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Genetic structuring of important medicinal species of genus *Warburgia* as revealed by AFLP analysis

A. Muchugi · G. M. Muluvi · R. Kindt · C. A. C. Kadu · A. J. Simons · R. H. Jamnadass

Abstract The genus *Warburgia* (Canellaceae) contains four tree species that are of valuable medicinal importance and are all found in Africa. Genetic diversity present in wild populations of these species is under great threat due to unsustainable harvesting for medicines and indiscriminate felling for timber and agricultural expansion. There is therefore an urgent need for conservation of these species. Some authors disagree about the taxonomy of the genus and list different species as synonyms. Amplified fragment length polymorphism (AFLP) technique was used to determine the genetic relationships between three species to resolve the taxonomic confusion. The amount of genetic variation within and among populations was assessed to guide strategies for their conservation and sustainable utilization. Four AFLP primer pairs (*Eco*RI/*Mse*I) generated a total of 185 amplification products. Analysis of molecular variance revealed most variation among individuals within populations (63%, $P<0.0001$), but variation among populations (37%, $P<0.0001$) was highly significant as well. Constrained analysis of principal coordinates based on the Jaccard distance confirmed the separation among populations (38.2%, $P<0.0001$). A phenetic tree and ordination graphs showed a clear distinction of *W. ugandensis* from *W. salutaris* and *W. stuhlmannii*. *W. ugandensis* populations from Uganda and western Kenya formed a subgroup that clustered away from the rest of the *W. ugandensis* populations. *W. salutaris* and *W. stuhlmannii* populations showed little genetic differentiation. An implication of the data to genetic management and taxonomic clarification is discussed.

Keywords AFLP · Genetic diversity · Genetic structure · Genus Warburgia · Taxonomy

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Introduction

The genus *Warburgia* (Canellaceae) includes four species: *W. salutaris* Berto f. Chiov found in southern Africa (Lesotho, South Africa, Swaziland, Mozambique and Zimbabwe*), W. elongata* Verd. and *W. stuhlmannii* Engl. found along the East African coastline, and *W. ugandensis* Sprague found within eastern Africa highlands. All these species are highly valued within traditional health systems for curing several ailments such as stomachache, constipation, toothache, common cold, cough, fever, muscle pains, weak joints, measles, and malaria (Beentje 1994; Kokwaro 1976; Watt and Breyer-Brandwijk 1962). The curative efficacy of extracts is linked to their antibacterial and antifungal activities, which has been scientifically proven in different in vivo and in vitro trials of the isolated major bioactive ingredients (Jansen and Groot 1991; Taniguchi and Kubo 1993; Treurnicht 1997; Haraguchi 1998; Mashimbye et al. 1999; Rabe and van Staden 2000; Olila et al. 2001). However, commercial processing of extracts is currently only being carried out for *W. salutaris* (Botha et al. 2004).

The taxonomy of some of these species is a subject to controversy, as some authorities refer to some of the four

distinct species as synonyms. For example, Palmer and Pitman (1972) and Noad and Birnie (1989) refer to *W. ugandensis* and *W. salutaris* as synonyms of the same species based on their morphological characteristics and localities. Dale and Greenway (1961) suggest that *W. stuhlmannii* and *W. ugandensis* are conspecific. *W. salutaris* was formerly described under the names of *Chibaca salutaris* Bertol.f. and *W. breyeri* Pott (Palmer and Pitman 1972; Verdcourt 1990), but taxonomists later agreed that these are both synonyms for *W. salutaris* whose description took preference according to taxonomic rules (Verdcourt 1990). So far, this classification has been based on morphological characteristics. According to Verdcourt (1954), the three east African species may be distinguished by the sizes of the flowers and fruits: *W. elongata* can immediately be distinguished by its elongate fruits; *W. stuhlmannii* has small flowers with a staminal tube about 3.7 mm long and 1.2 mm in diameter, ten ovules, anthers about 1.0 mm long, and small fruits about 1.5 cm in diameter; *W. ugandensis* has considerably larger flowers with a staminal tube about 5 mm long and 2–3 mm in diameter, 30 ovules, anthers of 2.0 mm, and much larger fruits.

Deductions from several literature citations and herbaria specimen locations show that the species distribution falls into two main ecological categories: dry upland forests (including wooded riverines) and coastal grasslands (Fig. 1). The natural forests that these species inhabit are currently under great threat of destruction by clearing for farming and timber harvesting. Unsustainable exploitation of the species for medicinal purposes, mainly by harvesting the bark and roots, has also resulted in notable destruction in their natural habitats. As such, the species are reported as becoming rare in areas where they were once plentiful (Palmer and Pitman 1972; Beentje 1994). *W. elongata* and *W. salutaris* are listed as endangered and *W. stuhlmannii* as vulnerable in the 'Red List' of threatened species of the International Union for Conservation of Nature and Natural Resources (IUCN 2006). Indeed for this study, it was not possible to sample a *W. elongata* population. With the current trend in the global use of herbal medicine (Lange 2002) and the reliance of local communities worldwide on traditional medicines (estimated at about 80% by the World Health Organization; WHO 2002), continued exploitation of *Warburgia* species raises concerns regarding conservation and the sustainability of utilization. Thus, urgent
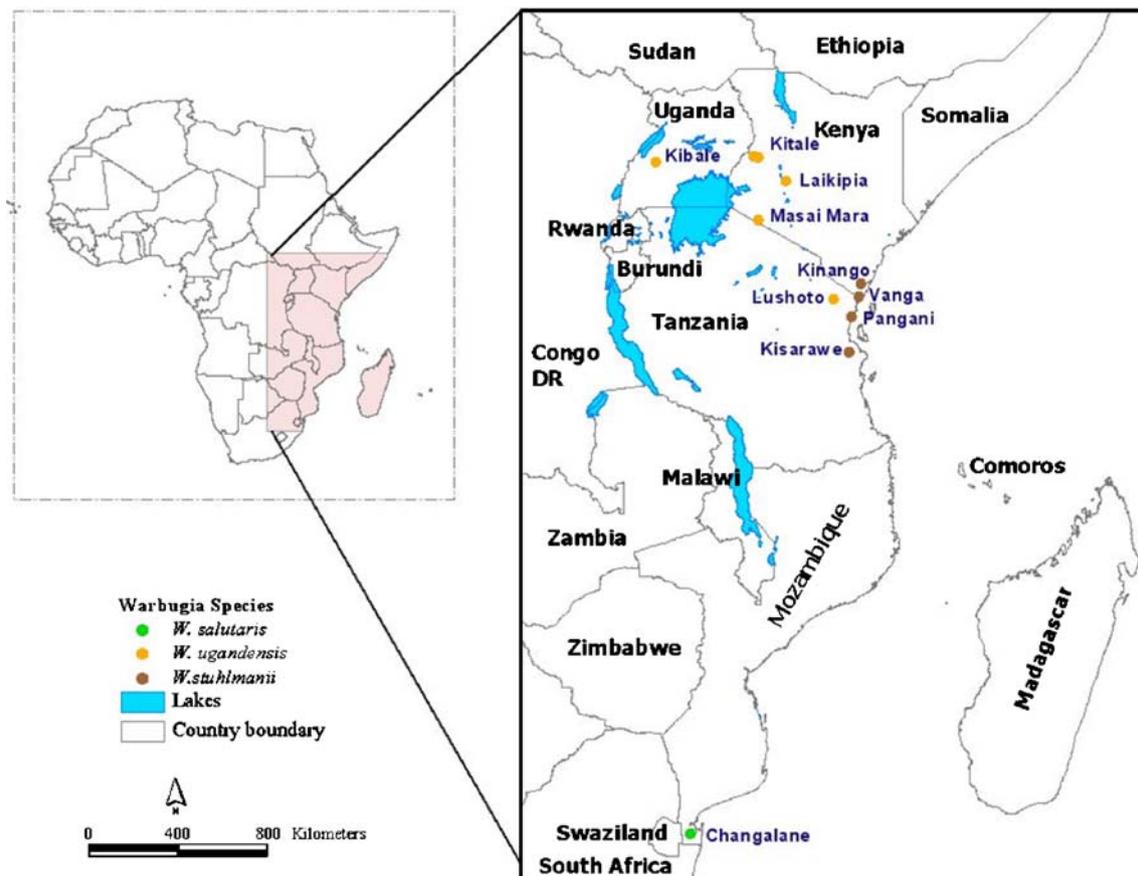

Fig. 1 Geographical distribution of populations of species in the genus *Warburgia* sampled for the AFLP analysis. Note that Kwale population samples were collected from Kinango and Vanga divisions shown in the map

measures to curb problems associated with overexploitation of both the trees and their habitats need to be addressed to ensure conservation of natural biodiversity and the traditional practices associated to its species.

Planting threatened medicinal tree species on farms and in plantations is one way of ensuring sustainability of production. This will reduce pressure exerted on natural forest stands while benefiting farmers with required products for their local use and generate income through commercial markets. There is great potential for commercial market development for extracts from *Warburgia* species. For example, in South Africa, *Warburgia* bark is highly valued, fetching more than 30 USD kg^{-1} in Zimbabwe (Cunningham 2003). Commercially processed *W. salutaris* in tablet form (to treat bronchitis, ulcers, chest infections, and thrush) are highly priced, ranging from 5.8 USD for 60 tablets (URL: http://www.vitalink. co.za) to 12.8 USD for 30 tablets (URL: http://www. biogenesis.co.za). A capsule form of the same species sells for US$ 60 per pack of ten tablets (URL: http://www. the-health-shop.net).

To develop sustainable management strategies for conservation and utilization, a clear taxonomic identification and a detailed understanding of the extent and distribution of genetic variation within a species are important. We employed the amplified fragment length polymorphism (AFLP; Vos et al. 1995) technique to evaluate the genetic structure of the morphologically defined species of the *Warburgia* genus to determine their phylogenetic relationships. We also assessed the amount of genetic variation within and among populations to guide genetic management. The AFLP technique is valuable for studying species where there is no prior sequence information and has been widely employed in taxa identification, determination of phylogenetic relationships as well as assessment of intraspecific diversity at molecular genetic level (Arens et al. 1998; Russell et al. 1999 and Muluvi et al. 1999 among others). This is the first study inclusive of all the *Warburgia* species where molecular markers have been used to assess genetic structuring and for validating their taxonomic relationships.

Materials and methods

Information from herbaria and literature citations on *Warburgia* species was used to outline their distribution to assist in identification of collection areas. Sampling was done for three species (*W. salutaris*, *W. stuhlmannii*, and *W. ugandensis*) from nine populations in Kenya (four), Mozambique (one), Tanzania (three), and Uganda (one), which were either natural or remnant on cultivated fields (Fig. 1 and Table 1). For the east African samples, identification of the specimens was based on the Flora of Tropical East Africa descriptions (Verdcourt 1954). A representative voucher specimen was also collected from

Table 1 Details of nine populations of *Warburgia* species sampled from Kenya, Mozambique, Tanzania, and Uganda for genetic variation analyses using 185 AFLP markers

Species	Population name	Population details	Longitude/latitude	% loci	H
W. ugandensis	Kitale (K)	Kitale forest near Kitale town, natural forest under the Forestry Department, Kenya.	35°01′ E/01°00′ N	48.7	0.13
W. ugandensis	Laikipia (K)	Marnanet North forest, a natural forest under the Forest Department, Kenya	36°25′ E/00°07′ N	50.3	0.16
W. ugandensis	Masai Mara (K)	Individual sampled along the river valley of Sekanani and Talek rivers in the Masai Mara Game Reserve	35°18′ E/01°31′ S	42.1	0.13
W. ugandensis	Lushoto (T)	Manolo natural forest reserve managed by the local community	38°12′ E/04°37′ S	38.9	0.12
W. ugandensis	Kibale (U)	Kibale Forest Natural park. Sampling done around Ngogo Camp	30° 44′E/00° 50′N	61.1	0.16
W. stuhlmannii	Pangani (T)	Msubugwe public land, wooded grassland under the local community management	38°53′ E/05°29′ S	54.6	0.17
W. stuhlmannii	Kwale (K)	Communal land (wooded grassland) in Kinango (ten samples) and Vanga (nine samples)	39°11′E/04°38′S	50.3	0.14
W. stuhlmannii	Kisarawe (T)	Mazungumanzi public land, natural forest managed by the local community	38°40′ E/06°54′ S	46.5	0.13
W. salutaris	Changalane (M)	Individuals are remnant trees on farms at Changalane in Namaacha District	32°21′ E/26°24′ S	45.4	0.13

Twenty individuals were sampled from each population except for Kwale (*n*=19). Percentage polymorphic loci (% loci) and mean diversity estimates (*H*, Nei 1978) are also shown. Longitude and latitude are the average of the coordinates of the individuals
Letter in parenthesis indicates country: Kenya (*K*), Tanzania (*T*), Uganda (*U*), and Mozambique (*M*)

each population and taken to herbarium in Nairobi (Kenya) and Lushoto (Tanzania) for confirmation of identity. Collection in Mozambique relied on local taxonomist for identification of the species. From each population, leaves were collected from 25 randomly sampled trees that were at least 100 m apart. The leaves were dried in snap-top plastic bags containing silica gel and stored at −20°C. Genomic DNA was extracted as described by Doyle and Doyle (1987). The AFLP method (Vos et al. 1995) from the *plant mapping protocol* of the Applied Biosystems (ABI), USA as designed for the AFLP Kit Module was used. Selective amplification was conducted using various combinations of two AFLP primers specific for *Mse*I and *Eco*RI primer adaptors on a test panel of representative samples. The best primer combinations that gave reliable amplifications and polymorphisms in repeated trials were picked for the complete set of samples analyses. The capillary system (*ABI PRISM 3730*™) was used to resolve selective amplification products.

Data analysis

From the automated sequencers, the sample data were analyzed using GeneMapper™ software, which displays the sizing results as electrograms. The data were converted into a Microsoft Excel spreadsheet of allele frequencies in form of product presence (1) or absence (0). Genetic distance (*D*; Nei 1978) between population frequency data sets was generated with POPGENE 1.31 (Yeh et al. 1999). POPGENE also calculated genetic diversity levels for each population (*H*) according to Nei's (1978) unbiased measure. Values were based on estimated allele frequency data assuming dominance and Hardy–Weinberg equilibrium. Analysis of molecular variance (AMOVA; Excoffier et al. 1992) based on Euclidean distances between individuals was undertaken with the ARLEQUIN 1.1 software package (Schneider et al. 2000).

A distance-based redundancy analysis (db-RDA; Legendre and Anderson 1999) was used to investigate the influence of geographical location (measured as population identity or as spatial coordinates of individuals) of the individual trees on differences in presence–absence of alleles measured by the Jaccard distance. db-RDA is a constrained ordination technique that combines ordination and regression approaches that allows for statistical investigation of explanatory variables on differences in distance among objects (Legendre and Legendre 1998; Legendre and Anderson 1999; Anderson and Willis 2003; Kindt and Coe 2005). The significance of ordination results was investigated by randomization test with 1,000 permutations. All the constrained analyses and ordination diagrams were obtained with the Biodiversity R software

developed for the R statistical software (Kindt and Coe 2005; R Development Core Team 2005). The distribution of individuals of the same population within ordination diagrams was investigated by spiderplots (which connect each individual to the centroid or multivariate mean of their population), ordination ellipses (which calculate the average two-dimensional spread of positions of the same population), and by randomization tests (which randomly change population affiliation of individuals in a number of permutations).

Results

Of the 24 primer combinations tested, four combinations (*Eco*RI-ACA/*Mse*I-CAA, *Eco*RI-ACT/*Mse*I-CAT, *Eco*RI-AGC/*Mse*I-CAT, and *Eco*RI-ACT/*Mse*I-CAG) that showed good amplification and polymorphisms across all populations were selected for use on the full set of individuals. A total of 185 polymorphic makers were scored across the nine populations. Estimates of Nei's unbiased genetic diversity (Table 1) showed two *W. ugandensis* populations (Laikipia from Kenya and Kibale from Uganda (*H*=0.16 for both) and one *W. stuhlmannii* population (Pangani from Tanzania [*H*=0.17]) being the most diverse, while one *W. ugandensis* population from (Lushoto from Tanzania [*H*=0.12]) was the least diverse. Overall, the levels of genetic diversity within the sampled populations do not vary greatly. The percentages of polymorphic loci corresponded to the diversity estimates, as populations with higher diversity estimates also showed higher percentages of polymorphic loci (Table 1).

Summaries of AMOVAs based on all surveyed populations are shown in Table 2. Unstructured analyses based on all nine populations indicated that most variation was found among individuals within populations (63%, *P*<0.0001) with significant variation partitioning among populations (37%, *P*<0.0001). A similar case was observed in nested analyses where the populations were also partitioned by species. Only 10% of the total genetic variation partitioned among the species, with 29% of the variation among populations within species and 61% of the variation among individuals within populations, respectively. Further AMOVA based on *W. ugandensis* and *W. stuhlmannii* populations separately showed the same pattern. However, genetic differentiation among populations was higher in *W. ugandensis* (34%) than in *W. stuhlmannii* (14%). The phenogram (Fig. 2) based on Nei's genetic distance showed three main groupings among the *W. ugandensis* populations, not corresponding to geographical distances. Two groups belonged to *W. ugandensis*, with one cluster of the two populations from the western side of the Rift Valley (Kitale from Kenya and Kibale from Uganda) and another

Table 2 AMOVA (Excoffier et al. 1992) based on 185 AFLP markers for 179 individuals sampled from nine populations of *Warburgia* species (*W. salutaris*, *W. stuhlmannii*, and *W. ugandensis*) from Kenya, Mozambique, Tanzania, and Uganda

Source of variation	df	Sum of squares	Variance components	% of total variance	P value
Unstructured					
Among all populations	8	1,389	8.0	37	0.0001
Among individuals within populations	170	2320	13.6	63	0.0001
Structured by *Warburgia* species					
Among species	3	669	2.2	10	0.0001
Among populations within species	5	720	6.5	29	0.0001
Among individuals within populations	170	2,320	13.6	61	0.0012
W. ugandensis only					
Among all populations	6	463	6.7	34	0.0001
Among individuals within populations	60	778	13.0	66	0.0001
W. stuhlmannii only					
Among all populations	2	119.5	2.3	14	0.0001
Among individuals within populations	56	792.9	14.2	86	0.0001

A nested analysis was undertaken on species basis. Degrees of freedom (*df*), mean squared deviation, and % variance are shown

cluster of populations from the eastern side of the Great Rift Valley. Interestingly, the Kibale/Kitale cluster of *W. ugandensis* formed an isolated out-group from all other species from this genus investigated in this study. *W. stuhlmannii* and *W. salutaris* populations clustered together, but formed two distinct subgroups: Kisarawe, a *W. stuhlmannii* population from the Tanzania, grouped together with Kwale, a *W. stuhlmannii* population from Kenya, while Pangani, a *W.*

Fig. 2 Phenogram based on Nei's (1978) genetic distance generated from 185 AFLP markers for 179 individuals sampled from nine populations of *Warburgia* species from Kenya (*K*), Mozambique (*M*), Tanzania (*T*), and Uganda (*U*)
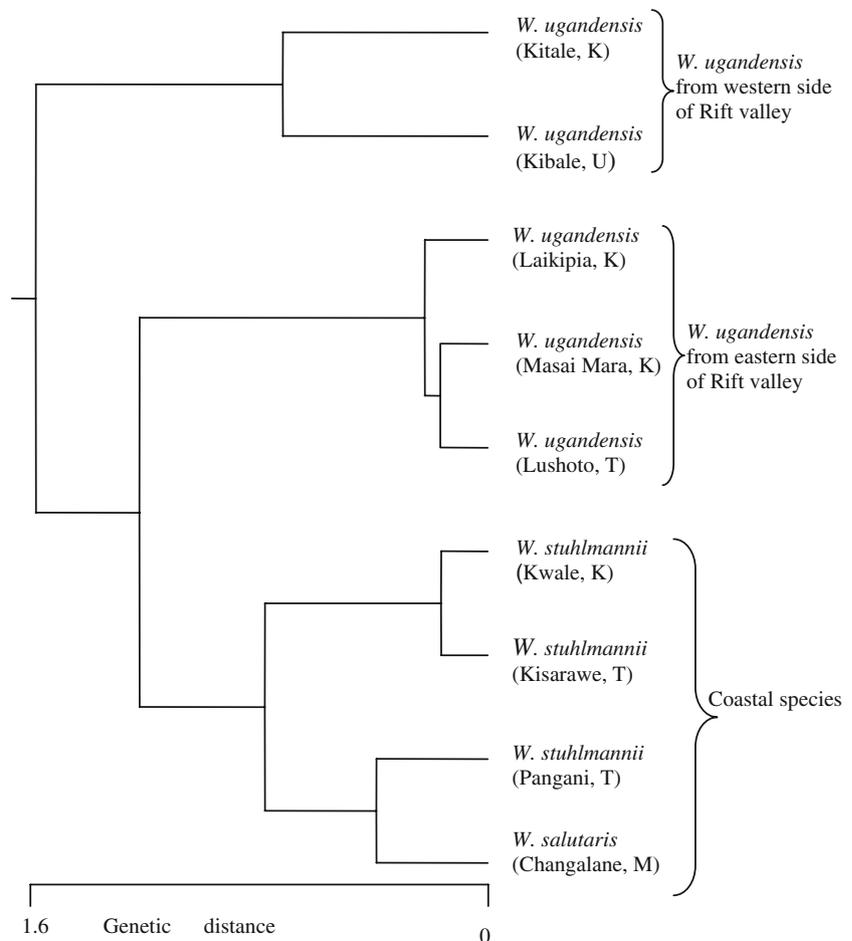
stuhlmannii population from Tanzania, grouped together with the geographically distant *W. salutaris* Changalane population from Mozambique.

The constrained ordination results for all individuals with population identities as explanatory variable show a clear differentiation between the various populations on the first two constrained ordination axes (Fig. 3; differentiation among populations explained 38.2% of squared Jaccard distance [$P<0.001$]; differences between centroid positions of the populations explain 88.9% of variance of positions of the individuals in the two-dimensional ordination diagram of the first two ordination axes [$P=0.01$]). Differentiation is quite clear between populations of *W. ugandensis* and those of *W. stuhlmannii*. The *W. ugandensis* populations are clearly split between a cluster of the Kitale (Kenya) and Kibale (Uganda) populations on the upper part of the ordination diagram and another cluster of the Laikipia (Kenya), Masai Mara (Kenya), and Lushoto (Tanzania) populations on the lower left-hand side of the diagram (Fig. 3). Although the Mozambican *W. salutaris* population has a centroid position within the same ordination quadrant as those for *W. stuhlmannii* populations, the overlap between positions of its individuals is less than for the latter species.

There was considerable overlap between the coastal *W. stuhlmannii* populations from Kisarawe (Tanzania) and Kwale (Kenya) as indicated by the overlap of ellipses and spiderplots of both populations (Fig. 3). For further insight,

constrained ordination analysis with population identity as explanatory variable was therefore carried out for the three *W. stuhlmannii* populations (Kisarawe, Kwale, and Pangani). The ordination results show that the three populations are significantly different, although population differences explained a smaller amount of variation (Fig. 4; differentiation among populations explained 11.6% of squared Jaccard distance [$P<0.001$]; differences between centroid positions of populations explained 40.8% of the variance of positions of the individuals in the two-dimensional ordination diagram of the first two ordination axes [$P=0.01$]).

Discussion

Genetic structuring in relation to the taxonomic units of the genus *Warburgia*

The phenogram and ordination analyses revealed interesting information on the genetic relationships of the species in the genus *Warburgia*. The ordination results showed that there are significant differences between the investigated species and populations. *W. ugandensis* (which occurs within highlands) formed a major cluster away from other species, showing it is indeed a distinct taxonomic group. There is very little genetic differentiation between W. *salutaris* and *W. stuhlmannii* ($D=0.10$; compared to $D=0.18$ for the most genetically distant *W. ugandensis* populations). The two species occur at lower elevations than *W. ugandensis*. However, *W. salutaris* populations are also found at slightly higher elevations from *W. stuhlmannii*. Two *W. stuhlmannii* populations, Kwale (Kenya) and Kisarawe (Tanzania), were found to be genetically closer. Indeed their centroids in ordination diagrams occurred within very close range. However, the other *W. stuhlmannii* population (Pangani, Tanzania) clustered together with *W. salutaris*, which is geographically further away. This low genetic differentiation among *W. salutaris* and *W. stuhlmannii* raises questions on their taxonomic identities. The results imply that these two coastal species may be indeed one species and that the little genetic differentiation shown may be due to ecological adaptations resulting from random genetic drift (Heywood 1991). Isolated populations evolve separately as they adapt to new ecological habitats leading to changes in the allele frequencies hence the genetic differentiations (Epperson 1992). However, further analysis employing different phylogenetic markers, such as those slower involving evolving cpDNA, may provide more information in this respect.

Based on these results, inferences based on morphology only must be treated with reservation considering the uniformity in features across the coastal species and the
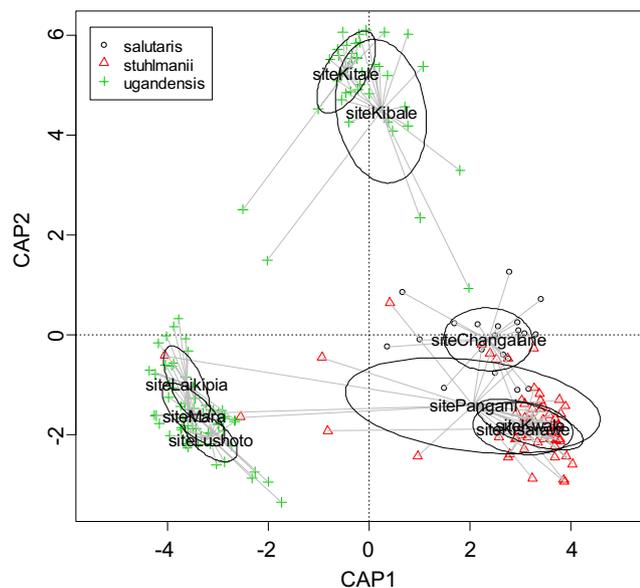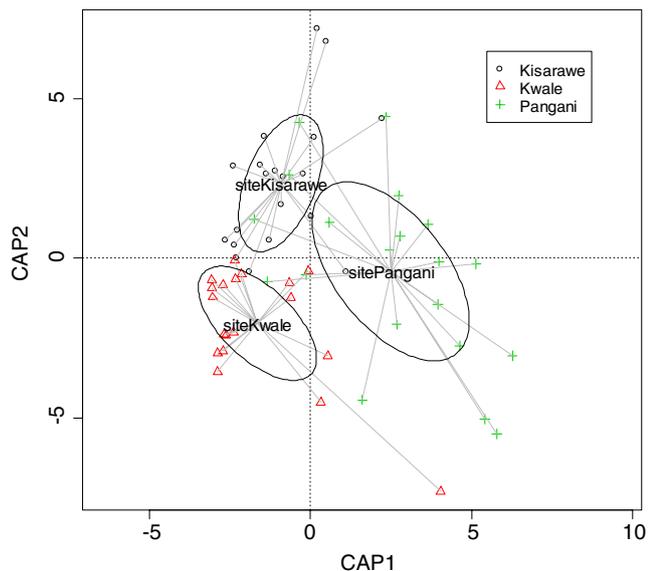

Fig. 3 Ordination diagram for nine populations of *Warburgia* species generated with Jaccard db-RDA using population identity as explanatory variable with 185 AFLP markers. These first two ordination axes show 26.6% of squared Jaccard distance ($P=0.001$ based on 1,000 permutations). Spiderplots connect each individual with the centroid of its population, whereas ellipses provide a summary of the distribution of individual positions for the same population

Fig. 4 Ordination diagram for three *W. stuhlmannii* populations derived by Jaccard db-RDA using population identity as explanatory variable with 185 AFLP markers. These two constrained ordination axes explain 11.6% of squared Jaccard distance ($P=0.001$ based on 1,000 permutations). Spiderplots and ellipses summarize the distribution of individuals of the same population

close genetic relatedness revealed in the ordination analysis. There is a need for intensively sampling individuals from different species that are in close geographical distance, preferably where positions of the different populations overlap in the ellipse in ordination so that this genetic structuring can be discerned conclusively. Molecular studies have shown species previously classified as distinct taxa to belong to the same species. Within the eastern African flora, two such studies on *Vitex* species (Ahenda 1999; Lengeek et al. 2006) found little genetic differentiation between *V. fischeri* and *V. keniensis* and thus suggested that the two may be synonyms. Still within the coastal species group, the taxonomic status of *W. elongata* could not be concluded from this study. The *W. stuhlmannii* Kisarawe (Tanzania) population collection took place from the same locality indicated for *W. elongata* in the herbarium voucher specimens. However, none of the specimens was identified as having the morphological characters described in Verdcourt (1954) as the distinguishing characteristic for *W. elongata* (elongate fruits). Future collections narrowing down to the region where the species was earlier found could help to validate the taxonomic identity. Though only one specimen type of *W. elongata* is available at the East African Herbarium (Nairobi), it would be worthwhile to include the sample in future molecular analysis.

Considering the case of *W. ugandensis* populations alone, the results suggest that there may be two species among these populations that are quite genetically distinct from each other. Despite the geographical proximity of the

Kitale (Kenya) population to other Kenyan *W. ugandensis* populations (Laikipia and Masai Mara, both <400 km away), it is genetically closer to Kibale from Uganda (>700 km away). This is the first time that such genetic distinction is being revealed for the species and suggests a possible case of speciation due to allopatry. However, a few other molecular works has been carried out on other trees species sampled across both central and western Kenya regions, which have shown similar genetic differentiation. While employing the random amplified polymorphic DNA (RAPD) analyses on an important timber species, *V. fischeri* (syn. *V. keniensis*), Lengkeek et al. (2006) found some limited differentiation in the species (19% of total variation among populations). Another RAPD study involving more populations across the two regions in Kenya on *Prunus africana* (Muchugi et al. 2006) and AFLP analysis of *Lobelia giberroa* (Mulugeta et al. 2007) showed a similar genetic disjunction between western and central highland Kenyan populations. These two species occur at slightly higher elevations than *W. ugandensis*. In the *P. africana* study, a substantially greater proportion of variation was partitioned among Kenyan populations (55%, $P<0.001$), than in the current study, most of which was attributed to the split between central and western Kenyan regions.

Some authors (e.g. Hedberg 1951) have suggested that the East African mountain forest bridges are important for species dispersal, but this theory seems not to hold for the forests across the west and central Kenyan highlands. Muchugi et al. (2006) and Mulugeta et al. (2007) propose the distribution of many species across isolated African highland 'islands' to be a combination of long distance dispersal events and direct overland migrations facilitated by climate fluctuations. The major unique geological feature between the western and central Kenyan highlands is the Great Rift Valley. The climatic fluctuations of the Pleistocene are said to have contributed to the shaping of species (Hamilton 1982). Within Kenya, *W. ugandensis* populations are found in the ravine at the floor of the Rift Valley (e.g., the Masai Mara population included in this study), thereby providing connectivity of the western and central Kenyan highlands. However, the clear genetic differentiation of *W. ugandensis* Masai Mara (Kenya) population from the Kitale (Kenya) and Kibale (Uganda) from the AFLP data disputes chances of long distance dispersal model and instead supports migratory theory.

Very little genetic differentiation is seen among *W. salutaris* and *W. stuhlmannii* populations ($D=0.10$ compared to $D=0.18$ for the most genetically distant highland populations). Interestingly, the geographically proximate *W. stulhmanii* populations from Kenya and Tanzania do not cluster together as expected (Fig. 2). As *W. salutaris* and *W. stuhlmannii* show considerable genetic similarity, the seed dispersal hypothesis seems more likely to play the major

role in the genetic exchange along the East African coast and southern region. According to the resolution of our AFLP markers, these two species seem not as genetically distinct as expected from their morphological characteristics that classified them into their individual taxonomic units.

It is important to note that morphological characters described in Verdcourt (1954) as distinguishing characteristic for *W. elongata* (elongate fruits) were not observed in the Kisarawe population, though collection took place from the same locality as the voucher specimens. The collector did not identify any specimen with distinguishing characteristic for *W. elongata* during the survey, concurring with the local community who claim not to have seen such specimen. *Has the species therefore become extinct?* This is quite an interesting question considering its 2006 IUCN classification as 'endangered'. If *W. elongata was found to be genetically close to the other two coastal species W. salutaris and W. stuhlmannii, this* may suggest that the elongate fruits in *W. elongata* were an adaptive feature that could not be sustained. Therefore, inferences based on morphology only must be treated with reservation considering the uniformity in features across the coastal species and the results of the statistical analysis. There is a need for intensively sampling individuals of different species that are in close geographical distance, preferably where positions of the different populations overlap so that this genetic structuring can be discerned conclusively. Other approaches such as cytology can also be used to validate the species status in the genus.

Implications of these findings to genetic management of the species in utilization and conservation

AMOVA and db-RDA showed that most of the genetic variation is retained within individuals rather than among populations, as previously found in other tropical tree species (Hamrick et al. 1992; Nybom and Bartish 2000; Jamnadass et al. 2005). There is also significant genetic differentiation among populations resulting from differences observed between the coastal and highland species as well as the differences between eastern and western highlands populations. As genetic diversity of populations was high, intensive sampling of a few populations for each species will capture most of the genetic variation. Considering international and national genetic management programs, the Kenyan *W. ugandensis* populations occupying both the eastern and western highlands require different management approaches that take into account the diverse genetic resources in the region. If phytochemical studies would show that individuals from either the eastern or from the western highlands to be superior in efficacy of their extracts, planting programs should ensure that their genetic

distinction is retained, as it may be related to ecological adaptation and transfers may erode genetic diversity. However, further studies can look at the possibilities of any existing genetic hybridization within the eastern and western populations and its implications to the conservation of the genetic resource.

Ecogeographical surveys during sample collection showed diminishing populations of *Warburgia* species. Population decline was especially critical along the coast. Based on these results, more emphasis should be placed on conservation strategies for the coastal species, despite the little genetic differentiation shown among them. As these species represent a diverse genetic resource from the highland groups, both cultivation and *circa situ* conservation should be encouraged in all countries. With respect to the cultural background of the local communities along the Kenyan and Tanzanian coasts, tree-planting practice is minimal unlike among communities living within the highlands. The few *W. ugandensis* trees that had been planted all occurred within farmer's fields within the highlands. Individual governments or nongovernmental organizations should therefore take the initiative in the conservation of coastal regions. Maybe in situ conservation of the species in designated protected lands or *ex situ* in public lands (such as parks or schools) can be undertaken. However, there is still the risk of subdivision of these protected and public lands in the future; this may lead to indiscriminate tree felling. The extension of conservation sites along the coastline (e.g. the sacred *Kayas* of Kenya), a few kilometers into the mainland, can also help in in situ conservation of the *Warburgia* species.

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