Bull. Iraq nat. Hist. Mus.
(2021) 16 (4): 509-533. https://doi.org/10.26842/binhm.7.2021.16.4.0509

A MULTIVARIATE MORPHOMETRIC ANALYSIS OF THE GENUS *LOTUS* L., 1753 (FABACEAE, LOTEAE) FROM EGYPT

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Received Date: 20 August 2021, Accepted Date: 23 October 2021, Published Date: 20 December 2021

ABSTRACT

This study aims at examining and confirming the patterns of phenetic relationships and the levels of variations within and among the species of *Lotus* L., 1753 in Egypt by using morphometric analysis techniques. We have evaluated 24 morphological characters from about 300 herbarium specimens representing 19 species of *Lotus* that are currently recognized. Based on numerical analyses of macromorphological characters (cluster analysis, principal coordinate analysis and principal component analysis), 19 species of *Lotus* were recognized from Egypt. These species were clustered in six species-specific groups: (I) *Lotus halophilus* Boiss. & Spruner, *L. angustissimus* L., *L. glinoides* Delile and *L. schimperi* Steud. ex Boiss., (II) *Lotus glaber* Mill. and *L. palustris* Willd., (III) *Lotus polyphyllos* E.D. Clarke, *L. creticus* L. and *L. cytisoides* L., (IV) *Lotus gebelia* Vent., *L. lanuginosus* Vent. and *L. arenarius* Brot., (V) *Lotus edulis* L., *L. tetragonolobus* L. and *L. conjugatus* L. and (VI) *Lotus ornithopodioides* L., *L. peregrinus* L., *L. arabicus* L. and *L. hebranicus* Hochst. ex Brand.

As a result of this study, we proposed that some characters, not previously examined in detail, showed significant characters in species delimitation: pod length, seed dimensions, features of upper and lower leaflets, calyx, length of corolla, length of style, numbers of flowers and ovules.

Keywords: Cluster analysis, Morphology, Numerical taxonomy, PCA, Phenetic analysis.

INTRODUCTION

The genus *Lotus* L., 1753 (Fabaceae, Loteae) is polymorphic and includes about 150 species native to Europe, Asia, Africa, Australia and some islands of Atlantic Ocean, Pacific Ocean and Socotra archipelago in the Indian Ocean. The greatest genetic diversity for *Lotus* occurs in the Mediterranean Basin (Grant, 1991; Sokoloff, 1998). Based on previous studies, this genus is a taxonomically difficult genus as it includes complexes of closely related groups with similar vegetative characters (Gillett, 1958; Heyn, 1967; Kramina, 1999, 2006; Kramina and Sokoloff, 2004; Kramina *et al*., 2016, 2018, 2020, 2021), including seasonal polymorphisms (Heyn, 1970), and it is difficult to distinguish among the species (Ojeda *et al*., 2009).

Historically there has been little agreement in the taxonomic literature regarding the generic limits of *Lotus* and its infrageneric subdivision (Degtjareva *et al*., 2006). All native New World species formerly placed in *Lotus* are now segregated in four genera (e.g. Arambarri *et al*., 2005; Sokoloff and Lock, 2005; Sokoloff *et al*., 2007) or two distinct genera (Brouillet, 2008). In the Old World, three monotypic segregate genera are accepted: *Kebirita* Kramina and Sokoloff, *Podolotus* Royle and *Pseudolotus* Rech. f.; while two commonly recognized genera: *Dorycnium* Mill. and *Tetragonolobus* Scop. are placed in the synonymy of *Lotus* (Degtjareva *et al*., 2006). However, this has changed considerably with the advent of phylogenetic studies based on nrITS sequences; these have clearly shown that the New World species of *Lotus* are not closely related to the Old World species (Allan and Porter, 2000), and in particular Degtjareva *et al*. (2006) revised sectional classifications proposed by Sokoloff (1999 a, b) and Kramina and Sokoloff (2003).

Some sections appeared as non-monophyletic, including the section *Lotus*, which was resolved as paraphyletic since *Lotus conimbricensis* Brot. (*Lotus* sect. *Erythrolotus* Brand) had ITS sequence type identical to those found in *Lotus subbiflorus* Lag. (*Lotus* sect. *Lotus*) (Faria *et al*., 2012).While several works dealt with the genus *Lotus* in Egypt, this genus was classified into six sections: *Lotus*, *Krokeria*, *Erythrolotus*, *Lotea*, *Pedrosia* and *Quadrifolium* (Muschler, 1912; Täckholm, 1974; Boulos, 1999). El Hadidy (2003, 2004) adopted the classification of *Lotus* L. into three subgenera *Pedrosia*, *Lotus* and *Tetragonolobus* and four sections *Krokeria*, *Loteae*, *Lotus* and *Erythrolotus* based on floral characters (style and stigma), fruit characters (pod and seed), as well as vegetation characters (basal leaflets) and geographical distribution. In Egypt, the taxonomy of the genus *Lotus* has always been problematic which has been reflected in the number of its species (Täckholm, 1974; Boulos, 2009). Several studies have demonstrated the use of micromorphological characters to differentiate between some taxa of Fabaceae (Stenglein *et al*., 2003; Zorić *et al*., 2009; Saheed and Illoh, 2010; Albert and Sharma, 2013; El-Gazzar *et al*., 2013).

Different techniques of multivariate analyses were increasingly applied to resolve some difficulties that may be confronted by a morphological overlap in flowering plants (e.g. Sokal and Sneath, 1963; Gilmartin, 1967; Jensen and Eshbaugh, 1976; McNeil, 1984; Jensen *et al*., 1993). Numerical taxonomy uses numeric algorithms to create groups of taxonomic units based on their character states. Two basic methodologies can be included within numerical analyses: phenetic and cladistic (phylogenetic); in phenetic analyses, classifications are Different techniques of multivariate analyses were increasingly applied to resolve some difficulties that may be confronted by a morphological overlap in flowering plants (e.g. Sokal and Sneath, 1963; Gilmartin, 1967; Jensen and Eshbaugh, 1976; McNeil, 1984; Jensen *et al*., 1993). Numerical taxonomy uses numeric algorithms to create groups of taxonomic units based on their character states. Two basic methodologies can be included within numerical analyses: phenetic and cladistic (phylogenetic); in phenetic analyses, classifications are formed based on the patterns of overall similarities, usually in exomorphology. On the other hand, cladistic (phylogenetic) analyses are based on the premise of estimating the pattern of evolutionary history (phylogeny) using shared derived characters (or synapomorphies); Morphometric techniques have long been established as valuable tools for exploring the development, population differentiation and systematics of plants (Wiens, 2000; Macleod and Forey, 2002; Jensen, 2003; Bateman and Rudall, 2006; El-Hadidy *et al*., 2011; Ellmouni *et al*., 2017).

The current study was carried out to examine and confirm the patterns of phenetic relationships and the levels of variations within and among the species of *Lotus* in Egypt by using morphometric analysis techniques.

MATERIALS AND METHODS

Plant specimens

Nineteen species of *Lotus* are used in the present study (Tab. 1). The data used for the morphometric analysis are recorded from about 300 herbarium specimens deposited in Herbarium of Cairo University (CAI), Herbarium of Agricultural Research Center (CAIM) and Assiut University Herbarium (ASTU) (acronyms sensu Thiers, 2017). Intact and well-preserved specimens are included in the analyses (Tab. 2). Species are collected from different bioclimatic zones of Egypt to represent as much as possible the entire distribution range of the taxa, as well as the morphological variation in each species. Species identification and nomenclature are made with the aid of the floras of Egypt and adjacent countries (Zohary, 1972; Boulos, 1999; Collenette, 1999).

Table (1): Classification of the studied taxa of *Lotus* (Callen, 1959) (A=Annual, P=Perennial. Abbreviations of species are used in Diagrams 1 and 2).

No.	Species	Abbreviation	Subgenus	Section	Duration
1	*Lotus arenarius* Brot.	*L. are*	*Pedrosia*	*Pedrosia*	A
2	*L. edulis* L.	*L. edu*	*Lotus*	*Krokeria*	A
3	*L. ornithopodioides* L.	*L. orn*	*Lotus*	*Lotea*	A
4	*L. halophilus* Boiss. & Spruner	*L. halo*	*Lotus*	*Lotea*	A
5	*L. peregrinus* L.	*L. pere*	*Lotus*	*Lotea*	A
6	*L. polyphyllos* E.D. Clarke	*L. poly*	*Lotus*	*Lotea*	P
7	*L. creticus* L.	*L. cret*	*Lotus*	*Lotea*	P
8	*L. cytisoides* L.	*L. cyt*	*Lotus*	*Lotea*	P

9	*L. glaber* Mill.	*L. gla*	*Lotus*	*Lotus*	P
10	*L. angustissimus* L.	*L. ang*	*Lotus*	*Lotus*	A
11	*L. palustris* Willd.	*L. pal*	*Lotus*	*Lotus*	P
12	*L. glinoides* Delile	*L. glin*	*Lotus*	*Erythrolotus*	A
13	*L. schimperi* Steud. ex Boiss.	*L. schim*	*Lotus*	*Erythrolotus*	A
14	*L. arabicus* L.	*L. arab*	*Lotus*	*Erythrolotus*	A
15	*L. hebranicus* Hochst. ex Brand	*L. heb*	*Lotus*	*Erythrolotus*	A
16	*L. gebelia* Vent.	*L. geb*	*Lotus*	*Erythrolotus*	A
17	*L. lanuginosus* Vent.	*L. lan*	*Lotus*	*Erythrolotus*	P
18	*L. tetragonolobus* L.	*L. tetra*	*Tetragonolobus*	*Erythrolotus*	A
19	*L. conjugatus* L.	*L. conj*	*Tetragonolobus*	*Erythrolotus*	A

Characters scored for morphometric analysis

The morphometric analysis is based on 24 quantitative continuous (17) and quantitative discrete cardinal (7) characters consisting of vegetative and reproductive structures are examined (Tab. 3). In order to avoid biased data due to variations in phenetic features, 10-15 specimens for each species are examined (Tab. 2).

For the data matrix, the quantitative cardinal characters are coded as binary/multi-state characters and the means of quantitative continuous characters are also coded as multi-state characters. Measurements in the herbarium specimens are conducted using digital calipers or a ruler. Each species is encoded as an Operational Taxonomic Unit (OUT) (Sokal and Sneath, 1963).

Table (2): The collection data for some examined specimens of *Lotus* taxa.

No.	Taxa	Locality	Habitat	Collection date	Collector
1	*L. are*	Ras el Hekma, Mariut	Sandy ground by the sea	2/5/1955	M.N.El Hadidi
2	*L. edu*	Burg el Arab	Field margin, roadsides, waste places, coastal sand dunes, rocky & limestone slopes.	15/3/1928	V. Täckholm
3	*L. orn*	Bahariya oasis, Bawiti, El Qasr.	Moist places by springs and streams; edges of cultivated ground and roadsides; rocky wastes	15/3/1968	Gun Romee
4	*L. halo*	Sinai, El Kharruba village	Sandy desert wadies, waste ground and roadsides; limestone rocks, in cultivation, dunes near sea shore	3/4/1988	El Hadidi *et al*.

5	*L. pere*	Bahariya oasis, Bawiti, El Qasr.	Coastal sand dunes, ; rocky calcareous slopes; in cultivated ground or by roadsides	15/3/1968	Gun Romee
6	*L. poly*	Sidi Kirir	Coastal sand dunes and adjacent desert plains.	23/3/1987	A.G. Famy
7	*L. cret*	Rosetta	Sand dunes and sand stone cliffs by the sea	20/4/1973	Ibrahim Mahdi & S. Sisi
8	*L. cyt*	El Rasool Village, Mersa Matruh – Salum road	Sandy desert places, dunes, wadies or in oolothic limestone rocks, usually by the sea	2/5/1988	A.G. Famy
9	*L.gla*	Cairo – Alexandria desert road (K48)	moist and cultivated ground, canal banks, lawns	7/3/1978	Merxmuülle r *et al*.
10	*L. ang*	Kafr Siman	Usually in humid soil	7/4/1927	N.Simpson
11	*L. pal*	Dakhla Oasis: Mutat Bir Asmant el Gedid	Near rivulets and ditches, in cultivated ground	11/2/1952	V. Täckholm & Kassas
12	*L.glin*	Wadi Iseili, Suez road	Sandy desert wadies and plains	8/1/1960	V. Täckholm *et al.*
13	*L.schim*	Wadi Idib, "*Panicum turgidum* community"	Sandy wadies and plains	4/3/1967	D. Oshorn & I. Helmy
14	*L. arab*	El Minya, Eastern side, Deir Al Azzra Qena	Weed on Nile banks and in field	2/2/1979 15/4/1977	M. Amry Kosinova & Slavicova
15	*L. heb*	Thamilat Al-shifa, Red Sea Coast	Sandy coastal plains; foot hills; wadies in calcareous and stony ground in hot desert areas.	28/11/1986	Hobbs
16	*L. geb*	Heliopolis, Cairo	Dry and rocky places	1820 - 1826	Ehrenberg
17	*L. lan*	Sinai: El – Arish – El Hassana, 7 km before El Hassana	Desert plains on sandy gravel; in fields	4/4/1988	El Garf
18	*L. tetra*	West Mersa Matruh, wadi el-Ramleh	Fields, roadsides, calcareous ground and waste ground	10/3/1965	V. Täckholm

19	*L. conj*	Sinai, Tarfa district	Fields and dry places	7/5/1982	H. Barakat

Statistical treatment of data

For morphological diversity, simple descriptive statistics for quantitative continuous parameters are calculated for each species included in the analyses using STATISTICA software version 8.0 (Weiß, 2007). A Shapiro-Wilk statistic was used (with $p < 0.05$) to test whether any morphometric variable deviated from a normal distribution and equality of variance (Cortinhas *et al*., 2015). Before further statistical tests, appropriate transformations (when required) were applied to each parameter did not follow a normal distribution. The Pearson's correlation coefficients between each character pairs are computed in order to reveal highly correlated characters and to ensure that no high correlations (> 0.90) (Španiel *et al*., 2017), are present that could potentially affect the results of further multivariate analyses. If the correlation coefficients for the correlated pairs of variables exceeded r=0.90, they are excluded from the multivariate analyses.

Procedures of multivariate analyses

In order to obtain general information about the relationships and similarities of the examined morphological traits, a cluster analysis is performed on a dataset of all the 19 OTUs using 24 characters. To assess the phenetic relationships between species (OUTs), the similarity between two OTUs is calculated on the basis of Gower's general similarity coefficient, and the dendrogram is prepared using un-weighted pair-group method with arithmetic means (UPGMA) clustering algorithms with PAST 3.25 (PAlaeontological STatistics) software package (Hammer *et al*., 2001). Gower distance is chosen since it can handle metric characters as well as nominal and ordinal-scaled ones (Gower, 1971).

The cophenetic correlations were then calculated between the tree matrix and the similarity matrix in order to estimate how well the dendrogram represents its corresponding pairwise distance matrix. High cophenetic correlation coefficient (more than 0.7) indicates that the hierarchic classification obtained by the clustering method is a reasonably faithful representation of the original resemblance matrix (Sokal, 1986). Based on the morphological characters, the species groups that resulted from cluster analysis are subjected to ANOVA to reveal significant differences between means of characters across the identified groups (Sokal and Rohlf, 1981) using SPSS version 16.0.

A principal coordinates analysis (PCoA) is performed on the basis of the 24 morphological characters, where it is more appropriate with mixed dataset (continuous and discrete cardinal). The distance matrix is often based on Gower's coefficient (Legendre and Legendre, 1998). The goal of PCoA is the positioning of species in a space of reduced dimensionality while preserving their distance relationships.

On the basis of 17 quantitative continuous morphological characters, a principal components analysis (PCA) is applied on the matrix of product-moment correlations, obtained from the standardized data, to provide further insight into structure in the data set. This method is wellsuited to revealing patterns of continuous variations in a data set (Sneath and Sokal, 1973). The PCA investigates the overall variation pattern along the first two components in order to find hypothetical variables (components) that can discriminate among groups.

Morphological characters are projected onto the eigenvectors, with a priori assignment to the groups of species obtained from the classification plotted in two dimensions for examination. Results of PCA analysis is performed using CANOCO version 4.5 for windows (Ter Braak and Šmilauer, 2003), and presented as a two-dimensional scatter plot where each point represents one taxon and an arrow for a character.

Table (3): Characters and character states used for morphological characterization of *Lotus* species, together with their abbreviations used in Diagram (3).

	Characters	Abbreviation	Character states	Coded as
Stem	1- Life history	H	Annual	1
			Perennial	2
Leaf	2- Shape of upper leaflet	SUL	Ovate	1
			Obovate	2
			Lanceolate	3
			Oblanceolate	4
	3- Length of upper leaflet	ULL	(>15 mm)	1
			(< 15 mm)	2
	4- Width of upper leaflet	ULW	(>7 mm)	1
			(< 7 mm)	2
	5- Shape of lower leaflet	SLL	Ovate	1
			Obovate	2
			Lanceolate	3
			Oblanceolate	4
	6- Length Lower leaflet	LLL	(>2-10 mm)	1
			(<10 mm)	2
	7- Width lower leaflet	LLW	(>2-5 mm)	1
			(< 5 mm)	2
	8- Length of rachis	R	(> 4 mm)	1
			(< 4 mm)	2
Flower	9- Number	NF	(> 2)	1
			(< 2)	2
	10- Bract length	BL	(> 6 mm)	1
			(< 6 mm)	2
Corolla	11- Length	CRL	(>10 mm)	1
			(< 10 mm)	2

	12- Color	CRC	Yellow	1
			Pink	2
Calyx	13- Length	CL	(> 7 mm)	1
			(< 7 mm)	2
	14- Tube length	CTU	(> 3 mm)	1
			(< 3 mm)	2
	15- Teeth length	CT	(> 5 mm)	1
			(< 5 mm)	2
Style	16- Shape	STS	Bifid	1
			Simple swollen	2
			Simple un-swollen	3
	17- Length	STL	(>5 mm)	1
			<5 mm)	2
Pod	18- Length	PL	(>30 mm)	1
			(< 30 mm)	2
	19- Shape	PWK	Winged	1
			Keeled	2
Seed	20- Length	SL	(>2 mm)	1
			(< 2 mm)	2
	21- Width	SW	(>1 mm)	1
			(<1 mm)	2
	22- Color	SC	Black	1
			Brown	2
			Orange	1
			Green	2
Seed/Pod	23- Seed/Pod	S/P	(>16 mm)	1
			(<16 mm)	2
Ovules	24- Number	NOV	0-9	1
			10-19	2
			20- 40	3

RESULTS

Variations of characters among species

Results of the basic descriptive statistics for quantitative continuous characters in all species are given in Table (4). None of the characters had a correlation coefficient above the threshold (0.90), and all characters show normal distribution where no transformations are performed (Tab. 5). Thus, all studied quantitative continuous (17) and quantitative discrete (7) characters are included in the analyses. The highest correlation coefficients -0.84 and 0.76 occurred between the characters style shape; STS vs. shape of lower leaflet; SLL and style length; STL vs. pod length; PL, respectively. The ANOVA test show that seven [Seed/pod length (4), seed color (7), shape of upper leaflet (8), shape of lower leaflet (11), calyx tube length (18), corolla color (21 and style shape (22)] out of the 24 examined characters are insignificantly different between species in all measured variables (Tab. 5).

Table (4): Basic descriptive statistics of quantitative parameters resulting from the morphometric analyses of the *Lotus* species (SD=Standard deviation, CV= Coefficient of variation, 25%-75%=percentile boundaries. For species and character abbreviations see Tables (1) and (3), respectively).

Species		*L. are*	*L. edu*	*L. orn*	*L. halo*	*L. pere*	*L. poly*	*L. cret*	*L. cyt*	*L. gla*	*L. ang*	*L. pal*	*L. glin*	*L. schim*	*L. arab*	*L. heb*	*L. geb*	*L. lan*	*L. tetra*	*L. conj*
Quantitative continuous characters																				
PL	Mean	23.6	25.1	35.0	22.5	35.1	12.5	32.5	35.0	25.0	17.5	19.1	16.1	11.0	27.5	22.5	25.0	18.5	45.0	45.0
	SD	5.3	1.7	6.0	4.7	15.5	1.6	1.8	3.4	6.9	1.7	2.8	3.0	2.8	5.2	4.7	3.1	5.9	14.1	14.5
	25%-75%	17.3-26.4	23.6-26.7	25.2-33.5	19.0-26.8	25.3-44.7	11.4-14.0	31.4-34.2	32.6-38.6	15.5-25.8	16.3-18.5	17.5-21.8	13.6-17.8	8.5-14.0	22.7-32.5	19.0-26.8	23.0-27.0	17.9-19.1	29.4-51.6	27.4-52.1
	CV	22.4	7.0	25.1	21.0	29.9	12.6	5.4	9.8	34.6	9.5	14.5	18.5	25.7	18.7	21.0	12.3	4.7	35.2	36.3
S/P	Mean	22.5	13.2	14.1	19.1	14.0	16.0	16.0	14.5	16.1	14.1	17.5	15.5	7.5	17.5	22.5	13.5	13.5	16.0	14.0
	SD	3.3	1.0	2.3	5.0	2.5	0.8	0.8	1.6	0.8	0.7	0.4	0.4	1.0	1.6	1.6	1.0	1.2	2.3	0.7
	25%-75%	21.3-23.9	12.7-13.8	12.6-15.4	16.0-21.0	11.9-15.5	15.2-16.7	15.1-16.6	13.5-15.6	15.5-16.9	13.6-14.6	17.1-17.8	15.2-15.9	6.6-8.2	16.3-18.5	20.8-23.6	12.4-14.4	12.2-14.3	14.8-16.7	13.4-14.6
	CV	14.8	7.5	16.3	26.5	17.7	4.9	4.9	11.3	4.7	4.7	2.3	2.7	13.0	9.4	3.7	7.7	8.7	14.5	5.0
SL	Mean	1.3	2.8	2.1	1.0	1.5	1.5	1.5	1.3	1.3	0.9	1.8	1.3	0.8	1.5	1.2	1.8	1.8	3.0	2.5
	SD	0.4	0.4	0.3	0.1	0.3	0.4	0.3	0.2	0.2	0.1	0.2	0.2	0.1	0.4	0.2	0.2	0.2	0.6	0.4
	25%-75%	1.0-1.3	2.5-3.1	1.8-2.3	0.9-1.1	1.3-1.6	1.2-1.9	1.4-1.7	1.1-1.4	1.1-1.3	0.8-1.0	1.5-2.0	1.1-1.4	0.7-0.9	1.1-1.9	1.0-1.4	1.5-2.0	1.5-2.0	2.5-3.6	2.2-2.8
	CV	33.7	14.7	16.8	14.9	22.0	25.7	19.1	15.7	13.2	9.1	12.4	15.2	15.6	25.9	20.0	12.4	12.4	21.0	14.7
SW	Mean	0.8	2.2	2.0	0.8	0.9	1.0	1.3	1.0	1.3	0.8	1.0	0.8	0.8	1.3	1.3	1.1	1.3	2.5	1.8
	SD	0.1	0.3	0.5	0.2	0.1	0.4	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.3	0.2
	25%-75%	0.7-0.9	2.0-2.4	1.6-2.3	0.6-1.0	0.8-1.0	0.6-1.4	1.1-1.5	0.9-1.2	1.1-1.4	0.6-0.9	0.8-1.2	0.7-0.9	0.6-0.9	1.1-1.4	1.1-1.4	1.0-1.2	1.1-1.4	2.3-2.8	1.5-2.0
	CV	18.6	14..8	23.0	28.3	10.5	41.9	16.1	15.1	14.7	21.1	18.9	22.0	25.3	13.7	14.6	8.6	14.2	12.5	12.4
ULL	Mean	11.4	14.5	12.5	5.5	13.5	5.3	10.0	10.0	11.0	8.5	8.5	6.5	6.5	16.0	10.0	8.5	15.0	19.0	12.5
	SD	1.8	1.2	3.4	1.6	2.3	1.3	3.2	3.1	2.7	2.3	1.0	2.7	1.0	4.3	4.7	2.2	3.7	7.3	1.5
	25%-75%	10.0-12.0	13.7-15.3	10-14.9	4.4-6.8	12.4-15.1	3.8-6.4	7.5-12.3	7.4-12.2	9.1-13.1	6.4-10.3	7.7-9.3	3.9-9.3	5.8-7.2	12.0-19.7	5.7-14.7	6.4-9.5	11.0-18.0	12.7-24.1	11.6-13.6
	CV	16.1	8.2	26.9	28.9	16.9	25.6	32.0	30.7	24.5	26.7	11.7	42.2	14.6	26.9	46.6	26.0	24.6	38.5	12.4
ULW	Mean	6.5	7.0	9.1	3.0	7.1	7.5	5.5	5.5	4.1	4.1	4.0	3.0	4.0	7.5	7.5	4.0	6.5	7.0	6.0
	SD	0.6	0.9	3.3	0.8	1.5	1.6	2.2	2.3	1.2	1.2	0.7	1.4	0.7	3.9	2.9	0.7	0.9	0.8	2.6
	25%-75%	6.0-7.1	6.3-7.8	7.7-11.2	2.3-3.8	6.2-8.0	6.5-8.2	3.6-6.8	3.3-7.1	3.1-5.2	3.1-4.6	3.4-4.6	1.2-3.8	3.6-4.6	4.7-11.4	4.7-9.5	3.6-4.6	6.1-7.1	6.3-7.8	4.2-8.1
	CV	9.6	13.1	36.4	25.5	21.4	21.9	40.1	40.6	30.1	29.4	17.2	46.7	17.6	52.6	38.1	17.6	13.4	10.9	43.8
LLL	Mean	3.3	7.0	6.1	5.0	6.5	3.5	4.5	6.0	6.5	5.0	3.5	4.0	3.5	10.0	7.0	5.0	4.5	11.0	7.0
	SD	0.6	0.5	1.7	1.9	2.0	1.1	1.5	2.8	2.5	2.0	0.9	0.7	1.0	6.0	1.8	2.0	1.5	4.8	2.2
	25%-75%	3.0-3.3	6.8-7.3	5.5-7.2	4.5-6.1	5.0-7.6	2.3-4.5	3.3-5.6	3.5-8.3	4.3-8.4	3.5-7.1	2.9-4.2	3.2-4.7	2.9-4.1	3.7-15.6	5.6-8.1	3.0-7.0	3.3-5.6	5.1-14	4.9-8.4
	CV	19.0	7.0	27.0	38.2	31.0	30.8	33.6	46.4	38.0	39.6	24.8	18.0	28.3	60.3	25.8	40.0	33.6	43.3	31.0
LLW	Mean	2.3	5.2	5.5	2.5	5.0	2.0	4.1	3.8	2.0	2.0	2.1	2.5	2.0	5.5	4.0	2.5	3.5	5.5	5.0
	SD	0.7	0.7	1.5	1.0	1.8	0.6	0.8	1.5	0.9	0.7	0.7	0.4	0.6	2.4	1.2	0.4	0.9	1.6	2.0
	25%-75%	1.9-2.2	4.8-5.9	4.9-6.4	1.8-3.3	3.9-6.2	1.6-2.5	3.1-4.9	2.9-5.2	1.1-2.8	1.3-2.6	1.5-2.6	2.1-2.8	1.5-2.4	4.1-7.5	3.1-5.0	2.1-2.9	2.9-4.2	4.1-6.5	3.4-7.1
	CV	30.1	14.3	26.7	39.1	35.5	32.1	20.7	40.2	42.4	36.4	33.9	14.6	32.1	43.4	30.1	15.7	24.8	29.2	39.8
R	Mean	8.1	7.0	5.0	2.0	5.5	2.5	0.8	2.8	3.5	4.5	6.5	3.5	3.5	5.0	2.8	10.0	3.0	7.5	8.5
	SD	0.8	2.0	0.7	0.6	1.6	0.4	0.2	1.7	1.1	1.0	2.4	1.0	1.0	2.0	1.2	2.3	0.8	1.5	1.2

	25%-75%	7.5-8.5	5.3-8.5	4.4-5.6	1.5-2.5	4.4-6.1	2.0-2.9	0.6-0,8	0.8-4.2	2.8-4.8	3.8-5.4	4.4-8.5	2.9-4.1	2.9-4.1	3.4-7.1	1.5-3.9	8.0-11.4	2.0-4.0	6.4-8.7	7.7-9.3
	CV	9.6	28.1	13.6	30.2	28.4	16.1	22.0	60.5	32.4	22.8	37.5	28.3	28.3	39.8	44.5	23.3	27.2	19.9	14.7
BL	Mean	9.0	7.0	9.5	5.5	6.5	5.5	5.5	4.5	6.5	7.0	9.5	4.0	3.0	9.0	9.0	10.0	5.0	10.0	11.0
	SD	1.0	1.3	1.0	1.6	1.1	0.4	0.4	1.0	1.1	0.8	0.4	0.7	0.6	0.7	0.7	0.7	0.7	2.6	1.5
	25%-75%	8.2-9.5	6.1-7.2	8.7-10.3	4.1-5.6	6.0-7.3	5.1-6.0	5.2-5.7	3.7-5.2	5.5-7.2	6.3-7.8	9.2-9.8	3.6-4.7	2.5-3.6	8.4-9.3	8.3-9.5	9.4-10.6	4.3-5.6	7.0-12.6	9.8-12.4
	CV	11.1	18.0	10.8	29.2	16.4	7.3	6.5	22.0	16.5	11.1	4.0	16.8	21.0	7.4	0.8	6.9	14.7	25.7	13.4
NF	Mean	4.3	1.5	3.6	2.5	2.0	4.0	4.5	5.0	2.0	2.0	3.5	3.0	3.0	3.0	3.5	3.5	3.0	1.5	1.5
	SD	0.5	0.5	1.0	0.5	0.8	1.2	1.1	2.0	0.7	0.8	1.6	0.9	0.8	0.9	0.5	1.1	0.9	0.5	0.5
	25%-75%	4.0-5.0	1.0-2.0	3.0-4.0	2.0-3.0	1.0-3.0	3.0-5.0	4.0-5.0	3.0-7.0	2.0-2.0	1.5-3.0	2.0-5.0	2.0-4.0	2.0-4.0	2.0-4.0	3.0-4.0	3.0-4.0	2.0-4.0	1.5-2.0	1.0-2.0
	CV	11.2	35.1	26.8	21.1	40.8	28.9	24.0	40.0	33.3	40.8	45.2	31.4	27.2	31.4	15.1	30.9	31.4	35.1	35.1
CL	Mean	8.0	8.5	6.5	5.1	6.5	5.5	8.0	7.5	5.5	5.5	7.5	4.0	4.0	7.5	6.5	8.5	7.5	12.5	12.5
	SD	0.7	1.3	1.0	0.7	1.1	0.8	0.6	1.1	1.1	0.4	1.1	0.7	0.7	1.0	0.9	1.0	1.1	2.7	1.6
	25%-75%	7.7-8.5	7.9-9.2	5.7-7.4	4.3-5.2	5.7-7.5	5.0-6.1	7.5-8.5	6.4-8.4	4.7-6.4	5.2-5.9	6.4-8.5	3.6-4.5	3.6-4.5	6.6-8.2	6.1-7.1	7.7-9.3	6.6-8.2	9.9-15.3	11.4-14.0
	CV	8.6	15.8	14.8	14.8	17.0	15.1	8.0	15.2	19.3	7.2	14.6	17.3	16.8	13.7	13.4	11.7	14.5	21.3	12.5
CTU	Mean	3.5	3.5	3.7	2.5	3.5	2.5	3.5	3.5	3.0	1.5	3.0	2.5	2.3	3.0	3.0	4.3	3.0	4.0	8.0
	SD	0.7	0.5	0.9	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.3	0.5	0.7	0.9	0.6	0.8	0.7	1.7
	25%-75%	3.0-4.2	3.1-4.0	2.9-4.4	2.1-3.0	3.2-4..0	2.1-2.9	3.1-3.8	3.1-3.9	2.6-3.2	1.1-1.9	2.6-3.2	2.3-2.8	1.9-2.7	3.0-3.0	2.0-4.0	3.7-4.9	2.0-4.0	3.4-4.5	6.4-9.8
	CV	18.4	13.4	23.0	16.5	11.6	15.7	11.9	11.1	12.5	25.9	12.7	12.5	24.4	22.2	31.4	14.0	27.2	17.0	21.5
CT	Mean	5.0	0.5	2.8	1.5	3.0	3.0	4.5	4.0	3.0	3.5	4.5	2.5	1.8	5.0	3.0	4.3	4.5	8.5	4.0
	SD	0.0	1.0	0.2	0.5	0.9	0.8	0.5	0.8	0.4	0.4	0.7	0.4	0.2	0.8	0.9	0.6	0.4	1.8	0.7
	25%-75%	5.0-5.0	5.0-6.0	2.5-3.0	1.0-2.0	2.0-4.0	2.0-4.0	4.0-5.0	3.0-5.0	2.7-3.5	3.1-3.8	3.9-5.0	2.2-2.8	1.6-2.0	4.2-5.7	2.0-4.0	3.8-4.9	4.2-4.9	6.9-9.8	3.4-4.5
	CV	0.0	17.7	8.2	35.1	31.4	27.2	11.7	20.4	13.3	11.3	15.0	14.7	11.2	15.5	31.4	14.9	8.2	21.0	17.0
CRL	Mean	12.6	12.5	9.5	6.5	9.0	6.5	15.0	11.0	8.5	6.5	9.0	6.0	4.0	8.5	9.5	12.9	14.0	16.0	13.5
	SD	0.4	1.5	0.6	1.1	0.6	1.0	2.0	2.1	0.9	0.9	0.7	0.5	0.7	0.9	2.0	2.5	3.1	2.5	1.2
	25%-75%	12.1-13.0	11.6-13.6	9.4-10.0	5.5-7.8	8.6-9.3	5.7-7.2	13.8-16.6	9.1-12.6	7.8-9.3	6.1-7.1	8.4-9.5	5.7-6.4	3.2-4.6	7.8-9.1	7.5-11.6	12.0-14.3	11.8-16.4	13.5-17.9	12.2-14.3
	CV	3.4	12.3	6.4	17.6	7.1	15.2	`13.2	19.1	10.7	13.4	7.6	9.0	18.7	10.9	21.0	19.7	21.9	15.7	8.7
STL	Mean	6.7	5.5	3.3	2.3	2.8	2.8	6.6	4.5	3.5	2.8	5.0	1.3	1.8	3.5	4.8	6.5	5.5	3.5	6.0
	SD	0.4	0.4	0.2	0.3	0.2	0.2	0.4	0.4	0.3	0.2	0.5	0.2	0.2	0.4	0.2	0.4	0.4	0.4	0.8
	25%-75%	6.5-7.0	5.0-6.0	3.0-3.5	2.1-2.4	2.5-3.0	2.5-3.0	6.2-7.0	4.1-4.9	3.3-3.8	2.6-3.0	4.8-5.4	1.1-1.4	1.5-2.0	3.1-3.8	4.5-5.0	6.1-6.8	5.2-5.8	3.1-3.8	5.5-6.4
	CV	5.7	8.1	6.5	12.4	7.9	7.5	6.3	10.0	9.4	7.3	9.6	13.7	12.4	10.9	4.8	5.7	6.9	11.9	12.8
NOV	Mean	39.3	16.0	25.9	18.0	29.5	19.0	16.0	20.0	24.0	16.0	16.5	16.9	8.0	21.0	29.5	34.0	34.0	18.4	16.0
	SD	4.4	0.8	0.9	0.8	1.3	0.8	0.8	0.9	1.8	0.8	1.1	0.9	0.8	0.9	1.1	0.7	0.9	1.3	0.8
	25%-75%	37.0-41.0	15.0-17.0	25.0-27.0	17.0-19.0	28.0-31.0	18.0-20.0	15.0-17.0	19.0-21.0	23.0-25.0	15.0-17.0	16.0-17.0	16.0-18.0	7.0-9.0	20.0-22.0	29.0-30.0	34.0-34.0	33.0-35.0	17.0-20.0	15.0-17.0
	CV	11.2	5.1	4.3	4.5	4.3	4.3	5.1	4.7	7.6	5.1	6.5	5.2	10.2	4.5	3.7	2.0	2.8	6.9	5.1

Table (5): Results of ANOVA for the means of 24 morphological characters between the species groups resulted from cluster analysis of *Lotus* species (For full names of character abbreviations, see Table (3). NS= Not significant, *= *P*<0.05, ** = *P*<0.01).

	Species groups						F-value	Total *P*
	I	II	III	IV	V	VI		
Number of species	4	2	3	3	3	4		
Quantitative cardinal characters								
H	NS	NS	NS	0.001**	NS	NS	13.41	0.001**
PWK	NS	NS	NS	NS	0.03*	NS	3.28	0.039*
SC	NS	NS	NS	0.001**	NS	0.001**	1.07	0.42
SUL	0.001**	NS	0.001**	0.001**	0.001**	0.001**	1.35	0.30
SLL	0.04*	NS	0.001**	0.001**	0.001**	NS	1.20	0.36
CRC	0.04*	NS	NS	0.001**	0.001**	NS	0.85	0.54
STS	0.001**	NS	NS	0.001**	NS	NS	1.83	0.17
Quantitative continuous characters								
PL	NS	NS	NS	NS	0.001**	NS	3.04	0.049*
S/P	NS	NS	0.001**	0.001**	NS	NS	0.40	0.084
SL	NS	NS	0.001**	0.001**	NS	NS	19.64	0.001**
SW	0.001**	NS	0.001**	NS	NS	NS	8.56	0.001**
ULL	NS	NS	0.001**	NS	NS	NS	5.11	0.008**
ULW	0.04*	NS	0.001**	0.001**	0.001**	NS	10.98	0.001**
LLL	NS	NS	NS	NS	0.001**	NS	4.10	0.019*
LLW	0.04*	NS	NS	NS	NS	NS	14.98	0.001**
R	NS	0.02*	NS	NS	NS	NS	4.42	0.014*
BL	NS	NS	0.001**	NS	NS	NS	3.52	0.031*
NF	0.05*	NS	NS	NS	NS	NS	9.00	0.001**
CL	NS	NS	NS	NS	0.001**	0.001**	10.78	0.003**
CTU	NS	NS	0.001**	NS	NS	NS	2.72	0.07
CT	NS	NS	NS	NS	NS	0.025*	3.54	0.031*
CRL	NS	NS	NS	NS	NS	NS	8.60	0.001**
STL	NS	NS	NS	0.003**	NS	NS	5.71	0.005**
NOV	NS	NS	NS	0.001**	0.001**	NS	15.06	0.001**

Cluster analysis

The UPGMA dendrogram (Diag. 1) is based on morphological similarity values (Gower's coefficient) with a cophenetic correlation of 0.704, demonstrating good consistency in the presented morphological patterns. The tree can be divided into three acceptable levels yielding six species-specific groups, beyond which recognition of the larger number of groups will be less significant. At the first level of classification, groups (V) and (VI) were separated. Group (V) comprised of *Lotus edulis* L. (Sect. *Krokeria*), *L. tetragonolobus* L. and *L. conjugatus* L. (the latter two species included in Subgenus *Tetragonolobus*), and group (VI) included *Lotus ornithopodioides* L., *L. peregrinus* L., *L. arabicus* L. and *L. hebranicus* Hochst. ex Brand. At the second hierarchical level, groups (III) and (IV) were recognized. Group (III) comprised of the perennials *Lotus polyphyllos* E.D. Clarke, *L. creticus* L. and *L. cytisoides* L., group (IV) consisted of *L. gebelia* Vent., *L. lanuginosus* Vent. and *L. arenarius* Brot. (subgenus *Pedrosia*). At the third classification level, groups (I) and (II) were separated.

Group (I) consisted of OUT's of *Lotus halophilus* Boiss. & Spruner, *L. angustissimus* L., *L. glinoides* Delile and *L. schimperi* Steud. ex Boiss., group (II) included the perennials *L. glaber* Mill. and *L. palustris* Willd. The results of ANOVA test between the six separated groups (I-VI) showed that habit of the plant (H; p=0.001) and pod shape (PWK; p=0.039) were the quantitative discrete cardinal characters that were significantly different among groups (Tab. 6). Except for the length of calyx tube (CTU) and the ratio between seed/pod (S/P), all the remaining quantitative continuous characters were significantly different among groups.

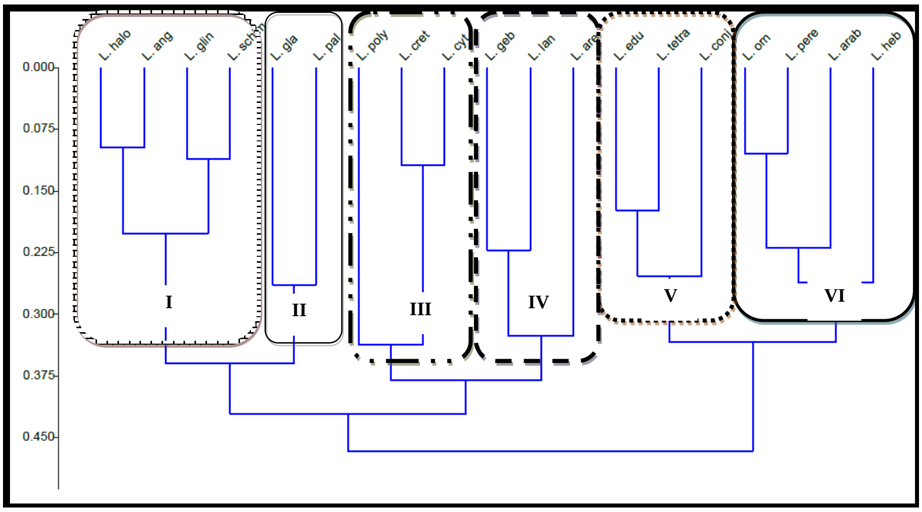

Diagram (1): Cluster analysis (UPGMA classification method and Gower's similarity coefficient) derived from the 24 characters of studied *Lotus* species (Species abbreviations are shown in Table (1), I-VI are the separated species groups).

Table (6): Pearson's correlations coefficients between characters (For full names of character numbers, see Table (3). **= p<0.01, *=p < 0.05).

2	-0.05							
3	-0.21	0.42						
4	0.22	0.28	-0.24					
5	-0.35	0.41	0.63	0.07				
6	0.46	0.28	0.22	0.27	0.35			
7	0.01	0.34	0.03	0.17	0.02	0.17		
8	0.20	-0.24	-0.45	0.09	-0.40	0.09	0.00	
9	-0.19	0.15	-0.09	0.42	0.29	0.19	0.16	0.01

10	0.05	0.58	0.25	0.42	0.39	0.42	0.17	-0.04	0.72															
11	0.37	-0.30	0.11	-0.08	-0.16	0.35	0.00	0.30	-0.08	-0.26														
12	-0.42	0.37	0.34	0.19	0.54	0.19	0.32	0.01	0.37	0.29	0.18													
13	-0.19	0.81	0.34	0.42	0.54	0.42	0.32	0.01	0.37	0.72	-0.35	0.58												
14	-0.36	0.21	0.19	-0.15	0.31	-0.15	0.01	-0.38	-0.15	-0.21	-0.05	0.33	0.09											
15	-0.51	0.05	0.22	0.27	0.35	0.03	0.17	-0.34	0.19	-0.05	-0.08	0.42	0.19	0.37										
16	0.29	-0.27	-0.75	0.02	-0.84	-0.29	0.01	0.42	-0.17	-0.33	0.04	-0.46	-0.46	-0.26	-0.29									
17	-0.03	0.52	0.22	0.27	0.35	0.51	0.02	-0.34	0.42	0.65	-0.37	0.19	0.64	0.11	0.27	-0.29								
18	0.22	0.28	0.22	0.27	0.35	0.51	-0.35	-0.20	0.19	0.42	-0.08	0.19	0.42	0.11	0.27	-0.29	0.76							
19	-0.15	0.04	0.05	0.41	0.28	0.15	0.18	-0.54	0.39	0.21	-0.10	0.15	0.15	0.36	0.41	-0.40	0.41	0.15						
20	0.04	0.15	0.34	-0.04	0.29	0.42	-0.32	-0.13	0.16	0.51	-0.22	-0.06	0.37	-0.15	-0.04	-0.46	0.64	0.64	0.15					
21	-0.28	-0.13	-0.02	-0.42	-0.13	0.05	0.02	0.18	0.15	-0.10	0.26	0.15	-0.07	-0.04	-0.19	0.03	0.05	-0.19	0.04	0.15				
22	-0.12	0.03	0.24	-0.29	-0.01	-0.29	0.30	-0.19	-0.46	-0.43	-0.08	0.12	-0.07	0.07	0.12	-0.06	-0.29	-0.29	-0.29	-0.46	-0.17			
23	0.18	-0.13	0.20	-0.19	0.14	0.28	-0.34	-0.52	-0.07	0.13	-0.16	-0.29	-0.07	-0.04	0.05	-0.27	0.52	0.52	0.29	0.59	-0.13	-0.17		
24	0.09	0.19	-0.23	0.29	-0.15	0.29	0.14	0.13	0.39	0.36	0.01	0.22	0.22	0.02	0.29	0.31	0.48	0.48	-0.02	0.22	0.01	-0.34	0.01	
Characters	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
Shapiro-Wilk test	0.59**	0.62**	0.36**	0.59**	0.51**	0.59**	0.69**	0.86**	0.64**	0.62**	0.77**	0.64**	0.64**	0.55**	0.59**	0.44**	0.59**	0.59**	0.55**	0.64**	0.62**	0.7**	0.6**	0.7**
p- ANOVA	0.001**	0.05*	0.04*	0.84	0.001**	0.001**	0.42	0.30	0.001**	0.001**	0.36	0.02*	0.001**	0.01*	0.03*	0.001**	0.001**	0.07	0.03*	0.001**	0.53	0.17	0.001**	0.001**

Principal Coordinates Analysis (PCoA)

Based on Gower's similarity coefficient of the 24 characters, a principal coordinates analysis (PCoA) is performed and visualized in Diagram (2). It supports the separation patterns of the six species groups (I-VI) along the first two axes that are responsible for 40.7% of the total variation (26.25% for axis 1, and 14.45% for axis 2). A clear separation between groups (I) and (II) positioned along the negative end of axis 1, and groups (V) and (VI) along its positive ends was indicated. An overlap occurred between groups (III) and (IV) positioned along the negative end of axis 2. Other projections confirmed the same general pattern, although less clearly because they are supported by axes that account for less inertia than the first two. Along axis 1 (results not shown), characters with the highest scores (more than 0.6) were pod length (PL), seed length (SL), upper leaflet width (ULW), lower leaflet width

(LLW), number of flowers (NF), calyx length (CL) and calyx tube length (CTU). Along axis 2, the habit of plants (H) and style shape (STS) had the highest scores. Thirteen out of the 17 quantitative continuous characters showed significant variations along the first three PCA axes (Tab. 7). Variations between PL, S/P, R and CT were insignificantly different along the three axes.

Principal Components Analysis (PCA)

The ordination diagram from the principal components analysis (Diag. 3) based on the 17 quantitative continuous characters showed a pattern similar to the results of the cluster analysis. The scores of the first three components explained 61.1% of the total variation accounted for 34.2%, 14.5%, and 12.4% of the total variance for axes 1, 2 and 3, respectively. Pod length (PL), seed width (SW), upper leaflet width (ULW), lower leaflet width (LLW), calyx length (CL), and calyx tube length (CTU) showed highest loadings in relation to PCA axis 1 (Tab. 7). Along PCA axis 2, length of lower leaflet (LLL), length of corolla (CRL) and length of style (STL) had the highest loadings (the latter character contributed weakly to PCA axis 1).

The seed length (SL), number of flowers (NF) and number of ovules (NOV) contributed essentially to the construction of PCA axis 3. The six species groups were distributed in the ordination plane, with some overlap, along the first two important PCA axes. Inspection to the PCA diagram, the number of flowers (NF) was correlated to group (I) that occupied the positive end of PCA axis 1, while groups (V) and (VI) occupied the negative end that were correlated to (LLL), (LLW), (ULW) and (CL). Whereas group (II), which occupied the center of the ordination plane, was not affected by any character, groups (III) and (IV) that positioned on the positive end of PCA axis 2 were correlated with (STL).

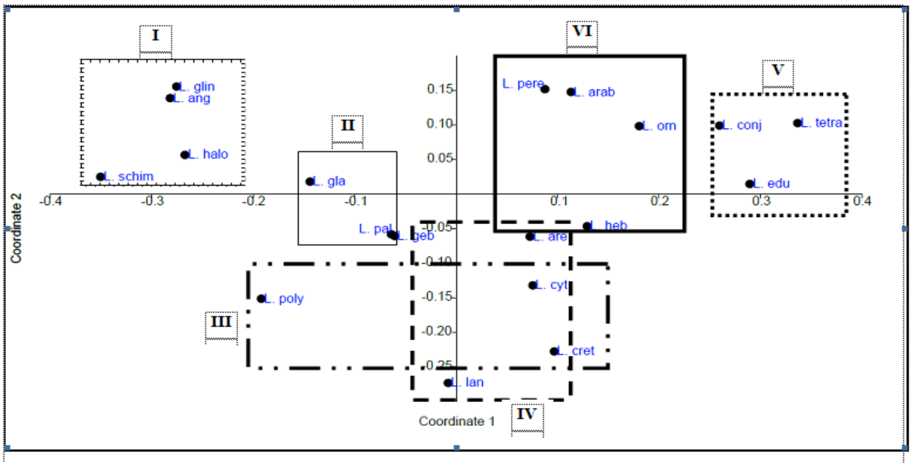

Diagram (3): Scatter plot of principal components analysis (PCA) performed on 17 quantitative characters along the first two PCA axes, with projection of the variables on the factor plane (For species and character abbreviations see Tables (1) and (3), respectively. Gr I-VI refers to the species groups).

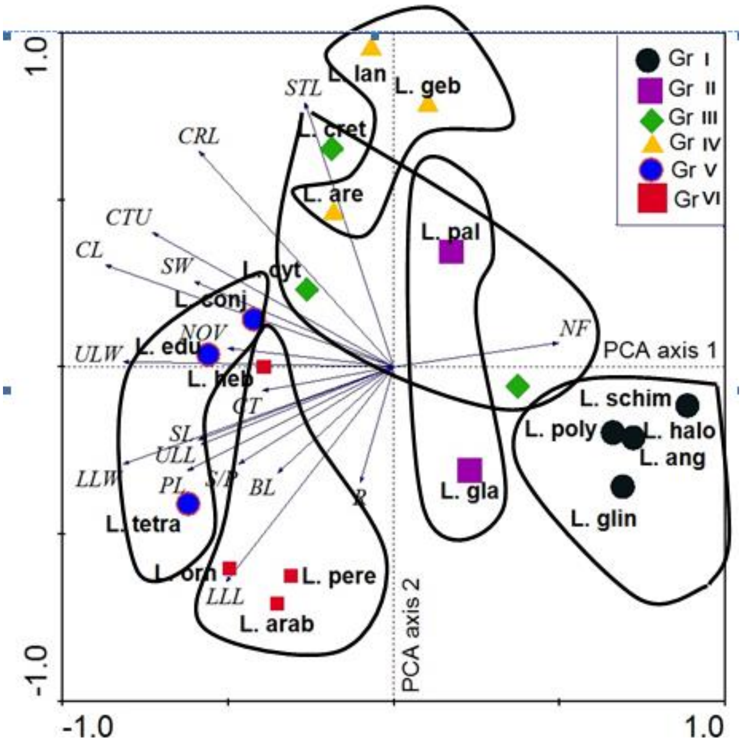

Diagram (2): Principal coordinates analysis (PCoA) scatterplot performed on 24 quantitative and qualitative characters along axes 1 and 2, I-VI are the species groups, for species abbreviations see Table (1).

Table (7): Results of the principal components analysis (PCA) for the species of the *Lotus* as OTUs-total variance and 17 morphological quantitative continuous characters showing the factor loadings on the first three principal components, and results of one-way ANOVA F- and P-values for characters with normal distribution (The numbers in bold are characters with high factor loading > 0.6. For character abbreviations, see Table (3), * = *P*<0.05, ** = *P*< 0.01).

Characters	PCA–factor loadings			ANOVA–F value	*P* value
	PC1	PC2	PC3		
PL	**-0.62**	-0.31	0.04	2.92	0.056
S/P	-0.47	-0.29	-0.38	2.52	0.083
SL	-0.58	-0.23	**0.61**	8.35	0.001**
SW	**-0.63**	0.26	-0.02	4.93	0.009**
ULL	-0.59	-0.22	-0.33	3.56	0.030*
ULW	**-0.81**	0.01	-0.21	14.64	0.001**
LLL	-0.50	**-0.64**	0.17	7.25	0.002**
LLW	**-0.82**	-0.29	0.08	15.88	0.001**
R	-0.10	-0.35	0.47	0.78	0.581
BL	-0.35	-0.32	0.17	3.80	0.024*

NF	0.50	0.07	**-0.76**	6.38	0.003**
CL	**-0.87**	0.30	0.0007	6.55	0.003**
CTU	**-0.73**	0.40	0.02	4.93	0.009**
CT	-0.40	-0.07	0.30	1.11	0.403
CRL	-0.59	**0.64**	0.16	6.09	0.004**
STL	-0.27	**0.79**	0.33	3.67	0.03*
NOV	-0.50	0.06	**-0.67**	6.40	0.003**

DISCUSSION

The genus *Lotus* possesses a difficult generic delimitation, the classification of this genus is always of controversy among taxonomists; Whereas Gillett (1958) and Ball and Chrtková-Žertová (1968) proposed subgenera, sections and subsections. On the other hand, Heyn (1970) and Heyn and Herrnstadt (1968) suggested species groups to place the taxa and describe the relationships among species. This study of *Lotus* species in Egypt was based on the results of numerical analyses of morphological characters (vegetative and reproductive), the current results showed significant characters that may help in the diagnosis of the studied taxa, which were significantly different concerning the analyzed morphological characters.

In the present study, the applications of multivariate morphometric techniques resulted in the delimitation of 19 well-separated species of *Lotus*, and are clearly distinguished from each other. As a result of the cluster, PCoA, and PCA analyses six clear clusters are obtained and they correspond quite well with the species of *Lotus* accepted in Flora of Egypt (El Hadidy, 2003; Boulos, 2009; El-Gazzar *et al*., 2013). UPGMA gives insight into the degree of similarity among the OUT's and whether they form groups/clusters. PCoA and PCA reflect which characters are important on the axes, and indicate the significant characters based on the highest factor loadings. For that reason, it becomes clear which characters are diagnostic and support the separation between groups, and can be useful to distinguish taxa. This study revealed the importance of pod length, seed dimensions, measurements of upper and lower leaflets, calyx, length of corolla, length of style, numbers of flowers and ovules as characteristics that determinate the studied 19 species of *Lotus*. Generally, our results confirm congruence between the UPGMA clustering, PCoA and PCA analyses, in suggesting six groups:

Group (I): *Lotus halophilus*, *L. angustissimus*, *L. glinoides* and *L. schimperi*

This group can be differentiated from the others by the width of the lower leaflets (LLW) and the variations in the number of flowers (NF) which showed significant differences within members of this group (*P*=0.04 and 0.05, respectively), and among other groups (*P*=0.001; Tab. 6). Despite not being included in the analysis, the morphological difference in the pod shape (PS) between species of this group was diagnostic: curved in *L. glinoides* but straight in the others. This group also occupied the extreme ends along the first axes of PCoA and PCA. Other significant quantitative continuous characters were seed width (SW), and upper leaflets width (ULW). Along PCA axis 1, it occupied the positive end that was affected significantly by the number of flowers (NF).

Group (II): *Lotus glaber* and *L. palustris*

Both species are annuals (Sect. *Lotus*) and can be distinguished by the length of rachis (R) that showed significant difference (P=0.02) between them (3.5 mm for the former and 6.5 mm for the latter), and among other groups (P=0.014). In PCoA ordination diagram, this group occupied a central position along axis 1, and was not affected by any character in PCA diagram. The number of lateral veins, not included in the analysis, can be used to distinguish both species from each other: 3 pairs in *L. glaber*, and 2 pairs in *L. palustris*. According to Zareh *et al*. (2017), *Lotus glaber* can easily be differentiated from all *Lotus* species by the absence of trichomes on the stem, leaf, and calyx.

Group (III): *Lotus polyphyllos*, *L. creticus* and *L. cytisoides*

Variations in the bract length (BL), upper leaflet length (ULL) and the length of calyx tube (CTU) were the significant quantitative continuous characters that differentiate among members of this group and between the others (Tab. 6). For quantitative cardinal character, between both species of *Lotus*, the shape of both upper (SUL) and lower (SLL) leaflets were of significant differences (P=0.001). In the lower leaflets, from lanceolate to ovate and in the upper leaflets ranged between obovate to lanceolate. This group occupied the negative end along PCoA axis1, and overlapped with group (IV), and positioned in the centre of PCA ordination plane overlapping with groups (II) and (IV) without any correlations to other variables. With respect to micromorphological characters, Zareh *et al*. (2017) found a high similarity coefficient (0.75) between *Lotus creticus* and *L. cytisoides* as both have the same type of trichomes on the stem, leaf, and calyx as well as the same shape of seeds. In our study, the latter two species were closely related with each other forming a cluster together (Diag. 1) which supports Zareh *et al*. (2017) results.

Group (IV): *Lotus gebelia*, *L. lanuginosus* and *L. arenarius*

The members of this group can be differentiated among and between the others by variations in style length (STL, Tab. 6). The length of rachis (R) was the longest (10 mm) in *Lotus gebalia*, while it was the shortest in *L. lanuginosus* (3 mm). As a quantitative cardinal character, the style shape (STS) played a significant role in the morphological discrimination between this and other groups. In *L. arenarius*, it was bifid, while simple in the remaining two species. *Lotus arenarius* formed a separate branch in this cluster (Diag. 1). Differences in the ratio between seed and the pod (S/P) and the length of seeds (SL) shared the significant characters that helped in delimitation of species of groups (III) and (IV). Along the positive end of PCA axis 2, this group occupied the highest scores and showed a correlation to the style length (STL; Diag. 3).

Group (V): *Lotus edulis*, *L. tetragonolobus* and *L. conjugatus*

The discriminating significant (P=0.001) quantitative continuous characters that can separate this group were the pod length (PL) and length of lower leaflet (LLL). It shared the significant variation in the number of ovules (NOV) with group (IV), and calyx length with group (VI). This can be illustrated in Diagrams (2) and (3) where this group occupied the highest positive scores along PCoA axis 1 and was affected by (PL), (NOV) and (LLL). Here, the variation in the shape of the pod (PWK) was significantly different and deliminates two species of his group: *L. tetragonolobus* with winged pod and *L. conjugatus* with a keeled pod. Along PCA axis 1, this group was spread at the negative end. The inclusion of *L. tetragonolobus* and *L. conjugatus* in this group is quite true and confirmed their taxonomic classification belonging to a separate Subgenus *Tetragonolobus* (Callen, 1959).

Group (VI): *Lotus ornithopodioides*, *L. peregrinus*, *L. arabicus* and *L. hebranicus*

Within the four species of this group, differences in seed color (SC) and shape of the upper leaflet (SUL) were the significant quantitative cardinal characters (Tab. 6). *Lotus arabicus* can be differentiated from the others by its green seed color, while the others have brown. The shape of upper leaflets varied from obovate to rhombic in *L. ornithopodioides* and *L. peregrinus*, and from obovate in *L. arabicus* and oblanceolate in *L. hebranicus*. The length of calyx teeth (CT) was the diagnostic character among the studied species. This fact becomes true when examining the PCA ordination diagram (Diag. 3). Together with a group (V), they positioned along the positive end of PCoA axis 1. The UPGMA dendrogram resulted from Zareh *et al*. (2017) placed *L. arabicus* and *L. hebranicus* as closely related branches in one cluster. In the current study, the latter two species separated into two close branches within the same cluster (Diag. 1).

CONCLUSIONS

Lotus is a taxonomically difficult genus. Using UPGMA clustering, PCoA and PCA analyses to both quantitative cardinal and continuous morphological vegetative and reproductive characters helped in the differentiation of the 19 species of *Lotus*. This study revealed the importance of pod length, seed dimensions, measurements of upper and lower leaflets, calyx, length of corolla, length of style, numbers of flowers and ovules as characteristics that discriminate between the studied taxa. Despite its being limited to some species of *Lotus* in Egypt, our results proposed diagnostic characters that were not previously used in the genus *Lotus*, and enabled the separation of six species-specific groups. Future more investigations and analyses using more characters to improve species delimitation are recommended. This becomes true especially to avoid overlapping of characters in closely related species.

ACKNOWLEDGMENTS

The authors would like to thank three anonymous reviewers for their revision and helpful comments on an earlier version of this manuscript.

CONFLICT OF INTEREST STATMENT

The authors have no conflicts of interest to declare.

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(2021) 16 (4): 509-533.

التحليل المورفومتري متعدد المتغيرات لجنس *LOTUS* L.,1753

(Fabaceae, Loteae)

في مصر

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تأريخ الاستلام: 2021/08/20، تأريخ القبول: 2021/10/23، تأريخ النشر: 2021/12/20

الخلاصة

هدفت الدراسة الحالية إلى فحص وتأكيد أنماط العلاقات التصنيفية المظهرية ومدى التباين فيما بين أنواع جنس *Lotus* L., 1753 المتواجدة في مصر و ذلك باستخدام تقنيات التحليل المورفومتري. تم تسجيل 24 صفة مظهرية لحوالي 300 عينة معشبية ممثلة لتسعة عشر نوعًا من جنس اللوتس. بناءً على التحليلات العددية للسمات المظهرية الأساسية (التحليل الشجري Cluster analysis، تحليل الإحداثيات الرئيسي PCoA وتحليل المكون الرئيسي PCA)، تم التأكيد على 19 نوعًا من اللوتس في مصر وتقسيمهم الى ست مجموعات وهى كالتالي: (1) *Lotus halophilus* و *L. angustissimus* و *L. glinoides* و *L. schimperi*، (2) *Lotus glaber* و *L. palustris*، (3) *Lotus polyphyllos* و *L. creticus* و *L. cytisoides*، (4) *Lotus gebelia* و *L. lanuginosus* و *L.*

arenarius، (5) *Lotus edulis* و *L. tetragonolobus* و *L. conjugatus*، (6) *Lotus ornithopodioides* و *L. peregrinus* و *L. arabicus* و *L. hebranicus*.

أظهرت النتائج ان بعض الصفات- التي تم فحصها للمرة الأولى في هذا البحث- كان لها تأثير معنوي في فصل الأنواع المختلفة مثل طول القرن، أبعاد البذور، سمات الوريقات العلوية والسفلية، طول كلا من الكأس والتويج والقلم، بالإضافة الى عدد الأزهار والبويضات.