Project Update: August 2023

A total of 47 faecal samples were transported from Bhutan on 30th December 2022, to IISc Bangalore. We did eight extractions of five samples each. Genetic data generated so far for the hybrids was intended to examine the hybridisation between golden and capped langurs using mitochondrial D-loop gene. Currently, we aim to examine the phylogenetic position of hybrids with respect to the golden and capped langurs. The following are the results for works carried out till now:

- Mitochondrial D-loop region was PCR amplified and subsequently sequenced for 33 individuals out of which 19 independent sequences were used for reconstructing phylogenetic tree and median joining network (Figure 1 & 2).
- The median joining network was constructed using 19 sequences with 518 bases. It shows that the hybrids cluster more towards *Trachypithecus pileatus* (capped langurs), than *Trachypithecus geei* (golden langur), samples collected from China and India respectively. However, adequate samples are required to confirm the maternal line of hybrids, and we are working more on it.
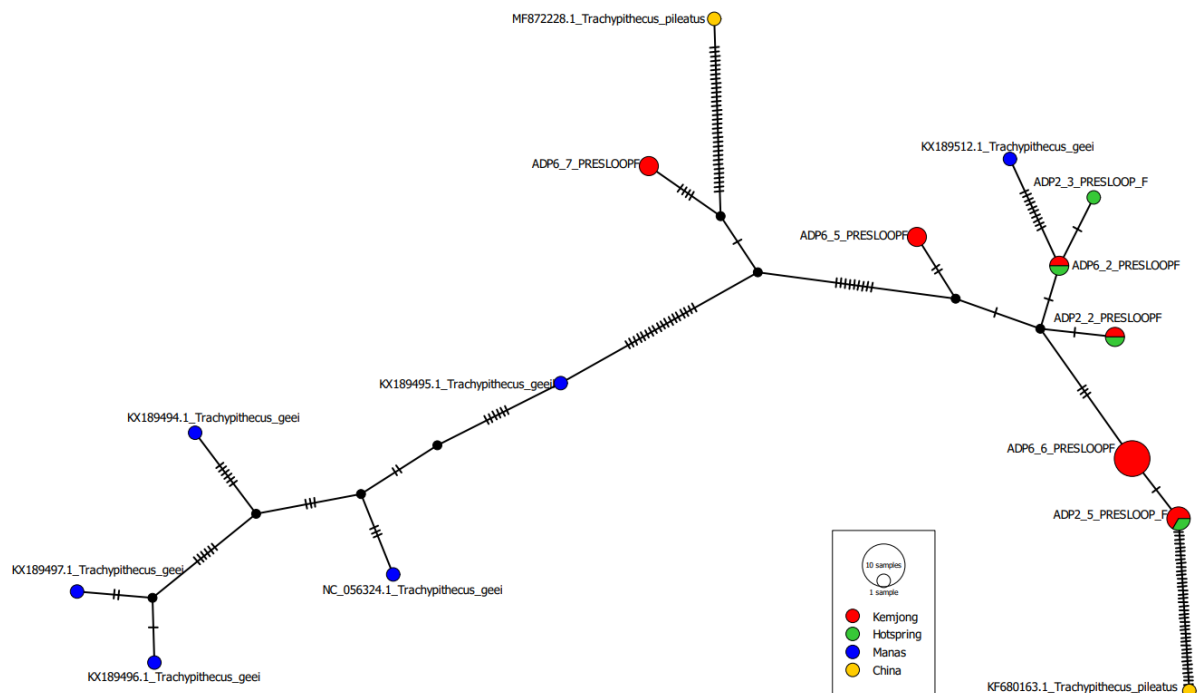

Figure 1: Median Joining Network of mtDNA D-loop haplotypes of hybrids between golden and capped langurs. The size of each circle represents the frequency of each haplotype, and the colours represent each fragment. Each bar on the lines connecting two haplotypes represent one mutational step. The names with ADP are the hybrid samples (N=19) collected from Zhemgang, Bhutan. All Trachypithecus geei sequences (N=6) are used from Ram et al. (2016). The sequences for Trachypithecus pileatus (N=2) are from Shi et al. (2015) and Roos et al. (2018).

- The 15 haplotypes differed from each other by between 1 and 51 bases. Three out of the 15 haplotypes were shared between utmost two fragments- all three haplotypes ADP6_2_PRESLOOPF, ADP2_2_PRESLOOPF, ADP2_5_PRESLOOPF shared between Kemjong and Dungmang Hotspring. In general, the haplotype network corresponded with the geographical origins of the samples,

however, there are some discrepancies. Firstly, the hybrids and *T. pileatus* clustering together, and most of *T. geei* separated by a massive nucleotide change.

- The 19 D-loop sequences of hybrids, along with GenBank sequences of *Trachypithecus geei, Trachypithecus pileatus* and *Semnopithecus schistaceus* as the out group were used to construct the Maximum likelihood tree (Figure 2).
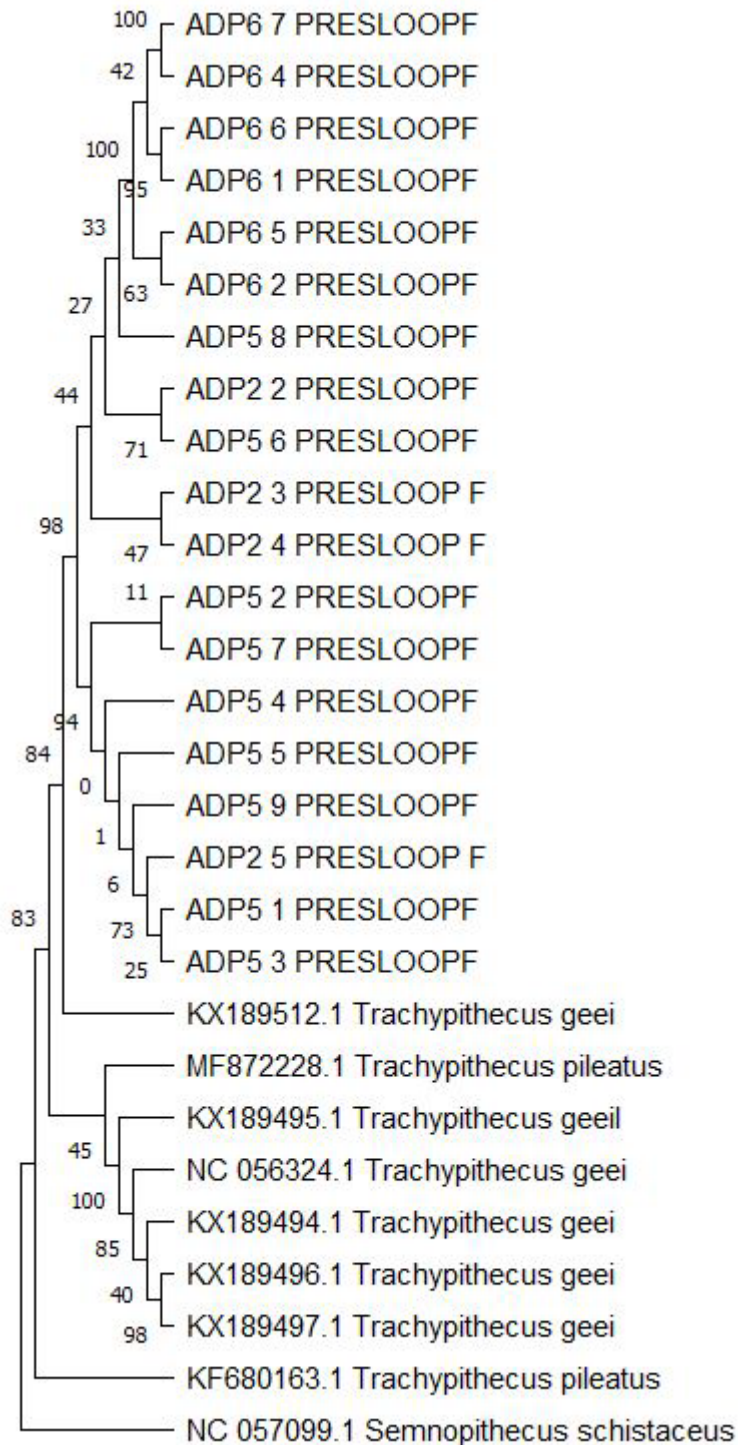

Figure 2: Maximum Likelihood tree reconstructed from 664 bases of D-loop. The numbers at each node are the bootstrap values of the corresponding nodes.

- The hybrids emerged as a monophyletic group, nested within *T. geei* with high bootstrap support. Interestingly, one of the *T. pileatus* sequences (Roos et al., 2018) also clustered with the *T geei* clade, while the other *T. pileatus* sequence separated out.
- Currently, more work is being carried out to genotype microsatellites to study the genetic structure of paternal component of the hybrids. The results and analysis would be shared to Rufford Foundation after completion.