

Project Update: March 2015

Objectives:

African wolf distribution pattern – Partially achieved - The most difficult task to fully address the spatial distribution of African wolf across its potential range that include 25 African countries (while African wolf confirmed in six African countries, its status in 19 countries remains unknown Table 1; Fig 1). The difficulties are both on collecting right samples of African wolf distinguishing from sympatric carnivores and processing export permit to laboratory facilities outside Africa).

Cryptic variation of Striped jackal of east Africa – Fully achieved - The cryptic variations of striped jackal between the west and east Africa (Genetic distance of 0.13%; Gaubert *et al.*, 2012) was not documented for samples of striped jackals from Ethiopia-Kenya border and west African countries (Fig 1; Fig 2). Striped jackals from Kenya (Wayne *et al.*, 1997) remain unique.

Genetic diversity of lions of Ethiopia – Fully achieved - From six lion populations sampled in Ethiopia (Fig 4), the lion population in western forest of Ethiopia (Gera forest) is the most distinctive population of all.

Unplanned discoveries – Fully achieved - While attempting in DNA exaction of lion samples, we end up on a pig sequence, this is likely from what the lion eats. The pig we came though is a closer species to the red river hog *Potamochoerus porcu*, a species not recorded in Ethiopia (based on mitochondrial DNA sequences including Cytb, 12s and 16s; Fig 5). Red river hog is known in the central and western African countries. Our first impression a red river hog which has never been spotted by researchers. Latter however, from the animal which was killed by the local people in protecting crops, we learn our pig was in fact from a cryptic bush pig and very different from the red river hog physical appearance (Fig 6). Whether this is a new pig species or rather a closer sub species of red river hog, I am working on the nuclear DNA sequencing with markers used by Gongora *et al.* (2011). I got over 50 samples representing bush pig and red river hog of central and western Africa from colleagues. With other wrong sample collected in Chewbahier, Ethiopia-Kenya border, I come to the bat-eared fox (*Otocyon megalotis*). Crab-eating fox is not related to most of the other fox species, and it is rather closer to the raccoon dog (*Procyon lotor*; Bardeleben *et al.*, 2005; Fig 6). Details will be submitted on detailed report of Rufford in April, 2015.

Getting samples for a DNA exaction is the most difficult task in this project. To fully acknowledge of the distribution pattern of African wolf, samples from the potential 16 African countries should be collected (Table 1; Fig 1). Getting export permit in some African countries are difficult by far than I originally imagined. The other difficulty is similarities of sympatric canid pellets that makes exclusive sampling of African wolf pellet difficult. Even small carnivores like serval cat which I expect to have much smaller pellet size and distinguishable from jackals remains difficult to be screened by their morphological features. Sequencing untargeted carnivore increase costs of lab work.

One of the most important findings of this project is the ever first report of the African wolf in southern Sudan. We also successfully samples Ethiopian lions from the western Ethiopia (Gambella) to the eastern Ethiopia and preliminary results indicates the lion population in the western Ethiopian forest is the most distinctive population. While not intended, the discovery of the cryptic pig species which is closer to the red river hog *Potamochoerus porcu* of Central Africa was also one of the most interesting results of this project. This species was the confused with bush pig (*Potamochoerus larvatus*) during the past. The result of the combination of 2010 bp of mitochondrial DNA however showed it is rather a closer species to the red river hog which has never been reported to exist in Ethiopia so far. Nuclear DNA sequencing is underway. Much more information will be available in the detailed report to be submitted for Rufford web site and published article in the coming couple of months

Local people were involved with this project as field assistance. But more importantly, the results of this project may open a new insight on the conservation effort of the lions and newly discovered cryptic red river hog which ultimately benefit the society.

The African wolf distribution pattern is something that needs collaboration of researchers and conservation managers across the potential ranges (Table 1). Further, this project reveals a cryptic red river hog in western Ethiopia. This is an important implication for conservation. Even large mammals taxonomic status remains unknown in Ethiopia. During this study, we also noticed the high level of lion-human conflict possibly because of increasing loss of the natural pray of lions as a result of habitat loss. Two lions were killed in Gera area and one in Maze National Park in southern Ethiopia as a response of livestock kill by lions. There is a viable population of lions in the western tropical rain of Ethiopia, in particular Gera, but needs an urgent conservation plan for the population to sustain.

I am writing two manuscripts on canids and the cryptic pigs of western Ethiopia to be submitted for international journals. Results however will also be published on local newspapers and web site of Rufford in the near future. I also hope researchers will take these results to discuss on its different aspects on their blogs as done before <http://carnivoraforum.com/topic/10096421/1/>

The RSG fund was used for a year so far and it is according to the plan. I however have samples to be sequenced with additional markers (region of mitochondria) and genotyping. I have laboratory supplies to be used. This work however will be completed in two months from now.

African wolf distribution pattern studies needs further work on wide range of African countries (Table 1). A full genome sequence on Africa wolf also may reveal the ultimate evolutionary history of the species. The lion-human conflict in Ethiopia is at the pic, three lions were killed since February 2014 in Gera and southern Ethiopia. We learn six to be killed during the last 3 years only in the western Ethiopia. In the western jungle forest of Ethiopia, habitat loss is increasing which deplete the natural prey population for lions. It is crucial to find a management in resolving the conflict. The bush pig in western Ethiopia were found rather a cryptic species closer to the red river hog. I am working on the nuclear DNA at the moment, further work to determine whether this is a unique small population or all

the “Bush pig” in Ethiopia including the population in the Bale Mountains are same cryptic pig species is important. There is also extensive hunting for food on this species in Ilibbabour zone while it is killed for crop protection in the adjacent zone in Jima that needs a conservation action plan. “Bush pig” are widely distributed in Ethiopia across the landscape that potentially can support a forest area (landscape with annual rain fall above 1270 mm; Fig 8).

The discovery of the new African wolf, cryptic red river hog and genetic distinctive lions of Addis Ababa zoo reveals, Ethiopia has a wide range of biodiversity which is still need to be studied and gets conservation effort. While limited effort is made for the research and conservation of biodiversity in the Ethiopian highlands, very little is known on the rest of the ecological zones of the country, and support by Rufford in wide range of ecological studies is greatly appreciated.

Table 1. African wolf status in their potential range that is the range of the golden jackal as indicated by IUCN (Jhala & Moehlman, 2008). Golden jackals are those which are confused with cryptic African wolf (Rueness et al., 2011).

No	Country	REGION	African wolf status
1	Tunisia	Northern Africa	Unknown
2	Algeria	Northern Africa	Confirmed
3	Morocco	Northern Africa	Unknown
4	Libya	Northern Africa	Unknown
5	Egypt	Northern Africa	Confirmed
6	Mauritania	Western Africa	Unknown
7	Mali	Western Africa	Confirmed
8	Niger	Western Africa	Unknown
9	Sudan	Northern Africa	Unknown
10	South sudan	Northern Africa	Confirmed
10	Chad	Middle Africa	Unknown
11	Ethiopia	Eastern Africa	Confirmed
12	Senegal	Western Africa	Confirmed
13	Burkina Faso	Western Africa	Unknown
14	Nigeria	Western Africa	Unknown
15	Cameroon	Middle Africa	Unknown
16	Djibouti	Eastern Africa	Unknown
17	Benin	Western Africa	Unknown
18	Somalia	Eastern Africa	Unknown
19	Central African Republic	Middle Africa	Unknown
20	Zaire	Middle Africa	Unknown
21	Kenya	Eastern Africa	Unknown
22	Uganda	Eastern Africa	Unknown
23	Tanzania	Eastern Africa	Unknown
24	Rwanda	Eastern Africa	Unknown
25	Burundi	Eastern Africa	Unknown

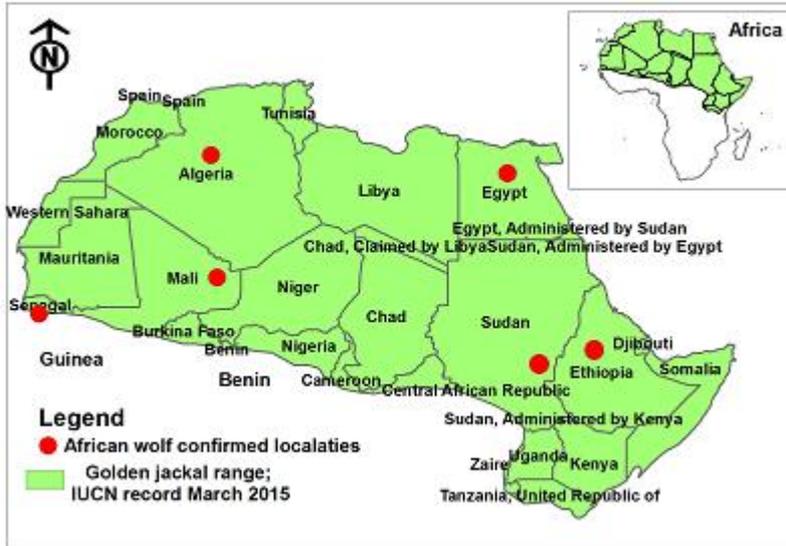


Fig 1. Golden jackal range (according to IUCN red list of endangered species on March 07, 2015) that potentially be the African wolf range.

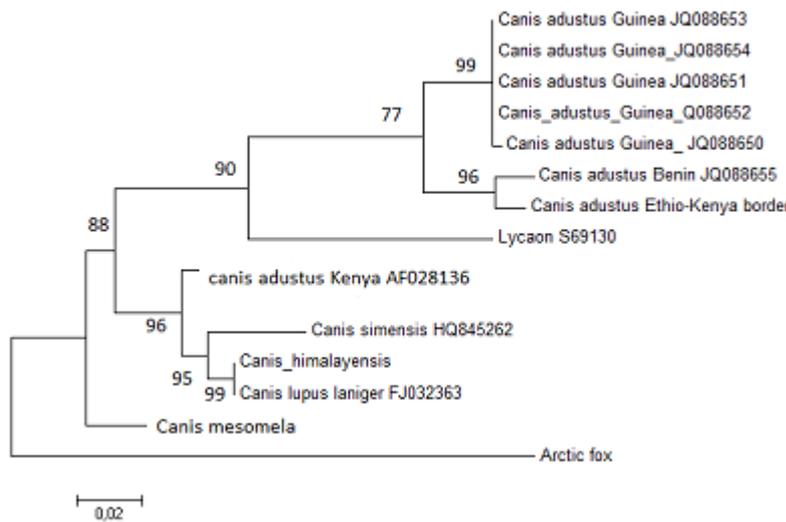


Fig 2. Maximum likelihood tree based on 420bp of Cytb analysed in MEGA. Striped jackals of Ethiopia-Kenya border grouped together with the rest of the striped jackal in the western African countries.

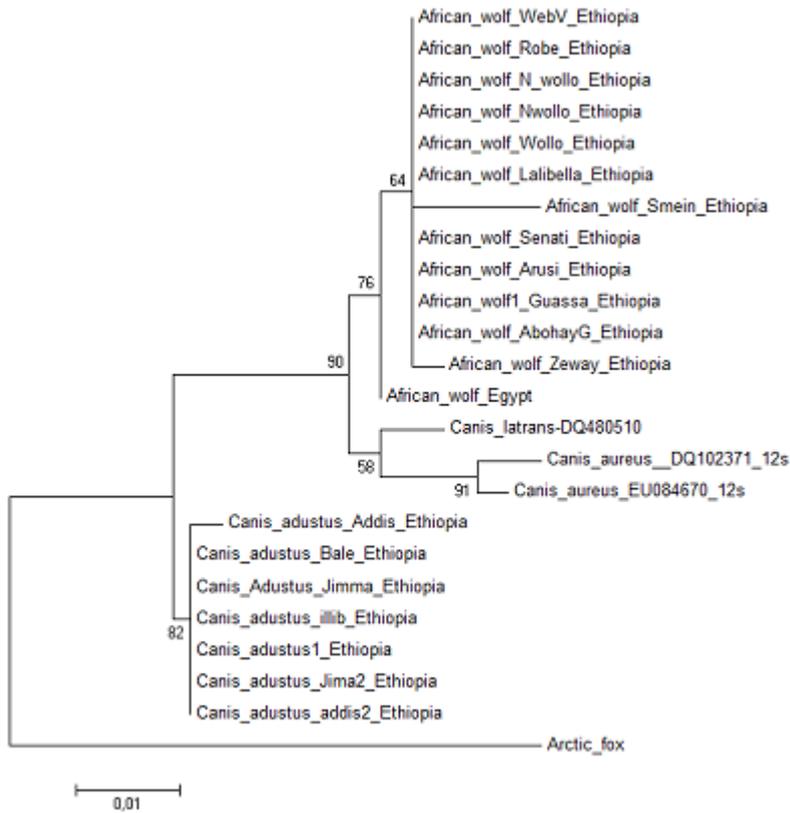


Fig 3. Maximum likelihood tree based on 380bp of 12S analysed in MEGA. Samples of striped jackal and Ethiopian wolf across the different regions of Ethiopia. We did not find any sample representing golden jackal sample so far.

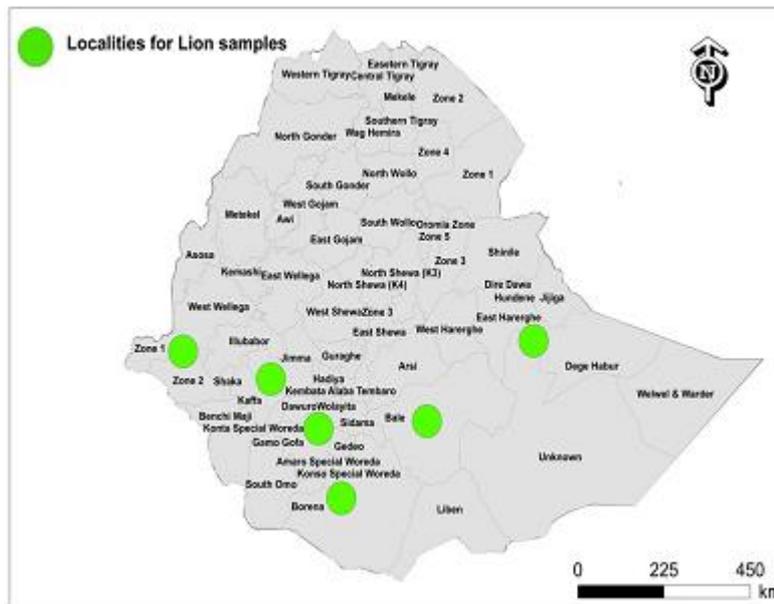


Fig 4. Lion populations sampled for genetic studies in Ethiopia

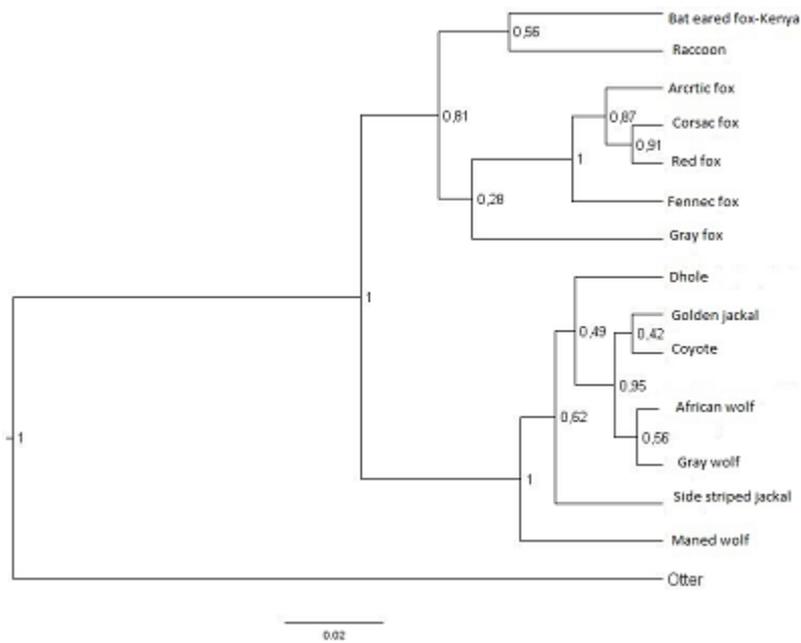


Fig 7. Maximum likelihood based on 380 bp of cytb.

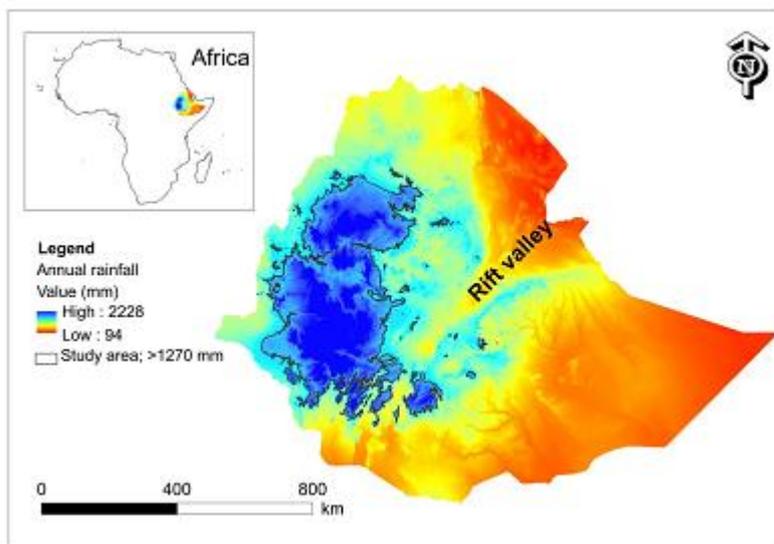


Fig 8. The potential “Bushpig” range in Ethiopia; landscape that potentially can support rain forest.

References

- Bardeleben, C., Moore, R.L., Wayne, R.K. (2005). A molecular phylogeny of the Canidae based on six nuclear loci. *Molecular Phylogenetic and Evolution* 37: 815–831.
- Bruche, S., Gusset M., Lippold S., Barnett R., Eulenberger K., Junhold J., Driscoll C.A. & Hofreiter M. A (2013). Genetically distinct lion (*Panthera leo*) population from Ethiopia. *Eur J Wildl Res* 59: 215-225.
- Gaubert, P., Bloch C., Benyacou S., Abdelhamid A., Pagani P., Adeyemi C., Djagoun M.S., Couloux A., Dufour S. (2012). Reviving the African Wolf *Canis lupus lupaster* in North and West Africa: A Mitochondrial Lineage Ranging More than 6,000 km Wide. *PLoS ONE* 7(8): e42740. doi:10.1371/journal.pone.0042740.
- Jhala, Y. & Moehlman, P.D. (2008). *Canis aureus*. The IUCN Red List of Threatened Species. Version 2014.3. <www.iucnredlist.org>. Downloaded on 13 March 2015.
- Rueness, Eli Knispel; Asmyhr, Maria Gulbrandsen; Sillero-Zubiri, Claudio; Macdonald, David W., Bekele, Afework; Anagaw Atickem & Stenseth, Nils Chr. (2011). The cryptic African wolf: *Canis aureus lupaster* is not a golden jackal and is not endemic to Egypt. *PLOS ONE* e16385:1–5.
- Wayne RK, Geffen E, Girman DJ, Koepfli KP, Lau LM, et al. (1997). Molecular systematics of the Canidae. *Systematic Biology* 46:622–653.