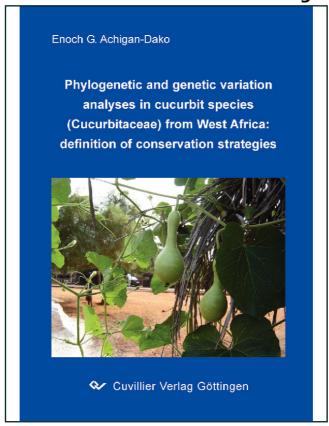Enoch G. Achigan-Dako (Autor)

Phylogenetic and genetic variation analyses in cucurbit species (Cucurbitaceae) from West Africa: definition of conservation strategies

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1. Introduction

1.1. Definition of conservation approaches for plant genetic resources: theoretical background

In a world with rapidly increasing human population pressures and accelerating exploitation it is imperative to utilize biological resources sagaciously on a sustainable basis. To this end we must foremost have an adequate knowledge of the flora (Hedberg 1978). The challenges faced by the world's biological and conservation scientists are then: 1) to classify the existing biological diversity, 2) to halt the rate of ecosystem, habitat, species and genetic loss, so that to feed the ever increasing human population and to conserve genetic diversity for upcoming generations (Maxted et al. 1997). The close relationship between genetic diversity and conservation is revealed through every aspect of human endeavours to steadily increase economic potential of plant genetic resources. Therefore, knowledge on plant genetic resources is of utmost importance for the definition of conservation strategies so as to ensure sustainable utilization.

Plant genetic resources include different types of direct or indirect utilitarian plants as well as ecosystems which house them (Brush & Meng 1998). Benefits from the existence and the utilization of plant resources are enjoyed by the society as a whole particularly in agriculture. The tight connections between livelihood of human populations and genetic resources cannot be any longer ignored and the multiple functions of plants (nutrition, health, shelter, nutrient cycling, culture, etc.) in human life are simply based on their diversity. This diversity expresses itself in various ways, most of which can be categorized as phylogenetic, morphological, or genetic variation (Owens & Bennett 2000). For instance, many plants that we already use as crops are still dependant upon the broad genetic base that exists in their wild relatives and wild relatives steady serve as a backup to improve cultivated plants. Unfortunately many species are going extinct and many others are threatened or endangered (Prance 1997). Tropical regions which hold more undescribed taxa than anywhere else are under pressure for deforestation and cultivation in developing countries. Prance (1997) indicated that 7% of the plant species of the world could be lost in the next decades and it is generally admitted that a catastrophic loss of plant genetic resources is occurring at this moment (Maxted et al. 1997). The loss of genetic resources has consequences not only for agriculture or medicinal potentials but also for local knowledge associated to their utilization.

Although one has to emphasize that most estimates of extinction rates suffer from a severe lack of knowledge and are thus highly speculative (Porembski 2003), human dependency on genetic resources and the threats on those resources call for research and development actions, particularly in the tropics and subtropics known as hot spots of biological diversity. This requirement is particularly important in West Africa where the flora is poorly known, under threat, and where plant genetic resources biologists are too few and often not adequately trained to classify, manage and utilize their native flora in sustainable ways. To reach a more informed use of plant genetic resources by breeding or conservation programmes foremost accurate knowledge on the species systematic position, genetic patterns and ecological requirements are required.

Systematics is the scientific study of the kinds and diversity of organisms and of any and all relationships among them (Simpson 1961). The term refers to the ordering of

organisms into groups to answer questions about classification, phylogeny and the processes of evolution (Stuessy 1990). Myers (1952) provided a broader definition of systematics as "the study of the nature and origin of natural populations of living organisms, both present and past". Systematics uses different approaches for biological classification as a result of historical progresses made in the field, the principal approaches being phyletics, phenetics and cladistics (Mayr 1982, Stuessy 1990). This dissertation combined the last two approaches on which I emphasize hereafter.

Phenetics, also known as numerical taxonomy, is here defined as classification based on numerous precisely delimited characters of equal weight and their comparison by an explicit method of grouping to obtain a measure of overall similarity among all operational taxonomic units (Cain & Harrison 1960, Sokal & Sneath 1963, Stuessy 1990). The importance of phenetics in taxonomic applications and plant breeding was demonstrated in the successful work of Goodman and Bird (1977) who used principal components and cluster analyses to delimit races of 219 Latin American maize (Poaceae: *Zea mays* L.) lines and Small (1978) who performed a numerical analysis on 783 herbarium specimens to clarify infraspecific variation of morpho-geographical taxa of *Humulus* (Cannabaceae). Characterization of genetic resources using phenetic approach revealed the existence of high-yielding lines in *Lathyrus sativus* L. (Leguminoseae: Polignano et al. 2005) or geographical distribution of characters in *Triticum aestivum* (Poaceae: Börner et al. 2005, Eticha et al. 2005). However, one of the difficulties of the phenetic method is the selection of homologous characters and states that have descended from modification of common ancestors (Stuessy 1990). Although I apply this method to both morphological and molecular data, ancestry relationships, however, were rather inferred, using cladistics methods.

Cladistics, an approach of classification also referred to as **phylogenetic systematics,** can be defined as the concepts and methods for the determination of branching patterns in evolution (Hennig 1966, Stuessy 1980). Rules governing this approach were outlined by Hennig (1966) and represented a fundamental shift concerning the role of the concept of evolution in systematics (de Queiroz & Gauthier 1992). The use of cladistics to reconstruct the evolutionary history of putative sister groups (including wild relatives of cultivated plants) of plant genetic resources is a powerful tool for the definition of conservation units and the search of useful genes in related taxa for breeding purposes. The reconstruction of evolutionary relationships among taxa is based on few concepts some of which deserve mentioning here: **monophyly** which refers to a group that include all the descendants of a common ancestor and **paraphyly,** which refers to a group that has a common ancestor but which does not include all the descendants of the ancestor (Hennig 1966). The application of these concepts to the definition of conservation units has great consequences on the composition of selected gene pools and it is worthy to revisit the implications of classification strategies based on one or the other concept. Hennig (1966)'s distinction between monophyly and paraphyly was a crucial first step in phylogenetic systematics with the precept that all taxa must be monophyletic. However, this precept is nowadays hotly debated, still stirring up the systematists' community, with new data coming in and revealing that true monophyly is sometimes unlikely to be achieved. The debate turns around 1) the compatibility between the Linnaean and the monophyletic hierarchy, and 2) the recognition of the 'hypothetical ancestor' of a taxon as real ancestor. To some systematists, paraphyly as a rule to define supraspecific taxa is inevitable (Brummitt 1997, Sosef 1997) and attempts to eliminate paraphyletic taxa from Linnaean classification are logically untenable (Brummitt & Sosef 1998). These adepts claim that Linnaean classification is the

optimal tool for cataloguing biological diversity and will inevitably be maintained, but this requires recognition of paraphyletic taxa and some rethinking of the practice and purpose of biological classification (Rieseberg & Brouillet 1994, Brummitt 2002, 2003, 2006, Nordal & Stedje 2005, Hörandl 2006). In addition, the concept of clades does not apply in many cases also at species level because of reticulate evolution through past interspecific hybridization when hybrids derive from more than one ancestor (Sosef 1997, Hörandl 2006). To others paraphyly is ''bad taxonomy'' and could be avoided by applying the principles of Hennig's system consistently (Freudenstein 1998, Nelson et al. 2003, Dias et al. 2005, Potter & Freudenstein 2005). The general trend is that some agree with the incongruence between Linnaean hierarchy and monophyly but prefer to develop a complete new system of classification and nomenclature on a pure phylogenetic basis (e.g. Schaefer 2007). Does this mid-way approach helps secure genetic resources? Brummitt (2002, 2003) warned "if you have good characters you should recognize good taxa; but if you have no characters you shouldn't recognize taxa at all"; characters cannot be ignored if we intend to understand relationship among organisms and to classify them (Hörandl 2006). In the course of the debate Stuessy and König (2008) suggest the use of **patrocladistic classification**, a method which incorporates patristic distances, or evolutionary divergence within lineages into an explicit method of producing diagram (patrocladogram). Principles of patrocladistic classification consist in: 1) completing a cladistic analysis using any specific algorithm such as parsimony, Bayesian probability or maximum likelihood; 2) using cladogram with high support levels as a structure for modification based on patristic distances. In other words cladistic and patristic distances are combined to construct a new tree using any phenetic algorithms. Stuessy and König (2008) recognized that patrocladistic is not strictly cladistic. They expect, however, a broad use of the new approach and argued further that classification should not be 'constrained by a branching topology solely in a cladistic context'. In this work I did not give any supremacy to any approaches but combined traditional classification (based on morphological traits) and molecular features and included as much as available taxa to make informed decisions. Various criteria have been applied to reflect the diversity of evolutionary processes at family and species levels (Stuessy 1987). The use of molecular data enormously helps clarify relationships among and within taxa, and to associate genetic differentiation with morphological features when possible. However, analyses of relationships within species were merely carried out using population genetics approaches.

Population genetics has a significant role to play in the analysis and description of biodiversity in plant communities and populations. It attempts to uncover the genetic variation present in a given population, in terms of different phenotypes and/or genotypes and establishes why these variations are present and what factors contribute to their existence (O'Neill et al. 2003). While phylogenetics mostly focuses on interspecific relationships, population genetics accounts heavily for analysis and comparison of variation among individuals from a single species. The prerequisite of analyses within species is the availability of **polymorphic traits**. Polymorphic traits can be either morphological or genetic. Although morphological traits have proved to detect polymorphism within populations (Gilliland et al. 2000, Achigan-Dako et al. 2008a,b), it has become clear that the level of information that can be realistically obtained from morphological markers is limited (Olmstead & Scotland 2005). The development of molecular markers in recent decades has allowed access to the wealth of information contained in the DNA sequences of all organisms and now allows plant breeders, ecologists, systematists, etc. to increase the power of their

studies on all botanical forms (O'Neill et al. 2003). Thus, to fully capture the available polymorphism in an organism, the combination of morphological markers and molecular markers is usually recommended. Correlation of morphological data to molecular markers can be a valuable tool for breeding purposes with the interpretation of morphological variation in the light of DNA-based analyses.

Genetic variation in organisms can be split into 1) genetic diversity, 2) genetic differentiation and 3) genetic distance. **Genetic diversity** (the amount of genetic variation exhibit by a taxon) is a commonly used expression to describe variation found within biological entities and can be measured at the individual, population and species level (Lowe et al. 2004). Basically, diversity statistics quantify two values: the proportion of heterozygosity and the level of polymorphism. **Genetic differentiation** is the partitioning of genetic variation among populations and is measured using Nei's G_{ST} (Nei 1973), Wright's F-statistics (Wright 1951), and gene frequency variance methods (Weir & Cockerham 1984). Currently, the analysis of molecular variance (AMOVA, Weir & Cockerham 1984) which is based on the hierarchical structuring of the diversity among and within populations is one of the most widely used since it enables *post hoc* statistical analyses to be undertaken to assess the validity of the results (Lowe et al. 2004). However, Chakraborty and Danker-Hopfe (1991) indicated that when sample size increases Nei's G_{ST} and Weir and Cockerham's F_{ST} result in similar results. **Genetic distance** is the amount of genetic variation between pairs of individuals or populations. It quantifies the degree of similarity/dissimilarity between two individuals, or groups of individuals. Basically, two groups of methods are used to calculate genetic distance: 1) methods using unordered data and which estimate genetic distance based on allele sharing criteria and 2) methods using ordered data that allow the phylogenetic information content of the study loci to be applied to evolutionary divergence questions (Lowe et al. 2004). The overall goal of conservation being to preserve representative samples of genetic diversity of species *in situ* and *ex situ*, measures of genetic statistics (diversity, differentiation and distance) are extremely important to build conservation units. One of the major long-term goals of conservation is the retention of enough genetic variation so that future adaptation, successful expansion, or reestablishment in natural populations is possible (Hedrick & Miller 1992). To this regard, the understanding of factors contributing to genetic variation is helpful.

The patterns and origins of variation in organisms are serious matters of concern if we need to develop sustainable conservation programmes. Investigation of genetic variation in relation with the environment helps develop appropriate long-term strategies for example for wild relatives of crop plants. From Turesson's (1922, 1925, 1930) point of view, habitat-correlated genetic variation is widespread among plant species. Genetic variation is continually being created and fixed by: mutation, selection or drift. These factors may be strongly influenced by a variety of ecological and evolutionary processes, historical events, and geographical circumstances (Schluter & Rickfels 1993). A quantitative assessment of these processes in populations is provided by knowledge about the spatial and temporal variation of gene frequencies which reflect population structure. It is widely known that species exhibit evolutionary stable limits to their geographical distributions (Hoffmann & Parsons 1997, Holt et al. 2005, Bridle & Vines 2007). How the amount of genetic diversity and its partitioning are revealed across population distribution ranges has been the subject of unresolved debates (Eckert et al. 2008). Under the **centre abundance assumption model** most authors stipulated that geographically peripheral populations should exhibit lower genetic diversity and higher differentiation than central populations, and populations situated

near the core of a species' geographic distribution exhibit greater abundance than those near the periphery. In this respect, many authors argued that peripheral population are not of interest when planning conservation programmes because they have reduced genetic potential to respond adaptively to potentially extreme environmental conditions (Millar & Libby 1991, Hoffmann & Parsons 1997). However, Vucetich and Waite (2003) argued that it depends much on whether those peripheral populations are of little or significant evolutionary potentials. Furthermore recent reviews of empirical evidences challenge this widely accepted biogeographical model (Sagarin et al. 2006) and indicate that peripheral populations do not always result in low genetic diversity (Lesica & Allendorf 1995, Eckert et al. 2008). Moreover, peripheral populations may play a key role in facilitating species range shifts in response to altered climate regimes and are therefore well worth conserving (Parmesan 2006). In this regard modelling the ecological niche of populations is a useful tool that helps for good conservation planning.

Distantly separated populations may exhibit low gene flow between themselves which will consequently lead to higher differentiation. This effect is termed isolation by distance (Wright 1943, 1946) and at a regional scale where this effect is suspected between widely spaced, discrete populations, a correlation between pairwise measures of geographic distance and genetic difference can be plotted and the closeness fit estimated using a Mantel's test (1967).

Genome size also may influence diversity and differentiation in species (Bennett 1973, Lowe et al. 2004). According to Bennett and Leitch (2005a) nuclear DNA amount and genome size (expressed in C-value) are important biodiversity characters, whose study provides a strong unifying element in biology with practical and predictive uses. Since the 1970s, inter- or intraspecific DNA content variation has been one of the important topics in plant biosystematic debates. Increasingly, genome size is being recognized as potentially important in the field of quantitative genetics, which aims to analyse and understand the genetic basis of characters showing continuous variation (Bennett & Leitch 2005a). Comparative studies of angiosperms indicated that DNA C-value is correlated with a wide range of phenotypic characters. Nuclear DNA can affect the phenotype not only by expression of its genic content but also by the physical effects of its mass, through the nucleotype (Bennett 1985, 1998). The term **nucleotype** was coined to define those conditions of the nuclear DNA which affect the phenotype independently of its encoded informational content (Bennett 1973). According to Bennett (1973, 1985) the nucleotype plays the controlling role in determining some phenotypic characters and hence has far-reaching biological consequences.

DNA amount was for long time thought to be constant in a species. Many examples of intraspecific DNA variation have been shown to be the results of experimental flaws or artefacts of measurement methods and should be treated with great caution (Greilhuber 1998, 2005, Dolezel et al. 2007). However, as new data are coming in, intraspecific C-value variation is not anymore regarded as an exception (Bennett 1985) and has been for example consistently described in *Zea mays* (Poaceae, Poggio et al. 1998), or in *Festuca pallens* (Poaceae, Smarda & Bures 2006). Although the majority of reports of intraspecific DNA amount variation make no mention of any variation of plant phenotype, intraspecific variation of C-value could bear taxonomic significance and indicate that there is more than one entity within a species (Murray 2005). The knowledge of the variation of C-values and its possible correlation with phenotype are additional tools for the definition of conservation units.

Any programme that focuses on genetic variation across large-scale geographic areas has obvious implications for conservation as a whole (Avise 2000, Lowe et al. 2004). The product of genetic structure and phylogeographic analyses provide a basis to formulate future conservation priorities and strategies for target taxa. There are two basic conservation strategies: *ex situ* and *in situ* conservation. *Ex situ* conservation means the conservation of components of biological diversity outside their natural habitats. *In situ* conservation means the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings and, in the case of domesticates or cultivated species, in the surroundings where they have developed their distinctive properties (UNCED 1992). Because both conservation approaches have advantages and inconveniences, it is usually recommended that for conservation priorities, one technique be used as back-up for another and the degree of emphasis on each will depend on the conservation resources available, and the aims and utilization potential for the target taxon (Maxted et al. 1997).

1.2. Systematics of Cucurbitaceae

Cucurbitaceae belong to the order Cucurbitales which include six other members: Anisophylleaceae, Begoniaceae, Coriariaceae, Corynocarpaceae, Datiscaceae, Tetramelaceae (Hutchinson 1973). Recent molecular investigation indicated the monophyly of the order (Zhang et al. 2006). Cucurbitaceae, a phenotypically plastic family, group scabrid herbs or rarely shrubs (e.g. *Dendrosyscos* Balf.f.) with scandent or prostrate stems. Tendrils are mostly present (except in *Acanthosicyos* Hook.f. and *Ecballium* A.Rich. where tendrils are spike-like or absent) and are spirally coiled. Flowers are monoecious or dioecious or very rarely hermaphrodite. Male flowers have a tubular calyx with lobes imbricate or open, the corolla are polypetalous or gamopetalous and the stamen (mostly three) are free or variously united. Female flowers have a calyx-tube adnate to the ovary which is inferior or very rarely free. The ovary usually shows three placentas with numerous ovules rarely few [e.g. *Ctenolepis cerasiformis* (Stocks) Naud.]. Fruits are very small with less than 1 cm diameter [e.g. *Zehneria scabra* (Linn.f.) Sond.] or very large with more than 50 cm width [*Cucurbita maxima* Duchesne, *Lagenaria siceraria* (Molina) Standl.]. Seeds also are various, often flattened (Keay 1954). To date circa 800 species from 125 genera are described within the family (Jeffrey 1990a, Jeffrey 2005, Kocyan et al. 2007, Schaefer 2007). Cucurbitaceae are present mainly in the tropical and subtropical regions (Africa and Madagascar, Central and South America, Southeast Asia and Malaysia) with disjunct distributions of many genera (Jeffrey 1990b, Kocyan et al. 2007). Of the 125 genera about 50 are monotypic and the inference of phylogenetic relationships among genera using morphological traits has been difficult (Jeffrey 1962).

The family has been subject to many taxonomic revisions and classification systems. The complete history of classifications in Cucurbitaceae from Linnaeus (1754) to Jeffrey (1964) is to be found in Jeffrey (1967). In the course of the revision of the family Jeffrey (1962) encountered confusions of the generic limits and experienced dissatisfaction about the hitherto taxonomic treatments of the family. One source of confusion raised by Jeffrey (1962) was the indiscriminate use of ovule orientation as classification character, which is likely to lead to misplacement of certain genera. He consequently proposed a rearragement of the classification system. This classification of Jeffrey (1962) was further confirmed by pollen morphology (Jeffrey 1964). Based on pollen characters, two subfamilies are usually distinguished: Nhandiroboideae Kostel (Syn. Zanonioideae C.Jeffrey) and Cucurbitoideae

Kostel. Nhandiroboideae is the smaller of the two subfamilies with 19 genera, (see Kocyan et al. 2007), and is characterized by a complete uniformity of striate pollen grains which are 3-colporate, prolate and comparatively small. Cucurbitoideae, by contrast, shows a considerable variety of types of larger pollen grains. Jeffrey (1964) concluded that no future work on the taxonomy of Cucurbitaceae can afford to ignore pollen morphology. Further tribal classifications within Cucurbitaceae were steadily improved in a remarkable series of papers using additional characters, mainly seed-coat anatomy, and newly collected material (Jeffrey 1971, 1975, 1978, 1980). Jeffrey's works appeared as landmark in modern classification for the following reasons: 1) he used pollen morphology and seed-coat anatomy to establish his classification; 2) he combined data of collections from all continents including wild material and horticultural types. However, chloroplast and nuclear DNA analyses within the family revealed serious deviation to monophyly of many tribes and subtribes based on the previous classification (Jeffrey 1990a, Jobst et al. 1998, Chung et al. 2003, Decker-Walters et al. 2004a). In a recent classification Jeffrey (2005) used additional seed coat data (Singh & Dathan 2001) to introduce major modifications into the make up of tribes and subtribes. The family now consists of eleven tribes within the two subfamilies: 1) Zanonieae Bl. (Nhandiroboideae) and 2) Joliffieae Schrad., Bryonieae Dumort., Trichosantheae C.Jeffrey, Herpetospermeae (C.Jeffrey) C.Jeffrey, Schizopeponeae C.Jeffrey, Luffeae (C.Jeffrey) C.Jeffrey, Coniandreae Endl., Sicyeae Schrad., Benincaseae Ser., Cucurbiteae Ser. as members of Cucurbitoideae. Major modifications were related to a) subtribes Luffinae C.Jeffrey, previously member of Benincaseae, which was raised to tribal rank; b) Cucumerinae Pax transferred from Melothrieae Endl. to Benincaseae; c) Trochomeriinae C.Jeffrey sunk into Benincasinae (Ser.) C.Jeffrey and d) the rest of Melothrieae transferred to Coniandreae Endl. Molecular evaluation of the new classification system using chloroplast sequences recovered eight tribes of Jeffrey's (2005) classification and revealed the poly- and paraphyly of Joliffieae and Trichosantheae (Kocyan et al. 2007). Furthermore, Benincaseae need some adjustments to reach monophyly and overall only few subtribes were monophyletic. Kocyan et al. (2007) suggested that the subtribes in Benincaseae for instance could be abandoned without noticeable detrimental effect on the current classification.

Cytogenetic analyses within the family, although hampered by technical constraints, reveal various basic chromosome numbers (x = 7, 8, 10, 11, 12, 13, and 20) (Beevy & Kuriachan 1996). The role of cytogenetic mechanisms in species differentiation in Cucurbitaceae has been reviewed by Singh (1990). Polyploidy is comparatively infrequent and does not seem to have played a significant role in the generic evolution of the family (Jeffrey 1980). Species differentiation, however, could result from structural rearrangement or differences in the morphology of complement chromosome or chromosome number in obligate sexual species while in facultative asexual or perennial species hybridization and polyploidy are additional factors involved in differentiation in Cucurbitaceae (Singh 1990).

1.3. Cucurbitaceae in phytochoria and agro-ecosystems of West Africa

In sub-Saharan Africa, there are strongly differentiated ecosystem types and, accordingly, the geographical distribution of plant species is not entirely random (White 1983, Linder 2001). In West Africa particularly, vegetation patterns indicate a gradient from the Atlantic coast towards the Sahara desert. The delimitation between floristic zones in the region is complex. Nonetheless, four major phytochoria – large areas (c. 10,000 km^2 or more) with largely homogenous plant species composition, which is different from that of other areas – could be recognized according to climate, vegetation types and dominant species (White

1983). These are: 1) the **Guineo-Congolian** regional centre of endemism which is a swath rainforest zone extending from south Guinea to the Togo Hills and from south-east Benin to the Congo forest block; between the congolian and the guinean forest blocks is a broad savanna corridor, known as **Dahomey gap**, which reaches the coast of southern Ghana, Togo and Benin between circa 0-3°E (Adomou 2005, Salzmann & Hoelzmann 2005); 2) the **Sudanian** regional centre of endemism, which is a woodlands and savannas region, partially surrounding the equatorial rainforests of Congo and Guinea with its own flora (Linder et al. 2005); 3) the **Sudano-Guinean** transitional zone between the Guineo-Congolian rainforests and the Sudanian woodlands and savannas which is characterized by a vegetation mosaic of forest islands, gallery forests and savannas, 4) the **Sahelian** regional transition zone, north to the Sudanian, which is characterized by grass and thorn shrub savannas; this zone is a pastoral zone where persistent rainfed agriculture is not possible due to low precipitation. Although there is a change in the flora from the coastal rainforests to the Sahara desert, there are no sharp floristic, geographical boundaries (Linder et al. 2005). Furthermore, in the last decades, the vegetation of West Africa was subject to strong changes with an extension of agricultural lands due to steady increase of population and simultaneous expansion of arid areas due to reduced precipitations (Nicholson 2001).

The last monography on Cucurbitaceae in West Africa goes back to forty years ago (Jeffrey 1965). Todate 65 cucurbit species (of which twelve are cultivated) are known for the region including two newly described species (Keay 1954, Jeffrey 1965, Jongkind 2002, 2004). Morphological diversity in West African species is shown by few selected samples in Figure 1. Most diverse genera are *Momordica* L., *Cucumis* L., *Zehneria* Endl. and *Coccinia* Wright & Arn. with five to nine species. Some species lack informative characters and consequently are not fully described yet. For instance female flowers and fruits of *Bambekea racemosa* Cogn. are unknown; *Diplocyclos palmatus* (L.) C.Jeffrey, *Euriandra formosa* Hook.f., *Luffa echinata* Roxb. *Zehneria gillettii* (de Wild.) C.Jeffrey, *Thlandiatha africana* C.Jeffrey need further collecting (Jeffrey 1964b) in the region; many samples of *Coccinia* are not completely identified and just referred to as *sp.* (Keay 1954, Jeffrey 1965).

Major cucurbit crops in West Africa are for instance *Citrullus lanatus* subsp. *vulgaris* Fursa (1972, 1981), watermelon, largely cultivated in the Sudanian region from Niger to Senegal, or *Citrullus lanatus* subsp. *mucosospermus* Fursa (egusi melon) mainly cultivated toward the coast from Nigeria to Côte d'Ivoire. Other largely cultivated species are *Lagenaria siceraria* (Molina) Standl., and *Cucurbita spp* which are subject to trans-country exchanges and for which specific data are still scarce.

2. Rationales, objectives and sampling strategies

2.1. Rationales and problem definitions

Cucurbitaceae are one of the most economically important flowering plant families (Kocyan et al. 2007). For example the genus *Cucumis* includes two major commercial vegetable or fruit crops, cucumbers and melons. *Citrullus lanatus* also is a worldwide cultivated plant which provides huge income to many industries. Many subspecies or varieties of the latter are grown as home garden crops in many areas. Many other wild species are used as medicinal resources. One well-know case in West Africa is the use of *Momordica charantia* L. as remedy against malaria and diabetes (Burkill 1985, Beloin et al. 2005). Seeds and leaves of *Momordica charantia* have been used in reproductive health as abortifacient, birth control agent or to treat painful menstruations. The plant also serves as