Aliso: A Journal of Systematic and Evolutionary Botany

Volume 23 | Issue 1 Article 31

2007

A Systematic Approach to Subtribe Loliinae (Poaceae: Pooideae) Based on Phylogenetic Evidence

Pilar Catalán
Universidad de Zaragoza, Huesca, Spain

Pedro Torrecilla
Universidad Central de Venezuela, Maracay, Venezuela

José A. López-Rodríguez
Universidad de Zaragoza, Huesca, Spain

Jochen Müller
Friedrich-Schiller-Universität, Jena, Germany

Clive A. Stace
University of Leicester, Leicester, UK

Aliso 23, pp. 380–405

A SYSTEMATIC APPROACH TO SUBTRIBE LOLIINAE (POACEAE: POOIDEAE) BASED ON PHYLOGENETIC EVIDENCE

PILAR CATALÁN,[1,6] PEDRO TORRECILLA,[2] JOSÉ A. LÓPEZ-RODRÍGUEZ,[1,3] JOCHEN MÜLLER,[4] AND CLIVE A. STACE[5]

[1]*Departamento de Agricultura, Universidad de Zaragoza, Escuela Politécnica Superior de Huesca, Ctra. Cuarte km 1, Huesca 22071, Spain;* [2]*Cátedra de Botánica Sistemática, Universidad Central de Venezuela, Avenida El Limón s. n., Apartado Postal 4579, 456323 Maracay, Estado de Aragua, Venezuela (torrecillap@agr.ucv.ve);* [3]*(cotepintin@eresmas.com);* [4]*Institut für spezielle Botanik, Friedrich-Schiller-Universität, Philosophenweg 16, 07743 Jena, Germany (jochen.mueller@uni-jena.de);* [5]*Department of Biology, University of Leicester, University Road, Leicester LE1 7RH, UK (stace@ullesthorpe.fsworld.co.uk)*
[6]*Corresponding author (pcatalan@unizar.es)*

ABSTRACT

Loliinae (Poaceae, Pooideae) encompass a large group of genera closely related to *Festuca,* the largest genus in the subtribe, which as traditionally circumscribed has been shown to be highly paraphyletic. In this investigation we combined molecular and morphological data representing 20 genera of Loliinae and closely related subtribes. Combined analysis of nucleotide sequences from the nuclear ITS and chloroplast *trn*L–F regions and structural characters recovered a consensus topology that shows Loliinae to be monophyletic and possessing two main clades—the fine-leaved *Festuca* clade that includes *Ctenopsis, Micropyrum, Narduroides, Psilurus, Vulpia,* and *Wangenheimia,* and the broad-leaved *Festuca* clade that includes *Lolium* and *Micropyropsis.* The presence of morphologically intermediate, unresolved, or poorly supported taxa (*Castellia, Festuca* subgen. *Subulatae* and subgen. *Leucopoa* p. p., and *Festuca* sect. *Amphigenes* p. p.) among the two groups points to a potential evolutionary trend from ancestral broad-leaved taxa to the more recently evolved fine-leaved taxa. Alternate classifications are evaluated for subtribes Loliinae, Cynosurinae, Dactylidinae, and Parapholiinae. We propose to maintain a paraphyletic *Festuca* as presently circumscribed and not to divide the polyphyletic *Vulpia* and *Festuca* infrageneric taxa until more phylogenetic data become available.

Key words: combined phylogenies, *Festuca,* ITS, Loliinae, morphology, systematics, *trn*L–F, *Vulpia.*

INTRODUCTION

Members of the grass tribe Poeae (Pooideae) typically are characterized by the possession of a pooid-type spikelet with short glumes, several florets, and 5-veined lemmas (Macfarlane and Watson 1982; Tzvelev 1982; Clayton and Renvoize 1986). Macfarlane and Watson (1982) included Hainardieae, characterized by an excavated inflorescence axis, within Poeae, and separated Poeae from tribes such as Aveneae and Agrostideae, with glumes longer than the florets, and Seslerieae, with capitate panicles. Clayton and Renvoize (1986) also distinguished Poeae from Aveneae (incl. Agrostideae), though they included Seslerieae, but not Hainardieae, within Poeae. Tzvelev (1982) further split Poeae, recognizing Seslerieae and Monermeae (= Hainardieae) plus a monotypic Scolochloeae, having coriaceous, 5–7-nerved lemmas. He distinguished seven subtribes within Poeae, the broadest being Festucinae and Poinae, and minor subtribes Brizinae, Cinninae, Coleanthinae, Dactylidinae, and Psilurinae.

According to Clayton and Renvoize (1986), three main lines can be separated within Poeae—*Festuca, Poa,* and *Sesleria,* each with their respective satellite genera. *Festuca* and *Poa* are the two largest genera in the tribe, each accounting for more than 500 species distributed worldwide and restricted to higher altitudes in subtropical and tropical regions (Kerguélen and Plonka 1989; Watson and Dallwitz 1992). Clayton and Renvoize (1986) considered *Lolium, Vulpia,* and other small genera (*Castellia, Cynosurus, Lamarckia, Micropyropsis, Micropyrum, Psilurus,* and *Wangenheimia* among others), as groups derived from *Festuca,* a genus characterized by its mostly dorsally rounded lemma and linear hilum. Tzvelev (1982) circumscribed nine genera in the festucoid lineage (*Bellardiochloa* Chiov., *Cutandia* Willk., *Festuca, Loliolum* Krecz. & Bobr., *Lolium, Nardurus, Scleropoa, Sphenopus,* and *Vulpia*). *Lolium* was placed within its own subtribe, Loliinae (Dumortier 1824), based on distinctive inflorescence traits (spikelets sunken in the excavated rachis of the spike, each covered by a single glume). Early botanists thought *Lolium* was closely related to the *Elymus* L.–*Triticum* L. group due to the similar ''spiculescences.'' However, other morphological data as well as karyology and hybridization indicated its closeness to Festucinae, where it was included by Tzvelev (1982). *Lolium* has been considered related to *Festuca* based on chromosome and breeding affinities (Jenkin 1933; Malik and Thomas 1966). It hybridizes spontaneously with representatives of *Festuca* subgen. *Schedonorus* (e.g., Lewis 1975). Nomenclatural priority favors Loliinae over Festucinae, as first pointed out by Soreng and Davis (2000). Loliinae presently encompass ca. 600 species distributed worldwide.

The systematic treatments proposed for *Festuca* have changed over the previous two centuries since description of the genus by Linné (1753), as new taxa have been incorporated or segregated. One of the most comprehensive studies of *Festuca* was that by Hackel (1882), who divided the European fescues into six sections based on characters associated with leaf vernation, the leaf sheath, auricles, spikelets and floral bracts (lemma and palea), presence or absence

of ovary pubescence, insertion of styles, adherence of caryopsis to palea, and hilum length. Hackel also separated infrasectional groups (series) based on the type of shoot innovation and was the first to establish the anatomical analysis of leaf cross sections as a useful approach to identify species and infraspecific taxa. The Hackelian system was broadly accepted by later festucologists although Hackel (1887, 1906) and other authors (Piper 1906; Krechetovich and Bobrov 1934; Krivotulenko 1960; Tzvelev 1971; Alexeev 1977, 1978, 1980, 1981, 1986) further divided the genus into several subgenera and sections. The most recent series of revisions of the world's fescues by Alexeev (1977, 1978, 1980, 1981, 1985, 1986) recognized up to 11 subgenera and several sections within each. *Vulpia* (Gmelin 1805), *Schedonorus* (Palisot de Beauvois 1812), *Leucopoa* (Grisebach 1852–1853), *Helleria* (= *Hellerochloa*) (Fournier 1886), and *Drymochloa* (Holub 1984) have been segregated from *Festuca* at different times. *Vulpia* has been recognized as a genus independent from *Festuca* by recent agrostologists (Cotton and Stace 1977; Stace 1981) whereas the other genera are synonyms of *Festuca* in most current Floras, while recent proposals favor the segregation of *Leucopoa* (Holub 1984) and *Schedonorus* (Holub 1998; Soreng and Terrell 1998, 2003; Tzvelev 1999, 2000).

Vulpia and other minor segregate genera of ephemerals mostly have been considered to be independent and more or less related to *Festuca* (Cotton and Stace 1977; Stace 1981). Cotton and Stace (1977) differentiated *Vulpia* from *Festuca* based on the annual habit, long unequal glumes, and long-awned lemma, though they indicated that none of these characters was absolute. The circumscription of *Vulpia* has changed depending on the inclusion or exclusion of different genera and infrageneric taxa (summarized in Stace 1981). Up to five different sections have been recognized within *Vulpia* (*Apalochloa, Loretia, Monachne, Spirachne,* and *Vulpia*) based on breeding system, number and size of anthers, spikelet structure, number of fertile/sterile florets, and the shape and size of the lemma callus (Cotton and Stace 1977; Stace 1978). *Vulpia* and another 11 annual genera (*Castellia, Catapodium, Ctenopsis, Cutandia, Desmazeria* Dumort., *Loliolum, Micropyrum, Narduroides, Sclerochloa, Vulpiella* (Batt. & Trab.) Andreansky, *Wangenheimia*) were grouped in the *Vulpia–Desmazeria* complex by Stace (1981). *Castellia, Ctenopsis, Micropyrum,* and *Wangenheimia* were considered to be close to *Vulpia*; *Loliolum* and *Narduroides* as intermediate between *Vulpia* and *Desmazeria*; and the remaining genera as more closely related to *Desmazeria. Desmazeria, Catapodium,* and *Cutandia* were recently classified by Soreng and Davis (2000) as belonging to their Parapholiinae subtribe, and *Sclerochloa* as a member of their *Puccinellia* complex. Species of *Vulpia* (sects. *Monachne* and *Vulpia*) have been shown to hybridize with species of *Festuca* sect. *Aulaxyper* (Ainscough et al. 1986).

Grasses, as with many other groups of plants, have been subjected to repeated taxonomic splitting and lumping. The recent advent of molecular phylogenetics has affected traditional classifications of subfamilies, tribes, subtribes, and genera (Davis and Soreng 1993; Clark et al. 1995; Hsiao et al. 1999; Grass Phylogeny Working Group [GPWG] 2001). Within Poaceae a single origin of the temperate grasses (Pooideae) is possible (GPWG 2001); however, the taxonomic limits of recently evolved pooid tribes become blurred when many taxa are sampled and analyzed (Soreng and Davis 2000). Phylogenetic analyses based on chloroplast and nuclear DNA sequences have supported the sister relationship of tribes Poeae and Aveneae within the core group of most recently evolved Pooideae (Soreng et al. 1990; Nadot et al. 1994; Hsiao et al. 1995; Catalán et al. 1997). However, Soreng and Davis' (2000) study, with the greatest sampling of Poeae–Aveneae taxa, based on combined analysis of chloroplast RFLP data and structural characters, showed intermingling of representatives of the two tribes in the optimal tree. Conflict between molecular data and morphology-based classifications of these two tribes (cf. Soreng and Davis 2000) moved the GPWG (2001) to subsume Aveneae within Poeae as a provisional proposal in need of confirmation from larger molecular studies of the two groups.

A series of molecular phylogenetic studies of *Festuca* and its closest relatives (Darbyshire and Warwick 1992; Charmet et al. 1997; Gaut et al. 2000; Torrecilla and Catalán 2002; Torrecilla et al. 2003, 2004; Catalán et al. 2004) demonstrated that *Festuca* s.l. is a large paraphyletic assemblage that encompasses not only *Lolium* and *Vulpia,* but also a number of other genera. The most exhaustive molecular study of festucoid taxa, conducted by Catalán et al. (2004) and based on combined analyses of nuclear ITS and chloroplast *trn*L–F sequences, found a likely evolutionary trend from more ancestral broad-leaved *Festuca* lineages toward more recently derived fine-leaved *Festuca* lineages; polyphyletic *Vulpia* and other Mediterranean genera of ephemerals were nested within the fine-leaved clade whereas *Lolium* and *Micropyropsis* were included within the broad-leaved clade. Also, revealed was that the sister clades Dactylidinae and Cynosurinae–Parapholiinae (sensu Soreng and Davis 2000) were the closest relatives of Loliinae. However, evolutionary rates within Loliinae and its closest relatives vary enormously, showing a general trend from slowly evolving perennial lineages toward rapidly evolving annual lineages (Torrecilla et al. 2004). Significant differences in nucleotide substitution rates seem to be correlated with the generation-time-effect hypothesis (Torrecilla et al. 2004). Highly heterogeneous sequences may be prone to higher rates of homoplasy, which could lead to undesirable long-branch attraction and site-saturation effects in phylogenetic reconstruction, thus increasing the risk of recovering potentially artifactual relationships.

The present study enlarges the phylogenetic survey of Loliinae and close allies to include the analysis of morphological characters. Structural characters are believed to have arisen through different gene regulatory mechanisms (Soreng and Davis 2000) and therefore would be expected to be congruent with molecular phylogenies. However, discrepancies in phylogenetic reconstruction between molecular and structural evidence are frequent within angiosperms (Hillis and Wiens 2000). Morphological data were shown to be congruent with and able to discriminate among subfamilies of grasses (GPWG 2001), but they failed to recover relationships below that level within Pooideae due to their homoplasious nature (Kellogg and Watson 1993). Nonetheless, a careful examination of a large set of morphological traits within particular groups (primary synapomorphies, sensu de

Pinna 1991) could help discover secondary synapomorphic characters of more inclusive groups.

Based on the general belief that morphological data remain essential to distinguishing taxa in a practical classification system, we conduct simultaneous cladistic analyses of morphological and molecular data for Loliinae and close allies to reconstruct their phylogenetic relationships and to improve the classification. Combined analysis of molecular and morphological data is also intended to evaluate the phylogenetic signal of the morphological characters by congruence with the molecular characters applying the principle of total evidence (Kluge and Wolf 1993). A taxonomic treatment of Loliinae and close subtribes is fashioned based on the resulting consensus tree. Because of the still-limited sampling within the large genus *Festuca,* the proposals presented here are preliminary; however, as most of the supraspecific groups studied include the type species, our treatment could be predictive of a more final classification. Discussions on the appropriateness of phylogenetic over evolutionary systematic methods and vice versa have been recently brought up with regard to this group of important forage grasses (Darbyshire 1993; Soreng and Terrell 1998). In this study, we examine alternative classification proposals based on the present phylogenetic knowledge of festucoids in search of a satisfactory systematic framework that could convey a natural classification system for these grasses.

MATERIALS AND METHODS

Materials

Included in this study were the representatives of Loliinae, Cynosurinae, Dactylidinae, and Parapholiinae sampled in the molecular survey by Catalán et al. (2004). The morphological survey was carried out for a total of 87 species representing 18 genera of Loliinae and close subtribes for which herbarium specimens and fresh collections were available. Of the 87 species studied, seven correspond to outgroup representatives of Brachypodieae (*Brachypodium*), Poineae (*Poa, Puccinellia,* and *Sclerochloa*), Seslerieae–Aveneae (*Sesleria* and *Parafestuca*), and Triticeae (*Secale*), as determined by Catalán et al. (2004). Two representatives of Cynosurinae (*Cynosurus*), three of Dactylidinae (*Dactylis* and *Lamarckia*), and four of Parapholiinae (*Catapodium, Hainardia, Parapholis,* and *Sphenopus*) comprised the sampling of the closest allies of Loliinae. Sampling of Loliinae covered 71 species, including 49 species of *Festuca* s.l., representing five subgenera (*Festuca, Drymanthele, Leucopoa, Schedonorus,* and *Subulatae*) and 12 sections; 11 species of *Vulpia* representing four of the five recognized sections (*Apalochloa, Loretia, Monachne,* and *Vulpia*); two species of *Lolium*; and nine species that correspond to other minor genera related to *Festuca* (*Castellia, Ctenopsis, Hellerochloa, Micropyropsis, Micropyrum, Narduroides, Psilurus,* and *Wangenheimia*). The list of the taxa studied with ploidy levels and geographic distributions is provided in Table 1.

Molecular Data

Analyses of molecular data were performed with a subset of samples included in Catalán et al. (2004). The ITS and *trn*L–F data matrices were trimmed for a common set of 87 taxa. The ITS data set consisted of 644 aligned nucleotide characters of which 46% were parsimony informative; 11 gaps that were potentially informative were also coded. The *trn*L–F data set was made up of 1089 aligned nucleotide positions of which 21% were parsimony informative; 15 potentially informative gaps were also coded.

Morphological Data

The analysis of morphological characters was conducted on 548 herbarium specimens from ARAN, BC, G, JACA, MA, MERC, MO, ORT, PRC, SEV, US, UZ, W, WA, and the private herbarium of J. Müller. The list of specimens studied is available from the first author upon request. A minimum of five specimens per species from different geographic localities were studied for most of the 87 species analyzed. In the few cases for which herbarium material was insufficient, the data were gleaned from the literature. Macro- and micromorphological traits were studied with the aid of a stereomicroscope. Transverse sections of innovation leaf blades were cut manually, mounted on slides, and studied under the light microscope (100× magnification) following the procedures in Kerguelén and Plonka (1989).

Characters were selected according to their diagnostic value at different hierarchical levels. Prominent characters were those proposed by Hackel (1882), Willkomm (1861), and Krivotulenko (1960) to separate taxa at subgeneric and sectional levels within *Festuca,* and by Cotton and Stace (1977) and Stace (1978, 1981) to differentiate sections of *Vulpia* and other related annual genera. Also included were diagnostic characters proposed by Saint-Yves (1922), Markgraf-Dannenberg (1980, 1985), and Chicouene (1999) to distinguish infrasectional groupings within *Festuca.* Another set of characters used to differentiate *Lolium* and other more distantly related genera of Poeae was chosen from Terrell (1968), Watson and Dallwitz (1992), and Kellogg and Watson (1993). Autapomorphies were excluded from consideration. Other potentially informative characters displaying a wide range of variation within some groups or species, such as the adherence of the caryopsis to the palea, were difficult to code into discrete homologous character states and also were excluded. Following the removal of continuous quantitative and invariant qualitative characters, a final set of 23 qualitative characters was selected for the cladistic analysis (Table 2).

Of the 23 selected characters, one refers to the habit of the plant (char. 1), 11 are vegetative traits (char. 2–12), and 11 are reproductive traits (char. 13–23). Sixteen traits were coded as binary characters and seven as multistate (Table 2). The morphological data matrix elaborated for the 87 species studied is provided in Table 3. Cataphylls are prominent in several broad-leaved groups and are absent in most of the fine-leaved lineages, but some fine-leaved taxa with extravaginal innovation shoots (e.g., the *F. rubra* group) possess reduced, less conspicuous cataphylls, hence the trait was coded as a three-state character. The amount of leaf sheath closure varies within several groups of *Festuca* and cannot be coded with confidence as a multistate character because of uncertainties in homology. Therefore, this character was coded as binary, differentiating taxa with leaf sheaths open or partially closed (closed <5%) from taxa with sheaths

Table 1. Taxa included in the phylogenetic study of subtribe Loliinae and close relatives. Indicated are the ploidy levels and geographic distributions. Ploidy levels are taken from Catalán et al. (2004). Information on herbarium vouchers and ITS and *trn*L–F GenBank accession numbers is provided in Catalán et al. (2004). Numbers in parentheses refer to sampled accessions indicated in Catalán et al. (2004).

Taxon	Ploidy level	Distribution
Tribe Poeae		
Subtribe Loliinae		
Festuca L.		
Subgen. *Festuca*		
Sect. *Festuca*		
Subsect. *Festuca* (*F. ovina* group)		
F. alpina Suter	2*x*	S Europe: Alps, Pyrenees Mts.
F. aragonensis (Willk.) Fuente & Ortúñez	4*x*	Spain: Moncayo
F. clementei Boiss.	2*x*	Spain: Sierra Nevada
F. frigida (Hack.) K. Richt.	2*x*	Spain: Sierra Nevada
F. glacialis Miègev. ex Anon.	2*x*	SW Europe: Pyrenees & Cantabrian mts.,
F. hystrix Boiss.	2*x*	W Mediterranean
F. longiauriculata Fuente, Ortúñez & L. M. Ferrero	2*x*	Spain: Sierra Filabres
F. ovina L.	2*x*	Central & N Europe, N Asia
F. plicata Hack.	2*x*	W Mediterranean
Subsect. *Exaratae* St.-Yves		
F. borderei (Hack.) K. Richt.	2*x*	SW Europe: Pyrenees Mts.
F. capillifolia Dufour	2*x*	W Mediterranean
F. querana Litard.	4*x*	Spain: Cantabrian Mts.
Sect. *Aulaxyper* Dumort. (*F. rubra* group)		
F. iberica (Hack.) K. Richt.	6*x*	W Mediterranean
F. juncifolia St.-Amans	8*x*	W Europe
F. nevadensis (Hack.) Markgr.-Dann.	10*x*	W Mediterranean
F. pyrenaica Reut.	4*x*	SW Europe: Pyrenees Mts.
F. rivularis Boiss.	2*x*	W & S Europe
F. rothmaleri (Litard.) Markgr.-Dann.	8*x*	Spain: Central & Cantabrian mts.
F. rubra L. (1)	6*x*, 8*x*	Europe, Siberia
Sect. *Eskia* Willk. p. p.		
F. burnatii St.-Yves	2*x*	Spain: Cantabrian Mts.
F. eskia Ramond ex DC.	2*x*	SW Europe: Pyrenees & Cantabrian mts.
F. gautieri (Hack.) K. Richt.	2*x*, 4*x*	Spain, Pyrenees Mts., Corbieres
F. quadriflora Honck. (1)	2*x*, 4*x*	S Europe: Alps, Pyrenees Mts.
Sect. *Pseudatropis* Krivot.		
F. elegans Boiss.	2*x*, 4*x*	W Mediterranean
Sect. *Scariosae* Hack.		
F. mairei St.-Yves	4*x*	NW Africa: Atlas Mts.
F. scariosa (Lag.) Asch. & Graebn.	2*x*	W Mediterranean
Sect. *Pseudoscariosa* Krivot.		
F. pseudeskia Boiss.	2*x*	Spain: Sierra Nevada
Sect. *Amphigenes* Janka		
F. agustinii Linding.	2*x*	Spain: Canary Is.
F. carpatica F. Dietr.	4*x*	E Europe: Carpathians
F. dimorpha Guss.	4*x*	S Europe: Alps, Apennines
F. pulchella Schrad. (1)	2*x*	S Europe: Alps, Carpathians
F. spectabilis Jan	6*x*	Italy, Balkan region
Sect. *Subbulbosae* Nyman ex Hack.		
F. baetica (Hack.) Richt.	2*x*	W Mediterranean
F. coerulescens Desf.	2*x*	W Mediterranean
F. durandoi Clauson	2*x*	W Mediterranean
F. paniculata (L.) Schinz & Thell.	2*x*	S Europe
F. triflora Desf.	2*x*	W Mediterranean
Subgen. *Drymanthele* Krech. & Bobr.		
F. altissima All.	2*x*	Central & S Europe, Central & SW Asia
F. drymeja Mert. & Koch	2*x*	Central & SE Europe, SW Asia
F. lasto Boiss.	2*x*	W Mediterranean
Subgen. *Leucopoa* (Griseb.) Hack.		
Sect. *Leucopoa* (Griseb.) Krivot.		
F. kingii (S. Watson) Cassidy	8*x*	W North America
Sect. *Breviaristatae* Krivot.		

Table 1. Continued.

Taxon	Ploidy level	Distribution
F. altaica Trin.	4*x*	N North America, N & Central Asia
F. californica Vasey	4*x*, 8*x*	W North America
Subgen. *Schedonorus* (P. Beauv.) Peterm.		
Sect. *Schedonorus* (P. Beauv.) Koch		
F. arundinacea Schreb. (1)	6*x*	Eurasia
F. fenas Lag.	4*x*	W Mediterranean
F. fontqueri St.-Yves	2*x*	NW Africa: Atlas & Rif mts.
F. pratensis Huds. (1)	2*x*	Eurasia
Sect. *Plantynia* (Dumort.) Tzvelev		
F. gigantea (L.) Vill.	6*x*	Eurasia
Subgen. *Subulatae* (Tzvelev) E. B. Alexeev		
F. subulata Trin.	2*x*, 4*x*	W North America
Lolium L.		
L. perenne L.	2*x*	Europe, Mediterranean
L. rigidum Gaudin (1)	2*x*	Mediterranean
Vulpia C. C. Gmel.		
Sect. *Vulpia*		
V. bromoides (L.) Gray	2*x*	W Europe, Mediterranean
V. ciliata Dumort. (1)	4*x*	Mediterranean
V. muralis (Kunth) Nees (1)	2*x*	Mediterranean
V. myuros (L.) C. C. Gmel. (1)	6*x*	W & Central Europe, Mediterranean
Sect. *Loretia* (Duval-Jouve) Boiss.		
V. alopecuros (Schousb.) Dumort.	2*x*	W Mediterranean
V. geniculata (L.) Link	2*x*	W & Central Mediterranean
V. sicula (C. Presl) Link	2*x*	NW Africa, Sicily, Sardinia
Sect. *Monachne* Dumort.		
V. fasciculata (Forssk.) Samp. (1)	4*x*	Mediterranean, W Europe
V. fontqueriana Melderis & Stace	2*x*	Spain: Cádiz, Segovia
V. membranacea (L.) Dumort.	2*x*	W Mediterranean
Sect. *Apalochloa* (Dumort.) Stace (= sect. *Nardurus* (Rchb.) Stace)		
V. unilateralis (L.) Stace (1)	2*x*	Mediterranean, W Europe
Castellia Tineo		
C. tuberculosa (Moris) Bor	2*x*	Mediterranean
Ctenopsis De Not.		
C. delicatula (Lag.) Paunero	2*x*	W Mediterranean
Hellerochloa Rauschert		
H. fragilis (Luces) Rauschert	unknown	N Andes
Micropyropsis Romero Zarco & Cabezudo		
M. tuberosa Romero Zarco & Cabezudo	unknown	SW Spain: Huelva
Micropyrum Link		
M. patens (Brot.) Rothm. ex Pilg.	2*x*	W Mediterranean
M. tenellum (L.) Link	2*x*	Mediterranean
Narduroides Rouy		
N. salzmannii (Boiss.) Rouy	2*x*	W Mediterranean
Psilurus Trin.		
P. incurvus (Gouan) Schinz & Thell.	4*x*	Mediterranean
Wangenheimia Moench		
W. lima (L.) Trin.	2*x*	W Mediterranean
Subtribe Cynosurinae		
Cynosurus L.		
C. cristatus L.	2*x*	Europe
C. echinatus L.	2*x*	Mediterranean
Subtribe Dactylidinae		
Dactylis L.		
D. glomerata L.	4*x*	Eurasia
D. hispanica Roth	4*x*	Mediterranean
Lamarckia Moench		
L. aurea (L.) Moench (1)	2*x*	Mediterranean

Table 1. Continued.

Taxon	Ploidy level	Distribution
Subtribe Parapholiinae		
Parapholis C. E. Hubb.		
P. incurva (L.) C. E. Hubb.	4*x*	W Mediterranean, W Europe
Catapodium Link		
C. rigidum (L.) C. E. Hubb.	2*x*	Mediterranean, W Europe
Hainardia Greuter		
H. cylindrica (Willd.) Greuter	4*x*	Mediterranean
Sphenopus Trin.		
S. divaricatus (Gouan) Rchb.	2*x*	Mediterranean
OUTGROUPS		
Tribe Poeae		
Subtribe Poinae		
Poa L.		
P. infirma Kunth	2*x*	Mediterranean, W Europe
Puccinellia Parl.		
P. distans (L.) Parl.	6*x*	Europe
Sclerochloa Beauv.		
S. dura (L.) P. Beauv.	2*x*	S & Central Europe
Subtribe Sesleriinae		
Sesleria Scop.		
S. argentea (Savi) Savi	4*x*	SW Europe
Tribe Aveneae		
Parafestuca E. B. Alexeev		
P. albida (Lowe) E. B. Alexeev	unknown	Madeira
Tribe Triticeae		
Secale L.		
S. cereale L.	2*x*	SW Asia
Tribe Brachypodieae		
Brachypodium P. Beauv.		
B. distachyon (L.) P. Beauv.	2*x*	Mediterranean

closed to the apex (≥95%). The shape of the leaf blade (flat vs. folded or setaceous) refers to blades of the innovation shoots; several taxa of intermediate shape were coded as polymorphic. The presence or absence of adaxial and abaxial sclerenchyma girders refers to those reaching the vascular bundles, whereas the structure of the abaxial sclerenchyma addresses whether it is separated into discrete bundles or distributed in a continuous ring. The inflorescence type was determined by considering the spikelet as the floral unit of grasses and interpreting the inflorescence as the arrangement of spikelets ranging from more or less open to condensed branched forms (panicle) to solitary spikelets inserted at the nodes of the rachis, these being either pedicellate (raceme) or sessile (spike).

Methods

Cladistic analysis.—Phylogenetic analyses were conducted for the three data sets (ITS, *trn*L–F, morphology) independently, for the molecular data (ITS + *trn*L–F) combined, and for the molecular and morphological data combined. All parsimony analyses were performed with PAUP* vers. 4.0 beta 10 (Swofford 2002) using two different heuristic search strategies as described in Torrecilla et al. (2004) (search 1: closest, tree-bisection-reconnection [TBR], MULPARS ON; search 2: random-order-entry of 10,000 replicates, TBR, MULPARS OFF, saving no more than five trees of score >10 per replicate). Analyses of the independent data sets were conducted first; all most-parsimonious trees obtained from the two heuristic searches were used to compute the final strict consensus tree for each data set. Support for internal nodes was estimated through 10,000 bootstrap replicates (Felsenstein 1985) using the fast bootstrap option provided in PAUP*. Simultaneous analyses were then performed on the combined molecular data matrix and the combined molecular+ morphological data matrix using the same methods described above. Support for nodes was also calculated with the fast bootstrap option of PAUP*.

Data heterogeneity, data decisiveness, and character evaluation.—The incongruence length difference (ILD) test of Farris et al. (1994) was used as an estimate of data heterogeneity among different combinations of data sets (ITS/*trn*L–F, ITS/morphology, *trn*L–F/morphology, ITS + *trn*L–F/morphology, ITS/*trn*L–F/morphology, *trn*L–F/ITS + morphology, ITS/*trn*L–F + morphology). The ILD test is based in the incongruence length metric (I_{MF}) of Mickevich and Farris (1981) that computes the differences in number of steps between trees constructed from random partitions of the same size as the original data sets and trees constructed from the original partitions. Significance for heterogeneity is achieved when 95% or more of the random partitions show an I_{MF} smaller than that of the original partition (Alvarez-Fernández et al. 2001). The ILD test has also been used as an indicator of combinability of data matrices that are not significantly heterogeneous (Johnson and Soltis 1998). How-

Table 2. Morpho-anatomical characters coded for the phylogenetic analysis of Loliinae and close allies. All characters were coded as unordered.

1. Habit: 0, annual; 1, perennial
2. Innovation shoots: 0, absent; 1, intravaginal; 2, extravaginal
3. Leaf sheath base: 0, not thickened; 1, thickened
4. Cataphylls: 0, absent; 1, reduced; 2, prominent
5. Leaf vernation: 0, conduplicate; 1, convolute; 2, supervolute
6. Leaf sheath: 0, open to partially closed (closed <5%); 1, closed (≥95%)
7. Falcate auricles: 0, absent; 1, present
8. Ligule apex: 0, acute; 1, obtuse; 2, truncate
9. Innovation leaf blades: 0, flat; 1, folded or setaceous
10. Innovation leaf blades, adaxial complete sclerenchyma girders: 0, absent; 1, present
11. Innovation leaf blades, abaxial complete sclerenchyma girders: 0, absent; 1, present
12. Innovation leaf blades, abaxial structure of sclerenchyma: 0, separate bundles; 1, continuous ring
13. Inflorescence type: 0, panicle; 1, raceme; 2, spike
14. Inflorescence axis (rachis): 0, not excavated or depressed; 1, excavated or depressed
15. Sterile spikelets: 0, absent; 1, present
16. Number of glumes: 0, two; 1, one
17. Number of veins on upper glume: 0, one; 1, three; 2, five or more
18. Back of lemma: 0, rounded; 1, keeled
19. Number of lemma veins: 0, one; 1, three; 2, five; 3, more than five
20. Lemma awn: 0, absent; 1, present
21. Number of anthers: 0, one; 1, three
22. Ovary apex: 0, glabrous; 1, pubescent
23. Ratio hilum/caryopsis length: 0, short (≤1/4); 1, medium to long (>1/3)

ever, there is disagreement about the appropriateness of this procedure to test for data combinability even at very low significance values (Barker and Lutzoni 2002). The GPWG (2001) found the ILD test to be misleading with respect to combinability of data sets in phylogenetic reconstruction of grasses. Nonetheless, heterogeneity among data sets was estimated from the number of extra steps required by trees constructed from random partitions with respect to trees constructed from the original partitions.

Another approach to estimate the quality of data sets used in cladistic analysis is data decisiveness (DD; Goloboff 1991), described as a measure of robustness of support by a data set for its most-parsimonious trees when compared to the average length of all possible trees (Davis et al. 1998). Davis et al. (1998) argued that despite evident incongruencies between data sets that provide strong support for different sets of phylogenetic relationships, these data sets are more valuable for phylogenetic inference than other less-conflicting character sets that provide little support for their own phylogenetic relationships. These authors used the Goloboff (1991) criterion to estimate the quality of different molecular data sets and combinations in monocotyledons, and concluded that less variable but more internally congruent data sets showed better quality attributes than more variable but less internally congruent data sets. We employed DD to estimate the potential decisiveness and quality of our molecular and morphological data sets. For this purpose, uninformative characters were removed from the three ITS, *trn*L–F, and morphological character sets and their combinations, and $\bar{S}$, S, and M values (Goloboff 1991) were computed using PAUP*. $\bar{S}$ was estimated as the average length of 100,000 randomly resolved trees (Davis et al. 1998).

Evaluation of the potential phylogenetic signal provided by the morphological characters was accomplished by superimposing their changes on the combined molecular + morphology optimal consensus tree using the trace character option provided in MacClade vers. 3.04 (Maddison and Maddison 1992).

RESULTS

Molecular Data

Cladograms obtained from the separate analyses of the ITS and *trn*L–F data matrices are a summary of those in Catalán et al. (2004). Names of clades also correspond to those indicated in Catalán et al. (2004). The heuristic search conducted on the ITS data set found 27,339 most-parsimonious trees (MPTs) of length (L) 1332 and with a consistency index (CI) of 0.411 and a retention index (RI) of 0.675. The strict consensus of all MPTs is shown in Fig. 1. Analysis of the *trn*L–F data set rendered 149,106 MPTs with L = 796, CI = 0.539, and RI = 0.801. The strict consensus of all MPTs is shown in Fig. 2. The two phylogenies are congruent in resolution of a moderately to poorly supported clade of fine-leaved *Festuca* + *Vulpia* + related ephemerals (FEVRE group, cf. Torrecilla et al. 2004) in which the strongest support is for subclades *Festuca* sect. *Aulaxyper* p. p. + *Vulpia* sect. *Vulpia* p. p. (2*x*), sect. *Festuca* p. p., and *Psilurus* + sect. *Vulpia* p. p. (4*x,* 6*x*). Representatives of *Festuca* sect. *Eskia* were resolved as basal paraphyletic assemblages of the FEVRE clade in both trees. *Wangenheimia* was resolved as the well-supported sister taxon of the sect. *Festuca* p. p. clade in the *trn*L–F tree (Fig. 2), whereas *Micropyrum* was unexpectedly resolved as sister but with no bootstrap support to the sect. *Aulaxyper* clade in the ITS tree (Fig. 1). A fourth resolved but unsupported lineage includes *Vulpia* sects. *Monachne* and *Loretia* plus *F. plicata*; the *trn*L–F tree also incorporates *Ctenopsis* and *Vulpia* sect. *Apalochloa.* Surprisingly, *Vulpia* was resolved as polyphyletic in both the nuclear and chloroplast trees.

Table 3. Data matrix for the morphological phylogenetic analysis of Loliinae and allies. See Table 2 for explanation of characters. Polymorphic characters are coded in brackets and missing data are indicated with a question mark.

Species	Character																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Outgroups																							
Brachypodium distachyon	0	0	0	0	2	0	0	2	0	1	1	0	1	0	0	0	2	0	3	1	1	1	1
Secale cereale	0	0	0	0	2	0	1	2	0	1	1	0	2	0	0	0	0	1	2	1	1	1	1
Sesleria argentea	1	[12]	0	0	0	1	0	2	0	1	1	0	0	0	0	0	0	0	2	1	1	1	0
Parafestuca albida	1	1	0	0	2	?	0	2	0	1	1	0	0	0	0	0	1	1	1	1	1	0	0
Sclerochloa dura	0	[12]	0	0	0	0	0	0	[01]	0	1	0	1	0	0	0	2	0	3	0	1	0	0
Puccinellia distans	1	[12]	0	0	0	0	0	0	[01]	1	1	0	0	0	0	0	1	0	2	0	1	0	0
Poa infirma	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	2	0	1	0	0
Dactylidinae																							
Dactylis hispanica	1	2	0	0	0	0	0	2	0	1	1	0	0	0	1	0	1	1	2	1	1	0	0
D. glomerata	1	2	0	0	0	0	0	2	0	1	1	0	0	0	1	0	1	1	2	1	1	0	0
Lamarckia aurea	0	0	0	0	0	0	0	2	0	1	1	0	0	0	1	0	0	0	2	1	1	0	0
Cynosurinae–Parapholiinae																							
Cynosurus echinatus	0	0	0	0	2	0	0	0	0	1	1	0	0	0	1	0	0	0	2	1	1	0	0
C. cristatus	1	1	0	0	2	0	0	0	0	1	1	0	0	0	1	0	0	0	2	1	1	0	0
Sphenopus divaricatus	0	0	0	0	1	0	0	1	[01]	0	0	0	0	0	0	0	0	1	1	0	1	0	0
Catapodium rigidum	0	0	0	0	2	0	0	1	[01]	0	0	0	1	0	0	0	1	0	2	0	1	0	0
Hainardia cylindrica	0	0	0	0	2	0	0	2	[01]	[01]	0	0	2	1	0	1	2	0	[12]	0	[01]	0	0
Parapholis incurva	0	0	0	0	2	0	0	2	[01]	[01]	0	0	2	1	0	0	[12]	0	0	0	1	0	0
Loliinae																							
Lolium perenne	1	2	0	0	2	0	1	2	0	[01]	[01]	0	2	1	0	1	2	0	3	1	1	0	1
L. rigidum	0	0	0	0	2	0	1	2	0	0	0	0	2	1	0	1	2	0	3	1	1	0	1
Micropyropsis tuberosa	1	2	0	0	2	0	1	2	0	1	1	0	1	1	0	0	1	0	2	1	1	0	1
Castellia tuberculosa	0	0	0	0	2	0	1	2	0	1	1	0	[12]	1	0	0	[12]	0	2	0	1	0	1
Hellerochloa fragilis	1	1	0	0	0	0	0	0	1	0	[01]	1	0	0	0	0	1	0	2	1	1	?	?
Festuca pratensis	1	2	0	0	2	0	1	2	0	[01]	1	0	0	0	0	0	1	0	2	1	1	0	1
F. arundinacea	1	2	0	[02]	2	0	1	2	0	1	1	0	0	0	0	0	1	0	2	1	1	0	1
F. fenas	1	2	0	2	2	0	1	2	0	1	1	0	0	0	0	0	1	0	2	1	1	0	1
F. gigantea	1	2	0	2	2	0	1	2	0	1	1	0	0	0	0	0	1	0	2	1	1	0	1
F. fontqueri	1	2	0	0	0	0	1	2	0	[01]	1	0	0	0	0	0	1	0	2	1	1	0	1
F. mairei	1	2	0	2	1	0	0	2	0	1	1	0	0	0	0	0	1	0	2	1	1	0	1
F. paniculata	1	1	1	0	0	0	0	2	0	1	1	0	0	0	0	0	1	0	2	0	1	1	1
F. durandoi	1	1	1	0	1	0	0	2	[01]	0	0	0	0	0	0	0	1	0	2	0	1	1	1
F. baetica	1	1	1	0	0	0	0	2	[01]	1	1	0	0	0	0	0	1	0	2	0	1	1	1
F. kingii	1	2	0	?	2	0	0	2	0	1	1	0	0	0	0	0	0	1	2	0	1	1	1
F. spectabilis	1	2	0	2	2	0	0	2	0	1	1	0	0	0	0	0	1	0	2	0	1	1	1
F. pulchella	1	2	0	2	2	0	0	2	0	1	1	0	0	0	0	0	1	0	2	0	1	[01]	1
F. dimorpha	1	2	0	2	0	0	0	2	[01]	[01]	1	0	0	0	0	0	1	0	2	0	1	1	1
F. drymeja	1	2	0	2	2	0	0	2	0	1	1	0	0	0	0	0	1	0	2	0	1	1	1

Table 3. Continued.

Species	Character																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
F. lasto	1	2	0	2	2	0	0	2	0	1	1	0	0	0	0	0	1	0	2	0	1	1	1
F. scariosa	1	[12]	0	0	0	0	0	0	1	1	1	[01]	0	0	0	0	1	0	2	0	1	1	1
F. coerulescens	1	1	1	0	1	0	0	2	0	1	1	0	0	0	0	0	1	0	2	0	1	1	1
F. pseudeskia	1	2	0	2	0	0	0	0	1	1	1	0	0	0	0	0	1	0	2	0	1	1	1
F. altissima	1	2	0	2	2	0	0	2	0	1	1	0	0	0	0	0	1	0	2	0	1	0	1
F. triflora	1	1	1	0	1	0	0	2	0	0	1	0	0	0	0	0	1	0	2	0	1	1	1
F. californica	1	[12]	0	0	0	0	0	2	0	1	1	[01]	0	0	0	0	1	0	2	1	1	1	1
F. altaica	1	?	0	?	2	0	0	2	0	1	1	0	0	0	0	0	1	?	2	1	1	1	1
F. subulata	1	2	0	2	1	0	0	2	0	1	1	0	0	0	0	0	1	0	2	1	1	1	1
F. carpathica	1	2	0	2	2	0	0	2	0	1	1	0	0	0	0	0	1	0	2	1	1	1	1
F. agustinii	1	2	0	1	1	0	0	2	0	0	1	0	0	0	0	0	1	0	2	1	1	0	1
F. rubra	1	[12]	0	1	0	1	0	2	1	0	0	0	0	0	0	0	1	0	2	1	1	0	1
F. querana	1	[12]	0	1	0	1	0	2	1	0	[01]	[01]	0	0	0	0	1	0	2	1	1	1	1
F. rothmaleri	1	[12]	0	1	0	1	0	2	1	0	[01]	0	0	0	0	0	1	0	2	1	1	0	1
F. juncifolia	1	[12]	0	1	0	1	0	2	1	0	[01]	[01]	0	0	0	0	1	0	2	1	1	0	1
F. nevadensis	1	[12]	0	1	0	1	0	2	1	0	[01]	[01]	0	0	0	0	1	0	2	1	1	0	1
F. rivularis	1	[12]	0	1	0	1	0	2	1	0	0	0	0	0	0	0	1	0	2	1	1	0	1
F. iberica	1	[12]	0	1	0	1	0	2	1	0	0	0	0	0	0	0	1	0	2	1	1	0	1
F. hystrix	1	1	0	0	0	1	0	2	1	0	0	1	0	0	0	0	1	0	2	1	1	0	1
F. longiauriculata	1	1	0	0	0	0	0	2	1	0	0	1	0	0	0	0	1	0	2	1	1	0	1
F. aragonensis	1	1	0	0	0	0	0	2	1	0	0	1	0	0	0	0	1	0	2	1	1	0	1
F. ovina	1	1	0	0	0	0	0	2	1	0	0	1	0	0	0	0	1	0	2	1	1	0	1
F. frigida	1	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	2	1	1	0	1
F. alpina	1	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	2	1	1	0	1
F. glacialis	1	1	0	0	0	1	0	2	1	0	0	0	0	0	0	0	1	0	2	1	1	0	1
F. borderei	1	1	0	0	0	0	0	2	1	0	0	[01]	0	0	0	0	1	0	2	1	1	0	1
F. pyrenaica	1	[12]	0	1	0	1	0	2	1	0	0	0	0	0	0	0	1	0	2	1	1	0	1
F. capillifolia	1	1	0	0	0	0	0	2	1	0	[01]	[01]	0	0	0	0	1	0	2	1	1	0	1
F. clementei	1	1	0	0	0	1	0	2	1	0	0	[01]	0	0	0	0	1	0	2	1	1	0	1
F. plicata	1	1	0	0	0	1	0	2	1	0	0	0	0	0	0	0	1	0	2	1	1	0	1
F. gautieri	1	1	0	0	0	0	0	2	1	0	0	[01]	0	0	0	0	1	0	2	1	1	1	1
F. eskia	1	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	2	1	1	1	1
F. elegans	1	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	2	0	1	1	1
F. burnatii	1	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	2	1	1	1	1
F. quadriflora	1	1	0	0	0	0	0	2	1	0	0	[01]	0	0	0	0	1	0	2	1	1	1	1
Vulpia bromoides	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	1	0	2	1	[01]	0	1
V. muralis	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	1	0	2	1	[01]	0	1
V. myuros	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	1	0	2	1	[01]	0	1
V. ciliata	0	0	0	0	1	0	0	2	[01]	0	0	0	0	0	0	0	[01]	0	1	1	[01]	0	1
V. alopecuros	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	1	0	2	1	[01]	0	1
V. geniculata	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	1	0	2	1	[01]	0	1
V. sicula	1	0	0	0	1	0	0	2	0	0	[01]	0	0	0	0	0	1	0	2	1	[01]	0	1
V. fasciculata	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	1	0	2	1	[01]	1	1
V. fontqueriana	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	1	0	2	1	[01]	0	1

Table 3. Continued.

Species	Character																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
V. membranacea	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	1	0	2	1	[01]	0	1
V. unilateralis	0	0	0	0	1	0	0	2	0	0	0	0	1	0	0	0	1	0	2	1	1	0	1
Ctenopsis delicatula	0	0	0	0	1	0	0	2	[01]	0	0	0	0	0	0	0	1	0	2	1	1	0	1
Psilurus incurvus	0	0	0	0	0	0	0	2	[01]	0	0	0	2	1	0	1	0	1	1	1	0	0	1
Micropyrum tenellum	0	0	0	0	0	0	0	2	[01]	0	0	0	1	0	0	0	[12]	0	2	[01]	1	0	1
M. patens	0	0	0	0	0	0	0	2	[01]	0	0	0	1	0	0	0	[12]	0	2	[01]	1	0	1
Narduroides salzmanii	0	0	0	0	0	0	0	0	1	0	0	0	2	1	0	0	[12]	[01]	2	0	1	0	1
Wangenheimia lima	0	0	0	0	0	0	0	2	[01]	0	0	0	0	0	0	0	1	1	2	0	1	0	0

The broad-leaved group was resolved as monophyletic (bootstrap 73%) in the *trn*L–F tree (Fig. 2), but not in the ITS tree (Fig. 1). The two topologies possess a moderately to well-supported clade of *Lolium* + *Micropyropsis* + *Festuca* subgen. *Schedonorus* s.l. that also includes *F. mairei* (cf. Catalán et al. 2004). Morphologically intermediate taxa (e.g., *F. altaica, F. californica, F. pulchella, F. subulata*) between the broad-leaved and the fine-leaved groups did not form clades in either phylogeny and were variously placed (Fig. 1, 2). Some well-supported clades in the ITS tree (e.g., *Festuca* subgen. *Leucopoa* + subgen. *Subulatae,* bootstrap 80%) were not recovered in the *trn*L–F tree. *Castellia* was resolved differently, though with bootstrap support <70%, in each topology, whereas *Parafestuca* fell outside of the festucoid clade confirming the separate treatment given to this genus by Alexeev (1985). *Lolium* was strongly supported as monophyletic in the ITS tree (Fig. 1), but not in the *trn*L–F tree (Fig. 2).

Loliinae were resolved as monophyletic (bootstrap 70%) in the *trn*L–F phylogeny, but not in the ITS phylogeny.

The simultaneous analysis of the ITS and *trn*L–F data rendered 8795 MPTs with L = 2208, CI = 0.431, and RI = 0.698; the strict consensus tree is shown in Fig. 3. The combined analysis provided better resolution than the separate analyses (cf. Catalán et al. 2004). Loliinae were resolved as monophyletic and consist of two main lineages, a well-supported clade of fine-leaved *Festuca* and relatives and a poorly supported clade of broad-leaved *Festuca* and relatives. Sister (basal) to these large clades, but lacking bootstrap support, is *Castellia,* a relationship unresolved in the larger study of Catalán et al. (2004). The clades of broad- and fine-leaved taxa become obscured when more samples are analyzed (Catalán et al. 2004). The presence of intermediate taxa at the base of or close to the broad-leaved clade indicates a trend from more ancestral broad-leaved *Festuca* lineages toward the more recently evolved FEVRE lineages, a finding that is correlated with the high mutation rates observed in most of the annual lineages of the fine-leaved group (cf. Torrecilla et al. 2004). The combined analysis also resolved, though with bootstrap support <50%, the sister clades Dactylidinae and Cynosurinae–Parapholiinae as the closest relatives of Loliinae. Resolution within the Loliinae clade is much the same as that recovered from the separate analyses for the best-supported clades: *Festuca* sect. *Aulaxyper* p. p. + *Vulpia* sect. *Vulpia* p. p. (2*x*) (bootstrap 85%), sect. *Festuca* p. p. + *Wangenheimia* (bootstrap 69%), *Psilurus* + sect. *Vulpia* p. p. (4*x,* 6*x*) (bootstrap 99%), and *Vulpia* sects. *Monachne* and *Loretia* + *F. plicata* (bootstrap 53%) within the fine-leaved lineage; and, within the broad-leaved lineage, *Lolium* + *Micropyropsis* + *Festuca* subgen. *Schedonorus* s.l. + *F. mairei* (bootstrap 98%), the *F. paniculata* group (bootstrap 99%), *Festuca* sect. *Leucopoa* (*F. kingii*) + *F. spectabilis* (of the polyphyletic *Festuca* sect. *Amphigenes*) (bootstrap 79%), *Festuca* sects. *Scariosae* and *Pseudoscariosa* + subgen. *Drymanthele* (bootstrap 70%), and *Festuca* sect. *Subbulbosae* p. p. (bootstrap 79%) (Fig. 3).

Morphological Data

The heuristic analysis of morphological and anatomical data rendered 453,300 MPTs with L = 145, CI = 0.275, and

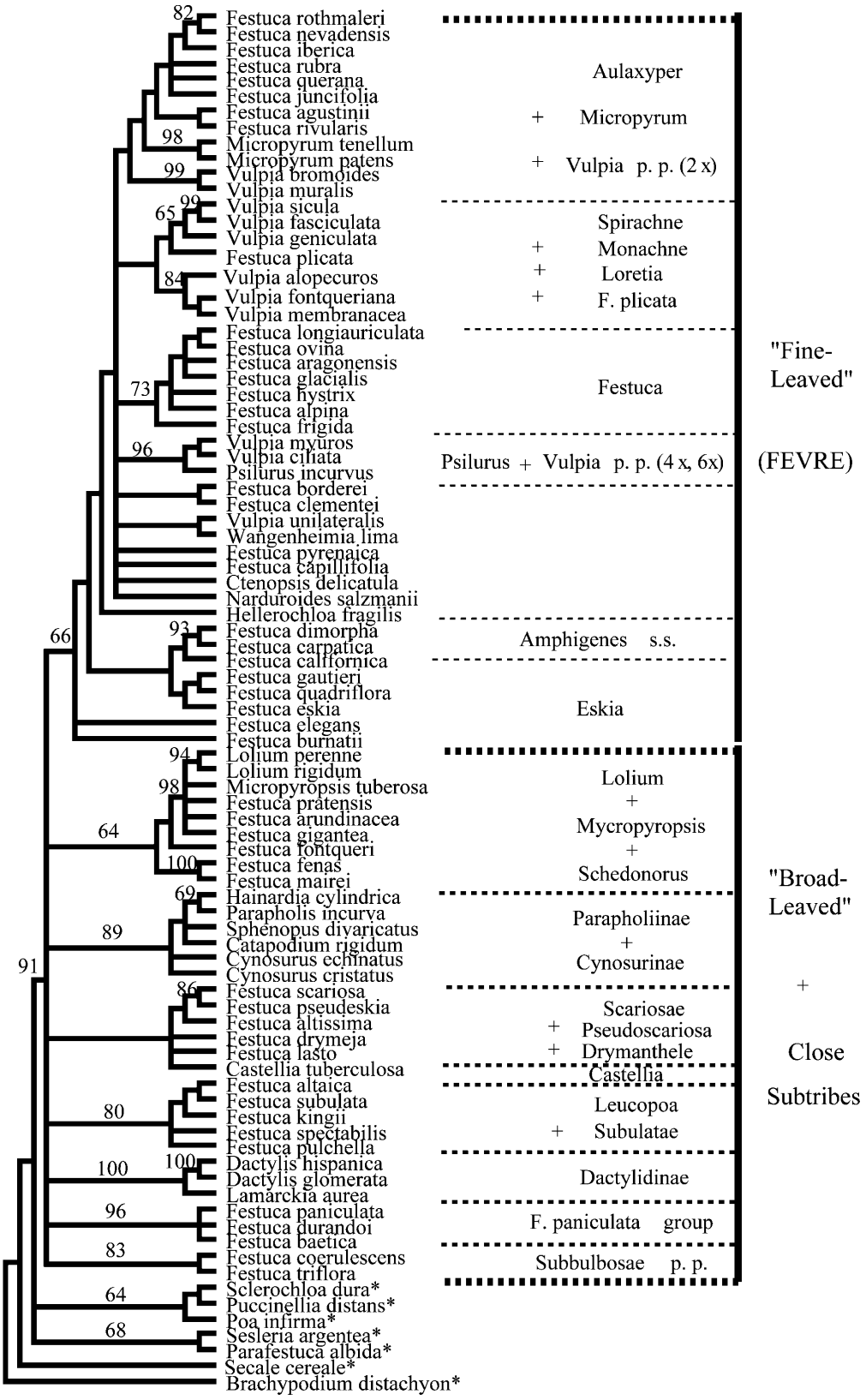

Fig. 1. ITS data set. Strict consensus of 27,339 most-parsimonious trees (L = 1332, CI = 0.411, RI = 0.675). Bootstrap percentages ≥50 are indicated. Outgroups are noted by asterisks.

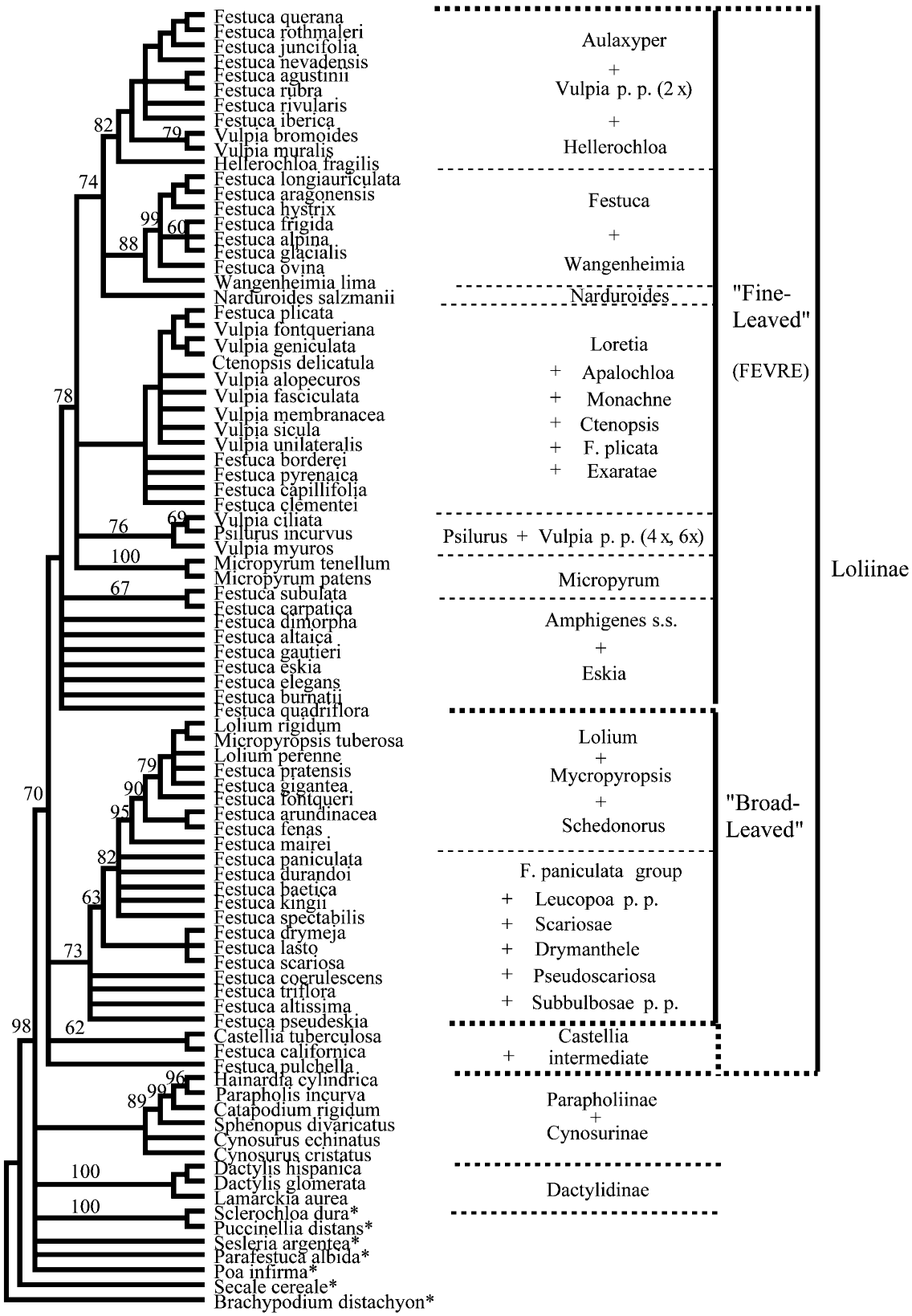

Fig. 2. *trn*L–F data set. Strict consensus of 149,106 most-parsimonious trees (L = 796, CI = 0.539, RI = 0.801). Bootstrap percentages ≥50 are indicated. Outgroups are noted by asterisks.

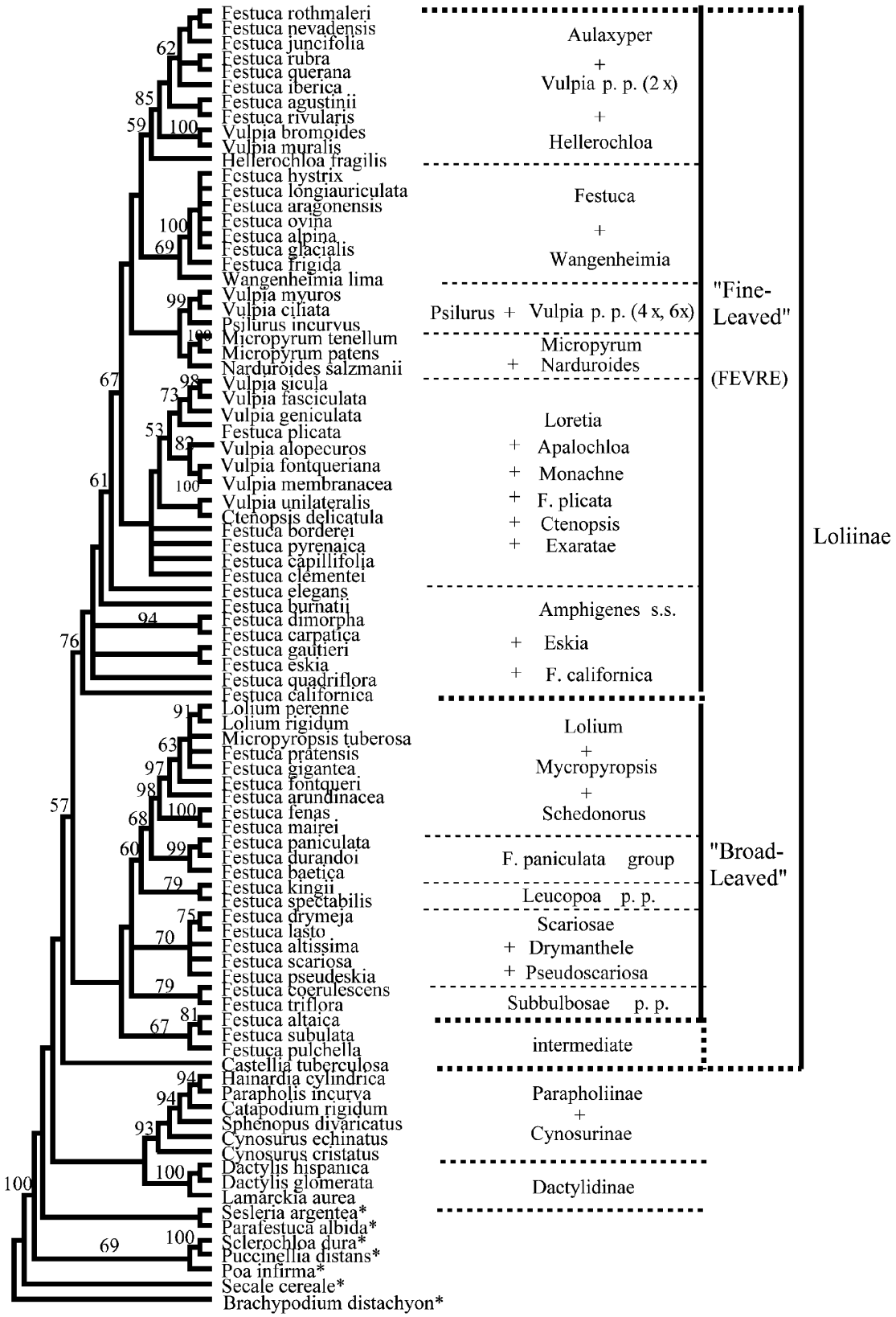

Fig. 3. Combined ITS + *trn*L–F data set. Strict consensus of 8795 most-parsimonious trees (L = 2208, CI = 0.431, RI = 0.698). Bootstrap percentages ≥50 are indicated. Outgroups are noted by asterisks.

RI = 0.774; the strict consensus is shown in Fig. 4. Lack of resolution characterizes the morphology tree except for some groups of fine-leaved taxa—*Festuca* sect. *Subbulbosae, Lolium, Dactylis, Lamarckia* + *Cynosurus,* and a clade of *Wangenheimia, Sphenopus,* and *Poa infirma.* All clades except *Dactylis, Lolium,* and sect. *Subbulbosae* lack bootstrap support greater than 50%. All recovered clades but one (*Subbulbosae*) are based on ambiguous synapomorphies. Despite the poor resolution obtained with this data set, the series of successive divergences observed within the clade of some fine-leaved *Festuca* and *Hellerochloa* (from basal assemblages of sect. *Eskia* + *Hellerochloa,* through the intermediate subsects. *Exaratae* and *Festuca,* toward a more recently evolved sect. *Aulaxyper*; Fig. 4) are similar with those obtained from the ITS analysis (Fig. 1).

Better resolution was obtained, however, when the analysis was restricted to Loliinae representatives. The 50% majority-rule consensus tree obtained when representatives other than Loliinae were pruned from the original set of MPTs is depicted in Fig. 5; *F. agustinii* was arbitrarily chosen to root the tree. The tree depicts two main and unsupported clades within Loliinae. One clade includes all annual genera and the other comprises the perennial genera *Festuca, Hellerochloa,* and *Micropyropsis.* Several ambiguous character states, related to the reduced habit and reproductive traits, group the ephemeral taxa in a poorly resolved clade. *Lolium* (including the perennial *L. perenne*) falls within this clade because of its contracted and reduced inflorescence and floral organs. The trend from a broad- to a fine-leaved morphological syndrome is supported in the perennial clade (Fig. 5). Some of the clades resolved in the Loliinae morphology tree, such as *Festuca* subgen. *Schedonorus* + *Micropyropsis,* and sect. *Aulaxyper* (Fig. 5), are similar to those obtained from the combined analysis of molecular data (Fig. 3).

All but one of the 23 structural characters studied are homoplasious across Loliinae, related subtribes, and the outgroups; however, their rescaled consistency index values (RC) vary considerably. The highest value (1.000) corresponds to character 3, thickening of the leaf sheath base. Other characters with moderate RC values are glume, lemma vein, and anther numbers (chars. 16, 19, and 21, respectively), presence of sterile spikelets (char. 15), and possession of adaxial and abaxial sclerenchyma girders (chars. 10 and 11, respectively). In spite of the differences in RC values, no secondary weighting scheme was applied for the cladistic analysis of the morphological data. Relationships recovered from this analysis are supported by different sets of synapomorphic character states. Only one, the thickened base of the leaf sheath that is a synapomorphy for *Festuca* sect. *Subbulbosae* (Fig. 4, 5), is unambiguous. The remaining character states are homoplasious, but constitute secondary synapomorphies. Thus, *Hainardia, Lolium,* and *Psilurus* share a spike inflorescence, an excavated rachis, and a single glume; the *Lolium* representatives, forming a weakly supported clade (bootstrap <60%), also bear more than five veins on the upper glume. The two *Dactylis* species form a moderately supported clade (bootstrap 82%) characterized by sterile spikelets, extravaginal innovation shoots, and conduplicate leaf vernation. *Cynosurus* and *Lamarckia* form an unsupported clade based on co-possession of a one-veined upper glume. These genera did not form a clade with *Dactylis* although the three possess sterile spikelets. The clade (bootstrap <50%) of fine-leaved *Festuca* and *Hellerochloa* (Fig. 4, 5) is based on a perennial habit, conduplicate vernation, and an awned lemma. The basal sects. *Eskia* and *Hellerochloa* have common traits such as a hairy ovary tip, and some of the intermediate representatives of subsects. *Exaratae* and *Festuca* have leaf blades that possess a continuous abaxial ring of sclerenchyma. The more recently diverged sect. *Aulaxyper* s.l. group was resolved as monophyletic based on their mixture of intravaginal and extravaginal innovation shoots and reduced cataphylls. This group also has sheaths closed to the apex, though this character state is also shared with some members of sect. *Festuca.*

In the Loliinae tree (Fig. 5), the *Festuca* subgen. *Schedonorus* + *Micropyropsis* clade is characterized by falcate auricles and awned lemmas, unique features within the broad-leaved group, whereas representatives of *Festuca* sect. *Amphigenes* and subgen. *Drymanthele, Leucopoa,* and *Subulatae* lack falcate auricles and awned lemmas, but with the subgen. *Schedonorus* + *Micropyropsis* clade share innovation leaves having complete abaxial and adaxial sclerenchyma bridges.

Data Heterogeneity, Data Decisiveness, and Character Evaluation

Attributes of the three data sets and combinations thereof are provided in Table 4. The ITS data set provided the greatest number of parsimony-informative characters and the longest MPTs. However, ITS yielded the second-lowest RI values of the three data sets and combinations. Conversely, the more conserved *trn*L–F data set possessed a lower number of informative characters and provided shorter MPTs, but the RI values were the highest. The morphological data set provided a small number of informative characters, yielding the shortest MPTs and the lowest CI values. In spite of that, RI values were intermediate between those from the ITS and the *trn*L–F data sets. Data decisiveness values corroborate these results, indicating that the chloroplast *trn*L–F character set is most decisive, followed by morphology and ITS. In terms of quality of data for cladistic analysis (based on DD, CI, and RI values; cf. Davis et al. 1998), it could be concluded that the chloroplast data carry a deeper phylogenetic signal (including insertions/deletions) for Loliinae and close allies than do the ITS and the morphological data. The morphological data also possessed some phylogenetic signal, though mostly in the form of secondary, homoplasious synapomorphies. The poorer quality of the ITS data when compared to *trn*L–F is probably related to the confounding homoplasy owed to a greater nucleotide substitution rate. Torrecilla et al. (2004) demonstrated that the annual lineages of the FEVRE group have experienced higher substitution rates than the perennial lineages, and, within the perennials, the rate is higher in polyploids than in diploids. Data decisiveness confirms the existence of noise created by both the highly heterogeneous ITS sequences and the highly homoplasious morphological characters in cladistic analysis. The DD values obtained for the different data set combinations reflect the values from the independent character sets. Thus, the three combinations that included the *trn*L–F data yielded

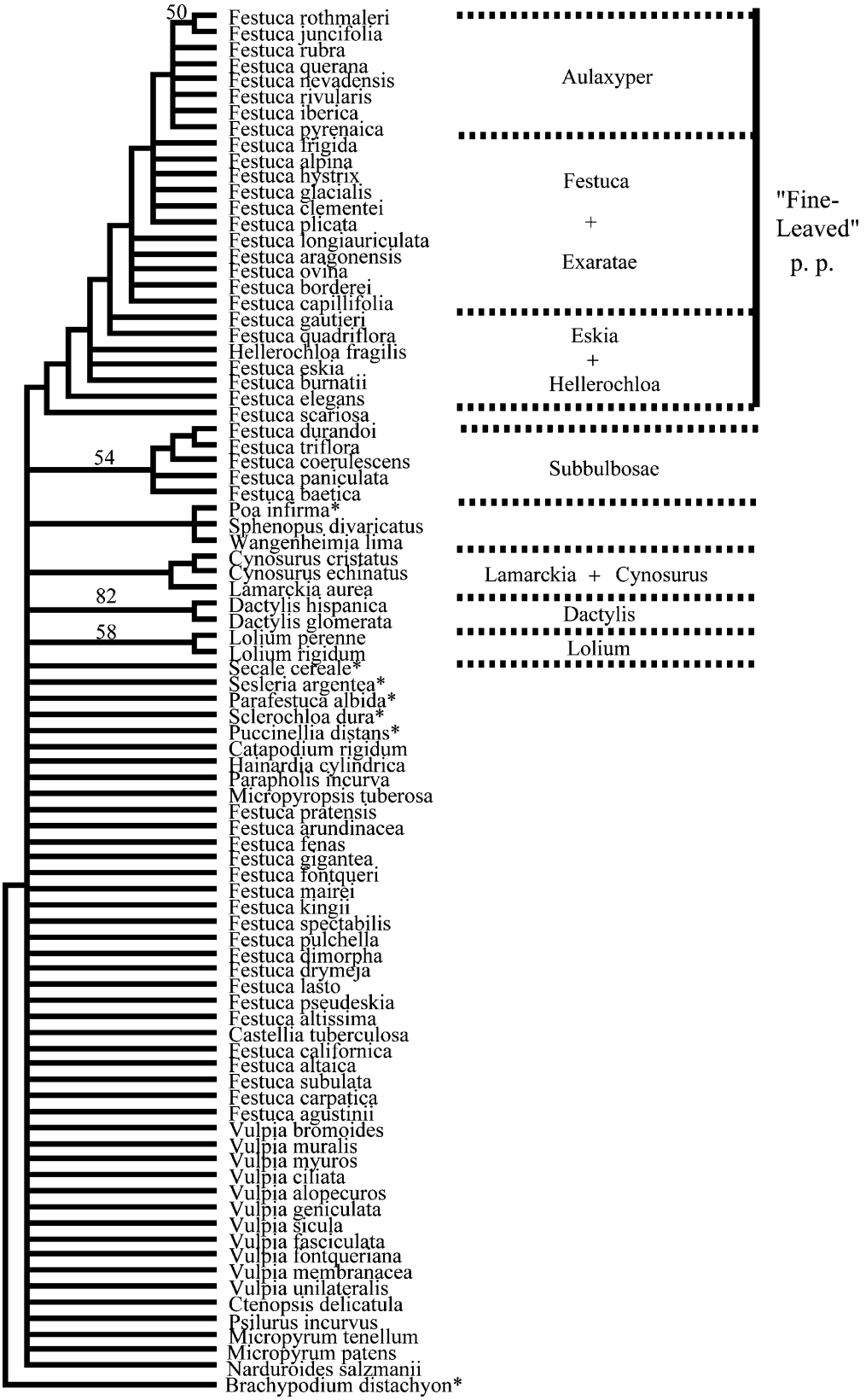

Fig. 4. Morphological data set. Strict consensus of 453,300 most-parsimonious trees (L = 145, CI = 0.275, RI = 0.774). Bootstrap percentages ≥50 are indicated. Outgroups are noted by asterisks.

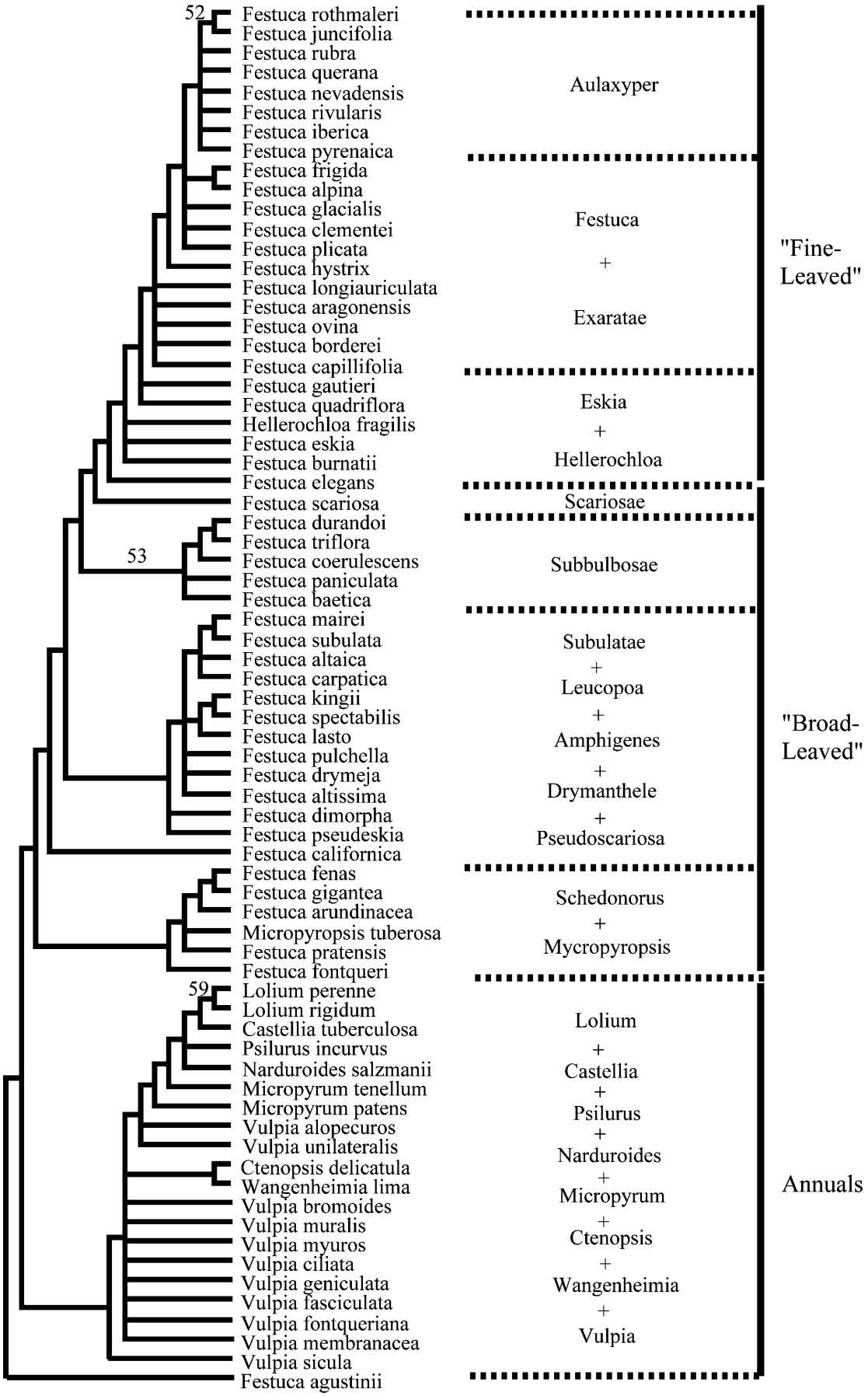

Fig. 5. 50% majority-rule consensus tree from the analysis of the morphological data set, with all non-Loliinae taxa pruned. *Festuca agustinii* was arbitrarily chosen to root the tree. Bootstrap percentages ≥50 are indicated.

Table 4. Attributes of the ITS, *trn*L–F, and morphological data sets and combinations thereof. Only parsimony-informative characters were included in the analyses. Tree length difference refers to the difference between the tree constructed from the original partitions and the shortest tree constructed from any random partition.

Data sets	Number of informative characters	Length of shortest tree	Consistency index	Retention index	Data decisiveness	Incongruence length difference test	Tree length difference (steps)
*trn*L–F	210	541	0.540	0.801	0.791		
ITS	280	1188	0.411	0.675	0.641		
Morphology	23	145	0.275	0.774	0.738		
*trn*L–F + ITS	490	1810	0.431	0.697	0.668	Incongruent ($P = 0.001$)	
*trn*L–F + Morphology	233	755	0.441	0.755	0.737	Incongruent ($P = 0.001$)	
ITS + Morphology	303	1415	0.382	0.665	0.634	Incongruent ($P = 0.001$)	
*trn*L–F + ITS + Morphology	513	2043	0.407	0.687	0.660	Incongruent ($P = 0.001$)	
*trn*L–F/ITS							50
*trn*L–F/Morphology							44
ITS/Morphology							44
*trn*L–F/ITS/Morphology							49
ITS + *trn*L–F/Morphology							50
*trn*L–F/ITS + Morphology							52
ITS/*trn*L–F + Morphology							53

higher CI, RI, and DD values than did the ITS + morphology data combination.

Tree length differences were calculated for all character set combinations as an estimate of the degree of heterogeneity present. The tree length differences (Table 4) agree with the previous results, in that the number of extra steps found between the length of the shortest trees obtained from the original partitions and that obtained from any random partition increases when discrepancies in the quality of the data sets are greater. The more decisive *trn*L–F data produced higher tree length differences in most combinations (e.g., *trn*L–F/ITS = 50) than the less decisive ITS and morphological data sets (e.g., ITS/morphology = 44) as ITS and morphology are, in fact, highly heterogeneous themselves.

Nonetheless, the phylogenetic analyses of Loliinae and close allies performed on the three independent data sets rendered topologies that are mostly congruent. That is, conflicts are not supported. Therefore, we believe that the analyses of the three separate data sets, despite differences in resolution of the resulting trees, recovered the same evolutionary history. Compared to the molecular data, the morphological data poorly resolved relationships when taxon sampling extended beyond Loliinae (Fig. 4). However, the clades resolved from analysis of Loliinae alone (Fig. 5) are mostly congruent with the topology recovered from the combined analysis of the two molecular data sets (Fig. 3). Therefore, we decided to jointly analyze the molecular and the morphological data sets as an epistemological approach to compare them and to evaluate the congruence of the morphological data in the combined tree.

The heuristic search conducted on the combined molecular/morphology data matrix rendered 13,596 trees with L = 2460, CI = 0.402, and RI = 0.683; the strict consensus is shown in Fig. 6. The topology of the morphology + molecular tree (Fig. 6) is almost the same as the combined (ITS + *trn*L–F) molecular tree (Fig. 3), as most of the informative characters were contributed by the molecular data. Differences mostly involve the placement of several ephemeral members of the FEVRE group (*Micropyrum, Narduroides,* and *Wangenheimia*) that form an unsupported clade including the polyploid *Psilurus* + *Vulpia* sect. *Vulpia* (4*x,* 6*x*) clade (bootstrap 98%). This clade forms a polytomy with the *Vulpia* sects. *Loretia* and *Monachne* + *Festuca plicata* clade, *Vulpia* sect. *Apalochloa,* and *Ctenopsis.* All sampled taxa in the monophyletic Loliinae represented by two or more species are non-monophyletic except for *Lolium* and *Micropyrum* (Fig. 6).

Morphological characters (Table 2) were evaluated applying the principle of total evidence by mapping their changes on the molecular + morphology tree. The most noteworthy character changes are shown in Fig. 7. All 23 characters were demonstrated to be homoplasious, though some changes are highly congruent with this topology. The possession of a long, linear hilum (char. 23) constitutes a synapomorphy for Loliinae (except for a reversal in *Wangenheimia*) and is the best trait to separate the subtribe from its closest relatives Dactylidinae and Cynosurinae–Parapholiinae, which bear a short, oval to punctiform hilum. Highly congruent character changes are possession of both adaxial and abaxial sclerenchyma girders (chars. 10, 11) that are common in most broad-leaved taxa (except *Lolium* and *Festuca durandoi*), but absent in most fine-leaved taxa (except for the intermediate *Festuca* sect. *Amphigenes* p. p. and *F. californica*). Other traits associated with the broad-leaved syndrome, such as robust extravaginal innovation shoots (char. 2) that possess large, conspicuous cataphylls (char. 4) and flat leaf blades (char. 9) with supervolute vernation (char. 5), are present in most of the broad-leaved taxa, but are lacking in the fine-leaved representatives except for the intermediate taxa. These character states are likely plesiomorphies. Reproductive characters are, in general, more homoplasious than vegetative characters. Development of a spike inflorescence

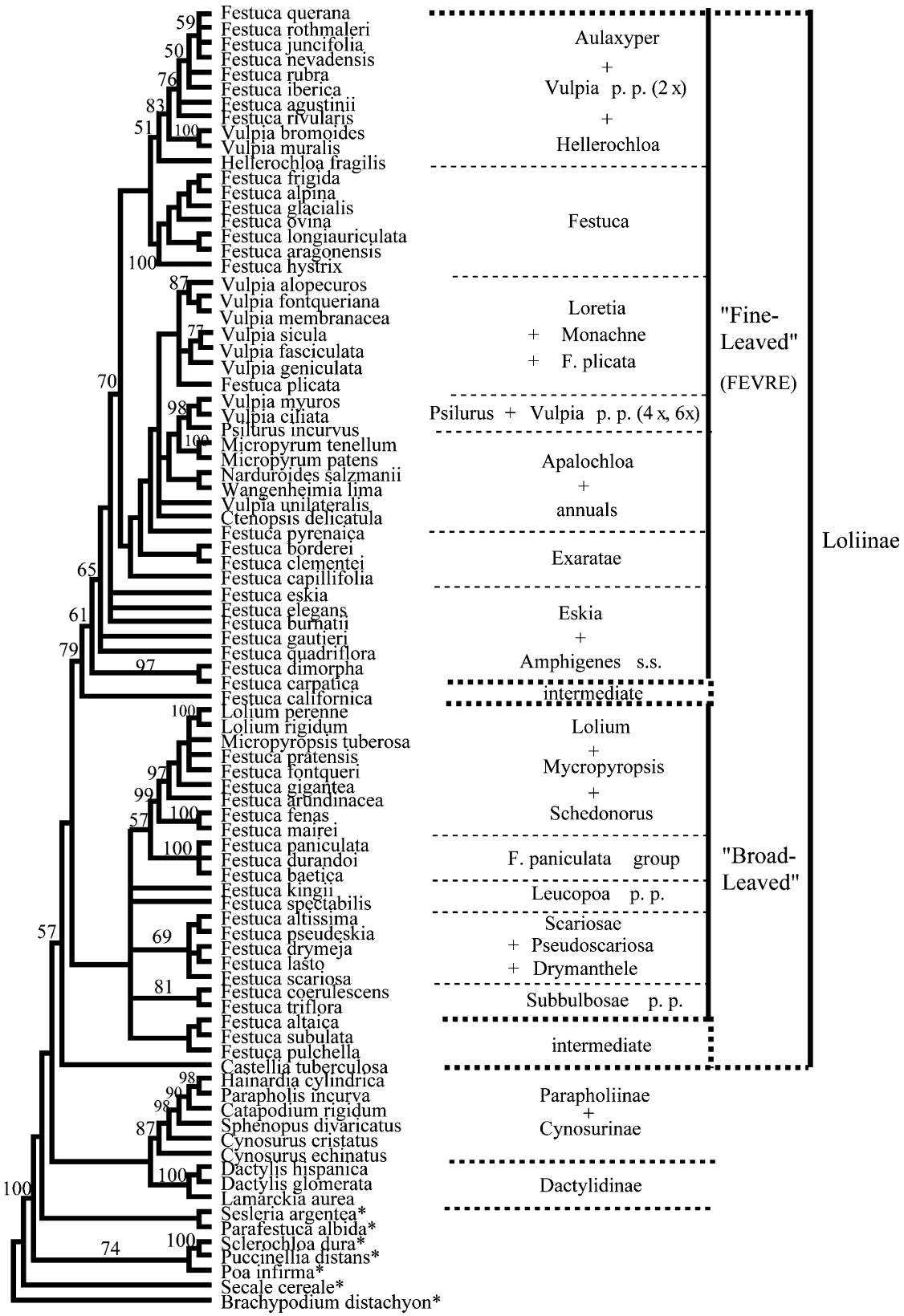

Fig. 6. Combined ITS + *trn*L–F + morphology data set. Strict consensus of 13,596 most-parsimonious trees (L = 2460, CI = 0.402, RI = 0.683). Bootstrap percentages ≥50 are indicated. Outgroups are noted by asterisks.

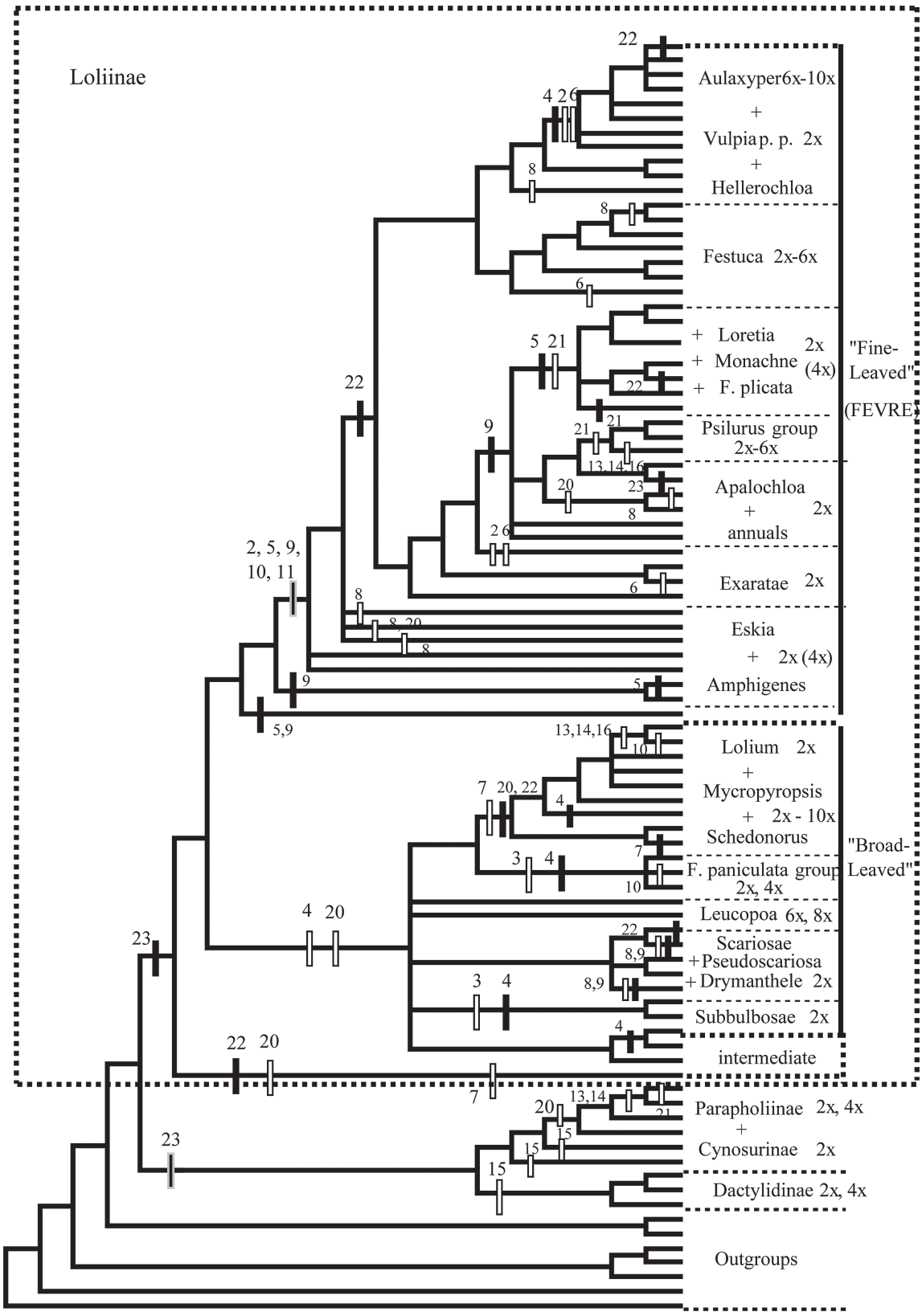

Fig. 7. Mapping of selected morphological character changes on the combined ITS + *trn*L–F + morphology strict-consensus tree. Solid bars correspond to unambiguous changes, gray bars to diagnostic, either unambiguous or ambiguous changes, open bars to parallelisms, and interrupted bars to reversals. Characters and states are explained in Table 2.

(char. 13) with a depressed or excavated rachis (char. 14), and loss of the lower glume (char. 16), is inferred to have taken place in parallel in both the broad- (*Lolium*) and fine-leaved (*Psilurus*) lineages, as well as in Parapholiinae (*Hainardia*). Falcate auricles (char. 7) are the best synapomorphy for the *Festuca* subgen. *Schedonorus* + *Micropyropsis* + *Lolium* clade (except *F. mairei*), though *Castellia* also bears them. Closed leaf sheaths (char. 6) have been acquired by several perennial groups of the fine-leaved clade (*Festuca* sect. *Aulaxyper,* subsect. *Festuca* p. p., and subsect. *Exaratae* p. p.). Members of the sect. *Eskia* and subsect. *Exaratae* and *Festuca* assemblages within the fine-leaved clade possess a continuous sclerenchyma ring along the abaxial side of the innovation leaf blade (char. 12) as opposed to the separate bundles found in the remaining taxa. Sterile spikelets (char. 15) are synapomorphic for Dactylidinae, but are also present in *Cynosurus.* Most Loliinae taxa possess a three-veined upper glume (char. 17), rounded lemma back (char. 18), and a five-veined lemma (char. 19). However, *F. kingii, Psilurus, Sphenopus, Vulpia ciliata,* and *Wangenheimia* lack some of these traits. Unawned lemmas (char. 20) are common in most members of the broad-leaved lineage except for the *Festuca* subgen. *Schedonorus* + *Micropyropsis* + *Lolium* clade and the intermediates *F. altaica* and *F. subulata.* Conversely, most fine-leaved taxa have awned lemmas except *Narduroides, F. elegans,* and the intermediate *F. dimorpha.* Single-anthered florets (char. 21) are autapomorphic for *Psilurus,* whereas transitions from one to three anthers have occurred in parallel in different *Vulpia* s.l. lineages and in *Hainardia.* A hairy ovary apex (char. 22) is common in the broad-leaved lineage except for the *F.* subgen. *Schedonorus* + *Micropyropsis* + *Lolium* group and *F. altissima.* Conversely, most fine-leaved taxa have glabrous ovary tips except for the basal sects. *Amphigenes* and *Eskia* and a few reversals (*F. querana, V. alopecuros,* and *V. fasciculata*). Ligule apex shape (char. 8) is highly homoplasious in Loliinae and close relatives.

DISCUSSION

Cladistic analysis of combined molecular and morphological data has provided a relatively well-resolved and supported phylogenetic hypothesis for Loliinae and close relatives (Fig. 6) to be used as the baseline evolutionary framework to postulate a natural classification system for these grasses.

In Loliinae, basal, relatively well-resolved broad-leaved lineages with flat leaves, convolute or supervolute vernation, and robust extravaginal innovation shoots diverged successively, giving rise to the less-divergent, fine-leaved groups that have folded or setaceous leaves with conduplicate vernation and mostly intravaginal (or less robust extravaginal) innovation shoots (Fig. 7). Basal lineages within both the broad- and fine-leaved groups are formed of diploids or low-level polyploids, whereas more recently evolved lineages underwent accelerated processes of increased polyploidy (e.g., *Festuca* subgen. *Aulaxyper* and *Schedonorus,* $2x$–$10x$) (Fig. 7). Recurrent hybridization coupled with occasional chromosome doubling are the invoked phenomena to interpret the observed evolutionary patterns of polyploidy in the festucoids (cf. Catalán et al. 2004).

With exception of the monotypic sect. *Apalochloa,* represented by *V. unilateralis,* no section of *Vulpia,* itself polyphyletic, is monophyletic. A close relationship of two sect. *Vulpia* polyploid species (*V. ciliata* and *V. myuros*) to *Psilurus* is well supported in both phylogenies, whereas two diploid species (*V. bromoides* and *V. muralis*), morphologically close to the polyploids, appear closely related to *Festuca* sect. *Aulaxyper,* although this relationship has less support. In light of the degree of concordance between the two phylogenies representing two genomes with different patterns of inheritance, parallel evolution seems quite plausible.

Analyses of data quality concur in showing better attributes of the *trn*L–F data set for cladistic inference of Loliineae and close relatives than the more resolutive but less decisive ITS data set and the poorly resolutive morphological data set. Despite differences in the number of informative characters provided by each character set that affect the lengths of their respective most-parsimonious trees (ITS > *trn*L–F > morphology; Table 4), DD, CI, and RI values should be interpreted as a likely consequence of differences in the intrinsic attributes of the three character sets rather than as a bias of sample size (cf. Davis et al. 1998). In contrast to previous findings, which indicate that small morphological data sets are not consistently swamped when combined with larger molecular character sets (Chippindale and Wiens 1994; Nixon and Carpenter 1996), our morphological data become obscured when combined with the molecular data set, probably due to their inherent homoplasy and relatively low incidence in overall levels of decisiveness. Despite significant incongruence found in all combinations of data sets, our analyses confirm that less decisive data sets (ITS, morphology) have less of a tendency than more decisive ones (*trn*L–F) to be incongruent with other data sets as corroborated by their shorter tree length differences detected across all classes of combinations (Table 4). Even if data decisiveness could be used as an informative index of overall robustness of support of relationships (Davis et al. 1998), combination of more decisive data with less decisive data is certainly possible when topologies are not in conflict with each other, which is the case for the molecular and morphological character sets analyzed here. Potential incongruence among data sets further allows refutation of evidence in one data set from the others and vice versa (cf. Davis et al. 1998); in our case, the most indecisive data set (ITS) shows the least evidence of incongruence with the others. Evaluation of data heterogeneity and quality could be a potentially valuable approach to estimate the accuracy of combined cladistic analysis of other molecular and structural character sets within this group of grasses.

Several factors in this study and our previous investigations of Loliinae and close relatives (Torrrecilla et al. 2003, 2004; Catalán et al. 2004) limit to different extents the recovery of phylogenetic relationships. Examples of these factors are reticulation and lineage sorting (Soreng and Davis 2000; Catalán et al. 2004), along with other undesirable effects such as the existence of potential paralogues of ITS (Gaut et al. 2000; Torrecilla et al. 2004) and significant heterogeneity in nucleotide substitution rates (Torrecilla et al. 2004), or potential effects related to chloroplast capture and phenotypic plasticity (Kellogg and Watson 1993; Mason-Gamer and Kellogg 1997; Catalán et al. 2004). These con-

founding factors may have altered the reconstruction of relationships among some festucoid lineages. Reticulation has probably occurred in the past and is operating today, as manifested by several spontaneous intergeneric crosses (×*Festulolium* Asch. & Graebn. and ×*Festulpia* Melderis ex Stace & R. Cotton) and by the highly introgressed polyploid assemblages found in both the fine- and broad-leaved lineages (Borrill et al. 1977; Ainscough et al. 1986). The negative impact of hybrids in cladistic analysis has been discussed extensively (McDade 1990, 1992; Soltis and Soltis 1999) and is usually the source of major conflict between nuclear- and chloroplast-based phylogenies in grasses (Kellogg et al. 1996; Mason-Gamer and Kellogg 1997; GPWG 2001). However, the relatively high congruence observed among the topologies recovered from the three independent data sets convinces us that a true evolutionary history of the festucoids and close relatives is represented.

Taxon sampling is another factor that might also affect the resolution of recovered phylogenies (Lecointre et al. 1993). Our sampling included most of the Loliinae genera recognized by Tzvelev (1982), Clayton and Renvoize (1986), and Watson and Dallwitz (1992), and almost all recognized sections of *Vulpia* (Cotton and Stace 1977; Stace 1981; cf. Catalán et al. 2004; Torrecilla et al. 2004). However, sampling is still insufficient for some subgenera of the large genus *Festuca* and for representatives of *Vulpia* sect. *Vulpia.* We sampled five of nine *Festuca* subgenera recognized by Clayton and Renvoize (1986), including some of the most important forage grasses native to Europe and the Mediterranean region, as well as several groups native to North and South America (Catalán et al. 2004). With this sampling we have attempted to establish a stable systematic framework for festucoids and close relatives that can be maintained as new phylogenetic data become available.

The information reported here and by Catalán et al. (2004) can be utilized to launch new systematic proposals for traditionally circumscribed taxa shown to be non-monophyletic, including *Festuca* and *Vulpia.* Four alternative scenarios can be envisaged for classification of these taxa:

Scenario 1—*Festuca* sensu latissimo. This scenario is based on both a monophyly criterion (Donoghue and Cantino 1998) and a synthetic taxonomic scheme (Judd et al. 1999; GPWG 2001). All Loliinae taxa would be subsumed under *Festuca,* including *Castellia, Ctenopsis, Hellerochloa, Lolium, Micropyropsis, Micropyrum, Narduroides, Psilurus, Vulpia,* and *Wangenheimia,* and probably other genera (e.g., *Loliolum, Vulpiella*) that have not been sampled. This scenario is bolstered by monophyly of Loliinae and some historical precedence, including authors (e.g., Hackel 1906; Piper 1906) who have classified *Vulpia* within *Festuca.* Inconveniences of this classification include a very complex and large genus that would be difficult to characterize by a congruent set of morphological traits and would require some nomenclatural changes against traditional use (e.g., *Lolium* would become a synonym of *Festuca*).

Scenario 2—*Festuca* sensu lato. This scenario is based on an evolutionary systematic criterion (Cronquist 1987; Takhtajan 1996; Brummitt 1997; Sosef 1997; Nixon and Carpenter 2000) that is nomenclaturally conservative. The traditional circumscription of *Festuca* would be maintained and subgenera recognized, and all other genera would be recognized except for *Vulpia,* which would be divided because of its polyphyly. An advantage of this scenario is to preserve the nomenclatural stability of *Festuca* until more complete phylogenetic studies are conducted. A disadvantage is the large number of morphologically derived segregate genera within a large and highly paraphyletic *Festuca.*

Scenario 3—*Festuca* sensu stricto. This scenario is based on employing a monophyly criterion (Donoghue and Cantino 1998; Cantino et al. 1999) for a less conservative classification. It would restrict *Festuca* to the fine-leaved taxa and treat broad-leaved lineages under separate genera. This approach is based on the relatively high support obtained for the fine-leaved clade from the combined nuclear and chloroplast data sets (Fig. 3) as well as from some morphological traits (e.g., innovation shoots mostly intravaginal or absent, leaf blades folded or setaceous, adaxial sclerenchyma girders absent, abaxial sclerenchyma girders mostly absent or incomplete, lemma awn mostly present). However, there would be difficulties circumscribing several of the lineages and placing many broad-leaved and intermediate species. Most of the present controversies surrounding the classification of *Festuca* and its segregates involve this scenario. Recognition of *Schedonorus, Leucopoa,* and *Drymochloa* (Palisot de Beauvois 1812; Grisebach 1852–1853; Holub 1984, 1998; Soreng and Terrell 1998, 2003, Tzvelev 1999, 2000) means accepting non-monophyletic entities according to our present state of knowledge (Catalán et al. 2004; this study). The search for a cladistic classification of the strongly supported *Schedonorus* + *Lolium* clade moved Darbyshire (1993) to subsume all *Schedonorus* taxa under *Lolium,* which has nomenclatural priority. However, noticeable morphological differences separating *Lolium* and *Schedonorus,* as well as tradition, persuaded Soreng and Terrell (1998, 2003) to classify *Schedonorus* as a paraphyletic genus separate from *Lolium,* thus opting for a practical evolutionary systematic approach.

Scenario 4—*Festuca* sensu strictissimo. This scenario is based on a relaxed evolutionary systematic or cladistic criterion for an even less conservative classification. It would restrict *Festuca* to most members of sect. *Festuca* (the *F. ovina* group, except *F. clementei* and *F. plicata*) and would recognize the other fine- and broad-leaved lineages as distinct genera. *Festuca* in this narrow sense would be monophyletic and well characterized morphologically. However, this scenario would mean recognition of many lineages, currently treated as *Festuca,* as separate genera. Further, many new nomenclatural combinations would be necessary.

Because of present uncertainties about the affinities of the intermediate taxa in Loliinae, nonrepresentation of several subgenera of *Festuca* and limited sampling, and limited and sometimes conflicting information from the ITS, *trn*L–F, and morphological character sets for particular groups, most of us (all except J. Müller) favor scenario 2. This scenario recognizes Loliinae as a monophyletic subtribe of Poeae (characterized by a *Festuca*-like spikelet, lemma, and hilum), and within it maintaining a paraphyletic *Festuca* (with subgenera and sections), and other traditionally recognized genera. Despite evidence of polyphyly for *Vulpia* and some groups in *Festuca* (e.g., subgen. *Leucopoa,* and sects. *Amphigenes, Aulaxyper, Festuca,* and *Subbulbosae*), we have decided not to

alter circumscriptions of these taxa until more phylogenetic information becomes available. J. Müller favors scenario 1.

Under our favored scenario 2, *Festuca* subgen. *Festuca* includes sect. *Festuca* (''ovina'' fescues), characterized by exclusively intravaginal innovation shoots, open sheaths, awned lemmas, and caryopses adherent to the paleas; sect. *Aulaxyper* (red fescues), characterized by mixed extra- and intravaginal innovation shoots, reduced cataphylls, closed sheaths, awned lemmas, and caryopses adherent to the paleas; subsect. *Exaratae,* mostly characterized by infolded sheaths; and sect. *Eskia,* characterized by hairy ovary apices, scariose lemma margins, and caryopses free from the paleas. Sections *Festuca* and *Aulaxyper* include some taxa that fall outside the clades that include their respective types. Taxa included in sect. *Amphigenes* by Hackel (1882) and Saint-Yves (1922) fall out in different lineages. The complex taxonomic history of *Amphigenes* and formal description of the group including *F. carpatica* and *F. dimorpha,* which is nested within the fine-leaved *Festuca,* needs to be addressed in further investigations. Other sampled species attached to the fine-leaved (*F. agustinii*) or broad-leaved (*F. pulchella* and *F. spectabilis*) clades fall under ''incertae sedis.'' *Hellerochloa,* related to the red fescues but with glumes longer than lemmas, is maintained as an independent genus.

Within the fine-leaved clade we could recognize as separate genera three segregates of *Vulpia* s.l.: (1) *Vulpia* s.s., characterized by cleistogamy and the small number and size of anthers; (2) *Loretia* Duval-Jouve, which would include representatives from *Vulpia* sects. *Loretia, Monachne,* and *Spirachne* (cf. Catalán et al. 2004), and would be characterized by perennials to annuals, florets mostly chasmogamous to half cleistogamous, anthers three, long, and exserted or one small and included, and sterile spikelets at the top of the inflorescence present or absent; and (3) the monotypic *Nardurus* (= *Vulpia* sect. *Apalochloa*), characterized by a reduced inflorescence. However, we abstain from proposing this segregation until a more complete sampling of sect. *Vulpia* is carried out. Polyphyly of *Vulpia* s.s., having diploid and tetraploid/hexaploid lineages, is not satisfactorily explained by our studies. The different positions of these groups in the ITS + *trn*L–F tree (Fig. 3) could be related to recurrent past introgression and polyploidization events (cf. Catalán et al. 2004; Torrecilla et al. 2004), so we have decided not to recognize them as separate taxa until more exhaustive phylogenetic studies are conducted. We also distinguish the independent lineages and recognize as genera *Castellia, Ctenopsis, Micropyrum, Narduroides, Psilurus,* and *Wangenheimia.*

With respect to the broad-leaved clade, we recognize *Festuca* subgen. *Schedonorus* and the monophyletic genus *Lolium,* characterized by inflorescence features (Terrell 1968), and the monotypic *Micropyropsis,* characterized by a swollen culm base (Romero-Zarco and Cabezudo 1983). These taxa plus *Castellia* share falcate auricles that are otherwise absent within Loliinae. The broad-leaved lineage also includes *Festuca* subgen. *Leucopoa,* monophyletic if restricted to sect. *Leucopoa* (*F. kingii*) plus its sister species *F. spectabilis* (formerly placed in subgen. *Festuca* sect. *Amphigenes*), characterized by broad leaf blades and a rounded to keeled lemma back; subgen. *Drymanthele,* monophyletic with the inclusion of *F. scariosa* (formerly placed in subgen. *Festuca* sect. *Scariosae*) and *F. pseudeskia* (formerly placed in subgen. *Festuca* sect. *Pseudoscariosa*), characterized by medium-wide to fine leaf blades; subgen. *Subbulbosae,* monophyletic if restricted to the *F. paniculata* group, characterized by the swollen base of the leaf sheaths and convolute to plicate leaf blades; and subgen. *Subulatae,* monophyletic if restricted to *F. subulata,* characterized by extravaginal innovation shoots, a lack of cataphylls, and large panicles (cf. Catalán et al. 2004).

Circumscriptions of the closest subtribes to Loliinae are mostly based on molecular characters and are similar to the results obtained by Soreng and Davis (2000). The close relationship of *Dactylis* to *Festuca,* discovered through the ITS-based studies of Charmet et al. (1997) and Torrecilla and Catalán (2002), is also supported here based on ITS (Fig. 1). Two species of *Dactylis* (*D. glomerata* and *D. hispanica*) form a clade sister to *Lamarckia* (Fig. 1–3), a relationship first recovered by Soreng and Davis (2000). *Dactylis* and *Lamarckia* share flat leaf blades with conduplicate leaf vernation, condensed panicles, spikelets with sterile florets and scariose lemma margins, although they differ in several other traits, such as a perennial vs. annual life cycle, keeled vs. rounded lemma backs, and round vs. linear hilum types, respectively. Dactylidinae, as described by Stapf (1898–1900), encompassed only *Dactylis.* Caro (1982) classified *Lamarckia* within Cynosurinae, whereas Tzvelev (1982) placed *Cynosurus* within Dactylidinae. None of these proposals agree with Soreng and Davis (2000) and our results. We conclude that circumscription of Dactylidinae should only include *Dactylis* and *Lamarckia.*

Cynosurinae were resolved as paraphyletic in the combined analysis (Fig. 6) and are represented by two species (*C. cristatus* and *C. echinatus*) characterized by dimorphic spikelets. Circumscription of Cynosurinae was originally limited to *Cynosurus* (Fries 1835–1837), but Caro (1982) later included *Lamarckia.*

Parapholiinae are characterized by few-veined lemmas, convolute leaf vernation, and papillose leaf epidermal cells. All except *Sphenopus* possess a spiciform-racemose inflorescence. The more recently diverged sister taxa *Hainardia cylindrica* and *Parapholis incurva* (Fig. 1–3, 6) also share a cylindrical inflorescence, spikelets sunken into the inflorescence rachis, and completely scariose lemmas. These remarkable inflorescence traits moved Hubbard (1948) to describe Monermeae (= Hainardieae), which contained *Hainardia, Parapholis,* and *Pholiurus.* His taxonomic treatment was followed by Caro (1982), Tzvelev (1982), and Clayton and Renvoize (1986). Caro (1982) restricted Monermeae to *Hainardia* and *Parapholis,* separating them into the monotypic subtribes Monerminae and Parapholiinae, respectively, based on the distinction between the single-glumed *Hainardia* and the two-glumed *Parapholis.* Results from Soreng and Davis (2000) and our study indicate that *Hainardia* and *Parapholis* are nested within a Parapholiinae s.l. clade that in turn shows close affinities to Loliinae.

The following is a synopsis of our proposed classification:

Tribe Poeae

Cynosureae Dumort., Observ. Gramin. Belg. 82 (1823).
Festuceae Dumort., Observ. Gramin. Belg. 82 (1823).

Lolieae Rchb., Consp. Regn. Veg. 47 (1828).
Psilureae Ovchinnikov, Fl. Tadzhik. 1: 117 (1957), nom. nud.
Hainardieae Greuter, *Boissiera* **13**: 178 (1967).

Subtribe LOLIINAE Dumort., Observ. Gramin. Belg. 95 (1823).—TYPE: *Lolium perenne* L.

Festucinae J. Presl, Reliq. Haenk. 1: 257 (1830).
Psilurinae Pilg., *Willdenowia, Beih.* **5**: 471 (1969).

LOLIUM L., Sp. Pl. 83 (1753).

FESTUCA L., Sp. Pl. 73 (1753).—TYPE: *Festuca ovina* L.

Subgen. FESTUCA

Sect. FESTUCA

Subsect. FESTUCA

Festuca sect. *Ovinae* Fr. *Intravaginales* Hack., Monogr. 80 (1882), p. p.

Subsect. EXARATAE St.-Yves, *Candollea* **1**: 21 (1922).—SYNTYPES: *Festuca algeriensis* Batt. & Trab., Fl. Algerie, Monoc. 212 (1895); *F. deserti* (Coss. & Durieu) Batt. & Trab., Fl. Algerie, Monoc. 215 (1895); *F. scaberrima* Lange, *Vidensk. Meddel. Dansk Naturhist. Foren. Kjøbenhavn* **1860**: 51 (1861), non Steud. 1854 [= *F. capillifolia* Dufour].

Festuca sect. *Ovinae* Fr. *Intravaginales* Hack., Monogr. 80 (1882), p. p.

Sect. AULAXYPER Dumort., Observ. Gramin. Belg. 104 (1823).—TYPE: *Festuca rubra* L., Sp. Pl. 74 (1753).

Festuca sect. *Ovinae* Fr. *Extravaginales* vel *Mixtae* Hack., Monogr. 80 (1882), p. p.

Sect. ESKIA Willk., in Willk. et Lange, Prodr. Fl. Hispan. 192 (1861).—TYPE: *Festuca eskia* Ramond ex DC., in Lam. et DC., Fl. Fr., Ed. 3, 3: 52 (1805).

Festuca sect. *Variae* Hack., Monogr. 80 (1882).

Subgen. SCHEDONORUS (P. Beauv.) Peterm., Deutschl. Fl. 643 (1849).—LECTOTYPE: *Festuca pratensis* Huds., Fl. Angl. 37 (1762).

Schedonorus P. Beauv., Ess. Agrostogr. 99 (1812).
Festuca sect. *Bovinae* (Fr.) Hack., Bot. Centralbl. 8: 413(1881).
Festuca sect. *Bromoides* Rouy ex Markgr.-Dann., Fl. Turkey 9: 407 (1985), pro syn.

Sect. SCHEDONORUS (P. Beauv.) Koch, Syn. Fl. Germ. Helv. 813 (1837).

Sect. PLANTYNIA (Dumort.) Tzvelev, Slaki SSSR 394 (1976).—TYPE: *Festuca gigantea* (L.) Vill., Hist. Pl. Dauph. 2: 110 (1787).

Schedonorus sect. *Plantynia* Dumort., Fl. Belg. Prodr. 159 (1827).

Festuca subgen. *Drymonaetes* V. I. Krecz. & Bobrov, Fl. SSSR 2: 533–534 (1934).

Subgen. LEUCOPOA (Griseb.) Hack., *Repert. Nov. Spec. Regni Veg.* **2**: 70 (1906).—TYPE: *Festuca sibirica* Hack. ex Boiss., Fl. Orient. 5: 626 (1884).

Leucopoa Griseb. in Ledeb., Fl. Ross. 4: 383 (1852).
Festuca subgen. *Hesperochloa* Piper, *Contrib. U. S. Natl. Herb.* **10**: 10 (1906).
Hesperochloa (Piper) Rydb., *Bull. Torrey Bot. Club* **39**: 106 1912).
Festuca sect. *Leucopoa* (Griseb.) Krivot., *Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk S.S.S.R.* **20**: 48 (1960).

Subgen. DRYMANTHELE V. I. Krecz. & Bobrov, Fl. SSSR 2: 572 (1934).—TYPE: *Festuca drymeja* Mert. & Koch, Deutschl. Fl., Ed. 3, 1: 670 (1823).

Festuca sect. *Montanae* Hack., Monogr. 80 (1882).
Drymochloa Holub, *Fol. Geobot. Phytotax.* **19**: 95 (1984).

Sect. PHAEOCHLOA Griseb., Spicil. Fl. Rumel. 2: 433 (1846).—TYPE: *Festuca drymeja* Mert. & Koch.

Sect. SCARIOSAE Hack. Monogr. 193 (1882).—TYPE: *Festuca granatensis* Boiss., Elench. Pl. Nov. 93 (1838) [= *F. scariosa* (Lag.) Asch. & Graebn.].

Sect. PSEUDOSCARIOSA Krivot. *Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk S.S.S.R.* **20**: 61 (1960).—TYPE: *Festuca pseudeskia* Boiss., Elench. 91 (1838).

Festuca sect. *Variae* Hack. *Extravaginales* Hack., Mon ogr. 183 (1882), p. p.

Subgen. SUBBULBOSAE Nyman ex Hack., Bot. Centralbl. 8: 407, 413 (1881).—SYNTYPES: *Festuca coerulescens* Desf., Fl. Atl. 1: 87 (1798); *F. costata* Nees, Fl. Afr. Austr. 1: 447 (1841); *F. scabra* Vahl, Symb. Bot. 2: 21 (1791); *F. spadicea* L., Syst. Veg., Ed. 12, 732 (1767); *F. triflora* Desf., Fl. Atl. 1: 87 (1798).

Subgen. SUBULATAE (Tzvelev) E. B. Alexeev, *Byull. Moskovsk. Obshch. Isp. Prir., Otd. Biol.* **82**: 96 (1977).—TYPE: *Festuca subulata* Trin., *Mém. Acad. Imp. Sci. St.-Pétersbourg, Sér. 6, Sci. Math* **2**: 173 (1832).

Festuca sect. *Subulatae* Tzvelev, *Ukrayins'k. Bot. Zhurn.* **56**: 1253 (1971).

Incertae Sedis

Festuca sect. AMPHIGENES (Janka) Tzvelev, *Ukrayins'k. Bot. Zhurn* **56**: 1253 (1971).—TYPE: *Festuca nutans* Host, Icon. Descr. Gram. Austriac. 4: 35 (1809), non Moench 1794 [= *F. pulchella* Schrad.].

Amphigenes Janka, *Linnaea* **30**: 619 (1860).
Festuca sect. *Variae* Hack. *Extravaginales* Hack., Monogr. 80 (1882), p. p.

Festuca sect. BREVIARISTATAE Krivot., *Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk S.S.S.R.* **20**: 57–58 (1960).—TYPE: *Festuca altaica* Trin. in Ledeb., Fl. Alt. 1: 109 (1829).

VULPIA C. C. Gmel., Fl. Bad. 1: 8 (1805).—TYPE: *Vulpia myuros* (L.) C. C. Gmel.

Sect. VULPIA

Sect. APALOCHLOA (Dumort.) Stace, *Nordic J. Bot.* **1**: 24 (1981).—TYPE: *Brachypodium nardus* (DC.) P. Beauv., Ess. Agrostogr. 101, 155, 180 (1812) [= *Vulpia unilateralis* (L.) Stace].

Nardurus Rchb., Fl. Germ. Excurs. 19 (1830).
Vulpia sect. *Nardurus* (Rchb.) Stace, *Bot. J. Linn. Soc.* **76**: 350 (1978).

Sect. LORETIA (Duval-Jouve) Boiss., Fl. Orient. 5: 630 (1884).—TYPE: *Vulpia geniculata* (L.) Link, Hort. Berol. 1: 148 (1827).

Loretia Duval-Jouve, *Rev. Sci. Nat. (Montpellier)* **2**: 38 (1880).
Vulpia subgen. *Loretia* (Duval-Jouve) Hack., *Flora* **63**: 477 (1880).

Sect. MONACHNE Dumort., Observ. Gramin. Belg. 1041 (1823).—TYPE: *Vulpia uniglumis* (Aiton) Dumort., Observ. Gramin. Belg. 101 (1823) [= *V. fasciculata* (Forssk.) Fritsch].

Sect. SPIRACHNE (Hack.) Boiss., Fl. Orient. 5: 630 (1884).—TYPE: *Vulpia inops* Hack., *Flora* **63**: 476 (1880) [= *V. brevis* Boiss. & Kotschy].

CASTELLIA Tineo, Pl. Rar. Sicul. 2: 17 (1846).—TYPE: *Castellia tuberculata* Tineo, Pl. Rar. Sicul. 2: 18 (1817) [= *C. tuberculosa* (Moris) Bor].

CTENOPSIS De Not., Ind. Sem. Hort. Gen. 26 (1847).—TYPE: *Ctenopsis pectinella* (Delile) De Not., Ind. Sem. Hort. Gen. 325 (1847).

HELLEROCHLOA Rauschert, *Taxon* **31**: 561 (1982).—TYPE: *Hellerochloa livida* (Kunth) Rauschert, *Taxon* **31**: 561 (1982).

Helleria E. Fourn., Mex. Pl. 2: 128 (1886), non Nees & Mart. 1824.

MICROPYROPSIS Romero Zarco & Cabezudo, *Lagascalia* **11**: 95 (1983).—TYPE: *Micropyropsis tuberosa* Romero Zarco & Cabezudo, *Lagascalia* **11**: 95 (1983).

MICROPYRUM (Gaudin) Link, *Linnaea* **17**: 397 (1844).—TYPE: *Micropyrum tenellum* (L.) Link, *Linnaea* **17**: 398 (1844).

Triticum L. sect. *Micropyrum* Gaudin, Fl. Helv. 1: 366 (1828).

NARDUROIDES Rouy, Fl. France 14: 301 (1913).—TYPE: *Narduroides salzmannii* (Boiss.) Rouy, Fl. France 14: 301 (1913).

PSILURUS Trin., Fund. Agrost. 93 (1822).—TYPE: *Psilurus nardoides* Trin., Fund. Agrost. 93 (1822) [= *P. incurvus* (Gouan) Schinz & Thell.].

WANGENHEIMIA Moench, Meth. 200 (1794).—TYPE: *Wangenheimia disticha* Moench, Meth. 200 (1794) [= *W. lima* (L.) Trin.].

Subtribe PARAPHOLIINAE Caro, *Dominguezia* **4**: 41 (1982).—TYPE: *Parapholis incurva* (L.) C. E. Hubb., *Blumea, Suppl.* **3**: 14 (1946).

Monerminae (C. E. Hubb.) Tzvelev, *Komarovskie Chteniya (Moscow & Leningrad)* **37**: 33 (1987).

PARAPHOLIS C. E. Hubb., *Blumea, Suppl.* **3**: 14 (1946).

CATAPODIUM Link, Hort. Berol. 1: 44 (1827).—TYPE: *Catapodium loliaceum* (Huds.) Link, Hort. Berol. 1: 45 (1827).

Scleropoa Griseb., Spicil. Fl. Rumel. 2: 431 (1846).

HAINARDIA Greuter, *Boissiera* **13**: 178 (1967).—TYPE: *Hainardia cylindrica* (Willd.) Greuter, *Boissiera* **13**: 178 (1967).

Monerma P. Beauv., Ess. Agrostogr. 116 (1812), nom. illeg.

SPHENOPUS Trin., Fund. Agrostogr. 135 (1822).—TYPE: *Sphenopus divaricatus* Rchb. (Gouan), Fl. Germ. Exc. 45 (1830).

Subtribe CYNOSURINAE Fr., Fl. Scan. 204 (1835).—TYPE: *Cynosurus cristatus* L., Sp. Pl. 72 (1753).

CYNOSURUS L., Sp. Pl. 72 (1753).

Falona Adans., Fam. Pl. 2: 496 (1763).

Subtribe DACTYLIDINAE Stapf, Fl. Cap. 7: 317 (1898).—TYPE: *Dactylis glomerata* L., Sp. Pl. 71 (1753).

DACTYLIS L., Sp. Pl.: 71 (1753).

LAMARCKIA Moench, Meth. 201 (1794), orthogr. cons.—TYPE: *Lamarckia aurea* (L.) Moench, Meth. 201 (1794).

ACKNOWLEDGMENTS

We thank J. Travis Columbus, Stephen Darbyshire, Paul M. Peterson, and an anonymous referee for comments that greatly improved an earlier version of the manuscript; Samuel Pyke, Carlos Romero-Zarco, and Robert Soreng for providing us with fresh and silica-gel-dried materials for some taxa; and the curatorial staffs of ARAN, BC, COLO, G, JACA, MA, MERC, MO, ORT, PRC, SEV, US, W, and WA for sending us herbarium specimens. This work has been subsidized by a Spanish Ministry of Science and Technology grant (BOS2000-0996 project) to PC and supported by a Universidad Central de Venezuela (CDCH) doctorate fellowship to PT.

LITERATURE CITED

AINSCOUGH, M. M., C. M. BARKER, AND C. A. STACE. 1986. Natural hybrids between *Festuca* and species of *Vulpia* section *Vulpia*. *Watsonia* **16**: 143–151.

ALEXEEV, E. B. 1977. K sistematike asiatskich ovsjaniz (*Festuca*). I. Podrod *Drymanthele, Subulatae, Schedonorus, Leucopoa*. *Byull. Moskovsk. Obshch. Isp. Prir., Otd. Biol.* **82**: 95–102.

———. 1978. K sistematike asiatskich ovsjaniz (*Festuca*). II. Podrod *Festuca*. *Byull. Moskovsk. Obshch. Isp. Prir., Otd. Biol.* **83**: 109–122.

———. 1980. Novye podrody i sekzii ovsjaniz (*Festuca* L.) Severnoj Ameriki i Meksiki. *Novosti Sist. Vyssh. Nizsh. Rast.* **17**: 42–53.

———. 1981. Novye taksoni roda *Festuca* (Poaceae) is Meksiki i Zentral'noj Ameriki. *Bot. Zhurn. (Moscow & Leningrad)* **66**: 1492–1501.

———. 1985. Novye rody slakov. *Byull. Moskovsk. Obshch. Isp. Prir., Otd. Biol.* **90**: 102–109.

———. 1986. Ovsjanizy (*Festuca* L., Poaceae) Venezuely, Kolumbii i Ekvadora. *Novosti Sist. Vyssh. Nizsh. Rast.* **23**: 5–23.

ALVAREZ-FERNÁNDEZ, I., J. FUERTES-AGUILAR, J. L. PANERO, AND G. NIETO-FELINER. 2001. A phylogenetic analysis of *Doronicum* (Asteraceae, Senecioneae) based on morphological, nuclear ribosomal (ITS) and chloroplast (*trn*L–F) evidence. *Molec. Phylogen. Evol.* **20**: 41–64.

BARKER, F. K., AND F. M. LUTZONI. 2002. The utility of the incongruence length difference test. *Syst. Biol.* **51**: 625–637.

BORRILL, M., M. KIRBY, AND W. G. MORGAN. 1977. Studies in *Festuca*. 11. Interrelationships of some diploid ancestors of the polyploid broad-leaved fescues. *New Phytol.* **78**: 661–674.

BRUMMITT, J. 1997. Taxonomy versus cladonomy, a fundamental controversy in biological systematics. *Taxon* **46**: 723–734.

CANTINO, P. D., S. J. WAGSTAFF, AND R. G. OLMSTEAD. 1999. *Caryopteris* (Lamiaceae) and the conflict between phylogenetic and pragmatic consideration in botanical nomenclature. *Syst. Bot.* **23**: 369–386.

CARO, J. A. 1982. Sinopsis taxonómica de las gramíneas argentinas. *Dominguezia* **4**: 1–51.

CATALÁN, P., E. A. KELLOGG, AND R. G. OLMSTEAD. 1997. Phylogeny of Poaceae subfamily Pooideae based on chloroplast *ndh*F gene sequences. *Molec. Phylogen. Evol.* **8**: 150–166.

———, P. TORRECILLA, J. A. LÓPEZ-RODRÍGUEZ, AND R. G. OLMSTEAD. 2004. Phylogeny of the festucoid grasses of subtribe Loliinae and allies (Poeae, Pooideae) inferred from ITS and *trn*L–F sequences. *Molec. Phylogen. Evol.* **31**: 517–541.

CHARMET, G., C. RAVEL, AND F. BALFOURIER. 1997. Phylogenetic analysis in the *Festuca–Lolium* complex using molecular markers and ITS rDNA. *Theor. Appl. Genet* **94**: 1038–1046.

CHICOUENE, D. 1999. Realité et perspectives dans la description morphologique des festuques, pp. 22–32. *In* R. Portal, *Festuca* de France. Published by the author, Vals-près-Le Puy, France.

CHIPPINDALE, P. T., AND J. J. WIENS. 1994. Weighting, partitioning, and combining characters in phylogenetic analysis. *Syst. Biol.* **43**: 278–287.

CLARK, L. G., W. ZHANG, AND J. F. WENDEL. 1995. A phylogeny of the grass family (Poaceae) based on *ndh*F sequence data. *Syst. Bot.* **20**: 436–460.

CLAYTON, W. D., AND S. A. RENVOIZE. 1986. Genera graminum: grasses of the world. *Kew Bull., Addit. Ser.* **8**: 1–389.

COTTON, R., AND C. A. STACE. 1977. Morphological and anatomical variation of *Vulpia* (Gramineae). *Bot. Not.* **130**: 173–187.

CRONQUIST, A. 1987. A botanical critique of cladism. *Bot. Rev.* **53**: 1–52.

DARBYSHIRE, S. J. 1993. Realignment of *Festuca* subgen. *Schedonorus* with the genus *Lolium* (Poaceae). *Novon* **3**: 239–243.

———, AND S. I. WARWICK. 1992. Phylogeny of North American *Festuca* (Poaceae) and related genera using chloroplast DNA restriction site variation. *Canad. J. Bot.* **70**: 2415–2429.

DAVIS, J. I, AND R. J. SORENG. 1993. Phylogenetic structure in the grass family (Poaceae) as inferred from chloroplast DNA restriction site variation. *Amer. J. Bot.* **80**: 1444–1454.

———, M. P. SIMMONS, D. W. STEVENSON, AND J. F. WENDEL. 1998. Data decisiveness, data quality, and incongruence in phylogenetic analysis: an example from the monocotyledons using mitochondrial *atp*A sequences. *Syst. Biol.* **47**: 282–310.

DE PINNA, M. C. C. 1991. Concepts and tests of homology in the cladistic paradigm. *Cladistics* **7**: 367–394.

DONOGHUE, M. J., AND P. D. CANTINO. 1988. Paraphyly, ancestors, and the goals of taxonomy: a botanical defense of cladism. *Bot. Rev.* **54**: 107–128.

DUMORTIER, B. C. J. 1824. Observations sur les graminées de la flore Belgique. J. Casterman, Tournay, Belgium. 153 p.

FARRIS, J. S., M. KÄLLERSJÖ, A. G. KLUGE, AND C. BULT. 1994. Testing significance of incongruence. *Cladistics* **10**: 315–319.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783–791.

FOURNIER, E. 1886. Mexicanas plantas, Gramineae, Vol. 2. Imprimerie Nationale, Paris, France. 160 p.

FRIES, E. M. 1835–1837. Corpus florarum provincialium Sueciae. I. Floram scanicam. Palmblad, Sebell and Company, Uppsala, Sweden. 394 p.

GAUT, B. S., L. P. TREDWAY, C. KUBIK, R. L. GAUT, AND W. MEYER. 2000. Phylogenetic relationships and genetic diversity among members of the *Festuca–Lolium* complex (Poaceae) based on ITS sequence data. *Pl. Syst. Evol.* **224**: 33–53.

GMELIN, C. C. 1805. Flora badensis alsatica. I. Müller, Karlsruhe, Germany. 768 p.

GOLOBOFF, P. A. 1991. Homoplasy and the choice among cladograms. *Cladistics* **7**: 215–232.

GRASS PHYLOGENY WORKING GROUP [GPWG]. 2001. Phylogeny and subfamilial classification of the grasses (Poaceae). *Ann. Missouri Bot. Gard.* **88**: 373–457.

GRISEBACH, A. 1852–1853. Gramineae, pp. 324–484. *In* C. F. Ledebour, Flora rossica, Vol. 4. E. Schweizerbarth, Stuttgart, Germany.

HACKEL, E. 1882. Monographia festucarum Europearum. T. Fischer, Kassel and Berlin, Germany. 225 p.

———. 1887. Gramineae, pp. 1–97. *In* H. G. A. Engler and K. A. E. Prantl [eds.], Die natürlichen Pflanzenfamilien . . . Teil II, Abteilung 2. W. Engelmann, Leipzig, Germany.

———. 1906. Gramineae novae. *Repert. Spec. Nov. Regni Veget.* **2**: 69–72.

HILLIS, D. M., AND J. J. WIENS. 2000. Molecules versus morphology in systematics. Conflicts, artifacts, and misconceptions, pp. 1–19. *In* J. J. Wiens [ed.], Phylogenetic analysis of morphological data. Smithsonian Institution Press, Washington, D.C., USA.

HOLUB, J. 1984. New genera in Phanerogamae (1–3). *Folia Geobot. Phytotax.* **19**: 95–99.

———. 1998. Reclassifications and new names in vascular plants 1. *Preslia* **70**: 97–122.

HSIAO, C., N. J. CHATTERTON, K. H. ASAY, AND K. B. JENSEN. 1995. Molecular phylogeny of the Pooideae (Poaceae) based on nuclear rDNA (ITS) sequences. *Theor. Appl. Genet.* **90**: 389–398.

———, S. W. L. JACOBS, N. J. CHATTERTON, AND K. H. ASAY. 1999. A molecular phylogeny of the grass family (Poaceae) based on the sequences of nuclear ribosomal DNA (ITS). *Austral. Syst. Bot.* **11**: 667–688.

HUBBARD, C. E. 1948. Gramineae, pp. 284–348. *In* J. Hutchinson [ed.], British flowering plants. P. R. Gawthorn, London, UK.

JENKIN, T. J. 1933. Interspecific and intergeneric hybrids in herbage grasses. Initial crosses. *J. Genet.* **28**: 205–264.

JOHNSON, L. A., AND D. E. SOLTIS. 1998. Assessing congruence: empirical examples from molecular data, pp. 297–348. *In* D. E.

Soltis, P. S. Soltis, and J. J. Doyle [eds.], Molecular systematics of plants, Vol. 2. Kluwer Academic Publishers, Boston, Massachusetts, USA.

JUDD, W. S., C. S. CAMPBELL, E. A. KELLOGG, AND P. F. STEVENS. 1999. Plant systematics: a phylogenetic approach. Sinauer Associates, Inc., Sunderland, Massachusetts, USA. 464 p.

KELLOGG, E. A., AND L. WATSON. 1993. Phylogenetic studies of a large data set. 1. Bambusoideae, Andropogonodae, and Pooideae (Gramineae). *Bot. Rev. (Lancaster)* **59**: 273–343.

———, R. APPELS, AND R. J. MASON-GAMER. 1996. When genes tell different stories: the diploid genera of Triticeae (Gramineae). *Syst. Bot.* **21**: 321–347.

KERGUELÉN, M., AND F. PLONKA. 1989. Les *Festuca* de la flore de France (corse comprise). *Bull. Soc. Bot. Centre-Ouest, N. S., Num. Spéc.* **10**: 1–368.

KLUGE, A. G., AND A. J. WOLF. 1993. Cladistics: what's in a word? *Cladistics* **9**: 183–199.

KRECHETOVICH, V. I., AND E. G. BOBROV. 1934. *Festuca* L. s. str., pp. 497–535. *In* V. L. Komarov, R. Y. Rozhevits, and B. K. Shishkin [eds.], Flora S.S.S.R., Vol. 2. Akademija Nauk S.S.S.R., Leningrad, Russia.

KRIVOTULENKO, U. 1960. Novye sekzii roda *Festuca* L. (Generis *Festuca* L. sectiones novae). *Bot. Mater. Gerb. Bot Inst. Komarova Akad. Nauk S.S.S.R.* **20**: 48–67.

LECOINTRE, G., H. PHILIPPE, H. LANH VAN LE, AND H. LE GUYADER. 1993. Species sampling has a major impact on phylogenetic inference. *Molec. Phylogen. Evol.* **2**: 205–224.

LEWIS, E. J. 1975. *Festuca* L. × *Lolium* L. = × *Festulolium* Aschers. & Graebn., pp. 547–552. *In* C. A. Stace [ed.], Hybridization and the flora of the British Isles. Academic Press, London, UK.

LINNÉ, C. 1753. Species plantarum, 2 vols. Laurentii Salvii, Stockholm, Sweden. 1200 p.

MACFARLANE, T. D., AND L. WATSON. 1982. The classification of Poaceae subfamily Pooideae. *Taxon* **31**: 178–203.

MADDISON, W. P., AND D. R. MADDISON. 1992. MacClade. Analysis of phylogeny and character evolution, vers. 3. Sinauer Associates, Inc., Sunderland, Massachusetts, USA.

MALIK, C. P., AND P. T. THOMAS. 1966. Karyotypic studies in some *Lolium* and *Festuca* species. *Caryologia* **19**: 167–196.

MARKGRAF-DANNENBERG, I. 1980. *Festuca* L., pp. 125–153. *In* T. G. Tutin, V. H. Heywood, N. A. Burges, D. M. Moore, D. H. Valentine, S. M. Walters, and D. A. Webb [eds.], Flora Europaea, Vol. 5. Cambridge University Press, Cambridge, UK.

———. 1985. *Festuca* L., pp. 400–442. *In* P. H. Davis [ed.], Flora of Turkey and the east Aegean Islands, Vol. 9. University Press, Edinburgh, UK.

MASON-GAMER, R. J., AND E. A. KELLOGG. 1997. Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Gramineae). *Syst. Biol.* **45**: 524–545.

MCDADE, L. A. 1990. Hybrids and phylogenetic systematics I. Patterns of character expression in hybrids and their implications for cladistic analysis. *Evolution* **44**: 1685–1700.

———. 1992. Hybrids and phylogenetic systematics II. The impacts of hybrids on cladistic analysis. *Evolution* **46**: 1329–1346.

MICKEVICH, M. F., AND J. S. FARRIS. 1981. The implications of congruence in *Menidia*. *Syst. Zool.* **30**: 351–370.

NADOT, S., R. BAJON, AND B. LEJEUNE. 1994. The chloroplast gene *rps*4 as a tool for the study of Poaceae phylogeny. *Pl. Syst. Evol.* **191**: 27–38.

NIXON, K. C., AND J. M. CARPENTER. 1996. On simultaneous analysis. *Cladistics* **12**: 221–241.

———, AND ———. 2000. On the other phylogenetic systematics. *Cladistics* **16**: 298–318.

PALISOT DE BEAUVOIS, A. M. F. J. 1812. Essai d'une nouvelle agrostographie; ou nouveaux genres des Graminées; avec figures représentant les caractères de tous les genres. Fain, Paris, France. 182 p.

PIPER, C. V. 1906. North American species of *Festuca*. *Contr. U.S. Natl. Herb.* **10**: 1–48.

ROMERO-ZARCO, C., AND B. CABEZUDO. 1983. *Micropyropsis,* género nuevo de Gramineae. *Lagascalia* **11**: 94–99.

SAINT-YVES, A. 1922. Les *Festuca* (subgen. *Eu-Festuca*) de l'Afrique du Nord et de les îles Atlantiques. *Candollea* **1**: 1–63.

SOLTIS, D. E., AND P. S. SOLTIS. 1999. Polyploidy: recurrent formation and genome evolution. *Trends Ecol. Evol.* **14**: 348–352.

SORENG, R. J., AND J. I. DAVIS. 2000. Phylogenetic structure in Poaceae subfamily Pooideae as inferred from molecular and morphological characters: misclassification versus reticulation, pp. 61–74. *In* S. W. L. Jacobs and J. Everett [eds.], Grasses: systematics and evolution. CSIRO, Collingwood, Victoria, Australia.

———, J. I DAVIS, AND J. J. DOYLE. 1990. A phylogenetic analysis of chloroplast DNA restriction site variation in Poaceae subfam. Pooideae. *Pl. Syst. Evol.* **172**: 83–97.

———, AND E. E. TERRELL. 1998. Taxonomic notes on *Schedonorus,* a segregate genus from *Festuca* or *Lolium,* with a new nothogenus, ×*Schedololium,* and new combinations. *Phytologia* **83**: 85–88.

———, AND ———. 2003. *Schedonorus* P. Beauv., pp. 605–607. *In* R. J. Soreng, P. M. Peterson, G. Davidse, E. J. Judziewicz, F. O. Zuloaga, T. S. Filgueiras, and O. Morrone [eds.], Catalogue of the New World grasses (Poaceae): IV. Subfamily Pooideae, *Contr. U.S. Natl. Herb.* **48.**

SOSEF, M. S. M. 1997. Hierarchical models, reticulate evolution and the inevitability of paraphyletic supraspecific taxa. *Taxon* **46**: 75–85.

STACE, C. A. 1978. Changing concepts of the genus *Nardurus* Reichenb. (Gramineae). *Bot. J. Linn. Soc.* **76**: 344–350.

———. 1981. Generic and infrageneric nomenclature of annual Poaceae: Poeae related to *Vulpia* and *Desmazeria*. *Nordic J. Bot.* **1**: 17–26.

STAPF, O. 1898–1900. Gramineae, pp. 310–750. *In* W. T. Thiselton-Dyer [ed.], Flora Capensis, Vol. 7. L. Reeve and Co., Ashford, UK.

SWOFFORD, D. L. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods), vers. 4.0 beta 10. Sinauer Associates, Inc. Sunderland, Massachusetts, USA.

TAKHTAJAN, A. 1996. Diversity and classification of flowering plants. Columbia University Press, New York, USA. 620 p.

TERRELL, E. E. 1968. A taxonomic revision of the genus *Lolium*. *Tech. Bull. U.S.D.A.* **1392**: 1–65.

TORRECILLA, P., AND P. CATALÁN. 2002. Phylogeny of broad-leaved and fine-leaved *Festuca* lineages (Poaceae) based on nuclear ITS sequences. *Syst. Bot.* **27**: 241–251.

———, J. A. LÓPEZ-RODRÍGUEZ, D. STANCIK, AND P. CATALÁN. 2003. Systematics of *Festuca* L. sects. *Eskia* Willk., *Pseudatropis* Kriv., *Amphigenes* (Janka) Tzvel., *Pseudoscariosa* Kriv. and *Scariosae* Hack. based on analysis of morphological characters and DNA sequences. *Pl. Syst. Evol.* **239**: 113–139.

———,———, AND P. CATALÁN. 2004. Phylogenetic relationships of *Vulpia* and related genera (Poeae, Poaceae) based on analysis of ITS and *trn*L–F sequences. *Ann. Missouri Bot. Gard.* **91**: 124–158.

TZVELEV, N. N. 1971. K sistematike i filogenii ovsjaniz (*Festuca* L.) flory S.S.S.R. I. Sistema roda i oshoriye naprav'enija evoljuzii. *Bot. Zhurn. (Moscow & Leningrad)* **56**: 1252–1262.

———. 1982. Poryadok zlaki (Poales). *Zhizn Rast.* **6**: 341–378.

———. 1999. Ob obeme i nomenklature nekotorykh rodov sosudistykh rastenii Evropeiskoi Rossii. *Bot. Zhurn. (Moscow & Leningrad)* **84**: 109–118.

———. 2000. Combinationes novae taxorum plantarum vascularium. *Novosti Sist. Vyssh. Rast.* **32**: 181–183.

WATSON, L., AND M. J. DALLWITZ. 1992. The grass genera of the world. CAB International, Wallingford, Oxfordshire, UK. 1038 p.

WILLKOMM, M. 1861. *Festuca* L., pp. 95–96. *In* M. Willkomm and J. Lange, Prodromus florae hispanicae, Vol. 1. E. Schweizerbarth, Stuttgart, Germany.