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A taxonomic study of *Carex* sect. *Phaestoglochin* and sect. *Stellulatae (Cyperaceae)* in Bulgaria

Abstract

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19 populations of 3 species of *Carex* sect. *Phaestoglochin* and 9 populations of *C. echinata* of *C.* sect. *Stellulatae* were studied karyologically and morphologically. The following chromosome numbers were found: $2n = 58$ in *C. divulsa* subsp. *divulsa* and subsp. *leersii, C. muricata* subsp. *lamprocarpa, C. spicata,* and *C. echinata;* $2n = 56, 55$ in *C. muricata* subsp. *muricata*. A change in the taxonomic scheme of *C.* sect. *Phaestoglochin* is proposed.

Introduction

Carex sect. *Stellulatae* (Kunth) H. Christ and sect. *Phaestoglochin* Dumort. belong to *C.* subgenus *Vignea* (P. Beauv. ex T. Lestib.) Peterm. The former is represented by a single species in Bulgaria, *C. echinata* Murray. The Bulgarian members of *C.* sect. *Phaestoglochin,* however, form a critical, taxonomically difficult group, as evidenced by the taxonomic treatments by various Bulgarian authors (Ahtarov 1957, Valev & Kitanov 1964, Stojanov & al. 1966, Kožuharov & Andreev 1980). Moreover Ahtarov (1957) included *C. echinata* in the synonymy of *C. muricata* L., thus reiterating the error made by some early European authors (for more information, see David 1976). For that reason, both sections were included in our study.

The aims of this study were:

- to determine the karyological and morphological differences within and between *C.* sect. *Phaestoglochin* and sect. *Stellulatae* in Bulgaria;
- using stepwise discriminant analysis (SDA; Lachenbruch 1975), to test the reliability of the a-priori classification of the studied populations in two groups corresponding to these two sections, and to establish which characters contribute most to their discrimination;
- employing SDA, to test the reliability of the a-priori classification of the studied populations of *Carex* sect. *Phaestoglochin* in five groups representing the five taxa

Table 1. Studied populations of genus *Carex* sect. *Phaestoglochin* and sect. *Stellulatae* from Bulgaria. – No. = population number. Ca: limestone; Si: silica.

	No.	2*n*	Locality and Habitat
Carex* sect. *Stellulatae			
C. echinata	1066	58	C. Balkan range: along the road from Kalofer to the peak Botev, 42°40'N, 24°57'E, 1700 m, mountain brook at the limit of the beech forest, Si.
	481	58[1]	Mt Vitoša: bog on the plateau between the peak Ušite and the rest home Aleko, 42°35'N, 23°19'E, c. 1850 m, subalpine scrub and grassland, Si.
	1443	58	Mt Pirin: Todorini Ezera lakes, 41°43'N, 23°27'E, c. 2500 m, wet grassland surrounded by *Pinus montana*, Si.
	1087	58	Mt Rila: swamp habitats along the route from Govedarci village to the rest home, 42°16'N, 23°28'E, c. 1200 m, wet grassland with dominant sedges, Si.
	1446	58	Mt Rila: the Sara Gjol lakes, 42°13'N, 23°36'E, c. 1950 m, subalpine grassland among *Pinus montana* and *Juniperus sibirica*, Si.
	1251	58	Mt Lozenska (Sredna Gora region): swamp habitats near the route from Dolni Lozen to the peak Polovrak, 42°33'N, 23°34'E, 950 m, temporary wet grassland with dominant sedges, Si.
	1005	58	W. Rodopi: Smoljanski ezera, behind the Hotel "Ezerata", 41°36'N, 24°40'E, c. 1150 m, wetland surrounded by *Pinus montana*, Si.
	1044	58	W. Rodopi: "Rozenski livadi" near the town Čepelare, 41°42'N, 24°42'E, c. 1600 m, peat bog surrounded by spruce, Si.
	1266	58	Tundza hilly region: water meadows between the villages Alexandrovo, Manolovo, and Tărničene, 42°35'N, 23°05'E, 420 m, wet grassland on slightly acid to neutral alluvial soils.
C.* sect. *Phaestoglochin			
C. spicata	785	58	N.E. Bulgaria: Srebărna reserve 44°06'N, 27°04'E, < 50 m, wet grassland at the Srebarna lake, on alluvial sediments.
	485	58	C. Balkan range: Trojanski Balkan, above the village Černi Osăm, 42°50'N, 24°46'E, c. 700 m, Si.
	1285	58	Mt Plana (Vitoša region): along the route from Simeonovo to Bistrica, 42°34'N, 23°22'E, c. 700-800 m, grassland, Si.
	1225	58	Struma valley: S. of the village Krupnik, 41°50'N, 23°08'E, c. 400 m, wet grassland, Si.
	422	58[2]	Mt Rila: near the Žabokrek tavern, 42°06'N, 23°16'E, c. 500 m, ditches and wet meadows, Si.
	746	58	Mt Lozenska (Sredna Gora region): wetlands along the route from Dolni lozen to the peak Polovrak, 42°33'N, 23°34'E, c. 950 m, wet grassland, Ca.
	1101	58	C. Rodopi: Červenata stena reserve, church above the holy spring "Ajazmoto", along the path from the Bačkovski monastery to the Marciganica rest house, 41°56'N, 24°52'E, c. 750 m, weedy areas among *Prunus cerasifera* trees, Ca.
C. muricata subsp. *muricata*	1210	56	Mt Belasica: "Bjalata češma", above the village Samuilovo, 41°22'N, 23°05'E, ~ 500 m, meadow among trees of *Platanus orientalis* L. Si.
	469	55	Mt Belasica: the Belasica rest house above the town Petrič, 41°22'N, 23°14'E, c. 720 m, forest meadow between the rest house and a chestnut wood, Si.
	1412	56	W. Sredna Gora: town Hisar, 42°30'N, 24 °42'E, 300-500 m, dry grassland within the "Kaleto" area, Si.
	1402	56	Thracian plain: "Kaleto" area above the village Mezek, 41°44'N, 26°06'E, 100-200 m, dry grassland at the margins of *Quercus pubescens, Q. virgiliana*, and *Carpinus orientalis* wood, Si.

Table 1 (continued).

	No.	2*n*	Locality and Habitat
subsp. *lamprocarpa*	1305	58	Mt Pirin: below the Bănderica rest house, 41°48'N, 23°26'E, c. 1950 m, rocky habitats by the river, Si.
C. divulsa subsp. *divulsa*	1275	58	C. Balkan range: Gabrovski Balkan, "Uzana" area, 42°49'N, 25°19'E, c. 1200 m, clearings in beech wood, Si.
	1288	58	E. Balkan range: Slivenski Balkan, Ardašläka reserve, 42°55'N, 26°12'E, c. 500 m, clearings in forest of *Abies alba, Carpinus betulus,* and *Quercus dalechampii,* Si.
subsp. *leersii*	1450	–	C. Balkan range: Tetevenski Balkan, "Dančova poljana area, 42°51'N, 24 °14'E, c. 1300 m, sunny meadow in beech forest, Si.
	1287	58	C. Balkan range: Trojanski Balkan, Steneto reserve, 42°46'N, 24°49'E, c. 1450 m, shady place in beech forest, Si.
	1277	58	C. Balkan range: Gabrovski Balkan, "Uzana" area, 42°49'N, 25°19 E, c. 1000 m, roadside in beech forest, Si.
Intermediate populations	1280	58	C. Rodopi: between the village Bačkovo and Červenata stena reserve, 41°56'N, 24°52'E, c. 750 m, among trees of *Carpinus orientalis* and *Quercus pubescens,* Ca.
	1214	56-57	Struma valley: Mt Malăk Kožuh, "Rupite", 41°25'N, 23°16'E, 50-100 m, among trees of *Q. pubescens* and *Q. virgiliana* and shrubs of *Paliurus spina-christi, Jasminum fruticans,* etc., Si.

[1] First published by Stoeva(1987). [2] First published by Stoeva(1992).

recognized by Chater (1980): *C. spicata* Huds., *C. muricata* L. subsp. *muricata* and subsp. *lamprocarpa* Čelak., *C. divulsa* Stokes subsp. *divulsa* and subsp. *leersii* (Kneuck.) W. Koch, and to determine the most important characters for discrimination between them;

– using the classification functions, to establish the most appropriate taxonomic disposition of some intermediate (ungrouped) populations of *Carex* sect. *Phaestoglochin.*

Material and methods

19 Bulgarian populations of *Carex* sect. *Phaestoglochin* and 9 of sect. *Stellulatae* were studied karyologically and morphologically. They were collected from different parts of the country (Table 1). Two populations of sect. *Phaestoglochin* (No. 1280 and No. 1214) were not classified a priori, because they combined features characteristic of different taxa within the section.

The chromosome numbers were counted on metaphase plates of root tips of living plants collected from nature and cultivated in the greenhouse of the Botanical Institute, Sofia. The root tips were treated and the squashes were made according to Stoeva & Štepankova (1990). Voucher specimens were deposited at SOM.

30 plants from each population were collected, 20 quantitative characters were measured on each, and the results of the measurements or their ratios were computed for numerical analysis. Some qualitative characters were considered, too: disposition of male and female flowers in bisexual spikes, presence or absence of a corky swelling at the utricle base, tint of the sheath, utricles and glumes. The coefficient of variation for

various characters was used for assessment of within-population variation. Euclidean distances between populations were computed for evaluating inter-population differences. SDA was applied as a multivariate method for solving the stated problems, based on the biometrical values of the quantitative characters of each plant examined, and continued stepwise until inclusion of additional variables did not any more improve the classification substantially.

Results and Discussion

Karyology. – Four chromosome numbers were counted: $2n$ = 55, 56, 57, 58 (Table 1). $2n$ = 58 chromosomes were found in all studied taxa except *Carex muricata* subsp. *muricata,* in which the number was $2n$ = 55 or 56. In one of the intermediate popula

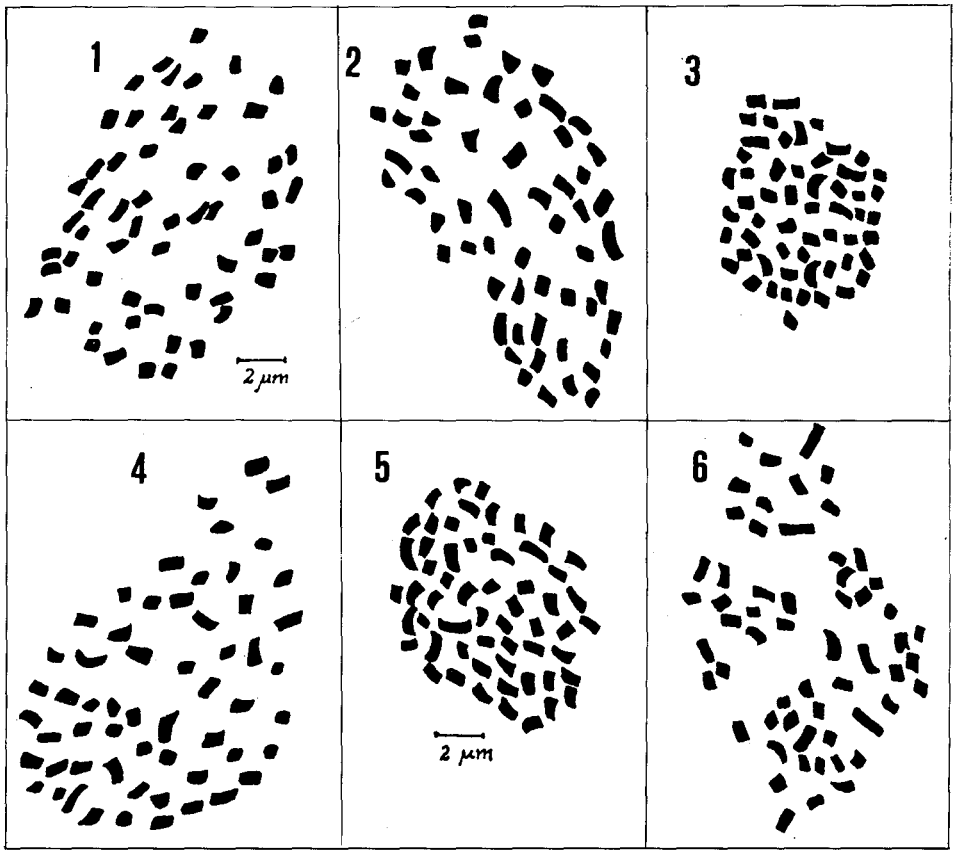

Fig. 1-6. Somatic metaphase plates showing the karyotypes of the 6 Bulgarian *Carex* taxa studied. – 1, *C. echinata* ($2n$ = 58); 2, *C. spicata* ($2n$ = 58); 3, *C. muricata* subsp. *muricata* ($2n$ = 56); 4, *C. muricata* subsp. *lamprocarpa* ($2n$ = 58); 5, *C. divulsa* subsp. *divulsa* ($2n$ = 58); 6, *C. divulsa* subsp. *leersii* ($2n$ = 58).

tions(No. 1280), $2n = 58$ chromosomes were counted; in the other (No. 1214), $2n = 56$ or 57, with the first number predominating. Our karyological results agree with most of the literature data. The constancy of the chromosome number $2n = 58$ counted in the studied Bulgarian populations of *C. divulsa* subsp. *leersii* contrasts, however, with the data from northern Europe and Switzerland where the chromosome number displays aneuploid variation, yet with $2n = 58$ predominating (Hartvig 1986). The number $2n = 58$ as established for Bulgarian *C.* muricata subsp. *lamprocarpa* disagrees with the results of Hartvig (1986), who considered the $2n = 52$ chromosomes counted by him in northern Europe as "the first unambiguous record for this taxon" and referred earlier reports to *C. muricata* s.l. In comparison, *C. echinata* shows a more homogeneous karyotype, in terms of relative length of the chromosomes, than the taxa of *C.* sect. *Phaestoglochin* (Fig. 1-6).

Morphology. – The coefficient of variation was used as a criterion of plasticity of the characters within the populations. *Carex echinata* shows less within-population variation than the taxa of *C.* sect. *Phaestoglochin*. The bract dimensions were the most variable characters in both sections. The number of female spikes, the length of the basal leaf, and the utricle tint were also very variable in *C. echinata,* while in most populations of *C.* sect. *Phaestoglochin* ligule length was among the most variable features. In both sections, the characters relating to reproductive structures were as a rule less variable than those of vegetative parts. The dimensions of the utricle and glumes were the least variable characters among those of reproductive structures. The disposition of the male and female flowers in the bisexual spikes, the sheath tint, and the presence or absence of a corky swelling at the utricle base were the least variable qualitative characters within populations.

Inter-population variation was estimated on the basis of the Euclidean dissimilarity coefficient matrix. The Euclidean distance values varied to different degrees in both sections, and in each taxon within *Carex* sect. *Phaestoglochin*. The range of these values was largest in *C. divulsa* subsp. *leersii* (10.15-42.21), in spite of the fact that only 3 populations were studied. In *C. muricata* subsp. *muricata* and *C. spicata* the Euclidean distance values varied from 2.55 to 30.08 and from 2.07 to 27.11, respectively. The Euclidean distance between the two populations of *C. divulsa* subsp. *divulsa* was 14.94. In *C. echinata* the Euclidean distance values varied less widely (1.84-20.18). No correlation whatever could be made out between the level of inter-population differences within individual taxa and the environmental variables of the respective habitats.

The results of SDA showed a perfect separation of the studied populations in two groups, coextensive with *Carex* sect. *Stellulatae* and sect. *Phaestoglochin*. This is visible from the distribution of the plants in the discriminant space, from the 100 % correct classification, and from the $F = 1106.2$ computed from the test of equality of group means at degrees of freedom 6 and 863. The classification function, with respect to the taxa of *C.* sect. *Phaestoglochin* (see Table 2 for variable designations), is:

$$38.28 + 4.21X_{15} + 20.87X_{16} + 10.49X_{17} - 22.15X_{20} + 8.19X_{23} - 68.51X_{27}$$

Besides by the characters listed in Table 2, the two sections differ from each other in the disposition of the male and female flowers in the bisexual spikes. Consequently, there is no reason to mix up *Carex echinata* with whatever taxon of *C.* sect. *Phaestoglochin*.

Table 2. The sequence of significance of 6 selected characters for discrimination between *C.* sect. *Phaestoglochin* and sect. *Stellulatae,* obtained by SDA.

Characters	Wilk's Lambda	degrees of freedom		
X_{23} male glume length	0.219	1	1	868.0
X_{17} utricle width	0.171	2	1	868.0
X_{15} distance between the two lower spikes	0.151	3	1	868.0
X_{27} utricle length : female glume length	0.138	4	1	868.0
X_{16} utricle length	0.128	5	1	868.0
X_{20} female glume length	0.115	6	1	868.0

Table 3. The sequence of significance of 11 selected characters for discrimination between 5 taxa of *Carex* sect. *Phaestoglochin,* obtained by SDA, and the corresponding discriminant functions (F_1-F_4).

Characters	Wilk's Lambda	F_1	F_2	F_3	F_4
X_{15} distance between the two lower spikes	0.473	−0.340	0.020	−0.903	0.090
X_{20} female glume length	0.263	2.235	0.472	0.572	1.192
X_6 length of the stem leaf	0.140	−0.078	0.031	0.064	0.031
X_4 length of the basal leaf ligule	0.106	0.497	0.317	−0.163	−0.139
X_{24} male glume width	0.084	0.452	−2.650	−0.435	1.846
X_{26} utricle length : utricle width	0.067	−0.006	0.953	−1.850	1.366
X_{27} utricle length : female glume length	0.057	1.277	0.296	2.954	3.206
X_{14} number of bisexual spikes	0.050	0.160	−0.394	0.036	0.224
X_1 stem height	0.044	−0.003	0.021	0.019	−0.020
X_{11} inflorescence length	0.039	−0.616	0.307	−0.241	0.132
X_7 width of the stem leaf	0.036	−0.126	0.862	0.549	−0.436
constant		−7.550	−3.250	−3.312	−13.974

SDA was also employed to determine the order of importance of the characters when used to discriminate between the five groups corresponding to the five taxa within *C.* sect. *Phaestoglochin,* and to classify individuals of the intermediate (ungrouped) populations, using the computed classification functions. The 11 characters with the greatest discrimination power are listed in Table 3 by decreasing Wilk's Lambda value and with their derived discriminant functions.

The relationships of the five taxa according to the first three discriminant functions and the classification of unknown populations are given in Fig. 7-8. Fig. 7 shows that function 1 separates the plants of *Carex spicata* from those of *C. muricata* and *C. divulsa.* The percent value of correct classifications for this species (94.8 %) is higher than for the other taxa. F-values computed from the test of equality of group means for each pair of taxa, given in Table 4, show that the group of populations corresponding to *C. spicata*

Table 4. F matrix (containing F-values computed from the test of equality of group means for each pair of taxa) from SDA for the 5 studied Bulgarian taxa of *Carex* sect. *Phaestoglochin*.

(No.)Taxon	F-values			
Taxon or (No.):	(1) *C. spicata*	(2)	(3)	(4)
(2) *Carex divulsa* subsp. *divulsa*	145.53			
(3) *Carex divulsa* subsp. *leersii*	93.67	49.77		
(4) *Carex muricata* subsp. *muricata*	95.14	69.26	55.23	
(5) *Carex muricata* subsp. *lamprocarpa*	43.96	33.44	21.96	24.29

Table 5. Results of the classification, by discriminant analysis, of individuals among 5 pre-defined taxa of *Carex* sect. *Phaestoglochin.* – The groups (coextensive with taxa) are numbered 1-5. Overall percent value of cases correctly classified: 91.57 %.

Actual group	No. of plants	predicted group membership				
		1	2	3	4	5
1 *C. spicata*	210	199	0	1	9	1
2 *C. divulsa* subsp. *divulsa*	60	0	57	0	1	2
3 *C. divulsa* subsp. *leersii*	90	5	1	76	5	3
4 *C. muricata* subsp. *muricata*	120	2	1	7	107	3
5 *C. muricata* subsp. *lamprocarpa*	30	0	0	1	1	28
intermediate populations (ungrouped)	60	1	19	22	6	2

clearly differs from the others. Bearing in mind the SDA results as well as the differences in qualitative features (the purplish-tinged basal sheaths and the corky swelling at the utricle base) we agree with Nilsson's (1985) statement that *C. spicata* is a good species. This taxon is recognized at species rank in most of the taxonomic treatments of *C.* sect. *Phaestoglochin* (Medovič 1960, Chater 1980, Kožuharov & Andreev 1980).

The numbers of correct classifications for each of the remaining four taxa, and the distribution of incorrect ones, is given in Table 5, which shows that the limits between *Carex divulsa* subsp. *divulsa* and subsp. *leersii* as well as between *C. muricata* subsp. *muricata* and subsp. *lamprocarpa* are more clear cut than the limits between *C. divulsa* subsp. *leersii* and *C. muricata* subsp. *muricata*. It is apparent from Table 4 that the difference between *C. divulsa* subsp. *leersii* and subsp. *divulsa* is much more significant than that between *C. divulsa* subsp. *leersii* and *C. muricata* subsp. *lamprocarpa*, for example. Even the difference between *C. muricata* subsp. *lamprocarpa* and subsp. *muricata* is larger than that between *C. muricata* subsp. *lamprocarpa* and *C. divulsa* subsp. *leersii*.

An attempt to classify the ungrouped populations makes it evident that the limits between the four taxa break down. This can be seen e.g. on Fig. 8, where the a priori groups *Carex divulsa* subsp. *divulsa* and subsp. *leersii* are well separated along the third canonical variable, but some plants mainly of population No. 1280 cause an apparent

continuum between the group clusters. While the plants of this population (No. 1280) fall within *C. divulsa* subsp. *leersii* at 46.6 %, *C. divulsa* subsp. *divulsa* at 33.3 %, and *C. muricata* subsp. *muricata* at 20 %, the majority (53.4 %) of the individuals of population No. 1214 falls within *C. muricata* subsp. *muricata,* 30 % within *C. divulsa* subsp. *leersii,* and only 16.6 % within *C. muricata* subsp. *lamprocarpa.* Bearing in mind the morphological features, the chromosome numbers (Table 1), the SDA classification, and the large number of sterile utricles observed, we may suppose population No. 1280 to be of hybrid origin, deriving from *C. divulsa* subsp. *divulsa* and subsp. *leersii;* and No. 1214 to probably comprise hybrid progeny of *C. divulsa* subsp. *leersii* by *C. muricata* subsp. *muricata.* Hence we cannot regard reproductive isolation between the four taxa of the *C. muricata-divulsa* complex to be effective. Hybridization was probably favoured by the panmictic population structure of the studied taxa and by their incomplete space and niche isolation. AS A result of our study; we therefore think it is appropriate to include *C. muricata* and *C. divulsa,* with their respective subspecies, in a single polymorphic species

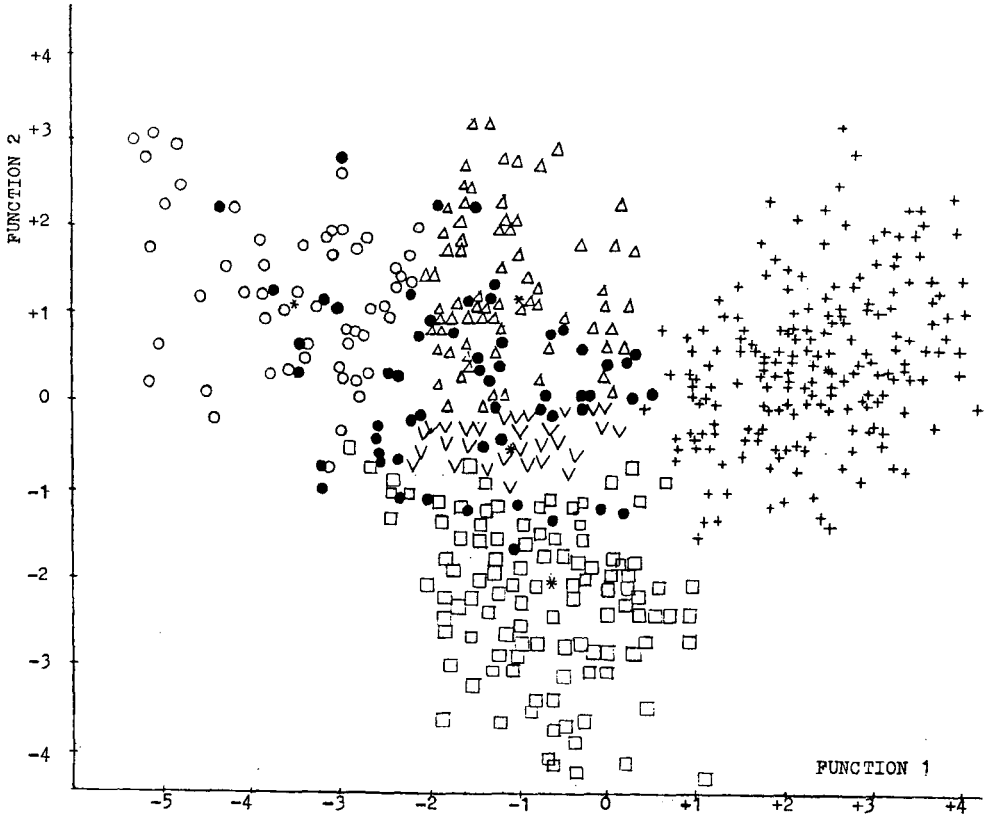

Fig. 7. Discriminant plot of individuals of the five a-priori defined groups corresponding to *Carex* taxa, and two intermediate populations, with respect to discriminant functions 1 and 2. – *C. spicata* (+); *C. muricata* subsp. *muricata* (□); subsp. *lamprocarpa* (V); *C. divulsa* subsp. *divulsa* (○); subsp. *leersii* (Δ); intermediate populations (●). – Group centroids denoted by asterisks (*).

named *C. muricata*. A similar opinion was held by Nilsson (1985) in Flora Turkey although he eventually chose to follow Chater's taxonomic scheme. We concur with Nilsson's statement, that it is necessary to investigate this complex group throughout its area, with emphasis on regions in which sympatric taxa occur.

Conclusion

Our results showed that *Carex* sect. *Stellulatae* and sect. *Phaestoglochin* are both karyologically and morphologically distinct and have different ranges of karyological and morphological variation.

In *Carex* sect. *Phaestoglochin* the variational picture is very complex. Based on our results, we propose the following taxonomic disposition for the 5 Bulgarian representatives of this section: *C. spicata* Huds., *C. muricata* L. subsp. *muricata*, *C. muricata* subsp.

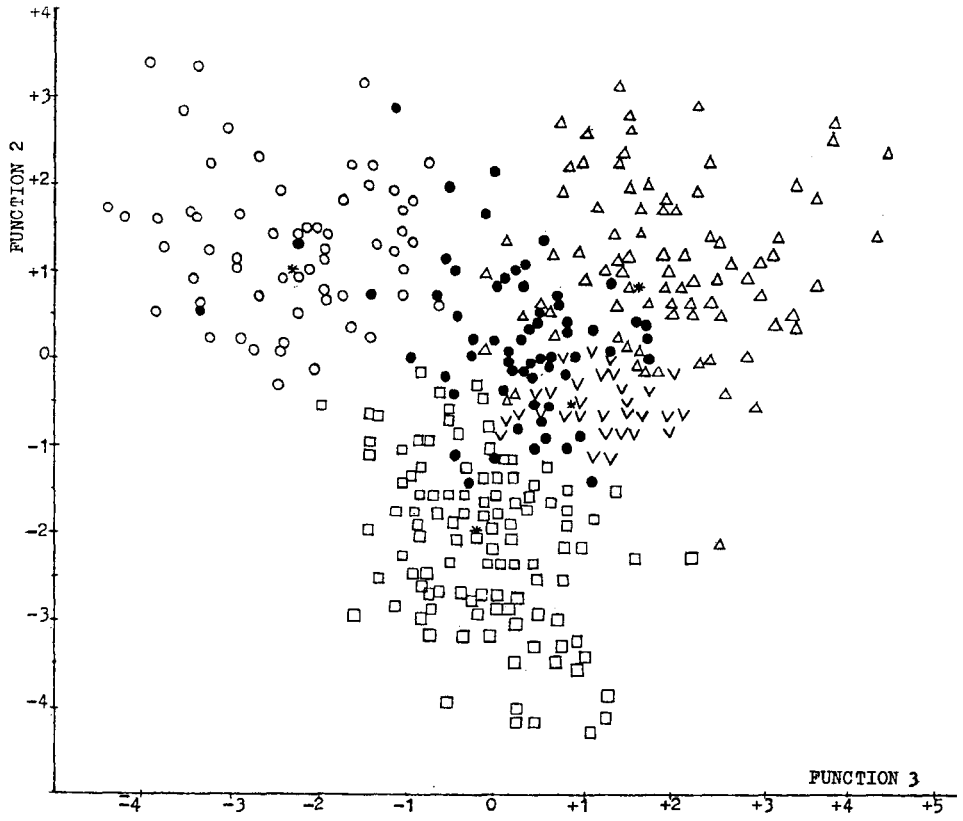

Fig. 8. Discriminant plot of individuals of the five a-priori defined groups corresponding to *Carex* taxa, and two intermediate populations, with respect to discriminant functions 2 and 3. – *C. muricata* subsp. *muricata* (□) and subsp. *lamprocarpa* (V); *C. divulsa* subsp. *divulsa* (○) and subsp. *leersii* (Δ); intermediate populations (●). – Group centroids denoted by asterisks (*).

lamprocarpa Čelak., *C. muricata* subsp. *leersii* (Kneuck.) Asch. & Graebn., and *C. muricata* subsp. *divulsa* (Stokes) Čelak.

The studied intermediate populations exhibit different chromosome numbers and a wide morphological variation. We suppose them to be hybrid derivatives from different subspecies of one polymorphic species, *Carex muricata.*

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