

BIOLOGICAL SCIENCES



ISSN 2090-0759

WWW.EAJBS.EG.NET

В

Vol. 13 No. 2 (2021)

www.eajbs.eg.net

Egypt. Acad. J. Biolog. Sci., 13(2): 65-75 (2021) Egyptian Academic Journal of Biological Sciences B. Zoology ISSN: 2090 – 0759 <u>http://eajbsz.journals.ekb.eg/</u>
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ARTICLE INFO Article History

Received:30/7/2021 Accepted:4/9/2021

*Keywords*: *Colobus guereza gallarum*, Diet, Harenna forest, Population Size

#### ABSTRACT

Population size, group composition and feeding ecology of the endemic and endangered Black-and-White Colobus Monkey (Colobus guereza gallarum) was investigated in Harenna Forest, South East Ethiopia by using the line-transect method in 10 selected blocks. During the dry and wet seasons, a mean number of 212 and 246 C. g. gallarum individuals was recorded, respectively. Also, the number of groups of the animal was higher in wet (36) than dry (31) season, but statistically insignificant (P=0.447). Out of the recorded C. g. gallarum, 25.98% were adult males; 32.53% adult females; 14.85% sub-adult male, 11.14% sub-adult female and 15.50% were juveniles/young. Young leaves were the largest (35.1%) contributor to the diet of guereza followed by shoots (22.1%) in both seasons. Other common items were included mature leaves (20.6 %), bark (17.7%), fruit (2.8%) and flower (1.7%). C. g. gallarum were observed feeding on 19 plant species belonging to 12 families. The highest contribution of the diet was from the family Rosaceae (41.67%), while family Fabaceae, Moraceae and Myrsinaceae were contributing 50% of the diet. Schefflera volkensii was the most consumed plant species which accounted for 7.96%, followed by Urera hypselodendron (7.83%) and Dombeya torrida (7.06%). Moreover, C.g.gallarum mainly forage on leaves particularly young leaves, and their foraging activities depend inside the forest. Thus, to maintain sustainable conservation of the endemic C.g.gallarum in the area, their habitats should be properly protected, and appropriate monitoring strategies should be designed.

## **INTRODUCTION**

Africa has the highest primate species abundance due to myriad heterogonous habitat types (IUCN, 1996; Jhon *et al.*, 1996). Black-and-White Colobus monkey (*Colobus guereza*, Ruppel, 1835) is among the most widely distributed of equatorial Africa's arboreal primate species (Kingdon *et al.*, 2008). It is large with a black and white coat. Glossy black fur covers much of the body, but contrasts with short, white hair surrounding the face, and a U-shaped, cape-like mantle of long white hair that extends down the shoulders and across the lower back (Groves, 2001; Arkive 2011; Jensz and Finley, 2011). Within their range, Black-and-White Colobus monkeys are reported in the

following countries: Cameroon, Central African Republic, Chad, Congo, The Democratic Republic of Equatorial Guinea, Ethiopia, Gabon, Kenya, Nigeria, Rwanda, Sudan, Tanzania and the United Republic of Uganda (Jensz and Finley, 2011).

According to Jensz and Finley (2011) and Fashing and Oates (2013), eight subspecies of black-and-white colobus monkey currently recognized based on morphological evidence, such as *Colobus guereza caudatus* (Mt. Kilimanjaro guereza), *C. g. kikuyuensis* (Mt. Kenya guereza), *C. g. guereza* (Omo River guereza) and *C. g. occidentalis* (Western guereza) are classified as Least Concern on the IUCN Red List. *C. g. gallarum* (DjaffaMountains guereza), *C. g. dodingae* (Dodinga Hills Guereza) and *C. g. matschiei* (Mau Forest Guereza) are classified as Data Deficient, and *C. g. percivali* (Mt. Uaraguess Guereza) is classified as Endangered on the IUCN Red List. These eight Guereza subspecies each occupy a distinctive range and exhibit slight variations in appearance. The main features that set the subspecies apart are the length and coloration of the mantle, which sometimes appears creamy or yellow, the length of the tail, and the extent of the tail tuft (Gron 2009). A comprehensive description of Guereza subspecies features was made by Groves (2001).

Two of the above subspecies (*C. g. guereza* and *C. g. gallarum*) are endemic to the Ethiopian highlands. *Colobus guereza guereza* is found in forested areas of the Ethiopian highlands west of the Rift Valley and down into the lowland reaches along the Awash River, the Omo River and the Blue Nile gorge. However, *C. g. gallarum* is restricted to the Ethiopian highlands east of the Rift Valley (Jensz and Finley, 2011). The proximal part of the tail is black with scattered grey hairs increasing distally; the distal part is 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). It exists in Munessa Forest, Wondo Genet forest and Dale forest and Bale Mountains National Park including the Harenna forest.

Clearance of forests for agriculture is a major concern for *C. g. gallarum* populations, and also has a relatively small range (Kingdon *et al.*, 2008, EOL, 2011). In the absence of recent survey work, it is not known how much pressure these populations are under and therefore it is currently listed as Data Deficient on the IUCN Red List as recorded in the 1960s (Arkive 2011). The only population estimate so far has been carried out only in Bale Mountains National Park (Petros *et al.*, 2018).

Furthermore, the total population size of C.~g.~gallarum has never been estimated and their distribution pattern has never been thoroughly mapped (Petros *et al.*, 2018). Given the lack of knowledge concerning the subspecies and the conservation priorities linked to its future existence in Ethiopia, this study aimed to 1) determine the abundance, distribution and group size of populations of C.~g.~gallarum within forest patches in Harenna and 2) examine the differences in diet, foraging behavior of C.~g.~gallarum the occupied patches. Hence, understanding the feeding ecology, population diversity and group size of this species were found to be essential for a better conservation effort.

## **MATERIALS AND METHODS**

## **Study Area:**

## **Description of the Study Area**:

Harenna Forest is a moist Afro-mountain Forest, located in the South-Eastern part of Oromia regional state, Ethiopia. It is situated on the southern slopes of Bale Mountain and is about 480 km from Addis Ababa. It is located approximately between latitude 60°20' and 60°50' N and longitudes 390 and 400E. Along with the adjacent State- and community-managed forest outside Bale mountains national park, it

constitutes an area of over 4,000km<sup>2</sup>. It is also the largest cloud forest in the country. It lies between altitudes of 3300 m to 1150 m above sea label (Zerihun *et al.*, 1988 cited in Tesfa Alemayehu, 2006).

Mountain bamboo grows within the forest, particularly on steep slopes. Rubussteuderi, a member of the blackberry family, grows along the road from April to June. The upper area of the Harenna forest is a wet cloud forest with an extensive bamboo belt, while the lower parts are drier mountain forests. At about 2,200m as the slopes become gentler, larger trees of up to 30m tall appear, and the canopy closes (GMP, 2007). These include fern pine (Podocarpus gracilior) and fig trees (Ficus spp.) of massive girth. Branches are covered with dense epiphytes. In the lower areas of the forest, wild forest coffee (Arabica sp.) grows. Because the forest is so dense and clearings are few and far between, the elusive animals of the forest have little trouble staying hidden. Black-and-white colobus monkey, olive baboon, warthog and Menelik'sbushbuck are common. With a little luck and perseverance, you might see a giant forest hog, a bush pig, or an endemic Bale monkey (Williams, 2002). Clearings are the best places to look for lions, leopards and African wild dogs. Genet, civet, porcupine, and hyena are all active at night. Birds of the Harenna forest are equally elusive. Look for the Abyssinian hill babbler, Abyssinian crimson-wing, Ayre's hawk eagle, silverycheeked hornbill, black-winged lovebird, Abyssinian oriole, yellow-fronted parrot, white-cheeked turaco and Narina trogon. A wide range of migrant birds can also be spotted, including Palearctic warblers (EWCA, 2013).

## **Data Collection**

## **Population Status and Group Size:**

The block counting method was used to estimate the population status and group size (Kifele et al., 2013). According to Harenna Buluk District agriculture and rural development office (2015), there are. Of the total 14 kebeles/local administrations in the District kebeles, 10 kebeles (Namly: Angettu, Kumbi, Hawo, Bulluk, Hero, Sorbira, Sudu Welmel, Shawe, Sodu Lalafto and Garba Galo) were selected based on preliminary survey and personal communication with an expert from Harenna District Agriculture office, the forest resources and C.g. gallarum highly available. So that existing these ten kebeles (local administrations) the forest areas were used as sites/ blocks for the count. The population size, group composition and feeding ecology of C.g. gallarum were carried out using a line-transect survey in each kebeles. During the transect survey, time of the sighting, location along census route, number of C. g. gallarum present, perpendicular transect to animal distance and observer to animal distance was recorded (Fashing and Cords, 2000). Each block or kebele was assessed two times for five continuous days in a month during both the wet (May to September) and dry seasons (December to April). Surveys were conducted on transects from 06:30 -06:45 to 10:30-10:45 h in the morning and from 14:00 to 18:00 h in the afternoon (Peres, 1999) at an average speed of 1km/hr in the forest or 2km/hr in the plantation (Wallace et al., 1998; Peres, 1999).

A total of 45 transects were placed at random keeping the transect survey principles. Each transect line had 100 to 300 m width in different sites of the forest. The survey was carried out using a compass to measure the perpendicular distance and to count the population within this site. The length of transects increases for large areas so as make them proportional. Every 1 km transect covers 10 km of the survey block. The counted individuals were categorized into adult male, adult female, sub-adult male, sub-adult female and young/unidentified juvenile. During individuals counting, the size of each troop of *C. g. gallarum* was also recorded. When the distance between individuals was less than 50 meters, they were considered members of the same group (Kibebew and

# Abie, 2017). **Feeding Ecology:**

Data on foraging and diet of *C. g. gallarum* were collected by applied instantaneous scan sampling method (Altmann, 1974). The Scan interval was set at 10 minutes with five minutes interval for five days per month for a total of 10 months. Ten study groups were observed, in each of the 10 study localities (kebeles). When the monkeys are observed feeding, plant parts, and species eaten were recorded and marked and identified later. Focal animals were selected on a rotating basis, according to age/sex class (Silver *et al.*, 1989).

The feeding behaviour of *C. g. gallarum* was analyzed by computing the percentage of foraging time devoted to a specific plants species and the parts consumed. The percentage of foraging time devoted to a specific plant item was calculated as the total time spent eating that item divided by the total amount of continuous observation time that monkeys were seen to feed (Chapman and Fedigan, 1990). The specific highly consumed plant species by *C. g. gallarum* were identified from a flora study at Addis Ababa University. Diet selection of the study group was determined from the relative proportion of the feeding time on different food items and species in their diet.

# Data Analysis:

SPSS software version 20 was used to analyze the allover collected data. One way ANOVAs, t-test, mean number of individuals of each transect were compared in each transect site. ANOVA also was used to compare the proportion of time that the different group members and individuals performed.

## **RESULTS**

## Population Size and Structure of C. g. gallarum:

The average number *C. g. gallarum* recorded from ten counting blocks during the study period was 229. The mean of *C. g. gallarum* individuals during the dry and wet seasons was 212 and 246, respectively. The variation in the mean number of individuals of wet and dry seasons was not statistically significant (F= 0.613 df = 1, P= 0.444). However, there was significant difference in the number of this animal among blocks/ sites (F = 8.55, df = 9, P = 0.001).

The number of troops/group size of *C. g. gallarum* was higher in wet (36) than dry (31) season (Table 1), but statistically insignificant (F =0.603, df= 1, P= 0.447). Similarly, number of troops were no significance difference between blocks (F = 1.34, df = 9, P = 0.328).

Table1.	Population	and	group	size	of	С.	g.	gallarum	recorded	during	wet	and	dry
	seasons in stu	udy ł	olocks.										

Name of the Site/block														
Seasons		Angettu	Kumbi	Hawo	Bulluk	Hero	Sorbira	Sudu Welmel	Shawe	Sodu Lalafto	Garba Galo	Total	Mean	± SE
Dry	Population size	34	23	12	36	14	16	28	21	20	8	212	21.2	2.93
	Group size	4	3	2	3	2	4	6	3	2	2	31	3.1	
Wet	Population size	42	18	18	43	20	23	22	18	28	14	246	24.6	3.21
	Group size	6	3	2	6	4	5	2	3	3	2	36	3.6	

#### **Population Structure:**

Out of the total (458) number of *C. g. gallarum* recorded during this study, 119 (25.98%) were adult males; 149 (32.53%) adult females; 68 (14.85%) sub adult male, 51(11.14%) sub adult female and 71 (15.50%) were juveniles/young (Table 2). The

pairwise comparison of the different age and sex groups through paired t test showed that, the number of adult females was not significantly different from the number of adult male (t= 1.884, df= 19, P= 0.075), but statistical different from sub-adult male (t= 6.698, df = 3, P< 0.05), sub-adult female (t= 8.876, df= 19, P< 0.05), and unidentified sex juveniles (t= 6.253, df = 19, P< 0.05).

There was no statistically significant difference among the various age and sex groups of *C. g. gallarum* counted between wet and dry season ( $\chi 2= 20.0$ , df = 16, P= 0.220) (Table3). However, the population structure during the dry study period was accounted highest percentage of adult males (33.21%) followed by adult females (25.47%), while the remaining 14.15%, 13.68% and 13.21% were juveniles, sub-adult females and adult males, respectively. On the other hand, during the wet season, 52.3% was accounted by adult females while adult males accounted for 54.6% which was the highest. The rest 16.67%, 8.94%, and 16.26% of the population were accounted for juveniles, sub-adult females and adult males, respectively. There was a significant difference among the different age and sex groups counted during the dry season (F = 9.366, df= P<0.05).

Seasons		Adult	Adult	Sub-adult	Sub-adult	Unidentified	Total
		Male	Female	Male	Female	juvenile	
Dry	Sum	54	71	28	29	30	212
	% of Total Sum	25.47%	33.49%	13.21%	13.68%	14.15%	100
Wet	Sum	65	78	40	22	41	246
	% of Total Sum	26.42%	31.71%	16.26%	8.94%	16.67%	100
Total	Mean	59.5	74.5	34	25.5	35.5	229
	% of Total Sum	25.98	32.53	14.85	11.14	15.50	100
	Std. Error of Mean	0.694	0.667	0.582	0.426	0. 473	

Table 2. Age and sex class composition and percentage of C. g. gallarum

## Age and Sex Ratio of C. g. gallarum:

The average sex ratio of the adult male to adult female was 1.00:1.25 and adult female to unidentified juvenile or young was 2.1:1.00 in the entire study area (Table 3). The age and sex ratio of sub-adult females to adult females were the highest during both the dry (1.00:2.45) and the wet (1.00:3.55) seasons in the study area. The male to female sex ratio was highest in the dry (1.00:1.22) season, but lowest during the wet (1.00:0.95) season. Unidentified juvenile to other age and sex individuals were highest during both dry (1.00:6.07) and wet (1.00:5.0) season.

Table 3. Age and sex ratio of C. g. gallarum during wet and dry seasons

Season	Age and sex ratio												
	AM:AF	SAM:AM	SAF:AM	UJ:AM	M:F	SAM:AF	SAF:AF	UJ:AF	UJ: Other				
Dry	1.00:1.31	1.00: 1.93	1.00: 1.86	1.00:1.80	1.00: 1.22	1.00: 2.54	1.00: 2.45	1.00: 2.37	1.00:6.07				
Wet	1.00:1.20	1.00: 1.63	1.00:2.95	1.00:1.59	1.00:0.95	1.00:1.95	1.00:3.55	1.00:1.9	1.00:5.0				
Total	1.00:1.25	1.00:1.75	1.00:2.33	1.00:1.68	1.00:1.07	1.00:2.19	1.00:2.92	1.00:2.1	1.00:5.45				

**\*Hint:** AM=adult male, AF=adult female, SAM= sub-adult male, SAF=sub adult female, M= Male, F= Female, UJ= Unidentified juvenile, Other= all age and sex except unidentified juvenile

## Feeding Ecology: Dietary Composition:

A total of 1558 feeding observations were recorded from scan sampling of *C. g. gallarum*. From these overall observations, 760 were observed during the dry season and 798 were observed during the wet season. The overall diet of *C. g. gallarum* during the study period is shown in Figure 1. Based on the overall percentage contribution of plant

parts to the diet of *C. g. gallarum* from each species, young leaves were the largest contributor to the overall diet followed by shoots, accounting for 35.1% and 22.1% of all feeding records, respectively in both seasons (N=1558). Other common items were included mature leaves (20.6 %), bark (17.7%), fruit (2.8%) and flower (1.7%).



**Fig. 1.** Overall percentage or observation of feeding time devoted to different food items by *C. g. gallarum* (N= 1558 feeding records)

In the dry season, the most frequently consumed food item by *C. g. gallarum* was young leaves, which accounted for 37.0% of feeding records (N=760) followed by mature leaves (22.6%), shoots (18.8%), barks (16.2%), fruit (3.2%) and flower (2.2%). Young leaves (33.3%), shoots (25.3%), barks (19.0%), mature leaf (18.7), fruit (2.4%), and flower (1.3%) made substantial contributions to *C. g. gallarum*'s diet in the wet season. However, there was no significant difference (P> 0.05) between seasons in time spent feeding on shoots, roots and flowers (Fig. 2).



**Fig.2.** Seasonal percentage contribution devoted to different food items consumed by *C*. *g. gallarum, feeding records* N=760 *and* 798 *in Dry and Wet season, respectively* 

#### **Dietary Diversity and Food Choice:**

According to the feeding activity recoded result, *C. g. gallarum* were observed feeding on 19 plant species belonging to 12 families. The percentage contribution and food items consumed are given in Table 4. The highest contribution of the diet was from the family Rosaceae (41.67%). Family Fabaceae, Moraceae and Myrsinaceae were contributing 50% of the diet and the other families shared 8.33%. Based on the overall percentage contribution, *Schefflera volkensii* was the most consumed plant species which accounted for 7.96%, *Urera hypselodendron* and *Dombeya torrida* ranked second and third of the overall diet of guereza (7.83% and 7.06%), respectively.

No.	Scientific Name	Family Name	Parts consumed	Frequency of	Time spent
				observation	(%)
1.	Schefflera volkensii	Araliaceae	ML, FR	124	7.96
2.	Urera hypselodendron	Urticaceae	YL, S	122	7.83
3.	Dombeya torrida	Sterculiaceae	YL, ML, FL	110	7.06
4.	Ficus vasta	Moraceae	YL,ML,FR	99	6.35
5.	Ficus sur	Moraceae	YL,ML,FR	94	6.03
6.	Prunes Africana	Rosaceae	YL,ML,BK	92	5.91
7.	Carissa spinarum	Cynaceae	Yl	91	5.84
8.	Myrsine Africana	Myrsinaceae	YL, ML	86	5.52
9.	Hygenia abyssinica	Rosaceae	Yl	85	5.46
10.	Maytenus gracilipes	Celasteraceae	YL, ML	84	5.39
11.	Albiza gummifera	Fabaceae	YL,ML,FL	82	5.26
12.	Myrsine melanophloeos	Myrsinaceae	YL	77	4.95
13.	Solanum adoense	Solanaceae	YL	74	4.75
14.	Galiniera saxifrage	Rubiaceae	YL, FR	73	4.69
15.	Rubus steudneri	Rosaceae	YL, FR	65	4.17
16.	Morus alba	Rosaceae	YL, ML, FR	57	3.66
17.	Rosa abyssinica	Rosaceae	FR	55	3.53
18.	Crotalaria agatitlora	Fabaceae	YL,ML	54	3.47
19.	Podocarpus falcatus	Podocarpaceae	FR	34	2.18
Total ob	oservation		1558		

Table 4. Percentage plant species contribution and items consumed by C. g. gallarum

\*\*Hint: YL: Young Leaf, ML: Mature Leaf, FR: Fruit, S: Shoot, FL: Flower

## DISCUSSION

The Guereza remains relatively widespread and abundant, and, owing to its tolerance of forest degradation, is considered to be one of the least threatened species of colobus monkey (Jensz and Finley, 2011). *Colobus guereza gallarum* is one of the endemic wild mammals found in Ethiopia. The sample count of *C. g. gallarum* in Harenna forest revealed that there were 229 average numbers of individuals observed. The total number of *C. g. gallarum* counted was 212 during the dry season and 246 during the wet season in the entire study area. The individual number of *C. g. gallarum* slightly increased during the wet season might be due to more quality and availability of food during the wet season than the dry season. Since, as Chapman and Chapman (2002) found that densities of primates are related to food quality and availability.

The number of groups or troops was greater during the wet (36) than the dry (31) season. Similarly, Ohsawa (1979) found that the largest multi-troop occurred more in the middle of the dry season than the wet season. During the dry season, the troop size increased and foraged together to an area where plenty of fruits and leaves were available. The group size may vary in seasons. During the dry season, food availability was limited to a given habitat. Therefore, two or three troops were mixed as one troop and foraged together; as a result, the group size increased, but the group number

decreased.

The average ratio of sub-adult female to adult female were 1.00:2.45 during the dry season and 1.00:3.55 during wet seasons in the study area. Unidentified juvenile to other age and sex individuals was 1.00:5.45. Adult females' number was higher than adult males due to the earlier maturation of females. The male to female sex ratio was 1.00:1.22 in the dry season, but 1.00:0.95 during the wet season.

All Guereza species and subspecies are highly folivorous and rely heavily on the leaves of plants (Harris and Chapman, 2007). It possesses a large and multi-chambered stomach which allows them to better digest plant fibers including foliage. This ability to digest plant material is also assisted by bacteria in the stomach. Together, these and other morphological adaptations allow Guereza to feed on large quantities of leaves (Gron, 2009; Jensz and Finley, 2011). However, in my study, C. g. gallarum were relied upon as foraging 55.7% for leaves (35.1% young leaves and 20.6% matured leaves), 22.1% for shoots, and 17.7% for barks. Fruits and flowers were the least consumed food items during the observation time. These results are agreed with the discoveries of Petros et al. (2018) stated that, of the overall diet of C. g. gallarum 82% leaves, 14% Fruits and 2% shoots and flowers were the least consumed food items during the observation time. My result is also in agreement with another study on other Guereza species/subspecies conducted in other areas as it shows that plant leaves constitute much of the guereza diet (Chapman et al., 2007). Thus, the major part of the species diet is heavily dependent on plant leaves. According to Oates and Davies (1994), Colobus monkeys in general rarely include more than 30% mature leaves in their diet unless they are of good quality. They eat high-quality young leaves and cannot maintain themselves on mature leaves of low quality for long periods. The reason for a high percentage of young leaves in their diets in my study could be because the troops were observed throughout the short and long rains period

In the present study, 35.1% of *C. g. gallarum*'s diet consists of young leaves. The finding is in line with Hussen and Ejigu (2017) study, *C. g. gallarum* fed mainly on young leaves of different plant species in order to maximize their physiological demand and minimize toxicity from mature leaves and other plants part. A similar study in Cameron indicated that about 35–75% of guereza's diet consists of young leaves which are easier to digest and are less toxic (Usongo and Amubode, 2001). *C. g. gallarum* spent 20.6% of their diet on mature leaves, which is in agreement with Hussen and Ejigu (2017) findings, as they stated that *C. g. gallarum* spent 26.91% of diet are matured leaves in Gidabo Forest, Sidama Zone, Ethiopia. Oates and Davies (1994) also examined colobus monkeys in general rarely include more than 30% mature leaves in their diet unless they are of good quality. Fleshy fruits are usually consumed by guereza when unripe, with consumption being reduced as they fully ripen, likely to avoid competition with other primate species that prefer ripe fruit (Fashing, 1999; Chapman *et al.*, 2006; Harris and Chapman, 2007). However, the diet of guereza is highly varied seasonally and geographically (Kim, 2002).

In total *C. g. gallarum* in Harenna forest was foraged from 19 plant species. Out of these food plant species in my study, 5 species were the same as Hussen and Ejigu (2017) study in Gidabo Forest and 7 plants were similar with Petros *et al.* (2018) study in Bale Mountains National Park. The total 19 plant species identified throughout the study period as being consumed by *C. g. gallarum* was higher compared to the findings of Hussen and Ejigu (2017) (15 species) and Petros *et al.* (2018) (8 species). Nonetheless, the difference in the number of plant species reported as consumed by *C. g. gallarum* in the study area and the former might be due to the duration of study periods and the availability of foraging plants. Therefore a higher number of different plant species foraged from is expected, as plants are of lower nutritional quality during the dry season and so colobus monkeys would have to forage from a greater variety to meet their nutritional needs (Lowe and Sturrock, 1998). Also, whole-day observations would cover much more of the foraging behaviour such as the midday peak (Teichroeb *et al.*, 2002).

The largest troop occurred in the wet season than the dry season. Due to a shortage of food during the dry season, troop size decreased and foraged together to an area where better fruit and leaves were less available. Generally, *C.g.gallarum* mainly forage on leaves of which young leaves was the most consumed plant food item and their foraging activities. Thus, to maintain sustainable conservation of the endemic *C.g.gallarum* in the area, their habitats should be properly protected and appropriate monitoring strategies should be designed. Government officials and concerned bodies also should be given to protect the species natural habitat and the primates that are living there. Other aspects of *C.g.gallarum* such as reproduction behaviour, home range ecology, and etc. should be conducted in further studies in the study area.

## Acknowledgment

The authors thank Madda Walabu University and Debre Berhan University for logistic and financial support. They are also grateful to the local administrators and people who helped them during the investigation period.

# **Authors' Contribution**

SM and MH equally conceived, designed the investigation and collected the data. SM analyzed the data and prepared the manuscript. SM and MH read and approved the final manuscript for publication and agreed to be held accountable for the work performed therein.

## **Consent for publication**

This manuscript does not contain any individual person's data, and further consent for publication is not required/ applicable.

# **Ethical Approval**

All applicable international, national, and institutional guidelines for the care and use of animals were followed. We respected the welfare of animals and excluded situations when animals were in pain.

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