# Linum flos-carmini (Linaceae), a new species from northern Morocco

José Ruiz-Martín<sup>1</sup>, Pedro Jiménez-Mejías<sup>2,3</sup>, Juan M. Martínez-Labarga<sup>2,4</sup> & Rocio Pérez-Barrales<sup>1,5,\*</sup>

- 1) Departamento de Biología Vegetal y Ecología, Universidad de Sevilla, ES-41012 Sevilla, Spain
- <sup>2)</sup> Departamento de Biodiversidad y Conservación, Real Jardín Botánico, CSIC, ES-28014 Madrid, Spain
- School of Biological Sciences, Washington State University, P.O. Box 644236, Pullman, WA 99164-4236, USA
- <sup>4)</sup> Departamento de Sistema y Recursos Naturales, Escuela de Ingeniería Forestal y del Medio Natural, Universidad Politécnica de Madrid, ES-28040 Madrid, Spain
- <sup>5)</sup> School of Biological Sciences, University of Portsmouth, Portsmouth PO1 2DY, UK (\*corresponding author's e-mail: rocio.barrales@port.ac.uk)

Received 9 Jan 2015, final version received 28 Aug. 2015, accepted 1 Sep. 2015

Ruiz-Martín J., Jiménez-Mejías P., Martínez-Labarga J.M. & Pérez-Barrales R. 2015: *Linum flos-carmini* (Linaceae), a new species from northern Morocco. — *Ann. Bot. Fennici* 52: 383–395.

Linum bicolor is a problematic North African taxon associated with L. setaceum. A consensus on the taxonomic status of a putative form (L. setaceum var. bicolor f. robusta) has not been achieved yet. We conducted a morphological study based on herbarium and field collections, combined with nuclear (ITS) and plastid (ndhF5-8 and trnL-F) phylogenies to clarify its status. The phylogenetic analysis did not reveal molecular divergence, but a comparative morphological study revealed substantial differences in traits previously used to distinguish the two taxa (stems, leaves, corolla and calyx size). In addition, we found that the indumentum of sepals, petal colour, and the arrangement of anthers and stigmas differed so clearly between the form robusta and L. setaceum s. stricto, that recognizing the former taxon as an independent species was justified. We provide an identification key to the yellow- and white-flowered Linum species in NW Africa. We also revised the unplaced name L. bicolor Schoubs. ex DC. and lectotypified it to avoid nomenclatural problems.

### Introduction

Linaceae is a cosmopolitan family of 14 genera, of which *Linum* is the most diverse (ca. 250 species). It has a wide geographic distribution, from temperate to tropical regions (McDill *et al.* 2009, 2011). The most recent taxonomic review of *Linum* recognized five sections (McDill *et al.* 2009), although the molecular data in that study

showed that some sections were not monophyletic. This illustrates how crucial it is to investigate the taxonomy and systematics of the *Linum* species and to disentangle the infrageneric classification.

The taxonomy of the *Linum* species in North Africa is particularly unclear. Linaceae was not included in the extensive (although incomplete) revision by Maire (1952–1987). His notes at the

genus level were recorded in the periodical fascicles of the *Contributions à l'étude de la Flore de l'Afrique du Nord* (cf. Lebrun & Stork 1978). More recent treatments and checklists adopted a conservative taxonomical and nomenclatural approach (cf. Quézel & Santa 1963, Greuter *et al.* 1989, Valdés *et al.* 2002, Fennane *et al.* 2007) and did neither review the genus profoundly nor include new combinations or synonyms.

Linum bicolor illustrates well the taxonomic confusion. De Candolle (1824: 428) described the species from near Tanger (Morocco) as "Species non satis notae" ("species not well known"), with a brief diagnosis in which he implicitly indicated that it was related to L. tenuifolium but distinguishable from it by the presence of "annual roots and flower colour". After that description, L. bicolor was treated by Ball (1878) as a doubtful taxon. He stated "Quid sit nescio" (literally "I do not know what it is"), and discussed its possible origin as a hybrid between L. setaceum and L. angustifolium (= L. bienne). Later, Maire (1933: 206) treated the taxon as L. setaceum var. bicolor. Maire (1933: 207) also described a new form based on a collection by Pius Font-Quer (L. setaceum var. bicolor f. robusta), presumably distinguishable by being larger than the typical form bicolor.

The field surveys conducted by the first author, and the herbarium material revised for the project Flora Iberica allowed identification of materials ascribable to L. setaceum var. bicolor f. robusta. In this study, we aimed at revisiting this taxon and clarifying its taxonomic status. We firstly conducted a morphological study revising Iberian and North African materials ascribable to L. setaceum s. lato (i.e. L. setaceum s. stricto and L. setaceum var. bicolor f. robusta). We studied vouchers from MA and SEV, which house the two main North African collections in Spain, as well as field material, and revised the digitalized material housed at JSTOR Global Plants (plants.jstor.org). We also carried out a phylogenetic analysis based on nuclear (ITS) and plastid (ndhF5-8 and trnL-F) markers to disentangle the phylogenetic relationships. Our results suggest that plants belonging to the form robusta are distinguishable from L. setaceum s. stricto at the species level. We provide a key to identify the yellow- and white-flowered Linum species from NW Africa, and revise the nomenclatural and taxonomic status of, as well as lectotypify, the name *L. bicolor* Schoubs. *ex* DC.

### Material and methods

### Morphological study

Morphological characters of nine specimens from eight collections of L. setaceum var. bicolor f. robusta, as well as of 25 specimens and collections of the Ibero-North African L. setaceum s. stricto were measured and compared (Appendix). We examined the important characters according to the taxonomic treatment in Europe and the Mediterranean (i.e. size of sepals, petals and stigmata; Quézel & Santa 1963, Ockendon & Walters 1969, López 1979, Valdés et al. 2002, Fennane et al. 2007), with particular emphasis on the characters already reported as distinctive between L. setaceum s. stricto and the form robusta (habit, leaves, corolla, calyx size; Maire 1933). Iberian and North African populations of L. setaceum s. stricto were evaluated separately to assess possible differences among them. We also searched for type materials of L. bicolor in eleven of the herbaria listed in Staffeu and Cowan (1985) as housing Schousboe's collections (B, BM, BR, C, FI, GOET, K, L, S, UPS and W).

### Molecular study

### Sampling

Our sampling covered the four sections recognized by McDill *et al.* (2009) (Table 1). We included 21 samples, 14 of which were taken from McDill *et al.* (2009) and 7 new samples from the specimens collected in the field and in the herbarium (Table 2). Two samples of *L. setaceum* (one from the Iberian Peninsula and one from Morocco), and a sample of the form *robusta* (hereafter *L. flos-carmini*) were studied, representing both known morphological extremes and distribution of *L. setaceum s. lato*. Three outgroups were included based on the previous phylogenetic studies (Savolainen *et al.* 

2000, McDill et al. 2009), namely Sclerolinon digynum, Anisadenia pubescens and Hugonia busseana (Table 2).

## DNA extraction, PCR amplification and sequencing

DNA was extracted from 15-20 mg of silicadried leaf tissue using the DNEasy Plant Minikit (QIAGEN Inc.) following the protocol of the manufacturer. We amplified and sequenced the nuclear ribosomal ITS region, and the plastid ndhF and trnL-F regions. We used the primers 17SE and 26SE (Sun et al. 1994) for PCR amplifications of ITS; for the plastid regions, we used the primers described by Jansen (1992) for ndhF and by Taberlet et al. (1991) for trnL-F. The three markers were amplified using the following PCR profile: 94 °C for 5 min; followed by 33 cycles of 94 °C for 1 min, 51 °C for 1-2 min and 72 °C for 2 min; with a final step of 72 °C for 10 min). Amplified DNA product was purified using the enzyme Exo SAP-IT® for PCR Product Clean-Up (USB laboratory, Cleveland) and sequenced in Macrogen sequencing service (Amsterdam).

### Phylogenetic analyses

We constructed a matrix for the ITS region (nuclear matrix), and another for the *ndh*F5-8 and *trn*L-F regions together (concatenated plastid matrix). To align sequences, we used MaffT 6.0 FFT-NS-I (Katoh *et al.* 2008) implemented in Geneious Pro<sup>TM</sup> 5.5.6 (Biomatters Development Team, Auckland). The resulting alignments

were checked manually and poorly aligned positions were eliminated using GBlock v0.91b (Castresana 2000), with less restrictive treatment to avoid losing information.

For each matrix, we performed maximum parsimony (MP) using TNT (Goloboff et al. 2008), maximum likelihood (ML) using RAxML ver. 7.3.1 (Stamatakis et al. 2006) implemented in Cipres Science Gateway bioportal (https:// www.phylo.org), and Bayesian inference using MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). For MP analyses, we set equal weighting of all characters. The ITS matrix was engaged with 5000 random addition replicates, and the plastidial matrix with 3000 replicates, according to computation time. Non-parametric bootstrap analysis under MP criterion took two trees per replication and TBR (tree bisection-reconnection) swapping to completion on a maximum of 20 000 minimal trees found. For the ML analysis, we performed 500 search replicates with starting topologies determined by the random stepwise addition sequence. We executed 1000 rapid bootstrap inferences to obtain bootstrap values. We performed thr Bayesian analysis under the best substitution model recovered by ModelTest 3.06 (Posada & Crandall 1998) using Akaike's information criterion. Models were calculated independently for each marker (Table 3). We ran four parallel Markov Chain Monte Carlo for 10 000 000 generations with trees sampled every 1000 generations. Two independent analyses were performed to check whether convergence to the same posterior distribution was reached. The first 1000 trees were discarded as burn-in. We estimated the 50% majority rule consensus of the remaining trees and used posterior probability as an alternative estimate of robustness.

Table 1. Sections recognized in Linum (McDill et al. 2009).

Section	Flower color	Distribution	Type species	Phylogenetic status
Syllinum Dasylinum Cathartolinum Linopsis	Yellow Rose-bluish White White or pale	Europe and W Asia E Europe to central Asia Europe and W Asia Europe, SW Asia, N, E & S Africa, N & S	L. flavum L. hirsutum L. catharticum L. quadrifolium	Monophyletic Monophyletic Monotypic Paraphyletic/
Linum	yellow Bluish	America N America, N Africa, Eurasia, Australia, New Zealand	L. usitatissimum	polyphyletic Monophyletic

Taxon	Distribution	Section	Country	Voucher	ITS	ndhF5-8	tmL-F
L. africanum	S Africa	Linopsis	South Africa, Cape Region	W.J. Hanekom 2630 (MO)	FJ169551	FJ160777	FJ160861
L. album	W Asia	Syllinum	Iran	<ol> <li>Meheregan 040948 (MJG)</li> </ol>	FJ169547	FJ160792	FJ160876
L. arboreum	Greece and Turkey	Syllinum	Turkey	U. Hecker TR0191 (MJG)	FJ169537	FJ160793	FJ160793
L. austriacum	Europe	Linum	Cultivated (MJG)	M. Repplinger 040933 (MJG)	FJ169522	FJ160799	FJ160883
L. bienne	Cosmopolitan	Linum	Italy	(MJG)	FJ169527	FJ160797	FJ160881
L. campanulatum	Spain, France	Syllinum	Spain	J. Ruiz-Martin 281542 (SEV!)	KJ194513	KJ194519	KJ194525
L. flos-carmini	N Africa	Linopsis	Morocco	J. Ruiz-Martin 281783 (SEV!)	KJ194514	KJ194520	KJ194526
L. hirsutum	E Mediterranean	Dasylinum	Cultivated (MJG)	M. Repplinger 040935 (MJG)	FJ169520	FJ160788	FJ160872
L. macraei	Chile and Peru	Linopsis	Chile	P. Aravena 33388 (MO)	FJ169544	FJ160782	FJ160866
L. mucronatum	E Mediterranean	Syllinum	Turkey	J. Ruiz-Martin 281543 (SEV!)	KJ194515	KJ194521	KJ194527
L. narbonense	Europe	Linum	Spain	J. Ruiz-Martin 281544 (SEV)	KJ194516	KJ194522	KJ194528
L. perenne	Europe	Linum	Cultivate (MJG)	M. Repplinger 040929 (MJG)	FJ169524	FJ160802	FJ160886
L. pubescens	E Mediterranean	Dasylinum	Syria	W. Licht SYR351 (MJG)	FJ169518	FJ160790	FJ160874
L. setaceum 1 (Fig. 5)	W Mediterranean	Linopsis	Spain	J. Ruiz-Martin 281545 (SEV)	KJ194517	KJ194523	KJ194529
L. setaceum 2 ("L. bicolor")	W Mediterranean	Linopsis	Morocco	J. Ruiz-Martin 251249 (SEV)	KJ194518	KJ194524	KJ194530
L. strictum	Cosmopolitan	Linopsis	Italy	W. Licht 040945 (MJG)	FJ169530	FJ160806	FJ160890
<ul><li>L. suffruticosum.</li></ul>	W Mediterranean	Linopsis	Spain	C. Navarro et al.CN 2339 (TEX)	FJ169532	FJ160807	FJ160891
L. tenue	W Mediterranean	Linopsis	Spain	M. Quint 040938 (MJG)	FJ169548	FJ160808	FJ160892
L. tenuifolium	Europe and	Linopsis	Germany	M. Kropf 040934 (MJG)	FJ169529	FJ160809	FJ160893
	E Mediterranean						
L. volkensii	E Africa	Linopsis	Tanzania	R.E. Gereau & C.J. Kayombo	FJ169531	FJ160813	FJ160897
				400Z (MO)			
Anisadenia pubescens	E Asia	ī	China	Bartholomew 1011 (GH)	FJ169513	FJ160772	FJ160856
Hugonia busseana	CE Africa	1	Malawi	M.W. 0029 (MJG)	FJ169512	FJ160773	FJ160857
Sclerolinon digynum	W USA	1	USA (California)	M.S. Taylor 3935 (TEX)	FJ169541	FJ160787	FJ160871

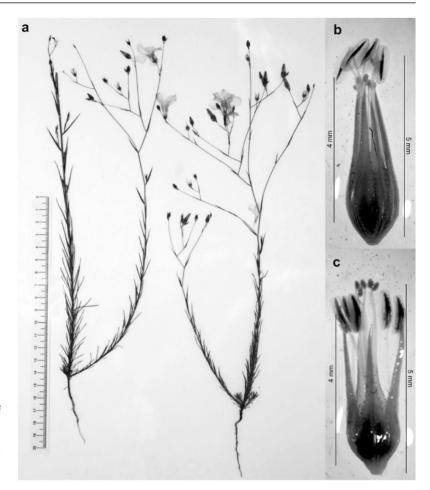


Fig. 1. Linum flos-carmini (holotype). — a: Holotype specimen. — b: Shortstyled flower without petals. — c: Long-styled flower without petals.

### Results

### Morphological study

The characters that distinguished *L. setaceum* from *L. flos-carmini* matched those reported by Maire (1933) to distinguish what he called the form *robusta* from *L. setaceum s. stricto*: stem length, leaf nervation and leaf length, sepal length and petal size (Figs. 1 and 2; *see* Table 4 with

measurements and ranges of variation). The variation thresholds did not overlap between the two species (petal size) or did so very slightly (leaf length, sepal length). In addition, we found other traits that allowed differentiation between the species: sepal indumenta, petal colour and arrangement of anthers and stigmas (Table 4). *Linum setaceum s. stricto* has sepals with the margin fimbriate in the lower half, yellow and weakly nerved petals, and homostylous flowers (anthers

Table 3. Sequence data information.

Parameters	ITS	ndhF5-8	<i>trn</i> L-F	Combined cpDNA matrix
Number of sequences	23	23	23	23
Missing data	n.a.	1.7%	n.a.	0.6%
Aligned length (bp)	623	440	970	1410
Variable characters	378	234	621	855
Model selected (AIC)	GTR + G	GTR+I+G	GTR + G	GTR+I+G



Fig. 2. Linum setaceum.

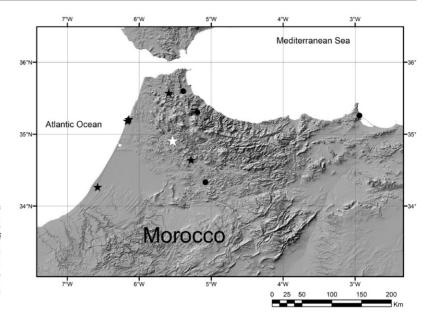
— a: Voucher J. Ruiz-Martin 281545 (SEV) with typical morphological characters. — b: Detail of the flower, where anthers and stigmas are placed at the same height.

and stigmas positioned at the same height). *Linum flos-carmini* has sepals with the margin fimbriate to glandulose-ciliate in the lower half, yellowish-whitish petals with purple nerves towards the base, and heterostylous flowers with two morphs, pin flowers with long-styled with stigmas above the anthers, and short-styled or thrum flowers with stigmas below the anthers. No significant differences were observed between the North African and Iberian populations of *L. setaceum* (Table 4).

Linum setaceum s. stricto is an Iberian—North African taxon, confined in Morocco to the Rif mountains (Fig. 3). Linum flos-carmini is a North African endemic in the Atlantic-facing plains and valleys of NW Morocco (Fig. 3). Below we provide an identification key to dis-

tinguish white- and yellow-flowered *Linum* species in North Africa, and detailed morphological descriptions of *L. setaceum s. stricto* and *L. floscarmini* (Fig. 3).

We could not find type specimens of the name *L. bicolor* Schoubs. *ex* DC. in B, BM, BR, C, FI, K, L, S, UPS or W. However, in GOET we found a voucher collected by Philipp Salzmann ("circa Tindigem"), a contemporary of Schousboe in North Africa (cf. Stafleu & Cowan 1985). That specimen might be more related to Schousboe's taxonomic concept of *L. bicolor* than other material so named later. Accordingly, *L. bicolor* is lectotypified on this specimen (*see* below) to ensure an accurate use of the name and prevent future nomenclatural problems.



**Fig. 3.** Distribution of *Linum setaceum* (black dots) and *L. flos-carmini* (stars: white = population sampled for the molecular study, black = all other known populations) in Morocco.

### Molecular study

Phylogenetic analysis of the ITS and the two combined-plastid regions (Figs. 4 and 5) gave similar topologies, both in accordance with the previous results (Savolainen *et al.* 2000, McDill *et al.* 2009). In both phylogenetic reconstructions (ITS and plastid) *Linum* was well-supported and it included *Sclerolinum dyginum*. The genus was divided in two main clades, one containing the sections *Linum* and *Dasylinum*, and the other

containing the sections *Linopsis* and *Syllinum*, as well as *Sclerolinum dyginum*. All sections were monophyletic except *Linopsis*. In the ITS phylogeny, this section was divided into a basal grade (*L. africanum* and *L. macraei*) and a well-supported clade in which the section *Syllinum* was nested. In the plastid phylogeny, three well-supported clades emerged: the first containing *L. africanum*, *L. macraei* (both section *Linopsis*) and *Sclerolinum digynum*, the second with most of the taxa in the section *Linopsis*, and the third

**Table 4.** Main morphological characters of *Linum setaceum s. stricto* populations from North Africa and the Iberian Peninsula, and of *Linum flos-carmini*.

Character	Linum setaceum (North Africa)	Linum setaceum (Iberian Peninsula)	Linum flos-carmini
Stems	10–40 cm, with hairs up to 0.3 mm long	6–55 cm, with hairs up to 0.3 mm long	20–75 cm, with hairs up to 0.5 mm long.
Leaves	1-nerved, those from upper third of stem 5–28.5 × 0.15– 1.25 mm	1-nerved, those from upper third of stem 6–28 × 0.1–1.3 mm	3-nerved, those from upper third of stem $16-42 \times (0.5)1-3$ mm
Sepals	$4.5$ – $6.5 \times 1.1$ – $1.55$ mm, 1-nerved, margin fimbriate in lower half	4.5–6.3 × 1.1–1.5 mm, 1-nerved, margin fimbriate in lower half	(5)6–7.8(8.4) × 1.3–2 mm, 3-nerved, margin fimbriate to glandulose-ciliate in lower half
Petals	8–19 $ imes$ 3–6 mm, yellow, rarely with bluish nerves	8–14 × 3–5 mm, yellow, weakly nerved	17–23 × (7)12–14 mm, white to yellowish-whitish, with purple nerves, more marked towards base, in blossom yellowish with bluish nerves
Flowers	homostylous	homostylous	heterostylous

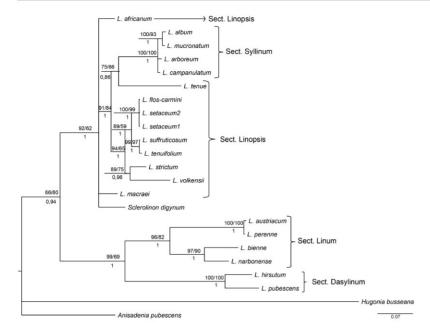


Fig. 4. Phylogeny of ITS from *Linum* and selected outgroup genera from Linaceae. Bayesian consensus tree. ML bootstrap/MP bootstrap values are given above the branches, posterior probability below the branches.

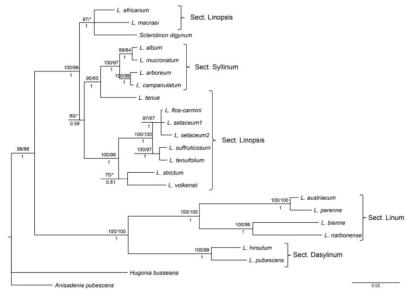


Fig. 5. Phylogeny of combined *ndh*F5-8 and *trn*L-F from *Linum* and selected outgroup genera from Linaceae. Bayesian consensus tree. ML bootstrap/MP bootstraps are given above the branches, posterior probability below the branches.

with the section *Syllinum* and *L. tenue* (section *Linopsis*).

The two *L. setaceum* samples and the *L. flos-carmini* sample were placed as sisters to *L. suffruticosum* and *L. tenuifolium* in all the phylogenetic reconstructions, within a well-supported clade with the section *Linopsis*. However, our phylogenetic reconstructions did not resolve the relationships between *L. setaceum* and *L. flos-*

carmini, which appeared unresolved in a polytomy. No autapomorphic molecular characters were found in the L. flos-carmini sample.

Sequence information for all markers, including aligned length, informative positions, indels, missing data, and best evolutionary model according to ModelTest (*see* Material and methods) is summarized in Table 4.

### Descriptions of Linum flos-carmini and L. setaceum s. stricto

**Linum flos-carmini** Ruiz-Martín, Mart. Labarga, Jim. Mejías & Pérez-Barrales, *sp. nova* (Fig. 1)

HOLOTYPE: Morocco. Ouazzane, Secteur forestiere de Bellouta, Comune rurale de Brickcha, Jbel Karkar, 34°54.555′N/5°32.193′W, 130 m a.s.l., 25 May 2011 *J. Ruiz Martín & M. Benabent Burguer 166JRM11* (SEV 162513; isotype SEV162513).

Linum setaceum var. bicolor f. robusta Maire, Bull. Soc. Hist. Nat. Afrique N. 24: 207. 1933. — LECTOTYPE (here designated): Morocco. Larache, Font-Quer, Iter marocc. 1930, n° 406 (MA; isotype MPU).

ETYMOLOGY: Dedicated to Carmiña Martín-Cacao, José Ruiz-Martín's mother. The epithet *flos-carmini* means "the flower of the song".

Annual, 20-75 cm, pubescent, with hairs up to 0.5 mm long, whitish, terete, striate, straight to ± curved. Stems single, erect, ± densely pubescent, with hairs irregularly distributed across stem surface, more abundant towards base. Leaves herbaceous, those from upper third of stem 16-42  $\times$  (0.5)1–3 mm, alternate, sessile, deciduous after fructification, erect to erect-patent, densely arranged, imbricate, from sublinear to narrowly lanceolate, subulate at apex, sometimes lowermost with rounded apex, 3-nerved, pubescent, with hairs in lower half, and antrorse prickles and shiny papillae towards the apex, frequently involute. Inflorescence a dichasium cyme, subcorymbiform, ± laxous, regularly zigzag branched, with (15)40-70 flowers. Bracts  $9-25 \times 1-2.5$  mm. sublinear to lanceolate, alternate. Pedicels erectpatent, growing up to 30 mm long in fructification. Flowers bisexual, heterostylous, with one morph having stamens below stigma level, and other with stamens above stigma level. Sepals (5)6- $7.8(8.4) \times 1.3-2$  mm, ovate-lanceolate, outer ones gradually attenuate into a long mucro, inner ones more abruptly contracted into an acumen, keeled, 3-nerved, central one strongly marked, with a hyaline margin, fimbriate to glandulose-ciliate in lower half, antrorsely-scabrid towards apex. Petals free,  $17-23 \times (7)12-14$  mm, ovate-spathulate, gradually attenuate towards base, mucronulate at apex, white to yellowish-whitish, yellow prior to antesis, with purple nerves, more marked towards base, in blossom yellowish with bluish

nerves. Stigmata 5, capitate. Fruit an ovate capsule with ten brown seeds, their measurements unknown. Flowering in May–June.

This species grows in Mediterranean shrub land on basic soils. It is distributed in the Atlantic drainage of NW Morocco (Fig. 3). Heterostyly was confirmed in field and herbarium material. A total of 100 flowers from the visited population in Ouazzane had 55 long-styled pin-flowers and 45 short-styled thrum-flowers.

### Linum setaceum Brot. (Fig. 5)

Fl. Lusit. 1: 484. 1804. — Type: "Hab. in collibus calcareis prope Conimbricam".

Linum bicolor Schousb. ex DC., Prodr. [A.P. de Candolle] 1: 428. 1824. — Lectotype (here designated): "In collibus apricis circa Tingidem", Salzmann s.n. (GOET). — Linum setaceum var. bicolor (Schoubs. ex DC.) Maire, Bull. Soc. Hist. Nat. Afrique N. 24: 206. 1933.

Annual, 6–55 cm, pubescent, with hairs up to 0.3 mm long, whitish, terete, striated, straight to ± curved. Stems frequently single, erect, sometimes ramified in up to 10 different branches from base, ± densely pubescent, with hairs irregularly distributed across stem surface, more abundant towards base. Leaves herbaceous, those from upper third of stem  $6-28 \times 0.1-1.3$  mm, alternate, sessile, deciduous after fructification, erect to erect-patent, densely arranged, imbricate, from sublinear to narrowly lanceolate, subulate at apex, sometimes lowermost with a rounded apex, 1-nerved, pubescent, frequently involute, with whitish antrorse prickets in margins. Inflorescence a dichasium cyme, subcorymbiform, ± laxous, regularly branched, with 5–40(55) flowers. Bracts  $5-15 \times 0.1-0.9$  mm, sublinear, alternate, sometimes up to 3 bracts under one pedicel. Pedicels erect-patent, growing up to 30 mm long in fructification. Flowers bisexual, homostylous, with stamens at same level as stigmata. Sepals  $4.5-6.3 \times 1.1-1.5$  mm, 1.2-1.7 times longer than fruit, ovate-lanceolate, outer ones gradually attenuate into a long mucro, inner ones more abruptly contracted into an acumen, keeled, 1-nerved, sometimes with two additional poorly-marked lateral nerves at base, with a hyaline margin, fimbriate in lower half, antrorsely-scabrid towards apex. Petals free,  $8-14 \times 3-5$  mm, spathulate, gradually attenuate towards base, mucronulate at apex, yellow, weakly nerved. Stigmata 5, capitate. Capsule 3–4 mm length, subglobose, abruptly contracted in base, with a mucro up to 0.3 mm long in apex, glabrous, yellowish-brown. Seeds  $1.8-2\times0.9-1$  mm, subreniform, brownish, shiny, weakly striate, with one side concave and slightly winged. Flowering in May–June.

This species grows in grasslands and clearings within Mediterranean shrub vegetation. It is distributed in S and W Iberian Peninsula and NW Morocco in the Rif Mountains (Fig. 3). The North African forms called var. *bicolor*, with yellow petals turning purplish-bluish toward the base, may deserve taxonomic recognition at infraspecific level.

#### Discussion

### Linum flos-carmini and L. setaceum s. stricto

The data obtained in the morphological study not only supported the morphometric differences reported by Maire (1933), but also showed that there is no or very scarce overlap between the distinguishing characters (Table 4, Figs. 1 and 2). Such a small morphological overlap between species (less than 25%) is thought to be acceptable (Valcárcel & Vargas 2010, Jiménez-Mejías *et al.* 2014).

We also found additional features that help to distinguish *L. setaceum s. stricto* from *L. flos-carmini*: sepal indumenta, petal colour, and especially the arrangement of anthers and stigmas (Table 4). This last character is vital for the sexual reproduction, and it has been reported as a key trait to characterize other *Linum* taxa such as South African *L. heterostylum* and *L. comptoni* (Rogers 1981). In an extensive study of the genera in the Mediterranean Basin, where the group is more diverse, we never detected any species with heterostylous and homostylous populations. The above evidence together supports the status of *L. flos-carmini* as a species distinct from *L. setaceum*.

The molecular study revealed a close relationship between *L. setaceum* and *L. flos-carmini*. The taxa are allopatric, *L. setaceum* s.

stricto being distributed mainly north of the Rif Mountains, while L. flos-carmini inhabits the Atlantic NW Morocco (Fig. 3). However, a similar lack of variation found for L. setaceum s. stricto and L. flos-carmini in the studied sequences also occurs in other closely-related species of Linum, such as L. tenuifolium aggr. Future studies with microsatellites or AFLPs could shed light on the deeper taxonomic relationships within the section Linopsis.

### Identity of Linum bicolor

Linum bicolor was described in De Candolle's Prodromus (1824) from near Tanger (Morocco) as "a not well known species". Later, Ball (1878) also considered the taxon doubtful. The most recent reference to L. bicolor is from Maire (1933), who considered that the taxon was a variety of L. setaceum. Maire distinguished it from the type variety by its slightly larger petals ("c. 1.5 mm longis"), which are yellow, turning purplish-bluish toward the base (instead of being entirely yellowish), as well as by the larger sepals (5 mm long). He cited the taxon from Tanger ("Tingidem") and from an additional unplaced location "in valle amnis Miqqes".

The voucher collected by Philipp Salzmann from North Africa is accurate material to typify the name Linum bicolor Schousb, ex DC, Several arguments support this view. First, Peter K.A. Schousboe was a contemporary of Salzmann's (cf. Stafleu & Cowan 1985), and it is likely that both botanists were in direct contact and probably exchanged plant specimens. Second, the collection site of Salzmann's voucher "in collibus apricis circa Tingidem" matches the indicatio locotipica cited by De Candolle (1824) as "prope Tanger". Third, the collection of the material by Salzmann could be coetaneous or predate the publication of the name in 1824, despite no date was provided in the herbarium label. Staffeu and Cowan (1985) established that Salzmann had visited North Africa in 1823–1825. Therefore, we designate Salzmann's specimen as the lectotype of L. bicolor, preventing further problems with the application of the name.

Regarding the species revalorized in this paper, based on the form *robusta*, the morpho-

logical evidence presented here shows that both Salzmann's specimen and the description provided Maire (1933), fit the variation observed in *L. setaceum*, with the single exception of the petals turning purplish-bluish towards the base. We consider that further studies are necessary to evaluate if these forms deserve taxonomic recognition within *L. setaceum*.

### Identification key

With this key it should be possible to distinguish *L. flos-carmini* from species and species complexes in *Linum* having yellow or whitish flowers (all from the section *Linopsis*) from NW Africa.

- 7. Petals 8-14 mm long, yellow; sepals 4.5-6.3 mm long,

- fimbriate in lower half; homostylous .......... *L. setaceum*7. Petals 17–23 mm long, white to yellowish-white, with bluish venation; sepals (5)6–7.8(8.4) mm long, fimbriate

### Acknowledgements

We thank curators and staff of B, BM, BR, C, FI, G, GOET, K, L, MA, SEV, UPOS, UPS and W for loan of specimens and search of type material of *L. bicolor*; F. Muñoz-Garmendia and S. Talavera for comments on *Linum* nomenclature; J. Arroyo for comments during the development of this work; M. Benabent-Burguer kindly collaborated in the field trip around Morocco. This research was supported by the Spanish Ministry of Science and Innovation through the project CGL2009-12565 and the associated FPI grant BES-2008-003946 to José Ruiz-Martín. The collection of samples complies with the current laws of the country in which it was performed.

#### References

- Ball J. 1878: Spicilegium florae maroccanae. *The Journal of the Linnean Society, Botany* 16: 281–772.
- Castresana J. 2000: Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis.

   Molecular Biology & Evolution 17: 540-552.
- De Candolle A.P. 1824: Prodromus systematis naturalis regni vegetabilis sive Enumeratio contracta ordinum generum specierumque plantarum huc usque cognitarium, juxta methodi naturalis, normas digesta, Pars prima: Sistens Thalamiflorarum Ordines LIV. Treuttel et Würtz, Parisiis.
- Engler A. 1908: Linaceae africanae. II. Botanische Jahrbücher fur Systematik, Pflanzengeschichte und Pflanzengeographie 40: 45–47.
- Fennane M., Ibntato M., Martez J., Ouyahaya A. & El Oualidi J. 2007: Flore pratique de Maroc, manuel de détermination des plantes vasculaires. — Institut Scientifique, Rabat.
- Goloboff P.A., Farris J.S. & Nixon K.C. 2008: TNT, a free program for phylogenetic analysis. — *Cladistics* 24: 774–786.
- Greuter W., Burdet H. & Long G. 1989: *Med-checklist*, vol. 4. Conservatoire et Jardin Botaniques, Genève.
- Griffith W. 1847–1848: Posthumous papers: Journals of Travels in Assam, Burma, Bootan, Afghanistan and the neighbouring countries: (Itinerary, notes on Plants col-

- lected in the Khasyah and Bootan Mountains, 1837-38, in Afghanistan and neighbouring countries, 1839-41).

   Bishop's College Press, Calcutta.
- Hudson W. 1778: Flora Anglica: exhibens plantas per Regnum Britanniae sponte crescentes, distributas secundum systema sexuale: cum differentiis specierum, synonymis auctorum, nominibus incolarum, solo locorum, tempore florendi, officinalibus pharmacopaeorum. Tomus II. — J. Nourse, Londini
- Jansen R.C. 1992: A general mixture model for mapping quantitative trait loci by using molecular markers. — Theoretical and Applied Genetics 85: 252–260.
- Jiménez-Mejías P., Luceño M. & Martín-Bravo S. 2014: Species boundaries within the southwest Old World populations of the Carex flava group (Cyperaceae). — Systematic Botany 39: 117–131.
- Katoh K. & Toh H. 2008: Recent developments in the MAFFT multiple sequence alignment program. — Briefings in Bioinformatics 9: 286–298.
- Lebrun J.P. & Sork A.L. 1978: Index general des "Constributions à l'étude de la flore de l'Afrique du nord" du Dr. René Maire. — IEMVT, Maisons-Alfort.
- López G. 1979: Algunas consideraciones sobre los linos del grupo *Linum tenuifolium* L. en España. *Bulletin de la Société Botanique de Genève* 1: 100–109.
- Maire R. 1933: Contributions à l'étude de la Flore de l'Afriqu du Nord 20. — Bulletin de la Société d'histoire naturelle d'Afrique du Nord 24: 194–232.
- Maire R. 1952–1987: Flore de l'Afrique du Nord, vols. 1–16.

   Paul Lechevalier. Paris.
- Miller P. 1768: Gardeners dictionary. John & Francis Livington, London.
- McDill J.R., Repplinger M., Simpson B.B., Kadereit J.W. & Schwarzbach A. 2009: The phylogeny of *Linum* and Linaceae subfamily Linoideae, with implications for their systematics, biogeography, and evolution of heterostyly. *Systematic Botany* 34: 386–405.
- McDill J.R. & Simpson B.B. 2011: Molecular phylogenetics of Linaceae with complete generic sampling and data from two plastid genes. — *Botanical Journal of the Lin*nean Society 165: 64–83.
- Ockendon D.J. & Walters S.M. 1968: *Linum L.* In: Tutin T.G., Heywood V.H., Burges N.A., Moore N.A., Val-

- entine D.H., Walters S.M. & Webb D.A. (eds.), *Flora Europaea*, vol. 2: 206–211. Cambridge University Press, Cambridge.
- Posada D. & Crandall K.A. 1998: ModelTest: testing the model of DNA substitution. — *Bioinformatics: Applica*tions Note 14: 817–818.
- Quezel P. & Santa S. 1963: Nouvelle flore de l'Algerie: et des regions desertiques meridionales. — Centre National de la Recherche Scientifique, Paris.
- Rogers C.M. 1966: Sclerolinon, a new genus in the Linaceae.
   Madroño 18: 181–184.
- Rogers C.M. 1981. A revision of the genus *Linum* in southern Africa. *Nordic Journal of Botany* 1: 711–722.
- Ronquist F. & Huelsenbeck J.P. 2003: MrBayes 3: Bayesian phylogenetic inference under mixed models. — *Bioin*formatics 19: 1572–1574.
- Savolainen V., Chase M.W., Hoot S.B., Morton C.M., Soltis D.E., Bayer C. & Fay M.F. 2000: Phylogenetics of flowering plants based on combined analysis of plastid atpB and rbcL gene sequences. Systematic Biology 49: 306–362.
- Staffeu F.A. & Cowan R.S. 1985: Taxonomic literature, vol. 5. Sal–Ste. *Regnum Vegetabile* 112: 1–1066.
- Stamatakis A. 2006: RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690.
- Sun Y., Skinner D.Z., Liang G.H. & Hulbert S.H. 1994: Phylogenetic analysis of Sorghum and related taxa using internal transcribed spacers of nuclear ribosomal DNA. — Theoretical and Applied Genetics 89: 26–32.
- Taberlet P., Gielly L., Pautou G. & Bouvet J. 1991: Universal primers for amplification of three non-coding regions of chloroplast DNA. — *Plant Molecular Biology* 17: 1105–1109.
- Valdés B., Rejdali M., Achhal El Kadmiri A., Jury J.M.S.L. & Montserrat M. 2002: Catalogue des plantes vasculaires du Nord du Maroc incluant des clés d'identification, vol. 2. — Consejo Superior de Investigaciones Científicas, Madrid.
- Valcárcel V. & Vargas P. 2010: Quantitative morphology and species delimitation under the general lineage concept. Optimization for *Hedera* (Araliaceae). — *American Journal of Botany* 97: 1555–1573.

### **Appendix**

### Specimens used in the morphological study

Linum setaceum: Morocco. Hab. in saxosis calc. Promontorii Ras Sidi-elAhbed (Bocoia), Font-Quer 290 (MA); 2ª caseta, Riff, 11 June 1915 A. Caballero (MA); Djebel Derza, Mas Guindal (MA); Riff, Vîdal & López 762 (MA); NE of Chefchaouen, S.L. Jury 19333 et al. (MA). Portugal. Algarve, Malato-Beliz & J.A. Guerra 15820 (MA); Beira Litoral, 21 June 1981, E. Rico s.n. (MA); Beira Litoral, June 1902 G. Sampaio s.n. (MA); Beira Litoral, June 1915 Sampaio s.n. (SEV); Estremadura, J. Malato-Beliz & J.A. Guerra 10961 (MA); Alto Alentejo, J. Malato-Beliz & J.A. Guerra 14364 (MA). Spain. Cádiz, 3 June 1922 E. Gros s.n. (MA); Cádiz, 10 July 1983 A. Aparicio (MA); Cádiz, 11 May 1980 A. Aparicio s.n. (SEV); Málaga, C. Navarro et al. CN2274 (MA); Málaga, 11 May 1931 C. Vicioso s.n. (MA); Granada, 6 June 1980 M. Ladero et al. s.n. (MA); Sevilla, 13 July 1978 J.A. Devesa et al. s.n. (SEV); Sevilla, 23 May 1982 J. Arroyo & J.M. Gil s.n. (SEV); Córdoba, J. Muñoz s.n. 6387/80 (SEV); Córdoba, 27 May 1978 J.M. Muñoz s.n. (SEV). — Linum flos-carmini: Morocco. Ouazzane, J. Ruiz Martín & M. Benabent Burguer 166JRM11, (type material SEV162513, SEV281783). Paratypes: Larache, Iter marocc. 1930, Font-Quer 405 (MA), MPU); L. setaceum var. bicolor f. robusta lectotypes); hab. In argillosis, pr. El Araix, 1. Txumix, 25 May 1930 Font-Quer 405 (MA); Djebala, Ouezzan, coteux calcaires, 5 May 1929 J. Jahandiez 131 (MA); de Tanger al Fondak 2 May 1921, C. Pau s.n. (MA); Lixus (Larache), 25 June 1923 A. Caballero s.n. (MA); Kenitra, 12 km S Ksar-elKebir an der Strasse nach Kenitra (P2), 100 m, sandige Flächen, 4 May 1987 D. Podlech 43616 (MA); Ouezzane, Mjara, entre Ain Dorij y Teroual, pastizal cerca del rio, 160 m, 22 May 2004 F.J. Márquez et al. s.n. (SEV251249).