The behavioural ecology of *Colobus guereza gallarum* in the Ethiopian highlands and its taxonomic positions in the colobines

1. Introduction

1.1 Habitat fragmentation and its implication for primate conservation

Human-induced habitat loss and habitat fragmentation in tropical forest ecosystems becomes an increasing threat for the survival of many species including primates (Laurance *et al.*, 1998; Daphne and Chapman, 2000; Cowlishaw and Dunbar, 2000; Riitters *et al.*, 2000 and IUCN 2012). Habitat loss that causes change in vegetation composition and structure which in turn it reduces habitat size, habitat quality and increase of fragment isolation (Dunn et al., 2009; Daphne and Chapman, 2000; Chapman et al., 2010; Fahrig, 2003; Arroyo-Rodríguez *et al.*, 2005; Chaves et al., 2012).

Habitat loss causes temporal and spatial variation in primates feeding ecology, home range size and activity pattern (Onderdonk and Chapman, 2000; Fashing *et al.*, 2007). Primates living in fragmented habitats have smaller home range due to limited resource availability (Arroyo-Rodríguez, 2007; Bracebridge et al., 2012), travel greater daily distances, and spend more time traveling and less time resting (Boyle et al., 2009; Boyle and Smith, 2010) which all affects its fitness. Ecological generalist taxa with their larger ecological flexibility in habitat use and diet preference have better chances to cope with the anthropogenic threat when compared to habitat and diet specialists (Onderdonk and Chapman, 2000; Irwin, 2008; Chaves et al., 2011; Chaves et al., 2012; Bicca-Marques, 2003; Chapman *et al.*, 2006a).

1.2 Aim of the study

- To determine the potential range of *C.g. gallarum* (i.e., possible areas of occupancy based on satellite imagery and ground survey)
- To study the behavioural ecology of *C.g. gallarum* including diet, foraging ecology, habitat use, activity pattern.
- To determine the phylogenetic relationships and intraspecific divergence patterns among the eight currently recognized subspecies of *C. guereza*
- To determine the current conservation status of C. g. gallarum

1.3 Study species

Black-and-white colobus (*Colobus guereza*) are an arboreal Old World primate taxon inhabiting the deciduous and evergreen forests of several African countries (Fashing and Oates, 2013). Eight subspecies of *Colobus guereza* are currently recognized based on morphological variation. However no molecular genetic study has been carried out in these species and the taxonomy remains vague and inconclusive (Kingdon et al., 2008). Two of the subspecies are endemic to the Ethiopian highlands, *C. g. guereza* west of the Rift Valley and *C. g. gallarum* east of the Rift Valley. The subspecies status is based on differences in morphological features according to Fashing and Oates (2013).

C. g. guereza: Omo River Guereza. Mantle hair relatively long, covering ca. 20% of tail. Tail much longer than head-body length (HB): proximal part of tail grey; distal part silvery white. *C. g. gallarum*: Djaffa Mountains Guereza. Ethiopian highlands east of Rift Valley. Proximal part of tail black with scattered grey hairs increasing distally; distal part white and bushy. Little is known about the ecology of this taxon and it is recognized as Data Deficient by IUCN by IUCN (Gippolliti and Butynski, 2008).

Study subjects

Three of the study groups have occupied different localities, which is no overlapping homerange among them. W–group was composed of 12 individuals during the end of this study, June 2016, including 2 adult males, 5 adult females, 3 sub-adults, 1 juvenile and 1 infant. M–group was composed of 8 individuals, including 1-2 adult male, 4-5 adult females, 1 sub-adult and 1 juvenile and D–group was composed of 10 individuals, including 1–2 adult males, 4–5 adult females, 2 sub-adults and 2 juveniles during the same period of time.

2. Study area

The study was carried out in three study localities of the Ethiopian highlands, Munessa Forest (7°17`N and 38°49'E), Wondo Genet (7° 6'N, 38° 37'E; Fig. 1) and Dale (6° 45`N, 38° 26`E).



Fig 1. C. g. gallarum from Wondo Genet

The region receives 1200 to 1314 mm rain per year and was covered with forest during the past. Munessa Forest covers approximately 120 km² and has an elevation from 2000 to 2700 m asl. Wondo Genet is a small forest island with an area of 27 km², Dale has about 10-20 km² but is heavily fragmented. This study focused on behavioural ecology, distribution pattern and phylogenetic position of C. g. gallarum.

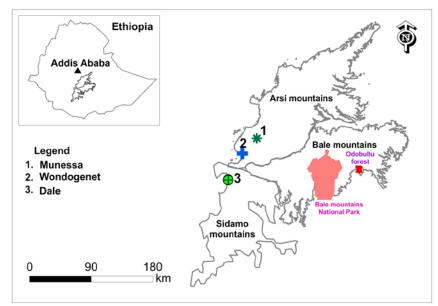


Fig 2. Study localities in the southern Ethiopian highlands

3. Methods

3.1 Forest Composition

I conducted forest composition study together with experienced botanists from Department of Biology, Arba Minch University. We created a series of vegetation transects on existing trails and new vegetation transects, mainly covering home ranges of each study groups. We identified and enumerated most tree species in the range, which have 20 cm girth at breast height (GBH) \leq 5m from the centre of the transect, assigned them to a particular 25x10m quadrat (Fashing, 2001b). I converted GBH to diameter at breast height (DBH), and calculated basal area (BA) for each tree from the DBH value using the formula:

BA = $[0.5 \times \text{DBH}]^2 \times \pi$

BA per hectare was used to estimate the biomass of each tree species found in the home range of each group per Kool (1989).

3.2 Foraging ecology

I applied instantaneous scan sampling (Altmann 1974) to collect data on foraging and diet of *C. g. gallarum*. Scan interval was set at 15 minutes for five days per month for a total of 12 months. I observed three study groups, one in each of the three study localities: Munessa continuous forest and moderately fragmented Wondo Genet and highly fragmented Dale. I defined the following food types: young leaves, mature leaves, root, stem, flower, fruit, shoot, bark, bud or animal prey and recorded the type and species consumed (Fashing, 2001b; Fairgrieve and Muhumuza, 2003; Di Fiore, 2004) whenever an individual in the focal group was observed feeding.

Diet composition was evaluated by using the proportion of different food items and type of species consumed by the monkeys. The daily food items and type of the species consumed by the groups were summed within each month to construct monthly proportion of food types consumed. The monthly proportion of each food type scans will be calculated as the total number of monthly individual scans for each food item divided by the total number of individual scans for all food item individual scans spent for the groups. The relative proportion of plant species used as food was calculated from the monthly percentage

contribution (Fashing, 2001b; Di Fiore, 2004). To assess dietary diversity over the study period, the Shannon-Wiener index of diversity was used to examine potential differences in diet (over time) by the species. Dietary diversity was measured via the Shannon-Wiener index, H' and calculated each month for each individual species (Krebs, 1989). Dietary evenness will be assessed by evenness index, J. Dietary preference of food species in the study groups will be calculated as the proportion of time spent feeding in certain species i divided by the density of that species i in the study group home ranges (Fashing, 2001a).

3.3 Activity pattern

Behavioural data was collected using instantaneous scan sampling method every 15 minutes interval for up to 5 minutes duration from 0700-1730 (Altmann, 1974; Fashing, 2001; Wong and Sicotte, 2007). Data was collected for the first 2-5 adults, sub-adults or juveniles (ignoring dependent offspring) at the time of each scan. The group was scanned each time from left to right to avoid possible biases on eye-catching activities like grooming, fighting and mating (Fashing, 2001). Group scans was recorded as performing one of the following behavioural records on the standardized data sheet: feeding, moving, resting, playing, aggression, grooming, sexual activity, and others such as vocalization, defecation, drinking, and looking towards the observer (Di Fiore and Rodman, 2001; Fashing, 2001). Activity time budgets were calculated by the total number of activity records each day. The grand mean proportions of the monthly budgets provide the overall wet and dry seasons, and also the overall time budgets during the study period (Di Fiore and Rodman, 2001).

3.4 Home range and ranging pattern

During scan sampling, the location of the geographic centre of the group was recorded at 15 minutes intervals using Garmin eTrex 10 GPS. Home range size and day ranges were estimated using adehabitat in R package and GIS software ArcGIS 10.2. You should add here how you calculated the home range size (MCP).

3.5 Phylogenetic relationship

The phylogenetic relationship and intraspecific genetic divergence were inferred from sequence information of the mitochondrial DNA cytchrome *b* gene. Pellet samples were used to extract DNA using Dynabeads MyOneTM SILANE (Atickem et al., 2013). DNA amplification will be carried out by polymerase chain reaction (PCR) and the resulting sequences were read and aligned using CLUSTAL (Thompson et al., 1997) as implemented in BIOEDIT v.7.09 (Hall, 1999). Phylogenetic relationship will be analyzed by using BEAST software (Drummond and Rambaut, 2007).

4. Preliminary Result

4.1 Forest composition

A total of 51 plant species in 26 families were enumerated with in the vegetation transects with designed. Of which, W–Group had 41 tree species in 25 families, D–Group had 31 tree species in 21 families and M–Group had 25 tree species in 18 families were recorded. Total number of tree density in Yirgalem was (680.0 stem/ha), Munessa (373.5 stem/ha) and Wondogenet (347.9 stem/ha). Individual tree species biomass or basal area per hectare is presented in table I. Tow tress have the highest basal area per hectare: *Syzygium guineense* (115642) and *Ficus vasta* (97587). *Podocarpus falcatus* and *Croton macrostachys* are the trees with highest basal area per hectare 147505 and 112731, respectively. In Dale, *Croton*

macrostachys and *Spathodea nilotica* are among the highest basal area per hectare with the value of 54709 and 49680, respectively.

WG-C	1		Basal área/ha	% total	
Rank			(cm ² /ha)	biomass	
1	Syzygium guineense	Myrtaceae	115642	20,9	
2	Ficus vasta	Moraceae	97587	17,7	
3	Celtis africana	Cannabaceae	50315	9,1	
4	Croton macrostachys	Euphorbiaceae	46603	8,4	
5	Anningeria adolfi fredricii	Sapotaceae	40608	7,3	
6	Albizia gummifera	Mimosacea	36629	6,6	
7	Prunus africana	Rosaceae	26385	4,8	
8	Ficus sur	Moraceae	23481	4,2	
9	Millettia ferruginea	Fabaceae	17267	3,1	
10	Spathodea nilotica	Bignoniaceae	13925	2,5	
11	Dracaena steudneri	Dracaenaceae	11619	2,1	
12	Acacia abyssinica	Fabaceae	10353	1,9	
13	Vepris dainelli	Rutaceae	10091	1,8	
14	Ekebergia capensis	Meliaceae	7017	1,3	
15	Olea welwitschii	Oleaceae	6527	1,2	
16	Jacaranda mimosifolia	Bignoniaceae	4553	0,8	
17	Podocarpus falcatus	Podocarpaceae	4335	0,8	
18	Polyscias fulva	Araliaceae	4145	0,7	
19	Fagaropsis angolensis	Rutaceae	2652	0,5	
20	Antiaris toxicaria	Moraceae	2590	0,5	

Table I. The 20 highest-ranking trees in terms of biomass or basal area/ha in the home ranges of groups WG (Wondogenet group)

M–Gro	up					
	•		Basal área/ha	% of total		
Rank	Species	Family	(cm ² /ha)	biomass		
1	Podocarpus falcatus	Podocarpaceae	147505	28,76		
2	Croton macrostachys	Euphorbiaceae	112731	21,98		
3	Bersama abyssinica	Melianthaceae	49443	9,64		
4	Millettia ferruginea	Fabaceae	35302	6,88		
5	Apodytes dimidiate	Icacinaceae	31361	6,12		
6	Prunus africana	Rosaceae	28799	5,62		
7	Maytenus senegalensis	Celastraceae	20667	4,03		
8	Ficus sur	Moraceae	16944	3,30		
9	Syzygium guineense	Myrtaceae	14721	2,87		
10	Celtis africana	Cannabaceae	13783	2,69		
11	Schefflera abyssinica	Araliaceae	13198	2,57		
12	Allophylus abyssinicus	Sapindaceae	12705	2,48		
13	Cassipourea malosana	Rhizophoraceae	2972	0,58		
14	Fagaropsis angolensis	Rutaceae	2524	0,49		
15	Ekebergia capensis	Meliaceae	2224	0,43		
16	Vernonia auriulifera	Asteraceae	2147	0,42		
17	Canthium oligocarpum	Rubiaceae	1953	0,38		
18	Teclea nobilis	Rutaceae	1558	0,30		
19	Brucea antidysentrica	Simaroubaceae	722	0,14		
20	Vernonia myriantha	Asteraceae	476	0,09		
D-Gro	up					
1	Croton macrostachys	Euphorbiaceae	54709	13.8		
2	Spathodea nilotica	Bignoniaceae	49680	12.6		
3	Polyscias fulva	Araliaceae	38173	9.7		
4	Podocarpus falcatus	Podocarpaceae	36247	9.2		
5	Millettia ferruginea	Fabaceae	36187	9.2		
6	Syzygium guineense	Myrtaceae	29927	7.6		
7	Sapium ellipticum	Euphorbiaceae	22434	5.7		
8	Maesa lanceolata	Myrsinaceae	20345	5.1		
9	Olea capensis	Oleaceae	16583	4.2		
10	Vernonia amygdalina	Asteraceae	15005	3.8		
11	Prunus Africana	Rosaceae	11825	3.0		
12	Schefflera abyssinica	Araliaceae	10472	2.6		
13	Albizia gummifera	Mimosaceae	10405	2.6		
14	Ficus thonningi	Moraceae	7743	2.0		
15	Pittosporum abyssinicum	Pittosporaceae	5548	1.4		
16	Ficus sur	Moraceae	5114	1.3		
17	Ekebergia capensis	Meliaceae	4398	1.1		
18	Psidium guajava	Myrtaceae	4371	1.1		
19	Euphorbia abyssinica	Euphorbiaceae	4233	1.1		
20	Dracaena steudneri	Dracaenaceae	3233	0.8		

Table The 20 highest-ranking trees in terms of biomass or basal area/ha in the home ranges of groups M and D (Munessa and Dale group)

4.2 Feeding ecology

The proportion of various food-tapes on the diet of *C. g. gallarum* is presented in Fig. 4. Young leaves, mature leaves and seeds contribute the highest proportion of the diet contributing 45.1%, 21.3% and 7.0%, respectively. There is however variations in the proportion among the three study locations. The group in the continuous forest (Munessa Forest, group M) spent more time feeding on leaves (79.9%) than the groups in the more fragmented habitats (61.5% Wondo Genet group WG, 58.0% Dale group D). Group D and WG have spent 16.3% and 13.4% of their time feeding on seeds, which is higher than group M (13%. Fruit is the most consumed diet (15.9%) in group YA when compared with 5.3% and 3.5% of time in group WG and group M, respectively. 13.9% of the overall time of group WG was used to consume flower and 5.5% and 1.2% of time in group YA and group M, respectively. Other plant parts were consumed less frequently by either of the study groups. Percent monthly plant parts consumed in all three groups are presented in Table 1.

Table 1. Diet of *C. g. gallarum* in three comparative study area (WG; Wondogenet, D; Dale and M; Munessa)

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Group	Young leave	Mature leave	Flower	Fruit	Seed	Leave bud	Shoot	Bark	Stem	Others
WG	43.3	18.0	13.8	5.3	13.6	0.4	0.0	1.3	0.0	4.3
D	45.8	12.9	6.2	14.0	16.7	0.1	0.0	0.4	0.0	4.0
М	47.3	32.6	1.2	3.3	6.5	6.2	0.2	0.3	0.8	1.6
Total	45.5	21.1	7.0	7.5	12.3	2.2	0.1	0.7	0.3	3.3

4.3 Homerange of Colobus guereza gallarum

Homerange of the three study groups in Wondogenet, Dale and Arsi mountains is estimated 41.62, 9.58 and 13.25 Hectar respectively for 100% MCP (Fig 1, Fig 2 and Fig 3).

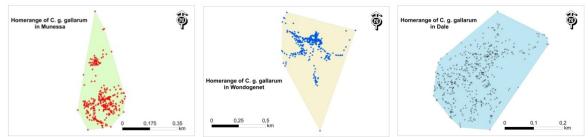


Fig 3. Homerange of *C.g.gallarum* in Munessa Wondogenet and Dale

4.4 Activity pattern

C. g. gallarum spent about 51.2% of their total active time resting, about 31.2% feeding, 9.6% moving and 5% grooming. Other activities were less frequently observed (Fig 4).

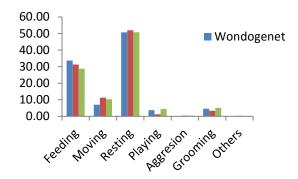


Fig 4. The time spent in different activity patterns in three different localities.

4.5 Phylogenetic relationship

While my laboratory work in determining phylogenetic relationship in *C. guereza* is still in progress, our prelminary result suggested that there is no clear geographic boundary between *C. g. guereza* and *C. g. gallarum*. *C.g. gallarum* samples from Bale Mts. grouped with northen Ethiopian *C. g. gureza*. On contrary, the *C. g. gallarum* sample from Wondo Genet clustered with *C.g. gureza* from Jima and Shako (Fig 5 and Fig 6).

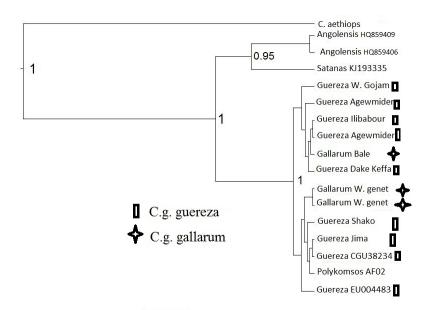


Fig 5. Phylogenetic relationship of black and white colobus taxa based on 400 bp of cytb; see

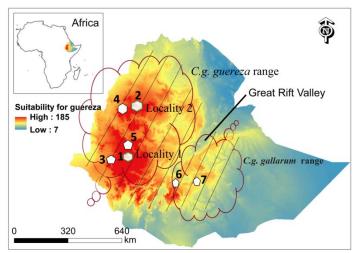


Fig 6. Range of *C. g. gallarum* and *C. g. guereza* separated by rift valley and localities where samples for the genetic study are collected (1. Jima; 2. Agewmider; 3. Shako and Dake; 4. West Gojamm; 5. Illibabour; 6. W.genet, 7. Bale).

Discussion

While Black-and-white colobus are consistently more folivorous than most other primates (Newton, 1992; Maisels *et al.*, 1994; Oates, 1994; Oates, 1988; Fashing *et al.*, 2007; Harris and Chapman, 2007). *Colobus guereza* is reported to feed primarily on young leaves (Oates 1977; Bocian, 1997; Oates, 1994), and this study provides data on more detail diet of the *C.g. gallerum* which feeds on young leaves but also matured leaves. Three of the black-and-white colobus—*Colobus satanas, C. polykomos,* and *C. angolensis*—appear to be more granivorous

than guerezas (Harrison, 1986; Maisels *et al.*, 1994). According to this study, *C.g. gallarum* spent more than 60 % of their time feeding on leaves of which about 40 % of the time feeding on young leaves. Similar to other studies on activity of black-and-white colobus spent much time resting and feeding (Fashing, 2001; 2001a).

The next steps

Taxonomic groupings at species and subspecies are foundation for the conservation priorities used by World Conservation Union (IUCN) Red List of Threatened Species and other international conservation efforts. However, taxonomy in animal Kingdome remains difficult and subject of dispute for decades. One challenge is that, traditional methods of subspecies taxonomy depends on morphological features including cranial and dental morphology and pelage coloration (Skinner et al., 2009) is increasingly controversial as it is sometimes appear to be a contradictory from molecular phylogenetic data (Burbrink et al., 2000; Koh et al., 2012). Morphological traits or colour variations may not necessarily represent actual genetic differences (Zink et al., 2000; Zink, 2004) or cryptic species may remain undetected and overlooked (Smith & Patton, 1988).

Eight subspecies of black-and-white colobus monkey are currently recognized based on morphological evidence (Fashing and Oates, 2013). Two of the subspecies are endemic to the Ethiopian highlands, *C.g. guereza* west of the Rift Valley and *C. g. gallarum* east of the Rift Valley (Kingdon et al., 2008). Our result highlight this taxonomic category does not fit to the molecular category. Here, we recommend further molecular work and revision of current black-and-white colobus monkey taxonomy clarify the taxonomic classification in the genus.

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