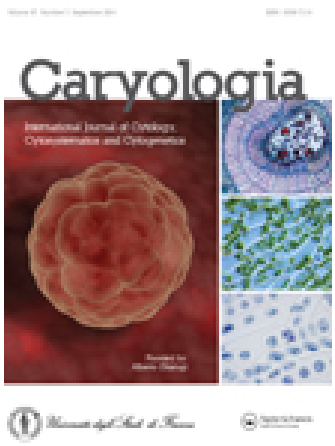

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A cytological study of four Sicilian Serapias (Orchidaceae)

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A cytological study of four Sicilian *Serapias* (Orchidaceae)

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Cytological analysis of four *Serapias* L. (Orchidaceae), of which two are Sicilian endemics, is carried out. In particular, the endemic *S. intermedia* subsp. *hyblaea* shows a triploid chromosome complement with $2n = 3x = 54$, representing natural nothotaxa arising from a hybridization process between supposed parental *S. vomeracea* ($2n = 2x = 36$) and *Serapias lingua* ($2n = 4x = 72$). For each of them the C-heterochromatin distribution, using Giemsa C-banding and karyotypes, was examined. The other endemic taxon (*S. orientalis* subsp. *siciliensis*) is characterized by a diploid chromosome number $2n = 2x = 36$, of which the C-heterochromatin distribution is examined as well. The taxonomical relationships among these taxa are discussed in relation to the literature data.

Keywords: chromosome number; endemic taxa; heterochromatin distribution; *Serapias*; Sicily

1. Introduction

Serapias L. is a genus of Orchidaceae distributed prevalently in the Mediterranean basin, Canaries and Azores. Currently, it is represented by *c*. 30 taxa, many of which are widespread, while few taxa are very localized. Basing on karyological investigations (Heusser 1938; Scrugli et al. 1976; Del Prete 1977; Scrugli 1978, 1980, 1982; Del Prete et al. 1980; Mazzola et al. 1981, 1982; Queiros 1983; Cauwet-Marc and Balayer 1986; Bianco et al. 1987, 1991, 1992; D'Emerico et al. 1990, 1992, 2000; Constantinidis and Kamari 1995; Bernardos et al. 2004; Bellusci and Aquaro 2008), most *Serapias* species show a diploid chromosome number ($2n = 2x = 36$), while a few species are tetraploid ($2n = 4x = 72$).

The taxa belonging to the genus *Serapias* occurring in the Sicilian territory are as follows: *S. nurrica* Corrias, *S. parviflora* Parl., *S. bergonii* E.G. Camus, *S. vomeracea* (N.L. Burman) Briquet, *S. orientalis* (Greuter) H. Baumann and Künkele subsp. *siciliensis* Bartolo and Pulvirenti, *S. cordigera* L., *S. cossyrensis* B. and H. Baumann, *S. lingua* L., *S. francavillae* Galesi and R. Lorenz and *S. intermedia* Forest. ex F.W. Schultz subsp. *hyblaea* Cristaudo, Galesi and R. Lorenz.

Cytological analysis in *Serapias*, considering the centromeric position and chromosome C-banding, gives important information on phyletic relationships. Giemsa C-bands are usually located at centromeric positions in numerous chromosome pairs (D'Emerico et al. 2000). Heterochromatic blocks, shown by C-banding, are known in several plants and the heterochromatin content was used to emphasize the relationships and the mechanism of genome restructuring within groups of species, genera and families (Guerra 2000).

The aim of this work is the analysis of the chromosome numbers and the pattern of heterochromatin distribution in *Serapias orientalis* subsp. *siciliensis* and *Serapias intermedia* subsp. *hyblaea*, Sicilian endemics not investigated to date from the karyological point of view. The results of this study are compared with the cytogenetic information available for other members of this genus.

2. Materials and methods

The specimens of the examined materials are stored at CAT herbarium of the University of Catania (CAT); see Table 1. The karyological investigation was performed using immature ovaries, pre-treated with 0.3% colchicine at room temperature for 2 h. For Feulgen staining ovaries were fixed for 5 min in 5:1:1:1 mixture of absolute ethanol, chloroform, glacial acetic acid and formalin. Hydrolysis was at 20°C in 5.5 N HCl for 20 min (Battaglia 1957a, 1957b), and stained in freshly prepared Feulgen stain (Feulgen and Rossenbach 1924). For C-banding, immature ovaries were fixed in ethanol-glacial acetic acid (3:1 v/v) and stored in the deep-freezer for up to several months. Subsequently they were squashed in 45% acetic acid; coverslips were removed by the dry ice method and the preparations air-dried overnight. Slides were then immersed in 0.2 N HCl at 60°C for 3 min, thoroughly rinsed in distilled water and then treated with 4% Ba(OH)2 at 20°C for 4 min. After thorough rinsing they were incubated in 2 × SSC at 60°C for 1 h. The stain used was 3–4% Giemsa (BDH) at pH 7 (D'Emerico et al. 1999). The chromosome numbers of examined taxa are reported in Table 2. Chromosome measurements were

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Table 1. Origins of the studied material of *Serapias* taxa.

Taxon name	Locality	Collector	Date
S. vomeracea	Monte Lauro (SR)	Bartolo, Brullo C. and S., Sciandrello (CAT)	17 May 2011
S. orientalis subsp. *siciliensis*	Monte Zinglino, Gela (CL)	Bartolo, Brullo S., Sciandrello (CAT)	4 April 2006
S. intermedia subsp. *hyblaea*	Monte Lauro (SR)	Bartolo, Brullo C. and S., Sciandrello (CAT)	17 May 2011
S. lingua	Monte Lauro (SR)	Bartolo, Brullo C. and S., Sciandrello (CAT)	17 May 2011

Table 2. Cytological data of four investigated *Serapias* taxa.

	Chromosome number	% Heterochromatin	% Euchromatin
S. vomeracea	$2n = 2x = 36$	40.82	59.18
S. orientalis subsp. *siciliensis*	$2n = 2x = 36$	30.46	69.54
S. intermedia subsp. *hyblaea*	$2n = 3x = 54$	35.11	64.88
S. lingua	$2n = 4x = 72$	38.87	61.13

made using the computer application MicroMeasure version 3.2 (Reeves and Tear 1999).

3. Results

According to data in the literature (Heusser 1938; Del Prete 1977; Scrugli 1978; Mazzola et al. 1982; Cauwet-Marc and Balayer 1986; Bianco et al. 1987; D'Emerico et al. 2000), the chromosome number observed in the Sicilian specimens of *Serapias vomeracea* is $2n = 2x = 36$, while that of *Serapias lingua* is $2n = 4x = 72$ (Figure 1A, D). The karyological investigation carried out on *Serapias orientalis* subsp. *siciliensis* verified that it is diploid with $2n = 2x = 36$, while *Serapias intermedia* subsp. *hyblaea* is triploid with $2n = 3x = 54$ (Figure 1B, C).

The banding patterns in chromosomes and the distribution of heterochromatin for *Serapias vomeracea* (Figure 2A) and *S. lingua* (Figure 3) are similar to those reported for the same species by D'Emerico et al. (2000). In *S. orientalis* subsp. *siciliensis*, the C-banded karyotype shows constitutive heterochromatin in the centromeric regions of numerous chromosomes. In particular, pairs 1, 3, 4, 5, 6, 7, 8, 9 and 13 are characterized by large centromeric bands; whereas pairs 2, 10, 14, 15, 17 and 18 have thin centromeric bands (Figure 2B). In

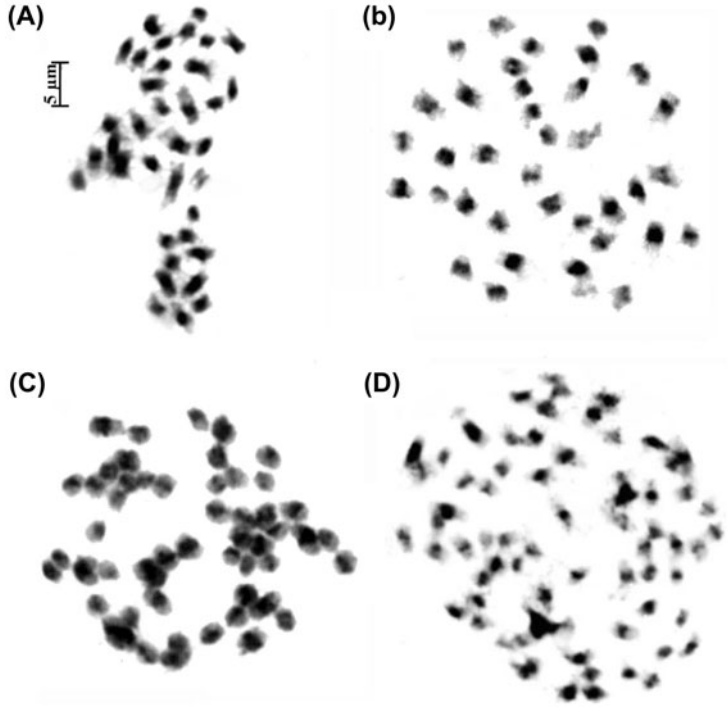

Figure 1. C-banded somatic metaphases of (A) *Serapias vomeracea* ($2n = 2x = 36$); (B) *S. orientalis* subsp. *siciliensis* ($2n = 2x = 36$); (C) *S. intermedia* subsp. *hyblaea* ($2n = 3x = 54$); (D) *S. lingua* ($2n = 4x = 72$). Scale bar = 5 µm.

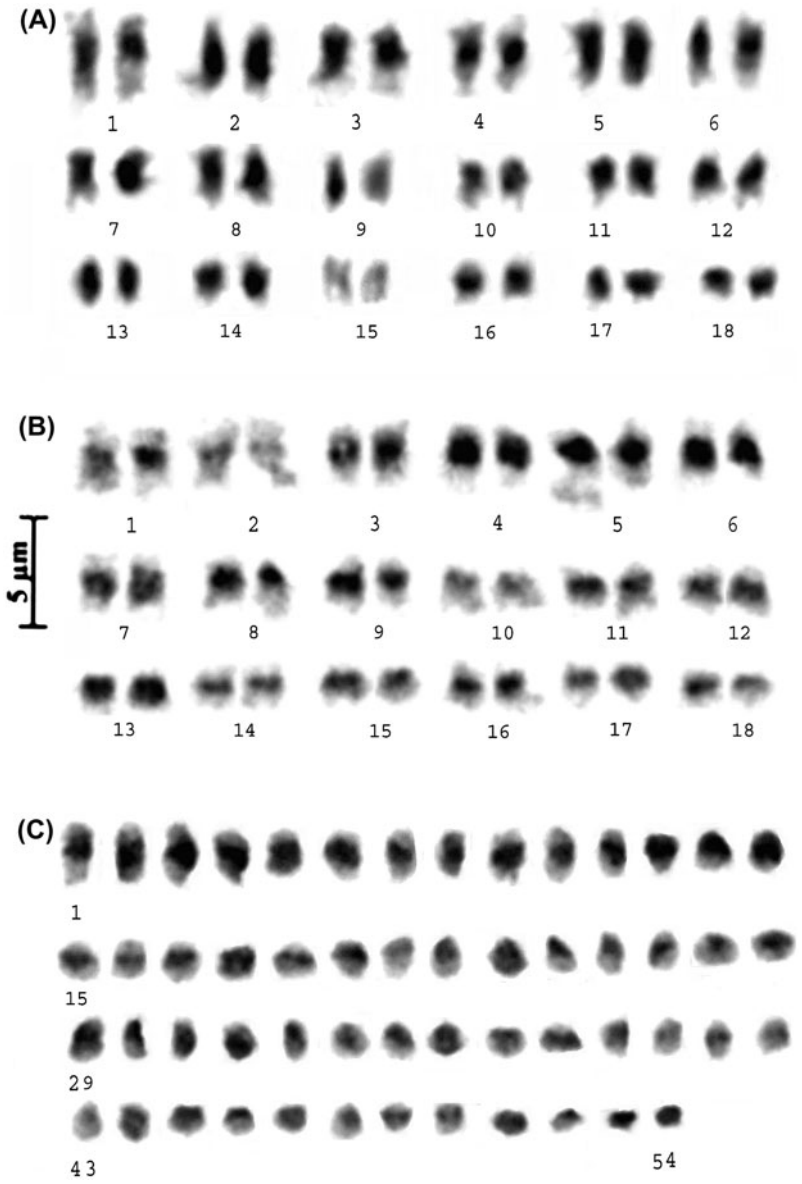

Figure 2. Giemsa C-banded karyotypes of (A) *Serapias vomeracea*; (B) *S. orientalis* subsp. *siciliensis*; (C) *S. intermedia* subsp. *hyblaea*. Scale bar = 5 µm.

the case of *S. intermedia* subsp. *hyblaea*, the heterochromatin in the C-banded karyotype forms large centromeric bands in many chromosomes (Figure 2C), as in the other *Serapias* taxa examined by D'Emerico et al. (2000). However, Giemsa C-banding analysis revealed that this taxon has the smallest amount of heterochromatin in many chromosomes. The amount of C-heterochromatin in the total chromosome length varies from 30% in *S. orientalis* subsp. *siciliensis* to 41% in *S. vomeracea* (Table 2).

4. Discussion

The chromosome numbers and C-banding of *Serapias orientalis* subsp. *siciliensis* and *S. intermedia* subsp. *hyblaea* are reported here for the first time. In this work the constitutive heterochromatin, which is a significant feature in the chromosome complement of eukaryotic karyotypes (Baimai 1988), is examined. The C-banding patterns of *Serapias* taxa analyzed in this study are quite similar, with the presence of very large centromeric bands occurring in most of the chromosomes. Similar banding patterns were previously observed in seven diploid and in one polyploid *Serapias* of the Italian flora (D'Emerico et al. 2000). On the other hand, small variations were observed among the *Serapias* taxa. Moreover, the comparison among the karyotypes of the investigated taxa of *Serapias* showed differences in number, position or amount of centromeric bands, while the similarity of the banding patterns suggests a certain homology among them. However, the analysis of microsatellite DNA in *Serapias* reveals significant genetic differentiation among

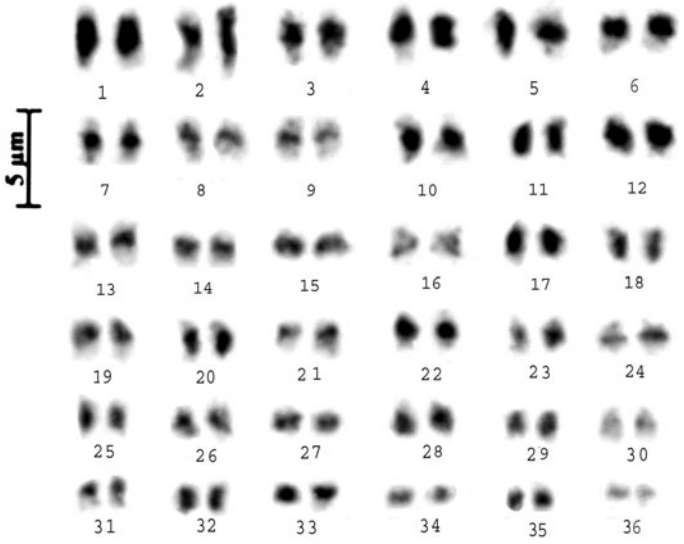

Figure 3. Giemsa C-banded karyotype of *S. lingua*. Scale bar = 5 µm.

the species (Pellegrino et al. 2001, 2005; Savo Sardaro et al. 2012).

As emphasized in Figure 2B, *S. orientalis* subsp. *siciliensis* ($2n = 2x = 36$) displays a lower amount of heterochromatin bands than other taxa, due to the occurrence of thin centromeric bands in seven chromosome pairs.

For *S. intermedia* subsp. *hyblaea* ($2n = 3x = 54$), Cristaudo et al. (2009) asserted that it represents a natural nothotaxa arising from a hybridization process between *Serapias vomeracea* and *Serapias lingua*. In fact, these supposed parental species are characterized by different chromosome complements, $2n = 2x = 36$ for *S. vomeracea* and $2n = 4x = 72$ for *S. lingua*. Grant (1981) reported evidence that most triploid plants are present within populations characterized by diploid and tetraploid species. Cytological analysis in the triploid *S. intermedia* subsp. *hyblaea* shows small amounts of heterochromatin in numerous chromosome pairs. This feature was observed also in the chromosomes of *S. lingua* as reported in this work and also by D'Emerico et al. (2000).

Unfortunately in this analysis we do not have meiotic plates of *S. intermedia* subsp. *hyblaea*, which would allow verification of whether this taxon is allotriploid. In fact, distinction between allopolyploidy and autopolyploidy in natural populations is quite difficult. Therefore, our study confirms in part the statements reported by Cristaudo et al. (2009), and new techniques such as banding with fluorescence *in situ* hybridization or molecular study are necessary for conclusive inferences.

These results indicate that the rearrangements in repetitive DNA sequences occurred at the centromeric regions during the differentiation of *Serapias* species is probable (D'Emerico et al. 2000). Therefore in this genus, Giemsa C-banded karyotypes can be useful to identify some chromosome groups, although they are not sufficient to resolve the phylogeny of these orchids.

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