

Morphological Relationships in Eurasian and North African Perennial Bromegrass (*Bromus* L., Poaceae)

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SUMMARY

Bromus L. is the largest genus in the Bromaceae Dum., including some 160 mesophytic or xerophytic species, living worldwide. *Bromus* subgen. *Festucoides* Hackel (= sect. *Pnigma* Dumort.) includes the perennial species having more or less developed rhizomes. Their spikelets are narrow and parallel sided, and have uni-nerved glumes (sometimes tri-nerved). The anatomy of the leaves is characteristic, slight ribbed, and with many vascular bundles associated to sclerenchyma strands. More than one hundred of morphological features were investigated in the perennial Eurasian and North African taxa to select a set of the most important features in the group. All them were treated as separate characters, and finally analysed to infer their taxonomic value, relations among the taxa, and to identify the evolutionary relationships. As a result of a multivariate analyses the taxa are grouped by the type of sheaths, indumentum, inflorescence type, spikelet size, florets number, and glumes and lemma characters including awn traits, etc. Morphological characters with evolutionary information were selected and used for a phylogenetic approach. The trees resulting from this study shows the primitive and the evolve species of the group. The main objective of this study is to explore patterns of morphological variation, and infer a phylogenetic approach.



RESULTS AND DISCUSSION

This study has attempted to resolve phylogenetic relationships among Eurasian and North African taxa belonging to *Bromus* subgen. *Festucoides* as well as to test the potential utility of the morphological characters. A previous multivariate analyses (UPGMA and PCA) with all the studied characters did not resulted in a good classification, and we preferred to select a set of 70 diagnostic characters to perform a phylogenetic analysis. Despite the low support of the clades, in all analysis resulted the same topology. The perennial *Festucoides* clade is resolved as monophyletic in all analyses by moderate bootstrap support (50 %) by 10 synapomorphies (1, 27, 34, 36, 37, 39, 42, 43, 44, 48, 53, 61). Among this clade several low supported clades are retrieved. Parsimony analysis of morphological data retrieved parsimonious trees with a length of 805 steps (CI 0.3565, RI=0.5256). One of the most parsimonious trees is presented in figure 1, and the neighbor joining tree in figure 2. The polytomy including the outgroup and other two clades (*Bromus* subgenus. *Ceratochloa* represented by *Bromus catharticus* and a second clade including members of *Bromus* subgen. *Festucoides*) revealed their simultaneous and primitive origin earlier than *Bromus* subgen. *Stenobromus* and *Bromus* subgen. *Bromus* that diverged recently. It is congruent with the suggestions made by Stebbins (1981) and partially with Pillay & Hillu (1995) studding chloroplast DNA variation concluding that subgenus *Ceratochloa* diverged at first while *Bromus* and *Stenobromus* diverged later, probably originated from similar ancestors than *Festucoides*. Within *Bromus* subgen. *Festucoides* six major clades are defined by morphological characters. The most primitive species (*B. ramosus*, *B. bennekenii*, *B. confinis*, *B. bifayensis* and *B. spilius*) diverged early. Our results do not support clearly a diverse group as *Bromus* subgen. *Festucoides* because the more diverse a group to be analyzed is, the more difficult is to find suitable characters to score, especially morphological ones (Endress 2003).

- Table 1. List of characters and states. Supported analyses in bold. Weighted characters are highlighted with * (Wagner) or † (Wagner). Uninformative characters between brackets.**
1. Rhizome Depressed (elongated) †-absent, short.
 2. Culm nodes at the base, forming an axoid cone †-absent, †-present.
 3. Sheaths auriculate at apex †-absent, †-present.
 4. Auriculae distinct †-absent, †-present.
 5. Head †-absent, 2-sterile when dead, 1-decaying into panicle fibres, 2-reticulate fibre.
 6. Head †-absent, 2-sterile when dead, 1-decaying into panicle fibres, 2-reticulate fibre.
 7. Leaves †-broad, 1-narrow.
 8. Triangles of Leaves †-oblong and narrow, 1-flat.
 9. Upper leaves wider than the lower †-absent, †-present.
 10. Ligula brown †-absent, †-present.
 11. Spike at lowest panicle node †-oblong and glaucous †-short.
 12. Spike †-oblong, 1-triangular.
 13. Panicle branches †-drooping, 1-drooping, 1-rigid, 1-patent.
 14. Branches †-oblong, 1-triangular.
 15. Branches †-oblong, 1-triangular.
 16. Length branches †-long, 1-short, 1-medium, 1-long.
 17. Length branches †-long, 1-short, 1-medium, 1-long.
 18. Length branches †-long, 1-short, 1-medium, 1-long.
 19. Length branches †-long, 1-short, 1-medium, 1-long.
 20. Length branches †-long, 1-short, 1-medium, 1-long.
 21. Length branches †-long, 1-short, 1-medium, 1-long.
 22. Length branches †-long, 1-short, 1-medium, 1-long.
 23. Length branches †-long, 1-short, 1-medium, 1-long.
 24. Length branches †-long, 1-short, 1-medium, 1-long.
 25. Length branches †-long, 1-short, 1-medium, 1-long.
 26. Length branches †-long, 1-short, 1-medium, 1-long.
 27. Length branches †-long, 1-short, 1-medium, 1-long.
 28. Length branches †-long, 1-short, 1-medium, 1-long.
 29. Length branches †-long, 1-short, 1-medium, 1-long.
 30. Length branches †-long, 1-short, 1-medium, 1-long.
 31. Awn †-absent, 1-present.
 32. Awn †-absent, 1-present.
 33. Awn †-absent, 1-present.
 34. Awn †-absent, 1-present.
 35. Awn †-absent, 1-present.
 36. Awn †-absent, 1-present.
 37. Awn †-absent, 1-present.
 38. Awn †-absent, 1-present.
 39. Awn †-absent, 1-present.
 40. Awn †-absent, 1-present.
 41. Awn †-absent, 1-present.
 42. Awn †-absent, 1-present.
 43. Awn †-absent, 1-present.
 44. Awn †-absent, 1-present.
 45. Awn †-absent, 1-present.
 46. Awn †-absent, 1-present.
 47. Awn †-absent, 1-present.
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 50. Awn †-absent, 1-present.
 51. Awn †-absent, 1-present.
 52. Awn †-absent, 1-present.
 53. Awn †-absent, 1-present.
 54. Awn †-absent, 1-present.
 55. Awn †-absent, 1-present.
 56. Awn †-absent, 1-present.
 57. Awn †-absent, 1-present.
 58. Awn †-absent, 1-present.
 59. Awn †-absent, 1-present.
 60. Awn †-absent, 1-present.
 61. Awn †-absent, 1-present.

SUMMARY OF PARSIMONY-TREE PARAMETERS
 Tree length = 805
 Consistency index (CI) = 0.3565
 Homoplasy index (HI) = 0.6435
 CI excluding uninformative characters = 0.3130
 HI excluding uninformative characters = 0.6870
 Retention index (RI) = 0.5256
 Rescaled consistency index (RC) = 0.1874

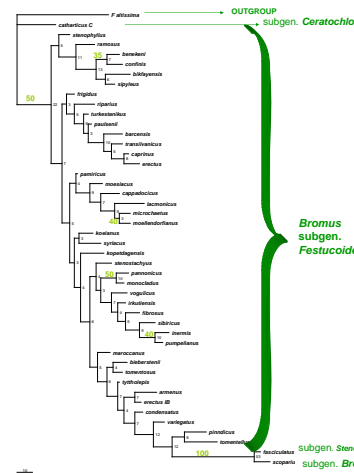


Figure 1. Phylogenetic analysis of *Bromus* subgen. *Festucoides* based on the morphological data. One of the most parsimonious trees obtained with the maximum parsimony method. The number of changes are represented in black. The bootstrap values appear along the branches. Scale bar = 10 steps. C = *Bromus* subgen. *Ceratochloa*, S = *Bromus* subgen. *Stenobromus*, B = *Bromus* subgen. *Bromus*.

MATERIAL AND METHODS

Taxa included in this study. Most Eurasian and North African taxa belonging to *Bromus* subgen. *Festucoides* (=sect. *Pnigma*) were studied (table 1). **Taxon sampling.** These investigations were based on collected material, and sheets mainly from K, but also by B, C, FCO, JACA, LEB, LISI, LISU, MA, P, SANT, SEV. More than 1000 specimens were examined in this study (a list is available on request). In some cases we didn't have available material and, it was necessary to compile the information in literature. **Morphology.** All the characters used in former studies Rechinger (1970), Smith (1980), Tselev (1983), Davis (1985), Acedo & Llamas (1999, 2001, 2008), Bacic & Logan (2001), Malyshev and Peschkova (2001), were checked in herbarium material and in our own specimens in order to assess their taxonomic value. Additional characters were included according to our observations. **Numerical Analyses.** -We carried out a hierarchical clustering with all the 46 taxa and 130 characters and 5 ratios, using Gower's coefficient for mixed data, and UPGMA algorithms of the SYN-TAX 2000 (Podani 2001). **Phylogenetic analysis.** All analysis were performed using PAUP* 4.0b10 (Swofford 2002). Only 70 (Table 1) of the 124 characters studied in previous numerical analysis were used. As we have few qualitative characters available for this study we have used quantitative data because one can recover phylogenetic information from these data (Thiele 1993). Values of some quantitative characters were coded using several ranges according to their variation (Table 1). **According to our knowledge of the genus *Bromus*, nineteen characters (highlighted with * in table 1) were weighted 3, and other five (** in table 1) were weighted 6. In a second analysis eleven characters (between brackets in table 1) parsimony-uninformative presenting some variation within taxa were excluded. *Festuca altissima*, was used as outgroup, and representatives from other *Bromus* subgenera have been introduced for comparison: *B. catharticus* (subgenus *Ceratochloa*), *B. fasciculatus* (subgenus *Stenobromus*), *B. scoparius* (subgenus *Bromus*). Gaps are treated as "missing". **Parsimony analysis (MPI)** were conducted with heuristic search and the default search options: 100 random addition replicates and the tree-bisection-reconnection (TBR) branch swapping algorithm. **Branch support** was assessed by the Bremer support index and the robustness of clades by means of bootstrap analysis (100 replicates and 10 heuristic search). **Trees** were drawn with TreeView (Page 2000).**

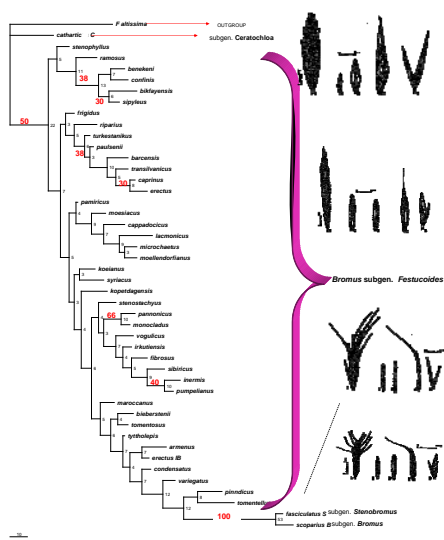


Figure 2. Neighbour joining tree inferred from the analysis of 59 of the 70 informative morphological characters used in MP analysis presented as phylogram. The number of changes are represented in black. The bootstrap (red) values appear along the branches. The scale bar indicates 10 changes. C = *Bromus* subgen. *Ceratochloa*, S = *Bromus* subgen. *Stenobromus*, B = *Bromus* subgen. *Bromus*.

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CONCLUSIONS

1. The overall evidence of this study suggest that *Bromus* subgen. *Festucoides* is a monophyletic taxon but evidently the groups of species within it are not strongly supported.
 2. Phylogenetic relations are not entirely resolved.
 3. Morphological data low support classical groups in *Bromus* subgen. *Festucoides*.
- To increase resolution and support within the phylogeny further efforts are:
- to increase taxon sampling.
 - to increase character sampling including molecular analysis.

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