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Short Title: Seedling Morphology and Phylogenetic Insights in *Euphorbia*



RESEARCH ARTICLE

Exploring seedling morphology for taxonomic delimitation and phylogenetic insights in the genus *Euphorbia*

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Abstract

This study investigates the taxonomic significance of seedling morphology in the genus *Euphorbia*, with emphasis on identifying and differentiating species during their juvenile stages. Key morphological traits including leaf shape, apex, base, venation pattern, and surface texture were analyzed across four *Euphorbia* species. The study employs both conventional morphological analyses and numerical taxonomic techniques, revealing distinct differences among species, namely *E. hirta*, *E. serpens*, *E. hypericifolia*, and *E. prostrata*. A dendrogram was constructed to illustrate phylogenetic relationships among the species, confirming the diagnostic value of seedling morphology for species identification. The results highlight the effectiveness of seedling morphological markers in species classification and offer new insights into taxonomic relationships within the genus. This study addresses a significant gap in *Euphorbia* seedling morphology research, providing a robust framework for future taxonomic and conservation studies, and encouraging further exploration of this diverse and complex genus.

Keywords: Cladogram analysis, *Euphorbia* species, Morphological variation, Phylogenetic relationships, Seedling morphology, Taxonomic differentiation

Introduction

The family *Euphorbiaceae*, commonly known as the 'spurge family,' is among the largest and most diverse families of angiosperms, comprising approximately 7,500 species distributed across 300 genera. Members of this family occupy a wide range of habitats from tropical and temperate regions to grasslands, deserts, and coastal zones contributing significantly to global floral diversity (Gilbert, 1987, Carter, 1994, Bruyns, et al. 2006, Riina, et al. 2013, Bolaji, et al. 2014). *Euphorbia*, the largest genus within the *Euphorbiaceae* family, represents one of the most taxonomically complex and ecologically versatile plant groups, with approximately 2,000 species distributed worldwide (Bruyns, et al. 2006, Horn, et al. 2012). In Pakistan, the genus comprises 111 species thriving across diverse ecosystems from arid deserts to fertile grasslands demonstrating remarkable ecological adaptability, ranging from arid deserts to fertile grasslands, and showcasing remarkable adaptability in terms of growth forms-ranging from small herbs to large trees and xerophytic forms (Fayed, et al. 2007, Dorsey, et al. 2013). This extensive species and habitat diversity has made *Euphorbia* an important

subject in plant taxonomy, with research focusing on molecular phylogeny, anatomy, and seed, capsule, and fruit morphology (Steinmann and Porter, 2002, Bruyns, et al. 2006, Gales and Toma, 2006, Aldhebani and Jury, 2013, Da Silva, et al. 2016, Genc, et al. 2018).

Pakistan's contribution to *Euphorbia* taxonomy is noteworthy, with 52 species described in the national flora (Euphorbiaceae, No. 172, 1986), accompanied by detailed descriptions of vegetative and floral traits and comprehensive identification keys. However, despite these efforts, a substantial knowledge gap persists regarding the genus's juvenile phase, particularly in seedling morphology, which has the potential to offer valuable insights for species identification and classification. Seedling morphology remains an under-explored yet valuable aspect of plant taxonomy, offering a cost effective and reliable approach to species differentiation at early developmental stages. It provides an alternative approach to more traditional, resource-intensive taxonomic methods and also contributes to plant conservation by facilitating the early detection and protection of endangered taxa (Lopez, et al. 1998, Tillich, 2000, Das, et al. 2001, Ibarra-Manriquez, et al. 2001, Linder and Caddick, 2001, Shulkina, et al. 2003, Sinjushin and Akopian, 2011, Lira Lopes and de Souza, 2015, Abozeid, et al. 2017, Bose, et al. 2017, Mehmood, et al. 2018, Mahmood, et al. 2018). The significance of seedling morphology in plant taxonomy has been emphasized by many researchers, underscoring its usefulness in both species' delimitation and evolutionary studies. The work of Verdus, 1976 serves as a pivotal contribution, as it explored seedling and cotyledonary characteristics of 131 Euphorbiaceae species, establishing a foundation for understanding evolutionary trends in this family. However, this research encompassed only a limited subset of species, indicating the need for further exploration. Additionally, early studies on Indian tree species by Lubbock, 1892 and Troup, 1921 expanded the understanding of seedling characteristics within the genus.

This study builds upon previous research by examining the role of seedling morphology in the taxonomy of *Euphorbia* and related taxa within the Euphorbiaceae family. By analyzing seedling morphological features, this study aims to enhance species identification accuracy and offer new insights into the evolutionary and taxonomic relationships within the family. Such studies are critical for advancing plant systematics and play a significant role in the conservation and management of biodiversity. Given the vast number of *Euphorbia* species yet to be thoroughly studied, this research provides a timely contribution toward addressing existing knowledge gaps within this taxonomically significant genus.

Materials and Methods

In this study, five species of the genus *Euphorbia*-*E. prostrata*, *E. cyathophora*, *E. hirta*, *E. serpens*, and *E. hypericifolia*-were examined for their seedling morphological characteristics. Multiple specimens of each species, representing various seedling stages, were collected from the M.Sc. Block, Federal Urdu University of Arts, Science and Technology, Gulshan Campus, Karachi. Collected specimens were maintained in a greenhouse under controlled conditions to monitor and document their developmental stages. The recorded data included detailed morphological descriptions of cotyledons, the first leaf pair, and subsequent leaves. Interrelationships among the species were analyzed using cluster analysis, and dendrograms were constructed with SPSS software. Photographs of the taxa were captured using a Nikon D3200 digital camera. The investigated taxa are listed below:

- *Euphorbia hirta* L. (NM 115)
- *Euphorbia hypericifolia* L. (NM 941)
- *Euphorbia prostrata* Ait (NM 329)
- *Euphorbia serpens* Kunth. (NM 361)

Results

Morphological characterization of *Euphorbia* species

The morphological traits of the four *Euphorbia* species-*Euphorbia hirta* L., *E. hypericifolia* L., *E. prostrata* Ait., and *E. serpens* Kunth-were analyzed in detail to differentiate among their seedling structures, paracotyledons, and leaf characteristics.

***Euphorbia hirta* L.:** The seedling of *E. hirta* exhibits epigeal, phanerocotylar germination. The hypocotyl is white, round, soft, and glabrous, measuring 0.1-0.3 cm in length. Paracotyledonary leaves are reddish-green, opposite, and oblong, with obtuse apices and bases, entire margins, and indistinct midrib. Internodes are red, round, pubescent, and 1-2 cm apart. The first two leaves are green, horizontal, and sub-sessile, with an obovate shape, obtuse-acute apex, and entire margins. Subsequent leaves are lanceolate-ovate, petiolate, and exhibit serrate margins with distinct pinnate venation.

***Euphorbia hypericifolia* L.:** The seedling of *E. hypericifolia* is also epigeal and phanerocotylar. Its hypocotyl is red, round, soft, and glabrous, about 2 cm long. Paracotyledonary leaves are green and obovate, with cuneate bases and entire margins. Internodes are red, round, glabrous, and spaced approximately 1 cm apart. The first two leaves are oblong, stipulated, and petiolated, with obtuse apex and entire margins. Subsequent leaves are narrow elliptic with serrulate margins and multicostate venation.

***Euphorbia prostrata* Ait.:** The seedling of *E. prostrata* is epigeal and phanerocotylar, with a red, round, erect, and glabrous hypocotyl about 1 cm long. Paracotyledonary leaves are green, ovate, with obtuse apex, entire margins, and indistinct midrib. Internodes are whitish-green, round, glabrous, and spaced up to 2 cm apart. The first two leaves are obovate, with cuneate base and entire margins, while subsequent leaves are obovate, with serrate margins and a distinct midrib.

***Euphorbia serpens* Kunth:** The seedling of *E. serpens* is epigeal and phanerocotylar, with a green, round, soft, and glabrous hypocotyl about 1 cm long. Paracotyledonary leaves are oblong, green, sub-sessile, and glabrous. Internodes are reddish-green, glabrous, and less than 1 cm apart. The first two leaves are ovate, retuse, and glabrous, while subsequent leaves are ovate-orbicular, emarginate, and exhibit entire margins with distinct midrib.

Comparative morphological analysis

The morphological data for paracotyledons, first two leaves, and subsequent leaves are presented in [Tabs. 1, 2, and 3](#), respectively. These analyses reveal distinct interspecific differences among the four *Euphorbia* species. The paracotyledons of *E. hirta* and *E. serpens* share an oblong shape, a trait that clusters them together morphologically. In contrast, *E. hypericifolia* exhibits obovate paracotyledons, while *E. prostrata* displays ovate ones. These variations serve as critical markers for species differentiation.

Table 1. Morphological characters of species' paracotyledons.

Plant Name	Shape	Apex	Base	Margin	Venation	Surface
<i>E. hirta</i>	Oblong	Obtuse	Obtuse	Entire	Indistinct	Glabrous
<i>E. hypericifolia</i>	Obovate	Obtuse	Cuneate	Entire	Indistinct	Glabrous
<i>E. prostrata</i>	Ovate	Obtuse	Obtuse	Entire	Indistinct	Glabrous
<i>E. serpens</i>	Oblong	Obtuse	Obtuse	Entire	Indistinct	Glabrous

Table 2. Morphological characters of species' first two leaves.

Plant Name	Type	Phyllotaxy	Shape	Apex	Base	Margin	Venation	Surface
<i>E. hirta</i>	Simple	Opposite	Obovate	Obtuse-cuneate	Obtuse	Entire	Pinnate alternate	Pubescent
<i>E. hypericifolia</i>	Simple	Opposite	Oblong	Obtuse	Obtuse	Entire	Pinnate opposite	Glabrous
<i>E. prostrata</i>	Simple	Opposite	Obovate	Obtuse	Cuneate	Entire	Distinct	Glabrous
<i>E. serpens</i>	Simple	Opposite	Ovate	Retuse	Oblique	Entire	Distinct	Glabrous

Table 3. Morphological characters of species' subsequent leaves.

Plant Name	Type	Phyllotaxy	Shape	Apex	Base	Margin	Venation	Surface
<i>E. hirta</i>	Simple	Opposite	Lanceolate-ovate	Acute	Oblique	Serrate	Pinnate alternate	Pubescent
<i>E. hypericifolia</i>	Simple	Opposite	Narrow elliptic	Acute	Obtuse	Serrulate	Multicostate	Glabrous
<i>E. prostrata</i>	Simple	Opposite	Obovate	Obtuse	Oblique	Serrate	Distinct	Glabrous
<i>E. serpens</i>	Simple	Opposite	Ovate-orbicular	Emerginate	Oblique	Entire	Distinct	Glabrous

Further morphological distinctions were evident in the first two leaves, particularly in their shape, apex, base, margins, and surface texture. For instance, the first two leaves of *E. hirta* are horizontal and sub-sessile with an obovate shape and a pubescent surface, whereas *E. serpens* has glabrous, ovate leaves. In *E. hypericifolia*, the first two leaves are characterized by an oblong shape with a cuneate base and entire margins, while *E. prostrata* exhibits broadly ovate leaves with rounded bases. These differences, coupled with unique venation patterns such as pinnate or multicostate venation, underscore the morphological diversity within the genus.

The subsequent leaves provided additional distinguishing features. *E. hirta* is notable for its lanceolate-ovate leaves, which contrast with the narrow elliptic leaves of *E. hypericifolia*. *E. serpens* exhibits ovate-orbicular leaves with entire margins, while *E. prostrata* displays broadly ovate leaves with a slightly serrated margin. Variations in venation, apex sharpness, and surface texture further contribute to the identification process, demonstrating the taxonomic significance of these morphological traits.

Artificial key and cladogram

An artificial key was constructed to facilitate the identification of these four species by focusing on distinctive morphological characteristics, particularly the shape, apex, and texture of paracotyledons and the first two leaves. The key emphasizes the unique obovate paracotyledons of *E. hypericifolia* and the pubescent surface texture of *E. hirta*, both of which serve as reliable identifiers.

Key to the species

- 1+ Paracotyledons oblong 2
- 1- Paracotyledons not oblong 3
- 2+ The first two leaves obovate, pubescent, size *E. hirta*
- 2- The first two leaves ovate, glabrous, size *E. serpens*

- 3+ Paracotyledons obovate, first two leaves oblong..... *E. hypercifolia*
3- Paracotyledons ovate, first two leaves obovate *E. prostrata*

Cluster II is also divided into two sub-clusters A and B. IIA presented variation among first two leaves and subsequent leaves of *E. serpens* on the basis of leaf shape (ovate, orbicular) and apex (retuse, emerginate) as well as among first two leaves and subsequent leaves of *E. prostrata* based on leaf base (cuneate, oblique) and margin (entire, serrate). IIB consist of single species *E. hirta* which is morphologically distinct from other species in Cluster II.

To complement the artificial key, a cladogram (Fig. 1) was constructed to visualize the morphological relationships among the studied species. The cladogram reveals two main clusters. Cluster I include sub-cluster IA, which groups *E. hirta* and *E. serpens* based on their shared oblong paracotyledons. Sub-cluster IB separates *E. hypericifolia* due to its narrow elliptic subsequent leaves and distinct venation pattern.

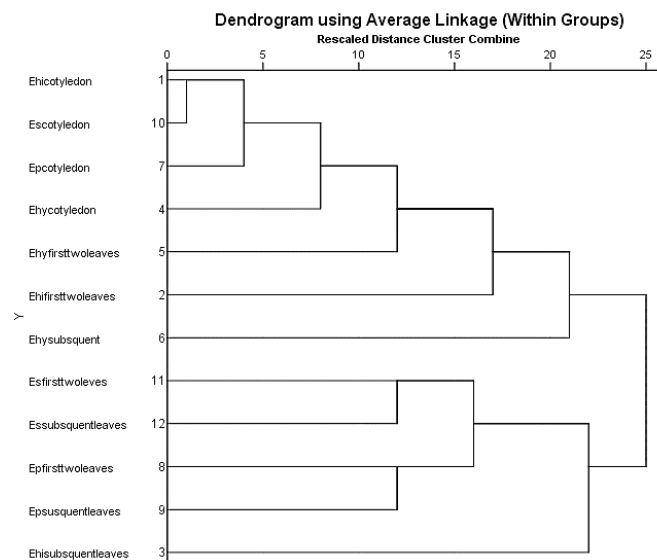


Figure 1. Dendrogram illustrating the phylogenetic relationships among four *Euphorbia* species. (Ehicotyledon-*E. hirta* cotyledons, Escotyledon-*E. serpens* cotyledons, Epcotyledon-*E. prostrata* cotyledons, Ehycotyledon-*E. hypericifolia* cotyledons, Ehifirsttwoleaves-*E. hirta* first two leaves, Esfirsttwoleaves-*E. serpens* first two leaves, Epfirsttwoleaves-*E. prostrata* first two leaves, Ehyfirsttwoleaves-*E. hypericifolia* first two leaves, Ehisubsequentleaves-*E. hirta* subsequent leaves, Essubsequentleaves-*E. serpens* subsequent leaves, Epsubsequentleaves-*E. prostrata* subsequent leaves, Ehyubsequentleaves-*E. hypericifolia* subsequent leaves).

Cluster II highlights the morphological divergence of *E. prostrata* and *E. serpens* based on differences in leaf base and apex characteristics. Sub-cluster IIB distinctly isolates *E. hirta*, characterized by its lanceolate-ovate leaves and unique surface texture. These clustering patterns highlight morphological affinities among *Euphorbia* species, providing a systematic framework for their classification.

Overall, this comparative morphological analysis highlights the taxonomic significance of paracotyledon and leaf traits, providing a strong framework for the identification and systematic understanding of *Euphorbia* species.

Discussion

The morphological attributes of the studied seedlings hold significant taxonomic importance, as seedling morphology serves as a valuable tool for identifying species at their juvenile stages. Accurate identification of species during early growth stages is crucial in both natural ecosystems and horticultural settings. Similar observations were reported by [Mangaly, et al. 1978](#), who examined two *Euphorbia* species-*E. hirta* and *E. thymifolia*-and highlighted distinct morphological differences in their seedlings. These differences can be effectively used for taxonomic classification, reinforcing the importance of seedling traits for species identification. The present findings align with those of [Ogie-Odia, et al. 2019](#), who emphasized that morphological diversity within *Euphorbiaceae* serves as a key criterion for taxonomic differentiation. Specific attributes such as paracotyledon shape, leaf morphology, and venation patterns have shown that species like *E. hirta*, *E. serpens*, *E. hypericifolia*, and *E. prostrata* possess unique seedling characteristics that make them identifiable even at early growth stages. This is in line with previous studies that have emphasized the utility of seedling morphology for identifying species in the *Euphorbiaceae* family, as noted by [De Souza, et al. 2014](#) and [Pujol, et al. 2004](#). These studies highlight the role of seedling morphology in discriminating between species, thus contributing significantly to taxonomic clarity.

Beyond the *Euphorbiaceae* family, the significance of seedling morphology has been widely explored in other plant genera and families. Numerous studies have examined how variations in seedling traits aid in species identification and classification, further

supporting the generality of these findings. [Martin, et al. 2011](#), [Sinjushin and Akopian, 2011](#), and [Singh, 2012](#) contributed valuable insights into how seedling characteristics can serve as reliable taxonomic markers. Similarly, [Karmakar, 2012](#), [Khalik, 2012](#), and [Gurgel, et al. 2012](#) demonstrated the importance of such traits in species identification within other genera. The breadth of research on seedling morphology across different plant families highlights its widespread applicability and value in resolving taxonomic ambiguities.

In addition to seedling morphology, the integration of numerical taxonomic techniques offers a more comprehensive approach to species classification. The use of numerical methods, which rely on the analysis of morphological traits, has been successfully employed in resolving taxonomic issues across various plant families. For example, [Bello, et al. 2013](#) applied numerical taxonomic techniques to the genus *Solanum*, successfully resolving taxonomic uncertainties. In the present study, numerical methods such as cluster analysis were applied to classify the *Euphorbia* species based on their seedling characteristics. The clustering patterns observed in the dendrogram ([Fig. 1](#)) further illustrate the effectiveness of combining numerical taxonomy with morphological analysis. The clustering of *E. hirta* and *E. serpens* based on their shared paracotyledon shapes, and the separation of *E. hypericifolia* due to its narrow elliptic leaves, exemplify the strength of this combined approach.

Numerical taxonomic techniques have been widely applied across diverse species by several researchers, including [El-Gazzar, 2008](#), who worked on Hyptis; [Folorunsho and Jayeola, 2009](#), who studied Polystachya; and Kolawole, Abdulrahman, and [Oladele, 2014](#), who focused on Jatropha. These studies highlight the utility of these techniques in examining morphological variations and resolving taxonomic uncertainties.

In addition to seedling traits, parameters such as seed and pollen morphology, along with ecological adaptations, also play vital roles in the delimitation of taxa. Research by [Abid and Qaiser, 2009](#), [Gontcharova, et al. 2009](#), and [Celep, et al. 2012](#) highlighted the significance of seed morphology in species identification, while studies by [Das, et al. 2013](#) and [Abid, et al. 2014](#) explored the role of pollen morphology in understanding phylogenetic relationships. Furthermore, ecological studies by [Song, Gu, and Liu, 2019](#) and [Erdtman, 2023](#) have illustrated how environmental factors contribute to species differentiation. These diverse factors underscore the multifaceted approach required for accurate taxonomic classification and provide additional support for the value of seedling morphology as one of the key taxonomic tools.

While seedling morphology has been explored in many species, the majority of taxa within the genus *Euphorbia* remain understudied, and many species have yet to be classified based on their juvenile characteristics. The present study represents an initial yet significant step toward addressing this research gap. By documenting and analyzing the morphological characteristics of seedlings within this genus, we contribute to a better understanding of its diversity and taxonomic structure. Moreover, the study emphasizes the critical role that seedling morphology plays at various taxonomic levels, providing a foundation for further research and exploration.

In conclusion, this study underscores the pivotal role of seedling morphological traits in species identification and classification within the genus *Euphorbia*. The integration of numerical taxonomic techniques with traditional morphological observations provides a robust framework for species classification, as exemplified by the dendrogram analysis. Given the large number of unexamined species within this genus, further research on seedling morphology is essential to unravel the complete taxonomic diversity of *Euphorbia* and to provide accurate identification tools for researchers and practitioners in the field.

Conclusion

This study highlights the critical importance of seedling morphology in the taxonomic identification and classification of species within the genus *Euphorbia*. Through the detailed examination of key morphological traits and the application of numerical taxonomic techniques, this study provides new insights into species differentiation at the juvenile stage, thereby enhancing the understanding of their taxonomic relationships. The findings demonstrate that integrating traditional morphological analysis with numerical methods effectively resolves taxonomic ambiguities, particularly among understudied species. Furthermore, this research addresses a notable gap in the current literature, and provides a foundational basis for future investigations into the largely unexplored seedling morphology of *Euphorbia* species. Overall, this study lays the groundwork for future research aimed at improving species identification, classification, and conservation strategies within the genus.

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