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Numerical taxonomic study of the genus *Crotalaria* L. (Crotalarieae, Fabaceae) in Nigeria

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Abstract. Numerical taxonomic study of the genus *Crotalaria* L. in Nigeria was conducted to identify and differentiate some of the species of the genus *Crotalaria* using numerical taxonomy based on quantitative and qualitative characters. Field work was conducted, where different species were collected and analyzed using multivariate analysis. The results showed that all the collected species are distinct at Euclidian distance of 0.41 in the cluster analysis with Cophenetic correlation (r)=0.964. The ordination analysis based on the results of the PCA, separated the specimens into 7 groups corresponding to the result of cluster analysis. The first two components of the PCA account for 81.5%. The length of petiole, width of leaflet and length of fruit contributed more to showing delimitation among the species.

Key words: *Crotalaria,* multivariate analysis, Nigeria, numerical taxonomy

1. Introduction

Crotalaria L. is one of the largest genera of Fabaceae consisting of ca. 700 species (Le Roux *et al*. 2013). It is the largest genus of vascular plants in tropical Africa (Polhill 1982). The genus common name is "rattlepod" or "rattlebox" and it is derived from the fact that the seeds become loose in the pod as they mature, and make a rattling sound when the pod is shaken.

Representatives of *Crotalaria* differ in habit that ranges from small shrubs to herbs and may be annual or perennial. They are easily recognised by their yellow, whitish to purplish or bluish coloured flowers. The leaves are simple or one to three foliate, alternate, lanceolate to obovate. The genus can also be recognised by a combination of the following five diagnostic characters: rostrate keel, 5+5 highly dimorphic anther arrangement (five long, basifixed anthers alternating with five short, dorsifixed ones), trichomes present on the style, inflated fruit, and macrocyclic pyrrolizidine alkaloids (Baker 1914; Polhill 1968; Van Wyk 2005)

Crotalaria is almost cosmopolitan in distribution across tropical and subtropical regions of the world (Lewis *et al*. 2005), with it centre of diversity in Africa and Madagascar (ca. 543 species) (Polhill 1968, 1982; Le Roux *et al*. 2013) and a secondary center in India (ca. 92 species) (Ansari 2008; Sibichen & Nampy 2007). The genus is also widely distributed across the southern hemisphere, extending into Asia and North America. There are ca. 51 species of *Crotalaria* in West Africa (Hutchinson *et al*. 1958) commonly distributed in tropical and sub-tropical regions.

Some of the species within the genus are widely used in agriculture, production of commercial products while some have medicinal and nutritional value (Polhill 1982; Van Wyk 2005; Pandey *et al*. 2010).

Morphometric analysis involves the multivariate analysis of a set of quantitative and qualitative morphological characters of individual specimens of the taxa of interest (sometimes referred to as operational taxonomic units, or OTUs). This is often used to determine whether closely related species have discrete or overlapping morphologies, which may be important in the taxonomic revision of a group. It is also used to ascertain the useful characters that can be used in classifying taxa of interest.

Morphometrics attempts to classify organisms based on morphological similarity. It can be used to describe

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the pattern of similarities among taxa by ordination or cluster analysis (James & McCulloch 1990). Several angiosperm taxa have been reclassified using numerical taxonomy (El-Gazzar 2008). Hussaini and Iwo (1992) worked extensively on *Crotalaria* and reported the genus phenological information which is based on conventional taxonomic methods.

The aim of this study is to identify and differentiate some of the species of the genus *Crotalaria* in Nigeria.

Table 1. List of studied species within the genus *Crotalaria*

S/No	Name of taxon	Collector	Number	Locality
1.	*C. macrocalyx*	Yaradua	SSY1	Jibia
2.	*C. macrocalyx*	Muhammad	MM1	Jibia
3.	*C. senegalensis*	Yaradua	SSY2	Kaita
4	*C. senegalensis*	Yaradua	SSY3	Kaita
5	*C. senegalensis*	Yaradua	SSY4	Kaita
6	*C. senegalensis*	Yaradua	SSY5	Mashi
7	*C. senegalensis*	Yaradua	SSY6	Mashi
8	*C. senegalensis*	Yaradua	SSY7	Mashi
9	*C. senegalensis*	Yaradua	SSY8	Mani
10	*C. senegalensis*	Yaradua	SSY9	Mani
11	*C. senegalensis*	Yaradua	SSY10	Daura
12	*C. senegalensis*	Yaradua	SSY11	Daura
13	*C. atrorubens*	Yaradua	SSY12	Sandamu
14	*C. atrorubens*	Yaradua	SSY13	Sandamu
15	*C. atrorubens*	Yaradua	SSY14	Kaita
16	*C. atrorubens*	Yaradua	SSY15	Kaita
17	*C. atrorubens*	Yaradua	SSY16	Kaita
18	*C. atrorubens*	Yaradua	SSY17	Mashi
19	*C. atrorubens*	Yaradua	SSY18	Mashi
20	*C. atrorubens*	Yaradua	SSY19	Jibia
21	*C. atrorubens*	Yaradua	SSY20	Jibia
22	*C. atrorubens*	Yaradua	SSY21	Jibia
23	*C. goreensis*	Mustapha	SSY22	Mani
24	*C. goreensis*	Yaradua	SSY23	Jibia
25	*C. goreensis*	Mustapha	SSY24	Jibia
26	*C. goreensis*	Yaradua	SSY25	Jibia
27	*C. goreensis*	Yaradua	SSY26	Jibia
28	*C. goreensis*	Yaradua	SSY27	Jibia
29	*C. retusa*	Yaradua and Bello	Bello395	Kaita
30	*C. pallida*	Yaradua	SSY28	Jibia
31	*C. pallida*	Yaradua and Bello	Bello396	Jibia
32	*C. pallida*	Yaradua and Bello	Bello397	Daura
33	*C. pallida*	Yaradua and Bello	Bello398	Daura
34	*C. pallida*	Yaradua and Bello	Bello399	Daura
35	*C. pallida*	Yaradua and Bello	Bello340	Daura
36	*C. pallida*	Bello	Bello341	Mani
37	*C. pallida*	Bello	Bello342	Mani
38	*C. pallida*	Bello	Bello343	Jibia
39	*C. pallida*	Yaradua	SSY28	Kaita
40	*C. pallida* var. *obovata*	Yaradua and Bello	Bello344	Kaita
41	*C. pallida* var. *obovata*	Yaradua and Bello	Bello345	Kaita
42	*C. pallida* var. *obovata*	Bello	Bello346	Kaita
43	*C. pallida* var. *obovata*	Yaradua and Bello	Bello347	Kaita
44	*C. pallida* var. *obovata*	Yaradua	SSY29	Kaita
45	*C. pallida* var. *obovata*	Yaradua and Bello	Bello348	Jibia
46	*C. pallida* var. *obovata*	Yaradua and Bello	Bello349	Jibia
47	*C. pallida* var. *obovata*	Yaradua and Bello	Bello350	Daura
48	*C. pallida* var. *obovata*	Yaradua and Bello	Bello351	Daura
49	*C. pallida* var. *obovata*	Yaradua and Bello	Bello352	Daura

2. Materials and methods

2.1. Taxon sampling

Specimens were collected in the field to study their vegetative and floral morphology. The specimens were pressed carefully, to not affect their vegetative and floral characters. Morphological measurements of the characters were made immediately. In addition, some dried specimens from the herbarium of Umaru Musa Yaradua University, Katsina (herbarium acronym) were used in the study. The total numbers of 49 individuals representing 7 species were found and used in the morphometric analyses. The details of the taxa included in the analysis are given in Table 1.

2.2. Morphological characters

A character set, consisting of 21 characters for morphometric analysis of *Crotalaria*, was adapted from the character set used previously by Le Roux *et al*. (2013) and Britto *et al.* (2011) with some slight modifications. Out of the 21 characters, 15 were quantitative and 6 were qualitative (Table 2).

2.3. Multivariate analysis

Multivariate analyses were carried out by cluster analysis (CA), principal components analysis (PCA) and Jaccards similarity coefficient using PAST 3 program (version 3.05) to determine the morphological similarity among the species and the characters that contribute to the variation of the taxa.

2.4. Cluster analysis

Cluster analysis is a multivariate data mining technique, whose goal is to group objects based on a set of user selected characteristics. The cluster analysis groups individuals that are very similar in one cluster and shows similarities and differences among and within the clusters by building similarity matrix between the studied individuals. Individuals in the same cluster will be recognized as belonging to the same species. The cophenetic correlation coefficient between the distance matrix and the tree matrix was calculated to examine how well the cluster analysis fits the distance matrix (Sokal & Rohlf 1997; Rohlf 1998).

2.5. Principal Component Analysis (PCA)

PCA is an accepted method to study multivariate character correlation. Its purpose is to study linearly correlated variables (Hotelling 1933, 1936). PCA enables the distinguishing of groups as well as identification of

Table 2. List of quantitative and qualitative characters and their characteristics used in morphometric analysis

S/No	Character	States
	Quantitative characters	
1	Number of leaflet (Nl)	
2	Length of petiole (Lp)	mm
3	Length of leaflet (Ll)	mm
4	Width of leaflet (Wl)	mm
5	Length of fruit (Lf)	mm
6	Length of seed (Ls)	mm
7	Length of pedicel (Lpd)	mm
8	Number of flowers per axis	
9	Length of calyx (Lc)	mm
10	Number of seeds (Ns)	
11	Length of standard petal (Lsp)	mm
12	Width of standard petal (Wsp)	mm
13	Length of wing petal (Lwp)	mm
14	Width of wing petal (Wwp)	mm
15	Length of keel petal (Lkp)	mm
	Qualitative characters	
16	Habit (H)	Herb (0), small shrub (1)
17	Life form (lf)	Annual (0), Perennial (1)
18	Presence of hair on stem(Ph)	Presence (0), Absent (1)
19	Shape of leaflet (Sl)	Lanceolate (0), Spatulate (1), Elliptic (2), Cuneate (3), Obovate (4)
20	Inflorescence position (Ip)	Terminal (0) Axial (1)
21	Pod size (Ps)	Slightly exceeding calyx (0), far exceeding calyx (1)

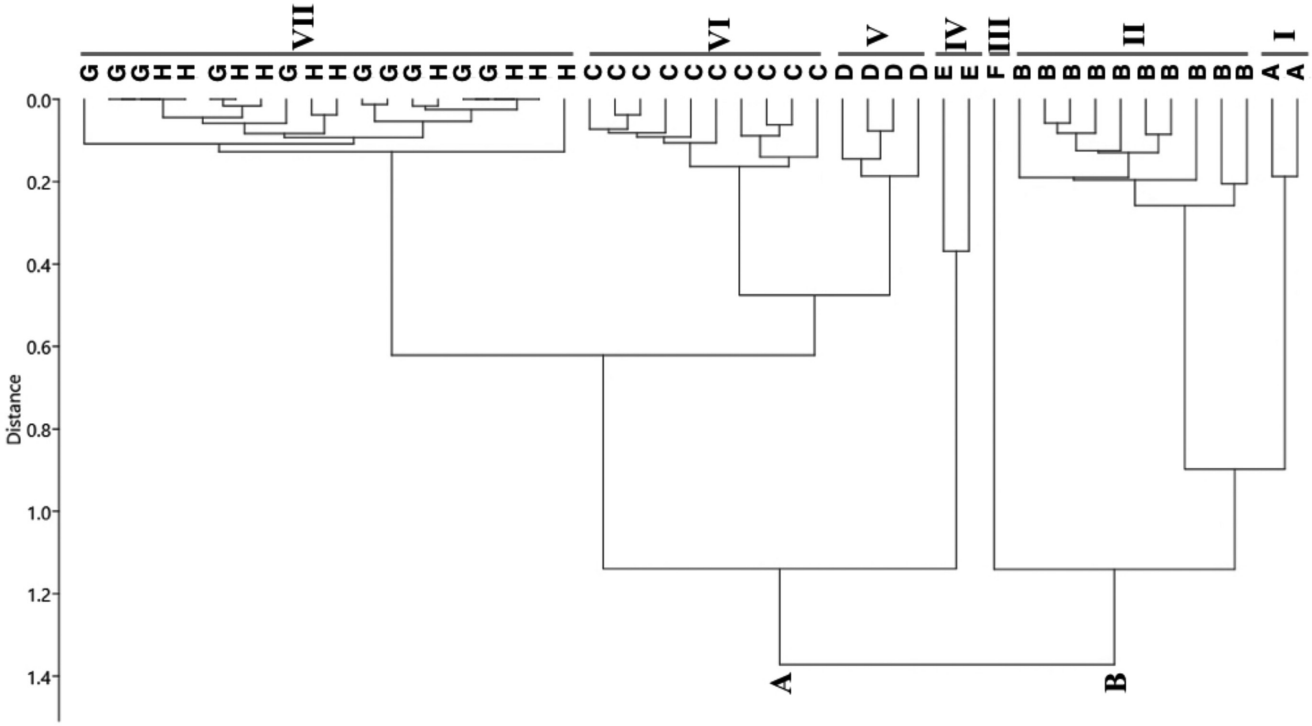

Fig. 1. Unweighted Pair Group Method with Arithmetic mean (UPGMA) phenogram resulting from cluster analysis. Cophenetic correlation (r)=0.964

the relative contribution of size dependent and size-independent variation to species discrimination (Humphries *et al*. 1981). Therefore, it will show those characters that are useful in showing delimitation among the species. The Jaccards similarity coefficient was calculated to found the morphological similarity among the species. All the 21 morphological characters, including both quantitative and qualitative traits, for all the 49 specimens were used for the analysis and each individual specimen was considered as an operational taxonomic unit (OUT).

In all the analysis, the data were first log10 transformed for the standardization of the data matrix.

3. Results

3.1. Cluster analysis

The result of the cluster analysis separates the data set into seven clusters (at Euclidian distance of 0.41; Fig. 1), and the cophenetic correlation coefficient value of r=0.964 indicates a perfect match between the triangular distance matrix and the phenogram (Sneath & Sokal 1973; Rohfl 1998). The groups were recognized as distinct taxa at different taxonomic hierarchies, if all their OTUs did not mix between clusters. All the a *priori* groups formed distinct clusters with the exception of G (*C. pallida* var. *pallida*) and H (*C. pallida* var. *obovata*)

Table 3. Similarity matrix based on Jaccard's coefficient

	C. macrocalyx	*C. atrorubens*	*C. senegalensis*	*C. goreensis*	*C. retusa*	*C. barkae*	*C. pallida*	*C. pallida* var. *obovata*
C. macrocalyx	1	0.933333	1	1	1	0.933333	1	1
C. atrorubens	0.933333	1	0.933333	0.933333	0.933333	0.866667	0.933333	0.933333
C. senegalensis	1	0.933333	1	1	1	0.933333	1	1
C. goreensis	1	0.933333	1	1	1	0.933333	1	1
C. retusa	1	0.933333	1	1	1	0.933333	1	1
C. barkae	0.933333	0.866667	0.933333	0.933333	0.933333	1	0.933333	0.933333
C. pallida	1	0.933333	1	1	1	0.933333	1	1
C. pallida var. *obovata*	1	0.933333	1	1	1	0.933333	1	1

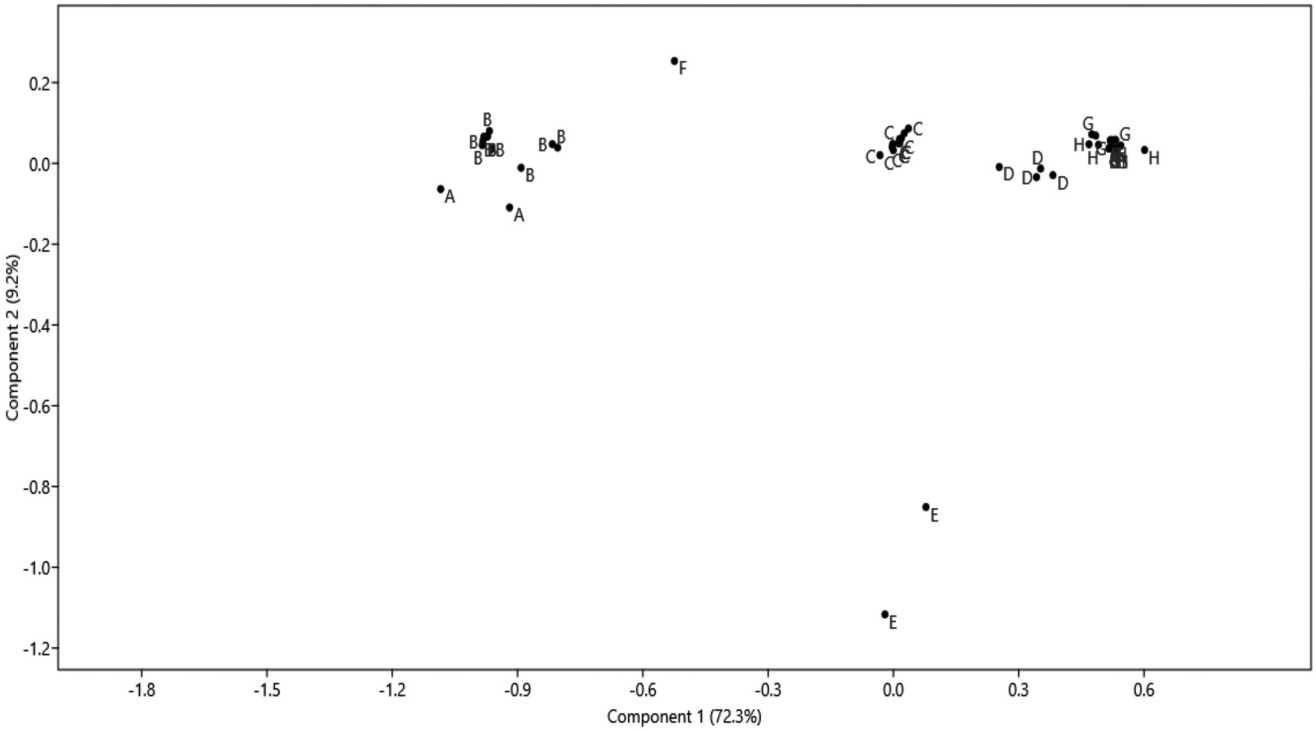

Fig. 2. Plot of the first two principal component analyses (PCA) obtained from the analysis of the morphological data set for specimens of the studied species within the genus *Crotalaria*. The first and second PCA axes explain 72.3% and 9.2% of the total variation among all the taxa, respectively

Explanations: A – *Crotalaria macrocalyx*, B – *C. atrorubens*, C – *C. senegalensis*, D – *C. goreensis*, E – *C. retusa*, F – *C. barkae*, G – *C. pallida* var. *pallida*, H – *C. pallida* var. *obovata*

whose specimens intermixed and formed a large cluster (cluster VII).This is because they are the same species, and H is a variety of G.

Result of the cluster analysis showed the relationship among the studied seven species. Two major clusters (A and B) were found in the UPGMA dendogram with sub-clusters within them. *C. macrocalyx* and *C. atrorubes* were clustered together in one sub-cluster. This showed that they are closely similar. This sub-cluster was connected with *C. barkae* forming a large cluster B.

In cluster A, *C. senegalensis* was grouped with *C. goreensis* in a sub-cluster, which indicates that the two species share some similarities.This sub-cluster toghether with a sub-cluster contaning *C. pallida* and *C. pallida* var. *obovata* and a sub-cluster of *C. retusa* formed the large cluster A. The grouping indicates that members of each group are more similar to each other than to the members of other groups.

The results of the Jaccard's similarity coefficient varied between 0.867 and 0.993,indicating closer relationships among the species (Table 3).

3.2. Ordination

The ordination analysis based on the result of PCA separated 49 specimens into 7 groups corresponding largely to those obtained in the cluster analysis (Fig. 2). Principal component 1 accounted for 72.3% of the variation, while principal component 2 accounted for 9.2% of the variation (Fig. 1). The loading of the PC 1 and 2 is presented in Table 4. The character mostly correlated with the first PCA axis (r>0.50) is the length of petiole – 0.77, while the character correlated with the second PCA axis (r>0.50) is the number of flowers per axis – 0.87.

The result of the PCA showed that the majority of the clusters in the ordination plot, corresponds largely to those obtained by cluster analysis. The length of petiole, width of leaflet and length of fruit contributed more to showing delimitation among the species (Fig. 3).

The *priori* groups identified in cluster analysis (Euclidian distance 0.41, Fig. 1) were supported by the ordination analysis.

3.3. Taxonomic description and revision

Crotalaria macrocalyx Benth.

London Journal of Botany 2: 572 (1843) Type: *Heudelot M. 205* (P, holotype)

Herb, annual; leaves 3-foliate, lanceolate, petiole length 3-5 mm, leaflet length 42-45 mm, leaflet width 8-10 mm, inflorescence terminal with 6-8 flowers, calyx length 9-11mm, standard petal length 6-8 mm, standard petal width 8-10 mm, wing petal length 9-11 mm, wing

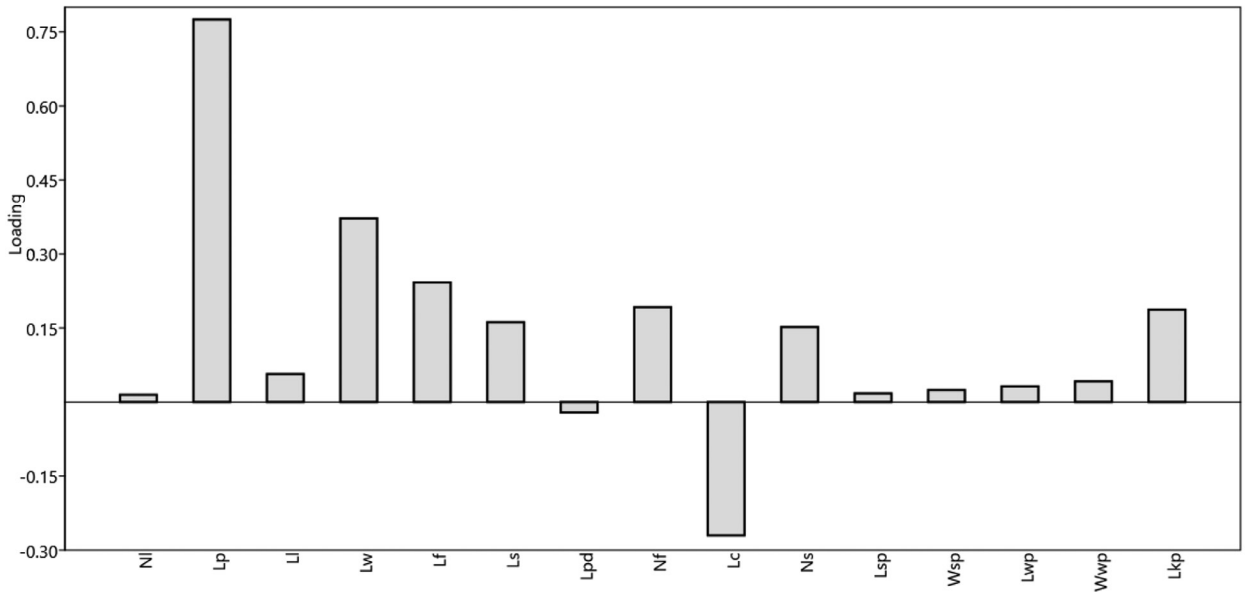

Fig. 3. PCA loading of the characters shows the PCA loading of the contribution of each character to similarities among the species. The length of petiole and length of leaflet have higher loading in the result

petal width 5-7 mm, fruit length 6-8 mm, pods slightly exceeding calyx, seed length 2-3 mm, hairs present on stem.

Crotalaria atrorubens Benth.

London Journal of Botany 2:572 (1843) Type (Isotype)

Shrub, annual, leaves 3-foliate; spatulate, leaflet length 30-33 mm, leaflet width 6-8 mm, petiole length 4-6 mm, inflorescence terminal with 7-8 flowers, calyx length 10-12 mm, standard petal length 8-10 mm, standard petal width 8-10 mm, wing petal length 7-9 mm, wing petal width 5mm, fruit length 8-10 mm, pods slightly exceeding calyx, seed number 9-11, seed length 1-2 mm, pedicel length 2-4 mm, hairs present on the stem.

Table 4. Loadings of the first and second components of the principal components analysis

Axis	Eigenvalue	% variance
1	0.3524410	72.337
2	0.0450442	9.2451
3	0.0368382	7.5608
4	0.0254375	5.2209
5	0.0179612	3.6864
6	0.0048452	0.99445
7	0.0012719	0.26105
8	0.0011449	0.23499
9	0.0007317	0.15018
10	0.0005689	0.11676
11	0.0003361	0.068992
12	0.0002474	0.050785
13	0.0001870	0.03839
14	0.0001280	0.026278
15	4.00E-05	0.0082159

Crotalaria senegalensis (Pers.) Bacle ex DC.

Prodromus Systematis Naturalis Regni Vegetabilis 2: 133 (1825) Type: *Adanson 24* (MPU, isotype).

Shrub, annual, leaves 3- foliate; elliptic, leaflet length 33-35 mm, leaflet width 10-12 mm, petiole length 20-22 mm, inflorescence terminal or axial with many flowers, calyx length 3-5 mm, standard petal length 10-12 mm, standard petal width 7-9 mm, wing petal length 5-7 mm, wing petal width 5-7 mm, fruit length 9-11 mm, pods far exceeding calyx, number of seed 10, seed length 1-2 mm, length of pedicel 3 mm, hairs present on stem.

Crotalaria goreensis Guill. & Perr.

Florae Senegambiae Tentamen 1: 165 (1832) Type: *Leprieur & Perrottet number* (P, protologue)

Shrub, annual, leaves 3-foliate; elliptic, leaflet length 40-42 mm, leaflet width 17-19 mm, petiole length 40-42 mm, inflorescence terminal or axial with many flowers, calyx length 6-8 mm, standard petal length 8-10 mm, standard petal width 10-12 mm, wing petal length 12-14 mm, wing petal width 7-9 mm, fruit length 15-17 mm, pods far exceeding calyx, seed number 10-12, seed length 3-5 mm, pedicel length 3-5 mm, hairs present on the stem.

Crotalaria barkae Schweinf

Bull. Herb. Boissier 4 (App.2): 226 (1896)

Shrub, annual, leaves 3-foliate; elliptic, leaflet length 30-32 mm, leaflet width 13-15 mm, petiole length 30-

32 mm, inflorescence terminal with 1-3 flower, calyx length 4-6 mm, standard petal length 8-10 mm, standard petal width 7-9 mm, wing petal length 6-8 mm, wing petal width 3-5 mm, fruit length 16-18 mm, pods size far exceeding calyx, seed number 10-12, seed length 2-4 mm, pedicel length 3-5.5 mm, hairs present on the stem.

Crotalaria pallida var. *obovate* (G. Don) Polhill

Kew Bull, 22: 265 (1968) Type: *Polhill* number (BM, holotype)

Shrub, annual, leaves 3-foliate; obovate, leaflet length 40-42 mm, leaflet width 23-25 mm, petiole length 38-40 mm, inflorescence terminal or axial with many flowers, calyx length 4-6 mm, standard petal length 7-9 mm, standard petal width 6-8 mm, wing petal length 7-9 mm, wing petal width 57 mm, fruit length 20-22 mm, pods far exceeding calyx, number of seed 12, seed length 1-2 mm, pedicel length 1-3 mm, hairs present on the stem 2 mm, length of pedicel 2 mm, hair presence.

Crotalaria pallida Aiton

Hortus Kewensis 3:20-21 (1789) Type: *James Bruce s.n* (BM, holotype)

Shrub, annual, leaves 3-foliate; elliptic, leaflet length 40-42 mm, leaflet width 23-25 mm, petiole length 38-40 mm, inflorescence terminal or axial with many flowers, calyx length 4-6 mm, standard petal length 7-9 mm, standard petal width 6-8 mm, wing petal length 7-9 mm, wing petal width 57 mm, fruit length 20-22 mm, pods far exceeding calyx, number of seed 12, seed length 1-2 mm, pedicel length 1-3 mm, hair present on the stem 2 mm, length of pedicel 2 mm, hair presence.

Crotalaria retusa L.

Species Plantarum 2:715 (1753) Type: *Herman 84* (BM, lectotype)

Shrub, perennial, leaves simple, cuneate, leaflet length 50-52 mm, leaflet width 12-14 mm, length of petiole 3-5 mm, inflorescence terminal with many flowers, calyx length 8-10 mm, standard petal length 12-14 mm, standard petal width 9-11 mm, wing petal length 12-14 mm, wing petal width 5-7 mm, fruit length 33-35 mm, pods far exceeding calyx, seed number 14-16, seed length 2-4 mm, pedicel length 6-8 mm, hair absent.

4. Discussion

The following representatives of *Crotalaria* were found during field work and included in this study: *C. senegalensis, C. goreensis, C. retusa, C. pallida, C. macrocalyx, C. pallida* var. *obovata*, *C. atrorubens* and *C. barkae*. Odewo *et al*. (2015) in their study on ecological distribution of the genus *Crotalaria* in Nigeria reported only two species from Katsina – *C. naragutensis* and *C. ononoides*. However, *C. ononoidesis* has not been found in this study; a species morphologically resembling *Crotalaria naragutensis*, i.e., *Crotalaria pallida* and 7 other species were reported from Katsina. Chromosome counts and cytomorphological studies of *Crotalaria* from Northern Nigeria conducted by Adelanwa *et al*. (2014) reported nine species. *C. ononoides* reported by Odewo *et al.* (2015) was wrongly identified. The specimens were not *C. ononoides*, because morphometric analysis showed that morphological features of these specimens did not match the type specimen of *C. ononoides.* For example, the leaf shape of the species in question is spatulate, while that of *C. ononoides* is lanceolate to elliptic or obovate. The species 3-foliate leaves are unequal in size, while that of *C. ononoides* are equal in size. The length of the fruit is 8 mm, while for *C. ononoides* is 12mm. The discussed species was correctly identified as *C. atrorubens.*

Britto *et al*. (2011) reported that phenetics proves its robustness in identifying species similarity, instead of relying on few vegetative characters, which creates a great confusion in identifying species. One of accepted infrageneric classification systems of the genus *Crotalaria* was based on morphometrics (Bisby 1973; Bisby & Polhill 1973). Morphological characters, both vegetative and generative, were used for constructing classifications (Agyeno *et al*. 2014a). Similarly, Jayeola (2001) reported the efficiency of utilizing vegetative and floral parts in numerical evaluation of similarities among taxa. Agyeno *et al*. (2014b) reported that morphology of leaf, habit and life span played a very important role in delimiting members of the genus *Crotalaria* due to their discontinuity or discreteness. Findings of this study agreed with his findings, because leaf morphology is the character that showed a great variation among the sampled species in this study. The study of Raj *et al*. (2011) also highlighted that qualitative characters, such as habit and leaf type, and quantitative characters, such as the pod length, seed number and petiole length, are phylogenetically important. Findings of this study also prove that morphological characters are effective in showing similarities among the species within the studied genus. This study considered some characters, such as petiole length, which were not reported earlier, and these characters were found to be effective in morphometric analysis of the genus.

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