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Proceeding Paper

Comparative Taxonomic Study of *Balanites aegyptiaca* (L.) Delile (Zygophyllaceae) †

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Abstract: *Balanites aegyptiaca* or Desert Date (Zygophyllaceae) is widely distributed in arid and semi-arid regions in Africa and South Asia. The current study aims to identify the infraspecific variation between *B. aegyptiaca* native to Egypt and Saudi Arabia. Detailed macro- and micromorphological analyses of leaflets, petioles, stems, spines, and fruits were performed using a light and Scanning Electron Microscope (SEM). Statistical analysis was performed by using the relevant R- software packages. Leaflet shape and apex, leaflet length/width ratio, leaflet indumentum density, and the petiole length were recorded. Based on those traits, the present study suggests the existence of one variety, "*B. aegyptiaca* var. *aegyptiaca*" in Makkah, Saudi Arabia. At the same time, two varieties were recorded in Egypt "*B. aegyptiaca* var. *aegyptiaca* and *B. aegyptiaca* var. *tomentosa*".

Keywords: Anatomical study; *Balanites aegyptiaca*; Macro- and micromorphology; SEM; Stomata; R-software; Zygophyllaceae

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1. Introduction

Balanites aegyptiaca (L.) Delile, commonly known as 'Desert Date', is a prickly evergreen tree up to 15 m high. It is a vital source of food and medicine, and it has an active role in conserving and renewing degraded vegetation cover in arid areas [1]. *Balanites aegyptiaca* is native to semi-arid regions, widely distributed in most African countries [2,3].

The genus *Balanites* Delile undergone numerous nomenclature and taxonomic changes. Alpinio first described the Desert Date in 1592 [4] under *Agihalid*. In 1753, Linnaeus recongnized the species *Balanites aegyptiaca* as *Ximenia aegyptiaca* L. [5]. Delile renamed the genus *Balanites*, which means the fruit in Latin [6]. Hegnauer and Cronquist placed *Balanites* in the family Zygophyllaceae based on the similarities between flavonoids of *Balanites* and that found in the other members of the Zygophyllaceae [7,8]. Sands treated *Balanites* in a separate monogeneric family called Balanitaceae [9]. Boesewinkel supported the separation of the family Balanitaceae according to the unique characters of ovule and seed [10]. However, information on anatomy, embryology, taxonomy, and pollen morphology was deduced by Singh [11]. In addition, molecular data of the chloroplast

and nuclear markers (*rbcL*, *trn*L-F and ITS) supported the genus placement within Zygophyllaceae rather than Balanitaceae [12,13].

Sands; carried out a taxonomic revision on the genus *Balanites*, and divided the polymorphic *B. aegyptiaca* into five varieties: *aegyptiaca*, *ferox*, *quarrei*, *pallida*, and *tomentosa*. He recognized these varieties based on spine length, leaflet shape, indumentum density of leaflet and flower, pedicel length, and the number of flowers per inflorescence [2,3]. In Egypt, Maksoud and El Hadidi; recognized eight flavonoids; six from the vegetative parts and two from the fruits of *B. aegyptiaca* [14]. Amer et al. performed a biosystematics study on eight Egyptian populations of *B. aegyptiaca* var. *aegyptiaca*, and recognized three ecotypes: Nile valley, Sahelian and Xerotype based on some morphological traits, physicochemical soil pattern, and RAPD markers [3]. In the Flora of Egypt and the Flora of Saudi Arabia, no taxonomic ranks were verified below the species level [15,16]. The current study aims to evaluate the infraspecific variation among *B. aegyptiaca* populations native to Egypt and Saudi Arabia, and to identify these infraspecific taxa using macro-and micromorphological traits.

2. Materials and Methods

2.1. Plant Materials and Statistical Analysis

Sixteen samples of *B. aegyptiaca* were collected from Egypt and Saudi Arabia from their natural habitats. The collected specimens were deposited at the herbarium of Suez Canal University, Ismailia, Egypt (SCUI). The macromorphological traits were investigated for three to five replicates of each sample. Twenty-two quantitative and qualitative characters were measured for the stem, leaflet and fruit; the description follows Sands [2] and Smith [17]. The coordinates of the collection localities were recorded using Handy GPS, then listed in Table A1. Analysis of variance (ANOVA) was performed and followed by a Post Hoc Tukey Honestly Significant Difference (HSD) test. The data matrix used for the cluster analysis was based on 16 quantitative characters. Hierarchical clustering with Euclidean distance was conducted after being scaled and standardized for the data matrix [18].

2.2. Anatomical Study and Scanning Electron Microscope (SEM)

For the anatomical study, three fresh samples of each stem, leave and petiole were fixed in FAA solution and then preserved in 70% ethyl alcohol until sectioning. The samples were dehydrated gradually in a series of ethyl alcohol, then the paraffin method of Johansen [19] was applied. The sectioning was performed with Reichert-Jung, Depew, NY 14043 USA rotary microtome, with a thickness of 10 to 12 μm. The sectioning was done at the same regions for the stem, leaflets, and petioles of all sample replicate. Sections stained with 2% crystal violet and 2% Erythrosin B (saturated in clove oil) then permanently mounted with Canada balsam.

For Scanning Electron Microscope (SEM), the stem, spine, leaflet and fruit of *B. aegyptiaca* were fixed onto cupper stubs with double-sided adhesive tape and were coated for 5 min with gold in a polaron JFC-1100E coating unit. The samples were investigated and photographed by using JEOL JSM-IT200, Japan. Thirteen micromorphological characters of the stem were investigated, and twelve quantitative and qualitative traits were studied for leaflet abaxial (AB) and adaxial (AD) surfaces. In addition, sixteen quantitative and qualitative characters were described for the fruit micromorphology. The trichomes were counted in an area of 5μm x 5μm, and the micromorphological description followed Barthlott [20], Weryszko-chmielewska & Chernetskyy [21], Koch & Barthlott [22], and Khokhar et al. [23].

3. Results

3.1. Vegetative and Fruit Macromorphology

Balanites aegyptiaca is a spiny tree or shrub, and its height ranges from 1.5 up to 4 m. The density of spines per branch varies within the same individual tree and among different trees. Spines are unbranched or rarely branched in samples 11 (Edfu-Marsa Alam, Red Sea, Egypt) and 16 (Aswan, Egypt). The spine length ranges from 0.42 to 4.7 cm, while the spinule length ranges from 0.16 to 0.84 cm, with an average size of 0.56 cm.

Leaves are spirally arranged and stipulate; the stipules are always caducous and vary in length from 0.156 to 0.28 cm. An interpetiolar stipule was remarked for all samples with an average length of 0.528 cm. The leaves are bifoliate and subsessile or petiolate, where petiole subtends the spines. The petiole length varies from 0.10 to 0.99 cm, and the petiolule length is in the range of 0.06 to 1.60 cm. The leaflet colour varies from yellowish-green or pale to dark green. The leaflet size is 0.89−6.40 x 0.33−3.55 cm, and its area ranges from 0.21 to 14.85 cm^2 with an average of 2.86 cm^2.

The majority of the examined samples show elliptic leaflets. Other leaflet shapes are recorded as follows: Three samples represent the narrowly elliptic leaflets sample 12, (Edfu- Marsa Alam, Red Sea, Egypt) and samples 15 and 16, (Aswan, Egypt). Ovate leaflet is recorded in one sample 7, (Wadi el Gemal National Park, Egypt). The orbicular leaflet was recognized in sample 10 (Edfu- Marsa Alam, Red Sea, Egypt). Finally, the suborbicular leaflet is recorded for two samples 1 and 2 collected from Makkah, Saudi Arabia.

The leaflet apex is generally obtuse, except sample 10, which is rounded, and samples 3, 7, 9, 11, 13, 15 and 16 attain acute apices; meanwhile, the leaflet base is cuneate.

The fruit is single-seeded with a leathery or wrinkled surface, and its dimensions are 2.44−3.66 x 1.00−2.71 cm. The shape of the fruit is subspherical, ellipsoid, or ovoid.

ANOVA indicate a significant variation of morphological traits among samples with p-value ($p= 6.27x10^{-10}$*, R-squared= 0.963). The conducted clustering Agglomerative cluster analysis Figure 1 split the samples into two clusters, differentiating samples from Egypt and Saudi Arabia. Cluster A contains all individuals collected from Saudi Arabia, with samples 11 and 10 from Egypt collected from Marsa- Alam, Red Sea, Egypt. In contrast, sample 10 divergent into a single branch. Cluster B is devoted to *B. aegyptiaca* collected from Egypt. The data matrix based on 16 quantitative characters was used for the cluster analysis is illustrated with descriptive data in Table A2.

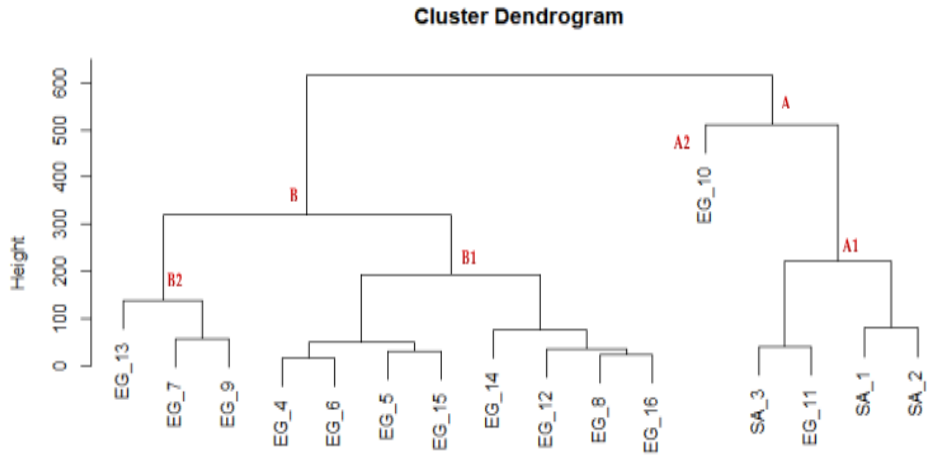

Figure 1. Agglomerative cluster analysis of *Balanites aegyptiaca* collected from Egypt (EG) and Saudi Arabia (SA).

3.2. Anatomical Characterization

The anatomical characterization of samples collected from Wadi El-Gemal (samples 4, 5, 6, 7, and 8) shows unique anatomical traits compared to those samples collected from

other localities. The leaflet's 'transverse section (T.S) shows more than one vascular bundle in the midrib region of sample 5 (Figure 2a); however, the others have only one vascular bundle (Figure 2b).

The palisade tissue constitutes >50% of the mesophyll layer compared to the small palisade layer <50% recorded in samples collected from other locations. Stem sections show differentiation into four regions: the epidermis, the hypodermis and cortex, and the pith. The epidermis shows anticlinal cutinized epidermal cells. The cross-section is composed mainly of parenchymatous cells. Three to four rows of lamellar collenchyma hypodermis filled with tannins present underneath the epidermis. Druses crystals spread, especially in the middle. Trichomes are non-glandular unicellular straight, reversed comma-shaped, in addition to little short compound hairs in specimens of the (Edfu- Marsa Alam, Red Sea) Figure 2b. Sample 14 was collected from High-dam region shows the presence of schizogenous glands in the cortex region Figure 2c. The anatomical traits of the petiole were alike in all samples. While sample 11 was gathered from Marsa Alam Aswan Road appears to have gap cavities underneath the hypodermis within the cortex region Figure 2d. The spine shows a unique anatomical pattern as it has no vascular bundles, and only fibers bands in the upper part of the cortex were detected. The parenchymatous loose cells in the pith region.

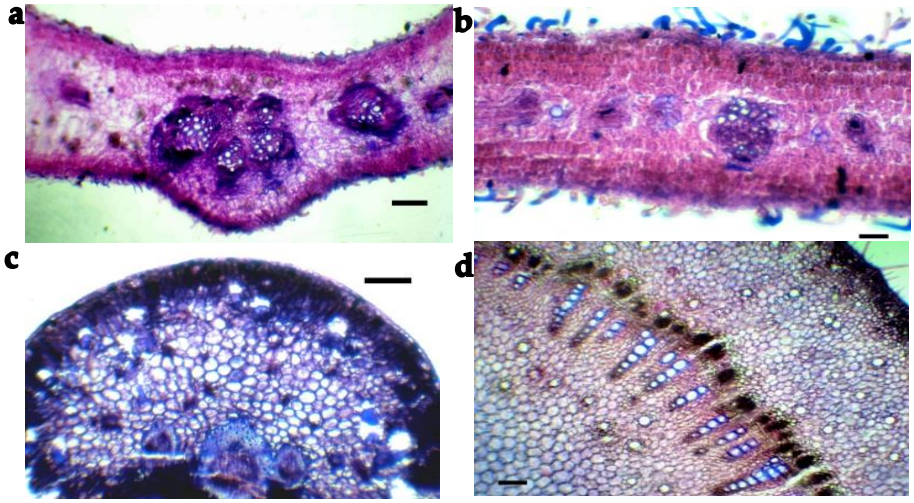

Figure 2. Light micrographs of stem, leaflet and petiole of *Balanites aegyptiaca*. **(a)** leaflet section with more than one vascular bundle; **(b)** leaflet section with one vascular bundle; **(c)** stem section showing the presence of schizogenous glands in the cortex region; **(d)** petiole section gap cavities underneath the hypodermis within the cortex region. Scale bar: **(a)** 225 μm; **(b)** 85 μm; **(c)** 55 μm; **(d)** 125 μm.

3.3. Vegetative and Fruit Micromorphology (SEM)

The vegetative organs (stem, spine, and leaflet) and the fruit of *B. aegyptiaca* are covered with unicellular non-glandular trichomes of different lengths Figure 3a; its length ranges from 28.31 to 429.21 μm. The surface of the trichome is smooth in most samples Figure 3b; while cuticular ornamentation in the form of dense striations is observed in sample 9 from Wadi el-Gemal Figure 3c. The trichomes have one lateral suture lying along the trichome length Figure 3c. Their density varies from sparse (6 trichomes per unit area) in sample 1 collected from South Al Jumum, Makkah, Saudi Arabia, moderate in sample 14 from Aswan (19 trichomes per unit area) and dense in sample 9 (30 trichomes per unit area).

Leaflets are amphistomatic with sunken stomata Figure 3f & 3g (indicated with stars) or deeply sunken Figure 3d-e & 3g actinocytic stomata on the stem, spine and leaflet of the studied samples. The stomata are surrounded by a ring of subsidiary cells of various numbers and shapes, where the number of cells ranges from five to eight cells Figure 3d-

j. For stem and spine, the superficial stomatal pore size is 9.53–21.35 x 6.78–22.76 µm, its area ranges from 55.12 µm² to 126.54 µm², and the stomatal opening size is 3.11–15.60 x 0.18–2.02 µm. The trichome density and the number of stomata on the spine decrease gradually toward the spine's tip. For leaflet, the superficial stomatal pore size is 8.54–16.73 x 5.02–11.98 µm, and its area is in the range of 27.06 µm² to 168.74 µm², and the stomatal opening size is 4.11–11.67 x 0.25–1.40 µm. The density of the trichome and the stomatal count on the abaxial leaflet surface is higher than that located on the adaxial surface.

Balanites aegyptiaca fruit surface is covered with trichomes (unicellular non-glandular trichomes) of length 22.16 to 232.97 µm, and it is characterized by the presence of lenticels Figure 3h. The superficial area of the lenticel varies from 54.87 to 112.50 µm², and its size is 9.97–14.62 x 6.52–10.13 µm. The epidermal cell size is 20.61–48.02 x 28.83–120.79 µm, and the epidermal cell area ranges from 544.99 µm² to 4148.23 µm². The epidermal cell outline is elongated rectangular in samples 9 and 14 collected from Egypt, or elongated elliptic in the sample collected from Saudi Arabia Figures 3i & 3j, respectively. The anticlinal wall is straight, the relief of cell boundary is channelled, and the curvature of the outer periclinal cell wall is convex Figure 3k. The fine relief of the cell wall exhibits scabrate ornamentation. In addition, waxy epicuticular secretions are noticed as secondary sculpture elements on the surface with various densities Figure 3k. The epicuticular secretions are in the form of waxy granules in sample 1 Figure 3l or non-entire flat crystalloids waxy platelets with sinuate or laciniate margin in samples 9 and 14, respectively Figures 3m & 3n, respectively.

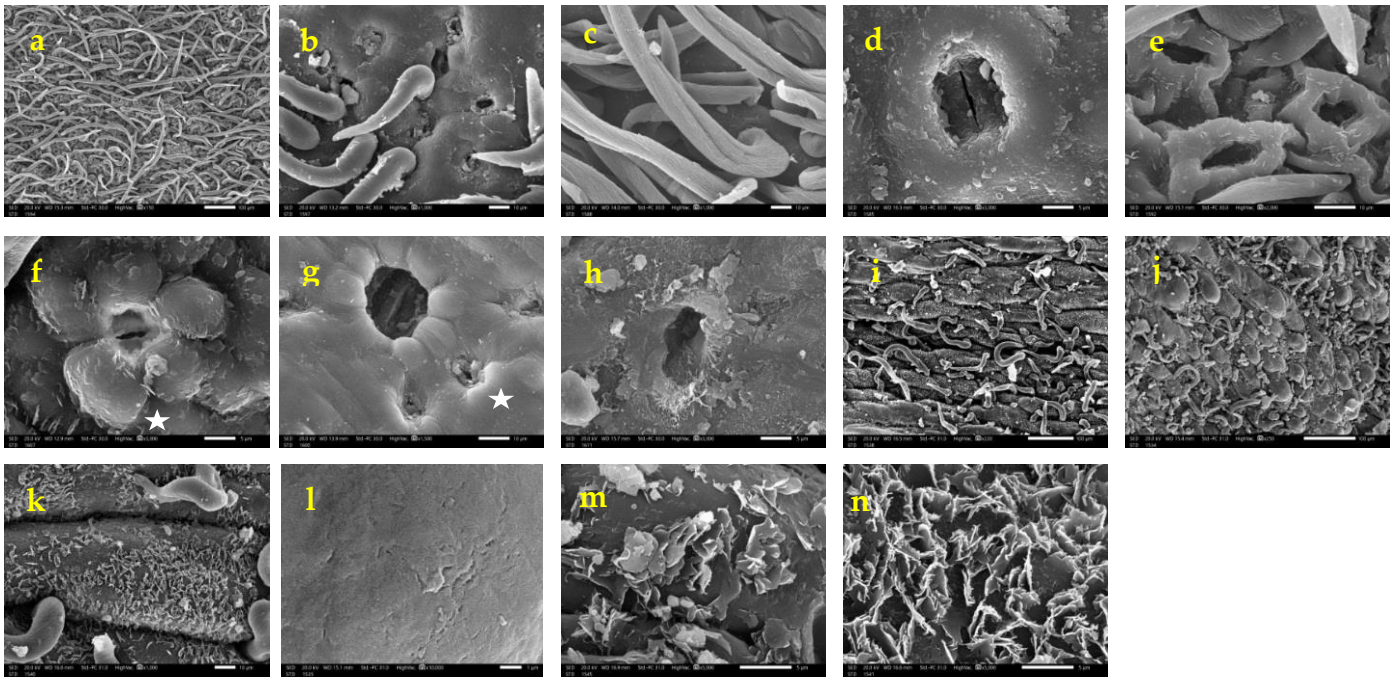

Figure 3. Scanning Electron Microscope (SEM) photomicrographs of *Balanites aegyptiaca*. (**a**) general view of the leaflet trichomes; (**b**) smooth trichome surface on the stem; (**c**) striate trichome surface with lateral suture on the stem; (**d–g**) sunken or deeply sunken stomata surrounded by a ring of subsidiary cells of various numbers and shapes; (**h–m**) fruit surface. (**h**) general view of the lenticel; (**i-j**) epidermal cell outline: (**i**) elongated rectangular, (**j**) elongated elliptic; (**k**) anticlinal wall, relief of cell boundary, the curvature of the outer periclinal cell walls, and the fine relief of the cell wall; (**l–n**) epicuticular secretions: (**l**) waxy granules, (**m**) non-entire flat crystalloids waxy platelets with sinuate margin, (**n**) non-entire flat crystalloids waxy platelets with laciniate margin. The stars indicate the sunken stomata. Scale bar: a, i, j= 100 µm; b, c, e, g, k= 10 µm; d, f, h, m, n= 5 µm; l= 1 µm.

4. Discussion

Balanites aegyptiaca is a polymorphic woody species with a wide distribution. Previously, many taxa have been described based on the floral characters of the species, which

is inconsistent and inconvenient to be used solely [2,3,24–28]. In the current study, an integrated taxonomic analysis was performed based on morphological data of the vegetative parts and the fruit; anatomical data of stem, spine, leaflet and petiole and the epidermal surface sculpture of stems, spines, leaflets (abaxial and adaxial surfaces), and fruits using Scanning Electron Microscope (SEM).

The conducted cluster analysis divided the studied samples into two clusters A and B. Both clusters A and B were subsequently divided into two subclusters 1 and 2, based on the petiole length, leaflet length/width ratio, leaflet shape and apex, and leaflet indumentum density. Cluster A includes samples characterized by their spine length that exceeded 2 cm, and sparse leaflet indumentum, which is congruent with the description of *B. aegyptiaca* var. *aegyptiaca* reported by Sands [2]. Whereas members of cluster B have spineless branches or shorter spines that reach up to 1.8 cm, and moderate or dense leaflet indumentum. The subcluster A1 has suborbicular or elliptic leaflet shape. While the subcluster A2 (contained sample 10 collected from Edfu- Marsa Alam Road, Edfu, Red Sea, Egypt) is characterized by the presence of orbicular leaflet shape (leaflet length/width ratio= 1.1) with rounded apex, the largest average leaflet area was 7.65 cm^2, and the longest petiole average value was 1 cm. The subcluster B1 attained overlapped character-states, whereas the subcluster B2 (containing samples 7, 9, and 13) is compatible with the description of *B. aegyptiaca* var. *tomentosa* mentioned by Sands [2], which has tomentose leaflets and their spines length is up to 3 cm long.

The present study suggests the presence of *B. aegyptiaca* var. *aegyptiaca* in Makkah, Saudi Arabia, while two varieties are identified in Egypt: *B. aegyptiaca* var. *aegyptiaca* and *B. aegyptiaca* var. *tomentosa*. Further confirmation is needed to identify these two varieties using molecular techniques such as molecular phylogeny, DNA barcoding, and whole-genome sequencing.

Appendix.

Table A1. Collection sites and sampling information of sixteen *Balanites aegyptiaca* samples collected from Egypt (EG) and Saudi Arabia (SA).

ID	Collection sites	Date	Latitude	Longitude
SA1	South Al Jumum, Makkah, Saudi Arabia	3 April 2021	21°34′27.0″ N	39°41′16.4″ E
SA 2	North Al Jumum, Makkah, Saudi Arabia	5 April 2021	21°35′56.1″ N	39°42′11.3″ E
SA 3	North Al Jumum, Makkah, Saudi Arabia	20 May 2021	21°38′51.5″ N	39°41′06.8″ E
EG4	Wadi el-Gemal National Park, Marsa Alam, Red Sea, Egypt	6 March 2021	24°38′30.88″ N	35°2′25.55″ E
EG 5	Wadi el-Gemal National Park, Marsa Alam, Red Sea, Egypt	6 March 2021	24°37′50.58″ N	35°1′43.186″ E
EG 6	Wadi el-Gemal National Park, Marsa Alam, Red Sea, Egypt	6 March 2021	24°37′37.03″ N	35°1′16.50″ E
EG 7	Wadi el-Gemal National Park, Marsa Alam, Red Sea, Egypt	6 March 2021	24°37′33.68″ N	35°1′11.06″ E
EG 8	Wadi el Gemal National Park, Marsa Alam, Red Sea, Egypt	6 March 2021	24°41′12.8″ N	35°4′59.64″ E
EG 9	Wadi el Gemal National Park, Marsa Alam, Red Sea, Egypt	6 March 2021	25°4′48.55″ N	34°53′12.22″ E
EG 10	Marsa Alam, Red Sea, Egypt	8 March 2021	25°04′43.3″ N	34°53′14″ E
EG 11	Edfu- Marsa Alam Road, Edfu, Red Sea, Egypt	8 March 2021	25°1′39.18″ N	33°3′13.79″ E
EG 12	Edfu- Marsa Alam Road, Edfu, Red Sea, Egypt	15 April 2021	25°1′39.40″ N	33°3′14.08″ E
EG 13	Edfu, Aswan, Egypt	15 April 2021	25°1′39.92″ N	33°3′12.54″ E
EG 14	High Dam region, Aswan, Egypt	30 April 2021	23°58′36.69″ N	32°53′50.28″ E
EG 15	High Dam region, Aswan, Egypt	30 April 2021	23°58′41.26″ N	32°53′53.52″ E
EG 16	Aswan University, Aswan, Egypt	30 April 2021	23°59′52.21″ N	32°51′37.07″ E

Table A2. Quantitative traits with descriptive data used for the agglomerative cluster analysis of *Balanites aegyptiaca*.

Variables	Mean ± StDev	SE Mean	Minimum	Q1	Median	Q3	Maximum	IQR
Leaflet length (cm)	2.693 ± 1.22	0.227	0.89	1.927	2.415	3.083	6.4	1.155
Leaflet width (cm)	1.273 ± 0.736	0.137	0.331	0.73	1	1.585	3.55	0.855
Leaflet length/ width ratio	2.321 ± 0.625	0.116	1.268	1.839	2.368	2.774	3.636	0.935
Leaflet area (cm^2)	2.864 ± 3.167	0.588	0.21	1.093	2.04	3.045	14.845	1.952
Petiole length (cm)	0.3936 ± 0.2466	0.0458	0.1	0.185	0.3	0.549	0.99	0.364

Petiolule length (cm)	0.377 ± 0.571	0.202	0.06	0.064	0.092	0.71	1.6	0.646
Apex angle (cm)	114.77 ± 21.38	4.04	58.5	107.13	117	127	171	19.88
Base angle (cm)	59.21 ± 26.36	4.89	26.5	39.25	54.5	69	124.5	29.75
Stipule length (cm)	0.2072 ± 0.0546	0.0223	0.156	0.156	0.2025	0.2545	0.28	0.0985
Interpetiolar stipule (cm)	0.5594 ± 0.1715	0.0767	0.275	0.4015	0.624	0.685	0.685	0.2835
Spines length (cm)	1.711 ± 1.291	0.358	0.42	0.662	1.37	2.457	4.7	1.794
Spinule length (cm)	0.4988 ± 0.2335	0.0953	0.1662	0.3328	0.461	0.7195	0.836	0.3867
Fruit length (cm)	3.0852 ± 0.3596	0.0848	2.438	2.819	3.048	3.4275	3.664	0.6085
Fruit width (cm)	1.751 ± 0.506	0.119	0.997	1.334	1.711	1.924	2.712	0.59
Fruit length/ width ratio	1.5792 ± 0.2597	0.0612	1.2142	1.3712	1.5348	1.8121	2.1509	0.4409
Fruit area (cm^2)	4.843 ± 1.88	0.443	2.59	3.521	4.229	6.137	9.49	2.616

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References

1. Abdelaziz, S.M.; Medraoui, L.; Alami, M.; Pakhrou, O.; Makkaoui, M.; Boukhary, A.O.M.S.; Filali-Maltouf, A. Inter simple sequence repeat markers to assess genetic diversity of the desert date (*Balanites aegyptiaca* Del.) for Sahelian ecosystem restoration. *Sci. Rep.* **2020**, *10*, 1–8, doi:10.1038/s41598-020-71835-9.
2. Sands, M.J.S. The Desert Date and Its Relatives: A Revision of the Genus *Balanites*. *Kew Bull.* **2001**, *56*, 1–128, doi:10.2307/4119431.
3. Amer, W.; Soliman, M.; Sheded, M. Biosystematic studies for *Balanites aegyptiaca* (Balanitaceae) populations in Egypt. *Flora Mediterr.* **2002**, 12, 353–367.
4. Alpinio, P. De plantis Aegypti liber. Franciscum de Franciscis Senensem. Venetiis, Ed. 1592.
5. Hansen, C.; Maule, A.F. Pehr 'Osbeck's collections and Linnaeus's Species Plantarum (1753). *Bot. J. Linn. Soc.* **1973**, *67*, 189–212, doi:10.1111/j.1095-8339.1973.tb01738.x.
6. De Roziere, M. Description minéralogique de la Vallée de Qoceyr in Description de l'Egypte, Histoire Naturelle, Vol. 2. Paris: L'Imprimerie Impériale 1813, 83–98.
7. Hegnauer, R. Chemotaxonomy der pflanzen.6. ; Basel & Stuttgart., 1973.
8. Cronquist, A.; Takhtadzhiăn, A.L. An integrated system of classification of flowering plants; Columbia university press: 1981.
9. Sands, M.J.S. Balanitaceae; Addis Abeba University and Sweden: uppsala University, 1989; Vol. Vol.3.
10. Boesewinkel, F.D. Ovule and seed characters of *Balanites aegyptiaca* and the classification of the Linales-Geraniales-Polygalales assembly. *Acta Bot. Neerlandica* **1994**, *43*, 15–25, doi:10.1111/j.1438-8677.1994.tb00730.x.
11. Singh, K.; Das, M.; Samanta, A.; Kundu, S.; Sharma, S. Evaluation of certain feed resources for carbohydrate and protein fractions and in situ digestion characteristics. *Indian J Anim Sci.* **2002**, 72.
12. Sheahan, M.C.; Chase, M.W. Phylogenetic relationships within Zygophyllaceae based on DNA sequences of three plastid regions, with special emphasis on Zygophylloideae. *Syst. Bot.* **2000**, *25*, 371, doi:10.2307/2666648.
13. Godoy-Bürki, A.C.; Acosta, J.M.; Aagesen, L. Phylogenetic relationships within the New World subfamily Larreoideae (Zygophyllaceae) confirm polyphyly of the disjunct genus *Bulnesia*. *Syst. Biodivers.* **2018**, *16*, 453–468, doi:10.1080/14772000.2018.1451406.
14. Maksoud, S.A.; El Hadidi, M.N. The flavonoids of *Balanites aegyptiaca* (Balanitaceae) from Egypt. *Plant Syst. Evol.* **1988**, *160*, 153–158, doi:10.1007/bf00936042.
15. Boulos, L. Flora of Egypt checklist, revised annotated edition. Al-Hadara Publishing, Cairo 2009, 198–201.
16. Migahid, A. Flora of Saudi Arabia. King Saud University Press. Riyadh: 1974.
17. Smith, T. Plant Systematics by Michael G. Simpson. *Syst. Bot.* **2006**, *31*, 631–632, doi:10.1600/036364406778388647.
18. Kassambara, A.; Mundt, F. Factoextra: extract and visualize the results of multivariate data analyses. R package version 2017, 1, 2017.
19. McCracken, E.; Johansen, D.A. Plant Microtechnique. *Trans. Am. Microsc. Soc.* **1940**, *59*, 405, doi:10.2307/3222557.

20. Barthlott, W. Epidermal and seed surface characters of plants: systematic applicability and some evolutionary aspects. *Nord. J. Bot.* **1981**, *1*, 345–355, doi:10.1111/j.1756-1051.1981.tb00704.x.
21. Weryszko-Chmielewska, E.; Chernetskyy, M. Structure of trichomes from the surface of leaves of some species of *Kalanchoë* Adans. *Acta Biol Cracov Ser Bot* **2005**, 47, 15–22.
22. Koch, K.; Barthlott, W. Plant Epicuticular Waxes: Chemistry, Form, Self-Assembly and Function. *Nat. Prod. Commun.* **2006**, *1*, 1934578 0600101123, doi:10.1177/1934578x0600101123.
23. Khokhar, A.L.; Rajput, M.T.; Tahir, S.S. Toxonomic study of the trichomes in the some members of the genus *Convolvulus* (Convolvulaceae). *Pak. J. Bot.* **2012**, 44, 1219–1224.
24. El-Banhawy, A.H.; Elkordy, A.; Farag, R.; Elbar, O.A.; Faried, A.; Ellamouni, F. Taxonomic Significance of the Leaf Geometric and Micrometric Attributes in the Discrimination of Some Cultivars of *Mangifera indica* L.(Anacardiaceae). *Egypt. J. Bot.* **2020**, *61*, 255–269, doi:10.21608/ejbo.2020.40870.1550.
25. El-Banhawy, A.; Al-Juhani, W. DNA barcoding and phylogeny of *Phlomis aurea* (Lamiaceae) endemic to Sinai peninsula, Egypt. *Pak. J. Bot.* **2019**, *51*, 1263–1271, doi:10.30848/pjb2019-4 (18).
26. Faried, A.; El-Banhawy, A.; Elqahtani, M. Taxonomic, DNA Barcoding and Phylogenetic Reassessment of the Egyptian *Ephedra* L. (Ephedraceae). *Catrina: Int. J. Environ. Sci.* **2018**, *17*, 1–13, doi:10.21608/cat.2018.14292.
27. El-Banhawy, A.; Kamel, W.M.; El-Din, E.M.G. *Salvia lanigera* var. *grandiflora* Benth: A New Record in the Flora of Egypt. *J. Ecol. Heal. Environ.* **2016**, *4*, 87–89, doi:10.18576/jehe/040205.
28. El-Banhawy, A.; Nour, I.; Acedo, C.; ElKordy, A.; Faried, A.; Al-Juhani, W.; Gawhari, A.; Olwey, A.; Ellmouni, F. Taxonomic revisiting and phylogenetic placement of two endangered Pplant species: *Silene leucophylla* Boiss. and *Silene schimperiana* Boiss. (Caryophyllaceae). *Plants* **2021**, *10*, 740, doi:10.3390/plants10040740.