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## Research article

### *Ornithogalum christinia* (Asparagaceae): a new species from an offshore islet on the Cretan area (S. Aegean, Greece).

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*Ornithogalum christinia* sp. nov., collected from the offshore islet of Agria Gramvousa (northwestern Crete, Greece), is described and illustrated as a locally endemic species. It is related to *Ornithogalum montanum*, *O. atticum* and *O. pumilum*, however, it differs from these in having wrinkled and corrugated leaf surface, fruiting pedicels thickened at base, flexuose rachis of infructescence and winged capsules. The relationships between the new species and the aforementioned are discussed, while information regarding its ecology, conservation status and karyotype is also given.

Keywords: Aegean Flora, island specialists, karyotypes, new species, *Ornithogalum*, taxonomy

## Introduction

The Aegean archipelago is a biodiversity hotspot especially for endemic species (López-Vinyallonga et al. 2015). High endemism is characteristic of southern Greece and the Island of Crete, while the latter is considered as the hottest island hotspot in the Mediterranean as far as endemic species are concerned (Médail and Quézel 1999, Blondel et al. 2010, Médail 2017, Kougioumoutzis et al. 2021). Small islands and offshore islets operate as evolutionary laboratories and can affect plant distribution patterns in the Aegean archipelago. They host a remarkable flora, characterized by the presence of plant species distributed exclusively on extraordinary and difficult habitats (Runemark 1971, Christodoulaki et al. 1991, Panitsa et al. 1994, 2006, Georgiou et al. 2006, Kougioumoutzis et al. 2014, 2016, Fois 2016, Kypriotakis et al. 2018). Similar examples are also known from the offshore islets of Crete, e.g. *Silene holzmannii* Heldr. ex Boiss., *Anthemis glaberrima* (Rech. f.) Greuter, *Scilla talosii* Tzanoud. and Kypr., *Allium platakissii* Tzanoud. and Kypr. and *Ornithogalum insulare* Kypr., Antal. and Tzanoud.

The genus *Ornithogalum* L. is distributed throughout Europe, Southwest Asia (Afghanistan, India, Sri Lanka), north and south Africa and Madagascar (Zahariadi 1965, Speta 1998, Stevens 2001 onwards) and is considered one of the largest genera



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in Asparagaceae (Stevens 2001 onwards). The genus has been thoroughly studied by many researchers in the past and present (Zahariadi 1965, 1982, Speta 1968, 1998, Garbari and Giordani 1984, Garbari et al. 2003, 2007, Wittman 1985, Landström 1989, Pfosser and Speta 1999, Coskuncelebi et al. 2000, 2002, Peruzzi and Passalacqua 2002, Manning et al. 2004, 2009, 2022, Martínez-Azorín et al. 2007, 2009, 2011, 2013, Peruzzi et al. 2007, Andrić et al. 2016, Rat et al. 2017, Rat 2019). It was previously divided into many sections and subgenera (Zahariadi 1965, 1977, 1982), however, Speta (1998) included all Eurasian and North African taxa of *Ornithogalum* subg. *Ornithogalum* in *Ornithogalum*, while the other subgenera were considered as separate genera e.g. *Albuca*, *Beryllis*, *Loncomelos* etc. Currently, there are two competing approaches advocated by different authors. While Manning et al. (2004, 2009) recognize *Ornithogalum* as a single genus containing 214 species worldwide (see also POWO 2024), Martínez-Azorín et al. (2011) divide it into 19 different genera.

In the Mediterranean and European region, *Ornithogalum* is mostly treated as a single genus with ca 120 species (Euro+Med 2006+, Martínez-Azorín et al. 2013, Dimopoulos et al. 2013, 2016, Strid 2016a, b, POWO 2024). According to recent research (Rat et al. 2017, Rat 2019) *Ornithogalum* species from the Balkan Peninsula are divided into three sections: *Ornithogalum* sect. *Cathissa*, *O.* sect. *Myogalum* and *O.* sect. *Heliochondros*, where the latter section includes all the Greek taxa of the genus.

The majority of *Ornithogalum* species are difficult to identify and distinguish, especially from herbarium specimens, as they tend to lose important morphological characters in the drying process. Additionally, many characters may overlap in different species, making its taxonomy even more problematic (Rat et al. 2017).

In Greece, the genus is represented by 35 species, 10 of which are also distributed on Crete and two are considered endemic to the Island (Dimopoulos et al. 2013, 2016, Strid 2016a, b). In this study, the taxonomy follows the 'Mediterranean' concept of a single genus *Ornithogalum* (Dimopoulos et al. 2013, 2016, POWO 2024). The material described here as a species new to science was found and collected from the Cretan offshore islet of Agria Gramvousa, located at the north-westernmost coast of Crete. According to its morphology, the new species mostly shows affinities with *O. montanum* Cirillo, and to a lesser extent with the broad-leaved species of the genus, such as *O. pumillum* Zahar. and *O. atticum* Boiss. and Heldr.

## Material and methods

All the morphological and anatomical studies were conducted on living material (ten bulbs) collected from Agria Gramvousa islet (locus classicus) and then cultivated in the experimental botanical garden of the Hellenic Mediterranean University of Crete (HMU). Living material has also been cultivated for cytological studies in the botanical garden of the University

of Patras. For comparative purposes, specimens of *O. montanum* Cirillo (1814) were used, collected and cultivated from Milos Island (Kiklades Aegean region) and Mt Ziria (NE Peloponnese). Herbarium specimens were also examined (UPA, ATHUM, TAU). For taxonomical purposes, relevant bibliographical sources were taken into consideration (Zahariadi 1965, 1978, 1982, 1980, Cullen 1984, Landström 1989, Martínez-Azorín et al. 2009, Dimopoulos et al. 2013, 2016, Rat et al. 2017, POWO 2024). For the leaf anatomy, a stereo-microscope was used on living material and the cross-section was conducted at the broadest part of the leaf when the plants were at anthesis. For karyological studies, root tips from potted bulbs were pretreated in  $\alpha$ -bromonaphthalene for ca 6 h at 4°C. Fixation, staining, chromosome measurements, and construction of the karyogram follow Tzanoudakis (1983) and Ioannidis and Tzanoudakis (2022).

## Taxonomic treatment

### *Ornithogalum christinae* sp. nov. (Fig. 1, 2).

#### Diagnosis

A species showing some similarities with *O. montanum*, and to a lesser extent with *O. atticum* and *O. pumillum*, in having bulbs with concrescent scales, leaves more than 5 mm wide without a white stripe, a short raceme that is corymbiform at anthesis, entire filaments and hypogean germination, however, it differs from these three species in having spherical to sub-spherical bulbs, wrinkled and corrugated leaf surface, basally thickened fruiting pedicel and winged capsules.

**Type:** Greece, Kriti (Crete), District Chania, Agria Gramvousa islet, 35°38'49.8"N, 23°34'39.2"E, 60–70 m a.s.l., 27 Mar. 2021, rocky places among phrygana, Z. Kypriotakis, (holotype: NHMC43-13423; isotypes: herb. Kypriotakis, UPA).

#### Etymology

The species epithet is dedicated to Dr Christini Fournaraki, Mediterranean Agronomical Institute of Chania (CIHEAM-MAICh), a well-known botanist and fellow in fieldwork.

#### Description

Geophyte. Bulb spherical to sub-spherical 2.0–2.7 cm in diameter; bulb scales concrescent, circular in transection; side bulblets 1–3, oblong-ovoid; outer tunic membranous, pale brownish. Scape (stem length excluding the inflorescence) glabrous, 2–5 cm long; flowering plants 8–14(17) cm tall. Leaves 6–10, proteranthus, glabrous, diverging almost horizontally or arching (rarely), persistent at anthesis, linear-tapering, broadest below the middle, 8–25(45) cm long and 0.6–1.1(1.5) cm wide, concolorous shiny green or rarely with a very narrow white line about 1 mm wide, thin-papyraceous, flexible, flat or slightly canaliculate, with corrugated and somewhat wavy surfaces and very narrow, smooth, translucent margins. Inflorescence a lax, 6–12(18) cm long, corymbiform to subcylindrical raceme with 5–12(15) flowers.

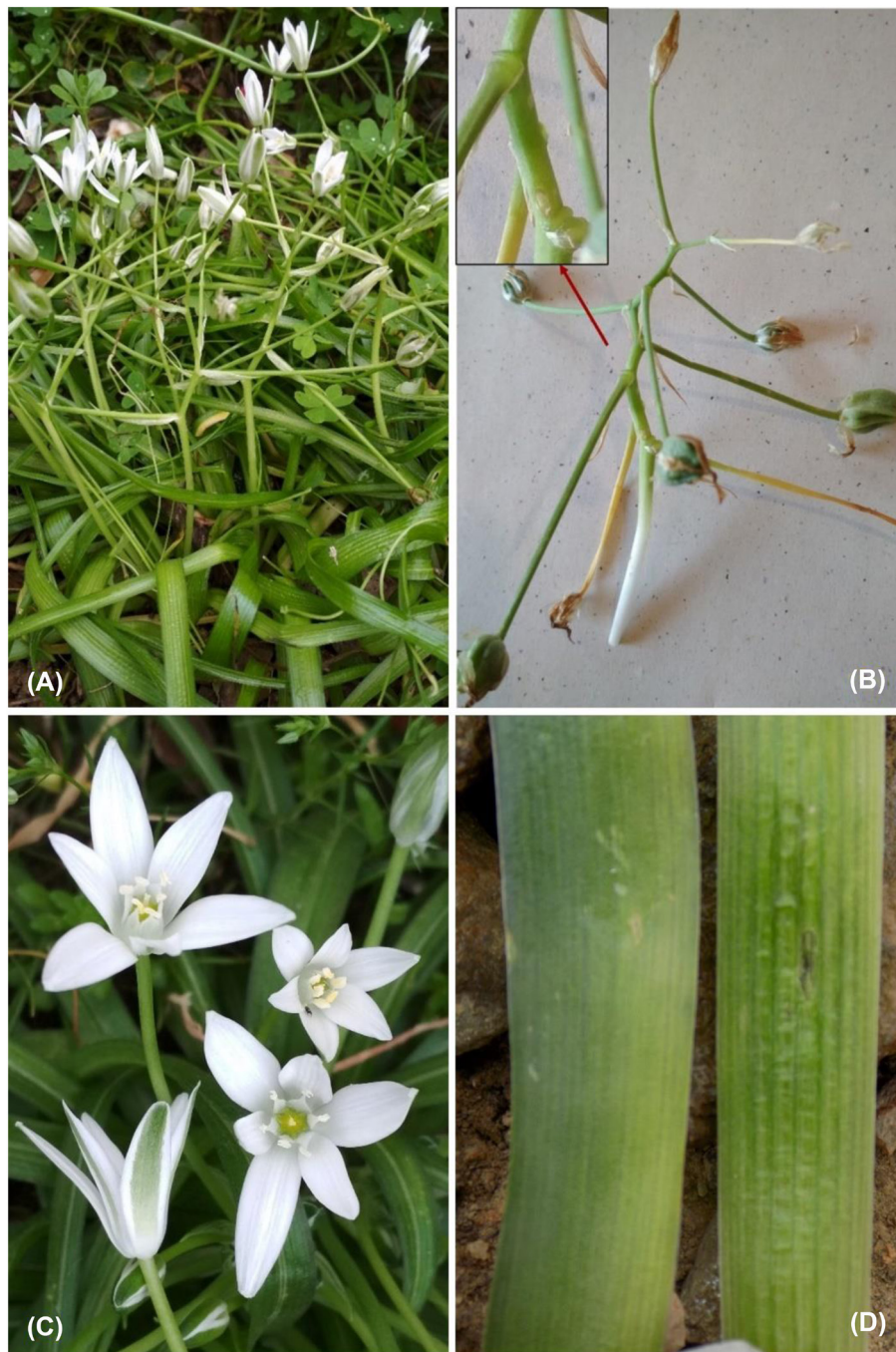


Figure 1. *Ornithogalum christinae* sp. nov. (Asparagaceae). (A) Plant habit at the stage of anthesis. (B) plant habit at the stage of fruit, (C) flower shape (apical and bottom view), (D) leaf surface structure showing the differences between *O. montanum* leaf (left) and *O. christinae* leaf (right).

Pedicels straight, 2–7 cm long, at first erecto-patent, becoming reflexed after anthesis, diverging up to an angle about 120°–130°, at fruiting stage elongating to about 20%, gradually thickening towards base and rachis of infructescence becoming flexuose (Fig. 1). Bracts membranous, whitish, lanceolate-acuminate 1.5–3.5 × 0.4–1.0 cm, 5–8-nerved and 0.3–0.5 times as long as the floral pedicels. Tepals white with a green stripe beneath, oblanceolate to narrowly elliptic, subacute at

apex, 5-nerved, patent, slightly canaliculate, 13.0–19.0(21.0) mm long × 3.6–5.6(6.0) mm wide, the outer three cucullate at apex, ending in a small sub-globose appendix, consisting of 50–80 small papillae; green stripe broad, occupying 4/5 of the outer tepal's width, while it occupies only the 1/4 of the three inner tepals. Ovary sub-cylindrical, depressed at apex, green with yellowish top, 4.0–5.0 × 2.0–3.5 mm; lobes contiguous in 3-pairs with 7–12 ovules per carpel. Style



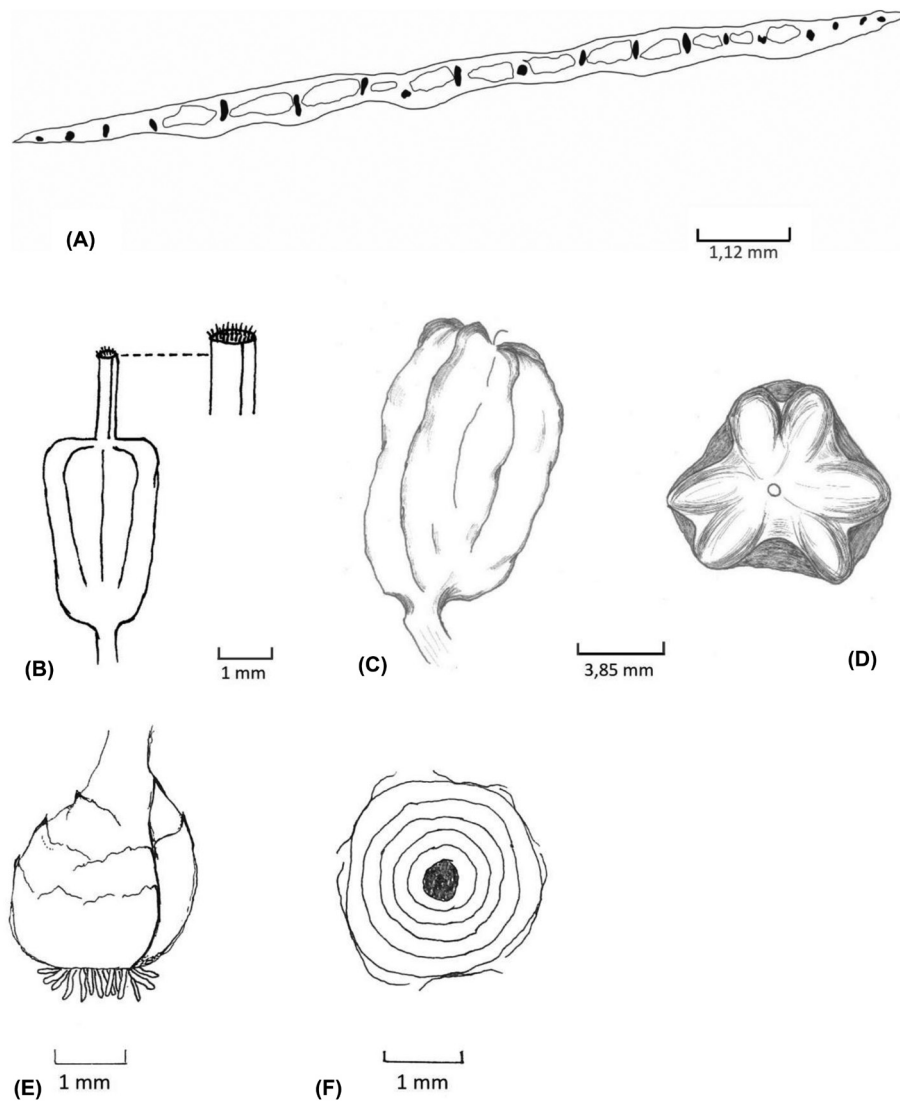


Figure 2. Diagnostic features of *Ornithogalum christinae* sp. nov. (A) Leaf cross-section with two types of vascular bundles, (B) ovary and style, (C, D) side and apical view of fruit capsule, (E) side view of bulb shape, (F) bulb cross-section.

white, 2.0–2.5(3.0) mm long, about 0.4–0.5 times as long as the ovary; stigma truncate, papillose. Filaments 6.5–8.5 × 1.5–2.5 mm, entire, oblong with parallel sides, abruptly contracted to a short filiform apex, on inner surface with three thickened and prominent nerves (Fig. 1). Anthers dorsifixed, dirty white, oblong 2.5–3.0 × 1.0–1.3 mm. Capsules oblong to ovoid 10–15(17) × 6–9 mm, emarginate at apex with apical lobes contiguous in three pairs and conspicuously winged (1–2 mm wide), when dry becoming coriaceous, yellowish-brown, venulose, minutely wrinkled and opening like a star. Seeds ovate to sub-globose, slightly apiculate 2.0–2.3 mm in diameter; testa black, prominently reticulate. Germination hypogeal.

#### Leaf morphology and anatomy

Leaf anatomy is an important vegetative character for species identification. Many specialists have studied the anatomy of

*Ornithogalum* leaves, such as Wittmann (1985), Lynch et al. (2006), Öztürk et al. (2014) and Bogdanović et al. (2020). Leaf cross-section of the new species revealed many and wide spaces with two types of vascular bundles: round bundles shaped like dots and oblong ones (Fig. 2A). Between those bundles, mucilaginous cells can be found interspersed with the large vascular bundles in immature leaves, but when mature, these cells are replaced by large lacunae. Similar leaf anatomy has been described for *O. montanum* by Peruzzi et al. (2007). However, there are 19 vascular bundles for a 9–10 mm leaf width in the new species, while in *O. montanum* there are 32 bundles, uniformly shaped (round) with much less and smaller spaces.

#### Phenology

*Ornithogalum christinae* flowers in early spring (end of February to end of March).

### Distribution and ecology

The new species is known only from the type locality, Agria Gramvousa islet, Kriti Island, Greece (Fig. 3). It grows on rocky, calcareous substrate among phrygana and several therophytes such as *Thymbra capitata*. It can also be found in shadowy places, hidden under bushes of *Pistacia lentiscus* L. where larger plants have been observed due to shady environment.

### Karyology

In all the material investigated, 20 chromosomes were counted in the metaphase plates. Combining chromosomes' size, centromeric position, and presence of nucleolar organizers on the chromosomes (SAT-chromosomes), a few chromosomes should be considered as markers in the chromosome complement of the new species. As these 'marker' chromosomes are always in pairs in the metaphase plates studied (Fig. 4), it is suggested that *Ornithogalum christiniaie* is a diploid species with  $2n = 2x = 20$  chromosomes and a haploid complement with  $x = 10$ . Considering the size and the morphology of the chromosomes, the new species does not belong to the bimodal karyotype category where other species of the

genus belong, i.e. with of a group of large and a group of evidently smaller chromosomes. According to our accounts, in a total mean average length of the haploid complement of  $54 \mu\text{m}$ , the mean length of the larger chromosome is  $9 \mu\text{m}$  (R-length = 16.90) and that of the smaller  $2.5 \mu\text{m}$  (R-length 7.61). However, all the remaining chromosomes of the haploid complement show intermediate values with only small differences (Table 1, Fig. 4). With regard to the chromosome morphology, in the haploid complement the anisobrachial chromosomes dominate, in fact only the largest chromosome (no. 1) is characterized by a centromere in a more or less median position (msm). All the other chromosomes have r-indices higher than 2 and are either submetacentrics (sm) or subtelocentrics (st) (Table 1). Some of them are clearly submetacentric and some other are clearly subtelocentric, a few others, however, show r-index values 3 and are not easily recognized and classified. One of the submetacentric chromosome pairs shows a secondary constriction in a median position of the shorter arm (SAT-chromosomes). A tentative karyotype formula of the species could be as follow:  $2n = 2x = 2\text{msm} + 2\text{sm}^{\text{SAT}} + 10\text{sm} + 6\text{st} = 20$ . In the case that the chromosomes are arranged according to their size, the



Figure 3. Geographic distribution of *Ornithogalum christiniaie* sp. nov. at the northwesternmost point of Crete. The star indicates the species population.

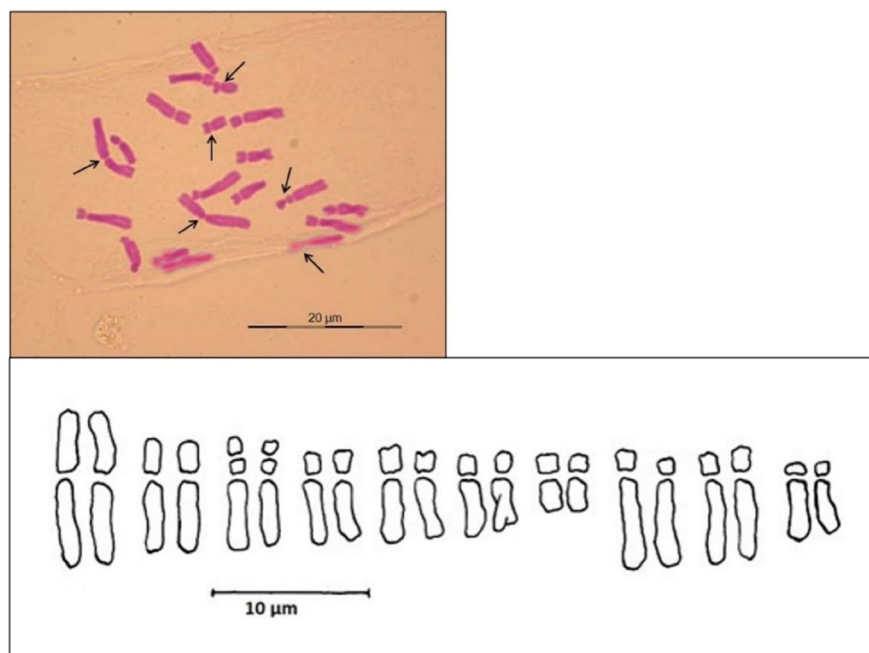


Figure 4. Metaphase plate and ideogram of *Ornithogalum christinae* sp. nov. Scale bar = 20 µm and 10 µm.

karyotype formula will be:  $2x = 2msm + 4st + 2sm^{SAT} + 6sm + 2st + 4sm = 20$ . Considering the karyotype diversity which characterizes the species of the genus *Ornithogalum* (Cullen and Ratter 1967) it is not easy to suggest a relationship of the new species among its relatives. *Ornithogalum pumilum* seems to be a diploid taxon with  $x = 7$  ( $2n = 14$ ) and for *O. atticum* a tetraploid chromosome number  $2n = 4x = 36$  is mentioned (Phitos 1980). Only in *O. montanum* plants and/or populations with  $2n = 20$  chromosomes (Phitos 1980) and  $2n = 16$  (Lungeanu 1972, Kožuharov et al. 1972) are reported, but rather as an exception since the most common chromosome number for the species is  $2n = 18$  (Cullen and Ratter 1967, Phitos 1980) and  $x = 9$  is considered as the basic chromosome number. It is to be pointed out, that the anisobrachial SAT-chromosomes observed in the new species are also present in both *O. montanum* and *O. atticum*. The same is also valid for the large chromosome with the median to submedian constriction. However, in the Greek populations

investigated by Phitos (1980), two chromosomes of this type were reported in the haploid complement of *O. montanum*.

#### Conservation status

*Ornithogalum christinae* is currently known only from the islet of Agria Gramvousa, NW of the Cretan area (Fig. 3). The islet is included in NATURA2000 site (GR4340001) and also in the protected area of the 'Plant Micro-Reserves' program (Fournaraki et al. 2004-2007), which both give indirect protection. The total occupied area does not exceed 10 km<sup>2</sup> and the total number of mature individuals is estimated to be ca 250. Regardless of its indirect protection, the plant is prone to accidental or deliberate grazing and trampling, hence in danger of population decline or even extinction in a very short time period. Therefore, according to the IUCN criteria (IUCN 2001), the species should be assessed as Endangered under D1 (number of mature individuals  $\leq 250$ ). Despite its small size, the island of Agria Gramvousa accommodates a remarkable flora, as it hosts eight south Aegean endemic plant species (Christodoulaki et al. 1991, Strid 2016a, b), emphasizing the importance of islets as evolutionary laboratories.

#### Similar species

*Ornithogalum christinae* shows some morphological similarities with *O. montanum* Cirillio, *O. pumilum* Zahar. and *O. atticum* Boiss. and Heldr., based on their leaf width with the absence of a white stripe, inflorescences longer than the scape and large seeds (Table 2).

However, *O. christinae* differs from *O. montanum* in having spherical to subspherical bulbs, wrinkled and corrugated leaf surface and fruiting pedicels thickened at base with flexuose rachis (Table 2).

Table 1. *Ornithogalum christinae* sp. nov. (Asparagaceae). Mean relative length (R-length) and arm ratio (r-index) values of the haploid chromosome complement. The SAT-chromosome is marked (\*).

Chromosome No	R-length	r-index	type
1	16.90	1.44	msm
2	11.07	2.07	sm
3*	10.61*	2.28*	sm*
4	8.28	3.00	sm
5	4.80	2.44	sm
6	11.32	6.00	st
7	11.00	3.30	st
8	9.55	3.12	st
9	8.90	5.44	st
10	7.60	3.28	st

Table 2. Main morphological differences between *Ornithogalum christinae* sp. nov. and *O. montanum*.

Character	<i>O. christinae</i>	<i>O. montanum</i>
Bulbs	spherical to subspherical 2.0–2.7 cm diam.	broadly ovoid 1.6–2.0 cm
Leaf shape	linear, shiny green 8.0–25.0 (–45.0) × 0.6–1.1 (1.5) cm	broadly linear to narrow elliptical, 10.0–25.0 × 0.4–2.0 cm, green or slightly glaucous
Leafcross section	wide spaces, less vascular bundles, different in shape (rounded and oblong)	no or small spaces, many and uniform in shape vascular bundles (rounded)
Leaf surface	wrinkled and corrugated	smooth, not wrinkled and corrugated
Pedicels	lower pedicels in fruit reflexed	pedicels in fruit, patent or ascending
Bracts	0.3–0.5 times as long as pedicels	0.5–0.8 times as long as pedicels
Tepals	green stripe broad, 4/5 as wide as outer tepal.	green stripe 2/3 as wide as outer tepal
Filaments	6.5–8.5 mm long, with parallel sides, abruptly contracted in short filiform apex	4.8–6.3 mm long, gradually contracted to a filiform apex
Style	0.4–0.5 times as long as ovary	0.6 times as long as ovary
Rachis of infructescens	flexuose and pedicels' base thickened	not the same
Capsules	oblong to ovoid, slightly oblique base, apical lobes contiguous in three pairs, winged	ovoid without oblique base, apical lobes equally spaced, not winged
Chromosome number	2n=20	2n=14, 18, 20, 22, 24 (2n=20, Landström 1989)

*Ornithogalum pumilum* differs from *O. christinae* in its narrowly ovoid bulbs, an inflorescence which is more or less on the ground, 10–15 mm long tepals and the chromosome number 2n = 14.

Lastly, *O. atticum* differs from *O. christinae* as the former has glaucous leaves, tepals 18–30 mm long, bracts equal to or exceeding pedicels, non-winged capsules, epigeal germination and the chromosome number 2n = 36 (Phitos 1980, Tan and Iatrou 2001).

## Conclusions

In conclusion, the genus *Ornithogalum* is an extremely difficult genus, due to its high variability and the difficulty of working on dry herbarium specimens. The few phylogenetic studies conducted have helped in some taxonomical clarifications (Pfosser and Speta 1999, Manning et al. 2009, Martínez-Azorín et al. 2011), but more research is still needed. *Ornithogalum christinae* is a distinct new species, with clear morphological differences and it can be regarded as a possible schizoendemic species, probably evolved as a result of geographical isolation.

## Key to the related species of *Ornithogalum christinae* sp. nov. in Crete

1. Small sized plants; leaves without a white stripe on upper surface; inflorescence close to the ground ..... 2
  - Larger plants; leaves without a white stripe on upper surface; inflorescence not close to the ground ..... 3
2. Dwarf plants; bulbs narrowly ovoid (diam. 1–4 cm); leaves (2) 3–5, broadest below the middle; inflorescence almost sitting on the ground; capsules unwinged ..... ***O. pumilum***
  - Dwarfish plants; bulbs ovoid (diam. 0.9–1.4 cm); leaves usually (1) 2–3, broadest above the middle; inflorescence with scape 4–6 cm; capsules strongly winged ..... ***O. oligophyllum***
3. Leaves glaucous, twisted; capsules truncate ..... 4

- Leaves green or scarcely glaucous, not twisted, suberect; capsules not truncate ..... 5
- 4. Leaves 8–22 mm wide; tepals 18–30 mm; seeds 3.0–3.5 mm ..... ***O. atticum***
  - Leaves 4–10 mm wide; tepals 13–20 mm; seeds 2.2–2.7 mm ..... ***O. oreoides***
- 5. Leaf surface wrinkled and corrugated; pedicels of lower fruits thickened at base and reflected; capsules winged with apical lobes contiguous in three pairs ..... ***O. christinae***
  - Leaf surface smooth and flat; pedicels not thickened at base and spreading; capsules unwinged with apical lobes equally spaced ..... ***O. montanum***

## Author contributions

**Zaharias Kypriotakis:** Conceptualization-Lead, Data curation-Equal, Investigation-Equal, Methodology-Equal, Supervision-Equal, Writing - original draft-Lead, Writing - review and editing-Equal. **Dimitrios Tzanoudakis:** Conceptualization-Equal, Investigation-Equal, Writing - review and editing-Equal. **Eleftheria Antaloudaki:** Data curation-Equal, Writing - original draft-Equal, Writing - review and editing-Equal

## Data availability statement

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.9kd51c5t8> (Kypriotakis et al. 2024).

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