Morphology and karyology of *Antirrhinum rothmaleri comb. & stat. nov.* (Plantaginaceae), a plant endemic to the NW Iberian Peninsula

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This study provides preliminary evidence of morphometric variation within and among species of the *Antirrhinum* section *Streptosepalum* (Plantaginaceae) that allows further development of hypotheses concerning species boundaries. Multivariate statistical analyses of the measurements of ten morphological characters suggest that the *Antirrhinum* occurring on the serpentine outcrops in the province of Trás-os-Montes, NE Portugal, is a species different from *A. braun-blanquetii* and *A. meonanthum*. Its ecological preferences are also different. *Antirrhinum rothmaleri* (Pinto da Silva) Amich, Bernardos & García-Barriuso *comb. & stat. nov.* is described and illustrated. Notes on its distribution, ecology, karyology, and taxonomic relationships are presented. The diagnostic differences among *A. rothmaleri*, *A. braun-blanquetii* and *A. meonanthum* are provided.

Introduction

Serpentinized ultramafic rocks outcrop in many parts of the world and are very often associated with an unusual flora, with rare and endemic plants of taxonomic, phytogeographic and ecological interest (Roberts & Proctor 1992). In NE Portugal, the serpentinized area covers about 8000 ha and it has a distinct geology and flora. This serpentine is inhabited by a smaller number of species than those known from other types of geological substrate, but the flora has a relatively high degree of endemism, the relict taxa differing in age and origin, and there are numerous edaphic ecotypes of species mostly growing on other substrates (Pinto da Silva 1970, Menezes de Sequeira & Pinto da Silva 1992).

Serpentinophytes present taxonomists with difficulties when they are attempting to distinguish between phenotypes representing

responses to nutritional imbalances and toxicity of ultrabasic soils, and those that represent genetic divergence and a certain degree of reproductive isolation, thus reflecting the existence of different varieties, subspecies, and even species.

The genus *Antirrhinum* (Sutton 1988, Güemes 2009) consists of 24 perennial species (2n = 16) with relatively large flowers that are native to the western Mediterranean. Most occur as narrow endemics on the Iberian Peninsula (Webb 1972, Sutton 1988, Güemes 2009), and several are under different forms of threat (Torres *et al.* 2003, Bernardos *et al.* 2006a). Speciation within the genus probably occurred first on the Iberian Peninsula as a result of drought during the Tertiary and the climatic changes following the last Ice Age (Rothmaler 1956, Vargas *et al.* 2004).

The section *Antirrhinum* subsection *Streptosepalum* (Rothmaler 1956) (= section *Streptosepalum* following Fernández Casas 1997) includes *A. braun-blanquetii*, *A. ambiguum*, and *A. meonanthum*. However, in a recent study Mateu-Andrés and Segarra-Moragues (2003) highlighted the great affinity between *A. ambiguum* and *A. graniticum* (subsection *Antirrhinum*) and proposed the combination *A. graniticum* subsp. *ambiguum*. In contrast, Güemes (2009) considered *A. ambiguum* to be a synonym of *A. meonanthum*. Accordingly, the subsection *Streptosepalum* would only contain *A. braun-blanquetii* and *A. meonanthum*.

Antirrhinum braun-blanquetii and *A. meonanthum* are endemic to the Iberian Peninsula, where they are restricted to the north and northwest (Güemes 2009). Pinto da Silva (1970) described *A. meonanthum* var. *rothmaleri* as growing on the serpentine outcrops in the Portuguese province of Trás-os-Montes (NE Portugal), and *A. braun-blanquetii* was observed growing on ultramafic rocks in the same province (Pinto da Silva 1970, Franco 1984). Güemes (2009), however, provided no record of the latter taxon in Portugal, and according to Laínz (2000), Pinto da Silva later became unsure of his own Portuguese observations. Thus, the populations of *Antirrhinum* subsection *Streptosepalum* growing on the serpentinized rocks in NE Portugal are not well identified and hence additional studies are needed (Mateu-Andrés & Segarra-Moragues 2003). It should also be borne in mind that the

sequence data for several genes support the hypothesis that all species of *Antirrhinum* are closely related (Vieira *et al.* 1999, Vieira & Charlesworth 2001).

The aim of the present work was to characterize the populations of *Antirrhinum* growing in areas of serpentinized rocks in NE Portugal and to compare them with Iberian populations of *Antirrhinum braun-blanquetii* and *A. meonanthum* by analysing their morphometry, seeds, and karyology.

Material and methods

Morphometric analyses

A Garmin e-map GPS was used to locate the populations geographically using 1 × 1 km UTM and geographical coordinates. A representative voucher specimen from each population was collected and deposited at SALA.

Twelve populations belonging to three putative species of *Antirrhinum* were sampled for morphometric studies. The study populations were located in several provinces of Portugal and Spain (Table 1 and Fig. 1). Each population sample consisted of 16–34 individuals (275 individuals in total). Six populations were sampled for *A. braun-blanquetii* (119 plants), two populations for *A. meonanthum* (represented by 49 plants), and four populations for *Antirrhinum* inhabiting ultramafic rocks in the NE of Portugal ("*A. rothmaleri*", 107 plants). The populations of *A. braun-blanquetii* and *A. meonanthum* studied grow near the type localities.

All measurements of the selected morphological characters (Table 2 and Fig. 2) were taken from living plants in the field using an electronic digital ruler, which allowed good precision (to the nearest 0.01 mm), especially with respect to flower characteristics. Only well-developed plants with no missing parts were considered. The characters evaluated in this study were selected following the previous *Antirrhinum* studies (Rothmaler 1956, Sutton 1988) and our initial field observations with a view to including characters that might be of discriminatory value. The data used in this study are available on request from F. Amich (amich@usal.es).

Table 1. List of populations and number of *Antirrhinum* plants used for morphometric analyses (MA), karyological analyses (KA), and voucher number sin SALA. Abbreviations: PO, Portugal; SPA, Spain. Collectors: FA, F. Amich; SB, S. Bernardos; MGB, M. García-Barriuso. + = analysed, − = not analysed.

| Taxon, number and code of populations, origin and collection data	Number of plants	MA	KA	Voucher no. in SALA
Antirrhinum braun-blanquetii				
ABB1. SPA, León, Barrios de Luna, 1125 m, 42°54′21.1′′N, 5°53′18.3′′W, 29.VI.2007, FA & SB	23	+	−	135487
ABB2. SPA, León, Busdongo de Arbás, 1205 m, 30TTN8062 (42°59′3.9′′N, 5°41′34.4′′W), 15.VII.2008, FA & SB	21	+	−	−
ABB3. SPA, Cantabria, desfiladero de La Hermida, 225 m, 30TUN6895, 1.VII.2007, FA & SB	16	+	−	−
ABB4. SPA, León, Valdelugueros, Tolibia, 1225 m, 42°58′10.60′′N, 5°23′21.20′′W, 29.VI.2007, FA & SB	16	+	−	135488
ABB5. SPA, Asturias, Ribadesella, 15 m, 43°27′00.2′′N, 5°03′18.27′′W, 1.VII.2007, FA & SB	22	+	16	135489
ABB6. SPA, León, Vegacervera, 1079 m, 30TTN9253 (42°54′9.8′′N, 5°32′21.2′′W), 29.VI.2007, FA & SB	21	+	−	135490
Subtotal	119			
Antirrhinum meonanthum				
AME1. SPA, Salamanca, San Esteban de la Sierra, 750 m, 30TTK5388 (40°30′13.03′′N, 5°54′25.78′′W), 1.VII.2008, FA & SB	33	+	−	135491
AME2. SPA, Cáceres, Hervás, 790 m, 30TTK5460 (40°15′29.7′′N, 5°52′33.7′′W), 1.VII.2008, FA & SB	16	+	16	116884
Subtotal	49			
Antirrhinum rothmaleri				
ARO1. PO, Trás-os-Montes, Macedo de Cavaleiros, Gralhos, near Nuestra Señora de La Salette, 625 m, 29TPF8799 (41°31′28.0′′N, 6°44′52.0′′W), 16.VII.2007, FA, SB & MGB	34	+	−	135485
ARO2. PO, Trás-os-Montes, Macedo de Cavaleiros, Chacim, Monastery de Balsemão, 525 m, 29TPF7993 (41°28′30.1′′N, 6°51′21′′W), 26.VII.2008, FA & SB	21	+	−	135484
ARO3. PO, Trás-os-Montes, Bragança, Alimonde, 730 m, 29TPG7429 (41°47′37.8′′N, 6°53′3.58′′W), 16.VII.2007, FA, SB & MGB	31	+	16	135483
ARO4. PO, Trás-os-Montes, Bragança, Alimonde, road to Vila Boa, 670 m, 29TPG7429 (41°47′54.7′′N, 6°54′12.9′′W), 3.VII.2008, FA & SB	21	+	−	135486
Subtotal	107			
Total	275			
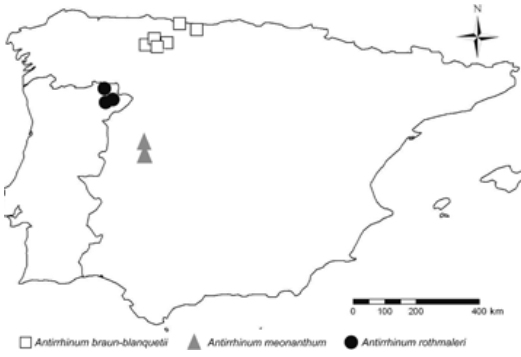
Fig. 1. Distribution of the sampled populations of *Antirrhinum* section *Antirrhinum* subsection *Streptosepalum* in the Iberian Peninsula.
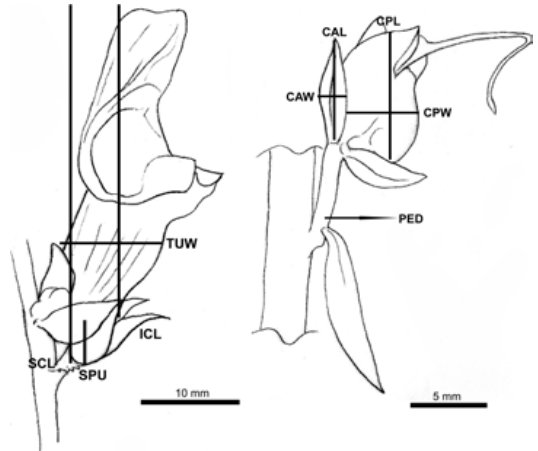
A cluster analysis (UPGMA, unweighted pair-group method using arithmetic averages; Everitt 1986), a principal component analysis (PCA) based on a Pearson correlation matrix

Fig. 2. Morphological characters examined in this study (*see* Table 2).

Table 2. Summary statistics for ten characters examined in *Antirrhinum braun-blanquetii* (ABB), *A. meonanthum* (AME), and *A. rothmaleri* (ARO). For explanation of abbreviations of morphological traits, *see* Fig. 2.

Character	Taxa	Mean	Median	SD	10–90 percentiles
CAL (calyx length, mm)	ABB	8.39	8.44	1.16	6.88–10.05
	AME	5.58	5.56	0.91	4.34–7.06
	ARO	8.64	8.44	1.31	7.13–10.43
CAW (calyx width, mm)	ABB	3.28	3.23	0.55	2.62–3.96
	AME	2.35	2.27	0.32	1.96–2.85
	ARO	3.12	2.86	0.71	2.43–4.29
PED (pedicel length, mm)	ABB	7.04	6.24	1.94	5.10–9.82
	AME	2.95	2.90	0.79	1.90–3.81
	ARO	5.82	6.15	1.02	4.55–6.76
SPU (spur length, mm)	ABB	6.28	6.46	2.15	3.60–8.94
	AME	2.92	2.85	0.49	2.36–3.67
	ARO	4.85	4.53	1.04	3.81–6.51
ICL (inferior corolla length, mm)	ABB	32.86	33.24	2.86	29.01–36.61
	AME	19.70	19.90	1.26	17.98–21.41
	ARO	24.93	24.82	1.94	22.53–27.63
SCL (superior corolla length, mm)	ABB	39.57	40.01	3.95	33.76–44.34
	AME	23.94	24.02	1.71	21.92–26.55
	ARO	29.37	29.40	1.76	27.32–31.62
TUW (corolla tube width, mm)	ABB	10.46	10.61	1.33	8.87–12.02
	AME	7.21	7.24	0.65	6.39–9.97
	ARO	9.20	9.34	1.06	7.67–10.30
CPL (capsule length, mm)	ABB	12.81	13.08	1.31	10.44–14.23
	AME	7.93	8.08	0.88	6.53–9.09
	ARO	11.18	11.06	0.96	10.02–12.74
CPW (capsule width, mm)	ABB	8.10	8.55	1.21	6.10–9.30
	AME	5.13	5.13	0.64	4.30–5.95
	ARO	6.69	6.48	1.07	5.31–8.09
NFL (number of flowers)	ABB	26.08	23.00	9.30	17.00–41.00
	AME	22.61	23.00	3.96	17.00–28.00
	ARO	41.94	44.00	13.47	21.00–57.00

(Sneath & Sokal 1973, Kraznowski 1990) and a canonical discriminant analysis (CDA; Klecka 1980) were performed on populations characterized by OTUs (operational taxonomic units; i.e., objects). Prior to the clustering, the data were standardized by zero mean and unit standard deviation, and City-block (Manhattan) distance was used to compute pairwise similarities among OTUs. UPGMA method amalgamation (analysis of variance was used to evaluate the distances of clusters) and the City-block distance metric (in contrast to the Euclidian distances, the distance measure for outliers produced by this method is dampened, since they are not squared) were used to study the similarity between the individuals, and presented graphically in a dendrogram. A biplot graphic design of the morphometric matrix was applied in PCA to analyze the Pearson correlations among the morphological variables and the individuals by factor analysis in order to obtain parsimonious descriptions of the total morphological variation (Morrison 1990). This methodology is a very useful tool for revealing cluster-unit distances and clustering between OTUs and individuals (Gabriel 1971). Finally, a discriminant canonical analysis (DCA) was used to reveal differentiations among possible groups (Hair *et al*. 1995).

Morphometric analyses were done using the SPSS version 15.0.1 (SPSS 2006) and STATISTICA software (ver. 7.0, 2004 edition).

Seed analyses

One plant sample was taken from each population for seed analyses. Some details of the seeds were studied using a scanning electron microscope (SEM). Samples were sputtered with gold in a BIO-RAD device and then observed using standard techniques under a ZEISS DSM 940 microscope.

Karyological analyses

Somatic chromosomes were studied from radicles. Seeds were collected between 2005 and 2007 from different natural populations present at several localities within the range of the species in Portugal and Spain (Table 1 and Fig. 1). Three populations (ABB5, AME2 and ARO3) were sampled for karyological analyses (*see* Table 1). Seeds were germinated on moistened blotting paper in Petri dishes at room temperature for 4–6 days. The radicles were collected and pretreated with ice-cold water for 24 h (1–2 h at 4 °C) for metaphase accumulation, and were fixed in absolute ethanol–glacial acetic acid (3:1). The fixed material was stored at 4 °C until staining with 2% acetic orcein. Mounting involved squashing in 45% acetic acid. The karyological techniques used in this study are described in detail in Bernardos *et al*. (2006b) and Amich *et al*. (2007).

At least three metaphases were drawn for each population (including 3–5 individuals) using a Kaiser camera lucida (Kaiser, Germany). A Nikon eclipse 50i microscope connected to a Nikon DS-Fi1 digital camera was used to take microphotographs. Drawings and microphotographs were deposited at the Department of Botany of the University of Salamanca. The ImageJ ver. 1.32j software was used to measure individual chromosome lengths.

Results

Morphometric characters

The characters examined and their values are listed in Table 2. The cluster and principal component analyses for all the Iberian material produced similar results at the population (Fig. 3) and at the individual (Fig. 4) levels. Three main groups, corresponding to *A. braun-blanquetii*, *A. meonanthum*, and *A. rothmaleri* as previously classified, were resolved. The three taxa were clearly separated along the first ordination axis, representing 56.75% of the total variation among populations and 52.01% of the total variation among individuals. Five out of the ten morphological traits contributed almost equally to the division along the first axis (SCL, ICL, TUW, CPL, and CPW), and four characters contributed to the separation of the groups along the second axis (CAL, CAW, SPU and NFL), as seen from the eigenvector values (*see* Table 3 and Fig. 4).

Canonical discriminant analysis (CDA, Fig. 5) based on three groups resulting from
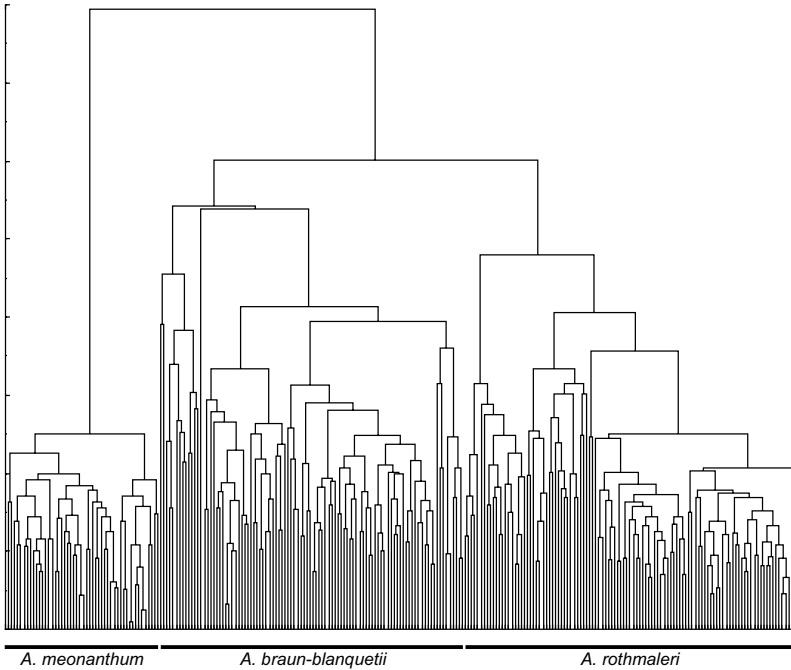
Fig. 3. Cluster analysis of Iberian individuals of *Antirrhinum* subsection *Streptosepalum*, based on ten morphological characters (*see* Table 2).
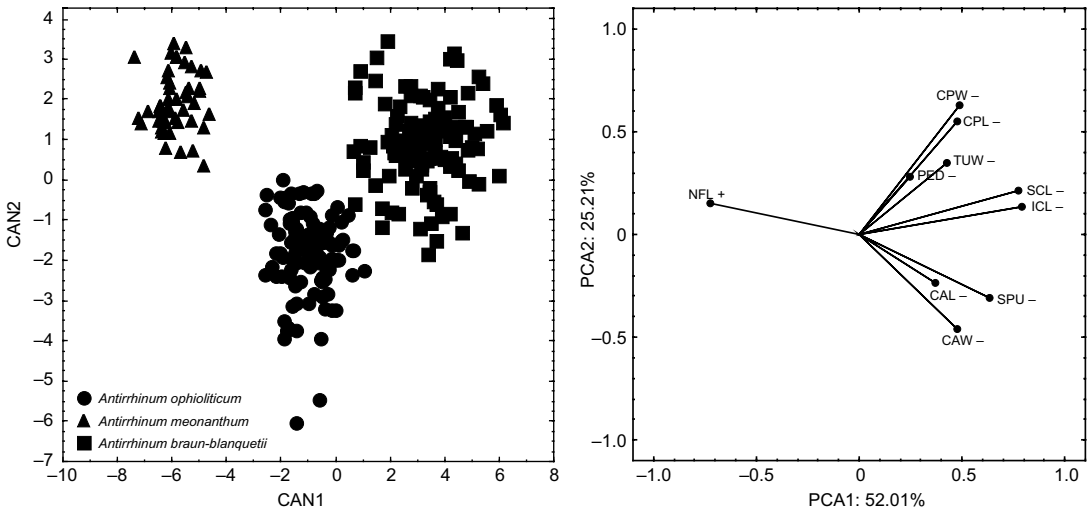

Fig. 4. Principal component analysis (PCA) of Iberian Peninsula individuals of *Antirrhinum* subsection *Streptosepalum*, based on ten morphological characters (*see* Table 2). The first two axes accounted for 52.01% and 25.21% of the total variation. For character abbreviations *see* Table 2.

PCA and cluster analyses revealed that there was enough information in the characters for a clear and unequivocal separation of all the taxa. The first canonical axis (82.84% of the variation for individuals) was the one most closely correlated with CAL, CAW, and SCL (Table 3). The characters most correlated with the second canonical axis (17.16% of the variation for individuals) were PED, SPU, and ICL (Table 3).

In order to visualise the variability in the most discriminating characters, we a created box-plots to compare their medians. As seen in Fig. 6, ICL and SCL provided sufficient discrimination among the species.

Table 3. PCA of populations of Iberian *Antirrhinum* based on ten morphological characters (*see* Fig. 4). Component loadings show contributions of the characters to principal components (PC1, PC2). CDA of populations of Iberian *Antirrhinum* based on 10 morphological characters (*see* Fig. 6). Total canonical structure expressing correlation of morphological characters with canonical axes (CAN1, CAN2) is presented. For character abbreviations *see* Table 2.

Character	PC1	PC2	CAN1	CAN2
CAL	0.015	0.836	0.683	0.610
CAW	−0.048	0.955	0.622	−0.313
PED	0.611	0.143	0.006	−0.896
SPU	0.303	0.879	0.182	−0.649
ICL	0.753	0.636	−0.005	−0.382
SCL	0.805	0.548	0.275	−0.195
TUW	0.824	0.162	0.140	−0.174
CPL	0.912	0.021	0.236	0.129
CPW	0.883	−0.058	0.088	0.147
NFL	−0.629	−0.741	−0.133	−0.004

Seed characteristics

Antirrhinum rothmaleri seeds (0.75–1 × 0.5–0.75 mm) are oblong-ovoid, irregularly crested, sometimes reticulated, black; longitudinal crests, very irregularly anastomosed and irregularly denticulate or lacerate. They are, therefore, similar in size to those of *A. meonanthum* (0.8–1.1 × 0.65–0.8 mm) but clearly smaller than those of *A. braun-blanquetii* (1–1.1 × 0.7–0.9 mm); for the main differences in seed size, morphology and ornamentation *see* Fig. 7.

● *Antirrhinum rothmaleri* (ARO3)
○ *Antirrhinum rothmaleri* (ARO2)
◔ *Antirrhinum rothmaleri* (ARO1)
◕ *Antirrhinum rothmaleri* (ARO4)
▲ *Antirrhinum meonanthum* (AME2)
△ *Antirrhinum meonanthum* (AME1)
□ *Antirrhinum braun-blanquetii* (ABB6)
■ *Antirrhinum braun-blanquetii* (ABB5)
✱ *Antirrhinum braun-blanquetii* (ABB4)
◇ *Antirrhinum braun-blanquetii* (ABB3)
★ *Antirrhinum braun-blanquetii* (ABB2)
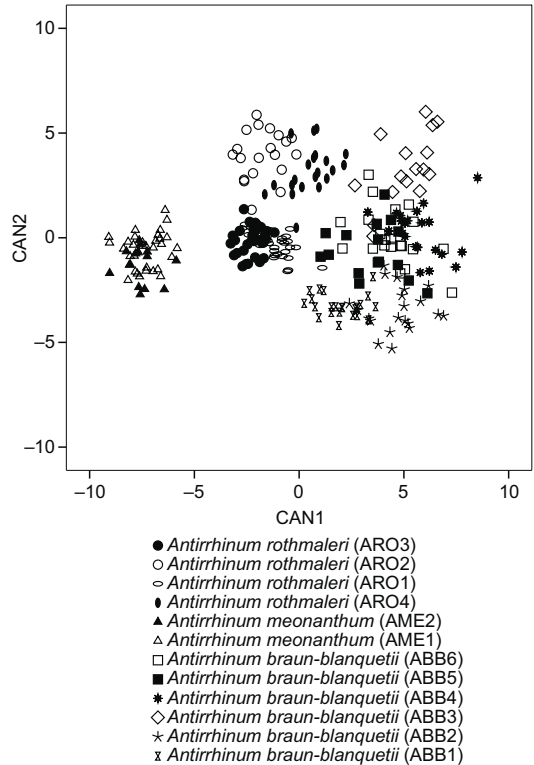⚹ *Antirrhinum braun-blanquetii* (ABB1)

Fig. 5. Canonical discriminant analysis (CDA) of individuals of Iberian representatives of *Antirrhinum* subsection *Streptosepalum*, based on ten morphological characters (*see* Table 2). The two axes explain 82.84% and 17.16% of the total variation.

Fig. 6. Variation in selected morphological traits of *A. meonanthum* (AME), *A. rothmaleri* (ARO), and *Antirrhinum braun-blanquetii* (ABB). Rectangles are 25 and 75 percentiles; squares show medians; whiskers are from 10 to 90 percentiles. For character abbreviations *see* Table 2.
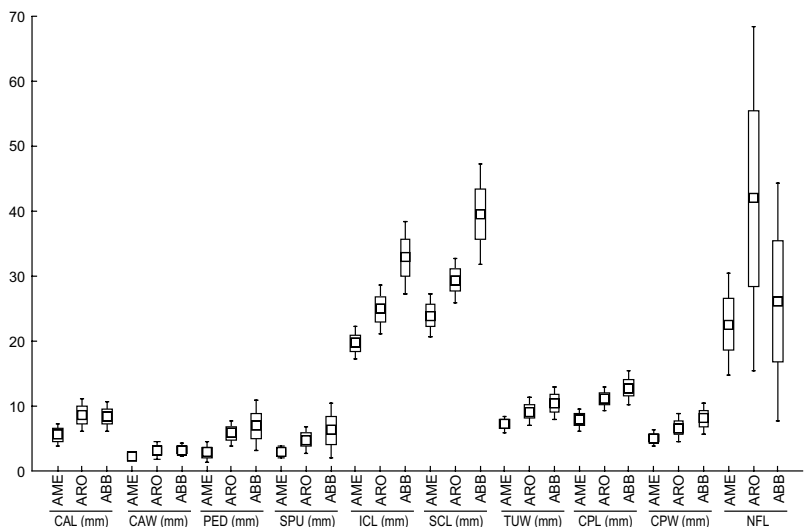
Karyology

In the subsection *Streptosepalum*, only one or two results per species have been reported (e.g. Boscaiu *et al.* 2000). *Antirrhinum rothmaleri*, like the other members of the section *Streptosepalum*, has a diploid number of $2n = 16$ (Fig. 8).

New combination

Antirrhinum rothmaleri (Pinto da Silva) Amich, Bernardos & García-Barriuso, *comb. & stat. nov.* (Figs. 9–10).

A. meonanthum var. *rothmaleri* Pinto da Silva, Agron. Lusit. 30(3–4): 223. 1970. — TYPE: Portugal. "Macedo de Cavaleiros: ad rupes in loco dicto Balsemão, solo serpentinoso et calcareo, 450 m.s.m. (P.S. & B.R. 7384, 1964, Jun. 30)." (LISE!).

A perennial herb, glabrous or glabrescent in basal and middle regions, glandular-pubescent in distal region and in inflorescence, with an indumentum of glandular hairs some 0.9–1.2 mm long; hairs multicellular, translucent, patent. Stems (40)70–80(90) cm, erect, simple or branched, glabrescent. Leaves $15–60 \times 5–20$ mm, generally opposite, occasionally uppermost leaves alternate, elliptical or lanceolate, acute or obtuse, glabrous or rarely glandular-pubescent. Petiole 2–7 mm. Inflorescence lax to dense, with 20–60 alternate flowers; bracts markedly different from leaves, $10–25 \times 2–8$ mm. Floral pedicel 5–8 mm, erect, straight, shorter than bract. Sepals $7–11 \times 2–3$ mm, ovate-lanceolate, acute, glandular-pubescent, with glandular hairs 1 mm long. Corolla 27–32 mm in length, yellow; tube 7–10.5 mm, yellowish, with yellow or purple veins, glandular-pubescent on exterior; upper labium erect and patent, with sinus 2–4 mm, and lobules 4.5–5.5 mm, ovate, convergent, with purple veins and a purple spot at base; lower labium yellowish, lacking purple veins, erect-patent; palate yellow; basal spur 2.5–5 mm, clearly standing out from sepals. Capsule $10–13 \times 5–8.5$ mm, obtuse, oblong-ovoid, glandular-pilous, with glandular hairs of up to 1 mm. Seeds 1.0–1.2 mm, oblong-ovoid, irregularly crested, sometimes reticulated, black; longitudinal crests very irregularly anastomosed and irregularly denticulate or lacerate.

SPECIMENS EXAMINED: **Portugal**. Trás-os-Montes, *Amich, Bernardos & García-Barriuso s.n.* (SALA 135482), *Amich & Bernardos s.n.* (HVR 17038, SALA 135484); Macedo de Cavaleiros, *Amich, Bernardos & Garcia-Barriuso s.n.* (HVR 17039, SALA 135485); Trás-os-Montes, *Amich & Bernardos s.n.* (SALA 135486).

Key to species of *Antirrhinum* subsection *Streptosepalum*

1. Corolla > 33(35) mm; width of tube > 10 mm; capsule > 12 mm; seeds $1–1.1 \times 0.7–0.9$ mm .... *A. braun-blanquetii*
1. Corolla < 30(32) mm; width of tube < 9.5 mm; capsule < 11 mm 2
2. Corolla < 25 mm; width of tube < 7 mm; capsule < 8 mm; seeds $0.8–1.1 \times 0.65–0.8$ mm .... *A. meonanthum*
2. Corolla (27)28–30 mm; width of tube 8–9(9.5) mm; capsule 10–11 mm; seeds $0.75–1 \times 0.5–0.75$ mm .... *A. rothmaleri*

Geographical distribution and ecology

Antirrhinum rothmaleri is known from only few locations in the *Concelhos* (local political regions) of Macedo de Cavaleiros and Bragança (Trás-os-Montes, NE Portugal). These areas are ophiolitic and are distributed over two massifs: Morais (Concelho de Macedo de Cavaleiros) and Bragança-Vinhais (Concelhos of Bragança and Vinhais), whose petrology is extremely complex and variable (Aguiar 2001). Biogeographically, they belong to the Lusitan Duriensean sector (Carpetan Leonese subprovince, Mediterranean West Iberian province). From a bioclimatic point of view they correspond to a Meso-SupraMediterranean subhumid-humid bioclimatic belt. *Antirrhinum rothmaleri* is found in thermophilous oak forests and cork-oak forests, characteristic of the Lusitan Duriensean biogeographical sector, corresponding to the associations *Genisto hystricis-Quercetum rotundifoliae* and *Junipero lagunae–Quercetum suberis*, respectively.

Discussion

Chromosome numbers have been reported for most species of *Antirrhinum* (Moore 1982), and their basic number is x = 8. Within the tribe *Antirrhineae*, this basic number is only shared
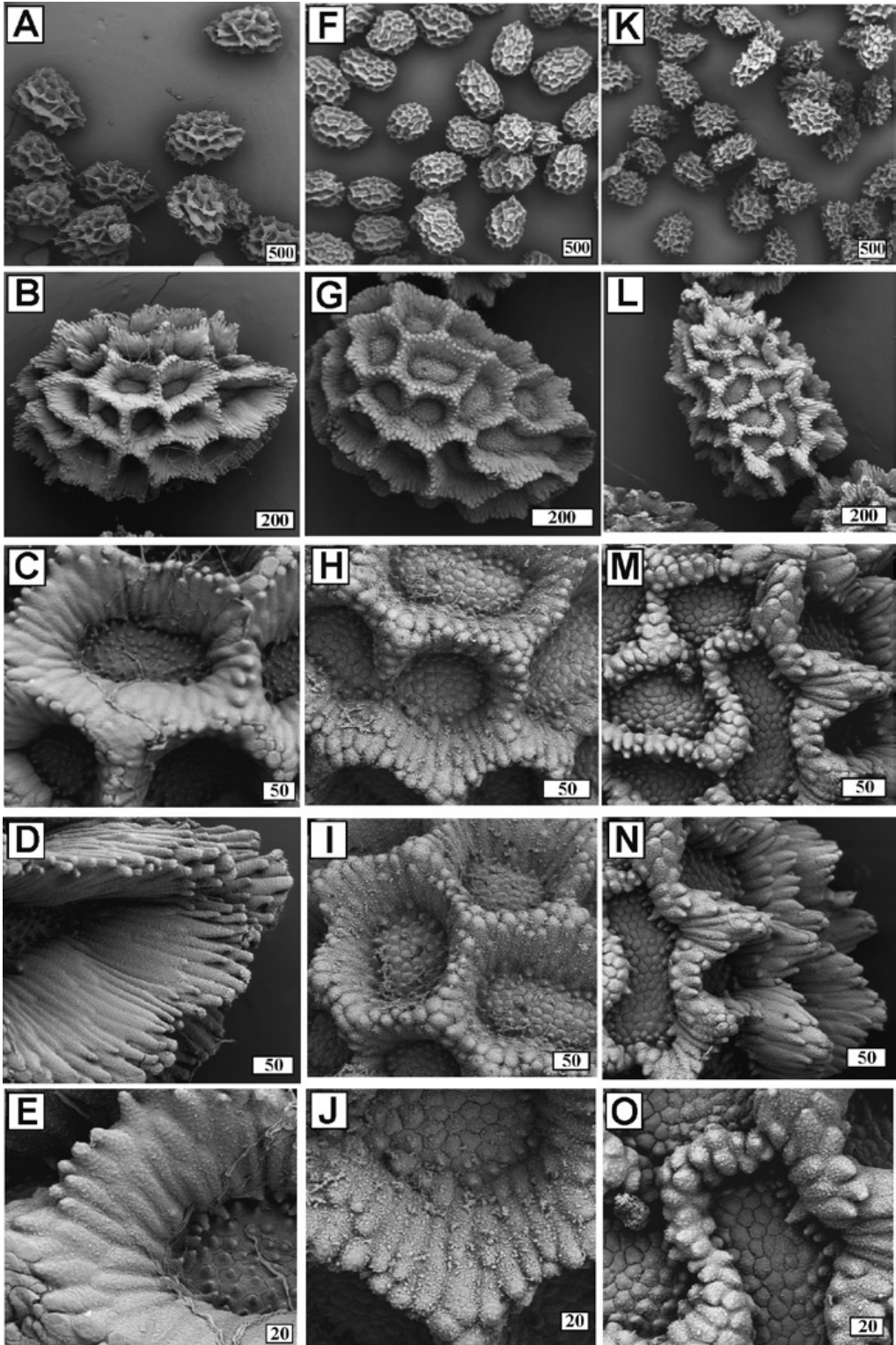
Fig. 7. SEM microphotographs showing types of ornamentation of *Antirrhinum* seeds. — **A–E**: *A. braun-blanquetii.* — **F–J**: *A. meonanthum.* — **K–O**: *A. rothmaleri.* Scale bars = μm.
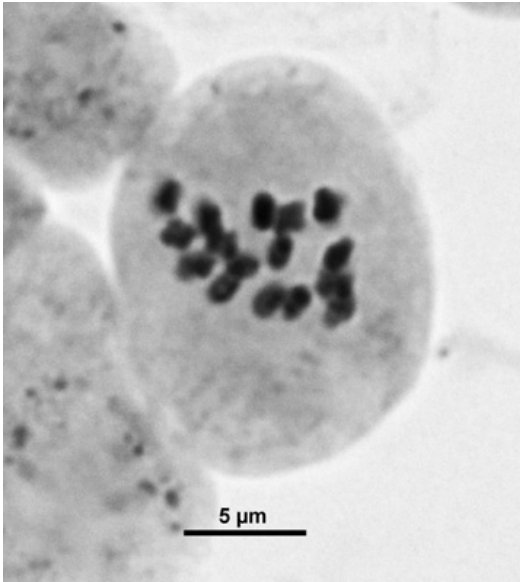
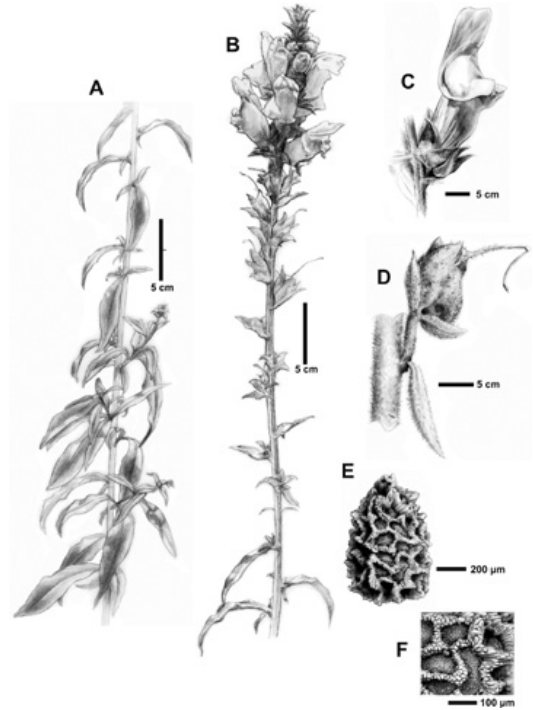**Fig. 8.** Microphotograph of *Antirrhinum rothmaleri* chromosomes. 2n = 16, mitotic prophase.

Fig. 9. *Antirrhinum rothmaleri* (from *Amich, Bernardos & García-Barriuso s.n.,* SALA 135482). — **A** and **B**: Habit. — **C**: Flower. — **D**: Capsule. — **E** and **F**: Seeds.
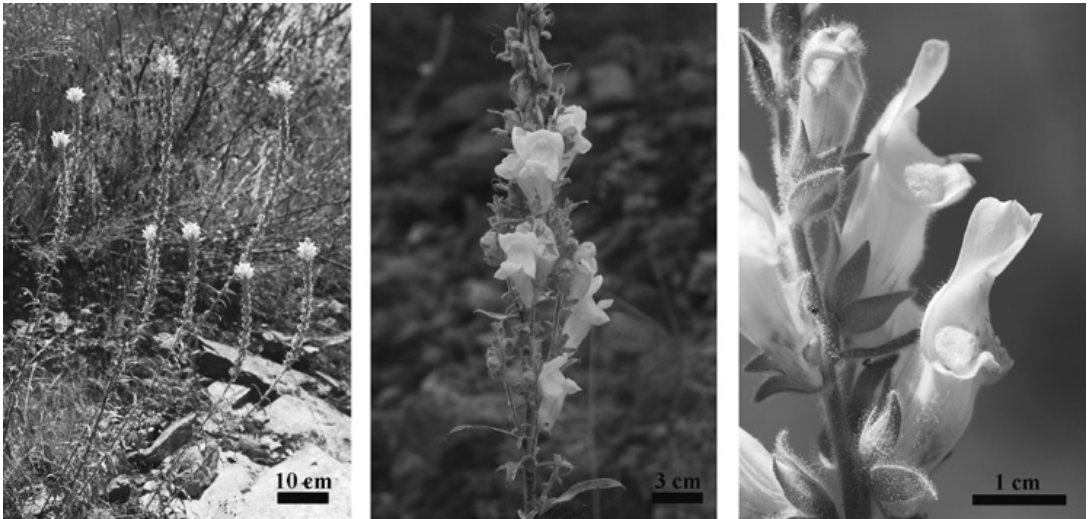

Fig. 10. *Antirrhinum rothmaleri* (from *Amich, Bernardos & García-Barriuso s.n.,* SALA 135482). — **A**: Habit. — **B**: Inflorescence and capsules. — **C**: Flowers.

with the genus *Misopates*, which is a good karyotaxonomic character (Valdes 2004). Although Fernandes *et al.* (1977) and Güemes (2009) indicated that no cases of polyploidy are known, at least in wild populations, Gallego Martín *et al.* (1984) reported a number of 2n = 24, although

this should be viewed with caution (Aedo *et al.* 2001). *Antirrhinum rothmaleri*, like the other members of the section *Streptosepalum*, is a diploid: 2n = 16. A karyological study over a broader distribution range would therefore be of great interest, especially with respect to the Iberian endemic taxa, to expand the taxonomic and geographic sampling of chromosome counts, for the numerical characterization of the karyotypes, and to examine the patterns of chromosome variation in relation to the taxonomic position.

The cluster and principal component analyses (Figs. 3 and 4, respectively) clearly separated *A. rothmaleri*, *A. braun-blanquetii* and *A. meonanthum*, which are characterized by a number of morphological traits (Table 2 and Fig. 6), the most important being the size of the corolla (ICL) and the width of its tube (SCL). Recognition of *A. rothmaleri* as a distinct species is clearly supported by the morphometric results.

Despite the morphological differences between *A. braun-blanquetii* and *A. meonanthum*, an allozyme study of six species of *Antirrhinum* by Mateu-Andrés and Segarra-Moragues (2003) revealed that the genetic distance between these two taxa was small. These authors analyzed populations growing in areas of ultramafic rocks in NE Portugal, and despite them being considered *A. braun-blanquetii s. stricto* they were clearly separable from the other populations of *A. braun-blanquetii* studied, as can be seen in the phenogram provided by Mateu-Andrés and Segarra-Moragues (2003). That phenogram also reveals clear distinctions between *A. meonanthum* var. *rothmaleri* and *A. meonanthum* var. *meonanthum*, and between *A. meonanthum* var. *rothmaleri* and *A. braun-blanquetii*. *Antirrhinum meonanthum* var. *rothmaleri* is at the same genetic distance from *A. meonanthum* var. *meonanthum* and *A. braun-blanquetii*, with the distance between the two latter being double that (Mateu-Andrés & Segarra-Moragues 2003). Thus, those authors observed clear genetic differences among the plants living in areas of ultramafic rocks in NE Portugal, which are now recognized as belonging to *A. rothmaleri*, *A. braun-blanquetii* and *A. meonanthum*. An analysis of genetic variability in different populations of *A. rothmaleri* using different molecular markers (García-Barriuso *et al.* 2009) produced similar results.

The present data clearly suggest that *A. rothmaleri* differs morphologically from *A. braun-blanquetii* and *A. meonanthum*, and this reflects the phenomena of genetic divergence and reproductive isolation rather than the nutritional imbalances and toxicity of the ultrabasic soil where *A. rothmaleri* grows.

As suggested by Kruckeberg (1992), an initial genetic discontinuity probably followed the pre-adaptation of some populations with respect to tolerance to serpentinites. The ensuing genetic-ecological isolation would then have been reinforced by further divergence of these populations. Serpentine tolerance is rare in *Antirrhinum*, and the close relationship between the three species suggests that obligate serpentinophytism in *A. rothmaleri* is an ecological autapomorphy originated *in situ* from non-serpentine ancestors, such as *A. braun-blanquetii* and *A. meonanthum*, as has been shown for other groups (Cecchi & Selvi 2009).

Ultramafic rocks support a distinctive flora with numerous uncommon or rare endemic taxa. Owing to the rarity and narrow area of distribution of some of the species studied in this work, a thorough knowledge of their reproductive system will be important for developing strategies for species management and conservation, as has been done for other taxa (e.g., Mateu-Andrés & Segarra-Moragues 2004). Nevertheless, conservation initiatives for the Portuguese serpentine flora and vegetation are urgently needed since the relatively limited extent and isolation of the ophiolitic outcrops exposes many rare and endemic species to a serious threat of environmental changes and certain events driven by human activities (e.g. mining). Many plant species inhabiting this habitat have a naturally fragmented range, and factors that will cause any further reduction of the populations are likely to lead to critical demographic thresholds.

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