TAXONOMY AND NUTRIENT COMPOSITION OF SPECIES IN THE *Vernonia hymenolepis* A. RICH. COMPLEX IN TROPICAL EAST AFRICA

VWONONI GERTRUDE EVUSA

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN PLANT TAXONOMY IN THE SCHOOL OF SCIENCE UNIVERSITY OF ELDORET, KENYA

DECLARATION

Declaration by the Candidate

This thesis is my original work and has not been submitted for any academic award in any institution; and shall not be reproduced in part or full, or in any format without prior written permission from the author and/or University of Eldoret.

Evusa Vwononi Gertrude Date_____Signature_____
SC/PGB/014/11

Declaration by supervisors

This thesis has been submitted with our approval as University supervisors.

Prof. Donald F. Otieno Date_____Signature_____
University of Eldoret

Prof. Violet K. Mugalavai Date_____Signature_____
University of Eldoret

Dr. Itambo Malombe Date_____Signature_____
East African Herbarium, National Museums of Kenya

DEDICATION

I dedicate this work to my parents Mr. and Mrs. Johnstone Evusa Lidambitsa, my beloved husband Eric Juma Khatete and my lovely children Faith Pendo, Prudence Neema, Joseph Baraka and Nathan Amani.

ABSTRACT

Three Stengelioid species forming a complex in the genus *Vernonia* Schreber. in Tropical East Africa viz., *Vernonia calvoana* (Hook.f.) Hook.f., *Vernonia hymenolepis* A. Rich. and *Vernonia tolypophora* Mattf. were investigated to establish their taxonomic relationships. Specimens were collected from various parts of Kenya and herbarium specimens sourced from the Royal Botanic Garden (Kew) and Missouri Botanical Gardens (MO) in addition to those deposited at the East African herbarium (EA) for the three East African countries. Leaf and floral characters obtained from 123 herbarium specimens covering almost the entire range of the complex were examined. Measurements were made of each quantitative character per specimen using a hand ruler or under a WILD M3 dissecting microscope. Qualitative characters were scored as binary characters. The data obtained was used to determine their relationships by subjecting it to multivariate analysis. In the Principal Component Analysis (PCA) the three species almost formed one coherent group while the Cluster Analysis (CA) produced no clear cluster of any of the species. Discriminant Analysis (DA) similarly did not clearly discriminate between them. Univariate analyses (UA) did not reveal any character among those investigated that is able to separate the species. The results showed that the three species overlap in many leaf and floral characters. However Independent t-test showed significant levels of statistical difference between the species in respect to some of the characters. There were also subtle differences in the inflorescence morphology of *V. hymenolepis* and *V. tolypophora* and achene pubescence of *V. hymenolepis* and *V. calvoana*. The results suggest that the three entities are largely indistinct and are therefore likely part of a morphologically variable species. It is therefore proposed that *V. calvoana* and *V. tolypophora* should be synonymized under the earliest name *V. hymenolepis*. Following this, there is need to make new combinations of the subspecies recognized under the synonymized *V. calvoana*. Nutrient composition of the the leaves of *Vernonia hymenolepis* was determined using atomic absorption spectrophotometer and flame photometer for mineral elements, Kjeldahl method for Nitrogen and titration using iodine and 1% starch solution for Vitamin C. The Nutritional data revealed a high quantity of nutritive elements. The leaves had a Vitamin C mean content of 1120.4mg/100g (SD 274.5) and 303.2mg (SD 191.0), 1207.5mg (SD 1262.5) and 133.8mg/100g (SD 153.3) dry weight (d.w) of Sodium, Iron and Zinc respectively. Protein levels in the leaves ranged from 11.2-18.1g/100g d.w.There is therefore need to explore possibilities of domesticating *V. hymenolepis* to be used as a vegetable.

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LIST OF ABBREVIATIONS

FAO- Agricultural Organisation of the United Nation

Ca – Cameroon

CA – Cluster Analysis

CP - Crude protein

Cu - Copper

DA – Discriminant Analysis

DISTR. - Distribution

EA – East African Herbarium

Fe - Iron

HAB - Habitat

K– Kenya

KK – Kakamega

Mo – Mozambique

ME – MT. Elgon

Mn - Manganese

N - Nitrogen

Na - Sodium

NR – Narok

OTU – Operational Taxonomic Unit

P - Phosphorus

PCA – Principal Component Analysis

SYN - Synonym

T – Tanzania

U – Uganda

UA- Univariate Analysis

UG – Uasin Gishu

Vit C - Vitamin C

Zn - Zinc

ACKNOWLEDGEMENT

I would like to thank the Almighty God because without him we can do nothing.Through his grace and divine guidance I have been able to do this work. My sincere gratitude goes to my supervisors Prof. Donald F. Otieno, Dr. Itambo Malombe and Prof. Violet K. Mugalavai for their mentorship and expertise guidance.

I appreciate the the National Commission for Science, Technology and Innovation for the research grant which enabled me to carry out the research. I thank the Teachers Service Commission for granting me a study leave.

I thank the National Museums of Kenya for allowing my usage of their facilities and staff whose availability, material and technical support contributed to the success of this research. I also thank the directors and curators of Royal Botanical Garden (Kew) and Missouri Botanical Garden Herbarium for loaning me material for study. I appreciate the Chemistry and Soil Science Departments at the University of Eldoret for providing facilities used in the nutritional analysis. The Department of Biological Sciences played a key role in monitoring the development and implementation of the research proposal.

I also acknowledge the valuable suggestions, material and moral support that I received from Prof. E. Njenga. Great ideas and sharing of experience by Dr. H. Beentje and Dr J. Mulei are also appreciated. Mr. B.K. Wanjohi was very handy during field work. I thank Mr. Kirika for his support during laboratory and field work. Dr. M. Muasya in coordination with Dr. I. Malombe supported the Scanning Electron Microscopy Work which was done in Cape Town, South Africa. I wholeheartedly thank them. Dr. Peris Kamau, Dr. Mutuku Musili, Dr. George Nderitu and Professor David Liti, encouraged and guided me on data analysis.

I also sincerely thank my husband and children for their patience, love, material, financial and moral support and not forgetting my parents, parents-in-law, siblings, brothers and sisters-in-law, principals, colleagues, friends, my church and all who supported me. Mr Ekeeya John, Mr. Froebel Karani, Jane Barasa, Ken Matheka, Simon Kang'ethe, Morris

Omondi, Mary Pepela, Margaret Maina, Rehema Hassan, Dorcus Shisoka, Nancy Kavochi, Edwin Ketoyo, Mr and Mrs Ihaji and Christine Andenyi are also appreciated.

CHAPTER ONE
INTRODUCTION

1.1 Background

Vernonia Schreber. (Ironweeds) is a core genus in the tribe Vernonieae which is one of the major tribes of the Asteraceae (Jeffrey& Beentje, 2000; Dematteis, 2002; Keeley *et al*., 2007). It comprises about 1000 species (Robinson, 1999a; 1999b; 2007; Yeap *et al.,* 2010) which are known for being mostly perennial herbs or shrubs with intense purple flowers. Identification of species in the genus has been a persistent problem and extreme forms and hybrids have frequently been regarded as distinct due to variation which occurs at and below the species rank and natural hybridization followed by introgression (Jones, 1966; Faust, 1972; 1977).

In tropical East Africa three Stengelioid species viz., *Vernonia calvoana* (Hook.f.) Hook.f., *V. hymenolepis* A. Rich. and *Vernonia tolypophora* Mattf. are known to form a complex (hereafter the *V. hymenolepis* complex) that is always in a state of flux (Smith, 1971). The three were considered by Jeffrey (1988) as belonging to his informal *Vernonia* group 4. He treated *V. hymenolepis* and *V. tolypophora* as distinct based only on the densely clustered capitula seen in *V. tolypophora*. However, he noted that *V. tolypophora* was probably a southern homologue or vicariant of *V. hymenolepis*. He therefore suggested that *V. tolypophora* was better off being treated as a subspecies of the latter as earlier done by Wild (cited in Jeffrey, 1988).

Isawumi *et al*., (1996) also maintained the two species as distinct based on micromorphological evidence obtained from what can only be considered as a very narrow specimen sample. They only examined two specimens of *V. hymenolepis* and one of *V. tolypophora*. *Vernonia calvoana* and *V. hymenolepis* have also been treated as distinct (Jeffrey, 1988; Isawumi *et al*., 1996; Jeffrey & Beentje, 2000) with *V. calvoana* often being considered different in the often larger and less densely pubescent leaves, usually larger and recurving outer involucral bracts and the more often glabrous fruits

(Jeffrey, 1988; Fomum, 2004). However the two species show considerable overlap in these characters (Fomum, 2004). *Vernonia calvaona* is also often confused with *V. hymenolepis* which replaces it in the uplands of Eastern Uganda, Kenya and Southern Ethiopia (Jeffrey, 1988) and sometimes it is treated as a synonym of the latter (Fomum, 2004).

The three species are widely distributed in parts of tropical Africa. The range of *V. calvoana* extends from Western Uganda to the Northern, Eastern, Southern highlands and Tanga regions of Tanzania and into Southern Nigeria, Western Cameroon, the Congo, Rwanda, Zimbabwe, Mozambique and Fernando Po (Burkill, 1985; Jeffrey & Beentje, 2000). The distribution of *V. hymenolepis* ranges from Kenya into Uganda (Jeffrey & Beentje, 2000) and extends into Ethiopia (Smith, 1971; Jeffrey & Beentje, 2000), Cameroon and Sudan (Isawumi *et al*., 1996; Jeffrey & Beentje, 2000). *Vernonia tolypophora* occurs in Kenya, Tanzania, Zambia, Malawi and Mozambique (Pope, 1992; Isawumi *et al*., 1996; Jeffrey & Beentje, 2000) (Figure 1.1). The species flower between December and July (Smith, 1971; Hyde *et al*., 2012).

It is clear from the foregoing and as aptly demonstrated in Table 1.1 on the next page, that the boundaries of these three species of *Vernonia* are rather blurred which raises the question of whether the three are really distinct species. Thus one of the aims of this study was to clarify the boundaries and taxonomic relationships between the three species. Several species of *Vernonia* are eaten as leafy vegetables (Afui *et al*., 2008). *Vernonia hymenolepis* is especially appreciated in Cameroon, where it is known as bayangi bitterleaf or 'ndole' (Schippers, 2000; 2002; Fomum, 2004). It is a vegetable which not only plays an important role in nutrition because of its quality and quantity of nutritive elements, but also possesses medicinal value (Schippers, 2000; 2002). It is commonly cultivated by resource – poor farmers in Cameroon and other parts of West and Central Africa where it is cherished for its leafy shoots (Gockowski & Moulende, 2003; Afui *et al*., 2008)

Table 1.1 Morphological characters distinguishing between *V. calvoana, V. hymenolepis* and *V. tolypophora*

Character	*V. calvoana*	*V. hymenolepis*	*V. tolypophora*
Achenes	Pubescent	Glabrous or nearly so	Sparsely pubescent on the ribs, glandular in between, rarely glabrous
Capitula	Capitula in corymbiform cymes; involucres 13-40 mm long	Capitula in corymbiform cymes; involucres 13-40 mm long	Capitula in dense round clusters; involucres 7-13mm long
Phyllary appendages	Appendages of outer phyllaries recurving	Appendages of outer phyllaries erect	Appendages of outer phyllaries erect

(Adapted from Jeffrey& Beentje, 2000)

In East Africa it is found in the wild and in Kenya it occurs in many parts of Western, Nyanza and Rift Valley Provinces. It is used as medicine for both human and livestock and locally is referred to as *musululitsa*/*luvilukitsa*/*ivindisi*/*shisavakwa*/*nandavulwa* (Luhya: Tiriki, Maragoli and Bukusu), *mucatha* (Kikuyu), *chesoliet* (Sabaot) and *ormojase*/*ormosakwa* (Maasai). The Maasai boil the roots in soup for preventive and curative medicinal purposes. Leaf decoctions and root extracts are used to treat fever, malaria, diarrhoea, dysentery, typhoid, eye defects, cough, headache, stomach-ache, gastrointestinal dis-orders, sexually transmitted infections (e.g. gonorrhea and syphilis) and as a fertility inducer (Pers. Comm. with locals). It therefore forms a rich herbal flora of highland Kenya. It has also been documented as a potential tumor inhibitor (Kupchan *et al*., 1968). *Vernonia calvoana* in Cameroon and Nigeria plays an important role in

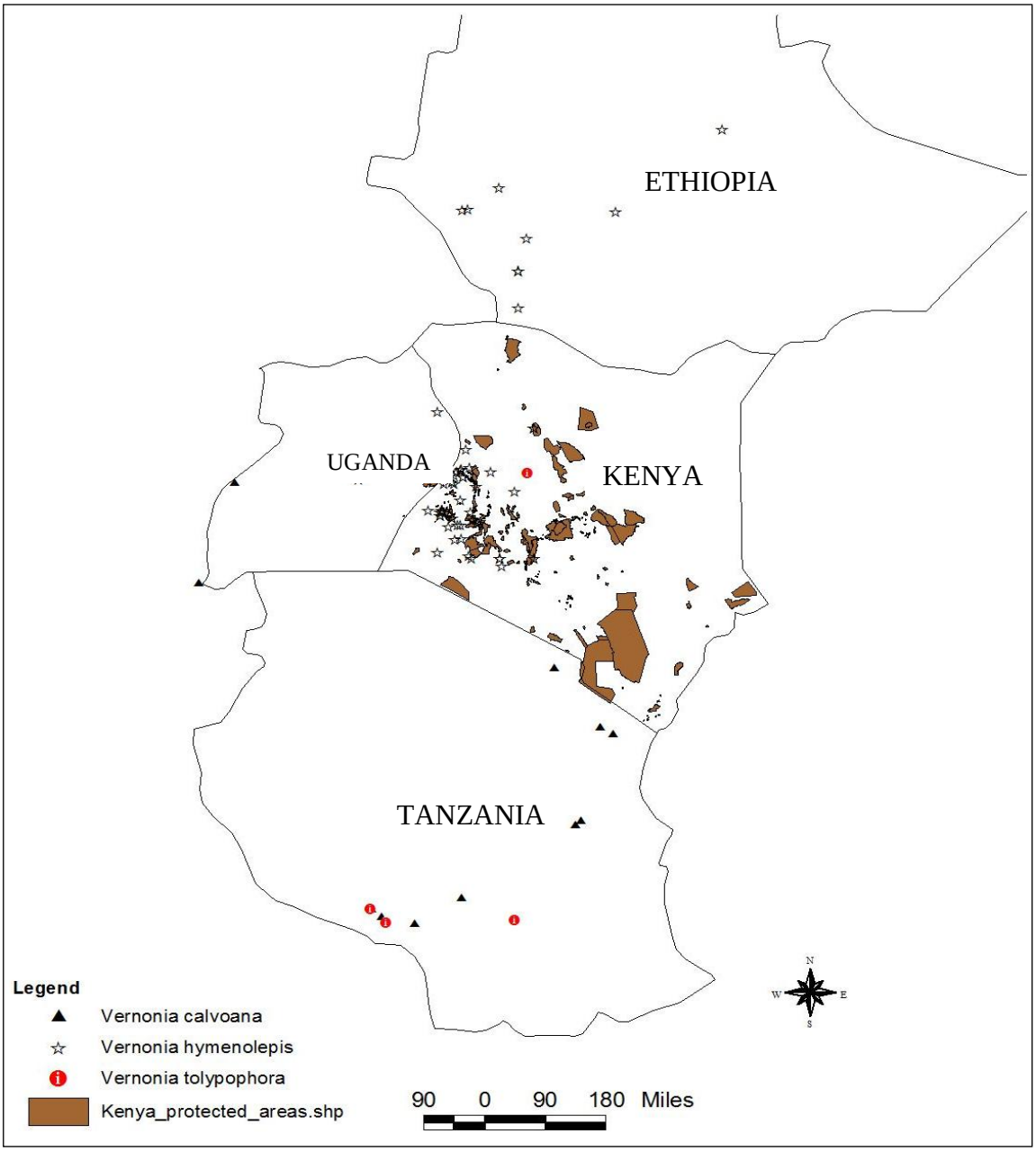

Figure 1.1 Distribution of *Vernonia calvoana-V. hymenolepis- V. tolypophora* complex in Eastern Africa (Kenya, Uganda, Tanzania and Ethiopia) Adapted from EA Herbarium.

human nutrition, because of its high mineral content and it is also important medicinally (Fube & Djonga, 1987). However it is often confused with *V. hymenolepis* (Jeffrey, 1988) and sometimes treated as its synonym (Fomum, 2004). In literature there is a dearth of information on the uses of *V. tolypophora.*

Unlike exotic vegetables, which have properly characterized cultivars, African indigenous vegetables exist as landraces with enormous heterogeneity that hinders the full exploitation of their genetic potential (Varalakshim, 2001; Abukutsa, 2010). Lack of taxonomy is often cited as a constraint to the production of indigenous leafy vegetables given that quite often the same local generic type name is applied to two or more cultivars (Smith & Eyzaguirre, 2007). For the three species forming the subject of this study, the first step that would lead to unraveling their true potential as vegetables is not only to clarify their taxonomic identities and relationships but also determine their nutrient composition. The second aim of this study was hence to determine the nutritive content of *Vernonia hymenolepis*. The use of local biodiversity to meet our food and nutritional needs requires not only accurate information on the identities of the plants that have the potential of being used as foods but also reliable and accessible data on their nutrient composition.

1.2. Statement of the problem

The boundaries and taxonomic relationships between the three species of *Vernonia* viz., *V. hymenolepis, V. tolypophora* and *V. calvoana* are unclear which raises the question of whether the three are really distinct species. The three species and several subspecies within the complex have been described but their taxonomy has remained complicated.Generally little has been done to resolve the taxonomy of the complex and similarly there is still a dearth of information on the nutrient composition of the species forming the complex. Identification and naming of lesser-known wild or locally cultivated plants used as food is often problematic and this hinders their development,

production and use as vegetables. Chronic malnutrition prevalent especially in one quarter of the children in Kenya is a challenge that requires sustainable strategic plans like utilization of locally available or cultivated plants. Globally micronutrient malnutrition is a problem that needs to be seriously addressed as it is much bigger than hunger and imposes huge costs on societies. Documentation of the correct taxonomy and nutrient data of taxa lays a basis for their monitoring and utilization.

1.3. Justification

Resolving the taxonomic boundaries and relationships of the three species forming the *Vernonia hymenolepis* complex and establishing their nutritional value are two aspects crucial to the full future exploitation of their potential as vegetables. Once the taxonomy of these species has been clarified, it will be possible and easier to characterize any existing landraces within the species for cultivar development and conservation in Kenya and within the East African region. The distribution of *V. hymenolepis* extends to some of the protected regions in Kenya. The removal of any hindrances to the exploitation of the entities in this complex as vegetables will not only increase the range of foods available for use locally but will also offer new sources of nutrition especially for the rural poor and thus help mitigate against micronutrition deficiencies that is known to affect a huge proportion of the population in Sub-Sahara Africa and more than two billion people in the world today. Chronic malnutrition places especially children at increased risk of mobility and mortality and is also related to impaired mental development as reported by Kenya Demographic and Health Survey 2014. It also results into unnecessary costs in terms of ill health, lives lost, reduced economic productivity and poor quality of life as observed by FAO (2011). Understanding the nutrient composition of the taxa is of economic importance and highly significant in Agriculture as this will contribute to food security.

1.4. Objectives

1.4.1 General objective

To determine the taxonomic relationship between *V. calvoana*, *V. hymenolepis* and *V. tolypophora* and their potential to be used as vegetables.

1.4.2 Specific objectives

1) To determine whether the three species in the *Vernonia hymenolepis* complex are conspecific.
2) To establish and compare the nutrient composition in the leaves of *Vernonia hymenolepis* from different contexts.

1.5. Research hypotheses

- *Vernonia calvoana*, *V. hymenolepis* and *V. tolypophora* are conspecific.
- The leaves of *V. hymenolepis* have high nutritional value.

CHAPTER TWO
LITERATURE REVIEW

2.1 Taxonomy of the Stengelioid Vernonias

The tribe Vernonieae is considered to be the most complex group in the Asteraceae and has been nicknamed the "evil tribe" (Funk *et al*., 2005; Keeley *et al*., 2007; Keeley, 2010) due to difficulties in taxonomic delimitation at various levels resulting from overlapping character states and intergrading morphologies for the majority of taxa (Keeley & Turner, 1990; Robinson, 1999a; 1999b; Dematteis, 2002; Keeley *et al*., 2007). The complexity of this tribe is centered on the delimitation and concept of the large genus *Vernonia* which consists of about 80% of the taxa in the tribe (Bremer, 1994; Dematteis, 2002; Yeap *et al*., 2010).

The current infra-generic division in the *Vernonia* follows the one proposed by Bentham and Hooker (1873) with further suggestions and contributions by Keeley (1978), Jones (1979; 1981) and Robinson (1987; 1988a; 1988b; 1990; 1999a; 2007). Robinson (1999a; 1999b; 2007) made some changes in the circumscription of *Vernonia*, limiting the genus to a small group of Eastern North American species that included the type species for the tribe - *Vernonia noveboracensis* (L) Willd. He then segregated the remaining species into new genera (Robinson, 2007). Before Robinson proposed these changes, Jones (1979; 1981) had made synoptic treatment of *Vernonia* in the New and Old World. Jeffrey (1988) in a treatment of African species noted some features that suggested separate lineages for New and Old World species. Although some authors such as Hind (1993), Keeley and Jansen (1994) hesitated to adopt the new classification proposed by Robinson (1999a; 1999b), Robinson and Funk (2011) point out that detailed morphological work and molecular phylogenies had finally forced the dismemberment of this unnatural genus.

All species of *Vernonia* are therefore now confined to the New World with the vast majority in continental North America (Vega & Dematteis, 2011). However, in tropical East Africa, the monographic studies needed to provide a sound basis for the transfer of

Vernonia species to other genera are yet to be undertaken and therefore the genus is still retained in the broad traditional sense (Jeffrey & Beentje, 2000). *Vernonia* Sect. *Stengelia* was recognized as a section within *Vernonia* by Bentham and Hooker (1873). Indeed Jones (1981) and Jeffrey (1988) also recognized it as a very natural group within *Vernonia*, with Jeffrey going as far as suggesting that it ought to be elevated to the generic level. However Jeffrey (1988) only gave it informal recognition as *Vernonia* group 4 in his classification of the Vernonieae in East Tropical Africa.

The Stengelioid species of *Vernonia* which include *V. hymenolepis*, *V. tolypophora* and *V. calvoana* among others have, taxonomically, been treated variously by different authors. Bentham and Hooker (1873) treated these species as *Vernonia* section *Stengelia* while Hoffmann (1889), using the presence of appendages on the phyllaries, treated the group as section 2 of *Vernonia*. Using macro-morphological characters several other authors e.g. Smith (1971), Jones (1981), Isawumi (1985; 1989; 1993) transferred many other species to this section. The definitive characters for species in this group include the unique corolla shape, flattened inner pappus bristles and the echinolophate, tricolpate, micropunctate pollen grains with long colpi and lacking mural projections (Robinson, 1990; Isawumi 1993; Isawumi *et al*., 1996). Isawumi (1993) transferred twelve species of *Vernonia* from section *Stengelia* to the new genus *Baccharoides* Moench defined by style bases that are surrounded by more or less cylindrical nectaries.

Jeffrey and Beentje (2000) on the basis of macro- and micro-morphological characters placed *V. hymenolepis*, *V. tolypophora* and *V. calvoana* in their *Vernonia* groups B and D in their account of the Compositae in Tropical East Africa. Afui *et al*., (2008) morphologically characterized four selections of *Vernonia hymenolepis* while trying to investigate intraspecific variations within the species. Their study revealed that morphological characters significantly differed between purple-stemmed and green stemmed selections.

2.2 Nutrient composition of *Vernonia*

Many studies on the nutrient composition of *Vernonia* have, to a large extent, been limited to *Vernonia amygdalina* Del. (Faboya, 1990). Leung *et al*., (1968) showed that 100g edible portion of the leaves of of *V. amygdalina* contained a substantial amount of water, energy, protein, fat, carbohydrate, fibre, ascorbic acid and minerals. Fube and Djonga (1987) in their study of *V. hymenolepis* and three other species of *Vernonia* revealed that the leaves of these species contained 22-27mg crude fibre, 15-20 mg ash, 10-13 mg cellulose and 15-885 ppm minerals such as iron, copper, zinc, manganese in 100g dry matter. They also reported that the quantity of protein in the four species of *Vernonia* eaten in Cameroon (i.e. *Vernonia colorata, V. calvoana, V. hymenolepis* and *V. amygdalina*) varied from 22.75–26.50 mg/100 of dry weight which indicated that it was a significant source of protein especially in the villages where children suffered from acute shortage of proteins. *Vernonia calvoana* was also found to be rich in minerals, particularly Iron and Manganese.

Faboya (1990) demonstrated that ascorbic acid decreased with storage time in *V. amygdalina* while Oshodi (1992) found that the dried leaves of *V. amygdalina* were rich in minerals, especially in phosphorus, and that the content of ascorbic acid was temperature dependent. Maundu *et al*., (1999) documented the nutrient composition of *V. cinerea* (L) Less. and also noted that *V. amygadlina* was used as a vegetable in Western Kenya where it is very common but not under cultivation. They further noted that *V. poskeana* Vatke and Hildebrandt, *V. appendiculata* Less, *V. colorata* and *V. perrottetii* Walp were also being used as vegetables. Ejoh *et al*., (2005) determined the effect of processing and preserving Vitamin C and total carotenoid in the leaves of *V. calvoana* and other species of *Vernonia*. Oboh, *et al*., (2008) compared the antioxidant properties of polar and non-polar constituents of some tropical green leafy vegetables among them *V. amygdalina*.

Ejoh *et al*., (2009) evaluated the effects of squeeze-washing, boiling in kanwa (a local alkaline salt) and drying of the leaves of *Vernonia colorata*, *V. calvoana* var. bitter, *V. calvoana* var. nonbitter and *V. amygdalina* on antinutritional factors. Their results showed that values for polyphenols were high in the unprocessed leaves. These values were reduced by 84.5% in *V. amygdalina* during processing. Sun-drying and oven drying at 75°C also caused slight losses in polyphenols. Saponin values were high in these four species but were considerably reduced by processing, rendering the nutrients in these leafy vegetables more bioavailable. Reductions were also observed for proteins as a consequence of processing by squeeze-washing and use of *kanwa*. With the exception of drying, significant losses in minerals were observed due to these processes (Ejoh *et al*., 2009).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study area

This study encompasses the *Vernonia hymenolepis* complex as occurring in the Flora of Tropical East Africa region (Figure 3.1).

3.2 Plant material

Herbarium specimens of *V. calvoana*, *V. hymenolepis* and *V. tolypophora* deposited at the East African Herbarium (EA), Nairobi and those obtained on loan from the Royal Botanical Gardens, Kew (K) and the Missouri Botanical Garden (MO) in St Loius were used in morphological studies. Specimens collected from the field were also incorporated in the studies. Voucher specimens of all materials collected from the field are deposited at EA. A list of all specimens examined is given in Appendix i.

3.3 Methods

3.3.1 Field studies

Field studies were confined to Kenya and were conducted in regions where the greatest variability of *Vernonia hymenolepis* is known to occur. The specific places visited included Kakamega, Malava, Nandi, Nyanza, Bungoma, Vihiga and Hamisi (all in K5); Trans Nzoia, Mt. Elgon, Uasin Gishu and Nakuru (all in K3) and Narok (in K6) (EA herbarium). The locality information and phenology details of the three species were obtained from herbarium specimens. However some localities were also identified from the lists of specimens examined by Smith (1971) which show the places where the specimens were collected and also from the list of specimens given in the Compositae account of the Flora of Tropical East Africa by Jeffrey and Beentje (2000). The Species in the complex tend to flower and/or fruit between the months of December and July

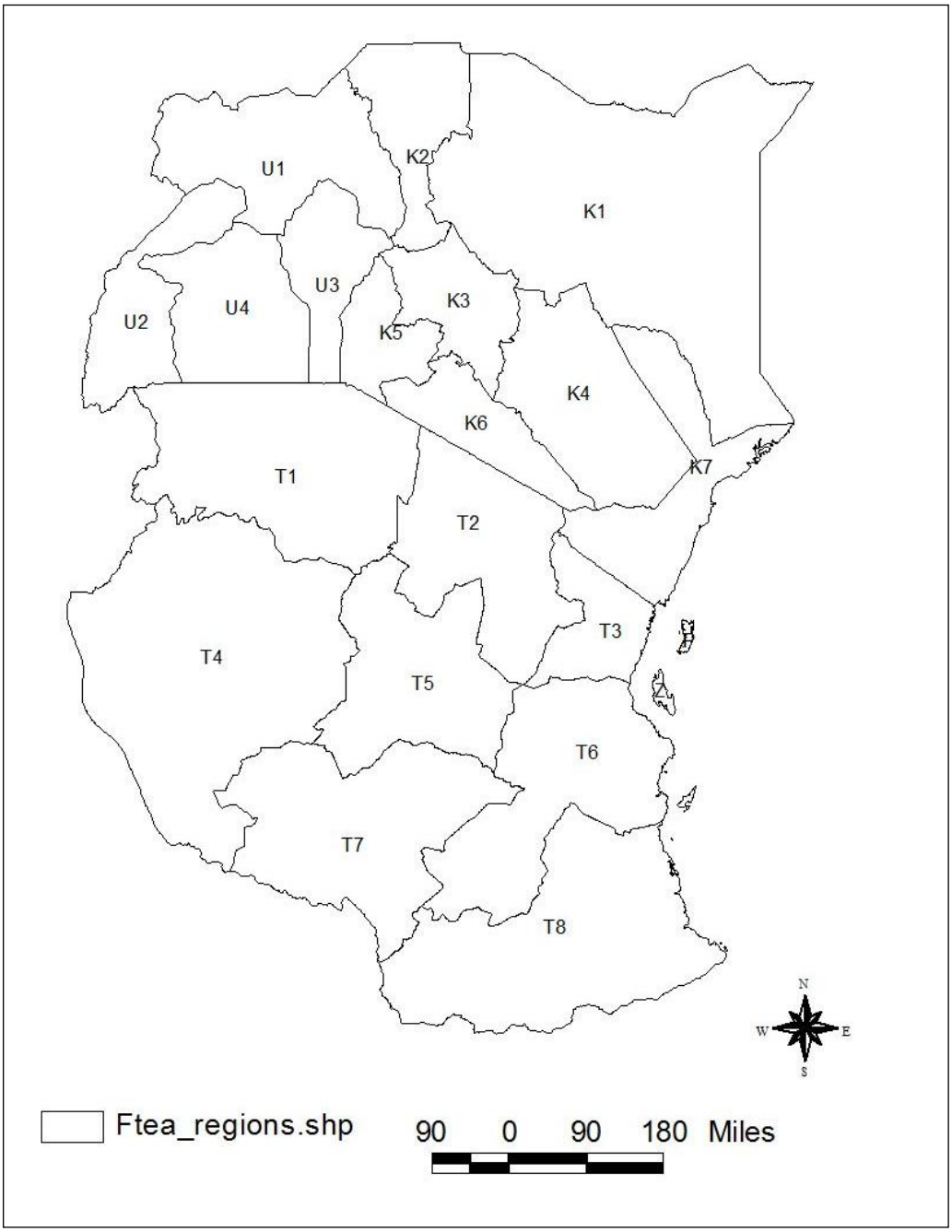

Figure 3.1 Regions of the Flora of Tropical East Africa (FTEA). K= Kenya, U= Uganda, T= Tanzania. Adapted from EA Herbarium, 2013.

(Smith 1971; Hyde *et al*., 2012). Thus, field work was conducted between January and May 2013 so as to collect specimens that were either in flower and/or fruiting.

Sampling of specimens was done randomly after every 50 km in the places where the species are distributed. Based on observation diagnostic features of the species were noted and recorded. The scientific identity, habit, habitat, altitude, latitude, collection number and date were also recorded (Appendix i).

3.3.2 Morphological studies

A total of 166 specimens were examined though only 123 selected on the basis of their relative completeness, i.e. possession of fully open florets and/or fruits and mature leaves were eventually included in the morphological analysis. The only exception was the type specimen of *V. hymenolepis* which was included despite having immature florets. Fourty two (42) of the 123 specimens selected for use in the morphological analysis belonged to the six subspecies of *V. calvoana*. The inclusion of only specimens in flower and/or fruiting and having mature leaves was done so as to avoid biasness that may arise from developmental plasticity and also to allow for standardized measurements to be made (Otieno *et al*., 2006). Material used was also qualified for inclusion by being matched to descriptions and key characters of *V. calvoana*, *V. hymenolepis* and *V. tolypophora* given in Smith (1971), Jeffrey (1988) and Jeffrey and Beentje (2000).

As far as was practicable, the specimens were selected to cover the entire range of distribution of each taxon in tropical East Africa as well as to reflect the morphological variability inherent in each one of them. A total of 48 quantitative characters were examined and 26 which were found appropriate, used in the subsequent phenetic analyses. Six qualitative characters scored as binary characters were also examined and only one was incorporated in the data matrix to be used in the analyses. Five measurements were made of each quantitative character per specimen using a hand ruler calibrated in mm or under a WILD M3 dissecting microscope at ×64 and ×400. Prior to being examined and measured, florets were rehydrated in warm water and and thereafter

dissected under the microscope. Characters that were scored are shown in Tables 3.1 and 3.2.

Table 3.1 List of quantitative characters

Character / Character State
Leaf
1. Length of leaf blade from the base to apex (LLB)
2. Width of leaf at the widest part of leaf blade (WL)
3. Petiole length from the point attached to stem to the leaf base (LP)
4. Length of serration at the middle of leaf measured from tip to base (LS)
5. Spherical index ratio of leaf length to width (SI)
Capitulum
6. Length of pedicel from base of lowest capitulum to junction with peduncle (LPD)
7. Width of involucre at broadest point (WI)
8. Length of involucre from base to the tip of longest phyllary (LI)
9. Length of the longest phyllary appendage (LPA)
10. Width of phyllary appendage at widest part (WPA)
11. Length/width ratio of phyllary appendage (LPA/WPA)
12. Length/width ratio of involucre (LI/WI)
Corolla
13. Diameter of corolla (CD)
14. Length of corolla tube and limb (LC)
15. Ratio of corolla length to diameter (LC/CD)
16. Length from base of corolla tube to point of filament attachment (CTFA)
Androecium
17. Length of anther filament (LAF)
18. Length of anther (LAN)
19. Length of anther spur (LASP)
Gynoecium
20. Length of style (LSTY)

Table 3.1 List of quantitative characters cont.

Character / Character State
Cypsela
21. Length of longest pappus setae on the cypsela (LPS)
22. Length of cypsela from base to where setae are attached (LSA)
23. Width of cypsela at widest point (WA 1)
24. Width of cypsela at mid-point (WA 2)
25. Ratio of cypsela length to width at widest point (LSA/WA 1)
26. Ratio of cypsela length to width at mid point (LSA/WA 2)

Table 3.2 List of qualitative characters

Character		Character state
I.	Pubescence on stem (SP)	Absence (0), Presence (1)
II.	Leaf attachment (LAT)	Sessile (0), Petiole (1)
III.	Pubescence on corolla lobes (CLP)	Absence (0), Petiole (1)
IV.	Style apical spur (SASP)	Absence (0), Presence (1)
V.	Filament collar (FTC)	Not constricted (0), Presence (1)
VI.	Cypsela pubescence (CP)	Absent (0), Presence

(Isawumi *et al*., 1996)

3.3.4 Scanning electron microscopy

Scanning Electron Microscope (SEM) micrographs of achenes were taken at 5keV using FEI Nova nanoSEM 230 Scanning Electron Microscope. Cypselas obtained from herbarium specimens were mounted directly on the adhesive surface of brass stubs and prepared for SEM study by being coated with a thin conductive layer for 3 minutes using a fine coat tweezers, 1.5 mm hex wrench. Carbon paint was applied on the portions of the specimen that were hard to coat.

3.4 Morphological data analyses

A data set was constructed from measurements taken from the specimens that were examined (Appendix ii). The matrix comprised all the 123 specimens representing the three species in the complex. The data was subjected to multivariate analyses using STATISTICA Release 7. The following analyses were carried out: Cluster Analysis (CA), Principal Component analysis (PCA), Discriminant Analysis (DA) and Univariate Analysis (UA). Before doing the PCA and CA the data was standardized to remove the effects of characters with large variances (Otieno *et al*., 2006). Cluster analysis was performed to show dissimilarity between OTU's and also to establish if the data grouped the classes to which the specimens had been assigned (Otieno *et al*., 2006). Pairs of OTUs were clustered based on Unweighted Paired-Group Method using arithmetic Averages (UPGMA) and their degree of similarity measured using the Euclidean distance coefficient.

Principal Component Analysis (PCA) was performed to show the relationships among groups of OTU's in a three dimensional space and to provide information on the characters defining these relationships. Discriminant analysis was also performed to determine the set of characteristics that allows for the best discrimination between the groups of OTU's (McCune & Grace 2002; Malombe *et al*., 2002; Otieno *et al*., 2006). Patterns of discontinuities in character variation among the groups were identified using boxplots and means and standard deviations computed for all quantitative characters used. The significance of individual character differences among the groups was tested

using an unpaired T-test. Data was arranged for the three species such that each column represented one group and each row the character being compared among the groups. The means of the dependent variable was compared between selected groups (*V. calvoana* versus *V. hymenolepis*, *V. calvoana* versus *V. tolypophora*; *V. hymenolepis* versus *V. tolypophora*) based on the specified values of the independent variable.

3.5 Nutrient composition

Various analytical methods were used to determine the moisture, ash, protein and mineral content of the leaves of *V. hymenolepis*.

3.5.1 Determination of moisture

Ten grams of shredded fresh leaves was dried in a Memmert thermostatically controlled ventilated oven at 105^{o}C for two hours to constant weight. The loss in weight was recorded as percentage moisture content (AOAC, 1990).

3.5.2 Sample preparation for protein, ash and mineral analysis

Leaves were cut into tiny pieces and dried in a Memmert ventilated oven at 60^{o}C for 5 days to constant weight. The dried leaves were then ground into powder and stored in airtight bottles for analysis (Okalebo *et al*., 2002).

3.5.3 Determination of crude protein

Crude protein content was determined using the Kjeldahl method. 0.2g of dried and pulverised leaf material was digested in 4.4 ml of a digestion mixture consisting of 2ml concentrated Sulphuric (VI) acid, Selenium catalyst, Lithium and Hydrogen peroxide until a clear digest was obtained (AOAC, 1984). The nitrogen content of diluted digest was determined colorimetrically at 630nm according to Charlot (1964). The Kjeldahl digestion converts organic Nitrogen compounds in the plant tissue to the ammonium form, which is then determined colorimetrically. Standard sample digest and the blanks were transferred into clearly labelled test tubes 0.20 ml of each using a micropipette. Five millilitres of reagent N1 (i.e. 34g Sodium salicylate, 25g Sodium citrate, 25g Sodium

tartrate and 0.12g Sodium nitroprusside) was added to each test tube using a 5 ml dispenser and mixed well using a vortex mixer. Five millilitres of reagent N2 (i.e. 30g Sodium hydroxide and 10ml Sodium hypochlorite) was added to each test tube and vortexed. The mixture was left to stand for 2 hours for full colour development and absorbency measured at 650nm using a spectrophotometer. Three recordings were made for each sample. Protein was calculated as: Nitrogen content x 6.25 ((AOAC, 1990).

Phosphorus content was determined colorimetrically without pH adjustment using Ascobic acid. Dried and pulverised leaf material (0.2g) was digested in 2ml concentrated sulphuric (VI) acid in the presence of Selenium catalyst, until a clear digest was obtained (AOAC, 1990). Five millilitres of the supernatant clear wet-ashed digest solution was pipetted into a 50ml volumetric flask. Twenty millilitres of distilled water and 10ml of Ascobic acid reducing agent were added to each flask, beginning with the standards. The mixture was then made up to 50ml with water and left to stand for 1 hour for colour to develop fully. The Phosphorus content of the standards and diluted digest was determined at 880nm using a spectrophotometer (Okalebo *et al*., 2002).

3.5.4 Determination of ash content

Ten grams of dried pulverised leaves were ignited slowly to a final temperatuate of 550^{o}C for three hours in an ALPHA 1 Phoenix muffle furnace after being pre-ashed on a hot plate for 2 hours. The crucible containing the grey ash was removed, cooled in a desiccator and weighed. Ash content was determined by calculating the difference in weight of the crucibles before and after combustion (Okalebo *et al*., 2002).

3.5.5 Determination of minerals

The content of Sodium, Potassium, Copper, Iron, Phosphorus, Manganese, Zinc, in the leaves was determined using the dry ashing procedure as described by Association of Agricultural Chemists (AOAC, 1990). About 2 g of the dried and ground sample was ashed as described in the procedure for determination of ash content above. After ashing the sample was cooled and weighed. The ash was then dissolved in 10ml of 30% HCl and

filtered through acid washed Whatman No. 541 filter paper into a 100ml volumetric flask. The filtrate was made up to the mark with deionised water. The solution was then used for individual mineral determination using VARIAN SPECTRA AA-200 atomic absorption spectrophotomer (Copper, Iron, Manganese, Zinc) and JENWAY PFP 7 flame photometer (Sodium and Potassium) (Okalebo *et al*., 2002; Oboh & Masodje, 2009; Yeap *et al*., 2010). Absorbance and concentration of the minerals was determined at 324.8nm, 213.9nm, 279.5nm, 248.3nm, 766.5nm wavelength for Copper, Zinc, Manganese, Iron and Potassium respectively. Flame type used was Air/Acetylene and Air/butane for atomic absoption spectrophotomer and flame photometer respectively.

3.5.6 Vitamin C

Twenty five millilitres of Ascorbic acid standard solution was placed in a 125ml conical flask and 10 drops of 1% starch solution added to it. This solution was then titrated with iodine until a dark blue colour, signifying the end point of the reaction, was attained (the dark blue colour was supposed to persist for 20 seconds while swirling the solution).

A hundred grams of leaf sample was then ground with 50ml distilled water and the mixture strained. The filtrate was then made upto 100ml in a volumetric flask by adding distilled water. The sample was titrated in the same way as the ascorbic acid sample described above until a dark blue colour, signifying the end point of the reaction, was attained (Fankhauser, 2009).

CHAPTER FOUR
RESULTS

4.1 Morphological analyses of the *Vernonia hymenolepis* complex

This chapter presents the research findings. Results from morphological data analysis of *V. calvoana*, *V. hymenolepis* and *V. tolypophora* are presented systematically to explicate the research objectives, statement of the problem and hypothesis. The clustering of the OTU's and the characters which contribute most to their distribution are elucidated. At the end of the chapter the results of the analysis of nutrient composition of the leaves of *V. hymenolepis* analysis are also presented.

4.1.1. Principal component analysis (PCA)

The first PCA done with all the OTU's (results not shown) presented the type specimen of *V. hymenolepis* as an outlier. As a result this specimen was removed from the subsequent analysis. In the second PCA, no distinct clusters of OTU's were revealed (Figures 4.1 and 4.2). The first two factor coordinates in this PCA explain 48.7% of the variation within the data while the third accounts for a further 7.7% (Table 4.1). The first factor mainly represents size variations in leaf, capitulum and fruit characters (Table 4.2). Along this factor-plane, the distribution of individuals of *V. hymenolepis*, *V. calvoana* and *V. tolypophora* is so interspersed there is nothing to suggest that three distinct species exist within the multivariate space.

Important characters which seem to have contributed the most to the spread along this plane include Characters 14 (length of corolla tube and limb), 21(length of longest pappus setae on the cypsela)**,** 16 (length from base of corolla tube to point of filament attachment), 8 (length of involucre from base to the tip of longest phyllary), 20 (length of style), 9 (length of the longest phyllary appendage), 7 (width of involucre at broadest point), 10 (width of phyllary appendage at widest part), 1 (length of leaf blade from the base to apex), 17 (length of anther filament), 22 (length of cypsela from base to where setae are attached)**,** 18 (length of anther), 6 (length of pedicel from base of lowest

capitulum to junction with peduncle), 15 (ratio of corolla length to diameter), 3 (Petiole length from the point attached to stem to the leaf base) and 25 (ratio of cypsela length to width at widest point) (Tables 3.1 and 4.2).

On the second factor coordinate, some degree of separation is apparent with a number of individuals of *V. calvoana* seeming to separate from the rest and aligning more to the left end of the multivariate space. The remaining specimens are intermixed with those of *V. hymenolepis*. Specimens of *V. tolypophora* are concentrated to the right end of the phenetic space but are interspersed with individuals of *V. hymenolepis* which largely occupy the intervening space between them and specimens of *V. calvoana* (Figure 4.1). However there is a greater overlap between individuals of *V. hymenolepis* and *V. tolypophora* than the OTU's of *V. hymenolepis* and those of *V. calvoana*. It is therefore still difficult to recognize distinct clusters along this factor-plane. The plot of the first and third factor coordinates does not also provide separation among the OTU's (Figure 4.2).

On the first factor coordinate, seventeen out of the twenty seven factor loadings are relatively high (greater than 0.5) with five of the highest being floral and fruit characters (Table 4.2). Of the four characters having the lowest loadings on this coordinate i.e. character 23 (width of cypsela at widest point), 24 (width of cypsela at mid-point), 5 (spherical index ratio of leaf length to width) and 26 (ratio of cypsela length to width at mid point) three (23, 24 and 26) have the highest positive loadings on the second factor coordinate (Tables 3.1and 4.2). Thus fruit characters contributed the most to the spread of OTU's on the second factor coordinate. Majority of the loadings on the third factor coordinate are relatively low except for character 13 (corolla diameter) which is the only character with a loading above 0.5 and hence had the most influence on the spread of OTU's along this coordinate.

Table 4.1 Table of Eigenvalues for the first three principal components, showing the total and cumulative variance of each component

	Eigenvalue	% Total variance	% Cumulative variance
1	10.12405	37.49647	37.4965
2	3.01558	11.16883	48.6653
3	2.08942	7.73858	56.4039

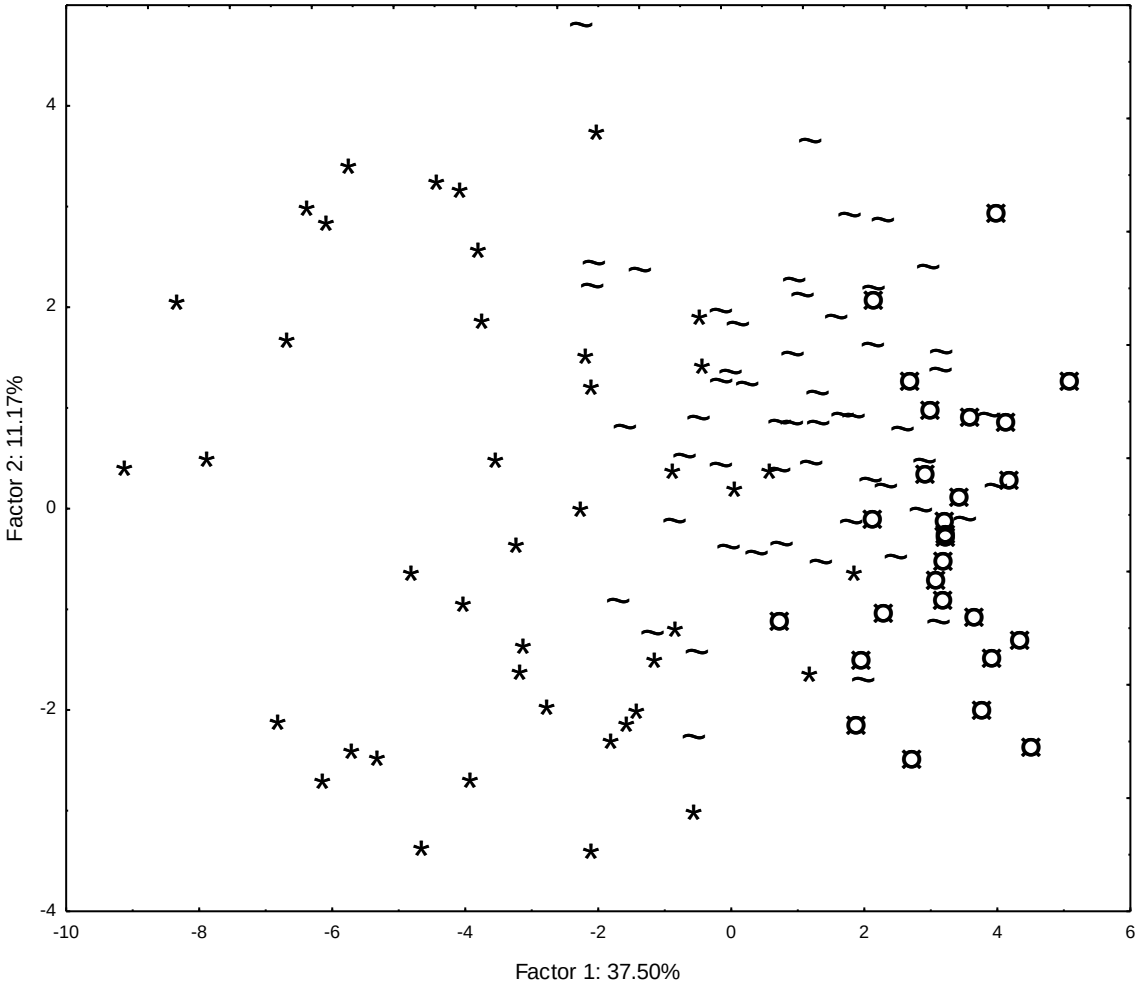

Figure 4.1 Scatter plots of the 134 OTU's. OTU's plotted against the first factor-plane by the second factor-plane. * = *V. calvoana,* ~ = *V. hymenolepis* and ¤ = *V. tolypophora*

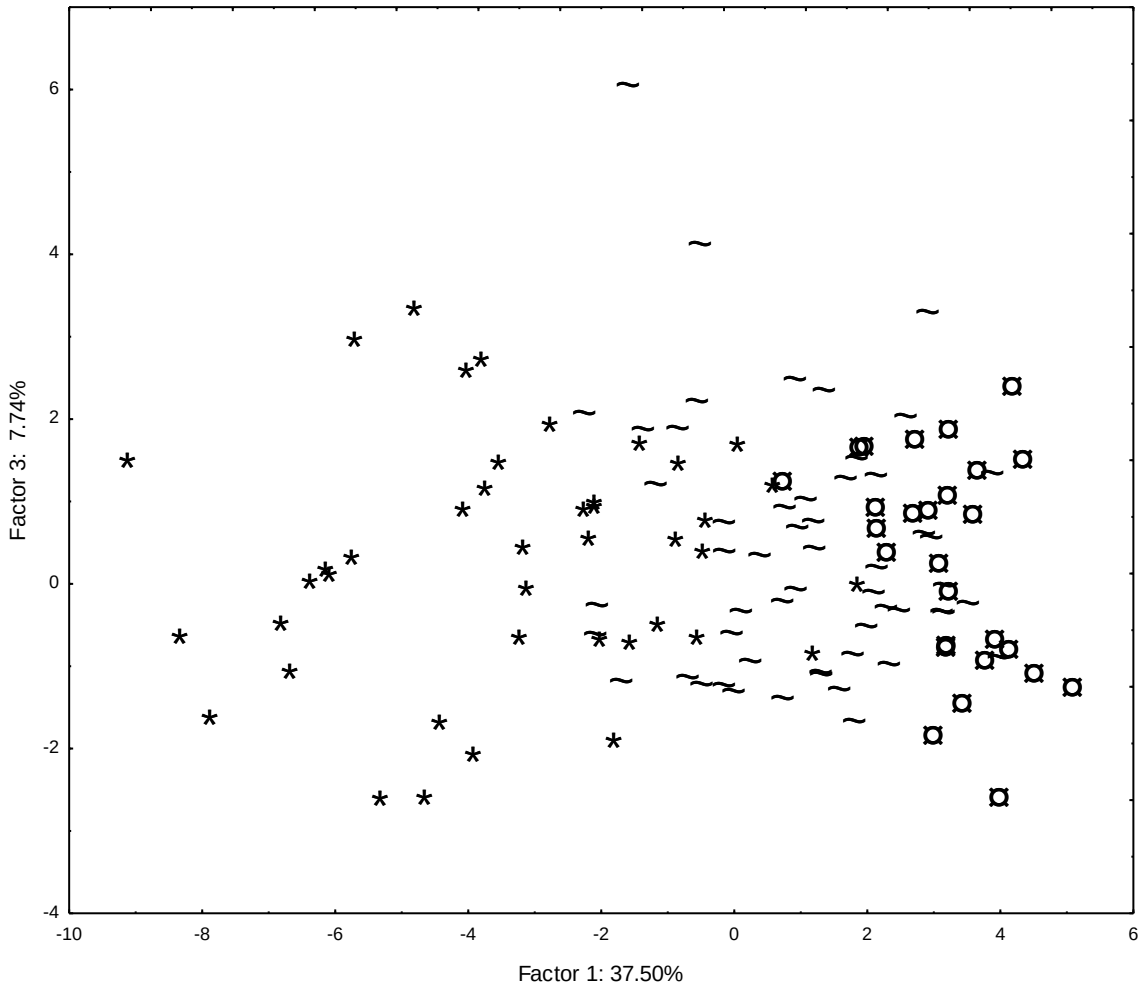

Figure 4.2 Scatter plots of the 134 OTU's. OTU's plotted against the first factor-plane by the third factor-plane. *= *V. calvoana*, ~ = *V. hymenolepis* and ¤ = *V. tolypophora*.

Table 4.2 Factor loading on the first three factor coordinates for 27 morphological characters of the *Vernonia hymenolepis* complex used in the final PCA. One qualitative character used is marked with an asterisk.

	Factor 1	Factor 2	Factor 3
LLB	-0.702641	-0.217148	-0.129353
WL	-0.568339	-0.451676	-0.187747
LP	-0.556892	-0.386124	-0.257365
LPD	-0.588013	0.256712	-0.122011
LS	-0.334215	-0.311026	0.013445
SI	-0.232871	0.298824	0.115314
LI	-0.860385	-0.003838	-0.143616
WI	-0.755325	0.387717	0.101393
LI/WI	-0.327143	-0.523880	-0.369178
LPA	-0.818943	-0.150500	-0.045997
WPA	-0.728533	0.047825	0.015621
LPA/WPA	0.350484	-0.089299	0.017081
LC	-0.886877	0.046637	0.145182
LPS	-0.873243	0.046329	0.045350
LSA	-0.658059	0.332831	-0.447775
WA1	-0.219490	0.778238	-0.284031
WA2	-0.285743	0.781500	-0.297532
LSA/WA1	-0.579845	-0.357204	-0.320284
LSA/WA2	-0.322517	-0.586486	-0.181451
*CP	-0.489036	-0.099191	-0.132989
LAN	-0.638422	-0.048412	0.392993
LAF	-0.653297	0.038631	0.402155
LASP	-0.456305	0.171794	0.356741
CD	-0.435909	-0.149800	0.650377
LC/CD	-0.543905	0.175349	-0.458647
LSTY	-0.814655	0.046362	0.313884
CTFA	-0.871768	0.064152	0.227266

4.1.2 Cluster analysis

The result of the cluster analysis is shown in Figure 4.3 in which two main clusters can be recognized. The main cluster A comprises almost exclusively of specimens of *V. calvaona* except for three of *V. hymenolepis* which are intermixed with them. Main cluster B is dominated by specimens of *V. hymenolepis* but with those of *V. tolypophora* and *V. calvoana* intermixed with them in almost equal number. No clear internal structure can be discerned in this cluster. This dendogram illustrating the morphometric relationships within the complex agrees with the PCA results (Figures 4.1 and 4.2) in which the three species do not separate out as distinct entities. It appears specimens of *V. calvoana* that grouped together in cluster A had characters with relatively higher measurement values than those that separated out in cluster B (Figure 4.4).

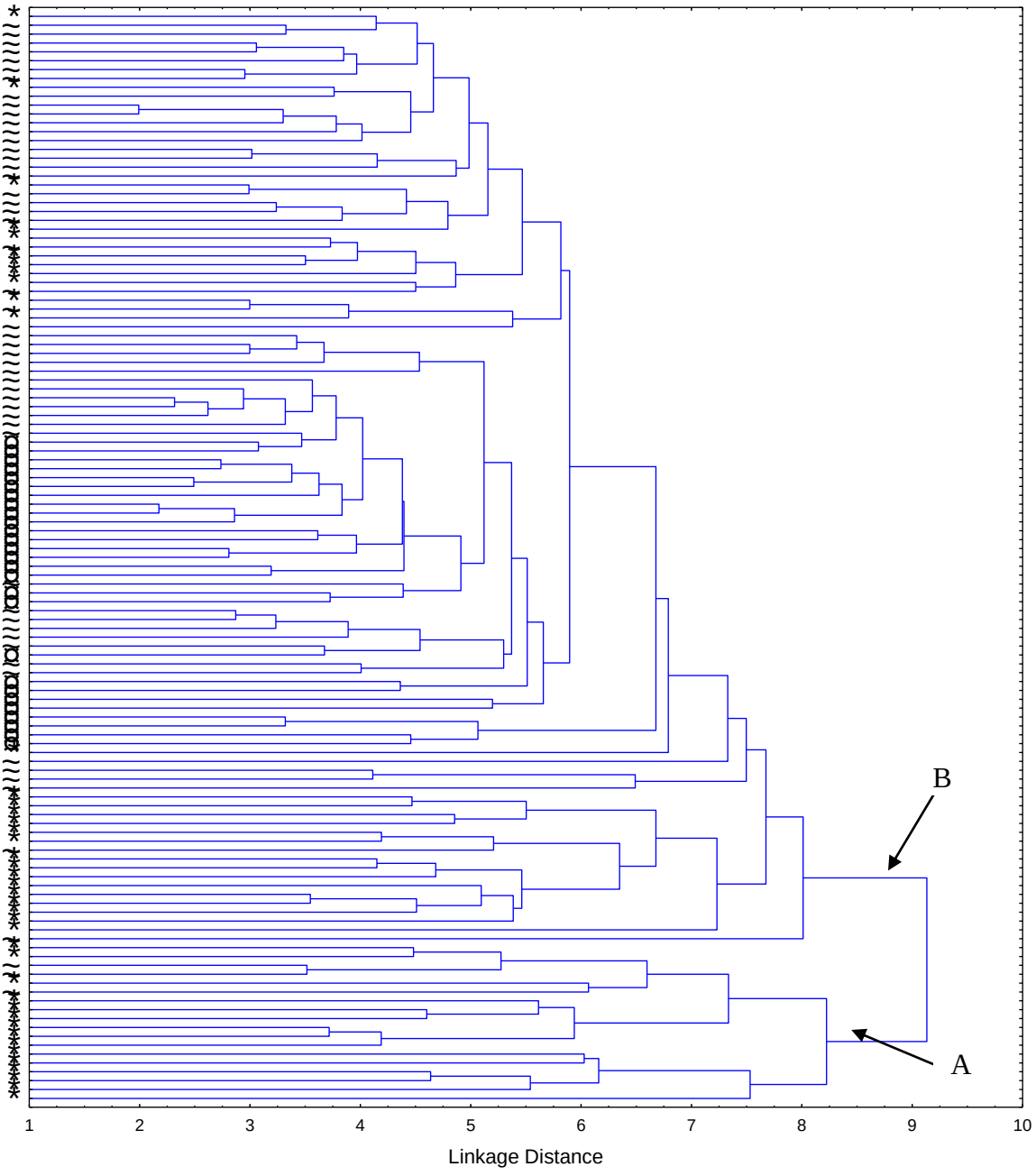

Figure 4.3 Phenogram from Cluster Analysis (UPGMA) of the *V. hymenolepis-V. calvoana-V. tolypophora* complex. * = *V. calvoana*, ~ = *V. hymenolepis* and ¤ = *V. tolypophora*.

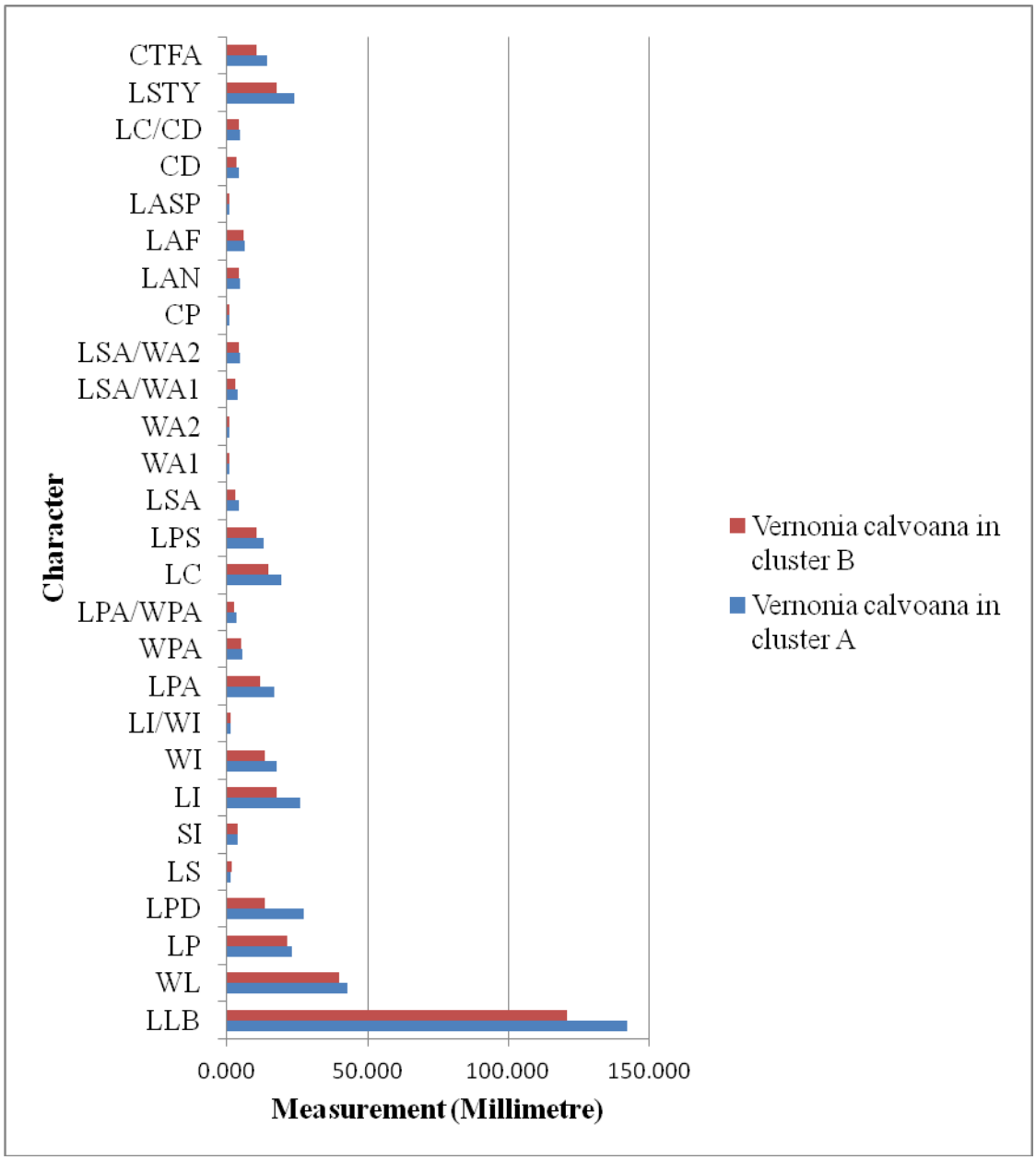

Figure 4. 4 Difference in the average values of measurements of morphological characters in specimens of *V. calvoana* belonging to Cluster A and Cluster B

4.1.3 Discriminant analysis

All the OTU's were plotted on the first and second roots of the discriminant analysis. The results show a conformation that depicts morphological continuity between the species (Figure 4.5) as also seen in the PCA and CA. A phenetic cluster to the left of the plot comprises specimens of *V. calvoana* which form a continuum of variation with the more-or-less bottom but centrally positioned specimens of *V. hymenolepis*. Specimens of *V. tolypophora* are spread largely to the right side of the plot but overlap along both roots with specimens of *V. hymenolepis*. The OTU's from all the three species do not show any sharp morphological distinctness as there is considerable overlap of the 95% confidence ellipses around them.

Characters that contribute the most to this variation pattern in order of importance according to their factor loadings (>0.6) on the first root were predominantly from the capitulum, fruit and leaf and included characters 8 (length of involucre from base to the tip of longest phyllary), 22 (length of cypsela from base to where setae are attached), 23 (width of cypsela at widest point), 25 (ratio of cypsela length to width at widest point), 12 (length/width ratio of involucre), 7 (width of involucre at broadest point), 14 (length of corolla tube and limb) and 5 (spherical index ratio of leaf length to width). On the second root the characters which had the greatest influence were from the leaves, fruit and florets and included characters 5, 24, 14, 23, 21 (length of longest pappus setae on the cypsela) and 26 ratio of cypsela length to width at mid point (Tables 3.1 and 4.3).

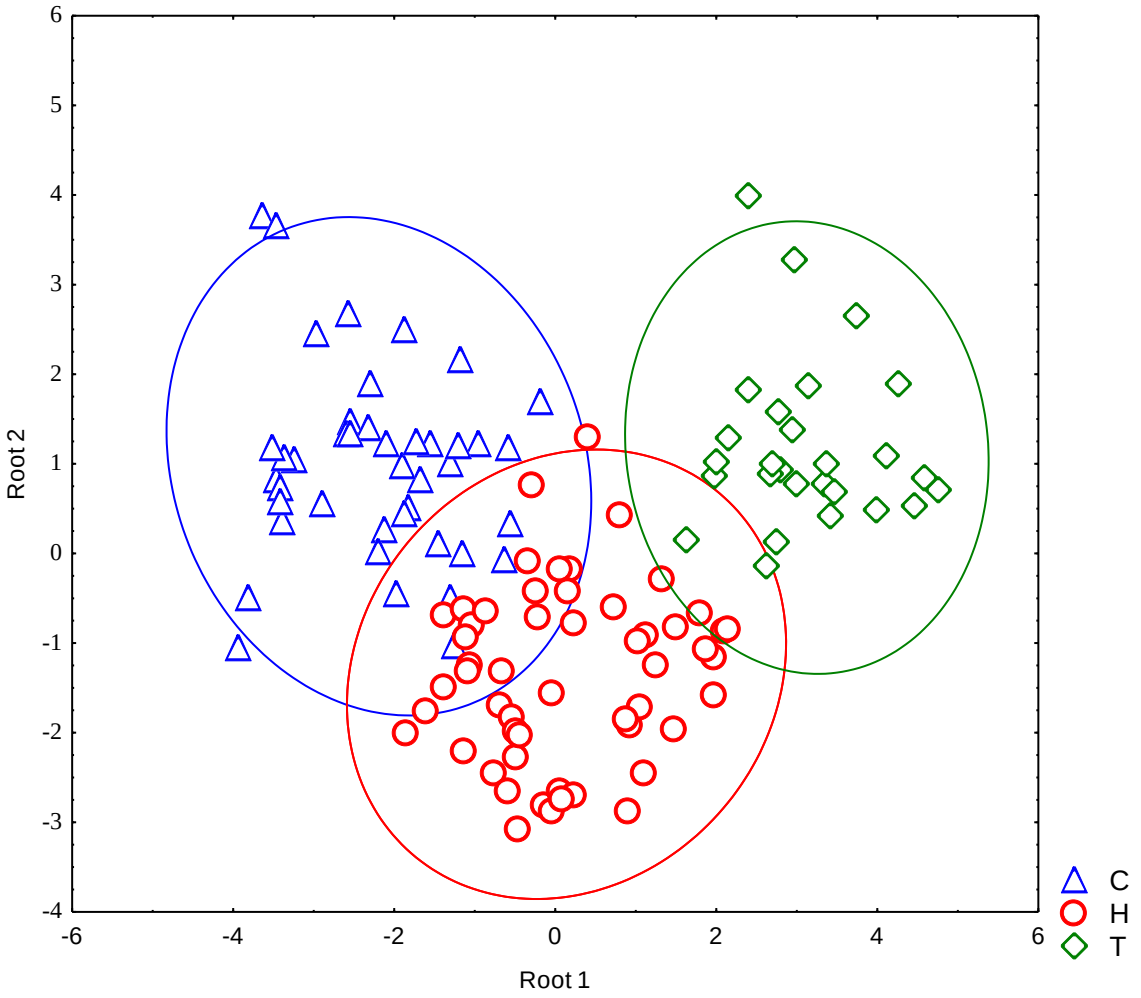

Figure 4.5 Discriminant analysis plot. Scatter plots of the two roots of the discriminant analysis of the *V. hymenolepis* complex. 95% confidence ellipses around group spread are shown for all the three groups. C = *V. calvoana*, H = *V. hymenolepis* and T = *V. tolypophora*.

Table 4.3 Standardized Coefficients for Canonical Variables loading on the first two roots for quantitative and qualitative characters used in DA. The only qualitative character used is marked with an asterisk.

Variable	Root 1	Root 2
LPS	-0.18649	0.698754
SI	-0.68053	-0.982595
WL	-0.32682	-0.403289
*CP	-0.31038	0.288742
WPA	-0.43364	-0.158757
WI	0.77427	0.259148
LASP	-0.06257	-0.293773
LPD	-0.17621	-0.236411
LS	-0.28791	0.097736
WA2	0.32387	0.817840
CD	-0.27982	-0.159579
CTFA	-0.25407	-0.369289
LC	0.55319	0.757442
LI/WI	0.91263	0.368058
LI	-1.16867	-0.227090
LAF	0.21752	0.210156
LSTY	-0.01729	-0.377622
WA1	-0.89308	-0.705720
LC/CD	-0.50683	-0.333224
LSA/WA2	0.14193	0.598439
LSA/WA1	-0.80983	-0.398532
LSA	0.95705	-0.062459
LAN	-0.16865	0.120595
LLB	-0.24956	0.197819
LPA	0.09701	-0.018989
LPA/WPA	0.03622	-0.062977

4.1.4 Univariate analysis

Univariate analyses using boxplots revealed that there is no single character among those investigated that is able to separate the species forming the *Vernonia hymenolepis* complex. All the characters showed continuity between the three species (Figure 4.6). Independent t-test done for all the characters and different combinations of the species showed significant levels of statistical difference between the species in respect to some of the characters (Table 4.4).

At significance level $P < 0.05$ fifteen of the twenty seven characters investigated differed significantly among the three species. These were characters 1(length of leaf blade from the base to apex), 3 (petiole length from the point attached to stem to the leaf base), 5 (spherical index ratio of leaf length to width), 6 (length of pedicel from base of lowest capitulum to junction with peduncle), 7 (width of involucre at broadest point), 8 (length of involucre from base to the tip of longest phyllary), 9 (length of the longest phyllary appendage) , 10 (width of phyllary appendage at widest part), 14 (length of corolla tube and limb), 15 (ratio of corolla length to diameter), 16 (length from base of corolla tube to point of filament attachment), 20 (length of style), 21 (length of longest pappus setae on the cypsela) and 22 (length of cypsela from base to where setae are attached). *Vernonia calvoana* differed from *V. hymenolepis* in all the characters except characters 23, 24 and 19 (length of anther spur), and from *V. tolypophora* in all the characters except characters 12, 23, 24 and 26. *Vernonia hymenolepis* and *V. tolypophora* differed in all the characters studied except characters 2, 4, 11, 26, 23, 18 (length of anther), 17 (Length of anther filament), 13 (diameter of corolla) and VI (cypsela pubescence) (Tables 3.1, 3.2 and 4.4).

Table 4.4 Comparison of quantitative variables used in final PCA and subsequently in CA and DA for species in the *Vernonia hymenolepis* complex. Sample size (n), mean and standard deviation (SD) are given. The results of independent t-test are summarized by the superscripts. Species having the same letters do not differ significantly for that character (P < 0.05).

Variable	*V. calvoana* Mean (SD) N=42	*V. hymenolepis* Mean (SD)N=54	*V. tolypophora* Mean (SD) N=27
LLB	0.820 (0.790)	0.090 (0.732)	1.095 (0.517)
WL	0.752 (0.939)	0.400 (0.772) [a]	0.369 (0.836) [a]
LP	0.683 (1.073)	0.102 (0.758)	0.860 (0.403)
LS	0.612 (1.166)	0.386 (0.768) [a]	0.180 (0.633) [a]
SI	0.132 (0.762)	0.520 (0.837)	1.245 (0.389)
LPD	0.572 (1.092)	0.0326 (0.870)	0.824 (0.179)
WI	0.685 (1.041)	0.029 (0.690)	1.009 (0.442)
LI	0.846 (1.132)	0.242 (0.507)	0.832 (0.390)
LI/WI	0.401 (1.212) [a]	0.343 (0.742)	0.062 (0.862) [a]
LPA	0.831 (1.055)	0.227 (0.628)	0.839 (0.425)
WPA	0.847 (0.750)	0.201 (0.900)	0.915 (0.208)
LPA/WPA	0.491 (0.656)	0.156 (1.128) [a]	0.452 (0.874) [a]
CD	0.447 (1.072)	0.234 (0.870) [a]	0.199 (0.919) [a]
LC	0.889 (1.036)	0.345 (0.623)	0.692 (0.432)
LC/CD	0.543 (1.089)	0.153 (0.827)	0.539 (0.776)
CTFA	0.836 (1.075)	0.287 (0.626)	0.727 (0.469)
LAN	0.614 (0.995)	0.189 (0.858) [a]	0.577 (0.774) [a]
LAF	0.692 (0.964)	0.228 (0.872) [a]	0.527 (0.763) [a]
LASP	0.305 (1.028) [a]	0.089 (0.996) [a]	0.651 (0.628)
LSTY	0.727 (1.111)	0.244 (0.690)	0.643 (0.607)
LPS	1.018 (0.932)	0.397 (0.451)	0.788 (0.511)
LSA	0.575 (1.157)	0.176 (0.814)	0.542 (0.570)
WA1	0.068 (1.057) [a b]	0.102 (1.048) [a c]	0.312 (0.749) [b c]
WA2	0.118 (1.181) [a b]	0.089 (0.873) [a]	0.361 (0.875) [b]
LSA/WA1	0.630 (1.112)	0.283 (0.833)[a]	0.413 (0.585)[a]
LSA/WA2	0.473 (1.000) [a]	0.344 (0.619) [b]	0.619 (1.3127) [a b]
CP	0.784 (0.432)	0.418 (0.969) [a]	0.383 (0.987) [a]

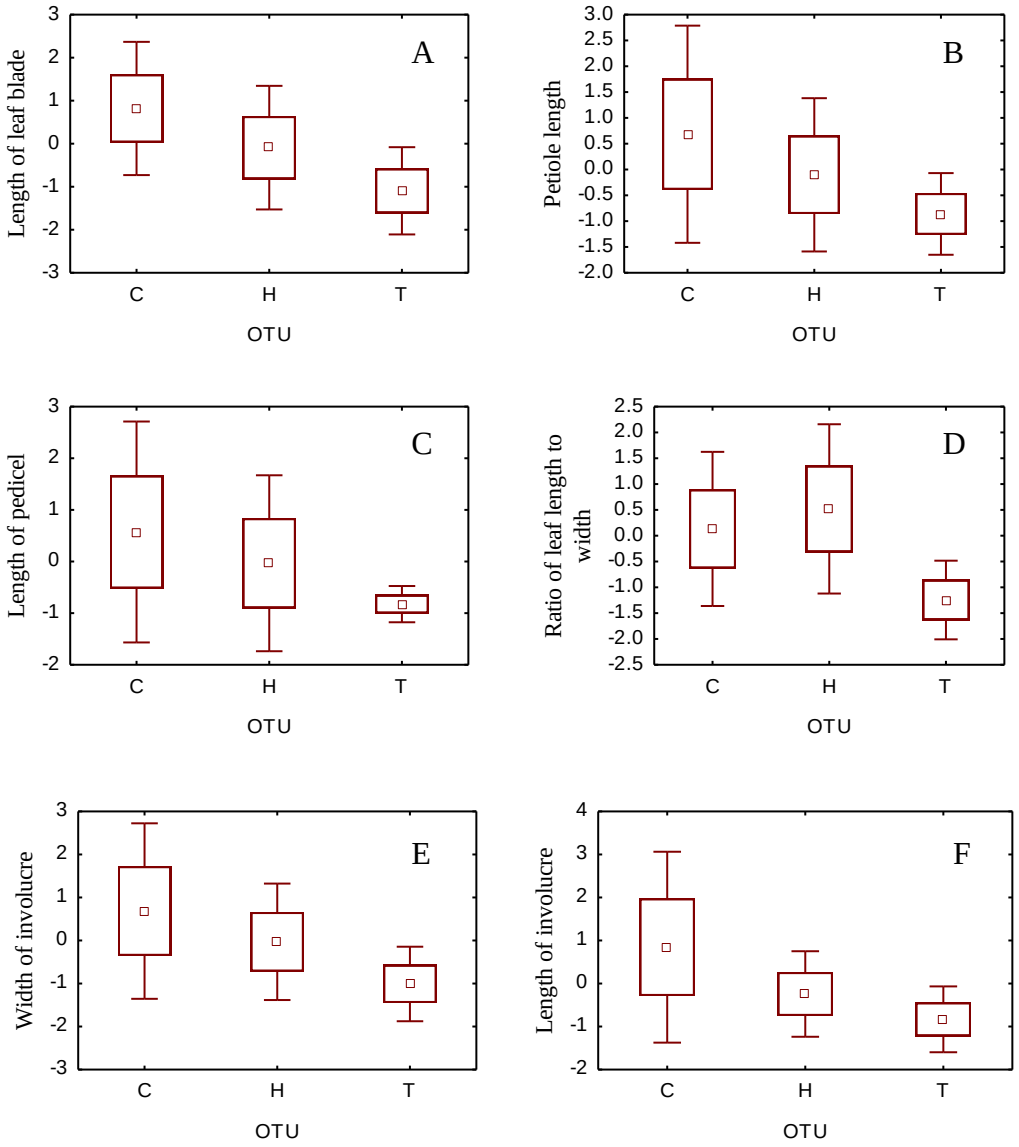

Figure 4. 6 Box plots of selected leaf and floral characters C- *Vernonia calvoana*, H- *V. hymenolepis* and T- *V. tolypophora.* Middle point–Mean, Box value – Mean ±SD, Whisker value - Mean±1.96*SD

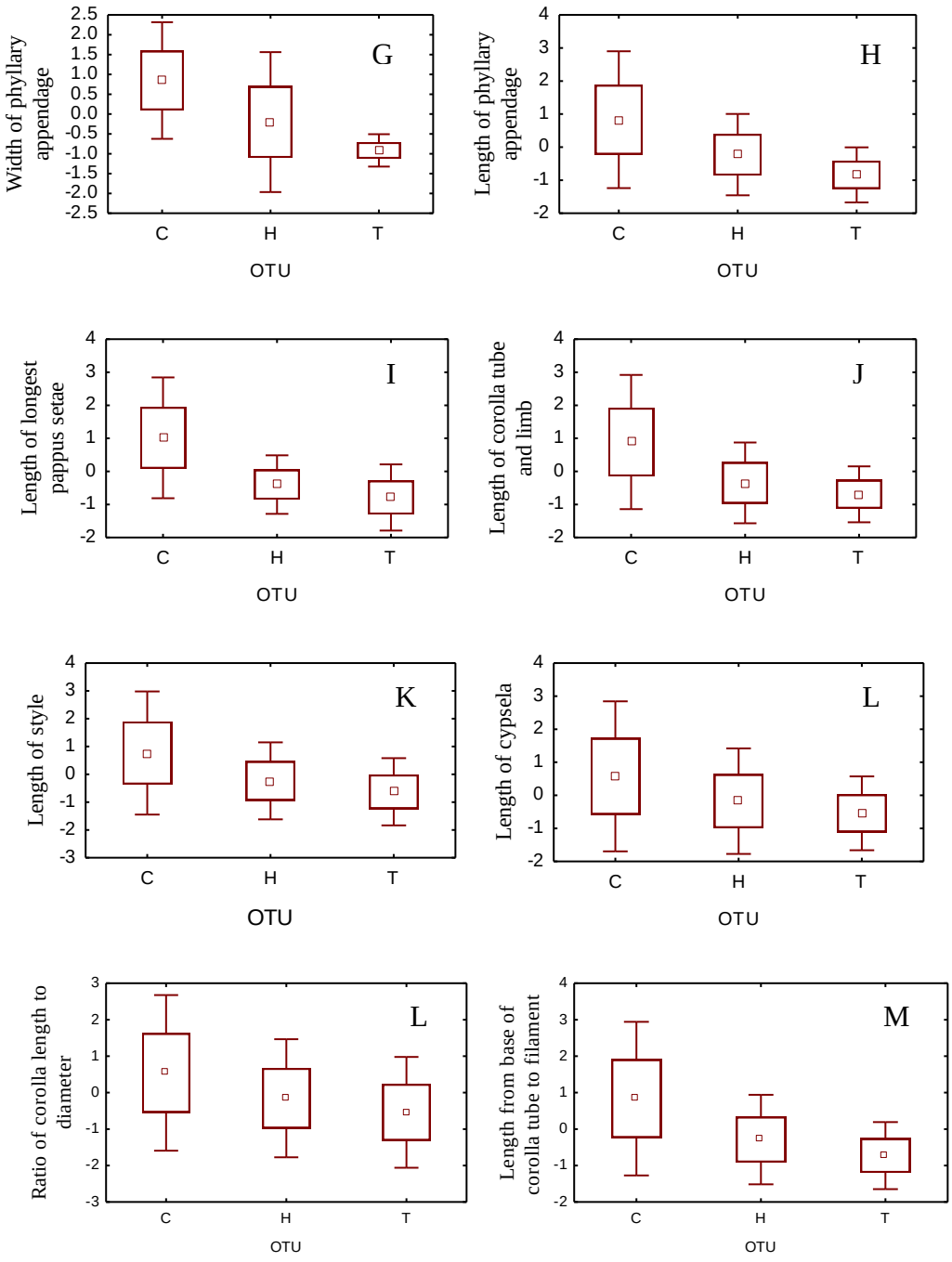

Figure 4.6. Box plots of selected leaf and floral characters, cont.

4.1.5 Scanning electron microscopy studies

Micrographs from the SEM revealed unique characters of cypsela indumentum. Twin hairs, idioblasts and/or glands were observed on cypselas from specimens representing the three species in the complex. Glabrous (Figures 4.7 A & B, Figures 4.8 A & B and Figures 4.9 A & B) as well as pubescent cypsela (Figure 4.7 C, Figure 4.8 C, Figures 4.9 C, D, E & F) were observed in specimens representing all the three species. The cypselas also showed a few or many idioblasts and/or glandular trichomes (Figures 4.7, 4.8 & 4.9). Within the same species there was gradation from glabrous (Figure 4.7 A & B, Figure 4.8 A & B and Figure 4.9 A & B), sparse (Figure 4.8 C and Figure 4.9 D & E), moderate (Figure 4.7 C and figure 4.9 C) to densely pubescent cypsela (Figure 4.9 F).

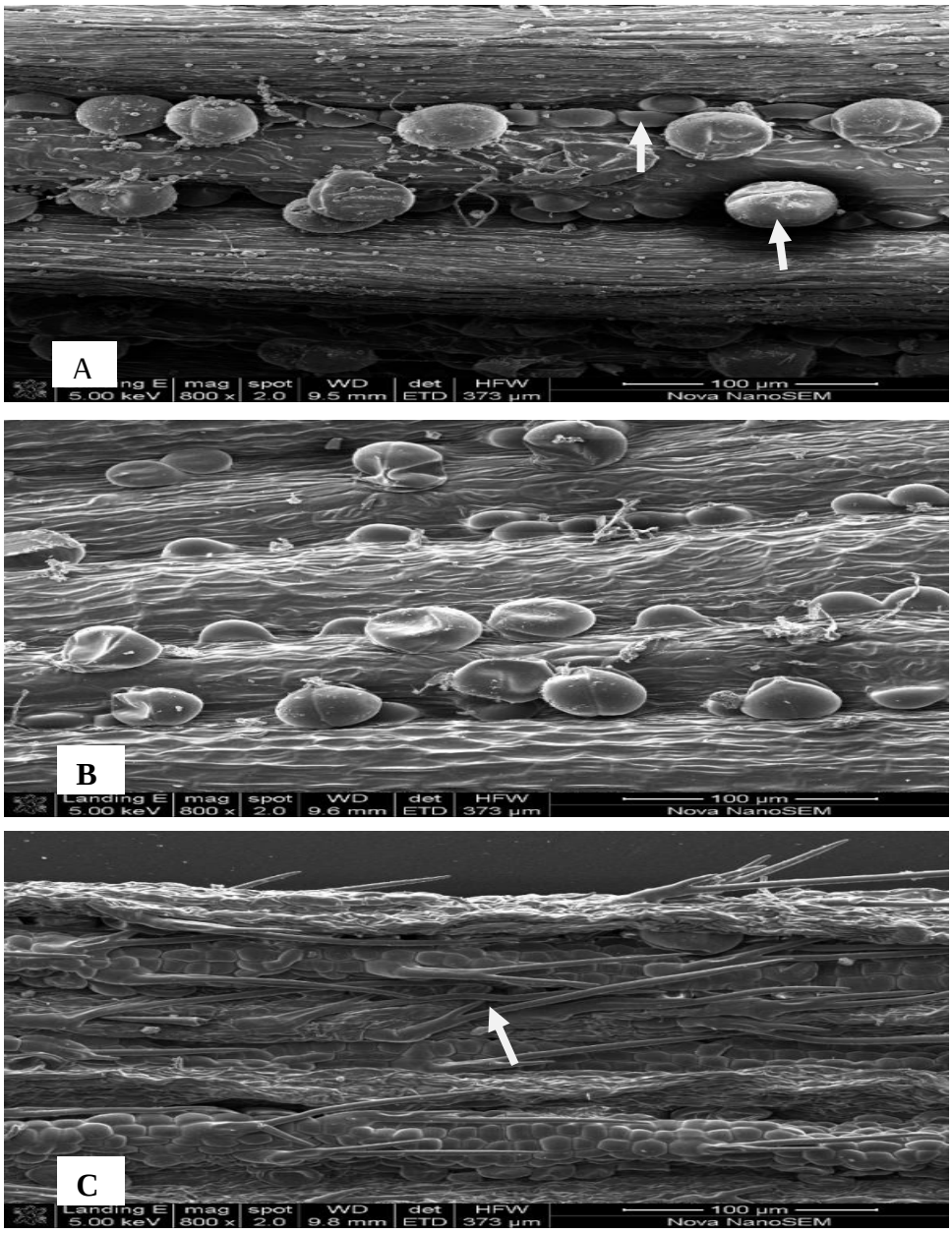

Figure 4.7 SEM micrographs of cypselas of *V. tolypophora*. A & B: Showing glabrous cypsela with idioblasts and capitate glands (arrow) (From Stolz 343 & Purdue *et al.* 11483 respectively. C: Showing cypsela densely covered between ribs with idioblasts and moderately so with twin hairs (arrow). From Gilbert *et al.* 5462 For all scale bar = 100µm. (Source: Author, 2013)

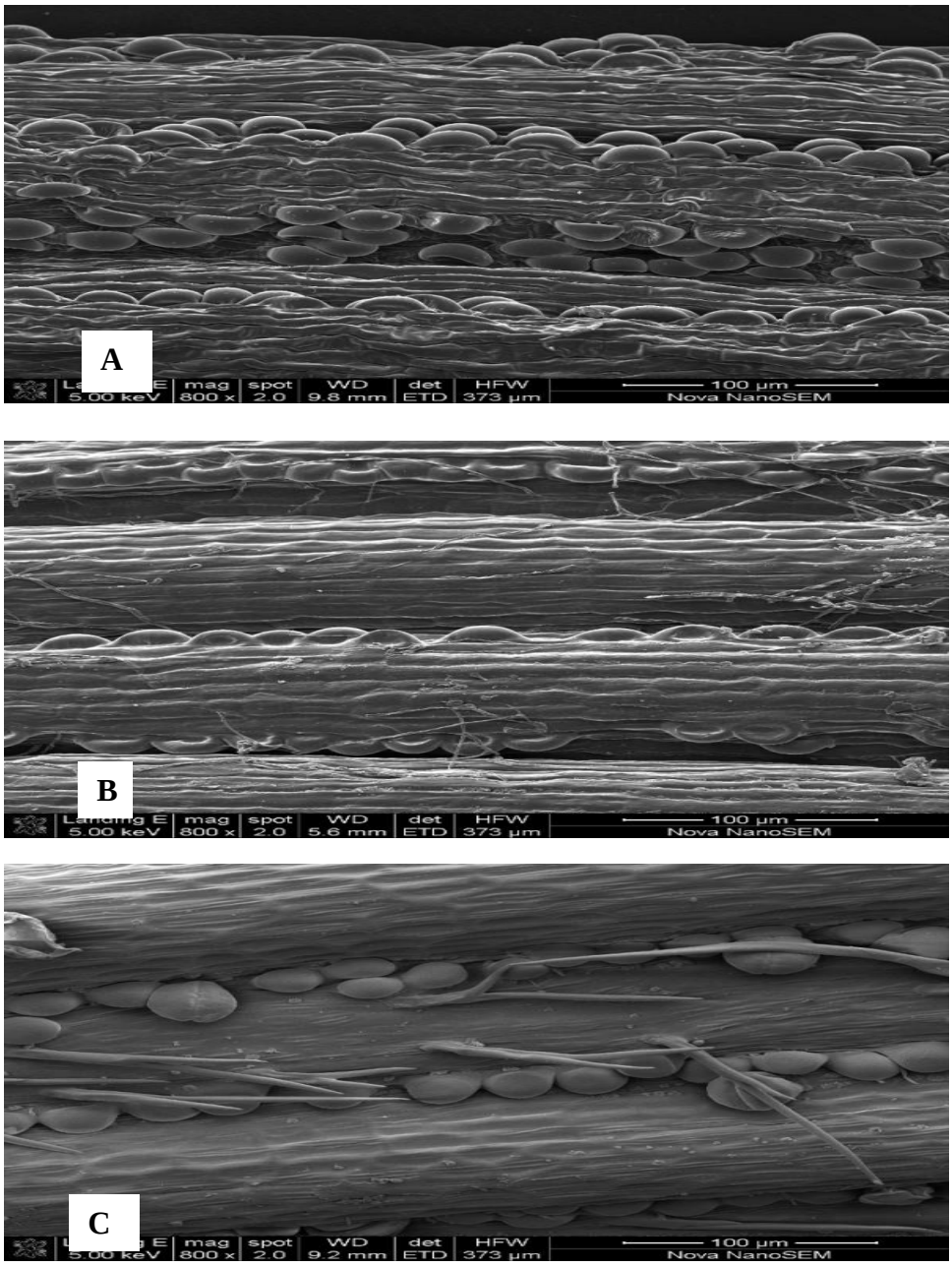

Figure 4.7. SEM micrographs of cypselas of *V. hymenolepis*. A & B: Showing cypsela with many (A) and few (B) idioblasts between ribs. From Witson 803 & Smith *et al.* 189. C: Showing cypsela with idioblasts, capitate glands and twin hairs. From Desta *et al.* 4557 For all scale bar = 100µm. (Source: Author, 2013)

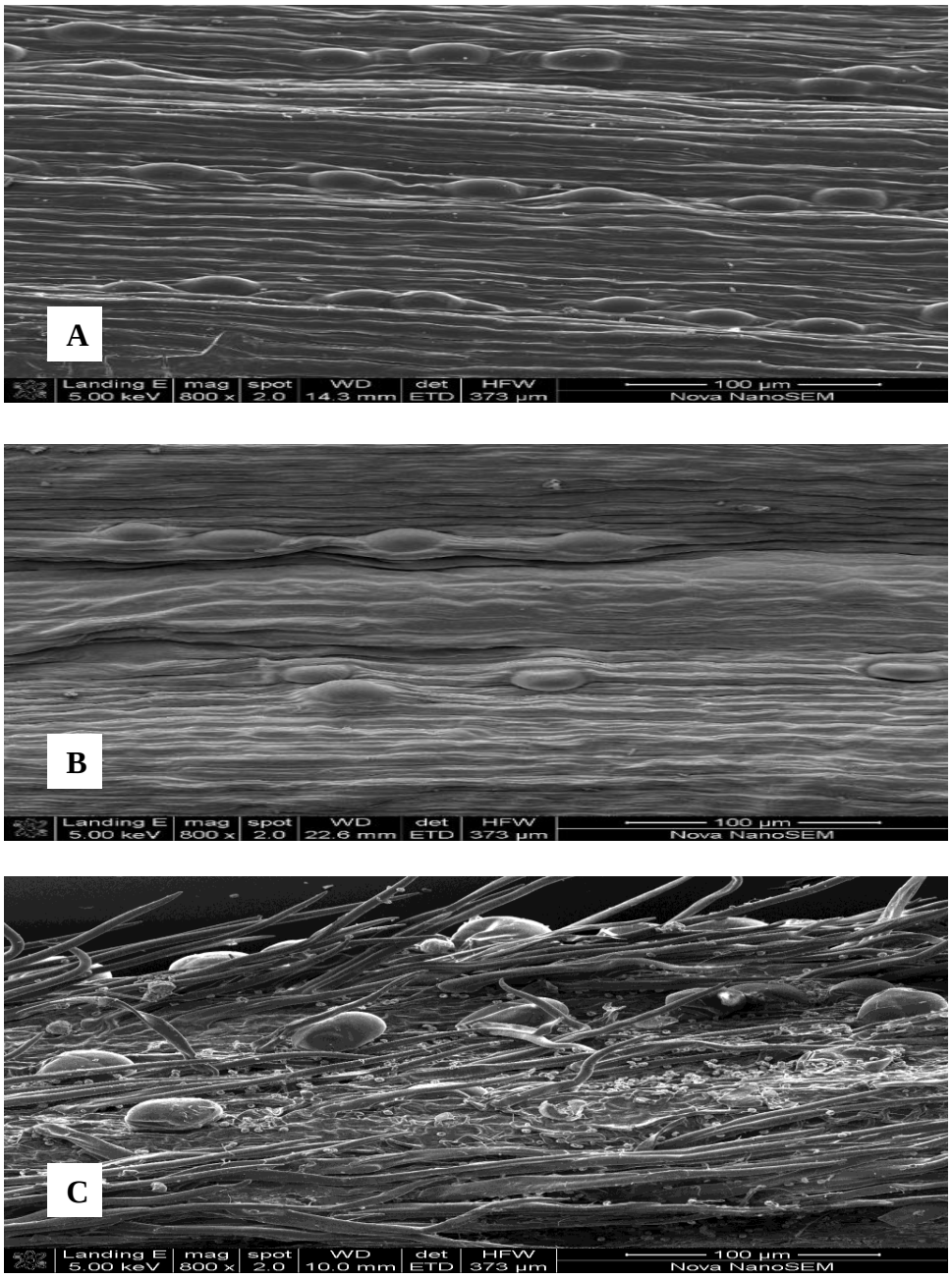

Figure 4.8. SEM micrographs of cypselas. A: *V. calvoana* subsp. *usambarensis* showing cypsela sparsely covered with idioblasts. From Benedicto 68. B: *V. calvoana* subsp. *oehleri* showing glabrous cypsela sparsely covered with idioblasts. From Freidberg T9. C: *V. calvoana* subsp. *oehleri* showing cypsela sparsely covered with capitate glands and moderately so by twin hairs. From Peter 43268. For all scale bar = 100µ. (Source: Author, 2013)

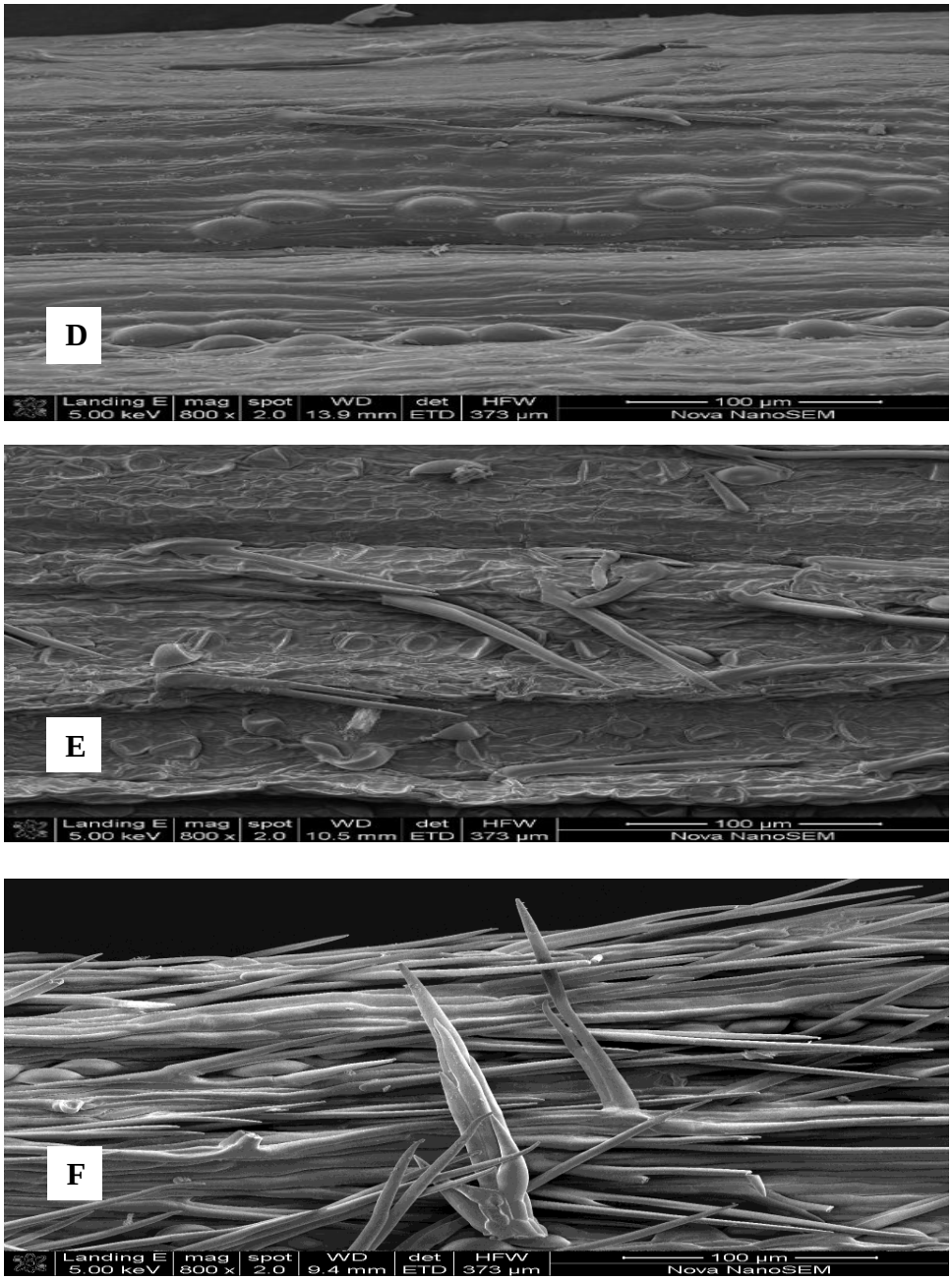

Figure 4.9 cont. SEM micrographs of cypselas. D: *V. calvoana* subsp. *uluguruensis* showing cypsela sparsely covered with twin hairs and idioblasts. From Harris DSMA2477 E: *V. calvoana* subsp. *leucocalyx* showing cypsela sparsely covered with idioblasts and twin hairs. From Goetze 928. F: *V. calvoana* subsp. *ruwenzoriensis* showing cypsela densely covered with twin hairs and a few idioblasts. Fyfee 21. For all Scale bar = 100µm.

4.2 Nutritional analysis

4.2.1 Proximate composition

The proximate composition of *Vernonia hymenolepis* leaves is shown in Table 4.5. The moisture content of the leaves ranged between 53.2% and 81.0% while that of proteins was on average 11.2g in 100g dry weight of leaves. The results also revealed a high ash content (11.7g), Phosphorus (284.1mg) and Nitrogen (8.7g) in 100g dry weight of leaves (see Table 4.5).

4.2.2 Vitamins and minerals

The Vitamin and mineral composition of *V. hymenolepis* leaves is also shown in Table 4.5. The Vitamin C content of the leaves was high, ranging from 671mg-1600mg/100g of raw sample and a mean of 1100mg/100g dry weight. The mineral content was equally high with a mean content of 303.2mg, 242.3mg, 19.6mg, 1200mg, 430.8mg and 133.8mg/100g dry weight for sodium, potassium, copper, iron, manganese and zinc respectively. Iron and manganese showed the biggest differences between the minimum and maximum value (Table 4.5).

Table 4.5 Nutrient composition of *Vernonia hymenolepis* leaves (mg/100g dry weight).

Variable	Min.	Max.	Mean (N=18)	Std. Dev.	RDA (ADULTS)
Moisture (%/mg)	53.2	81.0	73.4	7.2	2700000.0
Ash (mg)	8760.0	15080.0	11657.8	1841.5	
Phosphorus (P) (mg)	190.0	390.0	284.1	61.4	700.0
Sodium (Na) (mg)	87.7	620.7	303.2	191.0	1500.0
Potassium (K) (mg)	146.2	312.0	242.8	46.7	4700.0
Copper (Cu) (mg)	5.6	31.4	19.6	7.2	0.9
Iron (Fe) (mg)	271.0	4301.4	1207.5	1262.3	18.0
Manganese (Mn) (mg)	12.0	1199.8	432.2	333.6	2.3
Zinc (Zn) (mg)	16.1	521.5	133.8	153.3	11.0
Proteins (g)	6.6	18.1	11.2	2.7	56.0
Vitamin C (mg)	671.5	1585.2	1120.4	274.5	90

4.2.3 Comparison of the nutrient composition of *V. hymenolepis* leaves from different regions in Kenya

When compared, populations of *V. hymenolepis* from different regions in Kenya viz., Mt. Elgon, K3 (Bungoma, Trans Nzoia and Mt. Elgon), Kakamega, K5 (Kakamega, Vihiga and Kakamega-Nyanza, Kapsabet, Nabkoi and Nandi) and Narok, K6 (Nasampolai, Sakutik and Bomet) showed differences in their nutrient composition (see Table 4.6). Populations from Mt. Elgon had the highest amount of ash (12645.0mg/100g d.w) while those from Kakamega had the highest amount of copper (23.4mg/100g d.w) and Iron (1471.5mg/100g d.w). The Kakamega populations gave the highest amount of nitrogen (41713.7mg/100g d.w), protein (13036.5mg/100g d.w) and potassium (249.0mg/100g d.w) while those from Narok had the highest quantities of moisture (77.3%), phosphorus (302.4mg/100g d.w), sodium (564.1mg/100g d.w), zinc (284.7mg/100g d.w), manganese (687.7mg/100g d.w) and vitamins (1159.1mg/100g d.w). At $p < 0.05$ there was no significant difference in the moisture, ash, phosphorus, potassium, copper, iron, manganese and zinc content of leaves from the Mt. Elgon (K3) and Narok (K6) populations and in ash, potassium, copper and iron content of the leaves from the Kakamega (K5) and Narok (K5) populations. There was significant statistical difference in all the variables investigated in the leaves from the Mt. Elgon (K3) and Kakamega (K5) populations except in moisture, nitrogen, proteins and Vitamin C content at $P<0.05$ (see Table 4.7). In comparison with commonly used vegetables, *V. hymenolepis* is richer in minerals (Sodium, Potassium, Iron and Zinc), vitamins and proteins. (see Table 4.8).

Table 4.6 Comparison of the nutritive composition of *Vernonia hymenolepis* leaves (100g dry weight) from three FTEA regions in Kenya

Variable	Mt. Elgon (K3)	Kakamega (K5)	Narok (K6)
Moisture (%)	77.07	65.98	77.26
Ash (mg)	12645.00	11206.67	10360.00
Phosphorus (mg)	289.10	265.21	302.38
Nitrogen (mg)	29729.69	41713.66	38689.23
Sodium (mg)	224.79	233.79	564.05
Potassium (mg)	241.65	248.95	235.66
Copper (mg)	19.54	23.38	13.91
Iron (mg)	1330.69	1471.51	565.25
Manganese (mg)	454.13	232.74	687.68
Zinc (mg)	115.88	56.97	284.71
Proteins (g)	9.29	13.04	12.09
Vitamin C (mg)	1033.00	1141.00	1159.05

Table 4.7 Comparison of the nutritive composition of *Vernonia hymenolepis* leaves (100g dry weight) from three FTEA regions in Kenya

Variable	K3		K5		K6	
	Mean (N=8)	Std. Deviation	Mean N=6	Std. Deviation	Mean N=4	Std. Deviation
Moisture	0.509 [a]	0.340	1.035	1.110	0.534 [a]	0.361
Ash	0.536 [a b]	1.164	0.245 [a c]	0.544	0.705 [b c]	0.679
Phosphorus	0.082 [a b]	1.128	0.307 [a]	1.018	0.298 [b]	0.802
Nitrogen	0.688	0.871	0.690	0.945	0.342	0.200
Sodium	0.410 [a]	0.714	0.363 [a]	0.850	1.366	0.264
Potassium	0.024 [a b]	1.210	0.133 [a c]	0.919	0.151 [b c]	0.879
Copper	0.004 [a b]	0.932	0.532 [a c]	0.685	0.790 [b c]	1.208
Iron	0.098 [a b]	0.950	0.209 [a c]	1.358	0.509 [b c]	0.155
Manganese	0.066 [a b]	0.971	0.598 [a]	1.074	0.765 [b]	0.190
Zinc	0.117 [a]	1.161	0.501	0.447	0.985 [a]	0.601
Proteins	0.688	0.870	0.689	0.946	0.343	0.200
Vitamin C	0.319	0.340	0.075	0.00	0.141	1.366

Means sharing a common superscript are not significantly different at P<0.05

Table 4.8 Nutrient composition of *Vernonia hymenolepis* leaves. Included are the nutrient composition of Amaranth, cabbage, kale, jute and cowpeas (100g).

Vegetable	Moisture (%)	P (mg)	Na (mg)	K (mg)	Fe (mg)	Zn (mg)	Protein (g)	Vit. C (mg)
V. hymenolepis	73.4	284.1	303.2	242.8	1207.5	133.8	11.2	1120.4
Amaranth	91.69	50	20	611	2.32	0.90	2.5	43.3
Cabbage (*Brassica oleracea)*	92.18	26	18	170	0.47	0.18	1.3	36.6
Kale	84.04	92	38	491	1.47	0.56	4.3	120.0
Jute, potherb (*Corchorus olitorius*)	87.72	83	8	559	4.76	0.79	4.7	37.0
Cowpeas (Vigna *unguiculata*)	89.79	9	7	455	1.92	0.29	4.1	36.0

(National Nutrient Database for Standard Reference Release 26 Software v.1.4)

CHAPTER FIVE
DISCUSSION

5.1 Patterns of morphological variation

The results of PCA, UA, CA and DA suggest that the *V. calvoana- V. hymenolepis- V. tolypophora* complex consists of one morphologically variable species. None of the analyses done in this study recovered any group of OTU's that unambiguosly correspond to any of the species in the complex. Cluster analysis revealed two assemblages of OTU's; a big and small cluster which separated at a high level of dissimilarity. The big cluster took up approximately 84% of all the OTU's studied and comprised specimens of *V. calvoana, V. hymenolepis* and *V. tolypophora* intermixed but with those of *V. hymenolepis* and *V. tolypophora* dominating. The branches between the OTU's in the big cluster were very short indicating a very close morphological relationship among the OTU's (Sebola and Balkwill, 2013). The small cluster was made up largely of OTU's belonging to two subspecies of *V. calvoana* i.e., *V. calvoana* subsp. *ruwenzoriensis* and *V. calvoana* subsp. *ulugurensis* which occur in the Ruwenzori Mountains in western Uganda and Uluguru Mountains in eastern Tanzania respectively.

The higher measurement values found in most characters of the OTU's in the small cluster as opposed to those in the bigger cluster (Fig. 4.4) is an indication that those individuals probably had relatively more robust features than specimens from the big cluster which were collected from comparatively lower altitudes for example Kakamega, Kisii, Rungwe, Ulanga, Mbeya and Iringa. This same phenomenon has been observed in populations of *Cineraria deltoidea* Sond., from the tall mountains in East Africa which were found to have distinctly larger capitula when compared to specimens of the same species from lower altitudes (Cron *et al*., 2007). It is likely that the higher measurement values in specimens of *V. calvoana* in the smaller cluster could be the cause of their separation in CA from those in the big cluster. However in PCA, all specimens of *V.*

calvoana form a continuum of variation with *V. hymenolepis* and *V. tolypophora* (Figs. 4.1&4.2).

Discriminant analysis does not also separate *V. calvoana* specimens into different clusters (Fig. 4.5). Instead they all form a cluster which overlaps greatly with the group comprising specimens of *V. hymenolepis*. In appearance the specimens of *V. calvoana* in the small cluster in CA are morphologically consistent with those in the big cluster e.g. there is a conformity in the size of leaves and fruits, the size and shape of leaf serration and pores, the pubescence on cypsela and shape and size of capitula and it is not surprising that in the ordination space in DA they form a more or less homogeneous cluster (Fig. 4.5). This is the case also in PCA (Figs. 4.1&4.2). Logically, therefore, the clustering together of these specimens in CA separate from other *V. calvoana* specimens should not be accorded too much taxonomic weight because, as is evidently clear, they are not amenable to any kind of taxonomic recognition. It is likely that their clustering alone in CA could be an artefact of the analysis. In certain cases, linkage based CA techniques, like the UPGMA used in this study, are known to confer a heirarchical structure to data that is evidently continuous and this can often lead to wrong interpretation (Thorpe, 1983). Often they can show clusters that may not be recoverable in ordination analyses (Chandler & Crisp, 1998).

Since neither CA, PCA or DA recovered any groups that can outrightly be assigned to any of the three species forming the complex; the hypothesis that the three species are indeed inseparable as distinct taxa is supported by the results. The univariate analysis using box plots also showed no discernable morphometric distinction between the three species (Fig. 4.6) and hence further lends support to the hypothesis. The statistical difference demonstrated among the species for some capitulum, fruit and leaf characters as revealed by T-test unlike the box plots could be resulting from the small number of *V. tolypophora* and *V. calvoana* specimens used in the analysis as compared to *V. hymenolepis*. This was also noted with some characters in the *Cymopterus glomeratus*

(Nutt.) DC species complex where due to small sample sizes for varieties *greeleyorum*, *higginsii*, and *parvus*, statistical difference was observed (Sun *et al*. 2005).

Wild (1978) subsumed *V. tolypophora* in *V. hymenolepis* as a subspecies i.e. *V. hymenolepis* A. Rich. subsp. *tolypophora* (Mattf.) Wild and also transferred *V. calvoana* subsp. *leucocalyx* (O.Hoffm.) C.Jeffrey to *V. hymenolepis* as *V. hymenolepis* A. Rich. subsp. *leucocalyx* (O.Hoffm.) Wild. This revised treatment of *V. tolypophora* and *V. calvoana* subsp. *leucocalyx* provided the intial signs that there were difficulties in discerning morphological boundaries between the three species. Later studies, however (e.g. Jeffrey 1988; Isawumi *et al*., 1996; Jeffrey & Beentje, 2000) treated *V. hymenolepis* and *V. tolypophora* as distinct species though based only on a few morphological characters. For example Jeffrey (1988) and Jeffrey and Beentje (2000) recognised them as distinct but based only on the densely clustered capitula seen in *V. tolypophora* while Isawumi *et al*., (1996) maintained them as distinct based on micromorphological evidence obtained from only two specimens of *V. hymenolepis* and one of *V. tolypophora*.

In this study the densely clustered capitula cannot be used to separate the species due to inconsistency in the variability of the character. For example a specimen of *V. tolypophora* (Gilbert, Kanuri & Mungai 5462) collected from Samburu in 1979 did not have densely clustered capitula. In addition specimens of *V. hymenolepis* (e.g Bono 20, Kerfoot 2041) have also been collected from the same locality. Isawumi *et al*. (1996) observed that *V. hymenolepis* did not have twin hairs and glands on the cypsela and that *V. tolypophora* had cypsela which were seemingly glabrous without twin hairs but with idioblasts and capitates glands. However, this study has revealed that twin hairs, idioblasts and glands are found on the cypselas of both species in varying degrees and cannot therefore be used to distinguish the species (Figures 4.7 & 4.8). The earlier disparity observed in these characters by Isawumi *et al*., (1996) could be attributed to the small number of specimens that they examined. Jeffrey and Beentje (2000) used the length of the involucre to separate the same two species. However the results from this

study show that this character does not differ and is continous between the species (Fig. 4.6).

Vernonia calvoana and *V. hymenolepis* have also been treated as distinct (Isawumi *et al*., 1996; Jeffrey 1988). However, Fomum (2004) noted that the characters that have been used to distinguish between them viz., the often larger and less densely pubescent leaves, usually larger and recurving outer involucral bracts and the more often glabrous fruits in *V. calvoana*, showed considerable overlap. The current results agree with this observation. The size of the leaves and involucre was variable within the same species and continous among the species (Figure 4.6). The pubescence of leaves was also varied within the same species where younger leaves were seen to be more pubescent than older ones. The recurving of the outer involucral bracts was also observed in both species. The absence of and type of glands and hairs present on the cypsela which were thought to have been useful in separating the species showed a lot of intergradation. These results thus agree with observations made by Fomum (2004) that cypsela pubescence intergrades between the two species. It is worth noting that often *Vernonia calvaona* has been confused with *V. hymenolepis* (e.g. Jeffrey 1988) and therefore it could be that the subtle differences that have been observed between them are just the effects of phenotypic plasticity arising from differences in habitat. *Vernonia hymenolepis* occurs mainly in grassland openings in montane forests and high plateau areas while *V. calvoana* is often found in forested mountaneous places.

In this study the concept was that a species is the smallest group that is constistently and persistently distinct, and is distinguishable by ordinary means (Cronquist, 1978). The term ‘by ordinary means’ is taken to mean through visually observable morphological differences. The existence of gaps in the pattern of visually observable phenetic diversity is usually taken as evidence for reproductive isolation (Sites & Marshall, 2004) but since in this study the three species lack clear morphological discontinuities between them and thus do not seem to meet, to any reasonable degree, the three criteria of being consistently and persistently distinct and distinguishable by ordinary means as set by

Cronquist (1978), there is compelling reason to merge them. It is therefore proposed that they be merged into one morphologically variable species under the oldest name *Vernonia hymenolepis*.

5.2 Taxonomy

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calvoana subsp. *leucocalyx* (O.Hoffm.) Isawumi, El-Ghazaly & B.Nord. in Grana, 35(4): 227 (1996).

Perennial herb, shrub or rarely a small tree up to 12 m tall; stems unbranched at base, branching from higher up, often with small protuberances, densely pubescent or finely, velvety-tomentose, sometimes also with longer crispate or russet brown hairs intermixed and glabrescent. Leaves alternate, simple, elliptical, ovate, lanceolate or broadly so, 3.5–34.5 cm long, 1.0–9.5 cm wide, cuneate to attenuate to cordate to subtuncate and sometimes winged auriculate or exauriculate petiolate or pseudo-petiolate base, margins minutely to coarsely serrate, acuminate or shortly so at apex, finely thinly or minutely pubescent and glabrescent above, thinly pubescent mostly on veins and glabrescent to densely sometimes crispate pubescent or tomentose beneath, pinnately veined. Inflorescence a head, capitula numerous arranged in terminal, compound, corymbiform cymes or aggregated in dense terminal clusters; involucre ovoid to hemispherical, 8–36 mm long, phyllaries in several series, oblong or lanceolate, 1- 3.5 cm long, green, white, pink or purple with lanceolate to ovate appendages; appendages of phyllaries 3-28 cm long, 0.8-9.8 cm wide, green, white or rarely white with purple or faint pink tinge, outer recurved or inner erect. Flowers bisexual, regular, 5-merous, strongly exserted from the involucre; corolla tubular, 9–23 mm long, white, pale mauve, purple or white tipped with purple, glandular, with short, erect lobes; stamens with anthers united into a tube, with appendages at apex; ovary inferior, 1-celled, glabrous to pubescent, style hairy, 2-branched. Fruit a ribbed achene 1–6.9 mm long, 3-16 mm wide, glabrous, slightly pubescent to pubescent, dark brown or black or with both colours, crowned by the much longer pappus bristles. Pappus consisting of many-seriate caducous bristles 6-18.9 mm long pale brown (Appendix iii).

Kenya: Narok: Nasampolai, 2 May 2013, Evusa & Barasa 20; Narok: Sakutik, 2 May 2013, Evusa & Barasa 25; Trans Nzoia: Kipsain, 5 Jan. 2013, Evusa, Wanjohi & Barasa 3; Trans Nzoia: Mt. Elgon , 5 Jan. 2013, Evusa, Wanjohi & Barasa 5; Trans Nzoia: Saiwa, 5 Jan. 2013, Evusa, Wanjohi & Barasa 4; Kakamega: Malava, 6 Jan. 2013, Evusa,

Wanjohi & Barasa 6; Kakamega: Kakamega Forest, 6 Jan. 2013, Evusa, Wanjohi & Barasa 7; Kakamega: Kakamega Forest, 6 Jan. 2013, Evusa, Wanjohi & Barasa 8; 6 Jan. 2013, Nandi: Shiru, Evusa, Wanjohi & Barasa 9; Kakamega: Kaptik, 6 Jan. 2013, Evusa, Wanjohi & Barasa 10; Kakamega: Yala, 7 Jan. 2013, Evusa, Wanjohi & Barasa 12; Kakamega: Vihiga, 7 Jan. 2013, Evusa & Barasa 15; Bungoma: Misikhu, 7 Jan. 2013, Evusa & Wanjohi 1; Trans Nzoia: Kitale, 5 Jan. 2013, Evusa, Wanjohi & Barasa 2; West Pokot: Mount Sekerr, 4 Aug. 1968, Agnew, Kibe & Mathenge 10490; Trans Nzoia: Kitale, 24 Aug. 1986, Beentje 3002; Trans Nzoia: Hoeys Bridge, 25 Jan. 1967, Earle Smith & Njeroge 4632; Trans Nzoia: Hoeys Bridge, 27 Jan 1967, Earle Smith & Njeroge 4638; Trans Nzoia: Kitale, 23 March 1986, Ekkens 662; Trans Nzoia: Kitale, 2 Nov. 1963, Freidberg 19; Trans Nzoia: Kitale, 31 Jan. 1982, Gilbert 7008; Nakuru, 14 Oct. 1981, Gilbert & Mesfin 6807; Trans Nzoia: Kitale, 10 Oct. 1981, Gilbert & Mesfin 6576; Narok: Nasampolai, 8 July 1972, Greenway & Kanuri 15023; Kakamega Kaptik, 11 Nov. 1984, Hohl 121; Kakamega: Kakamega Forest, Holyoak 3022; Trans Nzoia, 22 Sept. 1957, 27 July 1960, Irwin 365; Samburu: Mount Nyiru, 27 July,1960, Kerfoot 2041; Kericho, 1 Feb. 2013, Kirika 3166; Kericho, 4 Feb. 2013, Kirika 3167, Kericho; West Pokot: Cherangani Hills, 31 March 1971, Kokwaro 2564; Uasin Gishu: Timboroa, 9 Feb. 1971, Livingstone , Thomas , Melack & LaBarbera 23; Trans Nzoia: Kitale, 11 Sept. 1984, Mungai 57/84; Trans Nzoia: Saiwa Swamp National Park, 9 Dec. 1993, Kirika 26; Trans Nzoia: Kitale, 28 Feb. 1984, Siemens 20; Elgeyo Marakwet: Elgeyo, 4 Nov. 2000, Smith , Beentje & Muasya 189; Elgeyo Marakwet: Cherangani, Feb. 1958, Symes 274; Elgeyo Marakwet, Oct. 1957, Symes 258: Cherangani; Elgeyo Marakwet: Cherangani, 3 Aug, 1969,Thulin & Tigids 125;Kakamega: Kakamega Forest, 10 Dec. 1956,Verdcourt 1706; Kisii, 30 Oct. 1974, Vuyk & Breteler 61; Samburu: Maralal, 2 June 1979, Gilbert , Kanuri & Mungai 5462.

Uganda: Toro: Ruwenzori Mountains, 4 Dec. 1930, Burtt 2831; Kigezi: Bufumbira, 24 April 1970, Katende 181; Kigezi: Bufumbira, 9 Sept. 1952, Norman 36; Kigezi, Sept. 1946, Purseglove 2195; Toro: Ruwenzori Mountains , Aug. 1931, Fishlock & Haucock

46; Toro: Ruwenzori Mountains, 5 Feb 1916, Fyffe 21; Toro, 9 Sept. 1951, Osmaston 1164; Toro: Ruwenzori Mountains, Jan. 1950,Osmaston 3681; Mbale: Mount Elgon , Dec. 1939, Dale 87; Toro: Ruwenzori Mountains , Nov. 1968, Scott 7058; 29 Aug. 1932, Thomas 364; Karamoja: Mount Moroto, Mar. 1960, Wilson 803; Mbale/Trans Nzoia: Mount Elgon, Jack 3507; Mbale/Trans Nzoia: Mount Elgon Dec. 1942, Tweedie 73,

Tanzania: Mbeya, 25 Nov. 1989**,** Lovett & Kayombo 3497; Rungwe, 12 June 1992, Mwasumbi 16298,; Ufipa, 16 June 1970, Sanane 1210; April 1937, Moshi: Kilimanjaro, Baldock 5; Mbeya: Mbeya Peak, 29/5/1958, Gaetan 39; Mbeya, 31 Aug. 1962, Kerfoot 4152; Mbeya: Ilembo, 17 June 1977, Leedal 4370; Mbeya/Chunya: Unyiha, 30 July 1972, Leedal 1224; Mbeya: Ipinda , 29/7/1974, Leedal 1915; Iringa: Mufindi, 7 Oct. 1936, McGregor 62; Njombe: Hagafilo, 8 July 1956, Milne-Redhead & Taylor 10789; Songea: Miyau, 19 May 1956, Milne-Redhead & Taylor 10252; Iringa: Mufindi, 22 July 1969, Paget-Wilkes 535; Iringa: Mufindi, 11 Aug. 1971, Perdue & Kibuwa 11020; Iringa: Mufindi, 18 Sept. 1971, Perdue & Kibuwa 11483; Rungwe: Tukuyu, 2 Oct. 1971, Perdue & Kibuwa 11664; Iringa: Mazombe, July 1965, Redmayne 13261; Mbeya: Mporotes, 10 Sept. 1954, Smith 1291; Mbeya, 9 Sept. 1954, Smith 1262; Rungwe: Ibaba, Nov. 1972, Stefanescu 411; Mbeya: Poroto Mountains, 1932, Thompson 673; Iringa: Idodi, Oct. 1936, Ward 18; Rungwe: Kiwira Forest, 2 April. 1983, Abdallah 1313; Mbeya , 5 June 1992, Gereau, Harder & Kayombo 4627; 3 Aug. 1989, Iringa: Mufindi, Kayombo 840; Rungwe: Unyakyusa , 18 Aug. 1977, Leedal 4514; Rungwe: Mwakaleli, 24 May 1954, Leedal 1857; Ndundulu Forest Reserve, 6 Sept. 2004, Luke & Butynski 10363; Luke & Iringa: Udzungwa mountain National park, 27 Sept. 2000, Luke & Bytebier 6714; Rungwe: Masoko, 19 Nov. 2008, Luke 12833; Mbeya, 16 June 1992, Mwasumbi 16313; 3 Jan. 1911, Goetze 928; Masai: Ngorongoro Crater, 23 Sept. 1932, Burtt 4294; Masai: Empakaai Crater, 8 Sept. 1972, Frame 15; Lushoto/Tanga: Usambara Mountains, 23 July 1996, Freidberg 9; Arusha: Mount Meru, 2 July 1961, Greenway 10394; Arusha , June 1927, Haarer 253; Moshi: Weru, 3 Dec. 2003, Hemp 3803; Masai: Lerai, 21 June 1965, Herlocker 139; Arusha: Meru, Aug. 1956, Ivens 764; Masai: Oldonyo Sambu, 14 March 1974, Richards 28961; Masai: Ngorongoro Crater, 14 July

1963, Verdcourt 3684; Masai: Ngorongoro Crater, 6 Aug. 1956, Verdcourt 1561; Arusha: Mount Meru, 17 Oct. 1968, Vesey-FitzGerald 5989; 24 July 1926, Peter 43268; Morogoro: Bondwa, 27 Sept. 1970, Harris, Pocs & Csontos 5185; Morogoro: Uluguru Mountains, 29 Sept 1971, Harris 2477; Morogoro: Uluguru Mountains, 8 May 1988, Pocs & Minja 88065; Morogoro: Uluguru Mountains, 20 Sept. 1970, Thulin & Mhoro 1077; Lushoto: Shume-Magamba Forest, 29 Sept. 1955, Benedicto 68; Lushoto: Shume Forest, July 1924, Mabes 149; Lushoto, 1 Sept. 1966, Semsei 4107.

DISTR. U1-3, K1-6, T2-8,

HAB. Mist, moist, disturbed or open parts of forests and forest glades in submontane rainforests, bamboo and *Juniper* forests; moist sites of montane woodland or bushland, bushed and grazed or fire swept grasslands, tree-grasslands, abandoned cultivations, hedgerows and margins of farmlands, riverine sites, fringes in damp areas near springs, lake side, valley sides, murram scrapes by roadside and rocky slopes, 518-3600m.

5.3 Nutrient composition

The dominant constituent of *Vernonia hymenolepis* leaves was water whose value was lower than the published value for amaranth, cabbage, kale, jute and cowpeas (see Table 4.8). However the fresh leaves had vitamin C content higher than published values for amaranth, cabbage, kale, jute and cowpeas, *V. amygdalina* and *V. calvoana* (Mensah *et al*., 2008). *Vernonia hymenolepis* leaves can thus be a good source of Vitamin C which cts as an anti-oxidant helping to protect against cancers, heart diseases and is essential for sperm production and collagen protein synthesis (Ejoh *et al*., 2005). However, its high moisture content also makes it vulnerable to microorganism spoilage (Numfor, 1997; Fidelia., 2000). To prevent this spoilage the plant can be grown locally so as to be used when still fresh or refrigerated (Natalie, 2010).

Vernonia hymenolepis leaves are rich in nitrogen (Table 4.5) and their protein content is higher than that of *V. calvoana* in Cameroon (22.75–26.50 mg/100 of d.w.) though less than that recorded in a non-bitter variety of *V. calvoana* (18.16 and 24.12 g/100g d.w)

(Fube & Djonga, 1987; Ejoh *et al*., 2007). *Vernonia amygdalina* also contains higher amounts of proteins than *V. hymenolepis* (Mensah *et al*., 2008). Athough *V. hymenolepis* has low amounts of proteins in the leaves compared to other commonly used vegetables it should not be considered as an insignificant source of proteins especially when one thinks of rural areas where people suffer from acute malnutrition due to lack of sufficient nutrients. Generally, green leafy vegetables have low amounts of proteins and are therefore usually taken in accompaniment with other protein rich foods.

Vernonia hymenolepis had a high ash and consequently a high mineral content, particularly of iron and manganese which were far much higher than the 0.200–0.300 mg/100g d.w and 0.580–0.885 mg/100g d.w recorded for *V. calvoana* respectively (Fube & Djonga, 1987). Iron was notably higher than published values for amaranth, cabbage, kale, jute and cowpeas (Table 4.8). Phosphorus, sodium and zinc content levels were also higher than those published for amaranth, cabbage, kale, jute and cowpeas. However levels of Potassium were lower compared to those published for the same vegetables. These levels of nutrients in *V. hymenolepis* are an indication that it can be used as a vegetable considering that it is also available throught out the year.

Most of the leaf samples of *V. hymenolepis* used in this study to test for the various nutrients were collected from the wild e.g. from roadsides, forest margins or disturbed environments and thus the statistical differences noted in the levels of some of the nutrients in the leaves (Table 4.7) could be due to differences in the physiological state of the plants before harvesting, habitats, edaphic and climatic conditions and human practices (Mbinglo, 1998; Ejoh *et al*., 2007). However, if *V. hymenolepis* were to be placed under cultivation under similar conditions and the leaves harvested at the same time using the same method, it is likely that the nutrient composition, when the leaves are analyzed, might be different from that obtained in this study.

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The morphometric analyses undertaken in this study resulted in the resolution of the taxonomic relationships between the taxa forming the *Vernonia hymenolepis* complex. The results presented revealed overall morphological similarities among the taxa forming the complex and thus invalidates the continued recognition of the three as distinct species. They are thus here merged to form one variable species with *V. hymenolepis* A. Rich., the earliest name of the three species, being adopted as the name of the new entity. *Vernonia calvoana* and *V. tolypophora* are placed under its synonymy.

Analyses of the leaves of *Vernonia hymenolepis* revealed a high quality and quantity of vitamins, ash, moisture, proteins, nitrogen, phosphorus and minerals. This therefore makes it a potential candidate for consideration as an alternative vegetable in the East Africa region.

6.2 Recommendations

1. Following the merger of the three species in the compex in this study to form one variable species under the name *V. hymenolepis*, new combinations should be made of the subspecies recognized under the synonymized *V. calvoana*.
2. Given the potential of *V. hymenolepis* to be used as a vegetable, there is need to explore possibilities of domesticating and adopting it in family agricultural systems to provide a cheap, readily available source of nutritive elements especially for rural populations. It could also be a source of income for resource poor people.
3. Molecular work to be done for further verification of the taxonomic relationship of the species.
4. Further nutrient analysis to be done to determine the nutrients which were not analysed in this study and identify the active components that have medicinal properties in *V. hymenolepis.*

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APPENDICES

Appendix I. List of specimens examined

Specimen No.	Species	Subsp.	Habitat	Country	Locality	FTEA Region	Latitude	Altitude	Collection Date
2831	*V. calvoana*	*adolfi-friderici*		U	Toro	U2	0.05N 45.30E	2769	4/12/1930
181	*V. calvoana*	*adolfi-friderici*	Moist areas.	U	Kigezi	U2	1.22S 29.39E	3600	24/4/1970
36	*V. calvoana*	*adolfi-friderici*		U	Kigezi	U2	1°12' S, 29°40' E	2987	9/9/1952
2195	*V. calvoana*	*adolfi-friderici*		U	Kigezi	U2	0°16' N, 31°37' E	2987	9/1946
803	*V. calvoana*	*adolfi-friderici*		U	Karamoja	U1	2°32' N, 34°46' E	2439	0/3/1960
1313	*V. calvoana*	*Leucocalyx*		T	Rungwe	T7	9°02' S, 33°38' E	1800	2/4/1983
4627	V. calvoana	*Leucocalyx*	Open disturbed place	T	Mbeya	T7	9.01S 33.33E	1980	5/6/1992
928	V. calvoana	*Leucocalyx*		T	Njombe	T7	9.1S 34.15E		3/1/1911
840	*V. calvoana*	*Leucocalyx*		T	Iringa	T7	8.35S 35.15E	1450	3/8/1989
4514	*V. calvoana*	*Leucocalyx*		T	Rungwe	T7	9°15' S, 33°40' E	2134	18/8/1977
1857	*V. calvoana*	*Leucocalyx*		T	Rungwe	T7	9°09' S, 33°49' E	1981	24/5/1954
10363	*V. calvoana*	*Leucocalyx*		T	Iringa	T7	7°47' S, 36°29' E	1480	6/9/2004
6714	*V. calvoana*	*Leucocalyx*		T	Iringa	T7	7°47' S, 36°32' E	1440	27/9/2000
12833	*V. calvoana*	*Leucocalyx*		T	Rungwe	T7	9°32' S, 33°74' E	915	19/11/2008
16313	*V. calvoana*	Leucocalyx	In forest	T	Mbeya	T7	8.5S 33.02E	2100	16/6/1992
4294	*V. calvoana*	*Oehleri*		T	Masai	T2	3°10' S, 35°35' E	2286	23/9/1932
15	*V. calvoana*	*oehleri*		T	Masai	T2	2°56' S, 35°49' E	2130	8/9/1972
9	*V. calvoana*	*oehleri*	Forest edge	T	Lushoto/Tanga	T3	4.5S 38.03E		23/7/1996
10394	*V. calvoana*	*oehleri*		T	Arusha	T2	3°14' S, 36°45' E		2/7/1961
253	*V. calvoana*	*oehleri*		T	Arusha	T2	3°20' S, 36°45' E	1829	6/1927
3803	*V. calvoana*	*oehleri*	Riverine	T	Moshi	T2	3.19S 37.15E		3/12/2003
139	*V. calvoana*	*oehleri*		T	Masai	T2	3°13' S, 35°29' E	2378	21/6/1965
764	*V. calvoana*	*oehleri*		T	Arusha	T2	3°14' S, 36°45' E		8/1956
6238	*V. calvoana*	*oehleri*		T	Masai	T2	3°01' S, 35°40' E	0	17/6/1981
28961	*V. calvoana*	*oehleri*		T	Masai	T2	3.07S 35.24E	2615	14/3/1974
28964	*V. calvoana*	*oehleri*		T	Arusha		3°14' S, 36°45' E	2591	14/3/1974
3684	*V. calvoana*	*oehleri*		T	Masai	T2	3°10' S, 35°35' E	0	14/7/1963
1561	*V. calvoana*	*oehleri*		T	Masai	T2	3°10' S, 35°35' E	0	6/8/1956
5989	*V. calvoana*	*oehleri*		T	Arusha		3°14' S, 36°45' E	2469	17/10/1968
43268	*V. calvoana*	*oehleri*		T					24/7/1926
46	*V. calvoana*	*ruwenzoriensis*		U	Toro	U2	0.05N 45.30E		8/1931

Specimen No.	Species	Subsp.	Habitat	Country	Locality	FTEA Region	Latitude	Altitude	Collection Date
21	*V. calvoana*	*ruwenzoriensis*		U	Toro	U2	0.05N 45.30E		5/2/1916
1164	*V. calvoana*	*ruwenzoriensis*		U	Toro	U2	0.55N 35.25E	2500	9/9/1951
3681	*V. calvoana*	*ruwenzoriensis*	Submontane rainforest	U	Toro	U2	0.05N 45.30E	3200	1/1950
5185	*V. calvoana*	*ulugurensis*	Cultivated area	T	Morogoro	T6	6.49S 37.49E	1500	27/9/1970
2477	*V. calvoana*	*ulugurensis*		T	Morogoro	T6	6°55' S 37°42' E	1200	29/9/1971
88065	*V. calvoana*	*ulugurensis*	Montane forest	T	Morogoro	T6	6.55S 37.42E	2100	8/5/1988
1077	*V. calvoana*	*ulugurensis*		T	Morogoro	T6	6°55' S, 37°42' E	2200	20/9/1970
68	*V. calvoana*	*usambarensis*		T	Lushoto	T3	4°40' S, 38°15' E	1829	29/9/1955
149	*V. calvoana*	*usambarensis*		T	Lushoto	T3	4.4S 38.13E	1538	7/1924
4107	*V. calvoana*	*usambarensis*		T	Lushoto	T3	4°47' S, 38°17' E	0	1/9/1966
*21	*V. hymenolepis*		Valley	K	Narok	K6	0.49S 36.06E	3040	11/11/1984
*20	*V. hymenolepis*		Forest edge	K	Narok	K6	0.49S 36.06E	3053	2/5/2013
*23	*V. hymenolepis*		Valley	K	Narok	K6	0.49S 36.06E	2896	2/5/2013
*24	*V. hymenolepis*		Roadside valley	K	Narok	K6	0.49S 36.05E	2896	2/5/2013
*25	*V. hymenolepis*		Roadside valley	K	Narok	K6	0.49S 36.05E	2753	2/5/2013
*3	*V. hymenolepis*		Roadside	K	Trans Nzoia	K3	0.06N 35.05E	1821	5/1/2013
*3	*V. hymenolepis*		Roadside	K	Trans Nzoia	K3	0.06N 35.05E	1821	5/1/2013
*5	*V. hymenolepis*		Roadside	K	Trans Nzoia	K3	1.00N 34.04E	2131	5/1/2013
*4	*V. hymenolepis*		Roadside	K	Trans Nzoia	K3	1.01N 35.54E	1887	5/1/2013
*6	*V. hymenolepis*		Roadside	K		K5	0.42N 35.85E	1648	6/1/2013
*5	*V. hymenolepis*		Roadside	K	Trans Nzoia	K3	1.01N 35.54E	2131	6/1/2013
*7	*V. hymenolepis*		Forest edge	K	Kakamega	K5	0.17N 34.52E	1613	6/1/2013
*8	*V. hymenolepis*		Roadside	K	Kakamega	K5	0.17N 34.52E	1637	6/1/2013
*9	*V. hymenolepis*		Roadside	K	Nandi	K5	0.17N 34.34E	1721	6/1/2013
*10	*V. hymenolepis*		Roadside	K	Kakamega	K5	0.14N 34.88E	1668	6/1/2013
*12	*V. hymenolepis*		Riverine	K	Kakamega	K5	0.01N 34.05E	1432	7/1/2013
*15	*V. hymenolepis*		Roadside	K	Kakamega	K5	0.04S 34.72E	1565	7/1/2013
*1	*V. hymenolepis*		Roadside	K	Bungoma	K5	0.72N 34.75E	1679	5/1/2013
*2	*V. hymenolepis*		Roadside	K	Trans Nzoia	K3	1.02N 35.00E		5/1/2013
10490	*V. hymenolepis*		Forest edges	K	West Pokot	K2	1.04N 35.23E	2615	4/8/1968
3002	*V. hymenolepis*		Riverine forest remnants	K	Trans Nzoia	K3	1.02N 35.00E	1850	24/8/1986
20	*V. hymenolepis*			K	Samburu	K1	2.01N 36.05E	2400	21/1/1977
125	*V. hymenolepis*			K	Elgeyo Marakwet	K3	1.13N 35.17E		3/8/1969
4632	*V. hymenolepis*			K	Trans Nzoia	K3	0.55N 35.05E		25/1/1967
4638	*V. hymenolepis*			K	Trans Nzoia	K3	1.10N 34.45E		27/1/1967

Specimen No.	Species	Subsp.	Habitat	Country	Locality	FTEA Region	Latitude	Altitude	Collection Date
61	*V. hymenolepis*			K	Kisii	K3	0.41S 34.46E		30/10/1974
662	*V. hymenolepis*		Grassland	K	Trans Nzoia	K3	1.02N 35.00E	1900	23/3/1986
19	*V. hymenolepis*		Roadside	K	Trans Nzoia	K3	1.02N 35.00E		2/11/1963
7008	*V. hymenolepis*		Roadside	K	Trans Nzoia	K3	1.02N 35.00E	1860	31/1/1982
6867	*V. hymenolepis*		High forest	K	Kakamega	K5	0.14S 34.05E	1600	24/1/1981
6807	*V. hymenolepis*		Scrape by roadside	K	Nakuru	K3	0.01S 35.35E	2730	14/10/1981
6576	*V. hymenolepis*		Roadside	K	Trans Nzoia	K3	0.53N 34.54E	1750	10/10/1981
15023	*V. hymenolepis*		Forest	K	Narok	K3	1.00S 36.09E	2462	8/7/1972
121	*V. hymenolepis*			K	Kakamega	K5	0.17N 34.52E	1750	11/11/1984
3022	*V. hymenolepis*		In scrub	K	Kakamega	K5	0.17N 34.52E	1846	
365	*V. hymenolepis*			K	Trans Nzoia	K3	0.53N 35.07E	615	22/9/1957
2041	*V. hymenolepis*			K	Samburu	K1	2.1N 36.05E	2462	27/7/1960
3166	*V. hymenolepis*		Disturbed riverine forest	K	Kericho	K3	0.46S 35.26E	1944	1/2/2013
3167	*V. hymenolepis*		Disturbed riverine forest	K	Kericho	K3	0.05S 35.03E	2010	4/2/2013
2564	*V. hymenolepis*		In scrub	K	West Pokot	K3	1.15N 35.27E		31/3/1971
23	*V. hymenolepis*		Lake side	K	Uasin Gishu	K3	0.04N 35.31E	2738	9/2/1971
57/84	*V. hymenolepis*		Riverine forest	K	Trans Nzoia	K3	1.02N 35.00E	1800	11/9/1984
26	*V. hymenolepis*		Open grassland	K	Trans Nzoia	K3	1.05N 35.07E		9/12/1993
20	*V. hymenolepis*			K	Trans Nzoia	K3	1.02N 35.00E		28/2/1984
189	*V. hymenolepis*		Streep roadside	K	Elgeyo Marakwet	K3	0.49N 35.36E	1980	4/11/2000
274	*V. hymenolepis*			K	Elgeyo Marakwet	K3	1.02N 35.19E	1938	2/1958
258	*V. hymenolepis*		Valley land near river	K	Elgeyo Marakwet	K3	1.02N 35.19E	1938	10/1957
1706	*V. hymenolepis*		Forest edge	K	Kakamega	K5	0.17N 34.52E		10/12/1956
1447	*V. hymenolepis*		Mixed forest	T	Mpwapwa/Kilosa	T6	6.25S 36.05E	1900	11/8/1972
87	*V. hymenolepis*		Disturbed ground.	U	Mbale	U3	1.08S 34.33E	2154	12/1939
26	*V. hymenolepis*			K	Trans Nzoia	K3	1.05N 35.07E		9/12/1993
7058	*V. hymenolepis*			U	Toro	U2	0.05N 45.30E		11/1968
364	*V. hymenolepis*		Roadside	U				2000	29/8/1932
803	*V. hymenolepis*		Forest edges	U	Karamoja	U1	2.32N 34.46E	2462	3/1960
3507	*V. hymenolepis*			K/U	Mbale/Trans Nzoia	K3	1.08N 34.33E	2154	
73	*V. hymenolepis*			K/U	Mbale/Trans Nzoia	K3	1.08N 34.33E		12/1942
16	*V. hymenolepis*		Cultivated field edges	K	Bungoma	K5		3500	30/5/2013
17	*V. hymenolepis*		Roadsode	K	Uasin Gishu	K3	0.31N 35.16E	2700	31/4/2013
5462	*V. tolypophora*		Hillside	K	Samburu	K1	1.09N 36.41E	1760	2/6/1979
3497	*V. tolypophora*		In maize field.	T	Mbeya	T7	8.05S 33.02E	1700	25/11/1989

Specimen No.	Species	Subsp.	Habitat	Country	Locality	FTEA Region	Latitude	Altitude	Collection Date
16298	*V. tolypophora*			T	Rungwe	T7	9.09S 33.04E	1560	12/6/1992
1210	*V. tolypophora*			T	Ufipa	T4	8.00S 31.30E	1846	16/6/1970
4056	*V. tolypophora*			M	Unknown			0	29/5/ 1948
5	*V. tolypophora*			T	Moshi	T2	3°04' S, 37°22' E	0	4/1937
39	*V. tolypophora*			T	Mbeya	T7	8°50' S, 33°18' E	2439	29/5/1958
4152	*V. tolypophora*			T	Mbeya	T7	8°50' S, 33°20' E	2439	31/8/1962
3292	*V. tolypophora*			T	Mbeya	T7	8°50' S, 33°18' E	0	30/11/1961
4370	*V. tolypophora*			T	Mbeya	T7	9°15' S, 33°22' E	2073	17/6/1977
1224	*V. tolypophora*			T	Mbeya/Chunya	T7	8°50' S, 33°00' E	0	30/7/1972
1915	*V. tolypophora*			T	Mbeya	T7	9°28' S, 33°54' E	518	29/7/1974
62	*V. tolypophora*			T	Iringa	T7	8°35' S, 35°15' E	0	7/10/1936
10789	*V. tolypophora*			T	Njombe	T7	9°19' S, 34°46' E	0	8/7/1956
10252	*V. tolypophora*			T	Songea	T8	11°01' S, 34°56' E	1620	19/5/1956
535	*V. tolypophora*			T	Iringa	T7	8°35' S, 35°15' E	1829	22/7/1969
11020	*V. tolypophora*			T	Iringa	T7	8°35' S, 35°15' E	0	11/8/1971
11483	*V. tolypophora*			T	Iringa	T7	8°35' S, 35°15' E	0	18/9/1971
11664	*V. tolypophora*			T	Rungwe	T7	9°15' S, 33°39' E	0	2/10/1971
13261	*V. tolypophora*			T	Iringa	T7	7°36' S, 36°00' E	0	7/1965
1291	*V. tolypophora*			T	Mbeya	T7		0	10/9/1954
1262	*V. tolypophora*			T	Mbeya	T7	8°50' S, 33°20' E	0	9/9/1954
411	*V. tolypophora*			T	Rungwe	T7	9°24' S, 33°22' E	2500	11/1972
673	*V. tolypophora*			T	Mbeya	T7	8°59' S, 33°38' E	0	1932
18	*V. tolypophora*			T	Iringa	T7	7°47' S, 35°11' E	0	10/1936

Appendix II. Data matrix used in morphometric analyses

OUT	LLB	WL	PL	LPD	LS	SI	LI	WI	LI/WI	LPA	WPA	LPA/WPA	LC	LP	LA	WA1	WA2	LA/WA1	LA/WA2	AP	LAN	LSTA	LASP	CD	LC/CD	LSTY	CTFA
C	128.0	35.7	15.0	15.9	2.3	4.0	17.4	15.9	1.1	9.6	4.4	2.2	15.1	10.0	4.2	1.1	1.1	3.8	3.8	0.0	4.5	6.0	0.8	3.8	4.0	21.1	12.5
C	131.0	50.3	11.0	36.3	2.3	2.8	17.5	16.1	1.1	10.8	5.7	1.9	17.9	11.0	3.4	0.8	0.7	4.3	5.0	1.0	4.7	6.8	0.8	4.9	3.6	21.9	14.3
C	83.0	21.0	5.0	31.0	1.5	4.2	24.5	16.2	1.5	14.2	6.6	2.1	18.9	11.6	4.5	1.5	1.4	3.0	3.2	1.0	4.5	6.8	0.6	4.5	4.2	24.9	13.0
C	150.3	46.7	39.7	29.7	1.5	4.1	30.3	16.3	1.9	22.9	3.4	6.7	16.6	10.6	3.6	0.8	0.5	4.5	7.9	1.0	4.5	5.3	0.8	3.0	5.5	17.4	11.3
C	133.0	49.5	25.0	24.0	2.3	3.2	33.0	16.6	2.0	22.3	4.4	5.1	19.9	12.5	3.8	0.8	0.8	4.8	4.8	1.0	4.5	6.0	0.8	3.8	5.2	27.9	16.6
C	110.5	42.5	24.5	22.8	1.5	3.2	36.2	22.8	1.6	28.1	6.2	4.5	21.9	11.6	5.7	1.2	1.1	5.0	5.2	1.0	4.5	6.0	0.8	3.8	5.8	22.7	15.9
C	137.0	45.0	36.0	17.3	0.8	3.8	30.3	14.7	2.1	20.9	4.3	4.9	18.5	10.7	3.4	0.8	0.6	4.3	5.3	1.0	3.9	4.5	0.6	3.8	4.9	21.9	12.1
C	111.0	33.3	18.7	16.0	3.8	3.9	19.0	16.0	1.2	14.9	4.5	3.3	15.1	11.9	2.5	0.8	0.5	3.1	5.0	1.0	2.3	5.3	0.6	3.4	4.4	19.3	11.3
C	134.3	55.0	22.0	10.0	2.3	2.8	19.8	15.3	1.3	13.0	7.3	1.8	16.1	10.3	2.0	0.8	0.5	2.5	4.4	1.0	4.5	8.3	0.6	3.8	4.2	20.4	11.3
C	121.0	33.0	30.0	10.0	1.5	4.6	17.4	13.8	1.3	15.1	4.2	3.6	13.1	11.2	2.3	0.8	0.6	2.9	4.2	1.0	4.5	6.0	0.8	3.8	3.4	15.9	9.1
C	144.0	56.0	31.0	11.5	2.3	3.1	25.7	16.5	1.6	18.1	8.7	2.1	14.8	9.8	2.3	0.8	0.8	2.9	2.9	1.0	4.5	6.0	0.7	3.8	3.9	18.1	10.6
C	122.7	44.3	11.7	14.7	1.5	3.0	14.0	13.5	1.0	9.6	2.8	3.5	12.2	10.2	2.5	0.8	0.8	3.1	3.1	1.0	3.8	6.0	1.1	3.4	3.6	17.4	9.8
C	130.7	28.0	25.0	28.0	1.5	5.6	20.2	17.0	1.2	15.9	7.7	2.0	17.4	11.9	3.0	0.9	0.9	3.5	3.5	1.0	3.8	6.0	0.6	4.5	3.9	23.4	12.8
C	71.0	23.5	11.5	15.8	1.5	3.5	18.6	15.4	1.2	12.7	4.7	2.7	13.6	9.8	1.5	0.8	0.6	1.9	2.7	1.0	4.5	6.0	0.6	3.0	4.5	15.5	9.8
C	111.0	34.7	13.7	10.0	2.3	3.6	19.4	16.6	1.2	11.6	6.6	1.8	13.8	9.8	3.0	1.0	1.0	3.2	3.2	1.0	3.8	6.0	0.6	3.8	3.6	17.4	9.4
C	75.0	24.3	11.0	19.3	1.5	3.5	17.0	14.0	1.2	10.6	3.3	3.2	14.3	9.8	3.0	1.1	1.1	2.7	2.9	1.0	4.5	6.0	0.8	3.0	4.8	15.9	12.5
C	110.0	34.0	20.0	15.3	2.3	3.8	21.8	17.4	1.3	13.2	8.3	1.6	16.6	11.3	3.5	1.2	1.2	2.9	2.9	1.0	4.5	7.6	0.8	3.8	4.4	18.1	12.8
C	129.3	44.3	22.0	13.5	2.3	3.4	16.0	12.0	1.3	9.1	3.5	2.6	18.1	9.8	2.3	0.8	0.5	2.9	4.6	1.0	4.5	6.0	0.6	4.5	4.0	18.9	12.1
C	121.0	51.0	21.5	16.4	2.3	2.8	17.2	15.5	1.1	11.0	5.7	1.9	16.1	9.5	3.4	1.5	1.1	2.3	3.1	1.0	4.5	6.0	0.8	3.8	4.2	19.6	10.6
C	151.0	49.0	8.0	43.0	0.8	3.2	26.3	18.0	1.5	14.1	6.6	2.1	16.9	12.1	4.7	1.5	1.5	3.2	3.2	0.0	4.5	6.0	0.8	3.0	5.6	20.4	12.1
C	165.7	43.3	15.3	13.8	1.5	4.2	11.3	9.2	1.2	6.0	1.5	4.0	13.6	10.6	3.8	0.8	0.8	4.8	4.8	1.0	3.0	4.5	0.6	4.5	3.0	13.6	6.8
C	142.5	42.5	16.0	11.0	2.3	3.7	18.5	10.5	1.8	11.6	3.8	3.0	14.3	10.0	4.0	0.8	0.7	5.0	5.7	1.0	3.8	6.0	0.8	3.0	4.8	15.9	10.6
C	132.3	42.0	30.0	4.6	0.8	3.9	29.0	14.6	2.0	17.3	6.3	2.7	16.6	11.2	2.3	0.8	0.8	2.9	2.9	1.0	4.2	5.7	0.6	3.8	4.4	21.1	13.6
C	107.7	27.3	26.0	1.5	0.8	4.9	24.0	18.5	1.3	17.0	4.4	3.9	16.6	8.8	2.4	0.8	0.6	3.0	4.0	1.0	5.3	6.8	0.8	3.0	5.5	16.6	10.6
C	131.7	51.7	30.0	8.3	0.8	3.1	34.6	18.7	1.9	18.4	7.8	2.3	23.1	14.8	5.4	1.4	1.1	3.9	5.1	1.0	5.7	6.8	1.1	4.9	4.7	27.9	17.4
C	146.5	53.5	24.0	12.7	1.5	3.2	28.0	13.5	2.1	15.9	5.0	3.2	18.1	12.7	4.3	0.8	0.8	5.4	5.4	1.0	4.5	6.8	0.6	3.0	6.0	23.4	12.4
C	179.7	55.3	50.0	12.4	0.8	4.2	20.6	13.8	1.5	19.3	5.0	3.8	18.1	12.4	4.0	0.8	0.7	4.9	6.1	1.0	5.3	6.8	0.8	3.8	4.8	26.4	14.3
C	177.0	58.3	40.0	10.6	2.3	3.7	14.5	11.3	1.3	10.1	6.1	1.7	14.9	11.2	4.5	1.2	1.2	3.8	3.8	1.0	4.5	6.0	0.8	3.8	3.9	18.9	11.3
C	89.0	20.3	11.0	4.0	0.8	4.9	14.7	11.1	1.3	11.0	4.5	2.4	12.8	11.0	1.5	0.8	0.3	1.9	5.0	1.0	4.2	5.3	0.6	2.6	4.9	12.1	7.6
C	84.0	17.5	11.0	13.6	0.8	5.4	21.7	15.9	1.4	12.1	3.2	3.7	20.4	12.4	3.5	1.2	1.2	3.1	3.1	1.0	4.5	6.8	0.8	4.5	4.5	27.9	16.6
C	196.7	36.3	13.7	26.6	0.8	5.8	20.6	15.6	1.3	16.1	4.7	3.4	18.0	12.8	3.3	0.8	0.7	4.1	5.0	1.0	4.5	7.6	0.8	5.3	3.4	25.7	13.0
C	153.3	33.7	16.3	32.7	1.5	5.0	23.4	22.3	1.1	15.0	4.6	3.2	21.1	14.3	4.9	1.3	1.2	3.8	4.3	1.0	4.5	6.0	0.8	3.8	5.6	22.7	15.9
C	113.0	56.0	35.0	15.3	0.8	2.6	16.0	9.7	1.7	10.8	5.5	2.0	14.7	10.4	3.0	0.8	0.5	3.8	6.7	1.0	4.5	6.0	0.8	4.5	3.3	21.1	10.9
C	101.7	31.0	15.7	18.0	1.5	3.8	14.5	11.6	1.3	9.5	5.3	1.8	14.3	10.6	3.1	1.4	0.9	2.3	3.5	1.0	3.8	6.0	0.8	3.8	3.8	18.1	9.8
C	110.7	47.7	37.7	19.7	1.5	3.1	15.7	10.9	1.4	10.8	6.0	1.8	15.5	10.7	2.7	0.8	0.6	3.4	4.9	1.0	4.2	5.3	0.6	3.4	4.5	18.9	11.3
C	115.5	28.0	10.5	25.8	0.8	4.5	19.4	15.3	1.3	12.1	4.9	2.5	15.1	11.9	4.8	1.6	1.6	3.0	3.1	1.0	4.2	4.9	0.8	3.8	4.0	18.1	10.6
C	149.0	47.7	13.0	51.0	1.5	3.4	25.7	18.1	1.4	16.4	6.8	2.4	21.1	18.9	5.8	1.3	1.3	4.4	4.6	1.0	4.5	7.2	0.8	3.8	5.6	25.7	16.6

C	190.5	50.0	13.5	62.2	1.5	4.1	26.1	16.1	1.6	16.8	5.8	2.9	15.9	14.5	5.9	1.4	1.4	4.3	4.3	1.0	4.5	7.6	0.8	3.8	4.2	24.2	11.3
OUT	LLB	WL	PL	LPD	LS	SI	LI	WI	LI/WI	LPA	WPA	LPA/WPA	LC	LP	LA	WA1	WA2	LA/WA1	LA/WA2	AP	LAN	LSTA	LASP	CD	LC/CD	LSTY	CTFA
C	155.3	52.3	31.0	17.3	2.3	3.6	17.0	13.8	1.2	13.4	5.8	2.3	21.4	15.7	3.0	0.8	0.5	3.8	6.0	1.0	4.5	6.8	0.8	5.3	4.0	24.9	16.6
C	146.3	52.3	22.0	22.0	1.5	3.2	25.0	24.7	1.0	9.1	5.5	1.6	20.4	13.4	4.3	1.5	1.4	2.8	3.0	1.0	4.5	6.0	0.8	3.8	5.4	27.9	13.6
H	107.3	23.8	10.3	15.6	0.8	4.9	12.0	12.0	1.0	8.0	1.0	8.0	12.0	9.0	2.5	1.1	0.8	2.3	3.1	0.0	4.5	6.0	0.8	3.0	4.0	15.9	9.0
H	136.3	35.3	19.3	16.3	0.8	4.4	15.0	15.0	1.0	10.0	2.6	3.8	12.0	8.2	3.0	1.1	1.0	2.7	3.2	0.0	3.4	5.7	0.8	3.0	4.0	17.4	9.1
H	86.7	29.3	10.7	13.0	0.8	3.3	15.5	15.3	1.0	7.0	1.0	7.0	11.8	9.0	3.0	1.5	0.8	2.0	3.8	0.0	3.8	5.3	0.8	3.0	3.9	15.1	9.8
H	121.0	50.3	41.7	10.8	0.8	3.2	17.0	12.0	1.4	10.5	9.8	1.1	12.0	9.0	3.0	1.5	1.0	2.0	3.0	0.0	3.8	6.0	0.8	3.0	4.0	14.3	8.3
H	75.3	15.3	6.7	16.3	0.8	5.3	14.7	13.4	1.1	7.0	1.0	7.0	11.0	8.6	3.4	1.5	1.0	2.3	3.4	1.0	3.8	5.3	0.6	3.0	3.7	17.4	9.1
H	90.3	32.0	10.3	4.2	0.8	3.1	14.5	11.5	1.3	5.1	1.0	4.9	11.1	6.8	2.6	1.5	0.9	1.7	3.1	0.0	3.8	6.0	0.6	3.0	3.7	16.6	8.3
H	76.3	28.3	11.7	77.5	0.8	3.1	16.3	15.0	1.1	9.8	5.3	1.9	12.8	9.5	2.3	0.8	0.6	2.9	3.8	0.0	3.8	6.0	0.8	3.0	4.3	15.9	9.8
H	79.0	16.0	13.5	13.5	0.8	5.8	19.8	19.5	1.0	12.3	7.2	1.7	17.4	11.1	3.0	1.5	1.4	2.0	2.1	0.0	4.5	6.8	0.8	3.8	4.6	21.1	12.1
H	88.7	33.3	13.0	5.3	0.8	3.1	12.0	12.0	1.0	8.6	1.5	5.7	11.8	8.6	2.3	0.6	0.6	3.8	4.2	0.0	3.4	4.5	0.6	3.0	3.9	14.3	9.1
H	127.5	39.0	34.0	19.0	0.8	4.1	16.5	14.8	1.1	7.6	3.2	2.4	13.6	8.1	3.7	1.0	0.9	3.8	4.1	0.0	3.8	5.3	0.8	3.0	4.5	15.1	9.8
H	106.7	38.0	10.0	8.0	2.6	3.1	20.0	12.3	1.6	15.9	2.5	6.3	15.1	8.3	1.8	0.6	0.5	2.9	3.5	0.0	4.5	6.8	0.8	3.8	4.0	20.4	11.3
H	68.0	20.3	7.7	10.0	0.8	3.7	17.5	15.0	1.2	15.1	3.8	4.0	15.8	9.1	1.5	0.8	0.6	1.9	2.5	1.0	5.3	6.8	1.1	4.9	3.2	25.7	15.1
H	135.7	28.0	9.7	9.0	0.8	5.2	19.0	15.0	1.3	10.0	4.5	2.3	16.6	10.4	2.8	1.3	1.0	2.2	2.8	0.0	4.5	6.8	0.8	3.8	4.4	20.4	11.3
H	145.0	33.5	10.0	12.3	1.9	4.6	19.3	16.5	1.2	13.3	1.8	7.5	16.3	9.8	1.1	0.4	0.4	2.8	2.8	0.0	4.9	6.0	0.8	4.5	3.6	20.4	11.3
H	109.7	26.0	8.3	8.1	0.8	4.5	18.0	15.4	1.2	11.6	4.2	2.8	13.8	7.9	1.8	0.8	0.7	2.3	2.8	0.0	3.8	5.3	1.0	3.8	3.6	17.4	9.8
H	70.5	18.0	9.0	10.0	0.8	4.4	15.2	14.0	1.1	11.5	4.3	2.7	14.3	8.0	3.0	1.1	1.1	2.7	2.8	0.0	3.8	5.3	0.6	3.8	3.8	19.6	10.6
H	122.3	36.0	22.0	15.6	0.8	4.0	19.6	15.6	1.3	12.1	4.7	2.6	14.7	10.7	2.5	0.7	0.5	3.8	4.9	0.0	4.2	5.3	0.8	3.8	3.9	19.6	11.3
H	85.7	24.3	6.3	9.8	0.8	3.8	15.4	15.0	1.0	9.8	3.0	3.3	16.4	9.1	4.5	1.5	0.8	3.0	5.6	1.0	4.8	6.8	0.8	2.6	6.3	24.2	13.6
H	103.3	28.3	15.0	14.8	2.3	4.2	16.3	12.0	1.4	10.6	3.2	3.3	14.1	9.1	3.0	0.9	0.8	3.3	3.8	0.0	3.8	5.3	0.6	3.8	3.7	18.1	10.6
H	60.7	21.0	4.3	9.0	0.9	3.1	14.2	14.0	1.0	8.3	3.8	2.2	11.2	7.0	2.3	1.1	1.1	2.1	2.1	0.0	3.8	5.3	0.6	3.0	3.7	17.4	8.3
H	75.3	25.3	26.7	6.8	0.8	4.0	17.8	14.7	1.2	10.8	4.6	2.3	13.1	9.4	3.9	1.1	1.1	3.6	3.6	1.0	3.8	5.3	0.8	3.0	4.4	15.9	7.6
H	69.0	15.0	8.7	11.0	0.8	5.2	16.5	15.0	1.1	9.7	3.5	2.7	13.0	8.2	1.5	0.8	0.5	1.9	3.0	0.0	3.8	4.5	0.6	3.8	3.4	17.4	9.1
H	110.0	22.0	11.0	16.2	0.8	5.5	18.0	14.6	1.2	10.7	4.0	2.7	12.8	9.7	3.8	1.1	1.1	3.5	3.5	0.0	3.8	6.0	0.8	3.8	3.4	17.4	9.8
H	65.5	14.0	10.0	8.0	0.8	5.4	13.0	13.0	1.0	8.0	1.0	8.0	12.3	8.5	1.5	0.8	0.5	1.9	3.0	0.0	3.4	5.3	0.7	3.0	4.1	14.3	8.3
H	67.5	20.5	7.5	3.0	0.8	3.7	15.3	14.0	1.1	12.3	3.8	3.2	13.8	8.8	1.9	0.6	0.5	3.2	4.2	0.0	3.8	5.3	0.8	3.8	3.6	19.6	10.6
H	93.0	24.7	14.3	8.8	0.8	4.4	13.0	10.8	1.2	7.7	1.3	6.1	11.3	7.6	2.5	1.1	0.6	2.3	4.2	0.0	3.0	4.5	0.6	3.0	3.8	12.8	6.8
H	90.7	34.0	11.7	13.5	0.8	3.0	15.3	12.0	1.3	10.5	5.7	1.8	13.2	8.9	1.8	0.8	0.5	2.2	3.5	1.0	4.5	6.0	0.8	3.0	4.4	14.3	9.1
H	75.3	24.7	13.7	14.0	1.5	3.6	14.4	11.3	1.3	10.4	3.3	3.2	13.2	8.2	3.1	1.1	1.1	2.8	2.9	1.0	3.8	4.5	0.6	3.0	4.4	17.4	9.8
H	107.3	23.3	26.7	17.5	1.5	5.7	17.6	10.9	1.6	10.6	3.5	3.0	12.3	7.7	2.9	1.1	1.1	2.6	2.6	1.0	3.4	6.0	0.6	3.0	4.1	14.3	9.1
H	135.7	39.0	25.0	12.0	1.5	4.1	18.9	11.3	1.7	10.8	3.2	3.4	10.7	8.6	2.9	1.0	1.0	2.9	2.9	1.0	4.5	5.3	0.8	3.0	3.6	17.4	9.1
H	60.7	16.0	7.7	15.0	0.8	4.3	19.8	15.5	1.3	9.5	2.5	3.9	12.7	8.5	3.4	1.2	1.1	2.8	3.1	1.0	4.2	5.3	0.8	3.8	3.3	15.9	8.3
H	88.0	23.3	9.0	7.0	0.8	4.2	16.9	10.8	1.6	14.7	3.4	4.3	15.7	9.5	3.9	1.0	1.0	3.9	4.1	1.0	4.2	5.3	0.8	3.0	5.2	18.1	10.6
H	98.3	30.0	12.7	8.8	0.8	3.7	13.2	9.8	1.3	7.7	1.8	4.4	13.3	7.6	3.0	1.0	0.8	3.0	3.8	0.0	3.4	5.3	0.6	3.0	4.4	15.9	9.1
H	128.7	52.0	18.7	6.4	0.8	2.8	11.7	8.0	1.5	6.2	2.9	2.1	12.1	6.6	2.5	1.0	1.0	2.5	2.5	0.0	3.4	4.5	0.6	3.8	3.2	18.1	9.1
H	142.7	35.3	17.7	14.8	0.8	4.5	14.7	9.8	1.5	9.2	1.9	4.8	11.8	7.6	3.3	1.0	1.0	3.5	3.5	0.0	3.4	5.3	0.6	3.0	3.9	15.1	7.6
H	128.0	38.3	15.0	9.5	0.8	3.7	12.5	9.4	1.3	8.0	1.1	7.6	13.4	8.3	2.3	1.0	0.9	2.4	2.6	0.0	3.8	6.0	0.6	3.0	4.5	17.4	9.8
H	142.0	33.7	13.7	13.8	1.2	4.6	13.2	10.2	1.3	7.0	2.1	3.3	14.0	8.8	3.0	1.1	0.9	2.9	3.3	1.1	4.2	5.3	0.6	3.4	4.1	19.6	8.3

H	78.3	28.3	12.7	8.0	0.8	3.2	12.7	11.0	1.1	8.3	1.7	4.9	12.1	7.4	2.5	1.1	1.1	2.3	2.3	0.0	3.8	4.5	0.6	3.0	4.0	15.9	9.1

OUT	LLB	WL	PL	LPD	LS	SI	LI	WI	LI/WI	LPA	WPA	LPA/WPA	LC	LP	LA	WA1	WA2	LA/WA1	LA/WA2	AP	LAN	LSTA	LASP	CD	LC/CD	LSTY	CTFA
H	170.3	47.3	29.0	8.3	1.1	4.2	14.1	9.5	1.5	8.3	2.3	3.6	11.8	8.2	1.5	0.8	0.5	1.9	3.0	1.0	3.8	4.5	0.8	3.0	3.9	12.8	6.8
H	94.3	28.0	22.0	10.0	1.5	4.2	15.0	12.3	1.2	5.6	2.5	2.2	11.8	10.6	4.3	1.3	1.0	3.3	4.3	1.0	3.8	6.0	0.8	3.0	3.9	15.9	9.1
H	66.5	15.0	13.5	14.0	0.8	5.3	14.0	13.3	1.1	8.0	2.6	3.1	13.3	7.4	3.5	0.8	1.3	4.4	2.7	1.0	3.8	4.5	0.6	3.0	4.4	15.9	8.3
H	135.0	32.0	20.0	10.0	1.1	4.8	24.0	16.0	1.5	17.4	5.2	3.3	16.9	8.5	3.8	0.9	0.7	4.2	5.4	0.0	3.4	5.3	0.6	3.8	4.4	16.6	10.6
H	96.5	30.0	10.5	9.5	1.5	3.6	15.8	13.4	1.2	10.0	1.7	5.7	12.8	9.1	3.5	1.0	0.9	3.4	3.9	0.0	3.8	4.5	0.8	3.8	3.4	17.4	9.8
H	67.3	20.0	10.0	20.7	1.5	3.9	14.8	14.0	1.1	9.8	3.9	2.5	15.1	9.8	3.0	1.4	1.1	2.1	2.8	0.0	3.0	3.8	0.7	3.4	4.4	14.3	8.3
H	98.0	24.7	23.0	15.3	1.5	4.9	16.6	13.3	1.2	11.6	3.5	3.3	14.7	8.6	3.1	0.9	0.8	3.5	3.9	1.0	4.5	6.0	0.8	4.5	3.3	19.3	9.8
H	70.3	20.0	11.7	9.1	0.8	4.1	13.3	10.1	1.3	8.6	1.6	5.2	13.4	7.8	3.0	1.0	1.0	2.9	2.9	1.0	3.8	5.3	0.8	4.5	3.0	18.5	8.3
H	51.3	10.3	6.7	7.2	0.8	5.7	11.3	9.6	1.2	5.3	1.0	5.3	14.7	8.6	1.7	0.6	0.6	2.7	2.8	0.0	4.2	5.7	0.8	4.2	3.5	17.4	9.1
H	72.7	14.3	15.0	6.8	1.5	6.1	11.6	9.1	1.3	6.0	1.2	5.1	11.3	8.8	3.0	0.9	0.8	3.5	3.9	1.0	3.8	4.5	0.8	3.8	3.0	13.6	6.8
T	33.3	17.3	4.0	4.0	1.5	2.2	11.0	7.3	1.5	5.6	1.3	4.4	12.1	7.3	3.0	0.8	0.5	3.8	6.7	0.0	3.8	4.5	0.6	3.0	4.0	14.7	8.3
T	46.7	19.3	4.7	3.3	0.8	2.7	14.3	10.8	1.3	7.5	2.2	3.4	12.6	8.5	2.8	1.1	0.9	2.5	3.1	0.0	3.8	5.7	0.8	3.4	3.7	15.1	9.1
T	42.7	19.7	4.3	3.8	0.8	2.4	14.0	12.0	1.2	9.7	2.0	4.9	14.3	8.3	1.9	0.8	0.6	2.4	3.5	0.0	3.8	5.3	0.8	3.0	4.8	15.5	8.3
T	39.3	17.0	1.7	3.8	1.1	2.4	11.3	11.0	1.0	5.7	2.0	2.8	12.9	7.2	1.9	0.8	0.8	2.5	2.6	1.0	3.4	5.3	0.8	3.0	4.3	15.1	8.3
T	52.7	27.3	7.0	9.6	0.8	2.2	14.5	12.0	1.2	11.1	1.4	7.9	11.7	8.1	3.3	1.1	1.0	3.0	3.3	1.0	3.8	4.5	0.6	2.6	4.5	13.6	6.8
T	89.0	38.0	6.3	2.3	1.1	2.5	15.3	12.3	1.2	9.6	1.4	6.9	12.3	8.7	2.3	0.8	0.6	2.9	4.2	0.0	4.5	5.3	0.6	3.0	4.1	15.9	9.1
T	41.7	19.3	3.0	3.8	1.5	2.3	10.0	10.0	1.0	7.4	1.3	5.7	13.3	7.7	2.7	0.8	0.3	3.3	8.8	1.0	3.8	5.3	0.6	4.5	3.0	16.8	9.8
T	50.3	22.0	4.3	3.0	1.5	2.5	16.8	10.0	1.7	9.1	2.1	4.3	9.8	7.9	3.0	1.1	1.0	2.8	3.0	1.0	3.4	4.5	0.6	3.0	3.3	13.6	6.8
T	47.7	23.7	5.0	4.0	0.8	2.2	15.3	10.0	1.5	8.2	1.9	4.3	13.1	7.9	1.9	0.8	0.8	2.4	2.4	0.0	3.8	4.5	0.6	3.8	3.4	19.6	9.1
T	54.0	25.0	4.0	3.8	0.8	2.3	11.7	11.0	1.1	6.2	1.5	4.1	14.0	7.9	3.4	1.5	1.0	2.3	3.4	1.0	3.9	5.7	0.6	3.8	3.7	19.3	10.0
T	71.7	46.7	6.0	2.3	1.1	1.7	11.8	9.4	1.3	7.6	2.1	3.6	14.2	8.3	2.2	0.8	0.5	2.7	4.3	0.0	4.5	5.3	0.7	3.8	3.7	20.4	9.1
T	58.0	29.0	6.0	3.8	0.8	2.2	12.1	11.2	1.1	3.2	1.1	2.9	11.3	8.3	3.1	1.0	0.5	3.1	6.9	1.0	3.4	4.7	0.6	3.0	3.8	15.1	9.4
T	63.3	23.3	7.0	4.3	1.1	3.0	11.6	9.8	1.2	6.3	1.6	4.0	12.5	6.8	3.0	1.5	1.4	2.0	2.2	1.0	3.0	3.8	0.6	2.6	4.8	11.3	7.6
T	74.3	31.3	11.0	2.4	1.5	2.7	14.5	8.5	1.7	9.3	2.0	4.8	14.4	12.3	2.0	0.8	0.8	2.5	2.7	1.0	3.8	6.8	0.6	3.8	3.8	19.6	11.3
T	70.0	22.7	8.0	1.9	0.8	3.4	14.2	9.3	1.5	6.8	1.5	4.5	12.3	8.3	2.6	0.8	0.8	3.1	3.3	0.0	3.8	4.5	0.6	3.4	3.6	15.1	9.1
T	100.3	58.0	12.3	2.3	2.3	1.9	13.6	9.6	1.4	8.2	2.2	3.7	13.4	7.6	1.9	0.8	0.7	2.4	2.9	0.0	3.9	5.3	0.6	4.5	3.0	18.1	9.8
T	94.0	45.3	8.0	2.3	1.5	2.3	12.3	9.1	1.4	8.2	1.5	5.5	12.3	7.6	2.2	0.9	0.9	2.5	2.5	0.0	3.8	5.3	0.6	3.4	3.6	14.3	9.1
T	44.3	21.0	5.3	3.0	1.1	2.4	10.0	9.1	1.1	7.8	1.1	6.9	12.8	7.6	1.5	0.8	0.7	1.9	2.1	0.0	3.8	5.3	0.7	3.8	3.4	17.4	9.1
T	33.3	21.0	3.0	2.3	1.1	1.7	11.3	8.5	1.3	6.4	2.3	2.8	11.4	7.6	2.6	0.9	0.9	2.9	3.1	1.0	4.4	5.3	0.6	4.5	2.5	16.6	8.3
T	83.0	39.3	7.7	2.3	1.1	2.3	11.6	8.3	1.4	4.2	2.7	1.6	11.0	7.4	2.5	0.9	0.9	2.9	2.9	1.0	3.4	5.3	0.6	3.0	3.7	14.3	8.3
T	52.0	22.7	9.7	1.5	1.1	2.7	15.1	8.3	1.8	6.6	0.9	7.2	11.0	6.0	2.3	0.8	0.4	2.9	5.8	0.0	3.4	4.5	0.6	3.0	3.7	13.6	6.8
T	42.0	20.0	4.0	2.3	0.8	2.3	8.1	6.2	1.3	4.7	1.5	3.1	12.8	7.4	2.6	1.1	1.0	2.4	2.6	0.0	2.6	4.2	0.6	3.0	4.3	13.6	7.6
T	86.7	34.7	13.7	5.0	1.5	2.9	12.8	10.0	1.3	6.7	1.4	4.7	12.6	7.4	1.5	0.8	0.5	1.9	3.0	0.0	3.4	6.0	0.6	3.8	3.3	14.3	6.8
T	62.0	29.3	10.3	5.0	1.5	2.5	9.2	7.5	1.2	5.3	1.5	3.5	11.6	7.1	1.7	0.8	0.5	2.1	3.8	0.0	3.8	5.3	0.6	3.8	3.0	14.3	7.6
T	75.0	31.7	19.7	10.3	1.5	3.0	11.5	8.0	1.4	7.3	1.8	4.1	11.6	7.1	1.9	0.8	0.5	2.4	3.8	0.0	3.4	4.5	0.6	3.0	3.9	13.6	6.8
T	71.7	28.7	9.7	4.3	1.5	2.8	11.0	8.0	1.4	7.0	1.6	4.5	14.4	7.4	3.0	0.9	0.9	3.2	3.3	0.0	3.8	6.0	0.8	3.8	3.8	17.4	8.7
T	46.7	22.7	5.0	5.0	0.8	2.3	12.6	10.3	1.2	6.0	1.4	4.3	11.3	6.6	3.0	0.9	1.0	3.2	3.0	0.0	3.0	5.3	0.6	3.0	3.8	14.3	7.6

Appendix III. Photograghs of different parts of *V. hymenolepis.*

Plate A.: Habit of *V. hymenolepis*. (Source: Author, 2013)

Plate B.: Shoot showing inflorescence with mature and immature florets. (Source: Author, 2013)

Plate C.: Specimen; inflorescence and mature leaves. (Photo by Evusa)

Plate D.: Lower (a) and upper (b) sides of the leaves. (Source: Author, 2013)

Plate E.: Capitulum. (Source: Author, 2013)

Plate F.: Disc floret and pappus. (Source: Author, 2013)

Plate G.: Phyllary appendage. (Photo by Evusa)

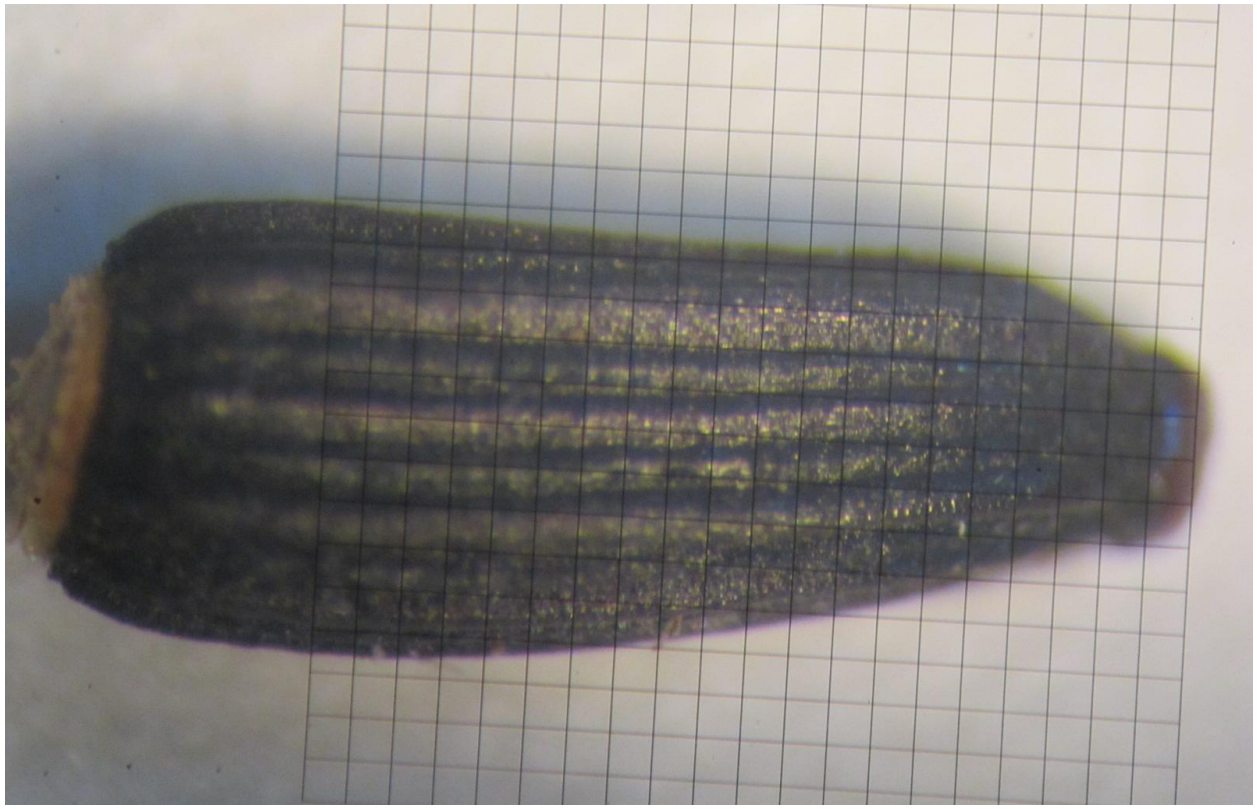

Plate H.: Cypsela (Mg ×160) (Source: Author, 2013)

Plate I.: Leaves and inflorescence of *V. calvoana* (zimbabweflora)

Plate J.: Leaves and inflorescence of *V. hymenolepis*

(Source: Author, 2013)