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Communication

An alternative key for identifying three prostrate species of the genus *Euphorbia*

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Abstract

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Plants are a valuable source of different folk and modern medicines. Therefore, correctly identifying the plant species is a prerequisite for using them in the health care system. *Euphorbia* species are of great medicinal importance but are difficult to distinguish due to the occurrence of ecotypes and a high degree of polymorphism within the species. Therefore, the present study was planned to explore the extent of variations in morphological characters among three *Euphorbia* species, namely, *Euphorbia prostrata* Aiton, *Euphorbia serpens* Kunth and *Euphorbia thymifolia* L. The edaphic ecotypes red and green forms of *Euphorbia prostrata* growing separately or adjacent to each other were reported during field surveys. To ensure identification accuracy, illustrated online key was prepared as a supportive tool for the traditional key.

Keywords: cyathium, ecotypes, medicinal plants, nomenclature, polymorphism.

INTRODUCTION

Euphorbia L. is the largest genus of the family Euphorbiaceae, with ca. 2150 species distributed worldwide and 195 in India (Aditya, 2010; Frajman & Schonswetter, 2011). These species grow in a variety of habitats and are used for the treatment of various ailments such as respiratory disorders (asthma), endocrine disorders (*Euphorbia prostrata* for diabetes), digestive disorders (*Euphorbia thymifolia* for diarrhoea, constipation), skin disorders (*Euphorbia serpens* for eczema, sores), parasitic infections (malaria), microbial diseases like tuberculosis and ringworm (Ernst et al., 2015). However, identifying *Euphorbia* species is also difficult due to species richness, phenotypic plasticity and similar morphological characteristics (Pahlevani, 2017).

The correct identification of plants is required for their utilisation in preparing herbal medicines (Odewo et al., 2020). The misidentification and misinterpretation of a plant species may result in adulterated herbal products and subsequently cause serious health concerns to consumers (Panter et al., 2019). There are some theoretical and practical issues associated with the identification of plants that need to be addressed.

The use of key lies in understanding the terminology for describing the characters and ease of recognition of specimens (Ebach et al., 2011). Due to a lack of expertise, identification keys can lead to erroneous plant species identification.

The intraspecific variations in morphological characters are likely due to ecological conditions, which result in the formation of ecotypes (Talebi et al., 2016). The ontogeny is also responsible for intraspecific variations (Henn & Damschen, 2021).

Many morphological resemblances between the species of the same genus may impede their identification (Whittall et al., 2004). For example, the species of the genus *Euphorbia* are often confused with each other due to their morphological closeness. The morphological similarity between species makes delimitation of taxa difficult.

Lombard et al. (2021) have prepared an interactive identification key to identify plant species with cryptic characters containing visual illustrations (images) and textual descriptions of important identifying features. The interactive keys with multiple images of each character and its states can help the user to assess the wide range of variations in that particular character (Ribeiro et al., 1999). Leggett & Kirchoff (2011) have discussed the use of images in field guides and identification keys and suggested that images should be standardised in a manner so that similar images can be compared across species. The keys should be able to zoom the images to observe the taxonomically critical morphological characters in detail. The present study is helpful in overcoming the problem of misidentification among three morphologically similar *Euphorbia* species, which might lead to a collection of faulty raw materials for the preparation of medicines.

MATERIALS AND METHODS

The present study was carried out on three prostrate *Euphorbia* species growing naturally in Chandigarh and its adjoining areas. Various field visits were undertaken to observe the morphological variations within *Euphorbia* species in their natural habitats in 2020–2021. Among the reported species, three prostrate species showed very few variations in their morphological characters. Several plant specimens of these three species, namely, *Euphorbia prostrata* Aiton (red form), *Euphorbia prostrata* Aiton (green form), *Euphorbia thymifolia* L. and *Euphorbia serpens* Kunth were observed and collected from different sites in the study area. Major morphological characters (stem indumentum, rooting at stem nodes, stipules, leaf margin, cyathia per axil, involucre indumentum, gland appendages, pedicel, capsule wall indumentum and seed coat ornamentation) were observed using the dissecting and stereomicroscopes.

The collected plant specimens were identified by consulting the available literature (Bamber, 1916; Nair, 1978; Batori et al., 2012; Silva et al., 2014; Sirbu & Susnia, 2018; Sharma, 2021; Souza & Silva, 2021). The authors' citations and botanical names of collected plant species were verified using the International Plant Names Index (IPNI). The specimens of identified species *Euphorbia prostrata* Aiton, *Euphorbia thymifolia* L. and *Euphorbia serpens* Kunth were deposited at the Herbarium (PAN) of the Department of Botany, Panjab University, Chandigarh, with accession numbers 22167, 22168 and 22170, respectively. One of the reported species, *Euphorbia prostrata* had two forms (red and green) growing in isolation or sometimes adjacent. The morphological characters of more than 100 specimens of each ecotype were studied in detail. An interactive key was prepared with the software Xper[3]

Table 1. Morphological characters and character states for the preparation of key for *Euphorbia* species

No.	Characters	Character states		
		1	2	3
1	Stem indumentum	Pubescent on adaxial surface	Glabrous	–
2	Rooting at stem nodes	Present	Absent	–
3	Stipules	Free and triangular	Fused and membranous	–
4	Leaf margin	Serrate	Entire	–
5	Cyathia per axil	Solitary	In groups	–
6	Involucre indumentum	Hairs only on the lobes	Glabrous	–
7	Gland appendages	Absent	Present	Generally, absent if present not clearly visible
8	Pedicel	Short and erect	Long and arching	–
9	Capsule wall indumentum	Glabrous	Pubescent	Hairs only on the angles of triangular capsule
10	Seed coat ornamentation	Sculptured with transverse ridges	Smooth	–

(online). At first, a knowledge base was created, and the names of three *Euphorbia* species entered in the Xper[3]. A set of ten morphological characters with their respective character states were considered for preparing paper-based and interactive keys (Table 1).

The characters not visible to the naked eye were observed using dissecting 10× and stereomicroscope. Textual descriptions for morphological characters and states were given wherever possible. Character states were manually coded for each *Euphorbia* species. Photographs were added to Dropbox to clarify each state and then uploaded in Xper[3] using the "Add from URL" tab. The key checked whether each morphological character provides full, partial, or no discrimination using the "Comparison tool". The prepared identification key was evaluated using a built-in tool named checkbase in Xper[3]. Checkbase automatically searches for various errors in the database, like descriptors without descriptions and two or more species with similar morphological accounts, if any. Xper[3] construct a profile for each species, including photographs, a short description, and a list of character and their character states for each species. A digital identification key was generated with the name "Identification key for three prostrate species of *Euphorbia* L." on Xper[3] software and was provided online through a link.

RESULTS

Morphological characteristics

Among the three studied species, *Euphorbia prostrata* was found common in the study area, with the red form more frequent than the green form, followed by *Euphorbia serpens* and *Euphorbia thymifolia* (Fig. 1). This study is an original survey and contains original illustrations.

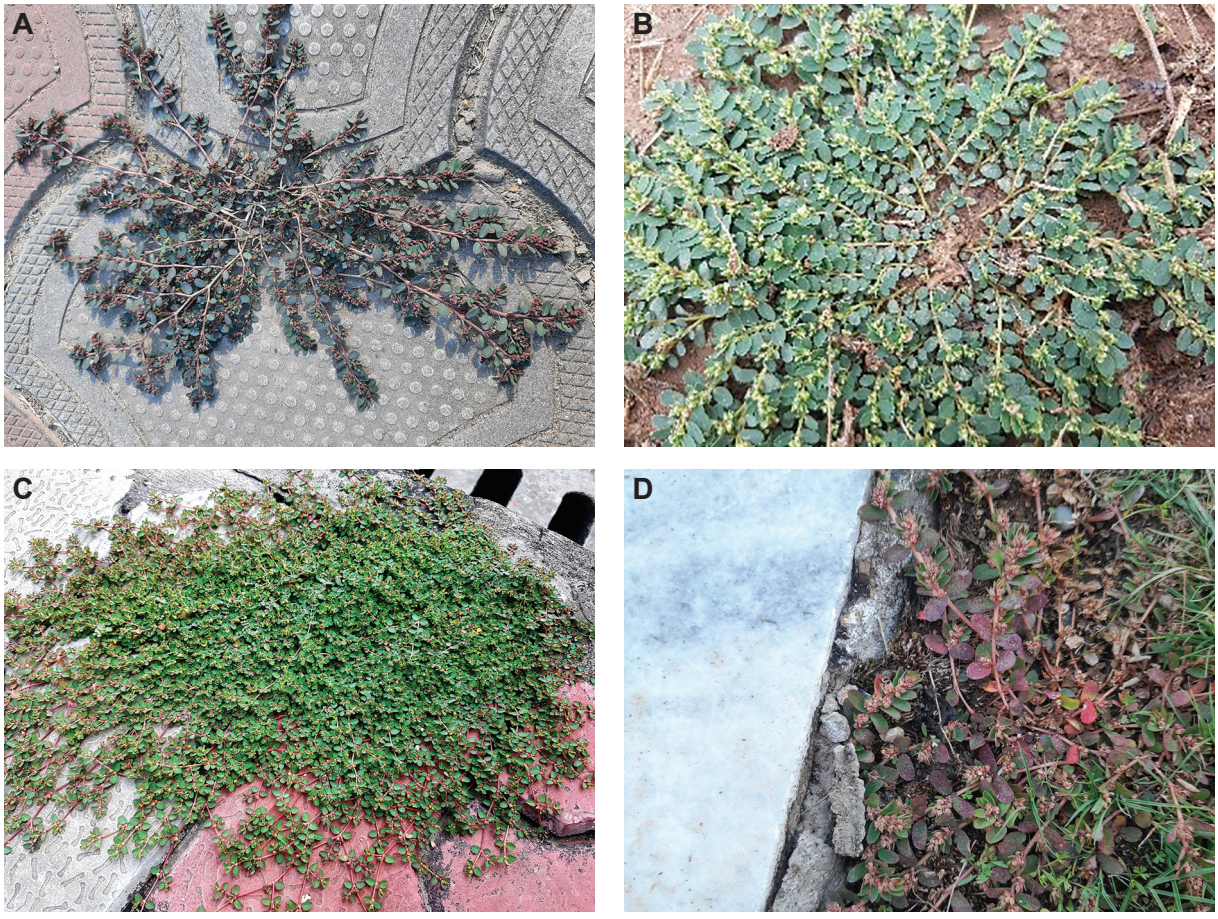

Fig. 1. Plant specimens of *Euphorbia* species: red form of *Euphorbia prostrata* (A); green form of *Euphorbia prostrata* (B), *Euphorbia serpens* (C), *Euphorbia thymifolia* (D)

Euphorbia prostrata **Aiton, Hort. Kew., 2: 139. 1789.**

Prostrate to ascending annual herb. Stems pubescent on the adaxial, glabrous on the abaxial surface, much branched from base bearing subsessile, opposite, oblong, serrate, larger leaves on the main stem and shorter leaves on branches. Involucre turbinate, elliptic glands four, each gland with minute appendages at the rim of the involucre, pedicel accrescent, partially pubescent triangular capsule with a line of hairs at each angle, seed surface with transverse ridges.

Ecotypes. Two forms (red and green) of *Euphorbia prostrata* were reported in the study area, growing individually or sometimes, adjacent to each other (Fig. 2). In the detailed morphological study of these ecotypes, the difference was observed only in colour while all distinguishing characters such as stem pubescence, cyathial glands, capsule indumentum and seed texture were similar.

Ramakrishnan (1961) has reported two edaphic ecotypes (red and green forms) in *Euphorbia thymifolia* in the same location as it was observed in the case of *Euphorbia prostrata* during the present study. However, no such forms were reported in *Euphorbia thymifolia*. Mishra & Sahu (1985) have reported the occurrence of two ecotypes (red and green) of *Euphorbia prostrata* in the same location, which validates the finding of two ecotypes of *Euphorbia prostrata* species in the present study.

Fig. 2. Red and green forms of *Euphorbia prostrata* growing adjacent to each other

Euphorbia serpens **Kunth, Nov. Gen. Sp. 2(5): 52. 1817.**

Prostrate to ascending, entirely glabrous annual herb. Stem much branched bearing opposite, subsessile, entire, orbicular leaves larger on the main stem and shorter leaves on branches. Roots are present at nodes on the abaxial surface of the stem. Glands four in number, each gland attached to white or light pink petaloid appendages. Seed surface smooth and lack transverse ridges.

Euphorbia thymifolia **L., Sp. Pl., 1: 454. 1753.**

Prostrate annual herb. Stem pubescent on the adaxial, glabrous on the abaxial surface, larger leaves present on the main axis and smaller on branches, rooting from stem nodes absent, leaves opposite, subsessile, pubescent, serrate. Involucre turbinate, petaloid appendages are generally absent, and present sometimes. Capsules are completely pubescent, rounded, pedicel, not accrescent breaks involucre at maturity. The seed surface is rough due to transverse ridges.

DISCUSSION

The available literature suggests the morphological similarities of the reported species with other closely related species of the genus *Euphorbia*. The prostrate *Euphorbia* species show a resemblance in having large leaves on the main stem and short leaves on the branches. The studied species are morphologically similar to other *Euphorbia* species (not reported during the present study), such as *Euphorbia maculata*, *Euphorbia granulata*, *Euphorbia chamaesyce* (Pahlevani & Riina, 2011), *Euphorbia heyneana* and *Euphorbia clarkeana* (Sharma, 2021). In *Euphorbia maculata*, leaves are oblong-ovate with reddish brown colouration in the centre, hairy capsules and seeds with 3–4 transverse ridges. In contrast, in *Euphorbia chamaesyce*, leaves are orbiculate-ovate with reddish brown colour in the centre, sometimes absent and seeds with irregular ridges. In *Euphorbia granulata*, plants are densely hairy; leaves are entire, leaf apex dentate without reddish colour in the centre. *Euphorbia prostrata* is similar to *Euphorbia clarkeana,* which is glabrous or sparsely hairy. *Eu-*

phorbia serpens resembles *Euphorbia heyneana* in various morphological characters, but the stipules only differ. Stipules are free on the adaxial stem surface and fused on the abaxial surface in *Euphorbia heyneana,* while fused stipules are observed on both sides in *Euphorbia serpens.* Visual illustrations of major identifying features through images could be used as a supporting tool to solve the ambiguity in the interpretation of main identifying characteristics.

The text-based key for the identification of three *Euphorbia* species

1A Stem indumentum only on adaxial surface, rooting at nodes does not occur, stipules free and triangular, leaf margin serrate, cyathia in groups, minute gland appendages, seed surface rough due to transverse ridges ……**2**

1B Stem indumentum absent, rooting at nodes, stipules fused and membranous, leaf margin entire, cyathium solitary, petal-like gland appendages visible to the naked eye, smooth seed surface …… ***Euphorbia serpens***

2A Pubescence all over the rounded capsule, pedicel short ……..…… ***Euphorbia thymifolia***

2B Pubescence only at the ridges of a triangular capsule, pedicel long ..... ***Euphorbia prostrata***

The online key has generated a profile for each species (*Euphorbia prostrata*, *Euphorbia thymifolia* and *Euphorbia serpens*) having photographs of species with highlighted characters and character states. The prepared online key (Fig. 3) can be accessed online (https://www.xper3.fr/xper3GeneratedFiles/publish/identification/5390739517649601485/mkey.html).

The interactive online identification keys can provide access to many images of different plant specimens of single species, thus helping to accurately identify species.

CONCLUSION

The occurrence of different ecotypes and infraspecific variations in morphological features amongst three prostrate, annual *Euphorbia* species (*Euphorbia prostrata*, *Euphorbia serpens* and *Euphorbia thymifolia*) is an obstruction to the correct taxonomic characterisation. In that case, traditional paper-based keys should be used with interactive image-based identification keys to enhance the general accuracy in identifying plants, which is a prerequisite for other disciplines such as horticulture, taxonomy, pharmacy and biotechnology.

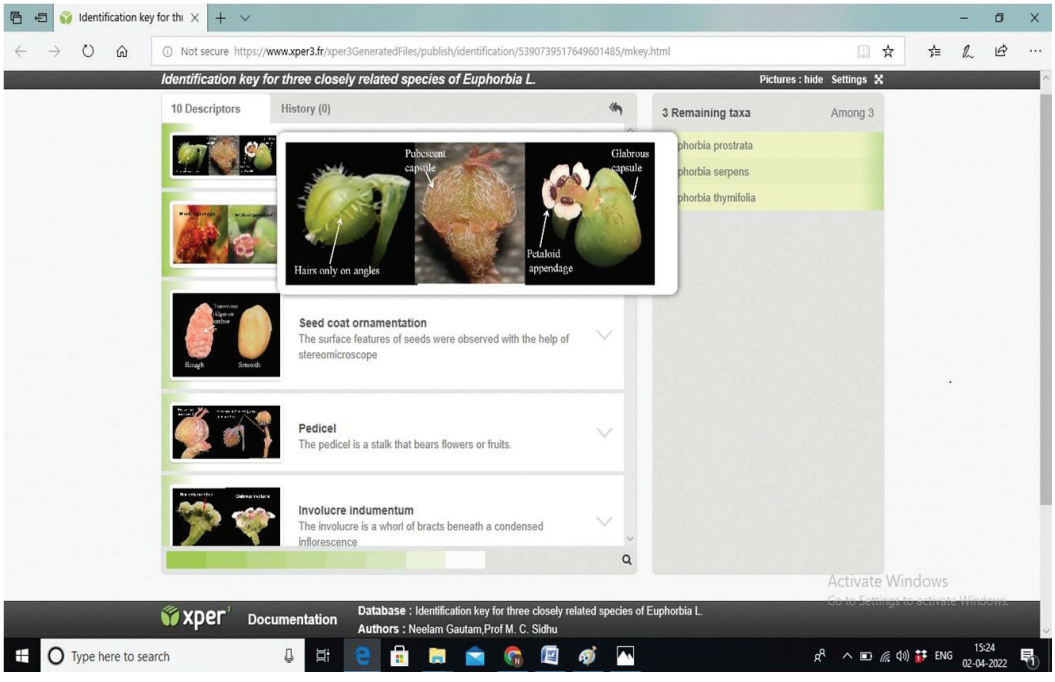

Fig. 3. Interactive identification key interface that appears after clicking on a link given above

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