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Abstract

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A new species of the recently established genus *Acis*, *A. ionica*, is described from the Ionian Islands and W Sterea Ellas in Greece and a restricted area of Albania, close to the city of Vlore. The morphological differences from its relatives are discussed and phytogeographical and karyological aspects of the group outlined. The chromosome number of the new species, $2n = 16$, and its karyotype are shared with its closest relative, *A. valentina* from the Iberian Peninsula.

Key words: taxonomy, *Leucojum*, karyology, phytogeography.

Introduction

The genus *Leucojum* L. s.l. (Amaryllidaceae) comprises about 10 species distributed mainly in the Euro-Mediterranean area. Most of them are cultivated for their ornamental value (Stern 1956). The genus has been divided into four subgenera originally by Baker (1888) and later by Stern (1956), stating that “the morphological characters work well with the geographical distribution of the species and also with the chromosome numbers”.

Contandriopoulos (1962) arranged these four subgenera into two groups. The first group consists of the subgenera *Leucojum* L. (with only one representative, *L. vernum* L.) and *Aerosperma* Stern (with *L. aestivum* L.). The members of this group are characterized by their hollow scape and wide leaves, the spring flowering time, a preference for wet and shady places and a wide, mostly European, distribution. Moreover, their basic chromosome number is $x = 11$. The second group, with a larger number of taxa (10), includes the remaining two subgenera, i.e. *Acis* (Salisb.) Baker and *Ruminia* (Parl.) Baker. Their main morphological characters are the solid scape and the filiform leaves. They have autumnal and spring flowering periods and prefer stony and rocky habitats around the Mediterranean. Most of the species are distributed in the W Mediterranean. The taxa of this group are characterized by a lower basic chromosome number of $x = 7, 8$ or 9 .

A recent phylogenetic analysis of *Leucojum* s.l. by Lledó & al. (2004), using plastid and largely non-coding nuclear ribosomal DNA sequences, supported the separation of *Leucojum* into two genera: the genus *Leucojum* L. s.str., which includes the subgenera *Leucojum* and *Aerosperma*, and the genus *Acis* Salisb., which includes the subgenera *Acis* and *Ruminia*. The phylogenetic classification provided by Lledó & al. (2004) is in full accordance with the distinction of two different groups in the genus proposed by Contandriopoulos (1962), based on morphological, ecological and cytological data. In our opinion, the harmonious cooperation of different data sets that all support two different, clearly circumscribed species groups fully justifies the splitting of the traditional genus *Leucojum* s.l. into two smaller, natural genera, *Leucojum* s.str. and *Acis*. This distinction shall be followed in the present study.

The first collections of *Leucojum* in Greece were made by Schimper & Wiest in 1834, on the island of Kefallinia. These plants were attributed to *L. autumnale* L. by Boissier (1882) and Heldreich (1882) and later on to *Acis cephalonica* by Gay (in sched.). The latter name, which appeared in a handwritten note on a specimen by Schimper & Wiest (herb. Gay, K), is a nomen nudum. The name *L. autumnale* was used for the plants from the islands of Kefallinia (Halácsy 1904, Hayek 1932, Contandriopoulos 1962) and Lefkas (Hofmann 1968) for several decades. Damboldt & Phitos (1975), after studying in detail the morphological, ecological and karyological characters of plants from Lefkas and Kefallinia, identified them as *L. valentinum* Pau, a member of subg. *Ruminia*. It also became clear that their chromosome number was $2n = 16$ and not $2n = 14$ as in *L. autumnale*. Müller-Doblies & Müller-Doblies (1975) also came, independently, to the same result.

The first doubt on the true identity of the Greek *Leucojum valentinum* populations is found in Aguilera & al. (1990) and Lledó & Crespo (1996). In both publications the distribution of *L. valentinum*, now *Acis valentina* (Pau) Lledó & al., in the E Mediterranean is questioned. The typical *A. valentina* is considered a rare endemic restricted to a limited coastal, calcareous area in E Spain (Valencia).

Extensive morphological and cytogeographical studies on the Greek plants from all over their known geographical range were made in the Botanical Institute of Patras during the last seven years (Bareka & Kamari 1999, Bareka 2001, Bareka & al. 2003). As a result it became clear that “the Greek populations of *L. valentinum* s.l., are morphologically not identical to Spanish specimens examined and probably represent an undescribed taxon” (Bareka & al. 2003). This opinion is reinforced by the highly discontinuous distribution range of *A. valentina* (E and W Mediterranean). Lledó & al. (2004: 239), based on sequence data, suggest a different status at species level.

The study of abundant material, including living and herbarium collections, allowed a detailed comparison of Greek, Albanian and Spanish *Acis* populations. Morphological and karyological data led us to recognize a new species, *Acis ionica*, which is described below.

Remarkably, Tan & al. (2004) recently described a new taxon from the Ionian area, *Leucojum ionicum*, which looks superficially similar and has a very similar distribution, but differs very significantly by the presence of hollow (“fistulose”) scapes, which exclude the species from the genus *Acis*. Our thorough search in ATH for the holotype (consisting of cultivated material) did not result in either a specimen or a photo of the species and therefore it was not possible for us to verify this difference.

Material and methods

Living plants of the investigated populations were cultivated in the experimental garden of the Botanical Institute, University of Patras.

The chromosome counts were obtained from root tip metaphases. Further details of the applied technique are described in Bareka (2001). Chromosome terminology follows Kamari (1984).

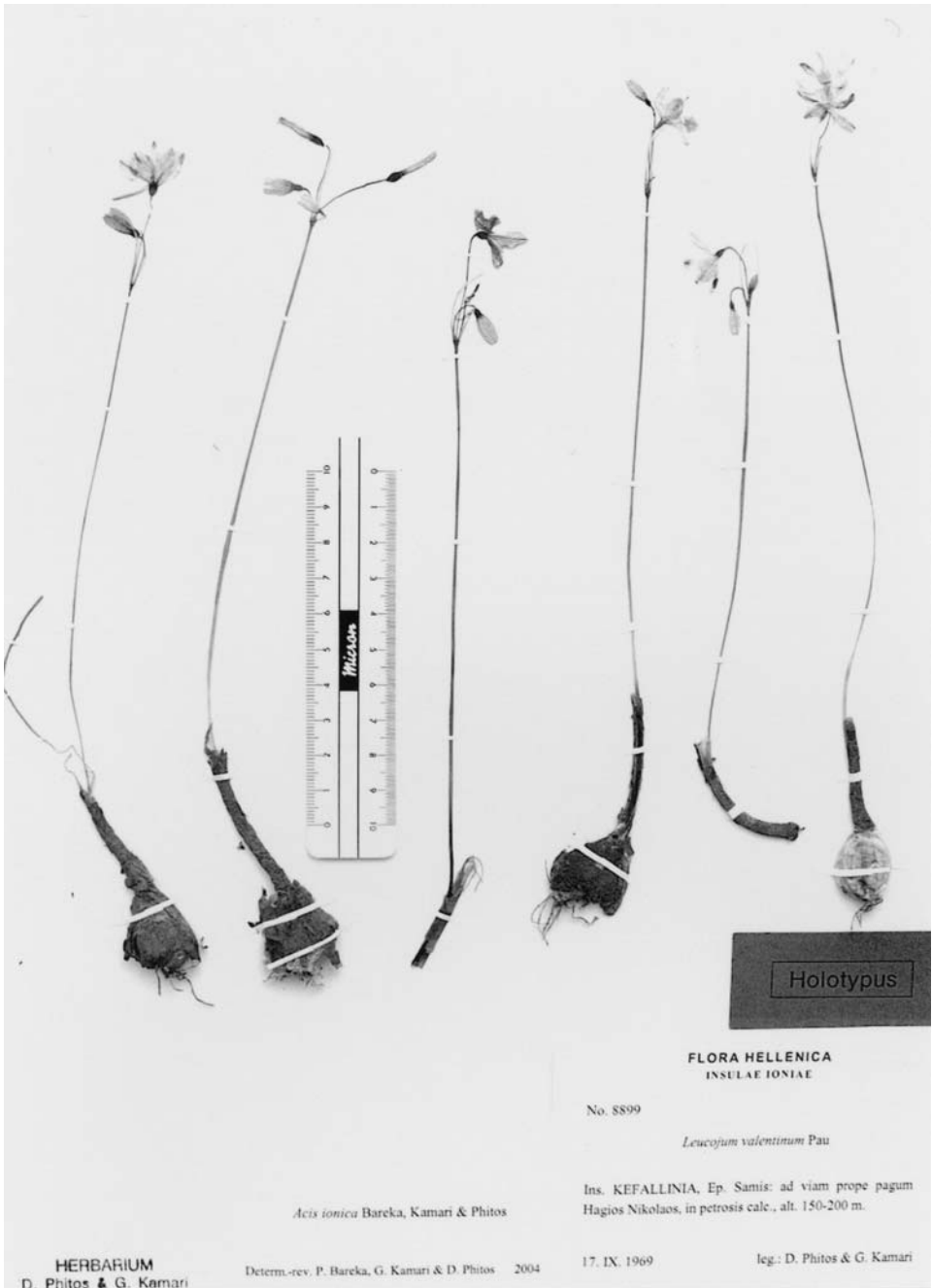


Fig. 1. *Acis ionica* – holotype at UPA.

Dried material was studied from ATH, K, MA and UPA (abbreviations according to Holmgren & Holmgren 1998-) as well as from the Museum of Natural History of Cefalonia-Ithaki (here abbreviated as MNHC-I).

Results

Key to *Acis ionica* and closely related species

1. Spring flowering 2
– Autumn flowering 3
2. Inner tepal apex obtuse; lobes of epigynous disc 0.2-0.3 mm long, triangular . . . *A. nicaeensis*
– Inner tepal apex rounded; lobes of epigynous disc 0.6-0.8 mm long, ovoid-lanceolate . . .
. *A. fabrei*
3. Inner tepal apex emarginate; lobes of epigynous disc 1.0-1.1 mm long . . . *A. valentina*
– Inner tepal apex obtuse; lobes of epigynous disc 0.8-0.9 mm long *A. ionica*

Acis ionica Bareka, Kamari & Phitos, **sp. nov.**

Holotype: [Greece, Isl. Kefallinia] “ad viam prope pagum Hagios Nikolaos, in petrosis calc., alt. 150-200 m”, 17.9.1969, *Phitos & Kamari* 8899 (UPA) – Fig. 1.

– *Leucojum valentinum* subsp. *vlorense* Papanisto & Qosja in Bul. Shkencat Nat. 1: 94. 1983, nom. inval.

– *Leucojum valentinum* auct. fl. graec. [non Pau in Bol. Soc. Aragon. Ci. Nat. 13: 42. 1914].

Bulbus ovoideus, 15-25 mm diametro, tunicis brunneis. *Vagina* membranacea, brunnea, (10-)15-25 mm longa. *Scapus* solidus, 8-20(-25) cm altus, gracilis, 0.8-1.2 mm diametro. *Folia* 2-3(-5), hysteroantheria, linearia, 12-22 cm longa. *Spathae* 2, membranaceae, subulatae, 17-28(-32) mm longae, basi 1-1.5 mm latae. *Flores* (1-)2-4(-6), nutantes, pedicellis filiformibus (10-)16-28(-35) mm longis, inaequalibus, longioribus spathis superantibus. *Perigonii phylla* alba, (8-)9-13(-15) mm longa, exteriora oblonga, mucronata, 3-4 mm lata, interiora late obovata, (3.5-)4.2-6.4(-7) mm lata. *Stamina* 5.5-8 mm longa, filamentis 1.5-3 mm longis, antheris 4-5 mm longis. *Discus* epigynus 6-lobatus, lobis late triangularibus 0.7-0.8 × 0.7-0.9 mm, filamentis brevioribus. *Semina* nigra, 2.5-3 mm longa, strophiole alba provisa.

Bulb ovoid, 15-25 mm in diameter, with brown tunica. *Sheath* membranous, brown, (10-)15-25 mm long. *Scape* solid, 8-20(-25) cm long, slender, erect, slightly sulcate, bending to the ground during fructification, 0.8-1.2 mm in diameter. *Leaves* 2-3(-5), appearing after anthesis, filiform, narrowly linear to filiform, 12-22 cm long, 2-3 mm broad. *Spathes* 2, membranous, subulate, unequal, 17-28(-32) mm long, the base 1-1.5 mm broad. *Flowers* (1-)2-4(-6) per inflorescence, pendent, in a terminal, unilateral umbel, pedicels filiform, (10-)16-28(-35) mm long at anthesis, (16-)28-40(-50) mm in fruit, unequal, the longest one usually extending beyond the spathe. *Perianth* conically campanulate; perianth segments white, (8-)9-13(-15) mm long, the outer three oblong, mucronate, 3-4 mm wide, the inner three broadly obovate, (3.5-)4.2-6.4(-7) mm broad. *Style* filiform, 5.5-8 mm long, filaments white, filiform, 1.5-3 mm long, anthers oblong, bright yellow, 4-5 mm long. *Epigynous disc* prominent, 6-lobed; lobes pale green, widely triangular, 0.7-0.8 × 0.7-0.9 mm, shorter than the filaments. *Seeds* black, 2.5-3 mm long, with strophiole. Flowering September-October.

Habitat and ecology. – Open, calcareous, stony and rocky places, hill slopes generally not far away from the coastline and often facing the sea, usually in phrygana or low macchia communities, at an altitude of 3-350(-450) m (Fig. 2).

Distribution. – W Greece: Ionian Islands (Zakinthos, Kefallinia, Lefkas) and W Sterea Ellas (Nomos Etolias-Akarnanias). S Albania: close to the city of Vlore (Fig. 3).



Fig. 2. *Acis ionica* in its natural habitat on Kefallinia island – A: among calcareous rocks; B: in phrygana.

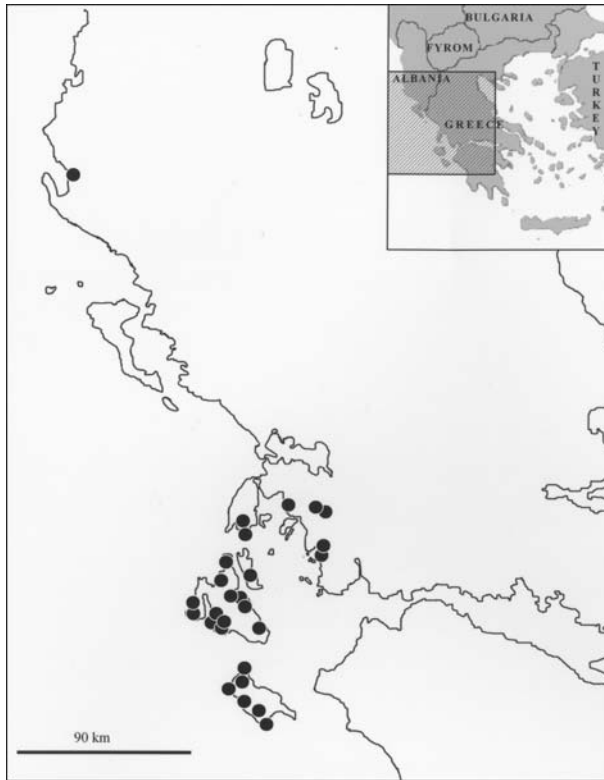


Fig. 3. *Acis ionica* – distribution.

Karyology. – The chromosome number $2n = 16$, counted in all Greek populations examined (Bareka & Kamari 1999, Bareka 2001 and Bareka & al. 2003), is in accordance with that given by Damboldt & Phitos (1975) and Müller-Doblies & Müller-Doblies (1975). A detailed analysis of many Greek populations gave a similar karyotype in all plants examined, also in the six new populations studied in this paper (Fig. 4). The chromosome number $2n = 16$, given by Paparisto & Qosja (1983) for plants from Albania, is confirmed here after karyological investigation of Albanian material (Fig. 4D).

We also analysed two populations of *Acis valentina* from Spain (Valencia, Sagunto, pr. Roman theatre, 30SYJ3359, 100 m, rocky limestone slope, 3.10.2000, *Crespo cult. L. 31*, UPA, Fig. 4E; Castellón; Artana, 30SYK3500, 210 m, open grassland with *Brachypodium retusum*, 3.10.2000, *Crespo cult. L. 30*, UPA), Fig. 4F. Their chromosome number proved to be equally $2n = 16$.

The karyotype morphology of *Acis ionica* (Fig. 4A-D) is similar to that of *A. valentina* (Fig. 4E-F) which is illustrated here for the first time. In both taxa the karyotypes are rather symmetrical, consisting of $2n = 8m + 4st + 4st-SAT = 16$ chromosomes, ranging in size between 10.8 and 3.2 μm . Both species also have small spherical satellites that are not always visible. Our count of $2n = 16$ for *A. valentina* agrees with the number provided by Boscaiu & al. (1997), while the chromosome number of $2n = 18$ given by Lledó & al. (2004) is probably erroneous.

Additional specimens seen. – (An asterisk indicates the populations studied karyologically in this paper). – GREECE: IONIAN ISLANDS: ZAKINTHOS: Between the villages Agios Nikolaos and Korithi, in stony, calcareous places among phrygana, c. 150 m, 22.9.1986, *Phitos & Kamari* (UPA); Agios Nikolaos, rock crevices along the road, 100 m (ex cultis), 37°44'N, 20°46'E, 8.10.1989, *Garbari cult. L. 10* (UPA); N of Korithi village on the way to cape Skinari, 37°55'N, 20°41'E, 13.10.1991,

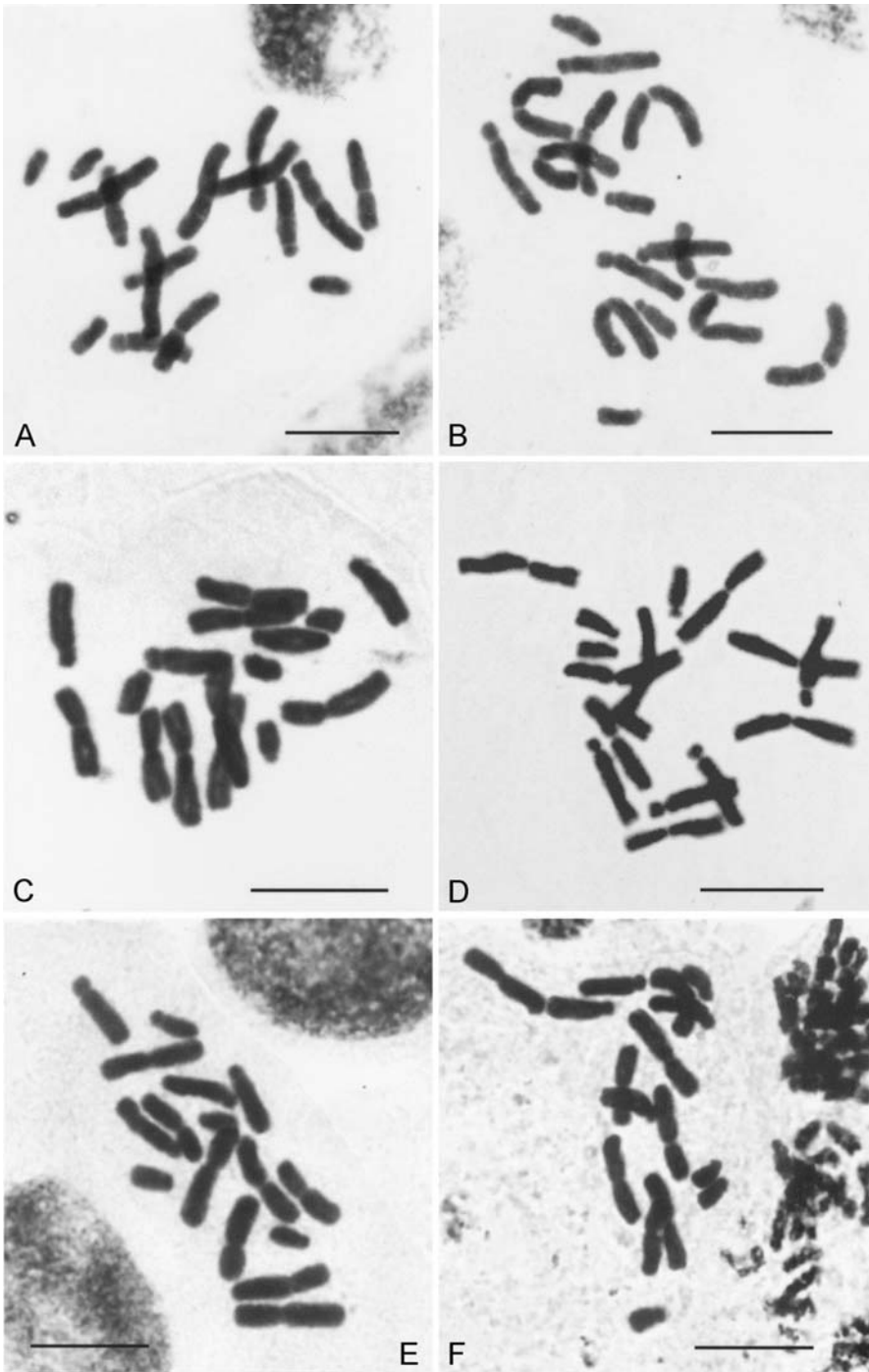


Fig. 4. Microphotographs of mitotic metaphase plates – A-D: *Acis ionic*, $2n = 16$ (Greece: A: Isl. Kefallinia; B: Isl. Levkas; C: Mt Boumistos; Albania: D: Vlore); E-F: *Acis valentina*, $2n = 16$ (Spain: E: Valencia; F: Castellón). – Scale bars = 10 μm .

Phitos & Kamari 26989 (UPA); between the villages Volimai and Korithi, rock crevices (ex cultis), 37°54'N, 20°40'E, 13.10.1991, *Phitos & Kamari cult. L. 12* (UPA); close to the village of Keri, 37°39'N, 20°48'E, 12.10.1997, *Phitos & Kamari cult. L. 27* (UPA); close to the village of Kambi along road towards Stavros (ex cultis), 12.10.1997, *Phitos & Kamari cult. L. 5* (UPA). — KEFALLINIA: Auf niederen Hügeln zwischen Steinen bei Argostoli, 6.10.1834, *Schimper & Wiest 1969* (K, Photo!); Ep. Samis, ad viam prope pagum hagios Nikolaos, in Petrosis, 17.9.1969, *Phitos & Kamari 8899* (UPA); Ep. Kraneas, Macchie c. 1 km vor Tzannata, Straße Sami-Poros, 1969, *Damboldt 685/69* (ATH); pagum Assos (ex cultis), 12.9.1973, *Phitos & Kamari* (UPA); Ep. Samis, supra vicum Sami, in apertis saxosis fruticetorum, 6.10.1973, *Phitos & Kamari 12113* (UPA); Ep. Samis, ad viam prope pagum Koulourata; in saxosis calc., 7.10.1973 *Phitos & Kamari 12114* (UPA); along coastal road S of Fiskardo (ex cultis), 38°21'N, 20°23'E, 12.4.1974, *Phitos & Kamari cult. L. 13* (UPA); Ep. Samis, supra vicum Sami, ad locum Agrilies, in saxosis calc., c. 150 m (ex cultis), 8.9.1985, *Phitos & Kamari cult. L. 14* (UPA); close to the village of Chavriata 38°11'N, 20°23'E, 25.10.1993, *Katsouni* (MNHC-I); NE of the village of Chavriata, Moni Kipoureon, 12.9.1973, *Phitos & Kamari* (UPA); Xirokampos, close to the village of Chavriata 38°11'N, 20°23'E, 1.11.1993, *Katsouni* (MNHC-I); between Argostolion and Davgata, limestone, 150-200 m (ex cultis), 38°12'N, 20°30'E, 31.10.1999, *Phitos & Kamari cult. L. 23* (UPA) (Fig. 4A); close to Argostolion on the way to Sami, locality Agia Varvara, 1.11.1993, *Katsouni* (MNHC-I). — ITHAKI: Islet Skartsoubonisi in Vathi bay, 5.10.2000, *Katsouni* (MNHC-I). — LEFKAS: S of Vasiliki village, c. 5 m, 38°36'N, 20°36'E, 21.10.1999, *Bareka & Lampropoulos 245* (UPA) (Fig. 4B); close to the village of Marantochori, *Kriemadi* (UPA). — STEREA ELLAS: NOMOS ETOLIAS-AKARNANIAS: Ep. Vonitsis-Xiromerou, inter vicos Astakos et Mytikas, 15 km a vico Astakos, in declivibus occidentalibus montis Veloutsa, 60-100 m, in rupestribus calc., 38°34'N, 21°02'E, 26.3.1995, *Phitos, Kamari, Greuter & Zimmer 24375* (UPA); Mt Boumistos, along road between Archontochori and Aetos, 4 km S of Aetos village, phrygana, 450 m, 38°41'N, 21°04' E, 20.9.1998, *Vlachos 58/820* (UPA) (Fig. 4C); Mt Boumistos, Xylogaidara, SE of the village of Archontochori, 450 m, 38°41'N, 21°01'E, 20.9.1998, *Vlachos 58/825* (UPA); Ep. Vonitsis-Xiromerou, c. 14.3 km S-SE Paleros along road to Mytikas, coastal stony places, limestone, c. 3 m, 38°42'N, 20°53'E, 13.10.1999, *Bareka & Constantinidis 250* (UPA); Ep. Vonitsis-Xiromerou, c. 17 km after Astakos, along coastal road to Mytikas, stony shady slopes of dry coastal area, limestone, 30-50 m, 38°42'N, 21°02'E, 13.10.1999, *Bareka & Constantinidis 249* (UPA). ALBANIA: Vlore, coastal stony places, limestone, macchie with *Quercus coccifera*, 50 m, 15.10.2001, *Gjini* (UPA) (Fig. 4D).

Discussion

Acis ionica brings the total number of species in the genus to 10. Two groups can be recognized, which correspond to the former subgenera *Acis* and *Ruminia*. The first group, characterized by an inconspicuous, epigynous disc and seeds without strophiole, includes *A. autumnalis* (L.) Herb., extending from Portugal eastwards to Sicily, and two Atlantic species, *A. tingitana* (Baker) Lledó & al. from Morocco and *A. trichophylla* Sweet from N Africa and the Iberian Peninsula. The remaining three species of this group are local endemics: *A. longifolia* J. Gay ex Salisb. is found only in Corsica, *A. rosea* (F. Martin) Sweet in Corsica and Sardinia, and *A. tingitana* (Baker) Lledó & al. in N Africa.

The second group comprises four taxa of the former subgenus *Ruminia* and are characterized by a 6-lobed epigynous disc and strophiolated seeds. Three are rare and extremely restricted: *Acis fabrei* (Quézel & Girerd) Lledó & al. and *A. nicaeensis* (Ardoino) Lledó & al. occur in S France, *A. valentina* (Pau) Lledó & al. in E Spain. *A. ionica* is the fourth species, found in the Ionian area, the easternmost known distribution range of any *Acis* species.

The most important morphological features of *Acis ionica* and its relatives are presented in Table 1. An obvious difference among the species of this group is in the flowering time: *A. ionica* and *A. valentina* are autumn-flowering, while *A. fabrei* and *A. nicaeensis* are spring-flow-

Table 1. The most distinctive features of *Acis ionica* and relatives.

Characters	<i>A. ionica</i>	<i>A. valentina</i>	<i>A. nicaeensis</i>	<i>A. fabrei</i>
Flowering period	autumn	autumn	spring	spring
Chromosome number	$2n = 16$	$2n = 16$	$2n = 18$	$2n = ?$
Inner tepal apex	obtuse	emarginate	obtuse	rounded
Outer tepal apex	mucronate	mucronate	mucronate	acuminate
Lobes of epigynus disc	triangular	triangular	triangular	ovoid-lanceolate
Length of lobes (mm)	0.8-0.9	1-1.1	0.2-0.3	0.6-0.8

ering. Moreover, *A. nicaeensis* differs significantly from the first two species in having $2n = 18$ chromosomes instead of $2n = 16$. The chromosome number of *A. fabrei* is unknown.

Besides its isolated geographical distribution, *Acis ionica* differs from *A. valentina* morphologically. It has a slender scape, 0.8-1.2 mm thick, the three inner perianth segments are widely obovate, (3.5-)4.2-6.4(-7) mm broad and the length of the lobes of the epigynous disc is 0.8-0.9 mm. In contrast, *A. valentina* has a more robust scape, 2-3 mm thick, the three inner perianth segments are 5.5-7.5(-8) mm wide, emarginate, broader than those of *A. ionica*, forming campanulate flowers that do not open fully, and the length of the lobes of the epigynous disc is larger (1-1.1 mm). In general, though, these two taxa exhibit a high morphological similarity; nevertheless their level of molecular divergence is equivalent to that of any pair of morphologically distinct species of *Leucojum* (Lledó & al. 2004).

Paparisto & Qosja (1983), based on dimension differences in some morphological characters between the Albanian plants and those given by Damboldt & Phitos (1975) for plants from Kefallinia and Lefkas islands, invalidly (without Latin description and *typus*) described the plants from Albania as *Leucojum valentinum* subsp. *vlorense*. An extensive and detailed comparison of the Greek and Albanian plants led us to the conclusion that they belong to one and the same taxon, since the numerical differences in the dimensions of morphological characters are not significant.

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