



Statistical Analysis of Selected Morphological Criteria of Some Taxa of Core Caryophyllales in Egypt

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THERE ARE still questions about the boundaries of numerous families, their evolutionary relationships, and the placement of several strange genera. The interrelationships between the families of the core Caryophyllales still need to be clarified, and there is an apparent discrepancy, even though there are many previous studies. In the current study, 43 studied taxa belonging to seven families were collected from their natural habitats and some botanical gardens in Egypt. This study aims to examine and extract several diagnostic characteristics; macromorphological, micromorphological, and the attributes of pollen grains using LM and SEM, to clarify and understand the interrelationships between the taxa under study as a new contribution to previous contributions in this approach. The obtained data showed they have diagnostic weight to help in the separation of the studied taxa, but some key characteristics are most influential in clarifying these relationships. All the obtained combined data were subjected to a statistical program (R programming) analyze the strength of these criteria in interpreting and understanding these relationships. The UPGMA cluster analysis showed that the taxa in the current study were categorized into two main groups, each of which gathered several taxa. The PCA divided the studied taxa into two dimensions (Dim. 1 & 2). The results obtained were reviewed and discussed considering the taxonomic concepts and what was achieved in previous studies.

Keywords: Anatomy, Caryophyllales, Morphology, Pollen grains, R Programming.

Introduction

Centrospermae has significant taxonomic diversity over the years, mainly because unique chemical and ultrastructural characteristics have been discovered to characterize several of these plants. The core members of the Caryophyllales lineage correspond to the old Centrospermae (central seeded), a group recognized for its unusual placentation and embryology (Braun, 1864; Eichler, 1878).

Caryophyllales are of cosmopolitan distribution. The members have unique characteristics that allow them to withstand environmental extremes. Caryophyllales include

29 families and 9000 species (APG III, 2009). They contain common horticultural plants such as *Bougainvillea*, carnations, cactus, four O'clock (*Mirabilis*), and many economically important species like beets, spinach, quinoa and *Amaranthus* (Bittrich, 1993).

Caryophyllales are hard to review because of their size: approximately 10,000 species are included within 'core Caryophyllales' (Behnke & Mabry, 1994). The core Caryophyllales correspond roughly to the former Centrospermae (Chenopodiales, Caryophyllales s.s.), which was used to an order of Dicotylodeneae in systems of Engler (1904) and Wettstein (1924). They comprise a clade of betalain-producing families

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and their close anthocyanin relatives (e.g., Caryophyllaceae, Molluginaceae). Caryophyllales *s.l.* is a large angiospermic clade containing approximately 12,500 species distributed among 749 genera and 40 families (APG, 2016, Walker et al., 2018). Benson et al. (2023) stated that the order includes 37 families, which contain 12,000 species in 722 genera.

Studies on the Caryophyllales lineage are interesting, as one of the first families whose circumscription was altered based on phytochemistry. Centrospermae came under investigation and argument in the 1960s. Cronquist & Thorne (1994) found that betalain pigments are present in ten families, while anthocyanine pigment is characterized by Caryophyllaceae and Molluginaceae which were then considered to be members of the Centrospermae. Cactaceae and Didiereaceae were moved to the Centrospermae based on these chemo-systematic markers; numerous families with uncertain membership were excluded.

Dahlgren (1975), Thorne (1976), Takhtajan (1980), and Cronquist (1981, 1988) considered several morphological, ultrastructural, and chemical characteristics; as a result, subsequent classifications recognized the Caryophyllales as a distinct group. Takhtajan (1980), Cronquist (1988), and Thorne (1992) identified the Caryophyllales *s. s.* comprised Phytolaccaceae, Achatocarpaceae, Nyctaginaceae, Aizoaceae, Didiereaceae, Cactaceae, Chenopodiaceae, Amaranthaceae, Portulacaceae, Basellaceae, Molluginaceae, and Caryophyllaceae before the utilization of DNA-based molecular systematics. Many systematists consider Polygonaceae and Plumbaginaceae to be closely correlated to these preceding 12 families, to cite but a few (Cronquist, 1988; Thorne, 1992).

Rodman et al. (1984) categorized the betalain taxa into three distinct clades: a primitive group of Nyctaginaceae and Phytolaccaceae, an intermediate group of Amaranthaceae and Chenopodiaceae in addition to the advanced group of succulents (Aizoaceae, Cactaceae, Portulacaceae, Basellaceae and Didiereaceae away from Caryophyllaceae and Molluginaceae of anthocyanin pigments) based on morpho-cladistics analysis. Rettig et al. (1992) distinguished four major clades: the first includes (Amaranthaceae and Chenopodiaceae); the second (Caryophyllaceae); the third one (Basellaceae,

Portulacaceae, Cactaceae, and Didiereaceae); and the fourth (Aizoaceae, Phytolaccaceae, Petiveriaceae, Nyctaginaceae, and *Gisekia*) based on rbcL sequencing.

There are still questions about the boundaries of numerous families, their evolutionary relationships, and the placement of several strange genera; Amaranthaceae, Chenopodiaceae, Portulacaceae, Nyctaginaceae, Phytolaccaceae, and Molluginaceae are six families with no apparent delimitation or real synapomorphies (Bittrich, 1993). Palmer (1985) showed that the molecular data combined with morphological, anatomical, ultrastructural, palynological, and phytochemical data (non-molecular) are strongly involved in interpreting the phylogenetic relationships within the order Centrospermae.

The macromorphological characteristics e.g., leaf arrangements, flowers, and fruits; micromorphological characteristics e.g., anomalous secondary thickening by successive cambia, the occurrence of idioblasts, Kranz syndrome, epicuticular waxes ultrastructure (Solereeder, 1908; Eckardt, 1976; Cronquist, 1981; Thorne, 1983; Gibson & Nobel, 1986; Engel & Barthlott, 1988; Bittrich, 1993; Sage, 2004; Thorne & Reveal, 2007; Carlquist, 2010; Tantawy et al., 2023); and the palynological characteristics e.g., pollen shape, size, number of pores, density, arrangement of microechini, surface sculpture, aperture structures, number of granules and spines on the operculum (Erdtman, 1952; Barth & Barbosa, 1972; Nowicke & Skvarla, 1979; Nyananyo, 1992; Perveen & Qaiser, 2006; El-Naga et al., 2014; Pramanick et al., 2015; Bayoumy et al., 2020; Dabbub et al., 2020) have great diagnostic weight helping in understanding the systematic placement of order Caryophyllales

R is a programming language and a software environment for computational statistics and visualizations (Team, 2013). It is extensively employed in the quantitative study of shape variation, known as geometric morphometrics (Savriama, 2018). This contrasts with the cladistic approach and other phylogenetic techniques, which seek to reconstruct evolutionary relationships among recognized taxa. The morphometric approaches are especially helpful in drawing distinctions between taxa and identifying the key characteristics that set them apart. In terms of protecting more morphological information and enabling analysis, geometric morphometric methods are more reliable

than classical morphometric methods (Marhold et al., 2011). UPGMA (Unweighted Pair Group Method with Arithmetic Mean) and PCA (Principal Component Analysis) are multivariate statistical methods used in geometric morphometrics to examine and visualize variation at specific and infraspecific levels (Marhold et al., 2011). A correlation is a statistical indicator of the direction and degree of a relationship between two variables (Ancona et al., 2019). The morphometric approaches have long been recognized as valuable tools for studying plant growth, population differentiation, and systematics (Elmouni et al., 2017; Savriama, 2018; El-Banhawy et al., 2021a, b; Wen et al., 2022).

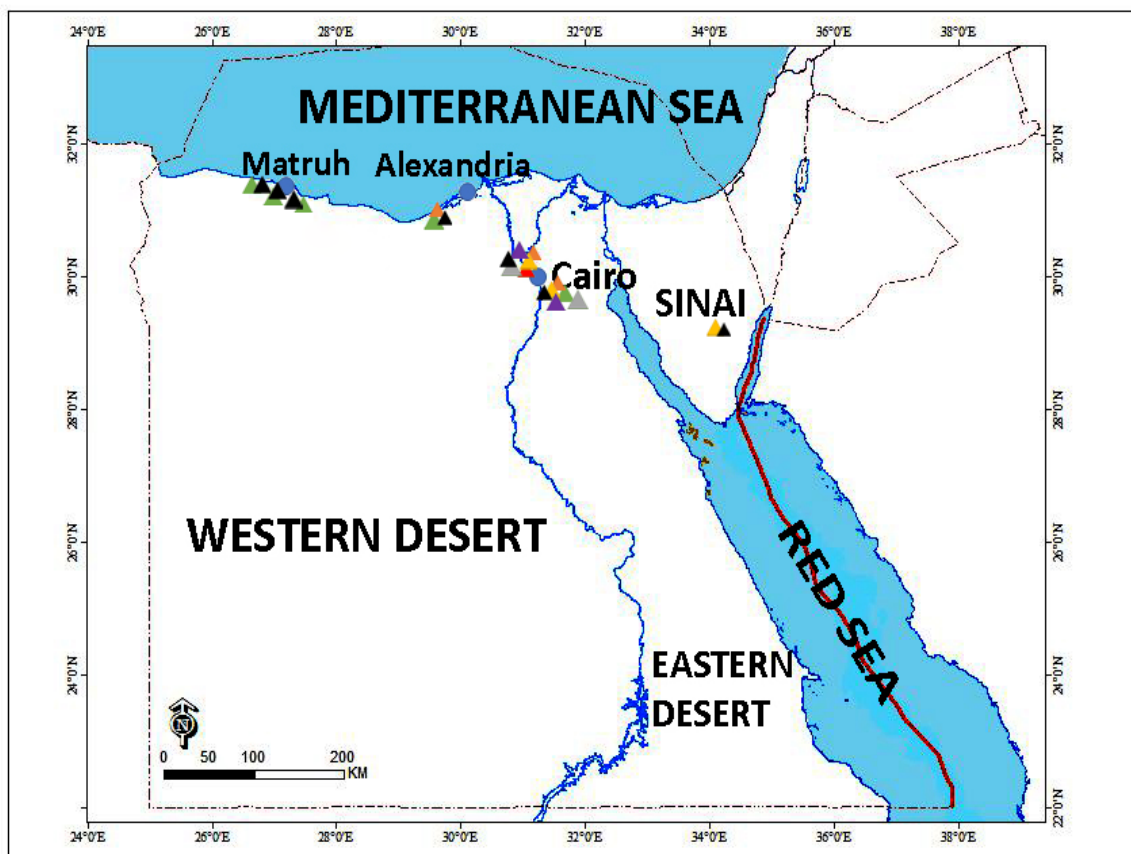
The current study aims to evaluate the systematic placement and the interrelationships between the studied taxa of the order based on macromorphological, micromorphological, and pollen characteristics. The obtained data are subjected to R Programming as a statistical program to obtain a phylogenetic tree of all

combined data (multivariate) and to discuss these interrelationships between the taxa under investigation, considering the previous studies and some available taxonomic concepts and systems.

Materials and Methods

Sampling and study area

The present study examines 43 wild and ornamental taxa collected from their natural habitats and botanical gardens in Egypt (Map 1), belonging to seven families viz. Aizoaceae (six taxa), Amaranthaceae (five taxa), Caryophyllaceae (13 taxa), Chenopodiaceae (13 taxa), Nyctaginaceae (three taxa), Portulacaceae (two taxa) and Phytolaccaceae (only *Phytolacca dioica* L.). The identification of the wild studied taxa takes place with the aid of Täckholm (1974) and Boulos (1999), while the ornamental taxa with the aid of Bailey (1949). Synonyms were derived from the PLANT LIST, GRIN, and IPNI. The collection data are listed as a Supplementary Table 1.



Map 1. The distribution of studied taxa in Egypt [Aizoaceae “orange”, Amaranthaceae “yellow”, Caryophyllaceae “black”, Chenopodiaceae “light green”, Portulacaceae “gray”, Nyctaginaceae “purple”, Phytolaccaceae “red”]

Morphological and anatomical analyses

The macro, and micromorphological characters of the whole plant of the studied taxa are examined directly from fresh specimens; some descriptions are derived from textbooks, some specific internet sites, and some are obtained from previous studies as a first attempt in this direction (Bayoumy et al., 2020; Tantawy et al., 2023). The floral parts are examined using a stereomicroscope. Thin cross sections of stem and lamina are prepared using hand microtome at 10-16 μm , double stained using a combination of safranin and light green, and then mounted using Canada Balsam (Johansen, 1940). The sections are examined using a light microscope (LM) and photographed by a digital camera (Canon power-shot G15, 12.1 megapixels). The anatomical characteristics are described following Eames's (1929) and Metcalfe & Chalk (1950) rules of anatomical description. The epidermal stripes are mechanically prepared, stained with safranin and iodine solution, and mounted on slides using pure glycerin (Johansen, 1940). The Canon PowerShot G15, which has 12.1 megapixels, is used for the examination.

Scanning electron microscopy (SEM)

For SEM investigation, small pieces of fresh or dried leaves are placed on a stub with double-sided sticky tapes, and then gold is sputtered onto them in the sputter coater (SPI-Module). The adaxial and abaxial surfaces of lamina are investigated using the high vacuum mode on the SEM (JEOL-JSM-5500LV). With the aid of prior research (Metcalfe & Chalk, 1950; Ash et al., 1999; Prabhakar, 2004; Stearn, 2005), epidermal characteristics terminology was finalized.

Palynological analysis

For pollen investigation, mature anthers at anthesis of 43 studied taxa are collected using a Stereomicroscope and are opened using a needle; pollen grains are left in air for four minutes to dry, mounted on a slide with a drop of glycerin, covered, and examined using BEL: B103 T-PL light microscope and photographed by a digital camera (Canon power-shot G15, 12.1 megapixels).

For SEM investigation: after grinding anther and removing residual parts, the soft powder is mounted on a stub with double-sided adhesive tape and then coated with gold sputter coater (SPI-Module). Examination and photographs of

samples are taken by SEM (JEOL-JSM-5500LV) using high vacuum mode. This work took place at the Central Laboratories of Chemical Warfare, Cairo, Egypt, and the Unit of Scanning Electron Microscopy, Faculty of Science, Alexandria University, Alexandria, Egypt. The description and terminology of pollen grains followed Erdtman (1952) and Punt et al. (2007).

Data analysis

R software (Vienna, Austria) is used with the required packages loaded (Team, 2013) Excel 365, and Minitab version 20. The macro-morphological, micro-morphological, and palynological data were extracted to generate qualitative descriptors that were handled for statistical analysis and coding, using the multistate data in the investigation. Using the Package "cluster," the Gower's dissimilarity distance was designed to standardize the qualitative or quantitative data for cluster analysis, and "The UPGMA" cluster was then created (Maechler, 2019). Principal component analysis (PCA) for examining a dataset containing continuous variables was made possible by installing the "factoextra" and "FactoMineR" packages in R (Kassambara & Mundt, 2020). The "Corrplot" package was utilized to obtain and display the correlation coefficients for the interaction between the two variables (Soetewey, 2022). Blue with a 1 indicates a strong positive correlation, while white with a 0 indicates no relationship between the two variables. A significant negative correlation is shown by a red value of -1 .

Results

Morphological characteristics

Although many macromorphological, anatomical, and pollen characteristics are examined and extracted, the key characteristics that have significant influence in distinguishing and separating the taxa under investigation are asterisked in Supplementary Tables 2, 3, 4, 5, 6, 7 and Plates I, II, III., IV, V. To cite but a few, we can refer to some of these characters *viz.* growth form, plant texture, leaf characters, inflorescence types, flower structures, sexuality, perianth structure, placentation type, trichomes, aspects of secondary growth, idioblasts, Kranz anatomy, stomatal types, epicuticular waxes, pollen class, pollen surface sculpture, pollen size, pollen shape, pore number, pore diameter and interpore distance.

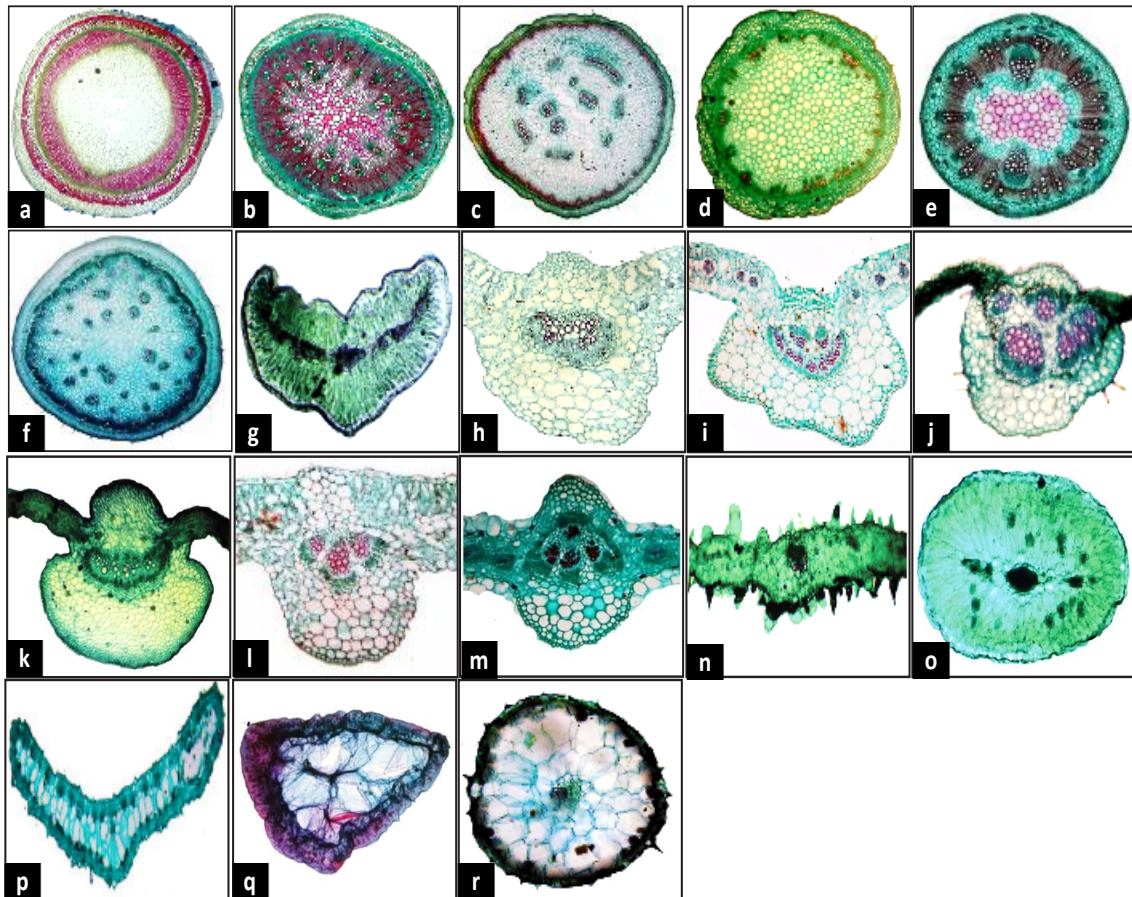


Plate I, Text Figs. a-f. Stem Microphotographs Showing Different Growth Aspects of The Studied Taxa a: Normal secondary growth (*Dianthus caryophyllus*); b: Abnormal secondary growth with successive cambial rings (*Atriplex leucoclada*); c: Abnormal secondary growth with successive cambial rings & medullary vascular bundles (*Boerhavia diffusa*); d: Abnormal secondary growth with successive cambial rings & included phloem (*Trianthema portulacastrum*); e: Abnormal secondary growth with included phloem at the opposite bipolar position (*Alternanthera brasiliana*); f: Abnormal secondary growth with successive cambial rings, included phloem & medullary vascular bundles (*Bougainvillea glabra*). Text Figs. g-r. Major Aspects of Lamina Anatomical Characteristics Showing Different Outline Shapes and Different Vascular Supplies. g: Lamina boat-shaped, one central bundle with numerous lateral traces (*Gypsophila capillaris*); h: Lamina wavy abaxially-convex adaxially, one kidney-shaped vascular strand (*Beta vulgaris* subsp. *ciela*); i: Lamina basin-like, one arc-shaped vascular bundle with phloem facing abaxially, two with phloem facing adaxially (*Amaranthus caudatus*); j: Lamina rounded ab- and adaxially, three large with phloem facing abaxially, one small with phloem facing adaxially (*Bougainvillea glabra*); k: Lamina broadly rounded abaxially-concave adaxially, expanded crescentiform vascular bundle with phloem facing abaxially (*Phytolacca dioica*); l: Lamina rounded abaxially-straight adaxially, one medium vascular bundle and two marginal with the phloem facing abaxially (*Chenopodium album*); m: Lamina convex abaxially-rounded adaxially, 3 vascular bundles with phloem facing abaxially, one with phloem facing adaxially (*Atriplex leucoclada*); n: Lamina flattened, differentiated into midrib and wings, one vascular bundle (*Silene succulenta*); o: Lamina ovate, one vascular bundle and two lateral traces (*Spergula fallax*); p: Lamina ribbon-like, one central vascular bundle with numerous peripheral vascular traces (*Bassia indica*); q: Lamina trigonous, one central vascular bundle (*Malephora crocea*); r: Lamina terete, one vascular bundle with peripheral vascular traces (*Salsola kali*)

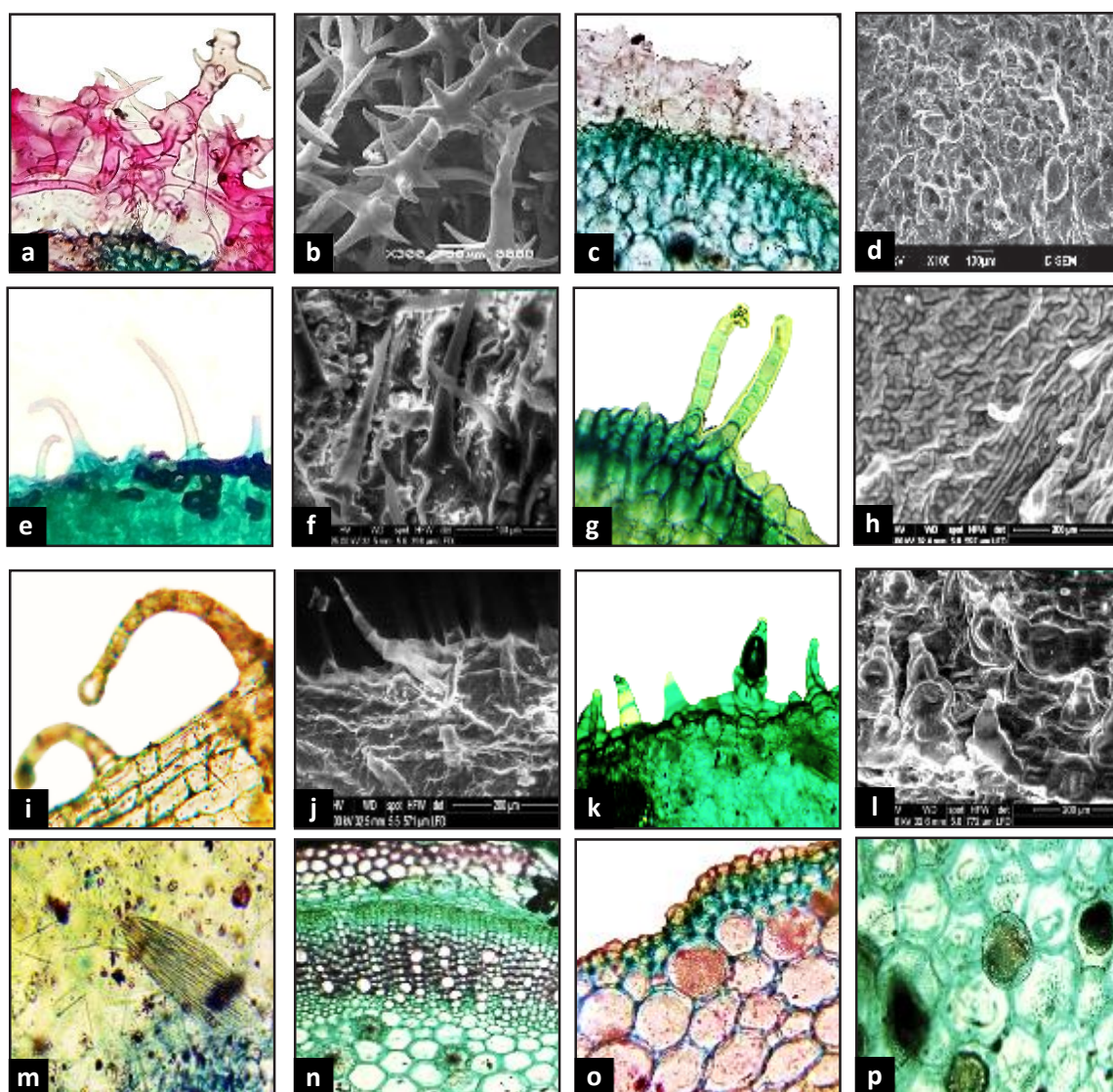


Plate II, Text Figs. a-l. Major Aspects of Trichomes. a,b: Candelabra type (*Aerva javanica*); c,d: Vesicular trichome (*Atriplex halimus*); e,f: E-glandular, uniseriate, unicellular trichome (*Paronychia argentea*); g,h: E-glandular & glandular, uniseriate, uni- & multicellular trichome (*Bougainvillea glabra*); i,j: E-glandular & glandular, uniseriate, multicellular trichomes (*Mirabilis jalapa*); k,l: E-glandular & glandular, uni- & multiseriate, uni- & multicellular (flask-shaped) trichomes (*Silene succulenta*). Text Figs. m-p: Major Aspects of Idioblasts. m: Raphides & pseudo-raphides (*Bougainvillea glabra*); n: Druses (*Silene succulenta*); o: Sandy crystals (*Amaranthus blitum* subsp. *oleraceus*); p: Tanniniferous idioblasts (*Lampranthus spectabilis*)

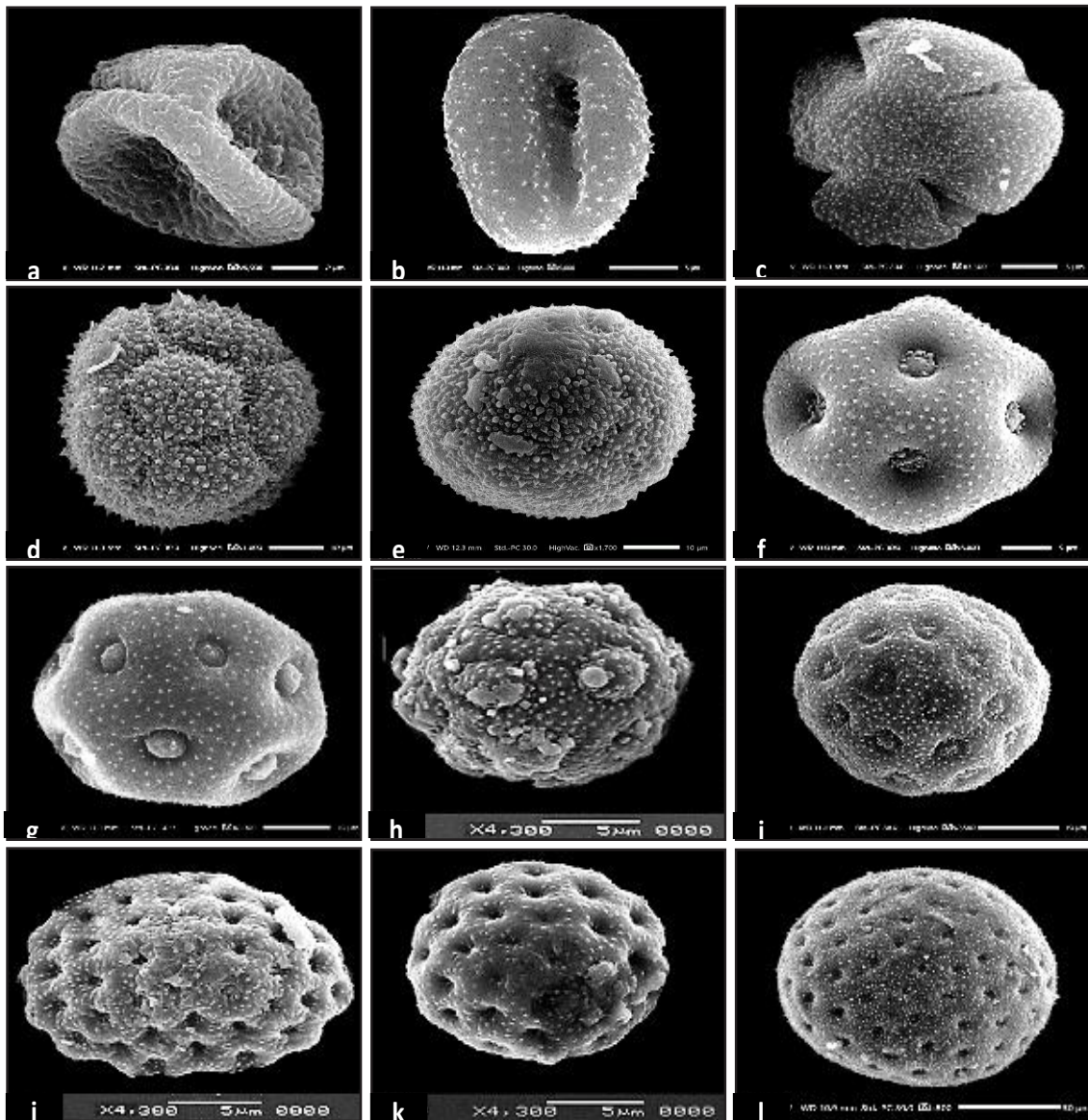


Plate III. Text Figs. a-l. Major Aspects of Pollen Grains Characteristics (Pollen Shape, Size, Polarity, Class, Characters of Aperture). a: Prolate, medium, isopolar, tricolpate with slit like colpi and developed margo (*Mesembryanthemum cordifolium*); b: Prolate, large, isopolar, tricolpate with oblong colpi and without margo (*Phytolacca dioica*); c: Oblate-spheroidal, medium, isopolar, tri- & tetracolpate (*Mesembryanthemum nodiflorum*); d: Prolate- spheroidal, large, isopolar, tetracolpate with short, fusiform colpi and without margo (*Portulaca grandiflora*); e: Oblate- spheroidal, large, isopolar, pantocolpate with short, fusiform colpi and without margo (*Portulaca oleracea*); f: Oblate-spheroidal, large, apolar, pantoporate with (7-10) deeply excavated pores and annulopunctate pore membrane covered by 6-10 spines (*Gypsophila elegans*); g: Prolate-spheroidal, large, apolar, pantoporate with (14-16) deeply excavated pores and pore membrane covered by 3-4 spines (*Dianthus caryophyllus*); h: Oblate-spheroidal, small, apolar (*Aerva javanica*); i: Prolate-spheroidal, large, apolar, pantoporate with (32-39) protuberant pores and membrane covered by few spines (*Silene succulenta*); j: Suboblate, medium, apolar (*Atriplex halimus*); k: Prolate-spheroidal, small, apolar, antoporate with (60-65) deeply excavated pores and spineless pore membrane (*Chenopodium album*); l: Prolate-spheroidal, large, apolar, pantoporate with (75-85) deeply excavated pores and pore membrane covered by 6-10 spines (*Mirabilis jalapa*)

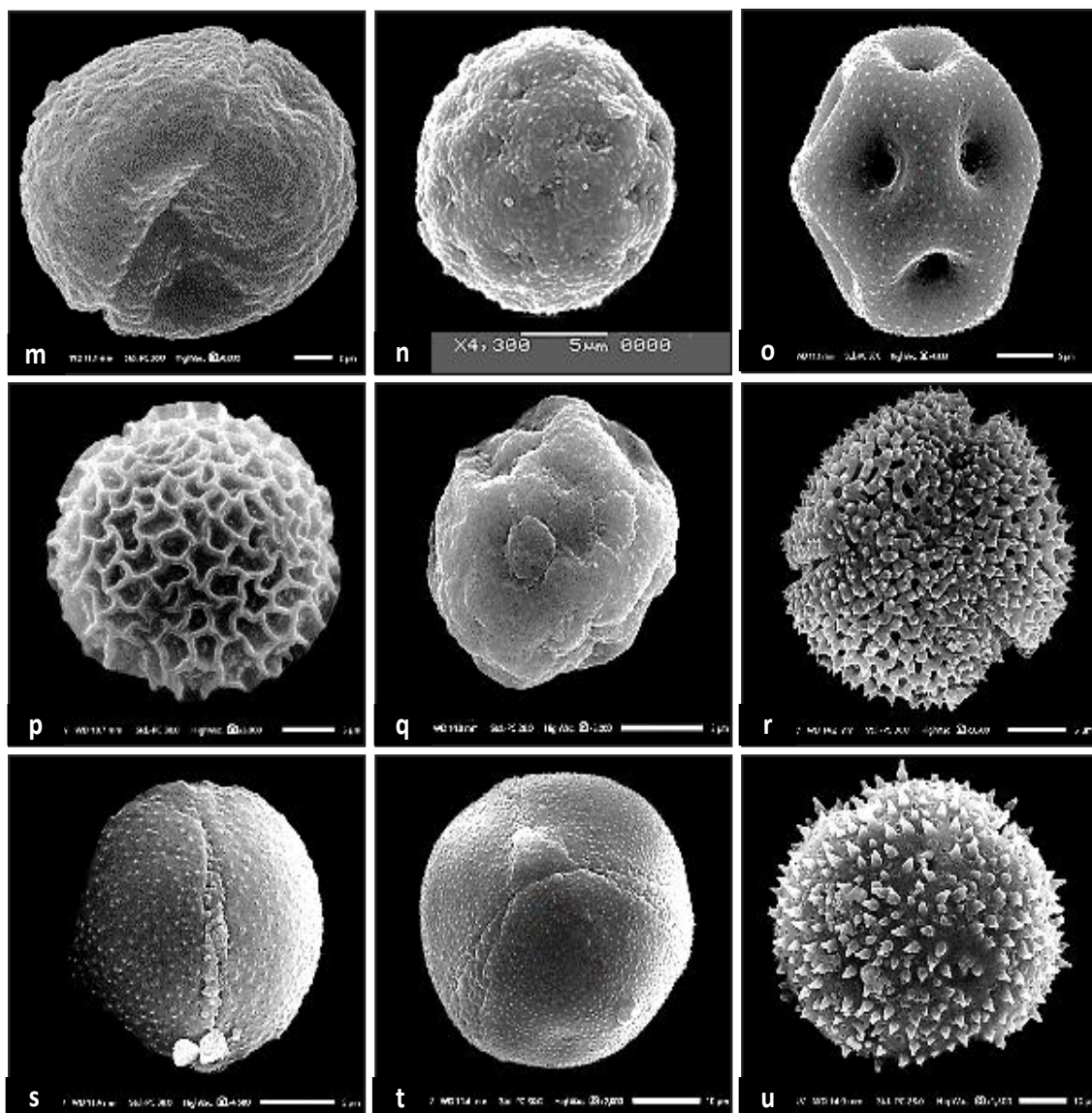


Plate III. Cont., Text Figs. m-u. Major Aspects of Pollen Grains Characteristics (Sculpture). m: Favulariate (*Mesembryanthemum cordifolium*); n: Scabrate (*Amaranthus blitum* subsp. *oleraceus*); o: Granulate (*Gypsophila capillaris*); p: Coarsely reticulate-granulate (*Bougainvillea glabra*); q: Verrucate (*Herniaria hirsuta*), Text Figs. r-u. Major Aspects of Echinulate-punctate Sculpture. r: Few, regular arranged echini with dense puncta (*Lampranthus spectabilis*); s: Medium, rather regular arranged microechini with sparse puncta (*Mesembryanthemum crystallinum*); t: Dense, irregular arranged microechini with medium puncta (*Trianthema portulacastrum*); u: Very dense, irregular arranged tubuliferous echini with sparse puncta (*Boerhavia diffusa*)

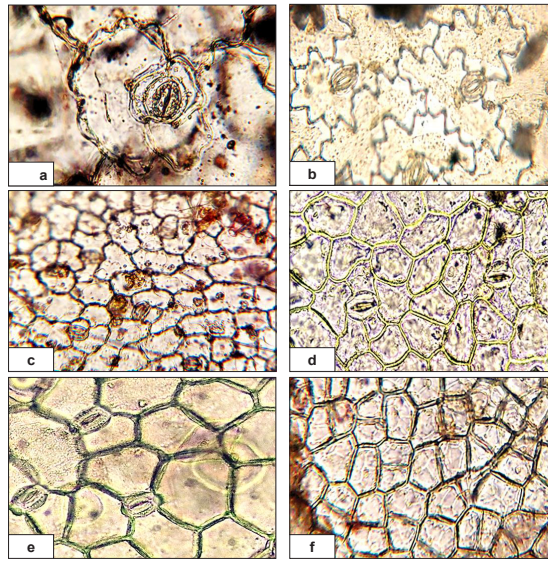


Plate IV, Text Figs. a-f. Major Aspects of Lamina Epidermal Characteristics (Ab- and Adaxial Surfaces, LM). a: Irregular cell, sinuate anticlinal wall with knobs and paracytic stomata (*Portulaca grandiflora*); b: Irregular cell, sinuate zig-zag shaped anticlinal wall, diacytic and anisocytic stomata (*Dianthus caryophyllus*); c: Polygonal cell, straight anticlinal wall and anisocytic, anomotetracytic, brachyparatracytic stomata (*Mirabilis jalapa*); d: Polygonal cell, straight anticlinal wall, anomocytic stomata (*Bougainvillea glabra*); e: Polygonal cell, straight anticlinal wall and brachyparacytic stomata (*Salsola kali*); f: Polygonal cell, straight anticlinal wall without stomata (*Phytolacca dioica*)

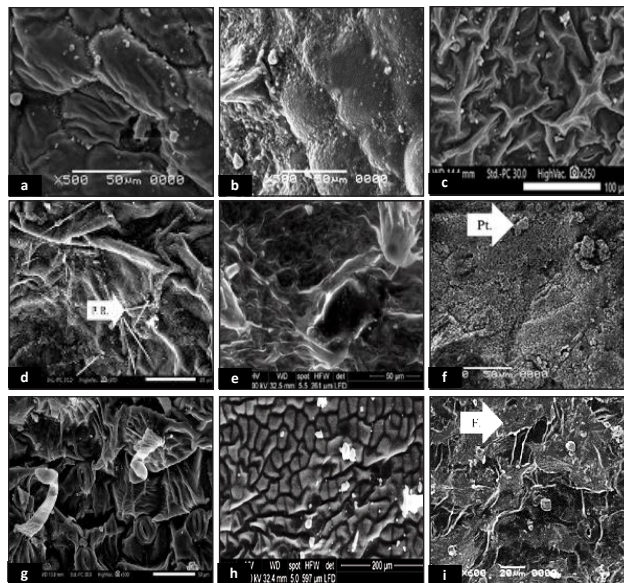


Plate V, Text Figs. a-i. Major Aspects of Lamina Epidermal Characteristics (Ab & adaxial Surface, SEM). a: Reticulate sculpture, stomata leveled and elliptic, rhomboid crystals and platelets wax (*Bassia arabica*); b: Colliculate sculpture, stomata elliptic and leveled, granules wax (*Salsola kali*); c: Favulariate sculpture, stomata elliptic and sunken, irregular platelets shape wax crystals and granules wax (*Polycarpon tetraphyllum*); d: Ruminant sculpture, stomata elliptic and sunken, platelets, granules and polyangular rodlets wax (*Malephora crocea*); e: Ruminant-reticulate, stomata elliptic and leveled, plates, platelets and granules wax (*Mirabilis jalapa*); f: Ill-defined sculpture due to the presence of dense layer of platelets wax, stomata slit-like and sunken (*Suaeda pruinosa*); g: Favulariate sculpture, stomata lens shaped to elliptic and elevated, granules wax, trichomes detected (*Spergularia diandra*); h: Reticulate sculpture, stomata lens shaped and sunken, granules and platelets wax (*Bougainvillea glabra*); i: Ill-defined sculpture due to the presence of dense layer of fissured crust and granules wax, stomata sunken and elliptic (*Chenopodium murale*) [Abbreviations: Pt.: Platelets. P.R.: Polyangular Rodlets. F.: Fissured Crust]

Morphometric analyses for macromorphological, micromorphological and palynological data using multivariate methods

Multivariate methods are used to analyse the data of 43 taxa, which included macromorphological, micromorphological, and palynological characters. The datasets are analysed individually and in combination (Supplementary Table 8). The data undergo standardization for Cluster analysis by utilizing Gower's dissimilarity distance, resulting in a UPGMA cluster. The cophenetic correlation coefficient was then measured between the distance matrix and the cophenetic matrix calculated for macromorphological, micromorphological, and palynological UPGMA. The resulting values of $r=0.86$, 0.62 , and 0.88 , respectively indicate good fitting for macromorphological and palynological data.

i. The taxa under investigation are divided into two series (I & II) based on 28 macromorphological traits (UPGMA cluster analysis, Fig. 1a). **Series I** include the studied taxa of Portulacaceae and Caryophyllaceae (11 taxa). **Series II** gather the studied taxa of Aizoaceae, Chenopodiaceae, Caryophyllaceae

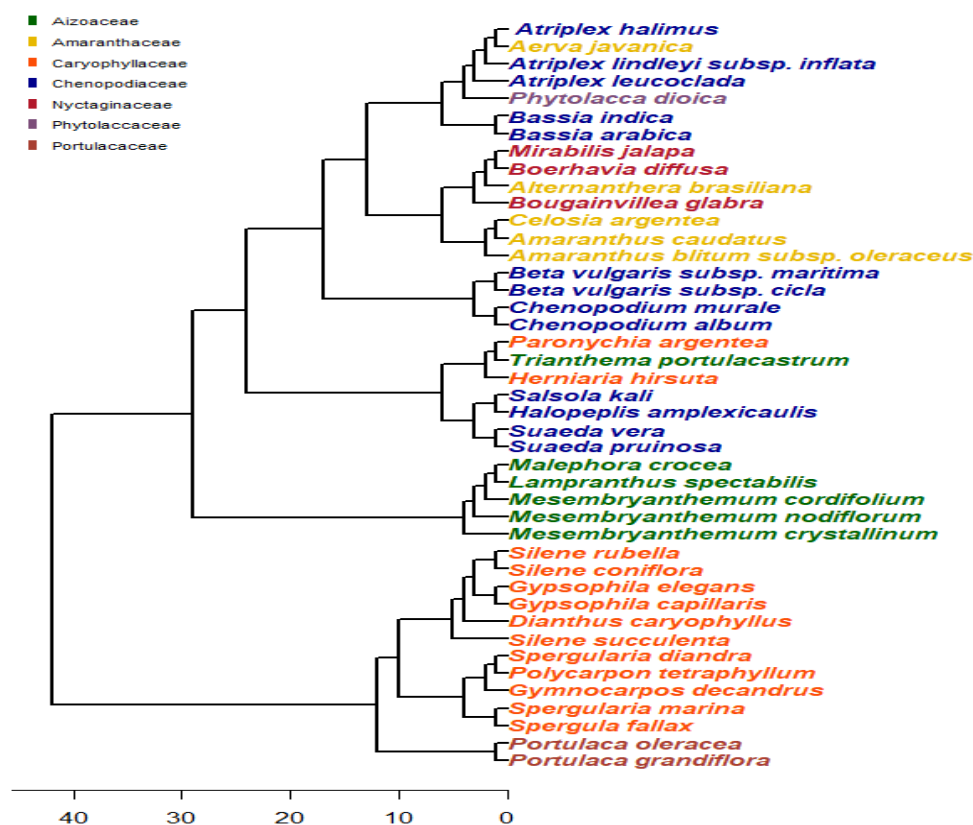
(two taxa), Amaranthaceae, Nyctaginaceae, and Phytolaccaceae.

ii. The taxa under investigation are categorized into two series based on 71 micromorphological traits (UPGMA cluster analysis, Fig. 1b). **Series I** include the studied taxa of Chenopodiaceae (seven taxa), Amaranthaceae (three taxa), Caryophyllaceae (seven taxa) and Aizoaceae (one taxon). **Series II** gather the studied taxa of Caryophyllaceae (six taxa), Chenopodiaceae (six taxa), Amaranthaceae (two taxa), Portulacaceae, Nyctaginaceae, Aizoaceae (five taxa) and Phytolaccaceae.

iii. The taxa under investigation are divided into two series based on 25 palynological traits (UPGMA cluster analysis, Fig. 1c). **Series I** include the studied taxa of Caryophyllaceae (four taxa), Aizoaceae, Phytolaccaceae, Portulacaceae and Nyctaginaceae (one taxon). **Series II** gather the studied taxa of Caryophyllaceae (nine taxa), Nyctaginaceae (two taxa), Chenopodiaceae, and Amaranthaceae.

a

UPGMA tree based on Macromorphological data



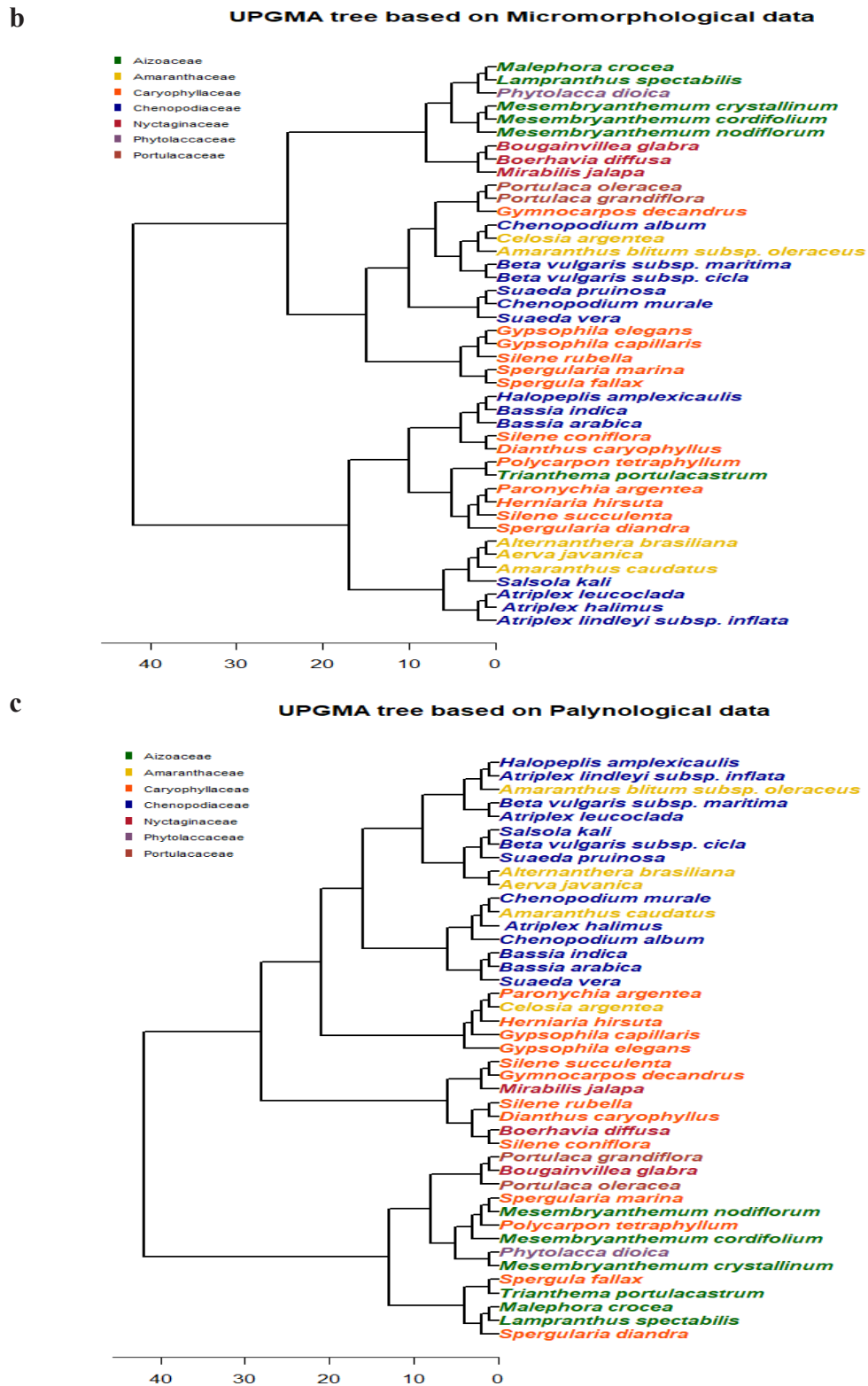


Fig. 1. UPGMA cluster analysis of 43 studied taxa based on a. macromorphological data, b. micromorphological data, and c. palynological data. Aizoaceae” green,” Nyctaginaceae “red”, Phytolaccaceae “purple”, Portulacaceae “mauve”, Caryophyllaceae “orange”, Chenopodiaceae “blue” and Amaranthaceae” yellow”

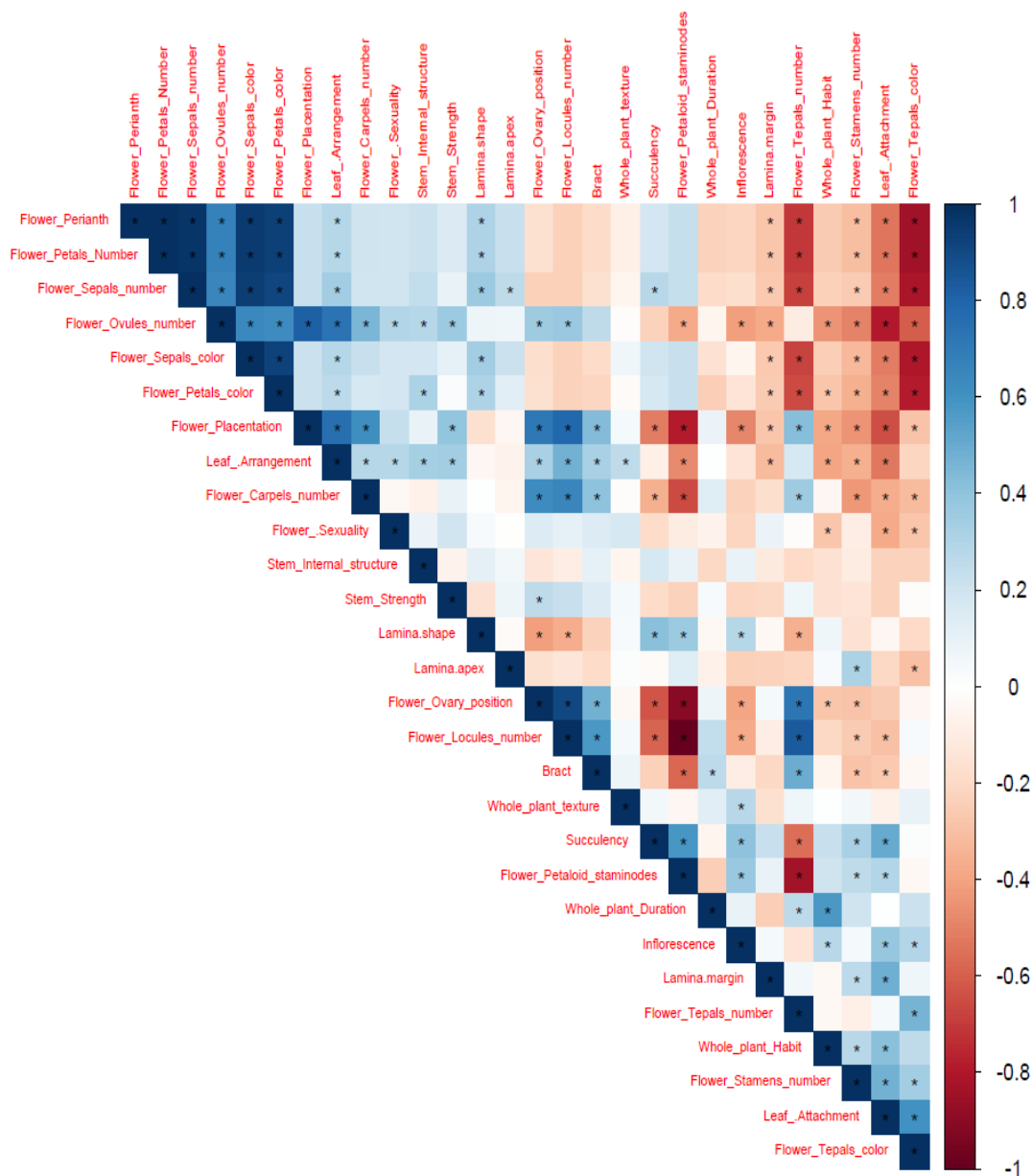
Pearson’s correlation analysis is conducted to explore the relationship and correlation between the macromorphological, micromorphological, and palynological features (Fig. 2a, b, c). The characteristics show a significant positive association ($P < 0.05$), suggesting the distinguishing variables of taxa.

Morphometric analyses for all combined data using multivariate methods (Cluster and ordination analyses)

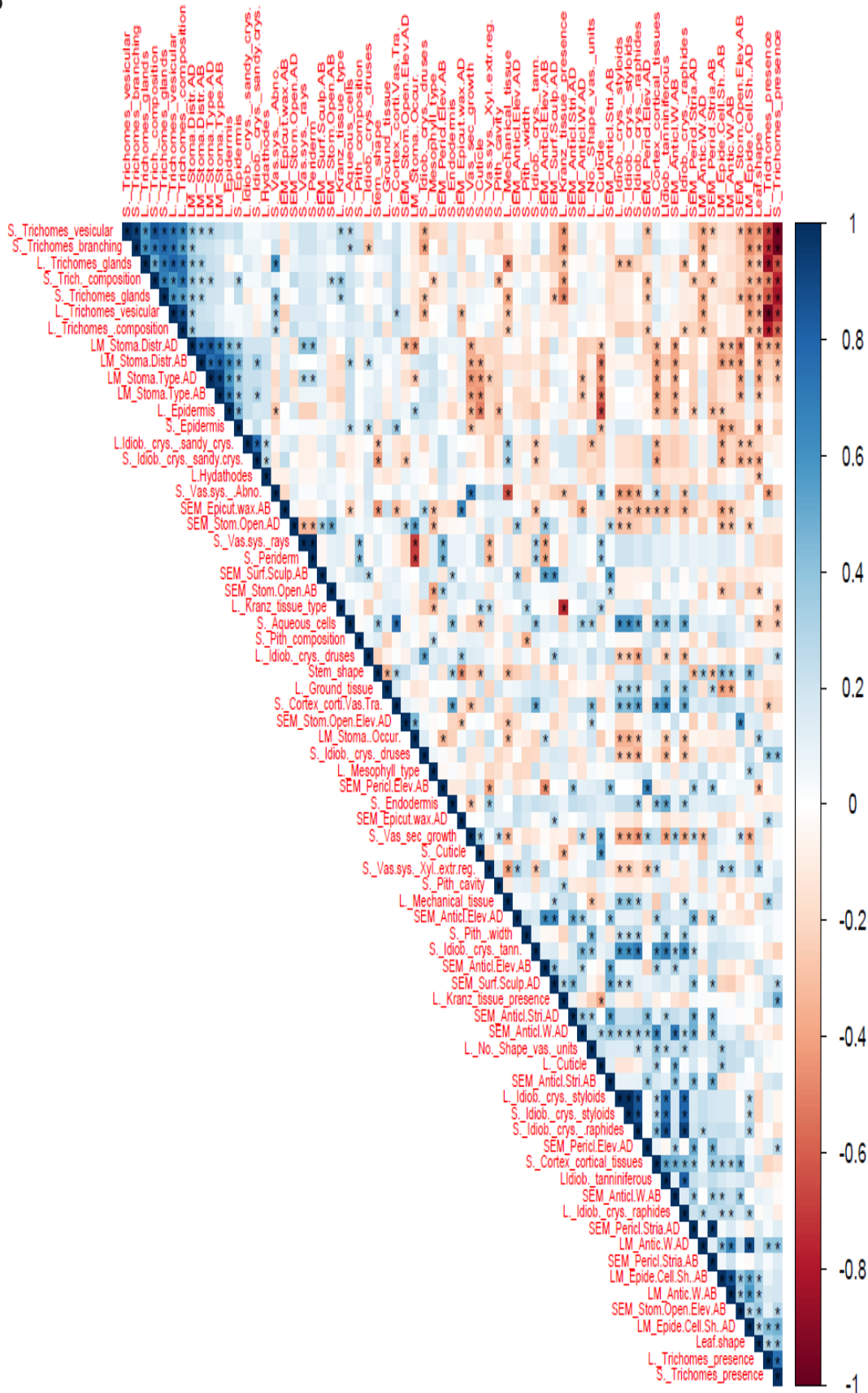
UPGMA cluster and ordination analyses (PCA) of 124 features (Figs. 3 and 4 a, b

respectively). The correlations for the most significant traits at the first and second dimensions with a cumulative variance of 45 % for the dimensions from 1 to 4, are illustrated in Table 1. The UPGMA cluster analysis divided the 43 studied taxa into two series (I and II). **Series I** include the studied taxa of Chenopodiaceae and Amaranthaceae. **Series II** gather the studied taxa of Portulacaceae, Caryophyllaceae, Aizoaceae, Phytolaccaceae, and Nyctaginaceae. The cophenetic matrix was calculated for UPGMA ($r = 0.66$).

a



b



c

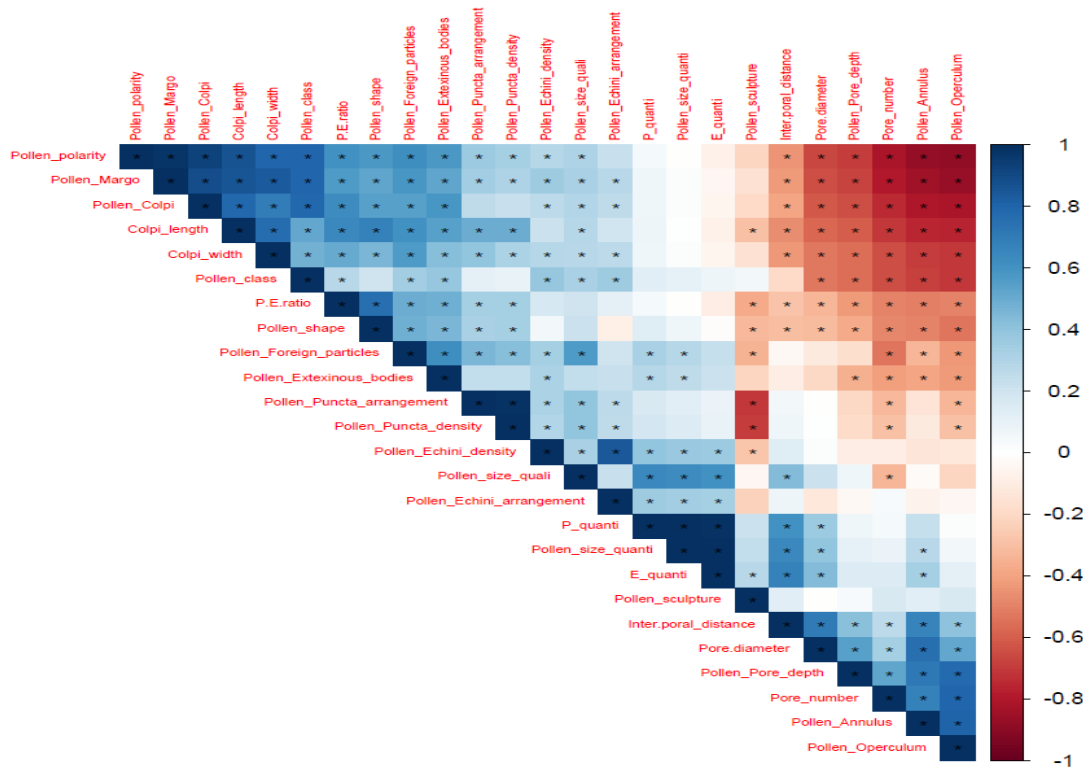


Fig. 2. Pearson’s correlation analysis based on the correlation coefficient of a. macromorphological features, b. micromorphological features, c. palynological features. Positive and negative correlations are displayed in blue and red color, respectively. Correlation coefficients are proportional to color intensity

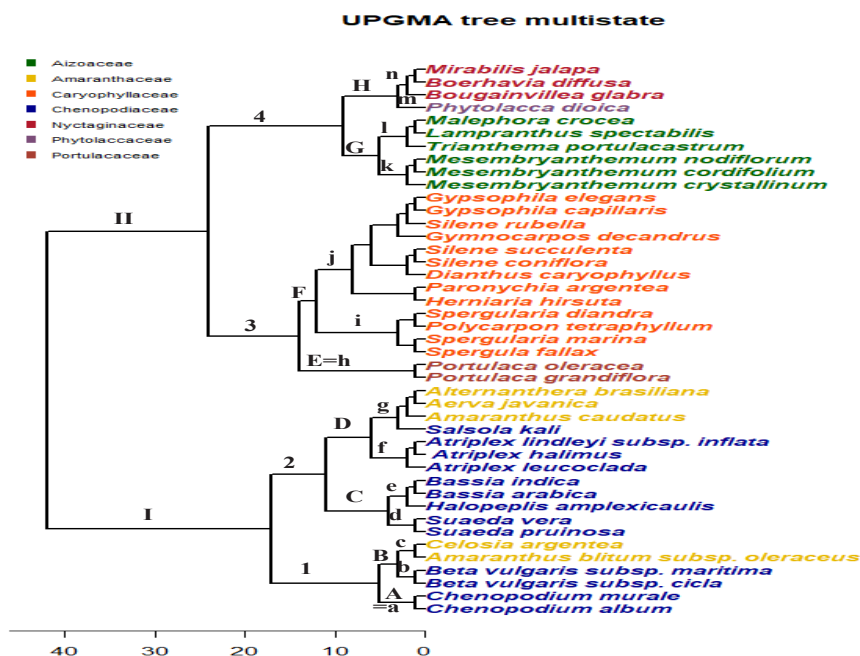


Fig. 3. UPGMA cluster of 43 studied taxa based on 124 combined traits. Chenopodiaceae “blue”, Amaranthaceae” yellow”, Portulacaceae “mauve”, Caryophyllaceae “orange”, Aizoaceae” green,” Phytolaccaceae “purple” and Nyctaginaceae “red”

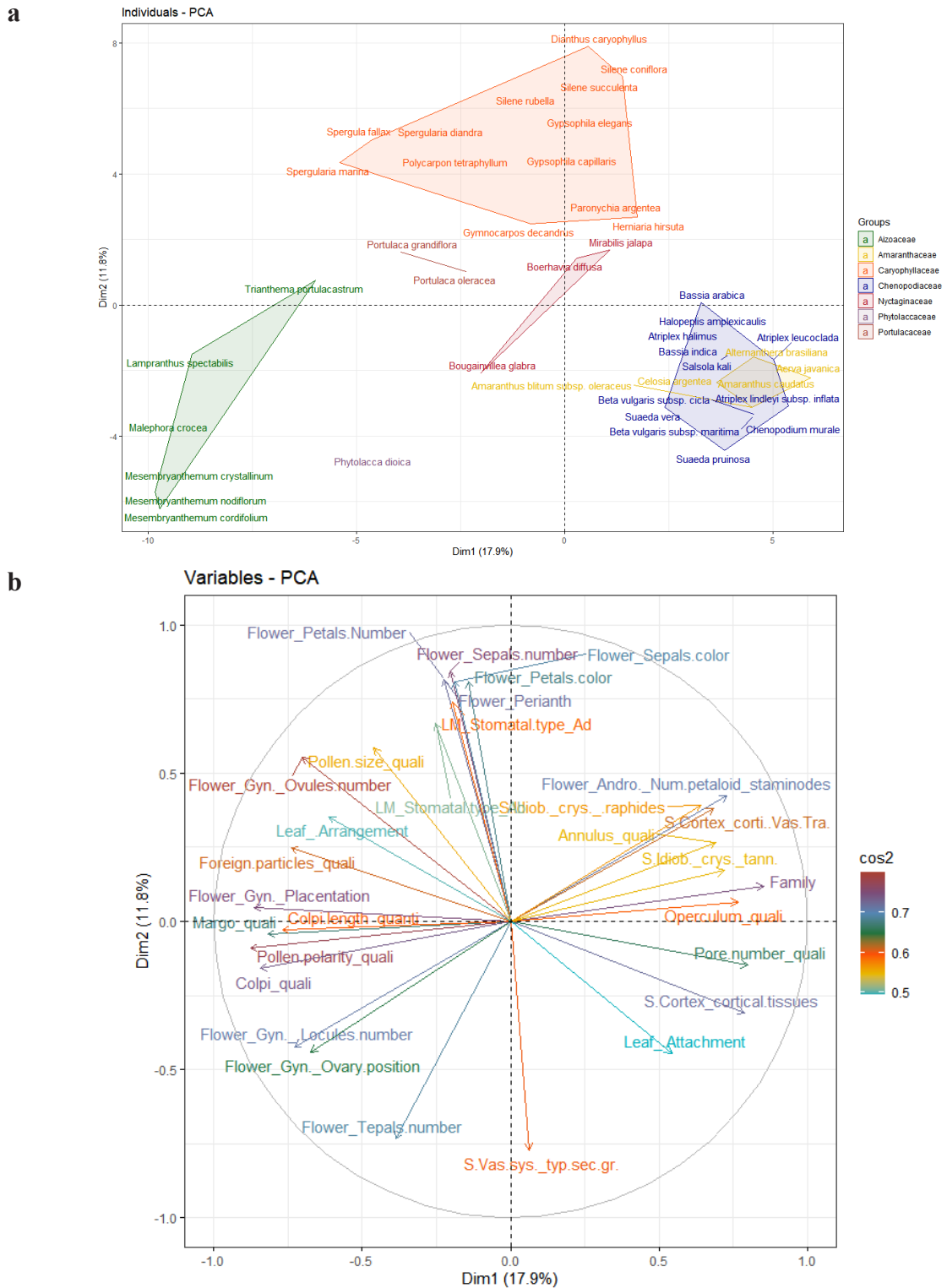


Fig. 4. a. Principal component analysis (PCA) of the 43 studied taxa with 17.9% total variance described along the first axis (Dim 1) followed by 11.8 % variance exhibited along the second axis (Dim 2). Chenopodiaceae “blue”, Amaranthaceae” yellow”, Portulacaceae “mauve”, Caryophyllaceae “orange”, Aizoaceae” green,”Phytolacca “purple “and Nyctaginaceae “red”, **b.** Correlation between the first two components and multiple variables. The variable plot helps to identify variables that are [the most correlated with dimension 1 and 2]

TABLE 1. Pearson's correlation (r) between the first two PCA components and the significantly correlated variables (P-value <0.001) [Based on the correlation value, the variables are arranged in descending order]

Dim. 1	R	Dim. 2	R
Macromorphological features			
Petaloid staminodes	0.728	Sepals number	0.844
Leaf attachment	0.543	Perianth	0.817
Succulency	0.501	Petals number	0.817
Inflorescence	0.481	Petals color	0.810
Stamens number	0.427	Sepals color	0.809
Lamina margin	0.342	Ovules number	0.554
Tepals number	-0.387	Lamina shape	0.427
Bracts	-0.433	Petaloid staminodes	0.423
Stem strength	-0.575	Stem internal structure	0.394
Leaf arrangement	-0.611	Succulency	0.389
Carpels number	-0.673	Leaf arrangement	0.353
Ovary position	-0.67	Lamina margin	-0.342
Ovules number	-0.701	Locules number	-0.423
Locules number	-0.728	Ovary position	-0.440
Placentation	-0.86	Leaf attachment	-0.446
		Tepals color	-0.587
		Tepals number	-0.733
Micromorphological features: stem anatomical features			
Cortical tissues	0.789	Epidermis	0.434
Tanniferous idioblasts	0.719	Aqueous cells	0.413
Cortical vascular traces	0.683	Raphides crystals	0.393
Raphides crystals	0.640	Cortical vascular traces	0.380
Aqueous cells	0.565	Trichomes composition	0.365
Endodermis	0.491	Styloids crystals	0.351
Styloids crystals	0.464	Vesicular trichomes	0.335
Trichomes branching	0.385	Cortical tissues	-0.30
Trichomes glands	0.342	Cuticle	-0.36
Types of vascular abnormality	0.318	Types of vascular abnormality	-0.423
Vesicular trichomes	0.311	Pith cavity	-0.427
Sandy crystals	-0.359	Type of secondary growth	-0.772
Presence of trichomes	-0.368		
Micromorphological features: lamina anatomical & epidermal features (LM & SEM)			
Tanniferous idioblasts	0.658	Adaxial stomatal type	0.740
Raphides crystals	0.60	Abaxial stomatal type	0.667
Adaxial anticlinal wall's width	0.574	Leaf type according to stomatal distribution	0.666
Styloids crystals	0.464	Epidermis	0.560
Abaxial anticlinal wall's width	0.455	Styloids crystals	0.351
Number and shape of vascular units	0.400	Abaxial anticlinal wall's width	-0.325
Abaxial epidermal cell's shape	0.391	Abaxial stomatal opening elevation	-0.342
Adaxial periclinal wall's striation	0.335	Leaf shape	-0.408

TABLE. 1. Cont.

Dim. 1	R	Dim. 2	R
Vesicular trichomes	0.307	Cuticle	-0.532
Abaxial periclinal wall's striation	0.306		
Epidermis	-0.334		
Sandy crystals	-0.349		
Presence of trichomes	-0.360		
Abaxial epicuticular wax	-0.472		
Palynological features			
Pore number	0.798	Pollen size	0.585
Operculum	0.765	Pore diameter	0.473
Annulus	0.690	Interporal distance	0.408
Pore depth	0.547	P	0.330
Sculpture	0.406	E	0.323
Pore diameter	0.398	Pore depth	0.306
Puncta density	-0.454		
Pollen size	-0.460		
Puncta arrangement	-0.481		
Pollen shape	-0.581		
P/E ratio	-0.589		
Colpi width	-0.643		
Extexinous bodies	-0.661		
Pollen class	-0.664		
Foreign particles	-0.740		
Colpi length	-0.768		
Margo	-0.817		
Colpi	-0.842		
Polarity	-0.876		
	Dim. 1	Dim. 2	Dim. 3
Eigenvalue	22.38	14.70	12.02
Variance %	17.90	11.76	9.61
Cumulative variance %	17.90	29.67	39.28

PCA at Dim. 1 and Dim. 2, with a cumulative variance of 29.67 %, declare the clarity separation of Chenopodiaceae, Amaranthaceae, Phytolaccaceae, Portulacaceae and the distribution of the studied taxa of Nyctaginaceae (*Boerhavia diffusa*, and *Mirabilis jalapa* at the side, away from *Bougainvillea glabra*). Dim. 1, with a cumulative variance of 17.9%, gathers all the studied taxa of Aizoaceae at the left side and the distribution of the studied taxa of Caryophyllaceae; *Dianthus caryophyllus*, *Herniaria hirsuta*, *Paronychia argentea* and *Silene coniflora* on the right side, while *Gymnocarpus decandrus*, *Gypsophila capillaris*,

G. elegans, *Herniaria hirsuta*, *Paronychia argentea*, *Polycarpon tetraphyllum*, *Silene coniflora*, *S. rubella*, *S. succulenta*, *Spergula fallax*, *Spergularia diandra* and *S. marina* on the left side. However, Dim. 2, with 11.8 % variance, gathers all the studied taxa of Caryophyllaceae at the upper center side and shows the distribution of the studied taxa of Aizoaceae (*Trianthema portulacastrum* at the upper side, while *Lampranthus spectabilis*, *Malephora crocea*, *Mesembryanthemum cordifolium*, *M. crystallinum*, *M. nodiflorum* at the bottom left side).

Discussion

Multivariate statistical analysis is applied to explore such concerns in the morphometric field. In the past, these methods were employed to study sets of angles or distances. Still, more recently, developments in theory, computing, and other areas have caused morphometric processes to focus on specific locations. Modern morphometric analysis has recently replaced conventional morphometric studies in biology and medicine. These investigations attempt to analyze the variations (Ocakoglu & Erca, 2013).

From the obtained UPGMA cluster and ordination analyses (PCA) based on all combined character states (macromorphological, micromorphological, and palynological multistate characters), the taxa under investigation are categorized into Series **I** and **II**, (Figs. 3 and 4 a, b).

Series **I** is divided into clusters **1** and **2** comprising all the studied taxa belonging to Amaranthaceae and Chenopodiaceae which are characterized by porate stenopalynous pollen grains. Cluster **1** is divided into groups **A** and **B**. Group **A** (= subgroup **a**) comprises only *Chenopodium album* and *C. murale*, which share a pollen pore diameter of less than one micron. Group **B** is divided into two subgroups, **b** and **c**, comprising four taxa sharing a pollen pore diameter of more than one micron. Subgroup **b** includes *Beta vulgaris* subsp. *cicla* and *B. vulgaris* subsp. *maritima* which have a sinuated lamina margin and semi-inferior ovary. Subgroup **c** includes *Amaranthus blitum* subsp. *oleraceus* and *Celosia argentea* which have entire lamina margin and superior ovary.

Cluster **2** is divided into groups, **C** and **D**. Group **C** is divided into subgroups **d** and **e** gathering all the studied taxa characterized by the absence of lamina trichomes in V.T.S. Subgroup **d** includes *Suaeda pruinosa* and *S. vera* which is characterized by a cylindrical lamina shape and four types of tissues in the cortex. Subgroup **e** includes *Halopeplis amplexicaulis*, *Bassia arabica*, and *B. indica*, characterized by trichomes on the stem epidermis and three types of cortical tissues. *Halopeplis amplexicaulis* appears as a separate entity due to the presence of an anomalous secondary thickening in the

stem cross-section, while *Bassia arabica* and *B. indica* share normal secondary thickening. Group **D** is divided into subgroups **f** and **g** including all the studied taxa characterized by lamina trichomes in V.T.S. Subgroup **f** includes *Atriplex leucoclada*, *A. halimus* and *A. lindleyi* subsp. *inflata* share stem and lamina vesicular trichomes and successive cambial rings in the stem cross-section. *Atriplex leucoclada* appears as a separate entity in this subgroup due to sexuality (polygamous). Subgroup **g** includes *Salsola kali*, *Amaranthus caudatus*, *Aerva javanica*, and *Alternanthera brasiliana* in having stem and lamina e-glandular trichomes. *Salsola kali* appears as a separate entity in this subgroup due to its succulence nature, and the fascicular and interfascicular xylem components are different (xylem vessels, xylary fibers, and xylem parenchyma at the fascicular regions and sclerenchymatous tissue inter-fascicularly). *Amaranthus caudatus*, *Aerva javanica*, and *Alternanthera brasiliana* are not succulent, and the fascicular and inter-fascicular xylem components are similar (xylem vessels, xylary fibers, and xylem parenchyma).

The obtained data in the present study gathered all the studied taxa belonging to Amaranthaceae *s.s.* and Chenopodiaceae in one direction (Series I). This is compatible and supported by the previous work of Eicher (1878), Engler (1886), Melchior (1964), Cronquist (1968), Rodman et al. (1984), and Thorne (1992), Takhtajan (1980), and Reveal (2012) who showed a close relationship between Amaranthaceae *s.s.* and Chenopodiaceae based on morphological criteria. The results achieved are consistent with the work of Bayoumy et al. (2020) and Tantawy et al. (2023), particularly on Chen-Am (Chenopodiaceae-Amaranthaceae) that gathered the studied taxa in one family (Amaranthaceae *s.l.*) based on macromorphological, micromorphological and palynological data. This is in accord with the concept of Judd et al. (2002), Brockington et al. (2009), APG I, II, III & IV (1998, 2003, 2009 & 2016 respectively), and Shipunov (2022) who placed Chenopodiaceae as a subfamily under Amaranthaceae *s.l.* based on molecular criteria. Walker et al. (2018) placed them under the AMAR clade and Rettig et al. (1992) placed both families in one clade, and considered them the basal groups of Caryophyllales *s.s.*

Series **II** is divided into clusters **3** and **4** including

all the studied taxa having colpate stenopalynous pollen belonging to Aizoaceae, Portulacaceae and *Phytolaca dioica* (Phytolaccaceae), eurypalynous (colpate and porate) pollen in the studied taxa of Nyctaginaceae and Caryophyllaceae. Cluster 3 is divided into groups, E and F including the studied taxa having normal secondary growth in the stem, central or free central placentation. In Group E (= subgroup h), *Portulaca grandiflora* and *P. oleracea* (Portulacaceae) are gathered due to the presence of semi-inferior ovaries. Group F is divided into two subgroups, i and j, comprising all the studied taxa of Caryophyllaceae that share the presence of superior ovaries. Subgroup i gathered *Spergula fallax*, *Spergularia marina*, *Polycarpon tetraphyllum* and *Spergularia diandra* based on the presence of tricolpate and isopolar pollen grains. Subgroup j gathered *Herniaria hirsuta*, *Paronychia argentea*, *Dianthus caryophyllus*, *Silene coniflora*, *S. succulenta*, *Gymnocarpus decandrus*, *Silene rubella*, *Gypsophila capillaris*, *G. elegans* based on the presence of polypantoporate and apolar pollen grains.

Although the previously available studies on the taxonomic relationships between Portulacaceae and Caryophyllaceae are rare, we were able to come up with some studies; some of which were traditional, some of which are recent; some of which we agreed with, and some of which we disagreed with. The obtained data achieved from the available taxa are reinforced by the work of Metcalfe & Chalk (1950) who stated that Portulacaceae and Caryophyllaceae have normal secondary growth instead of an anomalous nature in most taxa of Centrospermae. Rohwer et al. (1993) and Simpson (2019) reported that there is a close relationship Portulacaceae and Caryophyllaceae based on diplochlamydeous flower structure and free central placentation. This is compatible with the achieved data in the present work. The net results showed by UPGMA cluster analyses related to Portulacaceae and Caryophyllaceae are in contradiction with those of Rodman et al. (1984) who stated that Caryophyllaceae was placed in a distinct clade based on morphological cladistics analyses and placed Portulacaceae with Aizoaceae, Cactaceae, Basellaceae, Didiereaceae in advanced clade (succulent clade) of Centrospermae. Retting et al. (1992), Thorne (1992), Cronquist & Thorne (1994) and Clement et al. (1994) supported the placement of Caryophyllaceae in a separate clade with Molluginaceae and treated Portulacaceae as a relative group to Aizoaceae based on the presence

of anthocyanin pigments. Melchoir (1964) and Reveal et al. (2012) placed Caryophyllaceae in Caryophyllaceae and Portulacaceae in Portulacaceae. Based on molecular studies, Walker et al. (2018) separated Portulacaceae in the PORT clade and Caryophyllaceae in the CARY clade.

Cluster 4 is divided into groups G and H including all the studied taxa having anomalous secondary growth. Group G is divided into subgroups k and l gathering all the studied taxa of Aizoaceae having flowers with superior and inferior ovaries with parietal or axile placentation. Subgroup k includes *Mesembryanthemum crystallinum*, *M. Cordifolium* and *M. nodiflorum* with axile placentation. Subgroup i includes *Trianthema portulacastrum*, *Lampranthus spectabilis*, and *Malephora crocea*, with parietal placentation. *Trianthema portulacastrum* appears as a separate entity in a separate line due to the presence of Kranz anatomy as a unique character, superior ovaries, large-sized pollen grains, microechinate-punctate sculpture, presence of ektexinous bodies, fusiform colpi, colpi width more than one micron and the colpi length more than 20 microns. Group H is divided into two subgroups, m and n gathering all the studied taxa having basal placentation and stem medullary vascular bundles. Subgroup m includes *Phytolacca dioica* (Phytolaccaceae) as a separate entity based on unisexual flowers with numerous stamens and medium-sized pollen grains. Subgroup n includes *Bougainvillea glabra*, *Boerhavia diffusa* and *Mirabilis jalapa*, which have bisexual flowers with a definite number of stamens and large-sized pollen grains. *Bougainvillea glabra* is characterized by colpate pollen while *Boerhavia diffusa* and *Mirabilis jalapa* have porate pollen grains.

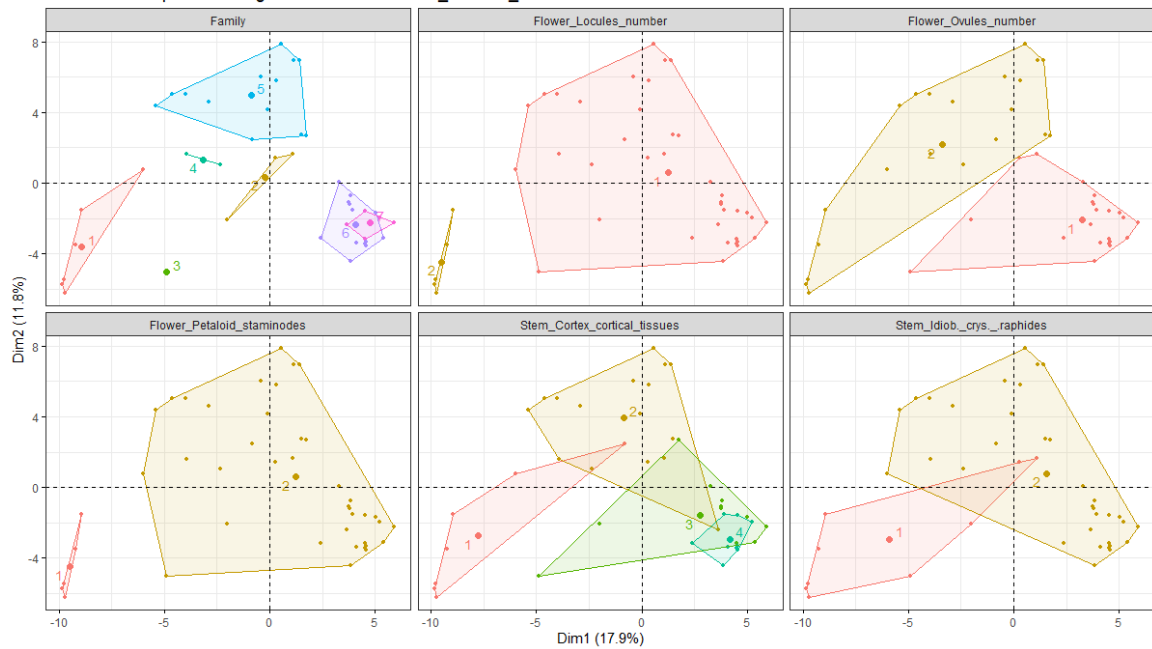
The obtained data in the present study related to Phytolaccaceae, Nyctaginaceae, and Aizoaceae show a close relationship between them. These findings are supported by Takhtajan (1980), who indicated a close relationship between Phytolaccaceae, Nyctaginaceae, Aizoaceae, and grouped them based on evolutionary relationships in the same suborder (Phytolaccineae). He considered Phytolaccaceae to be the most primitive family of the order. Melchoir (1964) and Reveal (2012) grouped Phytolaccaceae, Nyctaginaceae, and Aizoaceae in the same suborder (Nyctaginineae). These findings are in correspondence with the molecular studies

of Downie et al. (1997), Cuénoud et al. (2002), Brockington et al. (2009), and Hernández Ledesma et al. (2015) who placed them in the “raphide” clade due to the presence of raphides and pseudoraphides crystals and Walker et al. (2018) who grouped these families in the PHYT clade. Engler (1886), Eichler (1878), Thorne (1992), Bessey (1915), Cronquist (1981), and Rodman et al. (1984) placed Phytolaccaceae and

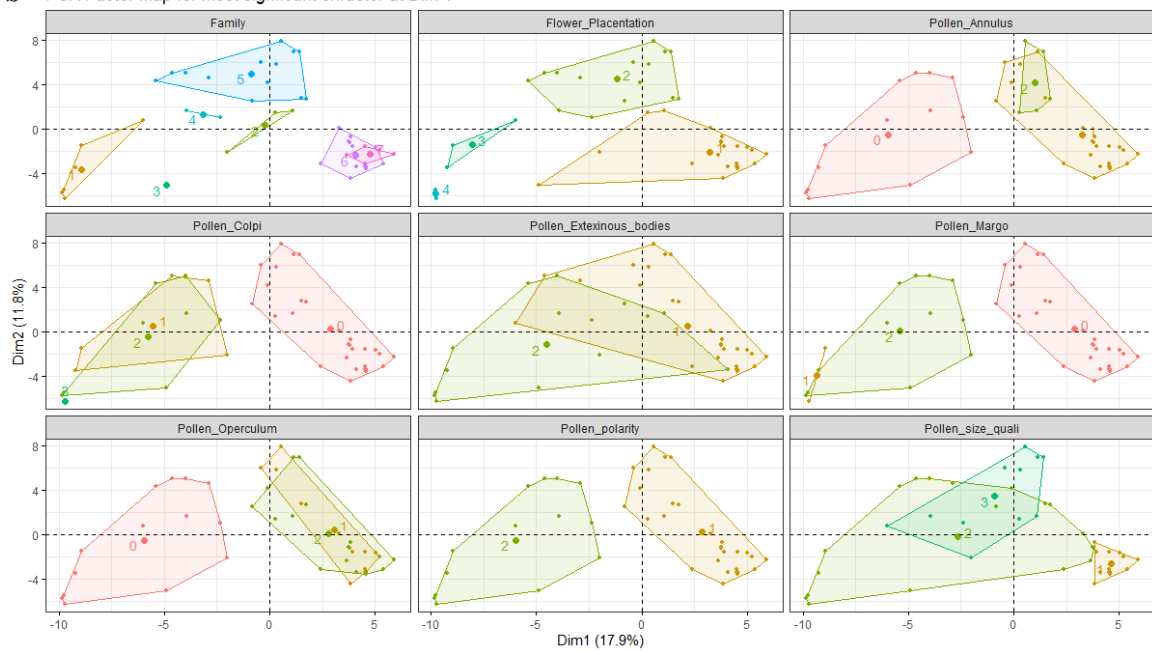
Nyctaginaceae in the same cluster and considered them as the primitive groups of the order.

The principal component analysis (PCA) factor map (Fig. 5), with convex ellipses, represents potential taxonomic groupings based on the taxa's response to the most crucial features. All variables were examined that show significance at two dimensions 1 and 2.

a PCA Factor map for most significant character at Dim_1 & Dim_2



b PCA Factor map for most significant character at Dim 1



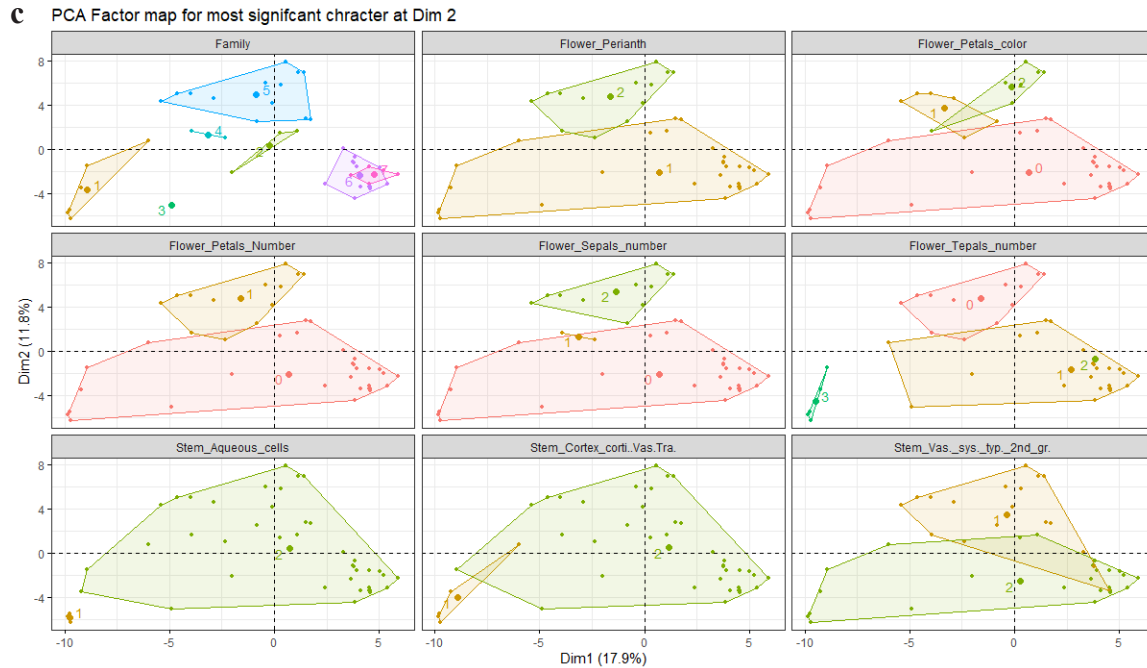


Fig. 5. Factor map ellipses from the principal component analysis (PCA) representing the most possible taxonomic traits based on the gathering response of families. a. the most significant characters at Dim. 1 & 2, b. the most significant characters at Dim. 1, c. the most significant characters at Dim. 2

Dim. 1 & 2 (ovules number, locules number, petaloid staminodes, stem aqueous cells, cortical vascular traces, stem cortical tissues, stem raphides crystals). At Dim. 1, the traits are (placentation, pollen size, pollen margo, pollen colpi, pollen polarity, pollen operculum, pollen annulus, pollen extenxious bodies), while at Dim. 2 (petals color, perianth, sepals number, petals number, tepals number, type of secondary growth).

For example, there is a clear divide between families based on placentation at Dim. 1 with free central (Caryophyllaceae, Portulacaceae), axile and parietal (Aizoaceae), or basal (Amaranthaceae, Chenopodiaceae, Nyctaginaceae, Phytolaccaceae) based on their positive or negative correlation with Dim. 1. The greater axis of each ellipse skews towards Dim. 1, indicating that it is the most influential dimension.

From the foregoing data, there are numerous overlapping characteristics, but there are also reliable and consistent characteristics in clarifying the distinctions between the studied taxa. To illustrate this,

the following Table 2 sheds light on some crucial criteria, which are regarded as diagnostic key features.

Conclusion

From achieved results in the current study, the interpretations of these results, the relationships between the taxa under investigation, and what these results agreed with and what disagreed with previous studies on this point, our results are in great compatibility with the general taxonomic concepts which stated that due to the significant variation within core Caryophyllales, the phylogenetic relationships between its families are still uncertain. To get a more precise review of the systematic placement of core Caryophyllales and to solve many of the taxonomic problems, we require further future studies, considering the increase in the number of OUTs and the collection of many characters from various sources, particularly molecular and phytochemical studies.

Conflict of interests: The authors confirm that there is no conflict of interest to disclose

TABLE 2. The most significant morphological & palynological characters of the studied taxa of Caryophyllales

Families	Characters																			
	Whole plant							Vegetative characters							Floral characters					
	Herb	Sub shrub	Shrub	Tree	Hollow stem	Heterophylly leaves	Inflorescence				Perianth			Placentation						
							So.	R.	C.	H.	Sp.	Pan.	M.	D.	A.	Par.	B.	F.		
Aizoaceae (6 taxa)	6	-	-	-	-	2	6	-	-	-	-	-	6	-	3*	3*	-	-		
Amaranthaceae (5 taxa)	3	2	-	-	-	-	-	1	1	1	2	-	5	-	-	-	5	-		
Caryophyllaceae (13 taxa)	12	-	1	-	3*	1	2	1	11	-	-	-	2	11	-	-	-	13		
Chenopodiaceae (13 taxa)	6	3	4	-	-	-	1	-	2	-	10	-	13	-	-	-	13	-		
Nyctaginaceae (3 taxa)	2	-	1	-	-	-	-	-	3	-	-	1	3	-	-	-	3	-		
Phytolaccaceae (1 taxon)	-	-	-	1*	-	-	-	1	-	-	-	-	1	-	-	-	1	-		
Portulacaceae (2 taxa)	2	-	-	-	-	-	2	-	-	-	-	-	-	2	-	-	-	2		

So.: Solitary; R.: Raceme; C.: Cyme; H.: Head; Sp.: Spike; Pan.: Pannicle; M.: Monochlamydeous; D.: Diplochlamydeous; A.: Axile; Par.: Parietal; B.: Basal; F.: Free Central.

(*) The asterisked numbers indicate the unique characters

TABLE 2. Cont. Stem anatomical characters

Families	Characters											
	Normal	Secondary growth					Idioblasts					
		Abnormal					Druses	Raphides		Styoids	Sandy crystals	Tanniferous idioblasts
		S. Ca.	I. Ph.	M. B.	G.T.	Ph.						
Aizoaceae (6 taxa)	-	6	6	-	2	5	-	3	-	6		
Amaranthaceae (5 taxa)	-	1	3	2	4	-	-	-	2	-		
Caryophyllaceae (13 taxa)	13	-	-	-	5	-	-	-	-	3		
Chenopodiaceae (13 taxa)	3	3	5	2	7	-	-	-	3	-		
Nyctaginaceae (3 taxa)	-	3	2	3	-	3	-	3	-	3		
Phytolaccaceae (1 taxon)	-	-	-	1	-	1	1*	1	-	1		
Portulacaceae (2 taxa)	3	-	-	-	2	-	-	-	-	2		

S. Ca.: Successive Cambial Rings; Ph.: Phloem; I. Ph.: Included Phloem; M. B.: Medullary Bundles; G.T.: Ground Tissue.

(*) The asterisked numbers indicate the unique characters

TABLE 2. Cont. Lamina anatomical characters

Families	Characters	Mechanical tissue	Water-bearing cells	Kranz						Hydathodes
				Atriploid type	Kochioid type	Kranz-ventrodorsal type	Salsoid type	Pilosoid type	Non-Kranz type	
Aizoaceae (6 taxa)		-	4	1	-	-	-	-	5	-
Amaranthaceae (5 taxa)		1	-	2	-	-	-	-	3	-
Caryophyllaceae (13 taxa)		-	-	2	-	-	-	-	9	-
Chenopodiaceae (13 taxa)		4	8	3	2*	1*	1*	-	6	2*
Nyctaginaceae (3 taxa)		3	-	2	-	-	-	-	1	-
Phytolaccaceae (1 taxon)		1	-	-	-	-	-	-	1	-
Portulacaceae (2 taxa)		-	2	1	-	-	-	1*	-	-

(*) The asterisked numbers indicate the unique characters

TABLE 2. Cont. Lamina epidermal characters (LM & SEM)

Families	Characters	Leaf type		Epidermal cell walls	Epicuticular wax	Fissured crust
		Hypostomatic leaf	Homostomatic leaf			
Aizoaceae (6 taxa)		-	3	-	-	-
Amaranthaceae (5 taxa)		-	5	-	-	-
Caryophyllaceae (13 taxa)		-	1	3	-	-
Chenopodiaceae (13 taxa)		-	10	-	3*	1*
Nyctaginaceae (3 taxa)		-	-	-	-	-
Phytolaccaceae (1 taxon)		1*	-	-	-	-
Portulacaceae (2 taxa)		-	2	1	-	-

(*) The asterisked numbers indicate the unique characters

TABLE 2. Cont. Pollen grains characters (SEM)

Families	Characters	Polarity			Pollen size			Pollen class		
		Apolar	Isopolar	Small	Medium	Large	Pantoporate	Tricolpate	Tetracolpate	Pantocolpate
Aizoaceae (6 taxa)		-	6	-	5	1	-	6	1	-
Amaranthaceae (5 taxa)		5	-	4	1	-	5	-	-	-
Caryophyllaceae (13 taxa)		9	4	-	8	5	9	4	-	-
Chenopodiaceae (13 taxa)		13	-	9	4	-	13	-	-	-
Nyctaginaceae (3 taxa)		2	1	-	-	3	2	1	-	-
Phytolaccaceae (1 taxon)		-	1	-	1	-	-	1	-	-
Portulacaceae (2 taxa)		-	2	-	-	2	-	-	1	1*

(*) The asterisked numbers indicate the unique characters

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التحليل الاحصائي للدلالات المورفولوجية المختارة لبعض وحدات تصنيفية من رتبة القرنفليات الأساسية في مصر

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لا تزال هناك الكثير من التساؤلات حول الحدود الفاصلة بين فصائل رتبة مركزية البويضات، وعلاقتها التطورية، ووضع العديد من الأجناس بداخلها. لا تزال العلاقات البيئية في الرتبة بحاجة إلى المزيد الفهم والتفسير على الرغم من وجود العديد من الدراسات السابقة. تم في الدراسة الحالية تجميع 43 وحدة تصنيفية تنتمي إلى سبع فصائل من بيئاتها الطبيعية وبعض الحقائق النباتية في مصر. تهدف تلك الدراسة إلى فحص واستخلاص العديد من الصفات المفتاحية الظاهرية والتشريحية الدقيقة وكذلك صفات حبوب اللقاح وذلك باستخدام الميكروسكوب الضوئي والميكروسكوب الإلكتروني الماسح وذلك لفهم وتفسير العلاقات البيئية بين الوحدات التصنيفية قيد الدراسة كمساهمة جديدة للمساهمات السابقة في هذا النهج. أظهرت النتائج التي تم الحصول عليها أن هناك بعض الصفات المفتاحية ذات التأثير الكبير في فهم هذه العلاقات. تم إخضاع جميع النتائج التي تم الحصول عليها لبرنامج إحصائي (برمجة R) لتحليل مدى قوة تلك الصفات في تفسير وفهم هذه العلاقات. أظهرت الشجرة التصنيفية الناتجة من تحليل UPGMA أن الوحدات التصنيفية قيد الدراسة تم تقسيمها إلى مسلسلتين رئيسيتين، كل منهما تجمع العديد من الوحدات التصنيفية. قام تحليل PCA الناتج بتقسيم الوحدات التصنيفية في بعدين (Dim1&2) ومناقشة النتائج التي تم الحصول عليها ومقارنتها مع ما توصلت إليه الدراسات السابقة.