Ann. Bot. Fennici 48: 49–56 ISSN 0003-3847 (print) ISSN 1797-2442 (online)
Helsinki 31 March 2011

Helianthemum motae (Cistaceae), a new species from southeastern Spain

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Received 2 Nov. 2009, revised version received 21 Dec. 2009, accepted 23 Dec. 2009

Sánchez-Gómez, P., Jiménez, J. F. & Vera, J. B. 2011: *Helianthemum motae* (Cistaceae), a new species from southeastern Spain. — *Ann. Bot. Fennici* 48: 49–56.

Helianthemum motae Sánchez-Gómez, J.F. Jiménez & J.B. Vera (Cistaceae) is described as a new species from southeastern Spain. It belongs to the section *Lavandulaceum* and appears to be close to *H. syriacum*. The diagnostic characters, a phylogenetic study as well as a full description, a dichotomic key, and illustrations are provided. Both macromorphological and micromorphological characters are discussed and compared with the morphologically related species. The conservational status of *H. motae* is discussed.

The genus *Helianthemum* is widespread throughout the Iberian Peninsula in the western Mediterranean context (Willkomm 1878–1880, Grosser 1903, Guinea 1954, López-González 1992, 1993). At present, around 45 taxa are known at the species and subspecies levels, belonging to the subgenera *Helianthemum* and *Plectolobum* (López-González 1993, Sáez & Rosselló 1999, Sánchez-Gómez *et al*. 2001). Of those taxa, 23 are Iberian endemics, 6 are Ibero-African, 11 have a wider Mediterranean distribution and 5 have a wide Euro-Siberian distribution. Many of the species are indifferent regarding the substrate, but several taxa prefer dolomite, gypsum, sand or brackish-water environment. Such species tend to be endemic and confined to narrow areas.

Numerous taxa of the genus *Helianthemum* occur in the southeastern Iberian Peninsula, of particular note being those belonging to the sections *Helianthemum* and *Pseudocistus* (López-González 1993). The exploration of botanically relevant areas (botanical microreserves) in the province of Murcia (southeastern Spain) revealed a population of *Helianthemum* on sandstones and in brackish environments near the sea. The plants present certain affinities with *H. syriacum*, a common Mediterranean species in these regions. An *a posteriori* morphological comparison identified some macromorphological affinities also with *H. sicanorum*, a species recently described from similar environments in southern Sicily (Brullo *et al*. 2007), and with other *Helianthemum* species of southeastern Spain and north Africa, such as *H. squamatum*, *H. almeriense* and *H. kahiricum*. However, the plants reported here differ from all of those species.

Material and methods

The morphological study was based on the material collected by the authors and on accessions

from MUB and MA. Scanning electron microscopy (SEM) was used to study the seeds and capsules in detail. For this, they were directly sputtered with gold (layer 200–300 Å thick). A Jeol JSM-6100 scanning electron microscope was used. For the DNA study, ten samples collected by the authors were used, including two samples of the new species (Table 1). In addition, six sequences of *Cistus* spp. from Genbank, representing several species of a phylogenetic study of *Cistus* (Guzmán & Vargas 2005), were used as an outgroup (Table 1). The total DNA was extracted using the DNAeasy Kit (Qiagen, Germany) and frozen at –20 °C until amplification. The PCR reactions were carried out in an Eppendorf Mastercycler thermocycler using the following procedure: initial cycle at 94 °C for 5 min, 35 cycles at 94 °C for 30 s, 52 °C for 45 s, 72 °C for 1 min, and a final extension step at 72 °C for 8 min to complete the PCR. The nuclear ribosomal internal transcribed spacer (ITS-2) was amplified with the primer pair 5.8S–26S (Sun *et al*. 1994). The reactions were carried out in a final volume of 50 μl using ca. 40 nanograms of the DNA, 0.4 μM of direct and reverse primers, 5 μl of polymerase buffer (provided by the supplier of the enzyme) 0.2 mM of each of dNTP, 2 mM of $MgCl_2$ and 2 units of Taq polymerase (Biotools). Finally, 2 ml of each amplification product were visualised on agarose gel at 1.5%, and successful amplifications were cleaned using the GenElute PCR Clean-Up kit (SIGMA). The primers used in the amplification were used in the sequencing reaction with the Big Dye sequencing kit and separated in an automatic ABI 3700 sequencer following standard protocols. For each DNA sample both strands were sequenced. The sequences have been submitted to GenBank (Table 1).

Sequences were aligned using CLUSTALX (Thompson *et al*. 1997). Bioedit (Hall 1999) was used to make minor alignment adjustments. The program PAUP 4.0b10 (Swofford 2002) was used to analyse the data under Maximum Parsimony (MP). Gaps were excluded from all analyses. The MP analysis was carried out as described in Harrison and Langdale (2006). Bootstrap analyses (Felsenstein 1985) were carried out with 1000 replicates using the heuristic search strategy as indicated above.

Results and discussion

Helianthemum motae Sánchez-Gómez, J.F. Jiménez & J.B. Vera, *sp. nova* (Figs. 1 and 2)

Differt a simili Helianthemo syriaco foliorum limbis minoribus (non ultra (10)12–23 × 2–6

Table 1. List of the species included in the analysis, with the voucher reference and GenBank accession number of the specimens.

Species	Voucher specimen or reference	Genbank accession no.
Cistus clusii	Guzman & Vargas (2005)	DQ092963
Cistus crispus	Guzman & Vargas (2005)	DQ092967
Cistus ladanifer subsp. *africanus*	Guzman & Vargas (2005)	DQ092956
Cistus ladanifer subsp. *sulcatus*	Guzman & Vargas (2005)	DQ092954
Cistus monspeliensis (1)	Guzman & Vargas (2005)	DQ092966
Cistus monspeliensis (2)	Guzman & Vargas (2005)	DQ092965
Helianthemum almeriense	Murcia, Cuatro Calas MUB 110981	GU327675
Helianthemum motae (1)	Murcia, Cuatro Calas MUB 110988	GU327668
Helianthemum motae (2)	Murcia, Cuatro Calas MUB 110989	GU327669
Helianthemum kahiricum (1)	Morocco, Guercif MUB 110983	GU327666
Helianthemum kahiricum (2)	Morocco, Guercif MUB 110984	GU327667
Helianthemum sicanorum	Sicily, Gela, MUB 110985	GU327670
Helianthemum squamatum (1)	Murcia, Molina MUB 111012	GU327671
Helianthemum squamatum (2)	Murcia, Molina MUB 111013	GU327672
Helianthemum syriacum (1)	Murcia, Gilico MUB 109122	GU327673
Helianthemum syriacum (2)	Albacete, Minateda MUB 100265	GU327674

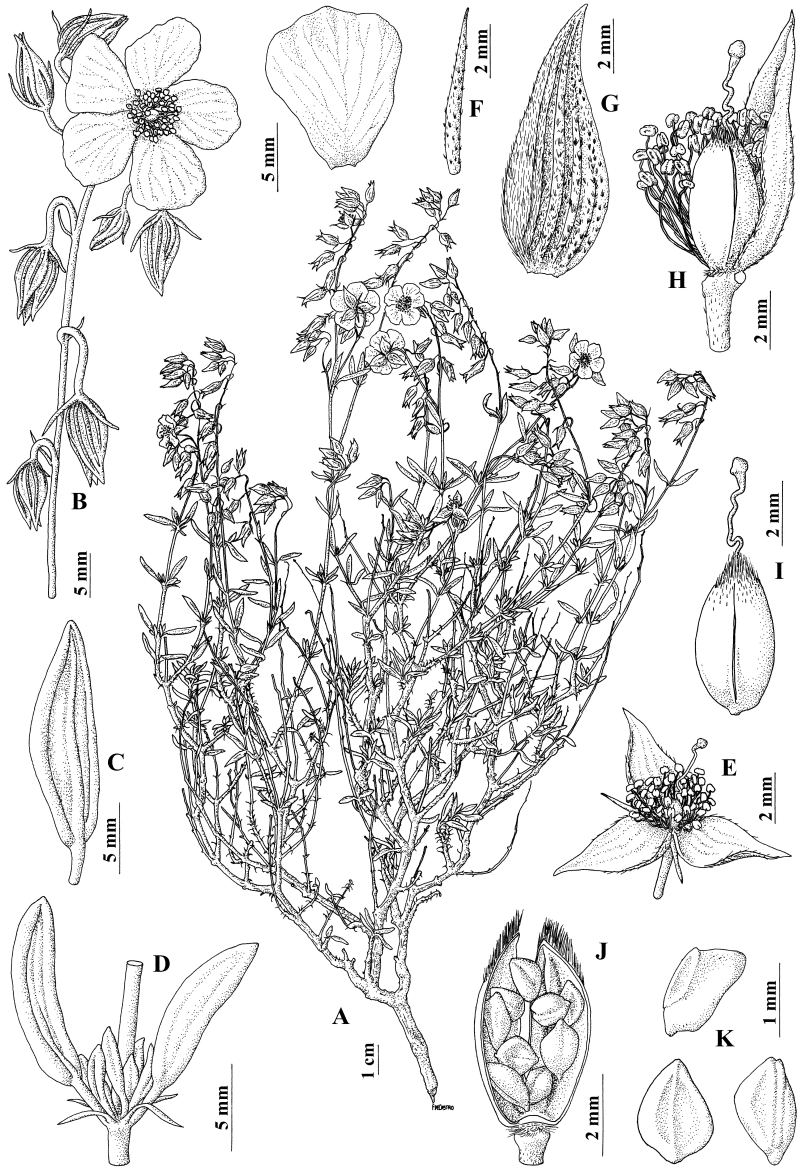

Fig. 1. *Helianthemum motae* (from the holotype). — **A**: Habit. — **B**: Detail of inflorescence. — **C**: Leaf (abaxial surface). — **D**: Insertion of leaves on stem. — **E**: Detail of flower without petals. — **F**: Outer sepal. — **G**: Inner sepal. — **H**: Detail of ovary and stamens. — **I**: Ovary. — **J**: Detail of open capsule and seed insertion. — **K**: Seeds.

mm longis latisque), inflorescentiis pauciforibus (ramosis aut simplicibus: hae, certe, non ultra 8(10) florae), sepalis internis pluries costatis (numquam bicostatis: costae uniuscuiusque 3–4(5)) atque sepalis externis linearibus, nec lanceolatis, numquam ciliatis. Species dicatur amicissimo Joanni Francisco Mota Poveda, botanico Aguilasensi.

HOLOTYPE: Spain. Murcia, Aguilas, Cuatro Calas UTM 30SXG2138, 10 m. Matorrales sobre areniscas y conglomerados, 17.V.2007 *P. Sánchez-Gómez, J. B. Vera & J. F. Jiménez* (MUB 111000; isotypes MA, MGC, VAL).

Perennial, 20–50 cm, suffruticose, erect with ascending branches. Stems reddish with adpressed stellate hairs. Leaves linear-lanceolate, lanceolate to oblong-elliptical, mucronate, slightly fleshy, greenish, with variable indumentum of stellate hairs on upper surface, denser on lower surface; limb (10)12–23 × 2–6 mm; petiole 2–5 mm. Stipules up to 4.5 mm long, reddish, from linear to triangular-subulate, with appressed indumentum. Inflorescences simple or branched (up to 3), each with (1)4–8(10) lax flowers. Bracts up to 4 mm long, linear,

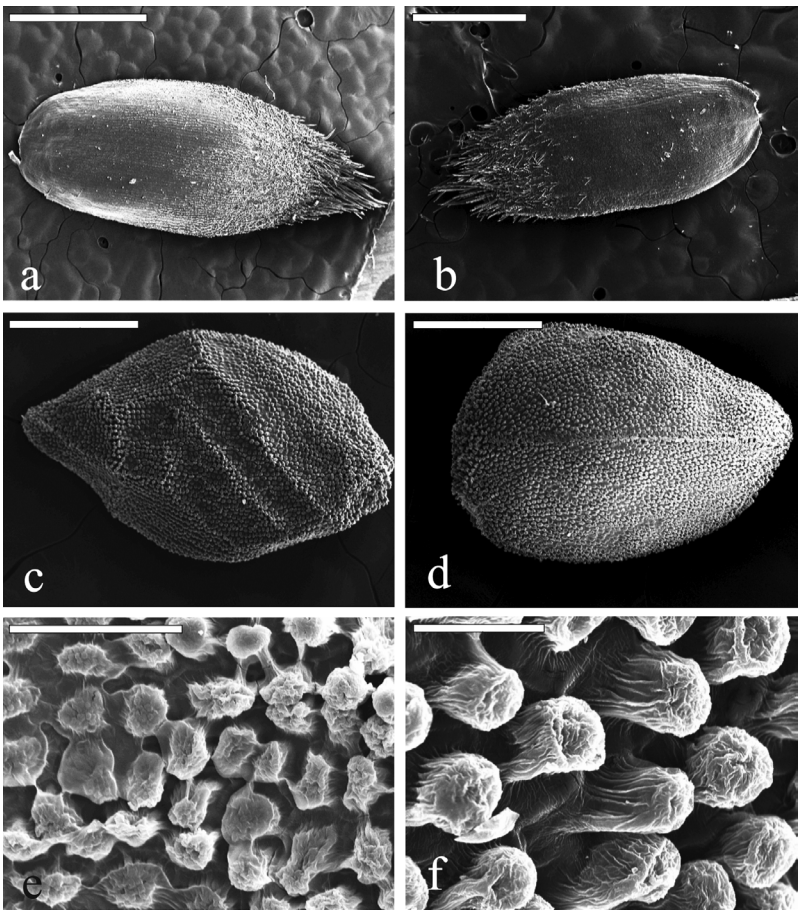

Fig. 2. SEM micrographs of *Helianthemum motae* and *H. syriacum*. — **a**: Capsule valve of *H. motae*. — **b**: Capsule valve of *H. syriacum*. — **c**: Seed of *H. motae*. — **d**: Seed of *H. syriacum*. — **e**: Detail of surface of *H. motae* seed. — **f**: Detail of surface of *H. syriacum* seed. Scale bars: in **a** ~2 mm, in **b** ~1 mm, in **c** and **d** ~500 μm, in **e** ~50 μm, in **f** ~20 μm.

reddish, with indumentum formed of bunches of appressed hairs. Outer sepals 3.5–6(7) mm, linear, reddish. Inner sepals (8)9–10 mm (up to 12 mm during fruiting), lanceolate, acuminate, with 3–4(5) slightly protruding nerves, indumentum stellate-tomentous, denser and with long hairs on larger lateral rib, shorter, frequently reddish hairs on the opposite side. Petals (7)10–11.5 × (5)10–12 mm, obovate, yellow. Stamens 45–65. Style exserted, filiform, sigmoid. Fruit pedicles 7–12 mm, reflexed, sigmoid. Capsule 4–5.7 mm, ellipsoid-trigonal, villous at apex. Seeds 9–12 per capsule, up to 1.6 mm, brown-reddish, angular; surface verrucous, verrucae up to 10–14 μm tall, joined by bridges.

Additional herbarium material studied. — *Helianthemum motae* (paratypes, all in MUB): **Spain**. *Almería*: Pulpí, Los Cocedores, 8 m, 30SXG2137, 5.V.2007 *P. Sánchez-Gómez, J. B. Vera & J. F. Jiménez* (111010, 111011). *Murcia*: Aguilas, Cuatro Calas, 5 m, 30SXG2138, 5.V.2007 *P. Sánchez-Gómez, J. B. Vera & J. F. Jiménez* (111006, 11007, 111008, 111009); Aguilas, Cuatro Calas, 10 m, 30SXG2138, 17.V.2007 *P. Sánchez-Gómez, J. B. Vera & J. F. Jiménez* (110988–110999, 111001, 111002). — *Helianthemum kahiricum:* **Algeria**. Montis Melias, prope Fignig, *R. Maire* (MA 81074). **Morocco**. Agadir, 15 km N Tiz nit en der Straβe nach Agadir, *D. Podlech* (MA 472225); Guercif, carretera de Guercif a Taourirt, *F. J. Saorín & J. B. Vera* (MUB 110983, 110984). **Tunisia**. Gafsa, entre Gafsa y Sened, 12 km al W de Sened, C. Benedí *et al*. (MA 557454); Gafsa, *C. J. Pitard* (MA 81075); Matmata, *C. J. Pitard* (MA 81076). — *Helianthemum sicanorum:* **Sicily**. Gela, prope Torre Manfria, *J. F. Jiménez* (MUB 110985–110987). — *Helianthemum syriacum* (all in MUB)*:* **Spain**. *Albacete*: Proximidades de Minateda, *P. Sánchez-Gómez & J. F. Jiménez* (100265); Embalse del Cenajo, *M. A. Carrión* (104484); Quebradas Bajas, *P. Sánchez-Gómez, J. L. Cánovas & D. López* (109206). *Almería*: Sierra de Gádor, *F. J. Pérez, F. Martínez & F. Sola* (101955). *Murcia*: La Pinilla, *J. B. Vera* (100266); Campus de Espinardo, *M. A. Carrión* (104482); Morrón de la Yesera,

M. A. Carrión (104483); Cola del Caballo, *J. F. Jiménez & M. A. Carrión* (104485); Monte Arabí, *J. B. Vera* (104486); Salinas de Molina, *E. Hernández* (104487); Majal Blanco, *J. Muñoz & R. Romero* (108078); Embalse del Mayés, *A. Cutillas* (108680); Baños de Gilico, *P. Sánchez-Gómez*, *J. L. Cánovas & D. López* (109122); Cruce carretera Calasparra-Moratalla-Socovos, *P. Sánchez-Gómez*, *J. L. Cánovas & D. López* (109164); Sierra de los Cucos, *P. Sánchez-Gómez et al.* (109626); Sierra de los Cucos, *P. Sánchez-Gómez et al.* (109627); Rambla de los Carboneros, *J. L. Cánovas* (109908); Huerta nueva, *J. L. Cánovas* (110151); Los Prados, *D. López* (110346); El Portús, *I. M. Martínez & P. Sánchez-Gómez* (110811). *Zaragoza*: carretera de Mequinenza a Caspe, *P. Sánchez-Gómez & J. F. Jiménez* (110982).

Morphological data

The main characters that differentiate *Helianthemum motae* from *H. syriacum* are listed in Table 2. The former also has various macromorphological and ecological affinities with *H. sicanorum*, although according to Brullo *et al.* (2007) that species, together with *H. kahiricum*, would belong to a different section (Sect. *Eriocarpum*), generally with plants with smaller flower parts and leaves, simple inflorescences, a reduced number of stamens (15–25 in *H. sicanorum*), a densely pilose capsule, cleistogamous flowers, etc.

Molecular data

Alignment of the 12 ingroup and 6 outgroup taxa for the nrITS-2 yielded 510 nucleotide sites. Of these, 441 sites were constant, 27 were variable but parsimony uninformative, and 42 were parsimony informative. The MP search revealed 214 most parsimonious trees of 238 steps, CI = 0.833, RI = 0.908. The strict consensus tree with bootstrap values for supported nodes is presented in Fig. 3.

Helianthemum species are well separated from *Cistus* species. The *Helianthemum* clade comprises three well supported (100% bootstrap) branches. The first branch is for *H. almeriense*, a clearly different species included in Sect. *Helianthemum*. The second branch, supported with moderate bootstrap values (83%), includes *H. sicanorum* and *H. kahiricum*, taxa placed in Sect. *Eriocarpum* (Brullo *et al.* 2007). The third branch is well supported (87% bootstrap value), and includes *H. motae* together with *H. syriacum* and *H. squamatum*, taxa belonging to Sect. *Lavandulaceum* and *Argyrolepis* (formerly Sect. *Polystachyum*), respectively (López-González 1993).

Table 2. Morphological differences between *Helianthemum motae* and *H. syriacum*.

	Helianthemum motae	*Helianthemum syriacum*
Height (cm)	20–50	10–90
Leaf length (mm)	(10)12–23 × 2–6	10–50 × 2–8
Petiole length (mm)	2–5	2–6
Stipules	up to 4.5 mm, linear to triangular-subulate	up to 1 cm, triangular-subulate to ovate-lanceolate, ciliate
Inflorescence	simple or with 1–3 branches	with 3–5 branches
Bracts	linear, up to 4 mm	linear-lanceolate, ciliate, up to 7 mm
Flowers per inflorescence	(1)4–8(10)	(5)15–30(34)
Outer sepals	3.5–5 mm, linear, glabrous margin	2.7–5.5 mm, lanceolate, often ciliate margin
Inner sepals	(8)9–10 mm up to 12 mm at fruiting, 3–4(5) nerves	5–7(9) mm up to 12 mm at fruiting, 2 nerves
Petals (mm)	(7)10–11.5 × (5)10–12	5–10 × 6–10
Stamens	45–65	> 25
Capsule (mm)	4–5.7, villose at the apex	2.7–4, villose at the apex
Seeds per capsule	9–12	3–6
Seed length (mm)	(1.2)1.3–1.6	(1.1)1.3–1.4(1.5)
Seed colour	brown-reddish	brown-reddish
Seed surface	verrucous, processes 10–14 μm high, joined by bridges	verrucous, processes up to 20 μm high, clearly separated

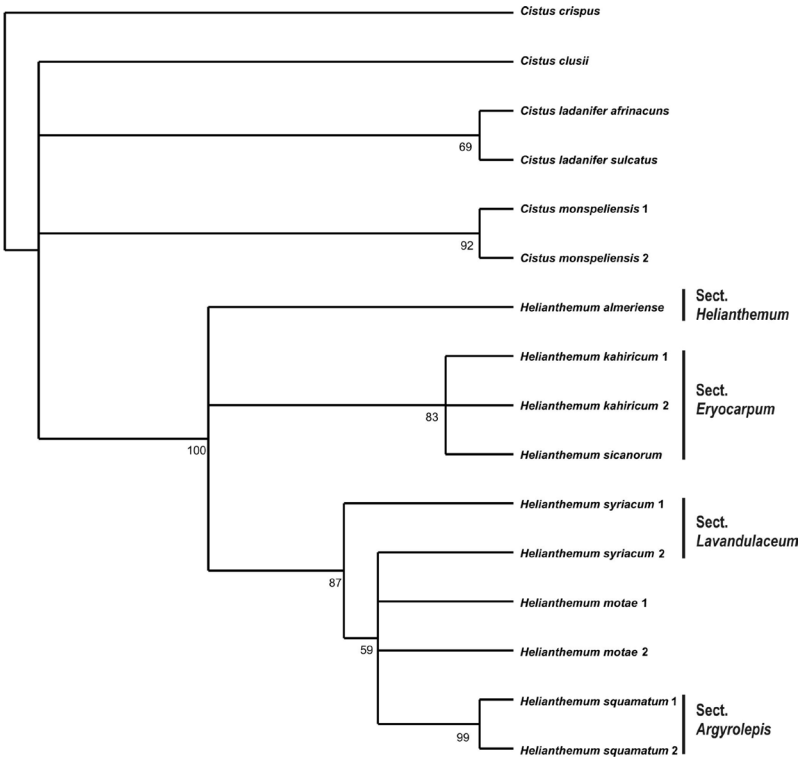

Fig. 3. Strict consensus of 214 most parsimonious trees of 238 steps (CI = 0.833, RI = 0.908), based on ITS sequences. Bootstrap values above 50% are given using Maximum Parsimony.

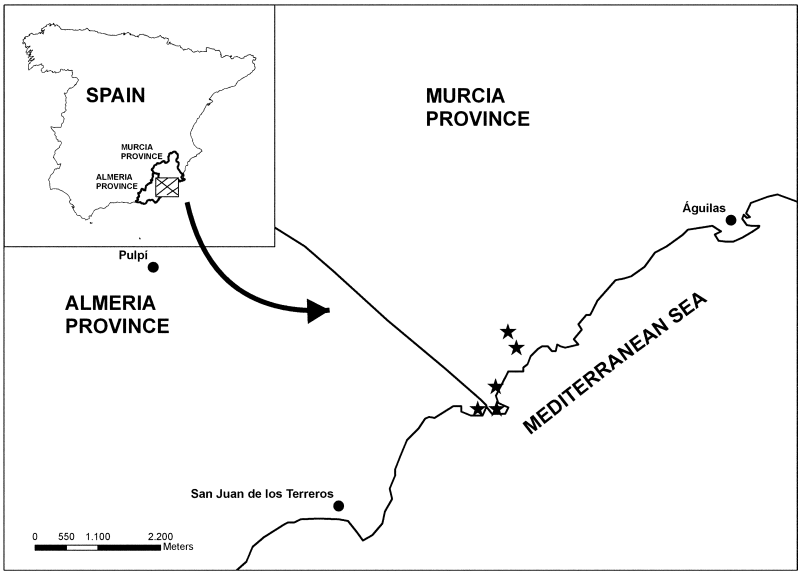

Fig. 4. Distribution map of known populations of *Helianthemum motae* (black stars).

Distribution and habitat

Helianthemum motae is endemic to southeastern Spain, where it grows in the provinces of Almeria (Pulpí) and Murcia (Águilas) in an enclave known as Cuatro Calas (Fig. 4). Biogeographically, the territories where it is found lie within the Murcian-Almeriensian chorological province, Almeriensian Sector, Oriental Almeriensian Subsector, characterised by a flora rich in

exclusive and Ibero-African endemics (Sánchez-Gómez & Guerra 2007).

At present only one population is known, consisting of small subpopulations that contain from 5 to 200 individuals. In all, the population is estimated at about 2000 mature individuals. Its area of occupancy does not exceed 4.6 ha, while the extent or occurrences covers 95 ha. *Helianthemum motae* grows almost exclusively on sandstones and conglomerates in a narrow strip next to the sea and in adjacent saline depressions. It forms a part of low cover matorrals accompanied by such species as *Anabasis hispanica*, *Asparagus horridus*, *Asteriscus maritimus*, *Frankenia corymbosa*, *Fumana ericifolia*, *Helianthemum almeriense*, *Launaea arborescens*, *L. lanifera*, *Limonium insigne*, *Lycium intricatum*, *Lygeum spartum*, *Salsola papillosa*, *Santolina viscosa*, *Sideritis ibanyezi*, *Teucrium carolipaui* subsp. *fontqueri*, *T. lanigerum*, etc. These matorrals correspond to low vegetation cover on special soils, frequently rich in heavy metals, with an optimum in territories of the Almeriensian Sector. The places where *H. motae* is found are close to saline depressions and coastal cliffs, so that some individuals even grow in transitory subhalophilous communities, forming part of the steppe vegetation or fleshy matorrals of *Halocnemum strobilaceum*, *Suaeda vera* and *Limonium* spp.

Taxonomic discussion

Taking into account the new subdivision of the section *Polystachyum*, *H. motae* shows greater affinity with the section *Lavandulaceum*, in which *H. syriacum* is at present included. Its position in the cladogram suggests that *H. motae* originated from a common ancestor with *H. syriacum*. The sequences of the nrITS-2 region show little divergence between *H. motae* and *H. syriacum*, but this could be common in the related *Helianthemum* species. In fact, the ITS-2 sequences of *H. sicanorum* and *H. kahiricum*, two clearly morphologically differentiated species, show no nucleotide divergence. Furthermore, Guzmán and Vargas (2005), in a previous phylogenetic study in *Cistus*, reported limited resolution of these ITS sequences. On the other hand, the presence of simple inflorescences suggests a possible hybrid origin with other species of the section *Helianthemum* (which are common in southeastern Iberia), or even of the section *Eriocarpum*, richest in the Saharo-Arabian Region. The molecular markers used do not permit us to affirm or reject this possibility, although using a greater number of species belonging to the mentioned sections and chloroplast DNA markers should throw more light on the phylogenetic relationships.

Implications for conservation

Administratively, the known distribution area of *H. motae* belongs to Andalucía and Murcia, which therefore share legislative responsibility from an environmental point of view, an inconvenience for the integrated management of the species. Most of the individuals identified to date (approx. 70%) grow in the province of Murcia. The area is considered to be protected at regional level while, at the European level, it forms a part of the Site of Community Importance within the Natura 2000 network denominated "Cuatro Calas"; moreover, part of the area has been proposed as a Botanical Microreserve (Sánchez-Gómez *et al*. 2005). The Almerian part (approx. 30%), however, is not included in any protected area. At present, the habitat of *H. motae* is susceptible to series of threats, especially anthropogenic: the expansion of built-up areas, rubbish and building material dumps, tourist visits, infrastructure construction or modification, especially roads and car parks related with access to beaches. There has been a steady decline in the number of *H. motae* individuals and the quality and quantity of their habitat. Applying the criteria of the IUCN (2001), the species should be considered as within the category CR B1ab (i, ii, iii, iv, v) + 2ab (i, ii, iii, iv, v), so that it should be immediately included in the list of legally protected species at regional, national and European levels.

Acknowledgements

We thank Dr. Juan Guerra Montes for help in studying the micromorphological characters observed by SEM, Francisco

J. Sánchez Saorin for field assistance in collecting seeds, Felicisimo de Castro for making the iconography of *H. motae*, M. Laínz for correcting the Latin diagnosis and Jaime Güemes and Carlos Aedo for a critical revision of the manuscript. This study was supported by an Agreement between the University of Murcia and the General Directorate of Universities and Scientific Policy of the Autonomous Community of Murcia.

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