



## *Limonium korakoniscum* (Plumbaginaceae), a new species from Zakynthos Island (Ionian Islands, Greece)

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### Abstract

*Limonium korakoniscum* (Plumbaginaceae), a new species from Zakynthos Island (Ionian Islands, Greece), is described and illustrated from the only known population (locality Korakonisi) located in the southwestern coast of the island. The hexaploid chromosome number ( $2n=6x=51$ ), the karyotype and the self-incompatible pollen-stigma combination A ('A' pollen and 'Cob' stigma), support that *L. korakoniscum* is an apomictic taxon originated through hybridization. This new taxon is related to the polyploid apomictic *Limonium* species which are prevalent in the Aegean area and especially to the recently described Cytherian endemic *L. spreitzenhoferi* Erben & Brullo. The morphological differences of *L. korakoniscum* from *L. spreitzenhoferi* as well as from the sexual diploid endemic *L. phitosianum*, which coexists at the same locality, are discussed. Data on the ecology and conservation status of the new species are also given.

**Key words:** Apomictic, breeding systems, endemic, Greek flora, Ionian Islands, karyology, taxonomy

### Introduction

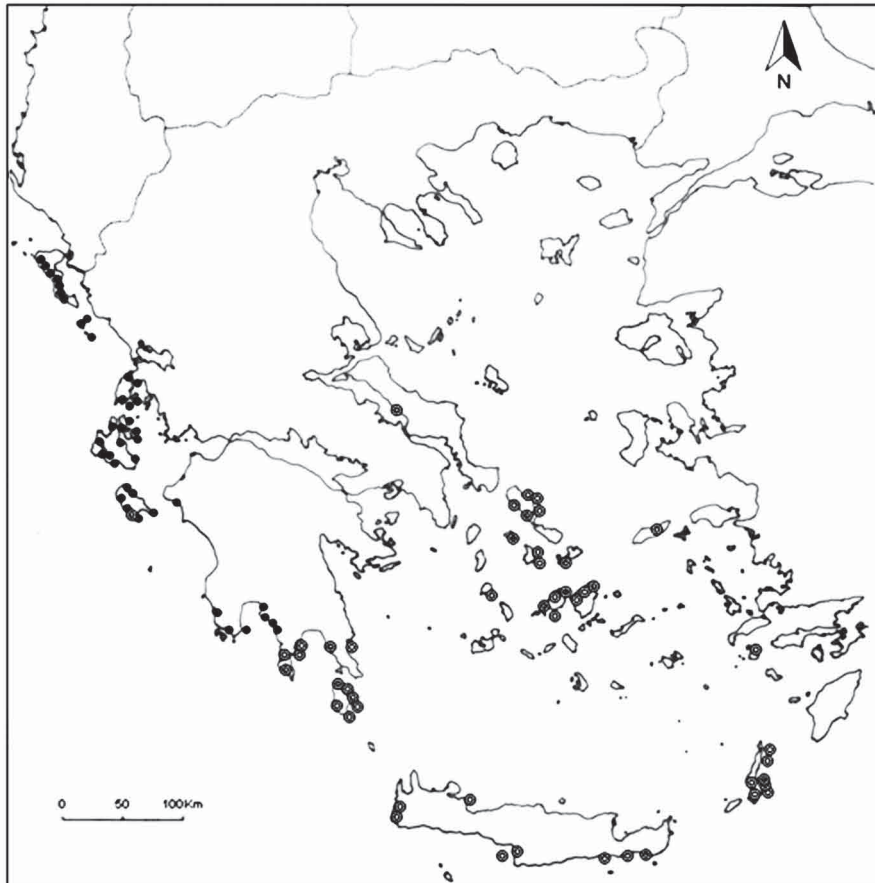
*Limonium* Miller (1754: 1328) is the largest and the most widespread genus of the Plumbaginaceae family including 400–500 species (Palacios & Gonzáles-Candelas 1997, Aparicio 2005, Brullo & Erben in press). The center of diversity is the Mediterranean basin (Cowan *et al.* 1998, Lledó *et al.* 2003, Palacios *et al.* 2000). The high taxonomic diversity and complexity of *Limonium* in the Mediterranean area is mainly due to its reproductive behaviour, i.e. the occurrence of both sexual and apomictic reproduction, as well as to the frequent occurrence of hybridization and polyploidy (Georgakopoulou *et al.* 2006). Thus, species delimitation in the genus is often a difficult task (Richards *et al.* 1996). This has as consequence the formation of numerous “microspecies” with local distribution.

In Greece, especially in the Aegean area, where a great number of islands and islets occurs, *Limonium* is represented by an high number of species. During the last 15 years, several taxonomic studies were published, increasing the number of endemic species in Greece (e.g. Artelari & Kamari 2000, Brullo & Guarino 2000, Crespo & Pena-Martín 2013). Dimopoulos *et al.* (2013), based in a recent paper of Brullo & Erben (in press), report 87 *Limonium* species most of them (79 species, 90.8%) endemic to Greece.

According to our data so far, in the Ionian Islands and the western coasts of the Greek mainland sexual diploid endemic species with  $2n=18$  are frequent, having the easternmost limit of their distribution range in the Messenian Mani (Kardamili) of South Peloponnisos (Artelari 1984a,b, Artelari & Kamari 1986, 1995, 2000) (Fig. 1). On the contrary, in the Aegean area and the eastern coasts of the Greek mainland polyploid apomictic *Limonium* species mainly occur (Artelari 1989a, 1989b, 1989c, 1992, Artelari & Georgiou 1999, 2000, 2003, Crespo & Pena-Martín 2013) having chromosome numbers with higher ploidy levels (pentaploid and hexaploid) than those known from the western and central Mediterranean taxa (Brullo & Pavone 1981, Arrigoni & Diana 1993, Erben 1993, Rosselló *et al.* 1994, 1998, Mayer 1995, Sáez *et al.* 1998a, 1998b, Sáez & Rosselló 1999).

Four species of *Limonium* were known to occur in the island of Zakynthos: *L. phitosianum* R. Artelari (1984b: 430) and *L. zacynthium* R. Artelari (1984b: 429), exclusively endemic to the island, *L. brevipetiolatum* R. Artelari & Erben (1986: 507) endemic to W-Greece (Kerkyra, Lefkada, Kefalonia, Zakynthos, and W coast of Peloponnisos), and *L.*

*virgatum* Fourreau (1869: 41) widespread in the Mediterranean area. It is important to mention that monitoring for the two exclusively endemic taxa (*L. phitosianum* and *L. zacynthium*) has been carried out to evaluate their conservation status (Valli 2013). In September 2014, during a field investigation on Zakynthos in the southwestern coast of the island, on the locality Korakonisi a single, very interesting *Limonium* population was found. Morphological and cytological study of this population revealed that it is well differentiated and belongs to a different taxon here described as a new species for science.



**FIGURE 1.** Distribution map of sexual diploid Ionian endemic species with  $2n=18$  (●) and of apomictic polyploid species with  $2n=34, 42, 43, 51$  and  $52$  (◎) in Greece. Symbols represent populations in which the chromosome numbers have been counted.

## Material and methods

This study is based on ten living plants collected from the only one *Limonium* population of the island of Zakynthos during the September of 2014 and on field observations (Fig. 2). This population was repeatedly visited for the estimation of its extension and size and for collecting mature seeds. Additionally, digital images of specimens from herbarium B as well as herbarium specimens from UPA were examined as comparative material. Voucher specimens are kept in the Herbarium UPA (acronym according to Thiers 2014+).

For the cytological study 70 seeds were germinated in Petri dishes on moistened filter paper and root tips were pretreated according to the method described in Artelari (1984a) and Artelari & Kamari (1986), while 15 metaphase plates were analyzed.

For assessing the reproductive mechanism, all reproductive mature individuals of the population were examined. Pollen and stigma type combination of the flowers were determined according to Erben (1978, 1979) and Artelari & Kamari (1986). The pollen stainability was estimated by using cotton blue as described in Artelari & Kamari (1986).

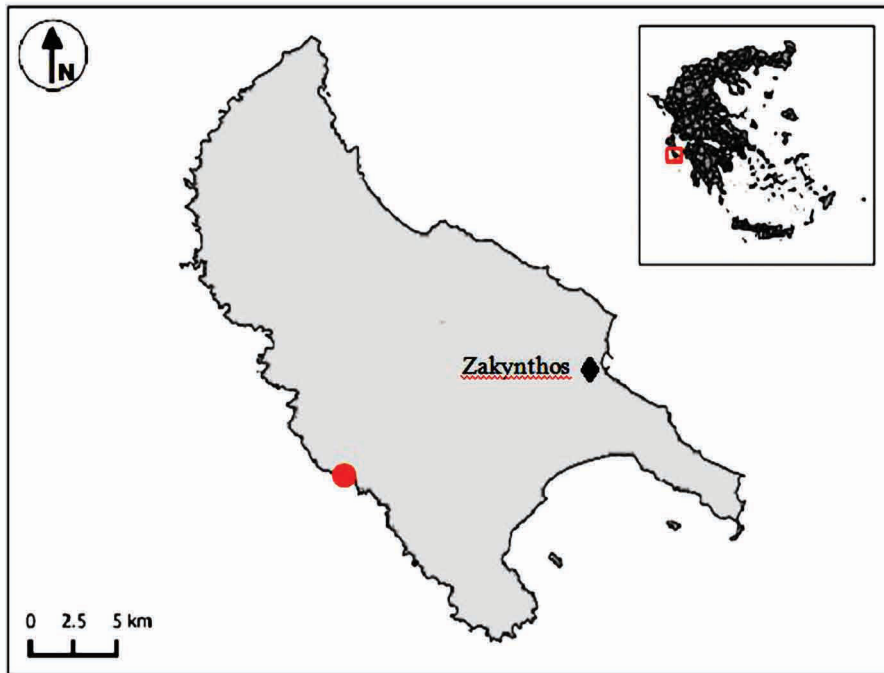


FIGURE 2. Map of Zakynthos Island where the only known population of *Limonium korakonisticum* R.Artelari & Valli is shown (red spot).

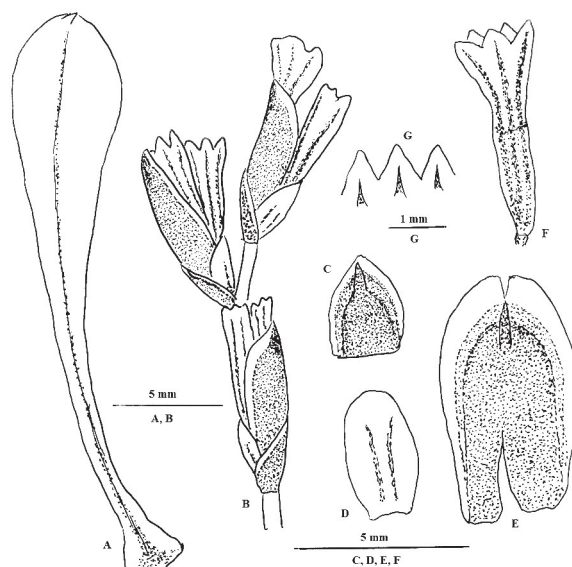
## Results and discussion

### *Limonium korakonisticum* R.Artelari & Valli *sp. nov.*

Type:—GREECE, Ionian Island: Zakynthos, locality Korakonisi, 37°43'08"N, 20°43'48"E, on calcareous maritime cliffs and rocks, 10 m a.s.l., 14 September 2014, leg. A.-Th. Valli no 1200 (holotype UPA!; isotype B!) (Figs. 3–4)



FIGURE 3. Holotype of *Limonium korakonisticum* R.Artelari & Valli (UPA!).



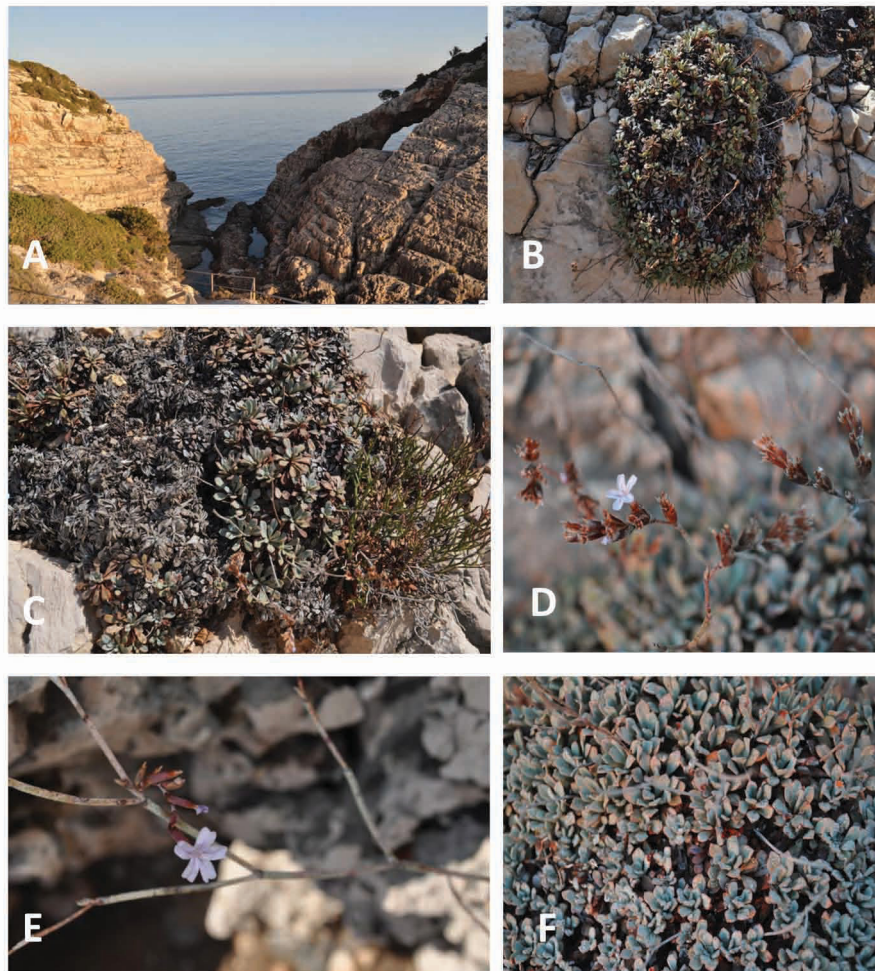
**FIGURE 4.** *Limonium korakonisicum* R.Artelari & Valli. **A.** Lower leaf surface. **B.** Spike. **C.** Outer bract. **D.** Middle bract. **E.** Inner bract. **F.** Calyx. **G.** Calyx lobes.

**Diagnosis:**—*Planta perennis, glabra, griseo-viridis, 9–25 cm alta, foliis rosulatis, densis, pulviniformis, 3.0–29.0 × (0.15–)0.3–0.6(–0.7) mm, oblanceolatis-spathulatis, obtusis, rugosis ad verrucosis, mucronulatis, 1-nerviis, caulibus paucis, valde fragilis, plerumque non ramosis, scabridis, interdum proliferis, ramis sterilibus absentibus vel solo 1–3 per caulem, spicis (1–)2–6.5(–7.0) cm longis, spiculis 7–9 mm longis, (1–)2–5(–6)-floris, ad 2–4(–5) in 1 centimetro dispositis, bractea inferiore 2.0–2.9 × 1.2–2.0 mm, triangulari-ovata, apice acuta, bractea media 2.7–3.4 × 1.0–2.0 mm, bractea superiore 6.0–7.5 × 3.0–4.0 mm, obovato-elliptica, apice obtusa, margine late membranaceo, calyce (5.0–)6.0–7.0 mm longo, ex bractea superiore 1.5–2.0 mm exserto, tubo glabro vel sparsim piloso, lobis calycis 0.6–0.7 mm, acutis.*

**Description:**—Perennial plant, 9–25 cm tall, glabrous, with very few and very fragile grey-green stems. Leaves 3–29 × (0.15–)0.3–0.6 (–0.7) mm on numerous small rosettes gathered to a very dense cushion-like formation up to 50 cm in diameter, uppermost leaves green at anthesis, lowermost ones brown and persistent after withering, oblanceolate-spathulate, fleshy, grey-green, flat or sometimes v-shaped in cross-section, rugose to verrucate, obtuse, without revolute margins, mucronulate with a mucro ca. 0.1 mm long sometimes curved backwards, with one central nerve, gradually tapering into a petiole shorter than lamina. Stems erect, scabrid, usually unbranched, when branched the branches begin almost from the base and form an acute angle; the branches sometimes proliferous (with small leaf rosettes); sterile branches absent or only 1–3 per stem. Inflorescence corymbose, with very fragile segments. Spikes (1.0–)2.0–6.5(–7.0) cm long, erect or slightly curved. Spikelets 7–9 mm long, composed of (1–)2–5(–6) flowers, 2–4(–5) per cm. Outer bract 2.0–2.9 × 1.2–2.0 mm, glabrous, triangular-ovate, acute, with a broad membranous margin, central part fleshy, brown, forming a point 0.4–0.5 mm. Middle bract 2.7–3.4 × 1.0–2.0 mm, glabrous, elliptical, hyaline-membranous. Inner bract 6.0–7.5 × 3.0–4.0 mm, glabrous, obovate-elliptic, obtuse, with a narrow membranous margin 0.4–0.5 mm wide; central part fleshy, forming a point 0.9–1.1 mm. Calyx (5.0–)6.0–7.0 mm long, exceeding the inner bract 1.5–2.0 mm; calyx tube glabrous or sometimes sparsely hairy; calyx lobes 0.6–0.7 mm, acute. Corolla pale lilac to white.

**Etymology:**—The specific epithet refers to Korakonisi, the type locality at the southwestern coast of the island of Zakynthos (Fig. 2).

**Ecology and distribution:**—*Limonium korakonisicum* is currently known only from the type locality in Korakonisi area (South-Western Zakynthos Island). Korakonisi is an isolated rock connected to the main island by a narrow land-bridge and it is characterized by impressive geological formations (Fig. 5A). The species forms a small population, which includes 100 individuals (complete census), while the 67 of them are mature (Fig. 6) according to IUCN definition (IUCN Standards and Petitions Subcommittee 2014). Flowering from August to October, fruiting from September to November. *L. korakonisicum* seems to be extremely restricted, a common feature among several agamosperous taxa, especially in the genus *Limonium* (Brullo & Pavone 1981, Artelari & Georgiou 1999). However, its occurrence in the neighbouring inaccessible rocks must not be excluded.



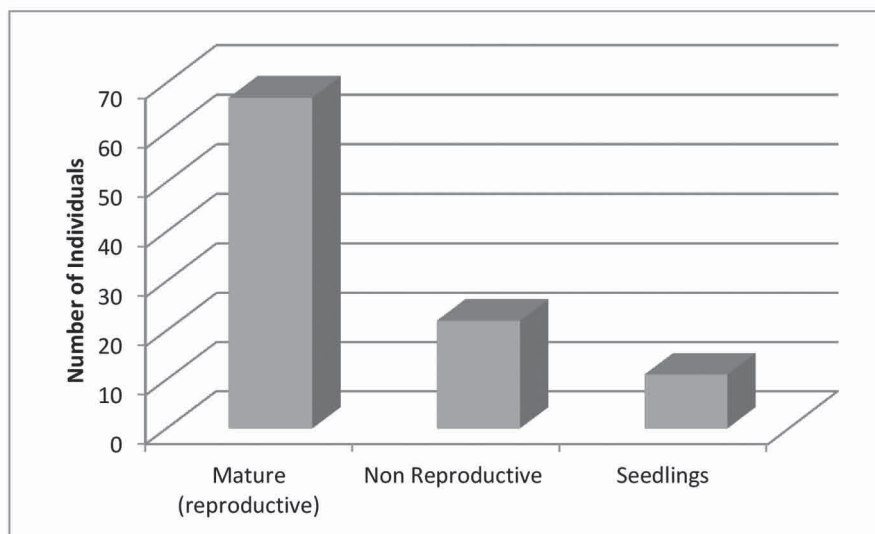
**FIGURE 5.** Habitat and morphological features of *Limonium korakonisicum*. **A.** Locality Korakonisi. **B.** A typical individual. **C.** The two local endemic taxa *L. korakonisicum* (left) and *L. phitosianum* (right) intermingled. **D.** Spikes, spikelets and flower of *L. korakonisicum*. **E.** A detail of the flower. **F.** Grey-green leaves.

*Limonium korakonisicum* (Fig. 5B, D, E & F) grows in crevices of high, calcareous, sunny maritime cliffs and rocks with terra rossa, at an altitude of about 10 m a.s.l., together with another endemic *Limonium phitosianum*. *L. phitosianum* forms a larger population in the area of Korakonisi which includes 245 individuals, 183 (74.7%) of them are mature. The two local endemic *Limonium* taxa not only coexist but sometimes are intermingled, producing an amazing sight (Fig. 5C).

Other cohabiting taxa in the area are *Crithmum maritimum* L., *Hypericum aegypticum* L. subsp. *webbii* (Spach) N. Robson and *Sarcocornia fruticosa* (L.) A. J. Scott.

**Conservation status:**—According to IUCN (2014), *Limonium korakonisicum* fulfils Criterion D, mainly due to its very limited expansion/ restricted distribution (Extent of Occurrence less than 100 km<sup>2</sup> and Area of Occupancy less than 10 km<sup>2</sup>), as well as its low number of mature individuals (less than 250). Thus, is here assessed as Endangered (EN). Moreover, in case of further touristic development in the area in the future, the species may be classified as Critically Endangered (CR). All the above reasons necessitate the long term monitoring of *L. korakonisicum*. Long term monitoring is essential so that required management measures can be taken to ensure the survivability of the species.

**Taxonomic relationships:**—*Limonium korakoniscicum* belongs to the subgenus *Limonium* (*sensu* Lledó *et al.* 2005). It is well differentiated from the group of the sexual diploid Ionian endemics with  $2n=18$ , which dominate in the Ionian Islands and the western coasts of the Greek mainland (Fig. 1), as well as from all the other *Limonium* taxa which are known so far from the same area. On the contrary, it is related to the polyploid apomictic *Limonium* species occurring in the Aegean area and the eastern coasts of the Greek mainland (Fig. 1). Among them, *L. korakoniscicum* is more closely related to the recently described Cytherian endemic *L. spreitzenhoferi* Erben & Brullo (Brullo & Erben in press). *L. spreitzenhoferi* has as type a specimen of G.C. Spreitzenhofer (Iter Ionicum IV num. 18: Cerigo, auf den Felsen bei den Häusern im Hafen von Kapsali, 15 June 1880, holotype B photo! no. 10-0294995), which as it seems from the label, was originally identified as *Statice sieberi* Boiss. Based on this, material which we have collected from the same locality of Kithira Island treated in a previous paper as *L. sieberi* Kunze (Artelari & Georgiou 2003: 495) is considered now as *L. spreitzenhoferi*. From the morphological point of view *L. korakoniscicum* (Fig. 5B, D, E & F) clearly differs from *L. spreitzenhoferi* in having leaves on numerous small rosettes gathered to a very dense cushion-like formation up to 50 cm in diameter, fewer and very fragile stems all of them reaching at about the same height, denser spikes, shorter spikelets with more flowers per spikelet, as well as shorter outer bracts and calyces.



**FIGURE 6 .** Population structure of *Limonium korakoniscicum*.

The coexisting species in the area, *L. phitosianum*, also has small leaf rosettes gathered to dense cushion-like formations which are much smaller (not more than 20 cm) than those of *L. korakoniscicum*. The two taxa are readily distinguished (Fig. 5C) as *L. korakoniscicum* has grey-green stems, grey-green leaves without revolute margins and with petiole shorter than lamina, segments of the inflorescence not articulate, fewer sterile branches, longer spikes and spikelets, longer outer and middle bracts, longer and obtuse inner bracts, and shorter calyx lobes. Furthermore, *L. korakoniscicum* is found to be an apomictic polyploid species with  $2n=6x=51$  (see cytology and breeding system below), while *L. phitosianum* is a sexual diploid with  $2n=2x=18$ . The main morphological diagnostic characters of *L. korakoniscicum*, *L. spreitzenhoferi* and *L. phitosianum* are given in Table 1.

**Chromosome number:**— $2n=6x=51$

**Cytology and breeding system:**—*Limonium korakoniscicum* was found to be hexaploid with  $2n=6x=51$ . The karyotype (Fig. 7) possesses three long metacentric ‘marker’ chromosomes which, according to Erben (1978, 1979) are characteristic for the karyotypes of the taxa of the subgenus *Limonium* with the basic number  $x=8$  and are missing in the taxa with  $x=9$ . Their occurrence indicates that the above chromosome number derived from the combination of  $3 \times 8 + 3 \times 9$  genomes (Erben 1978, 1979), therefore it can be assumed that *L. korakoniscicum* has a hybrid origin.

The study of breeding system of *L. korakoniscicum* was carried out on all 67 mature individuals of the known population of the species. The analysis of pollen and stigma combinations revealed that the population of this taxon is monomorphic. All studied plants have the self-incompatible ‘A’ combination, i.e. ‘A’ pollen and ‘Cob’ stigma (Baker 1948, 1953, Erben 1978, 1979) indicating self-sterility. Pollen stainability values are very low (0–2%) and pollen grains are irregular in size. Stainable pollen grains are well-shaped and much larger than the unstainable ones, which are small and misshaped. Such pollen and stigma features characterize apomictic species (Baker 1953). Although pollen stainability values are very low, relative reproductive success (RRS) of the species is high enough (75.6%). RRS

is defined as the percentage of all ovules maturing into seeds (Wiens 1984). The above data together with the hexaploid chromosome number support that *L. korakoniscum* is an apomictic species.

**TABLE 1.** Main morphological diagnostic characters of *L. korakoniscum*, *L. spreitzenhoferi* and *L. phitosianum* (measurements in mm).

	<i>L. korakoniscum</i>	<i>L. spreitzenhoferi</i>	<i>L. phitosianum</i>
Leaf size	3.0–29.0 × (0.15–)0.3–0.6(–0.7)	12–35 × 4–7	6.5–30 × 2.5–6.5
Leaf shape	oblanceolate-spathulate, without revolute margins	oblanceolate to subspathulate, without revolute margins	spathulate-oblanceolate, with revolute margins
Leaf apex	mucronate	rounded	obtuse, sometimes emarginate
Leaves on numerous small rosettes gathered to a cushion like formation	yes, with the formation reaching up to 50 cm in diameter	no	yes, with the formation reaching up to 20 cm in diameter
Sterile branches/individual	0–3	0–2	numerous
Spike length	(10–)20–65(–70)	(20)–40–100	0.7–10.0
Spikelet length	7.0–9.0	8.5–10.0	6.0–7.0
Spikelets per cm	2–4(–5)	1–2	2–4(–6)
Outer bract length	2.0–2.9	3.0–3.6	1.0–1.8(–2.0)
Middle bract length	2.7–3.4	3.0–4.0	(1.7–)1.9–2.6
Inner bract length	6.0–7.5	6.5–7.5	(3.7–)4.0–5.0(–5.7)
Inner bract shape	obovate-elliptic, obtuse	oblong-obovate, obtuse	elliptic, slightly curved, slightly acute to acute
Calyx length	(5.0–)6.0–7.0	7.0–8.0	5.0–5.8(–6.0)
Calyx indumentum	glabrous/ sometimes sparsely hairy	glabrous	very sparsely hairy
Calyx limb	slightly lacerate after anthesis	not or slightly lacerate after anthesis	lacerate after anthesis

It is important to note here the discovery, for the first time in the Ionian area, of an apomictic polyploid taxon with a chromosome number derived from the combination of  $x=8$  and  $x=9$ . According to our data so far such taxa are found in the Aegean area. A distribution map of the group of sexual diploid Ionian endemic species with  $2n=18$  and of apomictic polyploid species with  $2n=34, 42, 43, 51$  and  $52$  is presented in Fig. 1. This map is based on known data (Papatsou & Phitos 1975, Artelari 1984b, 1989a–c, 1992, Artelari & Kamari 1986, 1995, 2000, Artelari & Georgiou 1999, 2000, 2003, Georgakopoulou & *al.* 2006) as well as on other available data (Artelari & Georgiou unpublished).

## Acknowledgements

We wish to thank the curator of the herbarium B for the disposal of digital images. We also express our thanks to Ass. Prof. O. Georgiou and Ass. Prof. A. Tiniakou for their valuable help and criticism throughout this work and to Prof. G. Iatrou for his useful comments and suggestions. Thanks are also due to Dr. S. Spanou for her help in drawing the distribution map of diploid and polyploid taxa as well as helping us with the online submission of this article.



FIGURE 7. Mitotic metaphase plate of *Limonium korakoniscum* with  $2n=6x=51$ . Scale bar = 10  $\mu$ m.

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