uma editorial
Article
Acta Botanica Malacitana 51 (2026) 22810
DOI: https://doi.org/10.24310/abm.51.2026.22810

Limonium ×erbenii (*Plumbaginaceae*), new hybrid taxon for the Southeastern Iberian flora

Lucía Miranda-Hernández[1], Esteban Salmerón-Sánchez*[1], María J. Fernández-Cobo[1], Encarna Merlo[1], Carlos Salazar-Mendías[2], Antonio J. Mendoza-Fernández[3], Fabián Martínez-Hernández[3], Francisco J. Pérez-García[1], Juan F. Mota[1]

[1]Departamento de Biología y Geología, Universidad de Almería. Ctra. Sacramento s/n, 04120 Almería, España.
[2]Departamento de Biología Animal, Biología Vegetal y Ecología, Universidad de Jaén. Campus Las Lagunillas s/n, 23071 Jaén, España.
[3]Departamento de Botánica, Universidad de Granada, Avenida de la Fuente s/n, 18071 Granada, España.

*** Author for correspondence:** esanchez@ual.es

Received: 11 December 2025**. Accepted:** 4 February 2026. **Published:** 13 February 2026
Edited by: Marta Recio Criado

Abstract

Limonium ×erbenii, a hybrid resulting from the interspecific cross between *Limonium estevei* Fern. Casas and *Limonium cossonianum* Kuntze, is here described as a new nothospecies based on the morphological and molecular characterization of five individuals collected in Macenas, at the base of Sierra de Cabrera (Mojácar, Almería, SE Spain), where it coexists with the parental taxa. The morphological analysis included the measurement and comparison of 39 diagnostic characters to evaluate the intermediacy of the hybrid accessions relative to the parental species. For the molecular characterization, the ITS region of ribosomal nrDNA was amplified, sequenced and analyzed to detect potential additive polymorphic sites (APS). Results revealed a mosaic of parental and intermediate morphological traits, a typical pattern of a first-generation hybrid (F_1), and the presence of APS indicative of the coexistence of ribotypes inherited from both parental species within the hybrid's genetic background. Altogether, these findings provide strong evidence confirming *Limonium ×erbenii* as the product of natural hybridization between *L. cossonianum* and *L. estevei*.

Key words: interspecific hybrid, morphometrics, new nothospecies, nrDNA, sea lavenders, taxonomy.

Resumen

Limonium ×erbenii (Plumbaginaceae), nuevo taxón híbrido para la flora del sureste ibérico

Limonium ×erbenii, un híbrido resultante del cruce interespecífico entre Limonium estevei Fern. Casas y Limonium cossonianum Kuntze, se describe aquí como una notoespecie nueva, en base a la caracterización morfológica y molecular de cinco individuos recogidos en Macenas, al pie de la Sierra de Cabrera (Mojácar, Almería, SE España), localidad en la que coexiste con los taxones parentales. El análisis morfológico consistió en la medición y comparación de 39 caracteres diagnósticos para evaluar el carácter intermedio de los híbridos respecto a las especies parentales. Para la caracterización molecular, se amplificó, secuenció y analizó la región ITS del ADN ribosómico nuclear (nrDNA), con el fin de detectar posibles sitios polimórficos aditivos (APS). Los resultados mostraron un mosaico de *caracteres morfológicos parentales e intermedios, un patrón típico en híbridos de primera generación (F_1), así como la presencia de APS en el fondo genético del híbrido, indicativos de la coexistencia de ribotipos heredados de ambas especies parentales. En conjunto, estos hallazgos aportan evidencia sólida de que Limonium ×erbenii es el resultado de la hibridación natural entre L. cossonianum y L. estevei.*

***Palabras clave**: híbrido interespecífico, morfometría, nueva notoespecie, nrDNA, saladillas, taxonomía.*

Introduction and Objectives

Limonium Mill. is the largest genus within the family *Plumbaginaceae* Juss. According to Plants of the World Online (POWO, 2024), it comprises 606 accepted taxa, including perennial herbs, dwarf shrubs and, more rarely, therophytes, many of which are appreciated for their ornamental value (Kubitzki, 1993). A hallmark of the genus is its ecological specialization, as its species frequently occur in fragmented or marginal habitats, often associated with saline, gypsum-rich or arid substrates (Erben, 1993).

The evolutionary dynamics of the genus *Limonium* are strongly influenced by hybridization, polyploidy, and apomictic reproduction, processes that have generated remarkable morphological and taxonomic complexity (Erben, 1993; Palacios *et al*., 2000). In particular, Erben (1993) documented over 140 interspecific hybrids in the Iberian Peninsula and the Balearic Islands alone, emphasizing the genus's pronounced tendency toward hybridization.

At Playa Macenas (Mojácar, Almería), where the Iberian endemic *Limonium cossonianum* Kuntze (2n = 16, x = 8; Erben, 1978) and the critically endangered microendemic *Limonium estevei* Fern. Casas (2n = 16, x = 8; Erben, 1978; Fernández-Casas & García-Villaraco, 1979; García-Rodríguez *et al*., 2026) coexist in sympatry, individuals exhibiting morphological traits intermediate between these two taxa are relatively common. These have been identified as the interspecific hybrid *L. cossonianum* × *L. estevei*, first reported by Erben (1978) and later mentioned in *Flora iberica* (Erben, 1993). According to these references, this would be a diploid hybrid (2n = 16) exhibiting regular sexual reproduction, a feature that contrasts with the prevailing pattern in the genus, where most hybrids are triploid, predominantly apomictic, or display only facultative sexuality (Erben, 1979). These characteristics make this hybrid particularly noteworthy.

However, despite its biological interest and prior mentions in the literature, it is not considered a nomenclaturally valid or accepted taxon. The description in Erben (1978) fails to comply with several provisions of the International Code of Nomenclature for algae, fungi, and plants (Turland *et al*., 2025): the rank of the taxon was not explicitly indicated (Art. 37.1), no Latin diagnosis was then provided (Art. 39.1), and the nomenclatural type was not designated (Art. 40.1); instead, two specimens were merely cited as studied material, which also renders Art. 9.2 inapplicable. Accordingly, and based on both morphological and genetic evidence, we here proceed to formally describe the interspecific hybrid between *L. cossonianum* and *L. estevei* as a new nothospecies, named *Limonium ×erbenii*.

Material and Methods

The hybrid was characterized through a combined morphological and molecular approach. Morphological comparisons with the parental species were performed to assess its phenotypic intermediacy. Nuclear rDNA (ITS) sequences were analyzed to detect additive polymorphic sites (APS; Aguilar & Nieto-Feliner, 2003), indicative of the ribotypes inherited from the parental species (Aguilar *et al*., 1999; Baldwin *et al*., 1995).

For the morphological analyses, five complete individuals of each taxon (*L. cossonianum*, *L. estevei* and the hybrid) were collected in Playa Macenas (Mojácar, Almería), a coastal locality included within the Special Area of Conservation (SAC) "Sierra de Cabrera-Bédar" (ES6110005). An additional 44 individuals of *L. cossonianum* were collected from other localities covering its distribution range in Almería to ensure the accurate identification of this parental species in the study area. Herbarium vouchers were prepared from all collected specimens and deposited at the Herbarium of the University of Almería (HUAL; Thiers, continuously updated) (see Anex).

A set of 39 diagnostic characters (5 qualitative; 34 quantitative: 4 discrete, 30 continuous) was selected considering their usefulness in differentiating *L. cossonianum* from *L. estevei*, according to the descriptions in *Flora iberica* (Erben, 1993), and those reported by Iamonico *et al*. (2022) (see Table 1). Qualitative characters were observed *in situ* on fresh individuals. For quantitative traits, five measurements per character and voucher were taken using a digital caliper or from stereomicroscope images (EZ4 W; Leica Microsystems), which were subsequently analyzed using ImageJ 1.53t (Schneider *et al*., 2012).

To identify significant differences among the three taxa, a one-way ANOVA ($p < 0.05$) was applied to each character using the software PAST v.4.13 (Hammer, *et al*., 2001). Normality and homoscedasticity were assessed with Shapiro-Wilk and Levene tests, respectively. When ANOVA assumptions were violated, alternative approaches were applied, including the non-parametric Kruskal-Wallis test or Welch's ANOVA for unequal variances.

For molecular analyses, fresh leaves of the same individuals used in the morphological characterization were dried in silica gel (Chase & Hills, 1991) and stored until DNA extraction. Genomic DNA was isolated using the SpeedTools Plant DNA Extraction Kit (Biotools B&M Labs S.A., Madrid, Spain) following the manufacturer's instructions. DNA quality was assessed via 1% agarose gel electrophoresis stained with GelRed® (Biotium, Fremont, CA, USA), and quantification was performed at the Central Research Services of the University of Almería using a Qubit 4 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). These DNA samples were then used as template strands for the amplification of the nuclear rDNA ITS marker.

Each PCR was performed in a final volume of 20 µL, containing 2 µL of DNA and 18 µL of reaction mixture. The mix included 4 µL of 5X Colourless GoTaq® Flexi Buffer (Promega, Madison, WI, USA), 1.6 µL of dNTPs (2.5 mM), 1.5 µL of $MgCl_2$ (25 mM; Promega, Madison, WI, USA), 0.2 µL of each primer (10 µM; ITS-p5 and ITS-p4; Cheng *et al*., 2016), 0.05 µL of GoTaq® G2 Flexi DNA Polymerase (5 u/µL; Promega, Madison, WI, USA) and 10.45 µL of ddH_2O. Samples with recalcitrant amplification required the addition of 0.2 µL of BSA (20 mg/mL; New England Biolabs Inc., Ipswich, MA, USA; Farell & Alexandre, 2012). The volume of this reagent was subtracted from ddH_2O.

The PCR program consisted of a first denaturation step of 5 min at 94ºC followed by 40 cycles of 1 min at 94ºC, 1 min at 50ºC, 1 min at 72ºC and a final elongation step of 5 min at 72ºC. Amplicons were purified in 30 kD Filter Plates (Pall Corporation, New York, USA). Sequencing reactions were performed by means of the BigDye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and electrophoresed in an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Finally, forward and reverse sequences were assembled, edited and visualized using the software Geneious Pro v4.8.5 to identify additive polymorphic sites indicative of the hybrid origin of *Limonium ×erbenii*. All sequences were deposited in GenBank under the accession numbers PQ620361-PQ620371 and PQ623580-PQ623593.

Results and Discussion

Morphometric Analysis

Based on the set of quantitative characters listed in Table 1, specimens of *L. estevei* were characterized by longer spikes and spikelets, along with larger bracts, bracteoles and calyces. In contrast, *L. cossonianum* individuals showed larger leaves with thinner petioles and a greater number of lateral veins. Hybrid individuals exhibited a floral morphology, and certain foliar traits intermediate to that of the parental species, although their foliar architecture in terms of blade length and petiole dimensions (length and width), bore a close resemblance to that of *L. cossonianum*. In the hybrid individuals, 11 of the 34 quantitative characters measured were directly inherited from one of the parental species (four from *L. estevei*, seven from *L. cossonianum*), 12 exhibited intermediate values, and two were transgressive. The remaining nine characters were considered non-diagnostic due to the absence of significant differentiation across taxa (ANOVA, $p < 0.05$; Table 1). After excluding these, the proportional distribution of trait types was 44% parental, 48% intermediate, and 8% transgressive.

This distribution, in which hybrid individuals exhibit a mosaic of parental and intermediate traits rather than exclusively intermediate ones, together with the low frequency of novel or transgressive traits observed, suggests that these individuals represent first-generation (F_1) hybrids (Rieseberg *et al*., 1993).

Genetic analysis

Ribosomal sequences had lengths of 692 and 693 bp in *L. estevei* and *L. cossonianum*, respectively. The size of each amplified region was 211 bp (or 212) for ITS1, 163 bp for the 5.8S exon and 249 bp for ITS2. Partial sequences of the 18S (35 bp) and 26S (34 bp) genes flanked these amplicons.

The alignment of these sequences revealed 16 additive polymorphic sites, predominantly positioned within the internal transcribed spacers (eight in ITS1 and six in ITS2). The APS included both differential positions between the two parental species and intraspecific polymorphisms (positions 237, 258 and 653 in *L. estevei*; 439 in *L. cossonianum*).

Samples from the hybrid individuals exhibited additivities, consistent with the coexistence of divergent ribotypes inherited from the parental species, at all the identified positions (Table 2). This is a hallmark of interspecific hybrids where concerted evolution has not yet effectively occurred, although it could also result from alternative evolutionary mechanisms (Álvarez & Wendell 2003; Nieto-Feliner *et al*., 2004; Salmerón-Sánchez *et al*., 2014).

A similar pattern was observed in *L. cossonianum* samples from Macenas, which showed additivities in at least six of these sites, highlighting a unidirectional backcrossing event only involving this species and the hybrid forms (see Miranda-Hernández *et al*., 2026).

Taxonomic treatment

Limonium ×erbenii, L. Miranda-Hernández, E. Salmerón-Sánchez & J. Mota, *nothosp. nov.* [= *Limonium cossonianum* Kuntze × *Limonium estevei* Fern. Casas] (Figures 1 and 2, Tables 1 and 2).

SPAIN. Almería, rocky coastal slope between Mojácar and Carboneras (Macenas). 37º05’09”N,1º51’26”W. 13 July 2012. 58 m a.s.l. Legit. *F.J. Pérez-García, E. Salmerón-Sánchez, M.L. Rodríguez-Tamayo, A. Mendoza-Fernández, J.F. Mota.* Det. *J.F. Mota* (Holotype: MGC 97033; Isotypes: HUAL 30714, 30715, 30716, 30717).

Diagnosis: differs from *L. estevei* by its broader and longer leaf blades, longer and narrower petioles, shorter distance between spikelets and less developed inner bract apices. Differs from *L. cossonianum* by its finely undulate leaf margins, helicoidal foliar arrangement, wider average branching angle and broader middle and inner bracts. Differs from both parental species by its greater number and density of spikelets per spike. For characters such as leaf color, number of lateral veins, branching starting point, spike length, bract length (external, middle and inner), bracteole length, calyx length (tube and limb) and color of the flowers, *Limonium ×erbenii* exhibits intermediate values between *L. estevei* and *L. cossonianum*.

Etymology: Dedicated to Matthias Erben, a distinguished German botanist and the foremost global authority on the genus *Limonium*, who first identified and reported this hybrid that we have confirmed and formally characterized.

Description: Plant up to 90 cm tall, perennial, glabrous. Caudices branched, 3-12 cm long, erect to ascending, with leaves densely arranged in a helical pattern. Leaves 48-88 x 22-37 mm (length x width), bluish-green, non-coriaceous, with 3-5 pairs of arcuate lateral nerves, dry and persistent in the basal part of the caudex. Leaf blade elliptic-ovate to suborbicular, acute to obtuse at the apex, frequently conduplicate (folded upward along the midrib), with strongly undulate margins. Petiole 17-38 x 4-9 mm, with short leaf

Figure 1. Image from the holotype of *Limonium ×erbenii* (MGC 97033).
***Figura 1**. Imagen del holotipo de Limonium ×erbenii (MGC 97033).*

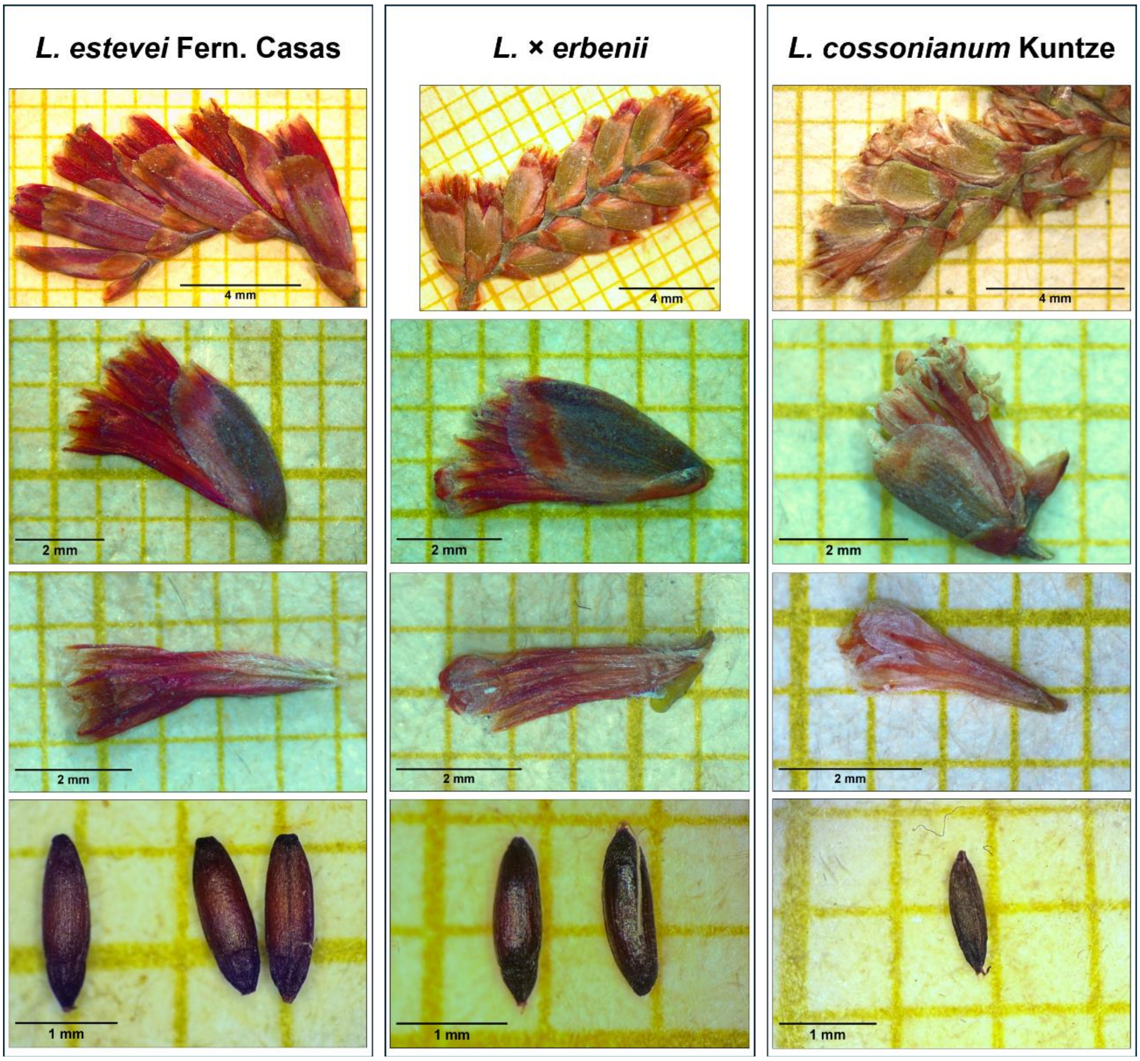

Figure 2. Comparison of diagnostic characters among *L. estevei*, *L. ×erbenii* and *L. cossonianum.* (From top to bottom): morphology/length of spikes, spikelets, calyces and seeds. Image: L. Miranda-Hernández & M.J Fernández-Cobo.

Figura 2*. Comparación de caracteres diagnósticos entre L. estevei, L. ×erbenii y L. cossonianum. (De arriba a abajo): morfología/longitud de espigas, espiguillas, cálices y semillas. Imagen: L. Miranda-Hernández & M.J Fernández-Cobo.*

sheaths. Floral scape 57-74 cm, ± erect, branched from the lower third. Inflorescence type C (according to Erben, 1978, 1993), without sterile branches. First-order branches 6-32 cm, laxly arranged in a bilateral pattern, straight to arcuate, from erect-patent to patent (branching angles of 35º-65º), laxly branched. Spikes 7.8-13.5 mm long, curved, dense (7-11 spikelets per spike). Spikelets 4.1-5.6 mm long, 6-8 per cm, 1-3 flowered. Outer bract 0.9-1.7 x 1.0-1.6 mm, triangular-ovate, acute to obtuse at the apex, broadly membranous at margins; central part slightly fleshy and keeled, with apex almost reaching the margin. Middle bract 1.2-1.6 x 0.9-1.4 mm, elliptic to oblong, membranous, with entire margin and round or asymmetrically bilobed apex. Inner bract 3.4-4.1 x 2.5-3.6 mm, obovate, obtuse at the apex, slightly keeled, whitish-membranous margin 0.3-0.7 mm wide; central part 2.5-3.5 mm long and 1.4-2.8 mm wide, fleshy, with an apex 0.4-0.9 mm long, not reaching the upper margin. Bracteoles 2.3-3.3 mm long, membranous, reddish. Calyx 3.4-4.2 mm long, exceeding the inner bract by 0.4-1.4 mm; calyx tube 1.4-2.1 mm long, heterogeneously hairy, with long dense hairs concentrated on the basal or middle half; calyx limb 1.7-2.4 mm long, with teeth 0.2-0.7 long and 0.4-0.9 mm wide; ribs of the tube ending above the base of the teeth. Petals 4.5-5.7 mm long and 1.1-1.4 mm wide, with emarginate apex, whitish violet. Corolla infundibuliform, 3.8-4.5 mm diameter. Seeds 1.0-1.4 mm long and c. 0.3 mm wide, narrowly ellipsoid, blackish brown, smooth-surfaced, one per mature capsule.

Table 1. Comparison of diagnostic characters among the hybrid *Limonium* ×*erbenii* and its parental species. Superscript letters (a, b, c) denote significant differences between taxon means within each row (i.e. for each character), according to the ANOVA's test ($p < 0.05$).

***Tabla 1**. Comparación de caracteres diagnósticos entre el híbrido Limonium × erbenii y sus especies parentales. Las letras en superíndice (a, b, c) indican diferencias significativas entre las medias de los taxones dentro de cada fila (es decir, para cada carácter), de acuerdo con la prueba de ANOVA ($p < 0,05$).*

	QUALITATIVE CHARACTERS	***Limonium estevei* Fern. Casas**		***Limonium × erbenii***		***Limonium cossonianum* Kuntze**	
1	Leaf color	Bluish green		Bluish green		Green	
2	Leaf blade shape	Oblanceolate-spatulate		Elliptic-ovate		Elliptic-ovate	
3	Leaf margin	Highly undulated		Highly undulated		Entire	
4	Leaf arrangement	Dense helical		Dense helical		Basal rosette	
5	Petal color	Pale violet		Whitish violet		White	
	QUANTITATIVE CHARACTERS	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**
6	Plant height (mm)	686 – 773	731.22 ± 38.85	643 – 857	725.86 ± 102.10	565 – 873	678.94 ± 122.24
7	Leaf length (mm)	39 – 63	52.72 ± 6.56	48 – 88	71.65 ± 10.20	36 – 100	64.89 ± 19.05
8	Leaf blade length (mm)	22 – 39	30.16 ± 4.83^{a}	29 – 53	42.85 ± 7.83^{b}	27 – 56	44.89 ± 6.65^{b}
9	Leaf blade width (mm)	15 – 30	22.63 ± 4.32^{a}	22 – 37	31.38 ± 4.34^{b}	14 – 36	26.46 ± 3.64^{ab}
10	Petiole length (mm)	17 – 26	22.56 ± 2.37^{a}	17 – 38	28.80 ± 4.83^{b}	10 – 46	28.27 ± 4.65^{b}
11	Petiole width (mm)	6 – 14	8.94 ± 2.34^{a}	4 – 9	6.41 ± 1.15^{b}	3 – 7	4.81 ± 1.09^{b}
12	No. lateral veins	3 – 5	4.00 ± 0.45^{a}	5 – 10	6.68 ± 1.14^{b}	6 – 11	8.64 ± 1.09^{c}
13	No. floral scapes	1 – 2	1.40 ± 0.55	1 – 2	1.20 ± 0.45	1	1.00 ± 0.00
14	Floral scape length (mm)	639 – 687	658.56 ± 20.37	571 – 738	651.72 ± 80.60	542 – 847	653.62 ± 122.90
15	Branching starting point (mm)	109 – 332	193.16 ± 87.28^{a}	143 – 342	259.36 ± 90.14^{ab}	212 – 446	351.42 ± 97.72^{b}
16	First-order branch length (mm)	69 – 243	167.24 ± 29.93	64 – 326	147.82 ± 41.85	34 – 276	105.03 ± 37.07
17	Angle between branches (°)	30 – 70	51.40 ± 7.96^{a}	35 – 70	54.88 ± 6.45^{a}	25 – 55	41.23 ± 6.96^{b}
18	Spike length (mm)	9.4 – 14.5	11.99 ± 0.27^{a}	7.9 – 13.5	10.19 ± 1.37^{ab}	5.8 – 9.6	7.50 ± 0.71^{b}
19	Spikelet maximum distance (mm)	1.3 – 2.4	1.67 ± 0.18^{a}	0.9 – 1.3	1.11 ± 0.06^{b}	0.7 – 1.7	1.02 ± 0.20^{b}
20	No. spikelets per spike	4 – 7	5.76 ± 0.62^{b}	7 – 11	8.00 ± 0.75^{a}	5 – 8	6.11 ± 0.66^{b}
21	No. spikelets per cm	3 – 6	4.16 ± 0.43^{a}	6 – 8	7.20 ± 0.32^{c}	5 – 8	6.11 ± 0.66^{b}
22	Spikelet length (mm)	5.8 – 6.8	6.13 ± 0.19^{a}	4.2 – 5.6	4.74 ± 0.22^{b}	3.1 – 4.0	3.57 ± 0.24^{c}
23	Outer bract length (mm)	1.1 – 1.9	1.49 ± 0.20^{a}	1.0 – 1.7	1.30 ± 0.07^{b}	0.8 – 1.2	0.99 ± 0.07^{c}
24	Outer bract width (mm)	0.9 – 1.6	1.29 ± 0.14	0.9 – 1.6	1.16 ± 0.08	1.0 – 1.3	1.13 ± 0.05
25	Middle bract length (mm)	1.5 – 2.0	1.77 ± 0.05^{a}	1.2 – 1.6	1.45 ± 0.06^{b}	0.9 – 1.3	1.02 ± 0.07^{c}
26	Middle bract width (mm)	0.8 – 1.5	1.12 ± 0.07^{a}	0.9 – 1.4	1.09 ± 0.05^{a}	0.5 – 1.1	0.71 ± 0.07^{b}
27	Inner bract length (mm)	4.6 – 5.5	4.99 ± 0.15^{a}	3.4 – 4.1	3.76 ± 0.19^{b}	2.5 – 3.3	2.87 ± 0.11^{c}
28	Inner bract width (mm)	2.5 – 3.5	2.97 ± 0.14^{b}	2.5 – 3.6	3.06 ± 0.19^{b}	2.1 – 3.4	2.69 ± 0.24^{a}
29	Inner bract central part length (mm)	3.4 – 4.0	3.70 ± 0.08^{a}	2.5 – 3.5	2.86 ± 0.20^{b}	1.9 – 2.7	2.24 ± 0.15^{c}
30	Inner bract central part width (mm)	1.3 – 2.5	2.04 ± 0.14	1.4 – 2.8	2.12 ± 0.20	1.6 – 2.7	2.07 ± 0.18
31	Inner bract margin width (mm)	0.4 – 2.2	0.93 ± 0.21^{a}	0.6 – 1.4	0.95 ± 0.11^{a}	0.2 – 1.1	0.62 ± 0.16^{b}
32	Inner bract apex length (mm)	0.4 – 1.0	0.69 ± 0.09^{a}	0.4 – 0.9	0.52 ± 0.06^{b}	0.3 – 0.6	0.45 ± 0.07^{b}
33	Calyx exceeding inner bract (mm)	0.9 – 2.1	1.29 ± 0.12^{a}	0.4 – 1.6	0.88 ± 0.13^{b}	0.3 – 1.3	0.81 ± 0.09^{b}
34	Bracteole length (mm)	3.0 – 4.1	3.62 ± 0.15^{a}	2.3 – 3.3	2.86 ± 0.22^{b}	1.9 – 2.6	2.18 ± 0.18^{c}
35	Calyx length (mm)	4.3 – 5.4	4.75 ± 0.27^{a}	3.4 – 4.2	3.70 ± 0.04^{b}	2.4 – 3.4	2.78 ± 0.22^{c}
36	Calyx tube length (mm)	1.9 – 2.8	2.33 ± 0.07^{a}	1.3 – 2.1	1.73 ± 0.05^{b}	1.0 – 1.9	1.35 ± 0.14^{c}
37	Calyx limb length (mm)	2.0 – 2.8	2.42 ± 0.20^{a}	1.7 – 2.4	1.97 ± 0.04^{b}	1.1 – 1.7	1.43 ± 0.11^{c}
38	Calyx teeth length (mm)	0.2 – 0.6	0.40 ± 0.05	0.2 – 0.7	0.41 ± 0.08	0.3 – 0.7	0.44 ± 0.08
39	Calyx teeth width (mm)	0.4 – 0.9	0.62 ± 0.03	0.4 – 0.9	0.62 ± 0.07	0.4 – 0.8	0.60 ± 0.04

Table 2. Variable sites in the ITS1 and ITS2 regions and the 5.8S gene of rDNA. APS are represented using IUPAC ambiguity symbols, with superscripts indicating the base with the strongest signal. For each sequence, the population code, GenBank accession number and herbarium voucher number are provided.

***Tabla 2**. Sitios variables en las regiones ITS1 e ITS2 y el gen 5.8S del ADN ribosómico. Los APS se representan mediante los códigos de ambigüedad de la IUPAC, con superíndices que indican la base con la señal más intensa. Para cada secuencia se proporciona el código de población, el número de accesión de GenBank y el número del pliego de herbario del que procede.*

		ITS1								**5.8S**		**ITS2**					
		49	**50**	**119**	**190**	**223**	**229**	**237**	**247**	**258**	**260**	**427**	**429**	**439**	**475**	**599**	**653**
***Limonium estevei* Fern. Casas (HUAL: 30718, 30719, 30720, 30721, 30722)**																	
EST1	PQ623584	C	C	Y	T	T	C	T	-	Y^{C}	A	A	C	T	T	G	C
EST2	PQ623585	C	C	Y	T	T	C	T	-	Y^{C}	A	A	C	T	T	G	C
EST3	PQ623586	C	C	Y^{C}	T	T	C	W^{T}	-	Y^{C}	A	A	C	T	T	G	C
EST4	PQ623587	C	C	Y	T	T	C	W^{T}	-	C	A	A	C	T	T	G	Y^{C}
EST5	PQ623588	C	C	Y^{T}	T	T	C	W^{T}	-	C	A	A	C	T	T	G	C
***Limonium ×erbenii* (MGC 97033; HUAL: 30714, 30715, 30716, 30717)**																	
HYB1	PQ623589	M	Y	Y	Y^{T}	K	Y	T	T/-	C	R	M	Y	Y	Y	R^{A}	C
HYB2	PQ623590	M	Y	Y	Y	K	Y	T	T/-	C	R	M	Y	Y	Y	R	C
HYB3	PQ623591	M	Y	C	Y	K	Y	W	T/-	C	R	M	Y	Y	Y	R	Y^{C}
HYB4	PQ623592	M	Y	Y^{T}	Y^{T}	K	Y	T	T/-	C	R	M	Y^{C}	Y	Y	R	C
HYB5	PQ623593	M	Y	C	Y	K	Y	W^{T}	T/-	Y	R	M	Y	Y	Y	R	C
***Limonium cossonianum* Kuntze (HUAL: 30723, 30724, 30725, 30727)**																	
MAC1	PQ623580	M^{C}	Y^{C}	Y^{T}	Y^{T}	K^{T}	Y	T	T/-	C	R^{A}	M^{A}	Y^{C}	T	Y^{T}	A	C
MAC2	PQ623581	M^{C}	Y^{C}	Y^{T}	Y^{T}	K^{T}	Y	T	T/-	C	R^{A}	M^{A}	Y^{C}	T	Y^{T}	A	C
MAC3	PQ623582	M^{A}	Y^{T}	C	Y^{C}	K^{G}	Y	T	T	C	G	M^{C}	Y^{T}	Y^{C}	Y^{C}	A	C
MAC5	PQ623583	A	T	C	C	K^{G}	T	T	T	C	R^{G}	M^{C}	Y^{T}	Y^{C}	Y^{C}	A	C
***Limonium cossonianum* Kuntze (HUAL: 30728, 30729, 30761, 30762, 30743, 30755, 30756, 30763, 30752, 30753, 30735)**																	
ADR1	PQ620361	A	T	C	C	G	T	T	T	C	G	C	T	C	C	A	C
ADR2	PQ620362	A	T	C	C	G	T	T	T	C	G	C	T	C	C	A	C
GAT1	PQ620363	A	K	C	C	K	Y	T	T	C	G	C	T	Y	C	A	C
GAT2	PQ620364	A	K	C	C	K	Y	T	T	C	G	C	T	Y	C	A	C
NIJ1	PQ620365	A	T	C	C	G	T	T	T	C	G	C	T	C	C	A	C
PIR1	PQ620366	A	T	Y	Y	G	Y	T	T	C	G	C	T	Y	Y	A	C
PIR2	PQ620367	A	T	Y	Y	G	Y	T	T	C	G	C	T	Y	Y	A	C
RIO1	PQ620368	M	Y	C	Y	K	Y	T	T/-	C	G	M	Y	Y	Y	A	C
VIL1	PQ620369	A	T	C	C	G	T	T	T	C	G	C	T	Y	C	A	C
VIL2	PQ620370	A	T	C	C	G	T	T	T	C	G	C	T	C	C	A	C
POB1	PQ620371	A	T	C	C	G	T	T	T	C	G	C	T	C	C	A	C

Phenology: Flowering occurs during July and August, simultaneously with that of the parental species. The formation of small capsule-shaped fruits has been observed, each containing a single seed.

Distribution and ecology: *Limonium ×erbenii* is narrowly restricted to the locality of Macenas, situated midway between Mojácar and Carboneras, at the base of Sierra Cabrera (SE Spain), where it coexists in sympatry with its parental species. Its ecology is similar to that of *L. estevei*. It grows on rocky ledges, slopes, and marine terraces crossed by dry streambeds (locally known as *ramblas*), on substrates dominated by graphitic schists with saline efflorescences, at elevations ranging from 0-100 m a.s.l.

Conclusions

Although species of *Limonium* often show a close morphological resemblance, which can hinder the delimitation of new taxa, the pronounced differences in vegetative and reproductive characters displayed by the parental species in this study facilitated the identification and characterization of the hybrid accessions. Comparative analysis showed that the hybrid is not purely intermediate but rather exhibit a mosaic of parental and intermediate features.

Molecular analyses further supported the results obtained from the morphometric approach, revealing the simultaneous presence of ribotypes inherited from both parental species within the genetic background of the hybrid. Therefore, based on the robustness and consistency of our evidence, we have proposed the recognition of this taxon as a new nothospecies: *Limonium ×erbenii*.

These findings contribute to a more comprehensive understanding of the taxonomy of *Limonium* species in southeastern Iberian Peninsula. The recognition of *L. ×erbenii* as a nothospecies also opens new perspectives for the conservation of this hybrid, which occurs exclusively in contact zones between *L. cossonianum* and *L. estevei*, and consequently exhibits an extremely restricted area of occupancy and distribution, comparable to that of the microendemic Mojácar sea lavender.

CRediT authorship contribution statement

E.S-S. and J.M. were responsible for the conceptualization of the study. L.M-H., E.S-S., and M.J.F-C. conducted the data curation, ensuring the organization and verification of all datasets. Formal analyses were performed by L.M-H., E.S-S., and J.M., while funding acquisition was carried out by J.M., E.S-S., E.MC., and L.M-H. The investigation, including field and laboratory work, was undertaken by L.M-H., E.S-S., M.J.F-C., J.M., F.M-H., A.J.M-F., and F.J.P-G. The methodological framework was developed and refined by L.M-H., E.S-S., E.MC., M.J.F-C., and C.S. Project administration was coordinated by E.S-S., E.MC., and J.M., and essential resources were provided by E.S-S., E.MC., J.M., and F.J.P-G. Supervision of the research process was carried out by E.S-S., E.MC., J.M., and C.S., whereas validation of the results was performed by E.S-S., E.MC., and J.M. Visualization and figure preparation were conducted by L.M-H., E.S-S., and J.M. L.M-H. and E.S-S. wrote the original draft of the manuscript, and all authors contributed to writing, critical review, and editing, and approved the final version.

Conflict of interest

The authors declare that they have no significant conflicts of interest in relation to the content of this manuscript.

Declaration of use of artificial intelligence (AI)

The authors declare that they have not used AI to generate the content of this manuscript.

Acknowledgements

This research has been partially funded by: Junta de Andalucía, through the agreement entitled "Specific Hybrid Identification of the *Limonium* population present in Baños del Carmen" (CONTR 2021 0000245363), Saint Gobain S.A., through the agreement with the code 001426, Explotaciones Río de Aguas S.L. though the agreement entitled ECORESGYP_2.0_, CEI.MAR through the research project SEALAV (CEI-JD-12) and University of Almería (UAL) through the research projects BARRIZOMINE (UAL2020-RNM-B2007), and PPITUAL, JJAA-FEDER 2021-2027. Program: 54.A. Lucía Miranda Hernández was awarded a collaboration grant by the Spanish Ministry of Education and Vocational Training at the University of Almería 2023-2024. We are also grateful to María Luisa Rodríguez Tamayo for her involvement in the early stages of this research, to Beatrice Antolin for reviewing the English translation of the manuscript, and to Hedwig Schwarzer for her assistance in her role as conservation technician at the El Albardinal Botanical Garden.

References

Aguilar, J. F., & Nieto-Feliner, G. (2003). Additive polymorphisms and reticulation in an ITS phylogeny of thrifts (*Armeria*, Plumbaginaceae). *Molecular Phylogenetics and Evolution, 28*(3), 430-447.

doi: https://doi.org/10.1016/S1055-7903(02)00301-9

Aguilar, J. F., Rosselló, J. A., & Nieto-Feliner, G. (1999). Nuclear ribosomal DNA (nrDNA) concerted evolution in natural and artificial hybrids of *Armeria* (Plumbaginaceae). *Molecular Ecology, 8*(8), 1341-1346.

doi: https://doi.org/10.1046/j.1365-294X.1999.00690.x

Álvarez, I., & Wendel, J. F. (2003). Ribosomal ITS sequences and plant phylogenetic inference. *Molecular phylogenetics and evolution*, *29*(3), 417-434. doi: https://doi.org/10.1016/S1055-7903(03)00208-2

Baldwin, B. G., Sanderson, M. J., Porter, J. M., Wojciechowski, M. F., Campbell, C. S., & Donoghue, M. J. (1995). The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. *Annals of the Missouri Botanical Garden, 82*(2) 247-277. doi: https://doi.org/10.2307/2399880

Chase, M. W., & Hills, H. H. (1991). Silica gel: an ideal material for field preservation of leaf samples for DNA studies. *Taxon, 40*(2), 215-220. doi: https://doi.org/10.2307/1222975

Erben, M. (1978). Die gattung *Limonium* im südwestmediterranen Raum. In H. Merxmüller (Ed.), *Mitteilungen der Botanischen Staatssammlung München* (Vol. 14, pp. 361–631). Munich: Botanischen Staatssammlung München.

Erben, M. (1993). *Limonium* Miller. In S. Castroviejo, C. Aedo, S. Cirujano, M. Laínz, P. Montserrat, R. Morales, F. Muñoz-Garmendia, C. Navarro, J. Paiva, & C. Soriano (Eds.), *Flora iberica* (Vol. 3, pp. 2-143). Madrid: Real Jardín Botánico-C.S.I.C.

Farell, E. M., & Alexandre, G. (2012). Bovine serum albumin further enhances the effects of organic solvents on increased yield of polymerase chain reaction of GC-rich templates. *BMC research notes*, *5*(1), 257.

doi: https://doi.org/10.1186/1756-0500-5-257

Fernández-Casas, J., & García-Villaraco, A. (1979). Números cromosomáticos de plantas occidentales, 38-45. *Anales del Jardin Botánico de Madrid*, *36*, 395-398.

García-Rodríguez, R., de Vega, C., Schwarzer, H., Rubio-Pérez, E., Martín-Hernanz, S., Fernández-Mazuecos, M., Blanco-Pastor, J. L., Aparicio, A., & Albaladejo, R. G. (2026). Conservation genomics of the critically endangered sea lavender Limonium estevei: a recent bottleneck and low introgression from a widespread congener. *Conservation Genetics*, *27*(1), 12. https://doi.org/10.1007/s10592-025-01741-y

Hammer, Ø., Harper, D. A. T, & Ryan, P. D. (2001). PAST: Paleontological Statistics software package for education and data analysis. *Palaeontologia Electronica 4*(1), 1–9.

doi: http://palaeo-electronica.org/2001_1/past/issue1_01.htm

Iamonico, D., De Castro, O., Di Iorio, E., Nicolella, G., & Iberite, M. (2022). Taxonomy Complexity of Some Tyrrhenian Endemic *Limonium* Species Belonging to *L. multiforme* Group (Plumbaginaceae): New Insights from Molecular and Morphometric Analyses. *Plants, 11*(22), 3163. doi: https://doi.org/10.3390/plants11223163

Kubitzki, K. (1993). Plumbaginaceae. In K. Kubitzki, J. G. Rohwer, & V. Bittrich (Eds.), *Flowering Plants · Dicotyledons. The Families and Genera of Vascular Plants, Vol. II*. (pp. 523-530). Heidelberg, Berlin: Springer. doi: https://doi.org/10.1007/978-3-662-02899-5_62

Miranda-Hernández, L., Salmerón-Sánchez, E., Fernández-Cobo, M. J., Merlo, E., Salazar-Mendías, C., Mendoza-Fernández, A. J., Martínez-Hernández, F., Pérez-García, F. J., & Mota, J. F. (2026). Hybridization, ecological niche, and conservation of the threatened endemic species *Limonium estevei*: an integrated approach in the genus *Limonium*. *Biodiversity and Conservation* 35, 54. doi: https://doi.org/10.1007/s10531-026-03254-7.

Nieto-Feliner, G., Gutiérrez-Larena, B., & Fuertes-Aguilar, J. (2004). Fine-scale geographical structure, intra-individual polymorphism and recombination in nuclear ribosomal internal transcribed spacers in *Armeria* (Plumbaginaceae). *Annals of Botany*, *93*(2), 189-200. doi: https://doi.org/10.1093/aob/mch027

Palacios, C., Rosselló, J. A., & González-Candelas, F. (2000). Study of the evolutionary relationships among *Limonium* species (Plumbaginaceae) using nuclear and cytoplasmic molecular markers. *Molecular Phylogenetics and Evolution, 14*(2), 232-249. doi: https://doi.org/10.1006/mpev.1999.0690

Plants of the World Online. (2024). *Limonium* Mill.
https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:331722-2. Accessed on 8 August 2025.

Rieseberg, L. H., Ellstrand, N. C., & Arnold, M. (1993). What can molecular and morphological markers tell us about plant hybridization? *Critical Reviews in Plant Sciences*, *12*(3), 213-241.
doi: https://doi.org/10.1080/07352689309701902

Salmerón-Sánchez, E., Pérez-García, F. J., Medina-Cazorla, J. M., Martínez-Nieto, M. I., Martínez-Hernández, F., Garrido-Becerra, J. A., Mendoza-Fernández, A. J., Merlo, E., & Mota, J. F. (2014). Genetic analysis based on plastidial and ribosomal sequences of the endemic bi-edaphic taxon *Jurinea pinnata* (Lag.) DC.(Compositae) in the Guadix-Baza Basin. *Plant Biosystems*, *149*(5), 922-932. doi: https://doi.org/10.1080/11263504.2014.983203

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods, 9*(7), 671–675. doi: https://doi.org/10.1038/nmeth.2089

Thiers, B. M. (continuously updated). *Index Herbariorum: A global directory of public herbaria and associated staff.* Electronic resource in https://sweetgum.nybg.org/science/ih/

Turland, N. J., Wiersema, J. H., Barrie, F. R., Gandhi, K. N., Gravendyck, J., Greuter, W., Hawksworth, D. L., Herendeen, P. S., Klopper, R. R., Knapp, S., Kusberg, W., Li, D., May, T. W., Monro, A. M., Prado, J., Price, M. J., Smith, G. F., & Zamora, J. C. (2025). *International Code of Nomenclature for algae, fungi, and plants (Madrid Code)*. Chicago: University of Chicago Press. doi: https://doi.org/10.7208/chicago/9780226839479.001.0001

Anex

Other material examined

Limonium estevei Fern. Casas

SPAIN: Almería, Mojácar, Macenas, between the urbanization and the Cueva Negra campsite. 37°05'04''N,1°51'31"W. 20 June 2012. 64 m a.s.l. Legit. *F.J. Pérez-García, E. Salmerón-Sánchez, M.L. Rodríguez-Tamayo, A. Mendoza-Fernández, J.F. Mota*. Det. *J.F. Mota* (HUAL 30718, 30719, 30720, 30721, 30722).

Limonium cossonianum Kuntze

SPAIN: Almería, Mojácar, Macenas, between the urbanization and the Cueva Negra campsite. 37°05'13"N,1°51'20"W. 13 July 2012. 31 m a.s.l. Legit. *F.J. Pérez-García, E. Salmerón-Sánchez, M.L. Rodríguez-Tamayo, A. Mendoza-Fernández, J.F. Mota*. Det. *J.F. Mota* (HUAL 30723, 30724, 30725, 30726, 30727). SPAIN: Almería, Levante Almeriense, between Garrucha and Mojácar, near Cortijo de los Granadinos. 37°9'46.25432"N,1°50'54.90164"W. 10 July 2012. 13 m a.s.l. Legit. *F.J. Pérez-García, E. Salmerón-Sánchez*. Det. *F.J. Pérez-García* (HUAL 30730, 30731, 30732, 30733, 30734). SPAIN: Almería, Adra, beach near the Albufera Nueva. 36°45'4.12521"N,2°56'28.76941"W. 10 July 2012. Legit. *F.J. Pérez-García, E. Salmerón-Sánchez*. Det. *F.J. Pérez-García* (HUAL 30728, 30729). SPAIN: Almería, Salinas de Cabo de Gata, next to the Bird Observatory. 36°46' 43.79510"N,2°14'1.44703"W. 17 July 2012. 3 m a.s.l. Legit. *F.J. Pérez-García, E. Salmerón-Sánchez*. Det. *F.J. Pérez-García* (HUAL 30761, 30762). SPAIN:

Almería, Salinas del Cabo de Gata, 30SWF6970, 22 June 2008, *F. Gómez Mercado* (HUAL 20150, HUAL 20151). SPAIN: Almería, Salinas del Cabo de Gata, 30SWF6870, 10 July 2008, *F. Gómez Mercado* (HUAL 20153, HUAL 20154). SPAIN: Almería, Campo de Níjar, near Rambla de las Palmillas. 36°56'24.91980"N,2°13'48.01385"W. 10 July 2012. 277 m a.s.l. Legit. F.*J. Pérez-García, E. Salmerón-Sánchez*. Det. *F.J. Pérez-García* (HUAL 30743, 30744, 30745, 30746). SPAIN: Almería, Mojácar, Torre del Pirulico, near Castillo de Macenas. 37°4'5.52658"N,1°51'2.62588"W. 10 July 2012. 16 m a.s.l. Legit. *F.J. Pérez-García, E. Salmerón-Sánchez*. Det. *F.J. Pérez-García* (HUAL 30755, 30756, 30757, 30758, 30759, 30760). SPAIN: Almería, Rioja, Rambla de Tabernas, next to km 462 of the N-340a road. 37°0'27.12653"N, 2°27'12.09784"W. 10 July 2012. 224 m a.s.l. Legit. *F.J. Pérez-García, E. Salmerón-Sánchez*. Det. *F.J. Pérez-García* (30763, 30709, 30710, 30711, 30712). SPAIN: Almería, Levante Almeriense, near Venta del Pobre, next to the course of Río Alías. 37°1'7.71559"N,2°2'52.39049"W. 10 July 2012. 211 m a.s.l. Legit. *F.J. Pérez-García, E. Salmerón-Sánchez*. Det. *F.J. Pérez-García* (HUAL 30735, 30736, 30377, 30738, 30739). SPAIN: Almería, near Faro de San Telmo, on the cliff of Cala del Lobo Marino. 36°49'42.65793"N,2°29'37.42119"W. 10 July 2012. 5 m a.s.l. Legit. *F.J. Pérez-García, E. Salmerón-Sánchez*. Det. *F.J. Pérez-García* (HUAL 30747, 30748, 30749, 30750, 30751). SPAIN: Almería, Levante Almeriense, San Juan de los Terreros, saltmarsh in the bed of the former saltworks. 37°21'36.08669"N,1°40'15.65730"W. 10 July 2012. 3 m a.s.l. Legit. *F.J. Pérez-García, E. Salmerón-Sánchez*. Det. *F.J. Pérez-García* (HUAL 30740, 30741, 30742). SPAIN: Almería, Levante Almeriense, Villaricos, near piedra del Medio Día. 37°15'59.97163"N,1°46'40.49647"W. 10 July 2012. 21 m a.s.l. Legit. *F.J. Pérez-García, E. Salmerón-Sánchez*. Det. *F.J. Pérez-García* (HUAL 30752, 30753, 30754).