TAXONOMIC REVISION OF THE GENUS *Phyllanthus* LINN. IN NIGERIA

BY

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A Thesis in the Department of Botany
Submitted to the Faculty of Science in Partial fulfilment of
the requirements for the Degree of

DOCTOR OF PHILOSOPHY

of the
UNIVERSITY OF IBADAN

MAY, 2019

ABSTRACT

The genus *Phyllanthus* (Phyllanthaceae) is of economic importance in herbal medicine and owing to its diversity, grows in similar habitats and shares common local names in Nigeria. These common local names give rise to misidentifications and taxonomic confusion. The only available taxonomic information on *Phyllanthus* is the Flora of West Tropical Africa which is not current. Therefore, this study was conducted to provide revised taxonomic information on *Phyllanthus* species and a reliable key for their identification.

One hundred and forty two specimens comprising 55 field collections covering major ecological zones in Nigeria and 87 representative herbaria materials from Forest Herbarium Ibadan, University of Ibadan herbarium and Obafemi Awolowo University herbarium were assessed for morphological characters using standard taxonomic methods. Light and Scanning Electron Microscopes were used to examine leaf epidermal and pollen morphology. Genomic DNA from 20 fresh and field collected young leaf samples was extracted, amplified using chloroplast *rbcL* primer and the product was sequenced using standard techniques. Phylogenetic trees were constructed using maximum composite likelihood based method. Principal Component Analysis (PCA) and cladograms of the assessed characters were used to establish taxonomic relationships among the taxa. Data were subjected to descriptive statistics.

Nineteen *Phyllanthus* species were recognised from the herbarium materials out of which 16 species were validated because *Phyllanthus floribundus*, *P. fraternus* and *P. physocarpus* were synonyms of *P. muellerianus*, *P. amarus* and *P. acidus*, respectively. The length and width of leaves ranged from 0.45 - 11.70 cm and 0.15 - 5.40 cm, respectively. The leaves were entire with alternate arrangement and the leaf shapes were mainly oblong, lanceolate and linear. Only *P. muellerianus* had recurved stipular spines at the nodal points. Perianth lobes ranged from 4-6 and were mainly green. Fruits were green except in *P. acidus* (yellow), *P. muellerianus* (red), *P. reticulatus* (black) and *P. urinaria* (reddish-brown). Epidermal cells were mostly irregular/polygonal on adaxial and/or abaxial surfaces but rectangular in *P. muellerianus* and *P. floribundus.* Stomata were anisocytic, anomocytic, laterocyclic and paracytic. Epicuticular wax deposits, sessile multicellular scales, unicellular trichomes, oil droplets, crystal sand and druses were recorded in all except *P. maderaspatensis, P. mannianus, P. nigericus, P. niruroides, P. pentandrus*, *P. sublanatus* and *P. urinaria*. Pollen was 3-colporate, finely reticulate, prolate, subprolate and oblate-spheroidal, while the size ranged from small (12.40 x 13.00 µm) to medium (31.50 x 23.25 µm). Four clusters and clades each were delineated from the PCA and cladograms, respectively indicating that the genus is paraphyletic, contrary to the presumed monophyletic relationship of the species. A dichotomous key was produced for easy identification of the species.

Sixteen *Phyllanthus* species were established and revised taxonomic information revealed clusters useful in understanding the relationships among the species of *Phyllanthus* in Nigeria.

Keywords: Herbarium specimens, *Phyllanthus* taxonomy, Leaf macro and micromorphology, Pollen morphology, Phylogeny

Word count: 448

CERTIFICATION

I certify that this work was carried out by Mrs. O. M. Wahab under my supervision in the Department of Botany, University of Ibadan, Ibadan.

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ACKNOWLEDGEMENTS

My utmost gratitude goes to Almighty Allah (SWT) who has given me life, sound health and the means to undertake this programme. My sincere gratitude also goes to my supervisor, Prof. A. E. Ayodele who also doubles as the Head of Department of Botany, University of Ibadan for his guidance, useful suggestions, tolerance and constructive criticism throughout the course of this work.

I wish to express my sincere gratitude to my mentor, Prof. A. Egunyomi who stood by me throughout this programme. I am grateful to the entire academic staff of the Department especially, Prof. A. A. Jayeola, Prof. S. G. Jonathan, Prof. O. J. Oyetunji, Prof. Taiye R. Fasola, Prof. Clementina O. Adenipekun, Dr S. K. Chukwuka, Dr A. A. Salaam, Dr Idayat T. Gbadamosi, Dr O. J. Olawuyi and Dr E. O. Shokefun for various assistance rendered during the course of this programme as well as Dr A. E. Orijieme and Mr. Okpara of Archaeology and Anthropology Department who assisted me in the pollen grain analysis. I appreciate Dr A. A. Sobowale and late Dr A. I. Adesoye, the present and immediate past Departmental PG co-ordinators for their understanding and assistance.

My gratitude goes to the Director General, Forestry Research Institute of Nigeria, Provost, Federal College of Forestry, Ibadan and the entire management staff for providing me the enabling environment to undertake this programme. I appreciate the entire staff of Crop Production Technology Department for their support especially Mr. J. O. Isola and Mr. Chukwuma Emmanuel of Forestry Research Institute of Nigeria, who helped me in the statistical analysis aspect of the work. I appreciate Prof. (Mrs.) A. F. Aderounmu, Dr O. O. Ajala, Mr. M. A. Amoo, Dr AbdulAzeez AbdulFatai, Dr G. T. Salaudeen and Dr R. A. Adegbite as well as the entire members of the Forestry Muslim Community, FRIN for their persistent encouragement and prayers.

I am grateful to people who assisted me during field trips made for this work, especially Mr. T. K. Odewo, Late Mr. Gabriel Ibhanesebor, Mr. Miftaudeen Taofeek and Mr. Donatus Esimekhuai, I appreciate Dr J. O. Akinloye as well as Mr. Taiwo Oluwasegun and Mr. Adisa Mayowa of the Anatomy unit of the Department for the support given to me in the laboratory aspect of the epidermal peels. Effort and assistance of members of staff of Forest Herbarium Ibadan, University of Ibadan Herbarium (UIH) and Obafemi Awolowo University Herbarium (IFE) in the use of their facilities are sincerely appreciated. My sincere appreciation goes to my friends and colleagues: Dr (Mrs.) J. Ibrahim, Dr (Mrs.) G. E. Ugbabe, Dr C. Goji, Mrs. Olayinka I. Adenle, Mrs. A. R. Aduloju and host of others for their persistent encouragement and contribution in one way or the other to the success of this study.

I also appreciate my siblings, most especially my one and only loving sister, Mrs. Medinat B. Adebanjo for her support, prayers and encouragement. I sincerely appreciate my wonderful niece, Miss. Morufat O. Faniyi for always being around me to give a helping hand.

Lastly, I appreciate my loving, caring, adorable husband and children for their patience, moral support, prayers, encouragement and understanding which spurred me on, you will forever remain my love.

DEDICATION

This work is dedicated to the glory of Almighty Allah (SWT) who sustained me throughout the course of this study and to my late parents, Alhaji and Alhaja Fasasi Oyelakin Faniyi who gave me the educational foundation to attain this level, May you continue to rest in perfect peace with the creator.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 The Genus *Phyllanthus*

Phyllanthus Linn. was first described by Linnaeus (1753) as a member of the family Euphorbiaceae (Chaudhary and Rao, 2002). However, based on molecular systematic studies over the years and according to Judd *et al*. (2002) APG II (2003), Samuel *et al.* (2005) and Wurdack *et al.* (2004; 2005), *Phyllanthus* is to be recognized as a member of a separate family, Phyllanthaceae instead of the former subfamily Phyllanthoideae of the Euphorbiaceae *sensu lato* which is now defined as a much smaller family than it had been (Tokuoka, 2007). Reveal *et al.* (2007) proposed that the name Phyllanthaceae should be conserved since it was validly published by Ivan Ivanovich in 1820 in a Russian book titled Tekhno-botanico Slovar.

Phyllanthus is one of the 79 genera of Phyllanthaeceae (http://www.the plantlist.org) which include *Margaritaria* L.f., *Flueggea* Willd, *Securinega* Comm.ex Juss, *Antidesma* Burm, *Bridelia* Willd, *Cicca* Baill, *Hymenocardia* Walli ex Lindi, *Uapaca* Baill. All of these genera are found in Nigeria. *Margaritaria* is sister to all genera of Phyllanthaceae with phyllantoid branching (Samuel *et al.*, 2005). *Phyllanthus* belongs to the tribe Phyllantheae and subtribe Flueggeinae which also include *Flueggea* and *Margaritaria*. *Securinega* is of the subtribe Securineginae of the tribe Phyllantheae. The genus *Phyllanthus* has a diversity of growth forms such as trees, shrubs, climbers, annual and perennial herbs, they may also exist as terrestrial or floating aquatic plants and pachycaulous succulents. Some species have flattened leaf-like stems or modified branchlets called phylloclades. These growth forms are distributed in all tropical and subtropical regions of both hemispheres (Webster, 1994). They are found in open and shaded conditions in rocky areas, waste grounds, roadsides, on termitaria, cultivated fields and swamps in different vegetational zones including the grassland, derived savanna and rainforest. According to Webster (1994) and Silva (2009), despite the variety of growth forms, almost all *Phyllanthus* species express a specific type of growth called "phyllanthoid branching" in which the leaves on the main (vertical) plant axes are reduced to cataphylls while leaves on the plagiotropic (horizontal) axes are deciduous and floriferous. Indeed, leaf flower is the common name for all *Phyllanthus* species and '*Phyllanthus'* means 'leaf and flower' because the flowers as well as the fruits are associated with the leaf (Cabieses, 1993). Govaerts *et al.* (2000) reported that with increasing knowledge from molecular phylogenetic studies and more genera being embedded in the genus *Phyllanthus*, the species number has increased tremendously making it a giant and heterogenous genus.

Some *Phyllanthus* species provide food, fruit, fuel, fodder, timber, dyes pharmaceutical and industrial products while others are extensively used in ethnomedicine (Rao, 2012). A survey of 300 ethnobotanical references of *Phyllanthus* species arranged taxonomically suggested some uses were clustered by subgenus (Holm-Nielsen, 1979). The genus forms one of the most important non-timber forest products in Southern India where a large number of forest dwelling and forest fringe communities depend on *P. embelica* L. and *P. indofischeri* Bennet (Ravikanth *et al*., 2012). As revealed by Sinha and Bawa (2002), unsustainable and destructive harvesting adversely affects regeneration of *Phyllanthus* species. To remedy the situation, domestication of the species and maintenance of *in-situ* gardens were suggested for long term conservation of the genetic resources. On the cultivation of *Phyllanthus* species, Kangsu Medical Institute (1975) recommended fertile, well-drained soil for growing *P. urinaria* L. To produce sufficient quantities for large scale extraction, a system was developed at the University of Florida Tropical Research and Education Center at Homestead, USA using black plastic mulch and trickle irrigation. Webster (1970) was of the opinion that the relative ease of growing herbaceous species of *Phyllanthus* in the greenhouse makes them to be attractive experimental objects for studying specialization in branching patterns. *Phyllanthus acidus* (L.) Skeel and *P. embelica* (*Emblica officinalis* Gaertner) are regionally cultivated for their fleshy edible fruits (Calixto *et al*., 1998). According to Murthy and Joshi (2007), *P. emblica* (Indian gooseberry) is grown in India, China, Taiwan, Indonesia, Malaysia, Thailand, Sri Lanka, Honduras and Costa Rica in orchards, home gardens, wastelands and forests. In these countries, *P. emblica* fruits are consumed and the plant parts utilized in local medicine systems. Tiwari *et al*. (2007) reported that well-drained deep fertile sandy loams are ideal for cultivation. *Phyllanthus acidus* (Malay gooseberry) raised in many parts of the world including Australia, Brazil and Venezuela prefers moist soil (Murthy and Joshi, 2007). Probably, the most economic importance of *Phyllanthus* species is their being used medicinally in various parts of the world.

1.2 Medicinal Uses of *Phyllanthus* Species

Unander and Blumberg (1991) reported that *Phyllanthus* species have been used in Ayuvedic medicine for over 2000 years. In this traditional medicinal system, various herbaceous *Phyllanthus* species are known as 'Bhuiamia', a name previously assigned to *Phyllanthus niruri* L. only. Bhuiamia is often prescribed for jaundice, gonorrhea, diabetes and skin problems. In Brazil, *P. niruri* is known as 'quebrapedra' or 'arranca-pedras' which translates to 'break-stone'. Bagalkotkar *et al.* (2006) recommended *P. niruri* for preventing and eliminating kidney stones. Apart from this use known in the Amazon for ages, Santos (1990) documented further ethnomedicinal uses of *Phyllanthus* species by the indigenous people of the region. In Africa, Murugaiyah and Chan (2007) reported that the bark of *Phyllanthus muellerianus* (O. Ktze) Exell commonly called 'mbolongo' in Cameroon is used by pygmies as a remedy for tetanus and wound infections. A considerable number of *Phyllanthus* species have been studied and some bioactive compounds reported. As an example, *Phyllanthus amarus* Schum & Thonn has been extensively used in pharmacological research due to its anti-HBV (hepatitis B virus) (Liu *et al*., 2001), anti-HIV (Notka *et al*., 2004), anti-mutagenic (Raphael *et al*., 2002), anti-inflammatory (Raphael and Kuttan, 2003) and anti-oxidant (Hari-Kumar and Kuttan, 2004) properties. Thyagarajan *et al.* (1982) reported that the extract from *P. amarus* produced consistent inhibition of hepatitis B surface antigen in *in vitro* studies. In addition, the extract was found to be capable of eliminating the virus from the sera of woodchucks (*Marmomata monax),* whether they are infected acutely or chronically with HBV (Jayaram *et al.*, 1987). Thyagarajan *et al.* (1988) and Blumberg *et al.* (1989) demonstrated that oral administration of *P. amarus* extract had little or no toxic effect on patients. Additionally, Wang *et al.* (1995) evaluated the effect of the extracts of *P. amarus, P. niruri* and *P. urinaria* L. on 123 patients with chronic hepatitis B. No evidence of side effects was reported in patients treated with *Phyllanthus* extracts and an improvement in the condition of patients was observed especially when *P. urinaria* was used. The studies of Unander and Blumberg (1991) also showed that aqueous extracts of *P. amarus, P. urinaria, P. debilis* Klein ex Wild and *P. niruri* inhibited viral DNA polymerase (DNAp) of hepadnaviruses *in vitro*. Hepadnaviruses include human hepatitis B virus and several animal hepatitis viruses.

The effect of *P. amarus* extract (PAE) on chemically induced carcinogenesis has been reported. Hepatic cancer was induced in rats using N-Nitrosodiethylamine resulting in 100% tumor within 18-20 weeks. Sequel to the development of tumor, PAE was administered and significantly elevated the life span of hepatic – tumor bearing animals (Jeena *et al.*, 1999; Rajeshkumar and Kuttan, 2000; Rajeshkunmar *et al.*, 2002). As uncontrolled proliferation is characteristic of cancer cells, the anti-proliferative effect of a substance refers to ability to halt cancer growth by inhibiting or killing cancer cells. The water extract of *P. urinaria* showed a selective anti-proliferative effect on leukemia cells without showing cytotoxicity on normal cells (Huang *et al.,* 2004). The antioxidant activity of *P. urinaria* has been reported. Fang *et al.* (2008) isolated numerous antioxidant compounds such as geraniin, phyllanthin and rutin from *P. urinaria*. Antioxidants protect the body against oxygen free radicals which have been implicated not only in aging but also in degenerative disorders like cancer, cataracts and atherosclerosis (Nordberg and Arner, 2001). Testing the toxicity of the plant, Huang *et al.* (2006) reported that there was no obvious toxicity detected in the mice group administered with *P. urinaria* 500 mg/kg body weight per day in drinking water for 4 weeks, as shown by histological examination of the liver, lung, spleen, heart and kidney.

Phyllanthus niruri is certainly one of the most widely used plants of the genus *Phyllanthus* in world folk medicine and it has been claimed to present beneficial therapeutic effects in the treatment of genitourinary infections and hepatitis B virus, in the management of airway diseases, besides its reported use as an antidiabetic (Chopra *et al.,* 1956; Perry and Metzger, 1980). Agarwal *et al.* (1992) demonstrated that *P. niruri* was effective in antagonizing the clastogenic effect induced by nickel chloride in mouse bone marrow cells. Experiments on mice and rats have shown that the extracts of *P. niruri* protected the liver from damage induced by various toxic chemicals like CCl_4.

(Reddy *et al.*, 1993; Prakash *et al*., 1995). The hepatoprotective effect of *P. niruri* against CCl_4 was found to be better than that of *P. urinaria* (Prakash *et al.*, 1995). Hypercholesterolemia is a risk factor associated with cardiovascular disease. Lukatta (2007) expressed the view that elevated plasma low density lipoprotein (LDL), the bad cholesterol, and low level of high density lipoprotein (HDL) – the good cholesterol is a major risk factor. Khanna *et al.* (2002) reported that *P. niruri* significantly increased levels of HDL and reduced LDL in induced hyperlipidemic rats, hence useful for cardiovascular problems.

Phyllanthus reticulatus Poir is used for the treatment of a variety of ailments including smallpox, syphilis, asthma, diarrhoea and bleeding from gums. It is claimed to have antidiabetic activity (The Wealth of India, 2005). Biplap *et al.* (2008) demonstrated the hepatoprotective activity of two partially purified organic fractions of the fat free ethanol extract of *P. reticulatus* against CCl_4-induced liver damage in rats. Maruthappan and Shree (2010) investigated the effects of *P. reticulatus* on lipid profile and oxidative stress in hypercholesterolemic albino rats. The results suggested that aqueous extract of the plant could be utilized for prevention of atherosclerosis in hypercholesterolemic patients. Saha *et al*. (2007) reported the anti-inflammatory activity of *P. reticulatus* aerial parts extracts evaluated in a carrageenan (CGN) – induced paw edema mice model. The methanol, ethyl acetate and petroleum ether fractions administered orally at 150 and 300 mg/kg decreased the paw edema induced by CGN. Also petroleum ether and ethanol leaf extracts of *P. reticulatus* had hypoglycemic effects at 500 and 1000 mg/kg in alloxan-induced diabetic mice (Kumar *et al*., 2008). At 1000 mg/kg the study noted a sustained decrease in blood glucose levels, on subchronic administration of extracts for 21 days.

Studies on the medicinal uses of *Phyllanthus maderaspatensis* L. have been carried out by various workers. Asha *et al.* (2007) established the antihepatotoxicity activity of *P. maderaspatensis* against induced liver injury in rats. Although the water and ethanol extracts showed modest activity, the n-hexane extract was very active even at a low dose of 1.5 mg/kg. The antihepatotoxicity activity of the hexane extract was better than that of silymarin, a standard hepatoprotective drug. Chemical mutagens or genotoxic agents as well as ionizing radiation are known to damage cellular DNA, causing chromosomal aberrations which often have implications for health (Jagetia, 2007).

Agents which inhibit such chromosomal aberrations are antimutagenic or antigenotoxic hence are said to be chemopreventive. The chemopreventive effect of *P. maderaspatensis* against chemotherapeutic drug, cisplatin has been attributed to its antioxidant potential (Chandrasekar *et al.,* 2006).

The medicinal uses of *Phyllanthus* species in parts of West Africa have been reported. In Nigeria, *P. amarus* extract showed antimicrobial activity by inhibiting the growth of *Staphylococcus aureus*, *Vibro spp.* and *Salmonella spp*. causal agents of urinary tract infection, cholera and typhoid fever respectively. Extracts compared favourably with attributes of floxacin and pefloxacin (fluoroquinolones) (Ohalete *et al.*, 2013). Also Adegoke *et al.* (2010) reported the antimicrobial activity of *P. amarus* against multiple antibiotic resistant bacteria; *Staphylococcus aureus, E. coli, Klebsiella spp.* and *Pseudomonas aeruginosa* at different minimum inhibitory concentrations (MIC). Ajala *et al.* (2011) reported that whole plant extracts of *P. amarus* demonstrated dose-dependent prophylactic and chemotherapeutic activity against the resistant strain malaria parasite, *Plasmodium yoelii* infection in mice. The aqueous extracts showed higher effect than the ethanol extract. The antiplasmodial effects of the extracts were comparable to standard drugs used in chloroquine resistant *Plasmodium* infection. Gbadamosi *et al.* (2012) reported *in vitro* antisickling activities of *Phyllanthus amarus* stating that the plant is a potential antisickling phytomedicine that could be developed as a novel drug. Shokunbi and Odetola (2008) reported gastroprotective and antioxidant activities of *Phyllanthus amarus* extracts on absolute ethanol induced ulcer in albino rats. In an acute toxicity study, *P. amarus* acetone extracts at doses of 250, 500, 1000, 4000 and 8000 mg/kg body weight were administered to the animals. According to the authors, there was no mortality at any of the tested doses at the end of 7 days, indicating the non-toxic nature of the plant extract. They suggested that *P. amarus* may offer protection against toxic effect of alcohol to the liver.

Nwanjo (2007) investigated the effects of aqueous extract of *P. niruri* on plasma glucose level and some hepatospecific markers in diabetic Wister strain rats. The author observed that the aqueous crude extract of the plant sample may have hypoglycaemic effect in diabetic rats and that no evidence of hepatotoxicity of the extract was established. The effects of aqueous extract of *P. niruri* on epididymal sperm characteristics, fructose and testosterone levels in male albino rats were reported by Ezeonwu (2011). The findings suggested that the aqueous crude extract of the plant sample has antifertility activity.

Phyllanthus muellerianus has been reported for the treatment of jaundice, skin diseases, stomach problems, fever, cough, insomnia and dysentery (Odugbemi, 2008). *Phyllanthus fraternus* Webster was reported to be effective in killing larval stage of *Dermestes maculatus* infesting smoked *Clarias gariepinus* (catfish) after 1st and 2nd week of infestation (Adesina *et al.*, 2014).

In Ghana, leafy tops of *Phyllanthus fraternus* Webster subsp. *togoensis* Brunnel & Roux (Syn: *P. niruri*) is used for treating septicemia, hyperglycemia and viral infection. Leaves are used for treating wounds, fracture, diarrhoea, fever and paralytic stroke while whole plant is used for abdominal pains, prenatal case, snake bite and dystocia. Roots of *P. muellerianus* are used for treating cough and dysentery, leaves for treating wound, diarrhoea, fever and visual disturbance while the stem is used for the treatment of conjunctivitis. All parts of *P. niruri var. amarus* (Syn. *P. amarus*) are used to treat abdominal pains, snake bite and dystocia. Whole plant of *P. capillaris* Schum & Thonn is used for the treatment of fever, poisoning and snakebite while the leaves are used for dysmenorrhea (OAU/STRC, 2000). In Cameroon, leafy shoot of *P. amarus* is used in the treatment of abdominal pains, juice of whole plant for snake bite while the leaves are for splenomegaly. The roots and leaves of *P. muellerianus* are used for treating fallopian tubal blockage while the leaves of *P. odontadenius* Mull. Arg are used for treating gastralgia (OAU/STRC, 1997).

Owing to increasing scientific knowledge validating indigenous uses of some *Phyllanthus* species, workers such as Rao (2012) have advocated the cultivation of proven economically important species. Giving an example, Rao (2012) reported that since *P. amarus* shot into prominence after its activity against hepatitis B and related hepadnaviruses was scientifically proven, it has been cultivated commercially in Southern India. Similarly, *P. urinaria* is cultivated in China in warm well drained sandy soils supplemented with fertilizers that are rich in nitrogen and potassium.

1.3 Classification

The following classification of *Phyllanthus* is after Cronquist (1981)

Domain:	Eukaryota
Kingdom:	Plantae
Subkingdom:	Viridaeplantae
Phylum:	Tracheophyta
Subphylum:	Euphyllophytina
Superdivision:	Spermatophyta
Division:	Magnoliophyta
Class:	Magnoliopsida
Subclass:	Rosidae
Order:	Euphorbiales
Family:	Euphorbiaceae
Subfamily:	Phyllanthoideae
Tribe:	Phyllantheae
Subtribe:	Flueggeinae
Genus:	*Phyllanthus* L.

Webster (1994) divided *Phyllanthus* into 10 subgenera, 68 sections and sub-sections. The subgenera are: *Isocladus* Webster, *Kirganelia* (Juss.) Webster, *Cicca* Linnaeus, *Emblica* Gaertner, *Gomphidium* (Baill.) Webster, *Phyllanthodendron*, Webster & Carpenter, *Xylophylla* Webster, *Botryanthus* Webster, *Eriococcus* (Hassk) Croiz & Metc. and *Phyllanthus* L. Out of these, only *Isocladus, Kirganelia* and *Phyllanthus* are represented in Nigeria. *Isocladus* differs from *Kirganelia* and *Phyllanthus* by having no phyllanthoid branching. Although *Isocladus* and *Kirganelia* are made up of herbs, shrubs or trees, *Phyllanthus* consists of only herbs or low woody shrubs (Botanical Survey of India, 2014). *Isocladus* is represented by *Phyllanthus maderaspatensis* which belongs to the section *Paraphyllanthus* and is regarded as sister to all other species of *Phyllanthus sensu lato* (Kathriarachichi *et al.,* 2006). Trees and shrub species, *P. reticulatus, P. acidus, P. muellerianus* and the herbaceous *P. pentandrus* Schum & Thonn belong to

Kirganelia. The subgenus *Phyllanthus* comprises the herbaceous species. *P. amarus, P. niruri, P. odontadenius* and *P. urinaria.* According to Kathriachichi *et al.* (2006) subgenera *Isocladus, Kirganelia* and *Phyllanthus* are paraphyletic whereas other subgenera appear to be monophyletic.

Based on molecular systematic studies, the following classification of *Phyllanthus* is recognized by the Angiosperm Phylogeny Group (APG II, 2003) and reported by Singh and Kalaiselvan (2012):

Domain:	Eukaryota
Regnum:	Plantae
Clade:	Angiospermae
Clade:	Eudicots
Clade:	Core cudicots
Clade:	Rosids
Clade:	Eurosids I
Order:	Malpighiales
Family:	Phyllanthaceae
Subfamily:	Phyllanthoideae
Tribe:	Phyllantheae
Subtribe:	Flueggeinae
Genus:	*Phyllanthus* L.

Phyllanthus has a wide variety of floral morphologies (Bancilhon, 1971) chromosome numbers (Webster and Ellis, 1962) and one of the widest ranges of pollen types of any seed plant genus which according to Webster and Carpenter (2002), rivals that of any genus of the flowering plant. The remarkable diversity of pollen in different groups of *Phyllanthus* is of systematic importance because the morphological types can be correlated with particular subgeneric taxa (Punt, 1967).

The circumscription of the genus has been a cause of much confusion and disagreement. As an example, Kathriarachchi *et al*. (2006) reported that the validly published and accepted sections and sub-sections of *Phyllanthus* by Webster (1956) were often with fluctuating, contradictory circumscriptions and complex synonymies. This situation according to these authors was due to the fact that Webster never synthesized his regional and sectional *Phyllanthus* treatments into a worldwide synopsis. However, the molecular phylogenetic studies of Kathriarachchi *et al.,* (2006) confirmed the paraphyly of *Phyllanthus* in its traditional circumscription with embedded *Reverchonia* Webster, *Glochidion* Webster, *Sauropus* Webster and *Breynia* Webster. These workers favour the inclusion of the embedded taxa in *Phyllanthus* over further generic segregation. Despite this view and based on molecular data *Glochidion, Sauropus* and *Breynia* have been recognized as distinct genera (Samuel *et al.*, 2005; The Plant List, 2010).

1.4 Justification

There has been increasing awareness worldwide of the economic importance of *Phyllanthus* species. As an example, the annual volume of *Phyllanthus* trade in India alone is estimated to be about 2,000-5,000 metric tonnes of herbaceous material and about 16,000-18,000 metric tonnes of fruits (Ved and Goraya, 2008). In spite of this, the *Phyllanthus* species trade in India is marred by the taxonomic confusion among closely related species (Elvin-Lewis *et al.*, 2002) and the fact that many species in trade share a common vernacular name (Srirama *et al.,* 2010). This situation has led to admixtures of herbal species with significant implication on the quality and efficacy of the eventual herbal drug (Song *et al.,* 2009). Although *Phyllanthus* species are not traded yet in Nigeria, some species such as *Phyllanthus amarus* are being used medicinally. Furthermore, the country is on the verge of deriving economic benefit from the booming world trade of medicinal plants. Towards this end, *Phyllanthus* species must be properly identified so as to avoid misuse and adulteration of plant drugs.

The fact that many herbaceous *Phyllanthus* species grow in similar habitats and share common vernacular names in Nigeria may give rise to misidentifications. Field and Herbarium observations of some *Phyllanthus* species show that there are similarities of highly conspicuous morphological features, making identification of the species difficult. Recently, Adesina *et al.* (2014) reported larvicidal activity of *Phyllanthus fraternus* powder against the larvae of *Dermestes maculatus* infecting stored smoked fish. As the plant was collected and identified at Owo (Ondo State) where it was reported to be common, the identification is suspect. *Phyllanthus fraternus* does not commonly grow in Nigeria as *P. amarus*. Khatoon *et al*. (2006) has observed that *P. amarus, P. fraternus* and *P. niruri* are often synonymously used in publications as the species look very similar. In fact, misidentification of plants by some workers including basic medical scientists and its implication for medicinal uses is a matter that deserves serious attention by taxonomists and all the relevant bodies in this regard. As an example, *Securinega virosa* (formerly: *Phyllanthus virosus*) has been wrongly identified as *Phyllanthus amarus* (Odeku, 2014). Also, Okpako (2015) extolled the importance of *P. amarus* and ethnomedicinally used for curing and preventing malaria in Delta State, Nigeria. Incorrectly, the plant identified and labelled as *Phyllanthus amarus* in the publication is clearly *P. odontadenius*. Jain *et al.* (2008) ascribed the confusion in identification of herbaceous *Phyllanthus* species largely to use of common vernacular names for all species, their similarity in gross morphology, a close proximity in their growth habitats and the range of diverse morphological features.

Unlike other parts of the world, the West African species of *Phyllanthus* have not been studied adequately; problems of identification and taxonomic confusion still persist. There is therefore the need to provide basic taxonomic information on these *Phyllanthus* species. Thus it is expedient to carry out a floristic search and taxonomic revision of the taxa of *Phyllanthus* in Nigeria with a view to ascertaining how many species there are and determine the species boundaries. It is expected that this study will contribute to their delimitation as distinct taxa. Additionally, the work will provide a means of ascertaining the evolutionary relationship among taxa which are not expressed morphologically and will enhance more complementary ways of identifying the plants scientifically.

1.5 Aim of the Study

The present study aims at revising the genus *Phyllanthus* in Nigeria with a view to contributing to the Flora of Nigeria project.

1.6 Objectives of the Study

(i) To determine the number of species in Nigeria with their synonyms.

(ii) To determine the species boundaries.

(iii) To improve understanding of the relationships and affinities of the species in the genus by using findings derived from macromorphological, micromorphological and molecular studies.

(iv) To generate a good picture database for the species in the genus in order to aid identification.

(v) To generate a reliable taxonomic key for the identification of taxa.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Status and Distribution of the genus *Phyllanthus* Worldwide

Phyllanthus is the largest and most diversified genus among the genera in the subfamily Phyllanthoideae of the family Phyllanthaceae (Santiago *et al*., 2004). As reported by Punt (1967), *Phyllanthus* has circumtropical distribution and is well represented on all continents and particularly offshore island groups like Madagascar and Cuba. The genus is not entirely a natural group as there are profound differences in vegetative structure, flowers and fruits. Owing to the high degree of variability, systematic treatment of the genus has always been controversial. Thus there has been multiplicity of opinions on the taxonomic status of many species as well as the specific and sectional relationships between the species and their pattern of distribution worldwide. These factors have given rise to taxonomic problems of *Phyllanthus* in parts of the world. Although the literature is replete with information on the status, distribution, opinions on speciation and interspecific relationships of members of *Phyllanthus* species worldwide, some of these are reviewed.

In the United States of America (U.S.A) *P. urinaria* described as a weed of gardens and roadsides is a circumtropical immigrant but not as ubiquitous as *P. amarus. Phyllanthus niruri* is the rarest in the U.S.A. and reports of the species from North Carolina by Ahles and Radford (1959) were based on misidentified specimens of *P. tenellus* Roxb. *Phyllanthus fraternus,* an annual herb resembling *P. amarus* and native to Pakistan and India was sporadically introduced into Africa and America (Webster, 1970). *Phyllanthus amarus,* presumably indigenous to the American tropics (Webster, 1957) is now a circumtropical weed and perhaps the most ubiquitous of all tropical Euphorbiaceae *sensu lato*. Webster (1970) opined that *P. amarus* is closely related to *P. abnormis* Baill. He also expressed the view that taxonomic relationship between highly advanced herbaceous *Phyllanthus* species of the Old and New world might be due to their remarkably long-distance overwater dispersal. Of the 12 species occurring in America, *Phyllanthus urinaria, P. niruri* and *P. amarus* have been recorded for Nigeria.

The herbs known as 'Bhumyamalaki' in Indian literature refer to a complex group of *Phyllanthus amarus, Phyllanthus fraternus, Phyllanthus aevius* Klein ex Wild and *Phyllanthus urinaria* (Chaudhary and Rao, 2002). Although these species closely resemble each other, they also show sufficient characters to maintain them as distinct species. However, ethnomedicinal uses and some aspects of pharmacological activities among these species are different (Theerakulpisut *et al.*, 2008). Webster (1955, 1957 and 1967) has provided detailed taxonomic accounts of West Indian *Phyllanthus.* He observed that true *P. niruri* is a native of New World and endemic to America and does not occur in India, despite this observation, there are many publications today on *P. niruri* from India. Mitra and Jain (1985) revealed after critical examination of Indian materials of *Phyllanthus* that the *P. niruri* described in Flora of British India (Hooker, 1887) is actually a mixture of three closely related but distinct species of *P. amarus, P. debilis* Klein ex Wild and *P. fraternus*. Studies on *P. niruri* done in India are actually pertaining to investigations in any one member of this '*niruri* complex' but not on *P. niruri*.

Dhandayuthapani *et al.* (2011) conducted a study to investigate the diversity of *Phyllanthus* species found in Tiruchirappalli district of Tamilnadu, India. They attempted to resolve the nomenclatural problem persisting in this genus by analyzing the morphological and anatomical characters of these plants and evolve a simple key for easy identification of the related species. Through distinct morphological features, the identity of *P. amarus* was confirmed but as an additional proof, the validity of Sequence Characterized Amplified Regions (SCAR) markers developed earlier was assessed (Jain *et al.,* 2008). Although SCAR markers (Jain *et al*., 2008) and DNA barcodes (Srirama *et al.,* 2010) are useful for distinguishing the *Phyllanthus* species, they are beyond the reach of a local herb collector. Hence the believe in simple morphological and anatomical character based keys for easy identification of herbs existing along with closely related allied species should be attempted (Dhandayuthapani *et al*., 2011).

As reported by Hunter and Bruhl (1997), the Phyllantheae, in particular *Phyllanthus* has presented considerable taxonomic problems within Australia. Webster (1956) compared the Phyllantheae to political boundaries which are superposed over the natural physiographic features of a region.

In a revision of the *Phyllanthus* of Eastern Melanesia (areas from New Hebrides to Fiji and Tonga), Webster (1986) reported that *Phyllanthus amarus, P. urinaria* and *P. debilis,* all weedy species, were introduced to Eastern Melanesia. Pollen morphological study revealed considerable similarity of some *Phyllanthus* species pollen types of eastern Melanesia and New Guinea.

Webster and Airy Shaw (1971) published a provisional synopsis of New Guinea taxa of *Phyllanthus* and provided keys for the identification of 35 species. These included *Phyllanthus reticulatus (P. microcarpus), P. urinaria* and *P. amarus*. Almost all the New Guinea reports of *P. niruri* were referable to *P. amarus.* Out of all the *Phyllanthus* species recorded for Australia, *P. amarus* and *P. maderaspatensis* are also found in Nigeria.

2.1.1 The genus *Phyllanthus* in West Africa

Phyllanthus is represented in the West African floristic region by about 22 species (Hutchinson and Dalziel, 1954) and at least 14 species are in Nigeria (Hutchinson and Dalziel, 1954; Burkill, 1985). Although 22 species are listed, 20 species are identified to the species level and *Phyllanthus discoideus* (Baill.) Mull. Arg. has since been named *Margaritaria discoidea* (Baill.) Webster. Out of the remaining 19 species, *Phyllanthus amarus, P. capillaris, P. maderaspatensis, P. muellerianus, P. pentandrus* and *P. reticulatus* were reported to be widespread in Tropical Africa. Curiously, *Phyllanthus niruri* with records from Senegal and Northern Nigeria was said to be common in India "but apparently rare elsewhere." Also *P. urinaria* was described as being rare in Africa, though recorded as being found in Sierra Leone, Ghana and Southern Nigeria.

In their contribution to the Flora of Nigeria, Ayodele and Yang (2012) published a comprehensive list showing the diversity and distribution of vascular plants. Out of the 15 *Phyllanthus* species listed, *P. reticulatus* var. reticulatus and *P. rotundifolius* Klein & Willd were recognized. That *Phyllanthus profusus* N.E.Br, *P. alpestris.* Beille, *P. dusenii*

Hutch and *P. petraeus* Chev. & Beille ex Beille were not recorded for Nigeria agreed with Hutchinson and Dalziel (1954). Although Burkill (1994) recognized 17 *Phyllanthus* species found in West Africa, *P. profusus, P. alpestris* and *P. petraeus* were reportedly not found in Nigeria. However, Arbonnier (2004) stated that *Margaritaria discoidea* (synonym: *Phyllanthus discoideus*), *Phyllanthus muellerianus*, (Kuntze) Exell., *P. reticulatus* and *P. welwitschianus* Mull. Arg. (synonym: *P. beillei* Hutch) are useful plants found in the dry zones of West Africa.

Using herbaceous plants collected from Cote d'Ivoire, Cameroon and Togo, Haicour *et al.* (1994) carried out experimental crossing within the *Phyllanthus* subsection *Odontadeni*. The workers recognized *Phyllanthus odontadenius* as a complex comprising *Phyllanthus braunii* Pax, *P. odontadenius, P. gagnioevae* Brunel & Roux, *P. bancilhonae* Brunel & Roux and *P. magnificens* Brunel & Roux. All these species except *P. odontadenius* have not been listed in the literature either for West Africa or Nigeria. In their opinion, *Phyllanthus odontadenius* complex originated from Africa just as *Phyllanthus urinaria* originated from eastern Asia (Haicour, 1983; Rossignol *et al*., 1987).

Over the years, workers have had different opinions on the taxonomic status of *Phyllanthus niruri*. In the opinion of Webster (1956), the broad concept adopted by Linnaeus led subsequent botanists to place at least a dozen different herbaceous *Phyllanthus* species under *P. niruri.* He argued that since *P. niruri* was native and restricted to the 'New world', all 'Old world' records of *P. niruri* must be referred to as *P. amarus.* However, Jain *et al.* (2008) reported that despite the fact that earlier workers grouped *P. amarus, P. fraternus* and *P. debilis* under the single species *P. niruri,* later described as '*niruri'* complex, Webster maintained that *P. niruri* was an American species and not found in India. Mitra and Jain (1985) showed that *Phyllanthus niruri* is actually represented by the three species; *P. amarus, P. fraternus* and *P. debilis*. A critical examination of Indian *Phyllanthus* species revealed that *P. niruri* described in the Flora of British India was a mixture of three closely related but distinct species namely *P. amarus, P. fraternus* and *P. debilis* (Mitra and Jain, 1985).

New *Phyllanthus* species have been recorded for West Africa. Brunel and Roux (1984) reported four species new to science. *P. nozeranianus* Brunel & Roux from Mt.

Nimba, Cote d'Ivoire, *P. caligatus* Brunel & Roux, *P. aspersus* Brunel & Roux and *P. raynalii* Brunel & Roux from Cameroon.

In Nigeria, confusion exists in identification of these herbaceous species mainly due to their similarity in gross morphology, close proximity in growth habitat and identification of two or more species with the samecommon name (Table 2.1). Uka *et al.* (2014) carried out leaf epidermal studies of *Phyllanthus* species occurring in Southeast Nigeria. The species were *Phyllanthus amarus, P. muellerianus, P. niruroides,* Mull. Arg., *P. odontadenius* and *P. discoideus* (already referred to as *Margaritaria discoidea*). The workers concluded that epidermal characters were of high taxonomic significance in the genus. Awomukwu *et al.* (2014) used anatomical characters of the leaf, stem and root to delimit *Phyllanthus amarus, P. urinaria, P. niruroides, P. muellerianus* and *P. odontadenius*. Using ITS genetic marker, Awomukwu *et al*. (2015) reported that *Phyllanthus niruroides* and *P. odontadenius* were closely related. Based on the dendrogram derived from the molecular study, *P. odontadenius* and *P. niruroides* showed the closest similarity at 96.9% in the ITS genome region. If this result is correct, then both plants cannot be maintained as distinct species. That high percentage of similarity obtained implies that in all probability both plant materials could not be *P. odontadenius* and *P. niruroides*, thus identification of the specimens may be suspect. In the same study, *P. amarus* and *P. muellerianus* displayed the least similarity while *P. urinaria* was closely related to *P. niruroides* and *P. odontadenius*.

Table 2.1: Vernacular names of some *Phyllanthus* species in Nigeria

***Phyllanthus* species**	**Yoruba**	**Hausa**	**Igbo**	**Bini**
Phyllanthus amarus	Eyin olobe, Dobi-sowo	Geron-tsuntsaye	Ngwu Ub'akufe	Iyeke-ebezukpe
P. muellerianus	Arunjeran, Asasa, Eegun-eja	Alambu kumcii	Egu eza	Asivin, Igba-ehen
P. niruri	Yoloba, Asasa	Majieyar-kurmii	Okwonwonazu, Enyikwonwa	Asivin, Igba-ehen
P. pentandrus	Eyin olobe	Geron-tsuntsaye, Geron-itaacee	Egu eza	Iyeke
P. reticulatus	Iranje	Alambu natudu	Ngwu	Igba- ehenata

(Gbile, 1980; 1984; Burkill, 1985; Gill, 1992)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Field work and sampling

Fifty-five specimens were collected during field trips undertaken to different parts of the country for the collection and study of *Phyllanthus* species. Fresh samples of these species were collected from seventeen states viz; Oyo, Osun, Ondo, Lagos, Kwara, Niger, Benue, Adamawa, Kaduna, Sokoto, Plateau, Edo, Abia, Akwa Ibom, Enugu, Rivers and Cross River covering major ecological zones in Nigeria (Table 3.1). Characters such as flower colour, fruit colour, number of perianth lobes as well as the colour of the leaf on both the adaxial and abaxial surfaces were recorded in the field notes as these might have changed or no longer available after the specimens had been processed. Identification of the species was based on the characters used by Hutchinson and Dalziel (1954). Voucher specimens were prepared for all collections and deposited in the Herbarium of the Department of Botany, University of Ibadan, Ibadan, Nigeria (UIH).

3.2 Picture database

Photographs of the specimens were taken during the field trips with Digital camera (Sony Steady Shot DSC W530) for the picture database.

3.3 Herbarium studies

Eighty-seven representative herbarium materials deposited at Forest Herbarium (FHI) of the Forestry Research Institute of Nigeria, University of Ibadan Herbarium (UIH), Obafemi Awolowo University Herbarium (IFE) were studied. Three specimens were taken on loan from Nigerian Institute of Pharmaceutical Research and Development Herbarium (NIPRDH) while one specimen each was taken from University of IlorinHerbarium (ILH) and Ahmadu Bello University Herbarium (ABUH) for assessment. The list of the specimens studied is presented in Table 3.2.

Table 3.1: List of *Phyllanthus* species collected for the study

Serial number	Taxa	Locality	Collector's name(s)/number	Date of Collection
1.	*Phyllanthus acidus* (L.) Skeels	Biological garden, Obafemi Awolowo University, Ile-Ife, Osun State	Wahab O.M & Omomoh B./IFE16595	07/06/12
2.	*P. acidus*	Biological garden, Obafemi Awolowo University, Ile-Ife, Osun State	Wahab O.M/058	04/03/15
3.	*P. amarus* Schum. & Thonn.	University of Benin campus, Edo State.	Wahab O.M/028	03/06/11
4.	*P. amarus*	Sokoto road, University of Ibadan. Ibadan.	Wahab O.M/006	11/08/11
5.	*P. amarus*	Tanke, Oke-odo, Ilorin, Kwara State.	Fatoba P./001	07/11/11
6.	*P. amarus*	University of Lagos campus, Akoka. LagosState.	Odewo T.K/003	17/11/11
7.	*P. amarus*	Old Oyo National park,Sepeteri, Oyo State	Wahab, O.M/007	08/12/11
8.	*P. amarus*	Abia State Polytechnic, Aba.	Awomukwu E./016	29/02/12
9.	*P. amarus*	University of Calabar Farmland.	Apejoye F.I./017	08/03/12
10.	*P. amarus*	University of Portharcourt,	Apejoye F. I./014	01/03/12
11.	*P. amarus*	Idu Industrial Area, Abuja	Ibrahim J.A/031	04/05/12
12.	*P. amarus*	Ajassor village, Ebong LGA, Cross River State	Adedeji S./037	15/05/12
13.	*P. amarus*	Agaie, Niger State	Adebola M.A/027	24/05/12
14.	*P. amarus*	Ikire, Osun State	Wahab O.M/034	06/06/12
15.	*P. amarus*	LAUTECH campus, Ogbomoso.	Wahab O.M/	18/06/12
16.	*P. amarus*	Otukpo, Katsina-Ala, Makurdi, Benue State	H.O.A. Oluma/041	20/06/12
17.	*P. amarus*	Shelta Afrique, Mbiabong, Uyo	M. Bassey/033	04/07/12
18.	*P. amarus*	Samaru, Zaria	Gallah U.S/046	20/07/12
19.	*P. amarus*	Ilorin, Kwara State	Fatoba P./048	01/08/12
20.	*P. amarus*	Okomu Natonal Park, Edo State	Obasan M.K/053	04/08/12
21.	*P. amarus*	Adamawa State Polytechnic, Yola.	Goji T.C/	10/09/12
22.	*P. amarus*	Department of Botany, U.I, Ibadan	Wahab O.M/	04/03/15
23.	*P. capillaris* Schum. & Thonn.	Obafemi Awolowo University, Ile-Ife, Osun State	Omomoh B./IFE16714	06/03/12
24.	*P. capillaris*	Obudu Cattle Ranch, Cross River State	Odewo T.K & Oyebanji W./LUH4870	19/04/12

25.	*P. capillaris*	Ajassor village, Ebong LGA, Cross River State	Adedeji S./036	15/05/12
26.	*P. capillaris*	Biological Garden, O.A.U, Ile-Ife, Osun State.	Wahab O.M & Omomoh B./057	07/06/12
27.	*P. capillaris*	Old Jos road, Zaria.	Gallah U.S/048	20/09/12
28.	*P. capillaris*	Federal college of Forestry campus, Ibadan.	Wahab O. M/	12/09/16
29.	*P. maderaspatensis* L.	Biological garden, Uthman Dan Fodio University, Sokoto.	Aliero, A.A/039	09/09/16
30.	*P. muellerianus* (O. Ktze) Exell	Odofin Agbegi village, Ikire, Osun State.	Odewo T.K/004	17/07/11
31.	*P. muellerianus*	Near 2nd gate, O.A.U, Ile-Ife, Osun State.	Omomoh B./IFE16712	06/03/12
32.	*P. muellerianus*	Idu Industrial Area, Abuja.	Ibrahim J.A/030	04/05/12
33.	*P. muellerianus*	O.A.U campus, Ile-Ife, Osun State.	Wahab O.M & Omomoh B./050	07/06/12
34.	*P. muellerianus*	Ife-Ibadan road, Osun State.	Ugbabe G.E/052	08/06/12
35.	*P. muellerianus*	Owena, Ondo State	Wahab O.M/060	12/06/12
36.	*P. muellerianus*	A.B.U Samaru, Zaria.	Gallah U.S/045	21/07/12
37.	*P. muellerianus*	LAUTECH campus, Ogbomoso, Oyo State.	Wahab O.M/049	18/08/12
38.	*P. muellerianus*	Cross River State National Park, Akamkpa LGA.	Apejoye F.I/070	10/09/12
39.	*P. muellerianus*	LAUTECH Botanical Garden, Ogbomoso, Oyo State.	Aworinde D.O/	04/03/15
40.	*P. niruri* Linn.	University of Calabar, Jos.	Apejoye F.I/069	28/08/12
41.	*P. niruri*	Dominican road, Samanda, U.I, Ibadan.	Taoheed, K.M/	28/08/14
42.	*P. niruri*	Dominican road, Samanda, U.I, Ibadan.	Wahab O.M & Taoheed, K.M/068	04/03/15
43.	*P. odontadenius* Mull. *Arg.*	Nursery section, Department of Botany, U.I, Ibadan.	Wahab O.M & Esimekhuai D./003	11/08/11
44.	*P. odontadenius*	Apete road, Ibadan	Wahab O.M/	24/02/12
45	*P. odontadenius*	Abia State Polytechnic, Aba	Awomukwu E./015	29/02/12
46.	*P. odontadenius*	Ibadan Polytechnic-Apete junction, Ibadan.	Wahab O.M/019	01/06/12
47.	*P. odontadenius*	Botanical Garden, University of Nigeria, Nsukka, Enugu state.	Onyeokwu C.J/040	22/06/12
48.	*P. odontadenius*	Department of	Wahab O.M/063	22/06/12

		Botany, U.I, Ibadan		
49.	*P. odontadenius*	Shelta Afrique, Mbiabong, Uyo	Bassey M./032	17/07/12
50.	*P. odontadenius*	Ibadan Polytechnic- Apete junction, Ibadan	Wahab O.M/	03/03/15
51.	*P. pentandrus* Schum. & Thonn.	A.B.U Water works, Zaria.	Gallah, U.S/044	20/07/12
52.	*P. pentandrus*	Biological garden, Uthman Dan Fodio University, Sokoto.	Aliero, A.A/038	27/07/12
53.	*P. reticulatus* Poir.	University of Lagos campus, Lagos State	Odewo, T.K/005	14/07/11
54.	*P. reticulatus*	By the 2nd gate, University of Lagos campus, Lagos State	Oyebanji W.	09/09/16
55.	*P. urinaria* Linn.	Osisioma, Aba, Abia State	Shokefun, E.O/	03/09/14

Table 3.2: Herbarium specimens of *Phyllanthus* species examined

Taxa	Reference/ Herbarium number	Locality	Collector(s)/Collector s' number	Date of Collection
Phyllanthus acidus	IFE 518	Biological garden,OAU, Ife.	B. O. Daramola/ B08	17.09.2000
	FHI 25674	Forestry hills, Ibadan	R. W. J. Keay	February,1950
Phyllanthus amarus	UIH 12922	Jericho reservation, Ibadan	J. Lowe/2212	20.05.71
	UIH 22022	Zoology Department,U.I, Ibadan	Kuteyi R. R/2	13.11.91
	UIH 11063	Old farmland, Ibadan	98	16.11.56
	UIH 14260	Bodija Cattleyard, Ibadan	G. Jackson	24.11.70
	UIH 19784	University of Portharcourt, Rivers state	R. A. Freemann/11A	January, 1982
	FHI 70064	Ankpa, Igala, Kwara	Olorunfemi & Ibhanesebor	21.05.73
	FHI 73377	Ajassor bridge, Nfum, S.E	Okeke, Ekwuno & others/ E&O 757	16.08.74
	FHI 27564	Quarters 680, Jericho, Ibadan	P. Wit/ PW 6	17.08.71
	FHI 103399	Wadata area, Makurdi	Daramola/Emwiogbon /Oguntayo/DEO 595	07.07.78
	FHI 89889	Along farmland, Gashaka, Gongola	Fagbemi F. A/326	12.08.77
	FHI 97140	Sapele, Bendel	Ariwaodo & Adesina / AA8	11.09.81
	IFE 13856	Borgu game reserve, Niger	B. O. Daramola	27.09.01
	NIPRDH 5884	-	-	29.08.06
Phyllanthus beillei	FHI 5636	Little Osse river, Owo, Ondo	A. C. Hoyle & J. P. M. Brenna	24.08.43
	FHI 61825	Iseyin, Oyo	D. P. Stanfield	02.05.65
Phyllanthus capillaris	FHI 84523	Jauro-Umar camp area, Gembu, Gongola	B. O. Daramola / D 233	26.08.77
	FHI 86495	Akoko south, Oka, Ondo	Daramola & Ihe /BO 550	30.05.78
	FHI 86973	Ogoja-Ikom road, Cross-river	Emwiogbon & Daramola/608	05.05.78
	FHI 78618	Akapabuyo beach, Calabar	Daramola, Macaulay & Oguntayo/C345	30.09.75
	UIH 17453	SHF hill, Yaounde, Cameroon	J. Lowe/3269	27.02.77
	UIH 12270	Umudike	Tuley & Redhead/705	17.08.64
	IFE 2779	CRIN station, Bende road, Umuahia	J. Medler/764	09.04.73
	IFE 2781	Roadside to Mayo-Ndaga, Mambilla	J. Medler/913	22.08.73

		Plateau		
Phyllanthus floribundus	FHI 104911	Iseyin-Oyo road, Oyo	B.O. Daramola/96	24.03.93
	FHI 32082	Forestry hill, Ibadan	C.F.A. Onochie	March 1953
	FHI 6284	Benin	A.P.D. Jones	08.03.42
Phyllanthus fraternus	NIPRDH 4096	-	-	06.11.97
Phyllanthus maderaspatensis	FHI 62771	Sokoto-Illela motor road, Gwadabawa, Sokoto	M.G. Latilo	03.08.69
	FHI 93997	Kauwa F.R, Kukawa, Borno	Ekwuno & Fagbemi/EF 222	29.09.80
Phyllanthus mannianus	FHI 77250	Ngeliyaki, Mambilla North-East	Ekwuno P. O/311	26.11.75
	IFE 2782	Obudu Cattle ranch, Ogoja	J. Medler	13.04.73
Phyllanthus muellerianus	FHI 97072	Okorshie, Obudu, C.R.S	Ekwuno & Others/E&O1001	19.09.81
	FHI 46275	Mambilla Plateau, N.E, Nigeria	J.D. Chapman	07.07.72
	FHI 65767	Zoo garden, Enugu	J.A. Emwiogbon	17.08.72
	FHI 88505	Isanlu, Kwara state	Olorunfemi/Oguntayo/ Ihe.284	04.10.78
	FHI 92098	Makurdi, Benue state	Daramola/Emwiogbon /Oguntayo DEO 658	03.01.80
	UIH 21638	Okomu F.R	J. Lowe/4937	10.03.91
	UIH 10235	Biological garden, University of Ife	D. Gledhill	10.01.68
	UIH 10892	Botanical garden, U.I, Ibadan	K.K. Agwu	21.08.62
	UIH 16816	10, Laird place, U.I, Ibadan	J. Lowe	23.10.75
	UIH 1927	South of Kishi, Oyo	J. F. Redhead	27.07.64
	IFE 2747	Igbetti rock, Oyo	J. Medler/577	06.02.71
	IFE 2746B	O A U Campus, Ife	D. P. M. Guide/597	January, 1967
	IFE 2745A	I A. R & T	J. Medler/1059	17.07.74
	NIPRDH 5559	Agricultural crop research station, Ilora		08.04.04
Phyllanthus nigericus	FHI 36162A	Akure, Ondo	J.P.M. Brennan & R. W.J. Keay	03.01.48
	FHI 70470	Enugu	Ekwuno P.O	16.10.73
	FHI 97017	Obudu, C. R. S	-	11.02.82
	FHI 56177	Adamawa division, Mambilla district, Plateau	-	29.01.58
	FHI 40000	Owo, Ondo	-	04.05.57

Phyllanthus niruri	FHI 89081	Duji F.R, Minna, Niger state	-	19.02.77
	FHI 95587	Bende F.R, Imo Jalingo, N.E	-	06.09.81
	FHI 60497	Odoba,Otupko road, Benue	-	08.05.72
	FHI 103424	-	-	17.06.78
	UIH 21438	Nursery, Botany Dept, U.I, Ibadan	J. Lowe/4866	23.11.89
	UIH 1928	Ibadan	A. J. C/598	10.08.33
	UIH 1930	Lagos	A. J. C/832	December, 1934
	IFE 16428	Ibadan road, Ile-Ife, Osun	Akinwande O.	27.06.11
	ILH 185	-	-	09.04.84
	ABUH 2522	-	-	02.08.88
Phyllanthus niruroides	FHI 42341	Igarra	-	22.09.58
	UIH 15562	Kolokuma area, Yenagoa Division, Rivers state	K. R. M. Williamson/339	06.11.73
Phyllanthus odontadenius	FHI 6217	Awka bathing pool, Awka, Onitsha	A. P. D. Jones/1800	14.06.42
	UIH 14259	Borgu	G. Jackson	03.10.72
	UIH 13797	Kiama,Yenagoa area, Rivers state	Dr Williamson's Assistant/A 13	March, 1970
	UIH 19713	Calabar	-	December,1981
	UIH 21320	Sapoba, Benin	J. Lowe/4812	03.04.88
	IFE 2785B	Idanre hills, Ondo	J. B. Hall/1258	20.04.69
Phyllanthus pentandrus	FHI 83311	Ohumbe F.R	-	13.06.77
	UIH 14257	Argungu road	G. Jackson	13.10.70
	UIH 12482	Igbetti, Oyo	Z.O. Gbile & J. Olorunfemi	22.10.68
	IFE 2794	Panyan, Plateau	J. B. Hall/2002	13.07.70
	IFE 2792	Borgu game reserve, near Kanji Dam, Ilorin	I.B. Faremi	15.10.76
Phyllanthus physocarpus	IFE 2795B	Road 7, OAU, Ile-Ife	I. B. Faremi/1262	21.03.77
	FHI 40876	Owena Akure F. R, Ondo, Ife	E. O. Bamgbala	07.05.60
	FHI 43451	-	M. G. Latilo	10.07.59
Phyllanthus reticulatus	FHI 19180	Kano town Forest	R. W. J. Keay	26.08.47

		Nursery, Kano		
	UIH 10866	Ikorodu	G. Jackson/2544A	09.05.62
	IFE 2796	Shagumu, near new village, Ilorin	J. B. Hall	11.06.69
		Shere Mountain, Bauchi		
Phyllanthus rotundifolius	IFE 2798B	Okitipupa, Ondo Oyo	J. B. Hall/2133	18.07.70
Phyllanthus sublanatus	FHI 96993	Shagumu, near new village, Ilorin	Ibhanesebor & Osanyinlusi	15.07.82
	UIH 2105	-	A. J. C/580	08.08.37
	IFE 2800	-	J. B. Hall/1304	11.06.69
Phyllanthus urinaria	UIH 21823	Odimodi, near Forcados, Delta state	A.Egunyomi/8	08.06.92
	IFE 2802B	Sha falls, Plateau	J. B. Hall/2034	15.07.70

UIH – University of Ibadan Herbarium
FHI - Forest Herbarium Ibadan
IFE – Obafemi Awolowo University Herbarium
ABUH – Ahmadu Bello University Herbarium
ILH – University of Ilorin Herbarium
NIPRDH – Nigerian Institute of Pharmaceutical Research and Development Herbarium, Abuja

3.4 Macromorphology

Vegetative and floral macromorphological features were assessed from 142 specimens studied. Vegetative characters were observed on shape, leaf apex, margin, leaf surface and base, while measurements were taken for the length, width at the widest part, petiole length, blade length, leaf length/width ratio and blade/petiole length ratio. The floral characters assessed were flower colour, number of perianth lobes and fruit colour. Descriptive statistics of the mean and standard deviation were done for each character using SPSS.

3.5 Micromorphology

3.5.1 Preparation of epidermal peels

Fresh samples as well as herbarium specimens of species that were not available for collections from the field were used for this study and cell description was based on Metcalfe and Chalk (1950), Stace (1965) and Esau (1977). The leaf specimens for study were obtained at standard median portion of the lamina and placing it in a water bath for 10 minutes to allow for softening and rehydration of the dried leaves. These were rinsed in ordinary water and put in a Petri-dish, 100% trioxonitrate (V) acid was added to each Petri-dish so that the cut leaves were covered with the acid. Formation of air-bubbles in the treated leaves indicated that the upper and the lower epidermis had separated from the mesophyll layer and were ready for peeling. The specimens were transferred into new Petri-dishes containing water for rinsing. The epidermal layers were separated by tearing them apart carefully with a pair of forceps and gently brushing the epidermal layer with soft artist hair brush to remove the residual mesophyll layer, these were then transferred into storage bottles containing 50% ethanol.

3.5.2 Preparation of slides

The leaf epidermal peels were stained in Safranin for about 3 minutes and rinsed in water to remove excess stain. They were dehydrated through series of alcohol of 50%, 70%, 80%, 90% and 100% ethanol for about 3 minutes each, the immersion in absolute ethanol (100%) was done twice. The epidermal layers were put in xylene for about 3 minutes to remove ethanol and then mounted in 25% glycerine on clean glass slides and covered with cover slips avoiding air bubbles. The edges of the cover slips were then sealed with nail polish to prevent dehydration. Observations and measurements were carried out using micrometer eyepiece on a pre-calibrated microscope. Measurements were converted by the ocular constant with respect to the power of the objective eyepiece under which they were taken and recorded. Twenty different measurements of each character were taken at random from each specimen and the mean and standard deviation calculated. Photomicrographs of the specimens were taken using Leica CME with a digital microscope eyepiece attached and photo explorer 8.0 SE basic software and stomata terminologies follow Dilcher (1974) and Stace (1989). The stomata index (SI) was calculated for each of the representative taxa using the formula adopted by Stace (1965).

$$S.I = \frac{S}{S + E} \times 100\%$$

Where S = number of stomata per unit area (mm^2)

E = number of epidermal cells in the same unit area.

3.6 Scanning Electron Microscopy (SEM)

This aspect of the study was carried out at the Scanning Electron Microscope unit of the Department of Material Science, Faculty of Engineering, Kwara State University, Ilorin, Kwara State. Scanning Electron Microscope was used to investigate the leaf surfaces of the herbaceous species in the genus *Phyllanthus* in order to complement the morpho-anatomical studies.

Portions of the leaves were taken from 14 specimens representing 14 species collected from the field as well as herbarium samples. The method used by Ayodele and Zhou (2008) was adopted. Small pieces (c. 5 mm^2) of each leaf taken from specimens were fixed to labeled stubs with double sided adhesive tape. Each sample was scanned and photographed using the ASPEX LLC – 3020 Analytical Scanning Electron Microscope with Energy Dispersive Spectrometer.

3.7 Pollen morphology

The method of acetolysis, slide preparation and terminologies used in the description of the pollens were based on Erdtman (1952) and Moore *et al*. (1991). To each numbered plastic centrifuge tube containing pollen samples from the flower buds of

18 field specimens representing 10 species preserved in 50% ethanol was added to 5 ml of acetolysis mixture (9 parts acetic anhydride to 1 part concentrated tetraoxosulphate (vi) acid) and heated in a water bath from 70^{o}C to boiling point, stirring occasionally. This was left in the boiling water for 5 minutes. Acetolysis mixture left over was then poured into the special bottle labelled 'Acetolysis waste'. It was centrifuged while still hot and decanted into the special bottle, distilled water was added and shaken vigorously with a mixer, centrifuged and decanted. This was repeated four times to rinse off the acetolysis mixture. Fifty percent aqueous glycerol was added and then left for at least 30 minutes. It was later vigorously mixed on the mixer, centrifuged for 10 minutes and then decanted. It was mixed thoroughly and stored in well labeled vials from where aliquots of it were mounted on slides.

3.7.1 Mounting of slides

Microscope slides were cleaned with cotton wool soaked in ethanol and labelled appropriately, 1-2 drops of the aliquot were then placed at the center of the slide and covered with a cover slip for it to spread evenly. The sides of the cover slip were sealed with nail varnish and then studied under the light microscope, Photomicrographs of the specimens were taken using Leica CME with a digital microscope eyepiece attached and photo explorer 8.0 SE basic software. All slides were deposited in the herbarium of the Department of Botany, University of Ibadan, Ibadan, Nigeria.

3.8 Molecular studies

The DNA extraction and sequencing analysis were carried out at the Biotechnology Research Laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan. These studies involved sequences from ten species of the genus *Phyllanthus* that were available on the field for study and one species each from the genera *Margaritaria* and *Securinega* used as outgroups.

3.8.1 DNA Extraction

Twenty freshly collected young leaf samples comprising 10 species in the genus *Phyllanthus* and one species each from the genera *Margaritaria* and *Securinega* were desiccated with silica gel in zip-lock plastic bags until use. Genomic DNA was extracted from dried leaf tissue using the DNeasy Plant Mini Kit (250) (QIAGEN) with catalogue number 69106.

3.8.2 Primers optimization

Extracted DNA was subjected to the following cocktail mix and condition for the PCR.

Condition for PCR	Values
10× PCR buffer	1.0
25mM Mgcl2	1.0
5pMol forward primer	0.5
5pMol reverse primer	0.5
DMSO	1.0
2.5Mm DNTPs	0.8
Taq 5u/ul	0.1
10ng/µl	2.0
H2O	3.1
	10µL

Purified PCR were quantified by estimation using a low DNA Mass Ladder1kb plus from Invitrogen and then cycle sequenced using the same primers as were used for purification. The base composition of *rbcL* was a typical gene profile with almost equal ratio of the bases.

3.8.3 PCR Amplification

The PCR amplification was performed using the cpDNA universal primer pairs, Hif: 5` CCA CAA ACA GAG ACT AAA GC 3` and Fofana: 5` GTA AAA TCA AGT CCA CCG CG 3`, amplicon size: 568 and *rbcL gene* (*rbcL* H535: 5` CTT TCC AAG GCC CGC CTC A3`and *rbcL* C705: 5` CAT CAT CTT TGG TAA AAT CAA GTC CA3` amplicon size: 140. Polymerase chain reactions were performed using 10.0 µL volumes as shown in the table above. The PCR profile used was 5 mins at 94ºC for initial denaturation, followed by 36 cycles of denaturation at 94ºC for 30 seconds annealing at

56ºC for 30 seconds and extension at 72ºC for 45 seconds. A final extension step was conducted for 7 mins. at 72ºC. The PCR product was purified by adding 2 vol (20 µL) of absolute ethanol to the PCR product and incubated at room temperature for 15minutes, spinned down at 10000 rpm for 15 minutes and supernatant then decanted. The supernatant was spinned down at 10000 rpm for another 15 minutes before 2 vol (40 µL) of 70% ethanol was added and supernatant decanted, air dried and about 10 µL of ultrapure water added. Amplicon was then checked on 1.5% agarose gel.

Purified PCR were quantified by estimation using alow DNA Ladder 1kb plus from Invtrogen and then cycle sequenced using the same primers as were used for purification. The base composition of *rbcL* was a typical gene profile with almost equal ratio of the bases.

3.8.4 Sequencing

Amplified fragments were sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Inc.), purified again and loaded on the 3130 X 1 Genetic analyzer. Alignment of the sequences was performed with the Clustal X program (Thompson *et al*., 1997) and manually corrected using Bioedit to refine DNA Sequences. The evolutionary history was inferred by using the Maxumum Likelihood method based on the Kimura 2-parameter model (Kimura, 1980). The trees with the highest log likelihood -1115,90 and -1405,85 are shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with superior log likelihood value. The analysis involved 18 nucleotide sequences and 27 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% allignment gaps, missing data and ambiguous bases were allowed at any position. There were a total of 439 positions and 470 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar *et al*., 2016).

3.9 Numerical taxonomy

3.9.1 Selection of Operation Taxonomic Units (OTUs)

Nineteen OTUs were selected for the numerical taxonomic study. The 19 OTUs were the species of the genus *Phyllanthus* identified in this study to occur in Nigeria. The nineteen OTUs are presented below:

1. *Phyllanthus acidus*……………………………………OTU1
2. *P. amarus*……………………………………... …….OTU2
3. *P. beillei*…………………………………………... OTU3
4. *P. capillaris*…………………………………………. OTU4
5. *P. floribundus*………………………………… OTU5
6. *P. fraternus*…………………………………… OTU6
7. *P. maderaspatensis*………………………………… OTU7
8. *P. mannianus*……………………………………….. OTU8
9. *P. muellerianus*…………………………………….. OTU9
10. *P. nigericus*……………………………….. OTU10
11. *P. niruri*………………………………................. OTU11
12. *P. niruroides*……………………………………….. OTU12
13. *P. odontadenius*…………………………………….. OTU13
14. *P. pentandrus*……………………………………….. OTU14
15. *P. physocarpus*………………………………. OTU15
16. *P. reticulatus*………………………………… OTU16
17. *P. rotundifolius*………………………………. OTU17
18. *P. sublanatus* ………………………………... OTU18
19. *P. urinaria*………………………………………….. OTU19

3.9.2 Selection of characters: A total of forty-six characters were selected for this analysis. These were 14 macromorphological characters, 27 epidermal characters and five pollen morphological characters. The characters were scored quantitatively and qualitatively. The characters used and their codes are listed in Appendix I. The data matrices were 46 characters by 19 OTUs which comprise combined macromorphological, leaf epidermal and pollen morphological characters; 27 characters by 19 OTUs which comprises leaf epidermal characters only; 14 characters by 19 OTUs which comprises macromorphological characters only and 5 characters by 19 OTUs which comprises pollen morphological characters only. The data matrices are presented in appendix II – V while the distance coefficients measures, eigenvalues and eigenvectors for the OTUs are shown in appendix VI-XVII.

3.9.3 Data analyses

3.9.3.1 Clustering analysis

Single linkage cluster analysis was used. This was performed for all the four different data matrices and cladograms were produced. The program was run on a computer using PAST Version 3 package.

3.9.3.2 Principal Component Analysis (PCA)

The four data matrices used for the clustering were also used for the PCA. The PAST Version 3 package was also used for this analysis. Data matrices that were used for the principal component analysis (PCA) were: 46 x 19 (combined macromorphological, epidermal and pollen morphological data matrix), 27x19 (epidermal data matrix), 14 x 19 (macromorpholgical data matrix) and 5 x 19 (pollen morphological data matrix). The four data matrices were first subjected to PCA and eigen values were calculated. The first four principal axes of each of the data matrix were selected for the character loading for 46, 27, 14 and 5 characters respectively and the characters that best accounted for the variation observed among the species were selected. A total of 46 combined characters (46 x 19), 27 epidermal characters (27 x 19), 14 macromorphological characters (14 x 19) and 5 pollen morphological characters (5 x 19) were assessed and subjected to PCA.

CHAPTER FOUR

4.0 RESULTS

4.1 Field collections

A total of 55 fresh specimens were collected from different locations across 17 states in Nigeria during field studies. These specimens represent ten species in the genus *Phyllanthus*: *Phyllanthus acidus, P. amarus, P. capillaris, P. maderaspatensis, P. muellerianus, P. niruri, P. odontadenius, P. pentandrus, P. reticulatus* and *P. urinaria.* A list of collected specimens has been presented in Table 3.1 while Figure 4.1 shows the collection sites of specimens of the genus *Phyllanthus* in Nigeria.

4.2 Picture database

Photographs of the specimens available during the field studies are presented in Plates 4.1–4.9.

4.3 Herbarium studies

A list of herbarium species studied has been presented in Table 3.2. Eighty-seven specimens representing 19 species in the genus *Phyllanthus* were studied: *Phyllanthus acidus*, *Phyllanthus amarus*, *Phyllanthus beillei*, *Phyllanthus capillaris*, *Phyllanthus floribundus*, *Phyllanthus fraternus*, *Phyllanthus maderaspatensis*, *Phyllanthus mannianus*, *Phyllanthus muellerianus*, *Phyllanthus nigericus*, *Phyllanthus niruri*, *Phyllanthus niruroides*, *Phyllanthus odontadenius*, *Phyllanthus pentandrus*, *Phyllanthus physocarpus*, *Phyllanthus reticulatus*, *Phyllanthus rotundifolius*, *Phyllanthus sublanatus* and *Phyllanthus urinaria.* Plates 4.10 - 4.19 show the photographs of the specimens in the genus from herbarium studies while Table 4.1 shows the distribution of members of the genus and the States where they occur in Nigeria.

Figure 4.1: Map of Nigeria showing collection sites of *Phyllanthus* species

Plate 4.1: Photographs of *Phyllanthus amarus* showing
A: growth habit;
B: the flowers (arrowed) on the abaxial surface;
C: fruits (arrowed)

Plate 4.2: Photographs of *Phyllanthus odontadenius* showing
A: growth habit;
B: alternate leaf arrangement and the flowers (arrowed) on the abaxial surface;

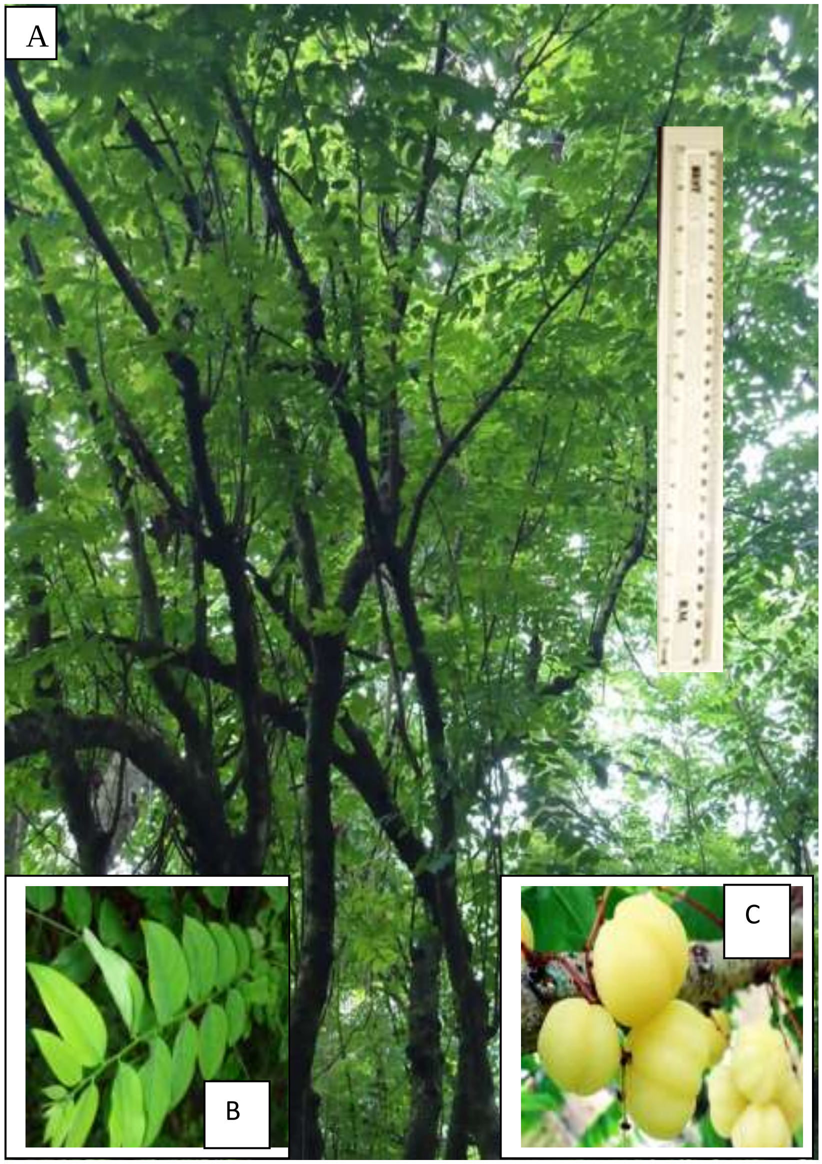


Plate 4.3: Photographs of *Phyllanthus acidus* showing
A: growth habit;
B: alternate leaf arrangement;
C: fruits

Plate 4.4: Photographs of *Phyllanthus capillaris* showing

A: growth habit showing the alternate leaf arrangement

B: fruits and flowers (arrowed) on the abaxial surface

Plate 4.5: Photographs of *Phyllanthus muellerianus* showing
A: growth habit;
B: alternate leaf arrangement;
C: flowers (arrowed);
D: fruits (arrowed)

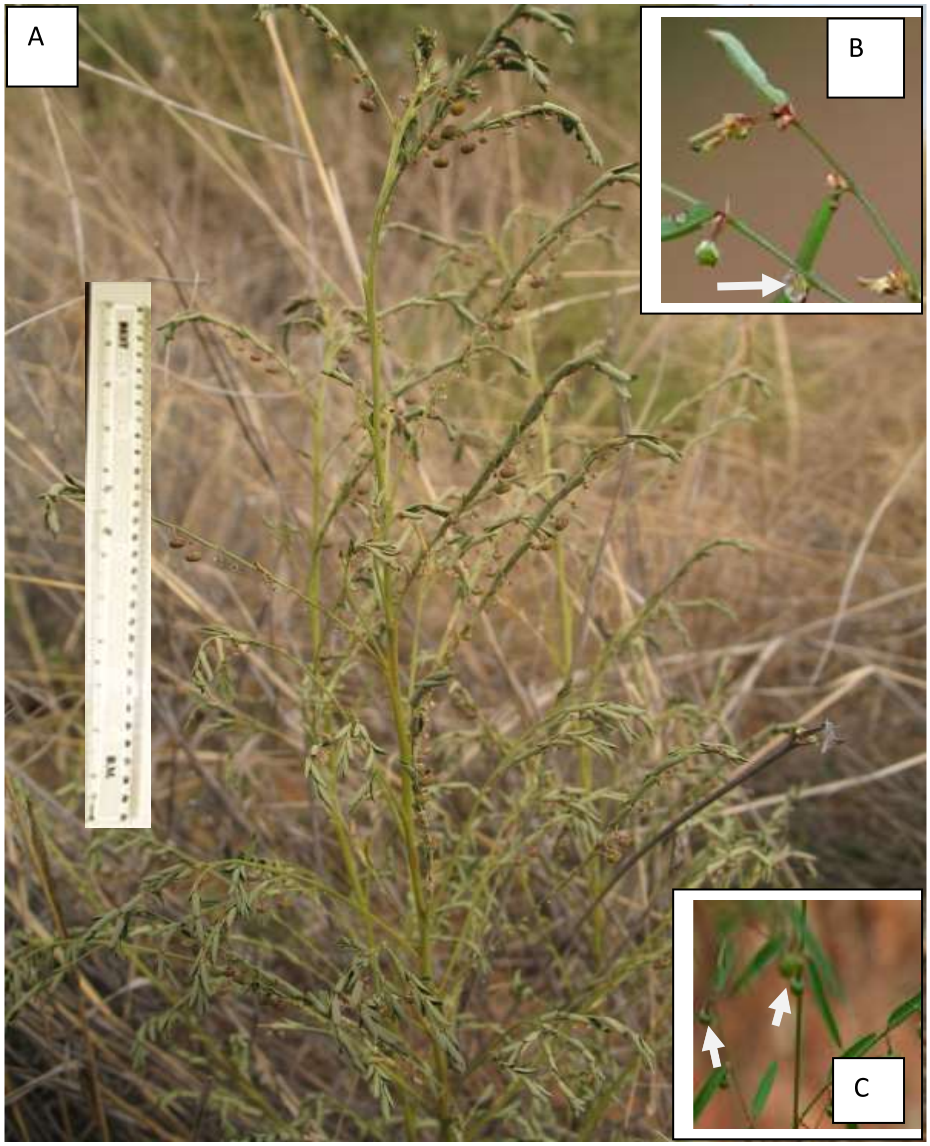


Plate 4.6: Photographs of *Phyllanthus pentandrus* showing
A: growth habit;
B: flowers (arrowed);
C: fruits (arrowed) and the linear shaped leaves

Plate 4.7: Photographs of *Phyllanthus niruri* showing
A: growth habit;
B: flowers (arrowed);
C: fruits (arrowed)

Plate 4.8: Photographs of *Phyllanthus urinaria* showing
A: alternate leaf arrangement and the reddish brown fruits (arrowed) on the abaxial surface;
B: growth habit;
C: flowers (arrowed);
D: fruits (arrowed)

Plate 4.9: Photographs of *Phyllanthus reticulatus* showing
A: growth habit;
B: fruits;
C: flowers (arrowed)

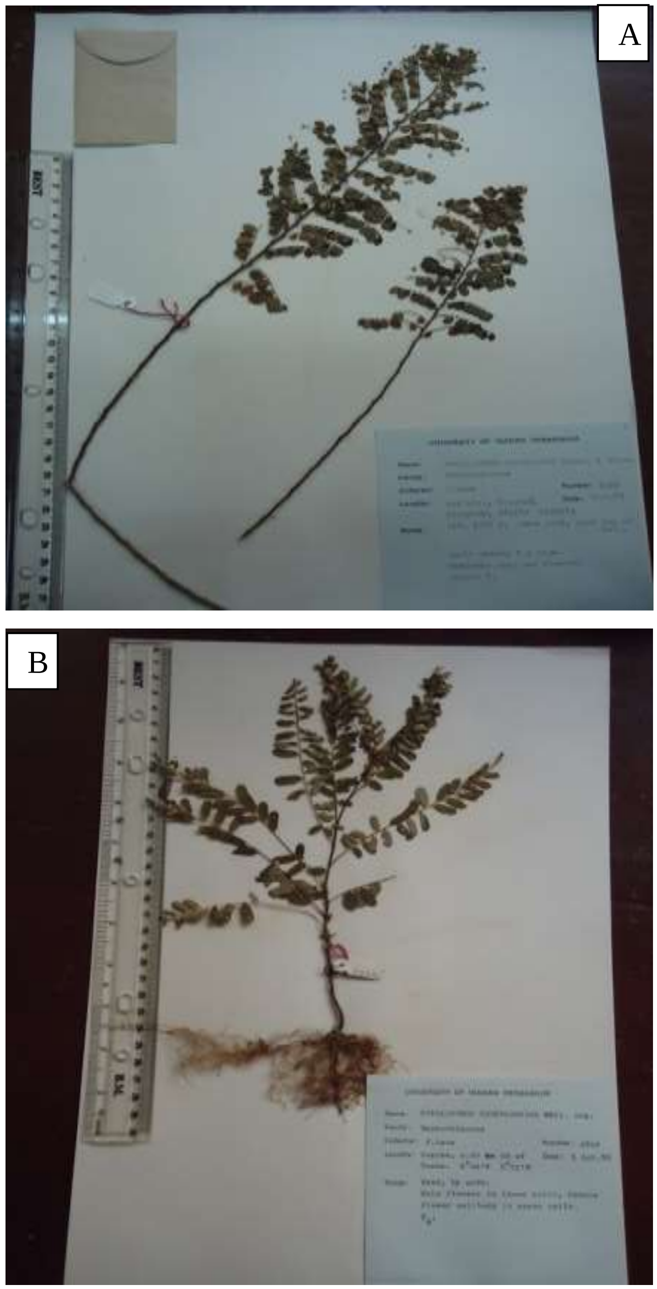


Plate 4.10: Herbarium specimens of *Phyllanthus* species
A: *Phyllanthus capillaris* J. Lowe 3269 (UIH)
B: *Phyllanthus odontadenius* J. Lowe 4812 (UIH)

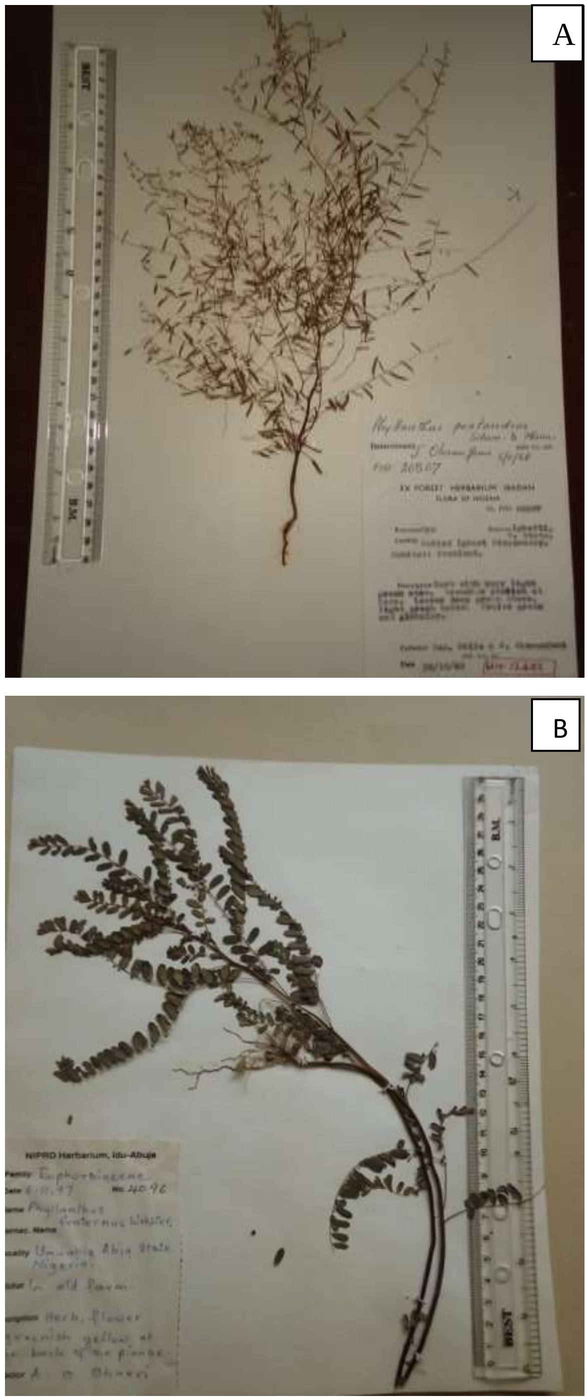


Plate 4.11: Herbarium specimens of *Phyllanthus* species
A: *Phyllanthus pentandrus* Gbile & J. Olorunfemi 20507 (FHI)
B: *Phyllanthus fraternus* A. O. Ohaeri 4096 (NIPRDH)

Plate 4.12: Herbarium specimens of *Phyllanthus* species
A: *Phyllanthus urinaria* A. Egunyomi 21823 (UIH)
B: *Phyllanthus reticulatus* Bamidele 21292 (UIH)

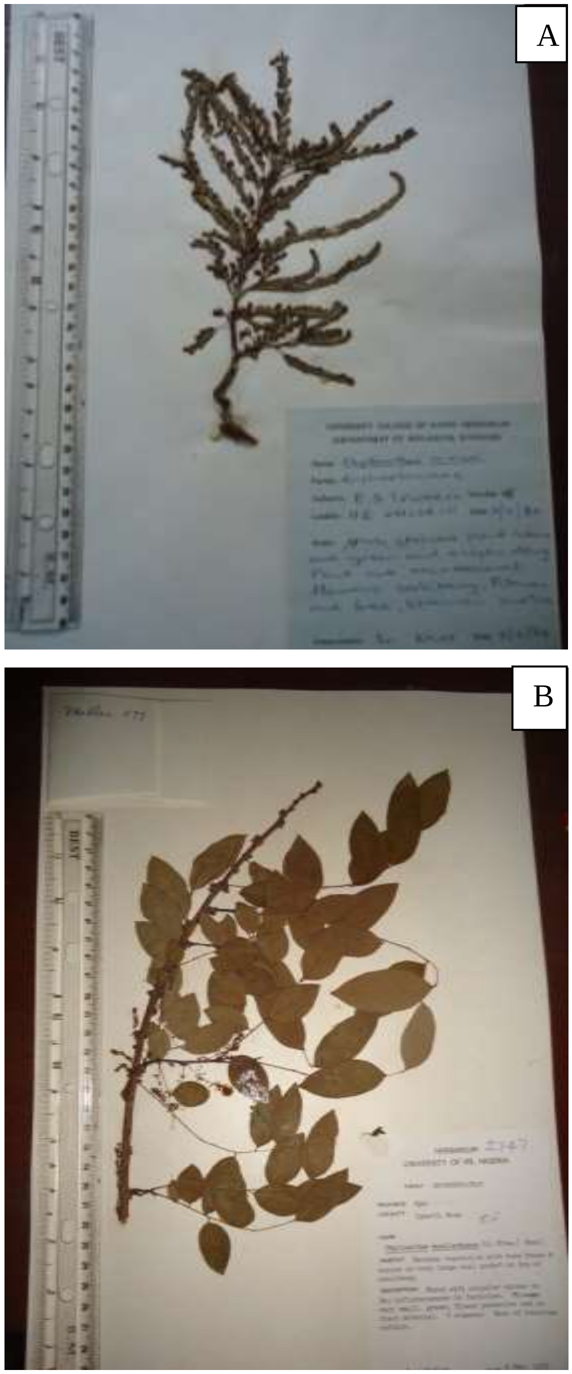


Plate 4.13: Herbarium specimens of *Phyllanthus* species
A: *Phyllanthus niruri* E. S. Irukera 185 (ILH)
B: *Phyllanthus muellerianus* J. Medler 2747 (IFE)

Plate 4.14: Herbarium specimens of *Phyllanthus* species
A: *Phyllanthus amarus* P. Wit 27564 (FHI)
B: *Phyllanthus rotundifolius* J. B Hall 27986 (IFE)

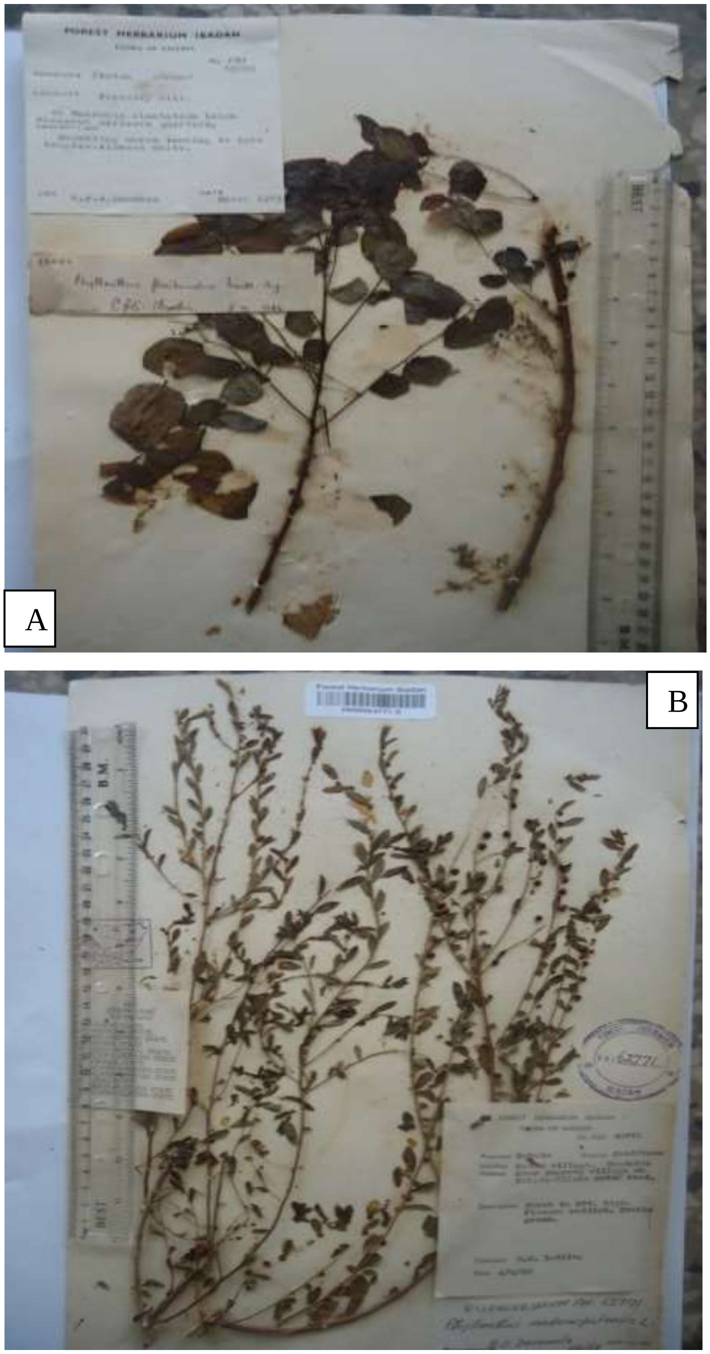


Plate 4.15: Herbarium specimens of *Phyllanthus* species
A: *Phyllanthus floribundus* C. F. A. Onochie 32082 (FHI)
B: *Phyllanthus maderaspatensis* M. G. Latilo 62771 (FHI)

Plate 4.16: Herbarium specimens of *Phyllanthus* species
A: *Phyllanthus mannianus* P. O. Ekwuno 77250 (FHI)
B: *Phyllanthus physocarpus* M. G. Latilo 43451 (FHI)

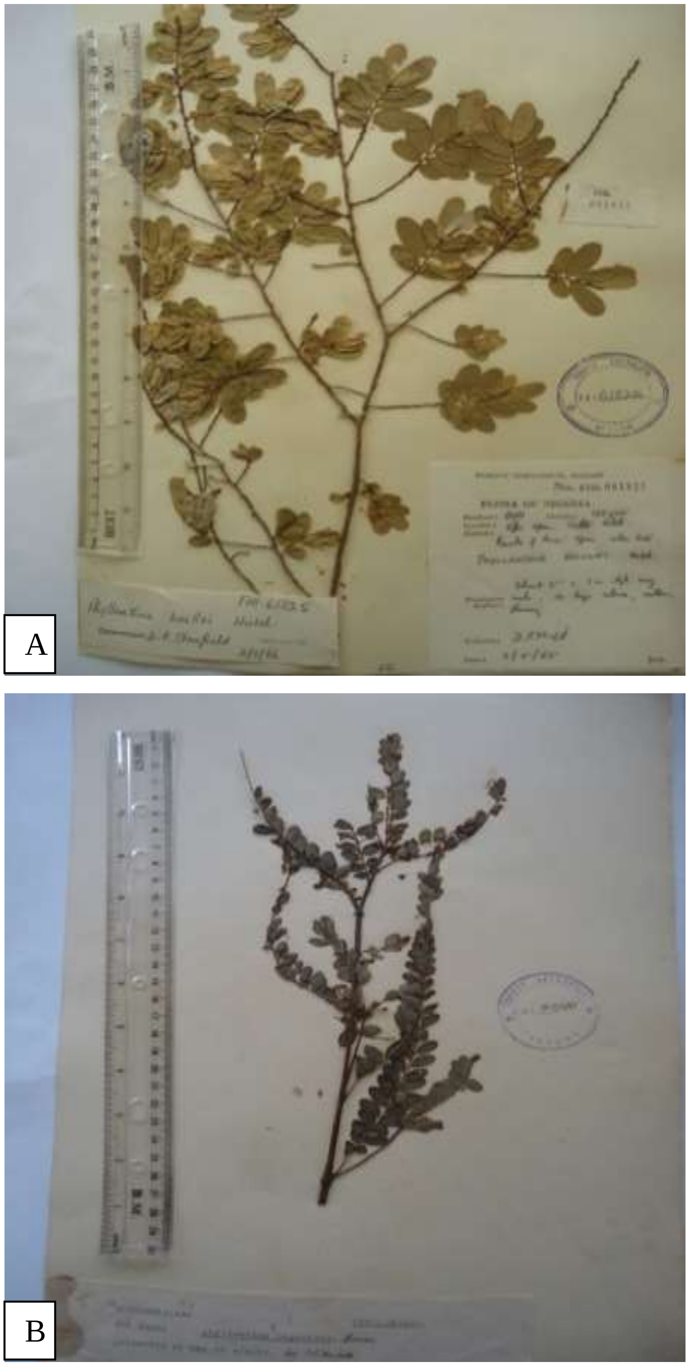


Plate 4.17: Herbarium specimens of *Phyllanthus* species
A: *Phyllanthus beillei* D. P. Stanfield 61825 (FHI)
B: *Phyllanthus nigericus* D. P. Stanfield 40000 (FHI)

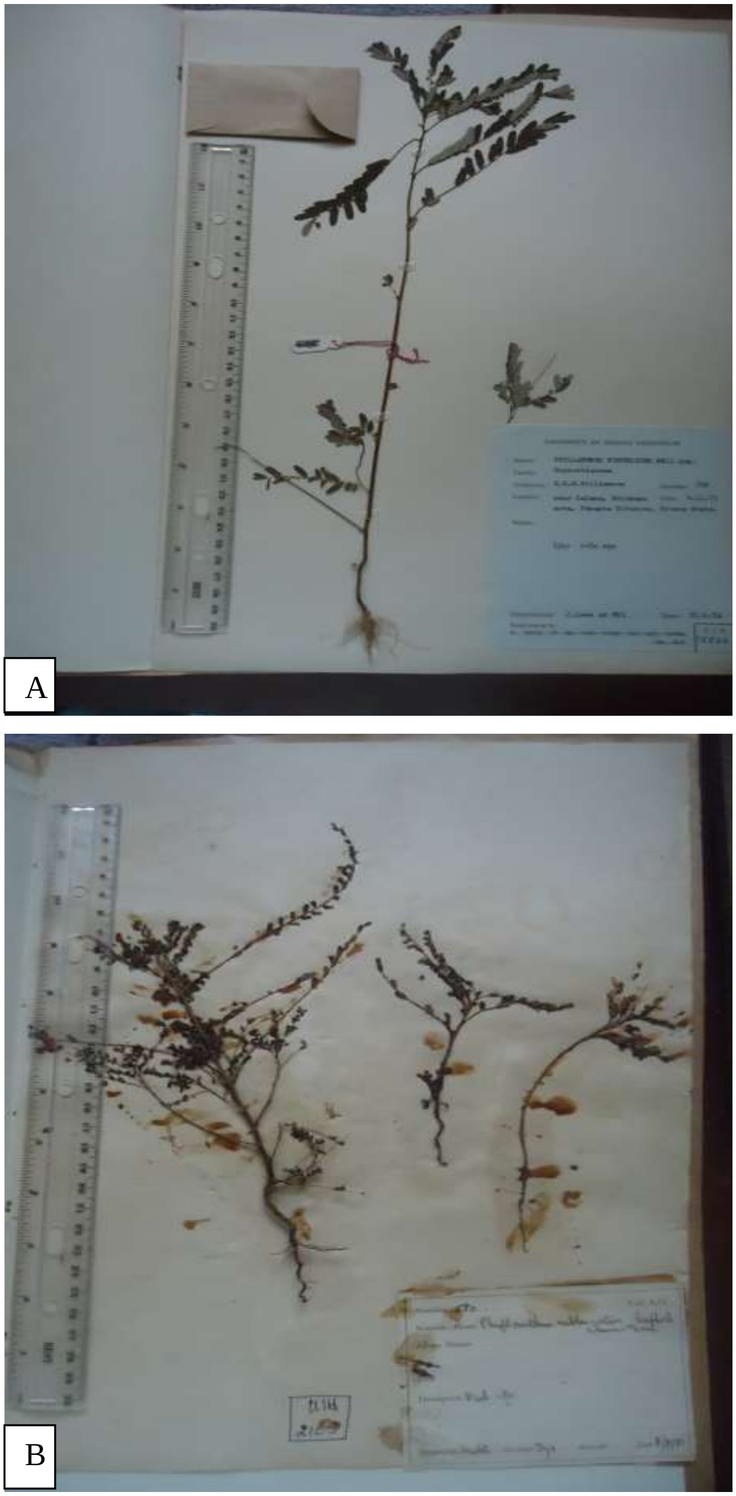


Plate 4.18: Herbarium specimens of *Phyllanthus* species
A: *Phyllanthus niruroides* K. R. M. Williamson 15562 (UIH)
B: *Phyllanthus sublanatus* A. J. C 2105 (UIH)

Plate 4.19: Herbarium specimen of *Phyllanthus* species
A: *Phyllanthus acidus* B. O. Daramola 518 (IFE)

4.3.1 Distribution of *Phyllanthus* species in Nigeria

As shown in Table 4.1, the most commonly distributed *Phyllanthus* species in Nigeria is *P. amarus* occurring in the northern to the southern states. Although *P. niruri* and *P. muellerianus* had no record of collection in the east and west part of northern states, they are also well distributed over the central or middle-belt of Nigeria to the southern states. *P. pentandrus* is the fourth most occuring species; records being from the west part of northern states through the central and extending to few southern states.

The species collected from the southern states only are *P. acidus, P. physocarcus* and *P. urinaria*. In contrast, *P. maderaspatensis* and *P. mannianus* are restricted to a few northern states. Records of collection revealed that certain species were found in the middle belt area and some southern states of Nigeria. The species are *P. floribundus, P. fraternus, P. niruroides, P. odontadenius* and *P. sublanatus.*

Phyllanthus species collected from some states in Nigeria characterized by highland and montane areas (Plateau, Taraba and Adamawa) are *P. beillei, P. capillaris, P. nigericus, P. rotundifolius* and *P. reticulatus*. The distribution of *Phyllanthus* species based on the ecological zones of Nigeria shows that 14 of the 19 species under study occur in the Guinea savanna, lowland rainforest and the mangrove forest. *Phyllantus amarus* and *P. pentandrus* occur in all the ecological zones hence have the widest ecological distributional range. The species that have narrow distributional range are *P. maderaspatensis* confined to the Sudan savanna, *P. physocarpus* restricted to the lowland rainforest and *P. urinaria* to the mangrove forest.

4.4 Macromorphology

The qualitative and quantitative leaf macromorphological features of *Phyllanthus* species are presented in Tables 4.2a and 4.2b respectively. The species in the genus *Phyllanthus* are mostly herbs, very few are shrubs while *P. physocarpus* is the only tree species. The leaves are small, generally entire and the arrangement is mostly alternate or spiral with the apices acute, shortly acute, obtuse, acuminate or mucronate. Leaf shapes are ovate, oblong, obovate, lanceolate, elliptic, linear, sub-orbicular, lanceolate-ovate or oblong-lanceolate. All the species are glabrous except the young leaves of *Phyllanthus muellerianus* which are glossy on the adaxial surface. *Phyllanthus beillei, P. capillaris, P. maderaspatensis, P. mannianus, P. niruri, P. pentandrus, P. physocarpus, P.*

odontadenius, P. reticulatus, P. rotundifolius and *P. urinaria* have cuneate bases while *P. acidus, P. amarus, P. floribundus, P. fraternus, P. muellerianus, P. nigericus, P. niruroides* and *P. sublanatus* have attenuate bases (Table 4.2a). Flowers are very small, in axillary clusters or solitary, disk in male flowers are of small glands and annular in female flowers. Flower colour was variable in the genus, white in *P. capillaris, P. niruroides* and *P. pentandrus*, yellowish-white in *P. amarus*, *P. beillei P. fraternus* and *P. niruri*, greenish-white in *P. urinaria, P. odontadenius*, *P. nigericus* and *P. mannianus* while it was pinkish-green in *P. acidus*, pale-yellow in *P. maderaspatensis*, green in *P. muellerianus,* pale-green in *P. rotundifolius* and cream in *P. reticulatus* (Table 4.2a). Perianth-lobes ranged from 4-6 in all taxa studied. The fruit colour was observed to be green in most of the species such as *P. amarus, P. beillei, P. capillaris, P. fraternus, P. maderaspatensis, P. niruri* (Plate 4.7c)*, P. niruroides, P. odontadenius* and *P. pentandrus* (Plate 4.6c). It was pale-yellow in *P. acidus* (Plate 4.3c), reddish-brown in *P. urinaria* (Plate 4.8d), red in *P. muellerianus* and black in *P. reticulatus* (Plate 4.9b) when ripe.

The smallest mean leaf length was obtained in *P. sublanatus* (0.45 cm) followed by *P. rotundifolius* (0.6 cm) while *P. physocarpus* had the highest mean leaf length (11.7 cm) followed by *P. floribundus*, *P. acidus, P. muellerianus* and *P. reticulatus* with mean leaf lengths of 5.45 cm, 4.56 cm, 4.13 cm and 2.8 cm respectively (Table 4.2b). *Phyllanthus physocarpus* had the broadest mean leaf width (5.4 cm) while *P. urinaria* had the narrowest of 0.15 cm. Differences were also obtained in the petiole length with the longest petiole of 0.45 cm recorded in *P. physocarpus* followed by *P. floribundus, P. muellerianus*, *P. acidus* and *P. reticulatus* with mean petiole lengths of 0.33 cm, 0.25 cm, 0.24 cm and 0.20 cm respectively. *Phyllanthus amarus, P. capillaris, P. maderaspatensis, P. nigericus, P. niruri, P. niruroides, P. rotundifolius, P. sublanatus* and *P. urinaria* were sessile (no petiole) while *P. beillei, P. fraternus, P. mannianus, P. odontadenius* and *P. pentandrus* were subsessile (</= 0.1 cm). The leaf length/width ratio was 2:1 for *P. acidus, P. amarus, P. beillei, P. capillaris, P. floribundus, P. mannianus, P. muellerianus, P. nigericus, P. niruri, P. odontadenius* and *P. physocarpus* while in *P. fraternus, P. niruroides, P. maderaspatensis* and *P. reticulatus,* the ratio was 3:1. *Phyllanthus pentandrus* and *P. urinaria* had a leaf length/width ratio of 6:1, 5:1 in *P. sublanatus* and 1:1 in *P. rotundifolius*. The blade/petiole length ratio of 25:1 was the highest in *P. physocarpus* followed by 18:1 in *P. acidus* while *P. reticulatus* had the lowest ratio of 13:1 (Table 4.2b).

Table 4.1: List of *Phyllanthus* species and the States where they occured in Nigeria

Zones	States in Nigeria	Pac	Pam	Pbe	Pca	Pfl	Pfr	Pma1	Pma2	Pmu	Pni1	Pni2	Pni3	Pod	Ppe	Pph	Pre	Pro	Psu	Pur
North west	Sokoto							X							X					
	Kebbi																			
	Zamfara																			
	Katsina		X												X					
	Kano																			
	Jigawa																			
North east	Taraba		X		X				X			X					X			
	Yobe																			
	Bauchi			X														X		
	Gombe		X																	
	Bornu		X					X												
	Adamawa				X															
North central	Nassarawa																			
	Niger		X				X					X		X	X					
	Abuja									X										
	Kaduna			X						X		X								
	Plateau			X	X				X	X	X	X			X			X	X	
	Kogi		X							X		X	X	X	X					
	Benue		X							X		X								
	Kwara		X			X				X		X					X		X	
South west	Oyo	X	X	X		X				X		X	X		X				X	
	Ogun		X							X		X					X		X	
	Osun	X	X		X					X		X		X		X				
	Ekiti				X					X										
	Ondo		X	X	X					X	X	X		X	X	X			X	
	Lagos		X							X		X			X		X			
South east	Enugu		X							X	X				X					
	Anambra									X					X				X	
	Ebonyi																			
	Imo		X									X								
	Abia				X		X					X		X						
South south	Edo		X			X				X		X		X						
	Delta																			X
	Bayelsa												X	X						
	Rivers		X										X	X						
	Akwa-Ibom																			
	Cross River		X		X				X	X	X	X		X						

Key:

Pac:*Phyllanthus acidus*; **Pam:***Phyllanthus amarus*; **Pbe:***Phyllanthus beillei*; **Pca:***Phyllanthus capillaris*; **Pfl:***Phyllanthus floribundus*; **Pfr:***Phyllanthus fraternus*; **Pma1:***Phyllanthus maderaspatensis*; **Pma2:***Phyllanthus mannianus*; **Pmu:***Phyllanthus muellerianus*; **Pni1:***Phyllanthus nigericus*; **Pni2:***Phyllanthus niruri*; **Pni3:***Phyllanthus niruroides*; **Pod:***Phyllanthus odontadenius*; **Ppe:***Phyllanthus pentandrus*; **Pph:***Phyllanthus physocarpus*; **Pre:***Phyllanthus reticulatus*; **Pro:***Phyllanthus rotundifolius*; **Psu:***Phyllanthus sublanatus*; **Pur:***Phyllanthus urinaria*

Table 4.2a: Qualitative macro-morphological features of *Phyllanthus* species in Nigeria

Species	Leaf shape	Leaf apex	Leaf base	Leaf surface	Flower colour	Perianth lobes	Fruit colour
Phyllanthus acidus	Lanceolate/Ovate	Acute	Attenuate	Glabrous	Pinkish green	4	Pale yellow
P. amarus	Oblong	Obtuse	Attenuate	Glabrous	Yellowish white	5	Green
P. beillei	Oblong/Obovate	Obtuse	Cuneate	Glabrous	Yellowish white	6	Green
P. capillaris	Obovate	Mucronate/Obtuse	Cuneate	Glabrous	White	5	Green
P. floribundus	Lanceolate/Ovate	Acute	Attenuate	Glabrous	NA	NA	NA
P. fraternus	Oblong	Obtuse	Attenuate	Glabrous	Yellowish white	NA	Green
P. maderaspatensis	Lanceolate/Obovate	Acute	Cuneate	Glabrous	Pale yellow	6	Green
P. mannianus	Obovate/Lanceolate	Obtuse/ Shortly acute	Cuneate	Glabrous	Greenish white	NA	NA
P. muellerianus	Ovate/Lanceolate	Acute	Attenuate	Glossy/Glabrous	Green	5	Red
P. nigericus	Elliptic	Obtuse	Attenuate	Glabrous	Greenish white	5	NA
P. niruri	Oblong/Elliptic	Obtuse/Shortly acute	Cuneate	Glabrous	Yellowish white	6	Green
P. niruroides	Oblong	Obtuse	Attenuate	Glabrous	White	5	Green
P. odontadenius	Oblong	Mucronate	Cuneate	Glabrous	Greenish white	6	Green
P. pentandrus	Linear/Lanceolate	Acute/Acuminate	Cuneate	Glabrous	White	5	Green
P. physocarpus	Lanceolate-ovate	Acute	Cuneate	Glabrous	NA	NA	NA
P. reticulatus	Lanceolate	Acute	Cuneate	Glabrous	Cream	5	Black
P. rotundifolius	Suborbicular	Obtuse	Cuneate	Glabrous	Pale green	6	NA
P. sublanatus	Oblong	Obtuse	Attenuate	Glabrous	NA	NA	NA
P. urinaria	Lanceolate/Obovate	Acuminate	Cuneate	Glabrous	Greenish white	6	Reddish brown

NA – Not Available for study

Table 4.2b: Quantitative leaf macro-morphological features of *Phyllanthus* species in Nigeria

Species	Leaf Length (cm)	Leaf width (cm)	Petiole length (cm)	Blade Length (cm)	Leaf length/Width ratio	Blade/Petiole length ratio
Phyllanthus acidus	3.3(4.56±1.02)5.8	1.6(2.4±0.50)3.0	0.2(0.24±0.05)0.3	3.1(4.32±0.98))5.5	2:1	18:1
P. amarus	0.3(0.71±0.20)2.2	0.2(0.31±0.07)0.5	Sessile	0.3(0.71±0.20)2.2	2:1	NA
P. beillei	1.6(2.32±0.56)3.4	0.8(0.97±0.13)1.2	Subsessile	1.5(2.22±0.56)3.3	2:1	NA
P. capillaris	0.5(1.29±0.51)2.2	0.4(0.81±0.27)1.3	Sessile	0.5(1.29±0.51)2.2	2:1	NA
P. floribundus	4.8(5.45±0.57)6.2	2.4(2.63±0.23)2.9	0.3(0.33±0.04)0.4	4.5(5.13±0.57)5.9	2:1	16:1
P. fraternus	0.5(0.82±0.16)1.0	0.2(0.33±0.07)0.4	Subsessile	0.5(0.82±0.16)1.0	3:1	NA
P. maderaspatensis	1.2(1.45±0.15)1.6	0.4(0.43±0.04)0.5	Sessile	1.2(1.45±0.15)1.6	3:1	NA
P. mannianus	0.9(1.63±0.47)2.2	0.6(0.9±0.21)1.2	Subsessile	0.8(1.53±0.47)2.1	2:1	NA
P. muellerianus	1.7(4.13±1.45)6.6	1.2(2.18±0.58)3.1	0.2(0.25±0.05)0.3	1.5(3.88±1.42)6.3	2:1	15:1
P. nigericus	0.8(1.07±0.17)1.3	0.5(0.6±0.08)0.7	Sessile	0.8(1.07±0.17)1.3	2:1	NA
P. niruri	0.4(0.85±0.34)1.8	0.2(0.4±0.19)0.8	Sessile	0.4(0.85±0.34)1.8	2:1	NA
P. niruroides	0.7(0.98±0.19)1.2	0.3(0.35±0.05)0.4	Sessile	0.7(0.98±0.19)1.2	3:1	NA
P. odontadenius	1.1(1.53±0.41)2.7	0.4(0.67±0.25)1.3	Subsessile	1.1(1.53±0.41)2.7	2:1	NA
P. pentandrus	0.7(1.4±0.37)1.9	0.1(0.24±0.16)0.6	Subsessile	0.7(1.4±0.37)1.9	6:1	NA
P. physocarpus	11.4(11.7±0.3)12.0	5.1(5.4±0.25)5.6	0.4(0.45+0.05)0.5	10.9(11.25±0.35)11.6	2:1	25:1
P. reticulatus	2.7(2.8±0.1)2.9	1.0(1.05±0.05)1.1	0.2	2.5(2.6±0.1)2.7	3:1	13:1
P. rotundifolius	0.5(0.6±0.08)0.7	0.4(0.45±0.04)0.5	Sessile	0.5(0.6±0.08)0.7	1:1	NA
P. sublanatus	0.4(0.45±0.05)0.5	0.2	Sessile	0.4(0.45±0.05)0.5	5:1	NA
P. urinaria	0.7(0.9±0.2)1.1	0.1(0.15±0.05)0.2	Sessile	0.7(0.9±0.2)1.1	6:1	NA

All measurements in cm [Min. (mean ±S.D) Max]

NA - Not Applicable

4.5 Micromorphology

The epidermal cell shape of most of the species were either irregular or polygonal on both the adaxial and abaxial surfaces. Irregular epidermal cell shape were observed in *Phyllanthus acidus, P. amarus, P. beillei, P. fraternus, P. maderaspatensis, P. nigericus, P. niruri, P. physocarpus, P. rotundifolius* and *P. sublanatus* with wavy or undulate anticlinal wall pattern while cells of *P. capillaris, P. odontadenius* and *P. urinaria* were observed with sinuate or deeply sinuate anticlinal wall pattern on both surfaces, however cells of *P. mannianus* and *P. niruroides* were with undulate and sinuate anticlinal wall pattern on abaxial and adaxial surfaces respectively. Only *P. reticulatus* had polygonal epidermal cell shape on both surfaces while *P. pentandrus* had it only on the abaxial surface with the characteristic straight anticlinal wall pattern (Table 4.3a, Plate 4.20b, 4.26a and 4.21c). *P muellerianus* and *P. floribundus* had rectangular epidermal cell shape on both surfaces (Table 4.3a, Plates 4.22a, 4.24a and 4.24b) with straight anticlinal wall pattern (Table 4.3a). The epidermal cells are moderately thick ranging from 1 μm in *P. nigericus, P. pentandrus, P. rotundifolius* and *P. urinaria* to 2 μm on the adaxial surfaces and 1μm in *P. amarus, P. floribundus, P. nigericus* and *P. urinaria* to 2 μm on the abaxial surfaces (Table 4.3b). However, the thickness ranged between 2 – 5 μm on the adaxial surfaces and 2 – 4 μm on the abaxial surfaces of other species (Table 4.3b).

Variations occur in the number of epidermal cells per field of view on both the adaxial and abaxial surfaces among the species. Both *P. rotundifolius* and *P. pentandrus* had the least values of epidermal cells of 21 and 22 on the adaxial and abaxial surfaces respectively while *P. acidus* had the highest mean values of epidermal cells of 286 and 171 on both adaxial and abaxial surfaces (Table 4.3b). In most of the species, there were more cells on the adaxial surfaces than the abaxial surfaces except *P. amarus, P. ninuri* and *P. rotundifolius* where greater number of epidermal cells were observed on the abaxial surface. Sessile multicellular scales and cell inclusions such as oil droplets were observed only on the adaxial surface of *P. acidus* (Table 4.3a, Plates 4.20a & b). Prismatic and styloid crystals, crystal sands and druses were observed on both the adaxial and abaxial surfaces of *P. amarus* and *P. physocarpus* while these were found only on the abaxial sunfaces of *P. acidus, P. capillaris, P. floribundus, P. muellerianus, P. niruri, P. odontadenius* and *P.*

rotundifolius. However the crystals and druses were observed only on the adaxial surface of *P. fraternus.* (Table 4.3a, Plates 4.28a, 4.25d).

Simple unicellular trichomes were found only on the adaxial surfaces of *P. rotundifolius* and *P. beillei* while both simple and dendritic uniseriate trichomes were found on both the adaxial and abaxial surfaces of *P. reticulatus* as well as the adaxial surfaces of *P. beillei* and *P. rotundifolius* (Table 4.3a, Plates 4.29a & 4.29c, 4.21a 4.30a). Eleven species out of all the taxa studied were amphistomatic with stomata on both surfaces of the leaves while only five other species were hypostomatic with stomata restricted only to the abaxial surface of the leaves (Table 4.3c). Fewer stomata were observed on the adaxial surfaces of all the species except in *P. amarus* in which the mean stomata density on the adaxial surface was 38 and 22 on the abaxial surface (Table 4.3c).

Four different types of stomata were recorded for the genus with anisocytic type (stomata surrounded by three cells, one of which is usually smaller than the other two) being the most common on both surfaces of *P. amarus, P. fraternus, P. niruri, P. niruroides, P. odontadenius* and *P. pentandrus*. Anisocytic stomata were recorded on the adaxial surface only in *P. mannianus* while they were found only in *P. acidus, P. floribundus, P. muellerianus, P. physocarpus* and *P. reticulatus* on the abaxial surface. Other types of stomata also recorded in the genus are Laterocyclic (in which the two lateral subsidiary cells surround the guard cells completely), anomocytic (where epidermal cells around the guard cells are not distinguishable from other epidermal cells) and paracytic (in which stomata are accompanied on either side by one or more subsidiary cells parallel to the long axis of the pore and guard cells).

Laterocyclic stomata were observed on both surfaces of *P. beillei, P. sublanatus* and *P. urinaria* as well as the abaxial surface of *P. maderaspatensis* (Table 4.3a, Plates 4.21a, 4.30c, 4.31a, 4.20c & 4.23d). *Phyllanthus rotundifolius* was the only species with anomocytic stomata on both the adaxial and abaxial surfaces while they were recorded only on the abaxial surface of *P. capillaris* and adaxial surface of *P. maderaspatensis* (Table 4.3a, Plates 4.21d & 4.23a). Paracytic stomata were recorded for both surfaces of *P. nigericus* (Table 4.3a, Plates 4.24d).

The number of stomata per field of view varied considerably in the genus. The lowest on the adaxial surface was in *P. nigericus* and *P. niruroides* with a mean value of six while *P. odontadenius* had the least number of stomata on the abaxial surface with a mean value of 15 (Table 4.3c). The highest number of stomata was recorded for *P. urinaria* on the adaxial surface with mean value of 71 while *P. sublanatus* had the highest number of stomata on the abaxial surface with a mean value of 140 (Table 4.3c). Stomata length ranged from 6.40 μm in *P. nigericus* to 10.15 μm in *P. beillei* on the adaxial surface and from 4.70 μm in *P. floribundus* to 9.95 μm in *P. mannianus* on the abaxial surface (Table 4.3c). Incidentally the least stomata width of 4.25 μm in *P. nigericus* on the adaxial surface and 2.25 μm in *P. floribundus* on the abaxial surface correspond to the taxa that had the least stomata length on both surfaces respectively. Moreover the highest stomata width of 5.80μm in *P. maderaspatensis* on the adaxial surface and 5.90 μm in *P. fraternus* on the abaxial surface were recorded (Table 4.3c).

Stomata index ranged from 2.35% in *P. nigericus* to 70.82% in *P. rotundifolius* on the adaxial surface while on the abaxial surface, it ranged from 10.03% in *P. odontadenius* to 77.43% in *P. sublanatus* (Table 4.3c).

Table 4.3a: Qualitative leaf epidermal and stomata features of *Phyllanthus* species in Nigeria

Taxa	Leaf surfaces	Epidermal Cell shape	Anticlinal wall pattern	Shape of G/cell	Stomata type	Trichome type/Sessile multicellular scales	Cell inclusions
Phyllanthus acidus	Adaxial	Irregular	Wavy	Absent	Absent	Sessile multicellular scales randomly and sparsely distributed	Oil droplets
	Abaxial	Irregular	Wavy	Suborbiculate	Anisocytic	-	Crystal sand & Styloid crystals
P. amarus	Adaxial	Irregular	Undulate	Suborbiculate	Anisocytic	-	Crystal sand & Prismatic crystals
	Abaxial	Irregular	Undulate	Elliptic	Anisocytic	-	Crystal sand & Styloid crystals
P. beillei	Adaxial	Irregular	Wavy	,,	Laterocyclic	Simple unicellular trichome	-
	Abaxial	Irregular	Wavy	,,	Laterocyclic	-	-
P. capillaris	Adaxial	Irregular	Sinuate	Absent	Absent	-	-
	Abaxial	Irregular	Sinuate	Elliptic	Anomocytic	-	Crystal druses
P. floribundus	Adaxial	Rectangular	Straight	Absent	Absent	-	-
	Abaxial	Rectangular	Straight	-	Anisocytic	-	Crystal druses
P. fraternus	Adaxial	Irregular	Undulate	Suborbiculate	Anisocytic	-	Prismatic & Styloid crystals
	Abaxial	Irregular	Undulate	,,	Anisocytic	-	-
P. maderaspatensis	Adaxial	Irregular	Undulate	Elliptic	Anomocytic	-	-
	Abaxial	Irregular	Undulate/Wavy	,,	Laterocyclic	-	-
P. mannianus	Adaxial	Irregular	Undulate/Wavy	,,	Anisocytic	-	-
	Abaxial	Irregular	Deeply sinuate	,,	Anomocytic	-	-
P. muellerianus	Adaxial	Rectangular	Straight	Absent	Absent	-	-
	Abaxial	Rectangular	Straight	-	Anisocytic	-	Crystals
P. nigericus	Adaxial	Irregular	Wavy	Suborbiculate	Paracytic	-	-
	Abaxial	Irregular	Wavy	,,	Paracytic	-	-

P. niruri	Adaxial	Irregular	Undulate	,,	Anisocytic	-	-
	Abaxial	Irregular	Undulate	,,	Anisocytic	-	Staloid crystals on the main vein
P. niruroides	Adaxial	Irregular	Undulate	Elliptic	Anisocytic	-	-
	Abaxial	Irregular	Sinuate	,,	Anisocytic	-	-
P. odontadenius	Adaxial	Irregular	Sinuate	,,	Anisocytic	-	-
	Abaxial	Irregular	Sinuate	,,	Anisocytic	-	Crystal sand
P. pentandrus	Adaxial	Irregular	Undulate	,,	Anisocytic	-	-
	Abaxial	Polygonal	Straight	,,	Anisocytic	-	-
P. physocarpus	Adaxial	Irregular	Undulate	Absent	Absent	-	Staloid crystals
	Abaxial	Irregular	Undulate	-	Paracytic	-	Prismatic & Staloid crystals
P. reticulatus	Adaxial	Polygonal	Straight	Absent	Absent	Simple unicellular trichome base	-
	Abaxial	Polygonal	Straight	Elliptic	Anisocytic	Simple unicellular trichome	-
P. rotundifolius	Adaxial	Irregular	Undulate	-	Anomocytic	Simple unicellular trichome	Stomata characteristically different
	Abaxial	Irregular	Undulate	-	Anomocytic	-	Crystal druses
P. sublanatus	Adaxial	Irregular	Undulate	Suborbiculate	Laterocyclic	-	-
	Abaxial	Irregular	Undulate	,,	Laterocyclic	-	-
P. urinaria	Adaxial	Irregular	Sinuate	Elliptic	Laterocyclic	-	-
	Abaxial	Irregular	Sinuate	,,	Laterocyclic	-	-

- = Absent

Table 4.3b: Quantitative leaf epidermal features of *Phyllanthus* species in Nigeria

Taxa	Number of epidermal cells per field of view (x40)		Cellwall thickness (µm)	
	Adaxial	**Abaxial**	**Adaxial**	**Abaxial**
Phyllanthus acidus	252(286.1±18.8)316	148(170.7±16.2)198	2.5(3.2±0.6)4.0	1.5(2.3±0.5)3.0
P. amarus	48(85.9±19.1)126	108(139.3±15.9)172	1.5(2.4±0.8)4.0	1.0(1.9±0.6)3.0
P. beillei	165(183.9±11.9)212	124(145.1±10.6)162	1.0(2.7±1.0)5.0	2.0(2.5±0.4)3.0
P. capillaris	186(205.9±12.6)230	94(116.9±11.7)132	3.0(4.5±1.1)7.0	2.0(2.5±0.4)3.0
P. floribundus	194(221.5±16.1)248	106(140.6±20.9)172	1.5(2.6±0.8)4.0	1.0(1.4±0.4)2.0
P. fraternus	62(75.7±8.1)91	24(33.8±4.9)42	2.0(3.0±0.7)5.0	2.0(3.1±0.7)5.0
P.maderaspatensis	39(49.8±6.1)59	23(30.5±3.9)38	2.0(3.0±0.7)5.0	2.0(3.1±0.7)5.0
P. mannianus	148(159.2±6.0)172	36(42.3±4.0)48	3.0(3.9±0.8)5.0	2.0(2.4±0.4)3.0
P. muellerianus	168(190.1±12.1)210	128(139.2±5.1)147	1.5(2.6±0.7)4.0	1.0(2.2±0.6)3.0
P. nigericus	250(259.8±5.6)272	44(50.5±3.9)58	1.0(1.7±0.4)2.0	1.0(1.6±0.5)2.0
P. niruri	25(35.5±6.3)44	31(38.2±3.5)44	1.0(2.2±0.6)3.0	1.0(2.1±0.7)3.0
P. niruroides	187(201.1±8.2)214	30(36.4±4.9)46	1.5(2.2±0.5)3.0	2.0(2.9±0.7)4.0
P. odontadenius	169(179.2±6.2)192	128(134.5±5.1)144	2.0(3.1±0.6)4.0	2.0(2.9±0.5)4.0
P. pentandrus	50(58.7±4.1)65	17(22.4±3.4)28	1.0(1.4±0.4)2.0	1.5(2.3±0.6)3.0
P. physocarpus	130(155.9±14.9)181	70(82.1±7.1)93	3.0(3.9±0.7)5.0	1.0(2.2±0.6)3.0
P. reticulatus	171(183.1±6.3)192	147(159.2±7.5)172	3.0(3.5±0.5)4.0	1.5(2.4±0.6)3.0
P. rotundifolius	10(20.5±4.1)28	24(31.1±4.4)40	1.0(1.3±0.4)2.0	2.0(2.7±0.4)3.0
P. sublanatus	64(74.7±7.7)90	31(40.8±5.7)50	1.5(2.2±0.4)3.0	1.5(2.2±0.4)3.0
P. urinaria	40(48.9±4.9)57	28(37.5±4.8)45	1.5(1.9±0.3)2.5	1.5(1.9±0.3)2.5

All Quantitative Characters Min (Mean±SD) Max

Table 4.3c: Quantitative stomata characters of *Phyllanthus* species in Nigeria

Taxa	Stomata density per field of view		Stomata length (µm)		Stomata width (µm)		Stomata index (%)	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
Phyllanthus. acidus	Absent	26(20.00±0.02	Absent	4(5.45±0.83)7	NA	1.5(2.43±0.67)4	NA	17.69
P. amarus	19(38.0±13.1)65	12(22.1±5.4)32	7(7.8±0.7)9	6(7.3±0.9)9	5(5.7±0.7)7	4(5.2±0.7)6	30.66	13.67
P. beillei	21(27.6±3.9)34	36(47.0±7.2)61	8(10.2±0.9)12	6(7.6±0.9)9	4(5.3±0.6)6	3(4.4±0.9)6	13.03	24.47
P. capillaris	Absent	17(28.5±7.7)44	Absent	7(8.4±0.9)10	Absent	4(4.9±0.8)6	Absent	19.58
P. floribundus	Absent	42(52.5±6.4)64	Absent	4(4.7±0.6)6	Absent	2(2.3±0.4)3	Absent	27.18
P. fraternus	12(17.1±3.4)24	39(51.1±5.8)59	7(8.3±0.7)9	8(9.2±0.6)10	5(5.8±0.6)7	5(5.9±0.6)7	18.44	60.19
P.maderaspatensis	26(33.3±4.4)42	41(49.0±4.7)58	8(9.8±0.8)11	9(9.9±0.6)11	5(5.8±0.5)7	5(5.8±0.6)7	40.07	61.67
P. mannianus	8(12.5±3.3)16	53(59.2±4.1)68	7(7.7±0.7)8	9(9.9±0.8)12	5(5.8±0.6)7	5(5.8±0.4)6	7.08	58.33
P. muellerianus	Absent	96(111.0±9.5)128	Absent	5(5.8±0.4)6	Absent	2(2.7±0.5)3	Absent	44.37
P. nigericus	4(6.3±1.9)10	92(100.3±5.4)110	6(6.4±0.5)7	8(9.0±0.7)10	3(4.3±0.6)5	5(5.7±0.5)6	2.35	66.53
P. niruri	22(27.3±4.0)35	28(38.8±5.7)47	9(9.9±0.7)11	8(9.4±0.8)10	5(5.5±0.5)6	5(5.6±0.5)6	43.47	50.39
P. niruroides	4(6.2±1.9)10	106(124.7±10.9)142	7(8.1±0.7)9	7(8.6±1.1)10	4(4.6±0.5)5	3(4.8±0.8)6	2.97	77.42
P. odontadenius	6(9.9±2.5)15	10(15.0±3.3)22	7(8.7±1.1)10	8(8.7±0.5)9	4(4.6±0.5)5	4(4.7±0.5)5	5.21	10.03
P. pentandrus	6(15.3±5.1)23	40(49.2±5.9)59	7(7.5±0.5)8	8(8.7±0.8)10	4(5.1±0.8)7	5(5.8±0.8)7	20.62	68.76
P. physocarpus	Absent	25(29.2±2.9)35	Absent	8(8.8±0.7)10	Absent	4(4.6±0.5)5	Absent	26.20
P. reticulatus	Absent	62(70.7±4.5)79	Absent	8(9.3±0.7)10	Absent	3(4.6±0.6)5	Absent	30.75
P. rotundifolius	41(49.8±5.1)58	93(105.6±6.3)115	7(7.6±0.5)8	8(8.9±0.8)10	5(5.6±0.5)6	5(5.6±0.5)6	70.82	77.25
P. sublanatus	52(62.5±6.5)73	120(139.8±7.9)152	8(9.1±0.9)11	6(6.4±0.5)7	5(5.6±0.5)6	3(3.6±0.5)4	45.53	77.43
P. urinaria	60(71.0±7.3)88	81(95.3±9.2)112	7(7.9±0.8)9	6(6.5±0.5)7	4(4.7±0.5)5	4(4.2±0.4)5	59.22	71.78

All Quantitative Characters Min (Mean±SD) Max

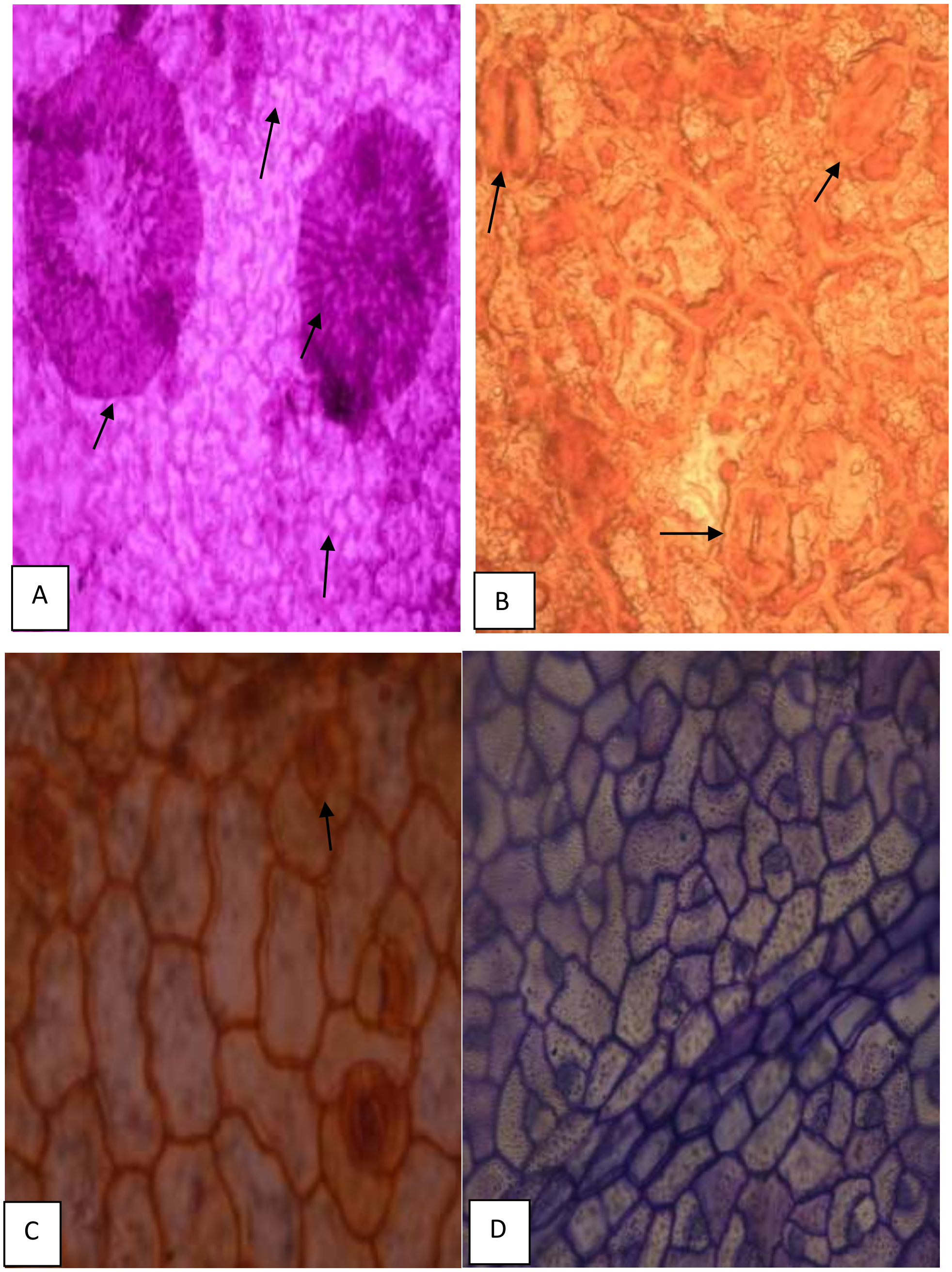


Plate 4.20: Photomicrographs of epidermal layers of leaves of *Phyllanthus amarus* and *Phyllanthus acidus*

A: Adaxial surface of *P. acidus* showing the epidermal cells and scales (arrowed)(x40)

B: Abaxial surface of *P. acidus* showing stomata (arrowed) (x100)

C: Adaxial surface of *P. amarus* showing stomata (arrowed) (*x*100)

D: Abaxial surface of *P. amarus* showing densely distributed stomata (x40)

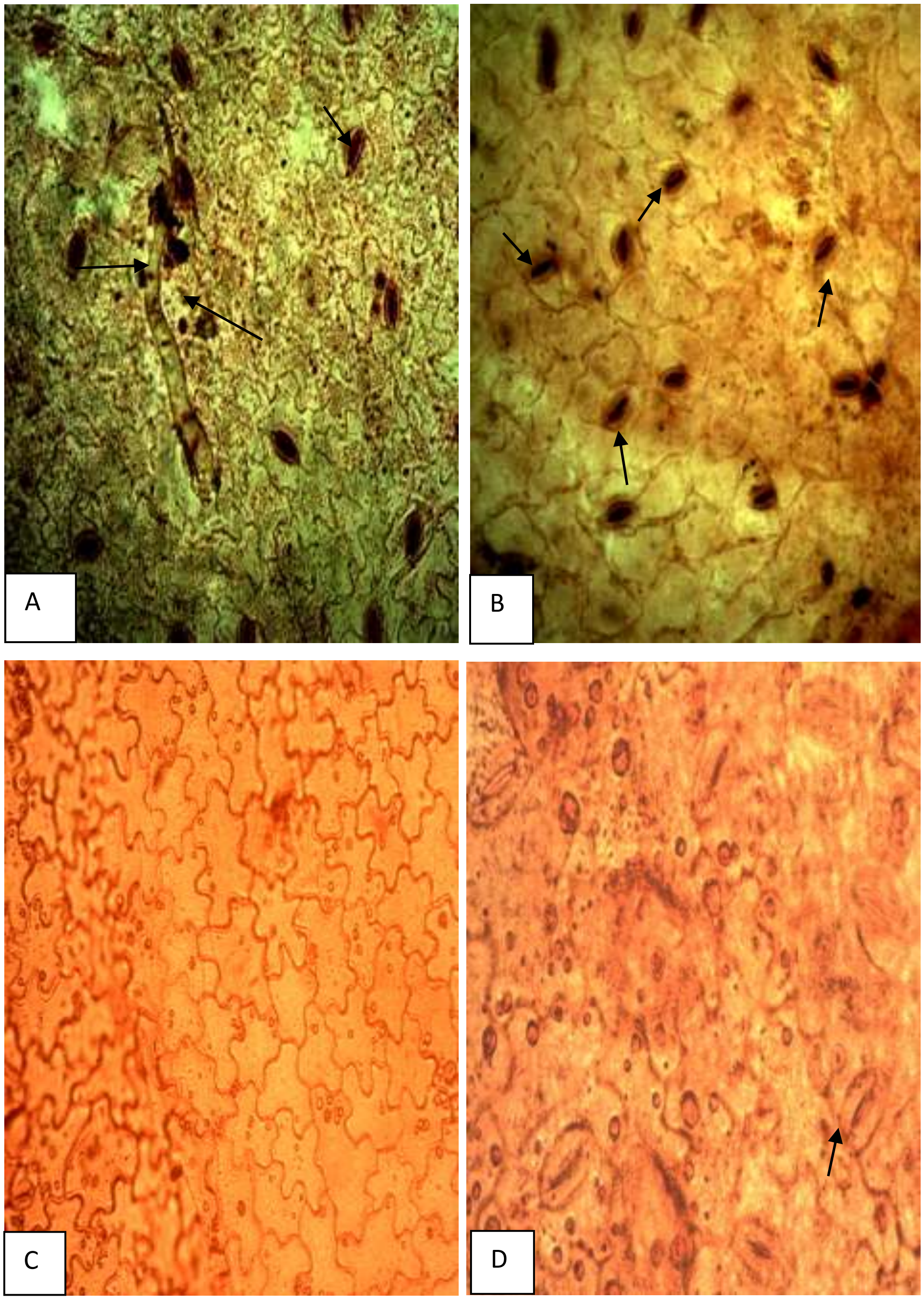


Plate 4.21: Photomicrographs of epidermal layers of leaves of *Phyllanthus beillei* and *Phyllanthus capillaris*

A: Adaxial surface of *P. beillei* showing trichome (arrowed) (x100)
B: Abaxial surface of *P. beillei* showing stomata (x100)
C: Adaxial surface of *P. capillaris* showing irregular epidermal cells (x40)
D: Abaxial surface of *P. capillaris* showing stomata (x100)

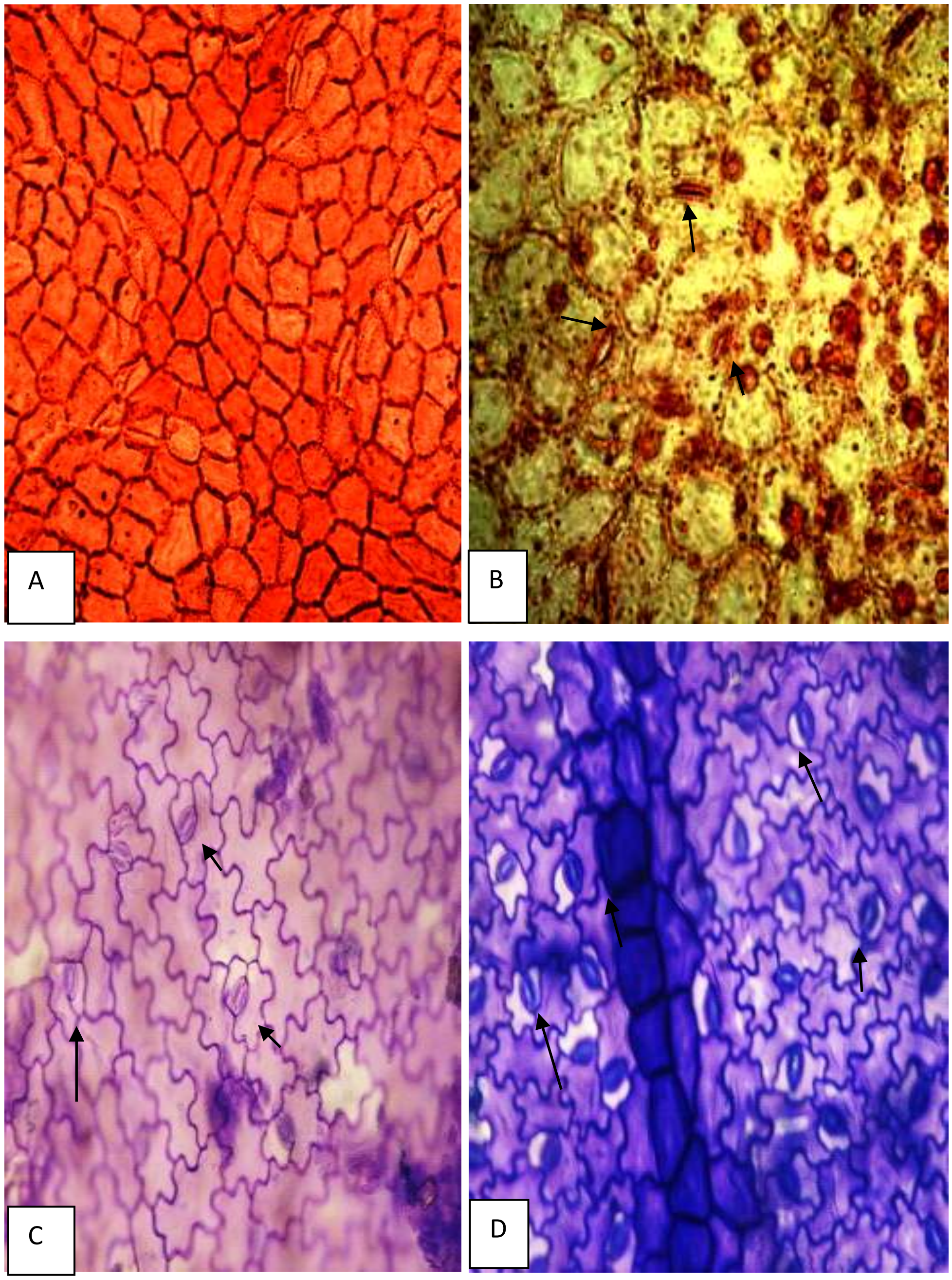


Plate 4.22: Photomicrographs of epidermal layers of leaves of *Phyllanthus floribundus* and *Phyllanthus fraternus*

A: Adaxial surface of *P. floribundus* showing the epidermal cells with no stomata (x100)

B: Abaxial surface of *P. floribundus* showing stomata (x100)

C: Adaxial surface of *P. fraternus* showing stomata (x40)

D: Abaxial surface of *P. fraternus* showing dense stomata (x40)

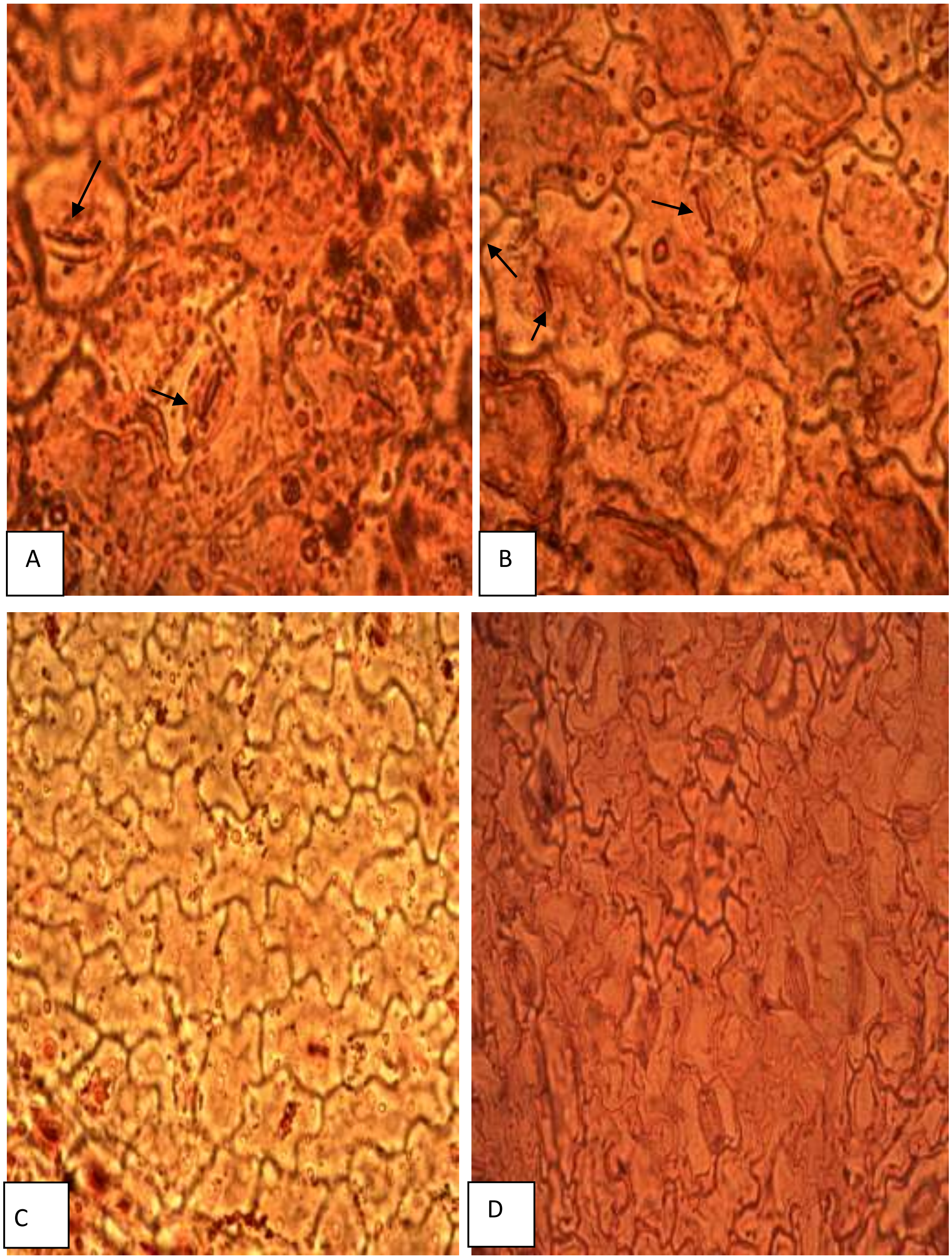


Plate 4.23: Photomicrographs of epidermal layers of leaves of *Phyllanthus maderaspatensis* and *Phyllanthus mannianus*

A: Adaxial surface of *P. maderaspatensis* showing stomata (x100)
B: Abaxial surface of *P. maderaspatensis* showing stomata (x100)
C: Adaxial surface of *P. mannianus* showing the epidermal cells(x40)
D: Abaxial surface of *P. mannianus* showing stomata (x100)

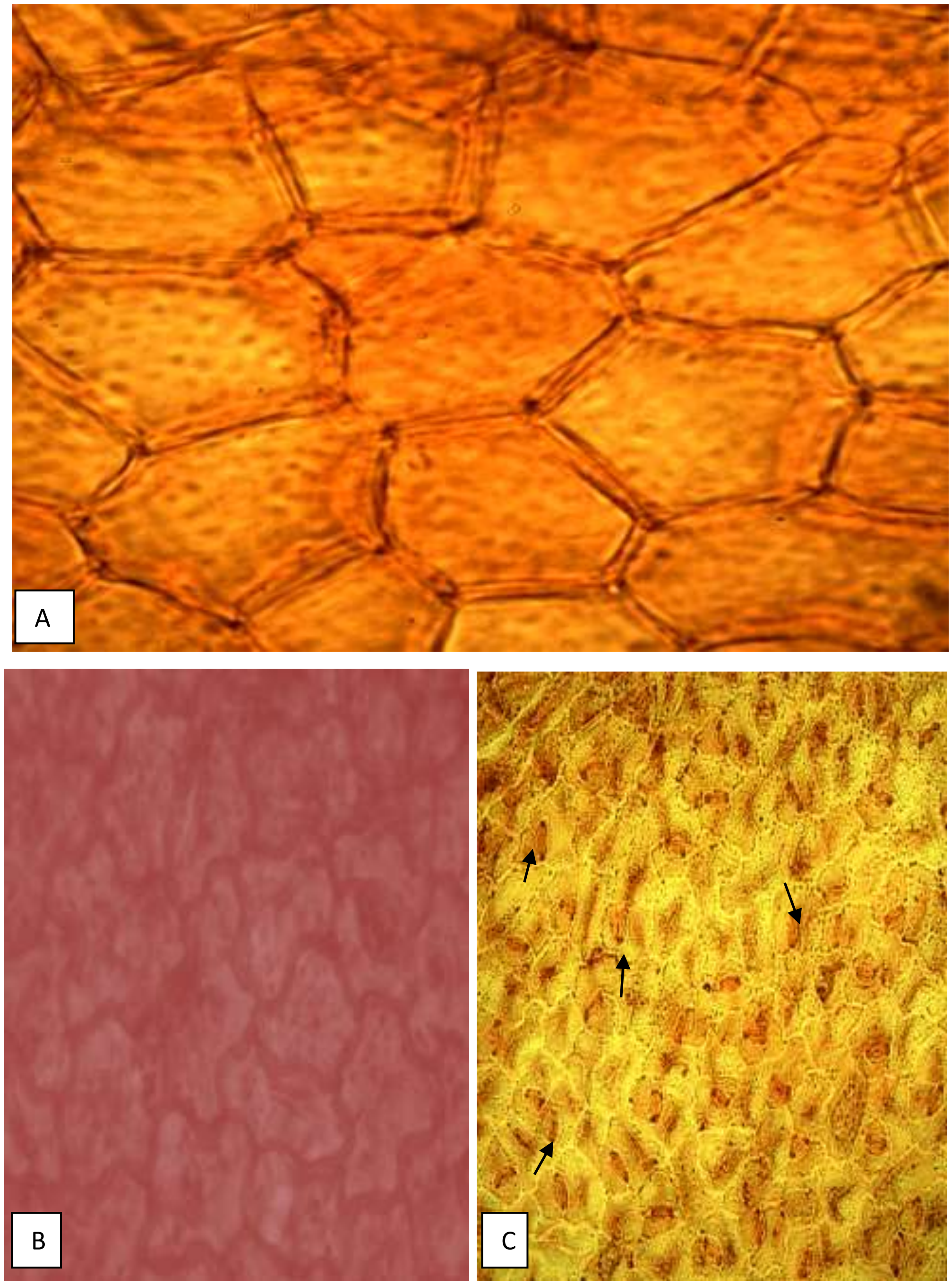


Plate 4.24: Photomicrographs of epidermal layers of leaves of *Phyllanthus muellerianus* and *Phyllanthus nigericus*

A: Adaxial surface of *P. muellerianus* showing epidermal cells with straight anticlinal wall(x400)

B: Adaxial surface of *P. nigericus* (x100)

C: Abaxial surface of *P. nigericus* showing stomata (x40)

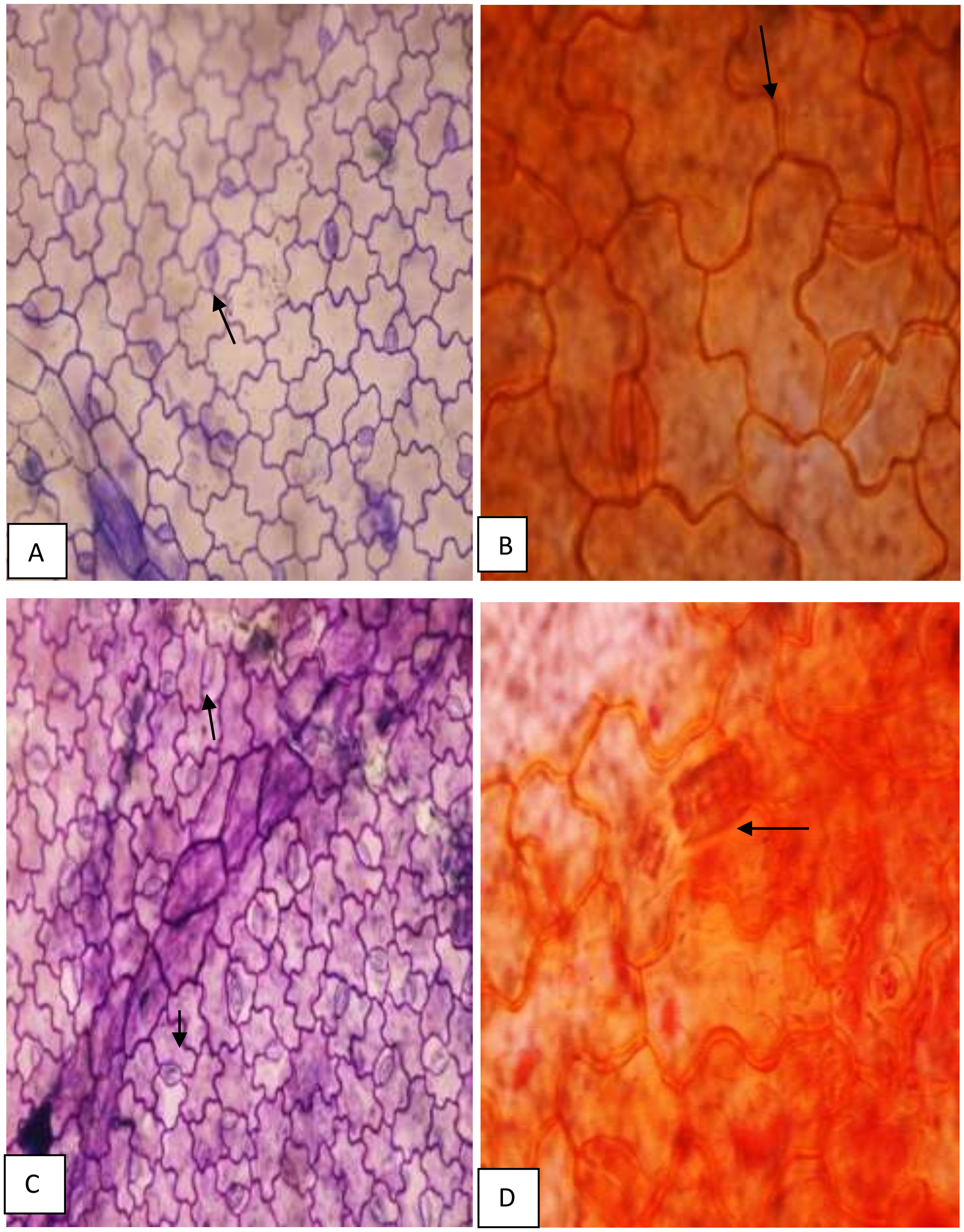


Plate 4.25: Photomicrographs of epidermal layers of leaves of *Phyllanthus niruri*

A: Adaxial surface of *P. niruri* showing anisocytic stomata (x40)

B: Adaxial surface of *P. niruri* showing irregular epidermal cell shape (x100)

C: Abaxial surface of *P. niruri* showing stomata (x40)

D: Abaxial surface of *P. niruri* showing prismatic crystal (arrowed) (x100)

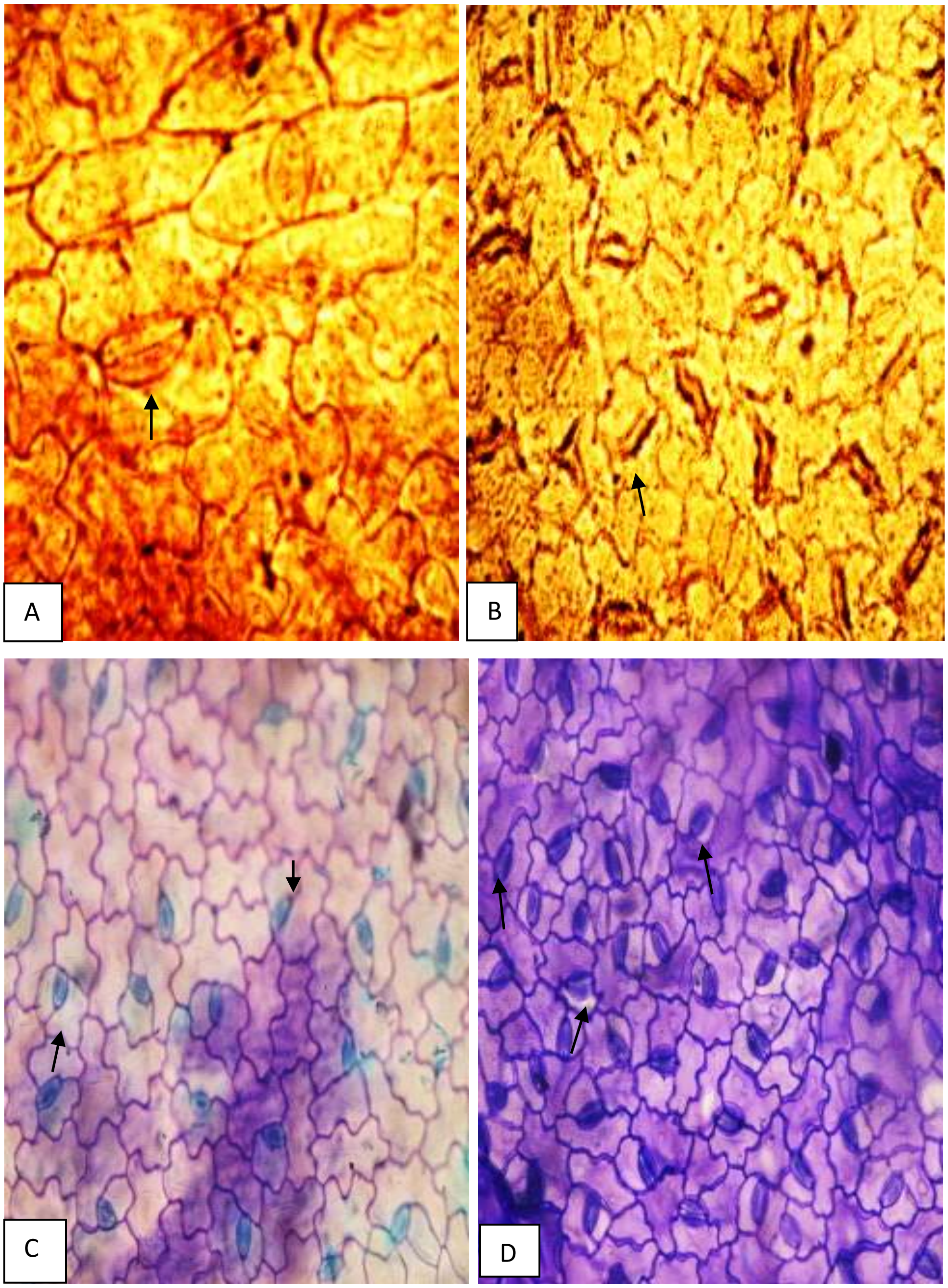


Plate 4.26: Photomicrographs of epidermal layers of leaves of *Phyllanthus niruroides* and *Phyllanthus odontadenius*

A: Adaxial surface of *P. niruroides* showing stomata (x100)
B: Abaxial surface of *P. niruroides* (x100)
C: Adaxial surface of *P. odontadenius* showing stomata (x40)
D: Abaxial surface of *P. odontadenius* showing dense anisocytic stomata (x40)

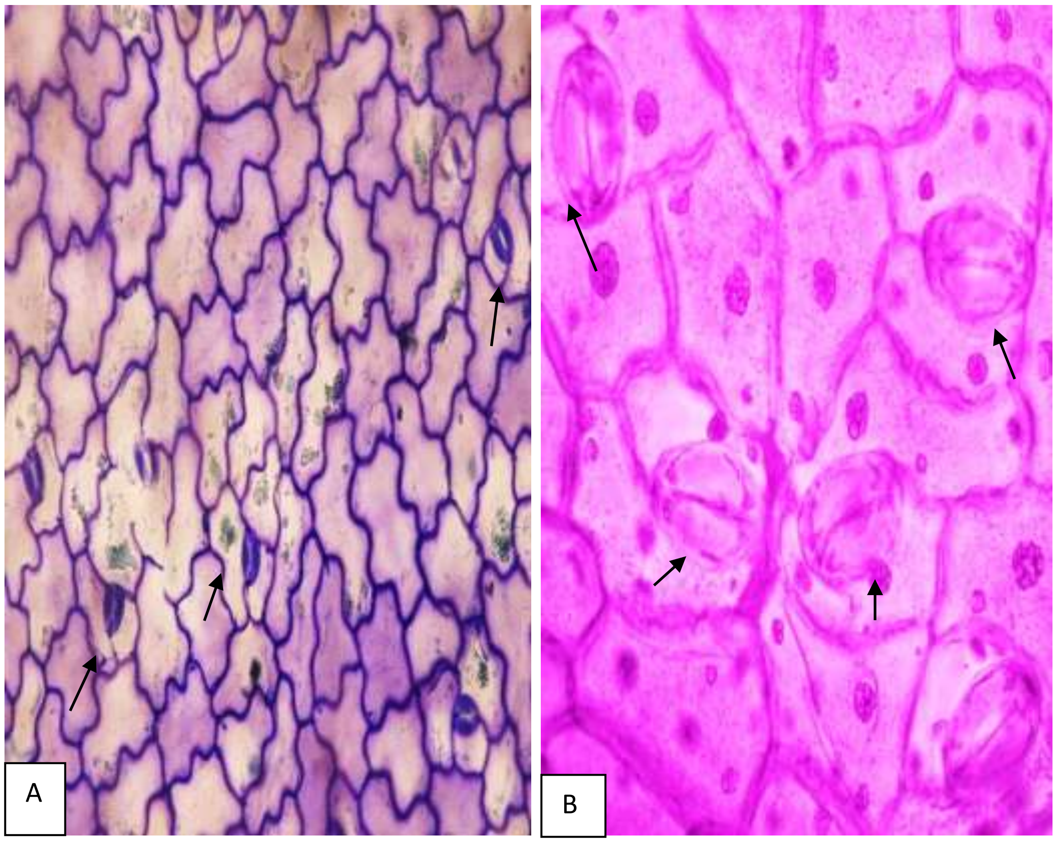


Plate 4.27: Photomicrographs of epidermal layers of leaves of *Phyllanthus pentadrus*

A: Adaxial surface of *P. pentandrus* showing stomata (x40)

B: Abaxial surface of *P. pentandrus* showing stomata (x100)

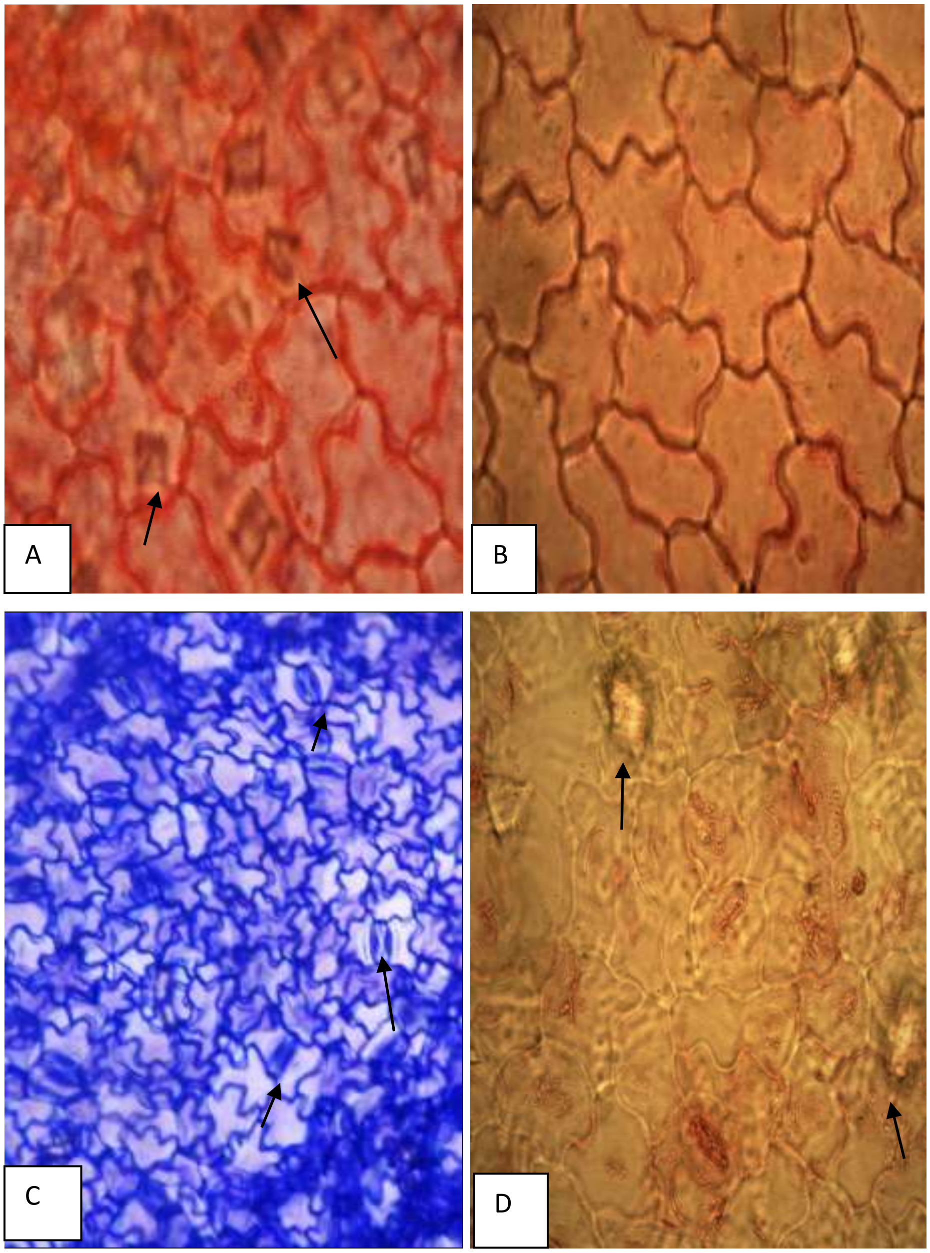


Plate 4.28: Photomicrographs of epidermal layers of leaf of *Phyllanthus physocarpus*

A: Adaxial surface of *P. physocarpus* showing crystals (arowed)(x100)

B: Adaxial surface of *P. physocarpus* showing epidermal cells with no stoma (x40)

C: Abaxial surface of *P. physocarpus* showing stomata (x40)

D: Abaxial surface of *P. physocarpus* showing crystals (arowed) (x100)

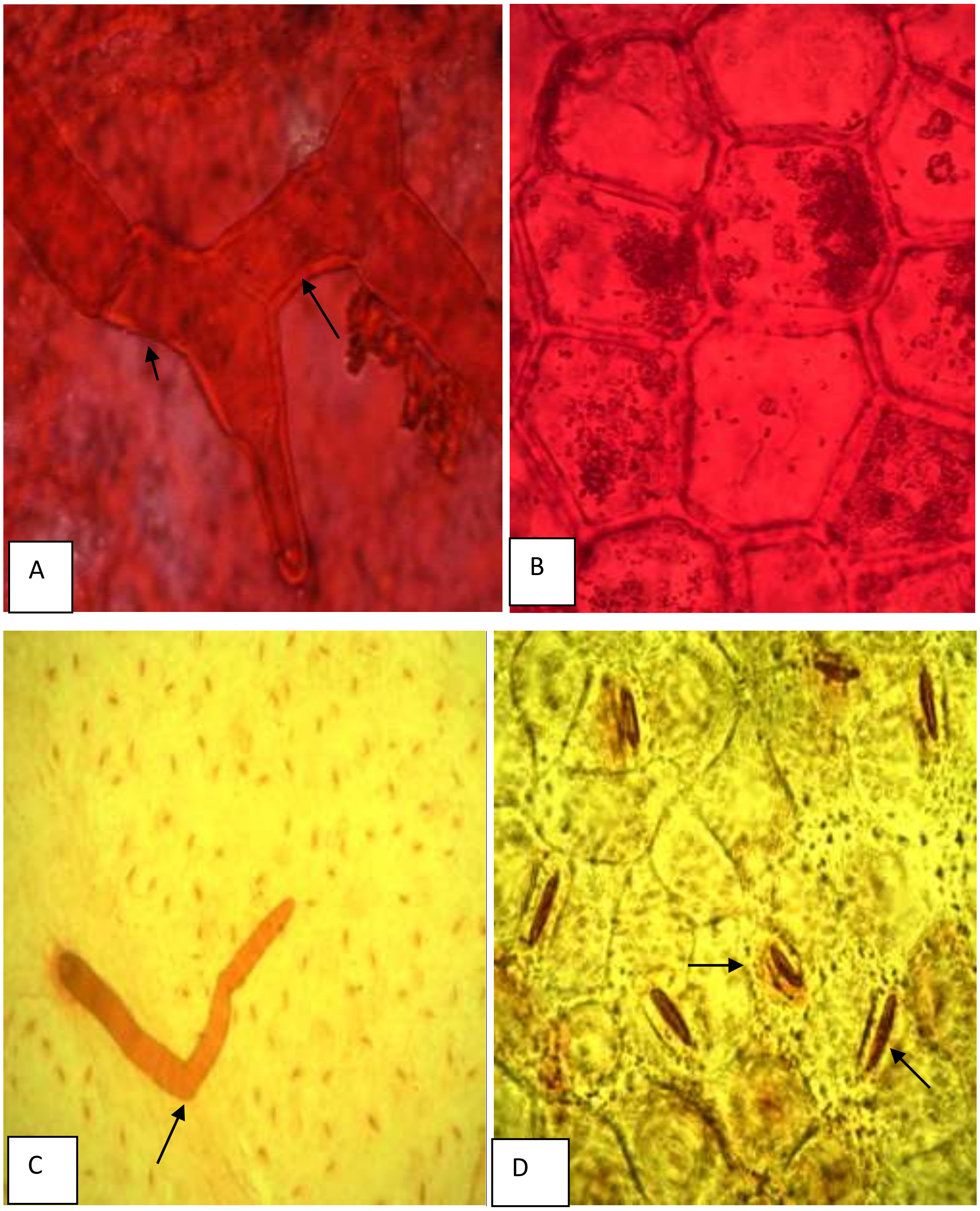


Plate 4.29: Photomicrographs of epidermal layers of leaf of *Phyllanthus reticulatus*
A: Adaxial surface of *P. reticulatus* showing dendric trichome (arrowed) x100
B: Adaxial surface of *P. reticulatus* showing polygonal epidermal cells x100
C: Abaxial surface of *P. reticulatus* also showing trichome (arrowed) x40
D: Abaxial surface of *P. reticulatus* showing stomata (arrowed) x100

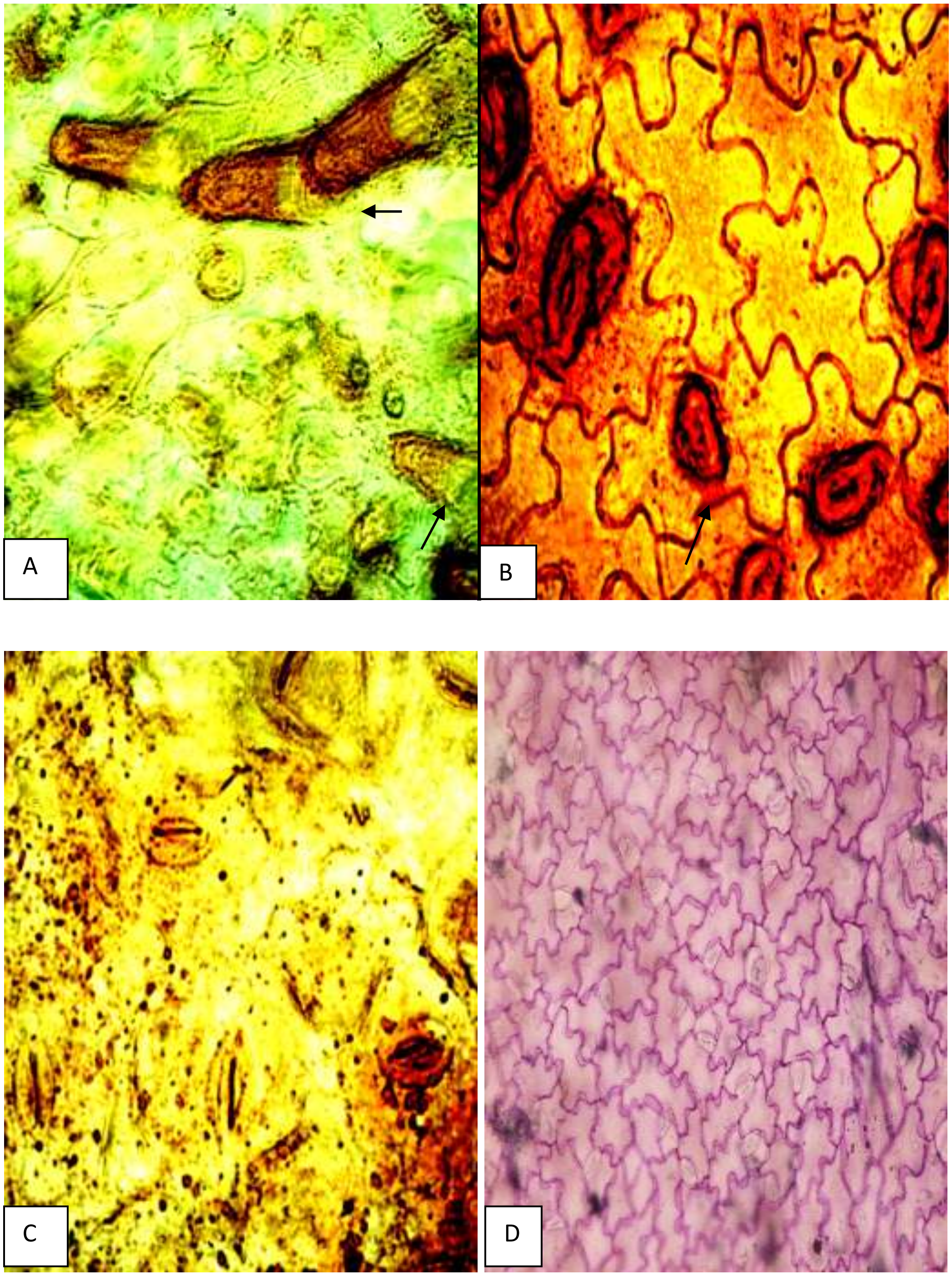


Plate 4.30: Photomicrographs of epidermal layers of leaves of *Phyllanthus rotundifolius* and *Phyllanthus sublanatus*

A: Adaxial surface of *P. rotundifolius* showing trichomes (arrowed)(x100)
B: Abaxial surface of *P. rotundifolius* showing stomata (arrowed) (x100)
C: Abaxial surface of *P. sublanatus* showing stomata (x100)
D: Abaxial surface of *P. sublanatus* showing undulate epidermal cell shape (x40)

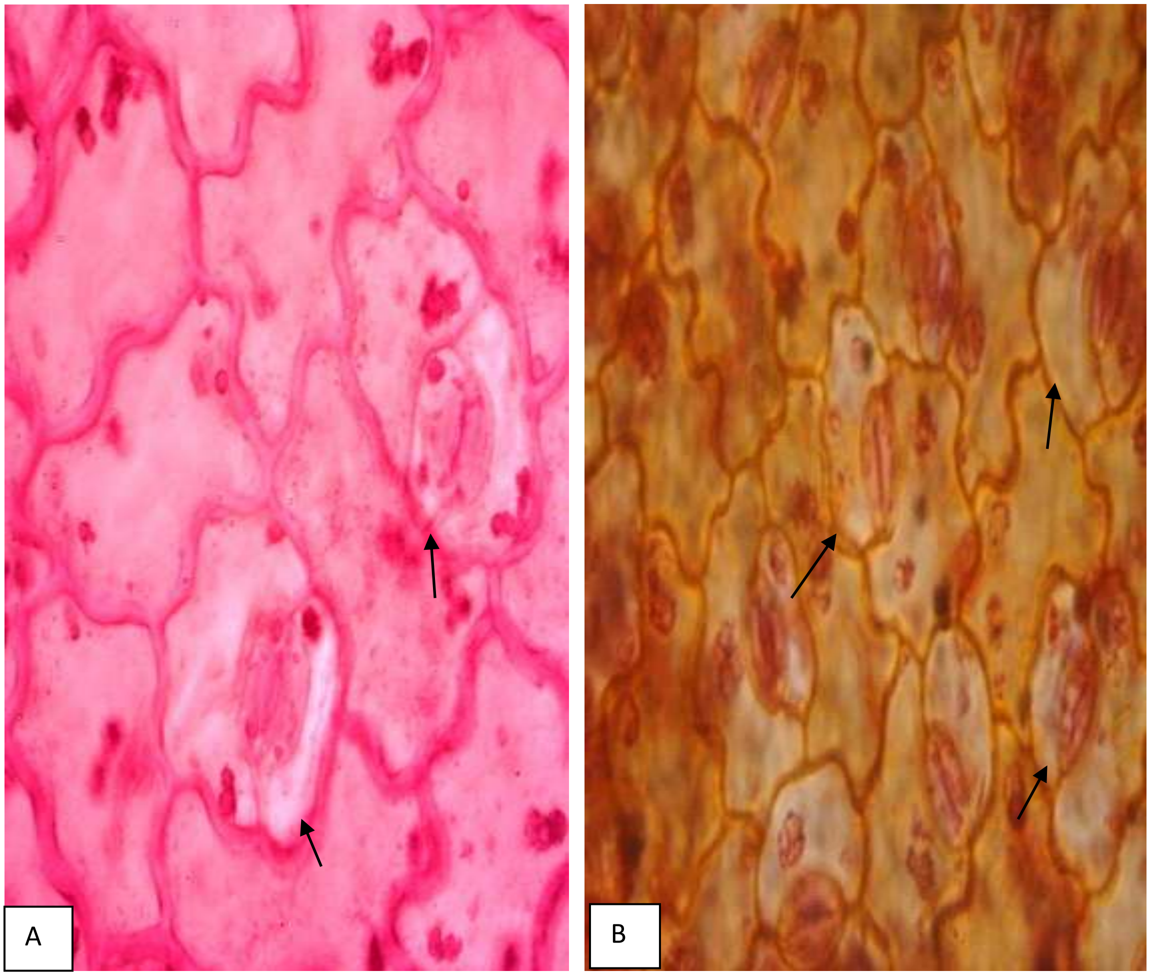


Plate 4.31: Photomicrographs of epidermal layers of leaf of *Phyllanthus urinaria*

A: Adaxial surface of *P. urinaria* showing stomata (arrowed) (x100)

B: Abaxial surface of *P. urinaria* showing laterocyclic stomata (x100)

4.6 Scanning Electron Microscopy (SEM)

Microcharacters of taxonomic significance obtained from selected features of the leaf surfaces using SEM are presented in Table 4.4. Leaf epidermal cells in all the species are irregular except *P. reticulatus* which is polygonal on both surfaces and *P. pentandrus* on the abaxial surface alone. Anticlinal walls are straight on both surfaces in *P. reticulatus* and abaxial surface of *P. pentandrus*, while the remaining taxa are thinly or thickly curved/wavy on both the adaxial and abaxial surfaces (Plates 4.32-4.43).

The stomata are restricted to the abaxial surface of lamina (hypostomatic) in *P. capillaris* and *P. reticulatus* while the leaves of all other taxa studied are amphistomatic. Stomata are dense or evenly distributed on the abaxial surface. Stomata type is mostly anisocytic except in *P. beillei, P. sublanatus* and *P. urinaria* with laterocyclic type of stomata (Table 4.4, Plates 4.40b, 4.43a and 4.43b) and *P. capillaris and P. mannianus* with anomocytic stomata on the abaxial surface and *P. maderaspatensis* on the adaxial surface alone while *P. nigericus* has paracytic stomata on both surfaces (Plate 4.41c). The outline of the pair of guard cells is usually wide elliptic to suborbiculate as seen on the surface view except in *P. beillei* where it is narrowly elliptic (Table 4.4). The outer stomatal ledge aperture ranged between 2.14 µm in *P. nigericus* to 5.88 µm in *P. niruri* on the adaxial surface and 2.52 µm in *P. urinaria* to 6.66 µm in *P. odontadenius* on the abaxial surface (Tables 4.4). The outer stomatal rims are flat in most of the species except in *P. fraternus, P. reticulatus* and *P. urinaria* where they are sunken (Plates 4.40d, 4.42d and 4.43b) while they are slightly raised in *P. beillei* (Plate 4.40b).

Epicuticular wax deposits were observed on the cells of *P. capillaris, P. fraternus, P. mannianus, P. niruri, P. odontadenius, P. reticulatus* and *P. urinaria* among the taxa studied (Table 4.4 with Plates 4.32c, 4.32d, 4.33b, 4.33d, 4.34b, 4.34d and 4.35b respectively).

Table 4.4: Characters of the leaf epidermis of *Phyllanthus* species under Scanning Electron Microscope (SEM)

Taxa		Shape of Epidermal cell	Shape of Guard cell	Pattern of Anticlinal wall	OSR	OSLA	Type of stomata
Phyllanthus amarus	Ad	Irregular	Suborbiculate	Thinly wavy	N/R	2.50	Anisocytic
	Ab	,,	W/Elliptic	,,	,,	4.43	Anisocytic
P. beillei	Ad	Irr. & wavy	N/Elliptic	,,	Raised	-	Laterocyclic
	Ab	,,	,,	,,	,,	-	Laterocyclic
P. capillaris	Ad	Irregular	Absent	,,	Absent	Absent	Absent
	Ab	,,	W/Elliptic	,,	N/R	3.41	Anomocytic
P. fraternus	Ad	,,	Suborbiculate	,,	Sunken	4.38	Anisocytic
	Ab	,,	,,	,,	,,	5.20	,,
P. maderaspatensis	Ad	,,	W/Elliptic	Thickly wavy	N/R	3.59	Anomocytic
	Ab	,,	,,	Thickly wavy	,,	4.70	Laterocyclic
P. mannianus	Ad	,,	,,	Thinly wavy	,,	-	Anisocytic
	Ab	,,	,,	,,	,,	4.51	Anomocytic
P. nigericus	Ad	,,	Suborbiculate	,,	,,	2.14	Paracytic
	Ab	,,	,,	Thickly wavy	,,	3.09	,,
P. niruri	Ad	,,	,,	Thinly wavy	,,	5.88	Anisocytic
	Ab	,,	,,	,,	,,	6.01	,,
P. niruroides	Ad	,,	Elliptic	Thinly wavy	,,	5.18	,,
	Ab	,,	,,	Thickly wavy	,,	5.25	,,
P. odontadenius	Ad	,,	,,	Thinly wavy	,,	4.78	Anisocytic

	Ab	,,	,,	,,	,,	6.66	,,
P. pentandrus	Ad	,,	,,	Thinly wavy	,,	4.38	,,
	Ab	Polygonal	,,	Thinly straight	,,	3.97	,,
P. reticulatus	Ad	,,	Absent	,,	Absent	Absent	Absent
	Ab	,,	W/Elliptic	,,	Sunken	5.83	Anisocytic
P. sublanatus	Ad	Irregular	Suborbiculate	Thinly wavy	N/R	4.90	Laterocyclic
	Ab	,,	,,	,,	,,	3.87	,,
P. urinaria	Ad	,,	W/Elliptic	Thinly wavy	Sunken	2.69	,,
	Ab	,,	,,	,,	,,	2.52	,,

OSR – Outer Stomata Rim

OSLA – Outer Stomata Ledge Aperture

W/Elliptic – Wide Elliptic

N/Elliptic – Narrow Elliptic

N/R - Not Raised

Irr. & Wavy – Irregular and Wavy

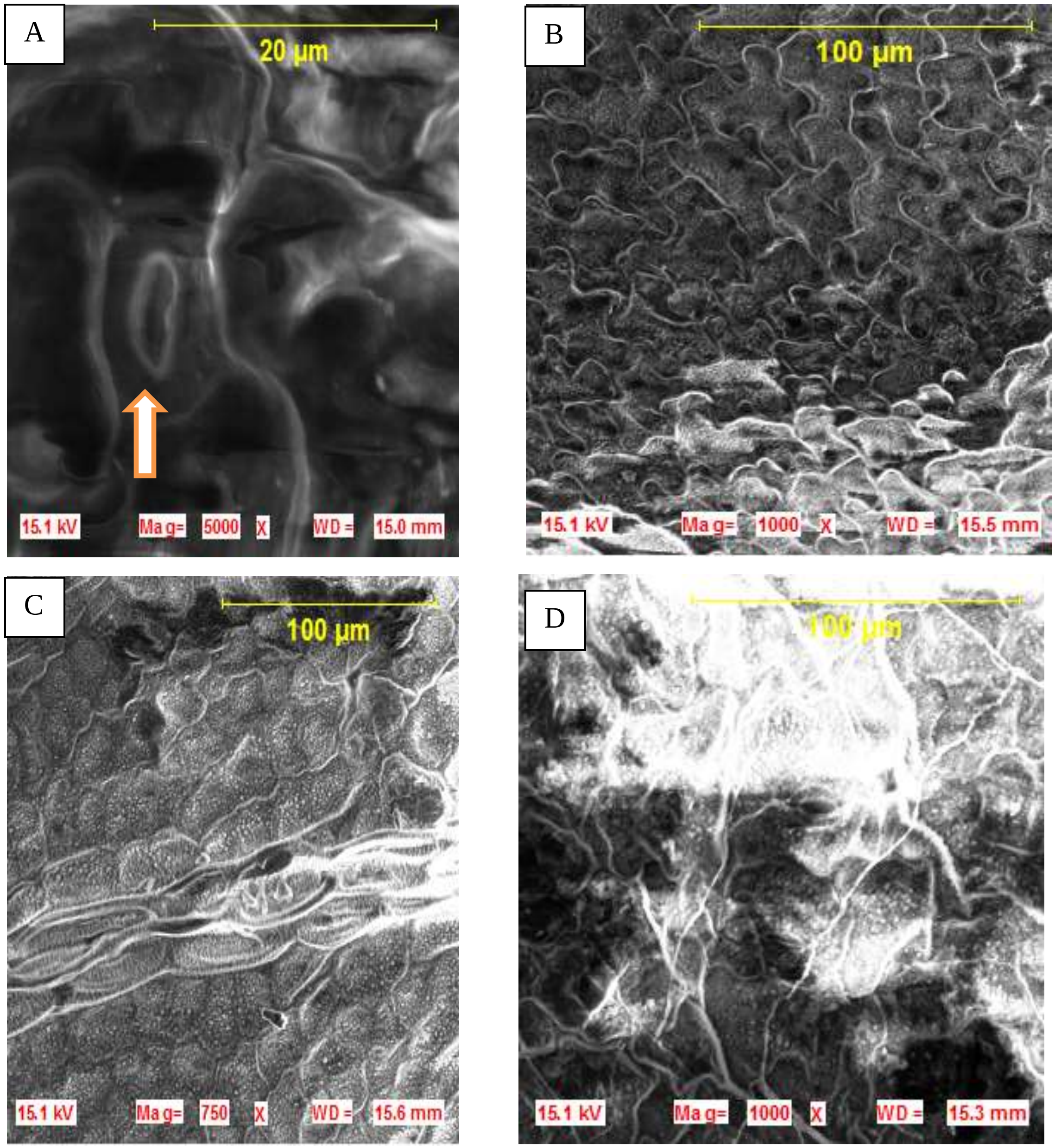


Plate 4.32: Scanning Electron Micrographs of adaxial epidermis of *Phyllanthus* species

A: *P. amarus* showing stoma (arrowed) [Daramola/Emwiogbon/Oguntayo 103399 (FHI)]
B: *P. beillei* showing irregular epidermal cells[D. P. Stanfield 61825 (FHI)]
C: *P. capillaris* showing wax deposits [Daramola/Macauley/Oguntayo 78618 (FHI)]
D: *P. fraternus* showing wax deposits [A. O. Ohaeri 4096 (NIPRD) Scale bar = 100µm]

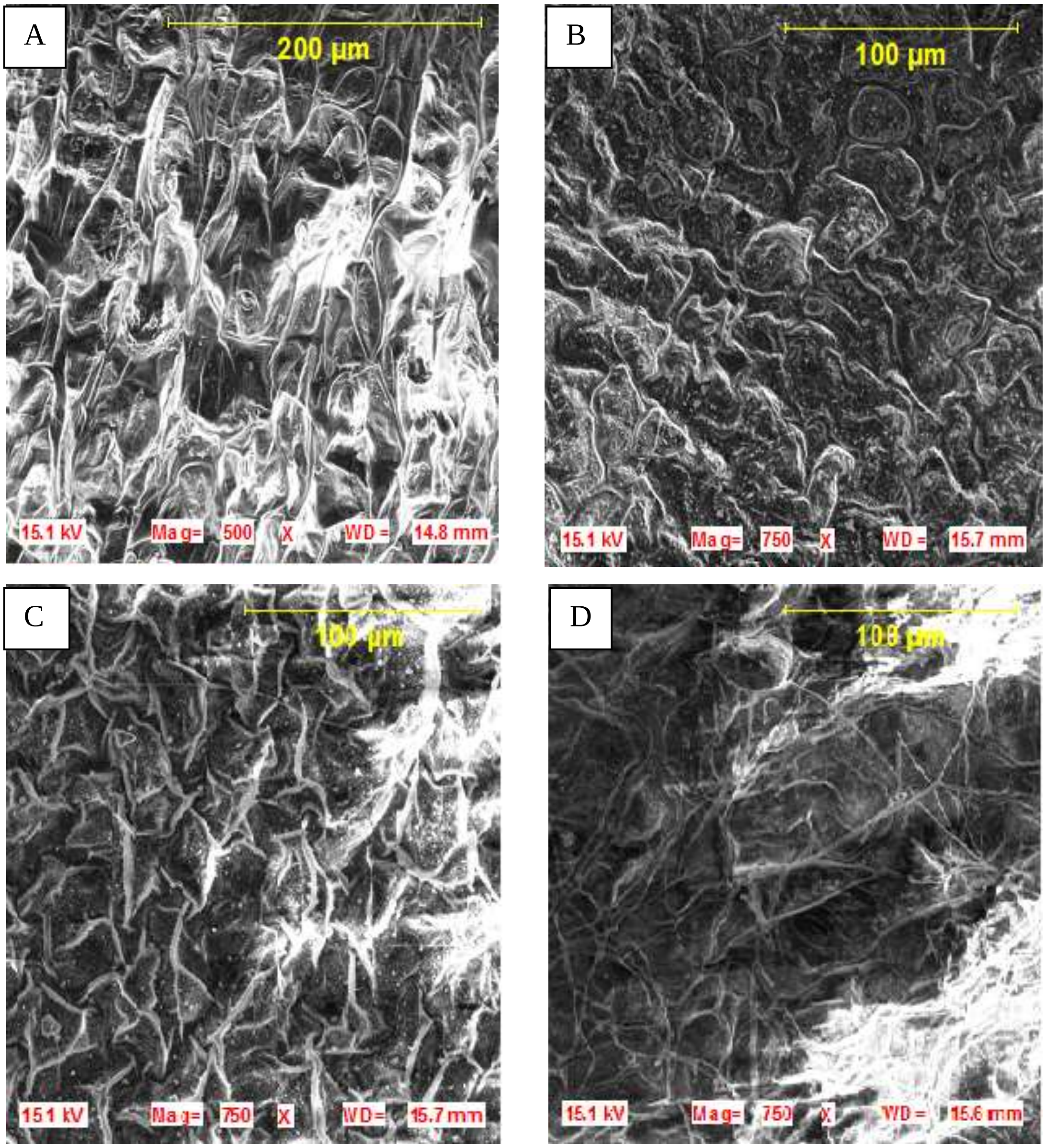


Plate 4.33: Scanning Electron Micrographs of adaxial epidermis of *Phyllanthus* species
A: *P. maderaspatensis* [M. G. Latilo 93997 (FHI)]
B: *P. mannianus* [P. O. Ekwuno 77250 (FHI)]
C: *P. nigericus* [P. O. Ekwuno70470 (FHI)]
D: *P. niruri* showing wax deposits [D. P. M. Guile 2784[C] (IFEH) Scale bar = 100µm]

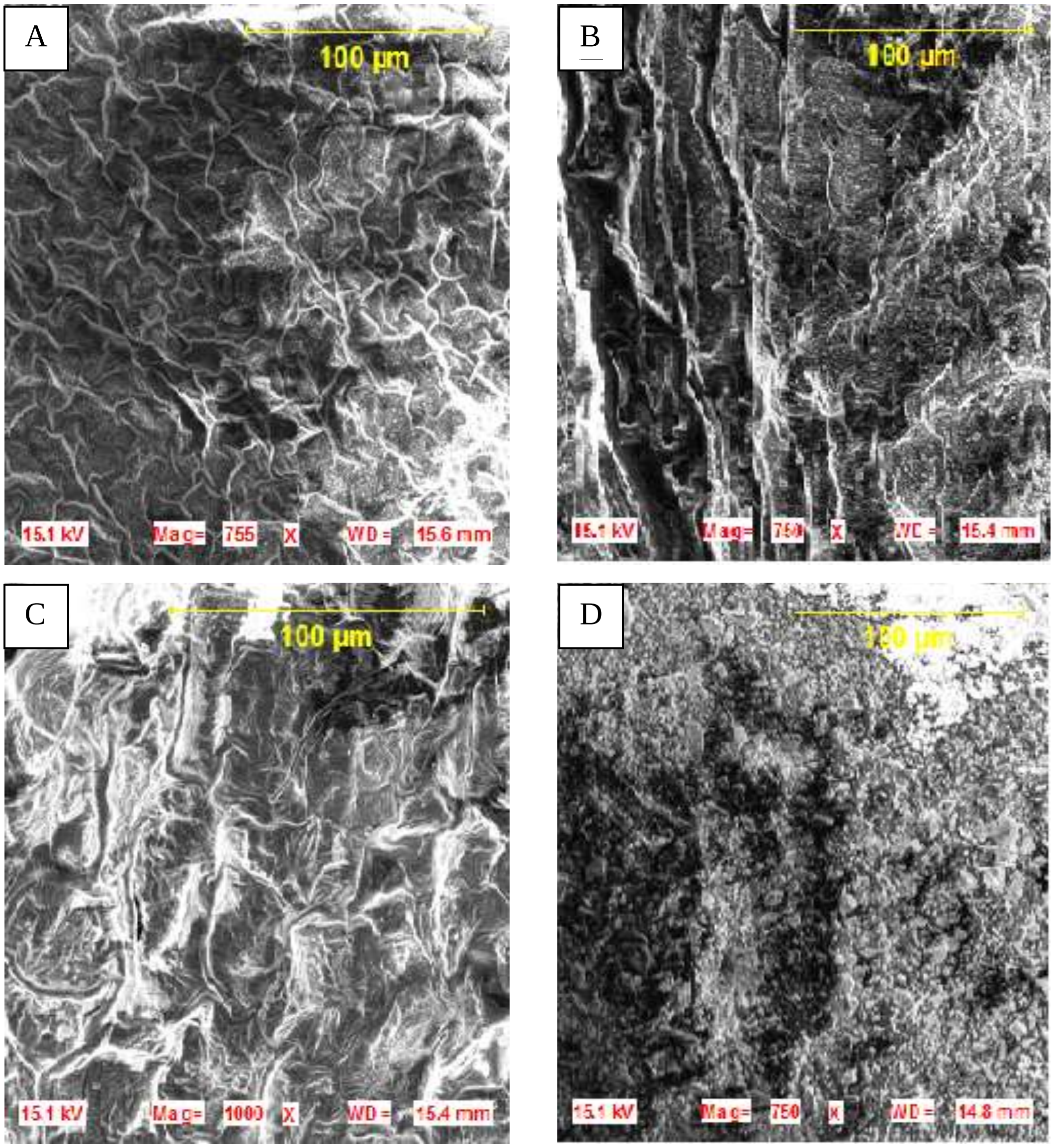
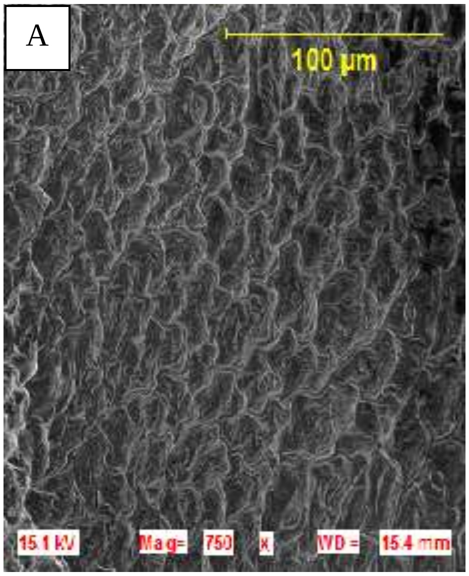


Plate 4.34: Scanning Electron Micrographs of adaxial epidermis of *Phyllanthus* species

A: *P. niruroides* [K.R.M. Williamson 15562 (UIH)]
B: *P. odontadenius* [A.P.D. Jones 6217 (FHI)]
C: *P. pentandrus* [J.B. Hall 2794 (IFEH)]
D: *P. reticulatus* showing wax deposits obscuring the epidermal features [J.B. Hall 2796 (IFEH) Scale bar = 100µm]



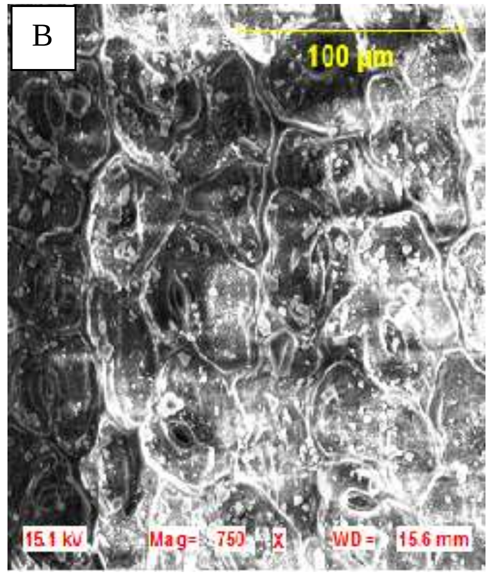


Plate 4.35: Scanning Electron Micrographs of adaxial epidermis of *Phyllanthus* species
A: *P. sublanatus* showing irregular epidermal cells [Ibhanesebor & Osanyinlusi 96993 (FHI)]
B: *P. urinaria* showing wax deposits [A. Egunyomi 21823 (UIH) Scale bar = 100µm]

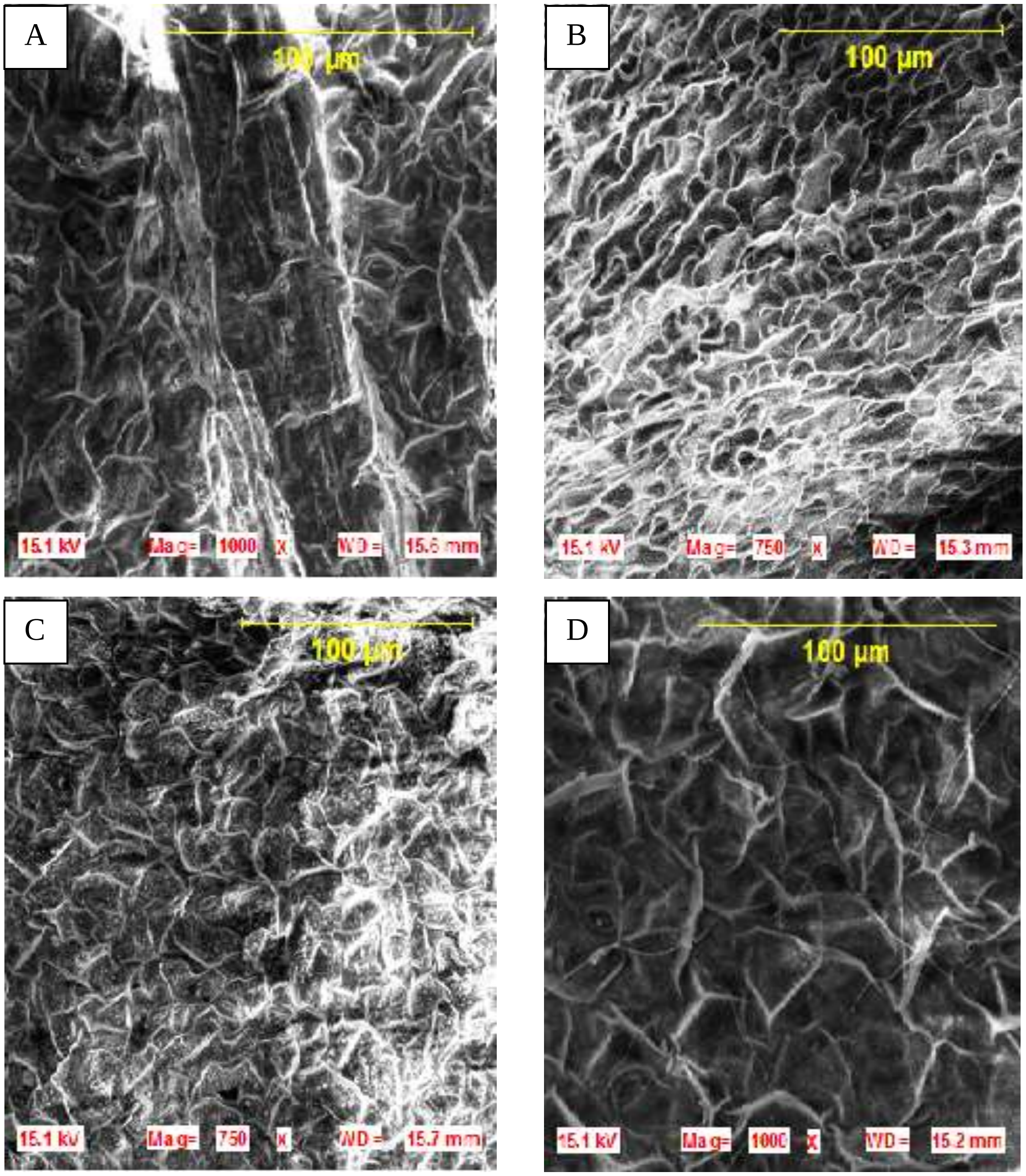


Plate 4.36: Scanning Electron Micrographs of abaxial epidermis of *Phyllanthus* species

A: *P. amarus* [Daramola/Emwiogbon/Oguntayo 103399 (FHI)]
B: *P. beillei* [D. P. Stanfield 61825 (FHI)]
C: *P. capillaris* showing wax deposits [Daramola/Macauley/Oguntayo 78618 (FHI)]
D: *P. fraternus* showing wax deposits [A. O. Ohaeri 4096 (NIPRD) Scale bar = 100µm]

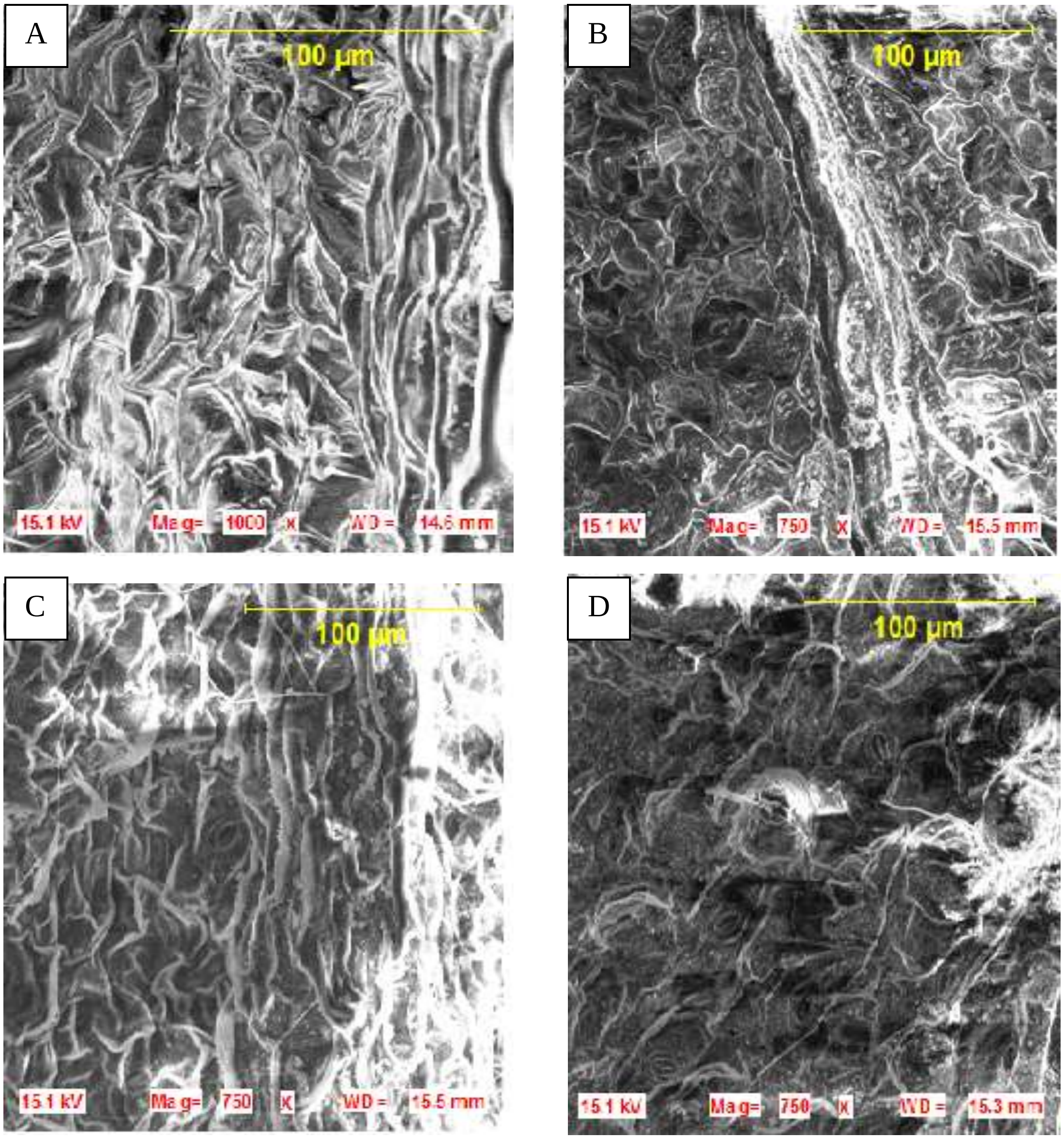


Plate 4.37: Scanning Electron Micrographs of abaxial epidermis of *Phyllanthus* species

A: *P. maderaspatensis* [M. G. Latilo 93997 (FHI)]
B: *P. mannianus* [P. O. Ekwuno 77250 (FHI)]
C: *P. nigericus* [P. O. Ekwuno70470 (FHI)]
D: *P. niruri* showing wax deposits [D. P. M. Guile 2784[C] (IFEH) Scale bar = 100µm]

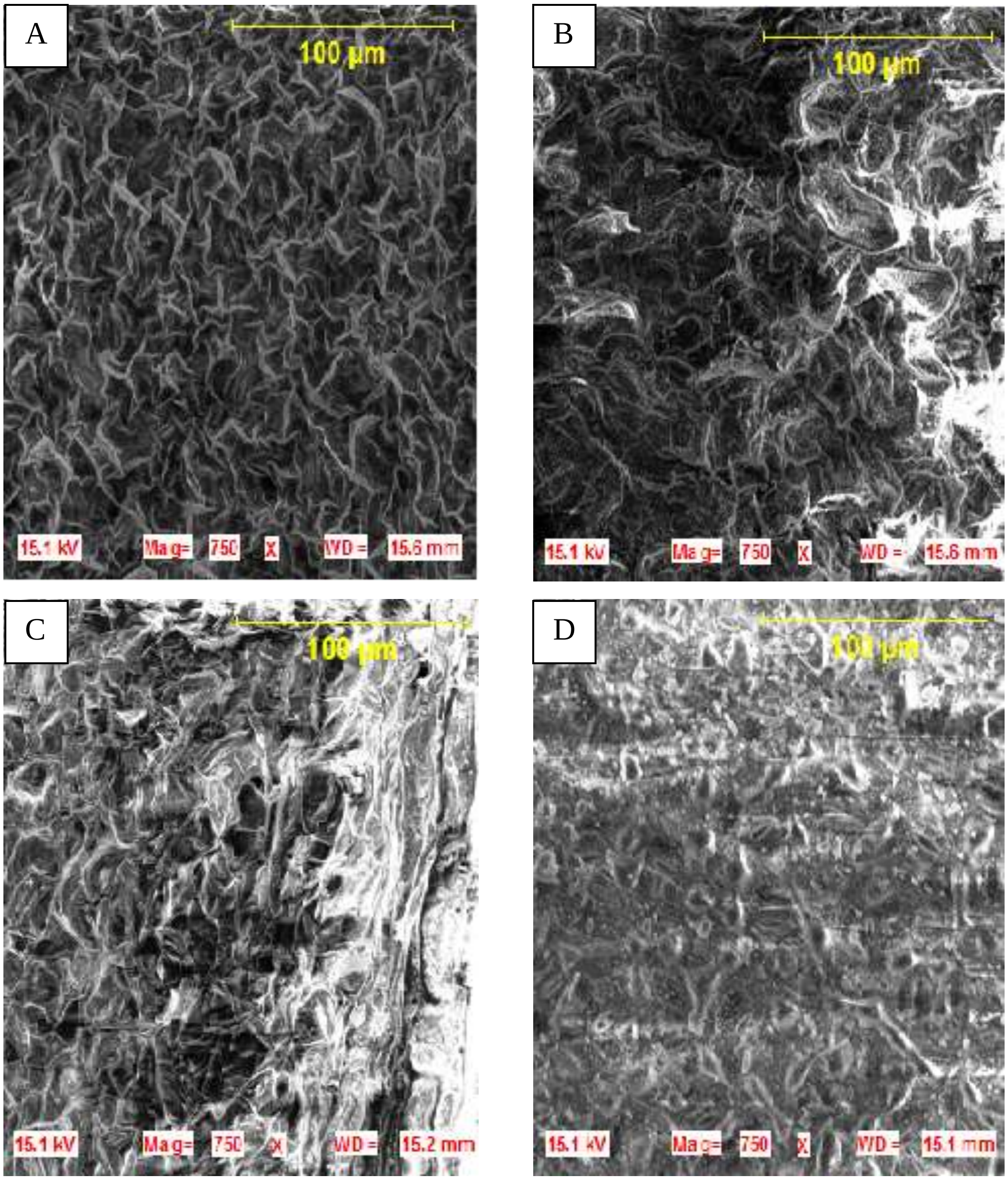


Plate 4.38: Scanning Electron Micrographs of abaxial epidermis of *Phyllanthus* species
A: *P. niruroides* [K. R. M. Williamson 15562 (UIH)]
B: *P. odontadenius* [A. P. D. Jones 6217 (FHI)]
C: *P. pentandrus* [J. B. Hall 2794 (IFEH)]
D: *P. reticulatus* showing wax deposits [J. B. Hall 2796 (IFEH) Scale bar = 100µm]

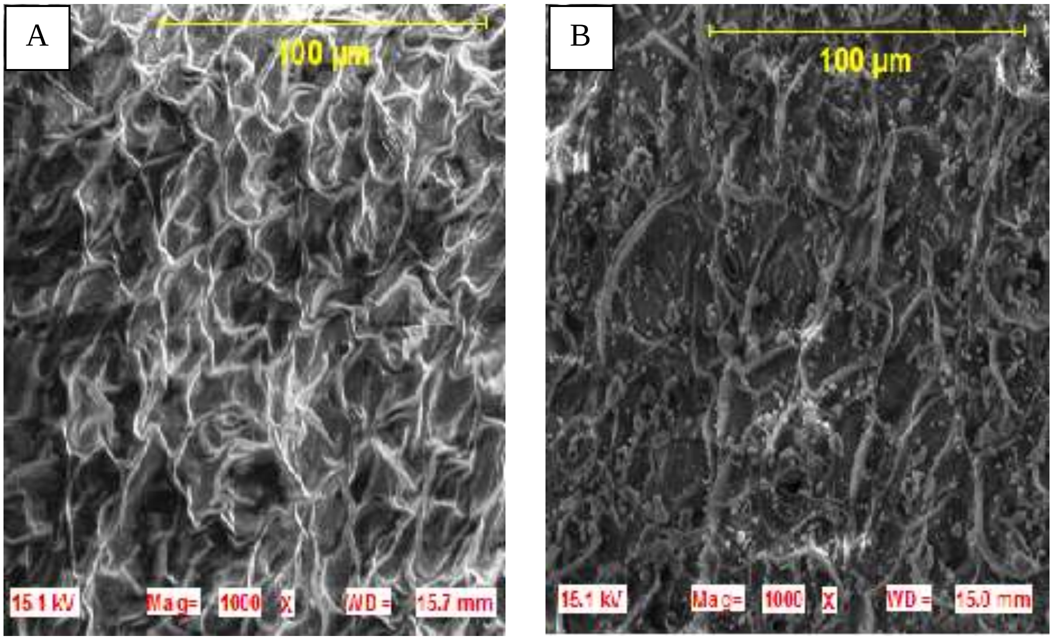


Plate 4.39: Scanning Electron Micrographs of abaxial epidermis of *Phyllanthus* species
A: *P. sublanatus* showing irregular epidermal cells [Ibhanesebor & Osanyinlusi 96993 (FHI)]
B: *P. urinaria* showing wax deposits [A. Egunyomi 21823 (UIH) Scale bar = 100µm]

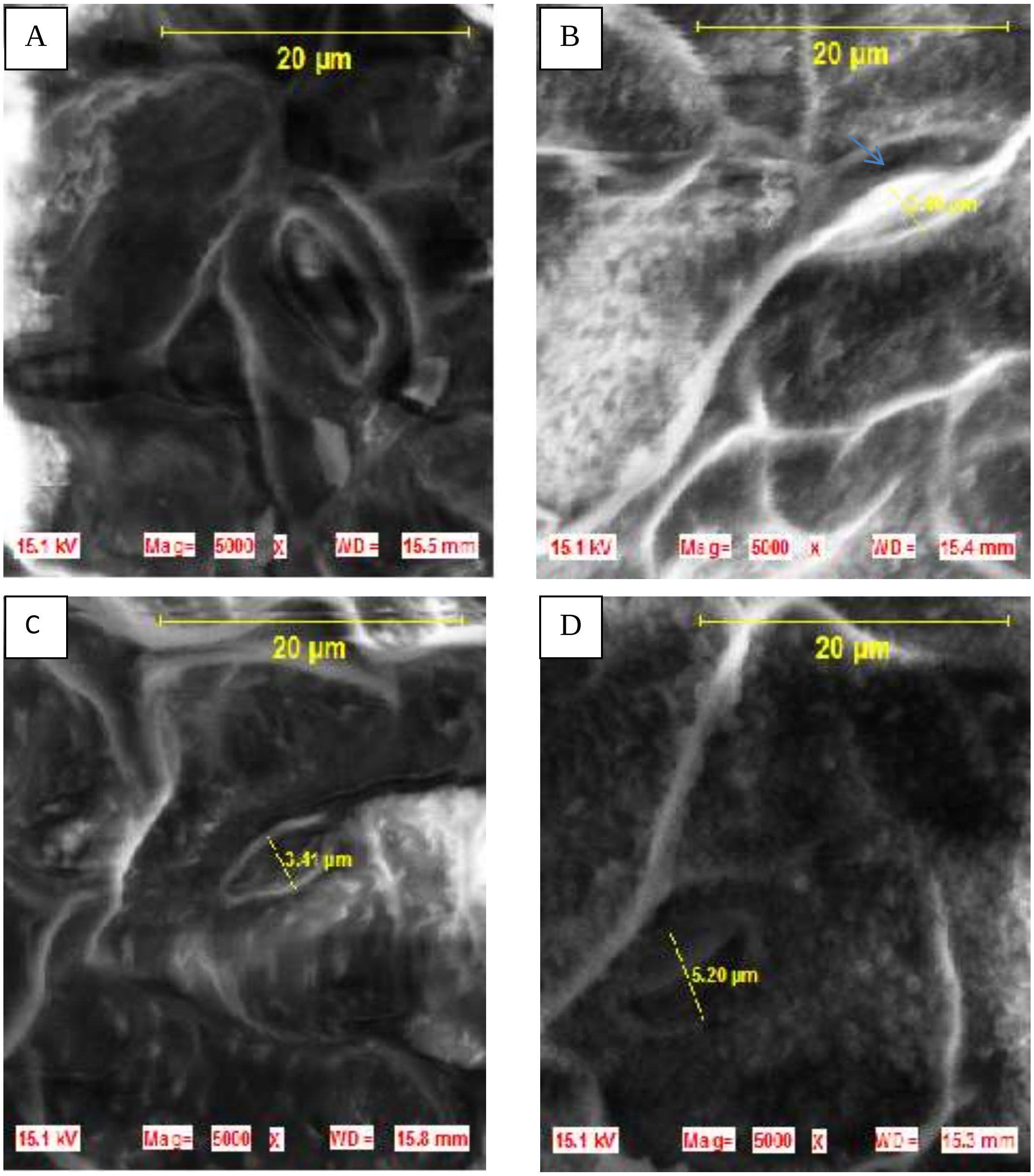


Plate 4.40: Scanning Electron Micrographs of abaxial stomatal morphology of *Phyllanthus* species

A: *P. amarus* [Daramola/Emwiogbon/Oguntayo 103399 (FHI)]

B: *P. beillei* showing narrow elliptic guard cell (arrowed) [D. P. Stanfield 61825 (FHI)]

C: *P. capillaris* showing outer stomata legde aperture [Daramola/Macauley/Oguntayo 78618 (FHI)]

D: *P. fraternus* showing wax deposits [A. O. Ohaeri 4096 (NIPRD) Scale bar: 20µm]

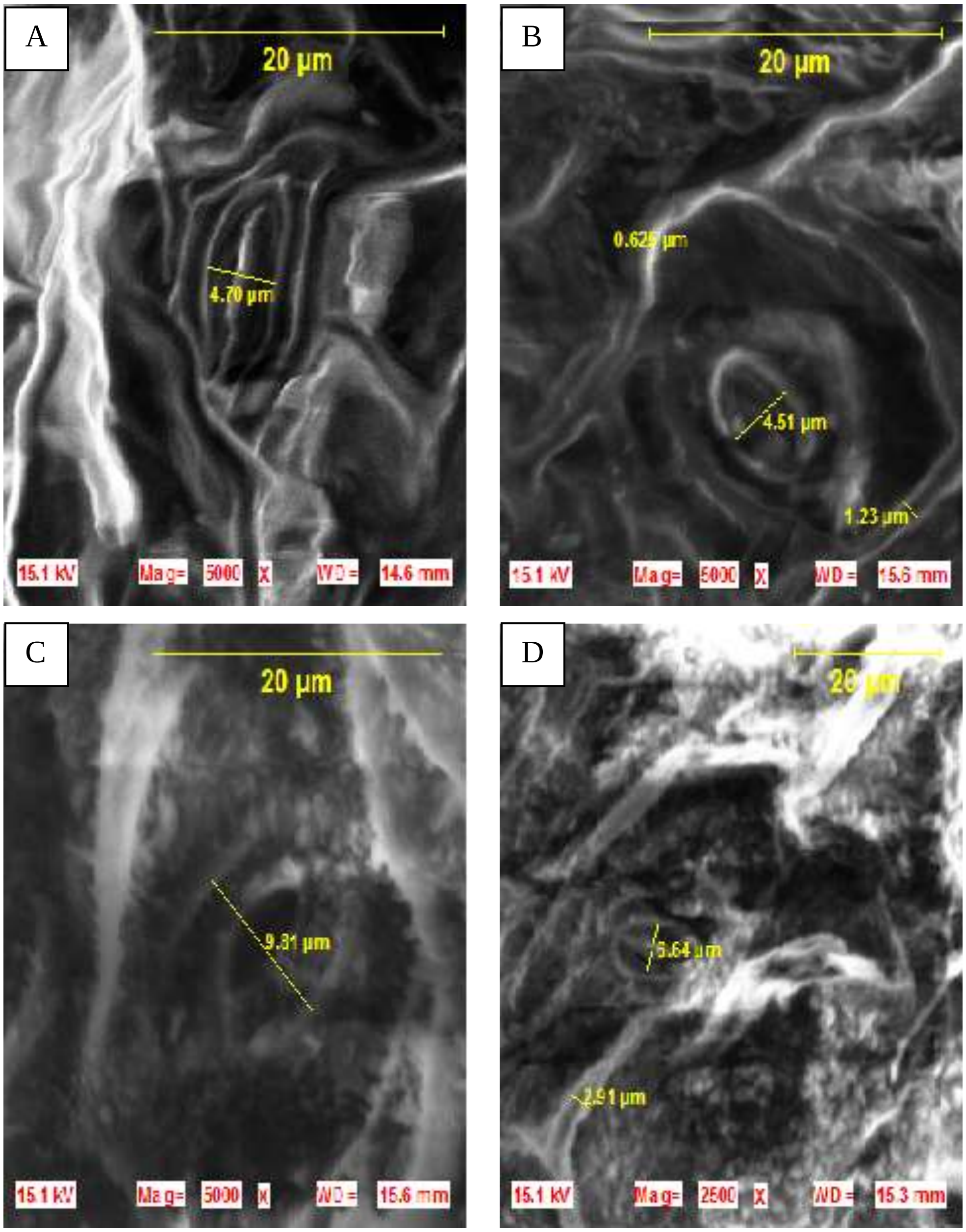


Plate 4.41: Scanning Electron Micrographs of abaxial stomatal morphology of *Phyllanthus* species

A: *P. maderaspatensis* showing outer stomata legde aperture [M. G. Latilo 93997 (FHI)]
B: *P. mannianus* ‘’ ‘’ ‘’ ‘’ ‘’ [P. O. Ekwuno 77250 (FHI)]
C: *P. nigericus* [P. O. Ekwuno 70470 (FHI)]
D: *P. niruri* showing wax deposits [D. P. M. Guile 2784^C (IFEH) Scale bar = 20µm]

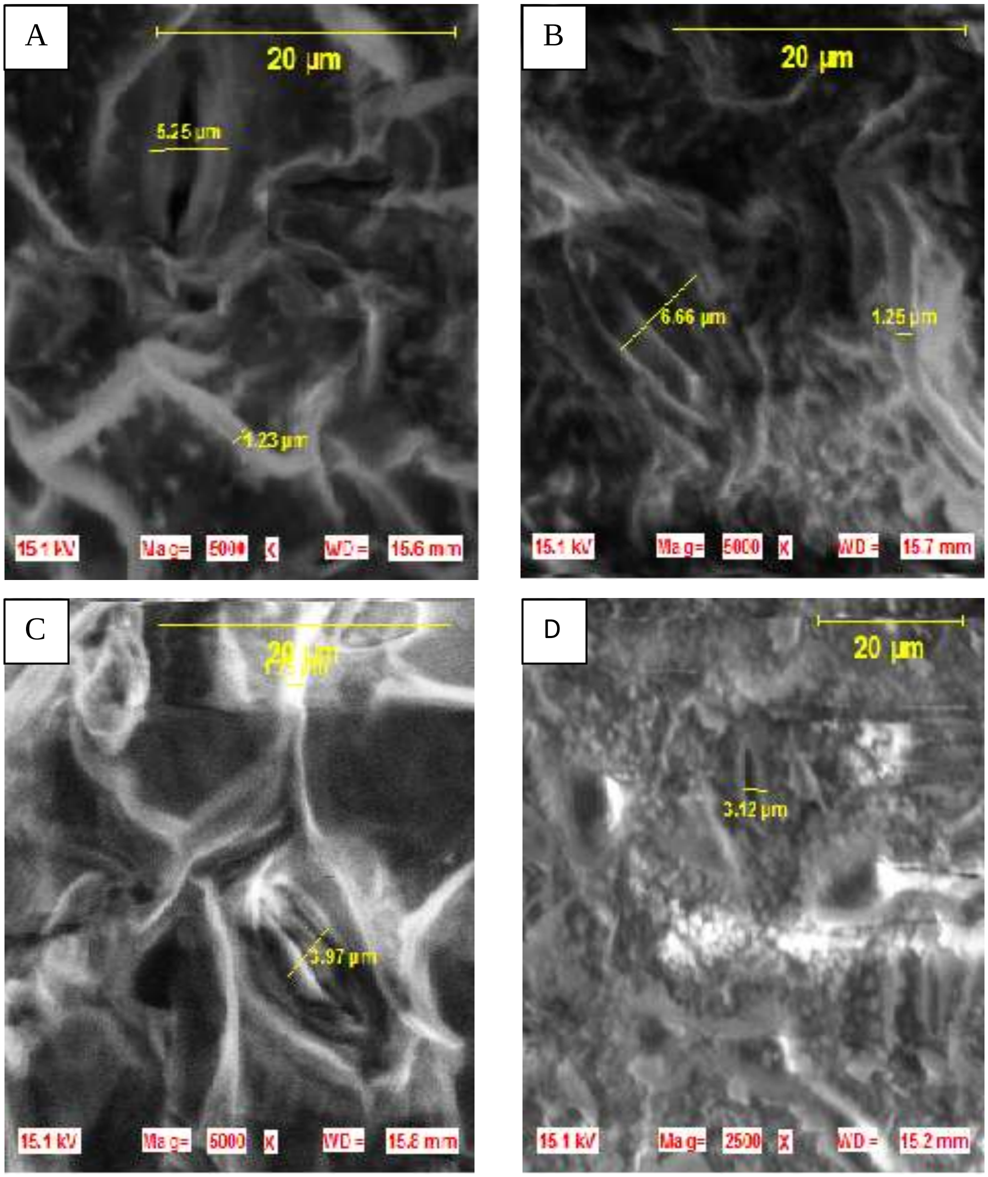


Plate 4.42: Scanning Electron Micrographs of abaxial stomatal morphology of *Phyllanthus* species

A: *P. niruroides* showing outer stomata legde aperture [K.R.M. Williamson 15562 (UIH)]

B: *P. odontadenius* [A.P.D. Jones 6217 (FHI)]

C: *P. pentandrus* [J.B. Hall 2794 (IFEH)]

D: *P. reticulatus* showing wax deposits [J.B. Hall 2796 (IFEH) Scale bar = 20µm]

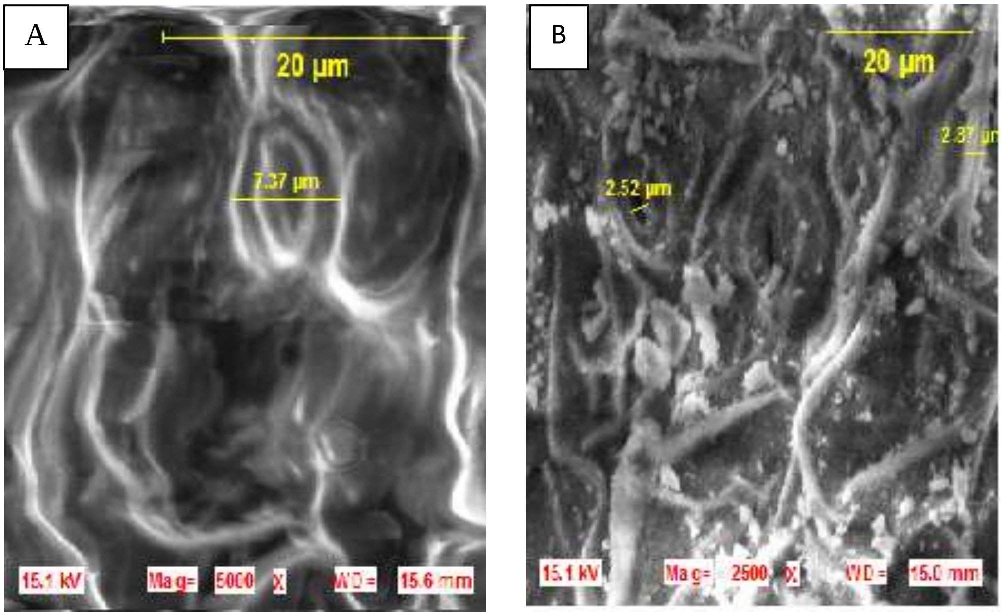


Plate 4.43: Scanning Electron Micrographs of abaxial stomatal morphology of *Phyllanthus* species

A: *P. sublanatus* showing outer stomata legde aperture [Ibhanesebor & Osanyinlusi 96993 (FHI)]

B: *P. urinaria* [A. Egunyomi 21823 (UIH) Scale bar = 20µm]

4.7 Pollen Morphology

The qualitative and quantitative pollen morphological characters of some of the species in the genus *Phyllanthus* are presented in Table 4.5. The pollen grains of the species studied were 3–colporate, finely reticulate pollen without much ornamentation. Pollens were prolate, subprolate in shape in all taxa studied except *P. muellerianus* which was oblate–spheroidal (Table 4.5, Plate 4.44d). The pollen grains ranged in size from small (10-25 µm) in *P. amarus*, *P. muellerianus*, *P. maderaspatensis*, *P. pentandrus* and *P. reticulatus* to medium (25-50 µm) in *P. maderaspatensis*, *P. capillaris*, *P. niruroides*, *P. odontadenius* and *P. urinaria* (Table 4.5). The smallest pollen size was observed in *P. muellerianus* being 12.4 µm X 13.0 µm while the largest pollen size was observed in *P. capillaris* being 31.5 µm X 23.25 µm. The colpi length ranged from 12.2 µm in *P. muellerianus* to 26.75 µm in *P. urinaria* (Table 4.5) while the percentage polar axis over equatorial axis ranged from 95.4% in *P. muellerianus* to 145.8% in *P. niruroides*.

Table 4.5: Qualitative and Quantitative pollen characters of *Phyllanthus* species in Nigeria

Taxa	Polar axis (P) (µm)	Equatorial axis (E) (µm)	Colpi length (µm)	Pollen class	P/E (%)	Pollen size
Phyllanthus amarus	17.5(20.75±2.06)22.5	15.0(16.75±1.21)17.5	15.0(18.5±1.75)20.0	Subprolate	123.9	Small
P. capillaris	25.0(31.5±3.16)35.0	20.0(23.25±1.69)25.0	22.5(25.75±2.65)30.0	Prolate	135.5	Medium
P. maderaspatensis	20.0(23.0±3.07)27.5	12.5(18.25±2.65)22	20.0(21.0±1.29)22.5	Subprolate	126.0	Small
P. muellerianus	11.0(12.4±0.84)14.0	12.0(13.0±0.67)14.0	11.0(12.2±0.79)13.0	Oblate-spheroidal	95.4	Small
P. niruri	25.0(30.75±4.42)37.5	17.5(22.25±3.22)27.5	17.5(24.0±4.12)30.0	Prolate	138.2	Medium
P. niruroides	25.0(26.25±1.32)27.5	15.0(18.0±1.97)20.0	22.5(24.5±1.97)27.5	Prolate	145.8	Medium
P. odontadenius	22.5(28.25±3.34)32.5	17.5(22.2±2.49)25.0	20.0(23.0±1.97)25.0	Subprolate	127.0	Medium
P. pentandrus	22.5(24.0±1.29)25.0	17.5(18.25±1.21)20.0	20.0(21.5±1.29)22.5	Subprolate	131.5	Small
P. reticulatus	15.0(16.5±1.75)20.0	12.5(14.0±1.29)15.0	15.0(16.25±1.32)17.5	Subprolate	117.9	Small
P. urinaria	22.5(25.5±2.84)30.0	12.5(18.25±2.37)20.0	22.5(26.75±2.90)30.0	Prolate	139.7	Medium

Size classes:10-25 µm - Small

25-50 µm - Medium

50-100 µm - Large

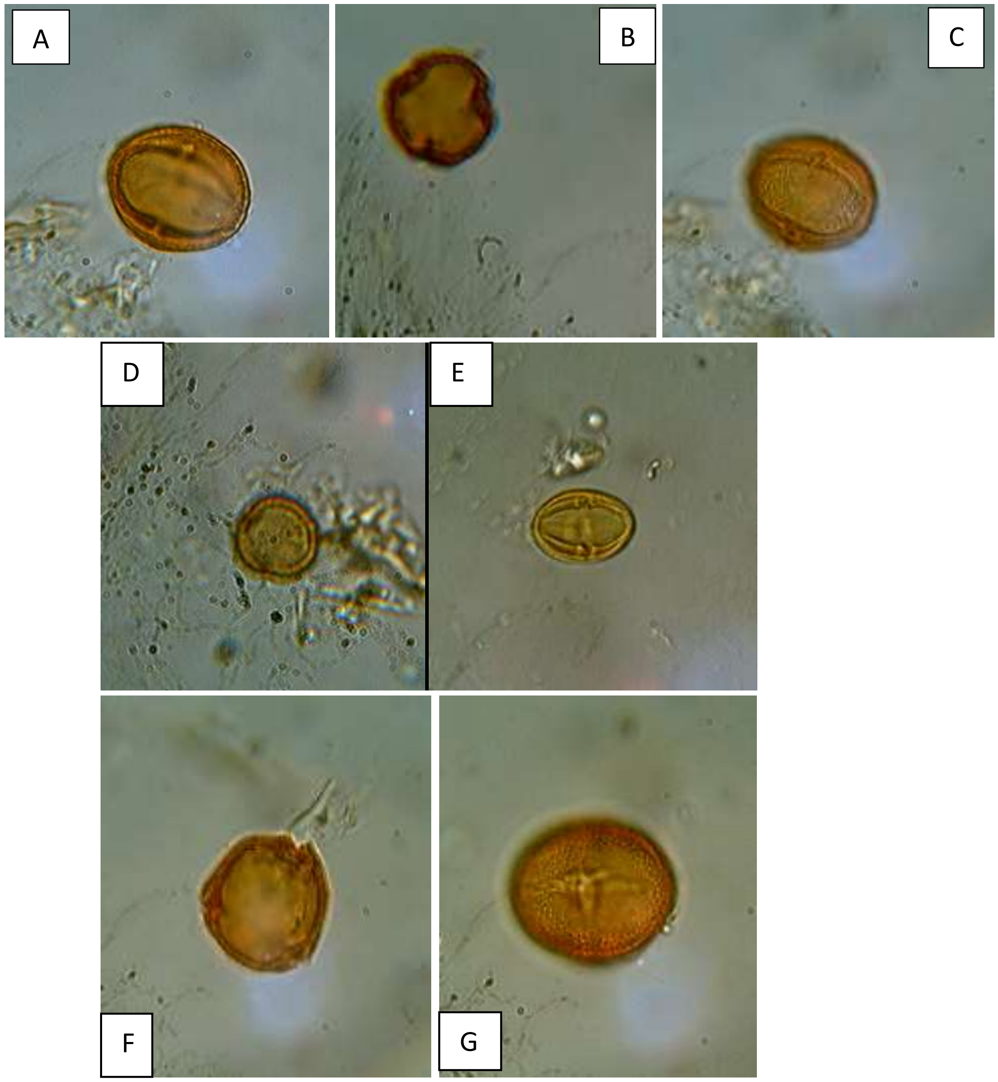


Plate 4.44: Photomicrographs of pollen grains of *Phyllanthus* species in Nigeria (x1000)

A: Equatorial view of *Phyllanthus amarus* showing colpi
B: Polar view of *Phyllanthus amarus showing* 3-colporate pollen
C: Equatorial view of *Phyllanthus amarus* showing reticulate pattern
D: Polar view of *Phyllanthus muellerianus*
E: Equatorial view of *Phyllanthus niruri*
F: Polar view of *Phyllanthus niruri* 3-colporate pollen
G: Equatorial view of *Phyllanthus niruri* showing reticulate pattern

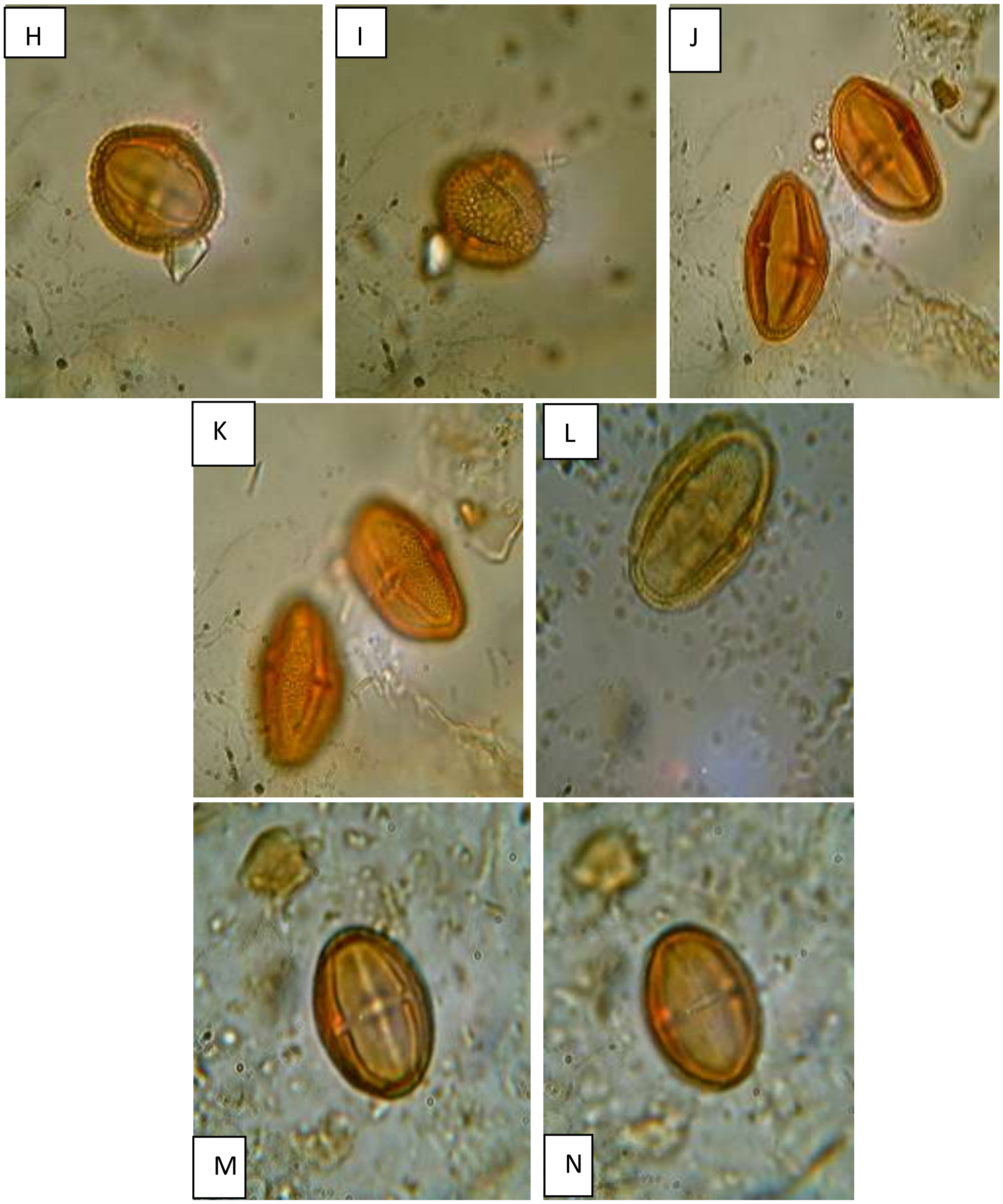


Plate 4.45: Photomicrographs of pollen grains of *Phyllanthus* species in Nigeria (x1000)

H: Equatorial view of *Phyllanthus pentandrus*

I: Polar view of *Phyllanthus pentandrus* showing reticulate pattern

J: Equatorial view of *Phyllanthus odontadenius* showing colpi

K: Equatorial view of *Phyllanthus odontadenius* showing reticulate pattern

L: Equatorial view of *Phyllanthus urinaria*

M: Equatorial view of *Phyllanthus capillaris* showing colpi

N: Equatorial view of *Phyllanthus capillaris*

4.8 Molecular studies

The quality of the genomic DNA when tested on 1% agarose gel showed high molecular weight bands following electrophoresis (Plate 4.46). This helped to confirm that the protocol used for the DNA isolation of twenty freshly collected young leaf samples of *Phyllanthus* and two allied species produced good quality and quantity DNA with little or no contaminants for further PCR based studies.

Two samples out of the twenty extracted genomic DNA seemed to contain PCR inhibitory components and failed to sequence. These were excluded from the study after several amplification attempts. The dendrogram (Figure 4.2a) generated by Maximum Likelihood method using the *rbcL* gene shows the molecular phylogenetic relationships among the *Phyllanthus* species in Nigeria. This revealed six clusters which contained sixteen ingroups and two outgroups namely *Magaritaria discodea* and *Securinega virosa*. Group 6 comprised only *M. discoidea* as an outgroup while Group 2 has only *S. virosa* embedded within the *Phyllanthus* species. Group 5 consists of *P. reticulatus* and *P. muellerianus* which are closely related with a boostrap percentage of 86% and could be regarded as sister taxa. In Group 1, *P. urinaria 1* and *P. urinaria* 2 had the closest affinity with boostrap value of 99% which shows that that the two are obviously same species but distinctly related to *P. maderaspatensis* which is more evolved. *Phyllanthus niruri* and *P. capillaris* also showed close affinity to one another in the same group when compared to *P. odontadenius*. The two specimens of *P. amarus in* Group 3 had close affinity and they form sister taxa. However, *P. amarus* is distinctly related to *P. pentandrus* and *P. acidus* in Group 4 which are more evolved.

Figure 4.2b shows the molecular phylogenetic analysis by Maximum Likelihood method of the *rbcL* gene locus obtained in the present study together with those retrieved from the DNA database. The datasets revealed seven clusters which contained twenty-five ingroups and two outgroups namely *Magaritaria discodea* and *Securinega virosa*. Group 7 has only *S. virosa* as an outgroup while Group 3 consists of only *M. discoidea* embedded within the *Phyllanthus* species. The two specimens of *P. amarus* 1 and 2, *GU441791 P. niruri* and *KF365994 P. amarus* in Group 6 had close affinity and they form sister taxa. *Phyllanthus acidus, GQ436329 P. acidus, P. reticulatus*, *P. muellerianus* and *KP094227 P. reticulatus* clustered together in Group 4 while *P. pentandrus* clustered with *KJ7737421 P. tenellus* in

Group 5 very close to Group 4 species. *Phyllanthus urinaria* 1 and 2 had a boostrap percentage of 100% which indicates that they are the same species but distintly related to *P. maderspatensis P. niruri, P. capillaris* and *P. odontadenius* which are more evolved.

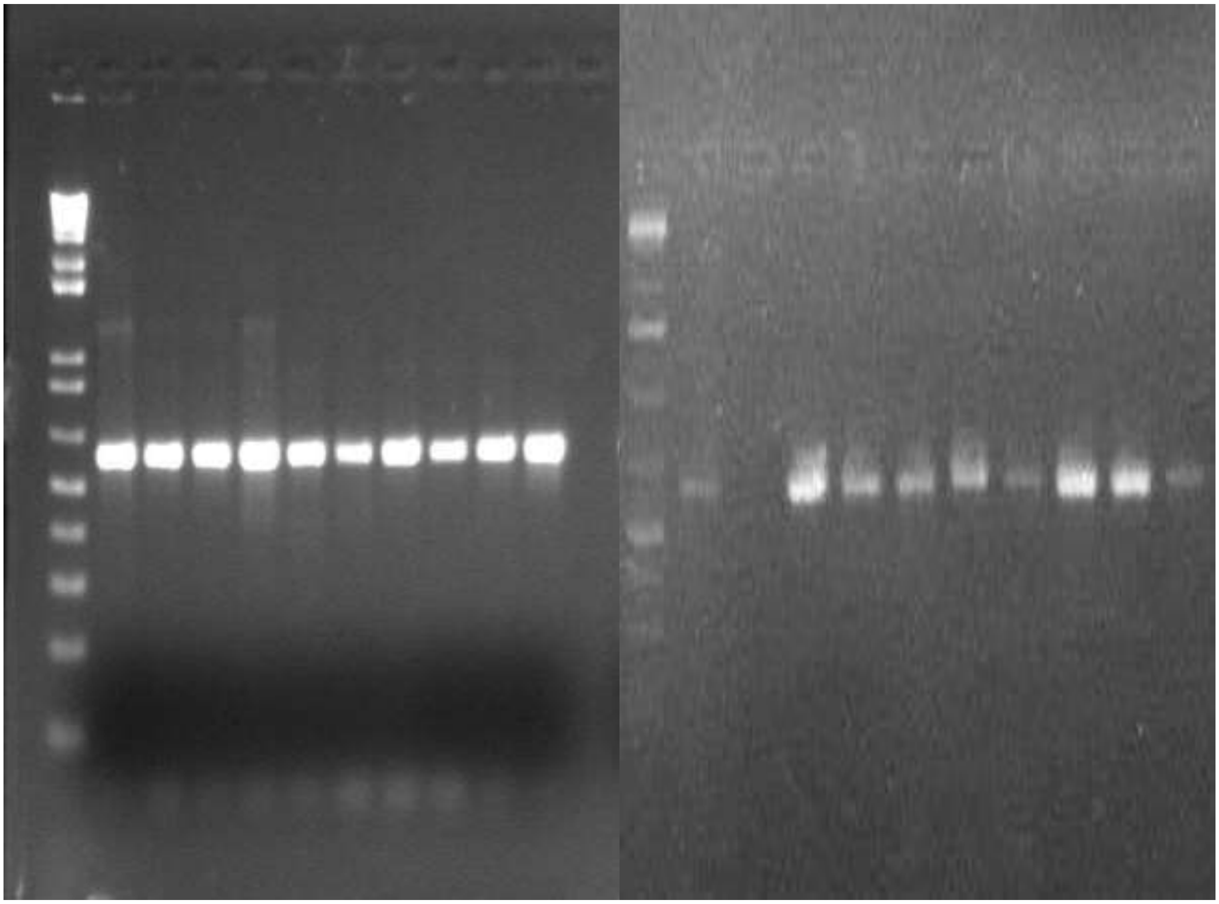


Plate 4.46: PCR profile of DNA at the rbcL gene region in the leaves of some *Phyllanthus* species as well as one species each of *Magaritaria* and *Securinega*

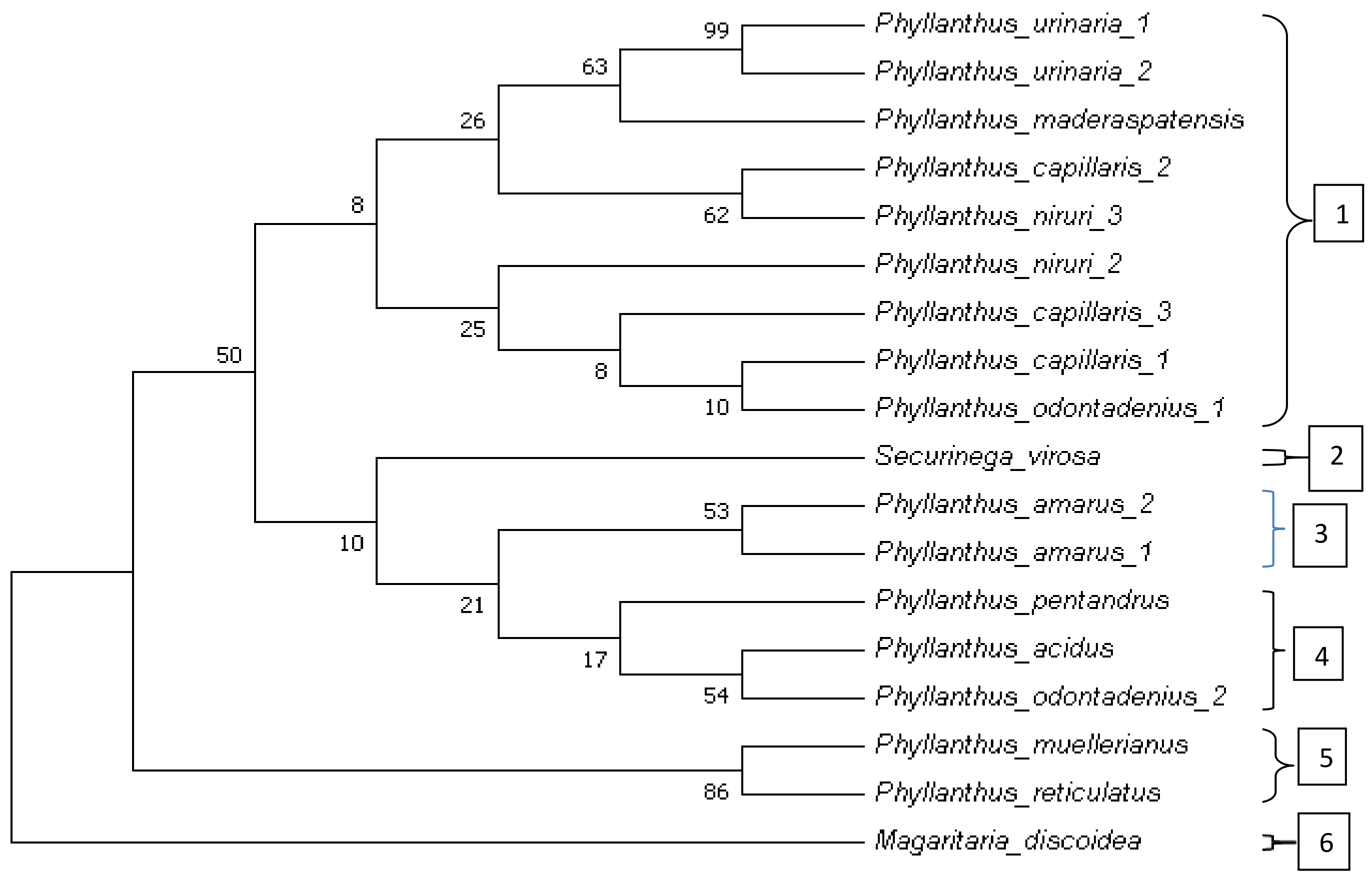


Figure 4.2a: Dendrogram of the molecular phylogeny of the *Phyllanthus* using the *rbcL* gene (with *Magaritaria* and *Securinega* species as outgroups)

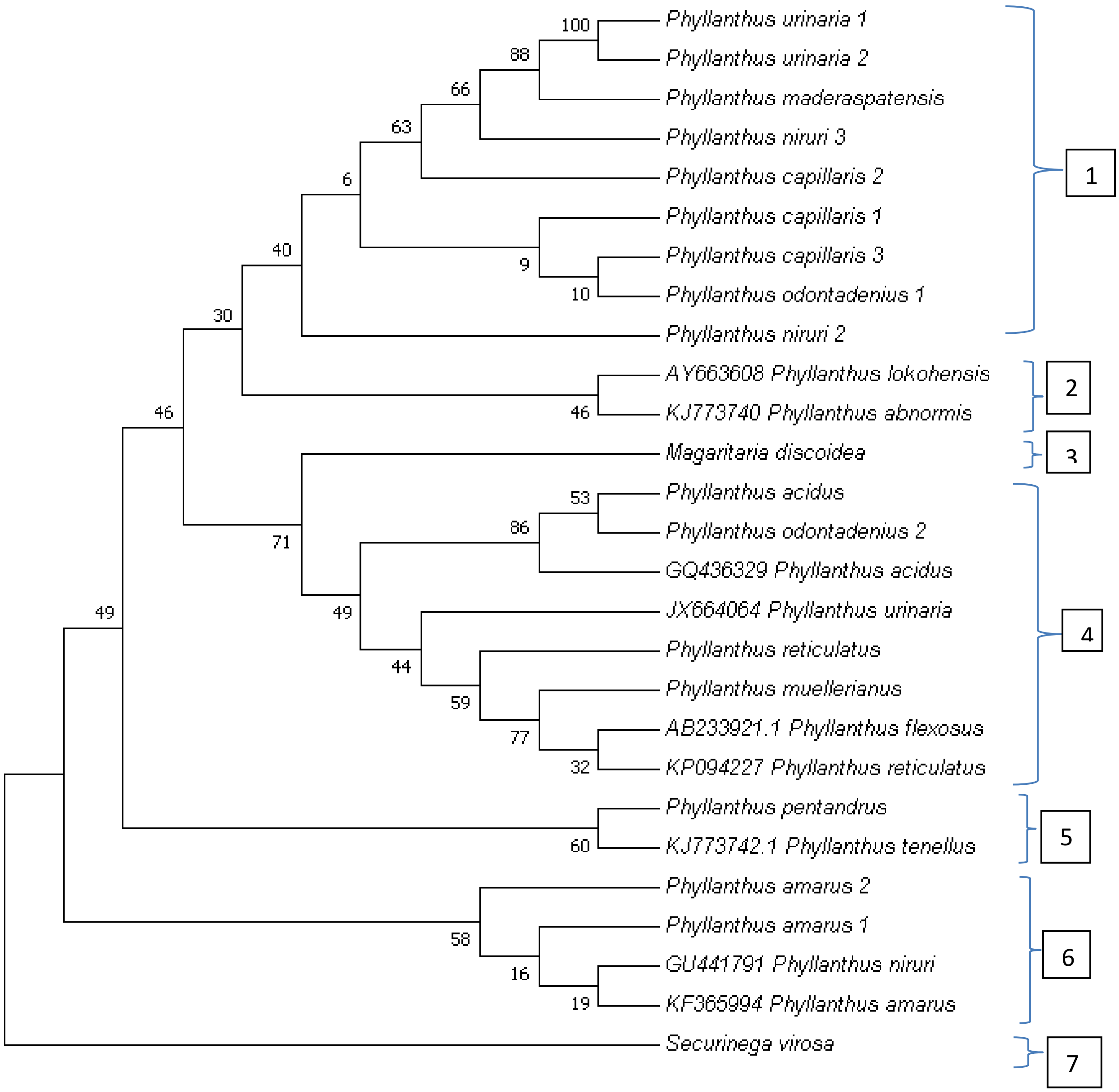


Figure 4.2b: Dendrogram of the molecular phylogeny of the *Phyllanthus* using the *rbcL* gene combined with datasets obtained from DNA database (with *Magaritaria* and *Securinega* species as outgroups)

4.9 Numerical Taxonomy

4.9.1 Cladograms from clustering analysis

The results of the clustering analysis are presented in form of cladograms which are diagrams of relationship. The taxa are represented by numbers as shown below:

(1)	*Phyllanthus acidus*	– OTU 1
(2)	*P. amarus*	– OTU 2
(3)	*P. beillei*	– OTU 3
(4)	*P. capillaris*	– OTU 4
(5)	*P. floribundus*	– OTU 5
(6)	*P. fraternus*	– OTU 6
(7)	*P. maderaspatensis*	– OTU 7
(8)	*P. mannianus*	– OTU 8
(9)	*P. muellerianus*	– OTU 9
(10)	*P. nigericus*	– OTU 10
(11)	*P. niruri*	– OTU 11
(12)	*P. niruroides*	– OTU 12
(13)	*P. odontadenius*	– OTU 13
(14)	*P. pentandrus*	– OTU 14
(15)	*P. physocarpus*	– OTU 15
(16)	*P. reticulatus*	– OTU 16
(17)	*P. rotundifolius*	– OUT 17
(18)	*P. sublanatus*	– OTU 18
(19)	*P. urinaria*	– OTU 19

The ordinate represents the degree of similarities among taxa.

Figure 4.3 shows the cladogram of 46 x 19 data matrix which comprised combined macro morphological, epidermal and pollen morphological characters, Figure 4.4 shows the cladogram of 27 x 19 epidermal data matrix, Figure 4.5 shows the cladogram of 14 x 19 macro morphological data matrix while Figure 4.6 shows the cladogram of 5 x 19 pollen morphological data matrix.

Tables 4.6, 4.7, 4.8, and 4.9 show the clusters obtained from the 46 x 19, 27 x 19, 14 x 19 and 5 x 19 data matrices respectively.

4.9.1.1: Data matrix of 46 x 19 combined macro morphological, epidermal and pollen morphological characters:

Four groups of clusters were obtained from the 46 x 19 data matrix (Figure 4.3). Cluster 1 contained OTUs 2 (*P. amarus*), 6 (*P. fraternus*), 11 (*P. niruri*), 13 (*P. odontadenius),* 12(*P. niruroides*), 4 (*P. capillaris*), 7 (*P. maderaspatensis*), 14 (*P. pentandrus*), 10 (*P. nigericus*) and 19 (*P. urinaria*). Cluster 2 contained OTUs 3 (*P. beillei)* and 8 (*P. mannianus*). Cluster 3 contained OTUs 17 (*P. rotundifolius*) and 18 (*P. sublanatus*) while cluster 4 had OTUs 1 (*P. acidus*), 15 (*P. physocarpus*) 5(*P. floribundus*), 9 (*P. muellerianus*) and 16 (*P. reticulatus*). All the species in cluster 4 have lanceolate or ovate leaf shape, petiolate with attenuate leaf base except OTUs 15 (*P. physocarpus*) and OTU 16 (*P. reticulatus*) which have cuneate leaf base. All the species are hypostomatic with polygonal or rectangular epidermal cell shape except *P. acidus* and *P. physocarpus* with irregular epidermal cell shape (Table 4.6) OTUs 11 and 13 showed the closest affinity to each other followed by OTUs 2 and 6 all in cluster 1 (Figure 4.3).

4.9.1.2: Data Matrix of 27 x 19 epidermal characters:

Four groups of clusters were also recognized from the cladogram. Cluster 1 had OTUs 2 (*P. amarus*), 6 (*P. fraternus*), 17 (*P. rotundifolius*) 18 (*P. sublanatus*) and 10 *(P. nigericus*), cluster 2 had OTUs 7, 14, 19, 11, 13 and 12. Cluster 3 had OTUs 1, 15, 3 and 8 while cluster 4 had three species OTUs 5, 9 and 16. (Figure 4.4, Table 4.7). Species in cluster 4 are all lanceolate-ovate in leaf shape with acute apex, glabrous surface with attenuate base except OTU 16 (*P. reticulatus*) which has cuneate base. OTUs 9 and 16 had five perianth lobes except OTU 5 which its perianth lobe was not available for assessment. They all had rectangular epidermal cell shape with straight anticlinal wall pattern as well as being hypostomatic with anisocytic type of stomata. OTUs 5 and 9 had the closest affinity in cluster 4 and this was followed closely by OTUs 7 and 14 as well as OTUs 11 and 13 all in cluster 2 (Figure 4.4).

4.9.1.3: Data matrix of 14 x 19 macro morphological characters:

Figure 4.5 shows the cladogram of 14 x 19 data matrix for macromorphological characters only and four clusters were recognized (Table 4.8). Cluster 1 had OTUs 2, 12, 6, 11, 13, 4, 3 and 14 (*P. amarus*, *P. niruroides, P. fraternus, P. niruri, P. odontadenius, P. capillaris, P. beillei* and *P. pentandrus* respectively). All the species in this cluster are herbaceous with OTUs 2 and 12 having the closest affinity with 5 as their number of perianth lobes respectively. Cluster 2 consisted of OTUs 1, 16, 7 and 9 all with acute leaf apex, cluster 3 had OTUs 8, 19, 17, 10 and 18 all with cuneate base except OTUs 10 and 18 with attenuate leaf base while cluster 4 had OTUs 5 and 15 (Figure 4.5, Table 4.8).

4.9.1.4: Data matrix of 5 x 19 pollen morphological characters:

Figure 4.6 shows the cladogram of 5 x 19 data matrix for pollen morphological characters only where four clusters were recognized (Table 4.9). Cluster 1 consisted of OTUs 5, 9 15, 16 and 2 (*P. floribundus, P. muellerianus, P. physocarpus, P. reticulatus* and *P. amarus* respectively).

Cluster 2 had OTUs 7, 10 and 14 (*P. maderaspatensis, P. nigericus* and *P. pentardrus* respectively) all with subprolate pollen shape and small pollen size except OTU 10 (*P. nigericus*) that was not available for assessment. Cluster 3 consisted of OTUs 1, 3, 4, 6, 11, 13, 8, 12 and 19 while cluster 4 had OTUs 17 and 18.

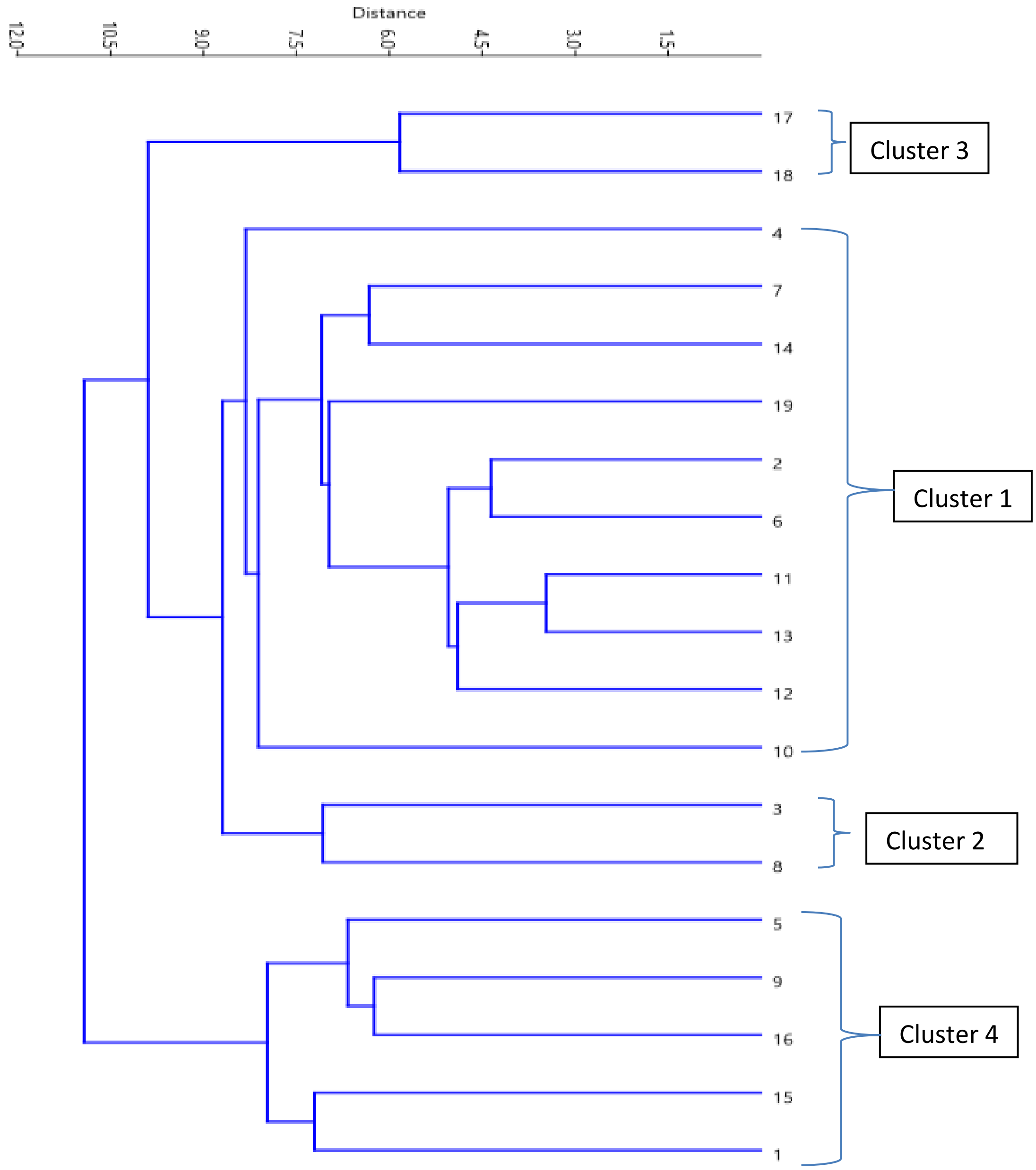


Figure 4.3: Cladogram from Hierarchical clustering of species of the genus *Phyllanthus* in Nigeria using paired group (UPGMA) based on 46 x 19 (combined macromorphological, epidermal and pollen morphological) data matrix

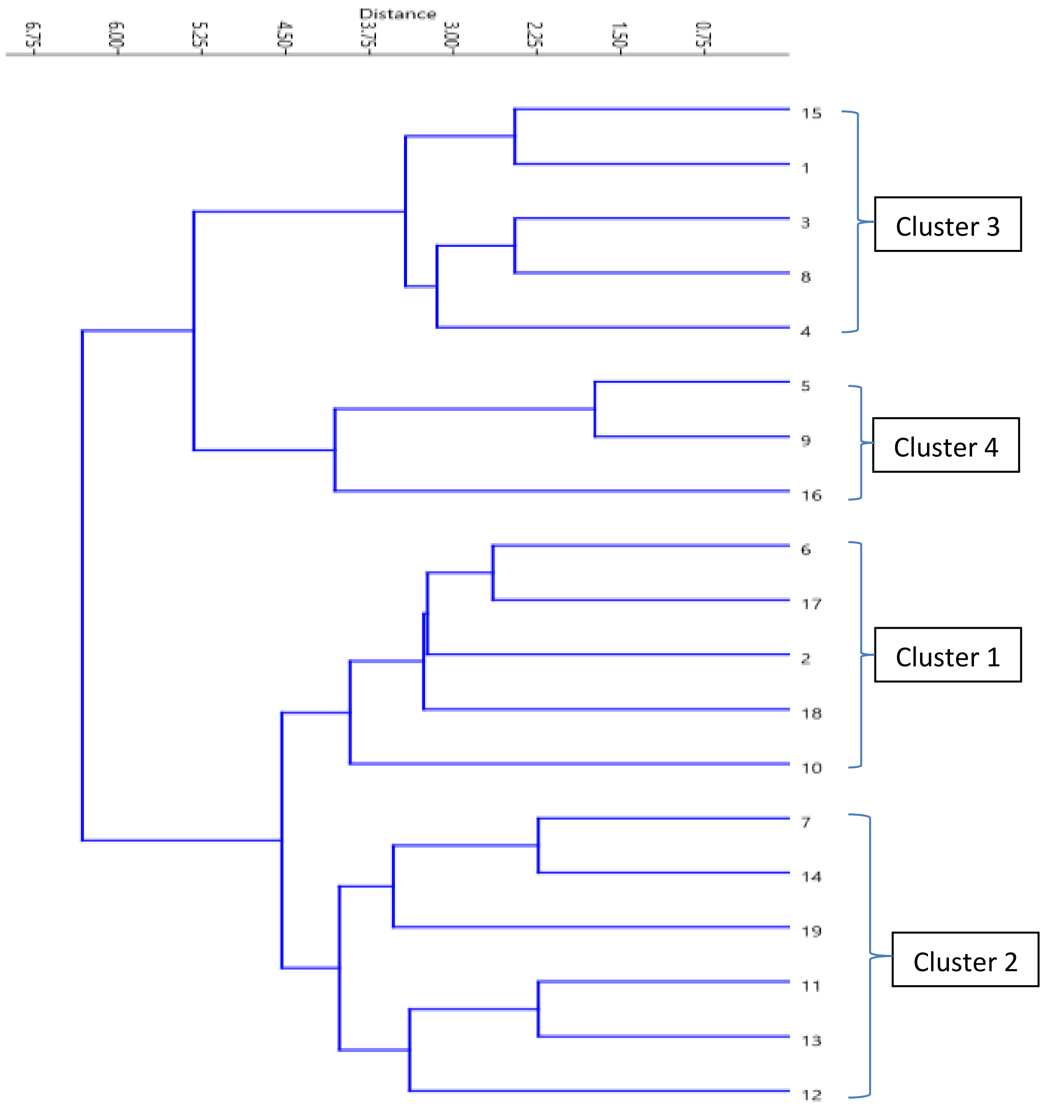


Figure 4.4: Cladogram from the Hierarchical clustering of species of the genus *Phyllanthus* in Nigeria using paired group (UPGMA) based on 27 x 19 data matrix (epidermal)

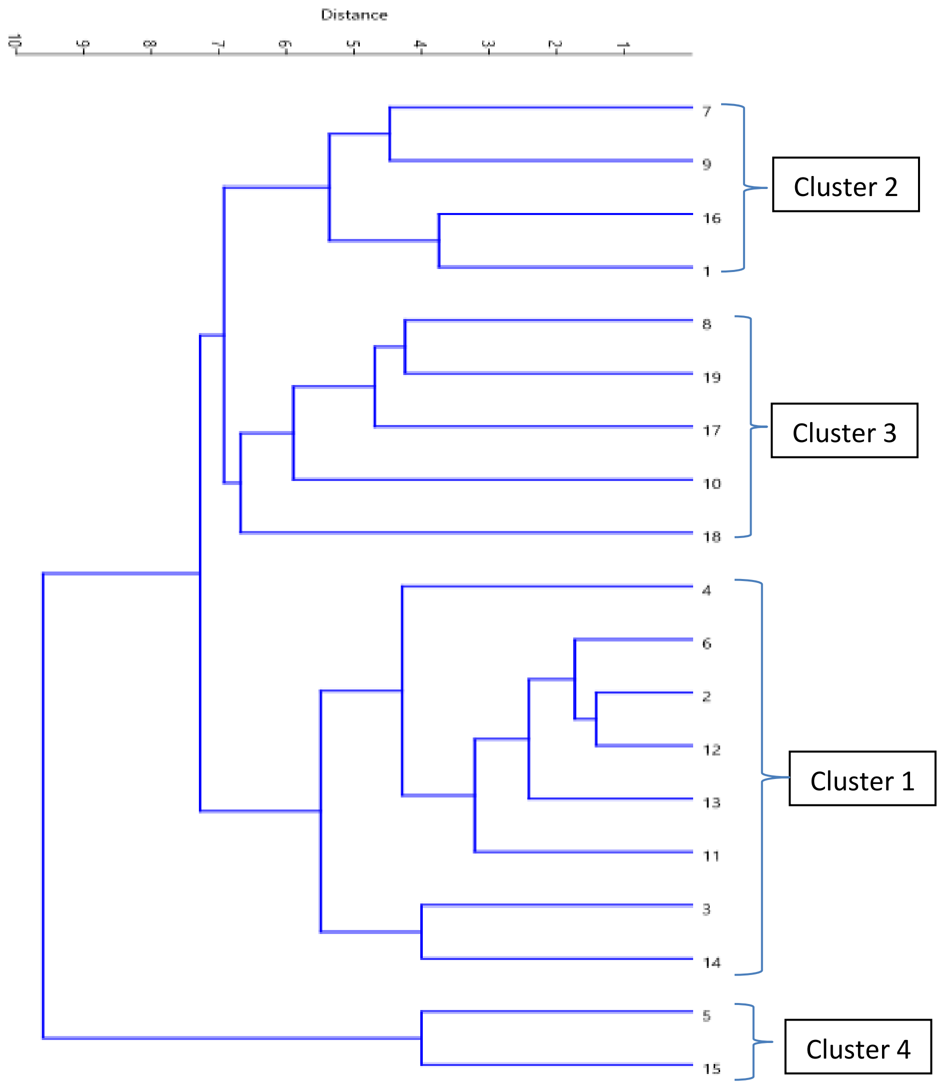


Figure 4.5: Cladogram from the Hierarchical clustering of species of the genus *Phyllanthus* in Nigeria using paired group (UPGMA) based on 14 x 19 data matrix (macromorphological)

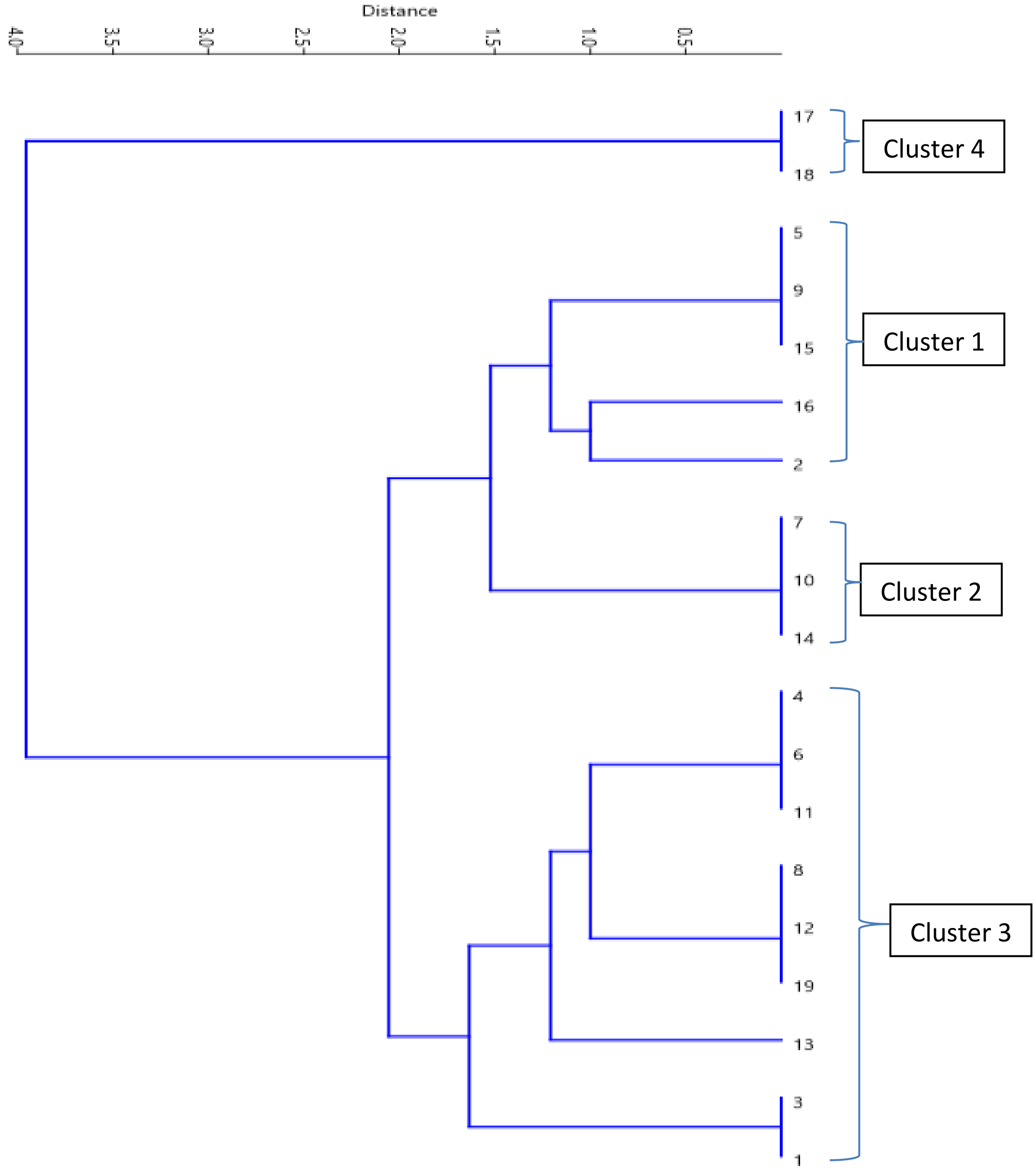


Figure 4.6: Cladogram from the Hierarchical clustering of species of the genus *Phyllanthus* in Nigeria using paired group (UPGMA) based on 5 x 19 data matrix (pollen morphological)

Table 4.6: Groups of OTUs recognized from hierachical clustering analysis using 46 x 19 macro morphological, epidermal and pollen morphological data matrix for *Phyllanthus* species in Nigeria

Cluster 1	**Cluster 2**	**Cluster 3**	**Cluster 4**
2–Phyllanthus amarus	*3–Phyllanthus Beillei*	*17–Phyllanthus rotundifolius*	*1–Phyllanthus acidus*
6–Phyllanthus fraternus	*8–Phyllanthus mannianus*	*18–Phyllanthus sublanatus*	*15–Phyllanthus physocarpus*
11–Phyllanthus Niruri			*5–Phyllanthus floribundus*
13–Phyllanthus odontadenius			*9–Phyllanthus muellerianus*
12–Phyllanthus niruroides			*16–Phyllanthus reticulatus*
4–Phyllanthus capillaris			
7–Phyllanthus maderaspatensis			
14–Phyllanthus pentandrus			
10–Phyllanthus nigericus			
19–Phyllanthus urinaria			

Table 4.7: Groups of OTUs recognized from hierachical clustering analysis using 27 x 19 epidermal data matrix for *Phyllanthus* species in Nigeria

Cluster 1	**Cluster 2**	**Cluster 3**	**Cluster 4**
2–Phyllanthus amarus	*7–Phyllanthus maderaspatensis*	*1–Phyllanthus acidus*	*5–Phyllanthus floribundus*
6–Phyllanthus fraternus	*14–Phyllanthus pentandrus*	*15–Phyllanthus physocarpus*	*9–Phyllanthus muellerianus*
17–Phyllanthus rotundifolius	*19–Phyllanthus urinaria*	*3–Phyllanthus beillei*	*16–Phyllanthus reticulatus*
18–Phyllanthus sublanatus	*11–Phyllanthus Niruri*	*8–Phyllanthus mannianus*	
10–Phyllanthus nigericus	*13–Phyllanthus odontadenius*	*4–Phyllanthus capillaris*	
	12–Phyllanthus niruroides		

Table 4.8: Group of OTUs recognized from Hierachical clustering analysis using 14 x 19 macromorphological data matrix for *Phyllanthus* species in Nigeria.

Cluster 1	Cluster 2	Cluster 3	Cluster 4
2–Phyllanthus amarus *12–Phyllanthus niruroides* *6–Phyllanthus fraternus* *11–Phyllanthus niruri* *13–Phyllanthus odontadenius* *4–Phyllanthus capillaris* *3–Phyllanthus beillei* *14–Phyllanthus pentandrus*	*1–Phyllanthus acidus* *16–Phyllanthus reticulatus* *7–Phyllanthus maderaspatensis* *9–Phyllanthus muellerianus*	*8–Phyllanthus mannianus* *19–Phyllanthus urinaria* *17–Phyllanthus rotundifolius* *10–Phyllanthus nigericus* *18–Phyllanthus sublanatus*	*5–Phyllanthus floribundus* *15–Phyllanthus physocarpus*

Table 4.9: Groups of OTUs recognized from hierachical clustering analysis using 5 x 19 pollen morphological data matrix for *Phyllanthus* species in Nigeria.

Cluster 1	Cluster 2	Cluster 3	Cluster 4
5–Phyllanthus floribundus	*7–Phyllanthus maderaspatensis*	*1–Phyllanthus acidus*	*17–Phyllanthus rotundifolius*
9–Phyllanthus muellerianus	*10–Phyllanthus nigericus*	*3–Phyllanthus beillei*	*18 – Phyllanthus sublanatus*
15–Phyllanthus physocarpus	*14–Phyllanthus pentandrus*	*4–Phyllanthus capillaris*	
16–Phyllanthus reticulatus		*6–Phyllanthus fraternus*	
2–Phyllanthus amarus		*11–Phyllanthus niruri*	
		13–Phyllanthus odontadenius	
		8–Phyllanthus mannianus	
		12–Phyllanthus niruroides	
		19–Phyllanthus urinaria	

4.9.2 Principal Component Analysis (PCA)

4.9.2.1: Data matrix of 46 x 19 combined macro-morphological, epidermal and pollen morphological characters

The PCA of the combined characters (macromorphological, epidermal and pollen morphological) are shown in Figure 4.7. Eigen values were calculated for the 46 selected characters (Appendix VII) and the first four component axes accounted for 61% total variation among all the characters. Factor or character loading of the 46 characters is presented in Appendix VI. Based on the principal axes I, II and III, scatter plots in form of 95% prediction eclipse was used to present the results and this permitted the visualization of the degree of affinity among the species. Four groups were recognized from the scatter plot diagram (Figure 4.7).

Group A: OTUs 11 *P. niruri*, 6– *P. fraternus*, 7 – *P. maderaspatensis*, 10 - *P. nigericus* 19 – *P. urinaria,* 13 – *P. odontadenius* all with irregular epidermal cell shape. They all have prolate or subprolate pollen shape and sessile or subsessile leaves with cuneate leaf base except *P. fraternus* and *P. nigericus* with attenuate leaf base.

Group B: OTUs 12 – *P. niruroides* 2 – 9. *P. amarus*, 14 – *P. pentandrus*, 4 – *P. capillaris*, 3 – *P. beillei.* All the species have oblong or obovate leaves with obtuse leaf apex and all sessile or subsessile except *P. pentandrus* with linear/lanceolate leaf shape with acute leaf apex. They all have prolate or subprolate pollen shape.

Group C: OTUs 8 – *P. mannianus*, 16 – *P, reticulatus*, 9 – *P. muellerianus*, 1 – *P. acidus*, 15 – *P. physocarpus*, 5 – *P. floribundus*. All the species have petiole except *P. mannianus* which is subsessile. They all have polygonal or rectangular epidermal cell shape with straight anticlinal wall pattern except *P. acidus, P. physocarpus* and *P. mannianus* with undulate or wavy pattern of anticlinal wall.

Group D: OTUs 17 – *P. rotundifolius*, 18 – *P. sublanatus*. These two species are both sessile with obtuse leaf apex. They both have irregular epidermal cell shape with undulate pattern of anticlinal wall.

4.9.2.2: Data matrix of 27 x 19 leaf epidermal characters:

The principal component analysis of 27 x 19 data matrix produced Eigen values of each principal axis of OTUs as shown in figure 4.8. eigenvalues were calculated for the 27 selected characters (Appendix X) and the first four component axes accounted for about 67% of the total variation among all the characters. Factors or character loading of the 27 characters is presented in Appendix IX. Based on the principal axes 1 and 2, scatter plot in form of 95% prediction eclipse was used to present the results (Figure 4.8). This permitted the visualization of the degree of affinity among the species and four groups were obtained from the plot (Figure 4.8).

Group A: OTUs 5 – *P. floribundus*, 9 - *P. muelllerianus* and 16 – *P. reticulatus*. All the species in this group are hypostomatic with the same type of anisocytic stomata on the abaxial surface. *Phyllanthus muellerianus* and *P. floribundus* have rectangular epidermal cell shape with straight anticlinal wall pattern while *P. reticulatus* has polygonal epidermal cell shape with straight anticlinal wall pattern.

Group B: OTUs 1 – *P. acidus* 3 – *P. beillei*, 8 – *P. mannianus*, 4 – *P. capillaris* and 15 – *P. physocarpus*. All the species in this group have irregular epidermal cell shape with wavy anticlinal wall pattern. *Phyllanthus acidus, P. physocarpus* and *P. capillaris* are all hypostomatic with *P. acidus* and *P. physocarpus* having same type of anisocytic stomata while *P. capillaris* has anomocytic stomata on the abaxial surface respectively.

Group C: OTUs 2 – *P. amarus*, 10 – *P. nigericus*, 6 – *P. fraternus*, 13 – *P. odontadenius*, 18 – *P. sublanatus*, 12 – *P. niruroides* and 11 – *P. niruri*. All the species in this group are amphistomatic with mostly anisocytic stomata except *P. nigericus* and *P. sublanatus* with paracytic and laterocyclic type of stomata respectively.

Group D: OTUs 7 – *P. maderaspatensis*, 14 – *P. pentandrus*, 17 – *P. rotundifolius* and 19 – *P. urinaria*. All the species are amphistomatic with different types of stomata. They all have irregular epidermal cell shape on both surfaces except *P. pentandrus* with polygonal epidermal cell shape on the abaxial surface.

4.9.2.3: Data matrix of 14 x 19 macromorphological characters:

The PCA produced eigenvalues from the selected fourteen (14) macromorphological characters (Appendix axes I, II, and III accounted for 80% of the total variation among the characters selected for factor loading (Appendix XII). Scatter plot presented for the first three principal axes is shown in Figure 4.9. The principal component axes produced eigenvalues of each principal component axis of OTU and four groups were obtained from this PCA:

Group A: OTUs 3 – *P. beillei* 14 – *P. pentandrus.* The PCA of the macromorphological characters identified members of group A as herbaceous which are sessile or subssile in nature with glabrous leaf surface.

Group B: OTUs 4 – *P. capillaris*, 12 – *P. niruroides*, 11 – *P. niruri*, 2 – *P. amarus*, 6 – *P. fraternus* 13 – *P. odontadenius*, 19 – *P. urinaria* and 10 – *P. nigericus*. Members of this group are also all herbaceous and sessile or subssile in nature with glabrous leaf surface.

Group C: OTUs 17 – *P. rotundifolius* 18 – *P. sublanatus,* 16 – *P. reticulatus* and 15 – *P. physocarpus*. Members of this group are all petiolate with the exceptions of *P. rotundifolius* and *P. sublanatus* which are sessile but with the common obtuse and acute leaf apex.

Group D: OTUs 7 – *P. maderaspatensis*, 8 – *P. mannianus*, 1 – *P. acidus*, 9 – *P. muellerianus,* and 5 – *P. floribundus*. Members of group D are petiolate with the exceptions of *P. maderaspatensis* and *P mannianus* which are sessile but with the common obtuse and acute leaf apex.

4.9.2.4: Data matrix of 5 x 19 pollen morphological characters:

The PCA for 5 X 19 data matrix produced eigenvalues of each principal axis of OTUs as shown in Figure 4.10, eigenvalues were calculated for the 5 selected characters (Appendix XV) and the first four component axes accounted for 99% of the total variation among all the characters. Factor or character loading of the 5 characters is presented in Appendix XIV and four groups were obtained from the scatter plot (Figure 4.10).

Group A: OTUs 1 – *P. acidus*, 3 – *P. beilllei*, 8 – *P. mannianus*, 19 – *P. urinaria* and 12 – *P. niruroides*. The PCA of the pollen morphological characters identified OTUs 1 and 3 to have close affinity to each other compared to their closeness to OTUs, 8, 12 and 19 in the same group.

Group B: OTUs 14 – *P. pentandrus*, 7 – *P. maderaspatensis*, 10 – *P. nigericus*, 2 – *P. amarus*, 16 – *P. reticulatus*, 15 – *P. physocarpus*, 5 – *P. floribundus* and 9 – *P.*

muellerianus. OTUs 5, 9 and 15 as well as OTUs 7, 10 and 14 appeared closely related to one another when compared to their closeness to OTUs 16 and 2 respectively in group B

Group C: OTUs 17 – *P. rotundifolius* and 18 – *P. sublanatus*. OTUs 17 and 18 clustered together in group C as observed in another data matrix (Figures 4.7 & 4.10)

Group D: OTUs 11 – *P. niruri*, 6 – *P. fraternus*, 4 – *P. capillaris* and 13 – *P. odontadenius*. OTUs 4, 6 and 11 have close affinity to one another compared to their closeness to OTU 13 (Figure 4.10).

Table 4.10: List of the 46 macro and micro characters from PCA of 46 x 19 data matrix of the genus *Phyllanthus* in Nigeria

PA1	PA2	PA3	PA4
Leaf apex	Leaf shape	Leaf base	Leaf surface
Leaf length/leaf width ratio	Leaf length	Petiole (+/-)	Perianth lobes
Adaxial shape of guardcell	Leaf width	Adaxial cell shape	Abaxial type of cell inclusion
Abaxial shape of guardcell	Petiole length	Adaxial trichome/base	Adaxial cell number
Adaxial type of cell inclusion	Blade length	Abaxial trichome/base	Adaxial cellwall thickness
Adaxial stomata type	Blade length/ Petiole length ratio	Adaxial anticlinal wall pattern	Abaxial cellwall thickness
Abaxial stomata width	Flower colour	Abaxial anticlinal wall pattern	Pollen equitorial diameter
Abaxial stomata length	Fruit colour	Abaxial cell number	Pollen size
Adaxial stomata length	Abaxial cell shape	Cuticular wax	-
Adaxial stomata width	Abaxial stomata density	-	-
Adaxial stomata density	Abaxial stomata index	-	-
Adaxial stomata index	Pollen shape	-	-
Adaxial outer stomata rim	-	-	-
Abaxial outer stomata rim	-	-	-
Pollen polar diameter	-	-	-
Colpi length	-	-	-

Table 4.11: List of the 27 selected leaf epidermal characters from PCA of 27 x 19 data matrix of the genus *Phyllanthus* in Nigeria

PA1	PA2	PA3	PA4
Adaxial shape of guard cell	Adaxial epidermal cell shape	Adaxial trichome/base	Adaxial cell inclusion
Abaxial shape of guard cell	Abaxial epidermal cell shape	Abaxial trichome/base	Abaxial cell inclusion
Adaxial stomata type	Adaxial pattern of anticlinal wall	Abaxial stomata length	Adaxial cell number
Abaxial stomata type	Abaxial pattern of anticlinal wall	Adaxial cellwall thickness	Abaxial cell number
Abaxial stomata width	Abaxial stomata density	Abaxial cellwall thickness	-
Adaxial stomata length	Abaxial stomata index	Cuticular wax	-
Adaxial stomata width	-	-	-
Adaxial stomata density	-	-	-
Adaxial stomata index	-	-	-
Adaxial outer stomata rim	-	-	-
Abaxial outer stomata rim	-	-	-

Table 4.12: List of the 14 selected macro characters from PCA of 14 x 19 data matrix of the genus *Phyllanthus* in Nigeria

PA1	PA2	PA3	PA4
Petiole (+/-)	Leaf shape	-	Leaf length/Leaf width ratio
Leaf length	Leaf surface	-	-
Leaf width	Leaf base	-	-
Petiole length	Leaf apex	-	-
Petiole length	Perianth lobes	-	-
Blade lenth/Petiole length ratio	-	-	-
Flower colour	-	-	-
Fruit colour	-	-	-

Table 4.13: List of the 5 selected pollen characters from PCA of 5 x 19 data matrix of the genus *Phyllanthus* in Nigeria

PA1	PA2	PA3	PA4
Pollen polar diameter	-	-	-
Pollen equitorial diameter	-	-	-
Pollen shape	-	-	-
Pollen size	-	-	-
Colpi length	-	-	-

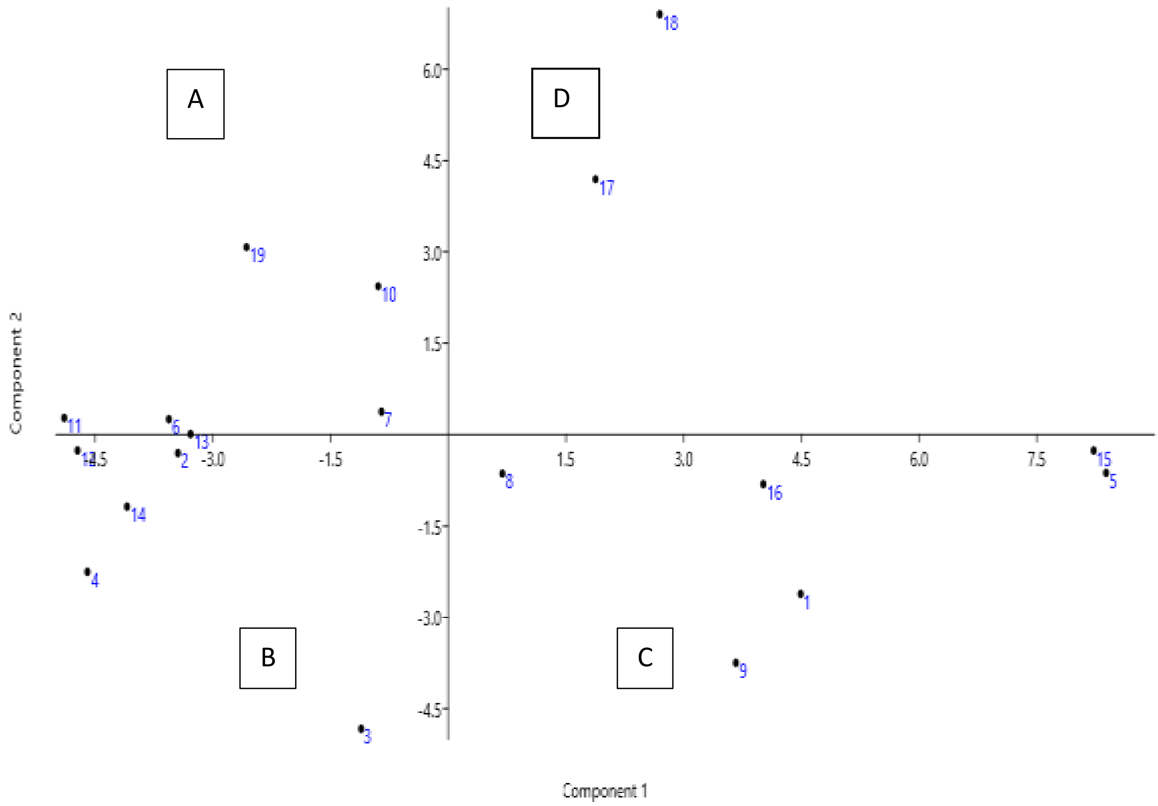


Figure 4.7: Scatter plot of *Phyllanthus* species obtained from principal component axes of 46 x 19 data matrix (macromorphological, epidermal and pollen morphological)

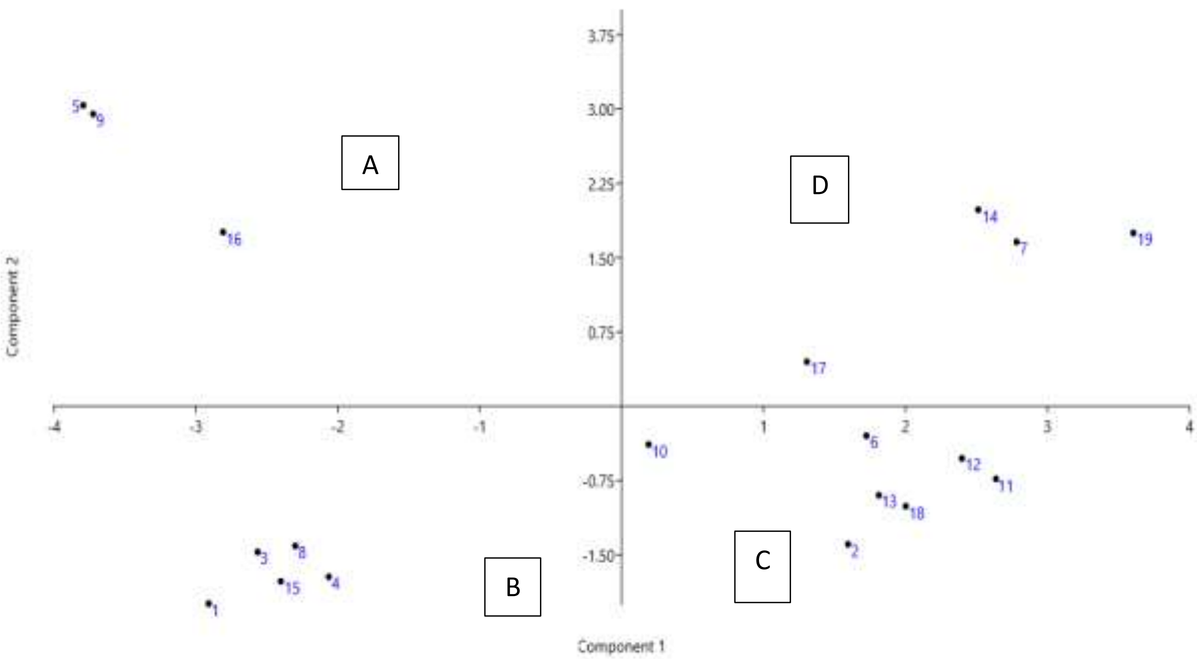


Figure 4.8: Scatter plot of *Phyllanthus* species obtained from principal component axes of 27 x 19 data matrix (epidermal)

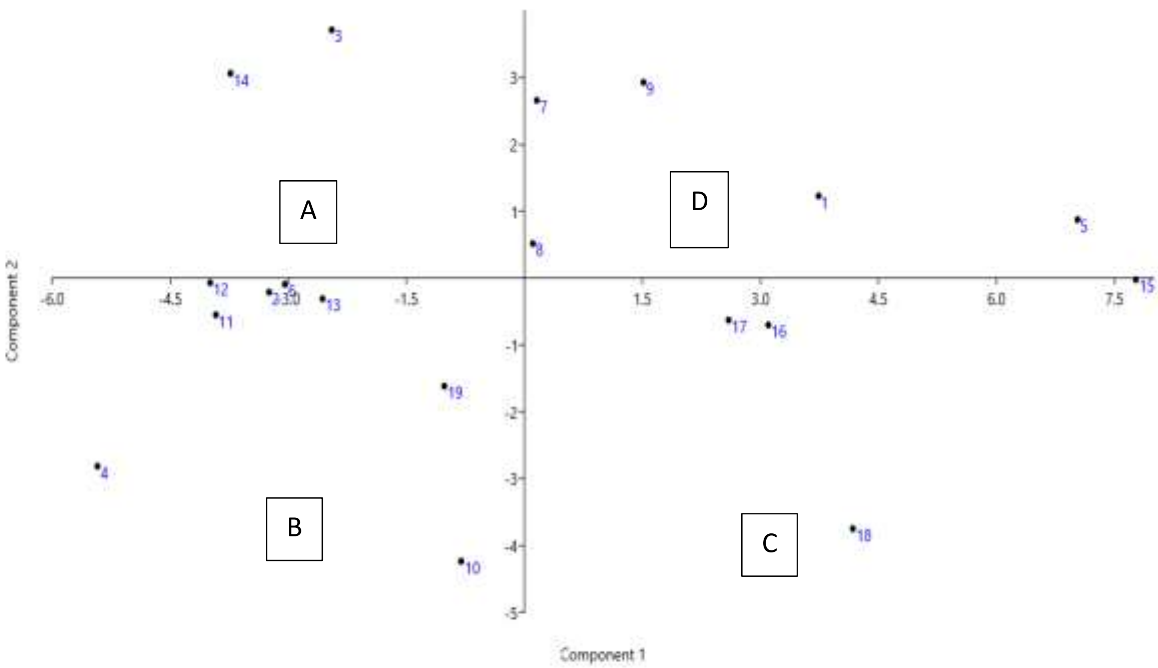


Figure 4.9: Scatter plot of *Phyllanthus* species obtained from principal component axes of 14 x 19 data matrix (macromorphological)

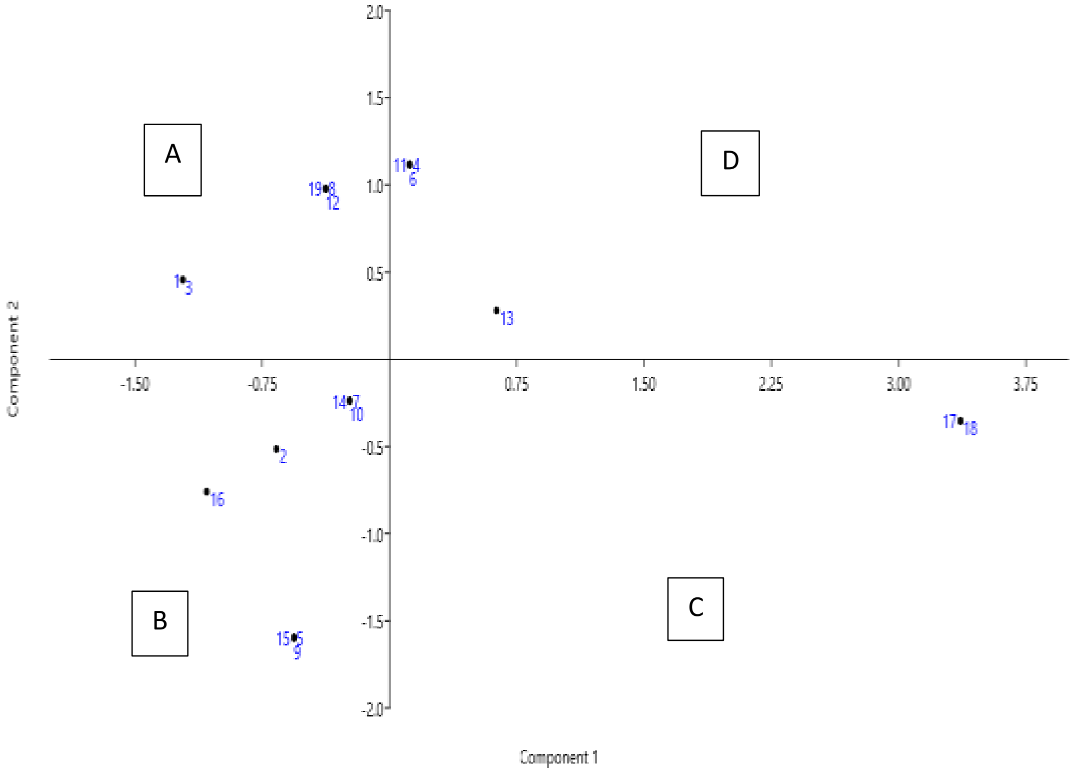


Figure 4.10: Scatter plot of *Phyllanthus* species obtained from principal component axes of 5 x 19 data matrix (pollen morphological)

4.9.3 Comparison of the features of synonymous species

Three herbarium specimens found to have been identified as *P. fraternus, P. floribundus* and *P. physocarpus* are cases of misidentification; they are supposed species of *P. amarus, P. muellerianus* and *P. acidus* respectively. These three species (*P. fraternus*, *P. floribundus* and *P. physocarpus)* share common features (Tables 4.14-4.16, Plates 4.47-4.49) in all the macro and micro characters considered for all the species studied with *P. amarus, P. muellerianus* and *P. acidus* respectively.

Table 4.14 shows the comparison of the common characters exhibited by *P. acidus* and *P. physocarpus,* table 4.15 shows that of *P. amarus* and *P. fraternus* while table 4.16 shows the comparison of the features between *P. muellerianus* and *P. floribundus.*

Table 4.14: Comparison of selected characters of *Phyllanthus acidus* and *P. physocarpus*

Characters	***Phyllanthus acidus***	***Phyllanthus physocarpus***
Leaf shape	Lanceolate/Ovate	Lanceolate/Ovate
Leaf apex	Acute	Acute
Leaf base	Attenuate	Attenuate
Leaf surface	Glabrous	Glabrous
Leaf length/Leaf width ratio	2:1	2:1
Blade length/Petiole length ratio	18:1	25:1
Crystal sand	Present	Present
Stomata index on abaxial surface	17.69%	26.20%
Stomata type	Anisocytic	Anisocytic

Table 4.15: Comparison of selected characters of *Phyllanthus amarus* and *P. fraternus*

Characters	***Phyllanthus amarus***	***Phyllanthus fraternus***
Leaf shape	Oblong	Oblong
Leaf apex	Obtuse	Obtuse
Leaf base	Attenuate	Attenuate
Leaf surface	Glabrous	Glabrous
Fruit colur	Green	Green
Leaf length/Leaf width ratio	2:1	3:1
Petiole	Sessile	Subsessile
Crystal sand	Present	Present
Stomata index on adaxial surface	30.66%	18.44%
Stomata index on abaxial surface	13.67%	60.19%
Stomata type	Anisocytic	Anisocytic

Table 4.16: Comparison of selected characters of *Phyllanthus muellerianus* and *P. floribundus*

Characters	***Phyllanthus muellerianus***	***Phyllanthus floribundus***
Leaf shape	Lanceolate/Ovate	Lanceolate/Ovate
Leaf apex	Acute	Acute
Leaf base	Attenuate	Attenuate
Leaf surface	Glossy/Glabrous	Glabrous
Stipular spines	Present	Present
Leaf length/Leaf width ratio	2:1	2:1
Petiole	Present	Present
Blade length/Petiole length ratio	15:1	16:1
Crystal sand	Present	Present
Stomata index on abaxial surface	44.37%	27.18%
Stomata type	Anisocytic	Anisocytic

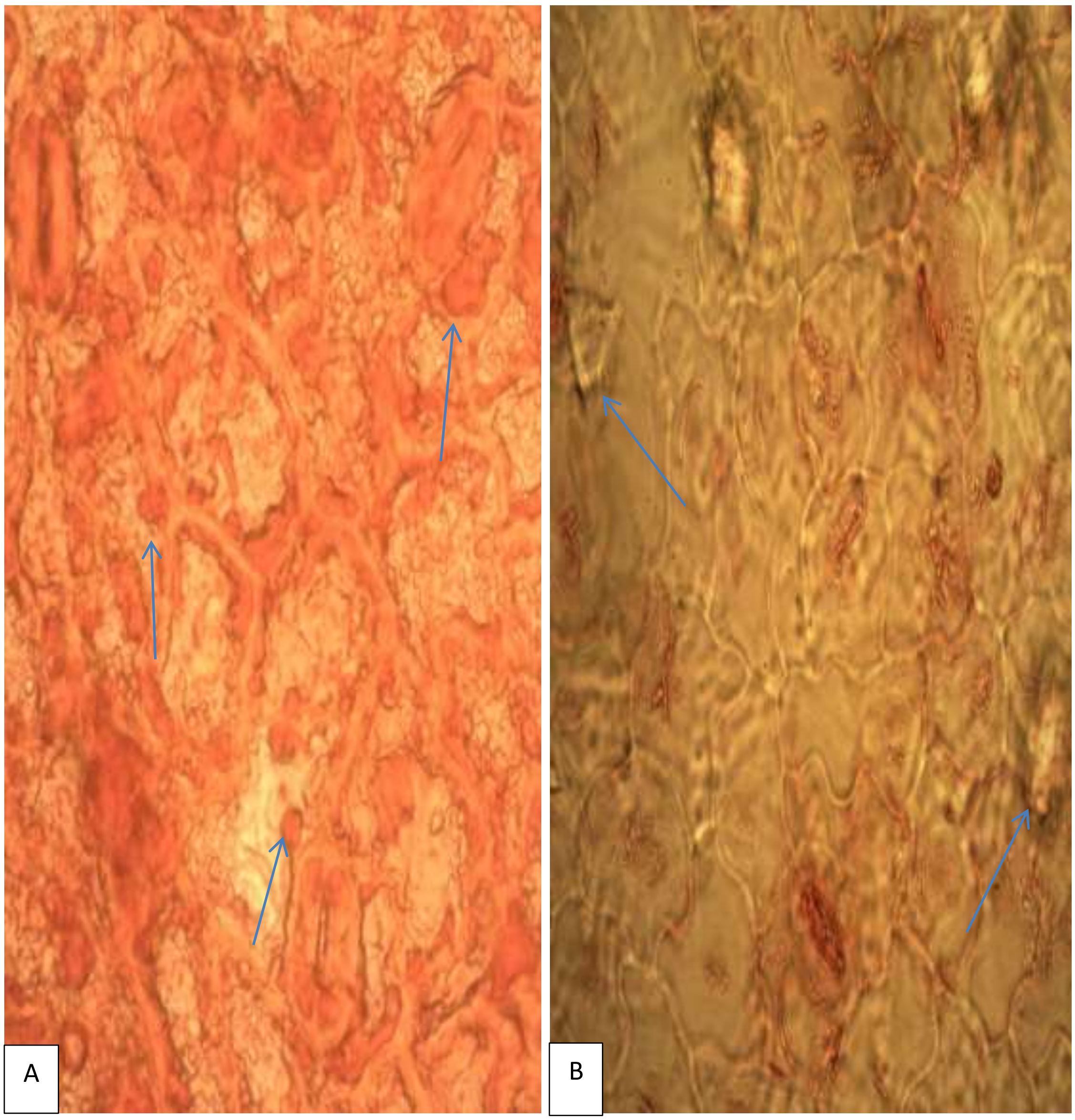


Plate 4.47: Photomicrographs of the epidermal layers of leaves of *P. acidus* and *P. physocarpus*

A. Abaxial surface of *P. acidus* showing crystals (arrowed) (x100
B. Abaxial surface of *P. physocarpus* showing crystals (arowed)(x100)

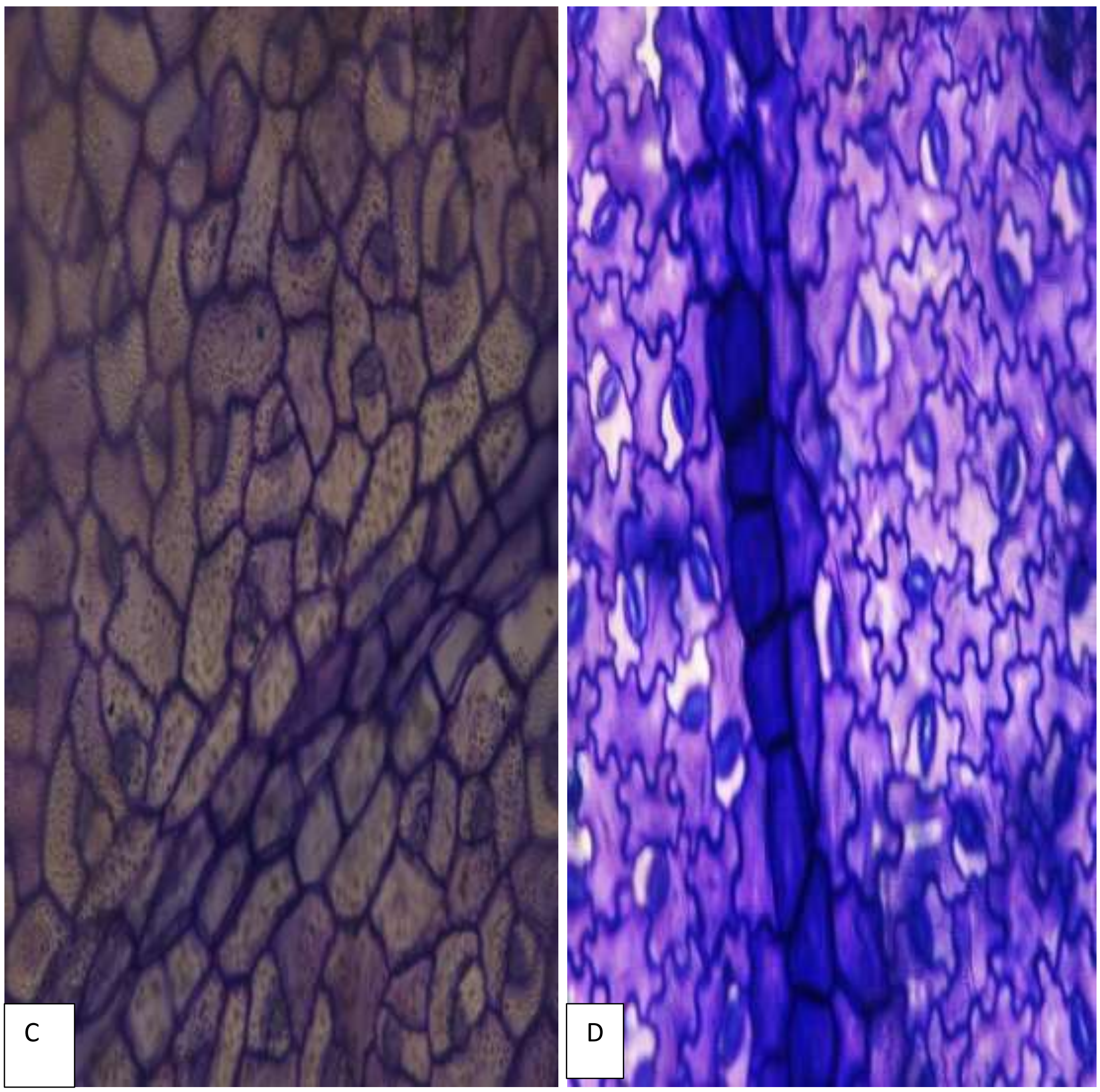


Plate 4.48: Photomicrographs of the epidermal layers of leaves of *P. amarus* and *P. fraternus*

C. Abaxial surface of *P*. *amarus* showing densely distributed stomata (x40)
D. Abaxial surface of *P*. *fraternus* showing dense stomata (x40)

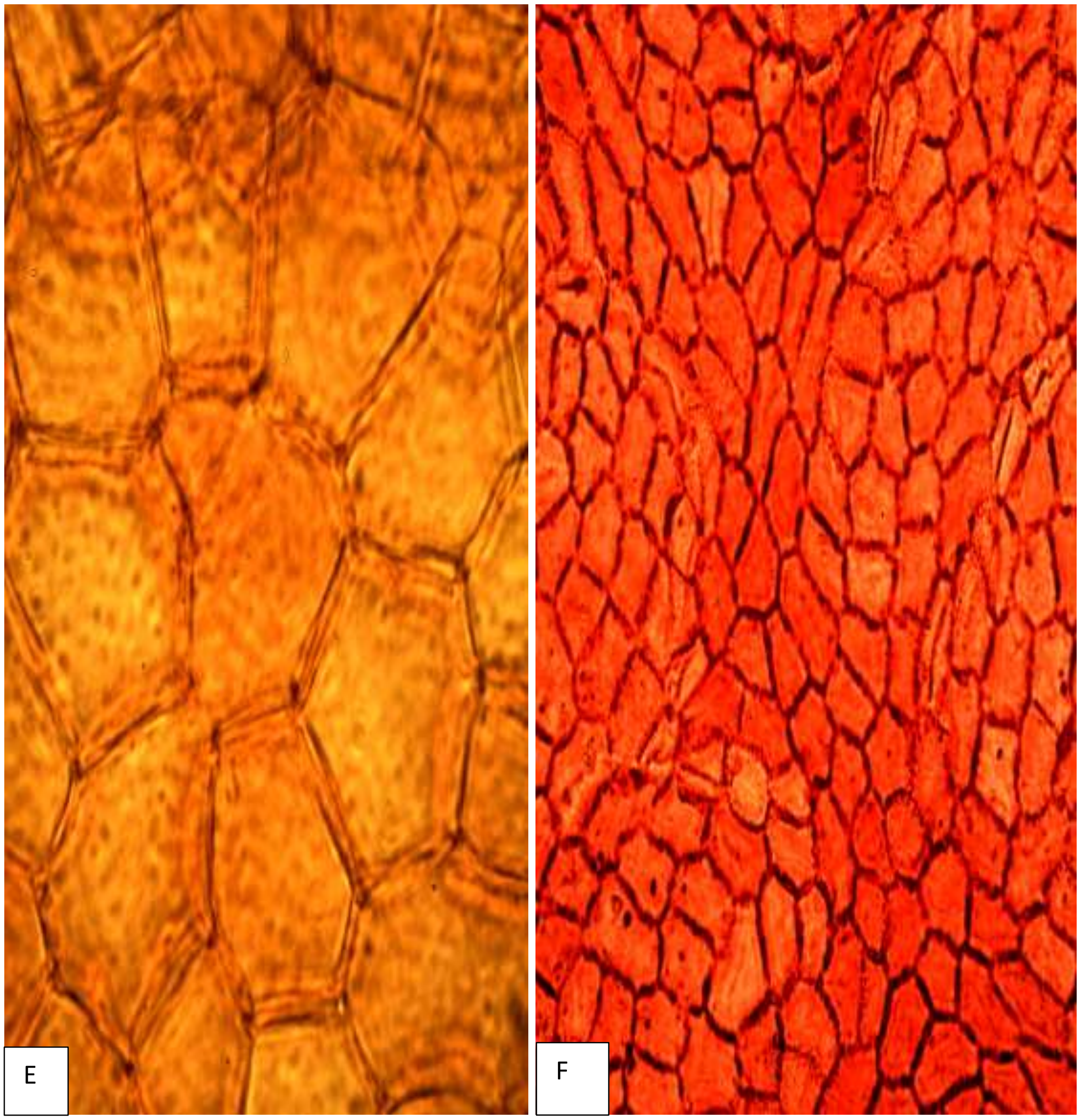


Plate 4.49: Photomicrographs of the epidermal layers of leaves of *P. muellerianus* and *P. floribundus*

E. Adaxial surface of *P. muellerianus* showing epidermal cells with straight anticlinal wall and no stoma (x400)

F. Adaxial surface of *P. floribundus* showing the epidermal cells with straight anticlinal wall and no stoma (x100)

4.10 Systematic Descriptions

Phyllanthus Linn. — F.T.A. 6, 1: 692; Pax & K. Hoffm. in E. & P. Pflanzenfam. 19C: 60 (1931).

Description: Herbs, woody herbs, shrubs and small trees. Leaves are small, simple, distichous, stipules narrow, alternate in arrangement and generally entire. Leaf shape oblong, elliptic, lanceolate, obovate, ovate to suborbicular. Leaves are generally green in colour and glabrous, leaf base either cuneate or attenuate while leaf apices are acute, obtuse, mucronate or shortly acute. Size 0.45 cm x 0.2 cm to 11.7 cm x 5.4 cm; mostly sessile or subsessile; petiole length when present between 0.24 cm-0.45 cm. Flowers very small, monoecious, in axillary clusters or solitary; flower colour green, greenish white, yellowish white to pale yellow. Perianth lobes ranged between 4-6; fruits a capsule and colour mostly green, sometimes red, reddish brown to black in some.

Leaf epidermal cell shape irregular or polygonal, sometimes rectangular in some species with sinuate, undulate/wavy or straight anticlinal wall pattern. Cell number per field of view 21 to 286 on adaxial, 22 to 170 on the abaxial surface. Cell wall thickness 1.28 µm to 4.45 µm on adaxial, 1.43 µm to 3.08 µm on abaxial. Stomata amphistomatic or hypostomatic, anisocytic, anomocytic, paracytic and laterocyclic stomata type; stomata number 6 to 71 on adaxial, 15 to 124 on abaxial; stomata index 2.35% to 70.82% on adaxial, 10.03% to 77.43% on abaxial. Pollen tricolporate; prolate, subprolate to oblate-spheroidal in shape with fine reticulate exine pattern; size small to medium; 12.4µm x13.0µm to 31.5µm x 23.25µm.

Distribution: *Phyllanthus* species widely distributed in the tropics all over the world and are found in all the geographical zones in Nigeria.

Ecology: Sudan and guinea savanna, lowland rainforest,mangrove forest.

Comment: Sixteen species in three subgenera are herein reported for Nigeria.

4.10.1 Bracketed key to the subgenera of *Phyllanthus* in Nigeria

1. Habit: Shrubs or small trees…………………………………………*Kirganelia*
1. Habit: Herbs or woody herbs…………………………………………2

2. Herbs or woody herbs with phyllanthoid branching…………………………………………………………*Phyllanthus*
2. Herbs or woody herbs with no phyllanthoid branching……………….*Isocladus*

4.10.2 Bracketed key to the subgenus *Kirganelia* based on macromorphological characters

1. Habit: Shrubs or small trees……………………………………...2
1. Habit: Herbs or woody herbs……………………………………….*P. pentandrus*

2. Stipular spines absent……………………….……………………3
2. Stipular spines present……………………………………………..*P. muellerianus*

3. Leaf shape lanceolate with attenuate base, perianth lobes 4…………….*P. acidus*
3. Leaf shape lanceolate with cuneate base, perianth lobes 5…………*P. reticulatus*

Phyllanthus acidus (Linn.) Skeel

Description: Shrub or small tree. Leaves lanceolate to ovate, apex acute, base attenuate, leaves glabrous, petiole length 0.2 cm–0.3 cm; leaf size 3.3 cm to 5.8 cm long; 1.6 cm to 3.0 cm broad; flowers monoecious, pinkish, perianth lobes 4. Fruits develop densely on the branches, fleshy drupe; pale yellow or greenish white.

Epidermal cell shape irregular on adaxial with sinuate or wavy anticlinal wall pattern, polygonal on abaxial with sinuate anticlinal wall pattern; adaxial cells 252 to 316 in number per field of view, abaxial cells 148 to 198 in number per field of view; adaxial cellwall thickness 2.5 µm–4.0 µm, 1.5 µm-3.0 µm on abaxial. Leaf hypostomatic, anisocytic stomata type; sessile multicellular scales randomly and sparsely distributed on the adaxial surface, oil droplets also present. Stomata density per field of view on abaxial 26-32, mean stomata size up to 5.45±0.83 µm long and 2.43±0.67 µm wide; stomata index 17.69%; guard cell shape ellipsoidal.

Distribution: Oyo, Osun

Ecology: Lowland rainforest to guinea savanna

Flowering and fruiting period: Flowers and fruits twice a year, March-May, November-January

Phyllanthus muellerianus (O. Ktze.) Exell in Cat. S. Tomé 290 (1944).

Synonyms: ***Diasperus muellerianus*** O. Ktze. (1891).

Kirganelia floribunda Baill. (1860), not of Spreng. (1826).

Phyllanthus floribundus Müll. Arg. (1863), not of Kunth (1817); F.T.A. 6, 1: 701; F.W.T.A., ed. 1, 1: 290; Chev. Bot. 556; Aubrév.

Description: Shrub; leaf glabrous with the young leaves glossy on the adaxial surface; shape ovate to lanceolate; leaf apex acute; leaf base attenuate; petiole length 0.2 cm to 0.3 cm long; recurved stipular spines present. Flower colour green; perianth lobes 5; fruit colour red when ripe.

Epidermal cells rectangular with straight anticlinal wall pattern on both surfaces; adaxial epidermal cell number 168 to 210 per field of view; abaxial cell number 128 to 147 per field of view; adaxial cellwall thickness 1.5 µm to 4.0 µm; abaxial 1.0 µm to 3.0 µm. Leaf amphistomatic; anisocytic stomata type on the abaxial surface; crystals present on the abaxial surface; stomata density 96 to 128 per field of view on abaxial surface; abaxial stomata size 5 µm to 6 µm x 2 µm to 3 µm; abaxial stomata index 44.37%. Pollen shape oblate-spheroidal; size small; P/E 95.4%; pollen size 11.0 µm to 14.0 µm x 12.0 µm to 14.0 µm.

Distribution: Abuja, Kaduna, Plateau, Kogi, Benue, Kwara, Oyo, Ogun, Osun, Ondo, Lagos, Imo, Abia, Edo, Cross River.

Ecology: Guinea savanna to lowland, mangrove rainforest.

Phyllanthus reticulatus Poir. **var. reticulatus** — F.T.A. 6, 1: 700; Aubrév. Fl. For. Soud.-Guin. 189.

Synonym: ***Phyllanthus prieurianus*** Müll. Arg. — Chev. Bot. 558.

Description: Straggling shrub; leaf shape lanceolate; leaf apex acute; base cuneate; leaf size 2.7 cm to 2.9 x 1.0 cm to 1.1 cm; petiole length 0.2 cm. Flowers in axillary fascicles; colour cream; perianth lobes 5; fruit berry and colour black when ripe.

Epidermal cells rectangular with straight anticlinal wall pattern on adaxial surface; polygonal with straight or undulate anticlinal wall pattern on abaxial surface; elliptic shape of guard cell on abaxial surface. Adaxial epidermal cell number 171 to 192 per field of view; 147 to 172 per field of view on abaxial surface; adaxial cellwall thickness 3.0 µm to 4.0 µm; 1.5 µm to 3.0 µm on abaxial surface. Leaf hypostomatic with anisocytic stomata type on abaxial surface; simple unicellular trichome present on both surfaces; abaxial stomata density 62 to 79 per field of view; stomata size 8 µm to 10 µm x 3 µm to 5 µm; abaxial stomata index 30.75%. Pollen tricolporate with subprolate pollen shape; size small; P/E 117.9%; Pollen size 15.0 µm to 20.0 µm x 12.5 µm to 15 µm.

Distribution: Taraba, Kwara, Ogun, Lagos.

Ecology: Guinea savanna to lowland rainforest.

Flowering and fruiting season: Throughout the year.

Phyllanthus pentandrus Schum. & Thonn. — F.T.A. 6, 1: 710; Chev. Bot. 558.

Synonym: *Phyllanthus reticulatus Poir. var. reticulatus*

Description: Herb; leaf shape linear to lanceolate; leaf apex acute to acuminate; base cuneate; leaf size 0.7 cm to 1.9 cm x 0.1 cm to 0.6 cm; subsessile. Flower colour white; perianth lobes 5; fruit colour green.

Epidermal cells polygonal with undulate anticlinal wall pattern on adaxial surface; rectangular or polyonal with straight anticlinal wall pattern on the abaxial surface; elliptic shape of guard cell; adaxial epidermal cell number 50 to 65 per field of view; 17 to 28 per field of view on abaxial surface; adaxial cellwall thickness 1.0 µm to 2.0 µm; 1.5 µm to 3.0 µm on abaxial surface. Leaf amphistomatic with anisocytic stomata type on both surfaces; adaxial stomata density 6 to 23 per field of view; 40 to 59 per field of view on the abaxial surface; adaxial stomata size 7 µm to 8 µm x 4 µm to 7 µm; 8µm to 10µm x 5µm to 7µm on abaxial surface; adaxial stomata index 20.62%; abaxial stomata index 68.76%. Pollen shape subprolate, size small; P/E 131.5%; pollen size 22.5 µm to 25.0 µm x 17.5 µm to 20.0 µm.

Distribution: Sokoto, Katsina, Niger, Plateau, Kogi, Oyo, Ondo, Lagos, Enugu, Anambra.

Ecology: All zones in Nigeria.

4.10.3 Bracketed key to the subgenus *Phyllanthus* based on macromorphological and epidermal characters

1. Leaves subsessile………………………………………...2
1. Leaves sessile…………………………………………..3

2. Perianth lobes 5…………………………………………...*P. amarus*
2. Perianth lobes 6…………………………………………...4

3. Leaf shape obovate…………………………………..   *P. capillaris*
3. Leaf shape variable………………………………………..5

4. Epidermal cells irregular with no trichome………………...*P. odontadenius*
4. Epidermal cells irregular with trichomes…………………….6

5. Leaf apex shortly acute………………………………………*P. mannianus*
5. Leaf apex acuminate………………………………………. *P. urinaria*

6. Crystal sand absent…………………………………………...*P. beillei*
6. Crystal sand present………………………………………… 7

7. Stomata type same on both surfaces…………………………*P. rotundifolius*
7. Stomata type variable on both surfaces…………………….. 8

8. Stomata Laterocyclic…………………………………………..9
8. Stomata Paracytic…………………………………………… *P. nigericus*

9. Leaf amphistomatic with laterocyclic stomata……………… *P. sublanatus*
9. Leaf amphistomatic with anisocytic stomata…………………10

10. Shape of guard cell Suborbiculate……………………………*P. niruri*
10. Shape of guard cell Elliptic……………………………………*P. niruroides*

Phyllanthus amarus Schum. & Thonn. — F.T.A. 6, 1: 717; Chev. Bot. 556.

Synonyms: *Phyllanthus niruri* Linn.
Phyllanthus fraternus G.L. Webster

Description: Slender herb, leaves oblong, leaf apex obtuse, base attenuate, surface glabrous, sessile. Leaf 0.3 cm-2.2 cm long; 0.2 cm-0.5 cm broad; flowers axillary, male flowers yellowish white, female flowers pale reen in colour, perianth lobes 5. Fruit capsule and green in colour.

Epidermal cell shape polygonal or irregular with sinuate anticlinal wall pattern on adaxial, polygonal with deeply sinuate anticlinal wall pattern on abaxial; shape of guard cell suborbiculate, cell number 48 to 126 per field of view on adaxial, 108 to 172 per field of view on abaxial surface; adaxial cellwall thickness 1.5 µm-4.0 µm, 1.0 µm-3.0 µm on abaxial. Leaf amphistomatic, anisocytic stomata type on both surfaces, prismatic and styloid crystal sand present on both sufaces. Stomata density per field of view on adaxial 19-65, abaxial 12-32, mean stomata size up to 7.80±0.70 µm long and 5.65±0.67 µm wide on adaxial; 7.30±0.92 µm long and 5.20±0.70 µm wide on abaxial; adaxial stomata index 13.03%, 24.47% on abaxial. Pollen tricolporate, shape subprolate; size small, P/E percentage 123.9%, pollen size 17.5 µm to 22.5 µm x 15.0 µm to 17.5 µm.

Distribution: Most commonly distributed all over Nigeria.

Ecology: Found in all ecological zones

Flowering and fruiting period: Throughout the year

Phyllanthus beillei Hutch. in F.T.A. 6, 1: 733 (1912).

Synonym: *Phyllanthus kerstingii* Brunel

Description: Woody herb, leaves glabrous and oblong or obovate, apex obtuse, base cuneate. Subsessile; leaf size 1.6 cm-3.4 cm long; 0.8 cm-1.2 cm broad; flower colour yellowish white; perianth lobes 6; fruit colour green.

Epidermal cells irregular with wavy anticlinal wall pattern on both surfaces; elliptic guard cell shape; adaxial cell number 165-212 per field of view, 124-162 per field of view on abaxial; adaxial cellwall thickness 1.0 µm to 5.0 µm; abaxial 2.0 µm to 3.0 µm; Leaf amphistomatic with laterocyclic stomata type on both surfaces; simple unicellular trichome present on the adaxial surface; adaxial stomata density 21 to 34 per field of view; abaxial 36 to 61 per field of view; adaxial stomata size 8 µm to 12 µm x 4 µm to 6 µm; abaxial 6 µm to 9 µm x 3 µm to 6 µm; adaxial stomata index 13.03%; abaxial 24.47%.

Distribution: Bauchi, Kaduna, Plateau, Oyo, Ondo.

Ecology: Guinea savanna to lowland rainforest

Flowering period: Not known

Phyllanthus capillaris Schum. & Thonn. — F.T.A. 6, 1: 709; Chev. Bot. 556.

Synonym: *Phyllanthus nummulariifolius* var. capillaris (Schumach. & Thonn.) Radcl.-Sm.

Description: Slender herb, leaves shape obovate; apex mucronate or obtuse, base cuneate; sessile; leaf size 0.5 cm to 2.2 cm long; 0.4 cm to 1.3 cm broad; flower colour white; perianth lobes 5; fruit colour green.

Epidermal cells polygonal on adaxial; irregular on abaxial with sinuate anticlinal wall pattern on both surfaces; elliptic guard cell shape on abaxial surface; adaxial cell number 186 to 230 per field of view; abaxial 94 to 132 per field of view; adaxial cellwall thickness 3.0 µm to 7.0 µm; abaxial 2.0 µm to 3.0 µm. Leaf hypostomatic with anomocytic stomata type on abaxial surface; crystal druses on abaxial surface;stomata density 17 to 44 per field of view on the abaxial surface; abaxial stomata size 7 µm to 10 µm x 4 µm to 6 µm; 19.58% stomata index on abaxial surface. Pollen tricolporate; pollen shape prolate; size medium; P/E 135.5%; pollen size 25.0 µm to 35.0 µm x 20.0 µm to 25.0 µm.

Distribution: Taraba, Adamawa, Plateau, Osun, Ekiti, Ondo, Abia, Cross River.

Ecology: Guinea savanna to lowland rainforest.

Phyllanthus mannianus Müll. Arg. — F.T.A. 6, 1: 730; Chev. Bot. 557.
Synonyms: Phyllanthus bancilhonae Brunel & J.P. Roux
Phyllanthus gagnioevae Brunel & J.P. Roux

Description: Herb; Leaf shape obovate or lanceolate; leaf apex obtuse or shortly acute; leaf base cuneate; leaf size 0.9 cm to 2.2 cm long; 0.6 cm to 1.2 cm broad; subsessile. Flower colour greenish white.

Epidermal cells polygonal or irregular on adaxial surface with undulate or wavy anticlinal wall pattern; polygonal cells with deeply sinuate anticlinal wall pattern on the abaxial surface; adaxial epidermal cell number 148 to 172 per field of view; 36 to 48 per field of view on abaxial surface; adaxial cellwall thickness 3.0 µm to 5.0 µm; 2.0 µm to 3.0 µm on abaxial. Leaf amphistomatic; adaxial stomata type anisocytic; anomocytic stomata type on abaxial surface; adaxial stomata density 8 to 16 per field of view; abaxial 53 to 68 per field of view; adaxial stomata size 7.65 µm x 5.75 µm; 9.95 µm x 5.75 µm on abaxial surface; stomata index 7.08% on adaxial; abaxial 58.33%.

Distribution: Taraba, Plateau, Cross River

Ecology: Guinea savanna to lowland rainforest

Phyllanthus nigericus Brenan in Kew Bull. 1950; 215.

Description: Herb; leaf shape elliptic; leaf apex obtuse; base attenuate; leaf size 0.8 cm to 1.3 cm x 0.5 cm to 0.7 cm; sessile. Flower colour greenish white; perianth lobes 5.

Epidermal cells irregular with wavy anticlinal wall pattern on both surfaces; suborbiculate shape of guard cell; adaxial number of epidermal cells per field of view 250 to 272; abaxial 44 to 58 per field of view; cellwall thickness 1.0 µm to 2.0 µm on both surfaces. Leaf amphistomatic; paracytic stomata type on both surfaces; adaxial stomata number 4 to 10 per field of view; abaxial number 92 to 110 per field of view; adaxial stomata size 6 µm to 7 µm x 3 µm to 5 µm; abaxial 8 µm to 10 µm x 5 µm to 6 µm; adaxial stomata index 2.35%; 66..53% on the abaxial surface.

Distribution: Plateau, Ondo, Enugu, Cross River.

Ecology: Guinea savanna to lowland rainforest.

Phyllanthus niruri Linn. — F.T.A. 6, 1: 731.

Synonyms: *Phyllanthus fraternus* G.L. Webster
Phyllanthus amarus Schumach. & Thonn.

Description: Herb,; leaf shape oblong or elliptic; leaf apex obtuse or shortly acute; base cuneate; sessile; leaf size 0.4 cm to 1.8 cm x 0.2 cm to 0.8 cm. Flower minute; axillary and unisexual; flower colour yellowish white; perianth lobes 6; fruit colour green.

Epidermal cells irregular with undulate anticlinal wall pattern on both surfaces; shape of guard cell suborbiculate; adaxial epidermal cell number 25 to 44 per field of view; 31 to 44 per field of view on the abaxial; cellwall thickness 1.0 µm to 3.0 µm on both surfaces. Leaf amphistomatic with anisocytic stomata type on both surfaces; styloid crystals present on main vein on the abaxial surface; adaxial stomata density 22 to 35 per field of view; 28 to 47 per field of view on the abaxial surface; adaxial stomata size 9 µm to 11 µm x 5 µm to 6 µm; stomata size 8 µm to 10 µm x 5 µm to 6 µm on abaxial; adaxial stomata index 43.47%; abaxial 50.39%. Pollen shape prolate; size medium; P/E 138.2%; pollen size 25.0 µm to 37.5 µm x 17.5 µm to 27.5 µm.

Distribution: Taraba, Niger, Kaduna, Plataeu, kogi, Benue, Kwara, Oyo, Ogun, Osun, Ondo, Lagos, Imo, Abia, Edo, Cross River.

Ecology: Guinea savanna, lowland, mangrove rainforest.

Phyllanthus niruroides Müll. Arg. — F.T.A. 6, 1: 715; Chev. Bot. 558.

Synonym: *Phyllanthus taylorianus* Brunel ex Radcl.-Sm.

Description: Herb; leaf shape oblong; leaf apex obtuse; base attenuate; leaf size 0.7 cm to 1.2 cm x 0.3 cm to 0.4 cm; sessile. Flower colour white; perianth lobes 5; fruit colour green.

Epidermal cells polygonal or irregular with sinuate or undulate anticlinal wall pattern on either surface; elliptic shape of guard cell; adaxial epidermal cells 187 to 214 per field of view; 30 to 46 per field of view on the abaxial; adaxial cellwall thickness 1.5 µm to 3.0 µm; 2.0 µm to 4.0 µm on the abaxial. Leaf amphistomatic with anisocytic stomata tpe on both surfaces; adaxial stomata density 4 to 10 per field of view; 106 to 142 per field of view on the abaxial; adaxial stomata size 7 µm to 9 µm x 4 µm to 5 µm; abaxial 7 µm to 10 µm x 3 µm to 6 µm in size; adaxial stomata index 2.97%; 77.42% on the abaxial. Pollen shape prolate, size medium, P/E 145.8%; pollen size 25.0 µm to 27.5 µm x 15.0 µm to 20.0 µm.

Distribution: Kogi, Oyo, Balyesa, Rivers.

Ecology: Guinea savanna to lowland rainforest.

Phyllanthus odontadenius Müll. Arg. — F.T.A. 6, 1: 727.

Synonym: *Phyllanthus gagnioevae* Brunel & J.P. Roux

Description: Herb; Leaf shape oblong; leaf apex mucronate; base cuneate; leaf size 1.1 cm to 2.7 cm x 0.4 cm to 1.3 cm; subsessile. Flower colour greenish white; perianth lobes 6; fruit colour green.

Epidermal cells polygonal on adaxial surface; irregular on abaxial surface with sinuate anticlinal wall pattern on both surfaces; elliptic shape of guard cell; adaxial epidermal cell number 169 to 192 per field of view; 128 to 144 per field of view on abaxial surface; cellwall thickness 2.0 µm to 4.0 µm on both surfaces. Leaf amphistomatic with anisocytic stomata type on both surfaces; crystal sand present on abaxial surface; adaxial stomata density 6 to 15 per field of view; 10 to 22 per field of view on abaxial surface; adaxial stomata size 7 µm to 10 µm x 4 µm to 5 µm; stomata size on abaxial 8 µm to 9 µm x 4 µm to 5 µm; adaxial stomata index 5.21%; 10.03% stomata index on abaxial surface. Pollen shape subprolate; size medium; P/E 127.0%; pollen size 22.5 µm to 32.5 µm x 17.5 µm to 25 µm.

Distribution: Niger, Kogi, Oyo, Osun, Ondo. Abia, Edo, Balyesa, Rivers, Cross River.

Ecology: Guinea savanna to lowland rainforest.

Phyllanthus rotundifolius Klein ex. Willd. — F.T.A. 6, 1: 731.

Synonym: ***Phyllanthus niruri*** **var. genuinus** Beille — Chev. Bot. 557.

Description: Prostrate or slightly ascending herb, leaf shape suborbicular; leaf apex obtuse; base cuneate; sessile. Flowers monoecious; colour pale green; perianth lobes 6; fruit capsule.

Epidermal cells irregular with undulate anticlinal wall pattern on both surfaces; adaxial cell number 10 to 28 per field of view; 24 to 40 per field of view on abaxial surface; adaxial cellwall thickness 1.0 µm to 2.0 µm; 2.0 µm to 3.0 µm on abaxial surface. Leaf amphistomatic with anomocytic stomata type on both surfaces; simple unicellular trichome present on the adaxial surface; crystal druses present on abaxial surface; adaxial stomata density 41 to 58 per field of view; 93 to 115 per field of view on the abaxial surface; adaxial stomata size 7 µm to 8 µm x 5 µm to 6 µm; 8 µm to 10 µm x 5 µm to 6 µm stomata size on abaxial surface; adaxial stomata index 70.82%; abaxial 77.25%.

Distribution: Bauchi, Plateau.

Ecology: Guinea savanna to lowland rain forest.

Flowering and fruiting season: July – September.

Phyllanthus sublanatus Schum. & Thonn. — F.T.A. 6, 1; 715 (excl. spec. Ansell); Brenan l.c. 217.

Description: Herb, leaf shape oblong; leaf apex obtuse; base attenuate; sessile; leaf size 0.4 cm to 0.5 cm x 0.2.

Epidermal cells irregular with undulate anticlinal wall pattern on both surfaces; adaxial epidermal cell number 64 to 90 per field of view; 31 to 50 per field of view on the abaxial surface; cellwall thickness 1.5 µm to 3.0 µm on both surfaces. Leaf amphistomatic with laterocyclic stomata type on both surfaces; adaxial stomata density 52 to 73 per field of view; 120 to 152 per field of view on abaxial surface; adaxial stomata size 8 µm to 11 µm x 5 µm to 6 µm; abaxial 6 µm to 7 µm x 3 µm to 4 µm; adaxial stomata index 45.53%; 77.43% on abaxial surface.

Distribution: Plateau, Kwara, Oyo, Ogun, Ondo, Anambra.

Ecology: Guinea savanna to lowland rainforest.

Phyllanthus urinaria Linn. — F.T.A. 6, 1: 721.

Description: Herb; leaf shape lanceolate; leaf apex acuminate; base cuneate; leaf size 0.7 cm to 1.1 cm x 0.1 cm to 0.2 cm; sessile. Flowers axillary, monoecious, colour greenish white; perianth lobes 6; fruit capsule and reddish brown in colour.

Epidermal cells polygonal with sinuate anticlinal wall pattern on both surfaces; shape of guard cell elliptic; adaxial epidermal cells 40 to 57 per field of view; 28 to 45 per field of view on the abaxial surface; cellwall thickness 1.5 µm to 2.5 µm on both surfaces. Leaf amphistomatic with laterocyclic stomata type on both surfaces; adaxial stomata density 60 to 88 per field of view; 81 to 112 per field of view on abaxial surface; adaxial stomata size 7 µm to 9 µm x 4 µm to 5 µm; abaxial 6 µm to 7 µm x 4 µm to 5 µm; adaxial stomata index 59.22%; abaxial 71.78%. Pollen shape prolate, size medium; P/E 139.7%; pollen size 22.5 µm to 30.0 µm x 12.5 µm to 20.0 µm.

Distribution: Delta, Abia.

Ecology: Mangrove and lowland rain forest.

Flowering and Fruiting season: July to December.

4.10.4 Subgenus *Isocladus*

Phyllanthus maderaspatensis Linn. — F.T.A. 6, 1: 722; Chev. Bot. 557.

Description: Herb, leaves glabrous; leaf shape lanceolate or obovate; leaf apex acute; leaf base cuneate; leaf size 1.2 cm to 1.6 cm long, 0.4 cm to 0.5 cm broad; sessile. Flower axillary; colour pale yellow; perianth lobes 6; fruit capsule; colour green.

Epidermal cells rectangular on adaxial surface with straight or undulate anticlinal wall pattern, polygonal cells on abaxial surface with undulate or wavy anticlinal wall pattern; shape of guard cell elliptic on both surfaces; adaxial cell number 39 to 59 per field of view; abaxial 23 to 38 cell number per field of view; cellwall thickness 2.0 µm to 5.0 µm on both surfaces. Leaf amphistomatic; anomocytic stomata on adaxial; laterocyclic stomata on abaxial; adaxial stomata density 26 to 42 per field of view; 41 to 58 per field of view on abaxial surface; adaxial mean stomata size 9.75 µm x 5.80 µm; 9.85 µm x 5.80 µm on abaxial; adaxial stomata index 40.07%; 61.67% stomata index on abaxial surface. Pollen shape subprolate; size small; P/E 126%; size 20.0 µm to 27.5 µm x 12.5 µm to 22.0 µm.

Distribution: Sokoto, Bornu.

Ecology: Sudan sahel

Flowering and fruiting season: Throughout the year

4.10.5 Bracketed key to the species of genus *Phyllanthus* in Nigeria

1. Habit: Shrubs or small trees………………………………..2
1. Habit: Herbs or woody herbs………………………………4

2. Shrubs or small trees with stipular spines…………………..*P. muellerianus*
2.Shrubs or small trees without stipular spines…………………3

3. Leaf shape lanceolate with attenuate base……………………*P. acidus*
3.Leaf shape lanceolate with cuneate base……………………...*P. reticulatus*

4. Leaves subsessille……………………………………….....5
4.Leaves sessile……………………………………..……..6

5. Perianth lobes 5…………………………………………...*P. pentandrus*
5.Perianth lobes 6…………………………………………...7

6. Leaf shape suborbicular…………………………………..*P. rotundifolius*
6.Leaf shape variable………………………… ……………..8

7. Epidermal cells irregular……………………………………..*P. niruri*
7.Epidermal cells polygonal………………………………………9

8. Leaf apex acute……………………………………………*P. maderaspatensis*
8.Leaf apex acuminate…………………………………… .*P. urinaria*

9. Crystal sand present……………………………………...*P. amarus*
9.Crystal sand absent………………………………………… ….10

10. Stomata type same on both surfaces……………………………11
10.Stomata type variable on both surfaces………….…………....12

11. Stomata Laterocyclic…………………………………………..13
11.Stomata Paracytic…………………………….………………*P. nigericus*

12. Leaf amphistomatic……………………………………........14
12. Leaf hypostomatic……………………………….………………..*P. capillaris*

13. Shape of guard cell suborbiculate……………………………..*P. sublanatus*
13. Shape of guard cell elliptic……………………………………*P. beillei*

14. Anisocytic stomata on both surfaces………………………….15
14. Anisocytic stomata on adaxial surface only…………………..*P. mannianus*

15. Pollen shape class prolate……………………………………...*P. niruroides*
15. Pollen shape class subprolate………………………………*P. odontadenius*

CHAPTER FIVE

5.0 DISCUSSION

Phyllanthus is the largest genus of all the genera in the family Phyllanthaceae. The species in the genus are widely distributed in Nigeria with the herbaceous members generating a great deal of confusion among scientists regarding their identification; in many cases, misidentification of the taxa makes evaluation of the published information difficult (Rao *et al*., 1999). Twenty-one species were recognized for this genus by Hutchinson and Dalziel (1954) and Hoffmann *et al*. (2006). Out of these 21 species, only one species was not identified to the species level and four other species (*P. dusenii*, *P. alpestris, P. petraeus* and *P. profusus*) were neither found in herbaria visited nor from the field study to occur in Nigeria. Consequently, nineteen species were studied based on gross macromorphology, epidermal and pollen characters as well as molecular phylogeny of the available field samples of some of the species. Ten species were found to be abundant from the field study while the remaining taxa were only represented by herbarium specimens. The most commonly distributed species in Nigeria is *P. amarus* occurring in the far northern to the southern States closely followed by *P. pentandrus* while the species collected from the southern States only are *P. acidus, P. physocarpus* and *P. urinaria*. In contrast, *P. maderaspatensis* and *P. mannianus* are restricted to a few northern states. Most of the species under study occur in the Guinea savanna, Lowland rainforest and the Mangrove forest with *P. amarus* occurring in all ecological zones, hence has the widest ecological distributional range while the species with narrow distributional ranges are *P. maderaspatensis* (confined to the Sudan savanna) and *P. urinaria* (restricted to the mangrove forest). That *P. amarus* was encountered in all ecological zones in the study was corroborated by the work of Webster (1986) which reported the species (*P. amarus*) among other species studied as a ubiquitous pantropical weed. In the present work, three herbarium specimens found to have been identified as *P. fraternus, P. floribundus* and *P. physocarpus* are cases of misidentification; they are supposed species of *P. amarus, P. muellerianus* and *P. acidus* respectively. These three species (*P. fraternus, P. floribundus* and *P. physocarpus*) were not listed in the flora (Hutchinson and Dalziel, 1954) but were found represented by single specimen and documented in three different herbaria as part of the collections for Nigeria. The present study did not also document them from the field in Nigeria.

The qualitative macromorphological characters of *Phyllanthus* species show variability within the same species and among species in the genus. While some of these characters are of great taxonomic importance especially in the identification of the species and delimiting species boundaries, some are not useful taxonomically. Variation observed in the leaf shape within the same species in *P. acidus, P. beillei, P. floribundus, P. maderaspatensis, P. mannianus, P. muellerianus* and *P. pentandrus* made the character of little or no diagnostic importance. However the peculiar suborbicular leaf shape of *P. rotundifolius* can be employed as diagnostic character for this species being the only one among all the species studied with this characteristic leaf shape. All the herbaceous species of the genus are sessile or subsessile in nature while other species that are shrubs or small trees have petiole with characteristic acute leaf apex; this character is stable and of high taxonomic importance hence can be employed in separating the species into two distinct groups. Among all the species that are shrubs, P. *muellerianus* and *P. floribundus* possess the characteristic recurved stipular spines at the nodal points which is a diagnostic feature that can be used to separate these species from others, as opined by Awomukwu *et al*. (2014) in which the same character was reported as a diagnostic feature when *P. muellerianus* was compared with four other *Phyllanthus* species in Southern Nigeria.

Flower colour is variable among the species studied and may not be of any useful taxonomic importance, however the fruit colour for most of the species is green except *P. acidus, P. muellerianus, P. reticulatus and P. urinaria* with pale yellow, red, black and reddish brown colours respectively. This can serve as a useful diagnostic floral feature that can be combined with other characters in distinguishing these species from others.

The quantitative macromorphological characters of the taxa in *Phyllanthus* may not be useful taxonomically because of the considerable variation and overlapping characters observed among the species. However the smallest leaf length and leaf width observed in *P. sublanatus* is a diagnostic feature for the species while the characteristic leaf length/width ratio of 1:1 observed in *P. rotundifolius* due to the orbicular leaf shape is a stable quantitative character of taxonomic value that can readily separate *P. rotundifolius* from other species. Highest blade/petiole length ratio of 25:1 obtained in *P. physocarpus* and closely followed by 18:1 in *P. acidus* is a diagnostic feature that can be used in distinguishing these species from other taxa among the petiolate ones in the genus.

Importance of leaf epidermal characters cannot be underestimated in this study. Variations in the shape of epidermal cells, types and arrangement of stomata, anticlinal wall pattern, cell wall thickness, stomata index as well as the presence of cell inclusions have been reported as useful taxonomic features to distinguish taxa by many authors (Gill and Keratela, 1982; Stace, 1984; Edeoga, 1991; Ogundipe and Wujek, 2004, Kadiri *et al*., 2005; Olowokudejo and Ayodele, 2007; Shokefun *et al*., 2014; Uka *et al*., 2014). Epidermal cell shapes are irregular or polygonal on both surfaces in most of the taxa with straight, undulate, sinuate or wavy anticlinal wall pattern except *P. floribundus* and *P. muellerianus* that are rectangular on both surfaces while *P. reticulatus* and *P. maderaspatensis* are only rectangular on the adaxial surface. This is regarded as an important taxonomic feature that can be used in delimiting these species from other taxa. The distribution of stomata in the present study revealed marked variation on both the adaxial and abaxial leaf surfaces of the taxa with the abaxial surface having more stomata than the adaxial surface (amphistomatic) except *P. amarus* with more stomata on the adaxial surface when compared to the abaxial surface. However, *P. acidus, P. capillaris, P. floribundus, P. muellerianus, P. physocarpus* and *P. reticulatus* were observed to be hypostomatic having stomata restricted to the abaxial surface; they have anisocytic type of stomata except *P. capillaris* with anomocytic stomata. Thus the type of stomata in *P. capillaris* can be used to separate this taxon from other hypostomatic taxa. The hypostomatic nature of *P. muellerianus* was reported by Uka *et al*. (2014) thus corroborating the observation in the present work. The amphistomatic nature of some of the taxa could be due to adaptation to water loss as suggested by Metcalfe and Chalk, (1950). The present study recognized four types of stomata which are anisocytic, anomocytic, paracytic and laterocyclic with anisocytic type the most common among all the taxa examined.

Presence of simple unicellular trichomes on both the adaxial and abaxial surfaces of *P. reticulatus* as well as the adaxial surface of *P. beillei* and *P. rotundifolius* is of taxonomic importance to separate these three taxa from other species which do not have trichomes on their surfaces. However, laterocyclic type of stomata recorded in *P. beillei* and anomocytic stomata in *P. rotundifolius* could be used to separate these species from *P. retculatus* with anisocytic stomata. Presence of crystal sands and druses in some of the taxa studied such as *P. acidus, P. amarus, P. capillaris, P. floribundus, P. fraternus, P. muellerianus, P. niruri, P. odontadenius, P. physocarpus* and *P. rotundifolius* is a diagnostic feature to separate them from other species, while the sessile multicellular scales recorded on the adaxial surface of *P. acidus* is diagnositic for the species.

The presence of stomatal ledge is of special note in most of the taxa studied while the presence of culticular wax deposits in *P. capillaris, P. fraternus, P. mannianus, P. niruri, P. odontadenius, P. reticulatus* and *P. urinaria* is also of great taxonomic significance which have been found helpful in separating these taxa from other species studied under SEM. These wax deposits are used by plants to prevent water evaporation and control hydration which reflected the type of environment from which these species were collected.

Pollen morphology and evolutionary characteristics in the genus *Phyllanthus* have been extensively studied (Punt, 1962; 1972; 1980; Punt and Rentrop, 1974; Bor 1979; Meewis and Punt, 1983; Lobreau-Callen *et al*., 1988; Webster, 1994). Webster (1956) reported pollen morphology and architectural pattern of the species to be considered the main characteristics useful in *Phyllanthus* taxonomy. He reported that a large number of *Phyllanthus* species showed 3–colporate and reticulate pollen grains which had been reported in the primitive tribes of Phyllanthoideae. Thus these structures were shown to be an important tool in understanding the evolution of *Phyllanthus* species at section and sub-section levels. This led Webster (1956; 1957 & 1958) to propose species of the section *Phyllanthus* as the ancestors of section *Chroretropsis*. In the present study, pollens were reported to be prolate or subprolate in shape except *P. muellerianus*, which was found to be oblate-spheroidal. This could be regarded as a diagnostic feature to distinguish *P. muellerianus* from other taxa studied. Webster (1956; 1957 & 1958) accumulated much phytomorphological, cytotaxonomical (Webster and Ellis, 1962) and other taxonomical data which enabled him to state that some species were primitive and others appeared to be advanced. Hence Punt (1967) reported that pollen morphology might give additional support for the position of Webster's primitive and advanced taxa. He was of the opinion that if their pollen morphology and taxonomy would lead to the same conclusion, that would be a real advance in understanding and delineating the subdivision of the genus. Webster (1956) suggested that there is a relationship between *Phyllanthus* and the presumably primitive genera *Securinega*, *Andrachne* and *Savia* of the same family having found out that some species of *Phyllanthus* e.g *Phyllanthus maderaspatensis* (also documented for Nigeria) which show relationships with *Securinega,* one of these primitive genera. Thus, Punt (1962); Webster (1956); Köhler (1965) logically concluded that the characteristics (tricolporate, distinctly prolate, reticulate and an elongated endoaperture) which the pollen grains of their taxa exhibit are also primitive. In addition, Punt (1967) also recognized that the so called 'primitive' pollen grains of the genus *Phyllanthus* are already 'advanced' in comparison to the other pollen types in the plant kingdom. A number of evolutionary trends within the genus *Phyllanthus* have already been presented by Punt (1967). Due to more recent and extensive investigations, a reconsideration with regard to the evolutionary trends within the *Phyllanthus* was presented by Meewis and Punt (1983). In their study, *P. amarus* type was reported to include only one species *P. pentandrus* with many more species (such as *P. odontadenius* from Africa and *P. fraternus* from other continents) from the subgenus *Phyllanthus*. As the information obtained from the present study on the pollen morphological characters of the *Phyllanthus* species do not only corroborate the existing information on the genus, they can also be used in conjunction with other characters to delimit the species in the genus. In addition, the reported presence of identical pollens can only be resolved when other lines of evidence are examined.

The phylogenetic trees obtained from this study showed the genetic relatedness of the species and these were constructed using the *rbcL* gene region alone together with those retrieved from DNA database. Dendrogram generated based on the DNA information of the *rbcL* gene region grouped the ten *Phyllanthus* species as well as

Magaritaria discoidea and Securinega virosa into six clusters. Group 6 comprised only *M. discoidea* as an outgroup while Group 2 has only *S. virosa* embedded within the *Phyllanthus* species. The separation of *M. discoidea* and *S. virosa* into different groups indicate their distinct relationship with other *Phyllanthus* species. These two species belong to separate genera *Margaritaria and Securinega* under different subtribes *Flueggeinae* and *Securineginae* respectively. This could have formed part of the reasons why *M. discoidea* formerly known as *Phyllanthus discoideus* is now found in the genus *Magaritaria.* Group 5 consists of *P. reticulatus* and *P. muellerianus* which are closely related with a boostrap percentage of 86% and could be regarded as sister taxa. They are shrubby in nature and are distantly related to other herbaceous species (except *S. virosa* and *P. acidus*) hence do not support a monophyletic lineage as taxa that can be traced directly to one node are said to be members of a monophyletic group. Separation of *P. reticulatus* and *P. muellerianus* from other taxa shows their distinctiveness and this agrees with the morphological characteristics of *P. muellerianus* and *P. reticulatus* as documented by Hutchinson and Dalziel (1963). In Group 1, *P. urinaria 1* and *P. urinaria* 2 had the closest affinity with boostrap value of 99%. This suggests that the two are obviously same species but distinctly related to *P. maderaspatensis* which is more evolved. *Phyllanthus niruri* and *P. capillaris* also show close affinity to one another in the same group when compared to *P. odontadenius*. Phylogenetic signals from *rbcL* gene become more apparent when datasets from DNA database were combined. It is assumed that all species evolve from a common ancestor and the branch length indicates the degree of evolution, the two specimens of *P. amarus, P. niruri* and *P. capillaris* had close affinity and they form sister taxa. The combined data provided a more reliable hypothesis of relationships than the gene tree alone. For example, *P. acidus, P. reticulatus* and *P. muellerianus* clustered together in Group 4 and this agrees with the earlier classification in which the three species were classified under same subgenus *Kirganelia* but different sections *Cicca, Anisonema* and *Floribundi* respectively. Also, *P. pentandrus* clustered with *KJ7737421 P. tenellus* in Group 5 very close to Group 4 species and distinctly related. These two species are also classified under the same subgenus *Kirganelia* but a different section *Pentandra.* Sections *Cicca*, *Anisonema*, *Floribundi* and *Pentandra* were classified in the paleotropical subgenus *Kirganelia* by Webster (1957) which was consistent with previous *MatK* and *PHYC* results (Samuel *et al*., 2005) and the analyses in the present study support polyphyly of this subgenus. Additionally, *P. amarus* 1 and 2, *GU441791 P. niruri* and *KF365994 P. amarus* in Group 6 show very close affinity. This corroborates earlier circumscription suggested by previous authors (Webster, 1994; Wurdack *et al*., 2005; Samuel *et al*., 2005; Kathriarachchi *et al*., 2006) for the two species under subgenus *Phyllanthus*, section *Phyllanthus* though different subsections *Swartziani* and *Niruri* respectively. Despite this closeness, they are regarded as distinct species. However, some authors (Bentham, 1880; Hooker, 1887) considered and treated *P. amarus* as a variety of *P. niruri* while in several reports (Elvin-Lewis *et al*., 2002; Khatoon *et al*., 2006; Srirama *et al*., 2010), both species were considered to be synonymous. However, Lee *et al.* (2006) in their investigation of phylogenetic relationships among 18 *Phyllanthus* species based on *nrITS* sequences along with chloroplast ATPB and *rbcL* sequences indicated that the genus is paraphyletic and strongly demarcates between the closely related species *P. ninuri* and *P. amarus*. The higher level of divergence observed among the species in the present study is in conformity with earlier reports in a variety of plant species (Kollipara *et al.,* 1997; Baldwin, 1993; Moller and Kronk, 1997; Awomukwu *et al.,* 2015). The present investigation on the molecular phylogenetic study of *Phyllanthus* species indicates that the DNA authentication using the gene *rbcL* sequence as a marker proposed and used as a potential plant barcode by several researchers (Chase *et al*., 2005; Newmaster *et al*., 2005) is a reliable method to identify and/or discriminate among the species distributed in Nigeria.

The groupings obtained from the clustering analysis conform to some extent with the existing classification of the taxa in the genus by previous authors like Webster, (1956); Levin, (1986) and Kathriarachchi *et al.* (2006). The clustering analysis of 46 x 19 combined data matrix clearly show a separation of the species in the genus into two major clusters by habit splitting all herbaceous species from the shrubs and small trees. Similarly, the 27 x 19 epidermal data matrix splitted the species into clusters that grouped the species by habit except cluster 3 where three herbaceous species *P. capillaris, P. beillei* and *P. mannianus* were clustered with *P. acidus* and *P. physocarpus* though distinctly related. In the two data matrices, two species each (*P. acidus* and *P.*

physocarpus; P. floribundus and *P. muellerianus; P. maderaspatensis* and *P. pentandrus; P. rotundifolius* and *P. sublanatus; P. odontadenius* and *P. niruri; P. amarus* and *P. fraternus as well as P. beillei* and *P. mannianus*) were observed to be closely related hence could be regarded as sister taxa. The display of close affinity by these species in this study is supported by Kathriarachchi *et al*. (2006) in their synopsis of the current classification from various sources. They reported *P. reticulatus*, *P. acidus*, *P. muellerianus* and *P. pentandrus* to belong to the same subgenus *Kirganelia* under different sections *Anisonema*, *Cicca*, *Floribundi* and *Pentandra* respectively. *Phyllanthus niruri*, *P. maninianus*, *P. amarus* and *P. urinaria* were grouped together in the subgenus *Phyllanthus* with *P. urinaria* in a separate section *Urinaria* while others belong to section *Phyllanthus* though different subsections. Only *P. maderaspatensis* belong to the subgenus *Isocladus* under section *Paraphyllanthus*. This species was reported to be common throughout the old world tropics and subtropics and at the same time sister to all other species of *Phyllanthus s.l*. The tricolporate, simply reticulate and slightly prolate pollens found in this species is considered to be the most undifferentiated pollen type in *Phyllanthus* (Punt, 1967; 1987).

The groupings obtained from the Principal Component Analysis (PCA) are almost similar to the clustering analysis obtained for all the data matrices with the scatter diagrams for the combined and epidermal characters i.e 46 x 19 and 27 x 19 splitting all the taxa in a way that conformed to the classification of the genus by Webster (1956) and Kathriarachchi *et al.*, (2006). This study has shown how numerical taxonomy justifies the classification of the genus using both macro and micro morphological characters as supported by several authors (Ayodele, 2000; Sonibare *et al*., 2004; Soladoye *et al.*, 2011).

CHAPTER SIX

6.0 CONCLUSION

This study revised the genus *Phyllanthus* for Nigeria based on macromorphological and micromorphological characters as well as molecular phylogeny. Sixteen species in three genera and seven sections have been identified to occur in Nigeria from field studies and authenticated herbarium specimens. The species are *P. acidus, P. amarus, P. beillei, P. capillaris, P. maderaspatensis, P. mannianus, P. muellerianus, P. nigericus, P. niruri, P. niruroides, P. odontadenius, P. pentandrus, P. reticulatus, P. rotundifolius, P. sublanatus* and *P. urinaria*. The most commonly encountered and widely distributed species among all the taxa studied is *P. amarus* which coincidentally is always misidentified for other herbaceous species probably due to similarities in growth habit and vernacular names hence being erroneously regarded as synonymous with one another by some researchers.

The taxonomy of the genus *Phyllanthus* is complex requiring the correlation of different characters for the identification of the specimens. Characters from the herbarium samples have to be combined with field characters for the identification, most especially the flowers of the herbaceous species because many of the field characters are lost or changed drastically during the preparation of samples. It was observed in the study that the species are very fragile and DNA extraction difficult for molecular studies hence further investigations of the molecular phylogeny to include more of the Nigerian species in the genus *Phyllanthus* is hereby suggested.

In general, the assumption by workers is that all the species in the genus *Phyllanthus* evolved from a common ancestor. Although the present study justified that these species bear some common morphological features and that they are closely related to one another; at the same time, some distinguishing morphological variations do not support the monophyletic lineage. Incidentally, three of the Webster's subgenera (*Kirganelia, Isocladus* and *Phyllanthus*) which are also documented for Nigerian species were found to be non-monophyletic hence maintained as paraphyletic groups (Kathriarachchi *et al*., 2006).

Although no new species were found in the course of this study, it has been established that *P. floribundus, P. fraternus* and *P. physocarpus* are synonyms of *P. muellerianus, P. amarus* and *P. acidus* respectively. All the species are described and species boundaries stated. A dichotomous key for easy identification of the species in the genus *Phyllanthus* is provided. Based on available information obtained from the study and taking into consideration the Nigerian taxa, a revised classification of the genus *Phyllanthus* is hereby presented.

6.1 Revised classification of the species of the genus *Phyllanthus* in Nigeria

Genus ***Phyllanthus***
- Subgenus ***Isocladus***
  - Section ***Paraphyllanthus***
    - Species **-** *Phyllanthus maderaspatensis*
- Subgenus ***Kirganelia***
  - Section ***Anisonema***
    - Species - *P. reticulatus*
  - Section ***Cicca***
    - Species - *P. acidus*
  - Section ***Floribundi***
    - Species - *P. muellerianus*
  - Section ***Pentandra***
    - Species - *P. pentandrus*
- Subgenus ***Phyllantus***
  - Section ***Phyllantus***
    - Subsection ***Niruri***
      - Species - *P. niruri*
P. niruroides
    - Subsection ***Odontadenii***
      - Species - *P. odontadenius*
P. mannianus
P. beillei
P. capillaris
    - Subsection ***Swartziani***
      - Species - *P. amarus*
P. nigericus
P. sublanatus
P. rotundifolius
  - Section ***Urinaria***
    - Species - *P. urinaria*

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APPENDIX I

LIST OF CHARACTERS AND THEIR CODES FOR NUMERICAL TAXONOMY

Macromorphological Characters

1.Leaf Shape

0. Obovate
1. Elliptic
2. Ovate
3. Oblong
4. Lanceolate
5. Lanceolate-ovate
6. Suborbicular
7. Shapes variable within species

2. Leaf pubescence/surfaces

0. Glabrous
1. Glabrous/Glossy

3. Leaf Apex

0. Acute
1. Obtuse
2. Mucronate
3. Acuminate
4. Apex variable within a species

4. Leaf Base

0. Attenuate
1. Cuneate
2. Attenuate/Cuneate

5. Petiole

0. Sessile
1. Sub sessile
2. Petiole present

6. Leaf Length

0. Less than 2 cm
1. 2.1cm - 4.0 cm
2. 4.1cm - 6.0 cm
3. 6.1cm - 8.0 cm
4. Above 8.1 cm

7. Leaf Width

0. Less than 2 cm
1. 2.1cm - 4.0 cm
2. 4.1cm - 6.0 cm

8. Petiole Length
    0. Absent
    1. Less than 0.4 cm
    2. 0.41cm - 0.60 cm

9. Blade Length
    0. Less than 2 cm
    1. 2.1cm - 4.0 cm
    2. 4.1cm - 6.0 cm
    3. 6.1cm - 8.0 cm
    4. Above 8.1 cm

10. Leaf Length/Leaf width ratio
    0. 1.1 - 2.1
    1. 3.1 - 4.1
    2. 5.1 - 6.1

11. Blade length/Petiole length ratio
    0. Absent
    1. 10:1 - 15:1
    2. 16:1 - 20:1
    3. 25:1 - 30:1

12. Flower colour
    0. White
    1. Yellowish white
    2. Greenish white
    3. Greenish
    4. Pale yellow
    5. Pale green
    6. Cream
    7. Pinkish green
    8. NA

13. Perianth lobes
    0. 4
    1. 5
    2. 6
    3. NA

14. Fruit colour
    0. Greenish
    1. Pale yellow
    2. Red
    3. Black
    4. Reddish brown
    5. NA

Epidermal Characters

1. Adaxial epidermal cell shape
    0. Irregular
    1. Irregular & Wavy
    2. Polygonal
2. Abaxial epidermal cell shape
    0. Irregular
    1. Irregular & Wavy
    2. Polygonal
3. Adaxial trichome base/Trichome
    0. Absent
    1. Present
4. Abaxial trichome base/Trichome
    0. Absent
    1. Present
5. Adaxial anticlinal wall
    0. Thinly wavy
    1. Thickly wavy
    2. Thinly straight
    3. Thickly straight
6. Abaxial Pattern of anticlinal wall
    0. Thinly wavy
    1. Thickly wavy
    2. Thinly straight
    3. Thickly straight
7. Adaxial shape of guardcell
    0. NA
    1. Suborbiculate
    2. N/Elliptic
    3. W/Elliptic
8. Abaxial shape of guardcell
    0. NA
    1. Suborbiculate
    2. N/Elliptic
    3. W/Elliptic
9. Adaxial type of cell inclusion
    0. NA
    1. Crystals
    2. Oil droplets

10. Abaxial type of cell inclusion
    0. NA
    1. Crystals
    2. Oil droplets
11. Adaxial type of stomata
    0. NA
    1. Anisocytic
    2. Anomocytic
12. Abaxial type of stomata
    0. NA
    1. Anisocytic
    2. Anomocytic
    3. Laterocyclic
13. Adaxial cell number
    0. Few - 20-200
    1. Many – 200 and above
14. Abaxial cell number
    0. Few - 20-100
    1. Many – 101-200
15. Adaxial cell wall thickness
    0. Short – 1-2 µm
    1. Long – 2 µm and above
16. Abaxial cell wall thickness
    0. Short – 1-2 µm
    1. Long – 2 µm and above
17. Adaxial stomata length
    0. NA
    1. Short – 1-5 µm
    2. Long – 5 µm and above
18. Abaxial stomata length
    0. NA
    1. Short – 1-5 µm
    2. Long - 5 µm and above
19. Adaxial stomata width
    0. NA
    1. Short – 1-5 µm
    2. Long – 5 µm and above

20. Abaxial stomata width
    1. NA
    3. Short – 1-5 µm
    4. Long – 5 µm and above
21. Adaxial stomata density
    0. NA
    1. Few – 1-50
    2. Many - 51 and above
22. Abaxial stomata density
    1. NA
    3. Few – 1-50
    4. Many - 51 and above
23. Adaxial stomata index
    0. NA
    1. Small – 1-20
    2. Large - 20 and above
24. Abaxial stomata index
    0. NA
    1. Small – 1-20
    2. Large - 20 and above
25. Adaxial outer stomata rim
    0. NA
    1. Raised
    2. Not raised
    3. Sunken
26. Abaxial outer stomata rim
    0. NA
    1. Raised
    2. Not raised
    3. Sunken
27. Cuticular wax
    0. Absent
    1. Present

Pollen morphological characters

1. Pollen Polar diameter
    0. Thin – 10-20 µm
    1. Thick – 21 µm and above
2. Pollen Equitorial diameter
    0. Small – 10-20 µm
    1. Large – 21 µm and above

3. Pollen shape

  0. Prolate
  1. Subprolate
  2. Oblate–spheroidal

4. Pollen size

  0. Small
  1. Medium

5. Colpi length

  0. Small – 10-20 µm
  1. Large – 21-30 µm

Key to character coding:

1. LSH = Leaf shape
2. LS=Leaf surface
3. LAP= Leaf apex
4. LB=Leaf base
5. P = Petiole
6. LL=Leaf length
7. LW= Leaf width
8. PL=Petiole length
9. BL=Blade length
10. LLLW_R= Leaf length/Leaf width ratio
11. BLPL_R= Blade length/Petiole length ratio
12. FLWCL = Flower colour
13. PTHLB = Perianth lobes
14. FC = Fruit colour
15. ADEPSH = Adaxial epidermal cell shape
16. ABEPSH = Abaxial epidermal cell shape
17. ADTR = Adaxial trichome type
18. ABTR = Abaxial trichome type
19. ADAWP = Adaxial Anticlinal wall pattern
20. ABAWP = Abaxial Anticlinal wall pattern
21. ADSHGC = Adaxial shape of Guardcell
22. ABSHGC = Abaxial shape of Guardcell
23. ADCIN = Adaxial type of cell inclusion
24. ABCIN = Abaxial type of cell inclusion
25. ADST = Adaxial Stomata type
26. ABST = Abaxial Stomata type
27. ADCN = Adaxial cell number

28. ABCN = Abaxial cell width
29. ADCWT = Adaxial cellwall thickness
30. ABCWT = Abaxial cellwall thickness
31. ADSL = Adaxial Stomata length
32. ABSL = Abaxial Stomata length
33. ADSW = Adaxial Stomata width
34. ABSW = Abaxial Stomata width
35. ADSD = Adaxial Stomata density
36. ABSD = Abaxial Stomata density
37. ADSI = Adaxial Stomata index
38. ABSI = Abaxial Stomata index
39. ADOSR = Adaxial outer stomata rim
40. ABOSR = Abaxial outer stomata rim
41. CW = Cuticular wax
42. PPD = Pollen polar diameter
43. PED = Pollen equitorial diameter
44. PSH = Pollen shape
45. PS = Pollen size
46. CL = Colpi length

APPENDIX II

46 x 19 Combined Macromorphological, Epidermal and Pollen Data Matrix

OTUS	LSH	LS	LAP	LB	P	LL	LW	PL	BL	LLLW_R	BLPL_R
1.	5	0	0	0	2	2	1	0	2	0	2
2.	3	0	1	0	0	0	0	0	0	0	0
3.	7	0	1	1	1	1	0	0	1	0	0
4.	0	0	4	1	0	0	0	0	0	0	0
5.	7	0	0	0	2	2	1	1	2	0	2
6.	3	0	1	0	1	0	0	0	0	1	0
7.	7	0	0	1	0	0	0	0	0	1	0
8.	7	1	4	1	1	0	0	0	0	0	0
9.	7	0	0	0	2	2	1	1	1	0	1
10.	1	0	1	0	0	0	0	0	0	0	0
11.	3	0	4	1	0	0	0	0	0	0	0
12.	3	0	1	0	0	0	0	0	0	1	0
13.	3	0	2	1	1	0	0	0	0	0	0
14.	7	0	4	1	1	0	0	0	0	2	0
15.	5	0	0	1	2	4	2	2	4	0	3
16.	4	0	0	1	2	1	0	1	1	1	1
17.	6	0	1	1	0	0	0	0	0	0	0
18.	3	0	1	0	0	0	0	0	0	2	0
19.	4	0	3	1	0	0	0	0	0	2	0

ADEPSH	ABEPSH	ADTR	ABTR	ADAWP	ABAWP	ADSHGC	ABSHGC	ADCIN	ABCIN	ADST	ABST	ADCN	ABCN	ABSW	ABSL
1	1	0	0	0	0	0	2	2	1	0	2	1	0	1	2
0	0	0	0	0	0	1	1	1	1	1	1	0	1	2	2
1	1	0	0	0	0	0	2	0	0	0	2	0	1	1	2
1	0	0	0	0	0	0	3	0	1	0	1	1	1	1	2
2	2	0	0	3	3	0	1	0	1	0	1	1	1	1	1
1	1	0	0	0	1	1	1	1	0	1	1	0	0	2	2
0	0	0	0	3	1	3	3	0	0	1	1	0	0	2	2
1	1	0	0	0	0	0	1	0	0	0	2	0	0	2	2
2	2	0	0	3	3	0	1	0	1	0	1	0	1	1	2
1	1	0	0	0	1	1	1	0	0	2	2	1	0	2	2
1	1	0	0	0	0	3	3	0	1	1	1	0	0	2	2
0	0	0	0	0	1	3	3	0	0	2	2	1	0	1	2
1	1	0	0	0	0	3	3	0	1	1	1	0	1	1	2
1	1	0	0	2	2	3	3	0	0	1	1	0	0	2	2
0	1	0	0	0	0	1	1	1	1	0	2	0	0	1	2
2	2	1	1	2	2	0	1	0	0	0	1	0	1	1	2
1	1	0	0	1	1	1	1	1	1	1	1	0	0	2	2
0	1	0	0	0	0	1	1	0	0	2	2	0	0	1	2
0	0	0	0	2	2	3	3	0	0	2	2	0	0	1	2

ADCWT	ABCWT	ADSL	ADSW	ADSD	ADSI	ADOSR	CW	ABSD	ABSI	ABOSR	FLWCL	PTHLB	FC	PPD	PED	PSH	PS	CL
1	1	0	0	0	0	0	0	1	1	0	7	0	1	0	0	0	1	0
1	0	2	2	1	2	2	0	1	1	2	1	1	0	1	0	1	0	0
1	1	0	0	0	0	0	0	1	2	1	0	2	0	0	0	0	1	0
1	1	0	0	0	0	0	1	1	1	2	0	1	0	1	1	0	1	1
1	0	0	0	0	0	0	0	2	2	0	8	3	5	0	0	2	0	0
1	1	2	2	1	1	3	1	2	2	3	1	2	0	1	1	0	1	1
1	1	2	2	1	2	2	0	1	2	2	4	2	0	1	0	1	0	1
1	1	0	0	0	0	0	1	2	2	2	2	3	5	1	0	0	1	1
1	1	0	0	0	0	0	0	2	2	0	3	1	2	0	0	2	0	0
0	0	2	1	1	1	0	0	2	2	2	2	1	5	1	0	1	0	1
1	1	2	2	1	2	2	1	1	2	2	1	2	0	1	1	0	1	1
1	1	2	1	1	1	2	0	2	2	2	0	1	0	1	0	0	1	1
1	1	2	1	1	1	2	1	1	1	2	2	2	0	1	1	1	1	1
0	1	2	2	1	2	2	0	1	2	2	0	1	0	1	0	1	0	1
1	1	0	0	0	0	0	0	1	2	0	8	3	5	0	0	2	0	0
1	1	0	0	0	0	0	1	2	2	3	6	1	3	0	0	1	0	0
0	1	2	2	1	2	2	0	2	2	2	5	2	5	2	2	3	2	2
1	1	2	2	2	2	2	0	2	2	2	8	3	5	2	2	3	2	2
0	0	2	1	2	2	3	1	2	2	3	2	2	4	1	0	0	1	1

APPENDIX III

27 x 19 Epidermal Data Matrix

OTUS	ADEPSH	ABEPSH	ADTR	ABTR	ADAWP	ABAWP	ADSHGC	ABSHGC	ADCIN	ABCIN	ADST	ABST	ADCN	ABCN
1.	1	1	0	0	0	0	0	2	2	1	0	2	1	0
2.	0	0	0	0	0	0	1	1	1	1	1	1	0	1
3.	1	1	0	0	0	0	0	2	0	0	0	2	0	1
4.	1	0	0	0	0	0	0	3	0	1	0	1	1	1
5.	2	2	0	0	3	3	0	1	0	1	0	1	1	1
6.	1	1	0	0	0	1	1	1	1	0	1	1	0	0
7.	0	0	0	0	3	1	3	3	0	0	1	1	0	0
8.	1	1	0	0	0	0	0	1	0	0	0	2	0	0
9.	2	2	0	0	3	3	0	1	0	1	0	1	0	1
10.	1	1	0	0	0	1	1	1	0	0	2	2	1	0
11.	1	1	0	0	0	0	3	3	0	1	1	1	0	0
12.	0	0	0	0	0	1	3	3	0	0	2	2	1	0
13.	1	1	0	0	0	0	3	3	0	1	1	1	0	1
14.	1	1	0	0	2	2	3	3	0	0	1	1	0	0

15.	0	1	0	0	0	0	1	1	1	1	0	2	0	0
16.	2	2	1	1	2	2	0	1	0	0	0	1	0	1
17.	1	1	0	0	1	1	1	1	1	1	1	1	0	0
18.	0	1	0	0	0	0	1	1	0	0	2	2	0	0
19.	0	0	0	0	2	2	3	3	0	0	2	2	0	0

ABSW	ABSL	ADCWT	ABCWT	ADSL	ADSW	ADSD	ADSI	ADOSR	CW	ABSD	ABSI	ABOSR
1	2	1	1	0	0	0	0	0	0	1	1	0
2	2	1	0	2	2	1	2	2	0	1	1	2
1	2	1	1	0	0	0	0	0	0	1	2	1
1	2	1	1	0	0	0	0	0	1	1	1	2
1	1	1	0	0	0	0	0	0	0	2	2	0
2	2	1	1	2	2	1	1	3	1	2	2	3
2	2	1	1	2	2	1	2	2	0	1	2	2
2	2	1	1	0	0	0	0	0	1	2	2	2
1	2	1	1	0	0	0	0	0	0	2	2	0
2	2	0	0	2	1	1	1	0	0	2	2	2
2	2	1	1	2	2	1	2	2	1	1	2	2
1	2	1	1	2	1	1	1	2	0	2	2	2
1	2	1	1	2	1	1	1	2	1	1	1	2
2	2	0	1	2	2	1	2	2	0	1	2	2
1	2	1	1	0	0	0	0	0	0	1	2	0
1	2	1	1	0	0	0	0	0	1	2	2	3
2	2	0	1	2	2	1	2	2	0	2	2	2
1	2	1	1	2	2	2	2	2	0	2	2	2
1	2	0	0	2	1	2	2	3	1	2	2	3

APPENDIX IV

14 x 19 Macromorphological Data Matrix

OTUs	LSH	LS	LAP	LB	P	LL	LW	PL	BL	LLLW_R	BLPL_R	FLWCL	PTHLB	FC
1	5	0	0	0	2	2	1	0	2	0	2	7	0	1
2	3	0	1	0	0	0	0	0	0	0	0	1	1	0
3	7	0	1	1	1	1	0	0	1	0	0	0	2	0
4	0	0	4	1	0	0	0	0	0	0	0	0	1	0
5	7	0	0	0	2	2	1	1	2	0	2	8	3	5
6	3	0	1	0	1	0	0	0	0	1	0	1	2	0
7	7	0	0	1	0	0	0	0	0	1	0	4	2	0
8	7	1	4	1	1	0	0	0	0	0	0	2	3	5
9	7	0	0	0	2	2	1	1	1	0	1	3	1	2
10	1	0	1	0	0	0	0	0	0	0	0	2	1	5
11	3	0	4	1	0	0	0	0	0	0	0	1	2	0
12	3	0	1	0	0	0	0	0	0	1	0	0	1	0
13	3	0	2	1	1	0	0	0	0	0	0	2	2	0
14	7	0	4	1	1	0	0	0	0	2	0	0	1	0
15	5	0	0	1	2	4	2	2	4	0	3	8	3	5
16	4	0	0	1	2	1	0	1	1	1	1	6	1	3
17	6	0	1	1	0	0	0	0	0	0	0	5	2	5
18	3	0	1	0	0	0	0	0	0	2	0	8	3	5
19	4	0	3	1	0	0	0	0	0	2	0	2	2	4

APPENDIX V

5 x 19 Pollen Morphological Data Matrix

OTUs	PPD	PED	PSH	PS	CL
1	0	0	0	1	0
2	1	0	1	0	0
3	0	0	0	1	0
4	1	1	0	1	1
5	0	0	2	0	0
6	1	1	0	1	1
7	1	0	1	0	1
8	1	0	0	1	1
9	0	0	2	0	0
10	1	0	1	0	1
11	1	1	0	1	1
12	1	0	0	1	1
13	1	1	1	1	1
14	1	0	1	0	1
15	0	0	2	0	0
16	0	0	1	0	0
17	2	2	3	2	2
18	2	2	3	2	2
19	1	0	0	1	1

APPENDIX VI

Factor or Character loading of 46 Macro morphological, Epidermal and Pollen morphological characters

Correlations

	LSH	LS	LAP	LB	P	LL	LW	PL	BL	LLLW_R	BLPL_R	ADEPSH	ABEPSH	ADTR	ABTR	ADAWP	ABAWP	ADSHGC	ABSHGC	ADCIN	ABCIN	ADST	ABST
LSH	1	.279	-.218	.189	.470*	.347	.299	.254	.302	.009	.272	.236	.383	-.052	-.052	.572*	.385	-.106	-.147	.007	-.062	-.421	-.039
LS	.279	1	.397	.201	.060	-.137	-.113	-.113	-.131	-.165	-.127	.056	.039	-.056	-.056	-.168	-.207	-.238	-.213	-.131	-.224	-.243	.276
LAP	-.218	.397	1	.483*	-.426	-.539*	-.473*	-.473*	-.512*	.152	-.528*	-.083	-.332	-.232	-.232	-.255	-.243	.327	.505*	-.362	-.089	.133	-.056
LB	.189	.201	.483*	1	-.088	-.093	-.174	.021	-.038	.030	-.147	-.042	-.140	.201	.201	.066	-.192	.265	.427	-.277	-.045	-.234	-.136
P	.470*	.060	-.426	-.088	1	.788**	.700**	.700**	.747**	-.244	.782**	.601**	.749**	.343	.343	.287	.346	-.503*	-.382	.253	.240	-.730**	-.040
LL	.347	-.137	-.539*	-.093	.788**	1	.960**	.872**	.979**	-.343	.953**	.209	.474*	.080	.080	.160	.155	-.433	-.369	.360	.419	-.599**	.191
LW	.299	-.113	-.473*	-.174	.700**	.960**	1	.824**	.933**	-.337	.943**	.113	.380	-.113	-.113	.146	.144	-.332	-.331	.411	.507*	-.496*	.174
PL	.254	-.113	-.473*	.021	.700**	.872**	.824**	1	.841**	-.209	.834**	.257	.530*	.318	.318	.309	.332	-.332	-.434	.071	.314	-.496*	-.021
BL	.302	-.131	-.512*	-.038	.747**	.979**	.933**	.841**	1	-.322	.963**	.131	.407	.095	.095	.074	.057	-.400	-.339	.403	.383	-.572*	.242
LLLW_R	.009	-.165	.152	.030	-.244	-.343	-.337	-.209	-.322	1	-.297	-.358	-.213	.149	.149	.271	.278	.469*	.269	-.267	-.664**	.558*	.112
BLPL_R	.272	-.127	-.528*	-.147	.782**	.953**	.943**	.834**	.963**	-.297	1	.216	.462*	.141	.141	.173	.173	-.400	-.357	.438	.447	-.554*	.147
ADEPSH	.236	.056	-.083	-.042	.601**	.209	.113	.257	.131	-.358	.216	1	.820**	.407	.407	.367	.515*	-.516*	-.293	-.146	.224	-.577**	-.436
ABEPSH	.383	.039	-.332	-.140	.749**	.474*	.380	.530*	.407	-.213	.462*	.820**	1	.407	.407	.326	.466*	-.491*	-.557*	-.053	.156	-.474*	-.193
ADTR	-.052	-.056	-.232	.201	.343	.080	-.113	.318	.095	.149	.141	.407	.407	1	1.000**	.231	.255	-.238	-.213	-.131	-.224	-.243	-.201
ABTR	-.052	-.056	-.232	.201	.343	.080	-.113	.318	.095	.149	.141	.407	.407	1.000**	1	.231	.255	-.238	-.213	-.131	-.224	-.243	-.201
ADAWP	.572*	-.168	-.255	.066	.287	.160	.146	.309	.074	.271	.173	.367	.326	.231	.231	1	.859**	.064	.025	-.318	-.052	-.153	-.427
ABAWP	.385	-.207	-.243	-.192	.346	.155	.144	.332	.057	.278	.173	.515*	.466*	.255	.255	.859**	1	-.020	-.128	-.306	-.109	-.028	-.330
ADSHGC	-.106	-.238	.327	.265	-.503*	-.433	-.332	-.332	-.400	.469*	-.400	-.516*	-.491*	-.238	-.238	.064	-.020	1	.713**	-.266	-.200	.662**	-.094
ABSHGC	-.147	-.213	.505*	.427	-.382	-.369	-.331	-.434	-.339	.269	-.357	-.293	-.557*	-.213	-.213	.025	-.128	.713**	1	-.304	-.065	.248	-.084
ADCIN	.007	-.131	-.362	-.277	.253	.360	.411	.071	.403	-.267	.438	-.146	-.053	-.131	-.131	-.318	-.306	-.266	-.304	1	.401	-.210	.089
ABCIN	-.062	-.224	-.089	-.045	.240	.419	.507*	.314	.383	-.664**	.447	.224	.156	-.224	-.224	-.052	-.109	-.200	-.065	.401	1	-.427	-.382
ADST	-.421	-.243	.133	-.234	-.730**	-.599**	-.496*	-.496*	-.572*	.558*	-.554*	-.577**	-.474*	-.243	-.243	-.153	-.028	.662**	.248	-.210	-.427	1	.234

ABST	-.039	.276	-.056	-.136	-.040	.191	.174	-.021	.242	.112	.147	-.436	-.193	-.201	-.201	-.427	-.330	-.094	-.084	.089	-.382	.234	1
ADCN	-.356	-.141	-.109	-.459*	.008	.093	.150	-.069	.127	-.259	.221	.141	-.088	-.141	-.141	-.122	.062	-.221	.101	.089	.151	.008	.217
ABCN	-.016	-.180	-.168	-.012	.324	.159	.031	.231	.099	-.390	.085	.506*	.296	.309	.309	.194	.186	-.423	-.105	-.233	.368	-.502*	-.430
ABSW	.060	.276	.299	.080	-.425	-.496*	-.410	-.410	-.474*	-.030	-.459*	-.117	-.193	-.201	-.201	-.066	-.121	.247	-.084	.089	-.169	.234	-.295
ABSL	-.279	.056	.232	.276	-.343	-.297	-.318	-.318	-.321	.165	-.408	-.407	-.407	.056	.056	-.430	-.486*	.238	.213	.131	-.248	.243	.201
ADCWT	-.006	.122	-.267	-.179	.335	.300	.248	.248	.287	-.325	.278	.071	.117	.122	.122	-.178	-.306	-.304	-.087	.060	.231	-.479*	-.083
ABCWT	.175	.122	.077	.344	.180	.063	.012	.012	.039	.018	-.015	.071	.117	.122	.122	-.178	-.306	.005	.189	.060	-.027	-.310	-.083
ADSL	-.310	-.276	.198	-.080	-.728**	-.682**	-.564*	-.564*	-.651**	.455	-.631**	-.519*	-.473*	-.276	-.276	-.114	-.088	.777**	.313	-.089	-.258	.879**	-.136
ADSW	-.153	-.252	.177	-.051	-.657**	-.621**	-.514*	-.514*	-.593**	.436	-.574*	-.457*	-.380	-.252	-.252	-.058	-.122	.630**	.181	.033	-.181	.681**	-.310
ADSD	-.308	-.247	.206	-.086	-.703**	-.609**	-.504*	-.504*	-.582**	.660**	-.563*	-.595**	-.457*	-.247	-.247	-.065	-.050	.682**	.264	-.157	-.348	.919**	.086
ADSI	-.126	-.252	.256	.070	-.728**	-.621**	-.514*	-.514*	-.593**	.515*	-.574*	-.545*	-.473*	-.252	-.252	.042	-.064	.725**	.308	-.072	-.181	.758**	-.190
ADOSR	-.161	-.240	.234	.025	-.577**	-.593**	-.490*	-.490*	-.566*	.581**	-.548*	-.520*	-.484*	-.240	-.240	-.021	-.031	.749**	.371	.004	-.225	.703**	-.212
CW	-.374	.309	.559*	.430	-.069	-.344	-.367	-.168	-.320	.046	-.287	.180	-.045	.309	.309	-.175	-.135	.101	.246	-.233	-.069	-.075	-.209
ABSD	.013	.224	-.192	-.382	.013	-.128	-.122	.071	-.181	.244	-.088	.248	.338	.224	.224	.230	.522*	-.222	-.500*	-.215	-.367	.290	.169
ABSI	.417	.122	-.095	.083	.024	.063	.012	.248	.039	.362	-.015	.071	.318	.122	.122	.368	.453	.109	-.226	-.396	-.544*	.195	.179
ABOSR	-.433	.076	.426	.270	-.600**	-.805**	-.830**	-.534*	-.752**	.513*	-.744**	-.237	-.390	.318	.318	-.134	-.086	.413	.234	-.295	-.557*	.544*	-.161
FLWCL	.245	-.095	-.604**	-.176	.477*	.609**	.611**	.577**	.638**	-.014	.720**	.095	.467*	.233	.233	.240	.131	-.349	-.502*	.325	.242	-.224	.139
PTHLB	.272	.351	.057	.238	-.004	.123	.149	.263	.172	.052	.096	-.166	.143	-.205	-.205	.011	-.093	.016	-.252	-.265	-.078	-.004	.139
FC	.143	.304	-.187	-.055	.153	.296	.320	.406	.311	-.002	.347	.081	.374	.094	.094	.125	.212	-.347	-.620**	-.026	-.044	.043	.387
PPD	-.325	.081	.394	.055	-.808**	-.748**	-.619**	-.619**	-.715**	.354	-.692**	-.465*	-.458*	-.303	-.303	-.264	-.287	.415	.126	-.111	-.190	.689**	-.055
PED	-.321	-.147	.167	.058	-.405	-.363	-.301	-.301	-.347	.082	-.336	-.086	-.019	-.147	-.147	-.313	-.318	.056	-.062	.065	.189	.274	-.216
PSH	.234	-.224	-.440	-.152	.113	.273	.314	.411	.232	.037	.268	.066	.403	.012	.012	.305	.253	-.158	-.518*	.029	.261	.054	-.169
PS	-.195	.114	.260	.077	-.413	-.387	-.357	-.504*	-.350	.124	-.380	-.234	-.205	-.247	-.247	-.474*	-.445	.037	.091	.127	-.025	.288	.249
CL	-.257	.098	.407	.150	-.701**	-.674**	-.557*	-.557*	-.644**	.400	-.623**	-.345	-.327	-.273	-.273	-.195	-.205	.418	.196	-.207	-.270	.642**	.018

ADCN	ABCN	ABSW	ABSL	ADCWT	ABCWT	ADSL	ADSW	ADSD	ADSI	ADOSR	CW	ABSD	ABSI	ABOSR	FLWCL	PTHLB	FC	PPD	PED	PSH	PS	CL
-.356	-.016	.060	-.279	-.006	.175	-.310	-.153	-.308	-.126	-.161	-.374	.013	.417	-.433	.245	.272	.143	-.325	-.321	.234	-.195	-.257
-.141	-.180	.276	.056	.122	.122	-.276	-.252	-.247	-.252	-.240	.309	.224	.122	.076	-.095	.351	.304	.081	-.147	-.224	.114	.098
-.109	-.168	.299	.232	-.267	.077	.198	.177	.206	.256	.234	.559*	-.192	-.095	.426	-.604**	.057	-.187	.394	.167	-.440	.260	.407
-.459*	-.012	.080	.276	-.179	.344	-.080	-.051	-.086	.070	.025	.430	-.382	.083	.270	-.176	.238	-.055	.055	.058	-.152	.077	.150
.008	.324	-.425	-.343	.335	.180	-.728**	-.657**	-.703**	-.728**	-.577**	-.069	.013	.024	-.600**	.477*	-.004	.153	-.808**	-.405	.113	-.413	-.701**
.093	.159	-.496*	-.297	.300	.063	-.682**	-.621**	-.609**	-.621**	-.593**	-.344	-.128	.063	-.805**	.609**	.123	.296	-.748**	-.363	.273	-.387	-.674**
.150	.031	-.410	-.318	.248	.012	-.564*	-.514*	-.504*	-.514*	-.490*	-.367	-.122	.012	-.830**	.611**	.149	.320	-.619**	-.301	.314	-.357	-.557*
-.069	.231	-.410	-.318	.248	.012	-.564*	-.514*	-.504*	-.514*	-.490*	-.168	.071	.248	-.534*	.577**	.263	.406	-.619**	-.301	.411	-.504*	-.557*
.127	.099	-.474*	-.321	.287	.039	-.651**	-.593**	-.582**	-.593**	-.566*	-.320	-.181	.039	-.752**	.638**	.172	.311	-.715**	-.347	.232	-.350	-.644**
-.259	-.390	-.030	.165	-.325	.018	.455	.436	.660**	.515*	.581**	.046	.244	.362	.513*	-.014	.052	-.002	.354	.082	.037	.124	.400
.221	.085	-.459*	-.408	.278	-.015	-.631**	-.574*	-.563*	-.574*	-.548*	-.287	-.088	-.015	-.744**	.720**	.096	.347	-.692**	-.336	.268	-.380	-.623**
.141	.506*	-.117	-.407	.071	.071	-.519*	-.457*	-.595**	-.545*	-.520*	.180	.248	.071	-.237	.095	-.166	.081	-.465*	-.086	.066	-.234	-.345
-.088	.296	-.193	-.407	.117	.117	-.473*	-.380	-.457*	-.473*	-.484*	-.045	.338	.318	-.390	.467*	.143	.374	-.458*	-.019	.403	-.205	-.327
-.141	.309	-.201	.056	.122	.122	-.276	-.252	-.247	-.252	-.240	.309	.224	.122	.318	.233	-.205	.094	-.303	-.147	.012	-.247	-.273
-.141	.309	-.201	.056	.122	.122	-.276	-.252	-.247	-.252	-.240	.309	.224	.122	.318	.233	-.205	.094	-.303	-.147	.012	-.247	-.273
-.122	.194	-.066	-.430	-.178	-.178	-.114	-.058	-.065	.042	-.021	-.175	.230	.368	-.134	.240	.011	.125	-.264	-.313	.305	-.474*	-.195
.062	.186	-.121	-.486*	-.306	-.306	-.088	-.122	-.050	-.064	-.031	-.135	.522*	.453	-.086	.131	-.093	.212	-.287	-.318	.253	-.445	-.205
-.221	-.423	.247	.238	-.304	.005	.777**	.630**	.682**	.725**	.749**	.101	-.222	.109	.413	-.349	.016	-.347	.415	.056	-.158	.037	.418
.101	-.105	-.084	.213	-.087	.189	.313	.181	.264	.308	.371	.246	-.500*	-.226	.234	-.502*	-.252	-.620**	.126	-.062	-.518*	.091	.196
.089	-.233	.089	.131	.060	.060	-.089	.033	-.157	-.072	.004	-.233	-.215	-.396	-.295	.325	-.265	-.026	-.111	.065	.029	.127	-.207
.151	.368	-.169	-.248	.231	-.027	-.258	-.181	-.348	-.181	-.225	-.069	-.367	-.544*	-.557*	.242	-.078	-.044	-.190	.189	.261	-.025	-.270
.008	-.502*	.234	.243	-.479*	-.310	.879**	.681**	.919**	.758**	.703**	-.075	.290	.195	.544*	-.224	-.004	.043	.689**	.274	.054	.288	.642**
.217	-.430	-.295	.201	-.083	-.083	-.136	-.310	.086	-.190	-.212	-.209	.169	.179	-.161	.139	.139	.387	-.055	-.216	-.169	.249	.018
1	.039	-.268	-.394	.015	-.278	-.217	-.369	-.260	-.369	-.399	-.209	.088	-.278	-.296	.050	-.378	.025	-.184	-.196	-.208	-.077	-.129
.039	1	-.430	-.309	.394	-.141	-.454	-.447	-.466*	-.447	-.394	.095	-.150	-.408	-.200	-.080	-.149	-.230	-.449	-.153	.040	-.299	-.542*

-.268	-.430	1	.201	-.344	-.083	.511*	.652**	.249	.531*	.351	.012	-.045	.179	.385	-.344	.013	-.087	.466*	.100	-.062	-.077	.353
-.394	-.309	.201	1	-.122	.456*	.276	.252	.247	.252	.240	.180	-.224	-.122	.407	-.398	-.351	-.304	.303	.147	-.248	.247	.273
.015	.394	-.344	-.122	1	.367	-.440	-.322	-.447	-.467*	-.269	.127	-.231	-.267	-.299	.163	.144	-.321	-.387	-.060	-.156	-.052	-.417
-.278	-.141	-.083	.456*	.367	1	-.179	-.031	-.250	-.176	-.042	.127	-.231	.050	-.035	-.017	-.008	-.321	.033	.323	-.027	.343	.192
-.217	-.454	.511*	.276	-.440	-.179	1	.911**	.893**	.911**	.869**	-.012	.045	.083	.598**	-.324	-.013	-.197	.750**	.375	.062	.241	.653**
-.369	-.447	.652**	.252	-.322	-.031	.911**	1	.789**	.933**	.844**	-.078	-.056	.115	.528*	-.224	.052	-.235	.753**	.477*	.175	.244	.629**
-.260	-.466*	.249	.247	-.447	-.250	.893**	.789**	1	.880**	.847**	.035	.187	.146	.587**	-.142	.135	.023	.753**	.422	.136	.383	.687**
-.369	-.447	.531*	.252	-.467*	-.176	.911**	.933**	.880**	1	.844**	-.078	-.056	.115	.528*	-.203	.052	-.129	.753**	.389	.175	.244	.629**
-.399	-.394	.351	.240	-.269	-.042	.869**	.844**	.847**	.844**	1	.182	.039	.072	.614**	-.298	.098	-.295	.651**	.394	-.039	.351	.567*
-.209	.095	.012	.180	.127	.127	-.012	-.078	.035	-.078	.182	1	.069	-.141	.582**	-.308	.108	-.133	.084	.170	-.506*	.202	.144
.088	-.150	-.045	-.224	-.231	-.231	.045	-.056	.187	-.056	.039	.069	1	.544*	.233	.199	.203	.608**	.190	.123	.267	.187	.270
-.278	-.408	.179	-.122	-.267	.050	.083	.115	.146	.115	.072	-.141	.544*	1	.097	.118	.448	.427	.033	-.060	.231	-.052	.192
-.296	-.200	.385	.407	-.299	-.035	.598**	.528*	.587**	.528*	.614**	.582**	.233	.097	1	-.452	-.037	-.153	.592**	.282	-.287	.256	.544*
.050	-.080	-.344	-.398	.163	-.017	-.324	-.224	-.142	-.203	-.298	-.308	.199	.118	-.452	1	.341	.634**	-.190	.129	.646**	-.001	-.121
-.378	-.149	.013	-.351	.144	-.008	-.013	.052	.135	.052	.098	.108	.203	.448	-.037	.341	1	.512*	.197	.286	.356	.230	.262
.025	-.230	-.087	-.304	-.321	-.321	-.197	-.235	.023	-.129	-.295	-.133	.608**	.427	-.153	.634**	.512*	1	.092	.110	.566*	.094	.167
-.184	-.449	.466*	.303	-.387	.033	.750**	.753**	.753**	.753**	.651**	.084	.190	.033	.592**	-.190	.197	.092	1	.723**	.240	.622**	.937**
-.196	-.153	.100	.147	-.060	.323	.375	.477*	.422	.389	.394	.170	.123	-.060	.282	.129	.286	.110	.723**	1	.424	.780**	.750**
-.208	.040	-.062	-.248	-.156	-.027	.062	.175	.136	.175	-.039	-.506*	.267	.231	-.287	.646**	.356	.566*	.240	.424	1	.055	.227
-.077	-.299	-.077	.247	-.052	.343	.241	.244	.383	.244	.351	.202	.187	-.052	.256	-.001	.230	.094	.622**	.780**	.055	1	.687**
-.129	-.542*	.353	.273	-.417	.192	.653**	.629**	.687**	.629**	.567*	.144	.270	.192	.544*	-.121	.262	.167	.937**	.750**	.227	.687**	1

Correlation is significant at the 0.05 level (2-tailed).

APPENDIX VII

Eigenvalues of the Correlation Matrix of 46 Combined characters

Components extracted from Principal Component Analysis (Total Variance Explained)

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	14.505	31.532	31.532	14.505	31.532	31.532
2	5.376	11.686	43.218	5.376	11.686	43.218
3	4.587	9.973	53.191	4.587	9.973	53.191
4	3.630	7.891	61.081	3.630	7.891	61.081

Average Linkage (Between groups) based on Agglomeration Schedule

Stage	Cluster Combined		Coefficients	Stage Cluster First Appears		Next Stage
	Cluster 1	Cluster 2		Cluster 1	Cluster 2	
1	11	13	3.464	0	0	3
2	2	6	4.359	0	0	4
3	11	12	4.690	1	0	4
4	2	11	4.796	2	3	6
5	17	18	5.831	0	0	18
6	2	14	6.083	4	0	8
7	9	16	6.245	0	0	10
8	2	7	6.325	6	0	9
9	2	19	6.403	8	0	11
10	5	9	6.557	0	7	12
11	2	4	6.557	9	0	13
12	5	15	6.856	10	0	14
13	2	10	7.000	11	0	17
14	1	5	7.000	0	12	16
15	3	8	7.071	0	0	16
16	1	3	7.141	14	15	17
17	1	2	7.348	16	13	18
18	1	17	7.810	17	5	0

APPENDIX VIII

Eigenvector of the Correlation Matrix of 46 Combined Macro morphological, Epidermal and Pollen characters

	Component			
	1	2	3	4
LSH	-.370	.321	.144	-.065
LS	-.023	-.100	.161	.639
LAP	.516	-.387	.196	.248
LB	.135	-.240	.301	.244
P	-.882	.049	.144	-.002
LL	-.885	.140	-.298	-.078
LW	-.795	.185	-.443	-.119
PL	-.786	.308	-.004	-.045
BL	-.847	.122	-.344	-.045
LLLW_R	.490	.409	.256	-.167
BLPL_R	-.859	.177	-.305	-.095
FLWCL	-.529	.574	-.275	.162
PTHLB	-.009	.457	-.109	.466
FC	-.258	.706	-.078	.448
ADEPSH	-.542	-.022	.562	.089
ABEPSH	-.640	.357	.343	.188
ADTR	-.275	.040	.671	.110
ABTR	-.275	.040	.671	.110
ADAWP	-.248	.455	.499	-.471
ABAWP	-.267	.521	.548	-.443
ADSHGC	.677	.047	-.024	-.427
ABSHGC	.453	-.477	.057	-.368
ADCIN	-.223	-.085	-.602	-.018
ABCIN	-.395	-.223	-.426	-.111
ADST	.795	.354	-.142	-.193
ABST	-.038	.085	-.397	.312
ADCN	-.221	-.194	-.221	-.177
ABCN	-.446	-.343	.349	-.147
ABSW	.495	.069	.053	-.005
ABSL	.443	-.333	-.064	.193
ADCWT	-.406	-.386	-.088	.164
ABCWT	-.022	-.258	.015	.395
ADSL	.874	.243	-.116	-.281
ADSW	.804	.270	-.130	-.218
ADSD	.837	.389	-.129	-.150
ADSI	.833	.308	-.113	-.277
ADOSR	.807	.194	-.055	-.234
CW	.223	-.383	.472	.389
ABSD	.020	.634	.328	.303
ABSI	.028	.672	.320	.126
ABOSR	.763	-.031	.474	.151
PPD	.856	.239	-.170	.272
PED	.448	.187	-.233	.473
PSH	-.173	.736	-.205	.068
PS	.464	.018	-.294	.606
CL	.788	.294	-.099	.370

APPENDIX IX

Factor or Character loading of 27 Epidermal characters

Correlations

	ADEPSH	ABEPSH	ADTR	ABTR	ADAWP	ABAWP	ADSHGC	ABSHGC	ADCIN	ABCIN	ADST	ABST	ADCN	ABCN	ABSW	ABSL	ADCWT	ABCWT	ADSL	ADSW	ADSD	ADSI	ADOSR	CW	ABSD	ABSI	ABOSR
ADEPSH	1	.820**	.407	.407	.367	.515*	-.516*	-.293	-.146	.224	-.577**	-.436	.141	.506*	-.117	-.407	.071	.071	-.519*	-.457*	-.595**	-.545*	-.520*	.180	.248	.071	-.237
ABEPSH	.820**	1	.407	.407	.326	.466*	-.491*	-.557*	-.053	.156	-.474*	-.193	-.088	.296	-.193	-.407	.117	.117	-.473*	-.380	-.457*	-.473*	-.484*	-.045	.338	.318	-.390
ADTR	.407	.407	1	1.000**	.231	.255	-.238	-.213	-.131	-.224	-.243	-.201	-.141	.309	-.201	.056	.122	.122	-.276	-.252	-.247	-.252	-.240	.309	.224	.122	.318
ABTR	.407	.407	1.000**	1	.231	.255	-.238	-.213	-.131	-.224	-.243	-.201	-.141	.309	-.201	.056	.122	.122	-.276	-.252	-.247	-.252	-.240	.309	.224	.122	.318
ADAWP	.367	.326	.231	.231	1	.859**	.064	.025	-.318	-.052	-.153	-.427	-.122	.194	-.066	-.430	-.178	-.178	-.114	-.058	-.065	.042	-.021	-.175	.230	.368	-.134
ABAWP	.515*	.466*	.255	.255	.859**	1	-.020	-.128	-.306	-.109	-.028	-.330	.062	.186	-.121	-.486*	-.306	-.306	-.088	-.122	-.050	-.064	-.031	-.135	.522*	.453	-.086
ADSHGC	-.516*	-.491*	-.238	-.238	.064	-.020	1	.713**	-.266	-.200	.662**	-.094	-.221	-.423	.247	.238	-.304	.005	.777**	.630**	.682**	.725**	.749**	.101	-.222	.109	.413
ABSHGC	-.293	-.557*	-.213	-.213	.025	-.128	.713**	1	-.304	-.065	.248	-.084	.101	-.105	-.084	.213	-.087	.189	.313	.181	.264	.308	.371	.246	-.500*	-.226	.234
ADCIN	-.146	-.053	-.131	-.131	-.318	-.306	-.266	-.304	1	.401	-.210	.089	.089	-.233	.089	.131	.060	.060	-.089	.033	-.157	-.072	.004	-.233	-.215	-.396	-.295
ABCIN	.224	.156	-.224	-.224	-.052	-.109	-.200	-.065	.401	1	-.427	-.382	.151	.368	-.169	-.248	.231	-.027	-.258	-.181	-.348	-.181	-.225	-.069	-.367	-.544*	-.557*
ADST	-.577**	-.474*	-.243	-.243	-.153	-.028	.662**	.248	-.210	-.427	1	.234	.008	-.502*	.234	.243	-.479*	-.310	.879**	.681**	.919**	.758**	.703**	-.075	.290	.195	.544*
ABST	-.436	-.193	-.201	-.201	-.427	-.330	-.094	-.084	.089	-.382	.234	1	.217	-.430	-.295	.201	-.083	-.083	-.136	-.310	.086	-.190	-.212	-.209	.169	.179	-.161
ADCN	.141	-.088	-.141	-.141	-.122	.062	-.221	.101	.089	.151	.008	.217	1	.039	-.268	-.394	.015	-.278	-.217	-.369	-.260	-.369	-.399	-.209	.088	-.278	-.296
ABCN	.506*	.296	.309	.309	.194	.186	-.423	-.105	-.233	.368	-.502*	-.430	.039	1	-.430	-.309	.394	-.141	-.454	-.447	-.466*	-.447	-.394	.095	-.150	-.408	-.200
ABSW	-.117	-.193	-.201	-.201	-.066	-.121	.247	-.084	.089	-.169	.234	-.295	-.268	-.430	1	.201	-.344	-.083	.511*	.652**	.249	.531*	.351	.012	-.045	.179	.385
ABSL	-.407	-.407	.056	.056	-.430	-.486*	.238	.213	.131	-.248	.243	.201	-.394	-.309	.201	1	-.122	.456*	.276	.252	.247	.252	.240	.180	-.224	-.122	.407
ADCWT	.071	.117	.122	.122	-.178	-.306	-.304	-.087	.060	.231	-.479*	-.083	.015	.394	-.344	-.122	1	.367	-.440	-.322	-.447	-.467*	-.269	.127	-.231	-.267	-.299
ABCWT	.071	.117	.122	.122	-.178	-.306	.005	.189	.060	-.027	-.310	-.083	-.278	-.141	-.083	.456*	.367	1	-.179	-.031	-.250	-.176	-.042	.127	-.231	.050	-.035
ADSL	-.519*	-.473*	-.276	-.276	-.114	-.088	.777**	.313	-.089	-.258	.879**	-.136	-.217	-.454	.511*	.276	-.440	-.179	1	.911**	.893**	.911**	.869**	-.012	.045	.083	.598**
ADSW	-.457*	-.380	-.252	-.252	-.058	-.122	.630**	.181	.033	-.181	.681**	-.310	-.369	-.447	.652**	.252	-.322	-.031	.911**	1	.789**	.933**	.844**	-.078	-.056	.115	.528*

ADSD	-.595**	-.457*	-.247	-.247	-.065	-.050	.682**	.264	-.157	-.348	.919**	.086	-.260	-.466*	.249	.247	-.447	-.250	.893**	.789**	1	.880**	.847**	.035	.187	.146	.587**
ADSI	-.545*	-.473*	-.252	-.252	.042	-.064	.725**	.308	-.072	-.181	.758**	-.190	-.369	-.447	.531*	.252	-.467*	-.176	.911**	.933**	.880**	1	.844**	-.078	-.056	.115	.528*
ADOSR	-.520*	-.484*	-.240	-.240	-.021	-.031	.749**	.371	.004	-.225	.703**	-.212	-.399	-.394	.351	.240	-.269	-.042	.869**	.844**	.847**	.844**	1	.182	.039	.072	.614**
CW	.180	-.045	.309	.309	-.175	-.135	.101	.246	-.233	-.069	-.075	-.209	-.209	.095	.012	.180	.127	.127	-.012	-.078	.035	-.078	.182	1	.069	-.141	.582**
ABSD	.248	.338	.224	.224	.230	.522*	-.222	-.500*	-.215	-.367	.290	.169	.088	-.150	-.045	-.224	-.231	-.231	.045	-.056	.187	-.056	.039	.069	1	.544*	.233
ABSI	.071	.318	.122	.122	.368	.453	.109	-.226	-.396	-.544*	.195	.179	-.278	-.408	.179	-.122	-.267	.050	.083	.115	.146	.115	.072	-.141	.544*	1	.097
ABOSR	-.237	-.390	.318	.318	-.134	-.086	.413	.234	-.295	-.557*	.544*	-.161	-.296	-.200	.385	.407	-.299	-.035	.598**	.528*	.587**	.528*	.614**	.582**	.233	.097	1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

APPENDIX X

Eigenvalues of the Correlation Matrix of 27 Epidermal characters

Components extracted from Principal Component Analysis (Total Variance Explained)

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	8.450	31.297	31.297	8.450	31.297	31.297
2	4.103	15.198	46.495	4.103	15.198	46.495
3	2.909	10.775	57.271	2.909	10.775	57.271
4	2.508	9.290	66.560	2.508	9.290	66.560

Average Linkage (Between groups) based on Agglomeration Schedule

Stage	Cluster Combined		Coefficients	Stage Cluster First Appears		Next Stage
	Cluster 1	Cluster 2		Cluster 1	Cluster 2	
1	5	9	1.732	0	0	16
2	7	14	2.236	0	0	12
3	11	13	2.236	0	0	11
4	1	15	2.449	0	0	7
5	3	8	2.449	0	0	7
6	6	17	2.646	0	0	9
7	1	3	2.646	4	5	8
8	1	4	2.828	7	0	17
9	2	6	3.000	0	6	10
10	2	18	3.162	9	0	14
11	11	12	3.317	3	0	12
12	7	11	3.317	2	11	13
13	7	19	3.464	12	0	15
14	2	10	3.606	10	0	15
15	2	7	3.606	14	13	18
16	5	16	3.873	1	0	17
17	1	5	4.000	8	16	18
18	1	2	4.123	17	15	0

APPENDIX XI

Eigenvector of the Correlation Matrix of 27 Epidermal characters

	Component			
	1	2	3	4
ADEPSH	-.703	.450	.078	.244
ABEPSH	-.651	.478	-.060	.017
ADTR	-.351	.535	.577	-.171
ABTR	-.351	.535	.577	-.171
ADAWP	-.175	.668	-.220	.417
ABAWP	-.215	.793	-.341	.260
ADSHGC	.800	.046	.076	.248
ABSHGC	.417	-.229	.245	.316
ADCIN	-.084	-.498	-.115	-.025
ABCIN	-.363	-.433	-.111	.607
ADST	.859	.165	-.213	-.180
ABST	.047	-.302	-.313	-.825
ADCN	-.280	-.226	-.438	-.056
ABCN	-.597	.063	.230	.447
ABSW	.496	.103	.005	.122
ABSL	.411	-.277	.529	-.355
ADCWT	-.451	-.342	.337	.053
ABCWT	-.104	-.211	.536	-.136
ADSL	.938	.121	-.040	.151
ADSW	.864	.106	.022	.247
ADSD	.906	.174	-.094	-.024
ADSI	.913	.135	-.042	.240
ADOSR	.874	.122	.110	.218
CW	.040	.136	.683	.002
ABSD	-.033	.670	-.274	-.424
ABSI	.119	.648	-.236	-.387
ABOSR	.624	.370	.534	-.147

APPENDIX XII

Factor or Character loading of 14 Macromorphological characters

Correlations

	LSH	LS	LAP	LB	P	LL	LW	PL	BL	LLLW_R	BLPL_R	FLWCL	PTHLB	FC
LSH	1	.279	-.218	.189	.470*	.347	.299	.254	.302	.009	.272	.245	.272	.143
LS	.279	1	.397	.201	.060	-.137	-.113	-.113	-.131	-.165	-.127	-.095	.351	.304
LAP	-.218	.397	1	.483*	-.426	-.539*	-.473*	-.473*	-.512*	.152	-.528*	-.604**	.057	-.187
LB	.189	.201	.483*	1	-.088	-.093	-.174	.021	-.038	.030	-.147	-.176	.238	-.055
P	.470*	.060	-.426	-.088	1	.788**	.700**	.700**	.747**	-.244	.782**	.477*	-.004	.153
LL	.347	-.137	-.539*	-.093	.788**	1	.960**	.872**	.979**	-.343	.953**	.609**	.123	.296
LW	.299	-.113	-.473*	-.174	.700**	.960**	1	.824**	.933**	-.337	.943**	.611**	.149	.320
PL	.254	-.113	-.473*	.021	.700**	.872**	.824**	1	.841**	-.209	.834**	.577**	.263	.406
BL	.302	-.131	-.512*	-.038	.747**	.979**	.933**	.841**	1	-.322	.963**	.638**	.172	.311
LLLW_R	.009	-.165	.152	.030	-.244	-.343	-.337	-.209	-.322	1	-.297	-.014	.052	-.002
BLPL_R	.272	-.127	-.528*	-.147	.782**	.953**	.943**	.834**	.963**	-.297	1	.720**	.096	.347
FLWCL	.245	-.095	-.604**	-.176	.477*	.609**	.611**	.577**	.638**	-.014	.720**	1	.341	.634**
PTHLB	.272	.351	.057	.238	-.004	.123	.149	.263	.172	.052	.096	.341	1	.512*
FC	.143	.304	-.187	-.055	.153	.296	.320	.406	.311	-.002	.347	.634**	.512*	1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

c. Cannot be computed because at least one of the variables is constant.

APPENDIX XIII

Eigenvalues of the Correlation Matrix of 14 Macromorphological characters

Components extracted from Principal Component Analysis (Total Variance Explained)

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	6.564	46.888	46.888	6.564	46.888	46.888
2	2.097	14.979	61.866	2.097	14.979	61.866
3	1.446	10.327	72.193	1.446	10.327	72.193
4	1.047	7.478	79.671	1.047	7.478	79.671

Average Linkage (Between groups) based on Agglomeration Schedule

Stage	Cluster Combined		Coefficients	Stage Cluster First Appears		Next Stage
	Cluster 1	Cluster 2		Cluster 1	Cluster 2	
1	2	12	1.414	0	0	2
2	2	6	1.732	1	0	3
3	2	13	2.000	2	0	4
4	2	11	2.449	3	0	5
5	2	4	3.317	4	0	14
6	1	16	3.742	0	0	10
7	5	15	4.000	0	0	18
8	3	14	4.000	0	0	13
9	8	19	4.243	0	0	11
10	1	17	4.472	6	0	15
11	8	10	4.472	9	0	15
12	7	9	4.472	0	0	13
13	3	7	4.583	8	12	14
14	2	3	4.583	5	13	16
15	1	8	4.690	10	11	16
16	1	2	4.796	15	14	17
17	1	18	4.899	16	0	18
18	1	5	5.196	17	7	0

APPENDIX XIV

Eigenvector of the Correlation Matrix of 14 Macro morphological characters

Component Matrix

	Component			
	1	2	3	4
LSH	.387	.417	.203	.413
LS	-.094	.748	.207	-.383
LAP	-.628	.424	.352	-.025
LB	-.152	.547	.455	.388
P	.802	-.020	.298	.113
LL	.967	-.072	.170	.026
LW	.936	-.064	.114	-.054
PL	.885	.061	.048	.082
BL	.954	-.035	.144	.031
LLLW_R	-.306	.066	-.493	.695
BLPL_R	.965	-.085	.071	-.012
FLWCL	.759	.117	-.491	.028
PTHLB	.218	.753	-.303	-.003
FC	.440	.526	-.545	-.264

APPENDIX XV

Factor or Character loading of 5 Pollen morphological characters

Correlations

	PPD	PED	PSH	PS	CL
PPD	1	.723**	.240	.622**	.937**
PED	.723**	1	.424	.780**	.750**
PSH	.240	.424	1	.055	.227
PS	.622**	.780**	.055	1	.687**
CL	.937**	.750**	.227	.687**	1

**. Correlation is significant at the 0.01 level (2-tailed).

APPENDIX XVI

Eigenvalues of the Correlation Matrix of 5 Pollen morphological characters

Components extracted from Principal Component Analysis (Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	3.351	67.015	67.015	3.351	67.015	67.015
2	.983	19.656	86.671			
3	.479	9.581	96.251			
4	.129	2.580	98.831			

Average Linkage (Between groups) based on Agglomeration Schedule

Stage	Cluster Combined		Coefficients	Stage Cluster First Appears		Next Stage
	Cluster 1	Cluster 2		Cluster 1	Cluster 2	
1	12	19	.000	0	0	5
2	17	18	.000	0	0	18
3	9	15	.000	0	0	8
4	10	14	.000	0	0	7
5	8	12	.000	0	1	13
6	6	11	.000	0	0	9
7	7	10	.000	0	4	14
8	5	9	.000	0	3	11
9	4	6	.000	0	6	12
10	1	3	.000	0	0	16
11	5	16	1.000	8	0	15
12	4	13	1.000	9	0	13
13	4	8	1.000	12	5	16
14	2	7	1.000	0	7	15
15	2	5	1.000	14	11	17
16	1	4	1.414	10	13	17
17	1	2	1.414	16	15	18
18	1	17	2.828	17	2	0

APPENDIX XVII

Eigenvector of the Correlation Matrix of 5 Pollen morphological character

Component Matrix

	Component
	1
PPD	.909
PED	.917
PSH	.367
PS	.826
CL	.931