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## Breeding systems and cytology in Cyprian populations of six *Limonium* species (*Plumbaginaceae*)

### Abstract

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The reproductive mechanisms and chromosome numbers have been investigated of the Cyprian endemics *Limonium cypricum* and *L. mucronulatum*, as well as of *L. aucheri*, *L. avei*, *L. meyeri* and *L. virgatum*, which also occur on the island of Cyprus. Their taxonomy is considered and the correlation between their breeding systems and cytology is discussed. *L. aucheri* and *L. cypricum* are apomictic and their chromosome numbers,  $2n=5x=43$  and  $2n=3x=27$  respectively, are reported here for the first time. For *L. mucronulatum* no chromosome number could be established, but pollen and stigma features indicate that it is apomictic too. *L. meyeri* is sexual with  $2n=2x=18$  (first report), while *L. avei* and *L. virgatum* are apomictic with  $2n=3x=27$ .

Additional key words: Cyprus, chromosome numbers, sexual reproduction, apomictic reproduction

### Introduction

The genus *Limonium* Mill., with about 400 species (Palacios & Gonzales-Candelas 1997) the largest genus of *Plumbaginaceae*, is one of the taxonomically most difficult plant taxa. This difficulty is due to its reproductive behaviour, i.e. the presence of both sexual and apomictic reproduction, as well as the frequent occurrence of hybridisation and polyploidy. Most of the *Limonium* taxa are concentrated in the Mediterranean region, which constitutes the centre of diversity of this genus (Cowan & al. 1998; Lledó & al. 2003).

In the island of Cyprus, *Limonium* is represented by eight species, a rather small number compared to the species numbers on other islands of the E Mediterranean.

According to Buttler & Hand (in Hand 2003), the Cyprus flora is relatively poor in *Limonium* species, e.g. in comparison to Crete, the genus being absent from many parts of the coast, especially from areas dominated by igneous rocks. Indeed, in Crete 18 *Limonium* species occur (Mayer 1995; Brullo & Guarino 2000), while from the much smaller island of Kithira still nine species are reported (Artelari & Georgiou 2002).

According to Bokhari & Edmondson (1982), Meikle (1985) and Greuter & al. (1989), the species known from Cyprus so far include the Cyprian endemics *Limonium cypricum* (Meikle) Hand & Buttler and *L. mucronulatum* (H. Lindb.) Greuter & Burdet, as well as *L. aucheri* (Girard) Greuter & Burdet, *L. avei* (De Not.) Brullo & Erben, *L. echioides* (L.) Mill., *L. meyeri* (Boiss.) Kuntze, *L. sin-*

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*uatum* (L.) Mill. and *L. virgatum* (Willd.) Fourr.

Very few cytological information exists for *Limonium* on Cyprus and there is a complete lack of data concerning their breeding systems. In the present work material from several localities of the island belonging to six of the eight above-mentioned species has been studied, focusing mainly on their cytology and reproductive mechanism.

## Material and methods

This study is based on plants collected mainly by the first author from several localities of Cyprus in the framework of her undergraduate diploma thesis. This material includes thirty-four populations from six of the eight species present on the island. No material of *Limonium echioides* and *L. sinuatum* has been found. Voucher specimens are kept in the Herbarium of the University of Patras (UPA).

All the collected populations were studied to assess their reproductive mechanism. Pollen and stigma type combinations of the flowers were determined according to Erben (1978, 1979). The pollen stainability was estimated by using cotton blue as described in Artelari & Kamari (1986). Fifteen populations were used for the cytological studies. For this purpose, seeds were germinated in Petri dishes on moistened filter paper and the root tips were pretreated according to the method described in previous papers (Artelari 1984; Artelari & Kamari 1986).

## Results and Discussion

**1. *Limonium cypricum*** (Meikle) Hand & Buttler in Willdenowia 33: 315. 2003  $\equiv$  *Limonium albidum* subsp. *cypricum* Meikle in Ann. Mus. Goulandris 6: 88. 1983.

*Distribution* — Endemic to Cyprus.

*Material examined* — Kato Pafos, “Tafi ton Vasileon”, on calcareous maritime rocks, *Christodoulou* 27 (UPA).

*Taxonomic notes* — *Limonium cypricum* was described by Meikle (1983) as a subspecies of *L. albidum* (Guss.) Pignatti, which is endemic to the island of Lampione, S Italy. According to Meikle (1985), it differs from the typical *L. albidum* in the smaller flowers with straighter calyx tubes and bracts. Greuter & al. (1989) placed this taxon as a subspecies into the *L. intermedium* group, which else comprises the endemics of S Italy (around Sicily) *L. albidum*, *L. hyblaeum* Brullo, *L. intermedium* (Guss.) Brullo, *L. lopadusanum* Brullo, *L. mazararum* Pignatti and *L. panormitanum* (Tod.) Pignatti, as well as *L. zembrae* Pignatti, endemic to Tunisia. Buttler & Hand (in Hand 2003), comparing the plants of Cyprus to the typical *L. albidum*, reported that they are very similar but all taxo-

nomically important parts of the spikelets and flowers of the Cyprian plants are smaller than those of typical *L. albidum*. They therefore came to the conclusion that the Cyprian taxon should be considered as a separate species, which probably constitutes a relict of the *L. intermedium* group.

*Cytology and breeding system* — Our study revealed the triploid chromosome number  $2n=3x=27$ . The karyotype (Fig. 1A) lacks the long metacentric “marker” chromosomes characteristic for karyotypes with the basic number  $x=8$ . The only previous report, by Yıldız & Güçel (2006), gives the diploid chromosome number  $2n=18$ .

Concerning the pollen and stigma combination, the population of this taxon is monomorphic with the self-incompatible combination B, indicating self-sterility. Pollen stainability is 0 % and the pollen grains are misshaped, irregular in size (conspicuously large or conspicuously small), with variable morphology (tri- and tetrapolypate). Such pollen and stigma features characterise apomictic species (Baker 1953c). Our data combined with the good seed production of the population and the triploid chromosome number indicate that *Limonium cypricum* reproduces apomictically.

**2. *Limonium mucronulatum*** (H. Lindb.) Greuter & Burdet in Willdenowia 19: 40. 1989  $\equiv$  *Statice mucronulata* H. Lindb. in Acta Soc. Sci. Fenn., Ser. B, Opera Biol. 2(7): 26.1946.

— *Limonium narbonense* sensu Meikle (1985).

*Distribution* — Endemic to Cyprus.

*Material examined* — Larnaka, Alikes area, on sand, *Kouzali* 31 (UPA), *Christodoulou* 26 (UPA).

*Taxonomic notes* — *Limonium mucronulatum* is only known from a single locality (area of Alikei), where it was rediscovered by Christodoulou & Tsintides (Tsintides & al. 2007) about 50 years after its first discovery by Lindberg in 1939 (Lindberg 1946).

Meikle (1985) erroneously used the name *Limonium narbonense* Mill. Greuter & Raus (1989), referring to it as *L. mucronulatum*, place it into the *L. sibthorpium* group, which comprises also *L. raddianum* (Boiss.) Pignatti from Egypt, *L. sibthorpium* (Guss.) Kuntze from Sicily as well as *L. teuchirae* Brullo and *L. vaccarii* Brullo from Libya.

*Limonium mucronulatum* is included in the “Red Book of the flora of Cyprus” (Tsintides & al. 2007) as “Critically Endangered” (CR), comprising only 600–700 plants distributed in an area of 3000 m<sup>2</sup> and its habitat being seriously threatened.

*Cytology and breeding system* — No cytological data are known so far for *Limonium mucronulatum*. In spite of



Fig. 1. Mitotic metaphase plates of *Limonium* from Cyprus – A: *L. cypricum*,  $2n=3x=27$ , material from Kato Pafos (*Christodoulou 27*); B: *L. aucheri*,  $2n=5x=43$ , material from Cavo Greco (*Kouzali 16*); C: *L. meyeri*,  $2n=2x=18$ , material from Agia Napa (*Kouzali 10*); D: *L. avei*,  $2n=3x=27$ , material from Cavo Greco (*Kouzali 36*). – Scale bar = 10  $\mu\text{m}$ .

our strenuous efforts, we did not succeed to obtain an accurate chromosome count.

Concerning pollen and stigma combination, the material collected belongs to two populations which were found to be monomorphic with the self-incompatible combination B, low pollen stainability (3–13 %) and good seed production. Pollen grains are tri- to tetra-colpate, irregular in size, the stainable ones being well-shaped, the unstainable ones being much smaller and misshaped. As this situation is characteristic for apomictic taxa, the pollen and stigma features allow us to conclude that *Limonium mucronulatum* reproduces apomictically, although no data for its chromosome number are available so far.

**3. *Limonium aucheri*** (Girard) Greuter & Burdet in Willdenowia 19: 39. 1989  $\equiv$  *Statice aucheri* Girard in Ann. Sci. Nat. Bot., ser. 3, 2: 328. 1844. = *Limonium ocymifolium* subsp. *bellidifolium* (Sm.) Meikle, Fl. Cyprus: 1070. 1985  $\equiv$  *Statice bellidifolia* Sm., Fl. Graec. Prodr. 1: 211. 1806 [non (Gouan) DC. in Lamarck & Candolle, Fl. Franc. ed. 3, 3: 421. 1805].

*Distribution* — Reported so far from the Greek islands of Kithnos, Rodos and Kriti as well as from Cyprus.

*Material examined* — Protaras area, “Fig Tree” bay, near the hotel, on calcareous maritime rocks, *Kouzali 17* (UPA); Protaras area, 300 m east of the “Fig Tree” bay, on calcareous maritime rocks, *Kouzali 18*, *Christodou-*

*lou 25* (UPA); Cape Cavo Greco, on calcareous maritime rocks, *Kouzali 16* (UPA).

*Taxonomic notes* — Greuter & al. (1989) placed this taxon into the *Limonium ocymifolium* group, which comprises allied taxa mainly distributed in the C and S Aegean and the S Peloponnisos. According to Greuter & al. (1989) and Artelari & Georgiou (1999), six species are included: *L. ocymifolium* (Poir.) Kunze, *L. hierapetrae* Rech. f., *L. creticum* Artelari, *L. cythereum* Artelari & Georgiou, *L. corinthiacum* (Boiss. & Heldr.) Kunze and *L. aucheri*. In addition, on the basis of morphological, cytological and breeding system features, we suggest that *L. runemarkii* Rech. f. and *L. doerfleri* (Halácsy) Rech. f. also belong to that group. The placement of *L. runemarkii* in the *L. palmare* group instead (Greuter & al. 1989) is not justified because of the morphological differences.

According to Greuter & Raus (1989), *Limonium aucheri* is a doubtful species corresponding to *Statice bellidifolia* Sm. This last taxon has been treated by Boissier (1879), Halácsy (1904) and Hayek (1928) as a variety of *S. ocymifolia* Poir., by Rechinger (1943a, b) as a variety of *L. ocymifolium* (Poir.) Kuntze and by Meikle (1985) as a subspecies of *L. ocymifolium*.

Mayer (1995) comments that the taxonomic status of *Limonium aucheri* concerns a case of great confusion, as the names *Statice aucheri* and *S. bellidifolia* are based on different types, the first one on a specimen collected by Aucher-Eloy from Kithnos, and the second on a specimen of Smith from Rodos, but that *L. aucheri* is possibly conspecific with *L. ocymifolium*. We also consider

*L. aucheri* and *L. ocymifolium* as very closely related. Smith's *Statice bellidifolia*, figured in Sibthorp & Smith (1821: t. 295), is very similar to *L. ocymifolium* and a detailed study of additional material of *L. aucheri* will probably lead to the fusion of these two species. This is also supported by the fact that they have the same chromosome number,  $2n=43$  (see below).

*Limonium aucheri* is classified by Tsintides & al. (2007) as "Endangered" (EN), being known from two localities only (Protaras area and Cape Cavo Greco).

**Cytology and breeding system** — Our cytological study of *Limonium aucheri* revealed the pentaploid chromosome number  $2n=5x=43$ , derived from the combination of  $2 \times 8 + 3 \times 9$  genomes. This is indicated by the presence of two long metacentric "marker" chromosomes (Fig. 1B), characteristic for karyotypes with the basic number  $x=8$  (Erben 1978, 1979) and shows that the taxon is of hybrid origin. The chromosome number of *L. aucheri* is given here for the first time.

The study of the pollen and stigma combination showed that all three populations are monomorphic, two with the self-incompatible combination B and pollen stainability 0–3 %, and one with the self-incompatible combination A and pollen stainability 0–24 %. Pollen grains of all populations are irregular in size. Stainable pollen grains are well-shaped and much larger than the unstainable ones which are small and misshaped. The above data combined with the good seed production of the plants and the pentaploid chromosome number indicate that *Limonium aucheri* is an apomictic species. The closely related *L. ocymifolium* has the same pentaploid chromosome number  $2n=43$  and is also apomictic (Artelari 1989; Artelari & Georgiou 2002).

**4. *Limonium meyeri*** (Boiss.) Kuntze, Revis. Gen. Pl. 1: 395. 1981  $\equiv$  *Statice meyeri* Boiss. in Candolle, Prodr. 12: 645. 1848.

– *Limonium gmelinii* sensu Osorio-Tafall & Seraphim (1973)

– *Limonium vulgare* sensu Osorio-Tafall & Seraphim (1973)

**Distribution** — Bulgaria, Crimea, Anatolia and Cyprus.

**Material examined** — Agia Napa, islet of the bay "Nissi", on calcareous maritime rocks, *Kouzali 10* (UPA); Cape Cavo Greco, Agii Anargiri, on calcareous maritime rocks, *Kouzali 13* (UPA); Larnaka, Alikes area, Aliki Spiros, on sand, *Kouzali 33* (UPA).

**Taxonomic notes** — *Limonium meyeri* belongs to the Mediterranean *L. vulgare* group, which also comprises *L. brevipetiolatum* Artelari & Erben, *L. effusum* (Boiss.) Kuntze, *L. gmelinii* (Willd.) Kuntze, *L. humile* Mill., *L. narbonense* Mill., *L. vanense* Kit Tan & Sorger and *L.*

*vulgare* Mill. (Greuter & al. 1989). Its closest relative is *L. gmelinii* from which it differs in the taller growth, the more open, loosely paniculate inflorescence and the remotely spaced spikelets (Bokhari & Edmondson 1982).

**Cytology and breeding system** — All three populations studied were found to be diploid with  $2n=18$  (Fig. 1C). The chromosome number of *Limonium meyeri* is reported here for the first time.

Concerning pollen and stigma combination, the populations are dimorphic and have the self-incompatible combinations A and B. This is in accordance with Baker's report (1953a) that *Limonium meyeri* is dimorphic. Pollen and stigma features are typical for sexual taxa, i.e. all pollen grains are regular in size, well-shaped and their stainability varies from 17 % to 100 %. These data, combined with the diploid chromosome number, indicate that *L. meyeri* is a sexually reproducing species.

According to available information, most species of the *Limonium vulgare* group are dimorphic and sexual with chromosome numbers derived from the basic number  $x=9$  and karyotypes not possessing "marker" chromosomes. More specifically, *L. brevipetiolatum* is dimorphic sexual and hexaploid with  $2n=54$  (Artelari & Erben 1986), *L. gmelinii* is dimorphic (Baker 1953a), *L. narbonense* is dimorphic sexual, tetraploid, hexaploid and octaploid with  $2n=36, 54, 72$ , respectively (Erben 1978, 1993; Brullo & Pavone 1981; Artelari 1992; Palacios & al. 2000; Georgakopoulou & al. 2006) and *L. vulgare* is dimorphic sexual and tetraploid with  $2n=36$  (Baker 1953a; Erben 1979, 1993; Palacios & al. 2000). The only exception so far is *L. humile*, which according to Baker (1953a, b) is secondarily monomorphic with the self-compatible type D, and according to Erben (1979) has the pentaploid chromosome number  $2n=54$ .

**5. *Limonium virgatum*** (Willd.) Fourr. in Ann. Soc. Linn. Lyon, ser. 2, 17: 141. 1869  $\equiv$  *Statice virgata* Willd., Enum. Pl. Hort. Berol.: 336. 1809.

= *Statice smithii* Ten., Fl. Napol. 3: 350. 1829.

– *Limonium oleifolium* Mill., Gard. Dict. Ed. 8, no 3. 1768, nom. amb.

– *Statice oleifolia* auct., non Scop., Del. Fl. Faun. Insubr. 1: 24, t. 10. 1786.

**Distribution** — Widely distributed in the Mediterranean region as well as in C and S Portugal.

**Material examined** — Agia Thekla area, on calcareous maritime rocks, *Kouzali 2* (UPA); in the small bay near the church of Agia Thekla, on calcareous maritime rocks, *Kouzali 3* (UPA); Agia Thekla area, on sand, *Kouzali 1* (UPA); Agia Napa, bay "Dome Hotel", on calcareous maritime rocks, *Kouzali 5* (UPA); Agia Napa, Makronisos, bay "Adamos", on calcareous maritime rocks, *Kouzali 6* (UPA); Agia Napa, bay "Nissi", on calcareous

maritime rocks, *Kouzali 8* (UPA); Agia Napa, bay “Yi-annoula Hotel”, on calcareous maritime rocks, *Kouzali 9* (UPA); Cape Cavo Greco, bay “Kamara tou Koraka”, on calcareous maritime rocks, *Kouzali 12* (UPA); Cape Cavo Greco, near the church of Agii Anargiri, on calcareous maritime rocks, *Kouzali 14* (UPA); Agios Thirsos, in the area of the church, on calcareous maritime rocks, *Kouzali 19* (UPA); in the area of the monastery Apostolos Andreas, on calcareous maritime rocks, *Kouzali 21* (UPA); Karpasia area, “Acheon Akti”, on sand dunes, *Kouzali 22* (UPA); Kato Pafos, “Tafi ton Vasileon”, on calcareous maritime rocks, *Christodoulou 28* (UPA); Protaras area, “Fig Tree” bay, on calcareous maritime rocks, *Kouzali 17* (UPA).

**Taxonomic notes** — *Limonium virgatum* is characterised by strongly dichotomously branched stems with numerous short sterile branches, compact, arcuate spikes and strongly banana-like curved spikelets. It often grows together with other *Limonium* species, forming intermediates (Pignatti 1972; Artelari 1984; Erben 1993). In Cyprus, in the area of Cavo Greco where *L. virgatum* coexists with *L. aucheri*, some plants present intermediate features such as sterile branches, somewhat curved spikelets (features of *L. virgatum*) and spatulate-obovate, ± emarginate leaves (features of *L. aucheri*), being the first record of intermediate forms of *Limonium* from Cyprus.

**Cytology and breeding system** — The triploid chromosome number  $2n=27$  was counted in two populations and confirms previous counts from Greek material (Artelari 1984, 1989, 1992; Artelari & Georgiou 2002; Georgakopoulou & al. 2006) as well as from elsewhere (D’Amato 1949; Baker 1952, 1953b, c; Erben 1978, 1979; Chichiricco & Tammara 1980; Brullo & Pavone 1891; Ingrouille 1984; Arrigoni & Diana 1993; Palacios & al. 2000). Dolcher & Pignatti (1971) reported  $2n=24-27$  and the tetraploid number  $2n=4x=32$ .

Concerning pollen and stigma combination, all studied populations are monomorphic with combination B. This confirms previous findings (Dulberger 1975; Ingrouille 1984; Artelari 1989a; Artelari & Georgiou 2002) with the exception of Baker (1953a), who states that *Limonium virgatum* is dimorphic and sexual but “some varieties of it may not be dimorphic”. Pollen stainability is low (0–8 %) and seed production is good. Pollen grains are irregular in size, the unstainable ones being misshaped and much smaller than the well-shaped stainable ones. These data and the triploid chromosome number confirm that *L. virgatum* reproduces apomictically, which was reported also by Erben (1979), Artelari (1984), Artelari & Georgiou (2002) and Georgakopoulou & al. (2006).

**6. *Limonium avei*** (De Not.) Brullo & Erben in Willdenowia 17: 17. 1988 ≡ *Statice avei* De Not., Prosp. Fl. Ligust.: 54. 1846.

= *Statice echioides* subsp. *exaristata* Murb. in Acta Univ. Lund. 35(3): 1. 1899 ≡ *Limonium echioides* subsp. *exaristatum* (Murb.) Maire in Jalandiez & Maire, Cat. Pl. Maroc: 571. 1934 ≡ *Limonium exaristatum* (Murb.) P. Fourn., Quatre Fl. France: 720. 1937.  
= *Limonium longispicatum* Erben in Mitt. Bot. Staats-samml. München 14: 555. 1978.

**Distribution** — France, S Sardinia, Italy, Sicily, Greece, Crete, Cyprus, Egypt, Tunisia and Libya.

**Material examined** — Cape Cavo Greco, near the church of Agii Anargiri, on calcareous maritime rocks, *Kouzali 36* (UPA); Protaras area, under “Cavo Maris” hotel, on calcareous maritime rocks, *Christodoulou 33* (UPA); Larnaka, Alikes area, Aliki Spiros, on sand, *Kouzali 40* (UPA).

**Taxonomic notes** — *Limonium avei* is characterised by its annual to biennial life form. Meikle (1985) refers to this taxon as *L. echioides* subsp. *exaristatum* (Murb.) Maire, indicating its close relationship to the annual Mediterranean *L. echioides* (L.) Mill., also occurring in Cyprus (Meikle 1985; Brullo 1988; Hand 2009). *L. avei* mainly differs from *L. echioides* in having mucronate to acute leaves, calyx ribs not or just shortly excurrent into an awn and a generally more robust habit. Moreover, *L. avei* is triploid with  $2n=27$  (see below), while *L. echioides* is diploid with  $2n=18$  (Dolcher & Pignatti 1971; Erben 1979; Artelari & Georgiou 2002).

**Cytology and breeding system** — The triploid chromosome number  $2n=27$  was found in the three populations of *Limonium avei* studied. This number has been already reported by Erben (1978, under *L. longispicatum*), Brullo (1988) and Brullo & Erben (1989). Its karyotype lacks the long metacentric “marker” chromosomes characteristic for karyotypes with the basic number  $x=8$  (Fig. 1D).

The examination of pollen and stigma showed that all individuals of the three populations of *Limonium avei* are male sterile and have a cob-like stigma. Nevertheless, they have good seed production and this, combined with the triploid chromosome number, indicates that *L. avei* reproduces apomictically. Existence of male sterile plants in *L. avei* has not been reported so far, but Baker (1953a) and Ingrouille (1984) stated the occurrence of male sterile colonies with cob-like stigmata in the closely related diploid *L. echioides*, which is a secondarily monomorphic species with the self-compatible combination C.

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