Taxonomic and Demographic Studies on Three Species Complexes within the Genus *Aloe L.* (Aloaceae) in Ethiopia

A Thesis Submitted to the School of Graduate Studies, Addis Ababa University in Partial Fulfillment of PhD Degree in Biology (Botanical Sciences)

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Dedication

To the late Dessalegn Boshe Birru, my father, who was deeply concerned with my education

May God bless his soul

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Abbreviations

ABI - Applied Biosystems Incorporated

AFLP - Amplified Fragment Length Polymorphism

CBD - Convention on Biological Diversity

CITES - Convention on the International Trade in Endangered Species

CSE - Conservation Strategy of Ethiopia

CTAB - Cetyldimethylethyl Ammonium Bromide

FEE - Flora of Ethiopia and Eritrea

FISH - Fluorescent In situ Hybridization

HPLC- High Performance Liquid Chromatography

ITS - Internal Transcribed Spacer

NTSYS - Numerical Taxonomy Systems

OTUs - Operational Taxonomic Units

PCO - Principal Coordinate Analysis

PCA - Principal Component Analysis

PCR - Polymerase Chain Reaction

RFLP - Restriction Fragment Length Polymorphism

SEM - Scanning Electron Microscopy

SSRs - Sequence-tagged Simple sequence Repeats

UPGMA - Un weight Pair Groups Method of Analysis

VNTRs - Variable Number of Tandem Repeats

Abstract

Taxonomic and demographic studies were conducted on three species complexes within the genus *Aloe* L. (Aloaceae) in Ethiopia. The studies aimed to contribute new additional knowledge necessary to delineate the *Aloe* species and their conservation in the Flora of Ethiopia and Eritrea (FEE).

In the taxonomic study, the status of 11 species was evaluated using morphological and molecular (AFLP) data. Morphological data were collected from herbarium specimens, fresh materials and literature. Data matrices were constructed for qualitative, quantitative and combined morphological data sets. Molecular (AFLP) data were obtained from leaves of randomly selected germinated seedlings. Total genomic DNA was extracted using a modified 2 x CTAB method described by Doyle and Doyle (1990). AFLP analysis was performed following a protocol modified from Vos *et al.* (1995). Fragments produced by each primer combination were analyzed and scored for the presence (1) or absence (0) of selected markers/fragments. Binary data matrices were constructed for each of the primer combinations and also for the combined data set. Each of the data matrices constructed for morphological and molecular (AFLP) data sets was subjected to multivariate analyses: cluster analysis (UPGMA) and principal coordinate analysis (PCO).

The results from the analyses of both morphological and AFLP data supported the present status of nine out of the eleven species: *A. harlana* Reynolds, *A. monticola* Reynolds, *A. debrana* Christian, *A. percrassa* Tod., *A. yavellana* Reynolds, *A. megalacantha* Baker, *A. gilbertii* Sebsebe & Brandham, *A. calidophila* Reynolds and *A. sinana* Reynolds. The boundary between the two caulescent species from the northern part of the FEE area: *A. camperi* Schweinf. and *A. adigratana* Reynolds was not very

clear and particularly the genetic delimitation displayed close relationship. The neighbor-joining tree constructed from combined AFLP data revealed thus weak support for *A. camperi* (51%) and *A. adigratana* (62%) but kept them together with high bootstrap support (94%).

In the PCO plot they constituted one single cluster. It is accordingly suggested that the two species should be recognized at subspecies level as *A. camperi* Schweinf. subsp. *camperi* and *A. camperi* subsp. *adigratana* (Reynolds) Fikre comb. et stat. nov.

In the demographic study**,** population structure and dynamics of three endemic species: *A. gilbertii, A. debrana* and *A. harlana*, representing the three complexes and different centers of endemism were analyzed. Nine permanent plots of 5 x 20 m², three plots per species, were established in selected sites. Each individual clone consisting of one genet and one or more ramet(s) were marked, mapped and recorded. For every genet, stem length and stem diameter were measured (when present) and for every ramet, rosette diameter, rosette height and number of inflorescence(s) were measured and counted. These measurements were undertaken in successive seasons (2003-2005) following their phenology. Recruitments and mortalities of genets and ramets were recorded in the second season. Based on the data recorded, population structure was described by the clone size and rosette diameter of ramets using descriptive and inferential statistics. Dynamics at the ramet and genet levels were analyzed using matrix model developed by (Caswell, 1998).

The results showed that the three species displayed different population structures. The population of *A. debrana* had the highest number of genets (221) as compared to *A. gilbertii* (208) and *A. harlana* (102). Including seedlings, 83.3% in *A. debrana*, 69.6% in *A. harlana*, and 54.3% in *A. gilbertii* of the genets were represented by a single ramet/genet. The *A. gilbertii* population was composed of a relatively greater proportion (45.7%) of multi-rameted genets (2-25). Thus, the three species vary significantly in the extent of clone size/formation ($P < 0.09$), and also in the size class distribution of the rosette diameter of ramets ($P < 0.20$). The number of ramets ‘born’ between seasons was

not correlated (r = 0.15) with the number of ramets died. Dynamics (growth rate) analysed at ramet-level indicated that *A. gilbertii* (λ = 1. 30) and *A. debrana* (λ = 1.28) populations were expanding whereas *A. harlana* (λ = 1.08) population was nearly stable or slightly increasing for the period of study.

A total of 83, 69 and 15 seedlings were recruited; and a total of 9, 4 and 5 genets died in *A. gilbertii, A. debrana* and *A. harlana* populations respectively. The number of seedlings recruited between seasons was not correlated (r = 0.42) to the number of genets died. The dynamics at genet-level was more apparent in *A. gilbertii* (λ = 1.38) with relatively high rejuvenation as compared to *A. debrana* (λ = 1.24) and *A. harlana* (λ = 1.16) populations even if mortality of genet is less pronounced in both species.

Isoenzyme data of six populations and 78 individuals representing the three species analysed demographically were generated. Genetic variation within and among the populations was analysed using the computer package GENEPOP (ver. 3.4) (Raymond and Rouset, 2003). Average levels of polymorphism (P = 60.9) and allelic richness (N_a = 2.18) were higher than that has been previously observed for other *Aloe* species. Observed heterozygosity (mean H_o = 4.5) was higher than expected under Hardy-Weinberg equilibrium (mean H_e = 4.2) resulted in negative fixation coefficient (mean F_{is} = -0.06), suggesting absence of inbreeding and an excess of heterozygosities. Since *Fst* values within populations of species are rather high (upto 25% in *A. gilbertii* and 18% in *A. debrana*, it is recommended that several populations of a species must be conserved in their natural habitat to keep the genetic diversity.

The germination success of field-collected seeds in the greenhouse was assessed and observations were made in the field to identify threats to aloes in the flora area. The percentage of germination was highest for *A. yavellana* (86%) and surprisingly very low success record was for *A. megalacantha* (18%). Despite variation in the success, the germination experiments indicated that most species are tolerant of the many soil types to be found in gardens and potting mixtures. Hence the cultivation of aloes in *ex-situ* conditions can be applied as effective conservation strategy. Clearing natural habitats for

agricultural lands, due to development construction such as roads and urbanization were found to be the major threats to aloes in the flora area. Urgent *in-situ* conservation measures to protect habitats inhabited by aloes, particularly the narrow endemics where their habitats cleared at alarming pace, are highly needed in Ethiopia.

1. Background and literature review

1.1. General introduction

1.1.1. Trends in plant systematics

Biology as a science consists of two major approaches to the study and understanding of living organisms. The first approach is largely experimental and reductionism. This approach focuses on finding general laws and phenomena that apply to all or many organisms. These include molecular, genetic, developmental or physiological aspects of the biological reality. Individual organisms used in such studies are just understood as "models", and their individual characteristics that cannot be generalized, are perceived as an irrelevant "noise" (Frankham, 2003). In contrast, the second approach to biology, which is largely holistic, comparative and mostly based on observations, is mainly concerned with variability and change among living beings. The term systematics applies to this approach, which deals with the diversity of organisms, their relationships and evolution. Under this approach, unique characters of some kinds of organisms (species or higher taxa) are not considered as "noise", but rather as basic facts on which our understanding of relationships and evolution are based (Lande, 1995; Newman & Pison, 1997).

Systematics is the science of biological classification. It embodies the study of organic diversity and provides the tools to study the historical aspects of evolution. It entails the discovery, description, and interpretation of biological diversity as well as the synthesis of information on diversity in the form of predictive classifications. In particular, it focuses on describing and classifying species (Stuessey, 1990; Judd *et al.*, 1999).

Man has used classification and some sort of nomenclature from the very beginning of his existence. There is no doubt that ancient people classified plants. However, the folk taxonomic groupings were much simpler and more robust than those used today (Stace, 1980). Typical mental abstractions (categories) into which plants were placed, were relating to whether they were useful (e. g. as sources of food or building materials),

poisonous, or could be worshipped. The species of *Aloe* L., particularly the widely known and used one, *A. vera* (L.) Burm.f., would without doubt have been included in the first of these categories (Smith & Steyn, 2004).

The formal taxonomy of plants dates back only to 1753 when Linnaeus proposed his sexual system of plant classification, based primarily on the number of stamens and pistils in a flower. The artificiality of the Linnean system was widely realised and various attempts at Natural classification of plants, thus classifying them to reflect overall similarity, were proposed. Natural classification began to appear in the late 1700s with Adanson and developed as a body of thought initially called 'numerical taxonomy' (Sokal and Sneath, 1963), and later replaced by 'phenetics' as introduced by Mayer in 1965. Recently, classifications following phylogenetic (evolutionary) relationships have been attempted (Henning, 1969, see e.g. Judd *et al.*, 1999). The work on presenting a complete phylogenetic taxonomy for all angiosperms is followed by the Angiosperm Phylogeny Group (APG, 1998).

Systematics itself consists of several complementary but distinct disciplines that include taxonomy (the definition and classification of the kinds of organisms), cladistics (the study of genealogical relationships between the units) and "evolutionary biology" (the study of the modalities and causes of organismal evolution, including historical biogeography) (Frankham, 2003). In taxonomy all living organisms are allocated to formal classificatory units (taxa) referred to a hierarchy of *categories* (e.g., species, genera, families, etc.) and to name all these units according to an internationally agreed nomenclatural system of Latin *nomina*, which constitutes a language common to all biologists (Stace, 1980). In the recent decades, cladistics and "evolutionary biology" have been largely flourishing; while taxonomy has according to Frankham (2003) suffered a continuous, and ultimately dramatic, decline in prestige, resources and number of professional positions. However, the recent focus on the importance of biodiversity and its conservation has again revitalized the field of taxonomy.

The use by some cladists, to designate cladogram-based classifications or "cladifications" (Lande, 1995) of the formula 'predictive classification' might have had another negative

impact on the perception of taxonomy by outsiders of this field. The discussion on whether paraphyletic groups should be accepted or not has created a heated debate among taxonomists these days (cf. e.g. Nordal & Stedje, 2005; Nelson *et al*., 2003).

1.1.2. Morphological and molecular data collections and analyses

Morphological data form the basis of virtually all systematic descriptions. The vast majority of systematic studies begin by grouping organisms on the basis of morphological similarity. Once they are grouped, the study of relationships among the groups can be initiated. Increasingly more often these morphologically defined groups are used as the basis for conducting studies of molecular variation (Mishler, 2000).

In phenetics one is concerned with forming classification based on overall similarity. This method is commonly used in morphological studies (Rohlf, 2000). Phenetic classification utilise all the attributes of the individuals under consideration, and hence is useful for a very wide range of purposes (Stevens, 2000). However, phenetic methodology, as an approach, has some criteria to be taken into account in the data collection phase. One of the operations involved in the approach is the selection of individuals for the study. For example, when one aims to assess relationships and delimitation of species, individuals representing various populations in their range of distribution have to be sampled. Moreover, a phenetic approach recommends certain number of individuals to be considered in the analysis "…at least 10 and preferably 25 or more" (Stuessey, 1990). Stuessey (1990) further emphasized the importance of objective selection of characters and definition of character states. The characters selected should not provide redundant information; i. e. phenetic characters should be non-correlated. As much as possible, characters selected should include qualitative and quantitative; vegetative and reproductive; and micro- and- macro characters.

A variety of different molecular techniques can be used for the study of botanical diversity. RFLP, arbitrary primed DNA, AFLP, VNTR, SSRs and PCR based DNA sequencing are methods for generating molecular markers (for abbreviations see p. XI). These techniques differ in the way that they resolve genetic differences, in the type of

data that they generate and in the taxonomic levels at which they may be most appropriately applied. For example, AFLP is one of the several methods for generating molecular markers that can be used to investigate variation in species and populations. For AFLP, genetic markers are generated by restriction enzyme digestion of the total DNA, then ligation of specific nucleotide sequences to ends of the restriction fragments followed by PCR amplifications using labeled primers specified to target the linked sequence. The advantages of this method are that no prior sequence information is required, and that it produces a large number of polymorphic bands. The AFLP technique has been patented and the licensed kits are available. Radioactive labeling of primers can be avoided by using automatic sequencers (Vos ***et al.***, 1995).

Isoenzyme analyses have also been successfully used to distinguish taxa at the level of species and subspecies (e.g. Soltis and Soltis, 1989; Brochmann ***et al.***, 1992; Marcussen & Borgen, 2000). This method is still widely used and remains among the most important tools in the analysis of population genetic structure, primarily due to the fact that a moderate number of loci can be screened quickly and economically (Bagley ***et al.***, 1997). The widespread use of isoenzyme techniques that began in the late 1960s has produced a large volume of data on genetic variation within and among populations and species. Patterns across a broad range of taxa are mostly consistent with our understanding of the effects of breeding systems (selfing species tend to possess lower levels of genetic variation within populations), life history (longer lived perennials tend to be more variable), and geographical range (endemic taxa tend to be less variable) (Hamrick & Godt, 1990). While isoenzyme markers are likely to continue to be the primary tool for population structure analyses, studies using DNA markers have increasingly begun to contribute to such data bases. For example, estimates of levels of genetic diversity and degrees of structuring among plant populations have been made using minisatellite fingerprinting (Muller & Wolfenbager, 1999).

Several methods of computer-assisted analyses of multivariate data are available and have become popular these days. The methods furnished in NTSYS, is largely associated with the field of phenetics. They are often used for morphological studies, but can be used also for binary molecular data sets (e.g. AFLP and isoenzymes). The methods

available in NTSYS programs can also be performed in other programs such as SPSS; SAS and PAST. There are also programs that are specialized for phylogenetic (cladistic) analysis. Some of the more well known ones are PAUP and PHYLIP.

Methods of computer-assisted analyses of multivariate data begin with a data matrix that contains information about the properties (features, characters, etc.) of a number of objects (individuals, species, OTUs, plot etc.). The data matrix can be prepared in Microsoft Excel and then imported, or can be prepared directly in the programs. For example, NTSYS has several modules and options for statistical analyses. Once the data matrix is ready, a module can be chosen to compute various measures of similarity or dissimilarity, usually between all pairs of objects, and then summarizes this information either in terms of nested sets of similar objects (cluster analysis) or in terms of spatial arrangement along one or several axes (ordination analysis) such as PCO and PCA (Rohlf, 2000).

1.1.3. Significance of taxonomy in conservation

It is predicted that the 21st century will be that of the crisis of biodiversity and of extinction. A majority of the planet's species might disappear before having even been studied. The discipline in charge of this study is taxonomy (and conservation biology). The significance of taxonomy to and its relationship with conservation is strongly recognized these days. Hence taxonomy and conservation biology would have much to gain by becoming allies rather than largely foreign disciplines (the secretariat of CBD, 2002).

Conservation biology has devoted most of its activity to the evaluation of the current threats on living organisms, populations and species and of the resulting extinctions. It is also involved in the study of the proximal and ultimate causes of these phenomena, and to the establishment of action strategies to try and stop or limit them (Ralls & Ballou, 1983). However, one major problem of this Endeavour is that such evaluations are necessarily incomplete, as their starting point, the inventory of the living species of our planet is largely unsatisfactory, both regarding the taxonomic completeness and taxonomic accuracy (the secretariat of CBD, 2002). Programs of action are doubtless needed to

struggle against identified threats on habitats and species, but biologists have to remain aware of our still very limited knowledge of basic facts such as the number of living species on our planet, their biological characteristics, their distribution and the status of their populations. Clearly, the development of a scientific, balanced and efficient policy in conservation biology would require a strong increase of our taxonomic knowledge of biodiversity, and therefore an important development of taxonomic research (Frankham, 2003).

A basic characteristic of taxonomy is the importance of its descriptive, observational and comparative components. Taxonomic research generates observational and descriptive data, that are meant to reply to "what?" questions, are the indispensable basis for any further scientific research (including conservation), and must be addressed before "why?" and "how?" questions can be considered (Lande, 1995).

Action programs for the conservation of biodiversity are of various kinds. Some of them, both ***in situ*** and ***ex situ***, are species-oriented, and can therefore be efficient only for well-known species, for which factors of threat have been identified. Other conservation actions are habitat-oriented, and are therefore liable to have positive consequences for the preservation of all or many species in these habitats, including those that are still unknown to biologists. In this respect, the particular attention recently paid to some areas of our globe, the hot spots characterised by high species diversity and/or high rates of endemism, provides some rationale for the choice of regions and habitats where particular efforts of biological conservation should be made (Ralls & Ballou, 1983).

An "alliance" between taxonomy and conservation biology would seem highly justified on several grounds: conservation biologists need taxonomic knowledge to act efficiently, and taxonomists are highly concerned by the accelerated extinction of their basic object of study, living species. It is crucial to recognise the need for taxonomic research as a basic scientific activity both for understanding the patterns and processes of biodiversity and evolution, and for a solid foundation of conservation biology programs. If this is acknowledged, it follows that there is a strong need for improving the current standards in taxonomic research. Most importantly, it is urgent to reconcile taxonomy and

conservation biology, in order for them to act as allies as they should naturally do (the secretariat of CBD, 2002).

1.1.4. Demography of plants

A population is defined as a group of actually or potentially interbreeding individuals of the same species (a genetical definition). It is also defined as the individuals of a species occurring within a defined homogeneous area; or a population is defined simply as the total number of individuals of a single species in an area circumscribed for the purpose of study, often a quadrate or a series of quadrates (an ecological definition) (Crawley, 1997).

For clonal plants one needs to decide what is an individual, Is it a ramet - i.e. the physiological individual or a genet - i.e. the genetic individual? The differentiation of genets from ramets is difficult if not impossible to do in the field, yet it is critical to understand population dynamics at both levels and the ability of a species to respond to changes in the environment (Harper, 1982). Clonal plants, unlike the non-clonal plants, are capable of producing potentially independent offspring's by means of vegetative growth and have the capacity to multiply by both sexual and vegetative reproduction (Harper, 1977). The spatial structure of the clonal plant depends on the relative magnitude of the two mode of reproduction. It is also influenced by dispersal capacity (Harada & Iwasa, 1996). In clonal plants a genet can be viewed as a population of ramets and the size of the genet is therefore determined by ramet dynamics (Harper, 1982).

The structure of a population of plants can be described in terms of sizes, life stages and ages of the individuals that compose it (Harper & White, 1974). These structures that we can identify in populations of plants result from the action of biotic and a biotic factors to which their members, and in some cases their ancestors, have been exposed in the past. The forces experienced by the ancestors of a population can clearly affect its genetic structures (although this may not be observable) (Hutchings, 1997).

The size of individuals of plant populations is far from uniform (Crawley, 1997). Even in a pure stand of a single species sown at the same time, in which plants are of similar age, a hierarchy of size develops, particularly if there is density stress (Harper & White, 1974). Many other factors may also promote variation in the size of individuals in a plant population. Firstly, seed size is rarely constant within a species (Hendrix & Sum, 1989), and seedling size at a given age is often correlated with the size of the seed from which it grew (Crawley & Nachapong, 1985). Secondly, relative growth rate is genetically determined. Thirdly, the time of germination relative to that of neighbours is a major determinant of the future of plants (Crawley, 1997). The plant population is often composed of a small number of large plants that account for most of the population's biomass and many small ones (Weiner & Solbrig, 1984).

In the words of Ross & Harper (1972), "an individual's potential to capture resources is dictated by the number and proximity of neighbours already capturing resources from the same resource pool". Thus, competition from neighbours can affect plant size and survival. Herbivory could also influence the size structures (Weiner, 1993). Since many aspects of performances such as the reproductive activity are correlated with size, they too will vary from plant to plant (Hutchings, 1997). In many species, the probability of flowering is also size dependent, so that plants must exceed a critical threshold size before flowering (Klinkhamer *et al.,* 1987), and the few dominant individuals contribute disproportionately to the total seed production (Harper & White, 1974).

Plant population can also be described by their life stage structure (Rabotnov, 1969); because plant species have certain consistently recognizable life stage in their life cycle. These may be called seed, seedling, juvenile, immature, vegetative, reproductive, sub-senile and senile (Silvertown, 1982). Each life stage may be characterized by a particular combination of quantitative and qualitative characteristics and differ in their response to environmental factors (Harper & White, 1974). Each succeeding stage is characterized by the appearance of new structures that were lacking in earlier stages of development and by the loss of previous ones. The average duration of each life stage of each plant species is fixed genetically, but since environmental condition may vary greatly, different

individuals will reach a certain life stage at different time (Silvertown, 1982). Unlike age, the succession of life stages is reversible and senile perennial plants may revert to reproductive activity if environmental conditions change (Rabotnov, 1969). The recognition of life stages has greater significance than calendar age in analysing the structure and dynamics of populations. Werner & Caswell (1977) compared the accuracy of prediction of number of individuals in a population by matrix models based upon age-related transition probabilities and stage-related transition probabilities. They found that the stage-related models predicted changes in number of seeds and vegetative or flowering rosette in experimental populations better than the age related models even though the transition probabilities for both types of models, were derived from the same population. Thus, the value of determining the life stage structure of a population is that the spectrum of developmental stages may be a better indicator of its condition than its age structure (Hutchings, 1985).

Size or life stage distributions are much easier to assemble than true age distributions (Harper & White, 1974). Attempts to correlate size or other easily measured aspects of performance with age tend to give inaccurate results (Hutchings, 1985). There are a few short-cut methods of determining age structure in populations of growing plants: Firstly, some type of annually produced morphological markers such as tree rings or bud scars can be counted. Secondly, individual plants can be recorded as they enter the population (as zero year old), and uniquely tagged or mapped so that their survival can be followed. For herbaceous plants, precise estimates of chronological age may be made if the plant has some organ (usually a rhizome) that persists for many years without decaying and show well-defined annual increments. The number of leaf scars on a rhizome may be a good indicator of age when the mean annual leaf production is known (Harper & White, 1974). By using such techniques Rabotnov (1969) and Harper & White (1974) were able to determine with some accuracy the age structure using year classes of some herbaceous perennials. Once determined, the age structure of populations must be interpreted with care; a structure dominated by young individuals may represent an expanding population with a few old early colonizers and a large number of descendants, or it may represent a stable population. An excess of old individuals may mean that a population is moving to

local extinction with no successful new recruits or that the system is stable (Harper & White, 1974).

Demography is the study of population changes with time. The relative change in the number of individuals at a given place and time is described by demographic parameters, where annual increase is described as $\lambda = N_t/N_{t-1}$. It can also be expressed by the relative increase between the ratio of the demographic factors that contribute to the dynamics of population; $\lambda = N_t (B + I)/N_{t-1} (D + E)$, where N_t = number at time t, N_{t+1} = number at time t + 1, B = births, D= deaths, I = immigration and E = emigration. The net increase result determines whether the population remains stable, ($\lambda = 1$), or increase ($\lambda > 1$), or decrease ($\lambda < 1$) (Caswell, 1998).

It is by quantifying demographic parameters that one can confront questions like what processes are responsible for fluctuation in number. Crawley (1997) suggested that the relative importance of various processes in plant population dynamics could be ranked as follows: interspecific competition > herbivory > intraspecific competition for micro sites > seed limitation. Moreover, the dynamics of plant population can also be affected by pathogens, weather, soil conditions and various environmental hazards like fire (Watkinson, 1997).

Demographic data collection begins with establishing permanent plots of appropriate size depending on the life cycle of plant species under investigation. The individuals in plots should be mapped, marked and measured or counted. This is followed by determining the size, stage (or age) structure of the population at a single point in time and follow up of the fates of plants of a given size, age or stage in successive seasons or years. Data collected also include individuals all ‘born’ and ‘die’ within a time interval.

With long-term demographic monitoring data, one can make a relatively realistic simulation model of population dynamics, preferably including information on levels of environmental stochasticity. The latter can be obtained by studying as many sites for as many years as possible (Oostermeijer *et al.*, 2003). There are several matrix methods deviced that allow one to model population changes and give insights into population

growth (Silvertown, 1982; Caswell, 1998). However, it is important to note that the value "λ" calculated from the matrix does not tell any thing about the mechanisms that controls the dynamics. This may be identified by further analysis and careful interpretation of transition matrix formulated based on the age or stage. When one population leaves more descendants than another because of superior ability to survive or to reproduce offspring or due to superiority in both of these characters; it has a relative evolutionary advantage of fitness. However, this fitness of a particular species is not a fixed value, but is determined in the context of prevailing ecological conditions and reproductive success of other population which occur in the same area (Silvertown, 1982).

The goals of almost all demographic studies are to understand and predict the dynamics of populations, and also identifying factors affecting the rate of change of population size (Lande, 1998). Such studies provide essential informations that are helpful in explaining ecology, evolutionary processes and in conservation decision making (Harper, 1982; Hartnett & Bazazz, 1985). For example, information about genet survivorship and age structure can distinguish between even-aged and multiple-aged populations, with important consequences for our understanding of natural selection (Harper, 1977). A population consisting of genets of various ages that have survived slightly different selective processes may be more tolerant of environmental variation or disturbance than a population consisting of clones that are all of a similar age. Comparing the dynamics of genets and individual plants (ramets) can also be useful in characterizing the genetic variability in a population as well as evaluating how well field studies represent the gene pool (Demetrius, 1975; Harper, 1977).

1.2. The genus *Aloe* L. (Aloaceae)

1.2.1. Description of the genus

The aloes are perennial plants that display a wide range of habits. Members of the genus vary from small herbs (e.g. sect. *Aloe* subsect. *Humiles* A. Berger); shrubs (e.g. *A. acutissima* Per.) and climbers (e.g., sect. *Aloe,* subsect. *Prolongatae* A. Berger ser. *Macrifoliae* Haw.) to trees (e.g. sect. *Dracoaloe* A. Berger). The genus also has species with a scandent habit (scramblers such as *A. tenuior* Haw. and *A. ciliaris* Haw.) (Reynolds, 1950, 1966; Smith & van Wyk, 1991; Adams *et al.*, 2000).

Aloes may be acaulescent or caulescent. The 'acaulescent' species are herbaceous forms having very short stems that are completely hidden by the leaves, whereas 'caulescent' species may be arborescent, shrubby, sprawling, climbing or pendulous. Arborescent species may be branched or unbranched. Pendulous plants, such as *A. kulalensis* L. E. Newton & Beentje and *A. veseyi* Reynolds, hang down rock faces, sometimes rooted only into rock crevices with little soil (Newton, 2004). The caulescent forms comprise ca. 10% of the species and the remaining ca. 90% are acaulescent (Holland, 1978). Members of the genus also exhibit secondary thickening, a character, which is much less pronounced

in herbaceous taxa than in well-known arborescent taxa. They are relatively unique within the monocots by the woody habit found in some of the species. However, they have a relatively poor root system like other monocotyledons (Court, 1981). Most of the species have underground rhizome except a few bulbous species, for example ***A. buettneri*** A. Berger and ***A. kniphofioides*** Baker, whose connection to the substrate is maintained by contractile roots (Smith & van Wyk, 1991; Newton, 2004).

Aloes occur in a wide range of sizes from a dwarf rosette about 30 cm high (e.g. ***A. dorotheae*** A. Berger) to the tall trees about 12-15 m in height (e.g. ***A. eminens*** Reynolds & P.R.O. Bally) (Adams ***et al.***, 2000).

Most aloes are recognized by fleshy, strongly cuticularized leaves usually with prickly (spiny) margins. The leaves may be arranged in rosettes, (i.e. tufted (congested) in terminal or basal rosettes) or they may be spaced out along the stems. Some species have solitary rosettes (e. g. ***A. rivae*** Baker), whereas others sucker from the base to form clumps of rosettes, in some cases with creeping stems so that the clumps can cover a large area (e. g. ***A. globuligemma*** Pole-Evans) (Smith & van Wyk., 1991; Newton, 2004). The leaves are sometimes spotted (maculated) more often in seedlings and young plants than mature plants. The leaves are dorsi-ventral and in most species are D-shaped in cross-section, but some have leaves more or less V-shaped (Sebsebe Demissew ***et al.***, 2003). The leaves are most often succulent. However, some miniature taxa, which mainly occur in high rainfall grasslands notably sect. ***Graminialoe*** G. Reynolds, (e.g. ***A. myriancantha*** Schult.f.) and sec. ***Leptoaloe*** A. Berger, (e.g. ***A. saundersiae*** Reynolds) seem to have a less succulent leaf consistency (Reynolds 1950; 1966; Smith & van Wyk., 1991).

Flowers are produced in racemose inflorescences. The inflorescences are usually branched (although occasionally simple), the lower branches sometimes branching again. The racemes may be dense or lax in different species. They are usually erect, but in some cases they are oblique or more or less horizontal, in one direction, becoming secund (e.g. ***A. mawii*** Christian and ***A. secundiflora*** Engl.). In a few species the peduncle grows

downwards and the branches bend upwards to present erect racemes (e. g. *A. penduliflora* Baker) (Newton, 2004). Aloes have long tubular flowers in most species, brightly coloured and conspicuously varying from white, yellow, pink, and greenish to red. Each flower is supported by a bract, the shape and size of which are important for identification. The tepals are fused to form a tube (but free to the base in *A. steudneri* Schweinf. ex Penz.). The outer parts of the tepals are more or less reflexed. The 3+3 stamens are free, inserted at the base of the ovary, exserted in the male flowering stage. Several species in northeastern Africa and southwest Arabia, such as *A. tomentosa* Deflers and *A. trichosantha* A. Berger., are unusual in having hairy flowers (Reynolds, 1966; Sebsebe Demissew & Gilbert, 1997; Sebsebe Demissew *et al.*, 2003). Fruit is a loculicidal capsule. The capsule wall is either papery or slightly woody when mature. The seeds are irregularly 3-sided to flattened, narrowly to broadly winged. The seed coat is covered with an arillus the colour of which varies from black and grey to brown (Reynolds, 1966; Sebesebe Demissew *et al.*, 2003).

In general, aloes are polymorphic both in their vegetative and reproductive characters. According to Reynolds (1966), "aloe plants vary so much in length and width of leaf, density and length of racemes, length of pedicels and perianths etc., that it is impossible to give precise measurements, or to devise any key to the groups and species that would be infallible, and from which the student could run any specimen to an exact identification".

1.2.2. Taxonomic review

The genus *Aloe* was described by Linnaeus (1753) when he proposed his sexual system of plant classification based on number of stamens and pistils. The familial position of aloes and their relatives has been changing several times over the past 250 odd years (Smith & van Wyk, 1991; Smith & Steyn, 2004).

The alooid plants were placed in Class Hexandria, Order Monogynia, due to the 6 stamens and a single pistil (Linnaeus, 1753). Subsequently, they have been treated as one

of the 28 tribes, namely Aloeae, in the family Liliaceae (Hutchinson, 1959). Later Cronquist (1981) included the alooid genera, along with *Kniphofia* Moench, in the family Aloaceae. Dahlgren *et al*. (1985) classified the alooid genera in the subfamily Alooidaeae in the family Asphodelaceae, with *Kniphofia* included in the subfamily Asphodeloideae. The currently widely accepted position of the alooid plants is in a separate family Aloaceae with seven genera: *Aloe* L., *Gasteria* Duval, *Haworthia* Duval, *Lomatophyllum* Willd., *Chortolirion* A. Berger, *Poellnitzia* Uitew., and *Astroloba* Uitew. (Cronquist, 1988; Smith & van Wyk., 1991).

According to Smith & Steyn (2004), "Aloaceae is a natural (monophyletic) group. All the genera in this family are therefore considered to have evolved from a common ancestor. The most obvious similarities amongst the genera are ± succulent leaves, crescentiform or cymbiform leaf outline in cross-section, markedly bimodal karyotype 2n = 14 chromosomes, and the presence of 1-methyl-8-hydroxyanthraqunones in the roots and anthrone-c-glucosides in the leaves; and leaf vascular bundles containing a parenchymatous inner bundle sheath". It has, however, been emphasized that Asphodelaceae *sensu str.* becomes paraphyletic without including Aloaceae. Cladists who do not wish to recognize paraphyletic groups formally, will still argue for a family Asphodelaceae *sensu lat.* including alooid taxa.

The genus *Aloe* occupies a central position in the taxonomy of Aloaceae. This is not only because it is the genus first described and with the largest number of species, but of all the genera in the family, it has been studied more extensively, both in terms of taxonomy and systematics. The other seven genera are generally considered segregates of the genus *Aloe* (Smith & van Wyk., 1991; Smith & Steyn, 2004).

The etymology of the name *Aloe* is not clear. Some authors have suggested that the word "aloe" is an old Arabic name, given by Linnaeus, which could have been derived from Arabic 'alloeh', referring to the medicinally use of the aloe plants (Johnson & Smith, 1982). Recently it has been proposed that the genus name *Aloe* is derived from the Greek

"aloe", the dried juice of aloe leaves, originally believed to be derived from earlier, Hebrew 'allal', i.e. bitter) (Stearn, 1983; Smith, 1993).

Since the generic name was first validly published by Linnaeus (1753), more than 15 names are available for the genus. However, only *Aloe* currently enjoys general recognition and the rests are accepted as synonyms (Smith & van Wyk., 1991):
Catevala Medik. (1786: 67) pro parate; *Kumara* Medik. (1786: 69); *Rhipidodendrum* Willd. (1811: 64); *Pachidendron* Haw. (1821: 35); *Bowiea* Haw. (1824: 299) non Hook. f. (1867: t. 5619); *Agriodendron* Endl. (1836-1840:144); *Papilista* Rafinesque (1840: 137); *Succosaria* Rafinesque (1840: 137); *Busipho* Salisbury (1866: 76); *Ptyas* Salisbury (1886: 76); *Chamaealoe* A. Berger (1905): 43); *Leptaloe* Stapf (1993: t. 9300); *Aloinella Lemee* (1939: 27) non Cardot (1909: 76); *Guillauminia* Bertrand (1956: 41).

The genus *Aloe* was monographed on a number of occasions in the 19 th and 20 th centuries (Baker, 1880; Berger, 1908; Reynolds, 1950; 1966). The currently most often accepted infrageneric classification of *Aloe* is that of Berger. His classification gained wide acceptance, mostly because his contributions on *Aloe* (Berger, 1908) was published as part of *Engler's Das Pflanzenreich.* Berger based his classification primarily on a combination of easily observable and readily available vegetative and reproductive characters. He established, e.g. groups such as "Maculata" for spotted-leaved aloes and "Graminaloe" for the grass-leaved aloes (Smith & van Wyk, 1991).
Subsequently, G. W. Reynolds revised the genus *Aloe* on a global scale. He monographed Southern African *Aloe* species (Reynolds, 1950) and Tropical African and Madagascan ones (Reynolds, 1966). His two books constitute a kind of monograph that is an important benchmark for the study of aloes. He traveled widely and studied various species intensively for more than 30 years. As the result of comprehensiveness, though not complete, his work on the genus became a standard works for the last 50 years and they are still the most cited in botanical literature on aloes (Smith & Steyn, 2004).

The two classification of Reynolds (1950, 1966) are not completely consistent, only his grouping/subdivision of Tropical African and Madagascar taxa is surveyed here. He

defined 20 groups based on common character (s) or character combinations and also on his personal knowledge of species as they grow in the wild:

Group 1 - Sect. *Graminialoe* Reynolds (grass aloes): small acaulescent plants with fuisform roots. **Group 2 -** Sect. *Leptoaloe* Berger: plants acaulescent or short-stemmed. **Group 3 -** Sect. *Bulbiformes* Christian: plants with underground "bulbs". **Group 4 -** plants with stripped perianth. **Group 5** - plants rather small with compact rosettes or larger with open rosettes. **Group 6 -** Series *Saponariae*: plants with perianth pronounced basal inflation, abruptly constricted above the ovary, thence enlarging to the throat. **Group -7** Series *Hereroenses*: plants acaulescent or short-stemmed. **Group 8 -** Series *Aethiopicae* Berger: plants with perianth trigonously indented above the ovary. **Group 9 -** Series *Verae* Berger: plants acaulous rarely caulescent, solitary or in group. **Group 10 -** plants pendent or semi-pendent. **Group 11 -** Series *Latebracteatae* Berger: plants with bracts large, broadly ovate or suborbicular. **Group 12 -** plants acaulescent or short-stemmed. **Group 13 -** plants with perianths clavate. **Group 14 -** Sect. *Orthologhae* Christian: plants with oblique racemes, flowers secund. **Group15** - plants with racemes densely flowered, bottle-brush-like, flowers sessile or very shortly pedicellate. **Group 16 -** medium to large plants with densely rosulate leaves forming rather compact rosettes. **Group 17 -** medium to large plants with leaves spreading to recurved, slightly or deeply canaliculated. **Group 18 -** Sect. *Pachydendron* Haw: plants tall-stemmed, simple or few branched from base. **Group 19 -** shrubs. **Group 20 -** tall trees dichotomously branched and rebranched.

Some of the groups and species of Reynolds are distinctive and can be recognized immediately, but others are heterogeneous. Reynolds (1966) himself stated "there are no fixation of characters in aloes and no measuring stick by which species can be determined. A reliable concept of a good species depends infinitely more on a worker with considerable field experience, who observed how species vary under different conditions of environment, soil, climate, rainfall, altitude, etc."

Not only the infrageneric taxonomy, but also the taxonomy on the species level is in flux. This can primarily be attributed to the fact that, the *Aloe* species display unusual patterns

of variation among populations and species and inconsistent integration among them. The morphology of leaves, flowers and inflorescence varies widely and inconsistently (Smith & van Wyk, 1991). Consequently, several post-Reynolds species have been described with authors 'forcing' their new taxa into an unwieldy system. Furthermore, the genus *Aloe* has fallen prey to immense taxonomic splitting resulting in many ill defined taxa (Vilijoen & van Wyk, 1996). For example, in a recent revision of *Aloe*, Glen & Hardy (2000), reduced the number of species recognized from Southern Africa from 150 to 119.

Recently, several new species concepts and classification systems have been adopted to solve the problems. Besides gross morphology, other characters have been employed by taxonomists to reveal the relationships in the genus. Some of these characters are pollen (Steyn *et al*., 1998); chemistry (Vilijoen & van Wyk, 1996; Vilijoen *et al.*, 2001) and isoenzyme (Van der Bank *et al.*, 1995). In many instances these characters were used for proposing new or confirming the existing relationships.

These days it is agreed by all researchers that the most accurate classification, as with many other plant groups, can be achieved from analyzing multi-variate matrices of numerous characters. However, for some time to be, in *Aloe* at least, gross morphology will remain the most important source of characters for identifying preserved and living material. As laboratory technology and methodology become more sophisticated and accessible, infrageneric relationships may be clarified by employing more obscure characters (Smith & Steyn, 2004).

1.2.3. Diversity, distribution and ecology

1.2.3.1. Diversity

Reynolds (1950) listed 132 species for South Africa and Reynolds (1966) listed 197 species for Tropical Africa and Madagascar, including also species occurring naturally in Arabia and Socotra. Nine species occur in both regions and four extra species are mentioned by Reynolds (1966) without being formally included, leading to a total number 324 (Reynolds, 1966). Since then, investigation of the *Aloe* species progressed

rapidly and resulted in discovery of several new taxa. For example, Smith & van Wyk (1991) reported more than 360 species. Adams *et al*., (2000) reported about 400 species and very recently about 450 species was reported by (Smith & Steyn, 2004; Newton, 2004).

Many countries have endemic species. The highest rate of endemism is in Madagascar (77 species (100%), and isolated Islands of Indian Ocean (e.g. Comoros (100%), Mauritius (100%) and South Africa (71 out of 119 (59.7%). A large number of endemic taxa also exist in Tropical East Africa (Kenya, Uganda, and Tanzania), for example, 50 out of the 83 species known (60%) are endemic to the region (Carter, 1994). Conversely, it is not surprising that very small countries, such as Burundi and Rwanda have no endemic species. Of the small countries on the African mainland only Lesotho and Swaziland have endemic species, with one each (Newton, 2004).

1.2.3.2. Distribution

The *Aloe* species are naturally distributed throughout mainland Africa, tropical and subtropical, except for the moist lowland forest zones and the western most part of the West Africa (Reynolds, 1966). The majority of species occur in Southern Africa and on the eastern side of the continent. Some species are found on the Arabian Peninsula and on Madagascar and a few, mostly formerly in the genus *Lomatophyllum*, are known from some of the smaller Indian Ocean islands (Smith & van Wyk, 1991; Newton, 2004) (see fig. 1). The genus has also been introduced to the West Indies and many other tropical countries. For example, *Aloe vera* has long been cultivated for its medicinal properties in many tropical and sub-tropical countries. The exact origin of *A. vera* is uncertain, but it seems likely that it is the Arabian Peninsula, home of the closely related, and possibly conspecific, *A. officinalis* Forssk. Others like *A. arborescens* and *A. saponaria* are frequently seen as ornamentals in European gardens (Sebesebe Demissew, 1996; Newton, 2004).

Some species are widespread, while some occupy just a single locality. Reynolds (1966) cites *A. buettneri* as the most widespread species, with the longest distance between any

two populations of at least 5,600 km, (i.e. from Mali to Zambia) and the species has even since been recorded from Nambia. Carter (1994), however, regards this as a West African species only and refers the Southern African populations to two related species. Another very wide spread species is *A. myriacantha*, with a range of about 4,800 km from Kenya and Uganda to the South Africa. Most other widespread species have more modest distribution ranges, amounting only to some hundreds of kilometers. At the other end of the scale, there are species with restricted distributions. They may be known only from a very limited area (e. g. *A. kulalensis* is known only from single isolated mountain (Mt. Kulal) in Northern Kenya (Newton, 2004).

Fig. 1 Geographical distribution of the genus *Aloe* L. (Adapted from Smith & van Wyk, 1991; Newton, 2004).

1.2.3.2. Ecology

The habitats occupied by aloes vary from forests (e.g. the dry coastal forests of eastern Africa including species such as *A. eminens* and *A. volkensii* Engl. subsp. v*olkensii*.);

wooded-grasslands (e.g. many acaulescent species, such as *A. lateritia* Engler and *A. secundiflora* Engler); and woodlands (e.g. species that are found in *Acacia* scrub and other thickets such as *A. morijensis* S. Carter and Brandham). Several species, including *A. chrysostachys* Lavranos and L.E.Newton and *A. classenii* Reynolds, occur on expanses of rocks, rooted into soil pocket or cervices. Others are also found in mountains, cliff faces, and beachs and even under the spray of waterfalls. However, the genus does not occur in moist lowland forests (Newton, 2004).

Aloe species are adapted to highly disturbed areas and areas with extreme environmental conditions; and are found flourishing on nutrient deficient soils. In the wild, aloes occur on a wide range of soil types and substrates. Some seem to be restricted to certain substrates, including dolomite (e. g. *A. alooides* (Bolus) van Druten), granite (e. g. *A. torrei* Verd. & Christain), gypsum (e. g. *A. berviscapa* Reynolds & Bally) and limestone (e.g. *A. calidophila* Reynolds) (Court, 1981; Willert *et al*., 1992). However, the success of most species in cultivation suggests that they are tolerant of the many soil types to be found in gardens and potting mixtures (Newton, 2004).

Most *Aloe* species have succulent xerophytic leaves, adapted to survive in areas of low or erratic precipitation. The fleshy leaves with sunken stomata and thick cuticular wax help them to resist drought. As the result, aloes are typical to semi-arid and dry deserts. They have also several adaptive characters that enable them to colonize wide range of habitats. For example, tough and spiked leaves with unpalatable juice protect them from grazing, brilliant flowers that have an excessive nectar production attract pollinators, and winged seeds aid them for wind dispersal (Court, 1981). These unique adaptations make the aloes the important groups of plant in such environments in providing important source of shelter, nectar food, and moisture, especially to the avifauna (Oldfield, 1997).

Some *Aloe* species are found as dense populations (e.g. *A. falcata* Baker), whilst others occur as scattered individuals (e.g. *A. variegata* L.). They are often prominent, but rarely dominant in the ecological sense, except where the vegetation is sparse and they are the only large plants around (e.g. *A. pillansii* Guthrie) (Oldfield, 1997; Newton, 2004).

1.2.4. Use, ethnobotany and conservation

1.2.4.1. Use

Members of the genus *Aloe* have been known for their use both in medicine, commerce, and horticulture; and have also provided a fascinating subject for research from chemical, pharmaceutical, economic and taxonomic points of view (Carter, 2001).

Since ancient times the medicinal use of aloe products has been documented in many ancient texts, including the Bible (Groom & Reynolds, 1987). For example, the drug was included in the Egyptian Book of Remedies (about 1500 B.C.), as well as in that of the Hebrews, as a laxative and dermatological preparation. Mesopotamians were also aware of its medicinal properties by that time (Swanson, 1995). Aloe was first reported in Greek literature before the first century as laxative. In the first century Dioscorides wrote of its use in treating wounds, chapping, hair loss, genital ulcers, haemorrohoides, boils, mouth irritation and inflammation (Hennessee, 1998). In the seventh century, aloe was also used in the Orient for eczema and sinusitis. When aloe was introduced into Europe, it was used for constipation and skin aliments and later on to treat radiation burns. At the end of the seventeenth century it was possible to find aloes from the Barbados (*A. barbadensis* Mill. which is a synonym of *A. vera*) in Europe, and towards the end of eighteenth century, the Cape Aloe (South African aloes) (Shelton, 1996). The curative nature of most aloes has been exploited in modern societies as well. For example, "*Aloe vera*" is known to kill *Mycobacterium tuberculosis*, the organism responsible for tuberculosis, and also the *Herpes virus* responsible for herpes. Other research has shown that it inhibits growth in many common organisms such as yeasts, fungi, and the bacteria associated with wound infection (Antherton, 1995).

Three distinct preparation of the aloe plants are used in medicin: aloe latex (= aloe); aloe gel (= aloe vera); and, whole leaf extract (= aloe extract). Aloe latex is used for its laxative effect. Aloe gel is used for skin ailments such as wound healing, psoriasis, herpes and internally by oral administration in diabetic and hyperlipidaemic patients and

to heal gastric ulcers. Aloe leaf extract is potentially useful for cancer and AIDS (Mascola *et al*., 2004).

The multitude of medicinal uses described and discussed over the centuries is sometimes diffcult to evaluate. However, authors such as Crosswhite & Crosswhite (1984) have given detailed accounts of the drug in use, concentrating on the species *Aloe vera* and *Aloe ferox* Miller which seem to be the main ones in use, with *Aloe perryi* from Socotra mentioned more rarely.

Some species of *Aloe* have further been used in a wide range of commercial uses. For example, leaves of *A. vera*, are used in the production of many cosmetic products. The many kinds on the market include after shaving gel, mouthwash, hair tonic and shampoo, skin-moistening gel, and even a 'health drink' (Groom & Reynolds, 1987). In recent years a brand of washing powder and a brand of toilet paper with '*Aloe vera*' have appeared on the market (Mascola *et al*., 2004). In South Africa, dried leaf exudates of *Aloe ferox* are exported (Van der Bank *et al.*, 1995).

Aloes are also popular as home and garden plants in both tropical and temperate regions and have become important in the horticulture industry (Carter, 1994). In many African countries aloes are used in gardens as decorative plants. For example, in Nariobi, the indigenous *Aloe ballyi* Reynolds and the exotic *A. barberae* Dyer are used as street trees (Newton, 2004). Smaller-growing species are popular pot plants for enthusiasts who grow succulent plants as a hobby in many temprate countries. The minature Madagascan species are used for this purpose. Hybrids of smaller-growing species, suitable for pot cultivation, and larger-growing species have been produced in Australia, the UK, the U.S.A. and South Africa to produce some spectacular garden plants for commercialization (Capasso *et al.*, 1998).

1.2.4.2. Ethnobotany

A number of ethnobotanical uses of various *Aloe* species have been recorded (e. g. Hutchings, 1989a, b; Pujol, 1990). A multitude of species are used throughout Africa in traditional medicine and for other purposes. For example, sap of *Aloe lateritia* Engl. is used in some communities in Kenya and Ethiopia for treatment of eye aliments (Wabuyele, 2000) and also some species have been used traditionally for treatment of constipation, burns and dermatitis in west Africa (Morton, 1961). A leaf sap of *A. buettneri* is said to be used as an ingredient of arrow poison in Mali. Several species are poisonous, because of the presence of the hemlock alkaloid alpha-conicience in leaves (Nash *et al*., 1992). These species have characteristic smell usually described as that of mice or rats, for which reason the East African *Aloe ballyi* is known as 'the rat aloe'. In Kenya and Somalia it is reported that *A. ruspoliana* Baker is used to kill hyenas by smearing meat with the leaf extract.

There are published reports of human deaths resulting from the use of aloe leaves (e.g. Drummond *et al*., 1975; Newton, 2001). An insect repellent can be made by drying and burning aloe leaves and similar preparations are used to protect animals against ticks and stored food against weevils (Reynolds, 1950; Newton & Vaughan, 1996). For many years, roots from species in the *A. saponaria* group have been used to make soap. In South Africa, leaf sap of *Aloe maculata* was used locally in the tanning of garments made from skins (Reynolds, 1950). The exudates from some species, such as *A. megalacantha* Baker and *A. confusa* Engler, are used to dye cloth and for making ink. The ash of dried *A. ferox* and *A. marlothii* A. Berger leaves is an ingredient in snuff prepared in some parts of South Africa (Newton, 2004). With the bitter compounds in the leaves, aloes are not regarded as edible, but Reynolds (1950) reported that in South Africa the leaves of *A. ferox* were used to make a jam and young flowering shoot of *A. kraussii* Baker and *A. minima* Baker are eaten as raw vegetables by the Zulus. The flowers of *A. zebrina* Baker have been used to make cakes. In West Africa, flowers of *A. macrocarpa* Tod., are eaten by various tribes, and used as seasoning herb in cooking (Reynolds, 1966). Newton & Vaughan (1996) report that dried leaf material may be mixed with tea leaves in South Africa.

Some shrubby species are grown as hedge plants in different parts of Africa (e.g. *A. arborescens* Miller, *A. ferox, A. dawei* A. Berger and *A. kedongensis* Reynolds (Newton, 2004). *A. rivae* plants have also been used as boundaries and *A. gilbertii* as hedge plant and for erosion control in Ethiopia (personal observation in the field). Stems of the Madagascan *A. vaotsanda* Decary are used locally for building huts. Branches of the South Africa *A. dichotoma* L.f. are used to make quivers for arrows, giving this species the vernacular name 'quiver tree'. In more modern times the hollow dead stems are cut into pieces to make various decorative items, such as ash trays and other small containers. The spiny leaves of *A. marlothii* A. Berger are used for scarping and thinning animal hides to prepare them for making garments (Reynolds, 1950). Aloes have been used in various activities relating to superstition due to their unique appreance (e.g. *A. aristata* Haw. is often hung over doors of houses as charms intended to ensure long life for the occupants and *Aloe maculata* is used to prepare a charm against lighting, Reynolds, 1966).

1.2.4.3. Conservation

Many species of *Aloe* are highly threatened (Carter, 2001) due to, e.g. agricultural expansion into marginal lands and habitat destruction due to new development schemes near urban and regional centers. Another reason might, however, be that many species, especially those with a very restricted distribution area and only small population, are much sought after by succulent enthusiasts for cultivation as rarities. Other species are collected for their use in medicine; and in the cosmetic and horticulture industries (Sebsebe Demissew, 1996).

According to Newton (2004), threats to species of *Aloe* can be placed in three major categories: over-collection of plants for cultivation, destruction of plants in harvesting leaf exudates and destruction of natural habitats. Some species, especially the miniature species of Madagascar and smaller-growing plants of South Africa, are possibly over collected by people supplying the nursery trade. The rising popularity of 'field trip holidays' by amateur growers as a new kind of tourism, may also result in some

collection of wild plants (Oldfield, 1997). There is also a lucrative trade in leaf exudates, required mainly for medicinal and cosmetic purposes, and these are frequently harvested from wild plants. Much of this activity is well organized, but there is also a large unofficial exploitation of wild plants (Newton, 1994; Newton & Vaughan, 1996). In South Africa 'aloe tapping' is a well-established industry, going back for more than 200 years. The main species used is *A. ferox*, with export records dating back to 1761. Newton & Vaughan (1996) estimated that a total of about 700 tons of crystalline bitters are harvested each year from about 17 million plants, 95% of which are in the wild. The harvesting of leaf exudates started in 1960s in Kenya, with local people being paid by 'outsiders' with no attempt to ensure sustainability. With no traditional or other controls in place, various species may be harvested without regard to chemical composition. In some areas this harvesting might be done on sustainable basis, but cases are known where whole populations are destroyed in the process (Newton, 1994). In many countries where aloes are native, the rise in human population levels results in an increased demand for land to use for agriculture, building, etc. This has led to wholesale clearing of natural habitat or vegetation. In some areas, the continued expansion of human populations is forcing people to move into arid areas, where many aloes occur. Another problem is overgrazing. Many people in arid areas have herds of domestic animals in numbers far greater than the carrying capacity of the land, and the land becomes increasingly denduded of vegetation (Newton, 2004).

Attempts to protect aloes as endangered species have been made at international and national levels. The most effective protection effort at the international level is provided by the Convention on the International Trade in Endangered Species of Wild Fauna and Flora (CITES), started in 1976. This convention aims at controlling the movement of endangered species and derivatives between countries, prohibiting trade in some species (listed in CITES Appendix I) and requiring official documentation of numerous other species (listed in CITES Appendix II).

Currently, 22 species of *Aloe* (mostly from Madagascar) are listed in CITES Appendix I and all other species of the genus in Appendix II, with the sole exception of *A. vera*, which is free from restrictions (Newton, 2004). The documentation requirement provides

an opportunity for monitoring the international trade in the listed plants, at least the legal trade (Oldfield, 1997). Most African countries have also national legislation aimed at protecting endangered species, animals and plants and preserving habitats in selected localities, such as national parks. Unfortunately, enforcement of the legislation is poor because of a lack of personel and a need to concentrate national effort in solving other immediate economic and social problems (Newton, 2004). However, in some countries like Madagascar and South Africa positive action has been initated in order to reduce the pressure on wild populations of rare species. For example, in Madagascar many nurserymen and individual growers propagate their stock plants from seeds or by vegetative means to meet the demand in the horticultural trade. There are also attempts to re-introduce some rare species to their natural habitats, using material propagated in cultivation (Smith & Swartz, 1999). The promising development is the use of tissue culture techniques, by means of which thousands of plants can be produced within a few months. Relatively few aloes have been propagated by tissue culture, but there are reports of success (Fay *et al.*, 1995; Barringer *et al.*, 1996).

1.2.5. Reproductive and population biology

Flowers of almost all *Aloe* species are tubular and brightly colored as mentioned earlier. They are unscented and produce abundant nectar. These features point to ornithophily as a pollination syndrome, and sunbirds (Nectariniidae) are frequent visitors to aloe flowers in the field and in African gardens (Smith & van Wyk, 1991; Stokes & Yeaton, 1995). The different *Aloe* species have different flowering periods; some flower over extended periods, thereby offering continuous food supply for nectar feeding birds which on the other hand are vital for the pollination of the flowers (Reynolds, 1966; Sebsebe Demissew *et al.*, 2003).

Although they are not typical melittophilous flowers, aloes are also visited by bees. In some areas, especially in South Africa, the flowering of aloes is important in apiculture, though it is reported that nectar and pollen of some species can affect the behaviour of bees, making them vicious (Newton, 2004). In West Africa some wasps were captured on

flowers of *A. buttneri*, and dissection of the gut revealed that they had been eating pollen. Pollination of flowers of different shapes, such as the white campanulate flowers of *A. albiflora* Guillaumuin, has yet to be investigated. The Madagascan *Aloe suzannae* Decary is exceptional in the genus in having nocturnal fragrant flowers, presumably pollinated by nocturnal animals such as bats and small lemurs (Newton, 2004). The abundance of nectar produced by the flowers of some species is such that even baboons have seen collecting flowers in order to suck the nectar (Reynolds, 1950). Stokes & Yeaton (1995) in their pollination experiments on populations of *A. candelabrum* Berger showed that birds are the only effective pollinator guild in the species.

Almost all aloes are self-incompatible, though oddly enough the flowers are protandrous (anthers ripen and pollen is dispersed before the stigma is receptive) and so self-pollination would not occur anyway. The species formerly included in the genus *Lomatophyllum* are reported as exceptions in being self-compatible (Lavranos, 1995). In an area where two or more species of *Aloe* flower at the same time, the main pollen vectors, sunbirds are flying indiscriminately from one species to another. Consequently hybridization is frequent. Reynolds (1950, 1966) reported many natural hybrids, especially in South Africa.

Most aloes produce dry dehiscent capsules that split open at maturity to release the seeds. The stiff erect capsules are only open in the upper parts. This hampers an easy fall out of the seeds. A strong wind or a strong kick is required to release or eject the seeds (ballistic dispersal). Seeds, in most species, are winged and accordingly mainly wind disppersed. The function of the thin arils covering the seeds is not clearly understood (Sebsebe Demissew *et al.*, 2003; Newton, 2004). It is interesting to note that aloe seeds in very dry climates have larger wings that would help them to be further dispersed.

In addition to reproduction by seeds, several species have the ability to produce lateral rosettes that may develop roots and thus independent individuals by fragmentation (suckering). Vegetative propagation might be an important factor for maintaining the populations. This is often seen in the spatial arrangement of the individuals, which occur

in clusters (Sebsebe *et al.,* 2003). Bulbil formation is reported as a regular occurrence in *A. bulbillifera* H. Perrier (from Madagascar) and *A. patersonii* B. Mathew (from Congo). The bulbils are formed in the axils of inflorescence branches. This phenomenon was also observed once on a cultivated plant of the Kenyan *A. lateritia* (Newton, 2004).

Stokes & Yeaton (1995) found that the reproductive fitness of *A. candelabrum* is dependent on the number of plants flowering per unit area. *A. candelabrum* was found to exhibit clumping in young populations, with clumps acting as nuclei from which plants spread slowly over time at maturity. Limited seed dispersal resulted in intense competition and consequently self-thinning within the stands.

Very few studies were conducted regarding the population biology of aloes. Midgley *et al.* (1997) studied the population structures of *A. dichotoma* L.f and *A. pillansii* Guthrie. in different localities (Richtersveld, Springbok and Nieuodtville) in South Africa. Their results showed that both *Aloe* species had high proportions of dead individuals in the Ritchtersveld compared to other localities and both species had only few seedlings there. Mortality here was attributed to destruction by baboons. The populations in the other localities had low levels of mortality.

1.2.6. Cytogenetic and chemotaxonomic studies

1.2.6.1. Cytogenetics

Cytological studies and surveys done so far on the genus *Aloe* indicate constancy (uniformity) of karyotypes with a basic number n = 7 (Cutler *et al.,* 1980). Most species of *Aloe* are diploid (2n = 14), although polyploidy is known from a few species (Brandham, 1971). All *Aloe* taxa share a conserved biomodal karyotype with a basic genome structure of one long sub-metacenteric, three long acrocentric; and three short acrocentric chromosomes (Adams *et al.,* 2000). Consequently chromosome structure and number, as taxonomic characters were found to be less important in identification of relationship within the genus *Aloe.*

The chromosomes in aloes are large compared with those of most angiosperms, with the short ones averaging 1.5-3 μm in length, and the long ones almost exactly 4.6 times larger in a sample of *Aloe* species (Brandham & Doherty, 1998). Neverthless, some interspecific variation in overall chromosome length and chromosome morphology was reported. For example, structural changes have been shown to be common in wild populations of some species, as *A. pubescens* Reynolds and *A. rabaiensis* Rendle (Brandham & Johanson, 1977). They explained this variation with the occurrence of paracentric or pericentric inversions or inter-chromosomal translocation of segments of different length. These phenomena are may be large enough to alter the gross morphology of the chromosome involved.

Polyploidy has recently been reported more frequent than was belived earlier. One record of triploidy (2n = 21) and several records of tetraploidy (2n = 28) in addition to the single wel-known example of hexaploidy in the genus (2n = 42) (Brandham, 2004). Polyploidy is not uniform in its geographical distribution in the genus. It is more common in East African aloes, with *A. inermis* Forssk. and *A. cremnophila* Reynolds from Somalia; *A. juvenna* P. Brandham and S. Carter from Kenya; and *A. jacksonii* Reynolds from Ethiopia being tetraploids with 2n = 28 (Brandham *et al.*, 1994) and can be regarded mostly as the products of sporadic doubling of the chromosome numbers of unrelated diploid species that have stabilized through natural selection to produce tetraploid species. The tetraploid group also comprises *A. cheranganiensis* S. Carter & P. Brandham, *A. dawei* Berger, *A. elgonica* Bullock, *A. kedongensis* Reynolds, *A. ngobitensis* Reynolds and *A nyeriensis* Christian, which occupy the Rift valley of central and northern Kenya. In southern Africa, where large numbers of species occur (Reynolds, 1950), only *A. ciliaris* Haw. is known to be polyploid with *A. ciliaris var. ciliaris* being hexaploid (2n = 42). This is the only known case of hexaploidy in the entire genus. There are a few reports of triploidy in *Aloe* (2n = 3x = 21), e.g. *A. humils* (L.) Mill. and *A. jucunda* Reynolds (Brandham, 1971). These might represent the hybridization events between diploid and tetraploid.

In an analysis of a population of *A. elgonica*, Brandham & Johnson (1977) found some specimens having 2n = 29, with either an extra pairs of long or an extra pairs of short chromosomes. This type of aneuploidy is occasional and is to be expected in tetraploid angiosperms as a result of irregular 3-1 segregation of quadrivalents during meiosis (rather than 2-2), with subsequent survival of the aneuploid gametes to fuse with normal ones.

1.2.6.2. Chemotaxonomy

The chemistry of the aloe plants have been studied intensively since 1980s. The leaf is the most frequently studied organ. When a typical aloe leaf is cut open, there appears an exudate arising from cells adjacent to the vascular bundles (Beaumont *et al*., 1985), which is usually yellow-brown, in a few species e.g. *A. confusa* Engl. changing into a deep blood red colour. These exudates contain phenolic compounds which can be distinguished chromatographically. Most of the exudates identified are chromones, anthraquinones or anthrones derivatives (Reynolds, 1985a). More than 80-chromatographic bands were revealed, when analyzing a large number of species (Reynolds, 1985b). According to Ermias Dagne (1996), anthrones are the most important class of compounds found in aloes. The most important constituents of commercial aloe are the anthrones, aloin A and B and the chromones, aloesin and aloeresin A. The roots of aloes are reported to contain 1-methyl-8-hydroxyantraquinones.

The distribution of such chemical compounds has been used for chemotaxonomic discussions. Some are widespread in the genus, and others are confined to few species and therefore of potential chemotaxonomic value.

Cutler *et al*. (1980) in their multidisciplinary study of 12 *Aloe* species from Congo, Kenya, Uganda and Tanzania analysed leaf exudates using TLC (Thin Layer Chromatography). Based on their results, nine exudate patterns were identified, which correlated with results from anatomy, cytology and geographical distribution of the taxa investigated. Viljoen *et al.* (1996) combined morphology, HPLC (High Performance

Liquid Chromatography) and horizontal starch gel electrophoresis to unravel the relationship between *A. candelabrum* and *A. ferox*. The results showed that *A. ferox* and *A. candelabrum* are closely related.

As part of a general chemotaxonomic survey of 380 taxa of aloes, Viljeon *et al.* (1999) demonstrated the presence of plicataloid (a naphthaline derivative) in 20 taxa, 17 of which were restricted to Eastern Africa and only 3 to South Africa. This compound was absent in species that are endemic to Madagascar. Some of the species associations that were suggested by this chemotaxonomic evidence conformed to morphological similarities, while many of the taxa had not been previously associated earlier. They concluded that chemical data are useful in providing evidence for taxonomic assessment within the genus *Aloe*. It should, however, be used in combination with other sources of evidences.

1. 2. 7. Isoenzyme and DNA studies

Relatively few isoenyzme and DNA studies were carried out on the genus *Aloe*.
South African *Aloe* species, as *Aloe ferox* and *Aloe marlothii* (Van der Bank *et al*., 1995); *Aloe ferox, Aloe arborescences* and their hybrids (Van der Bank & van Wyk, 1996); and *Aloe ferox* and *Aloe candelabrum* (Viljoen *et al*., 1996) have been analyzed genetically by isoenzyme electrophoresis. The results of these analyses showed low levels of genetic variation in the studied taxa. Some of the analyzed species are very close, indicating that the rank of species might be disputed. These observations led them to recommend more extensive electrophoresis survey in the genus *Aloe.* They explained the low levels of genetic variation found with ecological theory that species well adapted to their environment need less genetic variation. They proposed further that aloes have been selected for a xerophytic habit making them less sensitive to drought stress (Van der Bank *et al.,* 1995). The little variation revealed in South African aloes contrasts with the finding from two Eastern African species, *Aloe macrocarpa* and *Aloe lateritia* by (Wabuyele, 2000) that displayed variation in several isoenzyme loci.

Despite the uniform karyotypes in many *Aloe* species, they vary in DNA contents. The nuclear DNA C-value range in diploid species from 1C = 8.10 picograms (pg) in *A. haemanthifolia* to 35.95 pg in *A. dawei* (Zonneveld, 2002). Although the number of species examined was not large, there was strong indication that the primitive species, e. g. *A. tenuior*, had the lowest nuclear DNA amount (and thus the smallest chromosome set), and that the amount of DNA and overall chromosome size increased with evolutionay advancement (Brandham & Doherty, 1998). In addition, the physical organization of 18S-5.8S-26S and 5S ribosomal DNA (rDNA) was investigated for 13 *Aloe* species representing different geographical ranges: Arabian Peninsula, East Africa, South Africa, and Madagascar, ploidy levels: diploid, tetraploid and hexaploid, morphological types: mesophytes, rosettes, shrubs and tree. The Floresent *In-situ* Hybridization (FISH) analysis of 5S rDNA distribution showed a similar interstitial location on a large chromosome in all species examined. In contrast the distribution of 18S-5.8S- 26S rDNA was variable with differences in number, location, and size of loci found between species. The species considered closely related by Reynolds' (1950, 1966) morphological classification appear to be close. For example, *A. tenuior* and *A. ciliaris* (the mesophytic scramblers) showed similarity in sequence data of Internal Transcribed Spacer (ITS) of rDNA. Other species that occur in the same geographical area were also closely related (e. g. *A. bakeri* and *A. acutissima* from Madagascar) (Adams *et al*., 2000).

1.2.8. Micromorphometric studies

Initial studies of aloe leaf surfaces with the light microscope indicated a possible rich source of diagnostic characteristics. The very thick cuticle covering the epidermis had a rough interface with the outer wall of the epidermal cells made difficult to see a range of very fine surface sculpturing. However, with the discovery of the Scanning Electron Microscopy (SEM), the sculpturing of the cuticle and the varied nature of epicuticular wax has been determined (Cutler, 1979).

In aloes leaf cuticle is thick and covered with a layer of wax. In most species examined, the surface wax has distinctive patterns of ridges and/or micro-papillae, the size and

frequence of which can be of taxonomic value. Stomata are usual sunken, i.e. situated at the base of a stomatal cavity (Cutler, 1969). Newton (1972) studied the leaf relief patterns of *Aloe macrocarpa* var. *major*, *Aloe schweinfurthii*, *Aloe buettneri* and *Aloe keayi.* The results of this study indicated that relief patterns in the taxa were species-specific, and accordingly are useful taxonomic characters. Observations from specimens of *A. ferox* Miller and *A. africana* Miller, which have a very wide distribution, showed little variation between species in their leaf surface sculpturing. Experiments have shown that the epidermal sculpturing is under close genetic control (Cutler *et al*., 1980). In crossing experiments between diploid and tetraploid species, it was possible to demonstrate which arms of the particular chromosomes that coded for the normal sculpturing characters. Further experiments crossing *Aloe* with *Gasteria* and *Haworthia* species (Brandham & Cutler, 1978) confirmed the strong genetic control of the characteristic sculpturing feature. The appearance of this sculpturing revealed by SEM can be used to identify sterile plants or leaf fragments and to indicate relationships between species. Sometimes surface sculpturing may provide some information on habit preference of particular species. For example, it is easy to detect those species that grow in areas that are generally moist, such as *A. haemanthifolia* and *A. ciliaris* Haw. since their stomata are not sunken and have no overarching lobes.

The leaf anatomy of aloes was first studied for a taxonomic purpose (Cutler, 1969). Later, when it became clear that aloe exudates could be of medicinal interest, anatomy was used to try to locate the cells or tissues in which particular substances arose or were stored (Beaumont *et al.*, 1985). The internal anatomy of aloe leaves is fairly constant, regardless of the species; and provides little information of wider systematic significance. The leaves have large amount of parenchymatous ground tissue covered on both sides by chlorenchyme containing the green chloroplasts, and the flattened vascular bundles. Of particular interest are thin-walled bundle cap cells in the phloem pole. These strands contain exudates that may be coloured. The mucilage, which is of medicinal and cosmetic interest, is mainly found in the central cells of the parenchyme. Its function in the plant may be to store water (Cutler, 2004). Beaumont *et al.*, (1985) looked at the origins of substances present in the bundle cap cells. In a living plant, they are colourless, but on

exposure to air, they often become coloured, yellow, brown or purple. They found three types of cells at the phloem pole of the vascular bundles: aloin cells, outer bundle sheath cells and fibers. Species with fibers instead of aloin cells, such as *A. fibrosa* Lavranos & L. E. Newton, produce only sparse exudates, while the majority of species that contain aloin cells, produce in large amount.

Pollen grain also displayed variation relevant for taxonomic delimitation in *Aloe* (Steyn *et al.,* 1998). They investigated pollen character states in 36 species and showed that pollen grains were shed as monads, are of medium size, more or less elliptical in shape, heteropolar and monocolpate with distal colpus and a perforate to microreticulate tectum. Muri were smooth. Based on these parameters, species were categorized as belonging to three pollen types, *Aloe albida* type, *A. ciliaris*-type and *A. dinteri*-type. These compared well with some categories of Reynolds' (1950) classification of the genus. They therefore recommended more comprehensive SEM studies to evaluate their findings, and that pollen data should be used in combination with other sources for species diagnosis.

1.3. The aloes of Ethiopia and Eritrea

1.3. 1. Earlier taxonomic accounts

Taxonomic treatments or documentations of aloes in Ethiopia and Eritrea dates back to the mid-18th century. Berger's (1908) monograph in Engler's *Pflanzenreich* listed 181 species of *Aloe* then known around the world, out of these 14 species were from the flora area. They were described from materials collected during expeditions particularly by the Italian botanist Domenico Riva led by an Italian Nobel man, Eugenio Ruspoli. Most of the type collections of these species are kept in the Erabrio Tropicale in Florence (FT). The labels of the Riva collections were hand-written and difficult to read with minimal notes on habit, locality and other aspects. The type materials proved to be fragmentary and incomplete. As the result, the descriptions of many early species (i. e., Berger's taxa)

from the flora area were vague, incomplete, and sometimes misleading (Reynolds, 1966; Gilbert & Sebsebe Demissew, 1992).

The aloes of Ethiopia and Eritrea included in Berger's account were mostly described by Baker, Berger and Engler. These are: *A. trichosantha* Berger, *A. scholleri* Schweinfurth, *A. macrocarpa* Todaro, *A. lateritia* Engler, *A. ellenbeckii* Berger, *A. percrassa* Todaro, *A. steudneri* Schweinfurth, *A. rivae* Baker, *A. secundiflora* Engler, *A. ottalensis* Baker, *A. elegans* Todaro, *A. camperi* Schweifurth, *A. megalacantha* Baker, and *A. vituensis* Baker (Berger, 1908, Reynolds, 1966, Gilbert & Sebsebe, 1997, Sebsebe Demissew & Gilbert, 1997 and Sebsebe Demissew *et al.* 2003).

Reynolds (1966) revised the genus for tropical Africa and included 133 species found on the main land of Africa north of the Limpopo River. The account includes 26 species then known from the flora area (i.e. 14 Berger's taxa described earlier plus 12 newly described species mostly by the author). These are: *A. pubescens* Reynolds, *A. retrospiciens* Reynolds & Bally, *A. mcloughlinii* Christian, *A. harlana* Reynolds, *A. monticola* Reynolds, *A. debrana* Christian, *A. adigratana* Reynolds, *A. sinana* Reynolds, *A. calidophila* Reynolds, *A. schelpei* Reynolds, *A. yavellana* Reynolds, and *A. jacksonii* Reynolds.

Reynolds travelled very widely and saw most of the taxa in their natural settings. He also visited the cited type localities to solve the problems of identity of vaguely described Berger's taxa. However, Reynolds over-emphasized type localities at the expense of making full use of descriptions. He did not consult type collections, but relied entirely on Berger's account or second hand information (Gilbert & Sebsebe Demissew, 1997). Two new species were described from Ethiopia soon after Reynolds publication. These are: *A. trigonantha* Leach and *A citrina* Carter & Brandham (Sebsebe Demissew & Gilbert, 1997).

Gilbert & Sebsebe Demissew (1992, 1997) and Sebsebe Demissew & Gilbert (1997) in their review of Ethiopian taxa for the Flora of Ethiopia and Eritrea (FEE) described

various new species and subspecies, and also revealed a number of incompletely understood species, re-assessed the status of certain taxa and clarified some typifications. For example, *A. ankoberensis* Gilbert & Sebsebe, *A. tewoldei* Gilbert & Sebsebe, *A. gilbertii* Reynolds ex Sebsebe & Brandham, *A. gilbertii* subsp. *megalacanthoides* Gilbert & Sebsebe, *A. megalacantha* subsp. *alticola* Gilbert & Sebsebe, *A. pulcherrima* Gilbert & Sebsebe, *A. kefaensis* Gilbert & Sebsebe and *A. trichosantha* subsp. *longiflora* Gilbert & Sebsebe were described as new. *A. eumassawana* Carter, Gilbert & Sebsebe from the Red sea coast was also described as a new species. *A. percrassa* Todaro was redefined and *A. debrana* Christian resurrected as distinct species. *A. elegans* Todaro and *A. trichosantha* Berger were lectotypified. The misinterpreted *A. pirottae* Bereger later described as new, as *A. parvidens* Gilbert & Sebsebe. *A. boranensis* was interpreted as hybrid between *A. secundiflora* and *A. ottalensis* var. *elongata* Berger but was corrected and regarded to be conspecific with *A. ottalensis* Baker. *A. otallensis* var. *elongata* was later renamed as a new species, *A. rugosifolia* Gilbert & Sebsebe *and A. ruspolina* var *draceniformis* was identified as *A. retrospiciens* Reynolds & Bally. Altogether 38 species of which 3 were subdivided into subspecies are represented in FEE (Sebsebe Demissew & Gilbert, 1997). Two additional new species, *A. friisii* Sebsebe & Gilbert; and *A. bertemariae* Sebsebe & Doli have been discovered since the publication of FEE (Sebsebe Demissew *et al.*, 2001; Sebsebe Demissew *et al.*, 2003). Accordingly, 40 species of *Aloe* are known in Ethiopia and Eritrea so far. There are still taxonomic problems to be solved and new species are still being discovered. For example, a species not scientifically described, has been collected from the Wellega floristic region, is unique within the genus in having traits making it resistant to fires (Sebsebe Demissew *et al.*, 2003).

1.3.2. Distribution and endemism

The flora of Ethiopia and Eritrea is estimated to comprise between 6,500 and 7,000 species of vascular plants (Tewolde-Brehan Gebre Egzabher, 1991). The average percentage of endemic and near endemic species for all vascular plants in the flora area is approximately 27 percent (Friis *et al.*, 2001).

According to the analysis based on the flora account (Sebsebe Demissew & Gilbert, 1997) and later research (Sebsebe Demissew & Gilbert, 2000; Sebsebe Demissew & Doli, 2000), 35 (i.e., 87%) of the 40 species of *Aloe* found in the flora area are endemic or near endemic (i.e. have restricted distribution in one or few neighbouring countries) (Sebsebe Demissew *et al*., 2001). The degree of endemism in the genus *Aloe* in the flora is therefore nearly three times higher than the average figure for all vascular plants (Friis *et al.*, 2001; Sebsebe Demissew *et al*., 2001). Only five species are wide spread extending to East Africa or West Africa: *A. lateritia, A. macrocarpa, A. rivae, A. secundiflora* and *A. vituensis* (Reynolds, 1966, Sebsebe Demissew & Gilbert, 1997, Sebsebe Demissew *et al.*, 2001). Most species have very restricted distribution and three local centers of endemism are identified each of which has its own set of endemic taxa. These are: (1) Northern and central highlands north and west of the rift valley with 14 endemics (e.g. *A. adigratana, A. camperi, A. sinana, A. debrana, A. monticola* and *A. percrassa*, species further studied in this thesis,); (2) Eastern highlands with 5 endemics (e.g. *A. harlana*, and *A. megalacantha*); and (3) A southern highlands, lowlands and rift valley with 9 endemic taxa (e.g. *A. calidophila, A. gilbertii* and *A. yavellana*) (Sebsebe Demissew *et al*, 2001, 2003)(see fig. 2).

Most of the endemic and near endemic species occur mainly in two vegetation types (Sebsebe Demissew *et al*., 2001): Dry evergreen montane forest and grassland (including montane evergreen scrub) and *Acacia-Commiphora* bushland (woodland). *A. debrana* is typical in the evergreen forest and scrub of the central highlands, whereas, *A. ottalensis* is a typical example of a species mainly occurring in *Acacia-Commiphora* bush land. The altitudinal distribution of aloes in the flora area is wide from a few meters above sea level (e. g. *A. eumassawana* near Massawa in Eritrea in coastal vegetation) to above 3000 m (e. g. *A. steudneri* and *A. ankoberensis*), both of which reach the sub-afro-alpine vegetation. With the exception of three species mentioned above, all the species are concentrated between altitudes from 1000 to 3000 m. Hence the species of *Aloe* are mainly found in the highlands, at foot-hills and in the rift valley; and are rare in the semi-desert and desert. The Aloes frequently occur on rocky outcrops or on rocky faces and cliffs without much other vegetation. However, in the *Acacia-Commiphora* bushland the species of *Aloe* frequently occur in shade under bushes. Among the floristic regions defined in the

FEE, Wellega (WG), Illubabor (IL) and Keffa (KF), i. e., areas with high rainfall and with mainly evergreen forest, have few or no *Aloe* species. Moreover, the western lowlands with *Combretum-Terminalia* woodlands where grass fires are frequent are also more or less devoid of *Aloe* species (Sebsebe & Gilbert, 1997; Sebsebe *et al.*, 2001).

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1.3.3. Cytological and chemical studies

Very few *Aloe* taxa in the FEE area have been investigated cytologically. Brandham (1971) reported 2n = 14 in *A. harlana* Reynolds, *A. mcloughlinii* Christain, *A. megalacantha* Baker, and *A. pirottae* Berger. Fikre Dessallegn (1999) found the same number for *A. pulcherrima* Gilbert & Sebsebe; *A. debrana* Christian. Only one polyploidy (tetraploid) species has been reported so far from the flora area: *A. jacksonii* Reynolds (2n = 28, Adams *et al.*, 2000).

Chemical studies on several *Aloe* species of the FEE area were conducted from the extracts of leaves, roots and rhizomes by Ermias Dagne and his co-workers. These studies yielded chemical compounds of chemotaxonomic value.

Variation in the distribution of typical aloe compounds in leaf exudates (i.e. Aloenin, Barbaloin, Nataloin, Aloinoside, Homonataloin, 7-Hydroxyaloin, Aloesin and Microdontin) was reported among and between eleven species: *A. debrana, A. calidophila, A. camperi, A. elegans, A. sinana, A. megalacantha, A. pubescens, A. pulcherrima, A. rivae, A. secundiflora,* and one unidentified *A. species*. For example, *A. pulcherrima* revealed the conspicuous absence of Barbalion (a commonly occurring compound in most of the investigated species), but uniquely showed instead Nataloin and 7-Hydroxyaloin as its major constituents. Similarly, *A. pubescens* and *A. calidophila* yielded Aloesin and Homonatalion as their major constituents, respectively. Others, however, share one to three compounds between and among themselves, indicating some degree of relationships in their chemical composition (Ermias Dagne, 1996).

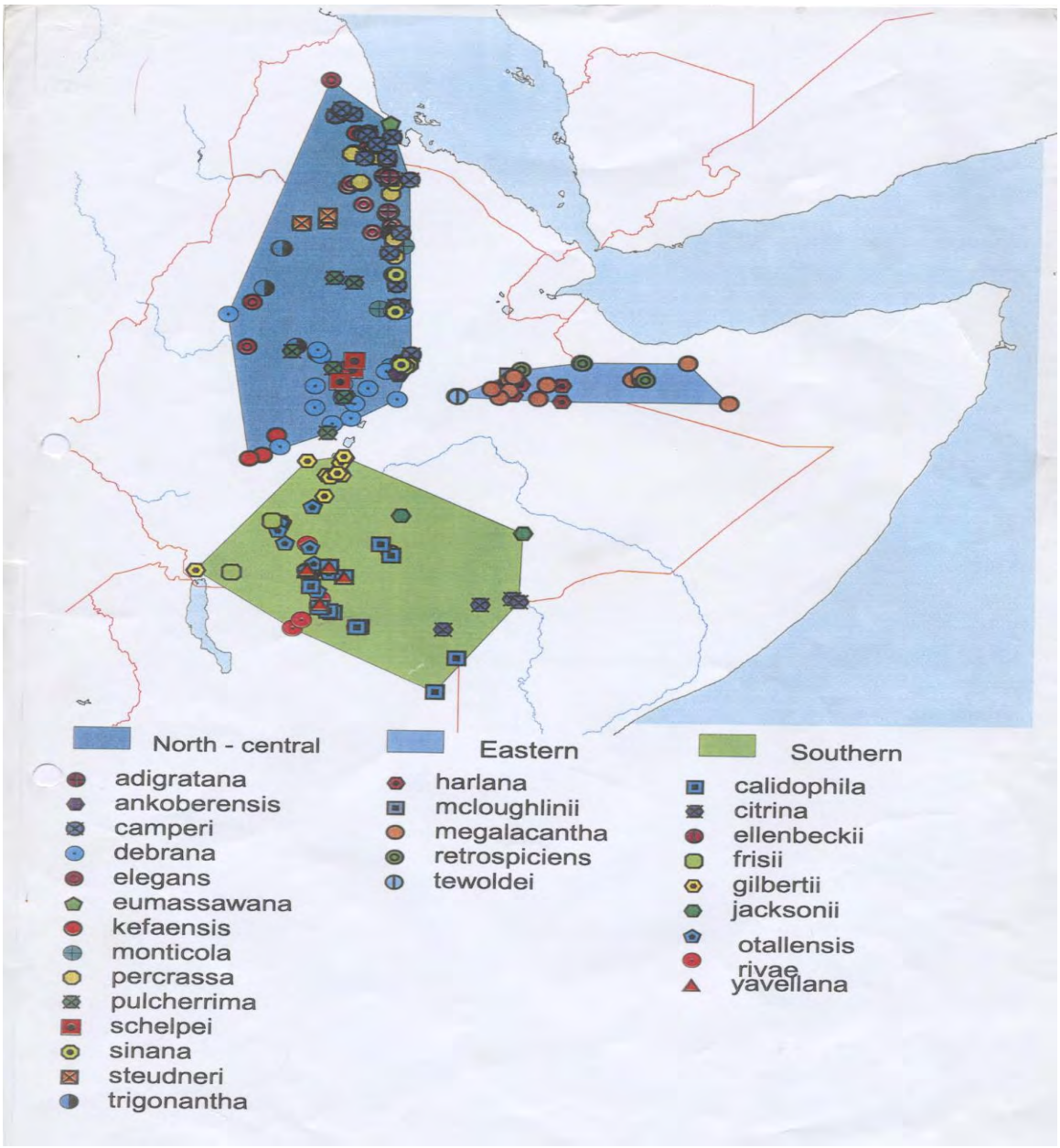


Fig. 2 Distribution of endemic and near endemic *Aloe* species in the FFE (Source: Sebsebe Demissew *et al.* 2001)

The root-chemistry of the investigated species (e.g. *A. calidophila, A. camperi, A. gilbertii, A. rivae, A. pulcherrima, A. schelpei, A. sinana and A. megalacantha)* was

similar, including aloechrysone, aloesaponarin I, aloesponol I, aloesaponarin II, aloesaponol II, aloesponol III, asphodelin, chrysophanol, chrysophanol-8-methyl ether, helminthoporin, and laccaic acid D-methyl ester. However, *A. secundiflora* differ in that it does not contain aloechrysone (Ermias Dagne *et al.*, 1994). Among the studied species were the morphologically similar species in the series *Saponaria*: *A. lateritia* var. *lateritia*, *A. lateritia* var. *graminicola, A. macrocarpa* and the recently described *A. kefaensis.* In the root extract of these species, the chemotaxonomically important 2-naphtoic acid derivative, isoeleutherol, might serve as chemotaxonomic marker for the group (Ermias Dagne *et al.*, 1994).

The species associations suggested by these chemical markers conform to the morphological similarities although some of the species had not been previously grouped together. These studies have shown that chemical data are useful in providing evidences to reveal species complexes within the genus *Aloe* in the flora area.

1.3.4. The *Aloe* species studied

Three of the major aloe complexes (groups) recognized in the flora area that comprises closely related species were considered. The species are listed in tab.1: the *Aloe camperi* complex (including 7 species), the *Aloe percrassa* complex (including 2 species), and the *Aloe harlana* complex (including 2 species).

1.3.4.1. Morphology and taxonomy

For the taxonomic study, all the 11 species of *Aloe* in three complexes were analysed. Morphological relationships are combined with geographical distribution to define the complexes (Reynolds, 1966; Sebsebe Demissew *et al.*, 2003).

Tab. 1 List of *Aloe* species selected for this study

Species	Center of Endemism	Complex
1. *Aloe camperi* Schweinfurth	north-central highland	*Aloe camperi* complex
2. *Aloe adigratana* Reynolds	north-central highland	
3. *Aloe sinana* Reynolds	north-central highland	
4. *Aloe megalacantha* Baker	eastern highland	
5. *Aloe calidophila* Reynolds	southern low lands	
6**Aloe gilbertii* Sebsebe & Brandham	southern rift valley	
7. *A. yavellana* Reynolds	southern lowland	
8. *Aloe percrassa* Todaro	north central highland	*Aloe percrassa* complex
9. **Aloe debrana* Christian	north central highland	
10.* *Aloe harlana* Reynolds	eastern highland	*Aloe harlana* complex
11. *Aloe monticola* Reynolds	north- central highland	

* *Aloe* species used for demographic studies

The *Aloe camperi* Complex

This complex (group) comprises 7 shrubby and caulescent *Aloe* species described in the FEE area, characterized by erect, ascending and sprawling stems. Reynolds (1966) in his treatment of species of Tropical Africa and Madagascar placed the shrubby and caulescent *Aloe* species of Ethiopia and Eritrea in different groups. For example, he grouped four of them in Group 13 (B). These are: *A. adigratana, A. camperi* and *A. sinana* occurring in the northern highlands with *A. calidophila* in southern lowlands. They are characterized by having clavate (club-shaped) perianth. He mentioned *A. sinana* to be closely related to *A. camperi* in general habit of growth

and character of leaves, but differs in having a less branched inflorescence, longer perianth and sub-laxly flowered, cylindric, slightly conical racemes with buds spreading horizontally to slightly downwards. *A. adigratana,* was also regarded as the closely allied to *A. camperi*, differing in having longer, thicker stems, longer leaves, denser and more

conical racemes much longer but less clavate perianth. *A. camperi*, however, was reported to be a polymorphic species having different geographical forms. *A. calidophila* was said to be unique among others by having large deeply channeled, very recurved leaves, a much branched inflorescence with erect subdense racemes and clavate perianth which are dull scarlet near base, turning orange towards the mouth. He related *A. calidophila* to *A. camperi* by close resemblance of clavate flowers.

Reynolds (1966) further placed *A. megalacantha* in Group 17 characterized by having leaves recurved and deeply canaliculate with thickened margins and large blunt teeth; and *A. yavellana* in Group 19 characterized by having bronze-brown leaves and capitate racemes, with pedicels only 10 mm long and perianth 27 mm in length. Another distinguishing character is that the dark scarlet buds are grey-striped in upper third, and minutely white flecked through out. Among the caulescent aloes in the flora area is, the recently described *A. gilbertii* from southern rift valley and lowlands. The species is morphologically very similar to the rest in the complex, particularly to *A. calidophila* in many of its vegetative and reproductive features.

Sebsebe Demissew *et al.,* (2003) combined geographical pattern of distribution with morphological characteristics to group the shrubby and caulescent aloe complex inhabiting in the flora area. They divided the complex into two subgroups. The first sub-group includes: *A. adigratana, A. camperi,* and *A. sinana,* which occur from northern Shewa towards Eritrea. They are characterized by clavate to sub-clavate perianth, and variously spotted leaves which are slightly to deeply canaliculate. The second sub-group includes: *A. calidophila, A. megalacantha, A. yavellana*, and *A. gilbertii* that occur from southern Shewa and extending to eastern and southern Ethiopia.

The *Aloe percrassa* complex

This complex (group) comprises 2 closely related herbaceous, acaulescent or semi-caulescent and rosulate leaved species in the FEE area. *A. debrana* was described from west of Debrebirhan, Shewa. Its relationship with *A. percrassa*, occurring in Eritrea, Tigray and Gonder, has been controversial. Reynolds (1966) treated *A. debrana* Christian

as a synonym of *A. percrassa* presumably on the basis of similar and small flowers. He stated *A. percrassa* (in the wide sense) is a variable species across its entire distribution range in characters of the raceme and the perianth. In the northern localities (Eritrea) perianths are 18-20 mm long, while in the southern localities (Shewa) they may be up to 25 mm in length, which Reynolds accepted as intraspecific variation only.

Sebsebe Demissew & Gilbert (1996), however, reassessed the circumscription of the species and have invoked certain new diagnostic features delimitating the two species. They found a marked discontinuity in bract size between true *A. percrassa* in north with bracts 8-13 mm long, and the plants from Shewa with bracts 3-5 mm long. They also found a difference in the inflorescence. *A. percrassa* (in the strict sense) has relatively few-branched inflorescences, very rarely with second order branching. *A. debrana*, on the other hand, has inflorescences nearly always with second order branching. As the result, they restricted *A. percrassa* to plants from northern Ethiopia and Eritrea and placed the closely related Shewan material in *A. debrana*.

The *Aloe harlana* complex

This complex (group) comprises 2 closely related herbaceous, acaulescent or semi-caulescent, and rosulate leaved species described in the FEE area. *A. harlana* was described from Hararghe and *A. monticola* from Tigray floristic regions. Despite difference in their geographical distribution, the group is particularly characterized by leaves with margins very prominent brown horny edge armed with paler brown teeth. Inflorescence is a branched panicle about 1 m high. However, they differ from each other by size of bracts, perianth, and size and shape of raceme.

According to Reynolds (1966) and Sebsebe Demissew & Gilbert (1997), the main morphological distinction between *A. harlana* and *A. monticola* are the following. *A. monticola* was characterized by bracts 6-8 mm long, perianth 38 mm in average in length, and racemes slightly conical with suberect buds. *A. harlana* was characterized by bracts slightly larger, 10 mm long, perianth shorter 33 mm long in average; and racemes conical-capitate to cylindrical-acuminate.

Key to the eleven species studied (after Sebsebe Demissew and Gilbert, 1997)

1. Plant stemless or stout, completely prostrate or pendent stems, rarely some old plants with short erect stems covered with persistent leaf-bases---------- 2
   - Plant soon developing obvious erect or ascending stems and eventually forming shrubs----------5
2. Leaves with horny margin continous between teeth; leaves glossy green, sometimes spotted; flowers commonly yellow flowered plants----------3
   - Leaves with pale margin, only the marginal teeth horny brown or red; leaves often glaucous, never glossy nor spotted except obscurely in seedlings; flowers pinkish to red---4
3. Bracts 9.5-15 mm long---------- *A. harlana*
   - Bracts 20- 2 mm long---------- *A. monticola*
4. Bracts (8-)10-16(-20) mm long; leaves usually slightly glaucose; inflorescences with just one level of branching----------....---------- *A. percrassa*
   - Bracts 3.5-6.5(-8.5) mm long; leaves not glaucos; inflorescences often with two level of branching---------- *A. debrana*
5. Leaves 50 cm wide or more, never linear; inflorescence usually much branched, perianth clavate, broad near tip---------- ----------6
   - Leaves 50 cm wide or more, never linear; inflorescence usually much branched, perianth cylindrical to cylindrical-trigonous---------- ----------9
6. Bracts 7-12 mm long; perianth 23-27(-33) mm long----------7
   - Bracts 3-6 mm long; perianth 17-22 mm long----------8
7. Racemes 12-22 cm long; bracts 9-12 x 2.5-4 mm---------- *A. adigratana*
   - Racemes 5.5-8.5 cm long; bracts 7-8 x 2 mm----------*A. sinana*
8. Leaves up to 60 cm long, often less, green, spotted whitish at least near base----------*A. camperi*
   - Leaves ca. 80 cm long, uniformly grey-green, not spotted ----------*A. calidophila*
9. Stems stout, 5 cm wide or more; perianth 23-28 mm long----------10
   - Stems slender, 3-4 cm thick; perianth 20-22 mm long----------*A. yavellana*
10. Marginal teeth 5-7 per 10 cm, 5-6 mm long; bracts (5-)7-12 mm long----*A. megalacantha*
    - Marginal teeth 7-12 per 10 cm, 3-5 mm long; bracts 4-6 mm long----------*A. gilbertii*

1.4. Statements and objectives of the study

1.4.1. Statements of the study

Most of the early taxa of aloes from the flora area were described in Europe or in South Africa based on materials collected during expeditions by visitors. In some cases, only a single specimen from the type locality was the basis for the described taxon. Especially in the 18^{th} and 19^{th} centuries species were described from single plants grown under artificial conditions in private collections (e.g. *A. percrassa* and *A. elegans*), especially in Europe, thousands of kilometers from their natural habitats. In cultivation succulent plants often become etiolated and chlorotic and may bear only a slight resemblance to plants in the wild and also authors of the new species had no idea of the variability of these taxa in their natural setting (Smith & Steyn, 2004). In some cases previous studies of the aloes of Ethiopia and Eritrea were contradictory and inconsistent in their delimitation of species even if some of the problems were solved in successive studies. Furthermore, the taxonomic treatments of the genus so far is mainly based on macromorphology and herbarium specimens (Berger, 1908; Reynolds, 1966; Sebsebe Demissew & Gilbert, 1997). The traditional system of classification or “Alpha taxonomy" used in the past has resulted in heterogeneous complexes with blurred demarcation of species boundaries.

Because of taxonomic problems encountered in the genus *Aloe*, Smith & van Wyk (1991), stressed the need to combine various modern approaches (e.g. molecular, biochemical and micromorphometry) with morphological characters in order to solve the problem.

Proper delimitation of taxa is fundamental for decision on the conservation of the taxa. In addition if a species may have unexploited economic potential, it is particularly important to define the species delimitation and genetical differentiation. Hence, it is important to analyse the genetic variation and particularly to settle how the variation is partitioned within and among populations to obtain the best conservation strategy for a given species. Besides taxonomic distinctiveness and genetic variability, valuable information on population structure, dynamics and identifying threats are crucial as tool to help conservation decisions.

In this study the focus was to answer the following questions:-

1. Are the closely related species, in the three aloe complexes identified in the flora area real or not?

2. How is the demographic structure and dynamics of the populations? Are the populations senile and stagnant or are they reproducing and increasing?

3. How is the genetic variation distributed among and within the populations?

4. What are the major threats to or environmental factors that threaten aloes of the flora area?

5. What is the best conservation strategy for a given species, single large or several small populations?

1.4.2. Objectives of the study

On the background stated above the objectives of the study were the following:-

General objectives

1. To gain new additional knowledge necessary to delineate *Aloe* species in Ethiopia.

2. To contribute new knowledge necessary for conservation of diversity and future sustainable use of aloes in Ethiopia.

Specific objectives

1. To investigate morphological variations by using taxonomic numerical analyses to evaluate the specific status of eleven *Aloe* species within three complexes.

2. To carry out DNA fingerprinting based on the AFLP technique in order to test species limits.

3. To determine the demographic structure and dynamics of populations of selected *Aloe* species.

4. To assess genetic variations of three demographic species by isoenzyme analysis

5. To identify and document major threats; and suggest the best conservation strategies to aloes of Ethiopia

2. Materials and methods

2.1. Morphology

Materials

Both fresh and dried herbarium materials were used as sources of data. Two important works: Reynolds (1966) and Sebsebe Demissew & Gilbert (1997) were used for consolidation purposes.

Fresh materials

Fresh specimens of studied *Aloe* species were obtained from field collections, garden and green house. About 5 individuals per population/species were randomly sampled to cover variation in habit, leaf, inflorescence and flower characters (see fig. 3 and appendix IV for species and populations sampled). Identification of closely related and sympatric species on their natural habitat was not simple. Specimens collected during fieldworks were deposited in the National Herbarium (ETH) and seedlings were transplanted to the aloe garden of the Chemistry Department at Addis Ababa University, Ethiopia.

Herbarium specimens

In addition to fresh materials, herbarium specimens deposited at the National Herbarium, Addis Ababa University (ETH) and the Herbarium Royal Botanical Garden, Kew (K) were used (see appendix I). Altogether 160 herbarium specimens of 11 species of *Aloe* were examined. Fifty specimens were excluded because of their poor quality and missing data mainly for quantitative characters, leaving a total of 110 specimens used for the analyses.

Data collection

Morphological traits considered in this study include both qualitative and quantitative characters in vegetative and reproductive organs. The selection of morphological traits was based on three criteria: a) their common use for taxonomic identification in the genus; b) their use in previous monographs and flora accounts for the construction of keys; and c) their variability among aloe complexes and species studied on a preliminary survey of the specimens.

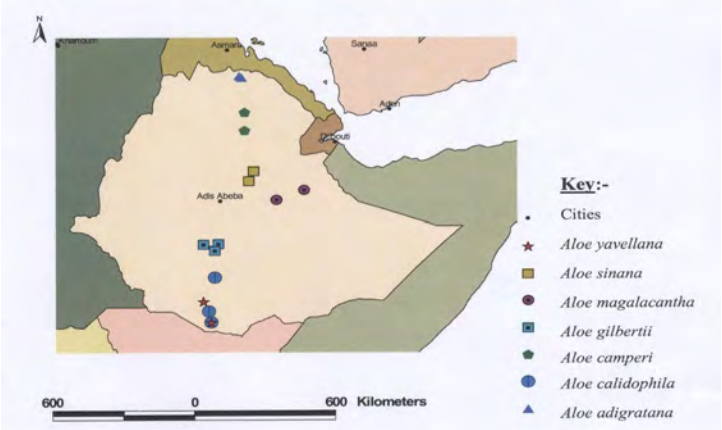
A selection was made for qualitative attributes in which two or more states of the attribute were present and scored unequivocally. Seven binary and thirteen multistate attributes were assessed. A selection was also made for quantitative characters that could be measured from specimens or taken from specimen labels (in case of ranges, median values were used). Characters were counted or measured with a ruler or protractor. Fifteen were continuous and five were discrete characters. For every species of *Aloe* studied, 22 reproductive and 18 vegetative (including seedling or juvenile) characters were assessed and scored. Fruit and seed characters not studied in Aloes within the FEE area erlier, were particularly included. When herbarium and field data were analysed together 40 characters and states of 165 individuals or specimens (i.e. 15 per species) were considered (see appendices IIa, b and III).

Data analysis

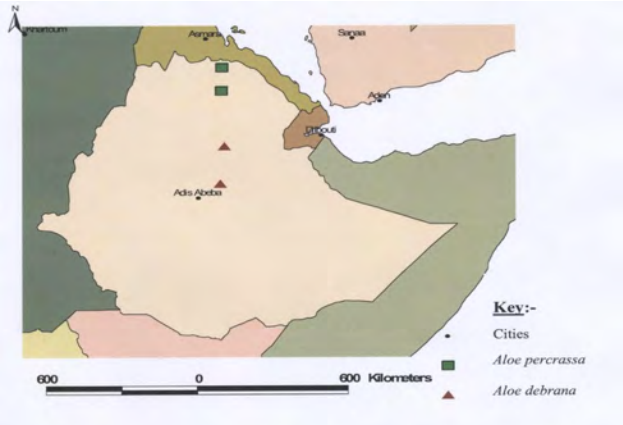
A data matrix was constructed with specimens/individuals (165) as columns and attributes/characters (40) as rows. Each individual of a species was considered as one OTU. Data matrices were generated for qualitative, quantitative; and combined

quantitative and qualitative data sets. The data matrices were then subjected to multivariate methods as cluster analysis (Unweighted Pair Group Method of Analysis, UPGMA) and ordination analysis (Principal coordinate analysis, PCO) using NTSYSpc program version 2.11f (Rohlf, 2000). For the cluster analysis (UPGMA), the SIMQUAL module of NTSYS with the simple matching coefficient was used to produce similarity matrices. Phenograms were constructed by using the SAHN option. The data matrices were standardized before generating similarity coefficient matrices. The same similarity matrices were also used for PCO using the DCENTER and EIGEN procedures; and used to construct scatter plots by Matrix Plot option. The cophenetic similarity values (ultrametric distances) were calculated from a tree matrix using COPH module and used to test the goodness of fit of clustering analysis to the data by using the MXCOMP module.

a) *Aloe camperi* complex



b) *Aloe percrassa* complex



c) *Aloe harlana* complex

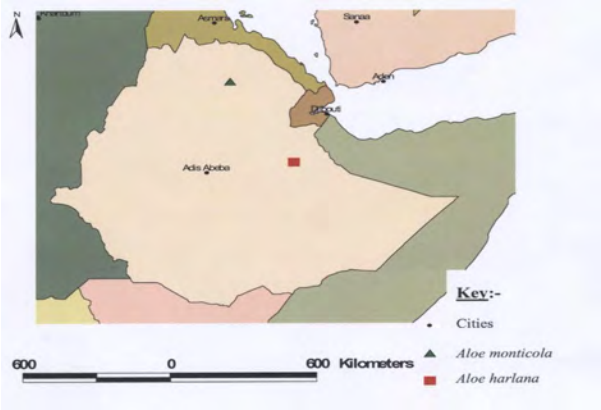


Fig. 3 Distribution of populations and species studied: a) *Aloe camperi* complex; b) *Aloe percrassa* complex and c) *Aloe harlana* complex

2.2. AFLP fingerprinting

We used 120 individuals representing eleven species and analysed them by Amplified Fragment Length Polymorphisms (AFLP) fingerprinting, which is a highly reproducible method for assessment of genetic variation at the intra-and interspecific levels (Vos *et al.*, 1995).

Sampling of seeds

Information from herbarium labels and avialable literature were consulted to determine phenology (i. e., time of flowering and fruiting) in order to collect seeds of the studied species. Seeds were collected from natural populations at various localities (see tab. 3 and appendix IV). Depending on the size of population and availability of individuals with seeds, 1-3 population(s) per species was/were included. Populations that are geographically isolated from each other were chosen to avoid the possibility of

hybridization and to represent the full range of genetic variation within the species. Seeds were collected from 10-20 individuals per population. Seeds from one individual were placed in a separate bag labeled with name of species, and its unique population and individual code. The seeds collected were transported to the University of Oslo and germinated in pots in the greenhouse (phytothrone chamber) (see section 2.5 for germination conditions), and leaves from young seedlings were used to undertake AFLP analysis.

DNA extraction

Leaves from randomely selected germinated seedlings, 5 individuals per population and 10 per population for species represented by one population, were carefully harvested, paranchymatous gel removed and the upper surface cut into pieces (4 x 4 mm). Then the cut leaf pieces were placed in labeled vials containing silica gel until use. Total genomic DNA was extracted using a 2 x CTAB method modified from Doyle and Doyle (1990). About 5 to 7.5 ml of CTAB extraction buffer (20 g CTAB, 81.9 g NaCl, 5.844 g Na_2EDTA, 12.114 g Tris, 4 g PVP, 2 ml 2-mercaptoethanol in 1 L dH_20) was preheated in a 30 ml glass tube in a water bath (60°C). While heating, 0.5 to 1.0 g of dried leaf tissue per sample was ground manually to a powder in liquid nitrogen using a chilled mortar and pestle. From this ground leaf material (powder) about 100-200 mg was scraped into labelled eppendorf tubes and kept in ice. 700 µl of the preheated extraction (CTAB) buffer was pippeted into each tube, vortexed (mixed), and incubated in a heating block (60 °C) for about 30 minutes. Into each tube, 500 µl of chloroform-isoamylalcohol (24:1; v:v) was added, shaked (mixed thoroughly) and allowed to stand for about five minutes. This was followed by centrifuging the tubes (13.000 rpm) for two minutes. Next, 500 µl of the supernatant (upper clear phase) was carefully removed to another eppendorf tube with 350 µl cold isopropanol, shaked and placed in a fridge for about five minutes. The tubes were centrifuged (13.000 rpm) for two minutes, and the supernatant decanted. The step was repeated by adding 1 ml ethanol (70%) to each tube, shaking, and centrifuging (13.000 rpm) for two minutes. The ethanol was again removed and tubes dried in a heating block (60 °C) for about five minutes. Alternatively, the tubes with the residue of nucleic acids/samples were left at room temperature for 30 minutes to an hour.

The dried sample was dissolved/resuspended in TE-buffer (100-150 µl) depending on the size of the residue/pellet and treated with 1 µl RNase. Finally, the tubes were vortexed, allowed to stand at room-temperature for 30 minutes or more, and kept in deep freezer (-20 °C) until use. The qualities of the DNA isolates were checked using 1% (w/v) agrose gels (0.50 g agrose powder, 50 ml 1 x TBE buffer, and 1.7 ml EtBr).

AFLP analysis

AFLP analysis was undertaken after the concentration of the sample DNA extracts were measured and checked using a NanoDrop® ND-1000 spectrophotometer. Working solutions of 50 ng/µl and 100 ng/µl were prepared for each sample analysed based on the resulting concentration from the measurement. AFLP analysis was performed following a protocol modified from Vos ***et al.*** (1995).

Restriction-ligation

For each sample, 5.5 µl sample/genomic DNA was digested/restricted using the restriction enzymes *Eco*RI and *Mse*I; and standard *Eco*RI and *Mse*I adapters were ligated onto the ends of restriction sites (for adapter and primer sequences, see Vos ***et al.*** (1995)). The restriction-ligation reaction mixture consisted of 1.1 µl 10x T4 DNA buffer (with ATP, Roche), 1.1 µl 0.5 M NaCl, 0.55 µl 1 mg/µl BSA (Roche), 0.02 µl *Mse*I (1U, New England Biolabs) and 0.125 µl *Eco*RI (5U, Roche) restriction enzymes, 0.2 µl T4 DNA Ligase (1U, Roche), 1 µl *Mse*I (10µM) and 1 µl *Eco*RI (10µM) adapter pairs; and 0.405 µl autoclaved distilled water, making up the total reaction volume to 11 µl per sample. The restriction-ligation mix was incubated for 3 hrs at 37 °C in an Eppendorf Mastercycler. The restriction-ligation products were diluted 10 times in autoclaved distilled water and mixed thoroughly for PCR amplification.

PCR amplification

The restriction-ligation products (fragments) were amplified with an *Eco*RI primer and a *Mse*I primer. Two steps of PCR amplification followed, each reducing the number of fragments. During the pre-selective PCR, primers with one and two nucleotide extension(s) were used, while primers with three or four nucleotide extensions were used

in the selective PCR. From initial trial of 60 primer combinations in order to find the most informative ones, three primer combinations were selected. *Eco*RI and *Mse*I primers were combined as follows: P14- *Eco*RI-AGA/*Mse*I-CACC, P56- *Eco*RI-AGAG/*Mse*I-CATA and P57- *Eco*RI-AGAC/*Mse*I-CAAG.

Pre-selective amplification of the adapter-ligated DNA with *Eco*RI and *Mse*I primers followed standard PCR conditions. The pre-selective PCR reaction mixture consisted of 2.5 µl Taq buffer/10×PCR buffer (Applied Biosystems); 1.5 µl 25 mM $MgCl_2$ (Applied Biosystems); 2 µl 10 mM dNTP (Applied Biosystems); 0.5 µl *Eco*RI-A/-AG and 0.5 µl *Mse*I-CA preselective primers, 0.1 µ AmpliTaq (Applied Biosystems), 14.9 µl outoclaved dH_20 and 3 µl diluted restriction-ligation product, giving a total volume of 25 µl per sample. The preselective PCR cycle started with 2 minutes at 72 °C, followed by 30 cycles of PCR amplification, (denaturing at 94 °C for 30 sec., annealing at 56 °C for 30 sec. and extensions at 72 °C for 1 min), and with a final extension at 72 °C for 10 minutes. The tempreture dropped to 4 °C at the end of amplification. The preselective PCR products (10 µl) were checked on a 1.5% agrose gel and the remaining products diluted 10 times with sterile water and used for selectivePCR

Selective amplification was done using *Eco*RI and *Mse*I primers with three or four selective nucleoitides. The 25 µl selective PCR mix consisted of 5 µl DNA (diluted preselective products), 0.2 µl 10 µM *Eco*RI primer, 0.5 µl 10 µM *Mse*I primer, 2 µl 10 mM dNTP mix, 0.2 µl AmpliTaq Gold (Applied Biosystems), 2 µl AmpliTaq Gold buffer (Applied Biosystems), 2.5 µl 25 mM $MgCl_2$, 0.2 µl 1 mg/ml BSA, and 11.9 µl outoclaved dH_20. The selective PCR started with 10 minutes at 95 °C, followed by 13 cycles of PCR amplification (denaturing at 94 °C for 30 sec., annealing at 65-56 °C for 1 min and extension at 72 °C for 1 min). The annealing temperature was decreased by 0.7° C for each of the 13 cycles. The remaining 23 cycles of PCR amplification started with denaturing at 94 °C for 30 sec., annealing at 56 °C for 1 min and extension at 72 °C for 1 min and was followed by a final extension at 72 °C for 10 minutes. The tempreture was dropped to 4 °C at the end of amplification. All the PCRs were run on an Eppendorf Mastercycler. Finally, 3.0 µl of each selective PCR product was mixed in 11.5 µl HiDi

(formamide) and 0.5 µl GeneScan Rox size standard (Applied Biosystems). The samples were denatured for 5 minutes at 95 °C and analyzed with an ABI 3100 automated sequencer (Applied Biosystems) with 30 sec. injection time and otherwise default conditions.

Scoring and data analysis

The selectively amplified DNA fragment data generated/collected by the ABI automated sequencer were visualized and sized using GeneScan Analysis Software, version 3.7 (Applied Biosystems). Two of the three selected primer combinations (P56- *Eco*RI-AGAG/*Mse*I-CATA; and P57- *Eco*RI-AGAC/*Mse*I-CAAG) had relatively clear cut/scorable profiles and were considered in the following analysis. The primer combination P14- *Eco*RI-AGA/*Mse*I-CACC was excluded at the scoring phase because it turned out to have complicated profiles with uneven intensity and disturbing background.

Fragments in the range 59-388 base pairs were scored using GeneMapper, version 3.7 (Applied Biosystems). The fragments produced by each primer combination were treated as characters and numbered sequentially. Genotypes/individuals were scored for the presence (1) or absence (0) of good quality peaks. Seven individuals out of 120 failed to be amplified by either one or the other primer pair. Bands not reproduced in two replicated samples were excluded from the analysis. Binary data matrices from each of the two primer combinations as well as a combined data were analysed in NTSYSpc version 2.11f (Rohlf, 2000). A matrix of genetic similarity between pairs of individuals was obtained using Jaccard's similarity coefficient by the SimQual option. The similarities matrix was the basis for cluster analysis (UPGMA) using the SAHN option. The result was shown as a phenogram representing the phenetic relationship between genotypes. A principal coordinate analysis (PCO) was performed on the similarity matrix using the DCENTER and EIGEN procedures; and two-dimensional scatter plots were constructed by the Matrix Plot option. The cophenetic similarity values (ultrametric distances) were calculated from a tree matrix using COPH module and used to test the goodness of fit of clustering analysis to the data by using the MXCOMP module. Nieghbor-joining (NJ) analysis was carried out on the combined AFLP data set using

TREECON software vers. 1.36 (Van de Peer & De Wachter, 1994). Nei & Li (1979) distance measure was used to construct a tree with midpoint rooting. A bootstrap analysis with 100 replicates was done.

2.3. Demography

Three endemic *Aloe* species representing three complexes and areas of endemism in Ethiopia: *A. gilbertii*, *A. debrana* and *A. harlana* were selected for demographic studies. The study was carried out in three sites inhabited by the above species in different parts of the country (see fig. 3).

For *A. gilbertii*, the study was carried out at at Alamura hill (type locality of the species), about 5 km South of Awass town; 7° 00' N, 38° 30' E at 1800 m. It is part of the rift valley system of Ethiopia situated in the rift valley lake region. As per the observation made during field work, the bed rock in the study area seems to be basalitic and volcanic in origin. The soils in the study site are extermly shallow (Leptosol), seldom exceeding 0.15 m in depth over the underlying rock. Plots were established in south east facing slopes of the hill, in wooded grassland classified as *Acacia* woodland/bushland.

For *A. debrana*, the study was carried out at Legatossa hill (close to type locality) 50 km from Addis Ababa in NE direction on Addis-Debre Birhan road; 9° 36' N, 39° 29' E at 2810 m. It is part of centeral highlands of Ethiopia classified as Shewan plateaue. As per the observation made during field work, the study area had thin soil overlying basalt rock. Plots were estabilished in south west facing slopes of the hill, in scrubland mixed with grasses, classified as dry evergreen montane vegetation.

For *A. harlana*, the study was carried out at a hill near Harla village, 16 km from Dire Dawa on the road to Harar; 9° 28' N, 41° 54' E, at 1880 m. It is part of south eastern highlands of Ethiopia grouped as Harerghe plataue. The locality is charcerized by dry steep slope with very little intact vegetation due to overgrazing. The studied population grows in limestone slopes with *Juniperus* remnants, now manily evergreen bushland with

Euclea schimperi, Euphorbia abyssinica, Carissa edulis, Dodonaea angustifola, Clerodendrum myricoides and Jasminum floribundum. Further up the slope *Dracaena schizantha* is also observed. Plots were estabilished on south eastern facing slopes of the hill.

To study demographic structure and dynamics, three permanent plots of 5 x 20 m^2 per species, i.e. altogether 9 plots were established in the study sites. Cattle exclosures were not built around the plots to protect them from grazing or any other human interference. Each individual clone consisting of one genet and one to several ramets was marked, mapped and recorded. Marking was made on the leaves with double numbers (G-R) by a water proof marker. Here 'G' stands for putative genet and 'R' stands for the individual ramet. A genet was defined as all of the individuals that derived from a single seedling. An individual ramet was defined as the vegetative offshoot connected to a single underground rhizome. Genetic individuals (genets) were also given coordinates (X-Y) within the plots measured from the left-side corner of the plot as origo.

For every genet of the population in the plots, stem length (SL) and stem diameter (SD) (when present); and for every ramet rosette diameter (RD), rosette height (RH) and number of inflorescence (s) (NI_s) were measured or counted. These measurements were undertaken in successive seasons following their phenology from Nov.2003 to Oct. 2005 for *A. gilbertii*, from Jan. 2003 to Oct. 2005 for *A. debrana* and from Jul. 2003 to Oct. 2005 for *A. harlana*. The reproductive phase was recorded by counting inflorescences/infrutescences of the reproductive individuals. Recruitments through vegetative propagation (offshoots) and seedling establishments were carefully recorded as new plants in the second season. Mortality of a genet occurred when all ramets belonging to that genet died. Mortality of a ramet occurred when it was no longer found in the clone to which it belonged.

Based on the data recorded, the demographic structure was analysed by the clone size and rosette diameter of ramets using descriptive and inferential statistics. Genets were characterized by life stage classes: seedling (SE), juvenile genet (J), mature sterile genet

with one ramet (M), mature flowering genet with one ramet (M*), senile sterile genet with ≥ 2 ramets (P), and senile flowering genet with ≥ 2 rametes (P*) (see fig. 14). The transition among stages classes were used to construct stage structured projection between years (see fig. 15a, b, and c). The probabilities of transition between stage classes and mortality parameters were calculated (see table 5). These transition probabilities were used to formulate a matrix incorporating demographic parameters. The demographic differences or growth rates were calculated for the monitored period according to the matrix model by Caswell (1998). As part of the demographic parameters, qualitative observations and record was made in the field to trace ecological factors that might threaten the actual population. Moreover, germination successes from field collected seeds of the studied species were assessed and compared (see section 2.5 for germination experments in the greenhouse).

2.4. Isoenzyme analysis

Seedlings germinated in the greenhouse at the University of Oslo were used to undertake isoenzyme analysis in three species: *A. gilbertii* (3 populations), *A. debrana* (2 populations) and *A. harlana* (1 population). Altogether 78 individuals were considered (tab. 2).

Approximately 1 x 1 cm² of leaf area was cut and dissected to remove the lower surface and gel-like substance. The upper surface was then sliced into smaller blocks. These were then put into microfuge tubes to which approximately 7 drops of homogenizing buffer and a few grains of sand were added. The leaf tissue was then ground manually by use of a glass pestle, resulting in a more or less homogenous ‘soup’. This was then stored at minus 70°C. Prior to each electrophoresis, the enzyme ‘soup’ was absorbed into filter paper wicks and the wicks inserted into horizontal starch gels. Enzyme separation was effected by electrophoresis and the gel is sliced and stained for different enzymes.

Tab. 2 Populations and individuals of three *Aloe* species used for isoenzyme analysis

Species	Population (s)	Individuals
A. gilbertii	3 (Shashemene; Alaba and Awassa)	37
A. debrana	2 (Debrebirahan and Dessie Zuria)	26
A. harlana	1 (Harla)	15
Total	6	78

Seven enzyme systems were initially screened for activity over two gel and electrode buffer combinations. The gel and electrode buffer combinations were categorized as D-system and AB-system. The D-system gels used an electrode buffer of 0.065 mol/L L-histiden and 0.02 mol/L citric acid at P^H 6.5 and a gel buffer that was a mixture of six parts water to one part electrode buffer (final concentration of 0.009 mol/L L-histiden and 0.002 mol/L citric acid) with 3% (w/v) sucrose at p^H 6.5. The D-system gels were stained for three enzyme systems: Phosphoglucomutase (PGM), Malate dehydrogenase (MDH), and 6-Phosphosphogluconate dehydrogenase (6-PGD). The AB-system gels used an electrode buffer of 0.19 mol/L boric acid and 0.04 mol/L LiOH at p^H 8.3 and a gel buffer that was a mixture of one part electrode buffer to nine part Tris-citric acid buffer (0.05 mol/L Tris and 0.007 mol/L citric acid at p^H 8.3). The AB-system gels were stained for four enzyme systems: Aspartate aminotransferase (AAT), Glucose-6-Phosphate isomerase (GPI), Triphosphate isomerase (TPI), and Aminopeptidase (AMP). The laboratory procedure followed above was a modification of standard procedures developed by Wendle & Weeden (1989).

The resulting banding patterns (enzyme phenotypes) were used to infer genotypes. For each enzyme, bands were assigned to putative loci. The alleles in a locus were designated by letters, the most anodal denoted as ‘A’ and the rest were assigned according to decreasing order of electrophoretic mobility in ascending order of the alphabet. Five enzymes with interpretable band phenotypes were scored. Genetic

variation analysis was done using the computer package GENEPOP (New version, ver. 3.4) (Raymond and Rouset, 2003). Four measures of genetic variation were estimated for each population: polymorphism (*P*), the percentage of loci exhibiting more than one allele; allelic richness (N_a), the number of alleles per polymorphic locus; heterozygosity (H_o) the proportion of individuals heterozygous for a locus; gene diversity (H_e), the expected heterozygosity at Hardy-Weinberg equilibrium. Wright's (1965) *F*-statistics, F_{is} (the fixation index arising from non-random mating within a population) was used to estimate deviation from random mating and genetic divergence among populations and species was estimated by F_{st} (the fixation index arising from population differentiation), following the formula of Weir & Cockerham's (1984).

2.5 Germination experiments

Pots of size 1.5 Lt with pores at the bottom were filled with mixture of humus, perlite, and sand (fine and gravel in mixture) so as to ensure good drainage and retention of water. Seeds were placed on top and covered with a layer of sand. 10 seeds collected from the same individual were sown in the same pot (see table 3). For the first three weeks, the pots with seeds were put in larger trays to water the medium from beneath. To compensate excessive evaporation, the pots were covered and given shade for parts of the day. The temperature in the green house was adjusted to 20° C (night) and 25° C (day) and day time 12 hrs light and 12 hrs night, so as to simulate the condtions in most parts of Ethiopia. Seedlings started to appear at different interval in time, i.e., from about two to four weeks. The first record for the number of germinated seeds per pot was made after a month from the date the seeds were sown.

Getting the right condition for seedlings was diffcult. When the seedlings started to develop they were watered twice a week and given liquid nutrients at weekly intervals (see appendix VI b for its composition). Some tiny stones were kept around the seedlings to support them from falling over. Seedlings were transplanted to separate pots when they had developed 3-4 leaves. The second record for the number of germinated seeds per pot was made after three months from the date the seeds were sown.

Tab. 3 Plants used in the germination experiment (for further details on locality see appendix IV).

Species	Floristic region	Population/locality	Number of mother plants
A. adigratana	Tigray	Adigrat A	15
	Tigray	Adigrat B	20
A. camperi	Tigray	Meionhi	17
	Wello	Kobo	18
A. sinana	Shewa	Debre Sina	20
	Shewa	Efeson	15
A. gilbertii	Shewa	Shashamene	15
	Shewa	Alaba	15
	Sidamo	Awassa	15
A. calidophila	Sidamo	Mega	15
	Sidamo	Dida Chenna	15
	Sidamo	Yabello	15
A. yavellana	Sidamo	Yabello	15
A. megalacantha	Harareghe	Harla	15
	Harareghe	Asebe Teferi	10
A. debrana	Shewa	Debre Birhan	18
	Wello	Dessie Zuria	12
A. percrassa	Tigray	Adigrat	10
	Tigray	Amba Alage	20
A. monticola	Tigray	Maichew	15
A. harlana	Harareghe	Harla	15

3. Results

3.1. Morphology

Cluster analysis (UPGMA) was performed on the following data matrices: qualitative, quantitative and combined morphological data sets. The phenograms obtained for each of the three data matrices showed similar tree topologies. The combined data set separated the species best and gave the phenogram shown in fig. 4, in which we can distinguish 11 clusters that are clearly separated into three groups in levels of similarity coefficients from 0.21 to 0.73.

The first group of clusters comprises clusters 1-4. In clusters 1 and 2, we find all specimens belonging to *A. harlana* and *A. monticola*, respectively (both placed in the *A. harlana* complex). Clusters 3 and 4 are composed of all specimens belonging to *A. debrana* and *A. percrassa*, respectively (both placed in *A. percrassa* complex). The clusters separate at similarity level of 0.47. The second group of clusters consists of clusters 5-8: 5 corresponding to *A. megalacantha*, 6 to *A. yavellana*, 7 to *A. gilbertii* and 8 to *A. calidophila*. The second group of clusters is well separated at similarity level of 0.42. The third group of clusters comprises; clusters 9, 10 and 11 representing *A. sinana*, *A. camperi* and *A. adigratana*, respectively. In this group, cluster 11 (*A. adigratana*) is well-separated from the other two at 0.37 level of similarity and cluster 9 (*A. sinana*) and cluster 10 (*A. camperi*) separate at similarity level of 0.47. The cophenetic correlation calculated to measure goodness of fit between the phenogram and combined morphological data set showed a good fit ($r = 0.88$).

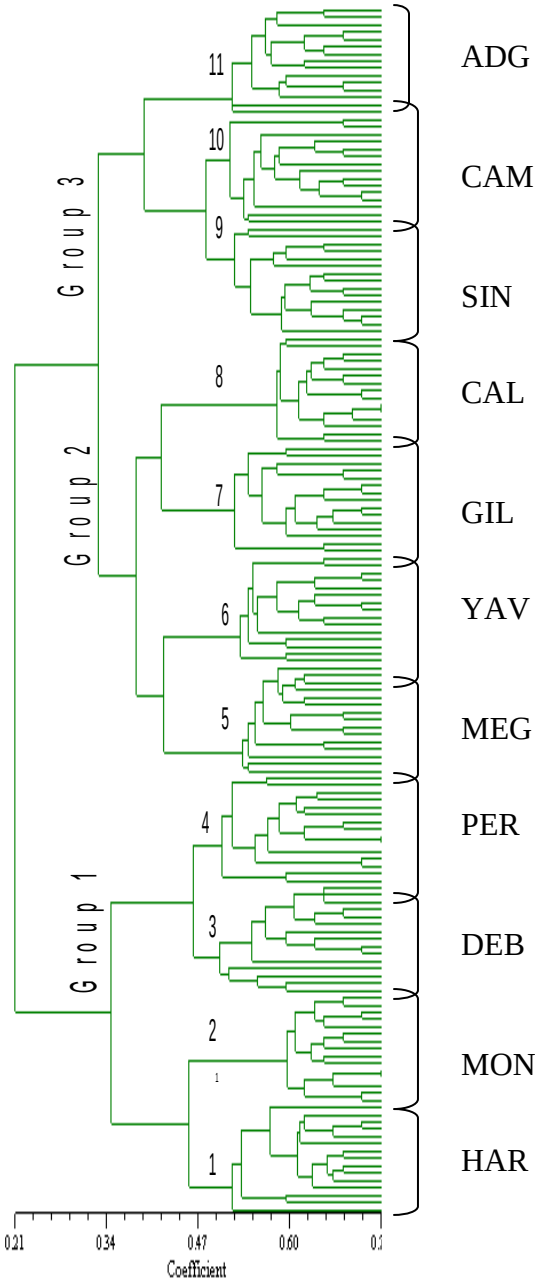


Fig. 4 UPGMA phenogram from combined morphological data showing the relationships among the studied species: (ADG = *A. adigratana* (Cluster 11), CAM = *A.. camperi* (Cluster 10), SIN= *A. sinana* (Cluster 9), CAL = *A. calidophila* (Cluster 8), GIL = *A. gilbertii* (Cluster 7), YAV = *A.*

yavellana (Cluster 6), MEG = *A. megalacantha* (Cluster 5), PER = *A. percrassa* (Cluster 4), DEB = *A. debrana* (Cluster 3), MON = *A. monticola* (Cluster 2), and HAR = *A. harlana* (Cluster 1).

A PCO analysis was carried out using the same similarity data matrices as in the UPGMA analysis. The PCO results support the UPGMA analysis as shown in fig. 5. The first two axes accounts for 36.5% and 20.6% of variation, giving a commulative value of 57.1%. A scatter plot of the first two axes components show 5 groups: cluster 1 corresponds to *A. harlana* and *A. monticola* (in *A. harlana* complex). Cluster 2 corresponds to *A. percrassa* and *A. debrana* (in the *A. percrassa* complex). Cluster 3 corresponds to *A. megalacantha, A. yavellana, A. gilbertii and A. clidophila*. Cluster 4 corresponds to *A. camperi* and *A. sinana*, and cluster 5 to *A. adigratana* Species clustered in 3, 4 and 5 belong to *A. camperi* complex.

Axis 1 separated most species of the shrubby and caulescent aloes (*A. camperi* complex) from the species of the herbaceous and acaulescent aloes (*A. harlana* and *A. percrassa* complexes). The second axis separated the *A. harlana* complex from *A. percrassa* complex and subdivided the *A. camperi* complex into three distinct clusters. *A. adigratana, A. camperi* and *A. sinana* (shrubby northern species) were fairly well separated from *A. megalacantha, A. gilbertii, A. calidophila* and *A. yavellana* (shrubby species in south and east). *A. adigratana* was the most distinct.

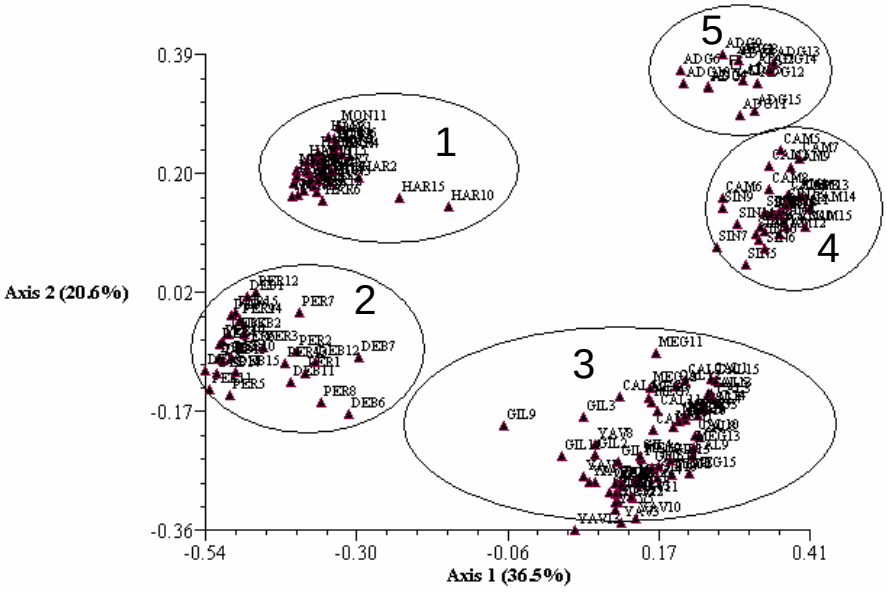


Fig. 5 Principal Coordinates Analysis (PCO) of combined morphological data for the studied species. The percentages given along PCO axis 1 and 2 represent the amount of variation explained by these two axes. For abbreviations of taxon names see fig. 3.

3.2. AFLP fingerprinting

A total of 91 markers (peaks/bands) were scored among 113 individuals analysed for the two primer combinations in the range between 59-388 base pairs. Average fragment length was 173.3 base pairs, but fragments shorter than 100 bp long were detected more often (25.3%). The primer combination *Eco*RI-AGAG/*Mse*I-CATA gave the largest number of markers (52) and the combination *Eco*RI-AGAC/*Mse*I-CAAG gave the smallest number of markers (39). In the combined data set 12.1% of the fragments/markers were monomorphic and detected in all individuals. The remaining (87.9%) detected fragments were polymorphic and varied among groups, species and/or among individuals of the same species. The proportion of polymorphic fragments did not correlate with the total number of markers (fragments) detected for the primer pairs. The markers generated from combination of *Eco*RI-AGAG/*Mse*I-CATA showed 78.8% polymorphism whereas all markers (100%) from the combination *Eco*RI-AGAC/*Mse*I-CAAG were polymorphic.

The total number of AFLP markers scored was highest for the acaulescent and herbaceous species in *A. harlana* complex (*A. harlana* and *A. monticola*) and *A. percrassa* complex (*A. percrassa* and *A. debrana*). On the contrary, the relatively highest level of polymorphism was exhibited by the shrubby and caulescent species in *A. camperi* complex (*A. megalacantha* and *A. adigratana*). Species specific bands were found for four of the species: *A. debrana*, *A. gilbertii*, *A. monticola* and *A. harlana* (tab. 4). Six diagonastic markers were shared by *A. monticola* and *A. harlana*; and three by *A. percrassa* and *A. debrana*.

Tab. 4 Summary of AFLP marker variation of eleven *Aloe* species studied (combined data set).

Species	Total number of markers	Mono-morphic markers	Poly-morphic markers	Species specific markers	Percentage polymorphism
A. adigratana	44	29	15	0	34.1
A. camperi	39	29	10	0	25.6
A. sinana	44	30	14	0	31.8
A. calidophila	36	27	9	0	25.0
A. gilbertii	38	27	11	2	28.9
A. megalacantha	38	22	16	0	42.1
A. yavellana	39	27	12	0	30.8
A. percrassa	49	39	10	0	20.4
A. debrana	48	35	13	3	27.1
A. harlana	46	42	4	1	8.7
A. monticola	51	43	8	2	15.7

Overall, the UPGMA phenograms from the three data matrices resulted in similar clustering. The UPGMA phenogram from the combined data set is presented in fig. 6.
Two major groups were generated, which were further divided into altogether eleven clusters. Each of these eleven clusters corresponded to a speices and individuals of a single species were always clustered together, showing a close genetic relationship.

Group 1, consisted of six clusters formed by *A. monticola, A. harlana, A. megalacantha, A. yavellana, A. percrassa* and *A. debrana* respectively. The first two species belong to the same complex (*A. harlana* complex) and were also well separated from other species of the group. The rest of the group was rather heterogenous, including variable species that have detectable morphological differences. Two of the species, *A. megalacantha* and *A. yavellana,* are recognized as caulescent or shrubby aloes whereas the other two species, *A. percrassa* and *A. debrana,* are herbaceous, acaulescent or semi-caulescent species like *A. monticola* and *A. harlana*.

Group 2 comprised five clusters formed by *A. adigratana, A. camperi, A. sinana, A. calidophila,* and *A. gilbertii* respectively, which are all identified as caulescent or shrubby aloes. Most clusters separated at levels of genetic similarity (Jaccards coefficients) ranging from 0.66 to 0.84. The two clusters formed by individuals of *A. adigratana and A. camperi* separated at 0.90. The cophenetic correlation calculated to measure goodness/degree of fit for the cluster analysis produced from combined AFLP data set (*r* = 0.80) showed a good fit between the cophenetic similarity value matrix and the similarity matrix upon which the clustering was based.

The result of PCO analysis was in agreement with what was found in the cluster analysis (fig. 7). The first three PCO axes explained 33.4%, 14.4% and 12.3% of the total variance in the data set, respectively, resulting in a cumulative value of 60.1%.

PCO axis 1 (33.4%) separated most of the shrubby *Aloe* species from herbaceous ones with *A. percrassa, A. yavellana* and *A. megelacantha* occupying an intermediate position. PCO axis 2 (14.4%), on the other hand, separated the herbaceous species into two groups with *A. harlana* and *A. monticola* occupying the lower part of the plot; and *A. debrana* and *A. percrassa* the upper part. It also separated the shrubby species of north (*A. adigratana, A. camperi* and *A. sinana*) from those in the south (*A. calidophilla* and *A. gilbertii*) of the flora area. The neighbor-joining (NJ) tree constructed from combined AFLP data set (fig. 8) revealed high level of support for most of the branches formed by a single species, with the exception of *A. camperi* (51%) and *A. adigratana* (62%). However, the relationship between these two species is rather strongly supported (94%). High support was also found for other pairs of the species within the species complexes,

e.g. *A. monticola* and *A. harlana* (99%), *A. calidophilla* and *A. gilbertii* (88%); and *A. debrana* and *A. percrassa* (81%).

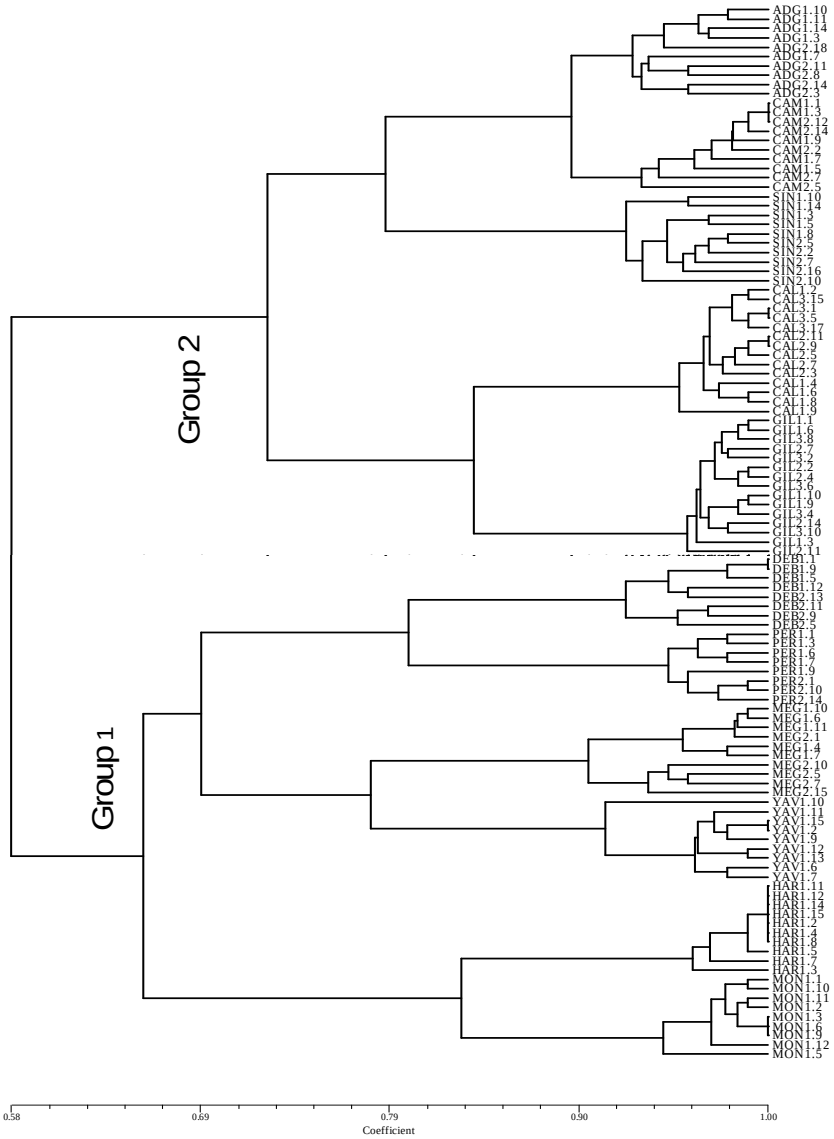


Fig. 6 UPGMA phenogram from the combined AFLP data matrix showing the relationships among the studied species (see fig. 4 for the abbreviation of taxon names).

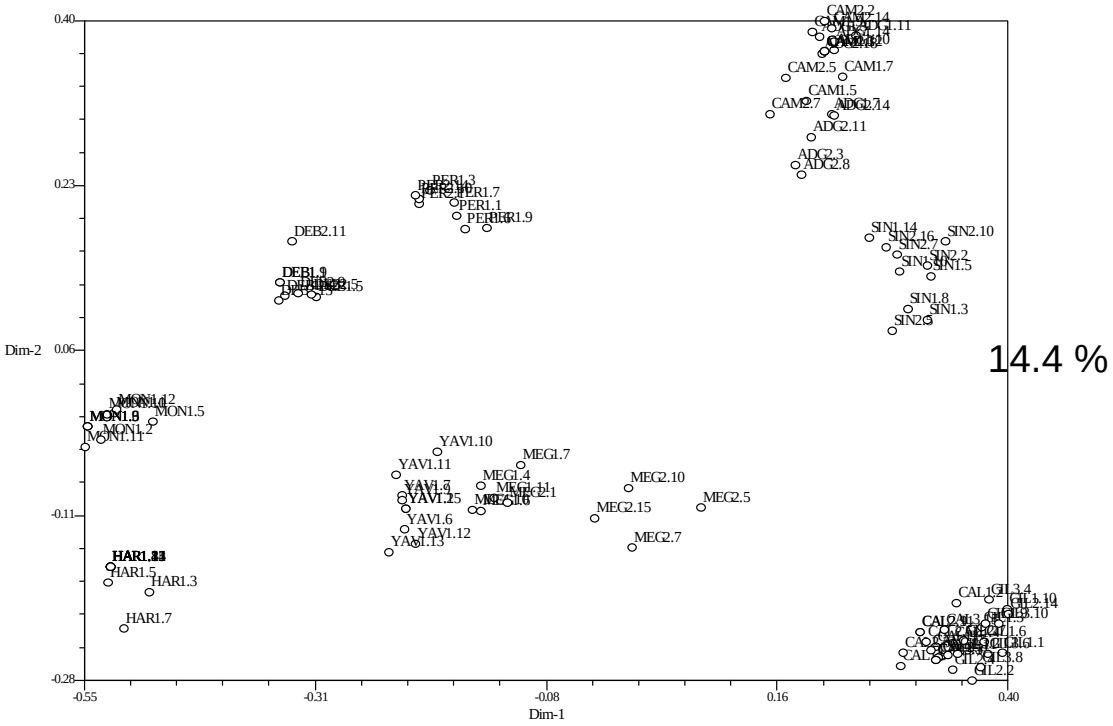


Fig. 7 Principal Coordinate Analysis (PCO) of the combined AFLP data for the studied species. The percentages given along PCO axis 1 and 2 represent the amount of variation explained by these two axes. For abbreviations of taxon names see fig. 4.

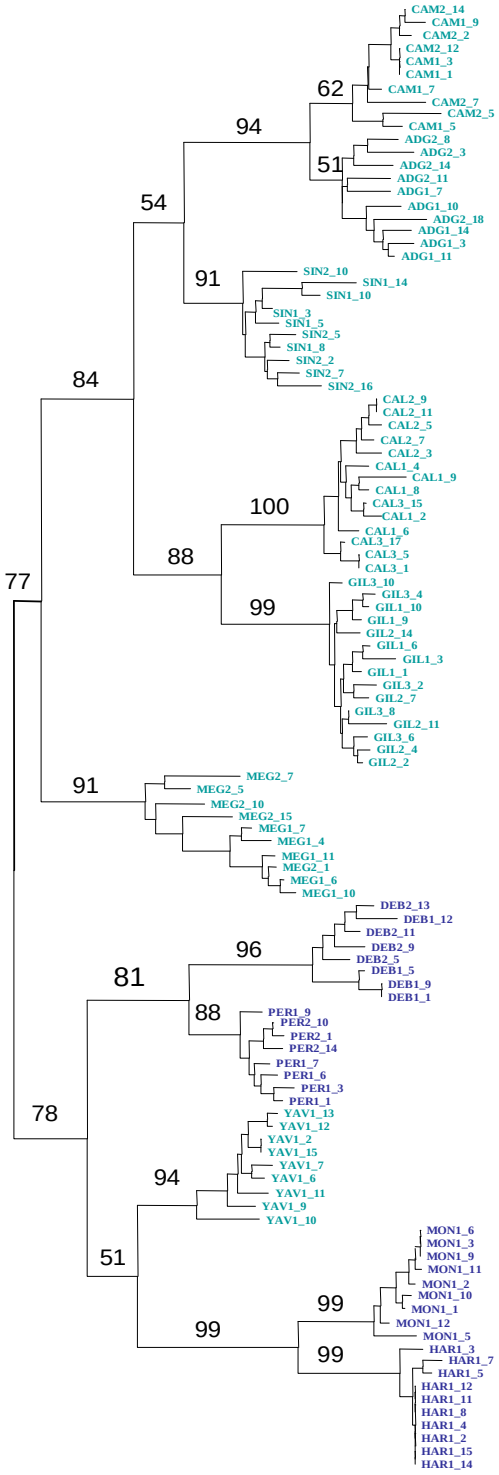


Fig. 8 Neighbor-joining (NJ) tree constructed from combined AFLP data set.

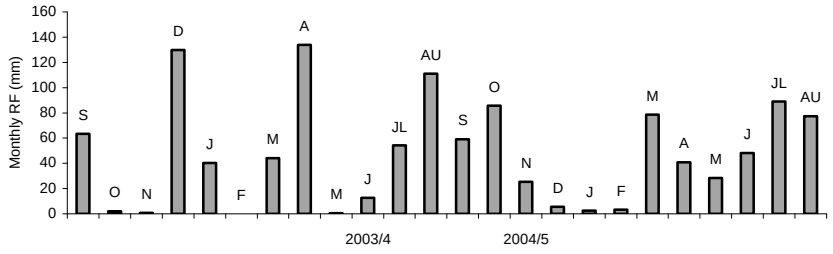
For abbreviations of taxon names see fig. 4.

3.3. Population structure and dynamics

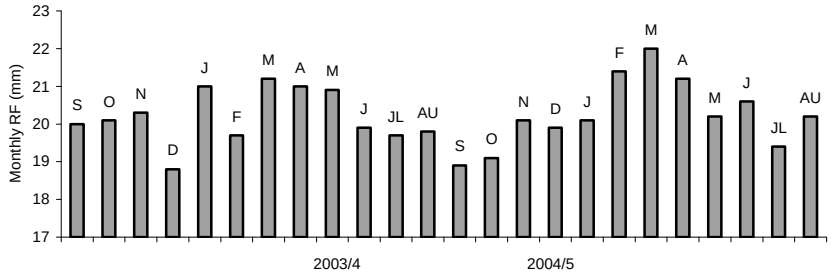
3.3.1. Climate

Monthly precipitations and mean monthly temperatures were variable during the study period and among the demographic sites (fig. 9a, b, c; and 10). The mean annual rainfalls showed no clear pattern but there were slight differences between years and sites. The year 2003/4 was wettest for Debre Birhan (85.5 mm) and relatively driest for Dire Dawa (49.4 mm). On the contrary, the year 2004/5 was wettest for Awassa (83.1mm) and again driest for Dire Dawa (45.3 mm). Mean annual temperatures were almost constant for the years 2003/4 and 2004/5. However, mean annual temperatures varied among the sites. The highest mean annual temperatures was found for Dire Dawa (26.1 °C), followed by Awassa (20.2 °C) and Debre Birhan (13.6 °C).

a) Dire Dawa station



b) Awassa station



c) Debre Birhan station

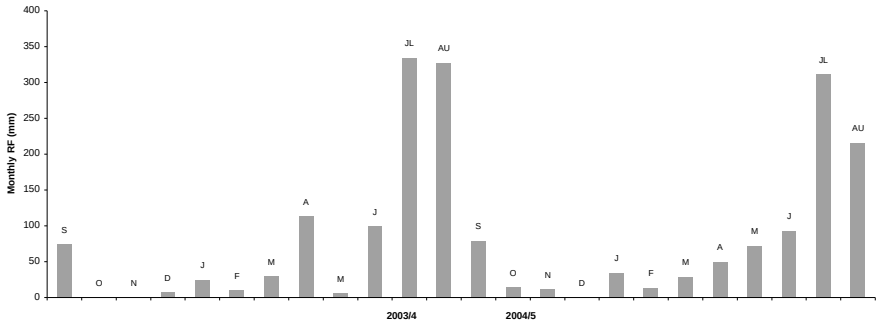


Fig. 9 Monthly rainfalls (mm): a) Dire Dawa, b) Awassa, and c) Debre Birhan stations (16, 5 and 20 kms from the demographic sites of *A. harlana*, *A. gilberti* and *A. debrana* respectively for the period of study.

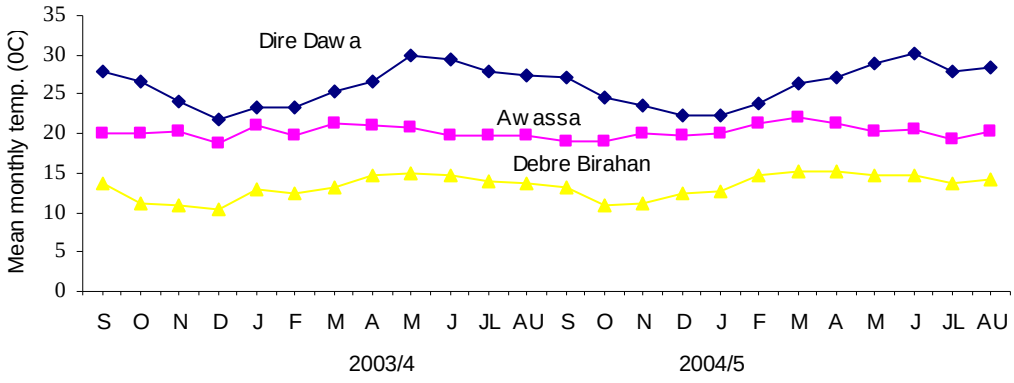
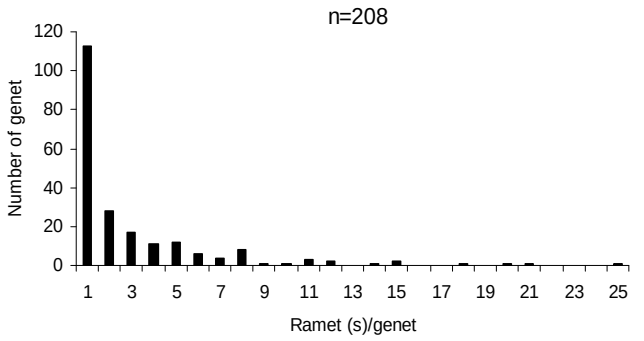


Fig. 10 Mean monthly temperatures (°C) of the three demographic sites for the period of study.

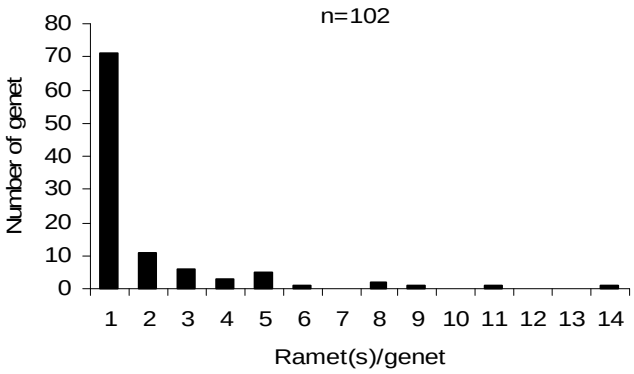
3.3.2. Population structure by clone size and rosette diameter

The population structure of the three endemic *Aloe* species (*A. gilbertii* from Alamura hill, *A. debrana* from Legatossa hill and *A. harlana* from Harla village*)* was described by the clone size and rosette diameter of the ramets that constituted the populations. As described by clone size (fig. 11a, b, c), the number of genets and ramets/genet was different for the three species. The total number of genets recorded in three plots (100 $m^{2)}$ varied from 221 in *A. debrana* to 208 in *A. gilbertii* and 102 in *A. harlana* in the first season. The average number of ramet s/genet varied from 12.3 (S.D.±7) in *A. gilbertii* to 6.3 (S.D.± 4.2) in *A. harlana* and 3.5 (S.D.±1.9) in *A. debrana.* In all of the three species, most genets consisted of only one ramet. Including seedlings, 83.3%, 69.6% and 54.3% in *A. debrana, A. harlana* and *A. gilbertii* populations, respectively were represented by a single ramet/genet. A few genets consisted of 14-25 ramets. Even though the frequency of genets with one ramet was very high, clonal growth by vegetative propagation cannot be dismissed as unimportant. For example, 69 out of 208 (33.2%), 25 out of 102 (24.5%), and 36 out of 221 (16.25%) genets had 2-5 ramets per genet in the *A. gilbertii, A. harlana* and *A. debrana* populations, respectively. 26 out of 208 (12.5%) were multi-rameted (6-25) genets in *A. gilbertii* as compared to *A. harlana* that had 6 out of 102 (5.9%) with (6-14) rameted genets, followed by *A. debrana* with only 1 genet (0.45%) that had 6 rameted genet. The three species thus vary significantly in the extent of potential for clone formation ($P < 0.09$).

a) *A. gilbertii*



b) *A. harlana*



c) *A. debrana*

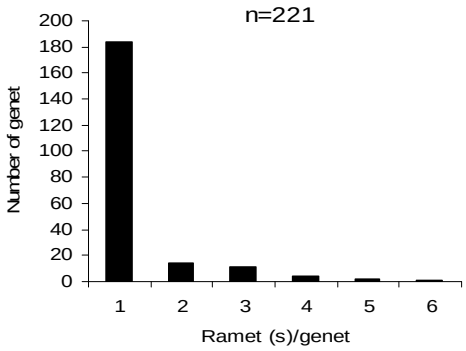


Fig. 11 Frequency distribution by clone size (the number of genets and ramets/genet) for a) *A. gilbertii* b) *A. harlana* and c) *A. debrana* populations.

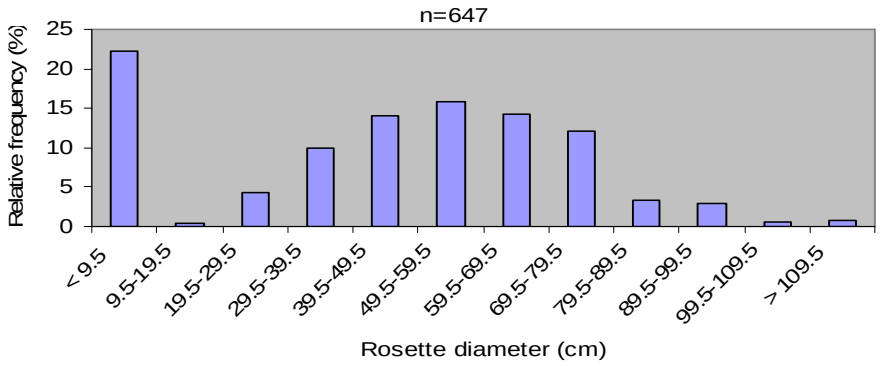
The populations of the three species also displayed different size class distribution, size class here defined by the rosette diameter of ramets in the populations. The relative frequency (proporition) of ramets whose rosette diameter is less than 30 cm (small sized, relatively young, either in seedling or juvenile phase) comprised 33%, 37.2%, and 43.6% in *A. gilbertii, A. debrana* and *A. harlana* populations, respectively. The ramets with rosette diameter between 30-90 cm (medium sized, relatively mature and that belong to the reproductive class) comprised 62.8%, 63.3% and 55.9% in the populations of *A. debrana*, *A. gilbertii* and *A. harlana,* respectively. Neverthless, only 3.7% (24 ramets) in *A. gilbertii* and one ramet in *A. harlana* populations consisted of ramet(s) with rosette diameter above 90 cm (large sized, relatively old or senile ramets); and there was no ramet recorded in this categorey for the *A. debrana* population (fig. 12 a, b, c; appendix V). Thus the three species differ significantly ($P < 0.20$) in the size class distribution of the rosette diameter of ramets.

Ramets that flowered in the three species studied were mostly the medium sized ramets (fig. 13). The majority of these ramets flowered, i.e. 100% in *A. debrana*, 94.7% in *A. harlana* and 91% in *A. gilbertii* populations. . The average number of ramets that flowered in the reproductive class was variable among species. It was highest for *A. gilbertii* 22.4 (S.D.±7.8) followed by *A. debrana* 14.7 (SD±10.6). Relatively few ramets

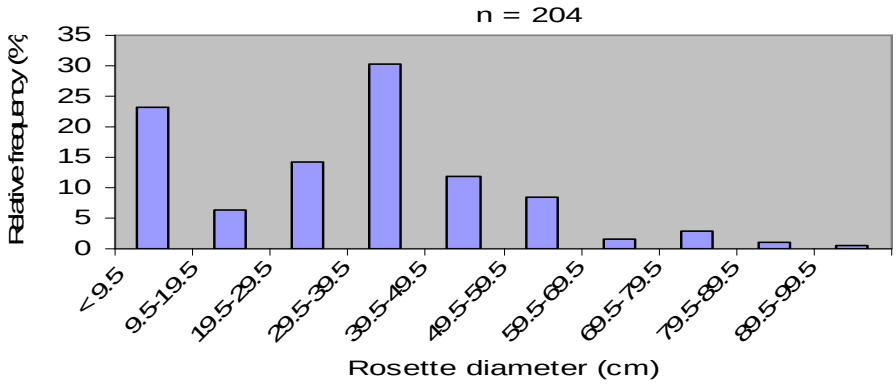
2.7 (SD±2.1) flowered in *A. harlana* population (fig. 13; appendix V). This shows that large number of ramets in this class failed to flower.

Relatively few, whose rosette diameter above 90 cm flowered: 14 (8.9%) in *A. gilibertii* and only one in *A. harlana*. None of the ramets with rosette diameter less than 30 cm flowered in the three species. The ramet size relationship to flowering showed that the minimum size that has to be attained by a ramet to flower is 30 cm rosette diameterin the three species studied

a) *A. gilbertii*



b) *A. harlana*



c) *A. debrana*

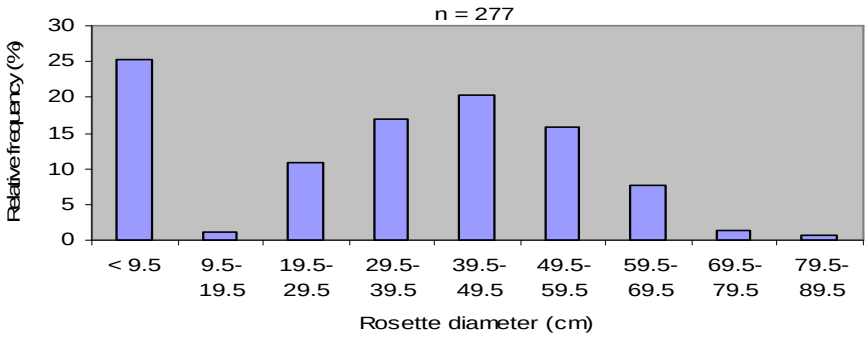


Fig. 12 The population structure in 10 cm size classes of rosette diameter of ramets in a) *A. gilbertii*, b) *A. harlana*, and c) *A. debrana* populations.

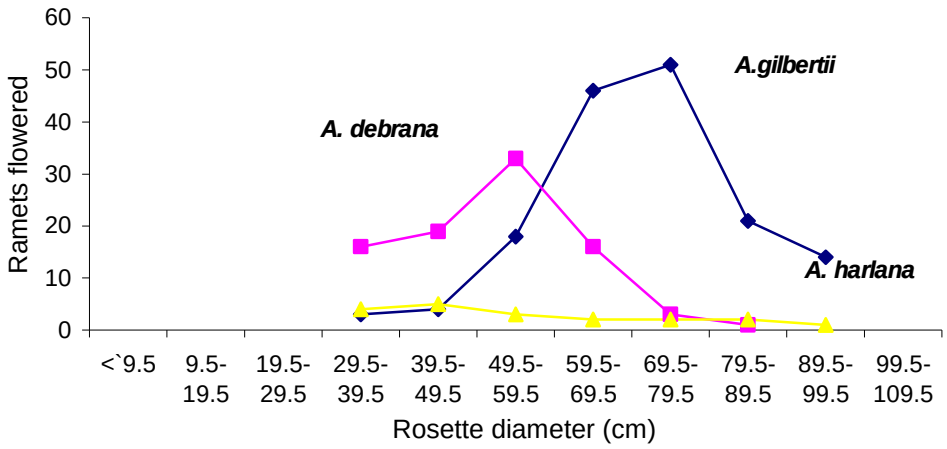


Fig. 13 Flowering ramets in relation to rosette diameter in the populations of *A. gilbertii*, *A. debrana* and *A. harlana.*

3.3.3. Population dynamics at ramet and genet levels

Ramet dynamics

The populations of the three *Aloe* species (*A. gilbertii*, *A. debrana* and *A. harlana)* had different densities and showed both similarities and differences in attributes of population dynamics at ramet-level in their respective plots (tab. 5 a, b, c).

The density of ramets was different between and among plots of the three *Aloe* species. The number of ramets in the 100 m² plot ranged from 50 in *A. harlana* to 297 in *A. gilbertii* in the first season. The mean number of ramets in the three plots analysed was 215.7 (S.D.±87.7) in *A. gilbertii*, 105 (S.D.±16) in *A. debrana* and 68 S.D.±21.2) in *A. harlana*. The density changed considerably in the second season particularly in *A. gilbertii* plots. On average 82.3, 28.3 and 8 new ramets had emerged between the seasons in *A. gilbertii, A. debrana*, and *A. harlana*, respectively.

Despite the major differences in density and recruitment of ramets, the mean percentage survival and mortality tend to be close in the three species: 95.2% in *A. gilbertii*, 97.4%

in *A. debrana*; and 94.3% survived in *A. harlana*. However, the number of ramets 'born' between seasons has no correlation (r = 0.15) with the number of ramets lost

The net increase (population growth rate) of ramets calculated for the populations of the three species on average was λ = 1. 30 for *A. gilberti*, λ = 1.28 for *A. debrana*; and λ = 1.08 for *A. harlana*. These values indicate that the *A. gilbertii* and *A. debrana* populations were expanding, whereas the *A. harlana* population was nearly stable or slightly increasing at ramet level in the period of study.

Tab. 5 Population flux (dynamics) at ramet-level in three (5 x 20 m²) plots between seasons in a) *A. gilibertii*; b) *A. harlana;* and c) *A. debrana*.

a) *A. gilbertii*

Attribute	Plot Number			
	1	2	3	Average
a) Number of ramets in Nov. 2003	113	297	237	215.7
b) Number of ramets in Oct. 2005	136	345	383	288
c) Net change (b-a)	23	48	146	72.3
d) Net increase (b/a)	1.20	1.16	1.61	1.30
e) Ramets 'born' b/n seasons	35	55	157	82.3
f) Ramets lost b/n seasons	8	7	11	8.6
g) Ramets surviving	105	290	226	207
h) Percentage of ramets surviving (g/a x 100)	92.9	97.6	95.3	95.2
i) Percentage mortality (f/g x 100)	7.6	2.4	4.8	4.8
j) Total ramets recorded	146	352	394	297.3

b) *A. harlana*

Attribute	Plot Number			
	1	2	3	Average
a) Number of ramets in July 2003	92	50	62	68
b) Number of ramets in Oct. 2005	92	58	68	72.6
c) Net change (b-a)	2	8	6	5.3
d) Net increase (b/a)	1	1.16	1.09	1.08
e) Ramets 'born' b/n seasons	2	13	9	8
f) Ramets lost b/n seasons	2	5	3	3.3
g) Ramets surviving	90	45	59	64.6
h) Percentage of ramets surviving (g/a x 100)	97.8	90	95.2	94.3
i) Percentage mortality (f/g x 100)	2.2	11	5.1	5.7
j) Total ramets recorded	94	63	71	76

c) *A. debrana*

Attribute	Plot Number			
	1	2	3	Average
a) Number of ramets in Jan. 2003	89	61	127	105
b) Number of ramets in Oct. 2005	150	64	141	118.3
c) Net change (b-a)	61	3	14	26
d) Net increase (b/a)	1.68	1.05	1.1	1.28
e) Ramets 'born' b/n seasons	62	5	18	28.3
f) Ramets lost b/n seasons	1	2	11	5

g) Ramets surviving	88	59	123	90
h) Percentage of ramets surviving (g/a x 100)	98.9	96.7	96.8	97.4
i) Percentage mortality (f/g x 100)	1.2	3.4	3.3	2 .6
j) Total ramets recorded	151	66	145	120.6

Genet dynamics

Recruitment of seedlings occurred frequently in *A. gilbertii* populations as compared to *A. debrana* and *A. harlana*. A total of 83, 69 and 15 seedlings were recruited in the three species, respectively (fig. 15 a, b, c and tab.5). The mortality of *A. gilbertii* genets was slightly higher than in the other two species. A total of 9, 5 and 4 genets died in the population of *A. gilbertii, A. harlana,* and *A. debrana,* respectively. Seedlings, juveniles and mature genets in the reproductive class have higher mortality than the senile genets in three species studied. The number of seedlings recruited in the seasons was not correlated ($r = 0.42$) to the number of genets that died.

The stage classes presented below (fig. 14) and the transition among them as shown in flow diagram (fig. 15 a, b, c) were used for analysis of population dynamics at genet level. As shown in the flow diagram, genets in seedling (SE) and juvenile (J) stages comprise relatively greater proportion in *A. debrana* (40.3%) as compared to *A. gilbertii* (30.3%) and *A. harlana* (36.3%). Similarly, mature sterile genets with one ramet (M) and mature flowering genets with one ramet (M*) comprised the largest proportion of the population in *A. debrana* (49.3%) as compared to *A. gilbertii* (23.1%) and *A. harlana* (38.2%). On the contrary, *A. debrana* (10.4%) and *A. harlana* (25.5%) had lower proportions as compared to *A. gilbertii* (46.6%) whose population had large number of senile sterile genets ≥ 2 ramets (P) and senile flowering genets ≥ 2 ramets (P*). No clear pattern of transitions among stage classes were observed in the species studied, but intermittent flowering is clearly demonstrated by exchange between M and M* and exchange between P and P*. The transition probabilities presented (tab. 6) constitute the

raw data for the Caswell matrix model (Caswell, 1989). The growth rate of the populations resulted from the matrix calculation was found to be $\lambda = 1.38$ for ***A. gilbertii***, $\lambda = 1.24$ for ***A. debrana,*** and $\lambda = 1.16$ for ***A. harlana.*** These values indicate that the populations of the three species were expanding at genet level in the period of study. The dynamics was more apparent in ***A. gilbertii*** with relatively high rejuvenation/recruitment as compared to the other two species.

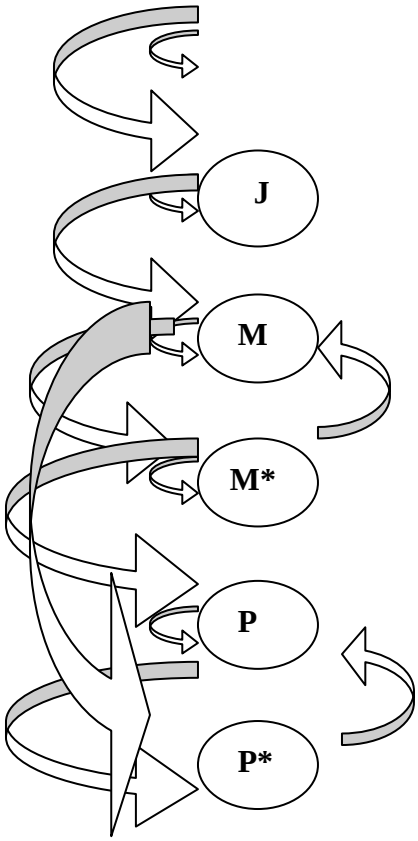
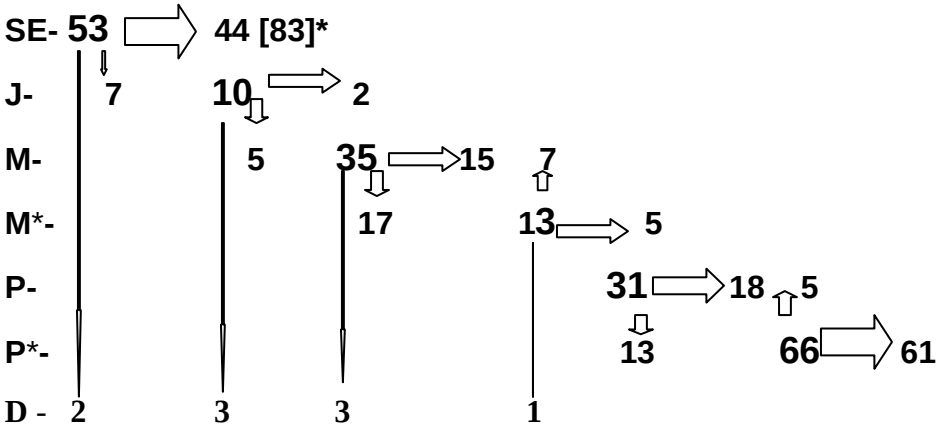
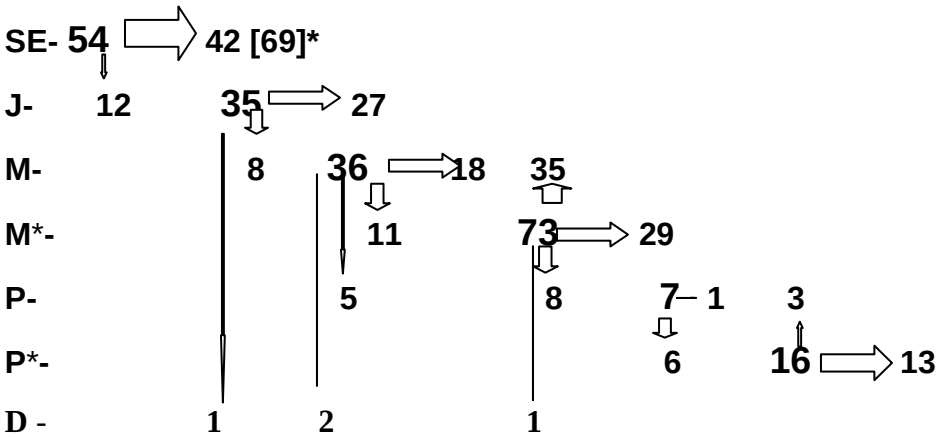


Fig. 14 Life stage classes: **SE**= Seedling; **J** = Juvenile; **M** = Mature sterile genet with one ramet; **M*** = Mature flowering genet with one ramet; **P** = Senile sterile genet with ≥ 2 ramets; **P*** = Senile flowering genet with ≥ 2 ramets. The realized transitions from one year to the next are visualized by the arrows.

a) *A. gilbertii* (from Dec. 2003 to Oct. 2005)



b) *A. debrana* (from Jan. 2003 to Oct. 2005)



c) *A. harlana* (from Jul. 2003 to Oct. 2005)

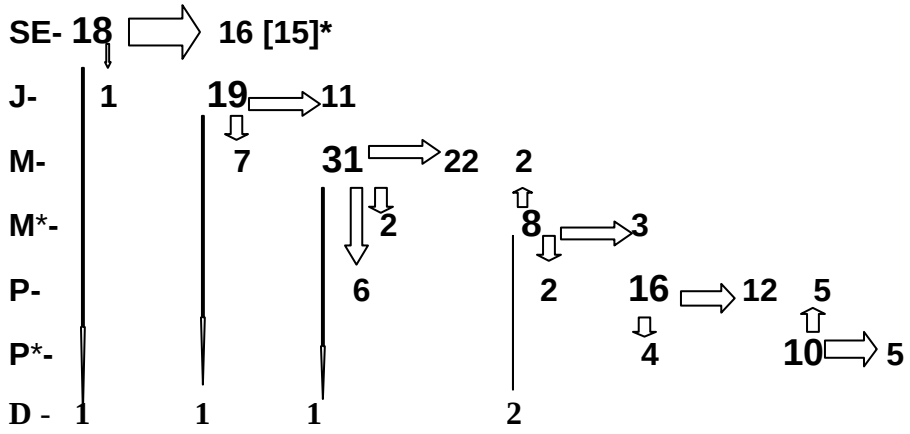


Fig.15 The fate of Seedlings (SE), Juvenile genets (J), Mature sterile genets with one ramet (M), Mature flowering genets with one ramet (M*), Senile sterile genets with ≥ 2 ramets (P), Senile flowering genets with ≥ 2 rametes (P*) of a) *A. gilberti* b) *A. debrana;* and c) *A. harlana* marked in season one. []* New seedlings recruited

Tab. 6 Transition probabilities between stage classes of genets of *A. gilbertii*, *A. debrana*; and *A. harlana* populations. Mortality between seasons equals the category of "Dead".

Parameter	Transition probabilities		
	A. gilbertii	*A. debrana*	*A. harlana*
Recruitment	**83**	**69**	**15**
SE-SE	0.83	0.78	0.90
SE-J	0.13	0.22	0.05
SE-Dead	0.04	-	0.05
N	**53**	**54**	**18**
J – J	0.2	0.77	0.58
J – M	0.5	0.22	0.37
J – Dead	0.3	0.02	0.05
N	**10**	**35**	**19**

M – M	0.44	0.5	0.71
M – M*	0.49	0.3	0.07
M- P	-	-	0.19
M – P*	-	0.14	-
M – Dead	0.08	0. 05	0.03
N	**35**	**36**	**31**
M* - M	0.54	0.48	0.25
M* - M*	0.38	0.40	0.38
M* - P	-	0.11	0.12
M*- Dead	0.08	0.01	0.25
N	**13**	**73**	**8**
P – P	0.58	0.14	0.75
P – P*	0.42	0.86	0.25
N	**31**	**7**	**16**
P* - P	0.07	0.19	0.5
P* -P*	0.93	0.81	0.5
N	**66**	**16**	**10**

3.3.4. Isoenzyme analysis

Interpretation of isoenzyme bands

Five out of seven enzyme systems were resolved clearly and consistently (i.e. had interpretable band phenotypes): Malate dehydrogenase (MDH), 6- Phosphogluconate dehydrogenase (6-PGD), Triphosphate isomerase (TPI), Glucose-6-Phosphate isomerase (GPI), and Phosphoglucomutase (PGM). The remaining two enzyme systems: Aspartate aminotransferase (AAT) and Aminopeptidase (AMP) had either weak or complicated ‘ghost’ band phenotypes difficult to interpret.

Malate dehydrogenase (MDH) is tetrameric with ± constant 6-banded pattern in the three species studied. Accordingly this isoenzyme did not give relevant information for population analysis.

6- Phosphogluconate dehydrogenase (6-PGD) is dimeric and showed a 3-banded with two monomorphic loci except in *A. harlana* where in 10 individuals either the anodal or the cathodal locus was sometimes silenced. In two individuals of *A. harlana*, the frontal (anodal) locus displayed slower bands than the rest.

Triphosphate isomerase (TPI) is also dimeric with two loci. The frontal (anodal) locus "TPI-1" is monomorphic with one rare 'A' allel in one *A. debrana* individual. The cathodal locus "TPI-2" is duplicated with fixed hetrozygosity in *A. debrana* and *A. gilbertii*, except in two of the individuals of *A. gilbertii* without this duplication and very weak cathodal bands. In *A. harlana*, though there is duplication, the cathodal bands were found to be extremely weak. Sometimes bands between the two loci were seen difficult to interpret, which might represent interlocus heterodimers (only in *A. gilbertii*).

Glucose-6-Phosphate isomerase (GPI) is dimeric with two loci. "GPI-1" is presumed to represent an instable chloroplast locus with 'ghost' bands; and "GPI-2" is locus which migrates slow and appears to be duplicated with fixed heterozygosity.

Phosphoglucomutase (PGM) is monomeric with three putative loci. "PGM-1" displayed two alleles (A and B) (fig. 15). The allele A is rare in *A. debrana*, relatively common in *A. gilbertii* and absent in *A. harlana*, whereas allele B is common in *A. debrana* and *A. gilbertii*, but rare in *A. harlana*. "PGM-2" displayed two alleles. Allele A is found in all populations and most individuals of *A. debrana* and *A. gilbertii* but absent in *A. harlana*. Allele B is absent in *A. debrana* and *A. gilbertii* but rare in *A. harlana*. "PGM-3" displayed five alleles (A-E). Alleles A, C and E are rare whereby A is found in only two individual of *A. gilbertii* and in one of *A. harlana*; C is only found in one individual of *A. debrana*, and E is found in some individuals of *A. gilbertii* only. Allele D is less common and found in *A. debrana* and *A. gilbertii*. Allele B is common and found in all populations and most individuals of the three species. Due to the observed polymorphism, only Phosphoglucomutase (PGM) was considered suitable for the population genetic analysis.

Genetic variation

The three PGM loci scored had more than one allele with between two ("PGM-1" & "PGM-2") and five ("PGM-3") alleles per locus. Population level genetic variation summary statistics are presented in tab. 7. Polymorphism ranged from ***P*** = 33.3% in population six (***A. harlana*** population) up to ***P*** = 66.6% in the remaining five populations surveyed with a mean of ***P*** = 60.9%. Allelic richness was generally high N_a = 1.3-2.6 with a mean of N_a = 2.18. The ***A. harlana*** population has relatively low level of genetic variability in all measures of genetic variation used. It was polymorphic only on one locus 'PGM-3' (33.3%) with mean number of alleles per locus (1.3). The observed heterozygosities (H_o, ranging from 1 to 7) with an average of 4.5 were generally greater than the expected heterozygosities (H_e, ranging from 1-4.7 with an average of 4.2). This resulted in negative fixation coefficients for three of the six populations with (mean F_{is} = -0.06) indicating variable levels of absence of inbreeding and an excess of heterozygotes in these populations.

Wrights (1965) F_{st} estimates for the genetic differentiation for the populations are given in tab. 8. Genetic differentiation values between all pairs of the populations of the three species ranged from (F_{st} = 0.02 to 0.73) with an average of (F_{st} = 0.37) overall pair wise comparisons indicating differentiation among the populations of the three species. The genetic differentiation between ***A. harlana*** population with the populations of ***A. debrana*** and ***A. gilbertii*** was (F_{st} = 0.66 to 073), higher than the mean values obtained from all pairs of populations. However, the values between the two populations of ***A. debrana*** (F_{st} = 0.18) and among the three populations of ***A. gilbertii*** ranged from (F_{st} = 0.02 to 0.25) with an average (F_{st} = 0.16) indicated that the genetic differentiation is moderate within the species.

Tab. 7 Estimate of genetic variability within each population studied. ***n,*** sample size; ***P,*** polymorphism; ***Na,*** allelic richness; ***Ho,*** observed heterozygosity, ***He,*** gene diversity; F_{is}, fixation coefficient.

Population (code)	*n*	*P*	N_a	H_o	H_e	F_{is}
1. Debre Birhana (DEB1)	16	66.6	2.3	5.5	4.7	-0.17
2. Dessie Zuria (DEB2)	10	66.6	2.3	4.5	4.5	0.006
3. Shashamene (GIL1)	10	66.6	2.3	4	3.9	-0.02
4. Alaba (GIL2)	14	66.6	2.6	5	5.7	0.12
5. Awassa (GIL3)	13	66.6	2.3	7	5.4	-0.32
6. Harla (HAR1)	15	33.3	1.3	1	1	0.00
Mean	13 (0.71)	60.9 (23.5)	2.18 (0.71)	4.5 (3.18)	4.2 (3.18)	-0.06 (0.12)

Tab. 8 Matrix of F_{st}-estimates for genetic differentiation among populations studied

Pop	1	2	3	4	5
2	0.18				
3	0.19	0.22			
4	0.45	0.17	0.25		
5	0.35	0.08	0.22	0.02	
6	0.73	0.66	0.68	0.73	0.69

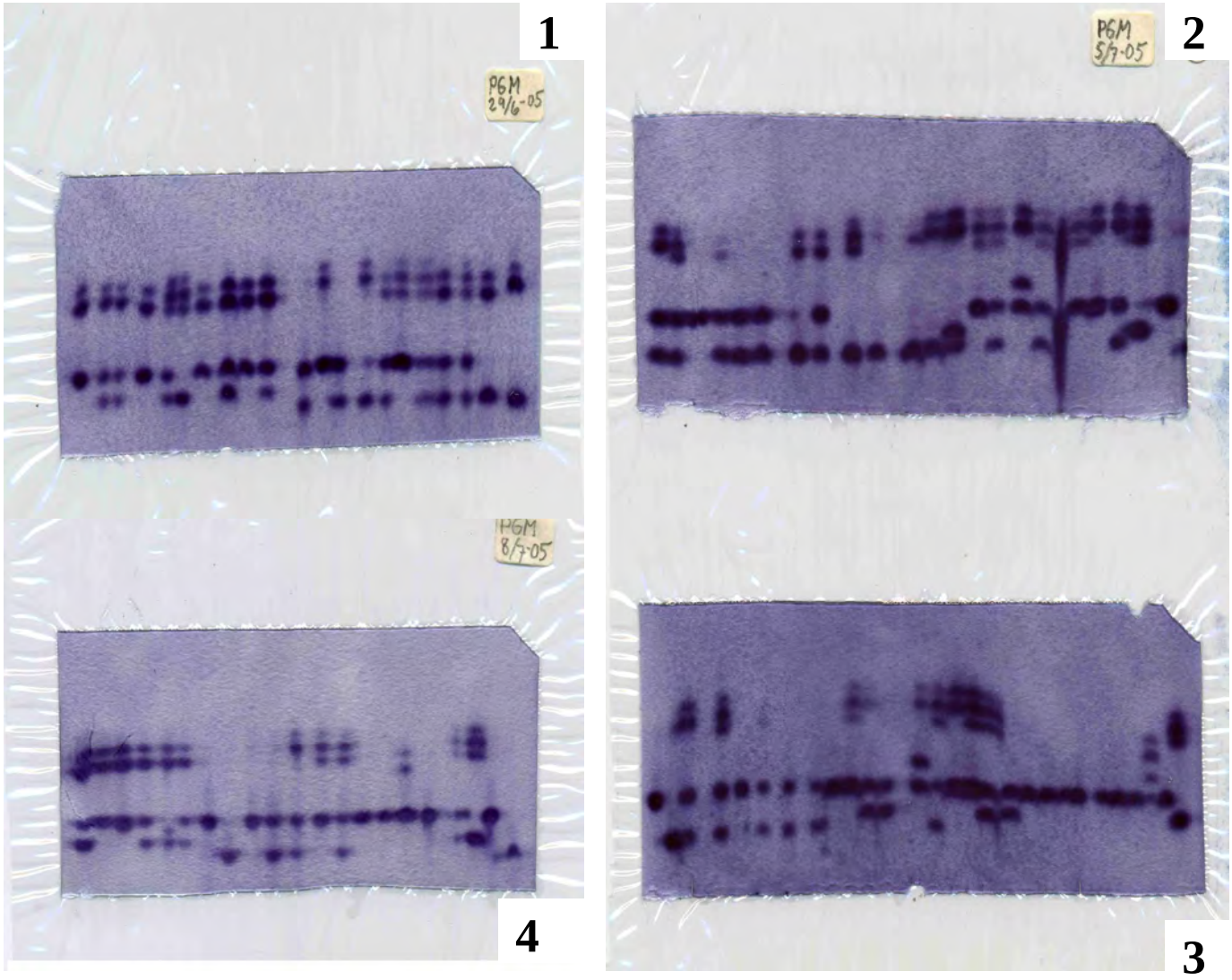


Fig. 16 Band phenotypes of PGM enzyme systems (Gel **1** = *A. debrana*, pop. 1(ind.1-10) & *A. gilbertii*, pop. 1 (ind. 1-10); Gel **2** = *A. debrana*, pop. 2 (ind.1-12) & *A. gilbertii*, pop. 2 (ind. 1-10); Gel **3** = *A. gilbertii*, pop. 3 (ind. 1-10) & *A. harlana* (1-10); and Gel **4** = *A. debrana*, pop. 1(ind.11-16), *A. gilbertii*, pop. 2 (ind. 11-14); and *A. harlana*, pop. 1 (ind. 11-15)).

3.4. Germination experiments

Germination experiments on field collected seeds of eleven *Aloe* species were conducted in a green house by providing similar and as optimal germination conditions as possible (i.e., nutrients, moisture, temperature, and light). The result showed that germination success varied not only of seeds of the same species and populations but even seeds from the same capsule. The mean number germinated from 10 seeds sown in each of the 15 pots per species was found to be highest for *A. yavellana* 8.6 (S.D.±1.5) followed by *A. harlana* 8 (S.D.±0.4), *A. camperi* 6.8 (S.D.±0.7), *A. adigratana* 6.6 (S.D.±2.1), *A. percrassa* 6 (S.D.±2.5), and *A. gilbertii* 5.8 (S.D.±3.5). Surprisingly, very low germination success was recorded for *A. megalacantha* 1.8 (S.D.±1.5), *A. monticola* 2.6 (S.D.±2.6), and *A. calidophila* 2.8 (S.D.±2.5). The last two species: *A. sinana* 3.6 (S.D.±1.4) and *A. debrana* 3.6 (S.D.±2.9), showed almost similar germination success (fig. 17).

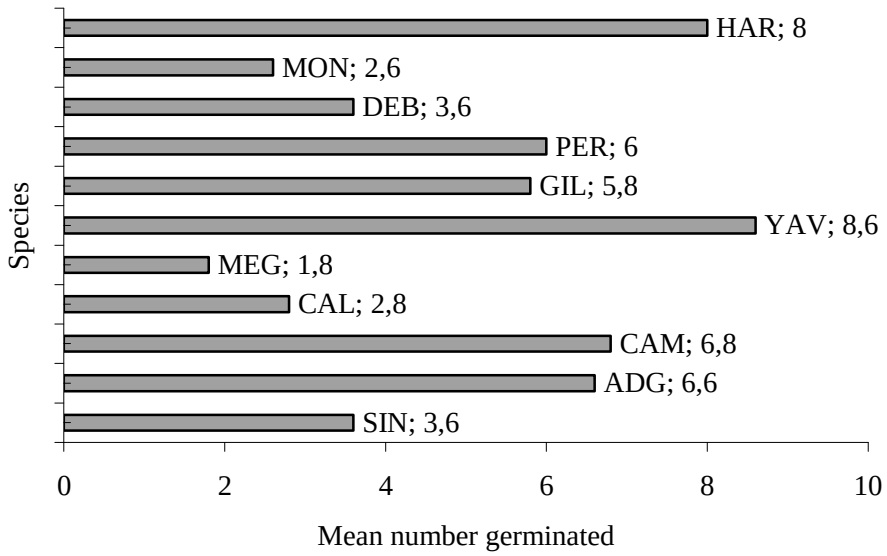


Fig. 17 Mean number germinated from 10 seeds sown in each of the 15 pots per species, (see fig. 4 for the abbreviation of taxon names).

3.5. Threats to aloes of Ethiopia

Field surveys done during the study period revealed the existance of threats to aloe plants in Ethiopia. It was observed and documented by photos that loss or destruction of natural habitats was found to be the major threat. Natural habitats inhabited by aloes in different parts of the country were destroyed for various reasons and purposes. As per qualitative observations made, clearing for agricultural lands (fig. 18 a, b), development construction such as road (figs. 19 and 20) and urbanization (fig. 20) were the major threats in decreasing order of frequency. Collections by the local community for different reasons and impacts from grazing animals such as cows, gots and monkeys were found to be of little concern. 'Unsustainable harvesting' through illegal collection for commercialization, a major threat to aloes in other parts of Africa such as South Africa, Madagascar and Kenya, seems to have little impact on aloes of Ethiopia. Some species of *Aloe* in Ethiopia inhabit special niches such as hills, escarpments and mountains; land sliding as natural phenomena, however, rarely destroys individuals in such places. A single observation made was in Alamura hill, near the demographic site of ***A. gilbertii***, where some of the individuals in the population had died due to land sliding.

a) *A. sinana* population

b) *A. debrana* population

Fig. 18 Threat to populations of a) *A. sinana* (near Debre Sinna); and b) *A. debrana* (near Beke-Mariam) where their habitats were cleared for agricultural lands. Photo: Fikre Dessalegn, December 2003.

Fig. 19 Threat to an *A. percrassa* population (at Alamata) due to road construction. Photo: Fikre dessalegn, December 2003.

Fig. 20 Threat to an *A. gilbertii* population (near Awassa town) due to urbanization (water pipeline) and road construction. Photo: Fikre Dessalegn, January 2003.

4. Discussion

4.1. Taxonomy

The clustering pattern and grouping in the UPGMA phenogram and PCO ordination from the combined morphological data (figs. 4, 5) of eleven *Aloe* species (*A. camperi*, *A. adigratana*, *A. sinana*, *A. megalacantha*, *A. calidophila*, *A. yavellana*, *A. gilbertii*, *A. debrana*, *A. percrassa*, *A. harlana* and *A. monticola*) reflected the levels of morphological relationships among and between the studied species of the three complexes. The first group of clusters (1-4) is formed by herbaceous, acaulescent or semi-caulescent, and rosulate forming species in the *A. percrassa* complex and the *A. harlana* complex, whereas, the second group of clusters (clusters 5-8: *A. megalacantha*, *A. yavellana*, *A. gilbertii*, and *A. calidophila*) and the third group of clusters (clusters 9-11: *A. sinana*, *A. camperi* and *A. adigratana*) are formed by caulescent and shrubby species in the *A. camperi* complex. The separation of species in this complex display two groups of clusters combining morphological relationships with geographical proximity/distribution; one of the groups being formed by species in southern and eastern parts of the country; and the other group by northern species. The values of dissimilarity coefficients (> 0.5) at which all eleven clusters separate are indicative of the identity of species level. The cophenetic correlation calculated to measure goodness of fit for a cluster analysis from the combined morphological data set (r = 0.88) showed a good fit between cophenetic similarity value matrix and the similarity matrix upon which the clustering was based. The clustering pattern/grouping and the species identity reflected in the UPGMA phenogram from the combined morphological data is in agreement with the

grouping followed in previous taxonomic treatments (Reynolds, 1966; Sebsebe Demissew & Gilbert, 1997).

In the DNA (AFLP) analysis (87.9%) of the markers detected were polymorphic, i.e. showed variability among or within groups. The advantage of this fingerprinting method is that it generally produces a large number of polymorphic markers helpful for the assessment of genetic variation at the intra- and interspecific levels (Vos *et al.*, 1995). The reduced number of scoreable markers in two of the selected primer combinations (with 4 + 4 selective nucleotides) and the even more complicated profiles of the third primer combination (with 3 + 4 selective nucleotides) which was eventually excluded during the scoring phase, might be attributed to the high DNA C-value observed in *Aloe* species. The nuclear DNA content measured in 83 species of *Aloe* is shown to range from 8.10 to 35.95 picograms in the diploids which indicate that the largest genome contains 3 x $10^{1°}$ more base pairs than the smallest (Zonneveld, 2002). Species with high C-values (1C > 15 pg) tend to produce AFLP traces or fragments with a large numbers of weakly amplifying and often co-migrating bands as the result of which only relatively few bands can be reliably scored (Fay *et al.*, 2005). For problematic species like *Aloe* species, however, modifications to the standard AFLP technique can generate reliable fingerprints, e.g. by changing the number of bases in one or both of the selective primers (Vos *et al.*, 1995). The total number of AFLP markers scored was highest for the acaulescent and herbaceous species (e.g. *A. monticola* and *A. harlana*) and lowest for shrubby and caulescent species (e.g. *A. calidophila* and *A. gilbertii*) (table 4). This might be explained with an observation by Brandham & Doherty (1998), stating that “although the number of species examined was not large, there was strong indication that the primitive species, with herbaceous habit, had the lowest nuclear DNA amount (and thus the smallest chromosome set), and that the amount of DNA and overall chromosome size increased with evolutionay advancement - caulescence”.

The percentage of polymorphic markers within species ranged from 8.7 % (*A. harlana*) to 42.1 % (*A. megalacantha*). The level of polymorphism in *A. calidophila* and *A. gilbertii* was not influenced by their high number of individuals and population representatives.

However, the low level of polymorphism in narrow endemics: *A. harlana* and *A. monticola* might be due to low numbers of populations and individuals. Lower genetic diversity is a general phenomenon in narrow endemics compared to their widespread relatives (Hamarik & Godt, 1990).

Cluster analysis of the combined AFLP data generated a UPGMA phenogram (fig. 6) with eleven clusters as in the UPGMA phenogram from the combined morphological data (fig. 4). However, the two phenograms differ with respect to the number of groups formed, i.e. the arrangements of species within the major groups, and levels of similarity/dissimilarity at which species cluster/separate. Group 1 in AFLP phenograms is heterogenous, including 6 species that have detectable morphological differences. Two of the species in this group, are recognized as caulescent or shrubby aloes belonging to *A. camperi* complex (*A. megalacantha* and *A. yavellana*). These two species are, however, nested between clusters of herbaceous, acaulescent or semi-caulescent species: *A. monticola, A. harlana, A. percrassa* and *A. debrana,* belonging to *A. percrassa* and *A. harlana* complexes. This result does not agree with the grouping of species based on morphlogy and geographical distribution, which has been followed in previous taxonomic treatments (Reynolds, 1966; Sebsebe & Gilbert, 1997). Generally, clusters form higher levels of similarity in the AFLP phenogram compared to that based on morphology. Another detectable difference between the two phenograms is the clustering patterns or relationships observed with regard to the three caulescent species from the northern part of the flora area: *A. sinana*, *A. camperi* and *A. adigratana.* In the UPGMA analysis of the morphological data, *A. sinana* and *A. camperi* appear close (0.47 similarity); whereas *A. adigratana* is well separated (0.37 similarity) from the two. On the other hand, *A. camperi* and *A. adigratana* are found to be very close (0.9 similarity); further clustering with *A. sinana* (0.79 similarity) as per the UPGMA analysis of AFLP data. The contradicting results from the two data sets are to some extent in agreement with earlier taxonomic works that appreciated the close resemblance and geographical proximity of all three species. According to Reynolds (1966) and Sebsebe Demissew & Gilbert (1997), *A. sinana* is closely allied to *A. camperi* in general habit of growth and leaf characters, but differs in having a less branched inflorescences, longer perianth (28

mm), and sub-laxly flowered, cylindric, slightly conical racemes with buds spreading horizontally to slightly downwards. *A. adigratana* is closely allied to *A. camperi* but differs in having longer, thicker stems, longer leaves, denser and more conical racemes and much longer but less clavate perianth. The above authorities have observed that *A. camperi* is a variable (polymorphic) species having different geographical forms. The stems vary in length and leaves vary in length and width, and occur with or without spots. Racemes vary from shorter and denser to longer and laxer. Perianths also vary in length and shape but are invariably clavate. Reynolds (1966) and Sebsebe Demissew & Gilbert (1997), also indicated that populations and individuals occurring at lower (drier) areas (towards the distribution area of *A. sinana*) are weaker forms, whereas robust forms occur at higher altitude (towards the distribution area of the narrow endemic *A. adigratana*). Consequently, further neighbor-joining analysis was done in order to check the level of support for branches formed by each species. In the tree constructed from the combined AFLP data (fig. 8), *Aloe camperi* and *Aloe adigratana* were kept together with high bootstrap support (94%) but the species status of the two species are only weakly supported with bootstrap values 51% and 62% respectively. However, separate and well supported branches were formed by the other nine species including *A. sinana* (91% bootstrap value). Based on the close resemblance in morphology, high level of genetic similarity (0.9) of *A. camperi* and *A. adigratana* and taking their geographical distribution into account (i. e., *A. adigratana* being narrow endemic and within the distribution range of *A. camperi* that streches from Wello in the south, across Tigray to Eritrea in the north), it is suggested that the two species should be recognized at subspecies level. These are: *A. camperi* Schweinfurth subsp. *camperi* and *A. camperi* subsp. *adigratana* (Reynolds) Fikre. comb. et stat. nov. Except for the above two, results from the analyses of both morphological and molecular data support the identity/species status of the species studied. These are: *A. harlana* Reynolds, *A. monticola* Reynolds, *A. debrana* Christian, *A. percrassa* Todaro, *A. yavellana* Reynolds, *A. megalacantha* Baker, *A. gilbertii* Sebsebe & Brandham, *A. calidophila* Reynolds and *A. sinana* Reynolds.

4.2. Demography

The population structure of plants can be described in terms of size, life stages (states), and ages of individuals that compose it (Harper & White, 1974). The populations of three *Aloe* species studied displayed different size structure as described by the clone size (number of genets and ramets/gent) (fig. 11a, b, & c); and also rosette diameter of ramets (fig.12a, b, & c).

The population of ***A. debrana*** had the highest number of genets (genetic individuals) as compared to ***A. gilbertii*** and ***A. harlana.*** On the other hand the ***A. gilbertii*** population displayed greatest number of multi-rameted genets (2-25) as compared to the other two species. This might be attributed to the relative magnitude of the two modes of reproduction found in the species. The genet, which grow from seeds once established, branches to form new independent physiological units; ramets, that might remain connected to a parent for a time. This constitutes the basic characterstics of clonal growth (Oborny, 1994). In aloes, like in other clonal plants, a genet can be viewed as a population of ramets (Harper, 1982), and the size of the clone/genet is therefore determined by ramet dynamics. Variations in the number of genets and ramets/genet in the studied species are indicative of whether the species rely more either on vegetative propagation or seedling recruitment or both as a strategy to maintain their population. All populations analysed had the highest number of genets consisting of only one ramet, showing that the populations have good juvenalization.

The size class distribution analysed on the rosette diameter of the ramets showed that the size of the individuals in the populations of three species is far from uniform. Small sized individuals (< 30 cm rosette diameter, RD) account greatest proportion in ***A. harlana*** population whereas ***A. gilbertii*** population was composed of relatively greatest proportion of large ramets (> 90 cm RD). Size structure is the most conspicuous aspect of population structures (Crawley, 1997); and many factors promote variation in the size of individuals in plant populations. These might be due to internal factors such as their ability to rejuvenate new ramets that contribute to the small size class of the population or strategic resource allocation for vegetative growth and reproduction such as flowering and fruiting. The relative growth rate and resource allocation for growth and reproduction are

genetically determined and thus size of individuals can vary from species to species (Crawley, 1997). The variation in size classes of RD of ramets identified in populations of three species might also be resulted from the action of external factors to which their members have been exposed during or even before the study period. For example, soil nutrients, size specific deaths, and such factors as competition (density stress) and size specific attack by natural enemies (e.g. herbivory and fungus) together can account for much of the variation in individual plant size (Hara, 1984; Weiner, 1993). In many species, the probability of flowering is also size dependent, so that plants must exceed a critical threshold size before flowering (Klinkhamer *et al.,* 1987). This is also true for three *Aloe* species where the minimum size that has to be attained by a ramet to flower is 30 cm RD. Despite variations among three species in the extent of flowering, highest number of ramets flowered belong to reproductive class whose rosette diameter ranges from 30-90 cm (fig. 13).

The populations of the three endemic *Aloe* species had different densities and shown remarkable similarities and differences in attributes of population dynamics such as recruitment, survival and mortality. Density changed appreciably in the second season in *A. gilbertii* plots as compared to *A.debrana* and *A. harlana*. Dynamics or population growth rate was also more apparent for *A. gilbertii* both at ramet and genet levels. This was attributed to relatively high recruitments both by vegetative propagation and seedling establishment in *A. gilbertii* plots. Variability in the recruitments among species was simply correlated to current year's annual precipitation or previous year's precipitation but it was not correlated to mean temperature in those years of demographic analysis (figs. 9 and 10). Mortality and survival (persistence) of ramets and genets in the populations of three species were tending to be close in the period of study with death of ramets more frequent in the *A. gilbertii* plots. This indicates that the demographic parameter contributed to the population growth rates obtained from matrix analysis was largely due to recruitment rather than to mortality or survival (persistence). Ramets and genets died in the populations of three species were from different size classes and life stages; and were caused by ecological factors such as destruction by man, grazing animals and land sliding. Land sliding occurred during the study period in the *A. gilbertii*

population killed both ramets and genets. Hence mortality of genets was not linked to size class or stage of development. No relationship was also found between mortality and climate variables. All populations analysed showed relative growth rate $\lambda > 1$, meaning that they have tendency to increase, which is good seen from a conservation point of view.

A. harlana, with its single population representation, exhibit different patterns of isoenzyme variation. It had displayed one polymorphic locus (PGM-3) and a relatively lower amount of within population variability as compared to *A. gilbertii* and *A. debrana* populations. This might be due to small population size and restricted distribution that result in founder effects, genetic drift and/or inbreeding in its smaller population (Hamrick and Godt, 1989). The inbreeding coefficients (*Fis*) were rarely deviating much from zero, meaning that outcrossing is prevalent within the populations. Three out of six populations displayed negative *Fis,* indicating that they produced more heterozygotes than expected from Hardy-Weinburg equilibrium. Isoenzymes do not appear to give clear taxonomic signals, as interspecific distances sometimes tend to be smaller than intra-specific distance. For example, genetic differentiation between population 2 of *A. debrana* (Dessie Zuria) and population 5 of *A. gilbertii* (Awassa) was *Fst* = 0.08 whereas population 1 (Debre Birahan) & 2 (Dessie Zuria) of *A. debrana* was *Fst* = 0.18. *A. harlana* population was more distinct with differentiation index ranging (F_{st} = 0.66 to 0.73) from the populations of *A. debrana* and *A. gilbertii.*

Germination success varied not only for seeds within the same species and populations but even seeds from the same capsule in the species studied. This variation in seed germination may be attributed to factors internal to the seeds such as level of maturity of seeds at harvesting time and/or other conditions linked to seed cover (testa) and the embryo. It may also be attributed to diverse environmental conditions in the wild where these species germinate and grow as compared to the conditions in the green house. The *Aloe* species studied differ in their natural habitats (i.e. in climate and soil types) ranging from hot lowland and the rift valley to the relatively cooler highlands. Species are found to grow in acid, neutral or basic soils. So the result was expected that they require different germination conditions. Neverthless, the germination experiment indicated that

most species are tolerant of the soil types to be found in gardens and potting mixtures. Hence cultivation of aloes in *ex-situ* can be applied as effective conservation strategy. However, in all cases good drainage is essential. One has to add humus around roots for young seedlings as they thrive on mature manure and plenty stones - especially dolomite stones. If manure is not available then use any of the artificial fertilizers during growing season (see appendix VI). As they are succulents, they do not require much water in the amount that impedes growth of the roots. It will, however, be necessary to provide shade for young aloes. Once past the young stage most aloes need sunshine to flower (http://www.made-in-afrika.com/aloes/tips.htm).

Field surveys conducted during the study period revealed the existence of threats to aloe plants in Ethiopia. The loss or destruction of natural habitats for agricultural expansion, due to road construction and urbanization were observed to be the major threats. Results from these field observations agree with threats identified to the entire flora of the country (IBC, 2004).

5. Conclusions and recommendations

1. Analyses of morphological and molecular (AFLP) data supported the species status of nine out of the eleven species studied. These are: *A. harlana* Reynolds, *A. monticola* Reynolds, *A. debrana* Christian, *A. percrassa* Tod., *A. yavellana* Reynolds, *A. megalachanta* Baker, *A. gilbertii* Sebsebe & Brandham, *A. calidophila* Reynolds and *A. sinana* Reynolds. Analysis of the AFLP data indicated that the remaining two species, *A. camperi* Schweinfurth and *A. adigratana* Reynolds are close genetically. Based on this close relationships combined with close resemblance in morphology and geographical proximity, it is suggested that the two species should be recognized at subspecies level. These are: *A. camperi* Schweinfurth subsp. *camperi* and *A. camperi* subsp. *adigratana* (Reynolds) Fikre. Hence, results from the taxonomic component of this study show that

the long-established morphological species concept is in agreement with molecular (AFLP) variation for most of the species evaluated.

2. The three species analysed demographically: *A. gilbertii*, *A. debrana* and *A. harlana* displayed variation in the population structures as described by clone size and rosette diameter of ramets. This might be attributed to the relative magnitude of the two modes of reproduction, i.e. vegetative and seedling recruitment. The three species also showed remarkable similarities and differences in demographic parameters such as recruitment, survival and mortality. Instant demography or population growth rate calculated at ramet and genet-levels for the season showed that *A. gilbertii* and *A. debrana* were increasing (expanding) whereas *A. harlana* was slightly increasing or nearly stable or stagnant. There was more variation in recruitment between seasons than mortality or survival (persistence) among species that contributed for the observed growth rate. Neverthless, it is diffcult to predict the future status of the studied species with data from only one population and one season. Consequently, further study should consider long-term demographic data from different populations that are extremely valuable to our understanding of the status and also in conservation decision making.

3. Both *in-situ* and *ex-situ* conservation strategies can be implemented for aloes of Ethiopia. Since *Fst* values within populations of species are rather high (upto 25% in *A. gilbertii* and 18% in *A. debrana* as per the result from isoenzyme analysis, it is recommended that several populations of a species must be conserved in their natural habitat to keep the genetic diversity. The success in the germination of field-collected seeds in the greenhouse suggests that cultivation of aloes *ex-situ* from seeds and also vegetatively can be applied as an effective conservation strategy. They can also be propagated by tissue culture as there are reports of success elsewhere. Neverthless, urgent measures are needed for the narrow endemic species such as *A. harlana*, *A. monticola*, and *A. yavellana* that are known from few localities and whose natural habitats are being cleared at alarming pace in Ethiopia. Several reports indicated that a number of species are threatened in the flora area. Obviously, much damage has been done so far not only to aloes but also to other plants, and it can be anticipated that if nothing is done to curb their

destruction, these species will become extinct in the wild. To ensure that wild plants (including aloes), continue to exist as constituenties of natural plant communities, it is important that the existing conservation legislations in the country should be enforced and have the whole-hearted and enthusisastic support of an enlightened and conservation conscious public. It is hoped that future conservation actions will ensure the survival of these fascinating plants.

6. Formal taxonomy

Both the morphology and molecular (AFLP) analysis have more or less supported the taxonomic delimitation as presented by Sebsebe & Gilbert (1997). Due to the morphometric work the species description can, however, be emendated. It will also be proposed to reduce *A. adigratana* Reynolds to *A. camperi* subsp. *adigratana* (Reynolds) Fikre.

1. *Aloe harlana* Reynolds

- type: Harerghe (HA), 15 km SE of Dire Dawa on the road to Harer, near Harla, *Reynolds* 8158 (PRE holo. not seen).

Mostly stemless but eventually developing a stem up to 30 cm long. Leaves numerous, 30-60 x 5-13 cm, glossy dark green, erect and spreading to slightly curved, sometimes spotted when young; marginal spines 6-12 per 10 cm, 3-5.5 mm long, dark brown with horny layer often continuous along margin between spines, dried sap colour deep brown. Inflorescence up to 110 cm high, with 5-8 racemes; racemes sub-capitate, conical to cylindrical, 4-9(-20) cm long, densely flowered. Bracts ovate, 6-20 x 3-6.5 mm, acuminate. Pedicels 11.5-18(-30 in fruit) mm. Perianth cylindrical-trigonous, 25-30 x 4.5-7 mm, bright yellow or red; outer lobes free for 10-15 mm. Capsule 20-25 x 6-9 mm, cylindrical, surface woody and rough at maturity. Seed irregular to 3-sided, 4.9-7 mm in diameter, arilus/testa colour grey.

Aloe harlana is a narrow endemic species in the flora area. The species mainly occur at the village of Harla (type locality), 15 km SE of Dire Dawa on the road up the mountain to Harer and for 3 km further on. During the field survey, a small population was found to occur about 30 km from Harer town on the road to Girawa in Hararghe floristic region together with *A. megalacantha*. In these localities the species grows on sparsely vegetated slopes, often on limestone between 1650 and 2100 m

Individuals of *Aloe harlana*; photograph taken at 17 km on the road from Dire Dawa towards Dengego. Alt.- 1880 m, Lat.- 41° 54’ E, Long.- 9° 28’ N.

2. *Aloe monticola* Reynolds

- type: Tigray (TU), 7 km N of Mayichew, *Reynolds* 8118 (PRE holo. not seen, K iso., EA iso. not seen)

Stemless, usually solitary. Leaves many, 33-52(-78) x 9-13.5(-18.5) cm, gently recurved, slightly canaliculated, glossy olive-green; marginal spines 5-16 per 10 cm, 3-6.5 mm long, brown with horny tissue usually forming continuous edge between spines, dried sap colour brown. Inflorescence upto 125 cm with 3-6(-14) racemes; racemes subcapitate to conical, 6-22.5 cm long, densely flowered. Bracts lanceolate, 16-30 x 6-7 cm, acute. Pedicels 11-18 mm long, 20 mm long or more in fruit. Perianth cylindrical-trigonous, 26-32(-42) x 6-7 mm, usually yellow, occasionally bright red; outer lobes free for 12-14 mm. Capsule

cylindrical, surface woody and rough at maturity. Seed irregular to 3-sided, 2.9-5.9 in diameter, testa colour black.

Aloe monticola is also a narrow endemic species known only from two localities in Tigray and Wello floristic regions. In Tigray, the species occurs at mountain slopes, 4, 7, and 30 km north of Mayichew. This very distinctive species occurs in the high mountains of northern Ethiopia from the upper northern slopes of Amba Alage to south of Adishew, and to about 5 km south of Mayichew. The largest numbers were found on mountain slopes, 268 km N of Dessie (Wello) at an elevation of 2360 m, with ***A. percrassa*** Todaro near by and 4 km S of Dessie at an altitude of 2460 m. The altitudinal range of distribution appears to be about 2370-2550 m. In these localities, the species grows on steep bare mountain slopes, in some places covered with grasses.

Individuals of *Aloe monticola*; photograph taken at 30 km from Maychew on the road to the town of Mekele. Alt.- 2730 m, Lat.- 39° 32’ E, Long.- 12° 53’ N.

3. *Aloe debrana* Christian

- type: *McLoughlin* 812A, live plant collected from SU, Debre Birhan, cultivated in Pretoria, specimen preserved under PRE 27173 (PRE holo. not seen)

A. berhana, Reynolds (1957)-type: Shewa (SU), 9 km SW of Debre Berhan, *Reynolds* 8135 (PRE holo. not seen)

Succulent herb, suckering from base to form small groups, mostly stemless but some old plants developing thick, prostrate stems up to 45 cm. Leaves in very dense rosette, spreading-recurved, 25-60 x 6-15 cm, dull-green, old leaves drying brown; marginal teeth 5-10(-14) per 10 cm, 2-4.5 mm long, red tipped, dried sap colour deep brown. Inflorescence *c* 106 cm high, compoundly branched; racemes 6-17, capitate to

cylindrical, 5-16 cm long, lax or dense (4-9 flowers per cm). Bracts ovate-triangular, 2.5-6.5(-8.5) x 1.5-3 mm long scarious. Pedicels 8-15(-22 in fruit) mm long. Perianth cylindrical, 17-35 x 4-6 mm; outer lobes free for 5-10 mm. Capsule cylindrical, surface woody and smooth at maturity. Seed irregular, 3.5-6.2 mm in diameter, testa colour black.

Aloe debrana is an endemic species known from Shewa, Gojam and Wello foristic regions. The species is named after the type locality where it is found in large numbers, on rocky slopes SW of Debre Berhan on the road to Dessie. The species also occurs in large numbers at 36, 65, 100 km North of Addis Ababa on the road to Blue Nile and Debre Markos. A small population also occurs above Bole Gorge on "Mul farm" which is about 50 km N of Addis Ababa. In Wello, it occurs around Dessie Zuria: 5 km from Dessie towards Addis Ababa. In these localities, the species commonly grow in areas of grassland on thin soil overlying basalt, usually on gentle slopes between 2000 and 2700 m.

Individuals of *Aloe debrana*; photograph taken at 30 km from Debre Berhan towards Addis Ababa. Alt.- 2810 m, Lat.- 39° 32' E, Long.- 9° 36' N.

4. *Aloe percrassa* Tod.

A. abyssinica var. *percrassa* (Tod.) Baker - lectotype: t.21 of *J. Linn. Soc.* 18: 175 (1880)- protologue based on plant grown from seeds sent by Schimper to St. Petersburg (Leningrad) from where seeds were sent to Todaro

A. oligospila Baker- type: cultivated plant, grown at Cambridge Botanical Garden from seed sent by Schinz of Zurch from Ethiopia.

Succulent herb, suckering from base to form small groups, mostly stemless but sometimes developing erect or decumbent stem to 80 x 10-15 cm. Leaves crowded, 36-75 x (3-)6-15 cm or longer, glaucous green or grey-green, often flushed red, old leaves drying brown; marginal spines 3-16 per 10 cm, (2-)3-5 mm long, tipped pale-pink to brown, dried sap colour yellow. Inflorescence 60-100 cm high; racemes (3-)6-21, cylindrical to conical, 6-28 cm long, with 2-5 flowers per cm. Bracts ovate-acuminate, (4.5)10-16(-20) x (2.5-)3-6 mm. Pedicels 11-17(-23) mm long. Perianth cylindrical, 16-29 x 4-6 mm; outer lobes free for 5-7 mm. Capsule cylindrical, woody and smooth at maturity. Seed flat to 3-sided, 3.5-6.1 mm in diameter, testa colour black.

Aloe percrassa is an endemic species with a relatively wide geographical range in Ethiopia and Eritrea. In Ethiopia, the species occur in mountain slopes 38-41 km North of Mayichew (78-81 km South of Mekele), on Ambalage both in Tigray floristic region. In the same region a small population of ***A. percrassa*** was found to grow at about 60 km from Mekele to Adigrat together with ***A. adigratana*.** In Gonder floristic region, the species occurs on 20 km from Debark on the Gonder-Axum road. In Eritrea, it occurs in large numbers on the Kohaito Plateau (presumed type locality), 7 km north east of the Adi Keyh-Senafe road. It also occurs in Saganeiti, Gorge of Goua, near Addingofom /Hill/ (Reynolds, 1966). Altitudinal distribution range is from 2100-2700 m.

An individual of *Aloe percrassa*; photograph taken at 20 km W of Adigrat towards Adwa. Alt.- 2500 m, Lat.- 39° 26’ E, Long.- 14° 16’ N.

5. *Aloe megalacantha* Baker

- type: Hararghe (HA), Ogaden, Milmil, *Puspoli & Riva* 905 (FT holo.).

Succulent shrub, 33-200 x (2.3-)7-14 cm high. Leaves crowded, 34-98 x (3.5-)13-17 cm, deeply canaliculated, recurved, dull light green to blue-green, marginal spines 5-10 per 10 cm, 3.5-6 mm long, pink-to red-brown, dried sap colour yellow. Inflorescence up to 110 cm high; racemes 6-23, cylindrical to conical, 5-18 cm long, lax or dense. Bracts triangular, (2.5-)4-12.5 x 2-4 mm. Pedicels (8-)10-15(-27 in fruit) mm long. Perianth cylindrical-trigonous, 19-31 x 4-7 mm, yellow orange or scarlet; outer segments free for 10-14 mm. Capsule oval to oblong, woody and rough at maturity. Seed irregular to 3-sided, 3.9-6 mm in diameter, testa colour grey.

Aloe megalacatha is a near endemic restricted to eastern Ethiopia (Hararghe floristic region) and Northern Somalia. In Eastern Ethiopia, the species occurs near Milmil in the Ogaden region (type locality), in large numbers 6-10 km west of Dire Dawa and 3 km from Asebe Teferi on the road to Awash. It also occurs in a small population about 30 from Harar town km on the road to Girawa. Moreover, the species is found in Bale floristic region, near Sof Omar. In the Northern Somalia, the species grows in considerable numbers for many miles around Hargeisa (Reynolds, 1966). The species grows on rocky hill sides and sandy alluvial plains in open *Acacia-Comiphora-Balanites* bushlands, frequently planted on graves and in margins of evergreen thickets on limestone slopes. Altitudinal distribution range is from 1650 to 1800 m.

Individuals of *Aloe megalacantha*; photograph taken at 14 km on the road from Dire Dawa towards Dengego. Alt.- 1880 m, Lat.- 41° 54’ E, Long.- 9° 28’ N.

6. *Aloe yavellana* Reynolds

- type: Sidamo (SD), 1.6 km (1 mile) W of Yavello, *Reynolds* 7063 (PRE holo. not seen, EA, K iso.).

Succulent shrub, stems erect to 1 m high or sprawling, up to 3 m long, 3-6 cm thick. Leaves spaced along stem, 30-68 x 4.5-12 cm, recurving towards tip, slightly canaliculated, brown to pale brown; marginal spines (4-) 14-17 per 10cm, *c* 2.5-4.5 mm high, tips reddish, dried sap colour yellow. Inflorescence up to 90 cm high, with 6-13 racemes; racemes capitate to cylindrical, (2.5-) 4-11 cm long, dense, 6-10 flowers per cm. Bracts triangular-ovate, 2.5-6 x 1-2 mm, scarious, Pedicels 8-10(-17.5 in fruit) cm long. Perianth cylindrical-triagonous, 15-28 x 4-6 mm, dull scarlet to orange; outer segments free for 8-10 mm. Capsule oval to oblong, woody and rough at maturity. Seed flat, 6.2-9.1 mm in diameter, testa colour grey.

Aloe yavellana is a narrow endemic restricted to two localities in Sidamo floristic region, near Yavello town and in the north-eastern slopes of Mega Mountain where it occurs in great numbers in forest, in clearings, and on rocks. The species was named after the first locality where it can easily be seen from the road where it grows in rocky slopes, in *Juniperus* forest, and also in more disturbed areas near roads between 1600 and 1900 m.

Individuals of *Aloe yavellana*; photograph taken at 2 km W of Yavello. Alt.- 1910 m, Lat.- 38° 04’ E, Long.- 4° 53’ N.

7. *Aloe gilbertii* Reynolds ex Sebsebe & Brandham

- type: Sidamo (SD), Alamura Hill, *c* 4 km S of Awassa on the main Addis Ababa road to Dilla, *Gilbert, Sebsebe D. & Ermias D.* 9307 (K holo., ETH iso.).

Usually a succulent shrub, stem erect to 22-200 x 4.9-28 cm long, occasionally stemless. Leaves crowded, 25-67 x 4-13 cm, canaliculated, recurved towards tip, dark green or glaucose, often flushed brown above and below; marginal teeth 5-10 per 10 cm, 3-5 mm high, tips brown, dried sap colour brown. Inflorescence up to 124 cm high, compoundly branched; racemes (9-)15-49 or more, cylindrical 6-15 cm long, lax, 2-4(-5) flowers per cm. Bracts ovate, ovate acute, 4-7.5 x 2-3 mm. Pedicels 7-12 mm long. Perianth cylindrical to subclavate, trigonously indented, 21-30 x 4.5-8 mm, orange to red; outer segments free for 8-11 mm. Capsule oblong, woody and rough at maturiy. Seed irregular to 3-sided, 5.3-9.5 cm in diameter, testa colour grey.

Aloe gilberti is recently described and is endemic in the flora area. It occurs in a number of places in Sidamo, Shewa, and Gamo Gofa floristic regions. In these regions it is confined in its distribution to rift valley and lowlands. In Sidamo floristic region, the species occur in Alamura Hill, 4-5 km from Awassa town on the road to Dilla (type locality) and on Tabor mountain slopes. It also grows in a large number from Morocho on the road to Billate. In Shewa floristic region the species occurs in different places near Arsi-Negelle, near Langano up to 30 kms northward from Shashemene on the road to Addis Ababa. South of Shashemene it also grows at 5, 20 and 60 kms (near Alaba town). In Gamo Gofa, it occurs at 3 km from Konso on the road to Yavello. In these localities, the species grows in *Acacia* woodland, often found in hedges and along field margins between 1300 and 1800 (-1900) m. It also grows in open or dense *Acacia-Commiphora* bushland, on rocky places with *Adenia venenata*.

Individuals of *Aloe gilbertii*; photograph taken at 5 km from

Awassa on the road to Dilla. Alt.- 1800 m, Lat.- 38 30 E, Long.- 7 00 N.

8. *Aloe calidophila* Reynolds

- type: Sidamo (SD), Dida Cheena Plains, 49 miles WNW of Moyale on the road to Mega, *Reynolds* 7029 (PRE holo. not seen, K iso.).

Succulent shrub, stems 59-200 cm, erect or basally decumbent. Leaves crowded, (22-50-106 x (3.5-) 6-16 cm, spreading with recurved tip, deeply canaliculated, uniformly dull green to grey-green; margin with *c* 3-13 spines per 10 cm, spines 2.5-5 mm high, dull white, dried sap colour deep brown. Inflorescence up to 123 cm high, branched; densely flowered with 6-7 flowers per cm, racemes (5-)10-32 or more, cylindrical 6-20 cm long. Bracts ovate, 2.5-5 x 1.5-2 mm, scarious. Pedicels 10-15(-21) mm long, extending to 22 mm in fruit. Perianth clavate, 17-20(-28) mm long, widest part 6-7 mm, scarlet turning orange towards throat; outer segments free for 8-10 mm. Capsule cylindrical, woody and rough at maturity. Seed irregular to 3-sided, 4.5-8 mm in diameter, testa colour grey.

Aloe calidophila is a near endemic species to the flora of Ethiopia and Eritrea with wide distribution in Southern Ethiopia, (i.e. in Sidamo and Gamo Gofa floristic regions). The species is also occurring in Northern Kenya bordering Ethiopia near Moyale. In Sidamo floristic region, it occurs in large number for about 100 km along the Moyale-Mega road, especially on the Dida Cheena plains (type locality); 5-8 km N of Mega; near Dubuluck and for about 35 km northwards to near Yavello. From Yavello, the species repeatedly occur for about 100 km along the Daua Parma road. In Borana, it occurs in numbers around the town of Negelle; northwards from Negelle on the road to Shakisho and then to Kibre-Mengist and eastward on the road to Filtu and Dollo Odo. In Gamo Gofa province, it occurs at 2 km from the town of Arbaminch on the road to Addis Ababa and southward on the road to Jinka in few places. In Kenya, the species was abundant in the area about 100 km N of Wajir (14 km S of Buna), to Korondil and near Moyale; along the Wajir-El Wak road (Reynolds, 1966). In these localities, the species grows in *Acacia-Commiphora* woodland/bushland or open wooded grassland often on dark soils. Altitudinal distribution range from about 1300 to 1600 m.

Individuals of *Aloe calidophila*; photograph taken at 31 km from Mega towards Moyale. Alt.- 1475 m, Lat.- 38° 30’ E, Long.- 5° 53’ N.

9. *Aloe sinana* Reynolds

- type: Shewa (SU), 18 km NE of Debre Sina, *Reynolds* 8126 (PRE holo. not seen, EA, K iso.).

Succulent shrub, stems (33-)50-200 x (3-)5-12 cm long, sprawling or ascending. Leaves crowded, 40-87 x (4.5-)7-15 canaliculate only towards tip, blue to grey-green; sometimes flushed reddish, with elongated white spots on undersides and towards base on the upperside; marginal teeth 4-8 per 10 cm, 2-4 mm long, dull white, dried sap colour deep brown. Inflorescence up to 112 cm high, with 4-10 racemes; racemes subcapitate to cylindrical, (3-)5-9(-14) cm long 6-12 floweres per cm. Bracts ovate, (3-)7-8 x 2 mm, tip attenuate. Pedicels 18-25 (-29 in fruit) mm long. Perianth clavate, (14-) 23-28 x 3-4 mm long, widest part 6-7 mm, orange to pink; outer segments free for 12-13 mm. Capsule cylindrical, woody and rough at maturity. Seed irregular to 3-sided, 4.5-8 mm in diameter, testa colour grey.

Aloe sinana is a narrow endemic only known from Northern Shewa and Southern part of Wello floristic regions in Ethiopia. The species was named after the locality where it was found in large numbers, namely Debre Sina (Shewa), a town 190 km NE of Addis Ababa. It occurs mainly in three small to medium sized populations at 10, 20 and 76-80 kms NE of Debre Sina. Some plants on mountain slopes between Kombolcha and Dessie (Wello) about 200 kms NE of Debresina are found growing with *A. camperi*. In the above localities the species grows on basaltic mountain slopes; often in areas of evergreen *Euclea-Rhus natalensis-Flueggea virosa* in scrubland. The altitude of occurrence ranges from 1250 to about 2120 m above sea level.

Individuals of *Aloe sinana*; photograph taken at 20 km from

Debre Sina towards Dessie. Alt.- 2119 m, Lat.- 39° 48’ E, Long.- 9° 52’ N.

10. *A. camperi* Schweinf.

- type: Eritrea West (EW), Ghinda, *Schweinfurth* 514a; Asmara, *Schweinfurth* 605; Arbashiko, *Schweinfurth* 668; Gheleb, *Schweinfurth* 1074; Acrur, *Schweinfurth* 1342, 1306 (all B syn. not seen).

Succulent shrub, stems erect or ascending (0.2-) 0.5-1 m long or decumbent to 2 m long, 6-10(-32) cm thick. Leaves crowded, (38-)40-60(-82) x (3-)5-8(-15) cm, recurved, slightly to deeply canaliculate dull to dark green or brownish; often spotted towards the base or on the lowered third to quarter of both surfaces; margin with 3-9 spines per 10 cm, 2.5-6 mm long, red with brown tip, dried sap colour deep brown. Inflorescence to 110 cm, branched; racemes 3-10(-14), cylindrical-conical, (3-)12-22 cm long, dense 8-12 floweres per cm. Bracts triangular-ovate, ovate-accuminate to almost triangular 2-8(-13) x (1-)2.5-4 mm. Pedicels (10-)12-25 mm long. Perianth clavate to subclavate, 17-27(-33) x 3-4 mm long, widest part 6-8 mm, yellow, orange, yellow-orange or scarlet; outer segments free for 7-16 mm. Capsule cylindrical, woody and rough at maturity. Seed irregular to 3-sided, 4.2-7.2 mm in diameter, testa colour grey to brown.

1. Raceme 3-10.5 cm long, Bracts 2-5(-7) mm long; perianth clavate, 17-22 mm long----------subsp ***camperi.***

2. Raceme 11-22 cm long, Bracts 7-12 mm long; perianth subclavate 23-27(-33) mm long----------subsp ***adigratana.***

Aloe camperi* Schweinf. subsp. *camperi

This subspecies has a relatively wide geographical range of distribution in the flora area. It occurs both in Ethiopia and Eritrea. In Ethiopia, it is known to occur in Wello and Tigray floristic regions. In Wello, the subspecies occurs abundantly near Woldia and as far South as near Dessie around Hayik town and 5 km of Dessie on the road to

Kombolcha. In Tigray, it was found in numbers at the northern foot of Mt. Amba Alage, near Alamata and Kobo in the rift valley. In Eritrea, it occurs in considerable numbers in many areas from Meschillit Pass, NE of Kern in the north, to the Ethiopian border in the south. The subspecies was common in the valley above Ghinda (i. e. the locality of one of the syntypes, to Nefasit following the mountain chain up to Asmara. It has further localities in Eritrea: near Gua Gorge, Adi Chaieh, on the Kohaito plateau (here with *A. percrassa* and *A. elegans*); and near Senafe (Reynolds, 1966). In general, the geographical range of distribution of *A. camperi* ssp. *camperi* streches from Kern in Eritrea to Dessie in Ethiopia more than 1000 km. It grows on rock slopes and sandy alluvial plain along the eastern escarpment of the NE African rift valley system from 550 to 2700 m.

Individuals of *Aloe camperi* subsp. *camperi*; photograph taken at 36 km from Maichew towards Alamata. Alt.- 1780 m, Lat.- 39° 38’ E, Long.- 12° 44’ N.

***Aloe camperi* subsp. *adigratana* (Reynolds) Fikre comb. et stat. nov.**

Basionym: *Aloe adigratana* Reynolds in *J. S. Afr. Bot.* **23**: 1 (1957). – type: Ethiopia, Tigray (TU), 10 km W of Adigrat, Reynolds 8076 (PRE, holo. not seen; K, iso.).

This subspecies has restricted distribution like the closely related *A. sinana*. It is known only from the Tigray floristic region around the town of Adigrat in Northern Ethiopia. It is found for 20 kms northward from Adigrat to the Eritrean boarder. It is seen in considerable numbers along the main road from Adigrat southward to Mekele

at 13 to 23 km; and also at 58 km near Agula and on rocky hills near Mekele (124 km south of Adigrat). West from Adigrat, a number of plants were found along the road for the first 10 km, at 23 km, 46 km and 85 km on slopy mountains to Adwa. In these localities the plants are found to grow in rocky places, mostly on sandstone basement complex. Altitudinal distribution range from 2100 to 2700 m.

Individuals of *Aloe camperi* subsp *adigratana*; photograph taken at 15 km from Adigrat towards Mekele. Alt.- 2650 m, Lat.- 39° 32’ E, Long.- 14° 11’ N.

7. References

Adams, S. P., Leitch, I. J., Bennett, M. D., Chase, M. W. & Leitic, A. R. (2000). Ribosomal DNA evolution and phylogeny in *Aloe* (Asphodelaceae). *American J. Bot.* **87**: 1578-1583.

APG (Angiosperm Phylogeny Group) (1998). An ordinal classification for the families of flowering plants. *Ann. Missouri Bot. Gard.* **85**: 531–553.

Atherton, P. (1995). First Aid Plant. Chemistry in Britain **34**(5): 33-36. London.

Bagley, M. J., Medrano, J. F. & Gall, G. A. E. (1997). Polymorphic molecular markers from anonymous nuclear DNA for genetic analysis of populations. *Molecular Ecology* **6**: 309-320.

Baker, J. G. (1880). A synopsis of Aloineae & Yuccoideae. *J. of Linn. Soc. London* **18**: 148-241.

Barringer, S. A., Mohammed-Yasseen, Y. & Splittstoesser, W. E. (1996). Micropropagation of endangered *Aloe juvenna* and *Aloe volkensii* (Aloaceae). *Haseltonia* **4**: 43-45.

Beaument, J., Cutler, D. F., Reynolds, T. & Vaughan, J. G. (1985). The secretory tissue of aloes and their allies. *Israel J. Bot.* **34**: 265-282.

Berger, A. (1908). Liliaceae-Asphodelaceae-Aloineae. In: Engler, A. (ed.), *Pflanzenreich* IV.38. III. Leipzig, Engelmann.

Brandham, P. E. (1971). The chromosome of Liliaceae: II. Polyploidy and karyotype variation in the Aloineae. *Kew Bull.* **25**: 381-339.

Brandham, P. E. (2004). The chromosome of *Aloe* - variation on a theme. In: Reynolds, T. (ed.), Aloes: The genus *Aloe*, pp 355-374. CRC Press, Boca Raton, London, New York, Washington, D.C.

Brandham, P. E. & Johnson, M. A. T. (1977). Population cytology of structural and numerical chromosome variants in the Aloineae (Liliaceae). *Plant Syst. Evol.* **128**: 105-122.

Brandham, P. E. & Cutler, D. F. (1978). Influence of chromosome variation on the organization of the leaf epidermis in a hybrid *Aloe* (Liliaceae). *Bot. J. Linn. Soc.* **7**: 1-6.

Brandham, P. E., Carter, S. & Reynolds, T. (1994). A multidisciplinary study of relationships among the cremnophilous aloes of north-eastern Africa. *Kew Bull.* **49**: 415-428.

Brandham, P. E. & Doherty, M. J. (1998). Genome size variations in the Aloeceae: an angiosperm family displaying karyotypic orthoselection. *Ann.Bot.* **82**: 67-73.

Brochmann, C., Soltis, D. E., & Soltis, P. S. (1992). Recurrent formation and polyphyly of Nordic polyploids in *Draba* (Brassicaceae). *Amer. J. Bot* **79**: 673-688.

Capaso, F., Borrelli, F. Capasso, R., & Izzo, A. (1998). Aloe and its threpuetic use. *Phytotherapy Research* **12**: 124-127.

Carter, S. (1994). Aloaceae. In: Polhill, R. M. (ed.) *Flora of Tropical East Africa.* Balkema.

Carter, S. (2001). Aloaceae. In: Pope, G. (ed.) *Flora Zambesiaca*, 12(3): 48-98. Royal Botanic Gardens, Kew. London.

Caswell, H. (1998). Matrix population model: construction, analysis and interpretation. Sinauer, Sunderland, Massachusets.

Court, D. (1981). Succulent Flora of South Africa. Academic Press, London.

Cox, A. V., Bennett, S. T., Parokonny, A. S., Kenton, A., Callimassia, M. A. and Bennett, M. D. (1993). Comparison of plant telomere locations using a PCR-genereted synthetic probe. *Annals of Botany* **72**: 239-347.

Crawley, M. J. (1997). Plant Ecology (2nd ed.). Blackwell Scientific Publication. Oxford.

Crawley, M. J. & Nachapong, M. (1985). The establishment of seedlings from primary and re-growth seeds of Ragwort (*Senceio jacobea*). *J. of Ecol.* **73**: 255-261

Cronquist, A. (1981). An integrated system of classification of flowering plants, pp. 1215. Columbia University Press. New York.

Cronquist, A. (1988). The evolution and classification of flowering plants, second edn., pp. 485, 516. New York Botanical Gardens, New York.

Crosswhite, F. S. and Crosswhite, C. D. (1984). *Aloe vera*, plant symbolism and threshing floor. *Desert Plants* **6**: 43-50.

Cutler, D. F. (1969). Cuticular markings in and other epidermal features in Aloe leaves. Notes from the Joderll Laboratory **6**: 21-27. London.

Cutler, D. F. (1979). Leaf surface studies in Aloes and Haworthia species (Liliaceae): taxonomic implications. *Tropisch und subtropisch Pflanzenwelt* **28**: 1-29

Cutler, D. F. (1982). Cuticular sculpturing and habitat in certain *Aloe* species (Liliaceae) from Southern Africa. In: Cutler, D. F., Alvin, K. L. and Price, C. E. (ed.), The plant cuticle, pp. 425-444. Academic Press. London.

Cutler, D. F. (2004). Aloe leaf anatomy. In: Reynolds, T. (ed.), Aloes: The genus *Aloe*, pp. 361-374, CRC Press, Boca Raton, London, New York, and Washington, D.C.

Cutler, D. F., Brandham, P. E. & Carter, S. J. (1980). Morphological, anatomical, cytological and biological aspects of evolution in East African shrubby species of *Aloe* L. (Liliaceae). *Botl J. Linn. Soc.* **80**(4): 293-317.

Dahlgren, R. M. T., Clifford, H. T. & Yeo, P. F. (1985). The Family of the monocotyledons: structure, evolution and taxonomy. Springer-Verlag. Berlin.

Demetrius, L. (1975). Reproductive strategies and natural selection. *American Naturalist* **109**: 243-249.

Dolye, J. J. & Dolye, J. L. (1990). Isolation of plant DNA from fresh tissue. *Focus* **12**: 13-15.

Drummond, R. B., Gelfand, M. and Mavi, S. (1975). Medicinal and other uses of succulents by the Rhodesian African. *Excelsa* **5**: 51-56.

Ermias Dagne, Abiy Yensew, Senait Asmellash, Sebsebe Demissew, and Mavi, S. (1994). Anthraquinones, pre-anthraquinones and isoleutherol in the roots of *Aloe* species. *Phytochemistry* **35**: 401-406.

Ermias Dange (1996). Review of the chemistry of aloes of Africa. *Bull. Chem. Soc. Ethiop* **10**(1): 89-103.

Fay, M. F., Gratton, J., and Atkinson, P. J. (1995). Tissue culture of succulent plants- an annotated bibliography. *Braddleya* **13**: 38-42.

Fay, M. F.; Cowan, S. R. & Leitch, J. I. (2005). The Effects of Nuclear DNA Content (C-value) on the Quality and Utility of AFLP Fingerprints. *Ann. Bot.* 95(1):237-246

Fikre Dessalegn (1999). Comparative study in population structure, reproductive biology and chromosome cytology of two endemic *Aloe* species: *Aloe debrana* Christian and *A. pulcherrima* Gilbert & Sebsebe in Ethiopia. M.Sc. Thesis, Addis Ababa University, Ethiopia.

Frankham, R. (2003). Genetics and conservation biology. *Comptes Rendus Biologies* **326**: 22-29.

Friis, I. Edwards, S., Ensermu Kelbesa & Sebsebe Demissew (2001). Diversity and endemism in the Flora of Ethiopia and Eriteria - what do the published Flora volume tell us? *Biol. Skr.* **54**: 173-193.

Glen, H. F. & Hardy, D. S. (2000). Aloaceae. In: Germishuizen, G. (ed.), Flora of southern Africa. Vol. 5, pp. 1-167. National Botanical Institute, Pretoria.

Gilbert, M. G. & Sebsebe Demissew (1992). Notes on the genus *Aloe* L. in Ethiopia: misinterpreated taxa. *Kew Bull.* **47**: 647-653.

Gilbert, M. G. & Sebsebe Demissew (1997). Further notes on the genus *Aloe* L. in Ethiopia and Eritrea. *Kew Bull.* **52**: 139-152.

Groom, D. & Reynolds, T. (1987). The *Aloe vera* phenomenon: a review of the properties and modern uses of the leap parenchyma gel. *J. Ethnopharmacology* **16**: 117-151.

Hamaric, J. L. & Godt, M. J. W. (1990). Allozyme diversity in plant species. In: Brown, A. H. D., Clegg, M. T., Kahler, A. L. and Weir, B. S. (eds.), Plant population genetics, breeding and genetic resources, pp. 43-64. Sinauer Associates. Sunderland

Hara, T. (1984). A stochastic model and the moment dynamics of the growth and size distribution in plant population. *J. Biol*. **109**: 173-190.

Harada, Y. & Iwasa, Y. (1996). Analysis of spatial pattern and population processes of clonal plants. *Res. Population Ecology* **38**: 153-164.

Harper, J. L., & White, J. (1974). The demography of plants: a review. *Ecol. & Syst.* **5**: 419-463.

Harper, J. L. (1977). Population Biology of Plants. Academic Press. London.

Harper, J. L. (1982). The plant community as working mechanism. In: Newman, E. I. (ed.) After Description, pp 11-25. Blackwell Scientific Publications. Oxford.

Hartnett, D. C. & Bazzaz, F. A. (1985). The genets and ramets population dynamics of *Solidago canadensis* in an abandoned field. *J. Ecol*. **73**: 407-413.

Hendrix, S. D. & Sum, I. F. (1989). Interspecific and itraspecific variation in seed mass in seven species of Umbellifers. *New phytol.* **112**: 445-451.

Hennessee, O. M. (1998). Some history about *Aloe vera*. Available from: http://www.aloe-vera.com/aloel.html. Accessed, 1998, Oct. 31.

Hennig, W. (1966). *Phylogenetic systematics.* University of Illinois Press, Urbana. 263 pp.

Holland, P.G (1978). An evolutionary biogeography of the genus *Aloe*. *J. Biogeography* **5**: 213-226.

Hutchinson, J. (1959). The families of flowering plants, Vol. 2, Monocotyledons, (2nd edn.), pp. 601-602. Clarendon Press. Oxford.

Hutchings, A. (1989a). A survey and analysis of traditional medicinal plants as used by the Zulu and Sotho. *Bothalia* **19**: 111-123.

Hutchings, A. (1989b). Observations on plant usage in Zulu and Sotho medicine. *Bothalia* **19**: 225-235.

Hutchings, M. J. (1985). Plant Population Biology. In: Moore, P. D. and Champman, S. B. (ed.), Introduction to plant ecology, pp. 377-435, Blackwell Scientific Publications. Oxford.

Hutchings, M. J. (1997). The Structure of Plant Population. In: Crawley, M. J. (ed.), Plant Ecology, pp. 325-358. Blackwell Scientific Publications. Oxford.

Institute of Biodiversity Conservation (IBC) (2004). Biodiversity Strategy and Action Plan, pp. 25-26. The Federal Demographic Republic of Ethiopia. Addis Ababa.

Johnson, A. T. & Smith, H. A. (1982). Plant names simplified. Their pronunciation, derivation and meaning. Buckenhill, Bromyard.

Judd, W. S., Campbell, S. C., and Stevens, P. F. (1999). Plant Systematic. A phylogenetic approach. Sinaure Associates, Inc., Sunderland, Massachusetts, USA.

Klinkhamer, P. G. L., Jong, T. D. and Meelis, E. (1987). Delay in flowering in the biennial *Cirsium vulgare*: Size effect and deveneralization. *Oikos* **49**: 303-308.

Lande, R. (1995). Mutation and conservation. *Conserv. Biol.* **9**: 782-791.

Lande, R. (1998). Genetics and demography in biological conservation. *Science* **241**: 1455-1460.

Lavranos, J. (1995). Aloaceae. In: Thulin, M. (ed.) *Flora of Somalia.* Vol. 4., pp. 35-42. Royal Botanic Gardens, Kew.

Linnaeus, C. (1953). *Species Plantarum*. Stockholm.

Marcussen, T. & Borgen, L. (2000). Allozymic variation and relationships within *Viola* subsection *Viola* (Violaceae). *Plant Syst. Evo* **223**(1-2): 29-57.

Mascola, N., Izzo, A., Borrelli, F. and Capasso, R. (2004). Healing powers of aloes. In: Reynolds, T. (ed.), Aloes: The genus *Aloe*, pp. 210-240, CRC Press. Boca Raton, London, New York, and Washington, D.C.

Mishler, B. D. (2000). Deep phylogenetic relationships among “plants” and their implications for classification. *Taxon* **49**: 661–683.

Morton, J. F. (1961). Folk uses and commercial exploitation of Aloe leaf pulp. *Economic Botany* **15**: 311-319.

Muller, U. G. & Wolfenbager, L. L. (1990). AFLP genotyping and fingerprinting. *TREE* **14**: 389-394.

Nash, R. J., Beaumont, J., Veitch, N. C., Reynolds, T., Benner, J., Hughes, C. N.G., Dring, J.V., Bennett, R. N. & Dellar, J. E. (1992). Phenylethylamine and piperidine alkaloids in *Aloe* species. *Planta Medica* **58**: 84-87.

Nei, M. & Li, W. H. (1979). Mathematical model for studying genetic variation interms of restriction endonucleases. Proceedings of the National Academy of Science (USA) **76**: 5269-5273.

Nelson G., Murphy, J. D., & Ladiges, Y. P. (2003). Brummitt on paraphyly: a response. *Taxon* **52**: 295-298.

Newman, D. & Pison, D. (1997). Increased probability of extinction due to decreased genetic effective populations of *Clarkia pulchella*. *Evolution* **51**: 354-362.

Newton, L. E (1972). Taxonomic use of the cuticular surface features in the genus *Aloe* (Liliaceae). *Bot. J. Linn. Soc.* **65**: 335-339.

Newton, L. E (1994). Exploitation and conservation of Aloe in Kenya. Proceedings of the XIIIth Plenary Meeting of A.E.T.F.A.T., Malawi 1: 219-222.

Newton, L. E. (2001). *Aloe*. In: Eggli, U. (ed.), Illustrated handbook of succulent plants: Monocotyledons, pp. 103-186. Springer Verlag, Berlin, Heidelberg, and New York.

Newton, L. E. (2004). Aloes in habitat. In: Reynolds, T. (ed.), Aloes: The genus *Aloe*, pp 1-14, CRC Press, Boca Raton, London, New York, and Washington, D.C.

Newton, D. J. & Vaughan, H. (1996). Soth Africa's *Aloe ferox* plant, parts and derivatives industry, pp 61. TRAFFIC Southern Africa, Johannesburg.

Nordal, I. & Stedje, B. (2005). Paraphyletic taxa should be accepted. *Taxon* **54**: 54-55.

Oborny, B. (1994). Growth rules in clonal plants and predictability of the environment: a simulation study. *J. Ecol.* **82**: 341-351.

Oldfield, S. (ed.). (1997). Status survey and conservation action plan. Cactus and succulent plants. 10: 214, IUCN, Gland.

Oldfield, S., Lusty, C. & MacKinven, A. (1998). The World list of threatened Trees. World Conservation Press. IUCN, Gland.

Oostermeijer, J., Luijten, S., and den Nijs, J. (2003). Integrating demographic and genetic approaches in plant conservation. *Biological Conservation* **113**: 389-398.

Pujol, J. (1990). Nature Africa. The herbalist handbook: African flora medicinal plants. Durban.

Rabotnov, T. A. (1969). Life cycles of perennial herbage plants meadow communities. *Bot. Ins. Acad. Science Series* **3**(6): 20-26

Ralls, K. & Ballou, J. (1983). Extinction: Lessons from zoos. In: Chambers, B. and Thomas, L. (ed.) Genetics and Conservation: a reference for managing wild animals and plant populations, pp. 164-184. Cummings, Menlo Park, CA.

Raymond M. & Rouset F. (2003). GENEPOP (Version 3.4): population genetics software for exact tests and ecumenicism. *J. Heredity,* **86**: 248-249

Reynolds, G. W. (1950). Aloes of South Africa. Aloes Book Fund, Johannsburg.

Reynolds, G. W. (1954). Notes on the aloes of southern Ethiopia and Somalia. *J. East Af. Nat. Hist. Soc.* **22**: 102-104.

Reynolds, G. W. (1966). The aloes of Tropical Africa and Madagascar. The Aloes Book Fund, Mbabane.

Reynolds, G. W. (1982). The aloes of South Africa, (ed. 4) Balkema, Cape Town.

Reynolds, T. (1985 a). The compounds in Aloe leaf exudates: a review. *Bot. J. Linn. Soc.* **90**: 157-177.

Reynolds, T. (1985 b). Observations on the phytochemistry of the Aloe leaf-exudate compounds. *Bot. J. Linn. Soc.* **90**: 179-199.

Rohlf, F. J. (2000). NTSYSpc: Numerical Taxonomy and Multivariate Aanalysis System, version 2.11f. Exeter software, New York, USA.

Ross, M. A. & Harper, J. L. (1972). Occupation of biological space during seedling establishment. *J. Ecol.* **60**: 77-88.

Sebsebe Demissew (1996). Botanical aspects of Aloes of North East Africa. *Bull. Chem. Soc. Ethiopia* **10**(1): 74-88.

Sebsebe Demissew & Gilbert, M. G. (1997). The family Aloaceae. In: Edwards, S., Sebsebe Demissew and Hedberg, I. (ed.), *Flora of Ethiopia and Eritrea*. Vol 6 Addis Ababa and Uppsala.

Sebsebe Demissew & Dioli, M. (2000). A new *Aloe* (Aloaceae) species from Ogaden (Southeastern Ethiopia). *Kew Bull.* **55**: 679-682.

Sebsebe Demissew & Gilbert, M. G. (2000). A new *Aloe* (Aloaceae) species from (Southwest Ethiopia). *Kew Bull.* **55**: 683-686.

Sebsebe Demissew, Nordal, I. & Stabbetorp, O. E. (2001). Endemism and patterns of distribution of the genus *Aloe* (Aloaceae) in the FEE. *Biol. Skr.* **54**: 194-203.

Sebsebe Demissew, Nordal, I. & Stabbetorp, O.E. (2003). Flowers of Ethiopia: Aloes and other Lilies. Shama Books. Addis Ababa, Ethiopia.

Shelton, R.M. (1996). *Aloe vera.* Its chemical and therapeutic properties. *Intern. J. of Dermatology* **30**: 679-683.

Silvertown, J. W. (1982). Introduction to Plant Ecology. Longman. London and New York.

Smith G. F. & Wyk, B. E. van (1991). Generic relationships in the Alooideae (Asphodelaceae). *Taxon* **40**: 557-582.

Smith, G. F. (1993). Familial orthography: Aloeaceae vs. Aloaceae. *Taxon* **42**: 87-90

Smith G. F. & Swartz, P. (1999). Re-establishing *Aloe suzannae* in Madagascar. *Brit. Succ. Jour.* **17**: 45-49.

Smith, G. F. & Steyn, E. M. A. (2004). Taxonomy of Aloaceae. In: Reynolds, T. (ed.), Aloes: The genus *Aloe*, pp. 15-30, CRC Press. Boca Raton, London, New York, and Washington, D.C.

Sokal, R. R. & Sneath, P. H. A. 1963. *Principles of numerical taxonomy.* W. H. Freeman and campany, San Francisco. 359 pp.

Soltis, D.E. & Soltis, P.E. (1989). Isoenzyme in Plant Biology. Disoscorides Press, Portlands.

Stace, C. A. (1980). Plant taxonomy and biosystematics. Edward Arnold, London

Steyn, E. M. A., Smith, G. F., Nilsson, S. & Grafstrom, E. (1998). Pollen morphology in *Aloe* (Aloaceae). *Grana* **37**: 23-27.

Stearn, W.T. (1983). Botanical Latin (3rd ed.). Newton Abbot.

Stokes, C. J. & Yeaton, R. I. (1995). Population dynamics, pollination ecology. and the significance of plant height in *Aloe candelabrum*. Afr. J. Ecol. **33**:101–113.

Stuessey, T. F. (1990). Plant taxonomy. Columbia University Press. New York.

Stevens, P. F. (2000). Botanical systematics, 1950–2000: change, progress, or both? – *Taxon* **49**: 635–659.

Swanson, L.N. (1995). Therapeutic value of *Aloe vera*. *US Pharmacy* **20**: 26-35.

Tewolde-Brehan Gebre Egzabher (1991). Diversity of Ethiopian Flora. In Engles, J. M. M., Hawkes, J. G. and Melaku Worede (ed.), Plant genetic resources of Ethiopia. Pp.75-81 Cambrige University Press, Cambrige.

The Secretariat of CBD (2002). The global strategy for plant conservation (GSPC), Decision vi/9. of the COPs to the convention, CBD and UNEP, Montereal, Canada. Website: http//www/biodiv/org.

Van der Bank, F.H., van Wyk, B.E. & van Der Bank, M. (1995): Genetic variation in two economically important *Aloe* species (Aloaceae). *Biochem. Syst. and Eco.* **23**: 251-256.

Van der Bank, F.H. & van Wyk, B.E. (1996). Biochemical genetic markers to identify hybrids between *Aloe arborescens* and *A. ferox* (Aloaceae). *S. Afr. J. Bot.* **62**: 328-331.

Van de Peer, Y. & De Watcher, R. (1994). TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. *Comput. Applic. Biosci*. **10**: 569-570.

Viljoen, A. M. & van Wyk, B. E. (1996). The evolution of aloes: new clues from their leaf chemistry. *Aloe* **33**: 30-33.

Viljoen, A. M., van Wyk, B.E., van Der Bank, H., Smith G. F. & van Der Bank, M. (1996). A chemotaxonomical and biochemical evaluation of the identity of the *Aloe candelabrum* (Aloaceae). *Taxon* **45:** 461-471.

Viljoen, A. M., van Wyk, B. E. and Newton, L. E. (1999). Plicataloside in Aloe- a chemotaxonomic appraisal. *Biochem. Syst. Eco* **27**: 507-517.

Viljoen, A. M., van Wyk, B. E. and Newton, L. E. (2001). The occurrence and taxonomic distribution of the anthrones aloin, aloinoside and microdontin. *Biochem. Syst. Eco* **29**: 53-67.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van de Lee, T., Hornes, M. Frijters, A., Pot, J., Pelemen, J., Kuiper, M., Zabeau, M. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* **23**: 4407-4414.

Wabuyele, E. (2000). A systematic assessment of the relationships between *Aloe macrocarpa* Tod. and *A. lateritia* Engl. M.Sc. Thesis, Addis Ababa University.

Watkinson, A. R. (1997). Plant population dynamics. In: Crawley, M. J. (ed.), Plant ecology. pp 359-400. Blackwell Scientific Publication. Oxford.

Watt, J. M., & Breyer-Brandwijk, M. G. (1962). The medicinal and poisonous plants of Southern and Eastern Africa, (2nd edn.), pp. 1457. Livingstone, Edinburg and London.

Weiner, J. (1993). Competition, herbivory and plant size variability in *Hypochaeris radicata* grazed by snails (*Helix aspera*). *Fun. Ecol.* **7**: 47-53.

Weiner, J. & Solbrig, O. T. (1984). The meaning and measurement of size hierarchies in plant population. *Oecologia* **61**: 334-336.

Weir, B. S. and Cockerham, C. C. (1984). Estimating *F*-statistics for the analysis of population structure. *Evolution* **38**:1358-1370.

Werner, P. A. & Caswell, H. (1977). Population growth rates and age vs. stage-distribution models for Teasel (*Dipsacus sylvestris* Hids) *Ecol.* **58**: 1103-1111.

Wendel, J. F. & Weeden, N. F. (1989). Genetics of plant izoenzymes. In: Soltis, D.E. and Soltis, P.E. (eds.), Izoenzymes in plant biology, pp 66-72. Disoscorides Press, Portland.

Willert, D. J., von Eller, B.M., Werger, M. J. A., Brinckmann, E. and Ihlenfeldt, H.-D (1992). Life strategies of succulents in deserts with special reference to the Namib Desert. xix, pp. 340 . Cambridge: Cambridge University Press.

Wright, S. (1965). The interpretation of genetic population structure by *F*-statistics with special regard to systems of mating. *Evolution* **19:** 395-420.

Zonneveld, J. M. (2002). Genome size analysis of selected species of *Aloe* (Aloaceae) reveals the most primitive species and results in some new combinations. *Bradleya* **20**: 5-12.

Appendices

Appendix 1: Herbarium specimens examined of the studied *Aloe species* (Source: National Herbarium (ETH), Ethiopia and Kew Herbarium (Kew), UK

Taxon	Coll. and N0.	Floristic Region	Alt.(m)/ft.	Lat.	Long.	Source

1. *A. camperi*	G. Schweinfurth 19	Eritrea	-	-	-	Kew
	G. W. Reynolds 8071	Eritrea	8200 ft	-	-	Kew
	G.W. Reynolds 8051	Eritrea	5200 ft	39° 04’ E	15° 23’ N	Kew
	G. W.Reynolds 8036	Eritrea	7600 ft	-	-	Kew
	Bally 6686	Eritrea	1800 m	-	-	Kew
	G.W.Reynolds 8105	Wello	5700 ft	-	-	Kew
	G. Schweinfurth 156	Eritrea	2600 m	-	-	Kew
	G. Schweinfurth 1350	Eritrea	1900 m	-	-	Kew
	G. Schweinfurth 155	Eritrea	1075 m	-	-	Kew
	G. Schweinfurth 1797	Eritrea	1900 m	-	-	Kew
	Sebsebe D. 2214	Eritrea	1700 m	-	-	Kew
	O. Ryding 1390	Eritrea	2400 m	-	-	Kew
	J. Cooper	-	-	-	-	Kew
	Sebsebe D. 2352	Wello	2400 m	-	-	Kew
	G. Schweinfurth 154	Eritrea	2700 m	-	-	Kew
	G. Popov 1380	Eritrea	2500 m	-	-	Kew
	Sebsebe D. 2971	Wello	2450 m	39° 58’ E	10° 05’ N	ETH
	Sebsebe D. 543	Wello	2400 m	-	-	ETH
	Sebsebe D. *et al.* 5734	Wello	2450 m	-	-	ETH
	Tewolde B.G.E. 28	Tigray	7400ft	-	-	ETH
	Sebsebe D. 46 43	Wello	1940 m	-	-	ETH
	P. R. O. Bally 6686	Eritrea	4500ft	-	-	ETH
	Sebsebe D. 2213	Eritrea	2250 m	-	-	ETH
	H.F.Mooney 9536	Eritrea	1250 m	-	-	ETH
	Getachew A. 3263	Wello	2000 m	-	-	ETH
	Sebsebe D 3192	Wello	2100 m	-	-	ETH
	Sebsebe D *et al.* 2352	Wello	2400 m	39° 47’ E	11° 07’ N	ETH
	Sebsebe D. *et al.* 2904	Wello	2440 m	38° 42’ E	9° 2’ N	ETH
	Sebsebe D. *et al.* 2214	Eritrea	1700 m	-	-	ETH
2.*A. sinana*	Sebsebe D. *et al.* 5188	Shewa	2100 m	-	-	ETH
	Sebsebe D. *et al.* 5187	Shewa	1620 m	-	-	ETH
	Sebsebe D. 4659	Shewa	-	-	-	ETH
	Getachew A. *et al.* 285	Shewa	1700 m	-	-	ETH
	K. Hildebrandt 284	Shewa	1000 ft	-	-	ETH
	Sebsebe D. *et al.* 2210	Shewa	1700 m	-	-	ETH
	G.W. Reynolds 8127	Shewa	4700 ft	-	-	Kew
	M. G. Gilbert 2323	Shewa	-	39° 49’ E	9° 50’ N	Kew
	J. Ash 1810	Shewa	1250 m	39° 47’ E	9° 50’ N	Kew
3. *A. adigratana*	Sebsebe D. *et al.* 3190	Tigray	2670 m	-	-	ETH
	J. Ash 1815	Tigray	2200 m	-	-	Kew
	G. W. Reynolds 8073	Tigray	7900 ft	39° 25’ E	14° 21’ N	Kew
	G. W. Reynolds 8076	Tigray	9000 ft	39° 23’ E	14° 21’ N	Kew

4. *A. calidophila*	Sebsebe D. *et al.* 2884	Sidamo	1450 m	38° 30’ E	3°55’ N	ETH
	Sebsebe D. *et al.* 2198	Sidamo	1500 m	-	-	ETH
	Getachew A. *et al.* 1507	G.Gofa	-	-	-	ETH
	Sebsebe D. 2941	Sidamo	550 m	35° 45’ E	5° 22’ N	ETH
	Sebsebe D. *et al.* 2197	Sidamo	1300 m	-	-	ETH
	Sebsebe D. *et al.* 2177	Sidamo	1710 m	39° 40’ E	5° 16’ N	ETH
	Sebsebe D. 2165	Sidamo	1620 m	-	-	ETH
	Sebsebe D. *et al.* 4213	Sidamo	1700 m	-	-	ETH
	H.F. Mooney 9772	Sidamo	1200 m	38° 10’ E	4° 30’ N	ETH
	G. W. Reynolds 7029	Sidamo	4400 ft	38° 35’ E	3° 53’ N	Kew
	J. B. Gillett 12944	Kenya	1080 m	39° 03’ E	3° 30’ N	Kew
	J. B. Gillett 13393	Kenya	360 m	40° 56’ E	2° 49’ N	Kew
5. *A. gilibertii*	M.G. Gilibert 1065	Shewa	-	-	-	ETH
	Friis *et al.*. 8222	Shewa	1700 m	38° 41’ E	7° 28’N	ETH
	Sebsebe D. *et al.* 2207	Sidamo	1850 m	-	-	ETH
	Friis *et al.* 1097	Shewa	1600 m	38° 41’ E	7° 28’ N	ETH
	D.A. Petelin 158	Shewa	1700 m	38° 42’ E	7° 20’ N	ETH
	Mesfin T. 502	Shewa	1970 m	-	-	ETH
	M.G. Gilbert 1097	Shewa	1600 m	38° 42’ E	7° 20’ N	ETH
	Friis *et al.* 9614	G. Gofa	850 m	36° 41’ E	4° 50’ N	Kew
	Friis *et al.* 8931	G. Gofa	600 m	36° 41’ E	4° 50’ N	Kew
	P. Brandham 1815	Shewa	1500 m	-	-	Kew
	W. J. J.O. dewilde 7063	Shewa	1500 m	-	-	Kew
	P. Brandham 1852	Shewa	-	-	-	Kew
	F.G. Meyer 8752	Shewa	5505 ft	38° 29’ E	7° 07’ N	Kew
	Friis *et al.* 1097	Shewa	1600 m	38° 29’ E	7° 28’ N	Kew
	M.G. Gilbert 1065	Shewa	-	-	-	Kew
	Friis *et al.* 8222	Shewa	1700 m	38° 41’ E	7° 28’ N	Kew
	M.G. Gilbert 9104	G. Gofa	1220 m	36° 35’ E	4° 52’ N	Kew
	J. Ash 2285	G. Gofa	1600 m	38° 06’ E	4° 50’ N	Kew
	J. Ash 2305	G. Gofa	1700 m	38° 45’ E	7° 35’ N	Kew
	J. Ash 2746	Shewa	6000 ft	38° 03’ E	7° 30’ N	Kew
6. *A. megalacantha*	Sebsebe D. *et al.* 2344	Harerghe	2100	41° 30’ E	9° 17’ N	ETH
	M.G. Gilbert 4071-80	Harerghe	2150	-	-	ETH
	M.G. Gilbert *et al.* 2263	Harerghe	1700	41° 55’ E	9° 30’ N	ETH
	Sebsebe D. *et al.* 2282	Harerghe	1830	40° 52’ E	9° 06’ N	ETH
	I. Friis *et al.*. 5763	Harerghe	1820	40° 36’ E	7° 00’ N	ETH
	J.J. Bos 1538	Harerghe	1200	-	-	ETH
	Berhanu M. A. 70	Harerghe	-	-	-	ETH
	Mr. X.	Harerghe	-	-	-	ETH
	W. Burger 2101	Harerghe	-	-	-	ETH
	I. Friis *et al.* 3708	Bale	1650	40° 41’ E	6° 58’ N	ETH
	M. Gilbert *et al.* 9104	G. Gofa	1220	36° 35’ E	4° 52’ N	Kew
	W. Burger 488	Harerghe	1100 m	-	-	Kew

	W. Burger 487	Harerghe	1100 m	-	-	Kew
	W. Burger 451	Harerghe	1700 m	-	-	Kew
	A.G. Mchoughlin 825	Harerghe	-	-	-	Kew
	G.W. Reynolds 8155	Harerghe	3000 ft	-	-	Kew
	W. Burger 2068	Harerghe	1520 m	42° 23’ E	9° 4’ N	Kew
	I. Friis *et al.* 3708	Bale	1650 m	40° 41’ E	6° 58’ N	Kew
	J. Ash 2555	Harerghe	-	-	-	Kew
	M.G. Gilbert 4080	Harerghe	2150 m	-	-	Kew
	W. Burger 3164	Harerghe	-	41° 50’ E	9° 15’ N	Kew
	E. Westphal 862	Harerghe	2200 m	41° 40’ E	9° 28’ N	Kew
	G. W. Reynolds 8332	Harerghe	3500 ft	-	-	Kew
	C.F. Hemming 1901	Harerghe	5300 ft	-	-	Kew
	P.R.O.Bally& Melville 16115	Somalia	1480 m	-	-	Kew
	P.R.O.Bally& Melville 16189	Somalia	-	-	-	Kew
	J.B. Gillett 3902	Somalia	4400 ft	-	-	Kew
	G.W. Reynolds 6244	Somalia	-	-	-	Kew
	J.B. Gillett 2841	Somalia	1250 m	44° 19’ E	9° 41’ N	Kew
7. *A. yavellana*	I. Friis *et al* .8570	Sidamo	1650 m	38° 46’ E	4° 30’ N	ETH
	Sebsebe D. *et al.* 3890	Sidamo	1905 m	38° 05’ E	4° 52’ N	ETH
	Sebsebe D. *et al.* 4218	Sidamo	1870 m	-	-	ETH
	H.F. Mooney 9867	Sidamo	1700 m	38° 05’ E	4° 53’ N	ETH
	G.W. Reynolds 7041	Sidamo	6300 ft	38° 20’ E	4° 05’ N	ETH
	P.R.O. Bally 7668	Sidamo	-	-	-	ETH
	P.R.O. Bally 9393	Sidamo	5300 ft	-	-	-
	G.W. Reynolds 7063	Sidamo	5600 ft	38° 06’ E	4° 55’ N	Kew
	I. Friis *et al.* 9340	Sidamo	1500 m	38° 20’ E	4° 48’ N	Kew
8. *A. percrassa*	Sebsebe D. 2989	Tigray	2180 m	39° 37’ E	12° 26’ N	ETH
	Tekle Hagos 95	Gonder	-	-	-	ETH
	M.G. Gilbert *et al.* 2313	Tigray	-	39° 35’ E	12° 55’ N	ETH
	Sue Edwards *et al* 5130	Tigray	2500 m	39° 31’ E	13° 56’ N	ETH
	Sue Edwards *et al* 5131	Tigray	2500 m	39° 31’ E	13° 56’ N	ETH
	Tewolde B.G.E. 1076	Tigray	2000 m	-	-	ETH
	Tewolde B.G.E. 1030	Tigray	1850 m	38° 54’ E	14° 06’ N	ETH
	Sebsebe D. 3025	Tigray	2270 m	39° 36’ E	12° 51’ N	ETH
	Sebsebe D. 3104	Tigray	2905 m	39° 26’ E	14° 18’ N	ETH
	Sebsebe D. 3020	Tigray	2930 m	39° 36’ E	12° 49’ N	ETH
	Mr. x (?)	Eritrea	1850 m	-	-	ETH
	G.W. Reynolds 8065	Eritrea	8300 ft	39° 16’E	14° 48’N	Kew
	G. Schweinfurth 152	Eritrea	2650 m	-	-	Kew
	G. Schweinfurth 722	Eritrea	2200 m	-	-	Kew
	Baker 7834	Eritrea	-	-	-	Kew
	G.W. Reynolds 8116	Tigray	9200 ft	-	-	Kew
	S.A. Robertson 1416	Eritrea	-	-	-	Kew

9. *A. debrana*	Sebsebe D. *et al.* 5732	Shewa	2450 m	-	-	ETH
	Sebsebe D. *et al.* 5733	Shewa	2450 m	-	-	ETH
	Sebsebe D. 4666	Shewa	2500 m	-	-	ETH
	Sebsebe D. *et al* 2209	Shewa	-	39° 35’ E	9° 47’ N	ETH
	Berhanu A.	Shewa	-	-	-	ETH
	H.F. Mooney 5769	Shewa	8000 ft	-	-	ETH
	Getachew A. 2794	Shewa	-	-	-	ETH
	Sebsebe D. *et al* 2903	Shewa	2750 m	38° 92’ E	9° 02’ N	ETH
	Sebsebe D. 5129	Shewa	2200 m	-	-	ETH
	Sebsebe D.2268	Shewa	2440 m	38° 45’ E	9° 02’ N	ETH
	Sebsebe D. 5142	Shewa	2650 m	-	-	ETH
	Sebsebe D. *et al* 5811	Wello	3200 m	-	-	ETH
	Sebsebe D. *et al* 2906	Shewa	-	-	-	ETH
	Mesfin T. *et al* 2866	Shewa	2650 m	-	-	ETH
	Afework K. 550	Shewa	2720 m	-	-	ETH
	Mesfin T. 7124	Wello	2450 m	-	-	ETH
	Sebsebe D. *et al* –	Shewa	2500 m	38° 16’ E	10° 20’ N	ETH
	J. Sutherland 320	-	2700 m	39° 37’ E	11° 07’ N	ETH
	Sebsebe D. 2216	Shewa	2440 m	38° 42’ E	9° 02’ N	ETH
	Sebsebe D. 2970	Wello	2450 m	39° 40’ E	11° 12’ N	ETH
	Sebsebe D. 4616	Wello	2720 m	-	-	ETH
	A.G. Mchloughlin 841	Shewa	-	-	-	Kew
	W.J.J.O. de Wilde 6098	Shewa	2500 m	-	-	Kew
	J. Ash 1853	Shewa	8000 ft	38° 41’ E	9° 21’ N	Kew
	E. Singer 6585	Shewa	5000 ft	-	-	Kew
	W.J.J.O. de Wilde 9772	Shewa	2000 m	-	-	Kew
	G.W. Reynolds 8135	Shewa	8900 ft	-	-	Kew
	J.Ash 1852	Shewa	8000 ft	38° 41’ E	9° 21’ N	Kew
	H. F. Mooney 5769	Shewa	8000 ft	-	-	Kew
	Sebsebe D. 2216	Shewa	2440 m	38° 42’ E	9° 21’ N	Kew
	Ensermu K. 2903	Shewa	2440 m	38° 42’ E	9° 02’ N	Kew
	Sebsebe D. 2268	Shewa	2440 m	38° 45’ E	9° 20’ N	Kew
	F.G. Myer 7606	Shewa	2530 m	38° 15’ E	10° 50’ N	Kew
	A.G. Mcloughlin 812	Shewa	-	-	-	Kew
	A.E. Sandford 1854	Shewa	8000 ft	-	-	Kew
	J.B. Giliett 14821	Shewa	2700 m	39° 10’ E	9° 15’ N	Kew
10. *A. harlana*	Gilbert *et al.* 2263	Harerghe	1700	41° 55’ E	9° 30’ N	ETH
	Sebsebe *et al* 2338	Harerghe	1720	41° 52’ E	9° 34’ N	ETH
	Sebsebe *et al* 2343	Harerghe	2100	41° 43’ E	9° 21’ N	ETH
	Sebsebe *et al* 2328	Harerghe	1500	42° 32’ E	9° 12’ N	ETH
	J. Ash 3330	Harerghe	1100 m	41° 52’ E	9 °35’ N	Kew
	J.B. Gillett 5272	Harerghe	6200 ft	41° 55’ E	9° 9’ N	Kew
	Bally 10076	Harerghe	1700 m	41° 55’ E	9° 30’ N	Kew
	G.W. Reynolds 8383	Harerghe	-	-	-	Kew

	G.W. Reynolds 8384	Harerghe	5500 ft	-	-	Kew
11. *A. monticola*	M.G. Gilibert 2311	Tigray	-	39° 35’ E	12° 57’ N	ETH
	Sebsebe D. 3010	Tigray	2500 m	39° 36’ E	12° 49’ N	ETH
	G.W. Reynolds 8117	Tigray	8200 ft	-	-	Kew
	M.G. Gilbert 2311	Tigray	-	39° 47’ E	12° 57’ N	Kew
	G.W. Reynolds 8118	Tigray	8200 ft	39° 47’ E	12° 41’ N	Kew

Appendix II: Morphological characters used for analyses.

a) Quantitative characters

A Stem:-

- Stem length (cm) - SL
- Stem diameter (cm) - SD

B Leaf:-

- Leaf length (cm) - LL
- Leaf diameter (cm) - LD
- Marginal teeth/10 cm - MT
- Marginal teeth size (cm) - MTS

C Inflorescence:-

- Inflorescence length (cm) - INL
- Inflorescence number/individual - INN
- Raceme number - RN
- Raceme length (cm) - RL
- Raceme width (cm) - RW

D Flower:-

- Perianth length (cm) - PL
- Pedicel length (cm) - Pd.L
- Bract length (cm) - BL
- Bract width (cm) - BW

E Fruit:-

- Fruit length (cm) - FL

F Seed:-

- Seed diameter (mm) - SD

G Seedling:-

- Number of leaf developed per month - NL
- Length of largest leaf of three month's seedlings (cm) - LLL
- Number germinated per month - NG

a) Qualitative characters

Character		State	Code
1. **Habit**:			
	A	Herb - (HE)	0
		Shrub - (SH)	1
	B	Acaulesent - (ACA)	0
		Caulesent - (CA)	1
	C	Solitary - (SO)	0
		Exist in Cluster – (CL)	1
2. **Leaf**:			
	Appreance - (LA)	Erect & spreading	0
		Slightly curved & canaliculated	1
		Deeply curved & canaliculated	2
	Colour – (LC)	Light/dull green	0
		Grey green	1
		Dark/glacuose green	2
		Gloosy/olive green	3
		Brownish/purple green	4
	Spot/maculation - (MA)	Absent	0
		Present	1
	Mariginal teeth colour - (MTC)	Brown	0
		Brown with white tip	1

		White with brown tip	2
	Dried sap colour - (DSC)	Deep brown	0
		Brown	1
		Yellow	2
3.	**Infloresence:**		
	Level of branching - (ILB)	First degree compound	0
		Second degree compound	1
	Raceme shape - (RS)	Cylinderical/sub cylindrical	0
		Conical/sub conical	1
		Capitate/ sub capitate	2
	Floral density/cm – (FD)	Dense (> 8)	0
		Sub dense (4-8)	1
		Lax (</= 3)	2
4.	**Flower:**		
	Colour - (FC)	Orange	0
		Yellow to orange	1
		Yellow	2
		Light red (scarlet)	3
		Deep red	4
	Perianth shape - (PS)	Clavate/sub clavate	0
		Cylindrical	1
		Cylindrical-triagonous	2
	Bract shape & tip - (BS)	Ovate-accuminate	0
		Ovate-acute	1
		Ovate-attiunated	2
		Triangiular-accuminate	3
		Triangular-acute	4
		Lanceolate	5
5.	**Fruit:**		
	Shape - (FS)	Oblong	0
		Cylinderical	1
		Cylinderical to oblong	2
		Oval to oblong	3
	Texture - (FT)	Woody & rough	0

	Woody & smooth	1
	Papery	2

6. **Seed:**

Testa colour - (TC)	Black	0
	Grey	1
	Brown	2
Shape - (SS)	Irregular to 3-sided	0
	Flat to 3-sided	1
	Irregular	2
	Flat	3

7 Seedling:

Leaf apex - (LA)	Cuspidate	0
	Acute	1
Leaf colour - (LC)	Dark green	0
	Light green	1

Appendix III: Data matrix for combined morphological (quantitative and qualitative) data set

Appendix IV Sources of fresh materials, fruits and seeds for taxonomic data

Taxon	*Floristic region/Province (s)*	*Population/Locality*	*Location*
1. *A. adigratana*	Tigray	1**. *Adigrat A:** 3-20 km West of Adigrat towards Adwa	Alt.- 2500 m Lat. 39 26 E Long.- 14 16 N
		2**. Adigrat B**: 3-15 km form Adigrat towards Mekele	Alt.- 2650 m Lat.- 39 32 E Long.- 14 11 N
2. *A. camperi*	Tigray and Wello	1**.Meionhi:** 23- 36 km from Maichew towards Alamata	Alt.- 1780 m Lat.- 39 38 E Long.- 12 44 N
		2. **Kobo**: 24 km from Kobo towards Dessie	Alt.- 1730 m Lat.- 39 39 E Long.- 11 58 N
3. *A. sinana*	Shewa	1. *** Debresina:** 10- 20 km from Debresina towards Dessie	Alt.- 2119 m Lat.- 39 48 E
		2. **Efeson:** 76 km from Debresina (near Efeson) towards Dessie	Long.-9 52 N Alt.-14 83 m Lat.- 39 59 E Long.- 10 17 N
4. *A. percrassa*	Tigray	1**. Adigrat:** 12 km from Adigrat towards Mekele (small population)	Alt.- 2605 m Lat.- 39 32 E
		2. **Ambalage:** 80- 102 km form Mekele towards Maichew	Long.- 14 12 N Alt.- 3000 m Lat.- 39 31 E Long.- 10 17 N
5. *A. monticola*	Tigray	1. **Maichew:** 30 km from Maichew towards Mekele	Alt.- 2730 m Lat.- 39 32 E Long.- 12 53 N
6. *A. debrana*	Shewa and Wello	1. *** Debrebirhan: 9**-30 km from Debrebirhan towards Addis Abeba	Alt.- 2810 m Lat.- 39 29 E Long.- 9 36 N
		2. **Dessie Zuria**: 5 km from Dessie towards Addis Abeba	Alt.-2460 m Lat. - 39 38 E Long.- 11 05 N

7.. *A. gilibertii i*	Shewa and Sidamo	1. **Shashamene:** 15 kms from Shashamene towards Addis Ababa	Alt.- 1980 m Lat.- 38 38 E Long.- 7 17 N
		2. **Alaba:** 3 kms from Alaba on the road to Arbaminch	Alt.- 1810 m Lat.- 38 03 E
		3*. **Awassa:** 5 kms from Awassa on the road to Dilla	Long. 7 15 N Alt.- 1800 m Lat.- 38 30 E Long.- 7 00 N
8. *A. calidophilla*	Sidamo	1. **Mega:** 10 kms from Mega towards Moyale	Alt.- 1615 m Lat.- 38 22 E
		2*. **Dida chenna:** 31 kms from Mega to Moyale	Long.- 4 00 N Alt.- 1475 m
		3. **Yabello:** 51 kms from Yabello to Mega	Lat.- 38 30 E Long.- 5 53 N Alt.- 1663 m Lat.- 38 16 E Long.- 4 28 N
9.. *A. yavellana*	Sidamo	1*. **Yabello:** 2 kms West of Yabello	Alt.- 1910 m Lat.- 38 04 E Long. 4 53 N
		2. **Mega:** 5 kms SW of Mega along small road into the mountain	Alt.- 1610 m Lat.- 38 22 E Long.- 4 00 N
10. *A. harlana*	Harerghe	1. ***Harla:-** 17 km from Dire Dawa towards Dengego	Alt.- 1880 m Lat.- 41 54’ E Long.- 9 28’ N
11.*A. megalachanta*	Harerghe	1. **Harla:-** 5-15 km from Dire Dawa towards Dengego	Alt.- 1750 m Lat.- 41 54’ E Long.- 9 29’ N
		2. **Asebe Teferi:-** 1-3 km from Asebe Teferi towards Awash	Alt.- 1800 m Lat.- 40 51 E Long.- 9 04 N

Appendix V: Demographic data for three species studied

Appendix VI Germination experiment:

a) Data from germination experiment

b) Nutrients applied for germination experiment in the green house

i) Medium of Growth

- Soil mixture

Composition**:**

- 75 (vol.) % Sphagnum peat little to medium decomposed
- 20 (vol) % Sphagnum peat medium to strongly decomposed
- 5 (vol) % Sand (0.5-4.0 mm)
- Dry matter 12 kg/ac
- Soil PH 5-6

Added pr. m^3 volum:

- 4 kg grinded calicium stone
- 2 kg grinded dolomite stone
- 1.5 kg 12-4-16 fulll fertilizer®
- 0.1 kg FTE nr. 36 micronutrients

- Content of plant nutrients in g pr. m^3 volume

- Nitrogen (N): 180
- Phosphorus (P): 55
- Potassium (K): 240
- Calcium (Ca): 240
- Magenisum (Mg): 260
- Sulfur (S): 100
- Boron (B): 0.8
- Copper (Cu): 2.5
- Iron (Fe): 9.0
- Magnesum (Mn): 2.4
- Molybdenum (MO): 0.7

- Zink (Zn): 2.0

ii) Nutrients given every week

Nutrient	Weight (%)
- Nitrogen (N)	7.3
- Nitrate ion (No3)	6.5
- Ammonium ion (NH4)	0.8
- Phosphorus (P)	4.3
- Potassium (K)	21.5
- Magnesium (Mg)	3.6
- Calcium (Ca)	19.0
- Sulfur (S)	5.3
- Boron (B)	0.025
- Copper (Cu)	0.010
- Iron (Fe)	0.15
- Manganese (Mn)	0.07
- Molybden (Mo)	0.004
- Zinc (Zn)	0.025

Declaration

I, the undersigned, declare that this thesis is my original work and has not been presented for the degree in any other university, and all sources of materials used for the thesis have been dully acknowledged.

Fikre Dessalegn Boshe _____

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