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PHENETIC RELATIONSHIPS WITHIN POPULATIONS OF *IRIS ILLYRICA*, *I. PALLIDA* AND *I. PSEUDOPALLIDA* (*IRIDACEAE*) WITH REGARD TO MORPHOLOGICAL CHARACTERISTICS OF EPIDERMIS

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The relationships within populations of *Iris illyrica* Tomm., *I. pallida* Lam. and *I. pseudopallida* Trinajstić, with regard to six morphological characteristics of the epidermis, by using cluster and PCA analysis, were established. The three species are mutually clearly separated, which confirms the otherwise controversial taxonomic rank of *I. pseudopallida*. The relative clarity is disturbed by undefined phenotypic plasticity of the registered characters, i. e. the possibility of their ecological conditions.

Introduction

Iris illyrica Tomm. and *I. pallida* Lam. are placed in the series *Elatae* (Dykes 1913, Lawrence 1953, Kohlein 1981), i.e. in the series *Pallidae* with *I. cengialti* Ambrosi and the newly described species *I. pseudopallida* Trinajstić (Trinajstić 1976). The most important characteristics for the whole of the series are dry spathe. According to Trinajstić (1976) *I. pallida* has silvery white spathe and pale-blue flowers, while *I. illyrica* and *I. pseudopallida* have a dirty-pale, rusty breath upon the spathe, and intensive violet, or pale-violet flowers. *I. pseudopallida* was registred earlier as a variety of the species *I. pallida* — *I. pallida* var. *dalmatica* Pamp. (Kohlein 1981), and as a special form of the species *I. illyrica* (Radić 1974). However, regardless of the taxonomic position

it is very difficult to distinguish these taxa from *I. pallida,* so, many authors have not considered it as a separated taxon (Hayek 1933, Webb and Chater 1980, Hećimović 1984, Schulze 1988). The distribution of the species *I. pseudopallida* would be south of Mosor (central and south Dalmatia with Montenegrin coast) (Trinajstić 1976, 1983).

Materials and Methods

Twenty specimens (OTU-operational taxonomic unit) of the populations of the species *Iris illyrica* (localities Grobničko polje, Rijeka, Croatia) and the species *I. pallida* (localities Zagreb, Croatia, from culture) and 18 specimens of the population of the species *I. pseudopallida* (localities Kotišina, Biokovo, Croatia) were collected and fixed in FAA mixture.

On the leaves of each individual measurements of the following six characteristics was performed:

1 — stoma length (μm)
2 — stoma width (μm)
3 — stomas number per mm^2
4 — length of epidermis cells (μm)
5 — width of epidermis cells (μm)
6 — number of epidermis cells per mm^2

Twenty measurements of the middle part of the leaves were done on every specimens and every characteristics by an ocular micrometar. The arithmetic mean was taken as a valid value for every OTU. For all populations and characteristics and for all OTU-s variabilities are presented by standard statistical tools. The difference between variables was tested by Kruskal-Wallis test (Sokal and Rohlf 1981).

Phenetic analysis

All variables were standardized by »z-score« method (Sneath and Sokal 1973). Euclidean distance was used to obtain the matrix of distance. UPGMA (unweighted pair group) method was used for hierarchic cluster analysis (Sneath and Sokal 1973). Cophenetic coefficient was used to show the validity of presentation by dendrogram (Everitt 1980). The PCA (Principal Component Analysis) was done on a matrix with standardized values of variables (Clifford and Stephanson 1975). A 3D scatter diagram was constructed from the first three PCA axes. Data processing was done with NTSYS-pc 1.6 program (Rohlf 1990).

Results

Statistical data processing

The results of statistical data processing are presented in Tab. 1.

The most variable characteristics in the population of *Iris illyrica* is stoma length (V=7.51%), and the most stable is the number of epidermis cell/mm^2 (V=1.00%). A similar type of variability, but different from the previous one is shown in the populations of *I. pallida* and *I.*

Table 1. Results of statistical data processing: $\bar{x}$ — arithmetical mean, S — standard deviation, V — coefficient of variability (%), $S_{\bar{x}}$ — standard error of $\bar{x}$

Iris illyrica	1	2	3	4	5	6 No. var
$\bar{X}$	40.66	43.29	54.05	302.43	47.01	92.3
S	0.784	0.920	1.099	22.713	1.501	0.923
V	1.918	2.125	2.033	7.510	3.192	1.00
$S_{\bar{x}}$	0.175	0.205	0.245	5.078	0.335	0.206
I. pallida						
$\bar{X}$	49.970	52.560	52.900	294.910	45.040	73.100
S	1.379	1.507	1.372	16.889	1.921	2.653
V	2.759	2.867	2.593	0.572	4.265	3.629
$S_{\bar{x}}$	0.308	0.336	0.306	3.776	0.429	0.593
I pseudopallida						
$\bar{X}$	48.821	45.220	64.111	272.272	44.298	98.833
S	1.454	1.633	1.906	15.323	2.012	1.855
V	2.978	3.611	2.973	0.452	4.542	1.876
$S_{\bar{x}}$	0.342	0.384	0.449	15.323	0.474	0.437
Total						
$\bar{X}$	48.534	47.092	56.775	290.482	45.493	87.706
S	1.978	4.303	5.194	22.335	2.128	11.181
V	4.075	9.137	9.149	7.688	4.677	12.749
$S_{\bar{x}}$	0.259	0.565	0.682	2.932	0.279	1.468

Table 2. The results of Kruskal-Wallis test.

Var	H
1	34.867
2	44.666
3	40.978
4	19.774
5	17.939
6	50.971

$H > x^2_{0.005(2)} = 10.597$

pseudopallida with most variable characteristic stoma width (V=4.26 and 4.54% respectively) and most stable characteristic stoma length (V=0.57 and 0.45% recpectively). If these three species are considered together, as the complex *I. pallida*, the most variable characteristic is the stoma number/mm² (V=12.74%), and the most stable is the length of epidermis cell (V=4.07%).

The results of Kruskal-Wallis test (Tab. 2) show siginificant differences for all six variables between population.

Table 3. The first five principal components with variability and cumulative variability.

Variable No.	PC axes No.				
	1	2	3	4	5
1	0.654	−0.594	0.412	0.083	−0.200
2	0.929	−0.293	0.065	0.139	0.113
3	−0.645	−0.614	0.385	0.082	0.225
4	0.318	0.724	0.463	−0.396	0.055
5	−0.077	0.824	0.228	0.514	−0.004
6	−0.949	−0.103	0.223	−0.038	−0.162
Var.	45.24	33.81	10.65	7.58	2.21
Cum. var.	45.24	79.05	89.69	97.27	99.49

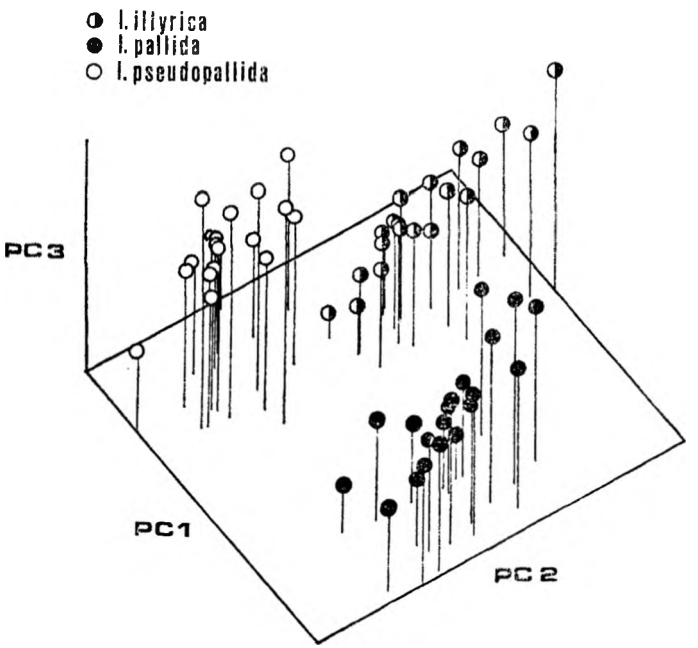

Fig. 1. PCA scattergram based on variance across six morphological characters of epidermis in 58 OTUs.

Principal component analysis (PCA)

The first three principal components accounted for 89.69% of the total variation among specimens. The Characteristics 2 and 6 yielded the highest variability connected with PC1. The characteristics 4 and 5

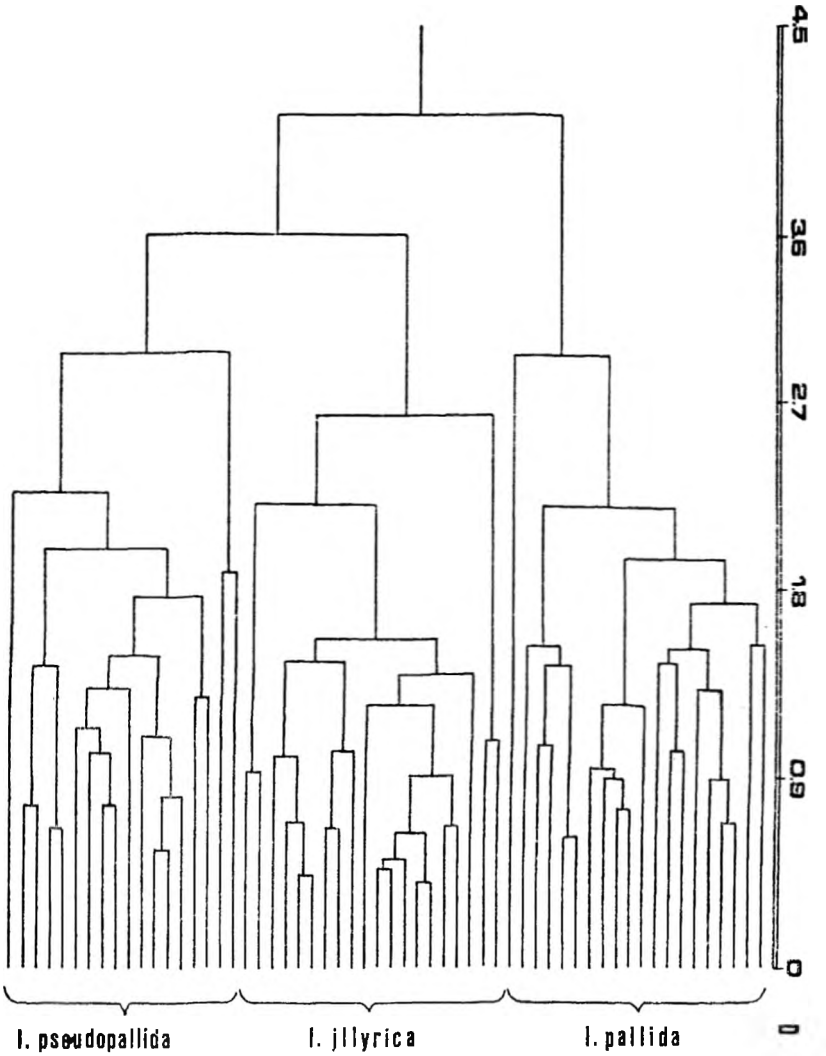

Fig. 2. UPGMA dendrogram based on six morphological characters of epidermis in 58 OTUs. Coefficient of cophenetic correlations r = 0.82036.

yielded the highest variability connected with PC2, and characteristics 1 and 4 yielded the highest variability connected with PC3 (Tab. 3).

Scatter diagram generated with first three PC axes (Fig. 1) clearly shows the presence of three groups. Each group completely comprises the specimens of three species *I. illyrica, I. pallida* and *I. pseudopallida.* This distribution is mostly influenced by the characteristics 2 and 6, and then 3 and 4.

Cluster analysis

Hierarchical cluster analysis mainly confirms the results of PCA analysis (Fig. 2). On the basis of all six variables three group have been formed: the specimens of *I. pallida* formed a separate branch of dendrogram at a distance coefficient DC = 4.063, and all other specimens formed separate branches of *I. illyrica* specimens and *I. pseudopallida* specimens at a distance coefficient DC = 3.409. There was no cases where a specimens from one species would be grouped with a specimens of a different species.

Discussion and Conclusion

Results of PCA and cluster analysis of morphological characteristics of the epidermis show with certainty the existence of population representatives of the controversial species *I. pseudopallida*, as a separate group of plants within *I. pallida* complex.

The validity of those results, however, are partlly disputed by the taxonomic value of the characteristics used: firstly, because of the unknown component of phenotypic plasticity as a part of the whole expressed phenotype (Mettler and Gregg 1969). According to Cutter (1978) stomatal index (which depends directly on the number of stomata and epidermal cells per mm^2) can vary in depending on humidity and light intensity, and also on the part of the plant on which it was measured (Metcalfe and Chalk 1979). According to Sharma and Buttler (1975) the number of stomata per mm^2 can vary also in dependence of the level of air pollution. Bačić (1982) noted that the number and dimension of stomata varied in dependence of the influence of light and age of plants. Maksimov (1952), Esau (1965), Fahn (1982), Wilder (1985) and Lucansky and Clough (1986) state the dependence of number and dimensions of stomata on xeromorphy, and mesomorphy of climatic conditions. Qi-Gen and Cutler (1985) emphasize that the diagnostic value of stomata is high because of their little variation within species, but the range of characters appears to be more related to ecological factors than to taxonomy. Metcalfe and Chalk (1979) state the dependence of stomatal dimensions on the poliploidy level (also Stebins 1971), correlations of stomatal dimensions with humidity and altitude. Rousteau (1981) states the variability of number of the epidermal cells and stomata per mm^2 in dependence of the position on the leaf, also the variability of stomatal index and number of epidermal cells in dependence of succulency of plants. Variability of stomatal frequency in dependence of light intensity have been shown by Pazourek experimentally (according to Lucansky and Clough 1986) and Knecht and O' Leary (1972).

However, there are examples of the usefulness of stomatal dimensions, stomatal frequency and stomatal index for diagnostic purposes (Metcalfe and Chalk 1979, Wilder 1985, Standley 1987). This presumes knowledge of phenotypic plasticity of all above mentioned characters of the respective group. Xeromorphy of stands of the populations *I. illyrica* and *I. pseudopallida* can be a cause of their grouping into a common branch of dendrogram, although in some other characters *I. pseudopallida* show similarity with *I. pallida*, whose exemplars were collected in a typically mesomorphic area. Those conclusion implicitly

include stipulated measured parameters with a set of climatic factors. If that hypothesis is accepted, the results obtained will be the classification of three populations on the basis of ecological conditions to the extent in which the phenotypic plasticity is present in the overall variability of the characters used. In other words, it is not possible to assing unambiguous taxonomic or other status to the established groups, without clearly defined phenotypic plasticity.

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SAŽETAK

FENETSKI ODNOSI POPULACIJA *IRIS ILLYRICA, I. PALLIDA* I *I. PSEUDOPALLIDA* (*IRIDACEAE*) S OBZIROM NA MORFOLOŠKE KARAKTERISTIKE EPIDERME

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Iris illyrica Tomm. i *I. pallida* Lam. svrstane su u seriju *Elatae,* odnosno u seriju *Pallidae* s *I. cengialti* Ambrosi i novoopisanom vrstom *I. pseudopallida* Trinajstić. Ta se svojta teško razlikuje od *I. pallida,* pa je mnogi autori ne smatraju posebnom vrstom. U svrhu jasnijeg definiranja međusobnih odnosa populacija vrsta *I. illyrica, I. pallida* i *I. pseudopallida* provedena je morfometrija nekih epidermalnih karakteristika listova. Rezultati mjerenja, obavljeni na statistički validnom uzorku, obrađeni su Kruskal-Wallisovim testom, klasternom analizom i PCA (Principal component analizom). Među populacijama ustanovljene su signifikantne razlike za sve varijable. Rezultati PCA i klasterne analize nesumnjivo pokazuju postojanje populacije, predstavnika sporne vrste *I. pseudopallida* kao odvojene grupe biljaka unutar kompleksa *I. pallida.* Kumulativna varijabilnost od 89,69% obuhvaćena je s prve tri od pet izračunanih PC osi. Širina puči i broj stanica epiderme pridonose u najvećoj mjeri varijabilnosti vezanoj uz PC 1, a dužina i širina stanica epiderme pridonose najviše varijabilnosti vezanoj uz PC 2. Dijagnostičku upotrebljivost ovih rezultata otežava zasad nepoznata fenotipska plastičnost korištenih epidermalnih svojstava.

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