

LEAF ARCHITECTURE AND ITS TAXONOMIC SIGNIFICANCE WITHIN THE GENUS *LYCIUM* L.

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ABSTRACT

Leaf architecture and micromorphology of leaves was studied for 6 taxa of the genus *Lycium* of the family Solanaceae viz., *L. depressum* Stocks *subsp. angustifolium* Schoenbeck-Temesy, *L. ruthenicum* Murray, *L. edgeworthii* Dunal, *L. makranicum* Schoenbeck-Temesy, *L. dasystemum* Pojark, and *L. europaeum* auctt. non L. by using Stereo-microscope, compound microscope and scanning electron microscope. Leaves were alternate, having petiole. Lamina shapes were varying but their margin was constantly same i.e. entire. Amongst all of the taxa, *L. depressum* showed tomentose type of indumentum. Variations in the leaf apices and bases and the vein categories i.e. 1°, 2°, and 3° vein categories were also observed. There were only two types of sunken stomata i.e. anisocytic and anomocytic. All of these characters were analyzed numerically which supports the affiliation of allied taxa.

Key-words: Leaf architecture, micromorphology, genus *Lycium*.

INTRODUCTION

The genus *Lycium* is a member of family Solanaceae, the nightshade family. It comprised of more than 90 species in the world (Kazbekovna *et al.*, 2018). In Pakistan, total 9 species of genus *Lycium* are found, named as, *L. ruthenicum*, *L. makranicum*, *L. chinense*, *L. shawii*, *L. dasystemum*, *L. edgeworthii* and *L. depressum*.

Leaf architecture refers to the placement and form of elements constituting the outward venation pattern, marginal configuration, leaf shape and gland position. Taia (2005) opined that leaf epidermal morphology and ornamentation played a key diagnostic role in phylogeny of species. Similarly, many workers studied stomata and trichomes and used the data for taxonomic delimitation of different taxa (Leelavathi, 1980; Gill *et al.*, 1982; Khalik, 2005; Shaheen *et al.*, 2009; Riaz & Abid, 2021). Regarding to the leaf architecture within family Solanaceae, there were various reports available such as; Ahmad (1963), Jouregui *et al.* (2001), Ibrahim *et al.* (2016). Hitchcock (1932) studied the vesture, cuticular sculpt, and stoma types of *Lycium* species. But he did not correlated the data taxonomically. Later Metcalfe & Chalk (1950) reported that the leaves of Solanaceae are usually dorsiventral and rarely some species isobilateral leaves were also reported. Similarly, Inamdar and Murthy (1981) studied the leaf architecture of twelve species of the Solanaceae; they found no correlation between the areole size and the number of vein endings in the leaves. Adedeji *et al.* (2007) studied 9 species belonging to 4 genera of the family Solanaceae for their trichomes from Nigeria and utilized the data for taxonomic delimitation of taxa within the family. So, many workers reaffirmed that micromorphological features of plants could be exploited in the biosystematics.

Moreover, multivariate techniques permit standardization of multiple types of information of a set of characteristics (Podani and Schmera, 2006). The most widely used methods are principal components and canonical variable analysis, as well as clustering methods (Blackmore, 1981; Diggs, 1987; Ather *et al.*, 2013; Kanwal *et al.*, 2015). The principle of clustering methods is to represent uniformity and heterogeneity among groups. These methods depend on estimates of dissimilarity measures derived from discrete and continuous variables. Several workers have given the importance to numerical taxonomic method in plants classification and delimitation.

Regarding to the leaf architecture of the genus *Lycium*. There is no detailed report available from Pakistan. In present studies 6 species of the genus were studied for their leaf architecture. The data obtained was also analysed numerically to find out the relationship among species.

MATERIALS AND METHOD

Leaves sample of 6 taxa of genus *Lycium* of family Solanaceae were collected from fresh plant material (Karachi University Campus) and from dried specimens from Karachi University Herbarium (KUH) and examined under stereomicroscope (Nikon XN model), (SMZ800).

For light microscopy dried leaves were directly placed on a glass slide and observed under stereomicroscope (Nikon XN model) and for scanning electron microscopy, a 1 cm² part of leaf was mounted on metallic stub by double adhesive tape and gold plated in sputtering chamber for 6 minutes followed by observation under scanning electron microscope (JSM-6380 A). Terminologies used, are in accordance to Metcalfe and Chalk (1950), Lawrence (1970) and Stearn (1983).

For numerical analysis, 6 species of the genus *Lycium* were studied to find out the phenetic relationship. Hierarchical clustering was performed using the Euclidean distance index with the computer package (IBM Corp., 2011). Each taxon was considered an operational taxonomic unit (OTU). Qualitative characters were recorded in binary state as 1, 2 and characters which were either absent or present were coded as 0 or 1 respectively. While, for quantitative characters average values were directly used.

OBSERVATIONS AND RESULTS

General leaf characters of the genus *Lycium*

Leaves 5-28 mm long, 1-8 mm broad, alternate, petiolate, tomentose towards margin, entire, unlobed, exstipulate, laminar symmetrical, or asymmetrical, shape mainly oblanceolate, lanceolate, elliptic and sometimes oblong. Apex acute, acuminate, obtuse, retuse, and attenuate, forming acute apex angle, base cuneate, obtuse, attenuate, cordate, and oblanceolate, were forming acute base angle. Similarly, we have also studied the vein categories i.e. 1°, 2°, and 3° vein categories. Multicellular, eglandular hairs were located at the petiole region of the leaves, having pointed heads, ribbon – like with irregular projections of different shapes and sizes, and the base of trichomes was flat. 2 types of stomata found i.e. anisocytic (*L. depressum*, *L. ruthenicum*, *L. edgeworthii*, *L. makranicum*, *L. dasystemum*, and *L. europaeum*) and anomocytic (*L. dasystemum*). Stomata were of sunken type, having elliptical aperture shape. No. of stomata were varied from species to species, (Table 1).

KEY TO THE SPECIES

- 1+ Leaves hairy. *L. depressum*
 - Leaves glabrous. 2
 2+ Stomata anomocytic. *L. dasystemum*
 - Stomata anisocytic. 3
 3+ Leaf base angle 0.2-0.4 °. *L. ruthenicum*
 - Leaf base angle >10 ° 4
 4+ Stomatal frequency 4/mm². *L. europaeum*
 - Stomatal frequency >15/mm². 5
 5+ Leaf apex obtuse or retuse. *L. edgeworthii*
 - Leaf apex acute. *L. makranicum*

Appendix- List of voucher specimens for leaf architecture.

S. No.	Species	Collector, Number and Herbarium
1-	<i>Lycium depressum</i> <i>subsp. Angustifolium</i>	Abdul Ghafoor and Rizwan Yusuf 1320 (KUH); S. Omer, M. Qaiser and Y. Nasir 2149 (KUH);Tahir Ali And G.R. Sarwar 2773 (KUH)
2-	<i>Lycium makranicum</i>	Schoenbeck and Temesy 1972 (KUH)
3-	<i>Lycium ruthenicum</i>	S.M.H. Jafri 1791,1805 (KUH); S.A. Farooqi and M. Qaiser 2292,2293 (KUH); M. Qaiser and A. Ghafoor 4356 (KUH)
4-	<i>Lycium europaeum</i>	Dr. S.A. Farooqi and Sultan-ul-Abedin 1205, 1207 (KUH); Dr.S.I. Ali and Sultan-ul-Abedin 699 (KUH); Dr. S. I. Ali, Sultan-ul-Abedin and A. Ghafoor 1295 (KUH); Sultan-ul-Abedin 5148 (KUH); M. Qaiser, Asad Raza and Abrar Hurani 963 (KUH); Sultan-ul-Abedin s.n (KUH)
5-	<i>Lycium edgeworthii</i>	S. Omer, M. Qaiser and Y. Nasir 2146 (KUH)
6-	<i>Lycium dasystemum</i>	Saood Omer, S. Nazimuddin and Abdul Wahid 892 (KUH)

Table 1. Leaf architecture and micromorphology of the genus *Lycium*.

S. No.	Name of Taxa	Length (mm)	Breadth (mm)	Laminar Shape	Laminar Symmetry	Laminar L:W Ratio	Indumentum	Midvein Length (mm)	Apical Extension Length (mm)	Basal Extension Length (mm)	Total Leaf Length (mm)	Base Angle
1	<i>Lycium depressum</i> subsp. <i>congestifolium</i>	5-12	1-6	Oblanceolate, Lanceolate, Elliptic	Symmetrical, Asymmetrical	9:2-17:8	Tomentose (towards margin)	11-18	1-3	1-3	11-26	17-35° acute
2	<i>Lycium zizanioides</i>	8-16	1-2	Oblanceolate	Symmetrical, Asymmetrical	2:1-12:1	-	4-10	1-2	1-3	8-13	0.2-0.4° acute
3	<i>Lycium edgenorthii</i>	6-9	1-3	Linear, Oblong, Oblanceolate	Symmetrical	4:1-9:2	-	8-16	1	1	10-18	12-25° acute
4	<i>Lycium madsenii</i>	8-9	2.5-3	Oblong, Oblanceolate	Symmetrical	2:1-9:2	-	6-9	1	1	8-11	10-20° acute
5	<i>Lycium doryzense</i>	8-9	2.5-3	Lanceolate, Oblanceolate	Symmetrical	3:1-8:2.5	-	8-9	1	1	10-11	28° acute
6	<i>Lycium europaeum</i>	8-28	2-5	Elliptic, Oblong, Oblanceolate	Symmetrical	4:1-28:3	-	8-28	1-3	1-2	11-31	10-20° acute

S. No.	Name of Taxa	Apex Angle	Leaf Base	Leaf Apex	1 st Vein Category	2 nd Vein Category	3 rd Vein Category	Type of Stomata	Aperture Shape	Stomatal Frequency (No. of Stomata /mm ²)	Aperture Length (µm)	Aperture Width (µm)
1	<i>Lycium depressum</i> subsp. <i>congestifolium</i>	0.4-20° acute	Obtuse	Acute, Acuminate	Acrocladonous Suprabasal	Brochidodonomous	Alternate Per Current	Anisocytic	Elliptic	8	1509 (8452) 37.7	0.375 (1.141) 2.64
2	<i>Lycium zizanioides</i>	0.5-0.6° acute	Cuneate	Obtuse	-	-	-	Anisocytic	Elliptic	20	0.52 (4.334) 19.062	0.104 (1.35) 5.937
3	<i>Lycium edgenorthii</i>	0.3-40° acute	Obtuse, Attenuate	Obtuse, Reuse	-	-	-	Anisocytic	Elliptic	5	7.457 (10.589) 12.931	0.847 (2.16) 3.189
4	<i>Lycium madsenii</i>	0.2-10° acute	Attenuate, Cuneate	Acute	Acrocladonous Basal	Pectonem Brochidodonomous	Dichotomous	Anisocytic	Elliptic	4	7.222 (9.145) 10.571	1.085 (1.858) 3.353
5	<i>Lycium doryzense</i>	0.7° acute	Cuneate, Cordate	Acute	-	-	-	Anisocytic	Elliptic	18	4.285 (11.481) 16.101	0.714 (3.518) 6.666
6	<i>Lycium europaeum</i>	22-38° acute	Attenuate, Oblanceolate	Acute, Obtuse, Attenuate	Acrocladonous Suprabasal	Pectonem Brochidodonomous	Random Reticulate	Anomocytic	Oblong, Elliptic	20	1.060 (7.485) 30.555	0.5 (2.903) 3

Table 2. List of characters scored for cluster analysis for the species of genus *Lycium*.

S.no.	Characters Description
	Leaves:
1	Simple (1)
	Size:
2	Length (mm)
3	Breadth (mm)
	Shapes:
4	Linear: Absent (0), Present (1)
5	Oblanceolate: Absent (0), Present (1)
6	Lanceolate: Absent (0), Present (1)
7	Elliptic: Absent (0), Present (1)
8	Oblong: Absent (0), Present (1)
	Apex:
9	Acute: Absent (0), Present (1)
10	Acuminate: Absent (0), Present (1)
11	Obtuse: Absent (0), Present (1)
12	Retuse: Absent (0), Present (1)
13	Attenuate: Absent (0), Present (1)
	Base:
14	Obtuse: Absent (0), Present (1)
15	Cuneate: Absent (0), Present (1)
16	Attenuate: Absent (0), Present (1)
17	Cordate: Absent (0), Present (1)
18	Oblanceolate: Absent (0), Present (1)
	Vesture Type:
19	Tomentose: Absent (0), Present (1)
	Laminar Symmetry:
20	Symmetrical: Absent (0), Present (1)
21	Asymmetrical: Absent (0), Present (1)
	Margin Type:
22	Entire: Absent (0), Present (1)
	Mid Vein Length:
23	mm
	Apical Extension Length:
24	mm
	Basal Extension Length:
25	mm
	Base Angle:
26	Acute: Absent (0), Present (1)
	Apex Angle:
27	Acute: Absent (0), Present (1)
	Type Of Stomata:
28	Anisocytic: Absent (0), Present (1)
29	Anomocytic: Absent (0), Present (1)
	Position Of Stomata:
30	Sunken: Absent (0), Present (1)
	Aperture Shape:
31	Elliptic: Absent (0), Present (1)
32	Oblong: Absent (0), Present (1)
	Size of Aperture:
33	Length (μ m)
34	Width (μ m)
	1° Vein Category:
35	Acrodromous Suprabasal: Absent (0), Present (1)
36	Actinodromous Basal: Absent (0), Present (1)
	2° Vein Category:
37	Brochidodromous: Absent (0), Present (1)
38	Festooned Brochidodromous: Absent (0), Present (1)
	3° Vein Category:
39	Alternate Per Current: Absent (0), Present (1)
40	Dichotomizing: Absent (0), Present (1)
41	Random Reticulate: Absent (0), Present (1)

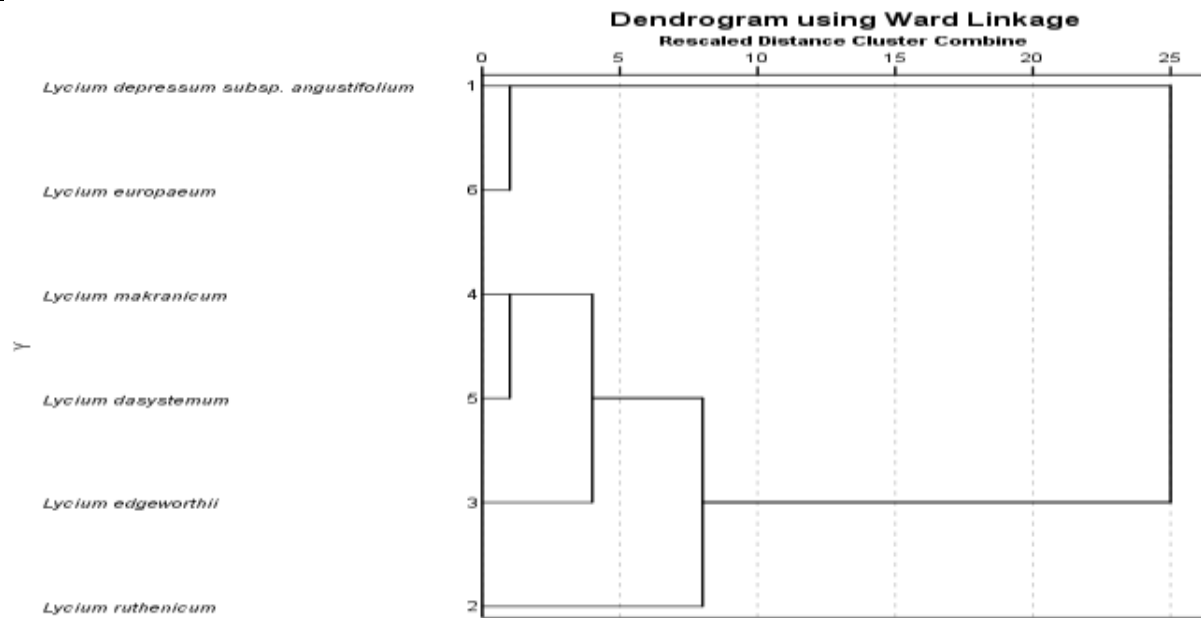
Fig. 1. Dendrogram showing the relationship of the taxa of the genus *Lycium*.

Table 3. Data matrix of the genus *Lycium* for characters presented in Table 2.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
<i>Lycium depressum</i> subsp. <i>quercifolium</i>	1	14	4.33	0	1	1	1	0	1	1	0	0	0	1	0	0	0	0	1	1	1	1	1	13.5	1.83
<i>Lycium rubenicum</i>	1	8.3	2.75	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	1	1	1	1	5.75	1.5
<i>Lycium edgenorthii</i>	1	10	1.8	1	1	0	0	1	0	0	1	1	0	1	0	1	0	0	0	1	0	1	1	10.4	1
<i>Lycium macrocarpum</i>	1	7	2	0	1	0	0	1	1	0	0	0	0	0	1	1	0	0	0	1	0	1	1	7	1
<i>Lycium dactyloctenium</i>	1	8.5	2.75	0	1	1	0	0	1	0	0	0	0	0	1	0	1	0	0	1	0	1	1	8.5	1
<i>Lycium europaeum</i>	1	13	3.5	0	1	0	1	1	1	0	1	0	1	0	0	1	0	1	0	1	0	1	1	12.9	1.9

Species	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41
<i>Lycium depressum</i> subsp. <i>quercifolium</i>	2.33	1	1	1	0	1	1	0	8.45	1.14	1	0	1	0	1	0	0
<i>Lycium rubenicum</i>	2.25	1	1	1	0	1	1	0	10.59	2.16	0	0	0	0	0	0	0
<i>Lycium edgenorthii</i>	1	1	1	1	0	1	1	0	3.51	3.51	0	0	0	0	0	0	0
<i>Lycium macrocarpum</i>	1	1	1	1	0	1	1	0	4.33	1.35	0	1	0	1	0	1	0
<i>Lycium dactyloctenium</i>	1	1	1	0	1	1	1	1	7.48	2.90	0	0	0	0	0	0	0
<i>Lycium europaeum</i>	1.2	1	1	1	0	1	1	0	9.14	3.5	1	0	0	1	0	0	1



Fig. 2. Light micrographs of leaves. A & B, *L. depressum*; C, *L. ruthenicum*; D, *L. edgeworthii*; E, *L. makranicum*; F, *L. europaeum*.

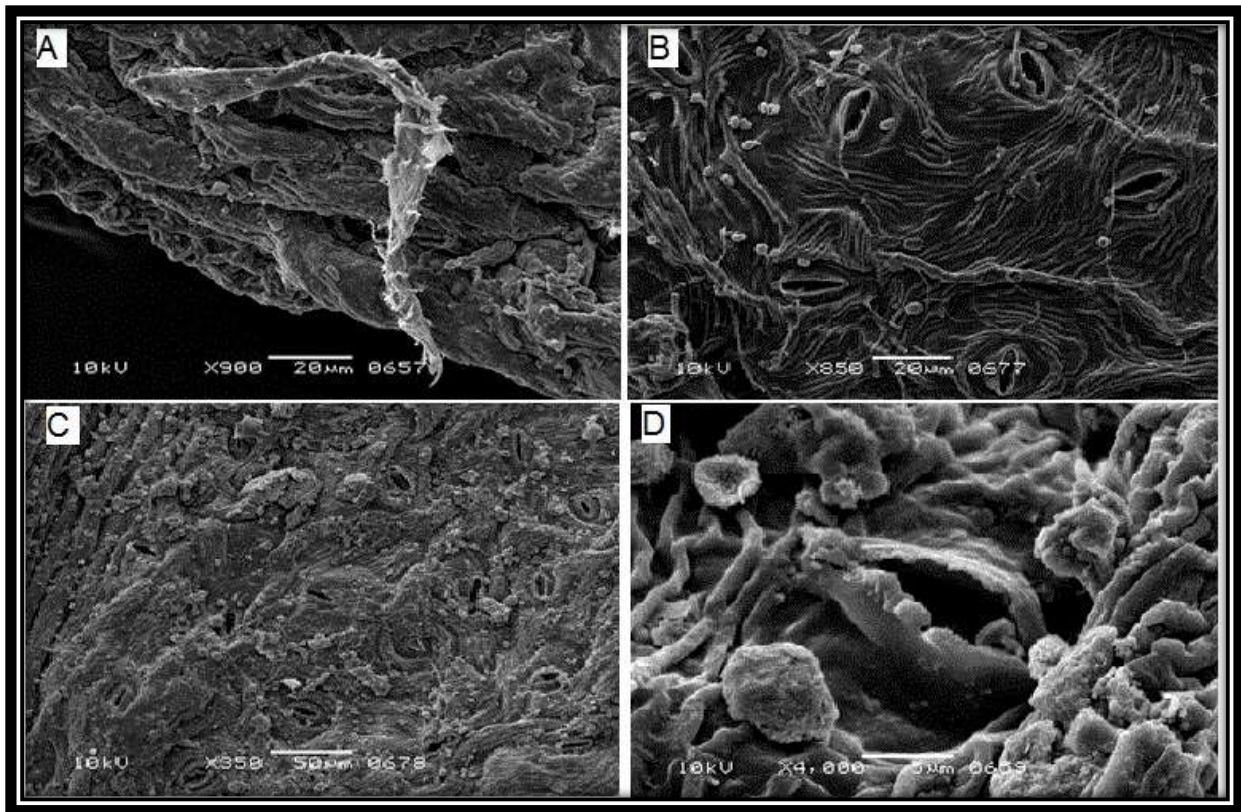


Fig. 3. Scanning electron micrographs showing. A, *L. depressum* trichome; B, *L. edgeworthii* stomata; C, *L. makranicum* stomata; D, *L. ruthenicum* stomata. (Scale bar: A, B = 20µm, C = 50µm, D = 5µm).

DISCUSSION

The genus *Lycium* belongs to the family Solanaceae. The family Solanaceae is one of the largest families of the flowering plants. Leaf architecture of the genus *Lycium* possesses great variations and due to their reliable constancy, these characters could be significantly used for taxonomic delimitation at various levels. The genus is characterized by alternate, petiolate, entire, unlobed leaves with sunken stomata. Anisocytic stomata were found to be dominant while anomocytic stomata were present in *L. dasystemum* only. Similarly, on the basis of trichomes, *L. depressum* remains distinct by having hairy leaves, while the remaining species have glabrous leaves.

Among the studied species, *L. ruthenicum* remains separate due to its small leaf base angle i.e. 0.2-0.4°. Other 3 species have leaf base angle >10°. On the other hand, *L. europaeum* remains separate by its stomatal frequency i.e. 4 Stomata/mm², while remaining two species i.e. *L. edgeworthii* and *L. makranicum* having higher stomatal frequency (>15/mm²) but both the species could be separated from each other by obtuse or retuse and acute leaf apices respectively.

The data obtained was also analyzed numerically, dendrogram obtained from numerical analysis of genus *Lycium* indicates the presence of two distinct clusters i.e., clad I and clad II.

Clad-I was represented by 2 species i.e., *L. depressum* subsp. *angustifolium* and *L. europaeum*. both the species were coupled by having similar vein 1° vein category i.e., acrodromous suprabaasal but still remain distinct by having different leaf base viz., obtuse in *L. depressum* subsp. *angustifolium* and attenuate, oblanceolate in *L. europaeum*.

The clad II was represented by remaining 4 species i.e., *L. ruthenicum*, *L. edgeworthii*, *L. makranicum* and *L. dasystemum*. They may be grouped together due to similar apical extension length (1-2mm). Among these *L. makranicum* and *L. dasystemum* were closed to each other due to their acute leaf apex but remain distinct due to anisocytic and anomocytic stomata respectively. Similarly, *L. ruthenicum* and *L. edgeworthii* were placed close to each other due to their obtuse leaf apex but remains distinct by their cuneate and obtuse leaf base in former and attenuate leaf base in later.

From the above discussion it can easily be inferred that leaf architecture and micromorphology of the genus *Lycium* can be used as an aid to taxonomic delimitation of the genus.

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