THE TAXONOMIC STATUS OF *SCILLA BEIRANA* SAMP. *(HYACINTHACEAE)*

by

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Resumen

ALMEIDA DA SILVA, R.M., F.B. CALDAS & J.A. ROSSELLÓ (1998). La posición taxonómica de Scilla beirana Samp. (Hyacinthaceae). *Anales Jard. Bot. Madrid* 56(2): 253-260 (en inglés).

Se muestreó *Scilla beirana* Samp. en diversas poblaciones del noroeste de Portugal y se comparó con dos táxones con los que se había relacionado previamente, *S. ramburei* Boiss. y *S. peruviana* L. La macromorfología, la anatomía de la hoja y escapo, el número cromosomático y el idiograma de *S. beirana* y *S. ramburei* fueron indistinguibles, pero diferentes de los de *S. peruviana*. Los caracteres diagnósticos que se habían utilizado previamente para discriminar a *S. beirana* –anchura foliar y número de flores– revelaron una variación continua y no permitieron diferenciarla de *S. ramburei*, en la cual debería ser incluida como sinónimo, tal como había sugerido COUTINHO (1935).

Palabras clave: *Hyacinthaceae*, *Scilla*, taxonomía, anatomía, cariología, endemismo, Península Ibérica.

Abstract

ALMEIDA DA SILVA, R.M., F.B. CALDAS & J.A. ROSSELLÓ (1998). The taxonomic status of Scilla beirana Samp. (Hyacinthaceae). *Anales Jard. Bot. Madrid* 56(2): 253-260.

Populations of *Scilla beirana* Samp. were sampled in NW Portugal and compared with its relatives *S. ramburei* Boiss. and *S. peruviana* L. Leaf and scape anatomy, morphology, chromosome number and idiogram were identical in *S. beirana* and *S. ramburei*, but differed from *S. peruviana*. Diagnostic characters previously used to discriminate *S. beirana* (width of leaves and flower number) showed continuous, but not clinal, variation, and failed to provide a clear-cut basis for identification and no other morphological attributes were found to separate the taxa. All available evidence suggests that *S. beirana* should be put into synonymy with *S. ramburei*, as was earlier suggested by COUTINHO (1935).

Key words: *Hyacinthaceae*, *Scilla*, taxonomy, anatomy, caryology, endemism, Iberian Peninsula.

INTRODUCTION

One of the least well-known bulbous species in Europe is *Scilla beirana* Samp., an Iberian squill of doubtful affinities. *Scilla beirana* was published on the basis of plants collected in Northwest Portugal (Beira Alta province), in Vila Nova de Paiva (SAMPAIO, 1931). This species was first related by the author to *S. peruviana* L., from which it was distinguished by the less vigorous habit, smaller bulb, glabrous leaves and blue

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anthers. Later SAMPAIO (1936) cast doubts about the taxonomic status of *S. beirana*, treating it as a synonym of *S. lusitanica* L. and *S. paui* Lacaita, and in the same group as *S. ramburei* Boiss, *S. verna* Huds. and *S. odorata* Link. The view that *S. beirana* was closely related to *S. ramburei* was also shared by COUTINHO (1935) who made *S. beirana* a synonym of *S. ramburei*.

MCNEILL (1980), in his account of the European squills, retained *S. beirana* as a distinct species endemic to Portugal and related it to *S. peruviana*. This was supported by SPETA (1987) who, when splitting *Scilla* in several satellite genera, transferred *S. beirana* under *Oncostema* Raf. subgenus *Oncostema*, in which he placed *S. peruviana*. Recently, FRANCO & ROCHA AFONSO (1994) included *S. beirana* at the subspecific rank within *S. ramburei*. These authors retain the endemic status of *S. beirana*, but expanded its range to NW Spain.

Thus the taxonomic status and relationships of *S. beirana* are by no means clear. In view of this a morphological, anatomical and cytological study was undertaken using *S. beirana*-like plants from the vicinity of the type locality, which were compared with the related *S. ramburei* and *S. peruviana*.

MATERIAL AND METHODS

Material

Specimens of *S. beirana*, *S. peruviana* and *S. ramburei* were studied from the following herbaria: AVE, BC, BCF, COA, COI, ELVE, HVR, ISA, LISU, MA, MAF, PO, SANT, VAB. The type specimen of *S. beirana* is kept at PO. Original material of *S. peruviana* was seen in microfiche (LINN 429.3) whereas no such material or type specimen is available for *S. ramburei*, at least at the Boissier herbarium at G (BURDET & *al.*, 1982). However, specimens of *S. ramburei* revised by Boissier (G) were examined. In addition, living material collected in Portugal and Spain (see Appendix) was cultivated at the Botanical Garden of Porto University.

Methods

Anatomy. Fresh leaves and scapes from 5 mature individuals of each population were collected after anthesis. Epidermal preparations were obtained by peeling off the epidermis of median portions of leaves, clearing in Sodium hypochlorite and staining in Vesuvin. They were then dehydrated through an alcohol series, placed in xylol and mounted in Canada Balsam. Median portions of fresh leaves and scapes were fixed in Formalin-Acetic Acid-Alcohol (FAA) (JOHANSEN, 1940), for 24 h at 0 °C. Fixation was followed by a thorough rinse in running water for 24 h and dehydration in an ethanol series at 0 °C, for 12 h in each step. Material was then transferred to a monomer mixture (GMA), modified from SEMBA (1979), containing 94 % Glycol methacrylate, 3 % 2-Butoxyethanol, 1,5 % Polyethylene Glycol 400, 0,75 % Divinylbenzene, 0,75 % Methyl Methacrylate and 0,72 g of Benzoyl peroxide for each 80 ml of monomer mixture, in two steps of 24 h each, at room temperature. Embedding was carried out in a mixture without PEG, but with 50 µl of 2,2'- azobis [2-methylpropionitrile] for each 3 ml of mixture, polymerizing at room temperature. After trimming, blocks were cut at 1-2 µm using glass knives in an adapted rotary microtome (BENNETT & *al.*, 1976; BUTLER, 1979; SEMBA, 1979). GMA sections were stained in two ways: with toluidine blue O in acetate buffer, pH 4.4 (FEDER & O'BRIEN, 1968), and with periodic acid - Schiff's reaction (PAS), using a 30 min aldehyde blockade in 2,4-dinitro-phenylhydrazine in 15 % acetic acid, and omitting the periodic acid oxidation step in controls (O'BRIEN & MCCULLY, 1981), followed by counter-staining with 1 % naphtol blue black in 7 % acetic acid (FISHER, 1968).

Karyology. Mitotic studies were carried out on root tips pre-treated with 0.2 % colchicine for 4 h at room temperature, fixed in ethanol: acetic acid (3:1) for 24 h, hydrolysed in 1N HCl at 60 °C for 5 min, stained with acetic orcein for 24 h and squashed in 45 % acetic acid. Chromosome

video image analysis was performed on a Macintosh IICi computer using the public domain NIH Image program. Five individuals from each population were studied, and at least 10 mitotic plates were used for karyotype and idiogram construction. For centromere position the nomenclature of LEVAN & *al.* (1964) is followed. In the tables, chromosome parameters are expressed in % of the karyotype (haploid karyotype = 100 %).

RESULTS

Morphology

Plants collected in NW Portugal, including those from the surroundings of the type locality (table 1), closely resemble the type specimen (fig. 1A) as well as the original description of *S. beirana* and were considered to be representative of this taxon. Macroscopic features were compared with *S. ramburei* and *S. peruviana* (table 1). *Scilla beirana* is very distinct from *S. peruviana* in several vegetative and reproductive attributes and no close relationship could be firmly established. By contrast, *S. beirana* is morphologically very close to *S. ramburei* (fig. 1B), and no single feature can differentiate between *S. ramburei* and *S. beirana*. FRANCO & ROCHA AFONSO (1994) separated *S. beirana* (populations north of Tagus River) from *S. ramburei* on the basis of its wider leaves (0.3-0.8 and 0.8-1.5 cm, respectively) and more dense inflorescences (8-25 versus 25-45 flowers). We have pursued this avenue to check for such quantitative discontinuities within *S. ramburei*. Over 150 individuals of *S. ramburei* s.l. throughout its range (N Africa, Spain) showed a continuous variation in both characters, with no discontinuities supporting the recognition of two morphs (fig. 2). A clinal (geographic) variation was also not evident from the results when the geographic origin of the samples was plotted on the scatter diagram. The correlation between leaf width and number of flowers is low ($r = 0.3505$, $p \leq 0.001$), thus supporting the view that most of the variation of these two characters is independent.

Anatomy

Leaf. All three taxa show a great similarity

TABLE 1

MORPHOLOGICAL, ANATOMICAL AND KARYOLOGICAL FEATURES OF *SCILLA BEIRANA*, *S. PERUVIANA* AND *S. RAMBUREI*

	Scilla beirana	*Scilla ramburei*	*Scilla peruviana*
Morphology			
Bulb (Ø)	13-27 mm	15-25 mm	up to 80 mm
Scape	up to 40 cm	up to 40 cm	up to 50 cm
Inflorescence	subcorymbose	subcorymbose	corymbose
No. flowers	5-30	5-35	(5)20-100
Bracts	7-25 mm	7-25 mm	50-80 mm
Leaves	100-400 × 2-16 mm	100-450 × 2-10 mm	40-60 × 10-40(60)
Indument	glabrous	glabrous	ciliate
Anatomy			
Epidermical extensions	(3)5 cells	(3)5 cells	9-10 cells
Mesophyll	homogeneous	homogeneous	heterogeneous
Mesophyll cavities	present	present	absent
Karyology			
Chromosome number	20	20	16

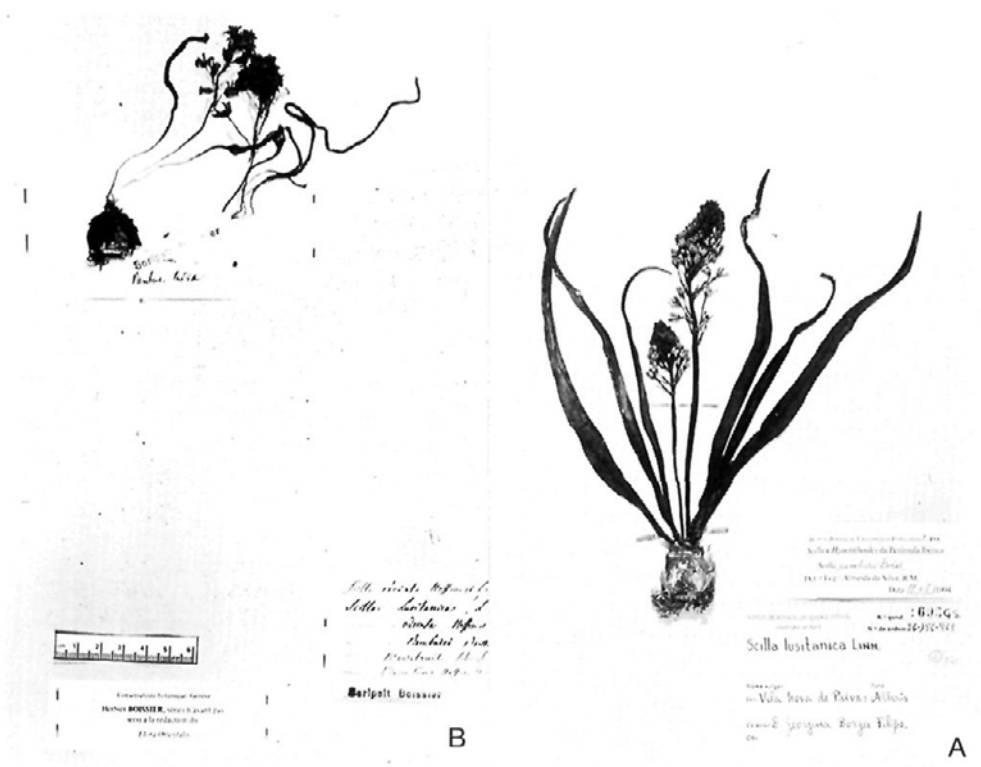

Fig. 1.–Type specimen of *Scilla beirana* Samp. (A) and Boissier's revised specimen of *S. ramburei* Boiss. (B).

in anatomical structure with the species belonging to the Scilla verna complex (ALMEIDA DA SILVA, 1997). Some differences exist between *S. peruviana* and the other two species (fig. 3). Trichomes are absent in *S. beirana* and *S. ramburei*, but *S. peruviana* has long cilia along the leaf margins. The margins show epidermal extensions of 3-5 cells in *S. beirana* and *S. ramburei*, and 9-10 cells in *S. peruviana*. Chlorenchyma is homogeneous and composed of spongy mesophyll in *S. beirana* and *S. ramburei*, while *S. peruviana* has an heterogeneous mesophyll with a somewhat loosely arranged palisade parenchyma in the subepidermal layers. Large cavities, sometimes surrounded by remnants of parenchyma cells, are found alternating with the vascular bundles in *S. beirana* and *S. ramburei*. Such cavities are not found in *S. peruviana*.

Scape. With the exception of the wider scapes of *S. peruviana*, no other internal feature could be used to differentiate the three species.

Karyology

Chromosome numbers and the statistics of the haploid complement of all taxa are depicted in table 2 and figure 4. Populations of *S. peruviana* sampled from the western Iberian peninsula have $2n = 16$, with three metacentric (no.1-3) and five telocentric (no. 4-8) pairs. Secondary constrictions were difficult to observe in every cell and therefore were not considered. The somatic chromosome number was $2n = 20$ for *S. beirana* and *S. ramburei*, with very similar karyotypes comprising two metacentric (no. 1-2), three submetacentrics (no. 3-5), four subtelocentrics (no. 6-9) and one

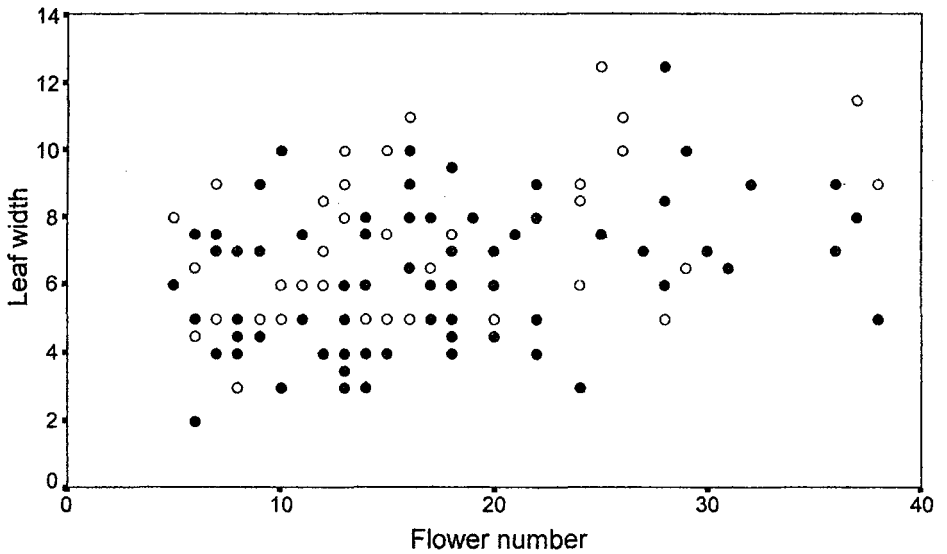

Fig. 2.–Scatter plot showing individuals of *Scilla ramburei* s.l. in relation to flower number and width of leaves (mm). ● populations situated to the North of river Tagus; ○ populations situated to the South of river Tagus.

telocentric pair (no. 10). This striking similarity is further supported by the presence of satellites located on the short arm of a subtelocentric chromosome pair.

Discussion

The *Scilla verna* complex is a group of western mediterranean taxa (*S. verna, S. ramburei, S. beirana, S. odorata, S. merinoi* and *S. paui*) of controversial taxonomic relationships at the generic and specific level. Overall morphology is very similar and without clear conspicuous gaps between all taxa, matering the delimitation of species borderlines difficult without a previous knowledge of the geographic origin of the plants. Individuals from near the type locality of *S. beirana* were indistinguishable from those from other Iberian populations of *S. ramburei*. Vigour and associated morphological characters (length and width of leaves, length of scape, number of flowers) are highly variable in this complex, and the type (*S. beirana*) and Boissier's revised specimens (*S. ramburei*) of these taxa are at

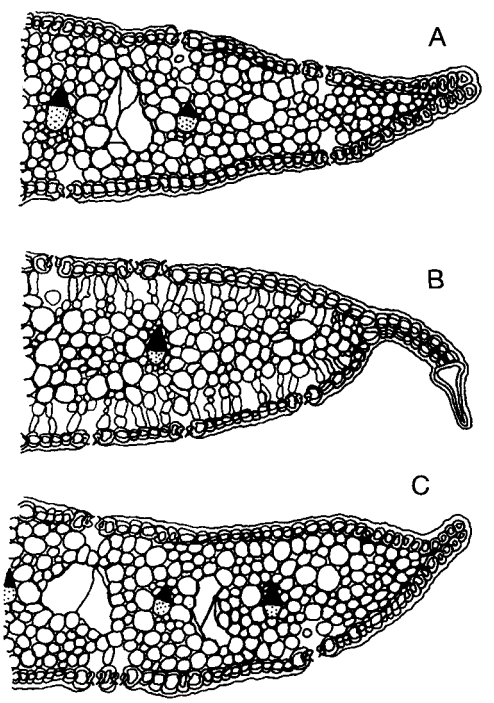

Fig. 3.–Portions of cross-sections of *Scilla* leaves showing epidermical extensions: A, *S. beirana;* B, *S. peruviana;* C, *S. ramburei*. Phloem - dotted; Xylem - solid.

TABLE 2

AVERAGE LENGTH AND STANDARD DEVIATION VALUES OF THE HAPLOID CHROMOSOME COMPLEMENT OF *SCILLA BEIRANA* ($2n = 20$), *S. PERUVIANA* ($2n = 16$) AND *S. RAMBUREI* ($2n = 20$)
(Populations sampled are given in the appendix. S: short arm; L: long arm; T: total chromosome length)

Taxon	No.	S	L	T
Scilla beirana	1	5.23 ± 0.33	5.61 ± 0.33	10.83 ± 0.56
	2	1.80 ± 0.46	2.76 ± 0.71	4.56 ± 0.95
	3	1.44 ± 0.43	2.53 ± 0.77	3.97 ± 0.96
	4	1.67 ± 0.35	3.77 ± 0.88	5.44 ± 1.16
	5	0.98 ± 0.25	2.30 ± 0.61	3.28 ± 0.83
	6	1.40 ± 0.20	5.48 ± 0.63	6.88 ± 0.59
	7	1.25 ± 0.34	4.40 ± 0.73	5.66 ± 0.91
	8	0.91 ± 0.45	3.30 ± 0.62	4.20 ± 0.75
	9	0.85 ± 0.12	2.66 ± 0.26	3.51 ± 0.33
	10	–	1.54 ± 0.21	1.54 ± 0.21
S. peruviana	1	7.95 ± 1.36	9.00 ± 1.03	16.95 ± 2.27
	2	0.89 ± 0.26	1.24 ± 0.23	2.13 ± 0.47
	3	1.08 ± 0.23	1.63 ± 0.16	2.71 ± 0.27
	4	1.10 ± 0.13	7.98 ± 0.70	9.07 ± 0.60
	5	0.95 ± 0.23	6.26 ± 0.59	7.21 ± 0.66
	6	0.96 ± 0.21	5.72 ± 0.56	6.68 ± 0.53
	7	0.62 ± 0.28	3.44 ± 0.68	4.06 ± 0.91
	8	–	1.61 ± 0.20	1.61 ± 0.20
S. ramburei	1	5.49 ± 0.46	5.88 ± 0.55	11.37 ± 0.95
	2	1.96 ± 0.16	3.32 ± 0.75	5.28 ± 0.80
	3	1.43 ± 0.61	2.26 ± 0.60	3.69 ± 1.17
	4	1.50 ± 0.43	3.76 ± 1.30	5.26 ± 1.72
	5	0.84 ± 0.11	2.13 ± 0.66	2.97 ± 0.73
	6	1.47 ± 0.20	5.81 ± 0.97	7.28 ± 1.00
	7	1.20 ± 0.13	4.70 ± 0.35	5.90 ± 0.36
	8	0.64 ± 0.16	3.04 ± 0.30	3.68 ± 0.35
	9	0.72 ± 0.11	2.52 ± 0.17	3.23 ± 0.25
	10	–	1.33 ± 0.28	1.33 ± 0.28

the opposite ends of a continuous variation. In addition, no geographic or ecological pattern has been observed. However, the taller and more vigorous individuals usually come from rainy or wet areas. *Scilla ramburei* grows in a high diversity of habitats, from fixed coastal dunes under a *Pinus pinea* L. canopy to mountain grasslands, from sea level to 1600 m. In the absence of common garden experiments or molecular data we cannot determine the nature of morphological variability, but it is possible that part of it is due to phenotypic plasticity. A close inspection of the *S. beirana* type has not revealed any feature, other than its size, which could link this taxon to *S. peruviana*. Apparently, SAMPAIO (1931) described *S. beirana* from plants cultivated at the Botanical Garden of Porto. Therefore, some characters, including the habit, might be expected to be modified thus mimicking the *S. peruviana* habit. The type specimen, which has two scapes, was prepared at the onset of flowering so the shape of its inflorescence (subcorymbose) is wider than usual, a diagnostic feature of *S. peruviana*. This may explain why such a relationship has been suggested (MCNEILL, 1980; SPETA, 1987). From our results, *S. ramburei* s.l. is fairly homogenous at the anatomical and

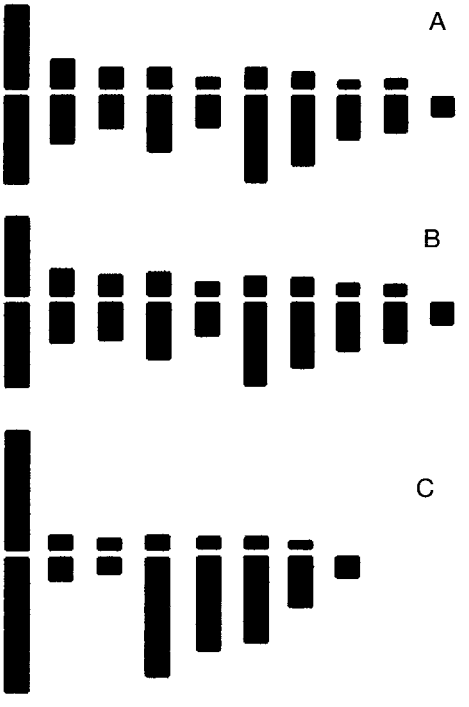

Fig. 4.–Karyograms of *Scilla ramburei* (A), *S. beirana* (B) and *S. peruviana* (C).

chromosomal level throughout its range (north African populations were not available for this study). Anatomical data, although of restricted taxonomic value within the *S. verna* complex (ALMEIDA DA SILVA, 1997), support the identity of *S. ramburei-S. beirana* and also point to their distinctiveness from *S. peruviana*, which differs from the *S. verna* complex by its hairy leaves, heterogeneous mesophyll and absence of cavities in the chlorenchyma.

Chromosome counts and karyotypes of *S. peruviana* were in accordance with previous records from Spain and Portugal (BATTAGLIA, 1949a, 1949b; BARROS NEVES, 1973; CARMONA & *al.*, 1984; PASTOR, 1985; LUQUE & *al.*, 1988). Chromosome numbers of *S. beirana* and *S. ramburei* confirmed earlier reports (FERNANDES & *al.*, 1948; GIMÉNEZ MARTÍN, 1959; BARROS NEVES, 1973; PASTOR, 1985; SPETA, 1987). PARKER (1981) attributed the same chromosome number ($2n = 20$) to *S. beirana* based on the record of *S. ramburei* by BARROS NEVES (1973) from Serra da Lapa, which is very close to the type locality of *S. beirana*. No karyotypic variation was detected when the idiograms of both taxa were compared. In conclusion, morphological, anatomical and karyological evidence show that the distinction of *S. beirana* and *S. ramburei*, even at the infraspecific level (FRANCO & ROCHA AFONSO, 1994), has no sound basis. Not a single character or combination of consistent characters can be used to differentiate between them throughout their range. From a taxonomic point of view, *S. beirana* is a mere synonym of the older name *S. ramburei*.

ACKNOWLEDGEMENTS

The authors are deeply grateful to A. Carvalho, M. Mayol, G. Nieto Feliner, J. Pitarch and L. Sáez for the helpful suggestions and criticism.

REFERENCES

ALMEIDA DA SILVA, R.M. (1997). *A taxonomic revision of some European Hyacinthaceae: the Scilla verna complex and Brimeura Salisb.* Ph. Thesis. University of Porto.

BARROS NEVES, J. (1973). Contribution à la connaissance cytotaxonomique des spermatophyta du Portugal. VIII. Liliaceae. *Bol. Soc. Brot.*, sér. 2, 47: 157-212.

BATTAGLIA, E. (1949a). Mutazioni cromosomiche in Scilla peruviana L. *Caryologia* 1: 144-174.

BATTAGLIA, E. (1949b). Nuove mutazioni cromosomiche in Scilla peruviana L. (secondo contributo). *Caryologia* 2: 85-99.

BENNET, H.S., A.D. WYRICK, S.W. LEE & J.H. MCNEILL (1976). Science and art in preparing tissues embedded in plastic for light microscopy, with special reference to glycol methacrylate, glass knives and simple stains. *Stain Technology* 51: 71-97.

BURDET, H.M, A. CHARPIN & F. JACQUEMOUD (1982). Types nomenclaturaux des taxa ibériques décrits par Boissier ou Reuter. II. Iridacées à Potamogetonacées. *Candollea* 37: 381-395.

BUTLER, J.K. (1979). Methods for improved light microscope microtomy. *Stain Technology* 54: 53-69.

CARMONA, M.J., R.I. DE DOMINICIS, G. SALVI & F. MAGGINI (1984). Ribosomal RNA genes in biotypes of Scilla peruviana (Liliaceae). *Plant Syst. Evol.* 146: 1-11.

COUTINHO, A.X.P (1935). Suplemento da Flora de Portugal - Plantas Vasculares. *Bol. Soc. Brot.*, sér. 2, 10: 70.

FEDER, N. & T.P. O'BRIEN (1968). Plant Microtechnique: some principles and new methods. *Amer. J. Bot.* 55: 123-142.

FERNANDES, A., J. GARCÍA & R. FERNANDES (1948). Herborizaçoes nos domínios da fundaçao da Casa de Bragança. I-Vendas Novas. *Mem. Soc. Brot.* 4: 5-89.

FISHER, D.B. (1968). Protein staining of ribboned Epon sections for light microscopy. *Histochemie* 16: 92-96.

FRANCO, J.A. & M.L. ROCHA AFONSO (1994). *Nova Flora de Portugal. Alismataceae - Iridaceae.* Vol. III, Fasc. I. Lisboa: Bertrand.

GIMÉNEZ MARTÍN, G. (1959). Cariologia de Scilla. II. *Phyton (Buenos Aires)* 13: 145-152.

JOHANSEN, D.A. (1940). *Plant microthecnique.* McGraw-Hill, New York.

LEVAN, A., K. FREDGA & A.A. SANDBERG (1964). Nomenclature for centromeric position on chromosomes. *Hereditas* 52: 201-220.

LUQUE, T., J.A. MEJÍAS & Z. DÍAZ INFANTE (1988). Números cromosomáticos para la flora española, 544-550. *Lagascalia* 15 (extra): 130-133.

MCNEILL, J. (1980). Scilla L. *In*: T. Tutin & *al.* (eds.), *Flora Europaea.* Vol. V. Cambridge University Press. Cambridge.

O'BRIEN, T.P. & M.E. MCCULLY (1981). *The Study of Plant Structure. Principles and Selected Methods.* Melbourne: Termacarphi.

PARKER, P.F. (1981). The endemic plants of metropolitan Portugal, a survey. *Bol. Soc. Brot.*, sér. 2, 53: 943-994.

PASTOR, J. (1985). Números cromosomáticos para la flora española, 368-372. *Lagascalia* 13: 296-299.

SAMPAIO, G. (1931). Adições e Correcções à Flora Portuguesa. *Bol. Soc. Brot.*, sér. 2, 7: 111-168.

SAMPAIO, G. (1936). Breves notas sôbre algumas plantas. *Anais Fac. Ci. Porto* 22: 5-6.

SEMBA, R. (1979). Contributions to semithin sectioning on a conventional rotary microtome. *Stain Technology* 54: 251-255.

SPETA, F. (1987). Die verwandtschaftlichen Beziehungen von Brimeura Salisb.: ein Vergleich mit der Gattungen Oncostema Rafin., Hyacinthoides Med. und Camassia Lindl. (Hyacinthaceae). *Phyton (Austria)* 26: 247-310.

APPENDIX

[Origin of the live material studied. Vouchers have been deposited at the Herbarium of the Porto Botanical Garden (PO)]

Scilla beirana Samp.

LU, DOURO LITORAL: Cinfães, Ferreiros de Tendais, Vila Boa de Baixo, RS035PO, RS039PO; ibidem, Pimeirô, RS040PO. BEIRA ALTA: Vila Nova de Paiva, between Granja do Paiva and V.N. de Paiva, Alhaes, RS046PO; ibidem, E.N. 225 at km 76, RS056PO. TRÁS-OS-MONTES e ALTO DOURO: Mogadouro, Santiago, RS057PO.

Scilla peruviana L.

HS, CÁDIZ: Villaluenga del Rosario, RS019PO. Garganta de la Barrida, RS023PO. Conil de la Frontera, Pinar del Colorado, RS024PO. Chaparral, RS026PO.

LU, BAIXO ALENTEJO: Distrito de Beja, Concelho de Ferreira do Alentejo, E.N. 121, from Beja to Ferreira do Alentejo, at km 62.3, RS032PO. Ferreira do Alentejo, Canhestros, RS054PO. ALGARVE: Faro, Coiro da Burra, RS051PO.

Scilla ramburei Boiss.

HS, CÁDIZ: Conil de la Frontera, Pinar del Colorado, RS025PO.

LU, DOURO LITORAL: Valongo, between Chão das Cavadas and Sra. das Chãs, RS036PO. TRÁS-OS-MONTES e ALTO DOURO: Mogadouro, between Castelo Branco and Lagoaça, RS058PO.

Editado por Ginés López González
Aceptado para publicación: 1-VI-1998