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Autor: Øvstedal, D.O.

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A multivariate comparison between Ornithogalum chionophilum Holmboe, O. lanceolatum Labill. and O. montanum Cyr., based on chemometric and morphometric data

D. O. ØVSTEDAL

RÉSUMÉ

ØVSTEDAL, D. O. (1991). Comparaison diversifiée entre Ornithogalum chionophilum Holmboe, O. lanceolatum Labill. and O. montanum Cyr., basée sur des données chémo- et morphométriques. *Candollea* 46: 399-406. En anglais, résumés français et anglais.

L'analyse du composant principal montre qu'*Ornithogalum chionophilum* et *O. montanum* sont différents. Les caractères qui les différencient le mieux sont la longueur de l'ovaire et du style, la longueur des pédicelles inférieurs, la longueur des filets staminaux externes et la largeur de la marge hyaline des feuilles. Tous ces caractères se chevauchent. *O. lanceolatum* semble être intermédiaire entre les deux autres espèces, mais le nombre insuffisant des données exclut toute conclusion définitive. La largeur des feuilles et la courte hampe distinguent ce taxon des autres. La morphologie pollinique ne révèle aucune différence entre les trois espèces.

ABSTRACT

ØVSTEDAL, D. O. (1991). A multivariate comparison between Ornithogalum chionophilum Holmboe, O. lanceolatum Labill. and O. montanum Cyr., based on chemometric and morphometric data. *Candollea* 46: 399-406. In English, French and English abstracts.

A principal component analysis showed that *Ornithogalum chionophilum* and *O. montanum* were different. The characters which differentiated best were length of ovary and style, length of lowest pedicel, length of outer filament and width of hyaline margin of leaf. All characters appear to overlap. *O. lanceolatum* appears to be intermediate between the other two, but too few data prevent definite conclusions. Width of leaves and short scape distinguishes this taxon from the others. Pollen morphology showed no difference between the three species.

Introduction

The endemic *Ornithogalum chionophilum* is fairly abundant on the top of the mountain Kionistra (alt. ca. 1900 m) on Cyprus (HOLMBOE, 1914). According to MEIKLE (1985), the species is related to *O. cuspidatum* Bertol., *O. lanceolatum* Labill. and *O. montanum* Cyr. Stedje & Øvstedal (in prep.) found that *O. chionophilum* has a chromosome number which makes it rather improbable that it is closely related to *O. cuspidatum*. While *O. lanceolatum* and *O. montanum* are widespread and partly sympatric (CULLEN in DAVIS, 1984), *O. chionophilum* is geographically isolated from the two others, and the purpose of this paper is to elucidate whether it might be regarded as an outlying, isolated population of one of the two mentioned species.

The nomenclature of plants follows MEIKLE (1977, 1985).

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Releve nr.	1	2	3	4	5
Ornithogalum chionophilum	5	2	2	2	2
Ranunculus cadmius subsp. *cyprius*	15	5	—	10	—
Alyssum troodi	1	1	—	—	1
Crocus cyprius	1	1	1	1	1
Minuartia subtilis subsp. *filicaulis*	1	1	1	1	1
Viola heldreichiana	1	—	—	—	—
Thlapsi perfoliata subsp. ?	1	—	—	1	—
Corydalis rutifolia	1	1	1	1	1
Herniaria micrantha	—	1	1	1	1
Cephalorrhynchus cyprius	—	—	1	5	—
Veronica ixodes	1	1	1	1	1
Pebbles and stones	75	80	90	75	85
Bare soil	0	10	5	5	10

Table 1. — Snow-bed community with *Ornithogalum chionophilum,* top of Mt. Kionistra, Troodos, Cyprus. Releve size 1 m^2. Values given as percent cover.

Habitat of O. chionophilum

The top of Kionistra has more than 1000 mm of annual precipitation, and about 10 weeks of snow in winter (MEIKLE, 1977). After snow has melted in February-March, the leaves and inflorescence start developing, and it flowers in April-May. Bumblebees (*Bombus* spp.) are the most probable pollinators (own observations). The species is found in three major types of habitat.

1). Among boulders in small periodical streams. The streams are active in only a short period of time (when the snow is melting), and the species profits from the moisture.

2). On NE-sloping, sand-, gravel- and pebble-covered and late-melting areas (Table 1). These areas cover each only a few square meters, and are broken by areas of solid rock or small boulders. These late-melting areas have a characteristic spectre of life-forms. The life-forms are annuals *(Veronica ixodes, Viola heldreichiana, Minuartia subtilis* subsp. *filicaulis, Thlapsi perfoliata* and probably *Herniaria micrantha)* and bulbous plants *(Ornithogalum chionophilum, Crocus cyprius* and *Colchicum troodii). Herniaria micrantha* is unique in this company, with its decumbent growth-form.

3). Below the canopy of specimens of *Pinus nigra* subsp. *pallasiana* and *Juniperus foetidissima.* In such habitats there is a pronounced upbuilding of humus, and bulbous plants such as *Ornithogalum chionophilum* profit on that, as well as perennials such as *Orthurus heterocarpus.*

Material and methods

Bulbs from the three taxa were grown in the botanical garden of Bergen under identical conditions. Collection data are found in Table 2. Material for chemical analysis was taken from ca. 1 cm below the tip in the youngest leaf in mid May.

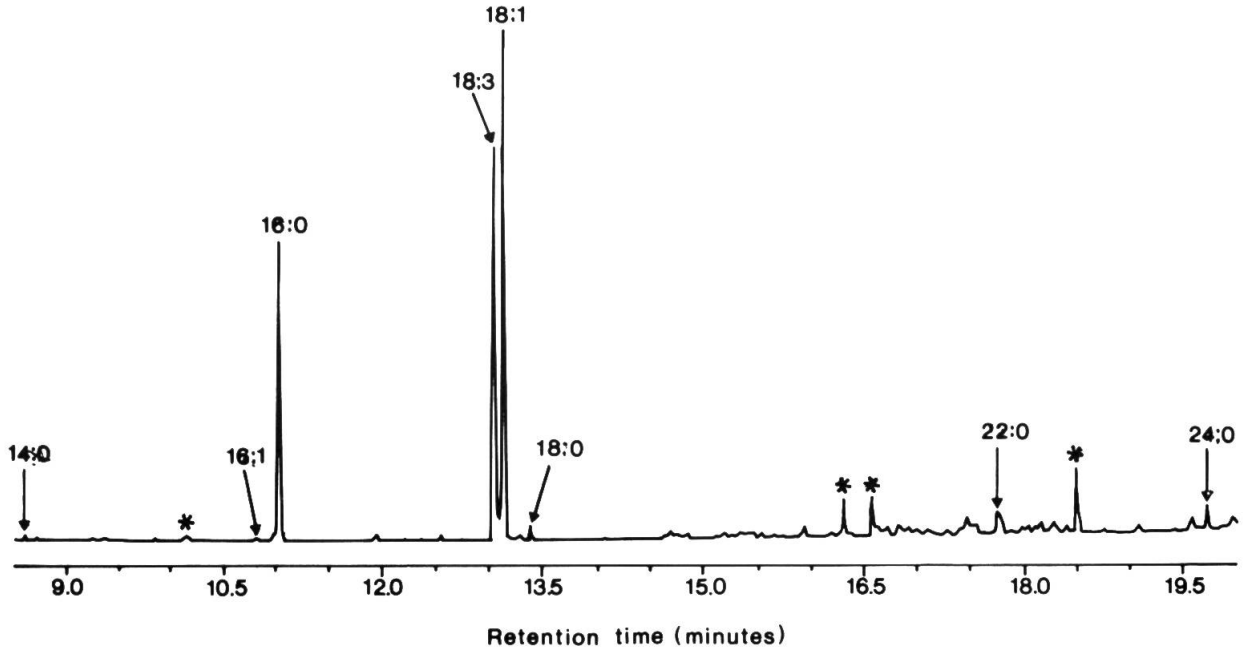
The methanolysis was performed on separate leaves in small glass ampoules by 0.5 ml anhydrous methanol containing 2 N HCl. After sealing the ampoules by melting, they are kept at 100°C for 15 hours. Thereafter the ampoules were broken open and $^3/_4$ of the methanol was evaporated under a stream of N_2 gas. One half ml of water was added to the remaining, dark brown methanolic solution. The mixture was extracted 3 times with 0.5 ml hexane by vigorous mixing on a whirl mixer. The coloured material remained in the methanol/water phase. The extracts, retrieved from the ampoule by a pipette, ware combined and evaporated by N_2 gas down to 0.2 ml. One microliter of the solution was chromatographed on a 25 m × 0.2 mm fused silica column with a stationary phase of 5% phenyl 95% methyl silicone of 0.33 micron thickness, and helium, at 1 ml/min, as carrier gas. The oven was programmed from 130°C to 280°C with 5°C/min. The chromatogram was digitalized, stored and integrated by a VG multichrome lab-data system.

The twelve peaks present in the chromatograms of all plants (Fig. 1), were selected for multivariate statistical treatment. This was performed by principal components analysis of the peak areas using the program SIMCA (ALBANO & al., 1987), available in the software-package SIRIUS

No. 1, *O. montanum*	Turkey, Adara District, Saimbeyli, Bozoglan Dag, above Saimbeyli, 1300 m, rocky south slope *P Davis 26691* (E acc. No. 644402).
No. 2, *O. montanum*	Italy, Sicily, Madonie Mts., 3 km N of Poli 33 i Generosa, *C. Cook 3* (E acc No. 612374).
No. 3, *O. montanum*	Turkey, Aydin, Karacasu, Aphrodiscas, alt. 600-700 m, *P. Davis 41614* (E acc No. 651455).
No. 4, *O. montanum*	Greece, Parnassus, above Arachova, alt. 1300 m, rocky limestone slopes facing north, *P. Davis 39388* (E acc No. 623145).
No. 5, *O. montanum*	Turkey, Bosphorus, Buyudere, in maquis, *Davis & Polunin 26001B* (E acc No. 734311).
No. 6, *O. montanum*	Turkey, Honazdag, alt. ca. 1300 m, under *Pinus nigra* etc. in NE position, *Pasche & Tauberheim s.n.* (E acc No. 772095).
No. 7, *O. montanum*	Turkey, Saimbeyli, Bozoglan Dag, above Saigeyli, 1300 m, rocky s. slope, *P. Davis 26691* (E acc No. 734138).
No. 8, *O. montanum*	Turkey, Prov. Bursa, alt. 1000 m, *T. R. Dudley 34729* (E acc No. 622322).
No. 9, *O. lanceolatum*	Turkey, Isauria, Kaldoken Dag above Anamur, 1500 m alt., turfy hollows where snow has lain, *Davis & Polunin 25895* (E acc No. 754196).
No. 9a, *O. lanceolatum*	Lebanon, *Polunin 9* (E acc. No. 593125).
No. 10, *O. chionophilum* ...	Cyprus, Troodos range, Kionistra, 1900 m, in open *Pinus nigra* subsp. *pallasiana* front, *Øvstedal 666a.*
No. 11, *O. chionophilum*	as 10, *Øvstedal 666b.*
No. 12, *O. chionophilum* ...	as 10, *Øvstedal 666c.*
No. 13, *O. chionophilum* ...	as 10, *Øvstedal 769.*

Table 2. — Localities for specimens used in the investigation. No. 9a is not used in the morphometic analysis. E acc no = accession number of Edinburgh Royal Botanic Garden.

Fig. 1. — Chromatogram of *Ornithogalum chionophilum.* The fatty acids have been designated by shorthand notation, e.g. 14:0, giving the number of carbon atoms before the colon, and the number of double bonds after the colon. Stars represent unidentified compounds used in the principal component analysis.



No. of specimens (see Table 2)	1	2	3	4	5	6	7	8	9	10	11	12	13
Number of flowers	7	9	17	17	11	14	3	8	18	12	11	11	12
Length of lowest pedicel	37	31	38	78	79	53	16	85	32	27	35	33	28
Length of uppermost pedicel	12	24	15	21	29	10	4	8	12	13	13	12	13
Length of lowest bract	23	20	26	22	24	42	9	25	20	24	22	22	25
Length of outer tepal	19	17	14	16	18	17	15	17	14	18	20	17	18
Width of outer tepal	5.0	4.7	6.5	5.3	5.0	6.5	5.2	6.3	4.6	5.5	5.4	5.5	5.5
Width of dorsal green band of outer tepal	3.0	2.8	4.5	3.5	2.8	3.7	2.5	3.8	2.8	2.5	2.5	2.5	2.5
Length of outer filament	6.5	6.0	6.0	5.8	7.0	5.2	5.2	6.5	5.4	5.4	5.6	5.5	5.4
Length of outer anther	1.5	1.3	1.5	1.5	1.8	2.0	2.5	1.5	2.6	1.4	1.5	1.6	1.6
Length of ovary + style	3.0	2.8	2.7	3.0	2.8	2.2	3.2	2.7	3.0	3.5	4.0	3.5	3.5
Width of hyaline margin of leaf	150	105	115	130	125	100	100	90	150	160	175	150	155
Width of longest leaf	9	—	12	—	—	—	9	7	21	9	8.5	9.5	10

Table 3. — Values of morphological characters used in the multivariate analysis (except width of longest leaf). Values are given as mm, except for the hyaline margin of the leaf which is given as µm.

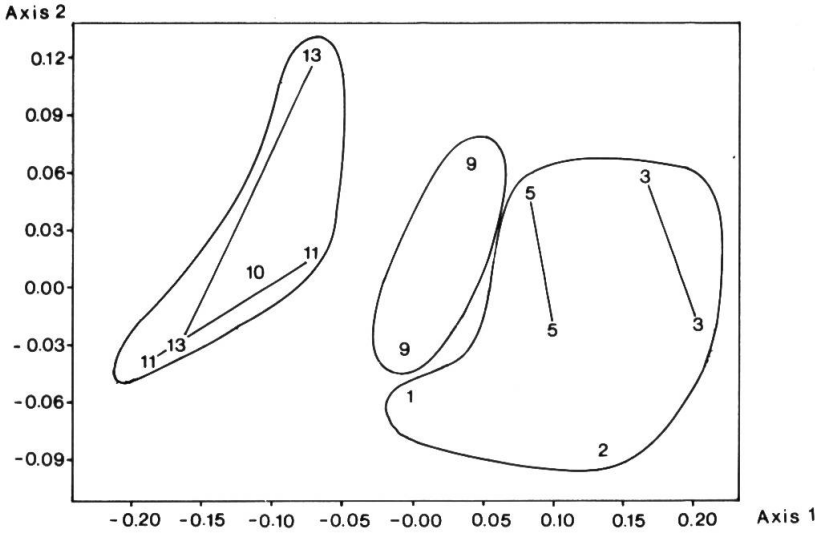


Fig. 2. — Plot of chemometric data from leaves of *Ornithogalum montanum* (1-5), *O. lanceolatum* (9), and *O. chionophilum* (10-13) againts the first two axes in a principal component analysis. The numbers denote localities (see Table 2). Straight lines connect samples from the same plant.

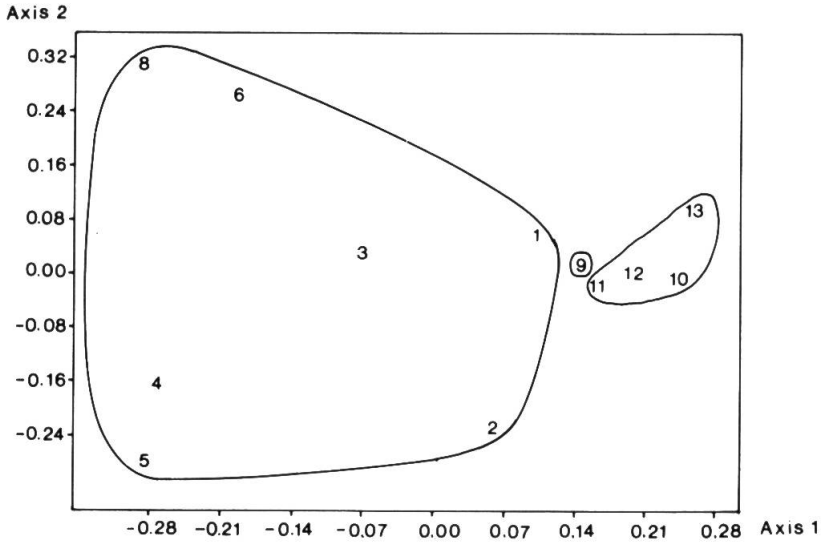


Fig. 3. — Plot of morphometric data of *Ornithogalum montanum* (1-8), *O. lanceolatum* (9) and *O. chionophilum* (10-13) against the two first axes in a principal component analysis. The numbers denote localities (see Table 2).

Photo 1. — *Ornithogalum montanum* (loc. 7), grown in the Botanical Garden of Bergen.
Photo 2. — *Ornithogalum chionophilum* on top of Kionistra, Cyprus, May 1989.

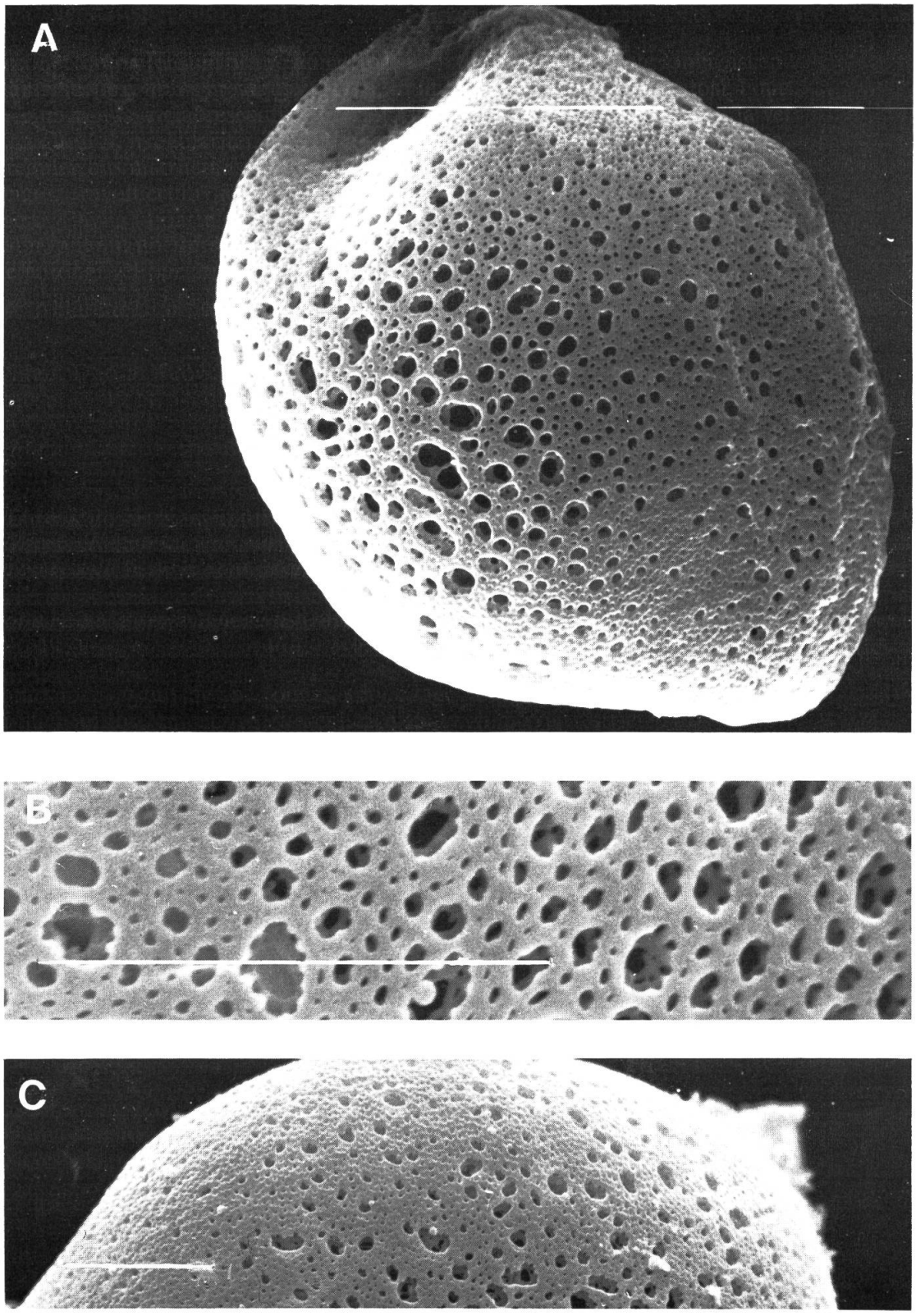


Fig. 4. — SEM pictures of *Ornithogalum montanum* (A), *O. lanceolatum* (B) and *O. chionophilum* (C). White bar = 5 µm.

(KVALHEIM & KARSTANG, 1987). The data were normalized after the average peak area for each sample. To prevent the largest peaks dominating the total variation in the data matrix, the data were scaled by taking the logarithm of the normalized numbers. The characters chosen for the morphometric analysis are found in Table 3. The SEM pictures were taken on a GEOL T 200.

Results and discussion

Five of the twelve labelled peaks in Fig. 1 represent straight chain, saturated fatty acids with even numbers, from 14 to 24, of carbon atoms. They have been identified by comparison with standards. In addition three peaks represent unsaturated fatty acids which have only tentatively been identified. The remaining peaks represent as yet undetermined compounds, but which according to the pretreatment procedure belong to the lipid fractation, and they have approximately the same number of carbon atoms as the identified compounds.

The result of the principal component analysis of the chemometric data are shown in Figure 2. Of the total variation in the data, 82% are in the direction of the two first components, with 68% along the first axis and 14% along the second. The results of the principal component analysis of the morphometric data are found in Figure 3. Of the total variation in the data, 75% are in the direction of the two first components, with 60% along the first axis and 15% along the second. The figures show that *O. chionophilum* and *O. montanum* are well separated in multivariate space, whether investigated by chemometry or morphometry. However, an inspection of the morphological data (Table 3) shows that it is difficult to find one character which absolutely distinguished between the two species. The only one which do not overlap in the present material is the length of ovary + style, which in *O. montanum* has a mean value of 2.8 mm and in *O. chionophilum* has a mean value of 3.6 mm. However, Zaharidani (in *Flora europaea* 5) reported a much larger variation in *O. montanum,* from 3 to 7 mm. Generally, in *O. chionophilum* the lowest pedicel is shorter, the leaves narrower, the length of the outer filaments shorter and the hyaline margin of the leaves broader than in *O. montanum. O. montanum* has a long scape and open inflorescense (Photo 1), while *O. chionophilum* has a shorter scape and more dense inflorescense (Photo 2). The few data on *O. lanceolatum* place it intermediate between *O. chionophilum* and *O. montanum* (Fig. 2 and 3), but do not permit a proper evaluation of its place in the multivariate space. The only flowering specimen investigated showed the characters used in keys in floras (*Flora europaea* 5, *Flora of Turkey* 8), viz. broader leaves and shorter scapes than *O. montanum.* However, these two characters were not used in the multivariate analysis due to incomplete data. The difference in flowering time between these two taxa may be responsible for keeping them apart, even if hybrids appear to occur in mixed populations in Turkey (CULLEN & RATTER, 1967).

O. montanum occurs from Turkey westwards to Italy and Sicily (*Flora europaea* 5), while *O. lanceolatum* occurs in Turkey, Syria and Lebanon. The material examined in this paper comprises *O. montanum* from Sicily, Greece (mainland and Aegean Islands), and Turkey, and *O. lanceolatum* from Turkey and Lebanon (Table 2), thus most of their distribution areas are covered. However, only one bulb from each locality has been grown, so that the insight in local variation is limited. In the present case, one might consider *O. chionophilum* as an isolated population of *O. montanum,* since there are no absolute differential characters, but as specimens of *O. montanum* from the whole distribution area make up a multivariate space different from that of *O. chionophilum,* it seems justified to maintain them as distinct species.

The pollen of the three species are prolate, with a heterobrochate and perreticulate exine. Number and size of lumina vary (Fig. 4), but no difference between the species could be found.

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Address of the author: Botanical Institute, Allégt. 41, N-5007 Bergen, Norway.