Creating an ID-catalogue as a tool for identifying individual giraffes at Entabeni Game Reserve as a prerequisite for collecting individual faecal samples for hormone analyses

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Abstract

The giraffe is an even-toed ungulate that lives on the African continent below the Sahara desert. Once widely distributed, the giraffe population is now in decline due to habitat degradation and poaching. In order to evaluate current management practices and environmental conditions, faecal hormone profiles can be monitored and used to assess reproductive success. Therefore an Identification Catalogue (ID catalogue) for the roaming giraffe population at Entabeni Game Reserve (EGR), Limpopo, South Africa, was initially compiled in February 2011. The catalogue includes photographs of both sides of each giraffe, age classification and bodily characteristics of in total 45 giraffes which were individually identified at the lower escarpment of EGR. As a subsequent objective, the possibility to collect individual faecal samples from the catalogued giraffe population was evaluated. Between February and April 2011, a total of 134 faecal samples from 45 individuals were collected; 56 samples from 14 males (n = 1-6 per individual), 69 samples from females (n=0-9 per individual), 3 samples from juveniles (n = 0-2 per individual) and 8 samples from the calves (n = 0-5 per individual). Overall, the collection frequency was on average 1 sample per 9-13 days for females and males, respectively. This study shows that in a small reserve of 6400 ha identification of individual giraffe is possible by the creation of an ID catalogue. Such a catalogue subsequently allows collection of individual faecal samples which e.g. could be used to monitor reproductive or stress-related activities within the population on an individual level.

Introduction

The giraffe is an even-toed ungulate that lives in arid and dry savannah forest areas on the African continent below the Sahara desert. The *Giraffidae* family includes the Okapi (*Okapi johnstoni*) and is part of the order Cetartiodactyla, family *Giraffidae*[1]. Currently, there are nine subspecies of giraffe described, based on differences in coat pattern, range and historic observations[2]. However, genetic research into mitochondrial DNA sequences and nuclear microsatellite loci has demonstrated that there may be at least six genealogically different lineages of giraffe, instead of just one[3]. Also, five of the six lineages contain genetically discrete populations, making the total of genetically distinct populations eleven[3]. The giraffe was once widely distributed throughout Africa[4], but is now restricted to the sub-Saharan region in discontinuous patches (Fig. 1.) due to habitat degradation and poaching[5].
The species is listed as Least Concern (LC) by the IUCN. The total population is estimated at less than 100.000 animals, with two of the subspecies, G.c. ssp. *Peralta* and G.c. ssp. *Rothschildi*, classified as endangered[1]. The subspecies living in Southern Africa are G.c. spp. *angolensis* and G.c. spp. *giraffe*.

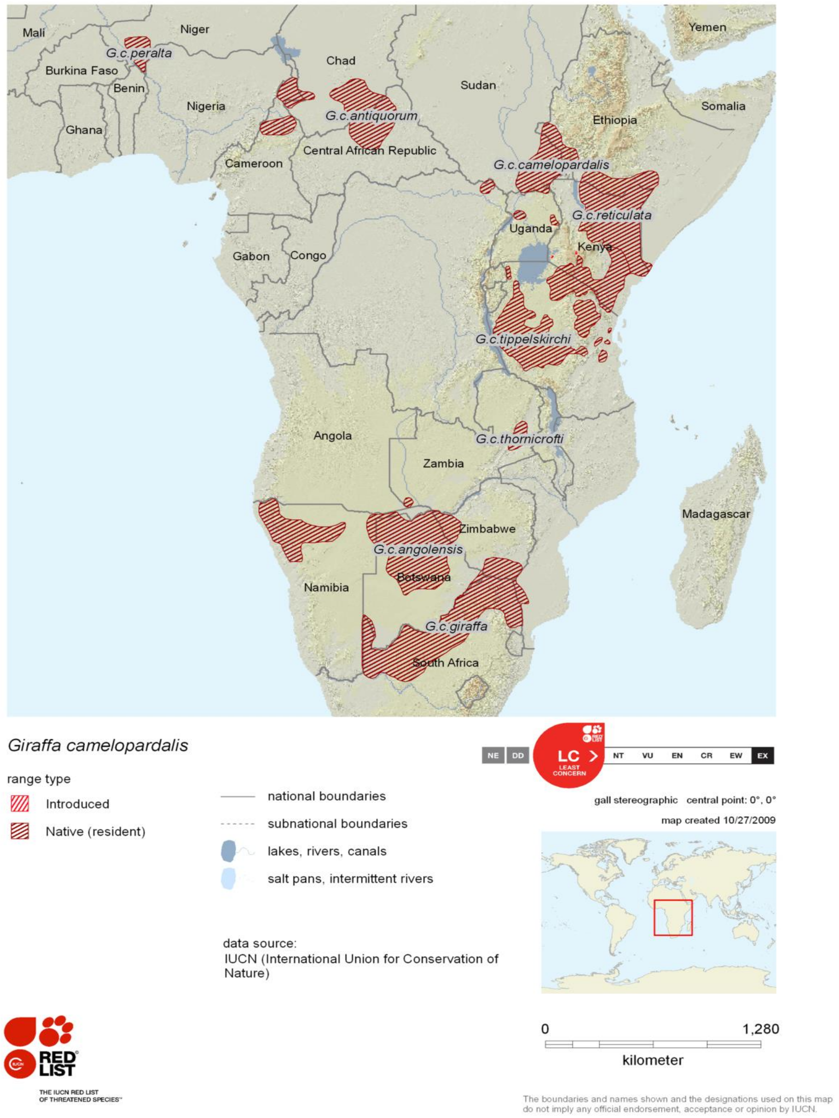

Figure 1. Distribution of the giraffe over the sub-Saharan African continent (IUCN)

The giraffe is typically associated with dry savannas, ranging from scrub to woodland where a particular range of food plants can be found to cover seasonal requirements. The giraffe is mainly a selective browser, feeding on leaves of *Acacia*, *Commiphora, and Combretum* species[1, 6, 7]. Giraffe have also been reported to graze on the herb layer in the dry season[8]. For a long time giraffe have been thought to live in open social herds of which the basis is formed by social relationships among individual adult female giraffes[2, 9-13]. The continuation of mother-daughter and allomothering relationships are the most important instead of peer bonds as observed in other ungulate species[14]. Giraffe males are solitary and move between female subgroups to investigate urine samples and assess the reproductive status of the females[2, 12]. The female giraffe is a non-seasonal polyestrous breeder with young being born throughout the year. Giraffe can be identified individually by their unique coat pattern and other morphological characteristics[9, 12]. The coat pattern does not change after birth, but the colour does with bulls going darker by age[9].

Giraffe populations have been monitored for a variety of reasons, e.g. determining population dynamics[2, 13, 15] and home range sizes[10, 16, 17] or for investigating behavioural questions[11, 13, 18] and reproductive events[19-26]. In order to monitor giraffe populations in the wild, they will need to be tracked. Satellite collaring as one form of tracking is difficult in giraffe due to their long neck, on which a normal collar would easily slide off. Special neck-chest harnesses exist and are being used to track giraffe[16, 27, 28], however, capturing the animals and applying the collars is a difficult operation as giraffe, unlike other large ungulates, have problems with anaesthesia because of their unique morphology[27, 29].

As an alternative to collaring, giraffes can be individually identified and subsequently monitored by using an ID catalogue for identification [9, 15, 30-32]. In this regard, Backhaus[31] used horn tufts, angle of the horns and scars for identification, but these marks can change over time and are not suitable for long term studies. Foster[9] found out that the spot pattern is a unique characteristic in giraffe as it does not change with age. In his study, Foster used a photograph of the left side of the neck in combination with sex and age, whereas Leuthold[30] photographed both sides of the neck and used a different age classification system. By being able to identify individuals, it is also possible to collect biological material like faeces, urine and blood on an individual basis, which would allow to e.g. monitoring of stress factors and reproductive cycle based on adrenocortical and reproductive hormones.

For example, faeces can be used for non-invasive measurements of both reproductive and stress hormones. This would make it possible to link observed behaviour to their subsequent biological mechanisms.

The overall aim of the study was to create an ID catalogue for the giraffe population at Entabeni Game Reserve, South Africa. As a second objective, we evaluated the possibility to collect individual faecal samples from the catalogued giraffe population.

Materials and method

Study site

Entabeni Game Reserve is situated in the Waterberg biosphere in the province of Limpopo, South Africa (fig. 2). The reserve occupies a total of 22 000 ha of land and has five separate ecosystems divided by electric fences or steep cliffs. Migration between the different areas is not possible. For this study, only the population at the lower escarpment (6400 ha) has been studied (fig. 3). The climate at Entabeni Game Reserve is moderate and has three seasons: a hot wet season from November to April, a cool dry season from April to August and a hot dry season from August to October (Entabeni resource inventory 2006).

The lower escarpment of Entabeni Game Reserve contains a variety of antelope species, zebra, hippo, elephant and rhinoceros. At the beginning of the study, the giraffe population was estimated at 25-35 animals. The population is predated on by a population of lions, killing on average 3-5 individuals per year (Entabeni game management pers. comm.).

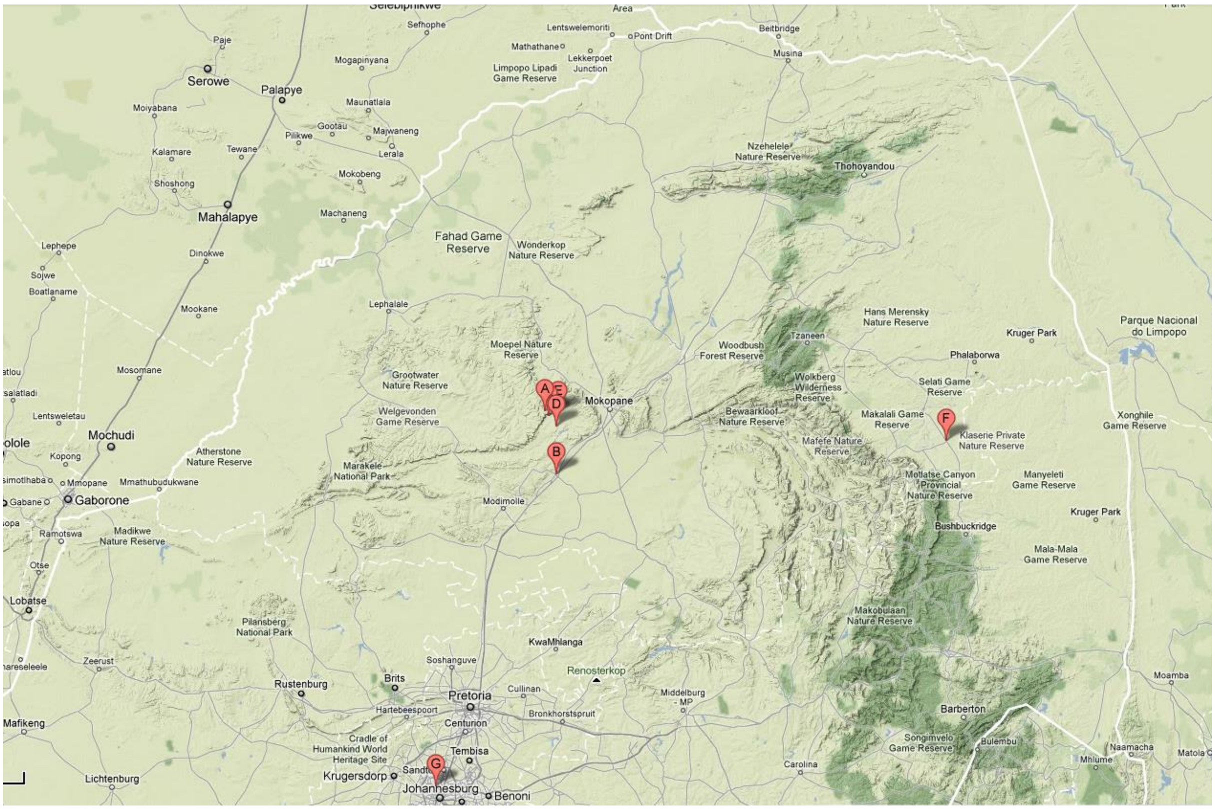

Figure 2. Locality map of Entabeni Game Reserve (A)

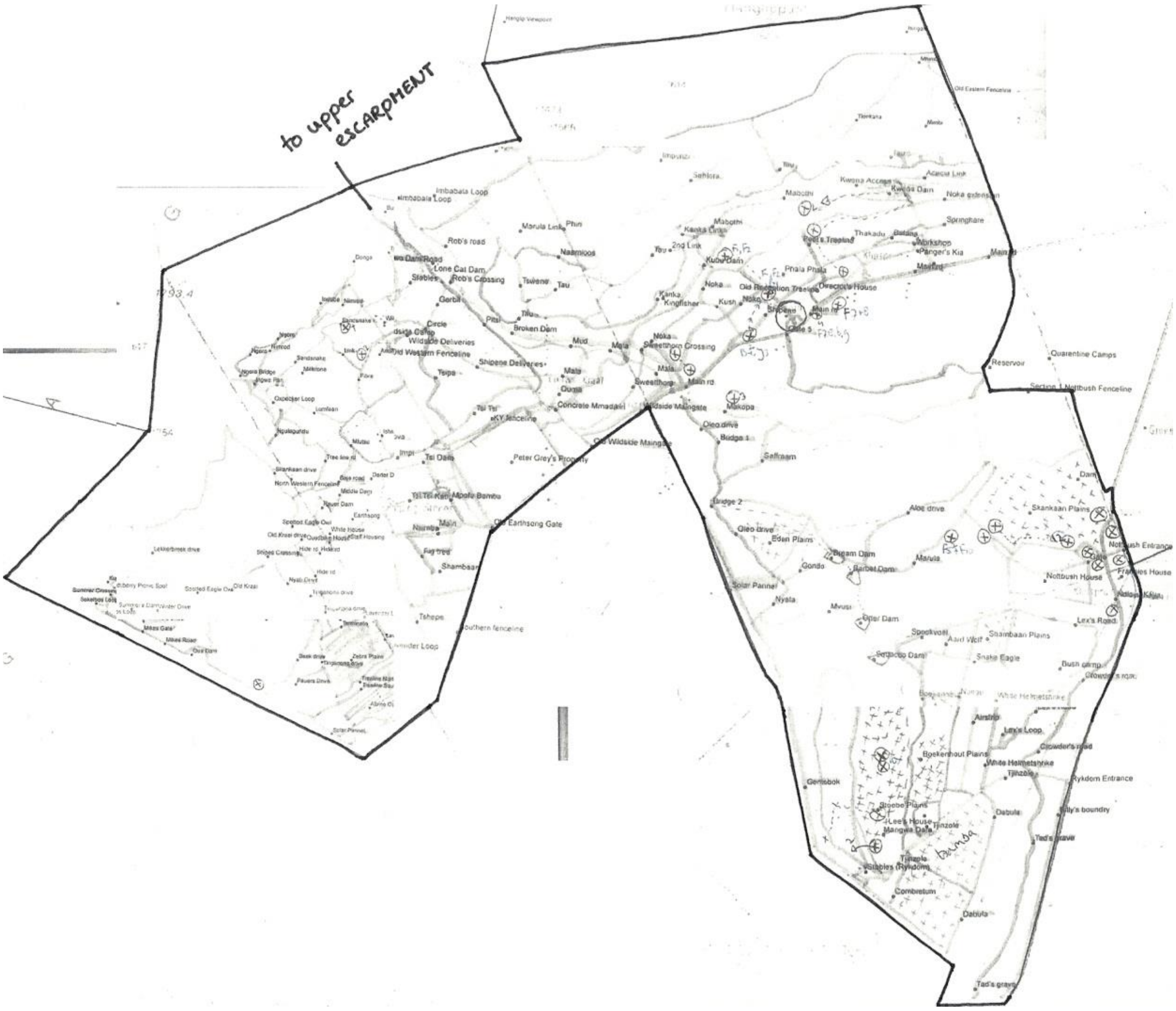

Figure 3. Map of the lower escarpment of Entabeni Game Reserve

Animal tracking and tracing

For 80 days between 08.02.2011 – 30.04.2011, 79 field trips were undertaken into the lower escarpment of Entabeni Game Reserve to track giraffe. Giraffe herds were observed together with experienced staff from Entabeni Game Reserve (EGR) during their daily routine in the field. Tracks were followed when encountered and different areas systematically searched. Giraffe were tracked, photographed, and faecal samples collected when defecation was observed.

Identification of giraffes

Observed giraffes could be either directly or subsequently identified using the intended Identification Catalogue. For each individual in the catalogue 90° angle photos of both the left and right side of each individual's unique coat pattern were taken. The photos were combined with a description of morphological characteristics, such as size, intensity of color, horn shape and size, the presence of scars, a missing tail and other special features[9, 11, 30, 31]. The age and classification of identified individuals were estimated based on criteria set by Pratt[32] (see table 1). At each encounter photos were taken of every single individual present in order to verify the number of individual giraffe counted.

Table 1. Classification and Age estimation of giraffe based on XYZ (REF)

Classification	Age	Criteria
Calf	Newborn – 1 year old	Regularly accompanied by mother 1st week: horns become erect Angle and appearance of ossicones State of umbilical cord Size relative to mother
Juvenile	1-3 years old	Leaves its mother in its second year of life
Cow	Young Middle Aged Old	Grow up to 5 years old
Bull	A	Larger than B class bulls stouter neck Massive horns with additional bone formation on the skull forming knobs ad extra horns. Eyes placed in the middle of the skull due to secondary bone formation
	B	Larger than adult female, heavier neck, longer and thicker horns
	C	Size of adult female Bulls grow until 7 years old

Faecal sample collection

Opportunistic faecal sample collection took place when defecation was observed and the area was clear of predators. Faecal samples were collected shortly after defecation and placed in a plastic zip bag or plastic/glass container. The bags/containers were marked and directly placed on ice before stored at -20°C until transport to the University of Pretoria[33, 34].

Results

Identified individuals

A total number of 45 individual giraffe were identified on the lower escarpment of Entabeni Game Reserve by the creation of an ID catalogue (Appendix 1-3). Of the 45 individuals, 14 bulls, 22 cows and 4 juveniles and 5 calves were identified. There were 4 A-class bulls, 5 B-class bulls and 5 C-class bulls. Of the 4 juveniles, 3 could be identified as male and 1 as female. The sex of the calves could not be determined. Most giraffe were identified within 3 weeks of the start of the research, after that the only addition was a new-born calf. Group size varied between single individuals that tended to be older bulls, to 20 or more. Group consistency was variable as well with observations made of single sex herds and herds of adult cows with their calves and unrelated juveniles. Habituation of giraffe over a period of approximately 2 weeks to the research vehicle made it possible to follow a herd for verification of identification as well as to establish faecal sampling on a regular basis. Compared to the bulls, the cows at EGR tended to move away from the research vehicle earlier than the bulls, making observation of defecation comparatively more difficult for them, which is reflected in a higher mean individuals sample amount for the bulls. By identifying presumably the entire giraffe population present at the lower escarpment of Entabeni Game Reserve it was possible to collect individual faecal samples.

Faecal sample collection

Success of individual faecal sample collection is usually influenced by a variety of factors, such as the group size, spreading, terrain, density of the vegetation, size and movement of the individual, safety conditions of the collectors and distance to research vehicle during defecation(18, 20, 33). From all classes faecal samples were collected. A total of 134 samples were collected in 79 days from identified individuals of the EGR giraffe population. Overall average of individual sampling frequency was 3.11±2.15 (mean +/- SD) samples per individual. The highest amount of samples collected for an individual female was 9 samples, giving an average frequency of 1 sample every nine days. For the males the highest amount of samples collected for one individual was six samples in 79 days, giving an average frequency of one sample every 13 days. From the males (N=14) a total of 56 faecal samples were collected, with a mean of 3.92 ± 1.85 samples per individual and a range from 1 to 6. From the females (N=22) we collected 69 samples with a mean of 3.09 ± 2.18 samples per individual ranging from 0-9. The total amount of samples collected from juveniles (N=4) was 3, with a mean of 1.6 ± 2.30 ranging from 0-2. From the calves (N=3) a total number of samples of 8 was collected with a mean of 2.66±2.51and a range from 0-5. Two calves were excluded from the research because one was born the day before the last day of the research and the other one was assumed deceased after it was never observed it again after the end of March and its mother had been observed without its calve since then.

Table 2. Number of faecal samples collected over a period of 79 days, mean number of samples per group and range of samples within each group.

Collected Samples	Males (N=14)	Females (N=22)	Juveniles (N=4)	Calves (N=3)	Total (N=45)
Total	55	68	3	8	134
Mean	3.92 ± 1.85	3.09 ± 2.18	1.6 ± 2.30	2.66±2.51	3.11±2.15
Range	1-6	0-9	0-2	0-5	

Discussion

This study explored the possibilities of identifying individuals of a population of wild giraffe at Entabeni Game Reserve, by setting up an ID catalogue (appendix 1-3). In this regard, the ID catalogue proved to be a reliable tool in collecting faecal samples from individual animals during the study period.

In order to create the ID catalogue and collect faecal samples it was necessary to observe the giraffe from a short distance for a period of time. At the start of the study the giraffe were quite skittish and would retreat into the bush shortly after the beginning of observation. After 2 weeks the giraffes would continue browsing for a longer period of time before retreating, giving us the opportunity to take good photographs for the ID catalogue and observe defecation more often. This habituation effect seemed to be more pronounced in male than in female giraffe at EGR and occurred only for the research vehicle, no for humans on foot. Miller 2010[35] also reports habituation of giraffes to the research vehicle, but without the difference between males and females. There was a difference in the total amount of samples collected at EGR from males compared to females, juveniles and calves (see table 2.). Only a few samples could be collected from juveniles. This difference might be explained by less hours of observation time for calves/juveniles in comparison to males and females (which was not recorded in this study) since giraffe cows hide their new-born calves away from the herd in dense vegetation, which makes observation difficult[36, 37].

Faecal sample collection is influenced by a variety of factors, such as A: season[10, 11, 18, 30, 32, 38], B: density of the vegetation[17, 18, 32], C: distance from the observer[18], D: unpredictability of herd composition[18], E: terrain[32] and F: the availability and accessibility of roads[17, 32]. In our case study, B, C, and E might have played the biggest influencing role as this study was performed in autumn, since the vegetation is much thicker in the hot rainy season than in winter, when it is cold and dry and the vegetation sparse. This is comparable to other studies such as Veasey[18], Leuthold[30] and Pratt[30, 32]. Also in flat, open terrain, defecation is observed easier and samples are found quicker. Therefore in our study, there might be a certain bias towards the collection from individuals who frequently travel open areas. Faecal sample collection was also restricted due to the presence of large predators in the area.

Faecal sample collection frequency of wild giraffe was one sample every 9 to 13 days for females and males, respectively. This frequency was achieved without focussing on collection from a specific individual or timeframe (i.e. once a week). Interestingly, the achieved rate would already allow some insight into the endocrine correlates of female reproductive activity as the oestrous cycle length for the giraffe is defined 14-15 days[39]. A higher collection frequency may be achieved if focus would lie on collecting samples from specific individuals in a set timeframe. In terms of distinguishing between pregnant and non-pregnant individuals, our approach almost provide the proposed necessary sampling frequency, as Dumonceaux[20] proposes a sample frequency of 2-3 samples per week for 2-3 weeks per individual and del Castillo[21] has shown that pregnancy can be diagnosed as soon as 2 weeks post conception by using only a single faecal sample. Bercovitch[19] focused on collecting samples from particular individuals in a set timeframe for 4-5 times per week. A longitudinal pregnane profile was created with the use of 7-10 samples per 15 days per individual. By increasing the collection frequency to 3-4 samples per individual per week it should therefore be possible to create longitudinal hormone profiles for assessing cyclicity and pregnancy in wild giraffes as well.

Being able to frequently collect faeces on an individual basis opens up a variety of possibilities not only in regard to monitor reproductive function. The collected faeces could be also used as an indicator for parasitological infection and also from an endocrinological point of view to monitor long-term stressful events (like e.g. injury)[40]. These approaches could subsequently also help to evaluate current management practices and environmental conditions, therefore positively influencing reproductive success in this declining species.

The present study shows that in a small reserve of approximately 6400 ha identification of individual giraffes is possible by creating an ID catalogue. Forty-five individuals were identified within a time span of three weeks and the resulting ID catalogue proved to be effective in field use. This allowed collection of individual faecal samples and demonstrates the potential for monitoring the reproductive cycle and adrenocortical status of wild giraffe.

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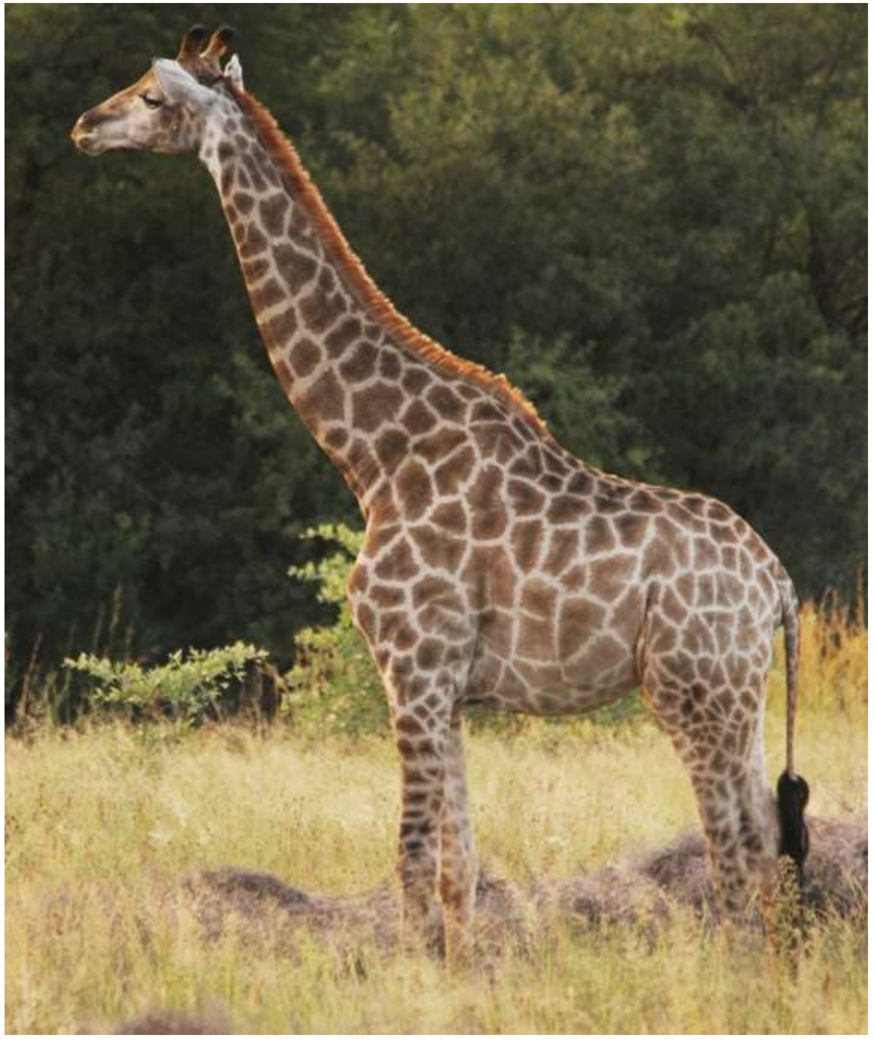
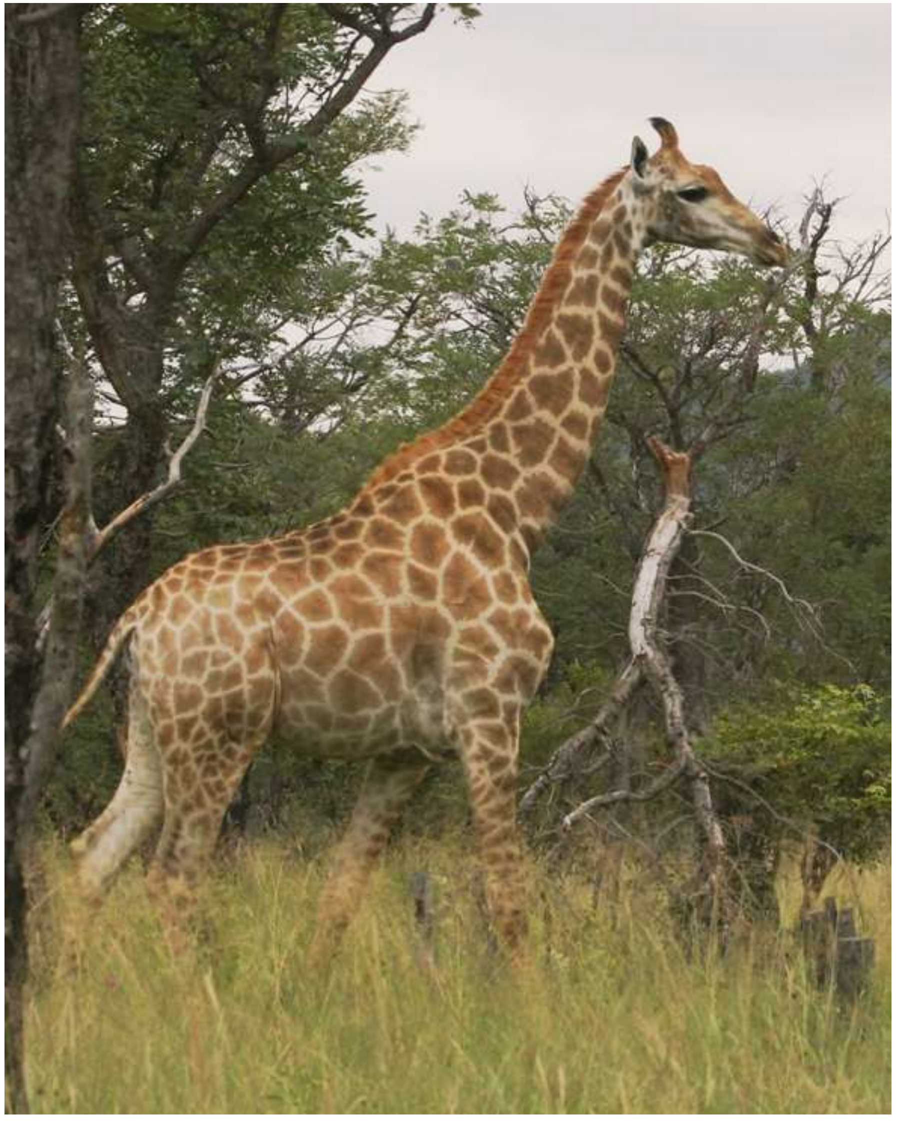
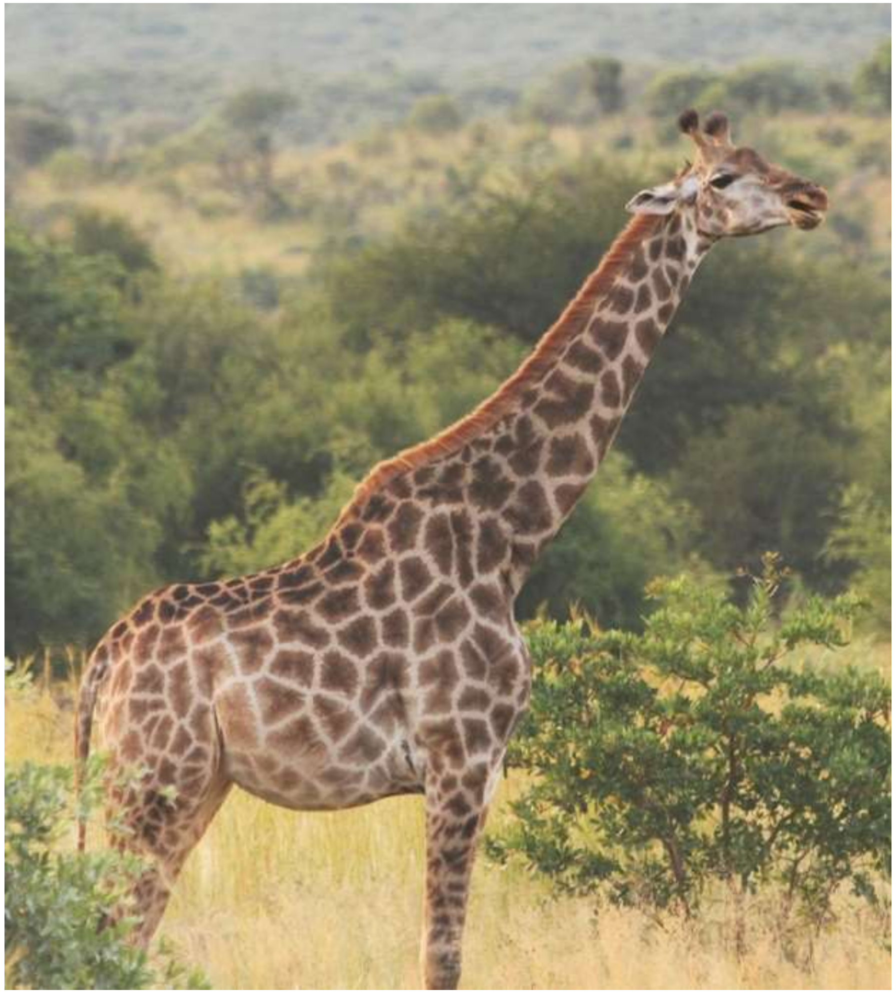
Appendix 1
Identification Catalogue
Females

Giraffe ID	F1
Size/Class	Medium
Features	



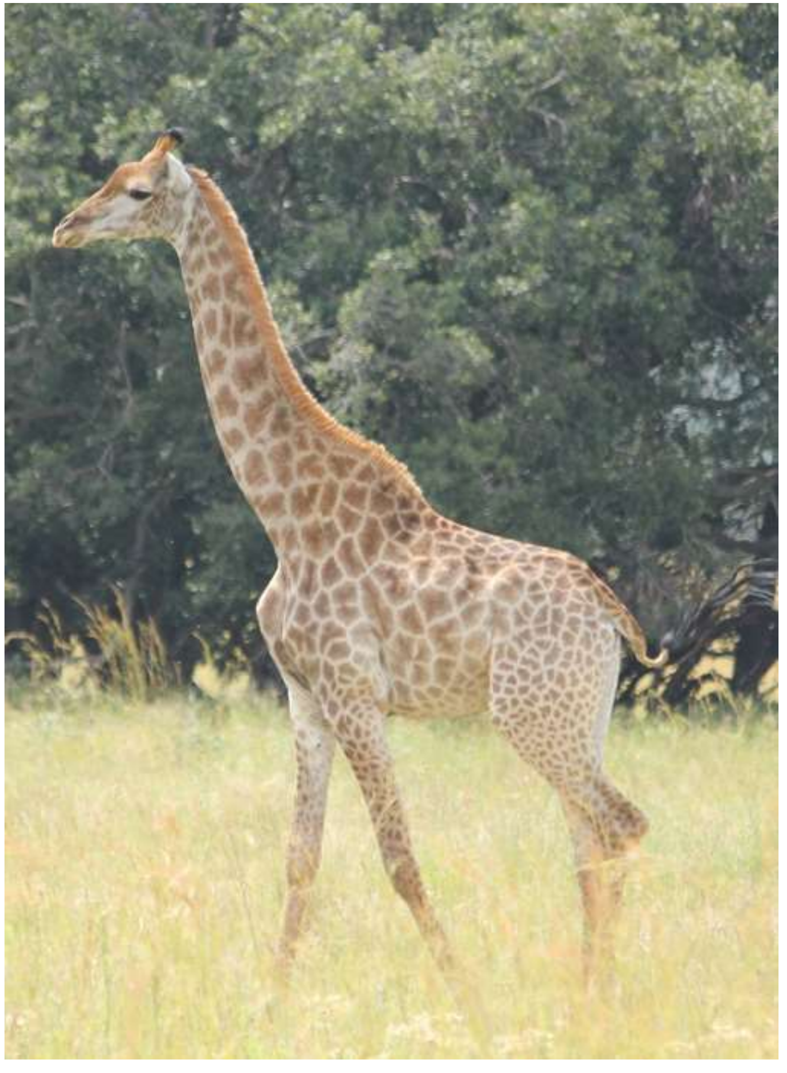


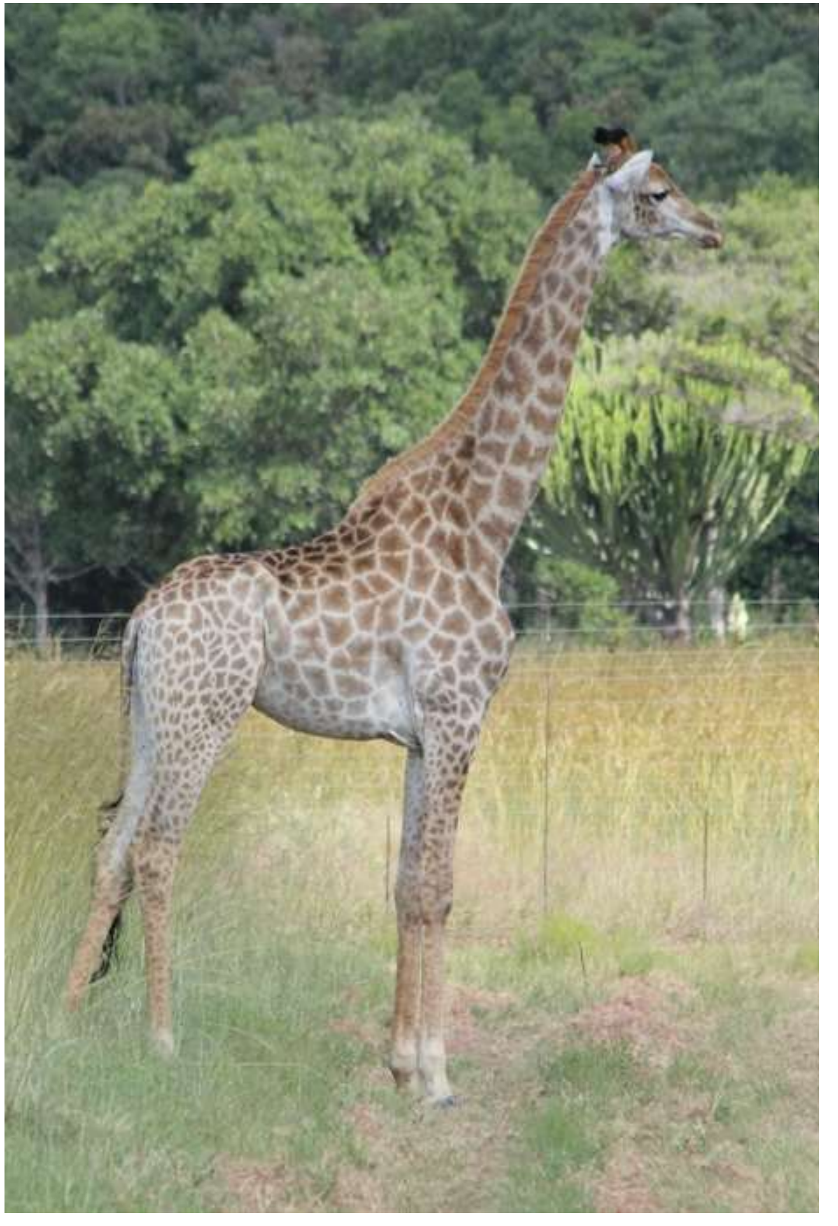
Giraffe ID	F2
Size/Class	Medium
Features	



Giraffe ID	F3
Size/Class	Small
Features	

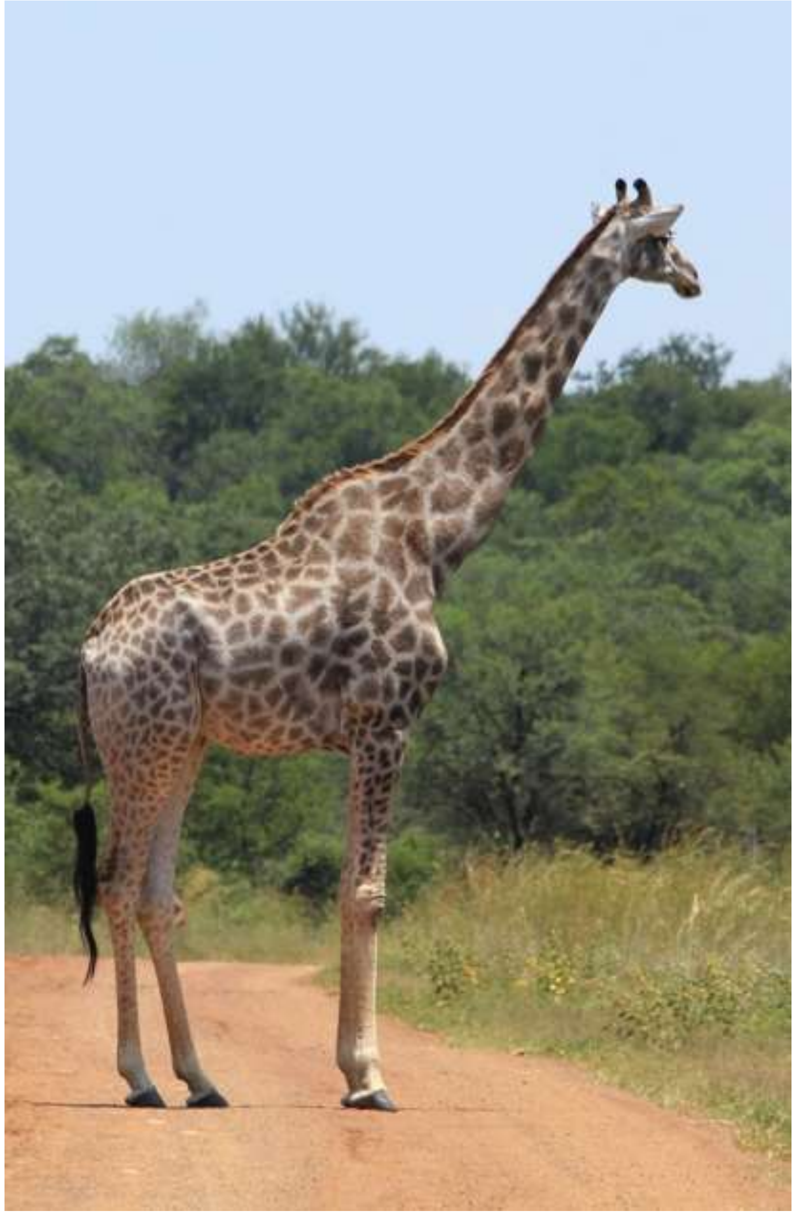
Giraffe ID	F4
Size/Class	Small to Medium
Features	



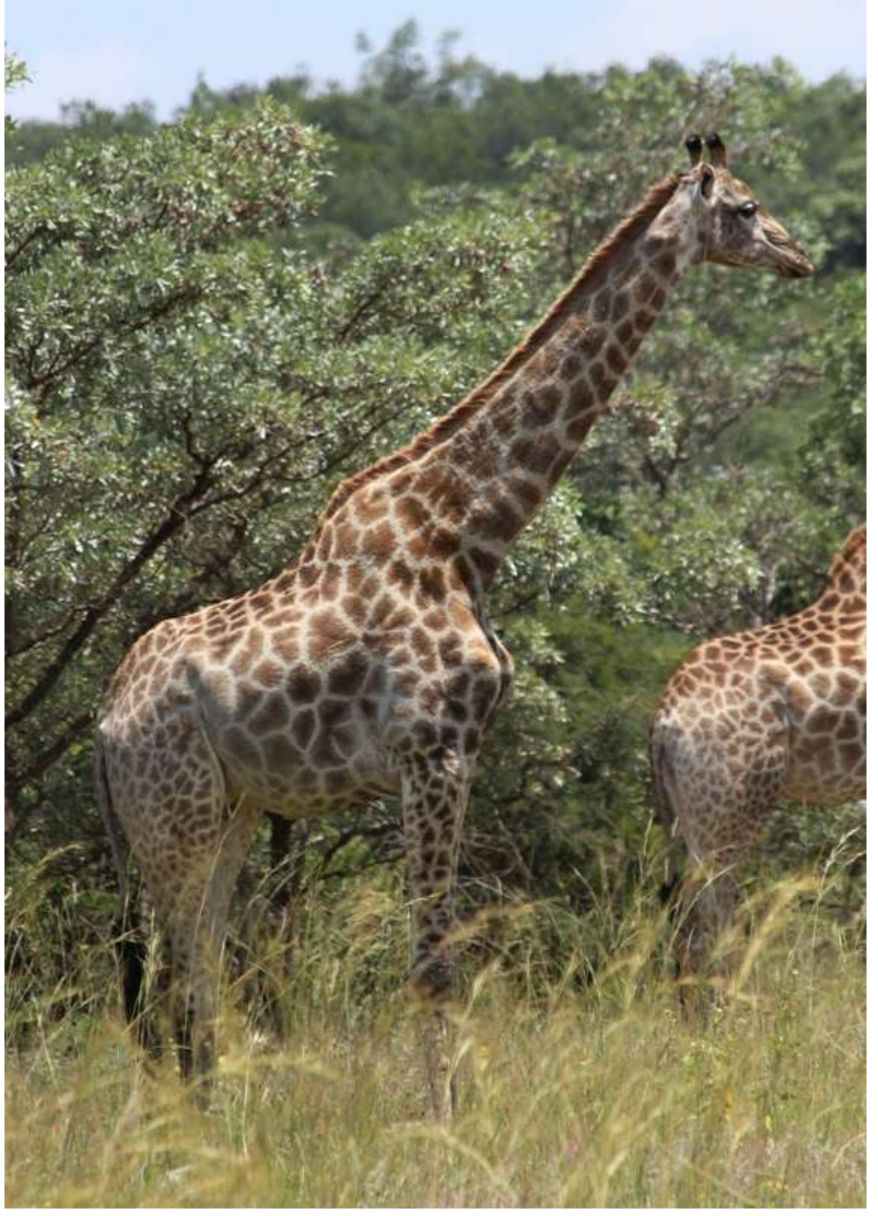


Giraffe ID	F5
Size/Class	medium
Features	

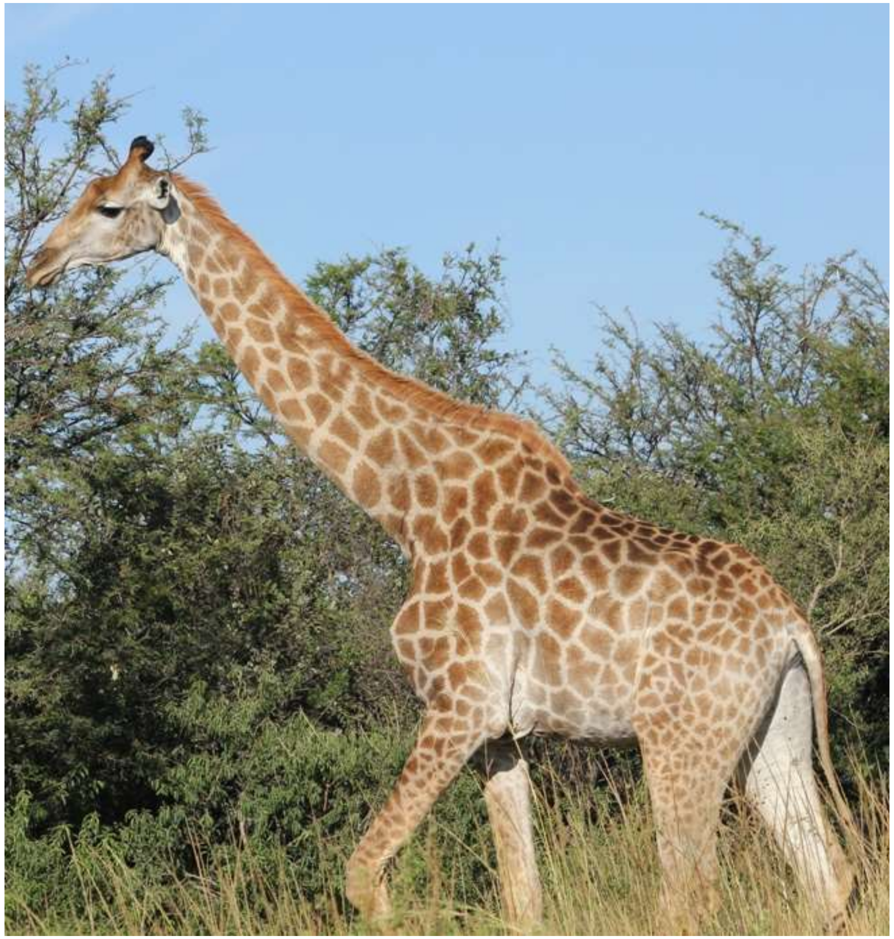
Giraffe ID	F6
Size/Class	Tall
Features	Very light colour

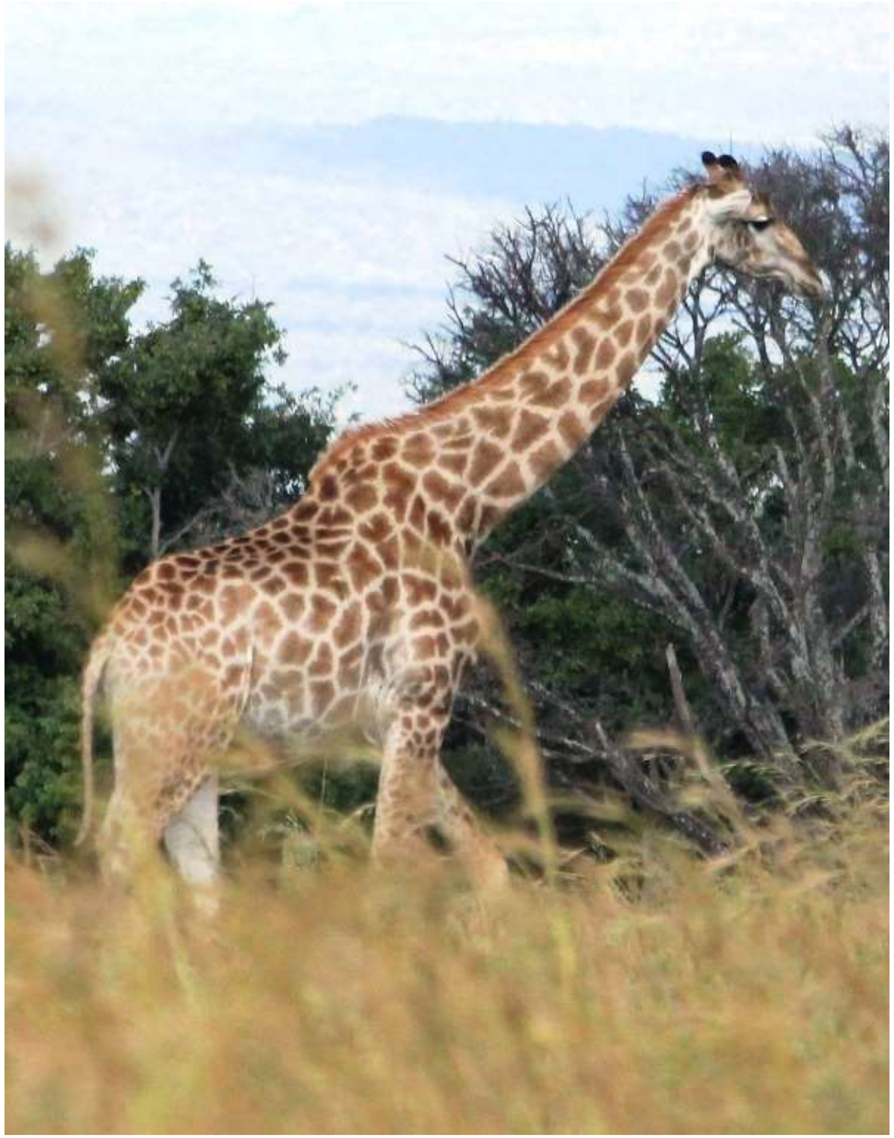


Giraffe ID	F7
Size/Class	Small - Medium
Features	



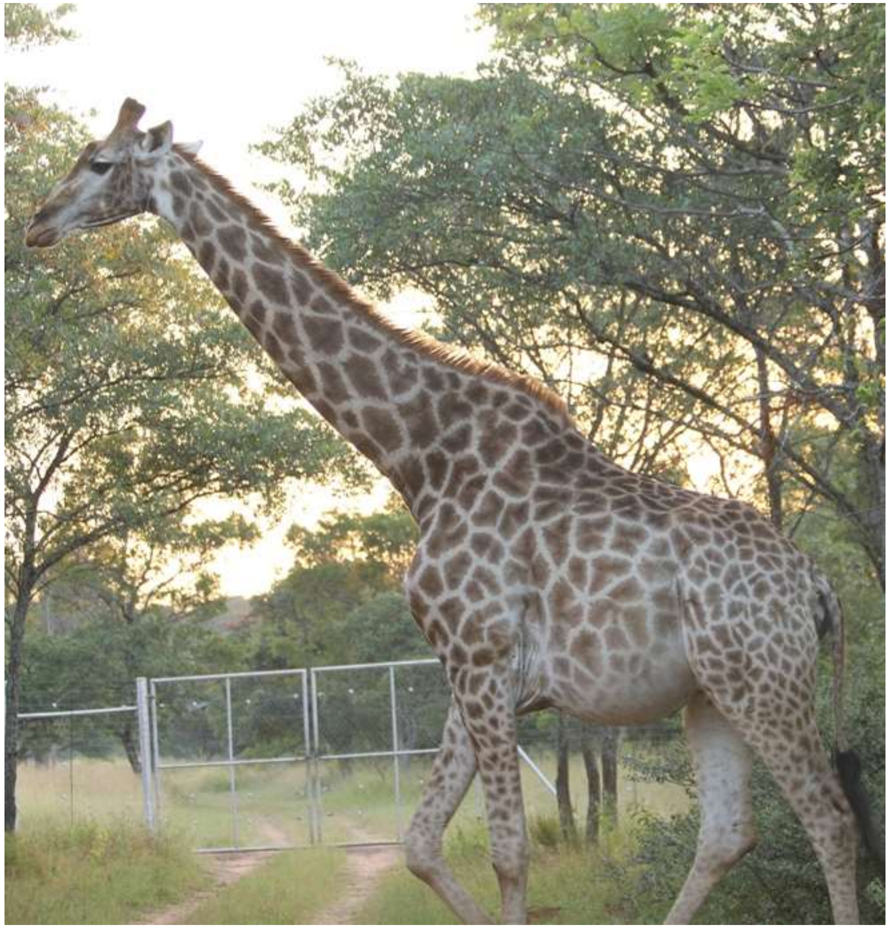
Giraffe ID	F8
Size/Class	Medium
Features	

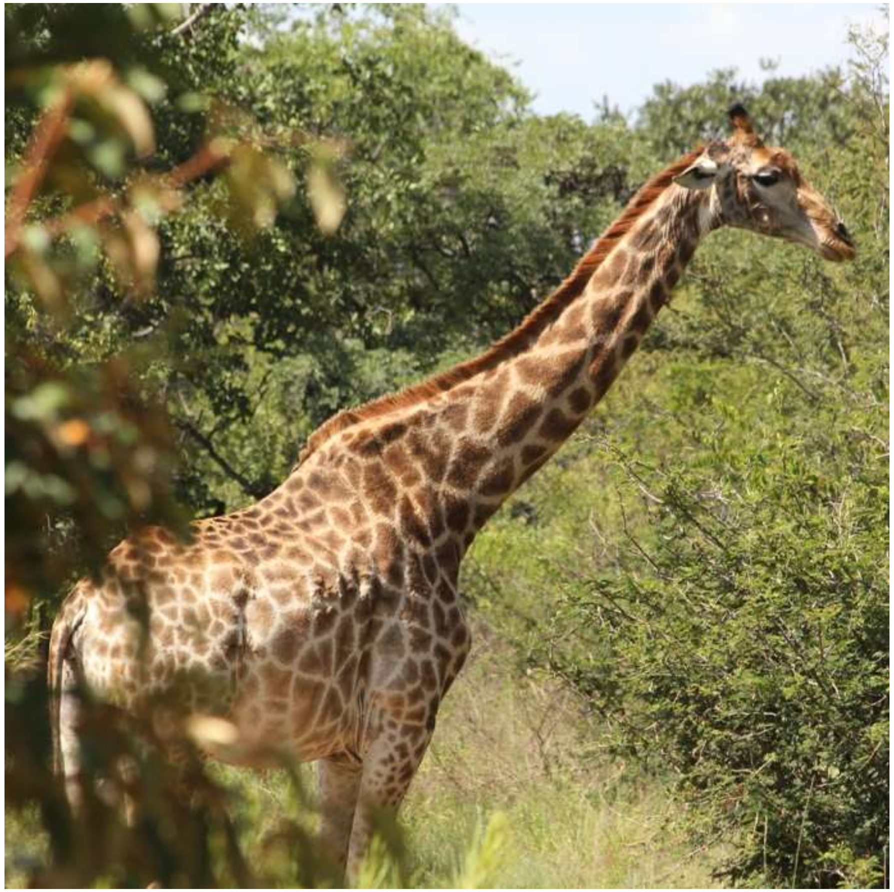




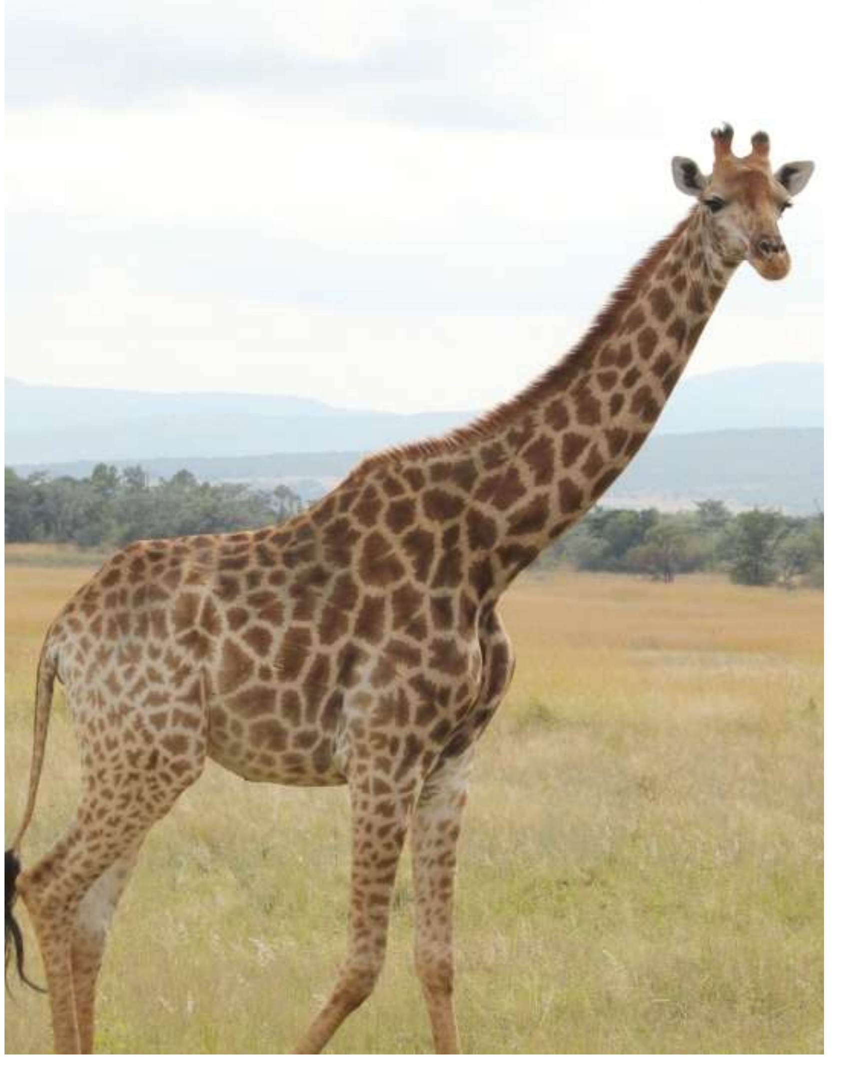
Giraffe ID	F9
Size/Class	Medium - Tall
Features	

Giraffe ID	F10
Size/Class	Tall
Features	Scar on right flank – mother of C4

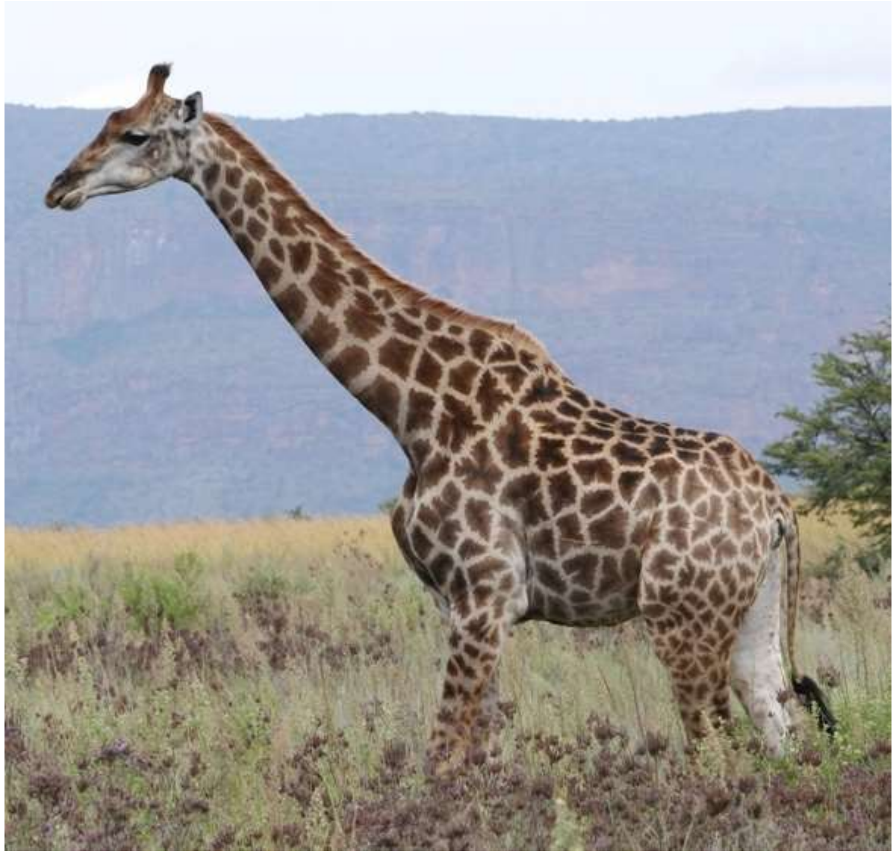


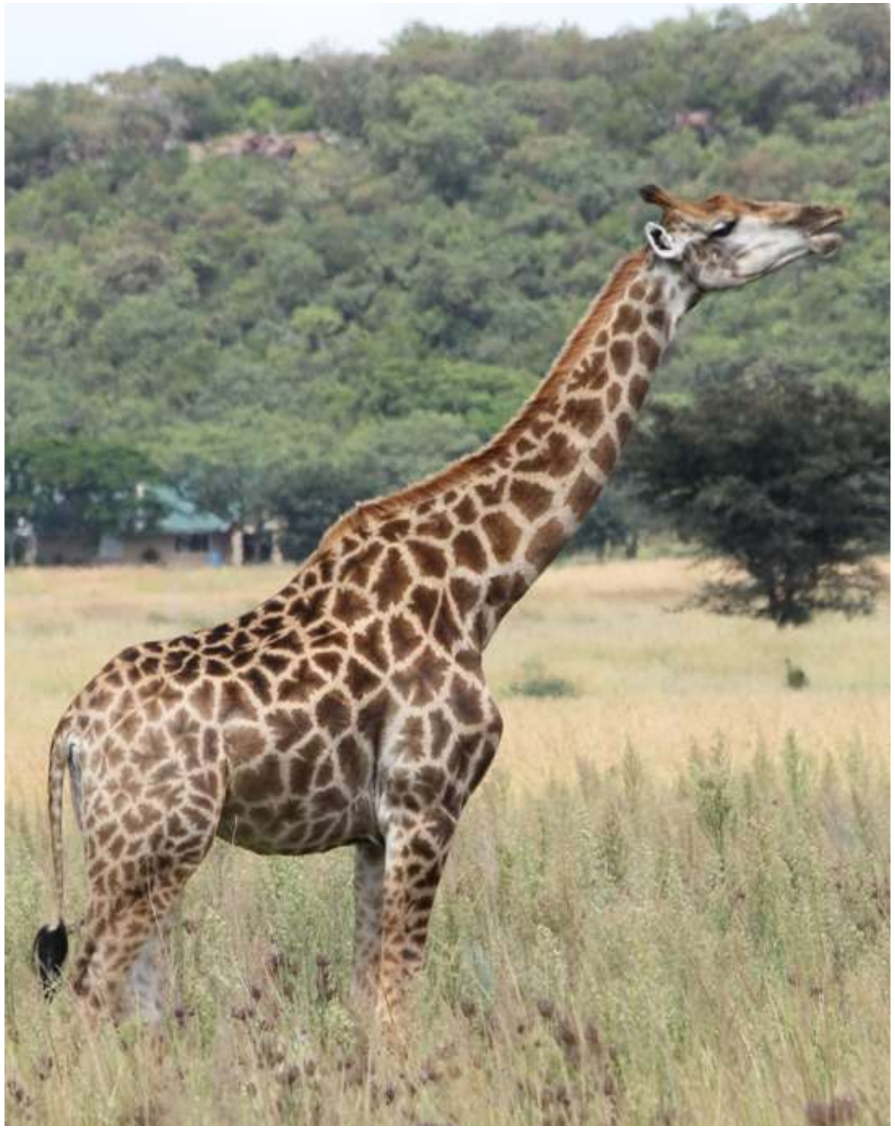


Giraffe ID	F11
Size/Class	Small - Medium
Features	

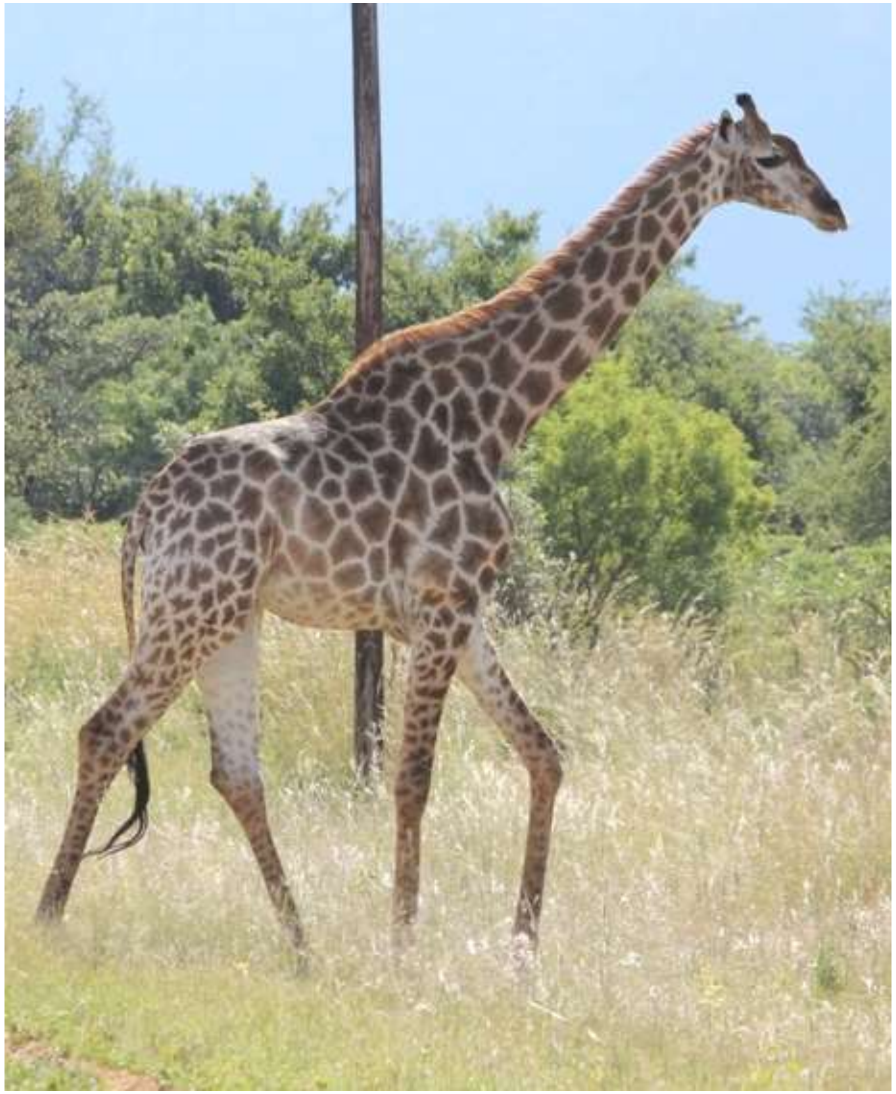


Giraffe ID	F12
Size/Class	Medium
Features	Mother of C3



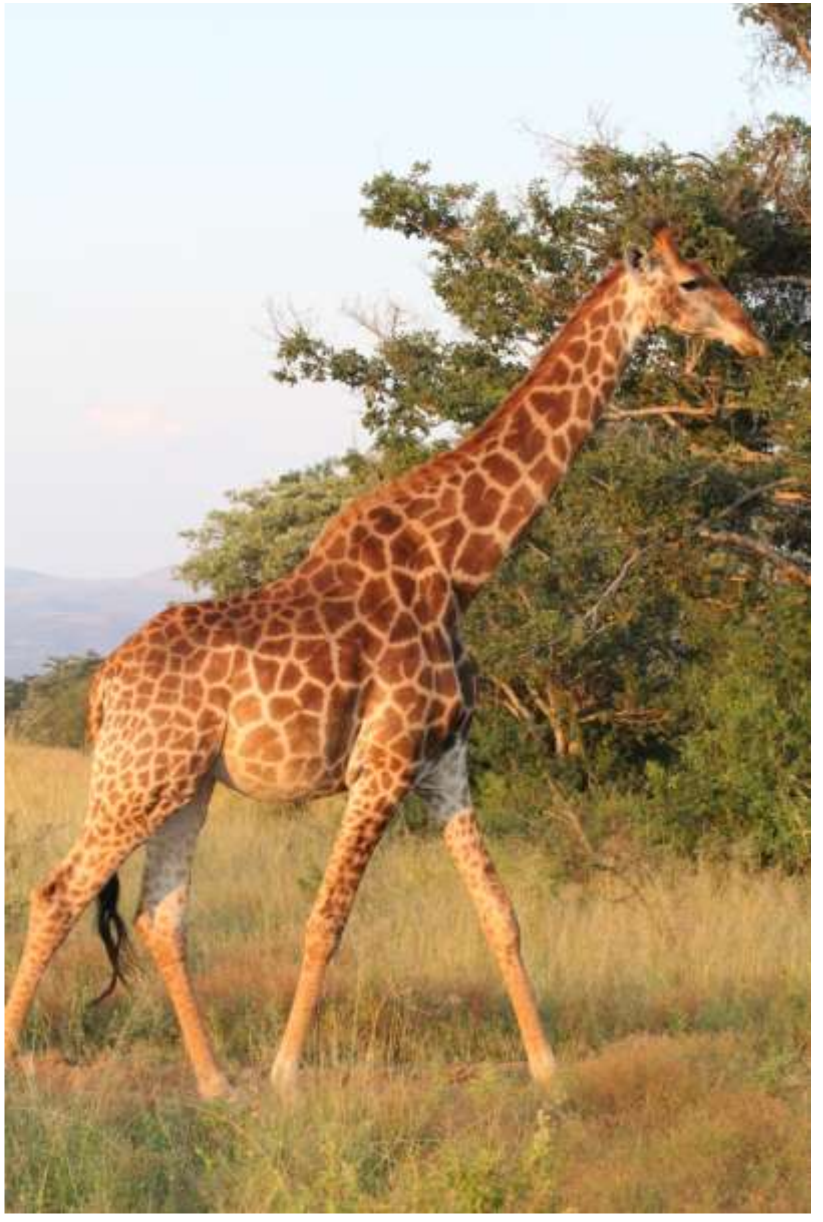


Giraffe ID	F13
Size/Class	Medium
Features	White mark on forehead

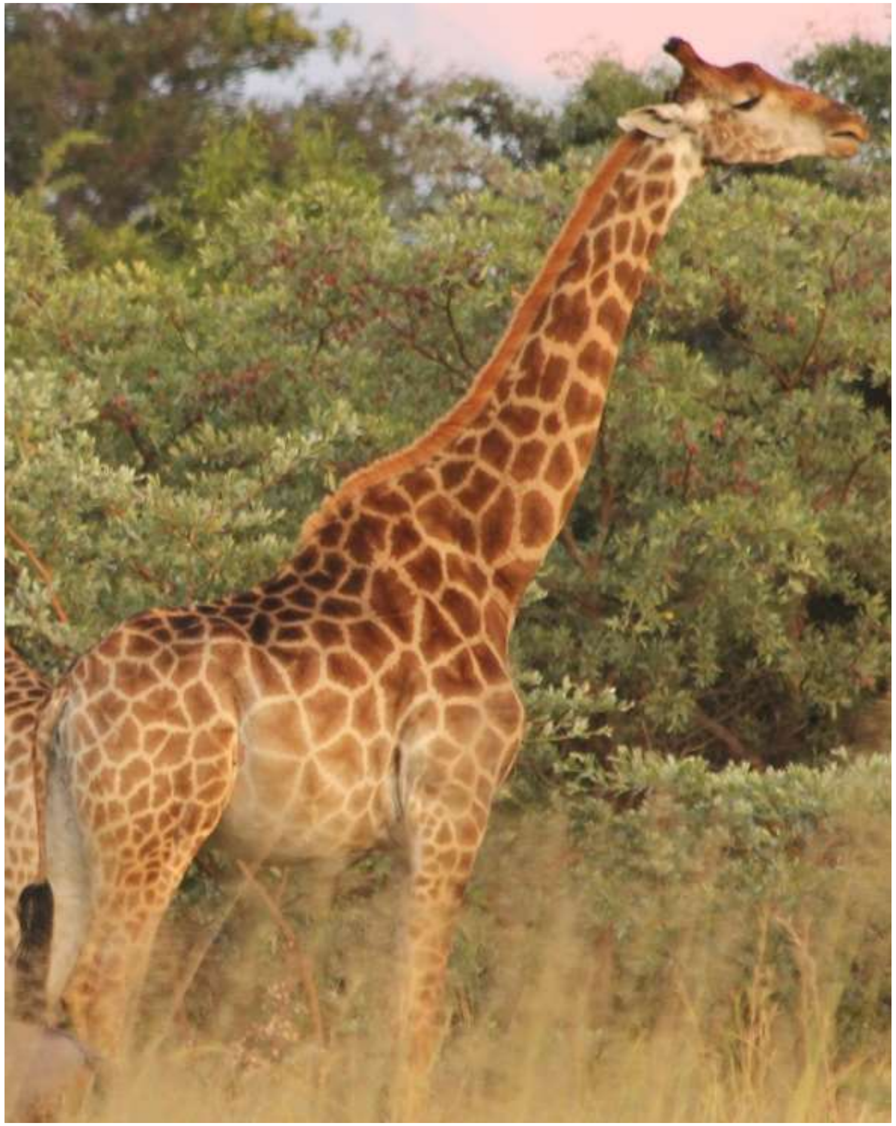


Giraffe ID	F14
Size/Class	Tall
Features	Flattend ossicones

Giraffe ID	F15
Size/Class	Tall
Features	

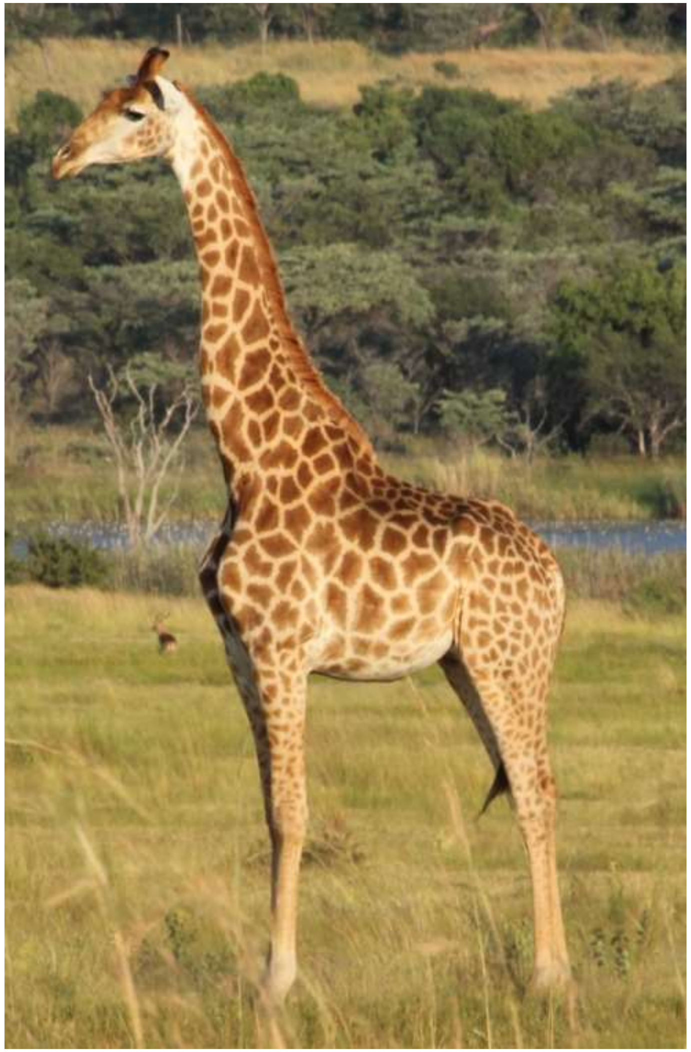


Giraffe ID	F17
Size/Class	Medium - Tall
Features	

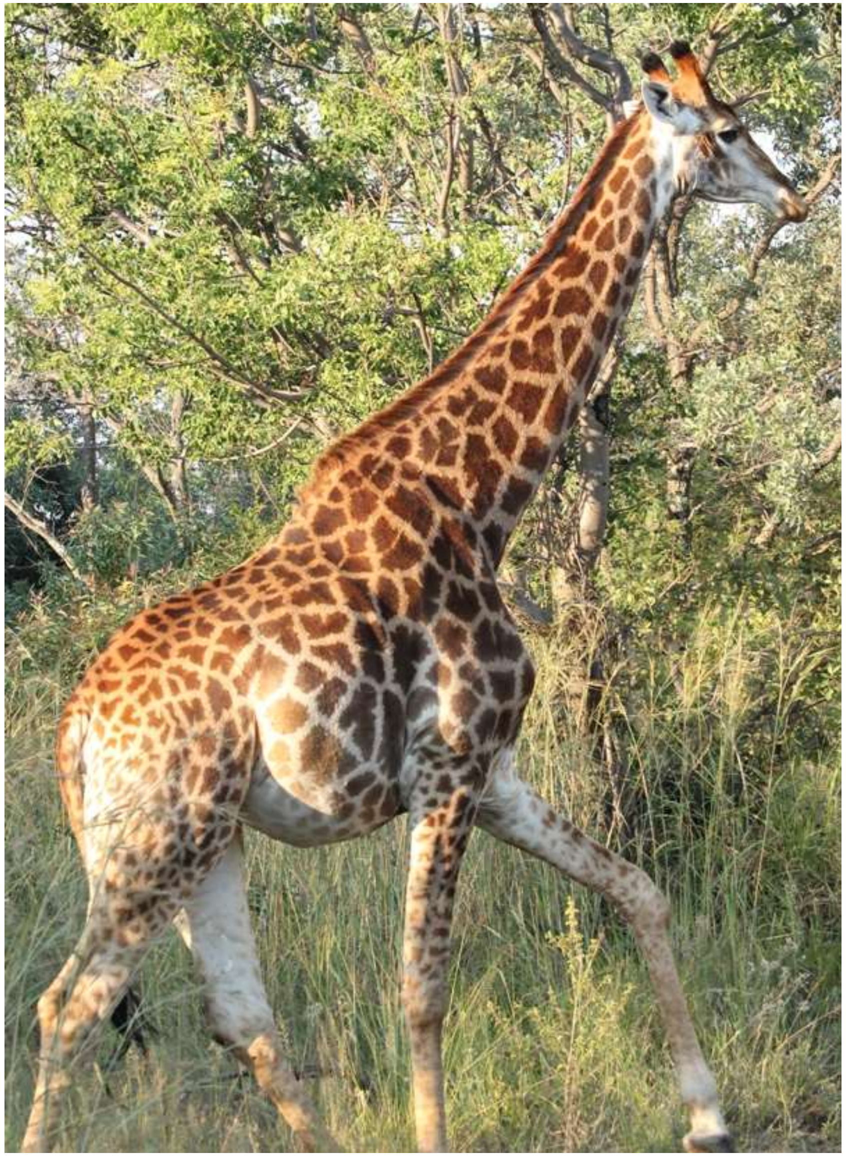


Giraffe ID	F18
Size/Class	Medium
Features	

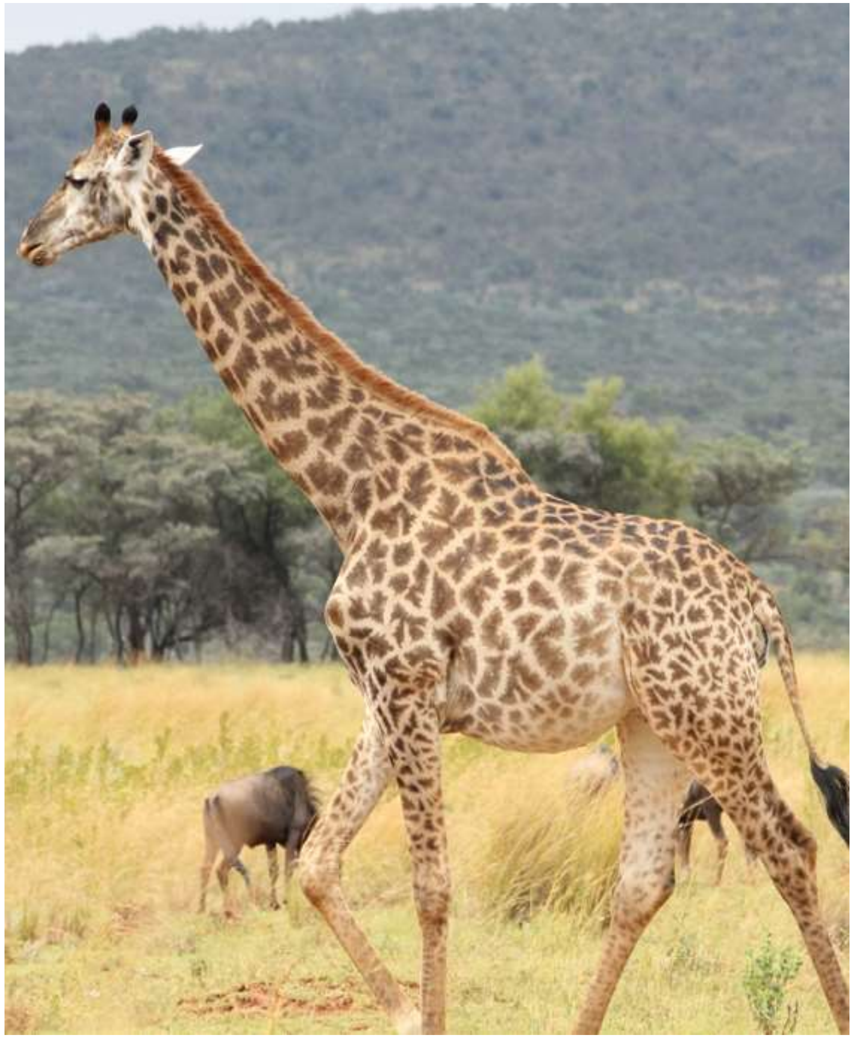
Giraffe ID	F19
Size/Class	Tall
Features	Mother of C1

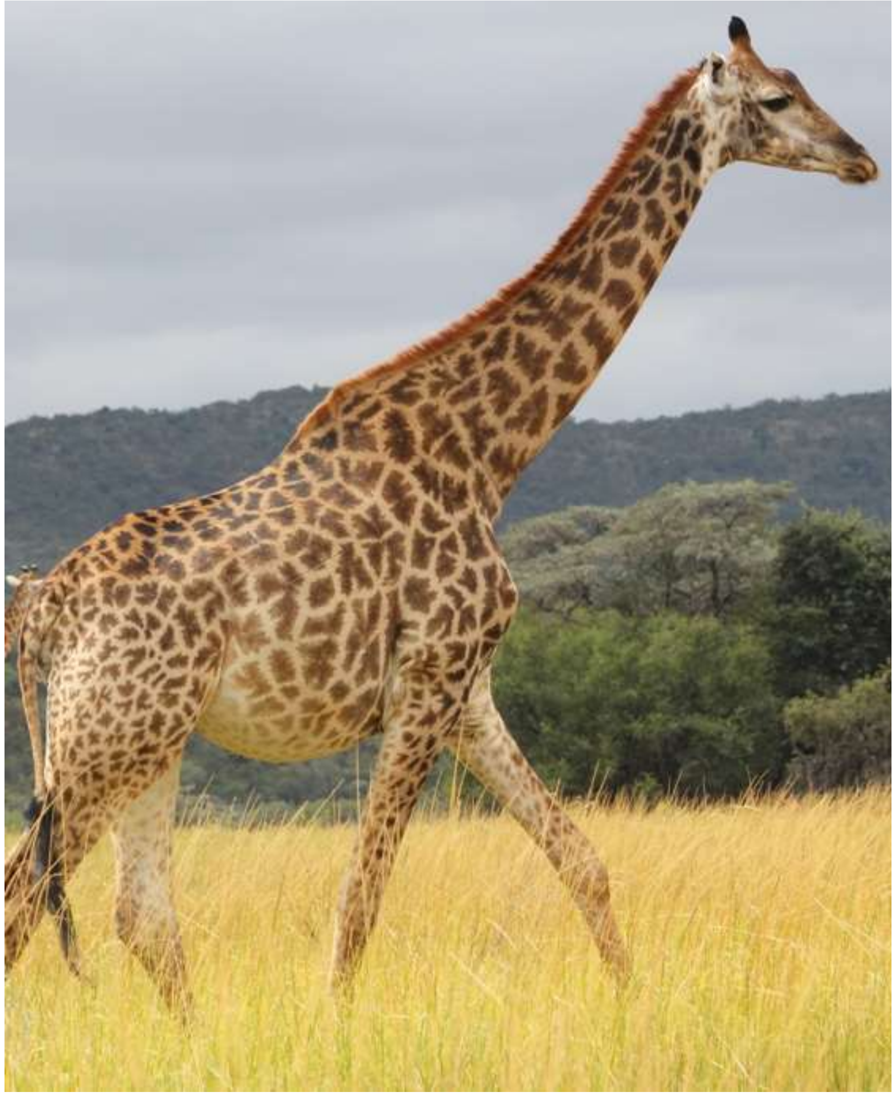


Giraffe ID	F20
Size/Class	Medium
Features	

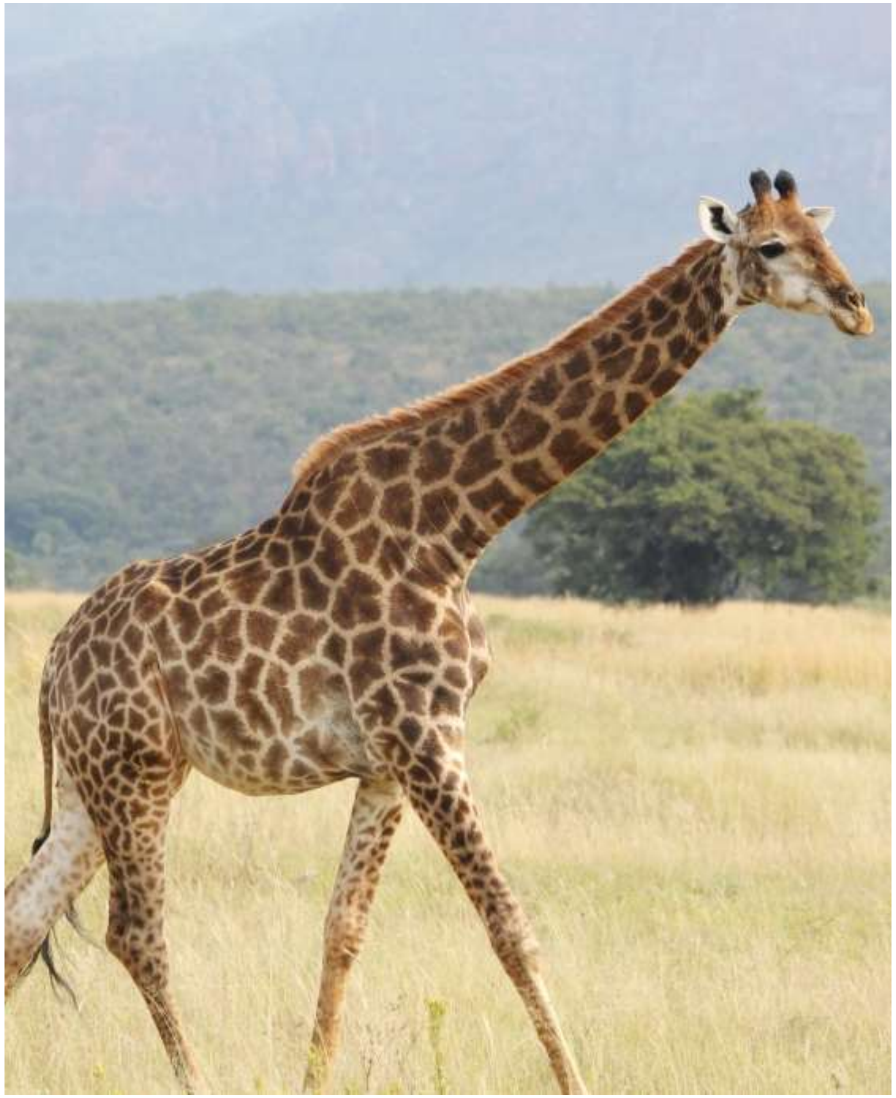


Giraffe ID	F21
Size/Class	Medium - Tall
Features	Mother of C5

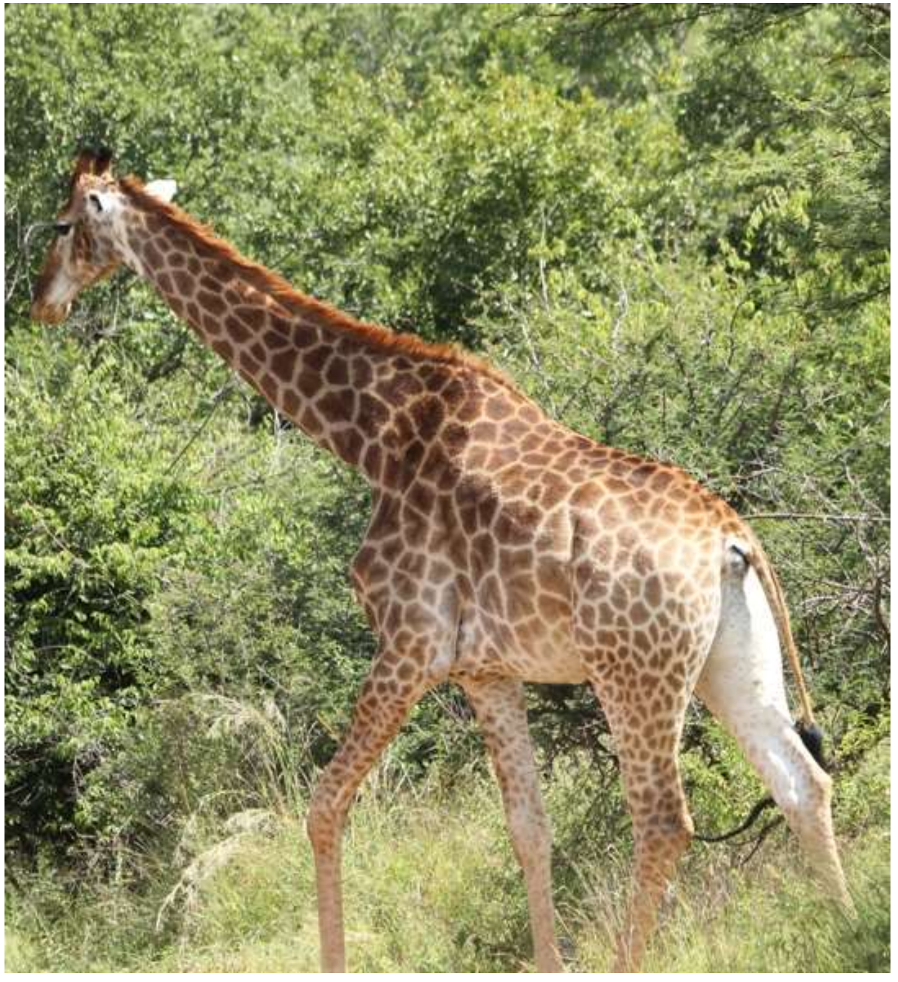


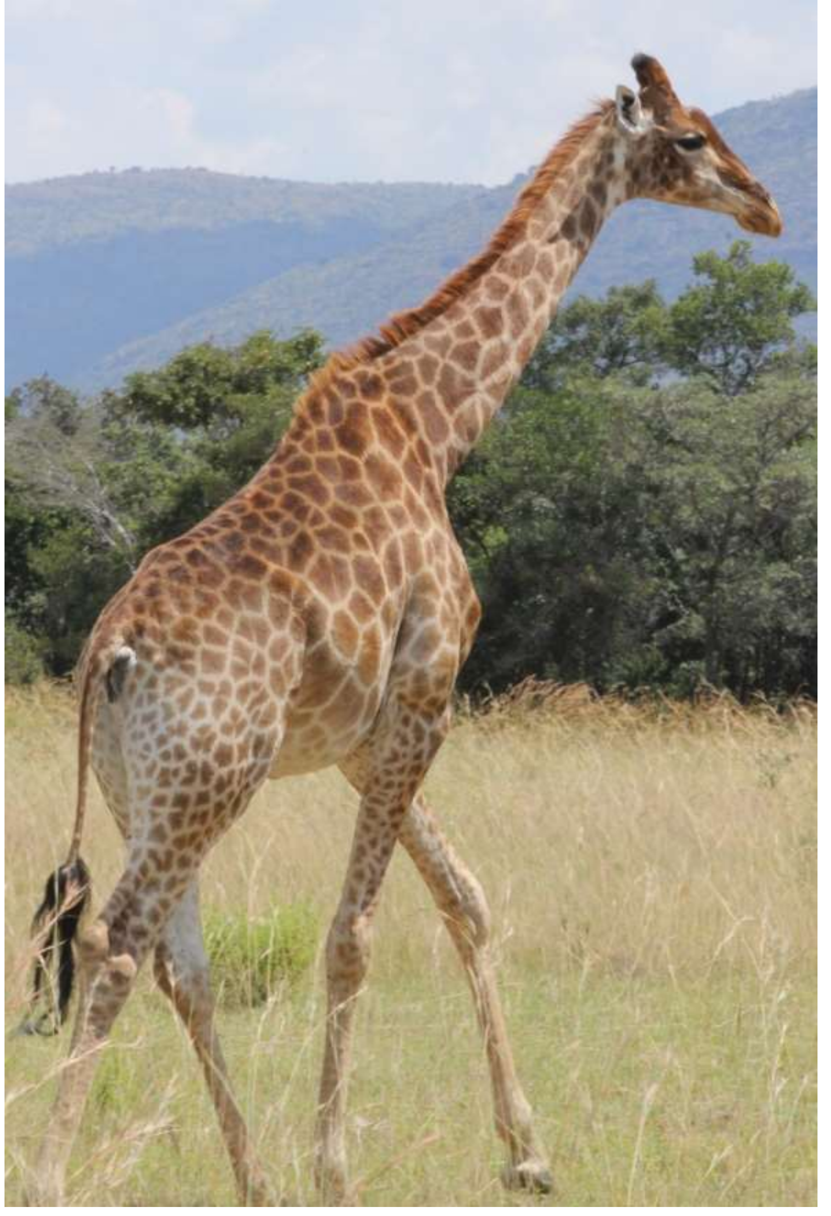


Giraffe ID	F22
Size/Class	Tall
Features	



Giraffe ID	F23
Size/Class	Tall
Features	Mother of C2



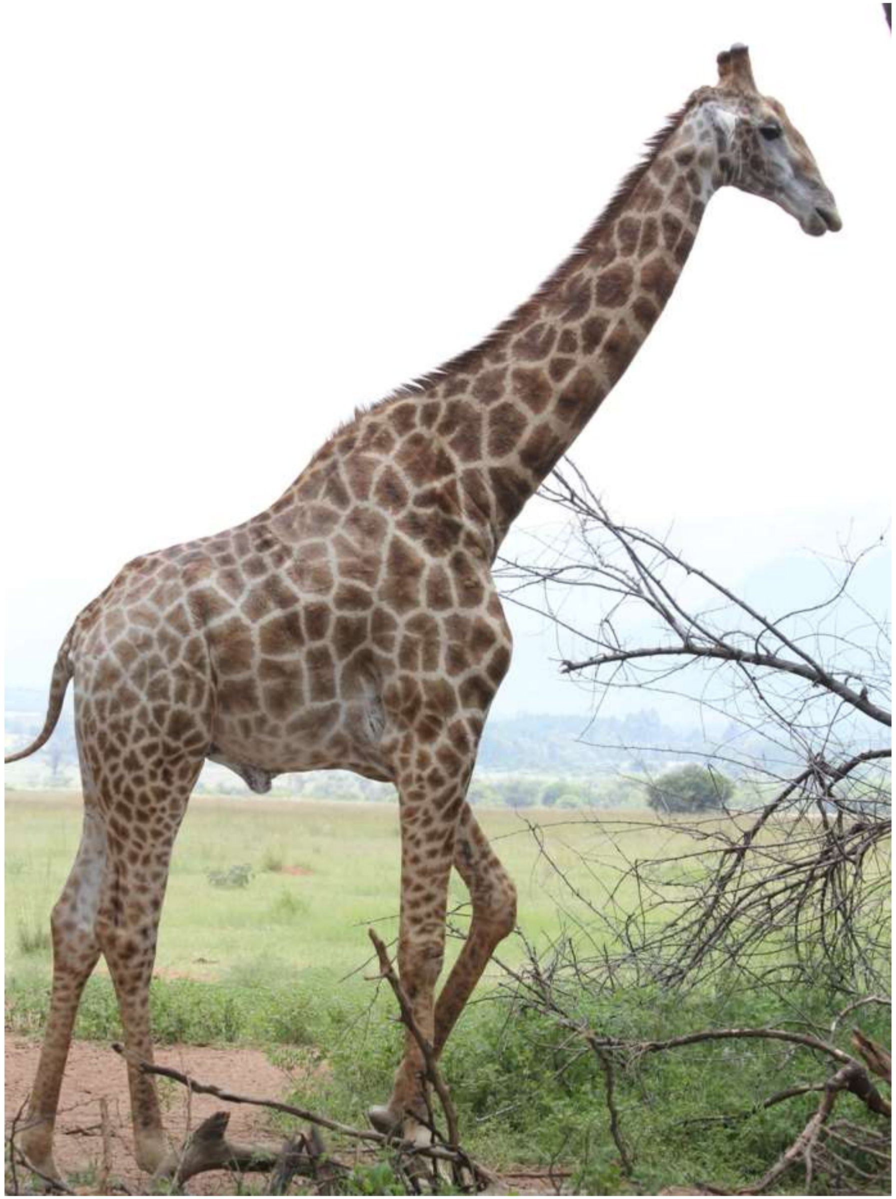


Appendix 2

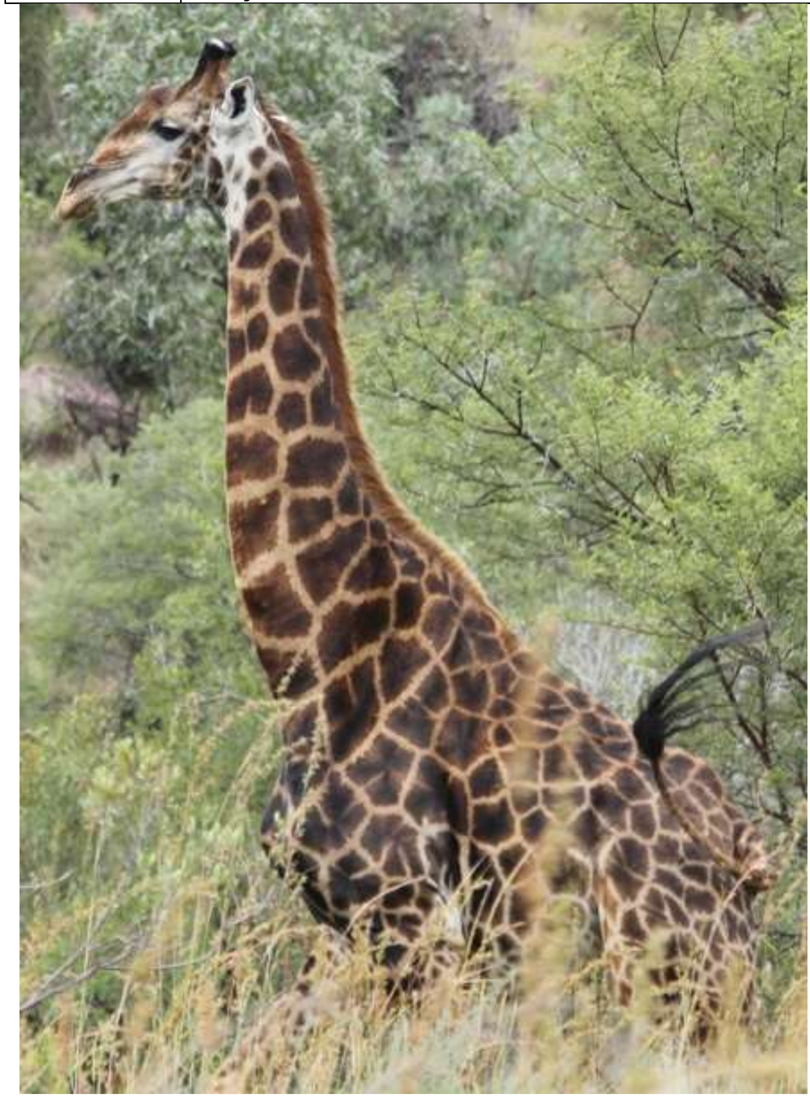
Identification Catalogue

Males

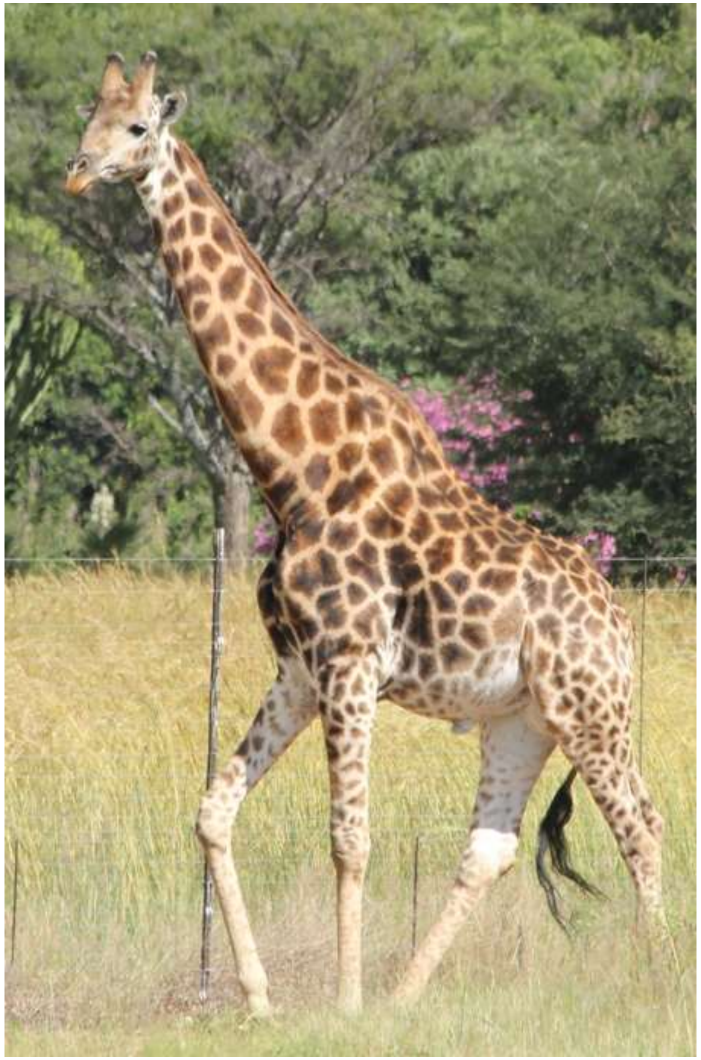
Giraffe ID	M1
Size/Class	Tall - A
Features	Right ear partly missing

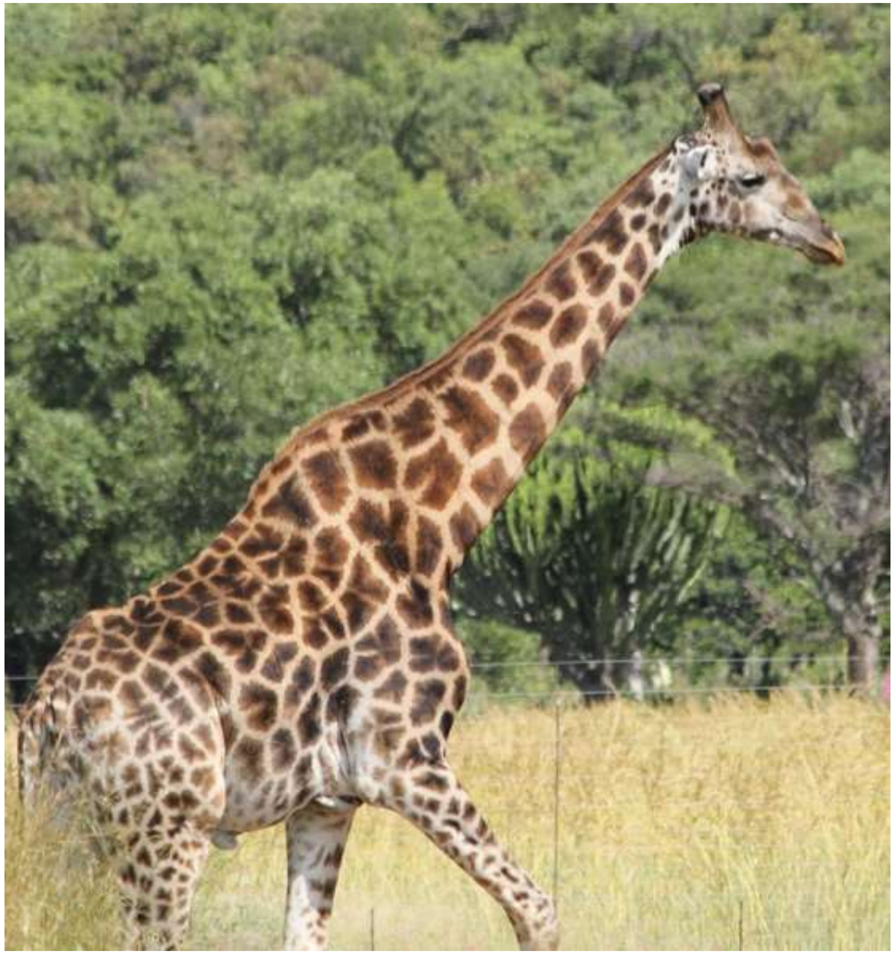


Giraffe ID	M2
Size/Class	Tall - A
Features	Very dark

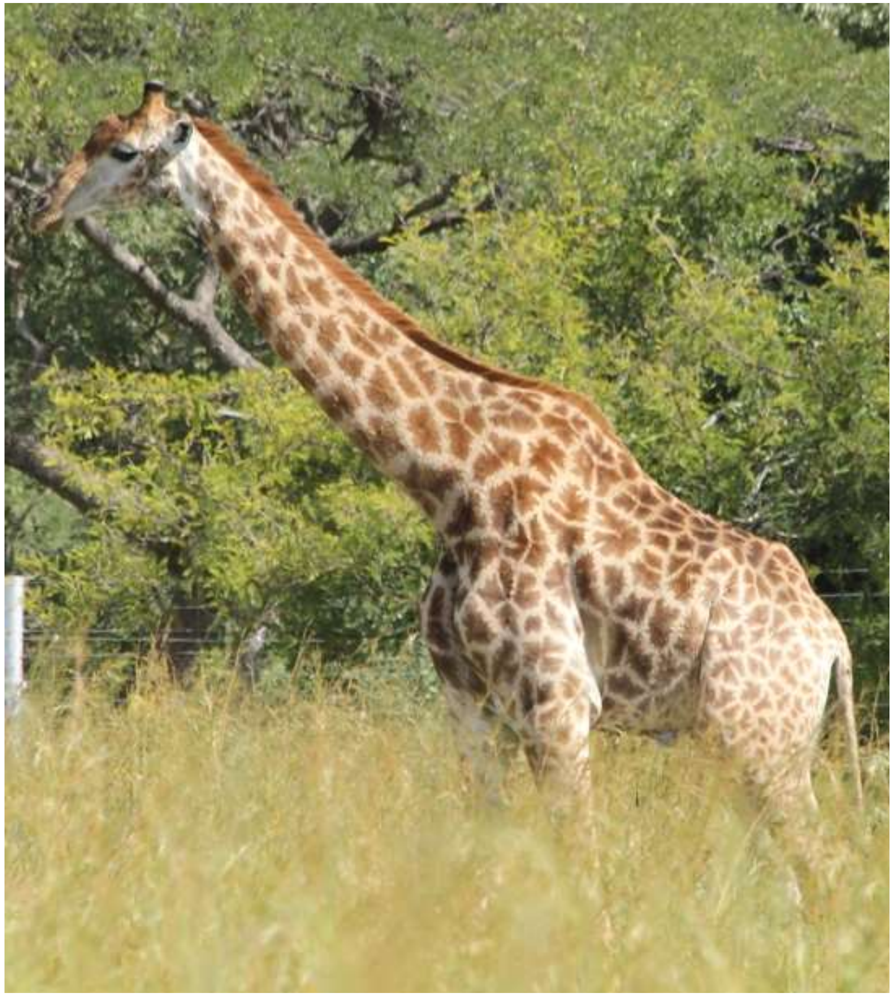


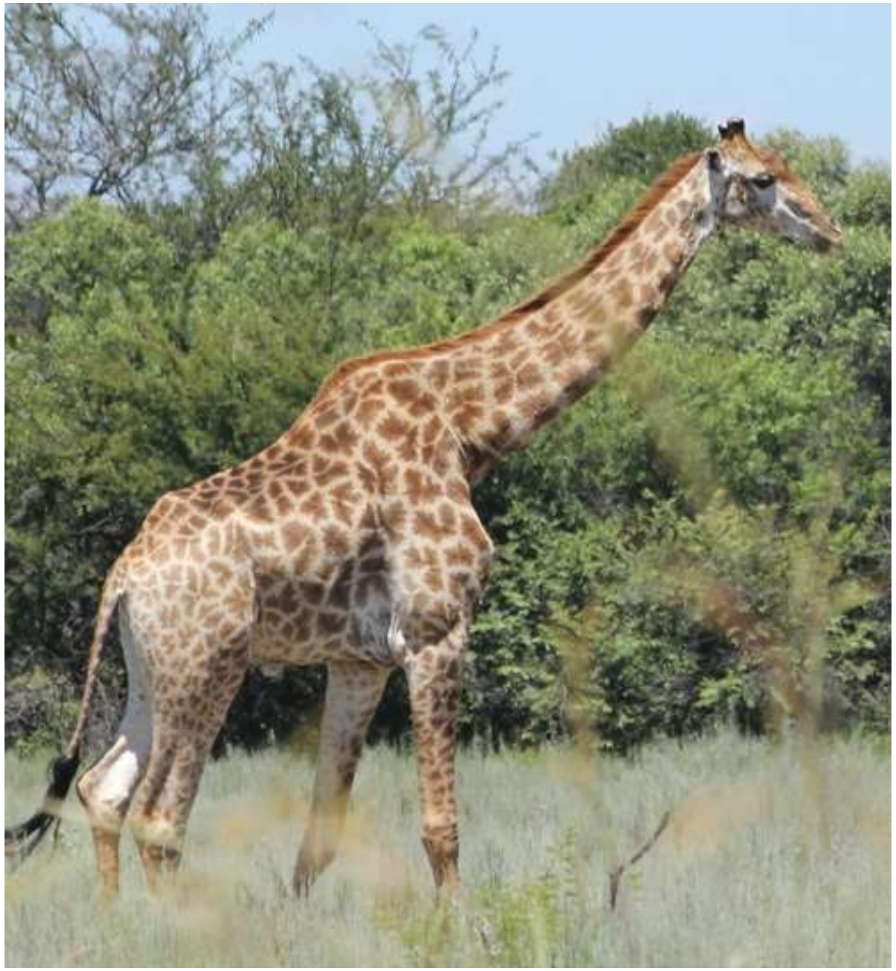
Giraffe ID	M3
Size/Class	Tall - B
Features	dark





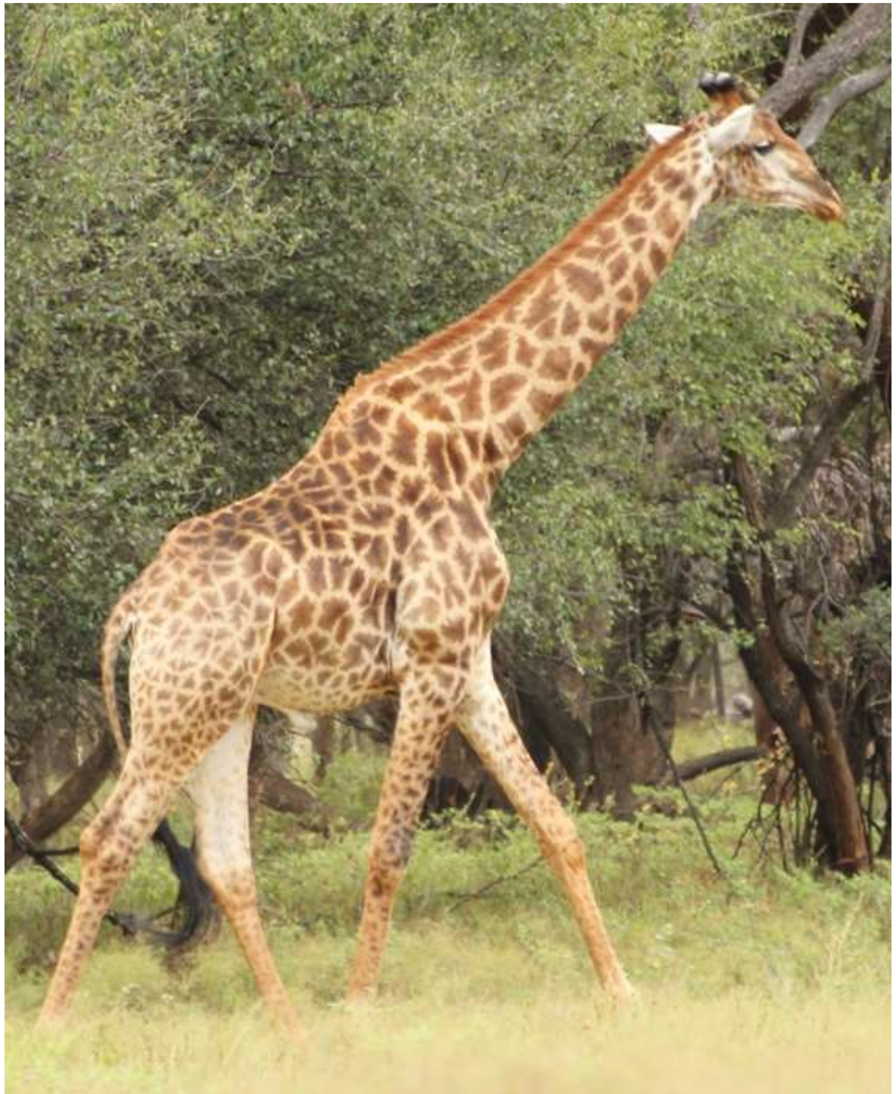
Giraffe ID	M4
Size/Class	Medium - C
Features	





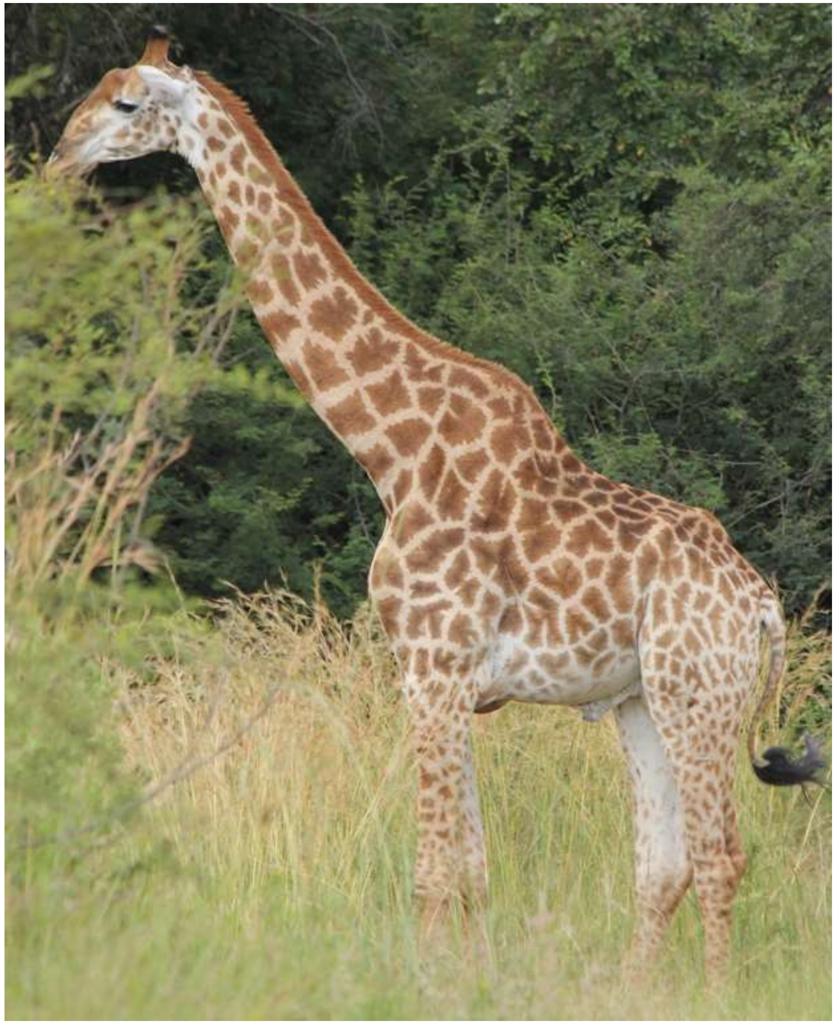
Giraffe ID	M5
Size/Class	Tall – A
Features	

Giraffe ID	M6
Size/Class	Tall - A
Features	

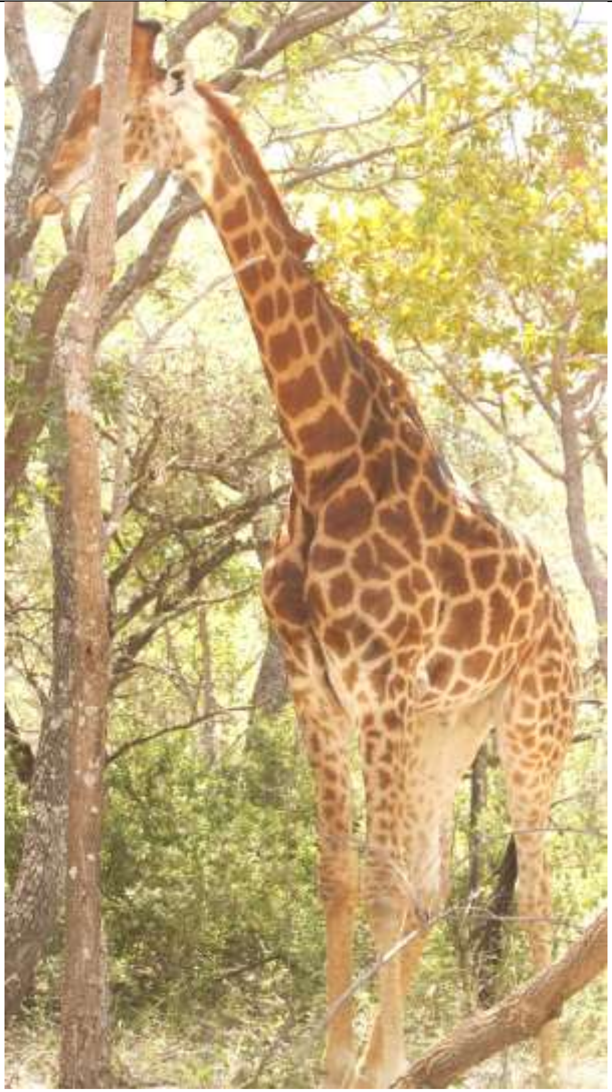


Giraffe ID	M7
Size/Class	Medium – B
Features	

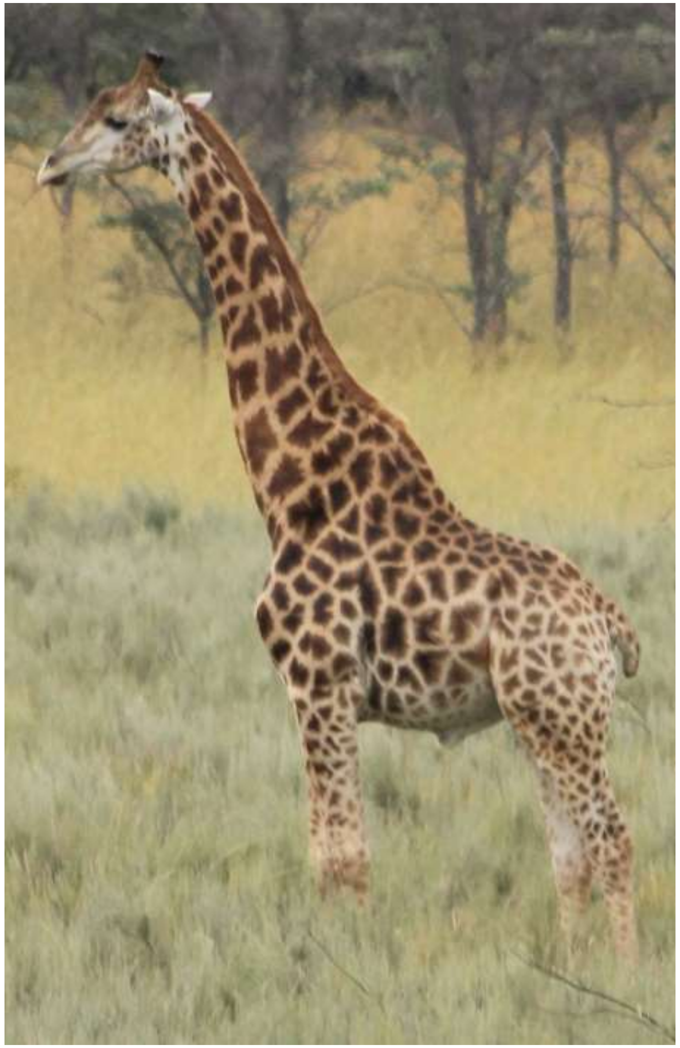
Giraffe ID	M8
Size/Class	Small - C
Features	



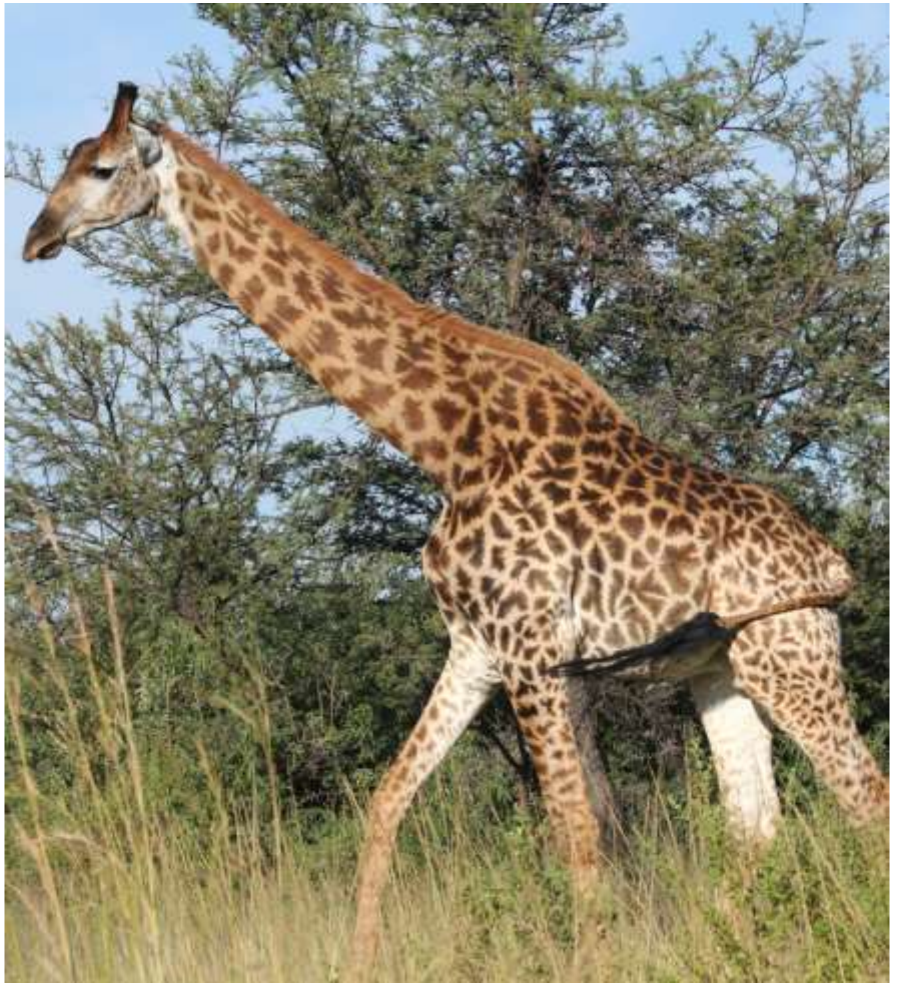
Giraffe ID	M9 (has not been seen since 03/03/2011)
Size/Class	Medium - B
Features	



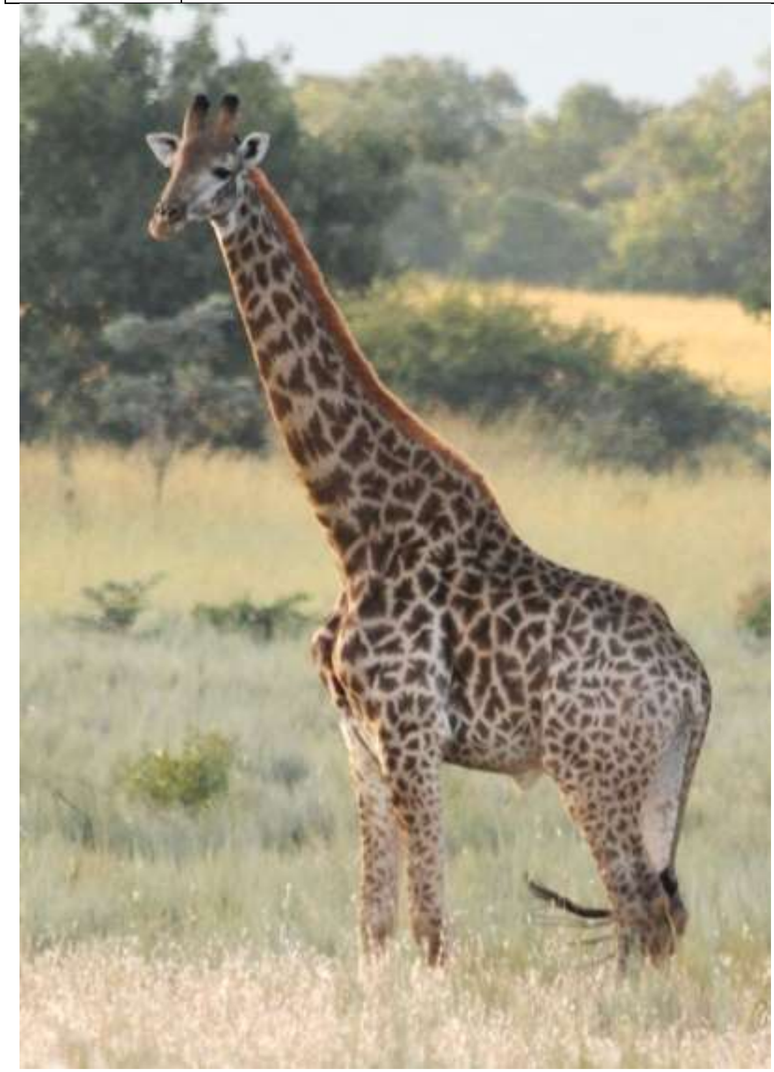
Giraffe ID	M10
Size/Class	Small - C
Features	Tail partly missing

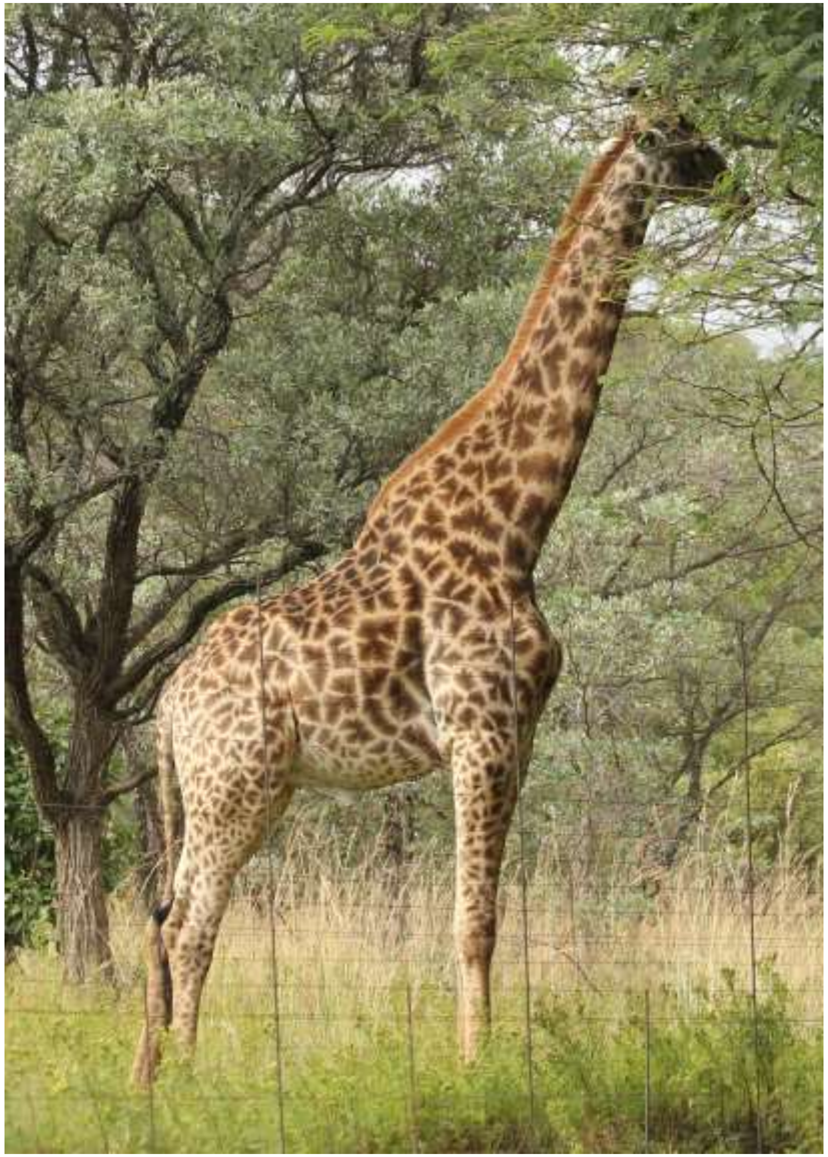


Giraffe ID	M11
Size/Class	Medium - C
Features	Very dark + black forehead

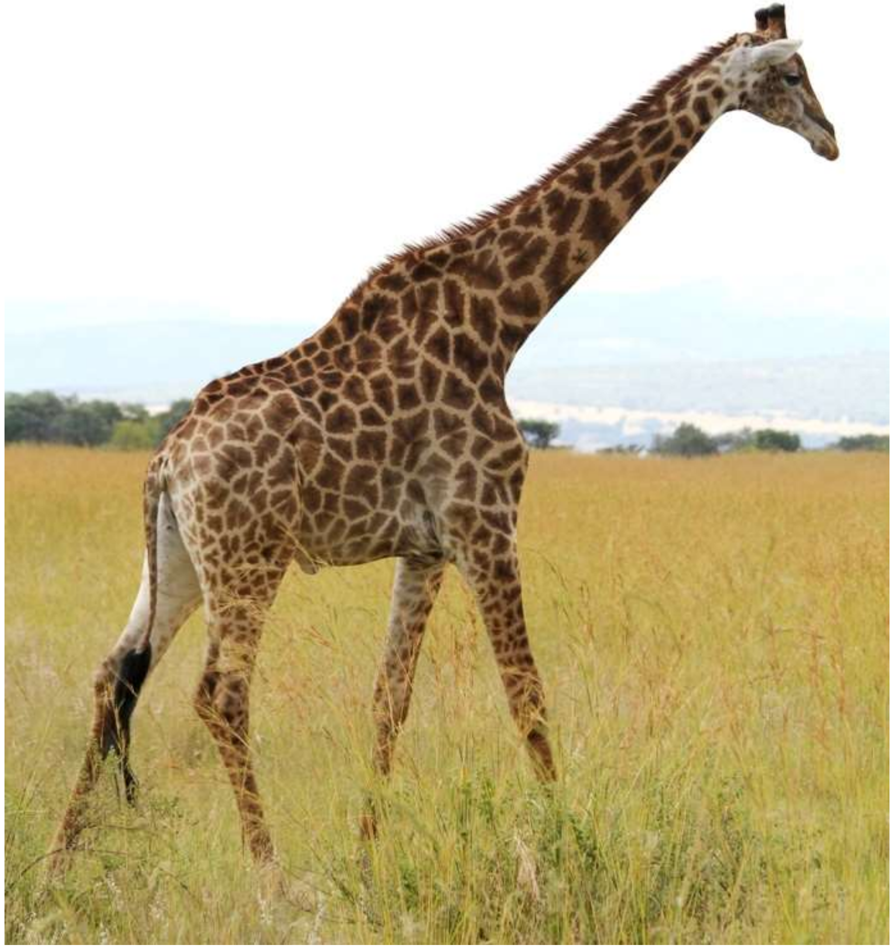


Giraffe ID	M12
Size/Class	Small - C
Features	

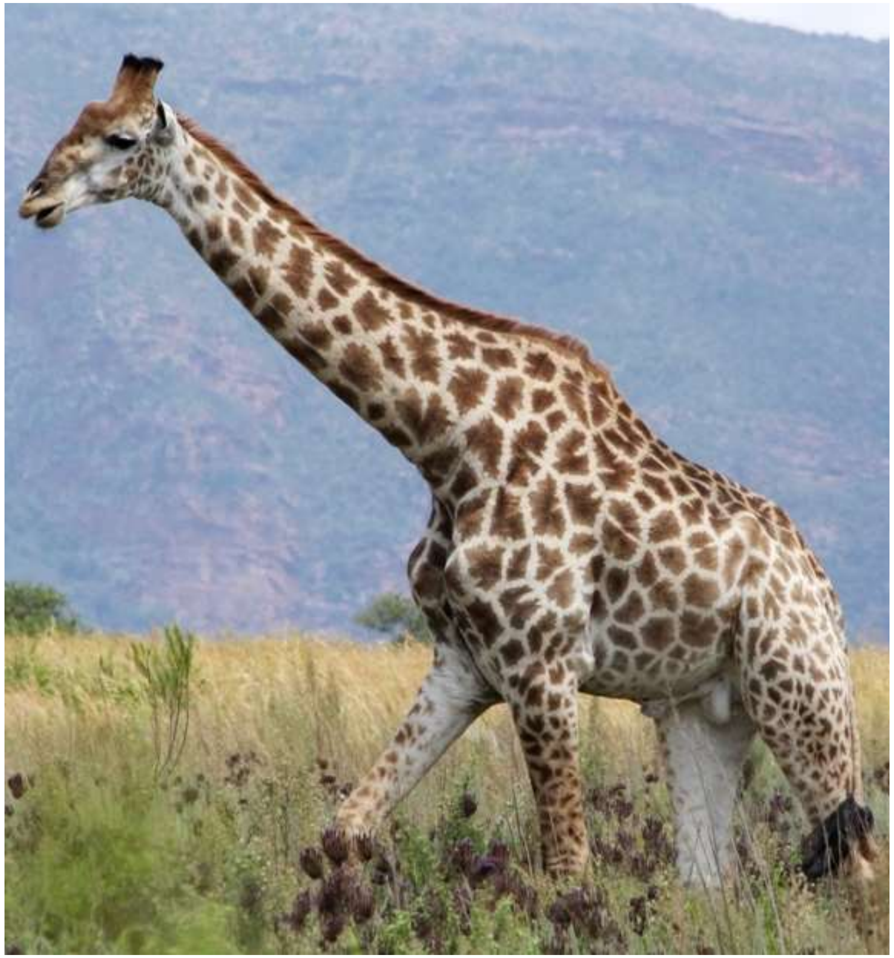


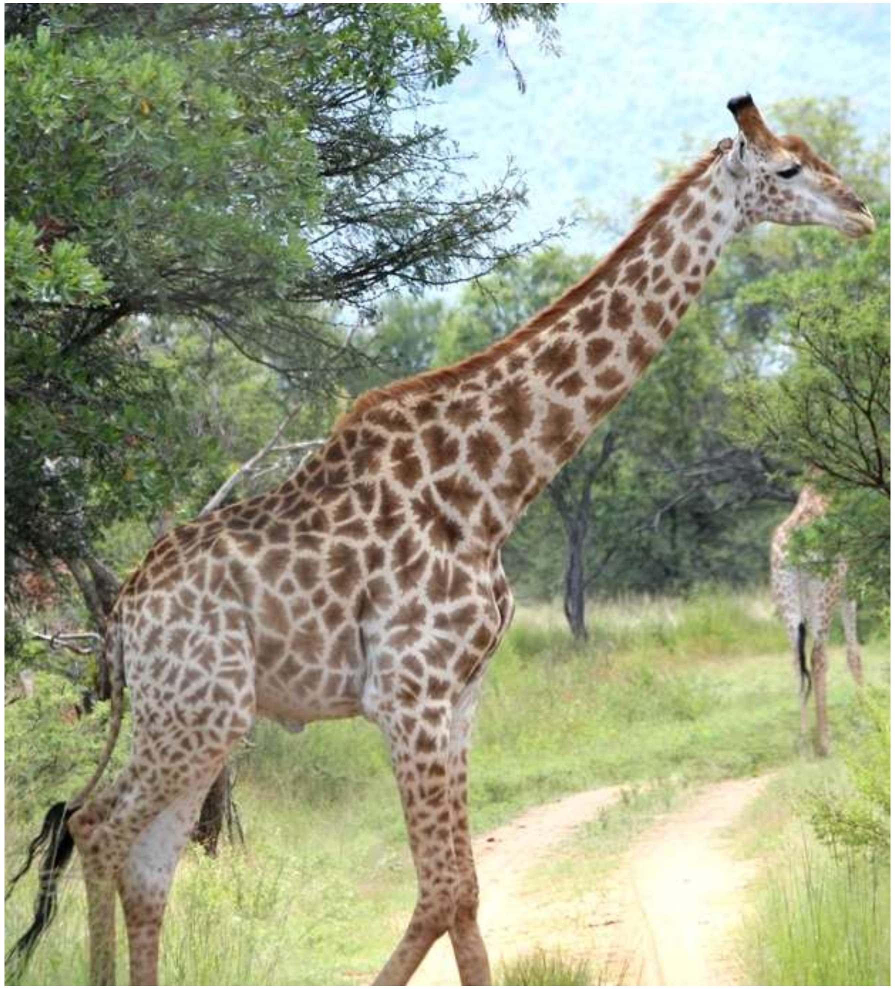


Giraffe ID	M13
Size/Class	Medium - B
Features	



Giraffe ID	M14
Size/Class	Medium – B
Features	Very white background colour

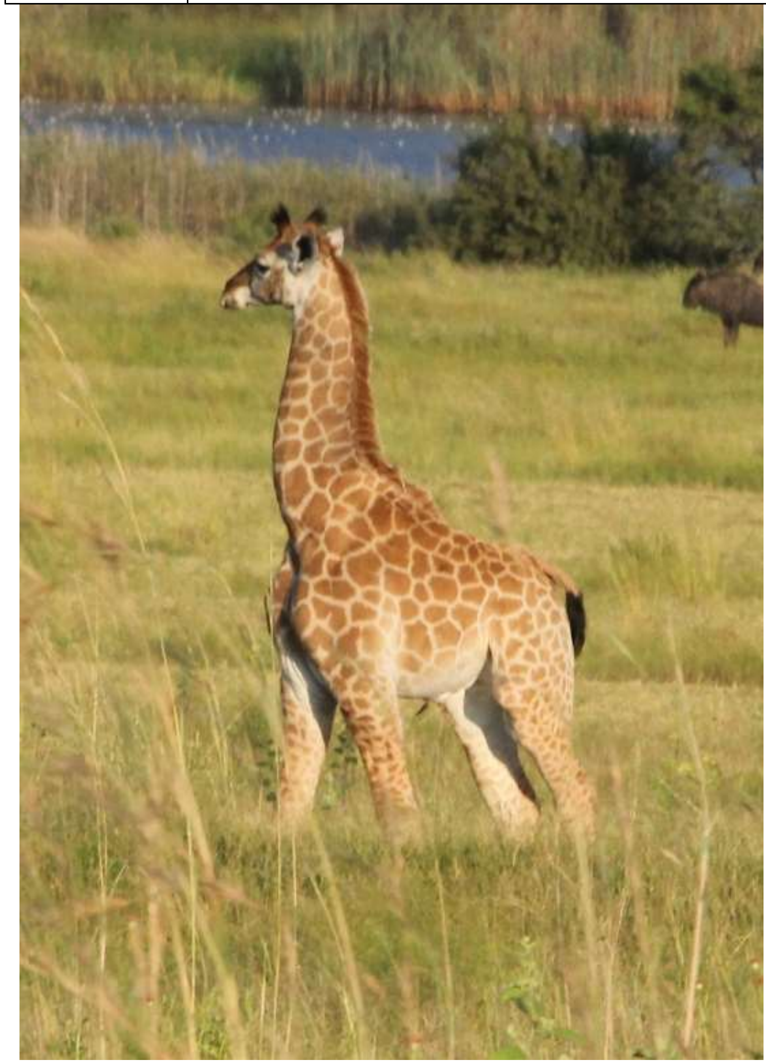




Appendix 3

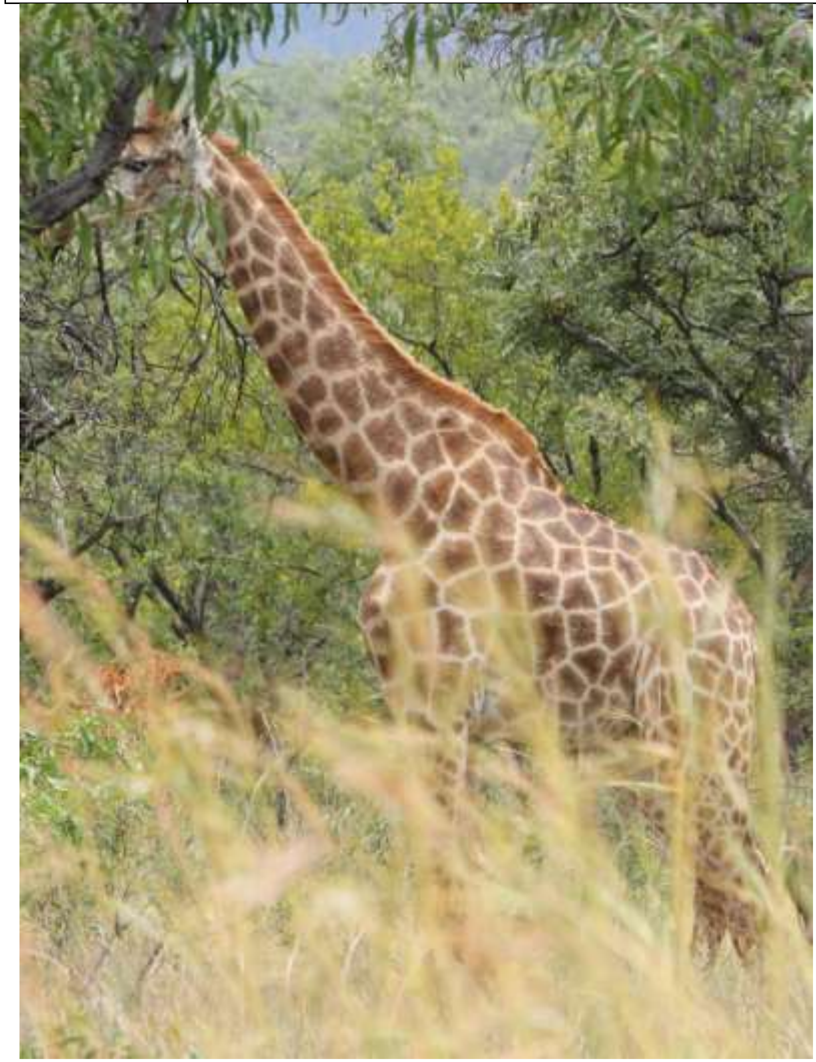
Identification Catalogue
Calves and Juveniles

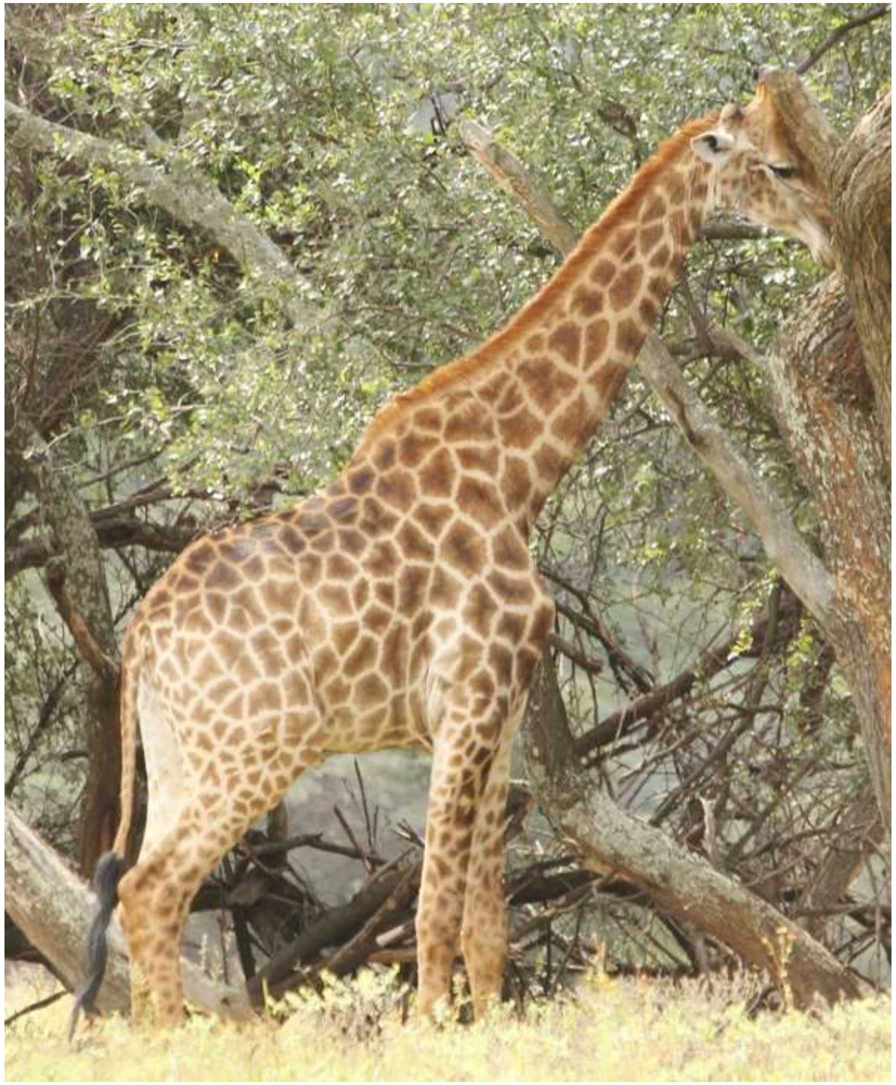
Giraffe ID	C1
Size/Class	Newborn – small
Features	Mother: F19



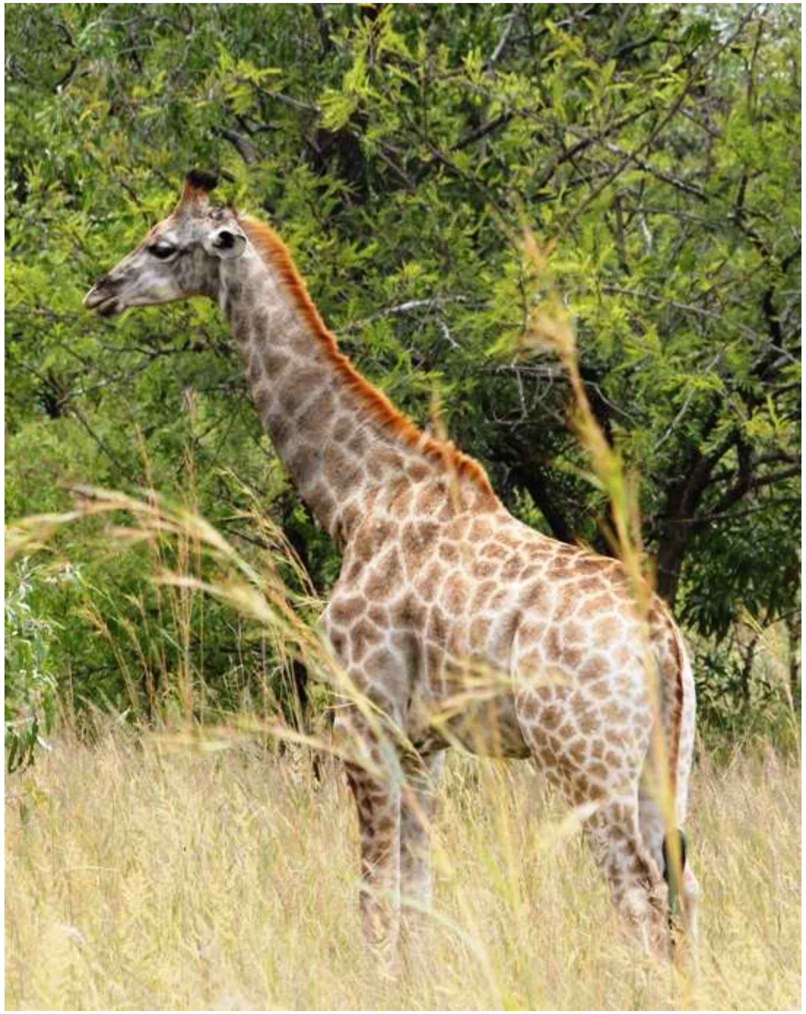
Giraffe ID	C2
Size/Class	Medium calf
Features	Mother: F23

Giraffe ID	J1
Size/Class	Subadult
Features	male

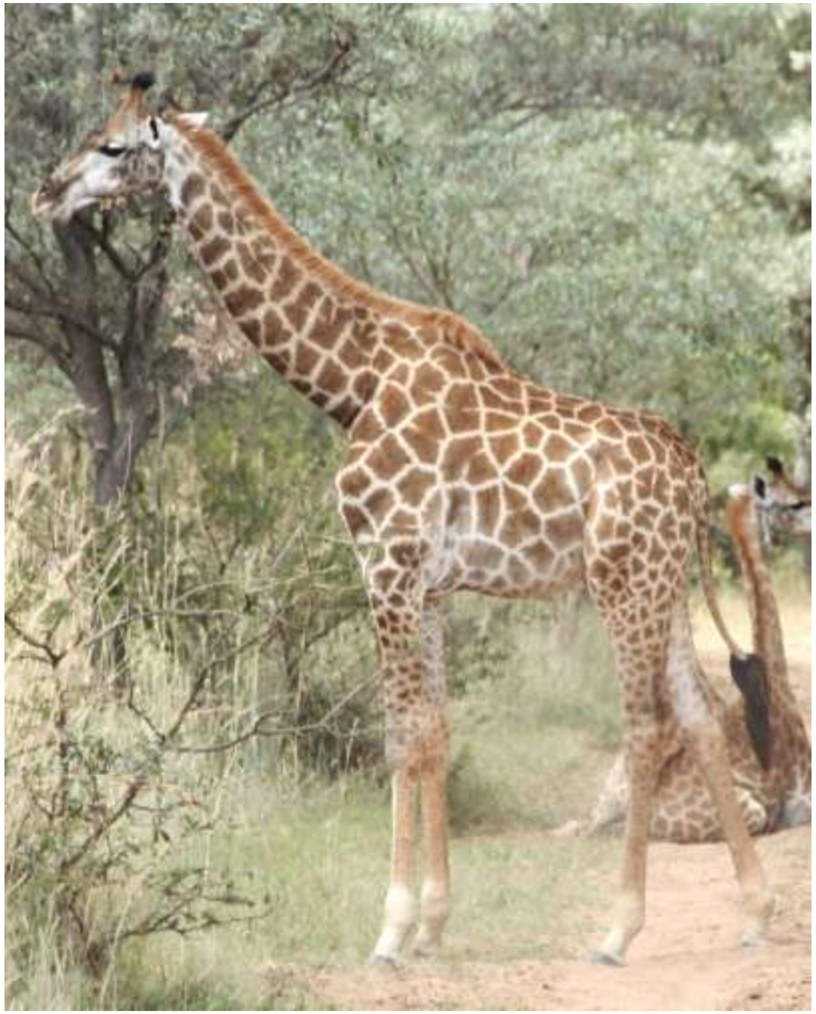


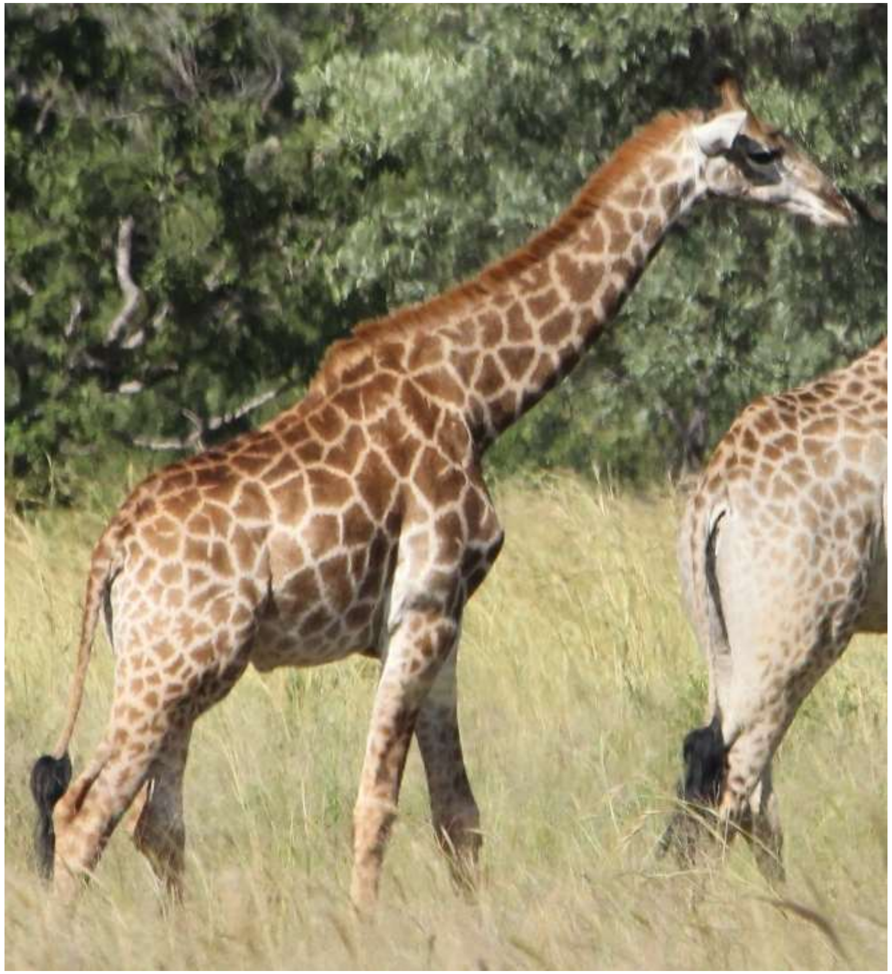


Giraffe ID	C3
Size/Class	Small juvenile (female)
Features	mother: F12

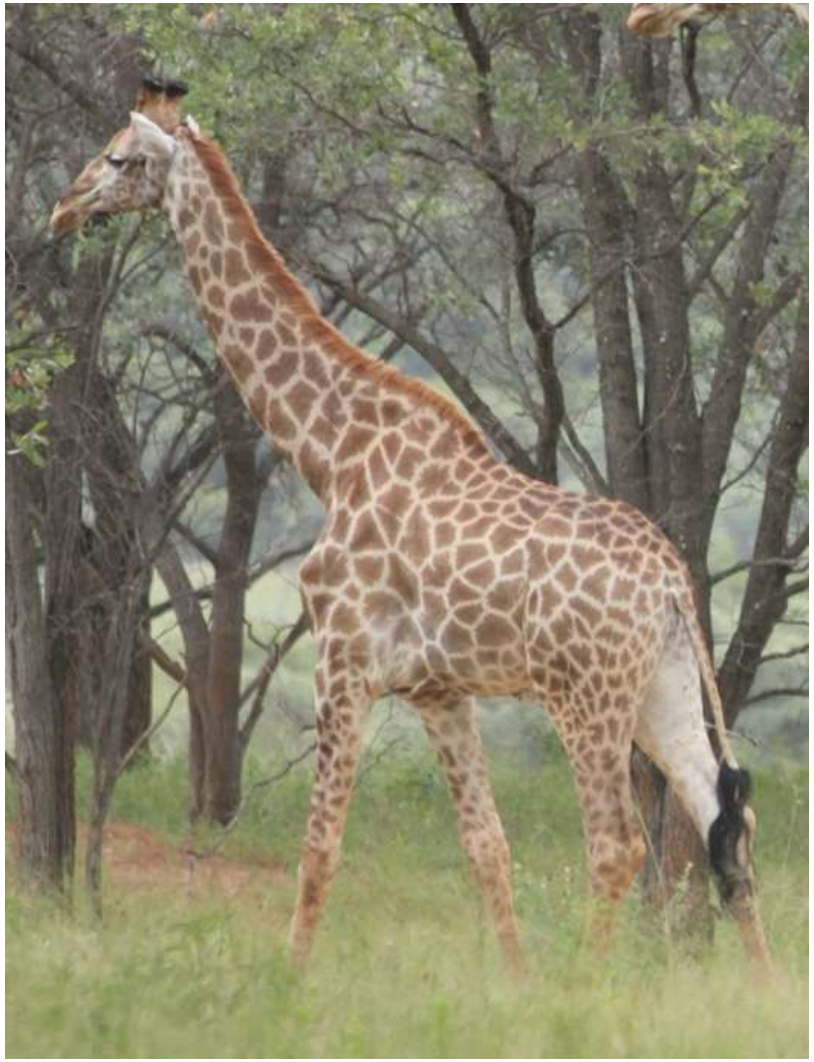


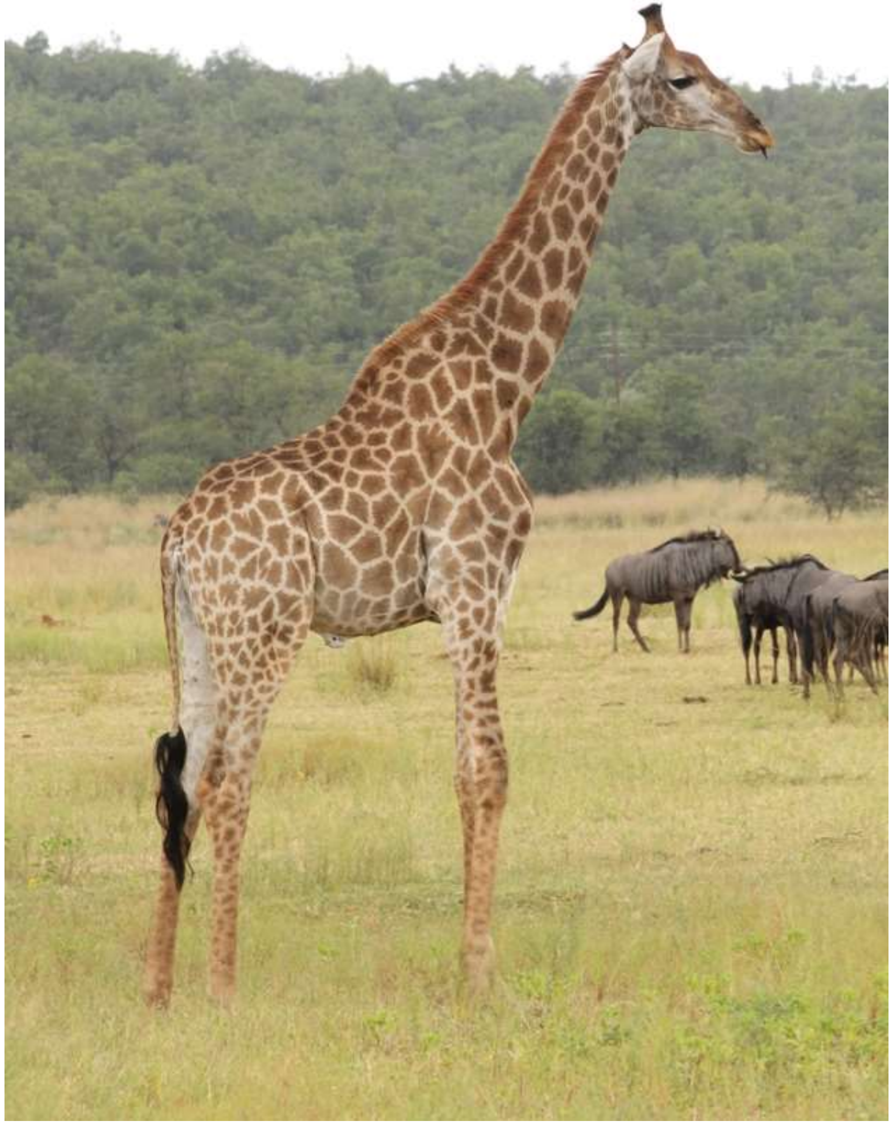
Giraffe ID	J2
Size/Class	Medium-Tall juvenile
Features	male



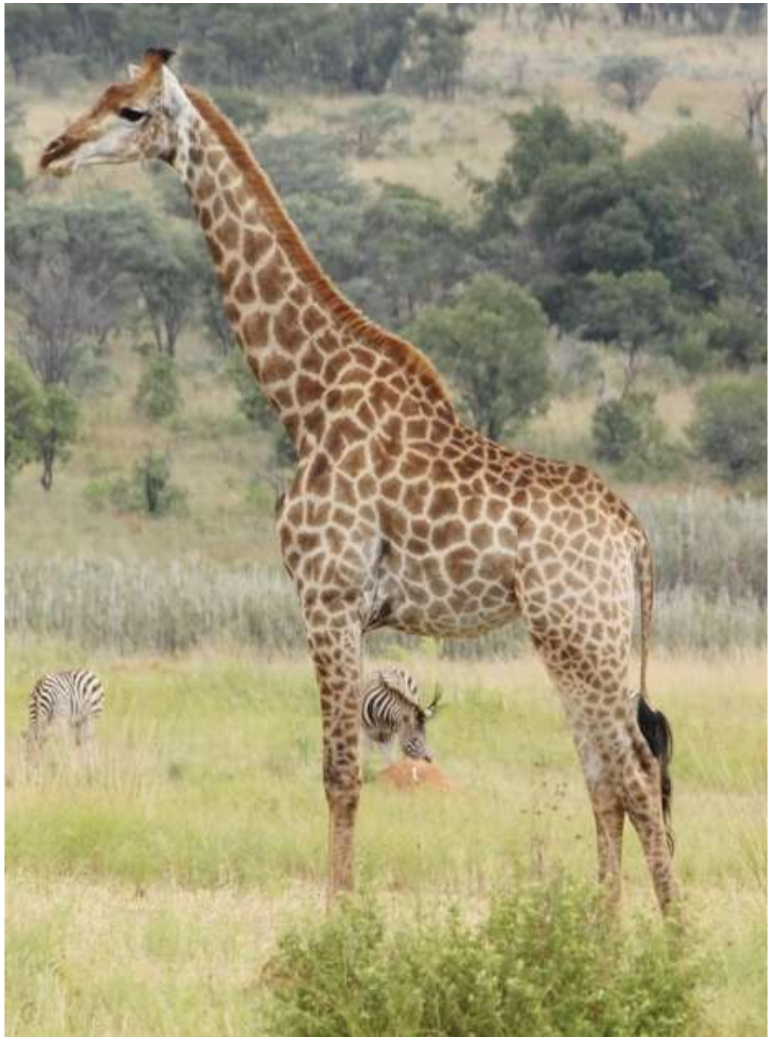


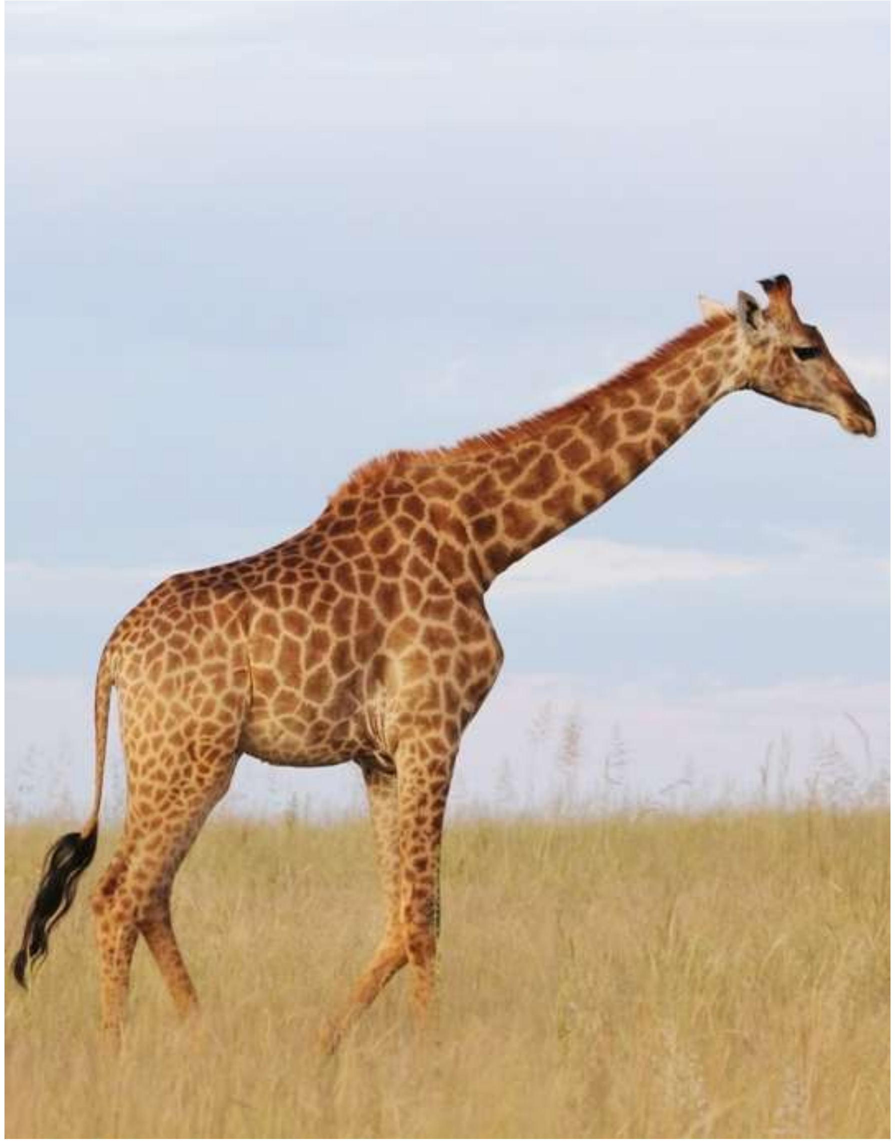
Giraffe ID	J3
Size/Class	Subadult
Features	male





Giraffe ID	J4
Size/Class	Subadult
Features	female





Giraffe ID	C4 (probably deceased - has not been seen since 29/03/2011)
Size/Class	Newborn
Features	Mother: F10

Giraffe ID	C5
Size/Class	Newborn (26th or 27th April 2011)
Features	Mother: F21