

## New cytological, morphological, and chorological data on *Prospero seisumsianum* (Rukšans & Zetterl.) Yıldırım (Asparagaceae) from the Zagros area

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**Abstract:** *Prospero seisumsianum* (Rukšans & Zetterl.) Yıldırım, a species recently described in southeastern Turkey, was recorded for the first time in the flora of Iraq based on samples collected from the Amadya and Duhok areas (northern Iraq). Our contribution here will be to find out more about its description, cytology, and chorology. The specimen collected from the Mesopotamian dry plain differs slightly from those collected from the mountainous Zagros forest area, but converges after a cultivation period. Both are treated here as *P. seisumsianum* and have the same genome size ( $2C = 8.77 \pm 0.24$  pg) corresponding to a diploid level. Three chromosome counts gave the same  $2n = 2x = 14$  karyotype. We hypothesize here that all the historical mentions of *P. "autumnale"* in Iraq actually correspond to *P. seisumsianum*. Comparative morphological description, genome size, ecological niche, and biogeography of the related taxa, and a revised key for *Prospero* taxa from the Irano-Anatolian enlarged to its Mediterranean border are given in the present study.

**Key words:** Taxonomy, cytology, karyology, *Prospero autumnale*, polyploid complex, flora of Iraq

### 1. Introduction

*Prospero* Salisb., described by Richard Salisbury in 1866, is a genus of bulbous flowering plants belonging to the family Asparagaceae. It contains 22 taxa, accepted or included in synonymy, distributed mostly around the Mediterranean basin and through the Levant to the Caucasus region (Speta, 1998, 2000; Govaerts et al., 2017). In spite of the fact that *Prospero* is an old generic concept, and after being rehabilitated and revised by Speta (1982, 1998), the acceptance of its generic level is still considerably debated by systematists (see Speta, 1998; Pfosser and Speta, 1999 versus Stedje, 1998, 2001; Valdès, 2004; Almeida da Silva et al., 2014). Furthermore, the traditional taxonomic definition of the complex genus *Scilla* sensu lato has been controversial due to its poor qualitative diagnostic characters and/or due to significant misinterpretation of its morphological variability. Thus, Speta (1998) and Pfosser and Speta (1999, 2004), after a morphological, cytological, and molecular phylogeny multiapproach, divided *Scilla* s.l. into many small genera, reassigned the complex group of *Scilla autumnalis* L. (among other species) to *Prospero*, and kept only in the genus *Scilla* s.s. the group of *Scilla bifolia*

L. (generic type of *Scilla* L.), which includes *Chionodoxa* Boiss.

In recent years, the number of species belonging to the genus *Prospero* has increased considerably based on morphological and cytological criteria. For example, Speta (2000) described six new species endemic to Crete and Greece; Brullo (2009) described one new species from Sicily; Yıldırım (2014) and Fırat and Yıldırım (2016) respectively reported one species and described another new one from Turkey. These new species described in the last few decades are usually narrow endemic (often with small populations) and they have different levels of polyploidy and/or controversial taxonomic delimitation. From an evolutionary standpoint, the *Prospero* species provide an excellent model system to study the trend of the diversification: this group of taxa exhibits (i) a particular pattern of polyploidy with important dysploidy (cf. Rothmaler, 1944; Speta, 1998; Hamouche et al., 2010); (ii) a contradictory geographical distribution pattern (often restricted endemic taxa while others have wide range areas); (iii) subtle morphological variations that need thorough observations (e.g., in cultivation); (iv) a specialization

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on hysteranthous autumn-flowering with one exception (*P. cudidaghense* Firat and Yildirim) and one derivative strategy (*P. seisumsianum*), both recently discovered (Rukšans, 2007; Yildirim, 2014; Firat and Yildirim, 2016). Furthermore, the evolution of *Prospero*, especially in the *P. autumnale* complex, has been driven by differentiation of an ancestral karyotype largely accompanied by discrete involvements of morphological changes (Jang et al., 2013; Emadzade et al., 2014).

According to *Flora of Iraq* (Wendelbo and Stuart, 1985), *Flora Iranica* (Rechinger and Wendelbo, 1990), and *Flora of Turkey* (Mordak, 1984), the genus *Prospero* was treated as a monospecific subgenus under the genus *Scilla*, the latter represented as a whole by 5 taxa for Iraqi territories (Wendelbo and Stuart, 1985). Northern Iraq, i.e. the southern side of the western Zagros, is a part of the Irano-Anatolian region comprising Iran and Turkey and considered a hotspot for biodiversity (Mittermeier et al., 2004) as well as a center of origin and diversity for diverse geophytes genus (Sheasby, 2007). However, it is a “coldspot” for floristic knowledge about the region: *Flora of Iraq*, the reference document at country level, is still incomplete (cf. Ghazanfar and McDaniel, 2016) and already outdated (cf. Townsend and Guest, 1966–1985), recurrently interrupted by the unstable political situation in Iraq. Therefore, for the last 5 years, diverse botanical field survey campaigns have been carried out to contribute to and update partially the knowledge about the flora of Iraq (e.g., Vela et al., 2013; Ahmad, 2013, 2016; Youssef et al., 2015, 2017).

During our botanical field surveys, two different types of *Prospero* specimens were collected in northern Iraq. Moreover, taxonomists currently recognize more and more new species in the Mediterranean territories, up to 8 species for Crete and also for neighboring countries such as Turkey with 3 species (Govaerts et al., 2017). The main aim of the present study was to add a new contribution to the flora of Iraq by (i) adding cytological data, taxonomic comments, laboratory iconographies, and field distribution data for the two observed morphotypes (ii) attributing a new identification key to the genus *Prospero* in the Irano-Anatolian and adjacent areas.

## 2. Materials and methods

During diverse botanical fieldwork (from 2012 to 2015, spring and autumn), in connection with other botanical surveys, particularly in the Amadya/Dêrelûk and Duhok/Mossul areas (northern Iraq), several individuals of *Prospero* specimens were collected in different places. Individuals collected from the field were cultivated first in Domiz (northern Iraq) and then in Montpellier (southeastern France). Some wild specimens were deposited in the herbarium of the Faculty of Agriculture at the University of Duhok (DPUH). Numerous specimens of *Prospero au-*

*tumnale* s.l. from France and neighboring countries were cultivated for comparison. Taxonomic identification was principally carried out on living specimens. Nomenclatural orthography followed the most recent *World Checklist of Selected Plant Families* for Asparagaceae (Govaerts et al., 2017).

Genome size was quantified by flow cytometry and ploidy levels were estimated on the basis of cytological references for the genus (Ebert et al., 1996; Jang et al., 2013). The total nuclear DNA amount was assessed by flow cytometry according to Marie and Brown (1993) for 13 *Prospero seisumsianum* individuals from 3 localities in Iraq. Additional taxa concerning diverse ploidy levels were assessed including i) 6 specimens of *Prospero cyrenaicum* (Pamp.) Speta (diploid: Bartolo et al., 1984) from the same locality in Libya, ii) 5 specimens of *Prospero autumnale* s.s. (tetraploid: Speta, 2010) from the same locality in sub-Mediterranean France, and iii) 6 specimens of *Prospero elisae* Speta (hexaploid: Speta, 1982; Ebert et al., 1996) from the same locality in Slovenia. The tomato *Lycopersicon esculentum* Mill. cv. “Montfavet” (2C = 1.99 pg), or *Petunia hybrida* E. Vilm.PxPc6 (2C = 2.85 pg) was used as an internal standard. For more details, see Fridlender et al. (2014).

The karyological study was carried out on three individuals from two localities in Iraq (see Table) and cultivated by us. Young root tips were pretreated in ice water at 1 °C for 24 h and fixed in a solution of absolute alcohol:glacial acetic acid (4:1, v/v) at 4 °C for 12 h. After 2 weeks at room temperature, the fixations were stored at –18 °C. Then the root tips were stained in 45% aceto-carmin-ferriacetate, boiled for 3 min, and squashed between slide and cover glass. Photomicrographs of five well-spread metaphases for each individual were taken with a Canon EOS 550D digital camera. The chromosomes analysis and the calculation of karyotype parameters were performed using KaryoType (Altınordu et al., 2016). For centromere position, the nomenclature given by Levan et al. (1964) was followed.

Specimens (flowered/fructified scapes and/or leafy bulbs) were deposited in the herbarium Errol Vela (incorporated into MPU) and the herbarium Sami Youssef (incorporated into DPUH).

Deposited material:

-*Prospero seisumsianum* (Rukšans & Zetterl.) Yildirim Iraq, Duhok (MAM): Amadiya, Sulav, 1440 m a.s.l., 24.09.2013, S. Youssef s.n. (DPUH: 3625, sub “*Scilla autumnalis*”); Iraq, Duhok (MAM): Dêrelûk, Merguê, 742 m a.s.l., 24.10.2015, S. Youssef s.n. (DPUH: 3626, sub “*Scilla autumnalis*”); Iraq, Duhok (MAM): Domiz, refugee camp, 425 m a.s.l., 04.01.2017, legit Ahmed Mahmood, S. Youssef s.n. (DPUH: 3627, sub “*Scilla autumnalis*”); Iraq, Duhok (MAM): Zawa foothill, Qassara, 525 m

**Table.** Genome size (total cellular DNA) of Iraqi and other reference *Prospero* species. For chromosome numbers, the counted specimens are followed by “!”.

Taxon	Sample/origin and number	2C (pg) ± SD	
	Pcyr_LY_2/1	10.39	
	Pcyr_LY_2/2	8.75	
	Pcyr_LY_2/3	8.75	
	Pcyr_LY_2/4	8.99	
	Pcyr_LY_2/5	9.12	
<i>Prospero cyrenaicum</i>	Al Kouf Nat. Park, Cyrenaica (Libya): n = 5	9.173 ± 0.615	2n = 2x ~ 14
	Paut_38_1/1	19.63	
	Paut_38_1/2	21.58	
	Paut_38_1/3	21.39	
	Paut_38_1/4	21.02	
	Paut_38_1/5	20.56	
<i>Prospero autumnale</i> s.s.	Crémieux, Isère (France): n = 5	20.836 ± 0.778	2n = 4x ~ 28
		26.27	
		25.46	
		25.23	
		29.24	
		25.87	
		27.86	
<i>Prospero elisae</i>	Gabrovica, legit J.M. Tison (Slovenia): n = 6	26.655 ± 1.572	2n = 6x ~ 42
	Pasl_IK_0	8.52	
<i>Prospero seisumsianum</i> “mountain”	Sulav, Duhok (Iraq): n = 1	(8.52)	2n = 2x ~ 14
	Psei_IK_0	9.25	2n = 2x = 14!
<i>Prospero seisumsianum</i> “mountain”	Merguê, Duhok (Iraq): n = 1	(9.25)	2n = 2x = 14
	Pass_IK_1/1	8.52	
	Pass_IK_1/2	8.74	
	Pass_IK_1/3	8.63	
	Pcfa_IK_1/1	8.75	
	Pcfa_IK_1/2	8.71	
	Pcfa_IK_1/3	failed	
	Pcfa_IK_1/4	8.41	
	Pcfa_IK_1/5	8.95	
	Pcfa_IK_1/6	9	
	Pcfa_IK_1/7	8.72	
	Pcfa_IK_1/8	9.11	
	Pcfa_IK_1/9	8.76	
	Psei_IK_0/1		failed
	Psei_IK_0/2		2n = 2x = 14!
	Psei_IK_0/3		2n = 2x = 14!
<i>Prospero seisumsianum</i> “lowland”	Domiz, Duhok (Iraq): n = 14	8.75 ± 0.20	2n = 2x ~ 14
<i>Prospero seisumsianum</i>	TOTAL Iraq (n = 14)	8.77 ± 0.24	2n = 2x = 14

a.s.l., 30.04.2017, legit Ahmed Mahmood, S. Youssef s.n. (DPUH: 3628, sub "*Scilla autumnalis*"); Iraq, Duhok (MAM): Amadiya, Sulav, 1440 m a.s.l., 08.2015 (in cultis Montpellier, France), *E. Véla* 0018 (MPU); Iraq, Duhok (MAM): Domiss, refugee camp, 425 m a.s.l., 29.09.2016 (in cultis Montpellier, France), *E. Véla* 0019 (MPU); Iraq, Duhok (MAM): Domiss, refugee camp, 425 m a.s.l., 08.01.2017, legit Ahmed Mahmood, *E. Véla* 0020 (MPU); Iraq, Duhok (MAM): Amadiya, Sulav, 1440 m a.s.l., 18.03.2017 (in cultis Montpellier, France), *E. Véla* 0021 (MPU); Iraq, Duhok (MAM): Domiss, refugee camp, 425 m a.s.l., 18.03.2017 (in cultis Montpellier, France), *E. Véla* 0022 (MPU); Iraq, Duhok (MAM): Domiss, refugee camp, 425 m a.s.l., 16.10.2017 (in cultis Montpellier, France), *E. Véla* 0023 (MPU); Iraq, Duhok (MAM): Dêrelûk, Merguê, 742 m a.s.l., 17.10.2017 (in cultis Montpellier, France), *E. Véla* 0024 (MPU).

### 3. Results

#### 3.1. Chorology

We found four localities hosting *Prospero* populations.

Two localities in the mountain/valley area are included in the "high folded zone" of the unstable shelf repulsed by the Arabian tectonic plate towards the Zagros suture zone. The geological substrate is dominated by Cretaceous massive calcareous bedrocks (Jassim and Goff, 2006).

-Sulav, Amadya: 37°07'09"N, 43°28'23"E; alt. 1440 m; more than 100 individuals; 24.09.2013; open oak forest steppe (*Quercus infectoria* Oliv., *Q. aegilops* Mill., *Crataegus azarolus* s.l., *Celtis tournefortii* Lam., *Juniperus oxycedrus* s.l.) species-rich in chamaephytes and geophytes.

-Merguê, Dêrelûk: 37°03'33"N, 43°41'01"E; alt. 782 m; around 50 individuals; 24.10.2015; open pistachio-oak forest steppe (*Pistacia khinjuk* Stocks, *P. eurycarpa* Yalt., *Q. aegilops*) species-rich in therophytes and geophytes.

The other two are close to one another and located on both sides of the border with the upper Mesopotamian plain/foothills (left riverside of Tiger), which correspond to the "foothill zone" of the unstable shelf. The geological substrate is dominated by Tertiary sediments like Mio-Pliocene molasses or Paleogene limestones (Jassim and Goff, 2006).

-Domiz (SW of the refugee camp): 36°46'16"N, 42°52'14"E; alt. 425 m; around 1000 individuals; 14.10.2014 and 04.01.2017; pseudosteppe grasslands species-rich in therophytes and geophytes.

-Qassara (above the village, southwestern slope of Zawa foothill): 36°50'08"N, 42°55'57"E; alt. 525 m; more than 200 individuals; 30.04.2017; pseudosteppe grasslands species-rich in therophytes and geophytes.

#### 3.2. Morphology

The vegetative morphology shows two distinct trends for *Prospero* plants observed and collected in the Duhok

governorate. The plants from the middle mountains and valleys with a forest-type soil have bigger bulbs than those from the Mesopotamian plains and foothills with a steppe-type soil (25–35 × 30–35 versus 12–24 × 12–21 mm) and leaves are more numerous (8–10 versus 3–6) and wider (2–4 versus 1–3 mm). Both have leaves spreading around December, at least 1 month later than the end of fruiting, and growing slowly until spring with a maximum length in April. The mountain plants have a scape relatively fragile and slender (1 mm at the base when fresh, more than 1.5 mm at maximum width) compared to the bulb robustness, and bear numerous (18–35) flowers. The lowland plants have an even more slender scape (less than 1 mm), with less numerous (9–12) flowers. Both show a cataphyll at the base of each stem, share flowers with a uniform bright pink color with pale pinkish ovary (except completely albino individuals), shortly pediculate (only 4–5 mm) and the pedicels are little or not growing during fruiting, and the fruits are 3-chambered capsules notched at the top and forming three distinct lobes (Figures 1 and 2). All these parameters are diagnostic of *P. seisumsianum* (but see discussion about cataphyll section 4.4.) as recently illustrated from the locus typicus in southeastern Turkey (Yıldırım, 2014). At this stage, the lowland plants fit perfectly with the description of typical *P. seisumsianum* from the Siirt area (Yıldırım, 2014) including for the vegetative quantitative characters, while the mountain plants belong also to *P. seisumsianum* for diagnostic criteria but with a quantitative variability of vegetative features.

#### 3.3. Cytology

*Prospero autumnale* s.s. shows a total amount of cellular DNA of 20.8 (min–max: 19.6–21.6) pg, while *P. cyrenaicum* shows 9.2 (8.8–10.4) pg and *P. elisae* 26.7 (25.2–29.2) pg. This original reference sampling confirms the presumed tetraploidy of *P. autumnale* s.s., the diploidy of *P. cyrenaicum*, and the hexaploidy of *P. elisae*.

The samples of *P. seisumsianum* from the steppe plains show a genome size very similar (8.41–9.11 pg), meaning they are also diploid (Table) and not tetraploid like *P. autumnale* s.s. Two chromosome counts for this locality revealed a diploid chromosome number  $2n = 14$ , which confirms and specifies the diploid level suggested by our genome size result. Chromosome lengths clearly show a bimodal karyotype with long chromosomes ranging from 5.6  $\mu\text{m}$  to 6.9  $\mu\text{m}$  (one submetacentric and one subtelocentric pairs) and shorter chromosomes ranging from 1.9  $\mu\text{m}$  to 3.5  $\mu\text{m}$  (two submetacentric and three metacentric pairs) (Figure 3). Some satellites have been detected. The mean values of karyotype parameters (Altınordu et al., 2016) are the following ones: THL =  $25.97 \pm 0.08$  (SD); CVCI =  $30.60 \pm 1.45$  (SD); CVCL =  $50.08 \pm 1.66$  (SD); MCA =  $28.59 \pm 2.1$  (SD).

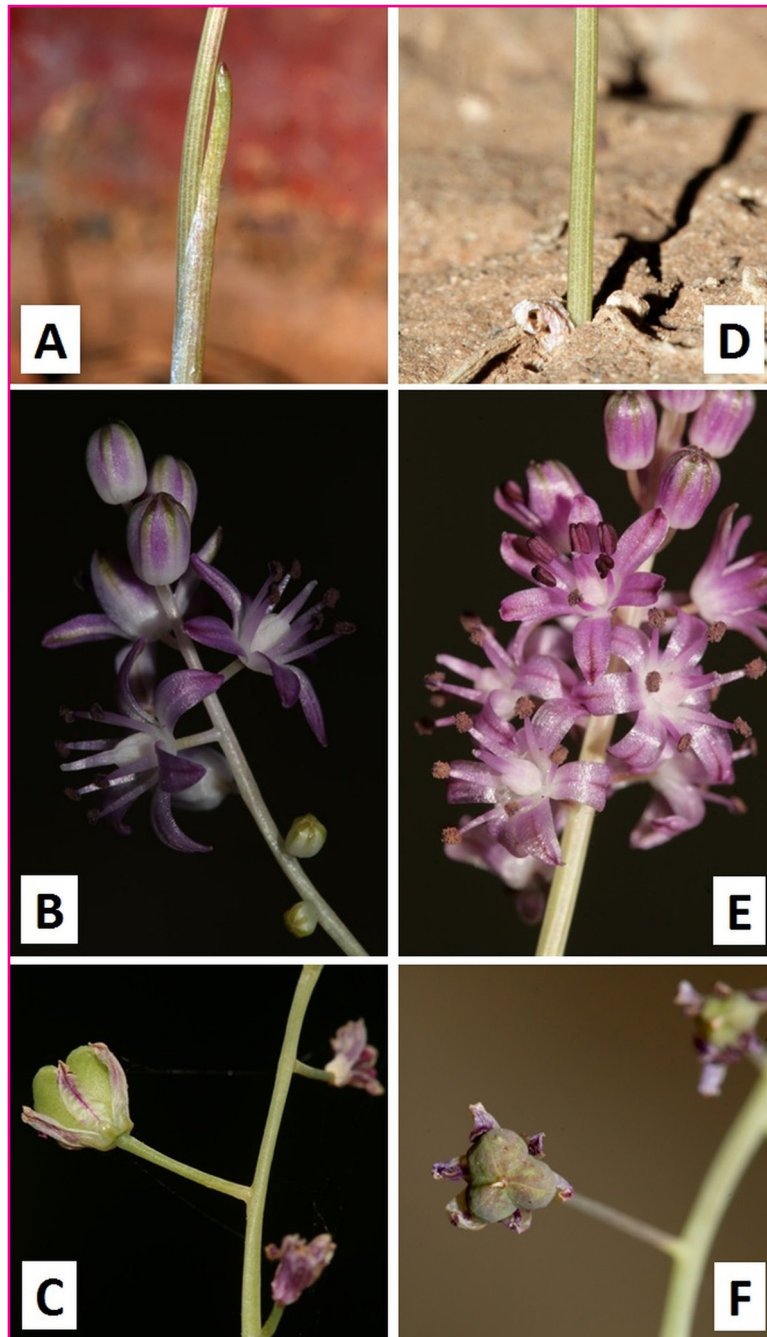


The two samples of *P. seisumsianum* from the mountainous valleys (Sulav and Merguê) show a genome size of respectively 8.52 and 9.25 pg, which means they are diploid individuals. The latter (Merguê) has been counted with  $2n = 14$  chromosomes and has a karyotype strongly conservative in comparison with the previous ones despite the morphological and ecogeographic differences.

#### 4. Discussion

##### 4.1. *Prospero seisumsianum* as a new record for the flora of Iraq

Since the winter of 2013/2014, and after several years of cultivation, both the *Prospero* specimen from the mountain area and those from the Mesopotamian plain remain morphologically strongly distinct from all the



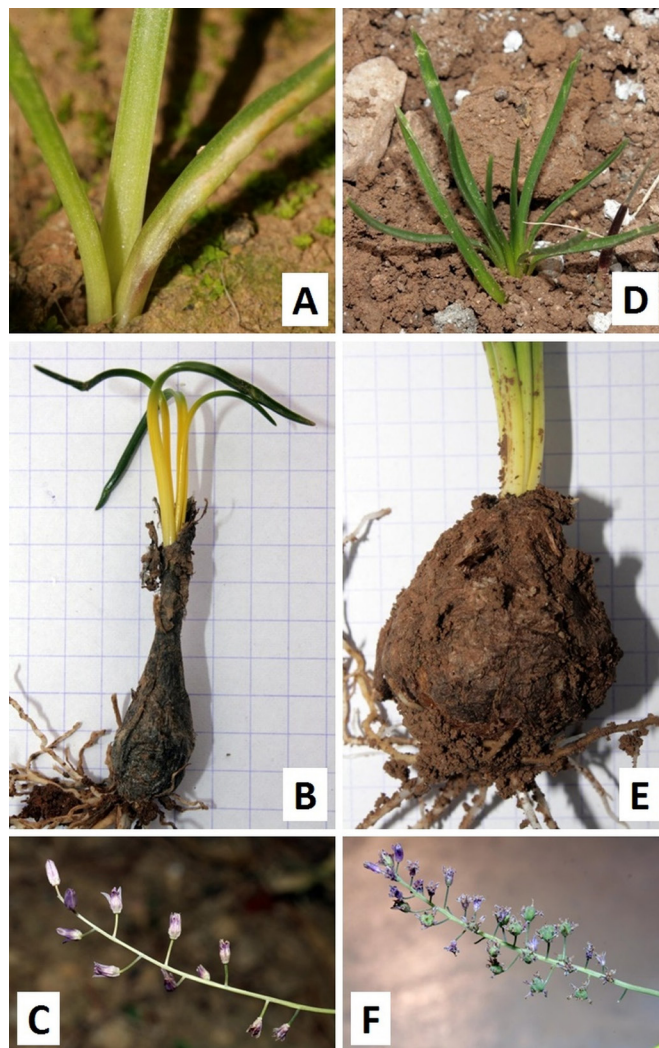
**Figure 1.** *Prospero seisumsianum* from Domiz (left column; A, B, C) and Sulav (right column; D, E, F), both in cultivation and at the same scale (each photo is 14-mm wide). From top to bottom: cataphyll (A: fresh versus D: dried), flowers (B and E), capsules (C and F).

Mediterranean and European known taxa (diploids and polyploids). However, *Flora of Iraq*, *Flora of Turkey*, and *Flora Iranica* are dealing with only one taxon under the name “*Scilla autumnalis*”. During this time, the recently described species “*Scilla seisumsiana*” from southeastern Turkey was recombined under *Prospero* and richly illustrated by Yildirim (2014). It was also easy to recognize it and thus enlarge its distribution area to the Iraqi side of the Zagros mountain chain, where it was consequently considered a new record for the country under this identification name.

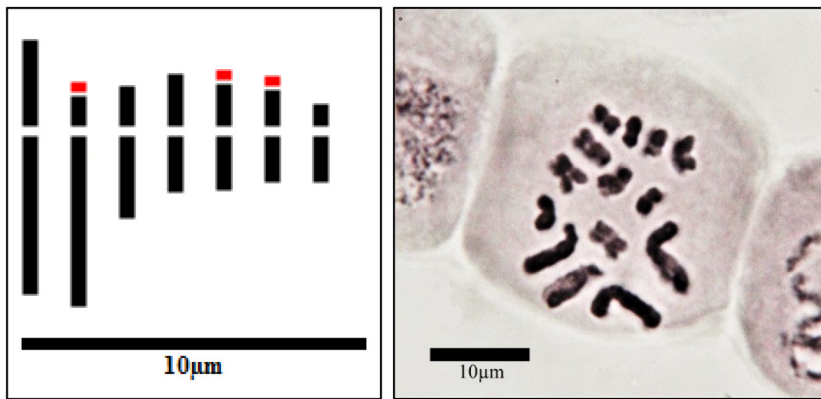
This finding is not surprising in view of both the high diversity of the Great Zab middle valley and the surrounding areas (Merguê, Sulav-Amadya, Gara mountain, etc.) and the biogeographic continuity between

both sides of the Turkish–Iraqi border. For example, four orchids previously known in southeastern Turkey were recently found as new for Iraq: *Ophrys cilicica* Schltr. (Véla et al., 2013), *Orchis spitzelii* subsp. *latiflora* B.Baumann & H.Baumann (Youssef et al., 2015), *Anacamptis papilionacea* subsp. *schirwanica* (Woronow) H.Kretzschmar, Eccarius & H.Dietr., and *Dactylorhiza romana* subsp. *georgica* (Klinge) Soó ex Renz & Taubenheim (Youssef et al., 2017). Inversely, *Allium calocephalum* Wendelbo, previously considered as endemic to northern Iraq (Townsend and Guest, 1985), was recently found as new for Turkey in the Hakkari area (Firat and Aziret, 2016).

Besides that, another new species, *Prospero cudidaghense* Firat & Yildirim, with extraordinary spring flowering, was discovered in southeastern Turkey (Cudi



**Figure 2.** *Prospero seisumsianum* from Domiz (left column; A, B, C) and Sulav (right column; D, E, F) both in cultivation. From top to bottom: leaves (A: spring close-up versus D: hibernial general overview), hibernial bulbs (B and E: grid on the sheet is 5 mm), autumnal inflorescence (C: anthesis versus F: fruiting).



**Figure 3.** Mean haploid idiogram of specimen Psei\_IK\_0, Psei\_IK\_0/2, and Psei\_IK\_0/3 (left) and microphotograph of a mitotic metaphase plate of specimen Psei\_IK\_0/3 (right) of *Prospero seisumsianum*:  $2n = 14$ .

mountain) near the Iraqi–Syrian border and has to be searched for in Iraq, especially around the Zakho and Sinjar areas.

#### 4.2. *Prospero seisumsianum* as a new diploid species at eastern limits

Recently, *Prospero* taxa have been studied well for their chromosomal diversification and evolution accompanied by little morphological variations (Jang et al., 2013). The new diploid status of *P. seisumsianum* from the western Zagros area extends the eastern geographical distribution range limits of diploid species and  $2n = 14$  cytotypes of the genus *Prospero*. This result highlights the important contribution of cytological data in the delimitation of the new species, which are mostly diploid species found in restricted areas. In this circumstance, all diploid *Prospero* taxa found on Zagros mountain and previously treated under the complex taxon “*P. autumnale* s.l.” according to the floras of the Levant countries (e.g., Turkey, Iraq, Iran) are more probably *P. seisumsianum*, including the eastern limit known from the bibliography at Khanaqin very close to the Iranian–Iraqi border (Rechinger and Wendelbo, 1990). Actually, the real *P. autumnale* s.s. occurring in southern and western Europe is tetraploid (Speta, 2010), but some Mediterranean Turkish and Israeli/Palestinian populations, which are morphologically similar, are known to be diploids (Battaglia, 1964).

#### 4.3. Two ecotypes or ecomorphosis from the same taxonomical entity?

Between Duhok and Nineveh/Mossul, our cytological and morphological analyses showed that the local *Prospero* has the typical appearance of *Prospero seisumsianum* from southeastern Turkey (the Siirt area) and is ecologically characteristic from the Mediterranean–Mesopotamian moist steppe area. Between Duhok and Soran, our

preliminary observations had suggested that the local *Prospero* could have a distinct morphological appearance because of their robustness (bulb, leaves, and stem). However, after 3 years of experimental cultivation in the same poor and dry soil from Mesopotamia, big bulbs of robust plants originating from Sulav, where they grow on rich forest soil, became slender and similar to those of the Domiz population. That is why we came to the conclusion that slender and robust plants from diverse ecological habitats were only ecomorphoses of the same taxonomic entity until evidence to the contrary becomes apparent. At this time, we have deeply analyzed only three individuals from two localities that showed no cytological difference. That is why we cannot make conclusions regarding ecotypic specialization or varietal taxonomic rank yet. Indeed, regardless of their robustness variation, the same diploid status excludes the hypothesis of potential autopolyploidy.

The whole morphological features exclude the other diploid *Prospero* known in the eastern Mediterranean as *Prospero hanburyi* (Baker) Speta from the Levant area (Syria, Lebanon, Israel/Palestine, Sinai) which differs by its long pedicles, growing after the flowering period (Mouterde, 1966). *Prospero autumnale* s.l. from Mediterranean Turkey (Firat and Yıldırım, 2016), where diploids are known (Ebert et al., 1996), differs from our diploids at least by its blue ovary (not pink) and the rapid development of the leaves just at the end of the flowering period, like for the tetraploid ones from Mediterranean Europe (Tison and De Foucault, 2014).

#### 4.4. The presence of cataphyll

About the cataphyll, the small hyaline bract situated at the base of the young flowering stem, Yıldırım (2014) wrote “*Prospero seisumsiana* [sic!] is related to *P. autumnale* [...]. It differs from *P. autumnale* by [...] cataphyll absent



(not frequently present) [...]”. In cultivation, we have not observed this surprising point, which moreover would seem inexplicable if it was maintained. As a matter of fact, in all specimens of hysteranthous autumn-flowering *Prospero* that we have cultivated, each flowering stem always emerges from a cataphyll. Consequently, if the bulb produces one stem, there is one cataphyll, if it produces two successive stems, there are two successive cataphylls, and so on. Generally, the cataphyll is fresh when the stem is just sprouting, but it withers rapidly during the inflorescence anthesis, and withers faster when the soil is not watered. It is the same phenomenon in wild specimens growing in the field, but depending on the microtopography and the competing vegetation, it is sometimes difficult to see the cataphyll, which can be very ephemeral and/or partially below the ground level or masked by dry herb litter. Considering this, we assume that *Prospero seisumsianum* is a typical hysteranthous autumn-flowering *Prospero* with one cataphyll at the base of each flowering stem. Contrariwise, the absence of cataphyll reported also for *P. cudidaghense* (Firat and Yildirim, 2016) is likely to be accurate and related to the synanthous spring-flowering characteristic of this peculiar species, unique within the genus.

#### 4.5. A comprehensive taxonomical key for *Prospero* in the Irano-Anatolian area

Hereafter, we provide a new comprehensive identification key for all the Irano-Anatolian species of *Prospero* plus the European-Mediterranean *P. autumnale* s.s.

A – Flowers in spring, after the complete development of numerous (8–16) leaves .....

..... *P. cudidaghense* [endemic to southeastern Turkey?]

A' – Flowers in autumn, before the emergence of few (3–10) leaves .....

B  
B – Pedicels briefly or not expanding after flowering (less than 5 times longer than perianth or capsule); perianth normally more or less pinkish-purplish (except abnormal albino individuals) .....

C  
B – Pedicels expanding quickly and long after flowering (more than 6 times longer than perianth or capsule); perianth mainly whitish with greenish-brownish dorsal veins.....

*P. hanburyi* [endemic to the Anti-Liban/Jordan river area]

C – Blue ovary (contrasting with the pinkish tepals), subglobulose capsule, leaves appearing just after flowering during the fruiting phase and growing rapidly at the maximum stage (before winter), scape more or less robust at the base (1–1.5 mm diameter), typically tetraploid ( $2n = 28$ ) in Europe but also diploid ( $2n = 14$ ) in the Mediterranean.....

*P. autumnale* s.l. [absent from the Irano-Anatolian]

C' – Pink/pinkish ovary (same color trend but lighter than tepals), 3-lobed notched capsule, leaves appearing after the

completion of flowering and fruiting, growing secondarily (during spring) after a nongrowth phase, scape slender at the base (1 mm diameter or less), diploid ( $2n = 14$ ).....  
*P. seisumsianum* [endemic to the southern side of Zagros mountains]

#### 4.6. Perspectives

These botanical field works limited to a small area (Duhok governorate) resulted in one new identification of the Iraqi *Prospero* species, reinforcing the status of the western Zagros mountain area (northern Iraq) as a part of hotspot for biodiversity but “coldspot for knowledge” (Véla, 2018). In this context, the results of this study may stimulate scientific researchers and naturalists to do more botanical field surveys, which in turn will update the knowledge about the flora of Iraq. Furthermore, these kinds of discoveries should be completed by an integrative approach based on morphology, cytology, and phylogeny in the future.

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