

## *Iris cengialti* Ambr. (*Iridaceae*) in Slovenia - karyological analyses

*Iris cengialti* Ambr. (*Iridaceae*) v Sloveniji - kariološka analiza

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**Izvleček:** Članek obravnava kariološke značilnosti alpske vrste *Iris cengialti* Ambr. iz serije *Pallidae* (A. Kern.) Trinajstić. Vzorci so bili iz Slovenije (Komarča, Kobarid), komparativni material pa iz Italije (Cengio Alto - *I. cengialti* s. s.). Rezultati so pokazali interpopulacijsku variabilnost znotraj vrste in upravičenost navajanja slovenskih populacij kot podvrste *I. cengialti* Ambr. subsp. *vochinensis* (Paulin) Trinajstić.

**Abstract:** The article discusses the karyological features of the Alpine species *I. cengialti* Ambr. from the series *Pallidae* (A. Kern.) Trinajstić. The samples were from Slovenia (Komarča, Kobarid) and comparative material was from Italy (Cengio Alto - *I. cengialti* s. s.). The results showed interpopulation variability within species and the justifiability of treating the Slovenian populations as subspecies *I. cengialti* Ambr. subsp. *vochinensis* (Paulin) Trinajstić.

### 1. Introduction

A group of connected taxa of the Genus *Iris* L., from the Alpine-Dinaric region of Europe characterised by dryskinned spathes was described by TRINAJSTIĆ (1976) as a separate series *Pallidae* (A. Kern.) Trinajstić. Following this opinion, the series comprehends the species *Iris cengialti* Ambr. (AMBROSI 1854), *Iris illyrica* Tamm. (TOMMASINI 1875), *Iris pseudopallida* Trinajstić (TRINAJSTIĆ 1976) and the horticultural *Iris pallida* Lam. (LAMARCK 1789).

The *Iris* with dryskinned spathe and violet flowers in the Alpine region of Italy was named after its "locus classicus" (Cengio Alto) *Iris cengialti* Ambr. (AMBROSI 1854). Botanists considered it a species (AMBROSI 1854, KERNER 1871, PAMPANINI 1909, DYKES 1913, LAWRENCE 1953, TRINAJSTIĆ 1976, KÖHLEIN 1981, PIGNATTI, 1982; SCHULZE, 1988, SCORTEGAGNA et al. 1996, MITIĆ 1998) or a subspecies from the species *I. pallida*

(WEBB & CHATER 1980, MATHEW 1981, WRABER 1998, 1999).

In the Slovenian Alps, plants that are different but nevertheless related to the Italian irises, have been described as *I. cengialti* f. *vochinensis* Paulin (PAULIN 1917). MAYER (1952) described these plants as a variety (*I. cengialti* Ambr. var. *vochinensis* (Paulin) Mayer), TRINAJSTIĆ (1976) as a subspecies (*I. cengialti* Ambr. subsp. *vochinensis* (Paulin) Trinajstić, SUŠNIK (1984) as *I. illyrica*, TRPIN & VREŠ (1995) take over the Paulin name, and WRABER (1998, 1999) considered them *I. pallida* Lam. subsp. *cengialti* (Ambr.) Forster, as did WEBB & CHATER (1980).

With regard to the controversy about the taxonomic status of the series *Pallidae* and subcategories (WEBB & CHATER 1980, MATHEW 1981, WRABER 1998, 1999), taxonomic investigations of its Alpine-Dinaric populations were carried out (MITIĆ 1998, MITIĆ et al. 1999). The presence of four separate groups was established, and to those groups a separate taxonomic status

can be ascribed, which tends to confirm the opinion of TRINAJSTIĆ (1976). One of those groups corresponds to the description of the *I. cengiali* species (Alpine populations from Italy and Slovenia).

Because of the taxonomical importance of cytological data for the genus *Iris* and the *Pallidae* series (SIMONET 1934, MITRA 1956, MITRA & RANDOLPH 1959, RANDOLPH & MITRA 1959, LAUSI 1964, RICCI 1970/71, TRINAJSTIĆ & LOVAŠEN-EBERHARDT 1977, SAUER & LEEP 1979, TRINAJSTIĆ et al. 1980, MITIĆ 1991), in this paper we present results of karyological analyses of the Alpine populations of *I. cengiali*, with the intention of further explanation of their relationships and completing the re-

classicus" for *I. cengiali*) were analysed (tab. 1).

For each population, four plants were analysed. A study of somatic chromosome morphology was made using root-tip squash technique and aceto-carmine stain, after pre-treatment with p-dichlorobenzene. The most satisfactory procedure was found to be a 3-hour pre-treatment in the p-dichlorobenzene at 4°C. Slides were made permanent by first removing the cover glass, with the freezing technique (CO<sub>2</sub>) and the mounting of the material in Canada balsam.

Morphometrical characteristics of chromosomes have been worked out based on the analyses of ten metaphase plates (according to RAFFINSKI & PASSAKAS 1976, SAUER & LEEP 1979). For each

Tab. 1: The populations sampled: name of locality; co-ordinates: Latitude (N) and Longitude (E).

Locality	Co-ordinates			
	N			E
Cengio Alto	45°	45'	30"	11° 40' 0"
Komarča	46°	17'	47"	13° 47' 40"
Kobarid	46°	16'	30"	13° 33' 5"

sults of morphological analyses (MITIĆ et al. 1999).

There are few data about *I. cengiali* chromosome morphology and number. We have only a few references about *I. cengiali* chromosome studies (SIMONET 1934, MITRA 1956, SUŠNIK 1962, SCORTEGAGNA et al. 1996). For Slovenia, only SUŠNIK (1962) has given any information about the number of chromosomes.

## 2. Material and methods

Two populations from Slovenia (Komarča and Starijski vrh, near Kobarid) and one from Italy (Cengio Alto - the "locus

population and for each chromosome pair the following parameters were estimated: the arithmetical mean of relative chromosome length (RL), arithmetical mean of relative length of longer chromosome arm (LA), arithmetical mean of relative length of shorter arm (SA), arm ratio (AR) and position of centromere (PC: M-metacentric, SM-submetacentric, ST-subtelocentric chromosome).

Photomicrographs were taken for the most successful metaphase plates with a microscope OPTON III, and camera lucida drawings of karyotypes were made with ZEISS apparatus. For idiograms the homologous chromosomes have been arranged in decreasing order of size.

### 3. Results

For all the populations analysed, the mitotic number of chromosomes  $2n=24$  and the interpopulation variability in chromosome morphology have been established. Two groups of karyotypes (fig. 1) and idiograms (fig. 2) have been established, but all karyotypes have relatively large chromosomes (tab. 2).

All karyotypes have one metacentric pair of chromosomes and the others are submetacentric or subtelocentric. Interpopulation variability in chromosome morphology is obvious in the number of chromosome pairs with satellites. That is, the Slovenian populations have shown uniformity and have two chromosome pairs with satellites, while the Italian population has three chromosome pairs with satellites.

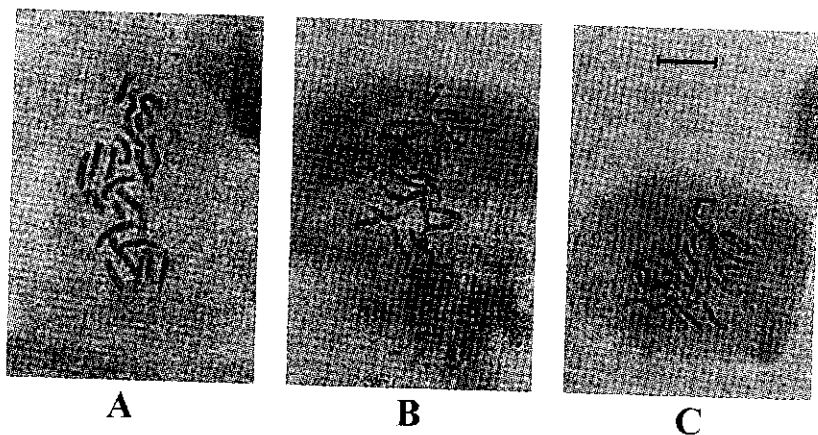


Fig. 1: Photomicrographs of somatic chromosomes of the species *I. cengialti* (010  $\mu$ m): A - Cengio Alto, B - Komarča, C - Kobarid.

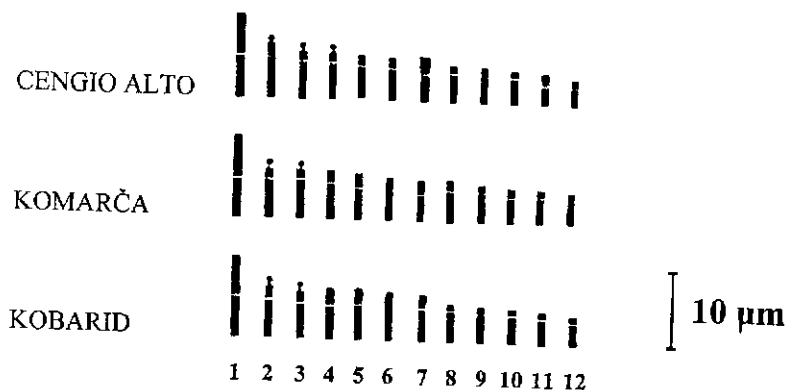


Fig. 2: Idiograms of somatic chromosomes of the species *I. cengialti* populations.

Tab. 2: Results of chromosome measurements of the *I. cengialti* populations: relative length of chromosomes (RL), relative length of longer chromosome arm (LA), relative length of shorter arm (SA), arm ratio (AR), position of centromere (PC: M-metacentric, SM-submetacentric, ST-subtelocentric chromosome), Chrom. no. - the ordinal number of chromosome pair.

Chrom. no.		1	2	3	4	5	6	7	8	9	10	11	12
Population													
C. Alto	RL	9,95	6,30	7,30	6,60	6,45	6,00	5,85	5,50	5,05	4,50	4,05	3,85
	LA	5,13	4,40	5,48	5,05	4,59	4,22	4,75	4,30	3,94	3,55	3,23	3,15
	SA	4,82	1,90	1,82	1,55	1,86	1,78	1,10	1,20	1,11	0,95	0,82	0,70
	AR	1,06	2,32	3,01	3,23	2,47	2,37	4,32	3,58	3,55	3,74	3,94	4,50
	PC	M	SM*	ST*	ST*	SM	SM	ST	ST	ST	ST	ST	ST
Komarča	RL	9,83	7,08	6,60	6,23	6,05	6,55	6,13	5,75	5,08	4,55	4,08	3,80
	LA	4,98	4,98	4,95	4,35	4,23	4,77	5,03	4,43	3,87	3,68	3,28	3,16
	SA	4,85	2,10	1,65	1,88	1,82	1,78	1,10	1,32	1,21	0,87	0,80	0,64
	AR	1,03	2,37	3,00	2,31	2,32	2,68	4,57	3,36	3,20	4,23	4,10	4,94
	PC	M	SM*	ST*	SM	SM	SM	ST	ST	ST	ST	ST	ST
Kobarid	RL	9,94	7,12	6,56	6,16	6,38	6,40	6,06	5,64	5,16	4,62	4,08	3,86
	LA	5,04	4,80	5,01	4,33	4,47	4,60	4,93	4,48	4,02	3,72	3,18	3,06
	SA	4,90	2,32	1,55	1,83	1,91	1,80	1,13	1,16	1,14	0,90	0,90	0,80
	AR	1,03	2,07	3,23	2,37	2,34	2,56	4,36	3,86	3,53	4,13	3,53	3,82
	PC	M	SM*	ST*	SM	SM	SM	ST	ST	ST	ST	ST	ST

\* - Chromosome pairs with satellite

Both Slovenian populations have the first chromosome pair metacentric; the second, fourth and sixth pairs submetacentric; the third, and seventh to twelfth pairs are subtelocentric. The second and third pairs have satellites.

In the Italian population the first chromosome pair is also metacentric. The second, fifth and sixth pairs are submetacentric; the third, fourth and seventh to twelfth pairs are subtelocentric. The second, third and fourth pairs have satellites.

#### 4. Discussion

For all three populations analysed, mitotic number of chromosomes  $2n=24$  has been established, which corresponds to pre-

vious studies of somatic chromosomes of the species *I. cengialti* (SIMONET 1934, MITRA 1956, SUŠNIK 1962, SCORTEGAGNA et al. 1996) and of the *Pallidae* series (SIMONET 1934, MITRA 1956, LÖVE & LÖVE 1974, MITIĆ 1991, MITIĆ 1998).

All karyotypes have been characterised as having relatively large, morphologically variable chromosomes, with one metacentric pair of chromosomes (the first pair), the others being submetacentric or subtelocentric. Also, morphometrical analysis of chromosomes has shown up variability at the population level, where two groups can be distinguished. That is, in one group there are the Slovenian populations, which have revealed uniformity and have two chromosome pairs with satellites, and one submeta-

centric pair more than Italian population. The other group is made up of the Italian population with three chromosome pairs with satellites.

Comparison of all the idiograms from this investigation with those shown in former papers (MITRA 1956, LAUSI 1964, MITIĆ 1991, SCORTEGAGNA et al. 1996), shows chromosome morphology similarities among some of the populations. For example, the idiogram of the Italian population Cengio Alto resemble that of the Italian population Passo di San Uboldo (SCORTEGAGNA et al. 1996). Idiograms of Slovenian populations resemble those of *I. illyrica* from the north Adriatic littoral (MITIĆ 1998). The Italian population of *I. cengialti* has an idiogram resembling those of *I. illyrica* from the northeast Adriatic littoral (MITIĆ 1998), with a very similar fourth satellite chromosome pair.

The existence of chromosome morphological variability has already been established for different species of the genus *Iris* (MITRA 1956, SAUER & LEEP 1979), the reason for which might be affinity of large chromosomes to structural mutations SAUER & LEEP 1979).

For the investigated populations of the *I. cengialti* species, five or six pairs of symmetrical (metacentric and submetacentric) chromosomes have been established. Because of the consideration that the number of (a)symmetric chromosomes can reflect more or less of a range of speciation (SIMONET 1934, MITRA 1956), and with regard to the more symmetric chromosomes in *I. cengialti* populations and the whole *Pallidae* series (MITIĆ 1998), we can presume that the speciation process within the series is not complete.

The results of our analyses complement the results of morphological analyses of the Alpine-Dinaric populations of the *Pallidae* series (MITIĆ et al. 1999) and tend to bear out the opinion about the taxonomical range

of the Slovenian dryskinned *Iris*es as constituting a separate subspecies named *I. cengialti* Ambr. subsp. *vochinensis* (Paulin) Trinajstić.

## 5. Summary

Within taxonomical investigations of the Alpine-Dinaric populations of the series *Pallidae* (A. Kern.) Trinajstić, Genus *Iris* L. (MITIĆ 1998, MITIĆ et al. 1999), the presence of four separate groups has been established. A separate taxonomic status can be ascribed to these groups, which tends to confirm the opinion (TRINAJSTIĆ 1976) that there are four species in this complex: *I. pallida* Lam. (LAMARCK 1789), *I. cengialti* Ambr. (AMBROSI 1854), *I. illyrica* Tomm. (TOMMASINI 1875) and *I. pseudopallida* Trinajstić (TRINAJSTIĆ 1976).

In this paper we present the results of karyological investigations of the Alpine species *I. cengialti*, with the accent on the Slovenian populations named *I. cengialti* Ambr. subsp. *vochinensis* (Paulin) Trinajstić.

Two populations from Slovenia – Komarča and Kobarid (Starijski Vrh) were analysed. The results were compared with those of investigations into typical *I. cengialti* from the Italian population from Cengio Alto (the "locus classicus" for *I. cengialti*).

A study of somatic chromosome morphology was made using root-tip squash technique and aceto-carmine stain, after pretreatment with p-dichlorobenzene. For each population and for each chromosome pair, chromosome length (with length of longer and shorter arm), arm index and type of chromosomes were estimated. The results are shown in a table (tab. 2), in photomicrographs of karyograms (fig. 1) and by idiograms (fig. 2).

For all the populations analysed, the mitotic number of chromosomes  $2n=24$

with relatively large chromosomes has been established. All karyotypes have one metacentric pair of chromosomes, while the others are submetacentric or subtelo-centric. Interpopulation variability in chromosome morphology and a different number of chromosome pairs with satellites have been established. The Slovenian populations revealed uniformity and have two chromosome pairs with satellites and one submetacentric pair more than the Italian population. The Italian population has an idiogram with three chromosome pairs with satellites. Those results tend to confirm the opinion that the taxonomical range of Slovenian dryskinned irises constitutes a separate subspecies, named *I. cengialti* Ambr. subsp. *vochinensis* (Paulin) Trinajstić.

Karyogram shape similarity in the populations analysed and in other populations of the *Pallidae* series point at their relationship and indicate that the procession of speciation within the series is still incomplete.

## 6. Sažetak

U okviru taksonomskih istraživanja alpsko-dinarskih populacija serije *Pallidae* (A. Kern.) Trinajstić, roda *Iris* L. (MITIĆ 1998, MITIĆ et al. 1999) utvrđena je prisutnost četiriju odvojenih skupina, kojima se može pripisati rang vrste, što potvrđuje mišljenje (TRINAJSTIĆ 1976) o postojanju četiriju vrsta tog kompleksa: *I. pallida* Lam. (LAMARCK 1789), *I. cengialti* Ambr. (AMBROSI 1854), *I. illyrica* Tomm. (TOMMASINI 1875) i *I. pseudopallida* Trinajstić (TRINAJSTIĆ 1976).

Ovim radom prezentiramo komparativna kariološka istraživanja alpske vrste *I. cengialti*. Istražene su dvije populacije iz Slovenije – Komarča i Kobarid (Starijski vrh), opisane kao *I. cengialti* subsp. *vochi-*

*nensis* (Paulin) Trinajstić. Rezultati su komparirani s rezultatima istraživanja tipične vrste *I. cengialti* iz talijanske populacije Cengio Alto ("locus classicus" za *I. cengialti*).

Analizirani su mitotski kromosomi iz meristema korjenovih vršaka, dobiveni standardnom "squash" metodom, bojenjem acetokarminom, nakon pretretmana p-diklorbenzenom. Za svaku populaciju i kromosomski par izračunati su relativna duljina kromosoma (s duljinom duljeg i kraćeg kraka), indeks krakova i tip kromosoma. Rezultati su prikazani tabelarno (tab. 2), te fotografijama kariograma (fig. 1) i idiogramima (fig. 2).

Za sve tri istražene populacije vrste *I. cengialti* utvrđen je mitotski broj kromosoma  $2n=24$ , a sve kariotipove karakteriziraju relativno veliki kromosomi varijabilne morfologije. Svi kariotipovi imaju po jedan metacentričan par kromosoma (prvi par), a ostali kromosomi su ili submetacentrični ili subtelo-centrici. Također je utvrđena interpopulacijska varijabilnost morfologije kromosoma i broja satelitskih kromosoma. Slovenske populacije pokazuju uniformnost idiograma u kojima su utvrđena dva para satelitskih kromosoma i jedan submetacentrični par više, dok su u talijanskoj populaciji utvrđena tri para satelitskih kromosoma. Ovakvi rezultati, uz rezultate morfološke analize (MITIĆ et al. 1999), doprinose mišljenju da se slovenskim populacijama perunika suhokožičastih spata može pripisati taksonomski status podvrste - *I. cengialti* Ambr. subsp. *vochinensis* (Paulin) Trinajstić.

Sličnost u izgledu kariograma istraženih populacija s kariogramima ostalih populacija serije *Pallidae* ukazuje na njihovu povezanost i nezavršen proces specijacije unutar serije.

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