# Contribution to the cytotaxonomical knowledge of four species of *Serapias* L. (Orchidaceae)

Bellusci Francesca[1,*] and Gabriella Aquaro[2]

[1]Department of Ecology, University of Calabria, via Bucci 6/B I-87036 Rende (CS), Italy.
[2]Natural History Museum of Calabria and Botanic Garden, University of Calabria, Rende, I-87036 Cosenza, Italy.

**Abstract** — *Serapias* L. includes about thirty entities, but just one (*S. lingua*) is presently known as polyploid. In this paper we reported, for the first time, the chromosome number of *S. gregaria, S. olbia* and *S. strictiflora*, three endemic species of Southwest Europe, that resulted tetraploid with $2n = 72$ chromosomes as *S. lingua*. The strong affinity between these four species revealed by molecular phylogenetic studies based on cpDNA is now supported by their common tetraploid level, so that polyploidy can be considered for this genus a possible mechanism of speciation. The karyotype of another endemic species of Iberian Peninsula, *S. perez-chiscanoi*, was carried out in order to add new karyological data on *Serapias* orchids.

**Key words**: chromosome number, karyotype, Orchidaceae, polyploid, *Serapias*.

## INTRODUCTION

The genus *Serapias* L. includes about thirty entities divided in two sections (Delforge 2006). The first one is represented by *S. lingua* L. group, with 6 species; the second one unites *S. vomeracea* (Burm.) Briq. group and *S. parviflora* Parl. group, with 21 and 3 species respectively.

*Serapias* has a predominantly Mediterranean distribution: its range extends from the Azores and the Canaries in the west to the Caucasus in the east, and as far north as Brittany (France) (Gölz and Reinhard 1980; Perez Chiscano *et al*. 1991; Delforge 2006). While *S. vomeracea*, *S. cordigera*, *S. lingua* and *S. parviflora* are widely distributed throughout the Mediterranean area, most of the other species are narrow endemic and characterized by small populations with a few individuals.

The genus is monophyletic as confirmed by molecular analyses based on the nuclear ribosomal internal transcribed spacers (nrITS), with low levels of divergence within its species that suggest a very recent origin (Bateman *et al.* 2003).

Previous karyological studies have shown that chromosome number of *Serapias* species belonging to *S. parviflora* and *S. vomeracea* groups is $2n = 2x = 36$ while we have no information on species belonging to the third group except for *S. lingua* that has $2n = 4x = 72$. Thus, the available karyological information on the genus *Serapias* is summarized in Table 1. This paper aims to add new karyological data on *Serapias* orchids proving the chromosome number of three endemic species belonging to *S. lingua* group (*S. olbia*, *S. gregaria* and *S. strictiflora*) and the karyotype structure of *S. perez-chiscanoi*, within *S. vomeracea* group.

## MATERIALS AND METHODS

*Plant material* - Karyological observations of *Serapias* species were based on material collected in the field, in April 2007. *S. gregaria* and *S. olbia* were collected in France; the first one near Puget sur Argens (43°28'N - 6°37'E, 40 m), the second one in Bormes le Mimosas, 43°09'N - 6°20'E, 150 m. Specimens of *S. strictiflora* and *S. perez-chiscanoi*, were collected in Spain near Gibraltar (La linea de la Conception, 36°12'N – 5°19'W 15 m) and in Aljucen, (39°01'N – 6°18'W, 300 m). Specimens were identified according to Delforge (2006) classification.

No voucher specimens were collected because population size of examinated species was too small, but photographic documentation of the studied units is available at request.

* Corresponding author: phone: +390984492955; fax: +390984492986; e-mail francescabellusci@libero.it

Table 1 — Summary of karyological knowledge for the genus *Serapias*.

| Group | Species | 2*n* | authors |
|---|---|---|---|
| S. parviflora | *S. parviflora* Parl. | 36 | DEL PRETE 1977, SCRUGLI 1978, MAZZOLA *et al.* 1981, QUEIROS 1983, BIANCO *et al.* 1991, D'EMERICO *et al.* 2000 |
| | *S. politisii* Renz | 36 | BIANCO *et al.* 1991, 1992, D'EMERICO *et al.* 2000 |
| | *S. nurrica* Corrias | 36 | SCRUGLI 1982, D'EMERICO *et al.* 2000 |
| S. vomeracea | *S. bergonii* E.G. Camus | 36 | D'EMERICO *et al.* 2000 |
| | *S. vomeracea* (Burm.) Briq. | 36 | DEL PRETE 1977, MAZZOLA *et al.* 1982, BIANCO *et al.* 1987, 1991, D'EMERICO *et al.* 1992, 2000 |
| | *S. perez-chiscanoi* Acedo | 36 | BERNARDOS *et al.* 2004 |
| | *S. orientalis* (Greuter)Baumann & Künkele | 36 | D'EMERICO *et al.* 1990, CONSTANTINIDIS and KAMARI 1995 |
| | *S. apulica* (Baumann & Künkele)Delforge | 36 | D'EMERICO *et al.* 2000 |
| | *S. neglecta* De Notaris | 36 | DEL PRETE *et al.*1980 |
| | *S. cordigera* L. | 36 | SCRUGLI et al. 1976, MAZZOLA *et al.* 1982, QUEIROS 1983, CAUWET-MARC and BALAYER 1986, BIANCO *et al.* 1991, D'EMERICO *et al.* 2000 |
| S. lingua | *S. lingua* L. | 72 | DEL PRETE 1978, SCRUGLI 1978, 1980, MAZZOLA *et al.* 1982, QUEIROS 1983, BIANCO *et al.* 1991, D'EMERICO *et al.* 2000 |
| hybrid | *S.* x *todaroi* Tineo (*S. lingua* x *S. parviflora*) | 54 | BIANCO *et al.* 1991 |

*Chromosome analysis* - Squash preparations were made from young ovules of plants collected *in situ*, according to the following schedule: pretreatment in 0.5% colchicine solution for 4 hours; Carnoy fixing for at least 1 hour; hydrolisis in HCl 1 N for 7 minutes at 60°C; staining with leuco-basic fuchsin for 3 hours. At least five plates were used in order to establish the chromosome number, or to build the idiogram; Chromosomes were drawn and microphotographs were taken through a Zeiss Ultraphot IIIB microscope equipped with Nomarski optics. Karyotype formula and terminology used are in accord to LEVAN *et al.* (1964). Intrachromosomal ($A_1$) and interchromosomal ($A_2$) asymmetry indexes were calculated according to the methodology defined by ROMERO ZARCO (1986). These indexes are a quantification of Stebbins's asymmetry categories: they ranges between 0 and 1 and are independent of chromosome number and size. No idiogram was built for *S. gregaria*, *S. olbia* and *S. strictiflora* because centromere position was no clear for each chromosome.

## RESULTS

The chromosome number of *S. gregaria, S. olbia* and *S. strictiflora* is here reported for the first time: these species are tetraploid with $2n = 72$ as shown in Fig. 1 (A-F).

Chromosome count on *S. perez-chiscanoi* confirms a previous finding for this species $2n = 2x = 36$ (BERNARDOS *et al.* 2004). Karyotype formula with a large proportion of metacentric chromosomes can be expressed for this species as follows: $2n = 2x = 36 = 2sm + 2m + 2sm + 6m + 2sm + 22m$ (Fig. 2). The mean lenght of haploid idiogram is 33.01 μm, while the chromosome size ranges between 1.16 and 3.1 μm. Intrachromosomal ($A_1$) and interchromosomal ($A_2$) asimmetry indexes are: $A_{1\,=}$ 0.33; $A_{2\,=}$ 0.25.

## DISCUSSION

Chromosome studies on *S. olbia*, *S. gregaria*, *S. strictiflora* have shown that polyploidy characterizes almost all the species of *Serapias lingua* group.

The strong affinity between these species pointed out by karyological data is not completely supported by morphological traits (DELFORGE 2006), rather it is confirmed by recent molecular studies on *Serapias* genus (BELLUSCI *et al.* 2008). In fact, phylogenetic tree based on four non-coding regions of cpDNA, divided the examined *Serapias* species in two main clades, each including two minor groups. *S. olbia*, *S. gregaria*, *S. strictiflora* and *S. lingua* are grouped together in the same clade pointing out that polyploidy can be consid-

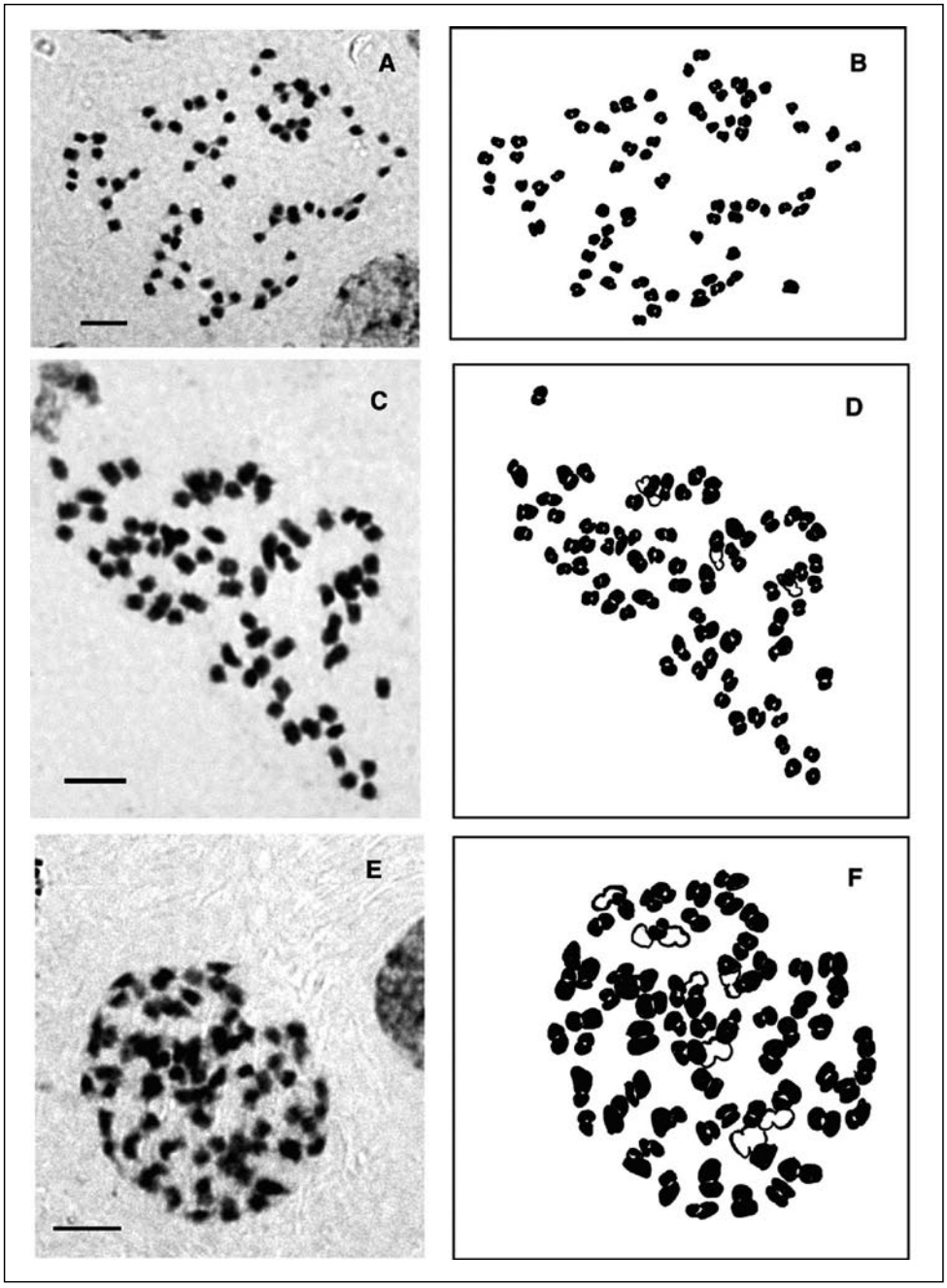


Fig. 1 — *S. gregaria* Godfery: microphotograph of chromosomes (A), drawing of methaphasic plate showing $2n$ = 72 chromosomes (B); *S. olbia* Verguin: microphotograph of chromosomes (C), drawing of methaphasic plate showing $2n$ = 72 chromosomes (D); *S. strictiflora* Welwitsch ex Veiga: microphotograph of chromosomes (E), drawing of methaphasic plate showing $2n$ = 72 chromosomes (F). Bars correspond to 5 μm.

ered a possible mechanism of speciation as demonstrated in other orchid genera (HEDRÉN *et al.* 2001; HEDRÉN 2003). For this reason, polyploidy within the subtribe Orchidinae is now no longer confined to the *Dactylorhiza–Coeloglossum* and *Gymnadenia–Nigritella* clades as suggested by PRIDGEON *et al*. (1997) but have to include also *Serapias* genus with four of the eleven species examined certainly tetraploid.

Chromosome numbers reported in this paper are, however, in contrast to those suggested by VENHUIS *et al.* (2007) for the same species. In fact, the latter authors estimated DNA content for *S. gregaria*, *S. strictiflora* and *S. olbia* of samples

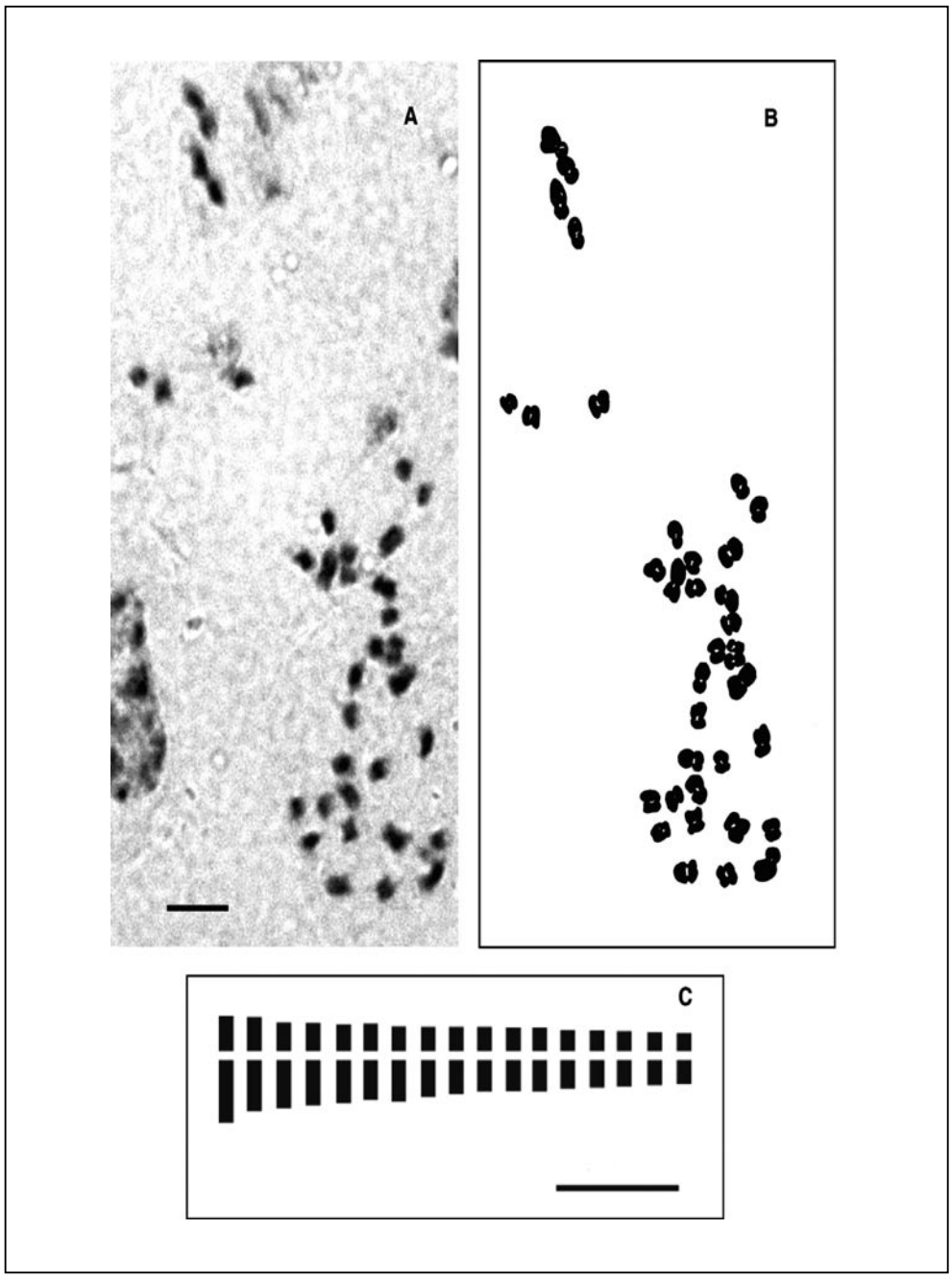


Fig. 2 — *S. perez-chiscanoi* Acedo: microphotograph of chromosomes (A), drawing of methaphasic plate showing $2n = 36$ chromosomes (B), haploid idiogram (C). Bar corresponds to 5 μm.

collected in the same sites inferring that the first two species are diploid, with $2n = 36$, while the third one is hexaploid, with presumably $2n = 108$. These results clearly point out that DNA content alone is insufficient to distinguish between $2x$ and $4x$ cytotypes, and that FCM measurements may not be an accurate indicator of ploidy level but should always be accompanied by chromosome counts (YEATER *et al.* 2004; LEONG-ŠKORNIČKOVÀ *et al.* 2007).

Chromosome count on *S. perez-chiscanoi* confirms a previous finding for this species (BERNARDOS *et al.* 2004); karyotype formula and asymmetry indexes, instead, are here reported for the first time. *S. perez-chiscanoi* has more metacentric chromosomes than those found in other species,

endemic or not, of *Serapias* genus such as *S. politisii*, *S. cordigera*, *S. apulica* (BIANCO *et al.* 1992). The intrachromosomal asymmetry index of *S. perez-chiscanoi* ($A_1$=0.33), shows a smaller variation in size of chromosome arms and its value is lower respect those of other species of this genus as *S. apulica* (0.52), *S. vomeracea* (0.46) and *S. politisii* (0.40) (D'EMERICO *et al.* 2000; D'EMERICO 2001).

Also the interchromosomal asymmetry index of *S. perez-chiscanoi* ($A_2$=0.25), is lower respect other species of *Serapias* genus as *S. politisii* (0.65) and *S. parviflora* (0.62) (BIANCO *et al.* 1992).

Karyotype asymmetry indexes can be considered, usually, a good expression of the general morphology of plant chromosomes and the magnitude of their difference can indicate the number of chromosome rearrangements that have occurred between species. Generally symmetrical karyotypes are regarded as more primitive and asymmetrical karyotypes as more specialized or longer chromosomes are considered more ancient than shorter ones (STEBBINS 1971). For these reasons *S. perez-chiscanoi* karyotype could be considered more primitive than those of the other related diploid species, even if recent chromosomal rearrangements could be occurred and fixed rapidly as happens in small, isolated populations exposed to random genetic drift and inbreeding (LEVIN 2002). Otherwise, because *S. perez-chiscanoi* often grows in sympatry with other *Serapias* species (i.e. *S. parviflora*) sharing pollinators and overlapping flowering phenologies, the different karyotypes could represent a valid postzygotic reproductive barrier (COZZOLINO *et al*. 2004).

**Acknowledgments** — The authors are grateful to Dr. Giuseppe Pellegrino for his help in plant sampling and to Prof. Aldo Musacchio and Dr. Lorenzo Peruzzi for their useful advises.

## REFERENCES

BATEMAN R.M., HOLLINGSWORTH P.M., PRESTON J., YI-BO L., PRIDGEON A.M. and CHASE M.W., 2003 — *Molecular phylogenetics and evolution of Orchidinae and selected Habenariinae (Orchidaceae)*. Biological Journal of the Linnean Society, 142: 1-40.

BELLUSCI F., PELLEGRINO G., PALERMO A.M. and MUSACCHIO A., 2008 — *Phylogenetic relationships in the orchid genus Serapias L. based on non-coding regions of the chloroplast genome.* Molecular Phylogenetics and Evolution, 47: 986-991.

BERNARDOS S., TYTECA D. and AMICH F., 2004 — *Cytotaxonomic study of some taxa of the subtribe Orchidinae (Orchidoideae, Orchidaceae) from the Iberian Peninsula***.** Israel Journal of Plant Sciences, 52: 161-170.

BIANCO P., MEDAGLI P., D'EMERICO S. and RUGGIERO L., 1987 — *Numeri cromosomici per la Flora Italiana*. Informatore Botanico Italiano, 138: 322-332.

BIANCO P., D'EMERICO S. and MEDAGLI P., 1991 — *Indagini citotassonomiche su alcuni taxa del genere Serapias (Orchidaceae)*. Giornale Botanico Italiano, 125(3): 243.

BIANCO P., D'EMERICO S., MEDAGLI P., RUGGIERO L. and LIVERANI P., 1992 — *Serapias polititsii Renz (Orchidaceae), nuova per la Flora Italiana*. Webbia, 46(2): 219-223.

CAUWET-MARC A.M. and BALAYER M., 1986 — *Les orchidées du bassin méditerranéen. Contribution a l'étude caryologique des espèces des Pyrénées orientales (France) et contrées limitrophes. II. Tribu des Ophrydae Lindl. pro parte*. Bulletin de la Société Botanique de France, 133: 265-277.

CONSTANTINIDIS T. and KAMARI G., 1995 — *Mediterranean chromosome number reports 5 (401-414)*. Flora Mediterranea, 5: 265-278.

COZZOLINO S., D'EMERICO S. and WIDMER A., 2004 — *Evidence for reproductive isolate selection in Mediterranean orchids: karyotype differences compensate for the lack of pollinator specificity*. The Royal Society, Biology Letters, 271: 259-262.

DELFORGE P., 2006 — *Orchids of Europe, North Africa and tha Middle East*. Timber Press Inc., Oregon, USA.

DEL PRETE C., 1977 — *Numeri cromosomici per la flora italiana: 358-365*. Informatore Botanico Italiano, 9(2): 135-140.

DEL PRETE C., 1978 — *Contributi alla conoscenza delle Orchidaceae d'Italia. VI. Tavole cromosomiche delle "Orchidaceae" italiane con alcune considerazioni citosistematiche sui generi "Ophrys", "Orchis" e "Serapias"*. Informatore Botanico Italiano, 10(3): 379-389.

DEL PRETE C., GARBARI F. and GIORDANI A., 1980 — *Numeri cromosomici per la Flora Italiana*: 690-695. Informatore Botanico Italiano, 12: 117-120.

D'EMERICO S., 2001 — Tribe Orchideae cytogenetics. In: Pridgeon A.M., Cribb P.J., Chase M.W. and Rasmussen F.N. (Eds.), *Genera Orchidacearum. 1. Orchidoideae*, vol. 2. pp. 216-224. Oxford University Press.

D'EMERICO S., BIANCO P. and MEDAGLI P., 1992 — *Caryological studies on Orchidaceae. Tribe Ophrydeae, subtribe Serapiadinae*. Caryologia, 45(3-4): 301-311.

D'EMERICO S., PIGNONE D., and SCRUGLI A., 2000 — *Giemsa C-banded kary-otypes in Serapias L. (Orchidaceae)*. Botanical Journal of the Linnean Society, 133: 485-492.

GÖLZ P. and REINHARD H.R., 1980 — *Serapias (Orchidaceae) Ergebnisse statistischer und chorologischer Untersuchungen*. Mitteilungsblatt Arbeitskreis Heimische Orchideen Baden-Württemberg, 12: 123-189.

HEDRÉN M., FAY M.F. and CHASE M.W., 2001 — *Amplified fragment length polymorphisms (AFLP) reveal details of polyploid evolution in Dactylorhiza (Orchidaceae)*. American Journal of Botany, 88: 1868-1880.

HEDRÉN M., 2003 — *Plastid DNA variation in the Dactylorhizaincarnata/maculata polyploid complex and the origin ofallotetraploid D. sphagnicola (Orchidaceae)*. Molecular Ecology, 12: 2669-2680.

LEVAN A., FREDGA K. and SANDBERG A.A., 1964 — *Nomenclature for centromeric position of chromosomes*. Hereditas, 52: 201-220.

LEVIN D., 2002 — *The role of chromosomal change in plant evolution*.Oxford University Press.

LEONG-ŠKORNIČKOVÀ J., ŠÍDA O., JAROLÍMOVA V., SABU M., FÉR T., TRÀVNÍČEK P. and SUDA J., 2007 — *Chromosome numbers and genome size variation in Indian species of Curcuma (Zingiberaceae). Annals of Botany*, 100: 505-526.

MAZZOLA P., GRISAFI F. and ROMANO S., 1981 — *Numeri cromosomici per la flora italiana: 850-859*. Informatore Botanico Italiano, 13(2-3): 182-188.

MAZZOLA P., GRISAFI F. and ROMANO S., 1982 — *Numeri cromosomici per la flora italiana: 919-928*. Informatore Botanico Italiano, 14(2-3): 268-274.

PEREZ CHISCANO J.L., GIL LLANO J.R. and DURAN OLIVA F., 1991 — *Orquídeas de Extremadura*. Madrid, Fonda Natural.

PRIDGEON A.M., BATEMAN R.M., COX A.V., HAPEMAN J.R. and CHASE M.W., 1997 — *Phylogenetics of subtribe Orchidinae (Orchidoideae, Orchidaceae) based on Nuclear ITS sequences. 1. Intergeneric relationships and polyphyly of Orchis sensu lato*. Lindleyana, 12: 89-109.

QUEIROS M., 1983 — *Números cromossómicos para a Flora Portugesa, 64-85*. Boletim da Sociedade Broteriana, 56: 79-98.

ROMERO ZARCO C., 1986 — *A new metod for estimating karyotype asymmetry.* Taxon, 35: 526-530.

SCRUGLI A., 1978 — *Numeri cromosomici per la flora italiana: 526-533*. Informatore Botanico Italiano, 10(3): 414-421.

SCRUGLI A., DE MARTIS B. and MULAS B., 1976 — *Numeri cromosomici per la flora italiana: 238-249*. Informatore Botanico Italiano, 8(1): 82-91.

STEBBINS G.L., 1971 — *Chromosomal evolution in higher plants*. London: Arnold.

VENHUIS C., VENHUIS P., OOSTERMEIJER J.G.B. and VAN TIENDEREN P.H., 2007 — *Morphological systematics of Serapias L. (Orchidaceae) in Southwest Europe*. Plant Systematics and Evolution, 265: 165-177.

YEATER K.M., BOLLERO G.A., BULLOCK D.G. and RAYBURN A.L., 2004 — *Flow cytometric analysis for ploidy level differentiation of 45 hairy vetch accessions*. Annals of Applied Biology, 145: 123-127.

Received March 1st 2008; accepted July 16th 2008