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Taxonomic characteristics of xylem anatomy in some species of *Euphorbia* in Nigeria

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Abstract

This study uses xylem-based traits to examine the anatomical and ecological adaptations of four *Euphorbia* species (*E. milii, E. hirta, E. heterophylla,* and *E. thymifolia*). The research aims to investigate the xylem anatomy of these species to provide valuable taxonomic data for classification and identification. By analyzing and comparing the xylem characteristics, this study highlights the effects of xylem structure on the species' adaptation and ecological strategies. Four species have been collected from the garden within the premises of the Department of Plant Biology, University of Ilorin and submitted after identification to the Herbarium. Thin transverse stem sections have been prepared using a manual microtome, then processed with graded isopropanol, embedded in paraffin wax, sectioned at 4 µm, stained with safranin, and observed under a light microscope. The results have revealed significant variations in vessel dimensions, fiber thickness, and parenchyma organization among the species. *E. milii* has exhibited large vessel lumens for rapid water conduction, while *E. thymifolia* has shown smaller vessels and thinner fibers, suggesting adaptation to water-limited environments. *Euphorbia heterophylla* has demonstrated the largest pit diameters and abundant ray parenchyma supporting efficient water transfer. A principal component analysis (PCA) has revealed that Principal Component 1 (PC1) (53.82% variance) and Principal Component (PC2) (40.03% variance) feature the species variation, with *E. milii* and *E. thymifolia* separated by PC1, and *E. heterophylla* distinguished by PC2. These findings underscore the ecological significance of xylem adaptations in water transport, mechanical stability and environmental resilience, offering insights into the taxonomy and functional anatomy of the genus *Euphorbia*.

Key words

anatomy, ecology, *Euphorbia*, taxonomy, xylem

Citation

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Introduction

Quantitative wood anatomy investigates the variability in xylem features in trees, shrubs and herbaceous species, linking these features to plant functioning, growth and environmental adaptation. The xylem performs critical roles such as transporting water, nutrients and hormones, and providing structural support essential for the plant survival (Arx & al. 2016). Plants exhibit various modifications and adaptation to survival under extreme conditions, which can be morphological, anatomical or physiological. Microscopic evaluation has revealed diagnostic features such as multicellular trichomes and anomocytic stomata. These features are crucial for the plants' survival under stress conditions and hold significant taxonomic importance (Lata & al. 2021). Water availability is important for plant survival and influences water uptake, loss and growth regulation. Research in plant physiology and soil science has enhanced the understanding of water movement through soil, its uptake by roots, and loss through transpiration. Soil texture and water content exercise impact on water uptake, with hydraulic resistance posing significant limitations. Plants respond to water potential gradients by regulating stomatal opening and hydraulic conductance via aquaporins. Under low water conditions, plants minimize water loss by closing their stomata and adjusting hydraulic conductance to maintain turgor (Scharwies & Dinneny 2019).

Understanding the macroscopic structure of wood is crucial for identifying wood types and assessing their properties and quality. Wood macroscopic structure varies significantly based on genetic factors, environmental conditions and the trees' developmental stage, leading to distinct macroscopic signatures (Ruffinatto & al. 2023). Wood anatomy, including vessel diameter, fiber length and parenchyma, often requires precise processing and quantification (Baas & al. 2004). It is a powerful tool for species classification, especially where reproductive organs are absent. The wood is semi-ring-porous to ring-porous, with solitary and grouped vessels. Vessels are smaller and fibers have thicker walls compared to other origins. Longitudinally, the vessel area and fiber size decrease top-wise, while vessel frequency, parenchyma and ray proportions increase. Radially, vessel size, fiber length and wall thickness grow with cambial age (Cardoso & al. 2015).

The *Euphorbiaceae* family, widely distributed in tropical regions, includes a variety of trees, shrubs and herbs. Such genera as *Euphorbia*, *Croton* and *Phyllanthus* possess significant ecological and medicinal properties (Dutta 1980). Earlier anatomical studies on the *Euphorbia* species have focused on the leaf epidermal characteristics and stomatal types, while internal stem anatomy remains understudied, particularly in species like *E. hirta*.

In spite of extensive use of the *Euphorbiaceae* species for medicinal and ecological purposes, little research has been done on their anatomical features for taxonomic classification. Earlier studies had primarily focused on growth, physiology and developmental anatomy (David & Ruth 1988; Kuhn & al. 1996), leaving a gap in understanding how anatomical characteristics can help delineate the species within that varied family.

Maheshwari & al. (2009) investigated the wood anatomical features of *Cocculus pendulus* and *Leptadenia arborea* to determine the average xylem vessel diameter and frequency. Both species exhibited cambial variation characterized by anomalous secondary growth. The mature stem of *C. pendulus* had successive rings of xylem alternating with phloem, while *L. arborea* had segmented groups of xylems separated by large rays and surrounded by fibers. Ray parenchyma cells in both species contained dense starch grains, though parenchyma cells were more abundant in *L. arborea*. Vessel frequency was low in both species, but there was no significant difference between them ($p > 0.05$). Hydraulic conductance was high in both species, although *L. arborea* had approximately six times higher conductance than *C. pendulus*. The perforation plates of the xylem vessels were simple, with circular wall thickening in both species, providing strength against collapse under tension. The vulnerability index was higher in *L. arborea* than in *C. pendulus* by 2.5 times.

Stomatal density was higher on the lower surface of *C. pendulus* leaves than on the upper surface ($p < 0.05$), whereas *L. arborea* exhibited no significant difference between the two leaf surfaces. The surface-to-volume ratio was higher in *C. pendulus* than in *L. arborea* ($p < 0.05$).

The study highlights the importance of wood anatomical adaptations to drought resistance in arid environments such as the Tihama plains, where water availability is the most limiting factor for plant growth. Lianas generally exhibit wide xylem vessels that enhance water conductivity, compensating for their narrow stem diameters (Carlquist & Hoekman 1985; Rowe & Speck 2005; Angyalossy & al. 2012). These wide vessels enable the efficient transport of large volumes of water, contributing to high hydraulic conductance (Mauseth 1988; Tyree & Zimmermann 2002) However, increased vessel diameter can also make plants more vulnerable to cavitation (Sperry & al.2002). *Cocculus pendulus* and *L. arborea* appear to have relatively stable groundwater supplies, as their deep root systems tap into the capillary fringe above the water table (Nilsen & Orcutt 1996; Andrade & al. 2005).

The present research examines the xylem structure and other anatomical features of some selected *Euphorbia* species, contributing to the taxonomic understanding of the family. It also aims at investigating the xylem anatomy of selected *Euphorbia* species, focusing on the significant effects on xylem structure. By analyzing and comparing the xylem characteristics, the study aspires to provide valuable taxonomic data that will help the classification, delineation and identification of these species.

Materials and methods

Four *Euphorbia* species (*Euphorbia hirta* L., *E. milii* Des Moul., *E. heterophylla* L., and *E. thymifolia* L.) were collected from the garden within the premises of the Department of Plant Biology, University of Ilorin and vouchers were submitted to the Herbarium of the Department of Plant Biology, Faculty of Life Sciences. The samples were collected aseptically by standard gardening tools and stored in polythene bags to preserve their integrity during transport to the Laboratory. Thin transverse sections of the stem were prepared using a manual sledge microtome. The plant tissues were first cut into smaller pieces and placed into tissue cassettes, which were labeled accordingly. The tissues were then subjected to fixation using an automatic tissue processor filled with graded concentrations of isopropanol (70%, 80%, 90%, and 100%) to gradually dehydrate them (Ruzin 1999).

After fixation, the tissues were embedded by an embedding machine. Melted paraffin wax was poured into steel base molds of various sizes, depending on the tissue dimensions, at 68°C–70°C (Bancroft & Gamble 2019). The wax-embedded tissues were then transferred to a CryoConsole for rapid freezing at -4°C to -5°C for 15 minutes to solidify the wax and form tissue blocks (Gerrits & Smid 2015).

The solidified tissues were sectioned at a thickness of 4 μm by a manual microtome. The tissue sections were carefully transferred with forceps into cool distilled water for a few seconds, then mounted on glass slides. The slides were briefly placed in a 42°C water bath to prevent wrinkling of the sections, after which they were labeled with a silver marker (Ruzin 1999).

The paraffin wax was removed by placing the slides on a hotplate at 65°C, after which the sections were stained with safranin to enhance the visibility of the anatomical features (Bancroft & Gamble 2019). Observations were made with a light microscope, focusing on such structures as vessels, vessel diameter, fibers, fiber length, rays, cell wall thickness, lumen diameter, and axial parenchyma. Measurements were recorded and photographs of the sections were made with a digital Olympus camera for documentation and analysis.

Results

Microscopic sectioning was performed on four species within the *Euphorbia* genus (*Euphorbia hirta*, *E. milii*, *E. heterophylla*, and *E. thymifolia*), focusing on the transverse sections to explore cambial variation and patterns of secondary growth. The analysis revealed important differences in the anatomical features of the species, with notable variations in the arrangement and dimensions of parenchyma cells, vessels, fibers, and pits, essential for the species' structural and functional adaptation. Representative transverse sections for each species are illustrated in Figs 1a–d.

Anatomical study of the four *Euphorbia* species has revealed significant variations in their wood structure, particularly in the arrangement and size of parenchyma, vessels and fibers. These differences reflect ecological adaptations and contribute to each species' water transport efficiency, mechanical support and resilience in varying environments. The transverse section of *Euphorbia hirta* (Fig. 1a) has shown well-defined parenchyma cells, vessels and fibers at 40× magnification. The parenchyma, which surrounds the vascular bundles, plays a critical role in storage and support. The vessels have shown different sizes, while the closely associated fibers have enhanced the plant's structural integrity. *Euphorbia hirta* has displayed the largest parenchyma area (7.57 μm^2) and substantial vessel lumen area (10.9 μm^2). The fiber lumen area (4.9 μm^2) and cell wall thicknesses suggest that *E. hirta* is well-adapted to environments where both storage and support are crucial, balancing water transport and mechanical strength (Table 1).

Euphorbia heterophylla in Fig. 1b shows a similar organization with the parenchyma, vessels and fibers, but with some distinct quantitative differences. That species

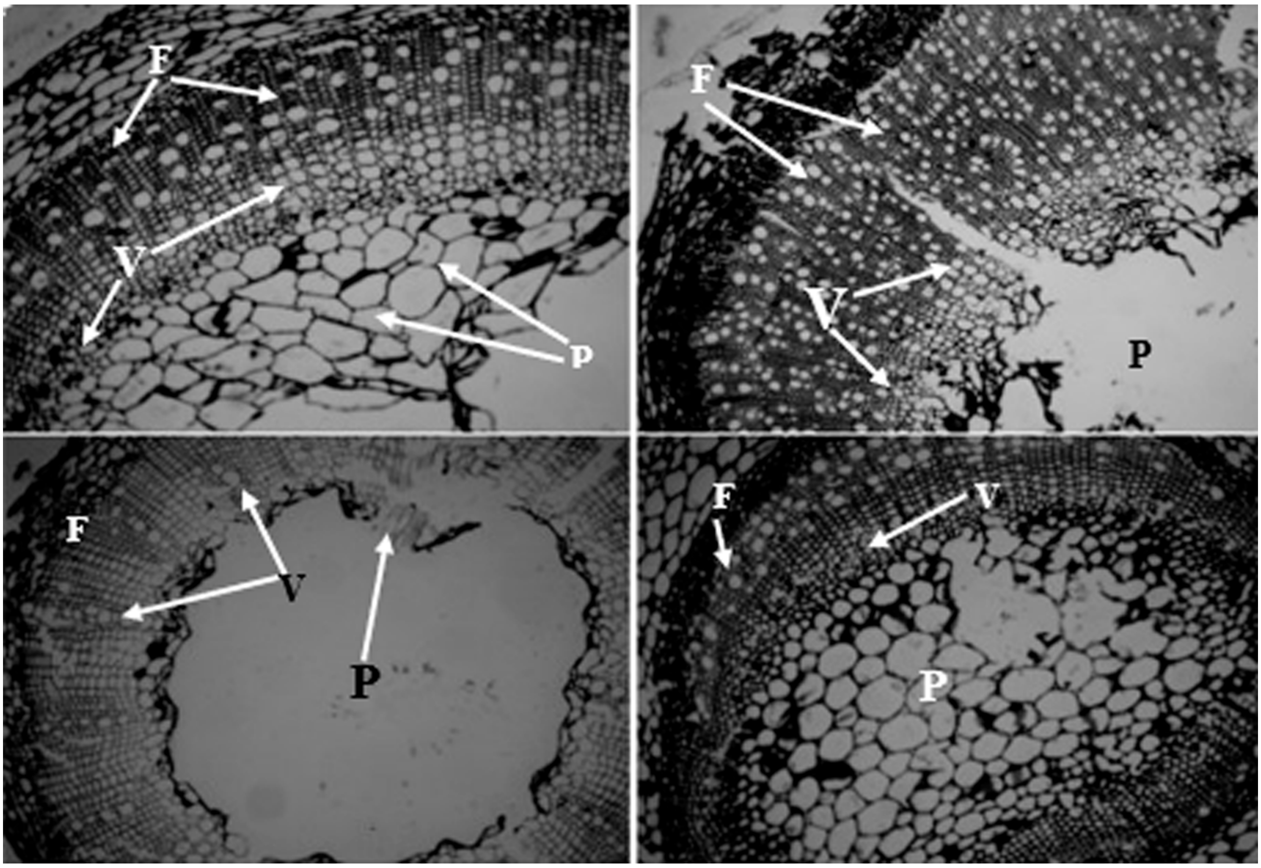

Fig. 1. Transverse section showing fiber (**F**), vessels (**V**), and parenchyma (**P**) of: **a**, *Euphorbia hirta*; **b**, *E. heterophylla*; **c**, *E. thymifolia*; **d**, *E. milii* (40×).

Table 1. Quantitative characters of the xylem features of four *Euphorbia* species.

Species	**Par (µm)**	**LVD (µm)**	**LFD (µm)**	**IP (µm)**	**CWV (µm)**	**CWF (µm)**	**SCW**
E. milii	5.6	18.66	4.23	3.86	1.83	3.9	Circular
E. hirta	7.57	10.9	4.9	3.7	4.43	5.43	Circular
E. heterophylla	6.6	11.8	3.1	7.37	11.1	4.2	Circular
E. thymifolia	4.63	5.5	1.76	2.31	1.48	1.06	Circular

Par, parenchyma; **LVD,** lumen vessel diameter; **LFD,** lumen fiber diameter; **IP,** intracellular pit; **CWV,** cell wall of vessel; **CWF,** cell wall fibers, **SCW,** shape of cell wall.

has exhibited a larger pit diameter (7.37 µm) and thicker vessel cell walls (4.2 µm), indicating high water conduction efficiency and mechanical support. The parenchyma cells in *E. heterophylla* have been tightly packed, suggesting adaptation to its specific growth environment. The fiber cell wall thickness has been the greatest among the four species (11.1 µm), contributing to the plant's structural rigidity and enhancing potentially its ability to withstand stress under varying conditions.

In contrast, *E. thymifolia* (Fig. 1c) has displayed smaller vessel lumen areas (5.5 μm^2) and thinner cell walls (vessel wall thickness of 1.48 µm and fiber wall thickness of 1.06 µm). Those anatomical characteristics suggest lower hydraulic conductance as compared to *E. hirta* and *E. heterophylla*, which possibly reflects that species' adaptation to environments with lower water availability. The smaller fiber lumen area (1.76 μm^2) and reduced mechanical strength indicate that *E. thymifolia* may rely more on flexibility than on structural rigidity.

Euphorbia milii (Fig. 1d), with the largest vessel lumen area (18.66 μm^2), is apparently adapted to conditions requiring high water transport efficiency. The thinner vessel and fiber cell walls (1.83 µm and 3.9 µm, respectively) suggest that the species may prioritize rapid water movement over mechanical strength, possibly as an adaptation to arid or semi-arid environments where efficient water uptake is critical. The fiber lumen area (4.23 μm^2) and the moderate parenchyma area (5.6 μm^2) balance the need in water storage and transport.

Among the four species, the presence of circular cell walls and varying levels of parenchyma, vessel and fiber development indicate specialized adaptation to their ecological niches. For example, *E. hirta* and *E. heterophylla* demonstrate features supporting mechanical strength and efficient water transport, such as thicker cell walls and larger parenchyma areas. In contrast, *E. thymifolia* and *E. milii* show traits suitable for rapid water transport or flexibility, such as larger vessel lumens and thinner cell walls, reflecting adaptation to environments with different water availability or stress conditions.

These observations suggest that the structural differences in wood anatomy of the four *Euphorbia* species are driven by their respective ecological requirements. The larger vessels and thicker fibers in *E. heterophylla* and *E. hirta* point to their ability to thrive in environments with higher mechanical demands, while the larger vessel lumens and thinner cell walls in *E. milii* support rapid water conduction in drier climates. Such anatomical features not only support the survival and growth strategies of these species but also provide taxonomically useful data that can help the classification and identification of the *Euphorbia* species.

Taxonomic implication of xylem anatomy in the four studied *Euphorbia* species

Investigation of xylem anatomy in the four studied *Euphorbia* species provides comprehensive information on their taxonomic and ecological dynamics. Variations in the xylem characteristics, such as vessel dimensions, fiber thickness and parenchyma organization, underpin the species classification and ecological adaptations. These findings have significant taxonomic implications, clarifying the interspecies relationships and environmental niches. Notably, *E. thymifolia* is the most distinct species, forming a separate cluster and indicating a substantial divergence. *Euphorbia hirta* and *E. heterophylla* form another cluster, distinct from *E. milii*, with moderate differences and shared traits (Fig. 2).

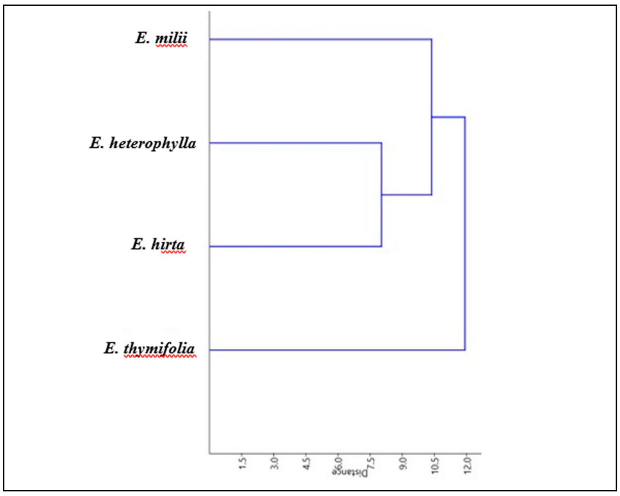

Fig. 2. Dendrogram of four *Euphorbia* species studied by the Unweighted Pair Group Method with Arithmetic Mean (UPGMA).

The principal component analysis (PCA) has revealed variations among the species based on the xylem traits. *Euphorbia milii* shows a high positive score on PC1 (5.43), indicating a strong influence of PC1 traits, and a high negative score on PC2 (-5.21), suggesting suppression of PC2 traits (Table 2). *Euphorbia hirta* has a very low score on PC1 (-0.13) and a low positive score on PC2 (0.095), reflecting limited influence from both. However, *E. hirta* scores high on PC3 (2.89), indicating a significant contribution. *E. heterophylla* shows moderate influence from PC1 (2.56), a high positive score on PC2 (6.59), and a slightly negative score on PC3 (-0.99), suggesting dominance of PC2 traits. *Euphorbia thymifolia* shows strong suppression of PC1 traits with a high negative score (-7.87) and slightly negative scores on PC2 (-1.47) and PC3 (-0.99) (Table 2).

Table 3 summarizes the eigenvalues and variance by three principal components from PCA: PC1, with an eigenvalue of 32.62, accounts for 53.8% of the total variance, showing the highest variability; PC2, with an eigenvalue of 24.26, explains 40.03% of the variance. Together, PC1 and PC2 account for 93.84% of the total variance; PC3, with an eigenvalue of 3.73, explains only 6.16%, thus contributing minimally (Table 3).

Euphorbia milii has a dissimilarity score of 8.58 in comparison with *E. hirta*, 12.15 with *E. heterophylla*, and 13.81 with *E. thymifolia*. *Euphorbia hirta* has a dissimilarity score of 8.58 in comparison with *E. milii*, 8.03 with *E. heterophylla*, and 8.79 with *E. thymifolia*, thus showing moderate dissimilarity. *E. heterophylla* scores 12.15 in comparison with *E. milii*, 8.03 with *E. hirta*, and 13.17 with *E. thymifolia*, indicating similarity with *E. hirta* but greater remoteness from the others. *Euphorbia thymifolia* is most dissimilar to *E. milii* (13.81) and *E. heterophylla* (13.17), and moderately dissimilar to *E. hirta* (8.79).

The smaller the dissimilarity score is, the closer related are the species. *Euphorbia hirta* and *E. heterophylla* have the smallest score (8.03), suggesting a closer relation. The highest score is between *E. milii* and *E. thymifolia* (13.81), indicating that they are the most distantly related (Table 4).

Discussion

The survival and distribution of any plant species are closely linked to its ability to adapt to its environment. The anatomical features observed in the four *Euphorbia* species reflect their adaptation to different ecological conditions. These dicotyledonous species exhibit wide xylem vessels in their stems, which enhance hydraulic conductance. Wider vessels compensate for the limited cross-section area of the stem by efficiently transporting large volumes of water over greater distances, even in narrow-diameter stems. This is especially notable in *E. heterophylla*, which displayed the largest vessel lumens and, consequently, the highest water conductance.

The large intercellular pits observed in *E. heterophylla* suggest a more efficient water transfer between vessels, facilitating better adaptation to environments with higher water demand. That species has also exhibited a more abundant ray parenchyma compared to the others. Ray parenchyma, present in all four species, plays a critical role in separating the xylem groups into distinct sectors and contributes to the mechanical stability and flexibility of the plant. The greater abundance of the ray parenchyma in *E. heterophylla* suggests that that species may be particularly well-adapted to support water and nutrient transport and structural flexibility, which is essential in its natural habitat.

Table 2. Principal component (PC) of the four studied *Euphorbia* species.

species	PC1	PC2	PC3
E. milii	5.43	-5.23	-0.91
E. hirta	-0.13	0.09	2.89
E. heterophylla	2.56	6.59	-0.99
E. thymifolia	-7.87	-1.47	-0.99

Table 3. Variance in the observed xylem traits using the principal component analysis.

PC	Eigenvalue	% variance
1	32.62	53.81
2	24.26	40.03
3	3.73	6.16

Table 4. Distance matrix of the four *Euphorbia* species based on their xylem characters.

species	*E. milii*	*E. hirta*	*E. heterophylla*	*E. thymifolia*
E. milii	0			
E. hirta	8.58	0		
E. heterophylla	12.15	8.03	0	
E. thymifolia	13.81	8.79	13.17	0

The presence of circular cell wall thickness in all four species enhances the mechanical strength of their stems, helping them resist collapse during periods of high-water tension or drought stress. That structural adaptation is crucial for maintaining metabolic functions under severe water deficit and during periods of high evapotranspiration, a common challenge in arid environments (Masrahi 2012).

The parenchyma tissue, particularly in *E. heterophylla*, also facilitates growth by allowing flexibility as the plant grows around such supporting structures like trees. Parenchyma cells also help the vascular repair by forming new cambia, which can replace the damaged conducting cells, thus ensuring continuous water transport (Sieber & Kucera 1980). That repair mechanism highlights the parenchyma tissue's ecological importance in maintaining the vascular system's functional integrity under environmental stress.

Moreover, the species in the present study have exhibited other various adaptive traits, such as thick cuticles, dense hair cover and a low stomatal index, all characteristic of plants adapted to water-stressed environments. These features reduce water loss and protect the plant from harsh climatic conditions. The presence of starch grains near the vessels, as observed in the xylem tissue, suggests that starch hydrolysis could be a mechanism for sustaining energy supply during periods of water scarcity.

From a taxonomic perspective, the anatomical differences observed in the wood structure of the four *Euphorbia* species provide valuable insight into the classification and identification of species within the *Euphorbiaceae* family. The unique wood characteristics, particularly the xylem adaptations, reflect the ecological significance and evolutionary strategies for thriving in their respective habitats. The present study contributes to a deeper understanding of the functional anatomy of the *Euphorbia* species, offering a basis for further taxonomic and ecological investigations.

The distinct patterns of variation observed among the species may reflect their ecological adaptations and evolutionary pathways. The strong influence of PC1 traits on *E. milii* and *E. thymifolia* suggests that these traits play a critical role in their ecological niches or functional adaptation. Similarly, the dominance of PC2 traits in *E. heterophylla* may indicate specialization in traits associated with that component. The unique influence of PC3 on *E. hirta* highlights its distinctiveness, which may be linked to ecological factors or evolutionary divergence uncaptured by the other components. Dissimilarity scores further suggest varying degrees of relatedness among the species, with *E. hirta* and *E. heterophylla* being closely related, and *E. milii* and *E. thymifolia* being the most distant. That information could be crucial for understanding the evolutionary relationships within *Euphorbia* genus and exploration of how the xylem traits have contributed to species diversification.

The present study underscores the utility of PCA and dissimilarity analysis in revealing the patterns of variation and relationships among the species based on morphological traits. Dominance of PC1 and PC2 in explaining the significance of variability highlights the importance of those components in differentiating the species. The clustering and dissimilarity analysis further confirms the distinctiveness of each species, providing a comprehensive picture of their relationships. Those findings contribute to the understanding of ecological and evolutionary dynamics within the *Euphorbia* genus and offer a framework for future studies exploring the trait-based species differentiation.

Conclusion

The present study reveals significant ecological adaptations and evolutionary strategies among the *Euphorbia* species based on the xylem traits. PCA identifies PC1 and PC2 as the primary contributors to the species differentiation, while clustering and dissimilarity analyses highlights the distinct interspecies relationships. Such adaptations like wide xylem vessels, circular cell wall thickness, and abundant ray parenchyma reflect ecological resilience to water stress and structural demands. Those findings advance the understanding of *Euphorbia*'s functional anatomy and offer a foundation for future research into trait-based differentiation and evolutionary pathways within that genus.

Conflict of interest

No conflict of interest has been revealed among the authors.

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