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A taxonomic review of the genus *Festuca* in Uganda: AFLP fingerprinting, chromosome numbers, morphology and anatomy

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Preface

This thesis is submitted in partial fulfilment of the requirements for the Philosophiae Doctor (PhD) degree at the Department of Ecology and Natural Resource Management, Norwegian University of Life Sciences (UMB). The research was funded by The Norwegian Agency for Development Cooperation (NORAD) Project to Makerere University Department of Botany, and The Norwegian Council of Universities' Committee for Development Research and Education (NUFU) Project 63/2003 – Biodiversity and Plant-Animal interactions in Uganda. Fieldwork was carried out in Uganda, where I personally collected the plant material. Permission to conduct fieldwork in the Ugandan national parks was given to me by the Uganda Wildlife Authority.

First and foremost, I express my gratitude to my supervisors, Prof. Kåre A. Lye and Prof. Dr. Manfred Heun for the guidance, teaching and encouragement throughout the study period. I am very grateful to Prof. Dr. Bernd Friebe of Kansas State University (KSU) for accepting me as his guest student at KSU and giving me a very enthusiastic introduction into the field of cytogenetics. I much appreciate the discussions I had with Prof. Dr. Remigius Z. Bukenya of the Department of Botany, Makerere University; Dr. Sylvia Phillips and Dr. Tom Cope of the Kew Herbarium, at the start of the PhD project. Special thanks go to Kari Vollan and Dr. Jørn Henrik Sønstebø for the technical assistance and guidance on the lab, and use of some data analysis software. Dr. Eunice Olet, thank you for introducing me to the SPSS data analysis software. Dr. Tryggve Krekling, of the Department of Plant and Environmental Sciences, UMB, facilitated the SEM analyses, and the staff at the anatomy laboratory of the Faculty of Veterinary medicine, Makerere University, facilitated most of the anatomy work. Dr. Stein Sæby translated some Russian literature.

To my fellow PhD students (Alex, Charlotte, David, Esther, Herbert, Jared, Paul and Ronnie), and the Ugandan community at Ås, thank you for the support – academic and social. The staff at INA (UMB), consulting and socializing with you has been a source of encouragement. Dr. James Kalema and Dr. Gerald Eilu, thank you for hiking the Ugandan mountains with me despite the 'malaria' as we searched for my *Festuca*. Finally, to my dear mother, sisters and brothers, and friends (JaneK, Joy, Julie, Justine, Kittel, Lanyero and Yanira), thank you for your love and prayers; sharing with you the ups and downs of this journey was so uplifting that I always managed to trek on.

Ås, October 2007 Mary Namaganda

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The thesis includes the following papers:

Paper I

Namaganda, M., Lye, K. A., Friebe, B. & Heun, M. (2006) AFLP-based differentiation of tropical African *Festuca* species compared to the European *Festuca* complex. Theoretical and Applied Genetics 113: 1529-1538.

Paper II

Namaganda, M., Lye, K. A. & Heun, M. The species distinction of three endemic narrow-leaved *Festuca* from East Africa. Submitted.

Paper III

Namaganda, M. & Lye, K. A. A taxonomic comparison between tropical African and related European broad-leaved species of *Festuca*. Submitted.

Paper IV

Namaganda, M., Krekling, T. & Lye, K. A. Leaf anatomical characteristics of Ugandan species of *Festuca* L. (Poaceae). Manuscript.

Paper V

Namaganda, M., Heun, M. & Lye, K. A. Should we recognise sibling genera among flowering plants? Manuscript.

Abstract

Seven species of *Festuca* and one species of *Vulpia* from Uganda and Ethiopia were analysed by AFLP fingerprinting and compared to seven selected European *Festuca*, one species of *Lolium* and two species of *Vulpia*. The five narrow-leaved African species (*F. abyssinica*, *F. richardii*, *F. claytonii*, *F. pilgeri* and *F. chodatiana*) were found to be genetically well separated from the rest of the species, with 100 % bootstrap support. The two broad-leaved African species (*F. africana* and *F. simensis*) grouped with the broad-leaved European species, and this group was separated from the narrow-leaved European species. *F. simensis* was found to be linked to the European group of species via *F. gigantea* and *F. africana* via *F. altissima*. The African *Vulpia* species were very closely related to the European *Vulpia*, and they grouped together with the narrow-leaved European *Festuca* species. *Lolium* grouped with the broad-leaved *Festuca*. In paper II the narrow-leaved African *Festuca* were re-analysed by AFLPs (and for the first time by morphology), and also including the rare *F. elgonensis*, as well as more samples of the uncommon *F. claytonii* and *F. pilgeri*. Better differentiation was obtained between *F. claytonii* and *F. pilgeri* although the two species are undoubtedly genetically closely related. However, *F. elgonensis* was intermixed with *F. abyssinica* and *F. richardii*. *F. abyssinica* and *F. richardii* were not separated in any of the two AFLP analyses, whereas *F. chodatiana* was always very well differentiated.

All the Ugandan species mentioned above are tetraploid, except *F. africana*, which is decaploid. Chromosome numbers alone cannot therefore be used to differentiate these species. However, the chromosome numbers confirmed the separation between the African *F. simensis* and the European *F. gigantea*. These species are morphologically rather similar but *F. simensis* is tetraploid whereas *F. gigantea* is hexaploid. Also the separation between *F. africana* and *F. altissima* is confirmed because the latter is diploid.

A morphological analysis of the narrow-leaved Ugandan *Festuca* gave similar results as AFLPs by merging *F. abyssinica*, *F. elgonensis* and *F. richardii*, having *F. claytonii* close to but differentiated from *F. pilgeri*, and showing *F. chodatiana* as a very distinct species. A morphological analysis of the broad-leaved species also confirmed the AFLP result in separating *F. simensis* and *F. africana*, showing a close relationship of *F. simensis* to *F. gigantea*, while *F. africana* was well differentiated from *F. altissima*. *F. engleri* and *F. mekiste*, the two other broad-leaved African species that were included in the morphological analysis, grouped close to *F. gigantea* and *F. africana* respectively.

Leaf anatomy in transverse section and in surface view, like AFLPs and morphology, does not support the distinction between *F. abyssinica*, *F. elgonensis* and *F. richardii*. However, leaf anatomy gives the best unambiguous distinguishing characters for *F. claytonii* and *F. pilgeri*, supporting them as well separated species. Also *F. chodatiana* is anatomically confirmed to be a distinct species. The broad-leaved species, *F. simensis* and *F. africana*, are also anatomically differentiated.

In conclusion, basing on AFLPs, morphological and anatomical evidence, the narrow-leaved African species *F. elgonensis* and *F. richardii* should be re-merged with *F. abyssinica*, but *F. chodatiana*, *F. claytonii* and *F. pilgeri* are good species. The narrow-leaved African species should possibly be included in a new genus because they are genetically, physiologically and geographically very distinct from *Festuca* s.s., although there are no observed differentiating morphological or anatomical characters. The broad-leaved African *Festuca* should be incorporated into the already established infrageneric ranks for the temperate species following the observed genetic and morphological similarities; hence *F. simensis* belongs to subgenus *Schedonorus*, and *F. africana* to subgenus *Drymanthele*.

Sammendrag

Sju arter av slekta *Festuca* samt en art av slekta *Vulpia* i Uganda og Etiopia ble analysert ved hjelp av AFLP fingerprinting og sammenliknet med sju utvalgte europeiske arter av *Festuca*, en art av *Lolium* og to arter av *Vulpia*. De fem smalbladete afrikanske artene (*F. abyssinica*, *F. richardii*, *F. claytonii*, *F. pilgeri* og *F. chodatiana*) ble funnet å være genetisk godt atskilte fra de øvrige artene (med 100 % bootstrap støtte). De to breibladete afrikanske artene (*F. africana* og *F. simensis*) grupperte seg sammen med de breibladete europeiske artene, og denne gruppen var godt atskilt fra de smalbladete europeiske artene. *F. simensis* ble funnet å være forbundet med de europeiske artene via *F. gigantea* og *F. africana* via *F. altissima*. De afrikanske *Vulpia* artene er nærstående europeisk *Vulpia*, og de grupperer seg sammen med de smalbladete europeiske *Festuca* artene. *Lolium* grupperte seg sammen med de breibladete *Festuca* artene. I den andre avhandlingen ble de smalbladete afrikanske *Festuca* artene analysert på ny ved hjelp av AFLP (og morfologi), og nå ble også den sjeldne arten *F. elgonensis* tatt med samt flere innsamlinger av de uvanlige artene *F. claytonii* og *F. pilgeri*. Bedre skjelning mellom *F. claytonii* og *F. pilgeri* ble oppnådd, sjøl om de to artene utvilsomt er nær beslektet. *F. elgonensis* kom ut sammen med *F. abyssinica* og *F. richardii*. *F. abyssinica* og *F. richardii* ble ikke skilt i noen av de to AFLP analysene. *F. chodatiana* var godt atskilt.

Alle artene fra Uganda nevnt ovenfor er tetraploide, unntatt *F. africana* som er decaploid. Kromosomtall kan derfor ikke alene brukes til å skille disse artene. Men kromosomtallene stadfesta skille mellom den afrikanske *F. simensis* og den europeiske *F. gigantea*. Disse artene er morfologisk ganske like, men *F. simensis* er tetraploid mens *F. gigantea* er hexaploid. Også skille mellom *F. africana* og *F. altissima* ble stadfesta siden den sistnevnte er diploid.

En morfologisk analyse av de smalbladete artene av *Festuca* fra Uganda ga samme resultat som AFLP ved at den slo sammen *F. abyssinica*, *F. elgonensis* og *F. richardii* til en art, ved at *F. claytonii* var nærstående men skilt fra *F. pilgeri*, og ved å vise at *F. chodatiana* er en godt atskilt art. En morfologisk analyse av de breibladete artene av *Festuca* ga også samme resultat som AFLP ved at den skilte *F. simensis* og *F. africana*, viste nært slektskap mellom *F. simensis* og *F. gigantea*, mens *F. africana* var godt atskilt fra *F. altissima*. *F. engleri* og *F. mekiste*, de to andre artene som ble inkludert i den morfologiske analysen, grupperte seg nær henholdsvis *F. gigantea* of *F. africana*.

På samme måte, som når det gjelder AFLP og morfologi, støtter ikke bladanatomi (tverrsnitt og overflate) skillet mellom *F. abyssinica*, *F. elgonensis* og *F. richardii*. Men

bladanatomi gir de mest entydige skillekarakterer mellom *F. claytonii* og *F. pilgeri*, og støtter dem som to godt avgrensete arter. Også *F. chodatiana* ble stadfestet som en god art med hjelp av anatomiske kriterier.

Som konklusjon, basert på AFLP, morfologiske og anatomiske bevis, bør de smalbladete afrikanske artene *F. elgonensis* og *F. richardii* slås sammen med *F. abyssinica*, mens *F. chodatiana*, *F. claytonii* og *F. pilgeri* er gode arter. De smalbladete afrikanske artene bør muligens inkluderes i en ny slekt fordi de er genetisk, fysiologisk og geografisk svært forskjellig fra *Festuca s. str*. til tross for at det ikke ble funnet morfologiske eller anatomiske skillekarakterer. De breibladete afrikanske artene bør føres til allerede etablerte infragenerske enheter for tempererte arter i følge deres genetiske og morfologiske likheter; derfor tilhører *F. simensis* underslekt *Schedonorus* og *F. africana* underslekt *Drymanthele*.

1.0 Introduction

Festuca is a very large cosmopolitan genus with about 450 species (Clayton & Renvoize 1986), and this makes it very difficult for a comprehensive taxonomic treatment. Since 1882 several infrageneric classifications have been proposed (summarised in Darbyshire & Warwick 1992), but a complete understanding of the genus will only be attained when studies from different biogeographic regions are conducted in a comparative way. Most of the species are temperate but some occur on mountain tops in the tropics.

In Uganda *Festuca* occurs in all the high mountains, i.e. Mt. Elgon, Rwenzori mts and the Virunga mts, and also in the Kigezi highlands in the southwest, e.g. at Bwindi Impenetrable National Park and Echuya swamp (Fig. 1). The high East African mountains display a marked vegetation zonation that is so different from that of the lowlands. It includes the montane forest belt on the lower slopes (1,800 – 3,000 m), the ericaceous or subalpine belt (3,000 – 3,500 m) and the afroalpine belt above 3,500 m (Hedberg 1955, 1965, 1969). The montane forest belt is characterized by tall timber trees, many of them broad-leaved. The ericaceous belt is mainly dominated by low forest or scrub of *Erica*, whereas the afroalpine belt varies from very open scrub-vegetation to dense *Dendrosenecio* forests and thick *Helichrysum* scrub like on the Rwenzori (Hedberg 1955). Most of the mountains with afroalpine zones are of volcanic origin (except Rwenzori which is an upthrust mountain), are of unequal ages (miocene to late pleistocene), and have evidently stood isolated from each other since their origin (Hedberg 1970). Although the flora of the afroalpine belt is obviously distinct from the surrounding lowlands, it is poor in species with only about 70 – 150 species of vascular plants per mountain (Hedberg 1970, 1973). This is because the plants have to adapt to a peculiar climate with "summer every day and winter every night" (Hedgerg 1964). In addition to being species poor, endemism in the afroalpine belt is very high. About 80 % of the afroalpine species of vascular plants are endemic to the high mountains of tropical East Africa and Ethiopia (Hedberg 1965, 1969). For example, 21 species of *Festuca* are native to tropical Africa, 18 of which are endemic to this region, and the remaining 3 species also occur in the temperate South Africa.

The broad-leaved species of *Festuca* mainly occur in the montane forest belt at the lower altitudes (below 3,000 m), whereas the narrow-leaved species mainly occur in the ericaceous and afroalpine belts at the higher altitudes above 3,000 m (Fig. 2). However, the narrow-leaved *F. chodatiana*, which has flat leaves, occurs below 3,000 m at an altitude range of 2,000 – 3,000 m. The species that grow above 3,000 m are characterised by possession of needle-like or thread-like leaves and dense tussocks, whereas the ones growing below 3,000

m have flat leaves and grow as single culms or in loose tussocks. Hedberg (1973) and myself (during fieldwork in 2003 – 2006) observed that *F. abyssinica* at low altitudes often occurs in solitary culms or loose tufts, whereas it forms dense tussocks at high altitudes; and *F. pilgeri* always forms dense tussocks and it only occurs above 3,500 m.

Figure 1. A map of Uganda showing the distribution areas of *Festuca* (shaded dark grey), which correspond to the study areas.

Big grass tussocks are a growth form adaptation of afroalpine grasses to temperature insulation (Hedberg 1964, 1973). In such a case the innovation shoots, which are formed mainly in the inner part of the tussock, are protected against environmental changes in temperature and moisture by a dense layer of dead leaves and culm bases. Márquez *et al*. (2006) term Hedberg's observation as an 'avoidance' mechanism to freezing and they experimentally prove 'tolerance' to be another strategy that grasses have adopted to withstand the thermal constraint present on tropical high mountains. They found the supercooling capacity of Andean grasses to be -6.2 – -2.9°C, hence qualifying these grasses as 'tolerant' to freezing because in the tropical environments 'tolerance' has been selected where night temperatures drop far below 0°C for several hours, whereas 'avoidance' is favoured when night temperatures do not drop far below 0°C or only do so for short periods of time. Clearly

the tropical African high mountain grasses have adapted a combination of strategies (morphological, functional or even anatomical) to survive in the peculiar climate. Hedberg (1964) recorded a temperature of -5°C on a frosty morning in the afroalpine belt on Mt. Kenya, yet at the same time the temperature in the central part of a *F. pilgeri* tussock was +2.5°C. Therefore, these grass tussocks are both morphologically and functionally (freezing tolerant) adapted.

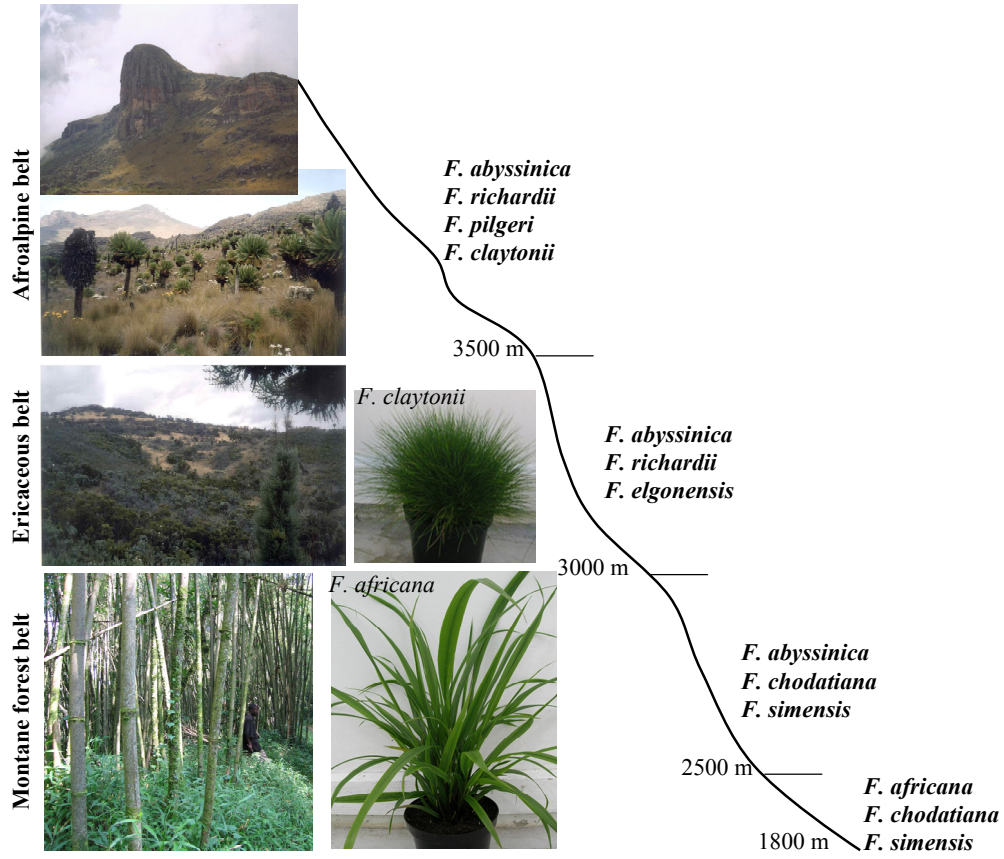

Figure 2. A schematic diagram of the altitudinal distribution of the Ugandan species of *Festuca*. The landscapes show the ericaceous and afroalpine belts of Mt. Elgon, Uganda (photographed by Mary Namaganda). The peak in the top picture is Jackson's summit (4,165 m). The montane forest belt is represented by a picture of the bamboo forest of Mt. Rwenzori (photographed by Maja Stade).

Reduced leaf dimension in plants is a xeromorphic feature. Therefore, the possession of needle-like leaves in *Festuca* and other grasses above 3,000 m is an adaptation to 'water economy' (Hedberg 1964). Needle-like leaves maintain the leaf temperature close to air temperature even under an intense solar radiation, meaning that the water vapour gradient between the leaf and the atmosphere is low (Gauslaa 1984). Low soil temperature at root

depth may result in slow absorption of water from the soil and its slow movement in the roots because water is more viscous at lower temperatures. Also in the nights and early mornings, the rate of movement of water through the roots may also be slow because parts of the plants may be frozen. However, because the high mountain grasses may be tolerant to freezing (Márquez *et al*. 2006), needle-like leaves seem to be an adaptation to reduce transpiration during the warm and windy days, and to reduce the amount of leaf surface exposed to frost during the nights.

This thesis includes both classical and molecular taxonomy with the main goal of revising the genus *Festuca* in Uganda (and later the rest of tropical Africa). The classical taxonomic component includes numerical morphological analyses, chromosome counts and an anatomical investigation. The molecular component is based on the amplified fragment length polymorphism (AFLP) fingerprinting technique (Vos *et al*. 1995). AFLP fingerprinting is known to be a robust and reliable DNA marker method, which generates fingerprints randomly covering the respective genome without prior sequence knowledge (Becker *et al*. 1995, van Eck *et al*. 1995, Vos *et al*. 1995). The AFLP is one of the most effective techniques for detection of polymorphism in plant genomes (Mohan *et al*. 1997, Botha et al. 2004). This makes it an ideal tool for discriminating between closely related taxa e.g. varieties (Mohan *et al*. 1997). For example, Ehrich *et al*. (2007) found much more genetic differentiation in the afroalpine *Arabis alpina* using AFLPs compared to that obtained with chloroplast DNA markers (Koch *et al*. 2006, Assefa *et al*. 2007). AFLP fingerprinting has been widely applied in plant breeding, genome mapping, map-based cloning and cultivar identification of different plants e.g. *Festuca* (Alm *et al*. 2003, Saha *et al*. 2005), *Lolium* (Bert *et al*. 1999), and *Triticum* (Medini *et al*. 2005). It has also been found useful in taxonomic studies investigating intra- and interspecific relationships of different plants: like bamboo (Loh *et al*. 2000), *Lactuca* (Koopman *et al*. 2001), *Vicia* ser. *Vicia* (van de Wouw *et al*. 2001), *Berberis* (Bottini *et al*. 2002), *Musa* (Ude *et al*. 2002), *Avena* (Drossou *et al*. 2004), *Solanum* sect. *Petota* (Lara-Cabrera and Spooner 2004), *Echinochloa* (Tabacchi *et al*. 2006), and *Festuca* ser. *Psammophilae* (Šmarda *et al*. 2007).

Objectives

The aim of this research was to streamline the taxonomy of the Ugandan *Festuca* and allies, and find out how they compare to the temperate species. This was achieved through the following objectives:

1. To determine the genetic relationships within and between the Ugandan species of *Festuca* and compare them to selected European *Festuca* using AFLPs.
2. To determine chromosome numbers of the species.
3. To carry out a numerical analysis of morphological characters.
4. To investigate whether anatomical characters are useful in separating the species as is implied in literature.

2.0 Materials and methods

Sampling

Sampling strategy was to capture as much genetic, morphological and anatomical variation as possible within a species. This was done by collecting samples from several populations at different altitudes and where applicable from different mountains, following the assumption that variation in a species could be related to the different climatic and edaphic factors, which are strongly influenced by the altitude (Hedberg 1969, Kebede *et al*. 2007). For the very rare species samples were collected from the only populations that I could find; i.e. two populations of both *F. claytonii* and *F. pilgeri* and only one population of *F. elgonensis*. When possible, all samples were collected from one individual plant; i.e. young leaves were picked and immediately dried in silica gel for use in molecular analyses, mature seeds when present were collected for chromosome counting, pieces from the middle part of mature leaves were fixed in 75 % ethanol for use in anatomical investigations, and herbarium vouchers were collected for morphological measurements as well as future reference. The herbarium vouchers are kept at the Makerere University herbarium (MHU) and duplicates will be deposited at the Oslo herbarium (O).

I identified the plants in the field and verified the identifications myself at the Kew (K) and East African (EA) herbaria. Sylvia Phillips of the Kew herbarium helped with some of the identifications. Type specimens of the narrow-leaved *Festuca* were included in the morphological analysis, and they were kindly provided by Kew. Some specimens of broad-leaved *Festuca*, including an old but originally misidentified collection of *F. engleri* from Uganda, were kindly provided by the British Museum (BM) herbarium.

Molecular analyses; – AFLP fingerprinting (Paper I & II)

DNA was extracted from finely ground silica-dried leaves using a DNeasy Plant Mini Kit. With the temperature and time profiles of Vos *et al*. (1995) and Becker *et al*. (1995), 400 ng of the DNA was digested with the restriction enzymes *Eco*RI and *Mse*I, and the digestion fragments were then ligated to the end-specific adapters *Eco*RI (GTC GTA GAC TGC GTA CC/AAT TGG TAC GCA GTC) and *Mse*I (GAC GAT GAG TCC TGA G/TAC TCA GGA CTC AT). The resulting fragments were PCR preamplified using the primers E01 (GAC TGC GTA CCA ATT CA) and M01 (GAT GAG TCC TGA GTA AA), which are complementary to the adapter sequences. Five +3/+3 primer pairs (1. *Eco*RI+AAT / *Mse*I+ACG, 2. *Eco*RI+AGA / *Mse*I+ACG, 3. *Eco*RI+ACA / *Mse*I+ACC, 4. *Eco*RI+ACG / *Mse*I+AGC and 5. *Eco*RI+ACG / *Mse*I+ACT) were used in the final PCR amplifications for Paper I, and the first three primer pairs listed above for Paper II. The e-primer was labelled with radioactive phosphorus (^{33}P). Polyacrylamide gel electrophoresis (PAGE) was used to reveal the patterns of the resulting fragments, viewed by exposure to x-ray films, and manually scored as present (1) or absent (0) to generate binary data matrices, which were analysed by principal coordinate analysis (PCoA) and neighbor-joining analysis (NJ).

Chromosome counts (Paper I & II)

Seeds were germinated in Petri dishes at room temperature. Roots were harvested when about 1.5 cm long and pretreated in ice water for 24 hours. The ice water pre-treatment helped to increase the number of cells at metaphase by arresting spindle formation, hence no further cell division beyond this stage. The pretreated roots were fixed in fresh Carnoy's solution I for three days at room temperature, stained in 1 % acetocarmine, and then squashes made. Chromosome painting by fluorescent in situ hybridization (FISH) was initiated but the preliminary results are not presented in this thesis because replication is needed and will be done at a later stage. The work was done at Kansas State University under the guidance of Prof. Dr. Bernd Friebe.

Morphology (Paper II & III)

As many morphological characters as possible were measured on the herbarium vouchers, i.e. 56 characters on the narrow-leaved species and 62 on the broad-leaved species. The character lists were generated from literature especially focussing on the Flora of tropical East Africa (Clayton 1970), Flora of Ethiopia and Eritrea (Phillips 1995), and Alexeev's

(1986, 1987) publications on the African *Festuca*. The resulting data matrices were standardised and then analysed by principal components analysis (PCA) and Unweighted Pair Group Method with Arithmetic mean (UPGMA). The data was also analysed by discriminant function analysis (DA) and box plots.

Anatomy (Paper IV)

The leaves fixed in ethanol were embedded in wax and sectioned using a microtome dissector. The sections were stained with 1 % safranin and mounted in DPX mountant to make permanent slides. The slides were viewed under a light microscope to determine the disposition of sclerenchyma tissue. In the fine-leaved *Festuca* the position of sclerenchyma tissue as seen in a cross-section of the leaf blade is an important aid to distinguishing the species (Clayton & Renvoize 1986, Phillips 1995), and has been used since 1882 (Aiken & Consaul 1995). Scanning electron microscopy (SEM) of the epidermis as seen in surface view was also done. Anatomy of the epidermis as seen in surface view offers a wide range of taxonomically important characters in the grasses (Ellis 1979, Metcalfe 1960). Energy dispersive x-ray analysis (EDXA) was done in order to view areas of high atomic number on the leaf surfaces, hence identifying the presence and distribution of silica bodies.

3.0 Results and discussion

Species distinction (see also appendix)

F. abyssinica, F. elgonensis and F. richardii – (*F. abyssinica* s.l.)

The AFLP results (Papers I & II), chromosome numbers (Papers I & II), morphology (Paper II) and anatomy (Paper V), do not support the recognition of these three species as separate taxa. In fact the four evidences are so congruent that the three species should be remerged. First of all, these species are all tetraploid. The AFLPs show these species to be genetically indistinguishable, following PCoA and NJ analysis. The morphological analysis included the vouchers corresponding to the AFLP analysed samples and also the type specimens. Interestingly the types, which served as controls to my own collections, grouped close together in the PCA, confirming the lack of morphological separation between the species as was shown by the rest of the samples that were analysed. Grouping in the PCA was confirmed by the DA. The main differentiating morphological characters of *F. abyssinica* s.l. from the rest of the narrow-leaved Ugandan *Festuca* are the large glumes with membranous

margins, which clasp around (envelope) the spikelets, and are more than 3/5 to sub-equaling the spikelet in length (Paper II).

The anatomy of the leaves in cross section as exhibited by *F. richardii* (with sclerenchyma only on the leaf underside) is also possessed by some typical *F. abyssinica* specimens, and is also the same as that described for *F. elgonensis* (Alexeev 1987). In *F. abyssinica* the sclerenchyma tissue can also be both on the leaf underside and on the rib tops. Anatomy of the leaf epidermis in surface view is basically the same for the three species. They all have long cells with sinuous cell walls, sparsely distributed prickles, crescent-shaped to round intercostal silica bodies, and both crescent-shaped and linear silica bodies in the costal regions. However, differences are in the density of silica bodies, i.e. highest in *F. elgonensis* (350 – 450 per mm^2), followed by *F. abyssinica* (300 – 350 per mm^2) and lowest in *F. richardii* (200 – 250 per mm^2). At this point, emphasising this as the difference between the species is not well supported until specimens from all over tropical Africa have been analysed.

F. abyssinica s.l. is a very widely distributed species in the tropical African high mountains, and is also abundant in many localities. In "Flora Zambesiaca" Launert (1971) described *F. abyssinica* as an extremely polymorphic species of which many but fully intergrading infra-specific taxa had been described. Earlier, in "Flora of Tropical East Africa" Clayton (1970) also regarded *F. abyssinica* as a polymorphic species and recognised two varieties, viz. *F. abyssinica* var. *abyssinica* and *F. abyssinica* var. *supina*, although he thought the latter to be a hybrid of var. *abyssinica* and *F. pilgeri*. Later, in his study of tropical African *Festuca*, Alexeev (1986, 1987) recognised *F. schimperiana* [awns (1)1.5 – 4(7) mm vs. *F. abyssinica* 0 – 1(1.7) mm], *F. richardii* (anther length 2 – 3 mm vs. *F. abyssinica* 0.6 – 1.8 mm), *F. elgonensis* (leaf blade with 5 or 7 vascular bundles, stiff, upright vs. leaf blade with 7 vascular bundles, soft, nodding in *F. abyssinica*), and several other species not present in Uganda, which he termed as the *F. abyssinica* aggregate. But Phillips (1995), in the Flora of Ethiopia and Eritrea, disregarded *F. schimperiana* as a separate species because the Ethiopian specimens fully intergraded with *F. abyssinica* in morphology as well as leaf anatomy in cross section, i.e. both forms had sclerenchyma tissue both on the leaf underside and on the rib tops. However, Phillips (1995) accepted *F. richardii* although she acknowledged that its specific status rests mainly on the slightly different leaf-anatomy, i.e. sclerenchyma tissue on the leaf underside only. I initially did not recognise *F. schimperiana* because I followed Phillips' taxonomy.

F. claytonii

F. claytonii was consistently well differentiated by AFLPs (Paper I & II), morphology (Paper II), and anatomy (Paper IV), which is in agreement with Alexeev (1986). However, like the above species it is also tetraploid. *F. claytonii* was genetically found to be close to *F. pilgeri* (see below). The two species also show superficial morphological resemblance, but *F. claytonii* can easily be distinguished from *F. pilgeri*, even in the field, by its smaller habit. In detail, it has shorter culms, shorter and narrower leaves, shorter spikelets, glumes and lemmas. Anatomy of the leaf blades as seen in surface view give the best distinguishing characters for *F. claytonii* from all the other Ugandan narrow-leaved species, i.e. the leaves are glabrous and smooth with neither hairs nor prickles, and the leaf surfaces are covered with numerous papillae. It was the only species found with these characters.

It also had the lowest density of silica bodies (60 – 80 per mm^2), which were also crescent-shaped (intercostal) and linear (costal) like in *F. abyssinica* s.l. Leaf anatomy in cross section was similar to one of the variations in *F. abyssinica* s.l., i.e. the leaf angled with 5 vascular bundles and sclerenchyma only present on the leaf underside.

F. pilgeri

F. pilgeri was also consistently well differentiated by AFLPs (Paper I & II), morphology (Paper II), and anatomy (Paper IV), and this agrees with Clayton (1970). It is also tetraploid, confirming Hedberg's (1957) count. *F. pilgeri*, *F. claytonii* and *F. chodatiana* (see below), are differentiated from *F. abyssinica* s.l. by the possession of narrow and herbaceous glumes, which are only up to 3/5 the length of the spikelet. Morphological differences between *F. pilgeri* and *F. claytonii* have been discussed above but no single morphological character distinguishes *F. pilgeri* from all the narrow-leaved Ugandan *Festuca*. However, anatomy of the leaf blade in cross section distinguishes *F. pilgeri* from all the other narrow-leaved species discussed here; *F. pilgeri* is the only species with an almost continuous ring of sclerenchyma tissue, which gives it a circular appearance (without angles) in transverse section, as is also reported by Clayton (1970). Also the leaf anatomy as seen in surface view is unique because *F. pilgeri* is the only species that is densely armed with prickles, which give it a very rough texture. And because its nerves are not prominent, being obscured by the continuous ring of sclerenchyma, it lacks the linear silica bodies that are placed along the nerves (costal) in the other narrow-leaved species. In addition, it has the highest density of prickles (500 – 600 per mm^2). Like *F. claytonii*, it has 5 vascular bundles and does not have sclerenchyma on the rib tops.

F. chodatiana

F. chodatiana is the most well differentiated of all the investigated narrow-leaved species as is shown by AFLPs (Paper I & II), morphology (Paper II), and anatomy (Paper IV). Like all the narrow-leaved species above it is tetraploid and is morphologically distinguished by its usually flat but sometimes folded leaves. It grows in solitary culms or very loose tussocks, and the culms are thin with very few leaves. It is the only narrow-leaved species with open inflorescences, which are long with many florets, and has the longest awns (6.6 – 11.6 mm). Anatomically it is unique because it has many vascular bundles in cross section (7 – 11), compared to the rest of the narrow-leaved species which have only up to 7 vascular bundles, and it also has the widest leaves (1.1 – 2.4 mm). The clear genetic, morphological and anatomical differentiation of *F. chodatiana* may possibly be explained by its adaptation to a different habitat. It is a forest species, not occurring beyond 3,000 m above sea level. Whereas *F. abyssinica* also occurs in the forest zone, it is at the same time adapted to the open, cooler, and drier ericaceous and afroalpine environments (above 3,000 m).

F. simensis

The broad-leaved *F. simensis* is genetically (Paper I), morphologically (Paper III), and anatomically (Paper IV) well differentiated. It was earlier documented to be similar to the European *F. gigantea* (Clayton 1970) and this was proved both genetically (Paper I) and morphologically (Paper III). Both species dwell in shady and moist habitats, and have large falcate auricles, which make them conspicuously similar. However, they are different cytologically with *F. simensis* tetraploid and *F. gigantea* hexaploid.

The leaf anatomy of *F. simensis* in transverse section is deeply furrowed, making the ribs to conspicuously stand out. It has sclerenchyma on both the leaf underside and on the rib tops and the sclerenchyma often touch the vascular bundles. The anatomy of the leaf epidermis in surface view shows presence of linear silica bodies along the nerves, which is a good feature differentiating the broad-leaved species from the narrow-leaved species that have either both crescent-shaped and linear silica bodies, or only crescent-shaped ones.

F. simensis is also morphologically and genetically well differentiated from *F. africana* (see below), which is a forest dweller without falcate auricles, with very wide and deep green leaves (leaves bright green in *F. simensis*), and often 1-flowered spikelets (2-several flowered in *F. simensis*).

F. africana

The broad-leaved *F. africana* is also genetically (Paper I), morphologically (Paper III), and anatomically (Paper IV) well differentiated. It is the only decaploid as the rest of the species investigated are tetraploid. It was genetically found to be most closely related to the European *F. altissima*, which is also a forest species with hairy ovaries like *F. africana* but without awns. The two species are also cytologically different because *F. altissima* is a diploid. They are also morphologically well separated (Paper III).

A comparison with *F. simensis* is given above. In addition, it is one of the few species of *Festuca* that often have 1-flowered spikelets, the other being the Malesian *F. monantha* (Clayton & Renvoize 1986). Like *F. simensis*, the *F. africana* leaf in cross section also has sclerenchyma both on the leaf underside and on the rib tops, often touching the vascular bundles, but in *F. africana* the furrows are very shallow making the ribs to appear like gentle undulations. It also has linear silica bodies along the nerves but their density is lower (1 – 5 silica bodies per mm of vein) compared to *F. simensis* (10 silica bodies per mm of vein).

Implications on the taxonomy of the narrow-leaved African *Festuca* (Paper I & V)

The AFLP analysis (Paper I) aimed at comparing, for the first time, the African species of *Festuca* and *Vulpia* to selected European *Festuca*, and the allied genera *Vulpia* and *Lolium*. Prejudiced by the earlier research on the temperate *Festuca*, which resulted in the separation of *Festuca* into two clusters (the narrow-leaved and broad-leaved; e.g. Charmet *et al*. 1997, Gaut *et al*. 2000, Torrecilla & Catalan 2002, Catalan *et al*. 2004), I expected the African species to fall well within the two temperate clusters. Unexpectedly, the narrow-leaved African *Festuca* formed a third clearly separated and very highly supported cluster (100 % bootstrap). However, the broad-leaved African *Festuca* grouped with their broad-leaved European counterparts, and the African *Vulpia* bromoides (from Ethiopia) grouped with the European *Vulpia* (*V. bromoides* and *V. myuros*). *Vulpia* species, like in earlier studies, grouped with the narrow-leaved European *Festuca*, whereas *Lolium* grouped with the broad-leaved *Festuca*.

The wide genetic separation of the narrow-leaved African *Festuca* from the rest of the *Festuca* species (*Festuca* s.l.) prompted a morphological and anatomical investigation into any possible differences between the narrow-leaved African *Festuca* and *Festuca* s.l. But *Festuca* being a very large and highly polymorphic genus, it was not possible to find morphological or anatomical characters that could separate the narrow-leaved African *Festuca* from the rest of the *Festuca*. Instead, physiological differences were emphasised and

these are based on the fact that the narrow-leaved African *Festuca* have evolved and become adapted to the peculiar climate in the tropical African mountains, with freezing temperatures in the nights followed by warm summer days (Hedberg 1964). The narrow-leaved African *Festuca* when planted outdoors did not survive the cold Norwegian autumn because they lacked survival strategies as they are not adapted to such climate.

Without doubting genetic differences as the basis for diversity (morphological, etc; e.g. Hansen et al. 2000, Fjellheim et al. 2001), and acknowledging the physiological differences (steered by the environment), the idea of sibling genera was born (Paper V). Like sibling species (Zohary 1996, 1999, Fiedorow *et al*. 2001), which are described as "*closely related and often sympatric taxa that are reproductively isolated but difficult to separate morphologically*" (Winker 2005), we in Paper V apply the concept of sibling genera to flowering plants, and describe sibling genera as "*any two or more genera which are morphologically very similar but shown by molecular, cytogenetic, or other extraordinary methods to be only remotely related to each other, with a barrier in place to ensure reproductive isolation between the genera*". We give the opinion that the narrow-leaved African *Festuca* and the rest of *Festuca* could be sibling genera. In this case we intend to recommend the narrow-leaved African *Festuca* to be recognised in a new genus '*Afrofestuca*'.

4.0 Conclusions and recommendations

F. elgonensis and *F. richardii* should be remerged with *F. abyssinica*, hence reducing the number of narrow-leaved *Festuca* in Uganda to four from six. There is need for a tropical Africa-wide study of *F. abyssinica* s.l. in order to check Alexeev's taxonomy, and determine species or infraspecific taxa that are well differentiated. Investigations into the cytogenetics of this widespread and highly polymorphic species may reveal baseline information on which morphological or anatomical differences could be based to define species or infraspecific taxa.

The narrow-leaved African species should possibly be included in a new genus because they are genetically, physiologically and geographically very distinct from the rest of the *Festuca*, although there are no observed differentiating morphological or anatomical characters.

The broad-leaved African *Festuca* should be incorporated into the already established infrageneric ranks for the temperate species following the observed genetic and morphological similarities; hence *F. simensis* belongs to subgenus *Schedonorus*, and *F. africana* to subgenus *Drymanthele*.

Except *F. africana*, which is decaploid, all African *Festuca* species investigated are tetraploid. Therefore, a search for the possible diploid species should be tried. The chromosome analyses should be deepened to establish if the tetraploid species are allo or autopolyploids through fluorescent in situ hybridisation (FISH) and meiotic pairing studies of the species, and their diallel crosses.

5.0 References

Aiken, S. G., Consaul, L. L. (1995) Leaf cross sections and phytogeography: A potent combination for identifying members of *Festuca* subgen. *Festuca* and *Leucopoa* (Poaceae), occurring in North America. American Journal of Botany 82: 1287-1299.

Alexeev, E. B. (1986) New narrow-leaved *Festuca* (Poaceae) members from Tropical and South Africa. Botanicheski Zhurnal 71: 1109-1117.

Alexeev, E. B. (1987) Fescues of the *Festuca abyssinica* s. l. (Poaceae) group. Botanicheski Zhurnal 72: 1260-1268.

Alm, V., Fang, C., Busso, C. S., Devos, K. M., Vollan, K., Grieg, Z., Rognli, O. A. (2003) A linkage map of meadow fescue (*Festuca pratensis* Huds.) and comparative mapping with other Poaceae species. Theoretical and Applied Genetics 108: 25-40.

Assefa, A., Ehrich, D., Taberlet, P., Nemomissa, S., Brochmann, C. (2007) Pleistocene colonization of afro-alpine 'sky islands' by the arctic-alpine *Arabis alpina*. Heredity 99: 133-142.

Becker, J., Vos, P., Kuiper, M., Salamini, F., Heun, M. (1995) Combined mapping of AFLP and RFLP markers in barley. Molecular and General Genetics 249: 65-73.

Bert, P. F., Charmet, G., Sourdille, P., Hayward, M. D., Balfourier, F. (1999) A high-density molecular map for ryegrass (*Lolium perenne*) using AFLP markers. Theoretical and Applied Genetics 99: 445-452.

Botha, A. M., Venter, E., van der Vyver, C., Kunert, K. J. (2004) Development and application of molecular DNA markers in Africa: a South African view. South African Journal of Botany 70: 152-166.

Bottini, M. C. J., De Bustos, A., Jouve, N., Poggio, L. (2002) AFLP characterisation of natural populations of *Berberis* (Berberidaceae) in Patagonia, Argentina. Plant Systematics and Evolution 231: 133-142.

Catalan, P., Torrecilla, P., Lopez Rodriguez, J. A., Olmstead, R. G. (2004) Phylogeny of the festucoid grasses of subtribe Loliinae and allies (Poeae, Pooideae) inferred from ITS and *trn*L-F sequences. Molecular Phylogenetics and Evolution 31: 517-541.

Charmet, G., Ravel, C., Balfourier, F. (1997) Phylogenetic analysis in the *Festuca-Lolium* complex using molecular markers and ITS rDNA. Theoretical and Applied Genetics 94: 1038-1046.

Clayton, W. D. (1970) Gramineae Part 1. In: Milne-Redhead, E., Polhill, R. M. (eds.) Flora of Tropical East Africa. Crown agents for oversea governments and administrations, London, pp. 56-63.

Clayton, W. D., Renvoize, S. A. (1986) *Genera Graminum: Grasses of the World*. HMSO, London, pp 374-375.

Darbyshire, S. J., Warwick, S. I. (1992) Phylogeny of North American *Festuca* (Poaceae) and related genera using chloroplast DNA restriction site variation. Canadian Journal of Botany 70: 2415-2429.

Drossou, A., Katsiotis, A., Leggett, J. M., Loukas, M., Tsakas, S. (2004) Genome and species relationships in genus *Avena* based on RAPD and AFLP molecular markers. Theoretical and Applied Genetics 109: 48-54.

Ehrich, D., Gaudeul, M., Assefa, A., Koch, M. A., Mummenhoff, K., Nemomissa, S., Consortium, I., Brochmann, C. (2007) Genetic consequences of Pleistocene range shifts: contrast between the Arctic, the Alps and the East African mountains. Molecular Ecology 16: 2542-2559.

Ellis, R. P. (1979) A procedure for standardizing comparative leaf anatomy in the Poacea. II. The epidermis as seen in surface view. Bothalia 12: 641-671.

Fiedorow, P., Odrzykoski, I., Szweykowski, J., Szweykowska-Kulinska, Z. (2001) Phylogeny of the European species of the genus *Pellia* (Hepaticae: Metzgeriales) based on the molecular data from nuclear $tRNA_{CAA}$ intergenic sequences. Gene 262: 309-315.

Fjellheim, S., Elven, R., Brochmann, C. (2001) Molecules and morphology in concert. II. The *Festuca brachyphylla* complex (Poaceae) in Svalbard. American Journal of Botany 88: 869-882.

Gauslaa, Y. (1984) Heat resistance and energy budget in different Scandinavian plants. Holarctic Ecology 7: 1-78.

Gaut, B. S., Tredway, L. P., Kubik, C., Gaut, R. L, Meyer, W. (2000) Phylogenetic relationships and genetic diversity among members of the *Festuca-Lolium* complex (Poaceae) based on ITS sequence data. Plant Systematics and Evolution 224: 33-53.

Hansen, K. T., Elven, R., Brochmann, C. (2000) Molecules and morphology in concert: tests of some hypotheses in arctic *Potentilla* (Rosaceae). American Journal of Botany 87: 1466-1479.

Hedberg, O. (1955) Altitudinal zonation of the vegetation on the east African mountains. Proceedings of the Linnean Society of London, session 165: 134-136.

Hedberg, O. (1957) Afroalpine vascular plants; a taxonomic revision. Symbolae Botanicae Upsalienses XV:1

Hedberg, O. (1965) Afroalpine flora elements. Webbia 19: 519-529.

Hedberg, O. (1964) Features of afroalpine plant ecology. Acta Phytogeographica Suecica, 49. Uppsala.

Hedberg, O. (1969) Evolution and speciation in a tropical high mountain flora. Biological Journal of the Linnean Society 1: 135-148.

Hedberg, O. (1970) Evolution of the afroalpine flora. Biotropica 2: 16-23.

Hedberg, O. (1973) Adaptive evolution in a tropical-alpine environment. In: Heywood, V. N. (ed) Taxonomy and ecology. The Systematics Association special volume no. 5. pp 71-92.

Kebede, M., Ehrich, D., Taberlet, P., Nemomissa, S., Brochmann, C. (2007) Phylogeography and conservation genetics of a giant lobelia (*Lobelia giberroa*) in Ethiopian and Tropical East African mountains. Molecular Ecology 16: 1233-1243.

Koch, M., Kiefer, C., Ehrich, D., Vogel, J., Brochmann, C., Mummenhoff, k. (2006) Three times out of Asia Minor: the phylogeography of *Arabis alpina* L. (Brassicaceae). Molecular Ecology 15: 825-839.

Koopman, W. J. M., Zevenbergen, M. J., Van den Berg, R. G. (2001) Species relationships in *Lactuca* s. l. (Lactuceae, Asteraceae) inferred from AFLP fingerprints. American Journal of Botany 88: 1881-1887.

Lara-Cabrera, S. I., Spooner, D. M. (2004) Taxonomy of North and Central American diploid wild potato (*Solanum* sect. *Petota*) species: AFLP data. Plant Systematics and Evolution 248: 129-142.

Launert, E. (1971) Gramineae. In: Fernandes, A., Launert, E., Wild, H. (eds) Flora Zambesiaca, vol. 10, part 1. Crown Agents for Oversea Governments and Administrations, London, pp 54-57.

Loh, J. P., Kiew, R., Set, O., Gan, L. H., Gan, Y. (2000), A study of genetic variation and relationships within the bamboo subtribe Bambusinae using amplified fragment length polymorphism. Annals of Botany 85: 607-612.

Márquez, E. J., Rada, F., Fariñas, M. R. (2006) Freezing tolerance in grasses along an altitudinal gradient in the Venezuelan Andes. Oecologia 150: 393-397.

Medini, M., Hamza, S., Rebai, A., Baum, M. (2005) Analysis of genetic diversity in Tunisian durum wheat cultivars and related wild species by SSR and AFLP markers. Genetic Resources and Crop Evolution 52: 21-31.

Metcalfe, C. R. (1960) Anatomy of the monocotyledons. I. Gramineae. Clarendon press, Oxford, pp 209-217.

Mohan, M., Nair, S., Bhagwat, A., Krishna, T. G., Yano, M., Bhatia, C. R., Sasaki, T. (1997) Genome mapping, molecular markers and marker-assisted selection in crop plants. Molecular Breeding 3: 87-103.

Phillips, S. (1995) Poaceae. In: Hedberg, I., Edwards, S. (eds.) Flora of Ethiopia and Eritrea, Vol. 7. Addis Ababa, Ethiopia; Uppsala, Sweden, pp. 23-27.

Saha, M. C., Mian, R., Zwonitzer, J. C., Chekhovskiy, K., Hopkins, A. A. (2005) An SSR- and AFLP-based genetic linkage map of tall fescue (*Festuca arundinacea* Schreb.). Theoretical and Applied Genetics 110: 323-336.

Šmarda, P., Šmerda, J., Knoll, A., Bureš, P., Danihelka, J. (2007) Revision of Central European taxa of *Festuca* ser. *Psammophilae* Pawlus: morphological, karyological and AFLP analysis. Plant Systematics and Evolution DOI 10.1007/s00606-007-0532-3.

Tabacchi, M., Mantegazza, R., Ferrero, A. (2006) Morphological traits and molecular markers for classification of *Echinochloa* species from Italian rice fields. Weed Science 54: 1086-1093.

Torrecilla, P., Catalan, P. (2002) Phylogeny of broad-leaved and fine-leaved *Festuca* lineages (Poaceae) based on nuclear ITS sequences. Systematic Botany 27: 241-251.

Ude, G., Pillay, M., Nwakanma, D., Tenkouano, A. (2002) Analysis of genetic diversity and sectional relationships in *Musa* using AFLP markers. Theoretical and Applied Genetics 104: 1239-1245.

van de Wouw, M., Maxted, N., Chabane, K., Ford-Lloyd, B. V. (2001) Molecular taxonomy of *Vicia* ser. *Vicia* based on amplified fragment length polymorphisms. Plant Systematics and Evolution 229: 91-105.

van Eck, H. J., van der Voort, J. R., Draaistra, J., van Zandvoort, P., van Enckevort, E., Segers, B., Peleman, J., Jacobsen, E., Helder, J., Bakker, J. (1995) The inheritance and chromosomal localization of AFLP markers in a non-inbred potato offspring. Molecular Breeding 1: 397-410.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., Zabeau, M. (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research 23: 4407-4414.

Winker, K. (2005) Sibling species were first recognized by William Derham (1718). The Auk 122: 706-707.

Zohary, D. (1996) The mode of domestication of the founder crops of southwest Asian agriculture. Pp. 142-158. In: Harris, D. R. (ed.), The origins and spread of Agriculture and pastoralism in Eurasia. Smithsonian Institution Press, Washington.

Zohary, D. (1999) Monophyletic vs. polyphyletic origin of the crops on which agriculture was founded in the Near East. Genetic Resources and Crop Evolution 46: 133-142.

Appendix

Key to the species

1. Leaves acicular or filiform, if flat then less than 2.5 mm wide; falcate auricles absent ….. 2
1. Leaves flat, usually more than 2.5 mm wide, but if less then leaves with falcate auricles ... 5

2. Glumes large with membranous margins, embracing the spikelets, and more than 3/5 to sub-equaling the spikelet in length …… *F. abyssinica*
2. Glumes narrow and herbaceous, up to 3/5 of the spikelet length ……. 3

3. Plant loosely tufted or spreading; leaves usually flat, 1 – 2.4 mm wide; ovary and caryopsis hairy on top ……..... *F. chodatiana*
3. Plants densely tufted; leaves acicular, 0.4 – 0.7 mm wide; ovary glabrous on top ……..... 4

4. Tussocks small, leaves soft and smooth, epidermis with numerous papillae; sclerenchyma discrete ……..…… *F. claytonii*
4. Tussocks large, leaves stiffly erect or curly, harshly scabrid, epidermis with numerous prickles, sclerenchyma a continuous ring ……..…….. *F. pilgeri*

5. Leaves 6 – 18 mm wide, with very shallow furrows on adaxial surface in transverse section; 1(-2)-flowered; ovary hairy on top …….. *F. africana*
5. Leaves 2 – 7.5 mm wide, with very deep furrows on adaxial surface in transverse section; 2 – 7-flowered, ovary glabrous ……..…… *F. simensis*

Festuca abyssinica A. Rich. in Tent. Fl. Abyss. 2: 432 (1850).

Type: Ethiopia, Tigre, Mt. Scholoda [Selleuda], 26 Oct. 1837, G. H. W. Schimper 410 (P holotype, K! isotype).

Syn. *F. schimperana* A. Rich. in Tent. Fl. Abyss. 2: 433 (1850). Type: Ethiopia, Demerk, G. H. W. Schimper 1384 (P holotype, B isotype).

Syn. *F. richardii* Alexeev in Bot. Zhurnal 71: 1109 (1986); *F. abyssinica* subsp. *eu-abyssinica* "f. inter var. *genuinam* et var. *intermediam* ambig." St. Yves in Bull. Candollea 4: 86 (1929). Type: Ethiopia, Ras Gunna, 12,000 ft, 15 Dec 1863, G. H. W. Schimper 1560 (B holotype, K! isotype).

Syn. *F. elgonensis* Alexeev in Bot. Zhurnal 72: 1266 (1987). Type: Uganda, Mt. Elgon, Madangi, alpine meadow, 10,000 ft, 6 September 1932, A. S. Thomas 644 (K! holotype).

Densely or loosely tufted; perennial or rarely annual. Culms 13 – 100 cm high. Leaf-blades filiform to acicular or flat if growing at lower altitudes, 6 – 35 cm long and 0.4 – 0.8 mm wide; intercostal silica bodies crescent-shaped, semicircular or round, costal silica bodies linear and some crescent-shaped; vascular bundles 5 – 7; sclerenchyma both on the leaf underside and capping the ribs, or only on the leaf underside; ligule 0.2 – 1.0 mm long. Panicle spiciform to linear, 6 – 20 cm long; spikelets green, tinged purple or purple; 2 – 6-flowered, 6.5 – 13 mm long (minus awn point), lanceolate. Glumes broad, with membranous margins, embracing the spikelet; lower glume lanceolate, 5.5 – 10.1 mm long, 1 – 3-nerved, 0.59 – 0.86 of spikelet length; upper glume lanceolate, 6 – 11.4 mm long, 3(-5)-nerved, 0.67 – 0.96 of spikelet length; lemmas scaberulous, second lemma 5-nerved, 6.0 – 9.2 mm long (minus awn point), awn-point 0.3 – 4.5(7.2) mm or sometimes awnless; palea as long as lemma, the keels scaberulous up to ¼ to ¾ of the length from the tip; anthers 0.4 – 4 mm long; ovary glabrous. Caryopsis elliptic to narrow elliptic, glabrous.

In upland grassland and moorland, usually above timberline, but occasionally in open parts of forests, such as near streams, 2130-4300 m in Uganda, elsewhere to 4700 m.

In Uganda known from all the mountains, i.e. Mt. Elgon, Rwenzori Mts. and the Virunga volcanoes. Also widely known from other African mountains, from Mt. Cameroun to the Ethiopian Highlands and south to Zimbabwe.

Festuca chodatiana (St.Yves) Alexeev in Bot. Zhurnal 71: 1113 (1986)

Type: Tanzania, Rungwe district, Kyimbila, Mbaka, 16 Dec 1938, A. F. Stolz 1162 (B lectotype; K! isolectotype:).

Syn. *F. camusiana* St. Yves subsp. *chodatiana* St. Yves in Bull. Soc. Bot. Genève, sér. 2, 18: 158 (1926).

Loosely tufted perennial; culms slender, 50 – 110 cm long. Leaf blades sparse, flat or folded, 6 – 40 cm long, 1 – 2.4 mm wide; intercostal silica bodies crescent-shaped, semicircular or round, costal silica bodies linear and some crescent-shaped; vascular bundles 7 – 11, sclerenchyma both on the leaf underside and capping the ribs on most vascular bundles; ligule 0.4 – 1.1 mm long. Panicle open, 10 – 33 cm long. Spikelets narrowly lanceolate, green to green with purple tinge, 3 – 5-flowered, 8 – 13 mm long; lower glume lanceolate, 2 – 4.5 mm

long, 1-nerved; upper glume lanceolate, (3.5) 4 – 6.5 mm long, 3-nerved. Lemmas lanceolate, 5-nerved, second lemma 5.5 – 7.6 mm long (minus awn), awn 6.6 – 12 mm long; palea about as long as lemma, the keels scaberulous up to ½ of the length from the tip; anthers 1.2 – 2.4 mm long; ovary hairy on top. Caryopsis linear-elliptic with persistent hairs on top.

In open parts of upland forests, more rarely in grassland and thickets above timberline, 2220-3070 m (in Uganda; elsewhere 2100-3500 m).

In Uganda known from Mt. Elgon and the highlands in the southwest. Also scattered in the highlands of Kenya and Tanzania, and also from Imatong Mts. in the Sudan, the eastern Congo mountains and Mt. Cameroun.

Festuca claytonii Alexeev in Bot. Zhurnal 71: 1117 (1986).

Type: Uganda, Mt. Elgon, 12,700 ft, 16 Dec. 1938, A. S. Thomas 2727 (K! holotype; K!, KAW! isotypes).

Densely tufted perennial forming small tussocks. Culms 23 – 52 cm high. Leaf blades acicular, 5.5 – 10.5 cm long and 0.4 – 0.6 mm wide, soft and smooth; sclerenchyma only on the leaf underside, vascular bundles 5; intercostal silica bodies crescent-shaped, semicircular or round, costal silica bodies linear and some crescent-shaped; leaf epidermis with numerous papillae; ligule 0.6 – 1.6 mm long. Panicle linear, 7.5 – 13 cm long. Spikelets 4 – 6-flowered, 7.8 – 9.4 mm long (minus awn), purple; lower glume lanceolate, 2.6 – 3.7 mm long, 1-nerved; upper glume lanceolate – oblong or lanceolate, 3.6 – 4.5 mm long, 3-nerved; second lemma 5-nerved, 5.2 – 6.8 mm long (minus awn), awn 1.9 – 4.8 mm long; palea keels scaberulous along the whole length; anthers 2.2 – 3 mm long; ovary glabrous. Caryopsis linear-elliptic, glabrous.

In grassland with sparse vegetation, often in poor sandy soil or shallow soil over rocks, 4140-4300 m.

A very rare endangered species endemic to Mt. Elgon, but it occurs on both the Ugandan and Kenyan side of the mountain.

Festuca pilgeri St. Yves in Notizbl. Bot. Gart. Berlin-Dahlem 9: 1130 (1927).

Type: Kenya, Mt. Kenya, 31 Jan. 1922, R. E. Fries 1316 (UPS lectotype; K! isolectotype).

Densely tufted, perennial with large tussocks. Culms 36 – 70(90) cm high. Leaf blades acicular, 10 – 30(45) cm long and 0.5 – 0.75 mm wide, stiffly erect or curly, harshly scabrid, unmarked by longitudinal striations on the outer surface; sclerenchyma almost a continuous

ring; vascular bundles 5; silica bodies crescent-shaped, semicircular or round; leaf epidermis with numerous prickles; ligule 0.4 – 1.3 mm long. Panicle linear, 8 – 20 cm long. Spikelets 3 – 6-flowered, oblong-elliptic, 8 – 12 mm long (minus awn), purple; lower glume lanceolate, 3 – 6 mm long, 1-nerved; upper glume lanceolate – oblong or lanceolate, 4 – 6.8 mm long, 3-nerved; lemmas minutely scaberulous, second lemma 5-nerved, 5.5 – 7.8 mm long (minus awn), awn 1 – 3.5 mm long; palea as long as lemma, the keels scaberulous up more than ¾ of the length from the tip; anthers 2.4 – 3.9 mm long; ovary glabrous. Caryopsis narrow elliptic, glabrous.

In grassland and moorland with sparse vegetation above the timberline, often in poor sandy soil or shallow soil over rocks, 3910-4100 m in Uganda, elsewhere 2700-4250 m.

An uncommon species endemic to the East African mountains; in Uganda from Mt. Elgon only; in Kenya from both Mt. Elgon and Mt. Kenya, and in Tanzania from the Kitulo Plateau.

Festuca africana (Hack.) Clayton in Kew Bull. 40: 727 (1985); *Brachelytrum africanus* Hack. in Bull. Herb. Boiss. 3: 382 (Aug 1895); *Pseudobromus africanus* (Hack.) Stapf in Dyer, Fl. Cap. 7: 763 (1900).

Type: South Africa, Transvaal, A. Rehmann 5732 (K! isotype).

Syn. *Pseudobromus silvaticus* K. Schum. in P. O. A. C: 108 (July 1895). Type: Tanzania, Kilimanjaro, G. L. A. Volkens 1285 (K! isotype).

Tufted perennial from a short knotty rhizome; culms 60 – 200 cm long. Leaf blades 20 – 43 cm long, 6 – 18 mm wide, with tranverse veinlets; vascular bundles 13 to 50; sclerenchyma supporting almost all vascular bundles on both the leaf underside and rib tops; silica bodies linear along veins; ligule 3.7 – 8.1(10.4) mm long. Panicle lax, spreading, 20.6 – 50 cm long. Spikelets 1(-2)-flowered with an additional awned sterile floret or rachilla-extension, green or rarely tinged purple, linear-oblong, 7 – 11(13) mm long; glumes lanceolate or oblong-lanceolate, the lower 1 – 3-nerved, 2 – 7.9 mm long, the upper 3-nerved, 3 – 8.2(9.6) mm long; lemmas oblong-elliptic or linear-lanceolate, 6 – 10 mm long, 3(5) nerved, awn terminal or subterminal, the tip then splitting into 2 filiform teeth 0.4 – 1.2 mm long; awn 7.2(9.3) – 20 mm long; palea 7.1 – 8.9(10.8) mm long; anthers 2.2 – 4.3(5.3) mm long; ovary hairy on top. Caryopsis elliptic to narrow elliptic, prominently hairy on top.

In shady habitats, often along margins of upland forests and bamboo thickets, very rarely reaching above the timberline, in Uganda usually from 2220-3000 m, but on Mt. Mgahinga I found this species at 3260 m (Namaganda 1407), and in Kenya I recorded it from a swamp forest at 1950 m (Namaganda 1727).

In Uganda it is a rare plant and grows in the lower parts of Mt. Elgon, the Virunga volcanoes and Rwenzori Mts. Elsewhere found scattered from the Sudan to South Africa, perhaps most abundant in Malawi.

Festuca simensis A. Rich. in Tent. Fl. Abyss. 2: 433 (1850).

Type: Ethiopia, Simen [Semien], G. H. W. Schimper 684 (P holotype, K! isotype).

Loosely tufted perennial with slender rhizomes. Culms slender, 40 – 160 cm long. Leaf blades 10 – 40 cm long, 2 – 7.5 mm wide, flat, with falcate auricles; vascular bundles 13 – 18; sclerenchyma supporting almost all vascular bundles on both the leaf underside and rib tops; silica bodies linear along veins; ligule 1 – 2.1(3.5) mm long. Panicle loosely ovate, 12 – 40 cm long.. Spikelets green tinged purple but sometimes without tinge, linear-oblong, 2 – 7-flowered, 1 – 2 cm long minus awn; lower glume lanceolate, 1(3)-nerved, 3 – 7 mm long, the upper lanceolate, 3-nerved, 4 – 7.6 mm long; lemmas oblong-elliptic or linear-lanceolate, 7 – 9.5 mm long, smooth or scaberulous, awned from 0.4 – 1.3 mm below the acute hyaline tip; awn 8 – 18.4 mm long; palea (6.6)7.2 – 9.6 mm long, keels scabrid; anthers 2.5 – 4.4 mm long; ovary glabrous. Caryopsis linear-elliptic, glabrous.

In shady habitats, often along margins of upland forests and bamboo thickets, sometimes on road verges, very rarely reaching above the timberline, usually at 1900-3100 m, but it was found up to about 3800 m on Mt. Kenya as well as on Mt. Elgon.

In Uganda it grows in the lower parts of all the higher mountain regions, i.e. Mt. Elgon, Rwenzori Mts. and the southwestern highlands. Elsewhere known from Kenya, Ethiopia, Sudan, Congo and Cameroun.

NOTES TO APPENDIX

Festuca engleri Pilger in Engl. Jahrb. 40: 85 (1907); *Pseudobromus engleri* (Pilg.) W. D. Clayton in Kew Bull. 23: 293 (1969). Type: Tanzania, West Usambara Mts., Magamba, E. Engler 1279 (B holotype).

This species was not reported from Uganda in "Flora of Tropical East Africa" (Clayton 1970), but during a search at the British Museum in London an originally wrongly identified plant of *F. engleri* was found. It was collected from the lower slopes of Mt. Rwenzori above Kasese in 1935. I searched for it unsuccessfully in this area in January 2007. In paper 3 this only Ugandan collection of *F. engleri* was included to compare with *F. africana*.

However, *F. engleri* is most probably at most an infraspecific taxon of *F. africana*. In the Kew herbarium *F. engleri* is now included in *F. africana*.

Festuca mekiste W. D. Clayton in Kew Bull. 23: 293 (1969). Type: Kenya, Mt. Elgon, A. Bogdan 5390 (K holotype, EA isotype).

This species is not known from Uganda. I searched for it unsuccessfully on the Ugandan side of Mt. Elgon, but in paper 3 two of my collections from the Kenyan side of Mt. Elgon was included to compare with *F. simensis*.

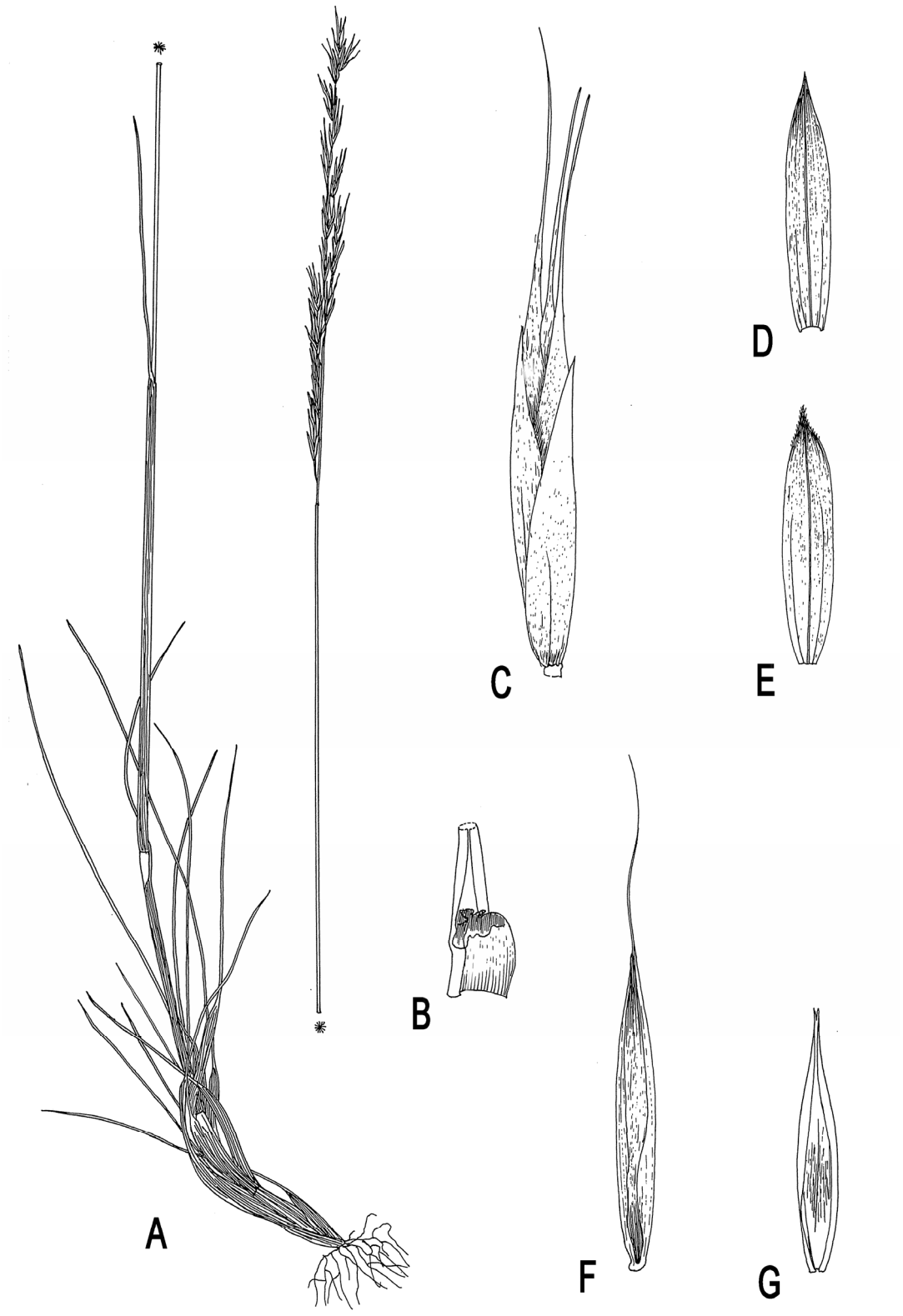

Figure 3. ***Festuca abyssinica*** A. Rich. A: Habit, ×½. B: Ligule, ×10. C: Spikelet, ×5. D: Lower glume, ×10. E: Upper glume, ×10. F: Lemma, ×10. G: Palea, ×10. Drawn by Janet Nabakooza from Namaganda 1670.

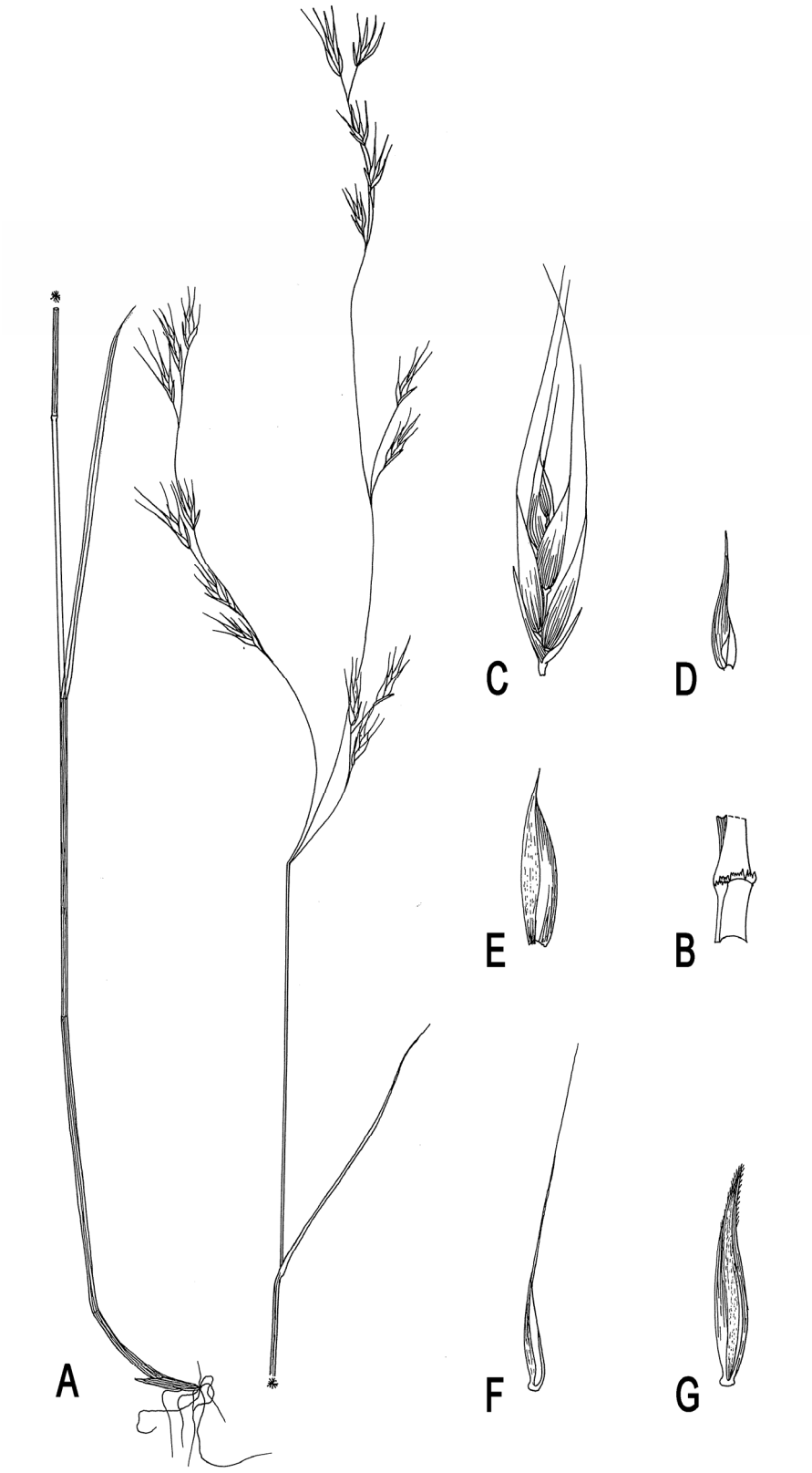

Figure 4. ***Festuca chodatiana*** (St. Yves) Alexeev. A: Habit, ×½. B: Ligule, ×10. C: Spikelet, ×5. D: Lower glume, ×10. E: Upper glume, ×10. F: Lemma, ×10. G: Palea, ×10. Drawn by Janet Nabakooza from Namaganda 1665.

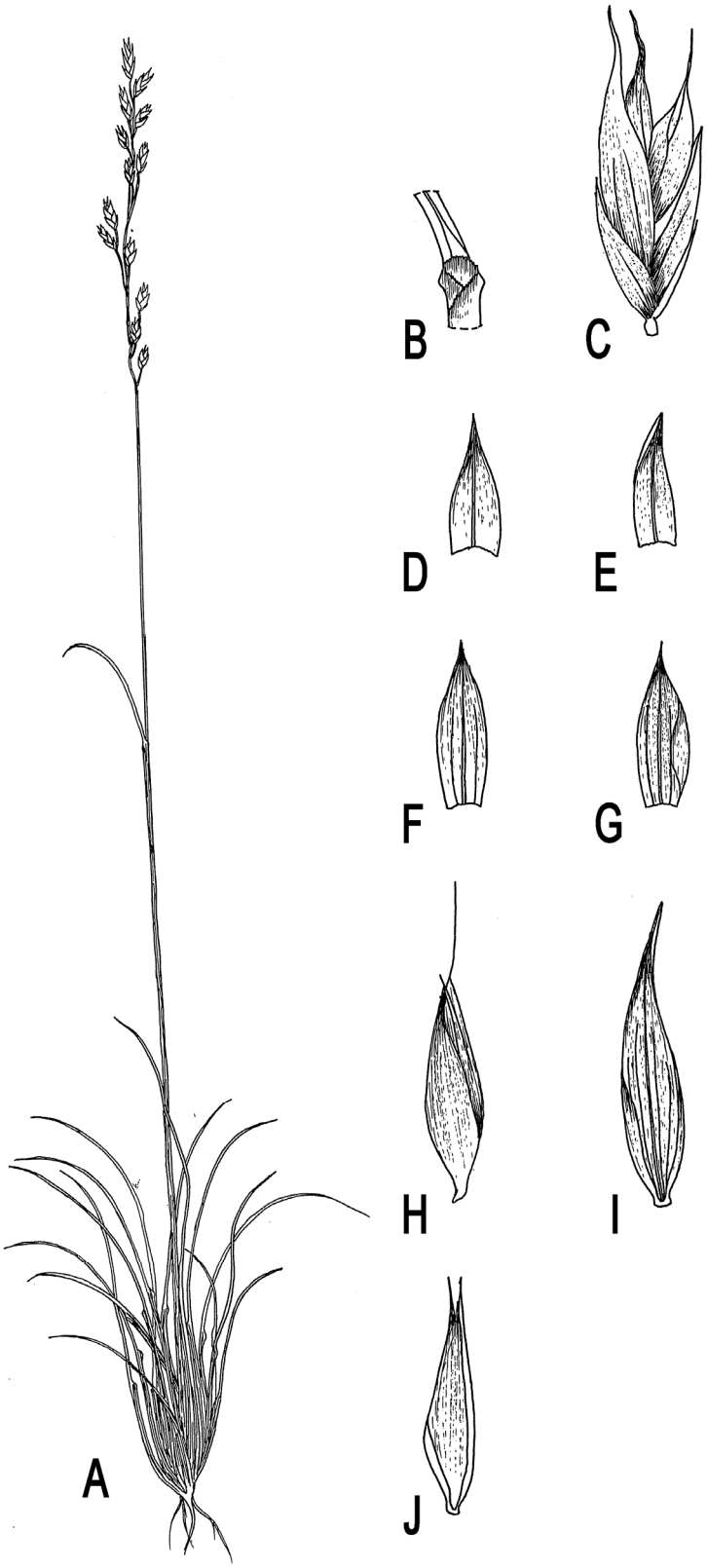

Figure 5. ***Festuca claytonii*** Alexeev. A: Habit, ×½. B: Ligule, ×12. C: Spikelet, ×5. D-E: Lower glume, ×8. F-G: Upper glume, ×8. H: Floret, ×5. I: Lemma, ×8. J: Palea, ×8. Drawn by Janet Nabakooza from Namaganda 1678.

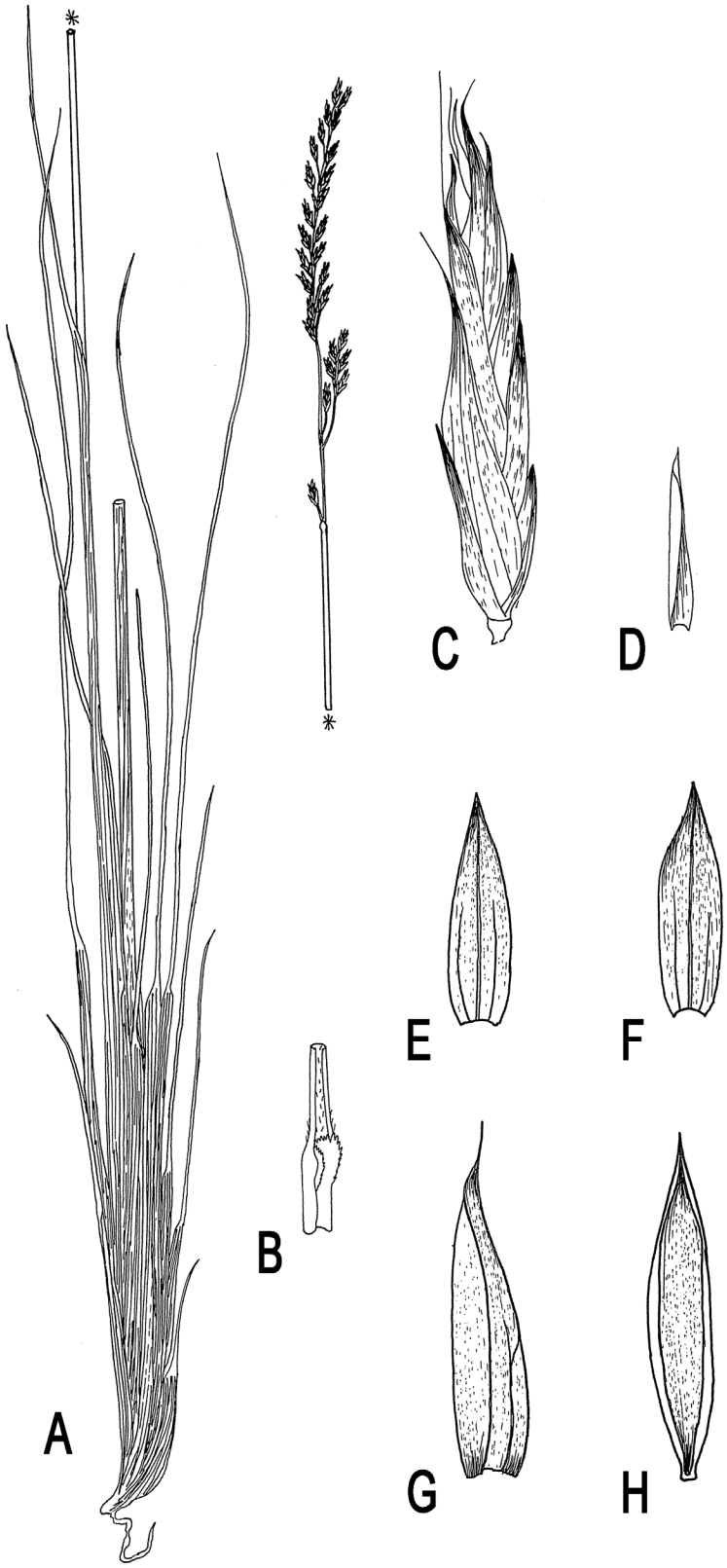

Figure 6. ***Festuca pilgeri*** St. Yves. A: Habit, ×½. B: Ligule, ×12. C: Spikelet, ×6. D: Lower glume, ×8. E-F: Upper glume, ×8. G: Lemma, ×8. H: Palea, ×8. Drawn by Janet Nabakooza from Namaganda 1675.

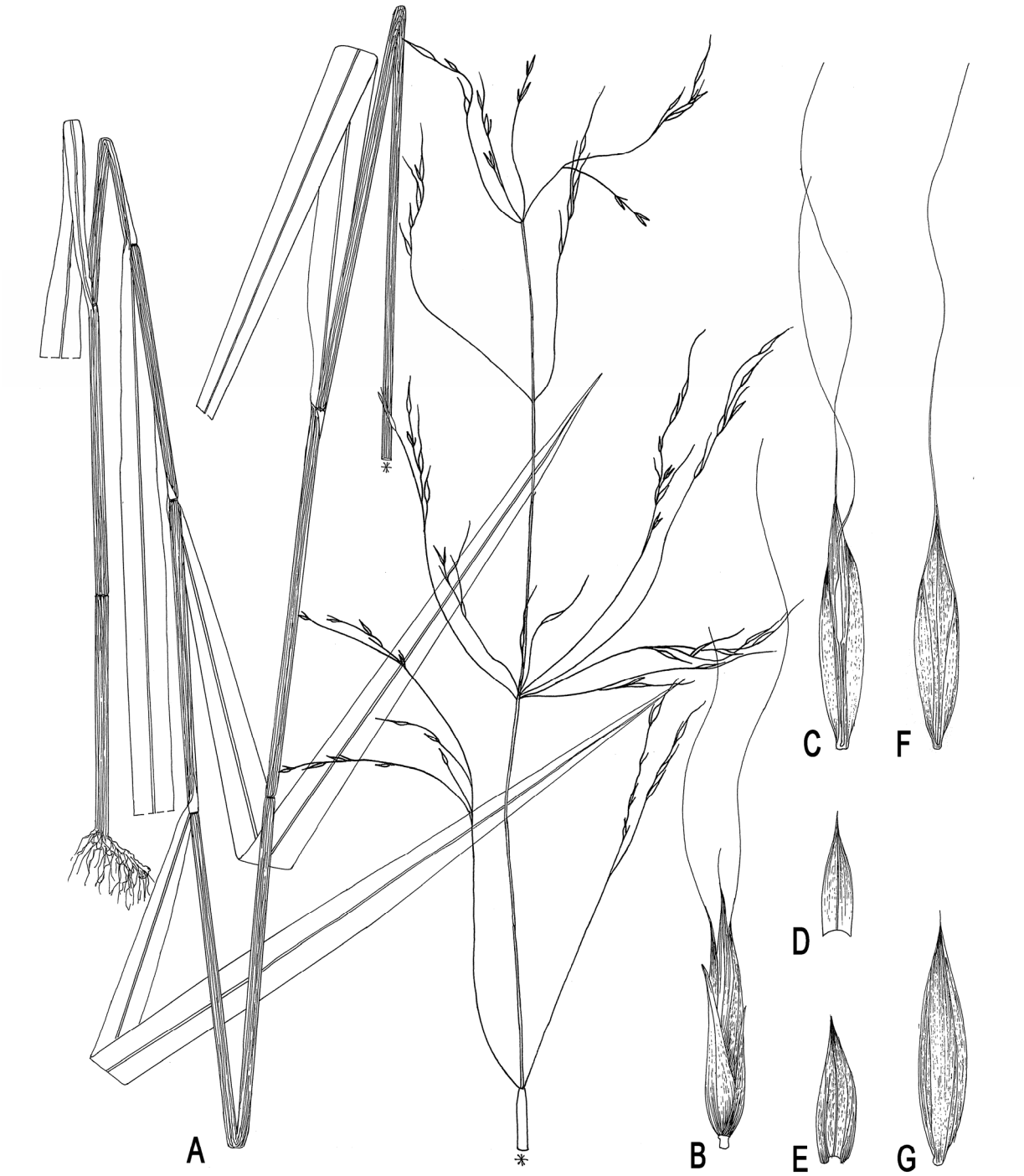

Figure 7. ***Festuca africana*** St. Yves. A: Habit, ×½. B: Spikelet, ×10. C: Spikelet without glumes, ×10. D: Lower glume, ×10. E: Upper glume, ×10. F: Lemma, ×10. G: Palea, ×10. Drawn by Janet Nabakooza from Namaganda 1688.

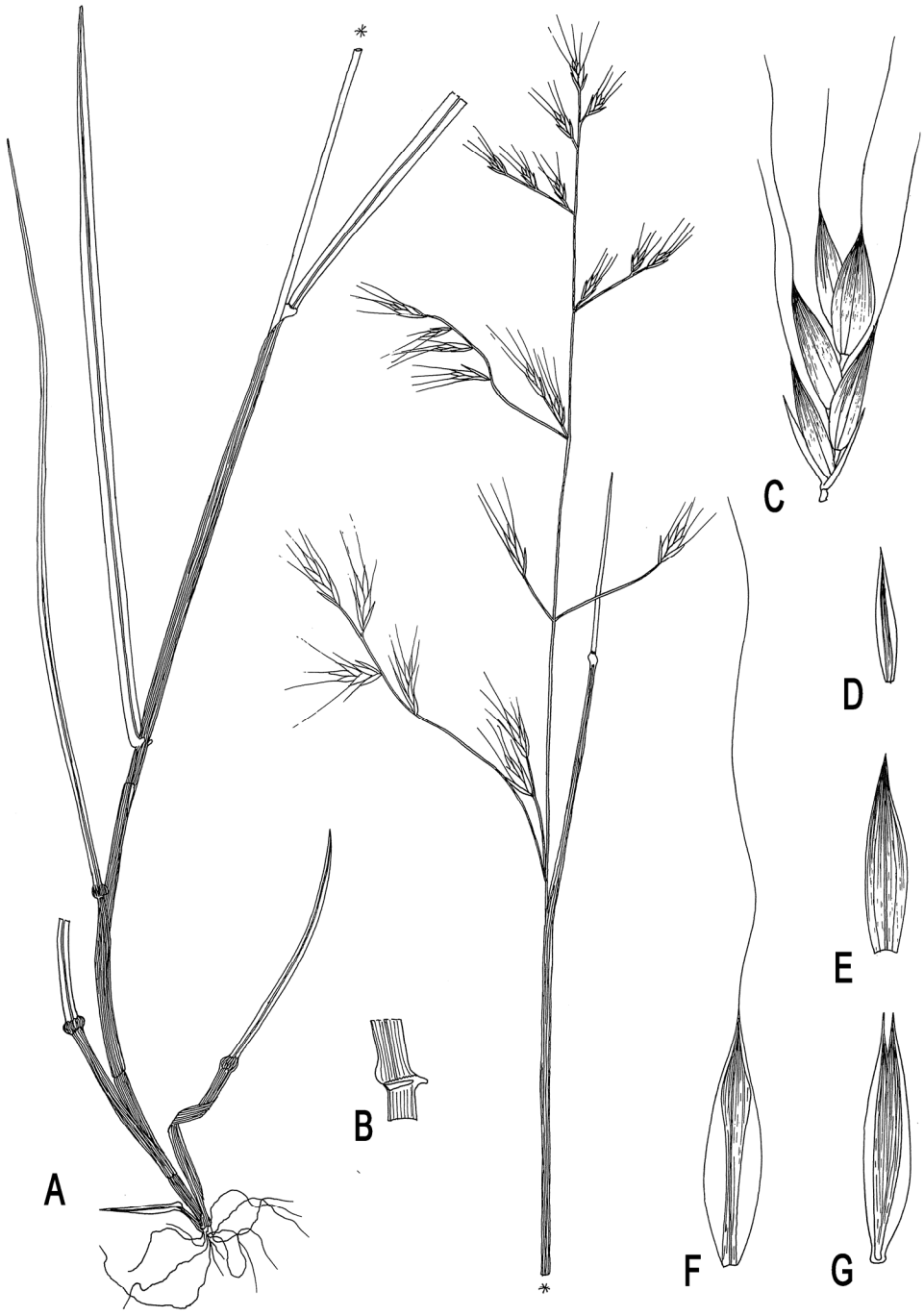

Figure 8. ***Festuca simensisi*** A. Rich. A: Habit, ×½. B: Ligule, ×5. C: Spikelet, ×5. D: Lower glume, ×5. E: Upper glume, ×5. F: Lemma, ×5. G: Palea, ×5. Drawn by Janet Nabakooza from Namaganda 1694.

Paper I

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ORIGINAL PAPER

AFLP-based differentiation of tropical African *Festuca* species compared to the European *Festuca* complex

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Abstract For the first time amplified fragment length polymorphism (AFLP) fingerprinting is applied to classify tropical African *Festuca* species. Five afro-alpine narrow- and two afro-montane broad-leaved species from Uganda and Ethiopia are compared to ten European grass species. A principal coordinate analysis (PCoA) accounts for 62.5% with its first three coordinates. The PCoA and the neighbor-joining (NJ) distinguish the five narrow-leaved African *Festuca* species from all other species. The broad-leaved African *Festuca africana* and *Festuca simensis* are linked to the broad-leaved European species through *Festuca altissima* and *Festuca gigantea*, respectively. The narrow- and broad-leaved European species are separated as expected in the NJ. One narrow-leaved African alpine species recently described appears merged (i.e. *Festuca richardii* with *Festuca abyssinica*). We provide chromosome numbers for all seven Ugandan species and compare taxonomy and AFLP classification. Our most striking result is that the narrow-leaved African *Festuca* species are unique and not clustering with the narrow-leaved European species.

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Introduction

Members of the genus *Festuca* L. (including *Lolium* L. and *Vulpia* C. C. Gmel.) are perhaps the most economically important forage grasses in temperate and cold regions. Some species are important fodder for wild as well as domestic animals, and others are widely sown in lawns. In tropical Africa, *Festuca* is important as forage for wild animals and for soil cover preventing erosion in the mountains. Eight *Festuca* species are known in Uganda; six are narrow-leaved (*Festuca abyssinica*, *Festuca chodatiana*, *Festuca claytonii*, *Festuca elgonensis*, *Festuca pilgeri* and *Festuca richardii*) and two are broad-leaved (*Festuca africana* and *Festuca simensis*). These fescues grow in the African mountains and highlands at an altitudinal range of 1,800–4,700 m above sea level (1,830–4,300 m in Uganda). *F. abyssinica* has the widest altitude distribution (2,130–4,700 m), but is most abundant above 3,000 m. *F. africana*, *F. chodatiana* and *F. simensis* mainly occur below 3,000 m, whereas *F. claytonii*, *F. elgonensis*, *F. richardii* and *F. pilgeri* grow above 3,000 m. *F. elgonensis* is very rare and was not found during our sampling seasons in 2003 and 2004. *F. claytonii* is also a rare species in Uganda and is only known from restricted areas on Mt. Elgon.

The genus *Festuca* is not only very polymorphic (Jenkin 1959), but it is also known to form hybrids with *Lolium*, *Vulpia* and possibly *Bromus* (Clayton and Renvoize 1986; Watson and Dallwitz 1992). Some species such as *F. abyssinica* show a very large variation in a variety of traits (Launert 1971), making the definition of species boundaries based on morphology very difficult to determine. Traditionally, the taxonomy of *Festuca* is largely based on morphology and anatomy

and to a lesser degree on cytogenetics. Moreover, morphologically very similar plants with different ploidy levels have usually not been distinguished as separate taxa, such as in *F. rubra*. To date, chromosome numbers in tropical African fescues have been established for only a few species.

The systematics and phylogeny of *Festuca* have been described using DNA markers such as random amplified polymorphic DNA (Charmet et al. 1997; Fjellheim et al. 2001), restriction fragment length polymorphisms (Xu and Sleper 1994; Charmet et al. 1997), variation of internal transcribed spacers (Gaut et al. 2000; Torrecilla et al. 2003; Catalan et al. 2004) and restriction site variation of chloroplast DNA (Darbyshire and Warwick 1992). Amplified fragment length polymorphisms (AFLPs; Vos et al. 1995) have also been used in *Festuca* diversity studies, breeding and genome mapping (Mian et al. 2002, 2005; Skibinska et al. 2002; Alm et al. 2003; Saha et al. 2005; Fjellheim and Rognli 2005a, b). However, the molecular studies have only concentrated on temperate, arctic and Mediterranean members of the genus, such as *F. ovina*, *F. pratensis* and *F. rubra*. None of the tropical African species have been investigated by DNA fingerprinting. We therefore focus on Ugandan material and describe the AFLP-based classification of seven Ugandan *Festuca* species in comparison with selected temperate species and some Ethiopian counterparts. We also provide chromosome numbers for seven African species; confirming Hedberg's (1957) counts for *F. abyssinica* and *F. pilgeri* whereas numbers for the remaining five species are reported here for the first time.

Materials and methods

Sampling procedures

Samples were collected from the mountains and highlands in eastern (Mt. Elgon), western (Rwenzori mountains) and southwestern (Mt. Gahinga, Mt. Muhavura, Echuya swamp and Bwindi forest) Uganda during 2003 and 2004. The sampled regions cover the known distribution of *Festuca* in Uganda. Three species were collected from the Semien Mountains in Ethiopia, nine from Norway and one from Denmark (Table 1).

The youngest leaves of individual plants (assumed to be free of fungal endophytes) were collected in the field and dried using silica gel. Three to five samples were collected from each population, ensuring spatial separation of at least 3 m between the samples when possible. Sample populations were chosen at different altitudes following transects up the mountains. Fifty-seven populations were sampled from Uganda and Ethiopia, and 67 populations were sampled in total. The European species served as controls for the African *Festuca* species. Narrow-leaved controls included accessions of *F. ovina* (type species of genus *Festuca*), *F. rubra*, *F. vivipara*, *Vulpia bromoides* and *V. myuros*. The broad-leaved controls included *F. altissima*, *F. arundinacea*, *F. gigantea*, *F. pratensis* and *Lolium multiflorum*. Herbarium vouchers were also collected and are kept at the Makerere University Herbarium (MHU; Table 1). Species identifications were made by the first author in the field and verified based on material at MHU and Kew Herbarium, on the floras ('Flora of Tropical East Africa and Flora of Ethiopia and Eritrea') and on Alexeev's taxonomy (Alexeev 1986, 1987). Sylvia Phillips (Kew) helped with some of the identifications.

DNA extraction and AFLP procedure

Silica-dried leaves were ground into a fine powder, and DNA was extracted using a DNeasy Plant Mini Kit (QIAGEN Inc., Valencia, CA, USA). The quality and quantity of the undigested DNA was checked on 0.8% agarose gels together with λ DNA. A total of 167 accessions representing 17 species (Table 1) were included in the final analysis. The AFLP fingerprinting procedure followed the original procedures of Vos et al. (1995) with minor modifications described by Becker et al. (1995). In short, 400 ng DNA per sample was digested with 5 U *Eco*RI and 5 U *Mse*I for 2 h at 37 °C. *Eco*RI (GTC GTA GAC TGC GTA CC/AAT TGG TAC GCA GTC), and *Mse*I (GAC GAT GAG TCC TGA G/TAC TCA GGA CTC AT) adapters were ligated to the obtained DNA fragments during an incubation period of 3 h at 37 °C. The obtained restriction/ligation products were selectively PCR pre-amplified with the primers E01 (GAC TGC GTA CCA ATT CA) and M01 (GAT GAG TCC TGA GTA AA) at the temperature–time profile given by Vos et al. (1995). The resulting preamplification products were checked on 1% agarose gels and diluted 100-fold prior to the selective +3/+3 PCR amplification. The E-primers were labeled with radioactive γ-[^{33}P] before being used in the selective amplification, which followed the PCR profiles described by Becker et al. (1995). An equal volume of loading buffer (99% formamide, 10 mM EDTA pH 8, 1 mg/ml xylene cyanol FF, 1 mg/ml bromophenol blue) was added to the final PCR products, and the mix was denatured at 94 °C for 10 min and immediately put on ice. A 3 μl of the mix was loaded on 5% polyacrylamide (PAGE)

Table 1 Species used in this study

Species	Chromosome number	Lab number	Herbarium number	Collection locality	Altitude [a]	Coordinates	Country
F. abyssinica A. Rich.	$2n = 4x = 28$[b,c]	058E	1369	Elgon, Wagagai peak	4300	01°07.5′N, 34°31.5′E	Uganda
		071E, 072E	1374	Elgon, in the caldera	3870	01°09.0′N, 34°33.3′E	Uganda
		073E, 074E, 075E	1376*	Elgon, hot springs	3560		Uganda
		076E	1377	Elgon, in the caldera	3770		Uganda
		226E, 227E, 228E	1566	Elgon, bamboo forest	3050	01°10.6′N, 34°27.0′E	Uganda
		278E, 279E, 281E	1576	Elgon, Mongongo cave	3780	01°09.3′N, 34°30.8′E	Uganda
		290E	1581	Elgon, Piswa, Sasa trail	3750	01°09.0′N, 34°30.0′E	Uganda
		291E, 292E	1582	Elgon, Piswa, Sasa trail	3740	01°09.0′N, 34°30.0′E	Uganda
		296E, 297E, 300E	1583	Elgon, Mude camp	3470	01°10.0′N, 34°29.3′E	Uganda
		–	1660*	Elgon, above Sasa R. camp	2980	01°10.6′N, 34°26.9′E	Uganda
		–	1662*	Elgon, 1.5 km E of Sasa camp	2990	01°10.6′N, 34°26.9′E	Uganda
		–	1663*	Elgon, Sasa trail, Kimoro	3050	01°10.6′N, 34°27.0′E	Uganda
		–	1670*	Elgon, Mude camp	3470	01°10.0′N, 34°29.3′E	Uganda
		113Vg, 114Vg	1406	Gahinga, montane forest	3070	01°22.9′S, 29°38.5′E	Uganda
		119Vg	1409	Gahinga, top	3470	01°23.0′S, 29°38.7′E	Uganda
		120Vg, 121Vg, 122Vg	1410	Gahinga, crater swamp	3460	01°23.0′S, 29°38.7′E	Uganda
		123Vg	1411	Gahinga, top	3470	01°23.0′S, 29°38.7′E	Uganda
		130Vg	1414	Gahinga, top	3470	01°23.0′S, 29°38.7′E	Uganda
		191Vg, 192Vg, 193Vg	1604	Gahinga, top	3470	01°23.0′S, 29°38.7′E	Uganda
		195Vg, 196Vg, 198Vg	1605	Gahinga, crater swamp	3460	01°23.0′S, 29°38.7′E	Uganda
		199Vg, 200Vg, 201Vg	1606	Gahinga, crater swamp	3460	01°23.0′S, 29°38.7′E	Uganda
		2*	–	Gahinga, top	3470	01°23.0′S, 29°38.7′E	Uganda
		146Vm, 147Vm, 148Vm	1592	Muhavura, forest	3080	01°22.3′S, 29°40.2′E	Uganda
		149Vm, 150Vm, 151Vm	1593	Muhavura, ericaceous zone	3800	01°22.0′S, 29°39.0′E	Uganda
		156Vm, 157Vm, 163Vm	1594	Muhavura, ericaceous zone	3800	01°22.0′S, 29°39.0′E	Uganda
		334T, 335T, 336T	1512	Semien Mts., Chenek	3530	13°15.0′N, 38°11.0′E	Ethiopia
F. chodatiana (St. Yves) Alexeev	$2n = 4x = 28$[b]	230E, 231E, 232E	1567	Elgon, bamboo forest	3070	01°10.6′N, 34°27.6′E	Uganda
		301E, 302E, 305E	1584	Elgon, Sasa River camp	2830	01°10.3′N, 34°26.3′E	Uganda
		322E, 323E, 324E	1587	Elgon, montane forest	2710	01°10.4′N, 34°25.4′E	Uganda
		–	1671*	Elgon, Mude camp	3470	01°10.0′N, 34°29.3′E	Uganda
		–	1687*	Elgon, Sasa trail	2750	01°10.5′N, 34°25.5′E	Uganda
		–	1689*	Elgon, Sasa trail	2600	01°10.5′N, 34°25.5′E	Uganda
		–	1695*	Elgon, Sasa trail	1770	01°10.5′N, 34°23.5′E	Uganda
		169Vg, 171Vg, 173Vg	1600	Gahinga, regenerating forest	2405	01°21.6′S, 29°37.4′E	Uganda
		182Vg, 183Vg, 184Vg	1602	Gahinga, regenerating forest	2610	01°22.0′S, 29°37.9′E	Uganda
		–	1696*	Gahinga, regenerating forest	2500	01°21.5′S, 29°37.0′E	Uganda
		137Vm, 138Vm, 139Vm	1590	Muhavura, regenerating forest	2620	01°21.8′S, 29°39.8′E	Uganda
		167Vm, 168Vm	1595	Muhavura, regenerating forest	2500	01°21.6′S, 29°39.8′E	Uganda
		210K, 211K, 212K	1609	Echuya, Kisoro, Kabale boarder	2280	01°15.4′S, 29°47.8′E	Uganda
		214B, 215B, 216B	1610	Bwindi forest, Ndeego	2220	01°06.5′S, 29°48.5′E	Uganda
		–	1717*	Bwindi forest, Ndeego	2220	01°06.5′S, 29°48.5′E	Uganda
F. claytonii Alexeev	$2n = 4x = 28$[b]	271E, 274E, 275E	1575*	Elgon, towards Wagagai	4140	01°08.7′N, 34°31.4′E	Uganda
		–	1678*	Elgon, towards Wagagai	4140	01°08.7′N, 34°31.4′E	Uganda

Table 1 continued

Species	Chromosome number	Lab number	Herbarium number	Collection locality	Altitude [a]	Coordinates	Country
F. pilgeri St. Yves	$2n = 4x = 28$[b,c]	033E, 034E	1365	Elgon, above Jackson's pool	4100	01°08.6′N, 34°31.3′E	Uganda
		255E, 256E, 258E	1572	Elgon, near Jackson's pool	3910	01°08.9′N, 34°30.6′E	Uganda
		264E, 265E, 267E	1574	Elgon, above Jackson's pool	4010	01°08.7′N, 34°31.0′E	Uganda
		–	1675*	Elgon, above Jackson's pool	4000	01°08.7′N, 34°31.0′E	Uganda
F. richardii Alexeev	$2n = 4x = 28$[b]	020E, 021E, 022E	1359	Elgon, above Mude camp	3900	01°08.9′N, 34°30.6′E	Uganda
		023E	1361	Elgon, above Jackson's pool	3965	01°08.8′N, 34°30.8′E	Uganda
		239E, 241E, 242E	1569*	Elgon, alpine grassland	3310	01°10.4′N, 34°28.3′E	Uganda
		244E, 245E, 248E	1570	Elgon, alpine grassland	3470	01°10.0′N, 34°29.0′E	Uganda
		249E, 250E, 251E	1571*	Elgon, alpine grassland	3730	01°09.5′N, 34°30.0′E	Uganda
		259E, 260E, 261E	1573*	Elgon, above Jackson's pool	4010	01°08.7′N, 34°31.0′E	Uganda
		288E	1579	Elgon, Piswa, Sasa trail	3750	01°09.0′N, 34°30.0′E	Uganda
		289E	1580	Elgon, Piswa, Sasa trail	3750	01°09.0′N, 34°30.0′E	Uganda
		–	1674*	Elgon, above Jackson's pool	4000	01°08.7′N, 34°31.0′E	Uganda
F. africana (Hack.) Clayton	$2n = 10x = 70$[b]	309E, 310E, 313E	1585	Elgon, Sasa River camp	2830	01°10.3′N, 34°26.3′E	Uganda
		186Vg, 188Vg, 190Vg	1603	Gahinga, *Hypericum* forest	3170	01°22.9′S, 29°38.5′E	Uganda
		219B, 220B, 222B	1612	Bwindi forest	2230	01°05.6′S, 29°48.7′E	Uganda
		089R	1394	Rwenzori Mts., Bwamba Pass	2430	01°40.0′N, 30°08.0′E	Uganda
		090R, 091R, 092R	1395	Rwenzori Mts., Bwamba Pass	2600		Uganda
		af2*	–	Elgon, Sasa trail	2500	01°10.5′N, 34°25.0′E	Uganda
		af3*, af4*	–	Bwindi, along forest road	2550	01°05.5′S, 29°48.0′E	Uganda
F. simensis A. Rich.	$2n = 4x = 28$[b]	234E, 235E, 237E	1568	Elgon, bamboo forest	3210	01°10.5′N, 34°27.9′E	Uganda
		282E, 283E, 286E	1577*	Elgon, Mongongo cave	3780	01°09.3′N, 34°30.8′E	Uganda
		317E, 318E, 319E	1586	Elgon, Sasa River camp	2830	01°10.3′N, 34°26.3′E	Uganda
		–	1664*	Elgon, Sasa trail	2800	01°10.0′N, 34°26.0′E	Uganda
		174Vg, 176Vg, 177Vg	1601	Gahinga, regenerating forest	2490	01°21.7′S, 29°37.7′E	Uganda
		131Vm, 133Vm, 134Vm	1589	Muhavura regenerating forest	2530	01°21.6′S, 29°39.8′E	Uganda
		141Vm, 142Vm, 143Vm	1591	Muhavura, montane forest	2990	01°22.2′S, 29°40.1′E	Uganda
		–	1698*	Muhavura regenerating forest	2330	01°21.5′S, 29°39.5′E	Uganda
		204K, 206K, 207K	1608	Echuya, Kabale, Kisoro boarder	2280	01°15.4′S, 29°47.8′E	Uganda
		096B, 097B, 098B	1398	Bwindi forest, Ndeego	2550	01°05.5′S, 29°48.5′E	Uganda
		–	1715*	Bwindi, along forest road	2550	01°05.5′S, 29°48.0′E	Uganda
		088R	1390	Rwenzori Mts., Bwamba Pass	2100		Uganda
		331T, 332T, 333T	1511	Semien Mts., Chenek	3530	13°15.0′S, 38°11.0′E	Ethiopia
F. ovina L.	$2n = 2x = 14$[d], $2n = 4x = 28$[e]	342N, 343N	1500	Besstrond	980		Norway
F. rubra L.	$2n = 6x = 42$[d]	345N, 346N	1501	Moss	0.5		Norway
F. vivipara L.	$2n = 3x = 21$[f], $2n = 4x = 28$[f]	360N, 362N	1506	Besstrond	1020		Norway
F. altissima All.	$2n = 2x = 14$[g]	357N, 359N	1505	Moss	120		Norway
F. arundinacea Schreber	$2n = 6x = 42$[h]	363N, 364N	1507	Rygge	0.5		Norway
F. gigantea (L.) Vill.	$2n = 6x = 42$[g]	352N, 353N	1503	Asker	150		Norway
F. pratensis Hudson	$2n = 2x = 14$[h]	354N, 355N	1504	Ås, Breivoll	0.5		Norway
Lolium multiflorum Lam.	$2n = 2x = 14$[i]	348N, 350N	1502	Ås	95		Norway
Vulpia bromoides (L.) S. F. Gray	$2n = 2x = 14$[j]	337N, 338N	1499	Rennesøy	5		Norway

Table 1 continued

Species	Chromosome number	Lab number	Herbarium number	Collection locality	Altitude [a]	Coordinates	Country
V. myuros (L.) C. C. Gmelin	$2n = 6x = 42$[k]	328T, 329T, 330T	1510	Semien Mts., Chenek	3530		Ethiopia
		340D, 341D	27761	Himmelbjerget	120		Denmark

Accessions marked with *asterisk* (*) were used for chromosome counting, and those without lab numbers were not used in the AFLP analysis. All accessions with lab numbers were used in the AFLP analysis. Voucher specimens are kept at the Makerere University Herbarium (MHU)

[a] Meters above sea level

[b] Reported in this study

[c] Hedberg (1957)

[d] Arohonka (1982)

[e] Malakhova and Markova (1994)

[f] Salvesen (1986)

[g] Lövkvist and Hultgård (1999)

[h] Jenczewski and Alix (2004)

[i] Spies et al. (1999)

[j] Morton (1993)

[k] Barker and Stace (1984)

gels. Electrophoresis was at 80 W for 1.5 h, with one and two times TBE in the upper and lower chambers, respectively. The gels were fixed in 10% acetic acid for 30 min, dried and exposed to X-ray film for 26–75 h depending on radiation intensity and then developed with an AGFA Curix 60. Five primer combinations were used: (1) E34/M37 (*Eco*RI+AAT/*Mse*I+ACG), (2) E39/M37 (*Eco*RI+AGA/*Mse*I+ACG), (3) E35/M36 (*Eco*RI+ACA/*Mse*I+ACC), (4) E37/M40 (*Eco*RI+ACG/*Mse*I+AGC) and (5) E37/M38 (*Eco*RI+ACG/*Mse*I+ACT).

Data analysis

AFLP fragments were manually scored as present (1) or absent (0). Because a high number of markers were generated per gel, only the clearest AFLPs were scored. Because of the selective amplification (Vos et al. 1995), each primer combination yields a unique, independent and randomly distributed set of fragments. Thus, each primer combination is an independent replication, and when these give similar results (as observed here), they can be merged and seen as a solid basis for a phenetic DNA marker-based analysis. Spreadsheet data matrices were prepared for each of the five primer combinations from which similarity matrices were computed using DICE derived distances. The five distance matrices obtained and the combined data set were analyzed by principal coordinate analysis (PCoA) using NTSYSpc 2.11f (Rohlf 2000). Neighbor-joining (NJ, Saitou and Nei 1987) and bootstrapping (1000 times) was done with TREECON (Van de Peer and De Wachter 1994). Normalized Mantel tests (Mantel 1967) were computed with the MxComp option of NTSYSpc.

Squash preparations and chromosome counts

Seeds collected from the same plants/populations sampled for leaf material or from nearby populations were germinated in Petri dishes at room temperature for 5–11 days, depending on the species. When about 1–1.5 cm long, the roots were cut and pretreated in ice water for 24 h. The pretreated roots were then fixed in fresh Carnoy's solution I (one acetic acid:three absolute ethanol) for about 3 days at room temperature and then stored at 4 °C. The roots were stained in 1% acetocarmine for at least 15 min and then heated until the acetocarmine started to boil. Using a razor blade, the root tip was cut off, placed in a drop of 1% acetocarmine on a glass slide, covered with a cover slip and the material was gently loosened by taping on the cover slip using a lancet needle. The loosened material

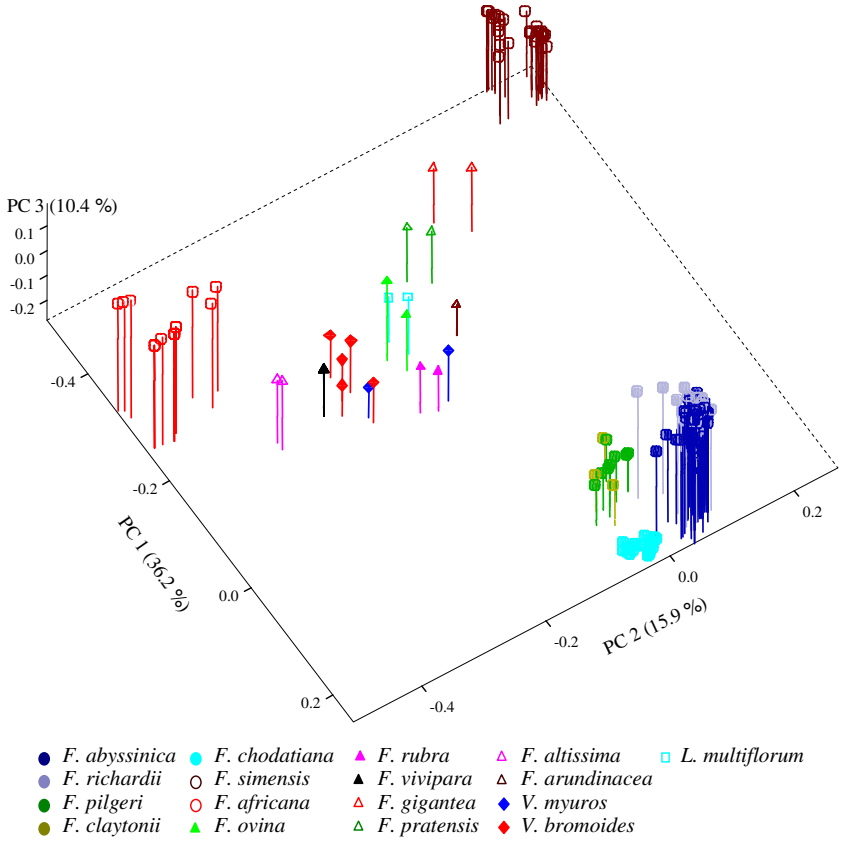

Fig. 1 Principal coordinate analysis of 167 *Festuca* accessions derived from 616 polymorph AFLPs. *Rings* represent the African fescues; *filled rings* are the narrow-leaved African fescues whereas the *empty rings* are the broad-leaved species. *Triangles* represent the European fescues; *filled triangles* are the narrow-leaved European fescues whereas the *empty triangles* are the broad-leaved species. *Diamonds* represent *Vulpia* species and squares represent *Lolium*. The first three coordinates (PC1, PC2 and PC3) account for a cumulative value of 62.5%

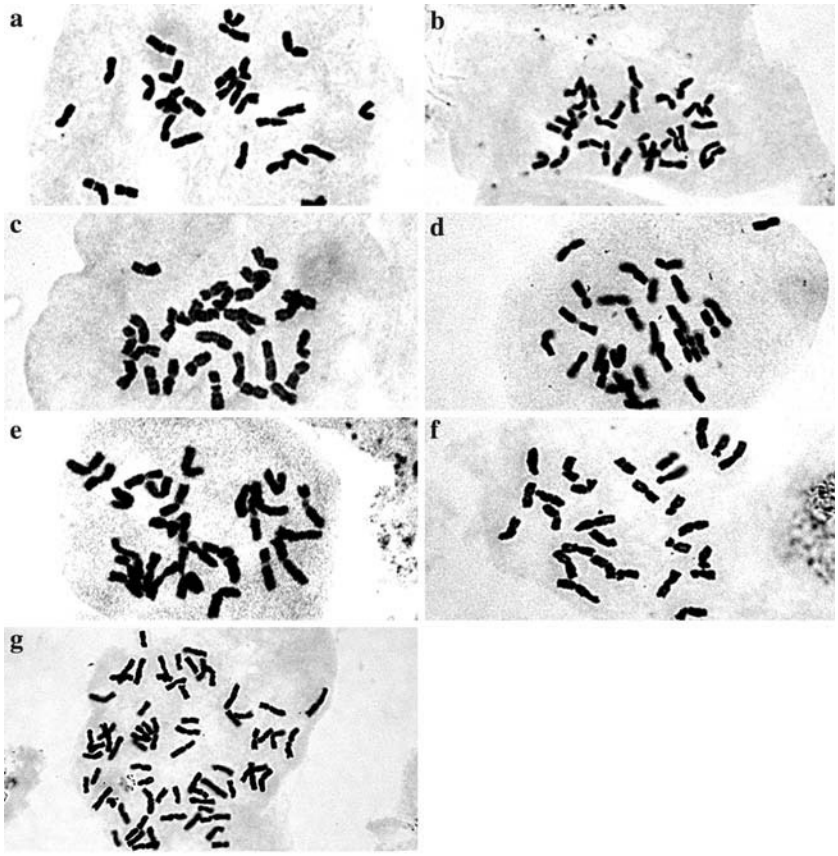

Fig. 2 Mitotic metaphase chromosomes stained with acetocarmine: **a** *Festuca abyssinica*; **b** *F. richardii*; **c** *F. pilgeri*; **d** *F. claytonii*; **e** *F. chodatiana*; **f** *F. simensis*; **g** *F. africana*

was heated to a point just below boiling and quickly squashed. It was generally difficult to get good chromosome preparations; hard squashing was found to be best. Mitotic metaphase chromosomes were viewed and counted under a phase-contrast microscope. Photographs were taken using a SPOT CCD camera and enhanced using Adobe Photoshop version 7. Ten roots were counted per accession analyzed (Table 1).

Results

The field collection was done in two consecutive years and targeted to get representative samples of all eight reported species from Uganda. The number of accessions for the individual species was variable due to the unequal frequency of occurrence of the species in the field. We therefore obtained few accessions for the rare East African endemics (*F. claytonii* and *F. pilgeri*). The endemic *F. elgonensis*, the eighth species, was not found. Table 1 shows the accessions of the species used for DNA analysis.

Each of the five AFLP primer combinations gave more than 100 polymorphic bands (i.e. 124 AFLPs with primer combination E34/M37, 119 with E39/M37, 116 with E35/M36, 122 with E37/M40 and 135 with E37/M38) and resulted in almost identical NJs and PCoAs (not shown). Mantel tests gave values ≥0.9 for the underlying data matrices (not shown). Therefore, the primer combinations were merged, and the resulting data set comprises 616 AFLPs and 167 accessions.

The PCoA obtained is shown in Fig. 1. Principal coordinate (PC) 1 accounts for 36.2% and mostly separates the narrow-leaved African species (represented by 103 accessions) from the remaining 64 accessions. PC 2 and 3 (accounting for 15.9 and 10.4%, respectively) provide further differentiation: the narrow-leaved African species with broad enveloping glumes about 3/4 as long as the spikelet, i.e. *F. abyssinica* and *F. richardii*, are intermixed and separate from the rest of the narrow-leaved African species that have short and narrow glumes only up to 1/2 the spikelet length. The later category of the narrow-leaved African species divides into species with acicular leaves (*F. claytonii* and *F. pilgeri*, which are intermixed) versus *F. chodatiana* with flat or folded leaves. These African narrow-leaved species are tetraploid (Fig. 2). The decaploid (see Fig. 2) *F. africana* and the tetraploid (see Fig. 2) *F. simensis* are also distinct. The ten European control species (each represented by two accessions, except for *Vulpia bromoides*, which is represented by five) are spread in the centre.

An NJ tree (based on individual accessions due to the unequal number of accessions per species) was done (Fig. 3) and the main differentiation separating narrow-leaved African species from the remaining species is again obtained and supported by a bootstrap value of 100%. Also, among European species, *F. gigantea* is the closest to the African *F. simensis* and *F. altissima* is the closest to the African *F. africana*. All broad-leaved species of *Festuca* (including *Lolium*) appear together on the NJ tree (Fig. 3), yet are separated from each other by bootstrap values of 100%. The same holds true for the European narrow-leaved species of *Festuca* (including *Vulpia*). A distinction between all *F. claytonii* versus all *F. pilgeri accessions* is obtained (bootstrap value 100%) but not for all *F. abyssinica* versus *F. richardii*.

Discussion

African fescue diversity

This being the first analysis of tropical African fescues using DNA markers, we anticipated results similar to those from earlier molecular investigations of European fescues, which support the old classical morpho-anatomical separation into a 'narrow-leaved' and a 'broad-leaved' clade (Charmet et al. 1997; Gaut et al. 2000; Fjellheim et al. 2001; Torrecilla and Catalan 2002; Torrecilla et al. 2003, 2004; Catalan et al. 2004). We found that the narrow-leaved African species are well separated from the broad-leaved African species, but not merged with the narrow-leaved European species. In fact, the first PC alone clearly separated the narrow-leaved African species from the remaining species. This wide separation of the narrow-leaved African species should be addressed from a taxonomic point of view with respect to the hierarchical placement of this group within the genus *Festuca*. The broad-leaved African *F. africana* and *F. simensis* are also distinct from the European fescues, but they are to some degree linked to the broad-leaved European species through *F. altissima* and *F. gigantea* (see below).

Morphology versus DNA classification

The taxonomic distinction (as described in the 'Flora of Tropical East Africa') between *F. abyssinica*, *F. chodatiana* and *F. pilgeri* is in full accordance with the distinction obtained by AFLP fingerprinting. All three species are tetraploid.

F. richardii (also $2n = 2x = 28$, reported here), a species recently separated from *F. abyssinica* by Alexeev (1987), is intermixed with *F. abyssinica* in the DNA analyses. The two species mostly show overlapping morphological traits in important characters, such as panicle shape, spikelet size and shape, and size and texture of the glumes. Distinctions are noted in habit (culms decumbent in *F. abyssinica* and erect in *F. richardii*) and leaf structure (1–3 mm wide, involute, filiform or acicular in *F. abyssinica*, and about 0.5 mm wide, conduplicate and acicular in *F. richardii*). Phillips (1995) described *F. richardii* as ''a high mountain segregate from the *F. abyssinica* complex, whose specific status rests mainly on its slightly different leaf-anatomy.'' The distribution of the sclerenchyma tissue is emphasized by taxonomists to support this species distinction, but the results presented by Alexeev (1986, 1987) and Phillips (1995) are conflicting. We suggest following our AFLP data and re-merging *F. richardii* with *F. abyssinica*.

The second incongruence with Alexeev occurs when the AFLPs do not separate *F. claytonii* (also $2n = 2x = 28$) and *F. pilgeri* with the PCoA. However, our NJ indicated such AFLP differentiation (Fig. 3). Again, the two species are mostly similar morphologically but can also be separated. *F. claytonii* is a generally a small plant with soft leaves and an open panicle, whereas *F. pilgeri* is a bigger plant with stiff leaves and a contracted panicle. Whether these morphological differences justify species distinction is uncertain, but since the NJ split of these two species is well supported by bootstrapping and since we have preliminary cytogenetic data (M. Namaganda et al. unpublished) indicating some differentiation, we conclude that Alexeev's classification is justified.

The broad-leaved African fescues *F. simensis* and *F. africana* are more similar to the European species of *Festuca*, *Lolium* and *Vulpia* than they are to the narrow-leaved African fescues. *F. simensis* is most closely related to the European *F. gigantea*, and this

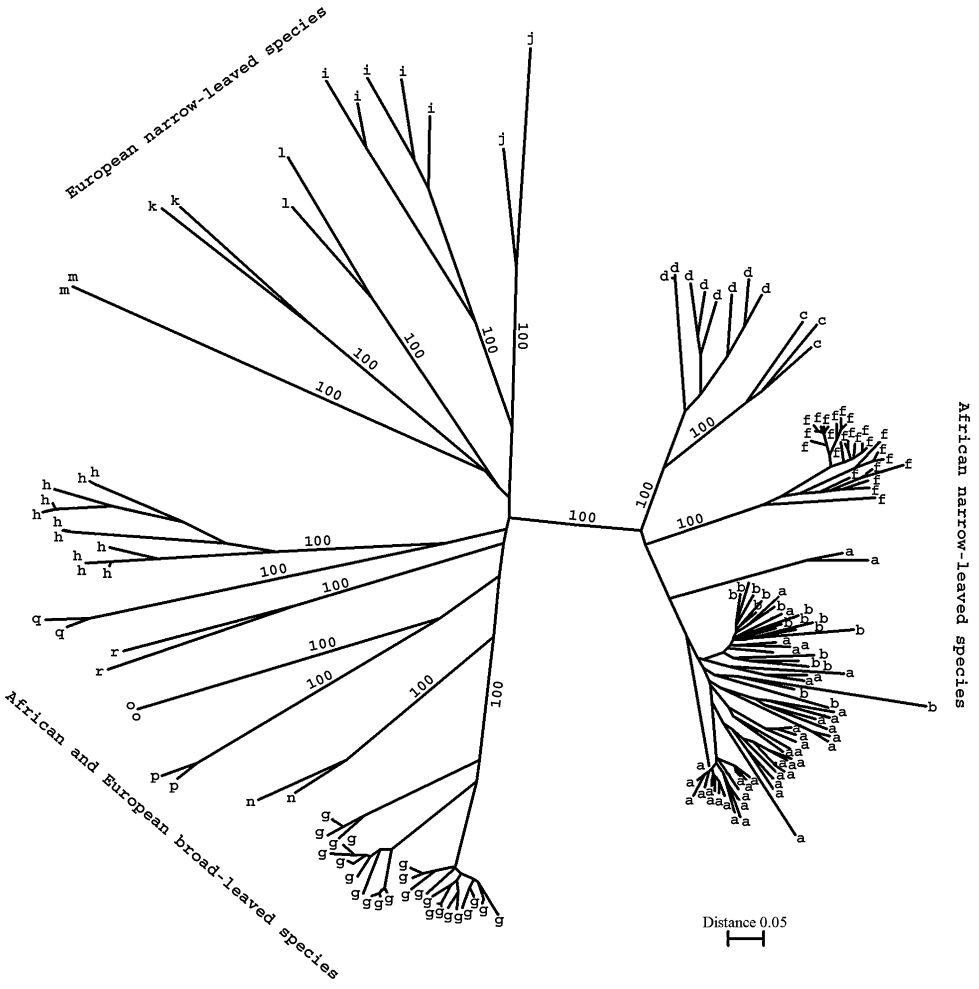

Fig. 3 Neighbor-joining of the whole data set: 167 *Festuca* accessions analyzed with 616 AFLPs. *Letters* at the end of each branch indicate the taxonomic classification of the respective accession: **a** *F. abyssinica*; **b** *F. richardii*; **c** *F. claytonii*; **d** *F. pilgeri*; **f** *F. chodatiana*; **g** *F. simensis*; **h** *F. africana*; **i** *Vulpia bromoides*; **j** *V. myuros*; **k** *F. ovina*; **l** *F. rubra*; **m** *F. vivipara*; **n** *F. gigantean*; **o** *F. arundinacea*; **p** *F. pratensis*; **q** *F. altissima*; **r** *Lolium multiflorum*. The *bar* represents a distance of 0.05. Bootstrapping values of 100% are only reported on those branches leading to species

agrees with the morphology (Clayton 1970). However, *F. simensis* is tetraploid, whereas *F. gigantea* is hexaploid ($2n = 6x = 42$, Lövkvist and Hultgård 1999). *F. africana* is most similar to *F. altissima* and this also corresponds to morphology. Both species are forest dwellers, lack auricles and have transverse veinlets in the leaves, but *F. africana* is decaploid whereas *F. altissima* is diploid.

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References

Alexeev EB (1986) New narrow-leaved *Festuca* (Poaceae) members from Tropical and South Africa. Bot Zhurnal 71:1109–1117

Alexeev EB (1987) Fescues of the *Festuca abyssinica* s. l. (Poaceae) group. Bot Zhurnal 72:1260–1268

Alm V, Fang C, Busso CS, Devos KM, Vollan K, Grieg Z, Rognli OA (2003) A linkage map of meadow fescue (*Festuca pratensis* Huds.) and comparative mapping with other Poaceae species. Theor Appl Genet 108:25–40

Arohonka T (1982) Chromosome counts of vascular plants of the island Seili in Nauvo, southwestern Finland. Biol Geogr 3:1–12

Barker CM, Stace CA (1984) Hybridization in the genera *Vulpia* and *Festuca* (Poaceae): the characteristics of artificial hybrids. Nord J Bot 4:289–302

Becker J, Vos P, Kuiper M, Salamini F, Heun M (1995) Combined mapping of AFLP and RFLP markers in barley. Mol Gen Genet 249:65–73

Catalan P, Torrecilla P, Lopez Rodriguez JA, Olmstead RG (2004) Phylogeny of the festucoid grasses of subtribe Loliinae and allies (Poeae, Pooideae) inferred from ITS and *trn*L–F sequences. Mol Phylogenet Evol 31:517–541

Charmet G, Ravel C, Balfourier F (1997) Phylogenetic analysis in the *Festuca–Lolium* complex using molecular markers and ITS rDNA. Theor Appl Genet 94:1038–1046

Clayton WD (1970) Gramineae, Part 1. In: Milne-Redhead E, Polhill RM (eds) Flora of Tropical East Africa. Crown Agents for Oversea Governments and Administrations, London, pp 56–63

Clayton WD, Renvoize SA (1986) Genera graminum: grasses of the world. HMSO, London, pp 374–375

Darbyshire SJ, Warwick SI (1992) Phylogeny of North American *Festuca* (Poaceae) and related genera using chloroplast DNA restriction site variation. Can J Bot 70:2415–2429

Fjellheim S, Elven R, Brochmann C (2001) Molecules and morphology in concert. II. The *Festuca brachyphylla* complex (Poaceae) in Svalbard. Am J Bot 88:869–882

Fjellheim S, Rognli OA (2005a) Genetic diversity within and among Nordic meadow fescue (*Festuca pratensis* Huds.) cultivars determined on the basis of AFLP markers. Crop Sci 45:2081–2086

Fjellheim S, Rognli OA (2005b) Molecular diversity of local Norwegian meadow fescue (*Festuca pratensis* Huds.) populations and Nordic cultivars—consequences for management and utilization. Theor Appl Genet 111:640–650

Gaut BS, Tredway LP, Kubik C, Gaut RL, Meyer W (2000) Phylogenetic relationships and genetic diversity among members of the *Festuca–Lolium* complex (Poaceae) based on ITS sequence data. Plant Syst Evol 224:33–53

Hedberg O (1957) Afroalpine vascular plants; a taxonomic revision. Symb Bot Upsal XV:1

Jenczewski E, Alix K (2004) From diploids to allopolyploids: the emergence of efficient pairing control genes in plants. Crit Rev Plant Sci 23:21–45

Jenkin TJ (1959) Fescue species (*Festuca* L.). In: Kappert H, Rudorf W (eds) Manual of plant breeding. vol 4. Breeding of forage plants. Paul Parey, Berlin, Hamburg, pp 418–434

Launert E (1971) Gramineae. In: Fernandes A, Launert E, Wild H (eds) Flora Zambesiaca, vol 10, part 1. Crown Agents for Oversea Governments and Administrations, London, pp 54–57

Lövkvist B, Hultgård UM (1999) Chromosome numbers in south Swedish vascular plants. Opera Bot 137:1–42

Malakhova LA, Markova GA (1994) Chromosome numbers in the flowering plants of Tomsk region. Monocotyledones. Bot Zhurnal 79:134–135

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

Mian MAR, Hopkins AA, Zwonitzer JC (2002) Determination of genetic diversity in tall fescue with AFLP markers. Crop Sci 42:944–950

Mian MAR, Saha MC, Hopkins AA, Wang ZY (2005) Use of tall fescue EST–SSR markers in phylogenetic analysis of cool-season forage grasses. Genome 48:637–647

Morton JK (1993) Chromosome numbers and polyploidy in the flora of Cameroon mountain. Opera Bot 121:159–172

Phillips S (1995) Poaceae. In: Hedberg I, Edwards S (eds) Flora of Ethiopia and Eritrea, vol 7. Addis Ababa, Ethiopia, Uppsala, pp 23–27

Rohlf FJ (2000) NTSYSpc: numerical taxonomy and multivariate analysis system, version 2.11f. Exeter Software, New York

Saha MC, Mian R, Zwonitzer JC, Chekhovskiy K, Hopkins AA (2005) An SSR- and AFLP-based genetic linkage map of tall fescue (*Festuca arundinacea* Schreb.). Theor Appl Genet 110:323–336

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Salvesen PH (1986) *Festuca vivipara* in southern Norway, infraspecific differentiation and systematic affinities. Symb Bot Upsal 27:155–167

Skibinska M, Kosmala A, Humphreys MW, Zwierzykowski Z (2002) Application of GISH and AFLP techniques for identification of *Lolium–Festuca* introgressions. Cell Mol Biol Lett 7:493–498

Spies JJ, Burger TH, van Wyk SMC (1999) Chromosome studies on African plants. 12. The tribes of subfamily Pooideae. Bothalia 29:335–341

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

Torrecilla P, Lopez Rodriguez JA, Stancik D, Catalan P (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. Plant Syst Evol 239:113–139

Torrecilla P, Lopez-Rodriguez JA, Catalan P (2004) Phylogenetic relationships of *Vulpia* and related genera (Poeae, Poaceae) based on analysis of ITS and *trn*L–F sequences. Ann MO Bot Gard 91:124–158

Van de Peer Y, De Wachter R (1994) TREECON for windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. Comput Appl Biosci 10:569–570

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Watson L, Dallwitz MJ (1992) Grass genera of the world: descriptions, illustrations, identification, and information retrieval; including synonyms, morphology, anatomy, physiology, phytochemistry, cytology, classification, pathogens, world and local distribution, and references. http://www.biodiversity.uno.edu/delta/, Version: 18th August 1999

Xu WW, Sleper DA (1994) Phylogeny of tall fescue and related species using RFLPs. Theor Appl Genet 88:685–690

Paper II

The species distinction of three endemic narrow-leaved *Festuca* from East Africa

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Abstract

Species distinction of *Festuca claytonii*, *F. elgonensis* and *F. pilgeri* (endemic species in East Africa) was assessed by comparison to the wide-spread narrow-leaved species *F. abyssinica*, *F. chodatiana* and *F. richardii*. The six species were analysed based on 56 morphological characters and 375 AFLPs. Principal components and discriminant analyses of the morphological data revealed that the endemic species *F. claytonii* and *F. pilgeri* are good species, but found *F. abyssinica, F. elgonensis* and *F. richardii* to be intermixed. Relevant morphological characters were identified for an overall species distinction and also for pairwise comparisons. A principal coordinates analysis of the AFLPs confirmed this above mentioned species distinction. In conclusion, four narrow-leaved species of *Festuca* occur in Uganda, viz. *F. abyssinica*, *F. chodatiana*, *F. claytonii* and *F. pilgeri*. *F. elgonensis* and *F. richardii* should be included in *F. abyssinica*.

Keywords: AFLP, Africa, alpine, *Festuca*, molecular, morphology, taxonomy, Uganda.

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Introduction

AFLP fingerprinting has shown that five tropical African narrow-leaved fescues are unique compared to the other species in the genus (Namaganda *et al*. 2006). But in total, six narrow-leaved species of *Festuca* are recorded from Uganda, three of which are endemic to East Africa viz. *F. claytonii*, *F. elgonensis* and *F. pilgeri*. *F. claytonii* is only known from Mt. Elgon. It is a rare species and was until our collection in 2003, last recorded in 1938 from Uganda and in 1967 from the Kenya side of Mt. Elgon. *F. elgonensis* (Alexeev 1987) is also rare and was until 2005 known from only one collection in Uganda (Mt. Elgon, 6 Sept. 1932, A. S. Thomas, 644, K), and another from southern Sudan (Mt. Kinyeti, 15 Nov. 1949, J. K. Jackson, 939, K). Basing on morphology and anatomy Alexeev (1987) named *F. elgonensis* from specimens originally identified as *F. abyssinica*. The *Festuca abyssinica* aggregate is a complex of highly variable species characterised by broad and enveloping glumes (Phillips 1995). *F. pilgeri* is a more widespread species known from Mt. Elgon, Mt. Kenya and the Kitulo plateau in Tanzania. *F. claytonii* and *F. pilgeri* are morphologically quite similar, but can easily be separated on the soft and smooth leaves in *F. claytonii* whereas F. *pilgeri* has stiff and scabrous leaves. A principal coordinate analysis of AFLP data could not differentiate between these two species but a neighbor joining analysis of the same data showed a good distinction (Namaganda *et al*. 2006). The other Ugandan narrow-leaved fescues include *F. abyssinica* and *F. richardii*, the two controversial species (Namaganda *et al*. 2006) and *F. chodatiana*. *F. abyssinica* is known from the high mountains in tropical Africa whereas *F. richardii* has been recorded from East Africa and Ethiopia. *F. chodatiana* is a distinct species (Namaganda *et al*. 2006), but is morphologically similar to the Madagascan *F. camusiana* St. Yves (Clayton 1970). *F. chodatiana* is known from many highland areas in tropical Africa. *F. elgonensis* was never before included in a molecular analysis and only recently we succeeded to collect it on Mt. Elgon.

Therefore, we aim at comparing AFLP-based and morphological classifications of all six narrow leaved species with a focus on the species distinction of three endemic species represented by as many accessions as possible, given their rare status. We redo AFLPs and make morphological measurements on the same accessions and compare the obtained results with the 'Flora of Tropical East Africa'. Morphological analyses were controlled by including type specimens of the species.

Materials and methods

Morphology measurements: A total of 56 morphological characters, 27 qualitative and 29 quantitative, were examined on 66 accessions (Table 1) of the six Ugandan narrow-leaved species of Festuca. The list of characters used (Tables 2 & 3) was built up from literature (Clayton 1970; Pavlick & Looman 1984; Dube & Morisset 1987; Aiken & Lefkovitch 1993; Phillips 1995) and were broadly divided into characters of the culm, leaf blade, sheath, ligule, inflorescence, spikelets and florets. Measurements were made on herbarium collections corresponding to material used by Namaganda et al. (2006) for DNA analysis and also on type specimens of the six species as well as the Sudanese collection of F. elgonensis. Identifications were verified at the MHU and KEW herbaria (acronyms according to Holmgren 1998).

Spikelet and floret measurements were made on spikelets from the middle portion of the inflorescence i.e. the second and third inflorescence nodes. Lemma, awn and where possible anther measurements were made on the second fertile florets. The awns were excluded when measuring lengths of spikelets and lemmas. Recorded numbers of florets per spikelet include the uppermost sterile or vestigial spikelet. Values recorded for each accession are means of up to three measurements.

AFLP procedure: Fifty two samples were analysed, taking selected accessions from Namaganda *et al*. (2006) and including the newly collected *F. elgonensis* (Table 1). Details of DNA extraction, digestion, ligation of adapters, pre-amplification and final PCR followed the procedure of Vos *et al*. (1995) as outlined in Namaganda *et al*. (2006). Different was the time for electrophoresis, which was at 80 W for 1 h 40 min to 2 h 30 min, the variation in time depending on how best the AFLP bands were separated. The gels were fixed in 10 % acetic acid for 30 min, dried and exposed to X-ray films for 22 – 173 h depending on radiation intensity and then developed with an AGFA Curix 60. Three primer combinations were used: 1.) E34/M37 (*Eco*RI+AAT / *Mse*I+ACG), 2.) E39/M37 (*Eco*RI+AGA / *Mse*I+ACG) and 3.) E35/M36 (*Eco*RI+ACA / *Mse*I+ACC).

Data analysis

Morphology: Following Norusis (1999) the 27 polymorphic quantitative characters (29 measured, but two monomorph) were analysed by box plots with the species predetermined based on taxonomic classification (Fig. 1). Next, a discriminant analysis (DA) was performed by selecting means and univariate ANOVAs as descriptives, with the 'equal prior probabilities for all groups', 'using a within groups covariance matrix' and 'combined groups' options under the classify option of SPSS release 10.05 (Fig. 2). Thereafter, following the procedures of Rohlf (2000), a principal components analysis (PCA) was done: the raw data were standardised, a correlation matrix computed, Eigen vectors calculated with the first three PC axes extracted (Table 4) and projected versus the morphology data (see NTSYSpc 2.11) (Fig. 3). Morphological variation in the measured 27 qualitative characters (by chance the same number as for the quantitative characters) and their importance in differentiating the species were assessed directly from the raw data.

AFLP: Presence/absence scores of the 375 polymorph fragments were generate for the three different AFLP primer combinations and later merged. A pairwise similarity matrix was obtained with the DICE option of the NTSYSpc 2.11f software (Rohlf 2000), which was converted into a distance matrix (by subtracting one and multiplying by minus 1). After using the DCENTER transformation, the EIGEN procedure of this software package generated a principal coordinate analysis (PCoA), see Fig. 4.

Chromosome counts: Following the procedures outlined in Namaganda *et al*. (2006), the chromosome number for *F. elgonensis* was counted from 10 root tips germinated from seeds of the three Ugandan accessions listed in Table 1 (excluding the type collection) and shown in Fig. 5.

Results

Quantitative morphology: Two quantitative characters, i.e. number of nerves on upper glumes (F6) and number of nerves on lemmas (G4) were abandoned because they were constant for all the species (three and five nerves respectively). Therefore, the results presented are after analysis of 27 quantitative characters.

Box plots (Fig. 1) revealed that only 15 of the analysed quantitative characters can be used to differentiate the species individually, group-wise (*F. abyssinica*, *F. elgonensis* and *F. richardii*) or only between *F. claytonii* and *F. pilgeri*. Individual differentiation is shown by A2 for *F. claytonii*; B2, E1, E2, E5, E6 and G6 for *F. chodatiana* (see Table 2 for character abbreviations). The *F. abyssinica* group (*F. abyssinica*, *F. elgonensis* and *F. richardii*) is best differentiated by LG, F3, UG, F4 and G2. No clear differentiation within this group was observed but B1 reveals *F. elgonensis* to have generally longer leaf blades. *F. richardii* was also shown to have generally longer anthers but with considerable overlap (figure not shown).

Differentiation between *F. claytonii* and *F. pilgeri* was shown by A2, B1, F2, LG, UG and G1; E2 shows some differentiation but with a lot of overlap. Other less distinguishing characters (figures not shown) like D4 reveal the *F. abyssinica* group to have the shortest ligules, whereas *F. claytonii* generally has longer ligules than *F. pilgeri*; A6 shows *F. claytonii* to have generally more nodes on the culm than all other species; A1 and C1 show F. chodatiana to respectively have generally longer culms and sheaths compared to all the other species.

The DA (Fig. 2) like the box plots does not differentiate between *F. abyssinica*, *F. elgonensis* and *F. richardii*, but differentiates between *F. claytonii* and *F. pilgeri* and separates *F. chodatiana* from all the others. Function 1, accounting for 61.8 % of the variation, correlates with the characters B2, E1, E2, E5, E6 and G6 all of which best differentiate *F. chodatiana* according to the box plots (Fig. 1). Function 1 clearly differentiates *F. chodatiana*. Function 2, which accounts for 30.6 % of the variation and correlates with characters D4 and G2, gives the differentiation between the *F. abyssinica* group, *F. claytonii* and *F. pilgeri*.

The PCA (Fig. 3) result is similar to the DA and box plots in not differentiating between *F. abyssinica*, *F. elgonensis* and *F. richardii*, but having *F. claytonii*, *F. pilgeri* and *F. chodatiana* differentiated. The first principal component (PC 1) accounts for 33.4 % of the observed variation and is highly correlated ($r \geq |0.7|$) with the characters A1, B2, C1, E1, E2, E5, E6, F3 and G6 (Table 4). The second principal component (PC 2) accounts for 22.3 % of the observed variation and is highly correlated ($r \geq |0.7|$) with UG and G2 (Table 4). The type accessions of *F. abyssinica*, *F. elgonensis*, *F. richardii*, *F. claytonii* and *F. chodatiana* grouped with our own collections but that of *F. pilgeri* did not. Also one accession *F. chodatiana* (1600) grouped close to *F. pilgeri*. According to the PCA, DA and box plot

analyses a total of 18 quantitative characters are important in differentiating between the narrow-leaved African *Festuca*. Table 5 summarises the quantitative data.

Qualitative morphology: Table 6 summarises the qualitative data. Five qualitative characters (B7, C5, F11, F12, and G3) were not variable across all the species (Table 6; see Table 3 for character abbreviations). Qualitative characters important in differentiating the species include D1, which differentiates *F. claytonii* from all the rest; B4, B5 and B6 differentiate *F. claytonii* from *F. pilgeri*; E7 and G8 differentiate *F. chodatiana* from all the other species, but no single character differentiates between *F. abyssinica*, F. elgonensis and *F. richardii*. The rest of the characters are variable and hence not useful.

AFLP: A total of 375 polymorphic AFLPs were scored and used to generate a PCoA (Fig. 4). Like the morphological analyses, the PCoA clearly differentiates *F. claytonii*, *F. pilgeri* and *F. chodatiana* whereas *F. abyssinica*, *F. elgonensis* and *F. richardii* are not. The first principal coordinate (PCo 1) and second principal coordinate (PCo 2) respectively account for 26.0 % and 18.4 % of the variation and are responsible for the separation into three groups; 1. *F. claytonii* and *F. pilgeri*, 2. *F. abyssinica*, *F. elgonensis* and *F. richardii*, 3. *F. chodatiana*. *F. claytonii* and *F. pilgeri* are especially differentiated by the third principal coordinate (PCo 3) which accounts for 13.9 % of the variation.

Chromosome numbers: *F. elgonensis* was found to be tetraploid (2n = 4x = 28; Fig. 5), like all the other narrow-leaved Ugandan fescues (Namaganda et al. 2006).

Discussion

In this study we report congruence between morphology and molecular analyses of some African narrow-leaved *Festuca*, and our result is that *F. abyssinica*, *F. chodatiana*, *F. claytonii* and *F. pilgeri* are good species whereas *F. elgonensis* and *F. richardii* are not. The strength of application of both morphology and molecular analyses in taxonomy has already been documented in grasses (e.g., Fjellheim *et al*. 2001; Roldan-Ruiz *et al*. 2001; Szczepaniak *et al*. 2002; Saarela *et al*. 2003; Torrecilla *et al*. 2003) and in other families (e.g., Hansen *et al*. 2000; Albarouki & Peterson 2007; Lee *et al*. 2007; Manns & Anderberg 2007). A combination of morphology and molecules in taxonomy is especially valuable in taxa that are closely related or those that are so much variable that choice of distinguishing morphological characters becomes difficult. In this case, genetic groups revealed by molecular analysis can be evaluated for 'good' morphological distinguishing characters (Hansen *et al*. 2000; Fjellheim *et al*. 2001), resulting in more natural taxonomic groups. Morphological variability for example below species level may not reveal intraspecific units like in Mizianty (2006) and Mizianty (2005) possibly because of inclusion of randomly variable characters that tend to conceal information in taxonomically significant characters (Fjellheim *et al*. 2001). But this can be verified via molecular analyses. Morphology and molecules have also been applied in evolutionary studies (e.g., Schmidt-Lebuhn *et al*. 2005; Ponsie *et al*. 2007; Sosa 2007).

F. elgonensis versus *F. abyssinica/F. richardii*

When Alexeev (1987) described *F. elgonensis*, he differentiated it from *F. abyssinica*, *F. richardii* and *F. scimperiana* by possession of 'leaf blades with five or seven conducting bundles (if seven then leaf blades stiff and upright) and acute-angled flanks with broad sclerenchyma bundles' as opposed to 'leaf blades with seven conducting bundles, relatively soft, more or less nodding, flanks with obtuse angles or without angles, with narrow

sclerenchyma bundles'. Alexeev obviously overemphasised leaf anatomy. In their studies on Canadian *Festuca*, Dube & Morisset (1986) and Aiken & Consaul (1995) showed that variation in anatomical characters is related to ecological factors. Leaf anatomy can be continuous within a species and should not be used alone as a distinguishing character.

Alexeev (1987) further separated *F. richardii* from *F. abyssinica* and *F. schimperiana* by anther length; 2 – 3 mm in *F. richardii* and 0.6 – 1.2 (1.8) mm in *F. abyssinica* and *F. schimperiana*. While we found it to be true that *F. richardii* generally has longer anthers (1.7 – 4.4 mm) than *F. abyssinica* (0.4 – 3.8 mm), the overlap is considerable for these two species and *F. elgonensis* anther length (0.6 – 3.3 mm) lies within the range for *F. abyssinica*. Alexeev further differentiated *F. richardii* by 'leaf blades smooth below or more or less rough, leaf sheaths closed halfway up' as opposed to 'leaf blades smooth below, leaf sheaths of vegetative shoots closed nearly all the way up'. We found *F. richardii* to have more rough accessions than smooth ones whereas *F. abyssinica* had more smooth accessions than rough ones (Table 6; B6). This agrees in part with Alexeev but again there is overlap in this character. We did not investigate Alexeev's leaf sheath character because Phillips (1995) found that this character could not be determined reliably from herbarium specimens. Awn length is one of the characters Alexeev used to separate *F. abyssinica* and *F. schimperiana* but Phillips (1995) found this character unreliable and disregarded *F. schimperiana* as a good species and we followed Phillip's taxonomy. However, we also did not find awn length to be a good character.

None of the 56 investigated morphological characters could differentiate between *F. abyssinica*, *F. elgonensis* and *F. richardii*, and this is confirmed by the close grouping of the type specimens. Since DNA (AFLP) also cannot differentiate these three species, we recommend that they should be remerged as *F. abyssinica* and again confirm our earlier

recommendation based on AFLPs alone (Namaganda *et al*., 2006) to merge *F. richardii* into *F. abyssinica*.

F. claytonii, *F. pilgeri* and *F. chodatiana*

Our data supports Alexeev (1986) in accepting these species. The most easily recognisable species is *F. chodatiana*, which usually has flat leaves, long and open inflorescences, long awns and ovaries with hairy tops. The one accession of *F. chodatiana* (1600) that did not group the rest of the *F. chodatiana* accessions in the morphology analysis, was a visibly smaller plant with shorter culms, shorter leaves, shorter ligules, less number of spikelets on the longest inflorescence branch, and low ratio of second lemma to upper glume. But in the DNA analysis this accession was in the centre of the *F. chodatiana* group, implying that the expressed deviation in morphological characters is most likely caused by environmental conditions. The type specimen of *F. chodatiana* from Tanzania is a rather small plant, but it is still grouped together with the Ugandan accessions (Fig. 3).

Based on the morphological analysis (Fig. 3), the type specimen of *F. claytonii* was placed well within the species' group. Very short flag leaf blades (1.4 – 3.2 cm) and rounded ligules differentiate *F. claytonii* from the remaining five species but no single morphological character solely differentiates *F. pilgeri*. The close relationship between *F. claytonii* and *F. pilgeri* is unquestionable given the close grouping in DNA (AFLP) and similarities in spikelet parts. The type specimen for *F. pilgeri* did not group closely with our *F. pilgeri* accessions from Uganda (Fig. 3), because it was found to be a generally bigger plant with longer culms, nodes, leaves, sheaths, ligules and inflorescences. Since the type collection from Mt. Kenya is geographically well separated from the Ugandan accessions, one may think this could be because they represent different subspecies or geographical races. However, the

differentiating characters mentioned above are easily modified by the environment as the type collection was exposed to different climatic and ecological conditions.

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References

Aiken, S. G. & Consaul, L. L. 1995. Leaf cross sections and phytogeography: A potent combination for identifying members of *Festuca* subgen. *Festuca* and *Leucopoa* (Poaceae), occurring in North America. - Am. J. Bot. 82: 1287-1299.

Aiken, S. G. & Lefkovitch, L. P. 1993. On the separation of two species within *Festuca* subg. *Obtusae* (Poaceae). - Taxon 42: 323-337.

Albarouki, E. & Peterson, A. 2007. Molecular and morphological characterization of *Crataegus* L. species (Rosaceae) in southern Syria. - Bot. J. Linn. Soc. 153: 255-263.

Alexeev, E. B. 1986. New narrow-leaved *Festuca* (Poaceae) members from Tropical and South Africa. - Bot. Zhurnal 71: 1109-1117.

Alexeev, E. B. 1987. Fescues of the *Festuca abyssinica* s. l. (Poaceae) group. - Bot. Zhurnal 72: 1260-1268.

Clayton, W. D. 1970. Gramineae Part 1. - In: Milne-Redhead, E., Polhill, R. M. (eds.) Flora of Tropical East Africa. Crown agents for oversea governments and administrations, London, pp. 56-63.

Dube, M. & Morisset, P. 1987. Morphological and leaf anatomical variation in *Festuca rubra sensu lato* (Poaceae) from eastern Quebec. - Can. J. Bot. 65: 1065-1077.

Fjellheim, S., Elven, R. & Brochmann, C. 2001. Molecules and morphology in concert. II. The *Festuca brachyphylla* complex (Poaceae) in Svalbard. - Am. J. Bot. 88: 869-882.

Hansen, K. T., Elven, R. & Brochmann, C. 2000. Molecules and morphology in concert: tests of some hypotheses in arctic *Potentilla* (Rosaceae). - Am. J. Bot. 87: 1466-1479.

Holmgren, P. K. & Holmgren, N. H. 1998. Index Herbariorum. New York Botanical Garden. http://sciweb.nybg.org/science2/IndexHerbariorum.asp

Lee, K. K., Harrison, D. K., Johnston, M. E. & Williams, R. R. 2007. Molecular taxonomic clarification of *Ptilotus exaltatus* and *Ptilotus nobilis* (Amaranthaceae). - Aust. Syst. Bot. 20: 72-81.

Manns, U. & Anderberg, A. A. 2007. Character evolution in *Anagallis* (Myrsinaceae) inferred from morphological and molecular data. - Syst. Bot. 32: 166-179.

Mizianty, M. 2006. Variability and structure of natural populations of *Hordeum murinum* L. based on morphology. - Pl. Syst. Evol. 261: 139-150.

Mizianty, M. 2005. Variability and structure of natural populations of *Elymus canis* (L.) L. based on morphology. - Pl. Syst. Evol. 251: 199-216.

Namaganda, M., Lye, K. A., Friebe, B. & Heun, M. 2006. AFLP-based differentiation of tropical African *Festuca* species compared to the European *Festuca* complex. - Theor. Appl. Genet. 113: 1529-1538.

Norusis, M. J. 1999. SPSS for Windows, release 10.0.5. SPSS Inc., Chicago.

Pavlick, L. E. & Looman, J. 1984. Taxonomy and nomenclature of rough fescues, *Festuca altaica*, *F. campestris* (*F. scabrella* var. *major*), and *F. hallii*, in Canada and the adjacent part of United States. - Can. J. Bot. 62: 1739-1749.

Phillips, S. 1995. Poaceae. - In: Hedberg, I., Edwards, S. (eds.) Flora of Ethiopia and Eritrea, Vol. 7. Addis Ababa, Ethiopia; Uppsala, Sweden, pp. 23-27.

Ponsie, M. E., Mitchell, A., Edwards, T. J. & Johnson, S. D. 2007. Phylogeny of *Bonatea* (Orchidaceae: Habenariinae) based on molecular and morphological data. - Pl. Syst. Evol. 263: 253-268.

Rohlf, F. J. 2000. NTSYSpc: numerical taxonomy and multivariate analysis system, version 2.11f. Exeter Software, New York.

Roldan-Ruiz, I., Eeuwijk, F. A., Gilliland, T. J., Dubreuil, P., Dillmann, C., Lallemand, J., De Loose, M. & Baril, C. P. 2001. A comparative study of molecular and morphological methods of describing relationships between perennial ryegrass (*Lolium perenne* L.) varieties. - Theor. Appl. Genet. 103: 1138-1150.

Saarela, J. M., Peterson, P. M., Soreng, R. J. & Chapman, R. E. 2003. A taxonomic review of the eastern North American and eastern Asian disjunct genus *Brachyelytrum* (Poaceae): Evidence from morphology, phytogeography and AFLPs. - Syst. Bot. 28: 674-692.

Saitou, N. & Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. - Mol. Biol. Evol. 4: 406-425.

Schmidt-Lebuhn, A. N., Kessler, M. & Muller, J. 2005. Evolution of *Suessenguthia* (Acanthaceae) inferred from morphology, AFLP data, and ITS rDNA sequences. Org. Divers. - Evol. 5: 1-13.

Sosa, V. 2007. A molecular and morphological phylogenetic study of subtribe Bletiinae (Epidendreae, Orchidaceae). - Syst. Bot. 32: 34-42.

Szczepaniak, M., Cieslak, E. & Bednarek, P. T. 2002. Morphological and AFLP variation of *Elymus repens* (L.) Gould (Poaceae). - Cell. Mol. Biol. Lett. 7: 547-558.

Torrecilla, P., Lopez Rodriguez, J. A., Stancik, D. & Catalan, P. 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.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. & Zabeau, M. 1995. AFLP: a new techinique for DNA fingerprinting. - Nucleic Acids Res. 23: 4407-4414.

Table 1. Accessions used in the study. MN = Mary Namaganda, WS = Wilhelm Schimper, AST = A. S. Thomas, ST = A. Stolz, JKJ = J. K. Jackson. Specimens collected by MN are kept at the Makerere University Herbarium (MHU), the rest were borrowed from the Kew herbarium. Type specimens are marked with an asterisk.

Species	**Collection data** (country, geographical division, locality, coordinates, altitude)	**Collector & collection number**	**No. of vouchers measured**	**Corresponding lab numbers (AFLP)**
F. abyssinica A. Rich.	Uganda. U3. Elgon, Wagagai peak, 01°07.5'N 34°31.5'E, 4300 m	MN 1369	1	-
	Uganda. U3. Elgon, in the caldera, 01°09.0'N 34°33.3'E, 3870 m	MN 1374	2	071E
	Uganda. U3. Elgon, hot springs, 3560 m	MN 1376	2	-
	Uganda. U3. Elgon, in the caldera, 3770 m	MN 1377	1	-
	Uganda. U3. Elgon, bamboo forest, 01°10.6'N 34°27.0'E, 3050 m	MN 1566	0	228E
	Uganda. U3. Elgon, Mongongo cave, 01°09.3'N 34°30.8'E, 3780 m	MN 1576	1	278E
	Uganda. U3. Elgon, Piswa – Sasa trail, 01°09.0'N 34°30.0'E, 3750 m	MN 1581	1	290E
	Uganda. U3. Elgon, Piswa – Sasa trail, 01°09.0'N 34°30.0'E, 3740 m	MN 1582	0	292E
	Uganda. U3. Elgon, Mude camp, 01°10.0'N 34°29.3'E, 3470 m	MN 1583	1	296E
	Uganda. U3. Elgon, Mude camp, 01°10.0'N 34°29.3'E, 3470 m	MN 1670	1	373E
	Uganda. U2. Gahinga, montane forest, 01°22.9'S 29°38.5'E, 3070 m	MN 1406	2	-
	Uganda. U2. Gahinga, crater swamp, 01°23.0'S 29°38.7'E, 3460 m	MN 1410	0	122Vg
	Uganda. U2. Gahinga, top, 01°22.9'S 29°38.5'E, 3470 m	MN 1411	1	-
	Uganda. U2. Gahinga, top, 01°23.0'S 29°38.7'E, 3470 m	MN 1604	1	193Vg
	Uganda. U2. Gahinga, crater swamp, 01°22.9'S 29°38.5'E, 3460 m	MN 1606	0	201Vg
	Uganda. U2. Muhavura, forest, 01°22.3'S 29°40.2'E, 3080 m	MN 1592	0	148Vm
	Uganda. U2. Muhavura, ericaceous zone, 01°22.0'S 29°39.0'E, 3800 m	MN 1593	1	150Vm
	Ethiopia. Tigray region. Mt. Scholoda (Selleuda)	WS 410*	1	-
F. chodatiana (St. Yves) Alexeev	Uganda. U3. Elgon, bamboo forest, 01°10.6'N 34°27.6'E, 3070 m	MN 1567	2	231E

	Uganda. U3. Elgon, Sasa River camp, 01°10.3'N 34°26.3'E, 2830 m	MN 1584	1	-
	Uganda. U3. Elgon, montane forest, 01°10.4'N 34°25.4'E, 2710 m	MN 1587	1	324E
	Uganda. U2. Gahinga, regenerating forest, 01°21.6'S 29°37.4'E, 2405 m	MN 1600	1	173Vg
	Uganda. U2. Muhavura, regenerating forest, 01°21.8'S 29°39.8'E, 2620 m	MN 1590	1	139Vm
	Uganda. U2. Muhavura, regenerating forest, 01°21.6'S 29°39.8'E, 2500 m	MN 1595	1	-
	Uganda. U2. Echuya, Kisoro – Kabale boarder, 01°15.4'S 29°47.8'E, 2280 m	MN 1609	1	211K
	Uganda. U2. Bwindi forest, Ndeego, 01°06.5'S 29°48.5'E, 2220 m	MN 1610	1	215B
	Uganda. U2. Bwindi forest, Ndeego, 01°06.5'S 29°48.5'E, 2220 m	MN 1717	1	374B
	Tanzania. Rungwe District. Kyimbila,	ST 1162*	1	-
F. claytonii Alexeev	Uganda. U3. Elgon, Wagagai, 01°07.5'N 34°31.5'E, 4300 m	MN 1370C	2	-
	Uganda. U3. Elgon, towards Wagagai, 01°08.7'N 34°31.4'E, 4200 m	MN 1367	2	038E, 039E
	Uganda. U3. Elgon, towards Wagagai, 01°08.7'N 34°31.4'E, 4140 m	MN 1575	2	269E, 271E, 274E, 275E, 276E
	Uganda. U3. Elgon, towards Wagagai, 01°08.7'N 34°31.4'E, 4140 m	MN 1678	2	366E
	Uganda. U3. Mt. Elgon, 3870 m	AST 2727*	1	-
F. elgonensis Alexeev	Uganda. U3. Elgon, below Sasa Patrol Hut, 01°10.4'N 34°28.5'E, 3000 m	MN 1666a	1	369E
	Uganda. U3. Elgon, below Sasa Patrol Hut, 01°10.4'N 34°28.5'E, 3000 m	MN 1666b	1	370E
	Uganda. U3. Elgon, below Sasa Patrol Hut, 01°10.4'N 34°28.5'E, 3000 m	MN 1666c	1	371E
	Uganda. U3. Elgon, Madangi, 3050 m	AST 644*	1	-
	Sudan. Torit District. Mt. Kinyeti, 03°57.0'N 32°54.0'E, 3180 m	JKJ 939	1	-
F. pilgeri St. Yves	Uganda. U3. Elgon, above Jackson's pool, 01°08.6'N 34°31.3'E, 4100 m	MN 1365	4	033E, 034E
	Uganda. U3. Elgon, near Jackson's pool, 01°08.9'N 34°30.6'E, 3910 m	MN 1572	2	255E, 256E, 257E, 258E
	Uganda. U3. Elgon, above Jackson's pool, 01°08.7'N 34°31.0'E, 4010 m	MN 1574	2	264E, 265E, 266E, 267E, 268E
	Uganda. U3. Elgon, above Jackson's pool, 01°08.7'N 34°31.0'E, 4000 m	MN 1675	2	367E
	Kenya. K4. Mt. Kenya.	CEF 1316*	1	-
F. richardii				

Alexeev	Uganda. U3. Elgon, near Sasa Patrol Hut, 01°10.0'N 34°29.0'E, 3450 m	MN 1355	1	-
	Uganda. U3. Elgon, above Mude camp, 01°08.9'N 34°30.6'E, 3900 m	MN 1359	2	020E, 022E
	Uganda. U3. Elgon, above Jackson's pool, 01°08.8'N 34°30.8'E, 3965 m	MN 1361	1	-
	Uganda. U3. Elgon, after Mude Hut above Simu gorge, 01°09.3'N 34°30.8'E, 3800 m	MN 1371	1	-
	Uganda. U3. Elgon, after Mude Hut above Simu gorge, 01°09.3'N 34°30.8'E, 3800 m	MN 1378C	1	-
	Uganda. U3. Elgon, alpine grassland, 01°10.4'N 34°28.3'E, 3310 m	MN 1569	1	239E, 242E
	Uganda. U3. Elgon, alpine grassland, 01°10.0'N 34°29.0'E, 3470 m	MN 1570	1	245E, 248E
	Uganda. U3. Elgon, alpine grassland, 01°09.5'N 34°30.0'E, 3730 m	MN 1571	2	250E, 251E
	Uganda. U3. Elgon, above Jackson's pool, 01°08.7'N 34°31.0'E, 4010 m	MN 1573	2	260E
	Uganda. U3. Elgon, Piswa – Sasa trail, 01°09.0'N 34°30.0'E, 3750 m	MN 1579	0	288E
	Uganda. U3. Elgon, Piswa – Sasa trail, 01°08.7'N 34°31.0'E, 3750 m	MN 1580	1	289E
	Ethiopia. Gonder region. Guna, 11°43.0'N 38°17.0'E, 3250 – 4000 m	WS 1560*	1	-

Table 2: List of 29 quantitative morphological characters measured

Feature	Abbreviation	Character
Culm	A1	Length (cm)
	A2	Length of flag leaf blade (cm)
	A3	Width of flag leaf blade (mm)
	A4	Length of uppermost internode (cm)
	A5	Length of uppermost internode : Length of culm ratio (A4:A1)
	A6	Number of nodes
Leaf blade	B1	Length (cm)
	B2	Width (mm)
Sheath	C1	Length (cm)
Ligule	D4	Length (mm)
Inflorescence	E1	Length (cm)
	E2	Length of lowermost internode (cm)
	E3	Length of lowermost internode : Length of inflorescence ratio (E2:E1)
	E4	Number of branches at lowermost node
	E5	Length of longest branch at lower most node (cm)
	E6	Number of spikelets on E5
Spikelet	F2	Length of spikelet minus awn (mm)
	LG	Length of lower glume (mm)
	F3	Length of lower glume : Length of spikelet ratio (LG:F2)
	UG	Length of upper glume (mm)
	F4	Length of upper glume : Length of spikelet ratio (UG:F2)
	F5	Number of nerves on lower glume
	F6	Number of nerves on upper glume
	F15	Number of flowers per spikelet
Floret	G1	Length of second lemma minus awn (mm)
	G2	Length of second lemma : Length of upper glume ratio (G1:UG)
	G4	Number of nerves on lemmas
	G6	Length of awn (mm)
	G9	Length of anthers (mm)

Table 3: List of 27 qualitative morphological characters scored

Feature	Abbreviation	Character	Character state	Score
Leaf blade	B3	Involution	Flat	0
			Folded	1
			Inrolled	2
			Flat and folded	3
	B4	Stiffness	Soft	0
			Stiff	2
	B5	Scabrousness (distribution)	None	0
			At apex only	1
			In top third	2
			Whole leaf	3
	B6	Scabrousness (intensity)	None	0
			Slight	1
			Strong	2
	B7	Abaxial pubescence	Absent	0
			Present	1
	B9	Colour (abaxial)	Bluish green	0
			Pale green	1
			Green	2
Sheath	C2	Abundance of anthocyanins	Absent	0
			Traces	1
			Abundant	2
	C3	Abundance of hairs	Absent	0
			Rare	1
			Abundant	2
	C5	Margins	Smooth	0
			Ciliated	1
Ligule	D1	Shape	Irregular	0
			Flat	1
			Rounded	2
			Notched	3
	D2	Margin	Entire	0
			Fimbriate	1
	D3	Hairs on margin	Absent	0
			Sparse	1
			Many	2
Inflorescence	E7	Type	Contracted	0
			Open	1
	E8	Scabrousness (lowest internode)	Absent	0
			Present	2
Spikelets	F7	Scabrousness on keel of lower glume	Absent	0
			Present	2
	F8	Scabrousness on keel of upper glume	Absent	0
			Present	2
	F9	Distribution in F7	None	0
			Up to ¼	1

			Up to ½	2
			Up to ¾	3
	F10	Distribution in F8	None	0
			Up to ¼	1
			Up to ½	2
			Up to ¾	3
	F11	Pubescence on lower glume	Absent	0
			Present	1
	F12	Pubescence on upper glume	Absent	0
			Present	1
	F13	Shape of lower glume	Lanceolate	0
			Oblong-lanceolate	1
	F14	Shape of upper glume	Lanceolate	0
			Oblong-lanceolate	1
	F16	Colour of spikelet	Purple	0
			Green tinged purple	1
			Green and purple	2
			Green	3
Floret	G3	Pubescence on lemmas	Absent	0
			Present	1
	G5	Shape of lemma	Oblong-lanceolate	0
			Lanceolate	1
	G7	Position of awn	Absent	0
			Sub-terminal	1
			Terminal	2
	G8	Pubescence on ovary top	Absent	0
			Present	2

Table 4. Correlation of the first three principal components (PC 1, PC 2, and PC 3) with 27 quantitative morphological characters measured on 63 narrow-leaved tropical African *Festuca* species. Coefficients $\geq |0.7|$ are marked in bold. See Table 1 for character abbreviations.

Character	PC 1	PC 2	PC 3
A1	**0.80**	0.38	0.20
A2	0.35	0.69	0.32
A3	0.52	0.44	-0.02
A4	0.44	0.11	0.69
A5	-0.21	-0.34	**0.71**
A6	0.28	-0.35	-0.16
B1	0.41	0.40	0.42
B2	**0.81**	0.28	-0.29
C1	**0.77**	0.29	0.25
D4	0.60	-0.45	0.25
E1	**0.84**	0.40	-0.19
E2	**0.70**	0.41	-0.23
E3	-0.20	0.13	-0.14
E4	0.32	0.31	0.30
E5	**0.77**	0.40	-0.19
E6	**0.72**	0.41	-0.30
F2	0.07	0.43	0.68
LG	-0.67	0.68	0.20
F3	**-0.80**	0.52	-0.15
UG	-0.53	**0.78**	0.20
F4	-0.69	0.61	-0.26
F5	-0.67	0.54	-0.13
F15	0.04	-0.53	0.57
G1	-0.18	0.60	0.57
G2	0.61	**-0.72**	0.13
G6	**0.85**	0.18	-0.31
G9	-0.03	-0.50	0.20

Table 5. Summarised quantitative data. See Table 2 for character definitions and units of measurement. Bold face values are the extreme measurements for the aberrant *F. chodatiana* (1600) in the PCA.

Char-acter	***F. claytonii***	***F. pilgeri***	***F. elgonensis***	***F. richardii***	***F. abyssinica***	***F. chodatiana***
A1	23.4 – 51.4	36.4 – 68.5 (90)	39.7 – 56.0 (67.6)	12.9 – 70.8	18.0 – 101.3	(**53.7**) 71.1 – 109.0
A2	1.4 – 3.2	4.3 – 8.2 (15.7)	3.4 – 8.6	3.9 – 7.2	3.1 – 16.0	3.6 – 13.7
A3	0.5 – 0.7	0.5 – 1.0	0.4 – 0.7	0.4 – 0.8	0.5 – 2.1	0.5 – 1.9
A4	12.6 – 38.2	20.6 – 55.6 (69.4)	23.2 – 34.2 (37.7)	2.5 – 42.1	9.2 – 41.9	**17.3** – 42.1
A5	0.52 – 0.76	0.57 – 0.81	0.42 – 0.67	0.16 – 0.81	0.28 – 0.68	0.25 – 0.46
A6	4 – 8	2 – 4	4	2 – 4	3 – 5	4 – 5
B1	5.5 – 10.5	11.5 – 28.0 (44.5)	16.3 – 34.9	5.8 – 21.2	6.4 – 33.7	**11.1** – 39.1
B2	0.4 – 0.6	0.5 – 0.7	0.5 – 0.6	0.4 – 0.8	0.4 – 0.8	1.1 – 2.4
C1	2.4 – 5.7	3.1 – 9.4 (18.9)	5.5 – 7.5	1.7 – 7.5	2.1 – 13.0	7.0 – 14.6 (17.7)
D4	0.6 – 1.6	0.4 – 1.3	0.3 – 0.5	0.2 – 1.0	0.2 – 0.7	**0.4** – 1.1
E1	7.4 – 12.9	8.0 – 15.4	9.3 – 14.9 (18.9)	6.9 – 14.8	5.7 – 20.3	(15.7) 23.8 – 32.9
E2	2.0 – 4.1	3.5 – 6.1	3.2 – 6.7	1.8 – 8.8	(1.6) 3.1 – 6.9	(4.9) 6.4 – 12.4
E3	0.25 – 0.36	0.29 – 0.44	0.32 – 0.42	0.30 – 0.59	0.28 – 0.66	0.24 – 0.39
E4	1 – 2	1 – 2 (3)	1 – 2	1 – 2	1 – 2 (3)	2
E5	2.9 – 6.3	3.9 – 8.9	4.0 – 7.6 (10.9)	2.9 – 7.1	2.5 – 9.8	(7.6) 9.1 – 17.0
E6	2 – 4	3 – 5	4 – 7 (10)	3 – 6	3 – 9	**7** – 18
F2	7.8 – 9.4	10.4 – 12.0	10.4 – 11.5	7.3 – 10.4 (12.6)	7.9 – 13.0	8.3 – 11.5
LG	2.6 – 3.7	3.9 – 5.7	6.7 – 8.7	5.5 – 8.4	5.7 – 10.1	2.6 – 4.0
F3	0.31 – 0.44	0.35 – 0.56	0.64 – 0.84	0.59 – 0.82	0.60 – 0.86	0.28 – 0.40
UG	3.6 – 4.5	5.1 – 6.8	7.7 – 10.0	6.3 – 8.7	6.6 – 11.4	4.7 – 6.1
F4	0.40 – 0.55	0.46 – 0.65	0.74 – 0.96	0.67 – 0.95	0.68 – 0.95	0.48 – 0.58
F5	1	1	3	(2) 3	1 – 3	1
F6	3	3	3	3	3	3
F15	4 – 6	4 – 6	3 – 4	3 – 5	3 – 6	3 – 4
G1	5.2 – 6.8	6.1 – 7.8	6.8 – 8.6	6.3 – 8.3	6.0 – 9.2	5.5 – 7.6
G2	1.31 – 1.63	1.09 – 1.38	0.84 – 0.95	0.86 – 1.02	0.73 – 0.99	**1.15** – 1.36
G4	5	5	5	5	5	5
G6	1.9 – 4.8	2.0 – 3.5	2.3 – 4.0	1.2 – 3.5	0.3 – 4.5 (7.2)	6.6 – 11.6
G9	2.2 – 3.0	2.4 – 3.9	0.6 – 3.3	1.7 – 4.0	0.4 – 3.8	1.2 – 2.4

Table 6. Frequency of qualitative characters. The data in the table are numbers of specimens. See Table 3 for character and codes' definitions.

Character	**B3**		**B4**		**B5**			**B6**			**B7**		**B9**			**C2**			**C3**			**C5**		**D1**				**D2**		**D3**			**E7**	
Codes	**2**	**3**	**0**	**1**	**0**	**1**	**3**	**0**	**1**	**2**	**0**	**1**	**0**	**1**	**2**	**0**	**1**	**2**	**0**	**1**	**2**	**0**	**1**	**0**	**1**	**2**	**3**	**0**	**1**	**0**	**1**	**2**	**0**	**1**
F. claytonii	9	0	9	0	9	0	0	9	0	0	9	0	7	0	0	7	1	1	9	0	0	9	0	0	0	9	0	0	9	0	0	9	9	0
F. pilgeri	11	0	0	11	0	0	11	0	0	11	11	0	10	0	0	11	0	0	11	0	0	11	0	11	0	0	0	0	11	0	0	11	11	0
F. elgonensis	4	0	3	1	2	1	1	2	2	0	4	0	2	1	0	3	1	0	4	0	0	4	0	3	1	0	0	0	4	0	1	3	5	0
F. richardii	14	0	14	0	1	0	13	1	6	7	14	0	0	11	3	2	9	3	14	0	0	14	0	13	1	0	0	0	14	0	0	14	14	0
F. abyssinica	11	5	14	2	12	0	4	12	3	1	16	0	1	9	6	3	6	7	16	0	0	16	0	12	3	0	1	0	16	0	0	16	16	0
F. chodatiana	0	11	11	0	11	0	0	11	0	0	11	0	0	0	10	5	6	0	5	2	4	11	0	11	0	0	0	1	10	3	4	4	0	11

Character	**E8**		**F7**		**F8**		**F9**			**F10**				**F11**		**F12**		**F13**		**F14**		**F16**				**G3**		**G5**		**G7**		**G8**	
Codes	**0**	**2**	**0**	**2**	**0**	**2**	**0**	**1**	**2**	**0**	**1**	**2**	**3**	**0**	**1**	**0**	**1**	**0**	**1**	**0**	**1**	**0**	**1**	**2**	**3**	**0**	**1**	**0**	**1**	**1**	**2**	**0**	**2**
F. claytonii	7	2	2	7	2	7	2	3	4	2	3	4	0	9	0	9	0	9	0	2	7	9	0	0	0	9	0	0	9	0	9	9	0
F. pilgeri	0	11	0	11	0	11	0	5	6	0	5	5	1	11	0	11	0	11	0	1	10	11	0	0	0	11	0	8	3	2	9	10	0
F. elgonensis	4	1	3	2	3	2	3	2	0	3	2	0	0	5	0	5	0	5	0	4	1	0	4	0	0	4	0	0	4	0	4	4	0
F. richardii	13	1	10	4	8	6	10	4	0	8	6	0	0	14	0	14	0	11	3	11	3	7	3	4	0	14	0	3	11	0	14	13	0
F. abyssinica	14	2	14	2	14	2	14	0	2	14	1	1	0	16	0	16	0	13	3	9	7	4	8	1	3	16	0	2	14	0	16	16	0
F. chodatiana	11	0	7	4	1	10	7	4	0	1	10	0	0	11	0	11	0	11	0	11	0	0	8	2	1	11	0	0	11	0	11	0	10

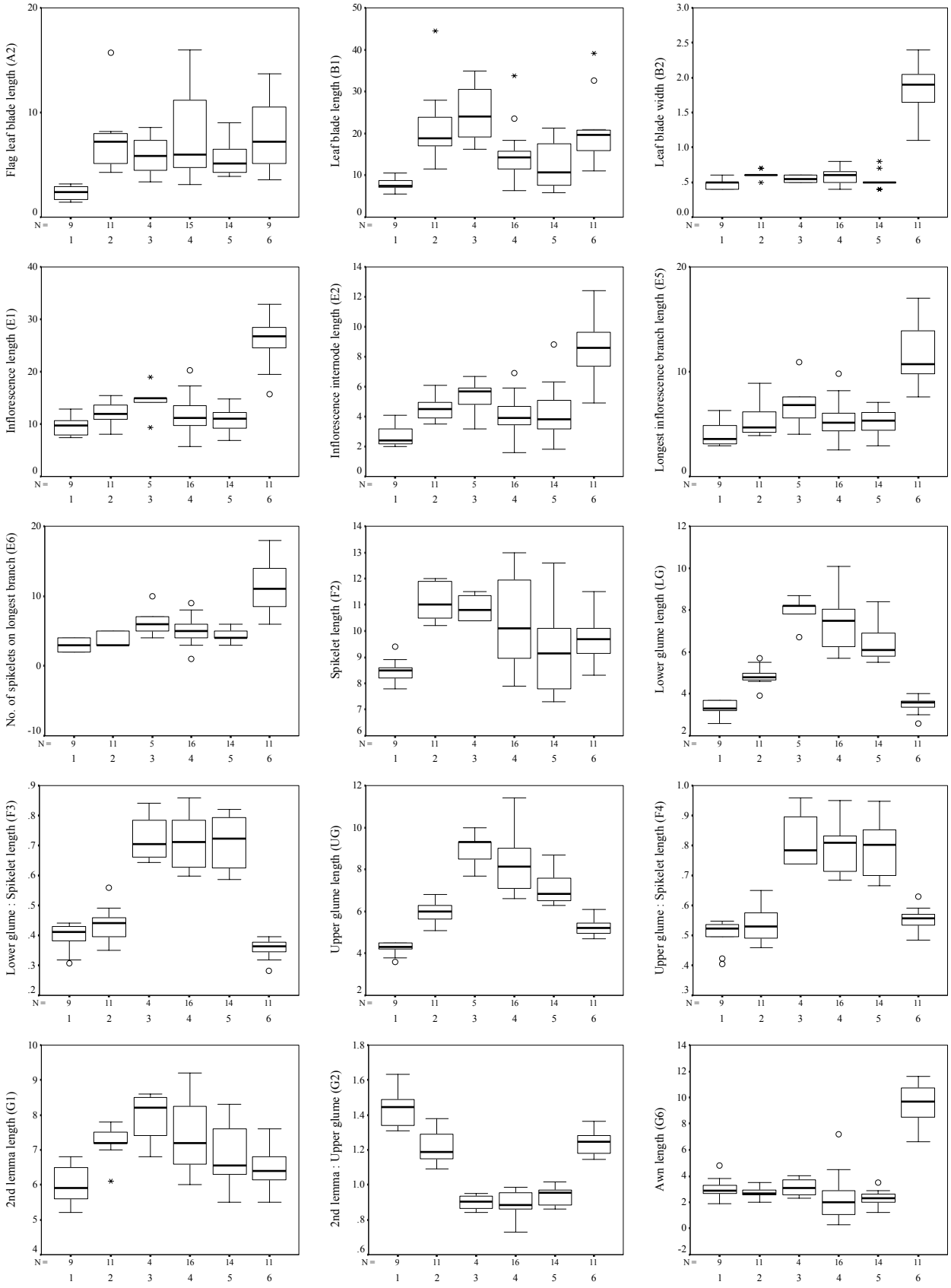

Figure 1. Box plots comparing six species of narrow-leaved African *Festuca* according to 15 quantitative morphological characters. Species' groups are given in the x-axis as 1 – *F. claytonii*, 2 – *F. pilgeri*, 3 – *F. elgonensis*, 4 – *F. abyssinica*, 5 – *F. richardii*, 6 – *F. chodatiana*. N – Number of accessions considered.

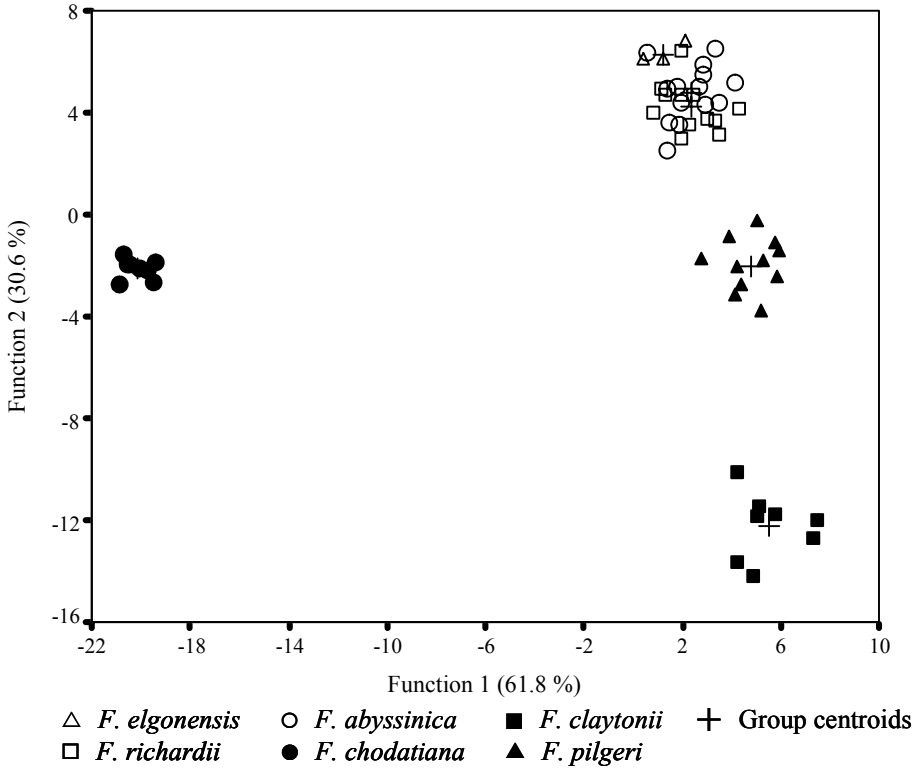

Figure 2. Discriminant analysis based on six groups of predicted species of narrow-leaved African *Festuca* and 27 quantitative morphological characters.

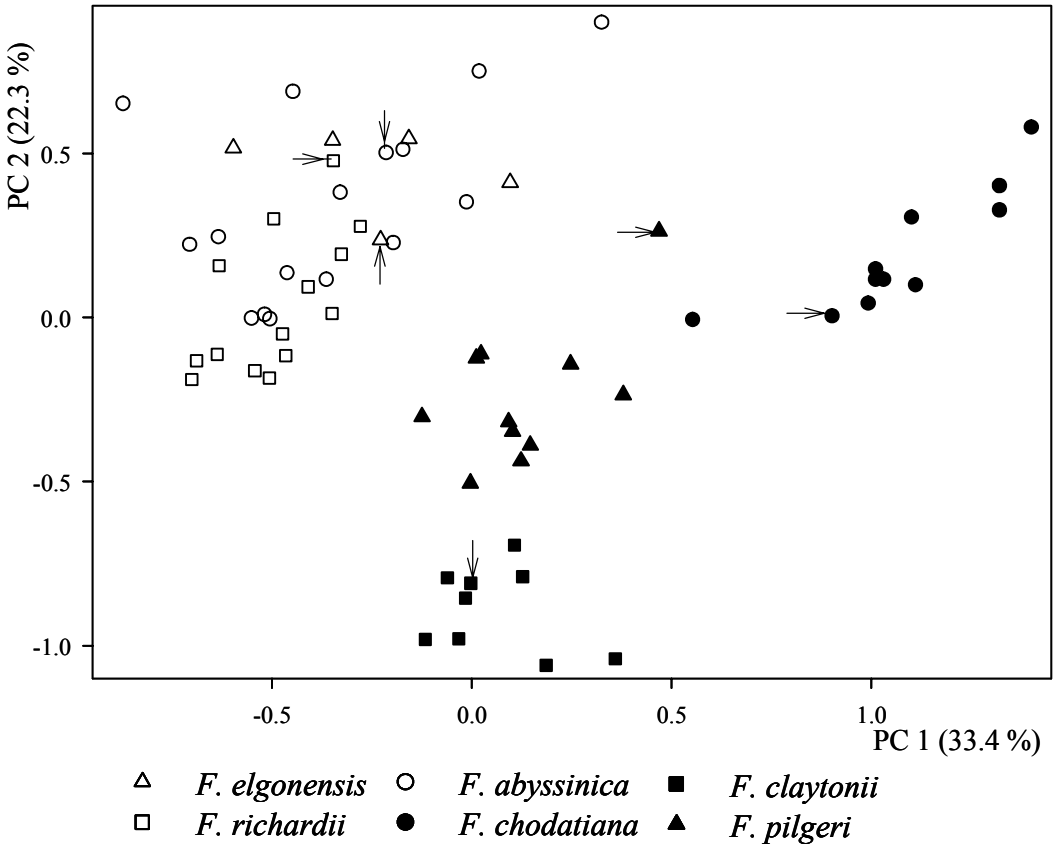

Figure 3. Principal components analysis of six species of narrow-leaved African *Festuca* based on 27 quantitative morphological characters. Arrows point to type specimens.

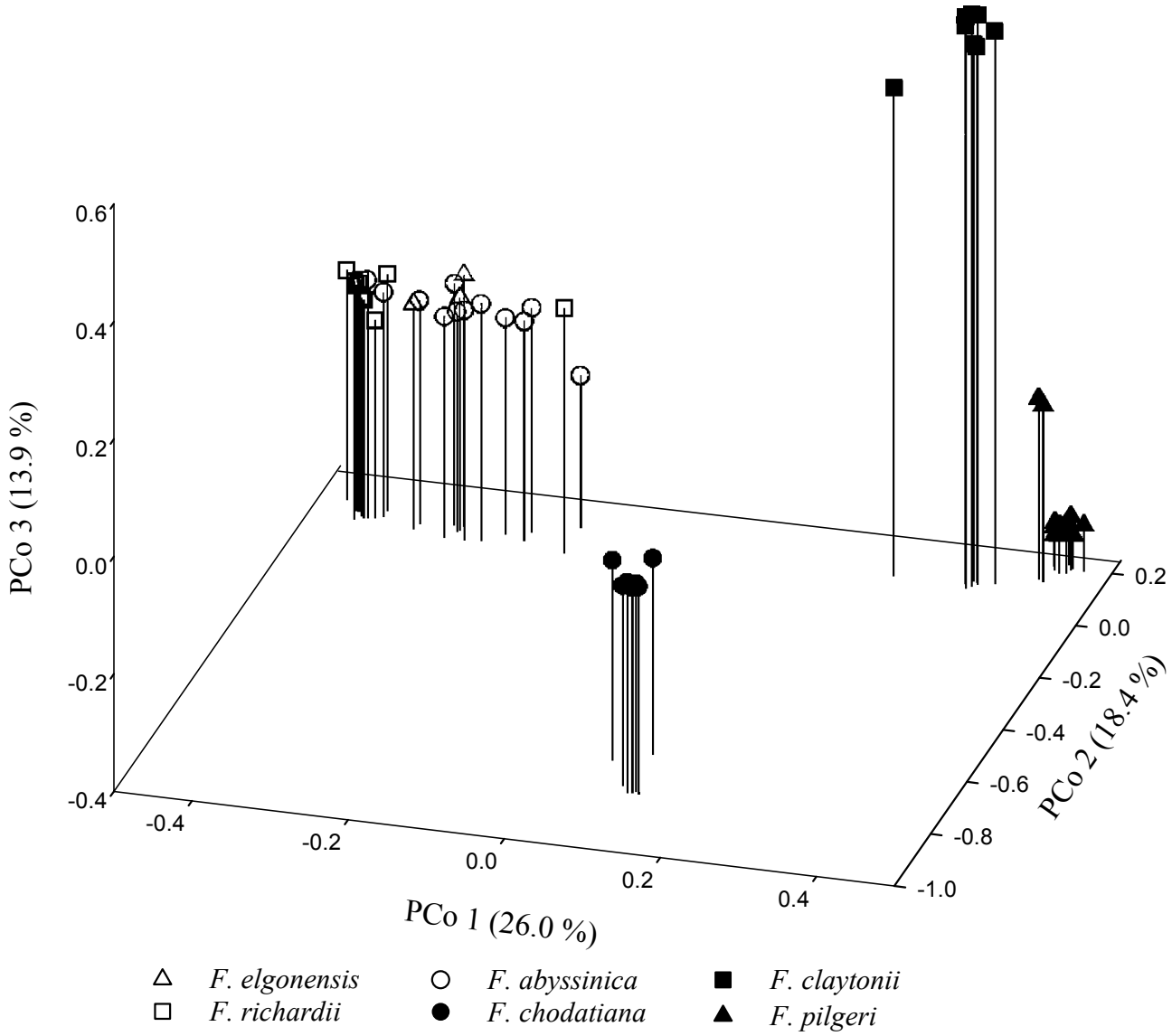

Figure 4. Principal coordinates analysis of 52 samples of narrow-leaved African *Festuca* based on 375 polymorphic AFLPs.

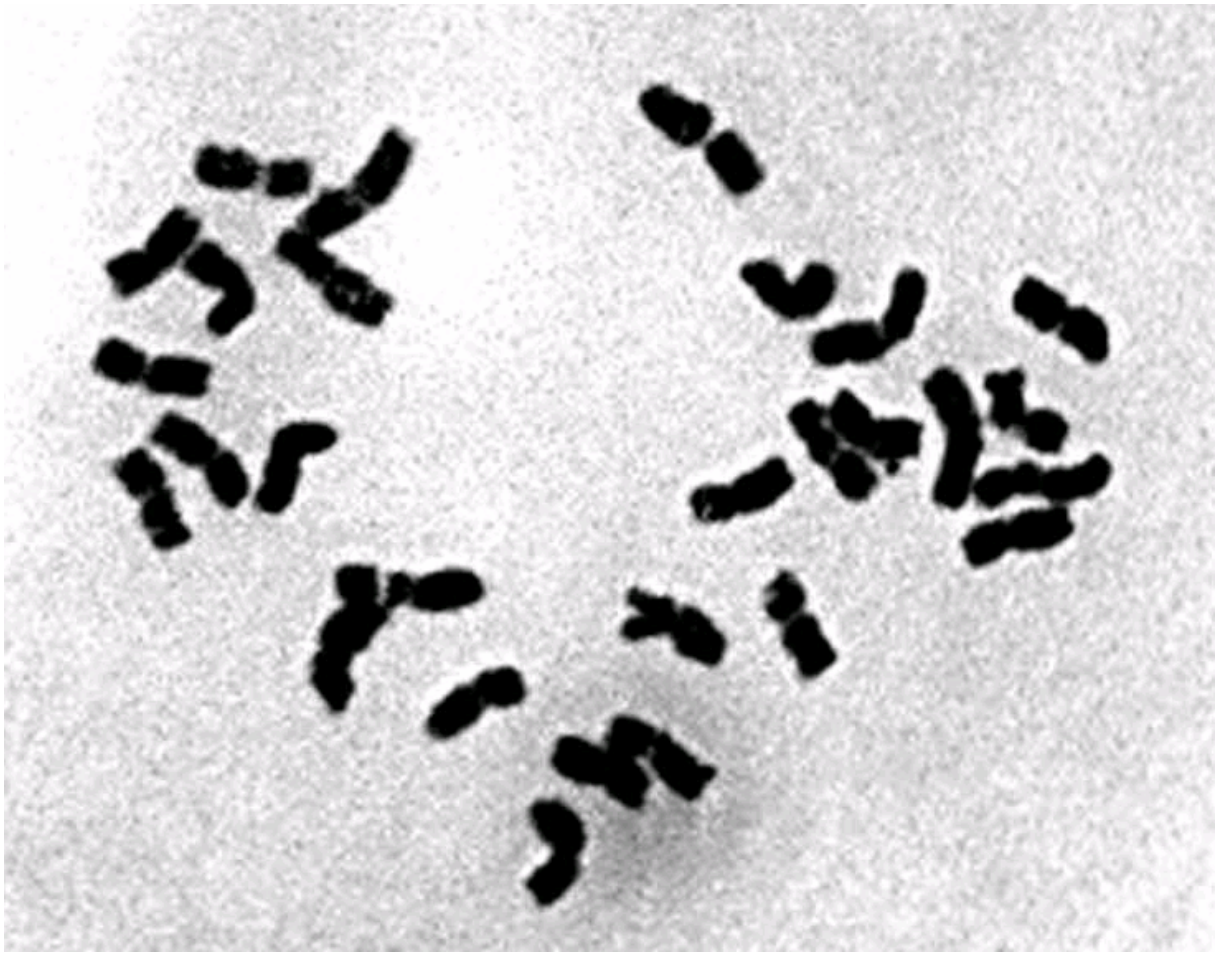

Figure 5. A cell showing mitotic metaphase chromosomes of *F. elgonensis* stained with acetocarmine.

Paper III

A taxonomic comparison between tropical African and related European broad-leaved species of *Festuca* L. (Poaceae)

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Summary

The four tropical African broad-leaved species *Festuca africana*, *F. simensis*, *F. engleri* and *F. mekiste* were analysed by morphological methods and compared to the European broad-leaved species *F. altissima* and *F. gigantea*. 31 qualitative and 31 quantitative morphological characters were measured and analysed by UPGMA, PCA, DA and box plots. *F. africana* and *F. altissima* were found to be morphologically clearly differentiated, whereas *F. simensis* and *F. gigantea* were quite close but distinct. As expected *F. engleri* was closely related to *F. africana*, and *F. mekiste* to *F. simensis* and *F. gigantea*. We confirm *F. africana* to be a good species in the genus *Festuca* and propose its inclusion into the subgenus *Drymanthele*, and *F. simensis* into the subgenus *Schedonorus*.

Keywords: Africa, Europe, broad-leaved *Festuca*, morphology, numerical taxonomy

Introduction

The genus *Festuca*, one of the largest grass genera with about 450 species, and with new species being periodically described (de Nova *et al*. 2006)is a morphologically variable genus for which no overall taxonomic treatment is available (Clayton and Renvoize 1986). Leaf-blade type is perhaps the most widely used line of variation, separating the 'fine-leaved' ('narrow-leaved') species from the 'broad-leaved' species. Use of the terms 'fine-leaved' and 'broad-leaved' in reference to *Festuca* was emphasised in the 1990s when molecular methods were found to separate *Festuca* into those two clades (e.g. Charmet *et al*. 1997). Before the molecular era in taxonomy, in for example floras (e.g. Clapham *et al*. 1962) *Festuca* was separated into those 'with setaceous leaves on sterile shoots' and those 'with flat or folded leaves of sterile shoots', but without defining the groups as fine- or broad-leaved. Markgraf-Dannenberg (1980) separated out species with 'wider leaves at least 5 mm wide' and in this category she only keyed out five species (*F. altissima* All., *F. drymeja* Mert. & W.D.J. Koch, *F. gigantea* (L.) Vill., *F. pratensis* Hudson and *F. arundinacea* Schreber) out of the 170 species she recognised for Europe, but she did not directly define these as the 'broad-leaved' species. To date, definitions for these terms are lacking despite being widely used, and that recent results show (e.g. Müller and Catalán 2006) the terms 'fine-' and 'broad-leaved' concur with how Clapham *et al*. (1962) separated the species. The term 'fine-leaved' includes those species with acicular, setaceous, rolled or filiform basal leaves and according to Clayton and Renvoize (1986) leaf-blades in *Festuca* are mostly rolled to filiform. The 'broad-leaved' species include those plants with flat or folded leaves but this can be misleading when dealing with dry herbarium material as narrow but flat leaves may roll up on drying.

Despite the opinions of Aiken *et al*. (1997), broad-leaved species of *Festuca* are genetically as well as morphologically more closely related to the genus *Lolium* than to *Festuca* s.s. (Darbyshire 1993, Lehväslaiho *et al*. 1987, Namaganda *et al*. 2006, Torrecilla and Catalán 2002), but for the sake of stability they are in this paper referred to as *Festuca*, although in future they may be transferred to either the genus *Lolium* or to smaller segregate genera. In tropical Africa, only four out of 21 species of *Festuca* (excluding the introduced *F. arundinacea*) are broad-leaved, that is with leaves wider than 2.5 mm. They are *F. africana*, *F. engleri*, *F. mekiste* and *F. simensis*. With a few exceptions, the genus *Festuca* is defined by 2 – several-flowered spikelets, 5-nerved lemmas and occasionally leaves with cross-nerves, e.g. in *F. gigantea* (Clayton & Renvoize, 1986). *F. africana* is rather anomalous in *Festuca*, commonly having 1-flowered spikelets, 3-nerved lemmas and cross-nerves in the leaves (Clayton and Renvoize 1986) and together with *F. engleri*, was earlier placed in the genus *Pseudobromus* (Clayton 1970, 1985, Schumann 1895). *F. engleri*, with its 2 – 4-flowered spikelets, 3 – 5-nerved lemmas and cross-nerves in the leaves, bridges *F. africana* to the rest of *Festuca* (Clayton, 1970; Clayton & Renvoize, 1986).

F. africana occurs in Sudan, Uganda, Kenya, Tanzania and southwards to South Africa, whereas *F. engleri* is recorded from Kenya, Tanzania and Malawi (Clayton, 1970). However, there is one collection of *F. engleri*, collected in 1935 from Uganda (Table 1) and kept at the British Museum in England which has not been documented before. Our efforts to re-collect it from its old locality (Namwamba valley, Mt. Rwenzori, Uganda) in 2007 were futile. *F. mekiste* occurs in Kenya, Ethiopia and Cameroon, and is rather similar to *F. engleri*, but the two species can easily be differentiated by the cross-nerves in the leaves of *F. engleri*, its obscurely scaberulous lemmas and hairy ovary-top, all characters lacking in *F. mekiste* (Clayton 1970, Phillips

1995). *F. simensis* occurs in Cameroon, the Democratic Republic of Congo, Sudan, Ethiopia, Uganda and Kenya. It is morphologically similar to *F. gigantea*, a species of Europe and temperate Asia (Clayton 1970). Via AFLP fingerprinting (Namaganda *et. al*. 2006), the latter two species were found to be fairly similar but with differences in chromosome numbers; *F. simensis* is tetraploid (2n = 4x = 28) whereas *F. gigantea* is hexaploid (2n = 6x = 42).

Namaganda *et al*. (2006) also found a close relationship between *F. africana* (2n = 10x = 70) and the European *F. altissima* (2n = 2x = 14). In this study we conducted a numerical taxonomic investigation to make a morphological comparison between *F. africana* and *F. altissima*, *F. simensis* and *F. gigantea*, and also to compare them to *F. mekiste* and *F. engleri*. According to molecular evidence, *F. altissima* links *F. africana* and *F. gigantea* links *F. simensis* to the European broad-leaved species: *F. pratensis*, *F. arundinacea* and *Lolium multiflora* (Namaganda et al. 2006). This is the first numeric taxonomic investigation on tropical African broad-leaved *Festuca*.

Materials and Methods

Plant material and morphological measurements

Six species represented by 88 specimens (Table 1) were used in this study. The plants were collected between the years 2003 and 2006 and are kept at the Makerere University Herbarium (MHU) except three collections, which were received on loan from the herbaria at the British Museum (BM) and the Norwegian University of Life Sciences (NLH). Herbarium acronyms are according to Holmgren (1998). The loaned specimens include *F. engleri* G. Taylor 3039 from BM, *F. altissima* A. Blytt s.n. and *F. gigantea* N. Wulfsberg s.n. from NLH (Table 1). Identifications of the specimens were verified based on material at MHU, KEW, NLH and EA. A total of 62 morphological

characters, 31 quantitative and 31 qualitative, were measured on the herbarium specimens (hereafter referred to as accessions). The list of characters (Table 2 & 3) was developed from literature (Aiken *et al*. 1997, Clayton 1970, Phillips 1995, Torrecilla *et al*. 2003) taking into consideration the characters that are used to differentiate the species especially those used for broad-leaved *Festuca* species. Spikelet measurements (F) were made on spikelets from either the second or third inflorescence nodes, that is the part which corresponds to the middle portion of the inflorescence. Floret measurements (G) were made on the first fertile florets of the spikelets measured in F. All florets in the spikelets are fertile except the uppermost one that is normally sterile or vestigial, represented only by a rhachilla extension. Awns were excluded when measuring the lengths of spikelets and lemmas. Recorded numbers of florets per spikelet include the uppermost sterile florets.

Data analysis

The qualitative and quantitative data were separately analysed by cluster analysis (Unweighted Pair Group Method with Arithmetic mean, UPGMA) using NTSYSpc 2.11f (Numerical Taxonomy and Multivariate Analysis System version 2.11f; Rohlf, 2000). The raw data were first standardised, then Manhattan and Euclidean distances respectively computed for the qualitative and quantitative data before generating the UPGMAs. Another analysis of the qualitative data was done with a Principal Coordinates analysis (PCoA) based on Manhattan similarities, and for the quantitative data a Principal Components Analysis (PCA) was generated from correlation coefficients. In both cases the analyses were done using NTSYSpc on standardised data. The quantitative data was further analysed by box plots and discriminant function analysis (DA) using SPSS (Statistical Package for Social Scientists, release 10.0.5;

Norusis 1999) in order to determine which quantitative characters best differentiate the species as well as determining how well the species fit in their designations.

Results

Qualitative morphology

The qualitative characters used were found to be useful in separating the species according to the UPGMA (Fig. 1). Figure 1 shows distinct clusters of the species except for *F. engleri*, which is embedded within the *F. africana* cluster. *F. mekiste* is shown to be more similar to the European *F. gigantea* than it is to *F. simensis* and these three species are more similar to *F. altissima* than they are to *F. africana* and *F. engleri*. According to the PCoA (Fig. 2) *F. altissima* is clearly differentiated, whereas *F. africana*, *F. gigantea* and *F. simensis* show continuous gradation into each other but with limited intermixing of accessions. *F. engleri* accessions are again embedded within *F. africana* accessions, and *F. mekiste* in *F. gigantea*. The first principal coordinate accounts for 44.1 % of the observed variation and is responsible for spreading out *F. simensis*, *F. gigantea* and *F. africana* whereas, the second principal coordinate accounts for 26.5 % of the observed variation and is responsible for separating out *F. altissima*.

Quantitative morphology

Although there was intermixing of a few accessions, the UPGMA based on quantitative data (figure not shown) was generally similar to the one based on qualitative data (Fig. 1) in showing *F. simensis* as similar to *F. gigantea*, *F. altissima* as more similar to both *F. simensis* and *F. gigantea* than to *F. africana* and *F. engleri* as similar to *F. africana*, while *F. mekiste* was shown to be very dissimilar from all the other species. The PCA (Fig. 3) is similar to the PCoA (Fig. 2) not only in showing *F. altissima* as a distinct

species but there is also a continuous gradation of *F. africana*, *F. gigantea* and *F. simensis*, again with minimum intermixing of accessions, but unlike in Fig. 2 *F. mekiste* is quite unique. The first principal component, which separates out *F. altissima*, accounts for 31.3 % of the observed variation and is highly correlated ($r \geq 0.75$, Table 4) with the characters E1, E2, E5, F3, F5, G7, G8 and G9 (see Table 2 character abbreviations). The second principal component, which spreads out *F. simensis*, *F. gigantea* and *F. africana*, accounts for 20.0 % of the observed variation and is highly correlated ($r \geq 0.75$, Table 4) with the characters B2, D4, F2 and F17.

Box plot analysis (Fig. 4) showed that the quantitative characters overlap a lot but the following interpretations can be made: D4 distinguishes both *F. simensis* and *F. gigantea* from the rest of the species but not between the two species. The most informative characters in differentiating between *F. simensis* and *F. gigantea* are F4 and F6. However, A3, B1, B2, E3, F2, G2, G7 and G12 can also be used although they show considerable overlap. According to the box plots, *F. africana* and *F. altissima* are clearly differentiated by the characters E1, E2, E5, F3, F17, G1, G7, G8 and G9, and although there is a small overlap, F5 is also a good character. The box plots reveal that *F. africana* and *F. altissima* are well differentiated unlike *F. simensis* and *F. gigantea*, which show considerable overlap in quantitative characters. Both *F. engleri* and *F. mekiste* mostly lie within the measurement ranges for the rest of the species.

The Discriminant Analysis (DA) (Fig. 5) shows that the species fit well within their taxonomic circumscriptions with *F. africana* and *F. altissima* clearly differentiated, but *F. engleri* is again shown to be close to *F. africana*. The close relationship between *F. simensis* and *F. gigantea* is confirmed, and *F. mekiste* is shown to be closely related to *F. gigantea*, which is in agreement with the qualitative morphology, Fig. 1. Function 1 of the DA accounts for 42.7 % of the observed variation

and correlates with the characters E4, G1, G8 and G9, whereas function 2 accounts for 36.3 % of the observed variation and correlates with characters D4, F2, F17 and G4. The DA is similar to the PCA in selecting D4, F2, F17, G8 and G9 as important characters in differentiating the six species.

Discussion

F. africana and *F. altissima*

According to our results, these two forest species are morphologically well differentiated although they showed a rather close genetic relationship (Namaganda *et al*. 2006). The morphological differences are the smaller inflorescence, spikelet and floret parts of *F. altissima* compared to *F. africana* as well as to the other species investigated as revealed by the box plots (Fig. 4). The most outstanding difference is that the lemmas of *F. altissima* terminate either into very short awn points or are awnless all together. Other qualitative differences are: in *F. altissima* the sheath margins are dentate, ligule margins fimbriate, and the callus is cylindrical. In all the other species, the sheath margins are smooth, ligule margins entire and the callus is wedge shaped. *F. africana* was the only species frequently found to possess hairy leaf sheaths, all the other species were glabrous. The 1(2)-flowered spikelets of *F. africana* compared to (1)2 – 3(4) florets (according to this study but 2 – 5 from literature) in *F. altissima* is not a good difference because of the overlap, yet the 1-flowered spikelets are emphasised in literature as an unusual character of *F. africana* in the genus *Festuca*. Moreover another species, *F. monantha* Stapf is also cited as having 1-flowered spikelets (Clayton and Renvoize 1986). In addition, *F. altissima* has generally smaller vegetative parts and this is perhaps because it is a diploid whereas *F. africana* is a decaploid.

The reference to *F. africana* as a somewhat aberrant species in *Festuca* (Clayton & Renvoize 1986) must be abandoned and instead *F. africana* should be accepted into the mainly temperate subgenus *Drymanthele* (which includes *F. altissima*). The subgenus *Drymanthele* is characterised by the absence of auricles, truncate ligules, hairy ovary top, keeled lemmas and anthers 2.5 – 3.5 mm long (Clayton and Renvoize 1986, Krechetovich and Bobrov 1934), all of which are characters possessed by *F. africana*. Noteworthy is that we found anther length in *F. africana* to be 2.2 – 4.3 mm in this study but it is 3 – 4 mm according to Clayton (1970). Characteristics of the subgenus *Drymanthele*, as given by Krechetovich and Bobrov (1934) and Clayton and Renvoize (1986), that are not possessed by *F. africana* are: awnless or obscurely awned lemmas (awns 7.2 – 17.7 mm long in *F. africana*), lemma length about 5 mm long (6.6 – 10 mm in *F. africana*), lemma prominently 5-nerved (3 – 5-nerved in *F. africana* and 3-nerved in *F. altissima*), and presence of scaly leafless sheaths on culm bases and rhizomes. Given the taxonomic account discussed above and also the molecular results of Namaganda *et al*. (2006), we propose following the genetic grouping revealed by molecular analysis as the basis for classifying *F. africana* and *F. altissima* in the same subgenus (Fjellheim *et al*. 2001, Hansen *et al*. 2000).

Both qualitative and quantitative characters show *F. africana* and *F. engleri* to be close and most interesting is the cluster analysis of qualitative characters, which shows both *F. africana* and *F. engleri* to belong to one cluster and the rest of the *Festuca* to another. This is in agreement with an earlier classification which included *F. africana* and *F. engleri* in a separate genus *Pseudobromus* (Clayton 1970); possibly this classification relied on qualitative characters alone. Like *F. africana*, *F. engleri* should also be included in subgenus *Drymanthele*.

F simensis* and *F. gigantea

Both the qualitative and quantitative characters are in accordance with Namaganda *et al*. (2006) and Clayton (1970), confirming the close relationship between *F. simensis* and *F. gigantea*. However, cytological evidence to determine the genome composition and relatedness of *F. simensis* to *F. gigantea* is lacking and is only limited to chromosome numbers: *F. simensis* is tetraploid whereas *F. gigantea* is hexaploid. It seems sufficient based on the available molecular and morphological data to propose the inclusion of *F. simensis* into the mainly temperate subgenus *Schedonorus*, which includes *F. gigantea* among other species. Krechetovich and Bobrov (1934) included *F. gigantea* in the subgenus *Drymonaetes*, but more recent authors (Catalán *et al*. 2004, Darbyshire and Warwick 1992) have followed the taxonomy of Alexeev (summarised by Darbyshire and Warwick 1992) that includes *F. gigantea* in the subgenus *Schedonorus* section *Plantynia*. The subgenus *Schedonorus* is characterised by the presence of auricles, the culm-bases not clad in papery scales, the rounded lemma and glabrous ovary (Clayton and Renvoize 1986) and *F. simensis* possesses all these characters.

The subgenus *Schedonorus* is the group of broad-leaved *Festuca* that is most closely related to the genus *Lolium* as shown by DNA (e.g. Darbyshire and Warwick 1992, de Nova *et al*. 2006, Müller and Catalán 2006, Namaganda *et al*. 2006) and seed protein electrophoresis (Bulińska-Radomska and Lester 1988). However, Aiken *et al*. (1997) argue based on morphological evidence, that the broad-leaved species of *Festuca* (subgenera *Leucopoa*, *Schedonorus* and *Subulatae*) should be retained in the genus *Festuca* and separate from the genus *Lolium*. Other authors (Clapham *et al*. 1962, Clayton 1970, Clayton and Renvoize 1986) emphasise morphological evidence from a single inflorescence character to keep *Festuca* and *Lolium* separate, but the use of a

combination of characters is definitely a more reliable approach in designating genera and species.

According to this study, although there is some overlap in morphology between *F. simensis* and *F. gigantea*, the two species are distinct. Some differences worth noting: *F. simensis* generally has narrower flag leaves and leaf blades, smaller ratios of lower glumes to spikelet length and upper glumes to spikelet length, and longer anthers (see box plots, Fig. 4). Qualitative differences include: absence of filiform teeth on lemmas of *F. simensis* (present in *F. gigantea*), shape of ligules (mainly irregular in *F. simensis*, rounded in *F. gigantea*), spikelets oblong in shape and tinged purple in *F. simensis* (elliptic and usually green in *F. gigantea*). The most outstanding similarities include: presence of falcate auricles, glabrous ovaries, and sub-terminal awns.

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References

Aiken, S.G., Dallwitz, M.J., McJannet, C.L., Consaul, L.L. 1997. Biodiversity among *Festuca* (Poaceae) in North America: diagnostic evidence from DELTA and clustering programs, and an INTKEY package for interactive, illustrated

identification and information retrieval. Canadian Journal of Botany 75, 1527-1555.

Bulińska-Radomska, Z., Lester, R.N. 1988. Intergeneric relationships of *Lolium*, *Festuca*, and *Vulpia* (Poaceae) and their phylogeny. Plant Systematics and Evolution 159, 217-227.

Catalán, P., Torrecilla, P., Rodríguez, J.A.L., Olmstead, R.G. 2004. Phylogeny of festucoid grasses of subtribe Loliinae and allies (Poeae, Pooideae) inferred from ITS and *trn*L-F sequences. Molecular Phylogenetics and Evolution 31, 517-541.

Charmet, G., Ravel, C., Balfourier, F. 1997. Phylogenetic analysis in the *Festuca-Lolium* complex using molecular markers and ITS rDNA. Theoretical and Applied Genetics 94, 1038-1046.

Clapham, A.R., Tutin, T.G., Warburg, E.F. 1962. Flora of the British Isles. Cambridge University Press, Cambridge, pp 1125-1130.

Clayton, W.D. 1970. Gramineae Part 1. In: Milne-Redhead, E., Polhill, R.M. (eds) Flora of Tropical East Africa. Crown agents for oversea governments and administrations, London, pp 56-63.

Clayton, W.D. 1985. Miscellaneous notes on Pooid grasses. Kew Bulletin 40, 727-729.

Clayton, W.D., Renvoize, S.A. 1986. *Genera Graminum: Grasses of the World*. HMSO, London, pp 374-375.

Darbyshire, S.J. 1993. Realignment of *Festuca* subgenus Schedonorus with the genus *Lolium* (Poaceae). Novon 3, 239-243.

Darbyshire, S.J., Warwick, S.I. 1992. Phylogeny of north American *Festuca* (Poaceae) and related genera using chloroplast DNA restriction site variation. Canadian Journal of Botany 70, 2415-2429.

de Nova, P.J.G., de la Cruz, M., Monte, J.V., Soler, C. 2006. Genetic relationships within and among Iberian fescues (*Festuca* L.) based on PCR-amplified markers. Genome 49, 1170-1183.

Fjellheim, S., Elven, R., Brochmann, C. 2001. Molecules and morphology in concert. II. The *Festuca brachyphylla* complex (Poaceae) in Svalbard. American Journal of Botany 88, 869-882.

Hansen, K.T., Elven, R., Brochmann, C. 2000. Molecules and morphology in concert: tests of some hypotheses in arctic *Potentilla* (Rosaceae). American Journal of Botany 87, 1466-1479.

Holmgren, P.K., Holmgren, N.H. 1998. Index Herbariorum. New York Botanical Garden. http://sciweb.nybg.org/science2/IndexHerbariorum.asp

Krechetovich, V.I., Bobrov, E.G. 1934. *Festuca* L. s.str. In: Komarov, V.L., Rozhevits, R.Y., Shishkin, B.K. (eds) Flora of the U.S.S.R. Vol. 2. pp 497-535. Translations for the National Science Foundation (1965), Israel Program for Scientific Translations, Jerusalem. pp 395-425.

Lehväslaiho, H., Saura, A., Lokki, J. 1987. Chloroplast DNA variation in the grass tribe Festuceae. Theoretical and Applied Genetics 74, 298-302.

Markgraf-Dannenberg, I. 1980. *Festuca* L. In: Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M., Webb, D.A. (eds) Flora Europaea, 5. Alismataceae to Orchidaceae (Monocotyledons). Cambridge University Press, Cambridge, pp 125-153.

Müller, J., Catalán, P. 2006. Notes on the infrageneric classification of *Festuca* L. (Gramineae). Taxon 55, 139-144.

Namaganda, M., Lye, K.A., Friebe, B., Heun, M. 2006. AFLP-based differentiation of tropical African *Festuca* species compared to the European *Festuca* complex. Theoretical and Applied Genetics 113, 1529-1538.

Norusis, M.J. 1999. SPSS for Windows, release 10.0.5. SPSS Inc., Chicago.

Phillips, S. 1995. Poaceae. In: Hedberg, I., Edwards, S. (eds.) Flora of Ethiopia and Eritrea, Vol. 7. Addis Ababa, Ethiopia; Uppsala, Sweden, pp 23-27.

Rohlf, F.J. 2000. NTSYSpc: numerical taxonomy and multivariate analysis system, version 2.11f. Exeter Software, New York.

Schumann, K. 1895. Reihe: Glumiflorae. In: Engler, A. (ed.) Die Pflanzenwelt Ost-Afrikas und der Nachbargebiete. Geographische Verlagshandlung Dietrich Reimer, Berlin, pp 95-130.

Torrecilla, P., Catalán, P. 2002. Phylogeny of broad-leaved and fine-leaved *Festuca* lineages (Poaceae) based on nuclear ITS sequences. Systematic Botany 27, 214-251.

Torrecilla, P., Rodriguez, J.A.L., Stancik, D., Catalan, P. 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. Plant Systematics and Evolution 239, 113-139.

Table 1. Accessions used in the study. Collectors: MN = M. Namaganda, GT = G. Taylor, AB = A. Blytt, NW = N. Wulfsberg. Voucher specimens collected by MN are kept at the Makerere University Herbarium (MHU). Accessions marked with an asterisk were used in the AFLP analysis of Namaganda *et al.* 2006.

Species	**Collection data** (country, geographical division, locality, coordinates, altitude)	**Collector & collection number**	**No. of specimens measured**
F. africana (Hack.) Clayton	Uganda. U3. Elgon, Sasa River camp, 1°10.3'N 34°26.3'E, 2830 m	MN 1585*	1
	Uganda. U3. Elgon, Sasa trail, 1°10.5'N 34°25.0'E, 2500 m	MN 1692	2
	Uganda. U3. Elgon, forest zone, 1°10.5'N 34°25.0'E, 2600 m	MN 1388	2
	Uganda. U3. Elgon, *Hagenia* forest, 1°10.5'N 34°25.0'E, 2550 m	MN 1338	2
	Uganda. U3. Elgon, Sasa trail, forest, 1°10.5'N 34°25.0'E, 2600 m	MN 1688	1
	Uganda. U3. Elgon, Sasa trail, forest, 1°10.5'N 34°25.0'E, 2550 m	MN 1691	2
	Uganda. U2. Gahinga, *Hypericum* forest, 1°22.9'S 29°38.5'E, 3170 m	MN 1603*	1
	Uganda. U2. Gahinga, 1°22.9'S 29°38.5'E, 3260 m	MN 1407	2
	Uganda. U2. Rwenzori Mts., Bwamba Pass, 1°40.0'N 30°08.0'E, 2430 m	MN 1394*	2
	Uganda. U2. Rwenzori Mts., Bwamba Pass, 1°40.0'N 30°08.0'E, 2600 m	MN 1395*	1
	Uganda. U2. Bwindi forest, roadside, 1°05.9'S 29°48.7'E, 2228 m	MN 1611	1
	Uganda. U2. Bwindi forest, roadside, 1°05.5'S 29°48.0'E, 2550 m	MN 1716	1
	Kenya. K4. Mt. Kenya, Sirimon, near camp site, 0°00.3'N 37°14.3'E, 2547 m	MN 1738	1
	Kenya. K3. Saiwa swamp National Park, forest, 1°10.3'N 35°04.2'E, 1950 m	MN 1727	1
F. simensis A. Rich.	Uganda. U3. Elgon, Mongongo cave, 1°09.3'N 34°30.8'E, 3780 m	MN 1577*	1
	Uganda. U3. Elgon, Sasa trail, 1°10.0'N 34°26.0'E, 2800 m	MN 1664	1
	Uganda. U3. Elgon, Bumagaba, towards Sasa trail, 1°08.5'N 34°25.5'E, 2000 m	MN 1333	1
	Uganda. U3. Elgon, Sasa trail, 1°10.6'N 34°27.0'E, 3100 m	MN 1345	1
	Uganda. U3. Elgon, Sasa trail, *Hagenia* forest, 1°10.5'N 34°25.0'E, 2550 m	MN 1690	1
	Uganda. U3. Elgon, Mongongo cave, 1°09.3'N 34°30.8'E, 3780 m	MN 1680	1
	Uganda. U3. Elgon, lower slopes, 1°08.5'N 34°25.5'E, 1900 m	MN 1693	1
	Uganda. U3. Elgon, Sasa trail near park boundary, 1°10.5'N 34°23.5'E, 1770 m	MN 1694	1

	Uganda. U2. Gahinga, regenerating forest, 1°21.7'S 29°37.7'E, 2490 m	MN 1601*	1
	Uganda. U2. Muhavura, regenerating forest, 1°21.6'S 29°39.8'E, 2530 m	MN 1589*	1
	Uganda. U2. Muhavura, montane forest, 1°22.2'S 29°40.1'E, 2990 m	MN 1591*	1
	Uganda. U2. Muhavura, regenerating forest, 1°21.6'S 29°39.8'E, 2350 m	MN 1699	1
	Uganda. U2. Echuya, Kabale – Kisoro boarder, 1°15.4'S 29°47.8'E, 2280 m	MN 1608*	1
	Uganda. U2. Echuya, near swamp, 1°15.4'S 29°47.8'E, 2280 m	MN 1401	1
	Uganda. U2. Echuya, near swamp, 1°15.4'S 29°47.8'E, 2280 m	MN 1710	1
	Uganda. U2. Bwindi forest, Ndeego, 1°05.5'S 29°48.5'E, 2550 m	MN 1398*	2
	Uganda. U2. Bwindi, along forest road, 1°05.5'S 29°48.0'E, 2550 m	MN 1714	1
	Uganda. U2. Bwindi, dense vegetation by roadside, 1°05.5'S 29°48.0'E, 2550 m	MN 1712	1
	Uganda. U2. Rwenzori Mts., Bwamba Pass, 1°40.0'N 30°08.0'E, 2100 m	MN 1390*	1
	Kenya. K4. Mt. Kenya, Met station, 0°11.3'S 37°15.5'E, 3800 m	MN 1750	1
	Kenya. K3. Mt. Elgon, Endebess bluff trail, ca. 1°05.9'N 34°41.9'E, 2460 m	MN 1730	1
F. mekiste Clayton	Kenya. K3. Mt. Elgon, bamboo forest, roadside, 1°04.0'N 34°43.0'E, 2710 m	MN 1734	1
	Kenya. K3. Mt. Elgon, Kambi Mtamaiwa, 1°03.0'N 34°42.0'E, 2828 m	MN 1736	1
F. engleri Pilg.	Kenya. K4. Mt Kenya, Sirimon, near stream, 0°00.3'S 37°14.0'E, 2535 m	MN 1739	1
	Uganda. U2. Rwenzori, Namwamba valley, ca. 0°14.3'N 29°56.5'E, 2740 m	GT 3039	1
F. altissima All.	Norway. Østfold. Moss kommune, Jeløya, 59°27.1'N 10°37.8'E, 120 m	MN 1505*	1
	Norway. Vestfold. Horten kommune, Vegge, 59°26.4'N 10°24.5'E, 85 m	MN 1761a	1
	Norway. Akershus. Frogn kommune, Hallangen, 59°41.9'N 10°36.5'E, 80 m	MN 1758a	1
	Norway. Østfold. Hobøl kommune, Bærøe, 59°33.8'N 10°56.3'E, 130 m	MN 1756a, b, c	3
	Norway. Vestfold. Larvik kommune, Kvelde, 59°11.6'N 9°56.5'E, 125 m	MN 1764a, b, c	3
	Sweden. Bohuslän. Strömstad kommune, Tveten, 59°03'N 11°17'E, 150 m	MN 1772a, b	2
	Norway. Østfold. Moss kommune, Jeløya, 59°27.1'N 10°37.8'E, 120 m	MN 1754a, b, c	3
	Norway. Buskerud. Hurum kommune, Sætre, 59°40.6'N 10°32.2'E, 5 m	MN 1767b, c	2
	Norway. Vest Agder. Søgne kommune, Ospedal, 58°05.8'N 7°40.2'E, 40 m	MN 1770a, c	2
	Norway. Telemark. Porsgrunn, Bjønnes-Solvika, 59°03.2'N 9°46.1'E, 30 m	MN 1763b	1
	Norway. Buskerud. Lier kommune, Glitreelven, ca. 59°51'N 10°10'E	AB s.n.	1
F. gigantea (L.) Vill.	Norway. Asker kommune, Semsvatn, 59°51.3'N 10°24.5'E, 150 m	MN 1503*	1
	Sweden. Bohuslän. Uddevalla kommune, 58°19.9'N 11°54.9'E, 15 m	MN 1773b	1

Norway. Vest Agder. Kristiansand, Ramnedalen, 58°09.4'N 7°58.5'E, 50 m	MN 1771a	1
Norway. Hordaland. Etne kommune, Stordalsvatn, 59°42.5'N 6°04.3'E, 85 m	MN 1769c	1
Norway. Telemark. Tokke kommune, Dalen, 59°26.8'N 8°01.0'E, 75 m	MN 1768a, b	2
Norway. Buskerud. Hurum kommune, Sætre, 59°40.6'N 10°32.2'E, 3 m	MN 1767a	1
Norway. Buskerud. Hurum kommune, Sagene, 59°32.2'N 10°31.4'E, 5 m	MN 1766c	1
Norway. Vestfold. Larvik kommune, Kvelde, 59°11.6'N 9°56.5'E, 125 m	MN 1765a, b	2
Norway. Telemark. Bamble kommune, Ris, 59°01.1'N 9°41.0'E, 25 m	MN 1762a, c	2
Norway. Vestfold. Horten kommune, 59°25.6'N 10°27.9'E, 3 m	MN 1760a, b	2
Norway. Akershus. Frogn kommune, Hallangen, 59°41.9'N 10°36.5'E, 80 m	MN 1759a	1
Norway. Østfold. Moss kommune, Jeløya, 59°26.6'N 10°38.1'E, 80 m	MN 1757a, c	2
Norway. Akershus. Frogn kommune, Drøbak, 59°40.2'N 10°38.2'E, 90 m	MN 1755b, d	2
Norway. Akershus. Asker kommune, Semsvatn, 59°51.3'N 10°24.5'E, 160 m	MN 1752a, c	2
Norway. Hordaland. Kvam kommune, Tangerås, 60°13.9'N 5°59.9'E	NW s.n.	1

Table 2: List of quantitative morphological characters measured

Feature	Abbreviation	Character
Culm	A1	Length (cm)
	A2	Length of flag leaf blade (cm)
	A3	Width of flag leaf blade (mm)
	A4	Length of uppermost internode (cm)
	A5	Ratio of length of uppermost internode : Length of culm (A4:A1)
	A6	Number of internodes
Leaf blade	B1	Length (cm)
	B2	Width (mm)
Sheath	C1	Length (cm)
Ligule	D4	Length (mm)
Inflorescence	E1	Length (cm)
	E2	Length of lowermost internode (cm)
	E3	Ratio of length of lowermost internode : Length of inflorescence (E2:E1)
	E4	Number of branches at lowermost node
	E5	Length of longest branch at lower most node (cm)
	E6	Number of spikelets on E5
Spikelet	F2	Length of spikelet – excluding awn (mm)
	F3	Length of lower glume (mm)
	F4	Ratio of length of lower glume : Length of spikelet (F3:F2)
	F5	Length of upper glume (mm)
	F6	Ratio of length of upper glume : Length of spikelet (F5:F2)
	F7	Number of nerves on lower glume
	F8	Number of nerves on upper glume
	F17	Number of florets per spikelet
Floret	G1	Length of second lemma – excluding awn (mm)
	G2	Ratio of length of second lemma : Length of upper glume (G1:UG)
	G4	Number of nerves on lemmas
	G7	Length of filliform teeth on lemma or lemma extension if awn subterminal (mm)
	G8	Length of palea (mm)
	G9	Length of awn (mm)
	G12	Length of anthers (mm)

Table 3: List of qualitative morphological characters scored

Feature	Abbreviation	Character	Character state	Code
Leaf blade	B3	Adaxial pubescence	Absent	0
			Present	1
	B4	Abaxial pubescence	Absent	0
			Present	2
	B5	Type of hairs	Micro	0
			Macro	1
			Prickles	2
			Trichomes	3
			Macro & Prickles	4
	B6	Colour (abaxial)	Dark green	0
			Lime	1
	B7	Colour (adaxial)	Pale dark green	0
			Pale lime	1
	B8	Falcate auricles	Absent	0
			Present	1
	B9	Cross-nerves	Absent	0
			Present	1
Sheath	C2	Abundance of anthocyanins	Absent	0
			Traces	1
			Abundant	2
	C3	Abundance of hairs	Absent	0
			Rare	1
			Abundant	2
	C4	Type of hairs	Micro	0
			Macro	1
			Prickles	2
			Trichomes	3
			Macro & Prickles	4
	C5	Margins	Smooth	0
			Dentate	1
Ligule	D1	Shape	Irregular	0
			Flat	1
			Rounded	2
	D2	Margin	Entire	0
			Fimbriate	1
	D3	Hairs on margin	Absent	0
			Sparse	1
Inflorescence	E7	Scabrousness (lowest internode)	None	0
			In upper part	1
			Everywhere	2
Spikelets	F9	Scabrousness on keel of lower glume	Absent	0
			Present	2
	F10	Scabrousness on keel of upper glume	Absent	0
			Present	2

	F11	Distribution of scabrousness in F9	None	0
			Up to ¼	1
			Up to ½	2
			Up to ¾	3
	F12	Distribution of scabrousness in F10	None	0
			Up to ¼	1
			Up to ½	2
			Up to ¾	3
	F13	Pubescence on lower glume	Absent	0
			Present	1
	F14	Pubescence on upper glume	Absent	0
			Present	1
	F15	Shape of lower glume	Lanceolate	0
			Linear-lanceolate	1
			Oblong-lanceolate	2
	F16	Shape of upper glume	Lanceolate	0
			Linear-lanceolate	1
			Oblong-lanceolate	2
	F18	Colour of spikelet	Green	0
			Green tinged purple	1
			Green and purple	2
	F19	Shape of spikelet	Linear	0
			Linear-oblong	1
			Oblong	2
			Elliptic	3
	F20	Shape of callus	Wedge shaped	0
			Cylindrical	1
Floret	G3	Pubescence on lemmas	Absent	0
			Present	1
	G5	Shape of lemma	Oblong-elliptic	0
			Linear-lanceolate	1
			Lanceolate	2
	G6	Filiform teeth on lemmas	Absent	0
			Present	1
	G10	Position of awn	Sub-terminal	0
			Terminal	1
	G11	Pubescence on ovary top	Absent	0
			Present, distinct	2

Table 4: Results of the first three principal components (PC 1, PC 2, PC 3) of the PCA for 88 accessions of six broad-leaved *Festuca* species and 31 quantitative morphological characters. Bold face values were highly correlated ($r \geq 0.75$) with the principal components. See Table 2 for character abbreviations.

Character	**PC 1**	**PC 2**	**PC 3**
A1	0.42	0.33	0.38
A2	0.43	0.62	-0.16
A3	0.38	0.73	-0.02
A4	0.36	0.26	0.36
A5	-0.08	-0.13	0.21
A6	0.69	-0.08	-0.08
B1	0.52	0.43	0.20
B2	0.27	**0.81**	0.07
C1	0.25	0.24	0.62
D4	0.05	**0.79**	-0.29
E1	**0.89**	0.17	-0.00
E2	**0.86**	0.13	-0.25
E3	0.68	-0.11	-0.32
E4	0.34	0.50	-0.47
E5	**0.87**	0.21	-0.13
E6	0.11	0.57	0.25
F2	0.57	**-0.75**	-0.08
F3	**0.81**	-0.20	0.25
F4	0.27	0.66	0.27
F5	**0.79**	-0.31	0.36
F6	0.20	0.61	0.44
F7	0.39	0.08	0.22
F8	0.60	-0.27	0.06
F17	0.14	**-0.78**	0.29
G1	0.68	-0.33	-0.28
G2	-0.27	0.12	-0.72
G4	0.48	-0.63	0.46
G7	**0.80**	-0.21	-0.23
G8	**0.89**	-0.20	-0.14
G9	**0.82**	-0.25	-0.17
G12	0.40	-0.02	-0.66

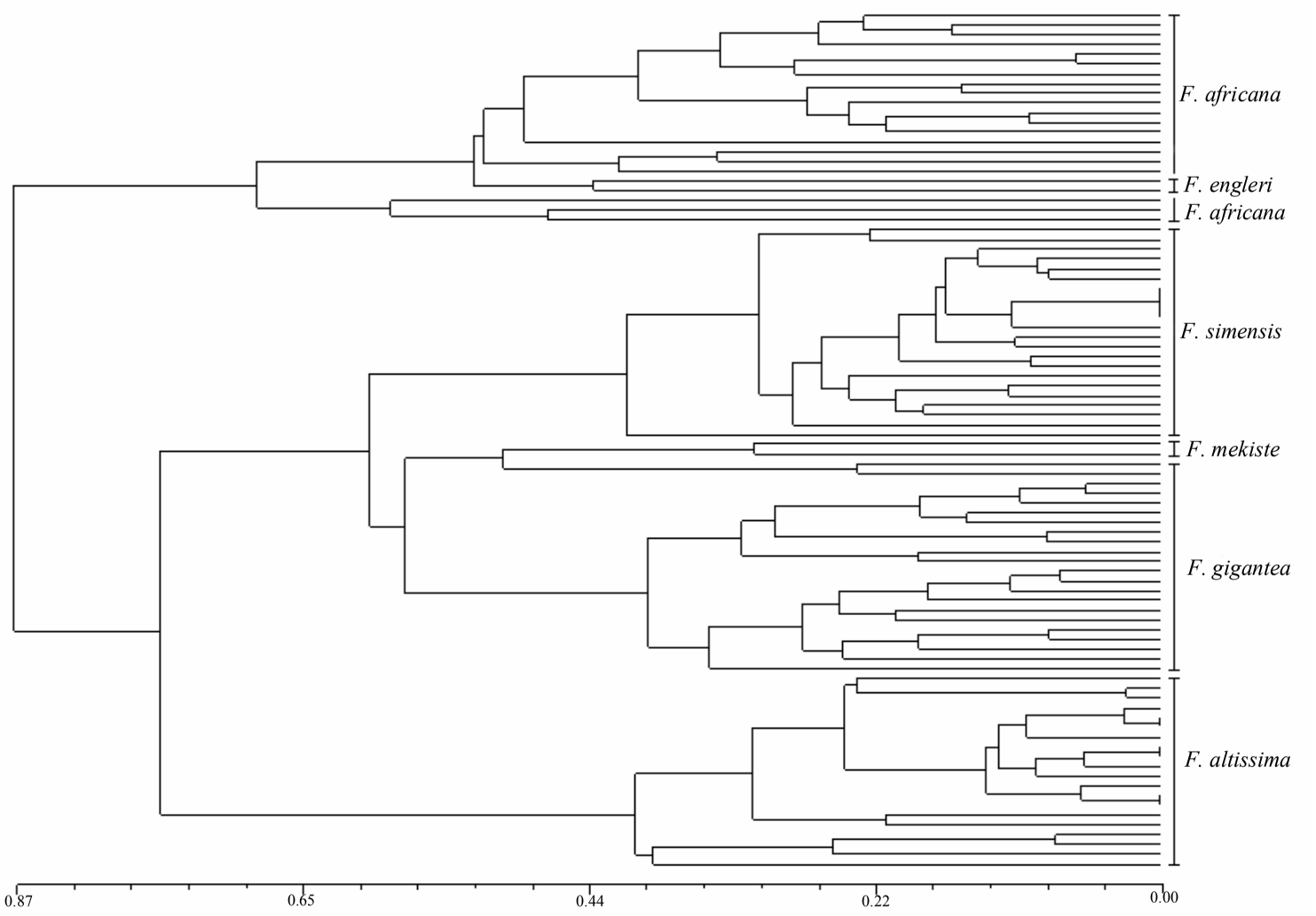

Figure 1. A UPGMA derived from qualitative data based on morphological measurements of 31 characters and 88 accessions. The scale represents Manhattan distance.

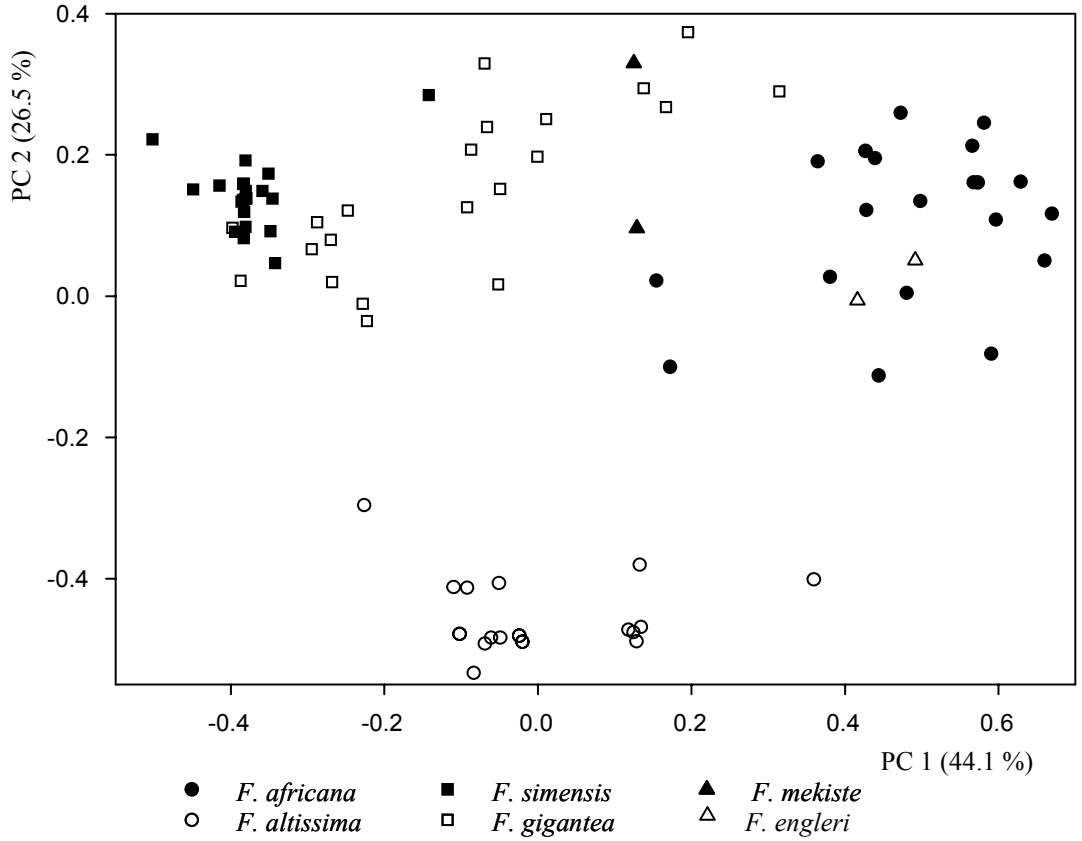

Figure 2. A Principal coordinate analysis (PCoA) of 88 accessions of broad-leaved *Festuca* derived from measurements of 31 qualitative characters.

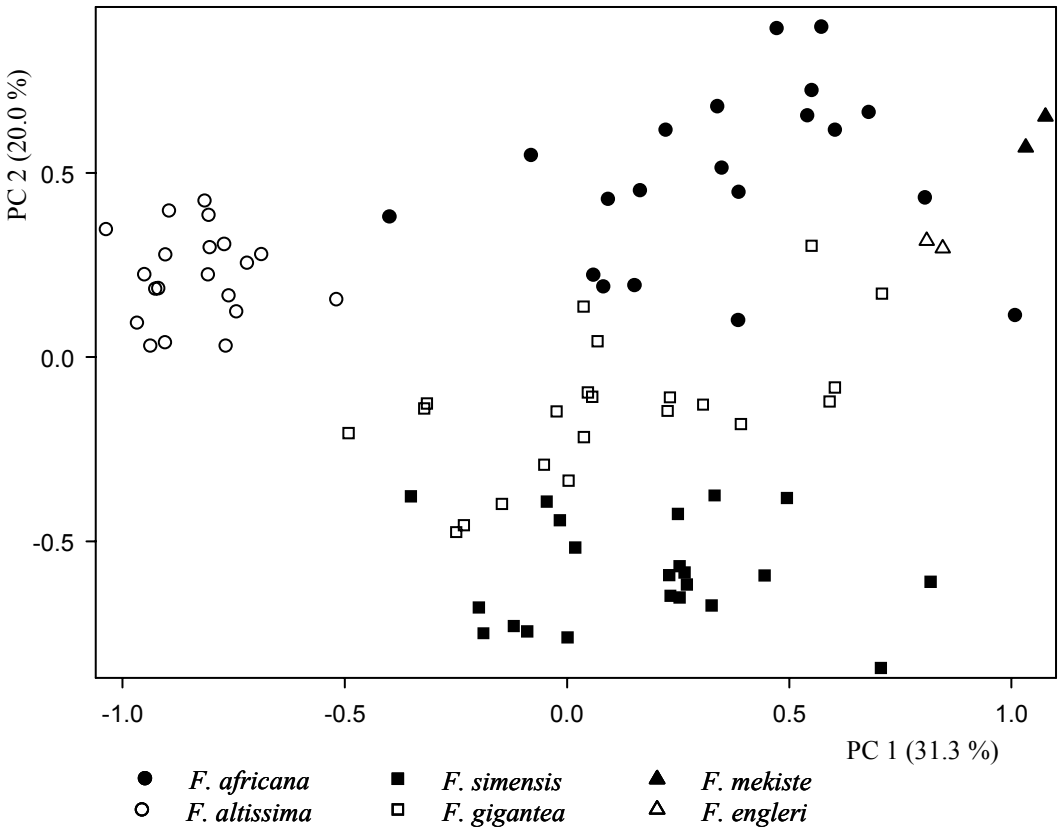

Figure 3. A Principal component analysis (PCA) of 88 accessions of broad-leaved *Festuca* derived from measurements of 31 quantitative characters.

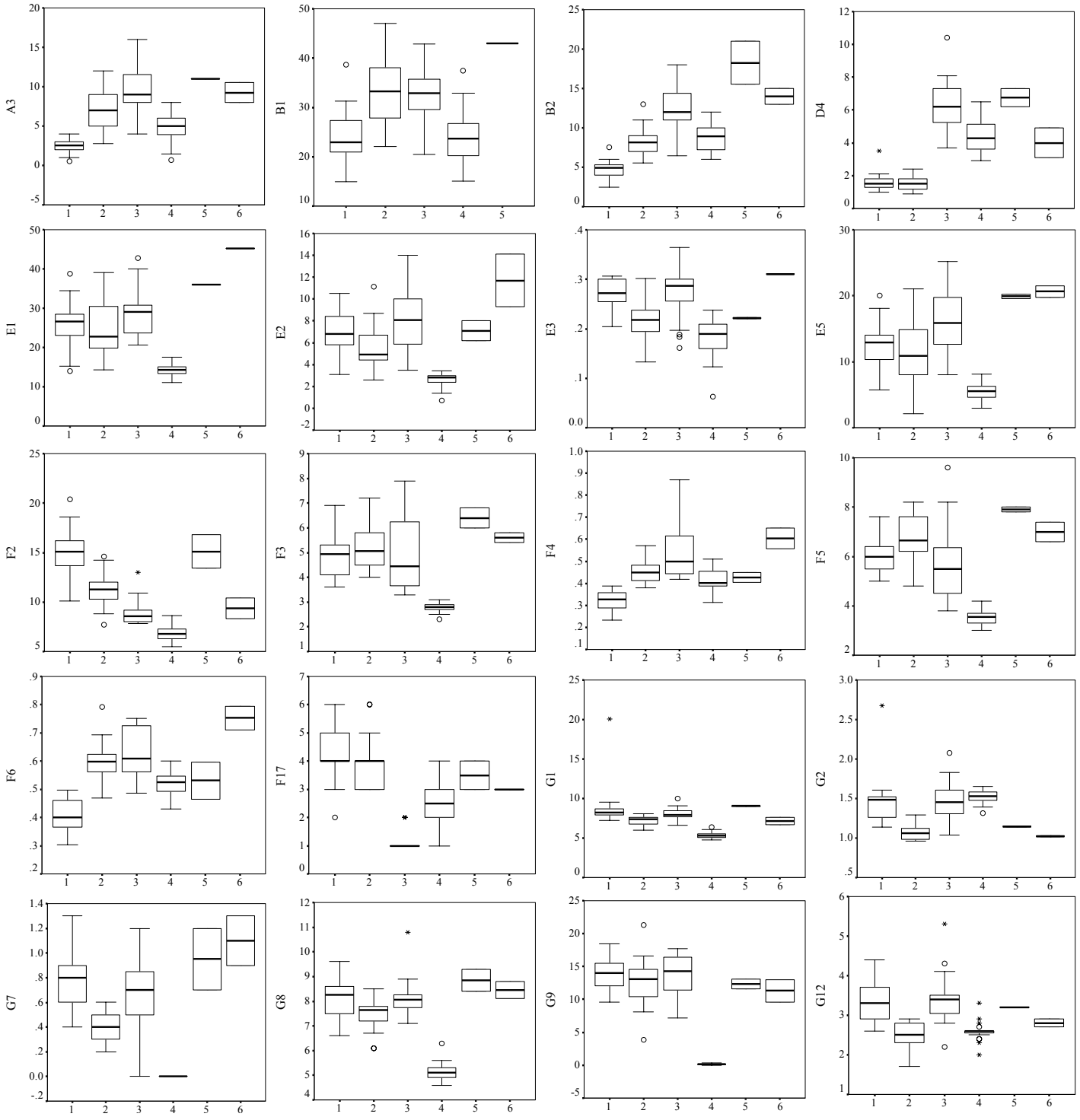

Figure 4. Box plot analysis showing some of the box plots based on quantitative characters (see Table 2 for character abbreviations). The x-axis represents species groups: 1 – *F. simensis*, 2 – *F. gigantea*, 3 – *F. africana*, 4 – *F. altissima*, 5 – *F. engleri*, 6 – *F. mekiste*.

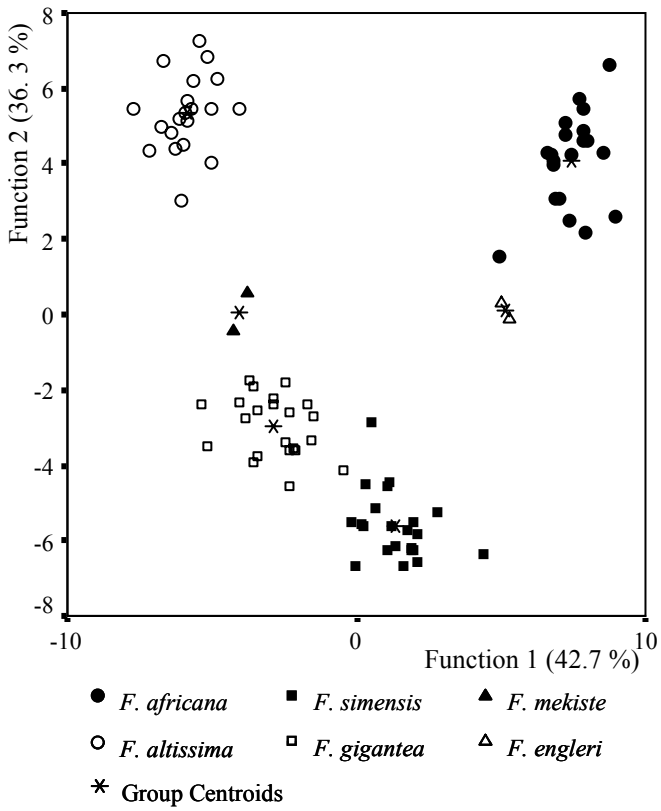

Figure 5. A discriminant analysis (DA) of 88 accessions of broad-leaved *Festuca* derived from measurements of 30 quantitative characters (leaf blade length (B1) was omitted because values were missing for *F. mekiste*).

Paper IV

Leaf anatomical characteristics of Ugandan species of *Festuca* L. (Poaceae)

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Abstract

The importance of leaf anatomical characters in the taxonomy of Ugandan *Festuca* species was investigated. In particular, leaf cross sections were investigated in order to study the variation in disposition of the sclerenchyma tissue and determine whether this is as taxonomically useful as is implied in much of the literature. Also the leaf epidermis was investigated for presence of diagnostic features. A lot of variation in disposition of sclerenchyma tissue was found in *F. abyssinica*, which did not support the recognition of *F. elgonensis* and *F. richardii* as good species, yet they were described mainly based on leaf anatomy. The ring of sclerenchyma in *F. pilgeri* and the presence of papillae on the epidermis of *F. claytonii* supported them to be good species. Presence of silica bodies on the epidermis of Ugandan *Festuca* was investigated for the first time and is confirmed using energy dispersive x-ray analysis. In conclusion, leaf anatomy should be used together with other non-anatomical characters before recognising species.

Keywords: anatomy, *Festuca*, sclerenchyma, silica bodies, taxonomy, Uganda

Introduction

Leaf anatomy as seen in cross section has been used in the taxonomy of *Festuca* since 1882 (Aiken & Consaul 1995). In particular, the disposition of sclerenchyma tissue in relation to the vascular bundles has been of key interest, although other characters such as number of vascular bundles, presence of bulliform cells, and number of ribs in the adaxial surface are also used (Clayton & Renvoize 1986, Ellis 1976, Markgraf-Dannenberg 1980, Metcalfe 1960). Its application has been mainly to streamline the taxonomy of the narrow-leaved (fine-leaved) fescues, which are often morphologically very similar. The anatomy of some broad-leaved species has also been studied (Metcalfe 1960), although they are taxonomically less difficult. The arctic and temperate fescues have been widely investigated and a large number of narrow-leaved species has been described, many characterized only by minor anatomical characters. For example in "Flora Europaea" (Markgraf-Dannenberg 1980) 151 of the 170 species described are in part recognized by characters of the sclerenchyma tissue. But such characters vary a lot in different leaves and different parts of leaves, and are strongly influenced by environmental conditions (Aiken & Darbyshire 1985, Aiken & Consaul 1995, Dube & Morisset 1987, Kjellqvist 1961, Metcalfe 1960, Ramesar-Fortner *et al*. 1995).

In East Africa the genus *Festuca* was revised by Clayton (1970), who recognized five species with acicular, involute or filiform leaf-blades. Mainly based on anatomical characters of the leaves in cross section, Alexeev (1986, 1987) described seven new narrow-leaved species from tropical and South Africa, of which three occur in Uganda (i.e. *F. claytonii*, *F. elgonensis* and *F. richardii*). Alexeev (1986, 1987) also upgraded some subspecies and varieties to full species, thus increasing the number of tropical African narrow-leaved *Festuca* by another five species, making the total number seventeen.

Leaf anatomy of the epidermis as seen in surface view is also taxonomically valuable in grasses (Metcalfe 1960, Ellis 1979, Palmer & Tucker 1981). Structures like stomata,

papillae, hairs, silica bodies, intercostal long and short cells are used. According to Metcalfe (1960) and Palmer & Tucker (1981) shapes and distribution of silica bodies are very important for taxonomic purposes. They are distinct structures, generally of constant shape and predictable pattern (Ellis 1979). However, Reimer & Cota-Sánchez (2007) did not find silica bodies informative in the subfamily Danthonioideae. Prychid *et al*. (2004) report the presence/absence of silica bodies to correlate with phylogeny in the monocotyledons, silica bodies being absent in the basal monocots. In the tribe Poaeae silica bodies are rounded or oblong (Markgraf-Dannenberg 1980), and they are round to elliptical or slightly crescent-shaped over the veins in *Festuca* (Metcalfe 1960). In this study we investigated some leaf anatomical differences between the species of *Festuca* in Uganda in order to establish which anatomical characters are useful taxonomically. Presence of silica in the African fescues is here investigated for the first time.

Methods

Leaf cross sections

Sampling strategy was aimed at capturing variation within the species investigated by picking sample leaves from several populations along different altitudes covering the distribution range of each species. Leaves from up to three separate plants were collected from each population. In total 90 sections were made represented as follows: 34 plants in 24 populations for *F. abyssinica*, 18 plants in 9 populations for *F. richardii*, 4 plants in 2 populations for *F. claytonii*, 6 plants in 2 populations for *F. pilgeri*, 16 plants in 8 populations for *F. chodatiana*, 10 plants in 5 populations for *F. simensis*, and 2 plants in 2 populations for *F. africana*.

Two mature leaves were picked from each plant and the middle portions of which were fixed in 75 % ethanol for 7 – 14 days. The samples were then embedded in wax before sectioning to 5 μm thick using a microtome dissector. The sections were dewaxed using two

changes of xylene, and then rehydrated using descending grades of alcohol (up to 70 %) and finally water. The sections were stained with safranin (1 %) for five minutes, and briefly dehydrated in ascending grades of alcohol. The alcohol was washed off (2 washes) in xylene before mounting using DPX mountant. The permanent slides are kept by the first author and can be availed on request.

Scanning electron microscopy (SEM)

Pieces of leaves corresponding to the middle part of mature leaves from both live and herbarium specimens were mounted on studs and studied by energy dispersive x-ray analysis (EDXA) before coating them with platinum and palladium. The EDXA was done in order to view areas of high atomic number, specifically silica, on the leaf surfaces and estimate the ion content in these areas. The metallic coating of platinum and palladium was aimed to help in studying the fine structure of the leaf surfaces. Leaves from four different plants were investigated for each species.

Results

Leaf cross sections

Fig. 1A, B and C show the variation found in the leaf sections of *F. abyssinica*. The characteristic feature of the type of variation represented in Fig. 1A is that sclerenchyma tissue in the sections is located both below the vascular bundles (abaxial) and on the ribs above the vascular bundles (adaxial). This is the most common arrangement of sclerenchyma tissue in *F. abyssinica* and is represented in several variations: the sections are mainly symmetrical with 7 vascular bundles, but asymmetrical forms with six vascular bundles were also found; the abaxial sclerenchyma tissue sometimes touches the vascular bundles, and some or all the rib tops may be capped with sclerenchyma tissue. In the second type (Fig. 1B),

the sections are symmetrical with 7 vascular bundles and abaxial sclerenchyma only. The third type (Fig. 1C) is similar to Fig. 1B in lacking sclerenchyma on the rib tops but this type is characterized by sections having 5 vascular bundles, with the abaxial sclerenchyma sometimes touching the vascular bundles. This type of arrangement (Fig. 1C) is more common than that in Fig. 1B.

In *F. richardii* (Fig. 1D) all sections had 5 vascular bundles with abaxial sclerenchyma but with none on the rib tops. In all the sections investigated for this species only one was found with a vascular bundle that touched the adjacent sclerenchyma tissue. This type of arrangement (Fig. 1D) is the same as that in *F. abyssinica*, represented in Fig. 1C.

In *F. claytonii* (Fig. 1E) the arrangement is like that in *F. richardii*: 5 vascular bundles with abaxial sclerenchyma, but none on the rib tops. Of the narrow-leaved species *F. pilgeri* (Fig. 1F) was unique in having an almost continuous ring of sclerenchyma on the abaxial side of the leaf section. The leaf in section is terete, with 5 vascular bundles and sometimes with sclerenchyma capping the rib tops. However, the sclerenchyma ring may be more frequently broken than the one presented in the Fig. 1F.

F. chodatiana (Fig. 1G) has 7 – 11 vascular bundles, the wider leaves having all the three orders: first, second and third as described in Ellis (1976). Sclerenchyma tissue is present on both abaxial and adaxial sides of the leaf-blades, and may touch the vascular bundles abaxially, adaxially or both ways regardless of the order of the vascular bundle. The other narrow-leaved species described above only have the first and second orders of vascular bundles.

The section of *F. simensis*, a broad-leaved species, is shown in Fig. 2A. Sclerenchyma tissue is present on both abaxial and adaxial sides, and it touches the first order vascular bundles both abaxially and adaxially. The number of vascular bundles varies from 13 to 18. The adaxial surface is deeply furrowed, hence the ribs appearing prominent.

The second broad-leaved species, *F. africana*, has sections similar to those of *F. simensis*, but the difference is that the adaxial surface in *F. africana* is not deeply furrowed, hence showing slightly protruding ribs (Fig. 2B). Vascular bundles vary from 13 to 50 (additional counts were made on herbarium specimens because it was not very successful to make complete sections with such wide leaves).

Scanning electron microscopy (SEM)

SEM studies revealed the presence of crescent-shaped, semicircular, round and linear silica bodies in the leaves of the Ugandan *Festuca* (Fig. 3 – 6). Presence of silica was confirmed by EDXA, which gave high ion content for silicon on the silica bodies (Fig. 3, spectrum 6) and the tips of the prickles (Fig. 3, spectrum 9), whereas other parts of the leaf had high content of chloride, potassium and carbon (Fig. 3, spectrum 1).

In the same leaf in the narrow-leaved species, shapes of the silica bodies vary from crescent-shaped to semicircular or rarely round (Fig. 4). From this point onwards we shall refer to all crescent-shaped, semicircular, and round silica bodies as 'crescent-shaped' because this is the most abundant shape of silica bodies, and there is no observable pattern in distribution of the three different shapes. The narrow-leaved species also have linear silica bodies. The linear silica bodies as well as some crescent-shaped ones are arranged longitudinally along the veins, whereas the crescent-shaped silica bodies are mainly transversely placed across the rows of cells, separating adjacent cells in the intercostal region. The broad-leaved species have only the linear silica bodies arranged longitudinally along the veins (Fig. 5). Figures 6A and 6B show the crescent-shaped and linear silica bodies respectively. We observed that the crescent-shaped silica bodies readily fall out of the leaf surfaces leaving small crescent-shaped or oval depressions, and linear depressions when linear silica bodies fall out.

Table 1 summarises the sizes and densities of the silica bodies in the different species. In the narrow-leaved species (*F. abyssinica*, *F. elgonensis*, *F. chodatiana*, *F. claytonii*, *F. pilgeri* and *F. richardii*) the crescent-shaped silica bodies are about the same size, i.e. 8 – 15 μm long and 5 – 12 μm wide. *F. claytonii* has the lowest density of silica bodies, whereas *F. pilgeri* has the highest. Fig. 4A shows the surface of *F. richardii*, which is similar to that of *F. abyssinica* and *F. elgonensis* but with different densities of silica bodies, *F. abyssinica* has a higher density and *F. elgonensis* the highest (Table 1). *F. claytonii* (Fig. 4B) is unique in having dense papillae on the abaxial surface, *F. pilgeri* (Fig. 4C) is unique with its dense prickles, whereas *F. chodatiana* (Fig. 4D) has the most strongly sinuose cell walls.

Silica bodies in the broad-leaved species are of the same length (ca. 20 – 40 μm) but are wider in *F. africana* and denser in *F. simensis* (Table 1). They are linear to rectangular in shape, strongly sinuose in *F. simensis* (Fig. 5A) and slightly sinuose in *F. africana* (Fig. 5B).

Discussion

Leaf cross sections of the narrow-leaved species

Leaf anatomy in cross section does not distinguish between the three species *F. abyssinica*, *F. richardii* and *F. elgonensis*, and this is in agreement with DNA analysis (Namaganda *et al*. 2006) as well as morphology (Namaganda *et al*. in prep.). The leaf sections in *F. abyssinica* are variable, broadly grouped into three main types; type 1, with seven vascular bundles and sclerenchyma both above and below the vascular bundles; type 2, with six or seven vascular bundles and sclerenchyma only below the vascular bundles; and type 3, similar to type 2 but with five vascular bundles. Type 3 is essentially the same as the sections of *F. richardii* according to this study. According to Alexeev (1987) *F. elgonensis* has the type 3 arrangement, but leaf sections for this species were not analysed in this study.

F. richardii, described from Ethiopia, was previously classified as *F. abyssinica*, but said to differ from *F. abyssinica* in its slightly different leaf anatomy. Phillips (1995) accepted this species for "Flora of Ethiopia and Eritrea", but noted that it is a high mountain segregate from the *F. abyssinica* complex. Her leaf sections for *F. richardii* have sclerenchyma only below the vascular bundles, whereas Alexeev's (1986, 1987) presentations have sclerenchyma both below and above the vascular bundles. Both Phillips and Alexeev's sections have seven vascular bundles. We found the Ugandan leaf sections of *F. richardii* to have five vascular bundles, but like the Ethiopian species, they have stiff and more scabrid leaves compared to the typical forms of *F. abyssinica*, purple-tinged spikelets and dense tussocks. We found no relationship in leaf anatomy characteristics with altitude but other ecological factors may possibly contribute to this observed variation and should therefore be investigated. In our opinion *F. richardii* is a synonym for *F. abyssinica*, and should thus not be accepted as a taxon at any level.

Similarly, *F. elgonensis* should also not be accepted as a taxon at any level. The leaf anatomy presented by Alexeev (1987) is not good enough to support its species status given that we found the same forms of variation in the *F. abyssinica* that we investigated. *F. elgonensis* was described from Uganda by Alexeev (1987), and was previously classified as *F. abyssinica*. Alexeev used the number of vascular bundles and size of sclerenchyma tissue to distinguish *F. elgonensis*, i.e. *F. elgonensis* should have five or seven vascular bundles and if with seven, then the leaf blade is stiff and upright, and has broad sclerenchyma tissue, whereas *F. abyssinica* (and *F. richardii*) should as a rule have seven vascular bundles, with relatively soft and almost nodding leaf blades and narrow sclerenchyma tissue. Alexeev's *F. elgonensis* fully intergrades with *F. abyssinica* in terms of number of vascular bundles and stiffness of leaf blades, and furthermore, the size of sclerenchyma tissue is environmentally

plastic (Kjellqvist 1961). Also neither morphology nor DNA (Namaganda *et al*. in prep.) supports the distinction of *F. elgonensis* as a species.

Sections of *F. claytonii* are of the type 3 described above. This is in agreement with Alexeev (1986). However, *F. claytonii* cannot be confused with the *F. abyssinica* complex because resemblance is only by the anatomy of the leaf cross sections. Otherwise differences are supported by DNA (Namaganda *et al*. 2006), morphology (Namaganda *et al*. in prep.) and anatomy of the leaf epidermis (see next section). Although some authors caution the use of characters of leaf cross sections in the taxonomy of *Festuca* because of their environmentally plastic nature (Dube & Morisset 1987, Kjellqvist 1961, Ramesar-Fortner *et al*. 1995), Ellis (1976) suggested that when used together with a wide spectrum of other diagnostic characters, anatomical details are essential for a satisfactory treatment of grass taxonomy.

F. chodatiana, another distinct species, has sections that are wider than all those described above. Its sections are characterised by the presence of 7 – 11 vascular bundles of first to third order. Since this species has flat leaves, about 1.1 – 2.4 mm wide, it is not usually confused with any of the other narrow-leaved species of *Festuca*.

The round leaf sections with an almost continuous ring of sclerenchyma give *F. pilgeri* a unique appearance different from all the other narrow-leaved Ugandan *Festuca*. The leaf sections alone very well differentiate *F. pilgeri* from the other species. The continuous ring of sclerenchyma gives mechanical support to the long leaves (11.5 – 44.5 cm long) of *F. pilgeri* making them stiff and hard, and this is possibly an adaptation against herbivory.

Leaf epidermis of the narrow-leaved species

Crescent-shaped, semicircular, round and linear silica bodies in Poaeae and *Festuca* have already been described from northern temperate regions (Metcalfe 1960, Markgraf-Dannenberg 1980). In Africa anatomy of the leaf epidermis separates the narrow-leaved

fescues from the broad-leaved species: silica bodies in the narrow-leaved species are crescent-shaped to round and linear, whereas broad-leaved species have only the linear type. *F. abyssinica*, *F. richardii* and *F. elgonensis* have the same types of silica bodies as well as arrangement, but with differences in density of the silica bodies. Highest density is in *F. elgonensis*, lowest in *F. richardii* with *F. abyssinica* in the intermediate position. *F. elgonensis* and *F. richardii* appear to be extreme forms of the highly variable *F. abyssinica*. These species do not have papillae and their prickles are only found along the veins. Prickle density was very low for the three species.

F. claytonii and *F. pilgeri* were found to be closely related but distinct via DNA analysis and morphology (Namaganda *et al*. 2006, Namaganda *et al*. in prep.), and leaf anatomy has revealed them to be very different. *F. claytonii* has no prickles but is instead densely covered with papillae, whereas *F. pilgeri* is densely covered with prickles (densest in all the species investigated) and has no papillae. Also the density of silica bodies is very low in *F. claytonii* (60 – 80 per mm^2) compared to *F. pilgeri* with a density of 500 – 600 per mm^2. Leaf anatomy therefore confirms the distinction between *F. claytonii* and *F. pilgeri*.

F. chodatiana is similar to the *F. abyssinica* group in anatomy of the leaf epidermis but with two main differences: *F. chodatiana* has more prominent sinuous cell walls and has hairs instead of prickles on the abaxial surface. Otherwise shape, size and density of silica bodies are comparable. The form and position of silica bodies is possibly genetically controlled because it is not greatly influenced by environmental factors (Prychid *et al*. 2004) and is constant within and characteristic to groups of species at higher level taxa (Metcalfe 1960). However, their size could be environmentally controlled by conditions like pH of soil and availability of silica (Ellis 1979). Therefore presence/absence and shape of silica bodies can be reliably used as taxonomic characters. We confirm the existence of inverted cone-shaped (three dimensional) intercostal silica bodies (Ellis 1979) as is shown in figure 6A.

Anatomy of the broad-leaved species

Leaf anatomy as seen in cross section shows some differences between the two Ugandan broad-leaved *Festuca*. *F. africana* with its very wide leaves possesses up to 50 vascular bundles, and it has very shallow furrows on the adaxial surface; they appear only as gentle undulations, whereas *F. simensis* has up to only 13 vascular bundles and has a deeply furrowed adaxial surface. The disposition of sclerenchyma tissue does not seem to be of much taxonomic value as there are no differences between the two species investigated, i.e. sclerenchyma subtends almost all vascular bundles on both surfaces. However, importance of the disposition of sclerenchyma is only emphasised for the narrow-leaved species (Metcalfe 1960, Clayton & Renvoize 1986, Phillips 1995).

Silica bodies on the abaxial leaf epidermis give us another character, in addition to leaf size, that distinguishes between the broad-leaved and narrow-leaved *Festuca*. The broad-leaved species only have linear silica bodies arranged longitudinally along the veins, whereas the narrow-leaves species have crescent-shaped silica bodies arranged transversely on the veins and between the veins. We also found linear silica bodies on the adaxial epidermis in the broad-leaved *Festuca*, but this surface was difficult to investigate in the narrow-leaved species. The density of silica bodies was higher in *F. simensis* than in *F. africana*.

Conclusion

In Uganda only one out of three Alexeevian species of *Festuca* (described mainly based on anatomical characteristics) were found to be of any taxonomic value. Therefore we should be very critical in accepting species merely or mainly defined on anatomical characters concerning number, shape and position of sclerenchyma tissue. We believe the number of fine-leaved species of *Festuca* should be drastically reduced also in other parts of the world.

Interestingly, for *F. claytonii* (the only valid Alexeevian species), a new microanatomical chracter was discovered to have good descriptive value towards all the other species. But *F. elgonensis* and *F. richardii* should be sunk back into *F. abyssinica*.

The reason for this lack of taxonomic value in certain anatomical characters is their enormous variation (not accounted for by taxonomists investigating a restricted number of leaves from a restricted number of specimens), and their being so strongly influenced by environmental conditions as is already documented by Kjellqvist (1961) and others.

Acknowledgements

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References

Aiken S. G., Consaul L. L. (1995) Leaf cross sections and phytogeography: A potent combination for identifying members of *Festuca* subgen. *Festuca* and *Leucopoa* (Poaceae), occurring in North America. Am. J. Bot. 82: 1287-1299.

Aiken S. G., Darbyshire S. J. (1985) Restricted taxonomic value of leaf sections in Canadian narrow-leaved *Festuca* (Poaceae). Can. J. Bot. 63: 995-1007.

Alexeev E. B. (1986) New narrow-leaved *Festuca* (Poaceae) members from Tropical and South Africa. Bot. Zhurnal 71: 1109-1117.

Alexeev E. B (1987) Fescues of the *Festuca abyssinica* s. l. (Poaceae) group. Bot. Zhurnal 72: 1260-1268.

Clayton W. D. (1970) Gramineae Part 1. In: Milne-Redhead E., Polhill R. M. (eds.) Flora of Tropical East Africa. Crown agents for oversea governments and administrations, London, pp. 56-63.

Clayton W. D, Renvoize S. A (1986) *Genera Graminum: Grasses of the World*. HMSO, London, pp 374-375.

Dube M., Morisset P. (1987) Morphological and leaf anatomical variation in *Festuca rubra sensu lato* (Poaceae) from eastern Quebec. Can. J. Bot. 65: 1065-1077.

Ellis R. P. (1976) A procedure for standardizing comparative leaf anatomy in the Poacea. I. The leaf-blade as viewed in transverse section. Bothalia 12: 65-109.

Ellis R. P. (1979) A procedure for standardizing comparative leaf anatomy in the Poacea. II. The epidermis as seen in surface view. Bothalia 12: 641-671.

Kjellqvist E. (1961) Studies in *Festuca rubra* L. 1. Influence of environment. Bot. Notiser 114: 403-408.

Markgraf-Dannenberg I (1980) *Festuca* L. In: Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA (eds) Flora Europaea, 5. Alismataceae to Orchidaceae (Monocotyledons). Cambridge University Press, Cambridge, pp 125-153.

Metcalfe C. R. (1960) Anatomy of the monocotyledons. I. Gramineae. Clarendon press, Oxford, pp 209-217.

Namaganda M., Lye K. A., Friebe B., Heun M. (2006) AFLP-based differentiation of tropical African *Festuca* species compared to the European *Festuca* complex. Theor. Appl. Genet. 113: 1529-1538.

Palmer P. G., Tucker A. E. (1981) A scanning electron microscope survey of the epidermis of East African grasses, I. Smithsonian Contributions to Botany, 49. Smithsonian Institution Press, Washington.

Phillips S (1995) Poaceae. In: Hedberg I, Edwards S (eds.) Flora of Ethiopia and Eritrea, Vol. 7. Addis Ababa, Ethiopia; Uppsala, Sweden, pp 23-27.

Prychid C. J., Rudall P. J., Gregory M. (2004) Systematics and biology of silica bodies in monocotyledons. Bot. Rev. 69: 377-440.

Ramesar-Fortner N. S., Aiken S. G., Dengler N. G. (1995) Phenotypic plasticity in leaves of four species of arctic *Festuca* (Poaceae). Can. J. Bot. 73: 1810-1823.

Reimer E., Cota-Sánchez J. H. (2007) An SEM survey of the leaf epidermis in Danthonioid grasses (Poaceae: Danthonioideae). Syst. Bot. 32: 60-70.

Table 1. Approximate sizes and density of crescent shaped silica bodies (in the narrow-leaved species) and linear silica bodies (in the broad-leaved *F. simensis* and *F. africana*).

Species	**Length (μm)**	**Width (μm)**	**Density**
F. abyssinica A. Rich.	10 – 15	5 – 12	300 – 350 per mm^2
F. richardii Alexeev	10 – 15	8 – 12	200 – 250 per mm^2
F. elgonensis Alexeev	8 – 12	8 – 10	350 – 450 per mm^2
F. claytonii Alexeev	10	10	60 – 80 per mm^2
F. pilgeri St. Yves	10 – 15	9 – 12	500 – 600 per mm^2
F. chodatiana (St. Yves) Alexeev	10	8	200 per mm^2
F. simensis A. Rich	20 – 40	5	10 per mm of vein
F. africana (Hack.) Clayton	20 – 40	8 – 12	1 – 5 per mm of vein

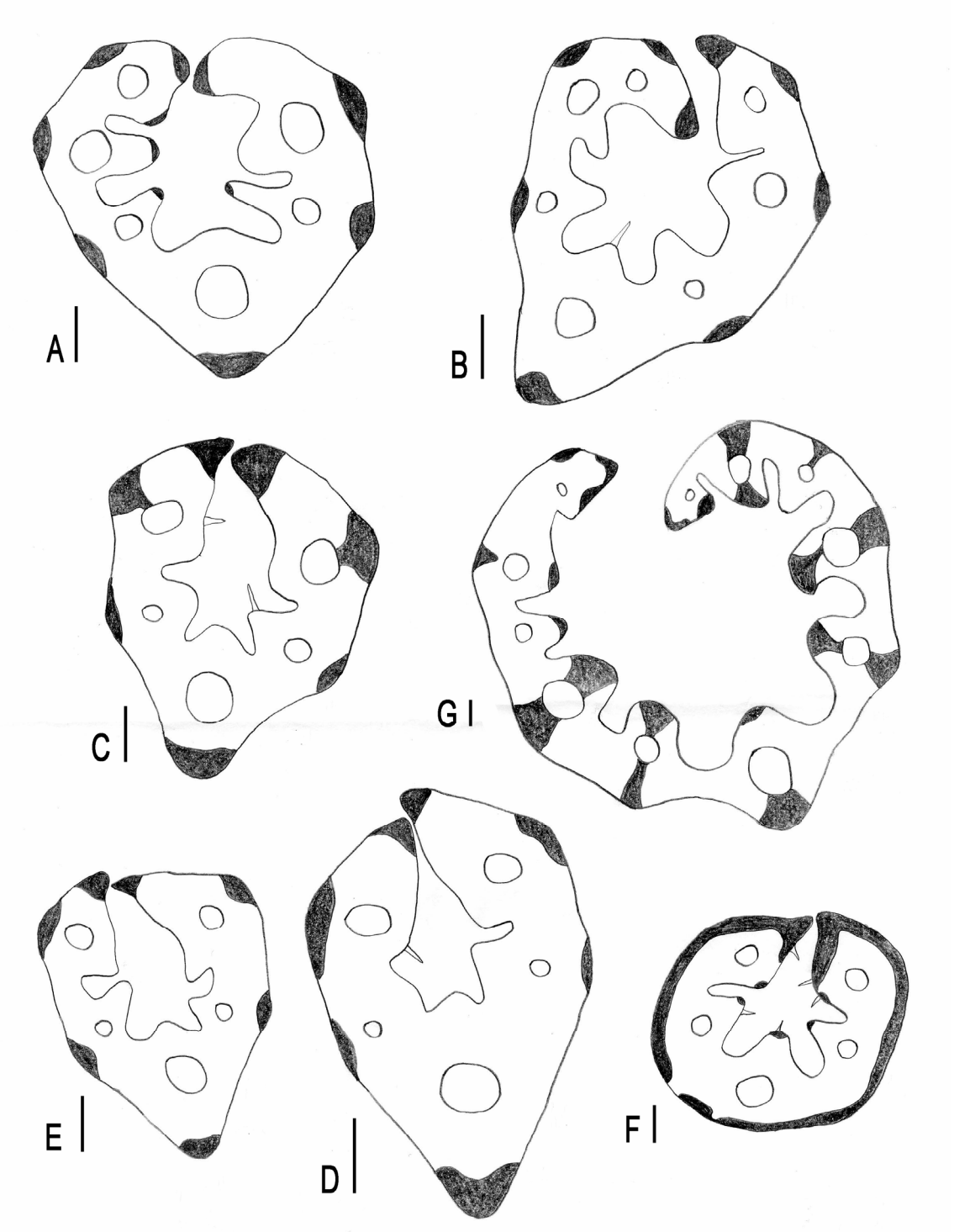

Figure 1

Leaf cross sections of narrow-leaved Ugandan *Festuca*; A – C: *F. abyssinica* from Namaganda 1593 (A); from Namaganda 1576 (B); from Namaganda 1581 (C), D: *F. richardii* from Namaganda 1569, E: *F. claytonii* from Namaganda 1575, F: *F. pilgeri* from Namaganda 1572, G: *F. chodatiana* from Namaganda 1590. Scale bars represent approximately 0.1 mm.

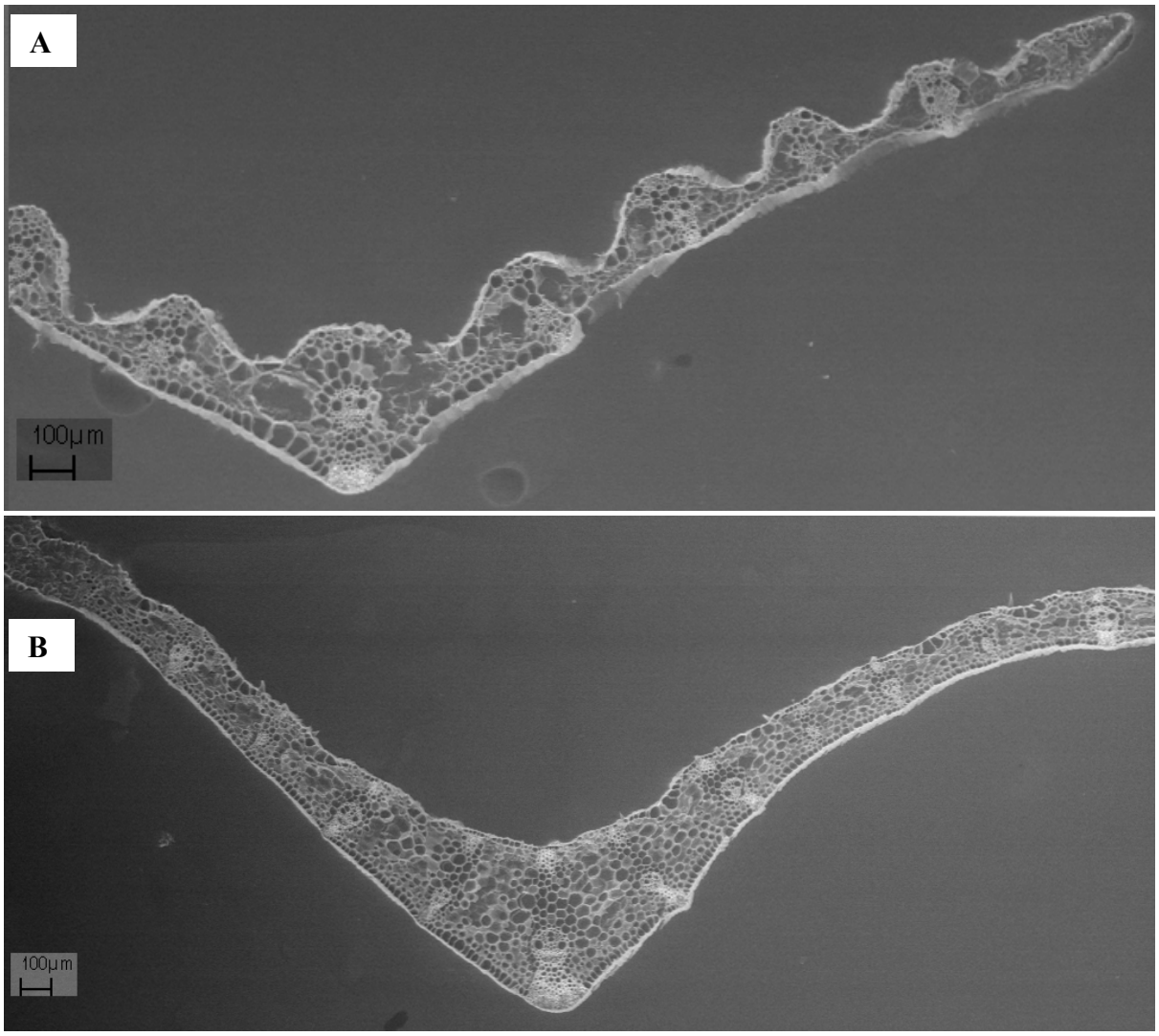

Figure 2

Leaf cross sections of broad-leaved Ugandan *Festuca*; A: *F. simensis* from Namaganda 1710, B: *F. africana* from Namaganda 1688.

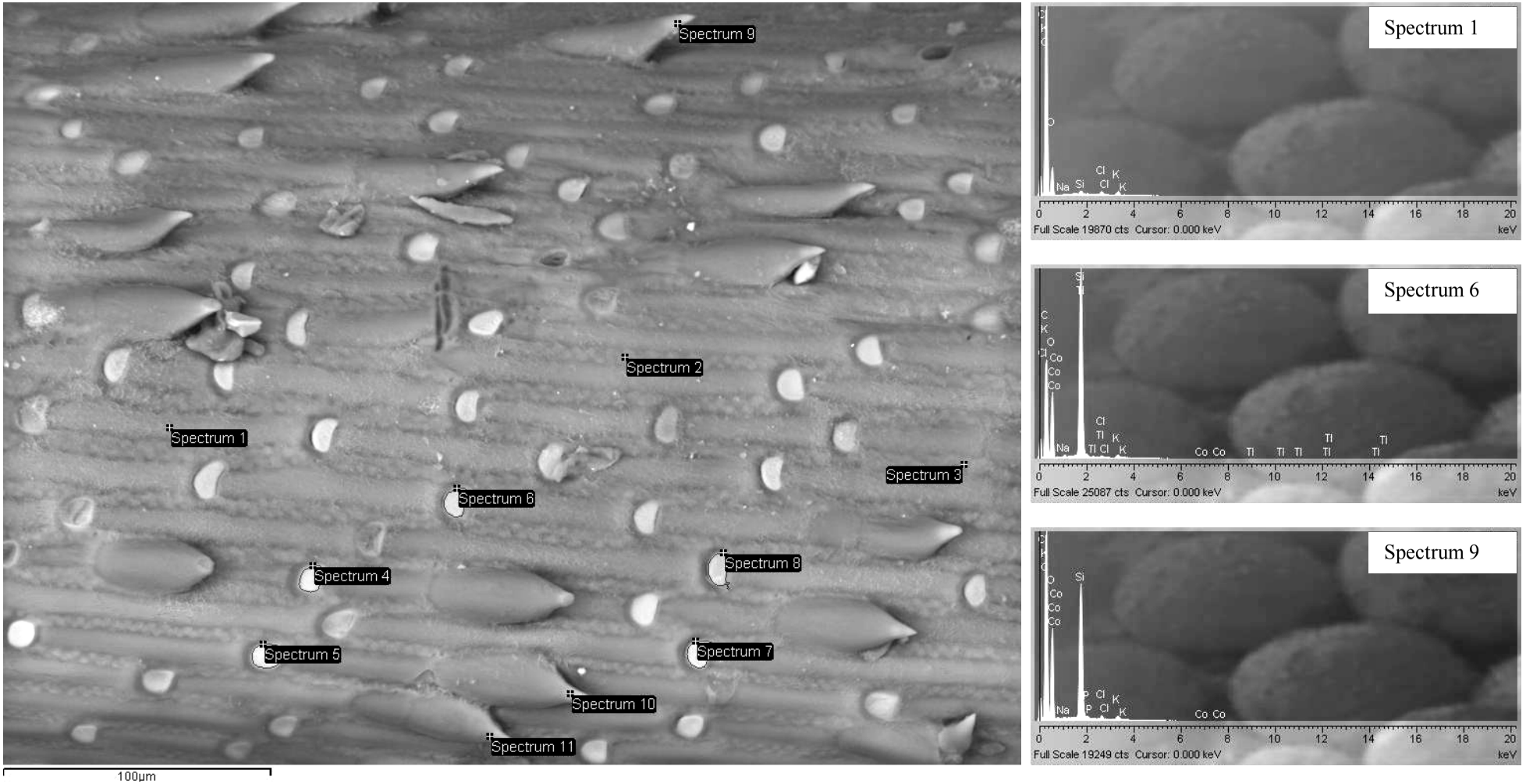

Figure 3

Energy dispersive x-ray analysis of the leaf surface of *F. pilgeri* showing the ion content of the lamina (Spectrum 1), silica body (Spectrum 6) and tip of prickle (Spectrum 9).

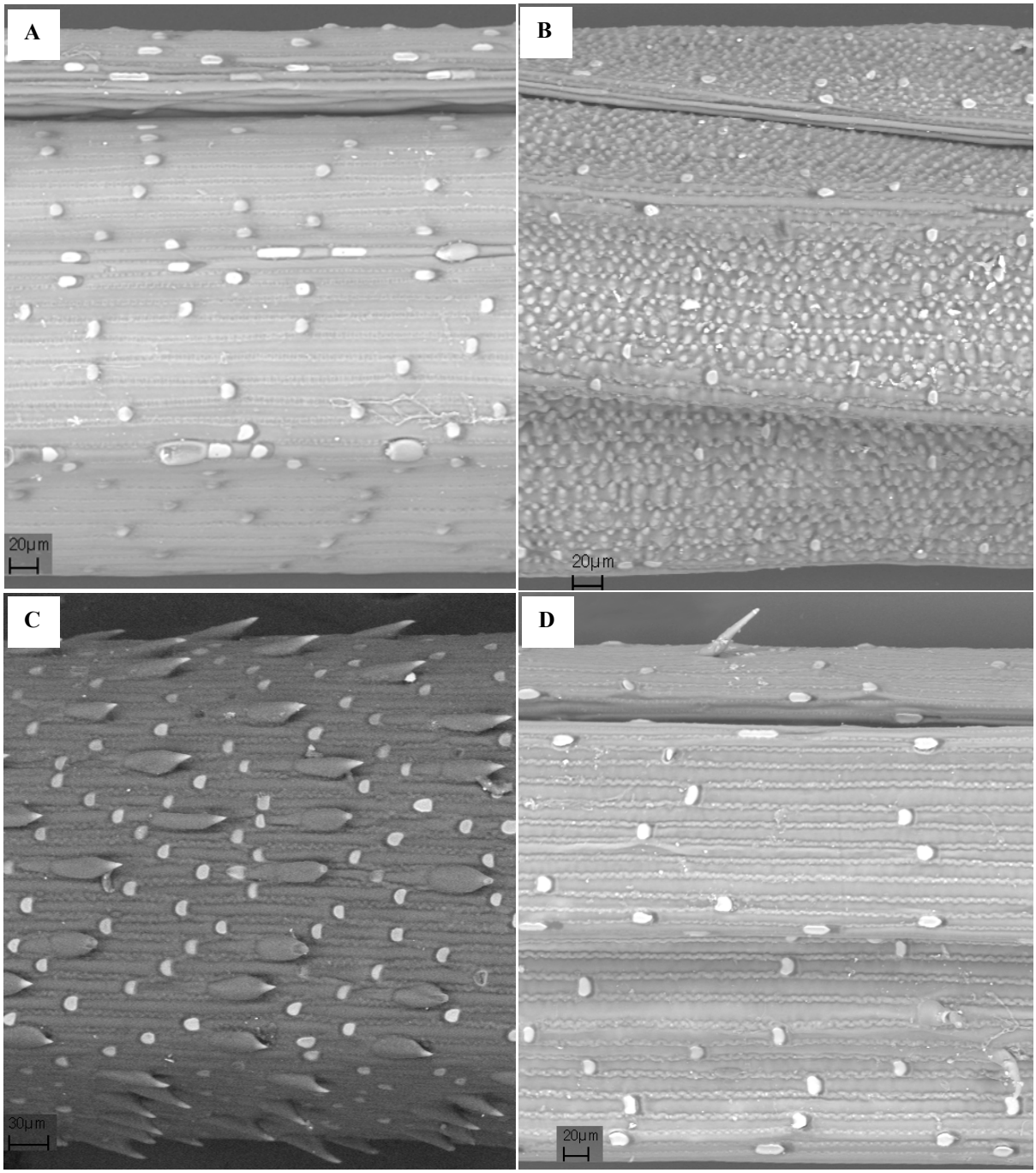

Figure 4

Scanning electron microscopy images of leaf surfaces of narrow-leaved Ugandan *Festuca*; A: *F. richardii* from Namaganda 1359, B: *F. claytonii* from Namaganda 1367b, C: *F. pilgeri* from Namaganda 1675, D: *F. chodatiana* from Namaganda 1567b.

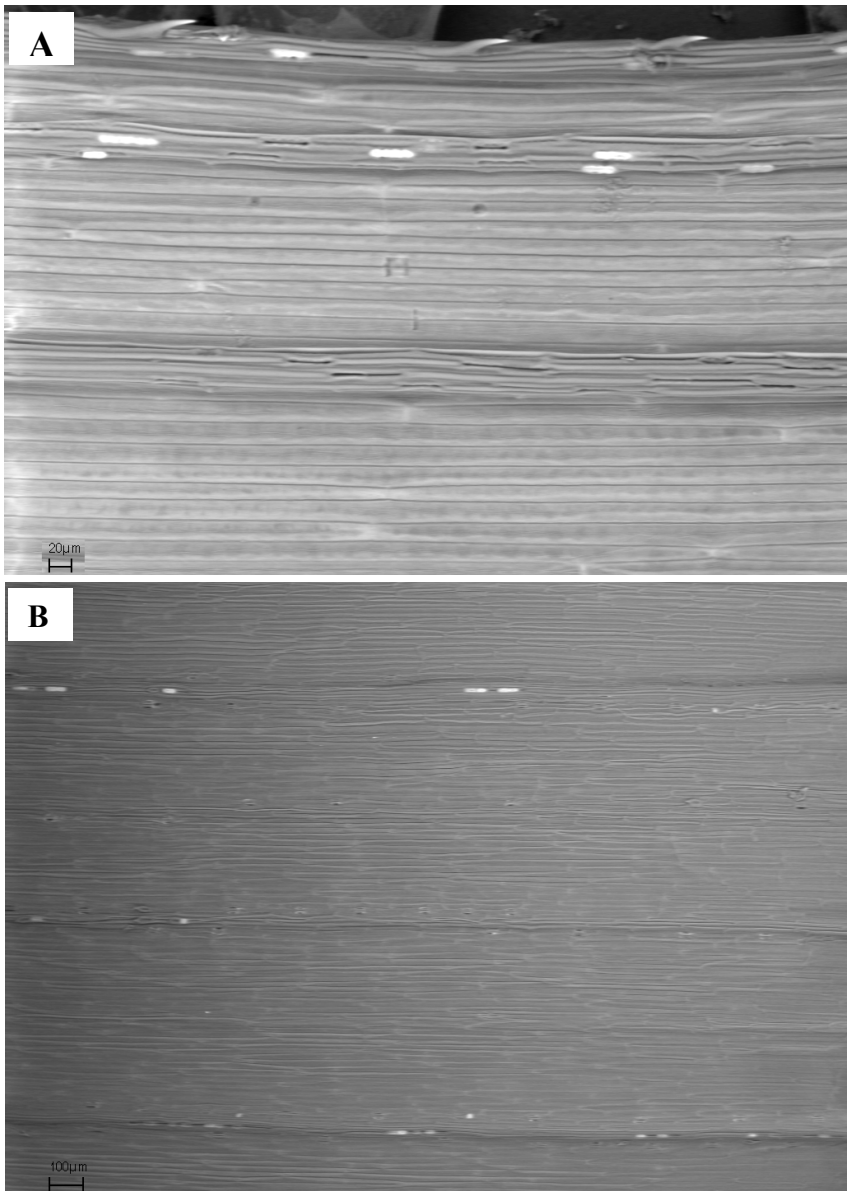

Figure 5

Scanning electron microscopy images of leaf surfaces of broad-leaved Ugandan *Festuca*; A: *F. simensis* from Namaganda 1698, B: *F. africana* from Namaganda 1688.

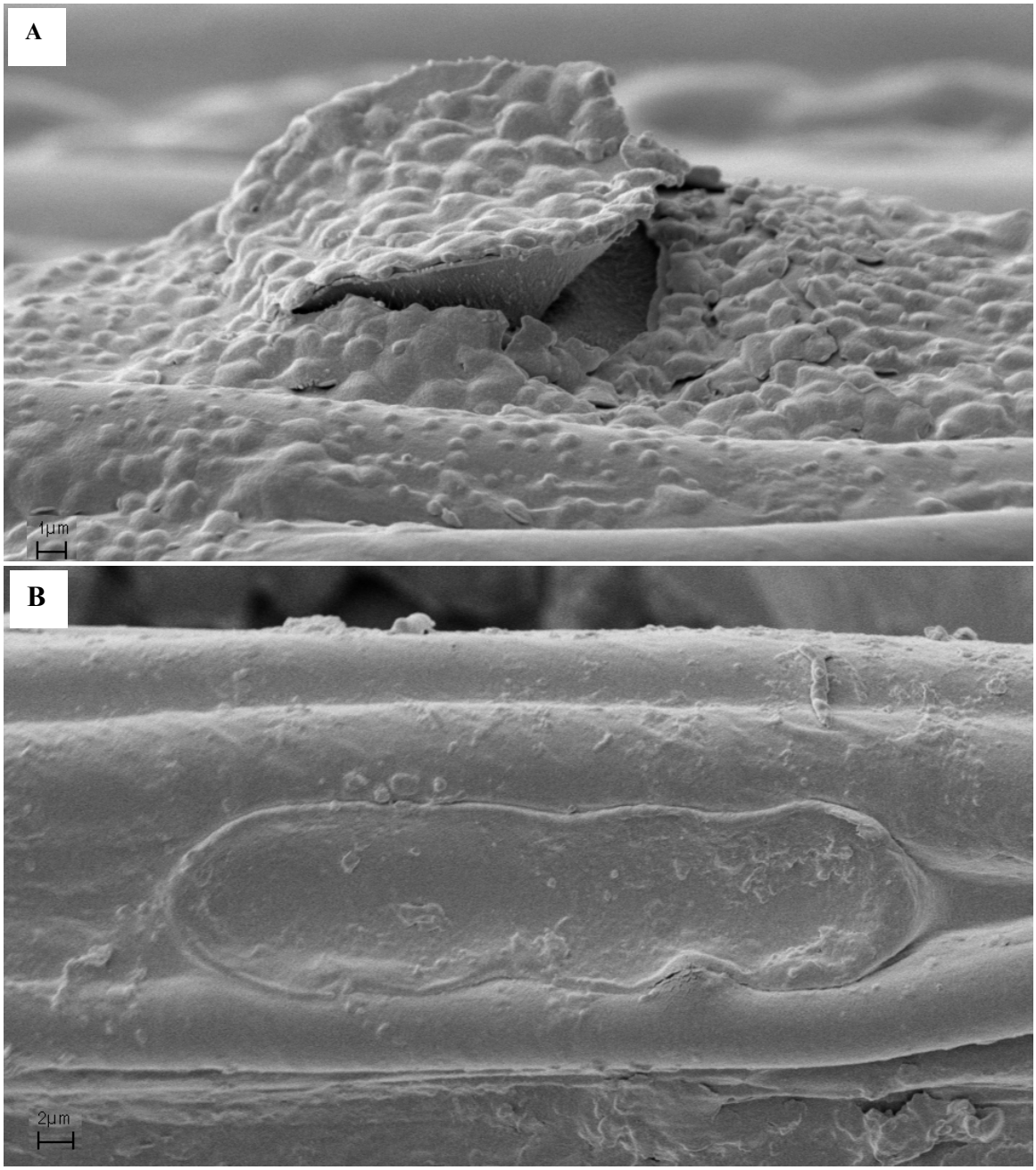

Figure 6

Silica bodies; A: Crescent-shaped silica body from Namaganda 1571, *F. richardii* from Mt. Elgon, showing the silica body pushing through the epidermis. B: Linear silica body on a vein in the leaf of Namaganda 1698, *F. simensis* from Mt. Muhavura (Virunga volcanoes).

Paper V

Should we recognise sibling genera among flowering plants?

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Although sibling genera are widely accepted in animals and lower plants, the term sibling genus has rarely been applied to flowering plants. Based on a molecular study of East African species of *Festuca* compared to European species it became evident that the African narrow-leaved species of *Festuca* belong to a separate group not closely related to *Festuca* s.s. although their morphology is very similar. We believe such morphological resemblance of flowering plant genera, hence sibling genera, may not be of very rare occurrence.

KEYWORDS: *Afrofestuca*, *Festuca*, sibling genera, sibling species, *Triticum*

INTRODUCTION

The concept of sibling species was first recognized in 1718 (Winker, 2005), and has been most widely used by zoologists to describe any of two or more related species that are morphologically nearly identical but are incapable of producing fertile hybrids. Among plants sibling species are known from bryophytes (e.g. the hepatic genus *Pellia* in Fiedorow & al., 2001) and vascular plants (e.g. the genus *Triticum* in Zohary, 1996; 1999). Most well documented is the genus *Triticum*, where *Triticum turgidum* L. subsp. *dicoccoides* (Kórn.) Thell. (wild emmer wheat, 2n = 4x = 28 AABB) and *T. araraticum* Jakubz. (2n = 4x = 28 AAGG) are sibling species. They are morphologically indistinguishable, yet they may form mixed stands, but have cytogenetically been proved to be reproductively isolated (Zohary, 1996; 1999). Reproductive barriers such as cross-incompatibility, hybrid inviability or hybrid sterility define sibling species.

The term sibling genus has also been used. Sibling genera are widely accepted in animals, e.g. mammals (Mackinnon & Read, 2003), fish (Oakley & Phillips, 1999; Waaland et al., 2004), gastropods (Kojima et al., 2002) and nematodes (Ryss et al., 2005). The term is also applied to fungi (Sanders, 1999), algae (Silva, 1993) and bryophytes (Ignatov & Huttunen, 2002). However, sibling genera are rarely applied to flowering plants, but Renvall & Niemelä (1993) mention that *Nectandra* Rolander (*Lauraceae*) is a sibling genus to *Ocotea* Aubl., and Rao & Naidu (1981) even mention sibling genera of the *Poaceae*.

DEFINITION

We use the following definition for extant sibling genera:

Any two or more genera which are morphologically very similar but shown by molecular, cytogenetic, or other extraordinary methods to be only remotely related to each other, with a barrier in place to ensure reproductive isolation between the genera. Whether species from sibling genera would hybridise can only be a speculation until proven, but possibilities of hybridisation are checked by isolating mechanisms such as geographic isolation, mechanical isolation, hybrid sterility, hybrid inviability, etc. However, it should be noted that palaeontologists have applied the term sibling genera to fossil genera, which are morphologically similar to extant genera (Butterfield, 2000; Grossman, 2005). It is acceptable that palaeontologists use the term sibling genus with a different perspective.

Example:

Afrofestuca [a possibly new genus comprising narrow-leaved tropical African species formerly included in *Festuca*] is a sibling genus to *Festuca* L. s.s. including *Vulpia* [but excluding the broad-leaved species such as *F. pratensis* Huds., which are generically misplaced].

In our revision of East African species of *Festuca* using AFLP fingerprinting (Namaganda & al., 2006) we found a close genetic relationship between broad-leaved native African species and broad-leaved European species (including *Lolium* L.). However, the narrow-leaved African species were not genetically close to the narrow-leaved European species (including *Vulpia* C. C. Gmel.). In fact they were genetically so well separated from both the narrow-leaved and the broad-leaved European species

that we intend to recommend that they should be classified in a separate genus, viz. *Afrofestuca* (Fig. 1).

We cultivated the species of '*Afrofestuca*' under controlled conditions in a phytotron and also carried out detailed investigations of morphological characters including scanning electron microscopy. Still we could not find single characters occurring in all East African species and lacking in the European species or vice versa. However, we found that one of the East African species (*F. abyssinica* A. Rich.) had both annual and perennial life forms. The annual form had more rapid germination (in the second and third weeks), flowered and died in the fourth month whereas the perennial form germinated in the fourth and fifth weeks and lived for more than one year. But *Vulpia* also has an annual life form, and since this genus is doubtfully distinct from *Festuca* s.s., this character alone does not carry much weight.

After the cultivation experiments were completed, the plants were planted outdoors in August 2006. With the low North European autumn temperatures the plants soon dried out, and unlike *F. ovina* L. and other European species of subgenus *Festuca*, the plants were not able to re-vegetate. Apparently '*Afrofestuca*' species are physiologically annual plants, which are not adapted to survive long periods of harsh conditions such as cold and dryness. They can survive very cold nights only when followed by a mild or warm day as is the case in the African mountains. Unlike temperate *Festuca* species, '*Afrofestuca*' species have probably not been able to cope with temperature below zero at their basal meristems. These meristems are protected by dense layers of dead leaves in the big grass tussocks, thus preventing the low night temperatures from reaching the meristems (Hedberg, 1964). Similarly, the dead leaves insulate the basal meristems from high temperatures hence creating a microclimate that is moist because evaporation is reduced. The major physiological distinction

between '*Afrofestuca*' and *Festuca* s.s. is that the former are not allocating energy to storage organs to survive unfavourable climatic periods.

Obviously, genetic divergence is sometimes more prominently steered by environmental conditions than morphological adaptations. In the case of '*Afrofestuca*' the major evolutionary force has probably been the climatic conditions. While *Festuca* s.s. probably evolved in cold or temperate regions with a prominent summer and winter, '*Afrofestuca*' gradually became adapted to a climate with "winter every night and summer every day" (Hedberg, 1964). According to Hedberg (1964) some arctic and temperate species (e.g. *Arabis alpina*, *Arabidopsis thaliana*, *Deschampsia caespitosa* and *D. flexuosa*, etc) that were possibly introduced into the afroalpine areas, remained morphologically conservative, but they became sufficiently adapted physiologically.

Whether or not to accept sibling genera is partly related to the theory of accepting or rejecting paraphyletic taxa (Nordal & Stedje, 2005; Williams & al., 2005; Brummitt, 2006; Ebach & al., 2006; Horandl, 2006), since the acceptance of the genus '*Afrofestuca*' transfers it from a paraphyletic to a monophyletic group. Although we will not involve ourselves in this discussion, we believe a monophyletic group like '*Afrofestuca*' should be accepted as comprising a separate genus despite its best distinguishing characters being physiological and genetical rather than morphological.

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LITERATURE CITED

Brummitt, R. K. 2006. Am I a bony fish? Taxon 55: 268--269.

Buttterfield, N. J. 2000. *Bangiomorpha pubescens* n. gen., n. sp.: implications for the evolution of sex, multicellularity, and the Mesoproterozoic/Neoproterozoic radiation of eukaryotes. Paleobiology 26: 386--404.

Ebach, M. C., Williams, D. M. & Morrone, J. J. 2006. Paraphyly is bad taxonomy. Taxon 55: 831--832.

Fiedorow, P., Odrzykoski, I., Szweykowski, J. & Szweykowska-Kulinska, Z. 2001. Phylogeny of the European species of the genus Pellia (Hepaticae: Metzgeriales) based on the molecular data from nuclear $tRNA_{CAA}$ intergenic sequences. *Gene* 262: 309--315.

Grossman, A. R. 2005. Path towards Algal Genomics. Plant Physiology 137: 410--427.

Hedberg, O. 1964. Features of Afroalpine plant ecology. Acta Phytogeographica Suecica 49. Almqvist & Wiksells Boktryckeri AB, Uppsala.

Horandl, E. 2006. Paraphyletic versus monophyletic taxa – evolutionary versus cladistic classifications. Taxon 55: 564--570.

Ignatov, M. S. & Huttunen, S. 2002. Brachytheciaceae (Bryophyta) – a family of sibling genera. Arctoa 11: 245--296.

Kojima, S., Ohta, S., Yamamoto, T., Miura, T., Fujiwara, Y., Fujikura, K. & Hashimoto, J. 2002. Molecular taxonomy of vestimentiferans of the Western Pacific and their phylogenetics relationship to species of the Eastern Pacific. II. Families Escrpiidae and Arcovestiidae. Mar. Biol. 141: 57--64.

Mackinnon, M. J. & Read, A. F. 2003. The effects of host immunity on virulence-transmissibility relationships in the rodent malaria parasite *Plasmodium chabaudi*. Parasitology 126: 103--112.

Namaganda, M., Lye, K. A., Friebe, B. & Heun, M. 2006. AFLP-based differentiation of tropical African *Festuca* species compared to the European *Festuca* complex. *Theor. Appl. Genet*. 113: 1529--1538.

Nordal, I. & Stedje, B. 2005. Paraphyletic taxa should be accepted. *Taxon* 54: 5--6.

Oakley T. H. & Phillips, R. B. 1999. Phylogeny of Salmonine fishes based on growth hormone introns: Atlantic (*Salmo*) and Pacific (*Oncorhynchus*) salmon are not sister taxa. Mol. Phylogen. Evol. 11: 381--393.

Rao. T. A. & Naidu, T. R. B. On the epidermal fiber-like sclereids in the 2 sibling genera of the Poaceae. Current Science 50: 958--959.

Renvall, P. & Niemelä, T. 1993. *Ocotea usambarensis* and its fungal decayers in natural stands. Bull. Jard. Bot. Nat. Belg. 62: 403--414.

Ryss, A., **Boström, S. & Sohlenius, B.** 2005. Tylenchid nematodes found on the Nunatak basen, East Antarctica. Ann. Zool. 55: 315--324.

Sanders, W. B. 1999. Fruticose Growth of *Dirina catalinariae* f. *sorediata* on Santa Cruz Island, Northern Channel Islands, California. Bulletin of the California Lichen Society, Vol. 6 (1). http://ucjeps.berkeley.edu/rlmoe/cals6_1.html

Silva, P. C. 1993. Proposal to conserve *Ahnfeltia* Fr. (Rhodophylaceae) with a conserved type. Taxon 42: 128--130.

Waaland, J. R., Stiller, J. W. & Cheney, D. P. 2004. Macroalgal candidates for genomics. J. Phycol. 40: 26--33.

Williams, D. M., Ebach, M. C. & Wheeler, Q. 2005. 150 reasons for paraphyly: a response. *Taxon* 54: 858.

Winker, K. 2005. Sibling species were first recognized by William Derham (1718). *The Auk* 122: 706--707.

Zohary, D. 1996. The mode of domestication of the founder crops of southwest Asian agriculture. Pp. 142--158. in: Harris, D.R. (ed.), *The origins and spread of Agriculture and pastoralism in Eurasia*. Smithsonian Institution Press, Washington.

Zohary, D. 1999. Monophyletic vs. polyphyletic origin of the crops on which agriculture was founded in the Near East. *Genetic Resources and Crop Evolution* 46: 133--142.

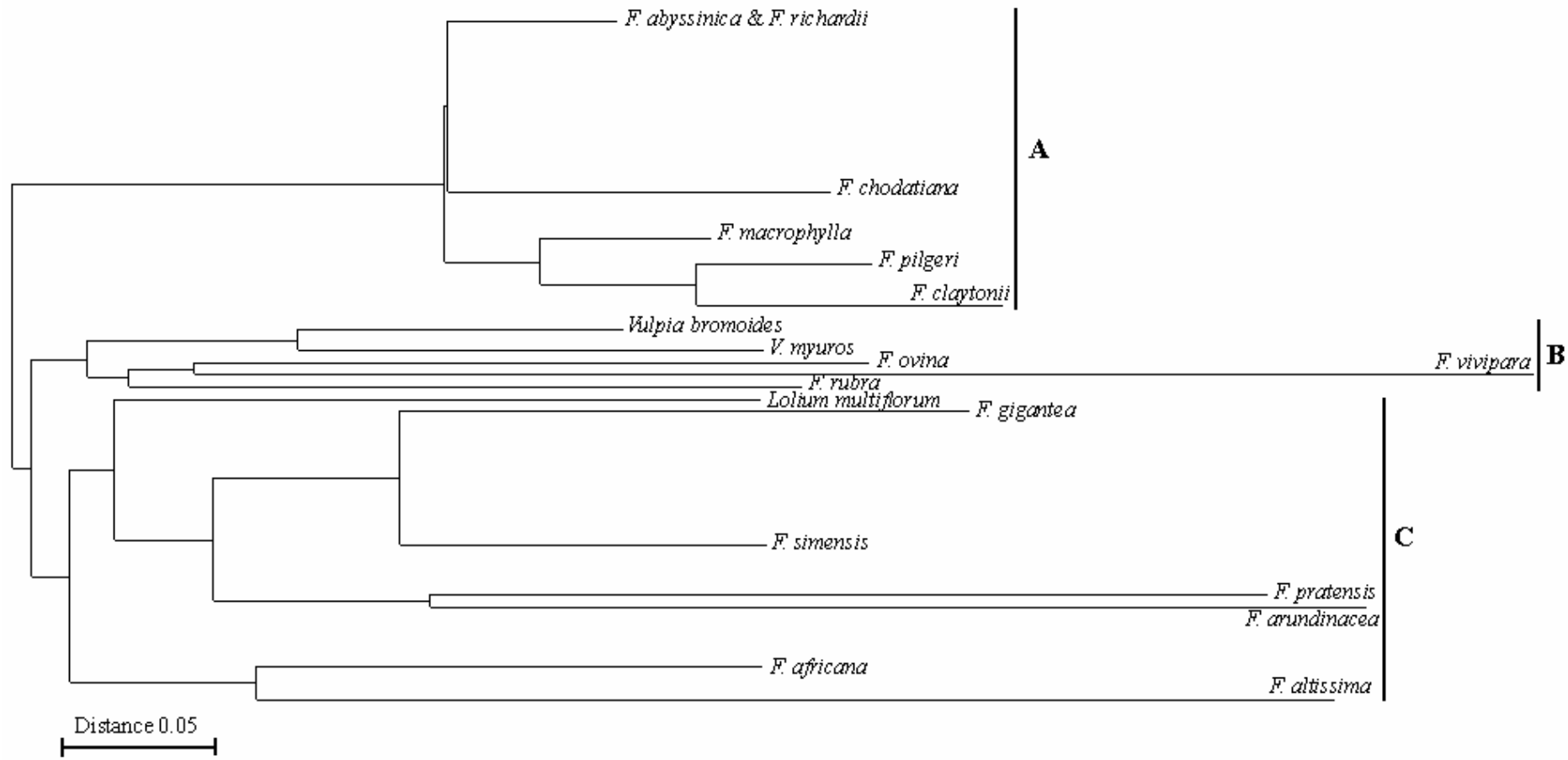

Figure 1. Neighbor-joining tree of 170 *Festuca* accessions analysed with 620 AFLPs (same data as in Namaganda & al., 2006; but with *F. macrophylla* added). The figure is edited to exclude the terminal branches leading to the individual accessions belonging to the same species. A – *Afrofestuca*, B – *Festuca* s.s. (narrow-leaved species), C – the broad-leaved species of *Festuca*.

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