



PROGRESS REPORT
SOKOINE UNIVERSITY OF AGRICULTURE
FACULTY OF FORESTRY AND NATURE CONSERVATION
DEPARTMENT OF FOREST BIOLOGY
PhD RESEARCH PROPOSAL



TITLE: POPULATION SIZE, THREATS AND CONSERVATION MEASURES OF *LOBARIA PULMONARIA* IN TANZANIA. A CASE STUDY OF FOREST LICHENS ON MOUNT KILIMANJARO.

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JUNE 2013

1.0 INTRODUCTION

1.1 Background Information

Lichens are mutualistic symbiotic organisms usually composed of a fungal partner (the mycobiont), and one or more photosynthetic partners (the photobiont), which are most often referred to either a green alga or cyanobacterium (Scheidegger and Goward, 2002). The two or more organisms live together in such a way that both are more successful within the partnership than they would have been living on their own (Nash III, 2008). Lichens occur practically everywhere on a wide range of substrates in most terrestrial ecosystems of the world and can survive in situations where other plants cannot grow including the bark of trees (corticolous lichens), tree leaves (foliicolous lichens), soil (terricolous lichens), rocks (saxicolous lichens) and deadwood (lignicolous lichens). Nevertheless, many particular lichen species are restricted to a narrow ecological niche with specific requirements concerning substrate (e.g. bark surface texture, pH range, moisture and nutrient content (Gauslaa, 1995; Boch *et al.*, 2013).

Lobaria pulmonaria (L.) Hoffm is an epiphytic foliose tripartite macrolichen species containing fungal (Ascomycetes), green-algal (*Dictyochloropsis sp.*) and cyanobacterial (*Nostoc sp.*) partners (Snäll *et al.*, 2005; Coxson and Stevenson, 2007; Larsson and Gauslaa, 2011). Recently, it has become an important model species for studies on the biological conservation of epiphytic lichens because of its wide distribution in Europe, Asia, North America and Africa, preferring habitats with high rainfall (Liska *et al.*, 1996; Walser, 2001; Dal Grande *et al.*, 2009). Studies show that some environmental variables such as forest site type, altitude, light regimes, and moisture of the habitat may affect the distribution of *L. pulmonaria* (Liska *et al.*, 1996; Mackenzie *et al.*, 2001; Aragon *et al.*, 2010). Another study has shown that *L. pulmonaria* mainly occurs on large host trees (Snäll *et al.*, 2005) and restricted to bark surface with relative pH (5.0 to 6.0) (Gauslaa, 1995).

Lobaria pulmonaria (L.) Hoffm has been used as a bio-indicator species to assess various types of environmental changes in the atmosphere and within different habitats, including air pollution, climate change and deforestation (Mistry and Berardi, 2005). It is one of the symbiotic and photosynthesizing cyanobacteria that can fix atmospheric nitrogen (Scheidegger *et al.*, 1995). Traditionally *L. pulmonaria* has been widely used for treatment of various diseases such as eczema, respiratory, pulmonary and arthritis (Atalay *et al.*, 2011). In Tanzania nearly 120 lichen species are known to exist on Mt. Kilimanjaro (Agrawala *et al.*, 2003), including *Lobaria pulmonaria* (L.) Hoffm around the altitude of 2800 m (Pócs, 1976; Scheidegger, C. personal communication, 2011).

However, the population size of this species on Mt. Kilimanjaro is not yet ascertained. In the recent decades, several studies have documented the reduction population of *L. pulmonaria* and therefore considered as endangered and earmarked it as among the red-listed species in many countries (Werth *et al.*, 2006; Nascimbene *et al.*, 2007; Carlsson and Nilsson, 2009; Catalano *et al.*, 2010) leading to several studies on its conservation biology and ecology (Gu *et al.*, 2001; Gauslaa *et al.*, 2006).

1.2 Problem Statement and Justification

1.2.1 Problem Statement

Lichens have been proven to play very significant roles in the ecosystems in terms of ecological functioning, socio-economic and in scientific researches (Giordani, 2012). However, their habitats, the forests on Mt. Kilimanjaro have been strongly altered by humans in the past and in the recent years (Schrumppf, 2004). Forest fires, expansion of agriculture activities and settlements in some areas have replaced forests (Nsolomo and Venn, 2000; William, 2003). The continuing melting of Kilimanjaro snow-cap which is a superlative natural feature, world natural heritage site and a powerful symbol of the country is an indicator of natural and anthropogenic disturbance regimes across the Montane forests (Hemp, 2005).

The global estimates of the number of lichen species vary from 13 500 to approximately 17 000 (Nash III, 2008; Scheidegger, 2010). This variation and lack of precise information on species composition and distribution of lichens is due to the fact that limited studies have been conducted in many regions of the world (Nash III, 2008). In particular the knowledge of African lichens species is still poor compared to all other continents (Scheidegger *et al.*, 1995; Wirth, 2010). Specifically, the knowledge of genetic diversity of lichen population, an important component of biodiversity within landscapes is limited (Werth *et al.*, 2006). Recent development of modern microsatellites (molecular markers) by Walser *et al.* (2004) allows the population genetic variability of *L. pulmonaria* to be investigated in order to formulate biologically sound conservation measures for this species (Zoller *et al.*, 1999).

1.2.2 Justification of the Study

In Tanzania, there still is limited knowledge on the 120 lichen species which are known to exist on Mount Kilimanjaro and information about their population size, composition and distribution in different habitats are scarce. Therefore, the model organism in this study, the epiphytic lichen *Lobaria pulmonaria* (L.) Hoffm, an indicator species of forests with long ecological continuity, air pollution and climate change, will be investigated to determine its distribution patterns across

different bio-climatic vegetation belts and altitudinal ranges on Mount Kilimanjaro. Also, the genetic differentiation of *L. pulmonaria* along different altitudinal ranges will be assessed and findings will contribute to new knowledge on whether *L. pulmonaria* is adapted only to certain environmental conditions and therefore cannot perform well when transplanted into other environmental conditions (e.g. at other altitudes) (Ensslin, 2011). The research results will contribute to the IUCN red listed species status assessment and enhance efforts on African tropical montane forest conservation so as to mitigate the effects of environmental changes especially climate change.

1.3 Research Objectives

1.3.1 Overall Objective

To assess the population size, threats and establish conservation measures of *Lobaria pulmonaria* on Mount Kilimanjaro.

1.3.2 Specific Objectives

- i. To assess the influence of altitudinal ranges on the population size of *Lobaria pulmonaria* (L.) Hoffm across different bio-climatic vegetation belts.
- ii. To assess the influence of habitat variables on the population size of *Lobaria pulmonaria* (L.) Hoffm.
- iii. To assess the influence of disturbed habitats on population size of *Lobaria pulmonaria* (L.) Hoffm.
- iv. To assess the genetic differentiation of *Lobaria pulmonaria* (L.) Hoffm across different bio-climatic vegetation belts along altitudinal ranges.

1.4 Hypotheses

H₀: Population size of *Lobaria pulmonaria* (L.) Hoffm do not vary with altitudinal ranges across different bio-climatic vegetation belts.

H₀: Habitat variables (phorophytes variables) and disturbed habitats have no influence on the population size of *Lobaria pulmonaria* (L.) Hoffm.

H₀: Genetic differentiation of *Lobaria pulmonaria* (L.) Hoffm do not vary with bio-climatic vegetation belts across altitudinal ranges.

2.0 LITERATURE REVIEW

2.1 Lichen Studies in Tanzania

In most regions of the world, lichens are poorly studied, and their decline is often not documented (Liska *et al.*, 1996). Historical as well as actual distribution of the majority of lichens in Tanzania is poorly known for various reasons. The number of lichenologists is very low in comparison with the number of botanists interested in the distribution of vascular plants. This truth is vindicated in the few lichens studies by Bertsch (1962), Vezda (1975), Pócs (1976), Krog and Swinscow (1986), Swinscow and Krog (1986), Farkas and Vezda (1987), Farkas (1987), Vezda and Farkas (1988), Krog (2000) and Ikingura and Akagi (2002). However, studies conducted by Agrawala *et al.* (2003), Schrumpf (2004), Hemp (2005) and Hermansen (2008) show different vascular vegetation belts and occurrence of lichens on the slopes of Mt. Kilimanjaro, but their findings did not reveal about the composition and distribution of lichen species across these bio-climatic vegetation belts along different altitudinal ranges. Therefore, in this research *L. pulmonaria*, a core species of a larger photobiont mediated lichen guild (Dal Grande, 2011), will be investigated to assess the population size in terms of distribution patterns and genetic differentiation across sub-montane, montane and upper-montane forests on Mount Kilimanjaro.

2.2 Human disturbances on the habitat of lichens

Previous study conducted by Gradstein *et al.* (1992) indicates that some epiphytes are disappearing from preserved forest fragments in East Africa due to large scale forest destruction in the region, and epiphytic lichens are the most seriously affected by this disturbance. Hemp (2005) shows that forests on southern part of Mt. Kilimanjaro are destroyed by fire lit by honey collectors or poachers and have become more aggressive on the higher slopes. In addition to the losses of upper-montane and subalpine forests by fire, clear cutting of lower elevation forests and massive logging still exist inside the forest belts as documented during an aerial survey by UNEP (2002). However, studies on the population size of *L. pulmonaria*, an indicator species of forest canopy continuity on Mt. Kilimanjaro remain an important part of lichenology during this decade of high levels of environmental changes.

2.3 Impact of human disturbances on the genetic differentiation of lichens

The genetic consequences due to habitat fragmentation have been studied in a wide array of taxa because of its importance on the distribution of species and the implications on species conservation (Werth *et al.*, 2006). The genetic differentiation of lichen populations, an important component of biodiversity is an accumulation of differences in allelic frequencies between completely or partially isolated populations due selection or genetic drift (Yamamoto *et al.*, 2004). Very little is known about genetic differentiation of lichens within landscapes. If a disturbance event leads to a significant reduction in the habitat of a particular population, the local population size generally declines. This may lead to a population bottleneck, involving an instant loss of rare alleles which may further be lost due to genetic drift, leading to a continuing decrease of genetic diversity over time (Werth *et al.*, 2006).

Zoller *et al.* (2009) have shown that in order to conserve threatened organisms particularly species with small and isolated populations, more information is needed about the genetic variation at different spatial scales. The development of microsatellites has been proven to be informative markers for population genetic studies and often show high levels of genetic variation (Walser *et al.*, 2003; Walser *et al.*, 2004). In this study the microsatellites developed by Walser *et al.* (2003), will be used to study genetic differentiation of *L. pulmonaria* at different altitudinal ranges on Mt. Kilimanjaro and the results will enhance conservation efforts for threatened and rare epiphytic lichen species.

3.0 MATERIALS AND METHODS

3.1 Study Area

3.1.1 Location and Topography

Kilimanjaro, Africa's highest mountain and a world heritage site, is located in Tanzania close to the Eastern Arc Mountains (UNESCO, 2010), 300 km south of the equator on the border with Kenya between 2°45' and 3°25' South and 37°0' and 37°43' East (IUCN 1987; UNEP 2011). It represents an eroded relic of an ancient volcano with three peaks; Kibo, Mawenzi and Shira that reaches an altitude of 5,895 m, 5,149 m and 3,962 m respectively (Agrawala *et al.*, 2003). This study will be conducted on the southern part of Mt. Kilimanjaro above Machame, Kibosho and Kidia (Old Moshi) between 1800 m and 4000 m a.s.l.

3.1.2 Climate

Southern part of Mount Kilimanjaro is characterized by a typical equatorial daytime climate. Two distinct rainy seasons occur in the study area: the long rains from March to May, and the short rains around November. The maximum annual precipitation reaches around 3 000 mm in the midmontane zone, between 1 800 m and 2 400 m. The mean annual temperature decreases linearly upslope with a lapse rate of 0.56°C per 100 m starting with 23.41°C at the foothills in Moshi (813 m) and decreasing to -7.11°C at the top of Kibo (Agrawala *et al.*, 2003; Hemp, 2005).

3.1.3 Vegetation

According to Agrawala *et al.* (2003), Schrupf (2004), Hemp (2005) and Hermansen (2008), the main bio-climatic vegetation belts across the slopes of Mt. Kilimanjaro are sub-montane, montane and upper-montane forests. The Sub-montane belt between 1 000 m and 1 800 m has been converted to coffee–banana plantations. Montane forests belt between 1 800 m and 2 000 m is dominated by *Ocotea* and *Agauria* forests. The forest belt between 2 100 m and 2 300 m is dominated by *Ocotea-Podocarpus* forests and the forest between 2 400 m and 2 700 m is dominated by *Erica*, *Podocarpus* and *Ocotea* forests with trees that reach heights of 40 m and DBH of 180 cm, and characterized by high density and diversity of epiphytes lichens. The upper- montane belt between 2 800 m and 4 400 m is dominated by *Erica*, *Hagenia*, *Rapanea* forests which gradually changes to shrublands.

3.2 Sampling Design

Southern part of Mt. Kilimanjaro above Machame, Kibosho and Kidia (Moshi rural district) is selected for this study because it receives much higher annual rainfall than any other parts of the mountain (Hemp, 2005) and which is a preferable habitat for lichens. Five transects will be situated by the aid of GPS across different altitudinal ranges (1800 m, 2100 m, 2400 m, 2700 m, 3000 m and 3300 m) following the distribution of bioclimatic vegetation belts (Appendix 1). In each transect of one hectare, phorophytes greater than 35 cm in diameter will be searched for *L. pulmonaria* (Mistry and Berardi, 2005; Wagner *et al.*, 2005). A random 30 thalli of *L. pulmonaria* will be collected from the trunk of different phorophytes and if there will be less than 30 colonized trees per transect, multiple thalli will be sampled from the same tree (Werth *et al.*, 2006). Nomenclature of all host tree species in each transect will be established by a botanist. For disturbed habitats measurements, a survey will be conducted purposively to search for disturbed and undisturbed areas by agriculture activities, fire and logging and then, five plots of similar size 10 m x 10 m (100 m²) in each transect along altitudinal ranges will be established and which is sufficient for investigating the incidence of *L. pulmonaria* on host trees.

3.3 Data collection and Analyses

3.3.1 Population size of *Lobaria pulmonaria* (L.) Hoffm across Altitudinal Ranges

The coverage of *L. pulmonaria* (L.) Hoffm on host trees from each transect across altitudinal ranges will be recorded. The relative species abundance scale used by Cameron and Richardson (2006) is modified to rate distribution patterns of *L. pulmonaria* on the trunk of host tree as follows;

1= $x \leq 5\%$; 2 = $5\% < x \leq 20\%$; 3 = $20\% < x \leq 50\%$; 4= $x > 50\%$. Where, x is the thallus coverage of *L. pulmonaria* on the trunk of the host tree.

3.3.2 Population size of *Lobaria pulmonaria* (L.) Hoffm across disturbed habitats

The incidence of *L. pulmonaria* will be observed on host trees across different disturbed habitats and altitudinal ranges. In each plot; burned areas will be classified by the following scale; 0=undisturbed; 1= $1\% < x \leq 25\%$; 2 = $25\% < x \leq 50\%$; 3 = $50\% < x \leq 75\%$; 4= $x > 75\%$. Logging measurements will be recorded as follows: 0= undisturbed; 1= old cut; 2=new cut and 3=live. Area covered by agriculture activities will be recorded by the following scale; 0= undisturbed; 1= small size; 2=medium size; 3=large size.

3.3.3 Population size of *Lobaria pulmonaria* (L.) Hoffm across habitat variables

The coverage of *L. pulmonaria* will be observed across different habitats (phorophytes) characteristics such as tree species, tree size (BDH), trunk shape and bark texture. Other characteristics include bark substrate variables (bark pH, moisture and nutrient content) which are known to be important factors for the establishment of lichen communities (Mistry and Berardi, 2005).

3.3.3.1 Phorophyte variables

Based on Mistry and Berardi (2005), the following phorophyte variables will be determined from each host tree of *L. pulmonaria*; (1) Bark texture will be categorized following the four-level scale: 0 = smooth; 1 = rough without marked crevices; 2 = rough with crevices; 3 = rough with deep crevices; (2) Host tree species in each transect will be identified with the help of a botanist; (3) Trunk shape will be categorized as; (straight=0; leaning=1; bent=2; crooked=3); (4) Tree size (DBH) (cm) will be measured by using calipers and measuring tape.

3.3.3.2 Bark substrate variables

In each transect, bark samples (200 g) will be randomly collected from 30 host trees and at a height of 1.5 m of the trunk where mostly lichens do establish. Samples will be taken to the laboratory for bark pH and nutrient content measurements (Mistry and Berardi, 2005; Marmor and Randle, 2007). For moisture content measurement, bark pieces (200 g) will be randomly cut from 30 host trees also at the height of 1.5 m of the trunk in each transect and immediately weighed in the field before taken to the laboratory for measurement.

3.3.4 Laboratory Analysis

3.3.4.1 Bark pH

The bark pH of different phorophytes will be measured using pH meter following the standard procedures described by Mežaka *et al.* (2008).

3.3.4.2 Moisture content

The moisture content will be measured as % oven dry weight following the standard procedures described by Nsolomo and Venn (2000).

3.3.4.3 Bark nutrient content

According to Mistry and Berardi (2005), high level of mineral contents in the bark phorophyte trunk is toxic and may deter the establishment of lichen community. In their study the following nutrients were studied: - magnesium (Mg), potassium (K), aluminum (Al), nitrogen (N), calcium (Ca), and phosphorus (P) which will be measured in this research using Kjeldahl procedures followed by spectrometric analysis described by Okalebo and Gathua (1993).

3.3.4.4 Molecular analysis

Fragments (lobe tips) of 5-10 cm² will be collected from the thallus of *L. pulmonaria* from 30 host trees in each transect, then stored in air dried place and prepared in the laboratory for DNA extractions (Zoller *et al.*, 1999; Werth *et al.*, 2006). Fungal samples (10–20) mg in dry weight will be used for DNA extraction and fragment length determination at six microsatellite loci (*LPu03*, *LPu09*, *LPu15*, *LPu16a*, *LPu20a*, and *LPu27a*), specific to the haploid mycobiont, using an ABI 3100-Avant automated sequencer (Applied Biosystems, Foster City, CA). The procedural Polymerase Chain Reaction (PCR) will follow protocols given by Zoller *et al.* (1999), Walser *et al.* (2003), and Werth *et al.* (2006) and alleles assignment will be performed using GENOTYPER 2.5 software (Applied Biosystems) (Wagner *et al.*, 2005).

3.3.5 Statistical Analyses

3.3.5.1 Genetic differentiation along altitudinal ranges

Walser *et al.* (2004) state that, genes on different chromosomes may deviate from random association due to different factors such as genetic drift, natural selection, clonal reproduction, and/or nonrandom mating. A Chi- test with p-values will be computed by Monte Carlo permutations with 1000 replicates to test for the presence of a significant association between pairs of loci within a single thallus, among thalli sampled from single trees and within and among populations across different altitudinal ranges. The genetic differentiation among and within populations will be estimated using the statistic developed by Werth *et al.* (2006) and the genetic diversity of each plot will be calculated using Nei's gene diversity (Nei, 1978) $H_I = N/N-1 (1 - \sum_{i=1}^n p_i^2)$.

Where **N** is the number of genes copies, **i** is the different number of alleles, and **p_i** is the frequency of alleles in a subpopulation. The value of **H_I** can range from 0 (no genetic differentiation, monomorphic population) to 1 (where all individuals within a subpopulation are unique).

3.3.5.1 Habitat variables and disturbed habitats

Many groups of organisms demonstrate a logarithmic distribution (Cameron and Richardson, 2006). Therefore, log-linear regression analysis in R version 2.15.3 will be used to describe the coverage of *L. pulmonaria* on phorophytes, altitudinal ranges and habitat types. Correlation analysis among substrate variables will be done using the Spearman's rank correlation coefficient.

4.0 EXPECTED RESULTS

In this study, we expect to find the reduction population size of *Lobaria pulmonaria* in disturbed habitats than in undisturbed habitats. Higher bark nutrient contents and lower bark pH level will limit the establishment of *Lobaria pulmonaria*. Host trees with rough bark texture and deep crevices will favour wide coverage of *L. pulmonaria*. Also, host trees with larger size of the trunk will be suitable site for the establishment of *Lobaria pulmonaria* than host trees with small size. The genetic differentiation of *Lobaria pulmonaria* will have higher frequency at higher altitudinal ranges.

5.0 PEOPLE

This project is supervised by Prof. Pantaleo K.T. Munishi from Sokoine University of Agriculture (SUA) in Tanzania and Prof. Dr. Christoph Scheidegger from the Swiss Federal Institute for Forest, Snow and Landscape Research (WSL) in Birmensdorf, Switzerland.

6.0 LOCATION AND DURATION OF THE STUDY

The study location is on Mt. Kilimanjaro and will be conducted within a period of 3 years starting from July 2012 to July 2015.

7.0 SOURCE OF FUNDS AND BUDGET

This study is funded by Rufford Small Grant Foundation and Tanzania Commission for Science and Technology (COSTECH). The total amount of funds is indicated in Appendix 4.

8.0 SCHEDULE OF ACTIVITIES

The schedule of activities is indicated in Appendix 3.

10. REFERENCES

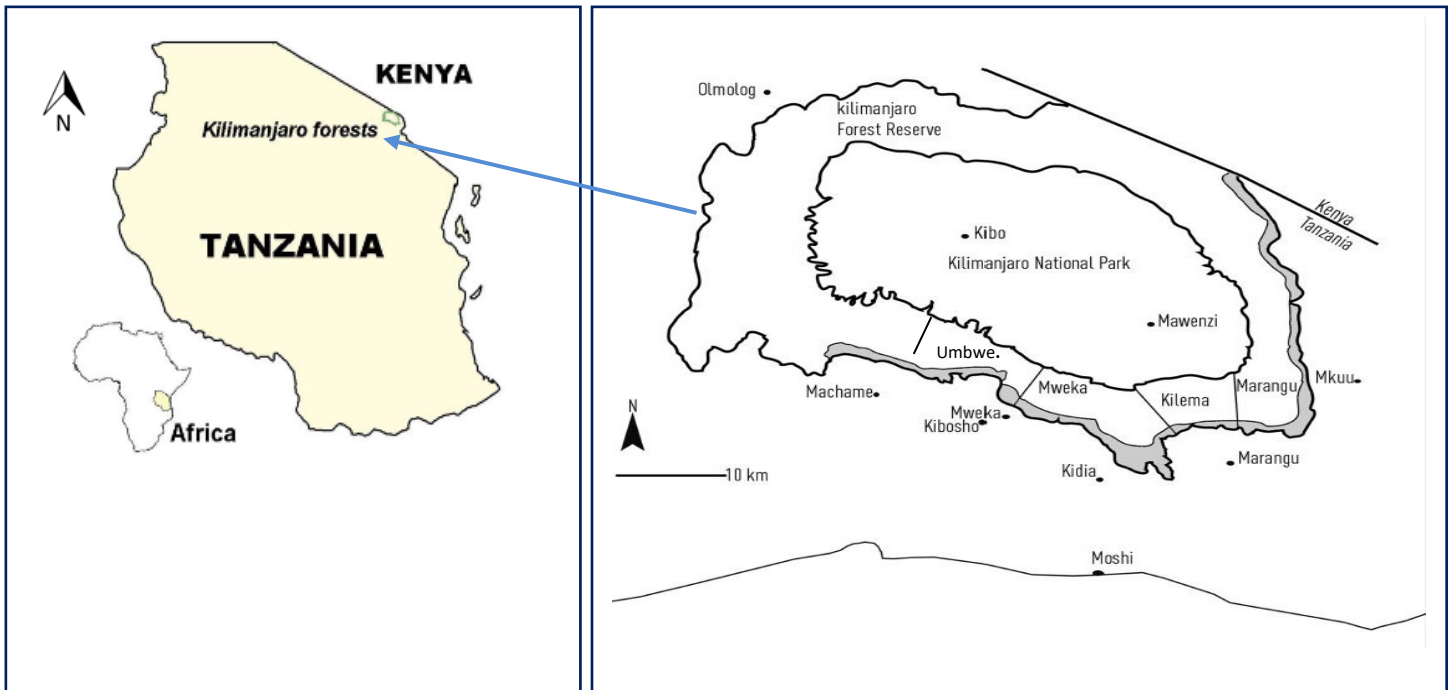
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APPENDIX 1

MAPS OF THE STUDY AREA




Source (UNEP, 2002)

APPENDIX 2

DISTRIBUTION AND DISTURBANCE TRANSECTS



KEY

 Undisturbed habitat

 Disturbed habitat

APPENDIX 3

SCHEDULE OF ACTIVITIES

Activities	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.
Year 2012												
Proposal development & Literature review												
Year 2013												
Proposal development & Literature review												
Research proposal presentation and submission												
Field data collection												
Laboratory sample preparation & analyses												
Year 2014												
Statistical analyses of data												
Paper writing, Manuscripts writing and Publication in international journals												
Year 2015												
Defence of research findings												

APPENDIX 4

RESEARCH BUDGET

No.	Budget category and description	Unit of measure	Units	Unit cost	Amount	Amount covered by Rufford	Amount covered by COSTECH
1.	Personnel costs					4,780,000	
	Field assistant (Botanist)	days	14	65,000	910,000		
	Field assistant (helper)	days	60	30,000	1,800,000		
	Park security guard	days	60	30,000	1,800,000		
	National park entrance fees for 3 researchers	days	180	1500	270,000		
2.	Meals					900,000	4,590,000
	Principal investigator during field work	days	60	15,000	900,000		
	Principal investigator in Switzerland during data analysis	days	90	51,000	4,590,000		
3.	Transport (domestic and international)					4,480,000	3,730,000
	Return bus ticket to Moshi	trips	3	60,000	180,000		
	Car rent	km	2,000	2,000	4,000,000		
	Maintenance	services	Lump-sum	300,000	300,000		
	VISA	document	1	200,000	200,000		
	Return ticket to Zurich-Switzerland	flight	1	2,000,000	2,000,000		
	Transport in Switzerland	Days	90	17,000	1,530,000		

4.	Lodging						
	Principal investigator during field work in Tanzania	days	60	30,000	1,800,000		
	Principal investigator in Switzerland during data analysis	days	90	850,000	2,550,000	1,800,000	2,550,000
5.	Equipment, Office supplies/ materials						
	Measuring tapes	pcs	2	40,000	80,000		
	Field note books	pcs	50	2,000	100,000		
	Field research diaries	pcs	6	5,000	30,000		
	Pens	pcs	50	500	25,000		
	Erasers	pcs	10	500	5,000		
	printer	pcs	1	500,000	500,000		
	Cartridge	pcs	4	150,000	600,000		
	Pencils	pcs	20	200	4,000		
	Marker pens	pcs	10	1,000	10,000		
	Laptop	pcs	1	2,500,000	2,500,000		
	Beam projector	pcs	1	2,000,000	2,000,000		
	External backup	pcs	1	200,000	200,000		
	Photo Camera	pcs	1	800,000	800,000		
	GPS (Garmin)	pcs	1	800,000	800,000		
	GPS batteries	pcs	10	2000	20,000		
	Binoculars	pcs	1	200,000	200,000		
	Knife	pcs	1	10,000	10,000		
	Altimeter	pcs	1	200,000	200,000		
	Weigh Balance	pcs	1	50,000	50,000		
Boots	pairs	4	50,000	200,000			
Rain coats	pairs	4	50,000	200,000			

	Calipers	pcs	2	400,000	800,000		
	Research permit from KINAPA	document	1	400,000	400,000		
	Paper bags (khaki)	pcs	200	300	60,000	1,970,000	7,824,000
6.	Laboratory data analyses						4,130,000
	Laboratory analyses of moisture, pH, Mg, N, P, Al, K, and Ca	specimens	1050	5,000	5,250,000	1,120,000	
7.	Publication						
	Papers	papers	4	200,000			800,000
8	PhD Thesis production						
	Thesis draft	copies	20	10,000	200,000		
	Soft Binding	copies	4	5,000	20,000		
	Hard binding	copies	5	50,000	250,000		470,000
	TOTAL					15,050,000	24,094,000

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