

# Patterns and levels of variation within section *Phacocystis* of genus *Carex* (*Cyperaceae*) in Bulgaria

Milka Stoeva<sup>1</sup>, Krassimira Uzunova<sup>2</sup>, Evgenija Popova<sup>3</sup> & Kalina Stoyanova<sup>4</sup>

<sup>1</sup>Department of Biology, Agricultural Faculty, Thracian University, 6000 Stara Zagora, Bulgaria

<sup>2</sup>Department of Botany, Biological Faculty, Sofia University St. Kliment Ohridski, 8, Dragan Tsankov Blvd., 1421 Sofia, Bulgaria

<sup>3</sup>Institute of Mathematics and Informatics, Bulgarian Academy of Sciences, Acad. G. Bonchev St., bl. 23, 1113 Sofia, Bulgaria

<sup>4</sup>Institute of Botany, Bulgarian Academy of Sciences, Acad. G. Bonchev St., bl. 23, 1113 Sofia, Bulgaria

Received: April 14, 2004 ▷ Accepted: May 26, 2004

**Abstract.** Morphological, karyological and foliar epidermal analyses have been made of the sedges from sect. *Phacocystis* in Bulgaria. The morphological data have been processed by cluster analysis, stepwise discriminant analysis and one-way ANOVA method. The results invited the conclusion that sect. *Phacocystis* is represented in Bulgaria with six taxa: *Carex buekii*, *C. elata*., *C. cespitosa*, *C. acuta*, *C. nigra* subsp. *nigra*, and *C. nigra* subsp. *dacica*. The intra- and interpopulation variations as well as the main evolutionary mechanisms and trends have been discussed.

**Key words:** *Carex*, epidermal structure, evolutionary mechanisms, karyology, morphology, variation

## Introduction

The section *Phacocystis* Dumort. in its morphology, leaf epidermal anatomy and cytology is one of the most variable in genus *Carex*. The species are not quite isolated geographically and genetically and produce interspecific hybrids. All that makes this section taxonomically very difficult and very interesting in regard to its evolution (Faulkner 1972, 1973; Standley 1985, 1987a, b, 1990; Cayouette & Morisset 1985, 1986; Luceño & Aedo 1994).

In Europe, sect. *Phacocystis* is represented by 16 species (Chater 1980). Six of them occur in the Balkan Peninsula: *C. acuta* L., *C. bigelowii* Torr. ex Schwein., *C. buekii* Wimm., *C. cespitosa* L., *C. elata* All. and *C. nigra* (L.) Reichard.

In his monograph Acharov (1957) reported four species as occurring in Bulgaria: *C. gracilis* Curtis

(= *C. acuta*), *C. buekii*, *C. acuta* (= *C. nigra* = *C. goodenowii* Gay), and *C. dacica* Heuff. According to him, *C. cespitosa* was not distributed in Bulgaria and had been erroneously reported by some authors (Pančić 1883; Velenovsky 1891; Stojanov & Stefanov 1924) for our country. The opinion of Acharov was reflected by Stojanov & al. (1966) and Vulev & Kitanov (1964) in their floras.

Some of the modern European authors have not accepted the rank of species for *C. dacica*. Thus, Egorova (1999) included it in *C. bigelowii* as subspecies: *C. bigelowii* subsp. *dacica* (Heuff.) T.V. Egorova. Chater (1980) referred it to *C. nigra* without any taxonomic rank. Nilsson (1985) considered it as a subspecies of *C. nigra*: *C. nigra* subsp. *dacica* (Heuff.) Soó.

In her study of genus *Carex* in Bulgaria, Stoeva (1994) added two species of sect. *Phacocystis* to those reported by Acharov (1957), namely *C. elata* and *C. cespitosa*.

The area of *C. elata* spreads almost throughout Europe and North Africa. It prefers wet places, fens and shallow standing waters at altitudes up to 1600 m. The population subject of this study was found in a habitat untypical for *C. elata* in the result of the drainage of Kazichene marsh. The area of *C. cespitosa* spreads from North Europe to France, N Balkans, S Russia, the Caucasus, W Turkey, Kazakhstan and W Siberia. It mainly occurs in wet meadows, swamps and along streams at altitudes above 2200 m. The studied population was found at an altitude untypical for this species (Table 1). The settlement and survival of the plants are due to an underground waters regime, close to the surface and rising aboveground. They are fed by precipitations in the adjacent mountains and sustain a soil thermal regime similar to that of the mountain soils of natural habitats of *C. cespitosa*. This also explains the presence of some other mountain species that have settled secondarily in the same habitat (Jordanov & al. 1972; Stoeva & Štěpánková 1990). The area of *C. buekii* spreads from Germany to E Bulgaria and Kazakhstan. It is comparatively rare in our country and mainly inhabits the banks of rivers and lakes, occasionally intermittent flooded places at altitudes up to 1600 m. *C. nigra* and *C. acuta* are the most widespread species of this section. The area of *C. nigra* covers most of Europe and W Asia, including Turkey, N Afrika (isolated localities) and N America (Luceño & Aedo 1994). This species is the most widespread in Bulgaria and inhabits a very wide range of wet habitats: marshy meadows, swamps, swamp peateries, lower places in forests, around lakes, rivers and springs. Its populations occur from the coniferous belt to the subalpine one. *C. nigra* subsp. *nigra* inhabits mainly shallow standing waters, at altitudes up to 2200 m. *C. nigra* subsp. *dacica* prefers fresh running waters and inhabits the banks of rivers, lakes and springs, at altitudes above 2200 m. *C. acuta* is widespread in Europe (in S Europe it is rare) and W Asia, including Turkey, W Siberia and the Caucasus, Lebanon, Syria and Morocco. In Bulgaria it occurs mainly along the watersides of rivers and lakes with fresh running waters, at altitudes up to 1650 m. and more rarely than *C. nigra*.

Studying the patterns and levels of morphological variation, chromosome numbers, leaf anatomy and habitats of the natural local Bulgarian populations of different taxa included in sect. *Phacocystis*, we have tried to resolve the following problems:

– to render more precise the taxonomical scheme of sect. *Phacocystis* and with the use of discriminant func-

tions to find the most appropriate taxonomic disposition of the two groups of plants, ungrouped preliminary;

– to elucidate how the intrapopulation and interpopulation variation contribute to the overall phenotype variation of *C. nigra* and *C. acuta*;

– to find some correlations between the morphological, anatomical and karyological variations at different levels of research;

– to find out some correlations between the phenotype differences of the studied populations and the ecological factors of their habitats;

– by using the results from different patterns and levels of variation to reveal the relationships between the taxa and some evolutionary trends in the section.

## Material and methods

Morphological, anatomical and karyological analyses were carried out on 22 natural Bulgarian populations of sect. *Phacocystis*, referred to six taxa: *C. acuta*, *C. buekii*, *C. cespitosa*, *C. elata*, *C. nigra* subsp. *nigra*, and *C. nigra* subsp. *dacica* (Table 1). Ten plants from each population were used in the overall research. Two groups of plants with previously uncertain taxonomic position were studied too. They combined inbred features in the different taxa of the section.

Chromosome numbers and karyotypes were estimated on permanent slides of metaphase plates from root tips of living plants collected from Nature and cultivated in the greenhouse of the Institute of Botany, Sofia. The root tips were treated and squashed according to the accepted methods (Stoeva & Štěpánková 1990). Voucher specimens were deposited in SOM.

Twenty-one quantitative characters were included in the morphological analysis: 1. stem height (cm); 2. leaf length (cm); 3. leaf width (mm); 4. bract length (cm); 5. inflorescence length (cm); 6. number of the male spikes; 7. length of the upper male spike (cm); 8. length of the lower female spike (cm); 9. distance between the lower two female spikes (cm); 10. utricle length (mm); 11. utricle width (mm); 12. beak length (mm); 13. length of the female glume (mm); 14. width of the female glume (mm); 15. length of the male glume (mm); 16. width of the male glume (mm); 17. leaf length to stem height ratio; 18. bract length to inflorescence length ratio; 19. inflorescence length to stem height ratio; 20. length of the female glume to utricle length ratio; 21. utricle length to utricle width ratio.

Table 1. Studied populations of genus *Carex* sect. *Phacocystis*.

Species, population No	2n	Locality
<b><i>Carex acuta</i></b>		
1230	4*	NE Bulgaria: Sreburna Reserve, 44°06' N, 27°04' E, 50 m, along Sreburna Lake.
710	84(83)	W Rhodopes: Beglika locality, near Beglika Reserve 41°50' N, 24°08' E, 1600 m, at the bank of a stream along the road to Banchev Chark. The population was neighbour-sympatric with <i>C. nigra</i> subsp. <i>nigra</i> .
1050	4	W Rhodopes: Rakovo Dere locality, 41°50' N, 24°08' E, 1600 m, at the bank of Rakov Dol River The population was neighbour-sympatric with <i>C. nigra</i> subsp. <i>nigra</i> .
1343	84(82)	W Rhodopes: along the road Smolyan-Dospat, before the road fork to Zmeitsa village, 41°38' N, 24°15' E, c. 1600 m, at the bank of a stream.
1346	84	W Rhodopes: along the road Smolyan-Beglika, after Shiroka Poljana water reservoir, 41°46' N, 24°09' E, 1650 m., in the ditch between the road and a <i>Picea abies</i> forest. The population formed a little open monodominant community without water cover and was neighbour-sympatric with <i>C. nigra</i> subsp. <i>nigra</i> .
<b><i>Carex buekii</i></b>		
1324	64	Sofia region: along the ring-road of Sofia city, 42°40' N, 23°16' E, 600 m., in the swampy places near Mototechnica.
<b><i>Carex cespitosa</i></b>		
1264	78**	Toundzha Hilly Country: between the villages Alexandrovo and Manolovo, 42°35' N, 25°56' E, 420 m, in inundated meadows.
<b><i>Carex elata</i></b>		
1314	74(76)**	Sofia region: Kazichene village, 42°39' N, 23°28' E, 550 m, in ditches covered by shallow water, almost annually after the drainage of Kazichene marsh.
<b><i>Carex nigra</i> subsp. <i>nigra</i></b>		
438	84**	Mt Vitoshka: on the plateau between peak Ushite and Aleko chalet, 42°35' N, 23°19' E, 1850 m, in the peat bog near Morenita Hotel.
1300	84(83)	Mt Vitoshka: between peak Cherni Vruh and Koumata chalet, 42°36' N, 23°14' E, 2000–2100 m, in the peat bog near Konyarnika locality.
1362	84	Rila Mts: along the road from Prodanovtsi village to Samokov town, near Prodanovtsi village, 42°20' N, 23°32' E, 950–1000 m, in a swamp habitat covered by shallow water.
1364	84	Rila Mts: along the road from Govedarts village to Ribarnika locality, 42°16' N, 23°28' E, 1200 m, in low places among the trees of a <i>Picea abies</i> forest.
1365	84	Rila Mts: along the road from Govedarts village to a children's rest home, 42°16' N, 23°28' E, 1200 m, in swamp habitats covered by shallow water.
1353	84(83)	Rila Mts: above Kostenets village, 42°14' N, 23°46' E, 1550 m, in swamp habitats along river Chavcha.
709	84(81, 82, 83)	W Rhodopes: Beglika locality, along the road to Banchev Chark near Beglika Reserve, 41°50' N, 24°08' E, 1600 m, in swamp habitats around the stream. The population was neighbour-sympatric with <i>C. acuta</i> .
1350	84(81, 82, 83)	W Rhodopes: Rakovo Dere locality, 41°50' N, 24°08' E, 1600 m, in a grassy draining place between a wood of <i>Pinus sylvestris</i> and river Rakov Dol, with western exposition, on marble basal rocks, in summer uncovered by water. The population neighbored on <i>C. acuta</i> .
1344	84(81, 82, 83)	W Rhodopes: along the road Smolyan-Beglika, after Shiroka Poljana water reservoir, 41°46' N, 24°09' E, 1600 m, in a swamp habitat between the road and a <i>Picea abies</i> forest. The population neighbored on <i>C. acuta</i> .
<b><i>Carex nigra</i> subsp. <i>dacica</i></b>		
272	80	Pirin Mts: Todorini lakes surrounded by <i>Pinus mugo</i> , 42°44' N, 23°27' E, 2500 m, along the lakes.
1361	80	Mt. Rila: between peak Malyovitsa and Malyovitsa chalet, 42°13' N, 23°21' E, 2100–2200 m, along rivers, surrounded by <i>Pinus mugo</i> communities.
1389	80	Rila Mts: Mousalenski Lakes, 42°13' N, 23°35' E, 2400 m, in a draining grassy habitat to the south of the lakes, with northern exposition and slope gradient 30–40°.
1394	80	Rila Mts: Mousalenski Lakes, 42°13' N, 23°35' E, 2400 m, at the lake bank nearby Mousala chalet.
1395	80	Rila Mts: Mousalenski Lakes, in the upper stretch of river Bistritsa, below Mousala chalet, 42°13' N, 23°35' E, 2400 m, on river banks and along river streams.
Undefined group 1	78(79, 80)	Toundzha Hilly Country: between villages Alexandrovo and Manolovo, 42°35' N, 25°56' E, 420 m, in inundated meadows. The group was nearby <i>C. cespitosa</i> .
Undefined group 2	84 (81, 82, 83, 85)	W Rhodopes: Beglika locality, along the road to Banchev Chark, near Beglika Reserve, 41°50' N, 24°08' E, 1600 m, between population No 709 of <i>C. nigra</i> subsp. <i>nigra</i> and population -710 of <i>C. acuta</i> .

\* First published by Stoeva (1992)

\*\* First published by Stoeva (1994)

Some qualitative characters were employed too: presence or absence of rhisomes; tint and leaved of the basal sheaths; tint, shape and veins of the utricle, etc.

The mean value and coefficient of variation (V in %) were calculated for each character of every population (Tables 5, 6). They have been used in the comparative analyses on different levels.

By the method of one-way ANOVA was evaluated the relative contribution of intra- and interpopulation variation to the overall variation of each feature within *C. acuta* and the two subspecies of *C. nigra* (Table 10).

The overall morphological variation pattern of the section was obtained by Cluster analysis (CA), using a centroid algorithm for clustering. The interpopulation differences within *C. acuta* and *C. nigra* were estimated by the values of Euclidean distance (Tables 7, 8, 9).

A stepwise discriminant analysis (SDA) was applied as a multivariate test for morphological separation of the taxa and in order to find the subset of characters contributing most to this separation. SDA was based on the biometrical values of the quantitative characters of each examined plant.

All morphological analyses were made with the help of BMDP statistical package (Dixon 1981).

The anatomical analysis was based on the following leaf epidermal features: stomatal and papillae distribution; intercostal epidermal cell sizes/ratio between length and width, stomatal peculiarities and presence or absence of prickles. These features have taxonomical value according to Metcalfe (1971). We also took into consideration the viewpoint of Standley (1990) that the foliar anatomy of sect. *Phacocystis* has considerable utility in examining the hybridization between different taxa and phylogenetic relationships among species within the section. The anatomical preparations were made from the middle part of the stem leaves. The leaves were boiled and then scraped out and the epidermal peels were washed in water and 70% alcohol and stained in saffranin. Semi-permanent glycerin and glycerin gelly slides were made. Twenty measurements of the stomata frequency and epidermal cell and stomatal sizes were carried out for each specimen. A mean value for every population was computed and the data are summarized (Table 11).

## Results and discussion

The results of our study demonstrate a very complex picture of variation in sect. *Phacocystis*. The var-

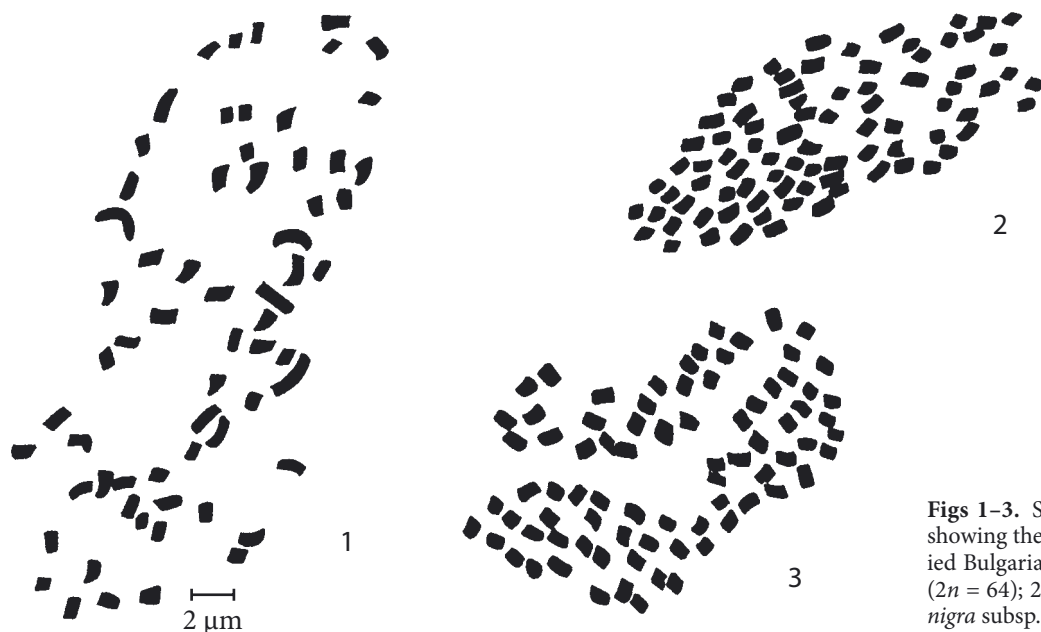
iation relates to different groups of features and to different levels within the section: interspecific, interpopulation and intrapopulation levels.

### Karyology

As a result of karyological study, 11 chromosome numbers have been found in the section:  $2n = 64, 74, 76, 78, 79, 80, 81, 82, 83, 84, 85$  (Table 1). They all, except  $2n = 64$ , form an aneuploid series of consecutive chromosome numbers.

The number  $2n = 64$  is the lowest number in the section in Bulgaria and somewhat isolated from the others. It has been counted in *C. buekii*. This species was studied karyologically in Bulgaria for the first time. Besides the result illustrated in Table 1, the same number was found in a West Rhodopean population near Surnitza village (Stoeva unpubl.). For *C. buekii*  $2n = 64$  is a new chromosome number. By now, only one chromosome number has been reported for it:  $2n = 40$  (Majovsky & al. 1987). The karyotype is somewhat more heterogeneous in the relative length of chromosomes than those of the other species studied. Two chromosomes of the karyotype are clearly longer than the others. Six chromosomes are a little shorter than the two longest but they are clearly longer than all the rest (Fig. 1).

Two chromosome numbers have been counted in *C. elata*:  $2n = 74, 76$  (Stoeva 1994). The first of them dominates. The numbers  $2n = 74$  (Davies 1956) and  $2n = 75, 76, 77$  (Faulkner 1972) have been reported from NW Europe and  $2n = 76$  was indicated as a dominant number there. Luceño & Aedo (1994) established  $2n = 73 - 77$  in polymorphic *Carex elata* from the Iberian Peninsula, and  $2n = 76$  from Morocco. By them,  $2n = 76$  dominates in subsp. *elata* and  $2n = 74$  in two Iberian subspecies: subsp. *reuteriana* (Boiss) Luceño & Aedo and subsp. *tartessiana* Luceño & Aedo. Faulkner (1972) and Luceño & Aedo (1994) considered  $2n = 76$  a typical eudiploid chromosome number in *C. elata*. The two latter authors regarded  $2n = 74$  in subsp. *reuteriana* and subsp. *tartessiana* as a secondary number evolved from  $2n = 76$  of subsp. *elata* by fusion of a pair chromosomes. The higher numbers  $2n = 75$  and  $2n = 76$  in the same two subspecies have agmatoploid origin from  $2n = 74$  cytotypes by fusion of chromosomes, and  $2n = 73$  by fusion of one chromosome. Bearing in mind that the population studied by us belongs to subsp. *elata*, it seems logical for the number  $2n = 74$  to have secondary origin from  $2n = 76$  by fu-



Figs 1–3. Somatic metaphase plates showing the karyotypes of three studied Bulgarian *Carex* taxa: 1, *C. buekii* ( $2n = 64$ ); 2, *C. acuta* ( $2n = 84$ ); 3, *C. nigra* subsp. *dacica* ( $2n = 80$ ).

sion of chromosomes, and the disturbed environments of the habitat have helped in one or another way its gradual stabilization. The karyotype of *C. elata* is more homogeneous than the karyotype of *C. buekii*. The chromosomes can be divided into three groups by relative length, but the limits between the groups are not as distinct as they are in *C. buekii* because of gradual transition from one group to another (Stoeva 1994).

In *C. cespitosa* only one chromosome number was established  $2n = 78$ . The same number was reported by Faulkner (1972) from Denmark and it was smaller than the other numbers known for the species (Stoeva 1994). With its chromosome number and karyotype *C. cespitosa* seems to be closer to *C. elata* than to the other *Phacocystis* species, but unlike *C. elata*, the intermediate chromosomes dominate in the karyotype: the shortest have a smaller number and the longest are only two (Stoeva 1994).

In *C. acuta* three chromosome numbers were ascertained:  $2n = 84, 83, 82$ . These numbers are equal to some numbers established for *C. nigra* and higher than those of *C. buekii*, *C. elata* and *C. cespitosa*.  $2n = 84$  was the number present in all studied populations. In three of them it was the only number and in the other two was the dominant one (Table 1). Our results correspond to most literature data illustrated by the cytochorological map (Fig. 4). On the basis of their own cytological results Faulkner (1972) and Luceño & Aedo (1994) reported  $2n = 84$  as the typi-

cal euploid chromosome number in *C. acuta*. Along with this, Faulkner noted the extensive distribution of aneuploidy in this species: 51 % of the specimens studied by him were aneuploid, with  $2n = 82, 83, 85$ , and  $2n = 83$  was dominant among them. Besides the euploid number  $2n = 84$ , Luceño & Aedo (1994) reported two hyperploid ones from the Iberian Peninsula:  $2n = 85$  and  $2n = 86$ , but they were not dominant. In Bulgarian *C. acuta*, the aneuploid numbers are comparatively rare:  $2n = 82$  was found in two plants of the Rhodopean population No 1343, and  $2n = 83$  only in one plant of another Rhodopean population No 710. The latter was neighbour-sympatric with the population No 709 of *C. nigra*. The karyotype of *C. acuta* is a little more homogeneous than the karyotypes of *C. elata* and *C. cespitosa* and insignificantly more heterogeneous than that of *C. nigra* (Fig. 2).

Karyologically, *C. nigra* was the most variable species of sect. *Phacocystis* in Bulgaria. In this study five chromosome numbers were found, which form an aneuploid series:  $2n = 80, 81, 82, 83, 84$ . The range of chromosome numbers variation was similar to that of Europe (Fig. 5) and of the whole area of *C. nigra*:  $2n = 80, 82, 83, 84, 85$  (Stoeva 1994). The lowest number  $2n = 80$  was established only in *C. nigra* subsp. *dacica*. This taxon has been studied karyologically for the first time and no other numbers have been found in it. The numbers  $2n = 81, 82, 83, 84$  were counted in *C. nigra* subsp. *nigra* and  $2n = 84$  predominated. It was



Fig. 4. Cytochorological map showing the chorology of the chromosome numbers of *C. acuta*: results of this study and from literature.

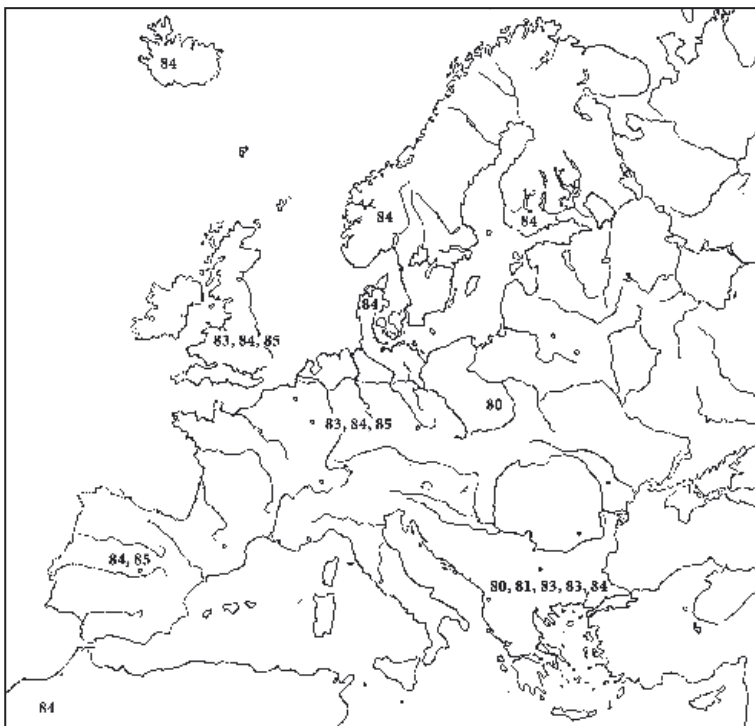


Fig. 5. Cytochorological map showing the chorology of the chromosome numbers of *C. nigra*: results of this study and from literature.

the only number in four populations and the predominating one in other five (Table 1). The number  $2n = 84$  was the widest spread in the area of *C. nigra*. Faulkner (1972) and Luceño & Aedo (1994) regarded it as the typical euploid chromosome number of *C. nigra*, like in *C. acuta*. On the basis of his results Faulkner (1972) concluded that aneuploidy of this species in N Europe was more limited in comparison to *C. acuta*. Only 17% of the studied specimens were aneuploid and  $2n = 85$  was the widest distributed among them. Contrary to Faulkner (1972), Cayouette & Morisset (1986) in N America and Luceño & Aedo (1994) in the Iberian Peninsula registered more aneuploid than euploid plants. The first two authors also indicated  $2n = 83$  as the widest distributed chromosome number in the region studied by them. The most frequent aneuploid chromosome number in Bulgaria was also  $2n = 83$ . It was counted in five populations. In three of them (No 1344, 709, 1350),  $2n = 83$  occurred together with  $2n = 81$  and  $2n = 82$ . These three Rhodopean populations could be pointed out karyologically as the most variable. Their karyological variability correlated with the neighbour-sympatricity of their areas and the areas of *C. acuta* populations, respectively No 1346, 710 and 1050. No hyperploid number has been observed in *C. nigra*. The hypoploid number  $2n = 81$  was a new one for it. The karyotype of *C. nigra* (Fig. 3) was the most homogenous in the section (Stoeva 1994).

The obtained results invited the conclusion that the main source of chromosome number variation in *C. nigra* was the intrapopulation variation in the populations neighbouring on populations of *C. acuta*. However, aneuploid numbers were also registered in the populations No 1300 and 1353 of *C. nigra* and in No 1343 of *C. acuta*, which were not sympatric with other species of sect. *Phacocystis*. These data suggest symploid origin of many hypoploid numbers of the two species, named partial symploidy by

Luceño & Guerra (1996). Along with this, on the basis of the larger chromosome number variation in sympatric populations No 1050 and 710 of *C. acuta* and No 1350 and 709 of *C. nigra* it could be assumed that some plants resulted from hybridization between the two species.

In the aberrant groups of plants the chromosome number was more variable than in their sympatric taxonomically distinct populations (Table 1). In Group 1 three chromosome numbers have been established:  $2n = 78, 79, 80$ . In most plants  $2n = 78$  was registered like in population No 1264 of *C. cespitosa*. In three plants  $2n = 79$  has been observed and  $2n = 80$  in other two plants. The karyotype of the plants with  $2n = 78$  seemed quite identical to the karyotype of *C. cespitosa*. In the karyotype with  $2n = 80$ , the longest pair of chromosomes was lacking, and in that with  $2n = 79$  one of the same chromosomes was lacking.

In the aberrant Group 2 five numbers were counted:  $2n = 81, 82, 83, 84, 85$ . It was very hard to decide which of them was dominant. The first four were the same as the ones registered in the sympatric population of *C. nigra* No 709, and  $2n = 83, 84$  were equal to the numbers of population 710 of *C. acuta* in the same locality. The possible reasons for this diversity were symploidy, hybridization and agmatoploidy.

### Morphology

Cluster analysis was performed as a criterion for the fusion of clusters. It was based on 21 quantitative features listed above. As it was evident from the dendrogram in Fig. 6, the most striking feature of the overall pattern was the presence of two wide clusters: **A** and **B**. Cluster **B** combined a set of populations of *C. acuta*, *C. buekii*, *C. cespitosa* and the aberrant Group 1. Cluster **A** consisted of two subclusters. Subcluster **Aa** included the populations of *C. nigra* subsp. *dacica*. Subcluster **Ab** amalgamated the set of populations of *C. nigra* subsp. *nigra*, *C. elata* and the aberrant Group 2. The two subclusters of cluster **A** were amalgamated on a higher level of similarity than the three species of cluster **B**, which was in accordance with the lower taxonomical rank of the taxa included in them. The species *C. elata* made exclusion. Its place in subcluster

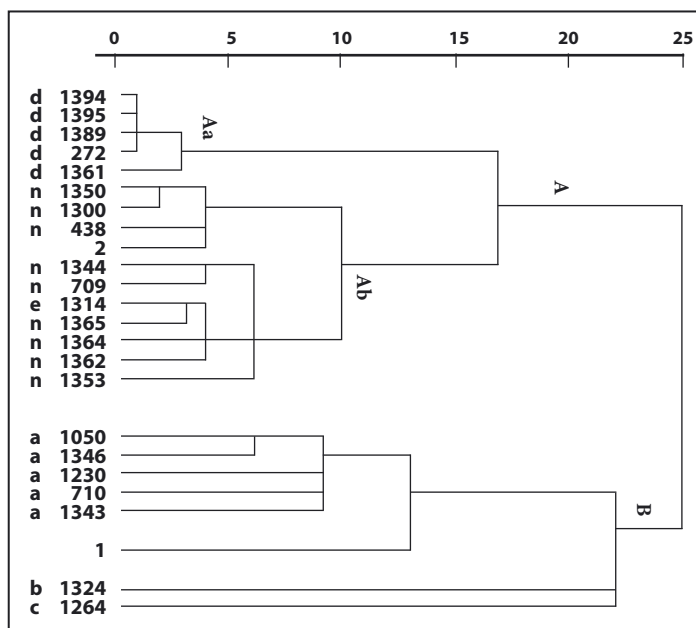


Fig. 6. Dendrogram of the analysis of *Carex* sect. *Phacocystis* using the Centroid method for clustering. No 1394–1361 = *C. nigra* subsp. *dacica*; No 1350–1353 (except No 1314 and Group 2) = *C. nigra* subsp. *nigra*; No 1314 = *C. elata*; No 1050–1343 = *C. acuta*; No 1324 = *C. buekii*; No 1264 = *C. cespitosa*; 1 and 2 undefined groups.

**Ab** on high level of similarity with *C. nigra* subsp. *nigra* could be explained with the uncommonly small measurements of some vegetative features in the studied population (stem height, leaf width, etc.). A probable cause of these lower measurements was the depressive growth of the plants in the ruined habitat of Kazichene village.

The obtained SDA results showed a good separation of the studied plants related a-priori to six taxa. This was obvious from the high total percent of correct classification 94.55% (Table 2), from the F-values for each pair of taxa (Table 3) and from the distribution of plants in the discriminant space (Figs 7, 8). The percentage of correct classification of individual taxa was also shown (Table 2). Thus, the plants belonging to *C. buekii*, *C. elata*, *C. cespitosa*, and *C. acuta* were 100% correctly classified. In *C. nigra* subsp. *nigra* the percentage amounted to 92.23% and in subsp. *dacica* to 90%. The computed classification functions D1-D5 are also illustrated (Table 4). Distribution of the studied taxa according to the first four functions in discriminant space is shown (Figs 7, 8). A very good separation of *C. cespitosa*, *C. buekii* and *C. acuta* and overlapping of *C. elata* and *C. nigra* subsp. *nigra* is demonstrated (Fig. 7). The species *C.*

**Table 2.** Results of the classification by SDA of individuals among six pre-defined taxa of *Carex* sect. *Phacocystis*. The overall percentage value of cases correctly classified is 94.55%.

Actual group	Number of plants	Predicted group membership					
		<i>C. acuta</i>	<i>C. buekii</i>	<i>C. cespitosa</i>	<i>C. elata</i>	<i>C. nigra</i> subsp. <i>nigra</i>	<i>C. nigra</i> subsp. <i>dacica</i>
<i>C. acuta</i>	50	50(100%)	0	0	0	0	0
<i>C. buekii</i>	10	0	10(100%)	0	0	0	0
<i>C. cespitosa</i>	10	0	0	10(100%)	0	0	0
<i>C. elata</i>	10	0	0	0	10(100%)	0	0
<i>C. nigra</i> subsp. <i>nigra</i>	90	0	0	0	3(3.33%)	83(92.23%)	4(4.44%)
<i>C. nigra</i> subsp. <i>dacica</i>	50	0	0	0	0	5(10%)	45(90%)
Undefined preliminary groups:							
1	30	1(3.33%)	0	22(73.33%)	2(6.66%)	5(16.66%)	0
2	10	0	0	0	1(10%)	9(90%)	0

**Table 3.** F-matrix (containing F-values computed from the equality test of group means for each pair of taxa) from SDA for six studied Bulgarian taxa of *Carex* sect. *Phacocystis*. Degrees of freedom: 8 and 149.

Taxa	F-values				
	<i>C. acuta</i>	<i>C. buekii</i>	<i>C. cespitosa</i>	<i>C. elata</i>	<i>C. nigra</i> subsp. <i>nigra</i>
<i>C. acuta</i>					
<i>C. buekii</i>	93.037				
<i>C. cespitosa</i>	134.030	69.845			
<i>C. elata</i>	33.727	70.079	62.448		
<i>C. nigra</i> subsp. <i>nigra</i>	101.570	138.010	135.080	25.024	
<i>C. nigra</i> subsp. <i>dacica</i>	162.130	167.430	177.110	39.753	13.309

**Table 4.** The sequence of significance of eight selected characters for discrimination between six taxa of *Carex* sect. *Phacocystis* obtained by SDA and the corresponding discriminant functions (D1-D5).

Characters	Approximate F-statistic	D1	D2	D3	D4	D5
X1 stem height	307.360	0.082	0.039	-0.054	0.028	-0.072
X2 bract length	205.787	0.003	-0.266	0.053	0.129	-0.244
X3 leaf width	137.110	0.855	-0.107	-0.775	-0.711	-0.983
X4 leaf length	117.856	0.031	-0.014	0.091	0.041	-0.002
X11 utricule width	104.222	7.023	-16.281	8.691	1.741	-17.680
X10 utricule length	89.452	-3.980	-9.687	-2.338	-4.798	-9.364
X21 utricule length to utricule width ratio	79.745	3.905	9.631	4.010	4.127	9.100
X7 length of the upper male spike	70.104	-0.019	0.025	0.545	-0.618	-0.852
constant		-14.018	-14.925	-10.996	0.685	-16.847

*elata* is separated well from all other taxa by function D4 (Fig. 8). The limits between the two subspecies of *C. nigra* were not quite clear. The F-values (Table 3) confirmed the smaller differences between subsp. *nigra* and subsp. *dacica*, as compared to the interspecific differences.

With the help of classification functions we have tried to find out the place of the two groups of plants not identified earlier. The results (Table 2) indicate that 22 plants of Group 1 were classified as *C. cespitosa*. The other eight plants fell respectively in: *C. nigra* subsp. *nigra* five, *C. elata* two and *C. acuta* one. All plants in Group 2 (excluding one) were classified as *C. nigra* subsp. *nigra*.

The most significant features for differentiation of taxa found by means of SDA were: stem height, leaf sizes, bract length, length of the upper male spike and utricule sizes (Table 4). Besides them, the taxa had some specific morphological characters which allowed their recognition:

– *C. buekii* was characterized with very high stems with wide leaves (Table 5), yellowish-green veinless utricule and blackish-brown basal sheaths getting conspicuously reticulate fibrous;

– *C. elata* differed to the greatest degree from the others with its long broadly elliptic and dark grayish-green utricule with a paler beak and three slender veins, as well as with yellowish-brown leafless basal sheaths;



– *C. cespitosa* could be distinguished easily by its very short bract, shorter than the inflorescence and than the lower female spike, long leaves, compact and comparatively short inflorescence, dense overlapping spikes which, single male spike, and pale suborbicular veinless utricle with a short beak (Table 5);

– The most distinctive features of *C. acuta* were: a very long bract, always longer than the inflorescence

**Table 5.** Mean (first line) and coefficient of variation in % (second line) of *C. acuta* (1230–1346), *C. buekii* (1324), *C. cespitosa* (1264), *C. elata* (1314) and undefined Group 1.

Population character number	1230	710	1050	1343	1346	1324	1264	1314	Gr. 1
1	73.49	65.63	57.03	57.61	50.31	102.30	95.06	43.20	73.97
	19.97	10.40	19.94	13.27	15.24	10.21	10.26	15.90	11.58
2	61.53	55.54	48.27	66.03	51.02	57.18	78.70	39.14	61.78
	15.27	20.21	12.59	9.16	17.46	33.45	14.43	13.15	17.10
3	3.79	2.80	3.48	3.72	3.49	5.80	2.80	3.08	2.62
	7.38	13.20	17.24	13.70	14.04	10.86	13.21	11.03	43.86
4	19.75	16.53	13.36	16.44	16.23	12.92	0.67	4.65	1.67
	19.13	29.82	32.13	25.72	19.10	33.12	17.91	39.13	43.86
5	17.94	13.72	15.91	14.26	14.17	14.66	3.27	7.93	4.91
	19.28	24.56	24.32	21.87	15.52	19.71	11.00	19.04	18.83
6	2.30	1.80	1.30	1.70	2.30	1.60	1.00	1.60	1.07
	20.86	23.33	51.53	39.41	29.13	31.87	0.00	31.87	11.67
7	4.33	2.94	4.13	3.19	3.93	3.51	1.88	3.23	2.58
	16.85	18.36	29.23	18.19	13.74	12.82	13.82	19.19	15.70
8	6.15	5.20	4.76	5.07	6.31	5.73	1.58	3.40	1.53
	19.34	19.03	28.36	18.14	18.06	19.37	17.72	30.88	16.10
9	4.91	4.94	3.87	3.37	4.54	4.79	0.70	2.61	1.21
	30.14	42.30	37.98	33.53	22.24	24.21	31.42	21.45	22.12
10	2.84	2.65	2.45	2.75	2.65	2.31	2.26	2.90	2.09
	9.85	7.54	7.34	5.45	4.15	4.76	6.19	4.48	7.31
11	1.32	1.39	1.28	1.20	1.22	1.39	1.84	1.71	1.62
	4.77	6.47	8.59	6.66	3.44	8.61	7.06	9.35	9.43
12	0.20	0.21	0.23	0.27	0.25	0.20	0.18	0.23	0.12
	24.00	26.66	20.86	17.77	20.80	24.00	23.33	20.86	33.36
13	2.84	2.94	2.47	2.07	2.59	2.09	1.94	3.03	2.15
	10.00	11.90	18.21	8.53	10.03	9.56	10.36	7.26	7.81
14	0.97	0.93	0.70	0.98	0.70	0.82	0.98	0.82	0.96
	15.36	10.75	15.71	11.22	11.57	14.97	9.28	19.51	9.05
15	4.15	4.08	4.09	3.05	3.68	3.44	3.54	4.09	3.37
	12.53	10.78	10.51	3.93	7.88	7.52	9.03	6.84	8.01
16	1.06	1.04	0.86	1.30	1.05	1.03	1.06	1.00	0.99
	9.05	15.09	23.25	7.23	8.09	9.12	9.05	11.50	9.75
17	0.86	0.84	0.87	1.16	1.01	0.57	0.83	0.91	0.85
	22.09	19.04	20.68	12.93	11.48	36.84	18.07	10.98	14.74
18	1.10	1.19	1.15	1.14	1.14	0.86	0.20	0.58	0.34
	7.09	8.40	16.52	9.64	4.38	16.27	23.50	31.03	37.00
19	0.24	0.20	0.28	0.25	0.28	0.14	0.03	0.18	0.06
	22.08	21.50	22.61	20.83	11.42	22.75	15.27	23.52	22.38
20	1.00	1.11	1.01	0.75	0.97	0.90	0.86	1.04	1.02
	8.92	12.47	22.77	7.86	8.17	9.50	12.02	10.40	10.32
21	2.15	1.91	1.92	2.30	2.17	1.66	1.23	1.70	1.31
	10.09	11.26	8.04	8.84	4.26	6.62	7.12	7.93	8.80

(Table 5), and brown (pale-brown, grey-brown to dark-brown) leaved basal sheaths;

– *C. nigra* could be distinguished by its slender, long, far creeping or shorter rhizomes; thin and comparatively short stems; narrow leaves; dark-purple or dark reddish-brown shining, leaved basal sheaths (the characters of the basal sheaths resembled *C. cespitosa*). The two subspecies of *C. nigra* differed from each other by the lengths of stem, inflorescence, female spike (Table 6), rhizomes, and density of tufts.

The interpopulation variation was evaluated only in *C. acuta* and *C. nigra*, owing to the fact that the other three species were investigated in single populations. The values of Euclidean distance (Ed) are illustrated (Tables 7, 8, 9). A comparison of these values has shown the following: Ed values varied within different limits in the individual taxa; the interpopulation differences were fewer and varied within much narrower limits in *C. nigra* subsp. *dacica* (Table 7), which correlated with the invariable chromosome number, smaller differences between the population habitats and similar altitude (2100–2500 m). The interpopulation differences within this subspecies were the least between the populations localized in the region of Musalenski Lakes and were the greatest between the Pirin population and that from Malyovitza (Rila Mts).

The largest interpopulation morphological variation was observed in *C. nigra* subsp. *nigra* (Table 8). It was in agreement with the large diversity of macro- and microecological conditions of the habitats from different regions, altitudes (950–2100 m) and ecosystems. The neighbour-sympatric areas of some populations of *C. nigra* subsp. *nigra* and *C. acuta* facilitated hybridization between some their plants and thus contributed to the large interpopulation variation too. The differences between the populations from one and the same region, as a rule, were less than those between the populations from different regions. Population No 1350 from Rakovo Dere (the Rhodopes) was an exception, because it resembled more the Vitosha populations than the other Rhodopean populations. A comparison of the populations within the different regions has shown that the lowest interpopulation differences were in Vitosha region: Ed = 5.35. The plants from these populations had short stems, leaves, bracts and inflorescence (Table 6). The smaller size of these plants could be explained with the macroecological conditions of their habitats localized at altitudes of 1850–2100 m. Interpopulation differences

Table 6. Mean (first line) and coefficient of variation in % (second line) of *C. nigra* subsp. *nigra* (438-1344), *C. nigra* subsp. *dacica* (272-1395) and undefined Group 2.

Population Character. number	438	1300	1362	1364	1365	1353	709	1350	1344	272	1361	1389	1394	395	Gr.2
1	35.37	30.38	38.45	43.75	42.84	51.78	40.13	28.26	42.34	15.67	19.05	14.96	15.81	15.5	33.29
	14.92	13.85	16.07	13.89	27.84	15.83	24.64	20.77	4.88	21.56	19.94	23.59	19.34	19.8	10.45
2	21.52	22.77	44.36	44.89	38.60	35.98	32.70	20.72	28.59	12.45	14.65	11.18	12.14	11.99	25.59
	20.16	16.64	18.52	11.47	39.93	15.56	23.14	32.14	31.19	26.74	33.44	32.28	29.38	27.77	26.61
3	1.66	1.38	1.55	1.08	0.97	2.01	1.78	1.57	2.71	1.74	1.66	1.62	1.79	1.54	2.13
	17.46	27.71	32.25	16.66	11.34	13.43	14.60	34.39	12.17	32.75	17.46	19.75	11.73	35.06	17.84
4	3.49	2.81	4.22	8.41	7.48	3.76	6.43	2.62	6.01	1.63	3.00	3.93	2.93	3.18	4.40
	37.82	31.67	44.78	14.50	18.71	51.59	37.01	67.93	34.10	29.44	44.00	52.16	53.63	35.53	38.86
5	5.10	4.10	7.31	8.18	8.45	4.90	7.13	4.45	7.30	2.53	3.90	4.92	3.84	3.70	5.70
	21.17	19.75	22.57	12.46	19.05	28.57	26.08	43.59	21.50	22.94	31.28	50.81	38.54	24.59	18.94
6	1.70	1.50	1.10	1.80	1.40	1.10	1.40	1.10	1.40	1.10	1.00	1.00	1.00	1.50	1.70
	28.23	34.66	28.18	23.33	36.42	28.18	36.42	28.18	0.49	28.18	0.00	0.00	0.00	34.66	28.23
7	1.83	1.61	2.45	1.99	2.33	1.36	2.21	1.86	2.06	1.09	1.42	1.60	1.38	1.33	2.39
	25.68	16.77	11.02	8.54	19.31	19.11	22.17	38.17	27.66	26.60	19.71	32.50	16.66	18.04	14.22
8	2.40	2.22	2.10	3.03	3.84	2.51	2.84	2.15	3.70	1.17	1.63	1.87	1.58	1.78	2.46
	23.75	27.47	42.38	24.09	16.92	23.10	17.6	51.62	15.40	23.93	17.79	28.27	46.83	24.71	23.98
9	2.28	1.86	1.55	2.67	2.49	2.23	1.97	1.43	2.60	1.11	1.66	1.72	1.78	1.38	1.54
	21.49	49.46	24.51	18.35	18.47	25.01	34.01	58.04	34.61	20.62	50.00	55.23	61.79	47.10	53.24
10	2.01	2.13	2.28	2.40	2.09	1.90	1.97	1.79	1.92	1.77	1.81	1.97	1.98	1.86	2.60
	6.40	12.20	16.22	5.41	4.30	9.47	3.40	7.82	11.45	14.68	9.39	7.10	7.02	15.59	5.11
11	1.02	1.19	1.31	1.22	1.31	1.16	1.17	1.04	1.25	1.07	1.03	1.00	1.14	1.04	1.28
	6.17	10.00	10.45	5.16	6.68	9.48	20.94	8.65	4.0	10.84	10.59	0.00	8.42	14.42	15.46
12	0.17	0.27	0.15	0.20	0.10	0.10	0.12	0.17	0.19	0.10	0.12	0.17	0.14	0.19	0.22
	39.41	17.77	46.66	77.50	24.00	24.00	52.50	28.23	16.31	24.00	35.00	28.23	36.42	16.31	9.09
13	1.80	1.98	2.17	2.42	2.06	1.72	2.09	1.95	2.04	1.78	1.61	1.64	1.99	1.80	2.40
	16.11	11.61	10.59	9.09	6.79	9.80	5.72	14.35	7.35	12.92	4.53	21.95	7.53	18.88	9.60
14	0.80	1.05	1.04	1.04	0.76	1.01	0.80	0.94	1.00	0.95	0.92	0.87	0.87	0.81	0.81
	5.87	8.09	17.07	11.53	9.07	8.61	21.25	10.21	9.40	10.52	11.95	14.36	15.36	9.11	17.28
15	3.03	3.01	3.23	2.75	3.56	2.64	3.44	2.96	3.45	3.01	2.59	2.77	3.09	2.85	3.68
	4.62	17.61	7.43	7.27	4.77	8.33	10.17	9.12	9.27	13.95	7.72	16.96	12.94	11.92	10.86
16	0.92	1.26	1.03	1.20	0.69	1.01	1.00	1.11	1.10	1.13	0.88	0.88	0.89	0.90	0.89
	10.86	10.00	16.05	14.16	10.69	30.69	13.33	14.32	12.72	17.69	13.63	18.18	21.34	15.55	19.10
17	0.60	0.75	1.18	1.04	0.89	0.71	0.82	0.72	0.67	0.80	0.76	0.73	0.77	0.77	0.76
	15.00	13.33	27.11	17.30	23.59	22.53	15.85	16.66	31.34	20.00	19.73	15.06	25.97	20.77	21.05
18	0.67	0.67	0.58	1.02	0.88	0.73	0.89	0.54	0.80	0.65	0.75	0.80	0.71	0.84	0.75
	31.34	20.89	43.10	7.78	11.02	27.39	26.62	29.43	22.50	27.69	15.73	27.25	30.56	17.50	22.66
19	0.14	0.13	0.19	0.18	0.20	0.09	0.17	0.15	0.17	0.16	0.21	0.33	0.24	0.24	0.17
	24.14	27.14	15.91	15.41	26.16	32.26	13.11	32.46	20.06	22.49	40.36	47.75	27.61	23.43	23.58
20	0.89	0.93	0.96	1.01	0.98	0.90	1.06	1.09	1.07	1.01	0.89	0.83	1.00	0.96	0.92
	12.12	14.10	16.72	9.59	6.12	5.02	7.41	16.45	13.41	17.09	9.11	21.60	7.76	8.05	12.88
21	1.97	1.79	1.74	1.97	1.60	1.66	1.75	1.73	1.53	1.66	1.76	1.97	1.75	1.79	2.07
	4.10	10.55	13.45	8.21	7.67	18.00	21.43	10.73	10.53	17.96	10.47	7.19	11.88	12.07	16.66

within the groups from Rila and the Rhodopes varied within nearly the same limits. The plants from Rila were tall, with longer leaves, inflorescences and female spikes (Table 6). The larger sizes were probably due to the lower altitude of their habitats: 950–1550 m. The Rhodopean populations have occupied an intermediate position between those of Rila and Vitosha with regard to their morphological characteristics correlating

with the intermediate altitude of the habitats: 1600–1650 m. The smaller sizes of population No 1350 mentioned above could be probably due to the untypical for subsp. *nigra* habitat: a relatively dry, marble sloping ground that was drained away and not under water in summer. *C. acuta* maintained an intermediate position in respect to its interpopulation differences (Table 9). Those differences were greatest between

**Table 7.** Values of Euclidean distance between the pairs of populations within *C. nigra* subsp. *dacica* based on 21 characters.

Number of population	272	1361	1389	1394
272				
1361	4.58			
1389	3.83	3.56		
1394	2.10	4.16	2.08	
1395	2.21	4.50	1.89	0.93

**Table 8.** Values of Euclidean distance between the pairs of populations within *C. nigra* subsp. *nigra* based on 21 characters.

Number of population	438	1300	1362	1364	1365	1353	709	1350
438								
1300	5.35							
1362	23.21	23.34						
1364	25.54	26.80	7.10					
1365	19.45	21.24	8.32	6.61				
1353	21.90	25.20	16.03	13.40	10.88			
709	12.71	14.74	12.04	13.00	6.86	12.66		
1350	7.33	3.09	25.97	29.59	24.03	28.09	17.54	
1344	10.65	14.23	16.52	16.70	10.36	12.57	4.91	16.89

**Table 9.** Values of Euclidean distance between the pairs of populations within *C. acuta* based on 21 characters.

Number of population	1230	710	1050	1343
1230				
710	11.40			
1050	21.38	11.78		
1343	17.44	13.43	18.04	
1346	25.98	16.09	8.03	16.83

the population from Srebarna Biosphere Reserve and the rest:  $Ed = 11.40 - 25.98$ . The longer stems, bracts, inflorescences and spikes of the Srebarna population (Table 5) were in accordance with the much lower situated habitat at 50 m a.s.l.

The intrapopulation variation was estimated on the basis of the coefficient of variation (Tables 5, 6). Data show that most morphological characters had  $V = 10-30\%$  in almost all populations. No character could be indicated as the most variable or the most constant in all taxa and populations. The most variable characters in most species were the distance between the two lower female spikes and the bract length. The sizes of utricle and glumes were among the most constant features. Occasionally, some characters were strongly variable in one taxon but relatively constant in the others. A similar picture could be observed in the different populations within one and the same taxon. Good ex-

amples of such features are the beak length and the number of male spikes.

A comparison of V-values in the different taxa has shown that they were the greatest and varied within larger limits in *C. nigra* subsp. *nigra*, followed by *C. nigra* subsp. *dacica* and *C. acuta*. The most invariable species was *C. cespitosa*. The narrow range of variation in *C. cespitosa*, *C. buekii* and *C. elata* could be explained with limited investigation of these species, but one should also note that it correlated with constant or less variable chromosome numbers.

A comparison of the intrapopulation variation in *C. acuta* and *C. nigra* has shown that the Rhodopean populations No 710 and 1050 of the first species and No 709 and 1350 of *C. nigra* subsp. *nigra* were morphologically the most variable. The large morphological variation in No 710, 709 and 1350 correlated with the variable chromosome numbers. The population pairs No 709–710 and No 1050–1350 had neighbour-sympatric areas. The immediate proximity of the two species enabled their plants to hybridize and produce hybrids and backcrosses between F1 hybrids and their parents. That could explain to some degree the large morphological and karyological variation. The Rhodopean populations No 1346 of *C. acuta* and No 1344 of *C. nigra* subsp. *nigra* were much less variable morphologically, despite their neighbour-sympatric areas. In the first of them, the small morphological variation correlated with the constant chromosome number. Thus, that population resembled a little a young clone unaffected by *C. nigra*. There has been no correlation between the small phenotype variation and variable chromosome number in No 1344. The populations of Rila and Vitosha Mts were less variable, probably by reason of their isolation from the other species of sect. *Phacocystis*.

The results obtained by the one-way ANOVA method have shown the dominant share of the intrapopulation variation in the overall morphological variation of *C. acuta*, *C. nigra* subsp. *nigra* and subsp. *dacica* (Table 10). That fact could be explained with the large numbers and biological structure of the populations which combined sexual and vegetative reproduction, as well as with the lack of effective genetic barriers between the species. The relative share of interpopulation variation in *C. nigra* subsp. *nigra* and *C. acuta*, in comparison with *C. nigra* subsp. *dacica*, was much larger than corresponded with ecological diversity of their habitats.

**Table 10.** Percentage of the interpopulation variation in the overall morphological variation for each character.

Character number \ Taxon	<i>Carex acuta</i>	<i>Carex nigra</i> subsp. <i>nigra</i>	<i>Carex nigra</i> subsp. <i>dacica</i>
1	41.03	49.69	17.61
2	39.36	57.78	9.46
3	38.37	67.76	4.76
4	9.43	59.98	23.16
5	20.69	55.72	22.35
6	31.27	22.61	35.61
7	35.81	37.77	21.16
8	24.82	44.88	21.13
9	15.89	27.89	13.03
10	32.88	47.92	14.63
11	42.76	41.43	18.16
12	25.47	40.76	43.18
13	50.41	47.51	23.07
14	57.98	51.65	16.76
15	55.34	56.41	19.09
16	53.96	49.23	27.27
17	37.29	50.17	1.93
18	6.14	42.21	12.66
19	25.07	44.28	28.05
20	45.22	26.96	25.27
21	43.07	31.07	18.95

### Leaf epidermis anatomy

The results of the leaf anatomy study have shown that all investigated taxa of sect. *Phacocystis* had papillose epidermis. Papillae were of different size and frequency but always connected to the surface with stomata. These structural peculiarities were observed by other authors too (Akiyama 1941; Claustres & Le Cohu 1965; Le Cohu 1971; Standley 1987a, b, 1990). In amphistomatal leaves the stomata density on the upper epidermis is higher than on the lower one. The epidermal cells of both surfaces are nearly equal in size like in Polish populations of *C. nigra* (Klimko 1981). In hypostomatal and epistomatal leaves the epidermal cells on the surface with stomata are shorter and narrower than those on the surface without stomata. In hypostomatal leaves the upper epidermis is without, or with very sparse papillae localized in the middle vein area. On the lower epidermis, papillae occur very frequently on every cell and are more prominent along the median vein. The species *C. acuta*, *C. cespitosa*, *C. elata*, *C. buekii* and Group 1 have hypostomatal and hypopapillose leaves (Table 11) with more oblong cells on the upper epidermis (Figs 9, 10). Klimko

(1981) reported the same type epidermal structure for the first three species and Standley (1990) for *C. acuta*, *C. cespitosa* and for some populations of *C. buekii*. According to the later author, other populations of *C. buekii* have amphistomatal leaves.

The investigated species of the hypostomatal group can be distinguished by the following features: *C. buekii* had less stomatal density, larger cell size on the lower epidermis and wider cells on the upper epidermis (Table 11; Fig. 9). *C. elata* had more oblong cells on the upper epidermis (l:w=6.8) and a lot of abnormal stomata: twins with a common subsidiary cell (Table 11; Fig. 11). The abnormalities in the stomatal apparatus could be explained by deteriorated water supply caused by the destroyed natural habitat. *C. cespitosa* showed some sclerophyllous features: high stomatal frequency and dense short papillae; thickened walls of the epidermal cells along the leaf margin and big veins, a lot of prickles (Fig. 12). The same leaf epidermis was observed in the aberrant Group 1. These sclerophyllous features are probably related to the untypical habitat.

The plants within the studied populations of *C. buekii*, *C. elata* and *C. cespitosa*, as well as of Group 1 were rather uniform in their leaf epidermis.

*C. acuta* combined populations which were not uniform in relation to the leaf epidermis structure. The interpopulation differences related to stomata and papillae distribution, epidermal cell sizes, presence or absence of prickles and abnormal stomata. Certain diversity was observed within most populations too. For example, in population No 1343 the cells on the upper epidermis were much more oblong than in the other populations l:w=6.4 (Table 11); the papillae were very variable from high and rough to quite short; there was many anomalies in the structure of the stomatal apparatus – a subsidiary cell “bridge” between two stomata (Fig. 10). Population No 1050 of the same species had a low number stomata and papillae and also of prickles and anomalies of the stomatal apparatus on the upper epidermis. Of special interest was population No 710. With its amphistomatal and amphipapillose epidermis this population resembled *C. nigra*, but unlike *C. nigra*, stomatal density is much higher on the lower epidermis. Its intrapopulation diversity was also very large and was related to stomata and papillae distribution, the presence or absence of prickles and anomalies in the stomatal apparatus. The same picture has been observed in aberrant Group 2. Some

Table 11. Results of leaf anatomical study of *Carex* sect. *Phacocystis*.

Taxa	N of the population	Upper epidermis						Lower epidermis					
		Mean of stomata number on 1 mm	Mean of epidermal cells length	Mean of epidermal cells width	Presence of papillae	Presence of prides	Presence of abnormal stomata	Mean of stomata number on 1 mm	Mean of epidermal cells length	Mean of epidermal cells width	Presence of papillae	Presence of prickles	Presence of abnormal stomata
1	2	3	4	5	6	7	8	9	10	11	12	13	14
<i>C. acuta</i>	1343	0.0	101.0	15.6	-	-	-	282.0	31.7	14.7	+	-	+
	1346	0.0	68.7	17.5	-	-	-	232.0	36.8	13.6	+	+-	-
	1050	11.9	63.7	16.8	+-	+	+-	285.8	35.6	15.2	+	-	-
	710	152.3	44.3	14.3	+	+	+	224.4	43.1	15.2	+	-	+
<i>C. buekii</i>	1324	0.0	115.7	23.1	-	-	-	135.6	50.4	17.5	+	-	-
<i>C. cespitosa</i>	1264	0.0	88.9	21.3	-	+	-	361.0	35.4	12.8	+	+	-
<i>C. elata</i>	1314	0.0	116.9	17.3	-	-	-	200.0	32.2	12.9	+	-	+
<i>C. nigra</i> subsp. <i>nigra</i>	1364	170.2	51.4	14.1	+	+	-	0.0	62.0	16.2	-(+)	+	-
	1365	232.4	46.9	20.0	-	+	-	0.0	86.7	16.7	-(+)	-	-
	1353	209.8	46.5	16.7	+	-	-	0.0	63.0	17.1	-	-	-
	1362	103.5	61.1	14.6	+	-	-	71.4	56.3	15.9	-(+)	+	-
	438	183.0	38.9	13.8	+	-	-	46.8	47.6	15.7	-(+)	-	-
	1300	193.0	28.2	10.0	+	-	+	55.3	35.7	13.0	-(+)	-	-
	1350	142.1	55.8	13.6	+	-	+	97.1	53.1	13.8	+	-(+)	+
	1344	149.5	46.9	15.3	+	-	+	94.4	53.7	16.6	-(+)	-	+
709	193.8	46.8	13.6	+(+)	+	+	134.7	48.6	12.1	+-	-	-	
<i>Carex nigra</i> subsp. <i>dacica</i>	272	164.3	37.8	14.9	+	-	+	88.5	41.7	16.1	+-	-	-
	1361	203.2	44.8	15.4	+	-	+	81.8	48.1	15.4	-(+)	-	+
	1389	185.6	39.7	16.2	+	-	+	94.1	44.3	16.8	-(+)	-	+
	1394	200.0	38.5	15.3	+	-	-	89.6	36.1	15.7	-	+	-
	1395	178.9	33.1	14.2	+	-	-	108.5	32.6	17.8	-	-	-
Undefined Group 1		0.0	87.1	23.8	-	+	-	328.9	38.8	13.7	+	+	-
Undefined Ggroup 2		181.6	47.3	14.6	+	+	+	217.6	44.2	15.5	+-	-	-

plants of population No 710, Group 2 and single specimens of population No 1050 in their leaf epidermal characteristics resembled hybrids between hypostomatal and amphistomatal species. Such hybrids were observed by Toivonen (1981) and Standley (1990). Population No 1346 was most invariable in its leaf epidermis which correlated to less morphological diversity and a constant chromosome number.

*C. nigra* with its two subspecies combined populations with amphistomatal and epistomatal leaves. Epistomatous were three Rila populations of subsp. *nigra* (No 1364, 1365 and No 1353), which corresponded to Standley's results (1990) about American *C. nigra*. The interpopulation differences related to the stomata and papillae density, papillae height and the sizes of epidermal cells. No anomalies in stomatal apparatus have been observed (Fig. 14). Only population No 1364 possessed prickles on the costal and

intercostal area. All plants within epistomatal populations had the same leaf epidermis. All other studied populations of subsp. *nigra* were amphistomatous (Table 11) like *C. nigra* in Poland (Klimko 1981). The populations of this group differed from each other in stomata and papillae density on the two surfaces, in the height of papillae and other peculiarities. Thus, the Rhodopean populations had more stomata and papillae than the others, variable height of papillae and many abnormal stomata. The Vitosha populations had up to four times more stomata and papillae on the upper epidermis than on the lower one. The two populations differed from each other mainly by the height and place of papillae and by the presence of anomalous stomata with conjugate subsidiary cells in population No 1300 (Fig. 13). The foliar epidermis within the amphistomatal populations of subsp. *nigra* was very variable, unlike that in the epistomat-

al group. Most variable were the Rhodopean populations, No 1300 from Mt Vitosha and No 1362 from Rila Mts. The diversity was mainly related to the frequency of stomata and papillae on the two surfaces. *C. nigra* subsp. *dacica* always had amphistomatal leaves (Table 11). The interpopulation differences related mainly to the stomata and papillae density on both surfaces and to papillae shape. In population No 1394 and No 1395 the papillae were delicate and only on the upper epidermis. Prickles were registered only in No 1394 (Fig. 16). There were anomalous stomata only in No 272, 1389 and 1361 and they were more numerous in the first population (Fig. 15). The intrapopulation variation of subsp. *dacica* was rather large and related to the ratio between stomata and papillae number on the two foliar surfaces, to papillae shape and epidermis cell size.

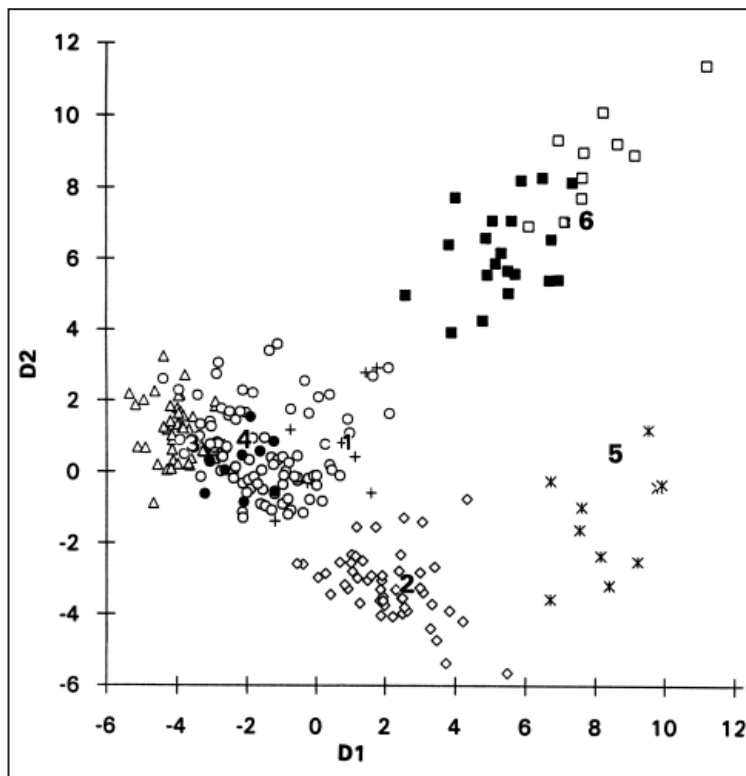


Fig. 7. Discriminant plot of the individuals of six a-priori defined *Carex* taxa and the two undefined groups with respect to discriminant functions D1 and D2.

- |   |                                      |     |                     |
|---|--------------------------------------|-----|---------------------|
| + | <i>C. elata</i>                      | □   | <i>C. cespitosa</i> |
| ◇ | <i>C. acuta</i>                      | 1-6 | Centres             |
| △ | <i>C. nigra</i> subsp. <i>dacica</i> | ■   | Group 1             |
| ○ | <i>C. nigra</i> subsp. <i>nigra</i>  | ●   | Group 2             |
| × | <i>C. buekii</i>                     |     |                     |

### Relationships and evolutionary trends in sect. *Phacocystis*

The results of multivariate statistics and leaf epidermis anatomy study of the Bulgarian species of sect. *Phacocystis* have led to the conclusion that all species can be divided in two groups. The first of them includes *C. buekii*, *C. elata*, *C. cespitosa*, and *C. acuta*. These species were characterized with high stems, comparatively rough habit, and hypostomatal hypopapillose leaves. The second group includes only *C. nigra*, which had short, more slender stems, and epistomatal epipapillose or amphistomatal amphipapillose leaves.

Standley (1990) considered the hypostomatal hypopapillose leaves as more primitive than the epistomatal ones and regarded amphistomatal leaves as derived from them. Then, the first group could be regarded as evolutionary older than *C. nigra*.

This viewpoint was supported by the karyological and morphological data. Of the species of the hypostomatal group, *C. buekii*

had the lower and constant chromosome number and a more heterogeneous karyotype. This species was characterized with the most high stems in sect. *Phacocystis*, and uniform leaf epidermal structure. The intrapopulation morphological variation was rather poor too. All these peculiarities gave us a reason to consider *C. buekii* the oldest member of sect. *Phacocystis*. In its morphological characteristics *C. buekii* was closer to *C. elata* and *C. cespitosa* than to *C. acuta* (Table 3). The greater phenotype differences between *C. buekii* and *C. acuta* correlated with the very different chromosome numbers. Taking into consideration the chromosome numbers, karyotypes, meiotic chromosome pairing, variability of the pollen grain size, morphology, ecological requirements and the ability for interspecies hybridization, Faulkner (1973) assumed that *C. elata* was closest related to *C. cespitosa*. He also regarded *C. elata* as a link between the pairs of species *C. acuta*–*C. nigra*, on one hand, and *C. aquatilis*–*C. bigelowii*, on the other. On the basis of the variable chromosome numbers ( $2n = 73 - 77$ ), and morphological and taxonomical diversity of *C. elata* in SW Europe, Luceño & Aedo (1994) regarded it as an active species with rapid diversification. They indicated also agmatoploi-

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dy with its two variants, fusion and fission of chromosomes, as the main mechanisms in the karyotype evolution of this species. Its ability for hybridization both between intraspecific taxa and with other species of the same section was mentioned as a significant factor for active diversification of this species. *C. elata* and *C. cespitosa* are rare species in Bulgaria and our study was very restricted. Nevertheless, the obtained results on the chromosome numbers, karyotype, foliar epidermis structure, and morphology have also shown that they are closer related to each other than to the other species of the section. Group 1, which grew in the immediate proximity of the population of *C. cespitosa* and possessed the same dominant chromosome number and the same invariable foliar epidermis, belonged to this species. The morphological variation in it correlated with the variable chromosome number. The absence of the longest pair of chromosomes in the cytotype with  $2n = 80$  suggested its agmatoploid

origin from the cytotype with  $2n = 78$ . The cytotype with  $2n = 79$  could have resulted from agmatoploidy of the cytotype with  $2n = 78$ , or from symploidy of the cytotype with  $2n = 80$ , or of hybridization between the cytotypes with  $2n = 78$  and  $2n = 80$ . On the basis of the higher chromosome numbers and of the more homogeneous karyotype, *C. acuta* could be regarded as the youngest member of the hypostomatal group. Relying on morphological characteristics, Kükenthal (1909) considered *C. acuta* and *C. nigra* as closely related. Taking into account the identical chromosome numbers and interfertility of the two species, Faulkner (1973) confirmed their close relationship. Along with this he pointed out their differences in morphology, ecology, cytology and interfertility. According to him, the most essential cytological differences were more often aneuploidy in *C. acuta* than in *C. nigra*, the higher interfertility of *C. acuta* in crosses with *C. paleacea* and *C. cespitosa*, and less effectively as a male parent in crosses with *C. elata*. Our results have led to the conclusion that in its morphology and leaf epidermal structure *C. acuta* was closer related to the species of the hypostomatal group. The chromosome numbers and the karyotype drew it together with *C. nigra*: the two species had one and the same typical eudiploid chromosome number, a rather homogeneous karyotype and a similar aneuploid series. They occurred at the same altitudes and their populations were often neighbour-sympatric, which helped the contacts between plants from the two species.

Analysing the similarity and differences between *C. acuta* and *C. nigra*, the question about the origin and relationships of the second species becomes very intriguing. On the basis of the foliar epidermis structure (epistomatal and amphistomatal leaves) and the phenotype characteristic, especially the short and slender stems, *C. nigra* should be regarded as a younger species than *C. acuta*. Bearing in mind the opinion

of Standley (1990) that amphistomatal leaves are derivative and that hybrids among species combine the parental anatomical characters, we could assume a secondary origin of the amphistomatal *C. nigra* from the epistomatal plants of the same species and from some hypostomatal species with high chromosome numbers of sect. *Phacocystis*. On the basis of the same chromosome numbers of *C. acuta* and *C. nigra*, their interfertility (Faulkner 1973) and their similar ecological lo-

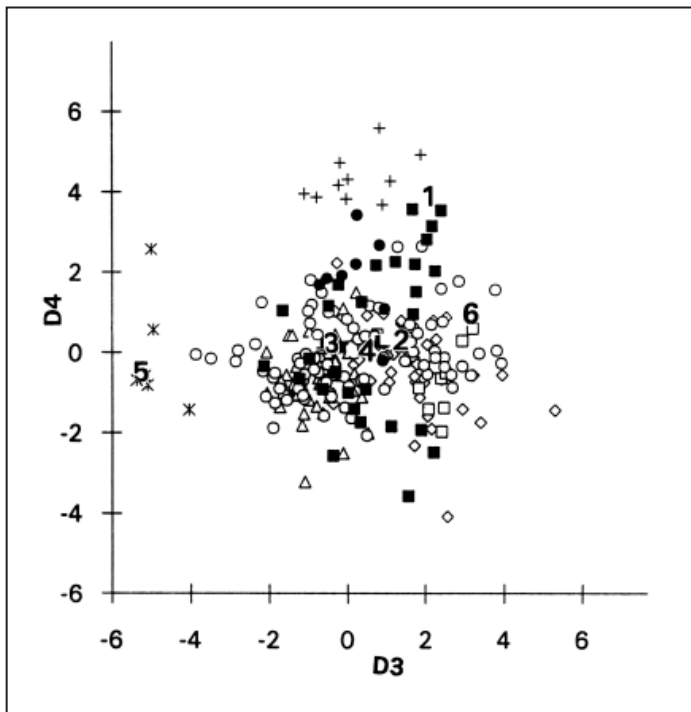
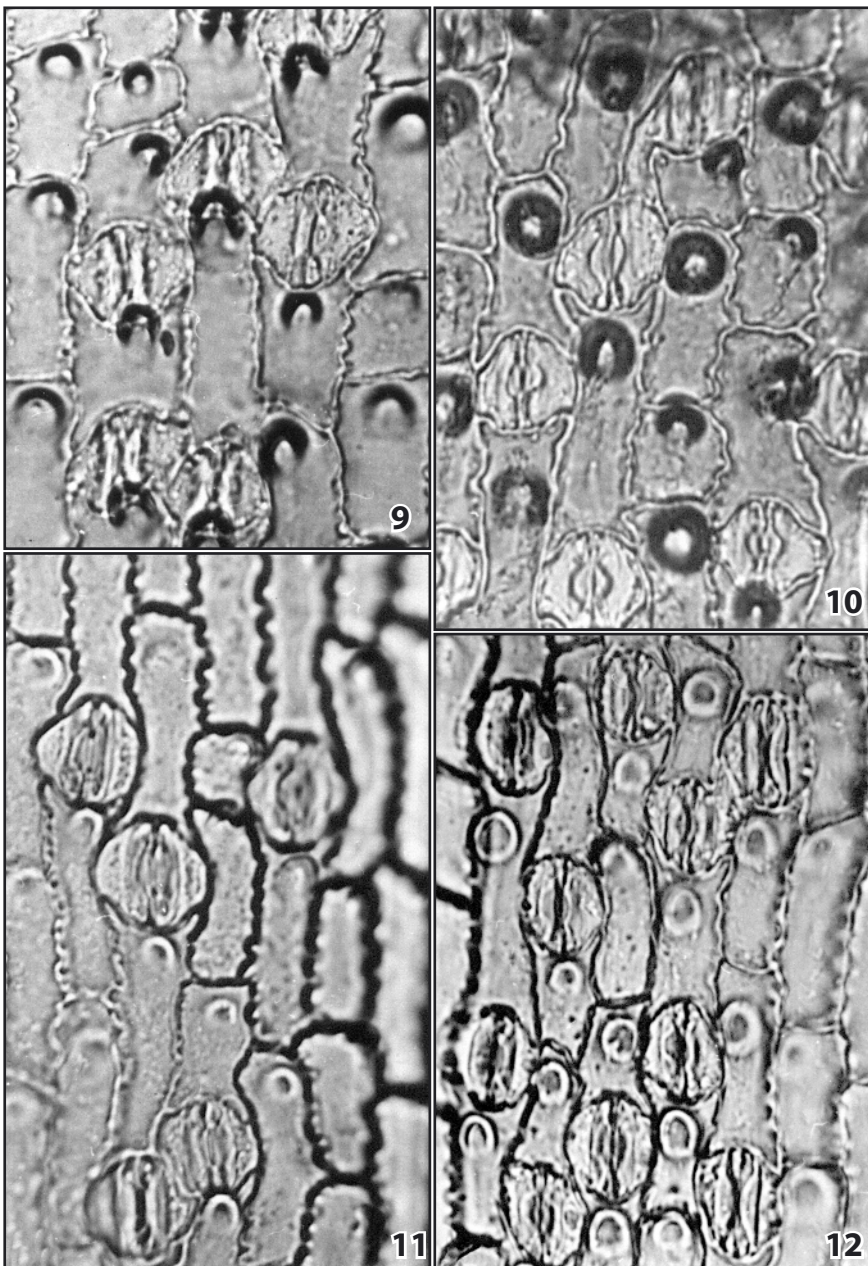


Fig. 8. Discriminant plot of the individuals of six earlier defined *Carex* taxa and the two undefined groups with respect to discriminant functions D3 and D4.

- |   |                                      |     |                     |
|---|--------------------------------------|-----|---------------------|
| + | <i>C. elata</i>                      | □   | <i>C. cespitosa</i> |
| ◇ | <i>C. acuta</i>                      | 1-6 | Centres             |
| △ | <i>C. nigra</i> subsp. <i>dacica</i> | ■   | Group 1             |
| ○ | <i>C. nigra</i> subsp. <i>nigra</i>  | ●   | Group 2             |
| × | <i>C. buekii</i>                     |     |                     |

calities, we could regard *C. acuta* as the hypostomatal parent of the amphistomatal *C. nigra*. The large anatomical, phenotypical and cytological diversity in the places where the plants of *C. acuta* and amphistomatal *C. nigra* grew side by side have shown that certain hybridization between the two species was very possible. Logically, the same process could be observed in places with sympatric populations of *C. acuta* and epistomatal *C. nigra* too. Stabilization of the hybrids, even when they were not fully fertile, was favoured by the K-strategy (combination of vegetative and sexual reproduction) of the two species, and especially of *C. nigra*.

*C. nigra* subsp. *dacica* could be a hybrid of epistomatal *C. nigra* and some hypostomatal species of the section with chromosome numbers smaller than  $2n = 84$ . It could also have a symploid origin from an amphistomatal *C. nigra*. On the basis of the closer similarity of subsp. *dacica* with *C. nigra*, and especially with the amphistomatal *C. nigra* populations, the symploid origin of this subspecies seemed more probable. No matter what was the origin of *C. nigra* subsp. *dacica*, the new cytotype with  $2n = 80$  had gradually occupied places at higher altitudes and thus a new ecotype has been formed.



**Fig. 9.** Lower epidermis ( $\times 600$ ) of *C. buekii* (No 1324).

**Fig. 10.** Lower epidermis with subsidiary cell "bridge" ( $\times 600$ ) of *C. acuta* (No 1343).

**Fig. 11.** Lower epidermis ( $\times 600$ ) of *C. elata* (No 1314).

**Fig. 12.** Lower epidermis ( $\times 600$ ) of *C. cespitosa* (No 1264).



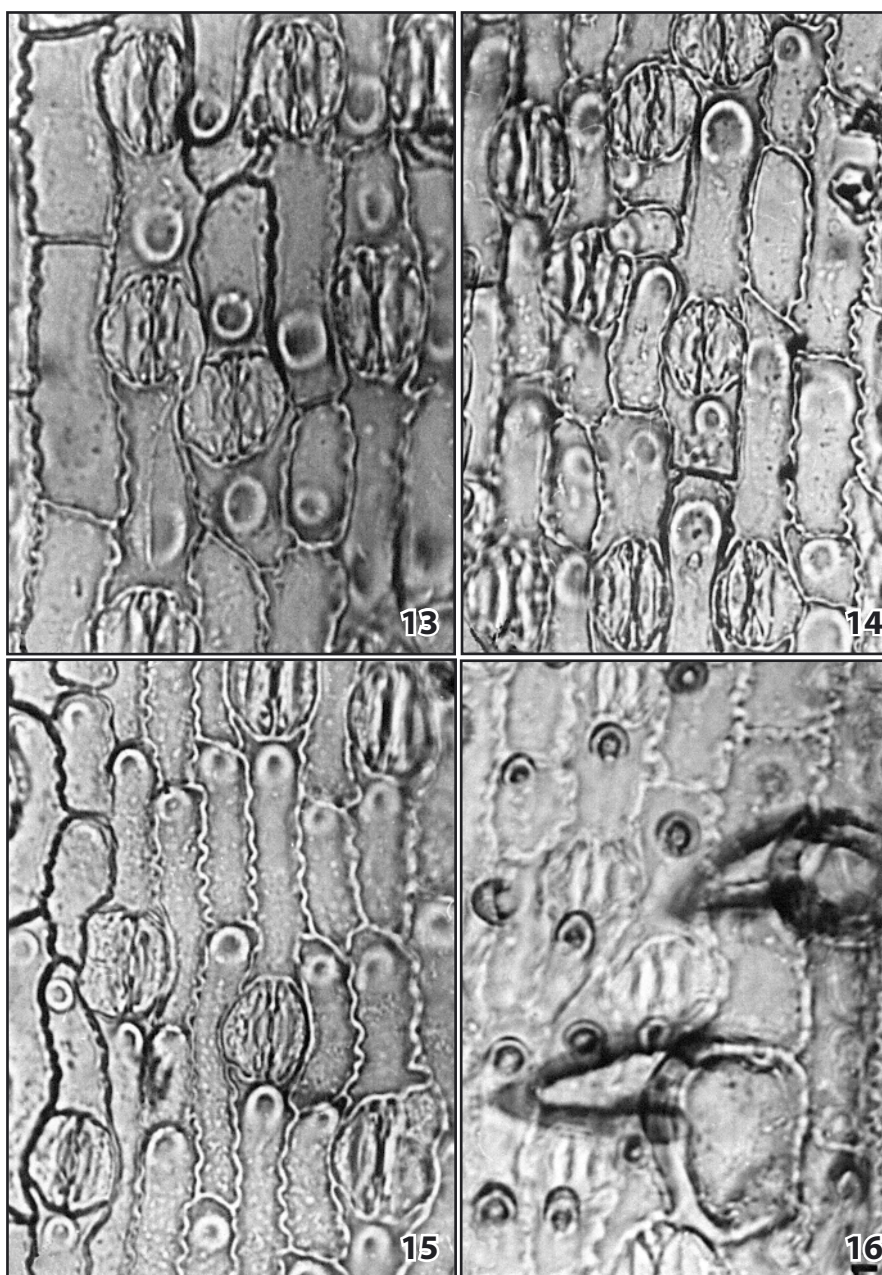
## Conclusion

The results of this study have shown that sect. *Phacocystis* is represented in Bulgaria by six taxa: *C. buekii*, *C. elata*, *C. cespitosa*, *C. acuta*, *C. nigra* subsp. *nigra*, and *C. nigra* subsp. *dacica*. The first four species were characterized with high stems and more or less rough habits, and hypostomatal leaves. They are evolutionary older than *C. nigra* having short, slender stems, and epistomatal or amphistomatal leaves.

The intrapopulation variation is the main source of phenotype variation in the species of this section.

In *C. nigra* subsp. *nigra* and *C. acuta* phenotype variation often correlates with variable chromosome numbers and more variable foliar epidermis structure. The interpopulation differences are greater in *C. nigra* subsp. *nigra* than in *C. acuta* and subsp. *dacica*. This corresponds to the more diverse habitats of its populations.

Symploidy and hybridization are the main evolutionary mechanisms in sect. *Phacocystis*. Hybridization between *C. acuta* and *C. nigra* has been favoured by the ineffective reproductive barriers. K-strategy of these species has contributed to a great extent to the stabilization of hybrids.



**Fig. 13.** Upper epidermis with conjugate subsidiary cells ( $\times 600$ ) of *C. nigra* subsp. *nigra* (No 1300).

**Fig. 14.** Upper epidermis ( $\times 600$ ) of *C. nigra* subsp. *nigra* (No 1365).

**Fig. 15.** Upper epidermis with third subsidiary cell ( $\times 600$ ) of *C. nigra* subsp. *dacica* (No 272).

**Fig. 16.** Upper epidermis with prickles of *C. nigra* subsp. *dacica* (No 1394).

**Acknowledgements.** The authors are grateful to E. Marinkova and R. Adzhiiska for their technical assistance. The study was made with the financial support of the National Science Fund, Ministry of Education and Science.

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