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The genus *Cochlearia* L. (Brassicaceae) in the Eastern Carpathians and adjacent area

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Most European scurvy grasses (including those of the Carpathian Mountains) belong to the nominate section *Cochlearia*. We analyse the status of two East Carpathian (Romanian) *Cochlearia* populations by comparing them with the two native species from the Western Carpathians, the diploid *Cochlearia pyrenaica* ($2n = 2x = 12$; 2C = 0.78 pg) and hexaploid *C. tatrae* ($2n = 6x = 42$; 2C = 2.09 pg). Using karyological methods and flow cytometry, differences between these taxa were detected. Because of differences in morphology, chromosome number ($2n = 8x = 48$) and DNA content (2C = 2.82 pg), we propose that the East Carpathian (Romanian) populations represent a separate species, *Cochlearia borzaeana* (Coman et Nyár.) Pobed. The lectotype of *C. borzeana* is designated. The new subassociation *Carici flavae–Cratoneuretum* Kovács et Felföldy 1958 *cochlearietosum borzeanae* is described. An isolated population of *C. pyrenaica s.l.* from Ukraine (outwith the Carpathian territory) ($2n = 2x = 12$; 2C = 0.91 pg) has been also studied, because of its unclear taxonomic position. The diploid chromosome number, $2n = 2x = 12$, is given for this single known population of *C. pyrenaica s.l.* in Ukraine. However, there is considerable difference in genome size and chromosome size between West Carpathian *C. pyrenaica s.s.* and Ukrainian plants, and taxonomic evaluation of the latter population needs further study. A comparative table with morphological characteristics and a short description of the phytosociological behaviour of *C. borzaeana* in Romania and taxa from the Western Carpathians are included. © 2006 The Linnean Society of London, *Botanical Journal of the Linnean* Society, 2006, **151**, 355–364.

ADDITIONAL KEYWORDS: chromosome number – chromosome size – Cruciferae – DNA content – endopolyploidy – flow cytometry – genome size – karyology – Maramureş – morphology – phytosociology – polyploidy – Romania – scurvy grass.

INTRODUCTION

According to traditional taxonomic classifications, e.g. Schulz (1936), the genus *Cochlearia* L., distributed in Europe, Asia and the circumpolar regions of North America, is represented by about 30 species. These species are divided into four sections: *Cochlearia*, *Glaucocochlearia* O.E. Schulz, *Pseudosempervivum* Boiss. and *Hilliella* O.E. Schulz. A different model of infrageneric classification was proposed by Pobedimova. She placed the taxa belonging to section *Glaucocochlearia* into a separate genus, *Glaucocochlearia* (L.) Pobed. (Pobedimova, 1969). She divided the three sections of *Cochlearia* (comprising 23 species in total) into seven series (Pobedimova, 1970, 1971). Pobedimova's model has not been followed by other authors (e.g. Markgraf, 1975; Vogt, 1985, 1987, 1993; Koch, Huthmann & Hurka, 1998).

A recently published phylogenetic study based on molecular analysis (Koch, Mummenhoff & Hurka, 1999), and also more traditional taxonomic considerations, has provided new insight into the infrageneric classification of *Cochlearia*. Koch *et al*. (1999) proposed

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classifying the Asian taxa (formerly included in sections *Hilliella* and *Pseudosempervivum*) into separate genera. They also proposed dividing the European taxa into four sections: *Cochlearia*, *Glaucocochlearia* O. E. Schulz, *Ionopsidium* DC. and the newly defined *Archaecochlearia* M. Koch. Most of the European taxa, including those of the Carpathian Mountains, belong to the nominate section *Cochlearia*. This section contains a heterogeneous group with many species exhibiting different cytotypes, ecological adaptations and habitats, and also distributions. From the karyological point of view, there are two groups in this section, characterized by two different basic chromosome numbers: $x = 6$ and $x = 7$. The first group includes the diploids *C. pyrenaica* DC., *C. macrorrhiza* (Schur) Pobed., *C. excelsa* Zahlbr. ex Fritsch and *C. aestuaria* (Lloyd) Heywood ($2n = 12$), the tetraploid *C. officinalis* L. ($2n = 24$), the hexaploids *C. polonica* E. Fröhl. and *C. bavarica* Vogt ($2n = 36$), and the polyploid *C. anglica* L. ($2n = 48, 60$). The other group includes the diploid *C. islandica* L. ($2n = 14$, unique 12) and the hexaploids *C. tatrae* Borbás and *C. danica* L. ($2n = 42$) (cf. Vogt, 1985; Koch *et al*., 1998).

Several recently published comprehensive taxonomic studies of the genus *Cochlearia* (Vogt, 1985, 1987, 1993; Koch, Hurka & Mummenhoff, 1996; Koch *et al*., 1998, 1999; Nordal & Stabbetorp, 1990) have been concerned with western and central European taxa, but only marginal attention was paid to Carpathian populations (Koch *et al*., 2003). Smejkal (1968) grouped all West Carpathian scurvy grasses into one species, *Cochlearia tatrae*. A karyotaxonomic and phytosociological revision (Valachovič & Kochjarová, 2000) revealed the existence of two native species in this part of the Carpathians, *C. pyrenaica* and *C. tatrae*.

The isolated population of *Cochlearia* (originally determined as *C. pyrenaica* DC.) in Ukraine (formerly Poland) was discovered in 1929 in the Zakhodni Buh river spring area near the village of Zolochiv east of the town of Lviv (Tymrakiewicz, 1931). The taxonomic classification of these plants was later changed to *C. polonica* (Pobedimova, 1971) and this treatment was followed in subsequent Ukrainian floras and determination keys (e.g. Kotov, 1979; Prokudin, 1987). In the comprehensive taxonomic study of central European *Cochlearia* taxa by Vogt (1985) the Ukrainian population was considered to be *C. pyrenaica* DC., based on measurement of pollen grains. This population was also classified as *C. pyrenaica* by Valachovič & Kochjarová (2000).

The occurrence of scurvy grass in the Romanian part of the Carpathians has been known since the end of the 18th century; Schur (1866) cited herbarium specimens from Transsylvania, collected by Lerchenfeld in 1780. However, probably the oldest published data from the Romanian territory come from the first half of the 19th century (Rochel, 1838). Nevertheless, the systematic position of Romanian *Cochlearia* has not yet been satisfactorily defined (cf. Jalas, Suominen & Lampinen, 1996: 114; Koch *et al*., 2003). The taxonomic classification of these populations has changed repeatedly. Most authors included them in *C. officinalis* or *C. pyrenaica*. The comprehensive Romanian flora (Nyárády, 1955) classified them as *C. pyrenaica* DC. var. *borzaeana* Coman et Nyár. The recently published checklist of the Romanian vascular plants (Popescu & Sanda, 1998) treated them as a separate species, *C. borzaeana* (Coman et Nyár.) Pobed., although the name *C. officinalis* ssp. *pyrenaica* (DC.) Rouy et Fouc. is used in the last published Romanian field key (Ciocârlan, 2000).

The main goals of our field research and subsequent data analyses were as follows: (1) to elucidate the taxonomic classification of the two East Carpathian (Romanian) populations of *Cochlearia*; (2) to define their relationship to the West Carpathian taxa; and (3) to give basic information on the ploidy level of the Ukrainian *Cochlearia* population, and to contribute to the taxonomic classification of this population.

MATERIAL AND METHODS

Plant material

Living plant material from six sites was studied: (1) Romania, Maramureş Mountains, Şalhoi, calcareous spring, N47°39′, E24°59′, 1226 m a.s.l. (*Cochlearia borzaeana*); (2) Romania, Obcina Meştecănisului Mountains, Rachitişul Mare hill near the village of Benia, N47°39′, E25°15′, 920 m a.s.l. (*Cochlearia borzaeana*); (3) Ukraine, the village of Zolochiv east from the town of Lviv, Zakhodni Buh river spring area, N49°50′, E25°06′, 297 m a.s.l. (*Cochlearia pyrenaica s.l.*); (4) Slovakia, Vel'ká Fatra Mountains, Bukovinka travertine hill near the town of Ružomberok, N49°00′, E19°17′, 650 m a.s.l. (*Cochlearia pyrenaica s.s.*); (5) Slovakia, Jazierce, south of the town of Ružomberok, hard water spring, N49°01′, E19°16′, 600 m a.s.l. (*Cochlearia pyrenaica s.s.*); and (6) Slovakia, Vysoké Tatry Mountains, Velická dolina valley, N49°09′, E20°09′, 1850 m a.s.l. (*Cochlearia tatrae*). Non-invasive methods were generally used because the species are rare and endangered. Altogether, 20–30 representatives of each population were measured *in situ* and only a few specimens were collected and dried. The Ukrainian population was not measured because flowers were absent when it was visited. Voucher specimens are deposited at the herbarium of the Comenius University Botanical Garden in Blatnica (BBZ) and at the herbarium of the Institute of Botany of the Slovak Academy of Sciences (SAV). Herbarium specimens of the whole genus *Cochlearia* from the collections BP,

BRA, BRNM, BRNU, CL, KRA, KRAM, PR, PRC, SAV, SLO and W (abbreviations according to Holmgren, Holmgren & Barnett, 1990) were also revised and several herbarium specimens obtained from the above-mentioned localities were used for measurement.

Chromosome numbers

Chromosome counts were made on young seedlings obtained from germinated seeds. Root-tip cuttings were pretreated with 0.002 M 8-hydroxychinoline for about 2 h at room temperature and then for *c*. 3 h at 4 °C. Subsequently, a mixture of absolute ethanol and acetic acid (3 : 1) replaced the hydroxychinoline. Root tips were kept in fixative solution for at least 1 h and then were hydrolysed for 5 min in 1 N hydrochloric acid at 60 °C. The squash and smear method followed Murín (1960) with cellophane replacing the glass covers. Giemsa solution in phosphate buffer was used as a stain. Selected permanent slides are stored at the Department of Botany, Institute of Biology & Ecology, P.J. Šafárik University, Košice. For two Romanian populations, chromosome counts were carried out on five seedlings of each locality, whereas for the Ukrainian population only two germinated seeds were used because of a lack of material.

Flow cytometry measurements of DNA content

A PA-I ploidy analyser (Partec GmbH, Münster, Germany), equipped with an HBO-100 mercury arc lamp, was used for the estimation of relative DNA content. Sample preparation involved a two-step procedure (Otto, 1990; Doležel & Göhde, 1995) in the Laboratory of Flow Cytometry at Masaryk University, Brno. Young leaf samples (0.5 cm^2) from two specimens were chopped with a new razor blade for about 20 s in a Petri dish containing 0.5 mL of ice-cold Otto I buffer: 4.2 g citric acid monohydrate + 1 mL 0.5% Tween 20 adjusted to 200 mL and filtered through a 0.22-μm filter; then 0.5 mL of Otto I buffer was added. The solution was filtered through a nylon cloth (50-μm mesh size). For DNA staining, 2 mL of Otto II buffer (0.4 M $Na_2HPO_4 \cdot 12H_2O$) with DAPI (4′,6-diamidino-2-phenylindole; 4 μg mL^{-1} final concentration) was used. A similar two-step protocol was employed for the determination of nuclear DNA in absolute units, using a CyFlow cytometer (Partec). For DNA staining, 1 mL of Otto II buffer supplemented with propidium iodide (50 μg mL^{-1} final concentration) and RNase II (50 μg mL^{-1} final concentration) was used.

Endopolyploidy in samples and standards resulted in interference between sample and standard peaks, so several internal standards were used for the various *Cochlearia* species (Table 1).

Phytosociology

The nine-point scale for assessment of the abundance and dominance of species (Barkman, Doing & Segal, 1964) was used. Nomenclature of vascular plants and mosses follows the checklist of Marhold & Hindák (1998). Nomenclature of syntaxa has been corrected in accordance with the International Code (Weber, Moravec & Theurillat, 2000).

RESULTS AND DISCUSSION

Chromosome numbers

The octoploid chromosome number ($2n = 8x = 48$) was determined for both Romanian populations (Figs 1, 2). Our result from the locus classicus of *C. pyrenaica* var. *borzaeana* Coman et Nyár. (Maramureş Mountains, Şalhoi) agrees with those of Lungeanu (1972: 682) and Ştefureac & Lungeanu (1976: 118) based on karyological study of the same population, while a different chromosome count of $2n = 42$ was reported from the Obcina Meştecănisului Mountains (Ştefureac & Lungeanu, 1976: 118). Our unique count $2n = 48$ does not confirm the existence of two cytotypes in Romania.

For the first time, the diploid chromosome number $2n = 2x = 12$ is reported for the only known population of *Cochelaria pyrenaica s.l.* in Ukraine (Fig. 3). This chromosome number is typical for *C. pyrenaica* DC. (cf. Vogt, 1985; Koch *et al*., 1996, 1998; Valachovič & Kochjarová, 2000). The diploid level for the Ukrainian population was assumed (Vogt, 1985: 24; Valachovič & Kochjarová, 2000: 482) on the basis of pollen size. However, there is considerable difference in genome size and chromosome size between *C. pyrenaica s.s.* and this isolated population (see under 'DNA content' below), and taxonomic evaluation of this population requires further study.

DNA content

Absolute DNA content of the *C. borzaeana* studied was 2.8 pg and there was no difference between samples from the Benia and Şalhoi localities. Relative DNA content measurements using AT-selective DAPI staining confirmed the absolute DNA measurements by intercalar PI staining (Table 1). The number of measurements and their precision (average CV of samples) are also given in Table 1; the CVs of internal standards for DAPI staining were similar to those of our samples (average CV of *Cirsium vulgare* was 1.57% and *Lycopersicon esculentum* 2.11%), but they were not as accurate as the results obtained with the CyFlow cytometer (which we calibrated using standard green beads prior to each measurement). Larger CVs were found with the results from PI staining than from DAPI staining (Table 1); the CVs of internal

Table 1. Nuclear DNA content of *Cochlearia borzaeana* and *C. tatrae* in relation to *C. pyrenaica* (C-value = 0.40 pg *)

Species, sample locality	Absolute DNA content (2C) (pg, mean ± SD)	Measurements (no./average CV of sample)	Chromosomes		Absolute DNA content ratios among samples					Relative DNA content (2C) (a.u., mean ± SD)	Measurements (no./average CV of sample)	Relative DNA content ratios among samples				
			2*n*	Average size (pg)	*C bo.* R : B	*C bo.* R : S	*C py.* S : B	*C py.* S : J	*C py.* U : Z			*C bo.* R : B	*C bo.* R : S	*C py.* S : B	*C py.* S : J	*C py.* U : Z
C. tatrae, Slovakia: Velická dolina	2.089 ± 0.087†	3/3.50	42¶	0.0497	0.739	0.741	2.665	2.846	2.296	1.005 ± 0.012†	8/1.98	0.719	0.720	2.659	2.776	2.354
C. borzaeana, Romania: Benia	2.827 ± 0.017‡	5/3.46	48¶	0.0587	1	1.003	3.606	3.851	3.107	1.398 ± 0.005†‡	5/1.65	1	1.001	3.698	3.862	3.274
C. borzaeana, Romania: Şalhoi	2.819 ± 0.101‡	5/3.78	48¶	0.0589	–	1	3.596	3.841	3.098	1.396 ± 0.008†‡	7/1.47	–	1	3.693	3.856	3.269
C. pyrenaica, Slovakia: Bukovinka	0.784 ± 0.008§	25/2.92	12	0.0653	–	–	1	1.068	0.862	0.378 ± 0.003†‡	22/1.84	–	–	1	1.044	0.885
C. pyrenaica, Slovakia: Jazierce	0.734 ± 0.013§**	15/3.04	11? **	0.0667	–	–	–	1	0.807	0.362 ± 0.003†‡	12/1.99	–	–	–	1	0.848
C. pyrenaica *s.l.*, Ukraine: Zolochiv	0.910 ± 0.039‡	7/2.98	12¶	0.0758	–	–	–	–	1	0.427 ± 0.003†‡	13/1.81	–	–	–	–	1

*1 a.u. (arbitrary unit) = fluorescence intensity of 2C DNA content of internal standard *Raphanus sativus* 'saxa' stained by DAPI.
†Internal standard *Cirsium vulgare*; 2C = 5.54 pg (Bureš *et al.*, 2004).
‡Internal standard *Lycopersicum esculentum* 'Stupické polní tyčkové rané'; 2C = 1.96 pg (Doležel, Sgorbati & Lucretti, 1992).
§Internal standard *Raphanus sativus* 'saxa'; 2C = 1.11 pg (Doležel *et al.*, 1992).
¶Samples in which chromosomes were counted by classical karyological methods.
**Probably aneuploid sample, not counted by classical method, but difference between samples equals approximately one chromosome.

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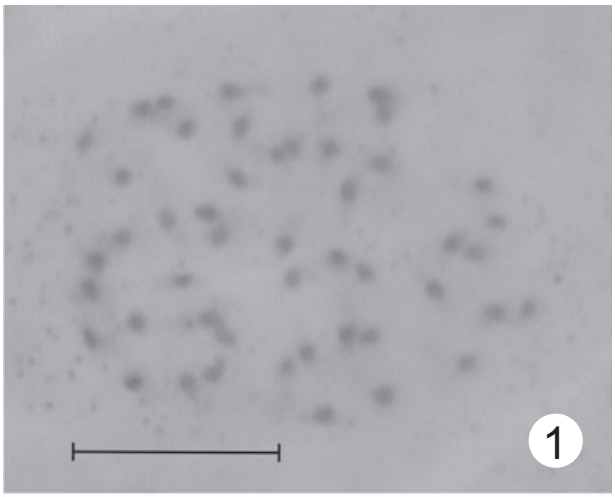

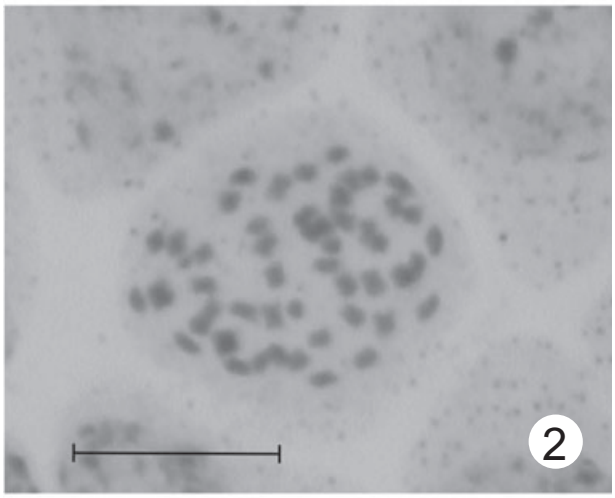

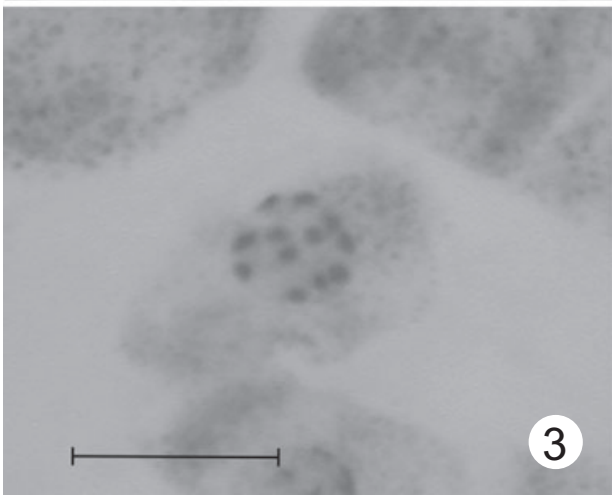

Figures 1–3. Mitotic chromosomes of *Cochlearia* species. Fig. 1. *Cochlearia borzeana*, $2n = 48$ (loc. Romania, Şalhoi). Fig. 2. *Cochlearia borzeana*, $2n = 48$ (loc. Romania, Benia). Fig. 3. *Cochlearia pyrenaica s.l.*, $2n = 12$ (loc. Ukraine, Zolochiv). Scale bar = 10 μm.

standards for PI staining were similar to those of our samples (average CV of *Cirsium vulgare* was 2.90%, *Lycopersicon esculentum* 3.21%, *Raphanus sativus* 3.47%).

Endopolyploidy was found for the leaf blades of *C. pyrenaica*, 2C + 4C + 8C + 16C, and for three other taxa, 2C + 4C + 8C. Such endopolyploidy is a frequent phenomenon, particularly in the family Brassicaceae (Barow, 2003; Barow & Meister, 2003). Polyploid species *C. tatrae* and *C. borzaeana* have 1.1–1.6 × smaller chromosomes than diploid taxa *C. pyrenaica* and *C. pyrenaica s.l.* (Ukraine) (Table 1); this polyploid–diploid difference, resulting from a loss of DNA following polyploidization, is probably a common phenomenon (Bennett, Bhandol & Leitch, 2000; Soltis *et al.*, 2003). However, there is also a more than 1.18-fold difference (Table 1) in the absolute DNA content or chromosome size between diploid species *C. pyrenaica* and *C. pyrenaica s.l.* (Ukraine). This relatively large 18% interspecific genome size difference is difficult to estimate based on measurement of pollen grains. Assuming a spherical shape of pollen grains and a correlation between nuclear and cell volume, this genome size difference equates to only a 5% difference in pollen grain diameter, perhaps therefore explaining why Vogt (1985) included a population of *C. pyrenaica s.l.* (Ukraine) in *C. pyrenaica* DC. on the basis of pollen size measurement. Taxonomic evaluation of *C. pyrenaica s.l.* (Ukraine) will require further comparative investigations. The difference between samples of *C. pyrenaica s.s.* from Slovakia as identified by both PI and DAPI staining is probably caused by aneuploidy; unfortunately, in these particular samples of *C. pyrenaica s.s.*, chromosomes were not counted. The absolute 2C DNA content of 0.784 pg of the *C. pyrenaica s.s.* sample from Bukovinka is in a good agreement with the genome size C = 0.4 pg reported for this taxon by Krisai & Greilhuber (1997).

Morphology

The three Carpathian *Cochlearia* species are distinguishable based on several morphological characteristics (Table 2). The most important for distinguishing between the species are: height of the main fruiting stem, thickness and surface area of the main flowering/fruiting stem, length of the petiole of the basal leaf, length and width of the basal leaf blade, length of the petal, length of the fruiting racemes, size of the silicules and pollen grains.

Distribution

Cochlearia borzaeana is found only rarely in the mountain belt of the Eastern Carpathians, namely in the Maramureş Mountains and Obcina Mestecanişu-

Table 2. Comparison of the morphological characteristics of *Cochlearia* species occurring in the western and eastern Carpathians (Slovakia and Romania)

	C. pyrenaica (2*n* = 12)	*C. tatrae* (2*n* = 42)	*C. borzaeana* (2*n* = 48)
Fruiting stem height (cm)	(20–)30–55(70)	(5–)7–15(–20)	(30–)53–85(–90)
Thickness of main stem (diameter) (mm)	(2–)2.2–3.4(–4)	(1.5–)1.8–2.8(–3)	(4–)5.5–8(–8.8)
Surface of main stem	Grooved	Nearly smooth	Expressive grooved
Length of petiole of basal leaf (cm)	(5–)7–16(–21)	(2–)3–6(–10)	(6–)10–29(–38)
Length of basal leaf blade (mm)	(15–)22.5–34(–45)	(6–)8.5–15.5(–24)	(32–)38–66.5(–78)
Width of basal leaf blade (mm)	(25–)28–44(–65)	(10–)12–17(–22)	(35–)41.6–68.9(–84)
Length of sepal (mm)	2–3	2–4	(2.2–)2.7–3.6(–5)
Width of sepal (mm)	1.5–2	2–3	(1.5–)2–2.5(–3)
Colour of petal	White	Pale yellowish	White
Length of petal (mm)	5–6	5–7	(4–)5.9–7.6(–8)
Width of petal (mm)	2–3	2–4	(2–)2.3–3.3(–4)
Length of pollen grain (μm)	(23–)25–27(–30)	(27–)28–31(–34)	(24–)27–30(–34)
Length of fruiting racemes (cm)	(12–)15–25(–30)	(2.5–)3–6(–10)	(12–)18–31(–35)
Length of silicule (mm)	(4–)4.5–6(–6.5)	(4–)4.5–6.3(–7)	(5–)5.6–7.3(–8)
Width of silicule (mm)	(2–)2.5–3.5(–4)	(3–)3.3–4.4(–6)	(3–)3.3–4.7(–5)
Length of seed (mm)	(1.5–)1.6–1.9(–2.0)	(1.7–)1.8–2.1(–2.2)	(1.7–)1.8–2.7(–3)
Width of seed (mm)	(1.2–)1.3–1.5(–1.7)	(1.3–)1.4–1.6(–1.7)	(1–)1.2–1.7(–2)

lui Mountains (both in northern Romania). Older literature records (Rochel, 1838; Heuffel, 1858) for the southern Carpathians (Banat region) have not been recently confirmed. They are not included in the list of localities given below, because we did not find any relevant herbarium specimen in public herbaria (see Material and methods) and thus the taxonomic identity of the published taxon (originally determined as *C. officinalis*) is unclear.

Index of localities (revised herbarium specimens and published data) in the Eastern Carpathians and northern Romania, respectively (all records are given with the original orthography): Siebenb. (Wolff 819, SAV) (Siebenbürgen = Transsylvania, 1819?]. – Transsylvania, von der Hochmooren der Alpen (Lerchenfeld, 1780s. Schur, 1866: 67; ut *C. pyrenaica s.s.*; Schur Verh. Naturforsch. Ver. Brünn 15 (1876): 94, 1877 ut *C. macrorrhiza*). – Marmaros, auf der Alpe Trojaga an quelligen Stellen (Müller Verh. Zool.-Bot. Ges. Wien 13, 1863: 559 ut *C. officinalis*). – '… sonst nur noch von der Trojaga in der Máramaros angegeben wird, ich selbst fand die Pflanze daselbst nicht…' (Pax Karpathen 1: 187, 1898 ut *C. officinalis*); '… das Vorkommen der *C. officinalis* an der Trojaga, wo ich selbst die Pflanze vergeblich suchte …' (Pax Karpathen 2: 220, 1908). – Maramureş, in aquis rivi montani inter montes Prislop et Măguricea vs. Şalhoi, prope pagum Borşa alt. cca 1285 m s. m. (Coman 1942 et 1945 CL, KRA, KRAM, W – Fl. Rom. Exs. 2760; Coman, 1946 CL no. 585208 lectotypus; Coman, 1946: 78). – Măgurice spre Şalhoi, 'n ape lin curgătoare, pe calcar, exp. S, alt. 1285 m (Coman 1945 BP). – Borsa (Coman 1945 BP). – Reg. Baia Mare, în ape lin curgătoare, pe calcar, în rîul dintre dealurile Prislop şi Măgurice spre Şalhoi, la alt. de 1285 m, aproape de Borşa (Nyárády, 1955: 380). – Moldavia, distr. Suceava, prope pagum Breaza, in monte Glodu ad 'Rachitişul Mare', alt. *c.* 1200 m (Topa, Tăbacăru, Ostaciuc et Coman 1969 CL – Fl. Mold. Dobrog. Exs. 426). – Obcina Mestecănişului, Benia, potok Tatarca v rezervácii Rachitişul Mare (Valachovič & Kochjarová, 2000 BBZ, SAV). – On the hill of Răchitişul Mare (Benia-Moldova-Sulita-Cîmpulung) in Bucovina (Ştefureac, 1972: 189). – Deux stations au nord des Carpates orientales, monts Maramures-Salhoi et Obcinile Mestecanis, Rachitisul Mare (Coldea, 1997: 147).

Taxonomic status of *Cochlearia borzeana*

One population of *Cochlearia borzeana* (Obcina Mestecănişului, Benia) was included in the molecular study of selected taxa from central Europe (Koch *et al.*, 2003). According to AFLP data, *C. borzeana* exhibits the highest number of unique and rare alleles of all analysed species and, based on cluster and PCA analyses, it is closely related to the hexaploid *C. tatrae*. Both these polyploid taxa form one subcluster (see figures 2 and 3 in Koch *et al.*, 2003). However, the genetic distance was strongly correlated with geographical distance. Thus, clustering may be influenced more by the geographical localization of the analysed population than by taxonomic relationships among particular taxa. As a result, one subcluster also contains two

populations of high alpine species of *C. excelsa* and two populations of *C. pyrenaica*, although other *C. pyrenaica* populations did not fall into this group (Koch *et al*., 2003). *C. borzeana* and *C. tatrae* are similar in respect of their high ploidy level; however, the basic chromosome number is different, $x = 6$ in *C. borzeana* and $x = 7$ in *C. tatrae*. Moreover, some qualitative differences were observed in morphological characters and ecological requirements (see Table 1 and text below). By contrast, only quantitative morphological differences were found between *C. pyrenaica s.s*. and *C. borzeana*. Both taxa have the same basic chromosome number and prefer similar habitats. It may be suggested that the octoploid *C. borzeana* evolved from diploid *C. pyrenaica s.s*. (see also Koch *et al*., 2003). It remains unclear if *C. borzeana* is autopolyploid (as with the octoploid *C. anglica* from *C. officinalis*, cf. Koch *et al*., 1998) or allopolyploid. A high number of rare and unique alleles may indicate either an allopolyploid origin with genomic contributions of *C. pyrenaica* and other (extinct?) putative parents or a very long period of geographical isolation of Romanian populations at the margin of the fragmented range of *C. pyrenaica s.s*.

Phytosociology and ecology

The first set of phytosociological relevés including *Cochlearia* from the eastern Carpathians (Rachitişul Mare Mts) was published by Ştefureac (1972). He designated stands in the swamps and brooks as the association *Carici flavae–Cratoneuretum* Kovács et Felföldy 1958 and provisionally proposed subassociation *cochlearietosum pyrenaicae* Ştefureac 1972 nom. prov. Coldea (1997: 142–144) considered ten relevés and constructed the synoptic table to which one relevé from the Şalhoi (Maramureş) Mountains was added. At the same time, he shifted the community into the association *Cochleario pyrenaicae–Cratoneuretum commutati* (alliance *Cratoneurion commutati*). From the published table as well as our own field observations at both localities, it seems that the swamp and brook stands represent a transition between true springs of the alliance *Cratoneurion commutati* and rich fens of the alliance *Caricion davallianae*. Ecological conditions are typical of those of the following relevé taken by M. Valachovič and J. Kochjarová in the Obcina Meştecănisului Mountains, Rachitişul Mare: Hill near the village of Benia, right bank of the Tatarca stream, elev. 919 m, alluvial deposits (serpentine prevailing with admixture of grey limestone), area 3 m^2, total cover 75%, 21 July 2000: *Cochlearia borzaeana* 3, *Deschampsia caespitosa* 2b, *Agrostis stolonifera* 2a, *Cardamine pratensis* agg. +, *Cerastium vulgatum* +, *Caltha palustris* ssp. *laeta* r, *Epilobium palustre* r, *Parnassia palustris* r, *Rumex obtusifolius* r, *Silene pusilla* r, *Cratoneurone filicinum* 2b, *Palustriella commutata* 1, *Bryum pseudotriquetrum* +.

Cochlearia pyrenaica prefers a slightly different habitat; hard water springs that contain numerous taxa typical of spring vegetation. A comparison with the coenological behaviour of the high mountain species C. *tatrae* is inappropriate because the latter taxon is representative of the plant communities on the alpine siliceous screes of the alliance *Androsacion alpinae* (Valachovič & Kochjarová, 2000).

According to the International Code of Phytosociological Nomenclature (Weber *et al*., 2000), the names of syntaxa based on newly defined taxa must be corrected (see below).

CONCLUSION

Because of different morphology and octoploid chromosome number (as opposed to the diploid number of *C. pyrenaica*), we propose classifying the East Carpathian (Romanian) populations of the genus *Cochlearia* as a separate species, namely *Cochlearia borzaeana* (Coman et Nyár.) Pobed.

***Cochlearia* L.**
Cochlearia L. Sp. Pl. 647, 1753.
sect. *Cochlearia*
***Cochlearia borzaeana* (Coman et Nyár.) Pobed.**
(Fig. 4)
Cochlearia borzaeana (Coman et Nyár.) Pobed. Novosti Sist. Vyssh. Rast. 7 (1970): 177, 1971.
≡ C*ochlearia pyrenaica* DC. var. *borzaeana* Coman et Nyár. in Coman Bull. Grad. Bot. University Cluj. 26: 78, 1946 (bazionymum). Ind. loc. In aquis rivi montani inter montes Prislop et Măgurice vs. Salhoi, prope pagum Borşa alt. *c*. 1285 m s. m.

Lectotypus (hoc loco designatus): Maramureş, distr. Maramureş. In aquis rivi montani inter montes Prislop et Maguricea, vs. Şalhoi, prope pag. Borşa. Alt. cca 1285 m s. m. 17. 6. 1946 leg. A. Coman (CL 585208).

– *Cochlearia officinalis* auct. (p. p.) non L. 1753: Müller Verh. Zool.-Bot. Ges. Wien. 13: 559, 1863; Neilreich Aufzählung: 262, 1866; Pax Karpathen 1: 187, 1898; Pax Karpathen 2: 220, 1908.

– *Cochlearia tatrae* auct. (p. p.) non Borbás 1895: Prodan Flora Rom. 475, 1923.

– *Cochlearia pyrenaica* auct. (p. p.) non DC. 1821: Schur Enum. Plant. Transsylv. 67, 1866; Ştefureac Stud. Comun. Ocrot. Nat. 2: 187, 1972; Chater & Heywood Fl. Eur. 1, 1. ed. 314, 1964; Wyse Jackson & Akeroyd Fl. Eur. 1, 2. ed. 379, 1993.

– *Cochlearia officinalis* var. *macrorrhiza* auct. (p. p.) non Schur Verh. Naturf. Vereins Brünn 15 (1876): 94, 1877: Schur Verh. Naturf. Vereins Brünn. 15 (1876): 94, 1877 (pro loc. Roman.).

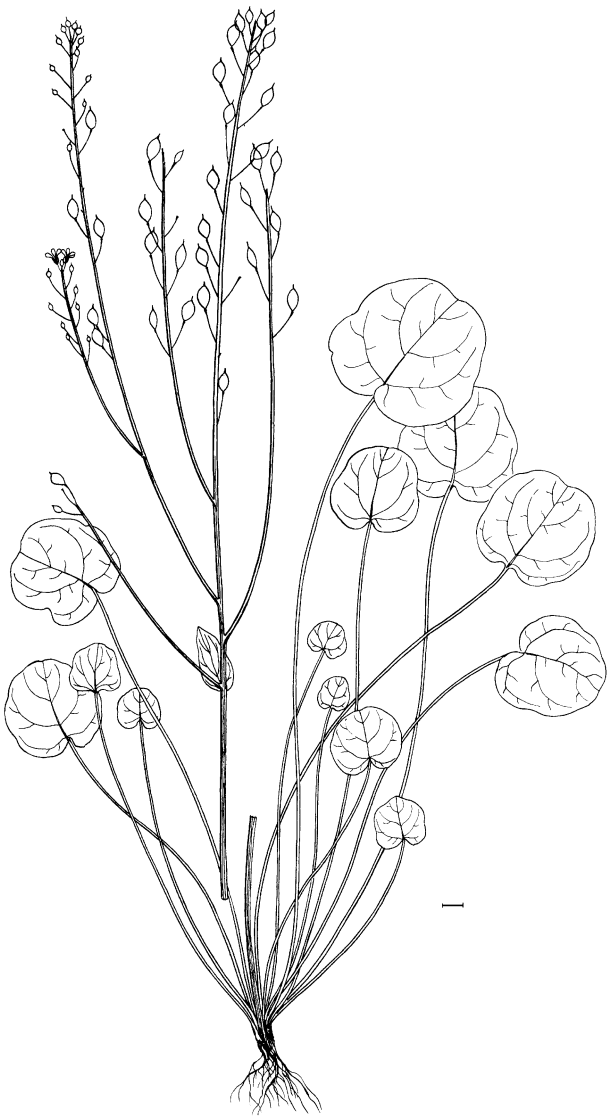

Figure 4. Habit of *Cochlearia borzaeana* (Coman et Nyár.) Pobed. (drawing by Z. Komárová). Scale bar = 1 cm.

Exssiccata visa: Fl. Romaniae Exs. no. 2760 – Fl. Moldaviae et Dobrogeae Exs. no. 426.

Icones: Savulescu (ed.) Flora Rep. Pop. Rom. 3: Pl. 43, Fig. 1, 1955. – Ştefureac et Pânzaru Ocrot. Nat. Mediului Inconj. Nat. Terra. 22: 39, 1978.

Icona nostra: Figure 4 (drawing by Z. Komárová).

The name of the plant community must be corrected accordingly. The name of the association, *Cochleario pyreanicae–Cratoneuretum commutati* (Oberd. 1957) Th. Müller 1961, proposed by Coldea (1997), is appropriate for the Alps and Western Carpathian Mountains only. For the Eastern Carpathians, with regard to the ecological features of stands, we propose to return to the initial classification of Şefureac (1972), namely to the association *Carici flavae–Cratoneuretum* Kovács et Felföldy 1958. The proposed subassociation *cochlearietosum pyrenaicae* Şefureac 1972 nom. prov. must be corrected according to the International Code of Phytosociological Nomenclature (Weber *et al*., 2000) for two reasons: provisional description (Art. 3b) and incorrect determination of the name-giving taxon (Art. 43). Therefore, for the new subassociation ***Carici flavae–Cratoneuretum* Kovács et Felföldy 1958 *cochlearietosum borzeanae* subass. nova hoc loco** the **nomenclatural type (holotypus)** is designated using recent relevé taken by G. Coldea: Locality Şalhoi, elev. 1288 m, area 16 m^2, total cover 98%, 13 August 2004: *Cochlearia borzaeana* 4, *Caltha palustris* ssp. *laeta* 2b, *Silene pusilla* 2b, *Deschampsia caespitosa* 1, *Cardamine rivularis* Schur 1, *Carex paniculata* 1, *Chaerophyllum hirsutum* 1, *Epilobium palustre* 1, *E. nutans* +, *Alchemilla glabra* +, *Crepis paludosa* +, *Leucanthemum rotundifolium* +, *Myosotis caespitosa* +, *Parnassia palustris* +, *Palustriella commutata* 3, *Bryum pseudotriquetrum* 1, *Cratoneuron filicinum* +.

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