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## Karyotype asymmetry in some Scilloideae (Hyacinthaceae) members from Algeria

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**Abstract.** This cytogenetic study attempts to shed light on the karyomorphological and asymmetry data of species in the subfamily Scilloideae previously included in *Scilla*, namely *Hyacinthoides lingulata* (Poir.) Rothm., *Prospero autumnale* (L.) Speta, *Prospero obtusifolium* (Poir.) Speta, *Barnardia numidica* (Poir.) Speta, and *Oncostema elongata* (Parl.) Speta. These taxa are predominantly from the Skikda region (north-eastern Algeria). *H. lingulata* had a somatic chromosome number  $2n=2x=16$ , *P. autumnale*  $2n=2x=14$ , *P. obtusifolium*  $2n=2x=8$ , *B. numidica*  $2n=2x=18$ , and *O. elongata*  $2n=2x=16$ . The indices of intrachromosomal ( $M_{CA}$ ,  $A_1$ , and  $AsI$ ) and interchromosomal ( $CV_{CL}$ ,  $CV_{CI}$ , and  $A_2$ ) asymmetry revealed that *H. lingulata* has the most asymmetrical karyotype (1C), while *P. autumnale* has the most symmetrical karyotype (3A). *P. obtusifolium* has a relatively symmetrical karyotype (4A), while *B. numidica* and *O. elongata* have both an asymmetrical karyotype (1B). These findings differ from those previously reported for the same taxa in Algeria, hence indicating the substantial genetic variation that exists within the country.

**Keywords:** chromosomes, bulbous, plants, genetic diversity, *Scilla*.

### INTRODUCTION

The Hyacinthaceae family comprises exclusively herbaceous species, most of which are bulbous. In phylogenetic classification, this family is now included in the Asparagaceae (APG IV 2016), and its genera fall under the subfamily Scilloideae. However, the nomenclature of its genera is still widely controversial. The species that belong to the former genus *Scilla* have also been split among several genera (Pfosser and Speta 1999). Three of them are related to the autumn squills of the North African flora, namely *Prospero*, *Hyacinthoides*, and *Barnardia*. The Hyacinthaceae family has a wide distribution in Algeria, where it occurs with a certain concentration in the Tell Atlas

region (Maire 1958; Quézel and Santa 1962). However, this family also includes endemic species shared between Algeria and neighboring countries, such as *Albuca amoena* (Batt.) J.C.Manning & Goldblatt, *Ornithogalum sessiliflorum* Desf. (both endemic to Morocco and Algeria), *Hyacinthoides aristidis* (Coss.) Rothm. (endemic to Algeria and Tunisia), and *H. lingulata* (Poir.) Rothm. (endemic to Morocco, Algeria, and Tunisia).

The cytogenetic research carried out by Hamouche *et al.* (2010), Véla and de Bélair (2016), and Azizi *et al.* (2016a), has shed light on the karyotypes of various species that are proliferating in Algeria. However, taking into account the variety of Algeria's ecosystems, it's possible that the genetic diversity found within Algeria's populations is much greater than what was earlier thought. Previous research has shown that the genus *Prospero* has a significant amount of polymorphism, both in terms of the ploidy level and the fundamental chromosomal number ( $2n=14$ ,  $2n=28$ , and  $2n=42$ ) (Hamouche *et al.* 2006; Hamouche *et al.* 2010; Jang *et al.* 2013; Jang *et al.* 2018). Two cytotypes,  $2n=8$  and  $2n=16$ , have been found for *H. lingulata*, which primarily proliferates in diverse biotopes in the northwestern coastal region of Algeria (Hamouche *et al.* 2006; Hamouche *et al.* 2010). This underscores the necessity to extend and vary the sample regions in order to cover the widest possible range of ecosystems.

In light of this, we carried out cytogenetic research on some species of the Hyacinthaceae that are thriving in the Skikda area (northeastern Algeria), which have not been previously investigated. The following species were considered for the cytogenetic analyses: *H. lingulata* (Poir.) Rothm., *P. autumnale* (L.) Speta, *P. obtusifolium* (Poir.) Speta, *B. numidica* (Poir.) Speta, and *O. elongata* (Parl.) Speta. This study will allow us, on the one hand, to learn about the chromosomal composition of the studied taxa, and on the other hand, to shed light on the genetic diversity and karyological variation that may exist within Algeria's populations.

## MATERIAL AND METHODS

The plants selected for cytogenetic analysis were collected from their natural habitat (Figure 1) and identified using the flora of North Africa (Maire, 1958), the GDB herbarium (<https://gdbelair.com/>), and confirmed Algerian occurrences documented on iNaturalist ([https://www.inaturalist.org/observations?place\\_id=7300](https://www.inaturalist.org/observations?place_id=7300)). The root tips were obtained from plants collected in nature (Table 1). Seed germination was not successful. After receiving a pretreatment with 0.05% colchicine,

the roots were subsequently fixed in a combination of ethanol and acetic acid (3:1). Roots were then placed in a solution of hydrochloric acid (HCl 1N) and heated to 60 °C. This step lasted for 10 min. Following that, they were stained for at least 1h with Schiff's reagent, and lastly, they were crushed in a drop of 3% acetic carmine. At least 10 plates per each population were studied. A series of photographs were taken of the best plates, and then different measures were carried out to determine the karyotype of each species.

The chromosomal type was determined based on the terminology used by Levan *et al.* (1964). The karyotypes were accurately categorized using the Stebbins (1971) method. Different approaches were used to estimate additional parameters of karyotype asymmetry. HCL: Haploid total length, TF: Total form percentage (Huzi-wara 1962), AsI: Karyotype asymmetry index (Arano and Saito 1980), Syi: Karyotype asymmetry index, Rec: Chromosome size similarity index (Greihuber and Speta 1976), CV<sub>CL</sub>: Coefficient of variation in chromosome length, CV<sub>CI</sub>: Coefficient of variation in centromeric index, AI: Asymmetry index (Paszko 2006), A<sub>1</sub>: Intrachromosomal asymmetry, A<sub>2</sub>: Interchromosomal asymmetry (Romero Zarco 1986), M<sub>CA</sub>: Mean centromeric asymmetry (Peruzzi and Eroğlu 2013). The Pearson correlation between several asymmetry indices was calculated using IBM SPSS Statistics 24 software. Additionally, the ideogram for each species was determined depending on their chromosomal size.

## RESULTS

The karyotypes of five species from the subfamily Scilloideae have been thoroughly examined. Originally classified as *Scilla* by Maire (1958), these species have recently been reassigned to four distinct genera. Table 2 provides details on chromosomal numbers, total chromosome length, the ratio of long to short arms, and the total size of the haploid complement. *H. lingulata*, *P. autumnale*, *P. obtusifolium*, *B. numidica*, and *O. elongata* are all diploids with respective chromosome counts of 16, 14, 8, 18, and 16.

The specific karyotypic formulas for each species are further documented in Table 2. The karyotypes of these species consist mostly of metacentric, submetacentric, subtelocentric, and even telocentric chromosomes, particularly in the case of *H. lingulata* and *B. numidica*. The size of the chromosomes in *B. numidica* and *H. lingulata* varied from 1.27  $\mu\text{m}$  to 6.49  $\mu\text{m}$  depending on the species. *H. lingulata* stands out for having the largest haploid complement, which has an average size of 29.80



**Figure 1.** Illustration of the studied taxa showing different parts of each plant: bulbs, flowers, and capsules, A: *Hyacinthoides lingulata*, B: *Prospero autumnale*, C: *P. obtusifolium*, D: *Barnardia numidica*, E: *Oncostema elongata*.

**Table 1.** Localities of the studied taxa.

Taxa	Locality	Habitat	Latitudes	Longitudes	Altitude	Month of collection
<i>Hyacinthoides lingulata</i>	Larbi Ben M'Hidi	Coastal dune	36°53'08"N	7°00'55"E	40 m	September
<i>Prospero autumnale</i>	Larbi Ben M'Hidi	Coastal dune	36°53'08"N	7°08'01"E	40 m	September
<i>Prospero obtusifolium</i>	Ramdan Djamel	Olive grove	36°45'18"N	6°54'40"E	90 m	December
<i>Barnardia numidica</i>	Filfilla	Rocky cliffs	36°53'01"N	7°00'55"E	340 m	November
<i>Oncostema elongata</i>	Ramdan Djamel	Olive grove	36°45'18"N	6°54'40"E	90 m	January

$\mu\text{m}$ . This size exceeds that of the other species. Following closely is *O. elongata*, which possesses a total haploid complement size of 28.28  $\mu\text{m}$ . *B. numidica* possesses chromosomes that are very tiny in size. However, its haploid complement measures 21.64  $\mu\text{m}$ , which is larger than that of *P. obtusifolium* (16.60  $\mu\text{m}$ ) and *P. autumnale* (14.85  $\mu\text{m}$ ), as shown in Table 2.

In order to assess the level of asymmetry in the karyotypes of the studied species, we used Stebbins' categorization together with the  $CV_{CL}$  and  $M_{CA}$  values. The specific asymmetry indexes used are outlined in Table 3. *H. lingulata* has much more prominent asymmetry characteristics compared to other species. The karyotype of this species is categorized as IC according to Stebbins'

**Table 2.** Karyotype features of the studied taxa.

Taxa	2n	SC-LC ( $\mu\text{m}$ )	LC/SC	p ( $\mu\text{m}$ )	q ( $\mu\text{m}$ )	CL ( $\mu\text{m}$ )	HCL	CI (min-max)	KF
<i>Hyacinthoides lingulata</i>	16	1.54-6.49	4.21	0.75 $\pm$ 0.1	2.97 $\pm$ 0.17	3.72 $\pm$ 0.26	29.80	0.05-0.44	1m+2sm+3st+2t
<i>Prospero autumnale</i>	14	1.52-2.66	1.75	0.61 $\pm$ 0.1	1.5 $\pm$ 0.10	2.11 $\pm$ 0.12	14.85	0.16-0.41	1m+m <sup>sat</sup> +3sm+2st
<i>Prospero obtusifolium</i>	8	3.17-4.82	1.52	1.08 $\pm$ 0.2	3.04 $\pm$ 0.1	4.14 $\pm$ 0.22	16.16	0.21-0.32	2sm+2st
<i>Barnardia numidica</i>	18	1.27-3.97	3.12	0.56 $\pm$ 0.1	1.83 $\pm$ 0.11	2.39 $\pm$ 0.17	21.64	0.05-0.46	3m+2sm+3st+1t
<i>Oncostema elongata</i>	16	1.79-5.38	3.00	0.85 $\pm$ 0.06	2.66 $\pm$ 0.12	3.51 $\pm$ 0.17	28.28	0.13-0.40	2m+1sm+5st

SC: shortest chromosome, LC: longest chromosome, p: mean short arm, q: mean long arm, CL: mean total chromosome length, HCL: total haploid chromosome length, CI: centromeric index, KF: karyotype formula, m: metacentric, sm: submetacentric, st: subtelocentric, t: telocentric.

**Table 3.** Asymmetry indices of the studies taxa.

Taxa	TF	AsI	Syi	Rec	CV <sub>CL</sub>	CV <sub>CI</sub>	AI	A <sub>1</sub>	A <sub>2</sub>	M <sub>CA</sub>	S
<i>Hyacinthoides lingulata</i>	20.27	79.72	25.33	4.58	48.40	59.23	28.65	0.66	0.48	59.40	1C
<i>Prospero autumnale</i>	28.98	71.01	37.33	5.57	18.53	29.23	5.40	0.56	0.18	44.54	3A
<i>Prospero obtusifolium</i>	26.50	73.49	35.85	3.44	17.14	20.12	3.43	0.64	0.17	47.10	4A
<i>Barnardia numidica</i>	23.77	76.22	30.76	5.43	42.01	51.89	21.79	0.57	0.42	52.57	1B
<i>Oncostema elongata</i>	24.44	75.55	31.95	5.24	45.69	42.20	19.28	0.60	0.42	51.45	1B

classification, with intrachromosomal asymmetry index  $M_{CA}$  values of 59.4 and interchromosomal asymmetry index  $CV_{CL}$  values of 48.4.

In contrast, *P. autumnale* and *P. obtusifolium*, have significantly reduced asymmetry values. The intrachromosomal asymmetry indices ( $M_{CA}$ ) for *P. autumnale* and *P. obtusifolium* are 44.54 and 47.10, respectively. Similarly, their interchromosomal asymmetry indices ( $CV_{CL}$ ) are 18.53 and 17.14, respectively. The karyotypes of these two species are categorized as 3A and 4A, respectively. Although belonging to different genera, *B. numidica* and *O. elongata* exhibit very comparable asymmetry indices, which are characterized by rather high values. The intrachromosomal asymmetry indices  $M_{CA}$  for *B. numidica* and *O. elongata* are recorded as 52.57 and 51.45, respectively. The interchromosomal asymmetry index  $CV_{CL}$  for *B. numidica* is determined as 42.01, whereas for *O. elongata* it is calculated as 45.69. Both karyotypes are classified as 1B according to Stebbins' classification (1971).

endemic taxon of North Africa. They were  $2n=8$  and  $2n=16$ . This holds true when compared to the findings of the current investigation, which indicated that  $2n=16$ . Furthermore, certain similarities in karyotype descriptions have been observed between the two populations. The main difference is that the Skikda population has two telocentric chromosomes but no satellite chromosomes. According to Weiss Schneeweiss and Schneeweiss (2013), variation in karyotypical configuration between populations is indicative of chromosomal rearrangements, particularly those involving subtelocentric or telocentric chromosomes that appear to result from chromosomal deletion or translocation. Previous research (Sato 1936) showed that members of the Scilloideae subfamily had various karyotypes even within the same species. It is worth noting that the presence of such variability in karyotype organization does not always imply a significant morphological difference (Thompson 2005; Thompson 2020).

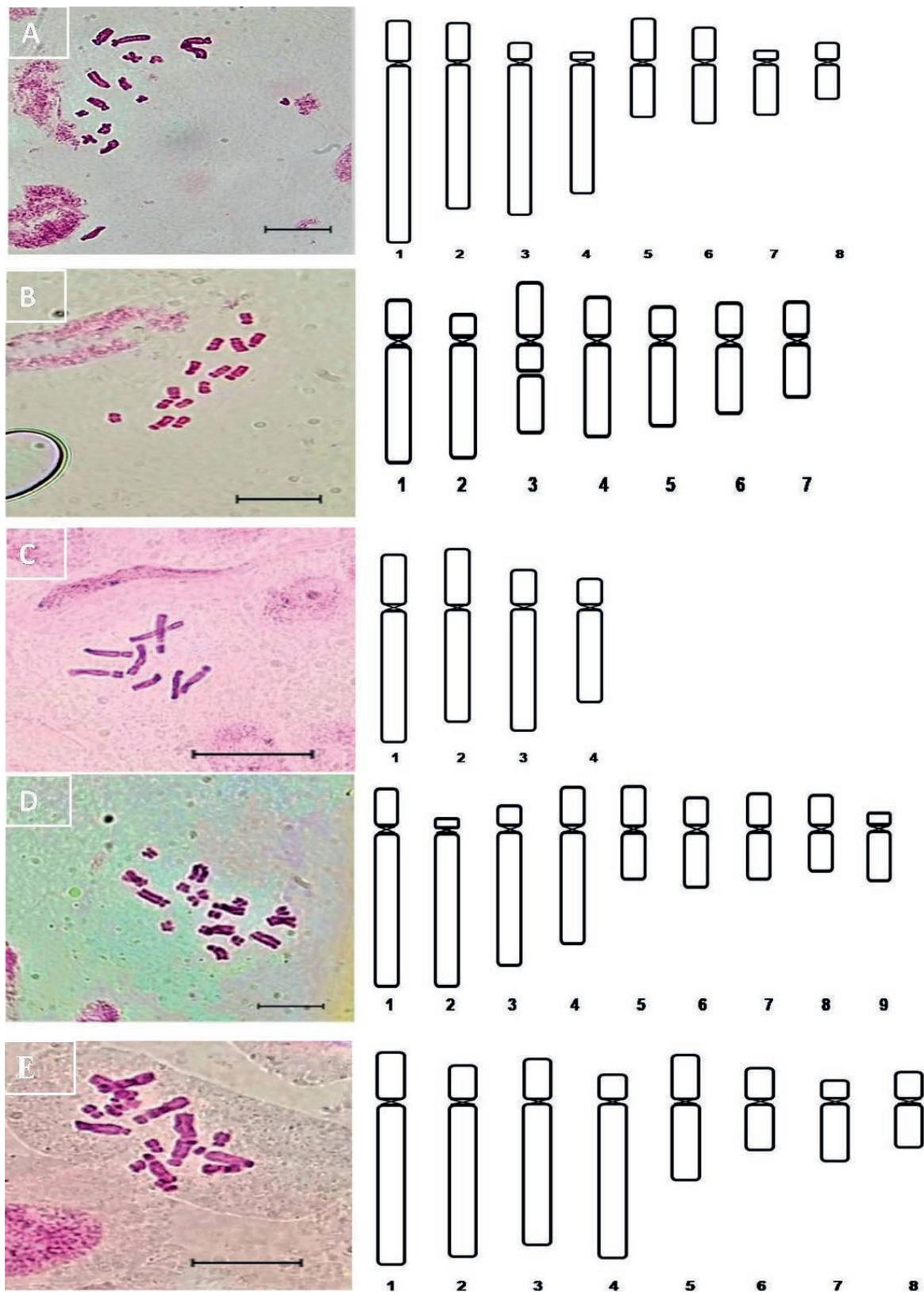
## DISCUSSION

### *Hyacinthoides lingulata*

The earlier research carried out by Hamouche *et al.* (2006) and (2010) on populations from western Algeria assigned two different chromosomal numbers to this

### *Prospero autumnale*

The research conducted by Hamouche *et al.* (2010) on multiple populations from various locations in central Algeria revealed a high level of diversity within the species, which is closely associated with the altitude and environment in which they grow. Diploid ( $2n=2x=14$ ),



**Figure 2.** Mitotic metaphase and idiograms of the studied taxa. A: *Hyacinthoides lingulata*  $2n=16$ , B: *Prospero autumnale*  $2n=14$ , C: *P. obtusifolium*  $2n=8$ , D: *Barnardia numidica*  $2n=18$ , E: *O. elongata*  $2n=16$ , scale bars=10  $\mu\text{m}$

tetraploid ( $2n=4x=24$ ), and hexaploid ( $2n=6x=42$ ) cytotypes were identified. The population of Skikda, which is located in northeastern Algeria, differs from the populations of Algiers, especially in terms of karyological description. It is distinguished by the presence of a metacentric chromosome (7) in addition to submetacentric and telocentric chromosomes, as well as a secondary constriction on the long arm of chromosome 3. In contrast, the diploid population ( $2n=2x=14$ ) from Algiers exhibits only submetacentric and subtelocentric chromosomes, in addition to a satellite on the short arm of chromosome 5.

This implies that there is considerable variation even among diploid cytotypes ( $2n=2x=14$ ) in Algeria, which might be due to chromosomal rearrangements such as inversions and translocations. Jang *et al.* (2013) and Jang *et al.* (2018) investigations both validated the existence of chromosomal rearrangements in the *P. autumnale* complex. In the *P. autumnale* complex, supernumerary forms such as B chromosomes and forms of aneuploidy, particularly in diploid cytotypes ( $2n=2x=14+1$ ) and hexaploid cytotypes ( $2n=6x=42+1$ ), have been documented (Rejoin *et al.* 1980; Hamouche *et al.* 2010; Jang *et al.* 2018). In addition, Jang *et al.* (2013) showed the existence of four diploid cytotypes with three distinct fundamental numbers,  $x=5$ ,  $x=6$ , and  $x=7$ , based on their study of seventeen individuals from various countries bordering the Mediterranean Sea. Individuals with the cytotype  $2n=2x=14$  were assigned two chromosomal descriptions, including one with submetacentric chromosomes (secondary constriction on chromosome 3), a subtelocentric chromosomes and a small metacentric chromosome. This is an exact match to the chromosomal description discovered in this investigation. The possibility cannot be ruled out for the presence of other *P. autumnale* complex cytotypes in Algeria, given the country's vast size and the vast diversity of its habitats and climates.

#### *Prospero obtusifolium*

Our findings regarding chromosomal number and formula are consistent with the studies conducted by Ebert *et al.* (1996), Hamouche *et al.* (2010), and Jang *et al.* (2013). These studies all identified a single cytotype ( $2n=8$ ) in populations throughout the Mediterranean basin. Overall, this indicates that the species has successfully maintained significant stability in its karyotype composition, even when dispersed over different habitats and a wide geographic area.

#### *Barnardia numidica*

Our population exhibits a chromosomal formula distinct from that of the central Algerian population ( $2n=2x=18=4m+2sm-sat+3st$ ) by possessing a telocentric pair but lacking a satellite. Both populations share metacentric and subtelo-centric chromosomes. The karyotype consists of two distinct sets of chromosomes, one containing small metacentric or submetacentric chromosomes and the other containing big subtelo-centric chromosomes (bimodal karyotype). The variation in size that was found might be attributed to hybridization, as shown by previous research (Speta 1979; Ebert *et al.* 1996; Hamouche *et al.* 2010). Other studies have indicated that this heterogeneity is linked to higher amplification of distinct forms of heterochromatin within specific chromosomal sets (De la Herrán *et al.* 2001). Indeed, the existence of a bimodal karyotype has already been observed in Hyacinthaceae species such as *Ornithogalum* (Stedje 1989) and *Galtonia* (Forrest and Jong 2004).

#### *Oncostema elongata*

There is still no taxonomic consensus on the specific delimitation within *O. peruviana* (*Scilla peruviana*). Some authors attribute the variability observed in the CW Mediterranean to a single species (Almeida da Silva and Crespi 2013; Almeida da Silva *et al.* 2014), others prefer to consider different species (Dobignard and Chatelain 2010; APD 2023). Previous research has pointed to the presence of different chromosomal counts of  $2n=16$ ,  $2n=15$ , and  $2n=14$  have been seen in different individuals of *O. peruviana* (Sato 1936).

The same is true for *O. hughii* (Tineo ex Guss.) Speta, which has four distinct numbers ( $2n=17$ ,  $2n=19$ ,  $2n=20$ , and  $2n=22$ ). It is worth noting that these chromosomal count differences were discovered in plants that shared the same habitat (Sato 1936). In addition, the same author has shown that *O. peruviana* (L.) Speta has a stable karyotype with  $2n=16=1M+4stSat+2m+1^{st}$ . Although  $2n=16$  is thought to be the fundamental chromosome number in *O. peruviana* (Sato 1936; Battaglia 1950, Barone *et al.* 2021).

Several asymmetry indices have been established since the work of Stebbins to evaluate karyotype asymmetry in its evolutionary state (Romero Zarco 1986). These are the first data on karyotype asymmetry for the several species included in this study. According to Peruzzi and Eroglu (2013), the intrachromosomal asymmetry index  $M_{CA}$  and the interchromosomal asymmetry index  $CV_{CL}$  are extensively utilized due to their abil-

ity to detect even the most subtle chromosomal variations. Various asymmetry parameters have disclosed karyotype diversity among the studied species. Species in the genus *Prospero*, such as *P. autumnale* and *P. obtusifolium*, classified as types 3A and 4A, respectively, appear to have the most symmetrical karyotypes, indicating relatively stable karyotypes, according to Stebbins' classification. The karyotypes of *B. numidica*, *O. elongata* and *H. lingulata*, on the other hand, belong to categories 1B, 1B and 1C, respectively, indicating asymmetrical karyotypes. High  $CV_{CL}$  and  $M_{CA}$  values for all three species, especially *H. lingulata*, support this result (Table 3). The intrachromosomal asymmetry index  $M_{CA}$  had a significant negative connection with  $Sy_i$  (-0.997\*\*) and TF (-0.996\*\*) and a positive correlation with  $AsI$  (0.996\*\*) and HCL (0.917\*\*) according to Pearson correlation. It also correlated positively with  $CV_{CL}$  (0.093) and  $CV_{CI}$  (0.516), although not significantly. The interchromosomal asymmetry index  $CV_{CL}$ , on the other hand, had a strong and positive relationship with  $CV_{CI}$  (0.83\*), AI (0.99\*\*),  $A_2$  (0.99\*\*), and HCL (0.91\*\*). Therefore, differences in asymmetry between chromosomes in the same set are associated with differences in size, which may result from changes in structure as well as differences in shape, particularly the location of the chromosomal centromere.

The karyotypes of *P. autumnale* and *P. obtusifolium* are notable for their symmetrical structure, but those of *B. numidica*, *O. elongata*, and *H. lingulata* have significant asymmetry. According to Stebbins (1971), asymmetric karyotypes are seen as derived traits that have emerged more recently in evolutionary processes. They are often linked to plants that display specific morphological features or are descendants of a more recent origin. In the Mediterranean flora, variations in chromosome size, base number, and symmetry between closely related species are not new. Many processes, such as polyploidy, aneuploidy, or dysploidy, can explain these changes (Stebbins 1971; Levin 2002; Guerra 2008; Choi *et al.* 2008) and morphological differences could result from the expression of these genetic variations (Thompson 2005; Thompson 2020). Species belonging to genera such as *Reichardia*, *Brachyscome*, *Crepis* and *Geranium* provide clear examples of this karyotypic variety (Siljak-Yakovlev 1996; Watanabe *et al.* 1999; Dimitrova and Greilhuber 2000; Martin *et al.* 2022). Interestingly, these variations are not limited to interspecific differences but extend to intraspecific levels, giving rise to the emergence of new chromosomal races (Levin 2002). *Prospero autumnale* (Parker *et al.* 1991, Ebert *et al.* 1996, Vaughan *et al.* 1997, Hamouche *et al.* 2010), *Ornithogalum tenuifolium* (Stedje 1989), *O. nutans* (Cullen and Rattert, 1967),

*Albuca abyssinica* (Stedje 1996), *Bellevalia mauritanica*, and *Muscari neglectum* (Azizi *et al.* 2016b) are notable examples in the Hyacinthaceae family.

## CONCLUSION

In terms of karyotypic constitution and asymmetry indices, the cytogenetic data related to the examined species belonging to four genera of the subfamily Scilloideae have revealed, on the one hand, that the populations of the Skikda region are different from other populations previously studied by other researchers, indicating the important genetic diversity that exists in Algeria and on the other hand, significant differences between the species. This suggests that *P. autumnale* is the most stable species and *H. lingulata* is the most evolved species. However, molecular phylogenetic investigations are required to fully comprehend the relationship between the species under issue.

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## SUPPLEMENTARY TABLES

**Table 4.** Karyomorphological analysis of *Hyacinthoides lingulata*.

N	L ( $\mu\text{m}$ )	S ( $\mu\text{m}$ )	LT ( $\mu\text{m}$ )	AR	R value	RL%	F%	CI	CT
1	5.28±0.26	1.20±0.07	6.49±0.33	4.37±0.49	0.22±0.02	21.7±0.63	4.05±0.25	0.18±0.01	st
2	4.30±0.37	1.11±0.20	5.42±0.55	3.93±0.40	0.25±0.03	18.1±1.20	3.73±0.06	0.20±0.01	st
3	4.49±0.20	0.49±0.13	4.98±0.33	9.15±0.69	0.10±0.01	16.7±1.14	1.64±0.06	0.09±0.01	t
4	3.85±0.15	0.22±0.06	4.08±0.20	17.0±0.66	0.05±0.01	13.6±0.50	0.76±0.01	0.05±0.01	t
5	1.54±0.15	1.24±0.15	2.79±0.30	1.24±0.03	0.80±0.02	9.37±1.01	4.18±0.50	0.44±0.01	m
6	1.73±0.01	0.98±0.04	2.71±0.05	1.76±0.07	0.56±0.02	9.12±0.12	3.29±0.12	0.36±0.01	sm
7	1.49±0.16	0.26±0.11	1.75±0.26	5.57±0.30	3.74±0.17	5.89±0.19	0.88±0.38	0.15±0.07	st
8	1.03±0.09	0.50±0.01	1.54±0.11	2.07±0.10	0.48±0.02	5.19±0.38	1.71±0.06	0.33±0.01	sm

Length of chromosome (L: long arm, S: short arm, LT: total length). F%: Form percentage of chromosome. CI: Centromeric index. AR: Arm ratio. R-value: S/L. RL: Relative length. CT: Chromosome type.

**Table 5.** Karyomorphological analysis of *Prospero autumnale*.

N	L ( $\mu\text{m}$ )	S ( $\mu\text{m}$ )	LT ( $\mu\text{m}$ )	AR	R value	RL%	F%	CI	CT
1	2.03±0.19	0.63±0.04	2.66±0.22	3.18±0.23	0.31±0.02	17.95±0.13	4.25±0.26	0.23±0.01	st
2	1.97±0.03	0.38 ±0.08	2.35±0.12	5.55±0.27	0.18±0.01	15.82±0.13	2.52±0.13	0.16±0.01	st
3	1.36±0.05	0.94±0.08	2.31±0.13	1.41±0.06	0.70±0.03	15.55±0.40	6.38±0.01	0.41±0.01	m-sc
4	1.61±0.10	0.67±0.08	2.29±0.18	2.41±0.12	0.41±0.02	15.42±0.53	4.52±0.01	0.29±0.01	Sm
5	1.42±0.07	0.51±0.01	1.93±0.08	2.76±0.15	0.36±0.02	13.03±0.53	3.45±0.39	0.26±0.02	sm
6	1.20±0.05	0.57±0.05	1.77±0.11	2.14±0.12	0.46±0.02	11.96±0.79	3.85±0.39	0.32±0.01	sm
7	0.92±0.15	0.59±0.03	1.52±0.18	1.53±0.20	0.65±0.08	10.23±0.13	3.98±0.26	0.39±0.03	m

Length of chromosome (L: long arm, S: short arm, LT: total length). F%: Form percentage of chromosome. CI: Centromeric index. AR: Arm ratio. R-value: S/L. RL: Relative length. CT: Chromosome type.

**Table 6.** Karyomorphological analysis of *Prospero obtusifolium*.

N	L ( $\mu\text{m}$ )	S ( $\mu\text{m}$ )	LT ( $\mu\text{m}$ )	AR	R value	RL%	F%	CI	CT
1	3.49±0.11	1.32±0.28	4.82± 0.17	2.67±0.69	0.37±0.10	29.0± 1.04	7.98±1.73	0.27±0.04	Sm-Sat
2	2.99±0.11	1.48±0.32	4.47± 0.44	2.05±0.38	0.48±0.09	26.9± 2.66	8.91±1.96	0.32±0.04	sm
3	3.22±0.04	0.90±0.17	4.13± 0.13	3.65±0.75	0.27±0.05	24.8± 0.81	5.43±1.04	0.21±0.03	st
4	2.47±0.13	0.69±0.03	3.17± 0.17	3.55±0.01	0.28±0.01	19.0± 1.04	4.16±0.23	0.21±0.01	st

Length of chromosome (L: long arm. S: short arm. LT: total length). F%: Form percentage of chromosome. CI: Centromeric index. AR: Arm ratio. R-value: S/L. RL: Relative length. CT: Chromosome type.

**Table 7.** Karyomorphological analysis of *Barnardia numidica*.

N	L ( $\mu\text{m}$ )	S ( $\mu\text{m}$ )	LT ( $\mu\text{m}$ )	AR	R value	RL%	F%	CI	CT
1	3.20±0.10	0.77±0.04	3.97±0.02	4.27±0.11	0.23±0.01	18.3±0.09	3.56±0.17	0.19±0.01	st
2	3.20±0.37	0.18±0.10	3.39±0.27	19.2±2.00	0.05±0.01	15.6±1.25	0.86±0.48	0.05±0.01	t
3	2.79±0.12	0.41±0.11	3.20±0.31	6.70±0.62	0.14±0.02	14.8±0.76	1.92±0.19	0.12±0.01	st
4	2.33±0.23	0.79±0.12	3.12±0.19	2.94±0.69	0.33±0.07	14.4±0.19	3.65±0.57	0.25±0.04	sm
5	0.97±0.10	0.83±0.04	1.81±0.11	1.15±0.13	0.86±0.10	8.37±0.10	3.84±0.19	0.46±0.02	m
6	1.14±0.06	0.58±0.16	1.72±0.10	1.92±0.72	0.51±0.17	7.98±0.48	2.69±0.76	0.33±0.07	sm
7	0.99±0.04	0.68±0.06	1.68±0.10	1.50±0.07	0.66±0.08	7.79±0.48	3.17±0.28	0.40±0.01	m
8	0.81±0.06	0.62±0.04	1.43±0.02	1.26±0.18	0.78±0.11	6.64±0.10	2.88±0.19	0.43±0.03	m
9	1.02±0.04	0.24±0.04	1.27±0.07	0.95±0.25	0.25±0.05	5.87±0.09	1.15±0.19	0.19±0.03	st

Length of chromosome (L: long arm. S: short arm. LT: total length). F%: Form percentage of chromosome. CI: Centromeric index. AR: Arm ratio. R-value: S/L. RL: Relative length. CT: Chromosome type.

**Table 8.** Karyomorphological analysis of *Oncostema elongata*.

N	L ( $\mu\text{m}$ )	S ( $\mu\text{m}$ )	LT ( $\mu\text{m}$ )	AR	R value	RL%	F%	CI	CT
1	4.11±0.12	1.20±0.10	5.38±0.20	3.29±0.38	0.30±0.03	19.0±0.21	4.49±0.35	0.23±0.02	st
2	3.91±0.60	0.88±0.02	4.79±0.62	4.40±0.68	0.22±0.03	16.9±2.13	3.13±0.60	0.18±0.02	st
3	3.64±0.06	1.02±0.02	4.67±0.08	3.60±0.01	0.27±0.02	16.5±0.28	3.63±0.07	0.21±0.02	st
4	3.97±0.06	0.62±0.02	4.59±0.04	6.50±0.30	0.15±0.07	16.2±0.14	2.20±0.07	0.13±0.02	st
5	1.95±0.02	1.14±0.10	3.10±0.08	1.71±0.16	0.58±0.05	10.9±0.28	4.06±0.35	0.36±0.02	sm
6	1.18±0.06	0.80±0.02	1.99±0.06	1.45±0.07	0.68±0.03	7.05±0.21	2.85±0.02	0.40±0.02	m
7	1.47±0.10	0.46±0.02	1.93±0.12	3.27±0.08	0.30±0.11	6.84±0.42	1.63±0.07	0.32±0.04	st
8	1.10±0.10	0.68±0.08	1.79±0.18	1.58±0.04	0.62±0.01	6.34±0.64	2.42±0.28	0.38±0.02	m

Length of chromosome (L: long arm. S: short arm. LT: total length). F%: Form percentage of chromosome. CI: Centromeric index. AR: Arm ratio. R-value: S/L. RL: Relative length. CT: Chromosome type.