

Integrating Quantitative Stem Anatomy and Multivariate Analyses to Resolve Taxonomic Boundaries within the *Caralluma* Complex (Apocynaceae)

Hanan A. Saleem *, Hassan M. Ibrahim and Arif S. Alhammadi

Department of Biological, Faculty of Sciences, Sana'a University, Sana'a, Yemen

*Corresponding author: h.saleem@su.edu.ye

ABSTRACT

Taxonomic delimitation of the genus *Caralluma* R.Br. from its segregated genera remains highly problematic due to severe morphological convergence and phenotypic plasticity in their vegetative characters. This study provides a quantitative anatomical characterization of four taxa within the *Caralluma* segregate complex: *Apteranthes tuberculata*, *Caralluma subulata*, *Desmidorchis penicillata*, and *Monolluma quadrangula*, to evaluate the systematic utility of the internal stem architecture. The findings revealed that these taxa possess succulent stems partitioned into distinct ribs and sulci, with a single epidermal layer, a specialized cortex, and a central pith. Furthermore, the vascular architecture exhibits a eustelic arrangement, with the internal phloem situated interior to the protoxylem. Quantitative analysis capitalized on these variations and revealed a clear evolutionary trade-off in water-storage strategies: larger, shrubby taxa maximized storage by increasing tissue-layer density, whereas smaller, low-growth taxa relied on cell hypertrophy. Statistical analysis demonstrated that rib cortical layers, pith parenchyma cell areas, and internal phloem distribution were highly significant taxonomic markers. Both cluster analysis (TWCA) and Principal Component Analysis (PCA) successfully resolved the taxa into distinct groups, distinguishing *C. subulata* as a basal lineage, *A. tuberculata* as a transitional group, and *D. penicillata* and *M. quadrangula* as closely related members of the derived southern clade. Ultimately, these internal structural variations provide a strong anatomical fingerprint for identifying and differentiating these segregated generic lineages.

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1. INTRODUCTION

The genus *Caralluma* R.Br. (*sensu lato*, including its segregate genera *Apteranthes* Mikan, *Desmidorchis* Ehrenb. and *Monolluma* Plowes) belongs to the Apocynaceae family (subfamily Asclepiadoideae) and comprises highly specialized succulent xerophytes. Its classification remains notoriously complex because of the morphological convergence and phenotypic plasticity often observed among succulent taxa [1, 2]. Although the genus has been extensively examined for its pharmacological value [3–7], it has a turbulent taxonomic history characterized by conflicting interpretations of its boundaries [8–14]. A striking example of this instability is the radical transfer of

numerous taxa to *Ceropegia* L. [15], a controversial move that is still rejected by major global botanical databases [16, 17]. Consequently, the true taxonomic status of these species remains a subject of intense debate [9]. Although external morphology and molecular data are standard in plant systematics, they often yield conflicting results in xerophytes because of rapid environmental adaptation.

In contrast, internal vegetative anatomy offers a highly stable alternative dataset that is less prone to superficial environmental pressures [18, 19]. Because succulent stems function as primary hydraulic and metabolic centers, quantitative anatomical traits, such as tissue layer distribution, pith cell architecture, and vascular cylinder



Map 1. Locality map showing the collection sites of the studied *Caralluma* complex specimens generated using QGIS (v.3.40). software.

configuration, can serve as reliable structural markers for delimiting closely related taxa [20–22]. However, despite the clear systematic value of internal structures [23–26], a critical research gap persists: comparative internal stem anatomy within the *Caralluma* complex remains almost completely unexplored. The existing literature is restricted to isolated species descriptions [27] or superficial Epidermal Micromorphology [9], and a detailed comparative analysis of deep internal tissue layers across these segregated groups is absent [28].

To fill this critical taxonomic gap, the present study provides a detailed comparative analysis of the internal stem anatomy of the genus *Caralluma* (represented by *C. subulata*) alongside its three primary segregated genera: *Apteranthes* (*A. tuberculata*), *Desmidorchis* (*D. penicillata*), and *Monolluma* (*M. quadrangula*). We evaluated the quantitative traits of these species to provide a robust structural resolution for these disputed generic lineages.

2. MATERIALS AND METHODS

From May to July 2024, four taxa of the *Caralluma* complex—*Caralluma subulata* and the segregated taxa *Angolluma tuberculata*, *Desmidorchis penicillata*, and *Monolluma quadrangula*—were collected from medium- and high-altitude mountainous areas in Yemen. Field photographs of the collected plants are presented in Fig. 1.

accurate geographic collection coordinates and localized mapping data are provided in Table 1 and Map 1, respectively. Available floristic and botanical references were used for taxonomic classification [29–31].

Voucher specimens for each species (*A. tubercu-*

lata BHSS733, *C. subulata* BHSS740, *D. penicillata* BHSS743, and *M. quadrangula* BHSS730) were deposited in the Herbarium of the Faculty of Science at Sana'a University for future reference.

To maintain anatomical uniformity and eliminate environmental phenotypic plasticity, succulent branches from each identified species were propagated in August 2024 at the experimental farm of the Faculty of Science, Sana'a University, under consistent nursery conditions. Following the approach of Ibrahim et al. [32], eight subpopulations were established, with two per species. Each subpopulation included 15 individuals to ensure a sufficient sample size for subsequent analyses. Sampling was performed in August 2025, exactly one year after controlled cultivation. To ensure strict developmental consistency and minimize age-related anatomical variation, sampling was standardized by harvesting one-year-old mature branches.

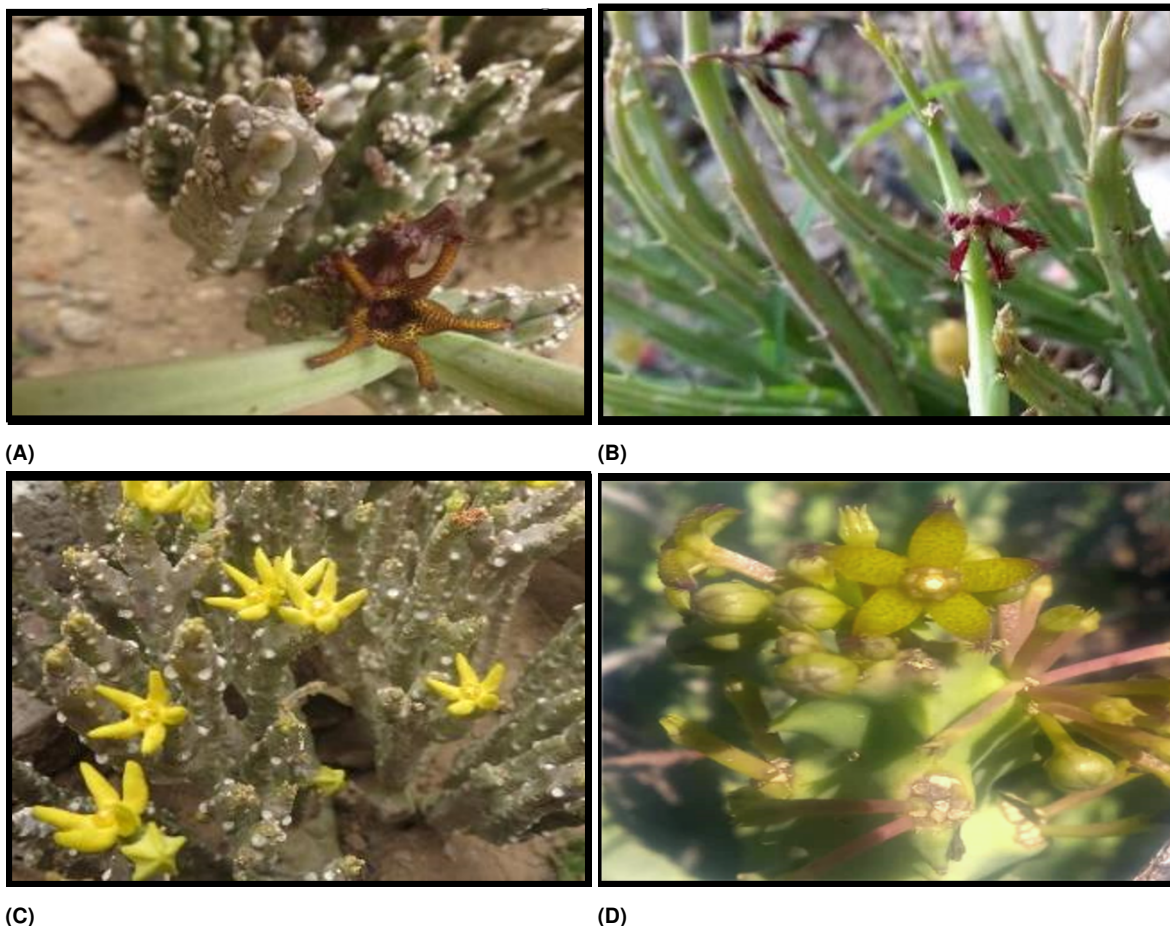
To investigate anatomical characteristics, stem cross-sections were prepared following a modified protocol adapted from Ibrahim et al. [33]. Transverse sections were precisely excised from the middle portion of the third internode (approximately 5–7 cm from the shoot apex) for all taxa studied. Six biological replicates were processed for each species to ensure the statistical reliability of the results. This specific sampling site was selected to fully represent

differentiated primary tissues in their mature functional state [34, 35].

Fresh stem segments were sliced into small pieces, measured for their stem width and rib lengths, and subsequently fixed in an FAA solution (containing 5 mL of 40% formalin, 5 mL of glacial acetic acid, and 90 mL of

Table 1. Locality and Sample collection of the four investigated taxa of the *Caralluma* complex.

No.	<i>Caralluma</i> species	Location	Coordinates		Date
			Longitude	Latitude	
1	<i>A. tuberculata</i>	Shibam Kawkaban District, Al Mahwit Governorate	43°53'57.20" E	15°32' 22.90" N	20 May 2024
2	<i>C. subulata</i>	Toor Al-Baha District, Lahaj Governorate	44°30'27.68" E	13°17'67.88" N	5 May 2024
3	<i>D. penicillata</i>	Toor Al-Baha District, Lahaj Governorate	44°29'35.46" E	13°17'73.19" N	2 May 2024
4	<i>M. quadrangula</i>	Bani Matar, District, Sana'a Governorate	43°58'53.08" E	15°14'12.15" N	15 May 2024

**Figure 1.** Flowering branches of the four investigated taxa of the *Caralluma* complex: A. *A. tuberculata*, B. *C. subulata*, C. *D. penicillata*, D. *M. quadrangula*.

95% ethyl alcohol) for 48 h to preserve the cellular structures. After fixation, the samples were thoroughly rinsed with distilled water to remove any remaining fixative and then subjected to a graded series of ethyl alcohol dehydrations (30%, 50%, 60%, 70%, 85%, 90%, and 100% solutions). The dehydrated tissues were cleared by passing them through a series of Xylene-95% alcohol (1:1) and pure xylene (100%) to facilitate paraffin infiltration. The specimens were embedded in paraffin wax, and transverse sections were cut at a thickness of 12 μ m using a rotary microtome. To visualize tissue differentiation, the sections were deparaffinized in xylene, rehydrated, and double-stained with Safranin O (1% w/v) Safranin O and Fast Green (0.5% w/v) Fast Green. This staining protocol provided high-contrast differentiation between lignified structures (stained red) and cellulosic tissues

(stained green). Finally, the sections were cleared in xylene and permanently mounted in DPX medium for microscopic investigation [33].

The anatomical characteristics of various stem tissues and layers were examined using a Leica (ATC 2000) microscope, following the authoritative anatomical literature of Fahn [36], Mauseth [27], and Esau [37]. Digital photographs of the stem cross-sections were captured using a Canon (IXUS255 HS) digital camera. The tissue thickness and number of cellular layers in the stem cross-sections were recorded at $\times 40$ magnification.

The detailed dimensions of the cells and crystals, including the area (length $\times$ width) within each layer, were obtained at $\times 400$ magnification, using optical light filters during microscopy to optimize boundary visibility and achieve maximum structural contrast. Measurements



were accurately recorded and expressed in micrometers (μm) using an ocular micrometer (where one ocular division equals $2.5 \mu\text{m}$) and ImageJ software [33].

Table 2. List of the quantitative anatomical stem characters examined for the four taxa of the *Caralluma* complex.

Layer		Characters
Epidermis		Width of the Stem (mm) Mean $\pm$ SD
		Length of the Ribs (Wings) (mm) Mean $\pm$ SD
	Ribs (Wings)	Cuticle Thickness of the Epidermal Cells (μm) Mean $\pm$ SD
		Mean Epidermal Cell Diameter (μm) Mean $\pm$ SD
		Area of Epidermal Cells (μm^2) Mean $\pm$ SD
	Sulci (Groove)	Cuticle Thickness of the Epidermal Cells (μm) Mean $\pm$ SD
		Mean Epidermal Cell Diameter (μm) Mean $\pm$ SD
		Area of Epidermal Cells (μm^2) Mean $\pm$ SD
Cortex	Ribs (Wings)	No. of Layers Mean $\pm$ SD
		Thickness (μm) Mean $\pm$ SD
		Mean Cortex Parenchyma Cells Diameter (μm) Mean $\pm$ SD
		Area of Cortex Parenchyma Cells (μm^2) Mean $\pm$ SD
	Sulci (Groove)	No. of Layers Mean $\pm$ SD
		Thickness (μm) Mean $\pm$ SD
		Mean Cortex Parenchyma Cells Diameter (μm) Mean $\pm$ SD
		Area of Cortex Parenchyma Cells (μm^2) Mean $\pm$ SD
		Mean Crystals Diameter (μm) Mean $\pm$ SD
		Crystals Area (μm^2) Mean $\pm$ SD
Vascular Cylinder	Ribs (Wings)	No. of Bundles Mean $\pm$ SD
		No. of Bundle Vessels Mean $\pm$ SD
		Thickness of Xylem Vessel Wall (μm) Mean $\pm$ SD
		Mean Diameter of Xylem Vessels (μm) Mean $\pm$ SD
		Area of Xylem Vessel Section (μm^2) Mean $\pm$ SD
		Thickness of Bundle Phloem (μm) Mean $\pm$ SD

		Sulci (Groove)	Thickness of Internal Phloem (μm) Mean $\pm$ SD
			No. of Bundles Mean $\pm$ SD
			No. of Bundle Vessels Mean $\pm$ SD
			Thickness of Xylem Vessel Wall (μm) Mean $\pm$ SD
			Mean Diameter of Xylem Vessels (μm) Mean $\pm$ SD
			Area of Xylem Vessel Section (μm^2) Mean $\pm$ SD
			Thickness of Bundle Phloem (μm) Mean $\pm$ SD
			Thickness of Internal Phloem (μm) Mean $\pm$ SD
Pith	Ribs (Wings)		Thickness of Pith (μm) Mean $\pm$ SD
			No. of Pith Layers Mean $\pm$ SD
			Mean Pith Parenchyma Cell Diameter (μm) Mean $\pm$ SD
			Area of Pith Parenchyma Cells (μm^2) Mean $\pm$ SD
	Sulci (Groove)		Thickness of Pith (μm) Mean $\pm$ SD
			No. of Pith Layers Mean $\pm$ SD
			Mean Pith Parenchyma Cell Diameter (μm) Mean $\pm$ SD
			Area of Pith Parenchyma Cells (μm^2) Mean $\pm$ SD

Approximately 40 quantitative anatomical stem characters (Table 2) were systematically evaluated for each taxon, selected based on their taxonomic relevance in previous literature [27, 28] and through preliminary exploratory screening.

All subsequent laboratory procedures, including tissue sectioning, staining, microscopic examination, and micrometric analysis, were conducted at the Botanical Laboratory of the Department of Biological Sciences, Faculty of Science, Sana'a University.

Statistical analysis

The significance of quantitative stem characteristics in taxonomic relationships was determined using one-way ANOVA in GraphPad Prism version 8.0.2 (p -values < 0.05 indicate significant differences). Prior to the one-way ANOVA, data normality was verified using the Anderson-Darling test (Minitab v. 14), and homogeneity of variance was assessed using the Brown-Forsythe test (GraphPad Prism 8).

The relationships among the four investigated taxa of

the *Caralluma* complex were illustrated in a dendrogram derived from stem anatomy data (Table 2) by generating a data matrix for numerical analysis using PC-ORD Windows version 7.09 (TWCA) [38]. Additionally, Principal Component Analysis (PCA) was applied to highlight the results of the cluster analyses using Paleontological Statistics (Past) version 5.3.

3. RESULTS AND DISCUSSION

According to Fig. 1, the four investigated taxa (*A. tuberculata*, *C. subulata*, *D. penicillata*, and *M. quadrangula*) lack leaves due to environmental stress; therefore, the stems are the main photosynthetic organs.

Morphologically, *Caralluma* (including its segregate genera) stems are characterized by the presence of ribs (wings) and sulci (grooves), which are typical morphological features of succulent stems that provide structural support and aid in water storage (Fig. 2). These ribs are longitudinal ridges on the stem surface. In contrast,

sulci are grooves or indentations between the ribs that allow for flexibility and expansion during water uptake [39]. However, *Caralluma* (including its segregate genera) stems are anatomically similar to dicot stems (Figs. 3 & 4) and are composed of an outer epidermal layer, cortex, vascular bundles (arranged in eustele rings), and a central pith [40].

As clearly demonstrated in the transverse sections in Fig. 3, the spatial arrangement of these tissue zones highlights the highly specialized functional compartmentalization. The uniseriate epidermis forms a tight outer boundary that encloses an expansive, chlorophyll-rich cortex. This cortical region is structurally segregated into distinct rib and sulcus sectors, showing varying densities in the parenchyma. Centrally, the vascular tissue is organized into a well-defined eustele ring composed of densely arranged bicollateral bundles that encapsulate an exceptionally wide water-storing central pith. This clear macro-anatomical organization, where a dense photosynthetic outer cortex shields a high-volume internal pith reservoir, maximizes the stem water-retention capacity while minimizing evaporative loss from the central core (Fig. 3). At a higher magnification, Fig. 4 reveals the fine-tuned microscopic elements that govern transport within these tissue layers. The protective outer cuticle exhibits prominent, localized thickening along the exposed rib epidermal cells, providing a dense mechanical barrier against solar radiation, while noticeably thinning within the humid microclimatic zones of the sulci grooves of the cicada.

Furthermore, the vascular architecture shown in Fig. 4 demonstrates a highly efficient bicollateral bundle configuration.

The xylem vessels were tightly organized in radial rows, flanked by distinct strands of both the external and internal phloem. This close structural association between the large, thick-walled water-conducting xylem vessels and the surrounding nutrient-transporting phloem elements ensures structural integrity under high negative pressure during severe droughts, protecting the transport system from vascular cavitation (Fig. 4) [27].

As in most succulent and photosynthetic stems, shoots not only store water but also act as photosynthetic organs, which agrees with Mauseth [27], who reported that the genus *Caralluma*, including *C. burchardii*, contains succulent and photosynthetic stems.

Based on Table 3 and Fig. 2, *D. penicillata* had the broadest stems and longest ribs, followed by *M. quadrangula* and *A. tuberculata*, whereas *C. subulata* had the smallest stem width and the shortest rib length.

According to González-Valenzuela *et al.* [41] and Tran *et al.* [42], the epidermis of succulent plants consists of a single layer of parenchyma cells covered by a cuticle, which supports water storage and protection. This aligns with our findings, in which the epidermis of the four investigated taxa comprised a single layer of epidermal

parenchyma cells covered by a cuticle (Figs. 3, 4, and 5). The stem ribs of *D. penicillata* had the thickest cuticle (Table 3 & Fig. 5), followed by the ribs of *A. tuberculata* and *M. quadrangula*, whereas *C. subulata* displayed the thinnest rib cuticle. The stem sulci of *C. subulata* had the thickest cuticle (Table 3 & Fig. 5), followed by *M. quadrangula* and *A. tuberculata*, whereas *D. penicillata* had the thinnest sulci cuticle. These results suggest that when the ribs in succulent stems are long, the sulcus cuticle is thin, whereas in stems with short ribs, it is thicker. This observation is consistent with that of Mauseth [27, 43], who reported that the rib length of succulent stems primarily affects the surface-to-volume ratio and capacity. Cuticle thickness is an indicator of protection against water loss and other environmental factors. In contrast, *A. tuberculata* exhibited (Table 3 & Fig. 5) the largest area of epidermal cell stem ribs (265.4 μm^2), followed by *D. penicillata* and *C. subulata*, whereas *M. quadrangula* had the smallest area of epidermal cell stem ribs. *A. tuberculata* showed (Table 3 & Fig. 5) the largest area of epidermal cell stem sulci, followed by *D. penicillata* and *M. quadrangula*, while *C. subulata* had the smallest area of epidermal cell-stem sulci.

As illustrated in Fig. 5, the four investigated taxa lack the hypodermis layer (a sub-epidermal supporting layer that strengthens and protects the stem, usually made of specialized cortical cells, collenchyma, and sometimes chlorenchyma or sclerenchyma, often with thickened walls and crystals, providing support and protection just under the epidermis [44–46]), which is a major structure in cactus stems [27]. This observation is consistent with Mauseth [27], who noted that the genus *Caralluma*, including *C. burchardii*, lacks a multiseriate, thick-walled hypodermis, which distinguishes it significantly from cacti.

As shown in Table 3 and Fig. 5, *C. subulata* exhibited the greatest thickness and largest parenchyma cell area within the rib cortex, followed by *A. tuberculata* and *M. quadrangula*, *D. penicillata* developed the thinnest rib cortex with the smallest parenchyma cell area. However, *M. quadrangula* demonstrated the highest thickness and largest parenchyma cell area within the sulci cortex, followed by *C. subulata* and *D. penicillata*, whereas *A. tuberculata* had the thinnest sulci cortex, with the smallest parenchymal cell area (Table 3 & Fig. 5). Additionally, the sulci cortex of *M. quadrangula* contained the largest crystals.

followed by *C. subulata* and *A. tuberculata*. Notably, *D. penicillata* lacked crystals in the sulcus cortex (Table 3 & Fig. 6). Crystals, typically in the form of calcium oxalate, are commonly found as druses or prisms in the cortex and pith of angiosperm stems, including close relatives of the genus *Caralluma* and its segregate genera, such as *Cynanchum acutum* (Apocynaceae/Asclepiadoideae), as documented by Doaigey [47] and Konyar *et al.* [48]. However, no specific studies have been identified that

Table 3. Quantitative anatomical stem characters of the four studied taxa (expressed as Mean $\pm$ SD)

Layer	Characters	<i>A. tuberculata</i>	<i>C. subulata</i>	<i>D. penicillata</i>	<i>M. quadrangula</i>
	Width of the Stem (mm)	16 $\pm$ 2	13 $\pm$ 2	27 $\pm$ 6 a ^{***} , b ^{****}	17 $\pm$ 5 c ^{**}
	Length of the Ribs (Wings) (mm)	7 $\pm$ 1	5 $\pm$ 1	14 $\pm$ 3 a ^{****} , b ^{****}	9 $\pm$ 3 b ^{*, c**}
	Cuticle Thickness of the Epidermal cells (μ m)	2 $\pm$ 0.4	1.9 $\pm$ 0.3	2.3 $\pm$ 0.5 b ^{**}	2 $\pm$ 0.4
Epidermis	Mean Epidermal Cell Diameter (μ m)	16.7 $\pm$ 3.5	15.2 $\pm$ 3.6	15.2 $\pm$ 2.8	13.4 $\pm$ 2.3 a ^{***}
	Area of Epidermal cells (μ m ²)	265.4 $\pm$ 113.8	206.7 $\pm$ 86.7 a ^{**}	234.1 $\pm$ 85.8	163.3 $\pm$ 54.2 a ^{****} , b ^{****} , c ^{****}
	Cuticle Thickness of the Epidermal cells (μ m)	1.1 $\pm$ 0.4	1.5 $\pm$ 0.4 a ^{****}	1 $\pm$ 0.2 b ^{****}	1.4 $\pm$ 0.2 a ^{***} , c ^{****}
	Mean Epidermal Cell Diameter (μ m)	16.2 $\pm$ 4.6	15.2 $\pm$ 3.7	15 $\pm$ 4.5	15 $\pm$ 2.3
	Area of Epidermal cells (μ m ²)	264 $\pm$ 139.3	187.8 $\pm$ 81 a [*]	224.3 $\pm$ 119.9	217.9 $\pm$ 66.7
	NO. of layers	18 $\pm$ 2	17 $\pm$ 1	17 $\pm$ 4	21 $\pm$ 5 a ^{****} , b ^{****} , c ^{****}
Cortex	Thickness (μ m)	141 $\pm$ 34.8	196.3 $\pm$ 15 a ^{****}	85.4 $\pm$ 26.7 a ^{****} , b ^{****}	132.5 $\pm$ 43.3 b ^{****} , c ^{****}
	Mean Cortex Parenchyma Cells Diameter (μ m)	51.8 $\pm$ 8.8	70 $\pm$ 26.56 a ^{***}	45.4 $\pm$ 11.5 b ^{****}	59.7 $\pm$ 16.3 c ^{**}
	Area of Cortex Parenchyma cells (μ m ²)	2606.6 $\pm$ 853.4	5390.3 $\pm$ 3744.7 a ^{****}	2138.8 $\pm$ 1041.7 b ^{****}	3704 $\pm$ 1938.3 b ^{*, c}
	No. of layers	20 $\pm$ 1	15 $\pm$ 2 a ^{****}	21 $\pm$ 1 b ^{****}	25 $\pm$ 2 a ^{****} , b ^{****} , c ^{****}
	Thickness (μ m)	202 $\pm$ 19.4	235.6 $\pm$ 25.7 a ^{**}	213 $\pm$ 19.7	289.3 $\pm$ 63.3 a ^{****} , b ^{****} , c ^{****}
Sulci (Groove)	Mean Cortex Parenchyma Cells Diameter (μ m)	51.6 $\pm$ 12.7	98 $\pm$ 34.5 a ^{****}	74.2 $\pm$ 22 a ^{**} , b ^{**}	74.9 $\pm$ 21.6 a ^{**} , b ^{**}
	Area of Cortex Parenchyma cells (μ m ²)	2574.7 $\pm$ 1157.5	10093.7 $\pm$ 6299.6 a ^{****}	5805.2 $\pm$ 3716.1 a ^{*, b}	5553.2 $\pm$ 2674.3 a ^{*, b}
	Mean Crystals Diameter (μ m)	5.2 $\pm$ 1.1	6.9 $\pm$ 0.9 a ^{****}	0 a ^{****} , b ^{****}	7.3 $\pm$ 1.2 a ^{****} , c ^{****}
	Crystals Area (μ m ²)	28 $\pm$ 9.8	47.7 $\pm$ 12.1 a ^{****}	0 a ^{****} , b ^{****}	53.7 $\pm$ 16.9 a ^{****} , c ^{****}
	No. of Bundles	10 $\pm$ 2	3 $\pm$ 1 a ^{****}	14 $\pm$ 3 a ^{****} , b ^{****}	9 $\pm$ 2 b ^{****} , c ^{****}
	No. of bundles vessels	60 $\pm$ 15	14 $\pm$ 5 a ^{****}	73 $\pm$ 15 a ^{**} , b ^{****}	44 $\pm$ 16 a ^{****} , b ^{****} , c ^{****}
	Thickness of xylem vessels' wall (μ m)	1.8 $\pm$ 0.4	1.6 $\pm$ 0.3	2.3 $\pm$ 0.4 a ^{****} , b ^{****}	2.1 $\pm$ 0.3 a ^{**} , b ^{****}
Ribs (Wings)	Mean Diameter of xylem vessels (μ m)	11 $\pm$ 1.4	9.6 $\pm$ 1.8 a ^{**}	14.1 $\pm$ 2 a ^{****} , b ^{****}	7.9 $\pm$ 1.2 a ^{****} , b ^{****} , c ^{****}
	Area of xylem vessels section (μ m ²)	118.9 $\pm$ 35	92.1 $\pm$ 33.7 a [*]	197.9 $\pm$ 57.7 a ^{****} , b ^{****}	63.1 $\pm$ 20.3 a ^{****} , b ^{****} , c ^{****}
	Thickness of bundle phloem (μ m)	16 $\pm$ 2.3	19.5 $\pm$ 4.4 a [*]	21.3 $\pm$ 4.4 a ^{***}	14.4 $\pm$ 5.8 b ^{***} , c ^{****}
	Thickness of internal phloem (μ m)	17 $\pm$ 3.2	17.8 $\pm$ 4	14.4 $\pm$ 2.6 a ^{*, b}	13.1 $\pm$ 2.8 a ^{****} , b ^{****}
Vascular Cylinder	No. of bundles	4 $\pm$ 1	2 $\pm$ 1 a ^{****}	6 $\pm$ 1 a ^{****} , b ^{****}	7 $\pm$ 1 a ^{****} , b ^{****}
	No. of bundle vessels	22 $\pm$ 5	9 $\pm$ 3 a ^{****}	27 $\pm$ 7 a ^{*, b}	35 $\pm$ 9 a ^{****} , b ^{****} , c ^{****}
	Thickness of xylem vessels' wall (μ m)	2.3 $\pm$ 0.6	1.4 $\pm$ 0.4 a ^{****}	2.3 $\pm$ 0.6 b ^{****}	1.9 $\pm$ 0.3 a ^{*, b} , c [*]
	Mean Diameter of xylem vessels (μ m)	15.4 $\pm$ 3.1	12.1 $\pm$ 2 a ^{****}	14.7 $\pm$ 2.4 b ^{****}	10 $\pm$ 1.3 a ^{****} , b ^{****} , c ^{****}
Sulci (Groove)	Area of xylem vessels section (μ m ²)	243.4 $\pm$ 97.1	145 $\pm$ 46.6 a ^{****}	216.2 $\pm$ 68 b ^{***}	98.4 $\pm$ 25.4 a ^{****} , b ^{****} , c ^{****}
	Thickness of bundle phloem (μ m)	18.3 $\pm$ 3.6	23 $\pm$ 5.7 a ^{**}	18.5 $\pm$ 4.9 b ^{**}	17.2 $\pm$ 5.9 b ^{***}
	Thickness of internal phloem (μ m)	15.3 $\pm$ 3.2	18.1 $\pm$ 3.5 a [*]	22.5 $\pm$ 4.9 a ^{****} , b ^{****}	13.5 $\pm$ 4.1 b ^{****} , c ^{****}
	Thickness pith (μ m)	527.4 $\pm$ 78.5	378.5 $\pm$ 30	625.7 $\pm$ 1317.6 a ^{****} , b ^{****}	3068.3 $\pm$ 793.5 a ^{****} , b ^{****} , c ^{****}
	NO. of pith layers	54 $\pm$ 9	21 $\pm$ 2 a ^{****}	110 $\pm$ 18 a ^{****} , b ^{****}	63 $\pm$ 21 b ^{****} , c ^{****}
Pith	Mean Pith Parenchyma Cells Diameter (μ m)	47.6 $\pm$ 11.2	82.4 $\pm$ 20.5 a ^{****}	51.2 $\pm$ 11.8 a ^{****}	39.7 $\pm$ 11.7 a ^{****} , c [*]
	Area of Pith Parenchyma cells (μ m ²)	2310.4 $\pm$ 1085.9	6877.3 $\pm$ 3162.1 a ^{****}	2672.9 $\pm$ 1134.5 b ^{****}	1616.8 $\pm$ 802.3 b ^{****}
	Thickness pith (μ m)	170.4 $\pm$ 31	221.6 $\pm$ 37.5	1302.1 $\pm$ 223.6 a ^{****} , b ^{****}	943.5 $\pm$ 162.5 a ^{****} , b ^{****} , c ^{****}
	NO. of pith layers	15 $\pm$ 2	12 $\pm$ 1 a ^{***}	27 $\pm$ 3 a ^{****} , b ^{****}	25 $\pm$ 4 a ^{****} , b ^{****} , c ^{**}
Sulci (Groove)	Mean Pith Parenchyma Cells Diameter (μ m)	75.5 $\pm$ 12.4	140.2 $\pm$ 24.8 a ^{****}	44.1 $\pm$ 16.4 a ^{****} , b ^{****}	63.9 $\pm$ 17.2 b ^{****} , c ^{****}
	Area of Pith Parenchyma cells (μ m ²)	5747.3 $\pm$ 1821.4	19954 $\pm$ 7188.4 a ^{****}	2159.3 $\pm$ 1545.2 a ^{****} , b ^{****}	4219.8 $\pm$ 2116.9 b ^{****}

a. Significant differences in quantitative stem anatomical characters compared to *A. tuberculata*, b. compared to *C. subulata*, c. compared to *D. penicillata*, *. P < 0.05, **. P < 0.01, ***, P < 0.001 and *****: P < 0.0001.

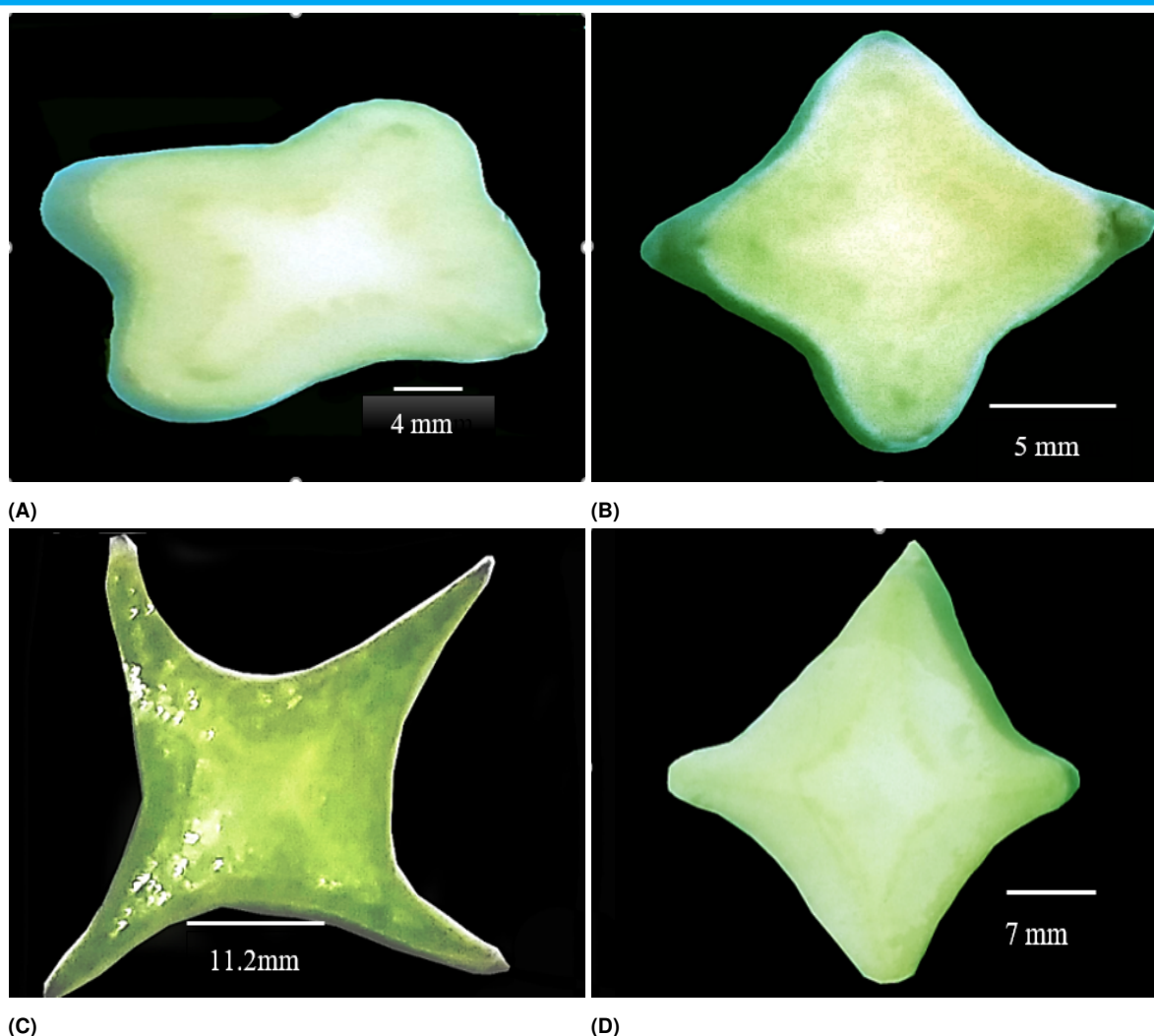


Figure 2. Transverse sections (outer outlines) of the four studied stem taxa, A. *A. tuberculata*, B. *C. subulata*, C. *D. penicillata*, D. *M. quadrangular*.

documented crystals in the stems of *Caralluma* taxa and its segregate genera.

In the studied taxa, vascular bundles are distinctly arranged in a eustelic ring that extends through the ribs and sulci, encircling a central parenchymatous pith (Figs 3 & 4). Narrow medullary rays separate these bundles and are typically collateral, with the primary xylem positioned internally toward the pith and phloem oriented externally toward the cortex (Fig. 7). Our observations are consistent with the structural patterns reported by Devadas and Beck [49] and Zhong et al. [50], confirming the efficient transport and structural integrity of the dicotyledonous frameworks. These findings align with Mauseth's [27] anatomical description of *Caralluma* (particularly *C. burchardii*), specifically regarding the primary vascular tissues (xylem and phloem).

As shown in Table 3, *D. penicillata* exhibited the highest number of vascular bundles along the ribs, followed by *A. tuberculata* and *M. quadrangular*, whereas *C. subulata* had the **lowest rib bundle count**. However, *M. quadrangular* had the highest number of sulci vascular

bundles, followed by *D. penicillata* and *A. tuberculata*. In contrast, *C. subulata* had the lowest number of vascular bundles within the sulci (Table 3). In addition, *D. penicillata* had the **highest number of vessels per rib bundle**, followed by *A. tuberculata* and *M. quadrangular*, while *C. subulata* had the **lowest vessel count** (Table 3). In contrast, *M. quadrangular* displayed the highest vessel frequency, followed by *D. penicillata* and *A. tuberculata*, whereas *C. subulata* showed the lowest mean number of vessels per sulcus bundle (Table 3). In terms of vessel size, *D. penicillata* maintained the **largest mean vessel area within the ribs**, whereas *A. tuberculata* exhibited the **largest mean vessel area within the sulci** (Table 3).

The quantitative data indicate a compensatory anatomical balance between the ribs and sulci in all four taxa. This regional partitioning ensures that a reduction in xylem elements in one area is counterbalanced by an increase in another area [51]. For example, *M. quadrangular* compensates for a lower rib vessel count by exhibiting the highest frequency of vessels within the

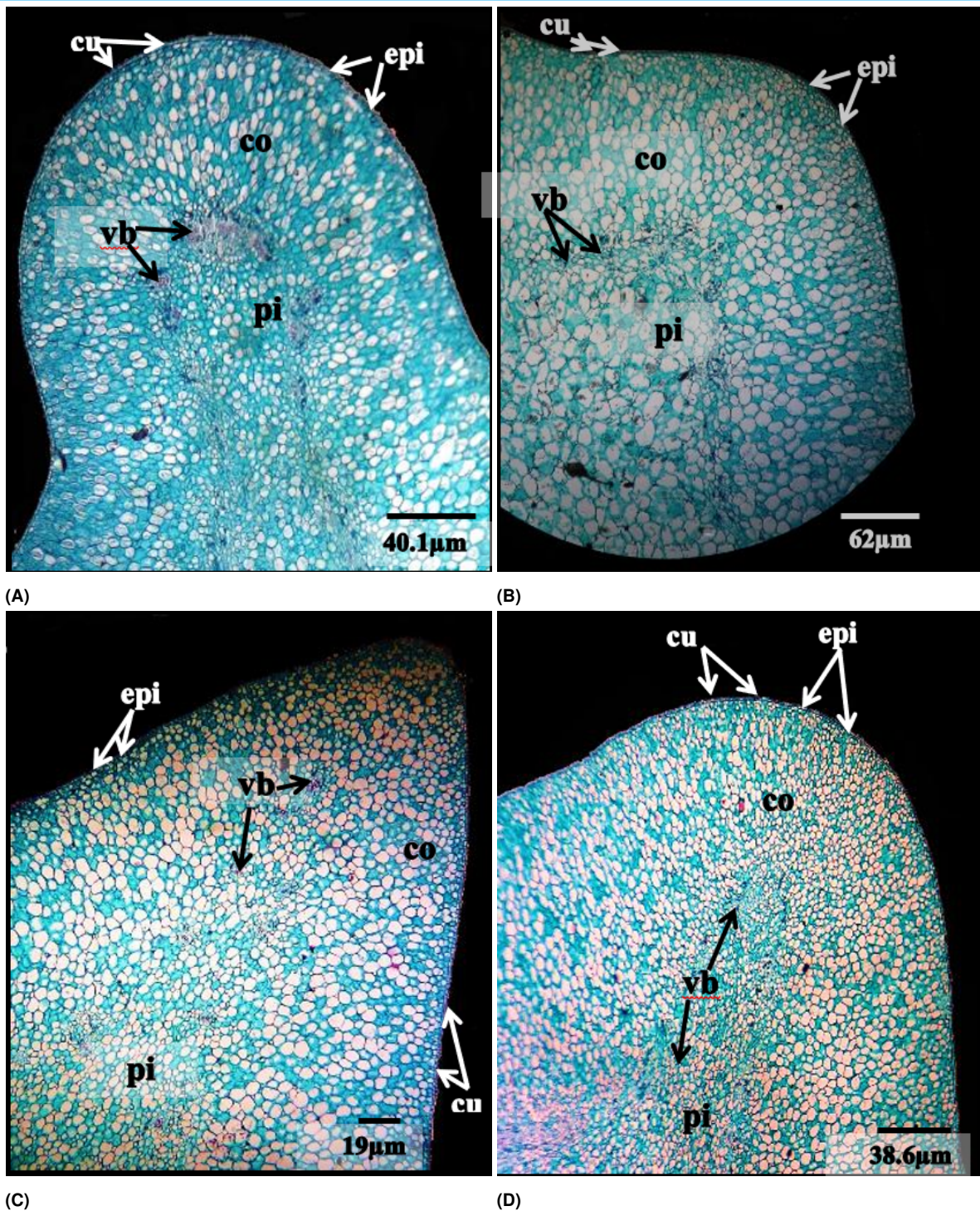


Figure 3. Anatomical layers of the stem rib, A. *A. tuberculata*, B. *C. subulata*, C. *D. penicillata*, D. *M. quadrangula*; cu: Cuticle; epi: Epidermis layer; co: Cortex; vb: Vascular bundles; pi: Pith.

sulci. In contrast, *A. tuberculata* employs a size-based balancing strategy while maintaining a moderate rib vessel count.

It possesses the largest mean vessel area within the sulci. This localized spatial partitioning is an important adaptive strategy for ensuring hydraulic safety in dry areas. By shifting most of the water transport capacity to the protected microclimatic zones of the sulci, these species effectively shield their main transport functions

from the intense sunlight and heat stress experienced by the exposed rib ridges. This structural adjustment maintains continuous hydraulic flow while lowering the risk of drought-induced embolism across varying microhabitats [27, 51].

Moreover, the minimal xylem elements observed in *C. subulata* strongly correlated with its reduced growth habit [14, 30]. As the taxon with the lowest habit in this study, *C. subulata* had a significantly lower transportation

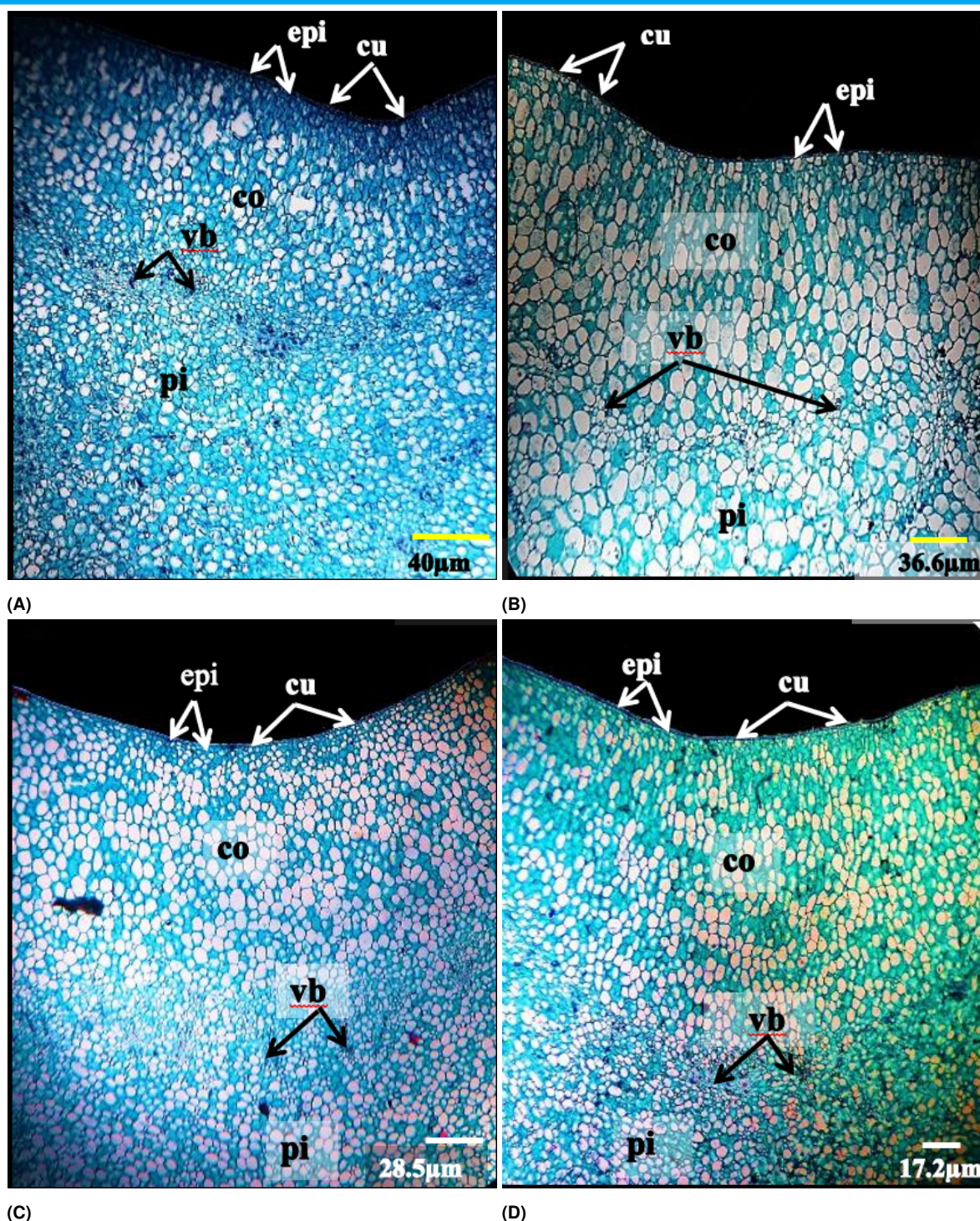


Figure 4. Anatomical layers of the stem sulcus, A. *A. tuberculata*, B. *C. subulata*, C. *D. penicillata*, D. *M. quadrangula*; cu: Cuticle; epi: Epidermis layer; co: Cortex; vb: Vascular bundles; pi: Pith.

demand than *D. penicillata*. This allows the species to maintain hydraulic homeostasis with fewer vessels [52, 53]. However, it maintains an internal balance by having a larger mean vessel area in the sulci than in the ribs, thereby optimizing the efficiency of its limited vascular networks.

Furthermore, Table 3 demonstrates that *D. penicillata* exhibited the highest external phloem thickness in the rib bundles, followed by *C. subulata* and *A. tuberculata*,

whereas *M. quadrangula* exhibited the lowest. However, *C. subulata* exhibited the highest external phloem thickness within the sulci bundles, followed by *D. penicillata* and *A. tuberculata*, whereas *M. quadrangula* exhibited the lowest thickness.

Additionally, all four studied taxa are characterized by the presence of internal phloem situated immediately interior to the protoxylem of both the rib and sulci bundles (Fig. 7). This observation aligns with the findings

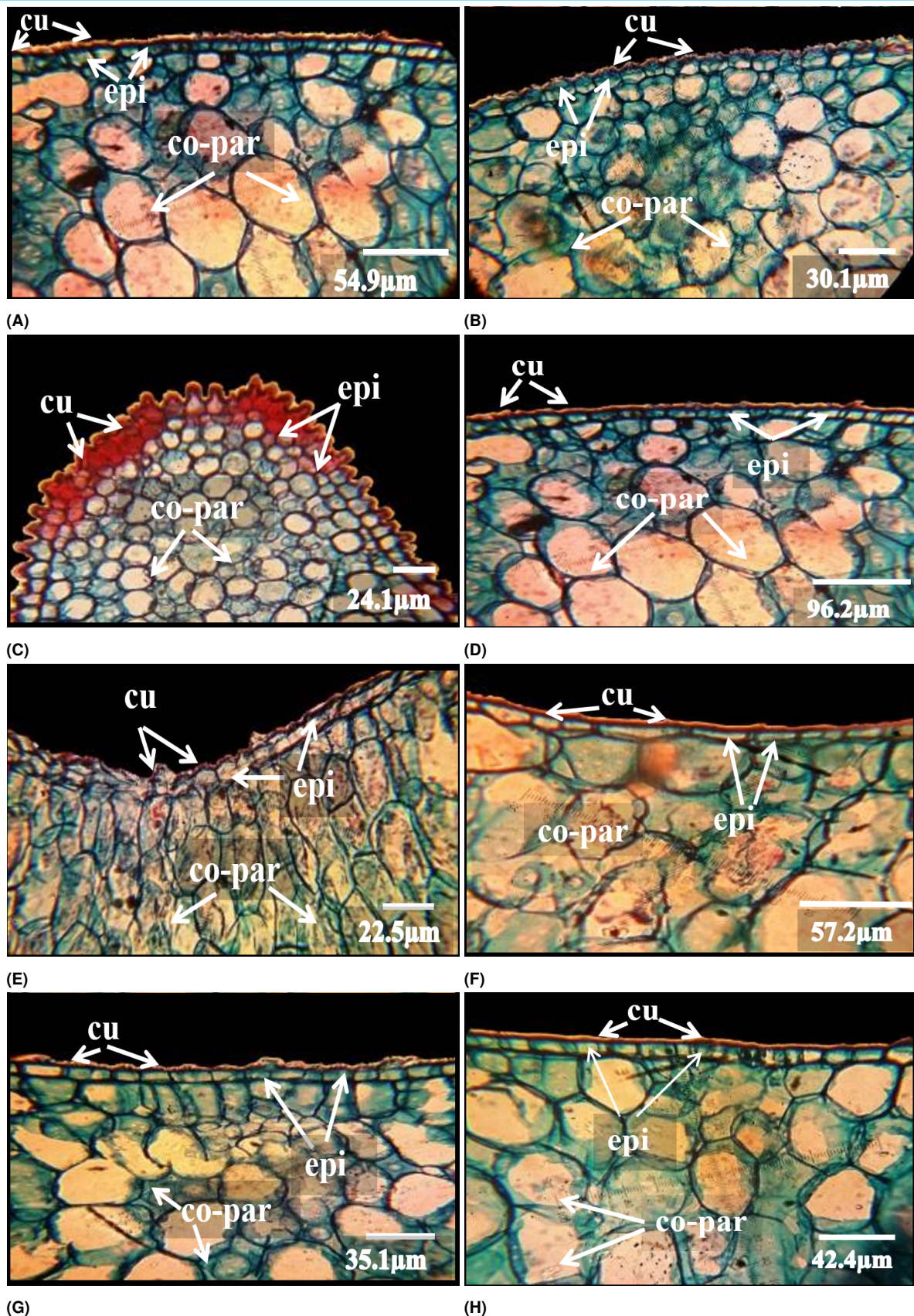


Figure 5. Epidermal and cortical layers of the stem, A–D: Stem rib epidermal and cortical layers of *A. tuberculata* (A), *C. subulata* (B), *D. penicillata* (C), and *M. quadrangula* (D), E–H: Stem sulcus epidermal and cortical layers of *A. tuberculata* (E), *C. subulata* (F), *D. penicillata* (G), and *M. quadrangula* (H); cu: Cuticle; epi: Epidermal cells; co-par: Cortical parenchyma cells.

of Mauseth [27], who reported the presence of internal

phloem in the genus *Caralluma*, specifically in *C. bur-*

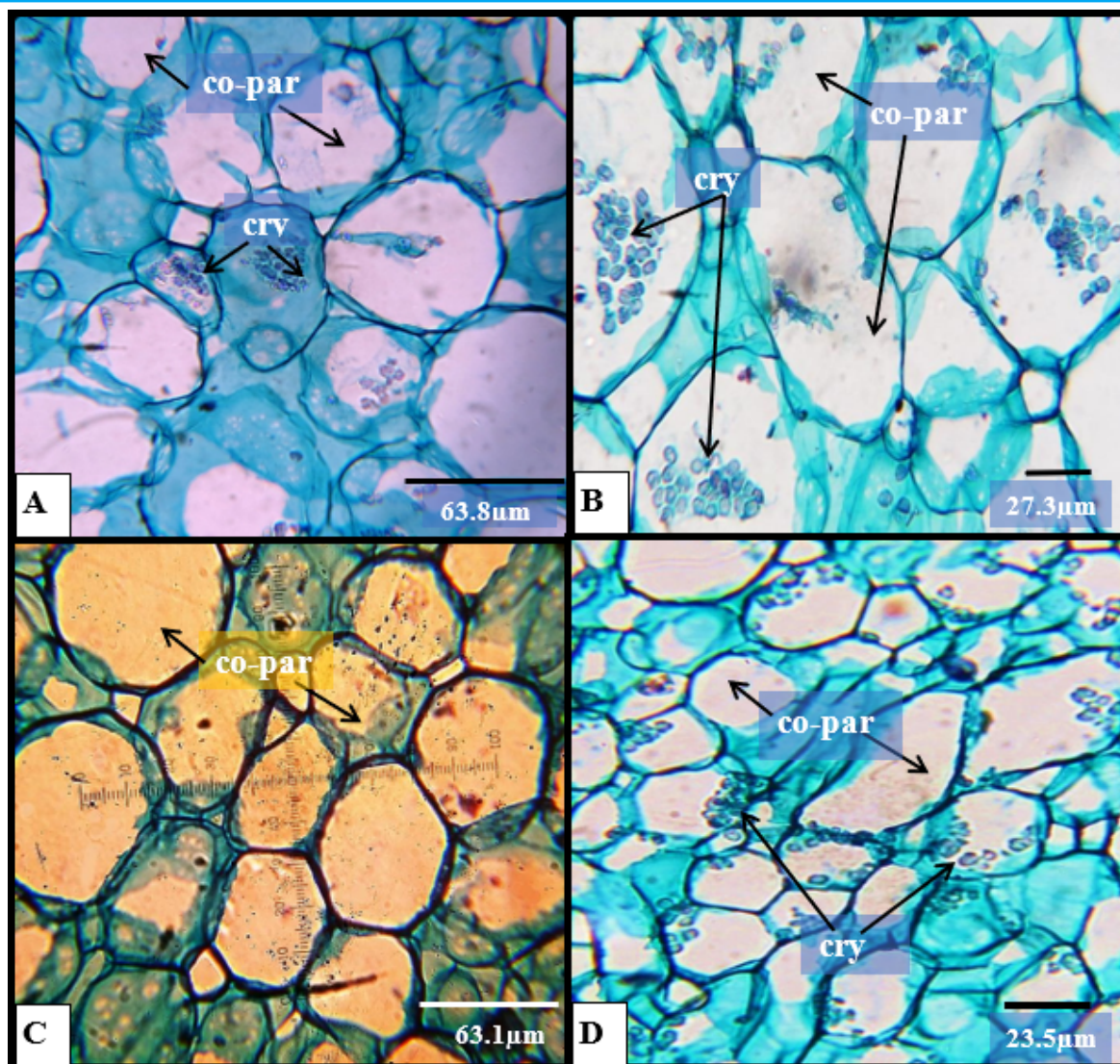


Figure 6. : Presence or absence of crystals in the sulcus cortex of the stems of the four studied taxa, A. *A. tuberculata*, B. *C. subulata*, C. *D. penicillata*, D. *M. quadrangula*.

chardii.

Regarding the internal phloem, Table 3 indicates that *C. subulata* had the thickest internal phloem in the rib bundles, followed by *A. tuberculata* and *D. penicillata*, whereas *M. quadrangula* had the lowest value. Conversely, *D. penicillata* exhibited the highest internal phloem thickness in the sulci bundles, followed by *C. subulata* and *A. tuberculata*, whereas *M. quadrangula* displayed the lowest thickness.

Table 3 illustrates that *D. penicillata* had the highest number of ribs, pith layers, and overall thickness, followed by *M. quadrangula* and *A. tuberculata*, whereas *C. subulata* exhibited the fewest layers and the thinnest thickness. Conversely, *C. subulata* possesses the largest individual rib pith parenchyma cells, the largest the largest individual rib pith parenchyma cells, followed by *A. tuberculata*, *D. penicillata*, and *M. quadrangula*. Regarding the sulci, *D. penicillata* recorded the highest number of pith layers and maximum thickness (Fig. 8), followed by *M. quad-*

rangula and *A. tuberculata*, whereas *C. subulata* presented the lowest layer count and thickness. However, *C. subulata* compensates for this structural reduction by developing significantly enlarged individual sulci pith parenchyma cells, which are substantially larger than those of the other three taxa.

Pith anatomy revealed significant differences in water-storage strategies among the species studied. *D. penicillata* displayed a model with a high storage capacity, marked by the greatest number of pith layers (in the ribs and sulci). In contrast, *C. subulata*, which had the fewest layers, showed a unique adaptation with the largest individual sulci pith parenchyma cell area ($19954 \mu\text{m}^2$). This indicates that although *C. subulata* has a smaller growth form [14, 30] and fewer tissue layers, its sulci pith parenchyma cells are significantly enlarged to enhance water retention. This anatomical setup likely enables the species to optimize its osmotic buffering and adapt to the effects of high environmental exposure in its natural

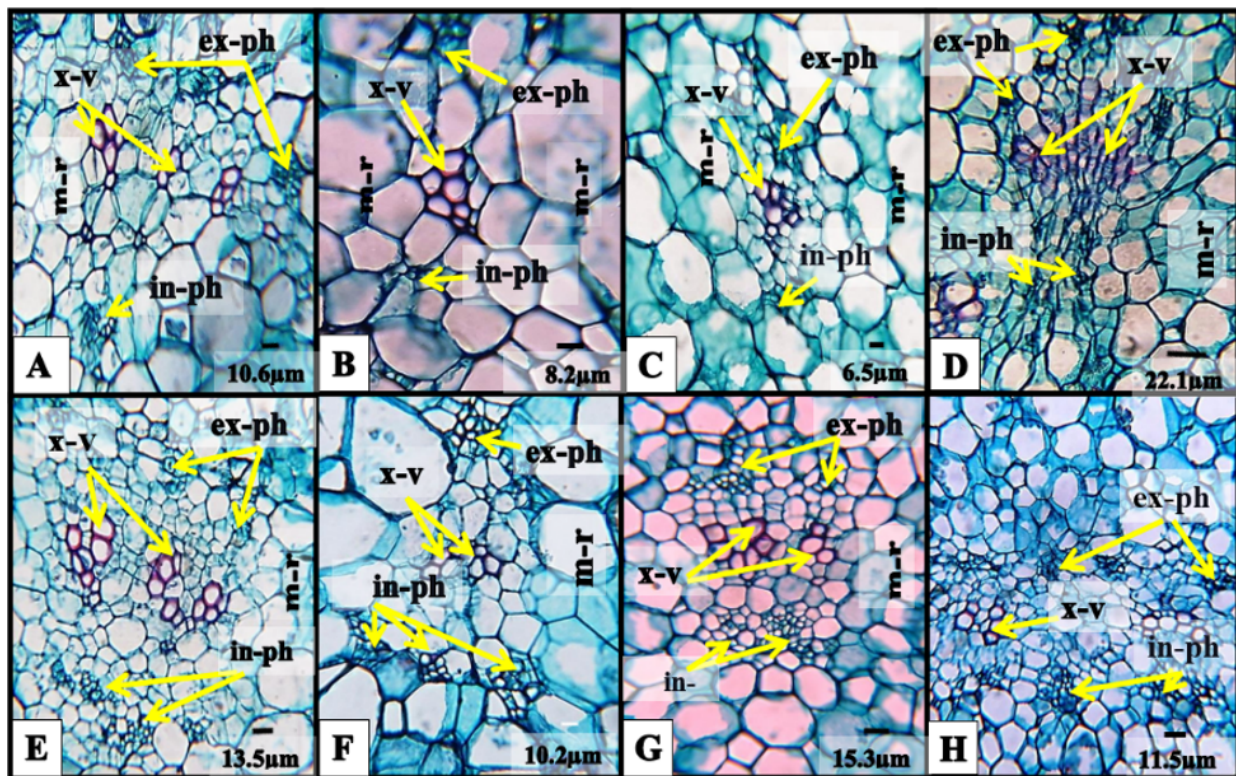


Figure 7. Vascular bundle components in stem ribs and sulci of the four studied taxa, **A–D:** Vascular bundles in the stem ribs of *A. tuberculata* (A), *C. subulata* (B), *D. penicillata* (C), and *M. quadrangula* (D), **E–H:** Vascular bundles in the stem sulci of *A. tuberculata* (E), *C. subulata* (F), *D. penicillata* (G), and *M. quadrangula* (H); **ex-ph:** external phloem, **x-v:** Xylem vessels; **m-r:** medullary ray; **in-ph:** internal phloem

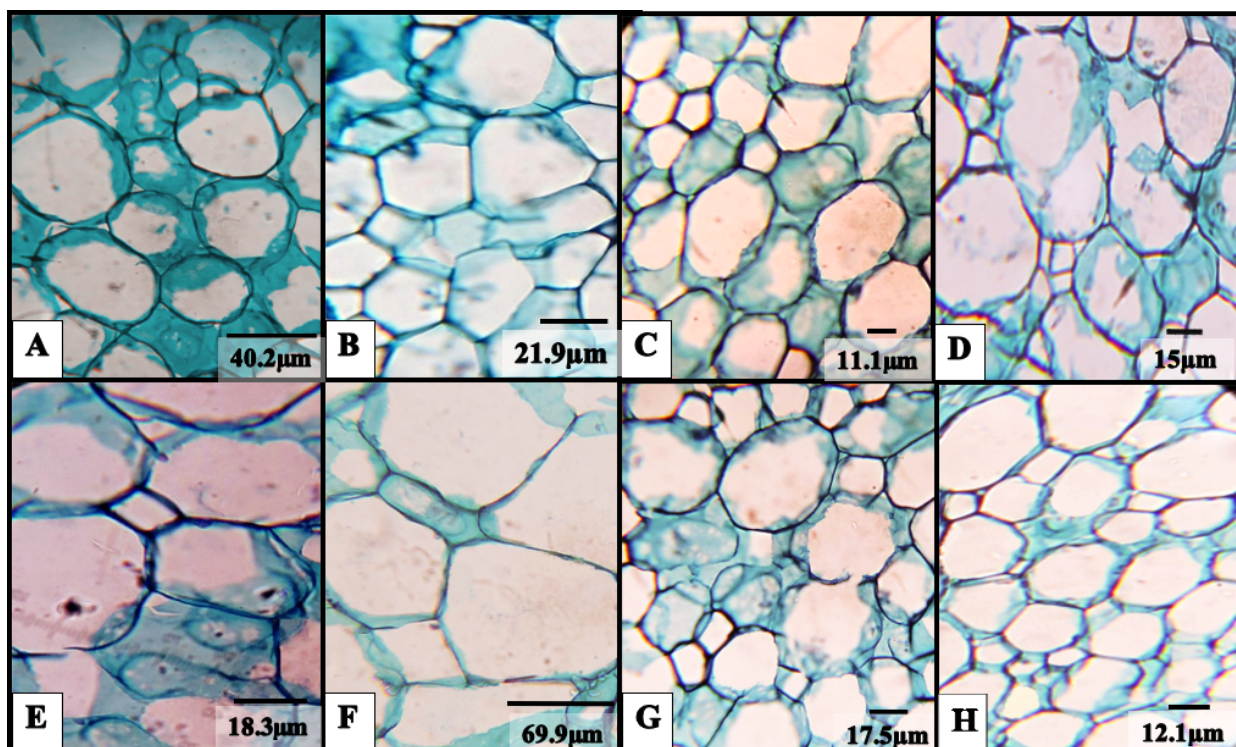


Figure 8. Pith parenchyma cells in stem ribs and sulci of the four studied taxa, **A–D:** Pith parenchyma cells in the stem ribs of *A. tuberculata* (A), *C. subulata* (B), *D. penicillata* (C), and *M. quadrangula* (D), **E–H:** Pith parenchyma cells in the stem sulci of *A. tuberculata* (E), *C. subulata* (F), *D. penicillata* (G), and *M. quadrangula* (H)

habitat [54].

Moreover, this structural divergence across tissue



layers reveals a critical evolutionary trade-off between cell proliferation (hyperplasia) and cell expansion (hypertrophy), which are linked to stem architecture and microclimate exposure. In taxa with long, prominent ribs, such as *D. penicillata*, water-storage capacity is achieved through a high density of organized tissue layers (mean of 110 rib pith layers), and the ribs bear the brunt of environmental exposure, demanding a significantly thicker protective rib cuticle (mean thickness of 2.3 μm). The deep, shaded sulci of these long-ribbed stems function as microenvironmental refugia, where humidity is naturally higher, allowing the plant to maintain a thinner sulci cuticle (mean thickness of 1.0 μm) [55]. Conversely, *C. subulata* adopts a metabolically conservative strategy; rather than expending significant energy on repeated cell divisions to build multiple tissue layers, it maximizes its water-storing volume by expanding individual cell vacuoles, achieving an immense sulci pith cell mean area of 19954 μm^2 . Its reduced, short-ribbed habit leaves the sulci significantly more exposed to direct environmental factors, driving the evolutionary necessity for a counter-balanced, thicker sulci cuticle (mean thickness of 1.5 μm) to mitigate boundary layer water loss from the sulci. Energetically, this cell hypertrophy is highly adaptive for a low-growth-habit succulent facing high-altitude solar radiation and drought, as it minimizes the metabolic resources required for cell wall synthesis while maximizing elastic water retention [27, 56].

Based on Table 3, several quantitative anatomical characteristics, such as the presence or absence of sulci, cortical crystals, number of ribs, cortical layers, rib pith parenchyma cell area, and dimensions, were used to differentiate these species. Analysis of variance (ANOVA) revealed exceptionally high statistical significance ($P < 0.0001$) across the evaluated quantitative traits. This extreme mathematical significance demonstrates that stem tissue variation within this *Caralluma* complex is not random phenotypic drift but rather the result of powerful evolutionary selective pressures driving structural adaptation in each species to its specific microhabitat.

Based on these statistically significant anatomical characteristics, a dichotomous key was developed to aid in the identification of the investigated taxa in the future.

1. Crystals were absent in the sulci cortex; the mean stem width was 27 mm and the mean rib length was 14 mm ***D. penicillata***
 + Crystals present in the sulci cortex; mean stem width < 20 mm, and mean rib length < 10 mm **2**
2. Mean number of cortex layers in ribs is high (21 layers) and mean xylem vessel diameter in ribs is small (7.9 μm) ***M. quadrangula***

+ The mean number of cortex layers in the ribs was lower than 21 layers (17–18 layers), and the mean xylem vessel diameter in the ribs was > 8 μm **3**

3. The pith parenchyma cell area in the ribs was extremely large (mean 6877.3 μm^2), and the mean number of pith layers in the ribs was low (21 layers)

..... ***C. subulata***

+ Pith parenchyma cell area in ribs is smaller (mean 2310.4 μm^2); mean pith parenchyma cell diameter in ribs is medium (47.6 μm)

..... ***A. tuberculata***

Using Two-way cluster analysis (TWCA), the relationships among the four studied taxa (*A. tuberculata*, *C. subulata*, *D. penicillata*, and *M. quadrangula*) were evaluated based on 40 quantitative stem anatomical characteristics (Table 3). A dendrogram was constructed based on the data in Table 3 (Fig. 9). The four taxa under investigation were separated into two distinct groups (Fig. 9) at a relative similarity level of 77.14%, based on 25 quantitative stem anatomical characteristics (Mean Stem width; mean rib length; mean cuticle thickness on rib epidermis cells; mean sulcus epidermal cell area; mean number of rib cortex layers; mean rib cortex thickness; mean rib cortex parenchyma cell diameter; mean rib cortex parenchyma cell area; mean number of sulcus cortex layers; mean sulcus cortex parenchyma cell diameter; mean sulcus cortex parenchyma cell area; mean number of rib bundles; mean vessel number in rib bundle; mean vessel wall thickness in rib bundle; mean number of sulcus bundles mean vessel number in sulcus bundle; mean vessel wall thickness in sulcus bundle; mean external phloem thickness in sulcus bundle; mean number of rib pith layers; mean rib pith thickness; mean rib pith parenchyma cell diameter; mean rib pith parenchyma cell area; mean number of sulcus pith layers; mean sulcus pith parenchyma cell diameter; mean sulcus pith parenchyma cell area), where Group I comprised *C. subulata* whereas Group II included the remaining three taxa (*A. tuberculata*; *D. penicillata* & *M. quadrangula*).

Biologically, this primary deep divergence reflects a fundamental ecophysiological split based on macromorphological growth habits and their exposure dynamics. The isolated clustering of *C. subulata* is driven by its highly specialized and low-growth architecture. Lacking the protective canopy of larger shrubs, its extreme adaptation is defined by a unique suite of hypertrophic cell traits and high boundary-layer protection, causing it to diverge significantly from the other three taxa at a 77.14% threshold.

This finding aligns with that of Plowes [14], who separated the four taxa into two groups: the first included *C. subulata*, which is characterized by slender stems and thin racemose or tapering flowering tips. However, the three remaining taxa (*A. tuberculata*, *D. penicillata*, and

M. quadrangula) were placed in one group characterized by flowers in terminal or sub-terminal pseudo-umbels and stem apices that were not slenderly tapered or deciduous.

Moreover, Group II was subdivided into two subgroups (Fig. 9) at a relative similarity level of 84.45%, based on 18 quantitative stem anatomical characteristics (mean stem width; mean rib length; mean rib epidermal cell diameter; mean rib epidermal cell area; mean sulcus epidermal cell diameter; mean sulcus epidermal cell area; mean number of sulcus cortex layers; mean sulcus cortex thickness; mean sulcus cortex parenchyma cell diameter; mean sulcus cortex parenchyma cell area; mean rib vessel wall thickness; mean rib internal phloem thickness; mean number of sulcus bundles; mean vessel number in sulcus bundle; mean sulcus pith thickness; mean number of sulcus pith layers; mean sulcus pith parenchyma cell diameter; and mean sulcus pith parenchyma cell area, where Subgroup A includes *A. tuberculata*, while Subgroup B compresses the two remaining taxa (*D. penicillata* & *M. quadrangula*). The 84.45% similarity split represents an evolutionary transition within the tissue suites, where *A. tuberculata* separates because it retains a more transitional and intermediate tissue thickness profile than do the other species. The high similarity pairing within Subgroup B indicates the presence of a highly coordinated 'anatomical syndrome' specifically optimized for robust shrubby habits. This agrees with Meve and Liede [56], who reported that *D. penicillata* and *M. quadrangula* belong to the derived "southern" clade (the *Stapelia* group), a monophyletic lineage characterized by advanced floral and vegetative specializations. Morphologically, members of this clade typically possess complex corolla structures, often featuring a prominent annulus or rugose surfaces, and a highly modified, frequently cup-shaped outer corona. These taxa also exhibit a high degree of stem succulence, with prominent tubercles and leaves reduced to minute caducous scales.

Phylogenetically, this clade represents a more recently evolved radiation that spread into the Arabian Peninsula and North Africa, placing *Desmidorchis* and *Monolluma* in a sister group relationship with core southern African genera, such as *Orbea* and *Stapelia*. In contrast, *A. tuberculata* resides in a more basal and distinct lineage that branched off earlier in the evolutionary history of stapeliads. Furthermore, Subgroup B was divided into two categories (Fig. 9) at a relative similarity level of 88.66%, based on 34 quantitative stem anatomical characteristics (Mean Stem Width; Mean rib length; mean cuticle thickness on rib epidermis; mean rib epidermal cell diameter; mean rib epidermal cell area; mean cuticle thickness on sulcus epidermis; mean number of rib cortex layers; mean rib cortex thickness; mean rib cortex parenchyma cell diameter; mean rib cortex parenchyma cell area; mean number of sulcus cortex layers; mean sulcus cortex thickness; mean sulcus cortex parenchyma

cell diameter; mean crystal diameter; mean crystal area; mean number of ribs bundles; mean vessel wall thickness in rib bundle; mean xylem vessel diameter in rib bundle; mean vessel area in rib bundle; mean external phloem thickness in rib bundle; mean rib internal phloem thickness; mean vessel number in sulcus bundle; mean vessel wall thickness in sulcus bundle; mean xylem vessel diameter in sulcus bundle; mean xylem vessel area in sulcus bundle; mean external phloem thickness in sulcus bundle; mean sulcus internal phloem thickness; mean number of rib pith layers; mean rib pith thickness; mean rib pith parenchyma cell diameter; and mean rib pith parenchyma cell area).

Mean sulcus pith thickness, mean sulcus pith parenchyma cell diameter, and mean sulcus pith parenchyma cell area, where Category i includes *D. penicillata*; and Category ii includes *M. quadrangula*. The exceptionally high similarity level (88.66%) between these two categories underscores that they share a heavily conserved internal tissue layout inherited from their common ancestors. However, the high number of quantitative traits (34 characteristics) that still statistically separate them reveals that generic differentiation within the core southern clade is heavily expressed through fine-tuned adjustments in internal tissue dimensions, such as cuticle variation and cell diameter, rather than restructuring the core stem ground plan.

These findings agree with those of Meve and Liede [56], who categorized *D. penicillata* and *M. quadrangula* as separate genera based on several crucial morphological and structural traits, even though both belong to the "southern" clade. The most notable diagnostic difference is found in the inflorescence structure, where *Desmidorchis* is identified by flowers arranged in terminal pseudo-umbels at the ends of the stems, whereas *Monolluma* has subaxillary inflorescences that do not form terminal clusters. Moreover, stem morphology varies, with *Desmidorchis* having a large, shrub-like form with distinctly crenate stem angles. In contrast, *Monolluma* stems are generally pungent or have ribs with unique grooves on their upper sides.

These distinct morphological and architectural divergence patterns, corroborated by molecular evidence, provide a robust basis for their recognition as separate generic lineages within the broader stapelioid complex. In contrast, the principal component analysis (Fig. 10) showed that the first component separated the studied taxa into two groups: The first group included *C. subulata*, whereas the second group comprised the other three taxa (*A. tuberculata*, *D. penicillata*, and *M. quadrangula*). However, the second component separated *A. tuberculata* from the two remaining taxa, *D. penicillata* and *M. quadrangula* (Fig. 10). The Principal component analysis (PCA) results (Fig. 10) matched the findings of the TWCA (Fig. 9).

In a broader taxonomic context, our quantitative stem-

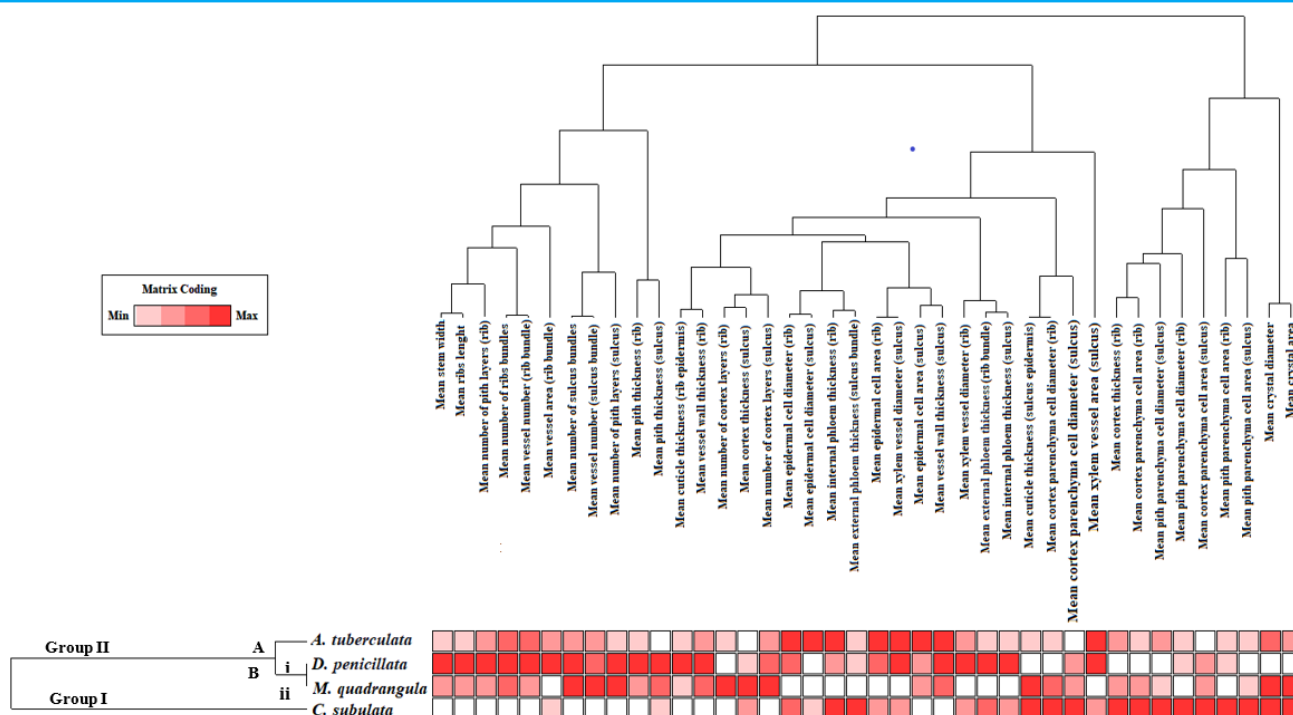


Figure 9. Cluster analysis illustrating the relationships among the four investigated taxa of the *Caralluma* complex (*C. subulata* and the segregated taxa *A. tuberculata*, *D. penicillata*, and *M. quadrangula*) based on 40 anatomical stem characters using Two-Way Cluster Analysis (TWCA) - Group average linkage method.

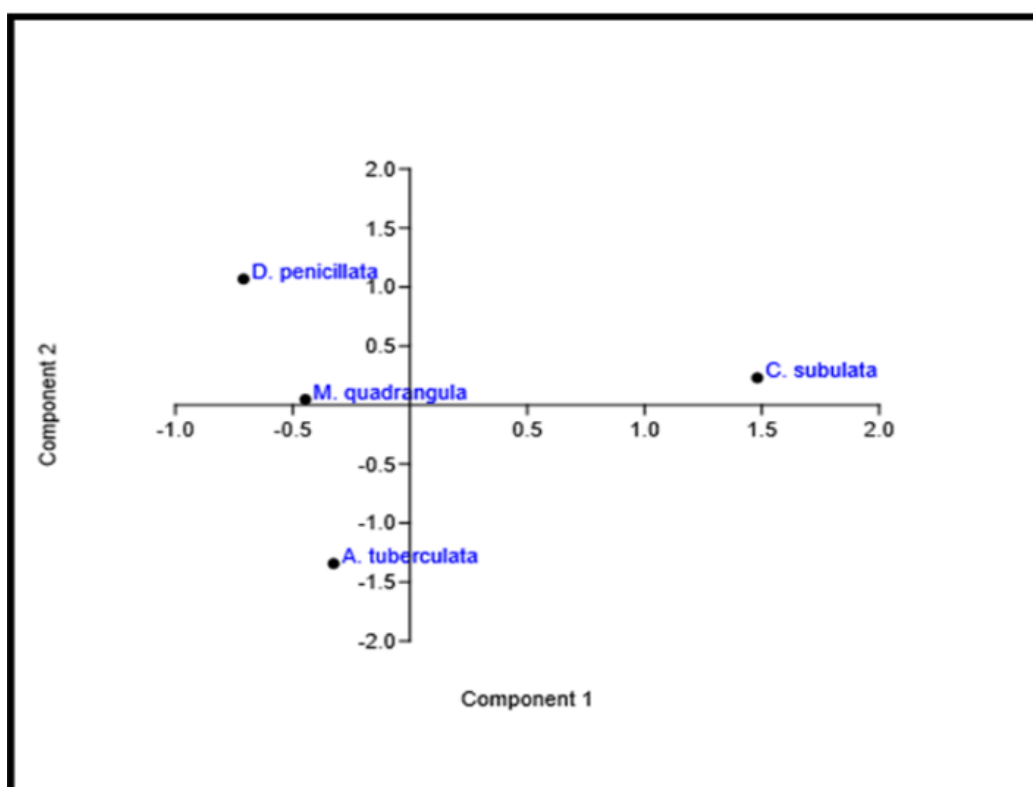


Figure 10. Principal Component Analysis (PCA) illustrating the relationships among the four investigated taxa of the *Caralluma* complex (*C. subulata* and the segregated taxa *A. tuberculata*, *D. penicillata*, and *M. quadrangula*) based on 40 anatomical stem characters

anatomical data strongly support the boundaries established by recent molecular phylogenetic reclassifications of the subtribe Stapeliinae [56]. This confirms that dis-

tinct structural modifications in the tissue layers closely align with the updated evolutionary lineages in modern systematic accounts of the genus in Yemen [4].

4. CONCLUSION

An anatomical study of *A. tuberculata*, *C. subulata*, *D. penicillata*, and *M. quadrangula* revealed that their succulent stems are highly specialized for photosynthesis and water storage.

This study highlights the anatomical balance in which each species optimizes its internal tissues according to its growth habit. For instance, *D. penicillata* has a high number of pith layers (110 ribs) compared to other species. In contrast, the smaller *C. subulata* was characterized by fewer pith tissue layers and larger pith parenchyma cells (with an area of 19954 μm^2). This regional partition between the ribs and sulci ensures hydraulic homeostasis and efficient nutrient transport through the eustelic arrangement of vascular bundles, supplemented by the internal phloem. Moreover, the quantitative anatomical data provide a strong basis for taxonomic differentiation, as evidenced by the high statistical significance ($P < 0.0001$) of characteristics such as the presence or absence of sulci, cortical crystals, and pith layer count in the cross-section.

The results of the Two-way Cluster Analysis (TWCA) and Principal Component Analysis (PCA) successfully categorized the four taxa into distinct groups that reflected their evolutionary specializations. These findings distinguish *C. subulata* from other taxa. However, *A. tuberculata* was distinct and separated from the southern clade members, *D. penicillata* and *M. quadrangula*.

Finally, these internal structural patterns, from cuticle thickness to xylem vessel distribution, provide a clear anatomical fingerprint for identifying and separating *Caralluma*-segregate genera.

5. DECLARATIONS

Ethical Approval

This article does not contain any studies involving animals or human participants performed by any of the authors. We declare that this manuscript is original and is not currently being considered for publication elsewhere. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

Conflict of interest

The authors declare no competing interests.

Competing interests

The authors have no financial or proprietary interest in any material discussed in this article.

Authors' contributions

All authors contributed to the conception and design of the study. All authors performed simulations, data collection and analysis, and commented on the present version of the manuscript. All the authors have read and approved the final manuscript.

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Availability of data and materials

No datasets is used in the present study.

Consent for publication

The authors confirm that informed consent was obtained for the publication of the data contained in this article.

Consent to participate

Informed consent was obtained from all authors.

Declaration of AI-Assisted Technologies

"The authors declare that artificial intelligence (AI) tools were used only for limited purposes, such as language editing, grammar improvement, and formatting assistance. All scientific content, data analysis, interpretations, conclusions, and final manuscript revisions were reviewed and verified by the authors, who took full responsibility for the integrity and originality of the work."

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