



A new polyploid species of *Limonium* (Plumbaginaceae) from the Western Mediterranean basin

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Abstract

A new species of Plumbaginaceae, *Limonium irtaensis*, is described and illustrated from the Western Mediterranean basin (Iberian Peninsula). The new species is triploid ($2n = 26$) and shows a papillate stigma and pollen with a fine reticulate exine (B type). A detailed morphological description is given, and its main diagnostic characters are compared with the related species. Conservation status has been assessed according to the IUCN protocol.

Key words: Castellón province, *Limonium*, Plumbaginaceae, Spain, taxonomy

Introduction

The genus *Limonium* Miller (1754: without page) (Plumbaginaceae Juss.) is one of the richest groups of the Mediterranean flora. Highly diversification rates occurred through hybridization and polyploidization processes, resulting in a challenging and difficult taxonomy throughout this area (Erben 1993).

The Iberian Peninsula is one of the center of diversity for *Limonium*, and about 100 species are recorded for this area (see e.g., Erben 1993). According to Mateo & Crespo (2014), and Crespo & Lledó (1998) at least 27 *Limonium* species occur in the Valencian Community. Field surveys carried out along the coastal cliffs (Castellón, Cerromar) allowed to verify the occurrence of a rare and endangered endemic species, *Limonium perplexum* L. Sáez & Rosselló (1999: 48). Unexpectedly, we found some scattered plants of *Limonium* showing a particular combination of morphological characteristics that did not fit any other species growing in the area, viz *L. perplexum*, *L. virgatum* (Willdenow 1809: 336) Fourreau (1869: 141), *L. girardianum* (Gussone 1843: 368) Fourreau (1869: 141) and *L. thiniense* Erben (1981: 485). A detailed study revealed that these specimens belong to a new polyploid species which is accordingly here described as *L. irtaensis*.

Material and Methods

The studied material is preserved at BC, MA, and VAL herbaria (acronyms according to Thiers 2015+). In order to verify the constancy in the morphological diagnostic features, 10 plants germinated from seeds sampled in the wild were cultivated and compared with 10 specimens collected from natural populations.

For chromosome number determination, root tips were pretreated with 0.002 M 8-hydroxyquinoline solution for 2 h at 4 °C and 2 h at room temperature, washed with distilled water, fixed in fresh Carnoy I solution (absolute ethanol: glacial acetic acid; 3:1) overnight and stored at 4 °C until used. For chromosome counts, the root tips were hydrolysed for 10 min in 1 N HCl at 60 °C, washed and stained in aceto-orcein for 4–6 hours. Stained meristems were squashed

in a drop of 45% acetic acid. Chromosome counts were made by direct observation and from the photomicrograph of at least five well-spread metaphases. The photomicrographs were taken with an Olympus Camedia C-2000-Z digital camera.

Description of the species

Limonium irtaensis P.P.Ferrer, A.Navarro, P.Pérez, R.Roselló, Rosselló, M.Rosato & E.Laguna, *sp. nov.* (Figs. 1–4, Table 1)

Type:—SPAIN. Castellón: Peñíscola, Sierra de Irta, Cerromar, 31SBC5695, 5 m, 29 July 2012, cliffs and coastal rocky outcrops, Navarro, Rosselló, Pérez & Laguna 013001 (holotype VAL-226810!, isotype BC-879803!).

Diagnosis:—*Planta perennis 40–60(–70) cm alt., glabra, paucicaulis, foliis ovatis–spatulatis orbiculatis, obtusis et apiculatis, inflorescentia triangulatis–lanceolatis et non ramulis sterilis, spiculis (8–)10–20 mm longis, bractea inferiore 0.9–1.0 × 1.0 mm longa, bractea media 1.0–1.3 × 0.9 mm longa, bractea superiore 3.0–3.2 × 1.9–3.2 mm longa, calyce 3.6–4.2 mm, calyce ex bractea superiore 1.0–1.6 mm exserto, petalo violaceo 7.2–7.5 × 2.4–2.5 mm, semina 1.5 × 0.5 mm. Combinatione pollen/stigma “B/papillate”. Chromosomatum numerum 2n=26.*

Description:—Perennial plant 40–60(–70) cm tall, with 1(–3) stems, glabrous, caudice thick and woody. Stem (20–)25–45 cm long, smooth, erect, straight, glabrous, 1.3–1.5 mm thick. Branching starts from the lower third of the total length of the stem. Basal leaves (3–)4–7(–9) × (0.5–)0.7–1.7(–2.5) cm, the upper ones green at anthesis, sometimes withered, the lower ones withered and persistent. Leaf margin ovate-spathulate to orbicular-elliptic, green, surface slightly rough with numerous papillae; marked and visible midrib to the apex of the leaf, 2–3 lateral nerves also visible but slenderer and less marked, reaching the upper third of the blade; apex obtuse to subobtuse; mucro (apiculum) quite visible, up to 2 mm; membranous margin, 0.1 mm; petiole up to 1.2–2.2 mm wide ($\frac{1}{2}$ – $\frac{3}{4}$ the length of the blade). Inflorescence (10–)15–30 cm long, open or with spikelets loosely arranged; shape type C (according to the forms indicated by Erben 1993), triangular-lanceolate, without sterile branches. Spikes (8–)10–20 mm, loose, straight or slightly arched downwards, erect-patent. Spikelets 1–2(–3) flowered, 4–6 mm long, 3–6 per cm; outer bract 0.9–1.0 × 1.0 mm, ovate to subcircular, blunt and obtuse apex, wide membranous margin; fleshy inner part and apex nearly reaching (occasionally reaching) the upper margin; middle bract 1.1–1.3 × 0.9 mm, oblong, membranous, emarginated; inner bract 3.0–3.2 × 1.9–3.2 mm, obovate to orbicular (occasionally even wider than longer), with blunt to rounded apex; margin widely membranous, central part 2.5 × 1.9–2.0 mm, fleshy, oblong-elliptical, with broad chestnut-coloured margin and apex 0.2–0.4 mm, not reaching the margin. Flowers 3–5 mm diameter; calyx 3.6–4.2 mm, surpassing 1.0–1.6 mm the inner bract; tube covered with hairs in all sides except in one of them, hairs densely arranged in one side, teeth 0.4–0.5 × 0.6–0.7 mm, widely triangular-ovate, obtuse; tube ribs not reaching the base of the teeth; petals 7.0–7.5 × 1.8–2.5 mm, emarginated, cuneiforms, violet; stigma papillate. Pollen showing a fine reticulum and narrow mesh, B type. Fruit 1.5 × 0.5 mm.

Etymology:—The specific epithet refers to the *locus classicus*, Irta mountains (Spain).

Phenology:—Flowering time: June–September. Fruiting time: July–October.

Chromosome number:— $2n = 26$.

Taxonomic remarks:—*Limonium irtaensis* is closely related to the polyploid species *L. perplexum*, *L. virgatum*, *L. thiniense*, and to some members of the *L. delicatulum* (Girard 1844: 327) Kuntze (1891: 395) complex.

On the basis of the cytogenetic grounds, the new species shows the same chromosome number of *L. thiniense* only, while *L. delicatulum* has $2n = 25$ and *L. perplexum*, and *L. virgatum* have $2n = 27$. According to Palacios *et al.* (2000) the $2n = 25$, $2n = 26$ and $2n = 27$ chromosome complements did not arise by aneuploidy events in *Limonium*, but from a contrasting hybrid origin involving reduced and unreduced $x=8$ and $x=9$ gametes. Thus, the origin of the new species should be traced from ancient hybridogenic processes (diploids with $2n = 16$ and $2n = 18$ are absent from the area where *L. irtaensis* occurs) or from anagenetic events involving $2n = 26$ ancestors. However, no closely related species having $2n=26$ chromosomes has been identified. Morphological features from the related *L. thiniense*, *L. girardianum* and *L. gibertii* (Sennen 1923: 113) Sennen (1936: 271) (all with $2n = 26$ chromosomes) did not support a progenitor-derivative origin from any of these species.

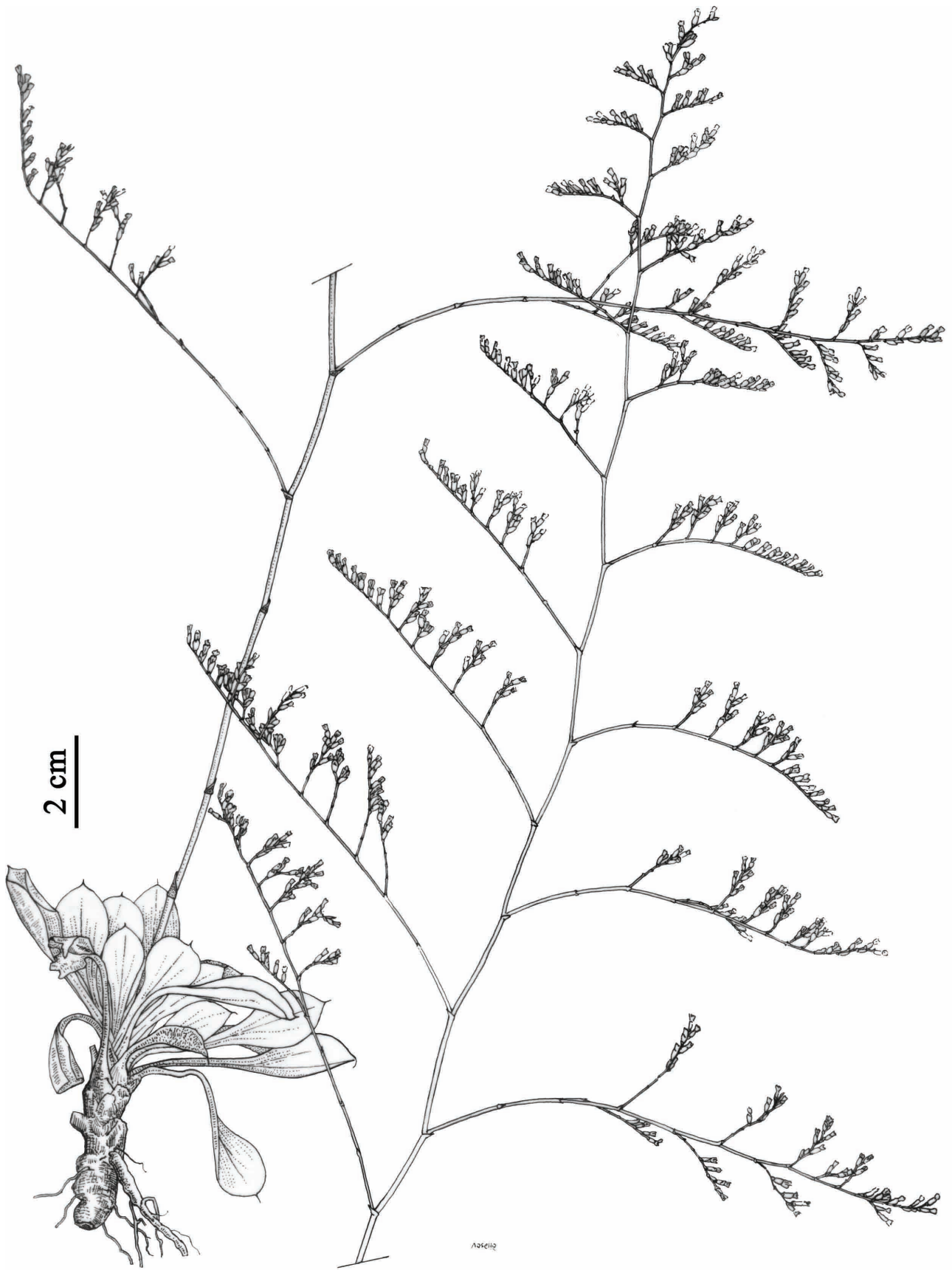


FIGURE 1. Habitus of *Limonium irtaensis*.

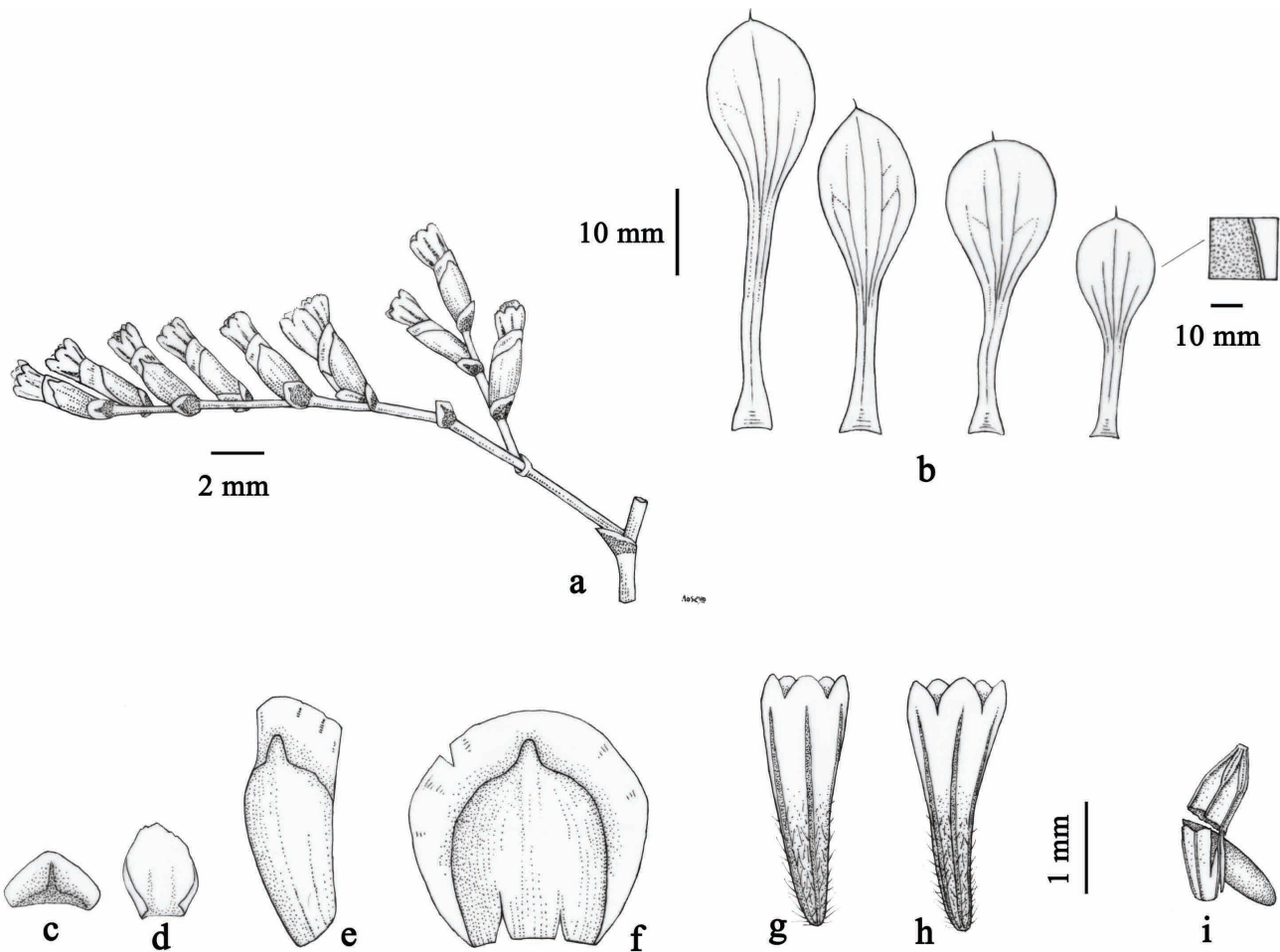


FIGURE 2. *Limonium irtaensis*: (a) spikes ; (b) leaves; (c–d) outer bract, adaxial and abaxial surfaces; (e) middle bract; (f) inner bract; (g–h) calyx; (i) fruit. Voucher: A. Navarro, J.A. Rosselló, P. Pérez Rovira & E. Laguna 013001 (Castellón: Peñíscola, Sierra de Irta, 31SBC5695, Castellón, Spain, VAL-226810, holotype). Illustration by Roberto Roselló.

From the morphological point of view, *L. irtaensis* can be distinguished from the related species by 1) its orbicular elliptical leaves, always green at anthesis, and with an obtuse apex, tapering in a long and conspicuous apiculum, and 2) a different inflorescence shape (type C), triangular–lanceolate, with first order branches up to 15 cm long, forming an angle of 50°–90° (Table 1). On the other hand, *Limonium irtaensis* is morphologically close to *L. delicatulum*, an endemic species from south-eastern Spain. The aggregate of *L. delicatulum* is highly diversified in the western Mediterranean region and specifically in the Iberian Peninsula (Erben 1993; Palacios *et al.* 2000; Lledó *et al.* 2005). *Limonium irtaensis* can be distinguished from *L. delicatulum* by the inflorescence type and its smaller size, as well as by the outer, middle and inner bracts which are always less. Therefore, both morphological and cytogenetical differences allows recognition of *L. irtaensis* at specific rank (Table 1).

Distribution, habitat and ecology:—*Limonium irtaensis* was found for the first time in July 2012, at Cerromar cliffs (Peñíscola), about 200 m northward to the site where specimens attributed to *L. perplexum* were formerly recorded (F. Royo, pers. comm.). It grows on an outcrop of calcareous conglomerate belonging to the Quaternary alluvian fans of gravels and clays (IGME 1973) rich in fossils and finely angulated gravels.

Conservation status:—During field investigations (2013–2014), a total of 19 individuals of *Limonium irtaensis* were found. Furthermore, a new growing site with 3 specimens was found in 2013 in Cala Ordí (Peñíscola), growing on a similar geological substrate but less rich in gravels. Taking into account the very scarce number of specimens and populations, both located a few meters from buildings or roads, the species is here classified as “Critically Endangered” (CR) according to IUCN Red List Category and Criteria (2001, 2011). According to the Valencian threatened species list provided by Aguilera *et al.* (2009), it should be added to the Valencian vascular species facing an extremely high risk of extinction. It should also be listed at first place according to the absolute Valencian endemism. In order to ensure its surviving, the Centre for Applied Forest Research (CIEF) started in 2012 a recovery programme for this species through the propagation by seeds in order to obtain new seeds/plants for its conservation in the Germplasm Bank at the CIEF.

TABLE 1. Morphological comparison among *Limonium irtaensis* and related species. *Data from Erben (1993), **Data from Sáez & Rosselló (1999).

	<i>L. irtaensis</i>	<i>L. delicatulum</i> *	<i>L. giberitii</i> *	<i>L. girardinianum</i> *	<i>L. perplexiun</i> **	<i>L. thiniense</i> *	<i>L. virgatum</i> *
Plant height (cm)	40–60(70)	25–105	15–60	(8) 10–80	15–50	30(40)	10–80(120)
Sterile branches	Absent	Absent	1–6 sterile lower branches	Absent, occasionally 1–5	Absent	Absent or few short sterile branches	Abundant
Leaf size (mm)	(30–)40–70 × (5–)7–17(–25)	35–150(–200) × 20–50	15–50 × 6–14	25–85 × 5–16	10–100 × 3–20	10–60 × 7–20	
Leaf margin	Ovate–spathulate to orbicular–elliptic; apex obtuse to subobtuse, apiculum	Ovate to orbicular–elliptic; apex acute to rounded, mucronated or very short mucro	Spathulate to cuneiform; apex rounded to blunt, non mucronated or very short mucro	Oblanceolate to oblanceolate–spathulate; apex acute to blunt, mucronate	Spathulate to subelliptical; obtuse apex, mucronated	Spathulate; round apex, mucrolunate	Narrow oblanceolated; acute or obtuse apex, rough and dark green
Inflorescence structure	C type; first order branches up to 15 cm, straight to arcuate, erect–patent (branching/ramification angle 50°–90°)	A or C type; first order branches up to 30 cm, erect to erect–patent (branching/ramification angle 50°–75°)	C, D or A type; first order branches up to 12(18) cm, straight to arcuate, erect–patent to patent (branching/ramification angle 45°–70°)	C or D type; first order branches up to 5 cm, short, uni or bilateral, near straight, erect–patent to patent (branching/ramification angle 50°–65°)	A type; first order branches up to 17 cm, straight to arcuate, erect–patent (branching/ramification angle 50°–90°)	A or G type, first order branches up to 9 cm, straight to pluriarcuate, erect–patent (branching/ramification angle 45°–55°)	A, B or C type; first order branches up to 12 cm, more or less dichotomous, straight to pluriarcuate, erect–patent (branching/ramification angle 40°–60°)
Inflorescence (cm)	(10–)15–30 × 20–30	25–90 × 20–45	10–60 × 10–30	10–40 × (8–)12–15	10–30 × 8–24	15–30 × 8–20	10–50
Spikes (mm)	(8–)10–20	5–30	10–40	8–16	20–140	25–90	20–80(–120)
Spikelets (mm)	4.0–6.0	4.5–5.0	5.0–6.5	5.0–6.0	5.0–6.0	5.6–7.0	6.5–7.5
Spikelets/cm²	3–6	5–8(–11)	3–6	10–14	1–3(–4)	1–3	3–5
Flowers/spikelets	1–3(–3)	2–5(–12)	1–4	2–7	2–3	1–3	1–4(–9)
Outer bract (mm)	0.9–1.0 × 1.0	1.3–2.0 × 1.4–2.0	1.5–2.0 × 1.7–2.1	1.8–2.3 × 2.0–2.6	1.3–1.6 × 1.5–1.6	1.3–2.0 × 1.4–1.9	1.9–2.8 × 1.9–2.5
Middle bract (mm)	1.0–1.3 × 0.9	1.4–2.0 × 1.1–1.6	1.6–1.9 × 1.1–1.5	1.8–2.3 × 1.2–1.8	1.6–1.8 × 1.1–1.3	1.5–2.2 × 1–1.3	2–2.6 × 1.5–2.2
Inner bract (mm)	3.0–3.2 × 1.9–3.2	3.0–4.0 × 2.6–3.6	3.7–4.2 × 2.8–3.4	3.8–4.6 × 3.8–4.4	4.3–5.0 × 2.9–3.1	(4.0–)4.2–5.4 × 2.5–3.3	5.1–6.5 × 2.9–4.0
Central part of inner bract	2.5 × 1.9–2.0	2.1–3.2 × 1.8–2.5	2.6–3.1 × 1.5–2.1	2.9–3.5 × 2.2–3.2	3.1–4.1 × 1.8–2.0	3.1–4.1 × 1.7–2.0	3.9–5.0 × 1.7–2.8
Flower diameter (mm)	3.0–5.0	4.6–5.2	4.5–5.0	4.8–5.6	3.0–4.0	3.8–4.7	8.5–9.5
Calyx (mm)	3.6–4.2	3.1–4.0	3.8–4.8	4.1–4.8	4.5–4.8	4.3–5.3	5.3–6.1
Calyx tube	Long hairs on all sides except one	Hairy on one side, long and loose hairs	Only on the basis scarcely to densely hairy, long hairs	Scarcely to densely hairy, long hairs	Densely hairy, long hairs	Densely hairy, long hairs	Hairy on one side, long and loose hairs
Calyx teeth (mm)	0.4–0.5– × 0.6–0.7	0.5 × 1.0	0.4 × 0.8	0.5 × 0.9	0.4–0.6 × 0.5–0.6	0.4 × 0.7	0.7 × 1.1
Petals (mm)	7.0–7.5 × 1.8–2.5	5.6–6.4 × 1.3–1.8	6.5–7.0 × 1.4–1.8	7.3–7.8 × 2.3–2.7	7.3–7.8 × 2.3–2.7	6.7–7.5 × 1.4–1.7	9.5–10 × 2.8–3.3
2n	26	25	26	26	27	26	27
Ecology	ledges of limestone at low coastal cliffs, warm and sunny areas	Dry limestone slopes close to coastline	Dry limestone slopes close to coastline	Coastal saline soils, sandy or rocky soils	Edges of rocky limestone at low coastal cliffs, warm and sunny areas	Compacted salty and ± sandy soils, in thermo– and mesomediterranean semiarid areas	Wide ecological range, from coastal cliffs to salt marshes, always in coastal sites
Corology	Iberian endemic, present only in Sierra de Irta (Castellón, Spain)	Southeast Spain endemic	Endemic from south Catalonia and Balearic Islands	Endemic from south France and NE Spain	Iberian endemic, present only in Sierra de Irta (Castellón, Spain)	South east Iberian endemic	Wide distributed in western coasts of Mediterranean basin

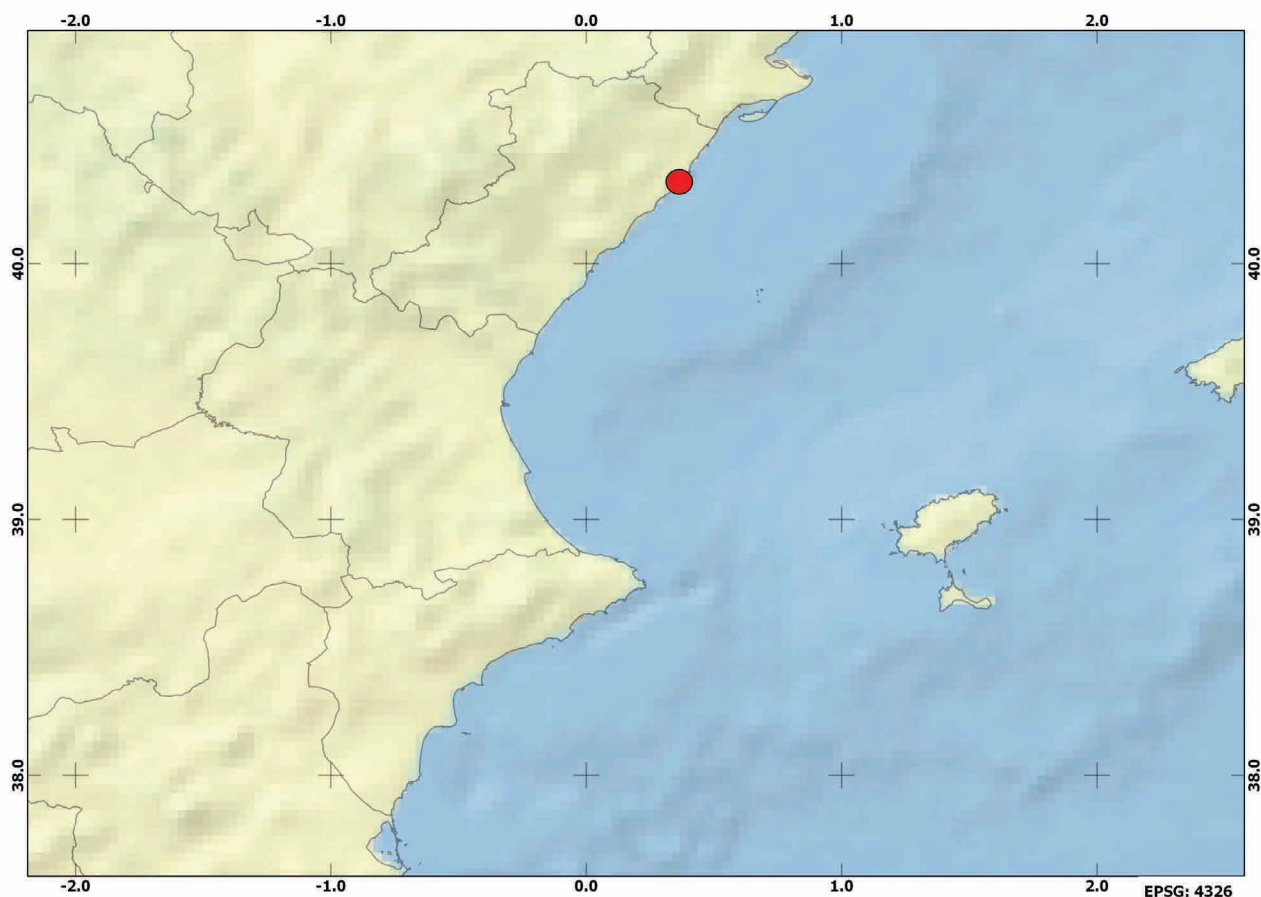


FIGURE 3. Distribution map of *Limonium irtaensis*; natural plant in the Irta mountains.

Specimina visa selecta:—*Limonium delicatulum*:—SPAIN. Alicante: Benidorm, Sierra Helada, 14 April 1982, *Aguilella & Mateo s.n.* (VAL-821125); Elche, Balsares del Altet, 8 May 1955, *Rigual s.n.* (MA-374703); Elche, cauce del Vinalopó, 28 May 1957, *Rigual s.n.* (MA-374676); El Campello, Playa de San Juan, 29 October 1956, *Rigual s.n.* (MA-374660); Santa Pola, circa de la Salinera Catalana, 30 Juny 1957, *Rigual s.n.* (MA-374665); Novelda-Villena, talud de la vía ferrea, 13 August 1982, *Mansanet & al. s.n.* (VAL-743); Villena, Salero de Penalba, 23 August 1961, *Rigual s.n.* (MA-374258).

***Limonium gibertii*:—SPAIN.** Formentera (Balearic Islands): Las Salinas, 27 July 1920, *Gros s.n.* (MA-701923); Estay Pudent, Les Salines, 9 November 1986, *Luceño & Pedrol 1613* (MA-333456); Sa Pedrera, April 1973, *Tarazona s.n.* (MA-507270). Ibiza (Balearic Islands). La Canal (playa), 3 May 1980, *Rivas Martínez, Costa & Regueiro s.n.* (MA-421103); *ibidem* (MA-421105); Ibiza, Es Codolar, 28 September 1995, *Rosselló, Sáez & Torres s.n.* (MA-597409); Ibiza, salinas de Llevant, 28 October 1995, *Rosselló, Sáez & Torres s.n.* (MA-597420). Mallorca (Balearic Islands). Palma de Mallorca, Santa Ponsa, 11 October 1989, *Erben 826, Morales Valverde & Rosselló* (MA-489228); Palma de Mallorca, Coll d'En Rabossa-San Juan de Dios, 11 October 1989, *Rosselló s.n.* (MA-489231); San Juan de Dios, Palma de Mallorca, 2 September 1993, *Rosselló. & Sáez s.n.* (MA-597415). Tarragona. Ametlla de Mar, c. estany Tort, May 1997, *Sáez s.n.* (MA-597709); Ampolla, cap Roig, May 1997, *Sáez s.n.* (MA-597707); Falaises du vieux Château de Salou, 14 July 1924, *Gonzalo s.n.* (MA-92301); L'Ampolla, Cap Roig, 27 August 2003, *Arán 5674 & Tohá* (MA-711702); Punta de la Creueta, 26 July 1993, *Hernández s.n.* (MA-541705); Punta de la Mora, May 1997, *Sáez s.n.* (MA-597708); Falaise de la Presqu' Île de Salou, June 1918, *Irlide 4477* (MA-92298); *ibidem* (MA-92300); Tamarit, 9 August 1935, *Sennen 9925* (MA-162718), (MA-92299); Tarragona, bei Tarragona, Felsküste, 4 September 1972, *Erben 51* (MA-212596).

***Limonium girardianum*:—SPAIN.** Alicante: Santa Pola (El Baix Vinalopó), platja Llissa, 30SYH1129, 8 October 1998, *Güemes, Riera & Estrelles s.n.* (VAL-39811); Teulada (La Marina Alta), Moraira, 15 November 1991, *Pérez s.n.* (VAL-149652). Castellón. Peníscola (El Baix Maestrat), Penyasegats de la Torre Badún, 31TBE76, 17 July 1996, *Villaescusa & Tirado 4664* (VAL-35402); Peníscola (El Baix Maestrat), Penyasegats de la Torre Badún, 31TBE76, 17 July 1996, *Villaescusa & Tirado 4665* (VAL-35403); Penyiscola (Baix Maestrat), Cap d'Irta, 31TBE76, 27 July 1988,

Aguilella, Baeza & Riera AAP-1878 (VAL-21247); Penyiscola (Baix Maestrat), Cap. d'Irta, 31TBE76, 27 July 1988, *Aguilella*, Baeza & Riera AAP-1877 (VAL-21246); Penyiscola (Baix Maestrat), Cap. d'Irta, 31TBE76, 27 July 1988, *Aguilella*, Baeza & Riera AAP-1879 (VAL-21248); Penyiscola (Baix Maestrat), Cap. d'Irta, 31TBE76, 27 July 1988, *Aguilella*, Baeza & Riera AAP-1881 (VAL-21250); Penyiscola (Baix Maestrat), Cap. d'Irta, 31TBE76, 27 July 1988, *Aguilella*, Baeza & Riera AAP-1880 (VAL-21249). Tarragona. Delta de l'Ebre, July 1980, *Boisset s.n.* (VAL-176324); Delta de l'Ebre, July 1980, *Boisset s.n.* (VAL-118351); Delta del Ebro, 30 July 1986, *Aguilella & Boisset s.n.* (VAL-175883). Valencia. Canet d'En Berenguer (El Camp de Morvedre), platja de L'Almardà, 30SYJ49, 18 July 1982, *Aguilella*, Mansanet & García-Fayos s.n. (VAL-175885); Puçol (L'Horta), platja, 30SYJ38, 23 July 1982, *Aguilella s.n.* (VAL-175884); Valencia (Horta), El Saler, Mallà de la Venta, 30SYJ35, 28 October 1981, *Costa, Beltrán & Peris s.n.* (VAL-200022); Valencia (L'Horta), Devesa de l'Albufera, Mallà del Garrofer, July 1984, *Mateu s.n.* (VAL-118352).

Limonium irtaensis:—SPAIN. Castellón: Peñíscola, Sierra de Irta, Cerromar, 29 July 2012, *Navarro, Rosselló, Pérez & Laguna 013001* (VAL-226810); *ibidem* (BC-879803!).

Limonium perplexum:—SPAIN. Castellón. Serra d'Irta, ubi loco dicto Torre Badum, pr. Peñíscola, 30 May 1997, *Mayol & Sáez s.n.* (MA-611342, holotype).

Limonium thiniense:—SPAIN. Alicante. Elche, Balsares del Altet, 8 May 1956, *Rigual s.n.* (MA-374679); Los Balsares del Altet, El Altet, 8 May 1956, *Rigual s.n.* (MA-374679). Albacete: Corral Rubio, laguna Hoya Rasa, 23 June 1995, *Cirujano & al. s.n.* (MA-593320); entrada al pueblo de Pétrola, 22 July 1976, *Cirujano & Castroviejo 58* (MA-458775); Higuera, laguna de Salobrejo, 24 September 1996, *Cirujano & al. s.n.* (MA-593318); Los Llanos, 18 September 1996, *Cirujano & al. s.n.* (MA-593321); Pétrola, laguna de Pétrola, 18 September 1996, *Cirujano & al. s.n.* (MA-593319). Almería. Mojácar, pr. locum dictum Lomos cantal, ad 5 m, secus viam, 16 April 1976, *Fernández Casas 1086 & García Guardia* (MA-394531).

Limonium virgatum:—SPAIN. Castellón. Cabanes, Prat de l'Albalat dels Ànecs, 30 November 1983, *Aguilella s.n.* (VAL-2645); Ribera de Cabanes, 23 July 1983, *Mansanet & al. s.n.* (VAL-787); Torreblanca, 23 July 1982, *Mansanet & al. s.n.* (VAL-783). Valencia: Cullera, Faro de Cullera, 24 November 1993, *Güemes s.n.* (VAL-1352).

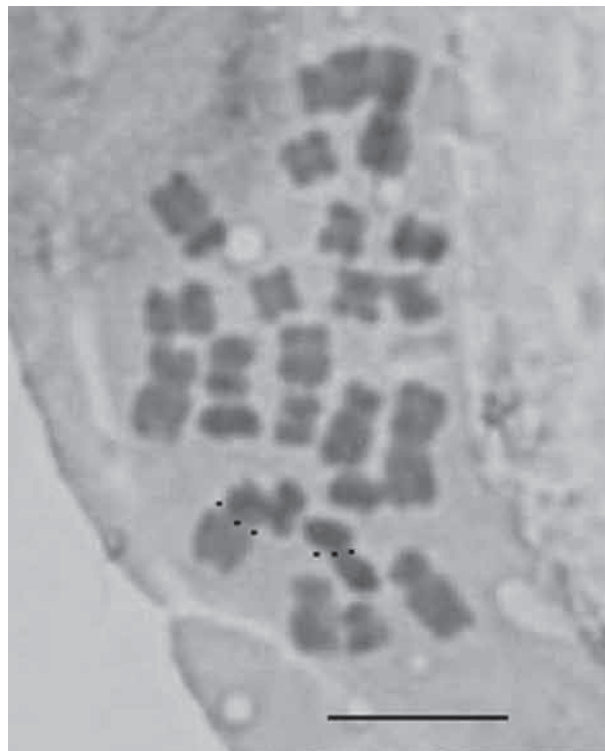


FIGURE 4. Somatic metaphase plate of *Limonium irtaensis*. Scale bar 5 μ m.

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