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# *CORONILLA MONTSERRATII*, A NEW HEXAPLOID ANNUAL SPECIES FROM THE EASTERN BALEARIC ISLANDS

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**SUMMARY**: A new species, *Coronilla montserratii*, is described from the coastal and inland sand dunes of Minorca (Balearic Islands). The new species is hexaploid (2n=36), the highest ploidy level so far known in annual *Coronilla* species. Morphological features suggest that *C. montserratii* is related to the tetraploid *C. repanda*, from which it could be discriminated by several leaf features. The join evaluation of morphological and molecular data suggests that *C. montserratii* is an allopolyploid species. Ribosomal ITS sequences identified *C. scorpioides* as a likely progenitor, but the other progenitor species remains elusive on molecular grounds. Based on the close morphology shared between *C. montserratii* and *C. repanda* it is hypothesized that the latter could be also involved in the origin of the new hexaploid species. ***Key Words***: Polyploidy, Loteae, Fabaceae, endemism, insular flora, taxonomy

**RESUMEN**: Se describe una nueva especie, *Coronilla montserratii*, de las dunas costeras y arenales interiores de Menorca (Islas Baleares). La nueva especie es hexaploide (2n=36), el nivel de ploidía conocido más elevado en las especies anuales de *Coronilla*. La morfología de la planta sugiere que *C. montserratii* está relacionada con *C. repanda* (tetraploide), de la que se distingue por diversos caracteres foliares. El análisis conjunto de datos morfológicos y moleculares sugieren que *C. montserratii* es una especie alopoliploide y que *C. scorpioides* y *C. repanda* pueden haber intervenido en su origen. ***Palabras clave***: Poliploidía, Loteae, Fabaceae, endemismo, flora insular, taxonomía.

*Coronilla* L. is a temperate genus of legumes belonging to the tribe *Loteae* which shows a mainly Mediterranean distribution with several species extending to northern European latitudes (UHROVÁ, 1935). The two annual species of *Coronilla*, *C. repanda* and *C. scorpioides*, have been included within sect. *Scorpioides* Bent. (UHROVÁ, 1935). A third entity, *C. dura* (Cav.) Boiss. is not unanimously recognized as a distinct species and has been usually included within *C. repanda* at the subspecific level (BALL, 1968; GARCÍA MARTÍN & TALAVERA, 2000). During field work aimed at increasing the floristic knowledge of the sandy areas of Minorca (Balearic Islands), we found several populations resembling *C. repanda* but showing a deviant morphology concerning the shape of leaves and leaflets. In addition, cytogenetic studies revealed that the Minorcan plants were highly polyploid and differed from previous chromosome numbers so far reported in annual species of *Coronilla*.

The combination of morphological characteristics of diagnostic value and ploidy level suggest that the plants from Minorca belong to an entity not previously described, and it is presented in this paper as a new species.

## MATERIALS AND METHODS

***Plant material*.** Living material from *C. repanda* and *C. scorpioides* was collected from populations across Minorca. Voucher specimens are preserved at VAL. Morphological observations were based on living material examined in the field and herbarium specimens from the herbaria BC, MA, VAL (abbreviations according to HOLMGREM & al. 1990).

***Chromosome preparations*.** Seeds were germinated on solid agar in Petri dishes in a constant temperature of 20ºC and 12 hours of white light daily. Root tips were pre-treated with 0.05% colchicine solution at room temperature for 2-3 hours, washed with distilled water, fixed in fresh Carnoy I solution overnight and stored in 70% ethanol at 4ºC until used. For chromosome counts root tips were hydrolysed for 5-10 min in 1M HCl at 60ºC, and washed and stained in Feulgen solution for 1-2 h. Stained meristems were squashed in a drop of 45% acetic acid and permanent preparations were made by mounting in Canada balsam. Photomicrographs of well-spread metaphases were taken with an Olympus Camedia C-2000-Z digital camera and processed with Adobe Photoshop 7.0. Chromosome counts were made from at least five well-spread metaphases by direct observation and from the photomicrographs. Chromosome measurements were made on digital images using the processing image software ImageTool 5.0.

## RESULTS

**Coronilla montserratii** P. Fraga & Rosselló, sp. nov**.** (Fig. 1)

**Diagnosis:** A *Coronilla repanda* similis, sed robustior, foliolis longiores et latiores, foliae composita cum foliolis heteromorphi, legumina latiores et robustior, et chromosomatum numerum (2n=36) differt.

**Holotype**: SPAIN: Balearic Islands. Minorca: Arenal de Macarelleta, Ciutadella de Menorca (31SEE7921), fixed calcareous sand dunes with low scrub, 20 m, 31-03- 1996, *P. Fraga* (VAL 190331). Isotype: herbarium *P. Fraga*.

**Description:** Ascending or erect, glabrous, glaucous annual. Stem 10-40 cm long, subterete or obscurely angled, branched from the base and upwards. Leaves slightly fleshy, heteromorphic. Basal leaves simple, unifoliate, petiolate, oblong, 0.5-3.5 cm long, 0.3-2.8 cm wide, apex rounded or truncate, base broadly cuneate, border entire. Medium leaves, petiolate, 3-7 foliolate. Leaflets heteromorphic, the terminal 0.7-2 x 0.4-1.4 cm, longer than the lateral ones, apex usually emarginated, mucronate, base narrowly cuneate, border entire; lateral leaflets orbicular to obovate, 0.5-1.5 x 0.3-1.2 cm, the lower pair bigger than the apical one, apex rounded, mucronate, base rounded to broadly cuneate, border entire; basal leaflets clearly distinct, obcordate to ear shaped, 0.2-1.2 x 0.15-0.8 cm wide, apex obtuse to emarginate, sometimes mucronate. Upper leaves with up to five leaflets, heteromorphic; stipules connate, 1.5-3 mm long, almost as long as wide, membranous, apex clearly bifid, teeth sometimes acuminate. Inflorescences 2-5 flowered, arising from the axils of the upper leaves. Peduncles 1-4 cm long, exceeding the subtending leaf and lengthening in fruit; bracts connate, up to 1 mm long, deltoid, membranous; pedicels short, curved, up to 2 mm long. Calyx cup-shaped, 2-2.5 x 1.8-2 mm wide, calyx-teeth widely deltoid, acute to subobtuse. Corolla yellow, the standard stripped brownish red; limb of standard ovate to somewhat orbicular, 3-5 x 2.5-5 mm, apex rounded or subacute, base narrowed suddenly to a claw 2-2.5 mm long;



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limb of wings obliquely oblong, concave, up to 5 x 2.5 mm, equaling or slightly shorter than the standard, apex rounded, base abruptly narrowed, auricula small and irregular, conjunctival tooth small or wanting, claw 2-2.5 mm long; limb of keel crescent-shaped, narrow, up to 5 x 1.5 mm, apex obtuse, claw 2-2.5 mm long. Stamens 10, all united, 5-6 mm long, connate for up to 4 mm, anthers basifixed, oblong, about 0.3 x 0.1 mm. Ovary linear, compressed, 3.5-4 x 0.4-0.5 mm, style 2-2.5 mm long, tapering towards the apex, stigma small, terminal, capitate. Pod linear, much curved downwards, 2-5 x 0.25-0.4 cm, glabrous, prominently articulated, and conspicuously narrowed between the articulations, dorsal and ventral sutures apparent, apex acute to shortly rostrate; seeds sausage-shaped, slightly curved, 3-4 x 1-2 mm; testa grayish-brown, minutely verrucose. Flowering season starts in early April and, with favorable climatic conditions, lasts until end of June.

***Representative specimens examined.*** **MINORCA**: 31SEE8021, Cala Macarella, Ciutadella de Menorca, 10-V-1959, *P. Montserrat,* (BC); 31SEE7921, Arenal de Macarelleta, Ciutadella de Menorca, 31-III-1996, *P. Fraga* (VAL, Herb. P. Fraga)*;* 31SEE7620, Arenal de Son Saura, Ciutadella de Menorca, 3-IV-1999, *P. Fraga* (VAL, Herb. P. Fraga); 31TEE 8333, Al Pilar, Ciutadella de Menorca, 25-IV- 1999, *P. Fraga* (VAL, Herb. P. Fraga); 31TEE7729, Ses Arenes, Ciutadella de Menorca, 19-V-2000, *P. Fraga* (VAL, Herb. P. Fraga); 31TEE7731, Binigafull, Ciutadella de Menorca, 6-V-2000, *P. Fraga* (VAL, Herb. P. Fraga); 31SEE 7619, Arenal de Son Saura, Ciutadella de Menorca, 31-III-2001, *P. Fraga* (VAL, Herb. P. Fraga); 31TEE8332, Alzinar d'Alforí, Ciutadella de Menorca, 1-V-2001, *P. Fraga* (VAL, Herb. P. Fraga); 31TEE7832, Algaiarens, Ciutadella de Menorca, 23 Jun 2001 *P. Fraga* (VAL, Herb. P. Fraga).

***Etymology***. The species is named after Pedro Montserrat, a Spanish botanist that first collected the species and made substantial contributions to the Balearic flora.

***Distribution.*** Currently, *Coronilla montserratii* is only known from the western half of Minorca.

***Habitat***. Usually, this species grows at low altitudes (between 5 and 80 m), on sandy soils derived from the inland migration of coastal dune systems. In fact, its known distribution area is mostly coincident with the relevant dune formations in the island. However, a large extent of potentially suitable soils is currently dedicated to the agriculture or sand extraction. In these areas the plant is rarely found or appears only in less altered spots like borders or outcrops. *Coronilla montserratii* appears in open and sunny spots where the sand is completely fixed or just slightly mobile, but with a significant content of organic matter. Here, it grows with other species like the perennial *Aetheorrhiza bulbosa* (L.) Cass. subsp. *bulbosa*, and the terophytes *Avellinia michelii* (Savi) Parl., *Cerastium semidecandrum* L., *Desmazeria rigida* (L.) Tutin, *Lagurus ovatus* L., *Lobularia maritima* (L.) Desv., *Lotus cytisoides* L., *Malcolmia ramosissima* (Desf.) Thell., *Medicago littoralis* Rohde ex Loisel., *Myosotis arvensis* (L.) Hill, *Polycarpon tetraphyllum* (L.) L., *Rumex bucephalophorus* L., *Senecio vulgaris* L., *Vulpia ciliata* Dumort., and *V. muralis* (Kunth) Nees, among others. The related *C. scorpioides* and *C. montserratii* grow together at several sites. Usually, the populations of *C. montserratii* show scattered, isolated individuals extending over large sandy areas. Dense populations have not been observed.

***Comparative morphology***. A summary of the morphological features used to discriminate annual species of *Coronilla* is shown in Table 1 and a representative



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specimen of each species is depicted in Figure 3. *Coronilla montserratii* is related to *C. repanda* but it is consistently more vigorous, showing longer and stout stems, and longer and wider dimensions in most of the leaf, leaflet, and legume features (Table 1). These quantitative differences are likely related to the different ploidy level shown by both species (hexaploid and tetraploid, see below). In addition, they differs by the lower number of leaflets in the upper leaves, the heteromorphic shape of terminal and apical leaflets, as well as leaflet shape (Table 1; Fig. 4). *Coronilla repanda* shows leaves with homomorphic leaflets, which are narrowly oblanceolate to elliptical and with rounded and obtuse apices. *Coronilla montserratii* has contrasting leaves: the leaflets are heteromorphic, the terminal being triangular-obovate (similar to the shape shown by *C. dura*), and the laterals, except the basal pair, are obovate with obtuse and mucronate apices. The number of leaflets in the upper leaves also differentiate the pair *C. dura* and *C. repanda* (leaves with 7-9 leaflets) from *C. montserratii* that consistently show leaves with up to (3)5 leaflets. No obvious morphological feature relates *C. montserratii* with *C. scorpioides*.

***Karyology.*** Individuals of *C. montserratii* from two populations (Minorca, 31TEE8332, Alzinar d'Alforí, calcareous scrub, 7-VI-2004, *P. Fraga*, VAL; Minorca, 31SEE7921, Macarelleta, fixed calcareous sand dunes with low scrub vegetation, 7-VI-2004, *P. Fraga*, VAL) have shown a constant somatic number of 2*n* = 36 chromosomes (Fig. 5). The chromosome complement of *C. montserratii* comprises small metacentric chromoso-mes (2-4.5 μm) similar in size. On the basis of the available cytogenetic knowledge in *Coronilla* suggesting a basic chromosome number of x = 6, our re-sults imply that the new species is hexaploid. The two accessions of *C. scorpioides* from Minorca (Minorca, 31TEE8333, Al Pilar, sandy soil, 18-V- 2008, P. Fraga, VAL; Minorca, 31SEE8918, Talis, cultivated fields, calcareous stony soil, 18-V-2008, *P. Fraga*, VAL) showed 2*n*=12. The other annual species of *Coronilla* are diploid (2*n*=12; *C. dura* and *C. scorpioides*; FERNANDES & SANTOS, 1971; FERNANDES & al. 1977) or tetraploid (2*n*=24; *C. repanda*, FERNANDES & al. 1977). Previously, the hexaploid cytotype was only known from the perennial *C. minima* subsp. *lotoides* (KÜPFER, 1972).

## DISCUSSION

*Coronilla montserratii* shows the greatest morphological similarities with *C. repanda* but clearly differs by the lower number of leaflets in the upper leaves, the heteromorphic shape of terminal and apical leaflets as well as leaflet shape (Table 1). These differences alone although constant could hardly justify its distinction as a new species, and the Minorcan populations could be better accommodated as a local race at any infraspecific rank, as has been done in the past (BOLÒS & VIGO, 1974) or, simply, subsumed under *C. repanda* (GARCÍA MARTÍN & TALAVERA, 2000).

However, the analysis of karyological and unpublished molecular data points to more complex evolutionary scenarios supporting the specific status for *C. montserratii*. This species is a highly polyploid (hexaploid) showing a chromosome number (2n=36) not shared by *C. repanda* (2n=24, tetraploid) and either *C. dura* and *C. scorpioides* (2n=12, diploids).

On a theoretical basis, the hexaploid level could have originated in *C. montserratii* by autopolyploidy from either 2n= 24 and 2n=12 ancestors through fertilization of reduced and unreduced gametes. However, invoking an origin from 2n=12 progenitors is less parsimonious than from tetraploid ones since it requires the formation of viable triploid intermediates (2n=18) before chromosome doubling.

On the basis of the strong morphological similarity usually displayed by diploid and autopolyploid cytotypes and the fact that most differences, when present, relate to quantitative rather than qualitative traits (SOLTIS & al., 2007; MANDÁKOVÁ & MÜNZBERGOVÁ, 2008), data from morphology clearly exclude the diploids *C. dura* and *C. scorpioides*, but not the tetraploid *C. repanda*, as likely parents of an hypothetical autopolyploid *C. montserratii*. Further, phylogenetic analyses of nuclear ribosomal ITS sequences (unpublished data) strongly suggest that *C. repanda* and *C. montserratii* are not sister taxa. Thus, available data do not support the hypothesis of an autopolyploid origin of *C. montserratii* from extant relatives.

The most parsimonious allopolyploid hypothesis for *C. montserratii* should involve an origin from tetraploid and diploid ancestors. The close morphology shared by *C. repanda* and *C. montserratii* would support the former as a likely tetraploid progenitor of the latter. On the other hand, ribosomal nuclear ITS sequences (unpublished data) strongly suggest that *C. scorpioides* was clearly involved in its origin and could be the diploid ancestor.

The fact that morphological features shown by *C. montserratii* are not midway between its proposed parental species or showed sorted discriminating features, but are biased towards a single progenitor (*C. repanda*), could be explained by its higher genomic contribution (four haploid genomes) than *C. scorpioides* (two haploid genomes) to its genesis.

In conclusion, available evidence best fits an allopolyploid origin for *C. montserratii* and molecular data identifies the diploid *C. scorpioides* as a likely progenitor. The identity of the second species involved in its origin remains elusive with the use of molecular markers. However, the close morphology shared between *C. montserratii* and *C. repanda*, if not due to convergence or to the retention of plesiomorphic features, tentatively suggests that a C. repanda-like ancestor could be the tetraploid parent.

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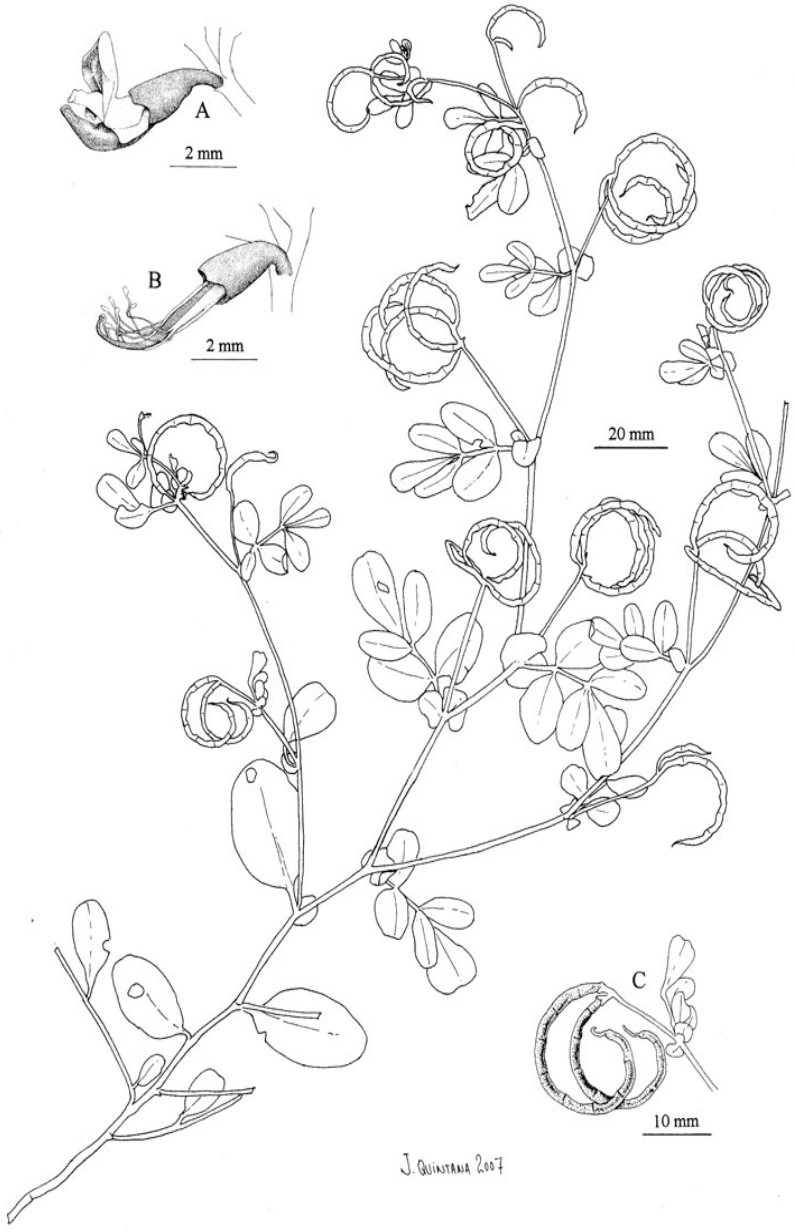


Fig. 1. Habit of *C. montserratii*, including details of flowers (A, B) and fruits (C).


Fig. 2. Representative specimens of annual species of *Coronilla*. (A) *C. montserratii* (Minorca, Arenal de Macarelleta, VAL 190331, holotypus); (B) *C. repanda* (Portugal, Torrao, SALA 42045); (C) *C. dura* (Spain, Arapiles, SALA 33906); (D) *C. scorpioides* (Spain, Usagre, SALA 3792).

| | *C. montserratii* | *C. repanda* | *C. dura* | *C. scorpioides* |
|---|---|---|---|---|
| Number of leaflets (upper leaves) | 5 | 7-9 | 7-9 | 3 |
| Length of the terminal leaflet (cm) | 1.5-2 | 1 – 1.8 | 0.4-1.5 | 0.6 – 4.5 |
| Length of basal leaflets (cm) | 0.5-1 | 0.4 – 0.8 | 0.2-0.7 | 0.1 - 1.3 |
| Leaflet shape (terminal and laterals) | Heteromorphic. Terminal leaflet obova-te-triangular, truncate. Lateral leaflets obovate, obtuse, and mucronate | Homomorphic. From narrowly oblanceolate to elliptical, apex rounded, obtuse | Homomorphic. Obovate triangular, truncate or emarginate | Ovate, elliptic to nearly orbicular |
| Calyx length (cm) | 0.2–0.25 | 0.2–0.3 | 0.15-0.26 | 0.15 – 0.25 |
| Corolla length (cm) | 0.3–0.5 | 0.3-0.8 | 0.4-0.55 | 0.4 – 0.6 |
| Legume width (cm) | 0.25–0.4 | 0.15-0.2 | 0.15-0.25 | 0.2-0.3 |
| Ploidy level | 6x | 4x | 2x | 2x |

Table 1. Diagnostic morphological features between annual *Coronilla* species.

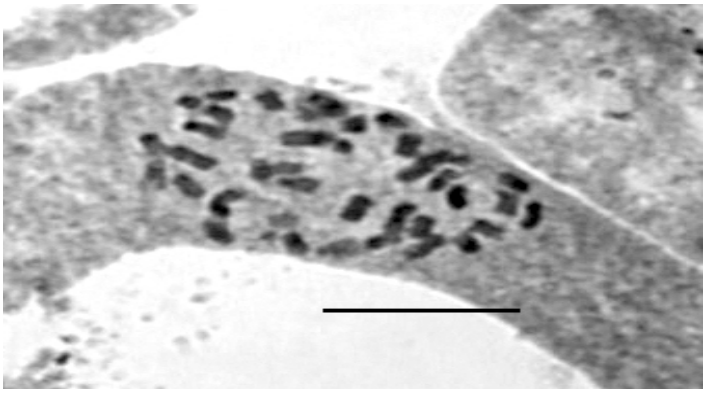

Fig. 3. Mitotic metaphase plate of *C. montserratii* (2n=36) from Alzinar d'Alforí. Scale bar= 10 μm.