# TAXONOMY, PHYTOGEOGRAPHY AND ETHNOBOTANY OF THE GENUS *ACALYPHA* L. (EUPHORBIACEAE) IN TANZANIA




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A THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN BOTANY AT THE UNIVERSITY OF DAR ES SALAAM

APRIL, 1995

**ABSTRACT**

The genus *Acalypha* L. (Euphorbiaceae) represented by 28 species and 4 varieties in Tanzania has been investigated with respect to taxonomy by employing a wide range of taxonomic data, inclusive of cytology, palynology and taximetrics. Gaps on the phytogeography of *Acalypha* and frontiers of knowledge on the ethnobotany of the genus are reported.

The study has resolved the taxonomic controversies of 12 problematical *Acalypha* species namely: *A. boiviniana; A. brachystachya; A. bussei, A. ciliata; A. engleri; A. fruticosa* vars. *fruticosa, eglandulosa, villosa; A. indica; A. lanceolata; A. neptunica* vars. *neptunica,* vars. *pubescens; A. paucifolia; A. psilostachya* vars. *psilostachya, glandulosa* and *A. villicaulis*. The study has also established a new record of the pollen morphology for 21 *Acalypha* species and 4 varieties, viz., *A. acrogyna, A. ambigua, A. bipartita, A. brachystachya, A. bussei, A. chirindica, A. echinus, A. engleri, A. gillmannii, A. hispida, A. lanceolata, A. neptunica group, A. nyasica, A. ornata, A. paucifolia, A. psilostachya* group, *A. stuhlmalnii, A. villicaulis, A. volkensii, A. welwitschiana* and *A. wilkensiana*. Of the 13 *Acalypha* taxa studied cytologically, the chromosome counts of 8 species are new records for genus *Acalypha* and include *A. bipartita; A. chirindica, A. fruticosa* vars. *fruticosa, eglandulosa* and *villosa; A. neptunica; A. villicaulis* and *A. volkensii*.

Taximetric analyses as a modern taxonomic tool has enabled the establishment of a classification of the genus *Acalypha* L. in Tanzania based on the latest

infrageneric classification of the genus *Acalypha* by Pax and Hoffmann (1924). In my classification, the latter's subgenus *Euacalypha* and all the subsequent series, except series *Oligogynae,* have been retained. Their subgenus *Linostachys* has been dropped and the author's series *Hypandre* replaced by Section *Monaxanthae* Muell.Arg. In the classification, four Sections are proposed namely: (I) *Polygynae-Pleurogynae*, comprising 8 species and 1 variety in 7 series. (II) *Pantogynae - Pleurogynae* comprising 7 species and 3 varieties in 6 series. (III) *Monaxanthae* Muell. Arg., comprising 3 species in a single series and (IV) *Pantogynae - Acrogynae,* comprising 9 species in 4 series.

On the basis of disjunct distribution pattern, Tanzanian *Acalypha* belong to 4 main categories namely, endemics (39.3%), moderates (42.4%), wides (12.1%) and cultivated (6.2%). The observed distribution pattern is dictated by altitude, edaphic factors, rainfall, temperature and vegetation types. Origin and evolution of Tanzanian *Acalypha* is associated with the disjunct afromontane regions and Lake Victoria regional mosaic area. The flowering period of *Acalypha* ranges from less than 6 months to throughout the year. Ethnobotanical studies have revealed that 71.0% of the *Acalypha* taxa have medicinal values and treat 46 human diseases and conditions, grouped as bacterial, viral, fungal, trypanosomal, molluscicidal, cytotoxins and protein synthesis inhibition. Also, 64.5% of *Acalypha* taxa have other non-medicinal uses, viz., as vegetables, fodder, ornamentals and in construction.

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## DECLARATION

I, Rogasian Lemmy Anselm Mahunnah hereby declare that the work submitted in this thesis, for the degree of Doctor of Philosophy in the University of Dar es Salaam, is the result of my own work except where acknowledged in the text. It has not been submitted, nor is it being concurrently submitted for a similar qualification in any other University.

Signed:..... Date..14/09/1995.....

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## CERTIFICATION

I certify that this work was carried out by Rogasian Lemmy Anselm Mahunnah in the Department of Botany of the University of Dar es Salaam. I have read the thesis and approve it for examination.

Signed: ..... Date 29/9/1995

**DR. Z.K. RULANGARANGA**

**(SUPERVISOR)**

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## DEDICATION

This work is dedicated to my late parents,

ANSELM AND SUSANA,

to my wife ELIAMBUYA for her patience, sacrifices and encouragement, and to our children: ANSELM, SUSANA, TEENDWA, MSURI and SUSIE, who were deprived of their father's presence and personal care in the course of the study.

## ACKNOWLEDGEMENTS

I take much pleasure to register my gratitude to the late Prof. K. Mtotomwema for his supervisory role during the research phase of the study. His constructive criticisms and valuable suggestions are highly appreciated. My subsequent supervisors, Dr. Z.K. Rulangaranga and the late Prof. A.J. Tesha, are also much thanked for their valuable criticisms and suggestions, which facilitated the completion of this work. I am additionally, greatly indebted to the Directors and Curators of the herbaria in Kew (England), Harvard (USA), Geveva (Switzerland) and Lucknow (India), for allowing me full use of their herbaria during my visits abroad. I am furthermore, grateful to the following scientists, through whom I was able to discuss, identify and develop my research topic: Mr. A. Radcliffe- Smith (Kew) and Prof. Bocquet (Geneva) on morpho-taxonomy; and Prof. R.E. Schultes (Harvard) and Prof. J. Maheshwari (Lucknow), for their expert advice on the ethnobotanical research component.

I am deeply indebted to the organizers and partners of the Tanzania Anti-cancer medicinal Plants Project, in whose design I was offered a fellowship to conduct further laboratory, herbaria and literature work in the U.S.A. and the U.K., during time which I benefitted from the rich herbaria, expertise and courtesy of scientists in the respective institutions. These include:

Dr. E. Forero, Director of Research, Missouri Botanical Garden, who is

much thanked for his expert advice and profound inspiration on my research endeavours. I would also like to express my sincere gratitude to Dr. Mick Ballick, Director, Institute of Economic Botany, New York Botanical Garden (Bronx), for his valuable suggestions on ethnobotanical work; Prof. Grady Webster, New York Botanical Garden (Bronx), for valuable discussions on morphometrics and palynology; Dr. Peter Goldblatt, Missouri Botanical Garden, for providing expert advice on my cytological studies; and Dr. Joan W. Nowicke, US National herbarium, Smithsonian Institution, Washington D.C., for introducing me to Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM) techniques, and expert suggestions on palynological studies. I am thankful to the Directors and Curators of these herbaria for affording me the opportunity to work there.

I am particularly indebted to Miss Mary Mellelo, Missiouri Botanical Garden, for furthering my knowledge on SEM techniques and her precious time she spent taking the SEM micrographs at the Monsanto laboratory of Life Sciences, Washington University at St. Louis.

Special thanks also go to Mr. Roy E. Gereau and Prof. Charlotte Taylor of Missouri Botanical Garden, for introducing and offering me the use of their word processor during my visit to Missouri Botanical Garden, and for their willingness to help whenever I had problems with my research work. I thank them further for their

profound encouragement and inspiration, especially during the write-up phase of the study.

I would like to express my sincere appreciation and gratitude to all members of the Department of Botany, University of Dar es Salaam, who, in one way or another facilitated my studies and helped me complete the research successfully.

Special thanks also go to Mr. E.N. Mshiu, Director, Institute of Traditional Medicine, Muhimbili College for his assistance and encouragement at various stages of my research, and also for allowing me to join some of his field trips, which facilitated my field data collection.

I am grateful to Mr. M. Omari, for preparing copies of the SEM micrographs; to Mr. G.G. Mkwemba for his skillful preparation of some of the figures, and to Ms. E.S. Muwanga, for so ably effecting the word processing of the manuscript. I also thank Prof. K.E. Mshigeni and Dr. M.P. Munyenyembe who spared their valuable time to assist in proof-reading and for offering valuable criticisms and suggestions.

My Employer, the Director General of the Muhimbili Medical Centre, is also much thanked for granting me part-time study leave, to pursue the study. My debt of gratitude additionally goes to my wife, Eliambuya, who inspired, supported and encouraged me while I was pursuing this study, and for having to bear with the

succession of months and years of my absence from home, during the study phase.

I am, furthermore, most grateful to the University of Dar es Salaam, which sponsored my entire research programme, through its Research and Publications Committee.

I am, additionally, very thankful to the British Council, which sponsored my visit to England, and the United Nations Industrial Development Organization (UNIDO), which provided me with a fellowship for my visits to the USA, Switzerland and India. During the visits I was able to able to undertake studies at the herbaria and libraries of the institutions in the respective countries.

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# CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

## 1.1 BACKGROUND

The genus *Acalypha* Linnaeus (1753; 1754) (*Acalyphes* Hassk.) (Willis, 1973) belongs to the family Euphorbiaceae, or the spurge family. The former name is conserved over all other names for the family (Lawrence, 1951). The Euphorbiaceae is one of the largest families in the angiosperms. The family is estimated to comprise 300 genera and 7,300 species (Webster, 1967; Webster & Ellis, 1962; Porter, 1967; Willis, 1973). The taxonomy of the family is subject to controversy due to the large number of species, diversification, and high magnitude of morphological variation.

The genus *Acalypha* belongs to the subtribe Acalyphinae Griseb., tribe Acalypheae Dumort., subfamily Acalphoideae Ascherson (Pax and Hoffman, 1931; Webster, 1975). *Acalypha* is one of the least studied genera of the Euphorbiaceae. The genus is also known to be taxonomically problematical, third only to the genera *Euphorbia* and *Phyllanthus* (Radcliffe-Smith, 1985).

## 1.2 BRIEF DESCRIPTION OF THE GENUS *ACALYPHA*

### Habit and Life Form

*Acalypha* species are monoecious or rarely dioecious (as in *A. hispida* Burm.f.) annual or perennial herbs, shrubs and rarely trees. Some species are prostrate, procumbent, ascending or scandent herbs, subshrubs or shrubs. Herbs,

mostly annuals include *A. bussei* Hutch., *A. ciliata*, *A. crenata* A. Rich., Forssk., Several *Acalypha* species are essentially perennial shrubs, for example *A. neptunica*, *A. fruticosa*, *A. bipartita, A. hispida* and *A. engleri* (Pax A few species are shrub-trees and include *A. chirindica*, *A. acrogyna* Pax and *A. boiviniana* Baill.

**Stem**

Stems vary from much branched in *A. chirindica* S. Moore to unbranched in *A. stuhlmanni* Pax. Stems range mostly from puberulous to pubescent, sometimes pilose or hirsute; usually green but later becoming reddish-brown in *A. neptunica* Muell. Arg. and, reddish-greyish or purplish -brown in *A. fruticosa* Forssk. Stems are simple but subquadrangular in *A. bipartita* Muell. Arg.

**Leaves**

Leaves are alternate on long or short petioles. The leaf margin varies from crenate or dentate to sub- entire. They also vary in shape from linear to ovate. Leaf venation is of two types namely, penni-nerved and palmate-nerved; with 5-9 nerves originating at the base and continuing to the leaf margin, and 4-8 pairs of lateral nerves. Leaves are mostly pubescent or subglobrous, rarely glabrous. All *Acalypha* are stipulate and the latter are lanceolate, subulate or setaceous.

**Inflorescence**

Inflorescences are either unisexual (for example in *A. villicanlis* A. Rich.) or bisexual (for example in *A. fruticosa*). When bisexual the sexes are very diversely arranged- axillary, terminally or both. Male inflorescences occur commonly in

slender dense-flowered spikes with one to few female flowers at the base; or the female inflorescences are found alone in relatively short and less dense-flowered racemes. The bracts are often lobed or dentate or accrescent in fruit. The inflorescence types vary from racemose in *A. echinus* Pax & K. Hoffm. and *A. acrogyna*, paniculate in *A. racemosa* Baill. and spicate in *A. welwitschiana* Muell. Arg.

**Flowers**

Male flowers are mostly minute, sessile, subsessile or pedicellate. The calyx is closed in bud, later spliting valvately into 4 segments. They lack petals and disc. The stamens are mostly 8 and free; anthers with free, narrow, twisted, vermiform loculi without pistil. Female flowers are conspicuous, sessile, subsessile or pedicellate. Sepals range from 3 to 5, shortly connate, imbricate; ovary with 3 locules, loculi one-ovulate, styles mostly conspicuous, laciniate, free or connate. Female allomorphic flowers are sometimes present.

**Fruits and Seeds**

The fruits are small, trilobed or bi-lobed. Seeds are round and smooth; sometimes with a conspicuous hilum or caruncle. The fruits are 3 -lobed in normal female flowers as in A. echinus and 1-2 lobed in allomorphic female flowers as in A. indica. The small seeds range from 0.8mm wide and 1.2mm long in *A. racemosa* to 2mm wide and 2.5mm long in *A. stuhllmanii*. Seed shape is also variable, ranging from ellipsoid- ovoid to ellipsoid in *A. bipartita*, *A. neptunica* and

*A. hispida* to ovoid -subglobose to subglobose in *A. gillmanii* and *A. acrogyna*.

## 1.3 TAXONOMIC STATUS OF *ACALYPHA*

### 1.3.1 Palynology

Many palyno-taxonomists like Lindau (1895), Wodehouse (1935), Erdtman (1943, 1952, 1956, 1969), Punt (1962,1967), Kohler (1965) and Webster (1975), have established that pollen morphology can be of great importance for plant taxonomy. Hans (1973) reported that in the three genera of subtribe Acalyphinae, to which *Acalypha* belongs, pollen morphology justifies the separation of the genera on the basis of pollen size but not on the basis of pollen structure.

Punt (1962) demonstrated the importance of pollen morphology in taxonomy in his treatment of the pollen morphology of the Euphorbiaceae. The author employed pollen variations in pollen shape, occurance of apertures and structure elements to study, based on pollen types, the taxonomy of the two subfamilies of the *Euphorbiaceae* namely, *Phyllanthoideae* and *Crotonoideae*. The author separated the *Phyllanthoideae* in three large groups of pollen types, the *Antidesma, Amanoa* and *Aristogeitonia* configurations. A remaining small group was assigned in taxonomic respect to the genera of the pollen types of the *Antidesma* configuration. In the *Crotonoideae*, Punt (Loc.cit) found out that may genera posses pollen grains with croton-pattern. Besides this natural group, the author found that the *Plukenetiinae* possess pollen grains which are clearly distinguished from other genera in the *Crotonoideae*, the observed two large configurations in the *Crotonoideae,* the *Mallotus* configuration which includes most genera of the *Acalypheae* and the

*Hippomane* configuration which comprises the tribe *Hippomaneae* and *Euphorbieae*.

In his commendable book, *Handbook of Palynology* Erdtman (1969) worked on pollen and spore morphology of a diversity of plants ranging from Mosses (*Pleurophascum* species) to advanced plants, for example the families Palmae and Proteaceae. On the basis of polarity of pollen grains, apertures, symmetry (size and shape) and structure, the author was able to establish, in taxonomic aspects, the pollen morphology and taxonomy of both lower plants and the angiosperms. He particularly based his work on what he called the NPC - system in relation to the appearance of apertures (N = aperture number , P = Position and C = character or form).

Embryological investigations on *Acalypha* have been carried out mostly by Indian workers. Arnoldi (1912) reported the *Peperomia* type of embryo-sac in *Acalypha* species and Maheshwari and Johri (1940, 1941) recorded it in *A. indica*. Thathacher (1952) worked on the embryo-sac and inflorescence of *A. lanceolata* and observed the embryo-sac to differ from that in *A. indica*. He also studied the embryo-sac in *A. fallax,* considered by Hooker (1885) and Gamble (1924) to be synonymous to *A. lanceolata*.

### 1.3.2 Cytology

Hans (1973) reported that the subtribe Acalyphinae, to which *Acalypha* belongs, is a taxonomically complex group and on cytological basis comprises many taxa having different base numbers. Miller and Webster (1966) pointed out that

Euphorbiaceous genera such as *Acalypha* and *Croton* were still poorly sampled cytologically.

Hans (1973) observed that the family Euphorbiaceae is believed to be polyphyletic having at least two base numbers x = 7 and 13. The other base numbers he encountered in the family are x = 6,8,9,10,11,12,15,16,17 and 18 coupling with the well known morphological and taxonomical complexity of the family. The author found out that both polyploidy and anaeuploidy are effective in speciation in Euphorbiaceae and that as much as 48 percent of the known taxa exhibit polyploidy.

Earlier Perry (1943) published a comprehensive report which allowed a cytotaxonomical appraisal or the determination of chromosomal interrelationships and evolution in the family Euphorbiaceae. However, there appears to be no consistent role which cytology, has played in circumscribing generic limits in various natural orders. Nevertheless, in demonstrating basic affinities or differences, the chromosomal knowledge can point out some unsuspected situations or endorse the existing opinion (Hans, 1973). Gaps in the chromosomal knowledge are many and some of the interpretations may change as the cytological data accumulate.

### 1.3.3 Taximetrics

The general description of the genus *Acalypha* was provided by Linneus (1754). More contributions based mainly on phytomorphology of *Acalypha* taxa

have been availed by Forsskal (1775), Aiton (1789;1880), Tent (1851), Jaub and Spach (1855), Mueller (1865, 1866), Baillon (1874), Bentham (1880), Bentham and Hooker (1880), Pax (1890) and Engler (1892;1896). Further descriptive work was availed by Knauf (1901), Moore (1911), Prain and Hutchinson (1912), Thiselton-Dyer (1913), Engler and Drude (1921) and, Pax and Hoffman (1924).

Further and more detailed descriptive work on *Acalypha* with rich representation of the Tanzanian *Acalypha* has been provided by Brenan and Greenway (1949), Dale and Greenway (1961), Leonard (1962) and, Palmer and Pitman (1972). Philips (1926), Gardner (1936), Robyns (1948) and White (1962) described *Acalypha* species, some of which are represented in Tanzania. The most elaborate treatment of the Tanzanian *Acalypha* species has been availed by Radicliffe-Smith (1987; 1989).

However, descriptions offered by floras and monographs are usually based on the same morphological characters, and sometimes on limited herbarium material. Hence a more objective approach based on larger plant material and sampled phytomorphometric data is needed. Therefore, this work will attempt to examine more objectively *Acalypha* taxa indigenous to Tanzania so as to justify their classification status on the basis of modern numerical taxonomic methods.

### 1.3.4 Problematical *Acalypha* species

The genus *Acalypha* is represented by 28 species in Tanzania, four of which were recently reported as new records for the region (Radcliffe - Smith, 1987).

However, even in such a recent treatment of the Flora of Tropical East Africa (FTEA) (Radcliffe-Smith, 1.c.), controversies still exist with respect to the taxonomy of *Acalypha* in the country. For example, the authenticity of 12 species, more than 1/3 of the Tanzanian *Acalypha,* is not definitive. The 12 *Acalypha* species are the problematic taxa being focused in the present study and are listed and discussed below:

*A. boiviniana* Baill.

*A. brachystachya* Hornem.

*A. bussei* Hutch.

*A. ciliata* Forssk.

*A. engleri* Pax

*A. fruticosa* Forrsk.

var. *fruticosa*

var. *eglandulosa* A.R.-Sm.

var. *villosa* Hutch.

*A. indica* L.

*A. lanceolata* Willd.

*A. neptunica* Muell. Arg.

var. *neptunica*

var. *pubescens* (Pax) Hutch.

*A. paucifolia* Baker & Hutch.

*A. psilostachya* Hochst.

var. *psilostachya*

var. *glandulosa* Hutch.

*A. villicaulis* A. Rich.

*A. boiviniana* appears to be conspecific with *A. engleri,* indicated by common distributional range. *A. bussei* appears to be a variety of or depauperate ecological form of *A. ciliata.* In *A. fruticosa,* only var. *eglandulosa* appears to be distinct from the two others, vars. *fruticosa* and *villosa,* which differ only in the latter possessing a densely pillose or villose midrib.

*A. neptunica* vars. *neptunica* and *pubescens,* though seeming to maintain their distinctness, are more or less sympatric. *A. paucifolia* appears to be a local race of *A. villicaulis. A. psilostachya* var. *glandulosa* appear to have good variations, but var. *psilostachya* seems variable. Agnew (1974) considers *A. psilostachya* as being synonymous with *A. brachystachya.* Airy Shaw (1975, 1980) observed that except for the difference in the all axillary inflorescence in *A. lanceolata* and broader bracts, and, both axillary and terminal inflorescence in *A. indica,* the two species are in resemblance.

A further intriguing problem is the occurrance of "allomorphic female flowers" in some species of *Acalypha.* Working on *A. segetalis,* Radcliffe-Smith (1978) reported the presence of allomorphic female flowers in the taxon for the first time. Earlier, Radcliffe-Smith (1973) had employed the criterion of diversification in female allomorphic female flowers in the series Polygynae-Pleurogynae and

Polygynae - Acrogynae Muell. Arg. to key 5 critical species of *Acalypha* on the accrescent female bracts and allomorphic flowers thus: *A. brachystachya, A. ciliata, A. crenata, A. lanceolata* and *A. indica.*

However, Kenoyer (1919) working on "dimorphic carpellate female flowers" of *A. indica* observed that right from the beginning of their development, these flowers are monocarpellary. Radcliffe-Smith (1978) reported that problematical *Acalypha* taxa still exist in the "allomorphic female flowers" of the series Oligognae Muell. Arg., examplified by two shrubby East African species, *A. volkensii* Pax and *A. psilostachya.* He concluded that the "homologies of the structures" found in the allomorphic female flowers in *Acalypha* taxa are not clear.

The doubts expressed by Radcliffe-Smith (1973, 1978) as to the relationship of some of the structures of the allomorphic female flowers in *Acalypha* species were later resolved by Seberg (1984) who established the homology between the allomorphic female and the female flowers in the genus by viewing it in line with the deviating apical flower found in genera of other families with monotelic inflorescence. Consequently, the aspect of allomorphic female flowers in *Acalypha* is not considered in this study.

The full list of the 28 Tanzanian *Acalypha* species are listed below including their synonyms. The herbaria where the type specimens of the various species are deposited are given in Appendix 1.1.

*A. acrogyna* Pax

*A. ambigua* Pax

*A. bipartita* Muell. Arg.

*A. boiviniana* Baill.

*A. brachystachya* Hornem. Syn. *A. elegantula* Hochst.

*A. bussei* Hutch.

*A. chirindica* S. Moore

*A. ciliata* Forssk. Syn. *A. fimbriata* Schumach. & Thonn.; *A. vahliana* Muell. Arg.

*A. crenata* A. Rich. Syn. *A. abortiva* Baill., *A. indica* L. lusus *abortiva* (Baill.) Muell. Arg.; *A. vahliana* sensu Oliv.)

*A. echinus* Pax & K. Hoffm.

*A. engleri* Pax

*A. fruticosa* Forssk.

var. *fruticosa; syn. A. betulina* Retz.

var. *villosa* Hutch.

var. *eglandulosa* A.R. -Sm.; Syn. *A. holtzii* Pax & K.Hoffm.; *A. kilimandscharica* Pax & K. Hoffm., *A.* sp. A. sensu Agnew (1974)

*A. gillmanii* A.R. -Sm.

*A. hispida* Burm. f.

*A. indica* L. Syn. *A. bailloniana* Muell. Arg., *A. chinensis* Benth.; A. somalium Muell. Arg.; *A. indica* L. var. *bailloniana* (Muell. Arg.) Hutch.

*A. lanceolata* Willd. Syn. *A. boehmerioides* Miq.;

*A. boehmerioides* Miq. var. *glandulosa* (Muell. Arg.) Pax & K. Hoffm.;

*A. crenata* A. Rich. var. *glandulosa* Muell.Arg.; *A. glomerata* Hutch.; *A. fallax* Muell. Arg.

*A. neptunica* Muell. Arg.

var. *neptunica*. Syn. *A. mildbraediana* Pax var. *glabrescens* Pax; *A. neptunica* Muell. Arg. var. *glabrescens* (Pax) Pax & K.Hoffm.; *A. subsessilis* Hutch. var. *glabra* Pax & K. Hoffm.

var. *pubescens* (Pax) Hutch. Syn. *A. mildbraediana* Pax var. pubescens Pax; *A. neptunica* Muell. Arg. var. vestita Pax & K. hoffm.; *A. subsessilis* Hutch. var. *mollis* Hutch.

*A. nyasica* Hutch.

*A. ornata* A. Rich. Syn. *A. grantii* Baker & Hutch.;

*A. swynnertonii* S. Moore

*A. paucifolia* Baker & Hutch.

*A. psilostachya* Hochst.

var. *psilostachya*. Syn. *A. johnstonii* Pax

var. *glandulosa* Hutch. Syn. *A. bequaertii* Staner

*A. racemosa* Baill. Syn. *A. paniculata* Miq.

*A. stuhlmanii* Pax

*A. villicaulis* A. Rich. Syn.*A. villicaulis* var. *minor*

Muell. Arg.; *A. senensis* Klotzsch; *A. sidaefolia* A. Rich.

*A. volkensii* Pax Syn. *A. psilostachyoides* Pax

*A. welwitschiana* Muell. Arg. Syn. *A. angolensis* Muell. Arg.

*A. wilkesiana* Muell. Arg. Syn. *A. godseffiana* Mast

*A. marginata* J.J. Sm.

*A.* sp. A. A.R. -Sm.

## 1.4 PHYTOGEOGRAPHICAL ASPECTS

The exact size and distribution of the genus *Acalypha,* as with the family Euphorbiaceae, is still indefinite. Thiselton- Dyer (1913) reported the genus to comprise 300 species occuring throughout the warmer parts of both hemispheres, few in tropical America. Fourty two species are represented in Africa and include most of the Tanzanian taxa. Pax and Hoffman (1931) observed that the genus, comprised of 320-430 species, is chiefly distributed in two centres, one in tropical America and the other in the Africa-Madagascar region. Hans (1973) and Radcliffe-Smith (1987) provided more reliable information that *Acalypha* is a large genus of 450 species, well represented throughout the tropics of both hemispheres, with a few species existing outside the tropics in the new world. There are about 50 species in Africa, of which 28 occur in Tanzanian, two of these being cultivated (Radcliffe-Smith, 1987).

The work of Hutchinson and Dalziel (1958) indicates that the size of the genus *Acalypha* lies between 400-450 species. Other contributions on the phytogeography of *Acalypha* include the work of Dale (1936), Troupin (1982) and White (1962). The work of Radcliffe-Smith's (1987) shows that the genus *Acalypha* is well distributed in Africa with about 30 species occuring in East Africa. Radcliffe-Smith's

(1987) recent treatment for the Flora of Tropical East Africa (FTEA) appears to shed light on the phytogeography of the Tanzania *Acalypha* species but is based on herbarium material.

## 1.5 ETHNOBOTANICAL STATUS

Webster (1967) reported that because of their chemical diversity, the members of the family Euphorbiaceae include genera of economic importance. The genus *Acalypha* is no exception to that generalisation, and contains taxa reported to be of medicinal and aesthetic values. For example, *A. indica* contains the chemical acalyphine and is used clinically as an expectorant, emetic and diaphoretic (Gibbs, 1974). Pharmacologically, it has irritant and poisonous effects due to cyanogenetic glycocides, and contains resins, tannins, and a volatile oil (Watt and Brandwijk 1962). *A. hispida* and *A. wilkesiana,* apart from being medicinally useful, are also important ornamental plants. However, apart from some scanty reports on the medicinal and other uses of Tanzanian species of *Acalypha,* no exhaustive work has so far been conducted with respect to the ethnobotany of Tanzanian *Acalypha* species.

## 1.6 STATEMENT OF THE PROBLEM

(i) The genus *Acalypha* is a taxonomically complex group and among the least studied genera of the family Euphorbiaceae (Hans, 1973). Most taxonomic work that has been carried out to designate Tanzanian *Acalypha* to their deserved taxonomic rank is based on descriptive phytomorphology. This has resulted

in controversies over the taxonomic status of the entire genus. Also, available literature indicates that a classification system for Tanzanian *Acalypha* is non-existent.

(ii) Cytologically, only 10 out of the 28 *Acalypha* species occuring in the country have been studied; and yet controversies still exist with regard to the chromosome counts and basic chromosome numbers of the respective species (Datta, 1967; Miller, 1964; Miller and Webster, 1966).

(iii) Palynologically, it has been established (Erdtman, 1969; Punt, 1962; Webster, 1975) that pollen morphology and pollen morphometrics can be of great importance for plant taxonomy. However only 5 *Acalypha* species out of the 28 occuring in the country have been treated with respect to pollen studies.

(iv) Information regarding the phytogeography of Tanzanian *Acalypha* species is not exhaustive. Moreover, the available information is mostly based on herbarium material.

(v) The ethnobotanical information available in the country with regard to the genus *Acalypha* is scanty and is mostly based on herbarium notes and literature.

## 1.7 OBJECTIVES

The following were the main objectives of the study:

(i) To study the pollen morphology of Tanzanian *Acalypha* taxa with emphasis on the taxonomically critical taxa.

(ii) To carry out cytological studies on the *Acalypha* taxa represented in Tanzania.

(iii) To conduct taximetric analysis on the genus in order to establish a classification for *Acalypha* taxa occuring in the country.

(iv) To carry out extensive phyto-geographical studies from diversified ecological habitats throughout the country.

(v) To broaden the knowledge on the ethnobotany of the genus *Acalypha* in Tanzania by conducting extensive field studies.

## 1.8 HYPOTHESES TESTED ARE:

(i) The general taxonomic controversies associated with the genus *Acalypha* have been eliminated by employing a wider range of taxonomic data, inclusive of palynology, cytology and taximetrics.

(ii) The application of palynological, cytological and taximetric data has facilitated the establishment of an informal classification system for the genus *Acalypha* in Tanzania.

(iii) Gaps of knowledge on the phytogeography of *Acalypha* in Tanzania have been eliminated by employing ecological and geographical data.

(iv) Frontiers of knowledge on the ethnobotany of *Acalypha* have been advanced through extensive field surveys and interviews with traditional healers and knowledgeable people in conjuction with literature search for chemical and biological activities of the *Acalypha* species.

# CHAPTER TWO: PALYNOLOGICAL STUDIES

## 2.1 INTRODUCTION

Punt's (1962) palynological treatment of the Euphorbiaceae established the *Acalypha* type pollen grains. He found out that the *Acalypha* type incorporates the genera closely allied to the tribe *Acalypheae,* viz., *Alcornea, Blumeodendron, Discocleidon, Mareya* and *Mallotus,* which he found to be closely related, with only minor differences. He found the *Acalypha* type to be the most related to the *Mallotus* type. However, the dimensions of the colpi transversalis and the colpi are so small that the composite aperture resemble pori. *Acalypha* type is typified by pollen grains which are tricolporate or stephanocolporate, and oblate spheroidal to suboblate. The pollen grains are quasi porate. With high magnification, the apertures are undoubtedly composite apertures. Colpi transevalis and colpus are of the same size; costae, tectate and psilate. Two of the *Acalypha* species reported (Punt, 1962) are represented in Tanzania, viz., *A. indica,* with Polar (P) to Equatorial (E) ratio (P:E) = 0.90 and *A. racemosa,* P:E = 0.89, 3-colporate.

Khan (1968) observed that pollen grains are oblate spheroidal in *A. ciliata* and suboblate in *A. indica,* 3- zonocolporate in both and, exine 1.5$\mu$m thick in *A. indica* and 2$\mu$m thick in *A. ciliata* (ectine = endine). Further, in both *A. indica* and *A. ciliata*, ornamentation is psilate with OL pattern, and colpus is small with pointed

ends. The endocolpium is elongate and longer than colpus in *A. indica* and dumb-bell shaped in *A. ciliata.*

Huang (1972) observed that pollen grains in *Acalypha* are 3 (-4)- colporate; suboblate to spheroidal, 10-16 x 10-16$\mu$m; amb semi-angular or circular; colpi very short, crassimarginate, 5.7 x 3. 5$\mu$m aperture drop type, ora transversally elliptic, 1-2 x 3-4$\mu$m exine less than 1$\mu$m thick; tectum psilate; sexine fine reticulate; nexine thinner than sexine.

Working on pollen grains of *A. crenata, A. fruticosa* and *A. indica,* Bonnefille and Riollet (1980) characterized pollen grains in *Acalypha* as isopolar, 3 (-4) -colporate, more or less equal axes, subcircular near meridian and near poles; 11-12 x 11 - 13$\mu$m; apertures complex (sensu Punt, 1962); costae, endoapertures present; ornamentation of exine simple. Apertures are dominantly 4-colporate in *A. crenata,* 3-colporate in *A. fruticosa* and 3 (-4) -colporate in *A. indica.*

Anand *et al.* (1982) worked on the pollen grains of *A. ciliata* and *A. indica.* The authors described pollen grains in *A. indica* as being suboblate (P.E 13/20$\mu$m = 0.65 , range 14-16/18$\mu$m); os lalongate (size 2.5/1$\mu$m); exine 2$\mu$m thick. Pollen grains in *A. ciliata* are 3-zonocolporate, oblate-spheroidal (P.E 33/37 = 0.90$\mu$m); os lalongate (size 2/1$\mu$m); colpi long with psilate membrane; exine 2$\mu$m thick, sexine slightly thicker than nexine, tegillate, psilate; nexine psilate.

It is therefore, evident that some differences exist in the pollen morphology of *Acalypha* species, as exemplified by *A. ciliata, A. crenata, A. fruticosa, A. indica* and *A. racemosa,* species which are represented in Tanzania. The current work is a further attempt to study the pollen morphology of Tanzanian *Acalypha,* with emphasis on the taxonomically critical taxa.

## 2.2 MATERIALS AND METHODS

### 2.2.1 Source and sampling of plant material

Flower material for the preparation of Acetolysed Pollen Slides (APS) and Scanning Elecron Micrographs (SEMGs) were obtained from authenticated herbarium material from Kew (United Kingdom) and Missouri Botanical Garden (USA). These were *Acalypha* plant materials collected from Tanzania and deposited in these herbaria. These plant materials were used to ensure that authentic *Acalypha* taxa are used in the study. The details on the localities of the collections are shown in appendix 5.1. Fresh plant materials were not used since fresh pollen material is not ideal for palynological work. The ASP and SEMGs were prepared at the Monsanto Laboratory of Life Sciences, Washington University (St. Louis), USA during my research visit in 199O.

### 2.2.2 Preparation of acetolysed pollen grains

Pollen grains were acetolysed using the procedures of Erdtman (1943, 1952), Nilsson (1974) and Pacini (198O). The anthers were placed in a marked centrifuge tube and crushed with a glass rod; a separate glass rod being used for each tube containing a different sample, and left the rod in the tube. Each tube was filled with

10 ml of a mixture of a 9:1 acetic anhydride and concentrated sulphuric acid (ACETOLYSIS LIQUID) and boiled in a water bath for a minute. Care was taken not to over-boil, as water in the tubes causes the acetolysis liquid to spatter. The tubes were removed from the water bath immediately after boiling time, rods were removed and the tubes were corked to avoid the potentially corrosive vapour. The tubes were immediately transferred to a centrifuge and centrifuged for one minute at 3/4 speed (approximately 2000 revolutions per minute) until the material was precipitated. The supernatant liquor was decanted in a waste bottle marked ACETOLYIS and discarded.

### 2.2.3 Filtration of pollen material

Distilled water was added up to 1/3 of each centrifuge tube, followed by a few drops of absolute alcohol and the tubes were corked. Each tube was shaken up well to suspend the material thoroughly and the material was filtered twice into a black tube through a copper net (filter) (approximately 5O$\mu$m wide) and funnel. Care was taken to wash the funnel and net between each filtration, and particularly where different material. The washed and filtered materials were filtered was centrifuged for one minute at 2000 revolutions per minute. The water was decanted and about 15 drops of a 1:1 glycerine: water mixture added to the tube, and allowed to stand for 30 minutes and again centrifuged. The glycerine-water mixture was decanted and, keeping the tube upside-down, placed on a filter paper for one hour to dry and left in that position to avoid contamination. The pollen material was now used for APS preparations. For tubes left much longer than an hour, glycerine-water mixture was added, covered tube with parafilm paper and re-centrifuged before

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decanting and drying procedure.

### 2.2.4 Preparation of acetolysed pollen slides (APS)

Permanent slides were prepared by embedding the pollen material in glycerine-gelatin jelly and sealing with paraffin wax (melting point 69-73° C). The glycerine-gelatin jelly was prepared by dissolving a mixture of phenol crystals (3.5 gm), gelatin grains (25.0 g), and glycerine (75.0 cc) in distilled water (87.0 g) in a steam bath under stirring allowing it to cool in the same steam bath.

In the process, two tiny pieces of plasticine were placed diagonally near one end of the glass slide. Glycerin-gelatin jelly was placed amidst the two pieces of plasticine. Pollen grains were tranfered onto the jelly from the tube using a dissecting pin. The slide was warmed gently on a bunsen flame to allow the jelly (hence the pollen grains) to spread evenly on the slide. Sometimes a pin was used to spread the grains. The preparation was covered by a slide cover. Then a small paraffin wax block was placed beside the slide cover and warmed gently to allow the ensuing liquid to spread under and round the slide cover, driving out any water bubbles. The slide was left to cool.

Exess parrafin was removed with a razor and the slide was cleaned with xylene soaked in cotton wool. Finally, the slide was cleaned with a good, clean soft paper and labelled as appropriate.

### 2.2.5 Measurement of pollen grains

Pollen grain measurements were taken and standard procedures were used to avoid the phenomenon of the "Cushing Effect"(Cushing 1961) and metric units were employed. The description of the pollen grains was made and follows closely that of Erdtman (1943; 1952) and Punt (1962).

### 2.2.6 Preparation of Scanned Electron Micrographs (SEMGs)

Non-acetolysed pollen grains were used to prepare Scanning Electron Micrographs (SEMGs). *Acalypha* flowers are very small, hence about 5-10 flowers of each taxon were transfered and applied onto a specimen stub, using a x 30-50 stereo-microscope and dissecting needles. The pollen was abstracted from the anthers and spread on the stub under the microscope using the needles. The specimen stubs, six at a time, were tranferred to a fine coating sputter equipment called POLARON-E5000, and coated with gold for 5 minutes. The specimen stubs representing the different material were fitted into the scanning elecron microscope, one at a time. The SEMGs were taken using a HITACHI Scanning Electron Microscope model S-450.

### 2.2.7 Characters of SEMGs studied

#### 2.2.7.1 Acetolysed pollen slides

The acetolysed pollen slides were used to study the structure of the pollen grains. The polar and equatorial axes, and their ratio were determined (Figure 2.1). Acording to Erdtman (1943), Polar axis (P) is the perpendicular line connecting the poles of a pollen grain, and the Equatorial axis (E) the line perpendicular to the Polar axis in the equatorial plane (Van Zinderen Baker, 1953).

On the basis of P:E ratio, Erdtman (1952) and later Punt (1962) distinguished the following classes:

P: E 1 - 0.88 : Oblate Spheroidal

0.88 - 0.75: Suboblate

0.75 - 0.50: Oblate

Measurements were taken using a calibrated light microscope, objective 1000x, oculair 8x in millimicrons. A total of 10 replications were made in each case. Standard error (SE) of the mean for each taxon and confidence limits (CL) at 95% were calculated using the formula: SE = SD/n; and CL = SE x 1.96.

#### 2.2.7.2 Scanned Electron Micrographs (SEMGs)

The SEMGs were used to study pollen shape and surface structure.

## 2.3 RESULTS

### 2.3.1 Pollen morphometrics

Mean values for polar and equatorial axes for all the *Acalypha* taxa studied including the P:E ratio, shape, apertures and exine thickness are given in Table 2.1.

The pollen morphometric data indicate significant variations in polar and equatorial dimensions. The polar to equatorial ratios also reveal significant variations in some of the taxa. Pollen grain size variation within each taxon at 5% is shown in Table 2.1. The reported pollen grain size in *Acalypha* species is shown in Table 2.2.

**Acalypha groups based on shape**

According to the pollen grain shape classes reported by Erdtman (1952) and Punt (1962), the *Acalypha* taxa studied in the present study fall into three classes namely; oblate, suboblate and oblate spheroidal pollen grains.

**Group I:** Oblate spheroidal pollen grains

This class comprises of pollen grains with a P:E index ratio ranging from 0.88 to 0.98. Most *Acalypha* (18 taxa) fall under this category (Table 2.1 Columns 5 and 6 and 2.3).

**Group II:** Suboblate pollen grains

This class comprises of pollen grains with a P:E index ratio range of 0.81 to 0.87. Nine *Acalypha* taxa belong to this group. (Table: 2.1 and 2.4).

**Group III:** Oblate pollen grains

Three *Acalypha* taxa belong to this class. It comprises of pollen grains with a P:E index ratio range of 0.64 to 0.74 (Tables 2.1 and 2.5).

A pictorialized scattergram illustrating the variations in equatorial axis in ralation to polar axis is given in Figure 2.2.

The scattergram elucidates a distinct separation of only three taxa namely *A. ciliata, A. lanceolata* and *A. stuhlmannii*. Otherwise, generally all the taxa in the genus depict an incoherent scattering, without a clear assortment of the taxa on the

basis of the two parameters employed in the study.

**Acalypha groups based on size**

The study reveals further that equatorial axis is always larger than the polar axis as indicated by P/E ratio always less than unity (1.00). When the equatorial size (P) component is used to determine grouping in the genus, three classes were identified (Table 2.6-2.8 and Figure 2.2).

(a) **Small sized pollen grains:** Range, E = 7.6 - 9.9$\mu$m. Eleven *Acalypha* taxa belong to this group and are listed in Table 2.6.

(b) **Medium sized pollen grains:** Range, E = 10.0 - 11.4$\mu$m. This group of *Acalypha* also comprises eleven taxa (Table 2.7).

(c) **Large sized pollen grains:** Range, E = 11.5 - 13.0$\mu$m. This group includes eight *Acalypha* taxa and are listed in Table 2.8.

On the basis of shape the Polar to Equatorial indices categorize the genus *Acalypha* into three groups. Most of the taxa (18 taxa) belong to the oblate spheroidal class with P:E indices ranging from 0.88 $\pm$ 0.04 in *A. wilkesiana* to 0.98 $\pm$ 0.03 in *A. bussei* (Table 2.3).

The second group encompasses 9 *Acalypha* taxa with suboblate pollen grains with P:E indices of 0.81 $\pm$ 0.06 in *A. palicifolia* to 0.87 $\pm$ 0.06 in *A. hispida* and 0.87 $\pm$ 0.01 in *A. chirindica* (Table 2.4).

The third group comprises of 3 *Acalypha* taxa with oblate pollen grains with P:E ratios ranging from 0.64 $\pm$ 0.08 in *A. lanceolata,* 0.70 $\pm$ 0.06 in *A. ciliata* and 0.74 $\pm$ 0.05 in *A. stuhlmanii* (Table 2.5).

### 2.3.2 Pollen morphology

In equatorial view, the pollen grains in *Acalypha* are mostly tri-colporate and rarely quadricolporate. In polar view, the *Acalypha* taxa are characterized by pollen grains which are mainly oblate spheroidal, less so suboblate and rarely oblate. The shape range for the genus is P:E = O.64 in *A. lanceolata* to 0.98 in *A. bussei.*

The *Acalypha* pollen grains are psilate and costae and belong to two structural categories, namely the tectate sub-type and reticulate sub-type. Only two species *A. ciliata* (Plate 2.1) and *A. lanceolata* (Plate 2.2) belong to the reticulate sub-type of pollen grains. The other *Acalypha* taxa studied belong to the tectate sub-type pollen grains. For example, *A. paucifolia* (Plate 2.3), *A. indica* (Plate 2.4), *A. bussei* (Plate 2.5), *A. villicaulis* (Plate 2.6) and the *A. fruticosa* group; viz., var. *fruticosa* (Plate 2.7), var *villosa* (Plate 2.8) and var. *eglandulosa* (Plate 2.9).

In the reticulate sub-type, the intra-reticulum is distinct in both species, *A. ciliata* and *A. lanceolata*. There is no distinction in the exine sculpture of the two reticulate sub-type of *Acalypha* species.

Similarly, the tectate sub-type pollen grains depict a homogenous tectum and costae in the respective *Acalypha* taxa. However, the muri of the pollen grains vary slightly in size and shape. For example, the muri in *A. paucifolia* are larger and more elongated than in the other tectate sub-type of *Acalypha* species (Plate 2.3). The muri structures in *A. bussei* have pointed elements (Plate 2.5). The other tectate sub-type *Acalypha* taxa have muri which appear to form a "continuum" of structure elements as examplified by *A. indica* (Plate 2.4), *A. villicaulis* (Plate 2.6 and the *A. fruticosa* group (Plates 2.7, 2.8 and 2.9).

The *Acalypha* pollen grains are typically quasiporate and have composite apertures. The amb is semi-angular, occasionally circular in shape. The colpus transversalis and the colpus are of the same size and possess a psilate membrane. The ornamentation of the exine is simple and is psilate with OL pattern. The exine is relatively thick and the thickness range for the *Acalypha* is between $< 1.0$ $\mu$m in *A. volkensii* and 2.5 $\mu$m in the *A. reptunica* group.

## 2.4 DISCUSSION

### 2.4.1 Pollen morphometrics

The P:E indices do not appear to provide a significant separation of the problematical taxa under study. For example in the *A. fruticosa* group, the three varieties have oblate spheroidal pollen grains. Indeed, var. *eglandulosa* (P:E = 0.95 $\pm$ 0.03) does not differ from var. *villosa* (P:E = 0.95 $\pm$ 0.02). There is, however,

appreciable departure from the two sister varietal taxa with regard to var *fruticosa* with P:E = 0.89 $\pm$ 0.07 (Table 2.1).

Similary the P:E index criterion has not been able to significantly separate varietal taxa in *A. neptunica* and *A. psilostachya*. However, the P:E index of 0.91 $\pm$ 0.02 in *A. psilostachya* var. *psilostachya* indicate a possibility to separate this variety from the sister variety *A. psilostachya* var. *glandulosa* (P:E = 0.95 $\pm$ 0.03). In *A. neptunica* there is insignificant difference in the P:E index of 0.86 $\pm$ 0.04 in var. *neptunica* compared to 0.86 + 0.007 in var. *pubescens*. The varieties have oblate spheroidal pollen grains (Table 2.3).

It has been reported that *A. ciliata* appears to be a variety or ecological form of *A. bussei* (Raddiffe - Smith, 1987). The P:E indices obtained in the present study resolve this controversy since P:E ratio of 0.98 $\pm$ 0.04 and exine thickness 1.5$\mu$m in *A. bussei* is significantly different from the P:E index of 0.70 $\pm$ 0.06 and 2.0$\mu$m exine thickness in *A. ciliata* to substantiate the distinctiveness of these *Acalypha* species. (Table 2.1, Plate 2.1). *A. ciliata* has oblate pollen grains whereas *A. bussei* has strongly oblate spheroidal pollen grains. The pollen grains of the two species are also different in that they have processes of different shapes as depicted by plates 2.1 and 2.5.

Agnew (1974) considered *A. brachystachya* as being synonymous to *A. psilostachya*. The present study appears to shed some light on the status of these two

*Acalypha* species. In *A. brachystachya* the P:E ratio is 0.89 $\pm$ 0.05 whereas in the *A. psilostachya* group, the P:E index is 0.91 $\pm$ 0.02 in var. *psilostachya* and 0.95 $\pm$ 0.03 in var *glandulosa*. The exine thickness is 1.6$\mu$m in *A.brachystachya* and slightly thicker (1.8$\mu$m) in the *A. psilostachya* group. This suggests that there are differences between the two species. The two *Acalypha* species posses oblate spheroidal pollen grains and are tricolporate.

In his authoritative work on the Flora of Tropical East Africa (Euphorbiaceae), Radcliffe-Smith (1987) observed that *A. paucifolia* appeared to be a local race of *A. villicaulis*. The present study tends to resolve this problem since the P:E index of 0.81 $\pm$ 0.06, 3-4 apertures and 2.0$\mu$m exine thickness recorded for *A. paucifolia* is significantly different to the P:E ratio of 0.85 $\pm$ 0.06, 3 apertures and exine thickness of less than 1.0$\mu$m in *A. villicaulis*. He also reported that there was resemblance in two species of *Acalypha* namely *A. indica* and *A. lanceolata*. It has been found in the present study that, with a P:E index of 0.82 $\pm$ 0.04, 3-4 apertures and 1.8$\mu$m exine thickness in *A. indica* and 0.64 $\pm$ 0.08, 3 aperturtures and 2.0$\mu$m exine thickness in *A. lanceolata,* these are definitely distinct species of *Acalypha*. *A. indica* has suboblate pollen grains and *A. lanceolata has* strongly oblate pollen grains.

Radcliffe-Smith (1987) stated that *A. boiviniana* seems to be conspecific with *A. engleri*. The worker also identified hybridization between *A. crenata* x *A. indica* and named the hybrid *A.* x *koraensis* A.R.-Sm. The pollen grains of the hybrid and

those of *A. boiviniana* could not be studied due to lack of flower material.

The pictorialized scattergram (Figure 2.2) depicts three classes of pollen grains on the basis of the equatorial size component. Eleven *Acalypha* taxa belong to each of two classes, namely small and medium sized pollen grains, and eight *Acalypha* taxa to the third class of large sized pollen grains.

On the basis of the equatorial size criterion, pollen grain size have been able to resolve the controversies existing with respect to some *Acalypha* taxa (Table 2.1). The differences in the equatorial sizes of *A. bussei* (E= 9.4$\mu$m $\pm$ 0.76$\mu$m) and *A. ciliata* (E = 12.2$\mu$m $\pm$ 0.38) appear to be significant to delimit them as distinct species despite the fact that previously *A. bussei* was considered to be a variety of *A. ciliata*. In the *A. fruticosa* group, E = 10.5$\mu$m $\pm$ 0.81 in var. *fruticosa,* E = 7.6$\mu$m $\pm$ 0.41 in var *eglandulosa* and E = 8.8$\mu$m $\pm$ 0.28 in var. *villosa*. The observations suggest a meaningful variation in var. *fruticosa* compared to the much closer vars. *eglandulosa* and var. *villosa*. It also concurs with previous work (Radcliffe-Smith, 1987) in which it was concluded that var. *fruticosa* is much closer taxonomically to var. *villosa* than to var. *eglandulosa*.

Although both species that follow fall under the category of large sized pollen grains, the equatorial size of *A. indica* (E = 11.8$\mu$m $\pm$ 0.63) is significantly different from *A. lanceolata* (E = 13.2$\mu$m $\pm$ 0.87), species which have been considered by Radcliffe-Smith (1987) to resemble one another. The equatorial size

of *A. paucifolia* (E= 11.8$\mu$m $\pm$ 0.77) is distinct from that of *A. villicaulis* (E = 9.6$\mu$m $\pm$ 0.64) which is sufficiently significant to refute a previous report by Radcliffe-Smith (1987) that *A. villicaulis* appears to be a local race of *A. paucifolia*.

The unravelling of the fore-going taxonomic controversies by the equatorial size component of the pollen grain clearly supports the observations indicated above by the P:E index criterion in resolving the same controversies reported by earlier workers with regard to the genus *Acalypha* (Radcliffe-Smith, 1987).

However, the equatorial size component as a taxonomic tool has not facilitated the revision of the varieties of *A. neptunica* and *A. psilostachya* groups. Also, unlike in the previous application of the P:E index in resolving the synonymy problem in *A. brachystachya* and *A. psilostachya*, the equatorial size criterion seems to support previous workers that *A. brachystachya* (E =10.6$\mu$m $\pm$ 0.61) appears to be a synonym of *A. psilostachya;* E = 10.4$\mu$m $\pm$ 0.58 in var. *psilostachya* and E = 10.2$\mu$m $\pm$ 0.53 in var. *glandulosa.*

### 2.4.2 Pollen morphology

The *Acalypha* pollen grains studied are tricolporate or stephanocolporate. The pollen grains shape range from oblate spheroidal, suboblate to oblate. These observations concur significantly with previous work (Punt, 1962; Bonnefille *et al;* 1980; Anand *et al.*, 1982). However, the P:E index range for the *Acalypha* species studied is 0.64 to 0.98; compared to the reported range of 0.84 to 0.96 (Tables 2.1, 2.2), that is oblate spheroidal to suboblate.

This study has, therefore, established that some *Acalypha* species have oblate pollen grains,as examplified by *A. lanceolata, A. ciliata* and *A. stuhlmanii*. The P:E index of 0.70 (*A. ciliata)* obtained in this work does not, however, accord with that reported by Anand *et al.* (1982) of P:E = 0.89. Otherwise the P:E index class for the other *Acalypha* species reported earlier viz., *A. crenata,A. fruticosa, A. indica* and *A. racemosa* accord with the observations made in this study (Table 2.1 and 2.2).

Pollen grains in the *Acalypha* species studied are quasi porate, costae and posses composite apertures. The colpus transversalis and the colpus are of the same size and the ornamentation is psilate. The reported exine range (< 1.O - 2.0 $\mu$m) accords to a great extent with the findings of this study (< 1.0-2.5 $\mu$m). Again, these findings accord significantly with the work of previous workers, notably Punt (1962), Bonnefille *et al.* (1980) and Anand *et. al.* (1982).

The study has established two categories of structures within the *Acalypha* pollen grains namely, the tectate subtype and the reticulate subtype. The latter subtype was first reported by Huang (1972) as occuring in the genus *Acalypha,* without indicating the specific taxon depicting the fine reticulum. This work has shown that the exine of two of the *Acalypha* taxa studied, *A. ciliata* and *A. lanceolata* have a distinct intrareticulum (Plates 2.1 and 2.2). The majority of the *Acalypha* species posses a tectate subtype of pollen grains; for example *A. paucifolia, A. indica, A. bussei, A. villicaulis* and the *A. fruticosa* group (Plates 2.3

to 2.9). The typical tectate subtype of *Acalypa* pollen grains, with indistinct intra-reticulum, is supported by the findings of earlier workers (Punt, 1962; Huang, 1972).

The *Acalypha* pollen grains closely related to the *Mallotus* type (Punt, 1962), particularly to the tectate subtype typified by *A. paucifolia* and the other *Acalypha* taxa which belong to this subtype. Within the *Mallotus* type, the genus *Adriana* with intra-reticulate pollen grains, a rare phenomenon in the *Mollotus* type, (Punt, 1962) relates the genus *Adriana* to the reticulate *Acalypha* subtype pollen grains, as exemplified by *A. ciliata* and *A. lanceolata.* The *Acalypha* type is also related, although to a lesser extent compared to the *Mallotus* type, to the *Alchornea* type, *Blumeodendron* type, *Discocleidon* type and *Mareya* type. The latter genera types and the *Acalypha* type share some common pollen character states. For example, the pollen grains are tricolporate or stephanocolporate, oblate to oblate spheroidal,colpus transversalis with costae (except *Mareya* type), tectate and psilate.

On the basis of shape and pollen grain structure, the hitherto distinct *Acalypha* species, *A. ciliata* and *A. lanceolata* do not justify their specific status. The observations suggest a taxonomic reduction of the two species to subspecies or varieties. This, however, demands further studies on other taxonomic attributes. For example, cytological studies indicate that n = 10 in *A. ciliata* whereas n = 14 in *A. lanceolata.*

Pollen structure in this study supports the pollen morphometric data in delimiting *A. paucifolia* from *A. villicaulis,* taxa previously considered (Radcliffe-Smith, 1987) as being local races. Although the two *Acalypha* species possess tectate subtype pollen grains, *A. paucifolia* has much larger and elongated muri compared to the muri in *A. villicaulis* (Plates 2.3 and 2.6). *A. paucifolia* and *A. villicaulis* seem also to differ in pollen size and number of apertures. Radcliffe - Smith (1987) considered *A. bussei* to be a variety or ecological form of *A. ciliata.* Pollen ultrastructure has resolved this problem since *A. ciliata* posses intra-reticulate pollen grains whereas the pollen grains in *A. bussei* are tectate (Plates 2.1 and 2.5). These observations are clearly supported by exine thickness and pollen morphometrics.

Similarly, pollen morphometric data support that *A. lanceolata* is distinct from *A. indica.* This is further supported by pollen ultrastructures since *A. lanceolata* possesses pollen grains with an intra-reticulum as opposed to the tectate pollen grains in *A. indica.* Working on phytomorphology of *Acalypha,* Radcliffe - Smith (1987) observed that the two *Acalypha* species resembled one another.

Hans (1973) reported that in the three genera of the subtribe Acalyphinae including *Acalypha,* pollen morphology justifies the separation of the genera on the basis of pollen size but not on the basis of pollen structure. This observation is only partly supported by this study since both pollen size and pollen ultrastructure have been applied in this work to separate problematical *Acalypha* species. However, the two parameters have not been able to significantly separate *Acalypha* varieties.

Nevertheless, pollen size has provided more light on the taxonomic status of the three varietal taxa in the *A. fruticosa* group viz., vars. *fruticosa, villosa* and *eglandulosa.*

Table 2.1. List of Acalypha taxa examined together with a summary of their major pollen morphological characters.

| Taxa | Collector | P± CL (μm) | E ± CL (μm) | P/E ± CL | Shape | No. of Apertures | Exine thickness (μm) |
|---|---|---|---|---|---|---|---|
| A. acrogyna | Ford 194 | 9.4 ± 0.43 | 10.2 ± 0.54 | 0.92 ± 0.04 | OBS | 3 | 1.7 |
| A. ambigua | Boaler 153 | 7.8 ± 0.26 | 8.6 ± 0.33 | 0.91 ± 0.02 | OBS | 3 | 1.5 |
| A. bipartita | Conrads 5781 | 8.2 ± 0.38 | 9.7 ± 0.47 | 0.84 ± 0.03 | SOB | 3 | 1.5 |
| A. boiviniana | Boivin s.n.1848 | - | - | - | - | - | - |
| A.brachystachya | Richards 21047 | 9.5 ± 0.49 | 10.6 ± 0.61 | 0.89 ± 0.05 | OBS | 3 | 1.6 |
| A. bussei | Vollesen 329(e) | 9.2 ± 0.72 | 9.4 ± 0.76 | 0.98 ± 0.04 | OBS | 3 | 1.5 |
| A. chirindica | Bally 4087 | 9.8 ± 0.58 | 11.2 ± 0.70 | 0.87 ± 0.01 | SOB | 3 | 1.7 |
| A. ciliata | St. Claire-Thompson 385 | 8.6 ± 0.41 | 12.2 ± 0.38 | 0.70 ± 0.06 | OB | 3 | 2.0 |
| A. crenata | Richards 21062 | 9.8 ± 0.24 | 10.8 ± 0.29 | 0.91 ± 0.07 | OBS | 4 | 1.5 |
| A. echinus | Drummond & Hemsley 3340 | 7.8 ± 0.58 | 8.5 ± 0.61 | 0.92 ± 0.02 | OBS | 3 | 1.9 |
| A. engleri | Magogo 758 | 9.6 ± 0.27 | 10.4 ± 0.43 | 0.92 ± 0.05 | OBS | 3 | 2.0 |
| A. fruticosa | St. Claire-Thompson 306 | 9.4 ± 0.67 | 10.5 ± 0.81 | 0.89 ± 0.07 | OBS | 3 | <1.0 |
| var.fruticosa | | | | | | | |
| var.eglandulosa | Richards 26393 | 7.2 ± 0.34 | 7.6 ± 0.41 | 0.95 ± 0.03 | OBS | 3 | < 1.0 |
| var.villosa | Procter 1511 | 8.4 ± 0.21 | 8.8 ± 0.28 | 0.95 ± 0.02 | OBS | 3 | < 1.0 |
| A. gillmanii | Vollesen 4250 | 10.4 ± 0.54 | 11.2 ± 0.65 | 0.93 ± 0.05 | OBS | 3-4 | 2.2 |
| A. hispida | Mahunnah 912 | 11.2 ± 0.67 | 12.8 ± 0.83 | 0.87 ± 0.06 | SOB | 4 | 2.3 |
| A. indica | Greenway & Kirrika 11165 | 9.7 ± 0.42 | 11.2 ± 0.63 | 0.82 ± 0.04 | SOB | 3-4 | 1.8 |
| A. lanceolata | Drummond & Hemsley 1826 | 8.5 ± 0.61 | 13.2 ± 0.87 | 0.64 ± 0.08 | OB | 3 | 2.0 |
| A. neptunica | | | | | | | |
| var.neptunica | Faulkner541 | 10.8 ± 0.44 | 12.6 ± 0.67 | 0.86 ± 0.04 | SOB | 3 | 2.5 |
| var.pubescens | Faden 74/344 | 10.7 ± 0.75 | 12.4 ± 0.90 | 0.86 ± 0.07 | SOB | 3 | 2.5 |
| A. nyasica | Milne-Redhead & Taylor 9463 | 7.7 ± 0.81 | 8.7 ± 0.69 | 0.88 ± 0.04 | OBS | 3-4 | 1.6 |
| A. ornata | Tanner 5588B | 9.0 ± 0.55 | 10.5 ± 0.46 | 0.86 ± 0.07 | SOB | 3 | 1.8 |
| A. paucifolia | Milne-Redhead & Taylor 7776 | 9.6 ± 0.83 | 11.8 ± 0.77 | 0.81 ± 0.06 | SOB | 3-4 | 2.0 |
| A. psilostachya | | | | | | | |
| var.psilostachya | Schlieben 2766 | 9.5 ± 0.36 | 10.4 ± 0.58 | 0.91 ± 0.02 | OBS | 3 | 1.8 |
| var. glandulosa | Speke & grant s.n. | 9.7 ± 0.64 | 10.2 ± 0.53 | 0.95 ± 0.03 | OBS | 3 | 1.8 |
| A. racemosa | Faulkner 946 | 8.6 ± 0.26 | 9.8 ± 0.42 | 0.88 ± 0.04 | OBS | 3 | 2.0 |
| A. stuhlmanii | Bullock 1915 | 8.5 ± 0.67 | 11.4 ± 0.83 | 0.74 ± 0.05 | OB | 4 | 1.4 |
| A. villicaulis | Bruce 31 | 8.2 ± 0.38 | 9.6 ± 0.64 | 0.85 ± 0.06 | SOB | 3 | <1.0 |

| Taxa | Collector | P ± CL (μm) | E ± CL (μm) | P/E ± CL | Shape | No. of Apertures | Exine thickness (μm) |
|---|---|---|---|---|---|---|---|
| A. volkensii | Richards 26403 | 7.4 ± 0.45 | 8.3 ± 0.34 | 0.89 ± 0.05 | OBS | 3 | <1.0 |
| A. welwitschiana | Richards 8501 | 8.8 ± 0.56 | 9.6 ± 0.73 | 0.92 ± 0.07 | OBS | 3-4 | 1.5 |
| A. wilkesiana | Ruffo 677 | 10.4 ± 0.76 | 11.8 ± 0.89 | 0.88 ± 0.03 | OBS | 4 | 1.9 |

**Key:** P = Polar axis; E = Equatorial axis; P/E = Polar/Equatorial axes ratio; CL at 95% (P = 0.05). OB = Oblate; SOB = Suboblate; OBS = Oblate spheroidal

**Table 2.2: Reported pollen grain size (μm) in Acalypha taxa represented in Tanzania.**

| Taxa | P | E | P/E | Shape | Reference |
|---|---|---|---|---|---|
| A. ciliata | 33 | 37 | 0.89 | OBS | Anand et al. (1982) |
| A. crenata | 11.5 | 12 | 0.96 | OBS | Bonnefille et al.(1980) |
| A. fruticosa | 11.5 | 12 | 0.96 | OBS | Bonnefille et.al.(1980) |
| A. indica* | 14.5 | 16 | 0.90 | OBS | Punt(1962) |
| | 13 | 20 | 0.65 | OB | Anand et al. (1982) |
| | 11.5 | 12 | 0.96 | OBS | Bonnefille et.al.(1980) |
| A. racemosa | 12 | 13.5 | 0.89 | OBS | Punt (1962) |

**Key:** A. indica* - P/E (average) = 0.84 (SOB).
: OB = Oblate; SOB = Suboblate; OBS = Oblate spheroidal.

**Table 2.3. List of *Acalypha* taxa possessing oblate spheroidal pollen grains.**

| Taxa | P:E Index |
|---|---|
| *A. acrogyna* | 0.92 ± 0.04 |
| *A. ambigua* | 0.91 ± 0.02 |
| *A. brachystachya* | 0.89 ± 0.05 |
| *A. bussei* | 0.98 ± 0.04 |
| *A. crenata* | 0.91 ± 0.07 |
| *A. echinus* | 0.92 ± 0.02 |
| *A. engleri* | 0.95 ± 0.05 |
| *A. fruticosa* var. *fruticosa* | 0.89 ± 0.07 |
| *A. fruticosa* var. *eglandulosa* | 0.95 ± 0.03 |
| *A. fruticosa* var. *villosa* | 0.95 ± 0.02 |
| *A. gillmanii* | 0.93 ± 0.05 |
| *A. nyasica* | 0.88 ± 0.04 |
| *A. psilostachya* var. *psilostachya* | 0.91 ± 0.02 |
| *A. psilostachya* var. *glandulosa* | 0.95 ± 0.03 |
| *A. racemosa* | 0.88 ± 0.04 |
| *A. volkensiii* | 0.89 ± 0.05 |
| *A. welwitschiana* | 0.92 ± 0.07 |
| *A. wilkesiana* | 0.88 ± 0.03 |

**Table 2. 4: List of *Acalypha* taxa possessing suboblate pollen grains.**

| Taxa | P:E Index |
|---|---|
| *A. bipartita* | 0.84 ± 0.03 |
| *A. chirindica* | 0.87 ± 0.01 |
| *A. hispida* | 0.87 ± 0.06 |
| *A. indica* | 0.82 ± 0.04 |
| *A. neptunica* var. *neptunica* | 0.86 ± 0.04 |
| *A. neptunica* var. *pubescens* | 0.86 ± 0.07 |
| *A. ornata* | 0.86 ± 0.07 |
| *A. paucifolia* | 0.81 ± 0.06 |
| *A. villicaulis* | 0.85 ± 0.06 |

**Table 2.5: List of *Acalypha* taxa possessing oblate pollen grains.**

| Taxa | P: E Index |
|---|---|
| *A. ciliata* | 0.70 ± 0.06 |
| *A. lanceolata* | 0.64 ± 0.08 |
| *A. stuhlmannii* | 0.74 ± 0.05 |

**Table 2.6: List of *Acalypha* taxa possessing small sized pollen grains.**

| Taxa | Equatorial axis (E) ($\mu$ m) |
|---|---|
| *A. ambigua* ..... | 8.6 ± 0.33 |
| *A. bipartita* ..... | 9.7 ± 0.47 |
| *A. bussei* ..... | 9.4 ± 0.76 |
| *A. echinus* ..... | 8.5 ± 0.61 |
| *A. fruticosa* var. *eglandulosa* ..... | 7.6 ± 0.41 |
| *A. fruticosa* var. *villosa* ..... | 8.8 ± 0.28 |
| *A. nyasica*..... | 8.7 ± 0.69 |
| *A. racemosa* ..... | 9.8 ± 0.42 |
| *A. villicaulis* ..... | 9.6 ± 0.64 |
| *A. volkensii*..... | 8.3 ± 0.34 |
| *A. welwitschiana*..... | 9.6 ± 0.73 |

**Table 2.7: List of *Acalypha* taxa possessing medium sized pollen grains.**

| Taxa | Equatorial axis (E) ($\mu$ m) |
|---|---|
| *A. acrogyna* ..... | 10.2 ± 0.54 |
| *A. brachystachya* ..... | 10.6 ± 0.61 |
| *A. chirindica* ..... | 11.2 ± 0.70 |
| *A. crenata* ..... | 10.8 ± 0.29 |
| *A. engleri*..... | 10.4 ± 0.43 |
| *A. fruticosa* var. *fruticosa*..... | 10.5 ± 0.81 |
| *A. gillmanii* ..... | 11.2 ± 0.65 |
| *A. ornata* ..... | 10.5 ± 0.46 |
| *A.psilostachya* var. *psilostachya* | 10.4 ± 0.58 |
| *A. psilostachya* var. *glandulosa* | 10.2 ± 0.53 |
| *A. stuhlmannii* ..... | 11.4 ± 0.83 |

Table 2.8: List of *Acalypha* taxa possessing large sized pollen grains.

| Taxa | Equatorial axis (E) ($\mu$ m) |
|---|---|
| *A. ciliata*..... | 12.2 ± 0.38 |
| *A. hispida* ..... | 12.8 ± 0.93 |
| *A. indica* ..... | 11.8 ± 0.63 |
| *A. lanceolata*..... | 13.2 ± 0.87 |
| *A. neptunica* var. *neptunica*..... | 12.6 ± 0.67 |
| *A. neptunica* var. *pubescens* ..... | 12.4 ± 0.90 |
| *A. paucifolia*..... | 11.8 ± 0.77 |
| *A. wilkesiana* ..... | 11.8 ± 0.89 |

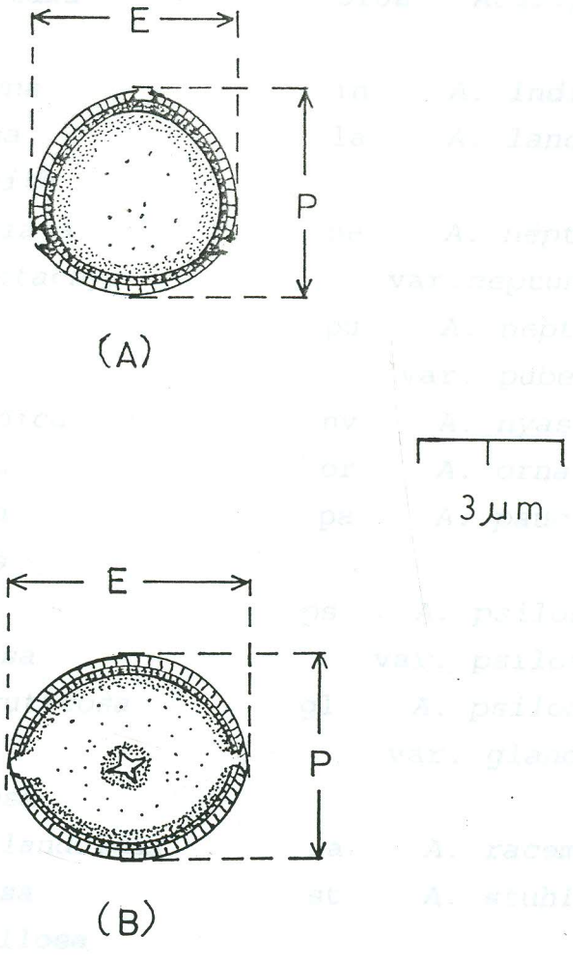



**Figure 2.1** Typical *Acalypha* pollen grain illustrating the dimensions used to study pollen morphology (oil immersion). (A) Polar view. (B) Equatorial view. P = Polar axis; E = Equatorial axis.

Key for Figure 2.2

| OTUs | *Acalypha* taxa |
|---|---|
| ac | *A. acrogyna* |
| am | *A. ambigua* |
| bi | *A. bipartita* |
| bo | *A. boiviniana* |
| br | *A. brachystachya* |
| bu | *A. bussei* |
| ch | *A. chirindica* |
| ci | *A. ciliata* |
| cr | *A. crenata* |
| ec | *A. echinus* |
| en | *A. engleri* |
| fr | *A. fruticosa* var. *fruticosa* |
| eg | *A. fruticosa* var. *eglandulosa* |
| vi | *A. fruticosa* var. *villosa* |
| gi | *A. gillmanii* |
| hi | *A. hispida* |
| in | *A. indica* |
| la | *A. lanceolata* |
| ne | *A. neptunica* var. *neptunica* |
| pu | *A. neptunica* var. *pubescens* |
| ny | *A. nyasica* |
| or | *A. ornata* |
| pa | *A. paucifolia* |
| ps | *A. psilostachya* var. *psilostachya* |
| gl | *A. psilostachya* var. *glandulosa* |
| ra | *A. racemosa* |
| st | *A. stuhlmannii* |
| vl | *A. villicaulis* |
| vo | *A. volkensii* |
| we | *A. welwitschiana* |
| wi | *A. wilkesiana* |

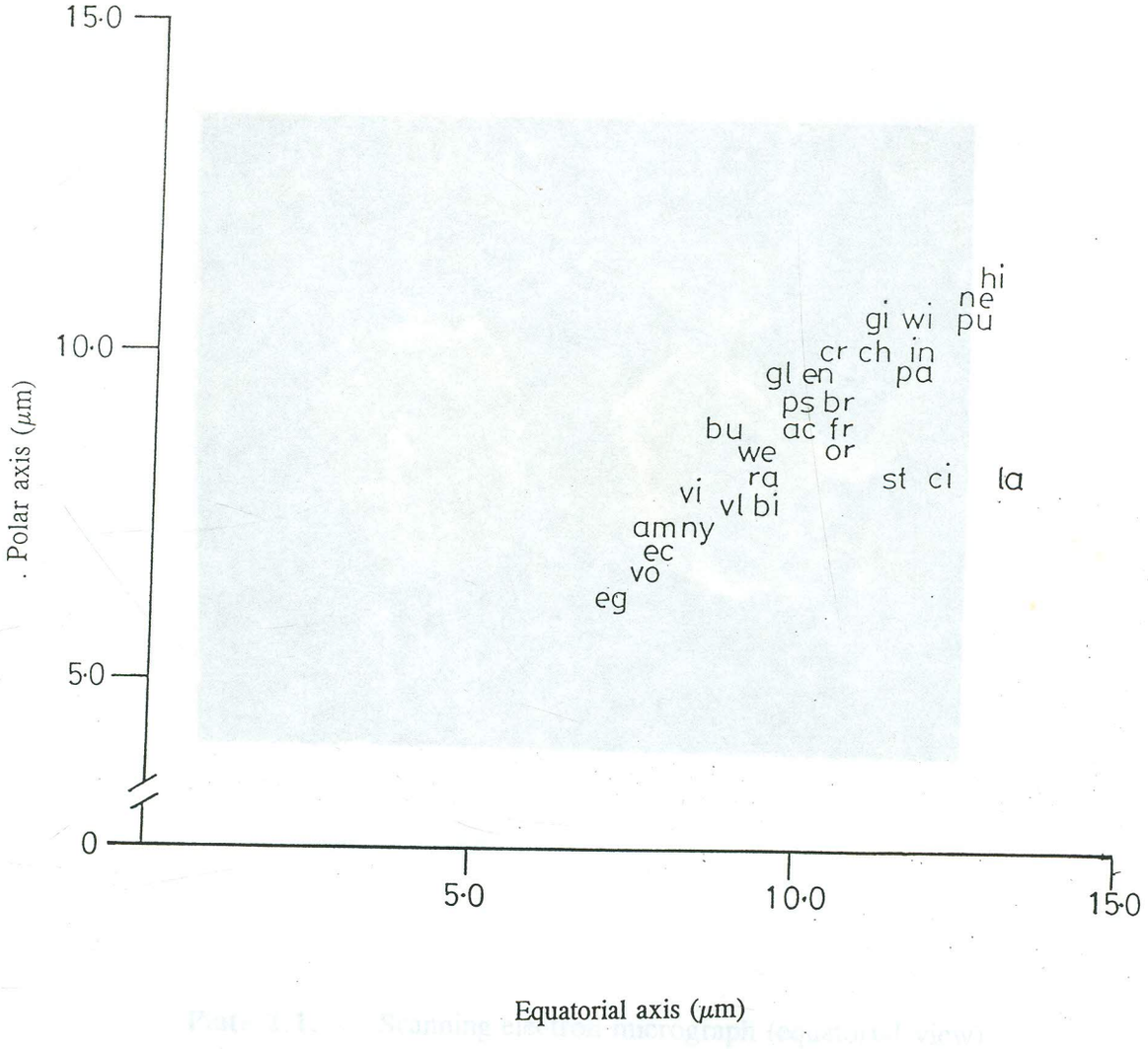



Figure 2.2. Pictorialized scatter diagram illustrating the variations in equatorial axis to polar axis in the genus *Acalypha*.



**Plate 2.1.** Scanning electron micrograph (equatorial view) pollen grain of *Acalypha ciliata*, St. Claire-Thompson 385, illustrating the surface structure; x10,000.

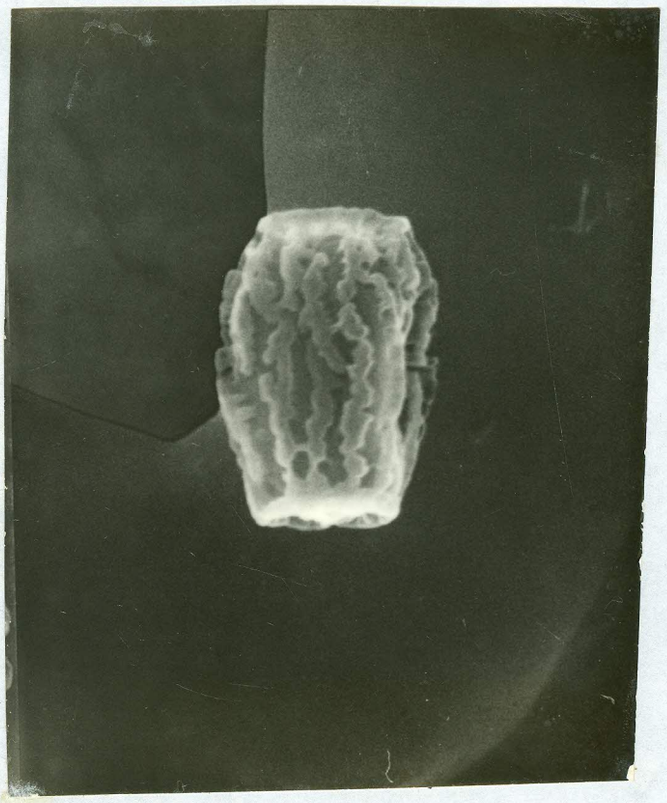


**Plate 2.2.** Scanning electron micrograph (equatorial view) of pollen grain of *A. lanceolata*, Drummond & Hemsley 1826, illustrating the surface structure; x10,000.

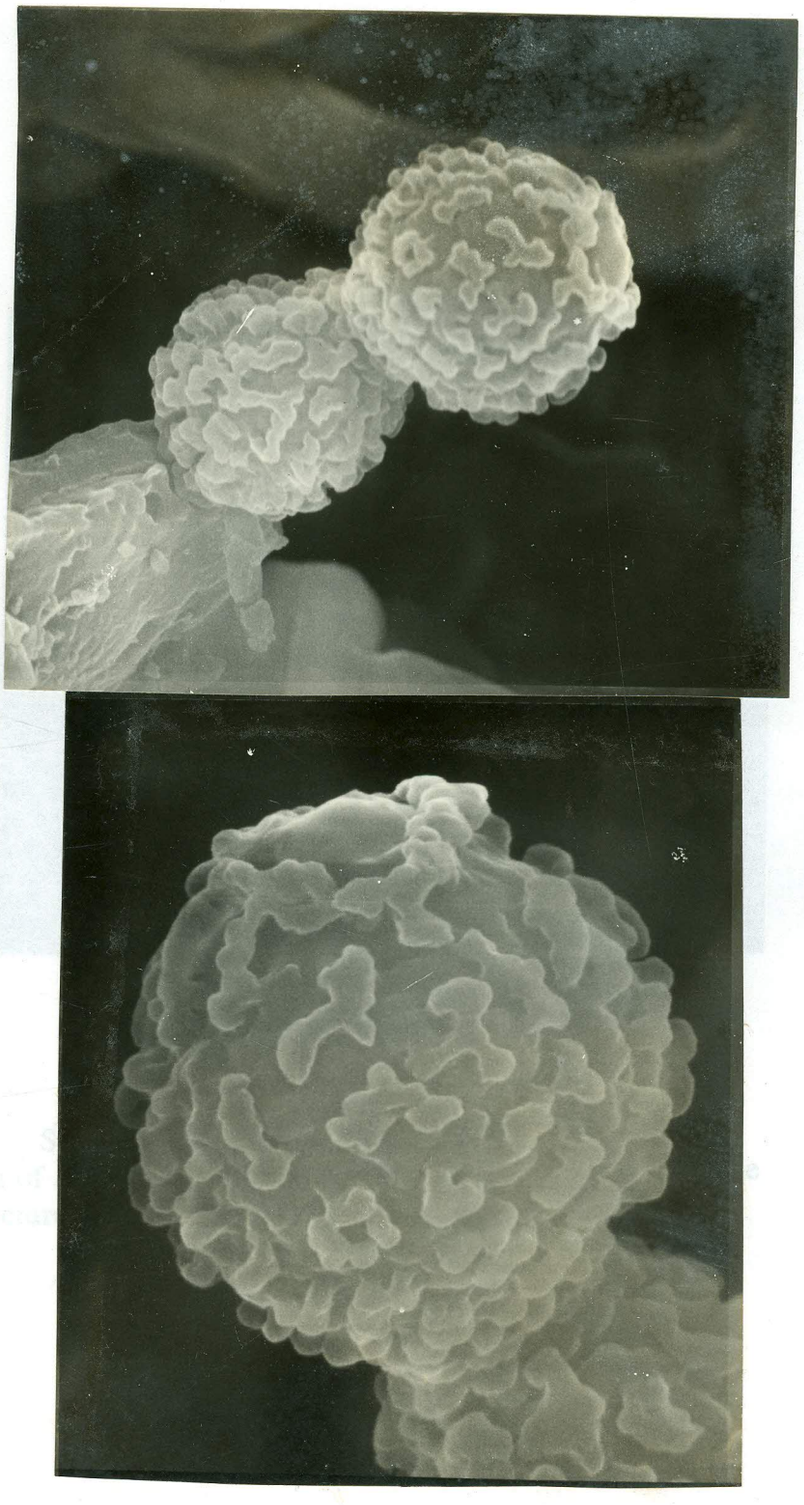


**Plate 2.3.** Scanning electron micrograph (equatorial view) of pollen grain of *A. paucifolia* Milne-Redhead & Taylor 7776. (A) surface structure, x10,000. (B) Detail of surface structure, x20,000.

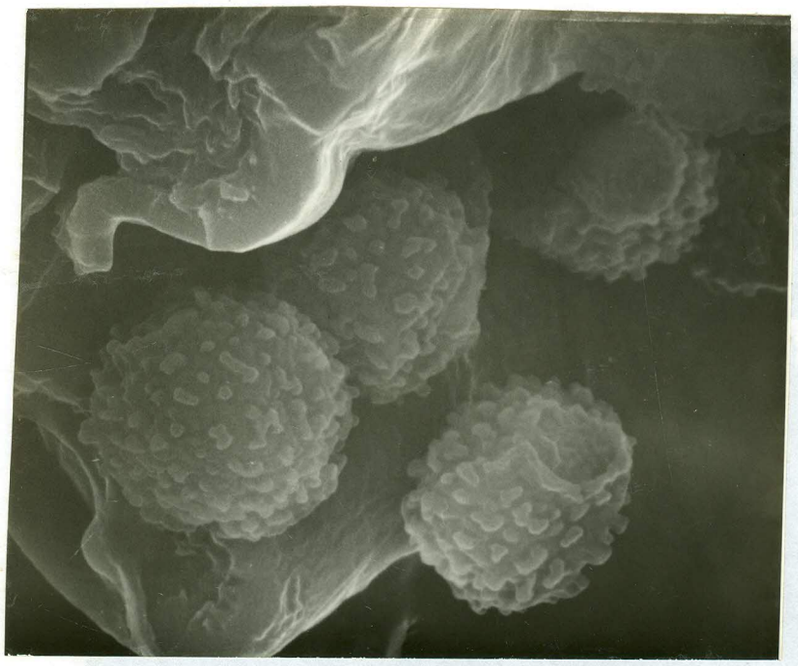


**Plate 2.4.** Scanning electron micrograph (equatorial view) of pollen grain of *A. indica* Greenway & Kirrika 11165, illustrating the surface structure, x10,000.

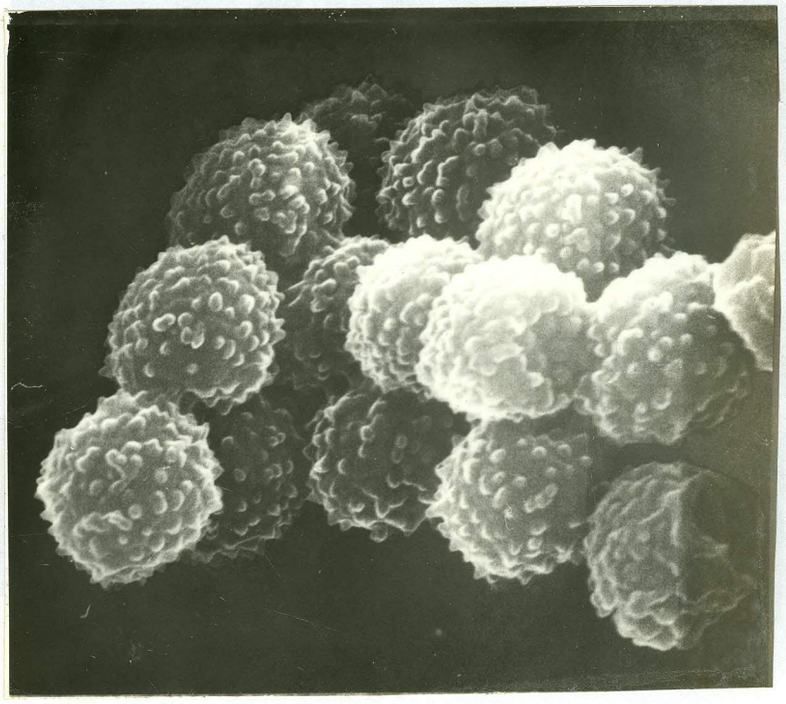


**Plate 2.5.** Scanning electron micrograph of pollen grain of *A.bussei* Vollesen 329 (e), illustrating the surface structure, x10,000.

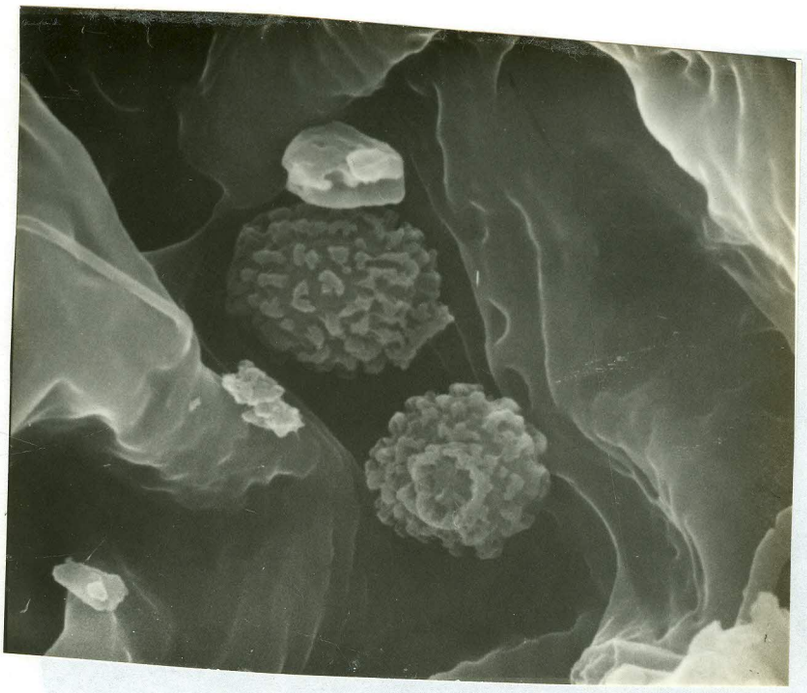


**Plate 2.6.** Scanning electron micrograph of pollen grain of *A. villicaulis* Bruce 31, illustrating the surface structure, x10,000.

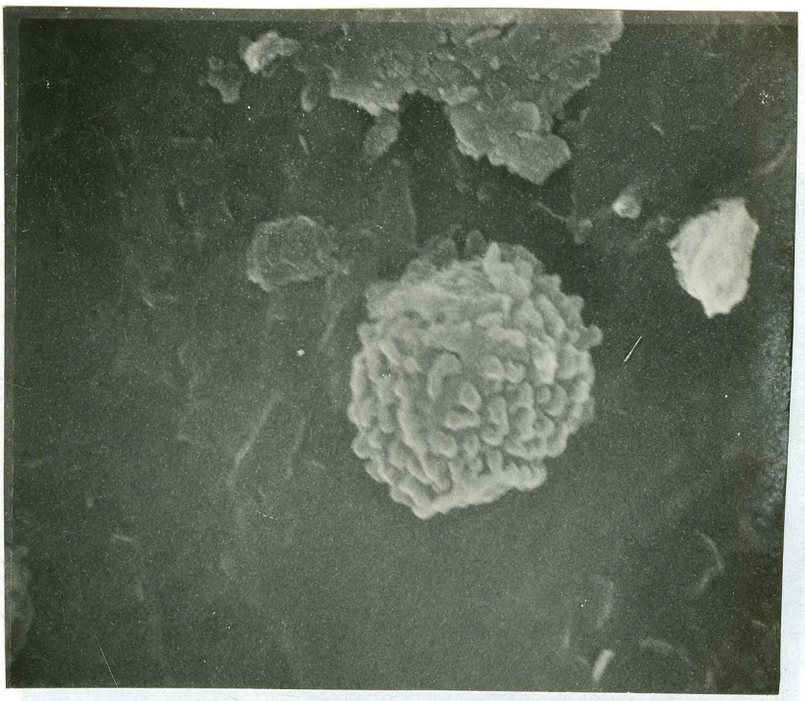


**Plate 2.7.** Scanning electron micrograph of pollen grain of *A. fruticosa* var. *fruticosa* St. Claire-Thompson 306, illustrating the surface structure, x10,000.

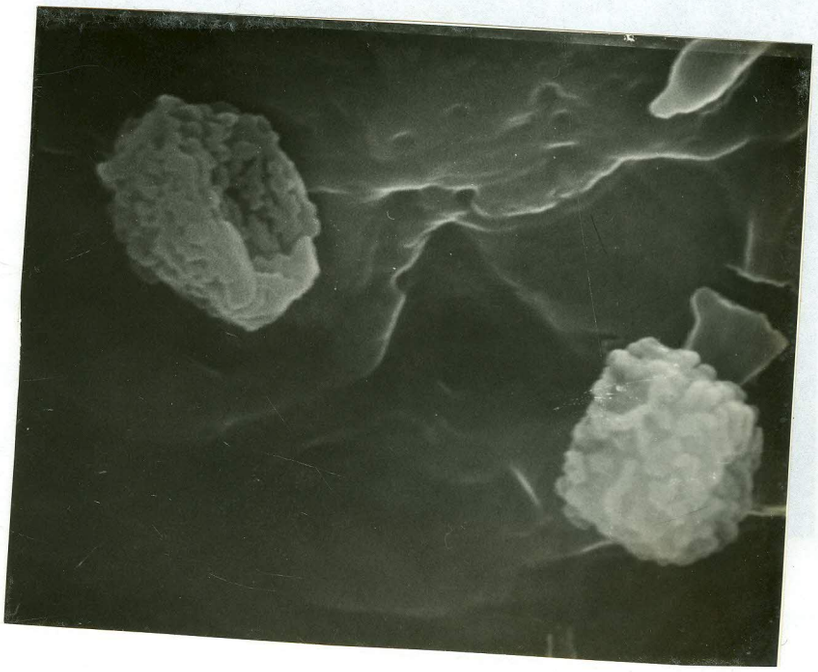


**Plate 2.8.** Scanning electron micrograph of pollen grain of *A. fruticosa* var. *villosa* Procter 1511, illustrating the surface structure, x10,000.

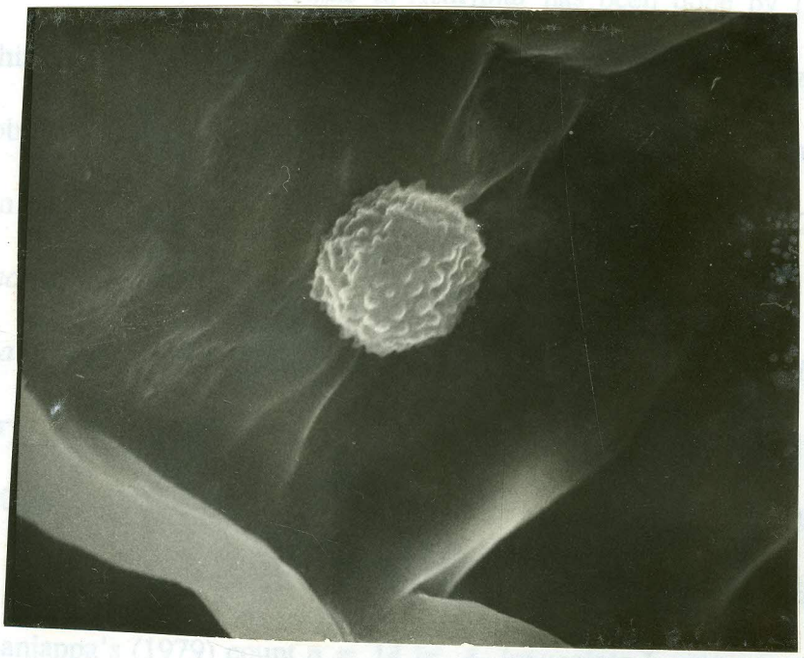


**Plate 2.9.** Scanning electron micrograph of pollen grain of *A. fruticosa* var. *egiandulosa* Richards 26393, illustrating the surface structure, x10,000.

# CHAPTER THREE: CYTOLOGICAL STUDIES

## 3.1 INTRODUCTION

Most work on chromosome counts in *Acalypha* has been done by Indian workers, and this work reviews those taxa which are represented in Tanzania. Banerji (1950) observed 2n = 42 and Datta (1967) n = 19 in *A. fallax*. Champault (1970) scored 2n =20 in *A. ciliata, A. crenata, A. ornata, A. racemosa* and 2n = 20, 24 in *A. brachystachya*. Champault's (1970) 2n = 24 in *A. brachystachya* accords with n = 12 (Datta, 1967), and 2n = 20 in *A. ciliata* concurs with Harvey's (1966) earlier record. In *A. racemosa,* Champault's (1970) count of 2n = 20 accords with n = 10 in *A. paniculata* (Gajapathy, 1961; Datta, 1967), the latter now considered to be a synonym of the former taxon (Radcliffe-Smith, 1987). Sanjappa's (1979) count n = 14 in *A. lanceolata* L. appears to be in disparity with *A. fallax* in which n = 19 (Datta 1967) and 2n = 42 (Banerji, 1950). *A. fallax* is hitherto considered to be a synonym of *A. lanceolata* (Shaw, 1980).

Cromosome counts in *A. indica* seem to be much more intriguing. Thombre (1959) and Sanjappa (1979) recorded n = 14, whereas Gajapathy (1961) and Datta (1967) had scored n = 10. Miller and Webster (1966) had observed that the counts for three neotropical species of *Acalypha, A. brachystachya, A. indica* and *A. paniculata* raised an intriguing problem, since they all showed n = 10, in contrast to previous accounts (Perry, 1943; Landes, 1946 and Barneji, 1950) in

which n = 7 was observed. However, Kapil (1960) and Gajapathy (1961) had also observed n = 10 in the three species. Miller (1964) and Raven, Khyos and Hill (1965) found n = 10 in North American species of *Acalypha.* It seemed then likely that the basic number for the genus is 10. Some more recent work appears to concur with these observations. Kothari *et al.*, (1981a; 1981b; 1982) found 2n = 20 in *A. indica, A. fruticosa* Forssk. and *A. panicalata* Micq. Also, Krishnappa and Reshme (1980; 1982) scored 2n = 20 in *A. paniculata* and n = 10 in *A. ciliata* Klein.

Polyploidy exists in *Acalypha* as demonstrated by the two cultivar, *A. hispida* and *A. wilkesiana*. In *A. hispida* 2n = 112 (Datta, 1967); 2n = ca. 112 (Perry, 1943) and 2n = 112, ca. 112, 168 (Champault, 1970). Gill *et al.* (1973) found 2n = ca. 200 in *A. wilkesiana.* Perry (1943) observed 2n = 28 in *A.wilkesiana* var. *triuphans,* and 2n = ca. 224 in *A. wilkesiana* var. *musaica.* Later, working on the two cultivated *Acalypha* Kothari *et al.* (1980; 1981b) recorded 2n = 80 in *A. godseffiana* Mast. var. *heterophylla* Hort., (= *A. wilkesiana)* 2n = 120 in *A. godseffiana* Mast., and 2n = 112 in *A. hispida* Burm. The two workers found 2n = 140 in *A. wikesiana* Muell. Arg. var. *macrophylla* Hort. and 2n = 180 *A. wilkesiana* Muell. Arg. var *macafeana* Hort. *A. godseffiana* Mast. is a synonym of *A. wilkesiana* Muell. Arg. They also found 2n = 40 in *A. lanceolata* Willd (non-cultivated).

A 2n count of ca. 224, a 32-ploid on n = 7 is among the highest number observed in the Euphorbiaceae. Perry (1943) had indicated that the occuring of

polyploidy bears little, if any, relation to the taxonomic divisions of the genus *Acalypha*. Later, Darlington (1963) advanced reasons that genera with short reproductive cycles tend to have variable numbers, often some of them high, and vice versa. Probably, this explains the case with *Acalypha*. This study is another attempt to contribute to the cytotaxonomy of the *Acalypha* represented in Tanzania.

## 3.2 MATERIALS AND METHODS

### 3.2.1 Source of plant material

Flower buds of *Acalypha* taxa were collected during my field surveys throughout Tanzania, T1-T8 (Chapter 5; Figure 5.9). The preliminary determinations of the plant voucher specimens were made in the field. Later, authentic determinations were made at the herbarium, Royal Botanical Gardens, London. The buds were fixed and preserved in Formalin Acetic Acid (Cornay's Fluid) solution immediately upon collection in the field.

### 3.2.2 Preparation of reagents and solutions

(a) Formalin acetic acid was prepared by mixing 6 parts of 70% alcohol with 3 parts of chloroform and 1 part of glacial acetic acid.

(b) A 4.4% Acetic-orcein stain was prepared by dissolving 4.4gms of the Orcein in 100 cc Glacial acetic acid and heated to aid take up of the stain. This makes the stock solution. A 2% acetic-orcein is prepared by mixing 9 parts of stock solution to 11 parts of distilled water and filtered.

(c) To prepare Rubber Solution, 45 cc glacial acetic acid are mixed with 55 cc distilled water and heated to boiling. Then, 2.0 gms of orcein were added and shaken well. On cooling, the mixiture is filtered to get Rubber Solution.

### 3.2.3 Preparation of chromosome slides

The ACETIC-ORCEIN method for FIXED bud material was used from the techniques developed by Snoad and Mcleish (1972) and Dyer (1979).

Slides and coverslips were thoroughly cleaned and placed on a white surface for better visibility. Flower buds (the *Acalyphas* have very small floral parts) had their anthers dissected out using a x 50 stereo-microscope, forceps and needles. Aided by the microscope, anthers equivalent to those produced by 10 to 20 flower buds were placed on a slide, 2% aceto-orcein added adjacent to the anthers, and not on anthers. Then the anthers were teased with a needle point to squeeze out the contents. This facilitates instantaneous fixation of pollen mother cells with minimal chromosome distortion. A coverslip was placed over the anthers, tapping very gently with a blunt pencil to spread cells, care being taken not to marsh the cells too much. Heat was applied on the underside of the slide, and coverslip pressed very gently with the thumb.

Semi-permanent slides were prepared by ringing the coverslip with Rubber solution to prevent the slide from "drying out". Permanent slides could not be prepared due to lack of facilities, viz: liquid/solid carbon dioxide ($C0_2$) and synthetic mountant Sandeural (or Euparal).

### 3.2.4 Chromosome counts

The counts were made accurately by drawing all the chromosomes seen (in an unbroken cell) on a piece of paper. Upon making sure that they are all there, the chromosomes were crossed off as they were being counted. Ten clear cells were counted per slide (per taxon) to optimize accuracy (Figure 3.1).

## 3.3. RESULTS

The findings of chromosome numbers of some *Acalypha* taxa investigated in the study is shown are Table 3.1. The observations of previous workers with respect to the chromosome numbers in *Acalypha* taxa represented in Tanzania are indicated in Table 3.2.

In the current study, the chromosome counts of 8 *Acalypha* taxa represent a new record for the genus. The taxa are *A. bipartita* (2n = 20); *A. chirindica* (2n = 24); *A. fruticosa* vars. *fruticosa; eglandulosa* and *villosa* (2n = 20); *A. neptunica* (2n = 24); *A. villicaulis* (2n = 20) and *A. volkensii* (2n = 20).

The scores of the other 5 *Acalypha* species studied, viz. *A. brachystachya, A. crenata*, *A. indica*, *A. ornata* and *A. racemosa* concur with the findings of previous workers. The count of *A. brachystachya* (2n = 24) accord with those of Champault (1970) 2n = 24 and Datta (1967) n = 12. However, these observations are in disparity with the scores of other workers notably Champault (1970) 2n = 20, n = 7 (Banerji, 1950; Landes, 1946; Perry, 1943) and n = 10 (Gajapathy, 1961; Kapil, 1960; Miller and Webster, 1966). However, the latter score n = 10 is

supported by Champault's (1970) score of 2n = 20.

In *A. crenata*, 2n = 20 accords with Champault's (1970) observation 2n = 20. The score in *A. indica* 2n = 28 corresponds to the score reported by Sanjappa (1979) and Thombre (1959) n = 14. However, it is in disparity with score n = 7 reported by Banerji (195O), Landes (1946) and Perry (1943). Also, it does not accord with n = 10 reported by Datta (1967), Gajapathy (1961), Kapila (1960) and, Miller and Webster (1966). The latter counts are, however, in parity with Kothari *et al.*'s (1981b) record of 2n = 20.

My count 2n = 20 in *A. ornata* conforms with 2n = 20 reported by Champault (1970). In *A. racemosa*, controversy similar to that in *A. indica* exists. The score of 2n = 14 in this work does conform with n = 7 reported by Banerji (1950), Landes (1946) and Perry (1943). However, it does not accord with n = 10 (Kapil, 1960), and 2n = 20 (Campault, 1970; Kothari *et al.*, 1982; Krishnappa and Reshme, 1980). The latter two reports (n =10 and 2n = 20), however, conform with each other.

**3.4 DISCUSSION:**

The current study has established the chromosome numbers of 8 *Acalypha* taxa which were hitherto unreported, viz. *A. bipastita: A. chirindica: A. fruticosa* vars. *fruticosa; eglandulosa* and *villosa; A. neptunica, A. villicaulis* and *A. volkensii*. The counts of the other 5 taxa studied have been reported by earlier workers, viz. *A. brachystachya, A. crenata, A. indica, A. ornata* and *A. racemosa* (Table 3.1 and 3.2). The *Acalypha* species studied are all wild taxa. The study has

also reviewed chromosome numbers of 18 *Acalypha* taxa, of which 10 are wilds and 8 are cultivated.

It has been observed in the study that there exist cytotypes both in some wild and cultivated taxa. In the wild *Acalypha* taxa cytotypes exist in *A. brachystachya, A. indica, A. lanceolata* and *A. racemosa* (Table 3.4). In *A. brachystachya*, both aneuploid and euploid forms appear to exist. For example, n = 7 against 2n =20, 24; n= 10 against 2n = 24; n =12 against 2n = 20 are aneuploid forms. Conversely, n= 10 and 2n = 20; n=12 and 2n = 24 are euploid forms of *A. brachystachya.*

Similarly, in *A. indica* the cytotype n = 7 and 2n = 20 is an aneuploid, whereas n = 7 and 2n = 28 could be an aneuploid form or a polyploid (4-ploid) cytotypes on base 7. The cytotype n = 10 and 2n = 20 is an euploid, but 2n= 28 is an aneuploid cytotype. The cytotype n=14 against n=20, 28 is an aneuploid and euploid form, respectively.

In *A. lanceolata,* two cytotypes exist 2n=40, 42 with the same base n = 14. Therefore there exist an aneuploid cytotype n = 14 against 2n = 40 and a polyploid 2n = 42.

There appear to exist two cytotypes in *A. racemosa* with base 7 and 1O. In the former, there is an euploid form 2n = 14 with base 7 combined with an aneuploid cytotype 2n = 20. Base 10 has the same trend, an aneuplod form 2n =

considered with base 7 as the basic chromosome number, all except *A. wilkesiana* var. *macrophylla* would be in the aneuploid state.

Therefore, in all the *Acalypha* taxa considered in this work, only *A. wilkesiana* var. *macrophylla* is a typical polypoid (allopolyploid) in both base 7 and 10 chromosome base numbers.

Table 3.3 categorizes the different possible base numbers (cytotypes) encountered in the study in both reported and studied chromosome counts.

The data indicate that the most encountered *Acalypha* cytotype has a chromosome base number of 10 which scored the highest (55.9%) percentage in the study. The second (20.6%) basic chromosome count in the category is 7. There is also a possibility of encountering other cytotypes with base numbers 12,14,19,20 and 21 in the genus *Acalypha*. Work on the cytology of temperate *Acalypha* species has revealed data which clearly supports the observations made hitherto. For example, Miller (1964) and Raven, Khyos and Hill (1965) found n = 10 in North American species of *Acalypha*. It is, therefore, most likely that the basic number for the genus *Acalypha* is 10.

With respect to polyploid in the cultivars, a count of 224, a 32 - ploid on n = 7 is among the highest number observed in the Euphorbiaceae (Miller and Webster, 1966). Earlier, Darlington (1963) advanced reasons that in general plants with short reproductive cycles tend to have variable chromosome numbers, often

some of them high, and vice versa. Probably, this explains the case with the situation encountered in the genus *Acalypha*. Another possible explanation with respect to polyploidy in cultivated *Acalypha* could be attributed to the application of horticultural chemical manipulation agents such as rooting agents and subsequent vegetative propagation.

There is a cyto-taxonomic implication on the chromosome counts, both in the gametophyte and sporophyte *Acalypha* taxa. The existence of cytotypes, aneuploids and euploids in the wild and cultivated *Acalypha* taxa has a cytotaxonomic significance.

For example, *A. fallax* has hitherto been considered a synonym for *A. lanceolata* (Shaw, 1980). Cytologically, this seems unlikely with n = 19 in *A. fallax* and n = 14; 2n = 40, 42 in *A. lanceolata*. It is not convincing to assume a limitation of an under/over count in the two *Acalypha* species. A similar doubt holds in *A. paniculata* n = 10 considered to be a synonym of *A. racemosa* n = 7 (Radcliffe-smith, 1987).

A critical look at the complexes in the basic chromosome numbers, including the polyploid counts indicate uncertainities in the synonymy of some *Acalypha*. For example, the *A. godseffiana* group has a typical base number 10 on 8- ploid (80) and 12- ploid (120). In the *A. wilkesiana* complex varieties, there are two chromosome base numbers 7 and 10. Thus, there is the base 7 series on 4-ploid (28) 20-ploid (140) and 32-ploid (224); and base 10 series on 14-ploid (140), 18-ploid (180) and

20-ploid (200). This raises a taxonomic controversy on the justification of the synonymy in *A. godseffiana* and *A. wilkesiana* on cytotaxonomic basis. Radcliffe-Smith (1987) reported that, though different, there is some resemblance between *A. indica* and *A. Lanceolata*. This work justifies the difference between the two taxa on cytological basis. In *A. indica* n = 7, 10, 14, 2n = 20, 28 whereas in *A. lanceolata* n=14; 2n = 40, 42. Thus, although Perry (1943) reported that the occuring of polyploidy bears little, if any, relation to the taxonomic divisions of the genus *Acalypha*, the observations in this work support that polyploid appear to divide some of the *Acalypha* taxa into their deserved taxonomic rank.

**Table 3.1:** Chromosome numbers of selected *Acalypha* taxa studied.

| Taxa | Locality/Collector | 2n |
|---|---|---|
| **A. bipartita* | T1, Biharamulo; 1300m Mahunnah 1102 | 20 |
| *A. brachystachya* | T7, Tukuyu, 2000m Mahunnah 1185 | 24 |
| **A. chirindica* | T6, Kidatu-Kilombero, 800m Mahunnah 1204 | 24 |
| *A. crenata* | T3, East Usambara; 1800m Mahunnah 1089 | 20 |
| **A. fruticosa* var. *fruticosa* | T6, Mikumi-Morogoro,900m Mahunnah 1208 | 20 |
| **A. fruticosa* var. *eglandulosa* | T2, Olmotonyi-Arusha; 1200m Mahunnah 1014 | 20 |
| **A. fruticosa* var. *villosa* | T3, Korogwe, 1100m Mahunnah 1094 | 20 |
| *A.indica* | T2, Tella-Old Moshi,1500m Mahunnah 1023 | 28 |
| **A. neptunica* | T6, Korogwe-Kibaha; 450 Mahunnah 1221 | 24 |
| *A. ornata* | T6, Kidatu-Kilombero;800m Mahunnah 1206 | 20 |
| *A. racemosa* | T3, Tella-Old Moshi, 1600m Mahunnah 1030 | 14 |
| **A. villicaulis* | T1, Rubya-Muleba, 1700m Mahunnah 1140 | 20 |
| **A. volkensii* | T3, Soni-Lushoto, 1450m Mahunnah 1076 | 20 |

* = New record for the genus *Acalypha*

**Key for Table 3.2**

- Unbracketed numbers indicate chromosome scores
- Bracketed letters/numbers indicate references.
- Asterisked taxa with corresponding number of star(s) are reported to be synonyms.
- **References**

B Banerji (1950)

C Champault (1970)

D Datta (1967)

G Gajapathy (1961)

Gl Gill *et al.* (1973)

H Harvey (1966)

K Kapil (1960)

Ko1 Kothari *et al.* (1980)

Ko2 Kothari *et al.* (1981a)

Ko3 Kothari *et al.* (1981b)

Ko4 Kothari *et al.* (1982)

KR1 Krishnappa and Reshme (1980)

KR2 Krishnappa and Reshme (1982)

L Landes (1946)

MW Miller and Webster (1966)

P Perry (1943)

S Sanjappa (1979)

T Thombre (1959)

**Table 3.2:** Reported chromosome numbers of some *Acalypha* taxa represented in Tanzania.

| Taxa | Gametophyte(n) (Reference) | Sporophyte (2n) (Reference) |
|---|---|---|
| *A. brachystachya* | 7(B,L,P,); 10 (G,K,MW);12(D) | 20, 24 (C) |
| *A. ciliata* | 10 (KR2) | 20 (C, H) |
| *A. crenata* | | 20(C) |
| **A. fallax* | 19 (D) | |
| *A. fruticosa* | | 20 (Ko2) |
| ***A. godseffiana* | | 120 (Ko3) |
| *A. godseffiana* var. *heterophylla* | | 80 (Ko3) |
| *A. hispida* | | 112 (C,D,Ko1, P); 168 (C) |
| *A. indica* | 7 (B,L,P); 10(D,G,K,MW);14(S, T). | 20 (Ko3) |
| **A. lanceolata* | 14 (T) | 40 (Ko3); 42(B) |
| *A. ornata* | | 20 (C) |
| ****A. paniculala* | 10 (D,G,MW) | |
| ****A. racemosa* | 7 (B,L,P);10 (K) | 20 (C,Ko4,KR1) |
| ***A. wilkesiana* | | 200 (GL) |
| *A. wilkesiana* var. *macafaena* | | 180 (Ko3) |
| *A. wilkesiana* var. *macrophylla* | | 140 (Ko3) |
| *A. wilkesiana* var. *musaica* | | 224 (P) |
| *A. wilkesiana* var. *triuphans* | | 28 (P) |

**Table: 3.3: Categorization of Acalypha taxa according to base number.**

| Taxa | status (Reported/Studied) | Base number category | | | | | |
|---|---|---|---|---|---|---|---|
| | | 7 | 10 | 12 | 14 | 19 | 20 |
| *A. bipartita* | Studied | | + | | | | |
| *A. brachystachya* | Studied/ Reported, | + | + | | | | |
| A. chirindica | Studied | | | + | | | |
| *A. Ciliata* | Reported | | + | | | | |
| *A. Crenata* | Studied/Reported | | + | | | | |
| *A. fallax* | Reported | | | | + | | |
| *A. fruticosa* | Reported | | + | | | | |
| *A. fruticosa* var. *fruticosa* | Studied | | + | | | | |
| *A. fruticosa* var.*eglandulosa* | Studied | | + | | | | |
| *A. fruticosa* var.*villosa* | Studied | | + | | | | |
| *A. godseffiana* | Reported | | + | | | | |
| *A. godesffiana* ar.*heterophylla* | Reported | + | + | | | | |
| *A. hispida* | Reported | | | | | | |
| *A. indica* | Studied | + | + | + | | | |
| *A. lanceolata* | Reported | | | + | | + | + |
| *A. neptunica* | Studied | | | | + | | |
| *A. ornata* | Studied/Reported | | + | | | | |
| *A. panicula* | Reported | | + | | | | |
| *A. racemosa* | Studied | + | + | | | | |
| *A. villicaulis* | Studied | | + | | | | |
| *A. volkensii* | Studied | | + | | | | |
| *A. wilkesiana* | Reported | | + | | | | |
| *A. wilkesiana* var.*macafaena* | Reported | | + | | | | |
| *A. wilkesiana* var.*macrophylla* | Reported | + | + | | | | |
| *A. wilkesiana* var. *musaica* | Reported | + | | | | | |
| *A. wilkesiana* var. *triuphans* | Reported | + | | | | | |
| Total | | 7 | 19 | 3 | 2 | 1 | 1 |
| Percentage | (100%) | 20.6 | 55.9 | 8.9 | 5.9 | 2.9 | 2.9 |

**Table 3.4: Chromosome numbers of reported and studied wild *Acalypha* taxa.**

| Taxa | n | 2n |
|---|---|---|
| *A. bipartita* | | 20 |
| *A. branchystachya* | 7, 10, 12 | 20,24 |
| *A. chirindica* | | 24 |
| *A. ciliata* | 10 | 20 |
| *A. crenata* | | 20 |
| *A. fallax* | 19 | |
| *A. fruticosa* | | 20 |
| *A. fruticosa* var. *fruticosa* | | 20 |
| *A. fruticosa* var. *eglandulosa* | | 20 |
| *A. fruticosa* | | |
| *A. fruticosa* | | 20 |
| var. *villosa* | | 20 |
| *A. indica* | 7, 10, 14 | 20, 28 |
| *A. lanceolata* | 14 | 40, 42 |
| *A. neptunica* | | 24 |
| *A. ornata* | | 20 |
| *A. paniculata* | 10 | |
| *A. racemosa* | 7, 10 | 14, 20 |
| *A. villicaulis* | | 20 |
| *A. volkensii* | | 20 |

**Table 3.5: Chromosome numbers of reported cultivated Acalypha taxa.**

| Taxa | n | 2n |
|---|---|---|
| *A. godseffiana* | | 120 |
| *A. godseffiana* var. *heterophylla* | | 80 |
| *A. hispida* | | 112, 168 |
| *A. wilkesiana* | | 200 |
| *A. wilkesiana* var. *macafaena* | | 180 |
| *A. wilkesiana* var. *macrophylla* | | 140 |
| *A. wilkesiana* var. *musaica* | | 224 |
| *A. wilkesiana* var. *triuphans* | | 28 |

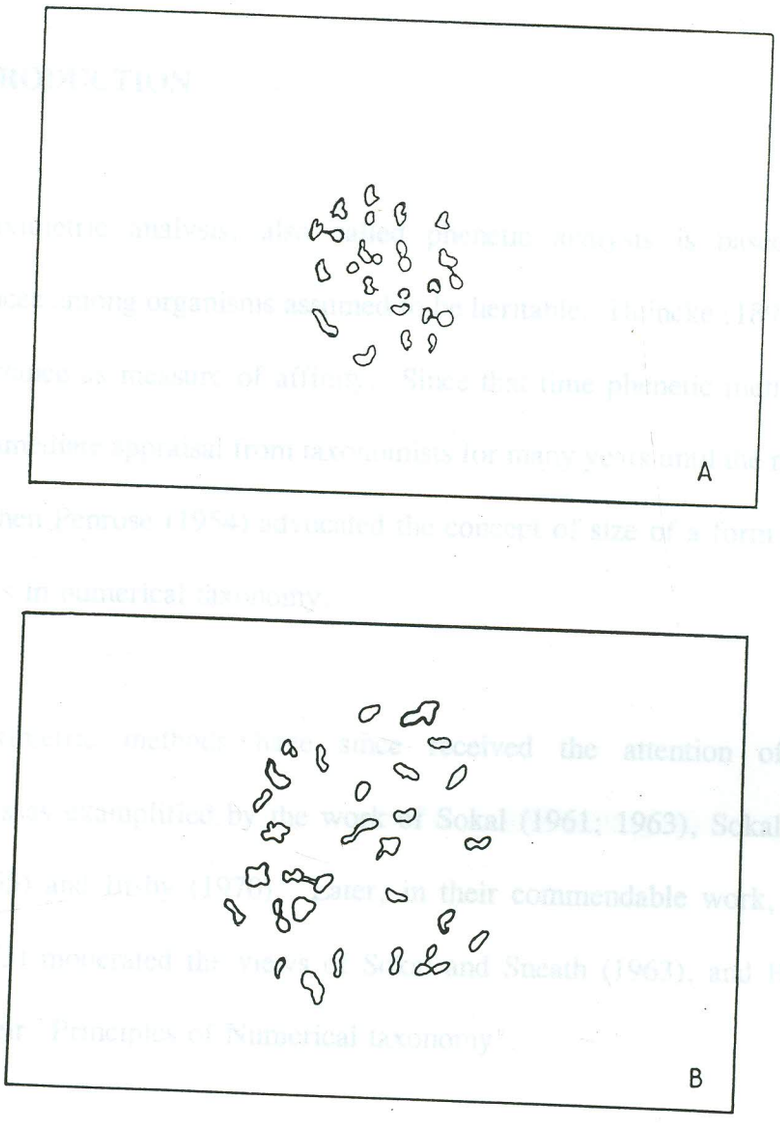



**Figure 3.1.** Anther smear chromosomes preparation. (A) A. *villicaulis,* Mahunnah 1140; 2n = 20. (B) A. *indica,* Mahunnah 1023; 2n = 28

# CHAPTER FOUR: TAXIMETRIC STUDIES

## 4.1 INTRODUCTION

Taximetric analysis, also called phenetic analysis is based on overall resemblances among organisms assumed to be heritable. Heincke (1898) used mean square distance as measure of affinity. Since that time phenetic methods did not receive immediate appraisal from taxonomists for many years until the middle of this century when Penrose (1954) advocated the concept of size of a form and distance coefficients in numerical taxonomy.

Taximetric methods have since received the attention of numerical taxonomists as examplified by the work of Sokal (1961; 1963), Sokal and Sneath (1963; 1965) and Bisby (1970). Later, in their commendable work, Sneath and Sokal (1973) moderated the views of Sokal and Sneath (1963), and Bisby (1970) through their "Principles of Numerical taxonomy".

Recent contributions with respect to taximetric studies are those of Mishra and Sahu (1984) on tribe Euphorbieae, Seberg (1984) on the *Acalypha* of the Galapogos Archipelago and Garofalo's (1988) revision of the *Axonopus* section *Cabrera* (Graminae: Paniceae).

In an attempt to establish a classification of the Tanzanian *Acalypha*, four

multivariate taximetric methods are employed in the study. The methods are the size of a Form, the Unweighted Pair Group Method Using Arithmetic Averages (UPGMA), the Single-Link and the Principal Coordinates Analysis (PCO). All these methods are said to be multivariate in that they analyse the collective pattern based on resemblance among many characters.

## 4.2 MATERIALS AND METHODS

### 4.2.1 Source of plant material

Plant material for taximetric studies were obtained from some international herbaria, several herbaria in Tanzania and personal collections as indicated in table 4.1(a), 4.1(b) and the explanation under 4.1.

**Table 4.1(a): List of international herbaria visited during the study.**

---

1. DSM: Herbarium, Botany Department,
   University of Dar es Salaam,
   Dar es Salaam, Tanzania.

2. EAH: East African Agriculture Research Organization,
   Nairobi, Kenya.

3. G : Herbarium, Conservatoire et Jardin
   botaniques de la Ville de Geneve.

4. K : Herbarium, Royal Botanic Gardens,
   Kew, Richmond, England.
5. MO : Missouri Botanic Gardens, St. Louis,
   Missouri, USA.
6. NBRI:Herbarium, National Botanical Research Institute,
   U.P., India.
7. NY :Herbarium, New York Botanical Garden, Bronx,
   New York.
8. UPS: Herbarium, Institute of Systematic Botany, Uppsala University, Sweden.
9. US :United States National Herbarium, Museum of Natural History,
   Smithsonian Institution, Washington D.C.

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**Table 4.1(b): List of Tanzanian herbaria visited during the study**

---

ITM : Herbarium, Institute of Traditional Medicine, Muhimbili University College of Health Sciences, Dar es Salaam.

NHT : National Herbarium of Tanzania, Tropical Pesticides Research Institute, Arusha.

SUA : Herbarium, Sokoine University of Agriculture, Morogoro.

TAFORI: Herbarium, Tanzania Forest Research Institute, Lushoto.

**Table 4.1(c):** Personal collections

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Plant voucher specimens of *Acalypha* were collected during my extensive field surveys throughout Tanzania, from T1 through T8 (Figure 5.9, Appendix 5.1).

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**PS:** The abbreviations of the international herbaria were taken from Holmgren *et al.* (1981). The abbreviations of the local herbaria are those used for the respective institutions.

### 4.2.2 Sampling of plant material

The total number of plant voucher specimens used for taximetric analysis was 310. The voucher specimens were selected from the about 6OO herbarium sheets examined for the other aspects of the study, viz., palynology, cytology, phytogeography and ethnobotany. The selection was based upon adequacy and completeness of the plant voucher specimens in relation to the variables being investigated. Wherever possible, plant specimens from the entire range of geographic distribution for each plant group / taxon were included in the numerical analysis.

All the plant voucher specimens used in the study are appended (Appendix 5.1). The nomenclature, classification and sequence of the plant specimens investigated is based on Turill (1956).

### 4.2.3 Selection of characters

Characters are the medium through which variation is surveyed, recorded, analysed and ultimately communicated. Hence characters have to be scrutinized carefully. Davis and Heywood (1963), writing on numerical methods, propounded that character selection is the weak link in the whole approach"... . In numerical taxonomy, many characters are selected from specimens chosen over a wide range of variation and geographical distribution (Sneath and Sokal, 1973; Abbott *et al.*, 1985). The emanating characters are called "classificatory characters" or "taxonomic characters" which are characters with high correlation value (Sneath and Sokal, 1973; Davis and Heywood, 1963; Abbott *et al.*, 1985).

There are two types of taxonomic characters namely intrinsic (genotypic) which are the properties of the plant, and extrinsic (phenotypic) which are modified by the environment, for example habitat, geographical distribution and climate.

In taximetric analysis, characters or descriptors are divided into two broad categories of data, namely quantitative and qualitative, the latter of which can be binary or multivariate/multistate. The multistate characters are either ordered or simple (unordered). The ordered multistate characters are further grouped to linear, meaning that their values are separated by equal intervals, or grouped as non-linear descriptors.

The binary descriptors are classified into symmetric and asymmetric, that is they are based on the presence or absence of a state.

The quantitative descriptors are either metric or discontinuous, in which the latter characters are either ranks or counts. A diagramatic representation of the data types explained above is given in Figure 4.2.

### 4.2.4 Character analysis

Taximetric analysis depends on measuring similarity by the relative number of similar character states. Therefore, much attention should be given to choice and formulation of characters. Balanced characters should be correlated with other balanced characters and the converse is also true. Similarly, rare-state characters should be correlated with other rare-state descriptors.

Cain *et al.* (1960) define a phenetic classification as an arrangement by overall similarity based on overall available characters without any weighing. All the characters selected are treated as of equal value and not given special *a-priori* weighing. That is, *a-posteriori* selection of characters is used whereby the value of characters are analysed after the characters have been scored. It has been established that stable classification can only be produced through the selection of characters with high taxonomic value (Abbott *et al.*, 1985; Bisby, 1981).

Correlations among characters produce the collective pattern used by taxonomists to make classifications (Abbott *et al.*, 1985). In quantitative descriptors, data which produce discontinuous groupings based on all possible combinations correlated and useful taxonomically. In the case of qualitative data (binary and qualitative multistate) including quantitative descriptors divided into discrete ranges,

the method designed by Eastabrook (1967) of character analysis was used for analysing the correlation values.

The procedure proposed by Abbott *et al* (1985) for character selection was adopted in the study in which there were 63 characters observed. However, uncorrelated crude characters were subsequently rejected to a balance of 49 characters.

Consequently, only descriptors with a bimodal or multimodal distribution pattern were selected and employed in the analysis.

### 4.2.5 Measurement of quantitative characters

Large characters like plant height, blade size, etc. were measured without using the microscope. However, with small attributes for example, stipules, flowers and bracts measurements were taken with the aid of a stereo-microscope (magnification : x 7-30) and, dissecting needles and forceps.

In working with herbarium material, small plant parts (floral parts and inflorescences) were softened in warm water or boiled to resume cell turgidity to facilitate accurate measurements. Plant material from personal collections was killed, fixed and preserved in Formalin Acetic acid (FAA) solution upon collection in the field. In each of the characters measured, 10 replications were made and averaged out. Most of the characters measured in the study are illustrated in the text (Figure 4.1).

### 4.2.6 Characters used in the study

A total of 49 characters were employed in the taximetric analysis. It is noted that this number is small compared to the recommended least number of characters used in phenetic analyses which is 60. This is due to the limited number of gross morphologiclal characters obtained in the study. The descriptors were categorized as 2 binary characters, 19 multistate characters and 28 quantitative characters. The characters were tabulated as summarized below:-

(a) Master character SET of all the 49 characters (Table 4.2 a,b).

(b) Character SUBSET of 28 quantitative characters (extrapolated on Table 4.2a)

(c Character SUBSET of 21 qualitative (2 binary and 19 multistate) characters (extrapolated on Table 4.2b)

**Table 4.2: Master character SET of all the 49 characters.**

---

(a) **Quantitative characters**

1. Plant height (m): Data were obtained from collector's notes on herbarium material, and in some cases supported with field observation in those taxa which were collected by the author.

2. Petiole length (cm): Distance from blade base to point of attachment to the stem node.

3. Blade length (cm): Distance from blade base to the blade tip.

4. Blade width (cm): Distance across the blade at its broadest part.

5. Blade length/Blade width: Ratio.

6. Number of nerves orginating from blade base (unit): Nerves were counted and recorded.

7. Number of pairs of blade lateral nerves (Unit): Nerve pairs were counted and recorded.

8. Stipule length (mm): Distance from point of attachment to the stem node to the tip of the stipule.

9. Male inflorescence length (cm): In unisexual inflorescences - length of the male thyrse; in bisexual inflorescences - the length of entire inflorescence less the female thyrse (including female allomorphic flower(s), when present).

10. Female inflorescence length (cm): In unisexual inflorescences-length of female thyrse (including female allomorphic flower(s), when present); in bisexual inflorescences - the entire inflorescence (including female allomorphic flower(s) when present), less the male thyrse.

11. Male bract length (mm): Distance from bract tip to point of attachment to base of (sessile or shortly pedicellate) male flower.

12. Female bract length (mm): Distance from bract tip to base of bract (excluding pedicel, when present) along meridian of the longest lobe.

13. Female bract width(mm): Distance across the broadest part of the bract.

14. Female bract length/female bract width: Ratio

15. Number of female flowers per bract (Unit): Bract was spread out, and the flowers were counted and recorded.

16. Number of sepals per female flower (Unit): Bract and calyx were spread out, and sepals counted and recorded.

17. Length of female flower sepal (mm): Sepals were abstracted from the flower; length was measured as the distance from sepal tip to point of attachment to the receptacle.

18. Ovary diameter(mm): Bract, calyx, sepals and style were removed from the flower, and ovary diameter was taken as the distance across the ovary.

19. Style length(mm): Style was spread lengthwise, and the distance from style tip

to point of attachment to ovary was taken.

20. Fruit length(mm): Distance along the meridian depression from point of attachment to the style down to the receptacle scar.

21. Fruit diameter(mm):Distance across the widest part of the trilobed fruit.

22. Fruit length/fruit diameter: Ratio.

23. Seed length (mm): Distance from seed tip to the end of the caruncle (when present).

24. Seed width(mm): Distance across the widest part of the seed.

25. Seed length/seed width: Ratio.

26. Polar axis ($\mu$m): The perpendicular line connecting the poles of a pollen grain.

27. Equatorial axis($\mu$m): The line perpendicular to the Polar axis in the equatorial plane.

28. Polar axis/Equatorial axis: Ratio.

(b) **Binary and Multistate characters**

Multistate characters include numbers 29-47 and Binary characters from numbers 48-49.

29. Pollen shape (class): 3 = Oblate; 2 = Suboblate; 1 = Oblate Sphereidol.

3O. Plant sex: 1 = Monoecious; 2 = Dioecious.

31. Plant duration: 1 = Annual; 2 = Perennial

32. Plant habit: 1 = Tree; 2 = Shrub; 3 = Herb.

33. Petiolation: 1 = Sessile/Subsessile; 2 = Shortly petiolate; 3 = Petiolate/Strongly Petiolate.

34. Venation: 1 = Palmi-nerved; 2 = Penni-nerved.

35. Shape of stipules: 1 = Linear-/Ovate - Lanceolate; 2 = Subulate; 3 = Filiform.

36. Inflorescence Sexes: 1 = Unisexual; 2 = Bisexual, 3 = Both Unisexual and Bisexual.

37. Type of female inflorescence: 1 = Spicate; 2 = Racemose; 3 = Paniculate; 4 = Spicate and Racemose.

38. Position of female inflorescence: 1 = Terminal; 2 = Axillary; 3 = Terminal and Axillary.

39. Position of male inflorescence: 1 = Terminal; 2 = Axillary.

40. Type of male inflorescence: 1 = Spicate; 2 =Racemose.

41. Pedicelation in female flower : 1 = Sessile; 2 = More or Less Sessile; 3 = Shortly Pedicellate; 4 = Strongly Pedicellate.

42. Presence of female accrescent bract: 0 = Absent; 1 = Present; 2 = Strongly Present.

43. Position of female allomorphic flowers: 0 = Absent; 1 = Terminal ; 2 = Axillary.

44. Nature of styles: 1 = Free; 2 = More or Less Free; 3 = Connate.

45. Shape of styles: 1 = Entire; 2 = Lobed/ Pectinate/ Laciniate.

46. Nature of female allomorphic fruit (when present): 0 = Absent; 1 = 1-lobed; 2 = Bilobed 3 = Trilobed.

47. Seed shape: 1 = Ellipsoid/ Ellipsoid - Ovoid; 2 = Subglobose;

3 = Ovoid/Ovoid-Subglobose..

48. Presence of female allomorphic flowers: 0 = Absent; 1 = Present.

49. Presence of caruncle: 0 = Absent; 1 = Present.

**Table 4.2(c): Character SUBSET of 28 quantitative characters.**

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Refer to Table 4.2(a): Character number 1-28.

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**Table 4.2(d): Character SUBSET of 21 binary and multistate characters**

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Refer to Table 4.2(b): Character number 29-49

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### 4.2.7 Choice of taximetric methods

Taximetric or phenetic classification is based on overall resemblances among organisms for characters assumed to be heritable. The methods employed in taximetric analysis are all multivariate in that they analyse the collective pattern

based on resemblance among many characters (Abbott *et al.*, 1985).

Three main taximetric methods are used in this study, namely the size of a form, cluster analysis and ordination.

### 4.2.7.1 Size of a form

It has been established (Penrose, 1954) that the size of a form is the mean of its quantitative character values. This mean is considered to be a measure of affinity or similarity between the Operational Taxonomic Units (OTUs) being considered.

In analysing the size of a form, the mean and standard deviation of each OTU's characters were worked out using a calculator by employing the formula;-

$$SD = \sqrt{\frac{\Sigma (X - X)^2}{n}}$$

Where SD = standard deviation, x = raw data for each character, x = mean and n = number of characters.

The standard error (SE) for each OTU mean was calculated from the formula:

$$SE = SD/\sqrt{n}$$

Confidence Limits (CL) at 95% (p = 0.05) for each OTU's population mean was calculated from the formula:

$$CL = SE \times 1.96$$

Then, histograms and scatter diagrams were prepared.

### 4.2.7.2 Cluster Analysis methods

Cluster analysis techniques, unlike ordination methods, analyse the variation pattern of given objects and construct a hierarchical classification based on it. Consequently, it has proved very useful to taxonomists.

There are two types of clustering methods; the agglomerative two-step cluster analysis method which has proved to be more appropriate to taxonomy, and the divisive method which has a wider application in ecological variation analysis than in taxonomic work. The Agglomerative two-step cluster analysis has more merit in that it constructs a similarity or distance table and synchronically constructs diagrams using a clustering procedure (Figure 4.3). A computer calculates the similarity or distance matrix known as the t x t or p x p matrix using appropriate measures of similarity or distance.

In the study two Agglomerative cluster analysis methods were used namely, the Group-Average Cluster Analysis and the Single -Link Cluster analysis (Figure 4.5).

#### 4.2.7.2.1 Group - Average Cluster Analysis

When they first introduced the method, Sokal and Michener (1958) called it

the "Unweighted Pair Group Method Using Arithmetic Averages" (UPGMA). This method, like the Single-Link method, employs the geometric model and presents results in dendrograms.

In this method, crossbars in dendrograms are slightly higher than in Single-Link Analysis. Also items and clusters or two clusters must be closer together to fuse at a given level on the dendrogram. The UPGMA has merits in that it is intermediate (compromises the "worst of both worlds"); the method lacks the precision of the Single-Link (extreme chaining property) axiom and the "powerful" tendency of space-dilating method of the Central Point Cluster Analysis, hence emphasizes gaps and forms a balanced hierarchy.

The UPGMA is used to analyse separately mixed data (quantitative, binary and multistate) and, binary and multistate characters using the Gower General Similarity Coefficient (GGSC). The Eucledian distance, a recommended measure of distance for quantitative data is used for quantitative characters.

#### 4.2.7.2.2 Single-Link Cluster Analysis

Single-Link clustering or NEAREST NEIGHBOUR procedurely uses the geometric model in the analysis and results in dendrograms. It was first established as the "Dendritic" method by Floreck *et al.* (1951 a,b). Single-Link Cluster Analysis is now based on Estabrook (1966) and the method is derived from axiomatic or assumed properties. Of all the cluster analysis methods, only the single-Linkage method always places an item in the same cluster as its nearest

neighbour (hence the name "Nearest Neighbour" Method) in the geometric model. However, single-Linkage method, apart from its likelihood to give a chained hierarchy, appears to be more stable than space dilating methods (for example, Group-Average Method) for minute changes in distances in some configurations. Users of Single-Linkage method, especially with Linkage diagrams for examining single linkage results seem to be happier with the results obtained with this method than with those obtained with other methods (Prance *et al.*, 1969; Stearn, 1971 and Bisby, 1973).

#### 4.2.7.3 Ordination

This method is used in taxonomy to produce scatter diagrams in two dimensions. There are mainly three ordination methods used in taxonomic analysis namely, Principal Components Analysis (PCA), Principal Coordinates Analysis (PCO) and Multi-dimensional Scaling method (MDS) (Abbott *et al.*,1985).

These methods have merits over the clustering methods since they do not assume that clusters are present. Also the scatter diagrams summarize the positions of the objects in a multidimensional space, hence the original variables are transformed into new independent variables called EIGENVECTORS. The MDS are usually used to construct a map on the basis of a prepared Distance Matrix, whereas the PCA is only used in analysing quantitative data.

The PCO has merits in that it projects points on a plane and identifies trends and clusters (also called clouds). The method is, however, subjective in that it is

difficult to identify boundaries, it is at the risk of the users subjectivity and scattering *vis a-vis* clustering is affected by changing axes (for example: Axes 1 x 2; 1 x 3; 2 x 3). The PCO analysis uses only the Gower (1971) Mixed Data Coefficient and the Multivariate Statistics Package (MVSP).

#### 4.2.7.4 Gower General Similarity Coefficient (GGSC)

The GGSC is used to calculate mixed data. Jacard (1908) provided the non-metric coefficient used to calculate the similarity matrix for multistate data as follows:

$$Si_{AB} = \frac{a}{a + b + c}$$

Where a, b and c are characters.

For Mixed data:

$$Si_{AB} = \sum_{i=a}^{n} Coi$$

This is called Gower's (1971) Mixed data Coefficient.

(i) For Simple Multistate data:

Coi = 1 for a match and O for any mismatch.

(ii) For Binary asymmetric data:

Coi = 1 for a double positive match and O

for a mismatch or a double negative match.

(iii) For quantitative or ordered multistate data:

Coi = x , where x is between 0 and 1, and is determined as:

$X = i_A - i_B$ after scaling the character i on its range.

### 4.2.7.5 The Euclidean Distance

This is recommended for calculating quantitative data only, and not for multistate or mixed data. The Eulidean distance is the distance between two points A and B in the geometric model calculated from Pythagora's theorem (Figure 4.4) extended to n dimensions (Abbott *et al.*, 1985).

For variables a and b (Figure 4.4), the general formula for distance between two objects, A and B, in two dimensions is given by the relationship:

$$Di_{AB} = \sqrt{(a_A - a_B)^2 + (b_A - b_B)^2}$$

When the formula is extended to n dimensions, it is given by:

$$Di_{AB} = (a_A - a_B)^2 + (b_A - b_B)^2 + \ldots..(i_A - i_B)^2 \ldots. + \ldots.(n_A - n_B)^2$$

$$= \sqrt{\sum_{i=a}^{n} (i_A - i_B)^2}$$

### 4.2.7.6 Computer Programmes

Working characters were coded into a form suitable for computer input and

subsequent analysis. Quantitative characters were entered by their respective integers. Qualitative character states were represented by code numbers which saved space, are more convenient and are acceptable to that particular computer programme.

The Computer Analysis programme called the Multivariate Statistics Package (MVSP), for example, uses codes of 0 and 1 for binary data; and integer codes for multistate data (Munyenyembe, 1993).

When the characters have been coded, they are arranged in a data matrix called the n x t or n x p table.

In both the Agglometative cluster Analysis and Principal Coordinates Analysis (PCO), only the Multivariate Statistics Package (MVSP) was employed.

#### 4.2.7.7. The Multivariate Statistics Package (MVSP)

According to Munyenyembe (1993), the MVSP is used on microcomputers with the MS-DOS operating system. It calculates distance and similarity matrices using all the well known distance and similarity measures, carries out hierarchical cluster analysis, principal coordinates analysis, etc.

The programme has its own data editor, although it can import ascii data files. Data are entered as numerals. Real numbers are used in quantitative data. Binary data are coded as 0 for absent and 1 for present. Multistate data are coded

as intergers.

The programme accepts data sets with missing values. The programme's output includes distance matrix files,free files from which dendrograms can be plotted and scatter diagrams that can be plotted using a dot matrix printer.

For calculating the similarity matrix using the MVSP, the Gower (1967) mixed data coefficient has been used throughout this study.

#### 4.2.7.8 Dendrograms

Dendrograms have been used in the study to present results of the two Agglomerative cluster analysis methods employed, the UPGMA and SINGLE linkage method.

Abbott *et al* (1985) writes that a dendrogram is a diagramatic representation of a strictly hierarchical classification. It is a tree showing progressive inclusion of items or groups of items into larger groups in a hierarchy.

Items or groups are depicted by stems. The inclusion of several items or groups into one group occurs at a node and is depicted by a crossbar (Figure 4.6).

At one hierarchic level or another, all items become included in groups, and these in turn all become members of the complete group. The height, measured on the ordinate, at which each node occurs is known as its rank. It is based on a

measure of resemblances which varies from study to study. At each node of the dendrogram, there is one larger group known as the supergroup, which is made up of two or more subgroups.

Divisive computer programmes produce dendrograms in a divisive order, starting from the largest cluster and finishing with the individual items. In most dendrograms the ordering of items within sets is arbitrary and not intended to give meaning either within or between sets. This is examplified in Figure 4.7 whereby the order of attachment to a crossbar can be shuffled without changed meaning. In (a) the juxtaposition of $B_3$ and $B_4$ does not mean that $B_4$ is more similar to $B_3$ than to $B_2$ . Similarly juxtaposition of $B_5$ and $B_4$ does not mean that $B_5$ is the most similar item of set $\alpha$ to $B_4$ in set ß. This, however, contrasts with the layout used by taxonomists where the most similar taxa are printed adjacent to each other.

## 4.3 RESULTS

### 4.3.1 Size of a form

Thirty one *Acalypha* taxa were analysed for the size of a form. The clusters or affinity groups are shown by a histogram (Figure 4.8) and a scattergram (Figure 4.9).

There are three major affinity groups which are better elucidated by the scattergram in Figure 4.9. The first largest cluster incorporates 23 *Acalypha* taxa, which is 74.2% of the population. The second and third groups are each comprised

of 4 *Acalypha* taxa both of which account for only 25.8% of the entire *Acalypha* taxa studied.

#### 4.3.1.1 The Affinity Groups

**Group 1**

This group has a size of a form ranging from 21.9± 5.6 in OTU **gi** to 35.2 ± 4.9 in OTU **pa** (Table 4.3). Other OTU members of this group are, on the basis of the observed subgroups, the **fr, vi, eg** subgroup; the **ch, vo, am, we, ny, la, bu, gi** subgroup; and the largest **pa, ac, bi, ps, bo, gl, ec, st, br, ci, cr, in** subgroup (Figure 4.8, 4.9). The general variation in form size and among the OTU in the entire group is moderately significant.

**The subgroups**

The smallest subgroup is comprised of three OTUs **fr, vi** and **eg**. There is insignificant variation within this subgroup in terms of both size of a form and within the OTUs . This is indicated by the range of the size of a form and the respective confidence limits in this group, that is 25.2 ± 1.9 in OTU **eg** to 26.2 ± 1.7 in OTU **fr**.

The medium sized subgroup comprises of eight OTUs namely **ch, vo, am, we, ny, la, bu** and **gi**. There is moderate to significant variation both within OTUs and in size of a form which is determined by the position of each OTU. This observation is reflected by the variations of the OTU size of a form and their respective confidence limits which range from 21.9 ± 5.6 **in gi** to 28.0 ± 3.3 in

OTU **ch.**

The third and largest subgroup consists of twelve OTUs, that is **pa, ac, ps, bi, bo, gl, ec, st, br,ci, cr** and **in**. The variation pattern within OTUs and in size of a form are reflected in this group than in the other two subgroups. For example, the range in the size of a form and their respective confidence limits are 25.6 $\pm$ 6.4 in OTU **in** to 35.2 $\pm$ 4.9 in OTU **pa**. The variation pattern within OTUs is indicated by OTU **bi** (31.1 $\pm$ 4.3) and OTU **cr** (26.4 $\pm$ 7.9).

**Group 2**

This is a small group which consists of four OTUs, that is **vl, pu, ne** and **eg**. The general variation in size of a form and within the OTUs is insignificant. For example, the size of a form ranges from 37.6 $\pm$ 9.5 in OTU **en** to 40.3 $\pm$ 8.5 in OTU **vl**, and the variation within OTUs ranges from 40.3 $\pm$ 8.5 in the latter OTU to 38.5 $\pm$ 9.8 in OTU **ne**.

**Group 3**

This is another small group consisting of four OTUs namely OTU **hi, wi, or** and **ra**. There is insignificant variation between OTUs **hi** and **wi** in form size, from 47.5 + 4.2 in OTU **hi** to 47.9 $\pm$ 9.5 in OTU **wi**. However, the size of a form variation is significant between OTU **ra** (44.5 $\pm$ 22.6) and OTU **or** (52.8 $\pm$ 12.8). An interesting feature is shown by the inter-OTUs variation pattern, as indicated by the histogram and scattergram (Figures 4.8; 4.9) and their respective confidence limits (Table 4.3).

### 4.3.2 The UPGMA, Single link and PCO methods

The results of the UPGMA and single-link cluster analyses of the 31 *Acalypha* taxa based on 21 (binary / multistate) characters using the MVSP (Figures 4.12 and 4.13) produced highly balanced dendrograms than when 49 mixed characters or 28 quantitative characters were used.

Therefore, for convenience of presentation, the groups produced by the 21 characters, which fortunately had the same taxa composition when both the UPGMA and single link cluster analyses were used, were treated as HYPOTHESIZED GROUPS.

The groups produced by the other character sets of 49 mixed characters and 28 quantitative characters using UPGMA and single link methods and the PCO are be presented later in relation to the hypothesized groups.

#### 4.3.2.1 The 21 character SUBSET

The results of the UPGMA cluster analysis of the 31 *Acalypha* taxa based on 21 binary/multistate characters using the MVSP are presented in Figure 4.12. The dendrogram produced a highly balanced hierarchy. Two sister supergroups were produced by the dendrogram namely, a small group D and a large balanced group comprising of subgroups A, B and C. The composition of the supergroups and subgroups will be discussed later on the basis of common character states of the respective groups.

The taxa composition of the groups in this analysis (Figure 4.12) remained more or less the same as in the dendrogram produced by 31 *Acalypha* taxa based on UPGMA cluster analysis using 49 mixed characters (Figure 4.10), except for a few taxa which inter changed groups.

The results of the single link cluster analysis of the 31 *Acalypha* taxa based on 21 binary/multistate characters using the MVSP are presented in Figure 4.13. A highly balanced dendrogram composed of 4 groups was produced namely A, B, C and D. The groups are identical in taxa composition to the 4 supergroup/subgroups produced by the same 21 characters using the UPGMA method (Figure 4.12).

However, the position of group/subgroup B and C in the two dendrograms (Figure 4.12 and 4.13) have been interchanged, possibly due to the 'nearest neighbour' axiom, typical of the single link method Estabrook (1966).

#### 4.3.2.2 The 49 master character SET

The results of UPGMA cluster analysis for the 31 *Acalypha* taxa based on 49 mixed characters using the MVSP are presented in Figure 4.10.

In the dendrogram, two sister supergroups came out clearly as group A, and a large group represented by 3 subgroups B, C and D.

The subgroups B,C and D represent distinct groups since the clusters stem

from low similarity values. The clusters are well balanced and will be interpreted and discussed later on the basis of common character states pertinent to each group.

The results of the single link cluster analysis of 31 *Acalypha* taxa based on 49 mixed characters using the MVSP are presented in Figure 4.11. A highly balanced dendrogram was produced with two well balanced sister supergroups comprising subgroups A, B, C,$D_1$ and $D_2$ . The subgroups branch from nodes of different similarity levels, yet at low similarity values which indicate that the subgroups represent distinct clusters. The much smaller subgroup $D_2$ came out more clearly than in the UPGMA method.

The composition of the clusters produced by this method were more or less identical to the taxa composition of clusters produced by the UPGMA method. This is evidenced by groups A and B of the two dendrograms (Figure 4.1O and 4.11). Slight variations exist in the taxa composition of the other clusters C,D, $D_1$ and $D_2$ in the two dendrograms. Interpretation of each cluster on the basis of common character states will be discussed later.

#### 4.3.2.3 The 28 character SUBSET

The results of the UPGMA cluster analysis of 31 *Acalypha* taxa based on 28 quantitative characters using the MVSP produced a chained dendrogram (Figure 4.14). The chaining axiom is characteristic of the Eucledian distance method which is preferred in cluster analysis using quantitative attributes. The Gower General Similarity Coefficient (GGSC) has so far been used in dealing with binary, multistate

and mixed characters.

It is not easy to identify clusters in this unbalanced hierarchy, particulary at low similarity levels. However, at high similarity values, balanced hierarchical clusters emerge progressively. The clusters are insignificant due to the high similarity among the taxa demonstrated by the high similarity values.

The foregoing explanation not withstanding, the dendrogram has 4 unbalanced clusters namely B,C and D, including a small supergroup A. The clusters produced by the dendrogram (Figure 4.14) have little bearing with respect to composition of taxa when compared to Figure 4.10 and 4.12 in which mixed and, multistate/binary attributes were used. The preceding observations indicate that quantitative characters have dismerit over the multistate/binary attributes.

The results of the single link cluster analysis of 31 *Acalypha* taxa based on 28 quantitative characters using the Eucledian distance coefficient and the MVSP produced the dendrogram presented in Figure 4.15.

A chained hierarchy was produced by the dendrogram based on the single link method. The dendrogram is much more unbalanced compared to the dendrogram produced by the UPGMA method (Figure 4.14) using the same 28 quantitative characters. However, the two dendrograms (Figures 4.14 and 4.15) are identical in terms of structure and composition of taxa, examplified by the 4 similar cluster characteristic to both dendrograms. The clusters are, however, not

easily identifiable and interpretable due to the chaining axiom.

#### 4.3.2.4 The PCO Method

The clusters or clouds produced in the Principal Coordinates Analysis (PCO) by the three axes (axes 1 x 2, 1 x 3 and 2 x 3) in the 2-Dimensional scatter diagrams (Figures 4.16a, 4.16b and 4.16c) are closely related to the clusters produced by UPGMA and single link analyses using the 49 mixed and 21 binary/ multistate attributes (Figures 4.10, 4.11, 4.12 and 4.13).

In clusters IQEHFR and abBVW, the taxa are in the same cluster in all the three diagrams (Figures 4.16a, 4.16b and 4.16c), indicating that the taxa are together in all the three dimensions. Similarly the subclusters XY, LMN, ST and EQ are also together in all the three dimensions. The subclusters AD and dG also came out clearly in the three diagrams and in all the three dimensions.

The trend in the rest of the clusters and subclusters is variable for the three diagrams. Hence some taxa are in the same cluster or subcluster in two diagrams, but not in the third, showing that they are separated in one plane only. The subclusters KLMN and KXY are present in one diagram only, indicating that the subclusters are separated in the other two planes.

Those taxa which are isolated in the three diagrams indicate that the respective taxa are also isolated in all the three dimensions; for example P,O,J,U, etcetera.

The first 3 axes account for 42.52%, six axes for 61.27% and 10 axes for 75.83% of the total dispersion (Table 4.4).

**Table 4.3: Mean values of size a form of 31 *Acalypha* OTUs. n= 31. CL at 95% (p=0.05).**

| OTUs | Mean ± CL |
|---|---|
| ac | 32.1 ± 4.4 |
| am | 26.7 ± 3.9 |
| bi | 31.1 ± 4.3 |
| bo | 29.0 ± 4.7 |
| br | 28.7 ± 6.7 |
| bu | 23.6 ± 4.8 |
| ch | 28.0 ± 3.3 |
| ci | 27.8 ± 7.3 |
| cr | 26.4 ± 7.9 |
| ec | 29.0 ± 5.2 |
| en | 37.6 ± 9.5 |
| fr | 26.2 ± 1.7 |
| eg | 25.2 ± 1.9 |
| vi | 25.5 ± 1.6 |
| gi | 21.9 ± 5.6 |
| hi | 47.5 ± 4.2 |
| in | 25.6 ± 6.4 |
| la | 24.1 ± 3.4 |
| ne | 38.5 ± 9.8 |
| pu | 38.3 ± 9.3 |
| ny | 24.8 ± 4.4 |
| or | 52.8 ± 12.8 |
| pa | 35.2 ± 4.9 |
| ps | 31.2 ± 6.5 |
| gl | 30.2 ± 5.8 |
| ra | 44.5 ± 22.6 |
| st | 28.3 ± 6.1 |
| vl | 40.3 ± 8.5 |
| vo | 26.7 ± 3.6 |
| we | 24.6 ± 3.7 |
| wi | 47.9 ± 9.5 |

## 4.4 DISCUSSION

### 4.4.1 Clusters produced by size of a form

The analysis based on size of a form was based on quantitative characters. The clusters produced by size of a form did not have any bearing in structure and taxa composition on the clusters produced by the UPGMA, single link and the PCO analytical methods. When 28 quantitative characters were analysed based on the UPGMA and single link methods using Euclidean distance coefficient, chained and uninterpretable dendrograms were produced. The unproductive histogram and 2-D scatter diagram produced by size of a form (Figures 4.8 and 4.9), and chained dendrograms (Figures 4.14 and 4.15) justify the conclusion that quantitative characters alone are not ideal in taximetric studies.

### 4.4.2 Clusters emanating from the analytical methods

#### 4.4.2.1 The 21 character SUBSET

The 4 clusters produced by taximetric analysis based on 21 characters when both the UPGMA and single link methods were employed the same in taxa composition although the clusters differ slightly in their structure (Figure 4.12 and 4.13).

Group A comprises of 9 *Acalypha* taxa namely: *A. volkensii*, *A. ciliata*, *A. bussei*, *A. psilostachya vars. psilostachya* and *glandulosa*, *A. lanceolata, A. indica, A. crenata* and *A. brachystachya.*

The taxa in this group share a number of common character states. Thus the group is characterized by being annual herbs, and having 4 pairs of blade lateral nerves, axillary male inflorescences, sessile female flowers and 1- to 2-lobed allomorphic flowers, mostly terminal but occassionally axillary.

Group B comprises of 10 *Acalypha* taxa: *A. hispida*, *A. neptunica* vars. *neptunica* and *glabrescens*, *A. fruticosa* vars. *fruticosa, villosa* and *eglandulosa*, *A. wilkesiana*, *A. echinus*, *A. engleri* and *A. bipartita.*

The common character states of the group are possession of short petioles, terminal male inflorescences, axillary female inflorescences, strongly accrescent female bract and ellipsoid to ellipsoid-ovoid seeds.

Group C comprises of 9 *Acalypha* taxa. They are *A. villicaulis, A. paucifolia*, *A. ornata, A. nyasica, A. racemosa*, *A. welwitschiana*, *A. chirindica*, *A. stuhlmannii* and *A. ambigua.*

The group is characterized by taxa with unisexual inflorescences and exceptionally long, free styles. Also, the male inflorescences and female bracts of the taxa in this group are very long.

Group D is a small group composed of 3 taxa namely; *A. gillmanii, A. boiviniana* and *A. acrogyna.* The taxa in this group have many characters in common,namely being perennial shrubs with short petioles, penni-nerved leaves,

racemose male and female inflorescences, more or less sessile female flowers, exclusively oblate spheroidal pollen grains and without accrescent female bract. The seeds are subglobose in shape and lack a caruncle.

#### 4.4.2.2 The 49 master character SET

Four clusters are also produced using 49 mixed characters based on the UPGMA and single link taximetric methods (Figures 4.10 and 4.11). However, when single link method is used (Figure 4.11) Group D divides to produce two subgroups $D_1$ and $D_2$, apparently due to the nearest neighbour axiom. This notwithstanding, the remaining groups A,B and C are identical in taxa composition in the two dendrograms.

An interesting observation is that the taxa composition of Group A and B are the same as when the UPGMA and single link taximetric methods are employed based on both 21 binary/multistate and 49 mixed characters. However, Group C and D or $D_1$ and $D_2$ produced by the 4 dendrograms do not conform in the composition of taxa within their respective groups. This suggests that the characters of the taxa in the respective groups are not highly correlated.

#### 4.4.2.3 The 28 character SUBSET

The clusters produced using the UPGMA and single link cluster analysis based on 28 quantitative characters using the Eucledian distance coefficient (Figures 4.14 and 4.15) produced identical chained dendrograms. The two dendrograms are identical in terms of structure and taxa composition. The clusters, steming mostly

from high similarity levels, have little bearing in structure and taxa composition when compared with the clusters produced based on 21 binary/multistate and 49 mixed characters using the Gower General Similarity Coefficient (GGSC) based on UPGMA and single link taximetric analysis methods. The chained clusters produced by the 28 quantitative characters are not easy to identify and interpret, hence the groups are not considered in the study.

### 4.4.3 The PCO method

The groups produced in the Principal Co-ordinates Analysis (PCO) by the three axes in the 2 - Dimensional scatter diagrams (Figures 4.16 a, b, c) are closely correlated to the clusters produced based on 21 binary/multistate and 49 mixed characters, using both the UPGMA and single link clustering methods. In particular, the clusters produced based on axes 1 x 2 and 1 x 3 (Figure 4.16a, b) conform in taxa composition to the clusters obtained based on UPGMA and single link analysis methods using 21 binary/ multistate and 49 mixed characters.

The clusters formed in the PCO based on axes 2 x 3 are not easily interpretable, hence they have not been useful in this discussion. These clusters are just as difficult to interpret as the chained clusters produced using 28 quantitative characters based on UPGMA and single link clustering methods using the Euclidean distance coefficient which have also been dropped in the discussion.

### 4.4.4 Clusters and character states

Group A appears to be the most stable cluster. The composition of the taxa in the group remained unchanged when all the three taximetric methods, UPGMA and single link, based on 21 binary/multistate and 49 mixed characters using the GGSC, and the PCO (axes 1 x 2, 1 x 3) were used (Figures 4.10-4.13, 4.16a,b). The stability of the group may be attributed to the stability of the common character states of the taxa within the group.

Group B is another equally stable cluster. The composition of the taxa did not change in the UPGMA and single link analysis based on GGSC using the 21 binary/multistate and 49 mixed characters. There have, however, been changes in the taxa composition of the clusters in the PCO based on the 49 mixed characters, unlike the case in the Group A category. Again, the extent of stability shown by the group is a reflection of the stability of the shared characters within the group.

Group C is an unstable cluster. The composition of the taxa have changed depending on the type of the taximetric analysis method used and nature of the characters employed (Figures 4.10-4.16a,b,c). The number of common characters within the group is relatively small and this might have contributed to the instability of taxa composition pertinent to the group.

Another uniquely stable but small cluster D comes out clearly in the three taximetric analysis methods specified under Group A. This applies to the two *Acalypha* taxa, *A. acrogyna* and *A. boiviniana* ( Figures 4.10- 4.13; 4.16a,b,c). *A.*

*echinus* (Figure 4.10) and *A. gillmanii* (Figures 4.12, 4.13) have interchangeably been included in the group.

The high stability shown by this small group is manifested in the many stable characters shared by the few members of the group, particularly *A. acrogyna* and *A. boiviniana.*

*A. echinus* has few characters that are shared by Group D: racemose male and female inflorescences and absence of an accrescent female bract. *A. gillmanii* shares all the common characters of the group except the absence of more or less sessile female flowers. *A. gillmanii* possesses short pedicelled female flowers. The distinction between more or less sessile and short pedicelled female flowers might not be distinct enough to warrant the justification for separating the two taxa namely, *A. echinus* and *A. gillmanii.*

### 4.4.5 Taxonomic outlook

The *A. fruticosa* group, *A. neptunica* group and *A. psilostachya* group retain their taxonomic and nomenclatural status as in Radcliffe-Smith (1987) since the taximetric methods used in the study have not provided sufficient evidence to the contrary.

In that respect the *A. fruticosa* group comprises vars. *fruticosa, villosa* and *eglandulosa;* the *A. neptunica* group consists of vars. *neptunica* and *glabrescens*, and the *A. psilostachya* group is composed of vars. *psilostachya* and *glandulosa.*

The following observations are also made in the light of the treatment of East African *Acalypha* by Radcliffe-Smith (1987).

The Group A taxa, *A. lanceolata* which was reported to resemble *A. indica, A. ciliata* which was considered to be a variety of *A. bussei,* and the Group C taxa *A. villicaulis* reported to be a local race of *A. paucifolia* are now considered to have this controversy resolved about their systematic classification and retain their specific and nomenclatural status as species.

Agnew (1980) considered *A. psilostachya* to be a synonym of *A. brachystachya.* This work has established that although both taxa belong to the same cluster (Group A) when 21 binary/multistate and 49 mixed characters were used, and in the same cluster in the PCO analysis, they stem (on the average) at such a relatively low similarity level (0.75) and dispersion that each taxon should retain a specific status in the taxonomic hierarchy.

A conspecificity between *A. boiniviana* and *A. engleri* was suggested by Radcliffe-Smith (1987). Although *A. boiviniana* was represented by limited material, yet the study shows that the two *Acalypha* taxa should each maintain a specific rank. This is clearly demonstrated by the PCO clusters (Figures 4.16a-c), and the observation that each taxon belongs to a different cluster in most of the dendrograms (Figures 4.10-4.13).

### 4.4.6 Areas for further research

In the PCO analysis, E (*A. brachystachya*) and Q (*A. indica*) appear to be locked in the same cluster in the 3 diagrams (4.16a-c). This indicates that the two taxa are together in all the three dimensions. However, this is not reflected by the dendrograms in Figures 4.10 - 4.15. The explanation to this is obscure and more work is needed.

There is 100% similarity observed between *A. brachystachya* and *A. crenata* (Figures 4.12 and 4.13) when 21 binary/multistate characters are analysed by both the UPGMA and Single link methods. This trend is not observed in the other taximetric methods. This may be explained on the basis that the two species share the same 21 common characters used. Moreover, the two species differ only in some quantitative characters viz., female bract length, width and their respective ratio which was reflected (figure 4.10 and 4.11) when 49 mixed characters were used. This requires revision in a taxonomic treatment of the genus with worldwide material.

Two taxa, A (*A. acrogyna)* and D (*A. boiviniana*) form a distinct cluster in all the 3 taximetric methods used, that is UPGMA, single link and PCO (Figures 4.1O-4.13; 4.16a,b,c) except when the Euclidean distance coefficient is used (Figures 4.14 and 4.15) based on 28 quantitative characters. Indeed, cluster AD is formed as a SUPERGROUP when the analysis was based on 49 mixed characters using single link analysis.

Each taxon in Group AD shares most of the 28 quantitative characters and almost all the 21 binary/multistate characters used in the study. The 2 *Acalypha* taxa call for revision using worldwide plant material.

In the PCO, K (*A. engleri)* forms a cluster with LMN (*A. frutiosa* group) in only one dimension in the 1 x 3 axes (Figure 4.16b), but are separated in the other two dimensions (Figures 4.16a,c).

Again, K (*A. engleri*) clusters with XY (*A. psilostachya* group) in one dimension (Figure 4.16c) but separated in the other 2 dimensions (Figures 4.16a,b).

Similar trends have not been observed in the UPGMA and single link clustering analysis (Figures 4.10 - 4.15), hence no explanation is apparent. This is another aspect which needs further research using worldwide plant materials.

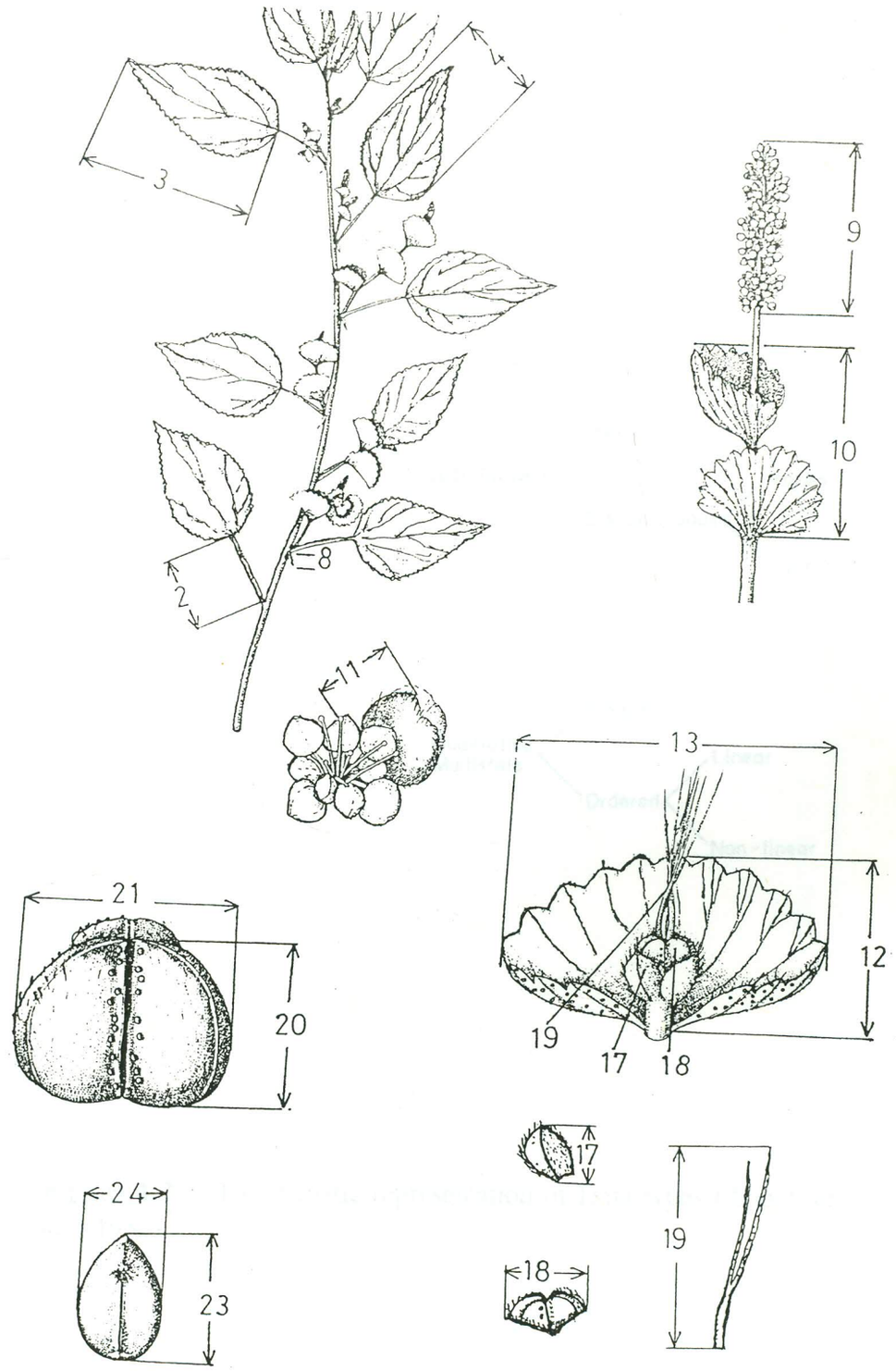



**Figure 4.1.** Diagramatic representation of quantitative characters employed in taximetric analysis of *Acalypha* taxa.

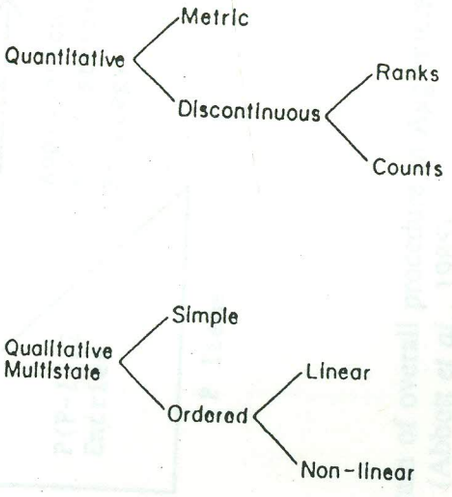



**Figure 4.2.** Diagramatic representation of Data types (Abbott *et al.*, 1985).

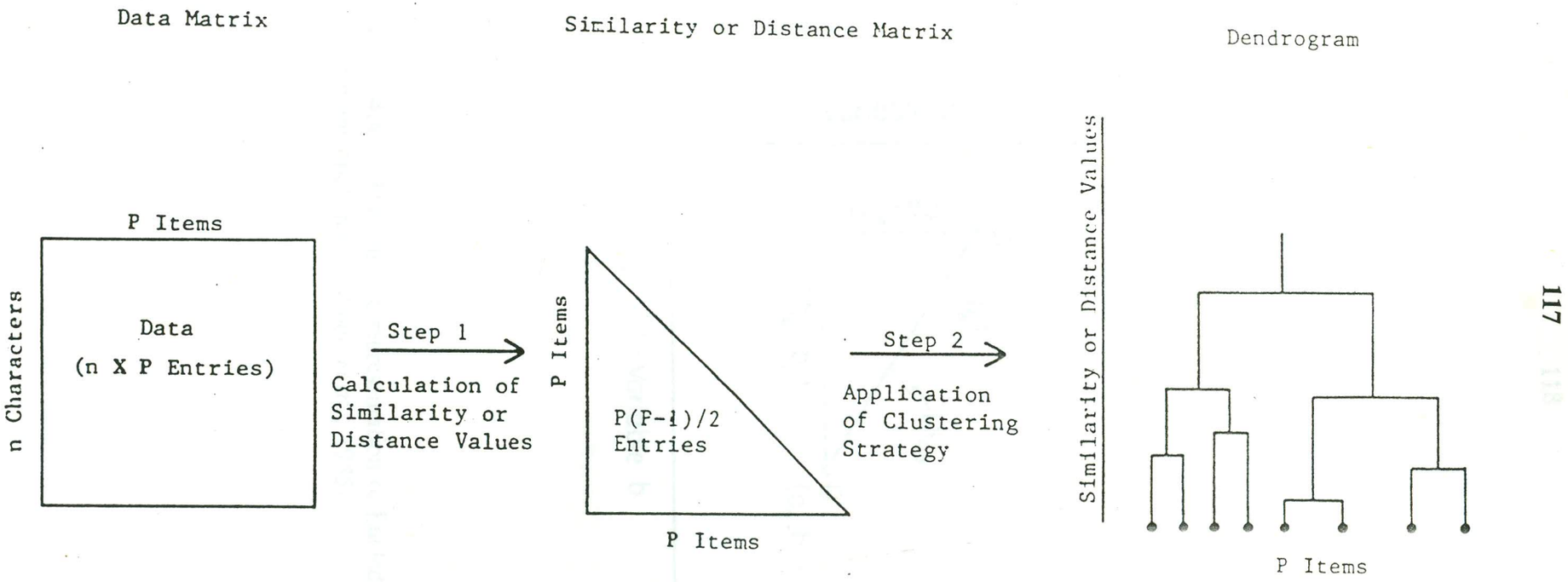



**Figure 4.3.** Diagram of overall procedure in Agglomerative two-step cluster analysis (Abbott *et al.*, 1985).

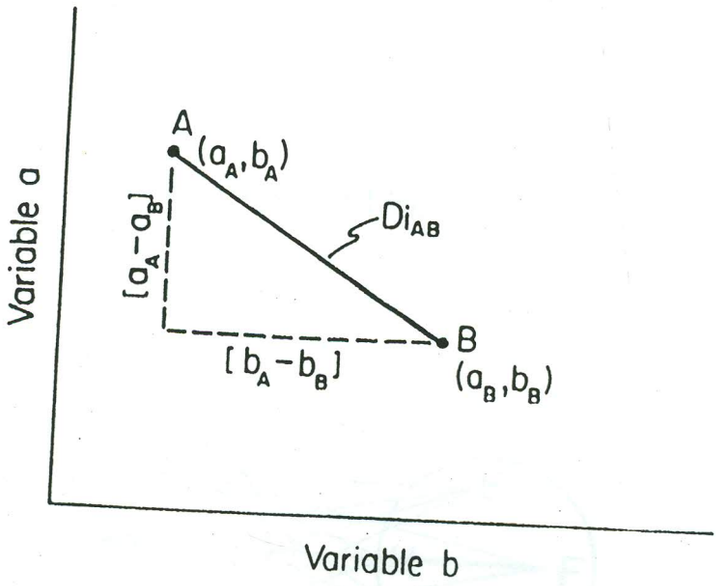



**Figure 4.4.** Diagramatic representation of Eucledean distance in two-dimensional space (Abbott *et al.*, 1985).

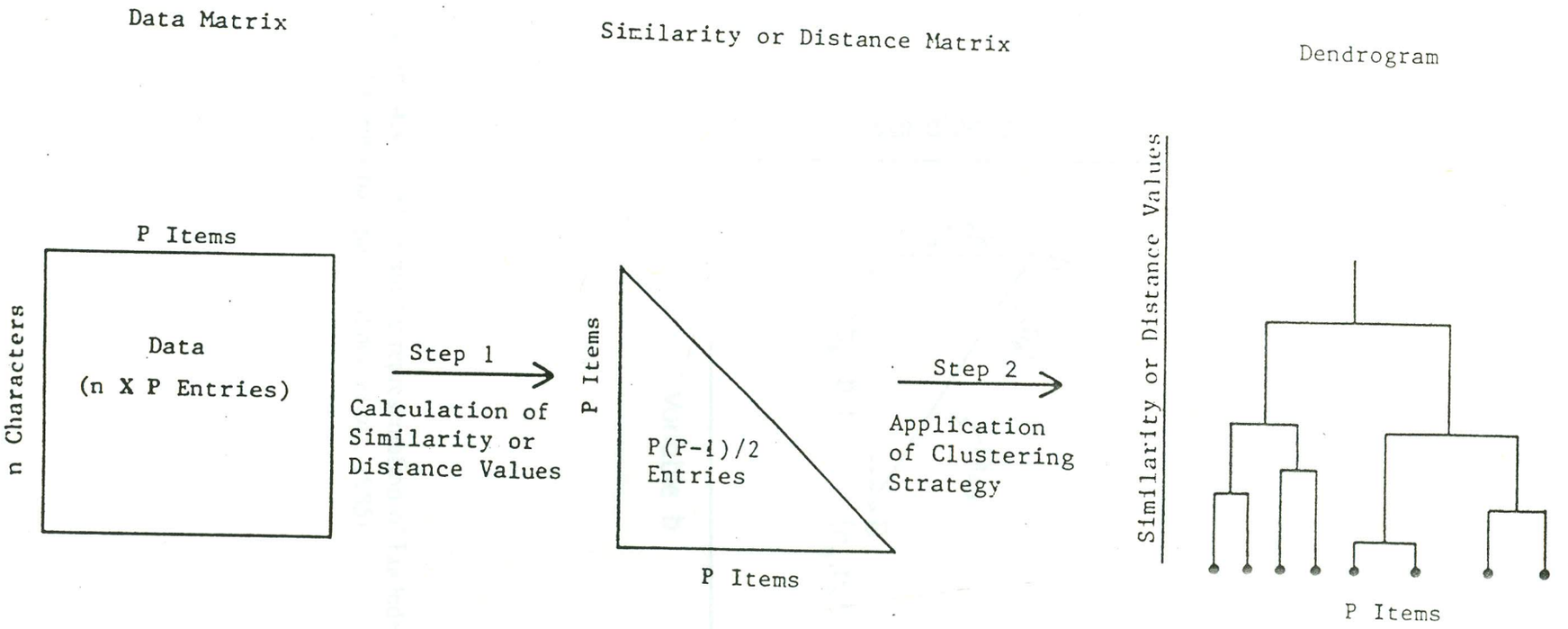



**Figure 4.3.** Diagram of overall procedure in Agglomerative two-step cluster analysis (Abbott *et al.*, 1985).

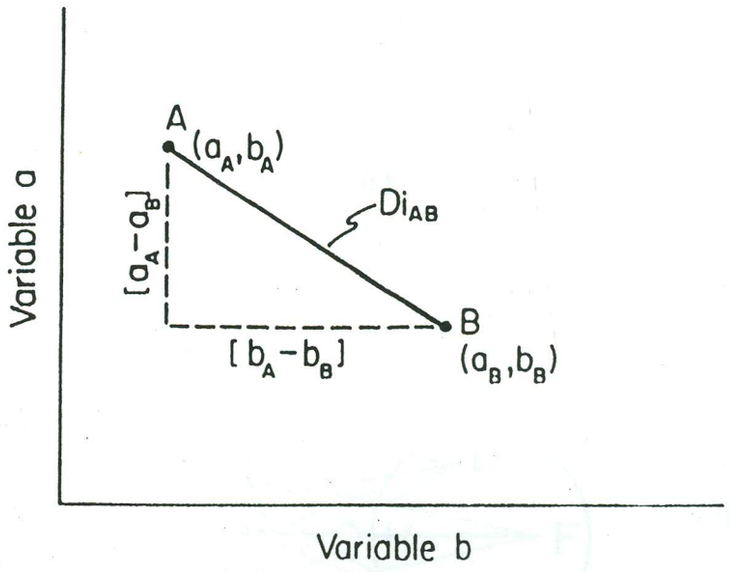
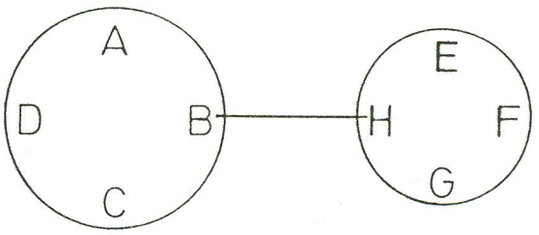
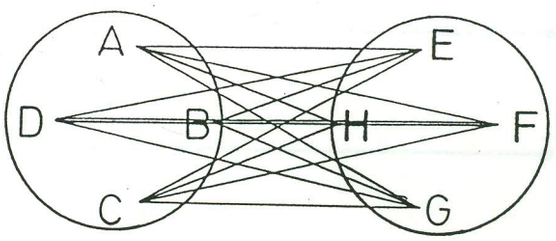



**Figure 4.4.** Diagramatic representation of Eucledean distance in two-dimensional space (Abbott *et al.*, 1985).




1. Single Link




2. UPGMA

**Figure 4.5.** Agglomerative methods of cluster analysis (Munyenyembe, 1993).

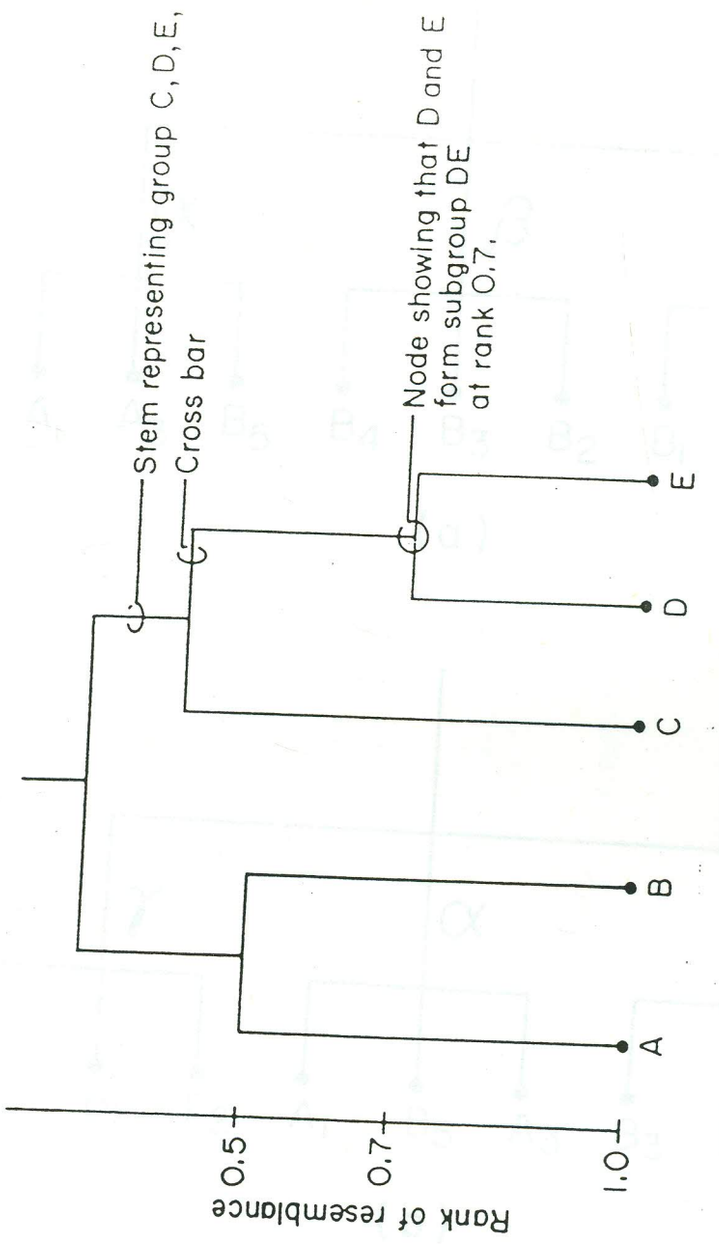



**Figure 4.6.** An explanatory diagram indicating the components of a dendrogram (Abbott *et al.*, 1985).

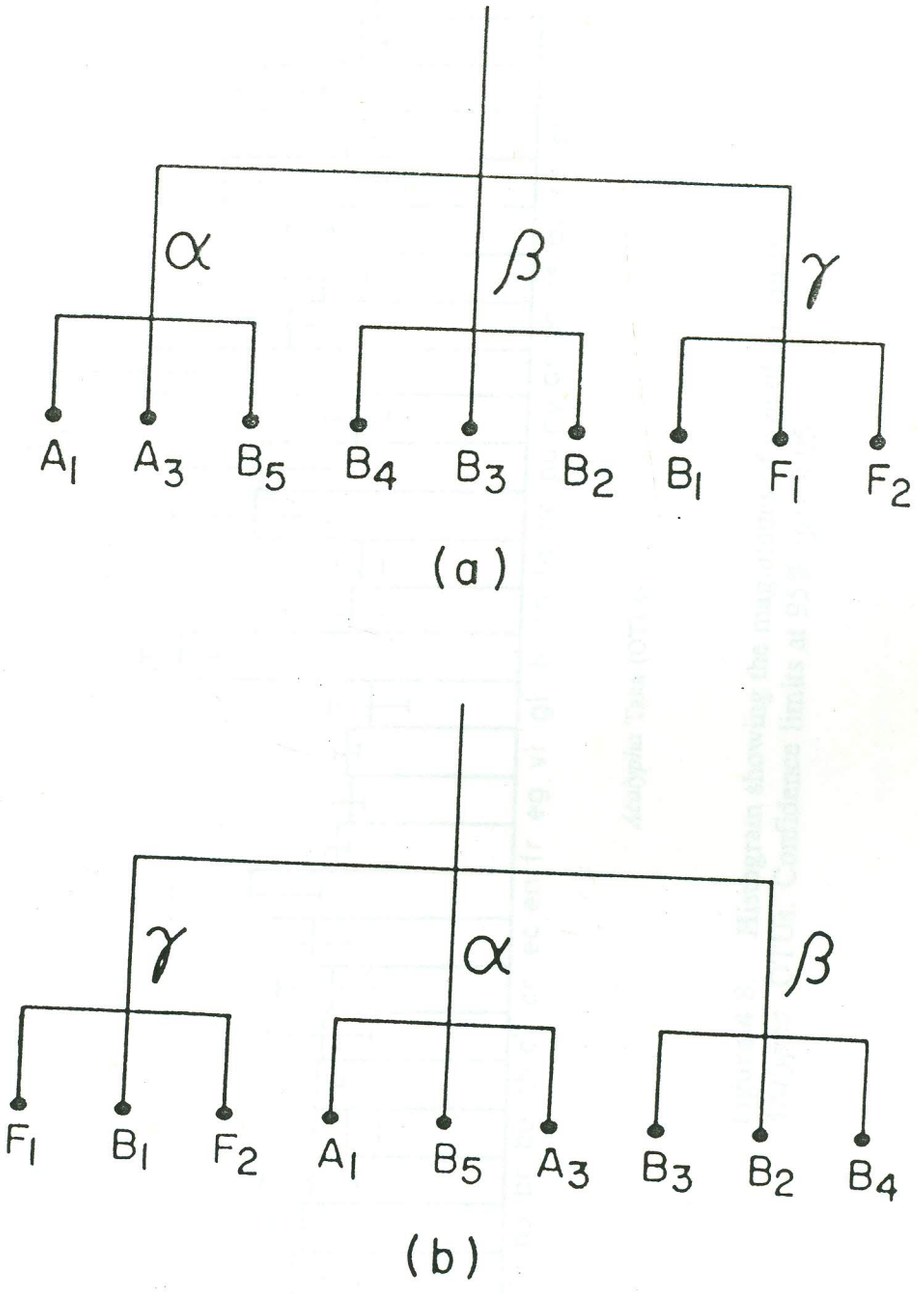



**Figure 4.7.** A diagramatic representation of two identical dendrograms (Abbott *et al.*, 1985).

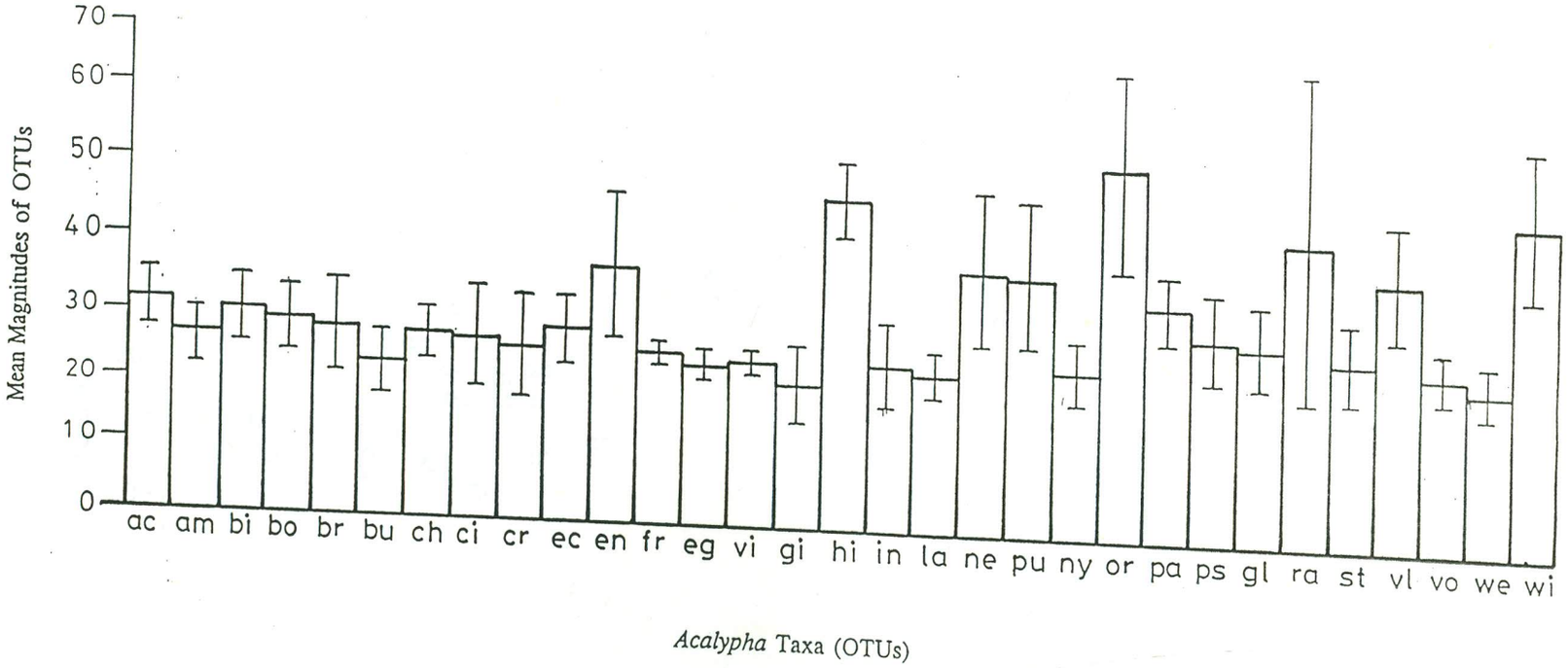



**Figure 4.8.** Histogram showing the magnitudes of size of a form in *Acalypha* OTUs. Confidence limits at 95% (p= 0.05).

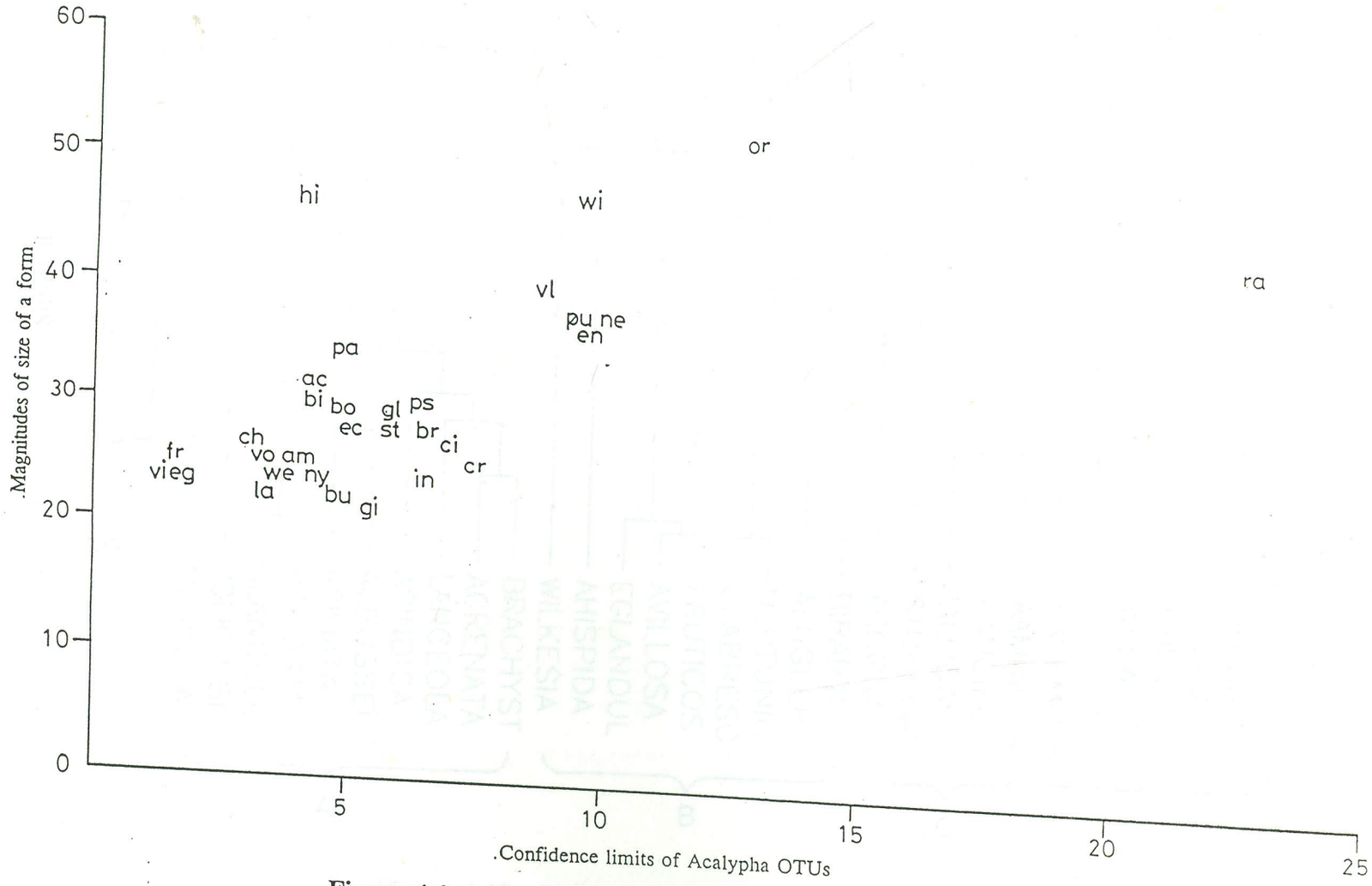



**Figure 4.9**. Pictorialized scattergram illustrating affinity groups produced by size of a form on analysing *Acalypha* OTUs. Confidence limits at 95% (p= 0.05).

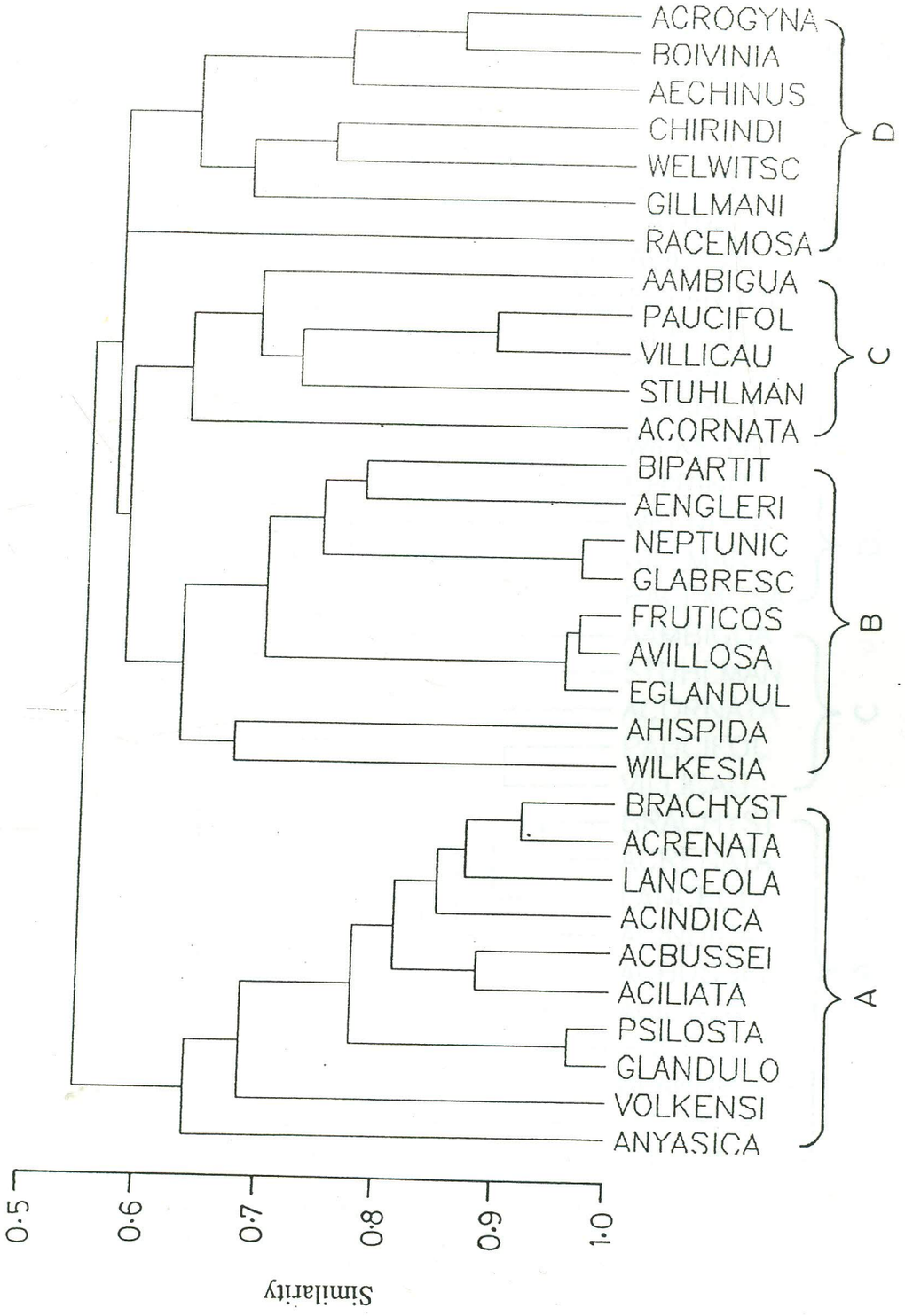



**Figure 4.10**. Dendrogram of 31 *Acalypha* taxa based on UPGMA cluster analysis using 49 mixed characters.

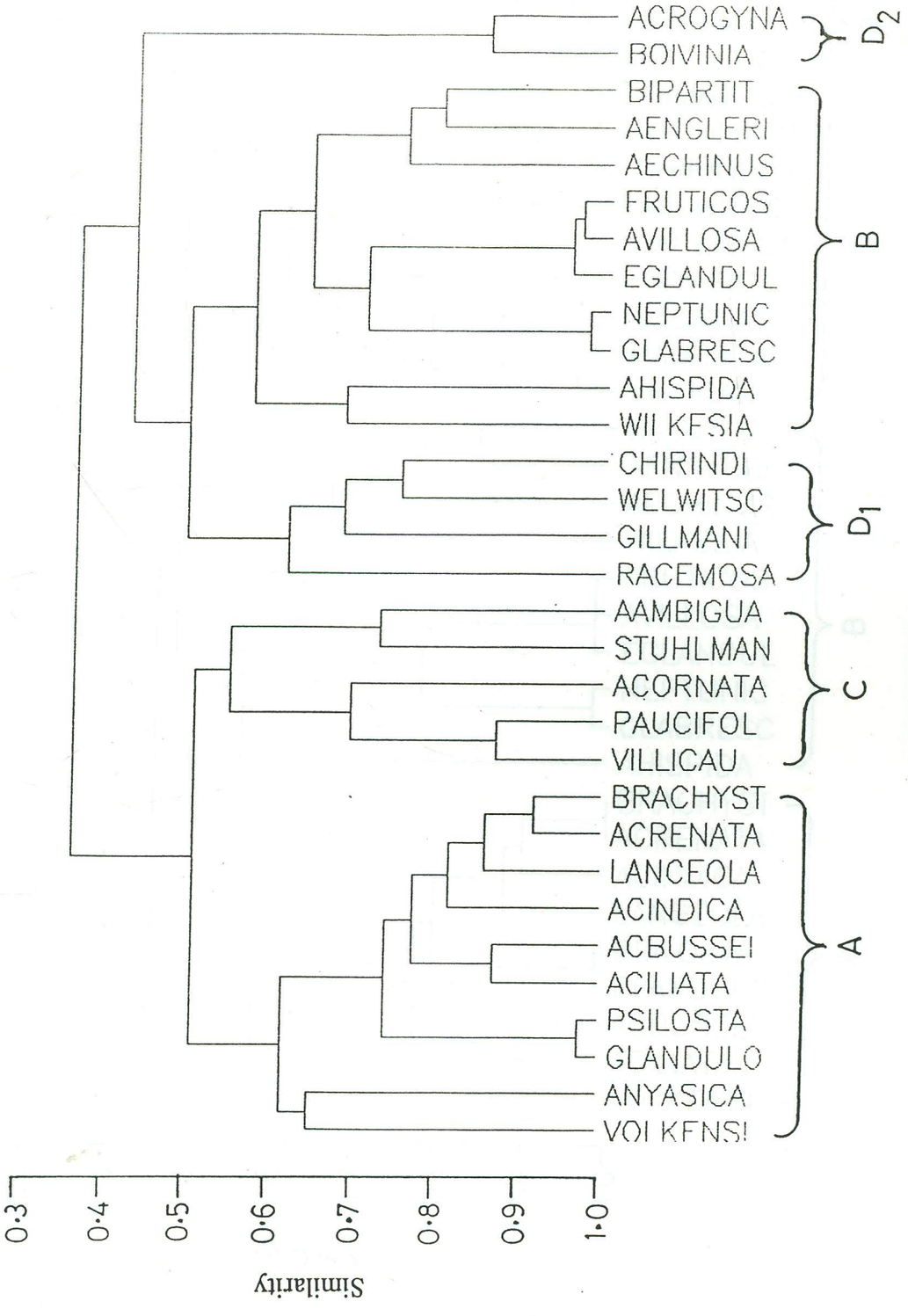



**Figure 4.11.** Dendrogram of 31 *Acalypha* taxa based on single link cluster analysis using 49 mixed characters.

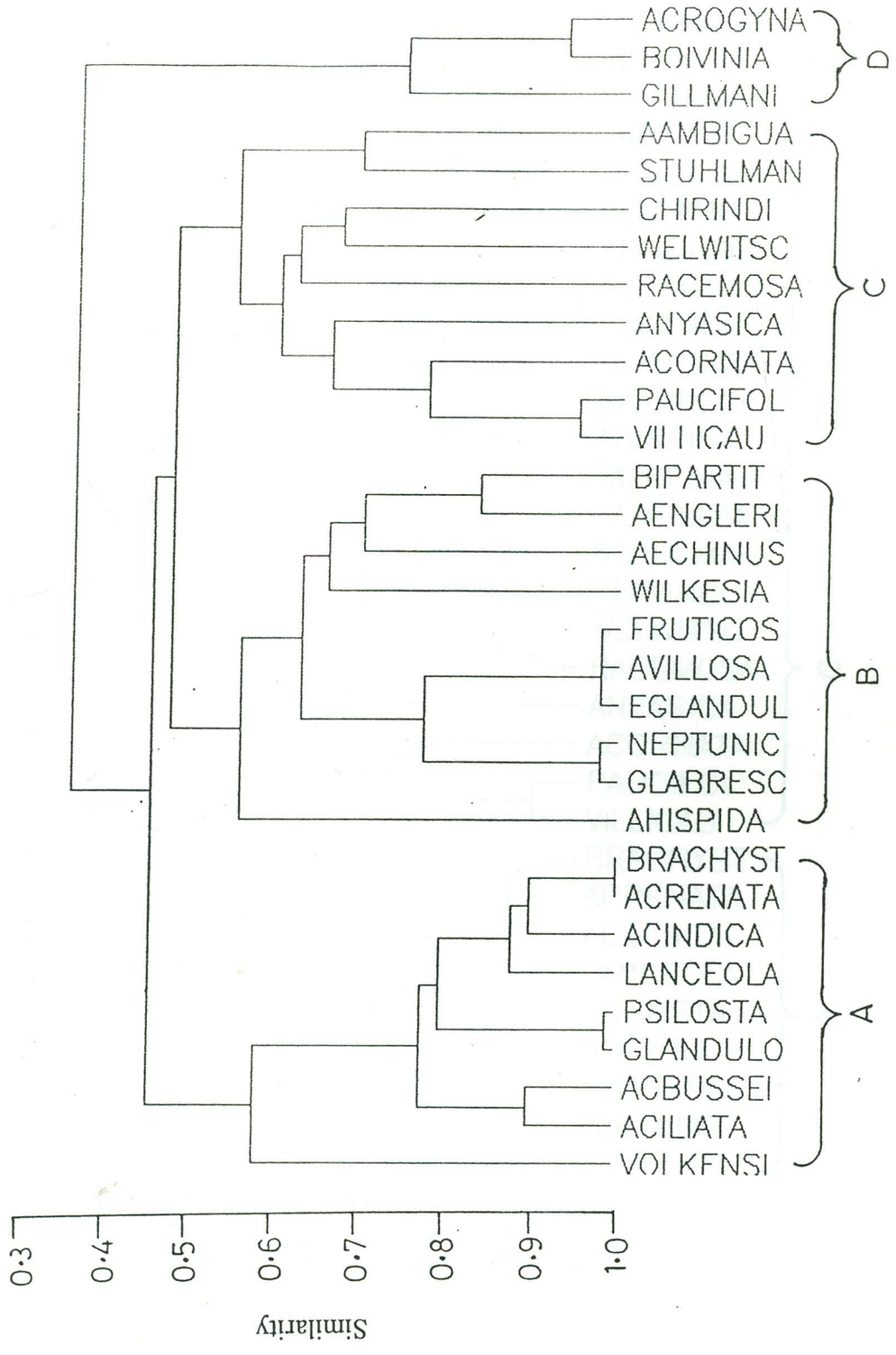



**Figure 4.12.** Dendrogram of 31 *Acalypha* taxa based on UPGMA cluster analysis using 21 binary/multistate characters.

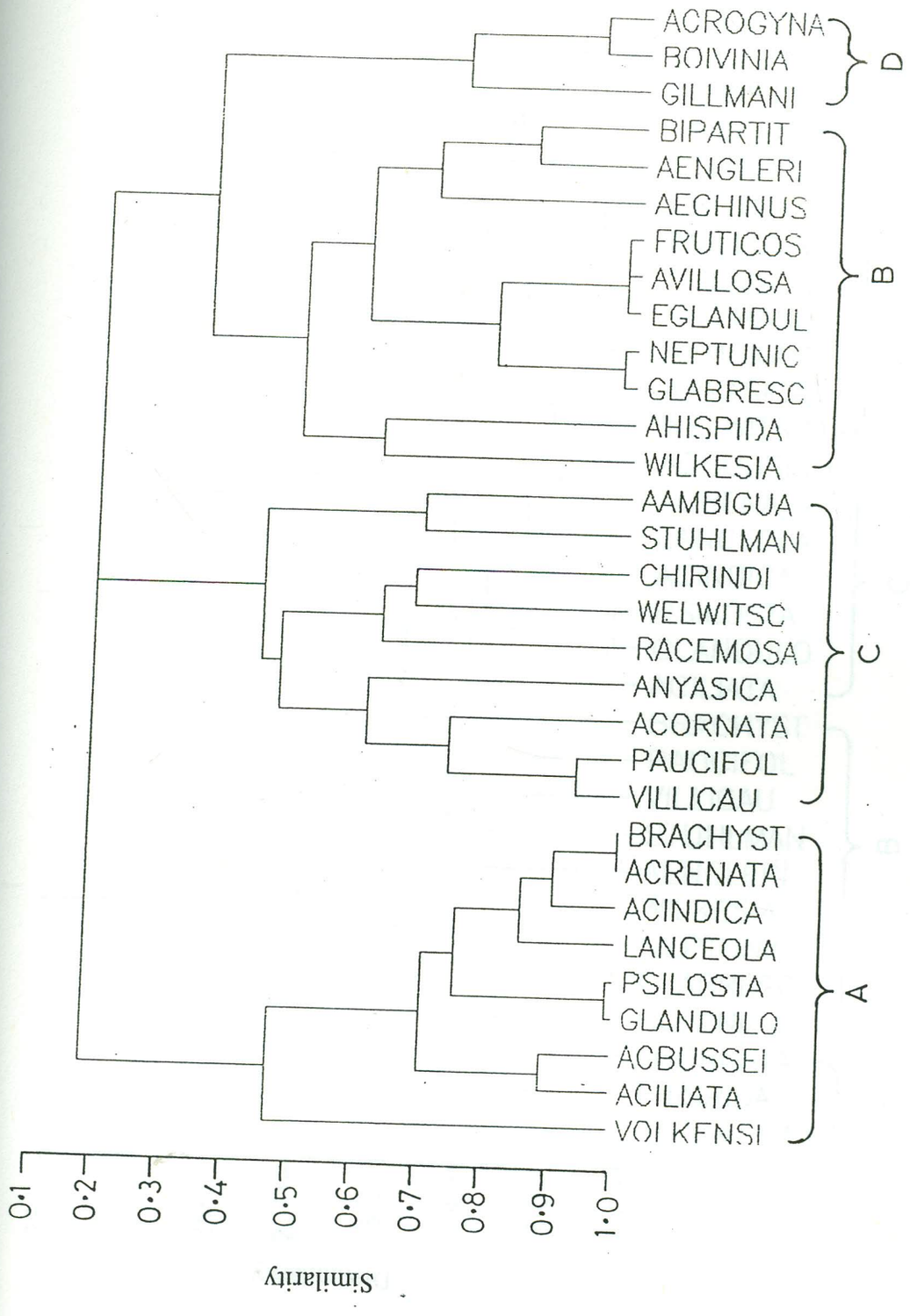



**Figure 4.13.** Dendrogram of 31 *Acalypha* daxa based on single link cluster analysis using 21 binary/multistate characters.

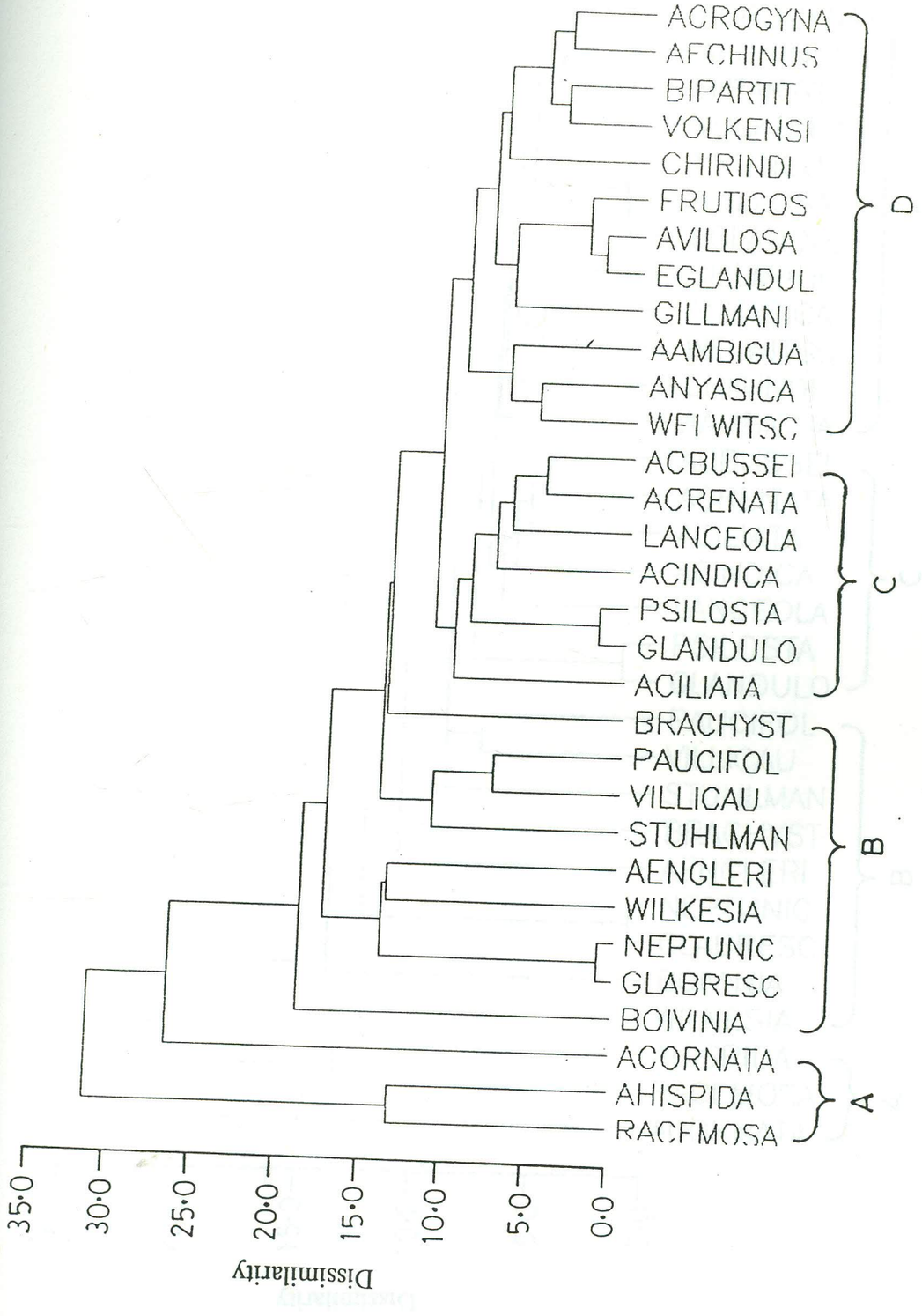



**Figure 4.14.** Dendrogram of 31 *Acalypha* taxa based on UPGMA cluster analysis using 28 quantitative characters.

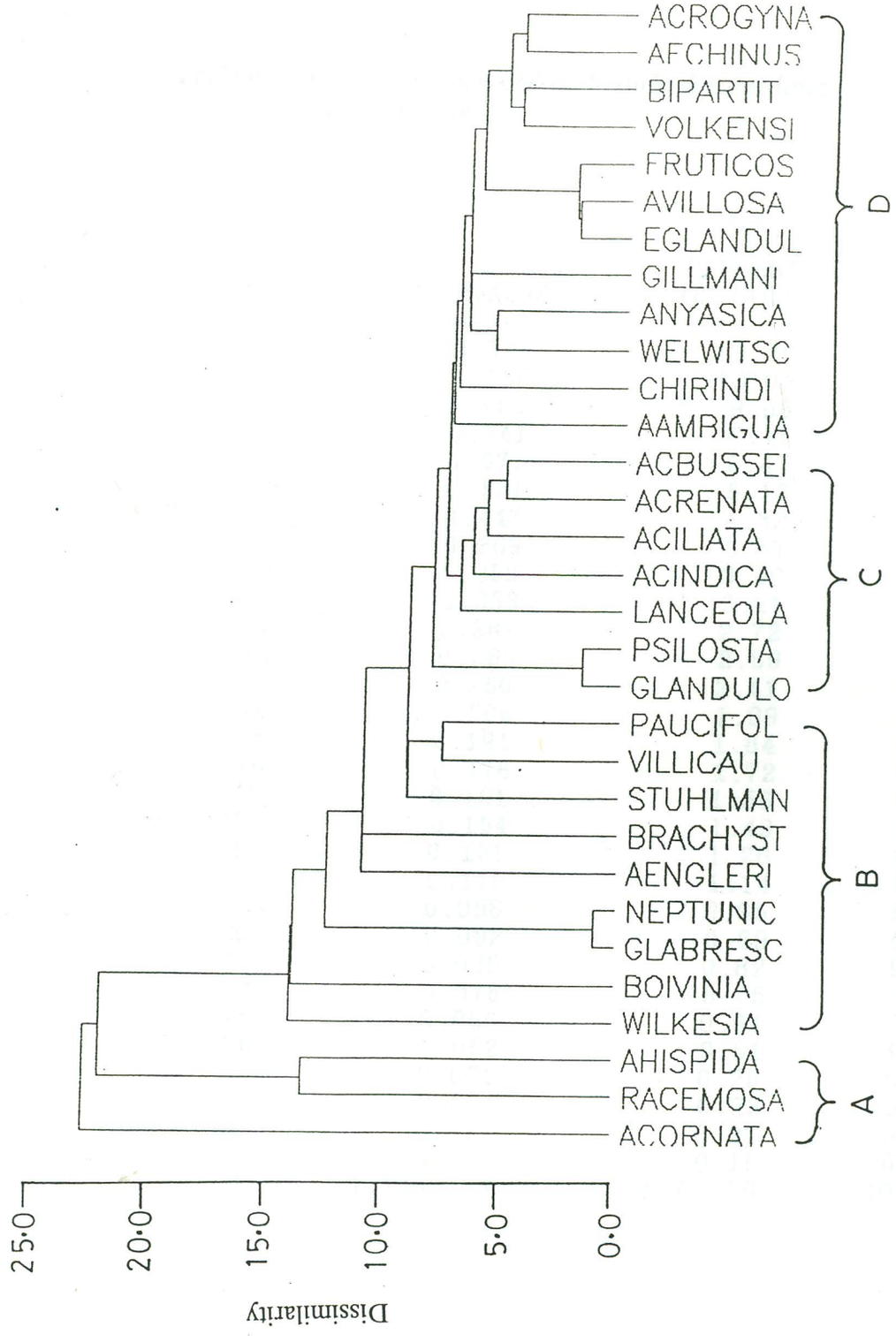



**Figure 4.15.** Dendrogram of 31 *Acalypha* taxa based on single link cluster analysis using 28 quantitative characters.

Table 4.4: Eigenanalysis based on Principal Coordinates Analysis (PCO) of 31 *Acalypha* taxa.

| AXIS | EIGENVALUE | PERCENT OF TOTAL | CUMULATIVE PERCENT |
|---|---|---|---|
| 1 | 2.047 | 19.76 | 19.76 |
| 2 | 1.356 | 13.09 | 32.86 |
| 3 | 1.000 | 9.66 | 42.52 |
| 4 | 0.741 | 7.15 | 49.67 |
| 5 | 0.671 | 6.47 | 56.14 |
| 6 | 0.531 | 5.13 | 61.27 |
| 7 | 0.447 | 4.32 | 65.59 |
| 8 | 0.369 | 3.57 | 69.15 |
| 9 | 0.359 | 3.46 | 72.62 |
| 10 | 0.333 | 3.21 | 75.83 |
| 11 | 0.281 | 2.72 | 78.55 |
| 12 | 0.269 | 2.59 | 81.14 |
| 13 | 0.250 | 2.41 | 83.55 |
| 14 | 0.206 | 1.99 | 85.54 |
| 15 | 0.191 | 1.84 | 87.39 |
| 16 | 0.178 | 1.72 | 89.10 |
| 17 | 0.161 | 1.56 | 90.66 |
| 18 | 0.154 | 1.49 | 92.14 |
| 19 | 0.131 | 1.26 | 93.41 |
| 20 | 0.118 | 1.14 | 94.55 |
| 21 | 0.098 | 0.95 | 95.50 |
| 22 | 0.092 | 0.89 | 96.39 |
| 23 | 0.085 | 0.82 | 97.21 |
| 24 | 0.078 | 0.75 | 97.96 |
| 25 | 0.066 | 0.64 | 98.60 |
| 26 | 0.052 | 0.51 | 99.11 |
| 27 | 0.031 | 0.29 | 99.40 |
| 28 | 0.024 | 0.23 | 99.64 |
| 29 | 0.022 | 0.21 | 99.85 |
| 30 | 0.015 | 0.15 | 100.00 |
| 31 | 1.145E-09 | 1.106E-08 | 100.00 |

## Key for Figures 4.16a - 4.16c

| | |
|---|---|
| ACROGYNA | A |
| AAMBIGUA | B |
| BIPARTIT | C |
| BOIVINIA | D |
| BRACHYST | E |
| ACBUSSEI | F |
| CHIRINDI | G |
| ACILIATA | H |
| ACRENATA | I |
| AECHINUS | J |
| AENGLERI | K |
| FRUTICOS | L |
| AVILLOSA | M |
| EGLANDUL | N |
| GILLMANI | O |
| AHISPIDA | P |
| ACINDICA | Q |
| LANCEOLA | R |
| NEPTUNIC | S |
| GLABRESC | T |
| ANYASICA | U |
| ACORNATA | V |
| PAUCIFOL | W |
| PSILOSTA | X |
| GLANDULO | Y |
| RACEMOSA | Z |
| STUHLMAN | a |
| VILLICAU | b |
| VOLKENSI | c |
| WELWITSC | d |
| WILKESIA | e |

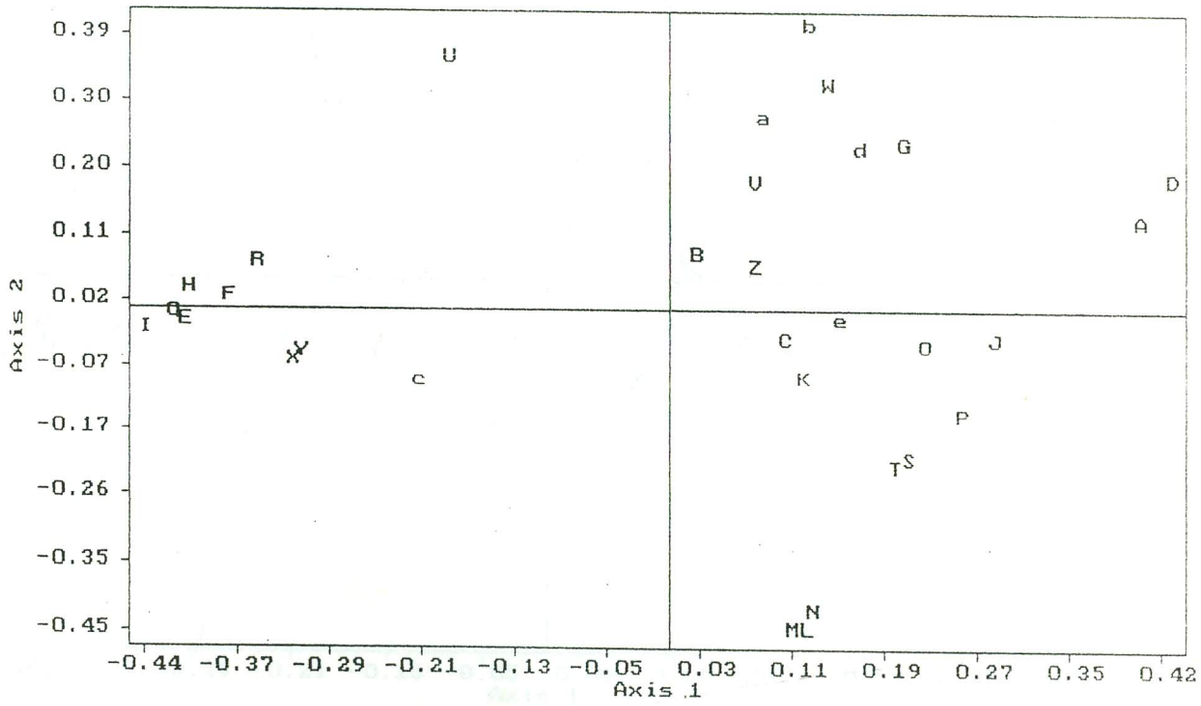



**Figure 4.16a**. A 2-Dimensional scatter diagram of 31 *Acalypha* taxa based on Principal Co-ordinates Analysis (PCO) using 49 mixed characters (Principal axis 1 compared with 2).

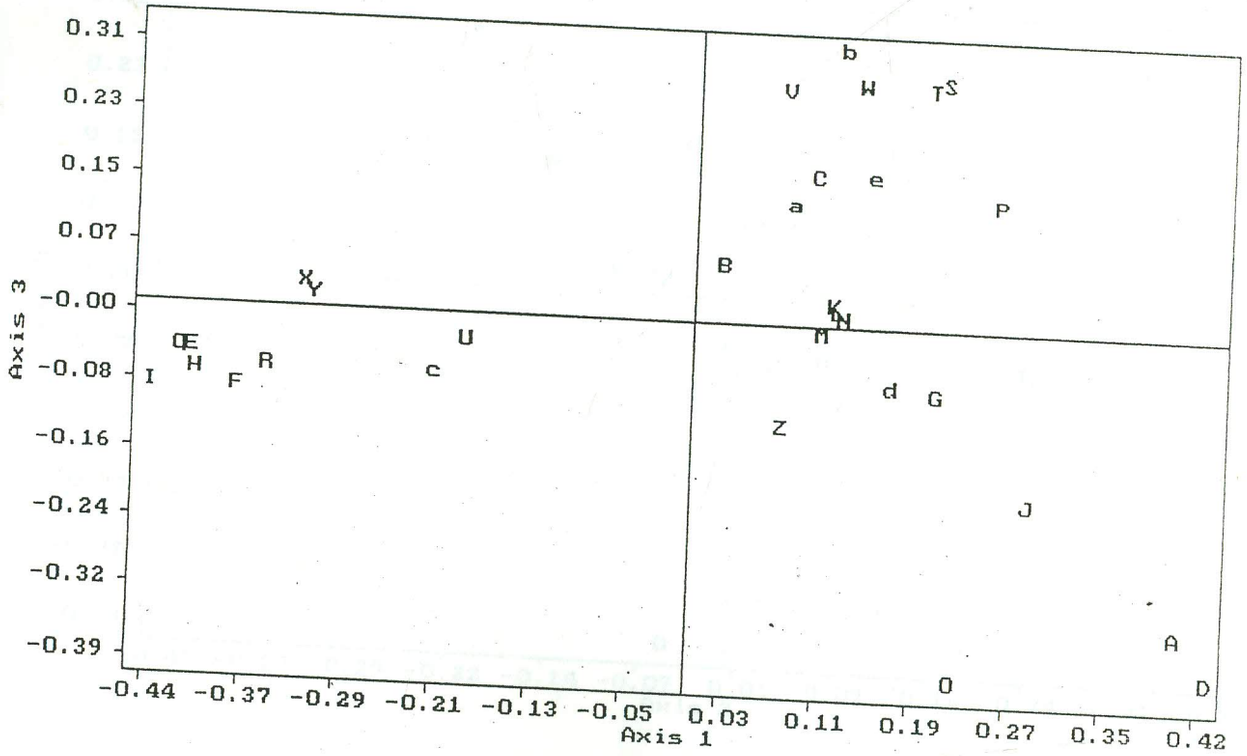



**Figure 4.16b.** A 2-Dimensional scatter diagram of 31 *Acalypha* taxa based on PCO using 49 mixed characters (Principal axis 1 compared with 3).

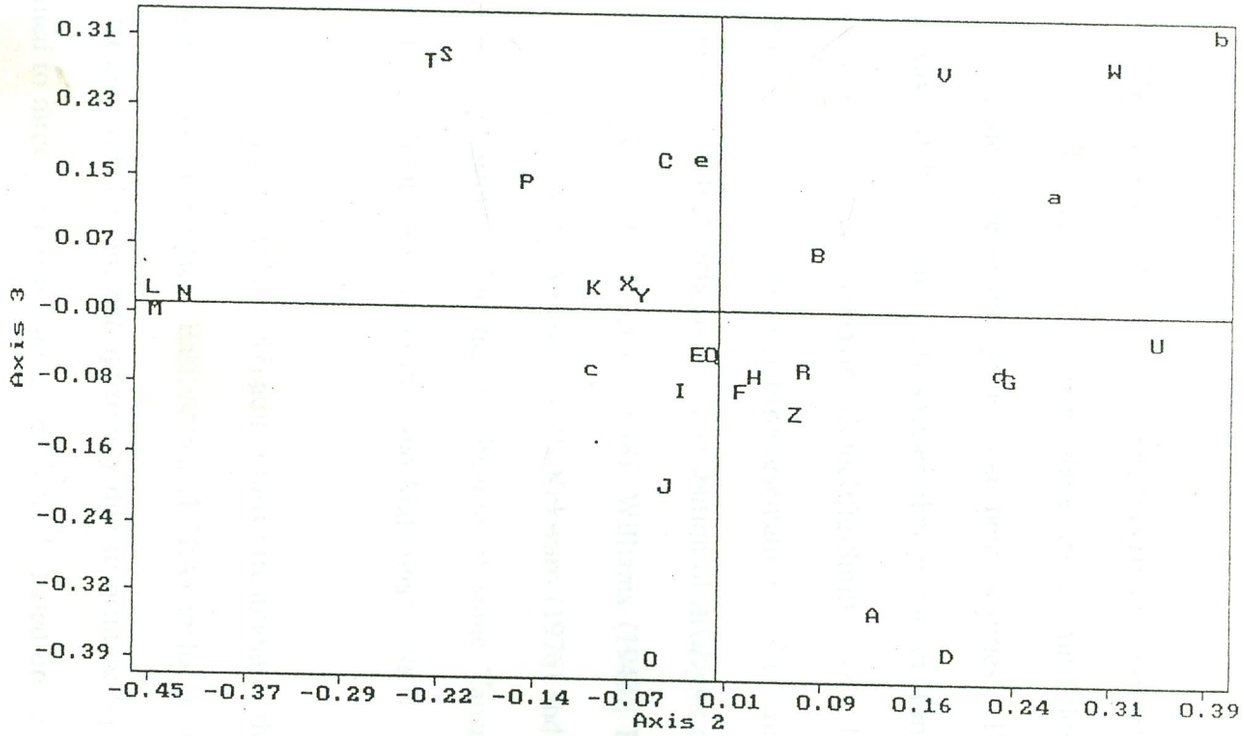



**Figure 4.16c.** A 2-Dimensional scatter diagram of 31 *Acalypha* taxa based on PCO using 49 mixed characters (Principal axis 2 compared with 3).

# CHAPTER FIVE : PHYTOGEOGRAPHICAL STUDIES

## 5.1 INTRODUCTION

The Tanzanian *Acalypha* are not properly mapped and their phytogeographical status is not definative. However, there are existing floras and allied literature which indicate some of the species and their localities. The work of Brenan and Greenway (1949) includes 20 species that occur in Tanzania, 4 of these now considered to be mere synonyms (Radcliffe-Smith, 1987; 1989). Also the work of Thiselton-Dyer (1913) has a rich representation of the Tanzania *Acalypha*. Other notable works which indicate the distribution of *Acalypha* taxa in Tanzania include those by Dale (1936), Robyns (1948), Williams (1949), Hutchinson and Dalziel (1958), White (1962), Agnew (1974), Kokwaro (1976) and Troupin (1982; 1983). A further contribution on the distribution of some Tanzanian *Acalypha* taxa is provided by Hedberg *et al.* (1983) and Mahunnah (1987).

Radcliffe-Smith's (1987) authoritative treatment of the family Euphorbiaceae for the Flora of Tropical East Africa (FTEA) includes information on altitude, habitat and distribution, but apparently due to printing logistics, the localities are limited to three per taxon, and is exclusively based on herbarium material. Also, the distribution pattern is based on the "Principle Geographical Regions of Tanzania", a model adopted for FTEA, which is based on the "Regional Administrative" boundaries during the pre-independence era. Therefore, a more

geographical approach, based on the possible factors which have contributed to the ecologically motivated distribution pattern (example; geology, climate, altitude, etc.) should be considered. These observations should facilitate the phytogeographical mapping of the *Acalypha* taxa occuring in Tanzania. Also, the flowering periods for the genus need to be studied as a retrieval source for future research workers.

## 5.2 MATERIALS AND METHODS

### 5.2.1 Source of information

Information on the distribution of Tanzanian *Acalypha* was obtained from three main sources namely:- (a) Field observations which were made and recorded during the author's excursions throughout the country (T1-T8) (Figure 5.9).

(b) Collector's notes on herbarium material studied in seven international herbaria, viz: DSM, EAH, G,K, Mo,NY and US (Chapter four: Table 4.1a). Also, other herbaria in the country, viz: Tanzania National Herbarium at Arusha; Tanzania Forest Research Istitute at Lushoto (Tanga); Traditional Medicine Research Institute, Dar es Salaam, and Sokoine University of Agriculture, Morogoro. (Table 4.1b) Data collected were in relation to habitat, locality and altitude.

(c) Available literature from floras and monographs, also with respect to altitude, locality and habitat notably: Thiseton-Dayer (1913), Bally (1937), Brenan and

Greenway (1949), Uphof (1968) and Radcliffe-Smith (1987).

Information on vegetation associated with the various *Acalypha* taxa was also obtained through personal field observation in T1-T8 (Figure 5.9). Valuable data were also secured from vegetation maps, notably; Polhill (1968), Morgan (1969;1972), Berry (1971), Greenway (1973), Atlas of Tanzania (1976), Hamilton (1982) and White (1983). Information with respect to geology, landform, soil type, rainfall (mm) and temperature (C.) was obtained from literature notably; Records of the Geological Survey of Tanganyika (1956-61), Scott (1962), Kenwothy (1966), Morgan (1969), Baker (197O), Osborne (197O), Berry (1971), East African Meterological Department (1972), Hathout (1973), Nieuwolt (1973) and Atlas of Tanzania (1976).

The literature sources indicated above also provided supplementary data on the flowering periods of some *Acalypha* taxa in Tanzania.

### 5.2.2 Information analysis

The information so obtained was pooled together to provide comprehensive data. The latter were used to prepare relevant tables, histograms and maps of Tanzania depicting the phytogeographical status of the genus *Acalypha* in Tanzania.

Information on flowering periods of the genus in months was recorded for each taxon from the various available sources. Then, the frequencies of flowering, indicating the respective months were tallied for the genus and relevant deductions were made.

## 5.3 RESULTS

### 5.3.1 The distribution groups

On the basis of the distribution pattern, the Tanzanian *Acalypha* may be grouped into four main categories (Table 5.2). The groups are listed below, including the taxa which fall under each category:

5.3.1.1 **Local endemic taxa**: These are the taxa with percentage distribution range of 10% (Endemic status 1) or 20 - 30% (endemic status 2) (Table 5.3; Figure 5.1).

*A. acrogyna*

*A. ambigua*

*A. bipartita*

*A. boiviniana*

*A. bussei*

*A. echinus*

*A. engleri*

*A. gillmanii*

*A. nyasica*

*A. paucifolia*

*A. volkensii*

*A. welwitschiana*

*A. x. cristata*

5.3.1.2 **Moderately distributed taxa**: Those taxa with distribution range of 40 - 60% fall under this group (Table 5.4; Figure 5.2).

*A. brachystachya*

*A. ciliata*

*A. fimbriata*

*A. fruticosa* var. *fruticosa*

*A. fruticosa* var. *eglandulosa*

*A. fruticosa* var. *villosa*

*A. indica*

*A. lanceolata*

*A. neptunica* var. *neptunica*

*A. neptunica* var. *pubescens*

*A. psilostachya* var. *psilostachya*

*A. psilostachya* var. *glandulosa*

*A. racemosa*

*A. stuhlmannii*

**5.3.1.3 Widely distributed taxa**: A few taxa show a distribution range lying between 70-80% and are considered to have a wide distribution range (Table 5.5; Figure 5.3).

*A . chirindica*

*A. crenata*

*A. ornata*

*A. villicaulis*

**5.3.1.4 Cultivated taxa:** There are two *Acalypha* species which are cultivated (Table 5.2).

*A. hispida*

*A. wilkesiana*

### 5.3.2 Observations on the distribution groups:

Valentine (1972) observed that in all larger taxa, the number of endemic species greatly exceeded the number of wides. This observation applies to the current study on *Acalypha* and will be treated later. The respective distribution groups are presented below in the light of Table 5.1 to 5.5; Figure 5.1 to 5.12.

#### 5.3.2.1 Local endemic taxa:

Five *Acalypha* species (15.1%) fall under endemic status 1 category, whereas 8 species (24.2%) belong to endemic status 2 group. Therefore, 13 species of the genus are endemic and represent 39.3% of the *Acalypha* taxa studied.

##### 5.3.2.1.1 Endemic status 1 species

The 5 endemic *Acalypha* species that belong to this group occupy a distinct ecological ranges (Figures 5.1 and 5.4). The species are *A. acrogyna, A. bipartita, A. boiviniana, A. bussei* and *A. nyasica.* The 2 cultivated species, *A. hispida* and *A. wilkesiana* have been included in Figure 5.4 but are not endemics *senso stricto.*