A phylogeographic study of the cosmopolitan genus *Phragmites* (Poaceae) based on AFLPs

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Abstract. Within the genus *Phragmites* (Poaceae), the species *P. australis* (the common reed) is virtually cosmopolitan, and shows considerable variation in ploidy level and morphology. Genetic variation in *Phragmites* was studied using AFLPs, and analysed with parsimony and distance methods. Groups of *P. australis* strongly supported in the analyses include one that comprises all South American clones, a distinct group from the US Gulf Coast, and a group of E. Asian and Australian octoploids. Among the other species, the paleotropical *P. vallatoria* is supported as monophyletic and most closely related to the paraphyletic *P. mauritanus* and to the Gulf Coast and S. American groups. The E. Asian species *P. japonicus* is closely related to a group of *P. australis* clones mostly from central North America. Tetraploidy predominates in the genus, and optimisation of chromosome numbers onto the phylogeny shows that higher ploidy levels have evolved many times.

Key words: *Phragmites*, common reed, phylogeography, AFLP, polyploidy.

The genus *Phragmites* Adans. is virtually cosmopolitan in distribution, ranging from the tropics to the cold-temperate regions of both hemispheres (Den Hartog et al. 1989).

Phragmites australis (Cav.) Trin. ex Steud., the common reed, is the most familiar and widespread species in the genus. This species shows high phenotypic, cytotypic and genetic variability, and has a wide ecological amplitude (Conert 1961, Björk 1967, Clevering and Lissner 1999). It forms dense, often nearly monospecific stands in the littoral zone of lakes, along rivers and in fresh- and saltwater marshes. Being an important component of such ecosystems, it has attracted considerable scientific interest and many aspects of its ecology have been studied extensively (Björk 1967, Den Hartog et al. 1989, Brix 1999, Cizkova et al. 2000, Brix and Cizkova 2001).

According to a recent review (Clevering and Lissner 1999), the genus *Phragmites* includes *P. australis*, *P. mauritanus* Kunth, *P. japonicus* Steudel and *P. vallatoria* (Plunk. ex L.) Veldk. (syn. *P. karka* [Retz.] Trin. ex Steud., according to Veldkamp [1992]). The species are all morphologically similar, and presumably closely related. The distribution of *P. australis* covers the entire range of the genus except parts of the Paleotropics. *Phragmites mauritanus* is a tropical African species, differing from *P. australis* in having woodier stems, more rigid leaves and

smaller glumes. The range of *P. japonicus*, different from *P. australis* in its much longer ligule, covers Eastern China, Japan and the Russian Far East. *Phragmites vallatoria*, intermediate between *P. australis* and *P. mauritanus* in many morphological characters, has its main distribution in tropical Asia but also occurs in Polynesia, Australia and Tropical Africa. A recently described species is *Phragmites frutescens* H. Scholz, which was initially considered endemic to the island of Crete, Greece (Greuter and Scholz 1996). It has subsequently been found also in other parts of Greece and in Turkey (Scholz and Böhling 2000, Mutlu 2002). Additional, poorly known species are *Phragmites dioica* Hackel ex Conert, recorded from Argentina and Uruguay (Conert 1961), and *P. berlandieri* E. Fourn. from North America.

Within *P. australis*, a large number of intraspecific units have been described, most of which seem to be merely local variants of little taxonomic value (Clayton 1967). Only two subspecies of *P. australis* are commonly recognised: *P. australis* ssp. *australis*, widespread in the temperate regions of both hemispheres, and *P. australis* ssp. *altissimus* (Benth.) Clayton in the Mediterranean area, Middle East and North Africa. The two subspecies are rather imperfectly separated by the shape of the upper glume (Clayton 1967), and ssp. *altissimus* is a taller plant with a larger panicle than ssp. *australis* (Clevering and Lissner 1999). Recently, the morphologically distinctive *Phragmites australis* ssp. *americanus* Saltonstall, P.M. Peterson & Soreng was described from North America (Saltonstall et al. 2004).

Considerable variation in chromosome number has been documented for *Phragmites*, and phenomena such as polyploidy, aneuploidy and mixoploidy have all been reported from the genus. *Phragmites australis* includes various euploidy levels (3x, 4x, 6x, 7x, 8x, 10x, 11x and 12x; Clevering and Lissner 1999), and may be an example of a polyploid complex, as defined by Stebbins (1971). A series of aneuploid tetraploids was found by Gorenflot et al. (1984, 1990), while mixoploid tetraploids and

octoploids were described by Connor et al. (1998). Remarkably, 22x cells were found by the latter authors in an octoploid specimen from New Zealand. The basic chromosome number is considered to be $x=12$ (Gorenflot 1986), but there is only a single report of a diploid plant (from Camargue, France; Dykijova and Pazourkova 1979). Worldwide, tetraploids predominate, but there is a West-East shift in dominance of ploidy level from mostly tetraploidy in the Americas, Europe and Africa, to predominating octoploidy in Asia and Australia (Gorenflot 1976, illustrated in the map by Clevering and Lissner [1999]). Chromosome numbers are known also for *P. vallatoria* (2x, 3x, 4x and aneuploid at all three levels; Shouliang and Phillips 2004, Clevering and Lissner 1999), for *P. japonicus* (4x and aneuploid 8x; Zong et al. 1991, Clevering and Lissner 1999, Ishii and Kadono 2001), and for *P. mauritanus* (4x; Clevering and Lissner 1999). It seems likely that this apparent cytotypic plasticity has been an important factor in the evolution and diversification of the genus, as has been shown to be the case in numerous genera of angiosperms (Grant 1981, Soltis and Soltis 1999, Otto and Whitton 2000).

A number of investigations of the genetic diversity of *P. australis* have been made, and these have revealed a very wide range of variation in terms of both overall genotypic variability and allelic polymorphisms (Kopitz 1999; Köhl et al. 1999; Pellegrin and Hauber 1999; Saltonstall 2002, 2003a). A study of sequences of two non-coding regions of the chloroplast revealed 27 haplotypes of *P. australis* world-wide (Saltonstall 2002). Eleven haplotypes were found to be unique to North America, while two, which the author defined as Haplotype M and Haplotype I, have a wide distribution on several continents. Haplotype M is the most common type in North America, Europe and continental Asia and, according to a parsimony network of chloroplast haplotype diversity, it could be ancestral to the other fourteen closely related haplotypes found in Europe,

Asia and Africa. Haplotype I predominates in South America and was found on several islands in the South Pacific and along the North American Gulf Coast. It is probably the same Gulf Coast group identified by Pellegrin and Hauber (1999) on the basis of isozymes. A subsequent study by Saltonstall (2003a), based on microsatellite and DNA sequence variation, provided further detail concerning North American populations, and confirmed that those of the Gulf Coast are clearly distinct from clones originating in other parts of North America. In the same study it was found that native North American clones from areas north of the Gulf Coast form another well-defined lineage, formally named *P. australis* ssp. *americanus* (Saltonstall et al. 2004). Within this subspecies, it is possible to distinguish Atlantic Coast, Midwest and Western populations, based on nuclear allelic variation (Saltonstall 2003a). A third, introduced lineage of European origin, (Haplotype M; Saltonstall 2003a), shows high allelic diversity. It is particularly common and dominating near the Atlantic coast, and appears to be expanding westwards.

While the large-scale genetic variation patterns of *Phragmites* in North America are now beginning to be elucidated, knowledge of the variation in the rest of the world is very poor. In addition, species other than *P. australis* have not been included in molecular studies so far, and generally, very little is known about species interrelationships in the genus. The present study is an attempt to investigate the phylogeography and species interrelationships of *Phragmites* based on collections covering large parts of its almost world-wide distribution. A phylogeographic model would make it possible to reconstruct the evolution of individual characters in the genus. Of particular interest is ploidy level, which has been considered a key factor in the evolution of the genus.

As a method for assessing the genetic variation, AFLPs (Amplified Fragment Length Polymorphisms) was chosen, as this has been successfully used in a number of phylogeographic

studies of individual plant species (Dodd et al. 2002, Muellner et al. 2005) as well as in studies of interrelationships in groups of closely related species (Kardolus et al. 1998, Després et al. 2003, Zerega et al. 2004).

Materials and methods

Plant material. AFLP fingerprinting was performed on 238 *Phragmites* accessions. Most of the examined material consisted of fresh specimens collected in the experimental area of the Institute of Biological Sciences, University of Aarhus. In addition, 24 specimens were sampled from the herbarium of the same university (AAU), in order to represent certain geographical areas from which fresh samples were not available.

The specimens used represent the species *P. australis*, *P. mauritanus*, *P. frutescens*, *P. vallis-litoria*, *P. japonicus*. *Phragmites karka* was treated as a synonym of *P. vallis-litoria*. Typical *P. australis* subsp. *altissimus* was unavailable for study. Species determinations are based on morphological characters as outlined by Clayton (1967) and Tsvelev (1983). Whenever the material was insufficient for certain species identification due to, e.g. lack of flowers, the determination by the collector was tentatively accepted.

For each sample the origin, species and ploidy level are indicated in Table 1. Chromosome counts were performed by Clevering and Lissner (1999), using flow cytometry. No counts were available for some of the *P. australis* clones nor for any specimens of the other species.

DNA extraction. DNA was extracted from apical leaves of fresh specimens and from leaves and/or inflorescences of herbarium samples, using Qiagen's DNeasy Plant Mini Kit. The Qiagen protocol was modified for the herbarium specimens in that the leaf tissue was ground dry with liquid nitrogen and a small amount of sand before addition of the extraction buffer. The DNA concentration was then measured with a fluorometer.

AFLP. The AFLP protocol was virtually the same as that described by Vos et al. (1995). Restriction digestion and adapter ligation were performed simultaneously on 100 ng of genomic DNA, using 2.5 units each of restriction enzymes EcoRI and of MseI, and 1 unit of T4 DNA ligase to ligate 5 pmol EcoRI and 50 pmol MseI double-stranded nucleotide adapters. Digestion and

Table 1. *Phragmites* clones sampled, arranged by country and sample number. Collection data generally refer to the origin of clones in cultivation at the experimental area of the University of Aarhus. Exceptions are DNA samples derived from museum specimens at the Herbarium of the University of Aarhus, and collections from wild populations in the Po Plain, Italy (sample labels starting with IT)

Sample label	Species	Country	Locality	Herbarium specimen	Date	Collector	Ploidy
Pa 68 AG	<i>P. australis</i>	Algeria	Guebbour, south of Hassi Messaoud		12/03/2001	G. Rivalta	
Pa 36 AU	<i>P. australis</i>	Australia	Cortina Lake, S.A.		01/03/1998	J. White	8x
Pa 76 AU	<i>P. australis</i>	Australia	Ginninderra Creek, A.C.T.		01/04/1998	R. Cruickshank	8x
Pa 88 AU	<i>P. australis</i>	Australia	Cutting Creek, Victoria		01/07/1998	K. Ward	10x
Pa 96 AU	<i>P. australis</i>	Australia	Botany Wetlands, N.S.W.		01/07/1998	P. Pistilla	8x
Pa 145 AU	<i>P. australis</i>	Australia	Telowie, Coorong, S.A.		01/07/1998	J. White	8x
Pa 148 AU	<i>P. australis</i>	Australia	Brewarrina, N.S.W.		01/07/1998	A. Amos	8x
Pa 149 AU	<i>P. australis</i>	Australia	Bora Channel, N.S.W.		01/07/1998	W. Martin et al.	8x
Pa 150 AU	<i>P. australis</i>	Australia	Old Willbriggie Road, N.S.W.		01/07/1998	G. McCorkelle	8x
Pa 156 AU	<i>P. australis</i>	Australia	Monkeygar Creek, N.S.W.		01/07/1998	W. Martin et al.	8x
Pa 157 AU	<i>P. australis</i>	Australia	Edward River, N.S.W.		01/07/1998	G. McCorkelle	8x
Pa 158 AU	<i>P. australis</i>	Australia	Lenewa Creek, Victoria		01/07/1998	M. Thomas	8x
Pa 162 AU	<i>P. australis</i>	Australia	Bonegilla, Victoria		01/07/1998	M. Thomas	8x
Pa 167 AU	<i>P. australis</i>	Australia	Hughes Creek, Victoria		01/07/1998	M. White	8x
Pa 173 AU	<i>P. australis</i>	Australia	Tullaroop Creek, Victoria		01/07/1998	J. Williamson	8x
Pa 176 AU	<i>P. australis</i>	Australia	Bethanga Creek, Victoria		01/07/1998	M. Thomas	8x
Pa H14 AU	<i>P. australis</i>	Australia	Nursery Belair, S.A.	Symon 1214	23/03/1961	D.E. Symon	
Pa 67 BE	<i>P. australis</i>	Belgium	Schelde-estuarie, Bureht, Antwerp		01/03/1998	W. Muylaert	
Pa 146 BE	<i>P. australis</i>	Belgium	Schelde, Konkelschoor, Berlare		01/03/1998	W. Muylaert	4x
Pm H1 BF	<i>P. mauritanus</i>	Burkina Faso	16 km E of Banfora	Lægaard 18386	28/10/1997	S. Lægaard et al.	
Pa 129 CA	<i>P. australis</i>	Canada	Cootes Paradise, Ontario		01/06/1998	T. Smith	
Pa 130 CA	<i>P. australis</i>	Canada	Lake Manitoba, Blind Channel, Manitoba		01/04/1998	R. Mead	
Pa 131 CA	<i>P. australis</i>	Canada	Huntingdon, Quebec		01/04/1998	D. Lauzer	
Pa 132 CA	<i>P. australis</i>	Canada	l'Islet sur Mer, Quebec		01/05/1997	C. Gervais	4x
Pa 151 CA	<i>P. australis</i>	Canada	Chemin de la Butte, Quebec		01/03/1998	C. Gervais	4x
Pa 152 CA	<i>P. australis</i>	Canada	Duvernay-est, Montreal, Quebec		01/04/1998	C. Gervais	4x
Pa 153 CA	<i>P. australis</i>	Canada	Ormstown, Quebec		01/04/1998	D. Lauzer	4x
Pa 154 CA	<i>P. australis</i>	Canada	Saint de Joliette, Quebec		01/04/1998	C. Gervais	4x
Pa 155 CA	<i>P. australis</i>	Canada	Ste-Martine, Quebec		01/04/1998	D. Lauzer	4x

Pa 204 CA	<i>P. australis</i>	Canada	Lake Manitoba III, Inkster Farm, Manitoba	01/04/1998	G. Goldsborough	4x
Pa 122 CN	<i>P. australis</i>	China	Mai Po, Hong Kong	01/11/1997	N. F. Y. Tam	8x
Pa 123 CN	<i>P. australis</i>	China	Lanzhou	01/05/1997	Zhang Cheng-Lie	4x
Pa 671 CY	<i>P. australis</i>	Cyprus	Coral Beach, Pafos	12/07/2003	H. Brix	
Pa 672 CY	<i>P. australis</i>	Cyprus	Afrodites Cad. Polis	11/07/2003	H. Brix	4x
Pa 620 CZ	<i>P. australis</i>	Czech Republic	Rozmberk			
Pa 49 DK	<i>P. australis</i>	Denmark	Norsminde Fjord	01/08/1992	J. Lissner	4x
Pa 50 DK	<i>P. australis</i>	Denmark	Knebel Vig	01/06/1995	J. Lissner	4x
Pa 609 DK	<i>P. australis</i>	Denmark	Vejlerne			4x
Pa 610 DK	<i>P. australis</i>	Denmark	Vejlerne			4x
Pa H8 EC	<i>P. australis</i>	Ecuador	Loja, W of church of Barrio Daniel Alvares	Lægaard 19975	S. Lægaard	
Pa H9 EC	<i>P. australis</i>	Ecuador	Los Rios, rd. Babahugo, Querredo Km 39	Lægaard 71482	S. Lægaard	
Pa H10 EC	<i>P. australis</i>	Ecuador	Los Rios, between Pueblo Viejo and Ventana	Lægaard 54845	S. Lægaard	
Pa H15 EC	<i>P. australis</i>	Ecuador	Imbabura, San Pablo del Lago	Lægaard 70985	S. Lægaard	
Pa H16 EC	<i>P. australis</i>	Ecuador	Prov. Esmeraldas: near Tunchique	Lægaard 54698	S. Lægaard	
Pv 273 EG	<i>P. vallatoria</i>	Eq. Guinea	Bioco, Luba	Carvalho 4458	Carvalho	
Pa 83 EE	<i>P. australis</i>	Estonia	Lake Vortsjärv	01/04/1998	T. Noges	
Pa 159 EE	<i>P. australis</i>	Estonia	Lake Peipsi	01/04/1998	T. Noges	4x
Pa 54 FI	<i>P. australis</i>	Finland	Husövikén, Åland	01/04/1998	J. Mattila	
Pa 160 FI	<i>P. australis</i>	Finland	Mariehamn, Åland	01/04/1998	J. Mattila	4x
Pa 217 FI	<i>P. australis</i>	Finland	Raisio-lahti, Turku	01/04/1998	J. Mattila	
Pa 70 FR	<i>P. australis</i>	France	Campagnol, Narbonne	01/01/1998	P. Grillas	4x
Pa 64 DE	<i>P. australis</i>	Germany	Chiemsee	01/04/1997	O. Clevering	4x
Pa 639 DE	<i>P. australis</i>	Germany	Lusatia lignite mining area, Schlabendorf Lake	1998	A. Chabbi	4x
Pa 640 DE	<i>P. australis</i>	Germany	Lusatia lignite mining area, Plessa Lake	1998	A. Chabbi	4x
Pa 641 DE	<i>P. australis</i>	Germany	Lusatia lignite mining area, Pleassa Lake	1998	A. Chabbi	4x
Pa 209 GB	<i>P. australis</i>	Great Britain	Thamesmead	01/10/1997	P. White	4x
Pa 670 GB	<i>P. australis</i>	Great Britain	Blacktoft Sands			4x
Pa 57 GR	<i>P. australis</i>	Greece	Georgioupolis, Crete	01/11/1998	H. Scholz	6x
Pf 196 GR	<i>P. frutescens</i>	Greece	Georgioupolis, Crete	01/08/1998	H. Scholz	4x
Pa 66 NL	<i>P. australis</i>	Holland	Slotermeer	01/04/1997	O. Clevering	4x
Pa 163 NL	<i>P. australis</i>	Holland	Verdronken Land van Saeftinghe	01/03/1998	W. Muylaert	4x
Pa 602 NL	<i>P. australis</i>	Holland	Slotermeer			4x
Pa 77 HU	<i>P. australis</i>	Hungary	Lake Ferto	01/04/1997	O. Clevering	4x

Table 1. (Contd.)

Sample label	Species	Country	Locality	Herbarium specimen	Date	Collector	Ploidy
Pa 663 HU	<i>P. australis</i>	Hungary	Lake Fertó				8x
Pa 664 HU	<i>P. australis</i>	Hungary	Lake Fertó				4x
Pa 668 HU	<i>P. australis</i>	Hungary	Lake Fertó				4x
Pa 58 IE	<i>P. australis</i>	Ireland	Kilcock		01/06/1998	T. Walsh	
Pa 164 IE	<i>P. australis</i>	Ireland	Lake Ree		01/06/1998	T. Walsh	4x
Pa 165 IE	<i>P. australis</i>	Ireland	Hazelhatch		01/07/1998	T. Walsh	4x
Pa 90 IL	<i>P. australis</i>	Israel	Yerokham, Negev Highlands		01/03/1998	A. Danin	4x
Pa 91 IL	<i>P. australis</i>	Israel	Dead Sea, south-west coast		01/03/1998	A. Danin	4x
Pa H13 IL	<i>P. australis</i>	Israel	Lake Huleh, Hulata	Zohary 483	16/09/1985	D. Zohary et al.	
Pa 75 IT	<i>P. australis</i>	Italy	Gorgona Island, Tuscany		01/06/1998	H. Brix	4x
Pa 207 IT	<i>P. australis</i>	Italy	Albano S. Alessandro, Bergamo		01/09/1998		
Pa IT3 IT	<i>P. australis</i>	Italy	Boscossa Wetland, Molinella, Bologna		28/06/2001	C. Lambertini	
Pa IT6 IT	<i>P. australis</i>	Italy	Boscossa Wetland, Molinella, Bologna		25/07/2001	C. Lambertini	
Pa IT9 IT	<i>P. australis</i>	Italy	Boscossa Wetland, Molinella, Bologna		25/07/2001	C. Lambertini	
Pa IT10 IT	<i>P. australis</i>	Italy	Boscossa Wetland, Molinella, Bologna		28/06/2001	C. Lambertini	
Pa IT11 IT	<i>P. australis</i>	Italy	Boscossa Wetland, Molinella, Bologna		28/06/2001	C. Lambertini	
Pa IT22 IT	<i>P. australis</i>	Italy	Tombe Wetland, Malalbergo, Bologna		11/07/2001	C. Lambertini	
Pa IT26 IT	<i>P. australis</i>	Italy	Tombe Wetland, Malalbergo, Bologna		11/07/2001	C. Lambertini	
Pa IT27 IT	<i>P. australis</i>	Italy	Tombe Wetland, Malalbergo, Bologna		11/07/2001	C. Lambertini	
Pa IT29 IT	<i>P. australis</i>	Italy	Tombe Wetland, Malalbergo, Bologna		11/07/2001	C. Lambertini	
Pa IT33 IT	<i>P. australis</i>	Italy	Tombe Wetland, Malalbergo, Bologna		11/07/2001	C. Lambertini	
Pa IT34 IT	<i>P. australis</i>	Italy	Tombe Wetland, Malalbergo, Bologna		11/07/2001	C. Lambertini	
Pa IT41 IT	<i>P. australis</i>	Italy	Bentivoglia Wetland, Medicina, Bologna		28/06/2001	C. Lambertini	
Pa IT56 IT	<i>P. australis</i>	Italy	Bentivoglia Wetland, Medicina, Bologna		28/06/2001	C. Lambertini	
Pa IT57 IT	<i>P. australis</i>	Italy	Bentivoglia Wetland, Medicina, Bologna		18/11/2002	C. Lambertini	
Pa IT58 IT	<i>P. australis</i>	Italy	Bentivoglia Wetland, Medicina, Bologna		18/11/2002	C. Lambertini	
Pa IT59 IT	<i>P. australis</i>	Italy	Bentivoglia Wetland, Medicina, Bologna		18/11/2002	C. Lambertini	
Pa IT60 IT	<i>P. australis</i>	Italy	Fracassata Wetland, Medicina, Bologna		25/07/2001	C. Lambertini	
Pa IT61 IT	<i>P. australis</i>	Italy	Fracassata Wetland, Medicina, Bologna		25/07/2001	C. Lambertini	
Pa IT69 IT	<i>P. australis</i>	Italy	Fracassata Wetland, Medicina, Bologna		25/07/2001	C. Lambertini	
Pa IT72 IT	<i>P. australis</i>	Italy	Fracassata Wetland, Medicina, Bologna		25/07/2001	C. Lambertini	
Pa IT75 IT	<i>P. australis</i>	Italy	Fracassata Wetland, Medicina, Bologna		25/07/2001	C. Lambertini	
Pa IT81 IT	<i>P. australis</i>	Italy	Ercolana Wetland, Malalbergo, Bologna		21/07/2001	C. Lambertini	
Pa IT84 IT	<i>P. australis</i>	Italy	Ercolana Wetland, Malalbergo, Bologna		21/07/2001	C. Lambertini	

Pa IT85 IT	<i>P. australis</i>	Italy	Ercolana Wetland, Malalbergo, Bologna	21/07/2001	C. Lambertini	
Pa IT86 IT	<i>P. australis</i>	Italy	Ercolana Wetland, Malalbergo, Bologna	21/07/2001	C. Lambertini	
Pa IT88 IT	<i>P. australis</i>	Italy	Ercolana Wetland, Malalbergo, Bologna	21/07/2001	C. Lambertini	
Pa IT90 IT	<i>P. australis</i>	Italy	Ercolana Wetland, Malalbergo, Bologna	21/07/2001	C. Lambertini	
Pa IT92 IT	<i>P. australis</i>	Italy	Ercolana Wetland, Malalbergo, Bologna	21/07/2001	C. Lambertini	
Pa IT102 IT	<i>P. australis</i>	Italy	Boschetti Wetland, Marmorta, Bologna	18/06/2001	C. Lambertini	
Pa IT105 IT	<i>P. australis</i>	Italy	Boschetti Wetland, Marmorta, Bologna	18/06/2001	C. Lambertini	
Pa IT107 IT	<i>P. australis</i>	Italy	Boschetti Wetland, Marmorta, Bologna	18/06/2001	C. Lambertini	
Pa IT111 IT	<i>P. australis</i>	Italy	Boschetti Wetland, Marmorta, Bologna	18/06/2001	C. Lambertini	
Pa IT114 IT	<i>P. australis</i>	Italy	Boschetti Wetland, Marmorta, Bologna	18/06/2001	C. Lambertini	
Pa IT116 IT	<i>P. australis</i>	Italy	Boschetti Wetland, Marmorta, Bologna	18/06/2001	C. Lambertini	
Pa IT121 IT	<i>P. australis</i>	Italy	Comune Wetland, Malalbergo, Bologna	21/06/2001	C. Lambertini	
Pa IT122 IT	<i>P. australis</i>	Italy	Comune Wetland, Malalbergo, Bologna	21/06/2001	C. Lambertini	
Pa IT123 IT	<i>P. australis</i>	Italy	Comune Wetland, Malalbergo, Bologna	21/06/2001	C. Lambertini	
Pa IT127 IT	<i>P. australis</i>	Italy	Comune Wetland, Malalbergo, Bologna	21/06/2001	C. Lambertini	
Pa IT130 IT	<i>P. australis</i>	Italy	Comune Wetland, Malalbergo, Bologna	21/06/2001	C. Lambertini	
Pa IT133 IT	<i>P. australis</i>	Italy	Comune Wetland, Malalbergo, Bologna	21/06/2001	C. Lambertini	
Pa IT135 IT	<i>P. australis</i>	Italy	Comune Wetland, Malalbergo, Bologna	21/06/2001	C. Lambertini	
Pa IT142 IT	<i>P. australis</i>	Italy	Quadrone Wetland, Medicina, Bologna	25/07/2001	C. Lambertini	
Pa IT143 IT	<i>P. australis</i>	Italy	Quadrone Wetland, Medicina, Bologna	25/07/2001	C. Lambertini	
Pa IT144 IT	<i>P. australis</i>	Italy	Quadrone Wetland, Medicina, Bologna	25/07/2001	C. Lambertini	
Pa IT145 IT	<i>P. australis</i>	Italy	Quadrone Wetland, Medicina, Bologna	25/07/2001	C. Lambertini	
Pa IT147 IT	<i>P. australis</i>	Italy	Quadrone Wetland, Medicina, Bologna	25/07/2001	C. Lambertini	
Pa IT149 IT	<i>P. australis</i>	Italy	Quadrone Wetland, Medicina, Bologna	25/07/2001	C. Lambertini	
Pa 120 JP	<i>P. australis</i>	Japan	Okayama	01/06/1996	Toru Matoh	8x
Pj 276 JP	<i>P. japonicus</i>	Japan	Nabara-kyo, Hiroshima	15/09/1979	Nakano s.n.	
Pa H12 JP	<i>P. australis</i>	Japan	Kyoto pref., Mt. Hiei-Zan, Yamasiro	09/09/1931	M. Tagawa	
Pa 92 KW	<i>P. australis</i>	Kuwait	Ahmadi	01/01/1998	R. Clayton	4x
Pa 85 LI	<i>P. australis</i>	Lithuania	Silute	01/07/1997	E. Steffensen	4x
Pv 269 MA	<i>P. vallatoria</i>	Malaysia	Sabah, Tenom, E. of Agricultural station	23/09/2001	S. Laegaard s.n.	
Pv 270 MA	<i>P. vallatoria</i>	Malaysia	Sabah, Kinabatangan, E. of Sukau	13/03/2001	S. Laegaard s.n.	
Pa 301 NA	<i>P. australis</i>	Namibia	Swakop River	01/04/1999	N. Yensen	
Pa 142 NZ	<i>P. australis</i>	New Zealand	Tutaekuri River, Napier	01/04/1998	P. Champion et al.	8x
Pa H17 PE	<i>P. australis</i>	Peru	La Libertade, km 27 Picasmayo - Cajamarca	19/03/1988	S. Lægaard et al.	
Pa 78 PL	<i>P. australis</i>	Poland	Krakow	01/05/1998	Copernicus BG	4x
Pa 105 ZA	<i>P. australis</i>	Rep. S. Africa	Keurboom Estuary	01/03/1997	J. Bates	8x
Pm 106 ZA	<i>P. mauritiamus</i>	Rep. S. Africa	Reunion, Durban	01/03/1998	Sageran Naidoo	

Table 1. (Contd.)

Sample label	Species	Country	Locality	Herbarium specimen	Date	Collector	Ploidy
Pa 168 ZA	<i>P. australis</i>	Rep. S. Africa	Papkuilsfontein		01/06/1998	D. Willert	8x
Pa 188 ZA	<i>P. australis</i>	Rep. S. Africa	Loeriesfontein		01/09/1998	D. Willert	8x
Pa 195 ZA	<i>P. australis</i>	Rep. S. Africa	Brandvlei		01/09/1998	D. Willert	8x
Pa 311 ZA	<i>P. australis</i>	Rep. S. Africa	Kalkgat		01/06/1998	D. Willert	8x
Pm H3 ZA	<i>P. mauritanicus</i>	Rep. S. Africa	Transvaal, Ellisras	Louw 4205	29/05/1967	W.J. Louw	
Pa 81 RO	<i>P. australis</i>	Romania	Lake Oborny		01/04/1998	O. Clevering	6x
Pa 84 RO	<i>P. australis</i>	Romania	Lake Obretinu-Mare		01/04/1997	O. Clevering	8x
Pa 624 RO	<i>P. australis</i>	Romania	Lake Obretinu-Mare				8x
Pa 625 RO	<i>P. australis</i>	Romania	Lake Obretinu-Mare				6x
Pa 643 RO	<i>P. australis</i>	Romania	Lake Razim				4x
Pa 647 RO	<i>P. australis</i>	Romania	Lake Razim				6x
Pa 650 RO	<i>P. australis</i>	Romania	Lake Razim				6x
Pa 652 RO	<i>P. australis</i>	Romania	Lake Razim				8x
Pa 654 RO	<i>P. australis</i>	Romania	Lake Razim				8x
Pa 655 RO	<i>P. australis</i>	Romania	Lake Razim				4x
Pa 656 RO	<i>P. australis</i>	Romania	Lake Razim				6x
Pa 657 RO	<i>P. australis</i>	Romania	Lake Razim				4x
Pa 658 RO	<i>P. australis</i>	Romania	Lake Razim				6x
Pa 659 RO	<i>P. australis</i>	Romania	Lake Razim				8x
Pa 660 RO	<i>P. australis</i>	Romania	Lake Razim				12x
Pa 661 RO	<i>P. australis</i>	Romania	Lake Obretinu-Mare				8x
Pa 62 RU	<i>P. australis</i>	Russia	Beregovoe, Korsakovsky Distr., Sakhalin		01/10/1998	A. Taran	10x
Pa 107 RU	<i>P. australis</i>	Russia	Moscow B.G. (RAS)		01/04/1998	O. Tkachenko	4x
Pa 110 RU	<i>P. australis</i>	Russia	Okhotsk, Dolynsky Distr., Sakhalin		01/10/1998	A. Taran	8x
Pa 138 RU	<i>P. australis</i>	Russia	Nazyvaevsk, Omsk		01/06/1998	O. Tkachenko	4x
Pa 169 RU	<i>P. australis</i>	Russia	St.-Petersbourg		01/06/1998	O. Tkachenko	4x
Pj 177 RU	<i>P. japonicus</i>	Russia	Lazovsky Reserve, Primorsky kraj, Sakhalin		01/08/1998	A. Taran	
Pa 178 RU	<i>P. australis</i>	Russia	Yuzhno-Sakhalinsk B.G., Sakhalin		01/08/1998	A. Taran	8x
Pa 183 RU	<i>P. japonicus</i>	Russia	Petrova, Primorsky kraj, Sakhalin		01/08/1998	A. Taran	
Pa 201 RU	<i>P. australis</i>	Russia	Pugachevo, Makarovsky Distr., Sakhalin		01/10/1998	A. Taran	
Pa 205 RU	<i>P. australis</i>	Russia	Novikovo, Korsakovsky Distr., Sakhalin		01/10/1998	A. Taran	4x
Pa 212 RU	<i>P. australis</i>	Russia	Laguna Busse, Korsakovsky Distr., Sakhalin		01/12/1998	A. Taran	
Pa 213 RU	<i>P. australis</i>	Russia	Voskhod, Tymovsky Distr., Sakhalin		01/12/1998	A. Taran	
Pa 214 RU	<i>P. australis</i>	Russia	River Manuy, Dolynsky Distr., Sakhalin		01/12/1998	A. Taran	
Pa 215 RU	<i>P. australis</i>	Russia	Pokrovka, Nayba, Dolynsky Distr., Sakhalin		01/12/1998	A. Taran	

Pa 216 RU	<i>P. australis</i>	Russia	Zaozernaya, Sakhalin					
Pa 102 SN	<i>P. australis</i>	Senegal	Potte d'Oie, Dakar					4x
Pa H4 SN	<i>P. australis</i>	Senegal	Marigot de Khar., Saint Louise	Madsen 2726	01/04/1997	F. Ervik		
Pa H5 SN	<i>P. australis</i>	Senegal	Lac de Guer at K Momar Saw, Saint Louise	Lægaard 17908	17/11/1993	J.E. Madsen et al.		
Pa H6 SN	<i>P. australis</i>	Senegal	Delta du Sloum National Park, Sine Saloum	Lykke 624	04/10/1995	S. Lægaard et al.		
Pa H7 SN	<i>P. australis</i>	Senegal	Louge, Keur Momar Sarr	Sonko 225	10-21/10/91	A.M. Lykke et al.		
Pa H11 SN	<i>P. australis</i>	Senegal	Lac Rose (Lac Retba) at S end of lake, Cap Vert	Lægaard 17833	04/10/1995	I. Sonko et al.		
Pa 79 SL	<i>P. australis</i>	Slovenia	Zadnji kraj, Lake Cerknisko		26/09/1995	S. Lægaard et al.		
Pa 170 SL	<i>P. australis</i>	Slovenia	Gornje jezero, Lake Cerknisko		01/04/1998	O. Urbanc-Berèè		4x
Pa 171 SL	<i>P. australis</i>	Slovenia	Pond drasa, Ljubljana		01/03/1998	O. Urbanc-Berèè		4x
Pa 172 SL	<i>P. australis</i>	Slovenia	Veèna pot, Ljubljana		01/04/1998	O. Urbanc-Berèè		4x
Pa 52 ES	<i>P. australis</i>	Spain	el Garxal, Ebro		01/03/1998	Ljubljana B.G.		4x
Pa 72 ES	<i>P. australis</i>	Spain	Gallocanta N		01/04/1996	J. A. Romero		4x
Pa 74 ES	<i>P. australis</i>	Spain	l'Encanyissada, Ebro		01/07/1997	J. Lissner		4x
Pa 95 ES	<i>P. australis</i>	Spain	Gallocanta NW		01/04/1997	O. Clevering		4x
Pa 300 ES	<i>P. australis</i>	Spain	Mallorca, Alcudia		01/07/1996	F. Comin		4x
Pa 141 SE	<i>P. australis</i>	Sweden	Luleå		01/04/1997	O. Lillelund		4x
Pa 615 SE	<i>P. australis</i>	Sweden	Tåkern			O. Clevering		4x
Pa 637 SE	<i>P. australis</i>	Sweden	Gammelstaden					4x
Pv 59 TH	<i>P. vallatoria</i>	Thailand	Maerim, Chiang Mai		01/10/1998	K. Larsen		4x
Pv 266 TH	<i>P. vallatoria</i>	Thailand	Satau Province	Larsen 41160	05/11/1990	K. Larsen		
Pv 271 TH	<i>P. vallatoria</i>	Thailand	Khaochong, Trang prov.	Larsen 41607	18/11/1990	K. Larsen		
Pv 272 TH	<i>P. vallatoria</i>	Thailand	Chiang Mai	Lægaard 21777	21/10/2001	S. Laegard		
Pa 53 TN	<i>P. australis</i>	Tunisia	Ras Taguermes, Djerba		01/07/1998	J. Lissner		4x
Pa 97 TN	<i>P. australis</i>	Tunisia	Ras Taguermes, Djerba		01/03/1998	J. Lissner		4x
Pa 174 TN	<i>P. australis</i>	Tunisia	Chenini (Gabès)		01/03/1998	J. Lissner		4x
Pm 73 UG	<i>P. maur-itanus</i>	Uganda	Entebbe		01/10/1998	A. Katende		4x
Pm 93 UG	<i>P. maur-itanus</i>	Uganda	Kampola, Kinawataka wetland		01/09/2002	F. Kansime		
Pa 55 US	<i>P. australis</i>	United States	Bluestem Prairie, Minnesota		01/11/1998	G. Yalch et al.		4x
Pa 65 US	<i>P. australis</i>	United States	Dayton Wet Prairie, Michigan		01/04/1999	C. Clappitt		
Pa 69 US	<i>P. australis</i>	United States	Burton's Island, Delaware		01/03/1999	C. Linder		
Pa 71 US	<i>P. australis</i>	United States	Buffalo, Great Baehre, New York		01/10/1998	C. Helk		
Pa 86 US	<i>P. australis</i>	United States	Ives Road, Adrian, Michigan		01/11/1998	C. Clappitt		4x
Pa 99 US	<i>P. australis</i>	United States	Avon, Pea Island, North Carolina		01/08/1998	M. S. Ailstock		4x
Pa 100 US	<i>P. australis</i>	United States	Mobile, Alabama		01/04/1999	J. Lissner		

Table 1. (Contd.)

Sample label	Species	Country	Locality	Herbarium specimen	Date	Collector	Ploidy
Pa 101 US	<i>P. australis</i>	United States	Dauphin Island, Alabama		01/03/1999	L. Stanton	
Pa 108 US	<i>P. australis</i>	United States	Panama Beach, Florida		01/04/2000	J. Lissner	
Pa 109 US	<i>P. australis</i>	United States	Santa Rosa Island, Florida		01/03/1999	L. Stanton	
Pa 111 US	<i>P. australis</i>	United States	Green River, Utah		01/06/1998	K. Haven	4x
Pa 112 US	<i>P. australis</i>	United States	Roosevelt Inlet, Delaware		01/04/1998	C. Linder	
Pa 114 US	<i>P. australis</i>	United States	Maumee Bay, Ohio		01/04/1998	D. K. Fall	
Pa 116 US	<i>P. australis</i>	United States	Moses Lake, Washington		01/03/1998	R. Royston	4x
Pa 117 US	<i>P. australis</i>	United States	SFWCA2A, Florida		01/06/1997	A. Allen	6x
Pa 118 US	<i>P. australis</i>	United States	Houma, Louisiana		01/05/1997	I. Mendelssohn	4x
Pa 119 US	<i>P. australis</i>	United States	Cocodrie, Louisiana		01/07/1996	B. Lorenzen	6x
Pa 121 US	<i>P. australis</i>	United States	Grayton, Florida		01/04/2000	J. Lissner	
Pa 125 US	<i>P. australis</i>	United States	Weeks Island, Louisiana		01/10/1999	J. Lissner	
Pa 134 US	<i>P. australis</i>	United States	New Orleans, Louisiana		01/11/1999	J. Lissner	
Pa 135 US	<i>P. australis</i>	United States	Morgan City, Louisiana		01/11/1999	J. Lissner	
Pa 143 US	<i>P. australis</i>	United States	Charenton, Louisiana		01/10/1999	J. Lissner	
Pa 179 US	<i>P. australis</i>	United States	Dover, Delaware		01/04/1998	C. Linder	4x
Pa 180 US	<i>P. australis</i>	United States	Lums pond, Delaware		01/04/1998	C. Linder	4x
Pa 181 US	<i>P. australis</i>	United States	Odessa, Delaware		01/04/1998	C. Linder	4x
Pa 186 US	<i>P. australis</i>	United States	Oyster, Delmarva, Virginia		01/06/1998	L. K. Blum	4x
Pa 187 US	<i>P. australis</i>	United States	Parramore Island, Virginia		01/08/1998	B. Truitt	
Pa 189 US	<i>P. australis</i>	United States	Mutton Hunk, Virginia		01/08/1998	B. Truitt	4x
Pa 190 US	<i>P. australis</i>	United States	Buffalo, Orchard Park, New York		01/10/1998	C. Helk	4x
Pa 191 US	<i>P. australis</i>	United States	N. Wheatfield, Bear Ridge, New York		01/10/1998	C. Helk	4x
Pa 192 US	<i>P. australis</i>	United States	Swash Bay, Virginia		01/08/1998	B. Truitt	4x
Pa 193 US	<i>P. australis</i>	United States	Upshur Creek, Virginia		01/08/1998	B. Truitt	4x
Pa 194 US	<i>P. australis</i>	United States	Virginia Beach, Virginia		01/07/1998	J. L. Rudnický	4x
Pa 197 US	<i>P. australis</i>	United States	Buffalo, Depew, New York		01/10/1998	C. Helk	4x
Pa 199 US	<i>P. australis</i>	United States	Bedford, Boston, Massachusetts		01/09/1998	C. Steinfeld	4x
Pa 200 US	<i>P. australis</i>	United States	Silver Spring Lake, Rhode Island		01/10/1998	P. P. O'Hearn	4x
Pa 206 US	<i>P. australis</i>	United States	Milford, Connecticut		01/07/1998	R. Chambers	4x
Pa 210 US	<i>P. australis</i>	United States	Jamestown Island, Virginia		01/06/1998	K. Haven	
Pa 211 US	<i>P. australis</i>	United States	Pipestone, Minnesota		01/10/1999	J. Lissner	
Pa 224 US	<i>P. australis</i>	United States	Christian, Mississippi		01/04/2000	J. Lissner	
Pa 225 US	<i>P. australis</i>	United States	Big Bend, Rio Grande, Texas		01/03/1966	Renvoize	
Pm 267 ZM	<i>P. mauritianus</i>	Zambia	Mote area, Luapula distr.	Renvoize 5747			
Pm 268 ZM	<i>P. mauritianus</i>	Zambia	Luapula distr.	Renvoize 5612	15/02/1996	Renvoize	

ligation were performed in a Peltier Thermal Cycler PTC-200 (MJ Research), programmed for 4 h at 37°C followed by a 0.1°C/s decrease to 16°C in 2 h, and 10 min at 70°C. The extracted DNA was diluted 4 times prior to preamplification and selective amplification.

A total of 68 primer combinations were tested for ten samples. Of these combinations, three were selected for amplifications of the full set of samples: E-ACTcy + M-CTT, E-CAGcy + M-ATG and E-CGTcy + M-CAG. The other primer combinations yielded fragments so numerous that peaks were poorly separated and difficult to identify (homologize). Nucleotide adapters and primers used are listed in Table 2. For pre-amplification one-nucleotide selective primers were used. Pre-amplification and amplification were performed in a Peltier Thermal Cycler PTC-200 (MJ Research) programmed for 20 cycles, each with 30 s DNA denaturation at 94°C, 1 min primer annealing at 56°C and 1 min DNA extension at 72°C for pre-amplification. For amplification the program was: 94°C for 30 s, 65°C for 30 s decreased by 0.7°C/cycle for the subsequent 12 cycles and 72°C for 1 min, followed by 23 cycles at 94°C for 30 s, 56°C for 30 s and 72°C for 1 min.

Electrophoresis was run in an ALF Express II DNA Analysis System (Amersham Pharmacia Biotech) on a 7% acrylamide gel (Reprogel™ Long Read), at 1500V, 60mA, 25W, 55°C for 400 min with 50–500 bp external sizars.

Data analysis. The presence or absence of fragments was scored on the chromatograms generated with the ALFwin Fragment Analyser Software Package (Amersham Pharmacia Biotech). The fragments were aligned on the basis of a number of monomorphic peaks (peaks present in all specimens examined). Fragments of the same size were considered homologous. All fragments between 50 and 500 bp for primers E-CAGcy + M-ATG and E-ACTcy + M-CTT, and between 50 and 318 bp for primers E-CGTcy + M-CAG, were manually checked. Only polymorphic fragments were scored. A total of 128 polymorphic markers were found for the three primer combinations used.

The results obtained were included into a binary matrix that was analysed using PAUP* 4.0b10 (Phylogenetic Analysis Using Parsimony; Swofford 2002). The matrix, available from the corresponding author, was subjected to a heuristic

search with TBR and random addition sequence. Inclusion of what may be the closest relative of *Phragmites*, the genus *Molinia* Schrank (Hsiao et al. 1998, Grass Phylogeny Working Group 2001), was attempted, but the AFLP pattern turned out to be highly divergent, with a high proportion of fragments not found in *Phragmites*. In view of this, the homology of the few peaks shared between the genera was deemed highly uncertain, and *Molinia* was excluded from the analyses. Trees were instead arbitrarily rooted with *Phragmites vallatoria* as a functional outgroup. The tree search was aborted due to an excessive number of trees retrieved (overflow). One possible explanation for the large number of trees is that the number of taxa (238) vastly exceeds the number of characters (128), in which case full resolution cannot be expected even with perfect character-fit (zero homoplasy). This problem was addressed in two ways. A reduced data matrix, including 98 terminals and the same 128 characters, was analysed with parsimony in order to make a complete tree search (100 replicates with random addition sequence) possible. This reduced set of terminals was selected based on the fully sampled consensus tree, through exclusion of clones with unresolved positions in the *P. australis* core group. In addition, a neighbour-joining (NJ) analysis, based on Nei and Li's (1979) genetic distances, was performed on the complete dataset. To evaluate support for groups, a jackknife analysis was performed, using "fast heuristic search", with 10000 replicates and "emulate jac-resampling" as implemented in PAUP. The character deletion percentage chosen was 36.8, equal to e^{-1} , as was first suggested by Farris et al. (1996). When this fraction of characters is deleted, a jackknife support of $1 - e^{-1}$ (63.2%) corresponds to the expected frequency of a group supported by one uncontradicted synapomorphy (Farris et al. 1996). This relationship between support value and number of synapomorphies is an advantage of this method, compared to, e. g. bootstrap analysis.

Finally, the correlation between genetic distances (Nei and Li 1979) and geographic distance (in km) was tested at three different geographical levels, using the Mantel test (Mantel 1967) as implemented in the R Package version R 3.02 (Legendre and Vaudor 1991). In a first test, 168 samples (of a total of 173; five were excluded due to uncertain coordinates) in the almost cosmopolitan,

Fig. 1. Strict consensus tree of 70000 most parsimonious trees retained. Numbers on branches are jackknife percentages. Terminals are labelled with species abbreviation (Pa = *Phragmites australis*, Pf = *Phragmites frutescens*, Pj = *Phragmites japonicus*, Pm = *Phragmites mauritianus*, Pv = *Phragmites vallisneria*), sample number, country acronym (see table 1 for explanations) and ploidy level

Table 2. Nucleotide adapters and primers used for AFLPs

Nucleotide adapters	
EcoRI ST+	5' – CTCGTAGACTGCGTACC – 3'
EcoRI ST-	5' – AATTGGTACGCAGTCTAC – 3'
MseI ST+	5' – GACGATGAGTCCTGAG – 3'
MseI ST-	5' – TACTCAGGACTCAT – 3'
Primers	
E-ACTcy	5' – AGACTGCGTACCAATTCACT – 3'
M-CTT	5' – GATGAGTCCTGAGTAACTT – 3'
E-CAGcy	5' – GACTGCGTACCAATTCCAG – 3'
M-ATG	5' – GATGAGTCCTGAGTAACAG – 3'
E-CGTcy	5' – GACTGCGTACCAATTCCGT – 3'
M-CAG	5' – GATGAGTCCTGAGTAACAG – 3'

monophyletic but largely unresolved group of *P. australis*. This group comprises most samples from Europe, North America and Australia. This correlation was tested also for a subsample of 97 collections originating in continental Europe, and again for 46 of these samples collected in a restricted (< 50 km²) area in the Po Plain, Italy.

Results

The initial parsimony analysis, which included all 238 terminals, was aborted when 70000 most parsimonious trees (length = 2492, CI=0.05, RI=0.58) had been found. The consensus tree is shown in Fig. 1. The NJ tree based on all terminals is shown in Fig. 2. A second parsimony analysis, based on the reduced set of 98 terminals, yielded 456 trees (length = 1223, CI = 0.10, RI = 0.64). The consensus tree is shown in Fig. 3.

Even though the sample sizes differ considerably between the two parsimony analyses performed, the relationships between the major groups are almost identical in the resulting trees.

Phragmites vallisneria is a monophyletic group (jackknife support 79 in the MP trees). A specimen from Equatorial Guinea (Pv 273

EG, from the island of Fernando Póo) is the basal branch of the clade, which otherwise includes specimens from Thailand and Malaysia. In the NJ tree *P. vallisneria* still forms a cluster (jackknife support 93), split into two branches. The specimen from Equatorial Guinea is the basal branch of one of these.

Phragmites frutescens is closely related to a *P. mauritianus* specimen from Uganda (jackknife support 97 in reduced analysis and 98 in full analysis). *Phragmites mauritianus*, including *P. frutescens*, comprises three clades that form a basal grade. In the NJ tree, *P. mauritianus* (with *P. frutescens*) forms a cluster (jackknife support 99) nested within *P. australis*, with a South American cluster as the closest relatives.

The three included specimens of *P. japonicus* form a monophyletic group with weak jackknife support, nested inside *P. australis*.

Phragmites australis, including *P. japonicus*, is a monophyletic group. The two basal-most branches are a clade of clones from the US Gulf Coast and a South American clade. Of these, only the South American clade has jackknife support. In the NJ tree, the two groups cluster with *P. mauritianus*, separately from other *P. australis*.

Fig. 2. Neighbour-joining tree of the complete set of terminals, labelled as in Fig. 1. Numbers on branches are jackknife percentages. The scale bar corresponds to a genetic distance of 0.005 (Nei and Li 1979)

Phragmites australis, including *P. japonicus* but excluding the South American and Gulf Coast clades, form a monophyletic group, the “core-group”. A basal grade in this group is formed by a number of clones from Africa, the Middle East and the Mediterranean area. In the NJ tree, these clones form two clusters, one basal in the core-group, and another, including the South African octoploids, nested well inside *P. australis*.

Another near-basal group present in the fully sampled parsimony tree and in the NJ tree comprises samples from Central North America (and a single sample from a Russian stand of probable East Asian origin). In the reduced tree the position is more deeply nested inside *P. australis*. The Central North American group, excluding the Russian specimen, is strongly supported (jackknife support 91 in reduced analysis and 93 in full analysis; no support in NJ tree). The relationship to the Russian sample is also well supported (jackknife support 67, 69 and 100, respectively). The sister-group of this clade is, in all trees, *P. japonicus*.

A large clade that consistently appears, albeit with different position in different trees, comprises octo- and decaploid clones from Australia and tropical and temperate East Asia. Within this group, there is an almost perfect separation of Australian and Asian samples into two groups in all analyses performed. Basal to this clade are tetraploid samples from France and Israel, but this relationship is not seen in the NJ tree.

Most of the clones of the core-group belong to a largely unresolved group within which, apart from the aforementioned Central North American and Australian-Asian groups, there are no supported major clades. There are, however, several minor groups with restricted distribution that are consistently identified. There are thus one Spanish, one Danish and a few Italian clades. The vast

majority of samples in this largely unresolved part of the tree are European, but there are also a number of North American and a few Asian clones represented. In the NJ tree, most clones from the North American Atlantic coast cluster together. Fragments of this cluster are also found in the full parsimony tree.

Most of the samples for which chromosome number is known are tetraploid. The only cases where higher ploidy levels (6x, 8x, 10x and 12x) occur consistently within monophyletic groups are the abovementioned South African and Australian-Asian clades. Other occurrences of higher ploidy levels are scattered throughout the tree. Even octoploids from a limited geographical area, such as those from Romania, do not cluster together.

The Mantel test showed a significant positive correlation between genetic and geographic distance in the “subcosmopolitan” group ($r = 0.0557$, $P(r) = 0.0559$ with 1000 permutations, $P(r) = 0.0538$ with 5000 permutations, Mantel $t = 1.70$, $P(t) = 0.0442$), whereas values for continental Europe and the Po Plain were not significant (Europe $r = 0.00530$, $P(r) > 0.05$ with 1000 permutations, Mantel $t = 0.164$, $P(t) > 0.05$; Po Plain $r = 0.00530$, $P(r) > 0.05$ with 1000 permutations, Mantel $t = 0.161$, $P(t) > 0.05$).

Discussion

Genetic diversity. The present study focuses on major interrelationships and biogeography of the genus *Phragmites*, but it may be noted that AFLP studies are frequently performed for the purpose of quantifying and characterising genetic diversity. The results of such studies are presented in the form of a variety of measures and indices such as F-statistics (Weir and Cockerham 1984), gene diversity (Nei 1987) and genetic distance (Nei 1978). Commonly, variation levels within and between populations are compared (Excoffier et al. 1992). In the present study, few populations

Fig. 3. Strict consensus tree of 456 most parsimonious trees from a parsimony analysis of a subset of terminals, labelled as in Fig. 1. Numbers on branches are jackknife percentages

are represented by more than one sample, the only exception being a set of populations from the Po plain (Italy), which are the subject of a focused study (Lambertini et al. unpubl. data). Genetic diversity of different geographical regions is likewise difficult to compare due to highly varying sample size and distance between sampled stands. Therefore, it was not deemed meaningful to report measures and indices of genetic diversity or heterozygosity, as they would give a false impression of pertinence and accuracy.

A rough idea of the distribution of genetic diversity can be gained from comparison of branch lengths in Fig. 2. The small sample size for some of the species (and for certain subgroups of *P. australis*) makes it, however, difficult to compare genetic variability between species, particularly as the few samples available for species other than *P. australis* may not be representative of their infraspecific variation.

Rooting the *Phragmites* phylogeny. In the absence of the sister-group of *Phragmites*, which may be the genus *Molinia* Schrank (Hsiao et al. 1998, Grass Phylogeny Working Group 2001) or *Hakonechloa* Makino ex Honda (Mathews et al. 2000), the question of the rooting point cannot be addressed in the present study. The rooting shown in Figs. 1–3 is but one of numerous possibilities. In order to address the problem of the rooting point through inclusion of taxa outside of *Phragmites*, it would be necessary to study phylogenetic markers more conserved than the AFLP fragments analysed here. With this as one of the aims, DNA sequence variation in a number of loci in *Phragmites* and its closest relatives is being investigated (Gustafsson et al. unpubl. data).

Until a rooting point has been established with certainty, no firm conclusions can be drawn regarding polarity of morphological characters or the geographical origin of the genus. Some geographical patterns do emerge, however. Many clades are restricted geographically to continents or countries. One notable observation is that the tropical species

P. mauritanus and *vallatoria* are closely related, and that their closest relatives, in turn, are tropical and subtropical clades of *P. australis*. The largely unresolved core group of *P. australis* consists predominantly of samples from northern hemisphere temperate areas, with a few southern African and southern temperate specimens, most of which belong to clades of octoploids.

Support for species. *Phragmites japonicus* and *vallatoria* are the only strictly monophyletic species in the present results. *P. mauritanus* is paraphyletic in the most parsimonious trees, and the same is true for *P. australis* if *P. japonicus* is recognized. These results cannot, however, be considered as arguments for revision of the species classification, as the monophyly criterion for accepting taxonomic units is difficult to apply at (or below) the species level (Davis 1999).

Phragmites mauritanus. *Phragmites mauritanus* (including *P. frutescens*) appears as a paraphyletic grade with considerable genetic variation, as indicated by branch lengths. Concerning *P. frutescens*, it is notable that our single sample from Crete is very closely related to a *P. mauritanus* specimen from Uganda. The close genetic relationship between *P. frutescens* and *P. mauritanus* is supported also by morphological similarities between the two species (Scholz and Böhling 2000), in characters such as woody, perennial stems and short rigid leaves. It may well be that *P. frutescens* represents a group of isolated, perhaps relictual, populations of *P. mauritanus*. The very rare and erratic sexual reproduction observed in the Greek populations (Scholz and Böhling 2000), also is to be expected in temperate outpost populations of an otherwise tropical species.

Phragmites vallatoria. *Phragmites vallatoria* is only represented by a few collections, but geographically these span the entire Palaeotropics. Nevertheless, the species appears as a genetically distinct and well-supported unit, but with a fairly high level of infraspecific variation in its AFLP pattern. Morphologically, it stands close to *P. mauritanus*, and is

poorly differentiated from it in most characters (Clayton 1967).

***Phragmites japonicus*.** The three samples of *P. japonicus* (from Japan and Sakhalin) form a monophyletic group nested inside *P. australis*. Morphologically, it is a distinctive species (Tsvelev 1983). It can be speculated that it was derived from *P. australis* ancestors under geographical isolation in some parts of its partly insular distribution range. Presently it co-occurs with forms of *P. australis* in most of its area of distribution, perhaps the result of a later expansion.

***Phragmites australis*.** *Phragmites australis* is far better represented in the sample than the other species, a reflection not only of its virtually cosmopolitan distribution but also of its common occurrence in areas easily accessible to European and North American collectors. The density of the sampling varies widely between geographical areas, however, with Europe in particular being far better covered than the other continents. Within the species, a number of more or less well-supported groups can be distinguished. The majority of the samples belong, however, to a mostly unresolved complex within the core-group of the species. It is evident that the variation level within this unresolved group is relatively low, and the number of characters variable within the group may simply be too small for phylogenetic resolution, even if hierarchic structure actually exists. Another possible explanation for this poor resolution is that a largely reticulate relationship may exist between populations, even on a large geographical scale. Such a relationship could have resulted from extensive interbreeding, which is possible over large distances in an outbreeding and wind-pollinated species like *P. australis*. Long distance dispersal by wind is another potentially important factor in this regard. The lack of correlation between geographic and genetic distances, both at the local and subcontinental scales, provides further support for a large-scale reticulate model of relationships between *Phragmites* clones. When the perspective is widened even more, to include different conti-

nents, a relationship between genetic similarity and geographical distribution is indicated by a statistical correlation (Mantel test) and is sometimes evident in the phylogenetic trees, where many clades are restricted to continents. There is apparently a limit to the dispersal potential of *Phragmites*, and if intercontinental dispersal does occur, it is apparently not frequent enough to entirely obfuscate this pattern.

Another important factor in the variation and dispersal of *Phragmites*, and one that potentially complicates biogeographical interpretations, is the introduction by humans. The recent introduction of the Eurasian Haplotype M in North America via the Atlantic Coast has been thoroughly documented by Saltonstall (2002, 2003a, 2003b). The general lack of resolution in the *P. australis* core-group could be, at least in part, the result of “blurring” of biogeographical patterns through human-aided introductions. In order to assess more accurately the influence of such introductions in the phylogeographic pattern of *Phragmites*, it is important in further studies to consider details regarding the ecological context of the populations sampled, evaluating the likelihood that individual clones have been introduced from other regions. The potential for wind-dispersal over long distances into undisturbed habitat makes, however, such assessments difficult.

***Phragmites in the Americas*.** The distinctive Gulf Coast clade within *P. australis* is most probably identical to the group that Saltonstall (2003a) referred to as Haplotype I, and to the group identified by Pellegrin and Hauber (1999) from the same area. Saltonstall (2003a) included a very wide sample from North America, and some samples from tropical America and other continents. Two distinctive groups were found by this author in South America, one of which belonged to Haplotype I, as did the clones from the Gulf Coast area. Haplotype I was very distinctive in its RFLP pattern (Saltonstall 2003b), compared to other North American clones. In the present results the South American clones appear as a mono-

phyletic group that is not in a sister-group relationship with the Gulf Coast clade in the parsimony trees, but constitutes the subsequent branch in the grade at the base of *P. australis*. In the NJ tree the two cluster together with *P. mauritanus*, indicating a closer genetic similarity to this species than to *P. australis*. The interrelationships of the South American and Gulf coast clades are clearly in need of further investigation, including a much denser sampling from the geographical areas concerned. Such a wide sample, along with data on actual and potential interbreeding with the core group of *P. australis*, will also make it possible to address the issue of possible species status for the Gulf coast and South American *Phragmites* clades. *Phragmites berlandieri* was described from Mexico and the Gulf Coast area, and this may prove to be the correct name for the Gulf coast taxon. The same epithet has, however, been used at the varietal level (*Phragmites australis* [Cav.] Trin. Ex Steud. var. *berlandieri* [Fourn.] C.F. Reed) to distinguish native North American populations from other areas from introduced *P. australis* phenotypes (Robichaud and Catling 2003, Catling et al. 2004). To avoid confusion regarding the application of the name, Saltonstall et al. (2004) recently lectotypified *P. berlandieri*, and used this name at the varietal level. As lectotype was chosen a collection by Berlandier from the Gulf coast, showing morphological characteristics of the Gulf Coast *Phragmites* lineage. This lineage can be distinguished from other North American *Phragmites* by its smooth and shiny culms (Saltonstall et al. 2004).

What is here referred to as the “Central North American clade” most likely represents what Saltonstall (2003a) identified as the native North American lineage, and which was later formally described as *P. australis* ssp. *americanus* (Saltonstall et al. 2004). In the phylogenetic tree, it forms a distinctive clade in the *P. australis* core group. The non-native Haplotype M of Saltonstall is instead most

likely represented by the specimens from the North American Atlantic Coast scattered in the *P. australis* core group. A study based on RFLP (Saltonstall 2003b) provided no evidence of hybridization between native and non-native strains. Personal observations by the same author (2003a) of several populations along the Atlantic Coast also indicated that native populations may mature, flower and senesce earlier in the growing season than introduced populations, which could prevent interbreeding. Native and introduced *P. australis* haplotypes thus could be considered to represent distinct biological species. The reproductive separation is accompanied by numerous morphological differences (Saltonstall et al. 2004, <http://www.invasiveplants.net/phragmites/phrag/morph.htm>), which can be said to further support species status for the two groups.

According to our study, the Central North American specimens are genetically more related to *P. japonicus* than to other *P. australis*. In addition, at least one morphological character, the relatively long ligule, is shared by the two groups. Perhaps the Central North American group is better assigned to *P. japonicus*, but this issue cannot be addressed until a wider sample of this species has been examined.

Chromosome numbers. Unfortunately, knowledge of ploidy level for species other than *P. australis* is limited, and within *P. australis*, the knowledge in many geographical areas is poor. The available data are, however, quite detailed concerning Europe, where most of the specimens examined are tetraploid, exceptions being a mixed cytotype population (4x, 6x, 8x and 12x) from Romania, octoploids from Hungary, and one hexaploid from Greece. European clones with higher chromosome numbers do not group together. In contrast, *P. australis* groups from the Southern hemisphere are, as far as investigated, octoploid or higher: one group of octoploid specimens from different parts of South Africa and one clade from the Far East and Australia. This obviously means that higher ploidy levels have arisen many times independently and in

different areas of the distribution range of *P. australis*. The fact that the octoploid number occurs within large and diverse clades like that of the Far East and Australia, is an indication that the condition is relatively old and that it may have been a factor important in the dispersal and success of *P. australis* in these areas. It is further interesting to note that in many of the areas where octoploids occur they are sympatric with other *Phragmites* species. In South Africa *P. australis* co-occurs with *P. mauritanus*, in easternmost Russia *P. australis* co-occurs with *P. japonicus* and in the Far East and Australia *P. australis* co-occurs with *P. vallatoria*. A barrier against hybridisation, such as a difference in chromosome number, may explain the coexistence of the species as distinct units in these areas. Another possibility worth mentioning is that the octoploids are actually allopolyploids resulting from hybridisation between tetraploid *P. australis* and the other species. The phylogenetic trees provide no support for this hypothesis, however, as the octoploids are nested within *P. australis* rather than occupying more basal positions, as would be expected if a major part of their genome had been contributed by other *Phragmites* species.

Origin- and dispersal scenarios. Even though the problem of rooting the *Phragmites* phylogeny has not been satisfactorily solved, it is still worthwhile at this stage to present a geographical scenario for the origin and dispersal of the genus, based on the available data. Out of the numerous possibilities, it is interesting to consider a rooting point inside the variable and paraphyletic *P. mauritanus*. This would mean that African *P. mauritanus* would be effectively ancestral to the other species, *P. vallatoria* and the *P. australis-japonicus* clade being independently derived from it. Such a scenario, with an African origin for the genus, would also minimize the number of inferred dispersal events, given that the basalmost branch in *P. vallatoria* is African, and considering the large, predominantly African, basal grade in the core-group (particularly pronounced in the reduced analysis). This would hold also with *P. vallatoria* at

the root, as shown in Figs. 1 and 3. Such a model would furthermore imply two early dispersal events to the Americas for the ancestors of the South American and Gulf Coast clades. *P. vallatoria* would have originated in Africa and later dispersed to Asia. *P. australis-japonicus* may have originated in Africa and dispersed to Eurasia via the Mediterranean area, and further to North America through the North Pacific Coast, twice to East Asia and finally to Australia.

Another possibility, which has some support from preliminary results of studies of plastid DNA sequence variation (M. H. G. Gustafsson et al., unpublished), is an origin in temperate East Asia, a region with high diversity of *Phragmites* genotypes. Interestingly, this area is also home to the genus *Hakonechloa* Makino ex Honda, which has been identified as a possible sister-group of *Phragmites* in one molecular study (Mathews et al. 2000).

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