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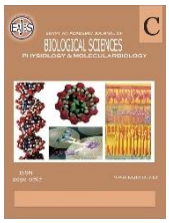

Macro-Morphological Leaf Variations Between Bombacaceae and Malvaceae withTheir Significance In Taxonomy and Phylogeny

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ABSTRACT

New taxonomic opinions of the Bombacaceae, with other families, downgraded the families into subfamilies under the Malvaceae s.l. These opinions are approved by molecular analysis data only. This work has been done to investigate the relation between the two groups on the basis of the external leaf macro-morphological characteristics. Eleven species covering both the two families subjected in this study. Sixteen macro-morphological characters have been investigated, measured, and subjected to clustering analysis using the UPGMA program. Results: The most important characters are those concerning the leaf type, leaf blade apex, leaf blade margin, number of teeth/cm, type of leaf blade venation, and number of main veins. The results obtained gave a clear separation between the taxa of the Bombacaceae from those of Malvaceae without the delimitations of the tribes and sub-tribes. Conclusion: This work confirmed the separation of the two families as separate entities and did not support their downgrades as sub-families. Meanwhile, characters of the Bombacaceae can be primitive to those of the Malvaceae which can be a step in the Malvaceous phylogeny.

INTRODUCTION

The Bombacaceae Family (Bombax, Baobab, or Kapok family) is a small family of about 28 genera with 200 species (Joly, 1991) with many economic and ornamental uses. Members of this family occur naturally in tropical and subtropical regions of the world, especially in tropical America (Benson, 1970). This family is considered one of the families subjected to many arguments considering their taxonomic position. The old traditional taxonomic treatments (Bentham & Hooker, 1867 and Engler & Prantl, 1896), beside recent works such as Cronquist (1981), Heywood *et al*. (2007) and Takhtajan (2009), treated members of the Bombacaceae as an independent family under order Malvales and this is the most acceptable taxonomical position of the family. Family Bombacaceae is considered the small flowering plant's family in contrast to Malvaceae; with perennial, deciduous, tall and woody trees (Benson, 1970; Joly, 1991 and Heywood *et al*., 2007). Trees of this family have showy and brightly coloured flowers, which make them ornamental plants besides their economically used benefits (Perez-Arbelaez, 1956 and Paula *et al*., 1997). Despite the morphological difference between members of the Bombacaceae with members of the Malvaceae, recent molecular works downgraded members of the Bombacaceae and other nearby families as subfamilies under the Malvaceae *sensu lato* (APG I, II, III, IV and Bayer & Kubitzki, 2003).

Some features are closely related between members of both families as internal structure, stamen and floral characters beside chemical constituents, but they were treated as separate, closely related families under order Malvales (Bentham and Hooker, 1867; Engler and Prantl, 1896). Recent taxonomic treatments (Cronquist, 1981; Heywood *et al*., 2007 and Takhtajan, 2009) approved the separation of the two families, besides Robyns (1971) and Sharma's (1993) opinions that the pollen grains of the two families are entirely different as those belonging to members of the Bombacaceae are smooth tricolpate while pollens of Malvaceae are echinate or spinulate porate. Besides the different habits and leaf morphological variations, this difference gives members of the two families their unique groupings. Although members of the two families can be well identified, some genera remain in dispute. Eldin (1935) considered members of Malvaceae as an evolutionary phylogenetic step from the Bombacaceae.

The present work is a taxonomical study of the leaf macro-vegetative characters in four common trees belonging to members of the Bombacaceae to clarify the main differences between them and some members of the different tribes under the family Malvaceae *sensu stricto*. Hibisceae, Gossypieae and Malveae as tribe Kydieae are represented in the Egyptian flora. These tribes are under Malvaceae *s.s.*, which become subfamily Malvoideae in the new classification systems under Malvaceae *s.l.* (APG systems).

MATERIALS AND METHODS

Fresh leaves and flowers collected from four trees of Bombacaceae are present in the different botanical gardens in Egypt, as mentioned in Table (1). These trees are of four species under three genera: *Bombax ceiba* L., *Ceiba pentandra* (L.) Gaertn. Fruct. Sem., *Ceiba speciosa* A.St.-Hil.

Pl. Usuel. Bras. and *Pseudobombax elipticum* (Kunth) Dugand Caldasia. These beside seven species belonging to three tribes of Malvaceae *s.s.*; Malveae / Abutilinae (*Abutilon*), Malveae / Sidinae (*Sida*), Malveae / Eumalvinae (*Althaea* and *Malva*), Hibisceae (*Hibiscus*) and Gossypieae (*Gossypium*); as representatives taxa for comparison. Each species was investigated carefully in at least five fresh specimens, and a few species herbarium sheets were used.

The macro-morphological leaf characters of the eleven studied species were carried out on ten leaves from the fourth top node of the branch. Measurements of the leaf petiole, leaf blade length, and width, as well as their ratio, are taken using a ruler. The number of lateral veins, as well as the number and state of teeth in the leaf margin per unit length, was counted under the stereomicroscope at a 1cm length from the margins. Description of the leaf blade, bases, margins, apices as well and venation was according to Stearn (1983).

The collected samples and their related information are summarized in Table (1). The samples were identified according to Kubitzki (2003), while the taxonomic authorization of Latin species names was according to datasets of the Global Biodiversity Information Facility (GBIF).

Clustering Analysis:

All the measured characters are subjected to the SPSS program to calculate the standard deviation. Clustering analysis of the sixteen characters studied in the different species under investigation with the aid of PAST program v.3.22 (Hammer *et al*., 2001).

Table 1: Samples collected with their families, sites, dates and collected specimens' locations.

Family	Tribe	Genus	Species	Site & date	Location
Bombacaceae		*Bombax* L.	*B. ceiba* L. Sp. Pl. 511. (1753)	Faculty of Agriculture botanic garden, Alex. Univ. (March 2020)	Alexandria University Garden (F)
		Ceiba Mill	*C. pentandra* (L.) Gaertn. Fruct. Sem. Pl. 2: 244 (1791)	Shehab Mazhar Botanic Garden, Giza, Cairo (June 2019)	Shehab Mazhar Garden (F)
			Ceiba speciosa (A.St.-Hil., A. Juss. & Cambess.) Ravenna 63.(1827) Onira 3: 46 (1998)	Orman Botanic garden, Giza, Egypt (November 1976)	Orman Botanical Garden (F)
				Faculty of Science Botanic Garden, Alex. Univ. (May 2018)	Alexandria University Garden (F)
		Pseudobombax Dugand	*P. elipticum* not italic *Caldasia, ii* 67. (1943)	Orman botanic garden, Giza, Egypt (April 2018)	Orman Botanical Garden (F)
				Shehab Mazhar Botanic Garden, Giza, Cairo (June 2019)	Shehab Mazhar Garden (F)
Malvaceae	Malveae / Abutilinae	*Abutilon* Mill.	*A. hirtum* (Lam.) Sweet. Hort. Brit. 53. (1826).	Faculty of Science Botanic Garden, Alex. University. June (2018)	Alexandria University Garden (F)
				Shehab Mazhar Botanical Garden	Shehab Mazhar Garden (F)
	Malveae / Sidinae	*Sida* L.	*S. alba* L. Sp. Pl., ed. 2, 960 (1763)	Wadi Feiran (St.123) N. 28 45' 48" E. 33 23' 448" A. 242 m (May 2005)	Tanta University Herbarium (H)
	Malveae / Eumalvinae	*Althaea* L.	*Al. ludwigii* L. Mant. 98 (1767)	Jebel Hareem, 1900 m, fallow field, Oman (May2002)	Shehab Mazhar Herbarium (H)
		Malva L	*M. neglecta* Wallr.	Burg El-Arab, Alexandria (April 2005)	Alexandria University Garden (F)
			M. parviflora L.	Movempick garden, Alexandria (August 2001)	Alexandria University Herbarium (H)
	Hibisceae	*Hibiscus* L.	*H. rosa-sinesis* L. Sp. Pl. 694. (1753).	Faculty of Science botanic garden, Alex. University. July (2018)	Alexandria University Garden (F)
	Gossypieae	*Gossypium* L.	italic L. Sp. Pl., ed. 1, 693 (1753)	Gebel G'afar, Aderar Amallel Ecolodge garden, Siwa Oasis, Egypt (July 2007)	Shehab Mazhar Herbarium (H)

RESULTS

The results are summarized in Tables (2 and 3) and illustrated in Plate 1(photos 1-11).

Table 2: Morphological characters of the studied taxa.

No.	Species	Habit	Life span	Petiole L (cm)	Leaf				
					Type	L (cm)	W (cm)	L/W	Shape
1	*Bombax ceiba*	Tree	Per	8.8-13.2 11.0 ±0.09	C.P	9.2-16.8 13.0 ±0.19	2.0-4.2 3.85 ±0.12	4.2	Lanc
2	*Ceiba pentandra*	Tree	Per	2.8-6.2 4.5 ±0.12	C.P	5.0- 6.2 5.6 ±0.12	1.2-3.5 1.8±0.16	3.5	Lanc
3	*Ceiba speciosa*	Tree	Per	2.0-3.4 3.0 ±0.15	C.P	5.2-7.4 6.2 ±0.22	3.4- 4.2 3.8 ±0.09	1.73	Ov
4	*Pseudobombax elipticum*	Tree	Per	8.7-12.4 10.2 ±0.07	C.P	6.5-8.0 7.8 ±0.12	3.8-5.2 5.9 ±0.26	1.5	Ob
5	*Abutilon hirtum*	Shr	Per	3.5-3.6 3.5 ±0.05	Sim	4.0-5.0 4.5±0.5	4.1-6.1 4.90 ±1.0	0.92	Cord
6	*Sida alba*	Her	An	0.6-1.3 0.85±0.0	Sim	1.4-1.6 1.5 ±0.1	0.7-1.6 1.2±0.15	1.76	Ov
7	*Althaea ludwigii*	Her	An	2.1-2.4 2.25 ±0.15	Sim	1.0-1.3 1.15 ±0.15	1.3-1.5 1.4 ±0.1	0.82	Pf
8	*Malva neglecta*	Her	An	3.8-5.2 4.3 ±0.05	Sim	4.2-5.6 5.0 ±0.22	5.8-6.5 6.6 ±0.12	0.75	Pl
9	*Malva parviflora*	Shr	An	1.5-2.5 1.8 ±0.06	Sim	2.2-4.4 3.6 ±0.16	2.8-4.8 3.9 ±0.26	0.90	Pl
10	*Hibiscus rosa-sinensis*	Shr	Per	2.5-3.2 2.75 ±0.15	Sim	7.6-9.8 8.25 ±0.35	3.2-4.0 3.15 ±0.15	2.5	Ov
11	*Gossypium herbaceum*	Her	An	1.7-3.0 2.1 ±0.12	Sim	2.8-3.5 3.2 ±0.32	3.0-3.8 3.6 ±0.21	0.90	Tl.

Table 2 (Cont.)

No.	Species	No. Leaf-lets	Leaf blade					Venation		
			Base	Apex	Margin	Texture	No. of teeth/cm	Type	No. of L. veins	State
1	*Bombax ceiba*	5	Att.	Acm	Entire	Leath	0	RPi	10-14	Cra
2	*Ceiba pentandra*	7	Cun	Acm	Entire	Papy	0	RPi	10-14	Cra
3	*Ceiba speciosa*	5	Att	Acm	serrate	Leath	6	RPi	18-28	Cra
4	*Pseudobombax elipticum*	5	Cun	Ac	Rug	Cor	0	RPi	18-20	RP
5	*Abutilon hirtum*	1	Cd	Ac	Dent	Velv.	12	Pal	8-10	Rad
6	*Sida alba*	1	Tr	Ac	Dent	Papy	8	RPi	1	Cra
7	*Althaea ludwigii*	1	Tr	Td	Entire	Cor	0	RPi	5	Cra
8	*Malva neglecta*	1	Tr	Td	Cr	Tar	4	Pal	5-7	Rad
9	*Malva parviflora*	1	Cd	Td	Cr	Tar	4	Pal	5-7	Rad
10	*Hibiscus rosa-sinensis*	1	Att	Acm	Dent	Cor	6	RPi	9	RP
11	*Gossypium herbaceum*	1	Cd	Acm	Entire	Cor	0	RPi	5	Rad

Abbreviations: Ac=Acute, Acm=Accuminate, Att=Attenuate, Alt=Alternate, An=Annual, C.P=Compound palmate, Cd=Cordate, Cor=Coriaceus, Cr=Crenate, Cra=Craspedromus Cun=Cuneate, Dent=Dentate, Her=Herb, Ins=Insertion, L=Length, Lanc=Lanceolate, Leath=Leathery, Ob=Oblong, Orb=Orbicular, Ov=Ovate, Pal=Palmate, Pap=Papyrous, Par=Parallel, Per=Perennial, Pl=Pentalobate, Pf=pinnatifidus, Rad=Radiatus, RP=Reticulato-pinnatus, RPi=Reticulate pinnate, Ru=Rugate, Shr=Shrub, Sim=Simple, Tar=Tartareus, Td=Tridentate, Tl=Trilobate, Tr=Truncate, W=Width.

Table 3: List of characters, character states and codes used for the eleven studied taxa.

No.	Character	Type	character states
1	**Habit**	MQO	1-Herb, 2-Shrub, 3-Tree
2	**Life Span**	Binary	1-Annual, 2-Perennial
3	**Petiole length**	Cont.	
4	**Leaf type**	Binary	1-Simple, 2-Compound palmate
5	**Leaf length**	Cont.	
6	**Leaf width**	Cont.	
7	**Leaf or leaflet shape**	MQU	1-Lanceolate, 2-Ovate, 3-Orbicular, 4-Cordate, 5-Pinnatifid, 6-Pena-lobate,7-Tri-lobate
8	**No. of leaflets**	Cont.	
9	**Leaf blade base**	MQU	1-Cordate 2-Trncate, 3-Attenuate, 4-Cuneate
10	**Leaf blade apex**	MQU	1-Acuminate, 2-Acute, 3-Tridentate
11	**Leaf blade margin**	MQU	1-Entire, 2-Serrate, 3-Dentate, 4-Crenate, 5-Rugate
12	**Leaf blade texture**	MQU	1-Papyraceus, 2-Leathery, 3- Coriaceus, 4-Velvety, 5-Tartareus
13	**No. of dentate/cm**	Cont.	
14	**Venation type**	Binary	1-Pinnate, 2-Palmate
15	**No. of veins**	Cont.	
16	**Venation state**	MQU	1-Craspedrtomous, 2-Radiatus, 3-Reticulato-pinnatus

Cont. = Continuous, MQO=Multistate qualitative ordered, MQU=Multistate qualitative unordered.

A-Family Bombacaceae:

Taxa belonging to this family are perennial large woody trees, having stipulated long petiolated compound leaves. The petiole length varied from 2.0 cm in *Ceiba speciosa to* 13.2 cm in *Bombax ceiba.* The leaves are of the compound palmate type, comprising five to seven leaflets. The leaflet lengths ranged from 5.0 cm in the two *Ceiba* species and reached 16.8 cm in *B. ceiba.* The net shape of the leaflets is lanceolate, ovate, or even oblate (Photos. 1, 2, 3, 4). The leaflet's texture is leathery in both *B. ceiba* and *C. speciosa*, papyraceous in *C. pentandra* and coriaceous in *P. ellipticum.*

The leaflet bases are either attenuate or cuneate while their apices are acuminate or acute and all have smooth margins. The venation in all of them is reticulate pinnate, but the number of lateral veins varied from 10 in *B. ceiba* and *C. pentandra* to 28 in *C. speciosa.* The lateral veins are in a craspedromus pattern, except in *P. ellipticum* they are reticulato-pinnatus pattern.

B-Family Malvaceae:

The studied taxa of this family are annual herbs, except both *Abutilon hirtum* (Lam.)Sweet. Hort. Brit. and *Hibiscus rosa-sinensis* L. are perennial shrubs. They have stipulated petiolated simple leaves. The petiole lengths vary from 0.6 cm in *Sida alba* L. to 5.2 cm in *Malva neglecta* Wallr. The leaf blade length differs between the taxa, the shortest leaves were recorded in *S. alba* (0.7 cm) and the longest leaf blade was recorded in *M. neglecta* (6.5 cm). The leaves are cordate in *Abutilon hirtum*, pinnatifid in *Althaea ludwigii* L. Mant., trilobate in *Gossypium herbaceum* L., pentalobate in the two *M.* species and ovate in both *S. alba* and *H. rosa-sinensis* (Photos. 5, 6, 7, 8, 9, 10, 11). The leaf blade texture is velvety in *Abutilon hirtum*, papyraceous in *S. alba*, tartareus i.e. having a rough crambled surface, in the two *Malva* species and coriaceous in both *H. rosa-sinensis* and *G. herbaceum*.

The leaf blade base is either cordate or truncate except in *H. rosa-sinensis* it is attenuate, while the apices are acute in both *Abutilon hirtum* and *S. alba*, tridentate in *Althaea ludwigii* and the two *Malva* species, while they are acuminate in both *Althaea*

ludwigii and *G. ellipticum*. The leaf blade margin is dentate in all the studied taxa, except in the two *Malva* species it is crenate and entire in both *Althaea ludwigii* and *G. ellipticum*. The number of teeth/cm varied greatly between the malvaceous taxa. Within the dentate leaf margins, it varied from 6 in *H. rosa-sinensis,* 8 in *S. alba* to 12 in *Ab. hirtum.* The two *Malva* species with crenate margins have 4 elevations ***of*** 1 cm. The venation type is of the reticulo-pinnatous type, except in the two *Malva* species, it is of the palmate type.

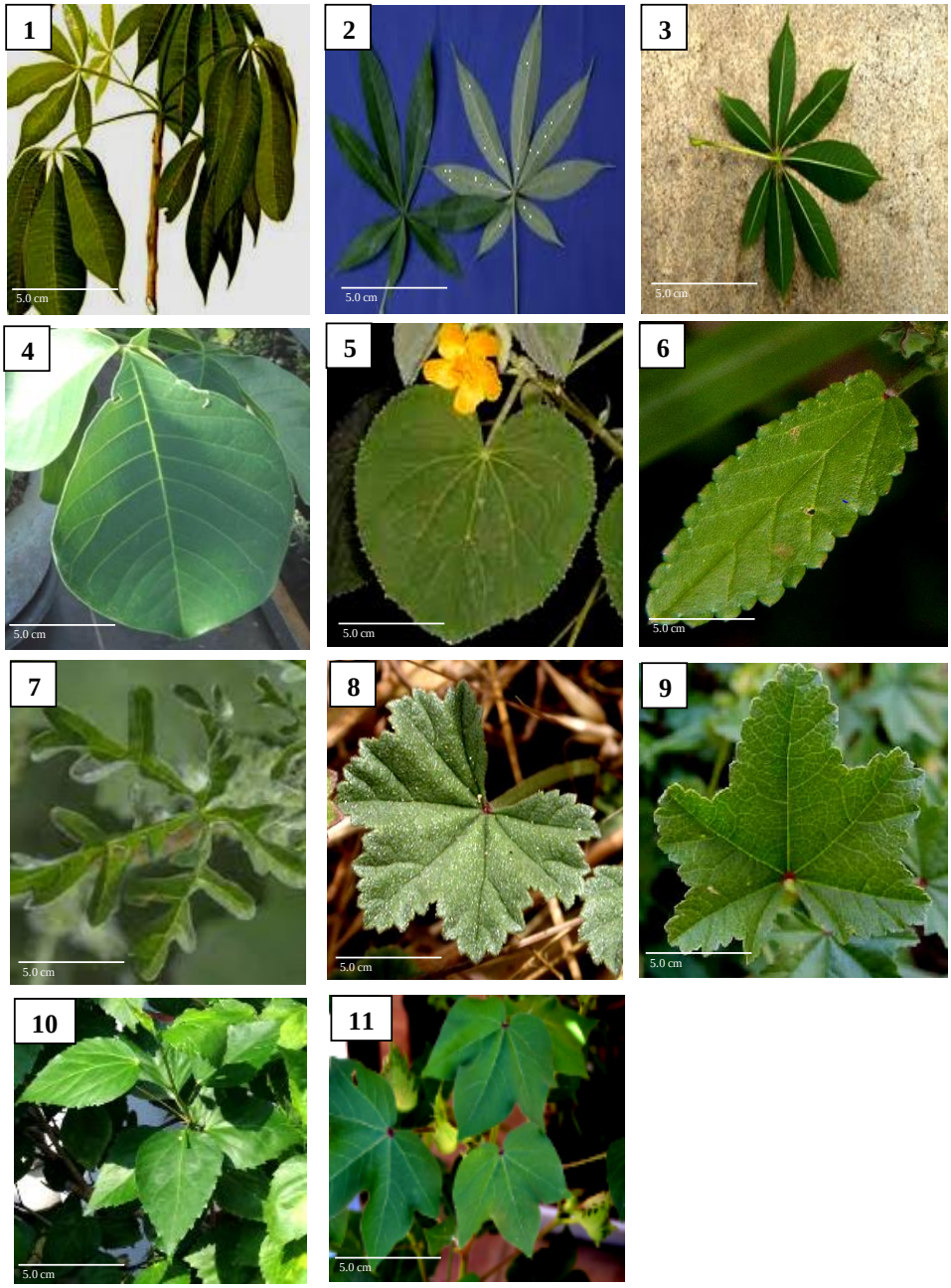

Plate 1: 1-11, The different leaf characters within the studied taxa. (1-4); Bombacaceae, compound palmate leaves, smooth leaflet margins and the number of main veins in the leaflets, (5-11); Malvaceae, simple leaves, leaf margins, apex, venation and number of main lateral veins.

1-*Bombax ceiba*, 2- *Ceiba pentandra,* 3- *Ceiba speciosa*, 4- *Pseudobombax ellipticum* , 5-*Abutilon hirtum*, 6-*Sida alba*, 7- *Athaea ludwigii,* 8- *Malva neglecta,* 9- *Malva parviflora*, 10-*Hibiscus rosa-sinesis* 11-*Gossypium herbaceum*.

C-Clustering Analysis:

All the obtained results subjected to clustering analysis are summarized in **Table (4)** and illustrated in Figure (1). The eleven studied species were used as Operational Taxonomic Units (**OUTs**). The sixteen employed characters are of four types; multistate qualitative unordered (MQU), multistate qualitative ordered (MQO), continuous and binary. The multistate qualitative unordered are those 4 characters with many unrelated states and the multistate qualitative ordered are characters with related states. The continuous characters are those measured ones, while the binary is two states characters. These characters have been coded and subjected to analyses (Table 4). The resulting clustering (Figure 1) divided the taxa into two major groups at Euclidean distance 17.5. The first group (I) gathers all the taxa belonging to the family Malvaceae *s.s.* (7 species), while group (II) has the Bombacaceae species (4 species). The first group (I) separates both *Ab. hirtum* and *H. rosa-sinensis* at a Euclidean distance of 11 in a separate subgroup (A) and the rest of the five species in the subgroup (B) which separates *S. alba* alone at Euclidean distance 10.5 and both *Al. ludwigii* and *G. herbaceum* from the two *Malva* species at Euclidean distance 7.5.

Group (II) separates *C. speciosa* into subgroup (D) while the other three species are in subgroup (C) at a Euclidean distance of 13.5. Group (II, C) separates *C. pentandra* from the *B. ceiba* and *P. ellipticum* at Euclidean distance 11.

Table 4: Basic data matrix (BDM) of the leaf macro-and micro-morphological characters and their coding in the studied taxa, 11 operational taxonomic units (OUT`s) × 16 characters.

No.	↓OUT`s/Char.↓	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	*Bombax ceiba*	3	2	11.0	2	13.0	3.9	1	5	3	1	1	2	0	1	12	1
2	*Ceiba pentandra*	3	2	4.5	2	5.6	1.8	1	7	4	1	1	1	0	1	12	1
3	*Ceiba speciosa*	3	2	3.0	2	6.2	3.8	2	5	3	1	2	2	6	1	24	1
4	*Pseudobombax elipticum*	3	2	10.2	2	7.8	5.9	3	5	4	2	5	3	0	1	19	2
5	*Abutilon hirtum*	2	2	3.5	1	4.5	4.9	4	1	1	2	3	4	12	2	9	2
6	*Sida alba*	1	1	0.9	1	1.5	1.2	2	1	2	2	3	1	8	1	1	1
7	*Althaea ludwigii*	1	1	2.3	1	1.2	1.4	5	1	2	3	1	3	0	1	5	1
8	*Malva neglecta*	1	1	4.3	1	5.0	6.6	6	1	2	3	4	5	4	1	6	3
9	*Malva parviflora*	2	1	1.8	1	3.6	3.9	6	1	1	3	4	5	4	2	6	2
10	*Hibiscus rosa-sinensis*	2	2	2.8	1	8.3	3.2	2	1	3	1	3	3	6	1	9	3
11	*Gossypium herbaceum*	1	1	2.1	1	3.2	3.6	7	1	1	1	1	3	0	1	5	2

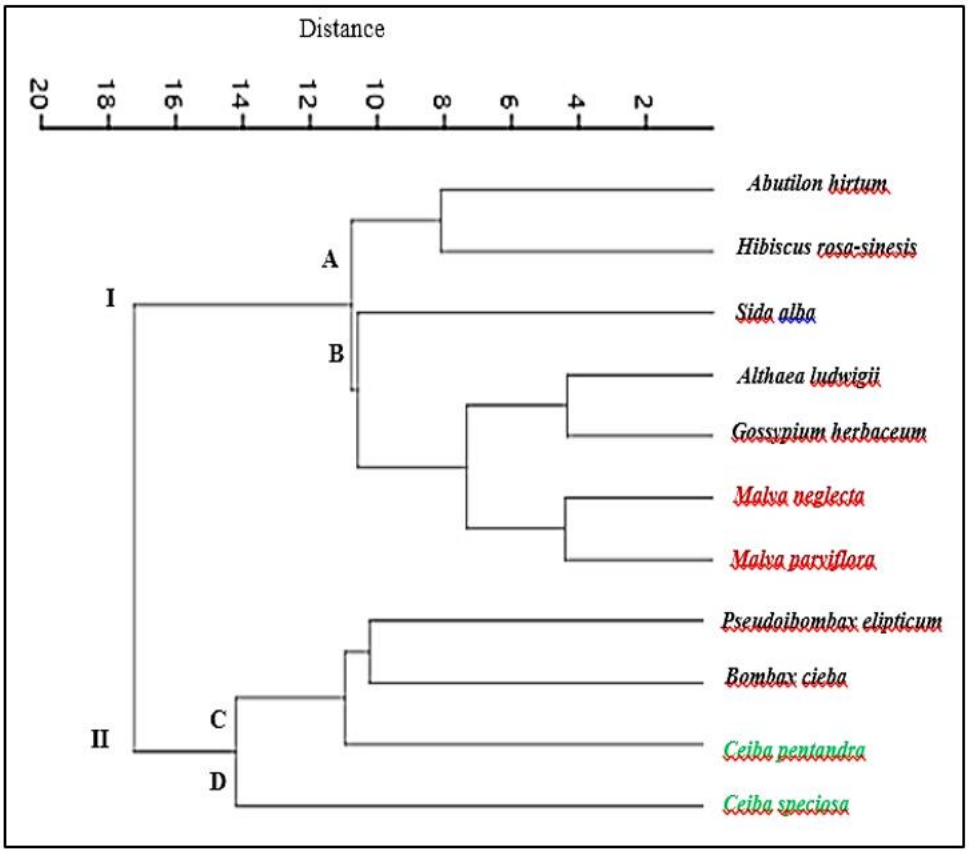

Fig. 1: Dendrogram based on the taxonomical characters of the eleven species belonging to Bombacaceae and Malvaceae.

DISCUSSION

The delimitation of taxa under the Bombacaceae is traditionally based on macro-morphological characters, and its separation as a separate group from the closely related families; Sterculiaceae, Malvaceae, and Tiliaceae seems to be rather a matter of convenience. Clear macro-morphological features, especially those related to leaf characters, permit differentiation between the families mentioned above and are very difficult to define. Family Bombacaceae is not represented in the Egyptian flora as wild trees, some species are cultivated for their ornamental flowers in a few places and gardens. Accordingly, only four taxa are included in this work. Taxa of this family were grouped in separate families for their morphological features, although phylogenetic research has shown that it is not a monophyletic group. Bombacaceae is no longer recognized by the APG I (1998); II (2003); III (2009); IV (2016) and Kubitzki system (2003) at the rank of family, most of the taxa being treated as subfamilies Bombacoideae and Helicteroidea e within family Malvaceae *s.l.* This taxonomic treatment is under dispute as most of the traditional taxonomic works are unsatisfied with this lumping of the taxa in the phylogenetic works. Accordingly, this investigation has been done to clarify the most observable leaf macro-morphological characteristics in distinguishing and separating the two families.

The results obtained clearly separated the taxa of the two families, as those of the Bombacaceae are perennial woody trees with compound palmate leaves while the taxa of the Malvaceae have annual or perennial shrubs or herbs with simple leaves. All the macro-morphological characters clearly separated the taxa of the two families. The clustering analysis of the sixteen morphological characters separates the two families each in a separate group, meanwhile, it separates the taxa under each tribe of

Malvaceae and this result approved that of Shamso and El-Khattab (2016). The most important characters within the studied taxa are the leaf type, leaf blade shape, leaf blade margin and several teeth\cm, type of venation and the number of main veins. These characters can be useful characters, not only in distinguishing members of the two families but also in identifying the members of the same family. The use of leaf macro-characters used in many taxonomical works since a long time ago. The most recent ones are those done by Taia & Mahdy (2021) and Taia *et al*. (2023) on the importance of leaf macro-characters in taxa identification. In fact, if we consider the data obtained from pollen descriptions done by Robyns (1971), Sharma (1993), Salma (2000), Bera *et al*. (2007) and Maity *et al*. (2018) besides this result, the taxa belonging to each family are completely different and must be treated as separate families.

Phylogenetic Perspective:

From the phylogenetic point of view, the leaf macro-characters support the conception of the Bombacaceae considered primitive taxa with its compound palmate leaves versus the simple leaves of the Malvaceae. The leaf morphological characters, in addition to the woody trees of the Bombacaceae versus the shrubs and herbs of the Malvaceae, support the primitiveness of the Bombacaceae. The palynological studies mentioned before considered the smooth tricolporate pollen grains of the Bombaceae as more primitive characters than the porate ornamented pollen grains. According to all the previous studies on both families and despite all the chemical and anatomical similarities.

Conclusion

We can conclude that both taxa are separate families from the taxonomic point of view, and taxa belonging to the Bombacaceae are evolutionary steps leading to the Malvaceae. Within the Malvaceae taxa, the type of venation besides the number of lateral veins supports the concept of evolution within the taxa as mentioned by Uhl & Osbrugger (1999) and Walls (2011). The palmate venation with the increase in the lateral veins is considered more advanced than the pinnate venation with one or few lateral veins (Taia *et al*., 2023).

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