



Dated historical biogeography of the temperate Loliinae (Poaceae, Pooideae) grasses in the northern and southern hemispheres

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Abstract

Divergence times and biogeographical analyses have been conducted within the Loliinae, one of the largest subtribes of temperate grasses. New sequence data from representatives of the almost unexplored New World, New Zealand, and Eastern Asian centres were added to those of the panMediterranean region and used to reconstruct the phylogeny of the group and to calculate the times of lineage-splitting using Bayesian approaches. The traditional separation between broad-leaved and fine-leaved *Festuca* species was still maintained, though several new broad-leaved lineages fell within the fine-leaved clade or were placed in an unsupported intermediate position. A strong biogeographical signal was detected for several Asian–American, American, Neozeylandic, and Macaronesian clades with different affinities to both the broad and the fine-leaved *Festuca*. Bayesian estimates of divergence and dispersal–vicariance analyses indicate that the broad-leaved and fine-leaved Loliinae likely originated in the Miocene (13 My) in the panMediterranean–SW Asian region and then expanded towards C and E Asia from where they colonized the New World. Further expansions in America (10–3.8 My) showed a predominant migratory route from North to South (N America ↔ the Andes ↔ Patagonia). This late Tertiary scenario of successive colonizations and secondary polyploid radiations in the southern hemisphere from the northern hemisphere was accompanied by occasional transcontinental long-distance dispersal events between South America and New Zealand. Multiple Pliocene dispersal events (3.6–2.5 My) from the near SW European and NW African continents gave rise to the Macaronesian Loliinae flora, while a more recent Pleistocene origin (2–1 My) is hypothesized for the high polyploid lineages that successfully colonized newly deglaciated areas in both hemispheres.

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1. Introduction

The theory of evolution for most of the continental biotas has been largely based on vicariance and speciation caused by geographical or genetic barriers (Raven and Axelrod, 1972, 1974; Morrone and Crisci, 1995; Sanmartin

et al., 2001), in contrast to that of oceanic islands for which long-distance dispersals have been compulsory prior to local radiations (Wagner and Funk, 1995; Francisco-Ortega et al., 1996). However, recent biogeographical studies have demonstrated that transcontinental dispersals have been more common than previously thought and that those events have affected a large number of angiosperm lineages (Soreng, 1990; Vargas et al., 1998; Vijverberg et al., 1999; Coleman et al., 2003; Blattner, 2006). These biogeographical patterns seem to have occurred in the northern

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hemisphere where species relationships are more complex and highly reticulate (Wendel et al., 1995; Coleman et al., 2003) as well as in the southern hemisphere where the species appear more geographically structured (Baum et al., 1998; Sanmartin and Ronquist, 2004). Compiled data indicate that the overwhelming predominance of long-distance dispersals over vicariances detected in austral plants, contrary to those observed in animals, might have been caused by the accumulation of those recent events in terminal taxa that could have obscured a deeper vicariance signal or just correspond to young-age lineages (Sanmartin and Ronquist, 2004). The biogeography of any angiosperm group should therefore be shaped within a timescale frame, allowing one to unravel the overlapping histories of those groups which commonly spanned the Tertiary and the Quaternary ages (Wikström et al., 2001; Bell et al., 2005).

Subtribe Loliinae, one of the largest groups of temperate grasses, is presently distributed in all continents except Antarctica (Catalán, 2006). The largest genus of the Loliinae, *Festuca*, encompasses more than 500 species which are widespread in the holarctic region but also inhabit cool and temperate areas in the southern hemisphere. Species of *Festuca* grow in a large variety of different habitats, from wetlands to xeric ecosystems, and are especially well adapted to extreme conditions in mountains, and arctic and subantarctic areas. Based on its large taxonomic diversity and wide geographical and ecological amplitude, different authors have speculated on the putative ancient origin of *Festuca* and postulated an evolutionary trend from more basal broad-leaved lineages to more advanced fine-leaved lineages (Hackel, 1882; Holub, 1984; Tzvelev, 1971). However, no attempt has been made to estimate the ages of those lineages.

Species of *Festuca* show an intricate history of past and recent hybridization and polyploidization (Jauhar, 1975; Catalán, 2006, and references therein), including intergeneric crosses with other Loliinae (Ainscough et al., 1986; Jauhar, 1993), that might obscure the recovery of divergence ages and geographical scenarios. Nonetheless, the recovered phylogenies have mostly shown a congruent systematics-based resolution (Catalán et al., 2004; Catalán, 2006). Our study aims at analyzing the biogeography of Loliinae, one of the largest and most ecologically and economically important groups of temperate grasses, within a temporal and geological framework. Loliinae have been traditionally considered to be of very recent, possibly late Miocene origin (Charvet et al., 1997). However, fossil studies (Linder, 1987; Jacobs et al., 1999; Prasad et al., 2005) support the past existence of open-habitat Pooideae-like grasses in the boundaries between the Mesozoic and the Cenozoic. Properly dated historical scenarios need to be reconstructed for the Loliinae that are based on more accurate nodal calibrations of the grass family. To comply with this objective, we have added to our previous Loliinae data set (Catalán et al., 2004) a large number of ITS and *trnLF* sequences of these grasses from the New World, a secondary centre of speciation of Loliinae, and from New Zealand and E Asia.

2. Material and methods

2.1. DNA sampling and sequencing

Our sampling scheme was designed to cover those taxonomic and geographic Loliinae groups that were underrepresented in previous analyses, especially from the New World and the southern hemisphere, and to build on previous studies (Torrecilla et al., 2004; Catalán et al., 2004). Seventy-eight taxa from North and South America (43), New Zealand (9), Eastern Asia (4) and the panMediterranean region (22) were incorporated in the study totaling 176 taxa in the present data set. Some taxa were represented by more than one sample (totalling 186 samples). Sampling was enlarged with 10 representatives of the closely related subtribes Parapholiinae/Cynosurinae (8) and Dactylidinae (2), and with 10 representatives of the more distant subtribes Poinae plus Puccinellinae (2) and tribes Aveneae (5), Seslerieae (1), Triticeae (1) and Brachypodieae (1) as outgroups. Our selection of Loliinae included 130 *Festuca* taxa corresponding to the seven major subgenera (out of 11; c. 70% of the total) and 22 sections, 14 *Vulpia* taxa corresponding to the five sections recognized in this genus, four taxa of *Lolium*, and eight taxa of other Loliinae genera (see Supplementary data in Table 1). Both fresh and herbarium materials were collected for this study, and 119 samples analyzed in our previous surveys (Catalán et al., 2004) were incorporated and used in the analysis. Systematic emendations followed those indicated in Catalán et al. (2007), Müller and Catalán (2006) and Catalán (2006) though several New World and New Zealand taxa have not been realigned.

DNA isolation and sequencing of the nuclear ribosomal ITS region, which included the ITS1 spacer, the 5.8S gene, and the ITS2 spacer, and of the plastid *trnTL* and *trnLF* regions, which included the *trnTL* spacer and the *trnL* intron, and the *trnLF* spacer, respectively, followed the procedures indicated in Catalán et al. (2004). One hundred and sixty three new *trnTL*, 70 new *trnLF* and 60 new ITS sequences have been deposited in GenBank. ITS and *trnLF* sequences of nine and seven taxa, respectively, were retrieved from GenBank and incorporated into the phylogenetic analysis (Table 1). The final data matrices consisted of 176 ITS sequences, 178 *trnTF* sequences, and 183 *trnLF* sequences. A concatenated plastid data matrix of 188 samples was constructed; 171 samples included both *trnTF* and *trnLF*, whereas 17 included only one of the two regions. A combined *trnTL*/ITS data set was created by union of the concatenated plastid *trnTL* region and the ITS region for 157 samples for which both sequences were available. Sequences were first aligned with Clustal X (Thompson et al., 1994) and then adjusted manually; the boundaries of each sequenced region correspond to those indicated in Catalán et al. (2004). Informative gaps were coded as binary data, incorporated in their respective data matrices, and used for parsimony-based analyses.

Table 1
List of taxa included in the phylogenetic study of Loliinae

Taxon (ploidy level)	Source	GenBank Accession No. ITS	<i>trnL-F</i>	<i>trnT-L</i>
Poeae Dumort.				
Loliinae Dumort.				
<i>Festuca</i> L.				
Subgen. <i>Festuca</i>				
Sect. <i>Festuca</i>				
Subsect. <i>Festuca</i> (“ <i>F. ovina</i> complex”)				
<i>Festuca alpina</i> Suter (2x)	Spain Huesca: Pyrénées: Vallibierna; Catalan P.2002 UZ	AF303415	AF478522	EF585001
<i>Festuca anethystina</i> L. (2x, 4x)	Germany: Bayern; Müller J. 6966	AF584919	EF592950	EF585004
<i>Festuca aragonensis</i> (Willk) Fuente & Ortúñez (2x) 1	Spain: Zaragoza: Moncayo; JALR 01174(A), UZ	AF519975	AF495884	EF585010
<i>Festuca aragonensis</i> (Willk) Fuente & Ortúñez (2x) 2	Spain: Zaragoza Moncayo; PC-17.2002, UZ	—	AF533062	—
<i>Festuca brachyphylla</i> Schult. & Schult. f. (6x)	USA: Alaska: North Slope Borough: Prudhoe Bay; Soreng R.J. 6243 US	EF584927	—	EF585016
<i>Festuca brevissima</i> Jurtzev (2x)	USA: Alaska: Denali Borough: Alaska Range; Soreng R.J. 6021 US	EF584928	EF592961	EF585018
<i>Festuca calligera</i> (Piper) Rydb. (4x)*	USA: New Mexico: Sangre Cristo Mts; Allred K. 8262	EF584929	EF592962	EF585021
<i>Festuca clementei</i> Boiss. (2x)	Spain: Granada: Sierra Nevada: Veleta; Catalán P & al., UZ-881.2000	AF478482	AF478524	EF585025
<i>Festuca edlundiae</i> S. G. Aiken, Consaul & Lefk. (4x)*	Canada: Nunavut: Báthurst island: Polar Bear Pass; Gillespie L.J., Consaul L.L. 6990	EF584936	EF592969	EF585036
<i>Festuca frigida</i> (Hack.) K. Richt. (2x)	Spain: Granada: Sierra Nevada: Laguna de Aguas Verdes; Catalán P. & al. UZ-80.2000	AF478481	AF478521	EF585043
<i>Festuca glacialis</i> Miègev. ex Anon. (2x)	Spain: Huesca: Pyrenees: Cotiella; Catalan P. 2002	AF303428	AF478523	EF585045
<i>Festuca guestfalica</i> Boenn. ex Reichenb. (4x)	Germany: Nordrhein-Westfalen; Müller J. 6857	EF584941	EF5929741	—
<i>Festuca halleri</i> All. subsp. <i>halleri</i> (2x)	Switzerland: Valais; Müller J. 8032	EF584942	EF592975	EF585047
<i>Festuca hyperborea</i> Holmen ex Frederiksen (4x)	Canada, Northwest Territories, Prince Patrick Island; Gillespie L.J., Consaul L.L. 6893	EF584946	EF592978	EF585050
<i>Festuca hystrix</i> Boiss. (2x)*	Spain: Almería: Sierra de Gádor; Catalán P. & al. UZ- 31.2000	AF478480	AF478520	EF585051
<i>Festuca idahoensis</i> Elmer (4x)*	USA: Oregon: Deschutes County; Wilson B. 533	EF584947	AF533064	EF585053
<i>Festuca indigesta</i> Boiss. (6x)	Spain: Granada: Sierra Nevada; Catalán P. & al. UZ-43.2000	—	AF478519	EF585054
<i>Festuca intercedens</i> (Hackel) Lüdi ex Becherer (4x)	Switzerland: Valais: Müller J. 7987	EF584948	EF592979	EF585055
<i>Festuca laevigata</i> Gaudin (8x)	Italy: Lombardia. Müller J. 8267	EF584950	EF592981	EF585059
<i>Festuca longiauriculata</i> Fuerte, Ortúñez et Ferrero (2x)	Spain: Almería: Sierra de los Filabres: Calar Alto; Catalán P. & al., UZ-59.2000	AF478479	AF478518	EF585062
<i>Festuca norica</i> (Hack.) K. Richt. (2x, 4x, 6x)	Italy: Trentino-Alto Adige; Müller J. 8422	EF584955	EF592987	EF585072
<i>Festuca ovina</i> L. (2x)*	Germany Thüringen: Saale-Holzland-Kreis; Müller J. 6879	AF532959	AF533063	EF585076
<i>Festuca pallens</i> Host (2x, 4x)	Germany: Bayern; Müller J. 6973	EF584958	EF592990	—
<i>Festuca plicata</i> Hack. (2x)	Spain: Granada: Sierra Nevada: Dornajo; Catalán P. & al., UZ-86.2000	AF478483	AF478525	EF585082
<i>Festuca saximontana</i> Rydb. (6x)	USA: Oregon, Soreng R. J. 6021-1	EF584969	EF593002	EF585098
<i>Festuca stricta</i> Host subsp. <i>trachyphylla</i> (Hack.) Patzke ex Pils (6x)	Germany: Bayern; Müller J. 7612	EF584972	—	—
<i>Festuca valesiaca</i> Schleider ex Gaudin subsp. <i>valesiaca</i> (2x)	Germany: Thüringen; Müller J. 6939	EF584978	EF593011	EF585112
<i>Festuca viviparoides</i> Krajina ex Pavlick (4x, 7x, 8x, 9x)	Canada Northwest Territories, Banks Island: Nelson Head; Gillespie L.J. & al. L 7204	EF5854980	EF593013	EF585114
Subsect. <i>Exaratae</i> St-Yves				
<i>Festuca borderei</i> (Hack.) K. Richt. (2x)	Spain: Huesca: Pyrenees: Vallibierna; Catalán P., 2000, UZ	AF303403	AF478510	EF585015
<i>Festuca capillifolia</i> L. Dufour (2x)*	Spain: Jaen: Cazorla; Cebollada, Rivas-Ponce M. A.	AF303419	AF478511	EF585022
<i>Festuca pyrenaica</i> Reut. (4x)	Spain. Huesca: Pyrenees: Cotiella; Catalán P., 2000	AF303423	AF478517	EF585088
<i>Festuca querana</i> Litard.(4x)	Spain: Lugo: Monforte de Lemos; López-Rodríguez J.A. 1326 UZ	AF532957	AF533057	EF585090
<i>Festuca rifana</i> Litard. & Maire (unknown)	Morocco: Al-Hoceima: 7 Azila, Koudinat Tighighime; Silvestre & Mejías 178/93 SEV-148594	-	EF592998	EF585092
<i>Festuca violacea</i> Gaudin subsp. <i>violacea</i> (2x)*	Switzerland: Bern; Müller J. 7907	EF584979	EF593012	EF585113
Sect. <i>Aulaxyper</i> Dumort. (“ <i>F. rubra</i> complex”)				
<i>Festuca agustinii</i> Linding. (2x) 1*	Spain: Canarias: Tenerife: Anaga: Bailadero; A.Santos	AY099005	AY099003	EF584999

Table 1 (continued)

Taxon (ploidy level)	Source	GenBank Accession No. ITS	trnL-F	trnT-L
<i>Festuca agustinii</i> Linding. (2x) 2	Spain: Canarias: La Palma: Bco. Los Tilos; Catalán P., 2002, UZ	EF584918	EF592949	EF585000
<i>Festuca ampla</i> Hack. (2x, 4x, 6x) 1	Spain: Cádiz: Grazalema; JARL, 1326, UZ	—	EF592951	EF585005
<i>Festuca ampla</i> Hack. (2x, 4x, 6x) 2	Spain: Huelva: Hinojos-Las Palomas; Talavera & Berjano s.n.	EF584920	EF592952	EF585006
<i>Festuca ampla</i> Hack. (2x, 4x, 6x) 3	Spain: Ávila: La Adrada; Montserrat & Rebollar s.n. JACA-56484	EF584921	EF592953	EF585007
<i>Festuca andicola</i> Kunth (unknown)*	Ecuador: Loja Saraguro; Stančík D. 3780 US-3428937	EF584922	EF592955	EF585009
<i>Festuca coxii</i> (Petrie) Hack. (8x)*	New Zealand: Chatham island: The Pinnacles; Lloyd K.M. 57956	AY524825**	AY528937**	EF585028
<i>Festuca francoi</i> F.Prieto, C.Aguiar, E.Dias & M.I.Gut. (2x)*	Portugal: Açores: Terceira: Caldeira de St. Bárbara; Sequeira M. 4403	EF584939	EF592972	EF585042
<i>Festuca heterophylla</i> Lam. (4x) 1	Germany: Bayern: Kreis Kelheim; Müller J. 7600	AF532958	—	—
<i>Festuca heterophylla</i> Lam. (4x) 2	France: Pyrénées-Orientales Forêt de Boucheville; Montserrat & al. s.n. JACA-152778	EF584944	—	EF585049
<i>Festuca iberica</i> (Hack.) K. Richt. (6x)*	Spain: Granada: Sierra Nevada: Borreguiles de S. Juan; Catalán P. & al., UZ-77.2000	AY118087	AF478516	EF585052
<i>Festuca jubata</i> Lowe (2x)*	Portugal: Madeira: Pico das Torres; Sequeira & Catalan s.n.	EF584949	EF592980	EF585056
<i>Festuca jumcifolia</i> Chaub. (8x)	Spain: Lugo Viveiro: Briero: Arenales de Area; López-Rodríguez J.A. 1366, UZ	AF478478	AF478515	EF585057
<i>Festuca luciarum</i> Connor (unknown)*	New Zealand: Maungaharuru Range: Hawkes Bay; Lloyd K.M. 57621	AY524828**	AY528939**	EF585064
<i>Festuca multinodis</i> Petrie & Hack.(8x)	New Zealand: Wellington: Baring Head; Lloyd K.M. 57958	AY524827**	AY528940**	EF585069
<i>Festuca nevadensis</i> (Hack.) Markgr.-Dann. (10x)*	Spain: Granada: Sierra Nevada: Dornajo; Catalán P. & al., UZ-69.2000	AF478477	AF478514	EF585071
<i>Festuca petraea</i> Guthnick ex Seub. (2x) 1*	Portugal: Açores: St. Maria, Maia; Sequeira M. 4393	EF584961	EF592993	EF585080
<i>Festuca petraea</i> Guthnick ex Seub. (2x) 2	Portugal: Açores: Flores, St. Cruz; Sequeira M. 4482	EF584962	EF592994	EF585081
<i>Festuca rivularis</i> Boiss. (2x) 1*	Spain: Granada: Sierra Nevada: Borreguiles de S. Juan; Catalán P. & al., UZ-78.2000	AF478475	AF478512	EF585093
<i>Festuca rivularis</i> Boiss. (2x) 2	Spain: Huesca Sallent de Gállego: El Petruso; Catalan P. & Müller J. UZ18.04	EF584967	EF593000	EF585094
<i>Festuca rothmaleri</i> (Litard.) Markgr.-Dann. (8x)	Spain: Madrid: Lozoya; López Rodríguez J.A. 1227B, UZ	AF478476	AF478513	EF585095
<i>Festuca rubra</i> L. (6x) 1	Romania; (cultivar at LEI) Stace C.A.	AF303422	AY118098	—
<i>Festuca rubra</i> L. (6x, 8x) 2*	Switzerland: Valais: Desses SSE Ferret; Müller J. 8060	AY118088	AY118099	EF585096
<i>Festuca rubra</i> L. (6x) 3	Finland: A.Kosonen (Hb.Univ.Oulu) JACA JA-474496	EF584968	EF593001	EF585097
<i>Festuca rubra</i> L. subsp. <i>arctica</i> (Hack.) Govor. (6x) 1	USA: Alaska: Denali N. Park: N side of Churchill Range of Alaska Range; Soreng, R.J. 6111 US	EF584965	EF592997	EF585091
<i>Festuca rubra</i> subsp. <i>arctica</i> (Hack.) Govor. (6x) 2	Canada: Northwest territories Banks island; Gillespie LJ, Bickerton H, Consaul L 7282-1	—	EF592956	EF585011
<i>Festuca rubra</i> subsp. <i>pruinosa</i> (Hack.) Piper	Spain: Pontevedra: Cangas de Morrazo, Donon; Sauquillo & Pimentel	EF584963	EF592995	EF585083
<i>Festuca ultramafica</i> Connor (unknown) Sect. <i>Eskia</i> Willk.	New Zealand: Nelson, Windy Point; Lloyd K.M. 57629	AY524826**	AY528938**	EF585110
<i>Festuca burnatii</i> St.-Yves (2x)	Spain: Cantabria: Picos de Europa; Catalán P. & Torrecilla P., UZ-44.2001	AY099007	AY099002	EF585019
<i>Festuca elegans</i> Boiss. (2x, 4x)	Spain. Granada: Baza; Cebolla & Rivas-Martinez s.n., UAM	AF303406	AF478509	EF585038
<i>Festuca eskia</i> Ramond ex DC. (2x)*	Spain: Huesca: Pyrenees: Benasque; Catalán P., Mirones V. UZ	AF303412	AF478508	EF585040
<i>Festuca gautieri</i> (Hack.) K. Richt. (2x)	Spain: Girona: Pyrenees: Nuria; Catalán P., Mirones V. UZ	AF303414	AF478507	—
<i>Festuca gautieri</i> (Hack.) K. Richt. (2x)	Spain: Huesca: Srra. Guara, P.Catalán s.n.	—	—	EF585044
<i>Festuca quadriflora</i> Honck. (2x, 4x)	France: Pyrénées: Col de Baroude; Catalán P.2000 UZ	AF303413	AF478506	EF585089
<i>Festuca scabricalmis</i> (Hackel) K. Richt. subsp. <i>luedii</i> Markgraf.-Dann. (6x) Sect. <i>Dimorphae</i> Joch. Müll. & Catalán	Italy: Lombardia; Müller J. 8224	EF584970	EF593003	EF585099
<i>Festuca dimorpha</i> Guss. (2x, 4x)*	France: Alpes de Haute-Provence Col des Champs; Korneck D. s.n, Herb. Müller J. 10969	AF519982	AF519987	EF585032
<i>Festuca pseudolaxa</i> Schur (syn. <i>F. carpatica</i> F. Dietr.) (4x)	Slovak Republic: Vysoké Tatry Mts.: Tisovnice; Marhold K. s.n.	AY099006	AY099001	EF585023

(continued on next page)

Table 1 (continued)

Taxon (ploidy level)	Source	GenBank Accession No. ITS	<i>trnL-F</i>	<i>trnT-L</i>
Sect. <i>Amphigenes</i> (Janka) Tzvel. <i>Festuca pulchella</i> Schrader subsp. <i>pulchella</i> (2x)	Switzerland: Bern; Müller J. 7807 UZ	AF519980	AF519985	EF585086
<i>Festuca pulchella</i> subsp. <i>jurana</i> (Gren.) Markgr.-Dann. (2x)	Italy: Trento: Passo di Sella N Canazei; Müller J. 8421, UZ	AF519981	AF519986	EF585085
Sect. <i>Scariosae</i> Hack. <i>Festuca scariosa</i> (Lag.) Asch. & Graebn. (2x)*	Spain: Almería Serón: Sierra Filabres: Las Menas; Catalán P. & al., UZ- 62.2000	AF519978	AY098999	EF585100
Sect. <i>Pseudoscariosa</i> Krivot <i>Festuca pseudeskia</i> Boiss. (2x)	Spain: Granada: Sierra Nevada: Collado del Diablo, Catalán P. & al., UZ-73.2000	AF519979	AY099000	EF585084
Sect. <i>Lojaconaa</i> Catalán & Joch. Müll. <i>Festuca coerulescens</i> Desf. (2x)*	Spain: Cádiz: Jerez de la Frontera; Catalán P. & al., UZ-91.2000	AF538363	AF533051	EF585027
<i>Festuca patula</i> Desf. (syn. <i>F. triflora</i> Desf.) (2x)*	Spain: Cádiz Grazalema: Bco. Ballesteros; Catalán P. & al., UZ 95.2000	AF538362	AF533052	EF585109
Sect. <i>Subbulbosae</i> Nyman ex Hack. <i>Festuca baetica</i> (Hack.) K. Richt. (2x)	Spain: Cádiz: Sierra de la Palma; Cebolla & Rivas-Ponce, UAM	AF303405	AF533049	—
<i>Festuca durandoi</i> Clouston (4x)	Spain: Segovia: Riaza; López Rodríguez J.A. 6-6-00, UZ	AF543514	AF533047	EF585035
<i>Festuca paniculata</i> (L.) Schinz subsp. <i>paniculata</i> (2x)*	France: Mont Aigoual; Cebolla & Rivas-Ponce, UAM	AF303407	AF533046	EF585077
<i>Festuca paniculata</i> subsp. <i>spadicea</i> (L.) Litard. (6x)	Spain: Lugo: Folgoso do Caurel; López Rodríguez J.A. 1346, UZ	EF584971	AF533048	EF585101
Subgen. <i>Drymanthele</i> V. I. Krecz. & Bobrov Sect. <i>Banksia</i> E.B. Alexeev <i>Festuca purpurascens</i> Banks & Sol. ex Hook. f. (6x)*	Argentina: Santa Cruz: Lago Argentino; Peterson P.M. 17147	EF584964	EF592996	EF585087
Sect. <i>Muticae</i> S.L. Lu <i>Festuca modesta</i> Nees ex Steud. (2x)*	China: Yunnan: Fugong (Bijiang); Soreng R.J.& al. 5227 US-3420887	EF584953	EF592985	EF585068
Sect. <i>Phaeochloa</i> <i>Festuca altissima</i> All. (2x)	France: Pyrenees: Aspe; Catalán P. s.n.	AF303411	AF478505	EF585003
<i>Festuca donax</i> Lowe (2x)*	Portugal: Madeira: Porto Moniz; Catalán & Sequeira MS4515	EF584935	EF592968	EF585033
<i>Festuca drymeja</i> Mert & Koch (2x)*	Hungary: Balaton; Stace C.A.	AF303425	AY098997	EF585034
<i>Festuca lasto</i> Boiss. (2x)*	Spain: Cádiz: Sierra Bermeja; Stace C.A.	AF303418	AY098998	EF585060
Sect. <i>Ruprechtia</i> E.B. Alexeev <i>Festuca amplissima</i> Rupr. (4x, one count 6x)	Mexico: Chihuahua: Barranca del Cobre; Peterson P.M., Catalán P. 17573	—	EF592954	EF585008
Subgen. <i>Subulatae</i> (Tzvel.) Alexeev Sect. <i>Glabricarpae</i> E.B. Alexeev <i>Festuca breviglumis</i> Swallen (unknown)	Mexico: Jalisco: Ciudad Guzman; Peterson P.M., Rosales O. 16078.	—	EF592960	EF585017
Sect. <i>Longiglumis</i> S. L. Lu <i>Festuca elata</i> Keng ex E. B. Alexeev (unknown)*	China: Yunnan, Lushui, Gaoligong Shan; Soreng R.J.& al. 5268, US-3420890	EF584937	EF592970	EF585037
Sect. <i>Subulatae</i> Tzvelev <i>Festuca cochabambana</i> E. B. Alexeev (unknown)	Bolivia: Cochabamba: Prov. Chapare; Müller J. 9277	EF584931	EF592964	EF585026
<i>Festuca cuzcoensis</i> Stančík & P. M. Peterson (unknown)*	Peru: Cuzco: Calca; Peterson P.M. & Refulgio-Rodríguez 16582	EF584932	EF592966	EF585029
<i>Festuca flacca</i> Hack. ex E. B. Alexeev (unknown)*	Ecuador: Pichincha Amaguana: Volcano Pasochoa; Stančík D., US-3428939	EF584938	EF592971	EF585041
<i>Festuca parodiana</i> (St.-Yves) Nicora	Bolivia: Tarija: Prov. O'Connor; (cult., Jena Univ.), Müller J. 9261	EF584959	EF592991	EF585078
<i>Festuca parvigluma</i> Steud.	China: Xizhang (Tibet): Miling; Soreng R.J. & al.5576, US-3420901	EF584960	EF592992	EF585079
<i>Festuca subulata</i> Trin. (2x, 4x)*	USA: Oregon: Clatsop County: Saddle Mnts; Wilson B. 10512	AF532953	AF533056	EF585104
<i>Festuca ulochaeta</i> Nees ex Steud. (6x)	Venezuela: Mérida: Tabay: laguna de Coromoto; Stančík D. 4179, US-3428928	—	EF933009	—

Table 1 (continued)

Taxon (ploidy level)	Source	GenBank Accession No. ITS	<i>trnL-F</i>	<i>trnT-L</i>
Subgen. <i>Subuliflorae</i> E.B. Alexeev Sect. <i>Subuliflorae</i> (Alexeev) Darbyshire <i>Festuca subuliflora</i> Scribn. (4x)	USA: Oregon; Wilson B. s.n.	—	EF593005	EF585105
Subgen. <i>Leucopoa</i> (Griseb.) Hack. Sect. <i>Breviaristatae</i> Kriv <i>Festuca altaica</i> Trin. (4x)*	Canada: Yukon Territory: Teslin Lake; Soreng R.J. 5996	AF532952	AF533055	EF585002
<i>Festuca californica</i> Vasey (4x, 8x)*	USA: Oregon: Benton County; Wilson B. 7014	AF532956	AF533054	EF585020
<i>Festuca thurberi</i> Vasey (4x, 6x)*	USA: New Mexico: Sangre Cristo Mts; Allred K. 8257	EF584975	EF593007	EF585107
Sect. <i>Leucopoa</i> (Griseb.) Krivot. <i>Festuca kingii</i> (S. Watson) Cassidy (8x)*	USA: Colorado: Boulder Co: Flat Irons; Catalán P. 1.93, UZ	AF303410	AY099004	EF585058
<i>Festuca spectabilis</i> Jan (6x)*	Italy: Lombardia: Bergamo: Passo della Presolana; Müller J. 8229	AF519977	AF519984	EF585102
Sect. <i>Obtusae</i> (E. Alexeev) E.B. Alexeev <i>Festuca subverticillata</i> (Pers.) E. B. Alexeev (6x)	USA: West Virginia: Grant Co.; Peterson P.M., Saarela J.M. 15784, US	EF584974	EF593006	EF585106
Subgen. <i>Schedonorus</i> (P.Beauv) Peterm. Sect. <i>Plantymia</i> (Dum.) Tzvelev <i>Festuca gigantea</i> (L.) Vill. (6x)	Spain: Navarra: Arce; Aizpuru & Catalán, UZ	AF303416	AF533043	EF379003
Sect. <i>Schedonorus</i> (P. Beauv.) Koch <i>Festuca apennina</i> De Not. (4x)	Switzerland: Valais: Gletsch: (N Oberwald); Müller J. 7965, UZ	AF548028	AF533041	EF378991
<i>Festuca arundinacea</i> Schreb. subsp. <i>arundinacea</i> (6x)*	Spain: Lugo: Lánacara: Santa Bárbara; López Rodríguez J.A. 1081, UZ	AF519976	AY098995	DQ367405
<i>Festuca arundinacea</i> var. <i>atlantigena</i> (St.-Yves) Auquier (8x)	(Charmet et al., 1997, only ITS)/Belgium cultivated seed ABY-BN 1304	AJ240155**	EF378956	EF378996
<i>Festuca arundinacea</i> var. <i>letourneuxiana</i> (St.-Yves) Torrecilla & Catalan (10x)*	Great Britain: Cultivated seed ABY-BN 400.1967U	EF379056	EF378960	EF378998
<i>Festuca fenas</i> Lag. (4x)	Spain: Segovia: Condado de Castilnovo; López Rodríguez J.A. s.n., UZ	AF532951	AF533042	EF379000
<i>Festuca fontqueri</i> St.-Yves (2x)	Morocco: Rif Mountains; Mejias J.	AF303404	AF533044	DQ631486
<i>Festuca mairei</i> St.-Yves (4x)*	Morocco: Marrakech: Oukaimeden; Cebolla & Rivas-Ponce 4064, UAM	AF303424	AY098996	EF379006
<i>Festuca pratensis</i> Huds. (2x)*	England: Wilshire: Calne; Stace C.A.	AF303421	AF478503	EF379007
Incertae sedis fine-leaved <i>Festuca actae</i> Connor (unkown)	New Zealand: Canterbury: Banks peninsula: Lake Forsyth; Lloyd K.M. 57589	AY524829**	AY528949**	EF584998
<i>Festuca arizonica</i> Vasey (6x)	USA: Colorado Park; Peterson P. M., Annable C.R. 12012 US	EF584924	—	—
<i>Festuca baffinensis</i> Polunin (4x)*	Canada: Northwest: Masik River Valley; Gillespie L.J. & al. 7116	EF584925	EF592958	EF585013
<i>Festuca chimborazensis</i> E. B. Alexeev (unkown)*	Ecuador: Cotopaxi: Chaupi; Stančík D. 4034, US-3428932	EF584930	EF592963	EF585024
<i>Festuca cundinamarcae</i> E. B. Alexeev (unkown)	Colombia: Bocaya: Aquitania: Vereda Suse; Stančík D., US-3428950	—	EF592965	—
<i>Festuca deflexa</i> Connor (unkown)*	New Zealand: Poverty Basin: Mt Owen; Nelson; Lloyd K.M. 57588	AY524838**	AY528942**	EF585030
<i>Festuca glumosa</i> Hack. ex E. B. Alexeev (4x)	Colombia: Imbabura Cayambe: Volcano Cayambe; Stančík D., US-3428930	EF584940	EF592973	EF585046
<i>Festuca hephaestophila</i> Nees ex Steud. (unkown)*	Mexico: Nuevo Leon: Sierra Madre Oriental; Peterson P.M., Knowless M.B. 13347, US	EF584943	EF592976	EF585048
<i>Festuca huntianiana</i> E. B. Alexeev (unkown)	Mexico: Nuevo Leon: Sierra Madre Oriental; Peterson P.M., Knowless M.B. 13343, US	EF584945	EF592977	—
<i>Festuca ligulata</i> Swallen (unkown)	Mexico: Cohauila: Saltillo; Peterson P.M. 10050	—	EF592982	EF585061
<i>Festuca madida</i> Connor (unkown)*	New Zealand: Central Otago: Rock & Pillar Range; Lloyd K.M. 57627	AY524833**	AY528943**	EF585065
<i>Festuca matthewsii</i> (Hack.) Cheeseman subsp. <i>matthewsii</i> (6x)	New Zealand: Fjorland: Tahake valley; Lloyd K.M. 57938	AY524836**	AY528948**	EF585066
<i>Festuca melanopsis</i> Foggi, Rossi and Signorimi (6x)	Switzerland: Valais: Müller J. 7966	EF584952	EF592984	EF585067
<i>Festuca nardifolia</i> Griseb. (unkown)*	Argentina: Salta Abra: El Acay; Peterson P.M. 10379	EF584954	EF592986	EF585070
<i>Festuca novae-zelandiae</i> (Hack.) Cockayne (6x)*	New Zealand: Fjorland: Lloyd K.M. 57940	AY524832**	AY528941**	EF585073

(continued on next page)

Table 1 (continued)

Taxon (ploidy level)	Source	GenBank Accession No. ITS	trnL-F	trnT-L
<i>Festuca occidentalis</i> Hook. (4x)	USA: Oregon: Klamath: Winema; Soreng R.J. 5971, US	EF584956	EF592988	EF585074
<i>Festuca orthophylla</i> Pilg. (unkown)	Bolivia: Tarija: Avilés: Copacabana; Müller J. 9245	EF584957	EF592989	EF585075
<i>Festuca rigescens</i> (J. Presl) Kunth (unkown)	Peru: Ancash: Recuay: Cordillera Blanca; Peterson P.M., Refulio Rodriguez N. 13809, US-3423058	EF584966	EF592999	—
<i>Festuca subantactica</i> Parodi (unkown)*	Argentina: Santa Cruz: Lago Argentino; Peterson P.M. 17163	EF584973	EF593004	EF585103
<i>Festuca toluensis</i> Kunth (6x)	Venezuela: Mérida: Páramo de Piedras Blancas: Catalán P. 99.2000 MERC	EF584976	EF593038	EF585108
<i>Festuca vaginalis</i> (Benth.) Laegaard (unkown)	Colombia: Pichincha Pifo; Stanóík D. US-3428946	EF584977	EF593010	EF585111
Broad-leaved				
<i>Festuca argentina</i> (Speg.) Parodi (4x)*	Argentina: Santa Cruz: Lago Argentino; Peterson P.M. 17158	EF584923	EF592957	EF585012
<i>Festuca dichoclada</i> Pilg. (unkown)	Peru: Junin: Tarma: Maraynioc; Peterson P.M., Tovar O. 14056, US-3421417	EF584933	EF592967	EF585031
<i>Festuca elviae</i> Briceño (unkown)	Venezuela: Mérida: Laguna de Coromoto; Catalán P. s.n. MERC	—	AF543517	EF585039
<i>Festuca longivaginata</i> Tovar (unkown)	Peru: Ancash: Pallasca: Huandoval; Peterson P.M., Refulio Rodriguez N. 13922, US-3423002	EF584951	EF592983	EF585063
<i>Castellia</i> Tineo				
<i>Castellia tuberculosa</i> Tineo	Spain: Cádiz: Sanlúcar de Barrameda: La Algaida; López Rodríguez J.A. s.n. UZ	AF532954	AF533053	EF584996
<i>Ctenopsis</i> De Not				
<i>Ctenopsis delicatula</i> (Lag.) Paunero (2x)	Spain: Madrid: Garganta de Los Montes; López Rodríguez J.A. s.n., UZ	AF478499	AF478537	EF584997
<i>Hellerochloa</i> Fourn.				
<i>Hellerochloa fragilis</i> (Luces) Rauschert (unkown)*	Venezuela: Merida: Páramo de Piedras Blancas; Catalán P. s.n. MERC	AF532960	AF533059	EF585115
<i>Lolium</i> L.				
<i>Lolium canariense</i> Steud. (2x)*	Spain: Canary Isles: Tenerife: Las Cañadas; Santos s.n. (ITS, trnLF) / Spain: Canary Isles: Tenerife: Teno; Santos, 13.3.02 (trnTL)	AY228161	AY228162	EF379014
<i>Lolium multiflorum</i> Lam. (2x)	Spain: Lugo: Sarria; López Rodríguez J.A. 01092 UZ (ITS, trnLF) / Spain: Zaragoza: Chodes; Martínez A. JACA 394790 (trnTL)	AF532946	AF533038	EF379023
<i>Lolium perenne</i> L. (2x)*	England (cv) (ITS, trnLF9 / Great Britain: Wales, cultivated seeds PI 619001 USDA-Pullman (trnTL)	AF303401	AF478504	EF379024
<i>Lolium rigidum</i> Gaudin (2x)*	Spain: Zaragoza: Vedado de Peñaflo; Catalán P. & al. 18.2000, UZ (ITS, trnLF) / France cultivated seeds ABY-BA 9166 (trnTL)	AF532944	AF533039	EF379034
<i>Lolium temulentum</i> L. (2x)	Ethiopia, cultivated seeds PI 195000,, USDA-Pullman	EF379087	EF378985	EF379037
<i>Micropyropsis</i> Romero Zarco & Cabezudo				
<i>Micropyropsis tuberosa</i> Romero-Zarco & Cabezudo (unknown)	Spain: Huelva: Almonte; Romero-Zarco s.n.	AF532943	AF533037	EF379013
<i>Micropyrum</i> Link				
<i>Micropyrum patens</i> (Brot.) Rothm. (2x)	Spain: Madrid: Cadalso de los Vidrios; López Rodríguez J.A. 01194, UZ	AF478495	AF495885	—
<i>Micropyrum tenellum</i> (L.) Link (2x)	Spain: Segovia: Navafría; López Rodríguez J.A s.n., UZ	AF478494	AF478534	EF585116
<i>Psilurus</i> Trin.				
<i>Psilurus incurvus</i> (Gouan) Schinz & Thell (4x)	Spain: Huesca: Estopiñan; JACA 236098	AF478493	AF478533	—
<i>Vulpia</i> C. C. Gmel.				
Sect. <i>Vulpia</i>				
<i>Vulpia bromoides</i> (L.) S. F. Gray (2x)*	Spain: Lugo: Láncara; López Rodríguez J.A. 01080, UZ	AF478485	AF487616	EF585119
<i>Vulpia ciliata</i> Dumort. (4x)*	Spain: Zaragoza: Vedado de Peñaflo; Catalán P. 19.2002, UZ	AY118094	AY118104	EF585120
<i>Vulpia microstachys</i> (unkown)*	USA: California: W of San Luis Obispo; Soreng R.J. 7406	EF584981	EF593015	EF585125
<i>Vulpia muralis</i> (Kunth) Nees (2x)*	Spain: Zaragoza: Actur; Pyke & Catalán 11.2000 UZ	AF478484	AF478526	EF585126
<i>Vulpia myuros</i> (L.) C.C.Gmel. (6x)*	USA: Washington: King Co: Seattle: Lake Forest Park; Catalán P. 54.2001 UZ	AY118092	AY118103	EF585127
<i>Vulpia octoflora</i> Rydb. (unkown)*	USA: Washington: Okanogan; Peterson P.M. 3263	EF584982	EF593016	EF585128
Sect. <i>Loretia</i> (Duval-Jouve) Boiss.				
<i>Vulpia alopecuros</i> (Schousboe) Dumort. (2x)*	Portugal: Algabe: Meia Praia: Lagos; Stace C.A., LEI	AF478491	AF487617	EF585117

Table 1 (continued)

Taxon (ploidy level)	Source	GenBank Accession No. ITS	trnL-F	trnT-L
<i>Vulpia geniculata</i> (L.) Link (2x)*	Spain: Sevilla: Constantina; JACA J29397	AF478490	AF478531	EF585123
<i>Vulpia sicula</i> (C. Presl.) Link (2x)*	France: Corse: Ponte Leccia; JACA 366589	AY118089	AY118100	EF585129
Sect. <i>Monachme</i> Dumort				
<i>Vulpia fasciculata</i> (Forsk.) Samp. (4x)*	Spain: Barcelona: Vilanova; Pyke S. 15.2000, UZ	AF478487	AF478528	EF585121
<i>Vulpia fontqueriana</i> Melderis & Stace (2x)	Spain: Segovia: Lastras de Cuellar: Nava del Pobo; López Rodríguez J.A. 16-6-2000, UZ	AF478488	AF478529	EF585122
<i>Vulpia membranacea</i> (L.) Dumort. (2x)	Spain: Cádiz: Sanlúcar de Barrameda: La Algaida; Catalán P. 8.2002, UZ	AY118090	AY118101	EF585124
Sect. <i>Apalochloa</i> (Dumort) Stace				
<i>Vulpia unilateralis</i> (L.) Stace (2x)*	Spain: Zaragoza: Vedado de Peñaflo; Catalán P. 18.2002 UZ	AY118095	AY118106	EF585130
Sect. <i>Spirachne</i> (Hack.) Boiss				
<i>Vulpia brevis</i> Boiss. & Kotschy (2x)*	Cyprus: SE Nicosia; Stace C.A., LEI	AF478489	AF478530	EF585118
<i>Wangenheimia</i> Moench				
<i>Wangenheimia lima</i> (L.) Trin. (2x)*	Spain: Zaragoza: Vedado de Peñaflo; Catalán P. & al. 17.2000, UZ	AF478498	AF478536	EF585131
Cynosurinae Fr.				
<i>Cynosurus</i> L.				
<i>Cynosurus echinatus</i> L. (2x)	Spain: Soria: Monte Valonsadero; López Rodríguez J.A. s.n., UZ	AF532937	AF533031	EF584993
Dactylidinae Stapf.				
<i>Dactylis</i> L.				
<i>Dactylis glomerata</i> L. <i>hispanica</i> (Roth) Nyman (2x)*	Spain: Zaragoza: Peñaflo; Catalán P. & al. s.n., UZ	AF393014	AF533027	EF584994
<i>Lamarkia</i> Moench				
<i>Lamarkia aurea</i> (L.) Moench (2x)	Spain: Zaragoza: Puenete de la Almozara; PC 14.2000, UZ	AF532935	AF533029	EF584995
Parapholiinae Caro				
<i>Catapodium</i> Link				
<i>Catapodium rigidum</i> (L.) C.E. Hubbard	Spain: Segovia: Sepúlveda; López Rodríguez J.A. s.n., UZ	AF532940	AF533034	EF584986
<i>Cutandia</i> Wilk.				
<i>Cutandia maritima</i> (L.) Barbey (2x)	Spain: Valencia: El Saler; Stace C.A., Catalán P. 5.04 UZ 5.04	EF584915	EF592946	EF584987
<i>Cutandia memphitica</i> (Spreng.) K. Richt.*	Western Mediterranean; Boulos L. & Cope T. 17676 (E)	EF584916	EF592947	EF584988
<i>Desmazeria</i> Dumort.				
<i>Desmazeria sicula</i> (Jacq.) Dumort.	Seed Bank, Royal Botanic Gardens Kew.17332; Soreng R.J. s.n. (BH)	EF584917	EF592948	EF584989
<i>Hainardia</i> Greuter				
<i>Hainardia cylindrica</i> (Willd.) Greuter	Spain: Zaragoza: Parque Tio Jorge; Pyke S. s.n., UZ	AF532941	AF533035	EF584990
<i>Parapholis</i> C.E. Hubbard				
<i>Parapholis incurva</i> (L.) C.E. Hubbard (4x)	Spain: Zaragoza: Vedado de Peñaflo; Catalán P. & al. 23,2000, UZ	AF532942	AF533036	EF584991
<i>Sphenopus</i> Trin.				
<i>Sphenopus divaricatus</i> (Gouan) Reichenb.	Spain: Zaragoza: Vedado de Peñaflo; Catalán P. & al. s.n., UZ	AF532939	AF533033	EF584992
Poinae Dumort.				
<i>Poa</i> L.				
<i>Poa infirma</i> Kunth (2x)*	Spain: Zaragoza: La Jota; Pyke S. s.n., UZ	AF393012	AF488773	DQ367407
Puccinellinae Soreng & Davis				
<i>Puccinellia</i> Parl.				
<i>Puccinellia distans</i> (L.) Parl.	Spain: Navarra: Lazagurría; JACA207897	AF532934	AF533024	EF584985
Aveneae Dumort.				
<i>Antinoria</i> Parl.				
<i>Antinoria agrostidea</i> (DC) Parl.	Spain: Zamora, Tábara; MA 651156	DQ539562	—	—
<i>Avena</i> L.				
<i>Avena eriantha</i> Durieu*	Spain: Madrid: Chinchón; López Rodríguez J.A. 032001, UZ	DQ336822	AF533021	EF584983

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Table 1 (continued)

Taxon (ploidy level)	Source	GenBank Accession No. ITS	<i>trnL-F</i>	<i>trnT-L</i>
<i>Deschampsia</i> Beauv. <i>Deschampsia cespitosa</i> (L.) Beauv.	USA: Colorado: Boukder Co: Rocky Mnts; Catalán P. s.n., UZ	AF532929	AF533026	EF584984
<i>Dielsiochloa</i> Pilg. <i>Dielsiochloa floribunda</i> Pilg.	Bolivia: Dpto. La Paz, Cumbre near La Paz, Müller J., MA 721312	DQ539563	DQ631428	DQ631494
<i>Koeleria</i> Pers. <i>Koeleria loweana</i> Quintanar, Catalán & Castrov. (syn. <i>Parafestuca albida</i> (Lowe) Alexeev)	Portugal: Madeira, Pico do Arieiro, Sequeira, MA 721307	AF532930	AF533022	DQ336869
Seslerieae Koch <i>Sesleria</i> Scop. <i>Sesleria argentea</i> (Savi) Savi (4x)	Spain: Navarra: Araxes; Catalán P. 21, UZ	AF532931	AF533030	DQ631544
Triticeae Dumort. <i>Secale</i> L. <i>Secale cereale</i> L. (2x)*	USDA (cv.)	AF303400	AF478501	DQ336856
Brachypodieae Harz <i>Brachypodium</i> P. Beauv. <i>Brachypodium distachyon</i> (L.) P. Beauv. (2x)*	Slovenia: Ljubljana, LE1 s.n.	AF303399	AF478500	DQ336855

Numbers correspond to different accessions of the same taxon. Asterisks indicate the 74 terminal taxa selected for divergence dating and biogeographical analyses. Double asterisks mark sequences downloaded from GenBank.

Bayesian and Parsimony analyses were conducted with both the separate *trnTF* and ITS data matrices and with the combined data matrix using Mr. Bayes 3.0 (Huelsenbeck and Ronquist, 2001) and Paup* 4.0 beta10 (Swofford, 2002), respectively. The two plastid and one nuclear data sets showed the same optimal nucleotide substitution model (GTR + G + I, 4 gamma rate categories) after the tests of goodness of fit for 56 nts substitutions models under the Likelihood Ratio Test (LRT) conducted with Modeltest 3.06 (Posada and Crandall, 1998), so this model was imposed in subsequent independent and combined analyses. Bayesian analyses were performed twice under the same parameters (2 million generations initiated with a random starting tree, three heated chains and one cold chain, sampling of trees every 100 generations, likelihood parameters (nucleotide frequencies, nucleotide substitution rates, gamma shape, proportion of invariable sites) estimated by the program, and sampled points collected prior to stationarity (approximately one fourth of the total, 250, discarded as burn-in). The posterior probability (PP) values of branches recovered in the 50% majority rule consensus tree constructed with the 750 trees of each Bayesian search were interpreted as an estimation of support. Clades with PP values of 95–100% were considered highly supported. Parsimony analyses were based on heuristic searches of 10,000 random-order-entry trees, with TBR branch swapping and saving no more than 10 trees of length equal or shorter than 10 per replicate (Catalán et al., 2004). All most parsimonious trees obtained from each separate search were used to compute their respective strict and 50% majority rule consensus trees. Bootstrap

support for branches of the parsimonious trees was calculated through similar heuristic searches of 1000 replicates but with a lower number of random-starting trees to reduce the computational time of the resampling analyses (DeBry and Olmstead, 2000). Clades with bootstrap support (BS) values of 90–100% were considered strongly supported, 70–89% moderately supported, and ≤50–69% weakly supported (Mason-Gamer and Kellogg, 1997). The Incongruence Length Difference (ILD) test of Farris et al. (1994) was calculated through 1000 random-order-entry replicates in Paup* to estimate if the nuclear and plastid data sets were significantly different from random partitions of the same size.

2.2. Divergence time estimation

Due to the lack of crown Loliinae fossils, we first estimated the divergence time of the grass family and that dated the divergence of the Triticeae tribe from the Aveneae-Poeae tribe (which include both our outgroup and ingroup taxa) using Bayesian dating methods (Thorne et al., 1998; Kishino et al., 2001; Thorne and Kishino, 2002) as indicated in Bell et al. (2005) and Forest et al. (2007). Following Gaut (2002), the inferred dates were derived from the plastid gene *ndhF* of nine Poaceae representatives [*Anomochloa* (Anomochloae), *Leersia* (Oryzaceae); *Avena* (Aveneae); *Poa* (Poeae); *Hordeum* (Triticeae); *Setaria* and *Pennisetum* (Paniceae); *Sorghum* and *Zea* (Andropogoneae) plus *Joinvillea* (Joinvilleaceae; outgroup)], and a phylogeny of the family concordant with that presented by Clark et al. (1995) and the Grass

Phylogeny Working Group (GPWG, 2001). We used as calibration lower and upper bounds previously reported on the age of the family based on pollen (Linder, 1987; Jacobs et al., 1999; Kellogg, 2002, 2001) and phytoliths (Prasad et al., 2005) fossil records that varied between 55–80 My and that were imposed as minimum and maximum constraints to the crown node of the Poaceae, respectively. Divergence times and additional parameters (based on the F84 + G model and 100,000 MCMC iterations, with *rttm* and *rtmsd* set at 6.0, *rtrate* and *rratesd* set at 0.02, *brownmean* and *brownsd* set at 0.16, and big time set at 100, see below) were calculated using the programs *Baseml* (PAML package, Yang, 1997), *Estbranches* (Thorne et al., 1998) and *Multidivtime* (Kishino et al., 2001).

To infer divergence times within Loliinae a reduced Bayesian tree consisting of 74 terminal taxa with complete sequences for both nuclear ITS and plastid *trnTL* and *trnLF* DNA regions that represented the main lineages of the subtribe and its allies (Table 1) was used. Extrapolations of divergence times across plastid genes could be done as most plastid regions (e. g., *ndhF*, *trnTL*, *trnLF*) show similar mutation rates in the Poaceae (Gaut, 2002) and the Loliinae (Catalán et al., 2007). The use of plastid data to infer dated biogeographical scenarios was considered to be partly misleading in *Hordeum* as the past reticulation history in barleys caused strong incongruence between plastid and nuclear topologies (Jakob and Blattner, 2006). Nonetheless, we followed the procedures suggested by other authors that supported the use of more resolved combined phylogenies, which could also be a better estimate of branch lengths and thus of divergence times, to infer the biogeographical history of the plant lineages (cf. Bell et al., 2005; Forest et al., 2007). As all the Loliinae sequences showed highly significant rate heterogeneity after a likelihood ratio test (χ^2 , $p < 0.001$), calculations were performed using the same Bayesian relaxed clock methods stated above. This also involved the use of the programs *Baseml*, *Estbranches* and *Multidivtime* as outlined in Rutschmann (2004). A basal calibration for the separation between *Secale cereale* (Triticeae) and *Avena eriantha* plus Loliinae and allies (Aveneae-Poeae) was set for minimum and maximum constraints of 16.0 and 25.8 My, which corresponded to the standard deviation of 20.9 ± 4.9 My obtained for that node in the preliminary dating analysis of the grasses. Model parameters related to nucleotide frequencies, kappa, and alpha shape parameter of the gamma distribution were estimated from the combined data set in *Baseml* imposing the most complex substitution model allowed by the program (F84 + G; Felsenstein, 1993). Branch lengths and their variance-covariance matrix were then estimated by maximum likelihood with the program *Estbranches*. The posterior distributions of substitution rates and the divergence times were calculated with *Multidivtime* through 100,000 generations of MCMC, sampling every 100 generations after an initial burnin of 10,000 generations. We used the following priors (in 10 My units): *rttm* and *rtmsd* (mean and standard deviation of the

expected number of time units between tip and root) set at 2.0; *rtrate* and *rratesd* (mean and standard deviation of rate of molecular evolution at the root node, derived from the median distance between ingroup root and tips) set at 0.005; *brownmean* and *brownsd* (mean and standard deviation of the Brownian motion constant “nu” influencing the change of logarithm of rate of molecular evolution over time; calculated as $1/rttm$ following J. Thorne (cf. Bell et al., 2005; Forest et al., 2007) set at 0.5; big time (number higher than time units between tips and root) set at 50.

2.3. Dispersal–vicariance analysis

Distribution areas of the Loliinae and its close allies were defined according to major taxonomic and geographical studies of these grasses (Tutin, 1980; Tzvelev, 1983; Soreng et al., 2003; Chen et al., 2006). However, due to the lack or limited sampling in some poorly studied areas some biogeographical regions could not be represented in the present study. For this reason our analysis was focused on recovering the historical patterns of the main Loliinae lineages in the major centres of all five studied continents. Potential biogeographical scenarios of the Loliinae were tested using dispersal–vicariance analysis implemented in DIVA 1.1 (Ronquist, 1996, 1997) and dispersal-extinction-cladogenesis (DEC) analysis implemented in LAGRANGE (<http://code.google.com/p/lagrange>; Ree and Smith, in press). Because the large analysis of 157 taxa and c. 13 preliminary areas could not be performed by DIVA, the optimization was performed in two consecutive steps using more reduced distribution data matrices. Although DIVA runs independently of any past history of reticulation (Ronquist, 1997; Sanmartin and Ronquist, 2004), we presumed that the complex inherent hybridization and polyploidy patterns that affect the Loliinae and their resulting topologies could distort the reconstruction of their biogeographical scenarios. To avoid this, a preliminary DIVA analysis was performed with a distribution matrix of exclusively 51 diploid Loliinae and allies plus four close outgroups of presumably more ancestral origin using the five geographical regions where they grow today (1-panMediterranean-SW Asia, 2-Macaronesia, 3-E&S Asia, 4-C-N Asia & circumarctic, and 5-N America), aiming at deciphering the basal events that occurred in the subtribe. Secondly, a distribution matrix of 74 terminal taxa used in the previous dating analysis and representing all geographical lineages and ploidy levels detected in the combined Bayesian tree was subjected to dispersal–vicariance analysis using eight geographical areas (the same five areas selected for the diploids plus three more areas that contain only polyploid taxa: 6-N South America, 7-S South America and 8-New Zealand). The nodal identities in the diploid reconstruction were chosen as priorities to interpret the most likely ancestral areas in the second DIVA analysis. Both analyses were run either with unconstrained and with maximally-two constrained ancient distribution areas.

In addition to the parsimony-based DIVA analyses, a likelihood was used to reconstruct ancestral range inheritance scenarios at internal nodes on the phylogeny estimated from the 74-taxon data matrix, using the DEC model as implemented in LAGRANGE (Ree et al., 2005; Ree and Smith, in press). The DEC model has free parameters for instantaneous rates of dispersal and local extinction along phylogenetic branches, and can be used to estimate likelihoods of ancestral areas (range inheritance scenarios) at bifurcating nodes. Unlike DIVA, vicariance of widespread ancestors is not enforced, lineage divergence can occur within an ancestral area, allowing a widespread range to be inherited and persist through a speciation event. Two DEC models were used (see Ree and Smith, in press). The first (M0) constrained ancestral ranges to a maximum of two areas, while the second (M1) allowed only a subset of these, excluding those representing disjunct (non-adjacent) area pairs. M1 also constrained rates of dispersal between areas to be inversely proportional to relative distance (Table 2). Both models were used to estimate rates of dispersal and of local extinction on the phylogeny.

Although the calculated dates of origin of Loliinae (15–13 My) and of its main lineages (11–2.5 My) imply that the colonized continents were in their present place and that the main land connections were already formed, some island colonizations and radiations in oceanic archipelagos occurred almost contemporaneously with the volcanic eruptions. Our hypothesis assumed that mainland continental migrations were more likely to occur than long-distance transoceanic dispersals based on a more parsimonious interpretation. This was also used as a converse way to test the extent of potential long intercontinental colonizations.

3. Results

3.1. Phylogenetic analysis

The separate *trnTL* and *trnLF* plastid data matrices consisted of 1094 and 1196 aligned nucleotides, respectively, with a concatenated 188-taxon *trnTF* data matrix containing 2290 positions. Four hundred and eighty-one ambiguously aligned characters were excluded from

analysis. Seven hundred and ninety-eight (44%) of the non-excluded characters were variable and 432 (24%) were potentially informative. The alignment included 28 potentially informative gaps, 16 in *trnTL* and 10 in *trnLF*, which were used in the Bayesian and parsimony analyses. The nuclear 174-taxon ITS data matrix included 641 aligned nucleotides of which 398 (62%) were variable and 282 (44%) were potentially informative. The combined 157-taxon *trnTF*/ITS data matrix was constructed with the plastid and nuclear data sets.

Bayesian and parsimony-based (not shown) analyses of each data set (*trnTF*, ITS, *trnTF*/ITS) produced similar topologies that were congruently resolved for the Loliinae and closed allies. The plastid and nuclear optimal trees were generally highly congruent with each other, though the plastid topology was better resolved basally than the ITS topology (Fig. 1). However, potential conflicts among the topologies were detected in some instances (the 10 cases reported in Catalán et al. (2004): *Castellia*, *Deschampsia*, *Festuca altaica*, *F. californica*, *F. pulchella*, *F. quadriflora*, *F. subulata*, *Hellerochloa*, *Micropyrum*, and *Sesleria*, plus six weakly supported cases affecting the distinct placement of the American clade I taxa (*F. chimboracensis*, *F. edlundiae*, *F. glumosa*, *F. purpurascens*, *F. subantarctica*, *F. vaginalis*) either within the American clade II (ITS tree; results not shown) or in a separate more basal clade (*trnTF* tree; Fig. 1). Despite this, resolution for the new data was highly consistent in the two data sets and showed strong systematic and geographical structure. The ILD test conducted on the combined data matrix of common ITS and *trnTF* accessions was significant ($p < 0.01$), indicating that the two data sets are heterogeneous. The data sets were also significantly different from random partitions ($p < 0.05$) when the sixteen conflictive taxa were excluded from analyses. Therefore, our rationale for combining the nuclear and plastid data was based on this apparent non-related heterogeneity of the data with phylogenetic histories, given that the congruent sections of the tree still give a significant ILD test. These results corroborated the irrelevance of the significant ILD values in terms of combinability, as proposed by previous authors (Barker and Lutzoni, 2002). The combined tree was better resolved than any of the separate plastid and ITS trees and is therefore the one explained here. Nonetheless, those taxa not included in this tree but strongly supported by either the plastid or the nuclear data have been manually added to the topology (Fig. 1) and are discussed below. This combined tree is based on the better-resolved Bayesian topology (Fig. 1a–f).

The data set supports a strong monophyletic Loliinae (97 BS/ 100 PP) sister to Parapholiinae/Cynosurinae (100/100) and Dactylidinae (100/100). Within Parapholiinae/Cynosurinae (Fig. 1a), resolution for genera in the grade (*Cynosurus* (*Sphenopus* ((*Parapholis*, *Hainardia*)(*Catapodium*, (*Desmazeria*, *Cutandia*)))) agreed with that indicated by Soreng and Davis (2000) and partially by Catalán et al. (2004). Affinity of *Cutandia* to Parapholiinae is confirmed by the strong nesting of the sister

Table 2
Probability transition matrix of dispersal between areas used in DEC analysis (Model 1)

	A	B	C	D	E	F	G	H
A	—	0.75	0.75	1.00	0.50	0.25	0	0
B		—	0	0	0.25	0	0	0
C			—	1.00	0.50	0	0	0.75
D				—	1.00	0.25	0	0.25
E					—	1.00	0.50	0.25
F						—	1.00	0.50
G							—	0.75
H								—

Area coding corresponds to that indicated in Fig. 3.

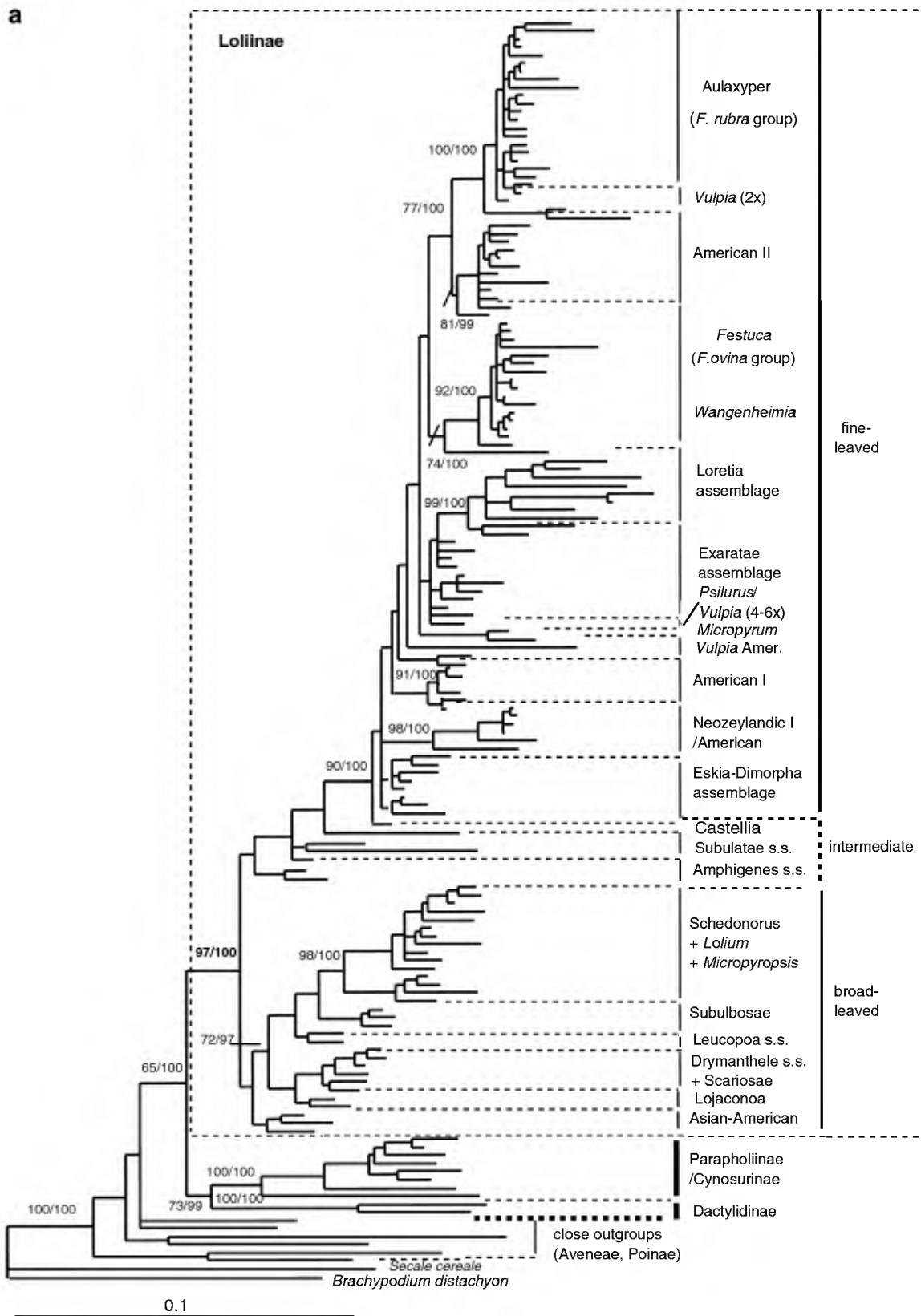


Fig. 1. Bayesian tree of Loliinae and close allies (data matrix of 188 terminal taxa). (a–f) Several parts of the tree are shown in detail. Numbers above and below branches indicate bootstrap (BS)/posterior probability (PP) support values. Grey branches indicate terminal samples analyzed only by *trnT* (*) or ITS (**) data. *Brachypodium distachyon* was used to root the tree.

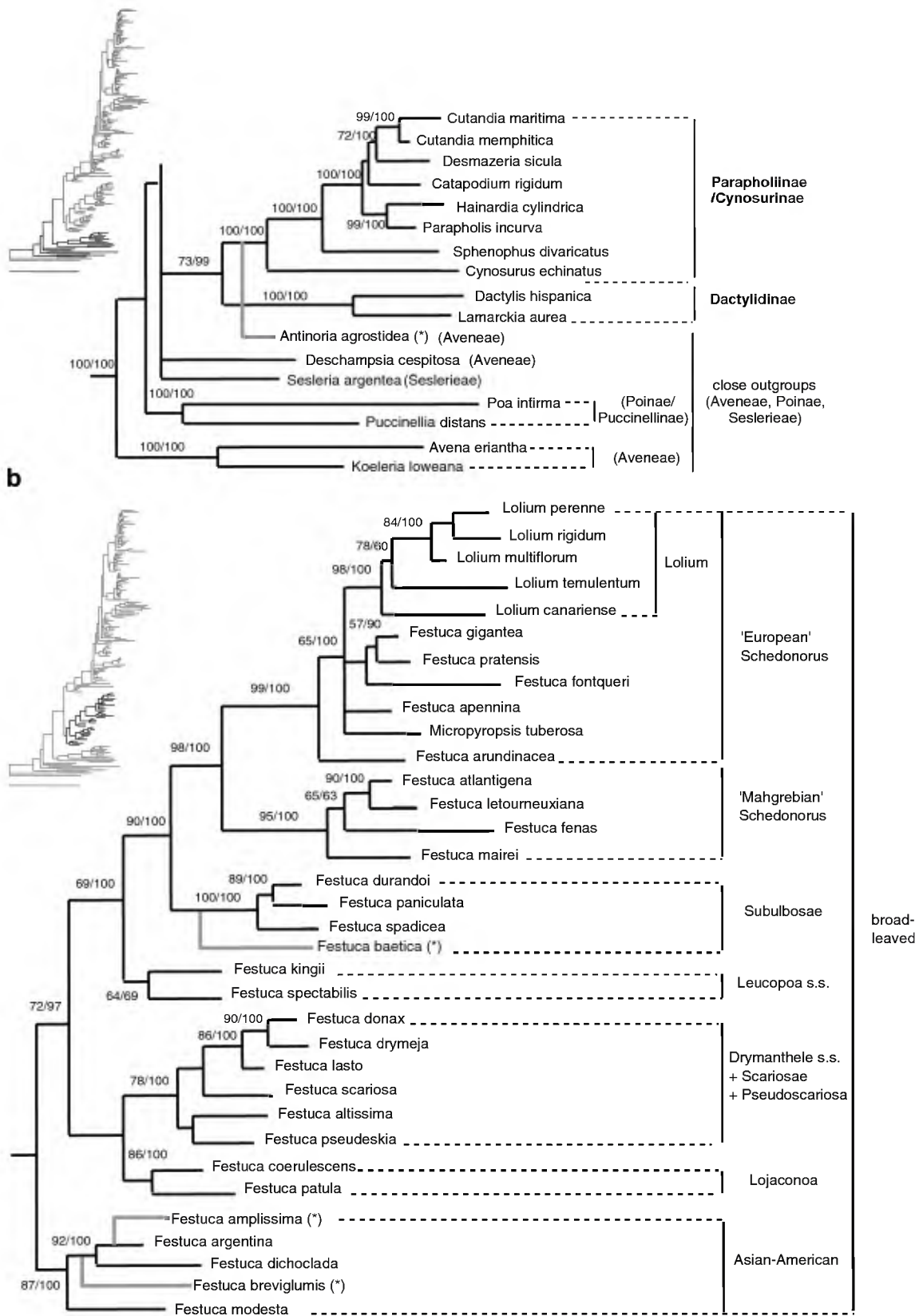


Fig. 1 (continued)

congeners *C. maritima*/*C. memphitica* (99/100) within this clade, thus correcting previous misattributions of this genus to *Vulpia* sect. *Loretia* 'assemblage' (cf. Torrecilla

et al., 2004; Catalán et al., 2004). The so-called avenoid *Antinoria agrostidea* is shown to be sister to the Parapholiinae/Cynosurinae in the ITS tree.

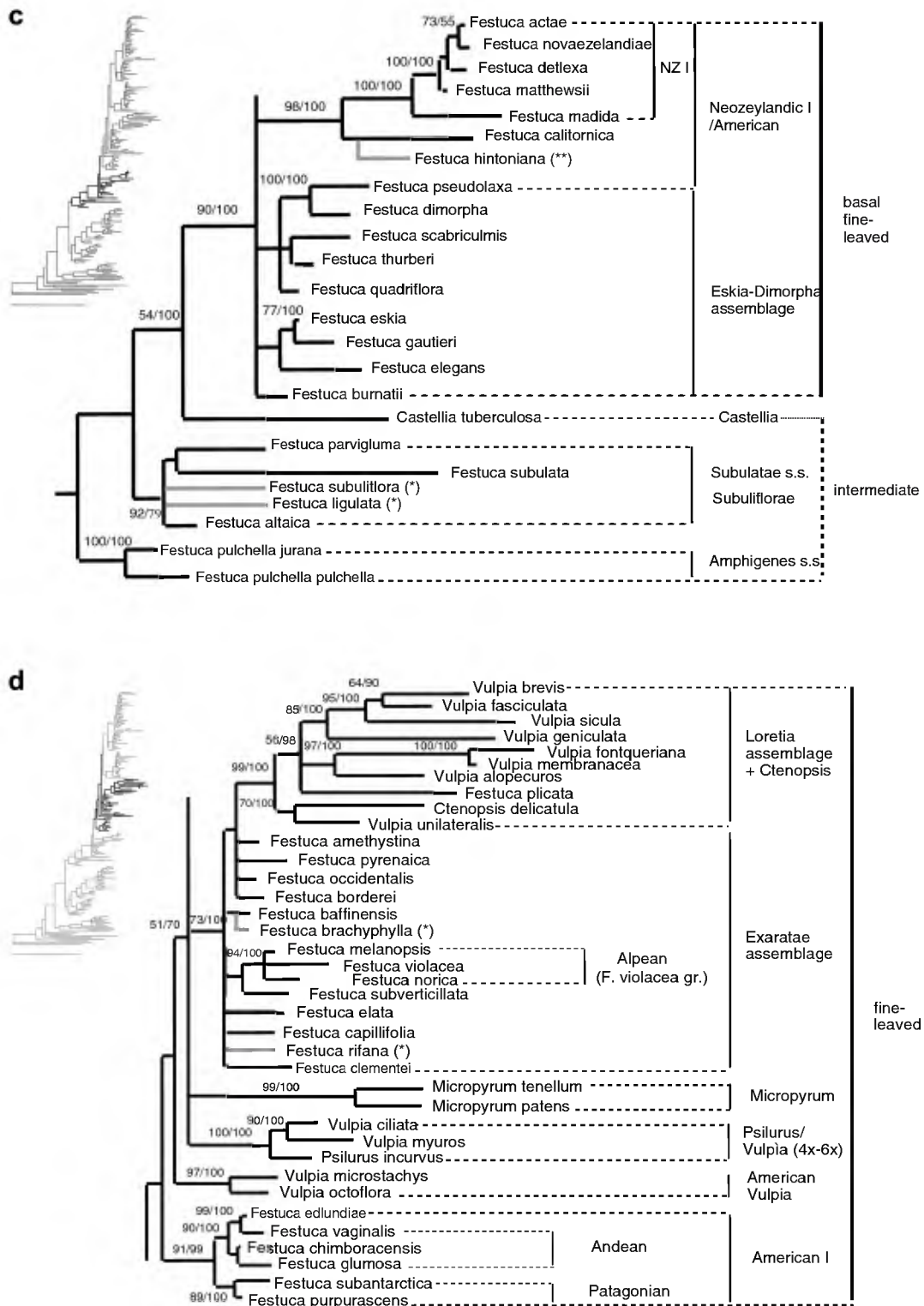


Fig. 1 (continued)

The classical divergence of the Loliinae into broad-leaved and fine-leaved lineages is maintained in the Bayesian tree; however, both clades were weakly supported for their respective basalmost branches due to uncertain placement of several broad-leaved (Asian–American clade, Amphiqenes clade) or morphologically intermediate (Subu-

latae s.str. clade) *Festuca* lineages, plus annual *Castellia* in one or the other split (Fig. 1). By contrast, the phylogenetic analysis recovered moderate to highly supported subbasal nodes for the core clades of broad and fine-leaved *Festuca* taxa, respectively (Fig. 1). These results concurred with previous hypotheses (cf. Catalán et al., 2004) on the

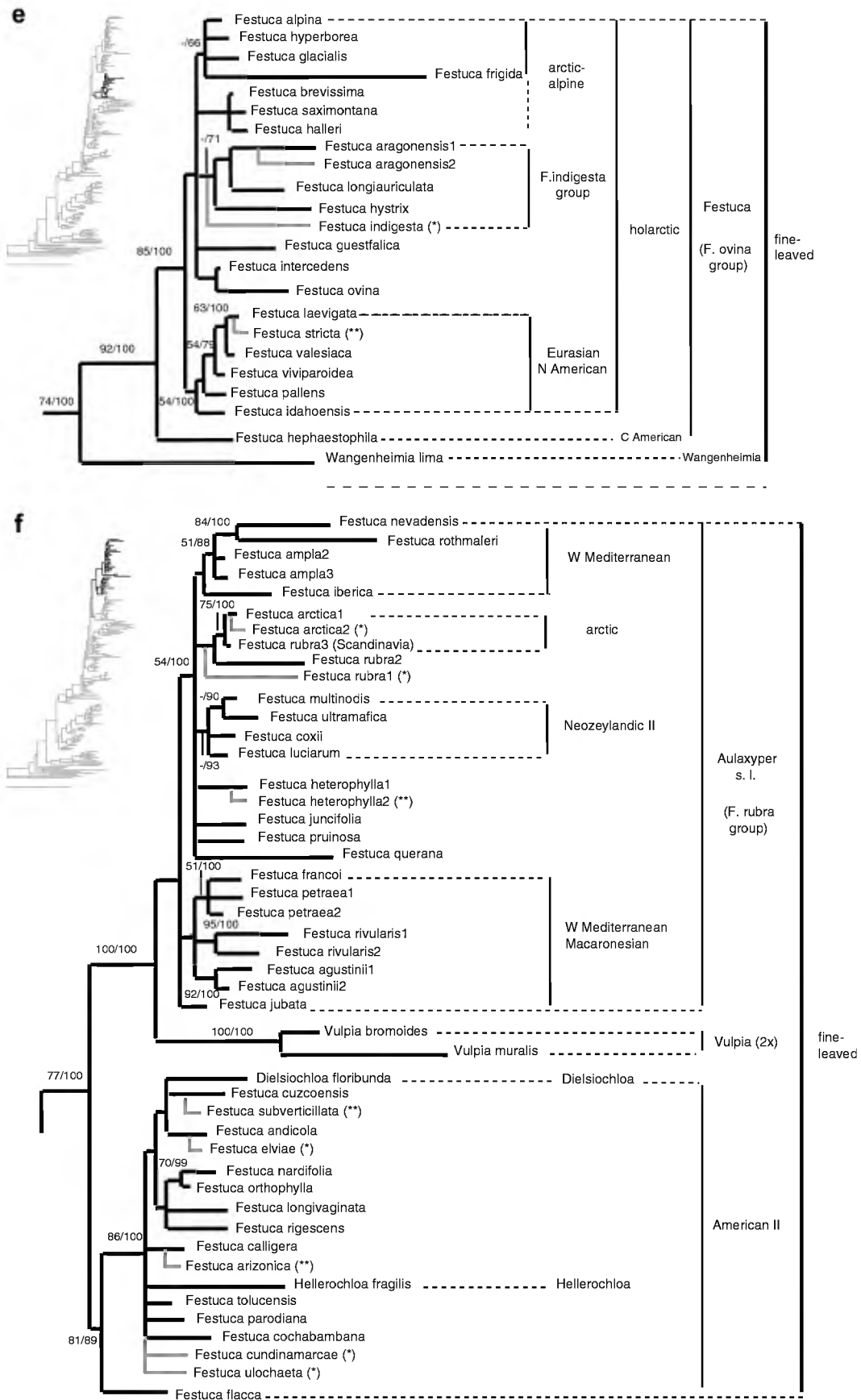


Fig. 1 (continued)

evolutionary trend from more ancestral broad-leaved *Festuca* lineages (*Drymanthele* + *Scariosa* + *Pseudoscariosa*, *Lojaconoa*) towards less ancestral broad-leaved groups (*Leucopoa*, *Subbulbosae*, *Schedonorus* + *Micropyropsis* + *Lolium*), and the successive divergences of lineages of *Eskia* + *Dimorphae*, *Exaratae*, the annual *Loretia* assemblage plus *Ctenopsis*, and the *Psilurus* + polyploid *Vulpia* group, that ultimately led to the most recently evolved *Festuca* + *Wangenheimia* and *Aulaxyper* + diploid *Vulpia* core clades, within the fine-leaved group (Fig. 1).

The Asian–American clade (87/100) (Fig. 1b), showed the sister relationship of *Festuca modesta* to the *F. argentina*/*F. dichoclada* clade (92/100). This group was enlarged by the inclusion of *F. amplissima* and *F. breviglumis* in the plastid tree. In the *Drymanthele* s.str. clade, *F. donax* was resolved as sister to *F. drymeja* (90/100). Both species joined with *F. lasto* into a strongly supported clade (86/100). Further relationships within the broad-leaved clade were similar to those indicated in Catalán et al. (2004), although geographic structure was refined within the *Schedonorus* group ('European' and 'Maghrebian' subclades; Fig. 1b).

The basal unsupported lineages of the fine-leaved *Festuca* clade include a range of apparently misplaced broad-to-intermediate-leaved European and Asian–American *Festuca* taxa (Fig. 1c). The Asian–American clade II (=Subulatae s.str. clade) united *F. parvighuma* with *F. subulata* and *F. altaica* into a well supported cladistic group (BS = 92) also enlarged with *F. subuliflora* in the plastid tree. However, representatives of this group and of *F. pulchella* (Amphigenes clade) joined into a larger and relatively well supported (BS = 86) broad-leaved lineage formed by other *F.* subgen. *Drymanthele* (Asian–American clade I) and *F.* subgen. *Leucopoa* s.str. (*F. kingii*, *F. spectabilis*) representatives in the ITS tree (Results not shown). The admixture also affected the basal and subbasal lineages of the highly supported core clade of fine-leaved *Festuca* that showed a range of paraphyletic and distinctly related *Eskia* and *Dimorpha* taxa with *F. thurberi* nested within (Fig. 1c). *F. ligulata* was resolved as a close relative of the Subulatae s.str. lineage in the plastid tree.

A notable finding was the recovery of a highly supported Neozeylandic I-American clade (98/100), intermediate between the broad and fine-leaved *Festuca* clades (Fig. 1c). *F. californica* was sister to a strong clade of New Zealand taxa (100/100) that showed the successive divergences of *F. madida*, *F. mathewsii*, *F. deflexa*, and *F. actae* and *F. novaezelandiae*. The central North American *F. hintoniana* joined *F. californica* in the ITS tree. The next more recent splitting corresponded to the well supported American clade I (91/99) (Fig. 1d), which divided into rather highly supported subclades of mostly Andean (90/100) and Patagonian (89/100) taxa. The first encompassed the fine-leaved *F. chimborazensis*, *F. glumosa*, *F. vaginalis* and *F. edlundiae* taxa, whereas the second showed the sister relationship of the broad-leaved *F. purpurascens* to the fine-leaved *F. subantarctica*. This clade was

mostly supported by plastid data, as the ITS data placed those taxa within the more recently evolved American clade II. Polyphyly of *Vulpia* sect. *Vulpia*, previously manifested in the distinct links of Mediterranean diploid and polyploid taxa to the *Aulaxyper* and the *Psilurus* clades, respectively, was extended in the present study through the finding of a new American lineage formed by the sister taxa *V. octoflora*/*V. microstachys* (97/100) which was intermediately placed between the American clade I and the Mediterranean *Psilurus*/*Vulpia* (4x–6x) clade (Fig. 1d). The annual Mediterranean *Loretia* assemblage plus *Ctenopsis* (99/100) joined with representatives of the *Exaratae* assemblage and other close taxa in a large unresolved and poorly supported clade (Fig. 1d). A strong group of Alpine representatives of *Exaratae* (*F. melanopsis*, *F. violacea*, and *F. norica*) (94/100), all belonging to the “*F. violacea* group” (Pils, 1980), was sister to *F. subverticillata* (65/97). The unresolved terminals also included *F. baffinensis*, *F. amethystina*, *F. occidentalis*, and *F. elata*.

The more recently evolved *Festuca* (*F. ovina* group) plus *Wangenheimia* (74/100) and *Aulaxyper* (*F. rubra* group) plus allies (77/100) clades showed different kinds of systematic boundaries (Fig. 1e and f). Whereas the *Festuca* clade (92/100) was enlarged with taxa morphologically close and unanimously identified under this rank (Fig. 1e), the *Aulaxyper* s. l. clade was broadened with a range of elements classified under different fine-leaved and broad-leaved taxonomic ranks (Fig. 1f). The *F. ovina* group showed the divergence of central American *F. hephaestophila* from the clade of holarctic taxa (85/100). Despite the homogeneity of this later group, there was poor resolution for an arctic-alpine clade (*F. alpina*, *F. frigida*, *F. glacialis*, *F. hyperborea*, enlarged by *F. hallaeri* in the plastid tree), a *F. indigesta*-group clade (*F. aragonensis*, *F. hystrix*, and *F. longiauriculata*, enlarged by *F. indigesta* in the plastid tree), and a Eurasian–North American clade (*F. idahoensis*, *F. laevigata*, *F. pallens*, *F. valesiaca*, and *F. viviparoidea*, enlarged by *F. stricta* in the ITS tree) (Fig. 1e).

Most noticeably, a poorly resolved American clade II (81/89) (Fig. 1f), including representatives of groups as diverse as *Hellerochloa* (*H. fragilis*), the so-called avenoid *Dielsiochloa* (*D. floribunda*) (cf. Quintanar et al., 2007), the broad-leaved *F. cochabambana*, *F. cuzcoensis*, *F. flacca* and *F. parodiana* (enlarged by *F. elviae* and *F. subverticillata* in the plastid and ITS trees, respectively), and the fine-leaved *F. calligera*, *F. longivaginata*, *F. nardifolia*, *F. orthophylla* and *F. tolucensis* (enlarged by *F. cundinamarca* and *F. ulochaeta* in the plastid tree, and by *F. arizonica* in the ITS tree) showed a close relationship to the *F. rubra* group. Within the core *Aulaxyper* + *Vulpia* (2x) clade (100/100) several diploid Macaronesian taxa (*F. agustinii*, *F. jubata*, *F. petraea* and *F. francoi*) aligned basally with another Mediterranean diploid congener (*F. rivularis*), and separated from the more recently derived polyploid clade (54/100) (Fig. 1f). Within this, there was a further differentiation of a Neozeylandic clade II

(*F. coxii*, *F. luciarum*, *F. multinodis*, and *F. ultranaftica*), an arctic clade (*F. rubra* subsp. *arctica*), and a western Mediterranean clade (*F. ampla*, *F. iberica*, *F. nevadensis*, and *F. rothmaleri*), from other non-resolved European red fescues (*F. heterophylla*, *F. juncifolia*, *F. pruinosa*, *F. querana*, and *F. rubra*).

3.2. Dates of divergence

Our estimates suggested that the grasses radiated 60.2 ± 5.2 My and that the separation between Triticeae and Aveneae-Poeae took place 20.9 ± 4.9 My (Fig. 2). The molecular data indicate that the Loliinae diverged in the mid-Miocene (c. 13 My) and that the two highly-supported main broad-leaved and fine-leaved groups radiated at approximately the same age (c. 10.5 My). The inconsistently resolved 'intermediate' lineages might have appeared earlier (c. 12 My) or might just reflect an artifactual hybrid placement. The successive divergences of the broad and fine-leaved lineages spanned in parallel along the late Miocene though the splits tended to be older in the first group (i.e. *Lojaconoa* c. 8 My, *Drymanthele* + *Scariosae* 7.3 My, *Leucopoa* s.str. c. 6 My, *Schedonorus* assemblage c. 6 My) than in the second group (i.e. *Eskia* + *Dimorpha* c 7 My, Neozeylandic clade I 5.3 My, *Loretia* + *Exaratae* 5.4 My). The most recently evolved lineages of both series differentiated during the Pliocene (i.e. core *Drymanthele* 4.9 My, European *Schedonorus* 3.5 My, Mahgrebian *Schedonorus* 2.9 My, American clade I 3.8 My, *Loretia* assemblage 3.6 My, *Festuca* + *Wangenheimia* 4.9 My, American clade I 4.2 My, *Aulaxyper* + *Vulpia* (2x) 3.5 My) or the Pleistocene (*Lolium* 2.1 My, *Psilurus* + *Vulpia* (4x–6x) 2 My, W Mediterranean *Aulaxyper* 1.6 My, Neozeylandic clade II 1.0 My).

3.3. Biogeographical analysis

The dispersal–vicariance analyses were initially conducted with the distribution matrix of diploid Loliinae representatives using DIVA and optimizing the search without constraints. These analyses indicated that the Loliinae originated in the panMediterranean-SW Asia area. The optimal reconstruction required 10 dispersal events, and the historical pattern indicated that expansion likely proceeded from the original Eurasian center towards E and S Asia and C-N Asia plus the circumarctic zone and then to N America, with several independent colonizations of Macaronesia at different nodes (Results not shown). The dispersal–vicariance analysis of the complete 74-terminal taxa distribution matrix provided several alternative optimal reconstructions though all of them unambiguously recognized the panMediterranean-SW Asian area as the center of origin of the Loliinae. To resolve the ambiguities of some of the internal nodes we imposed a limited constraint of a maximum of two ancestral areas and resolved the dubious cases assigning the nodal identities retrieved from the former diploid reconstruction.

The ancestral range inheritance scenarios inferred by the likelihood-based DEC analyses of the 74-terminal taxa distribution matrix were highly congruent with the optimal parsimony-based DIVA reconstruction. Both DEC models (M0 and M1) retrieved almost identical patterns of inherited ancestral ranges and of dispersal and extinction events across the studied Loliinae (all nodes but two). M0 gave higher likelihood values than M1, suggesting a better fit to the data and thus reason to prefer it. However, inferences of dispersal events under M1 are more realistic biogeographically and agree with the optimal scenario reconstructed from DIVA, so M1 is arguably preferable over M0. The inferred historical scenario requires 29 dispersal events (9 within the broad-leaved Loliinae: BI-BIX, and 20 within the fine-leaved Loliinae: FI-FXX) (Fig. 3) and exhibits some uncertainty at the basal Asian–American split of the broad-leaved clade and at the first split of the Exaratae assemblage in the fine-leaved clade (Fig. 3a).

4. Discussion

4.1. Phylogeny and systematics of the Loliinae

The more complete phylogenies of Loliinae, enlarged with newly studied southern hemisphere and holarctic taxa, have allowed us to analyze the historical events related to the subtribe within a more solid timescale-based evolutionary context. The results of our study are in general agreement with our previous surveys on the general evolutionary trends observed within the broad and fine-leaved Loliinae lineages (Torrecilla and Catalán, 2002; Torrecilla et al., 2004; Catalán et al., 2004). However, unexpected relationships have been recovered for some South America and New Zealand lineages. Previous evidence for the close relationships of some Southern American taxa to the Eurasian *Aulaxyper* lineage reported in Catalán et al. (2004) has been largely corroborated in our present work, notably for those broad-leaved taxa of *Festuca* subgen. *Subulatae*, *Leucopoa*, and *Drymanthele* (American clades I and II, Fig. 1) that are shown to be strongly nested within the fine-leaved clade. Phylogenetic uncertainty still exists at weakly supported basal and subbasal nodes of the combined tree, concerning the ambiguous attachment of the broad-leaved *Amphigenes* and *Subulatae* s.str. clades, plus *Castellia* to either the broad or the fine-leaved clades in the separate plastid and nuclear trees. Some of these apparent ambiguities might come from a poor taxonomic knowledge of many southern hemisphere and Asian *Festuca* taxa, but other unexpected placements are clearly taxonomically inconsistent. Despite the recent increase in taxonomic studies of New Zealandian (Connors, 1998), South American (Stančík, 2003; Soreng et al., 2003; Stančík and Peterson, 2007), and Asian (Chen et al., 2003) *Festuca*, their supraspecific assignment is unknown or still tentative (see Table 1). Moreover, the

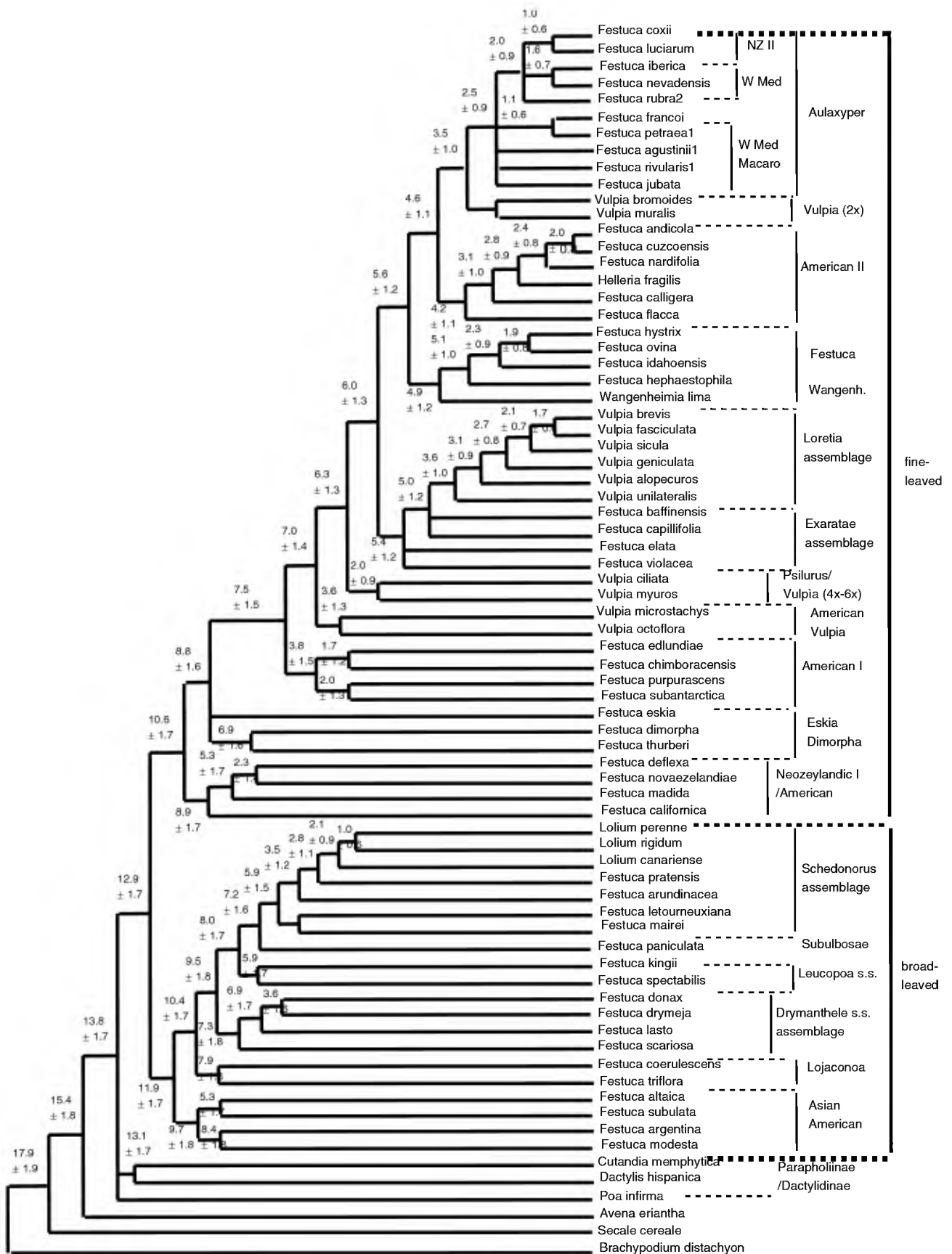


Fig. 2. Divergence times of the main Loliinae lineages and allies calculated using Bayesian relaxed-clock methods. Estimates and their associated standard deviations are indicated for the nodes of the Bayesian tree constructed with a data matrix of 74-terminal taxa. Upper and lower bounds for the age of the Loliinae were based on divergences between Triticeae and Aveneae-Poeae calculated from grass fossil records (see comments in the text).

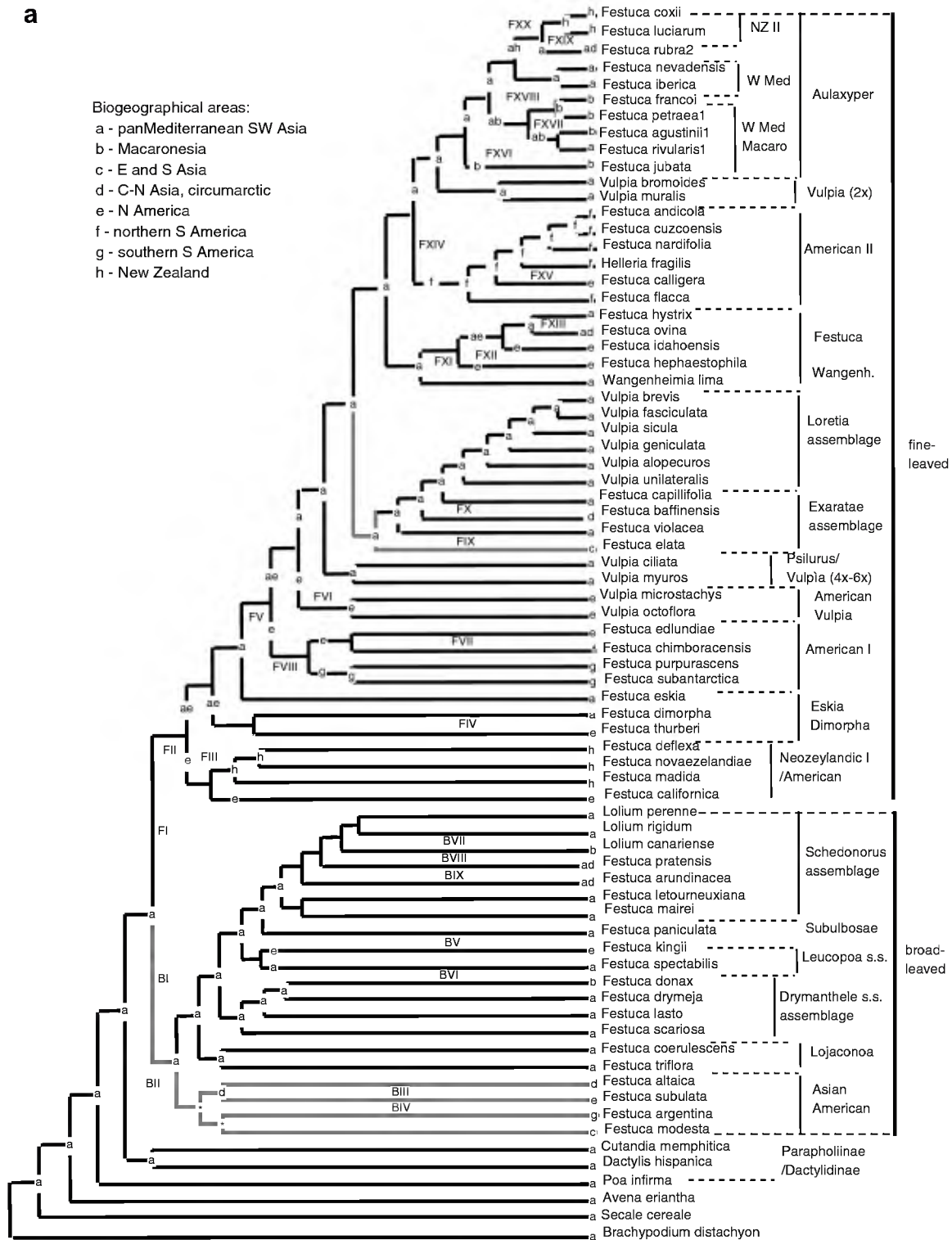


Fig. 3. Biogeographical scenario of Loliinae based on dispersal–vicariance and on dispersal-extinction-cladogenesis (DEC) analyses. (a) The optimization was performed onto an artificially (neighbour-joining) resolved tree that was congruent with the combined Bayesian tree used in the dating analysis, using eight geographical regions and imposing a constraint of two maximal ancestral areas and a between-adjacent-areas probability transition matrix (Table 2; M1 model). Grey lines indicate low probability support for the ancestral areas and for dispersals and extinctions events for those lineages; asterisks reflect conflicts in inherited ancestral ranges. Topological units correspond to molecular branch lengths used. (b) Roman numerals refer to the colonization routes of the broad-leaved (BI-BIX) and fine-leaved (FI-FXX) Loliinae lineages indicated in the map.

subgeneric and sectional assignments of some taxa have varied according to more detailed morphological surveys (Chen et al., 2003).

The unexpected placement of some broad-leaved lineages within the fine-leaved clade might have resulted from biological and historical phenomena other than simply sys-

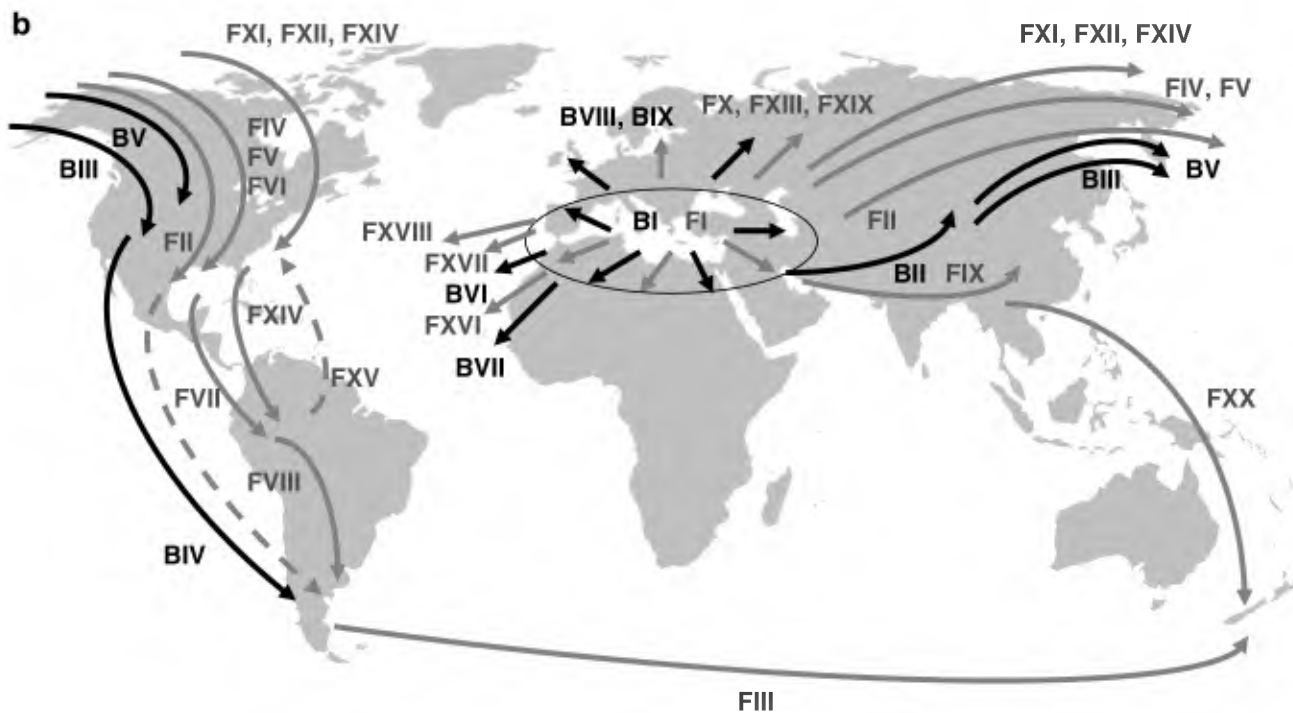


Fig. 3 (continued)

tematic misattributions. It could be possible that the traits related to the broad-leaved syndrome evolved secondarily within the fine-leaved clade, though these features seem to be rather plesiomorphic (Catalán, 2006; Catalán et al., 2007). Alternatively, a past history of colonization and hybridization might have been involved in the origin of the polyploid American clades I and II (Fig. 1).

Another remarkable finding was the distinct phylogenetic placements of the two morphologically close Neozeylandic *Festuca* clades within the fine-leaved clade (Fig. 1). The strongly supported group of apparently fine-leaved hexaploid unclassified *Festuca* species (Neozeylandic clade I) was resolved as sister to North American broad-leaved tetraploid *F. californica*, whereas the weakly supported group of extravaginal octoploid fine-leaved taxa (Neozeylandic clade II) was embedded within the most recently evolved *Aulaxyper* core clade, confirming previous attributions of most of these species to the red fescues (Connors, 1998). The unexpected sister relationships between *F. californica* and the Neozeylandic clade I might reflect a geological history of long intercontinental dispersals (see comment below).

Our combined study has extended the range of polyphyly previously indicated for the broad-leaved *Festuca* subgenus *Leucopoa* and *Subulatae* (Catalán et al., 2004, 2007) and has detected it within subgenus *Drymanthele*. Within *F.* subgen. *Leucopoa*, the two studied taxa of *F.* sect. *Leucopoa* were placed in an intermediate position within the broad-leaved core clade (*F. kingii* and *F. spectabilis*), whereas the three studied representatives of *F.* sect. *Breviaristulatae* were respectively attached to the Neozeylandic I clade (*F. californica*) to the basal region of the

fine-leaved clade (*F. thurberi*), and to a *Subulatae* s.str. clade of unclear placement (*F. altaica*) (Fig. 1). These results, together with the doubtful placement obtained for the *F.* sect. *Amphigenes* s.str. clade, confirm previous findings by Darbyshire and Warwick (1992) on the artificiality of this subgenus and its sectional divisions that are in need of revision. However, these taxa might have been influenced by some sort of horizontal plastid gene transfer, as the ITS phylogeny recovered a more coherent *Leucopoa* (+*Subulatae* s.str.) lineage (Results not shown). The strongly supported paraphyletic relationships of the tall and highly robust *Festuca* subgen. *Drymanthele* sect. *Phaeochloa* taxa with representatives of sects. *Scariosae* and *Pseudoscariosa* have been confirmed by the addition of *F. donax* to this group. By contrast, other species formerly attributed to *F.* subgen. *Drymanthele* fell into other clades (e.g., the broad-leaved Asian–American clade that includes a representative of *F.* subgen. *Drymanthele* sect. *Muticae* and the fine-leaved American clade I that includes the only studied species of *F.* subgen. *Drymanthele* sect. *Banksia*; Fig. 1). These results indicate that the systematics of *Festuca* subgenus *Subulatae*, reflected in the disparate placements of its members in the *Subulatae* s.str. clade and in the fine-leaved American clade II supported by both genome data (Fig. 1), but also in the broad-leaved Asian–American clade (*F. breviglumis*, supported by plastid data alone), also require a deeper systematic study. In agreement with the findings of Darbyshire and Warwick (1992), the close relationship recovered between the single representative of *Festuca* subgen. *Subuliflorae* (*F. subuliflora*) and *F. subulata*, two western North

American and morphologically similar taxa (Alexeev, 1980), would support the merging of both taxa under *F.* subgen. *Subulatae*.

The larger taxon sampling within the most recently evolved fine-leaved *Festuca* and *Aulaxyper* clades has allowed us to differentiate cryptic taxonomic and geographic groups within both assemblages; however, different biogeographical adaptive scenarios could be interpreted for each group. The strongly monophyletic *F. ovina* group shows a limited geographical coverage in the holarctic region, whereas the well supported *F. rubra* group broadly extends into the northern and southern hemispheres. This might be a consequence of the biological traits (cespitose habit) and limited crossing abilities of the *F. ovina* group, with many diploid elements that are morphologically similar to each other but do not easily form intergeneric crosses. By contrast, the rhizomatose and highly interfertile *F. rubra* taxa, usually with high ploidy levels, spontaneously hybridize with other Loliinae genera (Ainscough et al., 1986; Catalán, 2006). Past hybridization events within this group could be deduced from the close relationships found between the *Aulaxyper* core clade and the *Vulpia* (2x) and American I clades (Fig. 1). In addition, there is considerable morphological variation among the basal diploid Macaronesian lineages that were traditionally included as members of *Festuca* sect. *Amphigenes* (Saint-Yves, 1922). The adaptive success of the more aggressive *F. rubra* s. l. taxa might have played a role in their rapid inter and postglacial extension around the world.

4.2. Temporal and spatial biogeographical scenarios of the Loliinae

The origin of most of the Loliinae lineages and of other temperate grasses has been usually associated with the recent geological and climatic changes of the late Tertiary and the Quaternary periods (Stebbins, 1985; Charmet et al., 1997; Balfourier et al., 2000; Fjellheim et al., 2006). Previous molecular-clock estimates deduced from the divergence between rice and wheat indicate a late Miocene (9 My) origin of the broad-leaved and fine-leaved *Festuca* lineages (Charmet et al., 1997), supporting these recent historical scenarios. There have been several new developments, however, which might require re-dating of *Festuca* origins. First, evidence indicates that grassland ecosystems arose earlier in both hemispheres than previously believed (Jacobs et al., 1999; Kellogg, 2001), providing support for a more ancient origin of the grasses (Kellogg, 2001). Also, new molecular timescales that depart from constant rates of evolution have been proposed for the angiosperms (Wikström et al., 2001; Bell et al., 2005) and for the grasses (Gaut, 2002) based on non-parametric rate smoothing, penalized likelihood, and Bayesian-relaxed clock methods. The calculation of the tempo and mode of evolution of most grass lineages has been hampered, however, by the lack of reliable fossil records. The earliest grass pollen fossils dated back to the boundaries between the Upper Cre-

taceous (70 My) and the Paleocene (55 My) (Linder, 1987; Kellogg, 2002, 2001). The recent discovery of grass-like phytoliths in dinosaur coprolites points towards to an early evolution of grasses during the latest Cretaceous (80–75 My) in Gondwana (Prasad et al., 2005). Penalized likelihood estimates suggested that the Poaceae originated around 77 My (Gaut, 2002), assuming a 50 My divergence between maize and rice (Stebbins, 1981; Wolfe et al., 1989), an age apparently concordant with the upper bound estimates based on fossil data (Prasad et al., 2005). However, as it was not clear whether the Maastrichtian (Cretaceous) pollen grains attributed to the Poaceae correspond to the grass family or to other superficially similar sister families (Kellogg, 2002), we chose the 80–55 My time span as a feasible upper and lower bounding criterion for the origin of the grasses to calculate the time of divergence between the temperate Triticeae and Aveneae-Poeae split. The estimate of 20.9 ± 4.9 My covered that proposed by Gaut (25 My) for the divergence between oats and barley using non-parametric rate smoothing methods (Sanderson, 1997); this allowed us to set new upper and lower bounds for the timing of divergence of the Loliinae. According to our estimates, the Loliinae originated in the mid Miocene (c. 13 My) (Fig. 2), predating the ages proposed by Charmet et al. (1997) and concurring with similar dates calculated for the origin of *Hordeum* (Blattner, 2006). Also, the period of global climatic cooling and drying that expanded during the Miocene produced an extension of grassland open habitats on earth that likely fostered a fast diversification of the drought tolerant temperate pooid grasses (Kellogg, 2001) and eventually C₄ grasses in the chloridoids (ca. 7 My) and panicoids (Peterson et al., 2007).

The cores of broad and fine-leaved *Festuca* lineages radiated at approximately the same time (10.5 My) and this is in contrast to previous suggestions on an earlier origin of the broad-leaved group (Hackel, 1882; Holub, 1984; Tzvelev, 1971). Our analysis indicates that the broad-leaved Asian–American clade apparently diverged earlier (12 My). The successive splits of the basal and intermediate broad and fine-leaved lineages took part during the late Miocene (10–5 My), with diversification patterns of Asian–American (9.7 My) and Mediterranean (7.9–5.4 My) lineages that were likely affected by the earlier formation of the Eocene North Atlantic and Miocene Bering Land Bridges (Tiffney, 1985) and the aridity crisis of the Messinian period (6–5 My) that created new connections between Europe and Africa (Hsü et al., 1977). Evidence of those historical events is reflected in the present vicariant distribution patterns observed for several representatives of those groups on both sides of the Bering Land Bridge and groups near the Mediterranean sea after the uprising of the Gibraltar strait (5 My; Bocquet et al., 1978). The most recently evolved Loliinae lineages diverged during the Pliocene (5–2 My) and the Pleistocene (2–1 My) when the glacial and interglacial climatic oscillations forced plant populations to retreat to lower latitudes and altitudes

and to colonize newly deserted areas during the glacials and interglacials (Hewitt, 1996). Those events involved subsequent population contractions, genetic drift, and potential extinctions followed by expansions, secondary contacts and hybridization events (Hewitt, 1996, 2000). The origin of the European (3.5 My) and Mahgrebian (ca. 3 My) *Schedonorus* lineages could be dated back to those times, with ages for the split between meadow fescue and ryegrasses (2.8 My), and diversification of *Lolium* (2 My) slightly older than previously estimated (Charmet et al., 1997; Fjellheim et al., 2006). Nonetheless, caution should be taken about the estimates obtained for allopolyploid hybrid lineages of topological intermediate or subbasal placement (i.e. *F. arundinacea*, c. 3.5 My) which could not have predated the origin of their diploid genome-donor parents (i.e. *F. pratensis*, c. 2.8 My). As a consequence of the extended reticulation affecting most of the polyploid *Festuca* lineages, the estimates of those polyploid nodes should be considered approximate. The bursts of the phylogenetic annual Mediterranean *Loretia* (3.1 My) and *Psilurus* + *Vulpia* (4x–6x) (2.0 My), and American *Vulpia* (3.6 My) assemblages were nearly contemporaneous. The transition between the Tertiary and the Quaternary was the time interval for the diversification of the American clades I (3.8 My) and II (4.2 My), the holarctic *F. ovina* group (3.1 My), and the most recently evolved *Aulaxyper* s.str. group (2.5 My). The radiations of the youngest lineages of the Eurasian *F. ovina* group (1.9 My), the western Mediterranean red fescues (1.6 My), and the Neozeylandic red fescues (1.0) were coincident with the major postglacial expansion of the early Pleistocene (Hewitt, 1996).

The biogeographical analyses conducted with DIVA and LAGRANGE allowed us to reconstruct the first spatial and chronological scenario for the colonization patterns of the Loliinae. The complicated reticulate history of these grasses was unveiled through the successive analyses of diploid and more complete diploid + polyploid distribution data matrices and of between-adjacent-areas constrained dispersal DEC models (M1) (Fig. 3). The final results depicted a common scenario of origin for both broad-leaved and fine-leaved Loliinae lineage in the pan Mediterranean–SW Asian region followed by colonization of eastern and southern Asia, and different migrations to the New World and to the Old World Neozeylandic region (Fig. 3). These results are highly congruent with present ploidy level distribution patterns that indicate the presence of diploid *Festuca* races in the Mediterranean region, Europe and western–central Asia with only one diploid native species in North America and an absence of native diploids in the southern hemisphere (Dubcovsky and Martinez, 1992). Dubcovsky and Martinez (1992) also indicated that tetraploids predominate in North America, whereas hexaploids and octoploids are more common in South America and New Zealand. Our multiple dispersal pattern hypothesis intuitively matches the current distribution patterns exhibited by the polyploids (Fig. 3). Our geographical reconstruction also alludes to potential hybridization and

polyploidization events within the Loliinae lineages during the Tertiary and the Quaternary ages in both hemispheres.

Two biogeographical scenarios occurred in parallel in time but not in space among the broad-leaved (B series) and fine-leaved (F series) Loliinae lineages since their mid Miocene divergence in the pan Mediterranean–SW Asian region (Fig. 3). Within the broad-leaved series, multiple radiations from the centre of origin likely involved the differentiation of the relict diploid broad-leaved *Lojaconoa* and *Drymanthele* + *Scariosae* lineages (BI), though distinct range shifts due to climatic changes at that time (12 My) might have occurred. From this area two potential migrations of putative ancestral *Drymanthele* lineages to central–eastern and northeastern Asia (BII) probably occurred, with the later lineage successively differentiating into tetraploid species of *F.* subgen. *Leucopoa* and *Subulatae* and colonizing western North America (BIII) through the Bering Land Bridge in the late Miocene (5.3 My). However, a shorting sampling of Asian–American broad-leaved taxa might have caused the uncertainties of inherited ancestral areas detected within the basal splits of this broad-leaved clade in the DEC M1 likelihood model (Fig. 3a). The close phylogenetic relationships among *F. altaica* and *F. subulata* (Figs. 1–3) and the present distribution of taxa of *F.* subgen. *Leucopoa* in central Asia, Siberia, and Alaska (Aiken and Consaul, 1995; Chen et al., 2003) favour this hypothesis. Moreover, the colonization of N America from Asia by these taxa occurred at the same time that *Hordeum* crossed Beringia into western N America (Blattner, 2006). It is, however, more difficult to explain the apparent long-distance dispersal of tetraploid taxa of *F.* subgen. *Drymanthele* from eastern Asia to southern South America (BIV), as deduced from the basal Asian–American clade, although a similar colonization pattern was also found in *Gentianella* (von Hagen and Kadereit, 2001). A further colonization of N America from a separately evolved highly polyploid Eurasian *Leucopoa* lineage (BV) in the late Miocene (5.9 My) would be necessary to explain the distribution of *F. kingii*. The present unstable resolution observed between the nuclear and plastid trees for the *Leucopoa*–*Subulata* s.str. assemblage, as commented above, might be improved with further study.

The origin of the most recently evolved broad-leaved *Schedonorus* lineage occurred in the Mediterranean and dated back to the Messinian period (5.9 My) though the diploid ancestors of the polyploid western Mediterranean clade (2.9 My) probably went to extinction during the glacial ages or have not been found yet. Two Pliocene long-distance dispersal events from the western Mediterranean region would account for the colonization of Macaronesian archipelagos by broad-leaved Loliinae lineages. The first colonization event involved the relict diploid *Drymanthele* line that gave rise to the vicariant continental species *F. lasto* and *F. drymeja* (4.9 My), and the new founder Madeiran endemic *F. donax* (3.6 My) (BVI). The volcanic origin of the island of Madeira (5.3 My; Geldmacher et al., 2000) predated the estimated age of the colonization.

Our dating is in agreement with studies on some Macaronesian flora that have recently proposed an early origin of those angiosperm taxa (e. g. Lauraceae) (Chanderbali et al., 2001; Carine et al., 2004). The genetic similarity between the sister taxa *F. donax* and *F. drymeja* has been further corroborated by cytogenetic data (Dawe, 1989) and successful artificial crosses resulting in fertile offspring (Borrill et al., 1977). The second colonization event was facilitated by an ancestor of the young diploid Mediterranean–SW Asian *Lolium* (2.1 My) which probably migrated from the western Mediterranean area and colonized the already formed volcanic islands, speciating into *L. canariense* in the Canarian, Madeiran, and Cape Verde archipelagos (BVII). A larger sample of individuals per species might help discriminate between single versus multiple colonization scenarios for this Macaronesian taxon. The divergence estimates obtained for the most recently evolved Mediterranean ray-grasses (*L. perenne*/*L. rigidum*; 1 My) suggest an interglacial Pleistocene origin for these fodder grasses, contradicting the previous hypothesis that their origin was human-mediated (Balfourier et al., 2000) (Fig. 3). Two recent Pleistocene postglacial range expansions probably caused the continental dispersals of *F. pratensis* and *F. arundinacea* from circumMediterranean refugia towards more northern latitudes (BVIII, BIX).

The biogeographical patterns of the fine-leaved series also showed a large radiation of ancestral diploid lineages in the pan Mediterranean–SW Asian region of origin (FI) that were obscured by the addition of the putative early split of the polyploid Neozeylandic–American clade (Fig. 3). The interpretation of the complex historical distribution of this clade, strongly supported at its basal position by plastid but not by nuclear data, would require an early colonization event from Eurasia into North America (FII) in the late Miocene (8.9 My) followed by further transoceanic long-distance dispersals from America to New Zealand (FIII) in the transition towards the early Pliocene (5.3 My). This pattern implies that a putative morphologically-intermediate polyploid *Leucopoa* lineage colonized western North America through the Bering Land Bridge before expansion in N America. Assuming that the plastid relationships are correct (Fig. 1), colonization of New Zealand was more likely to have occurred from South America than from North America, implying that the southern American clade relatives are presently extinct or have not yet been sampled. The strong floristic affinities between the South American and the Neozeylandic regions, stressed by Takhtajan (1986), were later confirmed by Soreng (1990) in his biogeographical molecular (plastid) study of the temperate grass genus *Poa*. According to this author, the link between the New World and the New Zealand bluegrasses stemmed from dispersal from North America to South America and then to New Zealand rather than from Asia. With respect to the Neozeylandic–American clade I of Loliinae, even if the New Zealand taxa originated from a polyploid fine-leaved Asian migration, a long-distance transoceanic dispersal would be necessary to explain

the apparent New World maternal origin of those Neozeylandic plastid genomes.

A new Asian–North American migration (FIV) would have resulted in the present distribution of *F. thurberi*. The unsatisfactory phylogenies and geographical hypotheses near the basal *Leucopoa* lineages might be improved in future studies. Successive colonizations from the Old World to the New World and these subsequent radiations resulted in the American clade I (FV) and the North American *Vulpia* clade (FVI) in the late Miocene. The geographic trend for the American clade I fescues included two different migrations from North America to northern South America (FVII) and to southern South America (FVIII), respectively. Long-distance dispersals between North America and the Patagonian–Andean region have been proposed in *Hordeum* (Blattner, 2006) and other angiosperms (Vijverberg et al., 1999). However, a larger investigation of these American fescues would be required to clarify the patterns within this later lineage. Early Pliocene range expansion from the pan Mediterranean–SW Asian region to eastern Asia (FIX) and to the circumarctic zone (FX) gave rise to the *F. elata* (5.4 My) and the *F. baffinensis* (5.0 My) lineages, respectively, concomitantly with the radiation of the EuroMediterranean *Exaratae* (5.4 My) lineage. Speciation processes within the *Loretia* assemblage (3.6–1.7 My) apparently occurred from western to eastern Mediterranean (Fig. 3).

The radiation of the large and homogeneous *Festuca* group implied at least two independent colonizations from Eurasia into N America (FXI, FXII) in the late Pliocene–early Pleistocene times (3–2 My) followed by the more recent burst of Eurasian taxa of the *F. ovina* group, which probably moved southwards and then expanded northwards (FXIII) in the last glacial phases. A new Eurasian–American colonization event of a putative *Aulaxyper* ancestor (FXIV) is necessary to explain the biogeographical pattern of the American clade II. Although the elements of this clade are mostly restricted to South America, the presence of some of them (*F. calligera*, and *F. arizonica*—not sampled here) plus some other congeneric relatives (*Hellerochloa*) in North America would support a migration route via the Bering Land Bridge and a further expansion into South America (4.2 My). Otherwise, the origin of *F. calligera* would require a back-colonization of Southern American ancestors into North America (FXV). The intricate phylogeny of the core *Aulaxyper* group suggested multiple dispersals of late Pliocene–early Pleistocene (2.5–2.1 My) diploid basal lineages from the western Mediterranean region to Macaronesia and survival of the relic *F. rivularis* in SW Europe. From the phylogeny it can be deduced that probably three independent colonizations originated the Madeiran *F. jubata* (FXVI), the Canarian *F. agustinii* (FXVII), and the Azorean lineages (FXVIII) that further experienced a potential ecologically vicariant in-situ speciation event forming the sister taxa *F. francoi* and *F. petraea*. The Canarian and Azorean taxa apparently originated contemporarily with the volcanic eruptions of

the youngest western Canarian (2–1 My) and western Azorean (2.5–0.5 My) islands, though the first colonizations probably occurred in the central and easternmost older islands (12–6 My) of each archipelago (Carracedo, 1999; Valadao et al., 2002). The biogeographical patterns of the most recently evolved red fescues suggest a recent colonization of the holarctic (FXIX) and the holoantarctic (FXX) areas from Eurasia, the first causing the postglacial expansion of *F. rubra* into northern Eurasian latitudes and the latter the origin of the Neozeylandic clade II. This expansion likely occurred through southern Asia.

The biogeographical scenarios presented here are largely in accord with other historical floristic studies that indicate a Eurasian origin for temperate plants followed by the expansion towards the nearctic region and then to the southern hemisphere continents and subcontinents (Takhtajan, 1986; Bell and Donoghue, 2005). This has been particularly highlighted in other pooid genera such as *Brachypodium* (Schippmann, 1991), *Poa* (Soreng, 1990) and *Hordeum* (Blattner, 2006), and could be the general rule for the other members of the subfamily Pooideae. The phylogeographical patterns shown here are illustrative of the general geographical trends of the main *Festuca* lineages and its allies, however, a more detailed phylogenetic study will be required to clarify the biogeography and phylogeography of more inclusive groups.

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