Assessment of the primate community composition in the Kafa Biosphere Reserve

Andrea Schell & Karina Schell

1 HIGHLIGHTS

- This is the first broad assessment to determine the primate species composition of the Kafa BR conducted in a diverse set of habitats such as bamboo and montane forests or wetlands covering an altitudinal gradient from 1400m asl to 2700m asl.
- The Kafa BR is possibly home to 6 primate species of 5 different genera. We recorded all of them:
    - Olive Baboon (*Papio anubis*)
    - Guereza (*Colobus guereza guereza*)
    - Grivet Monkey (Chlorocebus aethiops aethiops)
    - Ethiopia lesser Galago (*Galago senegalensis dunni*)
    - De Brazza's Monkey (Cercopithecus neglectus)
    - Boutourlini's Blue Monkey (Cercopithecus mitis boutourlinii)
- We can confirm the presence of 1 vulnerable primate species endemic to the western side of the Ethiopian rift Valley: The Boutourlini's Blue Monkey (*Cercopithecus mitis boutourlinii*).
- The Boutourlini's Blue Monkey, just like the De Brazza's Monkey, is a forest-dwelling monkey that avoids colonising disturbed forest patches. These two primate species will hugely profit from the Biosphere Reserve and the permanent establishment of extended core areas and buffer zones.
- We present the first proof of the Ethiopia Lesser Galago (*Galago senegalensis dunni*) for the Kafa Biosphere Reserve; exact location: Sheaka Wild Coffee Forest, a PFM site, in the Awurada Valley. We further also provide the first loud-call recording, crucial for subspecies determination.
- We support the current choice of the Guereza as flagship species for the Kafa BR as it is a very common, easy to recognize and a widely appreciated primate species.
- All primate species mentioned hereafter are known as demanding species when it comes to habitat integrity and moderate agriculture and/or forestry. We therefore strongly recommend the following primate species to be treated as indicators for the intactness and diversity of a habitat, as well as for an environmentally sound agricultural and/or forest management system:

  - Intact and divers forest ecosystem: Boutourlini's Blue Monkey, De Brazza's Monkey, Ethiopian Lesser Galago
  - Environmentally sound (forest) farming: Guereza, Ethiopian Lesser Galago
- Olive Baboons and Grivet Monkeys are usually perceived as crop raiders, often causing conflicts with small-scale farmers. This bad reputation is confirmed by a variety of locals of the Kafa Biosphere Reserve, thus holding potential for participatory learning and action (PLA) based workshops on human-wildlife conflict management. Activities should be particularly directed at farmers that are reliant on plant cultivation.
- Along an altitudinal gradient we found Olive baboons, Guerezas and Grivet Monkeys in a broader altitudinal range than Boutourlini's Blue Monkeys, Ethiopia lesser Galagos and De Brazza's Monkeys.

2 INTRODUCTION

According to the relevant literature (Butynski et al. 2013; Berhan 2008) six primate species in the Kafa BR may occur: Olive Baboon (*Papio anubis*), Guereza (*Colobus guereza guereza*), Grivet Monkey (*Chlorocebus aethiops aethiops*), Ethiopia lesser Galago (*Galago senegalensis dunni*), De Brazza's Monkey (*Cercopithecus neglectus*) and the Boutourlini's Blue Monkey (*Cercopithecus mitis boutourlinii*).

2.1 Olive Baboon (*Papio anubis*)

The genus *Papio* includes five species that are also followed by the IUCN red list of endangered species (IUCN 2014): *Papio anubis* (or Olive Baboon), *Papio cynocephalus* (or Yellow Baboon), *Papio hamadryas* (or Hamadryas Baboon), *Papio papio* or (Guinea Baboon), *Papio ursinus* (or Chacma baboon) (Groves 2001).

The Olive Baboon is a common (IUCN 2014), an extremely adaptable and the most extensively distributed baboon species. It inhabits Sahelian woodlands and forest-mosaic habitats (e.g. Butynski et al. 2013). *P. anubis* occupies an enormous variety of vegetation and climate conditions from lowlands to high mountains from 500m asl to 3300m asl in e.g. Ethiopia (Yalden et al. 1977). Throughout its range it is considered as crop raider and continuing habitat loss intensifies conflicts with humans (Kingdon et al. 2008a; Butynski et al. 2013).

2.2 Guereza (*Colobus guereza*)

Guerezas (or *Colobus guereza ssp.*) belong to the Black-and-White Colobus monkeys of the genus *Colobus* (Groves 2005; 2007). They are distributed across forested areas in the centre of Africa ranging from Nigeria and Cameroon through the northern Democratic Republic of Congo and southern Sudan to Ethiopia, Kenya and Uganda and southwards into northern Tanzania (Oates 1977; Groves 2001). Their preferred forest habitats include lowland and medium-altitude moist forest, montane forest, swamp forest, dry forest and gallery forest but also disturbed forests (Oates 1994; Fashing et al. 2012), although highest population densities are found in fragmented and secondary forests (Oates 1977). Oates investigation reveals that even on an altitudinal scale they appear to be highly adaptable, as they are reported to occur from *ca.* 200m asl in Cameroon to at least 3300m asl in Ethiopia.

According to the latest IUCN (2014) assessment their conservation status is of "Least Concern". Although some population locally decline due to habitat loss, the generally widespread species is not thought to be declining fast enough to place it in a higher category of threat (Kingdon et al. 2008b).

As the Guereza taxonomy is subject of an ongoing debate we apply the provisional classification of Groves (2001; 2005) and Grubb et al. (2003) that lists eight subspecies: *C. g. occidentalis, C. g. dodingae, C. g. matschiei, C. g. percivali, C. g. kikuyuensis, C. g. caudatus, C. g. gallarum* and *C. g. guereza.*

The latter two subspecies are known to occur in Ethiopia (Butysnki et al. 2013). Whereas *C. g. gallarum* is restricted to the Ethiopian highlands east of the Rift Valley, the subspecies *C. g. guereza* is present in the forested areas west of the Rift Valley (Grubb et al. 2003). Döschner (2010) further confirms the presence of the subspecies *C. g. guereza* in the Kafa Biosphere Reserve. The study also suggests that Guerezas are not as capable of bearing habitat disturbance and degradation as well as they were thought to (Chapman et al. 2000; Fashing 2002; Lwanga 2006; Harris & Chapman 2007). Döschner further found that the population density of Guerezas negatively correlates with the intensity of coffee management in their potential forest habitats.

2.3 Grivet Monkey (*Chlorocebus aethiops*)

The genus *Chlorocebus* (or African Green Monkey) is widely distributed throughout sub-Saharan Africa (Butynski et al. 2013). In this report we taxonomically follow the most recent scientific findings of Groves (2001; 2005) that recognizes six species: *C. aethiops* (Grivet), *C. djamdjamensis* (Bale Monkey), *C. sabeus* (Green Monkey), *C. cynosuros* (Malbrouck Monkey) and *C. tantalus* (Tantalus Monkey; with subspecies *C. t. budgetti*, *C. t. marrensis*, *C. t. tantalus*) as well as *C. pygerythrus* (Vervet; with subspecies *C. p. hilgerti*, *C. p. excubitor*, *C. p. nesiotes*, *C. p. rufoviridis*, *C. p. pygerythrus*).

Excepting *C. djamdjamensis,* an endemic and "Vulnerable" species of the Ethiopian Bale Mountains (Butysnki et al. 2008; Mekonnen 2012) all other *Chlorocebus* species are abundant in a variety of habitat types and are listed as "Least Concern" (IUCN 2014). It is an extremely adaptable species that can live in both rural and urban environments. It is persecuted as a crop pest (Kingdon & Butynski 2008; Butynski et al. 2013) and the expansion of agricultural activities intensify the conflict between Grivets and Humans (Zinner et al. 2002).

For the Kafa BR we expect to find *C. aethiops* (Butynski et al. 2013; Haus & Zinner, pers. comm.) a common species also native to Djibouti, Eritrea, South Sudan and Sudan (Dandelot & Prevost 1972). This species is present in savannah, open woodland and forest-grassland mosaic, especially close to rivers (Dorst & Dandelot 1972; Zinner et al. 2002). Dandelot (1974) describes a geographic variation *C. a. matschiei* endemic to SW Ethiopia within and west of the Rift Valley (Kafa and Jimma districts). Haus (pers. comm.) also found the specimens of *C. aethiops* found in the Kafa region to differ from the typical "Savannah *aethiops*", being darker, with a woolly fur and a less pronounced brow band. It could resemble *C. Djamdjamensis*, but genetically it can be assigned to *C. a. aethiops* (Haus et al. 2013).

2.4 Lesser Galago (*Galago senegalensis* ssp.)

The Galagos (Family *Galagidae*) are nocturnal and often difficult to observe, and most species are phenotypically cryptic (Masters & Bragg 2000). They can best be identified by their species-specific advertisement calls (e.g. Butynski et al. 2013). For Ethiopia, two Galago species have been described: *G. gallarum*, for the north western Rift Valley (Butynski & de Jong 2004), and the *G. senegalensis* group. The latter group is possibly the most widespread small Galago in the whole of Africa (Butynski et al. 2013). This species, listed as "Least Concern", is found in all strata of savannah woodland, in dense to open bush land areas, in montane forest (e.g. Mau forest, Kenya, and Harenna forest, Ethiopia), and even in highly fragmented forests or cultivated areas (Bearder et al. 2008).

Apart from *G. s. senegalensis,* Grubb et al. (2003) recognizes three more subspecies: *G. s. braccatus*, *G. s. sotikae* and G. *s. dunni.* The latter subspecies has been described for the Ethiopian Plateau and

Somalia but its actual range limits are uncertain (Butynski et al. 2013). However, *G. s. dunni* is the only subspecies currently recognized for Ethiopia (pers. comm. Butynski).

2.5 Genus *Cercopithecus*

Both the De Brazza's Monkey (*Cercopithecus neglectus)* and the Blue Monkey (*Cercopithecus mitis spp.*) belong to the genus Cercopithecus (or Guenons). The De Brazza's Monkey is one of the most widespread of Africa's forest monkeys, though never very abundant (Brennan 1985; Decker 1995; Maisels et al. 2007; Mwenja 2007). This widespread species ranges from north-eastern Angola, Cameroon, Equatorial Guinea and Gabon in the west of its range to Uganda, Kenya and south-western Ethiopia in the east (Maisels et al. 2007). Hereby, Ethiopia is the northern limit of the species' range (Brown & Urban 1969).

It is known as the Swamp Monkey, as it is found close to rivers in lowland and submontane tropical moist forest, semi-deciduous forest and Acacia dominated forest (Kingdon 1971). The De Brazza's Monkey is considerably less conspicuous than most other Guenons (Gautier-Hion & Gautier 1978).

According to the IUCN (2014) the De Brazza's Monkey is probably not threatened in the main forest block of central Africa. But it probably is in East Africa, where its habitat is under severe threat of human encroachment through deforestation of habitats for agricultural land and timber (Brennan 1985; Butynski 2002b; Mwenja 2007). Although Brown & Urban (1969) find De Brazza Monkeys to be common in south west Ethiopia (near Godare), its actual status in Ethiopia is unknown (Butynski et al. 2013).

The Blue Monkey belongs to *Cercopithecus (nictitans)* group, in which three species are frequently recognized: *C. nictitans*, *C. mitis* (or Blue Monkey) and *C. albogularis* (or Sykes's Monkey). The great morphological variability and taxonomy of all monkeys of the *C. (nictitans)* group remain poorly understood (Grubb et al. 2003).

Generally, the Blue Monkey is a versatile and widespread African species (Colyn & Verheyen 1987; Lawes 1990; Colyn 1991; Gautier-Hion et al. 1999; Butynski 2002a/b). It is present in many different forest types, including lowland and montane tropical moist forest, riverine and gallery forest and bamboo forest (Lawes *et al.* 1990).

South western Ethiopia is the range of *C. m. boutourlinii* (Napier 1981), one of the 17 recognized subspecies of *Cercopithecus mitis ssp.* (Groves 2001; 2005; Grubb et al. 2003). The Boutourlini Blue Monkey is endemic to the area from Lake Tana southwards along the western side of the Ethiopian Rift Valley (Yalden et al. 1977; Butynski & Gippoliti 2008; Butynski et al. 2013). *C. m. boutourlinii is* categorized as "Vulnerable". According to the IUCN 2014 its greatest threats are destruction and fragmentation of forest habitat for agricultural land. Although this species tolerates low quality and disturbed habitat better than most Guenons (Lawes et al. 1990; Tesfaye et al. 2013), it nevertheless occurs in lower densities in these habitats (Chapman et al. 2000). It shows poor local colonizing ability in response to forest fragmentation and seldom occupies small forest patches (Lawes et al. 2000, Chapman et al. 2003).

First field studies on habitat requirements of *C. m. boutourlinii* in SW Ethiopia (Jibat forest) were conducted in 2009 (Tesfaye et al. 2013). Interestingly, according to the final report of the Kafa faunal survey, Prof. Afework Bekele of the Addis Ababa University suggests a possible hybrid of *C. neglectus* and *C. mitis* ssp. in the Kafa region.

This biodiversity assessment in the Kafa BR covers a wide range of different habitats, from bamboo and montane forests to wetlands, covering an altitudinal gradient from 1400m asl to nearly 3000m asl.

In terms of distribution patterns, we expected to find the generalist primate species like *Papio anubis*, *C. g. guereza* and *C. aethiops* in every of the above mentioned habitats including anthropogenically altered landscapes. Reasons for this are their ecological flexibility and extreme

adaptability. We expected a similar pattern for *Galago s. dunni*, although it might be present in a lower altitudinal range. We expected the forest monkeys *C. m. Boutourlinii* and *Cercopithecus neglectus* to be abundant in both lowland and montane forest, from riverine and gallery to bamboo forests. As the latter species prefers swampy habitats and is often found close to rivers we especially expected to find it in the wetlands of the Kafa Biosphere Reserve.

3 MATERIALS AND METHODS

3.1 Study sites

Due to time constraints this biodiversity assessment focused on surveying sites in two of the three National Forest Priority Areas, namely Bonga and Boginda forest.

3.1.1 Bonga forests

We explored the **Boka Wild Bamboo Forest**, which is a unique habitat covered by the monodominant species *Arundunaria alpina.* This is a mountain bamboo forming thickets on mountain slopes at 2400-3000 m and mostly in isolated patches. It is located in the very eastern stretch of the Bonga forest area, which is an example for the unique faunal composition of very dense bamboo undergrowth, homogeneous or mixed, as well as for a rather high altitude between 2400m to 3050m asl and almost sub-afroalpine conditions; the highest elevation during our assessment.

As reference area for lower altitudes and moderate forest management conditions, namely Participatory Forest Management (PFM) sites, we assessed montane and riverine habitats inside the **Sheaka Wild Coffee Forest.** These areas are located in the Awurada Valley, which is located in the southern most stretch of the Bonga forest area, as well as of the whole Kafa Biosphere Reserve. This forest occurs between 1500m and 2600m asl and is of global conservation significance, as wild Arabic coffee (*Coffea arabica*) still grows naturally in this area. Land conversion and timber extraction are causes for concern.

We also surveyed the **Komba Forest**, an evergreen montane forest and grassland complex distributed between 1900m and 3300m asl, which is located in the northern part of the Bonga forest area. It is a highly populated, fragmented and rather overgrown forests; already classified as a high priority core zone.

We also explored the forest habitats close to Bonga town and the Kafa Development Association Guesthouse (KDA GH). These sites are in the northern, more central part of the Bonga forest area. The so-called "**Guesthouse Forest**" is the part of a heavily disturbed, open woodland stretch. **Kayakela Forest**, however, is located even further out of Bonga city and represents a comparatively lesser disturbed area with an elevation of max. 1700m asl (Döschner 2010).

3.1.2 Boginda Forests

Inside Boginda forest areas we surveyed different sites that are all located in the southern, central part of this forest priority area. The furthermost point was again a moist evergreen montane forest, the **Saja Forest**, merging into the riverine, rather marshy habitats of the **Gojeb Wetland,** with altitudes ranging from 900m to 2600m asl. This whole ecosystem is highly at risk, due to intense harvesting activities and exploitation.

Tulla Forest ("Hot Spring" hiking trail) is situated towards the southern part of Boginda, towards Bonga city. It is characterized by a montane forest extending into an evergreen montane forest and grassland complex.

3.2 Sample methods

3.2.1 General data acquisition

We conducted a general survey throughout predetermined areas as our tool for an initial, general assessment of the primate community composition of the Kafa BR, rather than a rigorous standardization of distance or transect walks. The reason for that is the behavioural ecology of primate species, our target group. Primates, especially shyer species and species with large territories or home ranges, are extremely difficult to track in unknown and unexplored areas and within a very limited timeframe. However, we concentrated on obtaining very general indices of the primate species pool and conducted continuous field surveys during the field work period (covering several sites in Bonga as well as areas in Boginda forest (see Tab. 1). This data can be used as a basis for further, more rigorous research and monitoring activities.

3.2.2 Surveys and interviews

We firstly conducted interviews with five local field assistants, rangers and small farmers, as the basis for the selection of study areas. We showed photographs of a number of primate species that could occur in the Kafa BR and asked for information about them. Furthermore, we let them listen to sound recordings of nocturnal primate species e.g. Bushbabys (*Galagidae*) because we did not expect anyone to actually recognize a nocturnal species from a picture. This procedure was repeated at each sampling site with locals from the study area.

We included images of primate species that we knew could not occur in the Biosphere Reserve, such as Barbary macaques (*Macaca sylvanus*) or Chimpanzees (*Pan troglodytes*). We additionally formulated open questions ("tell us something about the primate species you recognize in the pictures") so that the respondents were able to tell us anything that he/she thought would be useful information to us. In that way we could minimize false statements and, moreover, learn about the peoples' interests and attitudes towards certain primate species (e.g. particularly Baboons are widely regarded as crop raiders).

We conducted day as well as crepuscular or night surveys, sampling each site just once and by following a rather opportunistic approach in terms of sample methods. We made used of the following methods: direct observations, camera traps, vocal recordings and live traps (collapsible squirrel/muskrat sized Tomahawk live traps, code 202 e.g. from Tomahawk live Trap, Hazelhurst, Wisconsin, U.S.A.); with the latter two mapping methods being mainly applied during crepuscular or night surveys to cover the occurrence of nocturnal primates, for example Bushbabys (*Galagidae*). Live traps were equipped with bait such as mashed ripe bananas and fermented honey wine (Pozzi pers. comm.).

Records of diurnal primate species were collected through direct observations and/or through their vocalising behaviour. General survey walks differed in length and time spent in the field. On average, we started early morning at sunrise, collecting live traps that we had set the night before, during a night survey which started at around sunset. Core surveys during the day, however, began in the morning and ended before dusk.

We determined geographic coordinates of each record we found with a GPS map 62s device from Garmin (GARMIN, Schaffhausen, Switzerland) set to the datum format WGS 84. Audio files were recorded using a Marantz PMD 660 sound recorder (Marantz Corporation, Kawasaki, Japan) equipped with a Sennheiser ME66 shotgun condenser microphone covered up with a windshield (Sennheiser GmbH & Co. KG, Wedemark-Wennebostel, Germany). Image files such as video footage and pictures were taken with a Nikon D90 SLR camera together with a Nikon 18-200mm Nikkor Lens (Nikon Corporation, Chiyoda/Tokyo, Japan).

Table 1: Survey timetable & sampling site selection. During a full 9 day biodiversity assessment we sampled different sites in the Bonga and Boginda forest areas. General survey walks differed in length and time. Night surveys began at dusk and were followed up. Legend: ✪ *night survey only,* ○ *day survey only,* ● *night and day surveys*

Sampled site and habitat type (number and code of study sites)			03.12.14	04.12.14	05.12.14	06.12.14	07.12.14	08.12.14	09.12.14	10.12.14	11.12.14
Bonga Forests	Sheaka Wild Coffee Forest (4, AW)	Moist evergreen montane forest, PFM* site					●	●			
	Boka Wild Bamboo Forest (1, BA)	High elevation, bamboo forest		●	●						
	"Guesthouse Forest" (11, KDA GH)	Montane forest remnants				○					
	Kayakela Forest (11, KDA GH)	Montane forest remnants	✪								
	Komba Forest (3, KO)	Evergreen Mountain Forest and Grassland Complex									○
Boginda Forests	Boginda Forest (10, BO)	Moist evergreen montane forest							○		○
	Tulla Forest (10, BO)	Moist evergreen montane forest									○
	Saja Forest (8, GO-wet)	Evergreen Mountain Forest and Grassland Complex							●	●	

***Participatory Forest Management (PFM) = initially established in 2005 at Kafa, the PFM is a forest management concept that includes a set of techniques and processes and the participation of the state forest departments and local communities. The logic of the PFM gives particular relevance to the level of knowledge of local communities as well as their key role as forest managers.**

3.3 Biological data collection

Faecal samples were collected opportunistically and measured, photographed and predetermined according to relevant literature such as "A Field Guide to the Tracks & Signs of Southern, Central & East African Wildlife" by Chris and Mathilde Stuart.

We used small branches and disposable gloves to collect faecal samplings, always considering a careful handling routine to avoid (cross) contamination. Each sample was stored in a 20ml collection tube filled with at least 90% undiluted ethanol and was further labelled with a clearly traceable number for further DNA analysis. All faecal samples were then kept for at least 24 hours in ethanol before transferred onto silica and dried until DNA extraction. We used Silica Gel Orange as drying agent (e.g. from Carl Roth GmbH & Co. KG, Karlsruhe, Germany). We then prepared 20ml storage tubes with the matching number of a collection tube and filled them halfway up full with silica and a piece of cotton to separate specimens from the drying agent. Dry tissue and hair samples, occasionally collected from road kills or killed animals by e.g. snare traps, were stored directly on silica.

3.4 Data analysis of image and sound files

Records of primates from image files and/or direct observations were determined using relevant classification literature as e.g. “The Kingdon Field Guide to African Mammals” by Jonathan Kingdon. For more detailed classification on the subspecies level, image and/or sound material were discussed with renowned primatologists familiar with species in this or in surrounding areas, e.g. Ph.D. Thomas M. Butynski (Wild Solutions) and Ph.D. Andrew Perkin (Nocturnal Primate Research Group) for Blue Monkeys (*Cercopithecus mitis* ssp.) and Lesser Galagos (*Galago senegalensis* ssp.), Dr. Dietmar Zinner (German Primate Center) for Baboons (*Papio anubis*) and Guerezas (*Colobus guereza* ssp.) and Dr. Tanja Haus (German Primate Center) for Green Monkeys (*Chlorocebus aethiops* ssp.).

Predetermined faecal samples were analysed in accordance with the national regulations of the Ethiopian Biodiversity Institute (EBI). All organic samples were prepared and exported properly and with no other objective than to complete a full species list for the Kafa Biosphere Reserve. Sample analyses was undertaken at the Primate Genetics Laboratory of the German Primate Center in Goettingen, Germany, and in collaboration with other experts: Christiane Schwarz (Technical Assistant), Dr. Rasmus Liedigk (Guest Scientist) and PD Dr. Christian Roos (Senior Scientist). See following chapter for further information on DNA analyses.

3.5 Data analysis of biological samples

3.5.1 DNA Extraction

We used the First-DNA-ALL-Tissue-Kit by GEN-IAL (GEN-IAL GmbH, Troisdorf, Germany) for DNA extraction of all faecal, tissue and urine samples, because it is suitable for various and especially degraded substrates and secondly because it is known for high yields of pure molecular DNA. We followed the standard protocol with minor changes (see Appendix 2). In terms of hair samples, we removed hair follicles of 3 hairs of each sample and amplified DNA by direct polymerase chain reaction (PCR) rather than prior DNA extraction.

3.5.2 DNA Amplification

In all reaction tubes we used wax pellets as a vapour barrier, separating 2 distinct layers with a lower layer comprising all dNTP’s and primers and an upper layer consisting of Taq-Polymerase, BT and template DNA. That way we delayed the reagents mixing as well as reducing the occurrence of non-specific products until the first heating step of the PCR amplification. We further used BioTherm Taq DNA Polymerase (Ares Biosciences GmbH, Cologne, Germany) for all samples in a 20 µl PCR mix (*premix 1:* 1 µl reaction buffer, 0,2µl dNTP’s, 1 µl for forward primers, 1 µl for reverse primers and 6,8 µl HPLC-purified water; *premix 2:* 2 µl reaction buffer, 4 µl BT, 0,2 µl Taq-Polymerase, 10,8 µl HPLC-purified water) with 10 µl of premix 1, 17 µl of premix 2 and 3 µl of template DNA for all faecal, tissue and urine samples, but 20 µl of premix 2 together with several hair follicles for hair samples. PCR reactions were conducted with one negative control (HPLC-purified water).

We generated two overlapping 700bp long fragments of the Cytochrom B region. Conditions for PCR amplification comprised a pre-denaturation step at 94°C for 2 min, followed by 40 cycles at 94°C for 1 min, annealing at 60°C for 1 min and extension at 72°C for 1 min and a final phase at 72°C for 5 min. Respective primers are available upon request.

3.5.3 DNA sequencing

PCR products were visualised on a 1% agarose gel. Sequences were run on an ABI 3130xL sequencer using the Big Dye Terminator Cycle Sequencing Kit (*both:* Applied Biosystems by Thermo Fisher Scientific, Waltham, Massachusetts, U.S.A.) and matching forward and reverse primers.

We assembled and aligned sequences with the program BioEdit 7.2.5 (Tom Hall, Ibis Biosciences, Carlsbad, California, U.S.A.).

4 RESULTS AND DISCUSSION

4.1 Assessment of the primate species composition

We have 57 records of 6 primate species (*Papio anubis, Colobus guereza guereza, Chlorocebus aethiops aethiops, Galago senegalensis dunni, Cercopithecus neglectus, Cercopithecus mitis boutourlinii*) comprising 19 biological samples (18 faecal and 1 urine sample) as well as 31 direct sightings, 1 foot print and 4 vocal recordings from 8 different sampling sites (see Tab. 2).

Due to data insufficiency we cannot clearly specify a particular area or habitat type with the highest or lowest primate species diversity. Detection frequency closely correlates with the behavioural ecology of a target species, as well as its social system or tolerance towards humans or landscapes altered by humans; its abundance and distribution and its detectability. Shy or rare species are almost impossible to track in unknown areas and within a very limited timeframe, whereas curious and common species are easy to find. Considering this bias, we are only able to vaguely highlight "primate rich areas".

Table 2: Species composition & sample collection. We have records of 6 primate species evident through a number of different detection methods (sightings, DNA samples, audible behaviours and vocal recordings, tracks and signs). Some primate species were detected

Sampled site and habitat type (number and code of study sites)			Cercopithecus mitis boutourlinii	Chlorocebus a. aethiops	Colobus g. guereza	Cercopithecus neglectus	Galago senegalensis dunni	Papio anubis
			vu	lc	lc	lc	lc	lc
Bonga Forests	Sheaka Wild Coffee Forest (4, AW)	UTM zone 37 N 7,093674 N 36,22671 E 1400m – 1800m asl			••• •		•	••• •• •
	Boka Wild Bamboo Forest (1, BA)	UTM zone 37 N 7,268285 N 36,455492 E 2000m – 2700m asl		•••	•••		••• •	••• •• • t
	"Guesthouse Forest" (11, KDA GH)	UTM zone 37 N 7,241035 N 36,45217 E 1800 – 1900 m asl	••• ••		•••			*
	Kayakela Forest (11, KDA GH)	UTM zone 37 N 7,314515 N 36,242543 E 1700m – 1800 m asl						*

	Komba Forest (3, KO)	**UTM zone 37 N 7,299871 N 36,090997 E 1800m – 2200m asl**	*	•••	•••			
Boginda Forests	**Boginda Forest (10, BO)**	**UTM zone 37 N 7,508285 N 36,061888 E 2100m – 2200m asl**						••
	Tulla Forest (10, BO)	**UTM zone 37 N 7,44789 N 7,44789 E 1600m – 1800m asl**		•••	•••	•••		••• ••
	Saja Forest (8, GO-wet)	**UTM zone 37 N 7,55529 N 36,060923 E 1500m – 2200m asl**		•••				••• ••

Some primate species, however, were detected more often than others. We found Olive baboons (*Papio anubis*) in 7 of 8 sampling sites. This is also the case with Guerezas (*Colobus guereza*). Records of Grivet Monkeys (*Chlorocebus a. aethiops*) were obtained from every second sample site, too (4 out of 8). Ethiopia lesser Galagos (*Galago senegalensis dunni*), Boutourlini's Monkey (*Cercopitecus mitis boutourlinii*) and De Brazza's Monkeys (*Cercopithecus negelctus*) were recorded considerably less and from fewer sample sites (in the order in which they are mentioned: 2 out of 8, 1 out of 8, 1 out of 8, respectively).

The same pattern applies to the detectability of relevant primate species along an altitudinal gradient. We found Olive baboons, Guerezas and Grivet Monkeys in a broader altitudinal range than Boutourlini's Blue Monkeys, Ethiopia lesser Galagos and De Brazza's Monkeys, the latter's range being even narrower. Concerning the fact that our total surveying activity covered a very wide altitudinal range from 1400m asl to up to 2700m asl we can confirm that both Olive baboons and Guerezas are present at every elevation we sampled at and similar results apply to Grivet Monkeys (1700m to 2600m asl). The Ethiopia Lesser Galago, as well as Boutourlini's Blue Monkey, were found within a similarly wide range (1500m up to 2200m), although less remarkable or elevated. The very shy De Brazza's Monkey was only recorded between 1600m and 1700m asl.

We can confirm the presence of 4 out of 6 species in both the dense and high-altitude Boka Wild Bamboo Forest and the moist evergreen montane forests in Boginda Forest areas such as Saja and Tulla Forest. The assessment further revealed the occurrence of 3 out of 6 primate species in the Sheaka Wild Coffee Forest, a PFM area in the Awurada Valley, as well as in Komba forest. Surveys in the forest stretches around the KDA guesthouse also came to the same result. The latter two forests, however, provided evidence of the only vulnerable primate species recorded, the Boutourlini's Blue Monkey. Surveys in the Boka Wild Bamboo Forest and the Sheaka Wild Coffee Forest, two very different habitats compared to each other, provided data on the occurrence of the Ethiopia Lesser Galago.

We can therefore confirm that the Olive baboon, Guereza and Grivet Monkey are very generalistic primate species which can cope with a variety of different habitats, including anthropogenically altered landscapes (Butynski et al. 2013; Döschner 2010; Zinner et al. 2002). They even seem to benefit from anthropogenic objects and land-use changes like the conversion of forests into agricultural land. Especially the Olive baboon appears to flourish in agricultural centres. Its adaptability and ecological flexibility are the causes for its bad reputation as a crop raider among small-scale farmers (Kingdon et al. 2008c).

These three species can be found throughout the study area. In contrast to this, the remaining three primate species are either stenoecious like the De Brazza's Monkey, whose very specific habitat requirements make it vulnerable to habitat destruction and loss (Brennan 1985; Butynski 2002b; Gautier-Hion & Gautier 1978; IUCN 2014; Mwenja 2007), already listed as vulnerable, e.g. like the Boutourlini's Blue Monkeys, or nocturnal, like the Ethiopia Lesser Galago, and therefore recorded considerably less. The latter is common but dependant on mostly undisturbed or moderately managed mature, primary forests.

4.2 More specific findings

4.2.1 *Papio anubis*

Molecular, phylogenetic analyses based on Cytochrom B DNA sequences indicate three different haplotypes detected for our study area (Boginda Forest Area (10, BO), Awurada Valley (4, AW), Boka Wild Bamboo Forest (1, BA)) which are already known for this area and which complement specimens from Uganda, DR Congo and north-western Tanzania. They can be further differentiated from central and southern rift Olive Baboons from eastern Ethiopia, Kenya and northern Tanzania.

4.2.2 *Galago senegalensis dunni*

The Ethiopia Lesser Galago (*Galago senegalensis dunni*) is, at present, the only recognized subspecies of G. senegalensis in Ethiopia (Butynski pers. comm.). We collected one sound recording of an individual from the Sheaka Wild Coffee Forest (4, AW), a honk call, which resembles previous recordings of *G. senegalensis dunni* calls. Further analysis with sound recordings of other populations, as well as additional surveys, may reveal more information on the sub-species level.

4.2.3 *Cercopithecus mitis boutourlinii*

The Boutourlini's Blue Monkey (*Cercopithecus mitis boutourlinii*) is currently the only recognized subspecies of *C. mitis* for Ethiopia (Butynski pers. comm.). We have video footage of a vocalising adult individual from the "Guesthouse forest" (11, KDA GH) as well sightings in the Komba Forest (3, KO) reported to us by other team members. This *C. mitis* call sounded similar to calls of individuals from coastal and eastern Tanzania, however it is shorter and more clipped (Perkins pers. comm.). Further analysis and surveys may reveal more details.

5 CONCLUSIONS AND RECOMMENDATIONS FOR CONSERVATION AND MONITORING

We recorded all primate species that are currently described for south-west Ethiopia. The Olive Baboon occurs in every sample site we visited, as do the Guereza and the Grivet Monkey. They are still very widespread and abundant, so that it appears no major threats are resulting in a range-wide population decline. All of them, however, could be perfect study subjects for enhanced human wildlife conflict management. They raid and ruin crops more or less strongly and are therefore in constant conflict with small-scale farmers. There is great potential for future conservation activities that focus on participatory learning and action (PLA) based workshops on human-wildlife conflict management. Activities should be particularly directed at farmers that rely on plant cultivation.

More importantly, we were also able to record primate species that are difficult to detect during a limited period in the field. We can therefore confirm that the Kafa BR provides suitable habitat conditions for primate species that have very strict habitat requirements; these include the Boutourlini's Blue Monkey, endemic to the western side of the Rift Valley, and the De Brazza's

Monkey. Both are forest oriented monkeys that avoid colonising forest patches but in turn are dependent on wide and structured forests. This is why habitat destruction and human encroachment represent their greatest threat in the future. In contrast to the already mentioned generalist primate species, these two representatives are a perfect example for future enhanced conservation activities and monitoring programmes. They will benefit from the Biosphere Reserve and especially from undisturbed core zones and their connectivity. Especially for the De Brazza's Monkey, classified as least concern, long-term research and monitoring is of high importance, as its conservation status in this, its northern most range, is still insufficiently assessed. Same is true for the remaining Boutourlini's Blue Monkey population in SW Ethiopia. Furthermore, for the conservation of the Boutourlini's Blue Monkey future phylogenetic studies are extremely important, because its taxonomy is only very poorly understood. We recommend that both primate species should be regarded as indicator species for the integrity of montane forests.

The Kafa BR with its habitat variation is extremely interesting for Galago research. Furthermore, Galagos have generally been studied less well, and therefore hold potential for the development of smart field research approaches for nocturnal, small-sized, arboreal primate species. Here again, phylogenetic research is of high importance, because the taxonomic substructure of *Galago senegalsenis* ssp is still far from being understood.

We support the current choice of the Guereza as flagship species for the Kafa Biosphere Reserve. Compared to the other five primate species, the Guereza meets all aspects of a flagship species: it is common, easy to recognize and popular, with no bad reputation, compared to e.g. the Olive Baboon.

Furthermore, the Guereza is a demanding species when it comes to bearing with habitat disturbances or habitat degradation (Chapman et al. 2000; Fashing 2002; Lwanga 2006; Harris & Chapman 2007) which is why its flagship species status could be expanded to include the additional status of that of an indicator species for healthy, more or less undisturbed habitats.

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8 Appendix

Appendix 1: Table 3: Primate species recorded during the Biodiversity Assessment in the Kafa Biosphere Reserve (December 2014)

No.	Scientific Name	Family	English name	Habitat, Forest Type	Study sites	Distribution	IUCN Threat Status	CITES Appendix	Endemism
1	*Cercopithecus mitis boutourlinii*	Cercopithecidae	Boutourlini's Blue Monkey	primary tropical deciduous and riverine forest at altitudes of 400-2,000 m asl	11, KDA GH 3, KO	R, Endemic to SW Ethiopia (western part of the Ethiopian Rift Valley)	vulnerable A2c, population decreasing	II	*
2	*Chlorocebus a. aethiops*	Cercopithecidae	Grivet Monkey	Savannah, montane forests (2000m asl), woodland, riverine landscapes and cultivation mosaics or urban areas; depends on Acacia trees, Fig trees, foliage and gum (highly adapted)	1, BA 3, KO 10, BO 8, GO-wet	W, from Khartoum (Sudan) in the north to Mongalla in the south, and in Djibouti, Ethiopia and Eritrea where it is found south of the River Omo and ranges as far east as the Ethiopian Rift Valley	least concern, population stable	II	-
3	*Colobus guereza*	Cercopithecidae	Guereza, Black-and-white Colobus	Wide ranging: montane forests, rainforest, Acacia-dominated riverine galleries. Prefers secondary over primary forests.	4, AW 1, BA 11, KDA GH 3, KO 10, BO	W, distributed in a band across the centre of Africa, from Nigeria and Cameroon east through the northern DR Congo, through southern Sudan to Ethiopia, Kenya and Uganda and south into northern Tanzania	least concern, population trend unknown	II	-
4	*Cercopithecus neglectus*	Cercopithecidae	De Brazza's Monkey,	River-oriented monkey, linear home range along river and streams; lowland, swamp forest	10, BO	(R)W rare in Ethiopia only distributed in southern Ethiopia, otherwise	least concern, population	II	-

No.	Scientific Name	Family	English name	Habitat, Forest Type	Study sites	Distribution	IUCN Threat Status	CITES Appendix	Endemism
			Swamp Monkey	(frequently flooded), semi-deciduous, Acacia-dominated, montane forests (2100m asl), lower montane galleries and bamboo forests		distributed from Angola, Cameroon, Central African Republic, DR Conga, Guinea and in small patches in Ethiopia, and Kenya	trend unknown		
5	*Galago senegalensis dunni*	Galagidae	Ethiopia Lesser Galago	Lowest level of mature primary forest, woodlands dominated by *Acacia, Isoberlinia, Combretum* and *Julbernardia*	4, AW 1, BA	W, distributed in a band across the centre of Africa, from Senegal in the west to Sudan, Somalia and the east as well as Kenya and Tanzania in the south	least concern, population trend unknown	II	-
6	*Papio anubis*	Cercopithecidae	Olive Baboon, Anubis Baboon	Most extensively distributed Baboon species Lowland into deep rain forest, occurs from 500-3000m asl, sometimes also above tree line, seldom found more than 2km into the forest; benefits from resent climatic changes and seems to have no clear ecological boundary. Hybridises with Hamadryas Baboon (*P. hamadryas*) e.g. in the Awash region, Ethiopia, or with Yellow Baboon (*P. cynocephalus*) e.g. in the Amboseli National Park, Kenya	4, AW 1, BA 11, KDA GH 10, BO 8, GO-wet	W, *very widespread* Throughout sahelian woodland from southern Mauritania and Mali to the Sudan and southwards to the Democratic Republic of Congo and Tanzania. Outlying populations inhabit the Tibesti and Air massifs in the Sahara. In East Africa, the distribution is actively changing also due to hybrid zones	Least concern, population increasing	II	-

*Yalden et al. (1977); Butynski & Gippoliti (2008)

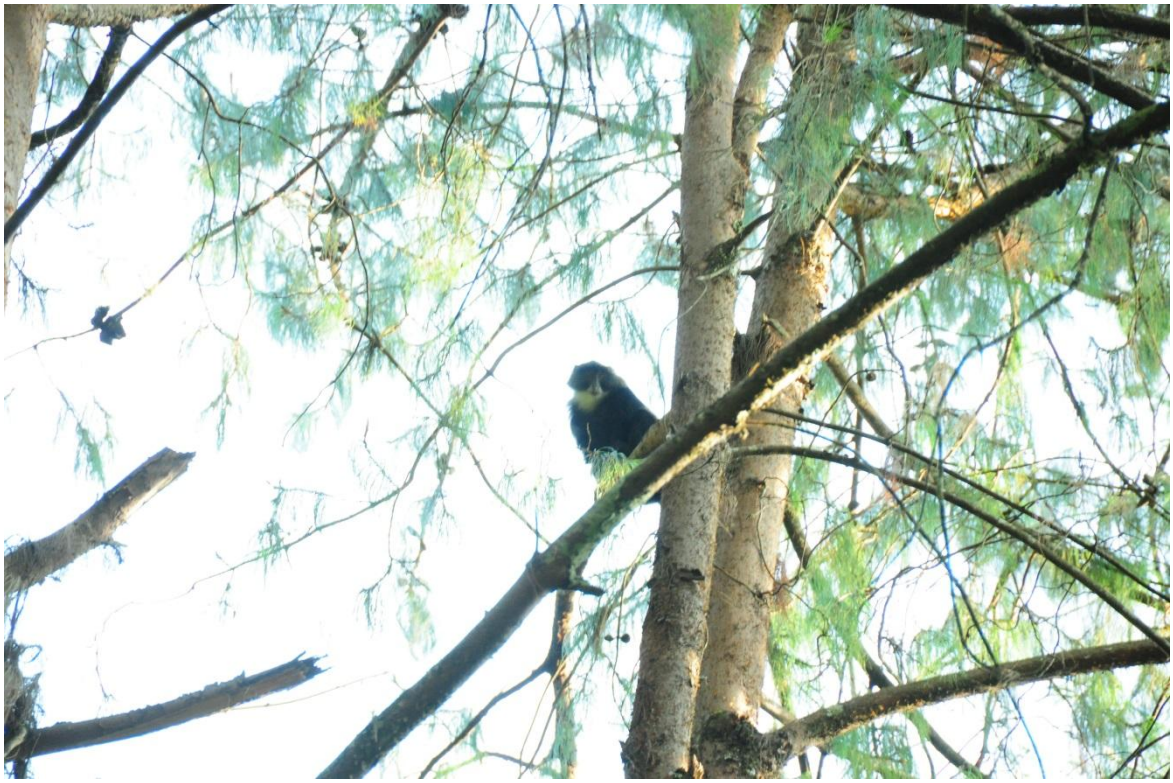
Appendix 2. DNA Extraction with the First-DNA-All-Tissue-Kit by GEN-IAL (GEN-IAL GmbH, Troisdorf, Germany)

lysis	01	Use low-binding tubes for all work steps as well as dual-filter pipet tips and change tips for every new sample.
	02	Switch on the sample heater and cool EtOH 70 % and DTT at -20°C. Sample racks need to be placed at +4°C and -20°C, too.
	03	Cut sample into tiny pieces and transfer it into a 2 ml tube.
	04	Add 1000 µl Lysepuffer 1, 100 µl Lysepuffer 2 and 20 µl Proteinase K (Enzyme).
	05	Add 10 µl 1 M DTT.
	06	Vortex samples.
	07	Incubate at 65°C at 1400 rpm for 1 hour on thermo mixer.
	08	Reduce temperature and incubate over night at 37°C at 1000 rpm.
	09	Spin at maximal speed for 10 min until sample is dissolved.
	10	Use time to label new tube: 2x 2 ml tube, 1x 1.5 ml tube.
seperation	11	Transfer 1000 µl supernatant into a new 2 ml tube. A galantine mass may occur at the bottom of the tube. Be careful not to transfer this mass. Do not use more than 1000 µl to ensure that there is enough space for add-on substances.
	12	Add Chloroform (80 % of volume, e. g. 400 µl for 500 µl supernatant).
	13	Invert several times in hand (8x).
	13	Spin at maximal speed for 10 min.
	14	Carefully transfer upper phase into a new 2 ml tube. Stop 1-2 mm before interface to avoid contamination.
	15	Add Lysepuffer 3 (75 % of volume, e. g. 375 µl for 500 µl supernatant).
	16	Vortex for 20 sec.
	17	Incubate at -20°C for 5 min.
	18	Spin at maximal speed for 20 min.
precipitation	19	Transfer 800 µl of supernatant into a new 1.5 tube.
	20	Add Isopropanol (2-Propanol 100 %) (80 % of volume, e. g. 640 µl for 800 µl supernatant).
	21	Invert several times in hand (8x).
	22	Incubate at +4°C for 30 min.
	23	Spin at maximal speed for 15 min.
	24	Remove supernatant by using a 1000 µl tip at least two times. Make sure not to touch or remove the pellet.
purification	25	Wash pellet with 300 µl EtOH 70 % (-20°C).
	26	Spin at maximal speed for 5 min.
	27	Carefully remove supernatant by starting with a 1000 µl tip and proceeding with a 100 µl tip. Try to absorb all of the alcohol without touching or removing the pellet.
	28	Dry pellet for 30 min with lid open to allow evaporation. Sample is ready once tube is completely free of any drops of fluid. (In order to fasten this step, sample can be heated up to 37°C. Tube will remain open.)
storage	29	Dissolve DNA in 50 µl HPLC water and freeze sample at -80°C. (In case you expect a high amount of DNA, e. g. in tissue samples, elute DNA in 100 µl HPLC water).

Appendix 3. Primate species catalogue

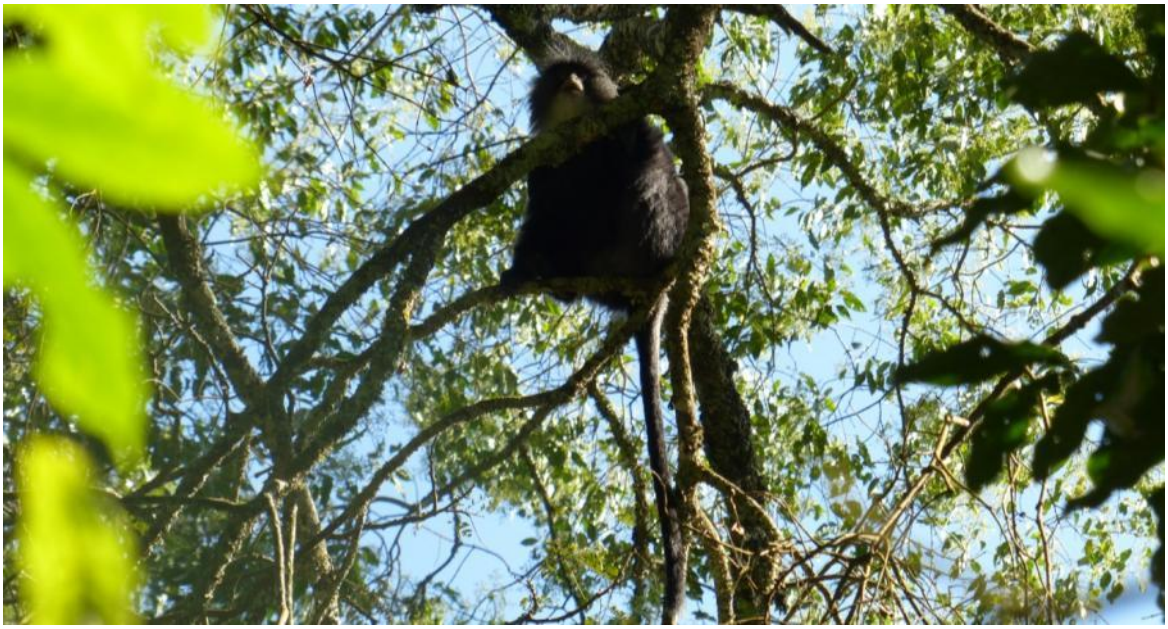
1. Boutourlini's Blue Monkey (Cercopithecus mitis boutourlinii)

IUCN vulnerable, CITES II, endemic to SW Ethiopia



Picture taken in „Guesthouse Forest" (11, KDA GH) 2014 by K. Schell

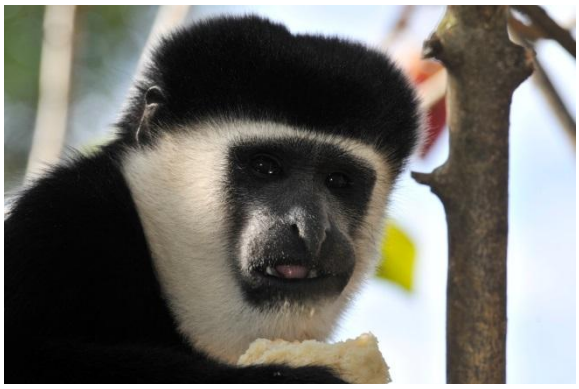
(Sound recording available here: https://drive.google.com/open?id=0BycmwOdoZGMJa0pkU0xqdUNBMVU&authuser=0)



Picture taken in Komba Forest (3, KO) by B. Walter

2. Guereza or Black-and-white Colobus (*Colobus guereza guereza*)

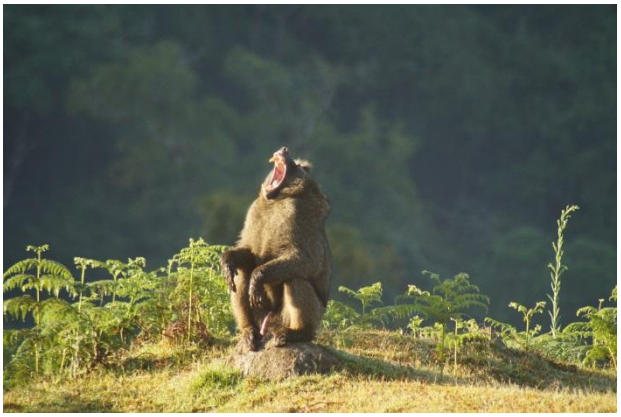
IUCN least concern, CITES II




Pictures taken in Waliso Negash Lodge, by Holger Meining

3. Olive Baboon or Anubis Baboon (*Papio anubis*)

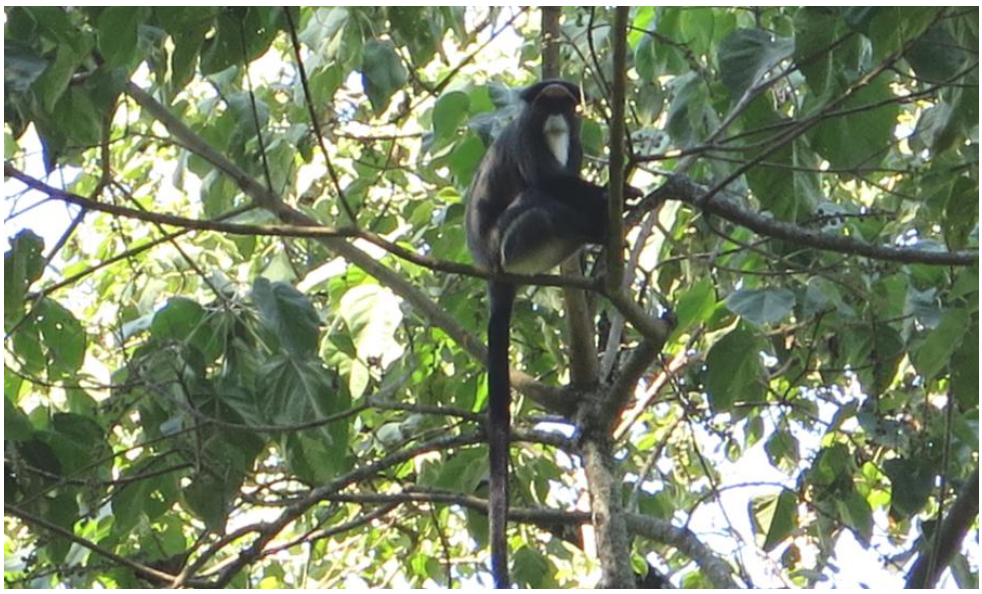
IUCN least concern, CITES II

Pictures taken by Tom Kirschey

4. De Brazza's Monkey or Swamp Monkey (*Cercopithecus neglectus*)

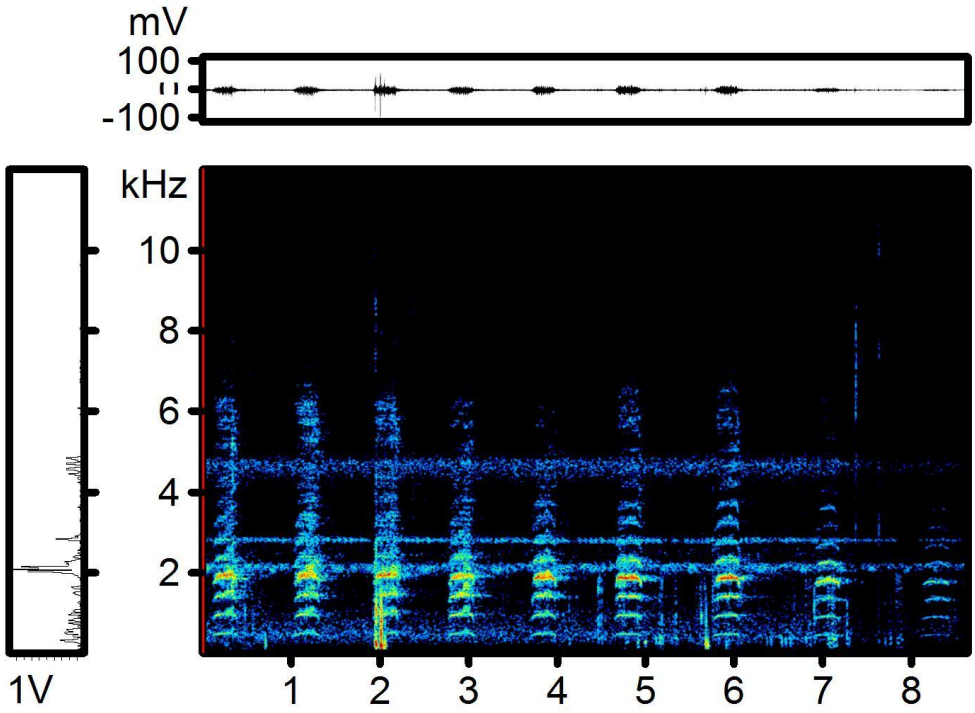
IUCN least concern, CITES II



Picture taken in Tulla Forest (10, BO) 2014 by F. Kölbl,

5. Ethiopia Lesser Galago (*Galago senegalensis dunni*)

IUCN least concern, CITES II



Sound recording in the Sheaka Wild Coffee Forest (4, AW) 2014 by K. Schell / Sonogram by A. Perkin

(Sound recording available here: https://drive.google.com/open?id=0BycmwOdoZGMJYjE2eTdvN3FwbDQ&authuser=0)

6. Grivet Monkey (Chlorocebus aethiops aethiops)

IUCN least concern, CITES II

Picture taken in Kafa 2008 by B. D'Amicis

(http://www.naturepl.com/search/preview/grivet--vervet-monkey-chlorocebus-/0_01229745.html)