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On the Delimitation of *Anagallis arvensis* L. (Primulaceae) 1. Evidence Based on Macromorphological Characters, Palynological Features and Karyological Studies

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Abstract: Macromorphological characters, palynological features and karyological criteria were investigated in two taxa of *Anagallis arvensis* growing in Egypt. Significant variations were recorded in palynological features in the two taxa, thus favouring the view held by some authors regarding the two taxa as distinct subspecies of *Anagallis arvensis*.

Key words: *Anagallis*, macromorphology, palynology, karyotype

Introduction

Anagallis arvensis L. is a common weed in the Egyptian flora, particularly in the cultivated areas. It produces either red or blue flowers in spring. The infra-specific taxonomy of *A. arvensis* L. has been controversial. Linnaeus (1753) described four species of *Anagallis* viz: *A. arvensis*, *A. monelli*, *A. latifolia* and *A. capensis*. Bailey (1935, 1949) reported that *A. arvensis* var *caerulea*, Gren. Godr. (*A. caerulea* Schreber), has blue flowers. Tutin *et al.* (1972) mentioned that *A. arvensis* L. included the following taxa: *A. phoenicea* Scop., *A. platyphylla* Baudo, *A. parviflora* Hoffmanns. and Link. In their opinion this is a species in which many variants have been described, (for example *A. parviflora* Hoffmanns and Link, which is sporadic and seems to be little more than a small-flowered variety). According to Tackholm (1974), stated that *Anagallis* is represented in Egypt by two species viz *Anagallis arvensis* L and *Anagallis pumila* Sw. In her opinion *A. arvensis* L. constitute two subspecies (subspecies *arvensis* with red flowers and subspecies *latifolia* with blue flowers) while the other species *(Anagallis pumila)* has white flowers. However, Bailey and Bailey (1976) mentioned, *A. arvensis* L. with scarlet or white flowers, while in forma *caerulea* (Schreb.) Baumg. = *A. caerulea* Schreb. the flowers are blue. Dothan (1978-1986) mentioned that *A. arvensis* has a blue or scarlet corolla, while in *A. foemina* the corolla is blue. Migahid (1978) has agreed Tackholm (1974). Beckett (1983) recorded that the cultivated species of *A. arvensis* have scarlet, red, pink, lilac, purple or blue flowers. According to him, the flowers of *A. arvensis foemina*, are always rich blue. Mabberley (1987) reported that *A. arvensis* L. has red flowers while forma *caerulea* (Schreb.) Baumg. has blue flowers. However, the same author stated (1997) that the level of forma is better raised to subspecies. Boulos (2000), reported that *A. arvensis* have two subspecies one of them *A. arvensis* ssp. *arvensis* var *arvensis* with red flowers and the other *A. arvensis* ssp. *foemina* with blue flowers.

In present work, macromorphological criteria, as well as the SEM of the pollen grains surfaces, in addition to certain cytological features were examined for the delimitation of *Anagallis arvensis* L.

Materials and Methods

The examined species were collected from different localities of cultivated areas of Egypt such as (Delta region, in April 1999, Mediterranean coast, in March 2000 and different localities at Cairo, in May 2001). The voucher specimens are kept at the Herbarium of Department of Biological Sciences and Geology of the Faculty of Education, Ain Shams University. Macromorphological aspects were studied from the fresh specimens as well as from relevant literature (Tutin *et al.*, 1972; Tackholm, 1974; Bailey and Bailey, 1976; Beckett, 1983; Boulos, 2000).

Samples of pollen grains were examined with light and SEM. For light microscopic investigation, pollen grains were acetolyzed according to Erdtman (1960). Measurements were based on at least 25 fully developed grains per specimen. Pollen descriptions were based on both optical and SEM. Lumina and Muri were measured in the mesocolpia, chiefly from SEMGs. For scanning electron microscopy, mature anthers were selected from dry specimens, then pressed. The pollen grains were then fixed on clean stubs, coated with gold and examined at an

accelerating voltage of 25 kv. with Jeol. JSM 5300 SEM at Electron Microscope Unit, Faculty of Science, Alexandria University. The terminology concerning the description of pollen mainly follows that of Erdtman (1952, 1969).

Cytological preparations were made from seeds germinated on moist filter paper in petri-dishes using the Feulgen squash technique. For karyotype analysis, root tips were pretreated with 0.05% colchicine for 3-4 h. fixed overnight in 3:1 ethanol: glacial acetic acid and stored in 70% ethanol at 4°C. C-metaphase chromosomes were counted and their features, such as somatic number, mean length (MCL), mean arm ratio (r-value), total form percent (TF%), intrachromosomal asymmetry index (A_1) and interchromosomal asymmetry index (A_2) were determined according to Zarco (1986). Karyotype formula types of chromosomes determined according to Levan *et al.* (1964), were also studied on photographic prints enlarged to a magnification of 2000 X using Carl-Zeiss-photomicroscope III.

Results

Macromorphological diagnostic features

***Anagallis arvensis* (scarlet flowers):** Glabrous annual herb, to 10-25 cm in length. Stems diffuse and ascending, tetragonal, trailing and much branched. Leaves 0.5-2.5 x 0.5-1.5 cm^2 exstipulate, simple sessile, opposite, occasionally in threes, ovate (Fig. 1). Flowers solitary in leaf axil, pedicellate, hermaphrodite, actinomorphic typically pentamerous; pedicels 1-3.5 cm slender, as long as subtending leaf or slightly longer fruiting pedicels recurved. Calyx gamosepalous, persistent, lobed, acuminate. Corolla scarlet, broadly obovate overlapping. Stamens 5, in a single whorl, opposite the corolla lobes, filaments free. Pistil 1; ovary superior; carpels 5, locule 1; ovules numerous; style with one capitate stigma. Fruit is a capsule, globose, 3-5 mm in diameter, dehiscing by circumscissile. Seeds many.

***Anagallis arvensis* (blue flowers):** Similar to *A. arvensis* (with scarlet flower) but differs in (Fig. 1):

The Upper leaves are lanceolate; often dotted with dull brown glands on lower surface.

The petals are blue, not overlapping.

The flowering pedicels as a rule are not longer than the leaf or only slightly so.

Palynological features

***Anagallis arvensis* (scarlet flower)** [Fig. 2 (a-d) and Table 1].

The pollen grains are solitary, tricolpate, radially symmetrical and isopolar. They are prolate, polar axis (P) = 18.2 μm., equatorial axis (E) = 13.5 μm. The equatorial outline is more or less elliptic; the amb is convex-triangle-aperturate. ectocolpi are elongate, its length is nearly 17.32 and 2.64 μm width with equator (Fig. 2c). The colpus margin is distinct, the sides are usually tapering with acute ends (Fig. 2b). The colpus exine is slightly roughened adjacent to the endoaperture. The endoaperture is an equatorial lolongate (Fig. 2a, d). Surface sculpturing (tectum) is microreticulate faveolated (Fig. 2d). Lumina vary in shape, its diameter is nearly 0.14 μm. Muri are sinous and latimurate (i.e. muri are thicker than the distance cross lumina).

***Anagallis arvensis* (blue flower):** The pollen grains are monad, radially symmetrical; isopolar; tricolporate; subspheroidal in shape and in the P/E ratio (polar axis/ equatorial axis) (Erdtman, 1952) (Fig. 2 e–h). The polar axis (P) length is 18.57 μm and the equatorial axis (E) is 18.25 μm (Table 1). Ectocolpi are elongate, nearly equal to the polar axis in length and its width at the equator is 3.82 μm. (Fig. 2e). The colpus margin is distinct and often raised at the equator. The sides are tapering with acute to more or less rounded ends (Fig. 2f). The endoaperture is an equatorial lalongate, extending beyond the boundaries of the ectocolpi and it is covered with conspicuous operculum (Fig. 2f,g). The exine sculpturing (tectum) is reticulate (Fig. 2h). Lumina are regular and polygonal in shape. Muri are straight and angustimurate (i.e. muri are narrower than the distance across the lumina).

Cytological criteria

***Anagallis arvensis* (scarlet flower):** Somatic

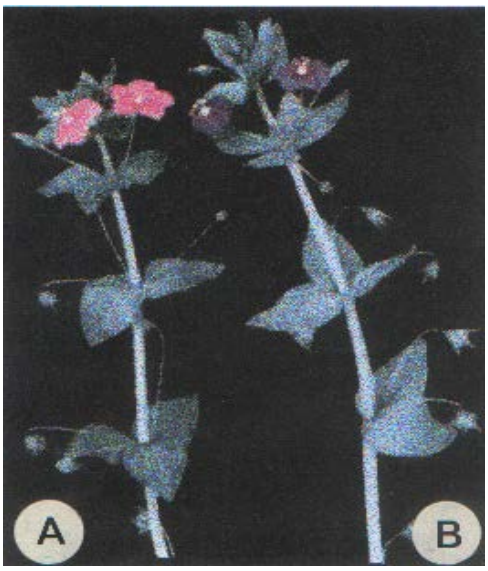

Fig. 1: Two taxa of *Anagallis arvensis*
a) *Anagallis arvensis* (Scarlet flowers)
b) *Anagallis arvensis* (blue flowers)

Table 1: Pollen morphological data of *Anagallis arvensis* (scarlet flower) and *Anagallis arvensis* (blue flower). All measurements are in microns

	Taxa	
	A. (scarlet flower)	A. (blue flower)
Character		
Shape	Prolate	Sub-spheroidal
Pollen size		
Polar axis (P) mean (range)	18.2 (17.3-19.2)	18.75 (17.2-20.3)
Eqatorial axis (E) mean (range)	13.5 (12.4-14.6)	18.25 (16.8-19.7)
P/E ratio	1.34	1.02
Colpus length	17.32	18.31
Colpus width at equator	2.64	3.82
Os type	Lolongate	Lolongate
Os length	3.25	5.5
Os width	2.08	6.11
Mesoco-lpium width	5.25	12.8
Exine sculpture (tectum)	Micro-reticulate faveolate	reticulate
Lumina diameter	0.14	0.57
Muri thickness	0.42	0.13

Os = Plural of ora: the inner pore like portion of a compound aperture

Table 2: Cytological features of *Anagallis arvensis* (scarlet flower)

Chr. pair	Chr. Length (μm)	Relative lengths	Short arm (μm)	Long arm (μm)	R-value	Relative R-value	Chromosome type
1	1.97	12.92	0.83	1.14	1.370	11.20	m
2	1.81	11.87	0.73	1.08	1.480	12.10	m
3	1.67	10.95	0.75	0.92	1.230	10.06	m
4	1.67	10.95	0.69	0.98	1.420	11.61	m
5	1.62	10.62	0.70	0.92	1.310	10.71	m
6	1.50	9.84	0.69	0.81	1.170	9.57	m
7	1.40	9.18	0.67	0.73	1.090	8.91	m
8	1.36	8.92	0.67	0.69	1.030	8.42	m
9	1.19	7.80	0.56	0.63	1.130	9.24	m
10	1.06	6.95	0.53	0.53	1.000	8.18	m
Tot.	15.250	100.00	6.820	8.430	12.230	100.00	-
Mean ± SE	1.525 ± 0.090	-	0.682 ± 0.030	0.843 ± 0.063	1.223 ± 0.052	-	-

chromosomal number of this species was found to be tetraploid of 2n = 40 (x = 10). The range of chromosomal length was 1.06 to 1.97 μm. It has one chromosome with median point centromere (M) and nine chromosomes with median region centromeres (m). The range of R-value (i.e. long arm/short arm) was 1 to 1.37. The total chromosome length (TCL) was 15.250 μm, the mean chromosome length (MCL) was 1.525±0.090 μm, the mean relative value was 1.223±0.052, the interachromosomal asymmetry index (A_1) was 0.018; while the interchromosomal asymmetry index (A_2) was 0.17 and the total form percent (TF%) was 44.72. The rest cytological features of this species are presented in Table 2; while the karyotypes are illustrated in Fig. 3A.

***Anagallis arvensis* (blue flower):** Somatic chromosomal number of this species also was found to be a tetraploid of 2n = 40 (x = 10). The range of chromosome length within was 1.09 to 1.95 μm. It has one chromosome with sub-metacentric chromosome (sm) and nine chromosomes with median region centromeres (m). The range of R-value was 1.42 to 1.41. The TCL was 14.980 μm.; the MCL was 1.498±0.081 μm, the mean relative value was 1.381±0.061,

Table 3: Cytological features of *Anagallis arvensis* (blue flower)

Chr. pair	Chr. Length (μm)	Relative lengths	Short arm (μm)	Long arm (μm)	R-value	Relative R-value	Chromosome type
1	1.95	13.02	0.81	1.14	1.41	10.21	m
2	1.79	11.95	0.73	1.06	1.82	13.18	sm
3	1.67	11.15	0.74	0.93	1.26	9.12	m
4	1.58	10.55	0.70	0.88	1.26	9.12	m
5	1.47	9.87	0.62	0.85	1.37	9.92	m
6	1.45	9.68	0.68	0.77	1.13	8.18	m
7	1.37	9.15	0.61	0.76	1.25	9.05	m
8	1.34	8.95	0.57	0.77	1.35	9.78	m
9	1.27	8.48	0.50	0.77	1.54	11.15	m
10	1.09	7.28	0.45	0.64	1.42	10.28	m
Tot.	14.980	100.02	6.41	8.570	13.81	99.99	-
Mean ± SE	1.498 ± 0.081	-	0.641 ± 0.036	0.857 ± 0.048	1.381 ± 0.061	-	-

Table 4: A survey of the studied taxa showing the perplexities of their delimitation levels

Levels of the category	Authors	Taxa: *Anagallis* (scarlet flower)	Taxa: *Anagallis* (blue flower)
Species	Linnaeus (1753)	*A. arvensis*	*A. latifolia*
	Dothan (1978-1986)	*A. arvensis*	*A. foemina*
	Beckett (1982)	*A. arvensis*	*A. foemina*
Sub species	Tackholm (1974)	*A. arvensis arvensis*	*A. arvensis* ssp. *latifolia*
	Mabberley (1997)	*A. arvensis*	*A. arvensis caerulea*
	Boulos (2000)	*A. arvensis arvensis*	*A. arvensis foemina*
Variety	Bailey (1935 and 1949)	*A. arvensis* var *phoenica*	*A. arvensis* var *cacrulea*
Forma	Bailey and Bailey (1976)	*A. arvensis*	*A. arvensis* forma *caerulea*
	Mabberly (1987)	*A. arvensis*	*A. arvensis* forma *cacrulea*

Table 5: Summary of the karyological criteria of the studied taxa

Criteria / Taxa	2n	n	TCL (μm)	MCL ±SE	M r-value ±SE	A_1	A_2	TF%	Karyotype formula
Anagallis (scarlet flower)	40	10	15.25	1.525 ± 0.090	1.223 ± 0.052	0.18	0.17	4.72	1 M + 9m
Anagallis (Blue flower)	40	10	14.98	1.498 ± 0.081	1.381 ± 0.061	0.17	0.25	42.79	9 m + 1sm

TCI = Total chromosomes lengths; MCL = Mean chromosome length; M r-value = Mean arm value; A_1 = Interachromosomal asymmetry index; TF % = Total form percent; M = Median point metacentric chomosome; m = Median region metacentric chromosome; sm = Sub-metacentric chromosome; A_2 = Interchromosomal asymmetry index

(A_1) was 0.17, (A_2) was 0.25 and the TF % was 42.79. The rest cytological features of this species are presented in Table 3, while the karyotypes are illustrated in Fig. 3B.

Discussion

Anagallis arvensis L. has long been a perplexing subject in the view of different authors (Table 4). Donoghue and

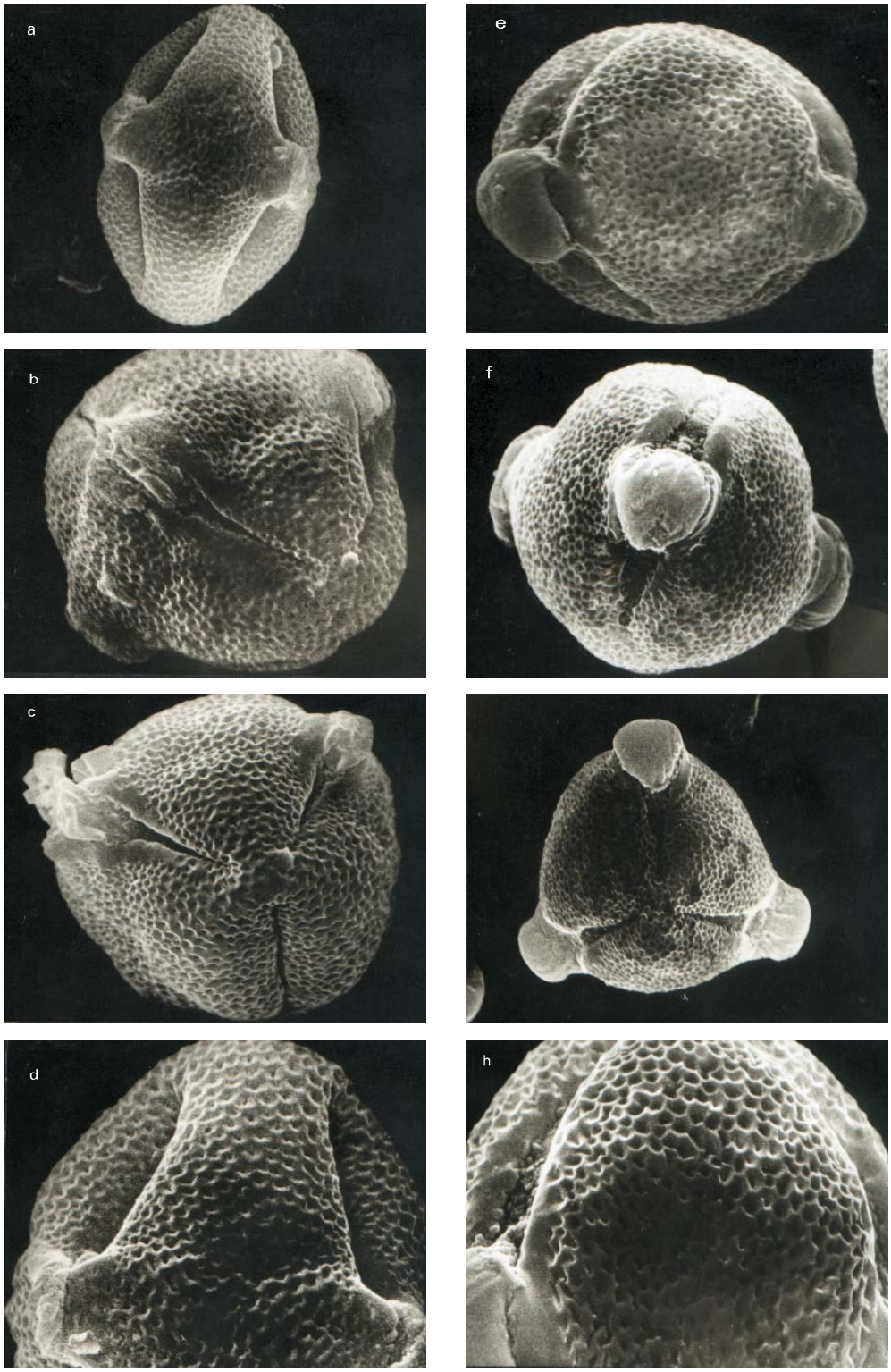

Fig. 2a-h: SEM micrographs of pollen grains

(a-d)	*Anagallis* (scarlet flower)	(e-h)	*Anagallis* (blue flower)
(a)	Equatorial view x 3,500	(e)	Equatorial view x 3,500
(b)	oblique view x 5,000	(f)	oblique view x 5,000
(c)	Subpolar view x 5,00	(g)	Subpolar view x 5,000
(d)	Exine detail (Tectum) x 7,500	(h)	Exine detail (Tectum) x 7,500

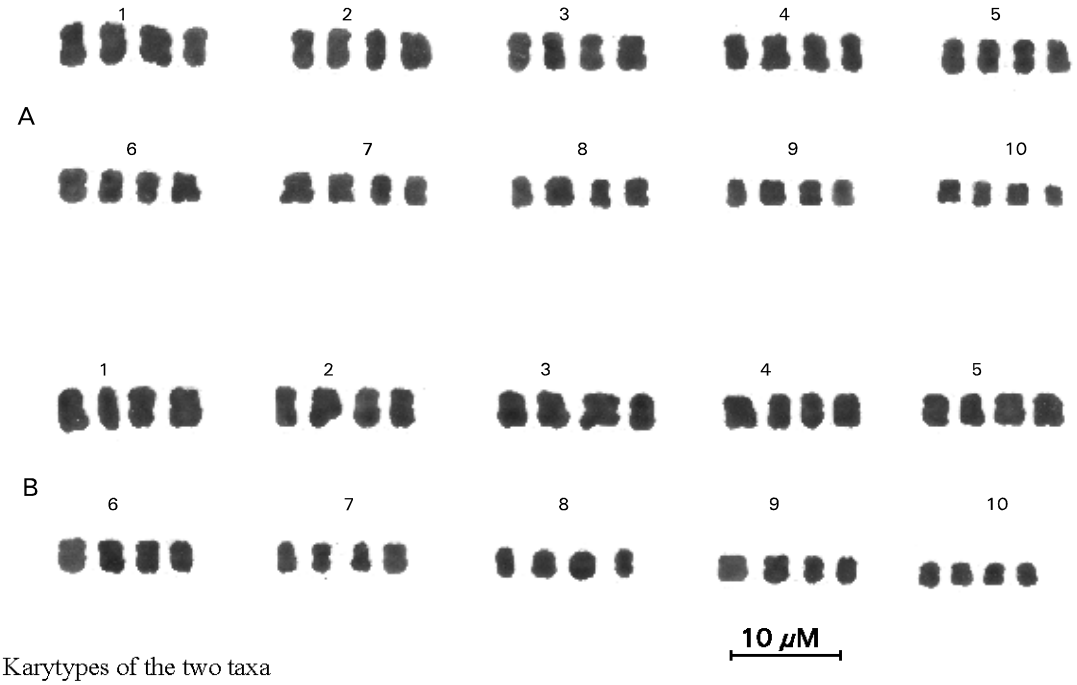

Fig. 3: Karytypes of the two taxa
(A) *Anagallis arvensis* (scarlet flower) (B) *Anagallis arvensis* (blue flower)

Sanderson (1992) have stressed on the importance of utilizing different criteria, both morphological and molecular, in reconstructing plant phylogeny and in the re-assessment of relationships between taxa. In their opinion, using only one or few criteria can be misleading. In present study, the morphological characters showed a closer relation between the two species studied. They have same habit, pentamerous flowers and fruits, but they are different in a few characters as: the upper leaves of *Anagallis arvensis* (blue flower) are lanceolate, have dotted dull brown glands on the lower surface of leaves (Dothan, 1978-1986), petals not overlapping and the pedicel of flower is not longer than the leaf or only slightly so, while they are ovate in *Anagallis arvensis* (scarlet flower) does not have that glands, petals overlapping and pedicel of flower is more or less equal to the leaf (Bailey and Bailey, 1935; Tutin *et al*., 1972; Boulos, 2000).

However, pollen micromorphology of the two studied taxa is shared in some characters as they are monad, radially symmetrical, isopolar and tricolporate. At the same time they are different in other as shape, which varied from prolate in *Anagallis arvensis* (scarlet flower) to subspheroidal in *Anagallis arvensis* (blue flower), the endoaperature of *Anagallis arvensis* (scarlet flower) is lolongate, while it is lalongate with distinct operculum in the other taxon and finally the fine sculpture of the exine surface (tectum) showed very good diagnostic characters. In *Anagallis arvensis* (scarlet) tectum is microreticulate and faveolated, muri are latimurate and *Anagallis arvensis* (blue) tectum is reticulate, muri are angustmurate.

Boulos (2000) stated that these two taxa are better placed as two subspecies. The same view was held earlier by Mabberley (1997).

As to the cytological criteria, little variations were recorded (Fig. 3, Table 5). Both taxa were found to be tetraploid with 2n = 40 and x = 10. Previous chromosome count reported for *Anagallis arvensis* L. by Fedorov (1969) was also 2n = 40. The same number was scored for *A. femina* Mill. Polyploidy is common among species of *Anagallis* L., for example, *A. serpens* Hochst. (2n = 60 and 66), *A. vaginatus* Turcz. (2n = 78 and 88) and *A. zosteriformis* Fernold. (2n = 52) (Fedorov, 1969).

Within the studied taxa, *Anagallis arvensis* (scarlet) was found to have the highest value of TCL (15.25 μm), MCL (1.525±0.090 μm), A_1 (0.18) and TF% (44.72%), while *Anagallis arvensis* (blue) have the highest values of; M r-value (1.381±0.061) and A_2 (0.25). More or less, both taxa were found to have short chromosomes (1.498 and 1.525 μm) and symmetric karyotypes as referred to by the TF% (42.79 and 44.72%). Chromosomes were varied between metacentric (M or m) as in *Anagallis arvensis* (scarlet) to submetacentric (sm) in *Anagallis arvensis* (blue).

It is worth mentioning that the no records of successful hybridization between the two taxa were stated. The two taxa seem to be reproductively isolated. Tutin *et al.* (1972),

mentioned that, *A. arvensis* appeared to be variable in several characters, including size of leaf and corolla; in their opinion though subspecific taxa have been described, they intergrate and it is difficult to recognize them over the whole range of the species.

Thus, the present study seem to support the view held by several authors (Dothan, 1978-1986; Beckett, 1983; Mabberley, 1997; Boulos, 2000 and several others) that the two taxa are better treated as two distinct subspecies or even more higher levels. However, the subject still needs more investigation and further studies, using cosmopolitan material and utilizing other criteria.

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