

The genus *Sternbergia* Waldst. & Kit. (Amaryllidaceae) in Italy. Contribution to the cytotaxonomical and morpho-anatomical knowledge

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Abstract — Morpho-anatomical and karyological studies were carried out on plants from S Italy of the three Italian species of the genus *Sternbergia*: *S. colchiciflora*, *S. lutea* and *S. sicula*. The morpho-anatomical features of the leaves are good taxonomic markers, especially concerning their colour, general shape, width and morphology of the median adaxial keel. Karyological analysis revealed the following chromosome complements: *Sternbergia colchiciflora*, $2n = 20$; *Sternbergia lutea*, $2n = 22$; *Sternbergia sicula*, $2n = 22$. Karyotype variability is discussed in the light of previous literature. According to our results *S. colchiciflora* is confirmed to be the most isolated species, while *S. lutea* and *S. sicula*, easily recognizable on morphological grounds, appear instead very close karyologically.

Key words: anatomy, leaf, karyology, taxonomy.

INTRODUCTION

The genus *Sternbergia* Waldst. & Kit. – extending from the Mediterranean to the Caucasus, N Persia and mountains of C Asia (MATHEW 1983) – belongs to the family Amaryllidaceae (order Asparagales) and it was recently considered sister to the genus *Pancratium* L., within a clade of Amaryllidaceae, which unites all the Eurasian species (MEEROW *et al.* 1999) or to the Mediterranean genus *Vagaria* Herb. (MEEROW and SNIJMAN 2006).

This genus (especially *S. lutea* (L.) Ker-Gawl.) is cultivated since ancient times as ornamental, but its cultivation is not very common (MORALES and CASTILLO 2004). UNVER *et al.* (2004) discovered antibiotic activity of extracts from *S. lutea* (L.) Ker-Gawl. and *S. sicula* Tineo ex Guss., since this genus is very rich in alkaloids (as many other Amaryllidaceae).

According to KAMARI and ARTELARI (1990) and ARTELARI and KAMARI (1991), the genus *Sternbergia* consists of 8 species, most of them having yellow flowers and autumnal flowering. Concerning Italy, two (PIGNATTI 1982) or three (PARLATORE

1858; ZANGHERI 1976; WEBB 1980; CONTI *et al.* 2005) taxa are quoted in literature: *S. colchiciflora* Waldst. & Kit., *S. lutea* and *S. sicula*. PIGNATTI (1982) did not recognise *S. sicula* Tineo ex Guss. as a species distinct from *S. lutea* (L.) Ker-Gawl. WEBB (1980) considered *S. sicula* as a subspecies of *S. lutea*, while ZANGHERI (1976) and CONTI *et al.* (2005) – as well as recent investigators of this genus (KAMARI and ARTELARI 1990; ARTELARI and KAMARI 1991) considered them as distinct species.

While several Italian populations of *S. colchiciflora* received attention by several authors (BRULLO *et al.* 2004; FRIGNANI *et al.* 2004; PERUZZI *et al.* 2006), no recent studies have been made on Italian plants of *S. lutea* and *S. sicula*. For this reason, we decided to carry out a first contribution towards a comparative cytotaxonomic and morpho-anatomic study of the Italian species, similarly to what has already been done for Greece (KAMARI and ARTELARI 1990; ARTELARI and KAMARI 1991) and for Iberian Peninsula (MORALES and CASTILLO 2004).

MATERIALS AND METHODS

Plant material. – *S. colchiciflora*: **Italy, Basilicata:** Timpa Falconara, 30/IV/1999, L. Bernardo,

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30/04/1999 (*cult.* Hort. Bot. University of Calabria, acc. n. 302-1, 464-3); **Calabria:** Piano Bellizzia, Civita (province of Cosenza), 19/III/2003, L. Peruzzi, D. Gargano, J.-M. Tison (*cult.* Hort. Bot. University of Calabria, acc. n. 250-s); *S. lutea*: **Italy, Calabria:** Fiume Celadi, Rossano, province of Cosenza, 12/X/2000, G. Cesca, L. Peruzzi (*cult.* Hort. Bot. University of Calabria, acc. n. 257-1); *S. sicula*: **Italy, Calabria:** Monte Sellaro, 17/X/1998, L. Bernardo (*cult.* Hort. Bot. University of Calabria, acc. n. 712-1); Calabria settentrionale: sotto Cerchiara di Calabria (prov. di Cosenza), loc. Costa del Ponte, ca. 300 m s.l.m., 13/X/2005, D. Gargano, L. Bernardo (*cult.* Hort. Bot. University of Calabria, acc. n. 595). Herbarium specimens are listed in Appendix 1.

Chromosome analysis. – Squash preparations were made from root tips from plants cultivated in pot in the Botanic Garden of University of Calabria, according to the following schedule: pretreatment in 0,5% colchicine solution for 4 hours; Carnoy fixing for at least 1 hour; hydrolysis in HCl 1 N for 7 minutes at 60°C; staining with leuco-basic fuchsin for 3 hours. Karyotype formulas and terminology are according to LEVAN *et al.* (1964). At least five plates were measured in order to build the idiogram. The graphic method proposed by PLUMMER *et al.* (2003) was used to match homologue chromosomes. We adopted the two coefficients proposed by PASZKO (2006) in order to compare the karyotype asymmetry among the studied species: CV_{CI} (Coefficient of Variation of Chromosome Index) and CV_{CL} (Coefficient of Variation of Chromosome Length).

Morpho-anatomical study. – Segments, 2-3 cm long, were taken from the basal third of leaves in springtime and fixed in a 50% ethylic alcohol 50% glycerol solution; afterwards, 40-50 µm wide cross sections were cut. Finally, camera lucida drawings were effected for each studied plant.

RESULTS

Sternbergia colchiciflora Waldst. & Kit. - The chromosome complement of the studied populations from Pollino Massif (S Italy) has proven to be diploid, with $2n = 20$ chromosomes (Fig. 1A). Karyotype formula can be expressed as follows: $2n = 20 = 2x = 6m + 2sm + 2t + 10st$ (Tables 1-2; Fig. 1B). Chromosome size ranges from 6.07 to 17.35 µm in average. Morpho-anatomical features of the leaves are reported in Table 3. Leaves in cross section (Fig. 2A) appear almost flat, a bit fistulose among vascular bundles. Leaf margins, obtuse in

Table 1 — Measurements made on 5 metaphasic plates belonging to studied *Sternbergia* species. Data were obtained from microphotographs, then reported in µm.
All measurement

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	THL
<i>S. colchiciflora</i>	S	8.28 ± 1.58	6.39 ± 1.07	5.45 ± 1.34	2.13 ± 0.28	1.14 ± 0.10	1.27 ± 0.19	1.15 ± 0.25	1.06 ± 0.17	0.90 ± 0.20	1.24 ± 0.07	/
	L	9.07 ± 1.47	8.51 ± 2.14	7.90 ± 0.74	8.91 ± 1.52	8.15 ± 1.23	6.84 ± 1.47	6.55 ± 1.12	6.21 ± 0.91	6.09 ± 0.63	4.83 ± 0.77	/
<i>S. lutea</i>	S	9.44 ± 2.18	4.63 ± 0.83	1.01 ± 0.05	2.03 ± 0.13	0.74 ± 0.09	1.05 ± 0.44	0.92 ± 0.12	0.87 ± 0.21	1.02 ± 0.36	0.85 ± 0.26	1.36 ± 0.53
	L	10.28 ± 2.55	11.00 ± 3.29	9.37 ± 1.67	6.94 ± 1.14	7.90 ± 1.20	7.30 ± 0.90	7.45 ± 1.14	7.02 ± 1.20	6.67 ± 0.64	6.40 ± 0.41	4.73 ± 0.68
<i>S. sicula</i>	S	8.87 ± 0.76	4.33 ± 0.36	1.18 ± 0.61	1.91 ± 0.48	0.93 ± 0.16	1.05 ± 0.20	0.84 ± 0.28	0.99 ± 0.26	0.84 ± 0.14	0.81 ± 0.25	1.20 ± 0.11
	L	9.45 ± 0.99	9.57 ± 0.61	8.37 ± 0.62	7.11 ± 0.89	7.61 ± 0.55	7.07 ± 0.33	7.12 ± 0.60	6.79 ± 0.42	6.68 ± 0.41	6.04 ± 0.28	4.57 ± 0.39

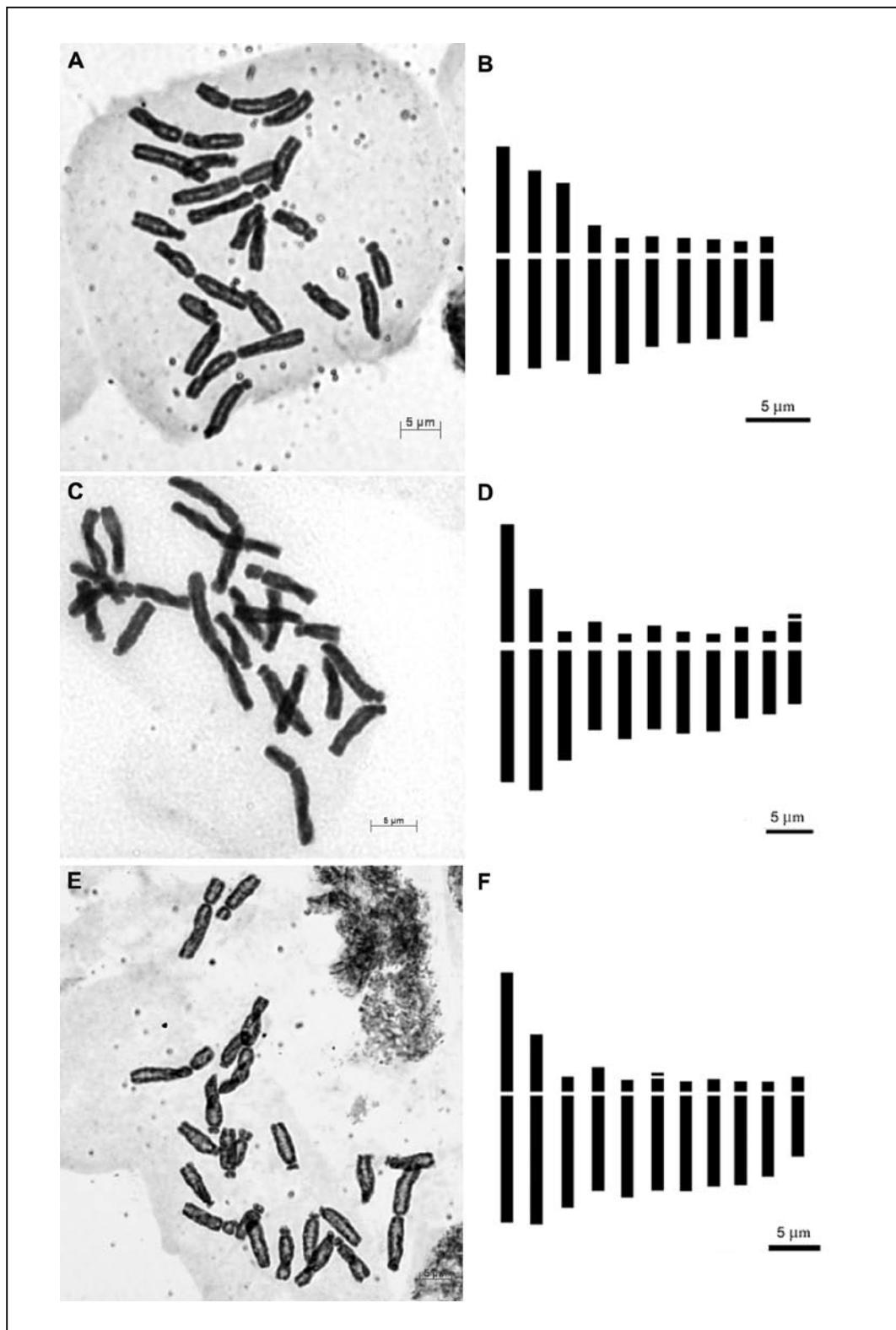


Fig. 1 — *Sternbergia colchiciflora* Waldst. & Kit. A. Metaphasic plate, showing $2n = 20$ chromosomes; B. haploid idiogram. *S. lutea* (L.) Ker-Gawl. C. Metaphasic plate, showing $2n = 22$ chromosomes; D. haploid idiogram. *S. sicula* Tin. ex Guss. E. Metaphasic plate, showing $2n = 22$ chromosomes; F. haploid idiogram. Scale bars = 5 μm.

Table 2 — Karyotype features of the studied *Sternbergia* species: number of chromosomes of terminal, sub-terminal, sub-median and median type (cfr. LEVAN *et al.* 1964); mean (\pm standard deviation) CVCI and CVCL values (cfr. PASZKO 2006).

	t	st	sm	m	CV _{CI}	CV _{CL}
<i>S. colchiciflora</i>	2	12	0	6	58.25 \pm 3.94	35.69 \pm 1.77
<i>S. lutea</i>	10	8	2	2	66.92 \pm 3.65	40.78 \pm 5.25
<i>S. sicula</i>	10	8	2	2	65.72 \pm 5.73	38.44 \pm 2.95

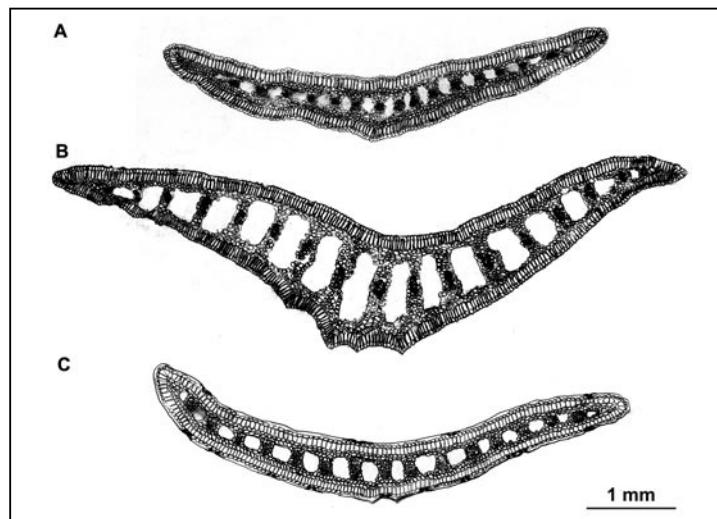


Fig. 2 — Cross sections of the leaves. A. *Sternbergia colchiciflora* Waldst. & Kit. B. *S. lutea* (L.) Ker-Gawl. C. *S. sicula* Tin. ex Guss. Scale bar = 1 mm.

cross section, can be indifferently smooth or ciliate (Fig. 3A-B).

Sternbergia lutea (L.) Ker-Gawl. - The chromosome complement of the studied populations from N Calabria (S Italy) has proven to be diploid, with $2n = 22$ chromosomes (Fig. 1C). Karyotype formula can be expressed as follows: $2n = 22 = 2x = 2m + 2sm + 2t + 2st + 2t^{sat} + 2st + 4t + 2st + 2t + 2st$ (Tables 1-2; Fig. 1D). Chromosome size ranges from 5.09 to 19.72 μm in average. Morpho-anatomical features of the leaves are reported in Table 3. Leaves in cross section (Fig. 2B) appear v-shaped, keeled, very fistulose among vascular bundles. Leaf margins, obtuse to sub-acute in cross section, show papillae generally wider than long (Fig. 3D).

show papillae generally as long as wide (Fig. 3C).

Sternbergia sicula Tin. ex Guss. - The chromosome complement of the studied populations from Pollino Massif (S Italy) has proven to be diploid, with $2n = 22$ chromosomes (Fig. 1E). Karyotype formula can be expressed as follows: $2n = 22 = 2x = 2m + 2sm + 2t + 2st + 2t + 2st^{sat} + 2t + 2st + 4t + 2st$ (Tables 1-2; Fig. 1F). Chromosome size ranges from 5.7 to 18.32 μm in average. Morpho-anatomical features of the leaves are reported in Table 3. Leaves in cross section (Fig. 2C) appear almost flat, fistulose among vascular bundles. Leaf margins, obtuse to sub-acute in cross section, show papillae generally wider than long (Fig. 3D).

Table 3 — Morphological features of the leaves in the studied *Sternbergia* specimens. All measurements are expressed as mean \pm standard deviation.

	<i>S. colchiciflora</i>	<i>S. lutea</i>	<i>S. sicula</i>
n° leaves	5.4 \pm 3.13	5.67 \pm 0.58	4.4 \pm 1.34
leaves length (mm)	38.3 \pm 11.54	121.67 \pm 24.83	96 \pm 21.71
leaves width (mm)	3.6 \pm 1.20	6.33 \pm 1.37	4.75 \pm 0.75
leaves colour	deep green - glaucous	bright green - glossy	deep green with an evident median glaucous stripe
leaves margin	smooth or ciliate	with papillae wider than long	with papillae as wide as long
leaves shape	linear, a bit twisted	linear, a bit v-shaped	linear, almost flat

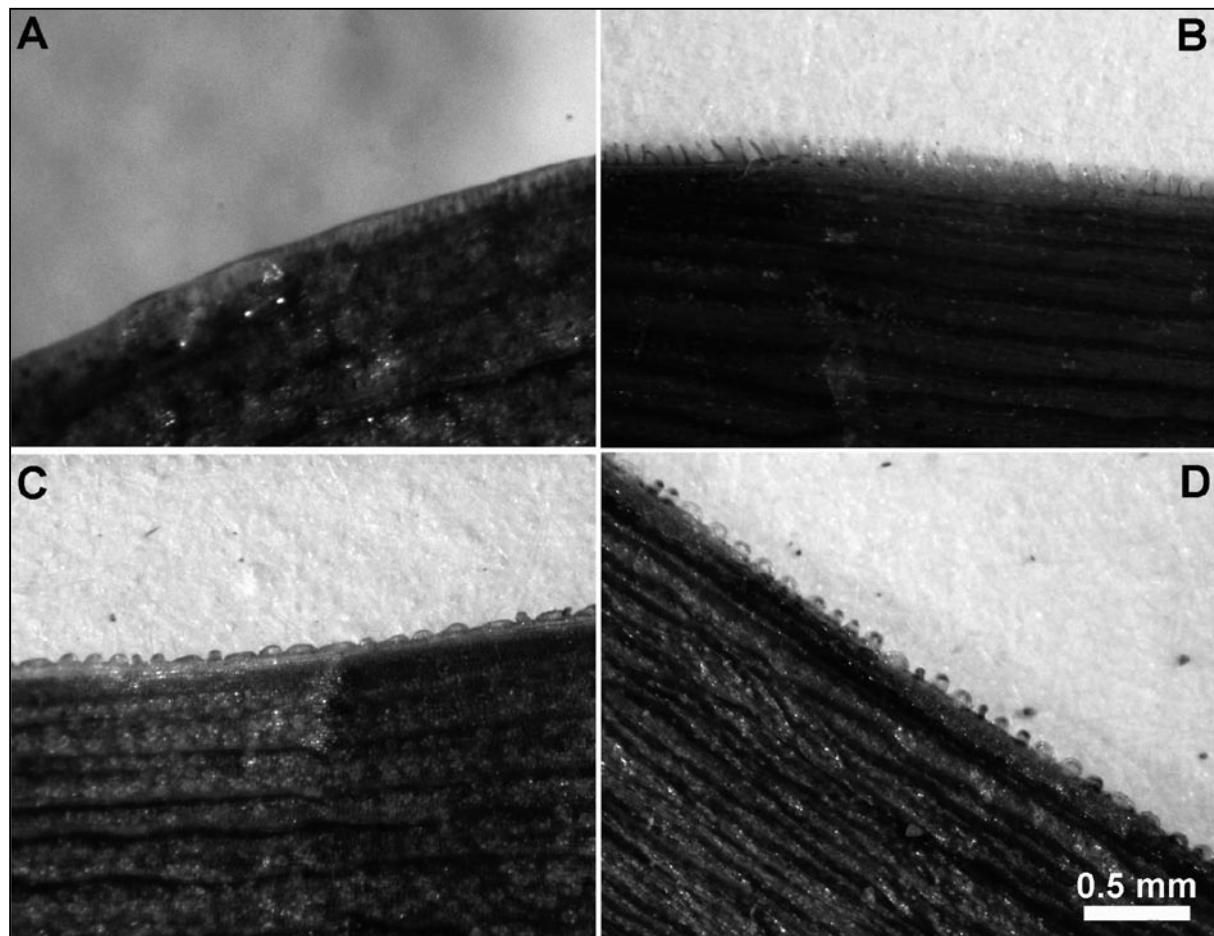


Fig. 3 — Margins of the leaves. A. smooth in *Sternbergia colchiciflora*. B. ciliate in *Sternbergia colchiciflora*. C. with papillae wider than long in *S. lutea*. D. with papillae as long as wide in *S. sicula*. Scale bar = 0.5 mm.

DISCUSSION

The chromosome number of *S. colchiciflora* found by us agrees with previous reports from other Italian localities (ARRIGONI and MORI 1976; FERRARELLA *et al.* 1978; D'AMATO and BIANCHI 1999; BRULLO *et al.* 2004; PERUZZI *et al.* 2006) and from elsewhere (ZACHARIYEVA and MATUSHENKO 1968; BEDALOV and SUSNIK 1971; FERNÁNDEZ CASAS *et al.* 1978; APARICIO 1987; YÜZBASİOĞLU *et al.* 1997; KAMARI and ARTELARI 1990; ARTELARI and KAMARI 1991; ÜNAL *et al.* 1997). On the contrary, TARNAVSCHI and LUNGEANU (1970) found $2n = 26$ for the same species.

As far as *S. lutea* is concerned, our counting confirm previous reports from Italy (BATTAGLIA 1949; PIZZOLONGO 1963; CAPINERI *et al.* 1978; MARCUCCI and TORNADORE 1996) and from elsewhere (BEDALOV and SUSNIK 1971; KRUSHEVA 1975; KAMARI and ARTELARI 1990; ARTELARI and

KAMARI 1991; YÜZBASİOĞLU *et al.* 1997). The $2n = 24$ counting given by AMICO (1947) is considered as an interpretative error, according to BATTAGLIA (1949), while an old $2n = 16$ counting (NAKAJIMA 1936) may also be erroneous or refer to another taxon. It is noteworthy to say that for this species autotriploidy ($2n = 33$) is also known to occur (BATTAGLIA 1949; PASTOR and VALDES 1986; YÜZBASİOĞLU *et al.* 1997). BLANCA and VALLE (1981) quote also a $2n = 30$ counting from Spain.

Finally, our $2n = 22$ counting for *S. sicula* is the first for Italy and agrees with previous reports from Greece (KAMARI and ARTELARI 1990; ARTELARI and KAMARI 1991) and Turkey (YÜZBASİOĞLU *et al.* 1997).

THL is more or less overlapping in all the three species, likely indicating a scarce differentiation in genome size. *S. colchiciflora* is the most distinct species for its different chromosome number, karyotype structure and asymmetry (especially

lower CV_{CI} values) respect to the other taxa. As far as *S. lutea* and *S. sicula* are concerned, they share the same chromosome number, basic karyotype structure and asymmetry, so that there is no clear separation between the two species. Indeed, *S. sicula* only tends to show lower CV_{CL} values.

Karyotype structure in our material substantially agrees with previous reports for all three species, differing only – in some cases – in minor features (i.e. visibility and position of nucleolar constictions, absolute lengths). D'AMATO and BIANCHI (1999) and YÜZBASIOĞLU *et al.* (1997) studied also the Giemsa banding karyotype pattern of *S. colchiciflora*, *S. lutea* and *S. sicula* respectively. Both authors found c-bands almost only in centromere positions on the chromosomes of these species.

Morphological features of the leaves in the studied plants perfectly agree with previous reports for the studied species (MATHEW 1983; KAMARI and ARTELARI 1990; ARTELARI and KAMARI 1991). Cross sections of the leaves, never performed before, enrich the known criteria for the discrimination of the three considered taxa, together with the features of leaf margins, already considered by LOJACONO POJERO (1908).

CONCLUSIONS

By a karyological point of view, the most important difference among the Italian species of *Sternbergia* is the basic chromosome number: $x = 10$ in *S. colchiciflora* and $x = 11$ in *S. lutea* and *S. sicula*. In spite of this, both karyotype asymmetry and total length of chromosome complement do not seem to be useful to clearly distinguish any of the species. A further little distinction is possible between the closely related *S. lutea* and *S. sicula*, on the basis of the nucleolar organizers position on the chromosomes. *S. colchiciflora* constitutes a peculiar species also on morpho-anatomical grounds, in having flat, scarcely fistulose twisted leaves with smooth or ciliate margins. *S. lutea* and *S. sicula* can be discriminated by leaf width and colour, other than by leaf anatomy and margins. All things considered, *S. colchiciflora* is an isolated species with respect to *S. lutea* and *S. sicula*. This matter is also well supported by recent molecular phylogenetic studies (MEEROW and SNIJMAN 2006).

Finally, according to our results and previous literature (MATHEW 1983; KAMARI and ARTELARI 1990; ARTELARI and KAMARI 1991), we propose the following analytical key for an easier identification of the Italian *Sternbergia* species:

1 Hysteranthous, with leaves typically twisted, margins smooth or ciliate; scape very short; perigon with long tube; $x = 10$*S. colchiciflora*

1 Synanthous at various degrees, with leaves not as above, margins distinctly papillose; scape well developed; perigon with very short tube; $x = 11$2

2 Leaves (5.5)7-12(17) mm wide, bright green and glossy, keeled, margins with papillae generally wider than long; obtuse tepals, 1-2.3 cm wide.....*S. lutea*

2 Leaves 2-6(7) mm wide, deep green with an evident glaucous median stripe, more or less flat, margins with papillae generally as long as wide; acute tepals, (0.4)0.6-1.5(2) cm wide.....*S. sicula*

Appendix 1. – Specimina visa selecta: *S. colchiciflora* Waldst. & Kit. – **Italy, Basilicata:** Timpa Falconara (Terranova di Pollino, Pz), 30/IV/1999, L. Bernardo (CLU 12303); **Calabria:** Piano Bellizzia, tra la Fagosa e il torrente Raganello, 18/III/2003, J.-M. Tison, L. Peruzzi, D. Gargano (CLU 7741); Timpa di Porace, 18/III/2003, J.-M. Tison, L. Peruzzi, D. Gargano (CLU 7742); Massiccio del Pollino versante meridionale di Timpa del Principe, loc. Salita Rascio, 13/V/2004, L. Peruzzi, D. Gargano, G. Aquaro (CLU 12304); *S. lutea* (L.) Ker-Gawl. – **Italy, Calabria:** Casino Drago Cassano allo Ionio (Cs), 26/IX/1993, A. Capparelli, L. Bernardo (CLU 1083); Torano nei pressi dello svincolo autostradale Prov. Cosenza, Calabria, 17/X/1998, L. Bernardo (CLU 2823); *S. sicula* Tineo ex Guss. – **Italy, Calabria:** Timpa di Cassano, Civita (Cs), 19/IX/1993, L. Bernardo, G. Maiorca (CLU 1087); Monte Sellaro (Cerchiara di Calabria) lungo il sentiero che sale dal Santuario, 17/X/1998, L. Bernardo (CLU 7017); Margine eucalipteto Sferracavallo dx Trionto, Crosia (Cs), 09/XI/1993, C. Gangale, L. Bernardo (CLU 1086); Timpa di Cassano, versante orientale del Massiccio del Pollino, Civita (Cs), s.d., L. Bernardo (CLU); Carrello superiore, S. Giovanni in Fiore (Prov. di Cosenza, Calabria), 28/IX/1999, L. Bernardo (CLU 2575).

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