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Festuca inops and *Festuca gracilior* (Poaceae): are they two different species?

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According to current systematics, *Festuca inops* and *F. gracilior* are two distinct species. However, they are hardly distinguishable from each other on the basis of their morphological characters. *Festuca inops* is considered a diploid species endemic to Italy, while *F. gracilior* has a discontinuous distribution area, apparently related to chromosomal levels: diploid populations in Italy and south-east France, tetraploid populations in north-east Spain. The diploid populations of both taxa from Italy and south-east France are investigated in the present study. Nearly 1000 exsiccata were examined and morphometric analysis was carried out on macro- and micromorphological features of 119 specimens (including type-specimens) and on 20 natural populations (including *loci classici*). All these data showed that the two species should be referred to a single taxon, for which the rank of species seems to be appropriate. This result is supported by karyological, ecological and chorological data and was confirmed by the results of ISSR analysis. According to nomenclatural rules, the legitimate name for the species is *Festuca inops* De Not. *Botanical Journal of the Linnean Society*, 2006, **151**, 239–258.

ADDITIONAL KEYWORDS: biosystematics – chromosome numbers – France – Italy – morphometric analysis – ISSR – type specimens.

INTRODUCTION

Since Hackel's fundamental *Monographia Festucarum Europaearum* (Hackel, 1882), the systematics of the complex *Festuca* genus in Europe have advanced thanks to the efforts of many authors. Among these Saint-Yves (1909, 1913, 1930) and other festucologists belonging to the francophone biosystematic school such as de Litardière (1923, 1945), Bidault (1964, 1969), Auquier (1974, 1977), and Kerguélen (1975 1983, 1987) presented valuable regional information and methodological contributions. In addition, monographs and general reviews were recently published by Wilkinson & Stace (1991), Kerguélen & Plonka (1989), Portal (1999), de la Fuente & Ortuñez (1998, on the section *Festuca*), de la Fuente, Ferrero & Ortuñez (2001), and Conert (1996). Unfortunately, with the exception of the latter, these studies were focused more on geographical regions than on natural groups of taxa, and involved investigations carried out within national political boundaries. This has led in some cases to abrupt interruptions in distribution areas of critical taxa, resulting from different interpretations of their systematic position in neighbouring countries. Other contributions (e.g. Al Bermani, Catalán & Stace, 1992; Foggi, Rossi & Signorini, 1999) have taken into consideration groups of closely related taxa as a whole and have been useful in clarifying the systematics of critical entities.

The case of *F. gracilior* and *F. inops* is a good example of how taxonomic confusion can arise from studies based on a limited distribution area within national limits.

BACKGROUND INFORMATION ON *F. INOPS* AND *F. GRACILIOR*

Festuca inops was originally described by De Notaris (1844) for Liguria, in north-west Italy. Hackel (1882) considered this taxon as a subvariety of *F. ovina* var.

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glauca, with a distributional range extended to a larger area in the northern Apennines (Liguria and Tuscany).

Festuca inops De Not., Repert. Fl. Ligust. 466 (1844)
Type: '*Festuca inops* Dntrs/Monte Gazzo/6.1843'; *lectotype:* designated by Mariotti (1995) in GDOR!
≡*Festuca ovina* L. var. *glauca* Hack. subvar. *inops* (De Not.) Hack. Monogr. Festuc. Eur. 95 (1882)

Festuca gracilior was described by Hackel (1882) as a subvariety of *F. ovina* L. var. *duriuscula* (L.) Koch, with a distributional area from eastern Spain (Montserrat) to south-east France and Italy (Tuscany). It was later raised to the rank of species by Markgraf-Dannenberg (1978).

Festuca gracilior (Hack.) Markgr.-Dann., Bot. J. Linn. Soc., 76: 325 (1978)
Type: *Festuca ovina* var. *duriuscula* subvar. *gracilior* Hack. 'Près Bouyon, Massif du Cheiron, Alpes Maritimes, leg. E. Burnat'; *lectotype:* designated by Kerguélen (1987) in G!, syntipi in W! (n. 9341, 9338, 9339)
≡*F. ovina* L. subvar. *gracilior* Hack., Monogr. Festuc. Eur. 90 (1882)

In Hackel's systematic opinion, the two *taxa* were fundamentally distinguished on the basis of the glaucous colour of leaf blades, as the main discriminating character between *F. ovina* var. *duriuscula* and *F. ovina* var. *glauca*. Hackel himself stated, however, that the two species are hardly distinguishable based on herbarium specimens and that the wax layer which causes the typical pruinosity of the leaf blades is often not maintained under cultivation (Hackel, 1882: 94). On this subject, Auquier & Kerguélen (1977), in a study on *F. glauca* Auct., reported that many taxa described within the *F. ovina* group merely correspond to individuals belonging to highly variable populations, including, for instance, both pruinose and not pruinose plants. In his recent monograph on the genus *Festuca* in France, Portal (1999) argued that *F. gracilior* can be more or less glaucous and pruinose. Our direct observations in the field on populations identified either as *F. inops* or *F. gracilior* showed that leaf colour and pruinosity can vary even within single populations and that these characters are not maintained in plants grown in Florence Botanic Garden 'Giardino dei Semplici' under controlled conditions. Consequently, in these taxa the glaucous colour of leaves appears to be of no systematic value.

In *Flora Europaea* (Markgraf-Dannenberg, 1980: 146, 148) and in *Flora d'Italia* (Pignatti & Markgraf-Dannenberg, 1982: 495–496), *F. inops* and *F. gracilior* are reported as two distinct species; however, the authors fail to indicate any macro- or micromorphological character which clearly discriminates between the two species. Furthermore, according to the cited Floras, the distribution areas of the two taxa partially overlap in north central Italy.

According to *Flora Europaea* (Markgraf-Dannenberg (1980), *F. gracilior* is a diploid species with $2n = 14$ (Parreaux, 1972, cited in Moore, 1982) growing in south-east France and north central Italy, but it is excluded from Spain. The karyological datum for the species in France was confirmed by Kerguélen (1975; sub *F. occitanica*), Bidault in Kerguélen [1975; sub *F. glauca* var. *exilior* (St.-Yves) Bidault and sub *F. duriuscula* L. var. *gracilior* (Hack.) Bidault], Kerguélen & Plonka (1989) and Portal (1999).

In a recent paper on *Festuca* sect. *Festuca* in Spain, Fuente & Ortuñez (1998) reported *F. gracilior* [including *F. tarraconensis* (Litard.) Romo and *F. valentina* (St.-Yves) Markgr.-Dann.] as growing in the north-east part of the country, but with $2n = 28$ (see also de la Fuente *et al*., 2001: 388, 393). More recently, another tetraploid species morphologically close to *F. gracilior* has been described for eastern Spain: *F. michaelis* (Cebolla & Rivas Ponce, 2001).

According to the most recent French contributions (Kerguélen & Plonka, 1989; Portal, 1999), *F. gracilior* surprisingly is not present in the area that spans the Spanish border and the Rhone: in this region it is apparently substituted by *F. occitanica* (Litard.) Auquier & Kerguélen, a tetraploid species ($2n = 28$) morphologically close to *F. gracilior*, but distinguished from it by its lemma awn always > 1.5 mm.

In Italy, *F. gracilior* grows in the north central part of the country (Liguria and Tuscany), cf. *Flora d'Italia* (Pignatti & Markgraf-Dannenberg, 1982), whereas *F. inops* De Not. is said to be endemic to the north and central Apennines and Apuan Alps (cf. *Flora Europaea* and *Flora d'Italia*). Bechi & Miceli (1995) reported the chromosome number $2n = 14$ for this last species.

All of the background information on these two species, as illustrated above, is summarized in Figure 1, where the distribution area of *F. gracilior* appears to be subdivided into two nonoverlapping subareas, correlated with a different chromosomal level: diploid populations east of the Rhone, and tetraploid populations west of it. No diploid species of *Festuca* morphologically close to *F. gracilior* has been reported to occur west of the Rhone. The diploid populations attributed to *F. gracilior* (eastern part of the distribution area) and to *F. inops* that grow in south-east France and Italy, including populations from both *loci classici*, are partially sympatric and appear to be indistinguishable on a morphological basis (see also Gherardi, Signorini & Foggi, 2003).

The aim of the present study is to verify whether these diploid populations from south-east France and north central Italy should really be referred to as two different taxa or not. The well-separated tetraploid populations from south-west France and north-east Spain are tentatively assumed to belong to a distinct taxon (or possibly even more than one taxon, as in

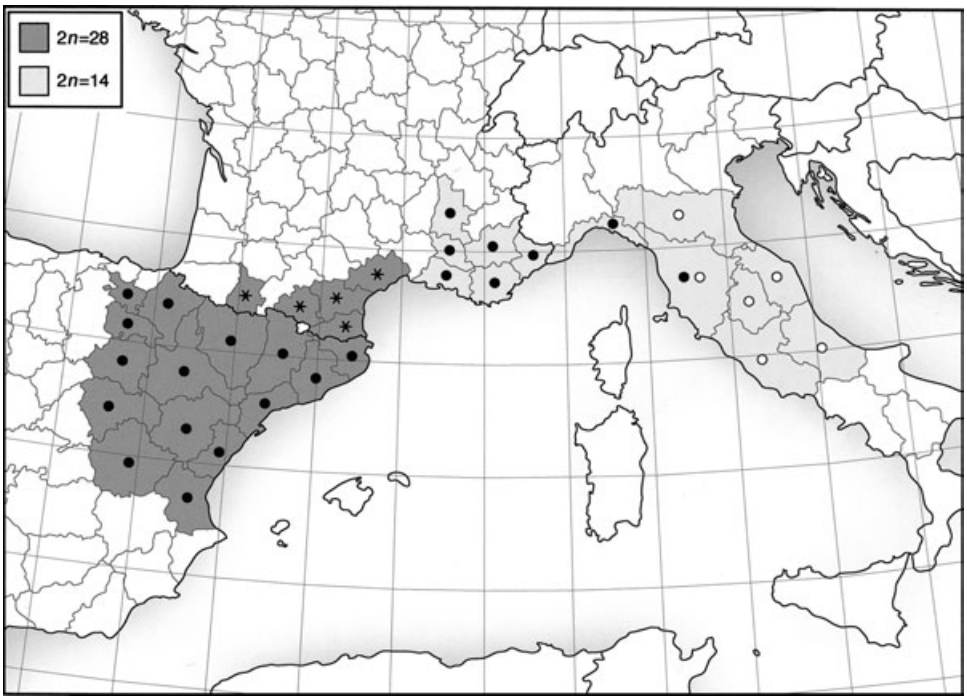


Figure 1. Geographical distribution and karyological data of *Festuca inops* (○) and *F. gracilior* (●), according to the current systematics. Asterisks: *F. occitanica.* Spain: data from de la Fuente & Ortuñez (1998), de la Fuente *et al*. (2001); France: data from Kerguélen & Plonka (1989), Portal (1999); Italy: data from Pignatti & Markgraf-Dannenberg (1982).

Cebolla Lozano & Rivas Ponce, 2003) and will be the subject of subsequent investigations.

As classical morphological analyses have proven in most cases to be insufficient to clarify the systematic status of many critical taxa, we decided to integrate them with investigations on genetic divergence among populations. Inter-simple sequence repeat (ISSR) is a PCR technique that uses repeat-anchored or nonanchored primers to amplify DNA sequences between two inverted SSR (Zietkiewicz, Rafalski & Labuda, 1994). ISSR markers are highly reproducible owing to their primer length and to the high stringency achieved by the annealing temperature, and have been found to provide highly polymorphic fingerprints (Zietkiewicz *et al*., 1994; Moreno, Martin & Ortiz, 1998). The applicability of ISSR-PCR for genomic fingerprinting at the interspecific level and for inferring genetic relationships among related species has been indicated by Zietkiewicz *et al*. (1994) and Wolff & Morgan-Richards (1998).

MATERIAL AND METHODS

Morphometric analysis

Phenotypic variability was investigated both in the field and on exsiccata identified either as *F. gracilior* or *F. inops* belonging to the following herbaria AQUI, BC, CAME, FI, FIAF, G (general herbarium, herb. Litardière, St.-Yves, Burnat), GE, GDOR, MAF, PAV, RO, SIENA, W, Z and from the following personal collections: S. Ballelli (Camerino), F. Conti (Camerino), D. Marchetti (Massa), R. Portal (Vals près Le Puy), O. Rinaldi (Perugia), L. Lombardi (Firenze). Furthermore, we personally collected plants from several localities in France and Italy (exsiccata in FI, FIAF and PAV). In total nearly 1000 exsiccata were examined. Localities of studied samples are listed in Table 1.

On 119 specimens, including type specimens of both species, the morphometric data were scored and subsequently used to perform a cluster analysis. With the exception of the types, the specimens were chosen randomly among those showing all the characters to be measured. Twenty morphological and anatomical characters considered as diagnostic in recent Floras and pertaining both to vegetative and reproductive organs were taken into account. They are listed in Table 2, where their respective range of variation in *F. inops* and *F. gracilior* are also included according to *Flora Europaea* (Markgraf-Dannenberg (1980) and *Flora d'Italia* (Pignatti & Markgraf-Dannenberg, 1982). Fifteen of these characters (marked with asterisks in Table 2) were also examined on 178 individuals belonging to 20 populations sampled in the field. These were later subjected to Canonical Discriminant Analysis (CDA) in order to assess the degree of differentiation of the populations by multivariate

Table 1. Specimens and populations of *Festuca gracilor* and *F. inops* tested in morphometric analyses (cluster analysis and CDA) and in molecular investigations. First column: individuals subjected to cluster analysis, number of specimens (OTUs) as in Figure 3. Last column: populations tested in CDA and molecular investigations, numbers of populations as in Figures 2, 4, 19, 20.

Specimens	Country	Region	Locality	Populations (population number/number of individuals)
1	Italy	Tuscany	Isola d' Elba, Madonna del Monserrato (LI)	(2/8)
2	Italy	Tuscany	Barberino del Mugello, Pimonte (FI)	
3	Italy	Tuscany	Oliveto dei Cavalleggeri (GR)	
4	Italy	Tuscany	Riparbella (LI)	
5	Italy	Tuscany	Pratofiorito (LU)	
6	Italy	Tuscany	Capo d' Uomo, Monte Argentario (GR)	
7	Italy	Tuscany	Balzo Nero (LU)	(3/9)
8	Italy	Tuscany	Rif. Gobie, Alpi Apuane (LU)	
9	Italy	Tuscany	Monte Nero, Ulignano (PI)	
10	Italy	Liguria	Passo Cento Croci, Varese Ligure (SP)	
11	Italy	Toscana	Castiglione, Mommio. (MS)	
12	Italy	Emilia Romagna	Passo Montevacà – Bedonia (PR)	
13	Italy	Tuscany	Monte Coronato – Monte Fegatesi (LU)	
14	Italy	Tuscany	Val di Lima (LU)	
15	Italy	Tuscany	Ponte Canigiano, Corfino (LU)	
16	Italy	Tuscany	Vecchiano (PI)	
17	Italy	Tuscany	Foce di Petrosciana, Alpi Apuane (MS)	
18	Italy	Tuscany	Carrodano Superiore, Val di Vara (SP)	(9/6)
19	Italy	Tuscany	Monte Alto, Vico Pancellorum (LU)	
20	Italy	Tuscany	Impruneta, Sassi Neri (FI)	(6/12)
21	Italy	Tuscany	Monte Beni (FI)	
22	Italy	Tuscany	Piazza al Serchio (LU)	
23	Italy	Tuscany	Ponte Coccia (LU)	
24	Italy	Liguria	Torrente Amola, Falcinello (SP)	
25	Italy	Liguria	Ponzano Maggiore, Ponzano Magra (SP)	
26	Italy	Liguria	Passo Cento Croci, Varese Ligure (SP)	
27	Italy	Tuscany	Arni, Alpi Apuane (LU)	
28	Italy	Tuscany	Tre Fiumi, Alpi Apuane (LU)	
29	Italy	Tuscany	Passo della Calla (AR)	(5/9)
30	Italy	Liguria	Capo Noli (SV)	
31	Italy	Umbria	Piano Grande, Norcia (TR)	
32	France	Alpes Maritimes	Peille	
33	France	Var	La Verdière	
34	France	Vaucluse	Mérindol	
35	France	Vaucluse	Faucon (Buis les Baronnies)	
36	Italy	Liguria	Vaze, Capo Noli (SV)	
37	Italy	Liguria	Strada per Manie, Capo Noli (SV)	
38	Italy	Liguria	Ponzano Superiore, Sarzana (SP)	
39	Italy	Tuscany	Rapolano (SI)	
40	Italy	Tuscany	Poggio di Firenze (FI)	
41	Italy	Tuscany	Vagli di Sotto, Alpi Apuane (LU)	
42	Italy	Tuscany	Vetta Pania della Croce (LU)	
43	Italy	Tuscany	Monte Pelato, Alpi Apuane (MS)	
44	France	Alpes Maritimes	Grasse – Pic de Courmettes – Kalkfels	
45	Italy	Umbria	Monte Acuto (PG)	
46	Italy	Tuscany	Torrente Edron – Poggio – A. Apuane – Garfagnana (LU)	
47	Italy	Tuscany	Vagli di Sopra – Giovo – A. Apuane – Garfagnana (LU)	

Table 1. *Continued*

Specimens	Country	Region	Locality	Populations (population number/number of individuals)
48	Italy	Tuscany	Molazzana, Alpi Apuane (MS)	(1/5)
49	Italy	Tuscany	Strada della Foce, Alpi Apuane (MS)	
50	France	Alpes Maritimes	Grasse – Pic de Courmettes – Gorge de Loup	
51	Italy	Umbria	Monte Tenetra (PG)	
52	Italy	Umbria	Pianlonia (PG)	
53	Italy	Tuscany	Pian della Fioba, Alpi Apuane (MS)	
54	France	Herault	Montpellier	
55	Italy	Tuscany	Camaldoli (AR)	
56	France	Hautes Alpes	Moùtiers	
57	Italy	Tuscany	Gli Scopeti (FI)	
58	France	Val d'Aosta	Alpi Graje, Cogne (AO)	
59	Italy	Val d'Aosta	Brissogne (AO)	
60	Italy	Tuscany	Alta Val Tiberina (AR)	
61	Italy	Liguria	Monte Gazzo (GE). Type specimen of *F. inops*	
62	Italy	Liguria	Portofino (GE)	
63	Italy	Liguria	Monte Fascio (GE)	
64	Italy	Marche	Monte Furlo (AN)	
65	Italy	Emilia Romagna	Boccassuolo (RE)	
66	Italy	Emilia Romagna	Montecalvario (MO)	
67	Italy	Emilia Romagna	San Marino (RSM)	
68	Italy	Emilia Romagna	Monte del Castellaccio (FC)	
69	Italy	Emilia Romagna	Casolo (FC)	
70	Italy	Abruzzo	Sirente – Prati di S. Maria sopre Ajelli	
71	Italy	Umbria	Castelluccio, Norcia (TR)	(4/11)
72	Italy	Tuscany	Monterufoli (PI)	(7/13)
73	Italy	Liguria	Monte Gazzo (GE)	(8/12)
74	Italy	Tuscany	Passo della Calla (AR)	
75	Italy	Tuscany	Monte Calvi (LI)	(10/10)
76	Italy	Tuscany	Monte Ferrato (PO)	(11/8)
77	Italy	Tuscany	Piglionico, Alpi Apuane (MS)	(12/9)
78	France	Alpes Maritimes	Bouyon	(13/7)
79	Italy	Tuscany	Cantagallo (PO)	(14/11)
80	Italy	Tuscany	Vallombrosa (FI)	(15/10)
81	Italy	Tuscany	Monte Prata, Massa Marittima (GR)	(16/11)
82	Italy	Tuscany	Monte Gazzo (GE). Specimen grown in pot.	
83	France	Alpes Maritimes	Rochers á formose près le Col de Tende	
84	France	Alpes Maritimes	Gorges des Saorgio	
85	Italy	Marche	Valleremita (MC)	
86	Italy	Marche	Monte Rotondo (PG)	
87	Italy	Marche	Monte Caccamillo (PG)	
88	Italy	Marche	Monte Catria (PG)	
89	Italy	Tuscany	Cornate di Gerfalco (GR)	
90	France	Alpes Maritimes	Près Bouyoun – Massif du Cheiron. Type specimen of *F. gracilior*	
91	France	Alpes Maritimes	Environs de Breil: Tete de Sapet	
92	France	Alpes Maritimes	Grasse quartier St Jacques	
93	Italy	Liguria	Environs d'Albenga (IM)	
94	France	Alpes Maritimes	Entre Signale et les clus du Riolan	
95	France	Alpes Maritimes	Descente du Col de Cuore sus Sospel	
96	France	Alpes Maritimes	Collet Saint André près Bonson	
97	France	Alpes Maritimes	Cime de la Graia, entre l'Escarène et Luceram	
98	France	Alpes Maritimes	Entre Contes et Chateauneuf (Nice)	

Table 1. *Continued*

Specimens	Country	Region	Locality	Populations (population number/number of individuals)
99	France	Alpes Maritimes	Montaigne de l'Audibergue	
100	France	Alpes Maritimes	Vallée de l'Esteron: au amont de Sigale	
101	France	Alpes Maritimes	Entre Gattiers et St. Laurent de Var	
102	France	Alpes Maritimes	Val de la Vesubie: Vallon de l'Infermet, près Lantosque	
103	France	Alpes Maritimes	Massif du Monnier: Cranger du Vignols	
104	France	Alpes Maritimes	Massif de L'Anthion. Vallon de Carios a Maurion	
105	France	Alpes Maritimes	La Turbie	
106	France	Alpes Maritimes	Montagne de l'Audibergue	
107	Italy	Liguria	Sopra Badalucco (IM)	
108	France	Alpes Maritimes	Tra Viève e Tende	(17/10)
109	France	Alpes Maritimes	From Col de Brus to l'Escarène	(18/6)
110	Italy	Emilia Romagna	Belforte (PR)	
111	Italy	Emilia Romagna	Bobbio (PC)	
112	Italy	Emilia Romagna	Val Nure (PC)	
113	Italy	Emilia Romagna	Pietra di Bismantova (RE)	
114	Italy	Emilia Romagna	Valle del Dardagna (BO)	
115	Italy	Emilia Romagna	Groppo di Goro (PR)	
116	Italy	Emilia Romagna	Carpineti (RE)	(19/6)
117	Italy	Emilia Romagna	Bobbio (PC)	(20/7)
118	Italy	Abruzzo	Lecce Vecchia (AQ)	
119	Italy	Molise	M. Mattone (IS)	

Table 2. Morphological characters used in cluster analysis. Characters marked with * were used also in CDA. Ranges of variation of some characters for *F. inops* and *F. gracilior* are indicated according to Markgraf-Dannenberg (1980) and Pignatti & Markgraf-Dannenberg (1982)

Character, units		*F. inops*	*F. gracilior*
CL*	Length of culms, cm (mean of 3 measures)	19–50	20–35
FL*	Length of tiller leaf blades, cm (mean of 5 measures)		
LW	Width of tiller leaf blades, mm	0.4–0.8	0.4–0.8
LT	Thickness of tiller leaf blades, mm		
BN	Number of schlerenchyma strands	7	7
RN	Number of ribs	5	3–5
RT	Thickness of the inner lateral rib (mm)		
PY*	Pruinosity of leaf blade (1 = not pruinose; 2 = slightly pruinose; 3 = pruinose)	2–3	1–3
PL*	Length of the longest panicle, cm	3.5–6.5	4.0–7.5
SK*	Pubescence of panicle branchelets (1 = glabrous; 2 = slightly pubescent; 3 = pubescent)	1–2	1–2
SL*	Length of spikelets, mm (mean of 5 measures)	6.0–7.7	6.5–7.5
PS*	Pubescence of spikelets (1 = glabrous; 2 = slightly pubescent; 3 = pubescent)		
PP*	Length of spikelet pedicel, mm (mean of 5 measures)		
G1*	Length of lower glumes, mm (mean of 5 measures)		
G2*	Length of upper glumes, mm (mean of 5 measures)	2.7–4.6	3.5–4.5
LL*	Length of lemma, mm (mean of 5 measures)	3.9–5.0	4.0–5.5
LP*	Pubescence of lemma (1 = glabrous; 1.5 very slightly pubescent; 2 = slightly pubescent; 2.5 = pubescent; 3 = highly pubescent)		
MP*	Ciliate portion of the palea keels, %		
AL*	Length of awn of lemma, mm (mean of 5 measures)	0–1.0	0.2–1.5
AS*	Length of anthers, mm (mean of 3 measures)		

measurements and to test the impact of individual variables on the discrimination. Specimens and populations tested for multivariate analyses (cluster analysis and CDA) are listed in Table 1. The geographical distribution of these populations is shown in Figure 2.

We adopted standard measurements and terminology (cf. Foggi *et al*., 1999), that comply with Hackel (1882), Saint-Yves (1913), Ellis (1976) and Wilkinson & Stace (1991). Floral characters were observed and measured through a Zeiss stereomicroscope (Stemi SR model) 8–20×. Observations of transverse sections of leaf blades were carried out under a Reichert microscope (Univar model) 100–600×, and their outlines were drawn on transparent paper placed directly on the 25 cm diameter screen of a viewer connected to the microscope.

Of the diagnostic characters used, most (16) were quantitative; two of these were quantitative discrete characters (number of schlerenchyma strands, number of ribs). The remaining four were qualitative discrete characters (pruinosity of leaf blades, pubescence of panicle branchelets, spikelets and lemma); they were coded as multistate characters with ranking scales. A total of 14 continuous and six discrete characters were studied. A matrix scoring 119 OTU (Table 1) for 20 characters was transformed into a dissimilarity matrix by the Euclidean Distance, after a standardization by range, i.e. setting the limits of all variables on the same scale (0 to 1). Agglomerative cluster analysis using average linkage (UPGMA) was performed on the dissimilarity matrix for use as an exploratory data method. Another matrix, composed of 180 specimens taken from the 20 sampled populations (Table 1), scored for 15 characters, was analysed using Canonical Discriminant Analysis (CDA). Both cluster and CDA were performed using the SPSS package (Norusis, 1993).

Characters belonging to the discriminant function were analysed with univariate analysis (median, 10%, 90% and extremes of the variation) by box plots (StatSoft, 1998) to show inter- and intrapopulation variability.

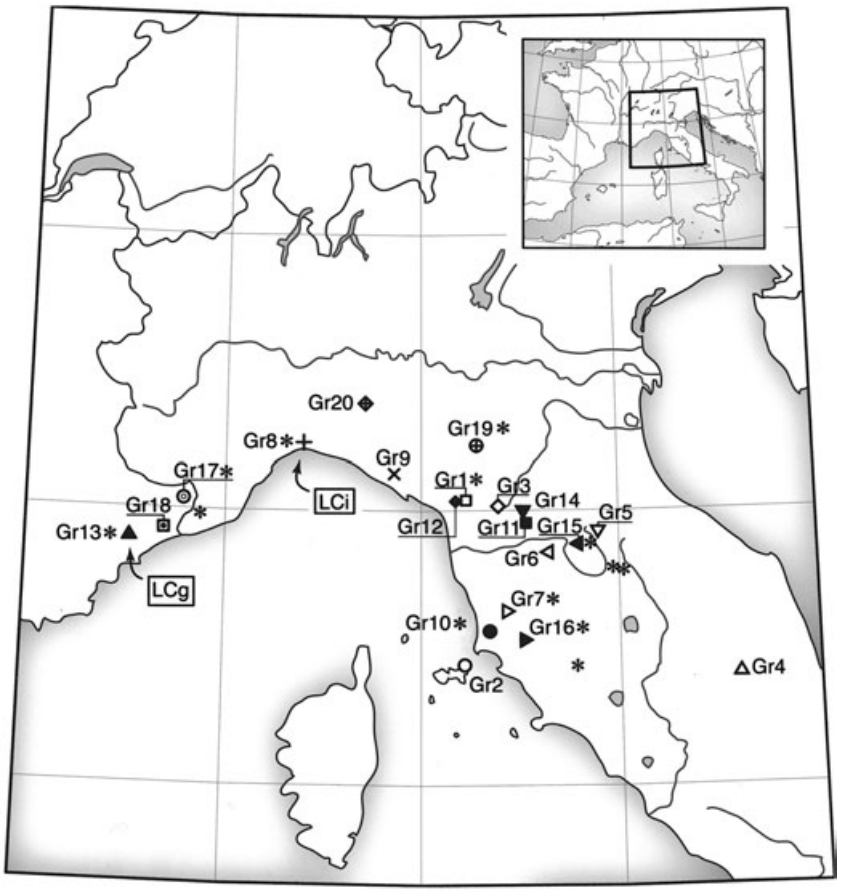


Figure 2. Location of the populations of *F. inops* and *F. gracilior* sampled for morphological and molecular data (Gr 1–20: population numbers as in Table 1) and for karyological analysis (*). LCg, *locus classicus* of *F. gracilior*; LCi, *locus classicus* of *F. inops*.

Observations of epidermal features at SEM

Micro-morphological characters of leaf epidermis (epicuticular waxes and epidermal cells) were observed using Scanning Electron Microscopy (SEM) on dried leaves taken from 20 living plants, one for each population sampled (Table 1). The middle portion of the blade from a mature, undamaged tiller leaf was selected for this analysis. In order to observe in detail the epidermal cells, the external waxes were removed by soaking fresh material in xylene for 24 h followed by ultrasonic treatment for 1 h (Palmer & Tucker, 1981). The samples were observed and photographed under a Philips XL30 SEM. Terminology follows Ellis (1979), Barthlott *et al*. (1998, 2003) and Barthlott & Theisen (2003).

Chromosome counts

Karyological observations were carried out on root tips of 18 plants collected from several localities, including the two *loci classici* (Fig. 2, marked with asterisks) and grown in pots in Florence Botanic Garden. The root tips were pretreated in a mixture of 8-hydroxyquinoline and alpha-Br-naphtalene (1 : 1) for 4 h, then fixed in Carnoy's solution (1 : 3 acetic alcohol) and subsequently transferred to 70° ethylic alcohol. After hydrolysis in 1 N HCl for 4 min at 60 °C, they were stained with Schiff's reagent and then placed on a slide with one or two drops of acetic orcein, mounted and examined under a light microscope (1000×).

ISSR analysis

In total, 180 specimens sampled from 20 natural populations from Italy and south-east France identified either as *F. gracilior* or *F. inops* were tested. These are the same populations that were used for the morphological analysis as described before (Table 1). Thirteen specimens, belonging to the same population from south-west France (Mt. Tauch-Gorges de la Vedouble, Aude) and identified as *F. occitanica*, were used as outgroups in the molecular analysis. The DNA was extracted from 5–10 mg of dry leaf tissue using a Qiagen DNeasy plant extraction kit. A subset of the samples was used to screen ISSR primers for polymorphism. PCR was performed in a 25-μL mixture containing 20 ng of template DNA, 200 μM each dNTP, 4 μM ISSR motif primer, 2.5 μL of 10× buffer and 0.5 U of Taq polymerase (SIGMA). The thermal cycling for all PCR reactions comprised 40 cycles, each with 20 s denaturation at 93 °C, 1 min annealing at 50 °C and a 20 s extension at 72 °C, followed by a final extension of 6 min at 72 °C. The amplification products (10 μL) were separated using standard 1.2% agarose gels in 1× TBE buffer and stained with ethidium bromide. The results were scored as presence (1) or absence (0) of bands and assembled into a data matrix table. Nei's unbiased genetic distances (Nei, 1978) were calculated among populations with NTSYS-pc software (Rohlf, 1993). Matrices of Nei's genetic distances were used to cluster the populations by the unweighted pair group method with an arithmetic mean (UPGMA, using SAHN in NTSYS) and by the neighbour-joining method (NJOIN in NTSYS). The goodness-of-fit of the phenograms to the distance matrix data was tested by cophenetic correlation (COPH in NTSYS). In order to check for a correlation between the Nei's genetic distance matrices and geographical distances (in km) among populations, Mantel tests (Mantel, 1967) were employed (MXCOMP in NTSYS). Principal coordinate analysis (PCO) was also applied to Nei's genetic distance matrices (DCENTER and EIGEN in NTSYS). An additional representation of genetic relationships among populations was expressed in a 3D graph based on the first three coordinates.

RESULTS AND DISCUSSION

Morphometric analyses

The results of the cluster analysis are shown in Figure 3. No distinct group can be spotted in the dendrogram. The only well-separated cluster includes specimens 41, 52, 3, 32, 8, 74 and 93, all characterized by high pubescence of lemmas and spikelets (level of pubescence generally 3, sometimes 2.5 on a scale from 1 to 3). These specimens appear to correspond to the characters of *F. ovina* subvar. *gracilior* Hack. f. '*spiculis villosis*', described by Saint-Yves (1913: 54) and not validly published under Art. 24.1 and 24.2 of the International Code of Botanical Nomenclature (Greuter *et al*., 2000). However, the specimens in the cluster come from populations scattered throughout the distribution range and growing under different ecological conditions. Moreover, some of the pubescent specimens belong to populations which also include individuals with glabrous spikelets and lemmas. This is in agreement with Auquier & Kerguélen (1977), who stated that some taxa described within the *F. ovina* group could correspond merely to more or less pubescent individuals belonging to variable populations. As the cluster is not related to any geographical and/or ecological range, we did not consider it as a systematic group.

The results of CDA were plotted in a 2D scattergram (Fig. 4). Here, all the specimens belonging to the analysed populations overlap in the plane described by the first two axes, the centroids of all the 20 groups lie close to one another and no groups appear to occupy a particular area on the plane. The best discriminant function is defined by nine characters: PY = 0.42772,

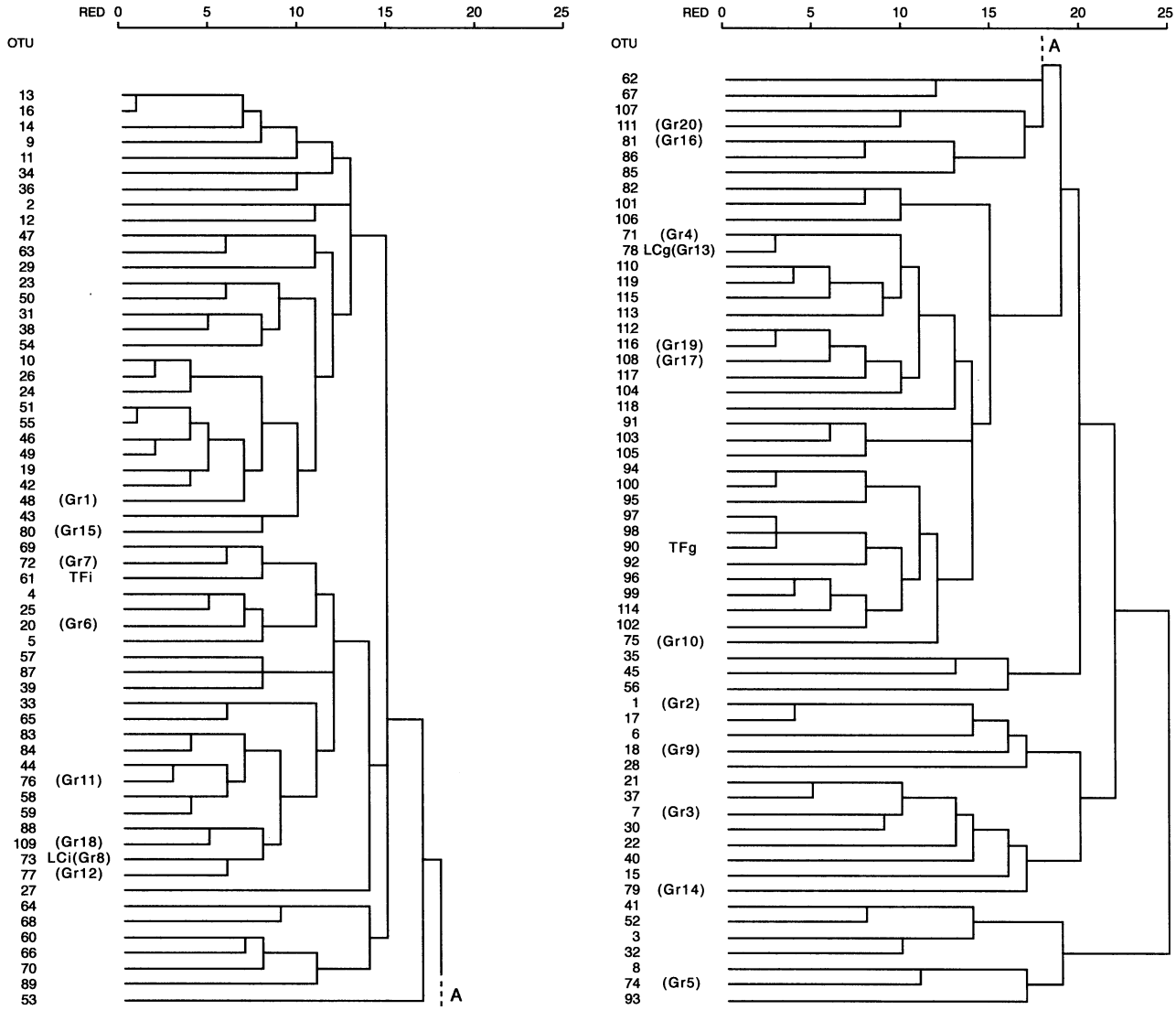


Figure 3. Dendrogram derived from the cluster analysis of the specimens of *F. inops* and *F. gracilior* that were scored in this study. LCg, *locus classicus* of *F. gracilior*; LCi, *locus classicus* of *F. inops*. TFi, type specimen of *F. inops*; TFg, type specimen of *F. gracilior*. Gr 1–20, specimens from sampled populations. OTUs numbers as in Table 1.

PS = 0.198821, °PP = 0.09613, LP = 0.04878, °AL = 0.02673, °CL = 0.0632, °PL = 0.00996, °G1 = 0.00596 and SK = 0.00394 (quantitative characters are marked with °; for character abbreviations, see Table 2). This function is responsible for only 63.6% of the cases. The best discriminant character is the value of pruinosity (PY), a qualitative character not maintained under cultivation. The irrelevant systematic value of this character has already been discussed above (see Introduction; Auquier & Kerguélen, 1977). The classification process gives a confusing matrix where only the population Gr5 shows 100% correct classification owing to high pubescence of spikelets of all the specimens.

Variation within the 20 tested populations of the five quantitative characters (PP, AL, CL, PL and G1) selected by CDA is represented in Figure 5. As shown in the figure, the ranges of variation of all these characters overlap to such an extent that none of them can be used to separate any population from the others.

Epidermal features

In the abaxial epidermis of the examined samples of leaf blades, long cells are very abundant, rectangular in shape and 53.9–100 μm long, with sinuous walls; short cells are also abundant, situated in costal and intercostal positions, mostly solitary, 29.3–44.2 μm long, and

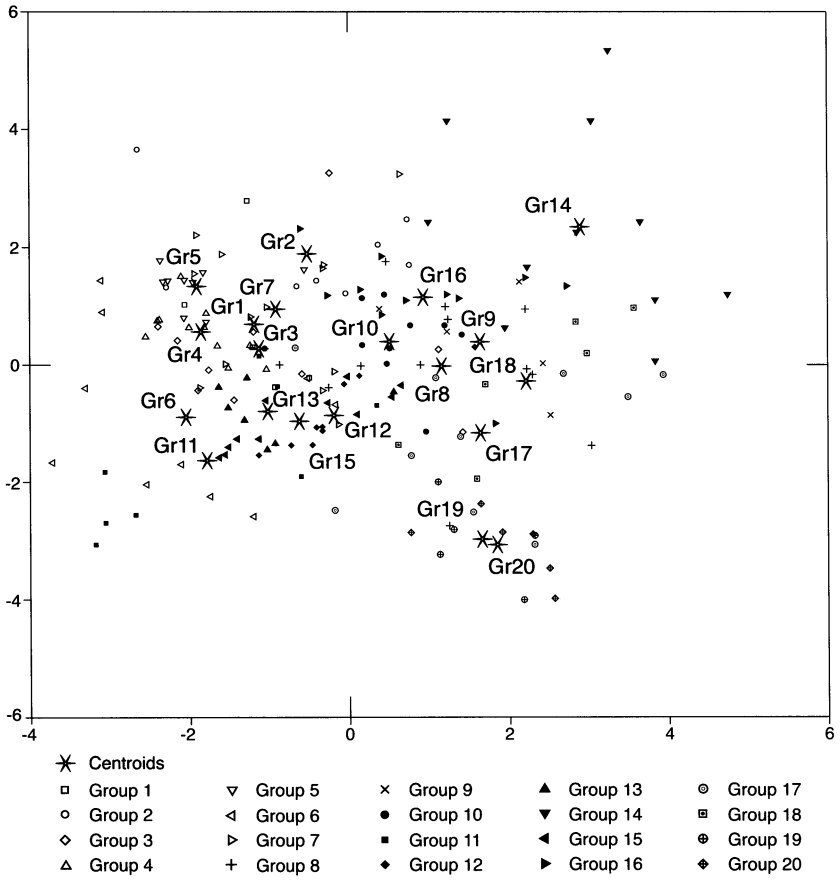


Figure 4. Scattergram of the Canonical Discriminant Analysis (CDA) performed on the investigated populations of *F. inops* and *F. gracilior*. Gr1–20 and symbols as in Figure 2.

rectangular to slightly oval in shape. Silica bodies are slightly crescent-shaped, placed in costal and intercostal positions and elliptical to round in shape; hairs, prickles and stomata are lacking (Figs 6, 7).

In the adaxial epidermis, stomata are rather common, arranged in bands, in costal and intercostal positions, 15.1–17.1 μm long and accompanied by dome-shaped subsidiary cells; prickles are abundant, patent to adpressed, in costal and intercostal positions, and 24.8–62.5 μm long (Figs 8–11).

In the 20 specimens examined, several different kinds of epicuticular waxes were observed, which can be referred to four main types and two transitional forms (Figs 12–17). According to K. Koch (pers. comm.), these types can be defined as platelets (Fig. 12); tubules (Fig. 13); coiled rodlets (Fig. 14); transitional forms between incomplete and membraneous platelets (Fig. 15); rodlets with intermediates to thinner threads (Fig. 16); and typical β-diketon tubules (Fig. 17).

Epidermal morphological features provided no evidence for differences among the investigated populations. As shown in Figures 6–11 (Figs 6, 8, 10: Bouyon in French Maritime Alps; Figs 7, 9, 11: Mount Gazzo near Genoa, Liguria), the specimens from the two *loci classici* show identical epidermal morphology. The differences in the structure and chemical composition of the epicuticular waxes appear not to be correlated with ecological and/or geographical features of the populations.

CHROMOSOMAL COUNTS

All the 17 populations analysed karyologically, including those growing in the two *loci classici* (Mount Gazzo and Bouyon), proved to be diploid with $2n = 14$ (Fig. 18). These data confirmed chromosomal counts previously reported for French and Italian populations of *F. gracilior* (Parreaux, 1972, cited in Kerguélen, 1975; Moore, 1982; Bidault in Kerguélen, 1975; Kerguélen & Plonka, 1989; Portal, 1999) and *F. inops* (Bechi & Miceli, 1995).

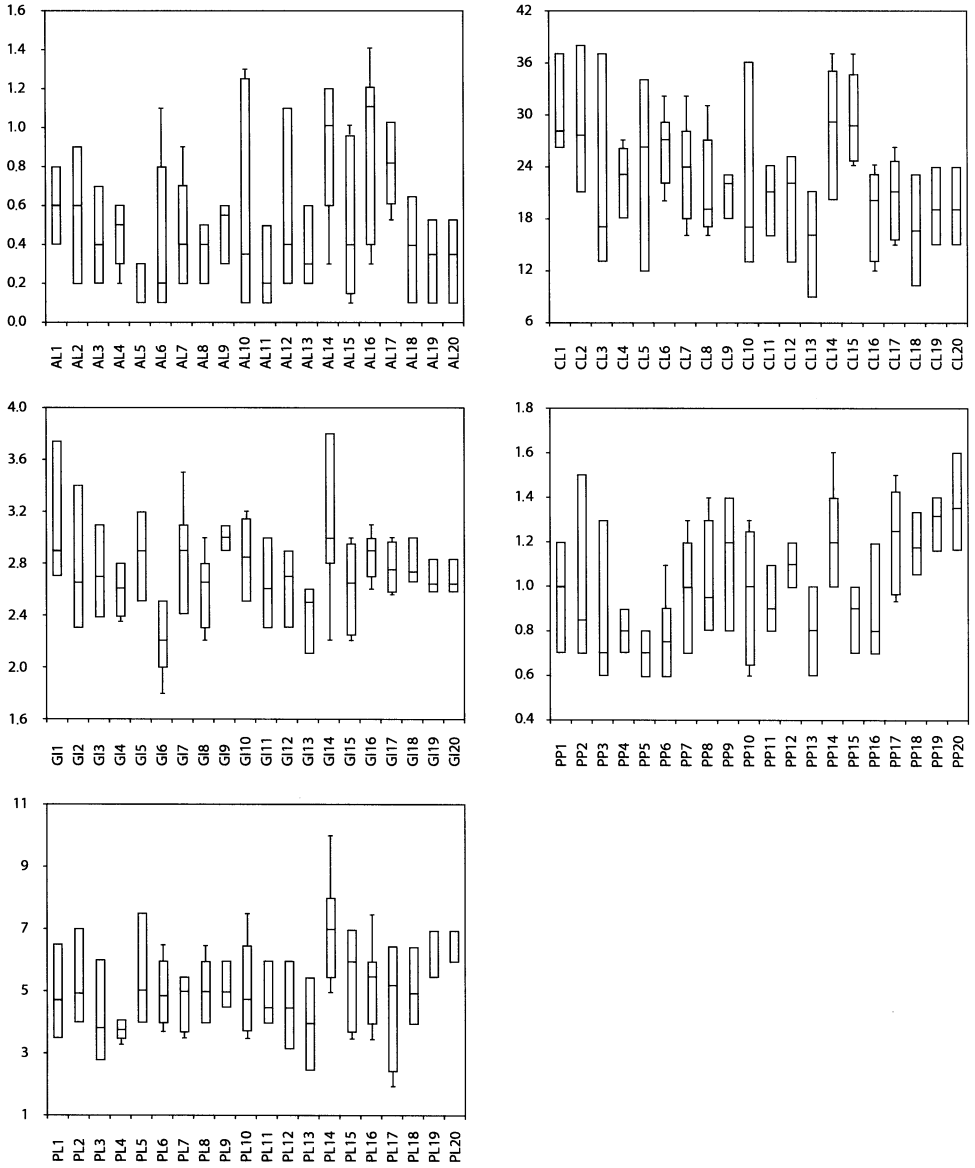
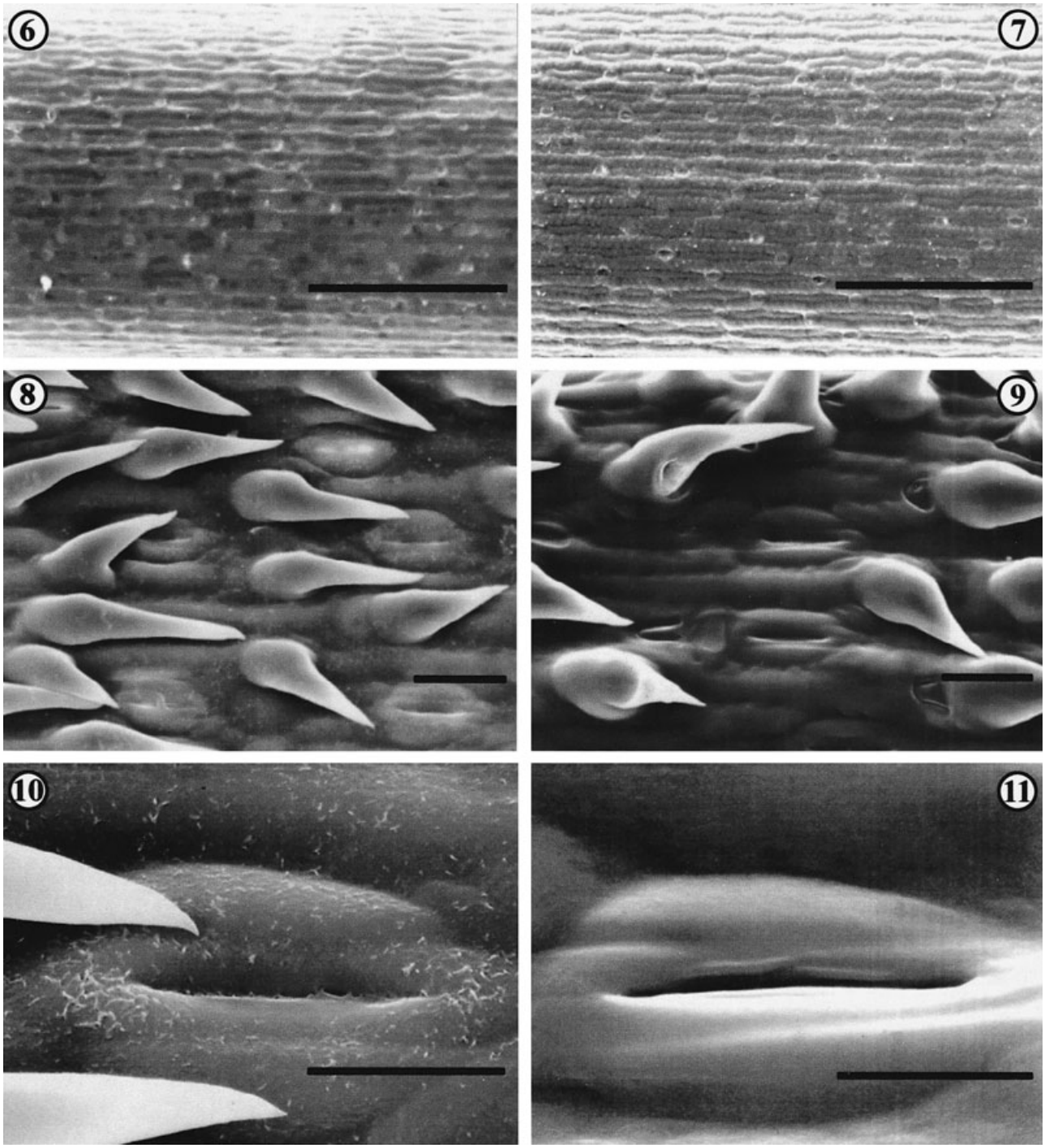


Figure 5. Box-plots describing the variation of quantitative characters in *F. inops* and *F. gracilior* defining the best discriminant function. PP, length of spikelets pedicel; AL, length of awn of lemma; CL, length of culm; PL, length of the longest panicle; G1: length of lower glume.

ISSR ANALYSIS

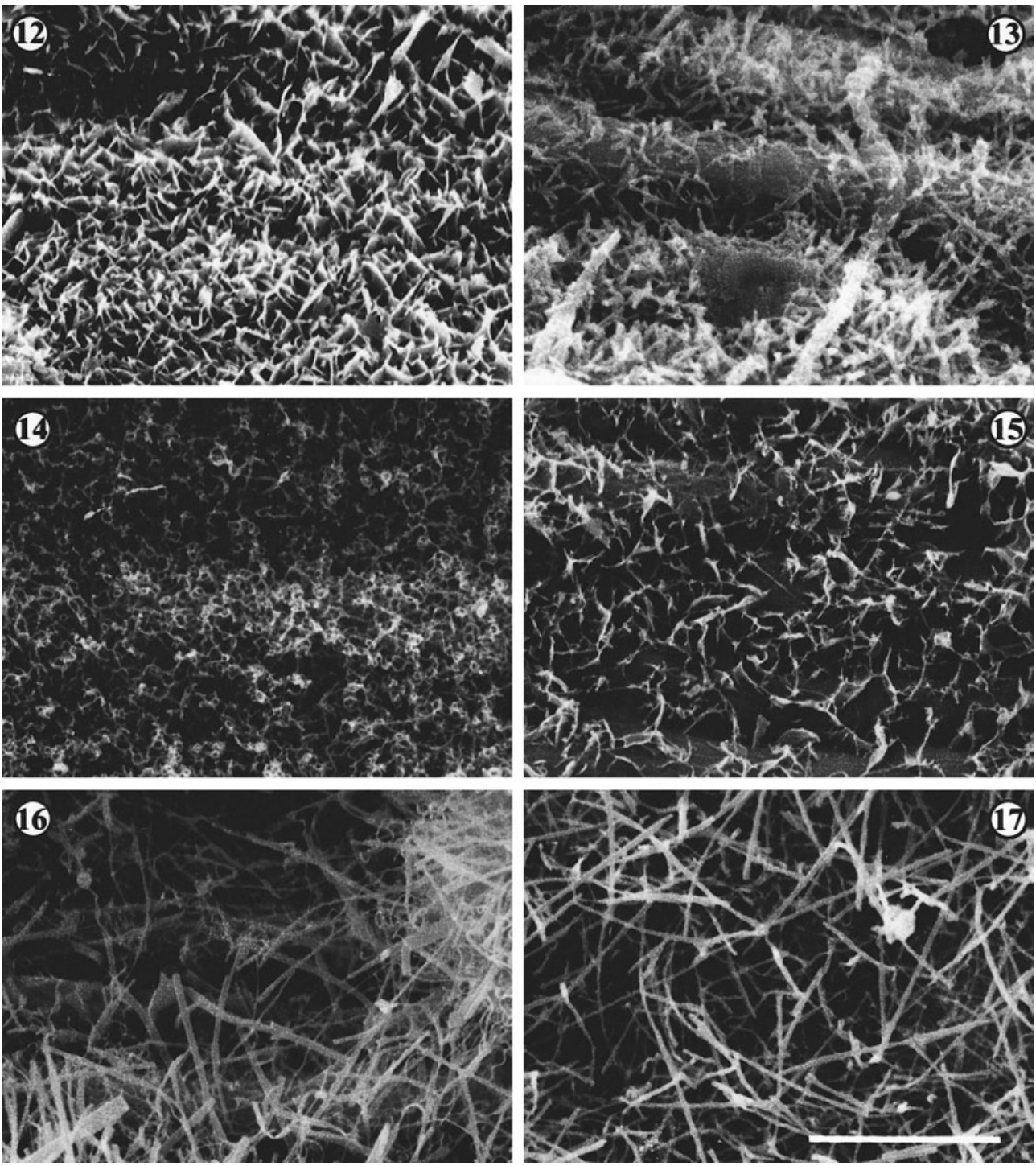
In a preliminary test of 16 available primers, five primers ([$(GA)_8$C], [$(AC)_8$G], [$(AG)_8$YC], [$(GA)_8$YG], [$(AC)_8$YT]) appeared to exhibit suitable band variation among individuals from different populations. These primers produced distinct, reproducible banding patterns among individuals and were used in this analysis. The five ISSR primers generated a total of 223 scorable bands, of which 64 (29%) were polymorphic in the 193 individuals examined. The number of polymorphic bands generated by a primer varied between 12 and 15. The size of the bands ranged from less than 100 bp to 3500 bp. Genetic similarities among different populations were represented with a dendrogram derived from the Nei's genetic distances matrix using the UPGMA method (Fig. 19).

Goodness-of-fit analysis suggests that UPGMA is highly useful for this kind of representation, with $r = 0.94$; the neighbour-joining method produced a considerably lower value ($r = 0.75$). The UPGMA dendrogram separates populations into two distinct clusters. The first one comprises all the populations from Italy and south-east France. Within this group, the



Figures 6–11. Analysis of the leaf blade epidermis in *F. inops* and *F. gracilior*. Figs 6, 7. Abaxial surface. Scale bar = 200 μm. Figs 8, 9. Adaxial surface. Scale bar = 20 μm. Figs 10, 11. Adaxial surface, stomata. Scale bar = 10 μm. (6, 8, 10: from Bouyon; 7, 9, 11: from Mount Gazzo).

pattern of clustering and the variation in genetic distance seems to follow a proximity-based trend, thus implying a more frequent gene flow among neighbouring populations than among those farther apart. The second cluster contains the population of *F. occitanica* (outgroup species). In order to prove a possible link between genetic relation and spatial location, we compared the matrix of Nei's distances



Figures 12–17. Different types of epicuticular waxes that could be found in *F. inops* and *F. gracilior*. Fig. 12. Platelets (France, Vaucluse). Fig. 13: tubules (Italy, Castelluccio). Fig. 14. Coiled rodlets (Italy, Ponti di Vara); Fig. 15. Transitional forms of platelets (France, Saint Louis). Fig. 16: Rodlets and intermediates to thinner threads (Italy, Molazzana). Fig. 17: β-diketon tubules (Italy, Balzo Nero). Scale bar = 10 μm.

belonging to the diploid French and Italian populations (first cluster) with a corresponding matrix of geographical distances. The matrices were moderately but significantly ($P < 0.05$) positively correlated: $r = 0.407$. This suggests a nonrandom linkage between the spatial distribution and the genetic similarity. In order to further analyse the genetic structure of the populations, we also performed a principal coordinate analysis (PCO). The first two principal components accounted for 53.4% of the existing variation (39.2% corresponding to component 1 and 11.2% to component 2), the third component accounted for only 3% of the total variation. Based on the effects of the first three components, a 3D plot of the sample scores was drawn to represent the interpopulation similarities (Fig. 20). The relationships among populations shown in this figure are similar to the results of the cluster analysis. The outgroup species is the most isolated group. Figure 20 also shows the relative isolation of the diploid French populations. This could be explained as consequence of intraspecific genetic variability spatially structured into geographical ranges. Therefore, although based on the limited sampling covered by this study, it seems that genomic affinity is very high between populations of the diploid *F. inops* and the diploid *F. gracilior* in Italy and south-east France. No correlation between groups of populations and ecological features could be detected.

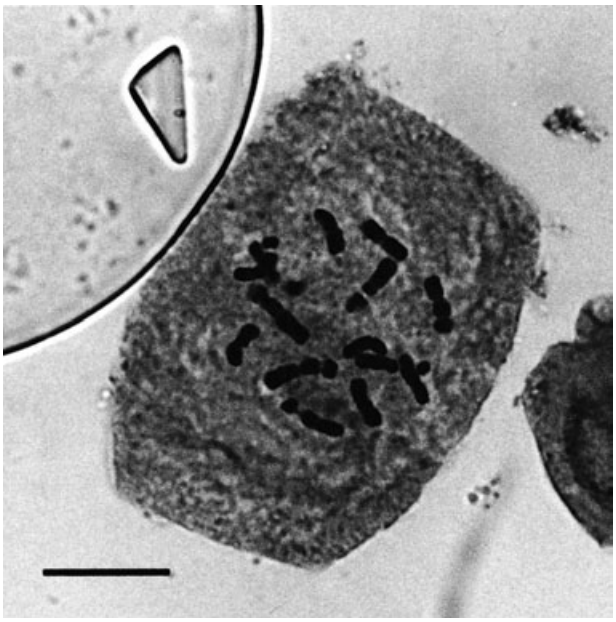


Figure 18. Chromosome metaphase plate of individuals from the *locus classicus* population of *F. inops* ($2n = 14$). Scale bar = 1 μm.

CONCLUSIONS

The analysis of macro- and micromorphological characters in *Festuca inops* and *F. gracilior* demonstrates that in the investigated populations no more than one distinct systematic unit may be recognized. This result is also supported by the rather high genomic affinity among the populations, as emerges from ISSR analysis. Furthermore, all the other data gathered from chromosomal counts, observations in the wild on eco-

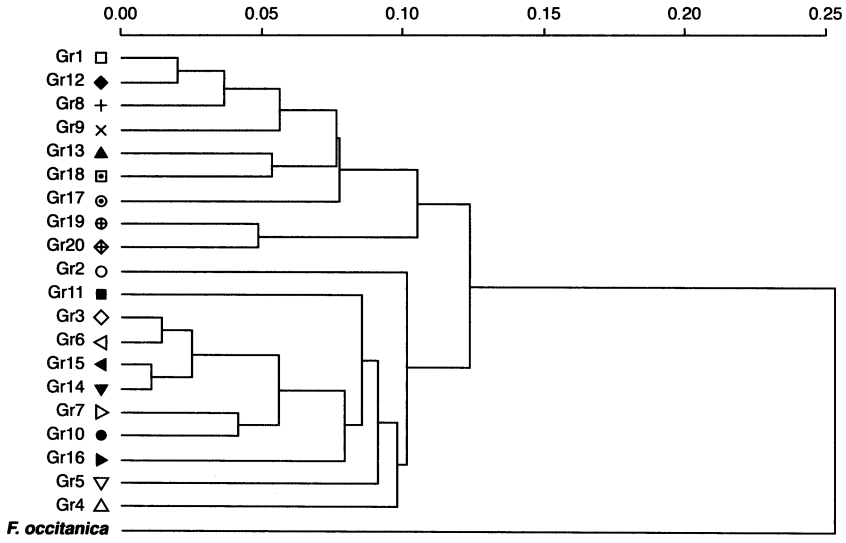


Figure 19. UPGMA dendrogram based on Nei's genetic distances between populations of *F. inops* and *F. occitanica*. Gr1–20 and symbols as in Fig. 2.

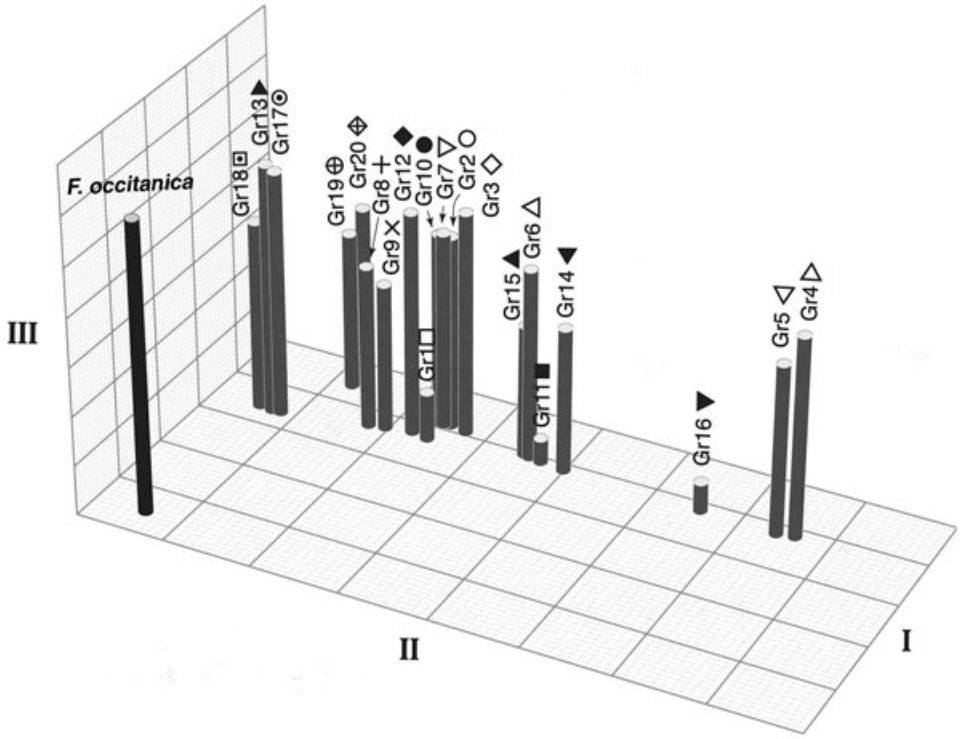


Figure 20. Results of the Principal Coordinates Analysis (PCO) of populations of *F. inops* and *F. occitanica*. The first three coordinates accounted for 56.4% of the variance. Gr1–20 and symbols as in Figure 2.

logical behaviour of the populations and distribution areas confirm that all the populations must be referred to one single taxon, for which the rank of species seems to be appropriate. According to nomenclature rules, the legitimate name for this taxon is *Festuca inops* De Not. Consequently, all previous records of *F. gracilior* from south-east France and Italy must be referred to *F. inops*.

However, further investigations might be useful to clarify the systematic relationships between this species and the morphologically similar tetraploid taxa growing in north-east Spain and south-west France, west of the Rhone, i.e. *F. occitanica*, *F. gracilior sensu* auct. hispan. (e.g. de la Fuente & Ortuñez, 1998) and possibly also *F. michaelis*.

FESTUCA INOPS DE NOT., REPERT. FL. LIGUST. 466 (1844)

Type: '*Festuca inops* Dntrs/Monte Gazzo/6.1843'. *Lectotype*: designed by Mariotti (1995) in GDOR!; *syntype*: in FI! The specimen kept in GDOR is very poor and not fully developed; the morphology of the species can be better observed on the syntype.

Locus classicus: M. Gazzo, Genova, Liguria (Italy).

≡*Festuca ovina* L. subvar. *inops* (De Not.) Hack. Monogr. Festuc. Eur. 95 (1882)

=*F. ovina* L. subvar. *gracilior* Hack., Monogr. Festuc. Eur. 90 (1882) *pro parte*, incl. type.

=*F. duriuscula* L. ssp. *gracilior* (Hack.) K. Richt., Pl. Europ., 1: 94 (1890) *pro parte.*

=*Festuca gracilior* (Hack.) Markgr.-Dann., Bot. J. Linn. Soc., 76: 325 (1978) *pro parte.*

=*F. occitanica* (Litard.) Auquier & Kerguélen ssp. *martinii* Kerguélen, Lejeunia, n. s. 110: 60 (1983). *Type*: 'Env. 1 km avant St-Marc-Jaumegarde (France: Bouches-du-Rhône) P. Auquier n° F.1137, W. Bellotte et E. Favaux'. *Holotype:* in LG.

= *F. ovina* L. var. *occitanica* Litard. f. *mucronulata* Litard., Bull. Soc. Bot. Fr. 95 (7–9): 281 (1948). *Type*: 'F. ovina L. ssp. eu-ovina Hack/. var. occitanica R. Lit./ subvar. eu-occitanica R. Lit./fa. mucronulata R. Lit.// Basses- Alpes: Gréoux-les-Bains, sables/rive gauche du Verdon, à çé m. env. en amont de la passerelle./4 août. 1947./Leg. G. Malcuit.' in G-Litardière!. *Lectotype*: here designated.

= *F. ovina* L. var. *duriuscula* subvar. *gracilior* f. *macilenta* St-Yves, Ann. Cons. Jard. Bot. Genève 17: 55 (1913). *Type*: 'Y187//F. ovina – ssp. eu-ovina – var. duriuscula –/subv. gracilior Hack.//f.a macilenta/Alpes de St. Etienne de Tinée: Tète de Gerpas. 14 VII 08/roccailles silice. 2000 m s. m./Iter Burn. 08', leg. St. Yves, G-St.-Yves! *Lectotype*: here designated. *Isotype*: Y188 G-St.-Yves!

=*Festuca duriuscula* var. *submutica* Parl., Fl Ital., 1: 437 (1850). *Type*: 'Festuca duriuscula var./Mte Antola Apenn. lig. Leg. Berti/F. duriuscula d submutica Parl. fl. it./DNtrs/Da De Notaris in Agosto 1847' FI! *Lectoype*: here designated. *Syntype*: in FI!
=*F. ovina* subvar. *glauca* f. *exilior* St. Yves, Ann. Cons. jard. Bot. Geneve, 17: 63 (1913) *Typus*: 'Entre Menton et Nice, Peille, La Turbie 500 m; St. Michel d'Eze: St-Arnoux prés Lantosque, calcaire, 550 m' A. St. Yves – Alpes Maritimes, France', G!. *Lectotypus* designated by Bidault (1969), Rev. Cytol. Biol. Vég. 31(4): 217–356 (Kerguélen, 1975).
–*F. occitanica sensu* Auquier & Kerguélen, Lejeunia, n. s. 75: 39–41 (1975) *pro parte*, non *F. ovina* L. var. *occitanica* Litard. f. *occitanica*, f. *aristata* Litard.
–*F. pallens sensu* Di Pietro & Catonica (1999a, b), non Host.

DESCRIPTION

Perennial herb densely tufted. Vegetative shoots intravaginal. Culms (8)15–35(50) cm, smooth and glabrous up to the inflorescence. Leaf-sheaths papiraceous, overlapping, fused for about 1/4–1/2 of their length, glabrous. Ligules very short (0.2–0.3 mm); auricles obtuse, evident. Leaves smooth and glabrous, slightly scabrid in the distal part, more or less pruinose (not maintained under cultivation). Leaf blades straight to more-or-less curved (2.5)5–15(30) cm, rather stiff, obtuse to acute but never pungent.

Panicles (3)4–8(10) cm, contract to lax, often interrupted, with pubescent-scabrid branches. Spikelets (4.3)6–7.5(8) mm, 4–7 flowered, more or less pruinose and scattered with purple, glabrous to densely pubescent. Lower glumes subulate (1.9)2.5–3(4) mm, 1-veined. Upper glumes short, acuminate, 3-veined (2.9)3.5–4(4.8) mm, 3-veined, glabrous to densely covered with long hairs. Lemmas acute (3.5)4–5(5.8) mm (excl. awn), 5-veined, often pubescent in the distal part, sometimes on the whole surface; awns short (0.1)0.3–0.7(2) mm, rarely completely absent. Palea generally ciliate on 1/4 of the margin, sometimes to more than 1/2. Anthers (1)2–2.3(2.8) mm.

Leaf blade sections: outline regularly oboval; diameter (0.4)0.6–0.9(1.1) mm; thickness (0.13)0.2–0.3(0.4) mm; sclerenchyma usually forming a complete ring, more or less uniform, consisting of 1–3 layers of cells, sometimes laterally thickened up to 5–6 layers of cells; veins 5–7(9); (1)3–5 ridges on adaxial surface; bulliform cells almost always present.

All these morphological characters were maintained under standard cultivation conditions.

Iconography: Figure 21 (by A. Maury)

Phenology: Flowering March–July

Karyology: $2n = 14$

Geographical distribution and ecology: Festuca inops is an endemic species growing from south-east France to north-west and central Italy. Figure 22 shows its distribution area based on our direct observations conducted in the field, on data derived from herbarium specimens and on the critical evaluation of floristic records.

The species shows a wide altitudinal range, occurring from sea level to mountain tops (south-west Alps, Apennines), up to 2000 m a.s.l.; it is most commonly found at lower altitudes and becomes rarer above the timberline. It shows great ecological tolerance, according to its wide distribution in the wild. It is mostly found in rocky and xeric habitats, in more or less stony open grasslands (Biondi *et al*., 1995) and, sometimes, at the border between wood and shrub vegetation. It is usually indifferent to aspects, but at higher altitudes it prefers south-facing slopes. It grows on base-rich substrata such as limestone, dolomite, clay, marble and ultramafic rocks (Foggi & Rossi, 1996, sub *F. gracilior, F. inops*; Castelli, Biondi & Ballelli, 2001, sub *F. gracilior*; Adorni & Tomaselli, 2002), as well as on acidic substrata such as sandstone and granite (Tomaselli & Rossi, 1989).

From a phytosociological point of view, in Italy this species can be found in several grassland associations of the class *Festuco-Brometea* (Biondi *et al*., 1995): it is a character species of the association *Seslerio nitidae–Brometum erecti* Bruno in Bruno et Covarelli 1968 and has been recorded also for *Brizo mediae-Brometum erecti* Bruno in Bruno et Covarelli 1968 corr. Biondi et Ballelli 1982. More recently, it has been indicated (sub. *F. gracilior*) as a character species of the association *Festuco gracilioris–Brometum erecti* Castelli, Biondi et Ballelli 2001 (Castelli *et al*., 2001).

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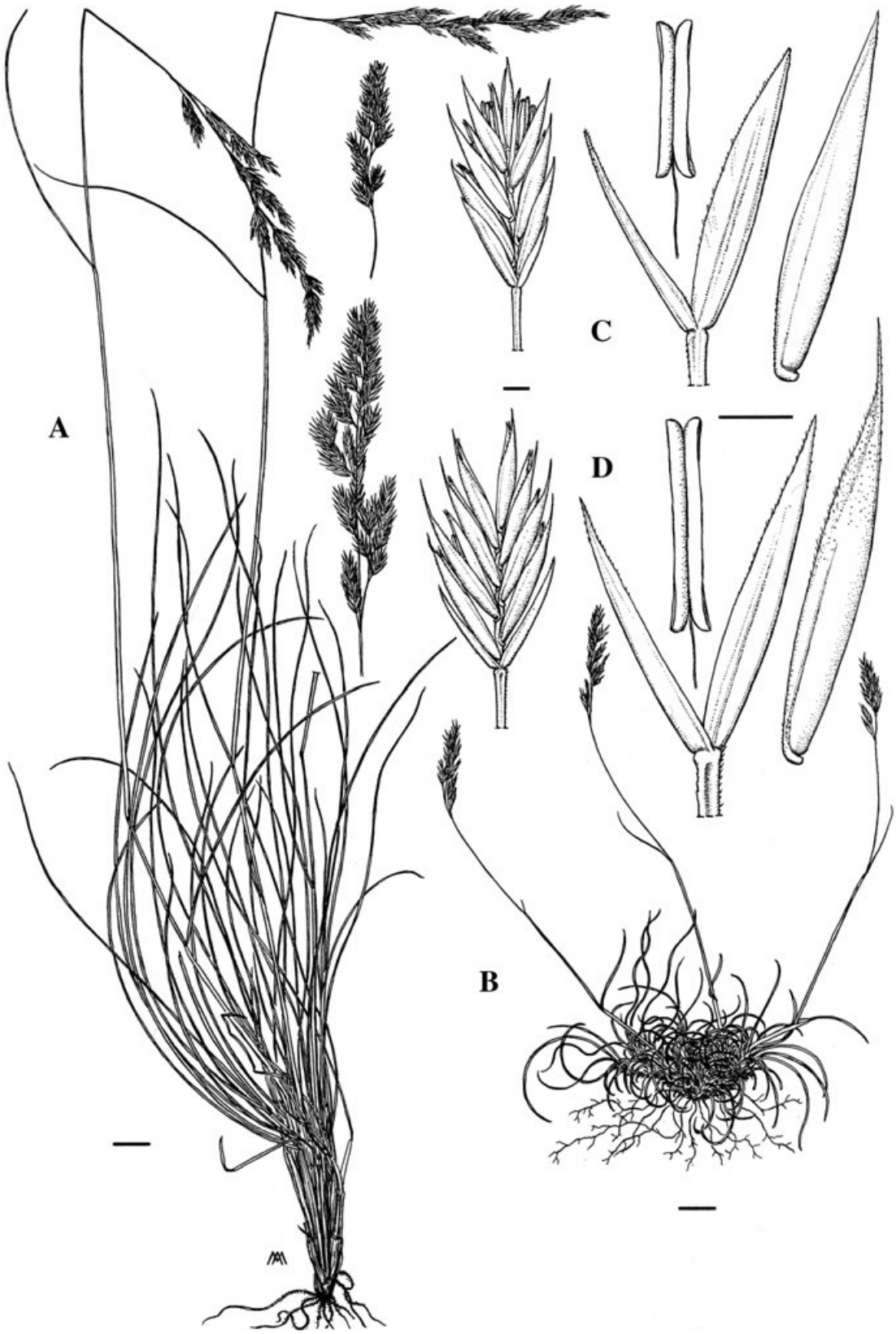

Figure 21. *F. inops*. A, Balzo Nero, Tuscany (Italy). Scale bar = 1 cm. B, Poggio delle Galbane, Tuscany (Italy). Scale bar = 1 cm. C, Monte Calvario, Emilia (Italy). Scale bar = 1 mm. D, Monte Argentario, Tuscany (Italy). Scale bar = 1 mm.

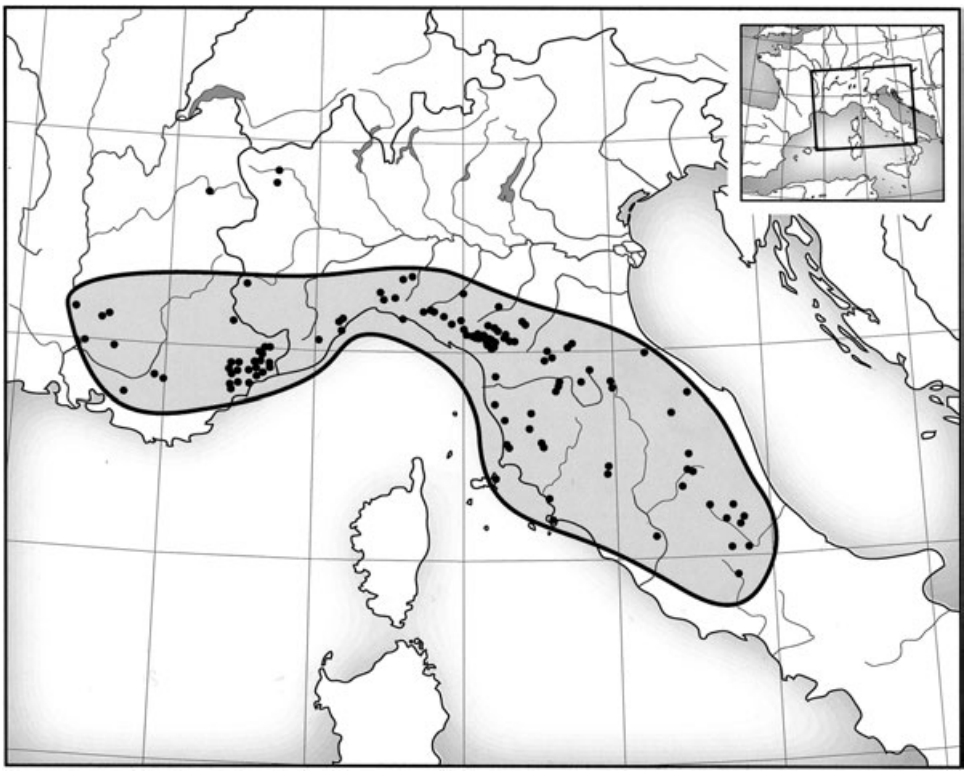

Figure 22. Distribution of *F. inops*, according to the results of the present study. Dots indicate measured specimens (see Table 1).

REFERENCES

Adorni M, Tomaselli M. 2002. Ricerche sulla vegetazione di un'area protetta con substrati ofiolitici: la Riserva naturale Monte Prinzera (Appennino parmense). In: Saccani A, ed. *Le ofioliti: isole sulla terraferma:. Per una rete di aree protette*. Atti del Convegno, Fornovo Val di Taro, 22–23 guigno 2001. Bologna: Regione Emilia-Romagna, 195–210.

Al Bermani A-KKA, Catalán P, Stace CA. 1992. A new circumscription of *Festuca trichophylla* (Gaudin) K. Richter (Gramineae). *Anales del Jardín Botánico de Madrid* **50**(2)**:** 209–220.

Auquier P. 1974. Biosystématique, taxonomie et nomeclature du groupe de *Festuca ovina* L. s.l. (Poaceae) en Belgique et dans quelques régions voisines. PhD thesis, Université Départment Botanique, Liége.

Auquier P. 1977. Taxonomie et nomenclature de quelques *Festuca* tétraploides de groupe de *F. ovina* L.s.1. (Poaceae) en Europe moyenne. *Bulletin Jardin Botanique Naturelless du Belgique* **47** (1–2)**:** 99–116.

Auquier P, Kerguélen M. 1977. Un groupe imbrouillé de *Festuca* (Poaceae): les taxons désignés par l'épithète 'glauca' en Europe occidentale et dans les régions voisines. *Lejeunia, n.s.* **89:** 1–82.

Barthlott W, Neinhuis C, Cutler D, Ditsch F, Meusel I, Theisen I, Wihlelmi H. 1998. Classification and terminology of plant epicuticular waxes. *Botanical Journal of the Linnean Society* **126:** 237–260.

Barthlott W, Theisen I. 2003. Epicuticular wax ultrastructure and classification of monocotyledons. In: Kubitski K, ed. *The families and genera of vascular plants III*. Berlin: Springer.

Barthlott W, Theisen I, Borsch T, Neinhuis C. 2003. Epicuticular waxes and vascular plant systematics: integrating micromorphological and chemical data. In: Stuessy TF, Mayer W, Horandl E, eds. *Deep morphology: toward a renaissance of morphology in plant systematics*. Regnum Vegetabile 141. Rugell, Lichtenstein: Ganter Verlag, 189–206.

Bechi N, Miceli P. 1995. Numeri cromosomici per la Flora italiana n. 1341–1345. *Informatore Botanico Italiano* **27**(1)**:** 21–25.

Bidault M. 1964. Étude expérimentale de quelques populations de *Festuca* du groupe *ovina* et ses conséquences taxonomiques. *Bulletin Société Botanique de France* **110**(9–10)**:** 372–380.

Bidault M. 1969. Essai de taxonomie expérimentale et numérique sur *Festuca ovina* L. s.1. dans les Sud-Est de la France. *Revue Cytologique Biologique Végetal (Paris)* **31**(4)**:** 217–356.

Biondi E, Ballelli S, Allegrezza M, Zuccarello V. 1995. La vegetazione dell'ordine *Brometalia erecti* Br. -Bl 1936 nell'Appennino (Italia). *Fitosociologia* **30:** 3–45.

Castelli M, Biondi E, Ballelli S. 2001. La vegetazione erbacea, arbustiva e preforestale del piano montano dell'Appennino piemontese (Valle Borbera e Curone, Italia). *Fitosociologia* **38**(1)**:** 125–152.

Cebolla Lozano C, Rivas Ponce MA. 2003. Catálogo del género *Festuca* L. (Poaceae) en la Peninsula Iberica. *Candollea* **58:** 189–213.

Cebolla C, Rivas Ponce MA. 2001. *Festuca michaelis* (Poaceae), une nouvelle espèce pour la Péninsule Ibérique. *Flora Mediterranea* **11:** 363–371.

Conert HJ. 1996. Gen. *Festuca* L. In: Hegi G, ed. *Illustrierte Flora von Mitteleuropa,* 1(3). Berlin: Blackwell Wissenschafts-Verlag, 561–633.

De Notaris G. 1844. *Repertorium Florae Ligusticae.* Taurini.

Di Pietro R, Catonica C. 1999a. *Festuca pallens* Host subsp. *pallens* (Gramineae). Segnalazioni Floristiche Italiane 929. *Informatore Botanico Italiano* **31**(1–3)**:** 79–80.

Di Pietro R, Catonica C. 1999b. *Festuca pallens* Host, a new species of the Italian flora in the central Apennines. Ecological and phytosociological considerations. *Plant Biosystems* **133**(2)**:** 173–183.

Ellis RP. 1976. A procedure for standardizing comparative leaf anatomy in the Poaceae. I. The leaf blade as viewed in transverse section. *Bothalia* **12**(1)**:** 65–109.

Ellis RP. 1979. A procedure for standardizing comparative leaf anatomy in the Poaceae. II. The epidermis as seen in surface view. *Bothalia* **12**(1)**:** 641–671.

Foggi B, Rossi G. 1996. A survey of the genus *Festuca* L. (Poaceae) in Italy. I. The species of the summit flora in the Tuscan-Emilian Apennines and Apuan Alps. *Willdenowia* **26:** 183–215.

Foggi B, Rossi G, Signorini MA. 1999. The *Festuca violacea* aggregate in the Alps and Apennines (central southern Europe). *Canadian Journal of Botany* **77:** 989–1013.

Fuente V de la, Ortuñez E. 1998. *Biosistemàtica de la secciòn. Festuca del género Festuca L. (Poaceae) en la Penìnsula Ibérica.* Madrid: ed. UAM.

Fuente V de la, Ferrero LM, Ortuñez E. 2001. Chromosome counts in the genus *Festuca* L. section *Festuca* (Poaceae) in the Iberian Peninsula. *Botanical Journal of the Linnean Society* **137:** 385–398.

Gherardi ME, Signorini MA, Foggi B. 2003. Il problema *Festuca inops-gracilior*: le entità diploidi. *Informatore Botanico Italiano* **35(1):** 250–252.

Greuter W, McNeill J, Barrie FR, Burdet HM, Demoulin V, Filgueiras TS, Nicolson DH, Silva PC, Skog JE, Trehane P, Turland NJ, Hawksworth DL, eds. **2000.** International Code of Botanical Nomenclature (Saint Louis Code). Adopted by the Sixteenth International Botanical Congress, St. Louis, Missouri, July–August 1999. *Regnum Vegetabile* 138: 1–474.

Hackel E. 1882. *Monographia Festucarum Europearum.* Berlin.

Kerguélen M. 1975. Les Gramineae (Poaceae) de la Flore Française. Essai de mise au point taxonomique et nomenclaturale. *Lejeunia, n.s.* **75:** 145–182.

Kerguélen M. 1983. Les Graminées de France au travers de 'Flora Europaea' et de la Flore du CNRS. *Lejeunia, n.s.* **110:** 1–79.

Kerguélen M. 1987. Données taxonomiques, nomenclaturales et chorologiques pour une révision de la flore de France (avec la collaboration de G. Bosc & J. Lambinon). *Lejeunia, n.s.* **120**(94–102)**:** 204–205.

Kerguélen M, Plonka F. 1989. Les *Festuca* de la flore de France (Corse comprise). *Bulletin de la Societé Botanique du Centre-Ouest, n.s.* **10:** 1–368.

Litardière R de. 1923. Contribution à l'étude des *Festuca* subgen. *Eu-Festuca* du Nord de la France et de la Belgique. *Bulletin Société Royale Botanique du Belgique* **55:** 92–123.

Litardière R de. 1945. Contribution à l'étude du genre *Festuca*. *Candollea* **10:** 103–146.

Mantel NA. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research* **27:** 209–220.

Mariotti MG. 1995. Osservazioni sulla vegetazione della Liguria. *La vegetazione italiana*. *Atti dei Convegni Lincei* **115:** 189–228.

Markgraf-Dannenberg I. 1978. New taxa and names in European *Festuca* (Gramineae). In: Heywood VH, ed. *Flora Europaea.* Notulae Systematicae 19. *Botanical Journal of the Linnean Society* **76:** 322–328.

Markgraf-Dannenberg I. 1980. Genus *Festuca*. In: Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA, eds. *Flora Europaea 5.* Cambridge: Cambridge University Press, 125–153.

Moore DM. 1982. *Flora Europaea check-list and chromosome index*. Cambridge: Cambridge University Press.

Moreno S, Martin JP, Ortiz JM. 1998. Inter-simple sequence repeats PCR for characterization of closely related grapevine germplasm. *Euphytica* **101:** 117–125.

Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**(583)**:** 590.

Norusis MJ. 1993. *SPSS for Windows. Professional Statistics* v.6.0. Chicago: SPSS Inc.

Palmer PG, Tucker AE. 1981. A Scanning Electron Microscope survey of the epidermis of East African grasses. *Smithsonian Contribution to Botany* **49:** 1–84.

Pignatti S, Markgraf-Dannenberg I. 1982. *Festuca* L. In: Pignatti S, ed. *Flora d'Italia* **3.** Bologna: Edagricole, 478–501.

Portal R. 1999. *Festuca* de France. Vals-près-Le-Puy: Portal.

Rohlf FJ. 1993. *NTSYS-Pc. Numerical taxonomy and multivariate analysis system*. New York: Applied Biostatistics Inc./ Exeter Software.

Saint-Yves A. 1909. Notes critiques sur quelques *Festuca* noveaux pour les Alpes-Maritimes. *Bulletin Societè Botanique de France* **56**(1–2)**:** 111–123.

Saint-Yves A. 1913. *Les Festuca* de la section *Eu-Festuca* et leurs variations dans les Alpes-Maritimes. *Annuaire du Conservatoire et Jardin Botaniques de la Ville de Genève*: 1–218.

Saint-Yves A. 1930. Aperçu sur la distribution géographique des *Festuca* (subgen. *Eu-Festuca*). *Candollea* **4:** 146–165.

StatSoft. 1998. *Statistica for Windows v.5.0*. Tulsa, OK: http://www.statsoft.com.

Tomaselli M, Rossi G. 1989. The rock-crevice vegetation of the Pietra di Bismantova (Northern Apennines, Italy). *Archivio Botanico Italiano* **65**(1–2)**:** 1–16.

Wilkinson MJ, Stace CA. 1991. A new taxonomic treatment of the *Festuca ovina* L. aggregate (Poaceae) in the British Isles. *Botanical Journal of the Linnean Society* **106**(4)**:** 347–397.

Wolff K, Morgan-Richards M. 1998. PCR markers distinguish *Plantago major* subspecies. *Theoretical and Applied Genetics* **96**(2)**:** 282–286.

Zietkiewicz E, Rafalski A, Labuda D. 1994. Genomic fingerprinting by simple sequence repeat (SSR) – anchored polymerase chain reaction amplification. *Genomics* **20:** 176–183.