CHAPTER ONE

1.0 INTRODUCTION AND BACKGROUND TO THE STUDY

Loganiaceae is a family of flowering plants classified in the Order Gentianales (Bendre, 1975). The family was first suggested by Robert Brown in 1814 and validly published by von Martius in 1827 (Nicholas and Baijnath, 1994). Members habits are in form of trees, shrubs, woody climbers and herbs. Some are epiphytes while some members are furnished with spines or tendrils (Bendre, 1975). They are distributed mainly in the tropics, subtropics and a few in temperate regions (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; Dunlop, 1996; Backlund and Bremer, 1998). Morphological studies have demonstrated that this broadly defined Loganiaceae was a polyphyletic assemblage and numerous genera have been removed from it to other families (sometimes to other Orders), e.g. Gentianaceae, Gelsemiaceae, Plocospermataceae, Tetrachondraceae, Buddlejaceae, and Gesneriaceae (Backlund and Bremer, 1998; Backlund *et al.*, 2000). The family has undergone numerous revisions that have expanded and contracted its circumscription, ranging from one genus at its smallest (Takhtajan, 1997; Smith *et al*., 1997) to 30 at its largest (Leeuwenberg and Leenhouts, 1980). One of the current infrafamilial classifications contains four tribes: Antonieae Endl., Loganieae Endl., Spigelieae Dum. (monotypic), and Strychneae Dum. (Struwe *et al*., 1994). The tribes Loganieae and Antonieae are supported by molecular data, but Strychneae is not (Backlund *et al*., 2000). Strychneae includes *Strychnos* Linn. and two Asian genera; *Gardneria* Wall and *Neuburgia* Blume. *Spigelia* Linn., which is restricted to the western hemisphere, was included in the same clade as Strychneae in Backlund *et al.* (2000). There are few molecular phylogenetic studies that

target Loganiaceae (Backlund *et al.,* 2000) and there is no publication that treats all Loganiaceae genera in a phylogenetic context (Bendre, 1975; Frasier, 2008). However, in the Flora of West Tropical Africa, Hutchinson and Dalziel (1972) reported six genera: *Anthocleista* Afzel. ex R.Br, *Spigelia, Mostuea* Bak**.**, *Strychnos, Nuxia* R. Br. Ex Fresen and *Usteria* Willd.. *Anthocleista* comprises nine species, *Mostuea* has five species, *Strychnos* has thirty five species, while *Spigelia, Nuxia* and *Usteria* genera are represented by a single species each in West Africa.

1.1.0 Deoxyribonucleic Acid (DNA)

Deoxyribonucleic Acid (DNA) was first isolated by the Swiss physician Friedrich Miescher who, in 1869, discovered a microscopic substance in the pus of discarded surgical bandages. As it resided in the nuclei of cells, he called it "nuclein" (Dahm, 2008). In 1919, Phoebus Levene identified the base, sugar and phosphate nucleotide unit. Levene suggested that DNA consisted of a string of nucleotide units linked together through the phosphate groups. He thought the chain was short and the bases repeated in a fixed order (Tamarin, 1999). In 1937 William Astbury produced the first X-ray diffraction patterns that showed that DNA had a regular structure (Ghosh and Bansal, 2003).

In 1928, Frederick Griffith discovered that traits of the "smooth" form of the *Pneumococcus* could be transferred to the "rough" form of the same bacterium by mixing killed "smooth" bacterium with the live "rough" form (Lorenz and Wackernagel, 1994; Tamarin, 1999). This experiment provided the first clear suggestion that DNA carries genetic information. DNA's role in heredity was confirmed in 1952, when Alfred Hershey and Martha Chase in the Hershey–Chase experiment showed that DNA is the genetic material of the T_2 phage (Lorenz and Wackernagel, 1994; Tamarin, 1999).

In 1953, James Watson and Francis Crick suggested what is now accepted as the first correct double-helix model of DNA. Their double-helix, molecular model of DNA was then based on a single X-ray diffraction image taken by Rosalind Franklin and Raymond Gosling as well as the information that the DNA bases are paired. This was obtained through private communications from Erwin Chargaff in the previous years. Chargaff's rules played a very important role in establishing double-helix configurations for B-DNA as well as A-DNA (Watson and Crick, 1953; Tamarin, 1999; Hartwell *et al.*, 2000).

Deoxyribonucleic acid (DNA) is a nucleic acid that contains the genetic instructions used in the development and functioning of all known living organisms (with the exception of RNA viruses). The main role of DNA molecules is the long-term storage of information. DNA is often compared to a set of blueprints, like a recipe or a code, since it contains the instructions needed to construct other components of cells, such as proteins and RNA molecules. The DNA segments that carry this genetic information are called genes, but other DNA sequences have structural purposes, or are involved in regulating the use of this genetic information (Lorenz and Wackernagel, 1994).

DNA consists of two long polymers of simple units called nucleotides, with backbones made of sugars and phosphate groups joined by ester bonds. These two strands run in opposite directions to each other and are therefore anti-parallel. Attached to each sugar is one of four types of molecules called bases. It is the sequence of these four bases along the backbone that encodes information. This information is read using the genetic code, which specifies the sequence of the amino acids within proteins. The code is read by copying stretches of DNA into the related nucleic acid RNA, in a process called transcription (Tamarin, 1999). Within cells, DNA is organized into long structures called chromosomes. These chromosomes are duplicated before cells divide, in a process called DNA replication. Eukaryotic organisms (animals, plants, fungi, and protists) store

most of their DNA inside the cell nucleus and some of their DNA organelles, such as mitochondria, ribosomes or chloroplasts. In contrast, prokaryotes (bacteria and archaea) store their DNA only in the cytoplasm. Within the chromosomes are chromatins, proteins such as histones compact and organized DNA. These compact structures guide the interactions between DNA and other proteins, helping to control which parts of the DNA are transcribed (Brenda, 2003).

The structure of DNA as described by James D. Watson and Francis Crick for all species comprises two helical chains each coiled round the same axis, and each with a pitch of 34 Ångströms (3.4 nm) and a radius of 10 Ångströms (1.0 nm). According to another study, when measured in a particular solution, the DNA chain measured 22 to 26 Ångströms wide (2.2 to 2.6 nm), and one nucleotide unit measured 3.3 Å (0.33 nm) long. Although each individual repeating unit is very small, DNA polymers can be very large molecules containing millions of nucleotides. For instance, the largest human chromosome, chromosome number 1, is approximately 220 million base pairs long (Mandelkern *et al.,* 1981).

In living organisms, DNA does not usually exist as a single molecule, but instead as a pair of molecules that are held tightly together. These two long strands entwine like vines, in the shape of a double helix. The nucleotide repeats contain both the segment of the backbone of the molecule, which holds the chain together, and a base, which interacts with the other DNA strand in the helix. A base linked to a sugar is called a nucleoside and a base linked to a sugar and one or more phosphate groups is called a nucleotide. If multiple nucleotides are linked together, as in DNA, this polymer is called a polynucleotide (Watson and Crick, 1953; Tamarin, 1999; Gregory *et al*., 2006).

The backbone of the DNA strand is made from alternating phosphate and sugar residues (Ghosh and Bansal, 2003). The sugar in DNA is 2-deoxyribose, which is a pentose (five-carbon) sugar.

The sugars are joined together by phosphate groups that form phosphor-di-ester bonds between the third and fifth carbon atoms of adjacent sugar rings. In a double helix the direction of the nucleotides in one strand is opposite to their direction in the other strand: the strands are antiparallel (Figure 1). The asymmetric ends of DNA strands are called the 5′ (*five prime*) and 3′ (*three prime*) ends, with the 5' end having a terminal phosphate group and the 3' end a terminal hydroxyl group. One major difference between DNA and RNA is the sugar, with the 2-deoxyribose in DNA being replaced by the alternative pentose sugar ribose in RNA (Hartwell *et al.*, 2000; Isaksson *et al.*, 2004).

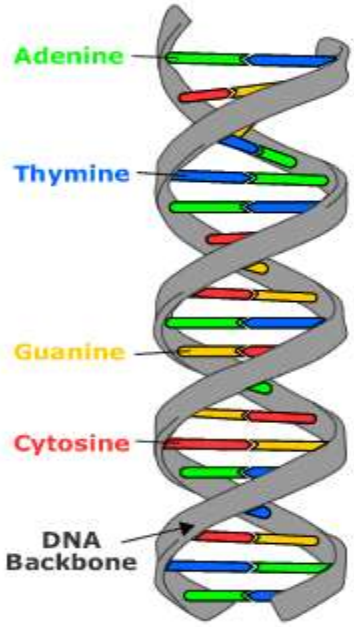


Figure 1: A section of DNA, the bases lie horizontally between the two spiraling strands.

Source: Ghosh and Bansal, (2003).

The DNA double helix is stabilized primarily by two forces: hydrogen bonds between nucleotides and base-stacking interactions among the aromatic bases. In the aqueous environment of the cell, the conjugated Pi (π) bonds of nucleotide bases align perpendicular to the axis of the DNA

molecule, minimizing their interaction with the solvation shell and therefore, the Gibbs free energy (Verma and Agarwal, 2003). The four bases found in DNA are adenine (abbreviated A), cytosine (C), guanine (G) and thymine (T). These four bases are attached to the sugar/phosphate to form the complete nucleotide, this refers to adenosine monophosphate. These bases are classified into two types; adenine and guanine are fused five and six-membered heterocyclic compounds called purines, while cytosine and thymine are six-membered rings called pyrimidines (Figure 1). A fifth pyrimidine base, called uracil (U), usually takes the place of thymine in RNA and differs from thymine by lacking a methyl group on its ring. Uracil is not usually found in DNA, occurring only as a breakdown product of cytosine (Gregory *et al*., 2006). In addition to RNA and DNA, a large number of artificial nucleic acid analogues have also been created to study the properties of nucleic acids, or for use in biotechnology (Tamarin, 1999; Hartwell *et al.*, 2000).

1.1.1 Molecular Systematics

Molecular systematics is the science of classifying organisms based on variations in protein and DNA sequences in order to make fine taxonomic categorizations not solely dependent on morphology (Ashton and de Queiroz, 2001). Analysis of sequences allows one to obtain a picture of how different populations have changed over time. It has been suggested that the amount of change that takes place in DNA over time can act as a molecular clock, gauging how much evolutionary time has passed (Bromham and Penny, 2003). The clock is set by first examining geological and historical records to determine how long two species have been physically separated (Hoelzer *et al.*, 1998; Bromham and Penny, 2003). The DNA sequences may be used to compare many different types of DNA: regions that encode for genes; do not encode for genes; reside in chloroplast DNA or reside in mitochondrial DNA (Soltis and Solits, 1998; Backlund *et al.*, 2000).

The real importance of molecular systematics is that it allows the examination of how species have changed over evolutionary time, as well as of the relationships between species that have no common physical characteristics (Kimura and Ohta, 1971; Bromham *et al.*, 2002; Brinegar, 2009). Molecular changes are used to explore phylogenetics - how populations are related evolutionarily and genetically (Soltis and Solits, 1998; Bromham *et al.*, 2002; Bromham and Penny, 2003). DNA analysis has become a tremendously useful tool in helping systematists differentiate between and declare new plant species, characterize the evolutionary relationships between lineages, and even identify the early stages of speciation (Kimura and Ohta, 1971; Bromham and Penny, 2003).

The smallest known haploid genome of a flowering plant contains over 63 million base pairs of DNA, and some contain over 100 billion base pairs (Soltis and Solits, 1998; Brinegar, 2009). Nevertheless, regulatory and coding sequences tend to be highly conserved and will generally only show variability when comparing plants belonging to different genera, families, orders, classes and divisions – with more sequence variation apparent at the higher taxonomic levels. Examples of these loci include the *rbc*L chloroplast gene and the nuclear 28S rDNA gene. However, major controversies regarding plant classification above the family level are uncommon where enough morphological differences exist to place plants in the proper categories (Bromham *et al.*, 2002; Brinegar, 2009). The greatest controversies in plant systematics occur at the lower taxonomic levels (genus and species) where morphological similarities can be so great as to confound proper classification (Bromham *et al.*, 2002). On the scale of geological time, these hierarchical units have only recently diverged from common ancestors and their DNA has had much less time to accumulate mutations. Therefore, when comparing plants at these levels it is necessary to study loci that can amass mutations very rapidly without having a deleterious effect on the organism (Dickerson, 1971; Frasier, 2008). These regions were formerly known as "junk" DNA and are

interspersed between the conserved structural genes. The Internal Transcribed Spacers (ITS-1 and ITS-2) of nuclear ribosomal DNA (rDNA), are nonstructural in nature and have much higher mutation rates relative to the 18S, 5.8S and 28S rDNA genes which they separate (Baldwin *et al.*, 1997). These ITS regions are generally conserved within a species but show enough variation between species and genera to be useful in the construction of phylogenetic trees (Baldwin *et al.*, 1997; Richard *et al.*, 2008; Brinegar, 2009). These two loci, usually amplified and sequenced together along with the small 5.8S rDNA region, have been tremendously helpful in delimiting plant taxa. The entire region is transcribed into precursor RNA which is then processed into 18S, 5.8S and 28S ribosomal RNA molecules by removal of the internal transcribed spacers (ITS-1 and ITS-2). Since the ITS sequences do not encode functional RNA, they accumulate mutations at a higher rate than most other regions of DNA and are therefore useful in assessing divergence between species (Drake *et al*., 1998).

Chloroplast DNA (cpDNA) is commonly used in assessing variation between populations, species, genera and sometimes even higher taxonomic levels (Downie and Palmer, 1992). Another most frequently used cpDNA locus for lower taxonomic comparisons is the intergenic spacer (IGS) found between the *trn*L and *trn*F genes which encode transfer RNAs for leucine and phenylalanine, respectively (Brinegar, 2009). Interrupting the two *trn*L exons is an intron sequence that also shows significant sequence variation. Another chloroplast IGS locus used for phylogenetic assessment is found between the *rbc*L and *acc*D genes. It is noteworthy that, no longer can only one genetic locus of a taxonomic group be sequenced and the reconstructed phylogenetic tree is assumed to reflect the true species tree (Brinegar, 2009). More than one locus is generally required in molecular systematics in order to be able to construct a reliable

phylogenetic relationship, this will minimize errors as well as wrong interpretation (Brinegar, 2009).

Since the chloroplast genome is haploid, unique sequences of cpDNA are referred to as *haplotypes*. Chloroplast haplotypes are usually inherited through the female in plants, although paternal inheritance (through pollen) is the norm in gymnosperms (Wolf *et al*., 1997; Soltis and Solits, 1998; Brinegar, 2009). As earlier mentioned, DNA maintains its unique print for every organism; this makes it a useful tool which could complement the morphological motifs in Systematics. Several longstanding controversies in plant classification have been resolved by DNA analysis (Brunsfeld *et al.,* 1994). Morphologically similar species have been delineated at their molecular level successfully based on the differences observed in their gene sequences (Brinegar, 2009). The Taxodiaceae (redwood family) when compared with Cupressaceae (cypress family), both have several morphological and developmental similarities and they have been grouped together for long a time until DNA analysis (chloroplast *rbc*L genes) finally convinced the botanical community to accept the revision (Levin, 2000; Kawase *et al.,* 2007; Brinegar, 2009).

1.2 STATEMENT OF PROBLEMS

It is expedient and obligatory for botanical samples to be accurately identified, named and classified. This guarantees the same result across the globe among scientists and makes possible reproducibility of findings and applications among stakeholders such as plant breeders, horticulturists, pharmacologists and local herbal medicine practitioners. Taxonomic problems of familial circumscription, generic delimitation and species identification exist within the family of Loganiaceae. Morphological phylogenetic studies have demonstrated that Loganiaceae is a polyphyletic assemblage and numerous genera have been separated from it and placed in other families, sometimes in other orders (Backlund *et al.*, 2000).

Ecological problems confronting wild plants have been compounded *inter alia*, by habitat destruction, over-exploitation, desertification and climate change. In order to ensure effective conservation and sustainable use of Loganiaceae species, the study seeks to address some ecological issues with respect to habitats and abundance. The diversity of their habitats environmentally, taxonomically and genetically are grossly challenged. They are no longer complementary; i.e. when they are rich in biodiversity, they are only represented by few common taxa. Furthermore, some of these habitats are currently under-represented in conservation efforts; they are threatened with genetic erosion while some are likely to be suitable as *in situ* conservation sites. In summary, these habitats are not well represented in current germplasm collections.

1.3 AIM AND OBJECTIVES

The aim of this research is to utilize both gross morphology and molecular motifs for the elucidation and delimitation of genera and species in the family Loganiaceae in West Africa.

The specific objectives are to:

1) Explore the diversity of species within the family, placing emphasis on collection from West Tropical Africa; identifying and preserving all voucher specimens in secured repositories;
2) Use West African climatic variables for the evaluation and interpretation of variation and distribution patterns of Loganiaceae species in West Africa;
3) Determine the number of genera and establish generic boundaries within the family by using selected representative species;
4) Generate Loganiaceae genetic materials suitable for deposition in a global gene bank;
5) Derive reliable taxonomic keys for the accurate identification of all the species.

1.4 SIGNIFICANCE OF THE STUDY

The genera and species of the family Loganiaceae are of economic, scientific, ecological and environmental importance; as food, medicine, shelter, ornaments, horticulture, furniture, windbreak, green manure, soil stability, dyes, pesticides, resin, gums etc. In addition, many of the taxa are used in herbal traditional medicine for the treatment of common diseases such as constipation, typhoid fever, abdominal pains, diarrhea, poison antidote, leprosy, edemas and elephantiasis of the scrotum; they have also been used as purgative, diuretic, contraceptives, abortifacients etc. The taxonomic problems of identification and circumscription have diminished the use of the species in this family. The continued existence of some of the species is under serious threat as a direct result of habitat destruction, deforestation, wildfires and climate change.

1.5.0 OPERATIONAL DEFINITION OF TERMS AND ABBREVIATIONS/ACRONYMS

Bootstrapping: This is a statistical method for estimating the sampling distribution of an estimator by sampling with replacement from the original sample, most often with the purpose of deriving robust estimates of standard errors and confidence intervals of a population parameter like a mean, median, proportion, odds ratio, correlation coefficient or regression coefficient.

DNA: Deoxyribonucleic acid is a nucleic acid that contains the genetic instructions used in the development and functioning of all known living organisms (with the exception of RNA viruses).

Exon: An exon (coding regions of DNA) is a nucleic acid sequence that is represented in the mature form of an RNA molecule either after portions of a precursor of RNA (introns) has been removed by cis-splicing or more precursor RNA molecules have been ligated by trans-splicing.

Heuristic Search: Heuristic Search designates a computational method that optimizes a problem by iteratively trying to improve a candidate solution with regard to a given measure of quality.

Intron: An intron (non-coding regions of DNA) is a DNA region within a gene that is not translated into protein.

IUCN: International Union for Conservation of Nature – engaging the private sector to help conserve the integrity and diversity of nature and ensure that any use of natural resources is equitable and ecologically sustainable.

Jackknifing: It is used to estimate the bias and standard error (variance) of a statistic, when a random sample of observations is used to calculate it i.e. estimating the precision of sample statistics (medians, variances, percentiles) by using subsets of available data.

matK: Maturase K gene - is found in the large single copy region of the chloroplast genome.

MAXENT: Maximum Entropy - is the least biased-estimate possible on the given information.

Maximum Parsimony: Maximum parsimony is a character-based method that infers a phylogenetic tree by minimizing the total number of evolutionary steps required to explain a given set of data, or in other words by minimizing the total tree length.

Molecular Phylogenetics: This is the use of the structure of molecules to gain information on an organism's evolutionary relationships.

NCBI: National Center for Biotechnology Information. It is a database for molecular biology.

Parsimony: Parsimony implies that simpler hypotheses are preferable to more complicated ones.

rbcL: Ribulose 1,5 - bisphosphate carboxylase large subunit gene of the photosynthetic enzyme rubisco (a carbon fixation enzyme of about 1428bp in size), is found in the chloroplast.

SEVAG: Ratio (24 chloroform**:** 1 Iso-amyl alcohol), is a DNA extraction reagent

BSA: Bovine Serum Albumin

BLAST: Basic Local Alignment Search Tools

CCDB: Canadian Centre for DNA Barcoding.

CTAB: Cetyltrimethylammonium Bromide

EDTA: Ethylene Diamine Tetra Acetate - is a DNA extraction reagent

ITS: Internal Transcribed Spacer gene - is found in the nucleus.

PAUP: Phylogenetic Analysis using Parsimony

PCA: Principal Component Analysis

PCR Polymerase Chain Reaction is the enzymatic synthesis of multiple copies of a specific DNA sequence in a cyclical manner.

SPSS: Statistical Package for Social Sciences

s.l.: *sensu lato* (in the widest sense)

s.s.: *sensu stricto* (in the strict sense)

TBE: Tris Borate EDTA

trnC: Transfer RNA gene for tRNA (Cys) found in the chloroplast

trnF: Transfer RNA gene for tRNA (Phe) found in the chloroplast

trnL: Transfer RNA gene for tRNA (Leu) found in the chloroplast

UPGMA: Unweighted Pair-Group Method with Arithmetic mean

CHAPTER TWO

LITERATURE REVIEW

2.0 Systematic history of Loganiaceae

The family Loganiaceae was first suggested by Robert Brown in 1814 and validly published by Von Martius in 1827 (Bendre, 1975; Frasier, 2008). The family belongs to the Order Gentianales which consists of the families Apocynaceae, Gelsemiaceae, Loganiaceae, Gentianaceae and Rubiaceae (Backlund *et al.*, 2000). Among these, Loganiaceae was considered to occupy a central evolutionary position (Bisset, 1980; Leeuwenberg and Leenhouts, 1980; Backlund *et al*. 2000). Bentham and Hooker (1862–1883), Solereder (1892-1895) were the pioneer taxonomists who attempted the classification of Loganiaceae after Martius had validly published the name of the family. Bentham and Hooker divided the family into seven tribes (Antonieae, Buddlejeae, Desfontainieae, Euloganieae including *Strychnos* and relatives, Gelsemieae, Potalieae and Spigelieae) (Leeuwenberg and Leenhouts, 1980). Solereder recognized two subfamilies: Loganioideae with six tribes (Antonieae, Fragraeae, Potalieae, Loganieae, Spigelieae, Strychneae) and Buddlejoideae with one tribe within the family, but he also excluded the two genera *Plocosperma* (of the Gelsemieae sensu Bentham and Hooker) and *Desfontainia* (of the Desfontainieae sensu Bentham and Hooker) (Backlund *et al*., 2000).

Almost 100 years later Hutchinson in 1973 divided the Loganiaceae of *sensu* Bentham and Hooker into seven distinct families and established a new Order (Loganiales) together with the family Oleaceae. The morphologically enigmatic genus *Plocosperma* was simultaneously placed as a monotypic family in the new Order Apocynales (Simões *et al.*, 2007).

In one of the latest acceptable classifications of Loganiaceae, (Leeuwenberg and Leenhouts, 1980), the family was circumscribed in its widest sense, classified into ten tribes (Table 1.0).

In the circumscription of Leeuwenberg and Leenhouts (1980), Loganiaceae consists of 600 species in 30 genera and included predominantly tropical, woody plants (Bendre, 1975; Mabberley, 1997). Cronquist (1981) reduced the circumscription of Leewenberg and Leenhouts to 21 genera in one tribe, grouped other six tribes to two families but removed three tribes completely from Gentianales (Table 1). 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 but said 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 genera completely from Gentianales (Table 1.0). However, Backlund *et al.,* (2000) which this study employs recognized 13 genera from Leewenberg and Leenhouts circumscription, the remaining genera were raised to nine different families but seven of them were completely excluded from Gentianales (Table 1.0). Loganiaceae is therefore herein treated to consist 13 genera worldwide (Backlund *et al*., 2000).

2.1.0 Classification of Loganiaceae

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Gentianales

Family: Loganiaceae

Genera: *Antonia, Bonyunia, Gardneria, Geniostoma, Labordia, Logania, Mitrasacme, Mitreola, Neuburgia, Norrisia, Spigelia, Strychnos, Usteria* (Backlund *et al*., 2000).

TABLE 1: Summary of most recent classifications of Loganiaceae by different authors

Taxa	**Leeuwenberg and Leenhouts (1980)**	**Cronquist (1981)**	**Thorne (1983)**	**Struwe *et al.* (1994)**	**Takhtajan (1997)**	**Backlund *et al.* (2000)**
Gelsemium	**Log**-Gels	**Log**	**Log**-Loga	Gel	Gel	Gel
Mostuea	**Log**-Gels	**Log**	**Log**-Loga	Gel	Gel	Gel
Anthocleista	**Log**-Pota	**Log**	**Log**-Loga	Gen	Gen-Pota	Gen
Fagraea	**Log**-Pota	**Log**	**Log**-Loga	Gen	Gen-Pota	Gen
Potalia	**Log**-Pota	**Log**	**Log**-Loga	Gen	Gen-Pota	Gen
Antonia	**Log**-Anto	**Log**	**Log**-Loga	Str	Ant	**Log**
Bonyunia	**Log**-Anto	**Log**	**Log**-Loga	Str	Ant	**Log**
Norrisia	**Log**-Anto	**Log**	**Log**-Loga	Str	Ant	**Log**
Usteria	**Log**-Anto	**Log**	**Log**-Loga	Str	Ant	**Log**
Gardneria	**Log**-Stry	**Log**	**Log**-Loga	Str	Str	**Log**
Neuburgia	**Log**-Stry	**Log**	**Log**-Loga	Str	Str	**Log**
Spigelia	**Log**-Spig	**Log**	**Log**-Loga	Str	Spi	**Log**
Strychnos	**Log**-Stry	**Log**	**Log**-Loga	Str	Str	**Log**
Geniostoma	**Log**-Loga	**Log**	**Log**-Loga	Geo	Geo	**Log**
Labordia	**Log**-Loga	**Log**	**Log**-Loga	Geo	Geo	**Log**
Logania	**Log**-Loga	**Log**	**Log**-Loga	**Log**	**Log**	**Log**
Mitrasacme	**Log**-Spig	**Log**	**Log**-Loga	**Log**	Spi	**Log**
Mitreola	**Log**-Spig	**Log**	**Log**-Loga	**Log**	Spi	**Log**
Buddleja	**Log**-Budd	Bud *	Bud	?	Bud *	Bud *
Emorya	**Log**-Budd	Bud	Bud	?	Bud *	Bud *
Gomphostigma	**Log**-Budd	Bud *	Bud	?	Bud *	Bud *
Nicodemia	**Log**-Budd	Bud *	Bud	?	Bud *	Bud *
Nuxia	**Log**-Budd	Bud	Bud	?	Bud *	Sti *
Retzia	**Log**-Retz	Ret	**Log**-Retz	?	Bud *	Sti *
Peltanthera	**Log**-Budd	Bud	Bud	?	Bud *	Ges *
Sanango	**Log**-Budd	Bud	Bud	?	Bud *	Ges *
Androya	**Log**-Budd	Bud	Bud	?	Bud *	Myo *
Plocosperma	**Log**-Ploc	**Log**	**Log**-Ploc	?	Plo	Plo *
Polypremum	**Log**-Spig	**Log**	**Log**-Loga	?	Bud *	Tet *
Desfontainia	**Log**-Desf	**Log**	**Log**-Desf	?	Des *	Col *
(30)	**30 Log**	**21 Log**	**22 Log**	**3 Log**	**1 Log**	**13 Log**

Note: The following abbreviations are used: for **families**, Ant = Antoniaceae, Bud = Buddlejaceae, Col = Columelliaceae, Des = Desfontainiaceae, Gel = Gelsemiaceae, Geo = Geniostomataceae, Gen = Gentianaceae, Ges = Gesneriaceae, Log = Loganiaceae, Myo = Myoporaceae, Plo = Plocospermataceae, Ret = Retziaceae, Spi = Spigeliaceae, Sti = Stilbaceae, Str = Strychnaceae, Tet = Tetrachondraceae; **for tribes** - Anto = Antonieae, Budd = Buddlejeae, Desf = Desfontainieae/Desfontainioideae, Gels = Gelsemieae, Loga = Loganieae/Loganioideae, Ploc = Plocospermeae/Plocospermatoideae, Pota = Potalieae, Retz = Retzieae/Retzioideae, Spig = Spigelieae, Stry = Strychneae, and for additional entries: ? =uncertain, and *= explicitly excluded from the Gentianales.

Since the description by Martius in 1827, both the circumscription of the family and the inter-familial relationships have been a matter of debate. Some authors accept one large 600 species family (Leeuwenberg and Leenhouts, 1980), whereas others prefer Loganiaceae split into 12 different families allocated to several distantly related orders (Takhtajan, 1997). According to many recent studies, Loganiaceae are polyphyletic (Downie and Palmer, 1992; Olmstead *et al*., 1993; Struwe *et al.*, 1994; Takhtajan, 1997; Oxelman *et al.*, 1999). This heterogeneity is also reflected in the classification by Leeuwenberg and Leenhouts (1980) in which the Loganiaceae was divided into ten tribes, some of which consisted of one or only a few species (Table 1.0). These genera share several characters such as valvate aestivation, coriaceous leaves, ability to accumulate aluminum (Leenhouts, 1962; Leeuwenberg and Leenhouts, 1980), and a homogeneous wood anatomy featuring absence of continuous rays, interxylary phloem of foraminate type, large cavities in rays, and vessels in tangential pairs or small clusters (Mennega, 1980; Metcalfe and Chalk, 1983; Bremer and Struwe, 1992; Bremer *et al.*, 1994; Struwe *et al.*, 1994). Additional features that could be interpreted as supporting this hypothesis are some indole alkaloid derivatives and loganine-type iridoids (Kisakürek and Hesse, 1980). The former Strychneae were united on the basis of their anther appendages and indehiscent fruits, features not reported for *Spigelia* (Leeuwenberg and Leenhouts, 1980). The other lineage, by contrast, is well supported both on morphological and molecular grounds. Characters include partly apocarpous carpels (possibly homologous, early from apex-splitting fruits), ochrea instead of stipules (Leeuwenberg and Leenhouts, 1980; Backlund *et al.*, 2000), a general change from the presumed plesiomorphic basal chromosome number of x = 11 to x = 10 (Gadella, 1980) and a general absence of alkaloids (Bisset, 1980).

The molecular studies have shown that combined sequence data from four plastid genes *rbcL*, *ndhF*, *mat*K, and *trn*L, placed Rubiaceae as the most basal family in the Gentianales (Bremer and

Thulin, 1998; Backlund *et al.,* 2000; Jiao and Li, 2007). Loganiaceae and Gelsemiaceae were the two subsequently diverging clades; Apocynaceae and Gentianaceae were sisters in the most nested position (Backlund *et al.,* 2000). The Gelsemiaceae is a relatively new family and the constituents have a history of being placed inside the Loganiaceae or the Apocynaceae based on morphological features (Struwe *et al*., 1994). Further works divided the Loganiaceae family to 15 genera (Dunlop, 1996) from four tribes: Antonieae, Loganieae, Spigelieae (monogeneric) and Strychneae. Strychneae includes three genera, *Gardneria*, *Neuburgia* and *Strychnos*. The first two genera are Asian endemics and have few species. *Strychnos*, on the contrary, has the largest number of species in the family with approximately 200 species pantropically distributed (Leeuwenberg and Leenhouts, 1980; Frasier, 2008). The relationships of the genera in Strychneae have been difficult to characterize as their placement in gene trees vary. The tribe seems to be an artificial grouping, and was paraphyletic in a study using two chloroplast genes, *ndhF* and *rbcL*, due to the inclusion of Spigelieae (Backlund *et al.,* 2000). Phylogenetic analyses of two faster evolving genes, *rps16* and the ITS were carried out to delimit the relationships between genera in the Loganiaceae with an emphasis on Strychneae (Olmstead *et al.,* 1998; Backlund *et al.,* 2000; Frasier, 2008). Analysis of the secondary structure of the ITS region resulted in possible new synapomorphies for tribes Antonieae and Spigelieae within the Loganiaceae (Sennblad and Bremer, 1996; Sennblad, 1997). Loganiaceae together with Geniostomataceae in Struwe *et al.* (1994) form a clad for which the name Loganiaceae has priority (Reveal, 1993; Frasier, 2008). Strychnaceae also can not be retained as they become paraphyletic due to the successive nesting of Loganiaceae and Geniostomataceae within them (Struwe *et al.,* 1994; Backlund *et al*., 2000). The Flora of West tropical Africa has reported six genera for Loganiaceae in Hutchinson and Dalziel (1972).

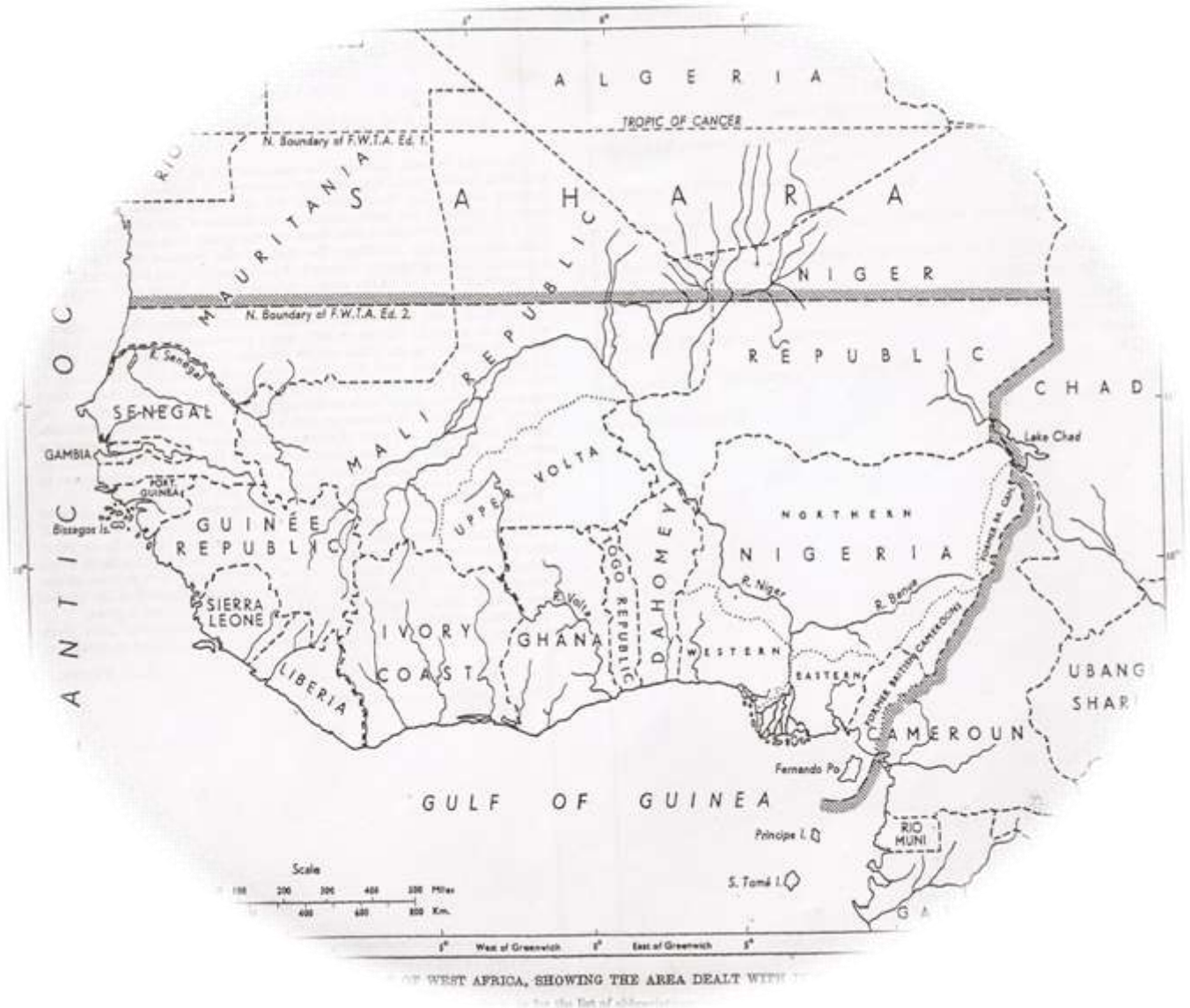


Figure 2: Map of West tropical Africa showing the regions where Hutchinson and Dalziel made their collections – the region enclosed by thick line.

Source: Hutchinson and Dalziel (1972).

2.1.1 Systematic positions of some genera formerly included in Loganiaceae

A number of genera previously regarded as members of the Loganiaceae have been found to belong to Lamiales. The positions of these genera are congruent with the results of Olmstead *et al.*, (1993), Oxelman *et al*., (1999) and Backlund *et al.,* (2000).

2.1.2 Buddlejaceae

Leeuwenberg and Leenhouts (1980) while citing Jussieu in 1789 and Bentham in 1856 stated that *Buddleja* was first placed in the Scrophulariaceae but was later moved to Loganiaceae and recently regarded as a tribe within Loganiaceae. Buddlejaceae were separated by Dahlgren (1975) from the rest of Gentianales and placed in Lamiales because of their seco-iridoids, biosynthesis route II (Jensen, 1991; Jensen, 1992). This issue has attracted attention and several features from wood anatomy, including lack of borders on pits of imperforate tracheary elements and embryological features such as cellular endosperm are characteristic of Lamiales (Mennega, 1980; Carlquist, 1986, 1992; Maldonado de Magnano, 1986; Engell, 1987). The *Buddleja* has been carved out of the family Loganiaceae based on the absence of intra-xylary phloem and the presence of glandular, stellate and scaly indumentums (Dahlgren, 1980). *Nuxia* is also now represented in the family Buddlejaceae and with just two genera, visibly: *Buddleja and Nuxia* (Alfarhan, 1992). The genus *Buddleja* can easily be separated from *Nuxia* by its stellate indumentums, densely clustered terminal spike and the included stamens (Dahlgren, 1980).

2.1.3 *Retzia*

Several recent studies indicate *Retzia* of the monotypic tribe Retzieae, sometimes recognized as the distinct family Retziaceae, differs in many characters from Loganiaceae (Dahlgren *et al.,* 1979; Bremer *et al*., 1994; Struwe *et al.,* 1994). *Retzia,* on phytochemical and anatomical grounds, had

been associated with the genus *Stilbe* (Dahlgren *et al.*, 1979; Carlquist, 1986), originally placed in the Verbenaceae. The systematic position for the genus is debatable due to its relationships with other families.

2.1.4 *Plocosperma, Polypremum, Peltanthera and Sanango*

Apart from *Retzia* and *Stilbe*; *Plocosperma, Polypremum, Peltanthera and Sanango* have also been excluded from Loganiaceae as well as from Gentianales (Jensen, 1992; Oxelman, *et al.*, 1999). The monotypic Central American genus *Plocosperma* was earlier the sole member of the tribe Plocospermeae of Loganiaceae (Leeuwenberg and Leenhouts, 1980). In some classifications, *Plocosperma* has been suggested to show a close relationship to Apocynaceae (Hutchinson, 1973; Cronquist, 1981; Takhtajan, 1987; Endress and Albert, 1995). In recent phylogenetic studies, these placements have been refuted (Struwe *et al*., 1994; Endress and Albert, 1995; Oxelman *et al.*, 1999). The likewise monotypic American genus *Polypremum,* former member of the tribe Spigelieae of Loganiaceae, is indicated to occupy a relatively early branch within Lamiales. *Peltanthera* and *Sanango,* two other monotypic American genera, were earlier placed in the tribe Buddlejeae of the Loganiaceae (Leeuwenberg and Leenhouts, 1980; Backlund *et al*., 2000).

As a result of these circumscriptional variations, Nicholas and Baijnath (1994) said "Classifications, including those of higher taxa, will continue to change as our knowledge of these groups and their relationships change. 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

2.2.0 Economic importance of Loganiaceae

Generally, all plants are useful except if the usefulness has not yet been discovered (Sofowora, 2006). The genera in Loganiaceae serve as source of food, medicine, shelter, ornaments, horticulture, furniture, windbreak, green manure, soil stability, dyes, pesticides, resin, gums etc. The entire genera found in the family Loganiaceae are very useful to wild, domestic animals and mankind for social purposes (Burkill *et al.*, 1995).

2.2.1 *Anthocleista*

The wood of *Anthocleista* is rarely used as timber because it is soft and the pith is thick. It is sometimes used as firewood (Leeuwenberg, 1969). The root, bark and leaves have medicinal value. A decoction of the roots is commonly taken for constipation, and the bark is sometimes used for fever (Musa *et al*., 2010). Musa *et al*. (2010) also demonstrated how aqueous and ethanolic extract of *A. vogelii* tree bark are potent medicine against all levels of typhoid fever. Their seeds and barks are used as purgative; but in small doses because they are regarded as toxic; they are also used as contraceptives and abortifacients (Asusu, 2007; Kadiri, 2009).

In southern Nigeria, the root is regarded as remedy for venereal disease (Asusu, 2007). In other West African countries like Sierra Leone, Ivory Coast and Liberia, *A. djalonensis* is used for treating various ailments. In Sierra Leone the leaf is used as a woman's medicine, a decoction being taken for any abdominal pain supposed to be of uterine origin and also a treatment against jaundice (Burkill *et al*., 1995; Musa *et al.,* 2010). In Liberia, the bark is applied as a poultice for sores and is given to dogs sick of diarrhea (Burkill *et al.,* 1995). The leaves are used to line kola-nut baskets for fermentation in several nations of West Africa. In Ivory Coast the root is used as a diuretic and a vigorous purgative and also as a poison antidote, against leprosy, in the treatment of

edemas and elephantiasis of the scrotum (Asusu, 2007). The root decoction is taken against chest pain, for constipation and against gonococci infection (Sofowora, 2006). Okorie (1976) isolated a novel compound jalonensis, a phthalide, from the stem bark of *Anthocleista djalonensis.* Dalziel in 1955 reported that the stem bark of this tree is used locally for curing fever, stomach ache and as a purgative and the root is used as a remedy for venereal diseases (Asusu, 2007**)**. Okoli and Iroegbu (2004) demonstrated that the root extract of *A. djalonensis* in combination with the root extracts of other plants showed positive activity against strains of *Staphylococcus aureus* and *Escherichia coli* isolated from cases of sexually transmitted disease. Iwu (1993) reported that the stem bark and roots of *A. djalonensis* are used to cure malaria, jaundice and diabetes. Etukudo (2003) and Asusu (2007**)** stated that the bark decoction of *A. djalonensis* when drunk cures painful menstruation and gonorrhoea, while the poultice of the bark cures ulcers, cuts, sores and wounds. He further noted that it is a very satisfying fodder for goats and sheep (Asusu, 2007).

2.2.2 *Strychnos* species

Strychnos is the largest genus in Loganiaceae with approximately 200 species that grow in tropical rain forests and savannas as lianas, shrubs, or small trees. In the neotropics *Strychnos* is distributed from Mexico down through Bolivia (Frasier, 2008). In the paleotropics it is found throughout tropical Africa and Madagascar, in India, Sri Lanka, Southeast Asia and the northern tropical part of Australia (Bisset *et al*., 1973; Leeuwenberg and Leenhouts, 1980). Multiple in-depth taxonomic studies have also been conducted on the genus (Leeuwenberg, 1969; Leeuwenberg and Leenhouts, 1980). *Strychnos'* generic classification has been divided into 12 sections based on morphological and anatomical characters in the most recent sectional treatment of the genus by Leeuwenberg and Leenhouts (1980). At the time of Leeuwenberg and Leenhouts' publication, Africa had the highest number of described *Strychnos* species and these were classified into 11 of the 12 sections. The

only section that does not have a representative in Africa is section *Strychnos*, whose species are American or Asian. Out of the 12 sections, six occur only in Africa: *Densiflorae* Duvign., *Dolichanthae* Duvign., *Spinosae* Duvign., *Aculeatae* Duvign., *Phaeotrichae* Duvign., and *Scyphostrychnos* (S. Moore) Leeuwenberg. The last three sections are monotypic. The sections with the greatest representation in Africa are *Lanigerae* A.W. Hill and *Breviflorae* Prog. with 12 species each. However, the bulk of *Lanigerae* is Asian (Leeuwenberg and Leenhouts, 1980), and the bulk of *Breviflorae* is American (Krukoff *et al.,* 1972). Section *Breviflorae* was divided into two subsections; *Breviflorae* and *Eriospermae* by Krukoff and Barneby (1969) in their treatment of the American species, but this was omitted in Leeuwenberg and Leenhout's (1980) classification. Therefore, the African species of this section are currently not assigned to either subsection. The American species of *Strychnos* are only in three sections: *Breviflorae, Strychnos* and *Rouhamon* (Aubl.) Prog. (Leeuwenberg and Leenhouts, 1980). The Asian species are divided into four sections: *Strychnos, Brevitubae* Hill, *Lanigerae,* and *Penicillatae* Hill (Leeuwenberg and Leenhouts, 1980). Unlike many of the African sections that are restricted to the African continent, none of the American and Asian sections are unique to their continents. For example, section *Strychnos* is divided between the Americas and Asia, and sections *Brevitubae* and *Penicillatae* are in both Asia and Africa. The primary purpose of Leeuwenberg and Leenhout's classification was for identification of *Strychnos* species and they utilized mostly gross morphological characters (Leeuwenberg, 1969). The sections group species with a common suite of cardinal characters relying heavily on the ratio of corolla tube length: corolla lobe length as well as the density and location of pubescence on the corolla characters (Leeuwenberg, 1969). However, many of the sections have overlapping character descriptions suggesting that the sections are heterogeneous, and some might be based on symplesiomorphies. For these reasons, the monophyly of many of the sections is in doubt (Frasier, 2008). *Strychnos* is probably most famous in popular science and

culture for its production of the toxin strychnine, which is commercially extracted from *S. nux-vomica* Linn. (Bisset *et al*., 1973; Samuelsson, 1992). More than 12 American species of *Strychnos,* such as *S. toxifera* Linn. are used as primary or secondary ingredients in the dart poison, curare (van Andel, 2000). In regions of Africa *Strychnos* species, such as *S. aculeata* Solereder, are used as fish poisons and for treating parasitic infections like guinea worm (Burkill *et al*., 1995). In Madagascar, *Strychnos myrtoides* Bak. has been combined with more conventional drugs to treat malaria (Rafatro *et al*., 2000), and in India, *S. potatorum* Linn. seeds are used to settle turbid water (Gupta and Chaudhuri, 1992). The various therapeutic and other ethno-botanical uses of *Strychnos* have made the genus an attractive subject to many chemical investigators (Bisset and Phillipson, 1971a; Yan *et al*., 2006). *S. spinosa* Lam. wood can be used for general carpentry. Timber from this tree is also used to produce implement handles, fighting sticks, hut poles and also used for carving. The leaves, fruit, seeds and roots are used medicinally. Some people use root infusions as a treatment for snake bite, others use the bark and unripe fruit. It is believed that the presence of strychnine in the bark and unripe fruit along with other alkaloids are responsible for helping overcome the venom of certain snakes, such as Mamba (Burkill *et al*., 1995). Strychnine is a powerful Central Nervous System stimulant that may be able to fight the respiratory depression caused be the venom of these snakes. It is also used as a purgative, for uterine problems and to treat sore eyes (Burkill *et al*., 1995). A decoction of the leaf or root is used as an analgesic in Central Africa (Burkill *et al*., 1995). The dried fruit, after the seeds are removed, are often used as sounding-boxes for musical instruments such as the ‘marimba’. They are also carved and sold as ‘curios’. The fruit is edible and often sun dried as a food preservative. The seeds must be avoided as they are poisonous though could have purgative effects (Watt and Breyer-Brandwijk, 1962).

Figure 3: *Strychnos spinosa* showing dried fruit: with the seed they are made to Decor Balls (1a - 1d); without the seed they are made to Promise Pots (2a - 2c); without the seed they are made to Ihlali candles (3 and 4); without the seed they are made to Salt & Pepper Sets (5a- b).

Source: Watt and Breyer-Brandwijk (1962).

The African *Strychnos* species have been examined for indole alkaloids, their antiplasmodial, cytotoxic actions and have been found to be involved in several ethno-botanical uses (Frederich, *et al.,* 2003). A few species are well known for their incorporation into arrow and ordeal poisons, but play more a role in ethno-medicine against fever, rheumatism, worms, ulcers, leprosy, snake-bites (Neuwinger, 1996). *Strychnos* alkaloids are examples of molecular and pharmacological biodiversity (Frederich *et al.,* 2003). More than 300 different *Strychnos* alkaloids have been isolated to-date and they present various biological activities in several fields: parasitology - amoebiasis and paludism (Frederich *et al.*, 2002); Cancer (Wright *et al.,* 1991); Neurology - tetanizing or curarizing effects (Krukoff *et al.*, 1972; Quetin-Leclercq *et al*., 1990); Inflammation (Quetin-Leclercq *et al*., 1990).

In an attempt to find new sources of natural antiplasmodial agents, pharmacologists and scientists have screened more than 50 plant extracts against *Plasmodium falciparum,* and three plants were particularly active: *S. usambarensis* Gilg, *S. icaja* Baill. and *S. variabilis* Linn. (Wright *et al.,* 1991; Frederich *et al*., 1999). The golden fruit pulp of *S. variabilis* is deliciously sweet but the root bark is said to be a violent poison from which twenty-one alkaloids have been isolated (Tits *et al*., 1980). The roots and leaves of these plants are used to prepare a curarizing poison that is applied to long arrows prepared for hunting lions, buffalos, and antelopes (Neuwinger, 1996). The root barks of these *Strychnos* species have been examined for tertiary and quaternary alkaloids and above twenty alkaloids products have been isolated. They include: C-dihydrotoxiferine which was also broken down to three: dihydrotoxiferine, curarine and calebassine (Tits *et al*., 1980). It has been found out that the family Loganiaceae contains taxonomically informative types of compounds like iridoids, xanthones, mangiferine and C-gIucofIavones (Carpenter *et al.*, 1969).

2.2.3 *Spigelia anthelmia*

Spigelia anthelmia is an herb found to be very potent against parasites in the digestive tract based on its anthelmintic properties (where it derived its specific epithet) (Hammond *et al.*, 1997; Waller, 1997; Akhtar *et al.*, 2000; Jackson and Coop, 2000; Jegede *et al*., 2007; 2009). Adegoke *et al.* (1968) who examined some medicinal plants for the presence of alkaloids reported that *Spigelia anthelmia* contained alkaloids in the leaves (Hammond *et al*., 1997). Some of the alkaloids have been isolated and identified (Asusu, 2007). Achenback *et al.* (1995) isolated two alkaloids: spiganthine and ryanodine. Hubner *et al.* (2001) isolated and identified twenty other minor constituents that have chemical structure and biological function similar to ryanodine-type compounds (Asusu, 2007). The result of the work of Assis *et al.* (2003) has demonstrated the possibility of using *S. anthelmia* in the control of goat and sheep gastro-intestinal nematode (Jackson and Coop, 2000; Jegede *et al*., 2007; 2009). They reported that ethyl acetate and methanol extracts of *S. anthelmia* inhibited 100% egg hatch and 81.2% larvae development of *Haemonchus contortus* (Hammond *et al*., 1997; Waller, 1997; Akhtar *et al*., 2000; Asusu, 2007).

2.2.4 *Usteria guineensis*

The leaves and stem of *Usteria guinensis* macerated with local black soap cures acute Malaria by bathing with the soap daily (Oladele and Adewunmi, 2008). Bierer *et al*. (1995) remarked that natural product chemists and phyto-chemists have recognized that plant species in the tropics contain a bewildering biological diversity of secondary metabolites. They suggested one of the most compelling explanations for the vast array of chemical diversity which resides with tropical plants species is the science of chemical ecology whereby plants in the tropics had to develop an extraordinary array of defenses, most of them are chemicals, to protect themselves from viral diseases, fungal pathogens, insect and mammalian pathogens (Asusu, 2007**)**.

2.3.0 Plant identification and Taxonomic Key

One of the primary objectives of Systematics is Identification. Although, identification is a separate process which in practice involves both classification and nomenclature (Radford *et al*., 1974). Identification is the determination of the similarities or differences between two elements (Heywood and Moore, 1984). Identification is, therefore a basic process in classification with nomenclature playing an essential role in the retrieval of information and as a means of communication (Heywood, 1968). Blackwelder (1967) stated it in this manner, "identification enables us to retrieve the appropriate facts from the system (classification) to be associated with some specimen at hand". One commonly identifies a plant by direct comparison or the use of keys and arrives at a name. The traditional methods of identification according to Radford *et al*. (1974) include: Expert determination, recognition, comparison and the use of taxonomic keys. Taxonomic key is a type of taxonomic literature, it is also a device useful in identifying an unknown (Lawrence, 1951). Keys are devices consisting of a series of contrasting or contradictory statements or propositions (a couplet) requiring the identifier to make comparisons and decisions based on statements in the key as related to the materials to be identified (Radford *et al*., 1974). Lawrence (1951) succinctly defined it as an artificial analytical device or arrangement whereby a choice is provided between two contradictory propositions resulting in the acceptance of one and the rejection of the other. In any key, each statement of a couplet is termed a lead. The currently conventional and most acceptable type of key is the dichotomous key; this could be indented (yoked leads) or bracketed (parallel leads). Preparation of a key usually is done by dividing the original taxa into two subgroups by the best possible character couplet, then considering each of these subgroups separately, dividing them similarly until every taxon is distinguished from all others (Radford *et al*., 1974; Heywood and Moore, 1984; Stace, 1989).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Herbarium study and sample collection

Specimens of Loganiaceae were studied in Forestry Herbarium Ibadan (FHI) Ibadan, Obafemi Awolowo University (OAU) Ile-Ife, University of Nigeria Herbarium (UNH) Nsukka, Bayero University Herbarium (BUH) Kano, Ahmadu Bello University Herbarium (ABUH) Zaria, University of Lagos Herbarium (LUH) Lagos and Gold Coast Herbarium (GCH) Accra, Ghana. All correctly identified specimens were observed for their vegetative and reproductive characters that were used for their identification before preservation in the herbaria. The habitat type and geographical distribution were also noted.

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, seeds and 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. Each sample was tagged as follows: (a) Name; Generic, Specific, Common and Local names temporarily for the purpose of identification, (b) Zone of Collection, (c) Location: Latitude, Longitude, Name of the Town/ Village of Collection, (e) Date of Collection. This follows the descriptions of Hutchinson and Dalziel (1958).

Photographs of samples from each species were equally taken with digital camera at their natural environment, and the exposure number of the photographs corresponding to each plant was recorded. On-field evaluation of vegetative and reproductive parts (leaves, stem, inflorescence and fruits) was carried out on each sample in their natural environment prior to sample collection or immediately after collection for tangled climbers.

3.1.1 Herbarium preparation

The complete plant (herb) or branch of shrubs, trees and climbers collected were spread separately in newspapers and this was kept in a plant press. The press was tied with twines and the newspapers were changed at intervals of two days in order to prevent the decomposition of the plant material. Moisture was removed as the newspapers were changed and this was carried out until the plant was thoroughly dried. Each of the dried specimens was then removed from the old newspaper and mounted on white cardboard papers with the aid of white gum (glue). Label of full description of the plant, its location, the date of collection and the name of collector was attached to each of the herbarium specimen. This was then authenticated at FHI and deposited both in FHI and LUH for reference purposes.

3.2.0 Biogeography

The distribution of Loganiaceae in West Tropical African forests was determined by plotting the coordinates obtained in the field throughout the period of collection. This was achieved by the use of Maximum Entropy (MAXENT) Programme for maximum entropy modeling of species' geographic distributions (Phillips *et al*., 2006).

The relationship for conversion of GPS values to decimal, recognizable by the software is given by: $X = \text{Degree} + \frac{\text{minute}}{60} + \frac{\text{seconds}}{3600}$

The following settings were used to run the programme: 18 presence records for training, 4 for testing, 10011 points used to determine the Maxent distribution (background points and presence points) as well as nineteen (19 - all continuous) environmental layers. They are adopted from the world climate database designed for West Africa.

3.3.0 Morphological and anatomical studies

3.3.1 Macromorphology

The qualitative features such as leaf apex, leaf base, leaf shape, surfaces indumentum, stem colour, inflorescence type and flower colour were visually assessed. Aided magnifying lens (x10) were sometimes used for minute organs. Quantitative features such as leaf size, petiole length, leaf blade length, plant height, corolla tube length and width were determined using thread and meter rule as described in Radford *et al*. (1974).

3.3.2 Micromorphology

Anatomical characters both qualitative and quantitative that have taxonomic significance were assessed for different plant specimens of five to eight replicates in the laboratory and the observations were recorded before they degraded on glass slides. An area of about 1cm square was removed from a central/standard position; midway between the base and apex of the mature leaf, fresh and herbarium leaves used (Olowokudejo, 1993). The herbarium leaves were revived by boiling in water before use. Samples were then soaked in domestic bleach or nitric acid to digest

the mesophyll layer after which they were carefully washed in water and the adaxial and abaxial epidermis teased from the mesophyll using fine forceps and dissecting needles. The membranes were transferred into different concentrations of ethanol; 50%, 60% and 70% in succession to harden and were later washed in distilled water to remove ethanol. They were later transferred into 10% aqueous solution of safranin-0 (dye) for 3 mins, removed and washed with water to remove the excess stain. They were mounted in glycerine, observed under the microscope and the good preparations were sealed with nail vanish for further assessment. The upper (adaxial) and lower (abaxial) surfaces were treated separately for each plant specimen. Photomicrograph of the epidermis was obtained with Olympus XSZ-N107 Model light microscope and Motic Camera 'Moticam 2300, 3.0 M.Pixel, USB 2.0 model. Twenty randomly selected epidermal cells and stomata were measured using a micrometer eye-piece, the trichome lengths were measured and types observed. Stomata Index was calculated using the formular:

$$\boldsymbol{Stomata\ Index}\ (SI) = \frac{\text{Stomata number}}{\text{Epidermal cell number} + \text{Stomata number}} \times 100$$

Counting of cellular structures was done at 640-Magnification, Measurement at 400-Magnification and image taken at 640-Magnification. The approaches adopted were according to Ogundipe, 1990; Ogundipe and Olatunji, 1991; Olowokudejo, 1993; Ogundipe and Akinrinlade, 1999; Olowokudejo and Pereira-Sheteolu, 1998.

3.4.0 Molecular studies

Fresh leaf samples were collected and stored immediately in a small cellophane silica gel bag. This was to dry the samples as soon as possible in order to avoid further enzymatic reaction and DNA

degradation (Chase and Hills, 1991). The samples in bags were later transferred to the laboratory domestic freezer until their DNA extraction was carried out (Doyle and Doyle, 1990).

3.4.1 DNA extraction

Total genomic DNA extraction was carried out using the modified (pinch of Silica gel used for grinding) Cetyltrimethyl-ammonium bromide (CTAB) extraction protocol described by Doyle and Doyle, 1990.

Mortar and pestles were preheated concurrently with 10 ml of Isolation Buffer (10 X CTAB) containing 80 ml of betamercaptoethanol (ratio 1000 µl : 8 µl) in 50 ml blue cap tubes in a 65 °C water bath. The mass of 0.15 - 0.2 g of dry or fresh leaf tissue in a mortar and pestle (preheated to 65°C) was ground using a portion (100 µl) of the isolation buffer. For tough tissues a pinch of sterile sand or silica gel was added. A volume of 600 µl of the buffer was added and swirl to suspend the slurry, poured into 1.5 ml Eppendorf tube and incubated at 65 °C for 15-20 minutes with optional occasional gentle swirling. An equal volume (700µl) of SEVAG mixing was added when cooled for about 4 minutes, gently mixed but thoroughly. The cap of the tube was opened to release entrapped gas, then re-tightened and the tubes were rocked using an orbital shaker (300-4000 rpm) for 60 minutes. Ultracentrifugation was carried out at 12,000 rpm, 0 °C to 25 °C for 7 minutes. Ideally the aqueous (top) phase, containing the DNA, was clear and colourless. The aqueous (top) phase containing DNA was removed with a plastic transfer pipette and transferred to a clean, newly- labelled 1.5 ml Eppendorf tube. The SEVAG and plant debris were disposed into the designated SEVAG waste container. The volume of the aqueous phase DNA typically was 300 µl. then equal volume of -20°C ethanol was added and mixed gently to precipitate the DNA. The DNA were kept in -20 °C freezer for a few hours for fresh material, at least one week for silica dried material and up to two weeks for herbarium samples to precipitate DNA. The tubes were

spun in centrifuge at 5,000 rpm for 2 minutes to collect DNA precipitate. The supernatant was discarded and 500 µl of 70 % ethanol was added to wash the DNA. Pellets were dislodged to facilitate "washing" and wash for 2 to 3 times. The DNA was spurn down at 5,000 rpm for 2 minutes and the pellet dried as the tube lied horizontally to allow alcohol evaporation. The DNA was re-suspend in 100 – 150 µl double distilled or Nuclease free water and store at -80 °C freezer for long periods.

3.4.2 Purification of extracted DNA

This was carried out in a Silica-column inserted into vacuum manifold connected to a vacuum pump using QIA quick purification kit. The following procedures were followed:

Labeled silica-columns were inserted into a vacuum manifold. The volume, 750 µl of Buffer PB (Binding buffer) was added to 150 µl of total DNA. The samples were loaded into the silica-columns and the buffer was allowed for 5 mins to bind with the DNA. Vacuum pump was connected to suck until entire liquid reagent had passed through the column but the DNA samples were practically suspended in the membrane of the column. The sample was washed by adding 750 µl Buffer PE (Washing Buffer/Ethanol) to each column and vacuum was applied. The columns were transferred into 1.5 µl collection tubes, Centrifuged at 13,000 rpm for 1 minute to remove residual ethanol (Washing Buffer/Ethanol). 50 µl Buffer EB (Elution Buffer) was added to the columns and left for 1 min at room temperature to dissolve the DNA in the elution buffer. The tube was finally centrifuged for 1 min at 13000 rpm from the column for further analysis.

3.4.3 Gel Electrophoresis

The purity and concentration of the DNA samples were checked by preparing 1% agarose gel (1.50 g agarose powder dissolved in 150 ml 1x TBE- tris borate ethylene diamine tetra acetate

buffer). This was allowed to electrophorese at 60 V for 60 min at 110 mAmp. The gel was then visualized under an ultraviolet transilluminator – UV Lamphouse, Cat. Number: 430-3350 attached to a computer system and photographed with the documentation units (UVitec) made by Liuyi Gel Documentation - model: WD- 9413A, Cat. Number: 130-1310.

3.4.4 Loading of samples and running the Gel

Two microliter of loading buffer (0.25 % bromophenol blue, 0.25% xylene cyanol and 30% glycol in water) was spotted on a parafilm mixed with 3 µl DNA. The mixture was pipetted into the well. However, the PCR amplicons require standard DNA range marker (1kb DNA lambda) as opposed to running the genomic DNA. This was allowed to electrophorese at 60 V for 30 mins at 110 mAmp. The gel was then visualized under an ultraviolet transilluminator attached to a computer system and photographed with the documentation units (UVitec).

3.4.5 Cleaning and quantifying PCR products

This was achieved using QIA quick silica column (QIAGEN Ltd) following manufacturers protocol. To 20 µl of the PCR reaction product, 5X volume of binding buffer (i.e. 100 µl PBB buffer) was added and loaded in a vacuum manifold and vacuum pump was applied. The samples were washed by adding 0.75 ml of wash buffer (buffer PE) to each column and vaccum was applied. The tubes were centrifuged for 2 min at 3000 rpm to remove excesss alcohol from the samples. Each of the columns was then transferred into a clean labeled lidless 1.5 ml Eppendorf tube and 20 µl of elution buffer was added directly into the membrane and incubated at room temperature for 10 mins to elute the DNA. The tubes were then centrifuged for 2 mins at 3000 rpm. To determine the concentration of the DNA obtained, 2 µl of the clean PCR product was added to 55 µl of sterile water in a cuvette and the quantity was verified on the spectrophotometer.

3.4.6 Spectrophotometry

Eppendorf Biophotometer Plus, was used for this analysis. The equipment was used to check the quality and quantity of the DNA; the quantity in terms of the concentration of the DNA in nanogram per microlitre *(ng/µl)* and the quality in terms of the absorbance of ultraviolet light through the cuvette measured in Armstrong *(A*). The dilution factor used was ratio (DNA: diluents; 1 µl: 20 µl). The results were recorded for concentration, absorbance at different wavelengths concurrently: 230 *A*, 260 *A*, 280 *A* and ratio of 260 *A* /280 *A*.

3.4.7 Polymerase Chain Reaction (PCR) for DNA amplification

Polymerase Chain Reaction (PCR) was used to amplify three gene regions: ITS 1 - the 5.8 S nuclear gene, two non-coding chloroplast DNA regions between trnl C – trnl D and trnl E – trnl F according to the methods of White *et al*. (1990) and Baldwin *et al.* (1997). The reaction conditions were as follows: initiation reaction at 94 ℃, denaturation reaction at 94 ℃, annealing reaction was at 65 ℃, elongation reaction at 72 ℃ and final elongation reaction was at 72 ℃. The reaction mixture was finally held at 4 ℃.

3.4.8 Amplification of the Internal Transcribed Spacer (ITS) region

Amplification of the Internal Transcribed Spacer (ITS) region of nuclear ribosomal DNA, composed of ITS1, the 5.8S gene, and ITS2 was carried out using two primers ITS leu1 (5'-GTC CAC TGA ACC TTA TCA TTT AG-3') and ITS4 (5'- TCC TCC GCT TAT TGA TAT GC-3') as described by White *et al*. (1990) and Baldwin *et al.* (1997). The fragment size amplified for ITS was between 500 bp – 800 bp. Reaction mixture of 25 µl included 22.5 µl of PCR Master Mix

(Red-top premix), 0.5 µl Bovine Serum Albumin (BSA), 0.5 µl of primer 1 (ITSleu1), 0.5 µl of primer 2 (ITS4), and 1µl of DNA sample. The reactions were performed in an Applied Biosystems thermal cycler (Gene Amp PCR System 2400). The DNA amplifications were carried out using the ‘‘Hot Start’’ PCR method: 94 ^{0}C for 1 min, 72 ^{0}C for 6 mins (initial strand separation); 30 cycles at 94 ^{0}C for 1 min (Denaturation), 50 ^{0}C for 1 min (annealing), 72 ^{0}C for 1 min and 30 s (primer extension); with a final extension at 72 ^{0}C for 7 mins. PCR products were cleaned using the QIAquick PCR purification kit (Qiagen, Valencia, California, USA).

3.4.9 Amplification of *trn*L-*trn*F Spacer Plus *trn*L Intron

The non-coding cpDNA regions *trn*L-trnF spacer plus *trn*L intron are adjacent and were amplified separately. TrnL includes region between trnl C – trnl D and trn F includes trnl E – trnl F. Primers used for amplification were UniC (5'-CGA AAT CGG TAG ACG CTA CG) and UniF (5'-ATT TGA ACT GGT GAC ACG AG) of Taberlet *et al*. (1991). The fragment size amplified was between 1100 bp – 1200 bp. Reactions of 25 µl were obtained with 22.5 µl of PCR Master Mix (Green-top premix), 0.5 µl Bovine Serum Albumin (BSA), 0.5 µl of primer 1 (UniC), 0.5 µl of primer 2 (UniF), and 1 µl of DNA sample, to complete a volume of 25 µl. The thermal cycler program included initial denaturing at 94 ^{0}C for 3 mins; 28 cycles at 94 ^{0}C for 1 min (Denaturation), 48 ^{0}C for 1 min (annealing), 72 ^{0}C for 1 min (primer extension); ending with a final extension at 72 ^{0}C for 7 mins and final hold at 4 ^{0}C.

3.4.10 Cycle sequencing

Cycle sequencing is a method used to increase the sensitivity of the DNA sequencing process and permits the use of very small amounts of DNA material (Sanger *et al*., 1977). Amplification of selected regions was carried out in a 10 µl reaction mixtures containing 0.5 µl pink juice (Big DyeTerminator, Applied Biosystems Inc.), 3.0 µl 5X sequencing buffer (Bioloine), 0.75 µl primer (1: 10 dilution forward or reverse for each primer pair) and varying quantities of purified PCR products depending on the strength of the reaction, final reaction was made up to 10 µl with sterile distilled water. The amplification of the ITS regions was improved by the addition of 4 % DMSO in the total volume of the sequencing mix. Cycle sequencing was carried out in a Gene Amp® PCR System 9700 thermocycler (Applied Biosystem Inc.) using the following programme; initial denaturation for 30 s at 95 ℃ followed by one cycle of denaturation for 1 min at 95 ℃, annealing for 30 s at 55 ℃ and extension for 60 s at 72 ℃. This was run for 30 cycles and the cycle was completed by holding the reaction mixture for 7 mins at 72 ℃ to allow complete extension of the PCR product with a final hold of 4 ℃.

3.4.11 DNA sequencing, editing and alignment

Deoxyribonucleic acid (DNA) sequencing is the determination of all or part of the nucleotide sequence of a specific deoxyribonucleic acid molecule. In this study, automated sequencer (ABI PRISM® 3100 DNA Analyzer) was used following the manufacturer's instruction. The program sequencher version 4.5 was used to assemble complementary strands and verify software base-calling on a Macintosh computer.

3.4.12 Sequencing of ITS Region

Sequencing of the purified cycle sequencing products was done in both directions on an automated sequencer (ABI PRISM® 3100 DNA Analyzer by Applied Biosystems, Foster City, California, USA) at the Royal Botanical Garden, Kew DNA Sequencing Core Facility using primers ITS 4 and ITS 5 HP (5'-GGA AGG AGA AGT CGT AAC AAG G-3').

3.4.13 Sequencing of trnL-trnF spacer plus trnL intron

These two regions were cleaned using the QIAquick PCR purification kit and sequenced on an ABI PRISM 3100 automated sequencer using the same primers (UniC and UniF) used for PCR.

3.4.14 Phylogenetic analyses

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. While in Distance method analysis; BioNJ, J-C distance, all gap sites ignored and 100 Bootstrap replicates were used.

3.5.0 Data Analysis

Statistical Package for Social Science Students- (SPSS, model 17) was used for descriptive statistics. Principal Component Analysis (PCA), extraction method was used and the rotation method was Varimax with Kaiser Normalization. Pair wise distances (similarity) matrices were computed for all the morphological and anatomical data using sequential, hierarchical and nested (SAHN) clustering option of the NTSYS –pc 2.02j software package (Rohlf, 1993). The program

generated dendrogram which grouped the test species on the basis of Nei morphological and anatomical variations using unweighted pair group method with arithmetic average (UPGMA) cluster analysis (Sneath and Sokal, 1973).

3.6.0 Taxonomic Key

Numerous data were generated from all evidences observed in this study and they were invaluable for the taxonomic key construction useful for the species identification. The key format – indented dichotomous key was strictly followed.

CHAPTER FOUR

RESULTS

4.0 Herbaria and field collection

4.1 Herbaria collection

Species of Loganiaceae assessed in various herbaria revealed that there are nine members of *Anthocleista* encounterd in repositories (Table 2). Mostuea species encountered were only four in number (Table 2). Some samples in the herbaria have lost their original shape due to a very long time since they were collected, poor handling by researchers and visitors to the repositories. The name of the collector, their accession numbers and collectors were recorded (Table 2). *Strychnos* species were reported to be common in the virgin forest with the exception of only two Savanna species; *Strychnos innocua* and *S. spiosa*. Important diagnostic features for each species such as type of venation, type and number of tendrils present. It was reported that they form dense canopy that could habour giant and poisonous snakes in the high forests.

4.1.1 Field collection

The six genera with twenty six species and several replicates were collected on the field for further study. *Spigelia* is represented by only one species; *Spigelia anthelmia*. It is an abundant, annual weed but scarce in the arid environment (Plate 3 a - d). Only two species in the genus *Mostuea* were encountered on the field and they are perennial shrubs with spike inflorescence (Plate 2 c – f). They are *Mostuea brunonis* and *M. hirsuta* with few representative species. *Anthocleista* are majorly trees only one species is a climber (Plates 1 and 2 a - b). *Strychnos* members are woody climbers and about four species are trees; *Strychnos dinklagei, S. innocua, S. spinosa and S. nux-vomica* (Plates 3, a-d and 4, a-c). Coordinates of collection were described in Table 3.

Members of the family have simple, opposite leaves, entire margin and leaf shape varies among the genera (Plates 1 to 12). The leaf surfaces encountered within the family are pubescent at various degrees: hirsute, pilose, tomentose and glabrous as found in *Mostuea hirsuta, Strychnos phaeotricha, Strychnos innocua, Strychnos spinosa* and members of *Anthocleista* species respectively (Plate 1 - Plate 12). One of the sterile *Strychnos* was also covered by glands. *Strychnos phaeotricha* was unique and could easily be recognized from afar as a result of its glandular hair covering both the entire leaf surface and the young stem or branches (Plate 10 c). *Strychnos spinosa* has long, straight and opposite spines at the internodal positions as its name implies. The *S. spinosa* leaves are also finely pubescent and thick but reduced because it is found in the arid environment only. *S. innocua* stems are always rough at their barks which could be an adaptation to the dry environment. These two species; *S. innocua* and *S. spinosa* are trees. Hence, *Strychnos* species found in the arid environment could only be trees while the woody climbers are in the rain forest vegetations. The inflorescence type within the family is either racemose or cymose. When racemose, it would be corymb as found in *Anthocleista*, *Mostuea, Nuxia* and *Usteria* genera (Plates 1, 2, 11 a and 12 b respectively). The cymose type however, is usually axillary and is common among *Strychnos* (Plate 8). One important and unique to *Strychnos* genus is the presence of tendrils in form of hooks at the axil of the stems. However, members in the arid regions lack this unique character. It is an adaptation for climbing to a very great height along their host trunks and branches. They also utilize old vegetations and become prolific climbers that exclude other vines aroud them as they choke others with their hooks in search for attachment. *Strychnos* hooks are of different types having specific numbers of hooks in a particular species, i.e. the hook type and number is species specific. For example, *S. floribunda* has only one hook, folded in one direction while *S. nigritana* has a pair (two directional folds). In *S. barteri,* we have two pairs and three pairs in other species in this genus. Another important feature encountered with

Strychnos was their inflorescence. The ratio of the length of the flower tube to its width is always diagnostic to each species.

Twelve samples of *Strychnos* species were collected from the field that did not match the herbaria collection during morphological characterisation. Hence, they were not fully identified and they were thus referred to as *Strychnos* Indeterminate (SID) in the studies (Table 8).

4.1.2 Herbarium preparation

Herbaria samples prepared were deposited in FHI and LUH. There are several observations made during the vouchers specimens' preparation. *S. camptoneura* dries black while *S. nigritana*, *S. barteri, S. spinosa* and others species dry brown.

The species of *Anthocleista* were difficult to maintain under room temperature without decaying. They rather required a higher temperature if one wants to retain the organs intact without falling off.

The tendrils or hooks on *Strychnos* are to be tied by rope to the herbarium sheet because they are very fragile (when dried) and easily break off the branches at a very slight pressure or friction on the herbarium paper. The hooks are one of the diagnostic feature for the genus.

Table 2: Herbaria collections assessed for this study

Name of plant specimens	Place of Collection	Accession N0	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
A. djalonensis	Abidjan, Ivory coast	FHI 12650	Leeuwenberg, A.J.M.
A. microphyla	Republic of Benin	FHI155021	Onochie, C.F.A
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

Table 2: Herbaria collections assessed for this study continued

Name of plant specimens	**Place of Collection**	**Accession no**	**Collector**
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.

Table 3: Loganiaceae collections on the field

I.D	Name of plant specimens	Place of collection	Coordinates of collection (GPS).	Name of collector/s
1	*Anthocleista djalonensis*	University of Lagos	N 06°30′55.4″ E003°23′56.7″	Oduoye, O.T
2	*A. nobilis*	Erokut station Forest Reserve, Calabar	N 05°22.835′ E008°25.21	Oduoye, O.T
3	*A. vogelli*	University of Lagos	N 06°30′53.14″ E003°23′46.7″	Oduoye, O.T
4	*A. vogelli*	Stubbs Creek Forest, Akwa-Ibom state	N 04°37′51.4″ E008°01′16.6″	Oduoye, O.T
5	*A. vogelli*	J_4 Omo Forest Reserve, Ogun State	N 06°48′16.4″ E004°21′54.2″	Oduoye, O.T.
6	*Mostuea brunonis*	Awi Forest Reserve	N 05°12′18 E08°21′31.9	Oduoye, O.T
7	*M. brunonis*	Ogoja/ Edondon Forest.	N 05°52′ E08°46′	Oduoye, O.T
8	*M. hirsuta*	Erokut station, Calabar.	N 05°22′19.2″′ E008°27.25.1″	Oduoye, O.T
9	*Nuxia congesta*	Mambella Plateau, Taraba state	N/A	Oduoye, O.T; Daramola, B.O
10	*Spigelia anthelmia*	University of Nigeria, Nsukka campus	N 06°51.835′ E007°24.58′	Oduoye, O.T.
11	*S. anthelmia*	University of Lagos	N 06°30′54.4″ E003°23′54.7″	Oduoye, O.T
12	***Strychnos*** *aculeata*	Cross River National Park (CRNP) Calabar.	N 05°21.835′ E008°26.21	Oduoye, O.T.
13	*S. afzeli*	Enugu to Nsukka rd., Enugu state	N 06°51.835′ E007°24.580′	Oduoye, O.T.
14	*S. angolensis*	Erokut station, Calabar.	N 05°22′39.28″ E008°25′44.1″	Oduoye, O.T
15	*S. asterantha*	Akure Forest Reserve, Owena.	N 07°12′07.1″ E005°01′43.9″	Oduoye, O.T.
16	*S. barteri*	J_4 Omo Forest R.	N 06°50′17.4″ E004°21′52.6″	Oduoye, O.T.
17	*S. boonei*	Onigambari Forest R.	N 07°11′.01″ E003°52′42.6″	Oduoye, O.T.

Note: N/A = Not available.

Table 3: Loganiaceae collections on the field continued

	Name of plant specimens	**Place of collection**	**Coordinates of collection (GPS).**	**Name of collector/s**
19	*S. congolana*	Edondon Forest Reserve	N 05°50′49.7″ E008°25′26. 3″	Oduoye, O.T
20	*S. congolana*	Akure Forest Rrserve, Owena	N 07°12′16.25″ E005°01′10.29″	Oduoye, O.T
21	*S. icaja*	Erokut station, Calabar	N 05°21′10.12″ E008°24′.20.3″	Oduoye, O.T
22	*S. innocua*	Bougu Park (Niger state)	N 09°54.851′ E003°57.364′	Oduoye, O.T; Daramola, B.O
23	*S.longicaudata*	Akure Forest Rrserve, Owena	N 07°12′07.1″ E005°01′.43.9″	Oduoye, O.T
24	*S.longicaudata*	Akure Forest Rrserve, Owena	N 07°12′17.2″ E005°01′.13.9″	Oduoye, O.T
25	*S. memecyloides*	Erokut station, Calabar	N 05°21′50.2″ E008°26′22.3″	Oduoye, O.T
26	*S. nigritana*	Erokut station, Calabar	N 05°23′46.22″ E008°24′02.3″	Oduoye, O.T
27	*S. nigritana*	Akure Forest Rrserve, Owena	N 07°12′19.4″ E005°42′.3″	Oduoye, O.T
28	*S. soubriensis*	Edondon Forest Reserve	N 05°46′49.2″ E008°25′25.33″	Oduoye, O.T
29	*S. spinosa*	Bougu Park (Niger state)	N 09°55.421′ E003°57.304′	Oduoye, O.T; Odewo, T.K
30	*S. staudtii*	Erokut station, Calabar	N 05°21′49.32″ E008°26′.23.13″	Oduoye, O.T
31	*S. tricalysioides*	Erokut station, Calabar	N 05°21′50.22″ E008°27′.23.11″	Oduoye, O.T
32	*S. urceolata*	Akure Forest Reserve, Owena	N 07°12′17.4″ E005°02′.33″	Oduoye, O.T
33	*S. usambarensis*	Erokut station, Calabar	N 05°23′36.22″ E008°26′20.3″	Oduoye, O.T
34	*Usteria guineensis*	Owo, Ondo State	N 07°11.835′ E005°14.58′	Daramola, B.O.
35	*U. guineensis*	Ibadan to Ijebu-Ode road, Ogun state.	N 06°52′ E003°56′03.9″	Oduoye, O.T., Daramola, B.O


a


b


c


d

e


f

Plate 1: Photographs of *Anthocleista* species physiognomy (a) *Anthocleista schweinfurthii* tree showing stipels and peduncle (b) *A. procera* showing the inflorescence (c) *A. vogelli* tree showing its branching pattern. (d) *A. nobilis* tree showing the large trunk (e) *A. djalonensis* tree showing long petioles and broad leaves (f) *A. vogelli* showing young inflorescence and fruit (Magnification × 0.05).

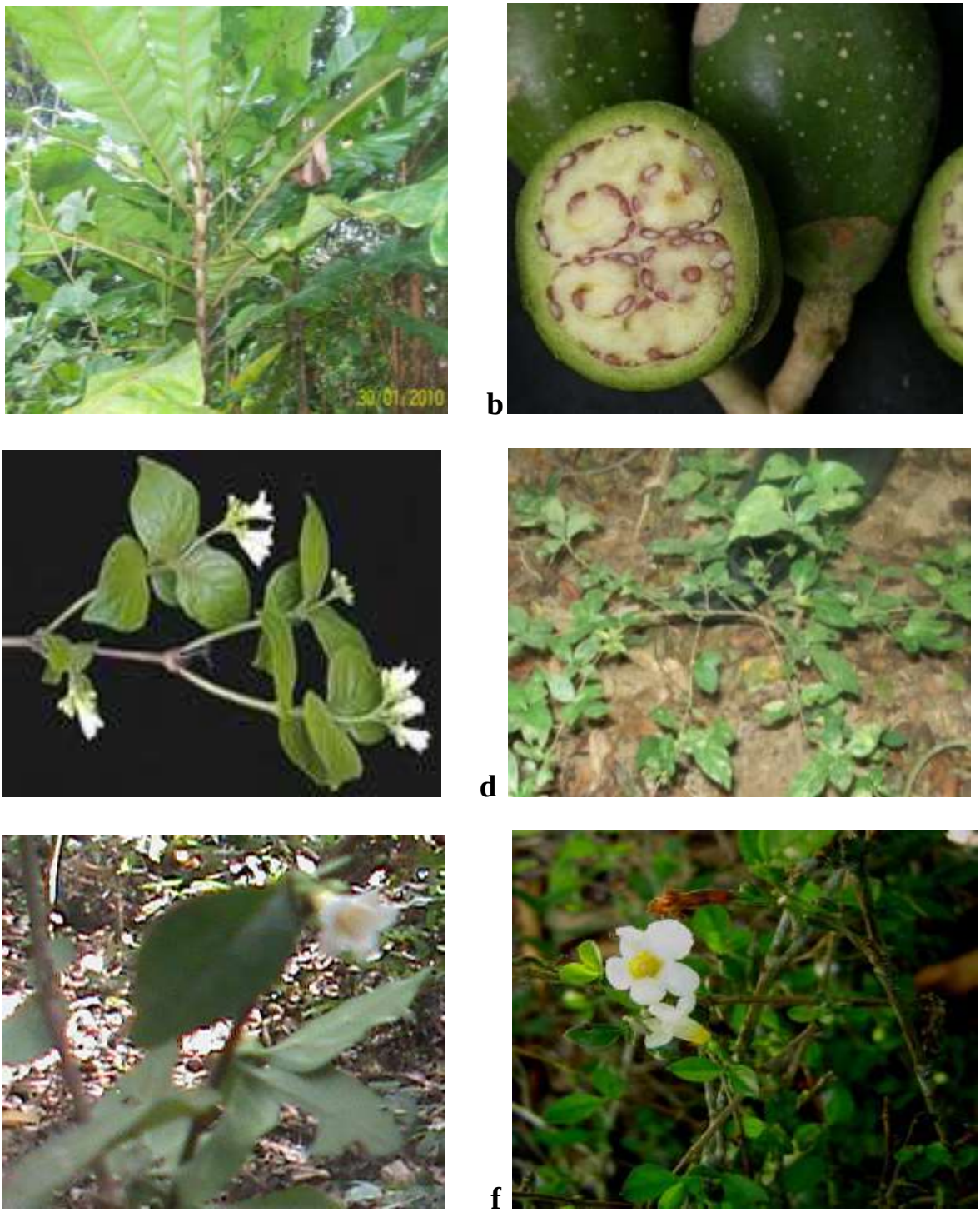


Plate 2: Photographs of *Anthocleista* and *Mostuea*: (a) *A. nobilis* showing the juvenile tree with large leaves (b) *A. vogelli* showing cut surface of globose unriped fruit and placentation (c) *M. hirsuta* showing inflorescence (d) *M. brunonis* showing its dwarf nature under forest trees (e - f) *M. brunonis* showing the flower coloration (Magnification × 0.1).

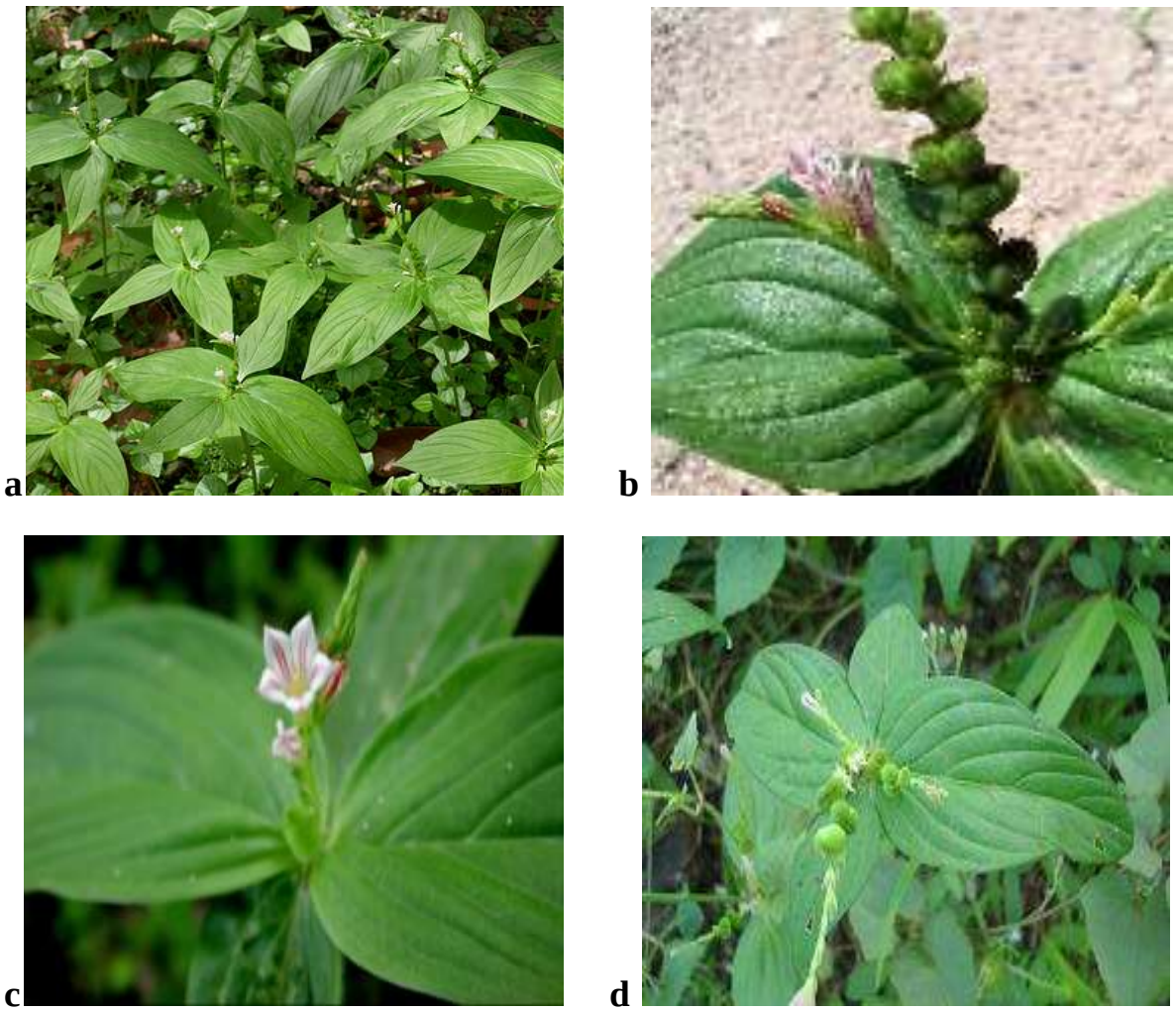


Plate 3: Photographs of *Spigelia anthelmia* (a) *S. anthelmia* showing its colonial growth (b) *S. anthelmia* showing its pinkish flower and globose fruit (c) *S. anthelmia* showing its flower and (d) *S. anthelmia* showing young fruit and pedicel. (Magnification ×0.1).

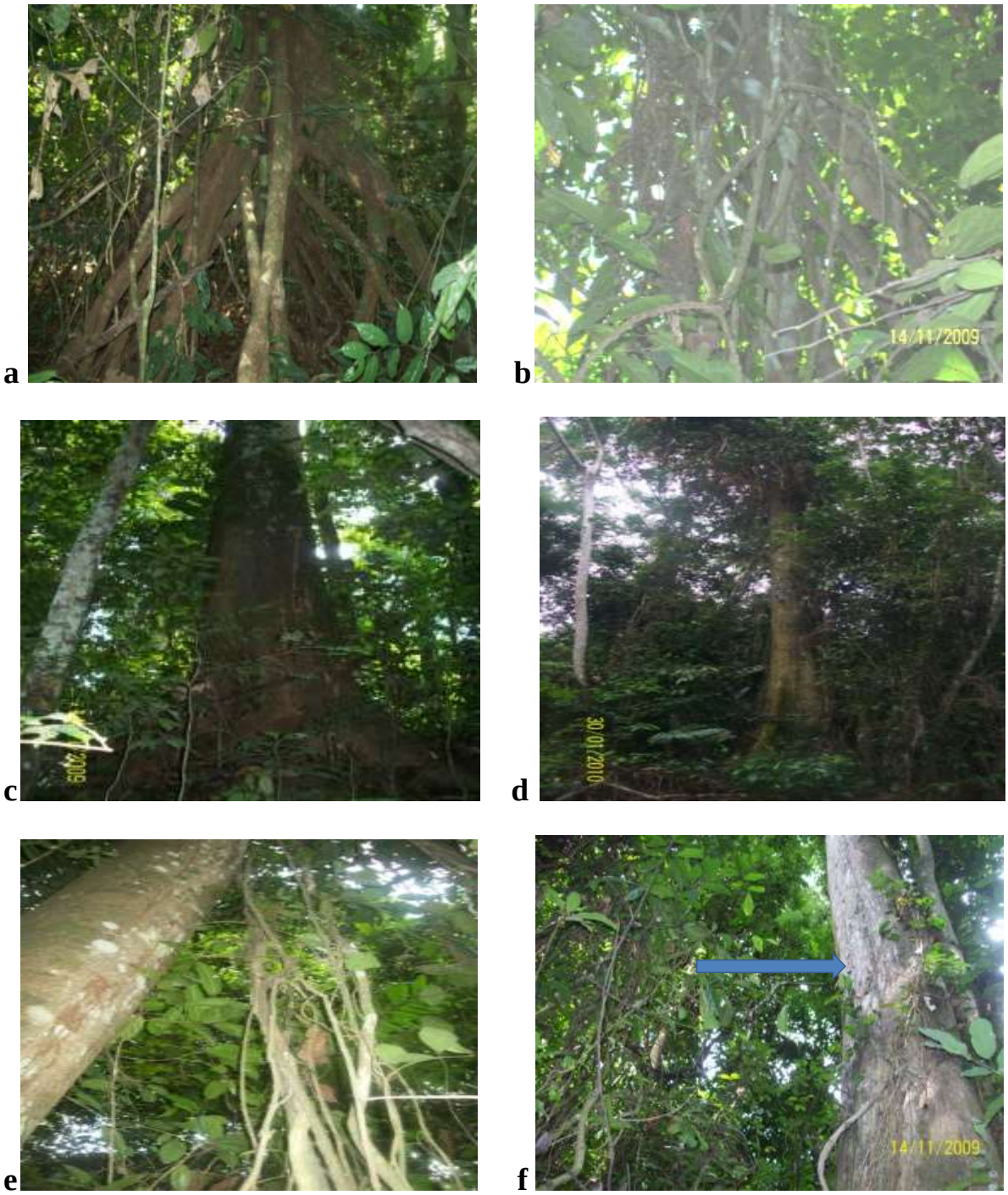


Plate 4: Photographs of *Strychnos* and support (host) in high forest. (a) *Haplormosia monophylla* – Papilionaceae showing its prop and stilt root that carries *Strychnos* (b) *Strychnos afzeli* climber showing the intertwining pattern along the host (c) *Afrormosia elata* – Papilionaceae showing its support of *Strychnos* canopy. (d) *Terminalia* species – Combretaceae showing (without basal branches) the climbing pattern of Strychnos (e) *Desbordesia glaucescens* – Irvingiaceae (f) Arrow pointing to *Cordia milleni* – Boraginaceae showing a great buttress for *Strychnos* support (Magnification × 0.05).

Plate 5: Photographs of *Strychnos* physiognomy (a) Arrow pointed to *Strychnos barteri* giant liana as it ascended the host (b) *S. boonei* leaves showing the flourishing pattern on its host – full of leaves (c) *S. usambarensis* showing the branch from a host. (d) *S. spinosa* tree showing the flourishing condition in the Savanna (e) *S. innocua* tree showing its growth pattern in the arid environment and its suprabasal venation type (f) *S. longicaudata* showing it stem cutting during collection in the high forest (Magnification × 0.05).

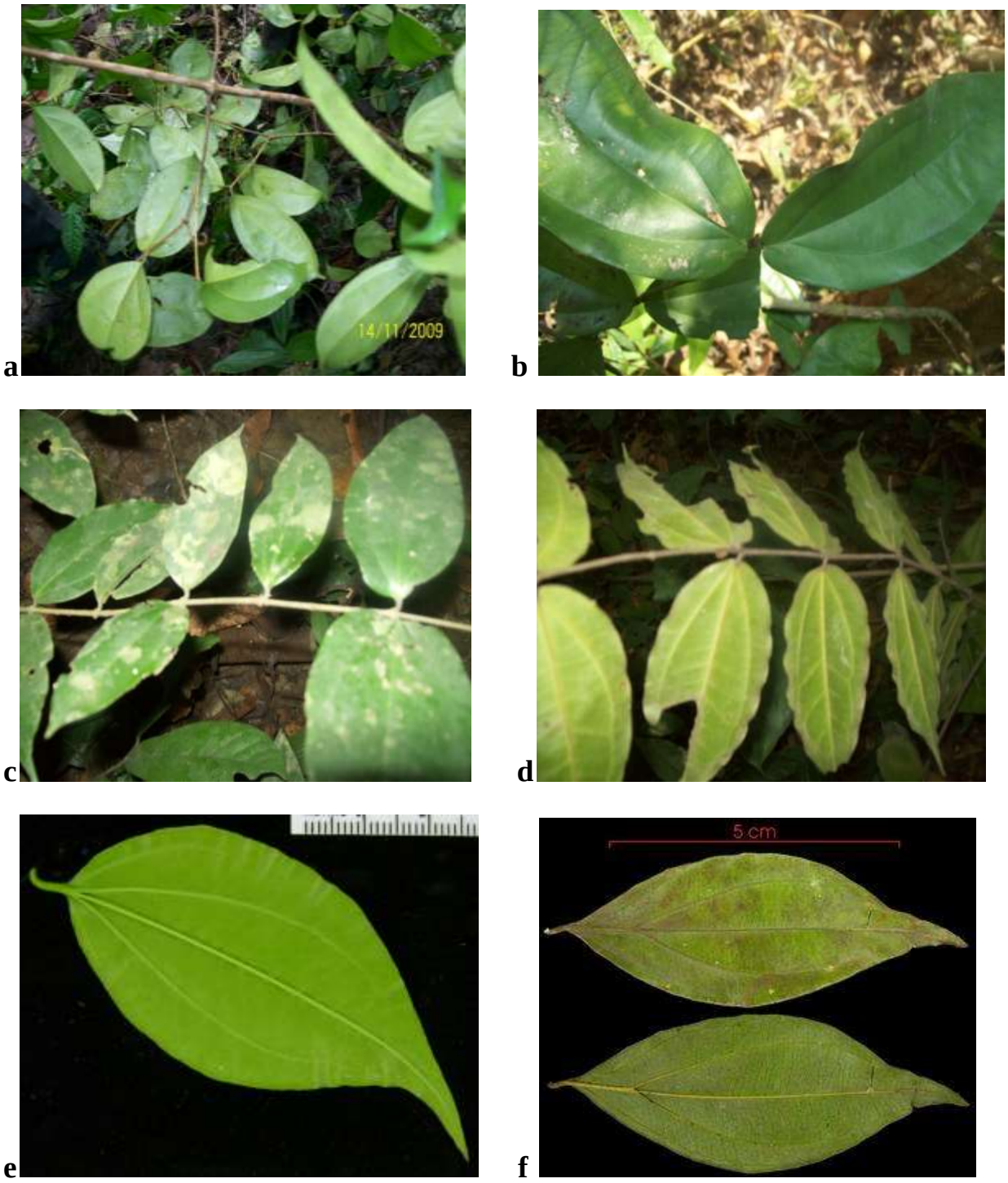


Plate 6: Photographs of *Strychnos* leaves: (a - b) *Strychnos usambarensis* showing leaf (c - d) *S. staudtii* showing Adaxial and Abaxial surfaces with their leaves arrangements (e) *S. dinklagei* showing leaf size with basal acrodromous venation (f) *S. dinklagei* showing leaf size with suprabasal acrodromous venation (Magnification × 0.1).

b 

c d 

Plate 7: Photographs of *Usteria guineensis* (a) young vegetative leaf showing the growth patten of the plant (b) *U. guineensis* showing the inflorescence and flower (c - d) *U. guineensis* showing fresh fruit, dry fruit and their arrangement (Magnification × 0.05).

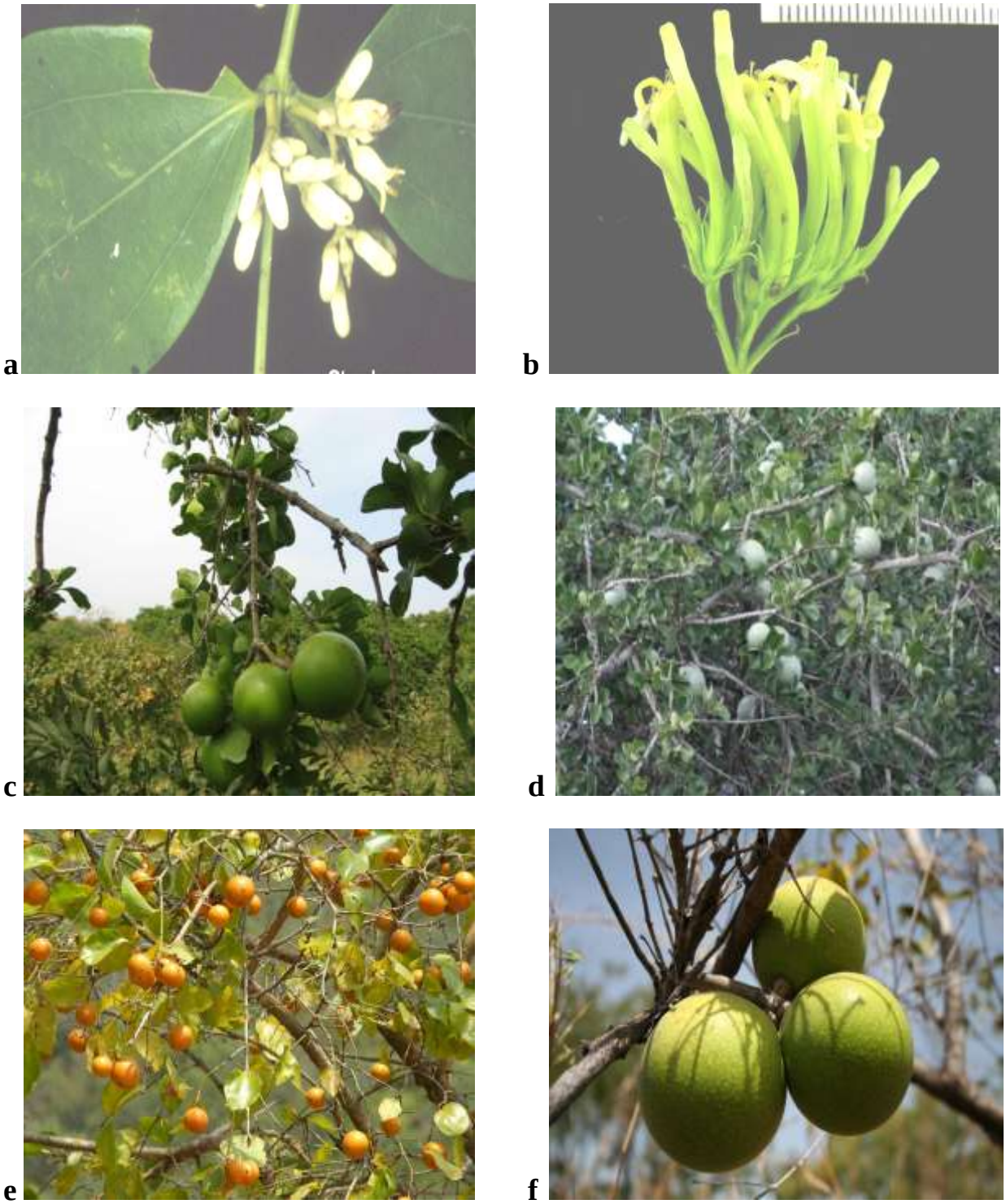
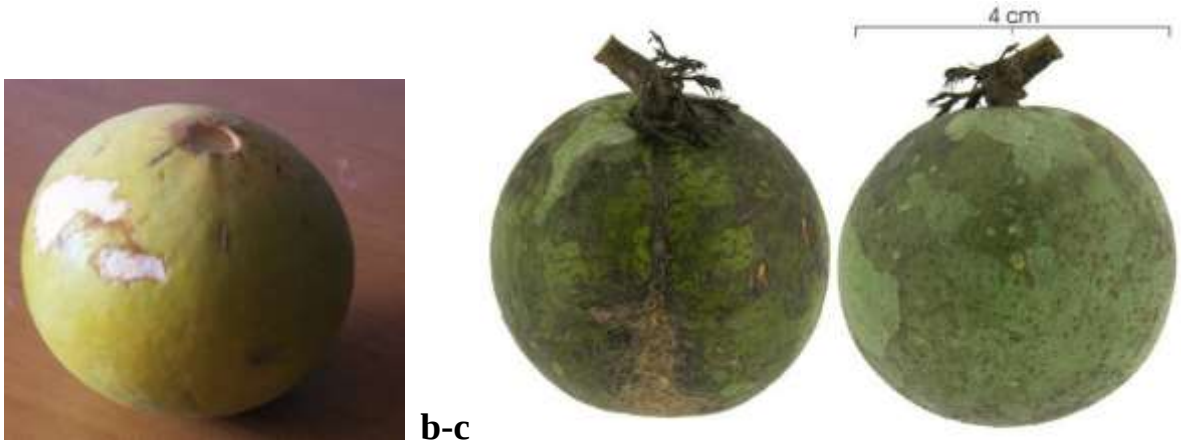


Plate 8: Photographs of *Strychnos*. (a) *Strychnos densiflora* showing the inflorescence (b*) S. dinklagei* showing flower corolla tube (c) *S. spinosa* showing unriped fruit (d) *S. staudtii* showing unriped fruit (e) *S. nux-vomica* showing riped fruit (f) *S. innocua* showing unriped fruit (Magnification × 0.05).



A b-c

d e f

g h I

Plate 9: Photographs of *Strychnos* (a) *Strychnos spinosa* showing riped fruit (b-c) *S. innocua* showing unripe fruit (d) *S. spinosa* showing unripe and ripped fruits opened (e-f) *S. nux-vomica* seeds showing different colour when dried (g) *S. potatorum* showing the small size of dried seeds (h) *S. nux-vomica* showing ripe fruit and its leaves (i) *S. staudtii* showing unriped fruit (Magnification ×1).

a 

b 

c 

d 

Plate 10: Photographs of *Strychnos* herbarium species showing their diagnostic features: (a-b) *Strychnos aculeate* showing short but wide basal spines (c) *S. phaeotricha* showing conspicuous hairy surface (d) *S. spinosa* showing conspicuous long internodal pair of thorns (Magnification × 0.1).

Plate 11: Photographs of Voucher specimens for Loganiaceae showing: (a) *Nuxia congesta* showing inflorescence (b) *Spigelia anthelmia* showing the size of the weed (c) *Strychnos afzeli* showing the cut branches (d) *Strychnos spinosa* showing branch, opened fruit and seeds (Magnification × 0.1).

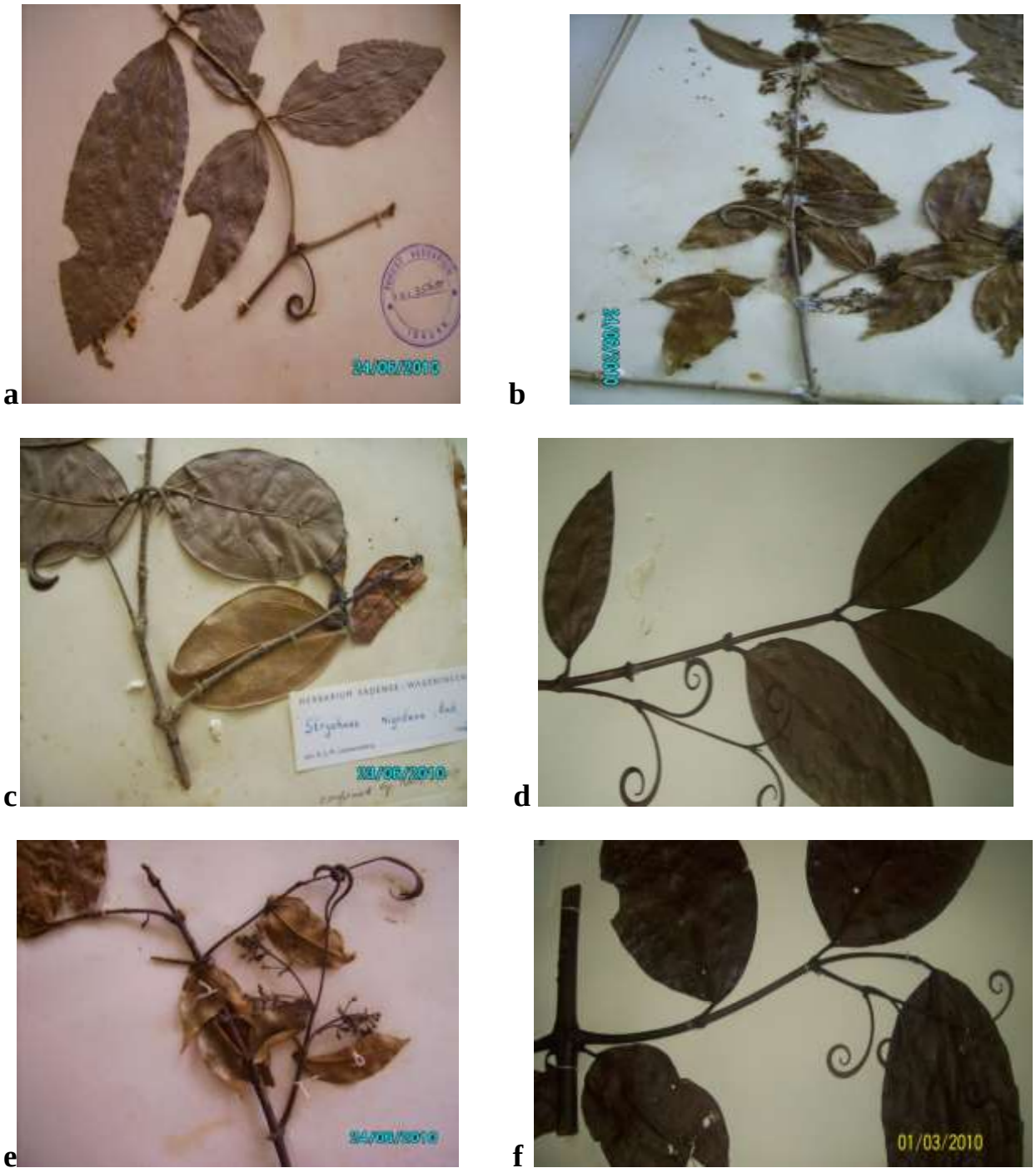


Plate 12: Photographs of *Strychnos* herbarium samples: (a-b) *Strychnos floribunda* and *S. usambarensis* showing single tendril or hook respectively (c) *S. nigritana* showing Paired hook (d*) S. aculeata* showing two pairs of hook (e) *S. nigritana* showing the orientation of *Strychnos* hook (f) *S. barteri* and *S. camptoneura* showing three pairs of hook (Magnification × 0.2).

4.2 Biogeography of Loganiaceae

The data for coordinates of collections made on the field was reported on Table 4A. Furthermore, the converted values of the coordinate obtained were also reported in the same table. The nineteen West Africa environmental variables or layers were represented on Table 4B.

The omission rate and predicted area as a function of the cumulative threshold is represented in Figure 4A. The omission rate is calculated both on the training presence records and on the test records. The omission rate is close to the predicted omission, because of the cumulative threshold used.

Figure 4B represents the Receiver Operating Characteristic (ROC) curve. The predicted area was defined by specificity rather than true commission. This makes the maximum achievable Area under Curve (AUC) less than 1.

The logistic prediction for Loganiaceae is represented by Figure 4C. Warmer colours (Yellow to Orange to Red) show areas with better predicted conditions. White dots show the presence locations used for training, while violet dots show test locations.

Figure 4D shows the result of the jackknife test of variable importance. The environmental variables with highest gain when used in isolation are Bio 6 and Bio 19. They therefore appear to have the most useful information by themselves. The environmental variable that decreases the gain the most when it is omitted is Bio 1, which therefore appears to have the most information that is not present in the other variables.

Figure 4E shows jackknife test, using test gain instead of training gain. However, the variable that is most important here is Bio 19. While the environmental variable that decreases the gain the most when it is omitted is Bio 14.

Table 4A: Coordinates of collection and their decimal Maxent values

S/N	SAMPLES NAMES	Coordinates of collection (GPS). Latitude &Longitude	ASCII, decimal coordinates	
			Lat	Long
1	*Anthocleista djalonensis*	N 06°30′55.4″ E003°23′56.7″	6.52	3.40
2	*Anthocleista nobilis*	N 05°22.835′ E008°25.21	5.33	8.42
3	*Anthocleista vogelli*	N 06°30′53.14″ E003°23′46.7″	7.39	3.39
4	*Anthocleista vogelli*	N 06°48′16.4″ E004°21′54.2″	6.80	4.37
5	*Anthocleista vogelli*	N 04°37′51.4″ E008°01′16.6″	4.62	8.02
6	*Mostuea brunonis*	N 05°52′ E08°46′	5.81	8.77
7	*Mostuea hirsuta*	N 05°22′19.2″ E008°27.25.1″	5.37	8.46
8	*Mostuea brunonis*	N 05°12′18 E08°21′31.9	5.21	8.36
9	*Spigelia anthelmia*	N 06°51.835′ E007°24.58′	6.86	7.41
10	*Strychnos aculeata*	N 05°21.835′ E008°26.21	5.36	8.44
11	*Strychnos afzeli*	N 06°51.835′ E007°24.580′	6.86	7.41
12	*Strychnos asteranta*	N 07°12′07.1″ E005°01′43.9″	7.20	5.03
13	*Strychnos barteri*	N 06°50′17.4″ E004°21′52.6″	6.83	4.35
14	*Strychnos boonei*	N 07°11′.01″ E003°52′42.6″	7.18	3.88
15	*Strychnos camptoneura*	N 05°21′49.2″′ E008°26.20.1″	5.36	8.44
16	*Strychnos congolana*	N 05°50′49.7″ E008°25′26. 3″	5.85	8.42
17	*Strychnos congolana*	N 07°12′16.25″ E005°01′10.29″	7.20	5.02
17	*Strychnos icaja*	N 05°21′10.12″ E008°24′.20.3″	5.35	8.41
18	*Strychnos innocua*	N 09°54.851′ E003°57.364′	9.91	3.96
19	*Strychnos longicaudata*	N 07°12′07.1″ E005°01′.43.9″	7.20	5.03
20	*Strychnos longicaudata*	N 07°12′17.2″ E005°01′.13.9″	7.20	5.02
21	*Strychnos memecyloides*	N 05°21′50.2″ E008°26′22.3″	5.36	8.44
22	*Strychnos nigritana*	N 05°23′46.22″ E008°24′02.3″	5.4	8.4
23	*Strychnos nigritana*	N 07°12′19.4″ E005°42′.3″	7.21	5.70
24	*Strychnos soubrensis*	N 05°46′49.2″ E008°25′25.33″	5.90	8.42
25	*Strychnos splendens*	N 09°55.421′ E003°57.304′	9.92	3.96
26	*Strychnos staudtii*	N 05°21′49.32″ E008°26′.23.13″	5.40	8.44
27	*Strychnos tricalysioides*	N 05°21′50.22″ E008°27′.23.11″	5.40	8.46
28	*Strychnos urceolata*	N 07°12′17.4″ E005°02′.33″	7.20	5.04
29	*Strychnos usambarensis*	N 05°23′36.22″ E008°26′20.3″	5.39	8.44
30	*Usteria guineensis*	N 07°11.835′ E005°14.58′	7.20	5.24
31	*Usteria guineensis*	N 06°52′ 45 E003°56′03.9″	6.88	3.93

Table 4B: West Africa nineteen environmental variables from Worldclim database

S/N	Environmental Variable	Legend
1	Bio 1	Annual mean temperature.
2	Bio 2	Means diurnal range; mean of monthly, max temp-min temp.
3	Bio 3	Isothermality (P_2/P_7) * 100.
4	Bio 4	Temperature seasonality (standard deviation 100).
5	Bio 5	max temperature of warmest month; March
6	Bio 6	Min temperature of coldest month; December.
7	Bio 7	Temp annual range (temp annual range (P_5-P_6).
8	Bio 8	Mean temperature of wettest quarter; June – August.
9	Bio 9	Mean temperature of wettest quarter; June – August.
10	Bio 10	Mean temperature of warmest quarter; February - April.
11	Bio 11	Mean temperature of coldest quarter; November - January.
12	Bio 12	Annual precipitation.
13	Bio 13	precipitation of wettest month; July
14	Bio 14	precipitation of driest month; March
15	Bio 15	Precipitation seasonality; coefficient of variation.
16	Bio 16	Precipitation of wettest quarter; June – August.
17	Bio 17	Precipitation of driest quarter; November – January.
18	Bio 18	Precipitation of warmest quarter; February – April.
19	Bio 19	Precipitation of coldest quarter; November - January.

Furthermore, jackknife test using AUC on test data is represented in figure 4F. The lowest bar in red colour shows that the environmental variables are complementary. Usually, sensitivity is 1 – specificity; thus the area under the curve is less than the sensitivity which is the entire area representing the environmental variables.

The response curves (Figure 4G (a-s) show the marginal effect of changing exactly one variable. The curves show how each environmental variable affects the prediction; how the logistic prediction changes as each environmental variable is varied, keeping all other environmental variables at their average sample value. As each environmental variable is varied, the probability of occurrence of the samples;

Decreases with varying Bio 2, 6, 7, 8, 13, 14, 15, 17 and 18;

Decreases but later increases with varying Bio 3; Remains constant with varying Bio 4, 5, 11, 12 and 16; Increases with varying Bio 1, 9, 10 and 19 (Figure 4G (a-s)

In contrast to the marginal response curves in (Figure 4G (a-s), Figure 4I. (a-s) model was created using only the corresponding variable. The plots reflect the dependence of predicted suitability both on the selected variable and on dependencies induced by correlations between the selected variable and other variables. A heuristic estimate of relative contributions of the environmental variables to the Maxent model shows that Bio 19 has the highest percentage contribution to the model (44.9 %), Bio 1 has the least contribution (0.2 %) while Bio 5, 12 and 16 did not contribute at all (Table 4C). Therefore, the species distribution of Loganiaceae in West Africa is most affected by the Precipitation of coldest quarter (November – January), followed by the precipitation of the driest month (March), minimum temperature of coldest month (December) and by precipitation of warmest quarter (February - April).

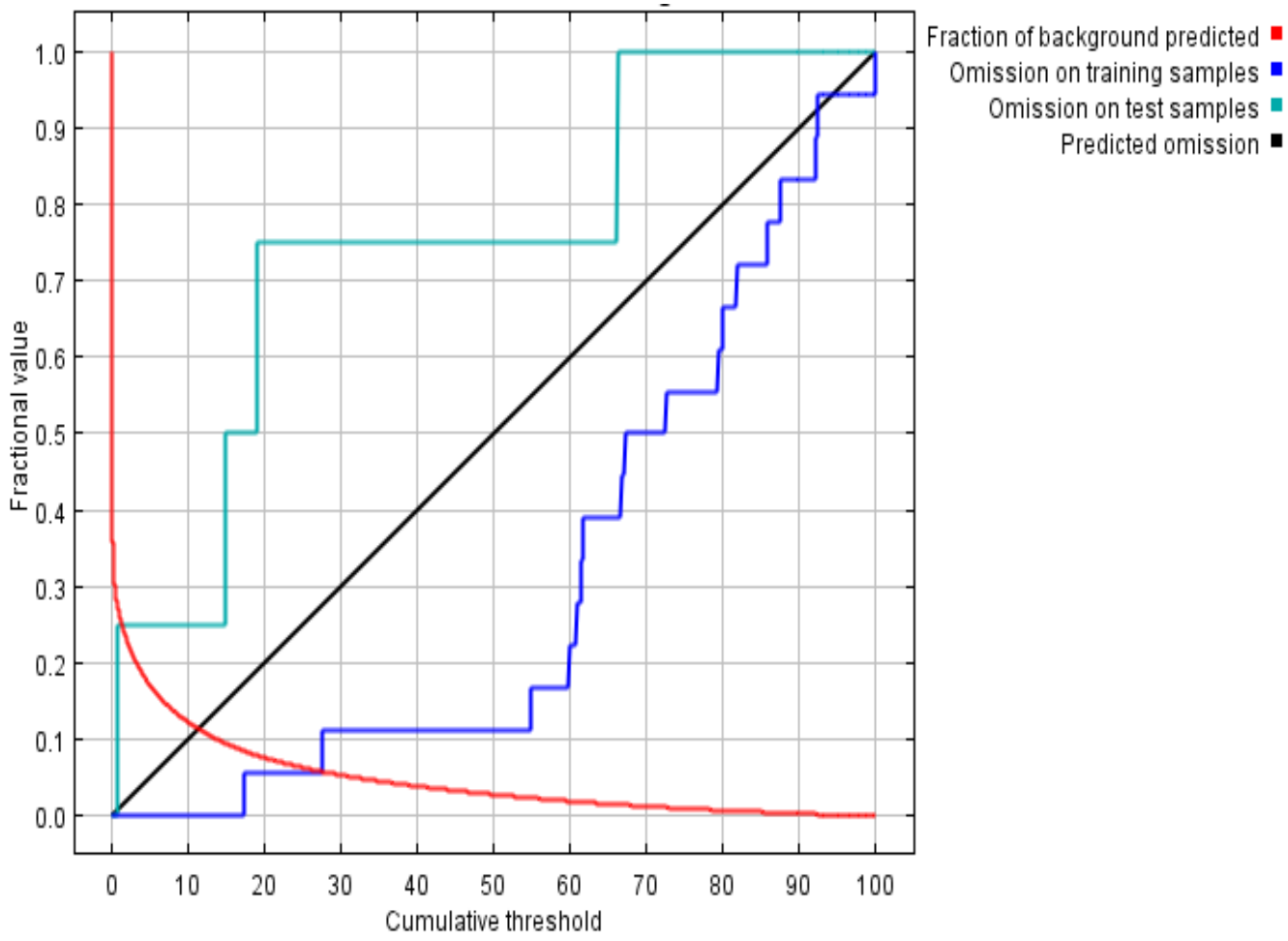


Figure 4A: Omission and predicted area for Loganiaceae.

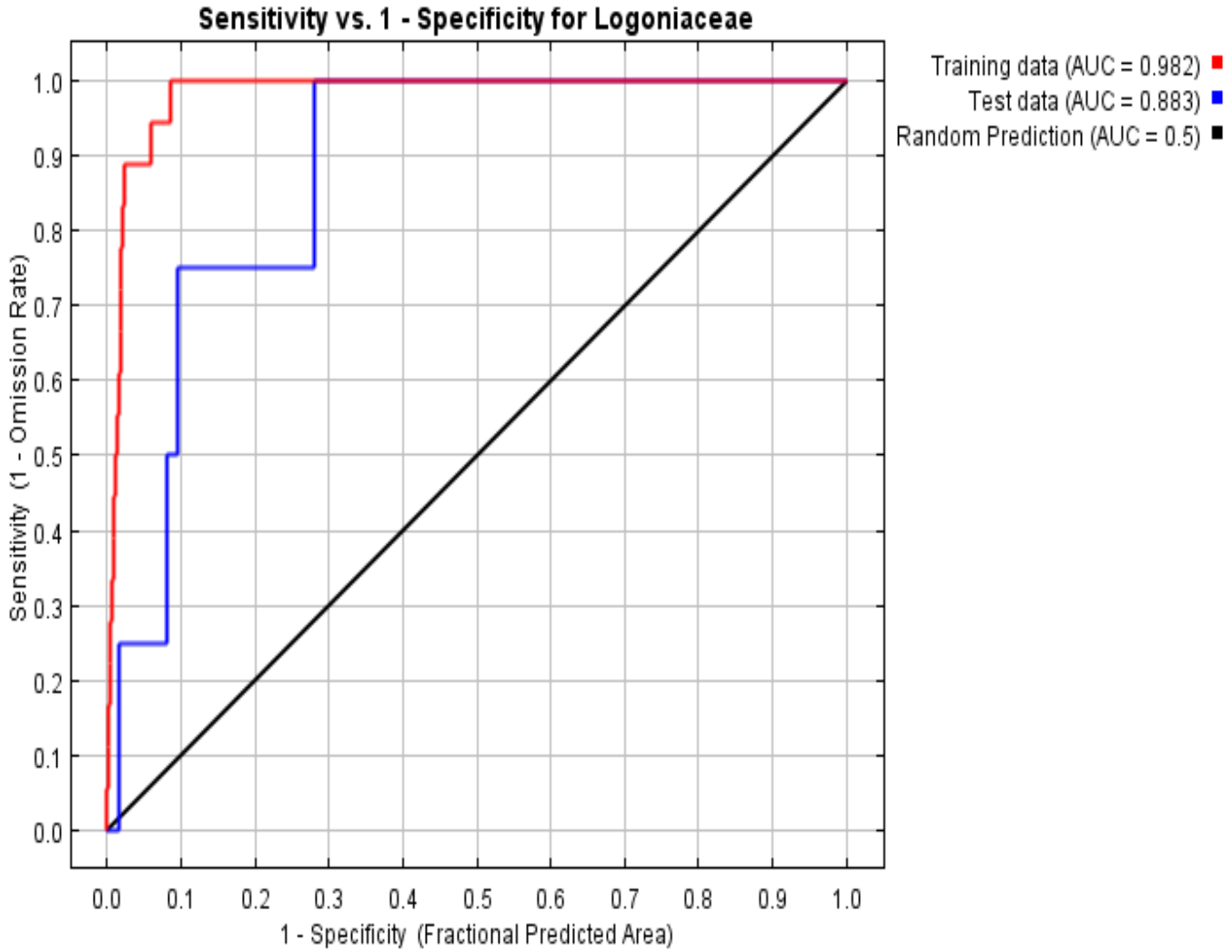


Figure 4B: The receiver operating characteristic (ROC) curve for Loganiaceae.

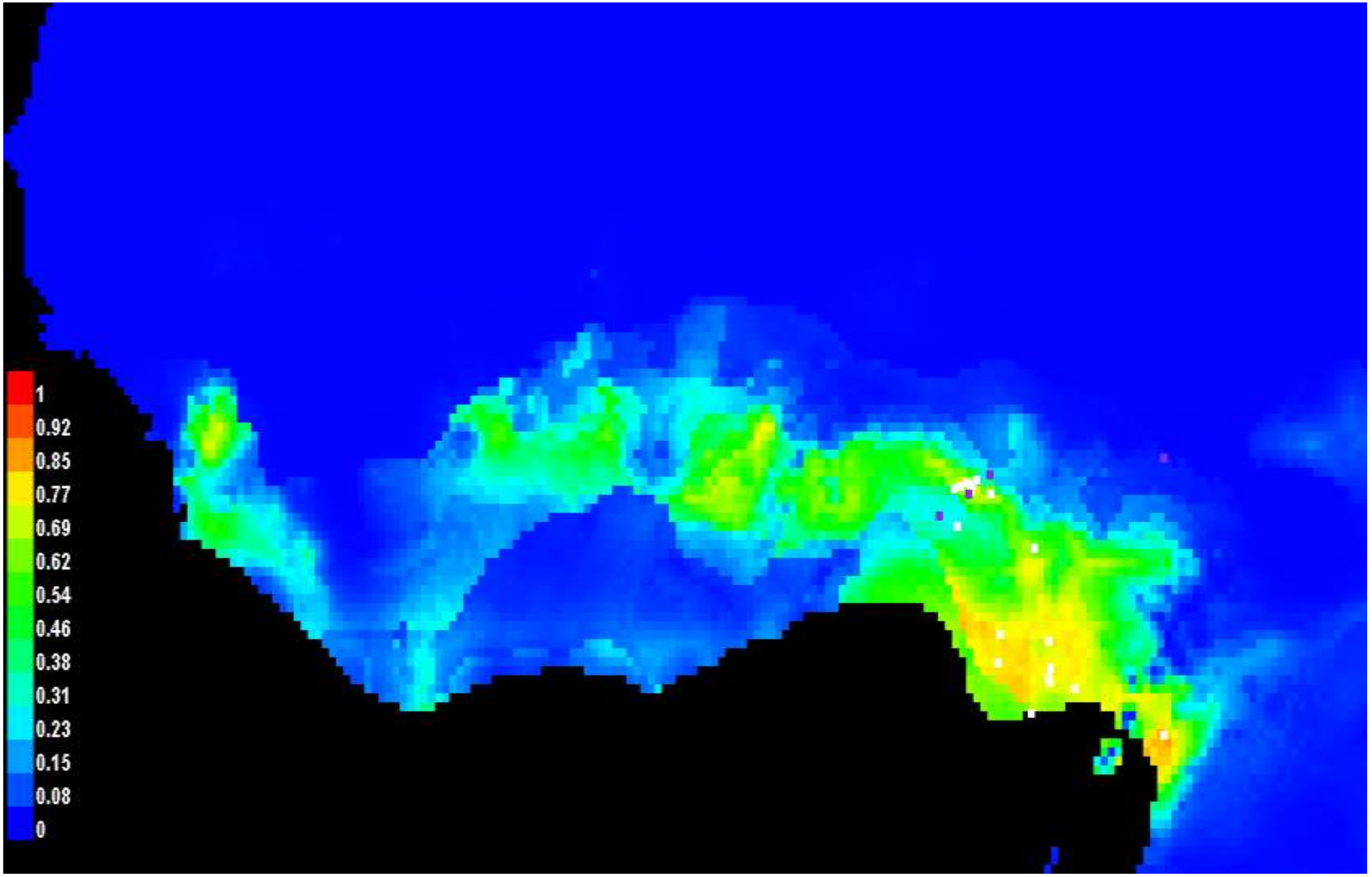


Figure 4C: Species distribution for Loganiaceae in West Tropical Africa using nineteen environmental variables.

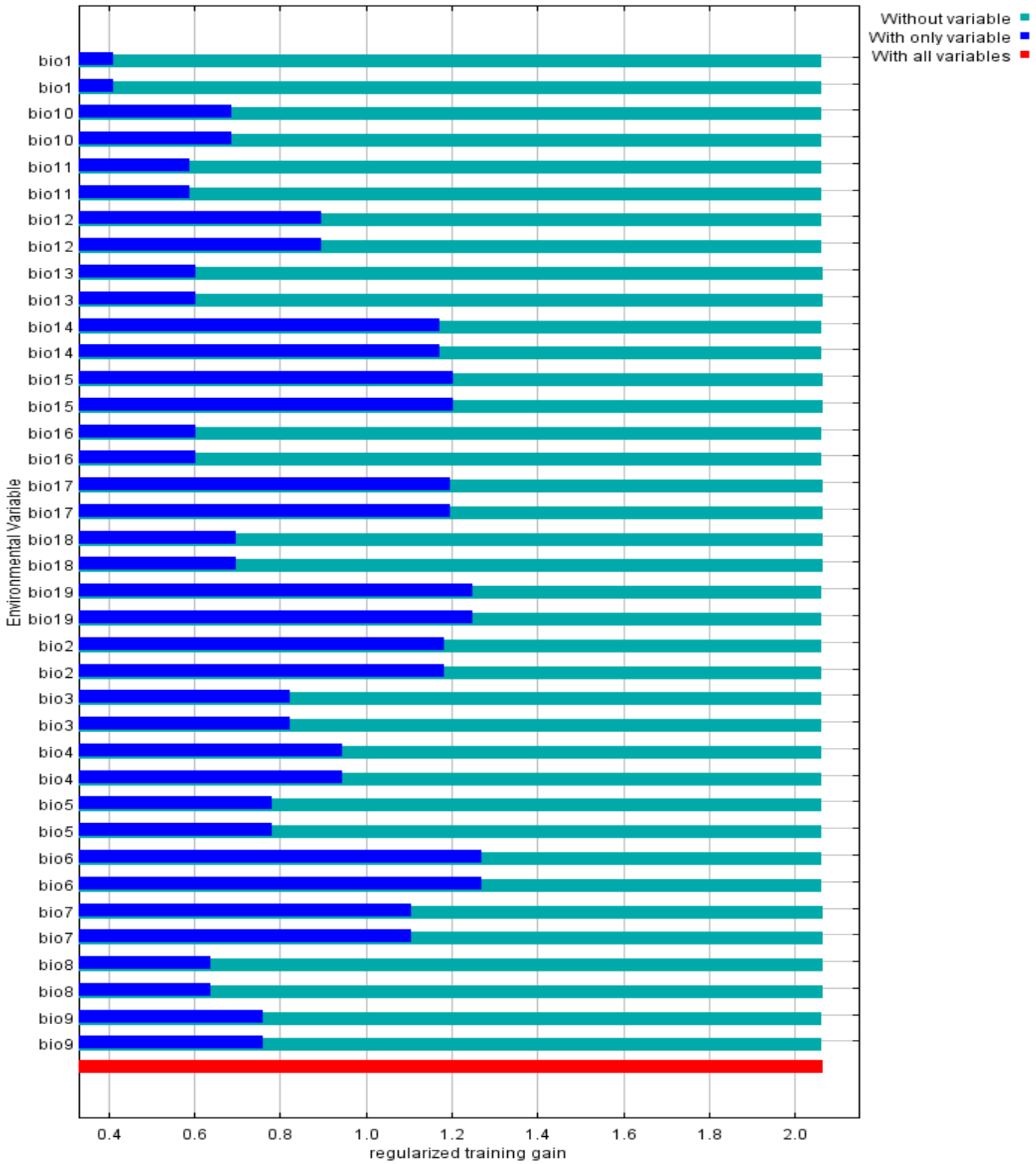


Figure 4D: Jackknife of regularized training gain for Loganiaceae

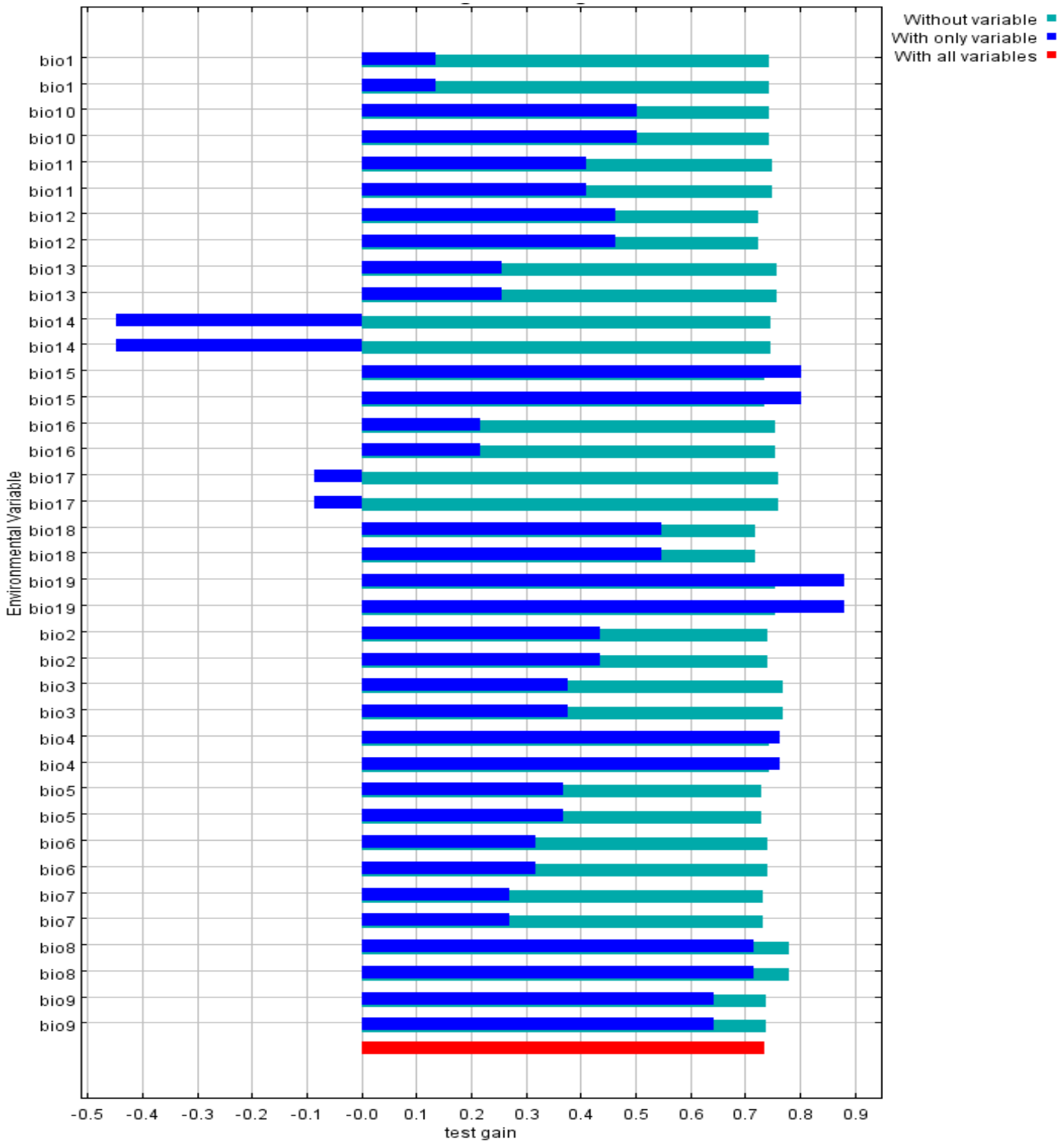


Figure 4E: Jackknife of test gain for Loganiaceae

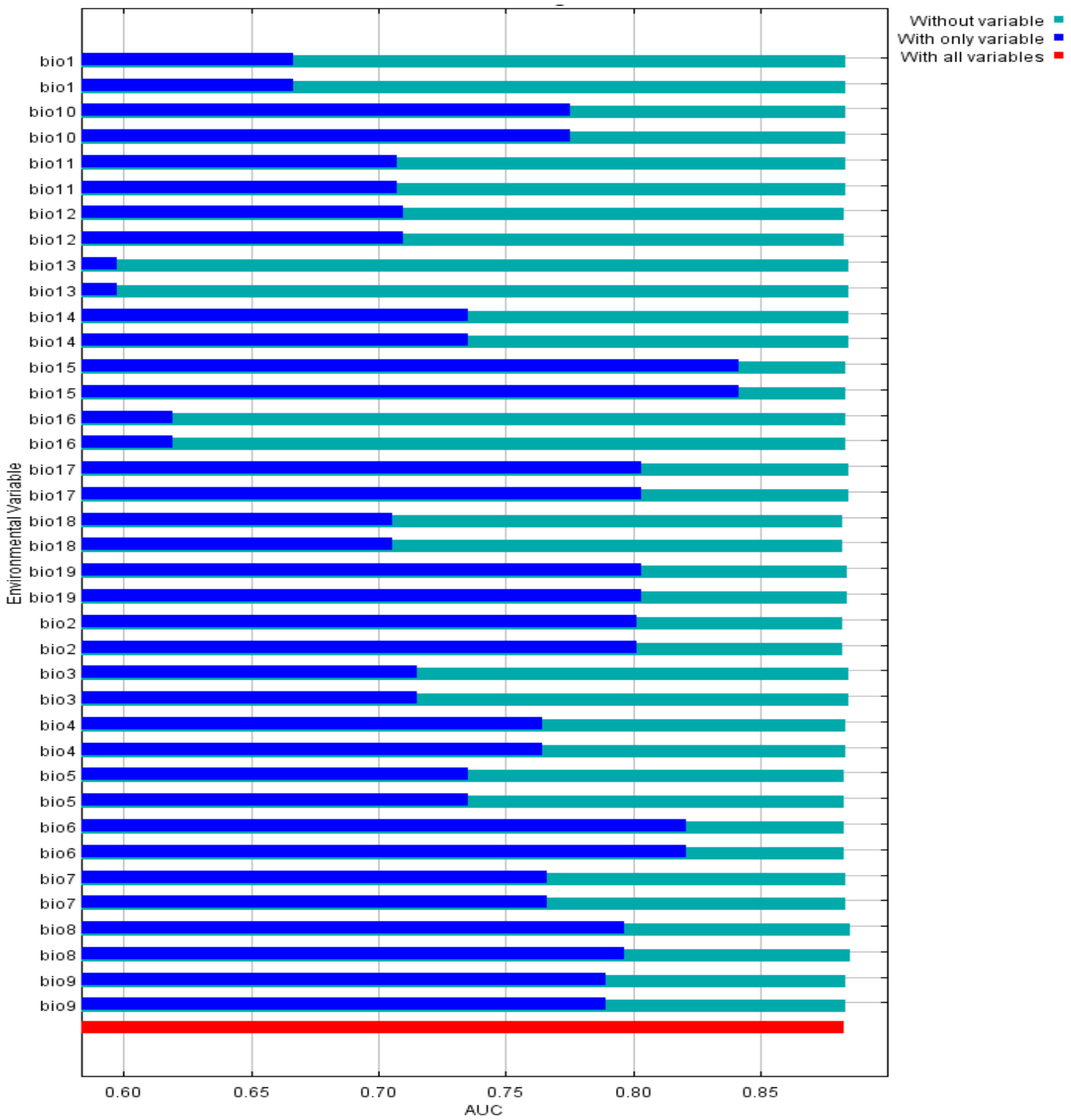
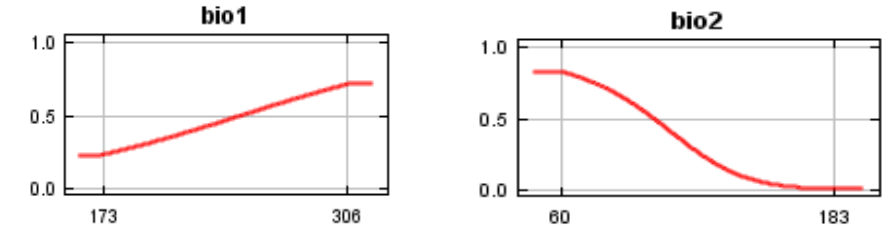
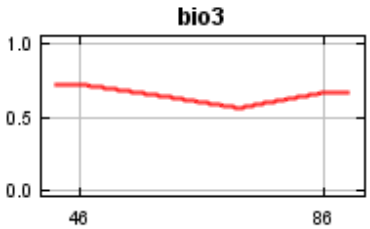


Figure 4F: Jackknife of Area Under Curve (AUC) for Loganiaceae



a

b



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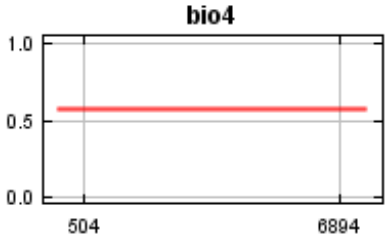

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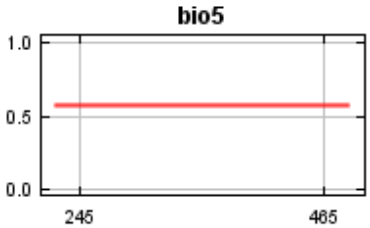

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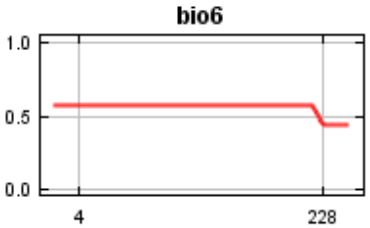

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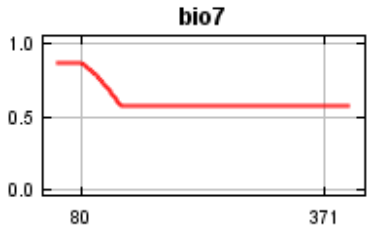

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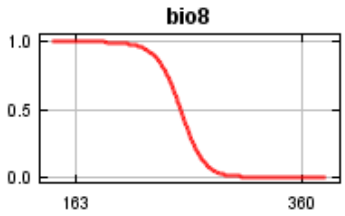

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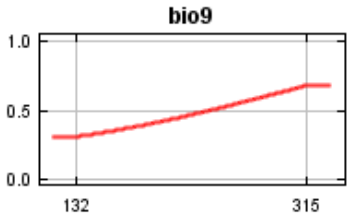

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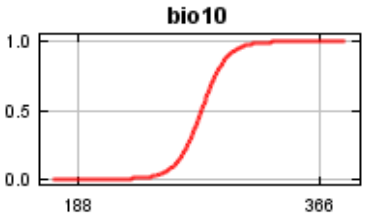
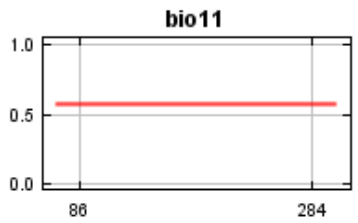


j

Figure 4G (a - j): The marginal effect of changing exactly one environmental variable



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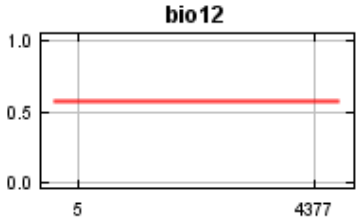

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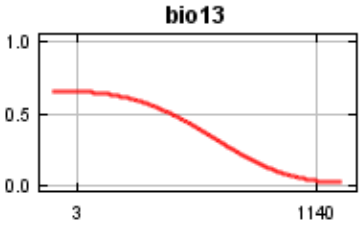

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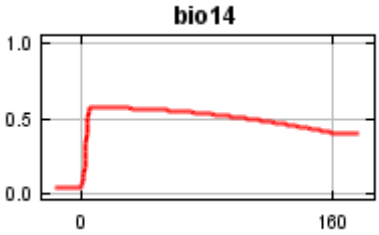

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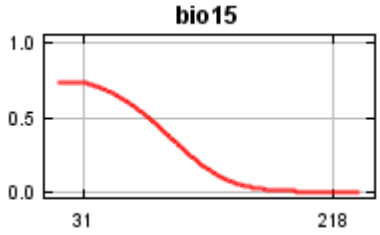

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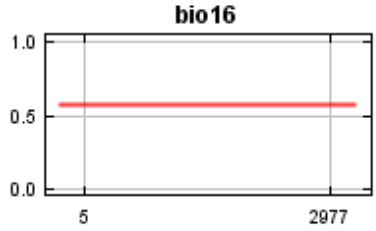

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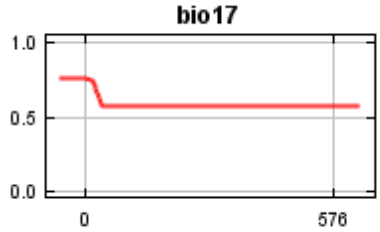

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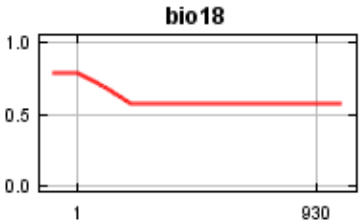

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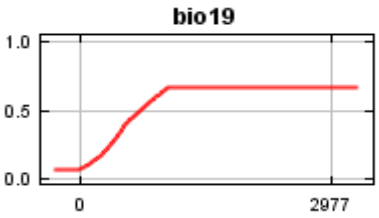
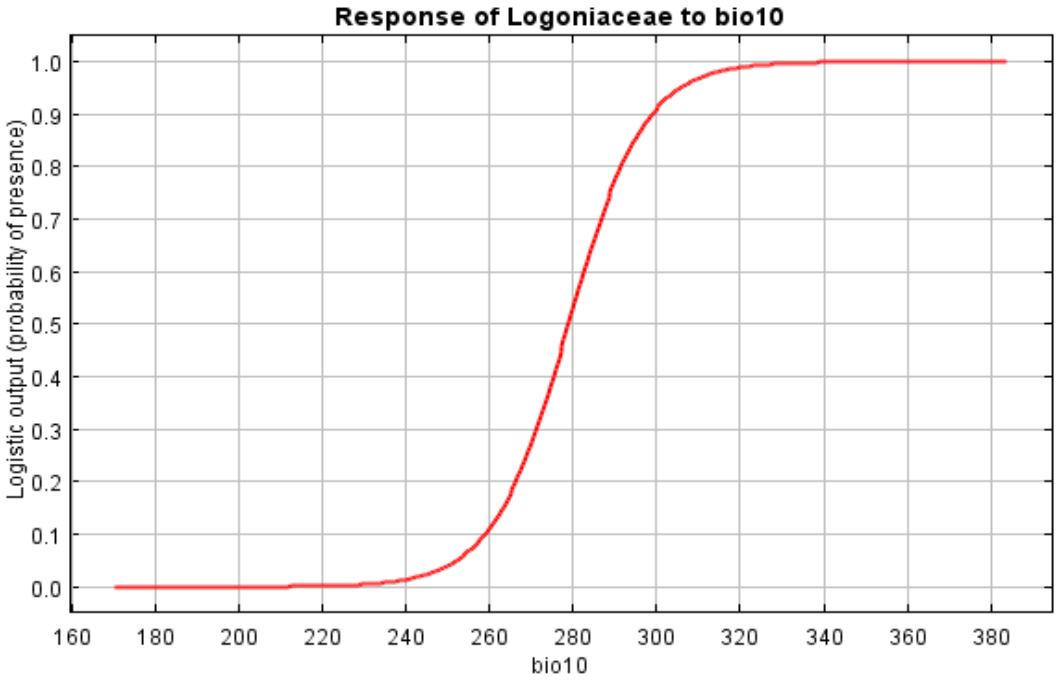


s

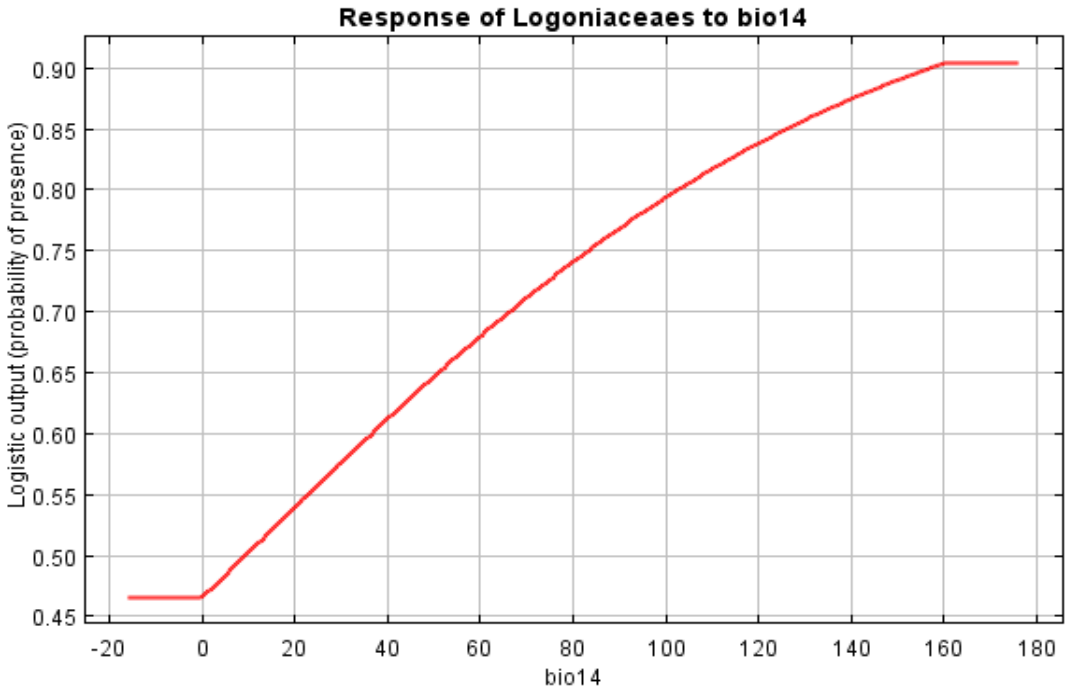
Figure 4G (k - s): The marginal effect of changing exactly one environmental variable continued

X axis: Environmental variable value

Y axis: Fractional value of Location



a



b

Figure 4H (a-b): Full view of response curves of Bio 10 and Bio 14.

X axis: Environmental variable values

Y axis: Fractional value of Location

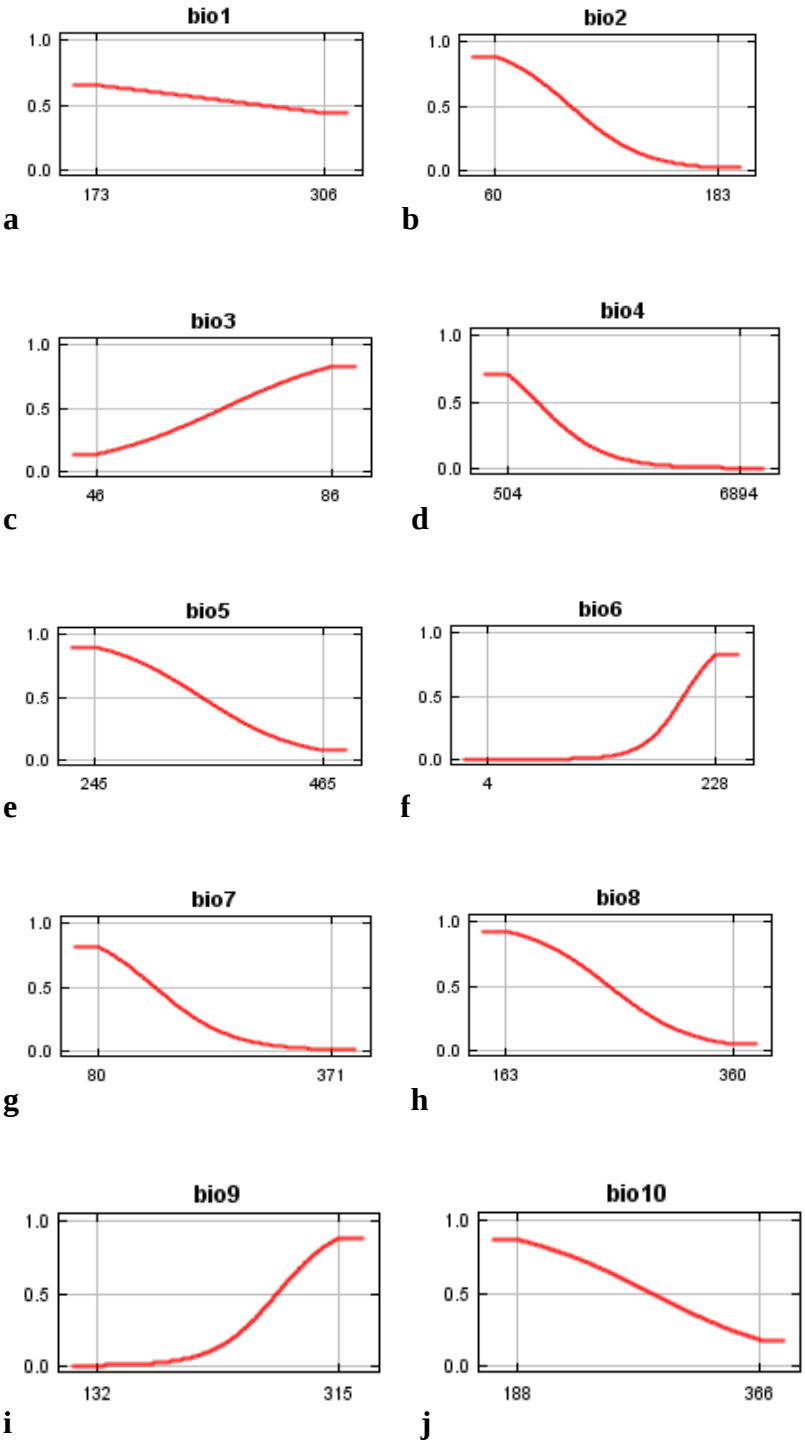
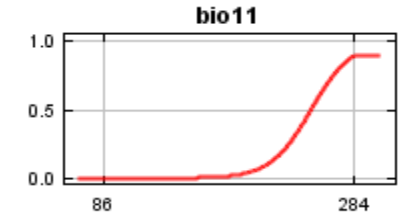


Figure 4I (a-j): The marginal effect of changing corresponding variables



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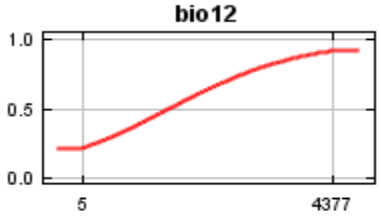

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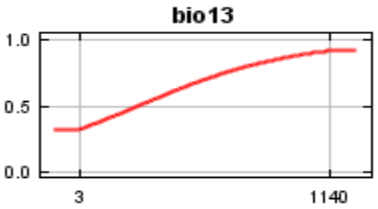

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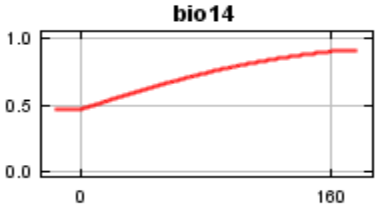

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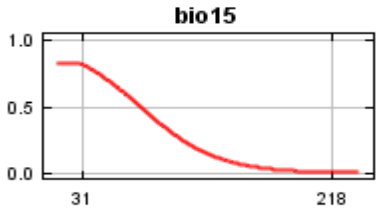

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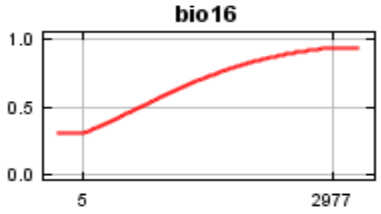

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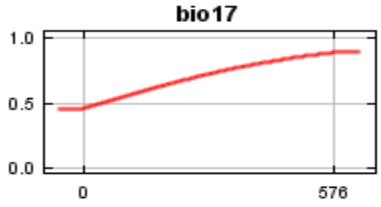

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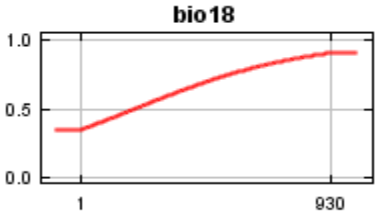

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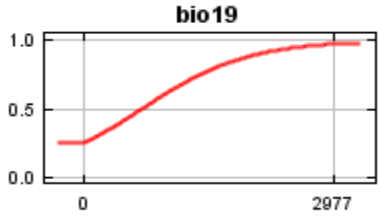

s

Figure 4I (k-s): The marginal effect of changing corresponding variables continued

X axis: Environmental variables value

Y axis: Fractional value of Location

Table 4C: Variable contributions to the Maxent Model

S/N	Variable	Percent contribution (%)	Environmental variables interpretation
1	bio19	44.9	Precipitation of coldest quarter; November - January.
2	bio14	31.5	precipitation of driest month; March
3	bio6	13.7	Min temperature of coldest month; December.
4	bio18	1.9	Precipitation of warmest quarter; February – April.
5	bio7	1.3	Temp annual range (temp annual range (P_5-P_6).
6	bio10	1.1	Mean temperature of warmest quarter; February - April
7	bio8	1.1	Mean temperature of wettest quarter; June – August.
8	bio4	1	Temperature seasonality (standard deviation 100).
9	bio2	0.9	Means diurnal range; mean of monthly, max temp-min temp.
10	bio13	0.5	precipitation of wettest month; July
11	bio15	0.5	Precipitation seasonality; coefficient of variation.
12	bio17	0.4	Annual precipitation.
13	bio9	0.4	Mean temperature of wettest quarter; June – August.
14	bio11	0.3	Mean temperature of coldest quarter; November - January.
15	bio3	0.3	Isothermality (P_2 /P_7) * 100.
16	bio1	0.2	Annual mean temperature.
17	bio12	0	Annual precipitation.
18	bio5	0	max temperature of warmest month; March
19	bio16	0	Precipitation of wettest quarter; June – August.

4.3.0 Morphological and Anatomical Studies

4.3.1 Macro-morphology of the leaves of the species of Loganiaceae

The plant samples were subjected to morphometric analysis; critical observation of the structures, counting and measuring of plant parts. Some of the observations were tabulated in Table 5 a – m. Among the nine *Anthocleista* species, five species have similar paired-spines from a common wide base. They are: *Anthocleista djalonensis, A. nobilis, A. scandens, A. schweinfurthii* and *A. vogelli.* This unique feature is only common to this genus in the family; the other genera do not have the type of spine observed in *Anthocleista.* All Anthocleista leaves have petiole except *A. vogelli* that possess sessile leaves. This is a diagnostic feature for the species among others in the genus. It was also observed that only *Mostuea* genus has inflourescence with wide bract, almost covering the inflorescence to the level of involucrate. This feature is reduced to small epicalyx in other genera.

The adaptation for climbing is present only in one genus, *Strychnos*. This tendril type is referred to as hook in this genus. They have single, paired, double paired and triple paired. This feature is unique across the species of the genus

The inflourescence type are basically terminal cymose in *Mostuea,* racemose in *Strychnos* corymbose in *Anthocleista* genus. *Mostuea* fresh flower colouration is generally white but ranges of fresh flower colouration are exhibited by the remaining genera; *Strychnos, Anthocleista, Spigelia, Usteria* and *Nuxia*. One striking feature that separated *Anthocleista* among others is their leaf size. Some leaves measured up to 120.0 cm while leaves could be as small as 2.0 cm in *Mostuea* or *Strychnos* genera. Leave shapes vary in the family. The major shapes are lanceolate, oblanceolate, ovate, obovate, elliptic etc. leaves arrangement is generally opposite while the leaf

bases are: rounded, cuneate, narrowly cuneate, attenuate etc. Their leaf apices vary as well, some are acute, acuminate, rounded apiculate, obtuse, etc (Table 5 a-k).

The qualitative characters were also assessed quantitatively by counting the features or measuring them. Cluster analysis for morphological variations and dendrogram were computed for all the morphological characters in Table 5. The dendrogram revealed the similarity among the species of Loganiaceae. The morphological evidence of 25 characters shows 3 clusters when working with a threshold of 36 % similarity coefficient (Figure 5A). Cluster 1 combined *Anthocleista* species with other tree species of *Strychnos* genus in the family. *Spigelia anthelmia, Nuxia congesta, Usteria gueineensis* were also grouped with *Anthocleista* at this low coefficient similarity. Cluster 2 - *Mostuea* species were not nested at all with any other genus but completely separated out from them indicating some level of dissimilarity among the genera. The savanna species of *Strychnos* are found nested together with *Nuxia* and *Usteria* species. This is because they are tree species and have several features in common (Cluster 1, Figure 5A).

At a higher similarity coefficient, using 47 % threashold, 7 clusters were generated with 3 ungrouped members. *Anthocleista* grouped together as clusters 1 and 2;

Spigelia anthelmia was ungrouped separately with its root at about 42 % coefficient similarities;

Nuxia and *Usteria* genera clustered together as cluster 3 with their root at 45 %;

Strychnos nux-vomica as well as *Strychnos innocua* were not grouped but stood as outliers;

The two varieties of *Strychnos spinosa* grouped together as cluster 4;

The two species of Mostuea genus were clustere together in the dendrogram as cluster 5;

Thirty three *Strychnos* species clustered together as cluster 6;

The Strychnos indeterminate also clustered together as cluster 7.

The significance of the 7 clusters and outliers are enoumous: for futher analysis, each cluster may be treated as a unit or use representative among them for further studies; fine taxonomic

inferences could be drawn to shwos the degree of similarity that exis t among the species of the family; a new hypothesis may be postulated for re-evaluation or re- recircumscription of the previous classification among other.

The leaf characters assessed quantitatively were subjected to Principal Component Analysis (PCA) which revealed that two components contributed about 64 % in the analysis (Table 5C). When several inflorescence leaves were assessed, Loganiaceae shows a considerable variation in their leaf shapes and sizes. In Tables 5B and 5C, the component 1 (C1) revealed leaf length, width and their ratio (shape) as the major characters that could delimit Loganiaceae when their leaves are considered for identification purposes.

The scatter plots in Figure 5C and 5D (group centroids) revealed that some genera are grouped together around the centre; example is *Strychnos* while 1, 4, 6, 9 (B-blue) and two others (*Anthocleista* genus – Table 5A) are scattered apart (outliers) in the space. This is due to unique features found in *Anthocleista* such as: Paired thorns in these four species, broad leaves with varying petiole length in the genus and massive garth when compared with strangling liana – the *Strychnos.* Figure 5D also showed similarity amonog other species: 3, 21, 52 and 53. They are *A. microphylla*, *S. afzeli*, *S. usambarensis* and *S. chrysophylla* respectively – (R-Red). Figure 5B is a scree plot showing the degree of significance when variance of the characters used are represented on a plot as revealed by PCA. The Eigen values of Component 1 and 2 were high enough to significantly delimit the entire population of Loganiaceae as revealed by the studies.

Table 5A: Morphological assessment of Loganiaceae

Plant specimen	CODE	Habit	Vegetation Zone	Bark texture (rough/ smooth)	Spine (present/ absent)	Branch (few/ many)	Branch (smooth/ spiny, rough)	Hook (paired/ single)	Inflorescent bract (large/ small)
Anthocleista djalonensis	**ADJ1**	tree	Man - Savana	rough	present	Few	spiny	nil	small
A. liebrechtsiana	**ALI2**	tree	Man - Savana	rough	absent	Few	smooth	nil	small
A. microphyla	**AMI3**	Epiphyte	Forest	rough	absent	Few	smooth	nil	small
A. nobilis	**ANO4**	tree	High Forest	rough	present	Few	spiny	nil	small
A. obanensis	**AOB5**	tree	Man - Savana	rough	absent	Few	smooth	nil	small
A. procera	**APR6**	tree	Swamps	rough	absent	Few	smooth	nil	small
A. scandens	**ASD7**	tree	mountain F.	rough	present	Few	spiny	nil	small
A. schweinfurthii	**ASF8**	tree	swamps to F.	rough	present	Few	spiny	nil	small
A. vogelli	**AVO9**	tree	Mangrove to Savana	rough	present	Few	spiny	nil	small
M. brunonis	**MBR15**	shrub	F. understory	rough	absent	Many	smooth	nil	large
M. hirsuta	**MHI16**	shrub	F. understory	smooth	absent	Few	spiny	nil	large
Nuxia congesta	**NCO18**	tree	mountain F.	rough	absent	Many	smooth	nil	small
Spigelia anthelmia	**SAT19**	herb	Cultivated plot	smooth	absent	Few	smooth	nil	small
Strychnos aculeata	**SAC20**	liana	High Forest	rough	present	Few	spiny	paired	small
S. afzeli	**SAF21**	liana	High Forest	rough	absent	Few	smooth	single	Small

Table 5A: Morphological assessment of Loganiaceae continued

Plant specimen	CODE	Habit	Vegetation Zone	Bark texture (rough/ smooth)	Spine (present/ absent)	Branch (few/ many)	Branch (smooth/ spiny, rough)	Hook (paired/ single)	Inflorescent bract (large/ small)
S. angolensis	**SAG22**	liana	High Forest, river banks	smooth	absent	Many	smooth	single	small
S. asteranta	**SAS23**	liana	High Forest	smooth	absent	Few	smooth	paired	small
S. barteri	**SBA24**	liana	High Forest	rough	absent	Many	smooth	paired	small
S. boonei	**SBO25**	liana	high forest	smooth	absent	Few	smooth	single	small
S. campicola	**SCP26**	liana	high forest	smooth	absent	Many	smooth	single	small
S. camptoneura.	**SCT27**	liana	high forest	smooth	absent	Few	smooth	paired	small
S. chromatoxylon	**SCH28**	liana	high forest	smooth	absent	Many	spiny	single	small
S. congolana	**SCO29**	liana	high forest	smooth	absent	Many	smooth	single	small
S. cuminodora	**SCU30**	liana	high forest	rough	absent	Many	smooth	paired	small
S. densiflora	**SDE31**	liana	high forest	smooth	absent	Few	smooth	paired	small
S. dinklagei	**SDI32**	liana	High Forest	rough	absent	Few	smooth	paired	small
S. floribunda	**SFL33**	liana	High Forest	smooth	absent	Many	smooth	single	small
S. gossweileri	**SGO34**	liana	High Forest	rough	present	Many	rough	paired	small
S. icaja	**SIC35**	liana	High Forest	smooth	absent	Many	smooth	single	small
S. innocua	**SIN36**	tree	savanna	rough	absent	Many	rough	nil	small
S. johnsonii	**SJO37**	liana	High Forest	rough	absent	Few	rough	paired	small

Table 5A: Morphological assessment of Loganiaceae continued

Plant specimen	CODE	Habit	Vegetation Zone	Bark texture (rough/ smooth)	Spine (present/ absent)	Branch (few/ many)	Branch (smooth/ spiny, rough)	Hook (paired/ single)	Inflorescent bract (large/ small)
S. longicaudata	**SLO38**	liana	High Forest	smooth	absent	Many	rough	single	small
S. lucens	**SLU39**	liana	High Forest	smooth	absent	Few	smooth	paired	small
S. malacoclados	**SMA40**	liana	High Forest	smooth	absent	Few	smooth	single	small
S. memecyloides	**SME41**	liana	High Forest	rough	absent	Many	smooth	single	small
S. nigritana	**SNI42**	liana	High Forest	rough	absent	Many	rough	paired	small
S. nux-vomica	**SNU43**	tree	High Forest	smooth	absent	Few	smooth	nil	small
*S. phaeotricha***.**	**SPH44**	liana	high forest	rough	absent	Many	rough	paired	small
S. soubrensis	**SSO45**	liana	high forest	smooth	absent	Few	rough	paired	small
S. spinosa	**SSN46**	tree	savanna	rough	present	Many	spiny	nil	small
S. spinosa var. pubescens	**SSN46B**	tree	savanna	rough	present	Many	spiny	nil	small
S. splendens	**SSD47**	liana	high forest	smooth	absent	Many	smooth	nil	small
S.staudtii	**SST48**	tree	high forest	rough	absent	Many	smooth	nil	small
S. talbotiae	**STA49**	liana	high forest	rough	absent	Many	smooth	paired	small
S. tricalysioides	**STR50**	liana	high forest	rough	absent	Few	rough	single	small
S. urceolata	**SUR51**	liana	high forest	smooth	absent	Many	smooth	paired	small
S.usambarensis	**SUS52**	liana	high forest	rough	absent	Many	smooth	single	small
S. ndengensis	**SND54**	liana	high forest	smooth	absent	Many	rough	single	small

Table 5A: Morphological assessment of Loganiaceae continued

Plant Name	CODE	Habit	Vegetation Zone	Bark texture (rough/ smooth)	Spine (present/ absent)	Branch (few/ many)	Branch (smooth/ spiny, rough)	Hook (paired/ single)	Inflorescent bract (large/ small)
S. indeterminate Edondon -2	**SID55**	liana	high forest	rough	absent	Many	smooth	single	Absent
S. indeterminate Edondon -3	**SID56**	liana	high forest	smooth	absent	Few	smooth	paired	Absent
S. indeterminate Erokut station -2	**SID57**	liana	high forest	smooth	absent	Many	smooth	single	Absent
S. indeterminate Erokut station -3	**SID58**	liana	high forest	rough	absent	Many	smooth	single	Absent
S. indeterminate Edondon -1	**SID59**	liana	high forest	rough	absent	Many	smooth	paired	Absent
S. indeterminate Edondon -8	**SID60**	liana	high forest	smooth	absent	Few	smooth	single	Absent
S. indeterminate Edondon -4	**SID61**	liana	high forest	rough	absent	Many	smooth	single	Absent
S. indeterminate Ipetu- Ijesha	**SID62**	liana	high forest	smooth	absent	Many	smooth	paired	Absent
S. indeterminate J_4 -3	**SID63**	liana	high forest	smooth	absent	Few	rough	single	Absent
S. indeterminate Erokut station -6	**SID64**	liana	high forest	rough	absent	Many	smooth	paired	Absent
S. indeterminate Edondon -6	**SID65**	liana	high forest	smooth	absent	Many	smooth	paired	Absent
S. indeterminate ENUGU	**SID66**	liana	high forest	smooth	absent	Few	smooth	single	Absent
Usteria guineensis	**UGU67**	tree	secondary f.	rough	absent	Many	spiny	nil	small

Table 5A: Morphological assessment of Loganiaceae continued

Plant Name	influore scence type	Flower fresh colour	Leaf shape	Leaf apex	Leaf margin	Leaf hairiness (indumentum)	Petiolate/ sessile	Leaf base	Leaf arrange ment
A.djalonensis	corymb	white	obovate	rounded	revolute & undulate	coriaceous	petiolate	rounded	opposite
A. liebrechtsiana	corymb	creamy	oblanceolate	rounded	entire	coriaceous	petiolate	cuneate	opposite
A. microphyla	corymb	white	oblong, elliptic, obovate	acuminate	entire	coriaceous	petiolate	narrowly cuneate.	opposite
A. nobilis	corymb	white	obovate, oblanceolate	rounded	revolute & undulate	coriaceous	petiolate	rounded	opposite
A. obanensis	corymb	Yellow	lanceolate	acuminate	entire	coriaceous	petiolate	attenuate	opposite
A. procera	corymb	white	obovate to oblanceolate	rounded	entire	coriaceous	petiolate	attenuate	opposite
A. scandens	corymb	white	obovate	acuminate	entire	coriaceous	petiolate	attenuate	opposite
A. schweinfurthii	corymb	creamy	obovate to oblanceolate	rounded	entire	coriaceous	petiolate	attenuate	opposite
A. vogelli	corymb	Creamy, yellow	obovate	rounded	revolute & undulate	coriaceous	sessile	cuneate, , auriculate.	opposite
M. brunonis	cymose	white	obovate	acute	entire	pilose	petiolate	rounded	opposite
M. hirsuta	terminal cyme	white	ovate	acute	entire	hirsute	petiolate	rounded	opposite
Nuxia congesta	corymb	white	elliptic	acute	entire	glabrous	petiolate	attenuate	opposite
Spigelia anthelmia	corymb	white	lanceolate	acuminate	entire	glabrous	sessile	attenuate	opposite
Strychnos aculeata	cymose	white	elliptic, oblong, lanceolate	acuminate , obtuse, acute	entire	glabrous	petiolate	attenuate	opposite

Table 5A: Morphological assessment of Loganiaceae continued

Plant Name	inflourescence type	Flower fresh colour	Leaf shape	Leaf apex	Leaf margin	Leaf hairiness (indumentum)	Petiolate / sessile	Leaf base	Leaf arrangement
S. afzeli	axillary cymose	white	obovate, spathulate	rounded, apiculate, acuminate	entire	Pubescent	petiolate	attenuate	opposite
S. angolensis	cymose; axillary	white	ovate, elliptic, oblanceolate	acuminate, obtuse, acute	entire	Pubescent	petiolate	attenuate	opposite
S. asteranta	cymose; axillary	yellow	elliptic, oblanceolate	acute	entire	Glabrous	petiolate	obtuse	opposite
S. barteri	cymose; axillary	white	oblong to obovate	rounded, apiculate, acuminate	entire	Glabrous	petiolate	attenuate	opposite
S. boonei	cymose; axillary	white	oblong, elliptic	acuminate	entire	pubescent at base	petiolate	attenuate	opposite
S. campicola	cymose; axillary	Yellow	oblong, ovate, lanceolate, elliptic.	acute	entire	Glabrous	petiolate	attenuate, obtuse	opposite
S. camptoneura.	cymose; axillary	white	elliptic, ovate	acute, acuminate, mucronate	entire	Pubescent	petiolate	obtuse, attenuate	opposite
S. chromatoxylon	cymose; axillary	Yellow	lanceolate, elliptic	cuspidate, acuminate	entire	Glabrous	petiolate	obtuse	opposite
S. congolana	umbellate	white	ovate, elliptic	cuspidate	entire	Glabrous	petiolate	attenuate	opposite
S. cuminodora	cymose; axillary	pink/ purple	elliptic, oblanceolate	acuminate, acute	entire		petiolate	obtuse	opposite
S. densiflora	cymose; axillary	Yellow	ovate, elliptic	acuminate	entire	Glabrous	petiolate	attenuate	opposite
S. dinklagei	cymose; axillary	white	elliptic, oblanceolate	acuminate, acute	entire	Coriaceous	petiolate	attenuate	opposite

Table 5A: Morphological assessment of Loganiaceae continued

Plant Name	inflourescence type	Flower fresh colour	Leaf shape	Leaf apex	Leaf margin	Leaf hairiness (indumentum)	Petiolate / sessile	Leaf base	Leaf arrangement
S. floribunda	cymose; axillary	white	oblanceolate	acuminate	entire	glabrous	petiolate	attenuate	opposite
S. gossweileri	cymose; axillary	white	ovate, elliptic	acuminate	entire	glabrous	petiolate	attenuate	opposite
S. icaja	raceme; axillary panicle	white	elliptic	acuminate, acute	entire	glabrous	petiolate	attenuate	opposite
S. innocua	raceme; panicle	Yellow	elliptic, obovate.	round, obtuse	entire	pubescent	petiolate	attenuate	opposite
S. johnsonii	cymose; axillary	white	ovate, elliptic	acuminate	entire	glabrous	petiolate	obtuse	opposite
S. longicaudata	axillary cymose	white	elliptic	acuminate, caudate	entire	glabrous	petiolate	attenuate	opposite
S. lucens	axillary cymose	Yellow	ovate	acute	entire	glabrous	petiolate	rounded	opposite
S. malacoclados	axillary cymose	orange	ovate, elliptic, oblanceolate	acuminate, obtuse, acute	entire	glabrous	petiolate	attenuate, curneate	opposite
S. memecyloides	axillary cymose	white	elliptic, oblong	acuminate, acute	entire	glabrous	petiolate	attenuate, curneate	opposite
S. nigritana	axillary cymose	Yellow	elliptic, ovate, obovate	acuminate, acute, mucronate	entire	glabrous	petiolate	rounded, attenuate, obtuse	opposite
S. nux-vomica	raceme; panicle	Yellow	ovate, elliptic, oblanceolate	mucronate, acuminate	entire	glabrous	petiolate	rounded, obtuse	opposite

Table 5A: Morphological assessment of Loganiaceae continued

Plant Name	inflourescence type	Flower fresh colour	Leaf shape	Leaf apex	Leaf margin	Leaf hairiness (indumentum)	Petiolate/ sessile	Leaf base	Leaf arrangement
S. phaeotricha.	axillary cymose	white	oblong, ovate, obovate, oblanceolate,	emerginate, caudate.	entire	pubescent	petiolate	attenuate	opposite
S. soubrensis	axillary cymose	white	elliptic, oblanceolate	acuminate, caudate	entire	glabrous	petiolate	attenuate	opposite
S. spinosa	compound cymose	Yellow	obovate, spathulate	rounded, mucronate, emerginate	entire	glabrous	petiolate	rounded, attenuate, obtuse	opposite
S. spinosa var. pubescens	compound cymose	white	obovate, spathulate	round, mucronulate, obcordate.	entire	pilose	petiolate	attenuate	opposite
S. splendens	cymose	white	elliptic, lanceolate	caudate, acuminate	entire	glabrous	petiolate	round	opposite
S.staudtii	axillary cymose	white	elliptic, lanceolate	acuminate	entire	glabrous	petiolate	attenuate	opposite
S. talbotiae	axillary cymose	white	lanceolate	acuminate	entire	glabrous	petiolate	attenuate	opposite
S. tricalysioides	axillary cymose	white	elliptic, ovate	acuminate	entire	glabrous	petiolate	attenuate	opposite
S. urceolata	axillary cymose	Yellow	ovate, lanceolate	acuminate	entire	glabrous	petiolate	attenuate	opposite
S.usambarensis	axillary cymose	Yellow	ovate, lanceolate	acuminate	entire	glabrous	petiolate	attenuate	opposite
S. chrysophylla	axillary cymose	white	elliptic, lanceolate	acuminate	entire	glabrous	petiolate	attenuate	opposite

Table 5A: Morphological assessment of Loganiaceae continued

Plant Name	inflourescence type	Flower fresh colour	Leaf shape	Leaf apex	Leaf margin	Leaf hairiness	Petiolate / sessile	Leaf base	Leaf arrangement
S. ndengensis	axillary cymose	white	elliptic, lanceolate	acuminate	entire	glabrous	petiolate	attenuate	opposite
S. indeterminate Edondon -2	Absent	Absent	ovate	acute	entire	glabrous	petiolate	attenuate	opposite
S. indeterminate Edondon -3	Absent	Absent	ovate	acute	entire	glabrous	petiolate	attenuate	opposite
S. indeterminate Erokut station -2	Absent	Absent	ovate	caudate	entire	glabrous	petiolate	attenuate	opposite
S. indeterminate Erokut station -3	Absent	Absent	ovate	caudate	entire	glabrous	petiolate	attenuate	opposite
S. indeterminate Edondon -1	Absent	Absent	ovate	caudate	entire	glabrous	petiolate	attenuate	opposite
S. indeterminate Edondon -8	Absent	Absent	ovate	caudate	entire	glabrous	petiolate	attenuate	opposite
S. indeterminate Edondon -4	Absent	Absent	elliptic, ovate	caudate, acuminate	entire	glabrous	petiolate	attenuate	opposite
S. indeterminate Ipetu- Ijesha	Absent	Absent	lanceolate	caudate	entire	glabrous	petiolate	attenuate	opposite
S. indeterminate J_4 -3	Absent	Absent	ovate	acuminate	entire	glabrous	petiolate	attenuate	opposite
S. indeterminate Erokut station -6	Absent	Absent	obovate	caudate	entire	glabrous	petiolate	attenuate	opposite
S. indeterminate Edondon -6	Absent	Absent	ovate	caudate	entire	glabrous	petiolate	attenuate	opposite
S. indeterminate ENUGU	Absent	Absent	ovate	caudate	entire	glabrous	petiolate	attenuate	opposite
Usteria guineensis	terminal panicle	yellow	elliptic	acute	entire	glabrous	petiolate	round	opposite

Table 5A: Morphological assessment of Loganiaceae continued

Plant Name	Leaf 2° Veins (bold/ faint)	leaf length (cm)	leaf width (cm)	LL A	LW A	L/W ratio	Plant height (m)	Petiole length (cm)	Apex length (cm)	Internode length (cm)
Anthocleista djalonensis	bold	8 - 70	3.5 - 28	45	19	2.37	5 - 40	7 - 12	0	9 - 15
A. liebrechtsiana	bold	11 - 75	3 - 15	20	8	2.50	5 - 20	4 - 7	0	10 - 17
A. microphyla	faint	6 - 20	2 - 16	13	10	1.30	6 -10	12 - 14	0	4 - 8
A. nobilis	bold	6 - 50	3 - 30	34	17	2.00	5 - 40	14	0	13
A. obanensis	faint	9 - 20	3 - 9	10	5	2.00	4 - 6	1 - 1.5	0.2 - 1	1.5 - 2
A. procera	bold	12 - 56	5 - 20	40	13	3.08	6 - 21	1.5 - 3	0.5 - 3	10
A. scandens	faint	12 - 18	4 - 9	15	6	2.50	7 - 12	2 - 3.4	0.5 - 2.0	4 - 6
A. schweinfurthii	bold	14 - 35	4 - 13	26	8	3.25	15 - 30	1.5 - 3	1 - 3	4 - 5
A. vogelli	bold	15 - 80	8 - 24	42	16	2.63	8 - 45	7 - 12	0	10 - 13
M. brunonis	faint	0.5 - 5.5	0.2 - 2.7	3.8	1.4	2.71	0.5 - 1.6	0.2 - 1.3	0	2.0 - 3.0
M. hirsuta	faint	0.7 - 5.8	0.2 - 2.8	4.0	1.9	2.11	0.7 - 2.0	0.1 - 0.2	0.5 - 0.9	2.3 - 5.0
Nuxia congesta	faint	4.5 - 14.5	1.5 - 4.5	10.2	3.5	2.91	6.0 - 9.0	0.6 - 2.3	0.5	1.0 - 2.5
Spigelia anthelmia	faint	3.2 - 12.5	0.8 - 3.3	8.5	1.9	4.47	0.1 - 0.43	0	0	5.5 - 19.6
Strychnos aculeata	bold	10 - 20	3.5 - 9.5	16.5	7.1	2.32	20 - 40	0.5 - 1.5	0.4 - 1.0	4.0 - 8.5
S. afzeli	bold	1.5 - 8.0	0.5 - 4.4	5.2	3.0	1.73	8 - 12	0.1 - 0.4	0.5	2.5
S. angolensis	bold	1.0 - 5.0	0.5 - 3.0	3.0	2.0	1.50	12	0.1 - 0.2	0.1	1.8

Table 5A: Morphological assessment of Loganiaceae continued

Plant Name	Leaf 2° Veins	leaf length (cm)	leaf width (cm)	LLA	LWA	L/W ratio	Plant height (m)	Petiole length (cm)	Apex length (cm)	Internode length (cm)
S. asteranta	bold	3.0 - 7.5	1.8 - 3.0	4.6	2.5	1.84	9	0.2 - 0.5	0.3 - 0.9	3
S. barteri	faint	3.0 - 8.0	2.0 - 4.0	5.8	3.2	1.81	23 - 30	0.2 - 0.9	0	3
S. boonei	bold	2.0 - 8.0	1.0 - 5.0	5.0	3.6	1.39	22	0.5 - 1.1	0.8 - 1.2	4
S. campicola	bold	5.2-11.0	3.1-3.8	8.0	3.5	2.29	31	0.6-0.8	0.7-1.1	8
S. camptoneura.	bold	6.0 - 22.0	3.0 - 10.0	15.2	7.5	2.03	28	0.8 - 1.5	0.5 - 0.8	9
S. chromatoxylon	bold	6.0-10.4	4.4-5.5	8.3	6.6	1.26	16	0.5	0.8	6
S. congolana	bold	4(6)10	3 (4.3)6	6	4.3	1.40		1	0.7 - 1.5	5
S. cuminodora	bold	4.0 - 8.0	1.8 - 3.0	6.7	2.6	2.58	34	0.2 - 0.5	0.3 - 0.6	4.5
S. densiflora	bold	4.0 - 10.0	3.0 - 6.10	8.0	5.0	1.60	10	1.2	0.6	8
S. dinklagei	bold	3.0 - 10.0	1.5 - 5.0	7.0	3.8	1.84	40	0.1 - 0.3	0.3 - 0.9	7
S. floribunda	bold	3.0 - 7.5	1.5 - 4.5	6.0	3.3	1.82	5	0.2 - 0.9	0.4 - 0.8	3
S. gossweileri	bold	1.8 - 5.0	2.0 - 4.0	3.4	3.1	1.10	7	0.1 - 0.4	0.1 - 0.2	2.4
S. icaja	bold	10 - 17	4.0 - -8.0	14.5	6.6	2.20	25	0.8 - 1.2	0.8 - 1.5	6.8
S. innocua	bold	3.0 - 7.0	1.5 - 4.2	5.4	3.0	1.80	4	0.2 - 0.9	0.1	10
S. johnsonii	bold	5.0 - 11.2	3.0 - 6.1	8.4	5.0	1.68	6	0.2 - 0.8	1.4	4.5

Table 5A: Morphological assessment of Loganiaceae continued

Plant Name	Leaf 2° Veins	leaf length (cm)	leaf width (cm)	LLA	LWA	L/W ratio	Plant height (m)	Petiole length (cm)	Apex length (cm)	Internode length (cm)
S. longicaudata	bold	6.0 - 14.0	2.0 - 4.7	11.5	3.6	3.19	17	0.3 - 0.7	1.5	4.7
S. lucens	bold	3.9-5.6	2.0-3.0	4.7	2.5	1.88	9	0.4-0.8	0	4
S. malacoclados	bold	3.0 - 10.2	1.4 - 3.0	7.3	2.4	3.04	10	1	0.8	4
S. memecyloides	bold	8.0 - 17.0	3.0 - 7.0	15.2	5.8	2.62	15	0.5 - 1.0	1.1	4.7
S. nigritana	bold	5.0 - 14.0	2.0 - 7.0	10.4	5.2	2.00	35	0.3 - 0.7	1	5
S. nux-vomica	bold	5.0-7.9	3.5-6.1	6.4	4.6	1.39	10	0.7-1.2	0.2	6
S. phaeotricha.	bold	5.0 - 12.5	2.2 - 5.0	9.4	4.3	2.19	10	5.2	0.6	5
S. soubrensis	bold	2.5 - 8.0	1.0 - 3.9	6.1	2.8	2.18	35	0.1 - 0.3	0.1 - 0.6	2.5
S. spinosa	bold	3.0 - 6.4	2.0 - 5.0	4.2	3.9	1.08	3 (4) 5	1	0.1 - 0.3	5
S. spinosa var. pubescens	bold	4.9-7.2	4.0-4.7	5.7	4.3	1.33	3 (5) 7	1 - 1.5	0 - 0.3	5.0
S. splendens	bold	1.5 - 9.0	1.0 - 4.5	6.5	3.1	2.10	12	0.1 - 0.4	0.1 - 0.5	3
S.staudtii	bold	6.0 - 25.0	4.0 - 7.9	18.8	6.2	3.03	14	0.5 - 1.2	1.0 - 2.3	5
S. talbotiae	bold	3.5-4.0	2.0-2.3	3.7	2.1	1.76	7	0.3 - 1.4	0.2 - 0.8	2.8 - 3.5
S. tricalysioides	bold	3.5 - 14.4	1.0 - 6.0	9.0	4.8	1.88	9	0.3 - 1.0	0.8 - 2.0	5
S. urceolata	bold	3.4-4.0	2.3-2.6	3.7	2.5	1.48	11	0.1 - 0.5	0.7 - 1.3	4.8 - 5.4

Table 5A: Morphological assessment of Loganiaceae continued

Plant Name	Leaf 2° Veins	leaf length (cm)	leaf width (cm)	LLA	LWA	L/W ratio	Plant height (m)	Petiole length (cm)	Apex length (cm)	Internode length (cm)
S.usambarensis	bold	3.0-9.0	2.0-5.8	6.2	4.0	1.55	23	0.3 - 1.0	0.6 - 1.4	3
S. ndengensis	bold	8 - 20	4 - 5	13	4.5	2.89	15	1	0.5	3
S. ndengensis	bold	5.4-9.5	3.9-5.0	8.1	4.5	1.80	10	0.5-0.8	0.3	4.0-6.0
S. indeterminate Edondon -2	bold	(2)5-11	(1.3)3.5-5.4	8.5	5	1.70	25	0.2 - 0.4	0.1	3.5-6
S. indeterminate Edondon -3	bold	(3.2)6-14	3.5-7.7	9.3	5.5	1.69	10	0.2 - 0.5	0.1-0.8	2.8-4
S. indeterminate Erokut station -2	bold	8-17.5	3.5-10	13	7	1.86	34	0.2-0.9	0.8-2.0	4-6
S. indeterminate Erokut station -3	bold	7-15	3-7	5	9	0.56	16	0.2-1.0	1.0-2.0	5-8
S. indeterminate Edondon -1	bold	7.5-12	4.4-7.0	8.2	5.3	1.55	11	0.6-1.3	1.0-2.0	6-9
S. indeterminate Edondon -8	bold	7 - 11.5	3.5 - 6.7	7	4.5	1.56	15	0.2-0.4	1.4- 1.7	5- 7
S. indeterminate Edondon -4	bold	6.0 -14.5	4.0 - 8.5	11	6	1.83	21	0.1 - 0.4	0.5-1.0	8 - 14
S. indeterminate Ipetu-Ijesha	bold	9.0-14.9	4.5-7.0	11.4	5.3	2.15	35	0.5-0.8	1.0-1.3	7.0-8.6
S. indeterminate J_4 -3	bold	6.4-10.6	4.0-7.0	8.1	5.2	1.56	12	0.2 - 0.5	0.1-0.8	5.8-6.8
S. indeterminate Erokut station -6	bold	5.0 - 10.5	3.0-5.6	8	4.5	1.78	8	0.2 - 0.5	0.6-1.3	4-6
S. indeterminate Edondon -6	bold	5.0 - 13.0	2.3-5.6	9.8	4.5	2.18	14	0.2-0.6	0.6-1.4	4.3-6.5
S. indeterminate ENUGU	bold	5.0 -9.0	2.7 - 5.3	7.4	4	1.85	3	0.5 - 1.1	0.6 - 2.0	6-9
Usteria guineensis	bold	3.0-9.0	2.0-8.0	6.5	5.2	1.25	6	0.2 - 1.0	0	10 - 18

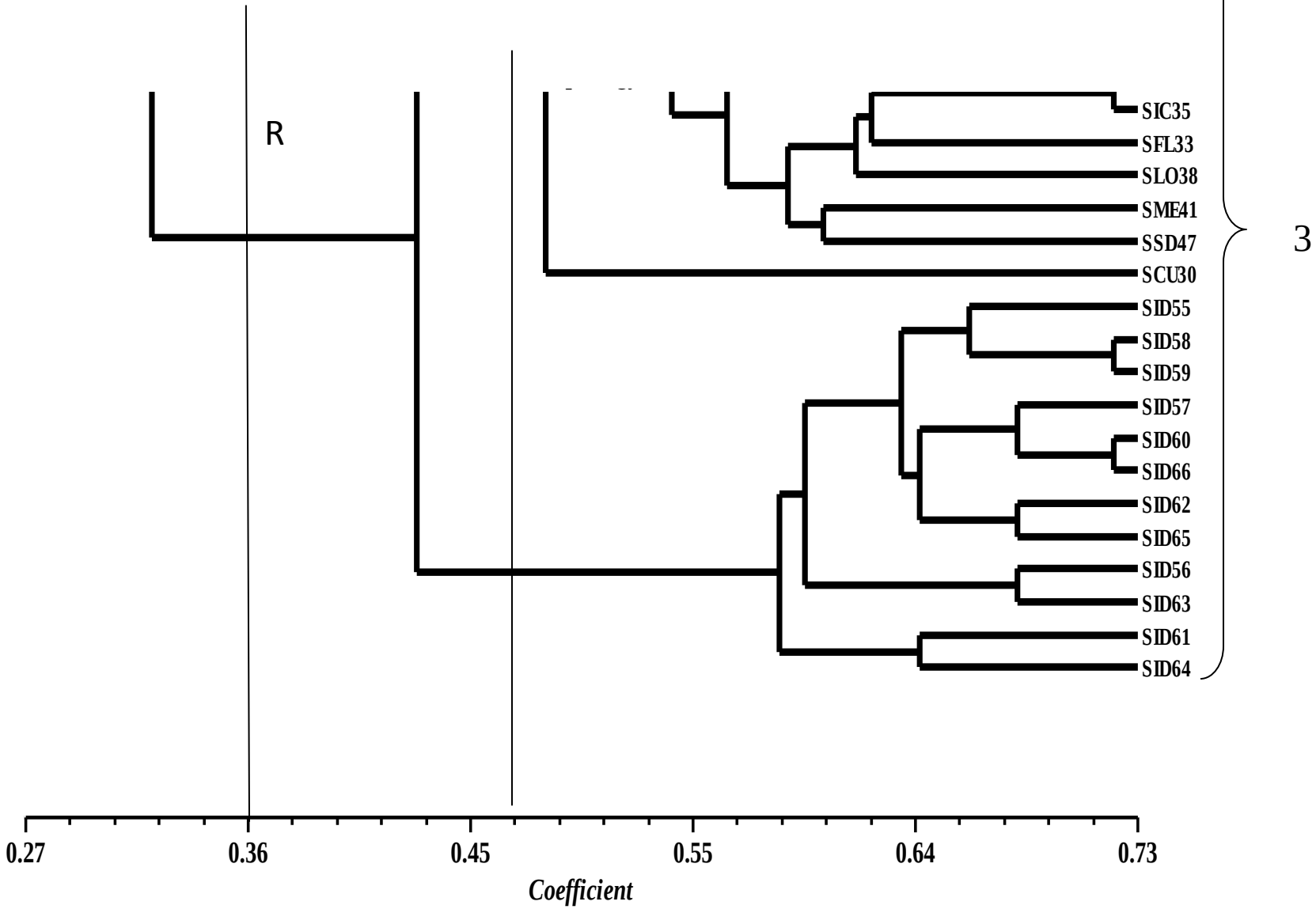

Figure 5A: Dendrogram showing relationship within Loganiaceae based on Morphological data. (R= reference line; 1 - 3= clusters one to cluster three).

Table 5B: Principal Component Analysis (PCA) showing communalities and component matrix for Loganiaceae morphology

Communalities			**Component Matrix**	
	Initial	Extraction	Component 1	Component 2
Leaf length	1.000	.914	.936	.194
Leaf width	1.000	.891	.944	-.016
Leaf L/W ratio	1.000	.308	.295	.470
Plant Height	1.000	.353	.306	.510
Petiole length	1.000	.802	.820	-.361
Apex length	1.000	.677	-.086	.818
Internode length	1.000	.527	.714	-.134

Table 5C: Two principal components from PCA contributed about 64 %

Total Variance Explained									
Component	**Initial Eigenvalues**			**Extraction Sums of Squared Loadings**			**Rotation Sums of Squared Loadings**		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	3.137	44.811	44.811	3.137	44.811	44.811	3.032	43.321	43.321
2	1.335	19.078	63.889	1.335	19.078	63.889	1.440	20.567	63.889
3	.985	14.069	77.957						
4	.741	10.590	88.548						
5	.590	8.428	96.976						
6	.193	2.757	99.733						
7	.019	.267	100.000						

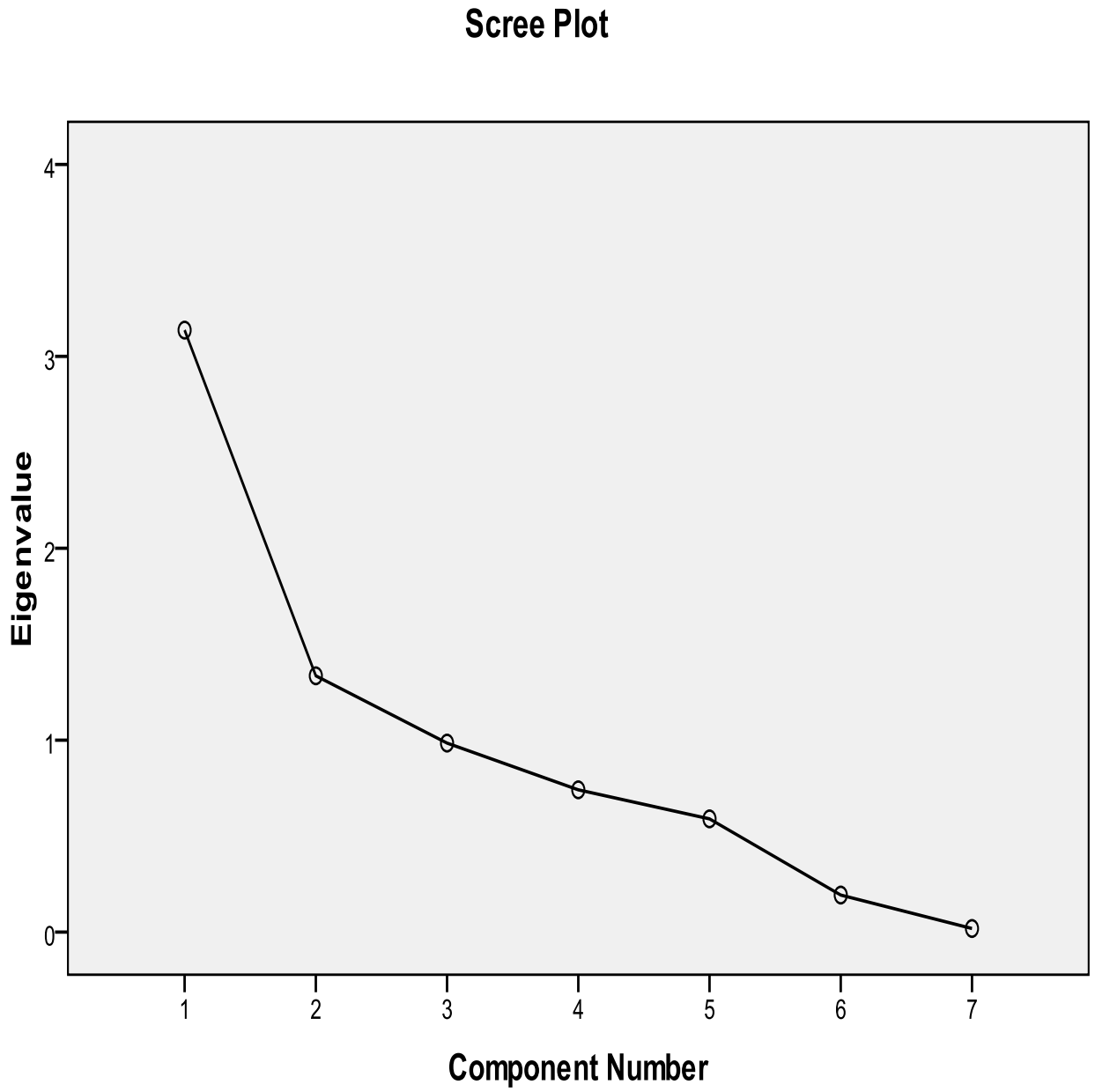

Figure 5B: Principal Component Analysis for scree plot of Eigen values for Loganiaceae morphology.

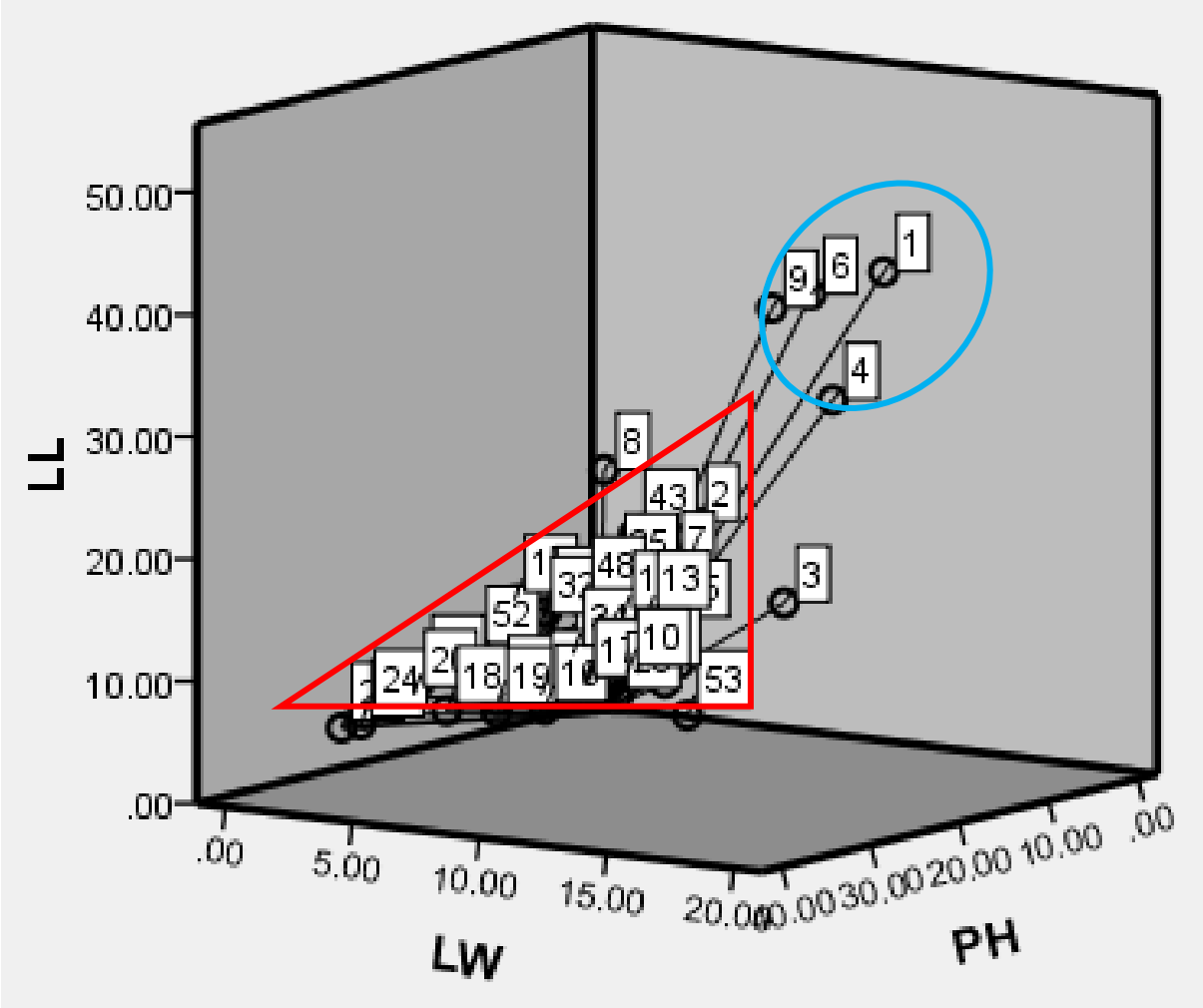

Figure 5C: Scatter plot of Leaf length (LL), Leaf width (LW) and Plant height (PH) of first component obtained from PCA (group centroids). R = Red; B = Blue - colours depicting the outliers from the centre R = Red

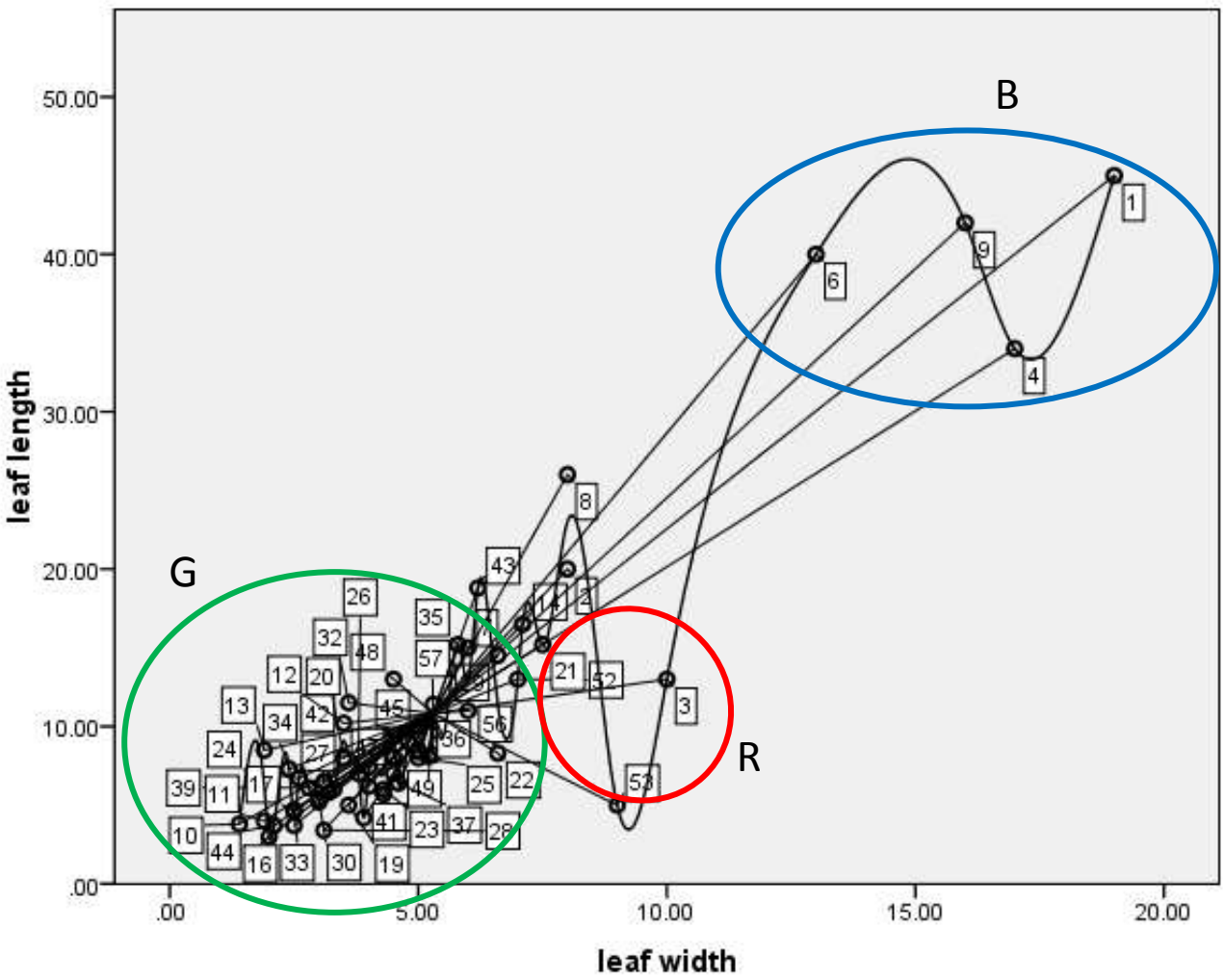

Figure 5D: The scatter diagram for Leaf length (LL) and Leaf width (LW) of first component obtained from PCA (group centroids). R = Red; B = Blue; G= Green colours depicting the outliers from the centre (Green).

4.3.2 Micro-morphology of the leaves of the species of Loganiaceae

The abaxial and adaxial surfaces were observed separately and variations between the species of Loganiaceae were recorded. *Anthocleista* species have anisocytic, anomocytic and staurocytic stomata (Plates 2A – 2F and Table 6A). The cell wall pattern in *Anthocleista* is slight straight to curved. They are however, sinuous in *A. vogelii*. Epidermal cell shape is generally polygonal in *Anthocleista* with the exception of few species that have only about tetragonal or pentagonal shape. The cell shape in *Mostuea* is perfectly irregular because of the sinuous nature of their walls. Epidermal cell length in *Spigelia anthelmia* is greater than any other species in Loganiaceae. They measure above 65 µm on most of the cells while *Strychnos* could at the same time measure just 15 µm. The epidermal cell in *S. asteranta* and *S. aculeata* are the smallest in Loganiaceae. Cell count in *S. asteranta* was above 600 per view of microcope. Hence, the dimension is expected to be small compared to others. There are two major types of trichomes: stellate and dendritic trichomes in *Anthocleista* (Plate 2A – 2D and Table 6A). Two *Anthocleista* species are amphistomatic: *A. djalonensis* and *A. procera* while the other seven are hypostomatic in nature. Peltate trichome is unique to *Nuxia congesta* just as conical trichome is unique to *Spigelia anthelmia* (Plate 2H (a-b and c-d respectively) and Table 6A). *Strychnos* species have non-glandular unicellular tichomes with the exception of two species: *Strychnos boonei* and *S. icaja*. They possess non-glandular multicellular tichomes (Plates 2F and 2M f respectively and Table 6A). Both adaxial and abaxial epidermal cells of *Mostuea* species are sinuous (Plate 2G and Table 6A). Generally, the anticlinal wall pattern curvatures in Loganiaceae are in this descending order: most curved in *Mostuea*, followed by *Anthocleista*, by *Spigelia*, by *Strychnos*, and least by *Nuxia* while near straight in *Usteria* (Plate 2A – 2W and Table 6A). *Mostuea* possesses anisocytic stomata and long non-glandular unicellular trichomes (Plate 2G and Table 6A). *Spigelia* possesses two types of trichomes: Anisocytic and Anomocytic. The species with the highest Stomata Index is *Strychnos*

lucens with 53.42 %. The species is hypostomatic with Paracytic stomata on the abaxial surface only. Starch deposition is common to most of the species studied but one epidermal character (ornamentation) not common in Loganiaceae is the presence of crystals. It is available with *A. vogelii, Strychnos spinosa* and one other *Strychnos* indeterminate. The abaxial surface is highly informative than the adaxial surface in Loganiaceae leaf epidermises (Table 6A and Figure 6B).

Table 6A: Micro-morphological characters of the species of Loganiaceae

Plant Name	Leaf surface	cell wall pattern	Cell wall thickness (µm). min.(mean ± s.e.) max	Epidermal Cell shape	Epidermal Cell number *min. (mean ± s.e.) max*	Epidermal Cell length min. (mean ± s.e.) max	Epidermal cell width min. (mean ± s.e.) max
Anthocleista djalonensis	Adaxial	curved	1 (1.68 ± 0.11) 3	polygonal	148(168.15 ± 4.81)215	9 (17.05 ± 0.85) 25	5 (8.28 ± 0.43) 11
	Abaxial	curved	1 (1.68 ± 0.11) 3	polygonal	140(169.55 ± 6.16)219	9 (17.05 ± 0.85) 25	5 (8.28 ± 0.43) 11
A. liebrechtsiana	Adaxial	straight	2 (3.13 ± 0.166) 4	polygonal	120 (246.30 ± 12.79)300	6 (17.6 ± 1.38) 26	4 (9.90 ± 0.64) 14
	Abaxial	straight to curved	0.5(1.05 ±0.070)1.5	Polygonal	300 (341.65 ± 5.94)380	8 (13.75 ± 0.86)20	4 (5.78 ± 0.33)8
A. microphyla	Adaxial	straight to curved	1(2.20±0.137)3	polygonal	68 (70.25 ± 0.37)74	18 (27.8 ± 1.02)37	11 (14.8 ± 0.44)18
	Abaxial	straight to curved	1(2.20±0.137)3	polygonal	68 (70.25 ± 0.37)74	18 (27.8 ± 1.02)37	11 (14.8 ± 0.44)18
A. nobilis	Adaxial	straight to curved	1(1.10±0.013)1.5	tetra to polygonal	380 (402.3± 2.12)420	10 (13.5 ± 0.47)16	5 (6.35 ± 0.24)9
	Abaxial	straight to curved	1(1.21±0.058)1.5	tetra to polygonal	270(295.47± 2.44)310	7(13.84± 0.83)20	5(6.63± 0.24)8
A. obanensis	Adaxial	straight to curved	1.5 (1.98 ± 0.044) 2.5	penta to polygonal	180(192.10 ± 1.41) 200	20 (26.1 ± 0.547)30	11 (17.45 ± 0.77)24
	Abaxial	curved	1 (1.43 ± 0.104) 2	penta to polygonal	78 (81.45 ± 0.47) 85	22 (29.55 ± 0.809) 36	1.5 (14.85 ± 0.18) 16
A. procera	Adaxial	curved	1 (1.13 ± 0.049)1.5	penta to polygonal	200 (215.3 ± 1.77) 230	11(16.3± 0.70) 20	7(9.8 ± 0.345) 12
	Abaxial	straight to curved	1 (1.13 ± 0.049)1.5	irregular	275(300± 3.06)321	5(11.85 ± 0.96)20	4(8.15 ± 0.55)12
A. scandens	Adaxial	straight to curved	1 (1.13 ± 0.050)1.5	penta to polygonal	100 (102.05 ± 0.328) 104	14(24.2 ± 1.77)32	12(16.2 ± 0.495)20
	Abaxial	curved	1 (1.13 ± 0.050)1.5	irregular	130 (137.80 ± 1.03)145	10 (14.60 ± 0.63)20	3 (8.45± 0.613)14
A. schweinfurthii	Adaxial	straight to curved	1 (1.13 ± 0.050)1.5	penta to polygonal	460 (473 ± 1.338)480	7 (8.75 ± 0.280) 11	4 (5.65 ± 0.0.233)8
	Abaxial	curved	1 (1.13 ± 0.050)1.5	irregular	248 (264.75 ± 1.252)270	8 (13.5 ± 0.848)20	5 (1.13 ± 0.34) 10
A. vogelli	Adaxial	wavy or undulate	2 (2.35 ± 0.109)3	irregular	40 (40.35 ± 0.109)41	20 (33.8 ± 1.400)40	13(16.6 ± 0.495)20
	Abaxial	wavy or undulate	2 (2.35 ± 0.109)3	irregular	40 (40.35 ± 0.109)41	20 (33.8 ± 1.400)40	13(16.6 ± 0.495)20
M. brunonis	Adaxial	straight to curved	1(1.175 ± 0.055)1.5	penta to polygonal	40(43.05 ± 0.505)46	20(31.15 ± 0.1.45)42	12(15.85 ± 0.494)19
	Abaxial	curved	1(1.175 ± 0.055)1.5	polygonal	40(43.05 ± 0.505)46	20(31.15 ± 0.1.45)42	12(15.85 ± 0.494)19
M. hirsuta	Adaxial	wavy / sinuous	1(1.175 ± 0.055)1.5	irregular	50(51.05 ± 0.198)52	20(12.55 ±1.647)46	8(12.55 ± 0.555)16
	Abaxial	wavy / sinuous	1(1.175 ± 0.055)1.5	irregular	75(76.8 ± 0.304)79	15(26.3 ± 1.42)36	6(9.5 ± 0.407)12
Nuxia congesta	Adaxial	straight	1(1.175 ± 0.055)1.5	penta to polygonal	190(197.00 ± 1.029)205	8(12.95 ± 0.716)18	5(7.85 ± 0.494)12
	Abaxial	straight	1(1.175 ± 0.055)1.6	polygonal	284(298.45 ± 1.565)310	6(11.95 ± 0.93)18	3(5.8 ± 0.45)9

Table 6A: Micromorphological characters of the species of Loganiaceae continued

Plant name	**Leaf surface**	**cell wall pattern**	**Cell wall thickness (µm). min.(mean ± s.e.) max**	**Epidermal Cell shape**	**Epidermal Cell number *min. (mean ± s.e.) max* (µm).**	**Epidermal Cell length min. (mean ± s.e.) max (µm).**	**Epidermal cell width min. (mean ± s.e.) max (µm).**
Spigelia anthelmia	Adaxial	straight to curved	1(1.175 ± 0.055)1.5	irregular	17(18.50 ± 0.295)21	45(54.60 ± 1.422)67	20(27.60 ± 0.916)35
	Abaxial	curved	1(1.175 ± 0.055)1.5	irregular	17(18.50 ± 0.295)21	45(54.60 ± 1.422)67	20(27.60 ± 0.916)35
S. aculeata	Adaxial	straight to curved	2(2.5 ± 0.115)3	polygonal	365(388.456 ± 14.75)668	4(10.0 ± 0.538)14	3(7.25 ± 0.507)10
	Abaxial	straight to curved	1(1.13 ± 0.0497)1.5	irregular	112(121.65 ± 0.958)128	12(22.25 ± 1.033)30	6(8.15 ± 0.264)10
S. afzeli	Adaxial	straight to curved	1(1.13 ± 0.0497)1.5	polygonal	420(433.5 ± 1.40)442	5(5.95 ± 0.276)13	3(5.95 ± 0.276)8
	Abaxial	wavy or undulate	1(1.13 ± 0.0497)1.5	irregular	248(254.8 ± 1.00)260	7(11.3 ± 0.612)15	3(5.95 ± 0.276)8
S. asteranta	Adaxial	straight to curved	1(1.13 ± 0.0497)1.5	irregular	380(394.90 ± 1.471)405	5(9.4 ± 0.455)13	4(6.150 ± 0.293)8
	Abaxial	straight to curved	1(1.13 ± 0.0497)1.5	polygonal to irregular	380(394.90 ± 1.471)405	5(9.4 ± 0.455)13	4(6.150 ± 0.293)8
S. barteri	Adaxial	curved	2(2.45 ± 0.114)3	polygonal to irregular	124(126.45 ± 0.950)128	10(17.05 ± 0.950)24	7(6.150 ± 0.293)22
	Abaxial	undulate	2(2.45 ± 0.114)3	irregular	96(97.70 ± 0.512)100	11(23.75 ± 0.369)46	8(9.15 ± 0.933)11
S. boonei	Adaxial	barbed	1(1.47 ± 0.092)2	penta to polygonal	240(246.90 ± 0.68)251	10(13.65 ± 0.342)16	5(8.75 ± 0.475)12
	Abaxial	barbed	2(2.45 ± 0.114)3	penta to polygonal	120(126.8 ± 0.618)130	13(17.550 ± 0.667)25	4(5.45 ± 0.211)7
S. campicola	Adaxial	curved	1(1.47 ± 0.092)2	penta to polygonal	118(123.35 ± 0.477)126	9(16.55 ± 0.953)23	7(10.00 ± 0.453)13
	Abaxial	curved	1(1.48 ± 0.0923)2	tetra to polygonal	104(104.95 ± 0.169)106	7(17.3 ± 1.23)25	4(7.25 ± 0.547)11
S. camptoneura.	Adaxial	undulate	1(1.48 ± 0.0923)2	penta to polygonal	128(135.2 ± 0.936)141	10(19.65 ± 1.435)30	6(7.48 ± 0.0226)10
	Abaxial	wavy	1(1.48 ± 0.0923)2	irregular	88(89.4 ± 0.152)90	10(17.4 ± 1.292)30	5(3.7 ± 0.336)10
S. chromatoxylon	Adaxial	straight to curved	1(1.48 ± 0.0923)2	polygonal	198(209.1± 1.706)220	10(16.9± 0.858)22	8(10.4 ± 0.413)14
	Abaxial	wavy	1(1.48 ± 0.0923)2	irregular	92(98.4 ± 0.623)102	12(24.5 ± 1.631)34	9(11.8 ± 0.388)15
S. congolana	Adaxial	straight	2(3.05 ± 0.170)4	polygonal	55(30.6 ± 2.391)60	12(30.60 ± 2.391)45	9(14.35 ± 1.064)32
	Abaxial	curved	2(3.05 ± 0.170)4	irregular	69(76.4 ± 0.796)80	15(21.4 ± 0.860)27	5(9.10 ± 0.518)15
S. cuminodora	Adaxial	straight	1.5(1.85 ± 0.053)2	polygonal	134(140.35 ± 0.634)144	10(15.65 ± 0.681)20	7(8.50 ± 0.256)10
	Abaxial	curved	1.5(1.85 ± 0.053)2	irregular	240(248.6 ± 0.697)225	10(16.45 ± 0.816)21	6(13.4 ± 0.346)16

Table 6A: Micromorphological characters of the species of Loganiaceae cotinued

Plant Name	Leaf surface	cell wall pattern	Cell wall thickness (µm). min.(mean ± s.e.) max	Epidermal Cell shape	Epidermal Cell number *min. (mean ± s.e.) max*	Epidermal Cell length min. (mean ± s.e.) max	Epidermal cell width min. (mean ± s.e.) max
S. densiflora	Adaxial	straight	2(2.65 ± 0.109)3	polygonal	72(74.50 ± 0.336)77	12(21.25 ± 0.986)27	9(11.55 ± 0.366)15
	Abaxial	curved	1.5(1.85 ± 0.053)2	irregular	90(93.75 ± 0.788)100	15(20.65 ± 0.898)26	8(10.5 ± 0.366)14
S. dinklagei	Adaxial	straight	2(2.65 ± 0.109)3	tetra to polygonal	148(153.7 ± 0.747)158	6(11.86 ± 0.772)17	5(7.55 ± 0.373)10
	Abaxial	curved	1(1.2 ± 0.056)1.5	tetra to polygonal	175(184.0 ± 1.079)190	10(17.90 ± 1.066)27	4(6.950 ± 0.450)10
S. floribunda	Adaxial	straight	2(3.05 ± 0.170)4	polygonal	55(57.5 ± 0.336)60	12(30.60 ± 2.391)45	9(14.35 ± 1.064)32
	Abaxial	straight	1(1.20 ± 0.056)1.5	polygonal	96(101.65 ± 0.662)106	15(20.45 ± 0.860)31	7(13.55 ± 0.999)20
S. gossweileri	Adaxial	straight	1(1.20 ± 0.056)1.5	tetra to polygonal	100(103.85 ± 0.595)108	14(19.10 ± 0.718)25	5(8.15 ± 0.379)11
	Abaxial	curved	1(1.20 ± 0.056)1.5	irregular	100(103.85 ± 0.595)108	14(19.10 ± 0.718)25	5(8.15 ± 0.379)11
S. icaja	Adaxial	straight	2(2.575 ± 0.098)3	polygonal	55(56.75 ± 0.239)58	15(23.7 ± 1.081)30	6(9.75 ± 0.547)15
	Abaxial	curved	2(2.575 ± 0.098)3	irregular	63(65.6 ± 0.358)68	15(26.05 ± 1.677)38	6(9.75 ± 0.547)15
S. innocua	Adaxial	straight	2(2.575 ± 0.098)3	polygonal	63(68.2 ± 0.451)71	15(23.70 ± 1.081)30	6(9.75 ± 0.547)15
	Abaxial	straight	1(1.70 ± 0.067)2	polygonal	110(115.45 ± 0.713)120	10(18.40 ± 1.350)27	5(9.15 ± 0.608)14
S. johnsonii	Adaxial	straight	2(2.575 ± 0.098)3	polygonal	98(101.70 ± 0.534)106	12(18.35 ± 0.841)24	6(9.95 ± 0.500)14
	Abaxial	straight	2(2.575 ± 0.098)3	polygonal	98(101.70 ± 0.534)106	12(18.35 ± 0.841)24	6(9.95 ± 0.500)14
S. longicaudata	Adaxial	curved	1.5(1.90 ± 0.046)2	irregular	160(163.75 ± 0.542)168	10(16.30 ± 0.932)23	5(6.60 ± 0.245)8
	Abaxial	curved	1.2(1.89 ± 0.054)2	irregular	160(163.75 ± 0.542)168	10(16.15 ± 0.930)23	5(6.60 ± 0.245)8
S. lucens	Adaxial	curved	1(1.20 ± 0.056)1.5	polygonal	165(169.30 ± 0.442)173	10(14.90 ± 0.684)20	5(8.20 ± 0.462)12
	Abaxial	straight	1(1.20 ± 0.056)1.5	penta to polygonal	120(125.20 ± 0.698)130	11(20.50 ± 1.146)30	4(9.90 ± 0.811)15
S. malacoclados	Adaxial	straight	1(1.23 ± 0.057)1.5	polygonal	95(97.50 ± 0.438)100	13(19.80 ± 1.012)27	8(11.35 ± 0.488)15
	Abaxial	straight	1(1.23 ± 0.057)1.5	irregular	75(77.70 ± 0.448)80	16(26.55 ± 1.468)35	10(12.90 ± 0.369)15
S. memecyloides	Adaxial	straight	1(1.23 ± 0.057)1.5	polygonal	105(108.95 ± 0.505)112	12(18.20 ± 0.893)25	7(10.65 ± 0.493)14
	Abaxial	curved	1(1.23 ± 0.057)1.5	polygonal	64(66.05 ± 0.312)68	15(30.90 ± 1.633)44	10(13.45 ± 0.478)18

Table 6A: Micromorphological characters of the species of Loganiaceae cotinued

Plant Name	**Leaf surface**	**cell wall pattern**	**Cell wall thickness (µm). min.(mean ± s.e.) max**	**Epidermal Cell shape**	**Epidermal Cell number *min. (mean ± s.e.) max***	**Epidermal Cell length min. (mean ± s.e.) max**	**Epidermal cell width min. (mean ± s.e.) max**
S. nigritana	Adaxial	straight	1(1.23 ± 0.057)1.5	tetra to polygonal	186(194.30 ± 1.073)200	10(16.35 ± 0.769)21	6(9.35 ± 0.412)12
	Abaxial	curved	1(1.23 ± 0.057)1.5	irregular	104(108.80 ± 0.647)115	10(23.60 ± 1.346)30	8(9.90 ± 0.355)13
S. nux-vomica	Adaxial	straight	2(2.50 ± 0.115)3	polygonal	98(101.20 ± 0.474)105	12(20.60 ± 1.012)27	10(14.05 ± 0.510)18
	Abaxial	straight	1(1.23 ± 0.057)1.5	polygonal	135(145.50 ± 1.082)150	4(11.45 ± 1.232)24	3(6.20 ± 0.506)10
S. phaeotricha.	Adaxial	undulate	1(1.63 ± 0.095)2	irregular	160(166.00 ± 0.703)170	8(13.55 ± 0.806)20	4(7.50 ± 0.521)12
	Abaxial	wavy	.5(0.825 ± 0.055)1	irregular	134(139.65 ± 0.916)147	7(13.85 ± 1.155)26	4(6.45 ± 0.394)9
S. soubrensis	Adaxial	straight	1.5(1.78 ± 0.057)2	polygonal	105(110.05 ± 0.647)115	10(20.60 ± 1.362)28	8(10.05 ± 0.3276)12
	Abaxial	curved	.5(0.78 ± 0.057)1	polygonal	175(180.40 ± 0.779)185	9(15.60 ± 1.047)24	5(7.25 ± 0.362)10
S. spinosa	Adaxial	straight	1(1.23 ± 0.057)1.5	polygonal	70(73.20 ± 0.579)78	10(17.85 ± 1.115)30	8(11.40 ± 0.499)16
	Abaxial	straight	1(1.23 ± 0.057)1.5	polygonal	110(115.00 ± 0.785)120	8(12.85 ± 0.734)18	4(7.20 ± 0.433)10
S. splendens	Adaxial	straight	1(1.23 ± 0.057)1.5	polygonal	130(135.40 ± 0.741)140	10(16.25 ± 0.729)21	5(7.25 ± 0.315)9
	Abaxial	curved	1(1.23 ± 0.057)1.5	polygonal	130(135.40 ± 0.741)140	10(16.25 ± 0.729)21	5(7.25 ± 0.315)9
S.staudtii	Adaxial	straight	1(1.23 ± 0.057)1.5	polygonal	198(205.65 ± 1.118)214	9(16.00 ± 0.798)21	6(9.00 ± 0.308)11
	Abaxial	curved	1(1.23 ± 0.057)1.5	irregular	130(135.10 ± 0.736)140	8(14.50 ± 0.803)20	5(7.95 ± 0.312)10
S. talbotiae	Adaxial	curved	1(1.20 ± 0.056)1.5	polygonal	165(169.30 ± 0.442)173	10(14.90 ± 0.684)20	5(8.20 ± 0.462)12
	Abaxial	curved	1(1.23 ± 0.057)1.5	polygonal	124(126.70 ± 0.442)130	12(17.60 ± 0.783)22	4(6.55 ± 0.467)10
S. tricalysioides	Adaxial	straight	1(1.23 ± 0.057)1.5	polygonal	198(205.65 ± 1.118)214	9(16.00 ± 0.798)21	6(9.00 ± 0.308)11
	Abaxial	straight	1(1.23 ± 0.057)1.5	irregular	130(135.10 ± 0.736)140	8(14.50 ± 0.803)20	5(7.95 ± 0.312)10
S. urceolata	Adaxial	straight	1(1.23 ± 0.057)1.5	polygonal	84(85.75 ± 0.307)88	13(18.10 ± 0.580)21	10(14.05 ± 0.505)18
	Abaxial	straight	1(1.23 ± 0.057)1.5	polygonal	108(110.05 ± 0.320)112	15(23.20 ± 1.065)30	8(11.20 ± 0.501)15
S.usambarensis	Adaxial	straight	1.5(1.98 ± 0.077)2.5	polygonal	168(172.75 ± 0.615)178	10(17.75 ± 0.909)23	4(7.95 ± 0.564)12
	Abaxial	straight	1.5(1.98 ± 0.077)2.5	polygonal	126(129.35 ± 0.443)132	7(14.55 ± 0.928)21	4(7.55 ± 0.450)10

Table 6A: Micromorphological characters of the species of Loganiaceae cotinued

Plant Name	**Leaf surface**	**cell wall pattern**	**Cell wall thickness (µm). min.(mean ± s.e.) max**	**Epidermal Cell shape**	**Epidermal Cell number *min. (mean ± s.e.) max***	**Epidermal Cell length min. (mean ± s.e.) max**	**Epidermal cell width min. (mean ± s.e.) max**
S. chrysophylla	Adaxial	straight	1(1.15 ± 0.053)1.5	polygonal	155(161.00 ± 0.665)165	10(16.20 ± 0.643)20	5(10.00 ± 0.729)15
	Abaxial	curved	1(1.15 ± 0.053)1.5	irregular	85(88.45 ± 0.495)92	10(22.80 ± 1.472)32	5(9.40 ± 0.655)15
S. indeterminate Edondon -2	Adaxial	straight	1(1.25 ± 0.057)1.5	polygonal	240(255.65 ± 1.813)270	7(11.6 ± 0.816)20	6(8.30 ± 0.317)10
	Abaxial	straight	1(1.25 ± 0.057)1.5	polygonal	240(264.35 ± 2.683)280	12(21.35 ± 1.16)28	5(11.25 ± 0.707)17
S. indeterminate Edondon -3	Adaxial	straight	1(1.25 ± 0.057)1.5	polygonal	240(255.65 ± 1.813)270	7(11.60 ± 0.816)20	6(8.3 ± 0.317)10
	Abaxial	straight	1(1.23 ± 0.057)1.5	polygonal	240(264.35 ± 2.683)280	12(21.35 ± 1.16)28	5(11.25 ± 0.707)17
S. indeterminate Edondon -4	Adaxial	undulate	1.5(1.8 ± 0.056)2	polygonal	150(155.95 ± 0.737)160	8(15.9 ± 0.959)20	5(9.75 ± 0.593)14
	Abaxial	curved	1(1.225 ± 0.057)1.5	polygonal	152(157.2 ± 0.627)162	10(20.55 ± 1.255)28	6(9.75 ± 0.491)13
S. indeterminate Ipetu-Ijesha	Adaxial	straight	1.5(1.8 ± 0.056)2	penta to polygonal	200(213.10 ± 1.306)220	7(15.05 ± 1.123)22	4(8.80 ± 0.555)14
	Abaxial	curved	1.5(1.85 ± 0.0526)2	polygonal to irregular	180(192.05 ± 1.639)205	9(18.65 ± 1.127)26	4(7.25 ± 0.464)11
S. indeterminate J4 -3	Adaxial	straight	2(2.575 ± 0.0908)3	polygonal	128(132.65 ± 0.646)138	12(19.2 ± 0.869)25	6(9.55 ± 0.535)14
	Abaxial	curved	1(1.25 ± 0.057)1.5	polygonal	246(251 ± 0.684)257	7(15.25 ± 1.279)25	4(6.3 ± 0.411)9
S. indeterminate Erokut station -6	Adaxial	curved	2(2.50 ± 0.096)3	polygonal	170(175.3 ± 0.788)180	9(14.25 ± 0.882)20	5(9.00 ± 0.637)15
	Abaxial	curved	1(1.25 ± 0.057)1.5	polygonal to irregular	90(94.25 ± 0.688)100	12(19.65 ± 1.212)30	4(8.050 ± 0.573)13
S. indeterminate Edondon -6	Adaxial	curved	1(1.875 ± 0.0497)2	polygonal	155(158.35 ± 0.466)162	11(20.25 ± 1.559)30	5(10.10 ± 0.657)15
	Abaxial	straight	1.5(1.775 ± 0.057)2	polygonal to irregular	87(90.1 ± 0.502)94	10(22.50 ± 1.688)38	8(10.45 ± 0.444)14
S. indeterminate ENUGU	Adaxial	straight	1(1.25 ± 0.057)1.5	polygonal	195(200.2 ± 0.742)205	8(11.75 ± 0.486)315	5(7.95 ± 0.359)11
	Abaxial	curved	1(1.25 ± 0.057)1.6	polygonal to irregular	152(157.85 ± 0.654)162	10(17.45 ± 0.869)23	5(8.65 ± 0.509)12
Usteria guineensis	Adaxial	straight	2(3.08 ± 0.116)4	polygonal	48(49.80 ± 0.287)52	20(32.55 ± 1.354)40	14(18.60 ± 0.655)23
	Abaxial	straight	1.5(1.88 ± 0.050)2	polygonal	40(41.50 ± 0.286)44	14(33.25 ± 1.727)45	13(17.30 ± 0.567)22
Fagrae fragrans	Adaxial	straight	1(1.23 ± 0.057)1.5	polygonal	100(104.35 ± 0.737)110	10(18.10 ± 1.220)25	8(10.45 ± 0.387)13
	Abaxial	curved	1.5(1.78 ± 0.057)2	polygonal	65(68.65 ± 0.488)72	10(20.60 ± 1.473)35	8(10.50 ± 0.500)15

Table 6B: Stomata distribution in Loganiaceae

Plant Name	**Leaf surface**	**Stomata length (µm)** ***min (mean ± s.e) max***	**Stomata width (µm)** ***min (mean ± s.e) max***	**Stomata number** ***min (mean ± s.e) max***	**Stomata type (subsidiary cell)**	**Stomata index %**
Anthocleista djalonensis	Adaxial	3(4.95 ± 0.185) 6	1(1.63 ± 0.10)2	2(2.85 ± 0.17) 4	Anisocytic	1.67
	Abaxial	9 (10 ± 0.14) 11	2 (2.4 ± 0.11) 3	3 (3.65 ± 0.18) 5	Anisocytic	2.10
A. liebrechtsiana	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	5 (7.50 ± 0.44)12	0.5 (1.28 ± 0.11)2	20(22.45 ± 0.37)25	Anisocytic	6.17
A. microphyla	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	3 (4.10 ± 0.33)5	1(1.63 ± 0.087)2	7 (8.15 ± 0.166)9	Anisocytic	10.40
A. nobilis	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	7(8.11± 0.19)9	1(1.5 ± 0.093)2	27 (29.62± 0.349)32	Anisocytic	9.11
A. obanensis	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	4(4.95 ± 0.169)6	2 (2.45 ± 0.11) 3	15 (16.05 ± 0.044) 17	Anisocytic	16.46
A. procera	Adaxial	5(7.35 ± 0.365)10	2 (2.87 ± 0.125)3.5	1 (2.45 ± 0.104) 5	Anisocytic	1.13
	Abaxial	5(6.6 ± 0.21)8	1 (1.85 ± 0.167)3	15(17.3 ± 0.17)19	Anisocytic	5.45
A. scandens	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	7 (7.45 ± 0.114)8	1 (2.0 ± 0.145)3	14 (15.45 ± 0.15)16	Staurocytic	10.08
A. schweinfurthii	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	6 (8.7 ± 0.309)10	1 (1.45 ± 0.114)2	16 (1.13 ± 0.050)17	Staurocytic	0.43
A. vogelli	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	7(8.6 ± 0.234)10	2(2.5 ± 0.102)2	7(7.5 ± 0.115)8	Anomocytic	15.67
M. brunonis	Adaxial	Absent	Absent	Absent	Absent	0
	Abaxial	4(5.0 ± 0.178)6	2(2.0 ± 0.0)2	5(5.9 ± 0.161)7	Anomocytic	12.05
M. hirsuta	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	3(3.6 ± 0.112)4	1(1.7 ± 0.105)2	3(4.7 ± 0.252)6	Anisocytic	5.77
Nuxia congesta	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	5(5.900 ± 0.19)7	1(1.75 ± 0.0769)2	25(27.05 ± 0.438)30	Anomocytic	8.30
Spigelia anthelmia	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	13(14.10 ± 0.161)15	3(3.60 ± 1.112)4	3(3.80 ± 0.1556)5	Anisocytic & anomocytic	17.04

Table 6B: Stomata Distribution in Loganiaceae Continued

Plant Name	**Leaf surface**	**Stomata length (µm)** ***min (mean ± s.e) max***	**Stomata width (µm)** ***min (mean ± s.e) max***	**Stomata number** ***min (mean ± s.e) max***	**Stomata type (subsidiary cell)**	**Stomata index %**
Strychnos aculeata	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	12(13.10 ± 0.176)14	3(3.55 ± 0.114)4	13(13.9 ± 0.161)15	Paracytic	10.26
S. afzeli	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	4(4.45 ± 0.114)5	1(1.53 ± 0.106)2	27(28.55 ± 0.246)30	Paracytic	10.08
S. astheranta	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	4(2.350 ± 0.109)3	1(1± 0.0)1	37(39.7 ± 0.300)42	Paracytic	9.14
S. barteri	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	6(7.65 ± 1.039)9	2(2.40 ± 0.503)3	4(5.35 ± 0.6708)6	Paracytic	5.19
S. boonei	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	5(5.35 ± 0.109)6	1.5(1.78 ± 0.057)2	11(12.35 ± 0.150)13	Paracytic	8.88
S. campicola	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	3(3.45 ± 0.114)4	1(1.775 ± 0.068)2	18(19.3 ± 0.179)20	Anomocytic	15.61
S. camptoneura.	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	3(3.7 ± 0.179)5	1(1.95 ± 0.102)3	9(9.600 ± 0.1123)10	Paracytic	9.70
S. chromatoxylon	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	4(4.85 ± 0.167)6	1(1.53 ± 0.092)2	8(9.4 ± 0.152)10	Anomocytic	8.72
S. congolana	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	7(8.90 ± 0.204)10	1.5(1.825 ± 0.055)2	14(15.1 ± 0.161)16	Paracytic	16.50
S. cuminodora	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	4(4.55 ± 0.114)5	2(2.60 ± 0.112)3	9(9.5 ± 0.115)10	Paracytic	3.68
S. densiflora	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	9(9.7 ± 0.164)11	1.5(1.85 ± 0.055)2	5(5.6 ± 0.112)6	Paracytic	5.64
S. dinklagei	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	4(5.00 ± 0.145)6	1(1.20 ± 0.056)1.5	9(9.500 ± 0.115)10	Anomocytic	4.91
S. floribunda	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	8(9.1 ± 0.176)10	2(2.45 ± 0.114)3	9(10.85 ± 0.310)13	Paracytic	9.64

Table 6B: Stomata Distribution in Loganiaceae Continued

Plant Name	**Leaf surface**	**Stomata length (µm)** ***min (mean ± s.e) max***	**Stomata width (µm)** ***min (mean ± s.e) max***	**Stomata number** ***min (mean ± s.e) max***	**Stomata type (subsidiary cell)**	**Stomata index %**
S. gossweileri	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	4(4.40 ± 0.112)5	2(2.55 ± 0.114)3	18(19.55 ± 0.170)21	Paracytic	15.84
S. icaja	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	6(7.15 ± 0.167)8	2(2.55 ± 0.114)3	8(9.10 ± 0.176)10	Paracytic	12.18
S. innocua	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	5(6.80 ± 0.236)8	2(2.55 ± 0.114)3	11(16.25 ± 0.615)20	Paracytic	12.34
S. johnsonii	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	4(5.25 ± 0.160)6	1(1.25 ± 0.057)1.5	9(10.00 ± 0.162)11	Paracytic	8.95
S. longicaudata	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	3(3.55 ± 0.114)4	1.5(2.00 ± 0.063)2.5	10(12.15 ± 0.357)15	Anomocytic	6.91
S. lucens	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	8(9.10 ± 0.191)10	2(2.40 ± 0.112)3	12(143.60 ± 89.802)15	Paracytic	53.42
S. malacoclados	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	4(5.15 ± 0.167)6	1(1.55 ± 0.088)2	10(12.25 ± 0.307)14	Para to anomocytic	13.62
S. memecyloides	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	5(6.20 ± 0.247)8	2(2.50 ± 0.115)3	10(11.95 ± 0.285)14	Para to anomocytic	15.32
S. nigritana	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	4(5.00 ± 0.162)6	1(1.48 ± 0.099)2	12(13.10 ± 0.191)14	Paracytic	10.75
S. nux-vomica	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	5(5.70 ± 0.179)7	2(2.60 ± 0.112)3	20(22.05 ± 0.312)24	Paracytic	13.16
S. phaeotricha.	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	5(5.95 ± 0.185)7	1(1.25 ± 0.057)1.5	11(13.25 ± 0.331)16	Anomocytic	8.67

Table 6B: Stomata Distribution in Loganiaceae Continued

Plant Name	**Leaf surface**	**Stomata length (µm) *min (mean ± s.e) max***	**Stomata width (µm) *min (mean ± s.e) max***	**Stomata number *min (mean ± s.e) max***	**Stomata type (subsidiary cell)**	**Stomata index %**
S. soubrensis	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	3(3.70 ± 0.128)5	1(1.50 ± 0.096)2	9(10.45 ± 0.256)12	Paracytic	5.48
S. spinosa	Adaxial	5(7.75 ± 0.298)10	1.5(2.10 ± 0.112)3	5(8.80 ± 0.408)11	Anomocytic	0.00
	Abaxial	5(7.75 ± 0.298)10	1.5(2.10 ± 0.112)3	5(8.80 ± 0.408)11	Anomocytic	6.96
S. splendens	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	3(4.10 ± 0.191)5	1.5(1.83 ± 0.055)2	8(9.75 ± 0.216)11	Paracytic	6.72
S.staudtii	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	2(2.13 ± 0.050)2.5	1(1.00 ± 0.000)1	95(98.30 ± 0.534)102	Anomocytic	42.12
S. talbotiae	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	5(5.90 ± 0.191)7	3(3.45 ± 0.114)4	12(14.45 ± 0.286)16	Paracytic	10.24
S. tricalysioides	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	2(2.13 ± 0.050)2.5	1(1.00 ± 0.000)1	95(98.30 ± 0.534)102	Paracytic	39.98
S. urceolata	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	7(9.60 ± 0.328)12	3(4.40 ± 0.169)5	7(14.65 ± 5.505)119	Anomocytic	11.75
S.usambarensis	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	5(5.55 ± 0.114)6	2(2.53 ± 0.099)3	18(20.00 ± 0.308)22	Anomocytic	13.39
S.chrysophylla	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	5(6.80 ± 0.247)8	2(2.53 ± 0.099)3	9(11.00 ± 0.299)13	Paracytic	11.06
S. indeterminate Edondon -2	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	5(6.80 ± 0.247)	1.5(1.825 ± 0.0547)2	24(29.90 ± 0.992)37	Paracytic	10.16
S. indeterminate Edondon -3	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	5(6.80 ± 0.247)	1.5(1.825 ± 0.0547)2	24(29.90 ± 0.992)37	Paracytic	10.20

Table 6B: Stomata distribution in Loganiaceae continued

Plant Name	**Leaf surface**	**Stomata length (µm) *min (mean ± s.e) max***	**Stomata width (µm) *min (mean ± s.e) max***	**Stomata number *min (mean ± s.e) max***	**Stomata type (subsidiary cell)**	**Stomata index %**
S. indeterminate Edondon -4	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	5(5.85 ± 0.167)7	2(2.45 ± 0.114)3	13(16.7 ± 0.534)20	Paracytic	9.60
S. indeterminate Ipetu-Ijesha	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	3(3.55 ± 0.114)4	2(2.5 ± 0.115)3	20(23.05 ± 0.48)26	Paracytic	10.72
S. indeterminate J4 -3	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	4(4.95 ± 0.170)6	1(1.675 ± 0.0908)2	15(17.45 ± 0.387)20	Paracytic	6.50
S. indeterminate Erokut station -6	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	5(5.90 ± 0.191)7	2(2.375 ± 0.095)3	5(10.900 ± 0.652)16	Paracytic	10.37
S. indeterminate Edondon -6	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	5(6.35 ± 0.274)8	2(2.60 ± 0.10)3	8(11.00 ± 0.0.340)14	Paracytic	10.88
S. indeterminate ENUGU	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	4(4.65 ± 0.109)5	3(4.15 ± 0.182)5	17(19.45 ± 0.352)22	Paracytic	10.97
Usteria guineensis	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	6(7.75 ± 0.216)9	1.5(2.15 ± 0.121)3	9(10.15 ± 0.244)12	Anisocytic	19.65
Fagrae fragrans	Adaxial	Absent	Absent	Absent	Absent	0.00
	Abaxial	5(8.20 ± 0.474)12	2(3.25 ± 0.204)5	12(13.80 ± 0.313)16	Anomocytic	16.74

Table 6C: Trichome distribution in Loganiaceae

Plant Name	Leaf surface	Trichome type	Trichome length (µm) min (mean ± s.e) max	Mean Trichome density
Anthocleista djalonensis	Adaxial	stellate & dendritic	75(106.25 ± 4.65)140	6
	Abaxial	stellate & dendritic	75(106.25 ± 4.65)140	6
A. microphyla	Abaxial	stellate & dendritic	62(104.2 ± 4.37)150	3
A. nobilis	Abaxial	stellate & dendritic	50(100.0 ± 5.21)143	13
A. obanensis	Adaxial	stellate & dendritic	47(97.7 ± 6.84)144	2
	Abaxial	stellate & dendritic	47(97.7 ± 6.84)144	6
A. vogelli	Adaxial	stellate & dendritic	73(201.7 ± 18.76)352	6
	Abaxial	stellate & dendritic	73(201.7 ± 18.76)352	6
M. brunonis	Adaxial	simple unicellular	60(110.8 ± 7.649)172	3
M. hirsuta	Adaxial	simple unicellular	100(301.2 ± 27.96)512	1
	Abaxial	simple unicellular	100(301.2 ± 27.96)512	2
Nuxia congesta	Adaxial	Peltate trichome	28(50.5 ± 2.11)76	3
	Abaxial	Peltate trichome	28(50.5 ± 2.11)76	3
Spigelia anthelmia	Abaxial	conical	43(58.5 ± 2.29)80	3
S. afzeli	Adaxial	simple unicellular	56(101.25 ± 6.966)150	2
	Abaxial	simple unicellular	56(101.25 ± 6.966)150	1
S. boonei	Abaxial	simple unicellular	43(58.5 ± 2.29)80	3
S. campicola	Abaxial	simple unicellular	100(189.8 ± 11.84)270	3
S. icaja	Abaxial	simple unicellular	90(114.35 ± 3.007)130	4
S. longicaudata	Adaxial	simple unicellular	24(39.25 ± 1.851)50	4
S. memecyloides	Abaxial	simple unicellular	40(42.60 ± 0.419)46	5
S. nigritana	Abaxial	simple unicellular	30(82.00 ± 10.655)240	4
S. soubrensis	Abaxial	simple unicellular	25(43.20 ± 2.086)60	2
S. spinosa	Abaxial	simple unicellular	25(48.50 ± 3.134)80	20
S. splendens	Abaxial	simple unicellular	40(42.60 ± 0.419)46	2
S. talbotiae	Abaxial	simple unicellular	7(13.15 ± 0.880)20	3
S.chrysophylla	Abaxial	simple unicellular	5(9.69 ± 0.771)13	1
S. ndengensis	Abaxial	simple unicellular	20(53.4 ± 4.05)75	1
S. indeterminate Edondon -3	Abaxial	simple unicellular	20(53.4 ± 4.05)75	1

Table 6D: Cuticular ornamentation and ergastic substances in Loganiaceae

Plant Name	Leaf surface	cuticular ornamentation	cuticular thickness	Type of Wax	Other ergastic substances
Anthocleista djalonensis	Adaxial	Absent	thin	plate and scales	Absent
	Abaxial	Absent	thin	plate and scales	Absent
A. liebrechtsiana	Adaxial	Absent	thin	soft wax coating	starch grains
	Abaxial	Guard cell striation	thick	soft wax coating	starch grains
A. microphyla	Adaxial	Absent	thin	plate and scales	Absent
	Abaxial	Absent	thin	plate and scales	Absent
A. obanensis	Adaxial	Absent	thin	soft wax coating	Absent
	Abaxial	Absent	thin	soft wax coating	Absent
A. procera	Adaxial	Guard cell striation	thick	soft wax coating	Absent
	Abaxial	Absent	thin	Absent	Absent
A. scandens	Adaxial	Absent	thin	Absent	Absent
	Abaxial	Absent	thin	soft wax coating	Absent
A. schweinfurthii	Adaxial	Absent	thin	Absent	starch deposit
	Abaxial	Guard cell striation	thick	soft wax coating	starch deposit
A. vogelli	Adaxial	radiating striae	thick	plate and scales	Crystals
	Abaxial	radiating striae	thick	plate and scales	Crystals
M. brunonis	Adaxial	Absent	thin	Absent	starch grains
	Abaxial	Absent	thin	Absent	Absent
M. hirsuta	Adaxial	radiating striae	thick	Absent	Absent
	Abaxial	radiating striae	thick	Absent	Absent
Nuxia congesta	Adaxial	Absent	thin	Granules	circular structures
	Abaxial	radiating striae	thick	Absent	circular structures
Spigelia anthelmia	Adaxial	Absent	thin	Absent	Absent
	Abaxial	radiating striae	thick	Absent	Absent
S. boonei	Adaxial	radiating striae	thick	Absent	Absent
	Abaxial	Absent	thin	Absent	starch grains
S. campicola	Adaxial	Absent	thin	Absent	Absent
	Abaxial	Guard cell striation	thick	Absent	Absent
S. camptoneura.	Adaxial	radiating striae	thick	Absent	Absent
	Abaxial	Absent	thin	Absent	starch deposit

Table 6D: Cuticular ornamentation and ergastic substances in Loganiaceae continued

Plant Name	Leaf surface	cuticular ornamentation	cuticular thickness	Type of Wax	Other ergastic substances
S. chromatoxylon	Adaxial	radiating striae	thick	Absent	Absent
	Abaxial	Absent	thin	Absent	Absent
S. congolana	Adaxial	radiating striae	thick	Absent	Absent
	Abaxial	radiating striae	thick	Absent	Absent
S. cuminodora	Adaxial	Absent	thin	soft wax coating	Absent
	Abaxial	Guard cell striation	thick	soft wax coating	Absent
S. densiflora	Adaxial	radiating striae	thick	Absent	Absent
	Abaxial	Absent	thin	soft wax coating	Absent
S. dinklagei	Adaxial	Absent	thin	soft wax coating	Absent
	Abaxial	Absent	thin	soft wax coating	Absent
S. floribunda	Adaxial	Absent	thin	Absent	Absent
	Abaxial	Absent	thin	soft wax coating	Absent
S. gossweileri	Adaxial	Absent	thin	Absent	Absent
	Abaxial	Absent	thin	soft wax coating	Absent
S. icaja	Adaxial	Absent	thin	Absent	Absent
	Abaxial	Guard cell striation	thick	Absent	Absent
S. innocua	Adaxial	radiating striae	thick	soft wax coating	Absent
	Abaxial	radiating striae	thick	soft wax coating	Absent
S. johnsonii	Adaxial	Absent	thin	Absent	Absent
	Abaxial	Guard cell striation	thick	Absent	Absent
S. longicaudata	Adaxial	Absent	thin	Absent	Absent
	Abaxial	Guard cell striation	thick	Absent	Absent
S. lucens	Adaxial	radiating striae	thick	Absent	Absent
	Abaxial	Guard cell striation	thick	Absent	Absent
S. malacoclados	Adaxial	radiating striae	thick	Absent	Absent
	Abaxial	radiating striae	thick	Absent	starch grains
S. nigritana	Adaxial	Absent	thin	Absent	Absent
	Abaxial	Guard cell striation	thick	Absent	Absent
S. nux-vomica	Adaxial	Absent	thin	Absent	Absent
	Abaxial	Guard cell striation	thick	Absent	Absent
S. soubrensis	Adaxial	radiating striae	thick	Absent	Absent
	Abaxial	radiating striae	thick	Absent	Absent
S. spinosa	Adaxial	Guard cell striation	thick	soft wax coating	Crystals
	Abaxial	Guard cell striation	thick	soft wax coating	Absent

Table 6D: Cuticular ornamentation and ergastic substances in Loganiaceae continued

Plant Name	Leaf surface	cuticular ornamentation	cuticular thickness	Type of Wax	Other ergastic substances
S.usambarensis	Adaxial	Absent	thin	Absent	Absent
	Abaxial	Guard cell striation	thick	Absent	Absent
S.chrysophylla	Adaxial	Absent -	thin	Absent	Absent
	Abaxial	Guard cell striation	thick	Absent	Absent
S. ndengensis	Adaxial	radiating striae	thick	Absent	Crystals
	Abaxial	Absent	thin	Absent	Absent
S. indeterminate Edondon -3	Adaxial	radiating striae	thick	Absent	Crystals
	Abaxial	Absent	thin	Absent	Absent
S. indeterminate Erokut station -6	Adaxial	Absent	thin	Absent	Absent
	Abaxial	radiating striae	thick	Absent	Absent
S. indeterminate Edondon -6	Adaxial	radiating striae	thick	Absent	Absent
	Abaxial	radiating striae	thick	Absent	Absent
S. indeterminate ENUGU	Adaxial	Absent	thin	Absent	Absent
	Abaxial	Guard cell striation	thick	soft wax coating	Absent
Usteria guineensis	Adaxial	radiating striae	thick	Absent	Absent
	Abaxial	radiating striae	thick	Absent	Absent

4.3.3 Data Analysis

The dendrogram for miromorphological variations for all adaxial and abaxial epidermal characters was represented by Figure 6A and 6B respectively. By using a threshold of 24 % similarity coefficient, Figure 6B shows a low level similarity among the species. There are four clusters with 2 ungrouped species. The ungrouped species are *Strychnos boonei* and *Anthocleista vogelli*. The dendrogram produced by abaxial data shows that SID55 and SID56 have above 95 % similarity coefficient and adaxial revealed 100 % similarity (Figure 6A) for the two species. The two species are therefore very close as revealed by this study.

The quantitative anatomical characters were analysed by Principal Component Analysis (PCA) which revealed four components contributing about 75 % to the delimitation of Loganiaceae (Table 7A). Table 7B further showed that epidermal cells dimension, stomata number and stomata indices are most useful to delineate Loganiaceae species in plant populations; they have the highest values. Figure 6E represents the Eigen value plot which shows the relevant components from the PCA while figures 6F is the scatter plots from the first two principal components. The plot revealed that samples 32, 41 and 43 did not group with others using the principal components generated from PCA. The species are: *S. nux-vomica, S.dinklagei* and *S. memecyloides*. Dendrogram for combined micro morphological characters revealed 9 clusters and one ungrouped species (Figure 6C). The dedrogram generated did not really resolve the genera into any picture of taxonomic interest. For example, cluster 9 grouped about four genera together even at a very low similarity coefficient of about 20 % on the dendrogram (Figure 6C). Most clusters generated nested genera together that were in no way depicting dissimilarity among the species of the family.

Moreover, both macro morphological and micro morphological evidences were combined as the gross morphological evidence to observe the degree of resolution possible for the family (Figure 6D). Interestingly, gross morphology was able to delineate the genera in the family to clusters meaningful to the familys taxonomy. Five clusters and two outliers or ungrouped were recovered at similarity coefficient of 33.5 %. *Anthocleista* genus formed the first 2 clusters and *Strychnos* occupied two separate clusters – clusters 3 and 4. *Usteria guineensis* was nested together with *Strychnos* in cluster 3 and others are *Strychnos* species showing a high coefficient of similarity with the two genera. *Mostuea* and *spigelia* were separated into a different cluster with little affinity to the other genera in the family.

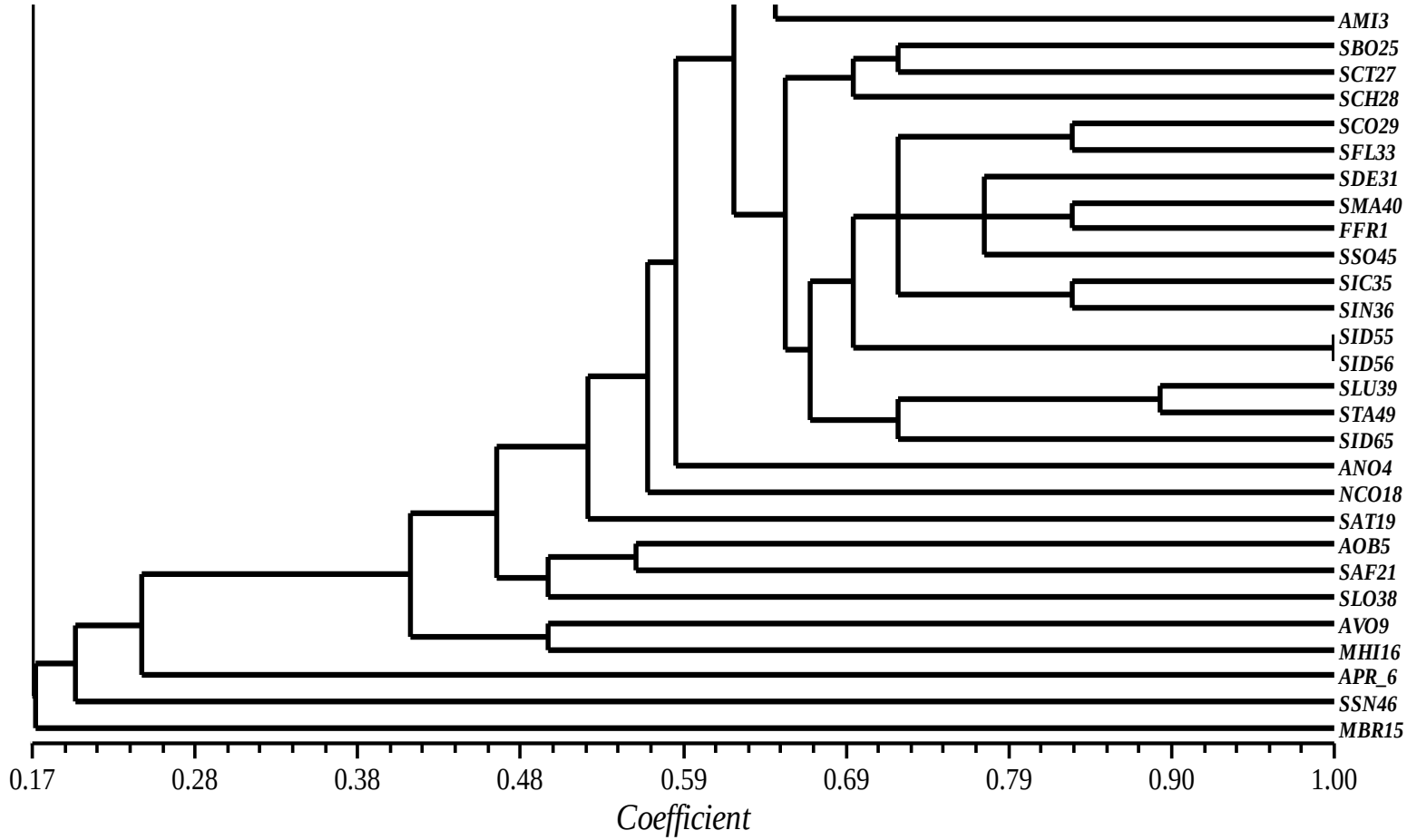

Figure 6A: Dendrogram showing relationship within Loganiaceae based on adaxial micro-morphological data.

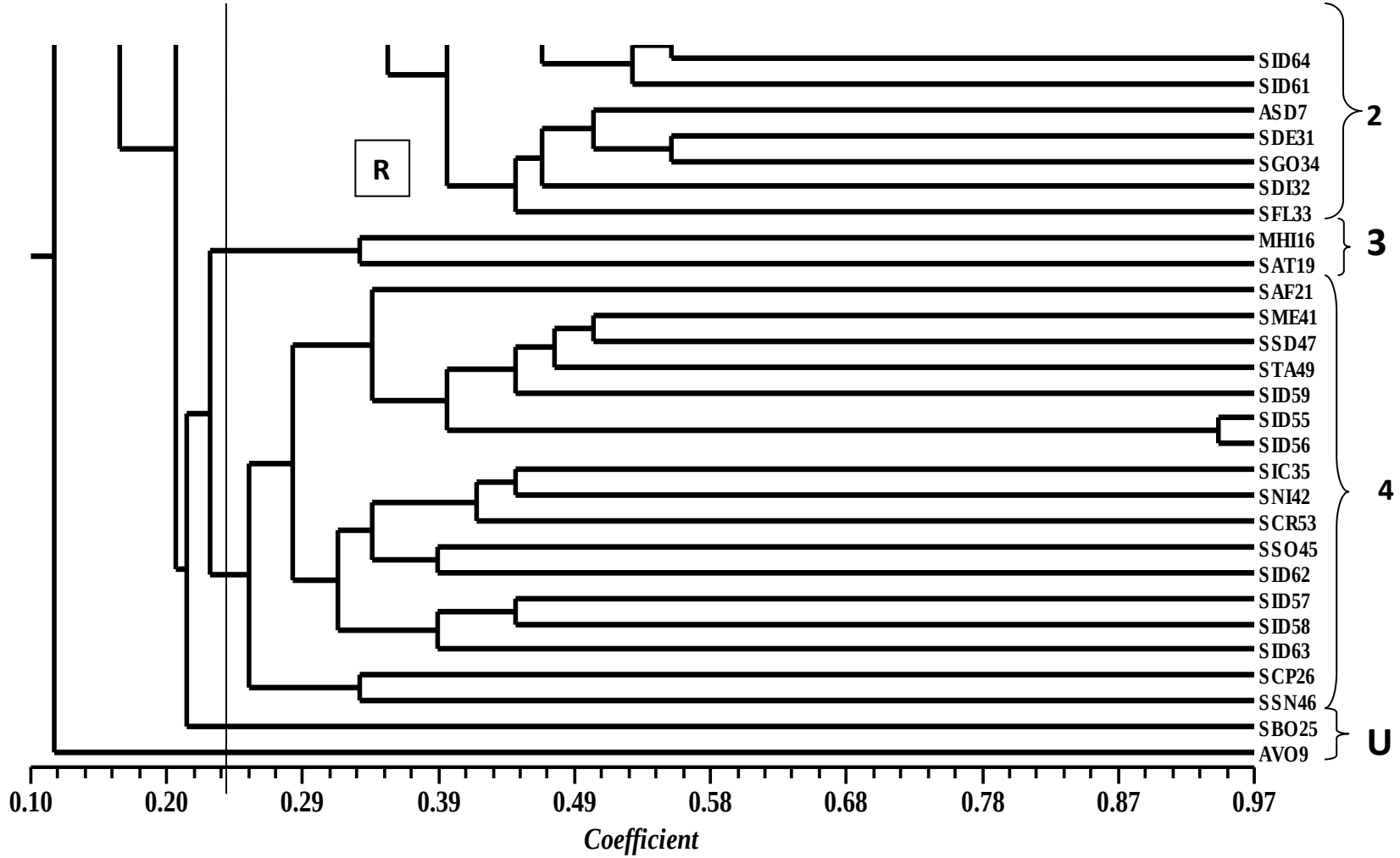

Figure 6B: Dendrogram showing relationship within Loganiaceae based on abaxial micromorphological data where U = ungrouped and R = reference line at a threshold of 24 % similarity coefficient.

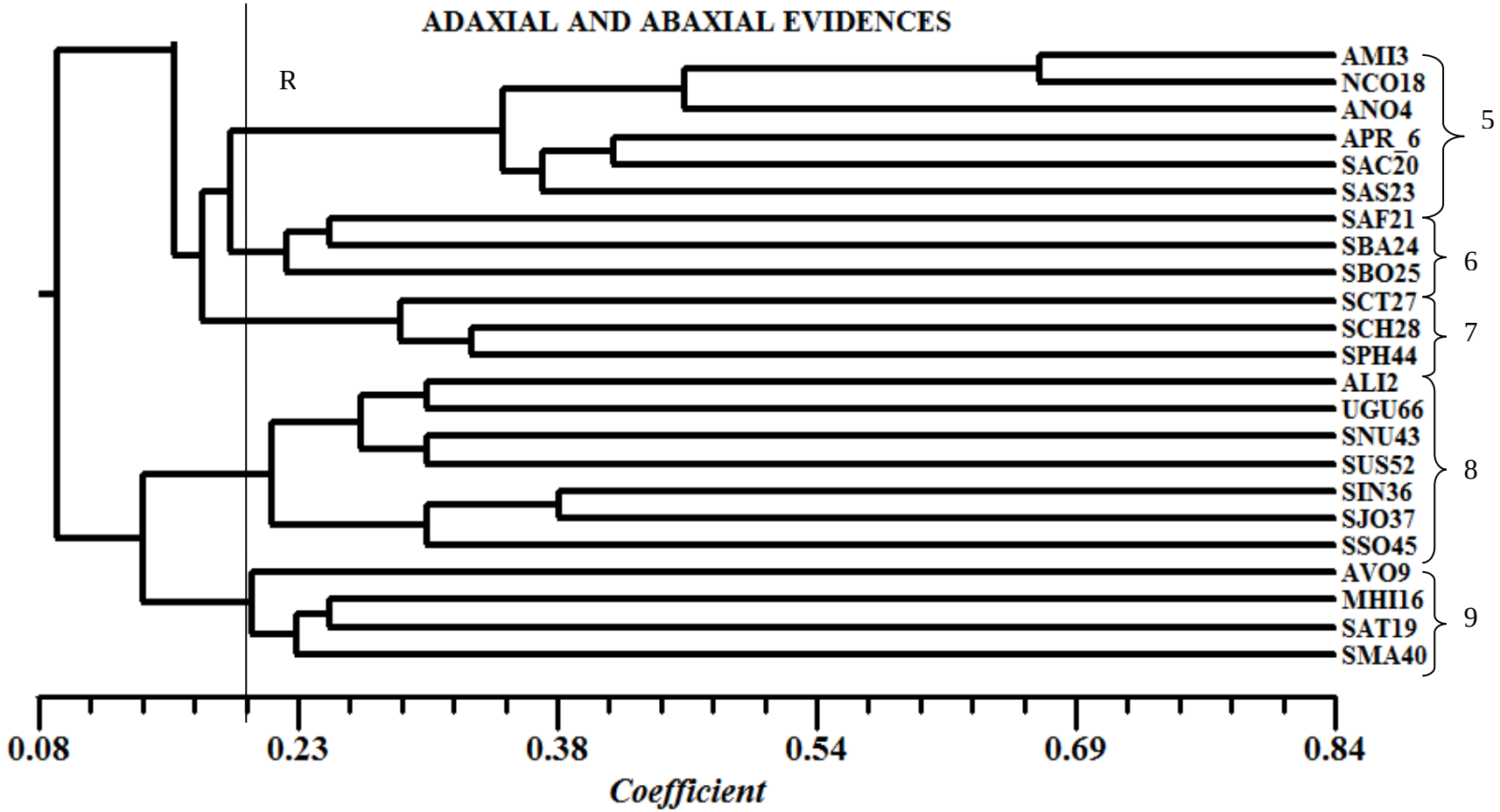

Figure 6C: Dendrogram showing relationship within Loganiaceae based on both adaxial and abaxial anatomical data where U = ungrouped and R = reference line at a threshold of 20 % similarity coefficient.

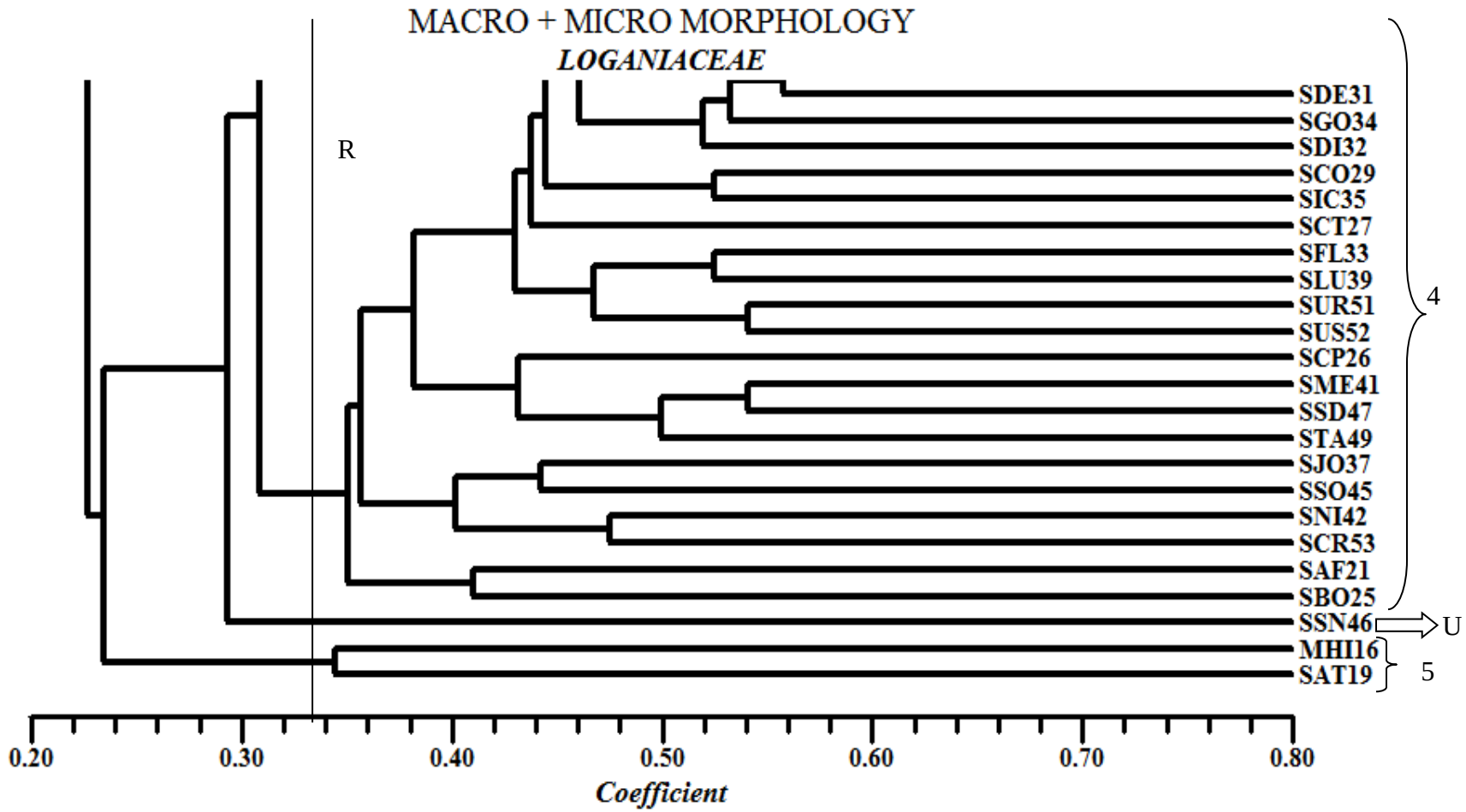

Figure 6D: Dendrogram showing relationship within Loganiaceae based on Macro and Micro (gross) morphological data where U = ungrouped and R = reference line at a threshold of 33.5 % similarity coefficient.

Table 7A: PCA of Loganiaceae micro-morphology showing the Eigenvalues and Major Four Components Contributing about 75%

Total Variance Explained									
Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	2.967	29.674	29.674	2.967	29.674	29.674	2.521	25.210	25.210
2	1.969	19.694	49.367	1.969	19.694	49.367	1.933	19.331	44.541
3	1.338	13.384	62.751	1.338	13.384	62.751	1.654	16.538	61.079
4	1.231	12.306	75.057	1.231	12.306	75.057	1.398	13.978	75.057
5	.954	9.536	84.593						
6	.587	5.869	90.462						
7	.543	5.430	95.892						
8	.307	3.068	98.961						
9	.077	.771	99.731						
10	.027	.269	100.000						

Table 7B: PCA of Loganiaceae micromorphology Showing Communalities and Component Matrix

Communalities			**Components Matrix**			
	Initial	Extraction	1	2	3	4
Cell wall thickness	1.000	.269	.330	-.162	-.133	-.342
Epidermal cell Number	1.000	.695	-.727	-.220	-.175	.294
Epidermal cell length	1.000	.880	.892	.188	.142	-.170
Epidermal cell width	1.000	.756	.805	.258	.110	-.171
Stomata length	1.000	.824	.543	-.048	-.495	.530
Stomata width	1.000	.727	.573	.019	-.503	.382
Stomata number	1.000	.955	-.386	.867	.068	.223
Stomata index	1.000	.953	-.022	.962	.094	.133
Trichome length	1.000	.697	.260	-.218	.725	.237
Trichome density	1.000	.750	.211	-.257	.470	.647

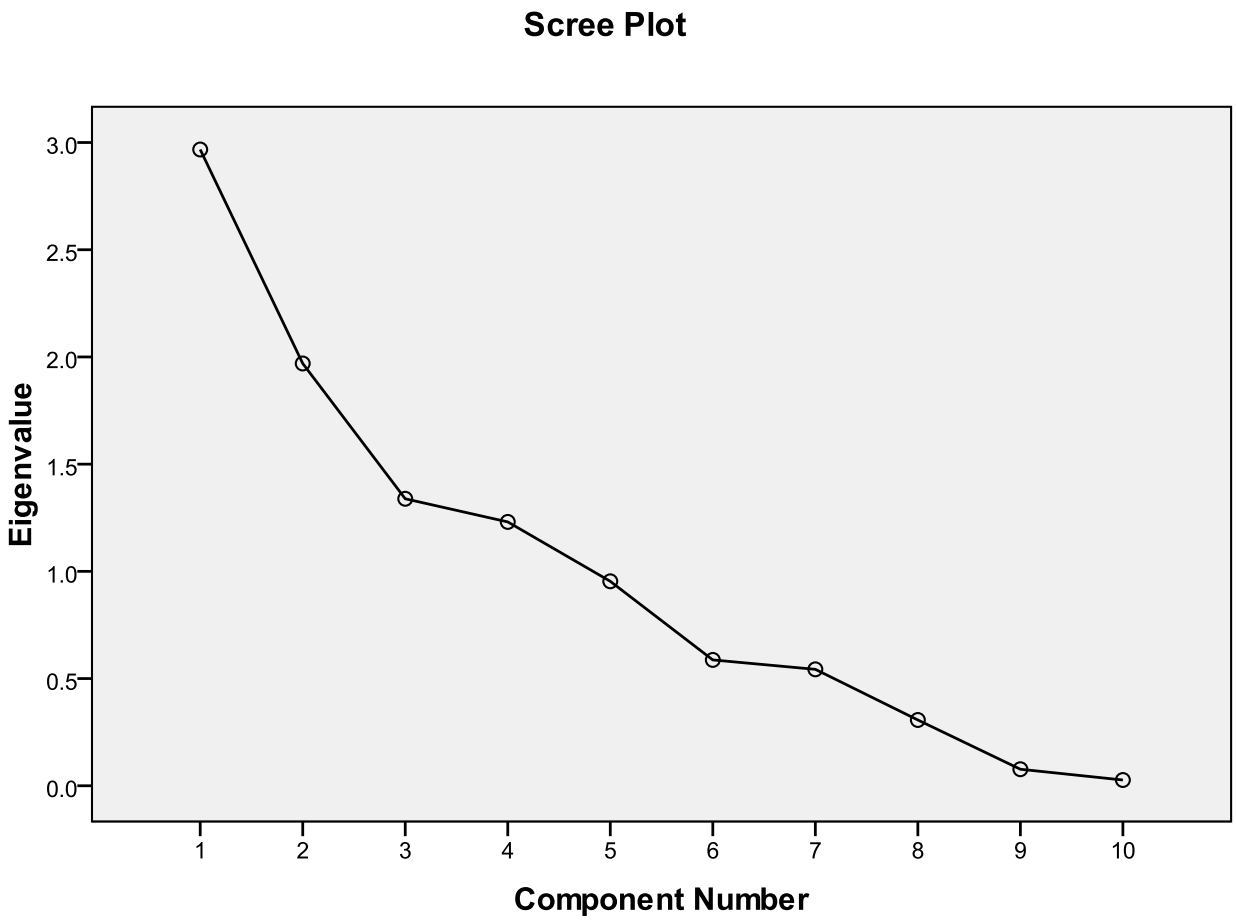

Figure 6E: PCA Scree Plot of Eigen values Loganiaceae micro-morphological showing the four major components high on the scale.

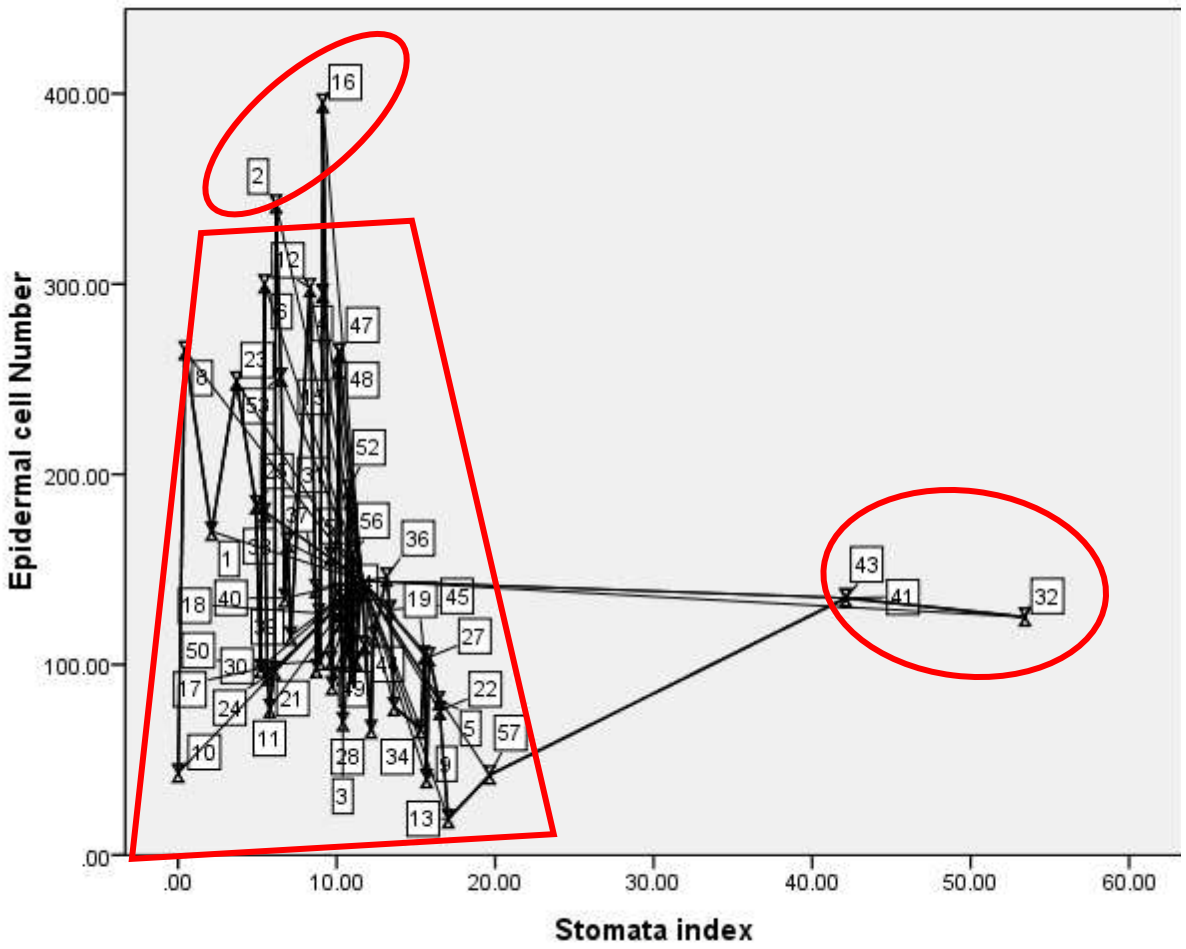

Figure 6F: Scatter plot of epidermal cell number versus Stomata index from first component obtained from PCA (group centroids) showing the distribution of species in Loganiaceae

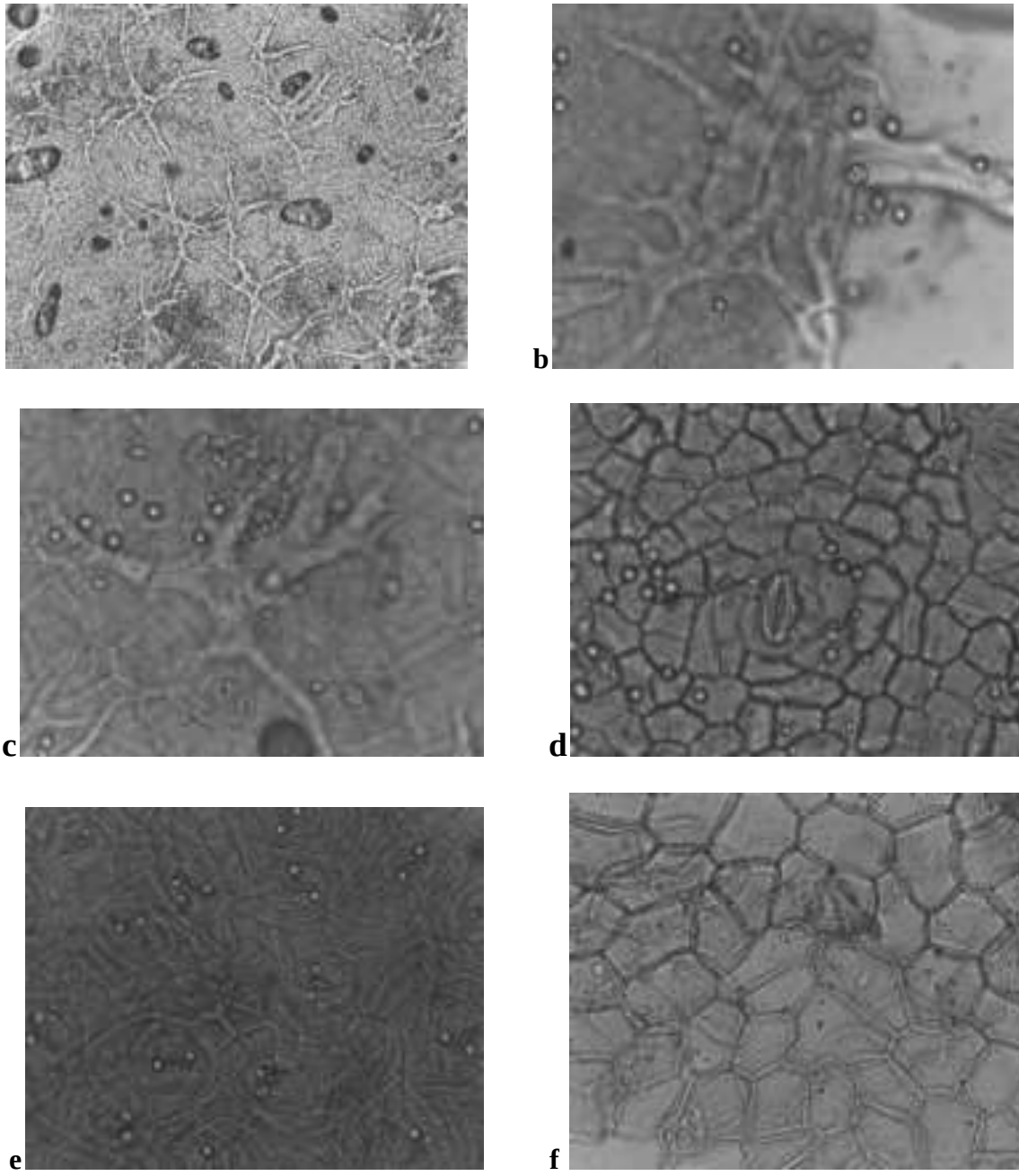

Plate 2A: Photomicrograph of the leaf surfaces of Loganiaceae; (a) Adaxial surface of *Anthocleista djalonensis* showing numerous stellate trichomes (b) Abaxial surface of *A. djalonensis* showing one giant stellate trichome (c) Adaxial surface of *A. liebrechtsiana* showing one stellate trichomes (d) Abaxial surface of *A. liebrechtsiana* showing anisocytic stoma. (e) Abaxial surface of *A. liebrechtsiana* showing anisocytic stomata (f) Adaxial surface of *A. liebrechtsiana* showing polygonal cell shape. Magnification: x 640

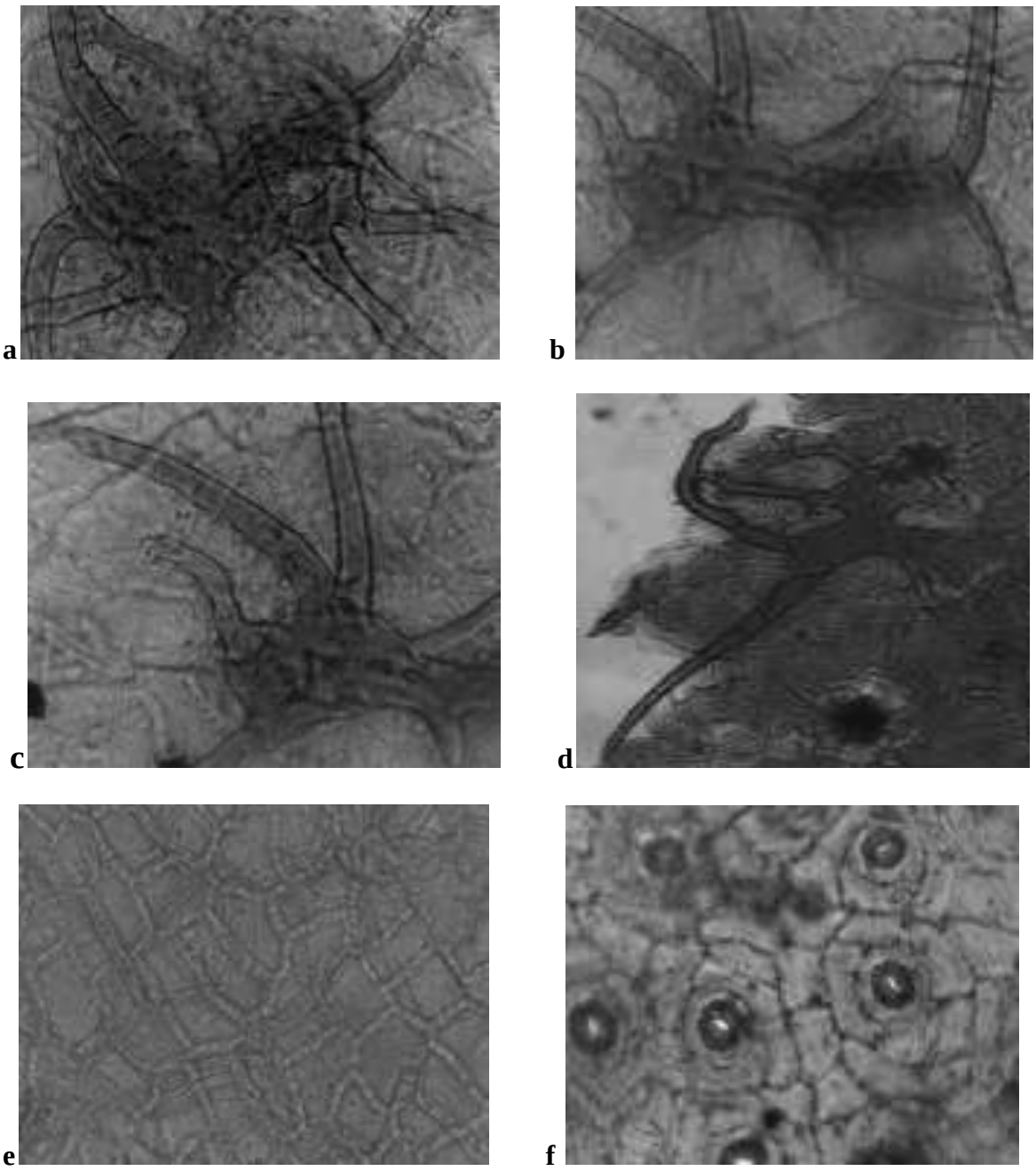

Plate 2B: Photomicrograph of the leaf surfaces of Loganiaceae; (a) Adaxial surface of *A. microphyla* showing complex trichome types (b) Adaxial surface of *A microphyla* showing stellate trichome (c-d) Adaxial surface of *A. microphyla* showing stellate trichome types (e) Adaxial surface of *A. microphyla* showing cell types (f) Abaxial surface of *A. microphyla* showing anisocytic stomata with starch grains scaled by wax deposition (f). Magnification: x 640

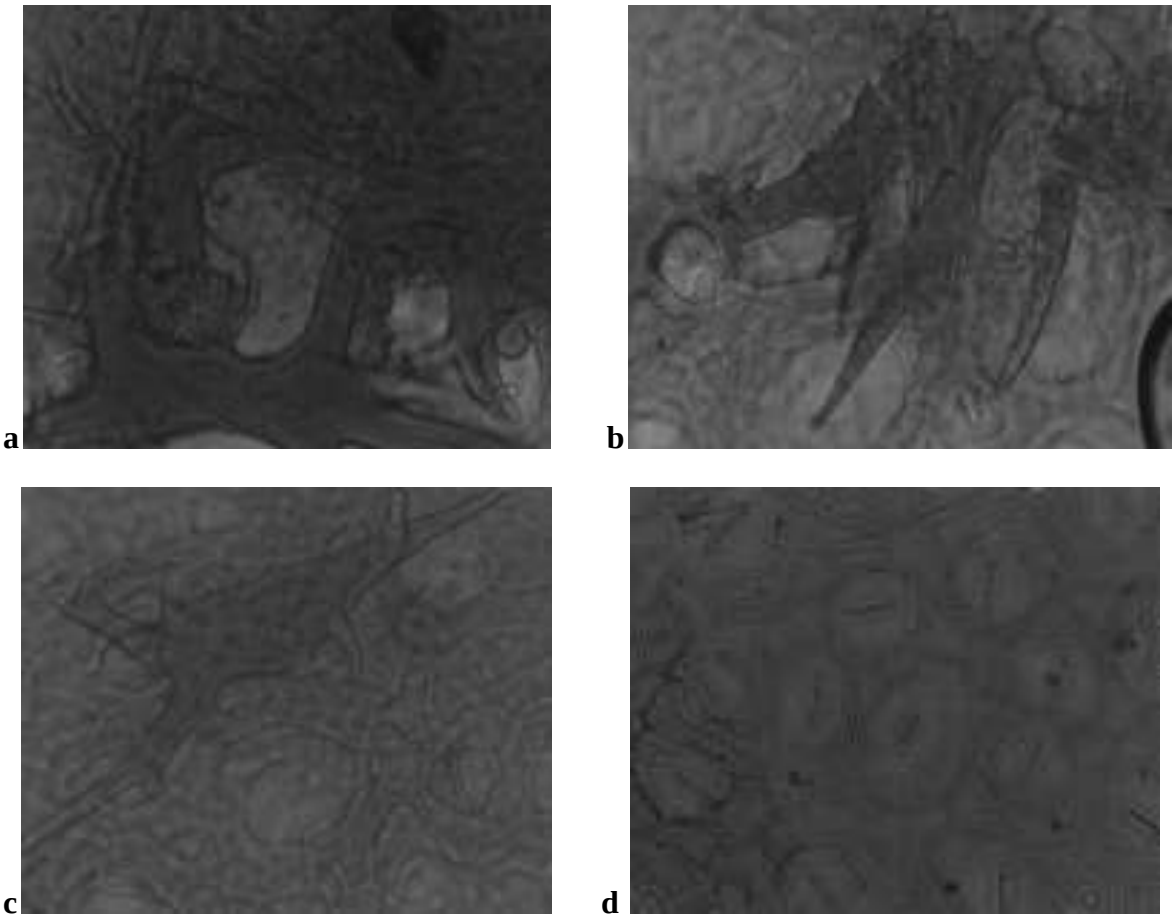

Plate 2C: Photomicrograph of the leaf surfaces of Loganiaceae; (a-b) Abaxial surface of *A. nobilis* showing dendritic trichomes showing complex trichome types (c-d) Abaxial surface of *A. nobilis* showing raised anisocytic stomata type. Magnification: x 640

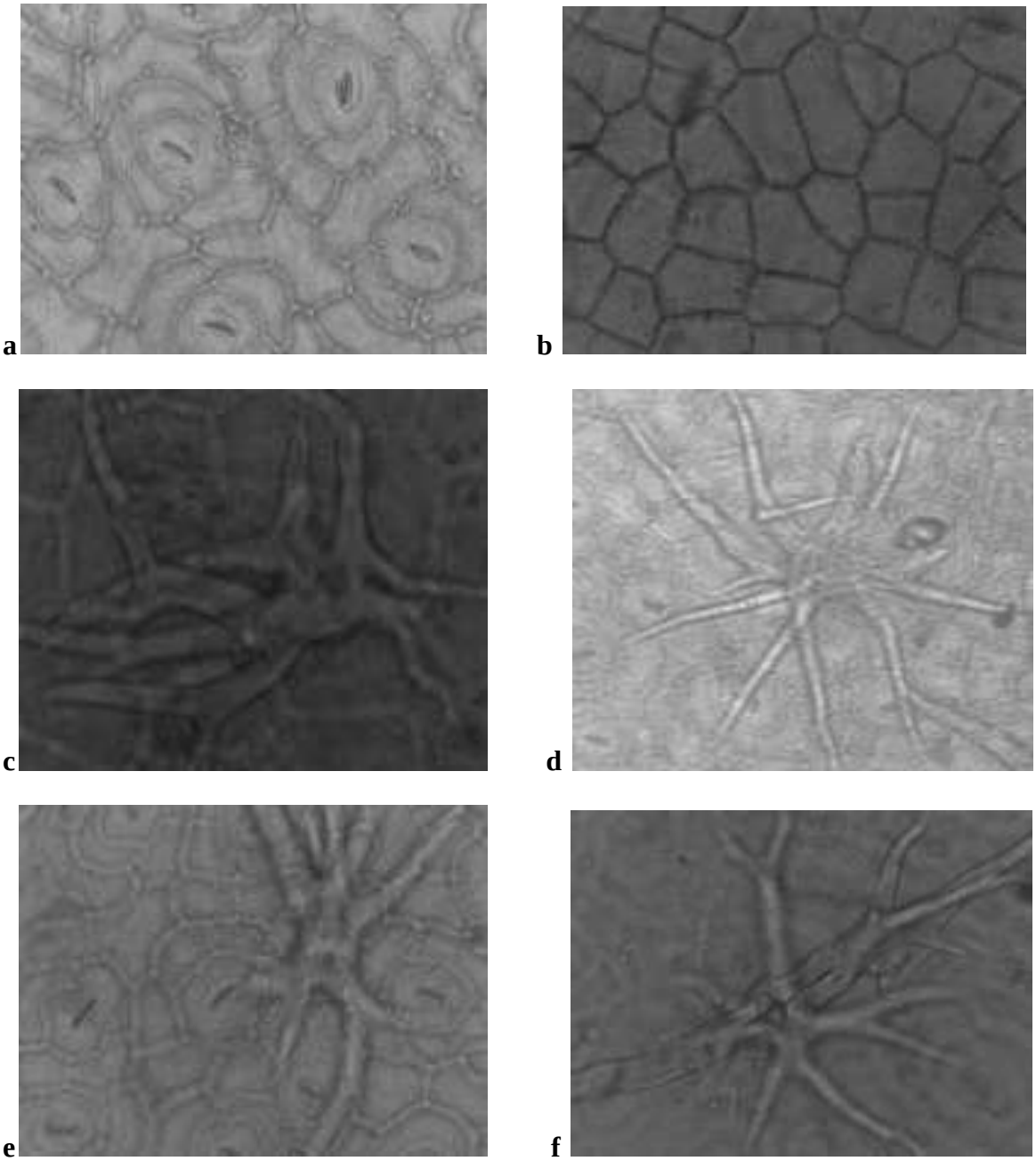

Plate 2D: Photomicrograph of the leaf surfaces of Loganiaceae; (a) Abaxial surface of *A. obanensis* showing raised anisocytic stomata. (b) Adaxial surface of *A. obanensis* showing polygonal cell shape. (c-f) Abaxial surface of *A. obanensis* showing raised anisocytic stomata and complex stellate trichomes. Magnification: x 640

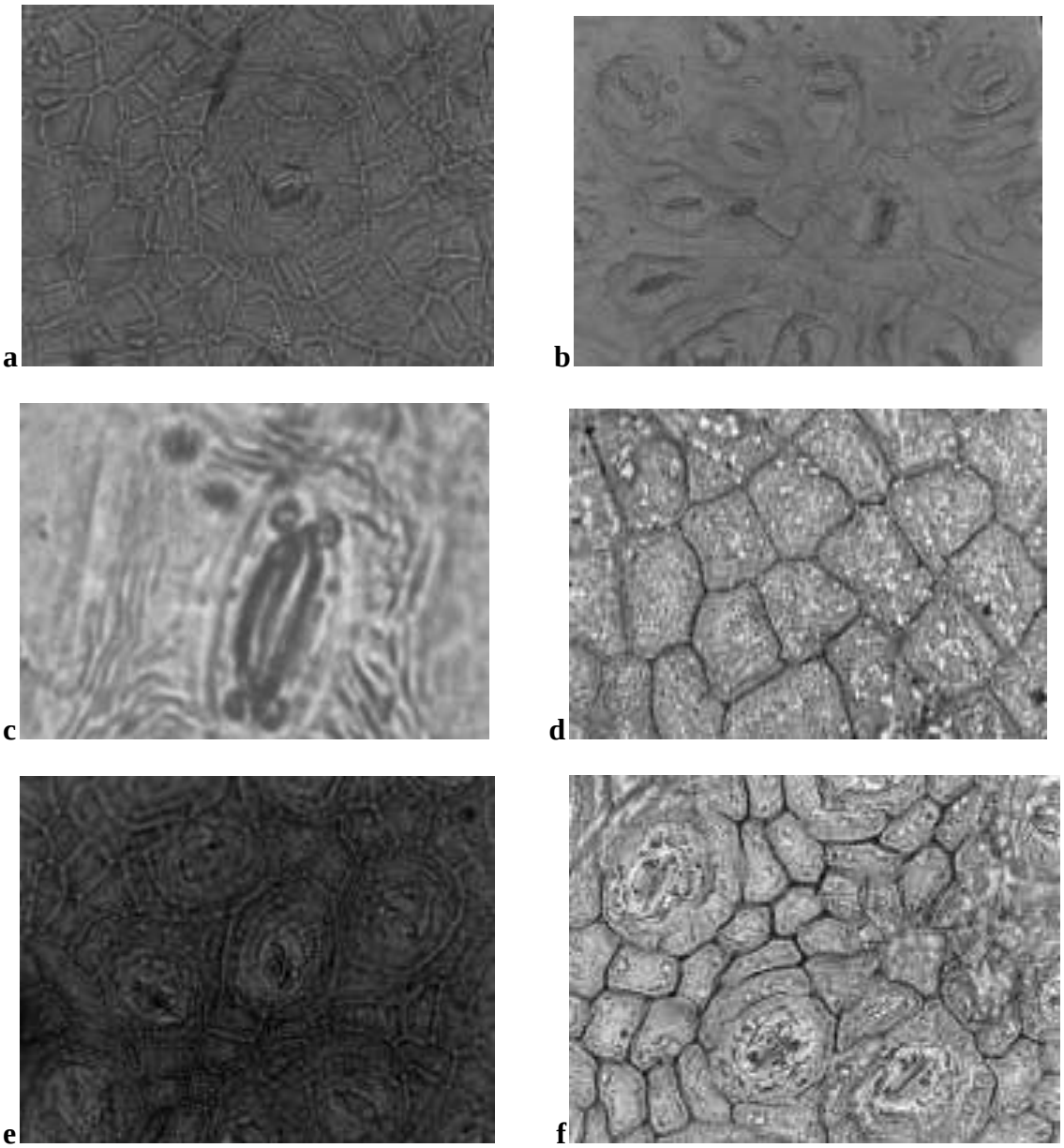

Plate 2E: Photomicrograph of the leaf surfaces of Loganiaceae; (a) Adaxial surface of *A. procera* showing anisocytic stomata and irregular cell shapes, (b-c) Abaxial surface of *A. procera* showing anisocytic stomata with scale wax deposition (d-f) Abaxial surface of *A. scandens* showing ornamentation of wax deposition and staurocytic stomata. Magnification: x 640

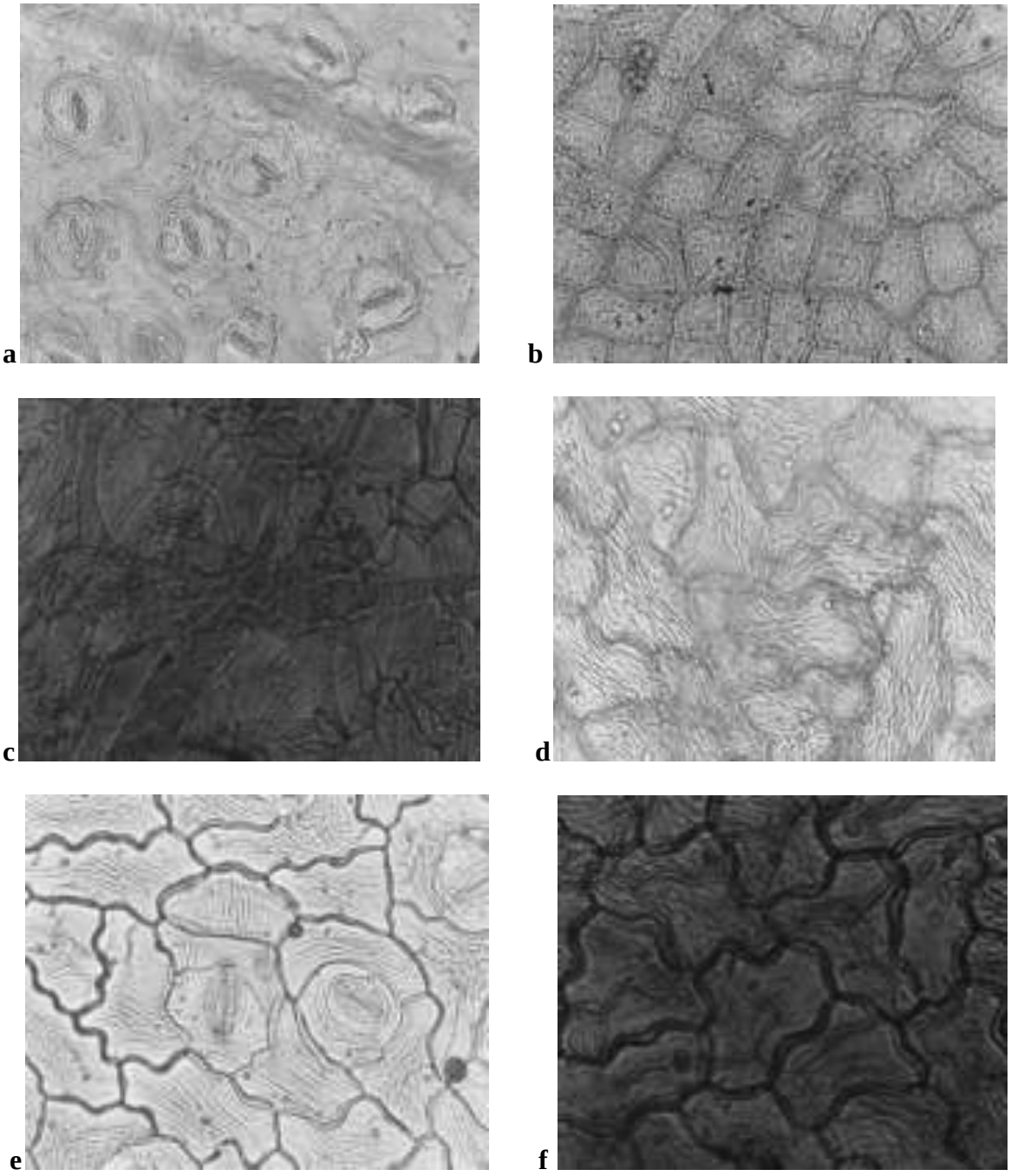

Plate 2.F: Photomicrograph of the leaf surfaces of Loganiaceae; (a) Adaxial surface of *A. scandens* showing anisocytic stomata and complex wax deposition (b) Adaxial surface of *A. scandens* showing regular polygonal cell shape (c-f) Abaxial surface of *A. vogelii* showing cuticular folding – striations, anomocytic stomata and sinuous cell shape. Magnification: x 640

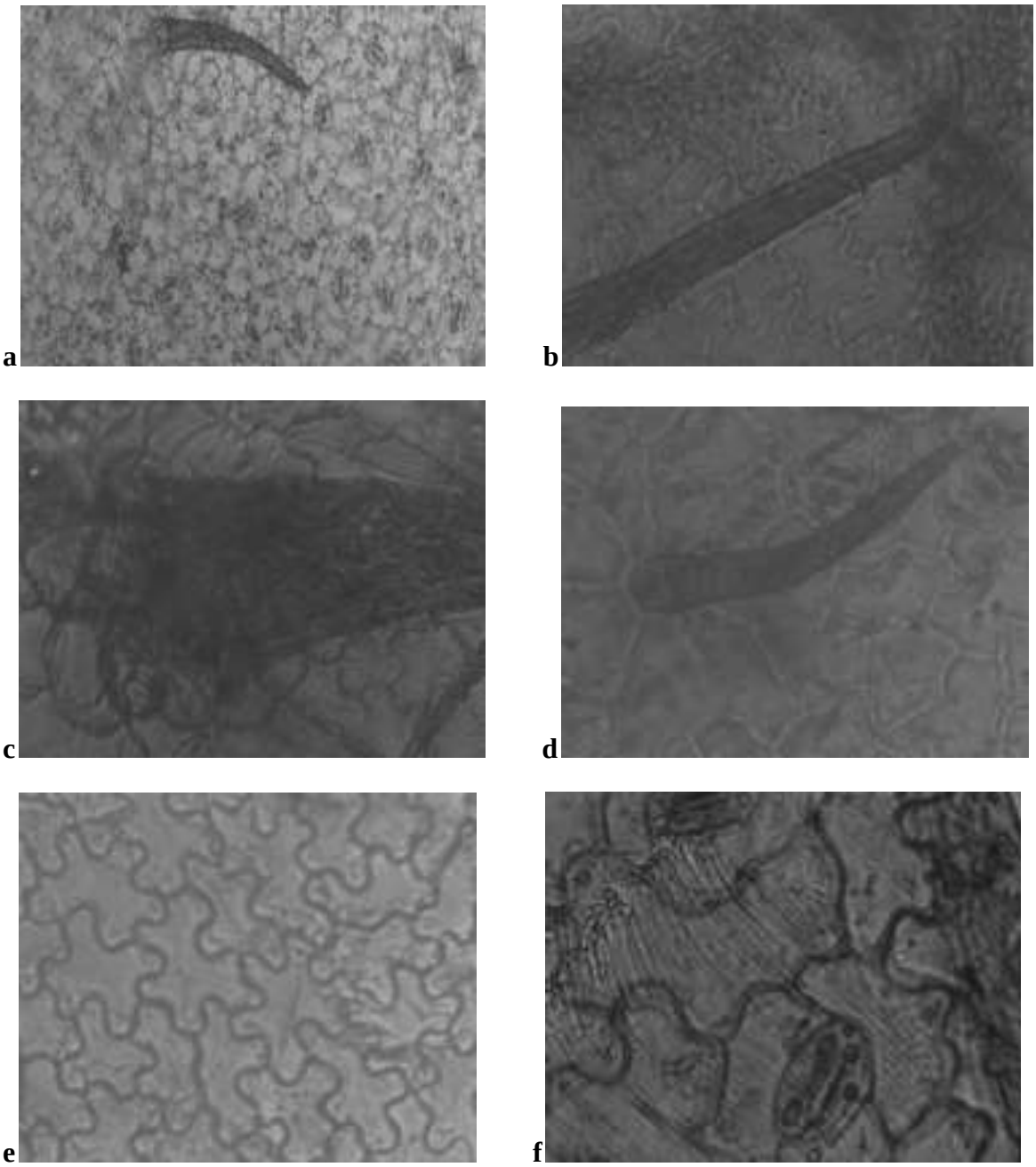

Plate 2G: Photomicrograph of the leaf surfaces of Loganiaceae; (a-b) Adaxial surface of *Mostuea brunonis* showing numerous but many anomocytic stomata with short and long unicellular trichome. (c - f) Adaxial surface of *Mostuea hirsuta* showing sinuous anticlinal wall with large anisocytic stomata. Magnification: x 640

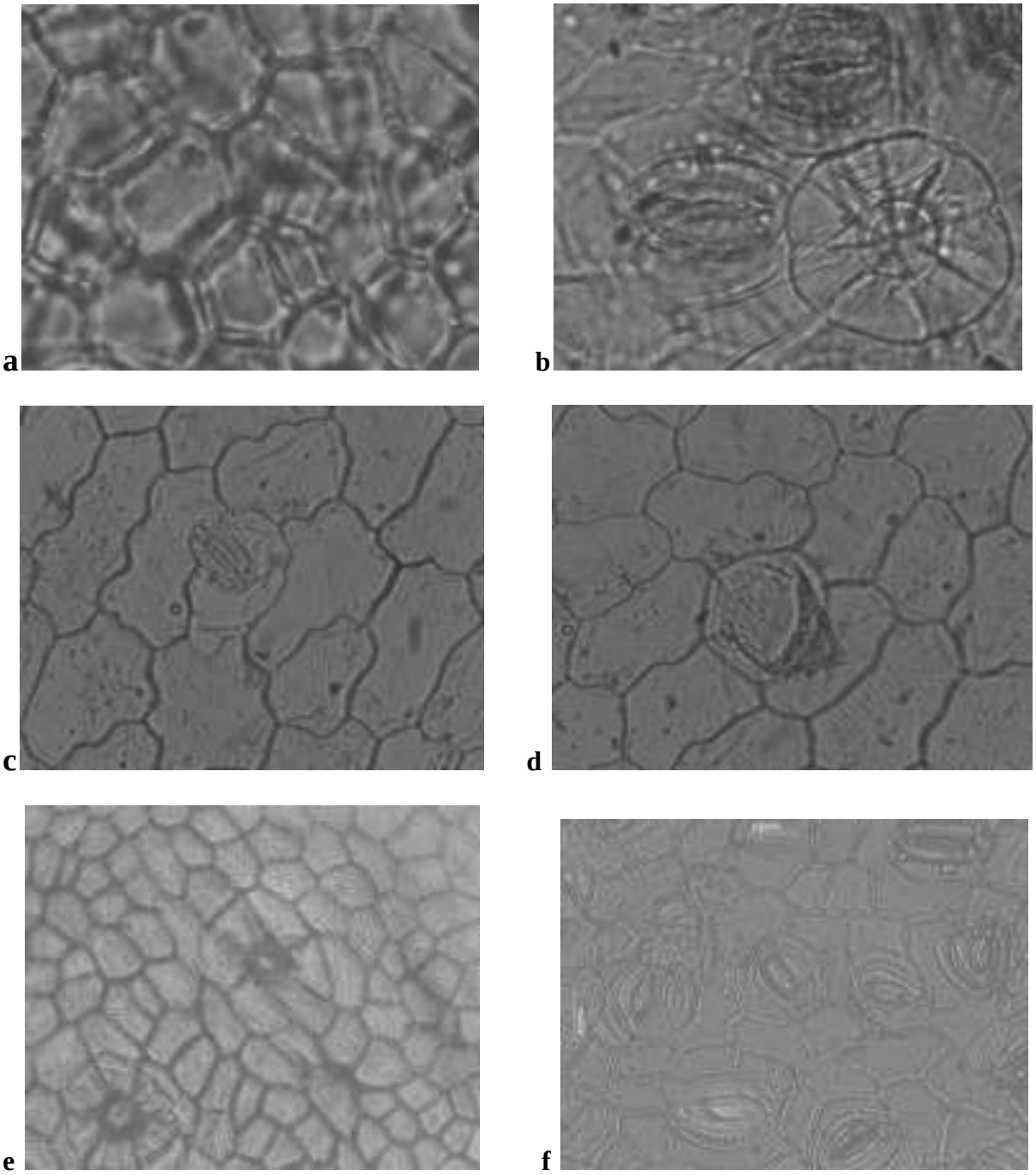

Plate 2H: Photomicrograph of the leaf surfaces of Loganiaceae; (a) Adaxial surface of *Nuxia conjesta* showing polygonal cell shape with wax deposition (b) Abaxial surface of *Nuxia conjesta* showing peltate trichome (c) Abaxial surface of *Spigelia anthelmia* showing large epidermal cells with anisocytic stomata of (d) Adaxial surface of *Spigelia anthelmia* showing large epidermal cells with conical trichome – (e-f) Abaxial and adaxial surfaces of *Strychnos aculeata* showing numerous cell with wax deposition with starch deposit on the abaxial large stomata Magnification: x 640

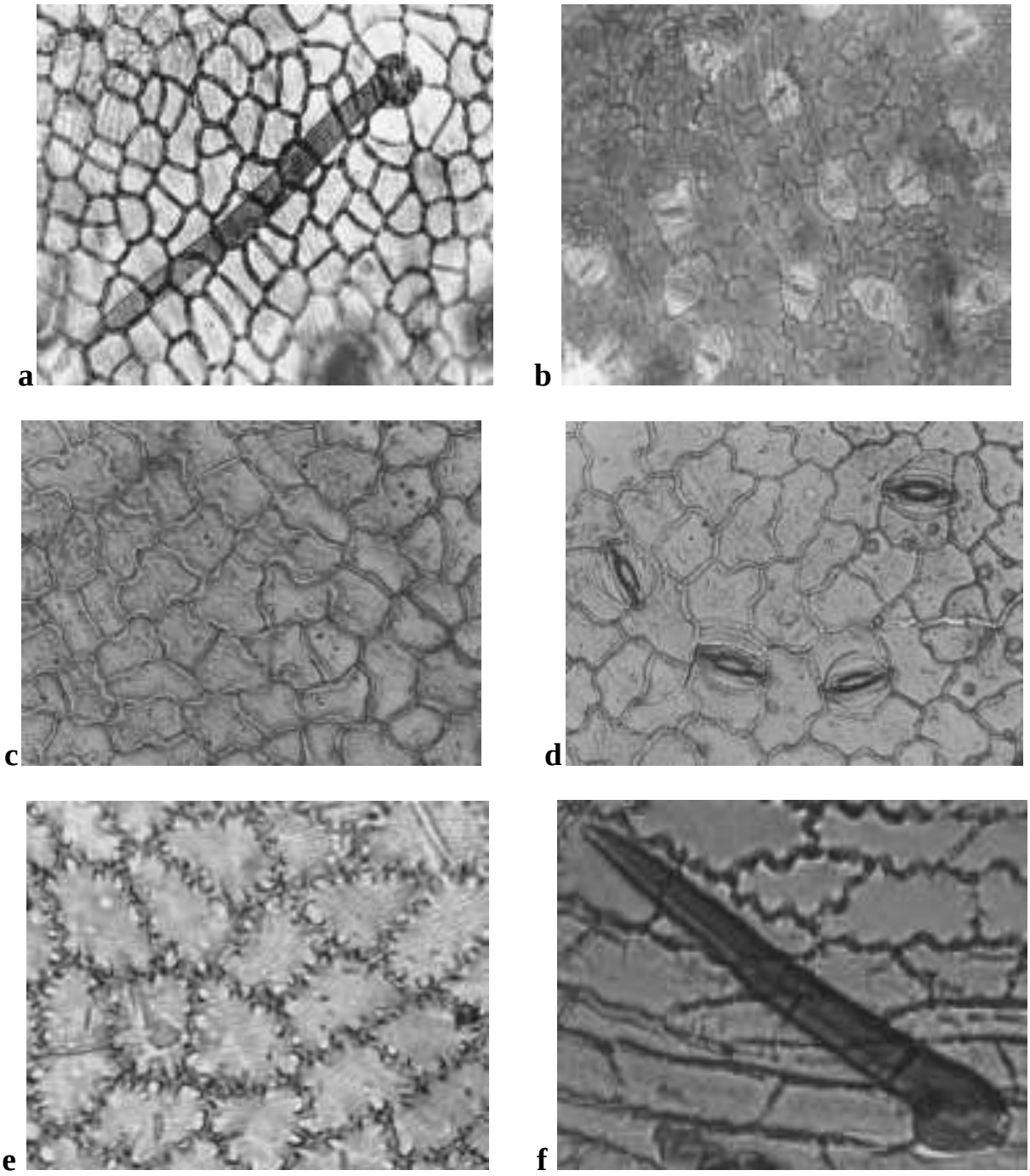

Plate 2I: Photomicrograph of the leaf surfaces of Loganiaceae; (a-b) Adaxial and Abaxial surfaces of *Strychnos afzeli* showing paracytic stomata and complex wax deposition (c-d) Adaxial surface of *S. barteri* showing irregular cell shape with paracytic stomata on the abaxial side only (e-f) Adaxial surface of *S. boonei* showing regular, polygonal with sinuous wall cells and long multicellular trichomes . Magnification: x 640

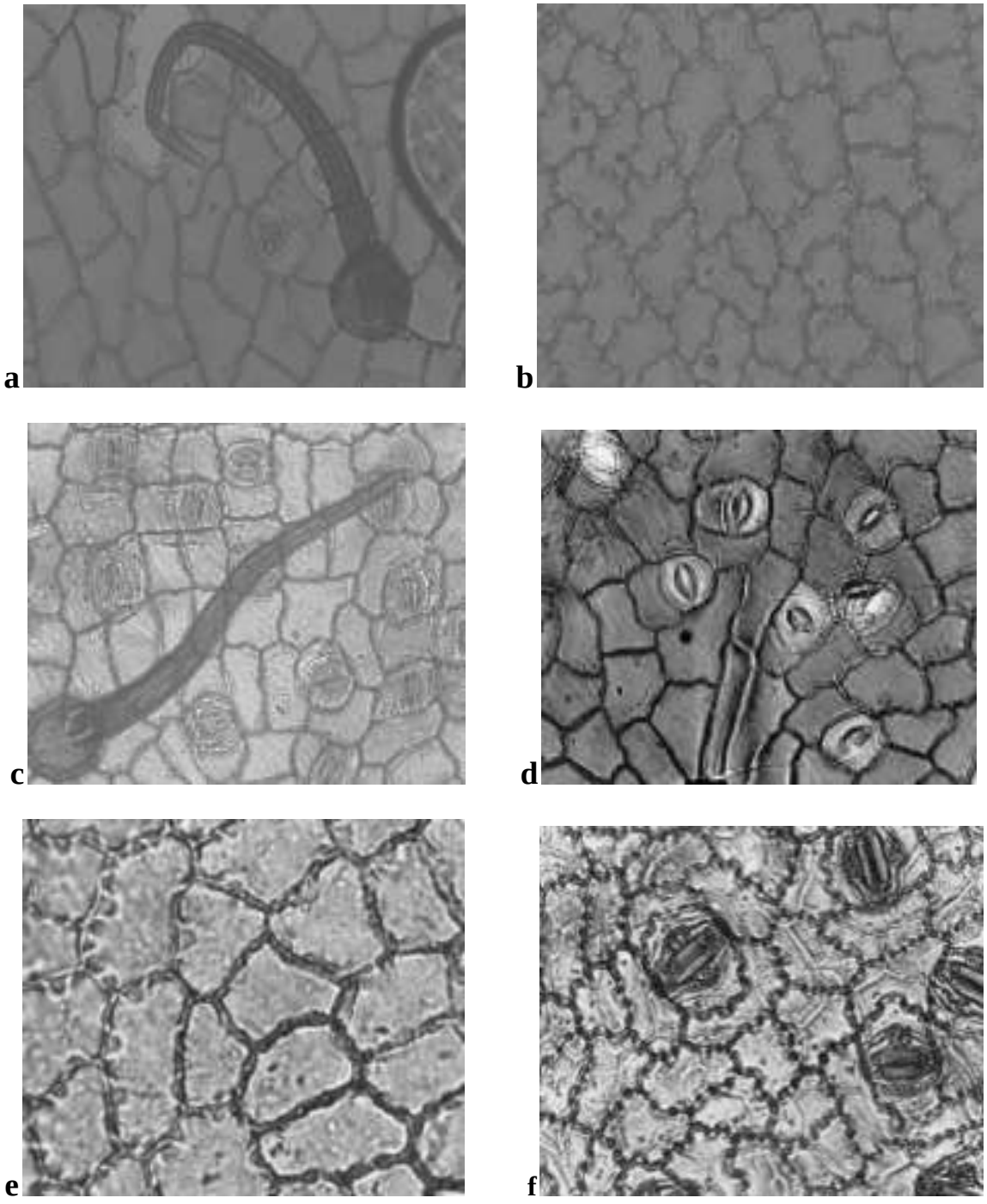

Plate 2J: Photomicrograph of the leaf surfaces of Loganiaceae; (a-d) The adaxial and abaxial epidermal surfaces of *Strychnos campicola* showing unicellular trichomes with paracytic stomata covered by starch grains and wax deposition. (e-f) The adaxial and abaxial epidermal surfaces of *S. camptoneura* showing undulating wall patter and sunken stomata. Magnification: x 640

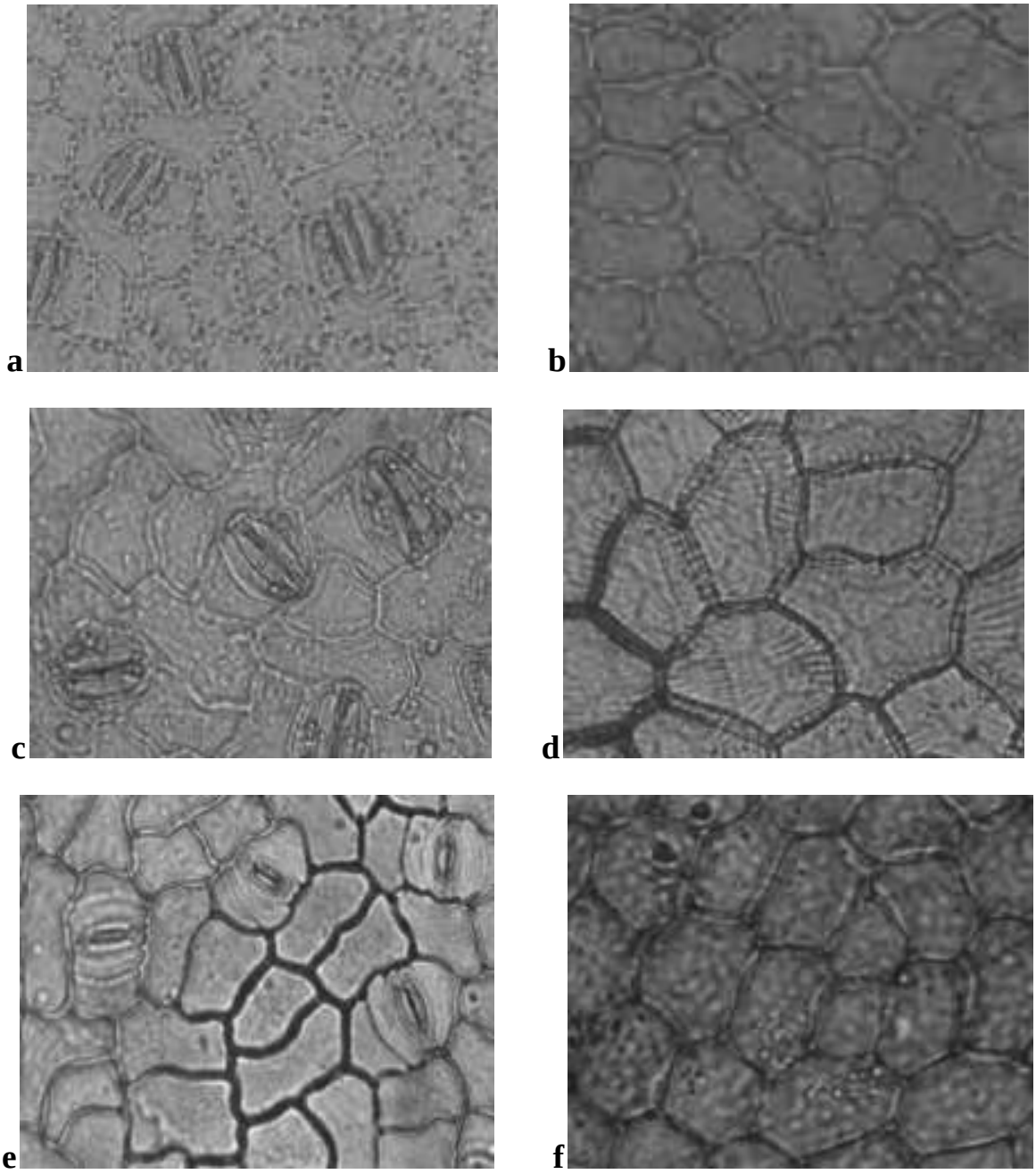

Plate 2K: Photomicrograph of the leaf surfaces of Loganiaceae. (a-b) The adaxial and abaxial epidermal surfaces of *S. chromatoxylon* showing large stomata under irregular wall shape, but with straight wall on the adaxial surface. (c-d), Adaxial and abaxial epidermal surfaces of *S. congolana* showing striation on the abaxial wall, irregular shape of cell on adaxial (e - f) Adaxial and abaxial surfaces of *S. cuminodora* showing enlarged stomata raised and regular polygonal cell shape on the adaxial wall.Magnification: x 640

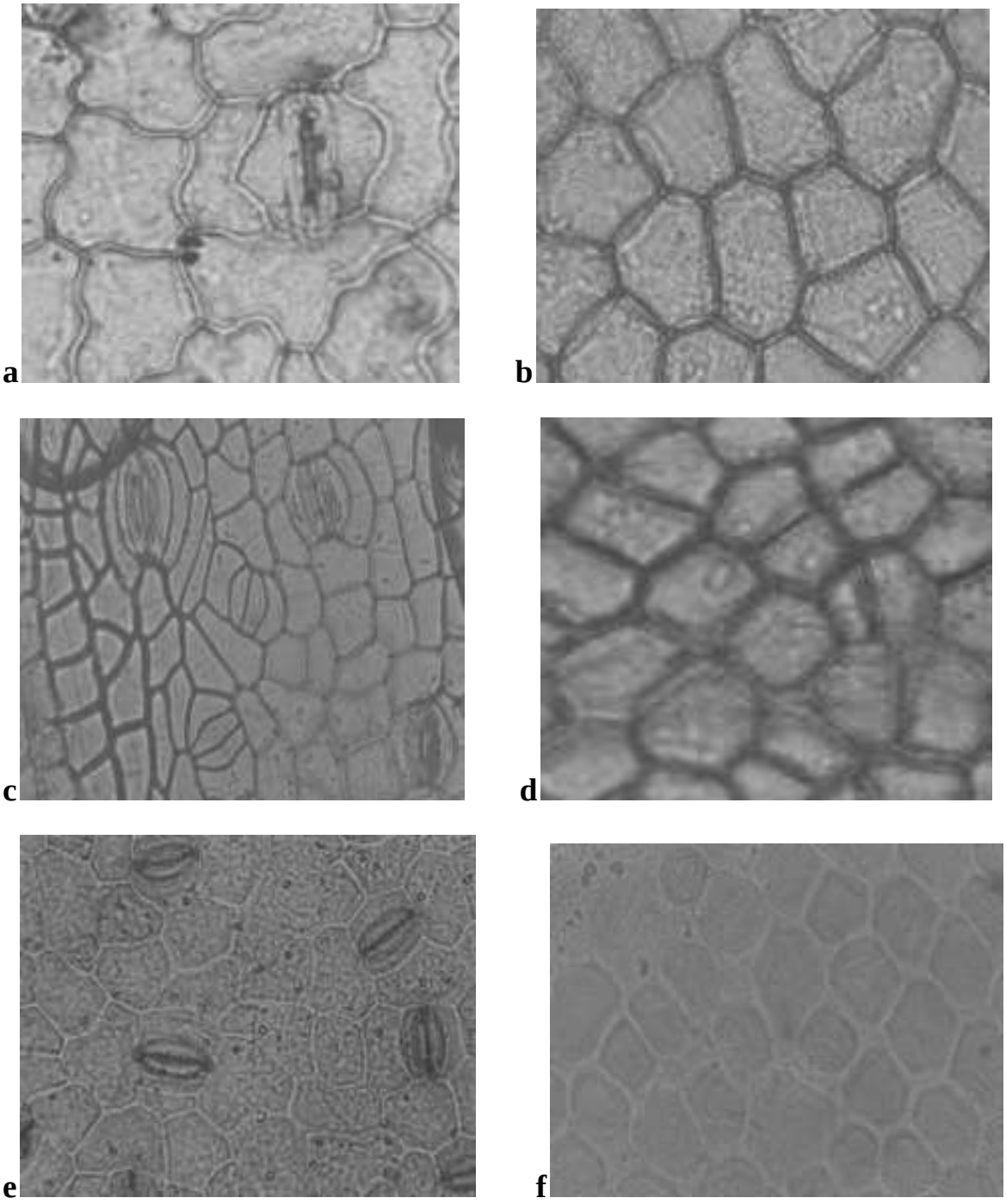

Plate 2L: Photomicrograph of the leaf surfaces of Loganiaceae. (a-b) The adaxial and abaxial epidermal surfaces of *S. densiflora* showing a single large stoma among a large epidermal cell. (c-d) the adaxial and abaxial wall of *S. dinklagei* showing large epidermal wall covered with wax deposit. (e-f) Adaxial and abaxial epidermal surfaces of *S. floribunda* showing regular polygonal cell shape and oblong stomata on the abaxial surface.

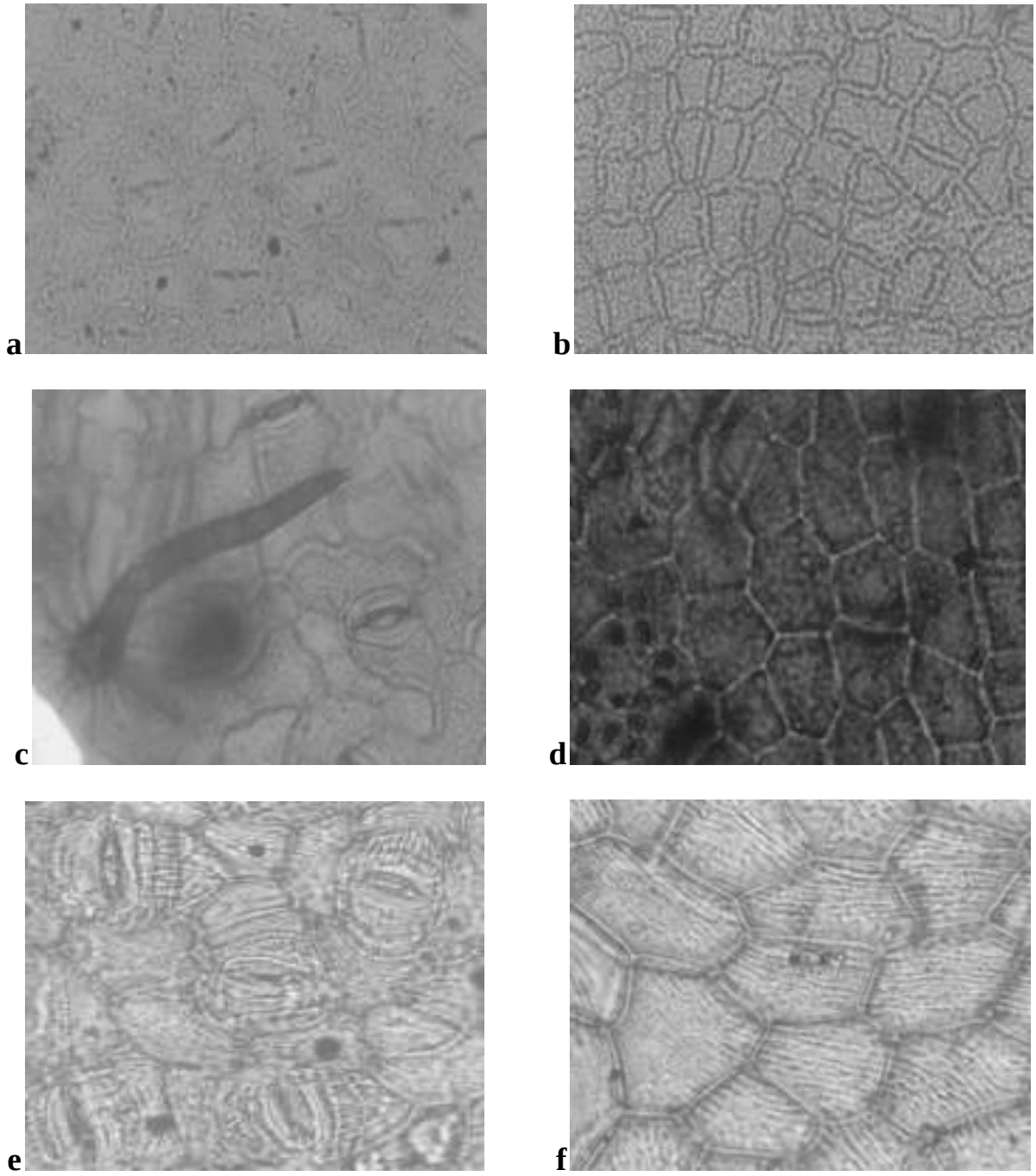

Plate 2M: Photomicrograph of the leaf surfaces of Loganiaceae (a-b) The adaxial and abaxial epidermal surfaces of *Strychnos gossweileri* showing tiny stomata with thin cell wall. (c - d) The adaxial and abaxial epidermal surfaces of *S. icaja* showing a long multicellular trichome on the abaxial surface. The wall on the adaxial surface are very straight (e-f) The adaxial and abaxial epidermal surfaces of *S. innocua* showing large cell wall with regular cuticular folding throught the cells, th stomata are raised, being partially covered by wall ornamentations. Magnification: x 640.

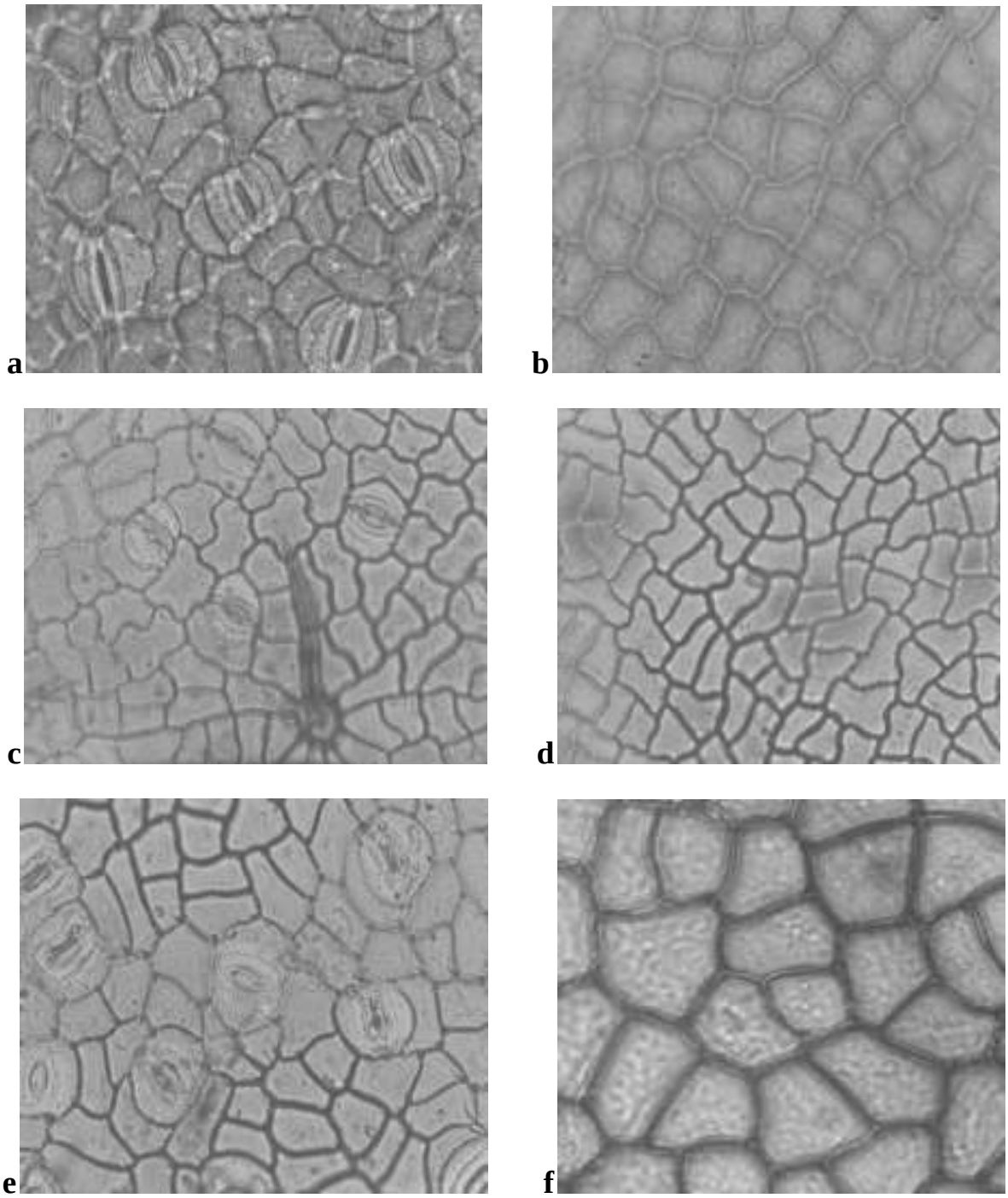

Plate 2N: Photomicrograph of the leaf surfaces of Loganiaceae (a-b) The adaxial and abaxial epidermal surfaces of *Strychnos johnsonii* showing starch deposition on the stomata of the abaxial side (c - d) The adaxial and abaxial epidermal surfaces of *S.longicaudata* showing uniform cell size on both surfaces but with stomata and unicellular trichomes on the abaxial surface. (e-f) The adaxial and abaxial epidermal surfaces of *S. lucens* showing larg wall size on the adaxial wall with wax deposit. Magnification: x 640

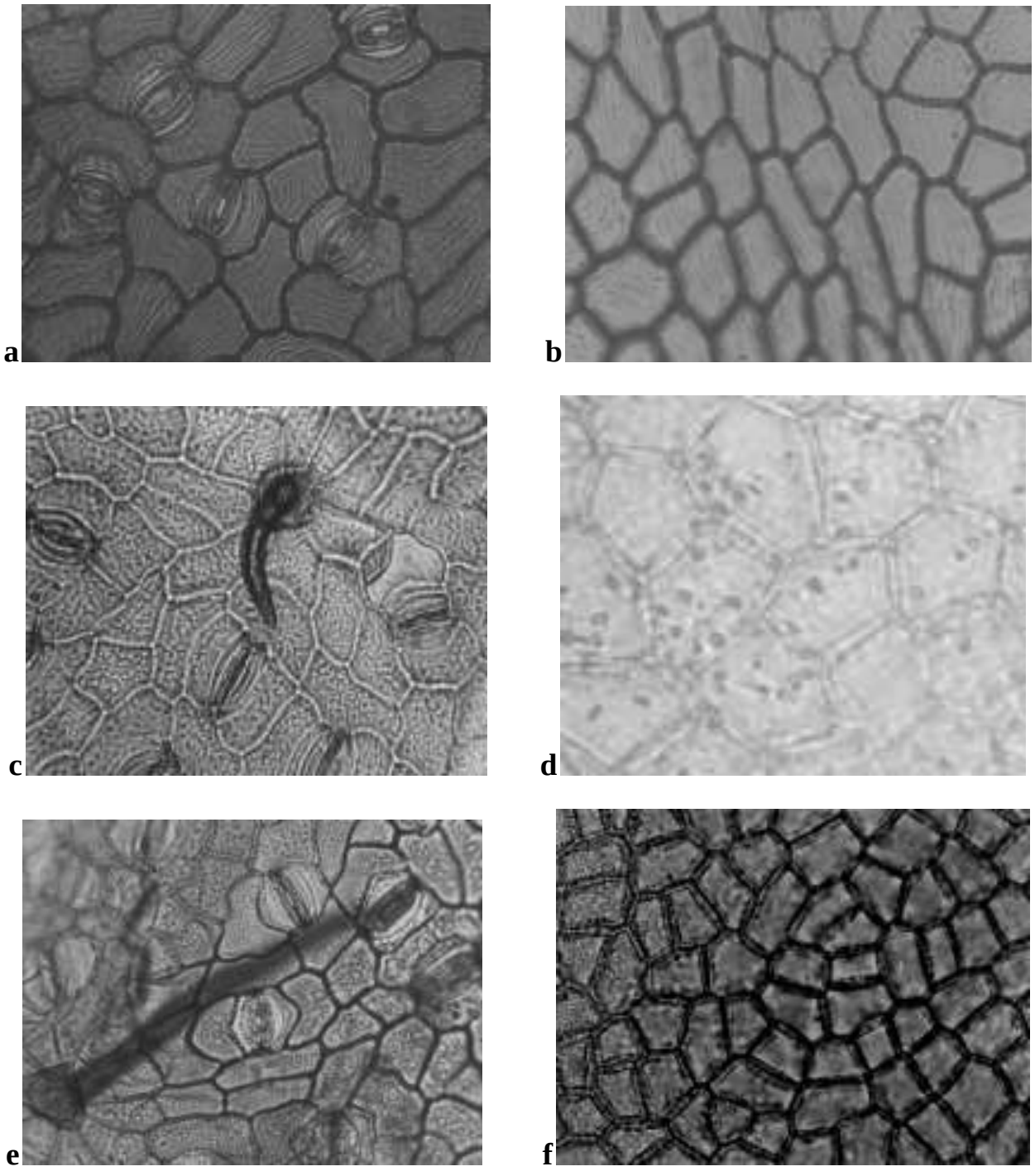

Plate 2O: Photomicrograph of the leaf surfaces of Loganiaceae (a-b) The adaxial and abaxial epidermal surfaces of *S. malacoclados* showing finely striated cells on both walls. (c - d) The adaxial and abaxial epidermal surfaces of *S. memecyloides* showing large wall on adaxial surface but stomata and short unicellular trichomes are on the abaxial surface. (e - f) The adaxial and abaxial epidermal surfaces *S. nigritana* showing long nonglandular trichome on the abaxial wall. Magnification: x 640

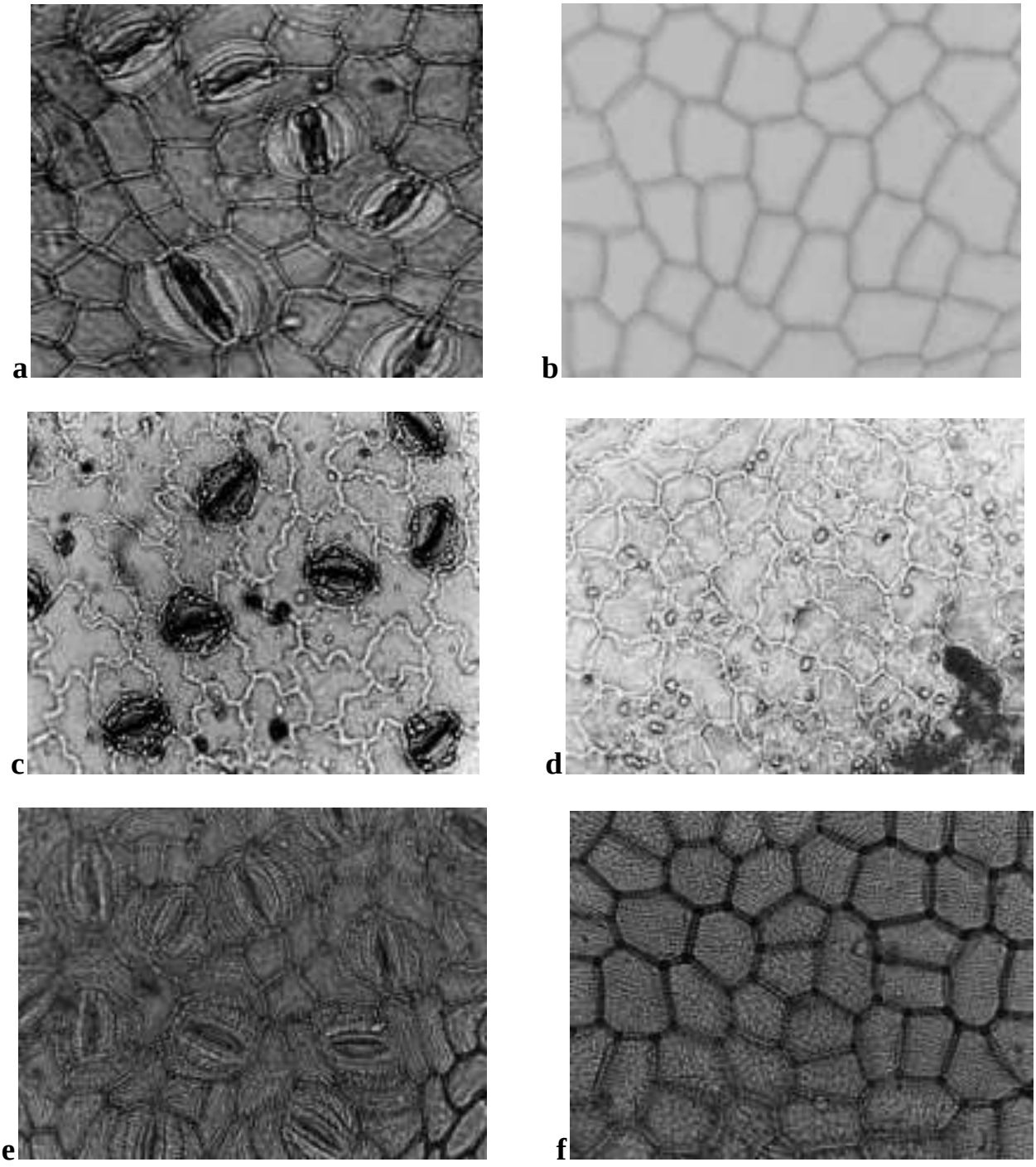

Plate 2P: Photomicrograph of the leaf surfaces of Loganiaceae (a-b) The adaxial and abaxial epidermal surfaces of *S. nux-vomica* showing very large stoma on the abaxial wall. (c-d) The adaxial and abaxial epidermal surfaces of *S. phaeotricha* showing sinuous anticlinal wall with sunken stomata on the abaxial wall; (e-f) The adaxial and abaxial epidermal surfaces of *S. soubrensis* showing uniform striation and regular polygonal walls. Magnification: x 640.

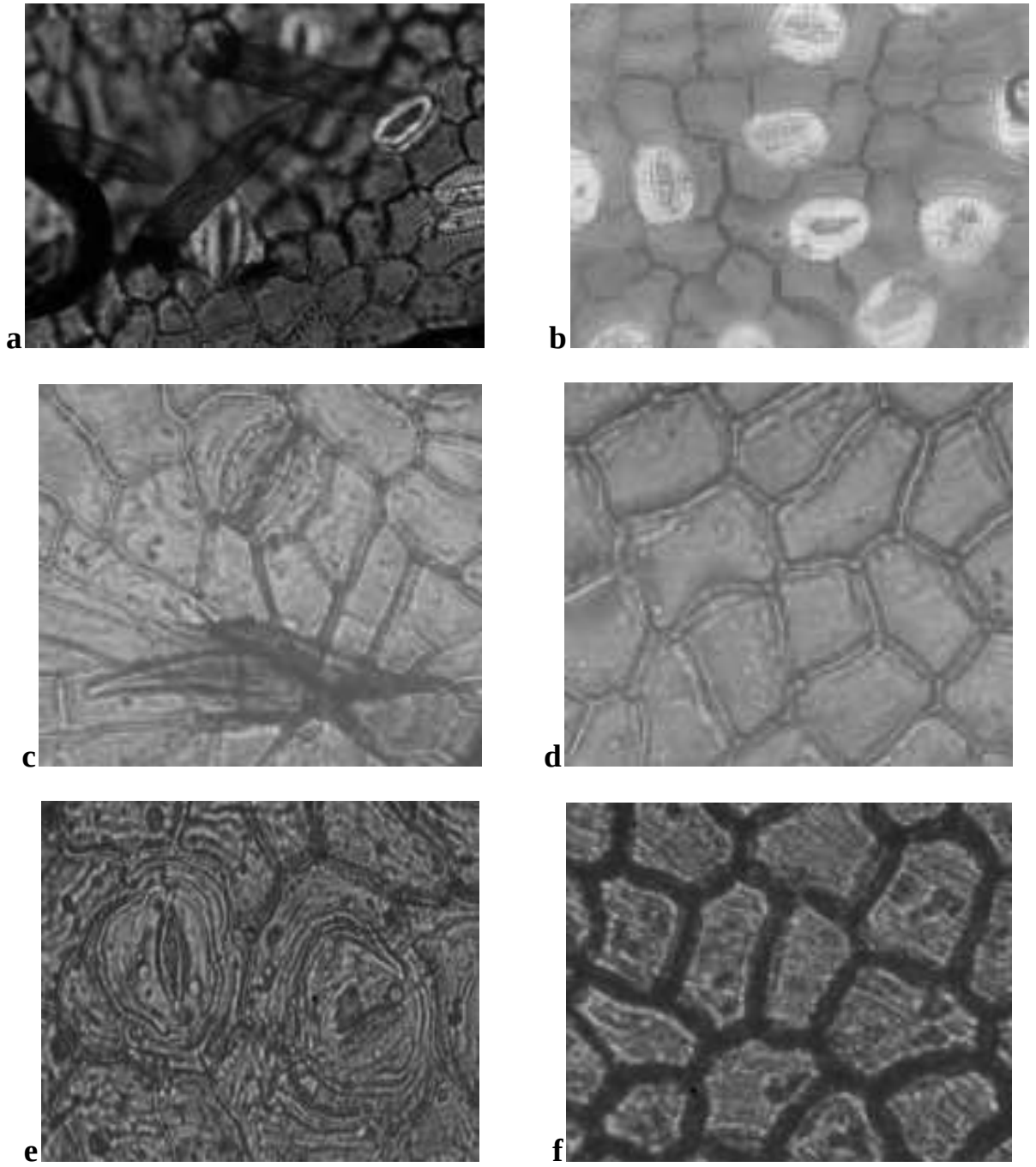

Plate 2Q: Photomicrograph of the leaf surfaces of Loganiaceae (a-b) The adaxial and abaxial epidermal surfaces of *S. spinosa* showing amphistomatic nature of the species with numerous trichomes on the cells (c - d) The adaxial and abaxial epidermal surfaces of *S. splendens* showing regular cell shape on the adaxial wall and short unicellular trichomes on the adaxial side and (e-f) The adaxial and abaxial epidermal surfaces of *Fagrae fragrans* showing a very large cavity of the stomata and plate like wax deposition of the wall. Magnification: x 640

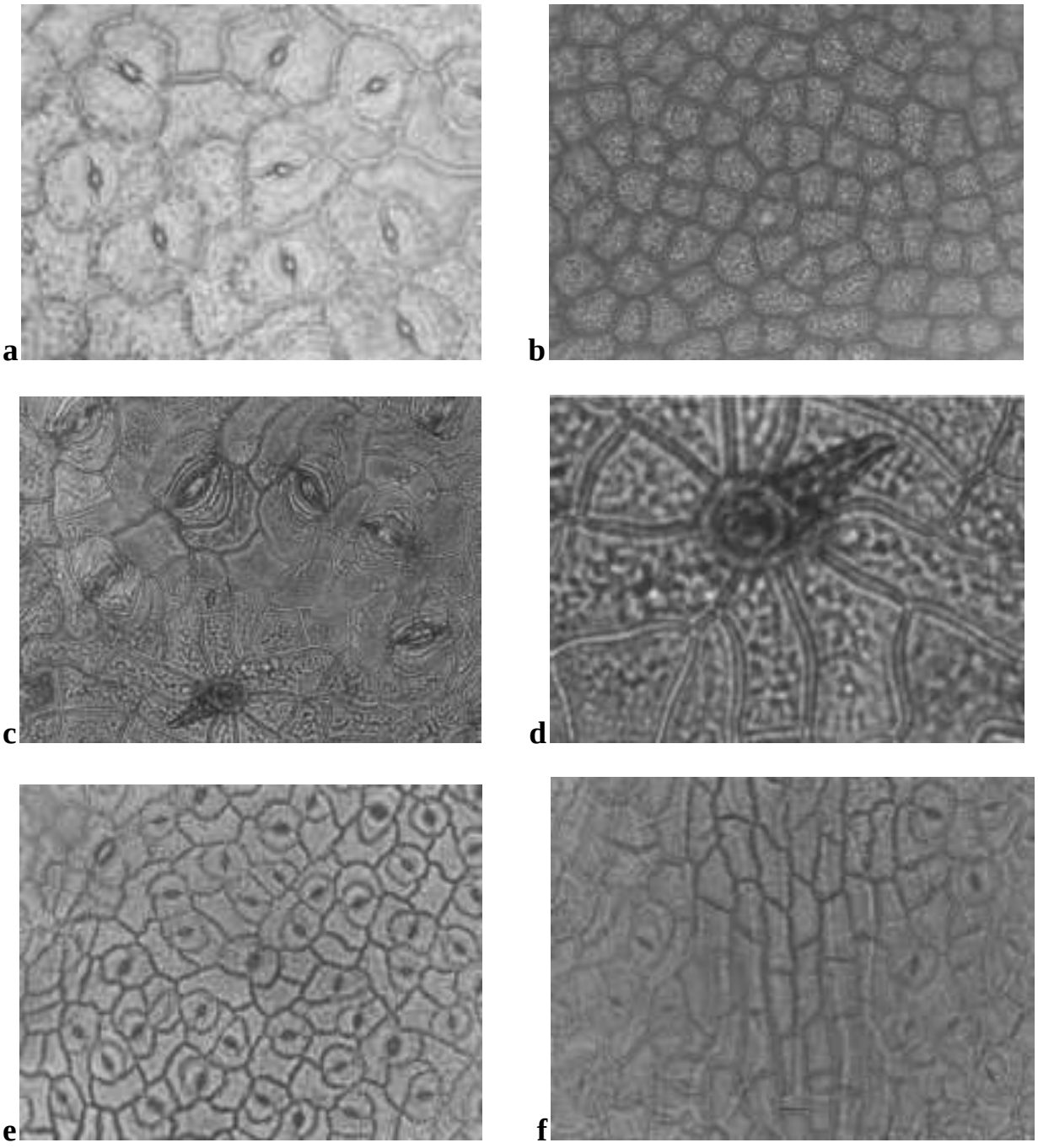

Plate 2R: Photomicrograph of the leaf surfaces of Loganiaceae (a-b) The adaxial and abaxial epidermal surfaces of *S. staudtii* showing large cell with vey tiny stomata on the abaxial side. (c-d) The adaxial and abaxial epidermal surfaces of *S. talbotiae* showing large wall with network of trichomes origin. (e-f) The adaxial and abaxial epidermal surfaces of *S. tricalysioides* showing numerous but tiny stomata on both sides – amphistomatic condition. Magnification: x 640

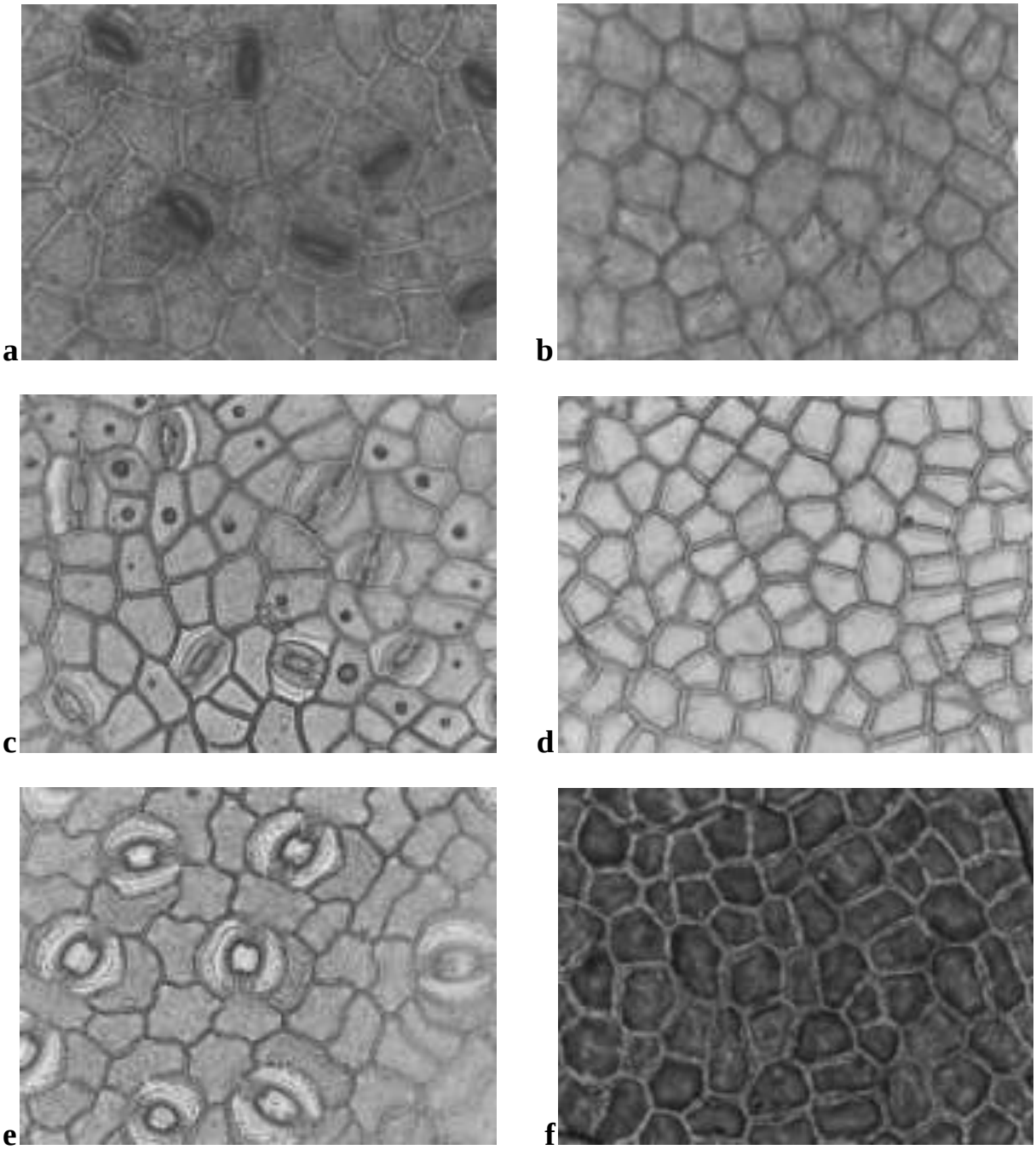

Plate 2S: Photomicrograph of the leaf surfaces of Loganiaceae (a-b) The adaxial and abaxial epidermal surfaces of *S. urceolata* showing wax covered epidermal wall with sunken stomata on the abaxial side.(c-d) The adaxial and abaxial epidermal surfaces of *S. usambarensis* showing irregular epidermal cell wall with starch grains on the stomata and (e - f) The adaxial and abaxial epidermal surfaces of *S. chrysophylla* showing oval shaped stomata fortified by starch grains. Magnification: x 640

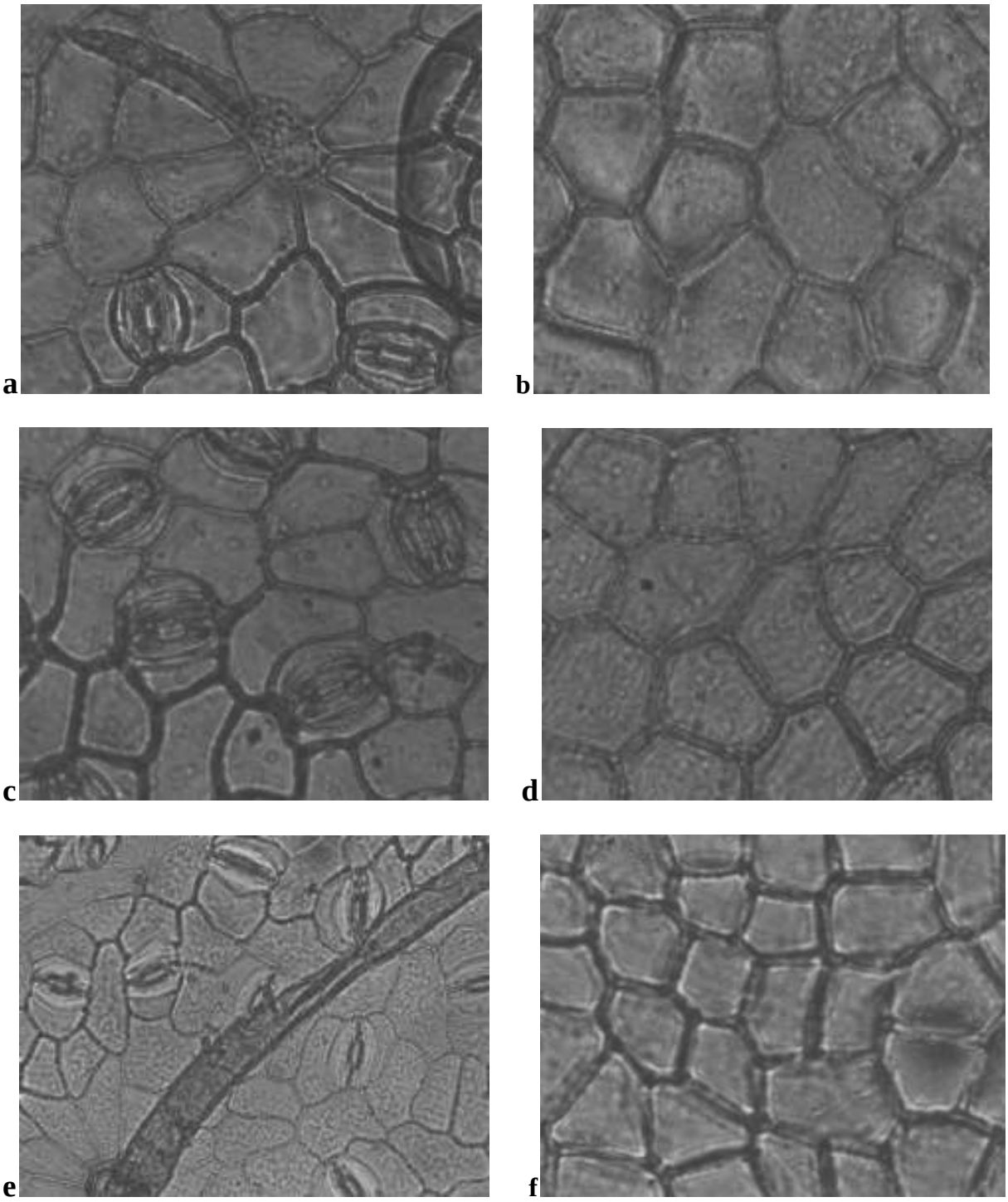

Plate 2T: Photomicrograph of the leaf surfaces of Loganiaceae (a-b) The adaxial and abaxial epidermal surfaces of *Strychnos* indeterminate SID 55 showing large epidermal cells with occaisional short trichome on the abaxial side. (c-d) The adaxial and abaxial epidermal surfaces of *Strychnos* indeterminate SID 56 showing very similar characters to the 55 but appear to have cuticular folding. (e-f) The adaxial and abaxial epidermal surfaces of *Strychnos* indeterminate SID 57 showing long unicellular trichomes on the abaxia side only

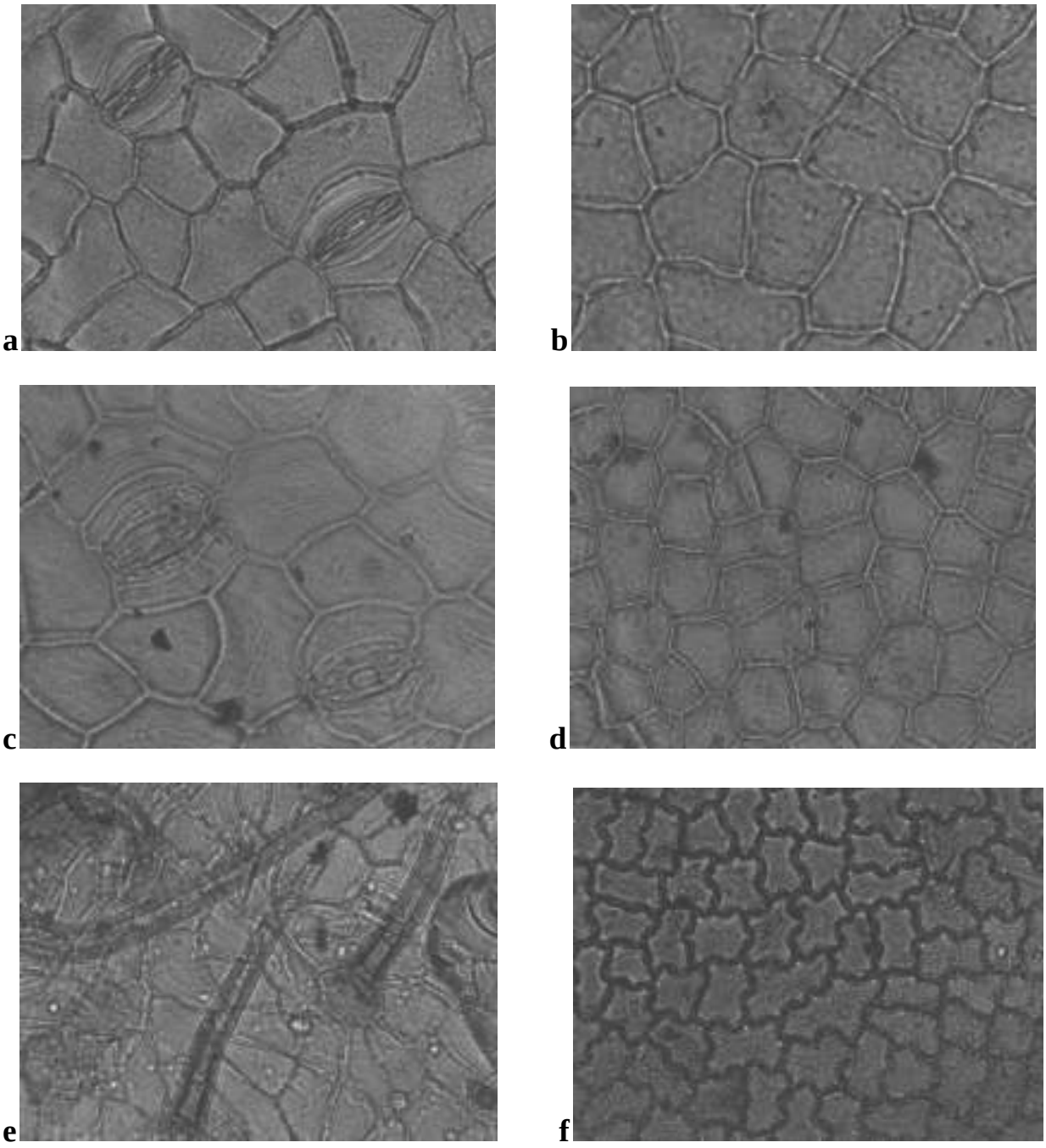

Plate 2U: Photomicrograph of the leaf surfaces of Loganiaceae (a-b) The adaxial and abaxial epidermal surfaces of *Strychnos* indeterminate SID 58 showing large epidermal cells with trichomes on the abaxial side only. (c-d) The adaxial and abaxial epidermal surfaces of *Strychnos* indeterminate SID 59 showing very large stomata on the abaxial surface with regular cell shapes (e-f) The adaxial and abaxial epidermal surfaces of *Strychnos* indeterminate SID 61showing abaxial cell with several trichomes and the cell wall is curved

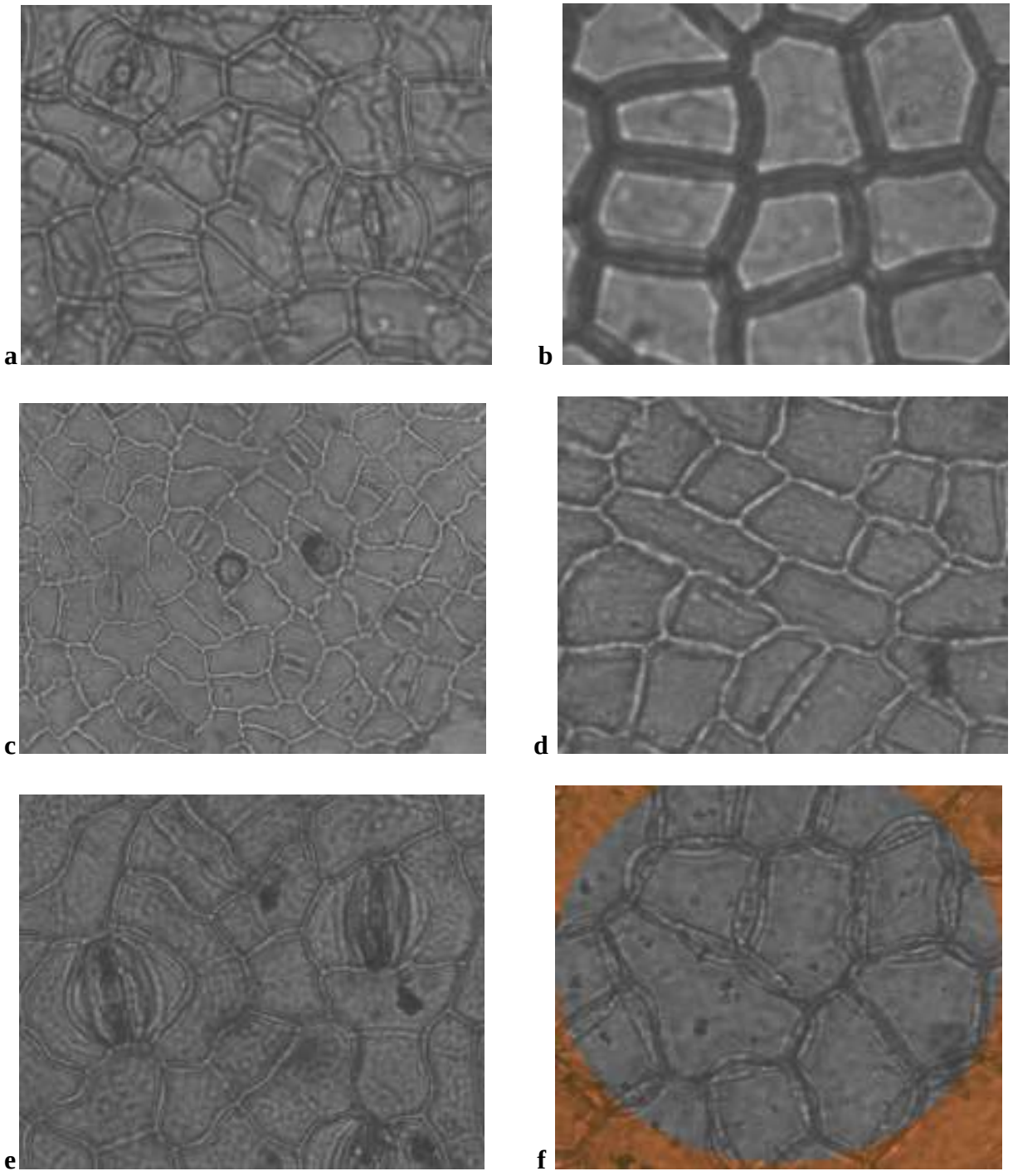

Plate 2V: Photomicrograph of the leaf surfaces of Loganiaceae (a-b) The adaxial and abaxial epidermal surfaces of *Strychnos* indeterminate SID 62 showing thick cell wall on the adaxial cell with irregular cell shapes on the abaxial cells (c-d) The adaxial and abaxial epidermal surfaces of *Strychnos* indeterminate SID 63 showing narrow stomata with elongated apertures (e-f) The adaxial and abaxial epidermal surfaces of *Strychnos* indeterminate SID 64 showing large aperturate stomata will cell wall network in tertwining each other. The cells are very large. Magnification: x 640

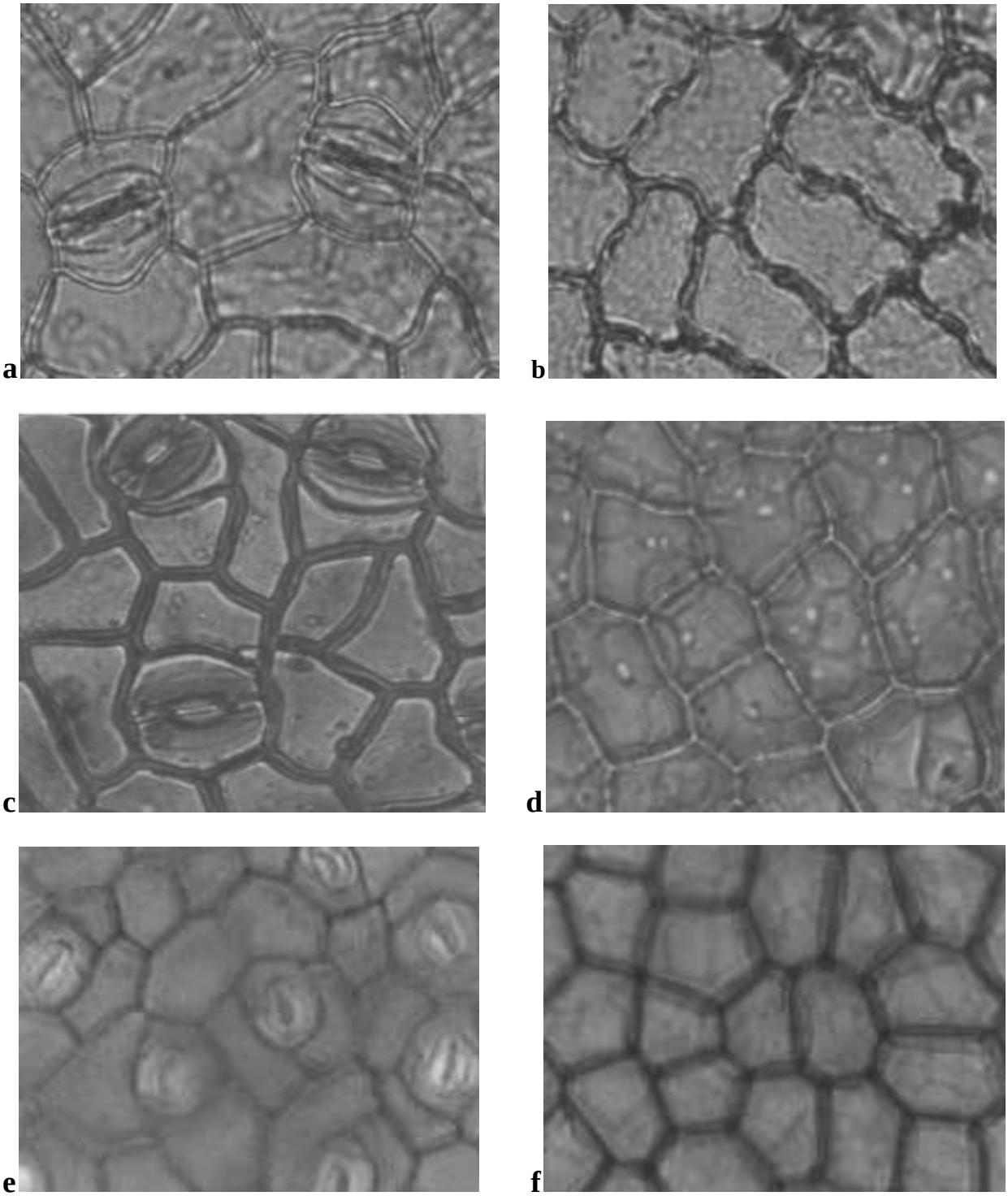

Plate 2W: Photomicrograph of the leaf surfaces of Loganiaceae (a-b) The adaxial and abaxial epidermal surfaces of *Strychnos* indeterminate SID 65 showing large rregular polygonal cell wall on the adaxial wall (c-d) The adaxial and abaxial epidermal surfaces of *Strychnos* indeterminate SID 66 showing thick cell wall on the adaxial side (e-f) The adaxial and abaxial epidermal surfaces of *Usteria guineensis* showing the wax coating on the wall making the stomata sunken and shaded the wall pattern. Magnification: x 640.

4.4 Molecular Analysis

4.4.1 Sample selection, DNA extraction and Gel – electrophoresis

Twenty one samples that totalled 17 species were selected across the six genera to represent the Loganiaceae population and were sent for sequencing at the Royal Botanic Gardens (RBG) Kew, London. The DNA were extracted and deposited at DNA Bank of the Royal Botanic Gardens Kew, London. Seven of the twelve samples of *Strychnos* species collected from the field which did not match the herbaria collections during morphological characterisation were included in the sequenced samples (Tables 8). Consideration was given to the 7 clusers as well as the ungrouped species generated from the morphological analysis at 47 % similarity coefficient (Figure 5A). Since *Anthocleista* genus clustered together in two successive clusters, a single representative species was selected. The outliers or the ungrouped from the morphological dendrogram species were included in the selection. Selection was made based on the clustering of species in the dendrogram. It was mentioned earlier that the species tree generated could guide a taxonomist on selecting representative species when working with a large number samples.

Total DNA extracted was cleaned and deposited at the Gene bank in Kew. The genomic DNA on 1 % Agarose gel-electrophoresis was verified (Plates 3A and 3B).

The DNA bands result from the gel was good enough to proceed on Polymerase Chain Reaction (PCR). The intensity of the glow of the DNA bands determines the quantity and quality of DNA present in the samples. There were few (five - 5) species that their DNA did not come out well (sharp band) in the gel that were re extracted and re-purified.

Table 8: The Selected Samples for Sequencing at RBG, Kew

S/N	General	Sample Sequenced	Code
1	**1**	*Anthocleista vogelli*	AVO9
2	**2**	*M. brunonis*	MBR15
3	**3**	*Nuxia congesta*	NCO18
4	**4**	*Spigelia anthelmia*	SAT19
5	**5**	*Strychnos boonei*	SBO25
6		*S. campicola*	SCP26
7		*S. icaja*	SIC35
8		*S. nigritana*	SNI42
9		*S. spinosa*	SSN46
10		*S. spinosa var*	SSN46b
11		*S.staudtii*	SST48
12		*S. urceolata*	SUR51
13		*S. usambarensis*	SUS52
14		*S.* indeterminate	SID 57
15		*S.* indeterminate	SID 58
16		*S.* indeterminate	SID 60
17		*S.* indeterminate	SID61
18		*S.* indeterminate	SID 62
19		*S.* indeterminate	SID64
20		*S.* indeterminate	SID65
21	**6**	*Usteria guineensis*	UGU66

Note: The selection of number of species represented in each Genus across the six genera was based on the clustering pattern in the dendrogram from the morphological evidence.

The quality and quantity of DNA for molecular downstream analysis – for phylogenetic studies of Loganiaceae are hereby reported. *Anthocleista vogelli* and other four *Strychnos* species DNA were re-extracted because of their poor quality – in terms of glowing of the gel bands and quantity – in terms of concentration in one nanogram. It was observed that DNA quality less than 1.00 ng/µL could not do well (amplify) in the PCR analysis. Furthermore, the DNA concentration was gradually reducing for all the samples as they were rechecked on biophotometer from time to time to ascertain their integrity. This was due to the handling during use. They are thawn before use, used on ice pellet and returned to the freezer immediately after use.

Table 9: Spectrophotometric check of the DNA quality and quantity

PLANT NAME	ng/ µL	OD $_{230}$	OD_{260}	OD_{280}	OD_{260}/ OD_{280}	$OD_{260/A\ 230}$
A. djalonensis	28	0.33	0.28	0.23	1.22	0.85
A. liebrechtsiana	35	0.41	0.35	0.29	1.21	0.85
A. microphyla	151	2.19	1.51	1.42	1.06	0.69
A. nobilis	77	1.24	0.77	0.73	1.05	0.62
A. obanensis	13	0.14	0.13	0.11	1.18	0.93
A. procera	24	0.26	0.24	0.18	1.33	0.92
A. scandens	30	0.3	0.3	0.24	1.25	1.00
A. schweinfurthii	43	0.55	0.43	0.37	1.16	0.78
A. vogelli	31	0.38	0.31	0.28	1.11	0.82
Mostuea batesii	12	0.14	0.12	0.1	1.20	0.86
M. brunonis	11	0.26	0.11	0.11	1.00	0.42
M. hirsuta	91	1.3	0.91	0.83	1.10	0.70
S. anthelmia	35	0.46	0.35	0.32	1.09	0.76
S. aculeata	76	0.81	0.76	0.62	1.23	0.94
S. afzeli	53	0.63	0.53	0.46	1.15	0.84
S. angolensis	7	0.09	0.08	0.06	1.33	0.89
S. asteranta	24	0.32	0.24	0.23	1.04	0.75
S. barteri	104	1.46	1.04	0.94	1.11	0.71
S. boonei	9	0.1	0.09	0.08	1.13	0.90
S. campicola	11	0.15	0.11	0.09	1.22	0.73
S. camptoneura	4	0.05	0.04	0.03	1.33	0.80
S. chromatoxylon	10	0.11	0.1	0.08	1.25	0.91
S. congolana	20	0.3	0.2	0.17	1.18	0.67
S. cuminodora	36	0.44	0.36	0.31	1.16	0.82
S. densiflora	50	0.55	0.5	0.4	1.25	0.91
S. dinklagei	13	0.18	0.13	0.1	1.30	0.72
S. floribunda	21	0.33	0.21	0.17	1.24	0.64
S. gossweileri	3	0.04	0.03	0.03	1.00	0.75
S. icaja	11	0.14	0.1	0.08	1.25	0.71
S. innocua	4	0.06	0.04	0.04	1.00	0.67
S. johnsonii	11	0.16	0.11	0.1	1.10	0.69
S. longicaudata	29	0.38	0.29	0.23	1.26	0.76

Table 9: Spectrophotometric check of the DNA quality and quantity continued

PLANT NAME	**ng/ µL**	**OD_{230}**	**OD_{260}**	**OD_{280}**	**OD_{260}/OD_{280}**	**$OD_{260/A\ 230}$**
S. lucens	5	0.08	0.05	0.04	1.25	0.63
S. malacoclados	34	0.49	0.34	0.28	1.21	0.69
S. memecyloides	20	0.22	0.2	0.15	1.33	0.91
S. nigritana	20	0.27	0.2	0.17	1.18	0.74
S. nux-vomica	34	0.58	0.34	0.29	1.17	0.59
S. phaeotricha	34	0.51	0.34	0.28	1.21	0.67
S. soubrensis	37	0.61	0.37	0.31	1.19	0.61
S. spinosa	72	0.91	0.71	0.61	1.16	0.78
S. splendens	53	0.85	0.53	0.46	1.15	0.62
S. staudtii	46	0.76	0.46	0.34	1.35	0.61
S. talbotiae	24	0.27	0.24	0.21	1.14	0.89
S. tricalysioides	45	0.57	0.45	0.39	1.15	0.79
S. urceolata	30	0.36	0.3	0.24	1.25	0.83
S. usambarensis	13	0.18	0.13	0.11	1.18	0.72
S. chrysophylla	20	0.268	0.2	0.17	1.18	0.75
S. ndengensis	53	0.59	0.53	0.48	1.10	0.90
SID 55	33	0.33	0.33	0.28	1.18	1.00
SID 56	48	0.61	0.48	0.39	1.23	0.79
SID 57	10	0.1	0.1	0.08	1.25	1.00
SID 58	18	0.24	0.17	0.15	1.13	0.71
SID 59	43	0.44	0.43	0.35	1.23	0.98
SID 60	52	0.61	0.52	0.45	1.16	0.85
SID 61	16	0.19	0.16	0.13	1.23	0.84
SID 62	7	0.07	0.07	0.05	1.40	1.00
SID 63	24	0.27	0.24	0.19	1.26	0.89
SID 64	6	0.08	0.06	0.05	1.20	0.75
SID 65	37	0.57	0.37	0.35	1.06	0.65

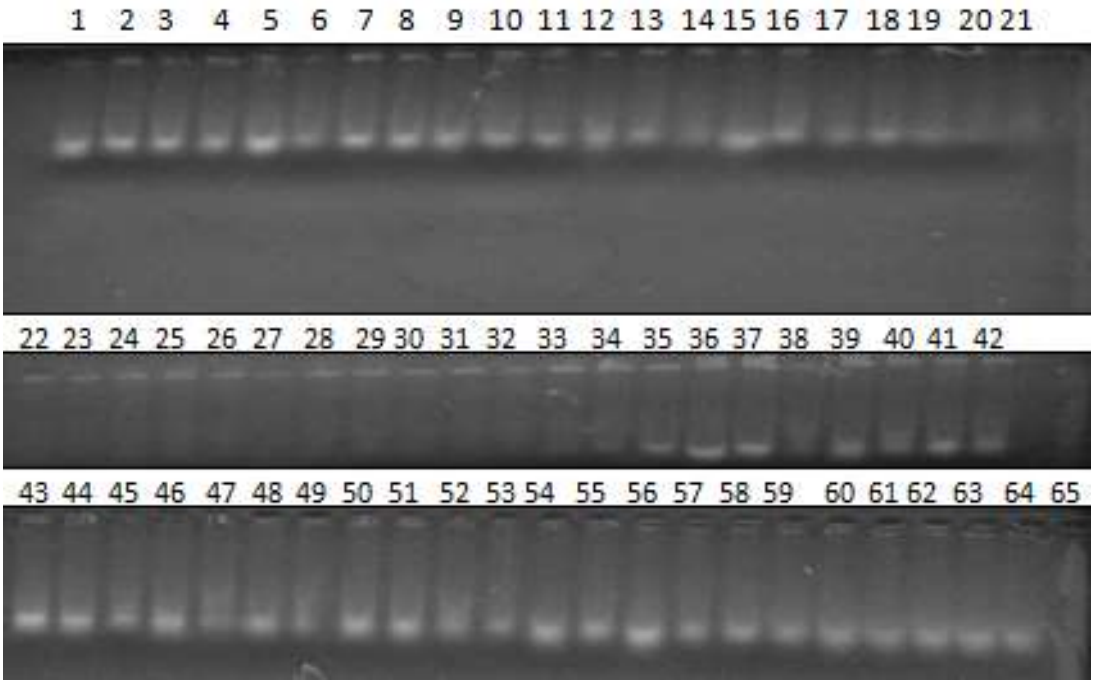

Plate 3A: Electrophorogram of Genomic DNA samples of Loganiaceae species.

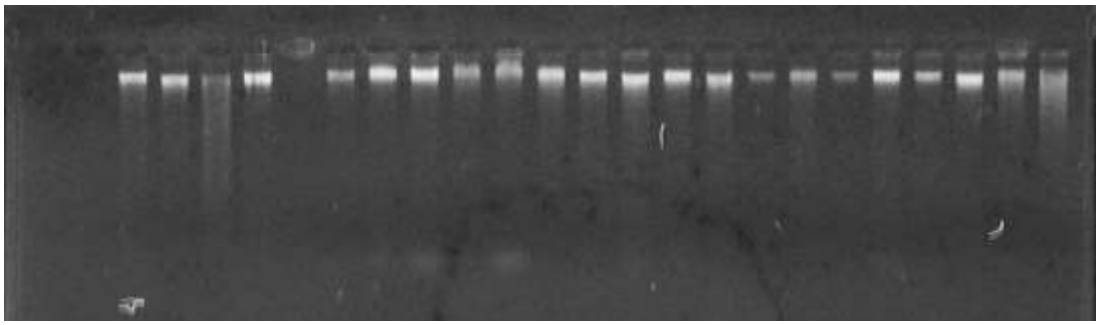

Plate 3B: Electrophorogram of Genomic DNA for 21 selected samples.

4.4.3 Amplification of gene regions and Sequencing

The amplification results of the two ITS genes: ITS3– ITS4 and ITS2–ITS5 were shown on Agarose gel electrophoresis (Plates 3C and 3D). Likewise, the non-coding chloroplast DNA regions amplified includes: trnLC-trnLD and trnLE-trnLF. The gel electrophorograms were represented in (Plates 3E and 3F). Further more, the product of amplification (amplicons) were recleaned in order to remove all reagents used for amplification and reconcentrate the DNA for further analysis – sequencing, and the results were shown on agarose gel electrophoresis (Plates 3G – 3J).

Plate 3C: Electrophorogram of ITS3– ITS4 amplification

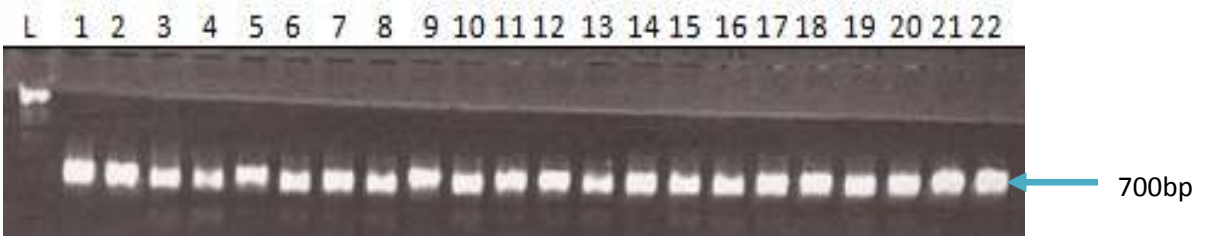

Plate 3D: Electrophorogram of ITS2–ITS5 amplification

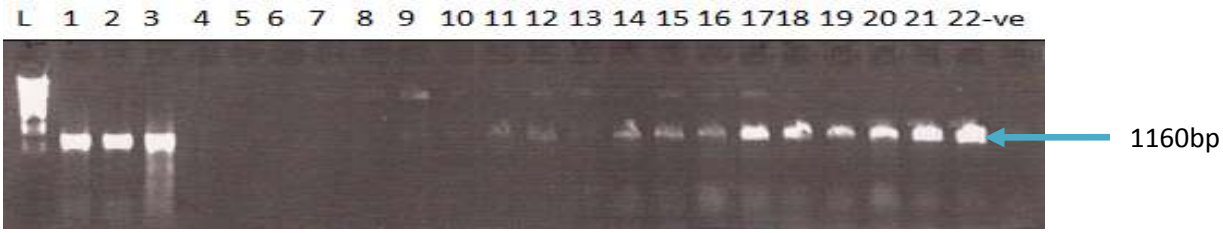

Plate 3E: Electrophorogram of trnlC – trnlD Amplification

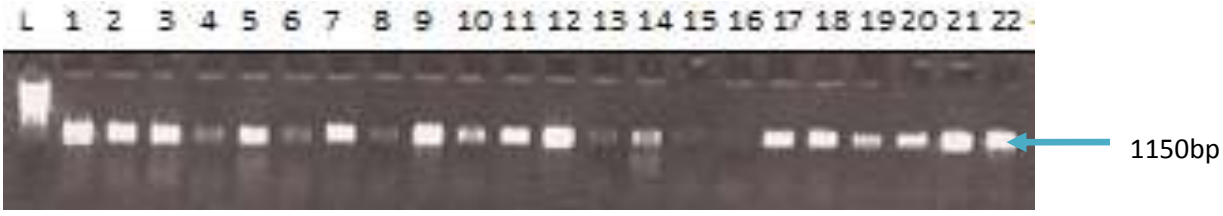

Plate 3F: Electrophorogram trnlE–trnlF amplification (L= Ladder, -ve = Negative control).

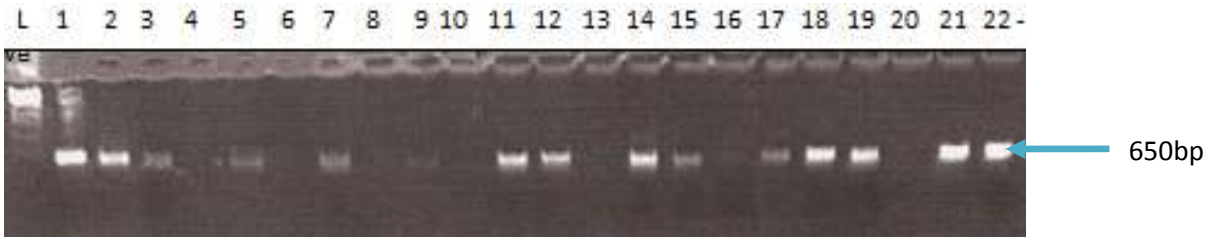

Plate 3G: Electrophorogram of ITS3– ITS4 purification

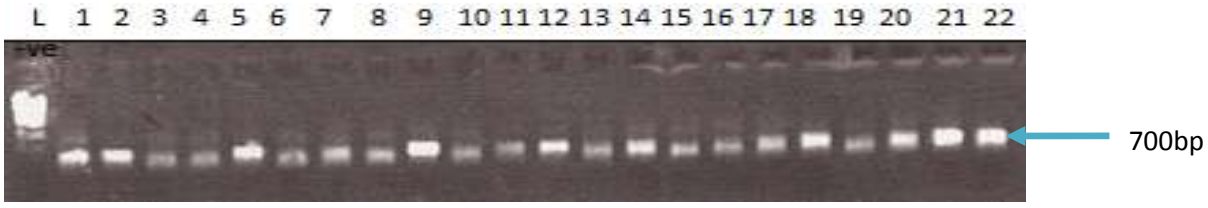

Plate 3H: Electrophorogram of ITS2–ITS5 purification

Plate 3I: Electrophorogram of trnlC – trnlD Purification

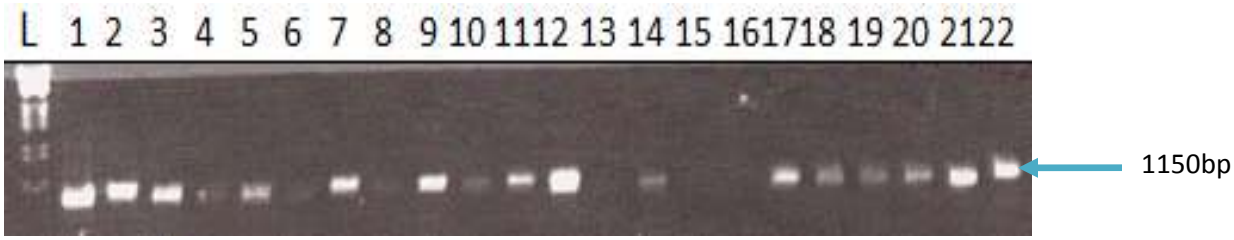

Plate 3J: Electrophorogram of trnlE –trnlF Purification (L= Ladder, -ve = Negative control).

4.4.4 Phylogenetic Analysis

The 21 samples yielded sequences for the various gene regions used. Parsimony and Distance method analysis carried out on each gene separately and combined show the evolutionary relationships among the genera of Loganiaceae (Figure 7A - 7E).

The sequences generated were aligned using both automated alignment and manual methods. The manully aligned sequences, though it took days but the phylogenetic result was meaningful more than the automated alignments. However, there were difficulty with the alignment of *Anthocleista, Mostuea* and *Nuxia* with *Strychnos*. Strychnos was used as the reference point because it is the most speciose in the family. Meanwhile, the alignment of *Spigelia, Usteria* with Strychnos did not encounter such difficulty. Hence, several gaps were deleted and in some cases, added in order to obtain the required positional homology.

As usual, a genus was taken out, as the reference point (outgroup) to which others were compared based on the amino acid sequences on the same gene sequenced. *Mostuea* was the out group used in figure 7A and the relationship revealed that *Usteria guineensis* was in the same clad with *Strychnos* with 36 % similarity coefficient. Within the same phylogenetic tree, *Anthocleista* did not show any relationship – when there is no bootstrap value. In the same way, *Nuxia congesta* and *Spigelia anthelmia* did not show any relationship with *Mostuea* (outgroup).

Meanwhile, the region of genes used was a nuclear ribosomal region called ITS. A singular gene region is not adequate to draw phylogenetic inference without being bias. Thus, the chloroplast region was used in turn.

Considering the phylogentic tree in Figure 7C, the outgroup used was *Nuxia congesta*. The relationships of this genus with others are as follows: *Anthocleista* and *Mostuea* did not show any relationship with others but *Usteria* and *Spigelia* did. In several other attempts of

phylogentic tree ran for the studies, *Anthocleista* was used as the outgroup and the result remained the same. Three genera agreed (*Mostuea*, *Anthocleista* and *Nuxia*) and the other three (*Spigelia, Usteria* and *Strychnos*) would not cluster with them.

This inference was finally tested with the combination of all matrices generated from all gene regions used for phylogenetic studies (Figure 7B). *Nuxia* was used as the outgroup for the analysis and it was found out that: *Strychnos* agreed with *Usteria* as they cluster together with similarity coefficient of 33 % in the species tree. Furthermore, *Spigelia* clustered with *Strychnos* at about 74 % similarity coefficient. Finally, *Nuxia* was taken out as the out group; *Mostuea* and *Anthocleista* did not have any relationship in the phylogenetic tree. Meanwhile, the combined parsimony with 2557 nucleotide character long was 1572 character informative and 985 uninformative.

In order to confirm the correct identification of the *Strychnos* Indeterminate (SID) used in the course of these studies, a phylogenetic analysis was carried out with all the *Strychnos* sequenced and *Anthocleista* was used as the outgroup (Figure 7E). All the *Strychnos* Indeterminate were connected with high similarity coefficient.

S. campicola has a similarity coefficient of 79 % with SID 58, SID 60 and SID 64;

S. boonei has a similarity coefficient of 52 % with SID 61;

S. urceolata, S. spinosa and *S. staudtii* all have similarity coefficient of 31 % with SID 57;

S. staudtii has a similarity coefficient of 96 % with SID 62.

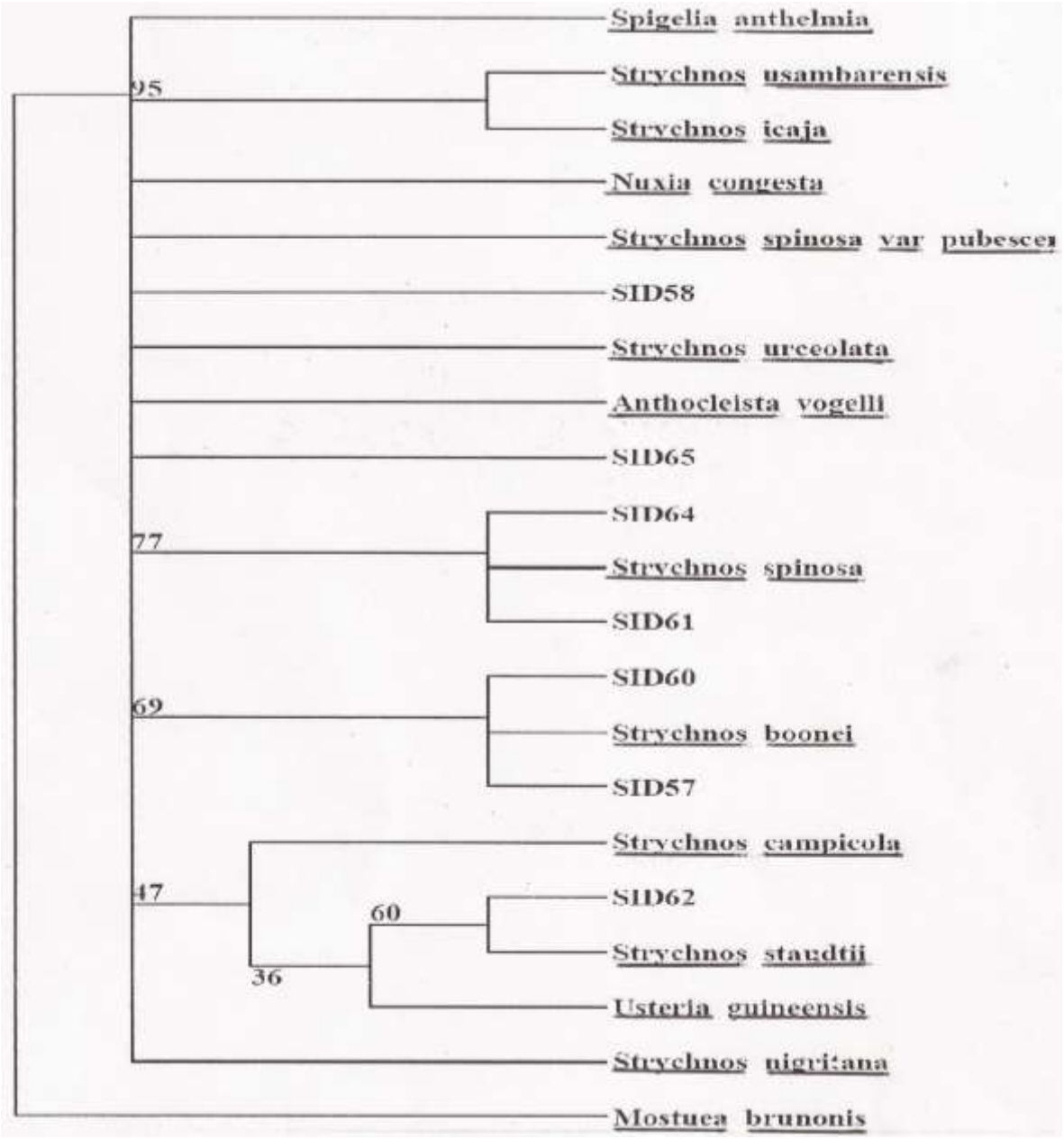

Figure 7A: Phylogenetic relationship of Loganiaceae showing their grouping by ITS matlrices with their bootstrap values.

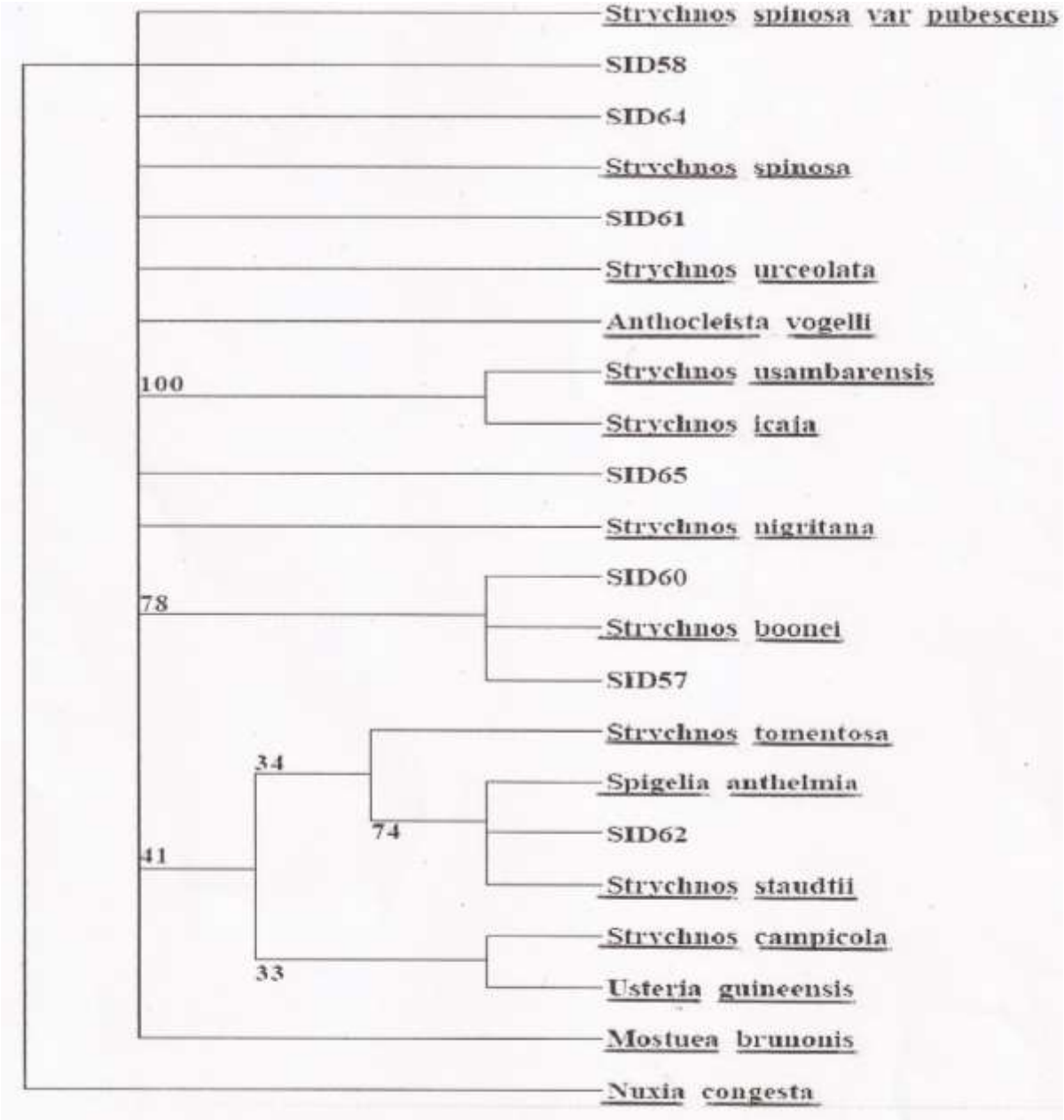

Figure 7B: Phylogenetic relationship of Loganiaceae showing their grouping by combined matrices; *Nuxia* re-rooted with their bootstrap values.

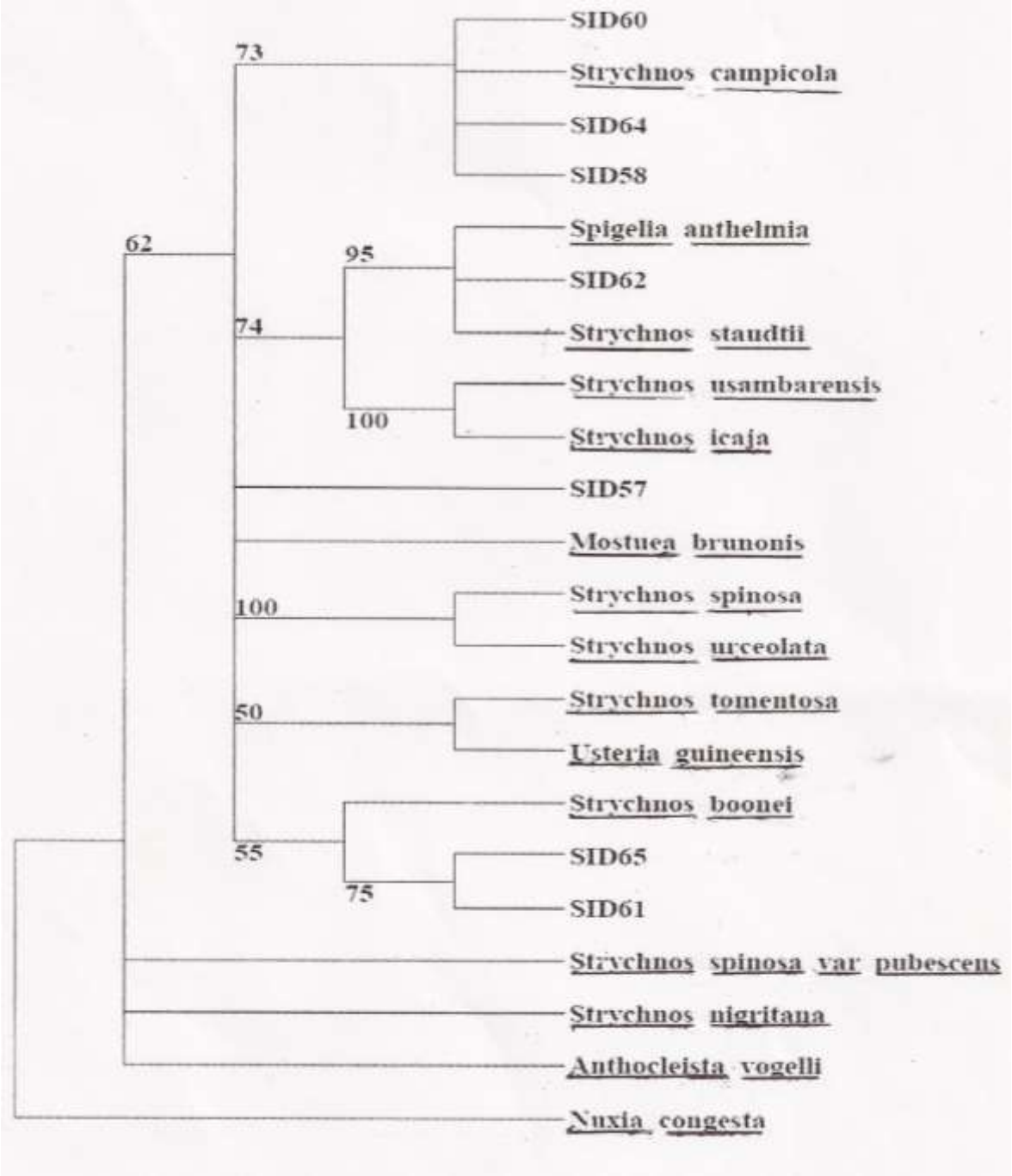

Figure 7C: Phylogenetic relationship of Loganiaceae showing their grouping by trnL E-F matrices; *Nuxia* re-rooted with their bootstrap values.

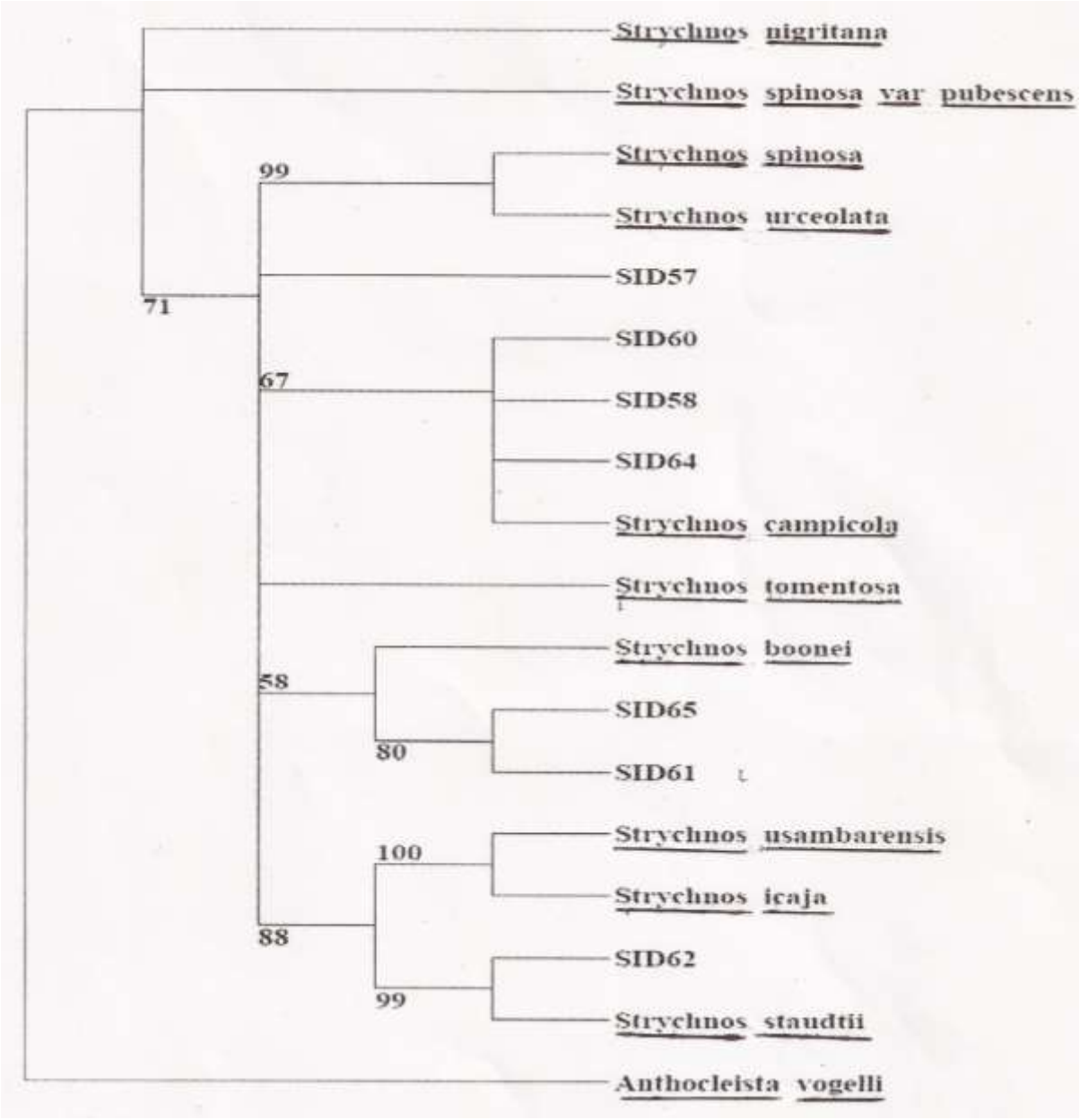

Figure 7D: Phylogenetic relationship of *Strychnos* showing their grouping by combined matrices (trnL c-d and trnL e-f); *Anthocleista* re-rooted with their bootstrap values.

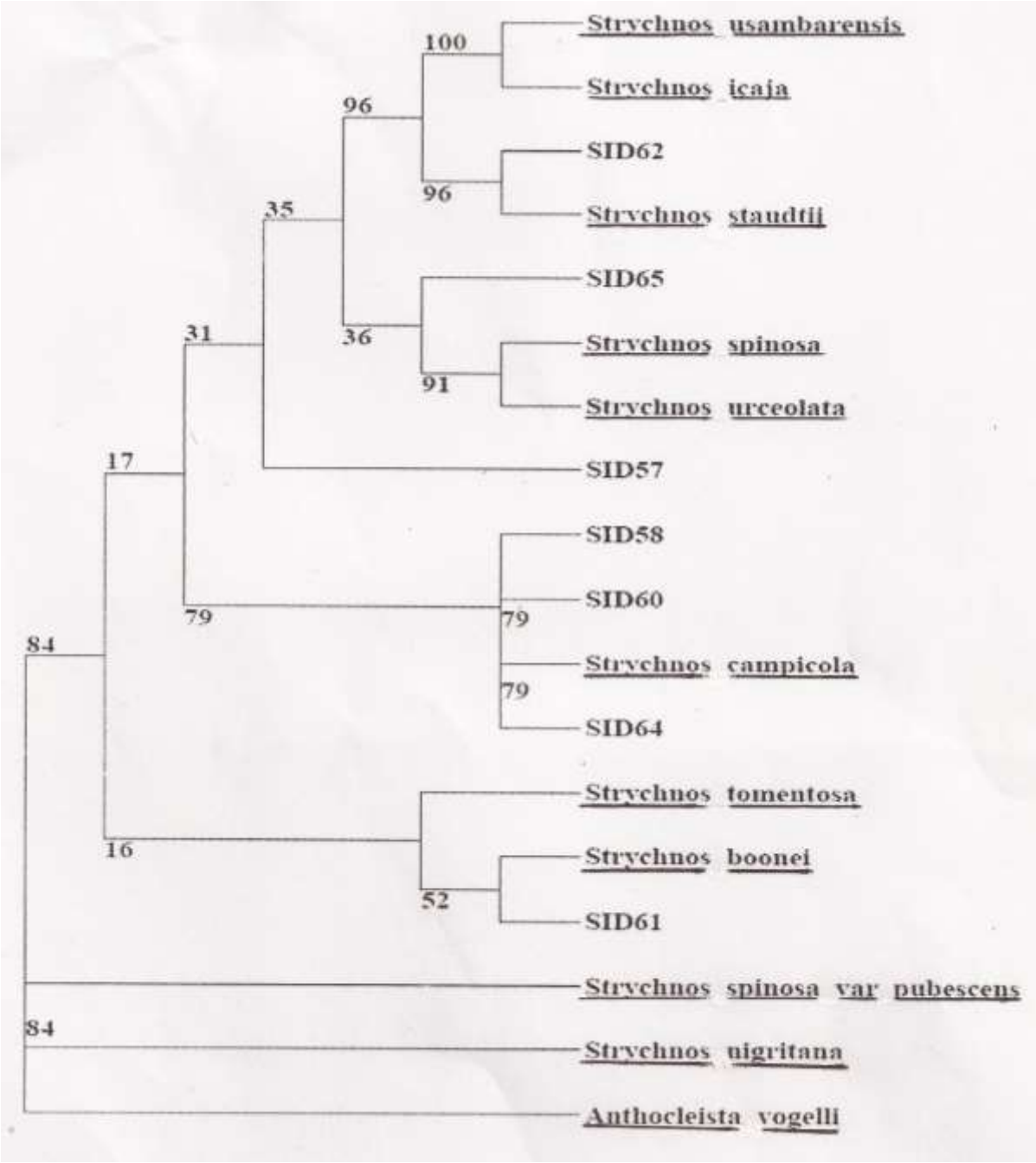

Figure 7E: Phylogenetic relationship of *Strychnos* based on trnL E-F matrices; *Anthocleista* re-rooted with their bootstrap values.

4.5 PLANT IDENTIFICATION AND TAXONOMIC KEY

4.5.1 Taxonomic Keys for Loganiaceae Species in West Africa

An indented dichotomous key to the West African species of Loganiaceae

1a. Herbaceous, annual plant ---------- *Spigelia anthelmia*

1b. Woody shrubs, trees, or climbers, perennial plant ---------- 2

2a. Leaf blade with 1–3 pairs of distinct longitudinal secondary veins from or near base; tendrils and thorns present, Stomata not anisocytic ---------- 3

3a. Savanna or secondary Forest, plant habit tree ---------- 4

4a. Savanna, plant bark rough ---------- 5

5a. Spine present and long, leaf surface glabrous ---------- *Strychnos spinosa*

5b. Spine absent, leaf surface pubescent ---------- *Strychnos innocua*

4b. Secondary Forest, plant bark smooth ---------- 6

6a. Flower colour yellow, Stomata paracytic ---------- *S. nux-vomica*

6b. Flower colour white, Stomata anomocytic ---------- *S. staudtii*

3b. High Forest, plant habit climbers ---------- 7

7a. Hook single, Bark smooth ---------- 8

8a. Leaf apex acuminate, Flower colour white ---------- 9

9a. Leaf surface pubescent, Cuticular striations absent ---------- *S. boonei*

9b. Leaf surface glabrous, Cuticular striations present ---------- 10

10a. Leaf more than 10 cm long, leaf shape obovate ---------- 11

11a. Stomata anomocytic, stomata index greater than 15.0 ------- *S. campicola*

11b. Stomata paracytic, stomata index less than 15.0 ---------- 12

12a. Trichome absent, epidermal cell number 192 ---------- *S. congolana*

12b. Trichome present, epidermal cell number 163 ---------- 13

13a. Leaf shape ovate, plant height 34 m --------- *Strychnos* sp. (P01860082)

13b. Leaf shape elliptic, plant height 15 m ---------- *S. chrysophylla*

10b. leaf less than 11 cm long, leaf shape ovate ---------- 14

14a. Epidermal cell number less than 100, stomata anomocytic ---------- 15

15a. Branches more than 4 in every 1 metre, leaf shape lanceolate ----- 16

16a. Leaf length 6-10 cm, internode length 7 cm ------ *S. chromatoxylon*

16b. Leaf length 3-9 cm, internode length 4-6 cm --------- *S. ndengensis*

15b. Branches less than 4 in every 1 metre, leaf shape oblanceolate ---- 17

17a. Trichome present, stomata index 12.18 ---------- *S. icaja*

17b. Trichome absent, stomata index 14 ---------- *S. malacoclados*

14b. Epidermal cell number greater than 100, stomata paracytic ---------- 18

18a. Leaf base attenuate, branches less than 4 in every 1 metre ------- 19

19a. Crystals found, trichome present ---------- *S. floribunda*

19b. Crystals not found, trichome absent ---------- *S. tomentosa*

18b. Leaf base round, branches more than 4 in every 1 metre ---------- 20

20a. Leaf length-width ratio 2.0, internode length 3 -------- *S. angolensis*

20b. Leaf length-width ratio 3.0, internode length 5 ------- *S. splendens*

8b. Leaf apex acute to caudate, Flower colour Yellow ---------- 21

21a. Leaf shape elliptic, anticlinal wall curved ---------- S. *longicaudata*

21b. Leaf shape ovate, anticlinal wall straight ---------- S. lucens

7b. Hook paired, Bark rough ---------- 22

22a. Anticlinal wall pattern straight, Epidermal cell shape polygonal ---------- 23

23a. Branches few, apex acute to caudate---------- 24

24a. Stomata paracytic, wax absent ---------- *S. aculeata*

24b. Stomata anomocytic, soft wax present ---------- *S. dinklagei*

23b. Branches many, apex rounded to acuminate ---------- 25

25a. Petiole length 0.1 cm – 0.5 cm, Leaf shape ovate ---------- 26

26a. Flower colour yellow, Stomata anomocytic ---------- *S. urceolata*

26b. Flower colour white, Stomata paracytic ---------- 27

27a. Leaf length 5 cm – 11 cm, stomata index 9 ---------- *S. johnsonii*

27b. Leaf length 7 cm – 12 cm, stomata index 6.5 ---------- *S. afzeli*

25b. Petiole length 0.6 cm – 1.0 cm, Leaf shape elliptic to lanceolate --------- 28

28a. Trichome absent, abaxial stomata number 40 ---------- *S. asteranta*

28b. Trichome simple unicellular, abaxial stomata number 14 --- *S. talbotiae*

22b. Anticlinal wall pattern curved, Epidermal cell shape irregular ---------- 29

29a. Leaf shape obovate, leaf base attenuate ---------- 30

30a. Branches more than 4 in every 1 metre, cuticle thick ---------- 31

31a. Trichome simple unicellular, stomata number above 13 ---------- 32

32a. Striations present, stomata index 13.39 ---------- *S. usambarensis*

32b. Striations absent, Stomata Index 10.75---------- *S. nigritana*

31b. Trichome absent, stomata number 5 ---------- *S. barteri*

30b. Branches less than 4 in every 1 metre, cuticle thin---------- 33

33a. Cell shape plygonal, cell number below 100 ---------- 34

34a. Soft wax present, stomata index 5.6 ---------- *S. densiflora*

34b. Soft wax absent, stomata index 9.7 ---------- *S. camptoneura*

33b. Cell shape tetra or pentagonal, cell number above 100 ---------- 35

35a. Striations absent, trichome absent ---------- 36

36a. Stomata width 1.0 µm, stomata index 39.98 ---------- *S. tricalysioides*

36b. Stomata width 1.3 µm, stomata index 8.67 ---------- *S. phaeotricha*

35b. Striations present, trichome present ---------- *S. soubrensis*

29b. Leaf shape ovate, leaf base obtuse ---------- 37

37a. Trichome absent, Petiole length 0.1 cm – 0.5 cm ---------- 38

38a. stomata index 3.7, flower pink ---------- *S. cuminodora*

38b. stomata index 15.7, flower white ---------- *S. gossweileri*

37b. Trichome present, Petiole length 0.6 cm – 1.0 cm ---------- *S. memecyloides*

2b. Leaf blade with network of veins, tendrils and thorns absent, Stomata anisocytic ----------

Usteria guineensis

CHAPTER FIVE

5.0 DISCUSSION

In this study, the analysis based on the morphological evidence of 25 characters revealed 7 clusters at a threshold of 47 % similarity. Clusters 1 and 2 (Figure 5A) clearly revealed *Anthocleista* species as being partly separated from other species of Loganiaceae. At this threashold, similarity coefficient, they are not completely grouped with other species of Loganiaceae. This agrees with previous findings (Leeuwenberg and Leenhouts, 1980; Struwe *et al*., 1994; Backlund *et al*., 2000; Frasier, 2008). Although, Cluster 5 - *Mostuea* species was nested with *Strychno*s species, they have their root completely separated from *Strychnos* even at about 31 % similarity, showing that they are distantly related (Backlund *et al*., 2000). The Savanna species of *Strychnos* - *Strychnos innocua* and *Strychnos spinosa* were also nested together with *Nuxia* and *Usteria* species. This is because; they are tree species with several similar morphological charaters in common. This grouping gave clues as to what species should be selected for sequencing at the Royal Botanic Garden, Kew, London. Those that were grouped together as a cluster have a high coefficient of similarity. Those that remained ungrouped were selected automatically since their separation is worthy of note in Loganiaceae taxonomy (Radford *et al*., 1974). The groupings at a threshold of 36 % similarity coefficient (Figure 5A) as depicted by another reference line (R) showed a different view because the coefficient of similarity is very low. *Anthocleista* species were combined with other tree species of *Strychnos* genus in the family. *Anthocleista* was also grouped with *Spigelia anthelmia, Nuxia congesta,*and *Usteria gueineensis*. *Mostuea* species were grouped separately and not with any other genus in the family. They are separated from them to indicate some level of dissimilarity among the genera. The removal of *Mostuea* from Loganiacae had been suggested by previous workers on the family (Strwue *et al.,* 1994, Takhtajan**,** 1997; Backlund *et al*., 200). The dedrogram generated

by the gross morphological data (Figure 6D) reveal that the higher the number of characters used for taxonomic deductions, the closer to the true species tree would the phylogenetic tree be

The Principal Component Analysis (PCA) of leaf characters that were assessed quantitatively revealed two components that contributed about 64% in the analysis; the leaf length and leaf width. From the field, the family shows a considerable variation in their leaf shapes and sizes. The component 1 (C1) revealed leaf length and width (size) as the major characters that could delimit Loganiaceae among the characters used. The extraction, under communality is a measure of the relative variations observed in the characters examined. Scatter plots based on grouped centroids and spatial distribution revealed *Anthocleista* species to be entirely different as they spread out in space, because they have unique foliage in the family.

In the biogeography of Loganiaceae, the omission rate and predicted area were tested as a function of the cumulative threshold and omission rate is calculated both on the training presence records and on the test records. The omission rate is close to the predicted omission because of the cumulative threshold.

The response curves show the marginal effect of changing exactly one variable. The curves show how each environmental variable affects the prediction; how the logistic prediction changes as each environmental variable is varied, keeping all other environmental variables at their average sample value. As each environmental variable is varied, the probability of occurrence of the samples:

Decreases with varying Bio 2, 6, 7, 8, 13, 14, 15, 17 and 18;

Decreases but later increases with varying Bio 3;

Remains constant with varying Bio 4, 5, 11, 12 and 16;

Increases with varying Bio 1, 9, 10 and 19.

In contrast to the marginal response curves in Figure 4 G, Figure 4 I (a-s) model was created using only the corresponding variable. The plots reflect the dependence of predicted suitability both on the selected variable and on dependencies induced by correlations between the selected variable and other variables.

A heuristic estimate of relative contributions of the environmental variables to the Maxent model shows that Bio 19 has the highest percentage contribution to the model (44.9 %), Bio 1 has the least contribution (0.2 %) while Bio 5, 12 and 16 did not contribute at all. Therefore, the species distribution of Loganiaceae in West Africa is most affected by the Precipitation of coldest quarter (November – January), followed by the precipitation of the driest month (March), minimum temperature of coldest month (December) and by precipitation of warmest quarter (February - April).

The task of a modeling Loganiaceae predicts environmental suitability of the species as a function of the given environmental variables (Pulliam, 2000; Anderson and Mart´ınez-Meyer, 2004). Therefore among many other things, the model's significance to exploration and collection of Loganiaceae are as follow:

Spigelia anthelmia: It is an annual herb that could be found during both the rainy and dry seasons but close to region of regular water supply during the dry season.

Anthocleista species: Tree species found close to water such as swamps. They grow stilt roots in search of water in semi dry zones.

Strychnos species: they are woody climbers found in thick forests; usually the understory of such forest is damp, moist with high humidity. When there is a river or stream in the forest, *Strychnos* form dense network of crowns (canopies) by the river course using a suitable host.

Usteria species: they are climbers growing at the forest edges, road sides and in cultivated lands near a stream or river. The family members generally flower from October and fruit until February the following year. The model – Bio 19 in particular, supportd this; if this environmental factor is altered in a year, the plant may not flower at all or may flower but not able to carry the fruit, hence, they senesce. It uses the available water to support its vegetative structures.

Carlquist (1961) stated that "the leaf is perhaps anatomically the most varied organ of angiosperms" (Radford *et al.,* 1974). The micromorphological features of the species of Loganiaceae show interconnection and overlapping of characters. The micromorphological features of each genus look very much alike than they are to another genus in the same family (Radford *et al.,* 1974). The epidermal cell shapes in *Anthocleista* species are mainly polygonal and irregular. Their cell wall patterns range from straight, curved to sinuous. *Anthocleista* are unique in Loganiaceae for their type of trichomes: they are stellate and dendritic with varying number of arms and arms' length. This is in line with the record of Metcalfe and Chalk (1979). The study revealed that *A. djalonensis, A. obanensis* and *A. vogelli* have stomata on both surfaces (amphistomatic); *A. microphyla* and *A. nobilis* have only on the abaxial surface while *A. liebrechtsiana*, *A. procera*, *A. scandens* and *A. schweinfurthii* are devoid of trichomes. These observations had earlier been made by Fahn (1977); Metcalfe and Chalk (1983) and Carlquist (1961). Trichomes have been known to be useful as an excellent tool for classification at subgeneric and specific levels (Cowan, 1950; Carlquist, 1961). In support of earlier studies, this study agrees with the fact that it is better to interpret the trichome distribution with respect to species in a genus and then delimit the genus from another within the same family. This study (Table 6 D) also revealed that cuticular ornamentations are of varying types within *Anthocleista*; hypodermis conferred sunken stomata, varying height of cuticle and various forms of striations

(Cowan, 1950; Metcalfe and Chalk, 1950, 1979; Carlquist, 1961; Moorea *et al.,* 2010). Previous workers observed that: *Anthocleista* leaves are usually coriaceous in texture, epicuticular waxes coat their epidermis to protect the plant from desiccation and pest attacks while also controlling leaf temperature and signaling between pollen and stigma etc (Taiz and Zeiger, 2002; Sonibare *et al*., 2007; Moorea *et al.,* 2010). In *Anthocleista*, stomata type is anomocytic, staurocytic or anisocytic and their indices vary between 0.43 % in *A. schweinfurthii* and 16.46 % in *A. obanensis*. The stomata types in the family also overlap because of their familial similarity but indices vary greatly. The most important component as revealed by Principal Component Analysis (PCA) among the quantitative characters assessed was the Stomata features; either stomata number or the stomata index. The PCA in Table 7B shows that stomata index has the highest contributing capacity (0.962) to the grouping of Loganiaceae. This taxonomic evidence was observed for classification by Metcalfe and Chalk (1983). The stomata are concentrated on the lower side of the leaf and it forms the most important character capable of delimiting plant species, it follows that the abaxial surface is more useful in leaf epidermal analysis.

The pioneer workers on this family clustered *Anthocleista* and *Mostuea* with Loganiaceae based on the characters they share such as valvate aestivation, coriaceous leaves, ability to accumulate aluminium and several other anatomical features (Carlquist, 1961; Metcalfe and Chalk, 1979; Leeuwenberg and Leenhouts, 1980). Similarity analysis showed that the adaxial surface is very similar in the species examined and less informative than the abaxial data when they are treated separately. The abaxial surface is highly informative and useful for delimitation of the species of Loganiaceae. The gross morphological data and dendrogram revealed that *Anthocleista* could separate out but at a very low affinity withing the family (Figure 6D). Notwithstanding, the genera in the family have not been totally resolved into separate clusters.

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* in particular (when there is no bootstrap value, Figures 7A and 7B). Hence, there is high propensity for them to be removed from the family (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* matrices requiring many gaps for alignment. Frasier (2008) reported that the ITS region is prone to more indels (insertions and deletions) 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 two genera are evolutionarily not similar with Loganiaceae. However, Brinegar (2009) commented that "more than one locus is generally required in molecular systematics in order to be able to construct a reliable phylogenetic relationship; this will minimize errors and wrong interpretation". The combined matrices presented a robust data that circumscribed out three genera which ITS matrix alone could not do. Previous workers have identified polyphyletic assemblage in this family (Leenhouts, 1962; Leeuwenberg and Leenhouts, 1980) because it occupies a central evolutionary position 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.

The *Strychnos* indeterminate matrixes were subjected to Basic Local Alignment Search Tools (BLAST) and the nearest matrixes were used for comparison in database (Cowan *et al*., 2006; 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 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* was 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 (Appendix 6).

CHAPTER SIX

SUMMARY OF FINDINGS AND CONCLUSION

6.0 Summary of Findings

- Majority of Loganiaceae species are abundant in forest vegetations. two new species of *Strychnos*; *Strychnos tomentosa* and *Strychnos* sp. (*species nova* - P01860082) were discovered and this is the first record in West tropical African flora. Voucher specimens were preserved and deposited in two herbaria in Nigeria: FHI and LUH.
- The distribution of Loganiaceae species in West Africa is affected most by the precipitation of coldest quarter of the year (November – January, 44.9 %); followed by the precipitation of the driest month (March, 31.5 %), followed by the minimum temperature of coldest month (December, 13.7 %) and by precipitation of warmest quarter (February – April, 1.9 %).
- Loganiaceae comprises 3 genera in West Africa (*Spigelia*, *Strychnos* and *Usteria*) with 39 species as opposed to the 6 genera with 52 species originally circumscribed in the family.
- Genomic DNA samples were successfully isolated and they have been deposited in the DNA Bank of the Royal Botanic Gardens Kew, London. PCR, gene Sequencing and Alignments were successfully carried out for Phylogenetic analysis.
- A reliable taxonomic key for the accurate identification of all species in Loganiaceae family has been successfully prepared.

6.1 Conclusion

It is clear from this studies that DNA characters provide an indispensable complement to traditional systematic analyses. In many cases they help to choose among different hypotheses of phylogenetic relationships not discriminable by traditional methods, or provide new notions on the location of misplaced taxa (Caputo, 1997). As observed by Nicholas and Baijnath (1994), 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.

6.2 Contributions to Knowledge

This work has contributed to knowledge in the following ways:

- Loganiaceae comprises 13 genera worldwide; three genera in West Tropical Africa (WTA). They are: *Spigelia, Strychnos* and *Usteria*; with 39 species. Three genera have been removed; *Anthocleista*, *Mostuea* and *Nuxia*. Two plant species: *Strychnos tomentosa* and *Strychnos* sp. (P01860082) one which is new to science and the other a new introduction to flora of Nigeria and West Africa.
- This study has affirmed that the distribution of Loganiaceae species in West Africa is most affected by the precipitation of coldest quarter of the year (44.9 %); followed by the precipitation of the driest month (31.5 %), followed by the minimum temperature of coldest month (13.7 %) and by precipitation of warmest quarter (1.9 %). This would enhance efforts and measures aimed at conservation, monitoring and sustainable use of Loganiaceae species.
- The genomic DNA for 21 samples were sequenced and matrices generated successfully for all gene regions used for the first time. Their genomic DNA have been deposited in the DNA Bank of the Royal Botanic Gardens, Kew, London.

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APPENDIX

Appendix 1: The code and common names of Loganiaceae species

Genera	Species	Scientific name	CODE NAMES	Common name
1	1	*Anthocleista djalonensis* **A. Chev.**	ADJ1	Cabbage tree
	2	*Anthocleista liebrechtsiana* **De Wild & Th. Dur.**	ALI2	Cabbage tree
	3	*Anthocleista microphyla* **Wernham**	AMI3	Cabbage tree
	4	*Anthocleista nobilis* **G. Don.**	ANO4	Cabbage tree
	5	*Anthocleista obanensis* **Wernham**	AOB5	Cabbage tree
	6	*Anthocleista procera* **Lepr. Ex Bureau**	APR6	Cabbage tree
	7	*Anthocleista scandens* **Hook.**	ASD7	Cabbage tree
	8	*Anthocleista schweinfurthii* **Gilg**	ASF8	Cabbage tree
	9	*Anthocleista vogelli* **Planch.**	AVO9	Cabbage tree
2	1	*Mostuea batesii* **Bak.**	MBA14	N/A
	2	*Mostuea brunonis* **Didr**.	MBR15	N/A
	3	*Mostuea hirsuta* **T. Anders. Ex Benth.**	MHI16	N/A
3	1	*Nuxia congesta* **R. Br. Ex Fresen.**	NCO18	Brittle-wood
4	1	*Spigelia anthelmia* **Linn.**	SAT19	Worm weed
5	1	*Strychnos aculeata* **Solered**	SAC20	Monkey orange
	2	*Strychnos afzeli* **Gilg.**	SAF21	Monkey orange
	3	*Strychnos angolensis* **Gilg.**	SAG22	Monkey orange
	4	*Strychnos asteranta* **Leeuwenberg**	SAS23	Monkey orange
	5	*Strychnos barteri* **Solered**	SBA24	Monkey orange
	6	*Strychnos boonei* **De Wild.**	SBO25	Monkey orange
	7	*Strychnos campicola* **Gilg.**	SCP26	Monkey orange
	8	*Strychnos camptoneura* **Gilg. et Busse**.	SCT27	Monkey orange
	9	*Strychnos chromatoxylon* **Gilg.**	SCH28	Monkey orange
	10	*Strychnos congolana* **C.H. Wright**	SCO29	Monkey orange
	11	*Strychnos cuminodora* **De Wild.**	SCU30	Monkey orange
	12	*Strychnos densiflora* **Bail.**	SDE31	Monkey orange
	13	*Strychnos dinklagei* **Gilg.**	SDI32	Monkey orange
	14	*Strychnos floribunda* **Gilg.**	SFL33	Monkey orange
	15	*Strychnos gossweileri* **Exell**	SGO34	Monkey orange
	16	*Strychnos icaja* **Bail.**	SIC35	Monkey orange
	17	*Strychnos innocua* **Del.**	SIN36	Monkey orange
	18	*Strychnos johnsonii* **Hutch. et M.B.Moss.**	SJO37	Monkey orange
	19	*Strychnos longicaudata* **Gilg.**	SLO38	Monkey orange
	20	*Strychnos lucens* **Bak.**	SLU39	Monkey orange
	21	*Strychnos malacoclados* **C.H. Wright**	SMA40	Monkey orange
	22	*Strychnos memecyloides* **S.Moore**	SME41	Monkey orange
	23	*Strychnos nigritana* **Bak.**	SNI42	Monkey orange
	24	*Strychnos nux-vomica* **Linn.**	SNU43	Monkey orange
	25	*Strychnos phaeotricha* **Gilg.**	SPH44	Monkey orange

Appendix 1: The code and common names of Loganiaceae species continued

Genera	Species	Scientific name	CODE NAMES	Common name
	26	*Strychnos soubrensis* **Hutch. et Dalz.**	SSO45	Monkey orange
	27	*Strychnos spinosa* **Lam.**	SSN46	Monkey orange
	28	*Strychnos splendens* **C.H. Wright**	SSD47	Monkey orange
	29	*Strychnos staudtii* **Gilg.**	SST48	Monkey orange
	30	*Strychnos talbotiae* **S.Moore**	STA49	Monkey orange
	31	*Strychnos tricalysioides* **Hutch.**	STR50	Monkey orange
	32	*Strychnos urceolata* **Leeuwenberg**	SUR51	Monkey orange
	33	*Strychnos usambarensis* **Gilg.**	SUS52	Monkey orange
	34	*Strycnos chrysophylla* **Gilg.**	SCR53	Monkey orange
	35	*Strychnos ndengensis* **Pellegr.**	SND54	Monkey orange
	36	*Strychnos* indeterminate Edondon -2	SID55	Monkey orange
	37	*Strychnos* indeterminate Edondon -3	SID56	Monkey orange
	38	*Strychnos* indeterminate Erokut station -2	SID57	Monkey orange
	39	*Strychnos* indeterminate Erokut station -3	SID58	Monkey orange
	40	*Strychnos* indeterminate Edondon -1	SID59	Monkey orange
	41	*Strychnos* indeterminate Edondon -8	SID60	Monkey orange
	42	*Strychnos* indeterminate Edondon -4	SID61	Monkey orange
	43	*Strychnos* indeterminate Ipetu- Ijesha	SID62	Monkey orange
	44	*Strychnos* indeterminate J_4 -3	SID63	Monkey orange
	45	*Strychnos* indeterminate Erokut station -6	SID64	Monkey orange
	46	*Strychnos* indeterminate Edondon -6	SID65	Monkey orange
	47	*Strychnos* indeterminate ENUGU	SID66	Monkey orange
6	1	*Usteria guineensis* **Willd.**	UGU67	N/A

N/A = Not Available

Appendix 2: Morphological descriptors and their codes used for Loganiaceae analysis

Habit = **HB**; based on their height from the soil surface	Vegetation Zone =**VZ**: from the sea level to the desert.	Bark texture **BT**: Smooth or rough.	Flower fresh colour **FC:** Based on their warmness.
Herb = 1	Mangrove = 1	Smooth = 1	White = 1
Shrub = 2	Swamp = 2	Rough = 2.	Creamy = 2
Tree = 3	Secondary forest = 3	**S**: present or absent	Creamy yellow = 3
Epiphyte = 4	High forest = 4	Present = 1	Yellow = 4
Liana = 5	Savanna = 5	Absent = 2	Orange = 5
	Mountain veg. = 6		Lemon = 6

Appendix 2B: Morphological descriptors and their codes used for Loganiaceae analysis continued

Leaf apex = **LA**	Leaf shape = **LS**	Branch **B**: smooth or spiny	Leaf hairiness = **LH**	Leaf base = **LB**
Acute = 1	Elliptic = 1	Smooth = 1	Glabrous = 1	Rounded = 1
Acuminate = 2	Oblong = 2	Spiny = 2	Coriaceous = 2	Cuneate = 2
Apiculate = 3	Ovate = 3	Hook **HK**: number present	Pubescent = 3	Attenuate = 3
Caudate = 4	Obovate = 4	Nil = 1	Hirsute = 4	Obtuse = 4
Cuspidate = 5	Lanceolate = 5	Single = 2	Pilose = 5	2 or 3 character = 5
Obtuse = 6	Oblanceolate = 6	Paired = 3	Others = 6	Leaf veins = **LV**
Round = 7	2 character = 7	Leaf margin = **LM**	Petiole = **P**	Bold = 1
2 characters = 8	3 or more charact. = 8	Entire = 1	Petiolate = 1	Faint = 2
3 or more = 9		Revolute & undulate = 2	Sessile = 2	

Appendix 3A: Anatomical characters assessed and their codes

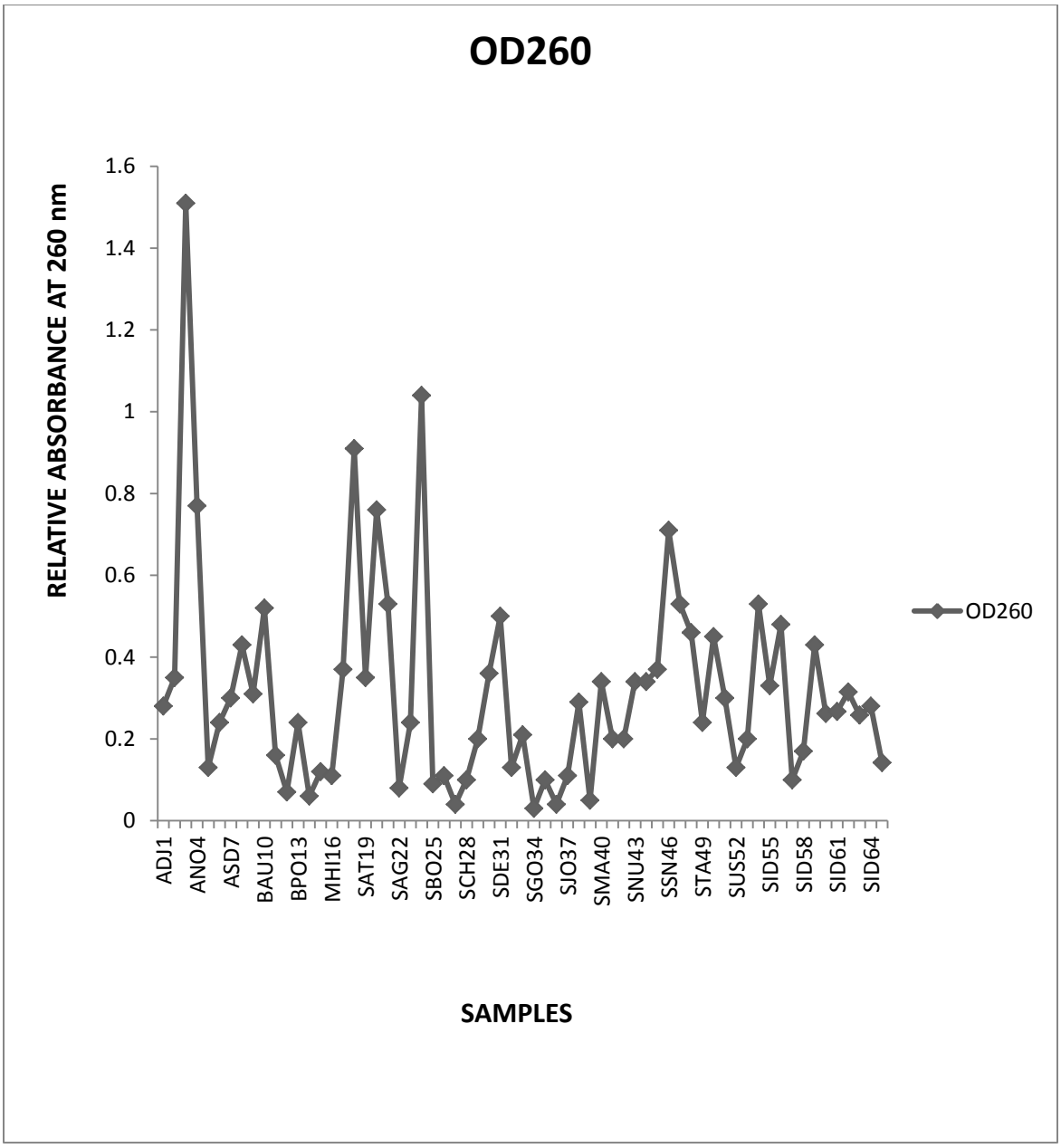
A. AWP = anticlinal wall pattern; due to the degree of curvature	SN = Stomata number	TL = Trichome length
CWT = Cell wall thickness	ST = Stomata type	TD = Trichome density
ECS = Epidermal Cell shape; according to the complexity and number of angles.	SI = Stomata index	CO = Cuticular ornamentation
ECN = Epidermal Cell number	SW = Stomata width	CT = Cuticular thickness
ECL = Epidermal Cell length	SL = Stomata length	TW = Type of wax present
ECW = Epidermal cell width	TT = Trichome type	OES = Other ergastic substance

Appendix 3B: Anatomical descriptors and codes used for Loganiaceae

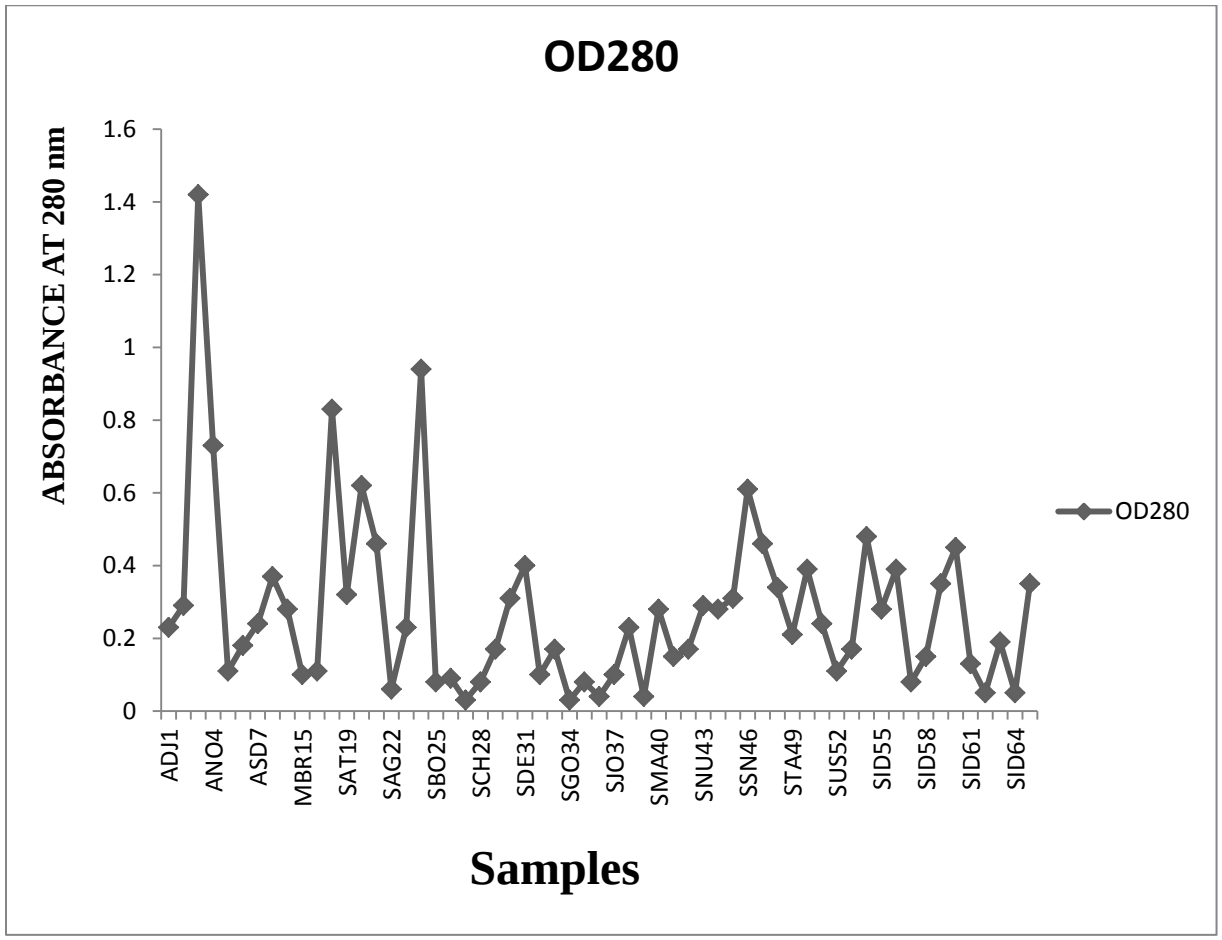
Score	**AWP**	**ECS**	**ST**	**TT**	**TW**	**OES**
1	Straight	Tetra to polygonal	Anisocytic	Simple unicellular	Granules	Starch grains
2	Straight to curved	Penta to polygonal	Anisocytic/anomocytic	Conical	Soft wax coating	Starch deposit
3	Curved	Polygonal	Paracytic	2 to 5 armed	Plate and scales	Crystals
4	Undulate	Polygonal to irregular	Para/anomocytic	Stellate		other structures
5	Undulate to wavy	Irregular	Anomocytic	stellate & dendritic		
6	Wavy		Staurocytic			
7	Barbed					

Appendix 4: Common thresholds and corresponding omission rates for Loganiaceae Maxent

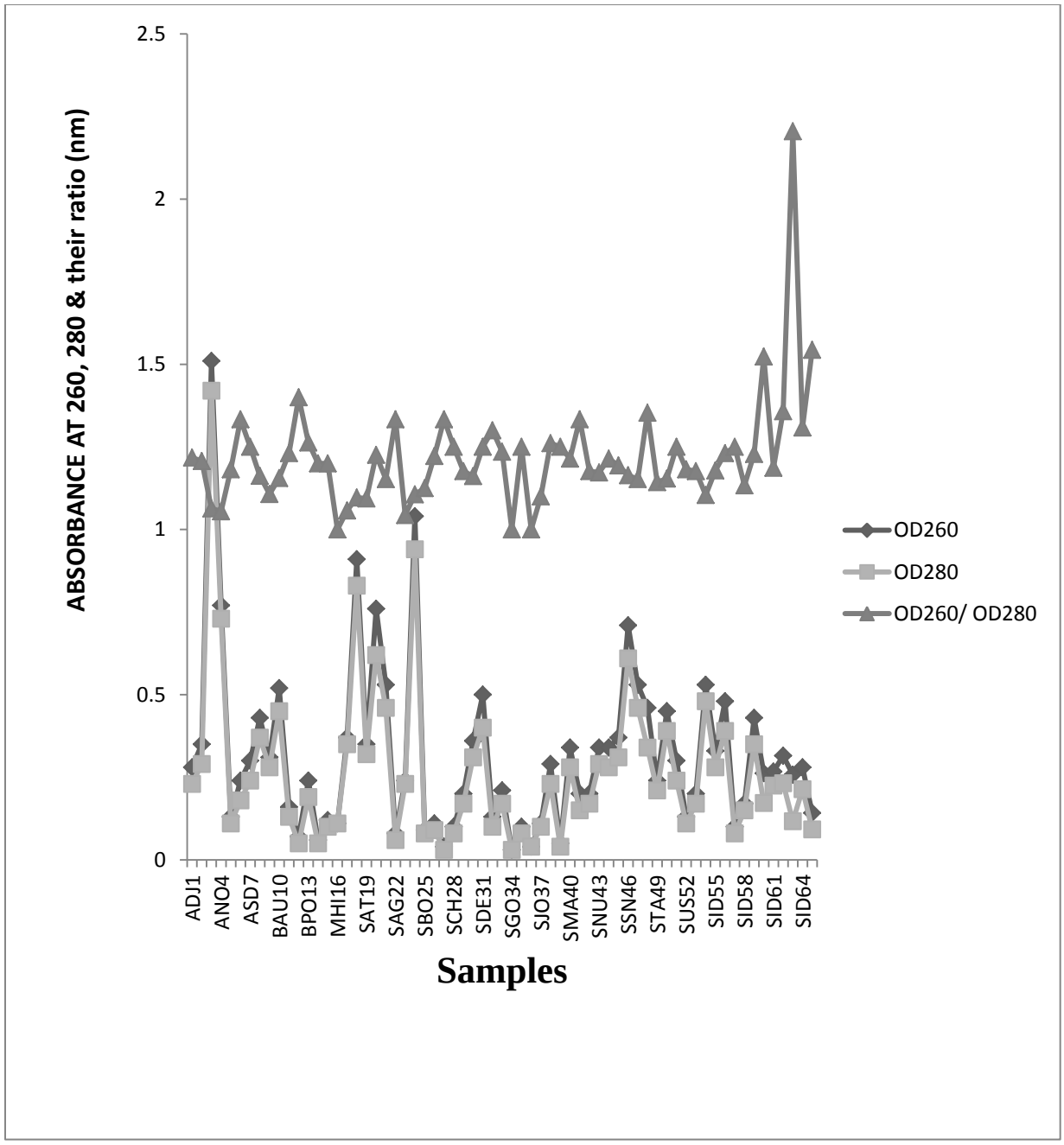
Cumulative threshold	Logistic threshold	Description	Fractional predicted area	Training omission rate	Test omission rate	P-value
1.000	0.030	Fixed cumulative value 1	0.263	0.000	0.250	5.86E-2
5.000	0.085	Fixed cumulative value 5	0.171	0.000	0.250	1.747E-2
10.000	0.153	Fixed cumulative value 10	0.123	0.000	0.250	6.735E-3
17.310	0.262	Minimum training presence	0.085	0.000	0.500	3.894E-2
27.528	0.392	10 percentile training presence	0.058	0.056	0.750	2.137E-1
27.528	0.392	Equal training sensitivity and specificity	0.058	0.056	0.750	2.137E-1
17.310	0.262	Maximum training sensitivity plus specificity	0.085	0.000	0.500	3.894E-2
1.348	0.035	Equal test sensitivity and specificity	0.250	0.000	0.250	5.08E-2
0.657	0.022	Maximum test sensitivity plus specificity	0.280	0.000	0.000	6.146E-3
2.386	0.049	Balance training omission, predicted area and threshold value	0.220	0.000	0.250	3.536E-2
9.295	0.142	Equate entropy of threshold and original distributions	0.128	0.000	0.250	7.611E-3



Appendix 5A: Relative absorbance of DNA samples of Loganiaceae family at 260 nm



Appendix 5B: Relative absorbance of DNA samples of Loganiaceae family at 280 nm



Appendix 5C: Relative absorbance of DNA samples of Loganiaceae at 260 nm, 280 nm and their ratio.

Appendix 6: 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%

Appendix 6: Basic Local Alignment Search Tools (BLAST) Results for the Matrixes Generated for *Strychnos* Indeterminate by Three Different Regions of Genes Continued

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%