Project Update: May 2019

Total count of cyt B results:

- 80 wolves/dogs (14 confirmed wolves with D-loop)
- 16 snow leopards
- three lynx (two in CGNP, one from near Hushe in Gilgit Baltistan)
- 34 red fox
- two Markhor (may have been prey DNA)
- one cow (may have been prey DNA)
- one golden Jackal
- one leopard cat

Total: 199 scat samples (including Hameed's and previous shipments from Gilgit Baltistan)

Laboratory Methods

To investigate what subspecies of wolves inhabit Pakistan, we sequenced the mitochondrial cytochrome-b and D-loop haplotypes of 199 scat samples collected from Gilgit-Baltistan, KPK, and FATA. This part of the work was done in the Mammalian Ecology and Conservation Unit in the Veterinary Genetics Laboratory at the University of California, Davis, USA. DNA was extracted from approximately 15 mg of faeces using the DNeasy Blood & Tissue Kit (Qiagen, CA). The total volume of DNA extracts from each scat sample was 50µL. Blank extractions were systematically performed to monitor possible contaminations.

We used Polymerace Chain Reaction (PCR) and performed sequencing in forward direction using cytochrome B primers RF14724 (5'-CAACTATAAGAACATTAATGACC-3') and RF15149 (5'- CTCAGAATGATATTTGTCCTC-3'), targeting about 400-bp of the cytochrome b of the mitochondrial DNA. To amplify DNA, we used a PCR thermal profile involving a 10 minute denature step at 94 °C, followed by 50 cycles.

The sequencing was carried out on the Illumina Genome Analyzer Iix (Illumina Inc.). The sequence reads were analyzed using Sequencher DNA Sequence Analysis Software 5.2.4 and we confirmed samples as wolf/dog species by using reference haplotypes in GenBank. Of the 80 scat samples that assign to wolf/dog based from cytochrome B, 65 of these samples still need to be sequenced at the D loop region.

For the samples that are identified as wolf/dog, we will perform D-loop PCR using the primers HV1A-F: CCCTGACACCCCTACATTCA and HV1C-R: TTATGTGTGATCATGGGCTGA to confirm *Canis lupus* and identify the phylogenetic position of the wolves in Pakistan. We have already sequenced the D loop region for 3 wolf samples and confirmed that they are grey wolf, *Canis lupus*.

All cytochrome B and D loop sequences that were obtained from the extracted samples were aligned and edited using the software Sequencher DNA Sequence Analysis Software 5.2.4.

Phylogenetic Analysis

Cytochrome B region

After sequencing the cytB region of the mitochondria for the 80 putative wolf samples, we obtained 39 worldwide samples from the genus *Canis* (Table xxx). We then aligned 350bp of the cytochrome B sequence of our canid samples using ClustalX 2.1 (Larkin et al. 2007). After alignment, a Bayesian coalescent-based phylogenetic analysis was performed using BEAST 1.7.5 (Heled and Drummond 2010) with applying the HKY+G+I model of nucleotide substitution (Hasegawa et al. 1985). This model was selected as the best-fit model based from maximum likelihood, which was computed in MEGA 7.0 (Kumar et al. 2016). We performed 50,000,000 generations, sampling every 5000 generations, which we then retained 10,000 samples. Based from the results using Tracer 1.5 (Rambaut and Drummond 2007), we designated 10% of the first trees as burn-in. The effective sample sizes (ESS) were above 200 for all parameters.

D-loop region

Similarly above, we constructed a phylogenetic tree using the D-loop region of the mitochondria for 3 wolf samples from Pakistan (2 from South Waziristan, 1 from Sadpara, Gilgit Baltistan) and 104 other worldwide grey wolves with one coyote as an outgroup (Table xxx). The same statistical methods as above were used to infer the evolutionary relationships among grey wolves. These results were previously published in Hamid et al. 2018.

Preliminary insight into the distribution of wolf subspecies in Pakistan

The preliminary results show all of the putative wolf samples from northern Pakistan fall within the Holarctic wolf clade, instead of the Tibetan or Indian wolf clade. This is an interesting result, since one previous sequenced grey wolf from Kargil (India) has a cytochrome B region that falls within the Tibetan wolf clade. Kargil is only ~40 miles from Sadpara, which we confirmed with cytochrome B and Dloop that the wolves from this region are Holarctic grey wolves, based from their mitochondria. This region along the India-Pakistan border may represent the extent of the Tibetan wolf's distribution.

We also sequenced wolf genetic samples from the Sulieman range of South Waziristan. This mountain range lies just west of the Indus River and has a different community of mammalian species compared to the high altitude mountainous region of Gilgit Baltistan. Based from Sharma *et al,* the wolves inhabiting the Indus river basin fall within the Indian wolf lineage and it remained unknown how far west this lineage was distributed into Pakistan and possibly Iran and Afghanistan. Our results show that the wolves of the Sulieman range of South Waziristan also fall within the Holartic wolf clade. Further research should aim to describe the extent of the distribution of the Indian wolf lineage in Pakistan.

Preliminary insight into the distribution of fox subspecies in Pakistan

From our 199 scat samples, our sequencing efforts identified 34 red foxes.

Table xxx. Canid samples included in the phylogenetic tree using 350bp of cytochrome B (figure xxx).

Genbank ID	Common Name	Species Name	Location of Sample
AF028140.1	Coyote	*Canis latrans*	USA
KT946970.1	Coyote	*Canis latrans*	USA
KT946974	Coyote	*Canis latrans*	USA
L29415	Ethiopian wolf	*Canis simensis*	Ethiopia
KT343783	Golden jackal	*Canis aureus*	India
KT343785	Golden jackal	*Canis aureus*	Israel
KT447712	Golden jackal	*Canis aureus*	Israel
KT343784	Golden jackal	*Canis aureus*	India
KT343786	Golden jackal	*Canis auerus*	Bulgaria
JQ088659	African wolf	*Canis anthus*	Algeria
JQ088663	African wolf	*Canis anthus*	Algeria
JQ088664	African wolf	*Canis anthus*	Senegal
KT447759	African wolf	*Canis anthus*	Morocco
JQ088665	African wolf	*Canis anthus*	Mali
KT447735	African wolf	*Canis anthus*	Kenya
AJ441334	Gray wolf	*Canis lupus*	Spain
DQ480503	Gray wolf	*Canis lupus*	Russia
DQ480504	Gray wolf	*Canis lupus*	Sweden
KT447701	Gray wolf	*Canis lupus*	Ukraine
HM222711	Gray wolf	*Canis lupus*	Mexico
KJ162937	Gray wolf	*Canis lupus*	Saudi Arabia
KU696392	Gray wolf	*Canis lupus*	Mongolia
KC461238	Gray wolf	*Canis lupus*	XinJiang, China
KT447705	Gray wolf	*Canis lupus*	Israel
DQ480508	Gray wolf	*Canis lupus*	Canada
DQ480507	Gray wolf	*Canis lupus*	Saudi Arabia
KT447703	Gray wolf	*Canis lupus*	Oman
KJ162934	Gray wolf	*Canis lupus*	Saudi Arabia
BH123	Indian wolf	*Canis lupus indica*	India
BH53	Indian wolf	*Canis lupus indica*	India
BH126	Indian wolf	*Canis lupus indica*	India
BH118	Tibetan wolf	*Canis spp.**	Ladakh, India
KY996533	Tibetan wolf	*Canis spp.**	Nepal
BH5	Tibetan wolf	*Canis spp.**	Ladakh, India
KY996534	Tibetan wolf	*Canis spp.**	Nepal
KT447688	Black backed jackal	*Canis mesomelas*	Africa
KT447689	African wild dog	*Lycaon pictus*	Africa
AY291430	Dhole	*Cuon alpinus*	India
MF196960	Side striped jackal	*Canis adustus*	Africa
MF196961	Side striped jackal	*Canis adustus*	Africa

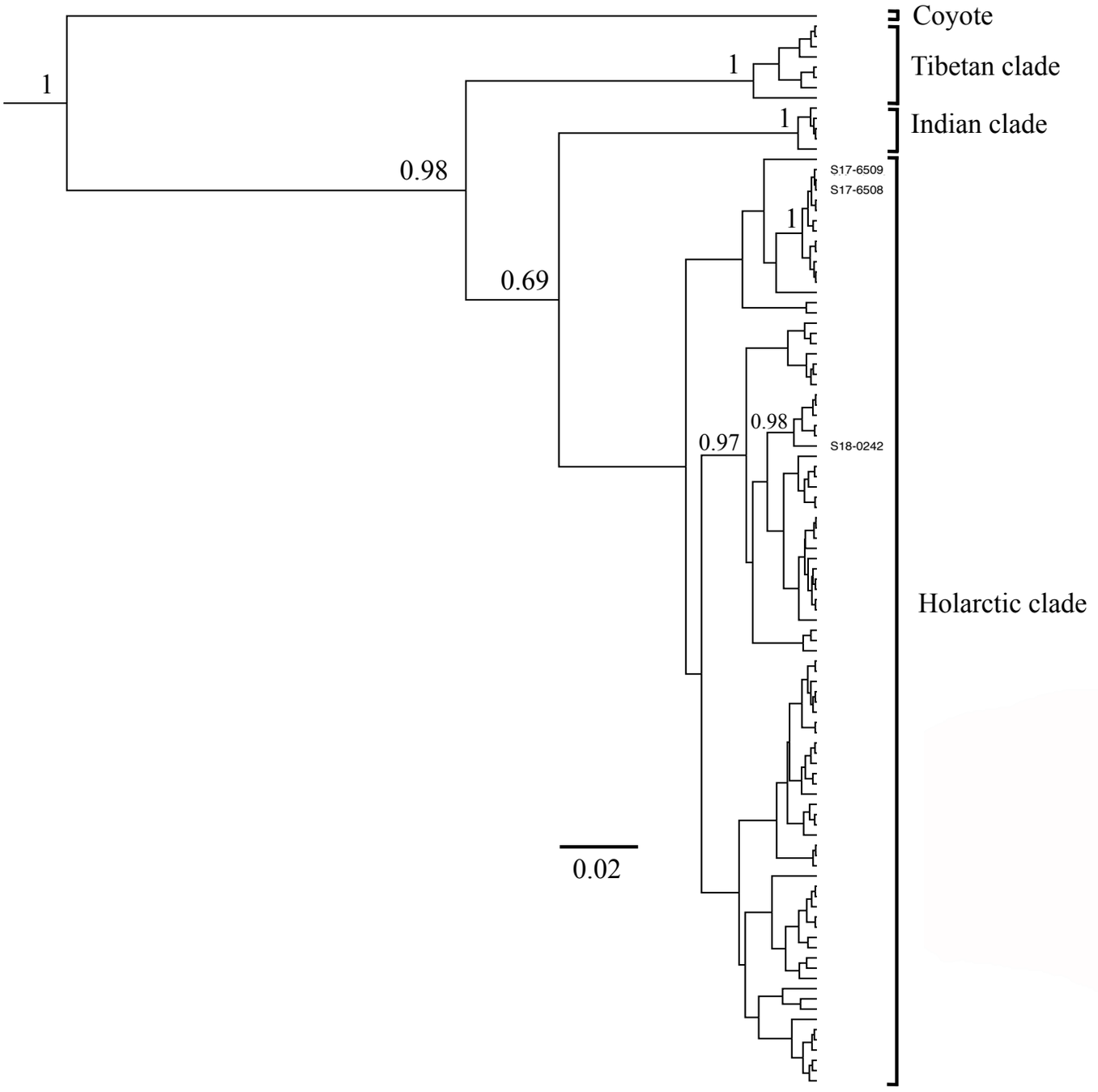
* Canis spp. refers to the Tibetan/Himalayan wolf lineage. Its scientific name needs to be formally described still, but see Werhahn et al. 2018.

Table xxx. Samples included in the phylogenetic tree using 402bp of the mitochondrial D-loop region, with the GenBank accession or ID number, and the geographic origin of the wolf. There are 104 gray wolf samples with the coyote as an outgroup.

GenBank accession/ID number	Species	Origin
AF008158	Coyote	North America
KY124130	Gray wolf	Sweden
KX898307	Gray wolf	Siberia (35000 BP)
KX898308	Gray wolf	Siberia (35000 BP)
KX898312	Gray wolf	Siberia
KX898313	Gray wolf	China
KX898314	Gray wolf	China
KX898315	Gray wolf	China
KX898316	Gray wolf	China
KX898317	Gray wolf	China
KX898318	Gray wolf	China
KX898319	Gray wolf	Iran
KX898320	Gray wolf	Iran
KX898321	Gray wolf	Russia
KX898322	Gray wolf	North America
KX898323	Gray wolf	Russia
KX898324	Gray wolf	Iran
KX898325	Gray wolf	Iran
KX898326	Gray wolf	Russia
KX898327	Gray wolf	Canada
KX898328	Gray wolf	Greenland
KX898329	Gray wolf	Greenland
KX898330	Gray wolf	Greenland
KX898331	Gray wolf	Greenland (1480 BP)
KX898332	Gray wolf	North America
KX898333	Gray wolf	China
KX898334	Gray wolf	North America
KX898335	Gray wolf	North America
KX898336	Gray wolf	North America
KX898337	Gray wolf	North America
KX898338	Gray wolf	North America
KX898339	Gray wolf	North America
KX898340	Gray wolf	North America
KX898341	Gray wolf	North America
KX898342	Gray wolf	North America
KX898343	Gray wolf	Russia
KX898344	Gray wolf	Siberia
KX898345	Gray wolf	Siberia
KX898346	Gray wolf	Russia
KX898347	Gray wolf	Russia

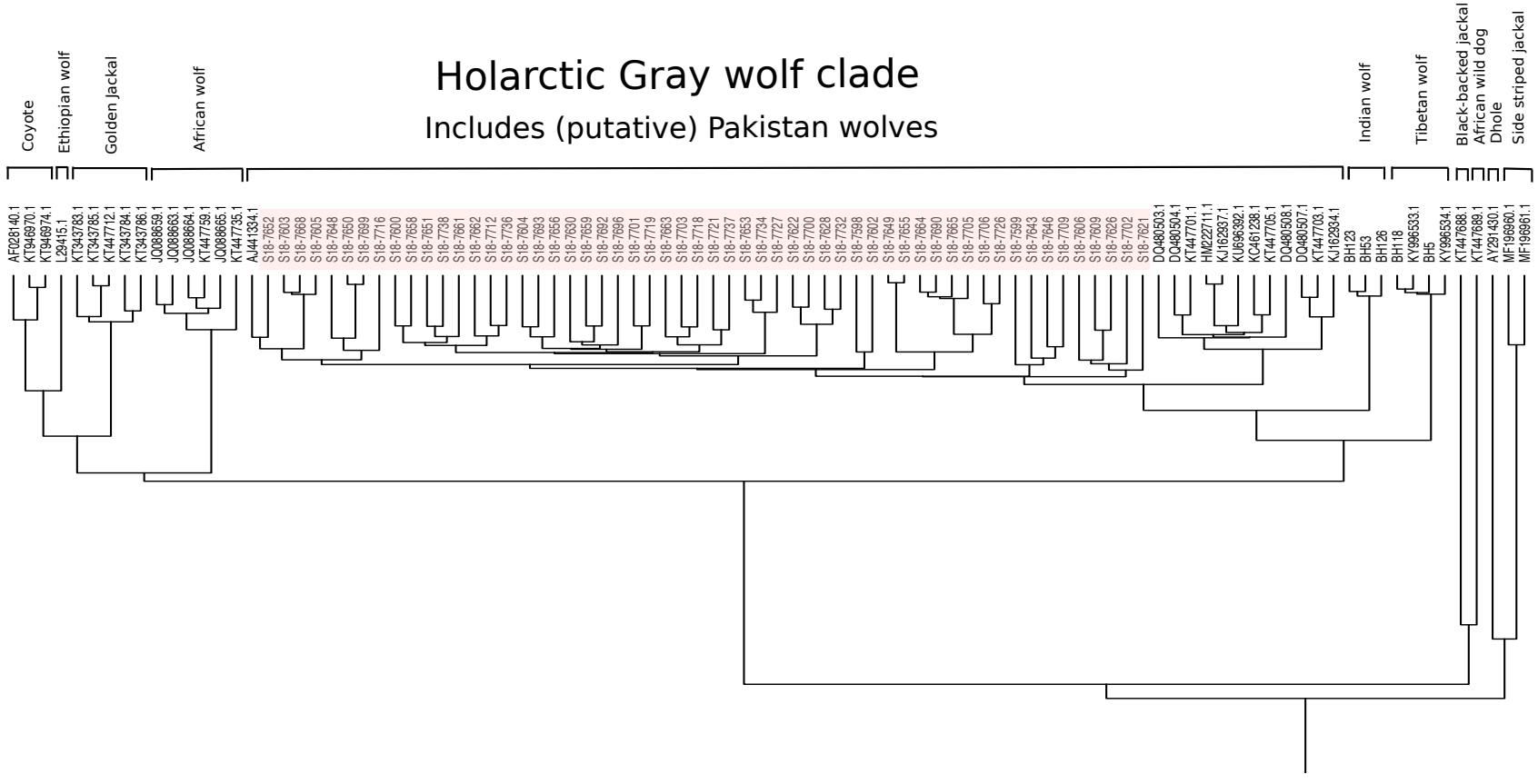
KX898348	Gray wolf	Russia
KX898349	Gray wolf	Russia
KX898350	Gray wolf	Russia
KX898351	Gray wolf	Iran
KX898352	Gray wolf	China
KX898353	Gray wolf	China
KX898354	Gray wolf	China
KX898355	Gray wolf	China
KX898356	Gray wolf	China
S17-6508	Gray wolf	Pakistan, South Waziristan
S18-6509	Gray wolf	Pakistan, South Waziristan
S18-0242	Gray wolf	Pakistan, Skardu
AY289986	Gray wolf	India
NC_010340	Gray wolf	Mongolia
AY289995	Gray wolf	India
JX415347	Gray wolf	China (Qinghai)
AY289973	Gray wolf	India
AY289974	Gray wolf	India
AY289975	Gray wolf	India
AY289984	Gray wolf	India
AY289976	Gray wolf	India
KF661041	Gray wolf	China
KF661043	Gray wolf	India
DQ480506	Gray wolf	Saudi Arabia
DQ480507	Gray wolf	Saudi Arabia
AB499818	Gray wolf	Japan
AB499825	Gray wolf	Japan
AF115690	Gray wolf	Greece
AF115696	Gray wolf	Palestine
AF115697	Gray wolf	Palestine
AY570178	Gray wolf	Iran (Tehran)
AY570181	Gray wolf	Iran (Hamedan)
AY570180	Gray wolf	Iran (Qazvin)
KF661051	Gray wolf	Iran
AY570179	Gray wolf	Iran (Mashad)
KC540929	Gray wolf	Iran
KC540927	Gray wolf	Iran
KC540926	Gray wolf	Iran
KC540925	Gray wolf	Iran
KC540924	Gray wolf	Iran
KC540923	Gray wolf	Iran
KC540922	Gray wolf	Iran
KC540922	Gray wolf	Iran
KC540920	Gray wolf	Iran
KC540919	Gray wolf	Iran
KC540918	Gray wolf	Iran
KC540917	Gray wolf	Iran

AY916822	Gray wolf	China
AY916821	Gray wolf	China
AY916820	Gray wolf	China
AY916819	Gray wolf	China
AY916818	Gray wolf	China
AY916817	Gray wolf	China
AY916816	Gray wolf	China
AY916815	Gray wolf	China
AY916814	Gray wolf	China
AY916813	Gray wolf	China
AY916812	Gray wolf	China
AY916811	Gray wolf	China
AY916810	Gray wolf	China
AY916809	Gray wolf	China
AY916808	Gray wolf	China
AY916807	Gray wolf	China
AY916806	Gray wolf	China
AY916805	Gray wolf	China
AY916804	Gray wolf	China


Coyote
Tibetan clade
Indian clade
Holarctic clade
1
1
1
0.98
0.69
1
0.98
0.97
S17-6509
S17-6508
S18-0242
0.02

Holarctic Gray wolf clade

Includes (putative) Pakistan wolves



Final-sample-ID	Sample type	Site	Lat	Lon	Elevation	FieldID	DateColl	Collector	Age/pelage	sex	Sp	Dup	Fresh?	storage	logged by	Comment	DateExtr
S18-7598	scat	Tormik valley, Gilgit-Baltistan, Pakistan	35°38.080'N	075°18.081' E	3000m	2	13-01-2018	Yasir Abbas						ethanol	SV		20190313
S18-7599	scat	Tormik valley, Gilgit-Baltistan, Pakistan	35°40.261'N	075° 16.160' E	2971m	3	13-01-2018	Yasir Abbas						ethanol	SV		20190313
S18-7600	scat	ASTK valley, Gilgit-Baltistan, Pakistan	35°42.216'N	075° 02.055' E	2505m	4	15-01-2018	Yasir Abbas						ethanol	SV		20190313
S18-7602	scat	Baghicha, Gilgit-Balistan, Pakistan	35°35.383'N	075° 20.091' E	2209m	6	01/11/2018	Yasir Abbas						ethanol	SV		20190313
S18-7603	scat	Baghicha, Gilgit-Balistan, Pakistan	35°35.273'N	075° 21.490' E	2507m	7	01/12/2018	Yasir Abbas						ethanol	SV		20190313
S18-7604	scat	Khomra, Gilgit-Baltistan, Pakistan	35°36.311'N	075°20.551' E	2542m	8	13-01-2018	Yasir Abbas						ethanol	SV		20190313
S18-7605	scat	Khomra, Gilgit-Baltistan, Pakistan	35°36.266'N	075° 21.221' E	2562m	9	14-01-2018	Yasir Abbas						ethanol	SV		20190313
S18-7606	scat	Khomra, Gilgit-Baltistan, Pakistan	35° 36. 561'N	075° 20.383' E	2866m	10	13-01-2018	Yasir Abbas						ethanol	SV		20190314
S18-7609	Scat	Hushay (Thang), Gilgit-Baltistan, Pakistan	35°26 .555'N	076°21.122' E	3114	13	02-02-2018	Yasir Abbas						ethanol	SV		20190314
S18-7621	scat	Skoyo (Nimoldoq), Gilgit-Baltistan, Pakistan	35°34 .823'N	075° 15.749' E	2129	25	21-01-2018	Ghulam Mohammad					3 weeks	ethanol	SV		20190412

S18-7622	scat	Stangokha, Gilgit-Baltistan, Pakistan	35°16 .683'N	075° 38.301' E	2369	26_SKY-1	30-12-2017	Ghulam Mohammad					1 week	ethanol	SV		20190412
S18-7626	scat	Skoyo (Hagokha), Gilgit-Baltistan, Pakistan	35°16 .683'N	075° 38.301' E	2369	30_Sky-02	30-12-2017	Ghulam Mohammad					1 week	ethanol	SV		20190412
S18-7628	scat	Skoyo (Chumik), Gilgit-Baltistan, Pakistan	35°34 .632'N	075° 15.749' E	1810	32_2	21-01-2018	Ghulam Mohammad					2 days	ethanol	SV		20190412
S18-7630	scat	Skoyo (Choshoqfa), Gilgit-Baltistan, Pakistan	35°34 .629' N	075° 17.412' E	2162	34_1	21-01-2018	Ghulam Mohammad					3 days	ethanol	SV		20190412
S18-7643	Scat	Khomra, Gilgit-Baltistan, Pakistan	35°36.246'N	075° 22.572' E	3420m	47	08-04-2018	Yasir Abbas						ethanol	SV		20190412
S18-7644	Scat	Khomra, Gilgit-Baltistan, Pakistan	35°36.257'N	075° 22.375' E	3198m	48	08-04-2018	Yasir Abbas						ethanol	SV		20190417
S18-7646	Scat	Khomra, Gilgit-Baltistan, Pakistan	35°36.308'N	075° 22.156' E	3060m	50	08-04-2018	Yasir Abbas						ethanol	SV		20190417
S18-7647	Scat	Khomra, Gilgit-Baltistan, Pakistan	35°36 .237'N	075° 22.205' E	3325m	51	08-04-2018	Yasir Abbas						ethanol	SV		20190417
S18-7648	Scat	Khomra, Gilgit-Baltistan, Pakistan	35°36.237'N	075° 22.205' E	3325m	52	08-04-2018	Yasir Abbas						ethanol	SV		20190417
S18-7649	Scat	Khomra, Gilgit-Baltistan, Pakistan	35°36.237'N	075° 22.205' E	3325m	53	08-04-2018	Yasir Abbas						ethanol	SV		20190417
S18-7650	Scat	Khomra, Gilgit-Baltistan, Pakistan	35°38.275'N	075° 16.648' E	3688m	54	08-04-2018	Yasir Abbas						ethanol	SV		20190417

S18-7651	Scat	Tormik valley, Gilgit-Baltistan, Pakistan	35°38.336'N	075° 16.427' E	3790m	55	08-04-2018	Yasir Abbas						ethanol	SV		20190417
S18-7652	Scat	Tormik valley, Gilgit-Baltistan, Pakistan	35°37.548'N	075° 17.411' E	3270m	56	08-04-2018	Yasir Abbas						ethanol	SV		20190417
S18-7653	Scat	Tormik valley, Gilgit-Baltistan, Pakistan	35°39.101'N	075° 16.265' E	3812m	57	08-04-2018	Yasir Abbas						ethanol	SV		20190417
S18-7655	Scat	Tormik valley, Gilgit-Baltistan, Pakistan	35°43.309'N	075° 11.439' E	3516m	59	08-04-2018	Yasir Abbas						ethanol	SV		20190417
S18-7656	Scat	Tormik valley, Gilgit-Baltistan, Pakistan	35°38.107'N	075° 19.913' E	3305m	60	08-04-2018	Yasir Abbas						ethanol	SV		20190417
S18-7658	Scat	Tormik valley, Gilgit-Baltistan, Pakistan	35°42.493'N	075° 12.248' E	3364m	62	08-04-2018	Yasir Abbas						ethanol	SV		20190417
S18-7659	Scat	Tormik valley, Gilgit-Baltistan, Pakistan	35°38.414'N	075° 77.916' E	3324m	63	08-04-2018	Yasir Abbas						ethanol	SV		20190417
S18-7660	Scat	Tormik valley, Gilgit-Baltistan, Pakistan	35°39.062'N	075° 17.078' E	3263m	64	08-04-2018	Yasir Abbas						ethanol	SV		20190417
S18-7661	Scat	Tormik valley, Gilgit-Baltistan, Pakistan	35°39.563'N	075° 18.243' E	3396m	65	08-04-2018	Yasir Abbas						ethanol	SV		20190417
S18-7712	Scat	Tormik valley, Gilgit-Baltistan, Pakistan	36°42.493'N	076° 12.248' E	3364m	66	08-04-2018	Yasir Abbas						ethanol	SV		20190417
S18-7663	scat	Gilgit Baltistan	N 352801.1 E 754112.1			67								ethanol	SV		20190417

S18-7664	scat	Gilgit Baltistan	N 352819.8 E 0754136.4			68								ethanol	SV		20190417
S18-7665	scat	Gilgit Baltistan	N 352738.7 E 0754309.6			69								ethanol	SV		20190417
S18-7668	scat	Gilgit Baltistan	N 352924.0 E 0754006.6?			72								ethanol	SV		20190417
S18-7671	scat	Gilgit Baltistan	E 750969.9 ? N ends with .6			75								ethanol	SV		20190417
S18-7672	scat	Gilgit Baltistan	N 352734.7 E 0754307.2			76								ethanol	SV		20190417
S18-7674	scat	Gilgit Baltistan	N 352737.0 E 075 43 09.0			78								ethanol	SV		20190417
S18-7680	scat	Gilgit Baltistan	N 353645.0 E 0753340.8			84								ethanol	SV		20190417
S18-7684	scat	Unknown	35°27. 590'N	075°41.130' E	7382	88	Might be somewhere in GB							ethanol	SV		20190417
S18-7685	scat	Unknown	35°28 .011'N	075°41.121' E	7367	89								ethanol	SV		20190417
S18-7686	scat	Unknown	35°28 .198'N	075° 41.364' E	7477	90								ethanol	SV		20190417
S18-7688	scat	Unknown	35°35 .523'N	075° 33.491' E	7919	92								ethanol	SV		20190417
S18-7690	scat	Unknown	35°28 .322'N	075° 33.426' E	1175	94								ethanol	SV		20190417
S18-7696	scat	Unknown	35°36 .450'N	075° 33.408' E	8787	96								ethanol	SV		20190417
S18-7693	scat	Unknown	35°38 .258'N	075° 33.380' E	11801	97								ethanol	SV		20190417
S18-7696	scat	Unknown	35°27 .359'N	075° 43.073' E	7719	100								ethanol	SV		20190417
S18-7699	scat	Astore				103	30-06-2018	Yasir Abbas						ethanol	SV		20190417
S18-7700	scat	CGNP (pencil 14)				104	30-06-2018	Yasir Abbas						ethanol	SV		20190417
S18-7701	scat	Astore				105	02-02-2018	Yasir Abbas						ethanol	SV		20190417
S18-7702	scat	Astore				106	02-02-2018	Yasir Abbas						ethanol	SV		20190417

S18-7703	scat	Astore				107	02-02-2018	Yasir Abbas						ethanol	SV		20190417
S18-7705	scat	Astore				109	24/11/2017	Raza khan						ethanol	SV		20190417
S18-7706	scat	Astore				110	24/11/2017	Raza khan						ethanol	SV		20190417
S18-7709	scat	CGNP (pencil 8)				113	25/01/2018	Raza khan						ethanol	SV		20190417
S18-7712	scat	CGNP (in pencil 3)				116	25/01/2018	Raza khan						ethanol	SV		20190417
S18-7716	scat	CGNP (in pencil 7)				120	25/01/2018	Raza khan						ethanol	SV		20190417
S18-7718	scat	CGNP (in pencil 6)				122	25/01/2018	Raza khan						ethanol	SV		20190417
S18-7719	scat	CGNP (in pencil 4)				123	25/01/2018	Raza khan						ethanol	SV		20190417
S18-7721	scat	CGNP (in pencil 2)				125	25/01/2018	Raza khan						ethanol	SV		20190417
S18-7726	scat	Shigar (says S in black sharpie)				130								ethanol	SV		20190417
S18-7727	scat	Shigar (says S in black sharpie)				131								ethanol	SV		20190417
S18-7732	scat	Unknown (says S) - Shigar?				136								ethanol	SV		20190417
S18-7734	scat	CGNP (says in pencil 20)				138								ethanol	SV		20190417
S18-7736	scat	Raghzai, South Waziristan, Federally Administered Tribal Areas, Pakistan				140								ethanol	SV	Contaminated with S18-7735, do not reextract	20190417

S18-7738	scat	Raghzai, South Waziristan, Federally Administered Tribal Areas, Pakistan				142								ethanol	SV		20190417
S18-0247	scat	Sadpara Skardu				2017 1216 MS11	20171216	Mehdi Sadpara						ethanol	SV		20180221
S18-0256	scat	Shigar CKNP	35°28.01'	075°41.11'	7363	2018 0111 YA04	20180111	Yasir Abbas						ethanol	SV		20180223
S18-0257	scat	Shigar CKNP	35°27.50'	075°41.18'	7366	2018 0111 YA05	20180111	Yasir Abbas						ethanol	SV		20180223
S18-0260	scat	Shigar CKNP	35°30.35'	075°28.24'	7375	2018 0112 YA08	20180112	Yasir Abbas						ethanol	SV		20180223
S18-0241	scat	Sadpara Skardu				2017 1216 MS05	20171216	Mehdi Sadpara						ethanol	SV		20180221
S18-0242	scat	Sadpara Skardu				2017 1216 MS06	20171216	Mehdi Sadpara						ethanol	SV		20180221
S18-0243	scat	Sadpara Skardu				2017 1216 MS07	20171216	Mehdi Sadpara						ethanol	SV		20180221
S18-0244	scat	Sadpara Skardu				2017 1216 MS08	20171216	Mehdi Sadpara						ethanol	SV		20180221

S18-0245	scat	Sadpara Skardu				2017 1216 MS09	20171216	Mehdi Sadpara						ethanol	SV		20180221
S18-0239	scat	Sadpara Skardu				2017 1216 MS03	20171216	Mehdi Sadpara						ethanol	SV		20180221
S18-0237	scat	Sadpara Skardu				2017 1216 MS01	20171216	Mehdi Sadpara						ethanol	SV		20180221
S18-0231	scat	Astor (GB)				2017 1218 AK01	20171218	Amjad Khan						ethanol	SV		20180220
S18-0223	scat	Kalam Mahoodand (Swat)	35°44.042'	072°38.050'	9570.2	2017 1124 RK03	20171124	Raza Khan						ethanol	SV		20180220
S18-0227	scat	Kalam Mahoodand (Swat)				2017 1124 RK07	20171124	Raza Khan						ethanol	SV		20180220
S18-0228	scat	Kalam Mahoodand (Swat)				2017 1124 RK08	20171124	Raza Khan						ethanol	SV		20180220
S18-0229	scat	Kalam Mahoodand (Swat)				2017 1124 RK09	20171124	Raza Khan						ethanol	SV		20180220
S18-0232	scat	Hotoo Skardu				2017 1221 MS01	20171221	Mehdi Sadpara						ethanol	SV		20180220
S17-6505	scat	S.Waziristan Jani Mela	32° 25.702' N	069° 31.677' E	8064	2017 0212 AH	20170212	Abdul Hameed						ethanol	SV		20171031
S17-6506	scat	S.Waziristan Jani Mela	32° 25.702' N	069° 31.677' E	8064	2017 0212 AH	20170212	Abdul Hameed						ethanol	SV		20171031

S17-6507	scat	S.Waziristan Jani Mela	32° 25.702' N	069° 31.677' E	7497	2017 0212 AH	20170212	Abdul Hameed						ethanol	SV		20171031
S17-6508	scat	S.Waziristan Raghazai	32° 28.065' N	069° 29.523' E	8190	2017 0305 AH	20170305	Abdul Hameed						ethanol	SV		20171031
S17-6509	scat	S.Waziristan Raghazai	32° 28.065' N	069° 29.523' E	8190	2017 0305 AH	20170305	Abdul Hameed						ethanol	SV		20171101 plateE
S17-6510	scat	S.Waziristan Tora Gola	32° 16.657' N	069° 21.931' E	5707	2017 0415 AH	20170415	Abdul Hameed						ethanol	SV		20171031
S17-6511	scat	S.Waziristan Tora Gola	32° 16.657' N	069° 21.931' E	5810	2017 0415 AH	20170415	Abdul Hameed						ethanol	SV		20171031
S17-6512	scat	S.Waziristan Tora Gola	32° 15.681' N	069° 22.433' E	6000	2017 0415 AH	20170415	Abdul Hameed						ethanol	SV		20171031
S17-6514	scat	S.Waziristan Tora Gola	32° 15.681' N	069° 22.433' E	5890	2017 0415 AH	20170415	Abdul Hameed						ethanol	SV		20171031

Extraction Method (e.g., PureGene?)	Extracted by	cyt b seq date	Cyt B haplo	DNALocation	Tissue sample location (small tube)	Reserve sample (e.g., carcass) loc	microsat run date (in Strand)	Y-microsats	Perm Plate	Dloop seq date	D-loop haplo	temp-ID	individ ID from micros	Volume(ul)	DNA concentration (ng/ul)	ProjectIdentifier
EURx (scat)	ELS	20190411_TMD_SppID	wolf?													
EURx (scat)	ELS	20190411_TMD_SppID	wolf?													
EURx (scat)	ELS	20190411_TMD_SppID	wolf?													
EURx (scat)	ELS	20190411_TMD_SppID	wolf?													
EURx (scat)	ELS	20190411_TMD_SppID	wolf?													
EURx (scat)	ELS	20190411_TMD_SppID	wolf?													
EURx (scat)	ELS	20190411_TMD_SppID	wolf?													
EURx	MPK	20190411_TMD_SppID	wolf?													
EURx	MPK	20190411_TMD_SppID	dog?													
EURx	LMH VS		wolf/dog													

EURx	LMH VS		wolf/dog													
EURx	LMH VS		wolf/dog													
EURx	LMH VS		wolf/dog													
EURx	LMH VS		wolf/dog													
EURx	LMH VS		wolf/dog													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf/dog poor													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf/dog poor													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													

DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf/dog poor													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													

DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf/dog poor													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													

DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													

DNeasy 96	SV, VS, LMH, MF, TMD	20190419_PakistanSppID_SF	wolf?													
	HF	20180302SppID02_HF_GK; 20180315SppID02_SV_HF	Wolf/Dog	2017-18 Pakistan Wolf/ Dog						20180322_wolfdog_Dloop	wolf					Wolf Pak Project
	HF	20180302SppID02_HF_GK; 20180315SppID02_SV_HF	Wolf/Dog	2017-18 Pakistan Wolf/ Dog						20180322_wolfdog_Dloop	wolf					Wolf Pak Project
	HF	20180302SppID02_HF_GK; 20180315SppID02_SV_HF	Wolf/Dog	2017-18 Pakistan Wolf/ Dog						20180322_wolfdog_Dloop	wolf					Wolf Pak Project
	HF	20180302SppID02_HF_GK; 20180315SppID02_SV_HF	Wolf/Dog	2017-18 Pakistan Wolf/ Dog						20180322_wolfdog_Dloop	wolf					Wolf Pak Project
	HF	20180302SppID02_HF_GK; 20180315SppID02_SV_HF	Wolf/Dog	2017-18 Pakistan Wolf/ Dog						20180322_wolfdog_Dloop	wolf					Wolf Pak Project
	HF	20180302SppID02_HF_GK; 20180315SppID02_SV_HF	Wolf/Dog	2017-18 Pakistan Wolf/ Dog						20180322_wolfdog_Dloop	wolf					Wolf Pak Project
	HF	20180302SppID02_HF_GK; 20180315SppID02_SV_HF	Wolf/Dog	2017-18 Pakistan Wolf/ Dog						20180322_wolfdog_Dloop	wolf					Wolf Pak Project
	HF	20180302SppID02_HF_GK; 20180315SppID02_SV_HF	Wolf/Dog	2017-18 Pakistan Wolf/ Dog						20180322_wolfdog_Dloop	wolf					Wolf Pak Project
	HF	20180302SppID02_HF_GK; 20180315SppID02_SV_HF	Wolf/Dog	2017-18 Pakistan Wolf/ Dog						20180322_wolfdog_Dloop	wolf* (dog came up close)					Wolf Pak Project

	HF	20180302SppID02_HF_GK; 20180315SppID02_SV_HF	Wolf/Dog	2017-18 Pakistan Wolf/ Dog						20180322_wolfdog_Dloop	wolf					Wolf Pak Project
	HF	20180302SppID02_HF_GK; 20180315SppID02_SV_HF	Wolf/Dog	2017-18 Pakistan Wolf/ Dog						20180322_wolfdog_Dloop	wolf					Wolf Pak Project
	HF	20180302SppID02_HF_GK; 20180315SppID02_SV_HF	Wolf/Dog	2017-18 Pakistan Wolf/ Dog						20180322_wolfdog_Dloop	wolf					Wolf Pak Project
	HF	20180302SppID02_HF_GK; 20180315SppID02_SV_HF	Wolf/Dog	2017-18 Pakistan Wolf/ Dog						20180322_wolfdog_Dloop	wolf					Wolf Pak Project
	HF	20180302SppID02_HF_GK; 20180315SppID02_SV_HF	Vulpes vulpes	2017-18 Pakistan Vulpes vulpes												Wolf Pak Project
	HF	20180302SppID02_HF_GK; 20180315SppID02_SV_HF	Vulpes vulpes	2017-18 Pakistan Vulpes vulpes												Wolf Pak Project
	HF	20180302SppID02_HF_GK; 20180315SppID02_SV_HF	Vulpes vulpes	2017-18 Pakistan Vulpes vulpes						20180322_wolfdog_Dloop	fail					Wolf Pak Project
	HF	20180302SppID02_HF_GK; 20180315SppID02_SV_HF	Vulpes vulpes	2017-18 Pakistan Vulpes vulpes												Wolf Pak Project
	HF	20180302SppID02_HF_GK; 20180315SppID02_SV_HF	Vulpes vulpes	2017-18 Pakistan Vulpes vulpes												Wolf Pak Project
	HF, EKA, SV	20171103SV_SppID	Vulpes vulpes	2017-18 Pakistan Vulpes vulpes												Pakistan wolf
	HF, EKA, SV	20171103SV_SppID	Vulpes vulpes	2017-18 Pakistan Vulpes vulpes												Pakistan wolf

	HF, EKA, SV	20171103SV_SppID	Vulpes vulpes	2017-18 Pakistan Vulpes vulpes												Pakistan wolf
	HF, EKA, SV	20171103SV_SppID	Wolf/Dog	2017-18 Pakistan Wolf/Dog						20180322_wolfdog_Dloop	wolf					Pakistan wolf
	SV, AL, CJS, HF	20171123_sppID_E	Wolf/Dog	2017-18 Pakistan Wolf/Dog						20180322_wolfdog_Dloop	wolf					Pakistan wolf
	HF, EKA, SV	20171103SV_SppID	Vulpes vulpes	2017-18 Pakistan Vulpes vulpes												Pakistan wolf
	HF, EKA, SV	20171103SV_SppID	Vulpes vulpes	2017-18 Pakistan Vulpes vulpes												Pakistan wolf
	HF, EKA, SV	20171103SV_SppID	Vulpes vulpes	2017-18 Pakistan Vulpes vulpes												Pakistan wolf
	HF, EKA, SV	20171103SV_SppID	Vulpes vulpes	2017-18 Pakistan Vulpes vulpes												Pakistan wolf