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"MICRO MORPHOLOGICAL CHARACTERIZATION OF *MURDANNIA* SPECIES"

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ABSTRACT

The study of micro-morphological characterization, which involves examining the microscopic features of plant parts, is essential for accurate species identification and key preparation, especially in taxonomically complex groups like *Murdannia.* Given this background, *Murdannia* species were collected and maintained in a botanical garden for further study. A proper herbarium specimen was identified using a standard reference book and the Flora of Maharashtra (1996). Micro-morphological characterization of 4 different species of *Murdannia*–viz. *Murdannia gigantea* (Vahl) G. Bruckn*, Murdannia lanuginosa* (Wall.) G. Bruckn, *Murdannia Nudiflora* (L.) Brenan, and *Murdannia semiteres* (Dalzell) Santapau were done based on different external characters and flower morphology. using SPSS V.20, conducted a correlation (dissimilarity matrix - Euclidean Distance) between 4 different species of *Murdannia*. Correlation shows how the *Murdannia* species are near to each other or vary from each other based on morphology. This study will help the researchers identify the correct species of *Murdannia*. The proximity matrix shows the difference between the four species.

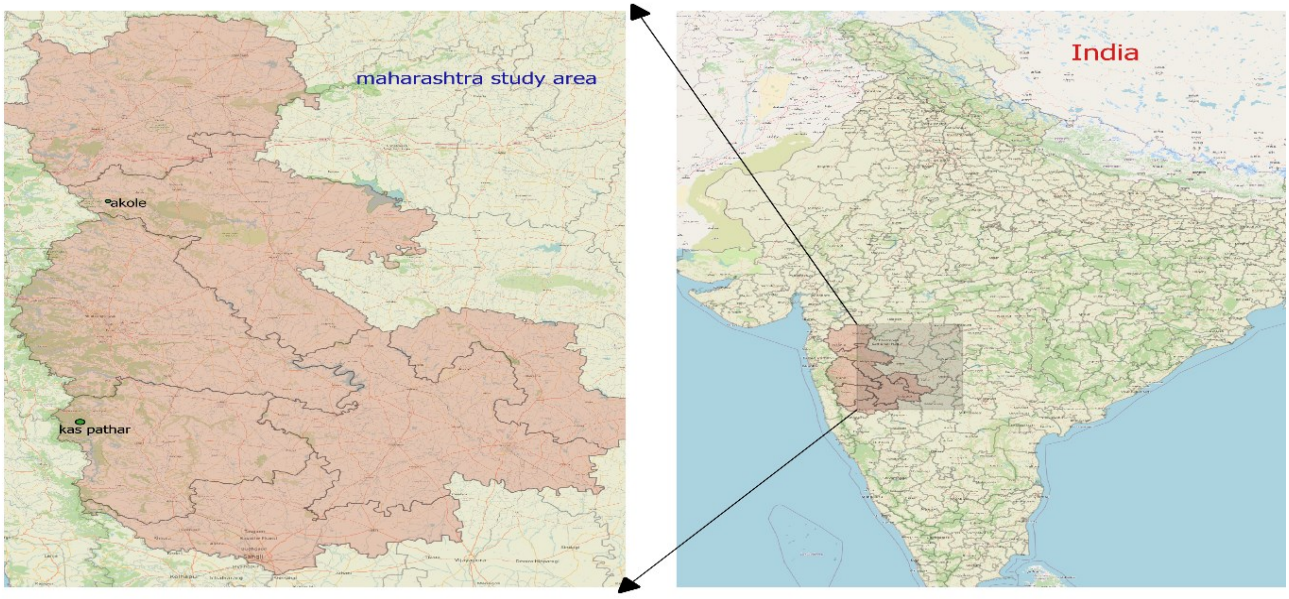
INTRODUCTION

The genus Murdannia belongs to the family Commelinaceae, with about 60 species distributed worldwide (Pellegrini et al., 2016) and 27 species in India (Nandikar and Gaurav, 2011). In "Flora of Maharashtra," it was reported that 15 species occurs in different parts of Maharashtra. (B.D.Sharma et al., (1996). These plants are characterized by their small, herbaceous habit and are often found growing in moist, shaded environments (Hong et al., 2014). Despite their relatively small size, *Murdannia* species have been found to possess a range of bioactive compounds, including flavonoids, phenolic acids, and terpenoids, which have been reported to exhibit various pharmacological activities (Liu et al., 2018). Traditional taxonomic classification relies on macroscopic morphological features, which can often be insufficient for fine and precise species delimitation, especially in complex genera such as *Murdannia*. This research examines the utility of micro-morphological characteristics to resolve the classical vagueness and brings an understanding of *Murdania.* In view of the background, the current study of the four species of *Murdania* was examined by micromorphological characters. The objective of this study is to provide a more detailed and stronger framework for species identification and phylogenetic analysis in species. Also, a proximity matrix is provided to show the interrelationship gap.

Materials and Methods

Collection and characterization of plants: Ideal branches with flowers and capsules from plant species collected in the Kas plateau (Lat 17.698822° Long 73.927047°) and Akola region (Lat 19.479011° Long 73.82284°) have been gathered and processed to create a herbarium using standard methods (B.D. Sharma et al., 1996).

Fig. No. 1 Study area map



Photographs of all dissected parts (photo plate), including habits, habitats, and plants, were captured using Oppo Reno cameras (48 megapixel) and a Leica binocular microscope equipped with a camera. (45 megapixel) These images were further processed with calibrated software such as ImageJ 1.54G version, Leica Application Suite, and the Measure Image Android application.

Identification: The identification process involved comparing specimens with those documented by Mayur Nandikar (2018) and relevant literature (Santosh Namphy and Manudev K.M., 2012). Final confirmation was achieved by comparing the plants with type specimens housed at the Botanical Survey of India, Western Regional Centre, Pune (MH). A detailed distribution map, encompassing all locations, was prepared using DIVA-GIS and QGIS Map Application

Examined four distinct *Murdannia* species: *Murdannia gigantea*, *Murdannia lanuginosa*, *Murdannia nudiflora*, and *Murdannia semiteres*, with particular emphasis on their external morphological characteristics. Furthermore, a comparative table no.1 of the four species was developed based on morphological characters in the identification process.

Table no 1. Micro morphological characters of *Murdannia* species.

Micro Morphological characterization	**species**			
	Murdannia gigantea	***Murdannia launginosa***	***Murdannia nudiflora***	***Murdannia semiteres***
Root	Fibrous roots	Fusiform tuberous	Fibrous roots	Fibrous roots
Stem	Creeping, rooting at nodes, **10 to 15** cm long, green in color, often vinaceous, glabrous with pubescent at the edge.	Angular and hairy Stem much branched from the base; **1 to 3**cm; glabrous, pale white to green color with brown or stramineous nodes	Creeping, rooting at nodes, often forming dense mats. Stem much branched from the base, internode **0.5 to 1**cm long, green to vinaceous, glabrous with sparsely pubescent edges	Slender, often reddish, with sheathing leaves. Simple or sparsely branched, tufted stem up to 20 cm. Stem size is **1 to 3** mm in diameter.
Leaf	Alternate, **5 to 30** cm long length and **1 to 5** cm broad, Lanceolate to ovate-lanceolate, Margin entire, Apex acute or acuminate, leaf sheathing **2 to 5** cm long, Glabrous or sparsely hairy	Alternate, **3 to 6** cm long length and 0.4 to 0.8 cm broad, apex acute-acuminate, base amplexicaul, Oblong-lance-shaped or lance-shaped, Margin wavy. Tip pointed or shortly tapering, densely hairy.	Leaves spiral, 2 to 10 cm long length and 2 to 5 mm broad, Linear-lanceolate to oblong-ovate, Entire Margin Acute or acuminate Apex Leaf Sheathing Size: 1cm long, glabrous or sparsely hairy Surface	Leaves alternate (often appear to be rosette), 3 to 12cm long and 0.1 to 0.2cm broad, with an acute apex. Thread-like, narrowly linear, Folded, Smooth.
Flower	Flowers are larger than other species; terminal or axillary inflorescences are blue or purple in color with 3 petals, 3 fertile stamens, and 3 staminode filaments 4mm long. Grows up to 100cm high, erect terminal, bisexual or male flower, bilaterally symmetrical, ovary	Small, typically blue or purple. solitary in leaf axils Inflorescence 3 petals symmetrical, 3 fertile stamens, and 3 staminodes filaments 3mm long, ovary ellipsoid, green, style 5mm long, stigma papillose, white in color	Terminal or axillary cymes, few-flowered Small, 5-6 mm in diameter, bisexual small, blue or purple flowers; 3 petals, 3 fertile stamens, 3 staminodes filament concolorous with petals, 6mm long ovary obovoid, style white 3mm long, stigma papillose.	Small, blue, or purple. Branched panicle Inflorescence, 3 petals, 3 fertile stamens, 3 staminode filaments 3mm long, bisexual flower, ovary ovoid to ellipsoid, style 4mm, stigma papillose.

	ovoid, green to lilac, style 8mm long, stigma simple.			
Seed	Capsule broadly ovoid, trilocular, seeds two per locule, 2.5 to 4 mm seed size, ovoid to sub-globose, larger than other species, smooth surface.	Capsule oblong-ellipsoid, Capsule, containing numerous small seeds Ovoid to globose, small, surface with fine reticulations, trilocular, 12 seeds per locule, 0.8 to 1mm seed size.	Capsule obovoid globose, Capsule, containing numerous small seeds Ovoid to globose, small, smooth surface, trilocular, 2 seeds per locule, 1.5 mm seed size.	Capsule ellipsoid-sub globose, Capsule containing numerous small seeds Ovoid to globose, small, smooth surface, trilocular, 2 to 8 seeds per locule, 1mm seed size.

Through the use of morphological studies on plants belonging to the *Murdannia* species, this study aims to generate dendrograms (single linkage) and perform correlation analyses. The statistical analyses were carried out using SPSS Version 20. Based on the variations in the morphological data, micromorphological characters were chosen for numerical analysis (Table 1); the selection was made because the micromorphological characters evaluated for the statistical study show variability among different taxa and can be commonly used for taxonomic identification and dendrogram preparation.

Micromorphological characteristics.

A: Habit: 1= Herb, 2= Shrub, 3= Tree, 4= Lianas, 5= Climber

B: Root: 1= Fibrous, 2= Tuberous

C: Leaf Sheath: 1= 1cm Long, 2= more than 1 cm

D: Leaf Arrangement 1= Rosette, 2= Spirally

E: Leaves: 1= Linear Semi -Terete, 2= Linear Oblong, 3= Linear Lanceolate

F: Leaf Apex: 1= Acute, 2= Acuminate

G: Leaf Base: 1=Attenuate, 2=Amplexicaul

H: Leaf Margin 1= Entire, 2= Undulate, 3= Lanuginose

I: Leaf Surface 1= Glabrous, 2= Not Glabrous

J: Flower 1 Unisexual, 2 = Bisexual

K: Inflorescences 1=Terminal Panicles, 2=Panicles, 3=Terminal & Axillary Panicles, 4=Thyrses

L: Flower Color 1= Blue Purple, 2= White Purple, 3= Purple Pink, 4= Orange Yellow

M: Bract: 1= Lanceolate, 2= Ovate to Lanceolate, 3= Elliptic Lanceolate

N: Sepal: 1=Obovate, 2=Unequal, 3=Oblong

O: Petal: 1= Obovate, 2= Orbicular, 3= Rhombic

P: Symmetry: 1 = Bilaterally Symmetrical, 2 = Unequal

Q: Stamen No: 1 = 2, 2 = 3

R: Stamen Filament: 1=Free, 2= Concolorous with Petals

S: Stamen Filament 1=Sparsely Bearded, 2=Not Bearded

T: Anther: 1= Ellipsoid, 2= Elliptic

U: Another 1=Bearded, 2=Not Bearded

V: Staminode No 1 = 2, 2 = 2 = 2=3

W: Staminode 1 = Bearded, 2=Not Bearded

X: Anthorodes 1=Globular, 2=Hastate, 3=Sagittate with Curved Margin

Y: Anthrodes Color 1=Yellow, 2= White, 3=Lemon, 4=Dotted White or Lack Colour

Z: Overy: 1=Ovoid, 2=Ellipsoid, 3=Obevoid

Z1: Style: 1=3mm 2=4mm, 3=5mm 4=8mm

Z2: Style: 1=Enantiostylous, 2=Sigmoid, 3=Incurved

Z3: Stigma: 1=Papillose, 2=Not Papillose

Z4: Capsule: 1=Oblong-Ellipsoid, 2=Ellipsoid Subglobase, 3=Ellipsoid Ovoid, 4=Obovoid Sub Globase

Z5: Capsule Locule: 1=Trilocular, 2=Bilocular

Z6: Capsule Seed Per Locule:1= 6 Seeds 2 Per Locule, 2= 8 Seeds 2 Per Locule, 3= 3=35seeds 12 Per Locule

Z7: Seed Shape: 1=Ovoid-Pyramidal-Deltoid, 2=Ovoid Trapezoidal, 3=Ovoid

Z8: Hilum: 1=Linear, 2=Oblong, 3=Punctiform

Z9: Habitat: 1=Wet Areas, 2= Variable, =Lowland Rice Field, 4=Laterite Soil

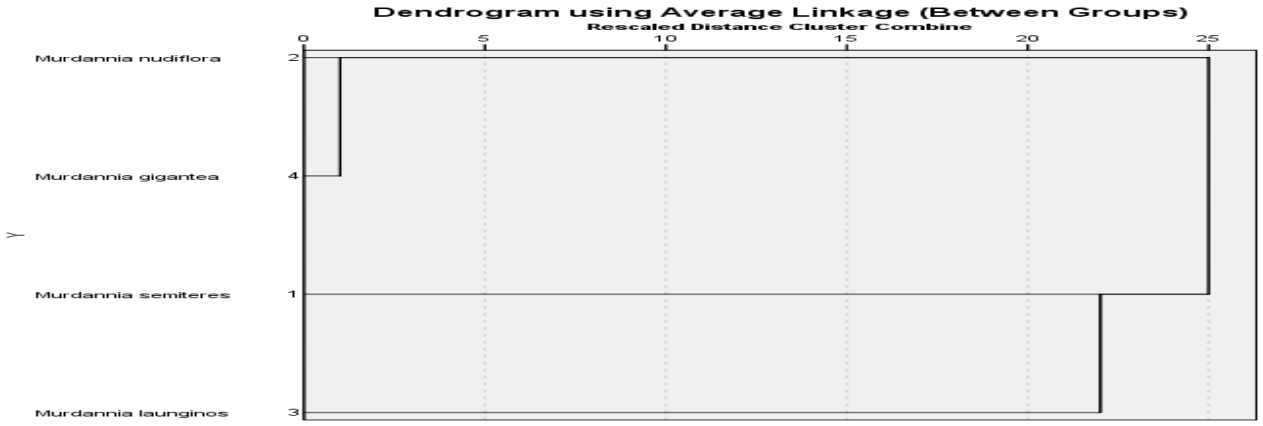
Taxa were coded as 1, 2, 3, 4 and the characters were coded as A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, R, S, T, U, V, W, X, Y, Z, Z1, Z2, Z3, Z4, Z5, Z6, Z7, Z8, Z9. (Table no.2).

Table No.2 - codes for micromorphological characters used for dendrogram and correlation.

Characteristics	*Murdannia semiteres*	*Murdannia nudiflora*	*Murdannia launginosa*	*Murdannia gigantea*
A	1	1	1	1
B	1	1	2	1
C	1	1	2	2
D	1	2	2	2
E	1	3	3	2
F	1	2	2	2
G	1	2	2	1
H	1	2	3	2
I	1	1	1	1
J	2	2	2	2
K	4	2	3	1
L	1	3	4	2
M	2	1	1	3
N	1	1	2	3
O	3	1	2	1
P	1	1	1	1
Q	2	1	2	1
R	2	1	1	1
S	2	1	1	1
T	2	1	1	1
U	2	1	1	1
V	2	2	2	2
W	2	1	1	1
X	3	1	2	1
Y	2	4	1	3
Z	1	3	2	1
Z1	2	1	3	4
Z2	1	3	1	2
Z3	1	1	1	1
Z4	2	4	1	3
Z5	1	1	1	1
Z6	2	1	3	1
Z7	2	3	1	3
Z8	3	1	3	1
Z9	1	3	4	2

A hierarchical cluster analysis using the average linkage (between-groups) method is displayed using the characters mentioned above and the data in Table 2. Dendrogram (Top) The "Dendrogram using Average Linkage (Between Groups)" clearly states the type of display and the clustering method. The vertical axis (Y) lists the names of the objects that are being grouped: *Murdannia gigantea* (labeled as 4), *Murdannia laounginosa* (labeled as 3), *Murdannia nudiflora* (labeled as 2), and *Murdannia semiteres* (labeled as 1).

Fig no.3



Correlation: - using SPSS V.20, conducted a correlation (dissimilarity matrix - Euclidean Distance) between 4 different species of *Murdannia*. Correlation shows how the *Murdannia* species are near to each other or vary from each other based on morphology

Proximity Matrix (Table no.3)

	Euclidean Distance			
	1:Murdannia semiteres	*2:Murdannia nudiflora*	*3:Murdannia launginos*	*4:Murdannia gigantea*
1:Murdannia semiteres	.000	-------	---------	-------
2:Murdannia nudiflora	7.616	.000	----------	---------
3:Murdannia launginos	6.856	7.000	.000	----------
4:Murdannia gigantea	6.928	5.477	6.856	.000

This is a dissimilarity matrix

Based on micromorphological characters, making a bracketed identification key
Identification Key for *Murdannia* Species:

1. * Leaves linear-lanceolate, sheathing at base; flowers blue or purple- *Murdannia nudiflora*

   * Leaves not linear-lanceolate; flowers not blue or purple. Go to 2
2. * Leaves ovate-lanceolate, sheathing at base; flowers pink. – *Murdannia semiteres*

   * Leaves not ovate-lanceolate; flowers not pink. Go to 3
3. Leaves broadly lanceolate to ovate, sessile; flowers yellow. *Murdannia gigantea*

   * Leaves elliptic-lanceolate, petiolate; flowers white or pale pink. – *Murdannia launginosa*

Result and discussion: -

Murdannia species shows the variation based on morphological characters like habit, flower structure, petal and sepal size, stamen number, staminode structure, number and pistil structure. (ref. photoplate of *Murdannia* micromorphological characters)

General Morphology (Panel 1.1): This panel shows the overall habit of the plants, likely including vegetative parts like stems and leaves, and possibly some inflorescences. Differences in growth form, leaf shape and size, and overall appearance can be observed across A, B, C, and D.

Flowers (Panel 1.2): The floral characteristics are crucial for *Murdannia* identification. This panel displays individual flowers, highlighting features such as petal color and shape, the arrangement of stamens and staminodes, and the overall floral symmetry. *Murdannia* flowers typically have three petals, often blue or purple.

Petals (Panel 1.3): Individual petals are shown here, allowing for a closer examination of their shape, size, color, and any surface ornamentation.

Sepals (Panel 1.4): The sepals, which enclose the flower bud, are depicted in this panel. Their shape, size, color, and texture can provide distinguishing features.

Stamens and Staminodes (Panel 1.5): This panel illustrates the fertile stamens (pollen-producing) and sterile staminodes (non-pollen-producing stamens). The number, size, shape, color, and the presence of hairs (bearded filaments) on these structures are key diagnostic characters in *Murdannia*. The genus is characterized by having staminodes opposite the petals and often 3-lobed antherodes on the staminodes.

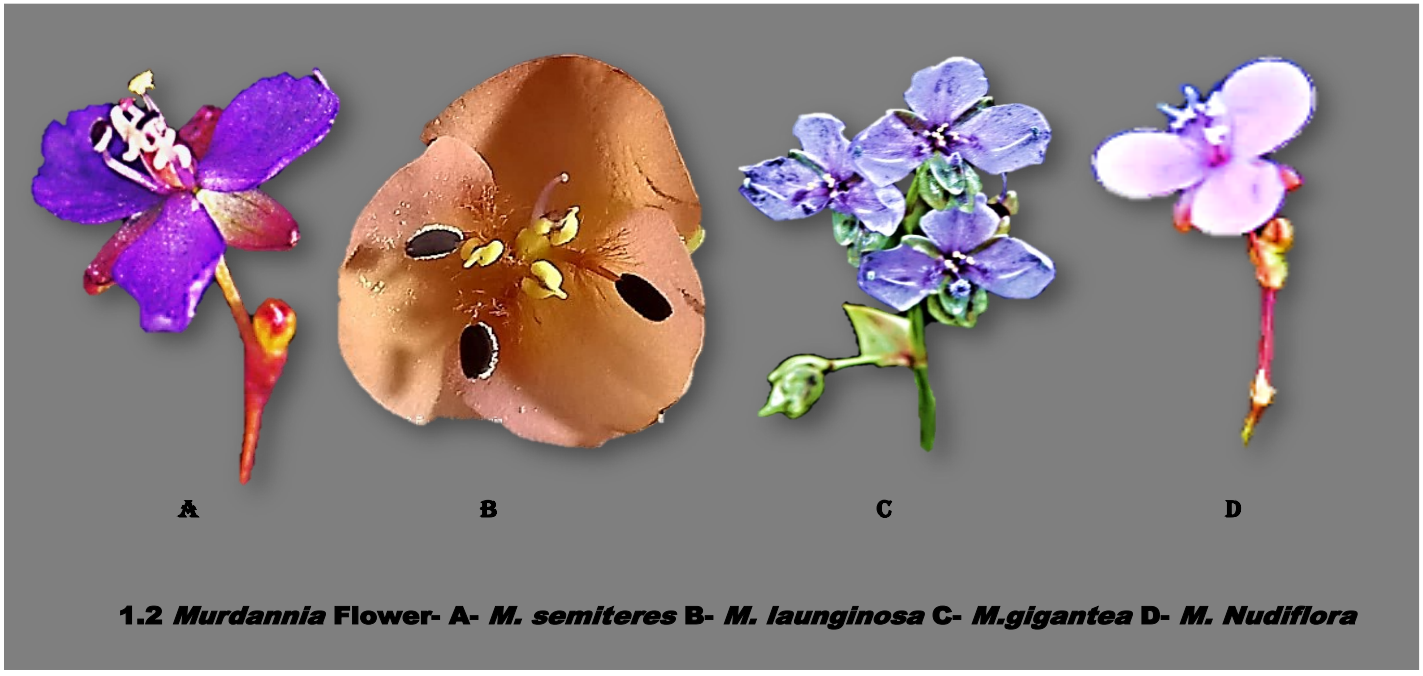
Staminodes (Panel 1.6): A closer view of the staminodes, emphasizing their morphology.

Pistils (Panel 1.7): The pistil, the female reproductive part consisting of the ovary, style, and stigma, is shown here. The

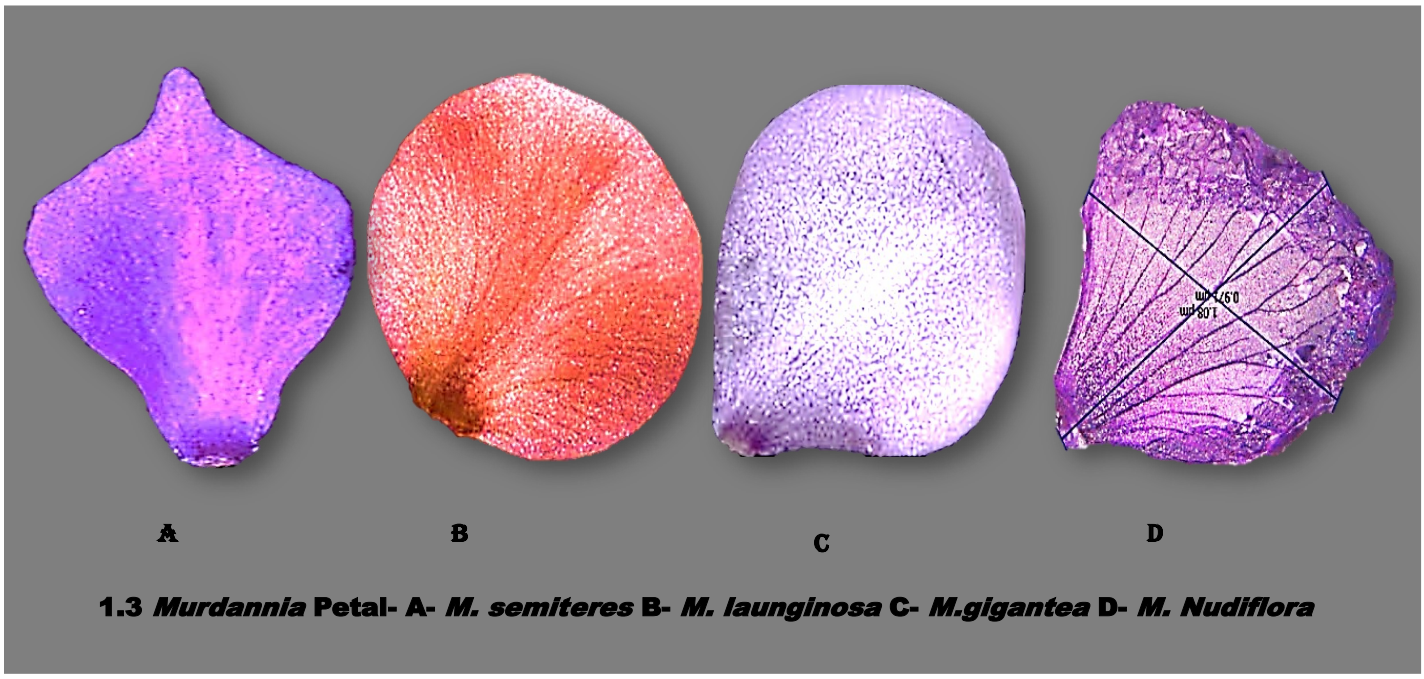
shape and size of the ovary and stigma, as well as the length of the style, can be important taxonomic features

Photoplates of *Murdannia* Micromorphological characters

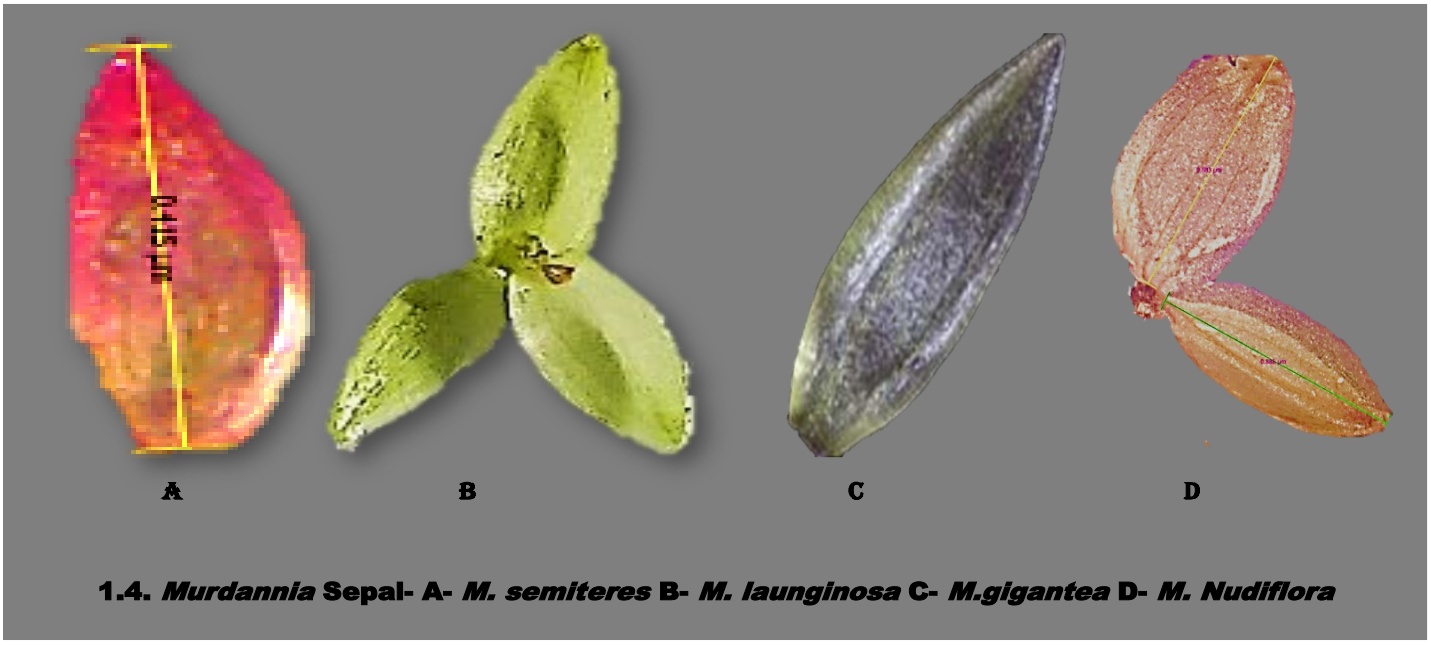
1.1 *Murdannia* habit- A- *M. semiteres* B- *M. launginosa* C- *M.gigantea* D- *M. Nudiflora*



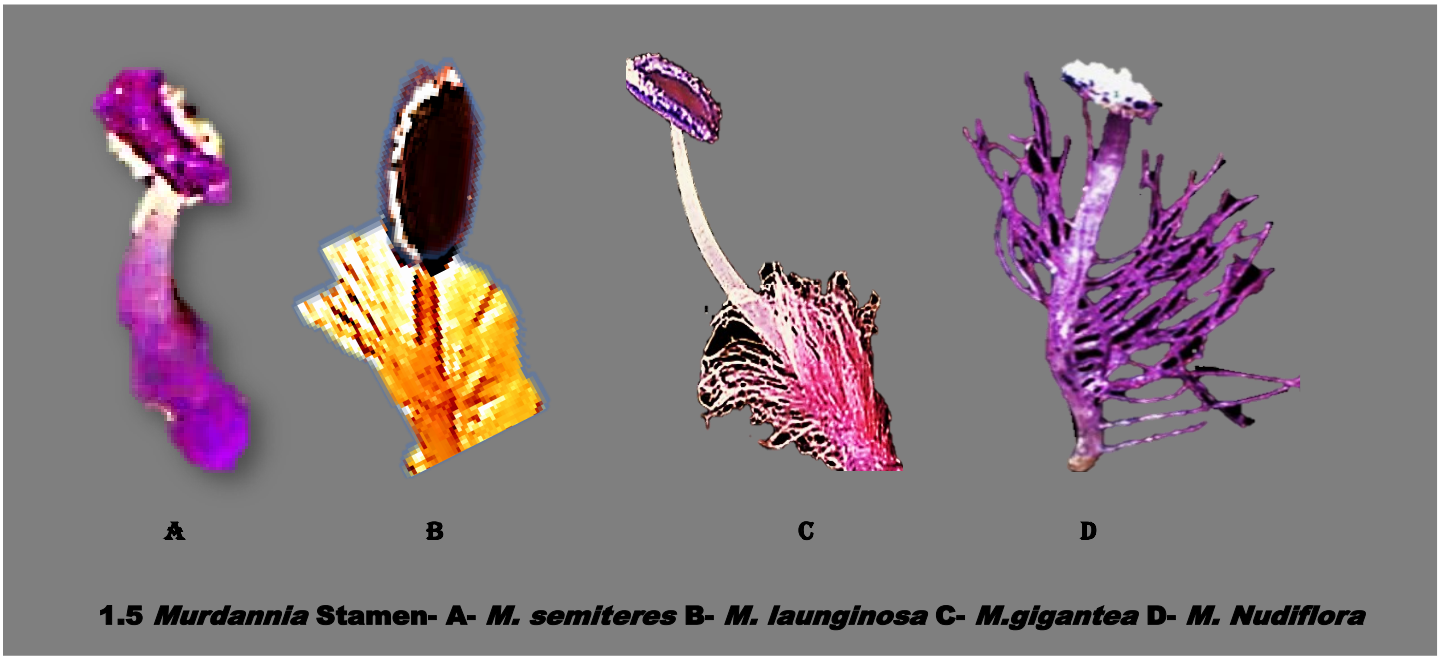
1.2 *Murdannia* Flower- A- *M. semiteres* B- *M. launginosa* C- *M.gigantea* D- *M. Nudiflora*



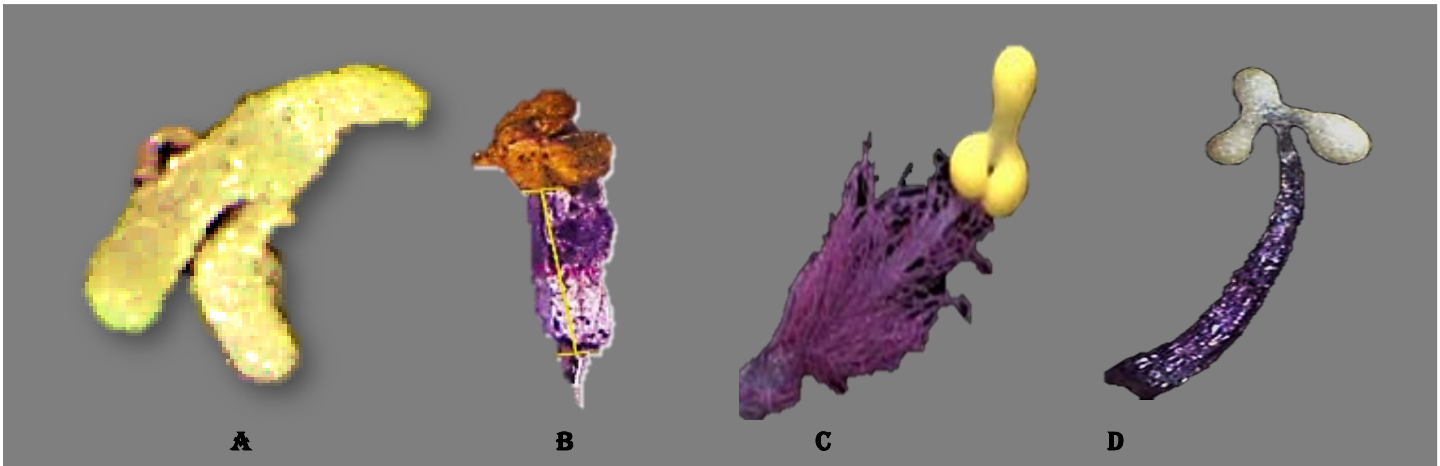
1.3 *Murdannia* Petal- A- *M. semiteres* B- *M. launginosa* C- *M.gigantea* D- *M. Nudiflora*



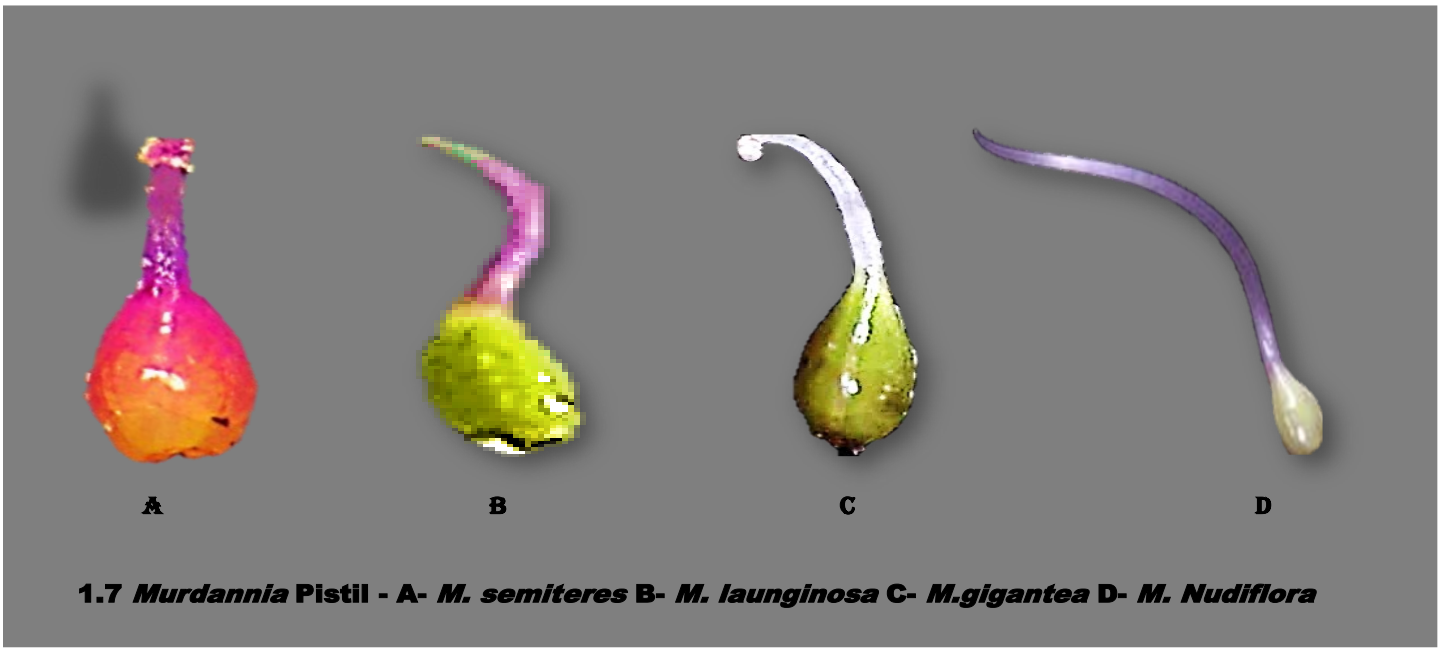
1.4. *Murdannia* Sepal- A- *M. semiteres* B- *M. launginosa* C- *M.gigantea* D- *M. Nudiflora*



1.5 *Murdannia* Stamen- A- *M. semiteres* B- *M. launginosa* C- *M.gigantea* D- *M. Nudiflora*



1.6 *Murdannia* Staminode - A- *M. semiteres* B- *M. launginosa* C- *M.gigantea* D- *M. Nudiflora*



1.7 *Murdannia* Pistil - A- *M. semiteres* B- *M. launginosa* C- *M.gigantea* D- *M. Nudiflora*

A dendrogram and an agglomeration method

As per Fig. 3, the horizontal axis, or rescaled distance cluster combine, shows how far apart or distinct the clusters are. The range of the scale is 0 to 25. The more dissimilar the clusters being merged, the further to the right the merge takes place. Branches (Nodes and Leaves): Each species is represented by the leaves on the left. Clusters that are merging are shown by vertical lines, whereas clusters that are merging are connected by horizontal lines. Based on the variables used for clustering, *Murdannia.nudiflora* (2) and *Murdannia.gigantea* (4) are connected at a very low distance (around 0), suggesting a high degree of similarity. In comparison to the first pair, *Murdannia.Semiteres* (1) and *Murdannia.laounginosa* (3) are merged at a greater distance (about 20), indicating a lower degree of resemblance. At an even greater distance (about 25), the cluster created by (*Murdannia.nudiflora, Murdannia.gigantea*) later merges with the cluster formed by (*Murdannia.semiteres, Murdannia.laounginosa*), suggesting that the two original pairs of species are not very similar to one another. With *Murdannia nudiflora* and *Murdannia gigantea* being the most closely related, followed by a cluster of *Murdannia semiteres* and *Murdannia laounginosa*, which are the most different from one another, the dendrogram graphically depicts a hierarchical relationship.

Euclidean Distance proximity matrix

As per Table No.3, the matrix shows the Euclidean distances between pairs of the four species. The diagonal elements are 0.000, indicating zero distance between a species and itself. The off-diagonal elements represent the pairwise Euclidean distances:

Distance between *Murdannia semiteres* and *Murdannia nudiflora*: 7.616

Distance between *Murdannia semiteres* and *Murdannia lanceolata*: 6.856

Distance between *Murdannia semiteres* and *Murdannia gigantea*: 6.928

Distance between *Murdannia nudiflora* and *Murdannia lanceolata*: 7.000

Distance between *Murdannia nudiflora* and *Murdannia gigantea*: 5.477

Distance between *Murdannia lanceolata* and *Murdannia gigantea*: 6.856

Larger values in this matrix indicate greater dissimilarity (difference) between the species based on whatever features were used to calculate the Euclidean distances.

This study elucidates the distinctions among *Murdannia* species. The descriptions provided will aid future researchers in their efforts to study and identify plants within the *Murdannia* group. Methodologies such as cluster analysis and correlation analysis significantly enhance our understanding of the relationships and patterns present within plant populations.

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