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SYSTEMATIC TREATMENT AND CONSERVATION OF LOGANIACEAE IN WEST AFRICA

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ABSTRACT
Loganiaceae is a family of trees, shrubs and tendril-bearing liana with 13 genera and about 350 species distributed mainly in the tropics, subtropics and a few in temperate regions of the world but has undergone numerous revisions that have expanded and contracted its circumscription. Herbaria studies preceded the collections made in several extant forests and National Parks in some countries of West Africa. Four gene regions from nuclear and chloroplast origin were amplified but three were sequenced at the Royal Botanic Gardens Kew, London. The study revealed that Loganiaceae is composed of 3 genera: *Spigelia, Strychnos* and *Usteria* with 39 species in West Africa as opposed to the 6 genera originally circumscribed in the family. Two new species: *Strychnos tomentosa* and *Strychnos* sp. (*species nova* - P01860082) are described in West Africa for the first time in this work.

KEY WORDS: Loganiaceae, Taxonomy, West Africa, species, *Strychnos.*

INTRODUCTION

The family Loganiaceae was first suggested by Robert Brown in 1814 and validly published by Von Martius in 1827 (Leeuwenberg and Leenhouts, 1980; Frasier, 2008). The family belongs to the Order Gentianales which consists of the families Apocynaceae, Gelsemiaceae, Loganiaceae, Gentianaceae and Rubiaceae. Among these, Loganiaceae was considered to occupy a central evolutionary position (Bisset, 1980; Leeuwenberg and Leenhouts, 1980; Backlund *et al*. 2000). Earlier treatments of the family have included up to 30 genera, 600 species (Leeuwenberg and Leenhouts, 1980; Mabberley, 1997) but were later reduced to 400 species in 15 genera, with some species extending into temperate Australia and North America (Struwe *et al*., 1994; Backlund and Bremer, 1998). Morphological phylogenetic studies have demonstrated that this broadly defined Loganiaceae was a polyphyletic assemblage and numerous genera have been removed from it to other families, sometimes in other orders (Downie and Palmer, 1992; Olmstead *et al*., 1993; Struwe *et al.*, 1994; Oxelman *et al.*, 1999). Leeuwenberg and Leenhouts, (1980) circumscribed the family in its widest sense, classified into 10 tribes in 30 genera and 600 species. Cronquist (1981) reduced the circumscription of Leewenberg and Leenhouts to 21 genera in one tribe, grouped other six tribes to two families but removed the remaining three tribes completely from Gentianales. Thorne (1983) recognized 22 genera in five tribes, raised other five tribes to family level but did not accept the removal of three families from Order Gentianales. Struwe *et al.*, (1994) recognized from Leewenberg and Leenhouts circumscription three genera, raised other 15 genera to family level and commented that the remaining twelve genera were not certain where to be placed. Takhtajan (1987) recognized only one genus from the same Leewenberg and Leenhouts circumscription but raised the remaining 29 genera to nine different families and removed two completely from Gentianales. In one of the most acceptable classifications of Loganiaceae, the family now consists of 13 genera worldwide: *Antonia, Bonyunia, Gardneria, Geniostoma, Labordia, Logania, Mitrasacme, Mitreola, Neuburgia, Norrisia, Spigelia, Strychnos* and *Usteria* (Backlund *et al*., 2000; Frasier, 2008). Some of the genera circumscribed in Loganiaceae family in the Flora of West Tropical Africa have been proved that they belong in another family with the advent of Molecular approach to taxonomy as opposed to the earlier gross-morphological classifications. Hutchinson and Dalziel (1958, 1972) in the Flora of West Tropical Africa, Loganiaceae consists of six genera which include: *Anthocleista, Spigelia, Mostuea, Strychnos, Nuxia and Usteria. Anthocleista* comprises nine species, *Spigelia* is monotypic, *Mostuea* has five species, *Strychnos* has 35 species, while *Nuxia* and *Usteria* are both monotypic. The aim of this study is to explore the diversity of Loganiaceae in West Africa, validate its familial circumscription and generic delimitation to enhance its diversity conservation.

MATERIALS AND METHODS

The approach adopted for this study includes: (a) Herbarium studies and samples collection (b) Molecular analyses. The taxa studied include: *Anthocleista, Spigelia, Mostuea, Strychnos, Nuxia* and *Usteria* according to Hutchinson and Dalziel (1972).Specimens of Loganiaceae were studied in

Forestry Herbarium Ibadan (FHI) Ibadan, Obafemi Awolowo University (OAU) Ile-Ife, University of Nigeria Herbarium (UNH) Nsuka, Ahmadu Bello University Herbarium (ABUH) Zaria, University of Lagos Herbarium (LUH) Lagos and Gold Coast Herbarium (GCH) Accra, Ghana. Plant samples were collected from several Forest Reserves and National Parks in Nigeria, Republic of Benin and Ghana with the aid of collection bags, cutlass, secateurs, ropes and Global Positioning System (GPS) device. The samples were authenticated at FHI and deposited in FHI and LUH. The material collected includes young fresh leaves, mature leaves with short stem cut, (for further studies and herbarium preservation), fruits and/or seeds and/or inflorescence when available. The fresh leaves were preserved in small and sealable polythene nylon; silica gelled to dry moisture from leaves gradually and preserves the DNA content. These follow the descriptions of (Hutchinson and Dalziel, 1958; Doyle and Doyle, 1987; Ogundipe and Chase, 2000). The DNA extraction followed the modified Cetyltrimethyl-ammonium bromide (CTAB) protocol (Doyle and Doyle, 1990). Twenty one samples were selected across the six genera to represent the sampled Loganiaceae population and were sent for sequencing at the Royal Botanic Gardens Kew, London. The DNA were extracted and deposited at DNA Bank of the Royal Botanic Gardens Kew, London. Seven sterile samples of Strychnos species (Strychnos Indeterminate - SID) were collected from the field that did not match the herbaria collection during morphological characterisation and were included in the sequenced samples (Tables 1). Amplification was carried out on four gene regions: nuclear genes; ITS2–ITS5, ITS3– ITS4 and two non-coding chloroplast DNA regions between trnlC – trnlD and trnlE – trnlF described by White *et al*., (1990), Taberlet *et al*., (1991) and Baldwin *et al.,* (1997). The total reaction mixture of 25µl was used. The reaction condition was: initial denaturation at 94 ℃ for four minutes, denaturation reaction at 94 ℃ for one minute, annealing reaction at 65 ℃ for one minute, elongation reaction at 72 ℃ for one and half minutes and final elongation reaction was at 72 ℃ for 10 minutes. The reaction mixture was finally held at 4 ℃. Automated sequencer (ABI PRISM® 3730 DNA Analyzer) was used in this study following the manufacturer's instruction. Phylogenetic Analysis was carried out using Parsimony (PAUP) version 4.0b10 (Swofford, 1993) and SeaView - multiplatform, graphical user interface for multiple sequence alignment and molecular phylogeny (Gouy *et al.,* 2010). The settings used were: Parsimony; 5 times randomized sequence order, all gap sites ignored, 10,000 equally best trees retained and 100 Bootstrap replicates used.

TABLE 1.0: The selected samples for sequencing at RBG, Kew

S/N	General	Sample Sequenced	Code	Location of species used
1	1	*Anthocleista vogelli* Planch.	AVO9	N 06°48´16.4″ E004°21´54.2″
2	2	*Mostuea brunonis* Didr.	MBR15	N 05°52´ E08°46´
3	3	*Nuxia congesta* R. Br. Ex Fresen.	NCO18	N6°43′ E11°15′ N6.719° E11.27°
4	4	*Spigelia anthelmia* Linn.	SAT19	N 06°51.835´ E007°24.58´
5	5	*Strychnos boonei* De Wild.	SBO25	N6°00', W5°16'
6		*S. campicola* Gilg.	SCP26	N 07°11´.01″ E003°52´42.6″
7		*S. icaja* Bail.	SIC35	N 05°21´10.12″ E008°24´.20.3″
8		*S. nigritana* Bak.	SNI42	N 05°23´46.22″ E008°24´02.3″
9		*S. spinosa* Lam.	SSN46	N 09°55.421´ E003°57.304´
10		*S. spinosa var. pub*. Lam.	SSN46b	N9°15', E 3°50'
11		*S. staudtii* Gilg.	SST48	N5°31' E8°41'
12		*S. urceolata* Leeuwenberg	SUR51	N 6°04', E5°50'
13		*S usambarensis* Gilg.	SUS52	N7°50' E4°50'
14		*S.* indeterminate	SID57	N 05°53´ E08°43´
15		*S.* indeterminate	SID58	N 05°22´19.2″´ E008°27.25.1″
16		*S.* indeterminate	SID60	N 05°12´18 E08°21´31.9
17		*S.* indeterminate	SID61	N 05°21.835´ E008°26.21
18		*S.* indeterminate	SID62	N5°25', E 8°35'
19		*S.* indeterminate	SID64	N 05°46´49.2″ E008°25´25.33″
20		*S.* indeterminate	SID65	N 5°25', E 8°36'
21	6	*Usteria guineensis* Willd.	UGU66	N 06°52´ 45 E003°56′03.9″

Note: The selection was based on the total number of species represented in each genus.

TABLE 2.0: The Sequences of the region of Gene used

REGION	SEQUENCES	DESCRIPTOR
*trn*C-*trn*D	5'-CGA AAT CGG TAG ACG CTA CG-3'	Taberlet *et al*., (1991).
*Trnl*E – *trnl*F	5'-ATT TGA ACT GGT GAC ACG AG-3'	Taberlet *et al*., (1991).
ITS 2	5'-GTC CAC TGA ACC TTA TCA TTT AG-3'	White *et al*. (1990)
ITS 4	5'- TCC TCC GCT TAT TGA TAT GC-3'	Baldwin *et al*. (1997)

TABLE 3A: Basic Local Alignment Search Tools (BLAST) results for the matrixes generated for *Strychnos* indeterminate by three different regions of genes

S/N	Sample code	Gene Bank Accessions number	Name of sample	Regions	Maximum score	Total score	Query average	Maximum Identity
1	SID 57 trnL e-f	HQ634604.1	*Strychnos* sp. P01860082	Trna-Leu (trnL) gene and trnL-trnF intergenic spacer	586	586	99%	97%
2	SID 57 trnL c-d	AF102484.2	*Strychnos tomentosa*	Trna-Leu (trnL) gene and trnL-trnF intergenic spacer	808	808	100%	98%
3	SID 57 trnL c-d	AF214301.1	*Strychnos tomentosa*	trnL gene, partial intron sequence ; chloroplast gene	782	782	99%	97%
4	SID 58 trnL e-f	AF102484.2	*Strychnos tomentosa*	Trna-Leu (trnL) gene and trnL-trnF intergenic spacer	566	566	99%	97%
5	SID 58 trnL e-f	HQ634604.1	*Strychnos* sp. P01860082	Trna-Leu (trnL) gene and trnL-trnF intergenic spacer	566	566	100%	96%
6	SID 58 trnL e-f	AF214147.1	*Strychnos tomentosa*	trnL-trnF intergenic spacer	401	401	74%	96%
7	SID60 trnL c-d	AF102484.2	*Strychnos tomentosa*	Trna-Leu (trnL) gene and trnL-trnF intergenic spacer	808	808	100%	98%
8	SID60 trnL c-d	AF214301.1	*Strychnos tomentosa*	trnL gene, partial intron sequence	782	782	96%	97%
9	SID 60 trnL e-f	AF102484.2	*Strychnos tomentosa*	Trna-Leu (trnL) gene and trnL-trnF intergenic spacer	566	969	99%	99%
10	SID 60 trnL e-f	HQ634604.1	*Strychnos* sp. P01860082	Trna-Leu (trnL) gene and trnL-trnF intergenic spacer	556	962	100%	99%
11	SID61 trnL c-d	AF102484.2	*Strychnos tomentosa*	Trna-Leu (trnL) gene and trnL-trnF intergenic spacer	782	782	100%	95%
12	SID61 trnL c-d	HQ634604.1	*Strychnos* sp. P01860082	Trna-Leu (trnL) gene and trnL-trnF intergenic spacer	761	761	96%	96%

TABLE 3B: Basic Local Alignment Search Tools (BLAST) results for the matrixes generated for *Strychnos* indeterminate by three different regions of genes contn'd

S/N	sample code	Gene Bank Accessions number	Name of sample	Regions	Maximum score	Total score	Query average	Maximum Identity
13	SID 62 trnL e-f	HQ634604.1	*Strychnos* sp. P01860082	Trna-Leu (trnL) gene and trnL-trnF intergenic spacer	237	237	92%	82%
14	SID 62 trnL e-f	AF102484.2	*Strychnos tomentosa*	Trna-Leu (trnL) gene and trnL-trnF intergenic spacer	237	237	92%	82%
15	SID 62 trnL e-f	AF214147.1	*Strychnos tomentosa*	trnL-trnF intergenic spacer	235	235	89%	83%
16	SID 62 trnL c-d	AF102484.2	*Strychnos tomentosa*	Trna-Leu (trnL) gene and trnL-trnF intergenic spacer	782	782	100%	95%
17	SID 62 trnL c-d	HQ634604.1	*Strychnos* sp. P01860082	Trna-Leu (trnL) gene and trnL-trnF intergenic spacer	761	761	96%	96%
18	SID 64 trnL e-f	AF102484.2	*Strychnos tomentosa*	Trna-Leu (trnL) gene and trnL-trnF intergenic spacer	566	566	99%	97%
19	SID 64 trnL e-f	HQ634604.1	*Strychnos* sp. P01860082	Trna-Leu (trnL) gene and trnL-trnF intergenic spacer	556	556	100%	96%
20	SID 64 ITS	AF102484.2	*Strychnos tomentosa*	Trna-Leu (trnL) gene and trnL-trnF intergenic spacer	782	782	100%	95%
21	SID 64 ITS	HQ634604.1	*Strychnos* sp. P01860082	Trna-Leu (trnL) gene and trnL-trnF intergenic spacer	761	761	96%	96%
22	SID 65 trnL e-f	AF102484.2	*Strychnos tomentosa*	Trna-Leu (trnL) gene and trnL-trnF intergenic spacer	451	451	91%	97%
23	SID 65 trnL e-f	HQ634604.1	*Strychnos* sp. P01860082	Trna-Leu (trnL) gene and trnL-trnF intergenic spacer	444	444	91%	96%
24	SID 65 trnL e-f	AF214147.1	*Strychnos tomentosa*	trnL-trnF intergenic spacer; chloroplast gene	412	412	91%	95%

RESULTS

Table 1.0 represents collections selected for sequencing at the Royal Botanic Gardens, Kew. The selection was based on the relative number of species present in each genus while Table 4.0 represents the herbaria collection assessed in the course of the studies. Plates 1.0 to 1.3 are the electrophorograms of the genes amplified for phylogenetic analysis. The ITS gene amplification, of the four regions show better on Agarose gel electrophoresis and the fragment size amplified for ITS was between 500bp (base pair) and 800bp (Plates 1.0 and 1.1). The sequences of the region are not long but they are highly informative during phylogenetic studies. *Strychnos* species have better information when they were sequenced on non-coding intergenic spacer and the fragment size amplified was between 1100bp and 1200bp (Plates 1.2 and 1.3). The sequences of the gene are described in Table 2 and the information produced by each gene is shown on Table 3A and 3B. Since only representative species were used for the analysis, the phylogenetic trees in Figures (1-3) are interpreted based on the various genera used in the study. *Mostuea* was used as the outgroup in Figure 1.0 while the outgroup in Figure 2.0 and Figure 3.0 was *Nuxia congesta*. In each case, *Anthocleista* was not nested with any of the members of Loganiaceae. It rather has a separate clad without a bootstrap value to show a low affinity in the dendrogram. In turn, when *Mostuea* was used as the outgroup, neither *Nuxia* nor *Anthocleista* was grouped with the remaining members of the family. Considering Figure 2, *Spigelia anthelmia* formed a clad with *Strychnos staudtii* and SID 62 with a bootstrap value of 95 %. Futher, *Strycnnos tomentosa* and *Usteria guineensis* have 50 % affinity but *Nuxia*, *Anthocleista* and *Mostuea* all have less than 40 % affinity.

TABLE 4.0: Some of the herbaria collections assessed for this study

Name of plant specimens	Place of Collection	Accession no	Collector
Anthocleista liebrechtsiana	Republic of Benin	FHI 30254	Onochie, C.F.A
A. obanensis	Iyekorhiomwon, Sapoba Forest R.	FHI 61734	Emwiogbon, J.A
A. procera	Abidjan, Ivory coast	FHI 30679	Leeuwenberg, A.J.M.
A. schweinfurthii	Republic of Benin	FHI 95075	Onochie, C.F.A
A. scandens	Cameroun	FHI 40516	Daramola, B.O
A. nobilis	Abidjan, Ivory coast	FHI 13655	Leeuwenberg, A.J.M.
A. vogelli	Forestry garden	FHI 107911	Daramola, B.O
Mostuea brunonis	Awi Forest	FHI 101156	Daramola, B.O
M. hirsuta	Zaria, Jamaa Nimbia	FHI 104567	Anders, T.
M. batesii	Yaoundé	FHI 69486	Leeuwenberg, A.J.M.
Mostuea thomsonii	West of Premises town, steep forest floor.	GCH 1802	Monton, J.K
Nuxia congesta	Victoria, cameroun mt.	FHI 40507	Daramola, B.O
N. congesta	Amed yote, Togo.	GCH 2871	Dewit and Morta.
Strychnos aculeata	Omo Sawmil, Ijebu-Ode	FHI 50221	Leeuwenberg, A.J.M.
S. afzeli	Owena river edge, Ondo state.	FHI 23012	Olorunfemi J.
S. angolensis	Oban F.R. Calabar	FHI 37221	Daramola, B.O
S. asterantha	Nigritana game Reserve, Plateau	FHI 10674	Gbile & Daramola
S. barteri	Nigritana game Reserve, Plateau	FHI 25601	Daramola, B.O
S. boonei	Benin city	FHI 25554	Olorunfemi J.
S. campicola	N/A	FHI 22110	Daramola, B.O
S. chrysophyla	Oban, CRNP	FHI 33768	Olorunfemi J.
S. congolana	Okeigbo, ondo state	FHI 15388	Onochie, C.F.G
S. densiflora	Ankasa Forest Reserve	GCH 3912	Enti, A.A
S. dinklagei	Abijan, Ivory Coast	FHI 13564	Leeuwenberg, A.J.M.
S. innocua	Igbeti- Ilorin road	FHI 89699	Ibhanesebhor, Adejimi
S. melacoclados	Ukpe-sobo Forest reserve	FHI 34792	Imwinogbon, J.A
S. memecyloides	N/A	FHI 10291	Olorunfemi J.
S. nigritana	Etemi, Owena River	FHI 92874	Ekwuno.
S. nus-vomica	Achimota School Aboretum.	GCH 638	Akpabla, G.K.
S. phaeotricha	Sapoba Forest Reserve	FHI 45344	Ibhanesebhor, Adejimi
S. soubriensis	Abidjan, Ivory coast	FHI 60566	Leeuwenberg, A.J.M.
S. spinosa	Igbeti- Ilorin road	FHI 58201	Ibhanesebhor, Adejimi
S. splendens	Ibadan South Forest R.	FHI 25366	Keay, R.W.J.
S. staudtii	Awi Forest Reserve	FHI 90351	Daramola, B.O
S. tricalysioides	Abeokuta, Ogun state	FHI 13536	Leeuwenberg, A.J.M.
S. urceolata	Calabar	FHI 66048	Daramola, B.O
S. usambarensis	Dakpa Inselberg, Agai, Kpedzeglo F.R.	GCH 42742	Hall and Enti.

DISCUSSION

The molecular pattern from the Phylogenetic analysis reveals that nuclear ITS, non-coding intergenic spacer and introns accord Bootstrap value of less than 40 % to *Mostuea*, *Anthocleista* and *Nuxia* genera in particular (when there is no bootstrap value, Figures 1.0, 2.0 and 3.0). Hence, there is high tendency for them to be removed from the family according to the report recorded in previous works (Backlund and Bremer, 1998; Backlund *et al.*, 2000; Frasier, 2008). The study also reveals that ITS gene for *Nuxia congesta* was difficult to align and so the difficulty experienced with *Anthocleista* and *Mostuea* matrices requiring many gaps for alignment. Frasier (2008) reported that the ITS region is prone to more indels than coding sequences thus, requires the insertion of gaps to maintain positional homology, which is critical for phylogenetic studies. Further, as the sampling of a group of species expands, it can become difficult to align ITS sequences between species that are evolutionarily more distant (Baldwin *et al.*, 1997). This is an indication that these three genera are evolutionarily not similar with Loganiaceae. Previous workers have identified polyphyletic assemblage in this family (Leenhouts, 1962; Leeuwenberg and Leenhouts, 1980) because it is central in Gentianales and therefore constitutes a link between the other families (Leeuwenberg and Leenhouts, 1980). Therefore, this study has revealed that among the six genera included in Loganiaceae of West Tropical Africa, three genera; *Anthocleista*, *Nuxia* and *Mostuea*, should be removed and placed in other families. Furthermore, the SID matrixes were subjected to Basic Local Alignment Search Tools (BLAST) and the nearest matrixes were used for comparison in database (NCBI database). The trnL-trnF intergenic spacer with 974 base pair has 99% query coverage and 99% maximum identity for *Strychnos* indeterminate (SID 58, 60, 64 and 65) with *Strychnos tomentosa* in the database. Hence, they are called *Strychnos tomentosa* based on this high level of similarity. Furthermore, *Strychnos* indeterminate (SID 57, 61 and 62) matrixes of trnL-trnF intergenic spacer have average of 860 base pair, 95% query coverage and 98% maximum identity with *Strychnos* sp. (*species nova* - P01860082). Hence, they are recognized in the database with no name given yet. These two species hitherto have not been reported in West Tropical African flora. *Strychnos tomentosa* has been thought to be endemic to British Guyana coastal rainforest (Sandwith, 1933) but this study has revealed that it flourishes also in the Coastal rainforest of Nigeria.

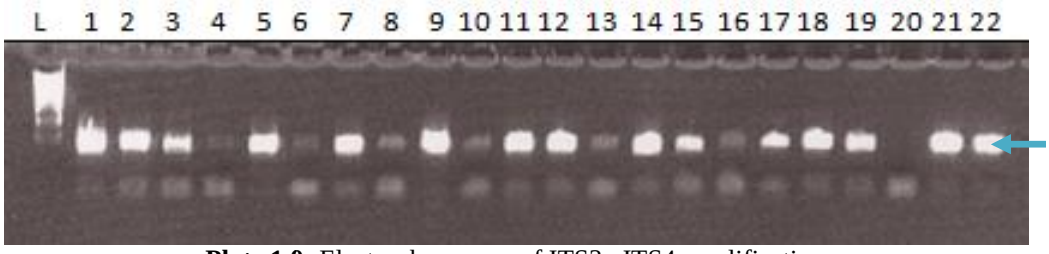

Plate 1.0: Electrophorogram of ITS3– ITS4 amplification

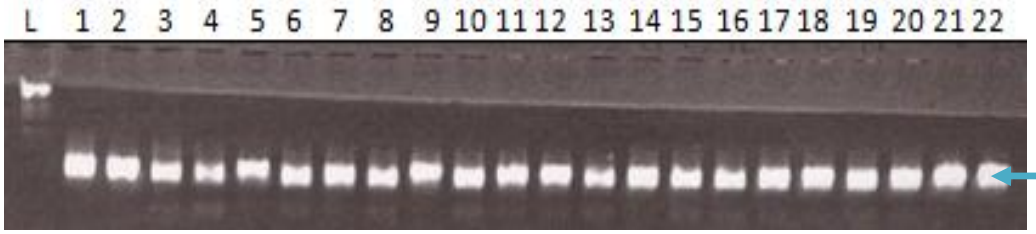

Plate 1.1: Electrophorogram of ITS2–ITS5 amplification

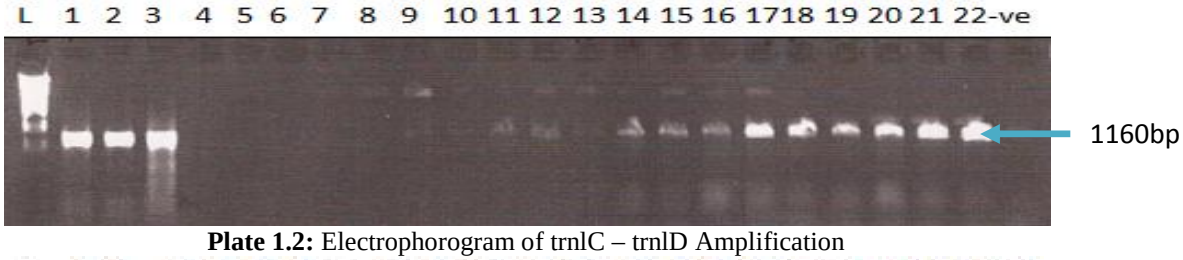

Plate 1.2: Electrophorogram of trnlC – trnlD Amplification

Plate 1.3: Electrophorogram trnlE–trnlF amplification

Plates 1.0 – 1.3: Electrophorogram of four gene regions amplified for Loganiaceae phylogenetic studies (L= Ladder, -ve = Negative control, bp = base pair).

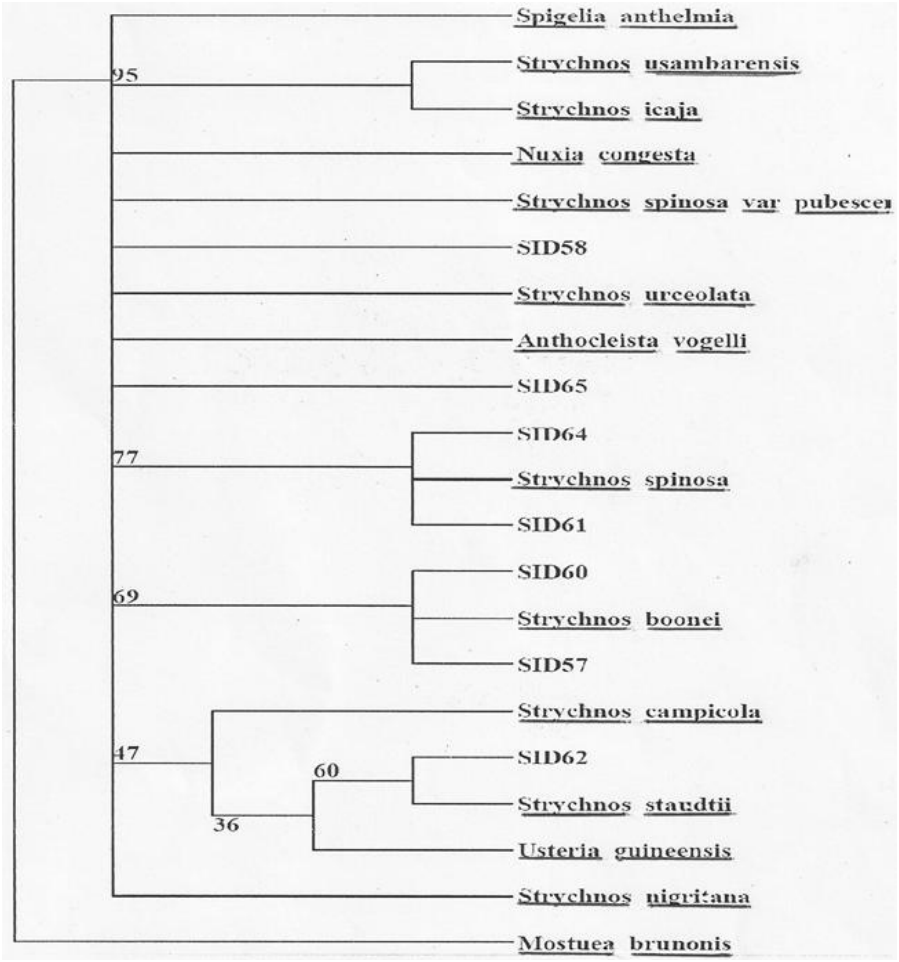

FIGURE 1.0: Phylogenetic relationship of Loganiaceae using ITS matrices with their bootstrap values.

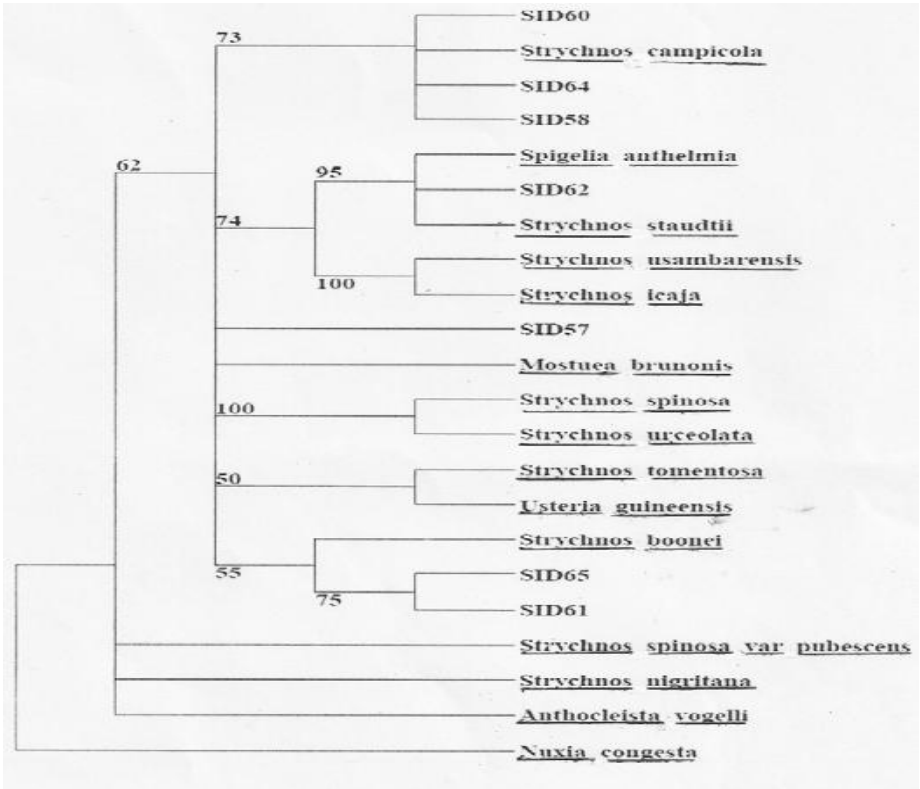

Figure 2.0: Phylogenetic relationship of Loganiaceae using trnL matrices; *Nuxia* re-rooted with their bootstrap values.

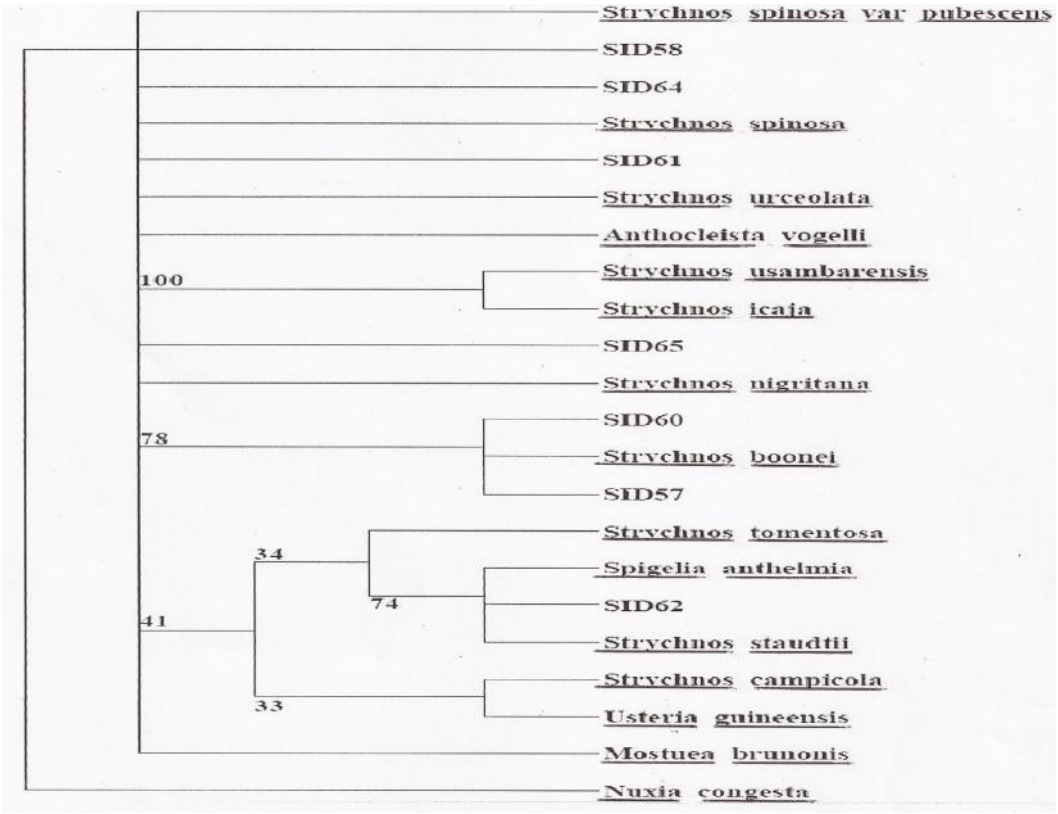

FIGURE 3.0: Phylogenetic relationship of Loganiaceae using combined matrices; *Nuxia* re-rooted with their bootstrap values.

CONCLUSION

The result above revealed that *Strychnos, Spigelia* and *Usteria* are related while *Anthocleista Mostuea* and *Nuxia* are not related to them. Hence, Loganiaceae in West Africa is composed of three genera: *Spigelia, Strychnos* and *Usteria* with 39 species as opposed to the six genera originally circumscribed in the family. "Classifications, including those of higher taxa, will continue to change as our knowledge of these groups and their relationships changes. Based on the accumulation of additional data and the way in which the data are interpreted, taxa are added, shuffled, or deleted. Classifications are therefore largely eclectic in nature and we seem to proceed toward a clearer picture of the plant world, past and present, by successive approximations toward a (probably unattainable) state of total knowledge"(Nicholas and Baijnath, 1994).

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