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## Remarks on *Medicago citrina* (sect. *Dendrotelis*, *Leguminosae*)

### Abstract

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*Medicago* sect *Dendrotelis* includes shrubby perennial plants which produce stipitate pods with 1-3 coils, flowers with the keel equalling or slightly longer than the standard, and embryos with the radicle about half as long as seed. Three taxa with Mediterranean distributions are known: *M. arborea*, *M. citrina* and *M. strasseri*. The tetraploid number  $2n = 32$  is reported for *M. strasseri*, and the lectotype of *M. citrina*, a taxon for which the species rank is accepted, is also designated. Diagnostic characters, ecology and distribution areas are commented for all of them. Conservation remarks on *M. citrina* are reported. Finally, an identification key is also presented.

### Introduction

The genus *Medicago* comprises about 80 species, mainly annual herbs and herbaceous perennial, though there are few taxa with a woody perennial habit (cf. Small & Jomphe 1989). Taxa of this genus present a cosmopolitan distribution, growing in quite different habitats (e.g. coastal sand dunes, pastures, rocky hillsides, and some different halonitrophilous and nitrophilous plant communities). Moreover, taxa of *Medicago* show a great economical importance in agriculture since including “alfafa”, one of the most important forage crops (Villax 1963; Hill 1965; Lesins & Lesins 1979; Small & Jomphe 1989), or other plants with curative uses such as the “tree medic” aggregate (Champeval 1947).

Among the species of *Medicago*, several morphological features such as the growing habit, indument, relative size of the petals or the pods are usually used to segregate infrageneric taxa. With regard to the woody plants related to *Medicago arborea*, several peculiar characters such as the shrubby perennial habit, stipitate pod with 1-3 coils, keel equalling or slightly longer than the standard and embryos with the radicle about half as long as seed, are unique as a whole in the genus. For this reason, many authors (cf. Willkomm 1877; Casellas 1962; Lesins & Lesins 1979; Greuter & al. 1982; Small & Jomphe 1989) have deserved recognition of this interesting group as a different section. This section is currently named *Dendrotelis* (Vassilcz.) Lassen ex Greuter & al., and includes three Mediterranean species: *M. arborea* L., *M. strasseri* Greuter, Matthäs & Risse, and *M. citrina* (Font Quer) Greuter. The two former are native to the littoral areas and islands from the eastern Mediterranean, whereas the latter is distributed on small islands from the western

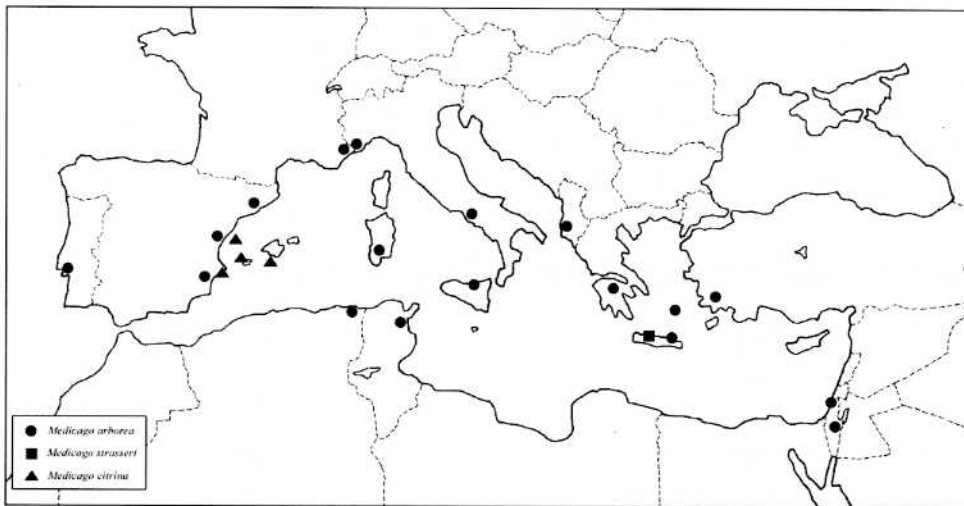


Fig. 1. Distributions of taxa of *Medicago* sect. *Dendrotelis*.

Mediterranean. Nevertheless, *M. arborea* presents a wider distribution through the western Mediterranean territories, in which it is commonly cultivated to improve soil conditions. As regards to *M. strasseri* and *M. citrina*, both show very reduced populations. The former is endemic to a few sites in Crete (Greuter & al. 1982), and the latter is only known from the Balearics, the Columbretes archipelago and a small islet in the northern Alicante province, near the eastern Iberian Peninsula (Bolòs & Vigo 1984; Serra & al. 2001).

The limited distribution area of *M. citrina* led the Spanish Government to regard this endemic as “threatened with extinction” (Spanish Royal Decree 439/1990), and Aizpuru & al. (2000) have recently labelled it as endangered (EN: B1+2e), according to the new categories of the IUCN (1994).

In the present contribution, some morphological, chorological, chromosomal and taxonomic data which complete the current knowledge of sect. *Dendrotelis* are reported.

## Material and methods

Observations of all morphological characters studied were carried out with a binocular microscope on both fresh and dried materials. Data on herbarium specimens were obtained from sheets of all taxa in the section conserved at ABH, B, BC and MA (Holmgren & al. 1990; Holmgren & Holmgren 1993). Observations of living specimens were carried out on 30 individuals per population. For *M. citrina*, wild plants growing in the natural populations of Columbretes, the Balearics and Alicante, were studied, and data were compared with those obtained from plants grown at the living collection of the CIDA (Centro de Investigaciones y Desarrollo Agroalimentario, Murcia, Spain). For both *M. arborea* and *M. strasseri*, data came mostly from plants growing at the living collection of the CIDA, though data were also taken from wild populations of *M. arborea* from Thira island (Greece). No significant differences were found in any case among wild or cultivated plants.

Karyological observations of *M. strasseri* are based on materials collected at the CIDA (Murcia, Spain). Somatic chromosomes were observed from root tips of mature plants grown in experimental pots. The root tips were fixed in glacial acetic acid: ethanol (1:3) after a pretreatment of 2-3 hours in 0,002 M hydroxyquinoline at 4 °C. After about 30 min of hydrolysis in 5 M HCl at room temperature, the root tips were briefly rinsed in distilled water. For staining, Feulgen method was used (Sharma & Sharma 1980), and after 1 hour in Schiffs reagent at room temperature, the root tips were squashed in carmin acetic on a slide.

Nomenclature of plant taxa follows Greuter & al. (1989), Castroviejo & al. (1986-2001) and Mateo & Crespo (2001).

## Results and discussion

### Taxonomic characters

*Habit* — Morphological features are relevant only after direct observation of living plants. On the one hand, *M. arborea* and *M. strasseri* show erect-patent branches, just from the beginning of their development, and the main stem grows indefinitely. On the other, *M. citrina* shows branches growing almost horizontally, since the main stem stops growth quite early and withers, as remarked by Robledo & al. (1993a). This latter pattern could be interpreted as an adaptation to the particular life strategy conditioned by hard marine winds blowing in the habitats *M. citrina* colonises.

*Leaf morphology* — Petiole length varies from c. 10-37 mm in *M. citrina*, almost 1,5-2 times longer than in the rest. In all taxa, leaves are mainly 3-foliolate, though 4- and 5-foliolate ones have been found rather frequently in plants of *M. citrina* from La Foradada island grown at Murcia (Robledo & al. 1993a). However, this character was never seen in the wild. Leaflets are greater (in both length and width) in *M. citrina* (13,0-31,0 × 8,0-18,8 mm), whereas they are the smallest in *M. arborea* (11,3-21,0 × 8,0-11,0 mm). Leaflet morphology is quite similar (obovate-cuneate) in all taxa, although *M. strasseri* shows obovate-spathulate leaflets (twice longer than wide). The apex is quite similar, excepting *M. citrina* which presents emarginate leaflets (Table 1).

*Leaf anatomy* — Relevant anatomical differences between *M. citrina* and its relatives have been observed (Chebbi & al. 1994). *Medicago citrina* shows a large layer of waxes covering the cuticular surface, which give its typical leaf glaucescence. However, the lower surface of leaflets of this species presents a higher number (8-10%) of trichomes. Moreover, parenchyma cells of leaflets are larger again in *M. citrina*, which makes the leaf section thicker.

*Corolla colour* — This feature is quite relevant to distinguish easily the two groups of *Medicago*, which is also correlated to a different Mediterranean distribution. Both taxa from the eastern Mediterranean (*M. arborea* and *M. strasseri*) show orange-yellow corollas, whereas the unique taxa in the western Mediterranean (*M. citrina*) shows distinctive lemon-yellow corollas (Table 1).

*Pods* — Taxa of sect. *Dendrotelis* are characterised by the fruit morphology (Greuter &

Table 1. Some morphological and karyological characters of *Medicago* sect. *Dendrotelis* based on the studied material (all measurements in mm)

	<i>M. citrina</i>	<i>M. arborea</i>	<i>M. strasseri</i>
Standard length	11,3-14,0	9,7-11,5	9,7-12,0
Corolla colour	Yellow-lemon	Orange	Orange
Petiole length	10-37	10-20	10-18
Leaflet shape	Obovate-cuneate and emarginate	Obovate-cuneate, not emarginate	Obovate-spathulate, not emarginate
Fruit diameter	11-20	11-17	6-9
Fruit surface	Weakly veined	Almost smooth	Strongly veined
Central opening diameter	0,5-1,0	1,7-2,0	0,3-0,5
Number of coils	1-2	1	2-3
Stipe length with regard to calyx	Twice longer	Shorter or equalling	Slightly longer
Ventral/dorsal pod suture	Usually similar	Usually thicker	Thicker
Number of ovules per pod	4-8	10-14	10-13
Seed size	4,2-5,9	3,5-4,5	2,0-2,5
Chromosome number	$2n = 48$	$2n = 32$	$2n = 32$

al. 1982; Robledo & al. 1993b). Pods are quite different in size. *Medicago strasseri* shows the smallest fruits (5,8-9,8 mm) whilst *M. citrina* has the largest ones (c. 11-20 mm). The fruit stipe is clearly visible in *M. citrina*, being up to twice longer than the calyx, whereas in *M. arborea* is equalling or shorter than the calyx. The central orifice varies from about 0,5-1 mm in diameter (*M. citrina*) to up to 2 mm (*M. arborea*). However, the number of the coils varies among the species, from one (*M. arborea*) to 2-3 (*M. strasseri*). The pod surface offers interesting differences on venation: *M. strasseri* shows strongly veined pods with a characteristic ornamentation, *M. arborea* usually bears pods almost smooth, and *M. citrina* shows an intermediate ornamentation between both cited types. The number of ovules per pod is also a relevant character: *M. citrina* produces only 4-8 ovules whilst *M. arborea* and *M. strasseri* produce 10-14 in all cases. All these features altogether are relevant in the taxonomy of the section (Table 1).

*Seeds* — Seeds of taxa of sect. *Dendrotelis* are kidney-shaped, and their size could be used with taxonomic value (Table 1). *Medicago citrina* produces the largest seeds (4,2-5,9 mm), whilst *M. strasseri* has the smallest ones (2,0-2,5 mm).

*Ploidy levels* — The basic chromosome number of sect. *Dendrotelis* is  $x = 8$  (Fernandes & Santos 1971). Counts made on materials of *M. arborea* from Portugal yielded a

tetraploid number  $2n = 32$  (Fernandes & Santos 1971). Greuter & al. (1982) reported that S'Espartar populations (Balearic Islands) of *M. citrina* conforms the hexaploid level ( $2n = 48$ ) of the aggregate, a number that has been confirmed for materials from the Columbretes populations (Boscaiu & al. 1997). However, no references were found about the chromosome number of *M. strasseri*. In our recent studies, we found this species to be tetraploid ( $2n = 32$ ), and not diploid as it had been thought up to date. Thus, *M. strasseri* should not be regarded as the closest putative ancestor of the whole section, as suggested by other authors (Greuter & al. 1986; Robledo & al. 1993a). After this new data, two ploidy geographical groups are found in the section: the tetraploid taxa, *M. strasseri* and *M. arborea*, native to the eastern Mediterranean, and the hexaploid, *M. citrina*, from the western Mediterranean.

*Molecular data* — By using RAPDs (random amplified polymorphic DNA), Chebbi & al. (1995) found that *M. arborea* had the highest intraspecific variation (mean frequency of polymorphism = 21,7%), whereas the endemic *M. citrina* showed the lowest (mean frequency of polymorphism = 9,4%). They also concluded that the eastern taxa (*M. arborea* and *M. strasseri*) were genetically much closer each other than they are to the western *M. citrina*. However, their studies were based on samples from a single seed accession for *M. citrina* and *M. strasseri*, which could justify the narrow variation observed for both taxa. Recent molecular studies (Juan & al. in press) based on AFLP™ (amplified fragment length polymorphism) and including samples from all known populations of *M. citrina*, have demonstrated that this taxon exhibits a much higher genetic variability than reported before, and microspeciation processes are currently active in each single population of the species. Other studies on sequences of chloroplast DNA (*trnL-trnF* region) and ribosome DNA (internal transcribed spacers, ITS) we are carrying out confirm the closer genetic relationship between *M. arborea* and *M. strasseri*, as reported by Chebbi & al. (1995). This points out to a longer shared evolutionary history.

*Ecological adaptations* — Experimental studies carried out on the three taxa of the section (Correal, pers. comm.; Robledo & al. 1993a; Chebbi & al. 1994) demonstrated that *M. citrina* endured better drought and freeze. This plant is a real xerophyte, since up to 85% of leaves remain on branches in the summer. Contrarily, *M. arborea* and *M. strasseri* can be regarded as semideciduous plants, since most of their leaves fall off within the dry period. Other remarkable characteristics pointing out to the adaptation of *M. citrina* to stressing conditions are that this plant presents a highly developed root system and produces the highest amount of hard seeds like most of the typical Mediterranean wild plants (Chebbi & al. 1994).

#### Taxonomic remarks

- Medicago* sect. *Dendrotelis* (Vassilcz.) Lassen ex Greuter, Matthäs & Risse in Willdenowia 12: 202 (1982).
- ≡ *Trigonella* sect. *Dendrotelis* Vassilcz. in Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk SSSR 14: 229 (1951).
- *Medicago* sect. *Arboreae* K. Lesins & I. Lesins, Gen. Medic.: 131 (1979), *nom. inval.*

As said before, taxa of *Medicago* with a shrubby habit or being woody only at the base

(e.g. *M. arborea*, *M. falcata*, *M. sativa*, *M. suffruticosa*, *M. marina*) were first grouped in a section called *Falcago* (Willkomm 1877; Casellas 1962). Later, Lesins & Lesins (1971), in a comprehensive study on the genus, rearranged sect. *Falcago* from which they excluded *M. marina*, *M. suffruticosa* and *M. arborea*. Moreover, these latter authors proposed the new section *Arboreae* to segregate *M. arborea* (incl. *M. arborea* var. *citrina*), which included shrubs up to 4 m, showing pods with 0,5-1,5 coils and a large central orifice, flowers with a yellow corolla, wings shorter than the keel and the standard shorter or equalling the keel. However, this new section proves to be an invalid name (Art. 36.1, ICBN), since lacking a Latin description. More recently, Greuter & al. (1982) validated a new combination attributed to Lassen on the basis of *Trigonella* sect. *Dendrotelis* previously described by Vassilczenko (1951). They included there three taxa with Mediterranean distributions: *M. arborea*, *M. arborea* var. *citrina* and *M. strasseri*. Later, Greuter & Raus (1986) raised var. *citrina* to species rank, with which the section definitely included three species corresponding to different levels of ploidy in the same chromosome series. However, Small & Jomphe (1989) treated *M. citrina* as a mere variety of *M. arborea*, and added some other diagnostic characters for the section (e.g. embryos with the radicle about half as long as seed, cotyledons epulvinate, and seeds smooth). The most important differences among them are summarised in Table 1.

***Medicago arborea* L., Sp. Pl.: 778 (1753).**

*Lectotypus*: Designated by Heyns (1959: 161). Herb. Clifford: 377, *Medicago* 6 (BM).

#### *Typification*

*Medicago arborea* was described by Linnaeus (1753) in *Species Plantarum* with the following protologue: “*MEDICAGO leguminibus lunatis margine integerrimis, caule arboreo*”, and adding a note about the locality as follows: “*Habitat in Rhodo, Neapoli*”. This diagnosis was based on several earlier works: Hort. Cliff., Hort. Ups., and Roy Lugdb, where this taxon had been already cited.

Typification by Heyn (1959) was based on materials from BM. As she indicated, there was a slight modification in the diagnosis phrase which appears in Hortus Cliffortianus and in *Species Plantarum*. This change affected the characteristics of the stem. In fact, in the former Linnaeus wrote “caule frutescente”, whereas in the latter he rewrote “caule arboreo”. Perhaps, as Heyn (*loc. cit.*) mentioned, this correction was due to a direct observation of the plants in the field and not on dried herbarium materials in which the collected material usually corresponds to young parts. Therefore, the sheet Hort. Cliff. 377-6 is to be regarded as the lectotype (not holotype as indicated by Heyn), since it is accompanied with the same phrase as in Hortus Cliffortianus.

#### *Description*

Perennial shrub (0,5-)1-3 m. Stem with erect branches, young twigs whitish-pubescent. Leaves 3-foliolate; leaflets generally obovate-cuneate, entire or slightly denticulate, sometimes retuse. Petiole length 10,0-20,0 mm. Stipules 3,8-7,5 mm long, entire, lanceolate. Inflorescence with 8-15 flowers arranged on dense racemes. Calyx 2,7-3,7 mm long. Corolla 9,7-11,5 mm long, orange-yellow. Fruit 11-17 mm in diameter, with one coil and a central opening 1,7-2,0 mm, stipe usually shorter than the calyx (seldom equalling

