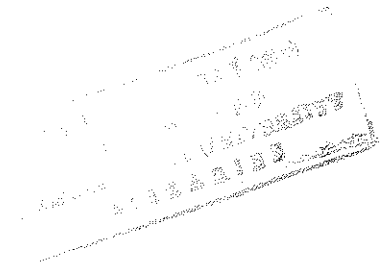


**A TAXONOMIC STUDY OF *BLEPHARIS EDULIS* (FORSSK.)
PERS. COMPLEX (ACANTHACEAE)**

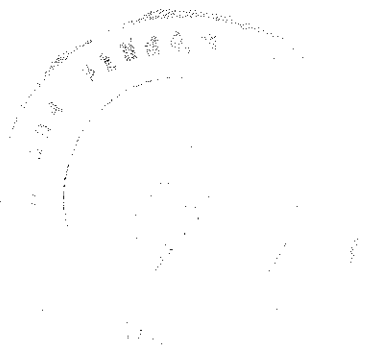


**A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE
STUDIES OF ADDIS ABABA UNIVERSITY IN PARTIAL
FULFILLMENT FOR THE DEGREE OF MASTER OF SCIENCE
IN DRYLAND BIODIVERSITY, DEPARTMENT OF BIOLOGY,
ADDIS ABABA, ETHIOPIA**

BY

ITAMBO MALOMBE

JUNE, 2000



Acknowledgements

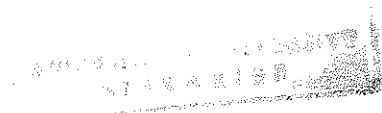
I am pleased to express my sincere gratitude to my supervisors Drs Ensermu Kelbessa (Biology Department, Addis Ababa University) and Abraham Muthama Muasya (East African Herbarium, National Museums of Kenya and Department of Biological Sciences Rutgers University, USA). They offered untold guidance and encouragement to me not forgetting their constant inspirations for discussion at every stage or time and the constructive criticisms of this report. Dr Ensermu Kelbessa constantly extended his strong knowledge of Acanthaceae and, in particular, genus *Blepharis* to me. Dr. Muasya explicitly displayed great perseverance in the harsh climate conditions of the drylands during the long field trips especially the Rift Valley and his tutorship on plant collection and identification. The company of Dr Dorothia Belgian, a visiting research scientist from Missouri Botanic Gardens, USA, working on a close relative family of Acanthaceae, Pedaliaceae, to the field trips enabled me to explore the most remote and extreme arid areas such as Turkana District. Her encouragement and material contribution are highly appreciated.

Much gratitude goes to the directors and all staff members of the following herbaria for sending their valuable specimens for my studies; ETH, WAG, MAL and NH. Also, much thanks to ICIPE, Kenya, for allowing me to use their Scanning Electron Microscopy equipment and accommodation during my field trips at Ngurumani, Kajiado, station.

I appreciate the moments shared with colleagues in both EA and ETH. The company of Mitiku Tiksa, Berihaun Gebremedene, Bayafers Tamene, Kenyatta, Mumin, Mbugua, Wanyama, E. Webuye, Ong'ang'i, Webala, Manoko, Ms J. Mary, Mushongi, Njau, Zerra and the entire Ethiopian Flora Project group, particularly Ms Sue Edwards and Melaku, is cherished. The assistance of Dr B. Khayota (Head, EA), P. Muthoka, Dr. J. Mutangah, Ms W. Musila, The Mwachallas, G. Mungai, P. Kirika, Mwamuye, Mwangangi, Halima, Mbale, Mugambi, Kang'ethe, Waiganjo, Laur, Hamida Hassan, J. Jefwa, P. Namachanja, Muoki, Mbatha, Muindi, Mathai, Rahab, Dr J. Mworia, Ms Muiruri and B. Kamondo, all of National Museums of Kenya (NMK) is of great contribution to the success of this study.

The proposal to study *Blepharis edulis* complex was floated to me by Dr. Kaj Vollesen of Royal Botanic Gardens, Kew through Ms Stella Simiyu of EA. Their concern is immensely acknowledged. The grant for the study was obtained from Swedish Agency for Research Cooperation with Developing Countries (SAREC) through the Research Programme on Sustainable Use of the Dryland Biodiversity (RPSUD) in Eastern Africa region. Particularly, I thank Drs Rashid A. Aman, J. Odera and Tamrat Bekele and Ms J. Kinyanjui for their untold facilitation. Moreover, the NMK through the EA granted the permission for my studies. Their kind assistance is also sincerely acknowledged. More so, the department of Ichthyology of the NMK is cherished for training me in "morphometric data analysis for taxonomy".

The last and not the least, I cherish the support from my family during the two year study stint for their patience and encouragement. My wife, Isabella Mwikali, and son, Bernard Muuo Itambo, provided moral and material support during my absence at home. *Ngai atutethie*.



Dedication

to my grandmother Mary Munono for her blessings

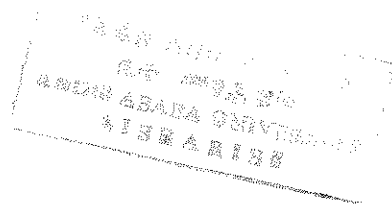
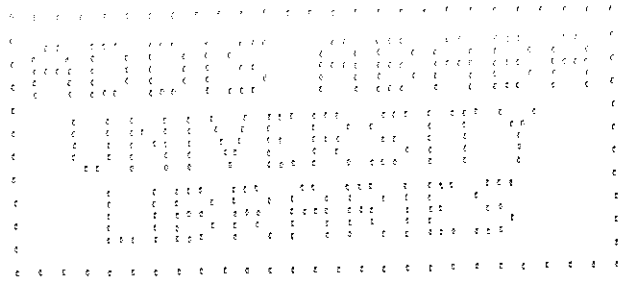


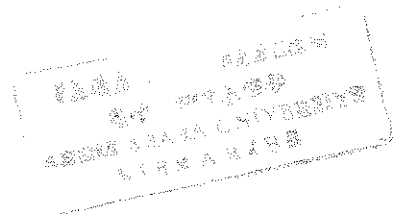
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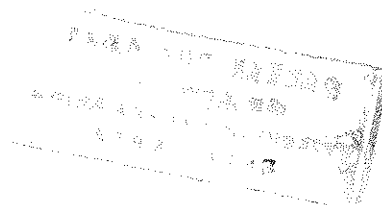
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Abstract

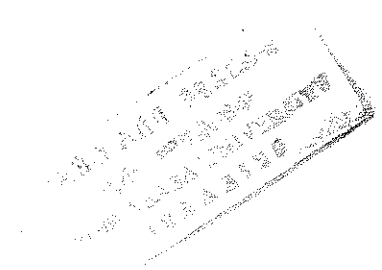
The species Blepharis edulis (Forssk.) Pers. has for long been mistaken with B. linariaefolia Pers. and B. ciliaris (L) B. L. Burt or B. boranensis Vollesen (ined.) and most of the collections identified indiscriminately. While revising the genus, attempt by Vollesen to separate the species, though with considerable observed morphological variation of specimens, was futile and recommended an infraspecific study based on morphology. This study, therefore, employs phenetic techniques on gross morphology, both mature and seedlings characters in conjunction with palynological evidence to disentangle B. edulis complex and related taxa. Cluster analysis segregated four groups, which were further verified by Principal Component Analysis, Discriminant Analysis and Non-Parametric Analysis. Importantly, vegetative and floral characters discontinuously or partially separated the accrued cluster groups. These results were further supported by palynology. Subsequently, these taxa were described as B. boranensis Vollesen (ined.), B. edulis var. edulis Pers., B. edulis var. glabra Malombe var. nov. and B. edulis var. isabellae Malombe var. nov. The species B. boranensis, a part from clear separation in the ordination space, it is morphologically discrete from all the other affiliated groups. B. edulis var. edulis is distinguished discontinuously from B. edulis var. isabellae and partially from B. edulis var. glabra by features of cotyledon colour, number and arrangement of leaf marginal spines. More so, the three varieties lack a wide geographical variation.

CHAPTER 1: INTRODUCTION

The genus *Blepharis* Jussieu, Acanthaceae, is composed of about 126 species (Furness, 1996, Vollesen, 1999) from the Old World tropics (mainly Africa), South Africa and the Mediterranean. Vollesen (ined.) has divided this genus into four subgenera namely *Oppositifoliae* (ined.), *Ebracteatae* (ined.), *Acanthodium* (Del.) Oberm. and *Blepharis*. Furthermore, he separates the subgenus *Acanthodium* into two sections; *Acanthodium* and *Biflorae* (Furness, 1996) and *Blepharis edulis* (Forssk.) Pers. sunk into the former. However, the work is still unpublished. Furthermore, comprehensive systematic analyses in Acanthaceae started recently (Daniel & Chuang, 1993).

According to Schilling (1992) and Furness (1996), data evidence like palynology and cytology have always been decoupled from gross morphological studies while studying species taxonomic status in Acanthaceae thus aggravating the problem. More so, palynology studies are poorly investigated in Acanthaceae.

Scanty taxonomic work in Acanthaceae has, therefore, resulted to unclear species boundaries. Particularly, *Blepharis edulis* subgenus *Acanthodium*, specimens in various herbaria have been lumped together or interchangeably identified as *B. ciliaris* (L.) B. L. Burrt, *B. linariaefolia* Pers or *B. boranensis* Vollesen (ined.). Such traces in records clearly show the difficult problems that plant taxonomists have wrestled with in their endeavour to deal with these taxa.

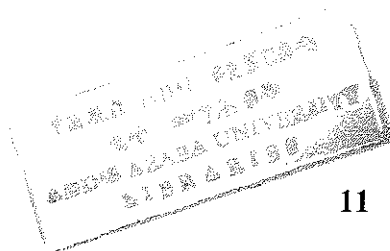


Subsequently, Vollesen (pers. comm.) has suggested allopatric distribution for these species. According to him, *B. linariaefolia* is distributed in southern Sudan westwards and *B. edulis* from Saudi Arabia through Ethiopia and Kenya to northern Tanzania. But *B. ciliaris* is only found in Asia. Earlier, using pollen morphology, Furness (1996) observed that *B. ciliaris* (from Kenya, Garissa, Gilbert *et al.* 5667) has smooth muri. A specimen of the same species from Yemen (Bisset 241) showed ridged muri and sometimes, irregular reticulum adjacent to the apertures and at the poles resulting from a probable disrupted pollen ontogeny. This, coupled with geographical distribution, suggests taxonomic differences between the two populations and a probable basic evidence of separating the taxon by Vollesen. Furthermore, the species has imbricate bracts in the inflorescence, a feature that hide important characters in the corolla and calyx. As Manktelow (1996) suggests, species in Acanthaceae that are very similar superficially are usually most clearly distinguished by the floral characters. Ensermu Kelbessa (1990), for example, used floral features in conjunction with pollen studies to study *Justicia* sect. *Ansellia*.

Ensermu Kelbessa (pers. comm.) has also observed that many taxonomic problems in this group are due to lack of seedling morphological studies in conjunction with mature plants. Moylan (1999) also points out that disagreement in the number of species recognized by different authors can arise, if widely different samples of variation are observed e.g. use of limited number of specimens. Importantly, missing data or mis-interpretation of minor variation, especially if living specimens have not been observed, can lead to many species being optimistically or provisionally proposed.

Besides, *B. edulis* is widely distributed in the extreme arid regions of Eastern Africa where it often occurs as pure stands of forbs especially on the overgrazed areas (White, 1983). Drylands are amongst the most threatened environments on Earth, although less publicized than the rain forests, always undergoing the processes of desertification being exacerbated by overuse of the vegetation cover by the ever increasing human population and their livestock (Pearce, 2000). The knowledge of the richness of biodiversity in the drylands and its importance to the survival and sustainable use of the natural resources is long overdue (Claridge *et al.*, 1997) and therefore, systematic studies, a possible way to identify species biodiversity status (Bates, 2000) by establishing their taxonomic stand and social –cultural values, in these vulnerable environments becomes imperative. Indeed, *B. edulis* as a complex species occupies an important ecological niche for the people of the Sub-Saharan region not only as an ecological indicator species or forage for livestock, but also as a source of medicine. Etymologically, ‘edulis’ is derived from a Greek word meaning edible (Stearn, 1967).

Whilst the Turkana and the Kalenjin people of Kenya use the root concoction to treat mouth wounds in children, the Maasai boil the dry inflorescence to treat kidney failure in human. The Somali people of Ethiopia use concoction from the plant for treatment against scorpion stings. Besides, a belt made from the branch is used to hasten delivery in women with prolonged labour the caution being that if the belt is not removed immediately after the delivery, the uterus will follow the baby.



The species epithet 'edulis' was coined by Forssk. (1775) under the genus *Ruellia* that was later transferred to the genus *Blepharis* Jussieu, by Persoon (1805). Earlier description of the species by Linnaeus as *Ruellia ciliaris* was reduced to a synonym of *B. edulis*. However, the genus *Blepharis*, and particularly the most widespread and variable taxon in the genus, *B. edulis*, has never been fully revised since the work of Clarke (1899–1900), a century ago, despite more material that have become available. This necessitates a re – evaluation of its infraspecific taxonomy as well as its delimitation from related species. Therefore, Vollesen has recommended, on the basis of gross morphology, an infraspecific study of *B. edulis* complex, a theme of this research. The study, besides field visits, pursued seedling morphology by collecting and germinating seeds in a green house with uniform environmental conditions at the Nairobi Botanic Gardens, National Museums of Kenya.

Generally, therefore, this study combines investigations on the features of gross morphology including seedling morphology and palynology in order to determine a full taxonomic relationship in *B. edulis* complex and closely related species in the sect.

Acanthodium.

Aims

- ❖ to undertake gross morphological (floral, vegetative and seedling morphology) study of *B. edulis* complex,
- ❖ to study pollen morphology of *B. edulis* complex and related species, and
- ❖ to establish a well circumscribed specific category for *B. edulis* complex.

CHAPTER 2: LITERATURE REVIEW

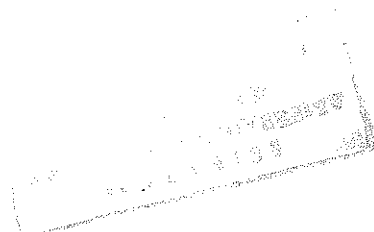
The Acanthaceae are a predominantly tropical family comprising about 3000 species in some 250 genera, making it one of the complex plant families, with centers of distribution in both the Old World and the New World (Dutta, 1979; Daniel, 1986). Broadly, this family is treated to include four subfamilies namely Acanthoideae, Thunbergioideae, Nelsonioideae and Mendoncioideae (Lindau, 1895). Recent classification by Cronquist, however, retained the first three groups in Acanthaceae and ranked Mendoncioideae as distinct family, Mendonciaceae (Mabberley, 1981, Daniel, 1986) on its own.

The genus *Blepharis*, erected by Jussieu (1789), is one of the eight African genera found in the sub-family Acanthoideae, tribus Acantheae, a tribe characterized by non-articulated stems and lacking cystoliths in stems and leaves. Napper (1970) and Vollesen (1990a, 1990b, 1991, 1994) have listed the other seven genera as *Acanthopsis* Harvey, *Acanthus* L., *Sclerochiton* Harvey, *Crossandra* Salisbury, *Crossandrella* C. B. Clarke, *Stenandrium* Nees and *Streptosiphon* Mildbraed. Further, the genera are divided into two groups of four based on both morphology and pollen features. The first three were grouped together with *Blepharis* characterized by long exserted stamens with thick bone-like filaments as opposed to the rest, last four, with included stamens, short thin filaments or with sub-sessile anthers. According to Furness (1996), the pollen of the first group possesses a distinct foot layer, which is absent or discontinuous in the pollen grains of the second set of genera.

Importantly, *Blepharis* has been found closely related to the genus *Acanthus*. The two genera are characterized by calyx, which is divided to the base into four sepals (Vollesen, 1991) and a distinctive hexagonal reticulum (Furness, 1996). However, they are distinguished, morphologically, by the floral features. *Blepharis* subg.

Acanthodium have 5-lobed corolla with the two outer lobes small and often tooth-like as opposed to the three lobed characters in *Acanthus*. Also spikes in *Blepharis* are secondarily reduced to a single flower, which is either supported, by single but mostly imbricate bracts, which lack in *Acanthus* (Vollesen, 1991). Further, the genera are delimited by ultra-structural differences in the exine, the infrequent occurrence of perforate sculpturing in *Acanthus*, and the usually larger size of *Acanthus* pollen. For example, whilst the pollen of *Blepharis* is recorded as P (32-) 46.2 (-67) μm , E (16-) 24.6 (-42) μm and P/E 1.36-2.61, *Acanthus* has shown the pollen sizes to be P (47-) 59.5 (-75) μm , E (22-) 29.3 (-45) μm and P/E 1.67 2.36). More so the pollen of *Blepharis* is more heterogeneous as compared to the one of *Acanthus* as to much the morphology, which prompts Furness (1996) to conclude that *Acanthus* is smaller, morphologically more uniform than *Blepharis*.

The morphological heterogeneity in the genus *Blepharis* aroused Vollesen to embark on compiling its gross morphological characters. Furthermore, the last and probably comprehensive infraspecific study in the genus was carried out, a century ago by Clarke (1899-1900). He treated close to fifty species in this genus and described thirty-one taxa based on vegetative and floral morphology. He divided the species into two groups based on absence or presence of spines on the leaf margins. Since then, plant



taxonomists have described more species in the genus *Blepharis*. Lind (1971), for example, indicated fifty species of *Blepharis*. Vollesen (ined.), has listed c. 126 species from the Old World tropics (mainly Africa) and South Africa. Based almost entirely on pollen morphology, Vollesen has divided the genus into four subgenera and five sections as shown in table 1.

Subgenus *Oppositifoliae* consists of only the Indian species, *Blepharis asperrima* characterized by pollen with rough hexagonal reticulum with ridged, inside depressed, muri. In Subgenus *Acanthodium*, Section *Acanthodium* the pollen, apart from having columellae in the perforations, is found to be abnormal i.e. pollen produced in extremely low quantities or entirely lacking. Examples of species with this pollen type include *Blepharis aspera* Obermeyer, *B. capensis* (L.f.) Persoon, *B. diversispina* (Nees) C.B. Clarke, *B. grossa* (Nees) T. Anderson, *B. mitrata*, *B. obmitrata* C.B. Clarke and *B. subvolubilis* C.B. Clarke. Subgenus *Blepharis* Section *Blepharis* has pollen that lack columellae in the perforations or rarely so, e.g. *B. chrysotricha* Lindau where columellae are found. Conversely, the species in Subg. *Blepharis* Section *Scorpioideae* possesses all the aforementioned pollen types and subtypes (Furness, 1996; 1997).

In the endeavour to accomplish his recommendation of unfolding infrageneric taxonomy of genus *Blepharis* by use of morphological characters, Vollesen (in prep.) was fascinated by the considerable morphological differences among *B. edulis* collections. For long, this species has been thought to be widely distributed in Eastern Africa and Asia (Clarke 1899-1900, Blatter, 1978), but Vollesen reckons that it occurs

only in Arabia through Ethiopia to Kenya and northern Tanzania. Its close affinities frequently confused and often used interchangeably i.e. *B. ciliaris* and *B. linariaefolia*, are delineated allopatrically to occupy southern Sudan westwards and in Asia (Oman, Iran, Pakistan and India), respectively.

Table 1: Infrageneric classification of the genus *Blepharis*

Subgenus	Section	Some taxa
<i>Oppositifoliae</i> ined.		<i>B. asperrima</i> Nees
<i>Ebracteatae</i> ined.		<i>B. spiculifolia</i> ined.
<i>Acanthodium</i> (Delile) Obermeyer	<i>Acanthodium</i>	<i>B. ciliaris</i> (L.) B.L. Burrt, <i>B. linariaefolia</i> Persoon, <i>B. hirtinervia</i> (Nees) T. Anderson, <i>B. attenuata</i> Napper, <i>B. capensis</i> (L.f.) Persoon, <i>B. noli-me-tangere</i> S. Moore, <i>B. pruinosa</i> Engler, <i>B. natalensis</i> Obermeyer, <i>B. diversispina</i> (Nees) C.B. Clarke, <i>B. subvolubilis</i> C.B. Clarke, <i>B. serrulata</i> (Nees) Ficalho & Hiern, <i>B. mitrata</i> , <i>B. dhofarensis</i> A.G. Miller.
	<i>Biflorae</i> ined.	<i>B. tricornuda</i> ined.
<i>Blepharis</i>	<i>Blepharis</i>	<i>B. hildebrandtii</i> Lindau, <i>B. jacamica</i> Bremekamp, <i>B. chrysotricha</i> Lindau, <i>B. integrifolia</i> (L.f) E. Meyer, <i>B. exigua</i> (Zollinger) Backer, <i>B. glumacea</i> S. Moore, <i>B. tanae</i> Napper, <i>B. maderaspatensis</i> (L.) Roth, <i>B. involucrate</i> Solms-Laubach, <i>B. cuanzensis</i> S. Moore, <i>B. tanganyikensis</i> (Napper) Vollesen, ined., <i>B. calcitrapa</i> Benoist, <i>B. glomerans</i> Benoist and <i>B. crinita</i> Benoist.
	<i>Inopinatae</i> ined.	<i>B. sinata</i> ined.
	<i>Scorpioideae</i> Obermeyer	<i>B. affinis</i> Lindau, <i>B. panduriformis</i> Lindau, <i>B. temiramea</i> S. Moore, <i>B. katangensis</i> De Wildeman, <i>B. mandandensis</i> S. Moore, <i>B. pungens</i> Klotzsch, <i>B. stuhlmanii</i> Lindau, <i>B. bainesii</i> S. Moore, <i>B. acanthodioides</i> Klotzsch, <i>B. ilicifolia</i> Napper.

More so, Vollesen (pers. comm.) noted a unique population in southern Ethiopia in Sidamo region, Borana Awaraja, whose collections deviates both morphologically, e.g. bigger bracts, and ecotypically, from *B. edulis*. Indeed, besides its seemingly

perennial nature this species, proposed as *B. boranensis* occupies higher altitudes ranging from 1550-1850 m a.s.l. as opposed to lower domination of the former in the same region. This complexity of *B. edulis* prompted Vollesen to call for an exhaustive infraspecific morphological study to further investigate its taxonomic rank, which in conjunction with palynological studies forms the basis of this study.

In taxonomy, morphological attributes taken collectively, give probably the best reflection of the overall genotype of the organisms, as well as ecological adaptation (Stuessy, 1994). However, Gornall (1997) observed that phenotypic differences between individuals of a taxonomic species could exceed those found between such species, thereby violating the grouping criterion of overall similarity, a phenomenon common in morphological species complexes. Therefore, collection of different types of data should be encouraged to attain a useful and a predictive classification.

Besides, all characters are not always useful in taxonomic delimitation. Stuessy (1994) expresses characters to be either good or bad. A good character should serve adequately to differentiate taxonomic groups while a bad character cannot be relied upon in such delimitations. In addition, Gornall (1997) writes that characters used to distinguish species are those with minimum variation within the species. Actually, as Moylan (1999) observes, the characters should not be polymorphous of a population but fixed (e.g. flower colour) or characteristics of a life cycle (e.g. hairiness of a leaf surface).

Studies on *Puccinellia spp.* within species, for example, variation involved characters such as inflorescence branching, a phenomenon interpreted as adaptive (Dutta, 1979), which also supports ecotypic differentiation of species. Indeed, the use of leaf size and shape to distinguish species can sometimes be very problematic. Studies by Moylan (1999) on *Hemigraphis spp.* (Acanthaceae) revealed that leaf characters mostly size are variably plastic phenotypic traits associated with particular localities and not fixed characters of different species *per se*.

In the taxonomic study of Acanthaceae, Zomlefer (1994) recommended the generic distinguishing features as the number and morphology of stamens, size and form of bracts, shape of corolla, number of ovules, presence of cystoliths and size, level and indumentum of the anthers. Importantly, some of these features are seemingly useful at all taxonomic levels in the family. For instance, Vollesen (1990b) worked on infraspecific taxonomy of African *Crossandra indundibulformis* (Nees) and deduced that subsp. *boranensis* and subsp(s). *eglandulosa* and *crocea* could be differentiated by the presence of fine appressed silvery indumentum and glabrous to densely pubescent hairs, respectively, which are located on young branches and leaves.

Elsewhere, based on trichome types, Sebsebe Demissew and Harley (1992) were able to divide the genus *Stachys* (Lamioideae, Labiatae) in Tropical Africa into three groups i.e. strictly simple hairs, dendroid simple and biramous and/or stellate hairs.

Importantly, while using morphological characters in the infrageneric classification of *Justicia*, Graham (1988) showed that characters of inflorescence, androecium and pollen

morphology played a pivotal role. In fact, Manktelow (1996) observes that species in Acanthaceae that are very similar superficially are usually most clearly distinguished by the floral characters. Ensermu Kelbessa (1990), for example, used floral features in conjunction with pollen studies to study *Justicia* sect. *Ansellia* and found out that corolla sizes marched with pollen size.

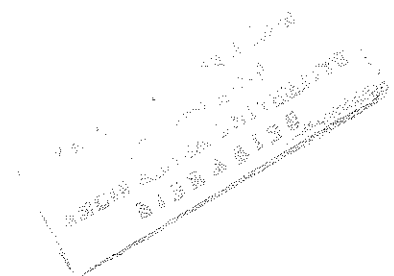
Furthermore, Scotland (1992) had mentioned the importance of pollen morphology and their mode of sculpturing as a basic diagnostic feature in generic delimitation of this family. Also Erdtman (1969) and Radford *et al.* (1974) emphasized the importance of pollen data at all taxonomic levels. Radlkoffer steered the first pollen morphology, of taxonomic value, in the classification of Acanthaceae. His work was carried over by Lindau (Erdtman 1969, Ensermu Kelbessa 1982, Schilling, 1992).

Other pollen investigations of this family have also made unprecedented contributions. For example, Bhaduri (1944) studied thirty species of Acanthaceae and reported that dimorphism in size and shapes have been found a character of the pollen grains of many of the species. Raj (1961) made detailed pollen morphological studies in the Acanthaceae, while Vishau-Mittre and Gupta (1966) investigated the pollen morphology of several species of *Strobilanthes* and divided all the species into two groups. The study was based on the apertures; porate, colporate types and exine pattern; the banded and the spinulose types. While dealing with the general morphology, vegetative and floral anatomy, palynology and geographical distribution

of thirty-four species of Taiwan Acanthaceae, Hsieh and Huang (1974) distinguished ten pollen types based on aperture and exine stratification.

Strictly at infraspecific level, pollen studies can also cast light into taxonomy. Furness (1996), for example, reported differences of pollen sculpturing within *Blepharis* species. In *Blepharis edulis* collections from Kenya and Saudi Arabia were found to have smooth and ridged muri, although unified by presence of columellae in the perforations, respectively. Pollen within *B. tanae* also had both simple evenly spaced regular perforations as well as fossulate form. Furness suggests that such infraspecific pollen variation is due to disrupted pollen ontogeny. Furthermore, the pollen morphological diversity found throughout the family coupled with pollen abnormality, explained above, imparts that pollen characters in Acanthaceae are plastic and actually evolving (Furness, 1997) probably to match morphological adaptation.

Palynology, as used in the classification of Acanthaceae is, however, still problematic (Schilling, 1992). Apart from scanty studies, many pollen investigations in Acanthaceae are established independently from taxonomic studies of other morphological characters and so the extent of character congruence between pollen and other character data types becomes difficult to appraise (Furness, 1989; Vollesen, 1989). Therefore, as Schilling (1992) and Furness (1997) advocate, general morphology and pollen morphological data ought to be integrated in order to acquire a full taxonomic relationship in Acanthaceae.



CHAPTER 3: MATERIALS AND METHODS

The study used both herbaria and field collections. Subsequently, specimens for gross morphology and palynology were obtained on loan from the following herbaria: the National Herbarium of Ethiopia (ETH), Herbarium Vadense (WAG) in Netherlands, The National Herbarium of Malawi (MAL) and National Herbarium of Durban (NH) in South Africa. These were examined in conjunction with East African Herbarium (EA) collections in Kenya, which houses duplicates of *Blepharis spp.* from most of the Eastern Africa countries, at the National Museums of Kenya. Herbaria Abbreviations follow Holmgren, *et al.* (1990).

Blepharis edulis is widely distributed in the extreme arid regions of Kenya. Therefore, several trips, based on the species phenological patterns, were conducted in most of the drylands of Kenya (fig. 2). These areas of study included the Rift Valley (Kajiado, Baringo/ Koibatek, West Pokot and Turkana Districts), Eastern (Makueni, Machakos and Mwingi Districts) and Coast (Voi and Taita Taveta Districts) Provinces. Besides, fresh specimens for parallel morphological and palynological studies, young flower buds and fruits/ seeds plus soil were collected for cytology and seed germination for seedling morphology studies, respectively.

3.1 Gross morphology

Morphological studies on herbarium specimens of *B. edulis* and other closely related (sect. *Acanthodium*) species were carried out. The vegetative and reproductive parts were measured using a hand ruler calibrated in mm and/ or under a WILD M3

dissecting microscope. Dry floral parts were soaked in wetting agent mainly absolute ethanol for up to 15 minutes to soften. Specimens with complete flowers, fruits and mature leaves were investigated in detail. Several measurements were taken for each part to be observed at standardized positions and an average taken. This holds for specimens with enough parts for measurements and/or observation. Therefore, those materials lacking adequate leaves or flowers were exempted for the rule and the parts available taken into consideration.

Generally, a total of 171 specimens were sampled out of the 400 specimens received and scored for about 62 characters. *Blepharis ciliaris*, *B. edulis*, *B. linariaefolia* and *B. boranensis* constituted 132, 30, 4 and 4 specimens respectively. As mentioned earlier, the identification of the specimens is a misnomer. Therefore, for the sake of this study, these taxa were treated synonymously as *B. edulis*. Furthermore, *B. ciliaris* is never found in Africa (Vollesen, pers. comm.).

3.2 Seedling morphology

The species/ populations studied were grown under similar environmental conditions. Seeds were germinated directly in plastic pots of 9 cm diameter and grown in the greenhouse, Nairobi Botanic Gardens at the National Museums of Kenya. Germination media composed of 70% sand and 30% forest soil.

Seedling description commenced after first week of germination. Description entailed features such as cotyledon color, stem color, internode size and indumentum; leaf blade, shape, color, size (length and width), number and size of marginal spines, indumentum and petiole characters.

3.3 Palynology

3.3.1 Light Microscopy (LM)

Acetolysis

Mature flowers were collected from herbarium and/or fresh specimens. Anthers were then picked out under a dissecting microscope and placed in mortar and gently crashed with a pestle after wetting in 7 ml of distilled water. The solution was then sieved in a 90 µm brass. The sediments were washed twice using distilled water, centrifuged and decanted. The solution was divided into two parts. One part was kept for Scanning Electron Microscopy (SEM) and the second for acetolysis in preparation for Light Microscopy (LM) samples.

Acetolysis was undertaken according to Erdtman (1969). Thus, a mixture of glacial acetic anhydride and concentrated sulphuric acid (3:1) was used.

Slide preparation

A pipette was used to transfer a drop or two of pollen material for each sample to a clean-labeled slide. The slides were then warmed for one minute. After drying, a drop of (Kaisers Glyceringelatine) gelatine was placed at the center of the dry pollen material using a clean needle. The slides were then covered by slide covers and immediately placed on the warmer. Finally, melted wax was placed at one side of the cover slide and spread to engulf the gelatine spot at the center. The permanent slides are kept for reference at the Palynology of the National Museums of Kenya and the duplicates stored at the National Herbarium (ETH).

Measurements and micrographs of the pollen were taken using ocular micrometer at x 400 and x 250/ 1000 respectively. At least ten pollen grains were measured under a calibrated microscope and average taken. However, some samples had fewer than ten pollen grains. In this case, measurements for all the grains present were taken.

3.3.2 Scanning Electron Microscopy (SEM)

Pollen grains for SEM (2.1.1 above) were unacetolysed. The pollen samples were washed and centrifuged twice in ethanol and oven dried for 10 minutes at 60°C to reduce the amount of ethanol. Samples of the grain were then transferred to a clean and labeled bronze (stub) using micropipettes under a dissecting microscope.

The pollen material was then spread over the surface of the stub and kept in a dust free area overnight to dry. The stubs with the pollen grains were then coated with gold in Geol Fine Coat. Scanning and analysis for micrographs were taken using a Jeol JSM 840 Scanning Electron Microscope.

3.4 Data analysis

Taxonomy (the act of identifying and classifying organisms into groups or taxa based on similarities and/or differences) coined by Augustin Pyramus de Candolle in 1813 (Stuessy, 1994), is seen as both 'queen' and 'servant' of biology. The former, because it is ultimate and as such, all other fields of biological research leading to the establishment or improvement of a classificatory system. The latter, because it

provides basic services of information on identity, probable close relatives and characteristics of organisms to the other units of biology (Sivarajan, 1991) but more properly limited to the study of the principles and methods of classification (Stuessy, 1994).

Classification which is defined by Stuessy (1994) as the ordering of organisms into groups based on observed similarities and/or differences, both artificial and natural systems, serve as an organized framework from which information about a particular organism or taxa can be logged and then retrieved for application. An artificial classification is based on one or few characters such as the Linnaean sexual system. Conversely, natural classification is based on sum total of all characters. For example, numerical taxonomic methods are useful in determining phenetic relationship.

Phenetic classification, unlike cladistics or phyletics which stress features arising directly from evolutionary ancestors, emphasis overall similarity of characters states (Stuessy, 1994). Therefore, a phenetic classification is produced by comparing as many characters as possible from an organism.

In cluster analysis, for example, a common phenetic method applied in taxonomy (Sneath & Sokal, 1973, Sneath 1995), many attributes are considered simultaneously and the objects referred to as OTUs (Operational Taxonomic Units) are clustered according to their overall statistical similarity or distance based on a matrix of affinity between pairs of OTUs. The two most similar OTUs are joined into a group and the similarities of this to all other OTUs are calculated. The closest groups are combined

repeatedly until only a single group remains and the results are usually expressed in a phenogram, a two-dimensional hierarchical tree (James & McCulloch, 1990) of clusters, sequentially formed starting with the most similar pair. Therefore, for instance, hierarchical clustering within a species with the specimens as OTUs produces a hierarchical pattern of specimen's resemblance to represent a hierarchical phenetic classification pattern of taxa within the species complex.

Subsequently, in order to group the 171 specimens (OTUs) having similar morphological and palynological characters together, clustering method was used prior to characters (variables) standardization. Unlike many other statistical procedures, cluster analysis methods are mostly used without any a priori hypotheses and they find the most significant solution possible to classify sizable information into manageable meaningful piles or clusters. They are therefore ideal for evaluating similarities and differences among OTUs as well as a method of delineating taxa. Importantly, clustering is seen as a process of sorting out OTUs according to their similarity, produced through a matrix of similarity or dissimilarity coefficients from which a phenogram can be generated. The matrix of affinities was calculated using Unweighted Paired - Group Method using arithmetic Averages (UPGMA) Euclidean distance linkage. Simple Euclidean distance was chosen as a similarity coefficient, because it is sensitive to additive, proportional, and minor image translation (Franceschinelli *et al.*, 1999).

Cluster analysis encompasses a number of different algorithms. Single linkage, for example, tends to exaggerate the similarity between groups whereas complete linkage (furthest neighbor) is a conservative method that shows the most cohesive clusters, but excludes those, which are less clearly defined. On the other hand, Ward's method, though efficient as it attempts to minimize the Sum of Squares (SS) of any two (hypothetical) clusters that can be formed at each step, leads to clusters of small size. Therefore, UPGMA, which is a compromise of these extreme methods, minimally distorts the overall dissimilarity among groups and appears to perform best in practice (Pankhurst, 1991, Franceschinelli *et al.*, 1999) was used in this study.

Clusters derived from cluster analysis were subsequently plotted on the map of Eastern Africa to test if the segregation complies on the basis of geographical distribution. Further, Principal Component Analysis (PCA), Discriminant Analysis (DA) and Non-Parametric Analysis (NPA) were performed on the accrued groups to test their separation and significance for taxonomic conclusions.

The PCA investigates the structure in a dataset and summarizes patterns of correlation and covariation among a set of variables and determines the most important variables in taxonomy. Principal components (axes) are extracted in descending order of the overall variance for which they account. The summary variables (axes) are completely orthogonal and each represents an independent description of the underlying variation among samples. The summary of correlation between two variables can be represented in a scatter plot diagram.

Discriminant Analysis on the other hand is a multivariate technique that may be used to test separation of previously defined groups in multivariate space. It also offers validation procedures to evaluate how well individual cases (e.g. specimens) are classified into their appropriate groups (Tabachnick & Fidell, 1989). Indeed according to Snoeks (pers. comm.), DA is used in study of hybrids.

Finally, since sampling involved samples of unequal size, Non – Parametric Analysis was carried out to determine the actual variables used in delimitation between two groups. Subsequently, multivariate statistical analysis used was STATISTICA version 4.1 software. This program standardizes the data, provides the calculation of the distance matrix, and does the cluster and the ordination analyses.

CHAPTER 4: RESULTS

4.1 Gross morphology

4.1.1 Cluster Analysis

Clustering of OTUs (cases = specimens) using UPGMA, a consensus tree (fig. 1) of 23 variables, yielded four clusters (groups) at 1.5 Euclidean linkage distance. Out of the 171 OTUs selected and scored for the 23 variables (table 2) and subjected for analysis, only 118 were accepted and otherwise deleted case wise following morphometric gaps. Leaf characters, for example, led to numerous gaps. Most of the specimens lacked complete, enough and even standard leaves as measurement was designed for the second node only. Even the leaves available were rolled up, culled or hidden under the multibranched or copious inflorescence.

Besides, the species are characterized by both short and long or unequal, 4- whorled, 2 or 3 (seedling) pairs of leaves, a feature also common in the genus *Blepharis* (Clarke, 1899-1900). During pressing, therefore, these leaves are frequently aligned interchangeably. The problem is exacerbated by the fact that on drying, the entire stem may turn white or brown. Therefore, for this consensus tree (fig. 1), some of the leaf and capsule characters were eliminated.

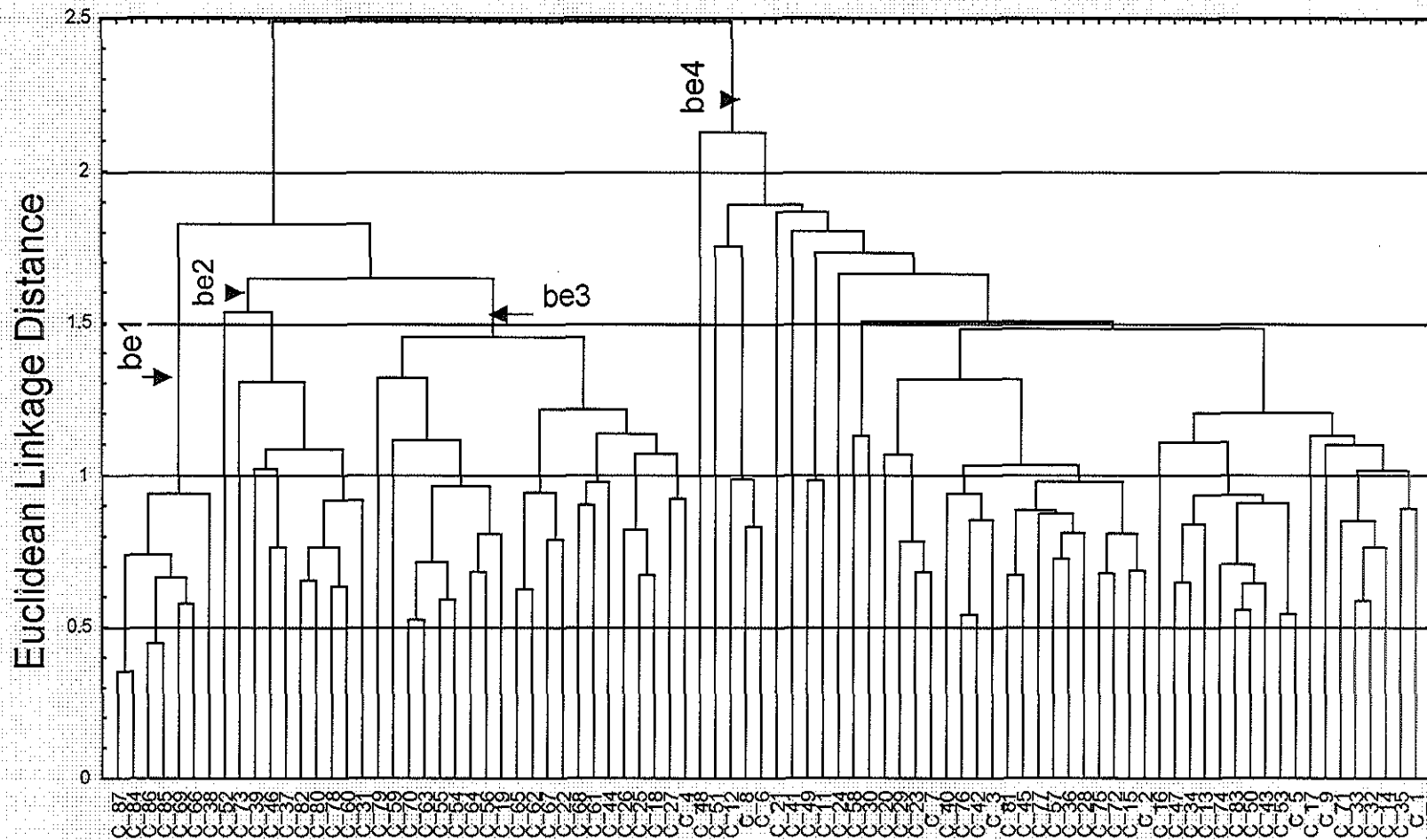


Fig. 1: Dendrogram of cluster analysis (UPGMA), specimens (C) applied as OTUs

In cluster be1 OTUs are those of *B. boranensis* OTUs. In clusters be2 & be4 OTUs are composed of *B. edulis*, *B. linariaefolia* and *B. ciliaris* while be3 is constituted of *B. ciliaris* & *B. edulis*. Specific clusters are shown by arrows.

Table 2: Variables used in the analysis (measurements in mm)

Variable abbreviation

SP-Species

CN-Collector

LBL-Leaf Blade Length

LBW-Leaf blade width

LBMC-Leaf Blade Marginal Spine Cover

LBMS1-Leaf Blade Marginal Spine number (Minimum)

LBMS2-Leaf Blade Marginal Spine number (Maximum)

BL-Bract Length

BW-Bract Width

BSN1-Bract Spine Number (Minimum)

BSN2-Bract Spine Number (Maximum)

BSLI1- Bract Spine Length (Minimum)

BSLI2- Bract Spine Length (Maximum)

BTL-Bractiole Length

BTB -Bractiole Width

BSC-Bract Spine colour

CLL-Calyx large (anticous) Length

CLB -Calyx large (anticous) Width

CML-Calyx Medium (posticous) Length

CM -Calyx Medium (posticous) Width

CSL-Calyx Smallest (ventral/innermost) Pair (inner) Length

COL-Corolla Length

COB- Corolla Width

COC-Corolla Colour

FL-Filament Length

PR-Process Length

PAB-Processed (anticous) Anther Breadth

PAL- Processed (anticous) Anther Length

AAL-Posticous Anther Length

AAB - Posticous Anther Breadth

SYL-Style Length.

These clusters were tentatively delineated as, from left to right of the dendrogram, be1, be2, be3 and be4. Cluster be4 is completely segregated from the first three clusters just below 2.5 Euclidean distance to form somewhat continuous clusters. Specimens 12, 48 and 51 in this group are, however, singled out in a small cluster. These specimens were collected from Turkana (Central Island), Njooro Maasai region of Tanzania and Werie Bridge (Tigray region) area of Ethiopia, respectively.

Cluster be1 branches out from be2 and be3 at Euclidean linkage distance of c. 1.8. Moreover, clusters be2 and be3 separate at c. 1.65 Euclidean distance. Although cluster be3 branch into two slightly below 1.5 Euclidean distance, the cases were found interchangeably listed at the amalgamation distance matrix and therefore designated as one cluster.

For each of the four identified clusters and per variable used for the analysis, the ranges (minimum and maximum), mean, standard deviation and variances were calculated (appendix 1). The number of spines on the leaf margins contributed immensely to accretion of the clusters. For example, discontinuities were observed between most of these groups. The number (minimum) of leaf marginal spine in particular showed discontinuous ranges amongst cluster be4, be3, be2 and be1, means in brackets, and are 2-(5)-9, 12-(18)-23, 4-(10)-13 and 25-(31)-33, respectively. For the same variable, be3 records the highest standard deviation (3.6) and hence the most diversified in terms of leaf marginal spine number otherwise, on average, the common highest number of

spine is 18. The leaf marginal spine cover shows that be1 spines cover is normally to the leaf tip, group be3 up to the leaf tip or sometimes to the three quarters.

For groups be2 and be4, the leaf spine cover is normally up to two-thirds and hardly to three-quarters of the leaf length in which the spines are usually loosely arranged.

Further, some OTUs in be4 showed leaf spines cover up to the first or second quarter and even in some cases, e.g. *Friis, S. Bidgood, P. Host, Melaku Wonderfrash & Shigulle Kebede 6735*, spines are lacking.

Clusters be1 and be3 are discontinuously segregated by number of spine on bracts recorded as 9–11 and 4–8. Cluster be1 differ from be2 by leaf spines recorded as 25-33 and 25-36 against 4-13 and 5-14 for maximum and minimum ranges, respectively.

Segregation between be2 and be3 is partially brought about by corolla colour and number of leaf marginal spines as depicted by high values of standard deviation in be3. However, group be3 records lower character values than be2 for most of the variables.

Geographically, be1 is concentrated mainly in Borana areas of Sidamo region of Ethiopia where altitude ranges from 1550 m to 1850 m above sea level. Cluster be3 occurs in southerly districts of Kenya such as Voi and Taita–Taveta (K7) up to northern Tanzania. This group is also found in Harer region and low altitude areas of Sidamo. Cluster Be4 is mainly a low altitude species occupying valley bottoms and shoreline as well as islands. These areas include Omo valley and Arba Minch (Gamo Gofa region) and Awash area in Ethiopia, Kenyan rift valley (Kajiado, Baringo, Turkana districts), Eastern and Northeastern provinces (K1, K2, K3 K4 and K6) up to western Somalia. It

is also collected at the coastal plains near Red Sea and Massawa area in Eritrea and Turkana Central Island in Kenya. Cluster be2 is concentrated at the Ethiopian Rift valley but ranging from around L. Turkana in Lodwar and Koobi Fora upwards through the rift up to Eritrea. However, in some parts it appears to coexist or found very close to group be3 and be4 such as in Kombolcha and Sodore localities.

Interestingly, be1 is composed of almost all the specimens, initially identified as *B. boranensis* by Vollesen, observed. Other OUTs making this group include 3 collections initially determined as *B. ciliaris*. The other three groups occupy a wider spectrum of altitude from about 100-1900m above sea level. The accompanying specimens were initially identified either as *B. ciliaris*, *B. edulis* or *B. linariaefolia*.

Generally, both vegetative and floral characters demonstrated strong delimitating capacity as discontinuities were recorded between clusters such as be1, be3 and be4. Differences between clusters be2 and be3, however, are not clear and only partial discontinuities were recorded in several variables. In order to check these results and determine the validity and which variables are the most important in discriminating among the four groups, Principal Component Analysis (PCA), Discriminant Analysis (DA) and Non-Parametric Analysis (NPA) were applied to the data of the four groups established.

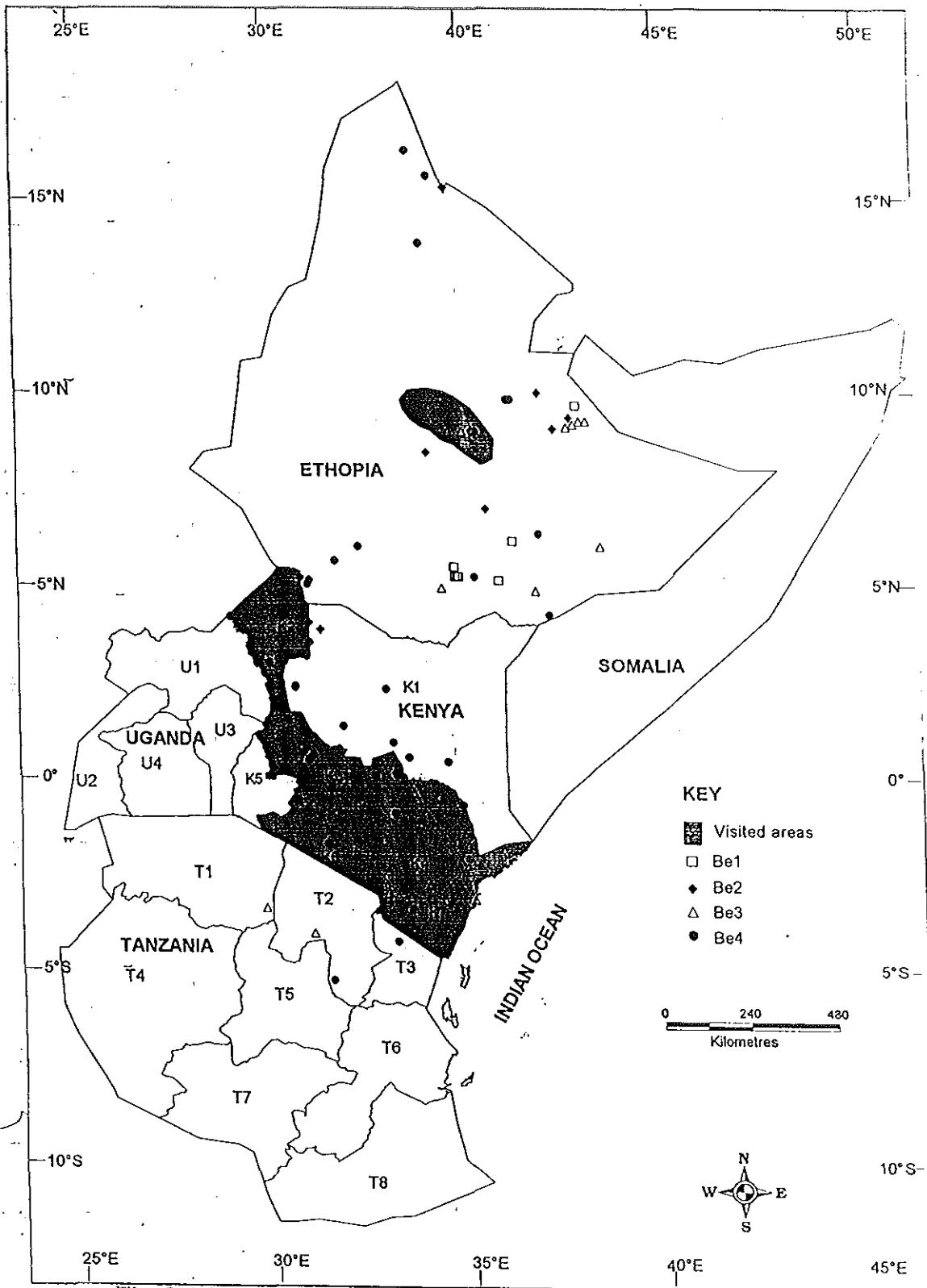


Fig. 2. Map of Eastern Africa showing distribution of *B. edulis* complex

4.1.2 Principal Component Analysis (PCA)

Four factors (axes) were selected for PCA. The variance attributed to each axis was found to be 53.56%, 9.43%, 6.42% and 5.24% for axes 1, 2, 3 and 4, respectively (table 3). Thus, axes 1 and 2 contributed to c. 63% of the total variance. A scatter plot of axis 1 against axis 2 (figs. 3 and 4) resulted to segregation of groups be1, be2 and be4. Group be3 had both ends embedded in clusters be2 and be4. When group be3 was excluded from the analysis, the other three groups were separated (fig. 4).

Factor loadings (table 4) indicated that most variables contribute significantly to axis 1 as majority were more than 0.450 and about two thirds over 0.7 factor loadings. For the latter, the variables included calyx (anticous) length (CLL) and width (CML), corolla colour (COL), anther (anticous) length (PAL), style length (SYL), corolla width (COB), anther (posticous) length (AAL), bract spines (BSN1 and BSN2), filament length (FL), number of leaf marginal spines (LBMS1 and LBMS2) and calyx (ventral) length (CSL), in order of importance. For the second axis, the important characters included the number of spines on both leaf and bracts. A scatter plot of axis 1 against axis 3 did not separate these groups (fig. 6).

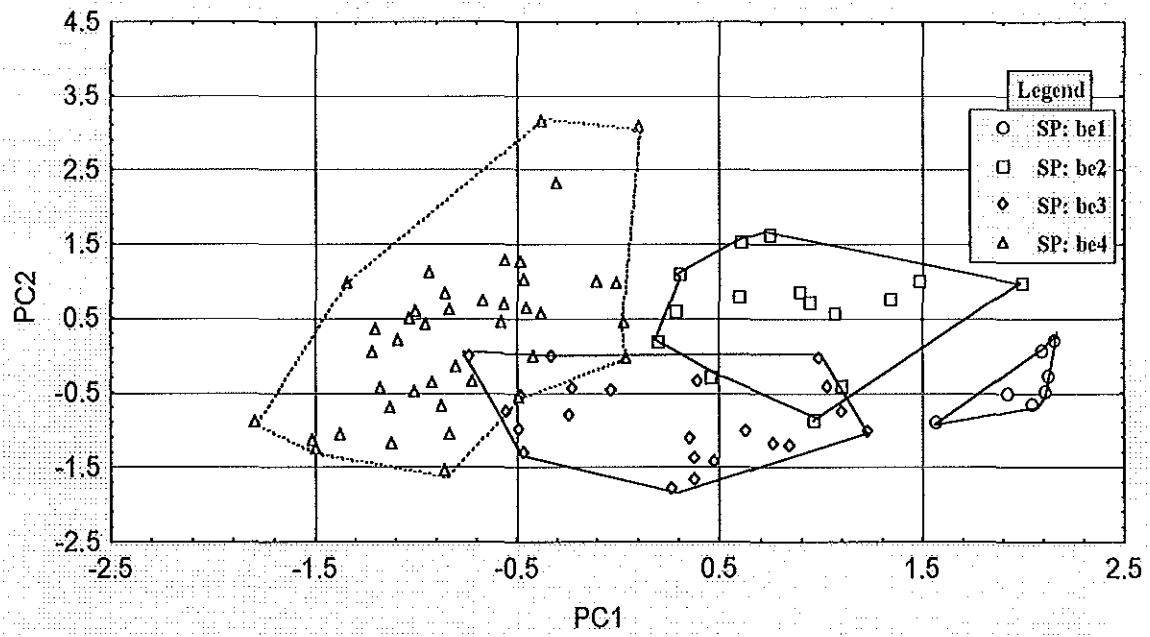


Fig. 3: Scatter plot (log-transformed data) of the four groups (be1, be2, be3 & be4)

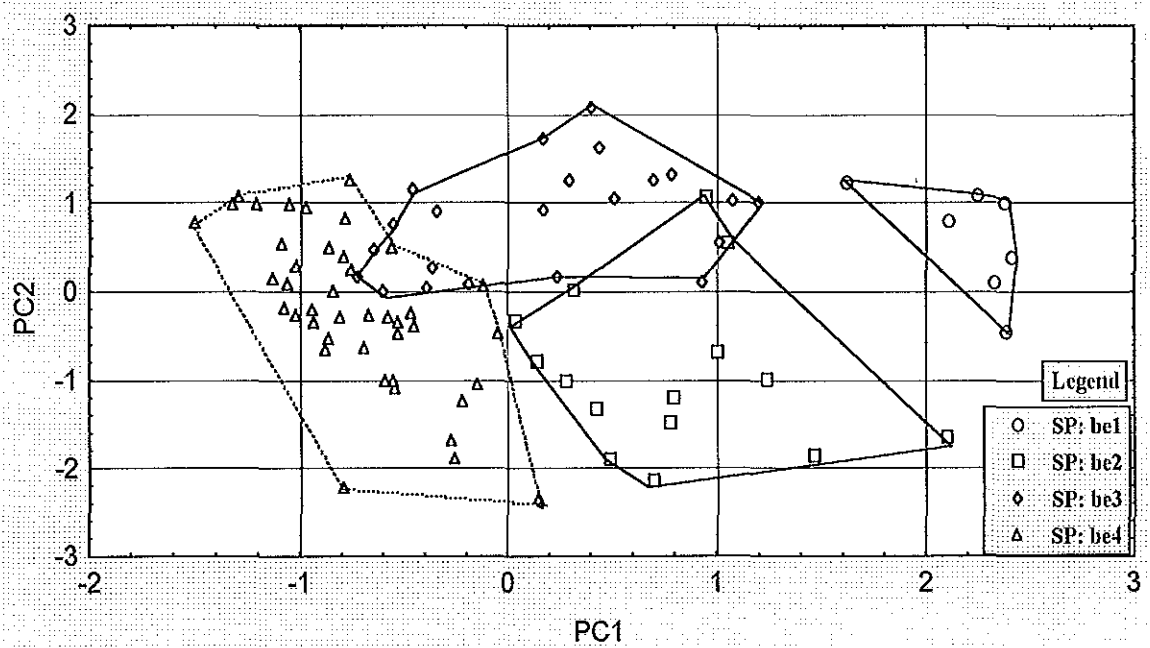


Fig. 4: Scatter plot (untransformed data) of the four groups on PC1 vs PC2 ordination

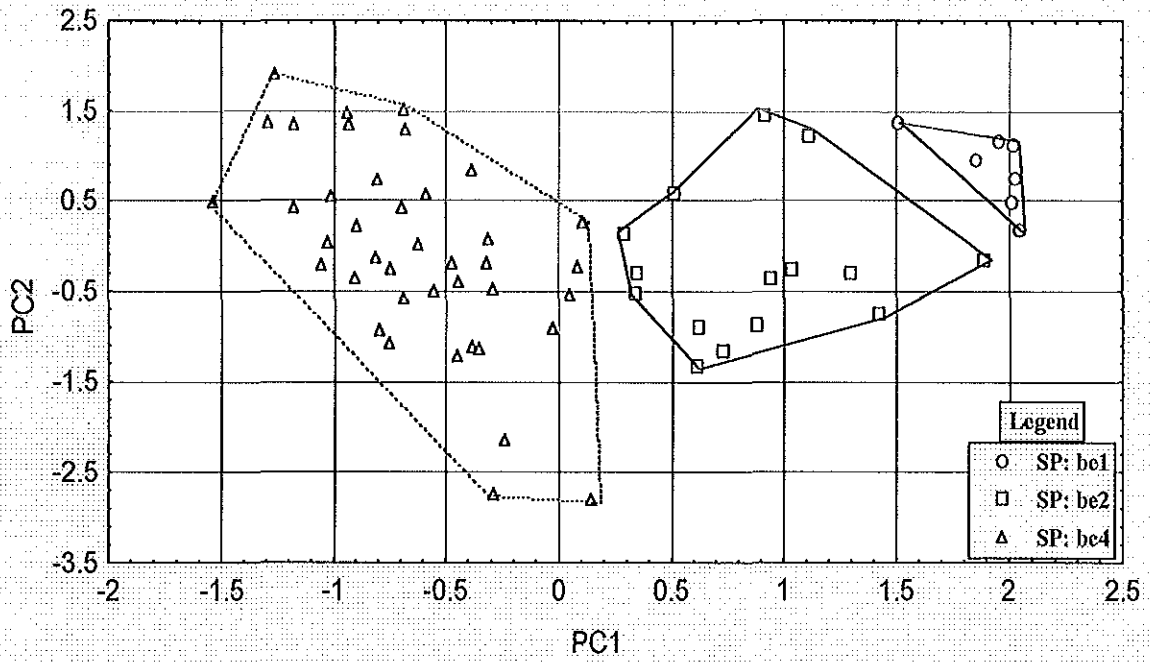


Fig. 5: Scatter plot (PC1 vs PC2) of clusters be1, be2 and be4 when be3 is excluded

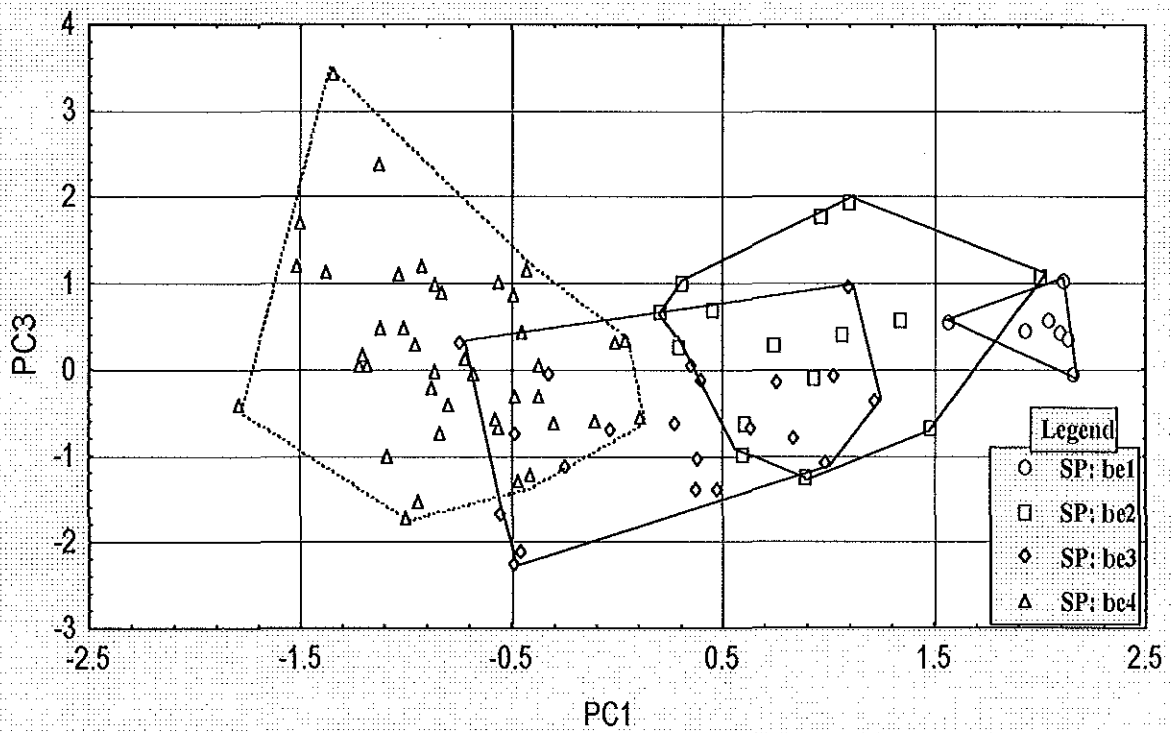


Fig. 6: Ordination of the four groups when PC3 is plotted against PC1

*SP represents the specimens in the individual groups

Table 3: Eigenvalues (variance) of Principal components 1 to 4

	Eigenvalue	% total Variance	Cumululative Eigenvalue	Cumul. %
1	9.641153	53.56196	9.64115	53.56196
2	1.697961	9.43312	11.33911	62.99508
3	1.155067	6.41704	12.49418	69.41212
4	.942584	5.23658	13.43677	74.64870

Table 4: Factor loadings of principal components (* loadings > .700000)

	Axes			
	1	2	3	4
GBL	.550458	.325119	-.148990	.581850
GBW	.647161	.268251	.147093	.496706
BSL2	.489674-	.436947	.340544	.164355
BTL	.699783	.033426	.383692	-.041596
CLL	.892735*	.059546	.036831	-.018594
CML	.864769*	.093722	.215515	.019190
CSL	.722349*	-.033892	.206788	-.170071
COL	.852332*	.271386	-.179102	-.128614
COB	.787473*	.388514-	.189221	-.018913
FL	.731385*	.016003	-.329610	-.211475
PR	.495588	.243009	.496612	-.402819
PAL	.834092*	.199769	-.010520	-.116882
AAL	.786342*	.227252	-.070425	-.010985
SYL	.814026*	.093646	-.278868	-.141026
BSN1	.661547	-.478132	.189202	.102140
BSN2	.735056*	-.405989	.134831	.165677
LBMS1	.716439*	-.517195	-.312151	-.046747
LBMS2	.726797*	-.519259	-.316351	-.036275
PAB	.45674	.185678	.162749	.048438
LBMSC	-.120191	-.170278	-.789426	.027186
COC	-.020120	-.101389	.726076	-.383757
AAB	.14379	.108799	.560501	.367023
BTB	.28553	.240986	-.595907	.067081
CLB	.49557	.250901	-.492473	.354512
CMB	.68532	.363977	-.246957	.105504
LBL	.29518	-.209330	-.363094	.483726
LBW	.53141	.228460	.092430	.565428
Expl. Var	10.42172	1.898938	1.652368	1.257329
Prp. Tot	1.45312	.082563	.071842	.054666

Generally, character states CLL, CML, COL, PAL, SYL, COB, AAL, BSN1, BSN2, FL, LBMS2, LBMS1, CSL and BSL1 were singled out as the major contributors to the



segregation of the clusters. However, due to persistent overlap of the two ends of be3 group with be2 group and be4 group, Discriminant Analysis that maximizes variability between groups while minimizing variability within them was undertaken.

4.1.3 Discriminant Analysis

Discriminant analysis (DA) of the 23 variables of both transformed and untransformed data separated all the four clusters (figs. 7 & 8). However, this was true when the first two axes were used in the analysis. For example, scatter plot between 1 and 3 axes showed overlap between group 2 and group 3 (fig. 9).

Probability level for the similarity of these groups was zero ($p < .0000$). Thus the chances that the probable taxa are similar are minimal implying that there is a high significant difference between the four groups. Classification matrix of observed classifications (rows) and predicted classification (columns) showed hundred per cent correct identification for the groups except in group be4 with ninety seven per cent identification (table 5) citing Kiboko collection by *J. Ossent 533* as a wrong identification to this group.

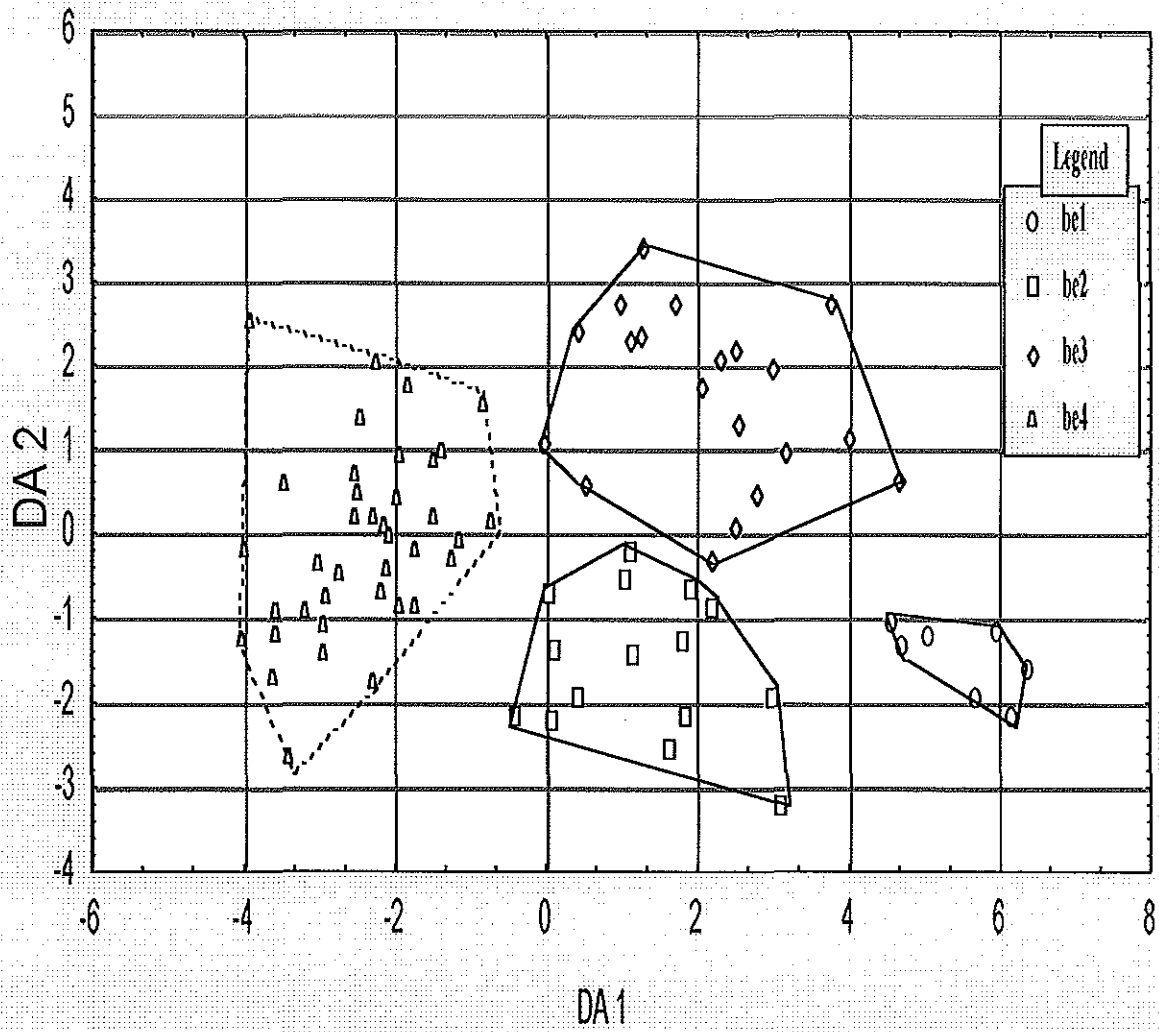


Fig. 7. Scatter plot of Discriminant Analysis between DA 1 vs DA 2

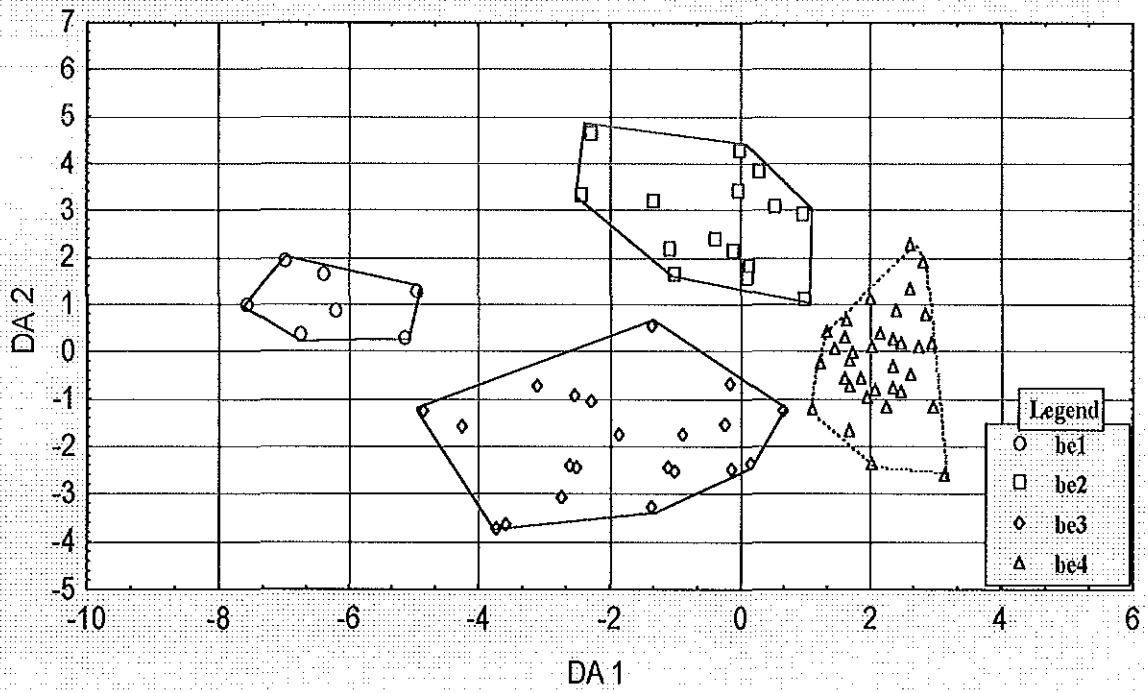


Fig. 8: Scatter plot of Discriminant Analysis (untransformed data) of DA 1 vs DA 2

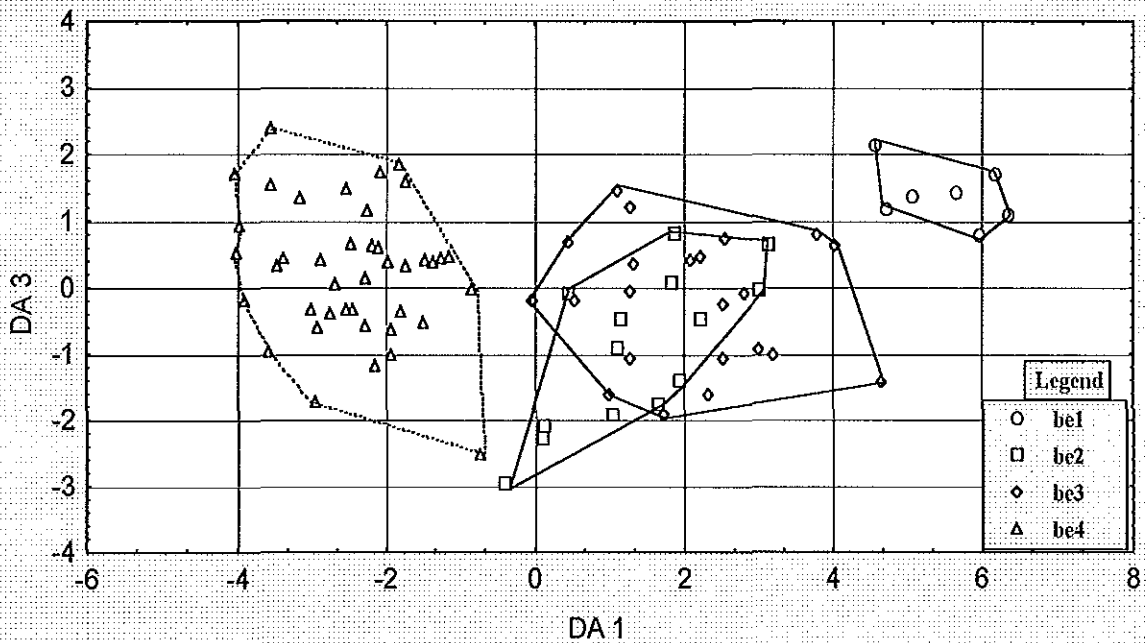


Fig. 9: Scatter plot of Discriminant Analysis of DA 1 vs DA 3

Table 5: Classification matrix of observed classifications (rows) and predicted classification (columns)

Percent	be1	be2	be3	be4
Correct	p=.08140	p=.17442	p=.25581	p=.48837
be1 100.0000	7	0	0	0
be2 100.0000	0	15	0	0
be3 100.0000	0	0	22	0
be4 97.6190	0	1	0	41
Total 98.8372	7	16	22	41

The DA results, therefore, affirms the findings in cluster analysis and PCA that these groups are different. Correspondingly, axes 1 and 2 short-lists the most important variables used in separating the four groups. However, because DA maximizes variability between groups, be1 and be4 are still located quite apart in the graph.

Similarly, groups that are phenotypically related are placed next to each other such as be2 and be3 and be1 (figs. 7, 8 & 9).

Sampling of the specimens, however, relied on availability of herbaria material.

Besides, cluster analysis and consequent PCA as well as DA used groups of unequal size. Therefore, Non- Parametric Analysis was conducted to further investigate the above findings.

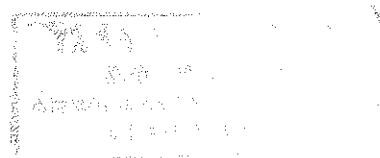


Table 6: DA factor structure matrix of correlations variables (Canonical Roots pooled-within-groups correlations)

	Root 1	Root 2	Root 3
BL	.144538	-.254090	-.287192
BW	.240877	-.499841	-.151913
BSL2	.237534	-.057711	-.226385
BTL	.240601	-.340943	.058813
CLL	.365904	-.343775	-.216532
CML	.351973	-.493820	.111721
CSL	.291045	-.187561	-.128985
COL	.336790	-.285876	.023233
COB	.290055	-.387024	-.005801
FL	.305311	-.118474	-.535239
PR	.142228	-.396456	-.041817
PAL	.356079	-.381049	-.151232
AAL	.321496	-.340820	-.114790
SYL	.309435	-.213788	-.262008
BSN1	.287163	-.166548	.182566
BSN2	.385932	-.295525	.355573
COC	-.041108	-.028952	-.028049
LBMSC	-.003251	.112673	.074188
LBMS1	.664868	.379615	.072372
LBMS2	.704051	.378884	.092885
LBL	-.124677	.039860	.190837
LBW	-.178691	-.215094	-.032667

4.1.4 Non-Parametric Analysis

The NPA reinforced extraction of the important taxonomic variables as found in the last three methods above. Importantly, variables used to separate between every two groups (6 combinations in table 7) have been outlined. The variables with p-values less than 5% significance levels were noted as the major contributors to the taxonomic delimitation for the groups. The less the relationship between any two groups, the fewer are the variables used to segregate the groups and the reverse is also true.

Group be1 and group be2 are differentiated largely by characters of, in the order of importance, number of spines on leaf and bract margins, style length, corolla length, anther (anticous) length, calyx (posticous) length, anther (posticous) length, calyx (anticous) length, corolla width, and bractiole length. Group be1 and group be3 are distinguished by anther (posticous) length, number of bract spines, corolla length and width, calyx (posticous) length, bract width, calyx (anticous) length, bractiole length, anther (anticous) length, style length, number of leaf marginal spines, calyx (ventral) length, processes length, calyx (anticous) width, anther (anticous) width, calyx (posticous) width and bractiole width. Group be1 and be4 are distinguished by almost all the characters, especially anther (anticous and posticous) length, bract spines, calyx (anticous and posticous) length, corolla width, number of leaf marginal spines and bractiole length, except (posticous) width.

In addition, group be2 and be3 are delimited by number of leaf marginal spines, bract width, processes length, anther (posticous) length, corolla width, calyx (posticous) length, anther (anticous) length, calyx (anticous) length, bract length, anthers (anticous) width, bractiole length, corolla length, filament length, leaf spine cover and corolla

colour. Group be2 and be4 are differentiated by anther (anticous) length, filament length, calyx (anticous) length, bract length and width, anther (posticous) length, style length, calyx (ventral and posticous) length, corolla length and width, number of leaf marginal spines, bractiole length, number of bract spines, bractiole width, calyx (anticous) width, anther (anticous) width, processes length and corolla colour. And finally, number of leaf marginal spines, filament length, calyx (anticous) length, style length, calyx (ventral) length, number of bract spines, corolla length, bract spine length, anther (anticous and posticous) length, calyx (posticous) length, corolla width and leaf spine cover separate group be3 from group be4. The actual sizes and the concomitant discontinuities for these characters of the groups are tabulated in appendix I.

Table 7: Adjusted Z p-values of Mann-Whitney U Test showing variables (p< 0.05) used to separate between groups

Species	be1 Vs be2	be1 Vs be3	be1 Vs be4	be2 Vs be3	be2 Vs be4	be3vs be4
Variables	p-level					
BL	0.887538	0.062314	0.004794	0.006362	1.46E-05	0.134635
BW	0.052417	0.000149	2.73E-05	2.13E-05	1.73E-07	0.687839
BSL2	0.127724	0.13655	0.000161	0.750017	0.000129	0.000514
BTL	0.019078	0.000301	0.000103	0.013743	0.000104	0.14215
CLL	0.014014	0.000202	4.77E-05	0.006327	1.02E-07	0.000106
CML	0.007418	0.000138	1.65E-05**	0.005214	1.97E-06	0.037019
CSL	0.027949	0.000929	1.71E-05	0.139526	1.34E-05	0.000343
COL	0.002597	8.92E-05	2.56E-05	0.032999	5.04E-05	0.000958
COB	0.01894	6.6E-05**	2.2E-05**	0.004177	8.92E-05	0.044954
FL	0.638069	0.055637	3.78E-05	0.035985	7.68E-08	2.09E-05
PR	0.203488	0.001016	0.000152	0.003576	0.000605	0.771325
PAL	0.00367	0.000318	8.56E-07**	0.006177	2.62E-08	0.00189
AAL	0.01107	3.82E-05	2.98E-06**	0.002637	1.27E-06*	0.001982
SYL	0.00157	0.000395	2.74E-05	0.020319*	6.42E-07*	0.000446
BSN1	0.000819**	7.32E-05**	9.13E-06**	0.847278	0.002871	0.000587
BSN2	0.000452**	6.18E-05**	1.06E-05**	0.490589	0.000149	0.000338
LBMS1	0.000201**	0.000879	2.29E-05**	5.48E-06	1.2E-05*	5.64E-11**
LBMS2	0.000199**	0.001545	2.26E-05**	1.61E-05	4.9E-06*	6.11E-11**
BTB	0.159996	0.025493	0.000583	0.319221	0.007068	0.06604
CLB	0.08163	0.003407	0.000793	0.273268	0.055753	0.123095
LBMSC	0.000874	0.516590	0.000712	0.000172*	.258241	0.000539*
COC	0.002745	0.802279	0.002136	0.008168*	.892440*	0.003861
CMB	0.179482	0.003487	0.00028	0.006623	2.99E-05	0.028156
PAB	0.455567	0.003415	0.001316	0.012155	0.005025	0.915974
LBL	0.000614	0.092048	0.002802	0.036598	0.726532	0.052948
LBW	0.152296	0.016324	0.001316	0.040187	0.001797	0.149273
AAB	0.286348	0.572706	0.340363	0.275328	0.744445	0.341197

** Discontinuous characters used for inter group's delimitation

* Partial between group's discontinuities

4.2 Seedling morphology

Observations of the seedling morphology entailed seedlings raised at the nursery from different populations under similar environmental conditions. In order to further investigate these features, field trips activities also incorporated seedling morphology studies as earlier indicated and results were marched with cluster analysis results as summarized in table 8 over slip.

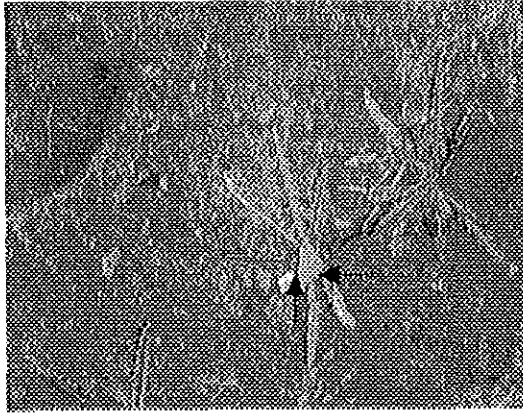
Importantly, seedling morphology records difference between groups. Whilst the cotyledons colour, leaf marginal spines and terminal spine at the lowest basal node character states are purple, about 23 and recurved downwards, respectively, in groups be2 and be4, these characters are green, up to 11 or absent for be4 and straight or recurved (upwards) terminal spines. More so, the leaves of group be3 are dark green and the branching almost to the ground as opposed to light green leaves and raised branching in groups be2 and be4 (fig. 10). However, they have shown some commonness for a record presence of six leaves at the base and four elsewhere. Also the leaves are unequal, the longer ones facing outwards.

Moreover, mature dry plant remains has shown that floral parts for groups be3 and be4 are smaller as compared to group be4. However, it was not possible to undertake seedling morphological studies for group be1 citing unavailability of seeds.

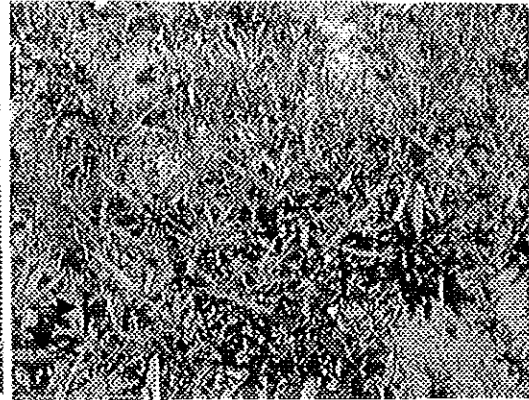
Table 8: Seedling morphological characters

Characters

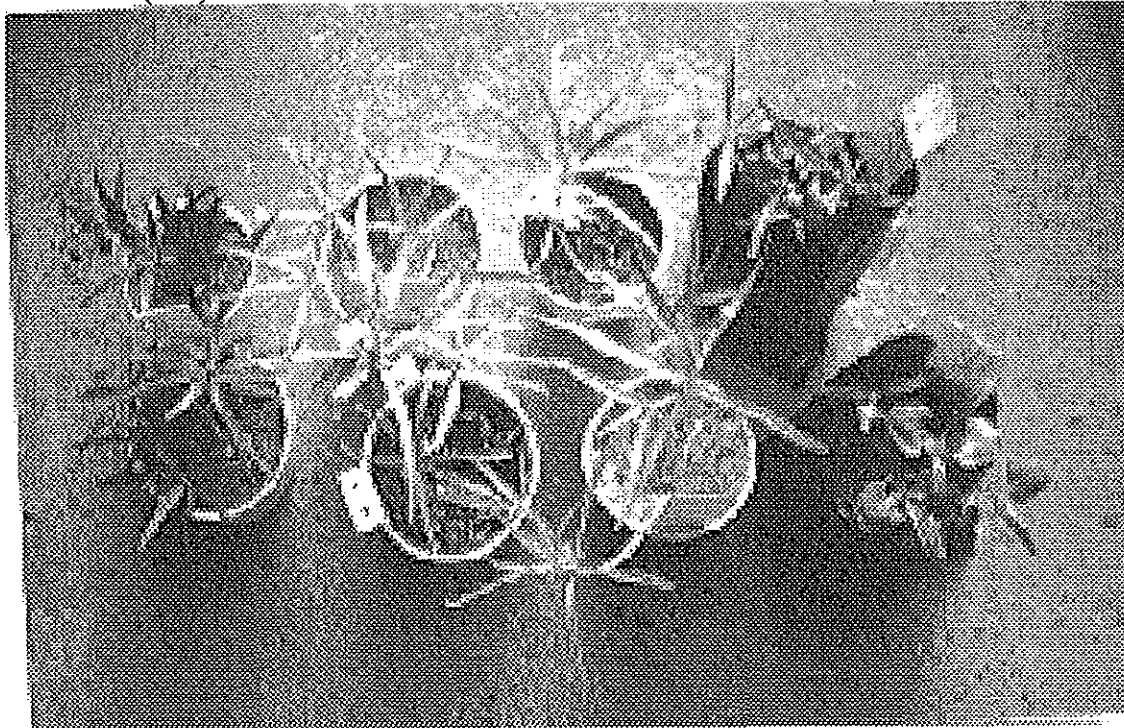
Group	Cotyledons				Stem/branch				Leaves			
	Colour below	Colour Above	Hair	Growth rate	No. base	No. other nodes	Size	Petiole length (mm)	Shape	Spine no.	Terminal spine	Hair
be2	Green	Purple	Glabrous or so	Slow	6	4	Unequal	2	Linear-lanceolate	6 -11	Straight/Recurved up	Glabrous
be3	Purple	Purple	Pubescent	Slow	6	4	Unequal	Sessile/1.4	Elliptic	20-23	Recurved down	Pubescent
be4	Green	Purple/green	Puberulent	High	6	4	Unequal	3mm	Linear – lanceolate	Abs/ <6	Straight/Recurved up	Puberulent



A (be3)



B (be4)



C be3

be2

be 4

be4

Fig. 10 Seedling morphology

Purple and green cotyledons of be3 (A) and be4 (B), respectively, where the seedlings are in their natural habitat. C shows rows of the three taxa potted and in greenhouse. Notice the dark green leaves in be3.

4.3 Palynology

Pollen is isopolar and tri-colpate. Outline in polar view is circular to triangular, elliptic in equatorial view (Figs. 11 B,D,C; 12A, D; 13 A & 14 A,C). There is a slight difference of the pollen shape observed amongst the groups. Where as group be1 and group be3 have perprolate shape the other two groups (be2 and be4) have prolate to subspheroidal shape (table 9). However, group be4 has a single specimen containing pollen with subspheroidal shape (Fig. 14 A & B).

For the sizes, group be4, on average, recorded the smallest pollen size at polar view (36.4 –42.6- 46.9 μm), colpus length (29–34.95-39.73 μm) and polar/ equatorial ratio (1.1–2.05- 2.51). The largest sizes for the polar view, colpus length and equatorial length, on average, were found in group be2 recorded as 47.1 μm , 38.9 μm and 24.07 μm , respectively. These features appeared to have a slight overlap for groups' be2 and be3 with group be3 recording slightly, on average, lower figures except at polar length. For example, the colpus length was found to be 37.12-(38.9)-40.6 μm in be2 where as in be3 was ranging from 32.69-(36.12)-37.7 μm (table 9).

Moreover, the colpus width character is found to create discontinuities between group be2 and be3. These sizes are, between the former, 2.83–(3.11)-3.54 μm and 2.04–(2.19)–2.6 μm .

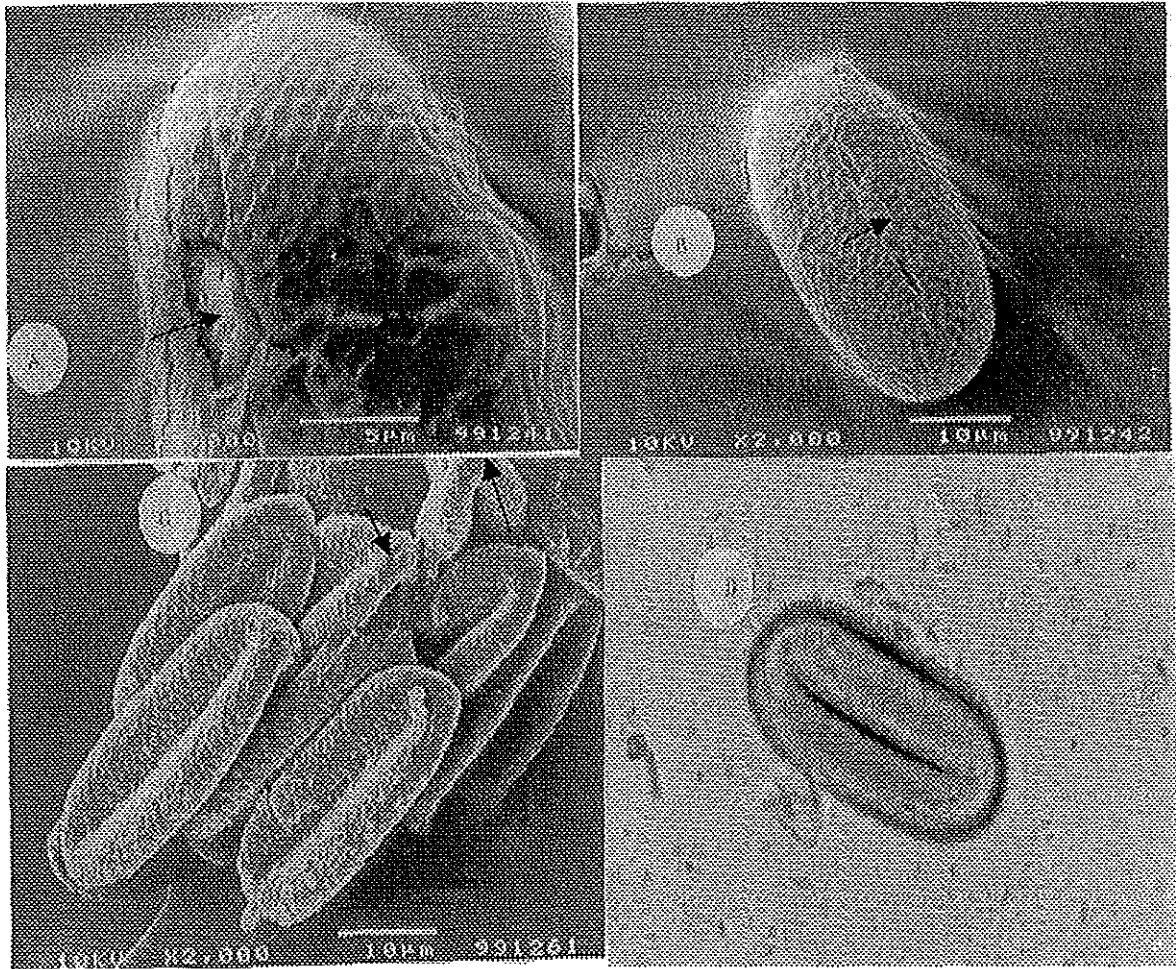


Fig. 11: Pollen morphology of *B. boranensis*.

A, B and C SEMgraphs and D LMgraph ($\times 1000$). A; details of a constriction along the colpus, B; equatorial view, isopolar and constriction as in A, C; polar view, triangular without constriction and D; equatorial view, elliptic and colpus not reaching the poles as in B and C.

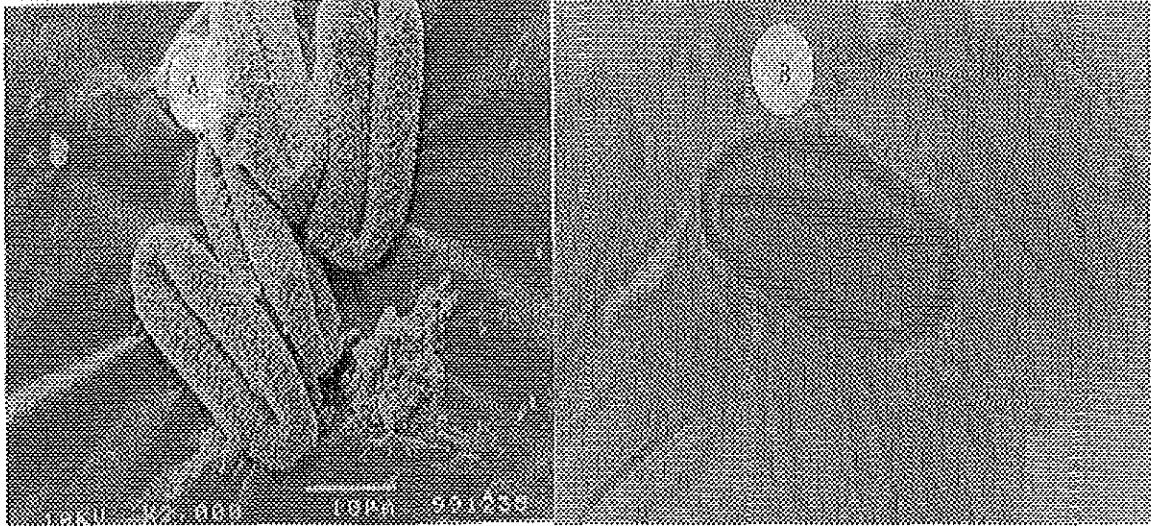


Fig. 12. Pollen morphology of *B. edulis* var. *glabra*

A, SEMgraph, triangular, B; LMgraph ($\times 1000$) equatorial view, colpus not reaching the poles

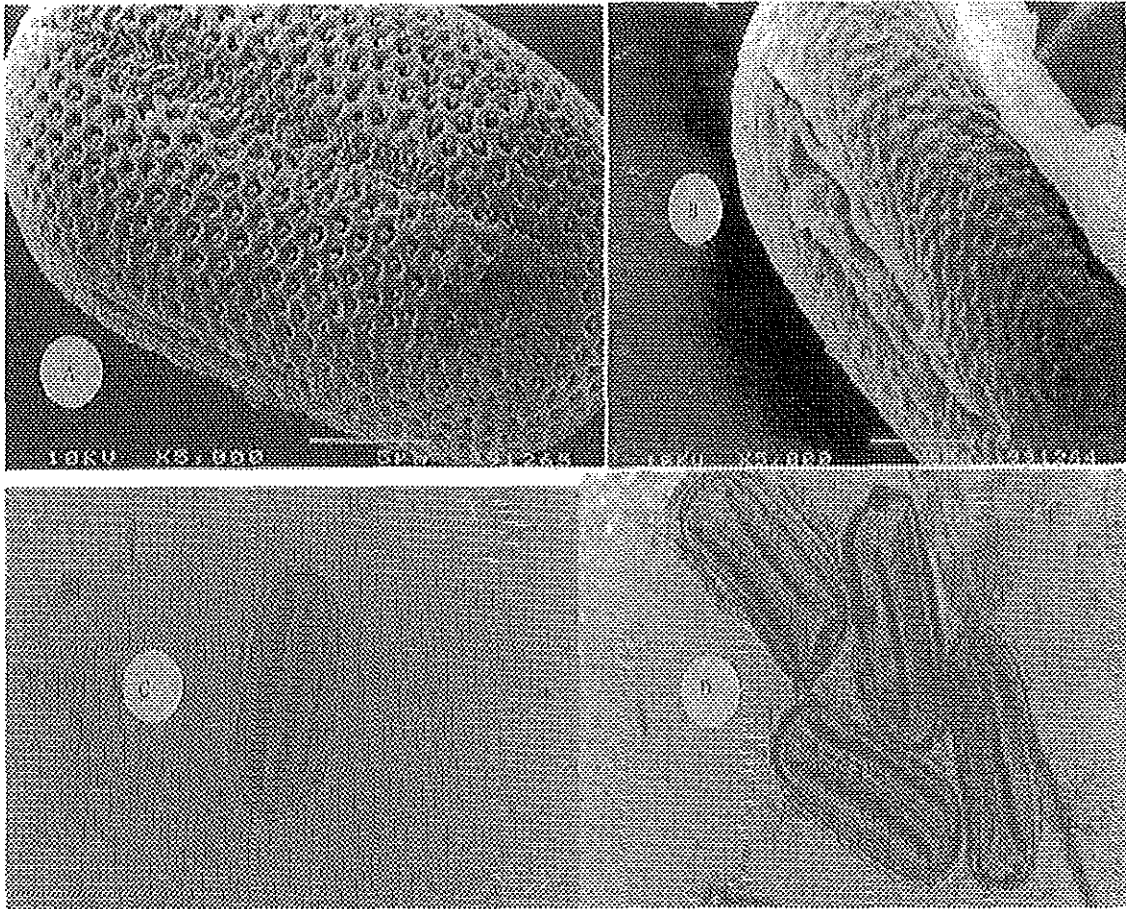


Fig. 13: Pollen morphology of *B. edulis* var. *edulis*

A and B (SEMgraphs) equatorial, isopolar, smooth muri, hexagonal reticulum, single columellae on perforations and colpus shallow and nonarticulate. C and D (LMgraphs $\times 1000$) equatorial view, colpi not reaching the poles and tips tend to tilt outside.

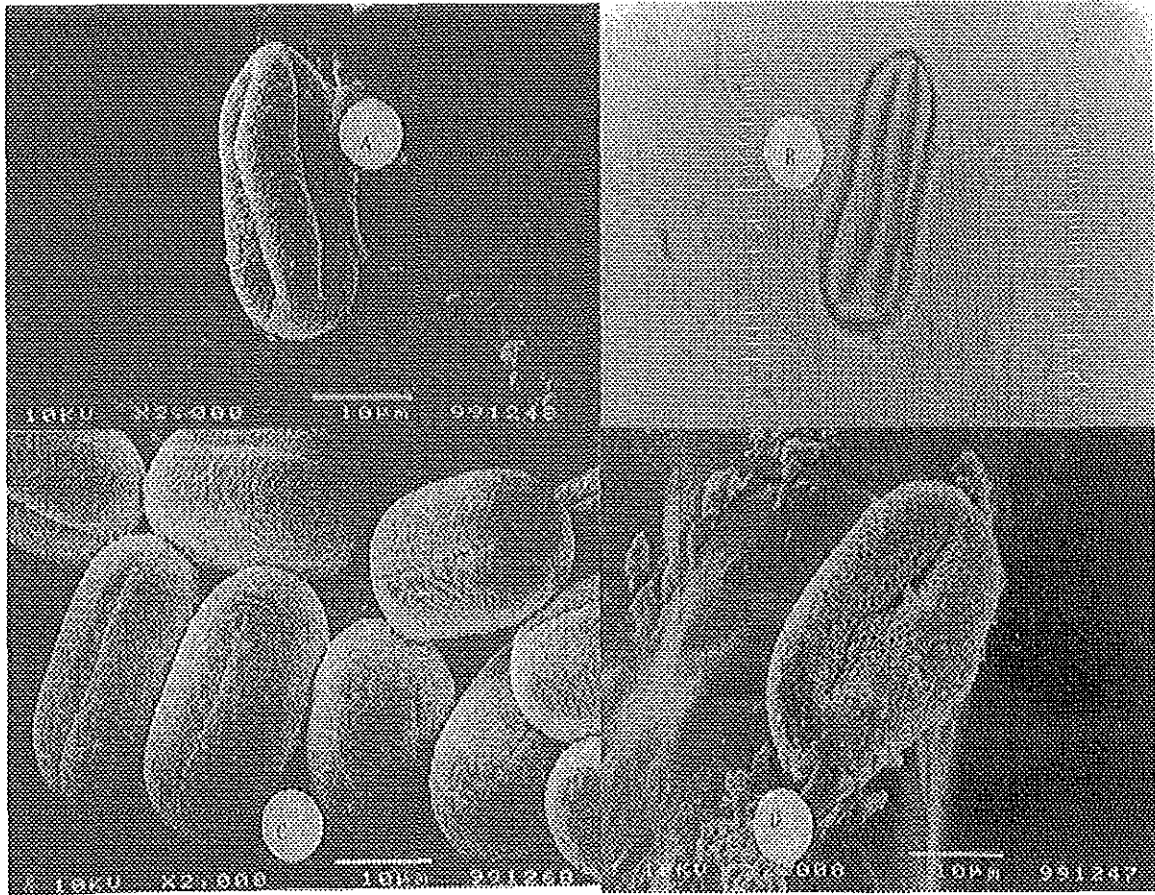


Fig. 14: Pollen morphology *B. edulis* var. *isabellae*

A (SEMgraph) and B (LMgraph (×1000).); equatorial view, depression/ narrowing of grains at the middle. C (SEMgraph); isopolar nature of the grains as in figs. 11-13 above and D (SEMgraph); constriction along colpi.

However, except the colpus length, the pollen sizes are in a continuum. As such, complete overlaps were observed between most of the groups except between group be2 and group be3 where the overlap was partial in several pollen character states.

The sculpturing of the four groups is also similar in several ways. For example, they are all characterized by a smooth muri and hexagonal reticulum in which single columella is visible in each perforation of the lumen (Figs. 11-14 above). Nevertheless, differences also occur. The diameter of the reticulum lumen, for instance, differs from group to group except between group be2 and group be3. Group be4 has the biggest size of lumen size of 1 μm where as be1 has, the medium reticulum lumen diameter, size of 0.6 μm . Group be2 and group be3 have the same and the smallest reticulum lumen diameter size of 0.5 μm . Besides, group be1 is further segregated from the other three groups by appearance of a constriction like embeddement, though not consistent, located at the center of the colpi as to block its stretch of the width.

Table 9: Pollen morphology data

Sp	Voucher and origin	Polar L μ m	Equatorial L μ m	P/E	Sh	Colpus L μ m	Colpus W
Be1	Sebsebe & Ensermu 870508-3/4(Et)ETH	40.31	18.85	2.11	perp	30.16	3.30
	Ensermu & Adchella 4012 (Et) ETH	46.95	22.33	2.155	perp	40.6	2.79
	Average	40.31-(43.6) -46.95	18.85-(20.34)-22.33	2.11-(2.13)-2.155	perp	30.16-(35.38) -	2.79 -(3.05)-
Be2	J. Bos & Jansen 10072, (Et) ETH	47.56	22.91	2.097	perp	38.57	2.98
	Mat Thulin 1309 ETH (Et)	45.82	24.07	1.926	pr	37.12	3.07
	W. J. deWilde 9722, (Et) WAG	48.43	23.78	2.062	perp	39.44	2.83
	S. A. Robertson 1089, (K7) EA	46.4	20.3	2.286	perp	40.6	3.54
	Average	45.8-(47.1) -48.43	20.3-(22.77)-24.07	1.934-(2.09)2.29	perp	37.12-(38.933) -	2.83 -(3.11)-
Be3	Ensermu & Tamrat 401, (Et) ETH	46.88	20.3	2.328	perp	37.7	2.19
	Ensermu & Patron 1869, (Et) ETH	40.02	17.11	2.358	perp	33.64	2.6
	Beentje 1860, (K4) EA	42.92	19.14	2.242	perp	35.96	2.12
	p.NappierTNP/R/66 (K7) EA	40.86	18.718	2.193	perp	32.691	2
	W. J. deWilde 5943, (Et) WAG	45.53	21.17	2.167	perp	37.7	2.04
	Average	40.9-(44.1) -46.9	18.56-(19.59) -21.17	2.17-(2.27) -2.43	perp	32.691-(36.12) -	2 -(2.19) -2.6
Be4	Bentjee 934, (K4) EA	43.21	19.72	1.857	pr	29	2.36
	Muasya & Malombe 1531 (K3) EA	42.92	20.59	1.911	pr	35.96	2.01
	H. F. Mooney 9523, (Et) ETH	45.82	24.09	1.954	perp	37.99	2.36
	Mesfir, T 2908, (Et) ETH	46.4	22.62	2.059	perp	38.28	2.83
	M.L.Modha 11, (K2) EA	45.34	21.75	2.089	perp	37.99	2.38
	M. S. Natrass 779, (K4) EA	45.82	22.91	2.038	perp	39.73	3.19
	D.L. Coppock 24, (K2) EA	46.4	20.3	2.286	perp	37.7	2.36
	A. Vincens 77 (K3) EA	39.73	15.95	2.513	perp	33.93	2.36
	Y. E.Symes 139 (K2) EA	36.44	18.64	1.973	pr	30.24	2.71
	J. B. Gillett 21201, (K1) EA	40.02	17.38	2.238	perp	31.32	3.07
	Vessey & Wales 16 (K2) EA	46.88	19.82	2.37	perp	38.67	2.95
	Gilbert et al 5667 (K1) EA	43.79	20.05	2.34	perp	35.67	3.07
	Bally J 1829 (K1) EA	36.44	18.64	1.97	pr	30.24	2.36
	A. abdikadir 37, (K1) EA	41.33	21.75	1.911	pr	31.5	2.66
	Sebsebe & Ensermu 2745, (Et) ETH	43.5	20.3	1.095	sbsprd	34.8	2.36
	J. Ament & et al 43, (K4) EA	38.86	20.3	2	perp	31.9	2.36
	Average	36.4- (42.6)-46.9	15.95-(20.4)-24.07	1.1-(2.05)-2.51	perp	29-(34.95)-	2.01 -(2.59)-
						39.73	3.19

Perp – perprolate: P(polar) axis more than twice as long as E(equatorial) axis ($P/E > 2.0$), **pr** – prolate: P longer than E ($P/E = 1.33 - 2.0$), **sbsprd** – subspheroidal: p about as long as E. ($P/E = 0.75 - 1.33$). Palynological terms adapted after Erdtman (1969).

CHAPTER 5: DISCUSSION

5.1 Gross Morphology

5.1.1 Cluster analysis

The UPGMA cluster analysis method shows that the 171 OTUs can be grouped into four clusters (be1 to be4) with the first three clusters closely linked. Comparison of these results with univariate analysis (appendix I) shows that both vegetative, especially the leaf marginal spines, and floral characters separate the clusters reaffirming the use of the former in the taxonomy of the genus *Blepharis* as employed by Clarke (1899–1900). Still based on leaf spine character, cluster be3 is closely associated with cluster be1 in which LBMS ranges as 20 ± 5 and 25 to 36 respectively and cluster be2 with cluster be4 with the same character ranges recorded as 4 to 14 and 2 to 10. In groups' be2 and be4 the spines are loosely arranged. Moreover, the leaf marginal spines arrangement overlaps (cover) partially as to form discontinuities within these, be1 and be3 and be2 and be4, combinations. Cluster be3 records the highest standard deviation (4) for the leaf spines and hence becomes the most diversified for this group as to differ with be1. In group be1, the leaf spine cover shows the spines are normally to the tip unlike in be3 where the spines are either to the tip or to three-quarters of the leaf length. Otherwise these two clusters, be1 and be3, are further distinguished by a discontinuous number of spines of the bracts which ranges from 9 to 11 and 4 to 8, respectively.

Geographically cluster be1 and cluster be3 are discrete where the difference is altitudinal. Cluster be1 is concentrated in Borana and rarely higher altitudes of Jijiga area of Ethiopia about 1500 m to 1850 m above sea level, where the soil is mainly limestone. Conversely, cluster be3 occupies lower altitudes of Borana Awraja (e.g. OUT *Ensermu Kelbessa* 1192) and Jijiga as well as the southerly Kenyan districts (Voi and Taita–Taveta) of K7 up to Northern Tanzania in Lushoto District where the soil is either alluvium or red and sandy on flattish grounds. Otherwise, OTUs contributing to be1 are found to be perennial herbs with woody base whereas be3 is normally an annual herb and rarely semi-perennial.

The relationship between group be2 and group be3 is depicted by partial overlap in most of the characters including the vegetative characters. They are specifically and partially distinguished by corolla colour, leaf marginal spines cover (LBMSC), number of leaf marginal spines and processes length. For group be2, whilst LBMSC is predominantly up to two-thirds and spines loosely arranged, in be3 the same character, spines are largely up to the tip and hardly at three-quarters leaf length and serrated or serrulate. Also, be3 corolla colour is blue or purple as opposed to purple or pink corolla of cluster be2.

5.1.2 Principal component Analysis

The PCA capacity to separate these groups is made possible by both vegetative and flora character states in support of the cluster analysis and concomitant purported taxonomic delimitation in *B. edulis* based on gross morphology (Vollesen, in prep.,

Furness, 1997). Particularly, important characters, in order of importance, are isolated as calyx (posticous) length, calyx (anticous) length, corolla length, anther processes length, style length, corolla width, anthers (anticous) length, number of spines on bracts margin, filament length, number of leaf marginal spines and inner calyx length. These characters are concentrated in the first axis. Furthermore, exception of this axis in PCA did not separate any of the above groups imparting that important taxonomic characters are concentrated in the first two axes (PCs).

These results also show that be1 is unique as it was always segregated from the other groups and furthest on PC1 axis followed by be2, be3, and be4 due to big morphological features, respectively. Moreover, the separation of groups' be1, be2 and be4 when be3 is excluded imparts that these groups are taxonomically autonomous and hence different. Group be1 is separated from groups' be2 and be4 mainly by leaf and bract character states. Quantitative and qualitative characters portray only partial discontinuity between group be2 and group be4. These characters include the seemingly important characters such as style length, anticous anther length and anther processes length. However, these results are interpreted with a lot of caution. According to Tabachnick & Fidel (1989), sample size of the smallest group should exceed the number of predictor variables, which is not the case of be1 and be2.

More so, the partial embeddement of both ends of group be3 to both premises of group be4 and group be2 emphasizes the ifraspecificity of the three groups and group one as separate but a related species. These could be supported by lack of complete

geographical descretion of some be2 OTUS in their distribution (Fig. 2). For example, be2 OTUs such as *P. Hucks* 1051 and *J. H. Padwa* 157 overlaps with be3 distribution. Furthermore, group be4 contains outliers such as OTUs 12, 48 and 51 implicating intermediaries between other close groups such as be2 and be4 hence lack of clear separation in ordination space. This suggests that group be2 might have arisen through hybridization as witnessed by its close location to either group be3 or group be4.

In summary, PCA seems to corroborate the cluster analysis, again showing that the diagnostic characters applied by Clarke (1899–1900) and advocated for by Vollesen (ined.) are useful in distinguishing taxa in *B. edulis* complex and probably for the whole genus. However, these characters are not enough to separate clearly between groups be2 and be4. The unclear geographical separation amongst the three groups, e.g. be2, be3 and be4 near Kombolcha and be2 and be3 in K1 and Eritrea coastal regions along Red Sea, might have influenced their partial distribution overlap.

However, these partial overlap of geographical regions can be interpreted as somewhat different. This is because, for example between be2 and be4, the localities of be4 might be slightly different citing close proximity to the water masses and obvious environmental influence such as Koobi Fora by Lake Turkana in Kenya and Massawa areas in Eritrea by the Red Sea and thus group be2 might be termed as occupying a slightly different ecological condition from the other three taxa.

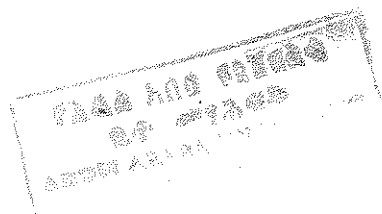
5.1.3 Discriminant Analysis

Both transformed and untransformed data in the DA using the same variables in PCA above separated all the four groups. This separation is supported by probability level of less than zero (0.000) implying that there is high significance difference between the groups. Subsequently, the individual groups are ordinated in accordance to their affinities. Groups be2, be3 and be4 are ordinated in a triangular manner to demonstrate their close affinities with be1 laid on their right and be4 to their left. It is therefore true to deduce that group be2 is clustered between be1 and be3 owing to its smaller and larger morphological character sizes, correspondingly, except the leaf spine characters which draw it near to be4 resemblance. This affinity alignment strategy is true for transformed data only. It is exactly opposite for untransformed data which shows negative allometry for previously positively ordinated groups.

Lack of separation between groups be2 and be3 when DA1 is plotted against DA3 axis shows that these groups have minimum characters, which are concentrated in the first three DA, separating them. It also imparts that the use of characters as in PCA above for infraspecific study of *B. edulis* complex.

5.1.4 Non - Parametric Analysis

Non-Parametric Analysis recapitulates the delimiting character states between groups. These are number of spines on both leaves and bracts margins, style length, corolla sizes and colour, anther (anticous and posticus) length and width, calyx characters (both width and width), bractiole length and width, bract sizes, processes length,



filament length, cover of spines on the leaf margins and length of spines on bracts. Importantly, combination of Non-Parametric Analysis results with descriptive statistical results (appendix i shows discontinuities, though partial in some groups, between groups in support of the results of Cluster Analysis, Principal Component Analysis and Discriminant Analysis.

Although group be3 and group be4 are discrete both morphologically (e.g. number of leaf marginal spines which is 4–10 and 12–25, respectively) and somehow geographically, sometimes altitudinal e.g. Jijiga. Differences between group be2 and group be4 and between group be2 and group be3 appear partial mainly supported by slight discrete characters such as style length, anther (anticous and posticus) length, number of leaf marginal spines and arrangement (cover), processes length and corolla colour. Besides, group be4 character description especially vegetative morphology concurs with that outlined by Clarke (1899–1900) that sometimes the leaf margins are devoid of spines.

Group be1 is discontinuously separated from all the other three groups by several morphological character states in favour of Vollesen (in press) OTUs by *Ensermu Kelbessa* 1052 and *Sebsebe Demissew & Ensermu Kelbessa* 2676) observations. This group seems to be endemic to the Borana Awaraja and few patches in Jijiga higher altitude zones of Ethiopia where the soil is mainly of limestone origin. However, more vigorous data collection in Borana Awaraja and its environs is required to capture extra data for this group.

5.2 Seedling morphology

Seedling morphology supports the phenetic evidence that infraspecificity delimitation is possible in this complex. However, it only separates group be3 from the other two (be2 and be4) consistently by the purple colour of the cotylendons, to add extra evidence to gross morphology characters, and the recurving of the terminal leaf spine and slow development of branches. Furthermore, the number of leaf marginal spines comply with the gross morphology evidence that group be3 is different from the others.

Indeed, it was interesting to record unusual character, common for the groups, not noted in the gross morphology. The leaf pairs at the lower most basal node are 3 (6 leaves) and four elsewhere as the plants continued to grow. The first pair may drop, in group be2 and be4, or be retained even after second or third internodes' development or else first spike flowering. This commonness or primitive behavior is here proposed as a sign of very close relationship of the groups probably the differences brought about by adaptation of the groups to suit the ever changing arid conditions.

5.3 Palynology

The shape of the pollen is found to deviate from prolate shape described by Furness (1997). Both group be1 and group be3 have shown only perprolate shape in which the polar axis is more than twice as long as equatorial axis. Group be2 and group be4 have both perprolate and prolate but on average indicating perprolate shape. However, group be4 contains a single specimen with subspheroidal shape.

The pollen sizes appear to be of medium size as outlined in Erdtman (1969) ranging from 36.44–48.43 μm. This agrees to the size spectrum of pollen in genus *Blepharis* with a range of P (32-46.2- 67) μm, E (16-24.6-42) μm and P/E 1.67–2.36 (Furness, 1996). For *B. edulis* complex, the ranges are P (36.4–48.43) μm, E (15.95–24.07) μm and P/E (1.1–2.51).

Thus the P/E ratio differ though in a single specimen collected from Sidamo region by *Sebsebe Demissew & Ensermu K. 2745* and designated as *B. edulis* because the average for the group is (2.05) quite within the expected bracket of the pollen size. The pollen grains in this specimen were very few and might have contributed to a low average. Moreover, the specimen has shown subspheroidal pollen shape deviating completely from the general shape of the observed pollen. This deviation can be explained as one of the abnormalities of pollen recorded in this genus especially sect *Acanthodium* whereby some species such as *B. mitrata*, *B. aspera*, *B. capensis*, *B. diversispina*, *B. grossa*, *B. obmitrata* and *B. subvolubilis* contain about 1 to 5 grains of pollen (Furness, 1997).

The pollen colpus width indicates discontinuities between groups be2 and be3 supporting their taxonomic delimitation. Therefore, pollen size has proven to be important as taxonomic evidence to delimit the four taxa. This is because, although showing similarity based on pollen length, equatorial view, P/E ratios and colpus length, they portray dissimilarities at colpus width. Further, partial discontinuities are

also found between group be2 and group be3 where the overlap is slightly partial suggesting some differences.

The pollen sculpturing also supports the separation of the above taxa. For instance, the lumen widths have proved to be different between the groups except again between group be2 and group be3. Group be2 and group be3 though with slight differences in pollen sizes records the same lumen diameter and the smallest size of 0.5 μm . For group be1, the width of the lumen is 0.6 μm where as in group be4, the largest size, is 1 μm . Group be1 is further distinguished from the rest by the conspicuous constriction along the colpi, a feature, observed in this genus for the first time, is probably an abnormality. Furness (1997) reported abnormal pollen grains in *Blepharis* species. According to her, abnormal pollen is usually rudimentary and their sculpturing patterns may be disrupted near the apertures. However, these result supports cluster analysis and the PCA that these groups are closely related. It also supports the taxonomic separation between group be1 and group be4.

Moreover, other sculpturing features of the pollen have been reported before in most of the *Blepharis* species such as Gillet *et al.*, 5667 (*B. ciliaris*). Furness (1996) observed roughly hexagonal reticulum with smooth muri and columellae appearing in the lumen perforations and therefore these results confirm her findings.

Abnormality of pollen features demonstrates that the pollen mother cell meiosis in Acanthaceae may be irregular, leading to the production of abnormal pollen. This

suggests that pollen characters in Acanthaceae are plastic and actively evolving. It may also imply that the species may be of hybrid origin (Furness, 1996, Furness, 1997). These support the purported infraspecific treatment of *B. edulis* complex as indicated by the complete or partial pollen differences in terms of shape and size among the four groups attesting to results arising from gross morphological differences. Breeding studies in *B. edulis* complex populations, therefore, might cast extra insights to this enigma. Indeed, Schilling (1992) decries that until palynology comprises rigorous anatomical comparisons that are fully integrated with other morphological characters, in character congruence studies, the relationship between pollen data and classification will remain disputable.

CHAPTER 6: TAXONOMY

6.1 Taxonomic implication

Clarke (1899–1900) used the characters of leaf; spines (number, density), shape, corolla, bracts and bracteoles to separate 50 species in genus *Blepharis*. Vollesen (ined.), an advocate of these features, probably used the same to delineate close to 126 species in the genus. Consequently, in this study, these vegetative and floral character states coupled with seedling morphology including pollen study have been employed to delineate *B. edulis* complex.

Modern taxonomic methods such as discontinuity in two or more independent characters of morphological character states by way of phenetic arguments have been employed for the species concept in this study. Species is the smallest natural populations permanently separated from each other by a distinct discontinuity in the series of biotypes (Davis & Heywood, 1963, Stace, 1980, Claridge, *et al.* 1997). For example, certain character correlations involving at least one consensus (or at least one of a very few) key characters. Subspecies rank is used when taxa are separated geographically on a large scale in association of a single morphological character difference. Varietal rank can be chosen where assemblages of specimens display different statistical values for the combination of characters as to form a complete discontinuity. The overlap can be either altitudinal and/ or ecological (Stebbins, 1950, Sileshi Nemomissa, 1994). Persoon (1805) regarded subspecies taxa as having major morphological variations within a species whereas a variety may be used for taxa with minor morphological characters.

Based on the above findings particularly gross morphological phenetic studies and evidenced by seedling morphology and palynology, therefore, group be1 is treated as *B. boranensis* Vollesen (ined.). It differs significantly from the other three in terms of independent characters of leaf marginal spines, trichomes type, bracts spines, corolla width, anther (posticous) length, anther (anticous) length and calyx (posticous) length conforming to Vollesen's suggestion e.g. specimen by *Puff et al.*, 870508-³/₄, *Friis et al.*, 3138, *Ensermu Kelbessa & Aschalew* 4012 and *Sebsebe Demissew & Ensermu K.* 2676.

Groups be2, be3 and be4 are circumscribed as *B. edulis* var. *glabra* Malombe var. nov., *B. edulis* var. *edulis* and *B. edulis* var. *isabellae* Malombe var. nov., respectively.

These taxa lack definite or wide geographical distribution and although are segregated in DA, they could not be ordinated differently in PCA until group be3 was excluded from the analysis.

Blepharis edulis var. *edulis* is distinguished discontinuously, though often partially, from *B. edulis* var. *isabellae* and *B. edulis* var. *glabra* by features of the cotyledons, trichomes, leaf marginal spines and leaf spine arrangements, filament processes length, corolla colour as well as pollen shape and colpus length.

6.2 Synopsis

Circumscription of the taxa based on both gross morphology and palynology results;

1. Bracts spines (maximum) 9-11; leaf maximum marginal spines 26-36, minimum marginal spines 25-33; stem hairs appressed ----- *Blepharis boranensis*
— Bract spines maximum 4-8; leaf maximum marginal spines normally 2-25, minimum marginal spines normally 2-23; stem hairs ascending or appressed ---2

2. Cotyledons purple below; leaf marginal spines 12-25, normally cover up to the tip -
----- *B. edulis* var. *edulis*
— Cotyledons green below; leaf marginal spines absent or 2-11 (-14), normally cover up to two-thirds -----3

3. Filament 6.8-(7.9)-11.5 mm long; style 8.1-(10)-11 mm long; anther (anticous) 2.6-(3.8)-5.1 mm long; corolla purple or pink ----- *B. edulis* var. *glabra*
— Filament 3.1-(5.6)-8.3 mm long; style 3.5-(7.1)-10 mm long; anther (anticous) 1.2-(3.2)-3.6 mm long; corolla mainly blue----- *B. edulis* var. *isabellae*

6.3 Taxa description

Blepharis boranensis Vollesen (ined.)

Creeping or ascending perennial herb up to 32 cm high. Internode 2.4-4.8 cm long; young branches densely pubescent, eglandular, appressed, conical, *c.* 0.1-0.2 mm long. Leaves light yellow when dry sometimes with purplish patches, elliptic or lanceolate, acute to cunate, acuminate or mucronate, sessile, $11.62 \pm 6.5 \times 1.6 \pm 0.7$ cm, glaucous beneath, marginal spines 25-36, fine and closely arranged up to the tip, sometimes 3, near base, at every serration, terminal spine 0.1-2.2 mm long, normally densely pubescent especially above, unicellular to conical ascending hairs. Inflorescence borne at the lower most node 1 to 2, erect, strobilate, $4.5-8.5 \times 1.7-4.7$ cm. Bracts glaucous, $1.8-3.3 \times 1.1-1.6$ cm, with 9-11 marginal spines, terminal spine 1.8-5.7 mm long, pubescent often cylindrical, 2.3 mm long; bractiole paired, linear, $1.5-1.9 \times 0.1-0.2$ cm. Calyx 4 lobed, unequal, 2 inner pair equal, anticous elliptic $2.0-2.4 \times 0.6-0.8$ cm, posticous oblong, $1.4-1.8 \times 0.5-0.8$ cm, inner pair conical, 0.8 - 1.1 cm long. Corolla blue, yellowish at base, $2.6-3.3 \times 1.4-1.9$ cm, terminal lobe 0.5-0.8 cm long, with dense brownish strip of indumentum from hind base of terminal lobe downwards; filament purple striped as the anthers, 8-9 mm long; capsule, elliptic, $9-10.4 \times 17-22$ mm. Seeds yellow, ovoid, $5.5-6 \times 3.9-4.3$ mm, covered by white branched hygroscopic hairs *c.* 1.75-7.5 mm long.

Distribution and habitat: Ethiopia, Sidamo region, Borana Awraja and probably Jijiga (Harerge region) hills. Altitude range 1550-1850 m above sea level found in limestone soil and *Acacia-Commiphora* woodland as the associated vegetation.

Specimens examined: Ethiopia: Lefa Isa, 1850 m, 9° 39' N 42° 55' E, *Peter K & Mahadi Kidar* 18435 (ETH 048464); Negelle, 33 km from Negelle, along the road to Filtu, Sidamo, 1500 m, 20.5.1982, *Friis I, Mesfin T. & K. Vollesen*, 3138, (ETH 048484); Negelle, Borana Awraja, Sidamo, 130 km Negelle to Filtu road, 1550 m, 8.5.1987, *R. H. Puff, Sebsebe Demissew & Ensermu K.* 870508-3/4, (ETH); Negelle, Borana Awraja, Sidamo, c.5 km from Negelle on the road to Addis Ababa, 1540 m, 27.7.1985, *Ensermu K.* 1052 (ETH 072438); Negelle, Borana Awraja, 31 km from Negelle towards Filtu, Sidamo, 1640 m, 5° 13' N 39° 47' E, 14.12.1990, *Sebsebe D. & Ensermu K.* 2676 (ETH 072430); Negelle, Borana Awraja, Sidamo, ca 32 km from Negelle on the road to Filtu, 1550 m, 5° 13' 26" N 39° 49' 53" E, 20.12.1998, *Ensermu K. & Aschalew G* 4012, (ETH 072919); Harar, Sidamo, *W. Burger* 3560 (EA259).

Notes: *B. boranensis* appears closely related to *B. edulis* var. *edulis* on the bases of appressed trichomes along the young stems and leaf marginal spine distribution. However, for the latter, the hairs are sparse and tend to pick a pattern of three hairs per spot and sometimes may be ascending. Also the two taxa are segregated in terms of corolla width, number of spines on the bracts and the geographical distribution. Whilst *B. boranensis* appears to be concentrated at the higher altitudes (1550–1850 m) of Borana Awraja in Sidamo Region of Ethiopia, *B. edulis* var. *edulis* is found at the

lower altitudes (400–1530) m of the same region and Harerge Region of Ethiopia and the coastal plains (Voi, Kwale and taita Taveta Ditrcts) of Kenya up to Lushoto district in Northern Tanzania. It is important to note that seedling morphology should be studied in this species to establish a more detailed morphological affinity to the *B. edulis* complex.

Blepharis edulis (Forssk.) Pers.

Annual or hardly perennial herbs up to 44 cm long, young branches grey pubescent, ascending or appressed, conical eglandular becoming glabrate with age. Cotyledons purple or green below. Leaves 4 or 6 (basal node), subequal longest pairs always facing outward, sessile to subsessile, lanceolate to elliptic, acute to acuneate, apiculate, glaucous beneath and above along midrib, marginal spines 2-25 or absent, serrulate or loosely arranged up to the two thirds or tip, terminal spine 0.2-4.5 mm long recurved), densely pubescent above or puberulent. Spikes, borne at the lowest node 1-5, strobilate, erect, 2-17.7×1.4-7 cm. Bracts 1.3-3.5×0.4-1.6 cm, glaucous, ovate, acuminate, recurved 1-2 cm long, marginal spines 3-8, terminal spines 0.5-8.4 mm long, glabrous or pubescent, cylindrical often on the veins above, c. 3 mm long; bractiole paired, linear, 6.5-20×0.4-7.5 mm. Calyx 4-lobed, unequal, 2 inner pair equal, densely pubescent, anticus broadly ovate 9.6-25×4.5-10 mm, posticus oblong, 7.5-19×2-7.5mm, inner pair conical, 6-9.8 mm long. Corolla blue or purple, 1.2-3.1×0.5-1.8 cm, veins blue conspicuous networked on lobes, 5 lobed, two lower side lobes vestigial, terminal lobe 1.4-6 mm long, dense strip of indumentum from base of terminal lobe downwards; filament purple stripped as the anthers, 5.3-12 mm long

with basal tuft of hair and the posticous pair broad and flattened possessed with apical processes next to the anther. Flowers, at least 2 per spike, after 3-4 months. Capsule 0.3-1×0.3-0.5 cm, elliptic or lanceolate. Seeds 2, yellow, discoid, 3-6.8×3-5.1 mm, covered with white hygroscopic hairs c. 2.9-8.7 mm long.

(A) *Var. edulis*

Annual or perennial herb up to 36 cm long, pubescent appressed, conical eglandular becoming glabrate with age. Cotyledons purple below, hardly green. Leaf marginal spines 12-25, serrulate up to the tip or so, terminal spine 4 mm ± 0.5 long recurved downwards especially on the first basal pair (seedlings), densely pubescent above. Corolla blue or purple, 17-26×7.5-12.3 mm, flower after 5 months, at least one flower per spike.

Distribution and habitat: Kenya; Voi and Taita-Taveta districts (K7) to Lushoto district (T3) in northeastern Tanzania and Ethiopia from Jijiga eastwards towards Somali mainly Harerge Region, Gursum Awaraja. Also lower ranges of Eastern Shewa, Sodere and Borana Awraja in Sidamo Region. Flower after 5 months at least one flower per spike. Prevalent in red alluvium or rocky soil on flattish open ground sometimes on slopes where the vegetation is mainly dry bushland of *Acacia spp.* and *Commiphora spp.*

Specimens examined: Ethiopia; Harerge Region; Jijiga Awraja, 30 km from Jijiga on the road to Kebri Beyah, 1640 m, 19.9.1987, *Ensermu K. & Petros* 1847, (ETH

072381); Jijiga Awraja, 1900 m, 20.9.1985, *Ensermu K.* 1371, (ETH 048490); Babile, Gursum Awraja, c. 11-13 km from Babile on the road to Jijiga, 19.9.1985, *Ensermu K.* 1357, (ETH); Ogaden Webbe Shibeli river, 400 m, 29.6.1969, *de Wilde* 5943, (WAG L606/29); Jijiga, 38 km from Jijiga on the road to Harer (596 km from Addis Ababa), Gursum Awr., 1500 m, 20.1.1987, *Ensermu K. & Petros* 1869, (ETH 072382). Shewa Region; Walenchiti, 26.9.1980, *Ensermu K. & Tamrat B.* 401, (ETH 048485); Sodere, 1400 m, 27.1.1980, *Mesfin T.* 925, (ETH 072385). Sidamo Region; Mega, Borana Awraja, 42 km N of Mega on the road to Addis Ababa, 1530 m, 14.8.1985, *Ensermu K.* 1192, (ETH); Malka Guba, 93 km from Negelle to Wachile and Mega, near Dawa Parma River, 800 m, 30.5.1996, *Ensermu K. & Dessalegn D.* 3798 (ETH 069416).

Kenya; Coast Province (EA K7): Kichwa Tembo, Tsavo N. Park, 600 m, 3° 04' S 38° 11', 26.01.1985, *Beentje* 1860; Voi district between Voi and Tsavo, Manyani area, 457 m, 3° 23' S 38° 35' E, 15.9.1967, *Ivens* 2236; Kora N. Reserve, 420 m, 0° 06' S 38° 45' E, 13.9.1968, *Kokwaro & Waitthaka* 1616; 12.12.1984, *Mungai G. & Rucina* 432; Manyani area 1067 m, 18.12.1974, *Williams* 8; Tsavo East N. P., Galana River, Bore slopes, 500 m, 28.2.1972, *Backens & Jonsson* 270; Taita -Taveta district, 30.4.1975, *Friis & Hansen* 2628; Voi, between Voi and Mombasa, 2.1.1957, *Rammbars* 11218; Tsavo N. P., 29.12.1961, *Treror* 18; Voi district, on road verges, 457 m, *W. Ivens* 2236; Tsavo East N. P., Galana River, on bare slopes, 500 m, K7, *Backens & Jonsson* 270; Voi, about 17 km S of Voi, near quarry, K7, *Assent J.* 32. **Tanzania;** Lushoto, *Drummond R B & Hemsley J H.* 2324 (EA).

(B) Var. *glabra* Malombe var. nov.

Annul herb up to 35 cm long with purple branches normally puberulent. Leaf marginal spines 4-14, very sparingly arranged up to two-thirds leaf length. Bracts 2.1-3.5×1-1.6 cm, broadly ovate. Corolla purple to pink. Style 8.1-11 mm long. Filament 3.1-8.3 mm long. Anthers; anticous 2.9-5.1×0.8-1.8 mm, posticous 2.6-5.1×0.4-1.4 mm.

Distribution and habitat: Ethiopia and Eritrea Rift Valley from Zula through Dire Dawa, Kombolcha sites to Northern Kenya in Lake Turkana area such as Koobi Fora and near the Ferguson Gulf. Habitat is mainly dry granitic or sandy soils on slopes where the vegetation is low *Acacia-Commiphora* bush land and annual grasses.

Specimens examined: Ethiopia (EA); Arsi Region, Sodore, Chilalo Awraja, at Awash River, near Sodore, 1600m, 3.5.1971, *Thulin* 1309; El Rago, 762 m, *Simmons* 13 (ETH/ 072384). Eritrea-Ocule, 15.4.1902, *A. Pappi* 1272, Eritrea-Zula, lower slopes of hills, 30.48 m, 15° 16' N 39° 40' E, 6.3.69, *Robertson* 1089; Zula, 6.3.1969, Bally B6909. Gahatli/ Ailet (ETH), 19.3.1989, *O. Ryding* 1816. Shewa, Koka Dam, 1350 m, 8° 27' N 39° 06' E, 14.3.1971, *J. W. Ash* 749, (EA 259); Harerge, Batie (WAG), c. 40 km E Kombolcha, 1900 m, 9.7.1966, *Wilde de*, 9722, Kombolcha, c. 60 km S Kombolcha, 1700 m, 8.1.1966, *Wilde de* 9620; Erergota, 46 km from Dire Dawa to Erergota, 1200 m, 11.7.1967, *Westphal* 542. Kenya: Laga Bura Hasuna 500 m, 13.7.1981, *Brown*; Central Isl., L. Trukana, 488 m, 17.5.1953, *Padwa* 157; Koobi Fora, 8.8.1974, *Jackson* 18,

(C) Var. *isabellae* Malombe var. nov.

Erect to creeping annual herb up to 44 cm long, gray pubescent ascending or appressed, conical up to 0.4 mm long. Leaf margin entire or spinose, 2-10, sparingly arranged normally up to two-thirds. Bracts 1.4-3×0.4-1.6 cm, lanceolate to elliptic, ovate, suddenly and shortly acuminate. Corolla 1.2-2.6×0.5-1.3 cm, colour blue or purple, style 3.5-10.6 mm long, filament 0.3-8.3 cm long, purple or entirely green, processes purple. Anther, anticus 1.9-3.8×0.6-2.7 mm, posticus 1.2-3.6×0.3-1.2 mm, flower after 3 months, at least 2 per spike.

Distribution and habitat: *B. edulis* var. *isabellae* occurs in the dry parts of Kenya provinces namely Eastern, North Eastern and Rift Valley. Elsewhere, it seems to extend to northwestern Kenya and northwards to Gamo Gofa Region of Ethiopia where it becomes scattered in drylands of Dire Dawa and southwest Sidamo region of Ethiopia up to Eritrea's northerly Region. Collections of this taxon have also been obtained from Usambara, Mkomazi in Tanzania. It grows in sandy or rocky, mainly granite lava, open grounds in flattish or gentle slopes in dry semi desert vegetation dominated by *Acacia spp.* or sometimes *Commiphora spp.*

Specimens examined: Ethiopia (EA); Gamo Gofa, Omo Valley, 21.3 m, 5° N 36° E, 6.1968, Carr 228; (ETH) Shewa, Arba Minch, 950 m, 15°35'N 39°15'E, Ensermu K & Girma Adugna 3956; Massowa, 300 m, Lower Omo Valley, west banks of Omo river, 400 m, 3.9.1971 Tornay 91; (EA) Korem/ Zobel, 20 km E of Zobel, 11.4.1976, Gelahun & Zerehun W.370, Hadar, 380 m, Sebsebe D 198, 2.11.1979. Harerge Region,

Isa, Dire Dawa, 1250 m, 18.9.1985 *Ensermu K.* 1349; Melka Jelda, Dire Dawa, 1250 m, soil sandy, 29.6.1982, *Mesfin* 2908; Dire Dawa Airport, Et Berger W. 936; Geleb & Hamerbako Awaraja, 5°37' N, 36°44'E, Puff & Ensermu K 8212; (WAG) Dire Dawa, Awash station 700 m, 5.4.1966, *Wilde de* 10483; Awash, *Jansen P. C. M* 6522
 Sidamo Region, Dolo Odo, 13 km from Dolo Odo to Dolo Bay, 330 m, 16.12.1990, *Sebsebe D & Ensermu K* 2745. (WAG), Eritrea, Af Abed, 800 m, 16°14'N, 38°43'E, *Mooney H. F.* 9523. Tigray, Werie Bridge, Abi Adi on the road to Adowa, 1450 m, 14.10.1995, *Friis et al* 6735. **Kenya (EA):** (K1) Garissa district, 29.5.1977, *Gillett* 21201; Isiolo, 2 km S of the township, 100 m of main road, 1200 m, 4.11.1979, *Jonsell & Moberg* 4511; Sigor hill site, 1189 m, *Mus* 181; Milgis, Siriwa River, Marsabit District, 21.5.1970, *Magogo* 1442; Modogash, 11 km W. Modogash, 330 m, 9.12.1977 *Standard & Gilbert* 878; Dololo Dertu, Wajir District. 259 m, 115.5.1960, *Piatt* 646; Barsaloi, Seya, 853.4 m, 1.20.1969, *Curry & Glen* 6; Garba Tula, c. 33 km W Garba Tula just N of hill marked, 2440 m, *Kuchar, Msafiri & Sheet* 6020; Surugei Killa, K1, *R. Watkins* 12. (K2) Turkana, 3° 00' N 35° 00' E, *Symes* 139; Lodwar, Turkana, 518 m, 4° 10' N 34° 00' E, 13.5.1953, *Padwa* 157; Kibish police post, c. SW 5 km of Kenya Kibish police post, 396 m, *Carr* 814; Central Isl. Turkana, 381 m, 23.4.1934, *Martin* 113; Kainuk, Turkana, 10 km S Kainuk, 30.12.1981, *Coppock* 24, N. W. Turkana, between Kakuma and Oropoi, 21.1.1989, *Ohta*; Lokichar, Turkana, 21.9.1999, *Muasya & Malombe* 1587; Central Isl., L. Trukana, *M. L. Modha* 11; Turkana, *Ohta* 134. (K3) Baringo District, Marigat, 6 km N of Marigat, 1067 m, 23.10.1964, *Leippert*, 5187; L. Bongoria, 991 m, 10.1978, *Vincens* 77; Ol Kakwa Is, L. Baringo, near Isl. Camp S of tip, 1050 m, 2.6.1977, *Gilbert* 4711; L. Bogoria Hannington – Mongotio,

9.1.1969 *Napper & Faden* 1809; L. Bogoria, 100 m to Hot Water View site, 991 m,
19.9.1999, *Muasya & Malombe* 1531. (K4) Rainkombe (Meru N. P.), 580 m,
14.3.1979, *Hamilton* 392: Mwingi, c. 8 km of Mwingi on road to Garissa, 850 m,
Sanagi 934, 4.3.1973; Kiboko, 0° 55'S 38° 8' E; *Ossent* 533; Tula, 140 m, *Faden et al*
74/988. (K6) Kajiado District, Nairobi–Magadi, *Bally* B9767; Nairobi–Magadi, 55
miles, 1067 m, 11.8.1951 *Bally* B8016 Ologosaille–Magadi 18 miles, 4.4.1969,
Napper, Greenways & Kanuri 1993; Nairobi–Magadi road, 1524 m, 18.6.39, *Bally*
10253; Nairobi–Magadi, *Bally P. R. O* B9767. **Tanzania;** (EA T3) Mkomazi, S poles
Usambaras, *Evens* 572; (T2) Usambaras, South Maasai, *Evens G. W* 572 and Njooro,
raised island in Mbuga, *Peterson D.* 433

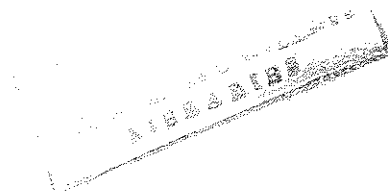
Notes: While observing both voucher specimens and seedling morphology characters,
some features in *B. edulis* var. *isabellae* were found to correlate with the species *B.*
linariaefolia described by Clarke (1899–1900). For example, Kajiado–Ologosaille
populations in Kenya and Tigray–Werie Bridge in Ethiopia showed some spineless
leaves. More so, the leaf marginal spines are less than 10 in most of the populations.
Therefore, a detailed morphological study of *B. linariaefolia* is required to ascertain
their correlation. Furthermore Clarke (1899–1900) was uncertain whether to place *B.*
linariaefolia on spineless or spinous groups in the genus. Importantly, cytological and
breeding systems data is required to further unfold the relationship among these three
varieties.



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CSL/CO	COB	OTEL	FL	PR	IPAL	PAB	AAL	AA	AB	BS	STSL	CPL	CPW	CPB	SEL	SEL	SEA	SC	LBP	LBS	LBF	LBMS	IFL	BSC	COO	CPS	CPT	CPV	COTL	LBMS	2-IN	BSNT	2-CPD	CNY	
7.2	30	14	7.8	5.1	4.8	1.5	4.4	1.4	11.3	0.45	9.15	4.05	6	3.9	7.5	1	0	0	1	1	1	1	1	1	0	2	2	25	28	1	10	10	1		
9.2	30	16.3	7.84	3.03	4.8	1.7	4.7	1.2	11.1	0.3	9.15	4.05	6	3.9	7.5	1	0	0	1	1	1	1	1	1	0	2	2	30	30	1	9	10	5		
9	30	15	7.795	4.5	4.7	1.5	4.5	1.1	12	0.35	9.15	4.05	3.90	1.75	1	0	0	0	0	0	0	0	0	0	2	1	1	0	28	30	2	11	11	9	
9	32	19	7.73	7.95	4.95	5.03	1.65	5.03	0.9	10.8	4.5	9.3	5.8	2.18	5.54	4.3	3.1	3	0	1	1	1	1	1	1	1	1	0	33	36	1	9	10	11	
10.2	26	15	4.5	6.33	4.5	4.65	1.35	4.5	0.9	11.1	0.45	10.4	4.9	2.18	5.56	4.2	3	0	1	1	1	1	1	1	1	1	1	1	33	36	1	9	11	5	
8.55	32	14.6	5.7	9	6.15	5.4	1.5	5.1	0.75	11	0.35	10.4	4.9	2.18	5.56	4.2	3	0	1	1	1	1	1	1	1	1	1	1	33	36	1	9	10	12	
8.7	30	15	5.1	7.8	4.85	4.85	1.44	4.5	1.28	10.5	0.3	9.68	5.63	1.65	5.5	4	3	0	1	1	1	1	1	1	1	1	1	1	33	35	1	8	10	12	
7.5	20	6.6	2.25	7.05	4.5	3.8	1.4	3.8	0.4	10.4	0.1	8.78	4.5	1.73	4.65	3.15	2.1	1	0	1	1	1	1	1	1	1	1	1	11	12	1	5	7	8	
6.8	23	11	5.25	7.5	4.65	3.8	1.4	3.2	0.7	9.21	0.05	10.7	5.1	6.75	3.6	5.25	1	1	0	1	1	1	1	1	1	1	1	1	5	5	3	4	4	5	
6.8	20	9	7.5	5.39	4.1	1.4	3.4	0.9	8.45	0.08	10.7	5.1	6.75	3.6	5.25	1	1	0	1	1	1	1	1	1	1	1	1	1	3	10	11	4	5	5	
7.3	21	17	8	9.98	5.23	5.1	1.5	4.5	1.02	10.5	0.4	10.7	5.1	6.75	3.6	5.25	1	1	0	1	1	1	1	1	1	1	1	1	3	10	11	4	5	5	
7.3	21	16	8	7.2	5.9	3.9	1.5	3.6	0.9	9.15	1.5	10.7	5.1	6.75	3.6	5.25	1	1	0	1	1	1	1	1	1	1	1	1	3	10	11	4	5	5	
7.3	25	12	5.1	7.77	3	3.6	1.5	3	0.9	10.4	0.25	10.7	5.1	6.75	3.6	5.25	1	1	0	1	1	1	1	1	1	1	1	1	3	10	11	4	5	5	
7.3	27	15	6	8.1	4.85	3.9	1.2	3.8	0.9	10.7	0.27	10.7	5.1	6.75	3.6	5.25	1	1	0	1	1	1	1	1	1	1	1	1	3	10	11	4	5	5	
9	22	8.55	3.9	6.75	3	2.9	0.8	2.8	0.4	10.3	0.15	10.7	5.1	6.75	3.6	5.25	1	1	0	1	1	1	1	1	1	1	1	1	3	10	11	4	5	5	
7.2	26	13.7	5.1	7.23	3.45	4.7	1.7	4.5	1.1	9.8	0.4	7.88	4.35	1.65	5.56	4.05	1.8	3	0	1	1	1	1	1	1	1	1	1	4	13	14	1	6	8	5
9.8	22	11	4.5	7.5	7.58	3	1.5	3	1.1	10.1	0.23	7.5	3	1.5	1	1	1	0	1	1	1	1	1	1	1	1	1	1	4	13	14	1	6	8	5
8.3	30	18	6	8.78	3.75	5	1.7	4.7	1.3	10.5	0.3	7.95	3.3	5.4	3.15	1	1	0	1	1	1	1	1	1	1	1	1	1	6	8	3	8	8	5	
8.7	28	11.8	4.95	7.58	4.5	1.8	4.3	0.9	9.9	0.3	7.95	3.3	5.4	3.15	1	1	1	0	1	1	1	1	1	1	1	1	1	1	6	8	3	8	8	5	
7.4	25	14.3	1.35	7.62	4.65	4.2	1.5	4.1	1.4	11	0.21	10.7	5.1	6.75	3.6	5.25	1	1	0	1	1	1	1	1	1	1	1	1	6	8	3	8	8	5	
7.5	24	9	3.75	7.5	3.65	2.8	0.8	2.8	0.6	12.2	1.5	7.35	3.6	1.5	4.5	3	2	1	1	1	1	1	1	1	1	1	1	6	8	3	8	8	5		
7.5	22	9	3.9	7.2	3.2	2.9	0.8	2.6	0.7	9.75	0.09	8.6	4.5	1.75	5.1	3.75	1	1	1	1	1	1	1	1	1	1	1	6	8	3	8	8	5		
7.7	21	9	3.68	6.88	3.11	3.2	1.4	3.1	0.8	8.3	0.11	9	4.5	1.75	4.8	3.3	3.2	1	1	1	1	1	1	1	1	1	1	6	8	3	8	8	5		
6.9	21.5	10.4	4.28	6.38	4.61	3	1.4	3	0.8	8.55	0.1	10.7	5.1	6.75	3.6	5.25	1	1	0	1	1	1	1	1	1	1	1	6	8	3	8	8	5		
8	22	8	4.28	6.38	4.61	3	1.4	3	0.8	8.55	0.1	10.7	5.1	6.75	3.6	5.25	1	1	0	1	1	1	1	1	1	1	1	6	8	3	8	8	5		
7.5	28	12	4.65	7.5	4.43	3.3	1.2	3.2	0.7	9.9	0.37	8.7	3.65	5.7	3.3	6.7	3	1	1	1	1	1	1	1	1	1	1	6	8	3	8	8	5		
7.2	23	12	4.5	7.23	4.73	3.7	1.3	3.2	0.6	9.9	1.28	10.7	5.1	6.75	3.6	5.25	1	1	0	1	1	1	1	1	1	1	1	6	8	3	8	8	5		
8	21	8.25	3.15	11.8	3.53	3.2	1.4	3.2	0.7	9.75	0.2	10.7	5.1	6.75	3.6	5.25	1	1	0	1	1	1	1	1	1	1	1	6	8	3	8	8	5		
8.5	24.5	12	5.8	6.78	3.38	4.5	1.6	4.4	1.4	10.4	0.23	9	4.35	1.95	5.55	3.35	3	0	1	1	1	1	1	1	1	1	1	6	8	3	8	8	5		
6.9	19.7	10.2	4.35	6	2.85	3	1.1	2.9	0.9	7.65	0.23	10.7	5.1	6.75	3.6	5.25	1	1	0	1	1	1	1	1	1	1	1	6	8	3	8	8	5		
7.7	19	9.15	3.5	6.12	3.15	3.2	1.1	3.2	0.7	7.5	0.38	10.7	5.1	6.75	3.6	5.25	1	1	0	1	1	1	1	1	1	1	1	6	8	3	8	8	5		
6	18	6.6	3	5.62	2.88	3	1.3	3	0.8	6.3	0.38	8.78	4.2	1.8	4.88	3.45	3	1	1	1	1	1	1	1	1	1	1	6	8	3	8	8	5		
6.2	16.5	7.5	3.15	12	3.15	2.9	1.4	2.8	0.7	7.13	0.09	10.7	5.1	6.75	3.6	5.25	1	1	0	1	1	1	1	1	1	1	1	6	8	3	8	8	5		
7.5	22	8.25	3.35	7.5	3.45	3.1	0.7	3.5	0.7	7.43	0.36	7.8	4.35	1.73	4.58	3.15	1	1	1	1	1	1	1	1	1	1	1	6	8	3	8	8	5		
9	21	9.6	3.23	8.16	3.15	3.2	1.4	3.2	0.9	9.9	1.5	10.7	5.1	6.75	3.6	5.25	1	1	0	1	1	1	1	1	1	1	1	6	8	3	8	8	5		
7.5	20	7.65	4.35	6	1.95	2.9	1.4	2.8	0.5	7.5	0.2	10.7	5.1	6.75	3.6	5.25	1	1	0	1	1	1	1	1	1	1	1	6	8	3	8	8	5		
8	22	9	4.2	6.08	3.3	3.9	1.4	3.3	0.8	9.45	0.19	10.7	5.1	6.75	3.6	5.25	1	1	0	1	1	1	1	1	1	1	1	6	8	3	8	8	5		
6.8	23	12.5	3.75	7.54	5.4	4.98	1.5	4.9	1.4	9	0.3	7.5	3.65	1.5	5.02	3	2.85	1	1	1	1	1	1	1	1	1	1	6	8	3	8	8	5		
7.5	25	11	4.65	7.68	4.2	4.3	1.3	4.3	0.5	10.5	0.3	10.7	5.1	6.75	3.6	5.25	1	1	0	1	1	1	1	1	1	1	1	6	8	3	8	8	5		
6.4	19	8.94	4.35	5.99	2.25	3	1.4	3	0.9	7.2	0.3	4.25	3.08	1.5	1	1	1	0	1	1	1	1	1	1	1	1	1	6	8	3	8	8	5		
7.5	18	9	2.7	5.25	3.68	2.4	1.1	2.4	0.7	6.45	0.18	10.7	5.1	6.75	3.6	5.25	1	1	0	1	1	1	1	1	1	1	1	6	8	3	8	8	5		
6.9	15.3	4.8	3	5.25	3.38	3	1.4	2.7	0.8	7.43	0.05	7.5	3.75	1.58	4.8	3.15	3.15	2	0	1	1	1	1	1	1	1	1	6	8	3	8	8	5		
6.5	19	9.53	4.2	5.85	4.05	2.9	1.1	2.9	0.4	7.13	0.15	8.25	4.5	0.38	5.25	3.75	2.63	1	0	2	1	1	1	1	1	1	1	6	8	3	8	8	5		
7.8	20	10.1	3.57	7.13	4.43	3.2	1.4	3	0.6	7.5	0.05	10.7	5.1	6.75	3.6	5.25	1	1	0	1	1	1	1	1	1	1	1	6	8	3	8	8	5		
6.3	18	7.5	3.06	4.8	2.63	3	1.4	3	0.7	5.55	1.2	7.73	4.2	2.1	4.5	3.3	2	1	1	1	1	1	1	1	1	1	1	6	8	3	8	8	5		
7.1	24	13.4	3.4	7.5	4.2	3.2	1.4	3.3	0.9	10.1	0.04	8.7	4.56	1.55	6	4.5	3.3	2	0	1	1	1	1	1	1	1	1	6	8	3	8	8	5		
6.7	16.5	6.45	3	5.78	4.73	3.8	1.4	3.6	0.7	10.6	0.04	10.7	5.1	6.75	3.6	5.25	1	1	0	1	1	1	1	1	1	1	1	6	8	3	8	8	5		
6.7	16.5	6.45	3	5.78	4.73	3.8	1.4	3.6	0.7	10.6	0.04	10.7	5.1	6.75	3.6	5.25	1	1	0	1	1	1	1	1	1	1	1	6	8	3	8	8	5		
7.8	22	12	3.7	6.15	3.1	1.2	3	0.6	6.77	0.11	10																								

Appendix ii: Specimens selected for observation

SP2	SP1	Collector	Co.N	Re	Locality	Alt (m)
be4	bc	J. Ament & F. C. Magogo	43	K4	Meru N. P.	
be4	bc	F.H. Hamilton	392	K4	Rainkombe (Meru N. P.)	580
bc	bc	G. W. Sanagi	934	K4	Mwingi	
be4	bc	J. Ossent	533	K4	Kiboko	
be3	bc	Beentje	1860	K4	Kichwa Tembo, Tsavo W.	600
bc	bc	Leippert	5187	K2	Marigat	1067
bc	bc	Vessey & Wales	16	K2	Ferguson gulf	457
be4	bc	A. Vincens	77	K3	L. Bogoria	991
be4	bc	W. Martin	113	K2	Central Isl. Turkana	381
bc	bc	M. G. Gilbert	4711	K2	OI Kakwa Is., L. Baringo	1050
bc	bc	J. R. Timberlake	296	K3	Chemolingot, Baringo	950
bc	bc	D. M. Napper & R. B. Faden	1809	K3	L. Bogoria (Hamington - Mongotio)	
bc	bc	A. Vicensus	229	K3	Laburu Delta, L. Bogoria	991
bc	bc	M.S. Natrass	779	K4	Makindu	1219
be2	bc	P. Hucks*	1051	K7	Tsavo N. P.	
be4	bc	M. L. Modha	11	K2	Central Is., L. Trukana	
be4	bc	B. Jonsell et al.	4511	k4	Isiolo	1200
be4	bc	Y. E. Symes	139	K2	Turkana	
be2	bc	J. H. Padwa	157	K2	Lodwar, Turkana	518
be4	bc	J. H. Padwa	157	K2	Central Isl., L. Trukana	488
bc	bc	D. L. Coppock	24	K2	Kainuk, Turkana	
bc	bc	S. Paulo	944	K2	Loro, Turkana	914.4
bc	bc	M. G. Gilbert, et al.	5667	K1	Isiolo	1050
bc	bc	I. Ohta	21	K2	N. W. Turkana	
be4	bc	N. Mus	181	K2	Sigor	1189
bc	bc	G. W. Jackson	18	K1	Koobi Fora	
bc	bc	J. Adamson	569	K1	Suguta	457
be4	bc	F. C. Magogo	1442	K1	Milgis - Siriwa R., Marsabit	
bc	bc	I. Ohta	183	K2	Turkana district	1067
bc	bc	N. Evans	100	K1	Koobi Fora	427
bc	bc	O. Mwangangi	1229	K1	Atapar, Kerio Valley	421
bc	bc	H. Aden	2	K1	Porr, Marsabit	
bc	bc	Joy Bally	1829	K1	Isiolo	914
be4	bc	L. Ohta	134	k2	Turkana	
be4	bc	Standard & Gilbert	878	K1	Modogash	330
bc	bc	D.J. Piatt	646	K1	Dololo Dertu, Wajir District	259
bc	bc	L. M. Gosling	8	K1	Isiolo G. R.	
bc	bc	Van Swinderen	29	K1	Did Sagallo, Marsabit	610
be4	bc	N. Curry & R. Glen	6	K1	Barsaloi	853.4
be2	bc	P.R.O. Bally	B690 9	Et	Werie Bridge	
bc	bc	V. C. Gilbert	C23	K7	Tsavo w. N. P. (Near Kivas water ole)	914
be3	bc	W. Ivens	2236	K7	Voi district	457
be3	bc	J.O. Kokwaro & Waithaka	1616	K7	Tsavo East	
be3	bc	G. M. Mungai & S. m. Rucina	432	K7	Kora N. res.	420
bc	bc	J. B. Newbould	6812	K2	Loya	
be4	bc	J. B. Gillett	21201	K1	Garissa district	
bc	bc	Z. J. Kimani	286	K1	Isiolo Township	
be3	bc	G. Powys	1972	K1	Koloba hill	
bc	bc	Shanty & Turner		K1	Archers Post N. F.	
bc	bc	S. Sato		K1	Korr, Marsabit	900
bc	bc	S. Sato	117	K1	Ilanto	

be2	bc	E. M. Nesbit		K1	Koobi Fora	427
bc	bc	C. R. Field	232	K1	Balesa Kulal, Marsabit	1800
be4	bc	R. Watkins	12	K1	Surugei Killa	
bc	bc	C. Esleme	15	K1	Koobi Fora	457
bc	bc	Ibrahim	7	K1	Samburu Game Reserve	3500
be3	bc	J. G. Williams	8	K7	Manyani Area	1067
be3	bc	Ingvar Backens & Jonsson	270	K7	Tsavo East N. P., Galana River	500
be3	bc	I. Friis & O. J. Hansen	2628	K7	Taveta, Taita district	
be4	bc	P.R.O. bally	17089	K7		
be2	bc	F. Brown		K1	Laga Bura Hasuna	
bc	bc	D. Stiles	82	K1	Kalacha	
bc	bc	S. Carter & B. Stannard	296	K1	Lodwar	700
bc	bc	Kazuhiro Shikano	23	K1	Bassaloi	1250
bc	bc	W. Shultka	49	K1	Ngare Ndare, Isiolo district	
bc	bc	J. P. M. Brenan, J. B. Gillett & Karuri	14747	K1	Jara Jila	225
bc	bc	A. Legese	6	K1	Mala Laman	
bc	bc	Dr Itani		K2	Kakuma	
be4	bc	P. Kuchar, F. Msafiri & R. Sheet	6020	K1	Garba Tula	2440
be2	bc	Mats Thulin	1309	Et	Chilalo Gwraja	1600
be4	bc	P. R. O. Bally	B801 6	K6	Nairobi - Magadi, 55 miles	1067
bc	bc	Bayner	95	K6	Olorgesaille Prehistoric site	3400
bc	bc	Verdcourt	3971	K6	Amboseli, Narok	1173.5
be4	bc	D. M. Napper, P. J. Greenways & Kanuri	1993	K6	Olorgesaille - Magadi.	
bc	bc	P. E. Glover & M. G. Cooper	3452	K6	Loopokilani	1371
bc	bc	V. Mackinnon	23	K6	Nairobi - Magadi	1219
bc	bc	A. D. Agnew & S. Agnew	5387	K6	Olorgesaille	
bc	bc	H. Horby	1027	T	Mkomasi desert	610
be4	bc	G. W. Evens	572	T	Usambaras	
bc	bc	D. m. Napper, P. J. Greenway & Kanuri	1881	K6	Nairobi - Magadi	1140
bc	bc	I. Ovent	510	K6	Olorgesaille	
be4	bc	P. R. O. Bally	B976 7	K6	Nairobi - Magadi	
be4	bc	P. R. O. Bally	10253	K6	Nairobi - Magadi	1524
bc	bc	B. J. Harris & J. Jenik	797	K6	Mombo	
bc	bc	H. B. Gilliland	14089	K1	Archilo	30.5
be2	bc	A. Pappi	1272	Er	Eritrea - Ocule	
bc	bc	M. Richards	25717	T2	Masai District	1462
bc	bc	Loutfy Boules		Et	Sodere, SE Nazaret	1500
be1	bc	W. Burger	3560	Et	Harar	
be2	bc	J. W. Ash	749	Et	Koka Dam	1350
be4	bc	C. J. Carr	228	Et	Omo Valley	21.3
bc	bc	M. Richards	25550	T2	Masai district	1005
bc	bc	J. Procter	33289	T3	L. Manka	457
bc	bc	Abdikadir A.	37	K1	Wajir Bor	230
be4	bc	D. Peterson	433	T2	S. Maasai, Njoor	
bc	bc	J. B. Newbould	6207	T	Saleh	1371
bc	bc	T. O. Robson		T	Burko - Rift Well	3500
bc	bc	N. H. Hansen	6040	S	Mogadesso	
bc	bc	Tsaha	92	S	Mogadesso	
bc	bc	B. J. Harris & J. Jenik	787	T	Himo	
bc	bc	S. A. Robertson	1076	T3	Buiko, Same district	2000
bc	bc	J. B. Newbould	5936	?	Longego - Olbalbal	1417

bc	bc	H. Leippert	5091	U	Karita	1400
bc	bc	Tweedie	163	U	Amudat	1219
bc	bc	Y. E. Symes	140	U?	Kara Suk	3000
be4	bl	C. J. Carr	814	K2	Kibish police post	396
bc	bl	S. A. Robertson	1194	Et	Elaboret Estate	1500
be4	bl	S. Tornay	91	Et	Lower Omo Valley	400
bc	be	E. Beals & M. Prosser	B 85	Et	L. Aisaita	
be3	be	Mesfin T.	925	Et	Sodere	1400
be4	be	Gehahun A. & Zerehun W.	370	Et	Korem/ Zobel	
be2	be	O. Ryding	1816	Et	Gahatli/ Ailet	
be4	be	A. Getahun	B 34	Et	Lecama (Dire Dawa	
be4	be	Friis I, etc	6735	Et	Werie Bridge	1450
bc	be	Tewolde B.G.E	825	Et	Adi Gebru	1375
be2	bl	S. A. Robertson	1089	Et/ Er	Zula	100
bc	be	Ensermu K & Petros E	1869	Et	Gursum Awr	1500
be4	be	Sebsebe D	198	Et	Hader	380
be4	be	Berger W.	936	Et	Dire Dawa Airport	
bc	be	*		Et		1175
be2	be	Simmons	13	Et	El Rago	762
bc	bc	P. Napier	TNP/ R/66	K7	Tsavo East N. P.	518
bc	be	Nystrom P.	s.r.	Et	Awash N.P.	1000
bc	be	Sebsebe D & Amha B	654	Et	Gamugoffa	680
bc	bc	Millne - Redhead E & Taylor	11176	T	Iringa	780
bc	bc	Gillman	761	K7	Ukunda	700
be4	bc	Ensermu K	8212	Et	Geleb & Hamerbako Awr	
be3	bc	Ensermu K., Dawe J & S Edwards	82091 9 -2/5	Et	Gursum Awr	1700
be3	bc	Ensermu K	1371	Et	Jijiga Awr	1900
be3	bc	Ensermu k & Tamrat B	401	Et	Walenchiti	
be4	bc	Ensermu K & Girma Adugna	3956	Et	Arba	950
bc	bc	Cunningham Van Sameren	398	K7	Kora N R	500
bc	bc	Rammbers Dr	11218	K7	Voi	
be4	bc	Faden R. B & A. J.	74/98 8	K7	Tula	140
be3	bc	Drummond R B & Hemsley J H.	2324	T	Lushoto	
be2	bc	W.J. de Wilde	9722	Net	Batie	1900
bc	bc	Muasya A. M & Malombe I	1531	K3	L. Bongoria	
bc	bc	Hucks	7	K7	Tsavo East N P	
be3	bc	Assent J	32	K7	Voi	
be3	bc	Treror S	18	K7	Tsavo N. P.	
be3	be	Ensermu K & Petros E	1869	Et	Gursum Awr	1500
be3	be	Ensermu K. & Petros E.	1847	Et	Jijiga Awr	1640
bc	be	Sebsebe & Ensermu K	87042 5 - 4/5	Et	Arero Awr	1050
bc	be	C. Puff, D. Mantell & Ensermu K	81092 4 - 3/1	ET	Awash Mulle	950
be3	bc	Ensermu	1192	Et	Borana Awr	1530
be1	bc	I. Friis, Mesfin T & K. Vollesen	3138	Et	Negelle	1500
be3	bc	Ensermu K & Dessalegn	3798	ET	Malka guba	800
bc	bc	J. W. Ash	1195	Et	Djigdjiga	
be3	bc	Sebsebe Demissew	4491	Et	Chachatu Kebele	1350
be1	bc	Peter K & Mahadi Kidar	18435	Et	Lefa Isa	1850
be3	bc	Ensermu K	1357	Et	Babile	
be4	be	Sebsebe D & Ensermu K	2745	Et	Sidamo	330
be4	bc	Ensermu K.	1349	Et	Dire Dawa Isa	1250

be2	bc	A. Igershiem, Sebsebe & Ensermu k	87051 2 -2/6	Et	Mendoyu Awr	1670
be4	be	Mesfin T.	2908	Et	Melka Jelda(Diri Dawa)	1250
be4	bc	W.J. de Wilde	10483	Net	Awash station	700
be4	bc	J. J.de Wilde	4648	Net	Massawa	300
be4	bc	P. C. M. Jansen	6522	Net	Awash	
be2	be	W.J. de Wilde	9620	Net	Kombolcha	1700
be3	be	W.J. de Wilde	5943	Net	Ogaden	400
bc	be	W.J. de Wilde	9873	Net	Dire Dawa	1000
bc	be	J. J. Bos	7712	Net		
be2	be	E. Westphal	542	Net	Dire Dawa-Ereergota	1200
be4	be	H. F. Mooney	9523	Net	Af Abed	800
be2	be	J. J. Bos & P. C. M. Jansen	10072	Net	Awale (Dire Dawa)	1470
bc	be	J. J. Bos	9035	Net	Dacata Valley	
be4	bc	Muasya A. M & Malombe I	1587	K2	Lokichar, Turkana	
be1	bb	RH. Puff, Sebsebe D & Ensermu K	87050 8-3/4	Et	Borana Awr	1550
be1	bb	Ensermu K	1052	Et	Borana Awr	1540
be1	bb	Sebsebe D & Ensermu K	2676	Et	Negelle	1640
be1	bb	Ensermu K & Aschalew G	4012	Et	Borana Awr	1550

SP2 = new species identification, SP1= previous species identification,
bb = *B. boranensis*, bc = *B. ciliaris*, be = *B. edulis* and be = *B. linariaefolia*