TAXONOMIC STUDIES ON THE GENUS *INDIGOFERA* Linn. (INDIGOFEREAE, FABACEAE) IN PARTS OF KATSINA AND KADUNA STATES, NORTH-WESTERN NIGERIA

Aliyu, H.[1]*, Sulaiman, S. K.[2] and Abubakar, B.[2]

[1]Department of Biology, Umaru Musa Yar'Adua University, Katsina, Nigeria
[2]Centre for Biodiversity and Conservation, Department of Biology, Umaru Musa Yar'Adua University, Katsina, Nigeria

Corresponding**:** aliyu.habib@umyu.edu.ng

ABSTRACT

Indigofera is a large genus in the family Fabaceae comprising about 750 species. In Nigeria, particularly the northwestern savanna region, identification and naming of *Indigofera* species have been very difficult, ambiguous and controversial for a long time. Thus, proper identification and naming of the species within this genus are paramount. Several field surveys were carried out in the study area and twelve species were successfully collected and identified. Multivariate analyses (CA and PCA) were used to determine whether the species are distinct or not, and to identify the most useful characters for the identification of the species in the region. Fresh specimens were collected purposely for this work and herbarium specimens were used. The result of the cluster analysis revealed 12 distinct clusters (at an Euclidean distance of 0.08) with a cophenetic correlation coefficient value (r) of 0.89. The result of the PCA revealed 12 distinct groups. The character loadings indicated that number of seeds per pod, petiole length, leaflet length, internode, fruit length and leaflet width contributed the highest variation among the species, which could be more useful in delimiting the species. These characters can, therefore, be used in the identification and distinguishing of *Indigofera* species in the region. We provided a key for the identification of the species.

Keywords: Legumes, Morphometric, Papilionoideae, Savanna, Taxonomy

INTRODUCTION

Indigofera L. is one of the largest genera in the family Fabaceae with over 750 species of flowering plants. (Paulino *et al.,* 2011). They are widely distributed throughout the tropical and subtropical regions of the world (Paulino *et al.,* 2011). Hutchinson and Dalziel (1968) recognised 78 species of *Indigofera* in West Africa. Sixty (60) species were recognised by Burkill (1995) while Soladoye and Lewis (2003) recorded 60 species in Nigeria with over 60% abundance in the northern region of the country and about 27 species distributed across the South-Western region of the country. Most of these species are in the Savanna ecological zone with a few present in the rainforest area (Hutchinson and Dalziel, 1968).

  Indigofera species are mostly shrubs, though some are small trees or annual or perennial herbs. Most of the species have pinnate leaves. The racemes of flowers grow in the leaf axils. Most of the species have flowers

*Author for correspondence

in hues of red, but there are a few white- and yellow-flowered species. The fruit is a legume pod of varying size and shape (Paulino *et al.,* 2011). *Indigofera* is a varied genus that has shown unique characteristics that make it an interesting candidate as a potential perennial crop. Specifically, there is diverse variation among species with a number of unique characteristics. Some examples of this diversity include differences in pericarp thickness, fruit type and flowering morphology.

Indigofera is a diverse genus that shows some unique characteristics in its flowering morphology. For example, one unique characteristic of *Indigofera* is that it demonstrates an open carpel which is not seen among many species. The types of fruit produced by different species of *Indigofera* can be divided into broad categories that show great variation. The three basic types of fruit categories can be separated by their curvature including straight, slightly curved and falcate. In addition, several of the species including *I. microcarpa* Desv., *I. suffruticosa* Mill. *and I. enneaphylla* L. have shown delayed dehiscent fruits (Leite *et al.,* 2009). Another way to categorise *Indigofera* is by its pericarp thickness. The pericarp can be categorised as type I, type II and type III with type I having the thinnest pericarp and fewest layers of schlerenchymatous layers and type III having the thickest pericarp and most layers of schlerenchymatous layers. Most fruits of *Indigofera* show normal explosive dehiscence to disperse seeds (Chauhan and Pandey, 2014). Economic and aesthetic uses have long been reported for members of this genus. Some are grown for ornamental purposes, but by and large major interest in the genus has focused on the economic use of *Indigofera* as a source of the deep blue dye, indigo.

The species within the genus *Indigofera* are very diverse. Even though they have many features in common; the species also differ in some of their morphological features. In northern Nigeria, *Indigofera* species are poorly collected and their classification seems very difficult, controversial and ambiguous. Some of the species are also wrongly classified and named while some bear similar names in most of the herbaria of the region. Also, the species within this genus are not easily identifiable and distinguishable. Some of the species also show close resemblance within the genus and with other species from related genus especially the genus *Tephrosia* L. because sometimes *Indigofera* species are classified as *Tephrosia* species and *vice-versa* by students, herbarium keepers and other plant collectors. Therefore, there is the need to identify common morphological characters that can be very useful in simplifying the identification of the species and in distinguishing between the species within the genus *Indigofera* in the region.

MATERIALS AND METHODS

Study area

All specimens were collected in Katsina and Kaduna States, North-Western Nigeria. Katsina State covers an area of 23,938 square kilometres and is located between latitude 12°15'N and longitude 07°30'E (Fig 1). Kaduna State covers an area of about 46,053 square kilometres and is located between latitude 10°20'N and longitude 07°45'E (Fig 1).
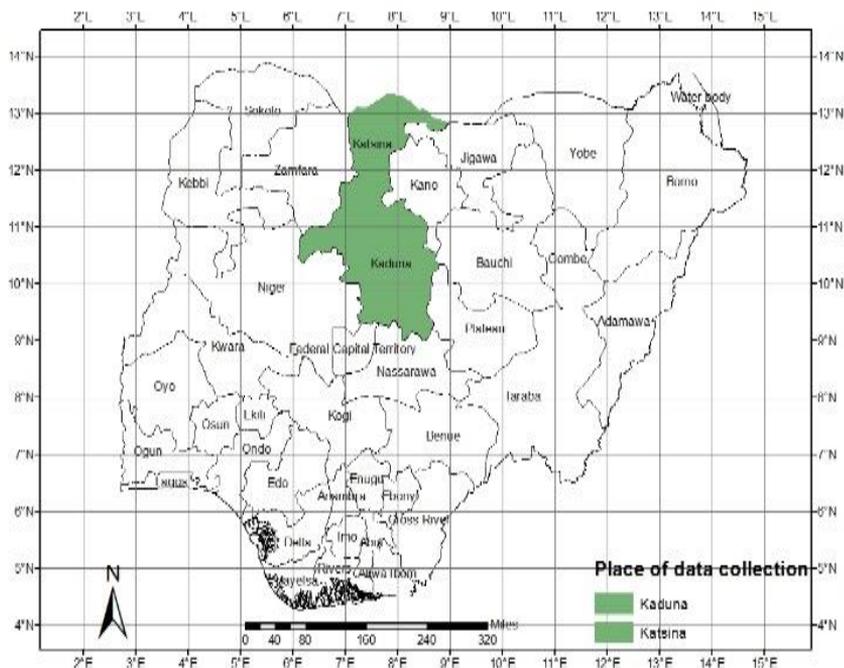
Figure 1: Map of Nigeria indicating the study area

Taxa sampling

Specimens were collected and identified using herbarium specimens (ABU and UMYU), identification keys (International Legume Database and Information Service (ILDIS); https://www.ildis.org/), online photographs ("African plants - A Photo Guide" (www.africanplants.senckenberg.de) and flora [Flora of West Tropical Africa (FWTA)]. A field record of the collected specimens was recorded which includes the date of collection, location, collection-number, name of the specimen, photos and GPS record. Immediately after collection, the specimens were pressed in the field and, where possible, identified. Some specimens of UMYU herbarium were included in this study. A total number of 60 specimens (Table 1) from 12 distinct species were used for morphological studies.

Table 1: List of specimens used for the study

	Species	Colllector	Voucher No	Location
1	*Indigofera arrecta*	Aliyu	AH 001A	S/Gari Zaria
2	*Indigofera arrecta*	Aliyu	AH 001B	S/Gari Zaria
3	*Indigofera arrecta*	Aliyu	AH 013A	R/Galma Zaria
4	*Indigofera arrecta*	Aliyu	AH 013B	R/Galma Zaria
5	*Indigofera arrecta*	Aliyu	AH 025	Hunkuyi
6	*Indigofera conferta*	Aliyu	AH 002A	ShikaDam Zaria
7	*Indigofera conferta*	Aliyu	AH 002B	ShikaDam Zaria
8	*Indigofera conferta*	Aliyu	AH 014A	Basawa Zaria
9	*Indigofera conferta*	Aliyu	AH 014B	Basawa
10	*Indigofera conferta*	Aliyu	AH 026	Samaru
11	*Indigofera nummulariifolia*	Aliyu	AH 003A	Kakiyayi
12	*Indigofera nummulariifolia*	Aliyu	AH 003B	Kakiyayi
13	*Indigofera nummulariifolia*	Nafisa	879	Katsina
14	*Indigofera nummulariifolia*	Aliyu	AH 015A	Malumfashi
15	*Indigofera nummulariifolia*	Aliyu	AH 027	Batagarawa
16	*Indigofera pulchra*	Aliyu	AH 004A	Zaria
17	*Indigofera pulchra*	Aliyu	AH 004B	Zaria
18	*Indigofera pulchra*	Aliyu	AH 016A	Kaduna
19	*Indigofera pulchra*	Aliyu	AH 016B	Kaduna
20	*Indigofera pulchra*	Aliyu	AH 028	Shika Dam
21	*Indigofera astragalina*	Aliyu	AH 005	Zaria
22	*Indigofera astragalina*	Surajo	124	Katsina
23	*Indigofera astragalina*	Aliyu	AH 017A	Malumfashi
24	*Indigofera astragalina*	Abdullahi	505	Katsina
25	*Indigofera astragalina*	Aliyu	AH 029	Kafur
26	*Indigofera stenophylla*	Aliyu	AH 006A	Zaria
27	*Indigofera stenophylla*	Aliyu	AH 006B	Zaria
28	*Indigofera stenophylla*	Aliyu	AH 018	Katsina
29	*Indigofera stenophylla*	Suleiman	808	Katsina
20	*Indigofera stenophylla*	Aliyu	AH 030	Malumfashi
31	*Indigofera secundiflora*	Aliyu	AH 007A	Tudun kusa
32	*Indigofera secundiflora*	Aliyu	AH 007B	Tudun kusa
33	*Indigofera secundiflora*	Aliyu	AH 019A	Zaria
34	*Indigofera secundiflora*	Aliyu	AH 019B	Zaria
35	*Indigofera secundiflora*	Aliyu	AH 031	Hunkuyi
36	*Indigofera bracteolata*	Aliyu	AH 008	Katsina
37	*Indigofera bracteolata*	Ibrahim	546	Batagarawa
38	*Indigofera bracteolata*	Aliyu	AH 020	Zaria

39	*Indigofera bracteolata*	Muhammad	691	Katsina
40	*Indigofera bracteolata*	Aliyu	AH 032	Funtua
41	*Indigofera polyphylla*	Aliyu	AH 009A	Katsina
42	*Indigofera polyphylla*	Aliyu	AH 009B	Katsina
43	*Indigofera polyphylla*	Aliyu	AH 021A	Katsina
44	*Indigofera polyphylla*	Aliyu	AH 021B	Katsina
45	*Indigofera polyphylla*	Aliyu	AH 033	Katsina
46	*Indigofera pilosa*	Aliyu	AH 010	Yar Haka
47	*Indigofera pilosa*	Olatunji	178	Batagarwa
48	*Indigofera pilosa*	Mani	1978	Katsina
49	*Indigofera pilosa*	Aliyu	AH 022	Tashar Bala
50	*Indigofera pilosa*	Aliyu	AH 034	Katsina
51	*Indigofera paniculata*	Aliyu	AH 011A	Katsina
52	*Indigofera paniculata*	Aliyu	AH 011B	Katsina
53	*Indigofera paniculata*	Aliyu	AH 023A	Yar Haka
54	*Indigofera paniculata*	Aliyu	AH 023B	Yar Haka
55	*Indigofera paniculata*	Aliyu	AH 035	Batagarawa
56	*Indigofera hirsuta*	Aliyu	AH 012	Katsina
57	*Indigofera hirsuta*	Tijjani	730	Katsina
58	*Indigofera hirsuta*	Aliyu	AH 024	Musawa
59	*Indigofera hirsuta*	Maryam	302	Batagarawa
60	*Indigofera hirsuta*	Aliyu	AH 036	Malumfashi

Morphometric measurements
A total number of 60 specimens from the 12 species collected were considered for the morphometric studies (Table 1). More than 20 characters were extensively analysed after which 17 characters were chosen for use (Table 2). These include ten (10) quantitative characters, five (5) qualitative characters and two (2) continuous characters. Leaves and flowers of the herbarium specimens were revived by soaking in warm water with a 2-3 drop of soap solution before measurements were taken. The length and width of the characters were measured using a 30 cm meter rule. Continuous characters were counted while qualitative characters were coded. The values were obtained by either measuring the quantitative characters, counting the continuous characters or by considering the code given to each qualitative character according to Bello (2015) (Table 2).

Multivariate analyses
Multivariate analyses were carried out by cluster analysis (CA) and principal component analysis (PCA) using PAST 3 program (version 3.05) (Sneath and Sokal, 1973). All the 17 morphological characters including both quantitative and qualitative were used in the cluster analysis while 12 quantitative characters were used in the principal component analysis and each individual specimen was considered as an Operational Taxonomic Unit (OTU). For all analyses, the data/values obtained were recorded in a Microsoft excel sheet and then transformed into Log10 in order to standardise the data matrix. Cluster analysis was used to cluster the specimens, as it is better in representing distances among similar specimens (Sneath and Sokal, 1973). The objectives of carrying out cluster analysis was to confirm the distinctness of the species and the principal component analysis was used to determine the characters that are most useful in identification or delimitation of the taxa.

Table 2: Morphometric characters used for multivariate analyses

S/N	Character	States/ code
1	Leaflet length (Ll)	mm
2	Leaflet width (Lw)	mm
3	Petiole length (Pl)	mm
4	Internode (In)	mm
5	Fruit length (Fl)	mm
6	Fruit width (Fw)	mm
7	Calyx length (Cxl)	mm
8	Corolla length (Crl)	mm
9	Rachis length (Rl)	mm
10	Pedicel length (Pl)	mm
11	Number of leaflets (Nl)	Continuous
12	Number of seeds per pod (Nsp)	Continuous
13	Leaflet shape (Ls)	oblong=1,elliptic=2, ovate=3, obovate=4,linear=5, lanceolate=6, oblanceolate=7, spatulate=8
14	Leaflets arrangement (La)	opposite=1, alternate=2
15	Fruit shape (Fs)	rounded=1, oval=2, sickle=3, tetragonal=4
16	Leaf type (Lt)	simple=1, pinnate=2

RESULTS

Clustering

The cluster analysis of the entire data set separated 60 specimens into 12 clusters (at a Euclidean distance of 0.08 (Fig. 2). The cophenetic correlation coefficient value of r = 0.89 obtained in the analysis indicates a very good fit between the triangular distance matrix and the phenerogam according to Sneath and Sokal (1973). All the groups were recognised as distinct taxa at different taxonomic hierarchies since all their Operational Taxonomic Units (OTUs) did not mix between clusters. All the *a priori* groups formed distinct clusters.

Ordination

The principal component analysis (PCA) presented in Fig. 3 also revealed twelve (12) distinct groups (Fig. 2). Principal component 1 accounted for 57.9% of the variation while principal component 2 accounted for 19.9% of variation among the taxa. The loadings of the PC 1 and PC 2 are presented in Figs. 4 and 5, respectively. The scree plot indicating the contribution of variation of PC 1-12 is also presented in Fig. 6. The loadings show that characters with higher contribution in the variation among the species are number of seeds per pod (NSP), petiole length (PL) , leaflet length (LL), internode (IN), fruit length (FL) and leaflet width (LW); these may be more useful in the identification and delimitation of the species. Pedicel length (PL), rachis length (RL), corolla length (CL), calyx length (CXL), fruit width (FW) and number of leaflets (NL) showed a high degree of similarities among the species and are, therefore, less useful in identifying and distinguishing the species within the genus. Eigen values and percentage of variance obtained from the principal component analysis are presented in Table 3.
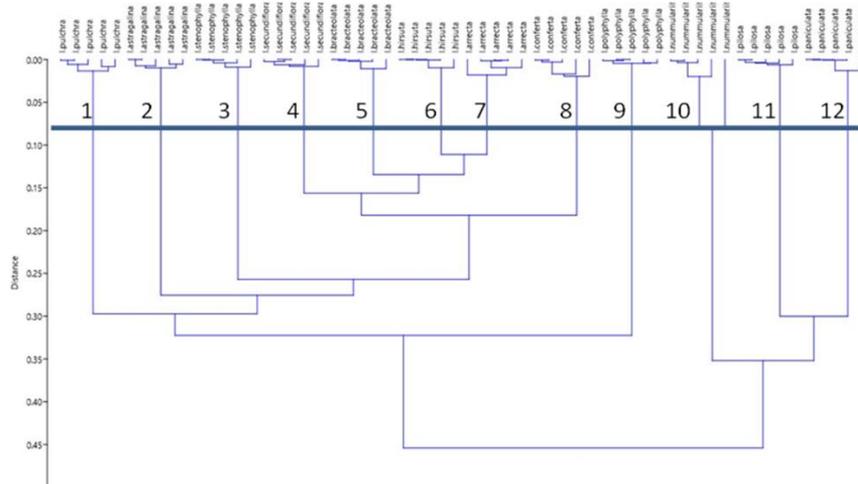
Figure 2. (UPGMA) Phenogram resulting from cluster analysis of the morphological data
(at an euclidean distance of 0.08). The vertical dark line indicates phenon line. The numbers
 (1-12) indicate distinct taxa recognisable at species level
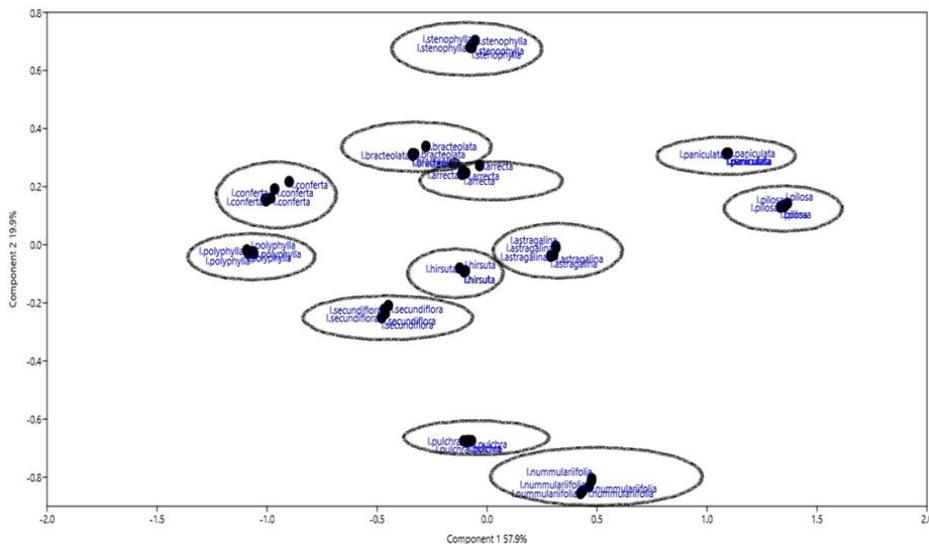

Figure 3. Scatter plot of the principal component analysis (PCA) of morphological variations among
the species obtained from the analysis of the morphological data. The circled black dots indicate distinct
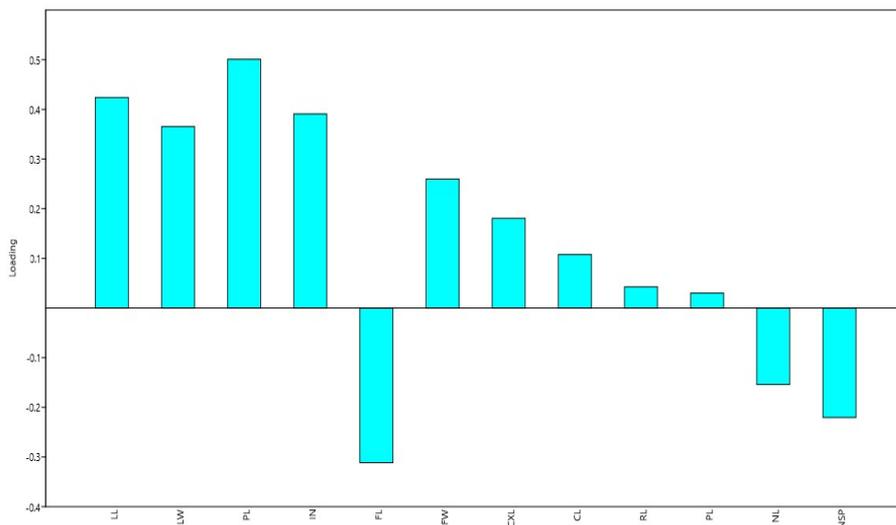clusters recognisable at species level.

Figure 4. PCA1 Loadings indicating morphological characters that have the highest and lowest contribution in the variation among the taxa.
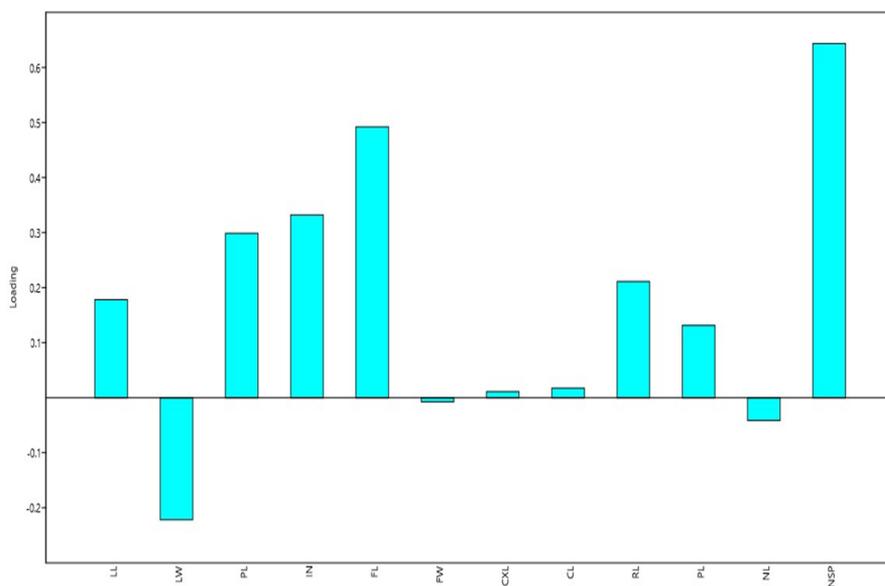

Figure 5: PC2 Loadings indicating morphological characters that have the highest and lowest in the variation among the species
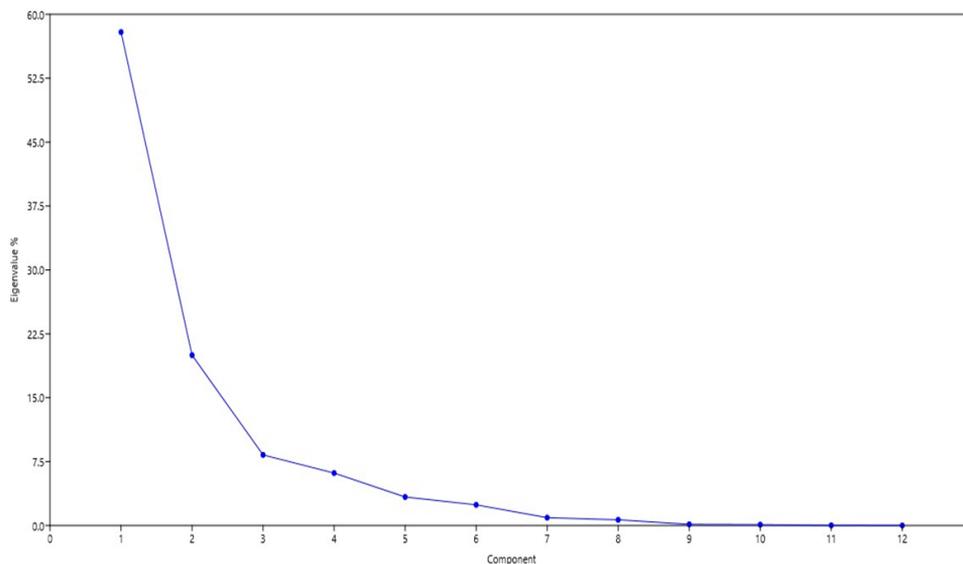
Figure 6: The Scree plot showing axes of Eigen values with percentage of variance and principal components indicating percentage contribution of principal components in the among the taxa

Table 3. Eigen values and percentage of variance obtained from PCA

PC	Eigen value	% variance
1	0.489057	57.913
2	0.16883	19.993
3	0.0699982	8.2891
4	0.0520036	6.1582
5	0.0283041	3.3517
6	0.0205275	2.4308
7	0.00781859	0.92586
8	0.00566268	0.67056
9	0.00118893	0.14079
10	0.000871961	0.10326
11	0.000181561	0.0215
12	2.05458E-05	0.002433

DISCUSSION

The result obtained from ordination analysis agrees with the findings of Soladoye *et al*. (2010) which revealed that number of leaflets, leaf length and leaf width have been found to significantly contribute to the delimitation of *Indigofera* species in South-Western Nigeria.

Multivariate analyses showed that *Indigofera bracteolata* is easily distinguished from other species by its suffruticose stem which is trailing or suberect, firm, slender branches, linear stipules, very reduced or short petiole, rounded or mucronate leaflet apex and a silky calyx (Plate 1), while *I. hirsuta* is a true perennial with conspicuous brown and rusty hairs on the stems and the stems are either striate or erect which become woody as the plant matures. Apical leaflets are longer. Flowers are purely papilionaceous, hairy, red to pink in colour, fruits are straight, cylindrical with dehiscent pods and cube-shaped, blotched seeds (Plate 2; Bello *et al.*. 2020 unpublished).

A=Habit, B=Flower and C=Fruit of *I. bracteolata*

Plate 1: Morphology of *Indigofera bracteolata* showing habit, flower and fruit

A=Habit and B=Flower of *I. hirsuta*

Plate 2: Morphology of *Indigofera hirsuta* showing habit and flower

Indigofera pilosa is the only prostrate branching species, short-lived perennial with lanceolate-acuminate stipules which is wide at the base, raceme of 5-20 flowers, divided calyx at the base, linear lobes and tetragonal-subcylindric pods (Plate 3) while *I. nummulariifolia* is unique for its creeping nature, a stem which is branched from the base, simple orbicular leaves, obcordate leaflet apex, hispidly hairy calyx which is deeply divided, sickle-shaped fruit and a falcate, thorny pod with spines (Plate 4; Bello *et al.,* 2020 unpublished).
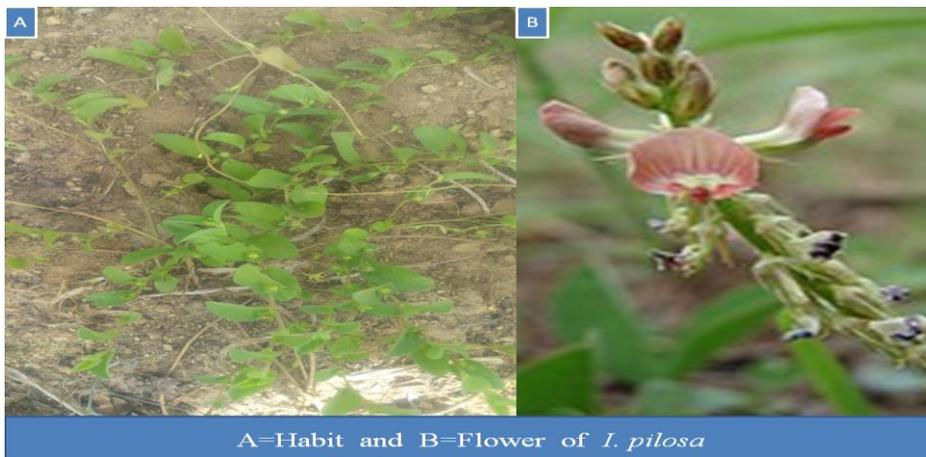

A=Habit and B=Flower of *I. pilosa*

Plate 3: Morphology of *Indigofera pilosa* showing habit, flower and fruit

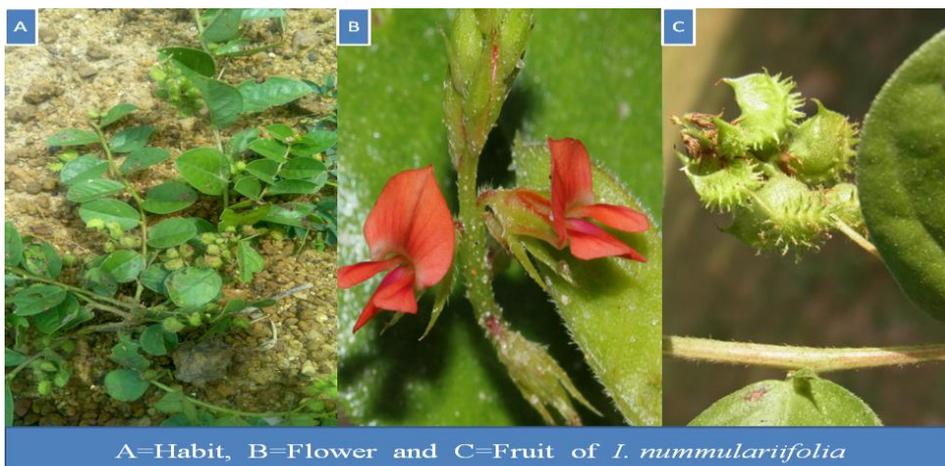A=Habit, B=Flower and C=Fruit of *I. nummulariifolia*

Plate 4: Morphology of *Indigofera nummulariifolia* showing habit, flower and fruit

Indigofera paniculata is unique due to its strigose stem, linear-lanceolate leaflets which are longer than in any other species up to 80 mm, foliaceous lower bracts, straght pedicels but jointed below the flower, longer style, hispid pod and not spotted endocarp (Plate 5) while *I. polyphylla* can be distinguished among the other related species for its striate stem, densely spreading trichomes covering all the body parts, very reduced petiole which is sometimes absent; absence of stipels, invisible secondary veins, cuneate leaflet base and obtuse leaflet apex, many seeded 10-14 per pod and gum property (Plate 6; Bello *et al.,* 2020 unpublished).

Plate 5: Morphology of *Indigofera paniculata* showing habit, flower and fruit

Plate 6: Morphology of *Indigofera polyphylla* showing habit, flower and fruit

Indigofera conferta can easily be distinguished by its glandular hairs on the stem, mucronate leaflet apex, stiffy erect pedicel and compact inflorescence which is sessile in the axils of the leaves (Plate 7) while *I. astragalina* differs from other species by its pilose stem, stiff hairs in all its parts, imparipinnately compound leaves, white inflorescence, caducuous bracts, pale corolla and tetragonal fruits in bunches (Plate 8).

Plate 7: Morphology of *Indigofera conferta* showing habit, flower and fruit

Plate 8: Morphology of *Indigofera astragalina* showing habit, flower and fruit

Indigofera arrecta is distinct by its slightly ridged and copiously branched stem which is covered with appressed, whitish or brownish hairs, bristle-like, 2–9 mm long stipules, spirally, imparipinnate leaves, Petiole which is thickened at base, sessile inflorescence, bisexual flowers, free and united stamens and superior ovary (Plate 9), while *I. stenophylla* is distinct being sparsely strigose with triangular-subulate stipules, leaflet narrowly linear, 10-30 flowered racemes, stiffly erect pedicels, calyx strigose which is divided at the base, corolla with white pubescent, stiffly erect pod with sutures, pitted seeds and a beautifully spotted endocarp (Plate 10; Bello et al. 2020 unpublished).
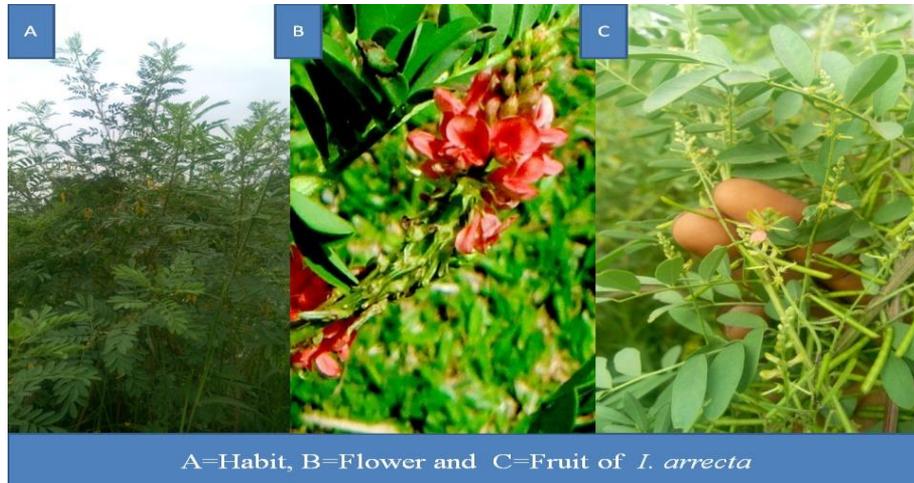
Plate 9: Morphology of *Indigofera arrecta* showing habit, flower and fruit

Plate 10: Morphology of *Indigofera stenophylla* showing habit, flower and fruit

Indigofera secundiflora is distinguishable due to its densely covered stem with white biramous hairs, subulate-setaceous stipules, leaflets with appressed hairs on both sides, many flowered racemes, linear, setose bracts, setose calyx with glandular hairs and triangular lobes, oval pods with hairs and a spotted endocarp at the septa (Plate 11) while *I. pulchra* is distinguished from other species by the possession of yellowish-brown calyx and bracts, dimorphic leaves, elliptic-oblanceolate leaflets, rachis which prolongs beyond the lateral leaflets and a spotted endocarp (Plate 12; Bello *et al.,* 2020 unpublished).

Plate 11: Morphology of *Indigofera secundiflora* showing habit and flower

Plate 12: Morphology of *Indigofera pulchra* showing habit and flower

Taxonomic (Bracketed Dichotomous) key for the identification of *Indigofera* species in parts of Kaduna and Katsina States, North-western Nigeria

1a Plants woody perennial shrubs...........……………………………………………………….........2
1b Plants annual herbs.…………………………………………………………………….....….3
2a Leaflet glabrous, shape ovate, apex emarginated.........……………….........1..*I. bracteolata*
2b Leaflet pubescent, shape oblong, apex mucronate……………......……….........2..*I. hirsuta*
3a Leaves simple, stem prostrate or sprawling…...4
3b Leaves compound, stem erect...…...5
4a Leaflet arrangement whorl……………………………….....……………....3..*I. pilosa*
4b Leaflet arrangement not whorl …………………………………………….....................6
5a Number of leaflets up to 15……………………..……….......…………….…...………........7

5b Number of leaflets less than 15…………………………………….…………,,,...............…8
6a Fruit spiny, broad leaves………......…………….……..................…4..*I. nummulariifolia*
6b Fruit not spiny, narrow leaves…...…………..……….......………….…..…....5..*I. paniculata*
7a Stem hairy, less than 0.4 m in height…...........…………….........……….....…6..*I. polyphylla*
7b Stem glabrous, up to 6 m in height………………………….......……..........7..*I. conferta*
8a Number of seeds per pod less than 6…………………………...............…..….........9
8b Number of seeds per pod more than 6……………….......…………….........…......10
9a Fruit densely hairy………………………………….........................8..*I. astragalina*
9b Fruit sparsely hairy………………………………………………......…....................11
10a Petiole length up to 2.5 mm………………………………..……...…….....9..*I. arrecta*
10b Petiole length less than 2 mm……………………………......…….........10..*I. stenophylla*
11a Fruit length up to 18 mm..11..*I. secundiflora.*
11b Fruit length 2-6 mm...12..*I. pulchra*

CONCLUSION

The results of the multivariate analyses revealed 12 distinct species. These are *Indigofera bracteolata*, *I. hirsuta*, *I. pilosa*, *I. nummulariifolia*, *I. paniculata*, *I. polyphylla*, *I. conferta*, *I. astragalina*, *I. arrecta*, *I. stenophylla*, *I. secundiflora* and *I. pulchra.* The number of seeds per pod, petiole length, leaflet length, internode, fruit length and leaflet width are the most useful diagnostic characters of *Indigofera* species in the region. These characters can, therefore, be effectively employed in the taxonomic treatment of other *Indigofera* species that were not included in the present study. This study covered two States of North-Western Nigeria. There is the need for an extensive field study of the genus to cover all the seven states in the region. It is anticipated that the remaining 48 species reported by Soladoye and Lewis (2003) would be discovered and many undescribed species may be uncovered.

ACKNOWLEDGEMENTS

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