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Numerical Taxonomy of some members of Acanthoideae *sensu* Scotland and Vollesen (Acanthaceae) from North 24 Parganas District, West Bengal, India based on Gross Morphology and Pollen Characters

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Abstract

Some members of Acanthoideae sensu Scotland and Vollesen from North 24 Parganas District, West Bengal, India have been classified following numerical taxonomic approach. Numerical taxonomic approach on Acanthoideae is rare. A total of 64 binary characters obtained from gross morphology and pollen morphology have been used for cluster analysis. Cluster analysis result shows remarkable differences of sub-tribal classification of Acanthoideae with that of previous phylogentic schemes. Besides Barleriinae no precise distinctions of Ruelliinae, Justiciinae and Andrographiinae are observed. Interestingly Phaulopsis-Rungia phenon has scored higher similarity value than the phenon of two species of same genus, i.e. Justicia adhatoda–J. gendarussa phenon. Finally two separate artificial keys are provided for identification purpose.

Keywords: Acanthoideae, numerical taxonomy, gross morphology, pollen morphology.

Introduction

Acanthaceae is a large pan-tropic family of herbs and shrubs. This family comprises a large number of species ranges from 2500-2600[1,2] to 3175[3]. The family divided into subfamilies Mendoncioideae, Thunbergioideae, Nelsonioideae and Acanthoideae[4]. Among these subfamilies only Acanthoideae possesses retinaculate fruits. Acanthoideae again divided into Contortae and Imbricatae- based on corolla aestivation. Acanthoideae alone contains more than 95 percent species of Acanthaceae. Explosive and retinaculate fruits are generally accepted as synapomorphies of monophyletic Acanthoideae[5]. Bremekamp[6] retained only the genera with retinaculate fruits under Acanthaceae and excluded all others. He further subdivides Acanthaceae into Acanthoideae, based on monothecate anthers and colpate pollens, and Ruelloideae, based on presence of cystoliths and articulated stem.

Molecular systematics on Acanthaceae[7-10] do not support the separation of Nelsonioideae, *Thunbergia* and *Mendoncia* from Acanthaceae as proposed by Bremekamp[6]. Scotland *et al.*[8] placed *Thunbergia* (Thunbergioideae) and *Elytraria* (Nelsonioideae) as sister to Acanthoideae *sensu* Lindau[4]. McDade[10] placed *Mendoncia* (Mendoncioideae) as sister to *Thunbergia*.

Pollens of Acanthaceae is tricolpate, triporate, diporate , pantoporate, or inaperturate[11]. Lindue[4] classified pollens of Acanthaceae into 11 types. Different authors[12-15] showed the importance of pollen characters for taxonomic treatments at generic and species levels.

Phylogenetic relationships among the members of Acanthaceae were demonstrated by several authors, while phenetic relationships or overall similarities got less attention although several taxonomists still rely on phenetics. Since its resurgence by Sneath and Sokal[16] it was widely applied in the field of biology to determine the natural relationships among taxa. Sneath and Sokal[16] suggested that the groups of OTUs (Operational Taxonomic Units) established in numerical taxonomy by cluster analysis method are not fully synonymous with taxa. They used the term 'Phenon' for such groups which can be of any hierarchic rank. Phenons were represented with a number indicating the level of resemblance at which they are formed. Another important aspect of numerical taxonomy is consideration of large number of characters for better natural relationships among OTUs[17].

With this above background knowledge the present study is conducted to elucidate the natural relationships through numerical taxonomic method among some members of the subfamily Acanthoideae *sensu* Scotland and Vollesen[14] available in North 24 Parganas District of West Bengal, India. Gross morphological and pollen characters have been considered for study.

Material and Methods

Operational Taxonomic Units (OTUs): Characters for numerical analysis were obtained from freshly collected plants. A list of taxa (OTUs) is given in table-1. Identification of the OTUs was confirmed following standard taxonomic literature. Correct names of OTUs were determined following Scotland

and Vollesen[14]. Herbarium specimens of OTUs were prepared and deposited at Barasat Govt. College Herbarium for future reference.

Gross morphology: Gross morphology was obtained from freshly collected worked out specimens. Freehand drawings were made for each specimen and descriptions were carried out following Harris and Harris[18].

Pollen morphology: Pollens were acetolyzed following Edtman's technique. Descriptions were made following Hesse *et al.*[19]. Pollen pictures were captured under Carl Zeiss Axio Lab.A1 Microscope fitted with camera. Pollen photos are given in figure-1.

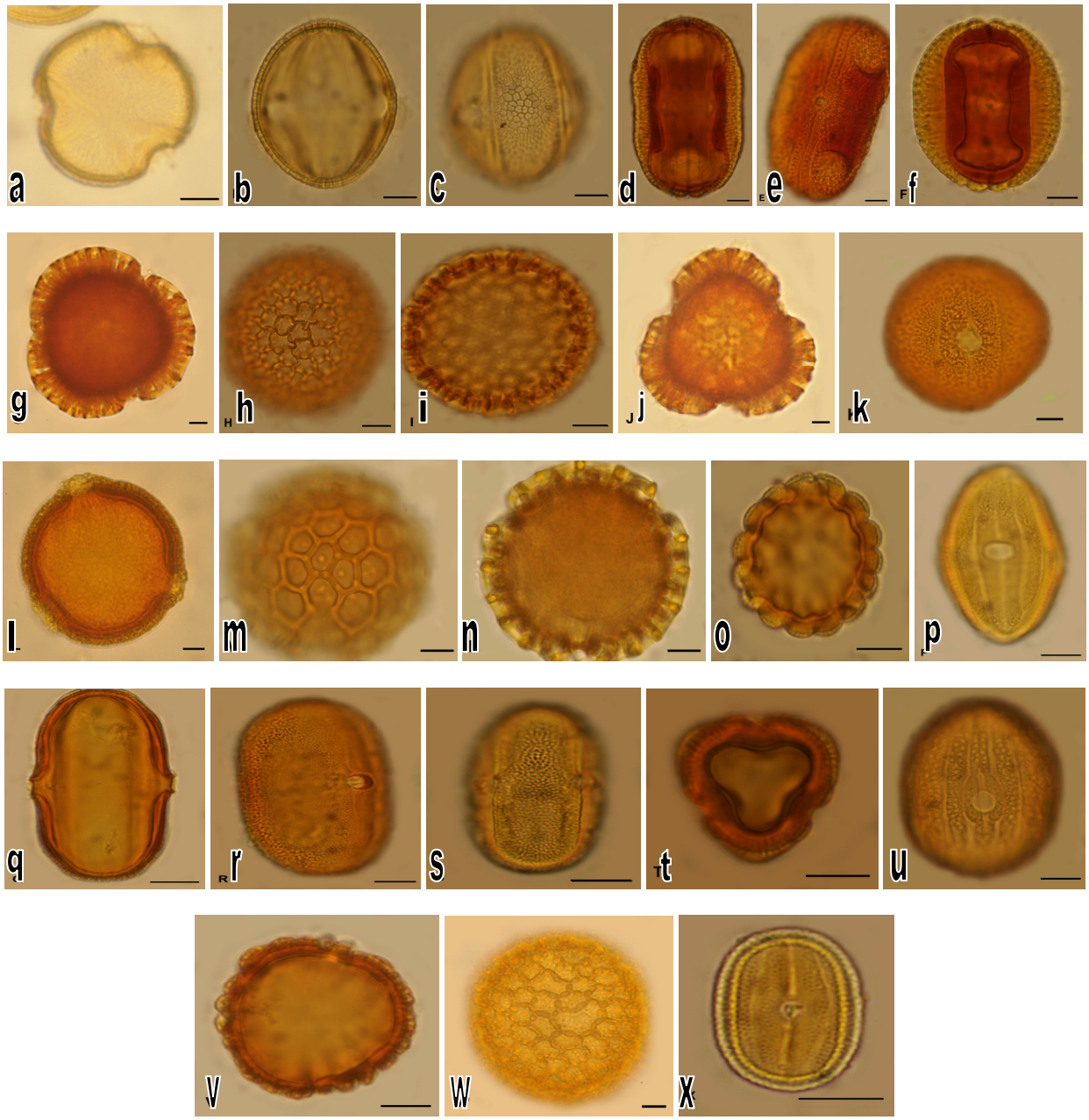

Figure-1

Pollens of studied species of Acanthoideae. a – c: Andrographis paniculata; d – f: Asystasia gangetica; g: Barleria cristata; h – j: Barleria strigosa; k – l: Ecbolium viride; m – n: Eranthemum pulchellum; o – p: Hemigraphis hirta; q – r: Justicia adhatoda; s – t: Justicia gendarussa; u – v: Phaulopsis parviflora; w: Ruellia tuberose; x: Rungia pectinata. Bars- 10 µm

Character coding: Characters were obtained from vegetative parts, floral parts and pollens. All total 64 characters were selected of which 57 from vegetative and floral parts and 7 from pollens. Binary character coding was employed for each character. A list of character and binary coding was given in table-2. Unavailable characters were presented as not assigned (NA).

Cluster analysis: Data matrix (table-3) obtained from character coding were used for cluster analyses to draw phenetic relationship among OTUs. Cluster analysis of OTUs was conducted by applying Jaccard's similarity coefficient or Jaccard Index (JI) and UPGMA methods in past software[20].

Results and Discussion

Two primary phenons are formed by cluster analysis at lower JI (nearly 0.35), one contains two species of *Barleria* and rest OTUs fall under the second (figure-2). Near 0.4-Phenon line three phenons are observed. The 0.5- and 0.6-phenon lines show five and ten phenons respectively. Except two species of *Barleria* all OTUs are segregated at 0.7-phenon line. So, the highest similarity is observed between *Barleria cristata* and *B. stigosa* near JI= 0.75. The next highest similarity is appeared between *Phaulopsis parviflora* and *Rungia pectinata* near JI= 0.65 followed by similarity between *Justicia adhatoda* and *Justicia gendarussa* near JI= 0.60. *Ruellia tuberosa* and *Hemigraphis hirta* also show resemblance near JI= 0.60. Two major phenons are observed above 0.4-Phenon line. One ended with *Ecbolium viride* and *Eranthemum pulchellum* and another one ended with eight OTUs' cluster, which further divides into three OTUs bearing Phenon and five OTUs bearing Phenon.

Table-1
List of OTUs collected from North 24 Parganas District (23º15' N to 22º11' N & 89º5' E to 88º20' E)

Sl. No.	OTU	Abbreviations	Collection Number	Collection places
1	*Andrographis paniculata* (Burm.f.) Nees	Apn	AC-003	Barasat, 24 Pgs (N), WB
2	*Asystasia gangetica* (L.) T.Anderson	Agn	AC-009	Barrackpore, 24 Pgs (N), WB
3	*Barleria cristata* L.	Bcr	AC-004	Barasat, 24 Pgs (N), WB
4	*Barleria strigosa* Willd.	Bsg	AC-014	Taki, 24 Pgs (N), WB
5	*Ecbolium viride* (Forssk.) Alston	Evr	AC-016	Taki, 24 Pgs (N), WB
6	*Eranthemum pulchellum* Andrews	Epc	AC-012	Duttapukur, 24 Pgs (N), WB
7	*Hemigraphis hirta* (Vahl) T.Anderson	Hhr	AC-006	Barasat, 24 Pgs (N), WB
8	*Justicia adhatoda* L.	Jah	AC-010	Barasat, 24 Pgs (N), WB
9	*Justicia gendarussa* Burm.f.	Jgd	AC-002	Barasat, 24 Pgs (N), WB
10	*Phaulopsis parviflora* Willd.	Ppr	AC-005	Barasat, 24 Pgs (N), WB
11	*Ruellia tuberosa* L.	Rtu	AC-001	Taki, 24 Pgs (N), WB
12	*Rungia pectinata* (L.) Nees	Rpc	AC-011	Birati, 24 Pgs (N), WB

Table-2
List of Characters and binary character states used in cluster analysis

Sl. No.	Characters	Character states (0/1)
1.	Plant:	Perennial (0), Annual (1)
2.	Plant habit:	Shrub (0), Herb (1)
3.	Stem:	Frutescent/Sub-frutescent (0), Herbaceous (1)
4.	Stem surface:	Glabrous (0), Pubescent (1)
5.	Stem shape:	Terete (0), Tetrangular (1)
6.	Leaf symmetry:	Symmetric (0), Asymmetric (1)
7.	Leaf petiole:	Sessile (0), Petiolate (1)
8.	Lamina shape:	Ovate/ Widely ovate (0), Lanceolate/ Elliptic (1)
9.	Lamina apex:	Narrowly acute/ Acute (0), Obtuse (1)
10.	Lamina base:	Narrowly cuneate/ Cuneate (0), Obtuse (1)
11.	Lamina margin:	Entire (0), Serrate (1)
12.	Lamina surface:	Glabrous (0), Pubescent (1)
13.	Leaf vein type:	Simple crespendodromous (0), Semi-crespendodromous (1)
14.	2º vein spacing of leaf:	Uniform (0), Irregular/ Increasing toward base (1)
15.	Inflorescence type:	Thyrse (0), Other than thyrse (1)
16.	Inflorescence:	Receme/ Spike (0), Dichasial cyme (1)
17.	Flowers position on inflorescence axis:	One-sided (0), All sided (1)

Sl. No.	Characters	Character states (0/1)
18.	Flowers density on inflorescence axis:	Flowers loosely arrange on inflorescence axis (0), Flowers densely arrange on inflorescence axis (1)
19.	Bract marginal growth:	Armed (0), Unarmed (1)
20.	Bract shape:	Ovate/ Widely Ovate/ Elliptic (0), Linear/ Lanceolate (1)
21.	Bract apex:	Acuminate/ Caudate (0), Acute/ Narrowly acute/ Obtuse (1)
22.	Bract margin	Entire (0), Serrate (1)
23.	Bract surface	Pubescent (0), Tomentose (1)
24.	Bract marginal special growth	Non-scarious margin (0), Scarious margin (1)
25.	Bractlets	Absent (0), Present (1)
26.	Flower symmetry	Actinomorphic (0), Zygomorphic (1)
27.	Flower stalk	Sessile/ Sub-sessile (0), Pedicellate (1)
28.	Calyx symmetry	Actinomorphic (0), Zygomorphic (1)
29.	Calyx lobes union	Connate at base (0), Connate at middle (1)
30.	Calyx lobes number	4 in number (0), 5 in number (1)
31.	Calyx lobes surface	Glabrous (0), Pubescent (1)
32.	Calyx lobes margin	Entire (0), Serrate (1)
33.	Calyx lobes surface hair type	Non-tomentose (0), Tomentose (1)
34.	Calyx lobes shape	Narrowly elliptic/ Linear (0), Lanceolate/ Ovate/ Lance-ovate (1)
35.	Corolla symmetry	Actinomorphic (0), Zygomorphic (1)
36.	Corolla type	Non 2-lipped corolla (1), 2-lipped corolla (0)
37.	2-lipped corolla type	Upper lip non-galeate (0), Upper lip galeate (1)
38.	Corolla aestivation	Imbricate (0), Twisted (1)
39.	Corolla tube length	Short (0), Long (1)
40.	Corolla tube bending	Straight (0), Bent (1)
41.	Corolla colour	White (0), Colourful (1)
42.	Corolla with purple spots	Absent (0), Present (1)
43.	Stamen Number	2 in number (0), 4 in number (1)
44.	Stamens-corolla adhesion	Adnate near the top or middle of the corolla tube (0), Adnate at the base of corolla tube (1)
45.	Anther cell shape	Oblong (0), Other than oblong (1)
46.	Didynamous stamens (4 in number) character	All fertile (0), Two rudimentary and two functional (1)
47.	Anther attachment with filament	Basifixed (0), Dorsifixed (1)
48.	Anther lobes attachment	Discrete (0), United (1)
49.	Anther base	Anther base without any appendage (0), Anther base with basal appendage (1)
50.	Stamen insertion	Cryptantherous (0), Phanerantherous (1)
51.	Filament length	Short (0), Long (1)
52.	Filament surface	Non-ciliated (0), Ciliated (1)
53.	Ovary surface	Glabrous (0), pubescent (1)
54.	Ovule number per chamber	2 in each chamber (0), 3-6 in each chamber (1)
55.	Style surface	Non-ciliated (0), Ciliated (1)
56.	Stigma character	Entire (0), 2-lobed or 2-fid (1)
57.	Capsule length	Short (0), Long/ Linear (1)
58.	Pollen type	Homocolpate (0), Heterocolpate (1)
59.	Pollen aperture number	2 to 3 in number (0), Many (1)
60.	Pollen aperture type	Colporate (0), Non-colporate (1)
61.	Pollen aperture type	Colporoidate (0), Colpoidorate (1)
62.	Pollen shape	Oblet-spheroidal (0), Other shapes (1)
63.	Pollen shape	Spheroidal (0), Prolate / Sub-prolate/ Prolate spheroidal (1)
64.	Pollen wall sculpturing	Reticulate/ Finely reticulate/ Coarsely reticulate (0), Lophoreticulate (1)

Table-3
Data matrix employed in the cluster analysis

OTUs	Characters																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Apn	1	1	1	0	0	0	0	1	0	0	0	0	0	1	1	0	1	0	0	1	1	1
Agn	1	1	1	1	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	1	1
Bcr	0	0	0	1	1	0	1	0	0	0	0	1	0	1	1	0	1	1	1	0	1	NA
Bsg	0	0	0	1	1	1	1	0	0	0	1	0	0	1	1	0	1	1	1	0	1	NA
Evr	0	0	0	1	0	0	1	0	0	0	0	1	1	1	1	0	1	1	0	1	1	1
Epc	0	0	0	1	1	0	1	0	0	0	0	1	1	0	1	0	1	1	0	0	0	0
Hhr	1	1	1	1	0	0	1	0	1	1	1	1	0	0	1	0	1	1	0	0	1	1
Jah	0	0	0	1	0	0	1	0	0	0	0	1	0	0	0	NA	1	1	0	0	1	1
Jgd	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	1	0	1
Ppr	1	1	1	1	1	1	1	0	0	0	0	1	0	1	1	0	0	1	0	0	1	0
Rtu	1	1	1	1	1	0	1	0	1	0	0	0	0	0	1	1	1	0	0	1	1	1
Rpc	1	1	1	1	0	1	1	1	1	0	0	0	0	1	1	0	0	1	0	1	1	0

OTUs	Characters																					
	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
Apn	0	0	1	1	1	0	0	1	1	1	0	1	1	1	0	0	0	0	0	1	0	1
Agn	0	0	1	1	0	0	0	1	1	1	0	0	1	0	NA	0	0	1	0	0	1	0
Bcr	0	0	1	1	0	1	0	0	1	0	0	0	1	0	NA	0	1	0	0	0	1	0
Bsg	0	0	1	1	0	1	0	0	1	0	0	1	1	0	NA	0	1	0	1	0	1	0
Evr	0	0	1	1	0	0	0	1	1	0	0	1	1	1	0	0	1	0	1	0	0	0
Epc	0	0	1	0	1	0	1	1	0	0	0	1	0	0	NA	1	1	1	1	0	0	0
Hhr	1	0	1	1	0	1	0	1	1	0	1	1	1	0	NA	1	1	0	1	0	1	0
Jah	0	0	1	1	0	0	0	1	1	1	0	0	1	1	1	0	0	0	0	1	0	0
Jgd	0	0	1	1	0	0	0	1	1	1	0	0	1	1	1	0	0	0	0	1	0	0
Ppr	0	0	1	1	0	0	0	1	1	0	0	0	1	0	NA	1	0	0	0	0	1	0
Rtu	0	0	1	1	0	0	0	1	1	1	0	0	1	0	NA	1	1	0	1	0	1	0
Rpc	0	1	1	1	0	0	0	1	1	0	0	0	1	1	0	0	0	0	1	1	0	0

OTUs	Characters																			
	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64
Apn	0	NA	0	1	0	1	1	1	1	1	1	1	1	0	0	0	NA	1	1	0
Agn	0	0	0	1	0	0	0	1	1	0	1	1	0	0	0	1	1	1	1	0
Bcr	0	1	1	1	0	0	1	1	0	0	0	1	0	0	0	0	NA	1	0	1
Bsg	0	1	1	1	0	0	1	1	0	0	1	0	0	0	0	0	NA	1	0	1
Evr	0	NA	0	1	0	1	0	0	1	0	1	1	0	1	1	1	1	0	NA	0
Epc	0	NA	0	1	0	1	0	0	1	0	1	1	0	0	0	1	0	1	0	1
Hhr	0	0	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0
Jah	1	NA	0	1	0	0	1	0	1	0	1	0	0	1	0	1	1	1	1	0
Jgd	1	NA	0	0	1	0	0	1	1	0	1	1	0	0	0	1	1	1	1	0
Ppr	0	0	0	1	0	0	0	0	0	0	1	1	0	1	1	1	1	1	1	1
Rtu	0	0	0	1	0	0	0	0	0	1	1	0	1	0	0	1	1	1	1	1
Rpc	0	NA	1	1	0	0	0	0	0	0	0	1	0	1	1	1	1	1	1	0

Acanthoideae is a eurypalynous taxon. Pollen morphological variations are observed even within species of a genus. Carine and Scotlnad[13] demonstrated that the genus *Strobilanthes* showed diverse type of pollen morphology. Therefore pollen characters are important for taxonomic delimitation of the members of Acanthoideae and useful for taxonomic key preparation up to species. Both Lindau[4] and Bremekamp[6] used pollen characters for classification of Acanthoideae. Similarly the aestivation pattern within Acanthoideae got importance for taxonomic delimitation[10]. Besides, other gross morphological features have been utilized in the taxonomy of Acanthoideae Therefore in the present numerical taxonomic study the use of gross morphological characters with special emphasis on pollen characters is appropriate.

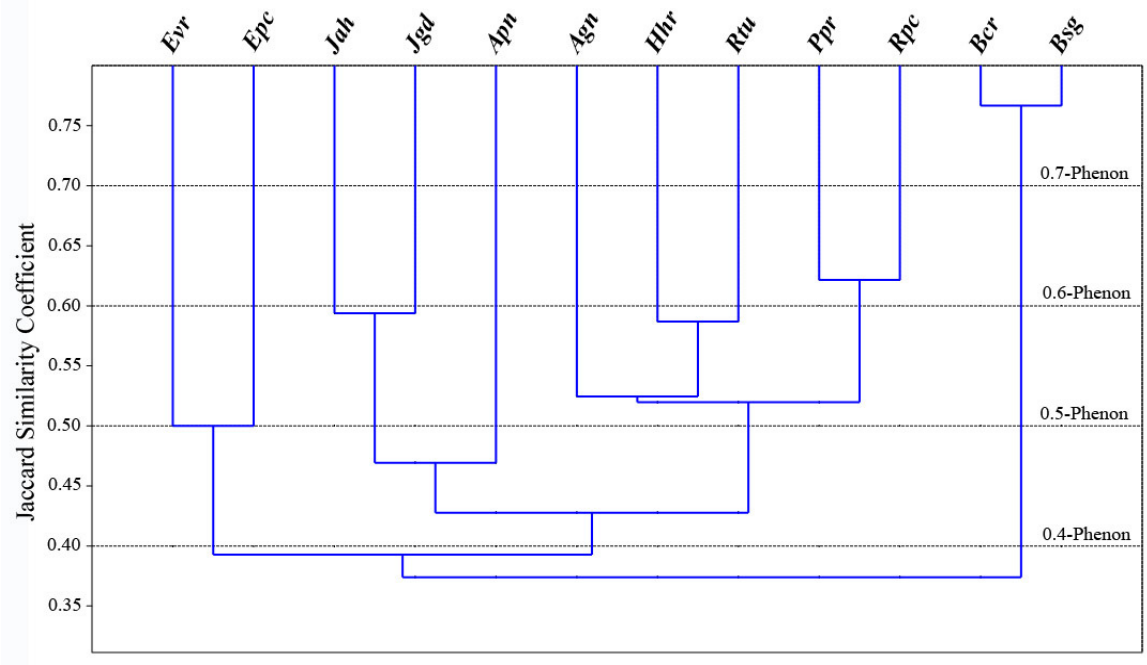

Figure-2
UPGMA dendrogram showing the resemblance among 12 species of Acanthoideae

The selected OTUs represent 12 species under 10 genera of Acanthoideae. Two species of *Barleria* show the highest resemblance near Jaccard Index (JI) 0.75. In contrast, two species of *Justicia* show much lower level of resemblance near JI 0.6. Interestingly even being the members of two distinct genera, *P. parviflora* and *R. pectinata*, form a phenon with higher resemblance than *J. adhatoda* - *J. gendarussa* phenon. *P. parviflora* has been treated under sub-tribe Ruelliinae *sensu* Scotnad and Vollesen and *Rungia* under Justiciinae *sensu* Scotnad and Vollesen based on phylogenetic evaluations.

The phenetic relationships among the taxa of Acanthoideae are varied with the result of cladistic analyses on Acanthaceae. Scotland and Vollesen [14] divided tribe Ruellieae into four sub-tribes, *viz.* Ruelliinae, Justiciinae, Andrographiinae and Barleriinae. *Eranthemus*, *Hemigraphis*, *Paulopsis* and *Ruellia* fall under Ruelliinae; *Asystasia*, *Ecbolium*, *Justicia* and *Rungia* fall under Justiciinae; *Andrographis* falls under Andrographiinae and *Barleria* falls under Barleriinae. But the dedrogram does not able to show such type of clear-cut distinction of tribes. Only near 0.4 Phenon line *Barleria* segregated from rest of the studied taxa. So the sub-tribe Barleriinae is supported. The closeness of *Ruellia* and *Hemigarphis* supported the placement of these two genera under Ruelliinae, but the grouping of *Eranthamus* with *Ecbolium* (Justiciinae) and *Phaulopsis* with *Rungia* (Justiciinae) are ambiguous. *Andrographis* phenon is close to *Justicia*.

Conclusion

This study indicates that the phenetic relationship among members of Acanthoideae is distinct with that of the result of phylogenetic analysis. Now it is our discretion that which method should we follow.

Two separate keys were prepared for identification purpose. Gross morphological characters key and pollen characters keys are given in table 4 and 5.

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Table-4
Artificial key to the specimens based on gross morphology

1	Herb; leaf asymmetric	*Phaulopsis parviflora*
1	Shrub; leaf symmetric	2
2	Inflorescence one-sided spike or raceme	3
2	Inflorescence other than one sided spike	4
3	Rachis short, bract orbicular-lanceolate; corolla 2-lipped, stamens 2	*Rungia parviflora*
3	Rachis long, bract triangular; corolla 5-lobed, stamens 4	*Asystasia gangetica*
4	Shrub; inflorescence thyrse; Flower white with purplish spots, stamens 2	*Justicia adhatoda*
4	Herb or shrub; inflorescence spike; flower blue, stamens 4	5
5	Leaf margin serrate; ovule 3-8 in each cell	*Hemigraphis hirta*
5	Leaf margin entire; ovule 2 in each cell	6
6	Corolla 2-lipped, corolla tube slender	7
6	Corolla 5 lobed, corolla tube funnel shaped	8
7	Upper lip and corolla tube narrow, flower light blue	*Ecbolium viride*
7	Upper lip and corolla tube broad; flower purplish	9
8	Calyx-4, unequal; stamen exserted, 2 fertile and 2 rudimentary anthers	10
8	Calyx-5, equal; stamen inserted; 4 fertile anthers	11
9	Anther cells discrete	*Justicia gendarussa*
9	Anther cells united	*Andrographis paniculata*
10	Flower purplish in colour	*Berleria strigosa*
10	Flower white in colour	*Barleria cristata*
11	Stamens 4; ovule 8-10 in each cell	*Ruellia tuberose*
11	Stamens 2; ovule 2 in each cell	*Eranthemum pulchellum*

Table-5
Artificial key to the specimens based on pollen morphology

1	Pollen colporate	2
1	Pollen other than colporate	3
2	Pollen shape spheroidal	4
2	Pollen sub-prolate	*Andrographis paniculata*
3	Pollen heterocolpate	5
3	Pollen homocolpate	6
4	Exine sculpturing finely lophoreticulate	*Barleria cristata*
4	Exine sculpturing lophoreticulate	*Barleria strigosa*
5	Aperture number 2-3	*Justicia adhatoda*
5	Aperture number more than 5	7
6	Exine sculpturing reticulate	8
6	Exine sculpturing lophoreticulate	9
7	Pollen shape oblate-prolate	*Ecbolium viride*
7	Pollen shape prolate or sub-prolate	10
8	Exine sculpturing coarsely reticulate, PA= 61.79 -83.5 µm	*Asystasia gangetica*
8	Exine sculpturing finely reticulate, PA= 30.06 – 41.75 µm	*Justicia gendarussa*
9	Pollen coporoidate, spheroidal in shape, exine c. 9.35 µm thick	*Eranthemum pulchellum*
9	Pollen colpoidorate, prolate-spheroidal in shape, Exine c. 5.84 µm thick	*Ruellia tuberose*
10	Aperture number ≥ 10	*Hemigraphis hirta*
10	Aperture number < 10	11
11	PA= 41.75 – 50.10 µm	*Phaulopsis parviflora*
11	PA= 18.37 – 28.39 µm	*Rungia pectinata*

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