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Morphometric Characteristics within *Festuca valesiaca* agg. *(Poaceae – Poeae)* in Istria and the status of the *F. illyrica* MARKGR.-DANN.

By

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With 6 Figures

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Summary

ALEGRO A. L. & ŠOŠTARIĆ R. 2005. Morphometric characteristics within *Festuca valesiaca* agg. *(Poaceae – Poeae)* in Istria and the status of the *F. illyrica* MARKGR.-DANN. – Phyton (Horn, Austria) 46(1): 113 – 128, with 6 figures. – English with German summary.

Festuca illyrica MARKGR.-DANNENB. has been considered as a commonly spread species in Dinaric area, to which Istria belongs. The precise analysis of the species descriptions and the determination key, as well as field work have showed that there are no clear borders between this species and other members of *F. valesiaca* agg. Herbarium material collected in 40 localities in Istria, from other parts of Croatia and vouchers from ZAHO and WU were taken into consideration. In the analysis 316 individuals of the *F. valesiaca* agg. and 20 morphological and anatomical characters were investigated. The data were analysed by using principal component analysis, discriminant analysis as well as some univariate statistical methods. It was possible to recognise only two groups corresponding with the species *F. rupicola* HEUFF. and *F. valesiaca* SCHLEICH. ex GAUDIN. Due to overlappings in the diagnostic characters *F. illyrica* has to be included in *F. rupicola*. The differentiation between the species *F. rupicola* and *F. valesiaca* is possible only on the basis of quantitative characters i.e. length of spikelets, lemmas, awns and diameter of leaf cross-sections. The most discriminative character in delimitation of *F. rupicola* and *F. valesiaca* was the length of stomata.

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Zusammenfassung

ALEGRO A.L. & ŠOŠTARIĆ R. 2005. Morphometrische Merkmale von *Festuca valesiaca* agg. *(Poaceae – Poeae)* in Istrien und der Status von *F. illyrica* MARKGR.-DANNENB. – Phyton (Horn, Austria) 46 (1): 113 – 128, mit 6 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Festuca illyrica MARKGR.-DANNENB. wurde als eine im dinarischen Raum inkl. Istrien weit verbreitete Art betrachtet. Eine genaue Analyse der Artbeschreibung, des Bestimmungsschlüssels und Feldstudien haben dagegen gezeigt, daß es keine klare Grenze zu anderen Sippen des *F. valesiaca* agg. gibt. Von 40 Lokalitäten in Istrien gesammeltes Herbarmaterial, Belege aus anderen Teilen Kroatiens und aus den Sammlungen ZAHO und WU wurden untersucht. Für die Studie wurden 316 Individuen aus dem *F. valesiaca* agg. und 20 morphologische und anatomische Merkmale berücksichtigt. Die Daten wurden mit Hilfe von Hauptkomponenten-Analyse, kanonischer Diskriminanzanalyse, sowie einiger univarianter, statistischer Methoden untersucht. Es konnten nur zwei Gruppen, die den Arten *F. rupicola* HEUFF. und *F. valesiaca* SCHLEICH. ex GAUDIN entsprechen, unterschieden werden. Wegen des Überlappens der diagnostischen Merkmale ist *F. illyrica* in *F. rupicola* einzuschließen. Die Unterscheidung zwischen *F. rupicola* und *F. valesiaca* ist nur auf der Basis quantitativer Merkmale wie Ährchen-, Deckspelzen- und Grannenlänge und der Dimension des Blattquerschnittes möglich. Das beste Unterscheidungsmerkmal war die Länge der Stomata.

1. Introduction

With 170 species (MARKGRAF-DANNENBERG 1980), the genus *Festuca* L. is the richest among the family of grasses in Europe and one of the taxonomically most difficult. The modern era of *Festuca* studies has begun with the fundamental works by HACKEL 1881 and 1882. He introduced anatomical characters of the transverse section of leaf blades and the first standard methods for measuring the different parts of the plants. During a century of research, new species have been described and the infraspecific status of many of HACKEL's taxa has been raised. As a result of these processes, the number of species has increased from 28 (HACKEL 1882) to 170 (MARKGRAF-DANNENBERG 1980).

The *F. valesiaca* agg. belongs to the very complicated *F. ovina* group. Young postglacial differentiation occurs in the aggregate, generating only quantitative differences in characters between the species. The group is comprised of series of species at different levels of ploidy. Diploids, tetraploids, hexaploids and octoploids have been known so far (cf. PILS 1984). With increasing ploidy level, the diameter of the leaves, the lengths of the spikelets, glumes, lemmas, fruit and stomata also increase. The overlappings of characters between the taxa can be significant, additionally caused by enviromental conditions in which the plants live (PATZKE 1968, HORÁNSKÝ & al. 1971). The *F. valesiaca* agg. is represented with the following species in the flora of Croatia («?» designates the species cited in literature but without evidence in the herbarium collections considered):

F. rupicola HEUFF., *F. valesiaca* SCHLEICH. ex GAUDIN subsp. *valesiaca*, *F. valesiaca* subsp. *parviflora* (HACK.) TRACEY (?), *F. stricta* HOST, *F. brevipila* TRACEY (?), *F. dalmatica* (HACK.) K. RICHT., *F. dalmatica* subsp. *panciciana* (HACK.) BELDIE and *F. illyrica* MARKGR.-DANNENB. (ALEGRO 2003). While the determination of most of these species can be based on the number of veins and/or the structure and number of sclerenchyma strands, the species *F. rupicola*, *F. valesiaca* and *F. illyrica* share almost the same pattern of leaf cross section resulting in an obscure determination key (cf. MARKGRAF-DANNENBERG 1980), which is discussed later. The «locus classicus» of the species *F. illyrica* MARKGR.-DANN. is Čelebić in the Livanjsko polje (Herzegovina), and the area of this species should comprise the whole Croatian coast and the Dalmatian hinterland to the dry grasslands in the karst of West Bosnia up to 1000 m a. s. (MARKGRAF-DANNENBERG 1972, 1973). There are only two additional shorter publications (PAVLETIĆ 1989, 1990) in which the relationships between *F. illyrica*, *F. pseudovina* (*F. valesiaca* subsp. *parviflora*) and *F. valesiaca* were studied. It was concluded in these papers that *F. pseudovina* does not appear in the Dinaric region at all, and that the «non valesiaca» specimens belong to the species *F. illyrica*. In these studies the species *F. rupicola* or any other species from this group were not taken into account. Therefore, the conclusion was, that except *F. valesiaca* there is another species from this group in the Dinaric region, and that should be *F. illyrica*. The phytosociological position of this species was discussed by TRINAJSTIĆ 1992.

During herbarium revisions and extent field work in Istria (theoretically part of the area of *F. illyrica*) it was impossible to recognise this species based on its descriptions (MARKGRAF-DANNENBERG 1972, 1973, 1980) because of significant overlappings of morphometric characters with *F. valesiaca* and *F. rupicola*. The only determination key (MARKGRAF-DANNENBERG 1980) is also questionable: *F. rupicola* on the one side and *F. valesiaca* and *F. illyrica* on the other are divided on the basis of stem length (more or less than 30 cm respectively) which is a very variable feature in the genus *Festuca*. Furthermore, the groups with *F. valesiaca* and *F. illyrica* are divided on the basis of the presence or absence of sheath hairs, also a very variable and inconstant character in the *F. valesiaca* agg.

The aim of this study was to analyse morphological and anatomical variation of Istrian populations of *F. valesiaca* agg. and to evaluate characters that have been used to delimit the taxa, especially the species *F. illyrica*.

2. Materials and Methods

2.1. Origin of the Material

The present study is based on herbarium material from the first author's collection sampled on forty localities through Istria (252 specimens) in the period from

1998 to 2000 (Fig. 1), on comparative material sampled in other Dinaric parts of Croatia (the island of Krk, Gorski kotar, Dalmatia) and on herbarium collections WU and ZAHO (64 specimens from different parts of Europe). Among them were ten herbarium specimens (ZAHO) determined as *F. illyrica* by MARKGRAF-DANNENBERG herself. A holotype of *F. illyrica* was not detected (in any of the considered herbaria), and MARKGRAF-DANNENBERG left behind no herbarium specimens from its "locus classicus".

For the anatomical analyses the transverse sections of tiller leaves were made using a razor blade. The sections were set in the glycerine-jelly (GERLACH 1977: 151–152). For vegetative characters five measurements on each specimen were made and three for reproductive characters. Each specimen was represented by arithmetical means of characters and treated as operational taxonomic unit (OTU). Altogether there were 316 OTUs included in the study. The full documentation of the OTU's is deposited at the library of the Botanical Department (Faculty of Sciences, University of Zagreb) as a part of a master thesis (ALEGRO 2002) and is available upon request from the corresponding author.

2.2. The Studied Characters

A list of 20 morphological and anatomical characters was prepared. The analysed characters were selected and studied after MARKGRAF-DANNENBERG 1980, PILS 1982, AIKEN & al. 1997 and FOGGI & al. 1999. The following characters were measured or scored:

Binary characters:

1. Colour of the plant (0 – plant green; 1 – plant bluish grey green)
2. Number of longitudinal ribs (in the leaf transverse section) (4, >4)
3. Number of sclerenchyma strands (in the leaf transverse section) (3, >3)

Attributive characters:

4. Glume-vestiture, presence (0 – glabrous; 1 – scattered pubescent; 2 – densely pubescent)
5. Glume-vestiture, position (0 – glabrous; 1 – at the apex and margins; 2 – over the most of the surface)
6. Lemma- vestiture, presence (0 – glabrous; 1 – scattered pubescent; 2 – densely pubescent)
7. Lemma- vestiture, position (0 – glabrous; 1 – at the apex and margins; 2 – over the most of the surface)
8. Number of veins (of the leaf transverse section) (<5, 5, >5)

Quantitative characters:

9. culm length (measured to the base of panicle; cm)
10. tiller leaf length (measured on uppermost well-developed leaf; cm)
11. panicle length (distance from the lowest node of panicle to the top, including the uppermost spikelet; cm)
12. spikelet length (distance from the base of lower glume to the tip of the lemma of the fourth fertile flower, awn excluded; mm)
13. number of florets in the spikelet
14. lower glume length (mm)
15. upper glume length (mm)

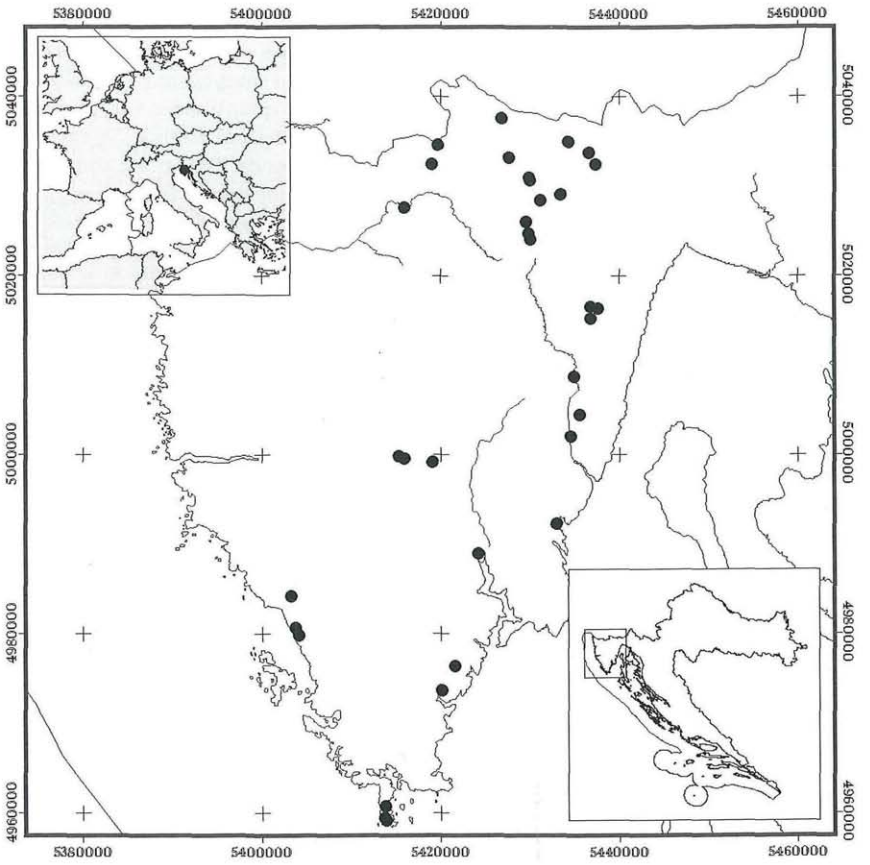


Fig. 1. Location and map of the study area. •: sampling localities.

16. lemma length (distance from the base to the apex, awn excluded, of the 2nd flower of the spikeket; mm)
17. awn of lemma length (measured from the lemma apex on the 2nd flower of the spikelet; mm)
18. height of leaf transverse section (diameter) (median measurements in cross-section; mm) (a in Fig. 2)
19. width of leaf (transverse measurement in cross-section; mm) (b in Fig. 2)
20. stomata length (μm)

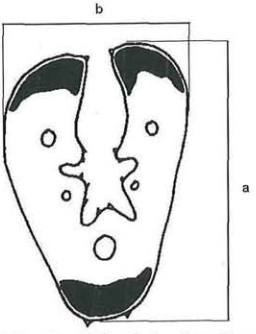


Fig. 2. Schedule for the measuremants of leaf cross-section. – a) height of leaf, b) width of leaf

2.3. Morphometric Analysis

In order to get an overall view of the phenetic relations among the species and to study morphological homogeneity of the species, principal component analysis (PCA) was done. The row data matrix with 316 OTUs and 12 quantitative variables was standardised by variables using "z-scores" as standardization method. A standardised data matrix was the input matrix for PCA. PCA was carried out by computing eigenvalues and eigenvectors from the variables correlation matrix. The two dimensions were retained. The eigenvectors matrix and eigenvalues, their percentages, and their cumulative percentages are listed. The spatial relationships between specimens are presented in the form of a two-dimensional scatter diagram.

With the same 12 variables the canonical discriminant analysis was carried out in order to find out if there is enough information in quantitative characters that would enable the separation into three groups corresponding to the three taxa. The two dimensions were retained and also presented as scatter diagram. The matrix of correlation between diagnostically most important variables and discriminant functions is also listed.

In order to study the discriminative power of the quantitative characters, descriptive statistics analysis was carried out on the data matrices of all three groups (species). For each species the minimum and maximum values, 1st and 9th percentiles were computed for each character. To evaluate the importance of the attributive and binary characters, the relative frequencies of the character states were calculated for each group.

Box-plots (median, quartiles, minimum and maximum values) of the diagnostically most important quantitative characters within each species are applied in order to show variability in an easily comparable way.

All calculating was done by the SPSS 10.0 package.

3. Results

The total variability of the first PC axis contributed as much as 30.98% of the total interspecies variability and the first three PC axes contributed as much as 69.61 % of cummulative variabilities, which indicated a relatively good representation of the relationships among the groups. The distribution along the first PC axis (Tab. 1) is in a large extent contributed by the length of upper and lower glumes, length of palea, length of spikelet and length of awn. The length and width of the leaf trensverse sections and the length of stomata are most correlated with the second PC axis. Less informative characters, the length of culms, tiller leaves and panicles, as shown in descriptive statistics, are most correlated with third PC axis.

The scatter diagram (Fig. 3) shows the presence of only two groups corresponding with the species *F. rupicola* and *F. valesiaca*. The marks representing the specimens determinated as *F. illyrica* are scattered throughout the group of *F. rupicola*. This result on the whole confirms the results of descriptive statistics, where the values of *F. rupicola* and *F. valesiaca* overlap.

Table 1. Principal component analysis. Correlation of the characters with the first three components

Characters	Component		
	1	2	3
heigth of culms	–0.080	0.087	0.848
length of tiller leaves	0.171	0.161	0.851
length of panicles	0.227	–0.040	0.847
length of spikelets	0.815	0.280	0.078
number of florets in spikelet	0.233	–0.060	0.104
length of lower glumes	0.870	0.066	0.067
length of upper glumes	0.904	0.082	0.169
length of lemma	0.860	0.238	0.070
length of awn of lemma	0.588	0.218	0.075
height of leaf cross-section	0.262	0.888	0.035
width of leaf cross-section	0.004	0.885	0.035
length of stomata	0.427	0.745	–0.060

Table 2. Canonical discriminate analysis. Canonical structure expressing correlation of some characters with discriminant axes. The strongest correlations are presented with bold numbers.

Characters	Axis	
	1	2
length of stomata	**0.839**	0.293
length of lemma	0.254	**–0.593**
height of leaf cross-section	0.211	**–0.484**
width of leaf cross-section	**0.438**	0.172
length of awn of lemma	0.225	–0.117
length of spikelets	0.216	–0.115
length of lower glumes	0.170	–0.099
length of upper glumes	0.210	–0.362
length of panicles	–0.032	–0.052

Canonical discriminant analysis (Fig. 4) shows that there is enough information in quantitative characters for clear separation of the two groups corresponding with the species *F. rupicola* and *F. valesiaca*. The group representing the species *F. illyrica* is continuosly connected with the group of *F. rupicola*. The first canonical axis is most correlated with the length of stomata, the hight of the leaf cross section and the length of awn. The characters most correlated with second axis are the length of lemma and the width of the leaf cross-section (Tab. 2).

The minimum and maximum values, 1st and 9th percentile of quantitative characters are listed in Tab. 3. Box plots of some diagnostically im-

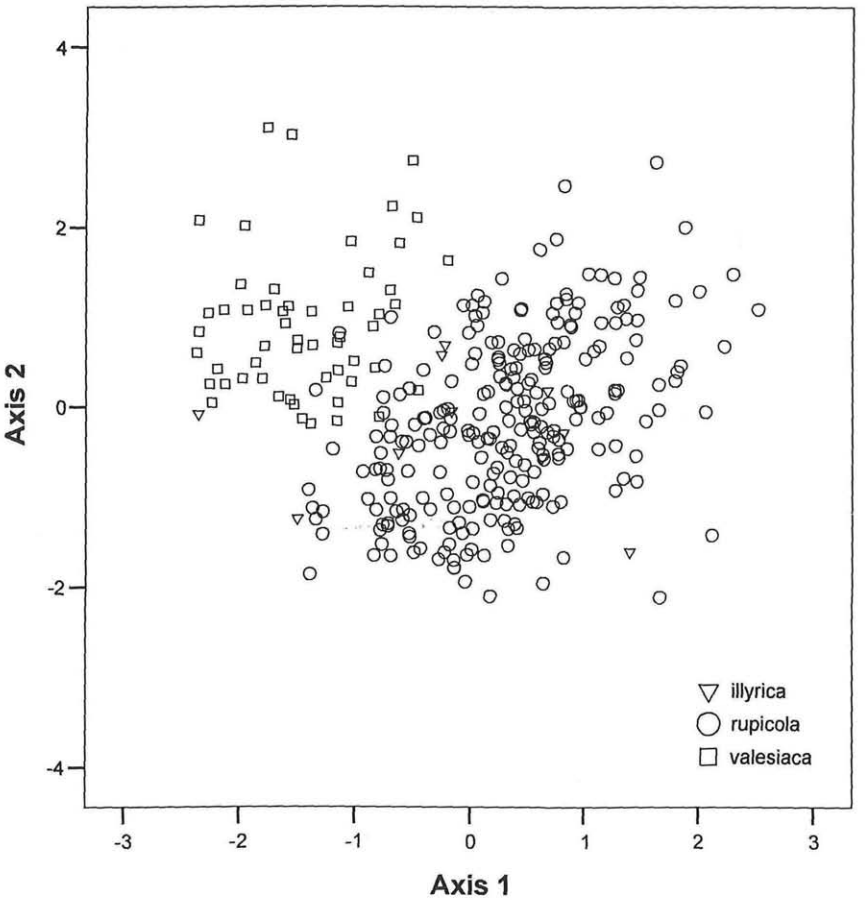


Fig. 3: Principal component analysis of *F. valesiaca* agg. in Istria

portant and some useless characters are presented in Fig. 5. Frequencies of states of the binary and attributive characters are listed in Tab.4. It is evident that is not possible to distinguish the mentioned species on the basis of nonquantitative characters. Generally, *F. rupicola* has more prominent indumentum of spikelets and *F. valesiaca* is more often bluish pruinose (clearly recognizable only on living plants).

Both statistical analyses, the multivariate and univariate, have shown that the studied specimens could be delimited only in two species, *F. rupicola* and *F. valesiaca*. *F. illyrica* could not be recognized as a separate species. This is illustrated in Fig. 6, where leaf cross-sections form two distinctive groups, one with *F. rupicola* and another with *F. rupicola* and *F. illyrica*.

Table 3. Quantitative characters; measures are stated as (minimum-) 1st percentile – 9th percentile (-maximum) value

Characters	*F. rupicola*	*F. valesiaca*	*F. illyrica*
length of culms (cm)	(13.4–)20.5–54.7(–82.3)	(21.1–)24.1–56(–66.3)	(22.7–)22.8–42.4(–42.6)
length of tiller leaves (cm)	(4.6–)8–25.7(–43.8)	(7.6–)9–21.9(–31.9)	(5.7–)5.7–17.4(–17.5)
length of panicles (cm)	(2.8–)3.8–7.9(–9.5)	(3.7–)4.6–7.6(–10.7)	(3.5–)3.7–8.2(–8.3)
length of spikelets (mm)	(4.7–)6.3–7.8(–8.5)	(4.9–)5.6–6.8(–7.6)	(5.8–)6–8.2(–8.3)
number of florets in spikelet	(3–)4–6(–8)	(4–)5–6(–9)	(4–)4.1–6.6(–7)
length of lower glumes (mm)	(1.9–)2.4–3.3(–3.8)	(1.9–)2.1–2.8(–3)	(1.7–)1.8–3.5(–3.6)
length of upper glumes (mm)	(2.9–)3.5–4.7(–5.5)	(2.8–)2.9–3.9(–4)	(2.6–)2.7–4.3(–4.4)
length of lemma (mm)	(3.6–)4.3–5.2(–6)	(3.5–)3.7–4.6(–5)	(3.8–)3.9–5.2(–5.3)
length of awn of lemma (mm)	(1.2–)1.8–3(–3.8)	(0.8–)1.2–2.2(–2.6)	(1.2–)1.4–3.7(–3.8)
height of leaf cross-section (mm)	(0.45–)0.56–0.69(–1)	(0.36–)0.38–0.53(–0.55)	(0.55–)0.56–0.74(–0.76)
width of leaf cross-section (mm)	(0.23–)0.28–0.51(–0.68)	(0.19–)0.21–0.35(–0.37)	(0.35–)0.37–0.57(–0.59)
length of stomata (*m)	(34.2–)36.6–41.5(–46.36)	(20.7–)22.9–29.3(–32.9)	(26.8)–27.6–45.1(–45.4)

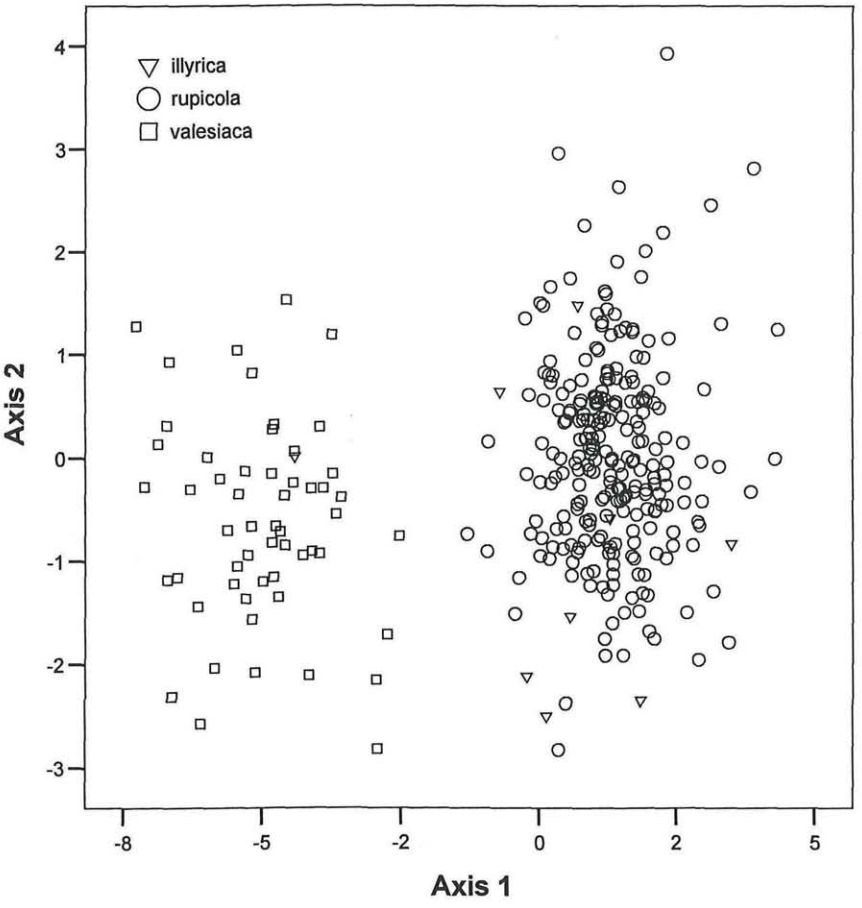


Fig. 4: Canonical discriminant analysis of the *F. valesiaca* agg. in Istria

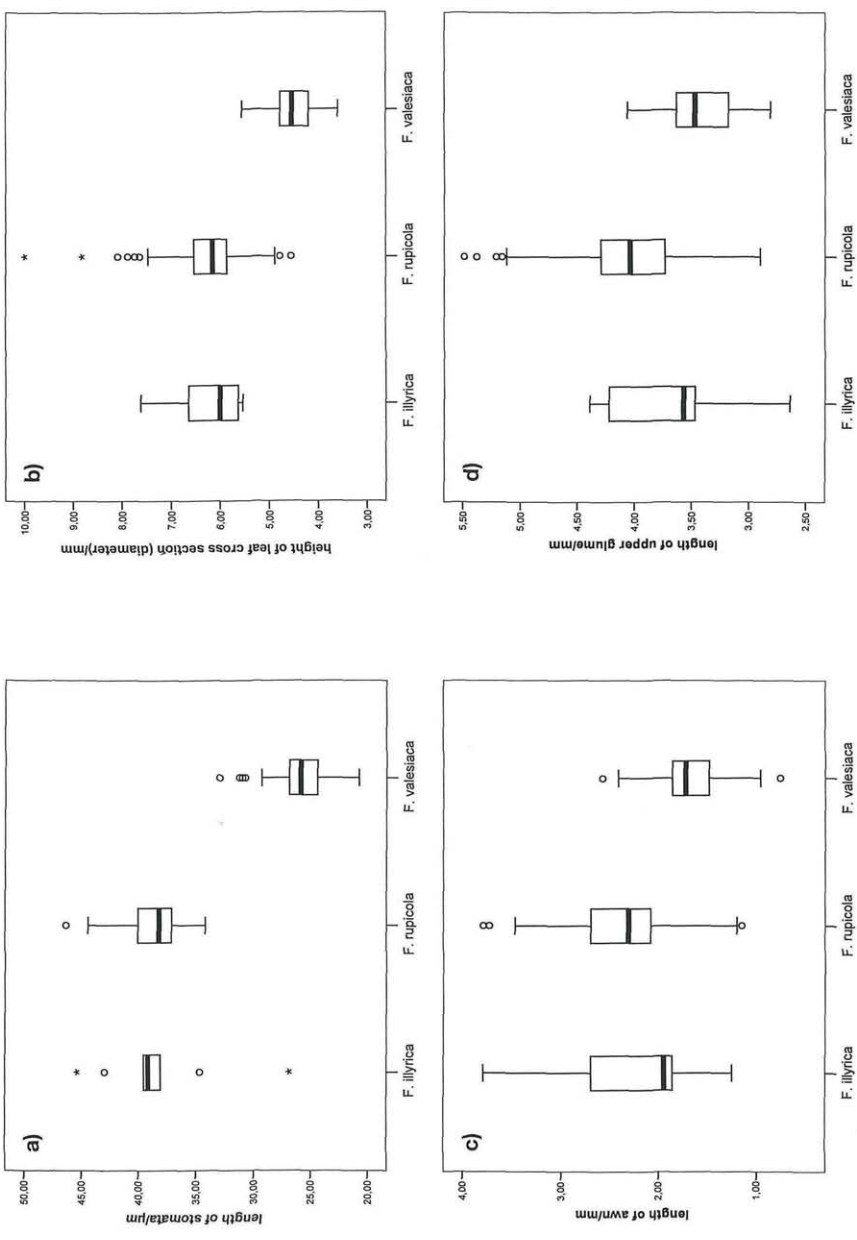
4. Discussion

The morphometric analysis showed that within the *F. valesiaca* agg. only two groups of specimens are determinable in the studied area, *F. rupicola* and *F. valesiaca*. All investigated specimens, determined by MARKGRAF-DANNENBERG as *F. illyrica*, were included into the group comprised of the specimens belonging to the species *F. rupicola*. Despite the fact that in the determination key (MARKGRAF-DANNENBERG 1980) the length of stem is considered as a differential character for *F. illyrica*, this study showed that this character, together with lengths of leaf blade and inflorescence, are not usable as determination characters due to great overlapping of values. These characters are also obviously determinated by enviromental influences such as soil and vegetation type, amount of water, grazing etc.

Table 4. Frequencies of states of the binary and attributive characters

Character	State	Frequency in the group (%)		
		F. rupicola	*F. valesiaca*	*F. illyrica*
glumes-vestiture, presence	0	75.2	94.6	50.0
	1	22.0	5.4	50.0
	2	2.8	0.0	0.0
glumes-vestiture, position	0	44.0	71.0	20.0
	1	34.0	23.2	20.0
	2	22.0	5.4	60.0
lemma-vestiture, presence	0	44.0	71.0	20.0
	1	34.0	23.2	20.0
	2	22.0	5.4	60.0
lemma-vestiture position	0	44.0	71.4	20.0
	1	35.2	26.8	60.0
	2	20.8	1.8	20.0
color of plants	–	2.0	0.0	80.0
	1	12.4	63.3	20.0
	0	85.6	35.7	0.0
number of veins	<5	0.0	5.4	0.0
	5	94.8	94.6	100.0
	>5	5.2	0.0	0.0
number of ribs	4	96.8	100.0	100.0
	>4	3.2	0.0	0.0
number of sclerenchyma strands	3	87.0	60.7	100.0
	>3	13.0	39.3	0.0

PATZKE 1968 and HORÁNSKÝ & al. 1971 came to a similar conclusion. Furthermore, there is no single qualitative character (e. g. presence or absence of sheat indumentum) that could be used as a determination character. The two remaining species are identifiable only on the basis of a number of quantitative characters as the lengths of spikelet, gluma, lemma, length and width of leaf transverse section. As an especially adequate determination character, the length of stomata can be used which, according to many authors (TRACEY 1980, PILS 1984, WILKINSON & STACE 1989, CONERT 1994, DENGLER 1998), represents the ploidy level. On the basis of the length of stomata all investigated specimens can be divided into two groups: one with mean stomata length of 26.2 μm and another with mean stomata length of 38.7 μm, what corresponds with the diploid species *F. valesiaca* and the hexaploid species *F. rupicola*. The mean stomata length of the specimens determinated as *F. illyrica* is 38.4 μm which probably indicates their hexaploid level and together with other characters belong to the species *F. rupicola*. The results of principal component analysis confirmed the division into two groups which corresponds with the species *F. vale-*



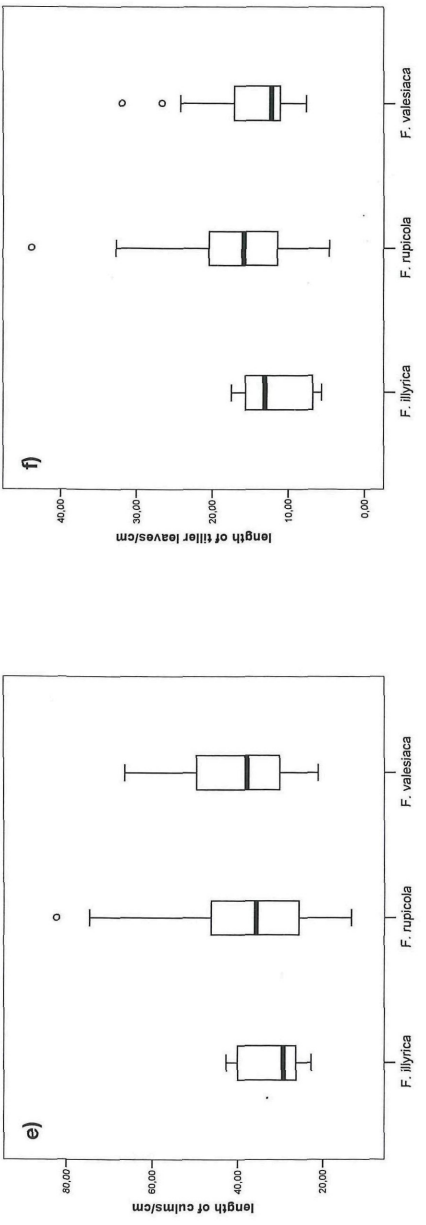


Fig. 5: Box-plots for characters of *Festuca valesiaca* agg. in Istria: a) length of stomata, b) height of leaf cross section, c) length of awn and d) length of upper glume are examples of useful determination characters. – e) height of culms and f) length of tiller leaves are examples of uninformative characters. – The box represents the interquartile range, the whiskers extend to the highest and lowest values, excluding outliers represented with circlets, while the extreme values are represented by asterisks. A line across the box indicates the median.

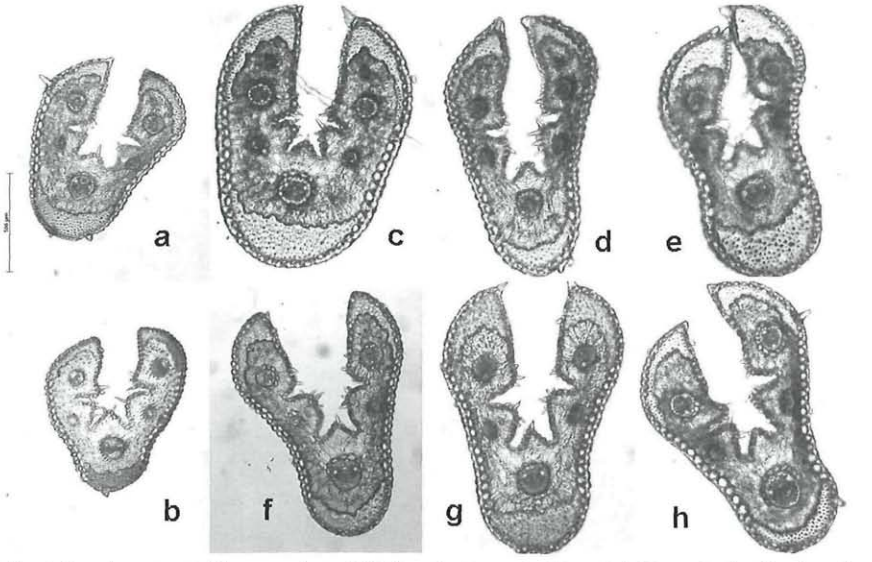


Fig. 6: Leaf cross-sections. – a) and b) *F. valesiaca*, c), d) and e) *F. rupicola*, f), g) and h) *F. illyrica* (determined by MARKGRAF-DANNENBERG). Scale bar equal = 0,5 mm.

siaca and *F. rupicola*. The third group corresponding with *F. illyrica* was not separated.

Therefore the synonymy is:

Festuca rupicola HEUFF. 1858, Verh. Zool.-Bot. Ges. Wien 8: 233.

= *F. illyrica* MARKGR.-DANNENB. 1972, Bot. Jahrb. Syst. 92: 151, nom. invalid.: typ. omiss.

= *F. illyrica* MARKGR.-DANNENB. 1973, Glasn. Zemalj. Muz., ser. nov. 11/12: 85–86. – The holotype is indicated as: Bosnien. Livansko polje (Livno) bei Čelebič. Unterhalb der Fahrstraße. Feinerde. Geschlossener *Festuca*-Rasen. 11. Jun. 1970: F. & I. MARKGRF-DANNENBERG. No relevant specimen could be found till now in any of the investigated herbaria.

In the investigated area the species *F. rupicola* is very variable in regard to the plant height, the length of tiller leaves and the indumentum of spikelets. This is a consequence of the broad spectrum of its habitats, from dry road sides and rocky places to upland grasslands. On the basis of spikelet indumentum a number of taxonomically irrelevant forms (CONERT 1994) could be recognised: f. *hirsuta* with hairs on the whole surface of lemma and ciliate margins, f. *glaucantha* with bluish spikelets, f. *sulcata* with smooth or only in the upper third scabrid lemmas, f. *barbata* with short hairs in the upper third of lemma and ciliate margins. The anatomical characters of leaves are much more constant. The specimens regularly had five vascular bundles and three bundles of sclerenchyma (Fig. 6). The specimens with seven vessels and additional sclerenchyma bundles were

also found, but even in this case the majority of leaves on the same specimen had five vascular bundles and three bundles of sclerenchyma.

The species *F. valesiaca* is less variable than *F. rupicola*. It has smaller spikelets, lemmas and glumes, which are glabrous or only ciliate on the margins. The leaves are slender, bluish, with five vascular bundles and three bundles of sclerenchyma. Some leaves have small additional bundles of sclerenchyma. *F. valesiaca*, in contrast to *F. rupicola*, is restricted to grassland vegetation developing predominantly on uneroded terra rossa. This habitat type is characteristic, especially for Central Istria, which can be considered as local centre of distribution of this species.

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