1 (1/1)
Aigeiros Duby	*P. deltoides* Marsh.	Eastern cottonwood	23 (21/21)
	P. fremontii Wats.	Fremont cottonwood	5 (4/0)[b]
	P. nigra L.	Black poplar	30 + 2 (21 + 2/23)[c]
Abaso Ecken.	*P. mexicana* Wesm.	Mexican poplar	1 (1/1)
Interspecific hybrids	*Populus* × *canescens* Smith (*P. alba* × *P. tremula*)	Gray poplar	4 (4/5)
	Populus × *berolinensis* Dippel (*P. laurifolia* × *P. nigra*)	Berlin/Russian poplar	2 (2/3)
	Populus × *canadensis* Dode (Syn *Populus* × *euramericana*; *P. deltoides* × *P. nigra*)	Euramerican poplar	1 (1/9)
Populus-unknown species (blind test)			2 (0)[c]

[a] Number of accessions per species; between *parentheses*, the first number corresponds to the number of accessions used for phylogenetic analysis, i.e., after removing misclassified and mislabeled accessions, and accessions with GS≥0.98; and the second number takes into account the misclassified accessions that could clearly be assigned to a species or hybrid based on the dendrogram and the AFLP patterns

[b] See discussion for explanation

[c] After genetic characterization of the two unknown clones as *P. nigra*

mainly in the Northern Hemisphere. All of them, except for one (*Populus lasiocarpa* Oliv.), are normally dioecious. The sections *Leuce* Duby, *Aigeiros* Duby, and *Tacamahaca* Spach. comprise species of economic importance; 90% of the commercially exploited poplars are eastern cottonwood (*P. deltoides* Marsh.), black poplar (*P. nigra* L.), and their interspecific hybrids (Food and Agriculture Organization, 1979).

Although *Populus* is the model tree species for biological research, information on intraspecific and interspecific phylogenetic relationships in the genus is rather limited. In fact, genus-wide phylogenetic relationships are not known in *Populus*. The placement of species within a section has traditionally been based on morphological and reproductive characters, as well as interspecific crossability (Zsuffa 1975; Food and Agriculture Organization 1979; Rajora and Zsuffa 1984). Members of the same section can hybridize with each other naturally or artificially (Zsuffa 1975; Rajora and Zsuffa 1984). Species of the sections *Aigeiros* and *Tacamahaca* are sexually compatible and natural hybridization occurs among several species of these sections (Zsuffa 1975; Rajora and Zsuffa 1984). However, classical taxonomic analysis, based on morphological characteristics, has proven to be very difficult because of wide intraspecific variability, high natural crossability among members of the genus, and the convergent morphology shown by hybrids and their parental species. For example, the classification of *P. nigra* and *Populus ciliata* Wall. in their respective sections is questionable and controversial. Moreover, the analysis of morphological characters has recently suggested the merging of some species (e.g., *Populus tremula* L., *Populus tremuloides* Michx., and *Populus davidiana* Schneid. into one species, and *Populus maximowiczii* Henry, *Populus koreana* Rehd., *Populus cathayana* Rehd., and *Populus suaveolens* Fisch. into another) (Eckenwalder 1996).

Various genetic markers have been used to examine relationships among a limited number of *Populus* species and hybrids (Keim et al. 1989; Rajora and Zsuffa 1990; Smith and Sytsma 1990; Faivre-Rampant et al. 1992; Barrett et al. 1993; Castiglione et al. 1993; Rajora and Dancik 1995a, 1995b; Khasa et al. 2003). So far, these studies have hinted that *Populus* species generally group along their classical section lines. However, notable exceptions have been observed, such as the placement of *P. nigra* in the *Aigeiros* section. Hence, Rajora and Dancik (1995a) have proposed a new section, *Nigrae* for *P. nigra*, which is separate from the other *Aigeiros* species.

We have studied intraspecific, interspecific, and intersectional genetic and phylogenetic relationships within the genus *Populus*, using AFLP (Vos et al. 1995). The high multiplex ratio of this technique and the relatively large genome coverage of AFLP markers (Powell et al. 1996) make it a useful tool for assessing such relationships (Arens et al. 1998; Winfield et al. 1998; Fay et al. 1999; Mougel et al. 2002). In contrast to previous studies, which mostly assayed a single or a few accessions for a limited number of species, a much higher number of accessions (178), belonging to 25 *Populus* species and several interspecific hybrids, as well as three accessions belonging to *Salix* species, were analyzed. The results, based on 151 AFLP markers, shed new light on the genetic and phylogenetic relationships among several species of the *Populus* genus and represent the first large-scale molecular phylogenetic analysis of the *Populus* genus.

Materials and methods

Plant material and DNA extraction

A collection of 171 accessions, originally thought to belong to 27 *Populus* species, and 7 accessions to 3 interspecific hybrids (*Populus* × *berolinensis* Dippel, *Populus* × *canescens* Smith, and *Populus* × *canadensis* Dode) was made (Table 1). Information on the accessions is provided in Table 2. Three *Salix* accessions (Table 2) were included as outgroups in the study. Total genomic DNA was isolated from fresh or frozen young leaves, obtained either from rooted woody cuttings grown in the greenhouse or from branches sampled in the field, as described by Dellaporta et al. (1983) and Rajora and Dancik (1995b).

AFLP analysis

AFLP analysis was performed as described by Cervera et al. (1996). Pre-amplification was carried out with *Eco*RI + A and *Mse*I + AC primers. To obtain a maximum number of polymorphic and scorable amplified DNA fragments for the *Populus* genome, a combination of one *Eco*RI and one *Mse*I primer, with three selective nucleotides each, was previously suggested (Cervera et al. 1996, 2000). However, due to the high level of interspecific and intraspecific polymorphisms observed, a combination of one *Eco*RI and one *Mse*I, with three and four selective nucleotides, respectively, was used to reduce the complexity of DNA fingerprints and facilitate scoring. The following five primer combinations were chosen for the final selective amplification: *Eco*RI + ATA/*Mse*I + ACAA, *Eco*RI + ATA/*Mse*I + ACAC, *Eco*RI + ATA/*Mse*I + ACAG, *Eco*RI + ATA/*Mse*I + ACAT, and *Eco*RI + AAA/*Mse*I + ACAT. From these primer combinations, 28, 45, 25, 28, and 25 clearly separated AFLP markers were scored, respectively, with a total of 151 polymorphic markers in 178 poplar accessions.

Data analysis

AFLP markers were scored as 1 (present) or 0 (absent). Only intense, consistently amplified fragments, which were clearly separated from other fragments, were scored. Genetic similarity (GS) among accessions was estimated from the number of shared amplified fragments by using the similarity coefficients Dice [Sneath and Sokal, 1973; $GS(ij) = 2a/(2a + b + c)$] and Jaccard (1908) [$GS(ij) = a/(a + b + c)$]; where GS(*ij*) is the measure of GS between the individuals *i* and *j*, *a* is the number of polymorphic fragments that are shared by *i* and *j*, *b* is the number of fragments present in *i* and absent in *j*, and *c* is the number of fragments present in *j* and absent in *i*. The unweighted pair-group method with arithmetic mean (UPGMA) and neighbor-joining (NJ) analyses were performed based on the similarity matrix and dendrograms were constructed with the TREE program (Rohlf, 1998). To test the goodness of fit of the cluster analysis to the similarity matrix, a co-phenetic matrix from the UPGMA tree file and the product-moment correlation between the similarity and the co-phenetic matrices were calculated. The Mantel test was also performed for matrix correspondence with 1000 permutations. A principal coordinates analysis (PCO) was performed to visualize interspecies relationships by means of the Dice GS matrix and the procedures DCENTER, EIGEN, and MXPLOT (Fig. S1, Electronic supplementary material). These statistical analyses were carried out with the software Numerical Taxonomy System (NTSYS-PC software package, Version 2.02i; Rohlf 1998). Some duplicated accessions were included as controls [accessions 27/29 (*P. ciliata* 72-085), 63/65 and 64/67 (*P. koreana* 105/66 and 77/65, respectively), 86/87 (*P. maximowiczii* branches 1 and 2), 140/142 and 141/143 (*P. suaveolens* 21/65 and 15/74, respectively), 164/166 (*Populus trichocarpa* 'Fritzi Pauley'), and 100/109 (*P. nigra* 'Vereecken')].

Cluster analysis (Fig. 1) revealed that a few samples did not group in the expected taxon. In this case, the GS values were compared and the potentially misclas-

Table 2 Information on individual poplar accessions analyzed

Accession number	Species	Variety or cultivar	Clone name	Origin	Provider	New tentative assignation[e]
1	*P. alba*	*boleana*			CN	
2	*P. alba*		603.02		FR, INRA	
3	*P. alba*		A.L05.010	IT	IT, ISP	
4	*P. alba*		BO-1	IT	IT, ISP	
5	*P. alba*		Villafranca	IT	IT, ISP	
6	*P. alba*		B	BE	BE, VIB-UG	
7	*P. alba*	*tomentosa*			FR, INRA	
8	*P. angustifolia*		46/69		DE, HLFWW	
9	*P. angustifolia*		ANG		FR, INRA	
10	*P. balsamifera*[c]		1-5	US, MN	BE, IBW	*P. szechuanica*
11	*P. balsamifera*		8-6	US, MN	BE, IBW	
12	*P. balsamifera*		21-7	US, WI	BE, IBW	
13	*P. balsamifera*		15-5	US, MI	BE, IBW	
14	*P. balsamifera*		19-2	US, MI	BE, IBW	
15	*P. balsamifera*	*subcordata candicans*		BE, IBW	*P. candicans*	
16	*Populus* × *berolinensis*				BE, arboretum Kalmthout	
17	*Populus* × *berolinensis*		19870019	FR	BE, arboretum Meise	
18	*P. candicans*	*aurora*	19860364		BE, arboretum Meise	
19	*P. candicans*		19810762		BE, arboretum Meise	
20	*Populus* × *canescens*		90000054		BE, arboretum Meise	
21	*Populus* × *canescens*		limbrichterbos		BE, arboretum Kalmthout	
22	*Populus* × *canescens*		Grauwe abeel 1		BE, IBW	
23	*Populus* × *canescens*		Grauwe abeel 2		BE, IBW	
24	*P. cathayana*[c]		E6		IE, Teagasc	unclassified
25	*P. cathayana*[b]		306-52		DE, HLFWW	
26	*P. cathayana*				US, Washington University	
27	*P. ciliata*		72-085		IT, ISP	
28	*P. ciliata*[c]		65-017		IT, ISP	*P. trichocarpa* × *P. maximowiczii*
29	*P. ciliata*[a]		72-085		FR, INRA	
30	*P. ciliata*[c]		102L7		IE, Teagasc	*Populus* × *canadensis*
31	*P. ciliata*[c]		D1D4E3		IE, Teagasc	intrasectional *Tacamahaca* hybrid
32	*P. davidiana*	*davidiana*			FR, INRA	
33	*P. deltoides*[c]		V12	US, IL	BE, IBW	*Populus* × *canadensis*
34	*P. deltoides*		V1	CA, ONT	BE, IBW	
35	*P. deltoides*		V2	CA, ONT	BE, IBW	
36	*P. deltoides*		V3	US, MN	BE, IBW	
37	*P. deltoides*		V7B	US, MO	BE, IBW	
38	*P. deltoides*		S174-1	US, ND	BE, IBW	
39	*P. deltoides*		S197-1	CA, ON	BE, IBW	
40	*P. deltoides*		S329-31	US, OH	BE, IBW	
41	*P. deltoides*[b]		S333-53	US, MI	BE, IBW	
42	*P. deltoides*		S235-3	US, IL	BE, IBW	
43	*P. deltoides*		S193-1	US, ND	BE, IBW	
44	*P. deltoides*		DO-006	US, TX	IT, ISP	
45	*P. deltoides*		DI-180A	US, OH	IT, ISP	
46	*P. deltoides*		S336-4	US, CT	BE, IBW	
47	*P. deltoides*	*deltoides*	D37	CA, ON[g]	CA, O.P. Rajora	
48	*P. deltoides*	*deltoides*	D43	CA, ON[g]	CA, O.P. Rajora	
49	*P. deltoides*	*deltoides*	D68	US, IN[g]	CA, O.P. Rajora	
50	*P. deltoides*	*deltoides*	D70	US, IL[g]	CA, O.P. Rajora	
51	*P. deltoides*	*deltoides*	D56	CA, ON[g]	CA, O.P. Rajora	
52	*P. deltoides*	*occidentalis*	D87	US, KS[g]	CA, O.P. Rajora	
53	*P. deltoides*	*deltoides*	D109	US, MS[g]	CA, O.P. Rajora	
54	*P. deltoides*	*deltoides*	D121	US, IL[g]	CA, O.P. Rajora	
55	*P. deltoides*		S9-2	US	BE, IBW	
56	*P. euphratica*			CN	VIB-UG	

Table 2 (Contd.)

Accession number	Species	Variety or cultivar	Clone name	Origin	Provider	New tentative assignation[e]
57	*Populus* × *canadensis*		Selys-Longchamps		BE, arboretum Kalmthout	
58	*P. fremontii*		F5706		FR, INRA	*Populus* × *canadensis*
59	*P. fremontii*	*wislizenii*	50-002		FR, INRA	*Populus* × *canadensis*
60	*P. fremontii*[b]	*wislizenii sargent*	28/51		CN	*Populus* × *canadensis*
61	*P. fremontii*				DE, HLFWW	*Populus* × *canadensis*
62	*P. fremontii*				DE, HLFWW	*Populus* × *canadensis*
63	*P. koreana*		105/66		DE, HLFWW	
64	*P. koreana*		77/65		DE, HLFWW	
65	*P. koreana*[a]		105/66		DE, HLFWW	
66	*P. koreana*[b]		143/65		DE, HLFWW	
67	*P. koreana*[a]		77/65		DE, HLFWW	
68	*P. koreana*[b]				US, Washington University	
69	*P. lasiocarpa*		Las		IE, Teagasc	
70	*P. lasiocarpa*[c]		LSC		FR, INRA	intrasectional *Tacamahaca* hybrid
71	*P. lasiocarpa*[c]		Q7		IE, Teagasc	*Populus* × *canadensis*
72	*P. lasiocarpa*		1		BE, IBW	
73	*P. lasiocarpa*[b]		2		BE, IBW	
74	*P. lasiocarpa*		4		BE, IBW	
75	*P. lasiocarpa*		19826411		BE, arboretum Meise	
76	*P. laurifolia*[c]		LRF		FR, INRA	intrasectional *Tacamahaca* hybrid
77	*P. laurifolia*[c]		KA14		IE, Teagasc	intrasectional *Tacamahaca* hybrid
78	*P. laurifolia*		13/65		DE, HLFWW	
79	*P. laurifolia*[b]		75/65		DE, HLFWW	
80	*P. laurifolia*[b]		69/65		DE, HLFWW	
81	*P. laurifolia*[c]				BE, arboretum Beveren	*P. berolinensis*
82	*P. maximowiczii*		3-20		FR, INRA	
83	*P. maximowiczii*[c]		4627		BE, IBW	*Populus* × *canadensis*
84	*P. maximowiczii*		mw03-093	JP	IT, ISP	
85	*P. maximowiczii*		mw05-283	JP	IT, ISP	
86	*P. maximowiczii*		branch 1		BE, VIB-UG	
87	*P. maximowiczii*[a]		branch 2		BE, VIB-UG	
88	*P. maximowiczii*		15/65		DE, HLFWW	
89	*P. maximowiczii*		121/66		DE, HLFWW	
90	*P. maximowiczii*		37/89		DE, HLFWW	
91	*P. maximowiczii*		M2	CN[f]	CA, O.P. Rajora	
92	*P. maximowiczii*[b]		M4	CN[f]	CA, O.P. Rajora	
93	*P. maximowiczii*		M5	CN[f]	CA, O.P. Rajora	
94	*P. maximowiczii*		M12	JP[f]	CA, O.P. Rajora	
95	*P. maximowiczii*[b]		M13	JP[f]	CA, O.P. Rajora	
96	*P. maximowiczii*		M15	JP[f]	CA, O.P. Rajora	
97	*P. mexicana*		MX	MX		
98	*P. nigra*		SRZ		FR, INRA	
99	*P. nigra*		72-501		FR, INRA	
100	*P. nigra*	'Vereecken'	ES		ES, SIA	
101	*P. nigra*		Fue6	ES	ES, SIA	
102	*P. nigra*		Luc2	ES	ES, SIA	
103	*P. nigra*		Yzer1	BE	BE, IBW	
104	*P. nigra*		Essene	BE	BE, IBW	
105	*P. nigra*[b]		Loire	BE	BE, IBW	
106	*P. nigra*[c]		Terwolde	BE	BE, IBW	interspecific *P. nigra* hybrid
107	*P. nigra*		73-081	YU	BE, IBW	
108	*P. nigra*	'Italica'	Irl1	IE	IE, Teagasc	
109	*P. nigra*[a]	'Vereecken'	Irl2	IE	IE, Teagasc	
110	*P. nigra*[b]	'Italica'	Aral	BE	BE, VIB-UG	
111	*P. nigra*[b]	'Italica'	Nogent-sur-Marne	FR	BE, VIB-UG	
112	*P. nigra*[b]	'Italica'	Reims	FR	BE, VIB-UG	
113	*P. nigra*[b]	'Italica'	PI88-002	IT	IT, ISP	

Table 2 (Contd.)

Accession number	Species	Variety or cultivar	Clone name	Origin	Provider	New tentative assignation[e]
114	*P. nigra*	'Italica'	PI88-058	TR	IT, ISP	
115	*P. nigra*	'Italica'	PI88-063	BG	IT, ISP	
116	*P. nigra*	'Italica'	Zaragoza	ES	ES, SIA	
117	*P. nigra*	*nigra*	N13	CZ[h]	CA, O.P. Rajora	
118	*P. nigra*	*nigra*	N19	NL[h]	CA, O.P. Rajora	
119	*P. nigra*	*nigra*	N20	FR[h]	CA, O.P. Rajora	
120	*P. nigra*	*nigra*	N29	NL[h]	CA, O.P. Rajora	
121	*P. nigra*	*nigra*	N40	NL[h]	CA, O.P. Rajora	
122	*P. nigra*[b]	*nigra*	N84	DE[h]	CA, O.P. Rajora	
123	*P. nigra*[b]	*nigra*	N85	DE[h]	CA, O.P. Rajora	
124	*P. nigra*	*nigra*	N96	CZ[h]	CA, O.P. Rajora	
125	*P. nigra*	*nigra*	N100	CZ[h]	CA, O.P. Rajora	
126	*P. nigra*	*nigra*	N102	CZ[h]	CA, O.P. Rajora	
127	*P. nigra*		Ghoy	BE	BE, IBW	
128	*P. sieboldii*[c]		Sie	GB	IE, Teagasc	*P. trichocarpa* × *P. balsamifera*
129	*P. simonii*		1/9		BE, IBW	
130	*P. simonii*		81-001-003	CN	IT, ISP	
131	*P. simonii*		81-002-003	CN	IT, ISP	
132	*P. simonii*		108/49		DE, HLFWW	
133	*P. simonii*[b]		57/65		DE, HLFWW	
134	*P. simonii*		147/65		DE, HLFWW	
135	*P. simonii*[b]		141/66		DE, HLFWW	
136	*P. simonii*		58/90		DE, HLFWW	
137	*P. simonii*		59/90		DE, HLFWW	
138	*P. simonii*		60/90		DE, HLFWW	
139	*P. simonii*	*fastigiata*			BE, VIB-UG	
140	*P. suaveolens*		21/65		DE, HLFWW	
141	*P. suaveolens*[c]		15/74		DE, HLFWW	*P. trichocarpa* × *P. balsamifera*
142	*P. suaveolens*[a]		21/65		DE, HLFWW	
143	*P. suaveolens*[a,c]		15/74		DE, HLFWW	*P. trichocarpa* × *P. balsamifera*
144	*P. suaveolens*[c]		20/65		DE, HLFWW	*P.* × *canadensis* × *P. nigra*
145	*P. szechuanica*		SZC		FR, INRA	
146	*P. szechuanica*		275/49		DE, HLFWW	
147	*P. szechuanica*[b]		67/65		DE, HLFWW	
148	*P. szechuanica*[c]		144/65		DE, HLFWW	*P. balsamifera*
149	*P. tremula*		130-19		FR, INRA	
150	*P. tremula*	*erecta*			BE, arboretum Beveren	
151	*P. tremula*		1H		BE, IBW	
152	*P. tremula*		2H		BE, IBW	
153	*P. tremula*		3H		BE, IBW	
154	*P. tremuloides*		210-22		FR, INRA	
155	*P. tremuloides*[c]		HI-10		IE, Teagasc	intrasectional *Tacamahaca* hybrid
156	*P. tremuloides*[c]				BE, arboretum Kalmthout	*Populus* × *canescens*
157	*P. trichocarpa*		FPL		FR, INRA	
158	*P. trichocarpa*		19-73		FR, INRA	
159	*P. trichocarpa*		36-77		FR, INRA	
160	*P. trichocarpa*		101-74		FR, INRA	
161	*P. trichocarpa*		S3-31		BE, IBW	
162	*P. trichocarpa*		V509		BE, IBW	
163	*P. trichocarpa*		V510		BE, IBW	
164	*P. trichocarpa*[c]	'Fritzi Pauley'	V235	US, WA	BE, IBW	*P. trichocarpa* × *P. maximowiczii*
165	*P. trichocarpa*[b]		212-161		FR, INRA	
166	*P. trichocarpa*[a,c]	'Fritzi Pauley'			BE, arboretum Kalmthout	*P. trichocarpa* × *P. maximowiczii*
167	*P. trichocarpa*	'Trichobel'			BE, IBW	
168	*P. trichocarpa*	'Columbia river'	V24	US, OR	BE, IBW	
169	*P. tristis*		24/65		DE, HLFWW	

Table 2 (Contd.)

Accession number	Species	Variety or cultivar	Clone name	Origin	Provider	New tentative assignation[e]
170	*P. violascens*		19860054	UK	BE, arboretum Meise	
171	*P. wilsonii*		19820416	DE	BE, arboretum Meise	
172	*P. yunnanensis*		82001		FR, INRA	
173	*P. yunnanensis*	*yunnanensis*			FR, INRA	
174	*P. yunnanensis*[b]		V535		BE, IBW	
175	*P. yunnanensis*[c]				BE, arboretum Beveren	*P. candicans*
176	*P. yunnanensis*				BE, arboretum Beveren	
177	*Populus-unknown*[d]		22616		BE, arboretum Kalmthout	*P. nigra*
178	*Populus-unknown*[d]		22031		BE, arboretum Kalmthout	*P. nigra*
179	*Salix*				BE, VIB-UG	
180	*Salix*				BE, VIB-UG	
181	*Salix*		22010		BE, arboretum Kalmthout	

Countries are abbreviated according to ISO 3166-1-Alpha-2 code (*BE* Belgium, *BG* Bulgaria, *CA* Canada [*ON* Ontario], *CN* China, *CZ* Czech Republic, *DE* Germany, *DK* Denmark, *ES* Spain, *FR* France, *IE* Ireland, *IT* Italy, *JP* Japan, *MX* Mexico, *NL* The Netherlands, *TR* Turkey, *GB* United Kingdom, *US* United States [*IL* Illinois, *IN* Indiana, *KS* Kansas, *MN* Minnesota, *MO* Missouri, *MS* Mississippi, *ND* North Dakota, *OH* Ohio, *OR* Oregon, *TX* Texas, *WA* Washington State, *WI* Wisconsin], *YU* Yugoslavia). *HLFWW* Hessian Forest Center for Management, Planning, Research and Ecology (Münden, Germany), *IBW* Instituut voor Bosbouw en Wildbeheer (Gerardsbergen, Belgium), *INRA* Institut National de la Recherche Agronomique (Orléans, France), *ISP* Istituto di Sperimentazione per la Pioppicoltura (Casale Monferrato, Italy), *SIA* Servicio de Investigación Agroalimentaria Diputacion General de Aragón (Zaragoza, Spain), *Teagasc* Irish Agriculture and Food development Authority (Dublin, Ireland), *VIB-UG* Vlaams Interuniversitair Instituut voor Biotechnologie-Universiteit Gent (Gent, Belgium). Accessions in bold were used to perform the phylogenetic analysis

[a] Samples known to be duplicates before the start of the analysis and confirmed by AFLP

[b] Accessions showing GS of ≥ 0.98 based on AFLP fragment similarities

[c] Possibly mislabeled and/or misclassified accessions based on AFLP analysis

[d] Based on morphological descriptors (blind test). These clones showed AFLP patterns typical of *P. nigra*

[e] Tentative assignation of misclassified accessions based on AFLP patterns, GS values and the dendrogram in Fig. 1

[f] Information on the origin of *P. maximowiczii* accessions is provided in Rajora (1988)

[g] Information on the origin of *P. deltoides* accessions is provided in Rajora (1989a)

[h] Information on the origin of *P. nigra* accessions is provided in Rajora (1989b)

sified accessions were assigned to a more likely species or hybrid, based on the AFLP patterns (Table 2). Four misclassified accessions could be assigned to certain species groups, two of which (148 and 175) shared a GS of > 0.98 with the other species (14 and 19, respectively). These accessions were included into the dataset to calculate interspecific as well as intraspecific GS values (Table 2; all, except those designated as unclassified or hybrid, were included). Interspecific GS ranges between two species *a* and *b* were calculated from all pair-wise GS values between all accessions from species *a* and all accessions from species *b*. Intraspecific GS values give the range of all pair-wise GS values among accessions of a single species. The GS matrix, based on the individual accessions, is available at http://www.psb.ugent.be/~vesto and the interspecific and intraspecific GS matrix is presented in Table 3. To verify the consistency of cluster analysis, a second dendrogram was constructed, using AFLP fragment similarities (Dice coefficient) with the UPGMA clustering method, without including the misclassified accessions and the accessions with GS ≥ 0.98 (Fig. S2, Electronic supplementary material).

Phylogenetic analysis, performed on the latter representative and non-redundant set of accessions (GS < 0.98), was carried out with the MIX program of the PHYLIP software package version 3.57c (Felsenstein 1993), in order to construct the single most parsimonious tree based on Wagner's method (Fig. 2). The data were bootstrapped, to assess how strongly phylogenetic data supported clades in this tree, with SEQBOOT (100 bootstrapped data files) and followed by the MIX and CONSENSE software packages of PHYLIP Version 3.57c. The single most parsimonious bifurcating unrooted tree was constructed with the TREEVIEW software package (Page 1996).

Results

Dendrograms obtained using Dice and Jaccard similarity coefficients were identical (data not shown). The correlation between the Dice and Jaccard similarity matrices and their co-phenetic matrices was very high (0.94 and 0.93, respectively), with an associated *p*-value of 0.002 (one-tailed) that indicated a very good fit of the

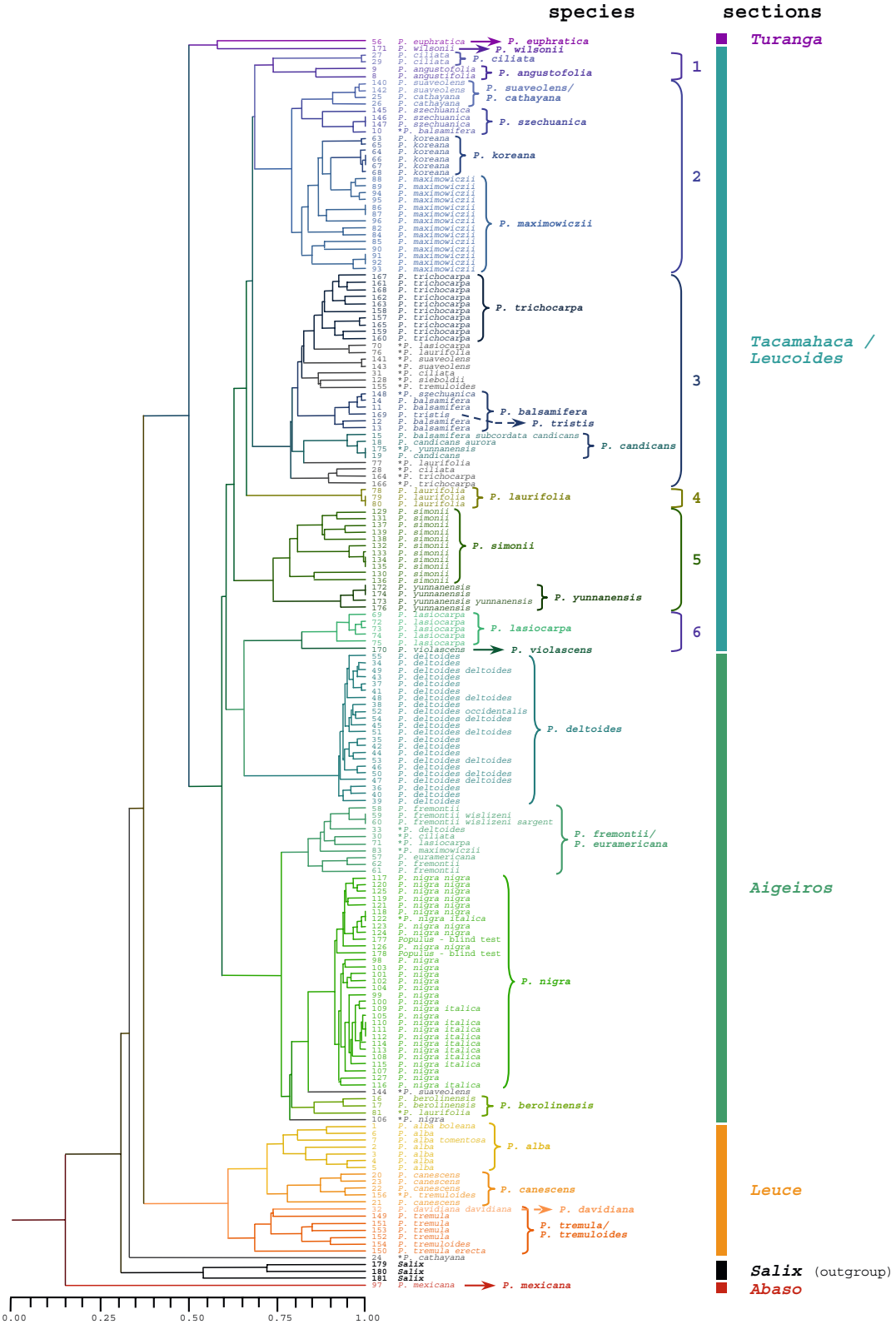

Fig. 1 Dendrogram of *Populus* and *Salix* accessions, constructed from AFLP fragment similarities (Dice coefficient), with the UPGMA clustering method, and based on AFLP markers resolved by five primer combinations (*Eco*RI + ATA/*Mse*I + ACAA, *Eco*RI + ATA/*Mse*I + ACAC, *Eco*RI + ATA/*Mse*I + ACAG and *Eco*RI + ATA/*Mse*I + ACAT, *Eco*RI + AAA/*Mse*I + ACAT). Accessions marked with an asterisk are potentially mislabeled species or hybrids (see text and Table 2). Species are marked by *brackets* and *arrows*, whereas lines group sections

cluster analysis. The combination of Dice similarity with UPGMA clustering yielded the highest co-phenetic correlation and is, therefore, considered the most suitable analysis for determining phenetic species relationships in this study. A dendrogram based on AFLP GS values, calculated for all accessions, is presented in Fig. 1. Up to 2% AFLP polymorphism was detected between ramets of the same clone (GS ≥ 0.98). A reproducibility error of 2% has been reported earlier for the AFLP markers (Arens et al. 1998; Chavarriaga-Aguirre et al. 1999). As expected, the two ramets of each of the eight clones clustered together (Fig. 1; Table 2). Putatively misclassified samples as well as entries with GS ≥ 0.98, were subsequently removed to obtain a second dendrogram (Fig. S2). The accessions with GS ≥ 0.98 represent either true duplicates that were passed from one germplasm collection to the other, or they might be somatic mutants (Tuskan et al. 1996) or genetically highly related samples. Since, in the latter dendrogram (Fig. S2), the same relative genetic relationships among species were retained as detected with all accessions, the phylogenetic analysis was conducted on the reduced set of accessions, by removing the misclassified accessions and those with GS ≥ 0.98 (Fig. 2). A NJ tree constructed from the Dice similarity matrix (data not shown) was very similar to the dendrogram obtained using UPGMA, indicating the robustness of the UPGMA results.

Intergeneric and intersectional relationships

With some notable exceptions, most of the sampled *Populus* and *Salix* accessions clustered along their species lines, and species from the same section generally clustered together (Fig. 1). GS values for the three *Salix* accessions, that were used as outgroups to the *Populus* species, ranged between 0.13 and 0.44 (Table 3). Intersectional GS values between the *Populus* species ranged from 0.22 to 0.55, whereas the intrasectional GS values were greater than 0.55. The single *Populus mexicana* accession had the highest differentiation from the others and its GS values with the other *Populus* accessions were even lower ($0.05 < GS < 0.26$) than those observed between *Salix* and the other *Populus* species. Besides the section *Abaso* Ecken., which was represented by *P. mexicana*, the most divergent section was *Leuce*, with *Populus alba* L., *P. tremula*, *P. tremuloides, P. davidiana*, and *Populus* × *canescens* (*P. alba* × *P. tremula*), forming a single distinct group. The next most divergent section was *Turanga* Bge., which was represented by a single accession of *Populus euphratica* Oliv. The *Aigeiros* and *Tacamahaca* sections were closely related genetically, as expected. Except for the *P. wilsonii* Schneid. accession, species from the *Leucoides* section (*P. ciliata*, *P. lasiocarpa*, and *Populus violascens* Dode) grouped with species from the *Tacamahaca* section or, in certain cases (*P. lasiocarpa* and *P. violascens* with *P. deltoides*), with those from the *Aigeiros* section (Fig. 1).

Interspecific relationships

At the interspecific level, species generally clustered with their consectional species (Fig. 1). *P. wilsonii*, from the *Leucoides* section, was the species most closely related to *P. euphratica* from the *Turanga* section, with a GS of 0.58. The remaining *Leucoides*, the *Tacamahaca*, and some of the *Aigeiros* species, formed a large meta-group that was further divided into six smaller clusters of related species: group 1 was formed by *P. ciliata* and *Populus angustifolia* James.; group 2 consisted of *P. suaveolens*, *P. cathayana*, *Populus szechuanica*, as well as *P. koreana* and *P. maximowiczii*, which showed a high genetic relationship. *P. ciliata* from group 1 was also highly genetically similar with the species of group 2. However, clustering of the *P. suaveolens* accessions was confusing (Fig. 1): one (clone 20/65, no. 144; Table 2) out of five accessions analyzed was potentially misclassified because it did not cluster in a group of accessions of any other *Tacamahaca* species and had an AFLP profile that was intermediate between the accessions classified as *Populus fremontii* and those of *P. nigra*. The two ramets of clone 15/74 (nos. 141 and 143) did not group with the two ramets of clone 21/65 (nos. 140 and 142) of *P. suaveolens*, which were grouped with *P. cathayana*. The AFLP pattern of clone 15/74 of *P. suaveolens* was intermediate between that of *P. trichocarpa* Torr. and Gray and *Populus balsamifera* (Table 3). Hence, the position of *P. suaveolens* remains unclear and the analysis of a larger number of accessions is required to clarify its relationship with other *Tacamahaca* species, especially with *P. cathayana*.

Group 3 consists of *P. trichocarpa*, together with *P. balsamifera*, *Populus candicans* Ait., and a single accession of *Populus tristis* Fisch. and *Populus sieboldii* Miq. (Fig. 1). *P. candicans* had AFLP profiles expected for *P. deltoides* × *P. balsamifera* interspecific hybrids. The AFLP banding pattern of the only *P. sieboldii* accession analyzed in this study was intermediate between that of *P. trichocarpa* and *P. balsamifera*. Since *P. sieboldii* is a Japanese aspen, described by Rehder (1947) as *P. tremula* var. *villosa*, this result is indicative that the *P. sieboldii* accession was misclassified.

Populus laurifolia Ledeb., which was represented by three accessions with GS values higher than 0.98, formed its own group 4, which was also more genetically similar to group 2. *Populus simonii* Carr. and *Populus yunnanensis*, both native to southwestern China (Dickmann and Stuart 1983), formed group 5. This group was genetically close to group 2. Finally, *P. lasiocarpa* and *P. violascens* formed group 6, which was linked to the group formed by *P. deltoides* accesions. *P. lasiocarpa* accessions were also genetically related to *P. trichocarpa*, *P. candicans*, and *P. tristis* (group 3), whereas the *P. violascens* accession was also genetically related to groups 2 and 1 (Table 3).

The species from the *Aigeiros* section formed two separate groups (Fig. 1). Accessions of *P. deltoides* clustered together to form a single species group (Fig. 1),

Table 3 Interspecific and intraspecific GS among pairs of *Populus* and *Salix*, with average similarities between *parentheses*

	P. euphratica (1)	*P. ciliata* (2)	*P. lasiocarpa* (5)	*P. alba* (7)	*P. davidiana* (1)	*P. sieboldii* (1)	*P. tremula* (5)	*P. tremuloides* (1)	*P. angustifolia* (2)	*P. balsamifera* (5)	*P. candicans* (4)	*P. cathayana* (2)	*P. laurifolia* (3)	*P. maximowiczii* (14)
P. euphratica	–													
P. ciliata	0.50–0.51 (0.51)	0.99												
P. lasiocarpa	0.46–0.47 (0.47 ± 0.01)	0.56–0.59 (0.58 ± 0.01)	0.90–0.99 (0.95 ± 0.03)											
P. alba	0.29–0.45 (0.36 ± 0.05)	0.29–0.4 (0.32 ± 0.04)	0.34–0.44 (0.40 ± 0.03)	0.70–0.95 (0.81 ± 0.06)										
P. davidiana	0.40	0.35	0.36–0.38 (0.38 ± 0.01)	0.57–0.65 (0.60 ± 0.03)	–									
P. sieboldii	0.48	0.62	0.60–0.62 (0.61 ± 0.01)	0.39–0.47 (0.44 ± 0.03)	0.43	–								
P. tremula	0.34–.047 (0.40 ± 0.04)	0.36–0.43 (0.39 ± 0.02)	0.35–0.49 (0.41 ± 0.04)	0.53–0.70 (0.59 ± 0.04)	0.71–0.78 (0.74 ± 0.03)	0.38–0.51 (0.46 ± 0.05)	0.67–0.86 (0.75 ± 0.07)							
P. tremuloides	0.39	0.37	0.37–0.43 (0.41 ± 0.02)	0.52–0.66 (0.59 ± 0.05)	0.71	0.47	0.63–0.90 (0.77 ± 0.11)	–						
P. angustifolia	0.54–0.60 (0.57)	0.72–0.76 (0.74 ± 0.02)	0.57–0.66 (0.62 ± 0.03)	0.34–0.55 (0.43 ± 0.06)	0.40–0.45 (0.43)	0.65–0.67 (0.66)	0.4–0.50 (0.45 ± 0.03)	0.42–0.43 (0.42)	0.86					
P. balsamifera	0.46–0.53 (0.50 ± 0.03)	0.58–0.67 (0.64 ± 0.03)	0.57–0.65 (0.62 ± 0.02)	0.33–0.47 (0.39 ± 0.03)	0.35–0.43 (0.40 ± 0.03)	0.80–0.88 (0.85 ± 0.03)	0.31–0.51 (0.43 ± 0.06)	0.40–0.47 (0.45 ± 0.03)	0.61–0.72 (0.67 ± 0.03)	0.89–0.99 (0.94 ± 0.03)				
P. candicans	0.51–.052 (0.51 ± 0.01)	0.67–0.72 (0.69 ± 0.02)	0.66–0.70 (0.68 ± 0.01)	0.37–0.52 (0.41 ± 0.05)	0.44–0.47 (0.45 ± 0.01)	0.80–0.85 (0.82 ± 0.02)	0.35–0.49 (0.45 ± 0.05)	0.44–0.45 (0.45 ± 0.01)	0.64–0.73 (0.68 ± 0.03)	0.75–0.87 (0.81 ± 0.03)	0.94–1.00 (0.97 ± 0.02)			
P. cathayana	0.44	0.67–0.73 (0.70 ± 0.03)	0.54–0.63 (0.58 ± 0.03)	0.30–0.45 (0.34 ± 0.05)	0.38–0.41 (0.40)	0.65–0.70 (0.68)	0.32–0.46 (0.40 ± 0.04)	0.40–0.43 (0.42)	0.59–0.69 (0.64 ± 0.04)	0.62–0.72 (0.68 ± 0.03)	0.65–0.73 (0.69 ± 0.03)	0.89		
P. laurifolia	0.39–0.40 (0.40 ± 0.01)	0.63–0.65 (0.64 ± 0.01)	0.47–0.54 (0.51 ± 0.02)	0.26–0.38 (0.31 ± 0.03)	0.33–0.35 (0.34 ± 0.01)	0.67–0.68 (0.68 ± 0.01)	0.31–0.41 (0.37 ± 0.03)	0.36–0.37 (0.37 ± 0.01)	0.56–0.61 (0.58 ± 0.02)	0.60–0.66 (0.64 ± 0.02)	0.61–0.67 (0.63 ± 0.02)	0.69–0.72 (0.70 ± 0.02)	0.99–1.00 (0.99 ± 0.01)	
P. maximowiczii	0.45–0.52 (0.47 ± 0.02)	0.69–0.74 (0.71 ± 0.02)	0.52–0.62 (0.58 ± 0.02)	0.27–0.49 (0.35 ± 0.05)	0.37–0.44 (0.42 ± 0.02)	0.60–0.69 (0.66 ± 0.03)	0.30–0.49 (0.41 ± 0.04)	0.40–0.44 (0.42 ± 0.02)	0.62–0.73 (0.68 ± 0.03)	0.59–0.73 (0.67 ± 0.03)	0.62–0.75 (0.68 ± 0.03)	0.73–0.86 (0.81 ± 0.03)	0.64–0.71 (0.68 ± 0.02)	0.73–1.00 (0.85 ± 0.06)
P. koreana	0.44–0.45 (0.45 ± 0.01)	0.67–0.69 (0.68 ± 0.01)	0.55–0.61 (0.58 ± 0.02)	0.31–0.47 (0.35 ± 0.05)	0.41–0.43 (0.42 ± 0.01)	0.63–0.67 (0.65 ± 0.01)	0.36–0.45 (0.41 ± 0.02)	0.41–0.45 (0.42 ± 0.02)	0.63–0.72 (0.68 ± 0.03)	0.60–0.71 (0.67 ± 0.03)	0.65–0.70 (0.67 ± 0.02)	0.78–0.84 (0.82 ± 0.02)	0.65–0.71 (0.68 ± 0.02)	0.79–0.91 (0.86 ± 0.03)
P. simonii	0.34–0.46 (0.39 ± 0.04)	0.48–0.62 (0.55 ± 0.05)	0.49–0.63 (0.56 ± 0.03)	0.27–0.45 (0.34 ± 0.04)	0.38–0.44 (0.41 ± 0.02)	0.53–0.62 (0.57 ± 0.02)	0.36–0.45 (0.45 ± 0.03)	0.33–0.41 (0.39 ± 0.02)	0.54–0.62 (0.59 ± 0.03)	0.50–0.69 (0.59 ± 0.03)	0.60–0.68 (0.63 ± 0.02)	0.56–0.73 (0.65 ± 0.06)	0.50–0.58 (0.55 ± 0.03)	0.49–0.72 (0.62 ± 0.06)
P. suaveolens	0.44	0.70–0.73 (0.72 ± 0.01)	0.57–0.63 (0.60 ± 0.01)	0.30–0.46 (0.35 ± 0.05)	0.41	0.70	0.37–0.47 (0.41 ± 0.03)	0.43	0.60–0.67 (0.64 ± 0.04)	0.63–0.72 (0.69 ± 0.04)	0.69–0.73 (0.70 ± 0.01)	0.91–0.98 (0.94 ± 0.04)	0.67–0.70 (0.69 ± 0.01)	0.75–0.84 (0.81 ± 0.03)
P. szechuanica	0.45–0.49 (0.47 ± 0.02)	0.67–0.70 (0.68 ± 0.01)	0.57–0.61 (0.59 ± 0.01)	0.30–0.47 (0.34 ± 0.05)	0.37–0.39 (0.38 ± 0.01)	0.64–0.71 (0.66 ± 0.03)	0.32–0.48 (0.39 ± 0.04)	0.39–0.44 (0.40 ± 0.03)	0.63–0.71 (0.66 ± 0.03)	0.63–0.73 (0.69 ± 0.03)	0.66–0.72 (0.68 ± 0.02)	0.82–0.86 (0.84 ± 0.02)	0.69–0.76 (0.71 ± 0.02)	0.70–0.82 (0.76 ± 0.03)
P. yunnanensis	0.42–0.45 (0.44 ± 0.02)	0.62–0.65 (0.64 ± 0.02)	0.58–0.66 (0.62 ± 0.03)	0.32–0.49 (0.39 ± 0.04)	0.45–0.48 (0.46 ± 0.02)	0.63–0.67 (0.65 ± 0.02)	0.36–0.51 (0.45 ± 0.05)	0.46–0.49 (0.48 ± 0.02)	0.61–0.68 (0.65 ± 0.03)	0.56–0.69 (0.64 ± 0.03)	0.66–0.72 (0.68 ± 0.02)	0.68–0.76 (0.73 ± 0.03)	0.55–0.61 (0.59 ± 0.02)	0.62–0.75 (0.69 ± 0.04)
P. trichocarpa	0.40–0.50 (0.48 ± 0.03)	0.62–0.70 (0.67 ± 0.02)	0.58–0.75 (0.65 ± 0.03)	0.30–0.48 (0.40 ± 0.04)	0.38–0.47 (0.41 ± 0.02)	0.82–0.89 (0.86 ± 0.02)	0.32–0.55 (0.45 ± 0.05)	0.41–0.51 (0.46 ± 0.04)	0.62–0.76 (0.70 ± 0.04)	0.71–0.87 (0.81 ± 0.04)	0.74–0.87 (0.80 ± 0.03)	0.63–0.78 (0.69 ± 0.03)	0.63–0.68 (0.66 ± 0.01)	0.58–0.76 (0.67 ± 0.04)
P. deltoides	0.41–0.49 (0.44 ± 0.02)	0.52–0.61 (0.56 ± 0.02)	0.61–0.72 (0.66 ± 0.02)	0.30–0.44 (0.37 ± 0.03)	0.37–0.45 (0.40 ± 0.02)	0.57–0.64 (0.59 ± 0.02)	0.28–0.43 (0.37 ± 0.04)	0.39–0.42 (0.41 ± 0.01)	0.54–0.69 (0.61 ± 0.04)	0.53–0.66 (0.60 ± 0.03)	0.68–0.81 (0.75 ± 0.03)	0.54–0.71 (0.59 ± 0.03)	0.49–0.60 (0.53 ± 0.02)	0.53–0.66 (0.60 ± 0.03)
P. × *euramericana*	0.37–0.45 (0.43 ± 0.03)	0.53–0.64 (0.59 ± 0.03)	0.53–0.64 (0.59 ± 0.02)	0.26–0.42 (0.35 ± 0.04)	0.35–0.42 (0.39 ± 0.02)	0.58–0.65 (0.63 ± 0.02)	0.27–0.46 (0.38 ± 0.04)	0.36–0.40 (0.39 ± 0.02)	0.58–0.71 (0.65 ± 0.04)	0.51–0.67 (0.61 ± 0.04)	0.64–0.79 (0.74 ± 0.03)	0.50–0.66 (0.60 ± 0.04)	0.50–0.56 (0.53 ± 0.01)	0.52–0.68 (0.61 ± 0.03)
P. nigra	0.41–0.48 (0.45 ± 0.02)	0.53–0.61 (0.57 ± 0.02)	0.45–0.53 (0.49 ± 0.01)	0.22–0.38 (0.29 ± 0.03)	0.30–0.40 (0.34 ± 0.02)	0.59–0.65 (0.62 ± 0.02)	0.31–0.42 (0.35 ± 0.03)	0.29–0.39 (0.32 ± 0.02)	0.58–0.67 (0.61 ± 0.02)	0.51–0.66 (0.59 ± 0.03)	0.57–0.67 (0.62 ± 0.02)	0.49–0.61 (0.55 ± 0.03)	0.50–0.55 (0.52 ± 0.02)	0.50–0.64 (0.57 ± 0.03)
P. violascens	0.49	0.69	0.79–0.83 (0.82 ± 0.02)	0.36–.46 (0.39 ± 0.04)	0.41	0.69	0.40–0.46 (0.42 ± 0.02)	0.43	0.67–0.70 (0.68)	0.61–0.69 (0.67 ± 0.03)	0.77 (± 0.01)	0.70 (0.70)	0.58–0.59 (0.58 ± 0.01)	0.65–0.75 (0.71 ± 0.04)
P. wilsonii	0.58	0.61–0.62 (0.61)	0.58–0.61 (0.59 ± 0.01)	0.38–0.47 (0.42 ± 0.03)	0.41	0.66	0.39–0.48 (0.44 ± 0.03)	0.41	0.65	0.59–0.68 (0.65 ± 0.04)	0.59–0.63 (0.61 ± 0.02)	0.56–0.59 (0.57)	0.46	0.55–0.65 (0.58 ± 0.03)
P. tristis	0.51	0.66–0.67 (0.66)	0.61–0.65 (0.63 ± 0.01)	0.37–0.45 (0.41 ± 0.03)	0.40	0.89	0.36–0.51 (0.45 ± 0.06)	0.50	0.71–0.72 (0.71)	0.88–0.96 (0.93 ± 0.03)	0.80–0.87 (0.83 ± 0.03)	0.67–0.72 (0.70)	0.63–0.64 (0.63 ± 0.01)	0.65–0.73 (0.69 ± 0.03)
P. berolinensis	0.41–0.43 (0.42 ± 0.01)	0.60–0.66 (0.63 ± 0.03)	0.49–0.54 (0.51 ± 0.02)	0.26–0.37 (0.33 ± 0.03)	0.31–0.37 (0.33 ± 0.03)	0.60–0.64 (0.62 ± 0.02)	0.33–0.40 (0.37 ± 0.02)	0.34–0.35 (0.35 ± 0.01)	0.60–0.64 (0.61 ± 0.02)	0.51–0.63 (0.59 ± 0.04)	0.62–0.67 (0.63 ± 0.02)	0.59–0.69 (0.65 ± 0.04)	0.64–0.68 (0.66 ± 0.01)	0.52–0.69 (0.63 ± 0.04)
P. × *canescens*	0.35–0.39 (0.37 ± 0.2)	0.31–0.41 (0.38 ± 0.04)	0.37–0.43 (0.40 ± 0.02)	0.63–0.79 (0.72 ± 0.04)	0.61–0.66 (0.64 ± 0.02)	0.44–0.46 (0.45 ± 0.01)	0.57–0.72 (0.65 ± 0.05)	0.58–0.64 (0.61 ± 0.03)	0.42–0.5 (0.47 ± 0.03)	0.34–0.46 (0.41 ± 0.03)	0.36–0.46 (0.42 ± 0.04)	0.33–0.42 (0.38 ± 0.03)	0.31–0.36 (0.34 ± 0.02)	0.29–0.41 (0.35 ± 0.03)
P. mexicana	0.14	0.13 (0.13)	0.13–0.17 (0.14 ± 0.02)	0.05–0.14 (0.10 ± 0.04)	0.26	0.16	0.12–0.20 (0.16 ± 0.03)	0.14	0.11–0.16 (0.13)	0.10–0.13 (0.12 ± 0.01)	0.14–0.16 (0.16 ± 0.01)	0.09–0.12 (0.11)	0.23–0.26 (0.25 ± 0.02)	0.10–0.16 (0.13 ± 0.02)
Salix	0.31–0.38 (0.34 ± 0.04)	0.17–0.29 (0.25 ± 0.05)	0.25–0.35 (0.31 ± 0.03)	0.26–0.44 (0.33 ± 0.06)	0.29–0.36 (0.32 ± 0.04)	0.29–0.34 (0.31 ± 0.03)	0.22–0.32 (0.28 ± 0.03)	0.26–0.35 (0.30 ± 0.04)	0.27–0.35 (0.31 ± 0.03)	0.26–0.37 (0.32 ± 0.04)	0.30–0.37 (0.33 ± 0.02)	0.24–0.30 (0.28 ± 0.02)	0.28–0.33 (0.31 ± 0.02)	0.23–0.37 (0.29 ± 0.04)

Table 3 (Contd.)

	P. koreana (6)	*P. simonii* (11)	*P. suaveolens* (2)	*P. szechuanica* (4)	*P. yunnanensis* (4)	*P. trichocarpa* (10)	*P. deltoides* (22)	*Populus* × *canadensis* (10)	*P. nigra* (31)	*P. violascens* (1)	*P. wilsonii* (1)	*P. tristis* (1)	*P.* × *berolinensis* (3)	*P.* × *canescens* (5)	*P. mexicana* (1)
P. euphratica															
P. ciliata															
P. lasiocarpa															
P. alba															
P. davidiana															
P. sieboldii															
P. tremula															
P. tremuloides															
P. angustifolia															
P. balsamifera															
P. candicans															
P. cathayana															
P. laurifolia															
P. maximowiczii															
P. koreana	0.85–1.00 (0.92 ± 0.07)														
P. simonii	0.51–0.74 (0.63 ± 0.06)	0.75–1.00 (0.83 ± 0.07)													
P. suaveolens	0.80–0.84 (0.83 ± 0.01)	0.57–0.73 (0.64 ± 0.06)	0.98												
P. szechuanica	0.74–0.79 (0.76 ± 0.01)	0.52–0.71 (0.61 ± 0.07)	0.80–0.83 (0.81 ± 0.01)	0.87–1.00 (0.93 ± 0.05)											
P. yunnanensis	0.67–0.71 (0.69 ± 0.01)	0.67–0.79 (0.74 ± 0.04)	0.71–0.76 (0.74 ± 0.02)	0.63–0.67 (0.66 ± 0.02)	0.91–1.00 (0.95 ± 0.03)										
P. trichocarpa	0.61–0.74 (0.68 ± 0.03)	0.54–0.72 (0.63 ± 0.04)	0.65–0.78 (0.70 ± 0.03)	0.60–0.74 (0.66 ± 0.03)	0.58–0.75 (0.69 ± 0.04)	0.91–1.00 (0.95 ± 0.03)									
P. deltoides	0.57–0.65 (0.60 ± 0.02)	0.51–0.66 (0.56 ± 0.02)	0.56–0.72 (0.61 ± 0.03)	0.57–0.72 (0.61 ± 0.02)	0.57–0.71 (0.62 ± 0.02)	0.54–0.68 (0.60 ± 0.03)	0.89–0.99 (0.94 ± 0.02)								
P. × *canadensis*	0.53–0.66 (0.61 ± 0.03)	0.54–0.73 (0.61 ± 0.04)	0.54–0.66 (0.62 ± 0.03)	0.50–0.64 (0.58 ± 0.03)	0.57–0.71 (0.65 ± 0.02)	0.54–0.71 (0.63 ± 0.03)	0.67–0.83 (0.75 ± 0.03)	0.78–1.00 (0.87 ± 0.02)							
P. nigra	0.53–0.64 (0.59 ± 0.02)	0.48–0.78 (0.59 ± 0.06)	0.52–0.61 (0.57 ± 0.02)	0.53–0.62 (0.57 ± 0.02)	0.53–0.63 (0.56 ± 0.02)	0.54–0.68 (0.61 ± 0.03)	0.45–0.60 (0.53 ± 0.02)	0.68–0.87 (0.76 ± 0.03)	0.84–1.00 (0.93 ± 0.03)						
P. violascens	0.71–0.72 (0.72 ± 0.01)	0.56–0.65 (0.61 ± 0.03)	0.70–0.72 (0.71)	0.65–0.69 (0.68 ± 0.02)	0.69–0.73 (0.72 ± 0.02)	0.66–0.76 (0.71 ± 0.03)	0.64–0.71 (0.67 ± 0.03)	0.59–0.68 (0.65 ± 0.03)	0.52–0.58 (0.55 ± 0.02)	–					
P. wilsonii	0.54–0.59 (0.56 ± 0.02)	0.43–0.55 (0.49 ± 0.05)	0.56–0.59 (0.58)	0.59–0.62 (0.61 ± 0.01)	0.50–0.54 (0.52 ± 0.02)	0.59–0.68 (0.64 ± 0.03)	0.46–0.55 (0.50 ± 0.02)	0.43–0.52 (0.49 ± 0.03)	0.45–0.54 (0.49 ± 0.02)	0.58	–				
P. tristis	0.67–0.71 (0.69 ± 0.01)	0.52–0.64 (0.58 ± 0.04)	0.70–0.72 (0.71)	0.69–0.71 (0.70 ± 0.01)	0.65–0.69 (0.67 ± 0.02)	0.80–0.87 (0.84 ± 0.03)	0.62–0.68 (0.64 ± 0.02)	0.58–0.68 (0.65 ± 0.03)	0.60–0.67 (0.63 ± 0.02)	0.69	0.66	–			
P. berolinensis	0.57–0.73 (0.67 ± 0.05)	0.55–0.74 (0.62 ± 0.05)	0.63–0.68 (0.66 ± 0.02)	0.55–0.63 (0.59 ± 0.03)	0.60–0.68 (0.63 ± 0.02)	0.57–0.69 (0.64 ± 0.03)	0.48–0.55 (0.52 ± 0.02)	0.67–0.78 (0.72 ± 0.03)	0.73–0.85 (0.79 ± 0.02)	0.61–0.65 (0.63 ± 0.02)	0.45–0.51 (0.48 ± 0.03)	0.59–0.64 (0.61 ± 0.03)	0.84–0.94 (0.88 ± 0.05)		
P. canescens	0.34–0.40 (0.37 ± 0.02)	0.27–0.46 (0.38 ± 0.04)	0.33–0.44 (0.40 ± 0.04)	0.31–0.41 (0.36 ± 0.03)	0.34–0.49 (0.42 ± 0.04)	0.37–0.53 (0.44 ± 0.04)	0.28–0.41 (0.34 ± 0.03)	0.27–0.41 (0.35 ± 0.03)	0.24–0.47 (0.34 ± 0.04)	0.38–0.44 (0.42 ± 0.02)	0.40–0.51 (0.46 ± 0.04)	0.39–0.46 (043 ± 0.03)	0.34–0.41 (0.37 ± 0.02)	0.73–0.94 (0.85 ± 0.07)	
P. mexicana	0.13–0.15 (0.14 ± 0.01)	0.14–0.23 (0.18 ± 0.03)	0.09	0.11–0.14 (0.11 ± 0.02)	0.16–0.18 (0.16 ± 0.01)	0.12–0.19 (0.17 ± 0.02)	0.13–0.18 (0.15 ± 0.01)	0.15–0.20 (0.17 ± 0.02)	0.15–0.22 (0.18 ± 0.02)	0.14	0.12	0.10	0.21–0.22 (0.22 ± 0.01)	0.08–0.14 (0.10 ± 0.03)	–
Salix	0.23–0.31 (0.26 ± 0.03)	0.20–0.34 (0.29 ± 0.05)	0.24–0.30 (0.27 ± 0.03)	0.27–0.36 (0.32 ± 0.03)	0.25–0.43 (0.35 ± 0.06)	0.24–0.38 (0.30 ± 0.03)	0.26–0.42 (0.34 ± 0.04)	0.22–0.40 (0.32 ± 0.06)	0.20–0.41 (0.30 ± 0.05)	0.28–0.36 (0.32 ± 0.04)	0.24–0.34 (0.30 ± 0.05)	0.29–0.37 (0.32 ± 0.04)	0.23–0.33 (0.28 ± 0.04)	0.24–0.38 (0.30 ± 0.05)	0.13–0.16 (0.15 ± 0.02)

The number of accessions analyzed per species is indicated between *parentheses* in the heading

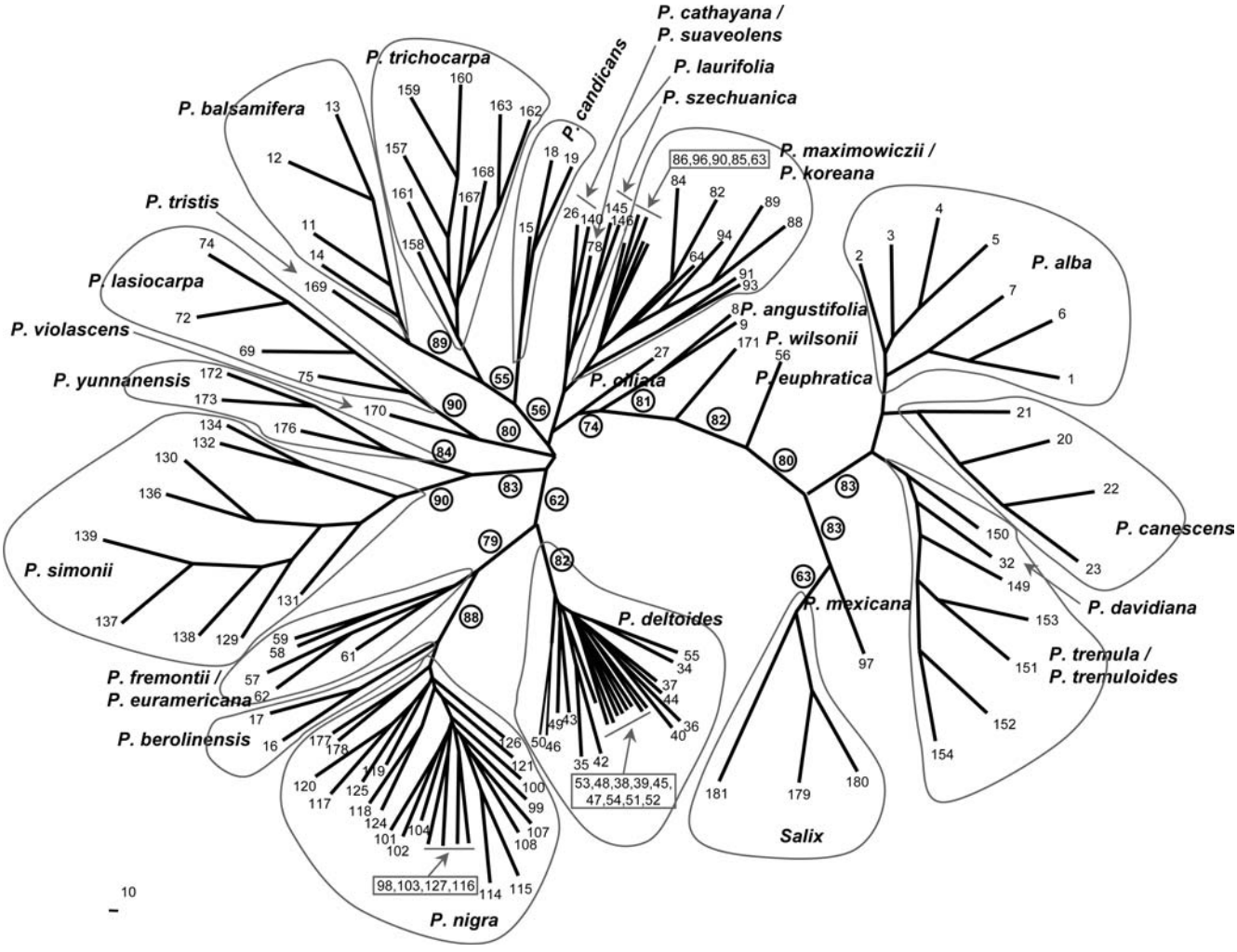

Fig. 2 The single most parsimonious bifurcating unrooted tree, based on the Wagner method, representing the phylogeny of *Populus*. Plain and circled numbers correspond to accession codes (Table 2) and bootstrap values (only those above 50% are shown for main branches, grouping several species), respectively

separate from *P. nigra* that was originally classified as a member of the *Aigeiros* section. The *P. deltoides* cluster was genetically closely related to the accessions classified as *P. fremontii* Wats. and *P. candicans* and, to a lesser extent, to *P. yunnanensis* (group 5) and species from groups 2, 3, and 6 of the *Tacamahaca*/*Leucoides* section. As mentioned previously, *P. candicans* represents interspecific hybrids of *P. deltoides* × *P. balsamifera* (Table 3). Remarkably, all *P. fremontii* accessions had AFLP patterns typical for *P. deltoides* × *P. nigra* hybrids and grouped with *Populus* × *canadensis* (synonym *Populus* × *euramericana* Moench), intermediate between the *P. deltoides* and *P. nigra* groups (Fig. 1). Therefore, these accessions were genetically associated with the group consisting of *P. nigra* and with *Populus berolinensis*, another interspecific hybrid of *P. nigra*.

The four species (*P. alba*, *P. tremula*, *P. tremuloides*, and *P. davidiana*) and interspecific hybrids (*Populus* × *canescens*) from the *Leuce* section, clustered in a single distinct group, which, with the exception of *P. mexicana* and *Salix* accessions, was the most distinct from the groups of the other *Populus* species (Fig. 1). *Populus* × *canescens* accessions clustered between *P. alba* and *P. tremula*, as was to be expected, since they are interspecific hybrids between the two species (Rajora and Dancik 1992).

Interspecific relationships were also studied with PCO (Fig. S1). The first PCO explains 18% of the total variation of the *Populus* species. The relative position of species and interspecific hybrids was consistent with the phenetic analysis. However, some of the species included in the previously described large meta-group were not distinguishable: *P. trichocarpa* and its associated hybrids, *P. balsamifera*, *P. tristis*, *P. laurifolia*, as well as *P. ciliata*.

Intraspecific relationships

As expected, the intraspecific GS values were higher than the interspecific ones, and their estimation depended on the number of accessions analyzed for each

species and the origin of the samples (Table 3). The most genetically divergent species were those from the *Leuce* section.

Different accessions from the same species clustered together in most cases. However, a few clones grouped with accessions corresponding to different species (Fig. 1), possibly because of a misidentification and/or mislabeling, such as accessions *P. sieboldii* (128), *P. lasiocarpa* (70), *P. laurifolia* (76, 77, and 81 accessions), *P. tremuloides* (155), *P. nigra* (106), and *P. ciliata* (31). *P. ciliata* (28) and *P. trichocarpa* (164 and 166) formed a separate cluster in group 3. Comparison of the AFLP profiles suggested that all these accessions were interspecific hybrids, rather than pure species. Other cases of clustering with species other than their own are: accession *P. yunnanensis* (175), which grouped with *P. candicans* and was completely identical to accession 19; accession *P. szechuanica* (148), which clustered with *P. balsamifera*; accessions *P. deltoides* (33), *P. ciliata* (30), *P. lasiocarpa* (71), and *P. maximowczii* (83), which grouped with *Populus* × *canadensis* and putative *P. fremontii* accessions (Fig. 1); accession *P. cathayana* (24), which remained individual, although it had the highest GS to *P. trichocarpa* and *P. balsamifera*; accession *P. balsamifera* (10), which clustered with *P. szechuanica*; accession *P. tremuloides* (156), which grouped with *P. canescens*; and the probable misclassification of *P. suaveolens* (141, 143, and 144), which was explained earlier.

A parsimony analysis, based on Wagner's method, allowed the construction of the single most parsimonious tree (Fig. 2). This tree, constructed from the dataset from which accessions known to be duplicates, accessions with GS ≥ 0.98, and putatively misclassified accessions were eliminated, is represented as a bifurcating unrooted tree because the GS values observed between *P. mexicana* and other *Populus* accessions are lower than between *Populus* and *Salix* accessions, which were initially included in this analysis as outgroups. The branching order and the grouping of species and accessions (clades) were consistent with and supported the phenetic analysis. The ordering of the sections was from the oldest *Abaso* to the newest *Aigeiros* (Fig. 2). The first branch is the genus *Salix*, followed by sections *Abaso*, *Leuce*, *Turanga*, *Tacamahaca*/*Leucoides*, and finally *Aigeiros* (Fig. 2). However, the following accessions did not follow the phenetic classification: *P. laurifolia* was associated with *P. szechuanica*, *P. cathayana*, and *P. suaveolens*, whereas *P. lasiocarpa* and *P. violascens*, from the *Leucoides* section, were not with *P. deltoides*.

Discussion

AFLP markers and DNA fingerprinting of *Populus* clones

Morphological traits as well as biochemical and molecular markers have been used, with different degrees of success, for genetic variability assessment and clonal identification in the *Populus* species (Rajora 1988, 1989a, 1989b; Rajora and Zsuffa 1989; Rajora and Dancik 1992; Castiglione et al. 1993; Dayanandan et al. 1998; Rahman et al. 2000; Rajora and Rahman 2001, 2003; Rahman and Rajora 2002). In this study, with a few exceptions, all *Populus* and *Salix* accessions could be uniquely identified, based on the AFLP markers. Of the 178 poplar accessions,24 were grouped with accessions of *Populus* species other than their own or with interspecific hybrids (Fig. 1). These accessions may potentially be mislabeled or misidentified or show greater AFLP divergence with the accessions of their own species along with higher coincidental AFLP similarities with the accessions of other species. Mislabeling or misidentification of species or clones in *Populus* is quite common and some accessions, commonly used in breeding programs as pure species, may actually be interspecific hybrids (e.g., Rajora and Zsuffa 1991). Interspecific hybrids were initially detected based on GS analysis and further confirmed by a direct comparison of their AFLP profiles. For example, clones 15/74 (nos. 141 and 143) and 20/65 of *P. suaveolens* (no. 144) are likely interspecific hybrids of *P. trichocarpa* × *P. balsamifera* and *P. canadensis* × *P. nigra*, respectively. Fritzi Pauley accessions (nos. 164 and 166), which are considered as pure *P. trichocarpa* and are used in breeding programs, correspond to AFLP patterns expected for *P. trichocarpa* × *P. maximowiczii* hybrids. Accession 81 was morphologically described as *P. laurifolia*; however, this accession clustered with *P. berolinensis* (*P. laurifolia* × *P. nigra* 'Italica') accessions. Similarly, accession 156 of *P. tremuloides* grouped with *Populus* × *canescens*. Furthermore, all five *P. fremontii* accessions clustered together with *Populus* × *canadensis*, and had AFLP (this study) and microsatellite (O.P. Rajora, unpublished results) patterns typical for *P. deltoides* × *P. nigra* interspecific hybrids.

Intersectional relationships in the genus *Populus*

Traditionally, *Populus* species have been grouped within their respective sections, based primarily on their interspecific crossability and morphological similarities (Food and Agriculture Organization 1958, 1979; Rajora and Zsuffa 1984). With certain notable exceptions, intersectional genetic and phylogenetic relationships, which have been observed from our AFLP data, are in agreement with earlier descriptions (Eckenwalder 1996). Our results suggest that the monospecific section *Abaso*, represented by one accession of *P. mexicana*, is the most differentiated from the other sections. The genetic differentiation of the *P. mexicana* accession from all other *Populus* species sampled was greater than that observed between the *Salix* and *Populus* species. The *Abaso* section was created by Eckenwalder (1977) to include *P. mexicana*, which has slight morphological similarities with poplars from the section *Aigeiros* (Eckenwalder 1977, 1996). Although based on a single accession of

P. mexicana, our data hint that *P. mexicana* may belong to another genus, which is different from that of *Populus* or *Salix*. Yet, the parsimony analysis does not withdraw the hypothesis that it could represent the most divergent and oldest lineage of *Populus* species supporting the fact that *P. mexicana* is a *Populus* species, that most closely resembles the oldest known poplar fossils (Eckenwalder 1996).

Among the five original *Populus* sections, the *Leuce* and *Turanga* sections were the most differentiated from the other three sections, based on both phenetic and phylogenetic analyses. The order of the sections in the phylogenetic tree more or less followed their known evolutionary patterns (Eckenwalder 1996), with the oldest *Leuce* section at one end and the most recent *Aigeiros* section at the other (Fig. 2). Thus, not only does the AFLP data support previously described evolutionary relationships in the genus *Populus* (Eckenwalder 1996), but also suggests close genetic relationships between the *Aigeiros* and *Tacamahaca* sections. These results are in agreement with the close relationships observed between these sections, which are based on morphology, evolutionary and crossability relationships, and on allozyme and DNA marker analyses (Zsuffa 1975; Eckenwalder 1984a, 1984b, 1996; Rajora and Zsuffa 1990; Barrett et al. 1993; Rajora and Dancik 1995a).

With the exception of the single accession of *P. wilsonii*, species from the *Leucoides* section (*P. ciliata*, *P. lasiocarpa*, and *P. violascens*) grouped with those from the *Tacamahaca* section. *P. lasiocarpa* and *P. violascens* were clustered together and their group was linked to that of *P. deltoides*. Phylogenetic analysis revealed branches comprising a mixture of species from three sections with low bootstrap values, thus suggesting close genetic relationships between the *Leucoides* section and the *Tacamahaca* and *Aigeiros* sections. These findings are new and in contrast with what is generally known about species crossability and the evolutionary relationships between these two sections (Zsuffa 1975; Eckenwalder 1996), although cross-compatibility of *P. ciliata* with *Populus* species of the *Tacamahaca* section is well established (Zsuffa 1975; Willing and Pryor 1976). However, the placement of *P. ciliata* in the *Leucoides* section is controversial: we propose that, in agreement with Eckenwalder (1996), this species might be classified in the *Tacamahaca* section (see below).

Interspecific genetic and phylogenetic relationships

Leuce

The results from both phenetic and phylogenetic analyses suggest close genetic relationships among the members of the *Leuce* section: *P. alba*, *P. tremula*, *P. tremuloides*, *P. davidiana*, and *Populus* × *canescens*. All the four species and *Populus* × *canescens* are part of the same branch of the phylogenetic tree and clearly distinguishable from the other *Populus* species (Figs. 1, S1, S2). *Populus* × *canescens* accessions formed a group intermediate to their parental species *P. alba* and *P. tremula*, supporting the earlier morphological classification (Food and Agriculture Organization 1979) and allozyme results (Rajora and Dancik 1992) that *Populus* × *canescens* represents interspecific hybrids between *P. alba* and *P. tremula*. Our results also suggest that *P. tremula* is very highly genetically similar to *P. tremuloides*. Accessions of these two species clustered in the same group. European aspen (*P. tremula*) is very similar to the North American trembling aspen (*P. tremuloides*), with respect to most of their morphological characters (Dickmann and Stuart 1983). These two species probably originated from a common ancestor and have been separated geographically. In addition, the *P. davidiana* accession showed very high genetic similarities with *P. tremula* and *P. tremuloides*. Our AFLP data lend some support to Eckenwalder's (1996) proposal of merging *P. tremula*, *P. tremuloides*, and *P. davidiana* into a single species. However, further analysis is required to verify these results with a larger sample size.

Tacamahaca

The species of the *Tacamahaca* section showed interspecific genetic similarities expected for consectional species (data not shown), with species clustered in a large meta-group of smaller groups of highly related species (Figs. 1, S1, S2). A similar grouping was also revealed by the phylogenetic analysis, where low bootstrap values at the branch of the *Tacamahaca* section were observed. Since *P. cathayana*, *P. suaveolens*, *P. szechuanica*, *P. koreana*, and *P. maximowiczii* clustered into one group or one clade, one can conclude that these species are highly genetically related. Within this group, the highest genetic similarities were found between *P. cathayana* and *P. suaveolens* clones 21–65 (accessions 140 and 142), and between *P. maximowiczii* and *P. koreana*, with accessions of these two species situated within the same clade. The high interspecific AFLP GS values observed between *P. cathayana* and *P. suaveolens* and between *P. koreana* and *P. maximowiczii*, together with phenetic and phylogenetic analyses, partially corroborate Eckenwalder's (1996) proposal (based on morphological classification), although they cannot substantiate the merging of all five species into a single species, despite the high GSs among them. Additional molecular, morphological and other analyses, with a larger sample size, would be required.

Another group of highly related species includes *P. balsamifera*, *P. candicans*, and *P. trichocarpa*. The high genetic similarities among these three species are in agreement with their known close relationship. *P. balsamifera* and *P. trichocarpa* are sometimes considered as sub-species: *P. balsamifera* subsp. *balsamifera*, and *P. balsamifera* subsp. *trichocarpa* (Brayshaw 1965). Although *P. balsamifera* and *P. trichocarpa* showed high

AFLP similarities, their accessions formed distinct species groups. Thus, *P. trichocarpa* and *P. balsamifera* should be treated as separate species. The status of *P. candicans* has been unclear. It has been described as a variety or cultivar of *P. balsamifera* [*P. balsamifera* var. *candicans*, var. *subcordata* cv. *candicans* (Stout 1929; Food and Agriculture Organization 1979), or var. C. Gray (Rehder 1947)]. However, *P. candicans* has also been considered as an interspecific hybrid between *P. balsamifera* and *P. deltoides* var. *missouriensis* (Little 1979). Based on the GS values (0.75–0.87), we propose that the *P. candicans* accessions analyzed be included in a separate group, which is genetically distinct from the *P. balsamifera* group. The genetic distinction observed was much higher than expected for accessions of the same species. It was further noticed that the *P. candicans* accessions had AFLP profiles expected for *P. deltoides* × *P. balsamifera* hybrids. Thus, *P. candicans* might, indeed, be considered a hybrid between *P. balsamifera* and *P. deltoides*, as described by Little (1979) and as supported by the PCO analysis (Fig. S1).

Leucoides

Heterogeneous relationships among species within the *Leucoides* section were identified, with *P. wilsonii* being the most distinct species and *P. lasiocarpa* and *P. violascens* the most closely related. *P. wilsonii* seemed related only to*P. lasiocarpa*, based on flower and fruit characteristics (D. Demeyere, personal communication). The high genetic similarities observed between *P. lasiocarpa* and *P. violascens* were consistent with their known high morphological similarities (Rehder 1947). *P. ciliata* clearly showed close genetic relationships with the species of the *Tacamahaca* section. Although AFLP analysis indicated a heterogeneous grouping of the *P. ciliata* accessions analyzed in this study, they were tightly linked with the accessions of balsam poplars (Table 3; see also http://www.psb.ugent.be/~vesto). The *P. ciliata* clone 72–085 (no. 27) clustered with *P. angustifolia*, and two other clones (65–017 and D1D4E3 represented by nos. 28 and 31, respectively) with *P. trichocarpa* hybrids. Eckenwalder (1996) proposed to include *P. ciliata* in the *Tacamahaca* section, on the basis of its crossability with balsam poplars and the lack of morphological similarities with the *Leucoides* species. The AFLP data supports this suggestion.

P. lasiocarpa and *P. violascens* were genetically closely similar to species from the *Tacamahaca* section, especially with *P. trichocarpa*, *P. candicans*, *P. simonii*, and *P. yunnanensis*, as well as with *P. deltoides* from the *Aigeiros* section (Table 3). Smith (1988) has shown that *P. lasiocarpa* is also closely related to *P. szechuanica*. Thus, *P. lasiocarpa* and *P. violascens* may have to be reclassified in the *Tacamahaca* section, if these species are found to be cross-compatible with the species of the *Tacamahaca* section and if their close genetic and phylogenetic relationships are ascertained with a larger sample size.

Aigeiros

Within the *Aigeiros* section, each of the three species formed its own group. Phenetic, PCO, and phylogenetic analyses indicate genetic distinctness of *P. nigra* from *P. deltoides*. These results are consistent with those reported earlier, based on chloroplast DNA (Smith and Sytsma 1990; Rajora and Dancik 1995a), mitochondrial DNA (Barrett et al. 1993), and Random Amplified Polymorphic DNA (Castiglione et al. 1993) analyses. Thus, our AFLP data support the previous suggestion that *P. nigra* should be either classified in a new section *Nigrae*, which is separate from *P. deltoides* (Rajora and Dancik 1995a), or as the most divergent species in a subsection of the *Tacamahaca* section, based on the crossability between its members. AFLP fragment pattern comparisons among *P. deltoides*, *P. nigra*, and *P. fremontii* accessions revealed that all the *P. fremontii* accessions sampled were actually *P. deltoides* × *P. nigra* hybrids. The clustering of *Populus* × *canadensis* and *P. fremontii* accessions and their relative position between the *P. deltoides* and *P. nigra* species in the PCO analysis, also support this result. Three of these putative *P. fremontii* accessions were confirmed as *P. deltoides* × *P. nigra*, based on microsatellite DNA markers (O.P. Rajora, unpublished data), since these accessions were heterozygous for species-specific alleles of *P. deltoides* and *P. nigra* (Rajora and Rahman 2003). *P. fremontii* is morphologically very similar to *P. deltoides* var. *occidentalis* (Food and Agriculture Organization 1958), but can be clearly distinguished from the described *Populus* × *canadensis* or *P. nigra* clones (S. Rood, personal communication). Also, *P. nigra* and *Populus* × *canadensis* do not occur in the USA, whereas *P. fremontii* is found naturally distributed there (S. Rood, personal communication). Therefore, the analyzed *P. fremontii* accessions could be misidentified or mislabeled. Its relationship with *P. deltoides* and *P. nigra* should be confirmed by analyzing samples obtained from its natural range in America.

Intraspecific genetic diversity and relationships

Since only a single or a few individuals were studied for several of the species analyzed, this study does not provide sufficiently accurate estimates of intraspecific genetic diversity. Nevertheless, moderate–to-high genetic similarities were observed among accessions within species. GS values suggest that the species belonging to the *Leuce* section have relatively high levels of intraspecific AFLP variability, whereas some species of the *Tacamahaca* and *Aigeiros* sections (*P. balsamifera*, *P. trichocarpa*, *P. deltoides*, and *P. nigra*) have relatively low levels of AFLP variability. The high levels of AFLP diversity observed in the species of the *Leuce* section are consistent with allozyme, Restriction Fragment Length Polymorphism, and microsatellite diversity observed in *P. tremuloides* and *Populus grandidentata* (Rajora and

Dancik 1992; Liu and Furnier 1993; Dayanandan et al. 1998). The progenitor or ancestral species are generally highly genetically diverse in contrast to the derived species. *Populus* species of the *Leuce* and *Aigeiros* section are considered to be the oldest and the most recent poplars, respectively (Eckenwalder 1996). Thus, our results seem to agree with the evolutionary relationships among the *Populus* species, as described by Eckenwalder (1996).

Conclusion

For the first time, intergeneric, intersectional, interspecific, and intraspecific genetic and phylogenetic relationships among 25 *Populus* species belonging to the six sections of the genus, a considerable number of interspecific *Populus* hybrids, and three *Salix* accessions have been determined, using AFLP markers. Our study has clarified genetic and phylogenetic relationships as well as the taxonomic placement of several *Populus* species, whose species status and taxonomic classification were earlier ambiguous.

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