



First Karyotype Report of *Arum cyrenaicum* Hruby (Araceae) from AL-Jabal AL-Akhdar, Libya.

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DOI: 10.37376/sjuob.v38i2 | Received:20/09/2025 | Accepted:28/11/2025 | Publishing: 23/12/2025

ABSTRACT

In this paper, somatic chromosome number and karyotype of the endemic species *Arum cyrenaicum* Hruby (Araceae), collected from the Tolmitha site in the Al-Jabal Al-Akhdar region of Libya, are reported for the first time. Our results revealed that all the studied populations were polyploid ($2n=4x=56$) with a karyotype formula (KF) of $6M+38m+10sm+2st(2SAT)$. Furthermore, the chromosome size of *A. cyrenaicum* was medium to small-sized, with lengths ranging from the largest $4.72\pm.21\mu m$ to the smallest $2.88\pm.04\mu m$. According to Stebbins' karyotype asymmetry classification, it was 1A type (symmetrical one). Satellites were observed on two chromosome pairs. The karyotypes of these species' findings contribute to the scientific understanding of this plant from a genetic perspective study. In addition to taxonomic characters, the analyzed karyotype features will contribute to the characterization of *A. cyrenaicum* varieties and to establish a base for future research.

KEYWORDS: Chromosome numbers, karyotype, endemic, *Arum cyrenaicum*.

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1. INTRODUCTION

Araceae is a large plant family that comprises 140 genera and about 4,075 known species⁽¹⁾. In Libya, there are 3 genera and 3 species: 1. *Arum* 2. *Biarum* 3. *Arisarum*⁽²⁾. *Arum cyrenaicum* (vernacular name: Renish) is an annual herbaceous plant with discoid-shaped tubers, which grows during the early fall season. It has simple sagittate and hastate leaves with long petioles (Fig. 1). The species produces a single inflorescence, which is sail-shaped and dark purple in color. The inflorescence consists of two parts: the spathe and the spadix, blooms during March-April. The fruits of *A. cyrenaicum* are red berries when ripe, and the seeds have an ovate shape⁽³⁾. Traditionally, *A. cyrenaicum* has been used in Libyan folk medicine for treating dermatitis, psoriasis, diarrhea, and diabetes^(4,5). Phyto-chemical studies have revealed the presence of flavonoids, alkaloids, terpenes, carbohydrates, and sterols⁽⁶⁾. *A. cyrenaicum* was first described as a Libyan endemic species by Hruby⁽⁷⁾, was previously misclassified under multiple names. Initially, Durand and Barratte⁽⁸⁾ identified it as *A. hygrophilum*. Later, in 1910, the same researchers referred to it as *Arum italicum*, while Pampolini⁽⁹⁾ assigned it the name *Arum pictum*.

Plant karyotype study is fundamental for understanding the origin and evolutionary pathways of plant species, as well as contributing to molecular genetics and floristic geography⁽¹⁰⁾. In addition, karyotype research is a fast, inexpensive approach to classify plant species by identifying the basic cytological parameters of a species, including chromosome number, size, morphology, ploidy levels, and karyotype coefficient of variation⁽¹¹⁾. These characteristics are considered vital sources of taxonomic information, as they serve as powerful tools for the authentication and identification of plant species after morphological studies⁽¹²⁾.

Although modern biosystematics relies heavily on molecular approaches, karyological data remain indispensable for understanding genome organization and evolutionary diversification⁽¹³⁾.

The genus *Arum* comprises both diploid ($2n = 28$) and polyploid taxa ($2n = 56$ and 84), with polyploidy playing a role in species diversification and adaptation⁽¹⁴⁾. The basic chromosome number for *Arum* is $x = 14$, and species exhibit significant variation in karyotype morphology and asymmetry⁽¹⁵⁾.

D'Emerico et al.⁽¹⁶⁾ studied chromosome morphology in five species of the genus *Arum*. Their results showed that the karyotypes of *A. orientale*, *A. alpinum*, and *A. nigrum* ($2n = 28$) were very similar. *A. pictum* ($2n = 28$) is composed of a higher number of asymmetric chromosomes. The karyotype of *A. maculatum* ($2n = 56$) shows great similarities to the karyotypes of *A. orientale* and *A. alpinum*.

Some studies have shown that certain *Arum* species share similarities in their morphological karyotype characteristics. *A. italicum* and *A. maculatum* share a similar karyotype morphology. On the other hand, *A. italicum*, with a higher intra-chromosomal asymmetry index ($A1 = 0.43$), exhibits a more asymmetrical karyotype than *A. maculatum* ($A1 = 0.39$). In comparison to *A. maculatum* and *A. italicum*, *A. apulum* ($A1 = 0.32$) has the most symmetrical karyotype⁽¹⁵⁾. According to reports, the majority of polyploid *Arum* taxa cover larger geographic areas than their diploid counterparts⁽¹⁷⁾. *A. italicum* is found throughout the Mediterranean region, the Atlantic coast, and the Caucasus⁽¹⁸⁾. Central and Western Europe are home to *A. maculatum*^(17,19,20). Therefore, the ability of *A. italicum* and *A. maculatum* to colonize new areas may account for their wider geographic range in comparison to diploids like *A. pictum*, or *A. orientale*⁽¹⁴⁾. However, the tetraploid *A. apulum* has a very limited distribution in Southern Italy⁽²¹⁾ compared with the diploid *A. alpinum* has a very wide distribution⁽²²⁾.

Despite the genus being relatively well studied in Europe, there is no previous report on the karyotype structure of *A. cyrenaicum*. Therefore, the present study aims to provide the first karyotype analysis of *A. cyrenaicum* from Al-Jabal Al-Akhdar, Libya, including chromosome number, size, morphology, ploidy level, and the occurrence of satellites, which will contribute to cytogenetic characterization and support future taxonomic and evolutionary studies.



Figure 1 *Arum cyrenaicum* Hruby in its natural habitat. (A)Habit of the plant, showing the sagittate leaves and long petioles.(B) Close-up of the dark purple, sail-shaped inflorescence consisting of the spathe and spadix.

2.MATERIALS and METHODS

2.1.Plant Material:

Plant samples of *A. cyrenaicum* Hruby were collected from natural habitats in the Tolmitha site, located in the Al-Jabal Al-Akhdar region, Cyrenaica, northeastern Libya, during 2021–2022. The site is characterized by reddish clay soil and a Mediterranean climate.

2.2.Experimental Method:

The seeds (isolated from the fruits) were germinated in petri dishes at 15°C for two weeks. Actively growing root tips were pretreated in 0.1% colchicine solution for 6 hours to arrest cells at metaphase. After pretreatment, the root tips were fixed in Carnoy's solution (glacial acetic acid: 95% ethanol, 1:3) for 24 hours at 4°C and subsequently stored in 70% ethanol at 4°C until further use. For slide preparation, fixed roots were hydrolyzed in 1 N HCl at 60°C for 20 minutes, rinsed in distilled water for 20 minutes, and then stained in 1% aceto-orcein solution (1 g orcein dissolved in 100 mL glacial acetic acid). Squash preparations were made by gently pressing the root tips under a coverslip for microscopic observation. At least ten metaphase cells were used to determine chromosome numbers and karyological characteristics. Photomicrographs were taken with a microscope at 1000 x magnification. 2.3karyotype Analysis:

Detected metaphase plates were recorded by computer; after that karyotypes were performed by cutting and arranging chromosomes with homologous pairs based on arm ratio and chromosome size using the ImageJ program⁽²³⁾ and Adobe Photoshop 2020 program.

The chromosome type was determined using the centromere position and arm ratio and classified according to Levan et al.⁽²⁴⁾. The degree of karyotype asymmetry was estimated with Stebbins's method⁽²⁵⁾. The following chromosomal measurements were recorded: chromosome length (CL), arm ratio ($AR = L/S$), Index of relative length of chromosome ($IRL\% = (CL/\Sigma CL) \times 100$), centromere index ($CI\% = S/CL \times 100$). Also, the following karyotype parameters were documented: degree of karyotype asymmetry ($A = \text{Mean} (L - S) / (L + S)$)⁽²⁶⁾, intrachromosomal asymmetry ($A_1 = 1 - \text{Mean} S / L$), interchromosomal asymmetry ($A_2 = sCL/xCL$)⁽²⁷⁾, coefficient of variation of chromosome length ($CVCL = A2 \times 100$)⁽²⁸⁾, coefficient of variation of centromere index ($CVCI = (sCI / x CI) \times 100$)⁽²⁸⁾, Arano index ($Ask\% = (\Sigma L/\Sigma CL) \times 100$)⁽²⁹⁾, symmetry index $S\% = (CL_{\min} / CL_{\max}) \times 100$ ⁽²⁶⁾, total form per centage of homologous chromosome pairs $TF\% = (\Sigma S/\Sigma CL) \times 100$ ⁽³⁰⁾ and Asym-metry index ($AI = (CVCL \times CVCI) / 100$)⁽²⁸⁾.

The Ideogram was drawn automatically by the Ideokar program, and the mean and standard deviations were analyzed for all chromosomal and karyotype parameters using the SPSS (version 13.0) statistical program.

3.RESULTS

All ten individuals/plants studied were identified as tetraploid $2n = 4x = 56$ (Fig. 2), with a basic chromosome number of $x = 14$ and the ideogram is shown in (Figure.3). The karyotype analysis of this species revealed the existence of three distinct types of chromosomes, with the metacentric (M-m) type being more frequent than the submetacentric (sm) and subtelocentric (st) types (Table1). The karyotype formula (KF) includes 22 M-m pairs, 5 sm pairs, and 1 st pair. The formula was $6M + 38m + 10sm + 2st$ (2SAT). This is the first study to document the karyotype of *A. cyrenaicum*, which is considered an endemic species of Al-Jabal Al-Akhdar.

The analysis of 28 chromosomes from *A. cyrenaicum* revealed significant variation in the arm lengths and their ratios. The average length of the short arm (S) was 1.62 μm , while the average length of the long arm (L) was 2.20 μm , resulting in an average total chromosome length of 3.82 μm . The S/L ratio, which indicates the degree of symmetry between the two arms, ranged from 0.28 (chromosome 18) to 1.000 (chromosome 2), with an overall average of 0.74. Further analysis using the (L-S)/(L + S) index, which reflects the relative difference be-

tween arm lengths, revealed a mean value of 0.15, reinforcing the presence of moderate arm length asymmetry in most chromosomes. The (L-S)/L index also averaged 0.24, which similarly supports this observation. Out of the 28 chromosomes, three chromosomes (2, 4, and 10) showed perfect symmetry ($S \approx L$), classifying them as metacentric(M). In contrast, several chromosomes, such as 3, 7, 18, 23, 25, and 26, had noticeably low S/L ratios (below 0.65), indicating submetacentric to telocentric configurations. Chromosome 18 was identified as the most asymmetrical, with an S/L ratio of 0.28 and a (L-S)/(L + S) value of 0.56 (Table 1). The relative length of the chromosomes, indicated by the index of IRL%, ranged from 2.53% to 4.29%. The CI% ranged from 21.94% to 50.00%, with an average centromere position of 43.68%. The AR ranged from 1.00 to 3.55, with an average AR of 1.42 (Table 1).

The karyotype of this species is classified as a symmetry type 1A according to Stebbins classification. The average value of the A_1 and the average value of the A_2 for *A. cyrenaicum* are 0.26 and 0.19, respectively. The value of the CVcL = 19.31 and the value of the CVcl = 5.82.

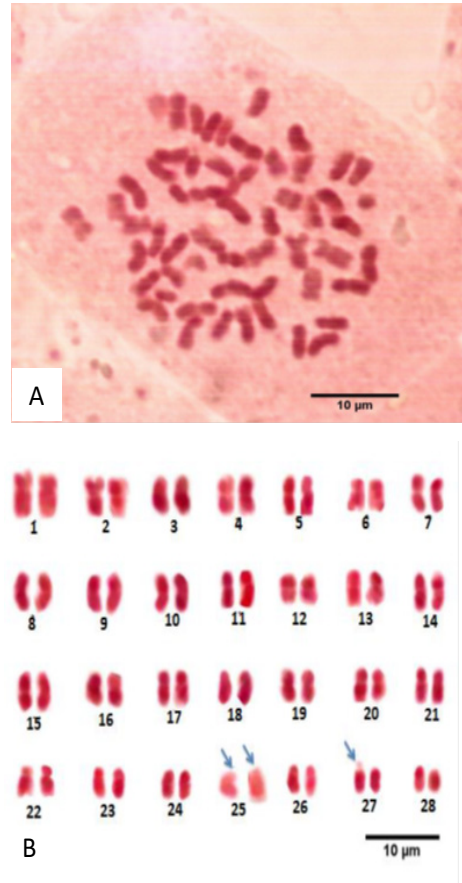


Figure 2. Metaphase chromosomes spread (A), and karyogram of *A. cyrenaicum*(B). Bar = 10 µm.

The values of the AI = 1.12, Ask% = 57.46, and the degree of A = 0.15, the values of the total S% = 61.01, and the total form percentage of TF% = 42.31 (Table 2).

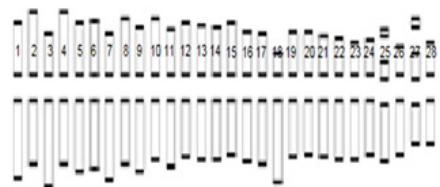


Figure 3. Idiograms of *A. cyrenaicum* based on chromosome length and primary and secondary constrictions (Satellites shaded on the short arms of pairs 25 and 27). Bar = 10 µm.

5.DISCUSSION

5.1.Chromosome Number and Morphology:

Determining chromosome numbers has been a very useful approach for researchers to study evolutionary relationships and assess the impact of inbreeding depression (31). *Arum* has a basic number of $x=14$, and three ploidy levels have been reported: diploid, tetraploid, and hexaploid. *A. orientale*, *A. alpinum*, *A. hygrophilum*, and *A. pictum* are diploid ($2n=28$), *A. maculatum* and *A. apulum* are tetraploid ($2n = 56$), while *A. italicum* is hexaploid ($2n=84$) (15,16,21,32). The tetraploid ($2n = 4x = 56$) observed here in *A. cyrenaicum* is consistent with Boyce’s (33) findings but differs from that of Marchant (34), who reported a somatic chromosome number of $2n=28$. This difference in chromosomal number could be due to the difficulty of counting or the small number of individuals studied. In the aforementioned works, only chromosome numbers have been reported. *A. cyrenaicum* ($2n = 56$) shares its chromosome number with *A. apulum* (supporting its placement in subsection *Dischroochiton*; Boyce³³) and *A. maculatum*. However, *A. cyre-naicum* differs

from both species in terms of its unique karyological features (e.g., high ratio of metacentric chromosomes and distinct chromosome size and morphology) may represent a unique adaptation to its local environment in Libya. Additionally, its chromosome number and morphology are inconsistent with *A. hygrophilum*, *A. pictum* and *A. italicum*. Polyploidy is a common phenomenon in plants that occurs naturally and spontaneously. It results in the increase in genome size caused by the presence of three or more chromosome sets. Polyploidy is an important mechanism regarding speciation and evolution of plants, that occurs in two ways: autopolyploidy with genome duplication in only one species and allopolyploidy with genome duplication between species. D’Emerico et al. (16); Bianco et al. (21); and Turco et al. (15) state that the tetraploid *Arum*’s chromosomes can be arranged in sets of two, and since it is impossible to arrange them in a series of four, it can be assumed that it is likely allopolyploid.

Table 1. Karyomorphometric measurements of *Arum cyrenaicum* ($2n = 56, 4x$) Short arm length (S), long arm length (L), total chromosome length (CL = S+L), arm ratio (S/L), relative length (CL/ΣCL ×100), centromeric index (S/(S+L)×100), chromosome type according to Levan et al. (1964).

Chr. No	S (µm)	L (µm)	(S+L)	S/L	L/S	(L-S)/L	(L-S)/(L+S)	(CL/ΣCL)*100	S/(S+L)* 100	Chr. Type
1	1.93±.05	2.79±.16	4.72±.21	0.69±.31	1.44±.04	0.3±.68	0.18±.52	4.40±.15	40.88±.15	m
2	2.27±.03	2.27±.03	4.54±.06	1.00±1.0	1.00±.00	0.00±0.00	0.00±.00	4.23±.01	50.00±.00	M
3	1.53±.04	2.99±.05	4.52±.09	0.51±.8	1.96±.02	0.48±.2	0.32±.11	4.21±.03	33.84±.23	sm
4	2.19±.02	2.25±.06	4.44±.08	0.97±.3	1.03±.01	0.01±.67	0.01±.5	4.04±.03	49.32±.35	M
5	1.91±.02	2.50±.05	4.41±.08	0.76±.4	1.31±.01	0.23±.6	0.13±.37	4.11±.02	43.31±.22	m
6	1.97±.06	2.41±.07	4.38±.13	0.81±.85	1.22±.00	0.18±.14	0.1±.07	4.08±.07	44.97±.03	m
7	1.53±.01	2.77±.02	4.30±.01	0.55±.5	1.81±.03	0.44±.5	0.28±1.00	4.01±.04	35.58±.42	sm
8	2.02±.02	2.23±.03	4.25±.01	0.9±.67	1.10±.01	0.09±.3	0.04±1.00	3.96±.04	47.52±.55	m
9	1.73±.02	2.46±.05	4.19±.03	0.7±.4	1.42±.01	0.29±.6	0.17±1.00	3.91±.07	41.28±.25	m
10	2.01±.01	2.08±.02	4.09±.03	0.96±.5	1.03±.00	0.03±.5	0.01±.3	3.81±.00	49.14±.06	M
11	1.64±.00	2.37±.07	4.01±.06	0.69±.00	1.44±.04	0.3±1.00	0.18±1.16	3.74±.02	40.92±.73	m
12	1.91±.02	2.02±.02	3.93±.01	0.94±1.0	1.05±.03	0.05±.00	0.02±.00	3.66±.04	48.60±.59	m
13	1.83±.01	2.08±.01	3.91±.03	0.87±1.0	1.13±.00	0.12±.00	0.06±.00	3.64±.02	46.80±.06	m
14	1.71±.05	2.16±.07	3.87±.02	0.79±.71	1.26±.08	0.2±.28	0.11±1.00	3.61±.02	44.18±1.56	m
15	1.87±.04	1.97±.05	3.84±.02	0.94±.8	1.05±.05	0.05±.2	0.02±.5	3.58±.09	48.69±1.17	m
16	1.58±.03	2.19±.03	3.77±.01	0.72±1.0	1.38±.05	0.27±.00	0.16±.00	3.52±.05	41.90±.87	m
17	1.55±.06	2.17±.06	3.72±.01	0.71±1.00	1.40±.10	0.28±.00	0.17±.00	3.47±.1	41.67±1.74	m
18	0.79±.03	2.81±.03	3.60±.05	0.28±1.00	3.55±.08	0.71±.00	0.56±.00	3.35±.09	21.94±.41	st
19	1.52±.06	2.04±.06	3.56±.01	0.74±1.00	1.34±.10	0.25±.00	0.14±.00	3.32±.04	42.69±1.85	m
20	1.54±.03	1.99±.03	3.53±.01	0.77±1.00	1.29±.05	0.22±.00	0.12±.00	3.29±.04	43.62±1.00	m

Chr. No	S (µm)	L (µm)	(S+L)	S/L	L/S	(L-S)/L	(L-S)/(L+S)	(CL/ΣCL)*100	S/(S+L)* 100	Chr. Type
21	1.51±.06	1.95±.11	3.46±.04	0.77±.54	1.30±.12	0.22±.45	0.12±1.25	3.23±.07	43.64±2.41	m
22	1.37±.02	2.04±.05	3.41±.02	0.67±.4	1.48±.06	0.32±.6	0.19±1.5	3.18±.05	40.17±0.97	m
23	1.24±.02	2.12±.02	3.36±.01	0.58±1.00	1.70±.03	0.41±.00	0.26±.00	3.13±.03	36.90±.46	sm
24	1.29±.01	1.97±.01	3.26±.02	0.65±1.00	1.52±.02	0.34±.00	0.2±.00	3.04±.01	39.57±.26	m
25	1.11±.05	2.04±.06	3.15±.12	0.54±.83	1.66±.12	0.45±.17	0.29±.08	2.93±.07	35.23±.39	sm
26	1.08±.02	2.01±.11	3.09±.14	0.53±.18	1.83±.06	0.46±.81	0.3±.64	2.88±.09	34.95±.77	sm
27	1.41±.03	1.61±.15	3.02±.18	0.87±.2	1.14±.08	0.12±.8	0.06±.67	2.82±.14	46.68±1.90	m
28	1.34±.02	1.54±.03	2.88±.04	0.87±.67	1.10±.00	0.12±.3	0.06±.25	2.68±.02	46.52±0.07	m
Average	1.62±.03	2.20±.05	3.82±.05	0.74±0.64	1.42±.04	0.24±.31	0.15±.42	3.56±.05	42.16±0.69	

Polyploids frequently have a wider geographical range than their diploid parents ^(35, 36), probably because they are preadapted to habitats and resources off limits to their parents ⁽³⁷⁾. They have a diversity of alleles that can confer a greater ecological niche than that of diploid progenitors ⁽³⁸⁾. Polyploidy associated with structural changes in chromosomes is involved in bringing about further diversification of karyotype morphology ⁽²⁵⁾. Therefore, on this basis, it is suggested that *A. cyrenaicum* is characterized by more rearrangement in its chromosome complement; these rearrangements may enhance the adaptive capacity of certain species. This exceptional tetraploid population of *A. cyrenaicum* could represent the starting point of evolutionary differentiation in this species. An average centromere position and arm ratio indicate a predominantly median position for the centromeres and predominantly metacentric chromosomes in the karyotype

of *A. cyrenaicum*. The karyotype formula of this species consisted of 22 pairs metacentric, 5 pairs submetacentric, and 1 pair subtelocentric. Satellites were observed on the short arms of pairs 25 and 27 in *A. cyrenaicum*, the analyses show that the karyotype is similar to the previous reports for *A. maculatum* (26m+24sm+6st; microsatellite on the short arm of pair 27) and *A. apulum* (40m+16sm; secondary constriction on the short arm and a microsatellite on the long arm of pair 27 ⁽¹⁵⁾).

This is useful in characterizing a tetraploid *Arum* and studying the relationship between them. In general, the Araceae family presents metacentric, submetacentric, and sub-telocentric chromosomes ⁽¹⁾. In *A. cyrenaicum*, no B-chromosomes were observed during the study period.

Table 2. Karyomorphometric data for the studied plant taxa

Ch.size variation(µm)	THCL	CVci	Interchromosomal index		Intrachromosomal index				Symmetry Index S%	Asymmetry Index AI
			CVci	A ₂	TF%	AsK%	A	A1		
2.88-4.72	107.21±1.51	5.82±.45	13.55±.195	0.19±1.62	42.31±.56	56.131±.091	0.15 ±.39	0.26±.40	61.01±.19	1.12±.00

5.2 Chromosome Size

Chromosome length is useful for distinguishing individuals, samples, populations and species. It is also an indirect indicator of the total DNA content. Measurement of chromosome size correlates with evolutionary age, which provides an estimate of genome size using the chromosomal data ^(31,39).

Most species in the genus *Arum* possess small to medium-sized chromosomes, typically ranging from 2.65 to 5.40µm. In this study, the chromosome size of *A. cyrenaicum* (2.88-4.72µm) was comparable to that of *A. alpinum* (2.95-4.80µm) and *A. maculatum*(2.00-4.80µm) ⁽¹⁶⁾. The THCL, a proxy for genome size ^(40,41), is calculated as

the sum of the lengths of all the chromosomes in a metaphase plate, divided by the ploidy level ⁽⁴²⁾. The THCL of *A. cyrenaicum* (107.21µm) further confirms that this species has medium to small chromosomes.

5.3 Karyotype Asymmetry

Karyotype asymmetry is an important parameter in karyological studies ⁽⁴³⁾. It is further considered one of the most popular, inexpensive, and widely used approaches, especially by botanists. Karyotype symmetry has two components, one related to variation among chromosome size and the other to variation in centromere position ⁽⁴⁴⁾.

This study revealed that most chromosomes in *A. cyre-*

naicum are either metacentric or submetacentric. The karyotype symmetry indices exhibited low heterogeneity and variation values. The value of the intrachromosomal asymmetry index plays a role in finding variations in chromosome types within a karyotype. The range of values for the A_1 is from zero to one. The low value of (A_1) for *A. cyrenaicum* signifies a value close to zero and this, in turn, indicates a predominantly metacentric chromosome in the karyotype of *A. cyrenaicum*. However, the value of the A_2 is used to assess the deviation (dispersion) in chromosome length within a karyotype (45). The small value for *A. cyrenaicum* shows that the chromosomal length deviation in *A. cyrenaicum* is relatively small (Table 2). Furthermore, the value of the CVcL and the value of CVci indicates low variation in chromosome length and centromere index, respectively. According to Uysal et al. (46), the larger the values of and, the greater the asymmetry in the karyotype. Therefore, the low values of both and in *A. cyrenaicum* indicate that its karyotype is predominantly composed of symmetric chromosomes.

The karyotype of this species is classified as a symmetry type 1A according to Stebbins classification, indicating that the karyotype of *A. cyrenaicum* is symmetrical. The low values of the AI, Ask%, and degree of A indicate a relatively low level of asymmetry. Meanwhile, the high values of the total S%, total form percentage of TF%, and CI% indicate a symmetrical structure of the karyotype. These findings demonstrate that *A. cyrenaicum* exhibits a distinct, symmetrical chromosomal pattern. Combined with morphological and geographical evidence, these cytological results support Hruby's (7) proposal that *A. cyrenaicum* warrants recognition as a distinct species. Although this species was recently recorded in 1992 in south-west Crete (Kakodikianos valley north of Paleohora as far as Kandanos), marking the first record of *A. cyrenaicum* outside Libya (47). The analysis offered by the current study reveals that its symmetric karyotype is predominantly composed of meta-centric chromosomes closely resembling that of *A. apulum*. According to Stebbins (25), a high proportion of metacentric chromosomes may indicate early evolutionary divergence in a species.

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