The tail of the horse´s tail – stable isotope analysis of tail hair to address ecological differences in three sympatric equid species in the Mongolian Gobi

1. Progress report, December 2012

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Background

In the Dzungarian Gobi of Mongolia, three equid species, Asiatic wild ass (*Equus hemionus*), domestic horse (*Equus caballus*), and re-introduced Przewalski´s horse (*Equus ferus przewalskii*) share the same habitat and thus provide a unique opportunity for comparative ecological studies. In the current project, stable isotope analysis of sequentially sampled tail hair form the three sympatric equid species will be used as an indirect measure for seasonal feeding ecology (carbon and nitrogen isotopes), water use (hydrogen and oxygen isotopes), movement pattern (carbon, nitrogen, sulphur, oxygen and hydrogen isotopes) and metabolism (nitrogen isotopes). In order to better understand the relationship between animal tissue and feed, important equid food plants as well as various water points and precipitation are also sampled and analyzed.

The current progress report presents the work that has been done in the first part of the project and encompasses first sampling expedition to Mongolia (tail hair, plant and water sampling), sample preparation in the laboratory and first isotopic measurements with short discussion of results. The report consists of four different parts:

I. Plant Sampling
II. Water Sampling
III. Tail hair growth
IV. Sample preparations & first analysis

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I. Plant Sampling

During the period of 6 weeks, from 17th of June until 30th of July, my activities in the Strictly Protected Area **Great Gobi B** (GGB), Kohvd Aimag, Mongolia and **Takhin Tal** Camp, Govi-Altai Aimag within the project mentioned above included:

1. Collection of a reference herbarium of important forage plants
2. Vegetation surveys, especially in the desert steppe zone in Khovd Aimag west of GGB (Fig. 12)
3. Sampling of potential Equid's forage plants according to a special sampling grid (Fig. 13)

Timetable

06/17/2012	Flight Berlin-Moscow-Ulaanbaatar with Aeroflot
06/18/2012	Arrival in Ulaanbaatar
06/21/2012	Domestic flight to Khovd and car ride to Takhin Tal
06/22/2012	Arrival in Takhin Tal
06/23/2012 to 07/03/2012	Instruction of the activities and the conservation area GGB by Dr. Petra Kaczensky; Joint excursion, sampling and vegetation surveys E of GGB and in the mountains NO of GGB Plot1 - Plot24 (forage plant samples), V001 - V018 (chronological numbering of relevés)
07/04/2012 to 07/06/2012	Travel to Khovd, accompanying Mss. Dr. Kaczensky to the airport, Grocery shopping for the month of July 2012, Application for a permission to enter the border region S and SW of GGB
07/07/2012	Plot 25
07/08/2012	Plot 26 - Plot 30; V019 - V024
07/09/2012	V025 - V041
07/10/2012	Plot 31; V042-V050
07/11/2012 to 07/12/2012	Determining the plant species collected in field, Preparing and planning the excursion to the west
07/13/2012 to 07/22/2012	Excursion W and N of GGB; Plots 32 - 41; V051 - V149
07/23/2012 to 07/28/2012	Determining the plant species collected in field, Preparing a Herbarium, Short Excursions to the southern part of GGB, Introducing the monthly sampling-methods to Ganbaatar and the Rangers, Determining the plots to be examined monthly (K and Q; Fig. 13)
07/29/2012	Travel to Altay, Altay-Khovd Aimag
07/30/2012	Domestic Flight to Ulaanbaatar

1. Reference herbarium

Dried and pressed individuals of the most important forage plants of equids can be inspected in a reference herbarium in the ITG office in Takhin Tal Camp.

Collected species are:

Achnatherum splendens
Ajania fruticulosa
Allium mongolicum
Anabasis brevifolia
Artemisa sublessingiana
Caragana leucophloea
Haloxylon ammodendron
Kraschenninikovia ceratoides
Leymus chinensis
Reaumurea songarica
Stipa glareosa

Fig. 1. *Achnatherum splendens* (in the foreground)

Fig. 2. *Ajania fruticulosa*

Fig. 3. *Allium mongolicum*

Fig. 4. *Anabasis brevifolia*

Fig. 5. *Artemisia sublessingiana*

Fig. 6-7. *Caragana microphylla*

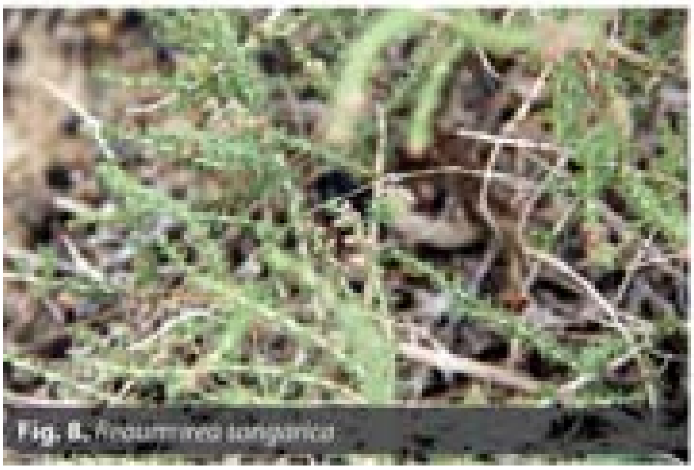


Fig. 8. *Reaumuria songarica*

Fig. 9. *Haloxylon ammodendron* (flowering)

Fig. 10. *Krascheninnikovia ceratoides*

Fig. 11. *Stipa glareosa*

Photographs by
Petra Kaczensky, Tereza Weissova, Jan Treiber

Table 1. Sampled Plots Summer 2012

Name	Date	Long	Long	Elevation (m)	*Achnatherum splendens*	*Allium mongolicum*	*Anabasis brevifolia*	*Artemesia sublessingiana*	*Ajania fruticulosa*	*Caragana leucophloea*	*Elymus spec.*	*Kraschenínnikovia ceratoides*	*Haloxylon ammodendron*	*Reaumuria soongarica*	*Stipa glareosa*	*Zygophyllum spec.*	*Festuca spec.*	*Agropyron cristatum*	*Poaceae*	*Grass mix*
PLOT1	11.6.12	45,2083	93,4895	1.658	1		1	1		1	1	1	1	1	1	1				
PLOT2	15.6.12	45,1996	93,3807	1.686	1		1	1		1	1	1	1	1	1					
PLOT3	15.6.12	45,4101	93,3744	1.491	1	1	1	1		1		1	1	1	1					
PLOT4	16.6.12	45,5792	93,3878	1.643	1	1	1	1		1	1	1		1	1					
PLOT5	23.6.12	45,4146	93,8212	1.743	2	1	1	1		1	1	1		1	1					
PLOT6	24.6.12	45,6179	92,9265	1.471			1	1		1		1	1	1	1					
PLOT7	24.6.12	45,4213	92,9101	1.334			1	1		1		1	1	1	1					
PLOT8	24.6.12	45,2371	92,9020	1.541		1	1	1		1		1	1	1	1					
PLOT4 (2)	24.6.12	45,5792	93,3878	1.643	1	1	1	1		1		1		1	1					
PLOT9	26.6.12	45,7800	93,3800		1			1		1	1	1			1					
PLQT11	26.6.12	45,8772	93,5262	2.269		1		1		1							1	1		1
PLOT12	26.6.12	45,9163	93,5754	2.525				1									1	1		
PLOT13	26.6.12	45,9014	93,5897	2.735				1									1			
PLOT14	26.6.12	45,8369	93,6323	2.208																1
PLOT15	26.6.12	45,8379	93,6886	2.365				1									1			
PLOT16	26.6.12	45,8479	93,7716	2.285				1							1			1		
PLOT17	26.6.12	45,8015	93,8338	2.240						1					1				1	
PLOT18	26.6.12	45,7668	93,8976	2.250						1		1					1	1	1	
PLOT19	27.6.12	45,8120	94,0200	2.802																1
PLOT20	27.6.12	45,7986	94,1643	2.674				1									1			1
PLOT21	27.6.12	45,6991	94,1105	2.595				1												2
PLOT22	27.6.12	45,7100	94,0748	2.670																1
PLOT00	27.6.12	45,6805	93,9880	2.253																1
PLOT23	27.6.12	45,6071	93,9760	2.056	1	1		1		1		1					1	1		1
PLOT25	7.7.12	45,2250	92,3789	1.655				1		1		1		1	1					
PLOT26	8.7.12	45,2565	92,4327	1.626			1	1	1	1		1		1	1					
PLOT27	8.7.12	45,4217	92,3610	1.285									1	1						
PLOT28	8.7.12	45,6070	92,4116	1.164						1			1	1						
PLOT29	8.7.12	45,6395	92,3549	1.153									1	1						
PLOT30	8.7.12	45,7801	92,3593	1.392				1				1		1	1					
PLOT31	10.7.12	45,7823	92,8714	1.647				1		1	1	1			1					
PLOT32	13.7.12	45,7572	91,8565	1.108	1								1	1						
PLOT33	13.7.12	45,7760	91,8479	1.089	1									1						
PLOT34	14.7.12	45,5626	91,8349	1.090	1							1	1	1						
PLOT35	14.7.12	45,4166	91,8658	1.090									1	1						
PLOT36	16.7.12	45,7738	91,3271	1.031										1						
PLOT37	16.7.12	45,5989	91,3436	1.159									1							
PLOT38	17.7.12	45,4153	91,3404	1.171	1					1			1	1	1					
PLOT39	17.7.12	45,2571	91,2780	1.542		1	1		1						1					
PLOT40	17.7.12	45,1712	91,3694	1.388		1	1		1					1	1					
PLOT41	18.7.12	45,2381	91,8471	1.425		1			1	1		1	1	1	1					

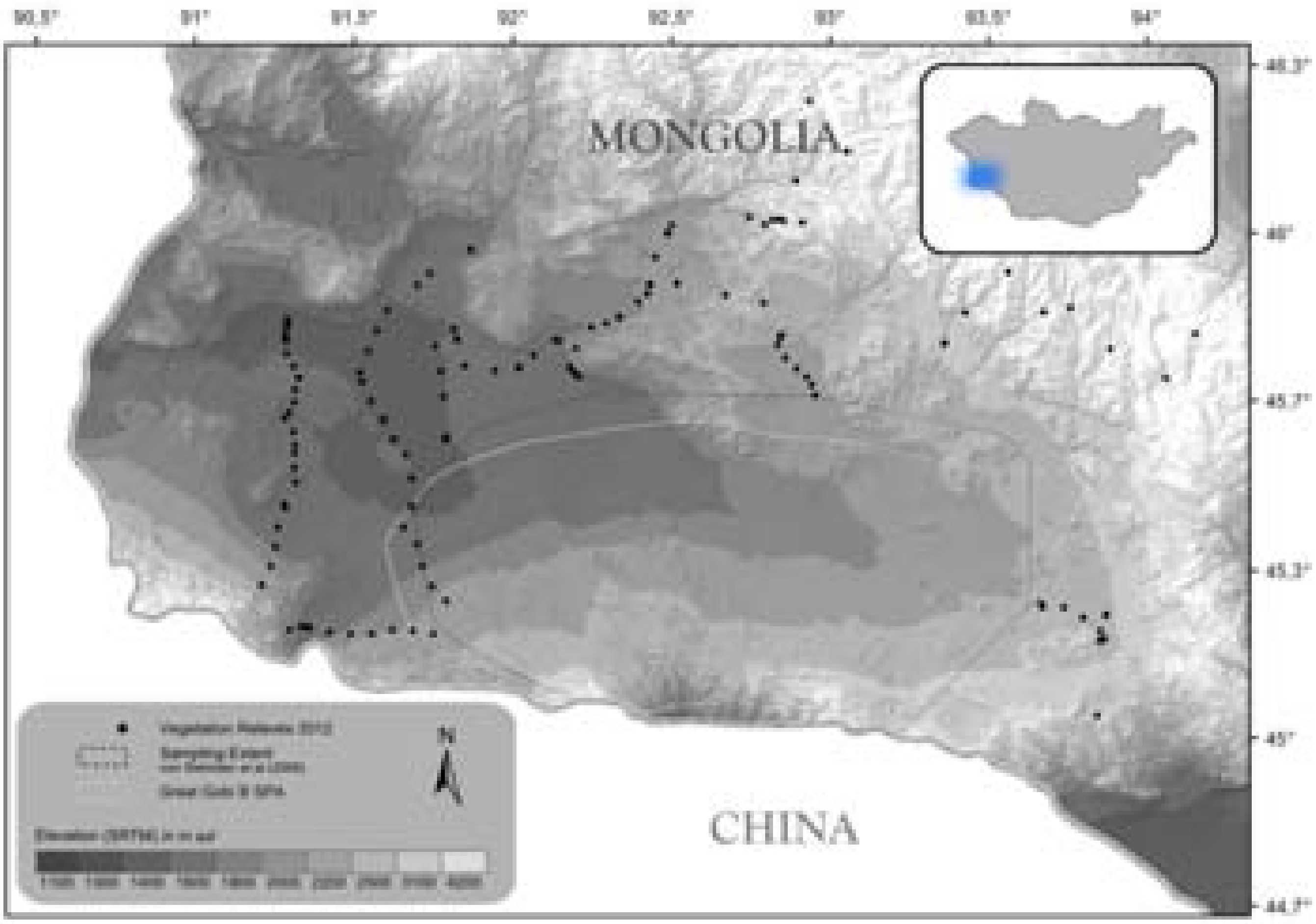


Fig. 12. Vegetation Relevés west and north of the Strictly Protected Area (SPA) "Great Gobi B"

2. Vegetation survey

149 Vegetation relevés according the methods of ***von Wehrden et al. (2006) Plant Communities of the Great Gobi B Strictly Protected Area, Mongolia. Mongolian Journal of Biological Sciences 2006 Vol. 4(1): 63-66*** were carried out. The area of mapped plant formations (von Wehrden et al., Fig. 12 dashed line) has been expanded to the west and northwest.

The determination and analysis of this vegetation survey is still in progress. Reference individuals will be accurately determined in Germany and if necessary expert opinions will be requested.

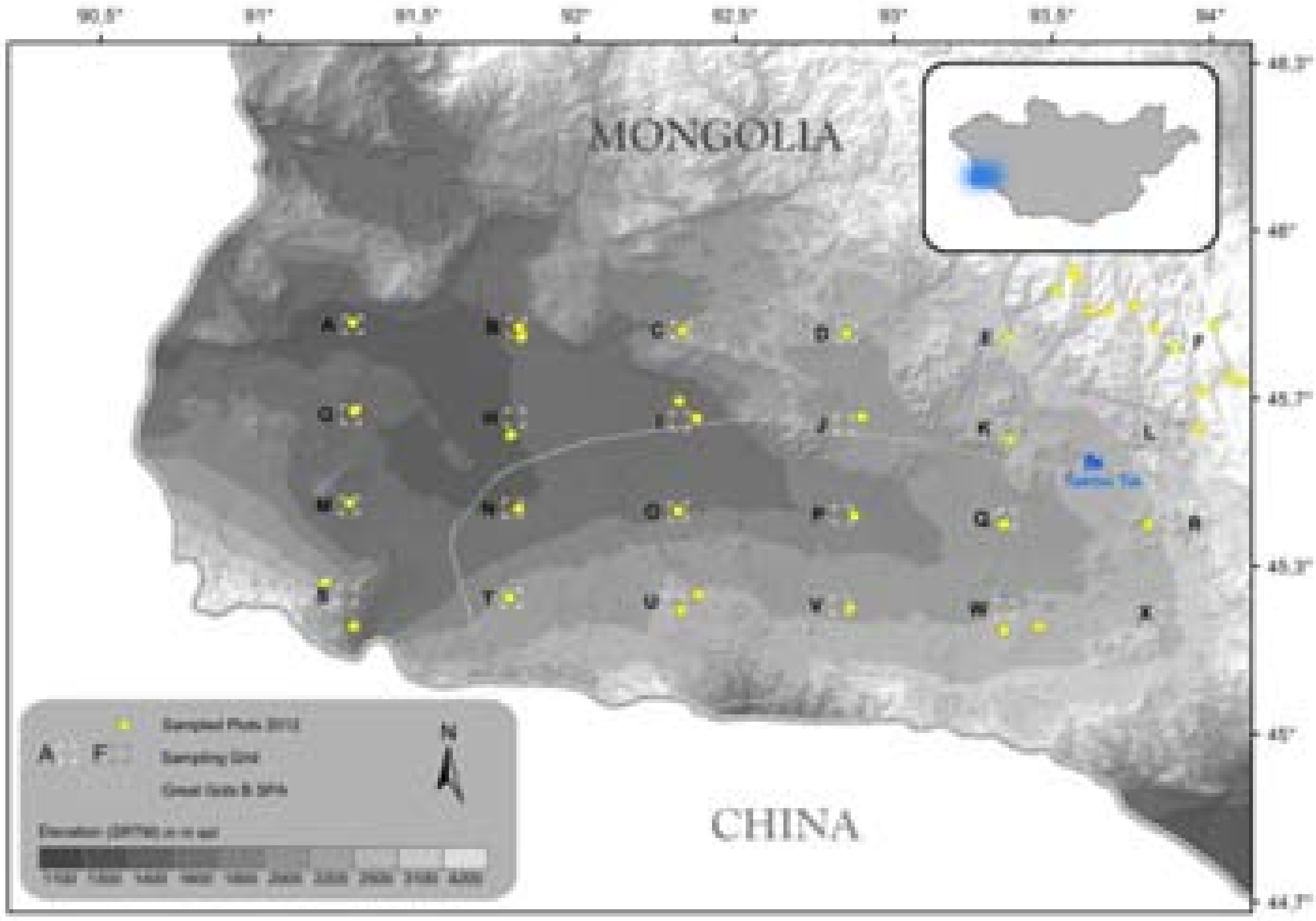


Figure 13. Sampling Grid and sampled Plots with potential forage plants of equids. Monthly plant samples will be collected from Plots K and Q.

3. Forage plants

The sampled forages plant meet the species listed in the reference herbarium. Due to the lack of roads not every planned sampling plot could be reached. In these cases an reachable area close to planned coordinates was chosen (Fig. 13, yellow dots). Of each potential forage species a mixed sample of 5 individuals per plot was collected.

Remark:

*Leymus chine*nsis should be considered as a further potential forage plant in this area. It often occured together with *Achnatherum splendes* and was equally grazed. According to ***Damiran (2005) Palatability of Mongolian rangeland plants*** *Leymus chinensis* is a preferred forage plant for cattle and horses in the spring and summer months.

Fig. 14. Mountain steppe N of GGB

Fig. 15. *Reaumurea songarica* – stands near Altay/Khovd

Fig. 16. Desert vegetation W of GGB

Fig. 17. Desert steppe vegetation N of Altay/Khovd with *Krascheninnikovia ceratoides* and *Allium mongolicum*

Fig. 18. Wetlands W of GGB

Fig. 19. *Clematis songorica* – stands W of GGB

II. Water sampling

We checked all known water points in and around the Great Gobi B Strictly Protected Area (SPA), including the summer pastures of the upper Bij river. In addition, we (1) checked clusters of GPS locations of Asiatic wild asses that suggested potential additional water points and (2) all areas marked as temporal wetlands on the 1:50.000 Russian maps. In total we collected water samples at 49 different locations: rivers, springs, high elevation mountain lakes, swamps, rain water puddles and from one well. We additionally set up a water sampling station for rain and snow at Takhin Tal research camp and organized weekly sampling of the lower Bij river near the village of Bij.

Fig 1: Water sampling points in Great Gobi B SPA in June / July 2012.

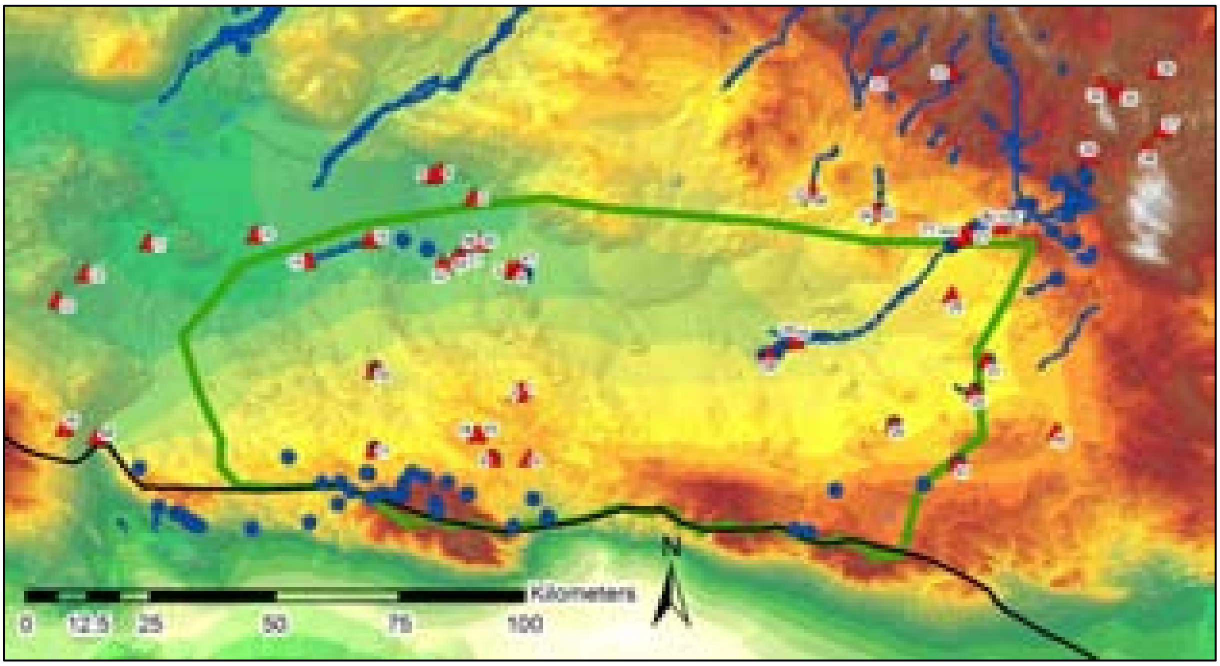
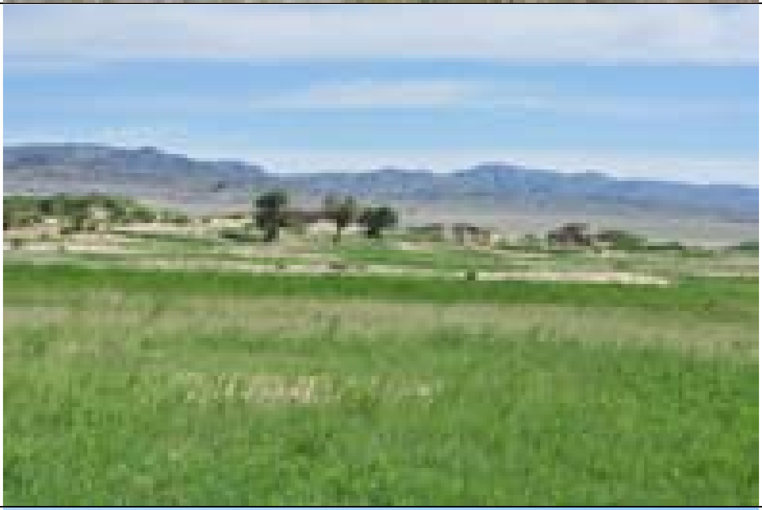


Fig. 2: Photo documentation of water sampling sites.

	1 – new water point
	2 - well
	3 – Takhi us 2

	4 – Takhi us 1
	5 – Khairchin Khuchin Tunge
	6 – Argali Shant

	7 – Takhiin Tsavchaa
	8 – Tooroin Tugul
	9 – Uzuur us

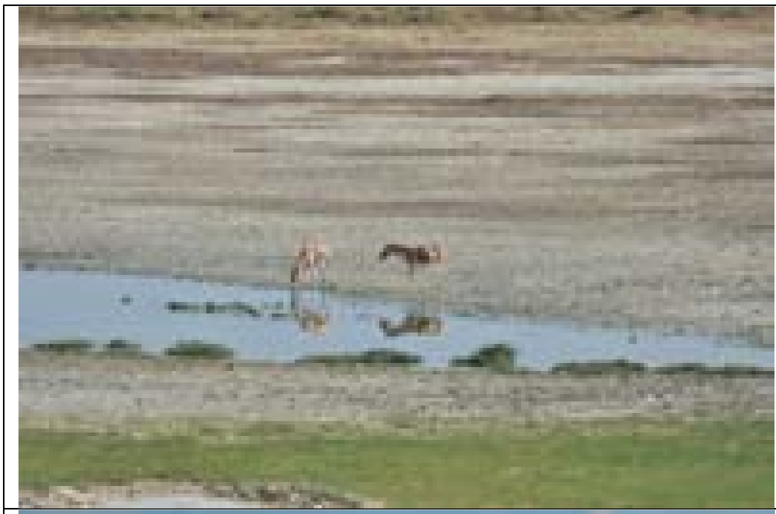
	10 – Tsaagan us
	11 – Baruun Khuurai
	12 - Damjig

	13 – no name
	14 – Khoolin Dud us (15 also looks like this)
	16 – Gakhai Bulag

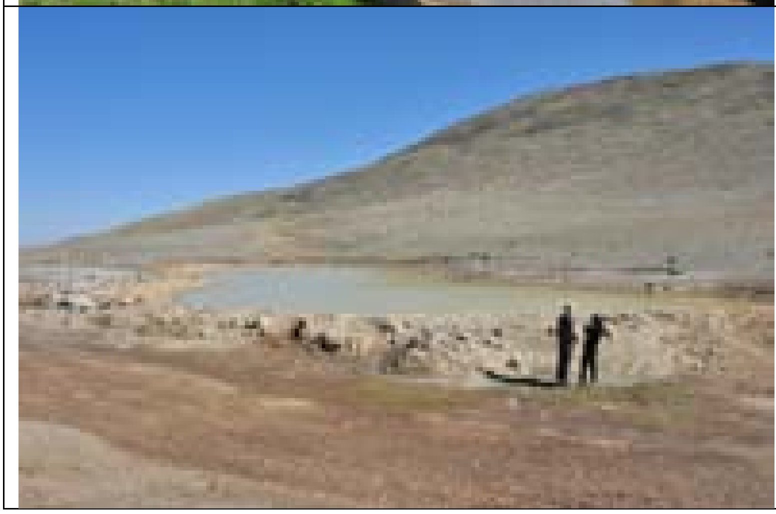
	17 – Gashurn us 2 (SW edge of park)
	18 - Zadgai
	19 – Surhaitin Dund Us

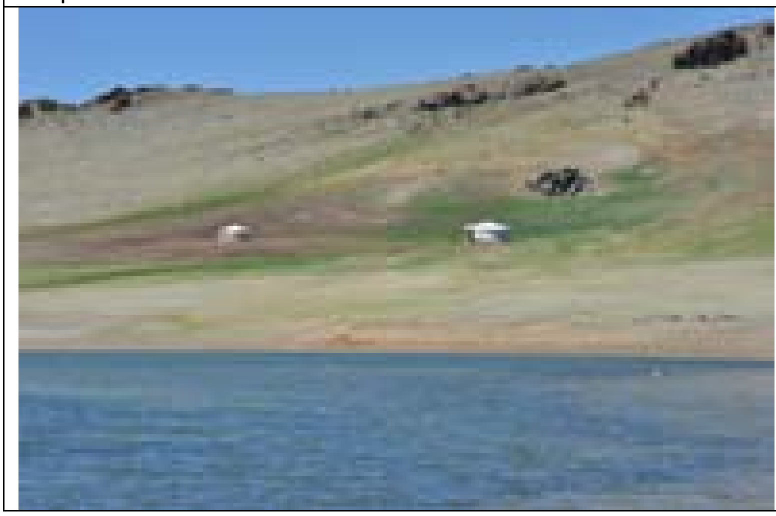
	20 – Gashuun us 3 (Western part of park)
	21 – Bosgo us

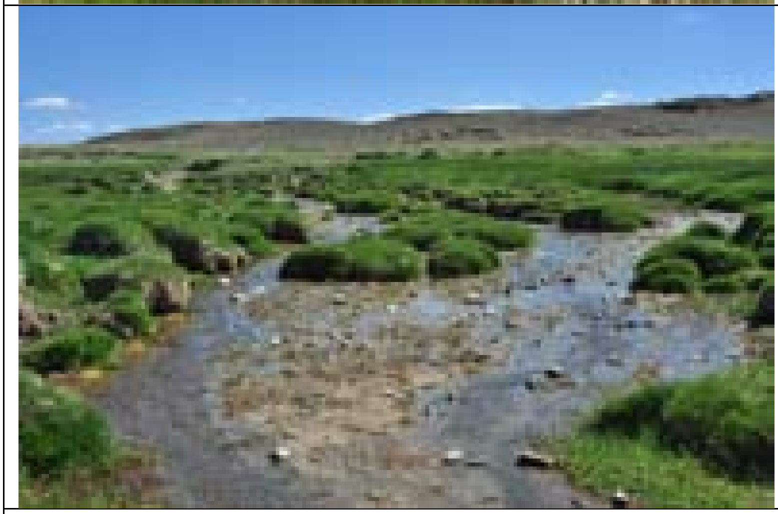
	22 – Shanagin us
	23 – Uvchoo river
	24 –Shirin us
Only slides	25 – Gun Tamgaa

	26 - Toodog
	27 – Bij River (at Ranger camp)
	28 – rain puddle

	29 – Chonin us - W
	30 –Chonin us - middle
	31 - Gashuun

	32 – Upper Uvchoo river
	33 – Little spring
	34 – dammed lake on pass

	35 – Hagin Nor
	36 – Kharch Tolgoi
No picture	37 – dry riverbed
	38 – Dirchin Noor

	39 – Upper Bij
	40 – no name (Waypoint W83)
	41 – Khairchen Bulag
No picture	49 – rain water in salty depression

	Takhin Tal research station – rain sampling -
	Bij river at Bij – weekly sampling site -

III. Annual tail hair growth in free-ranging domestic horse

In order to be able to relate the observed pattern from the sequentially sampled tail hair to actual seasonal events based on Julian dates, information on hair growth speed and patterns are required for the three species. The mane and tail hair of domestic horses has shown to grow continuously at a rather constant rate of 0.0792 mm/day, 2.3-2.3 cm/month or 28.9 cm/year (Dunnett 2002, Dunnett & Lees 2003; Fig. 1). Although hair growth varies slightly from month to month, no seasonal pattern, sex or age differences or any correlations with climate or diet have been demonstrated for domestic horses in temperate environments (Dunnett & Lees 2003). However, to our knowledge no measurements of hair growth have been conducted in other equid species or outside of the temperate zone.

Measuring tail growth in free-ranging Przewalski's horses or Asiatic wild asses would require repeated capture and handling of individual animals which would be logistically extremely challenging and ethically questionable as we are dealing with endangered species. Consequently we aim to address potential species-specific differences in tail growth speed and pattern by making use of trained animals in captive facilities. To test whether extreme seasonality does have an influence on tail hair growth we will use the same approach on free-ranging domestic horses in Mongolia.

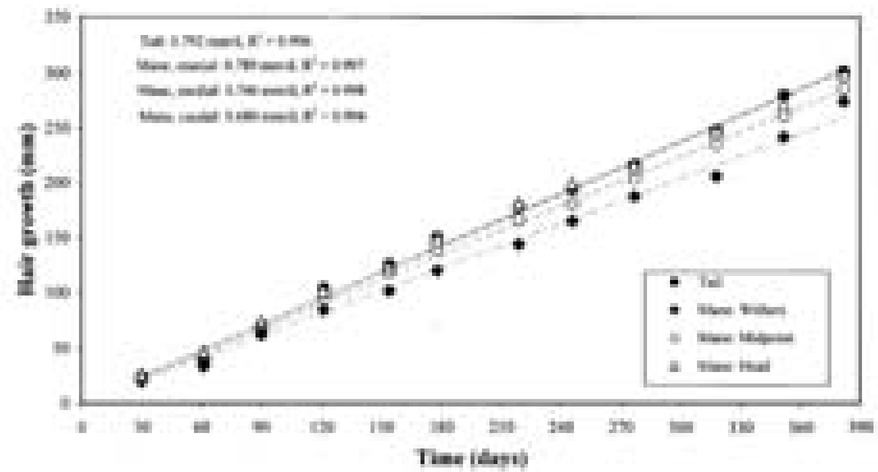


Fig. 1. Mean cumulative mane and tail hair growth for a group of continuously grazed native ponies (n = 5). Regions of the mane and tail were shaved to the skin and subsequent re-growth was measured monthly over the subsequent 12 months.

Source: Dunnett & Lees 2003

Methods field work June / July 2012

Unsuccessful attempts to bleach horse tail hair

In a first attempt we tried to use a simpler and less invasive way by using H_2O_2 to bleach part of the tail hair. However, it turned out that it was actually not possible to bleach horse tail hair. Although accidentally spilled H_2O_2 rapidly bleached clothing and attacked unprotected skin, horse hair seemed totally unaffected. Even cut off hair soaked overnight in H_2O_2 did not result in any color change in the horse hair.

Cutting tail hair of domestic horses

The herder Zaya felt that tail hair growth in domestic horses may be different close to the base as compared to lower down along the base of the tail. Thus he cut two areas:

1) High up on the base of the tail where hair does not grow that long
2) In the middle of the tail – about the same area, but under the other hair

Fig. 1: Herder Zaya cutting tail hair.

Fig. 2: The two areas cut for measuring tail hair growth.
The second area can be easily seen when lifting up the tail hairs.

Data will be collected using the following form:

Horse	**Date**	**length area 1 (mm)**	**length area 2 (mm)**	**Cut again (yes/no)**	**Comments**
Horse 1 (mare with foal)					
Horse 2 (mare without foal)					
Horse 3 (gelding)					
Horse 4 (stallion)					

We selected one mare with a foal, one mare without a foal, one gelding and one stallion (Fig. 3). Because horse graze freely in the mountains and cannot be easily captured – it takes multiple hours to find them and drive them back to camp or lasso them in the field – it was agreed to measure tail growth once at the beginning of each month (1 day of each month +/- 2 days).

Fig. 3: The study horses for measuring tail hair growth.

Note:
Zaya estimated that the re-growth of the tail of one horses, which had been chewed off to the base by cows in 2009/2010 took until now (2.07.2012).
At this point the horse's tail was 65 cm long
From March 2010 to July 2012 = ~822 days

- ➔ Tail grows: 65 / 822 = 0.079 cm/day or 2.37 cm / month

This value is almost identical to what has been previously measured for domestic horses in temperate climates (Dunnett and Lees 2003, Schlupp et al. 2004).

Literature

Dunnett, M., P. Lees. 2003. Trace element, toxin and drug elimination in hair with particular reference to the horse. Research in Veterinary Science, 75: 89-101.

Schlupp, A., P. Anielski, D. Thieme, R.K. Müller, H. Meyee and F. Ellendorf. 2004. The ß-agonist clenbuterol in mane and tail hair of horses. Equine Veterinary Journal, 36(2):122-118.

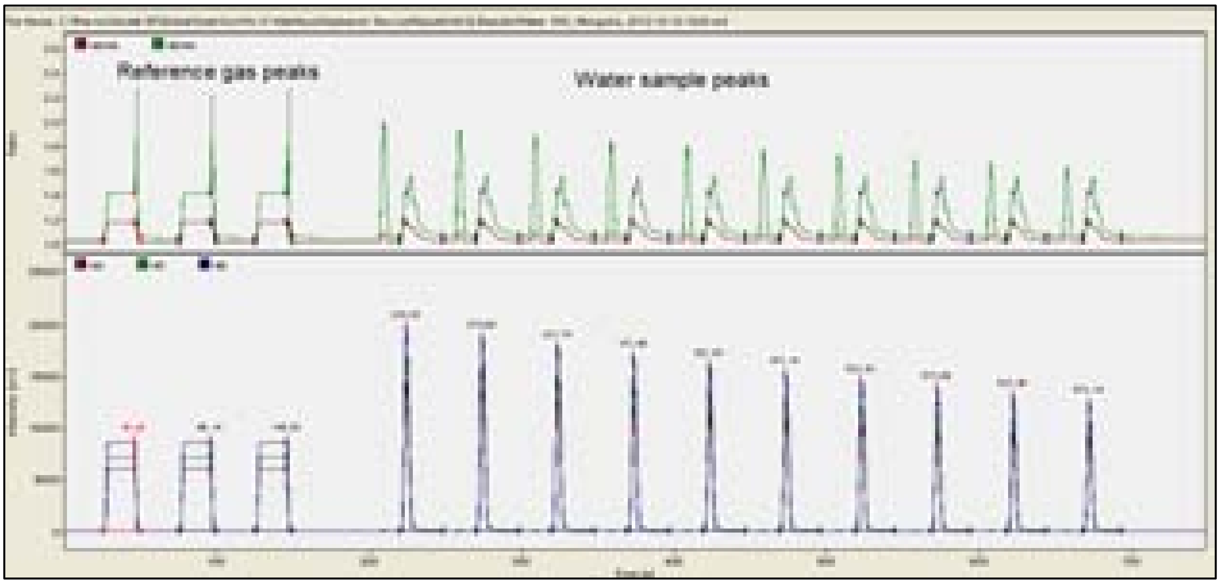
IV. Historic Przewalski's horse tail samples

Natalia Spasskaja, Zoological Museum of Moscow Lomonosow State University, organized and coordinated the sampling and transfer of seven historic Przewalski´s horse tail samples from Moscow Lomonosov State University (1 sample, shot in 1895) and from St. Petersburg Zoological Institute RAS (6 samples, shot between 1889 and 1899)(Table 1).

Table 1: Museum samples of autochthonous Przewalski's horses from the Mongolian Gobi.

Lab sample number	Museum sample number	Origine	Species	Sex	Age	Year of arrival in collection	Comments
12-0988	Equus prz., 5212-3088	Zoological Institute RAS, St. Petersburg	Przewalski´s horse	female	10	1899	from Klemenz, from Dzungarian Gobi btw. Nursu and Semitschendse
12-0989	Equus prz., 5213-3090	Zoological Institute RAS, St. Petersburg	Przewalski´s horse	female	11-12	1897	from the Russian consul J.P. Shismaryo at Urga (Ulan Bator)), from Dzungarian Gobi near Kobdo
12-0990	Eguus prz., 5214-3074	Zoological Institute RAS, St. Petersburg	Przewalski´s horse	male	9	1889	from Shismarev Ya., from Dzungarian Gobi
12-0991	Eguus prz., 5215-3087	Zoological Institute RAS, St. Petersburg	Przewalski´s horse	male	1.5-2	1899	from Clemenz, from Dzungarian Gobi btw. Nursu and Semitschendse
12-0992	Equus prz., 5216-3092	Zoological Institute RAS, St. Petersburg	Przewalski´s horse	male	13-14	1889	from Grum-Grzhimailo G. & M., from Dzungarian Gobi, Gashun
12-0993	Equus prz., 1055-3073	Zoological Institute RAS, St. Petersburg	Przewalski´s horse	male	pul	1899	from Clemenz, from Dzungarian Gobi
12-0994	Equus prz., S-1772	Zoological Institute RAS, St. Petersburg	Przewalski´s horse	female	6	1895	from Roborovksiy V., Kozlov P., from Dzungarian Gobi, Guchen

V. Sample preparations and first analysis



Contents

Sample preparations and analysis

1.1 Plant samples

2.1.1 Sample preparation

In laboratory, plant samples (192 samples in total) (Tab.. 1) were re-dried in a ventilation drying oven at 50 °C for 24 h. For isotopic analysis, samples were ground to fine powder and homogenized either by using a mortar and pestle or an oscillating mill MM 200 (Retsch GmbH, Germany), using 10 mL agate milling cups and 1 cm balls. Unfortunately, complete grinding of the wooden parts (mostly bark) of some samples could not be achieved with any of the grinding methods applied.

Table 1: Overview of the plant samples taken in the Mongolian Gobi in 2012 sampling campaign.

Species	Description	N
Achnaterum	*Achnatherum splendens*	13
Alium	probably mostly *Allium mongolicum*	10
Anabasis	*Anabasis brevifolia*	12
Artemisia spec.	*Artemisia sublessingiana*	22
Ajania	*Ajania fructiculosa*	4
Caragana	*Caragana leucophloea*	21
Elymus	*Elymus secalinus* also called *Leymus secalinus*	6
Eurotia	*Krascheninnikovia* oder *Eurotia ceratoides* also called *Ceratoides latens*	19
Haloxylon	*Haloxylon ammodendrum*	15
Reaumuria	*Reaumuria soongonica*	22
Stipa	in the Gobi probably mostly *Stipo glareosae*, in the mountains also other species possible	21
Zygophyllum	we probably so far collected only *Zygophyllum pterocarpum*	1
Festuca	only down to genus possible	7
Agropyron	only down to genus possible	5
Grass	a grass species that looks like ordinary Austrian lawn grass - species to be determined	3
Grass mix	riverside grass mix - impossible to determine species, does also include sedges	9
Reed	not sure about species - to be determined	1

After grinding, about 0.3-0.5 mg of each sample was weighed and closed in a tin capsule (8 x 5 mm, Thermo Fisher Scientific, UK) for stable carbon ($\delta^{13}C$) analysis. For hydrogen (δ^2H) analysis, about 0.1-0.2 mg of each sample as well as reference material (IAEA-CH3, cellulose) were weighed and closed into silver capsules (3.3 x 5 mm, IVA Analysentechnik e.K., Germany) and left at room temperature for a minimum of 48 hours to allow for equilibration with atmospheric vapor. Thus the Principle of Identical Treatment by which the laboratory reference material with known δ^2H value and same H exchange characteristics as the samples, is used for determination of δ^2H of nonexchangeable H of the sample (Wassenaar and Hobson, 2000), was followed.

2.1.2 Stable isotope analysis

Isotopic measurements of the plant samples will be determined using Flash 2000 HT Elemental Analyzer (Thermo Fisher Scientific, Germany) for solid samples are interfaced via ConFlo IV (Thermo Scientific, Germany) to an isotope ratio mass spectrometer (DELTA V Advantage, Thermo Fisher Scientific, Germany) (Figure 1). Samples will be analyzed in duplicate for CNS and in triplicate for hydrogen.

Results will be reported in δ notation in units of per mile (‰) with respect to international standards, i.e. Vienna Pee Dee Belemnite (V-PDB) for carbon and Vienna Standard Mean Ocean Water (V-SMOW) for hydrogen, Cañon Diablo Troilite (CDT) for sulphur, N_{air} for nitrogen, according to the equation:

$$\delta_{sample}\ (‰) = (R_{sample}/R_{standard}) \times 1000 \qquad (1)$$

where R denotes $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{34}S/^{36}S$ and $^{2}H/^{1}H$ ratios of the sample and standard, respectively. The repeatability and reproducibility of the isotopic measurements are checked with certified reference material IAEA-CH-3 ($\delta^{13}C$ = -24.7 ‰, $\delta^{2}H$ = -35.5‰; cellulose) and several in-house reference materials, i.e. Melon ($\delta^{13}C$ = -27.6 ‰, $\delta^{15}N$ = +12.6‰), Cherry Laurel ($\delta^{13}C$ = -28.7 ‰, $\delta^{15}N$ = -0.45 ‰), Sunflower ($\delta^{13}C$ = -30.1 ‰, $\delta^{15}N$ = +7.6 ‰).

Figure 1: Stable Isotope Ratio Mass Spectrometer connected to different peripheries.

2.2. Equid tail samples

For the purpose of the project, in the Mongolian Gobi tail hair of the three sympatric equid species, i.e. Przewalski´s horse (27 samples), domestic horse (40 domestic) and Asiatic wild ass (14 samples) ware collected.

2.2.1. Sample preparation

First Przewalski´s and domestic horse tail samples have been prepared as follows: tail samples were repeatedly wiped with 95 % ethanol and allowed to air dry. To estimate diet over time, tail samples were cut from root to tip in sections of 1 cm. The corresponding sections of hair were gathered to obtain a sufficient amount for analysis.

2.3 Water samples

2.3.1 Sample preparation

Water samples, i.e. precipitation, river, lake and other water point samples were stored in plastic bottles in a cool room at a temperature of about 9-11 °C. Before the analysis, the samples were left to achieve room T. Afterwards 400 µL of each sample was pipetted into 12 mL glass vials, closed with caps with pierceable rubber septum (Labco Exetainer, Labco Limited, UK) and placed into a Gas Bench autosampler tray (T = 22-24 °C) connected to a Delta V Advantage isotope ratio mass spectrometer (both Thermo Fisher Scientific, Germany) (Figure 1).

2.3.2 Stable isotope analysis

Before the stable oxygen isotope ($\delta^{18}O$) analysis, each sample was automatically flushed with a He/CO_2 mixture and left at least 20 hours to equilibrate. During isotopic equilibration, ^{18}O transferred from the sample H_2O was equilibrated with CO_2 in the headspace. After equilibration, CO_2 from the headspace was transferred via He carrier gas into the IRMS for the determination of $\delta^{18}O$. Each sample analysis consisted of three consecutive reference gas measurements followed by ten consecutive sample measurements (Figure 2). Each sample was prepared and analyzed in duplicate.

The repeatability and reproducibility of $\delta^{18}O$ determinations was checked by using WBW in-house reference material (Wieselburg water; $\delta^{18}O$ = -10.5‰) and was better than 0.1 ‰.

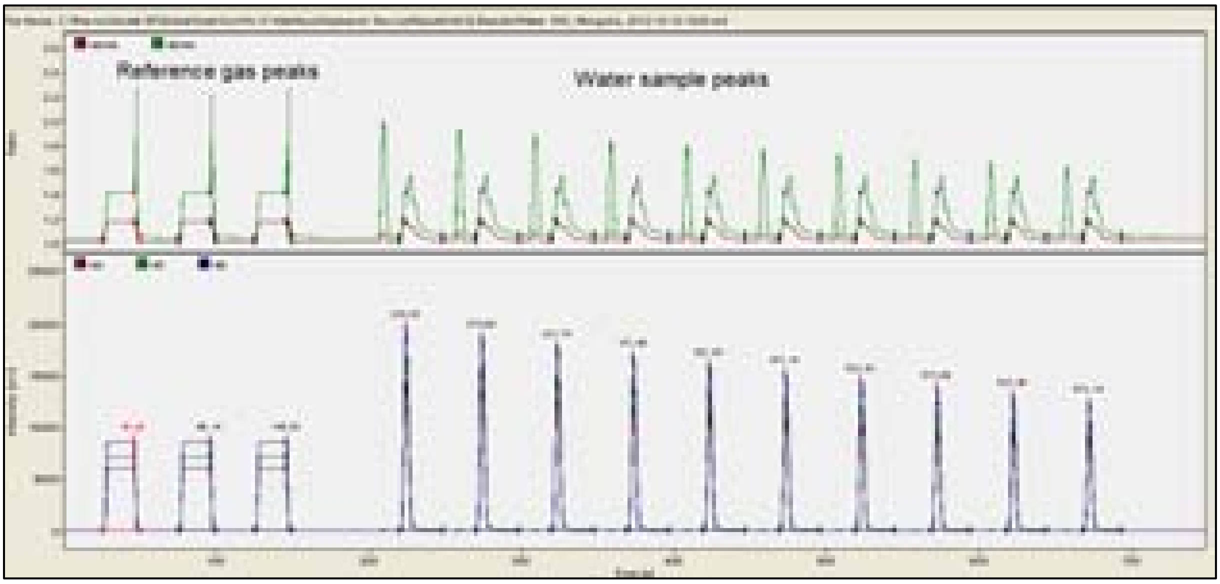


Figure 2: A typical chromatogram of water equilibration ($^{18}O/^{16}O$) indicating three reference gas peaks followed by ten sample peaks.

2. Results and discussion

2.1 Water samples

Results of stable oxygen isotope determinations ($\delta^{18}O$) of the different water samples, i.e. Tachin Tal rain samples, Bij river samples, and various water point samples sampled in June and July, 2012 are given in Tables 1-3, respectively.

Tachin Tal rain $\delta^{18}O$ values varied between -11.9 and -5.8 ‰ in June and between -10.1 and -4.2 ‰ in July samples (Table 2), which is in accordance with weighted June $\delta^{18}O$ values reported by global network for isotopes in precipitation (GNIP) (Figure 3, figure insertion in the upper left corner), established by the International Atomic Energy Agency in cooperation with the World Meteorological Organization (WMO; URL: http://www.isohis.iaea.org), which comprises monthly data from more than 500 stations world-wide, clearly displaying the geographical distribution on a global scale. In Mongolia, however, there is no station having at least one full-year record on $\delta^{18}O$ (or $\delta^{2}H$) values in precipitation. Similar values were obtained by Yamanaka et al. (2006), who investigated the atmospheric water cycle over eastern Mongolia and its surrounding regions by monitoring stable hydrogen and oxygen isotopes in precipitation. Authors report the mean monthly $\delta^{18}O$ values of about -7 ± 2 ‰ for June and -10 ± 2 ‰ for July, 2003 (reported monthly $\delta^{18}O$ values ranged between -7.1 and -33.3 ‰ from April 2002 to September 2003 with higher values in summer and lower values in winter). They have observed a considerable coherence in isotopic composition in space which is characterized by a strong correlation with air T and a weak correlation with precipitation.

Table 2: $\delta^{18}O$ (‰) values of Tachin Tal rain samples.

Lab. No.	#	Id	Date	XCO	YCO	Comments	Sampling bottle	$\delta^{18}O$ (‰)	std (n=2)
12-0666	1	TT camp	15.06.2012	93.65163	45.53879	Rain	TT camp & date	-5.84	0.04
12-0667	2	TT camp	16.06.2012	93.65163	45.53879	Rain	TT camp & date	-9.05	0.02
12-0668	3	TT camp	22.06.2012	93.65163	45.53879	Rain	TT camp & date	-8.03	0.01
12-0669	4	TT camp	23.06.2012	93.65163	45.53879	Rain	TT camp & date	-11.89	0.01
12-0670	5	TT camp	25.06.2012	93.65163	45.53879	Rain	TT camp & date	-9.59	0.01
12-0840	6	TT camp	08.07.2012	93.65163	45.53879	Rain	TT camp & date	-10.10	0.00
12-0841	7	TT camp	22.07.2012	93.65163	45.53879	Rain	TT camp & date	-4.24	0.01

Mean $\delta^{18}O$ value of Bij river samples taken weekly at same location was -13.7 ± 0.1 ‰ (Table 3). Two Bij river samples were additionally taken at the Ranger camp and in the mountains before dam (Upper Bij sample) (Table 4) and had the $\delta^{18}O$ values depleted compared to the three Bij river samples (-14.3 and -14.7 ‰, respectively). The $\delta^{18}O$ value of a river sample is determined by the altitude effect of precipitation, i.e. isotopic composition of precipitation changes with the altitude and becomes more and more depleted in ^{18}O at higher elevation (thus having lower $\delta^{18}O$ values) and the effect of distillation through evaporation from the river surface (higher evaporation appearing at higher T, in which the lighter isotopes are preferentially evaporated, leaving the remaining water enriched with the heavy isotopes, thus having higher $\delta^{18}O$ values) (Tsujimura et al., 2007 and references therein). These effects explain the depletion of the Upper Bij river sample taken in the mountain area as compared to the three Bij river samples taken at lower altitude and being exposed to higher evaporation. Same effects are observed for example in Ovchoo River with $\delta^{18}O$ values of -16.8 ‰ in the mountain area (Mount 1) and -15.9 ‰ downstream (identical value measured for two sampling dates, 20. and 26. 6.2012), as well as in Chonin Us with $\delta^{18}O$ values of -15.4 ‰ and -15.9 ‰ in the middle stream and -10.5 ‰ downriver.

Table 3: $\delta^{18}O$ (‰) values of Bij River samples.

Lab. No.	#	Id	Date	XCO	YCO	Comments	Sampling bottle	$\delta^{18}O$ (‰)	std (n=2)
12-0671	1	Bij	16.06.2012	93.75634	45.55622	Ranger Ombayer	Bij & date	-13.70	0.05
12-0672	2	Bij	22.06.2012	93.75634	45.55622	Ranger Ombayer	Bij & date	-13.82	0.02
12-0673	3	Bij	29.06.2012	93.75634	45.55622	Ranger Ombayer	Bij & date	-13.68	0.02

$\delta^{18}O$ results obtained for different water points (Table **4**, Figure **3**) indicate that large water surfaces, e.g. mountain lakes ($\delta^{18}O$ values -6.7, -3.8 and +2.4 ‰) and broken wells ($\delta^{18}O$ values between -11.4 and +8.3 ‰, mean value -6.1 ‰), are relatively enriched with the heavy isotope which is attributed to evaporation from the open surfaces. The extreme $\delta^{18}O$ value of +8.3 ‰ was obtained at the water point 64, which is a huge clay pan in which the water have likely been standing for a very long time and was hence subjected to long evaporation resulting in a very high ^{18}O enrichment.

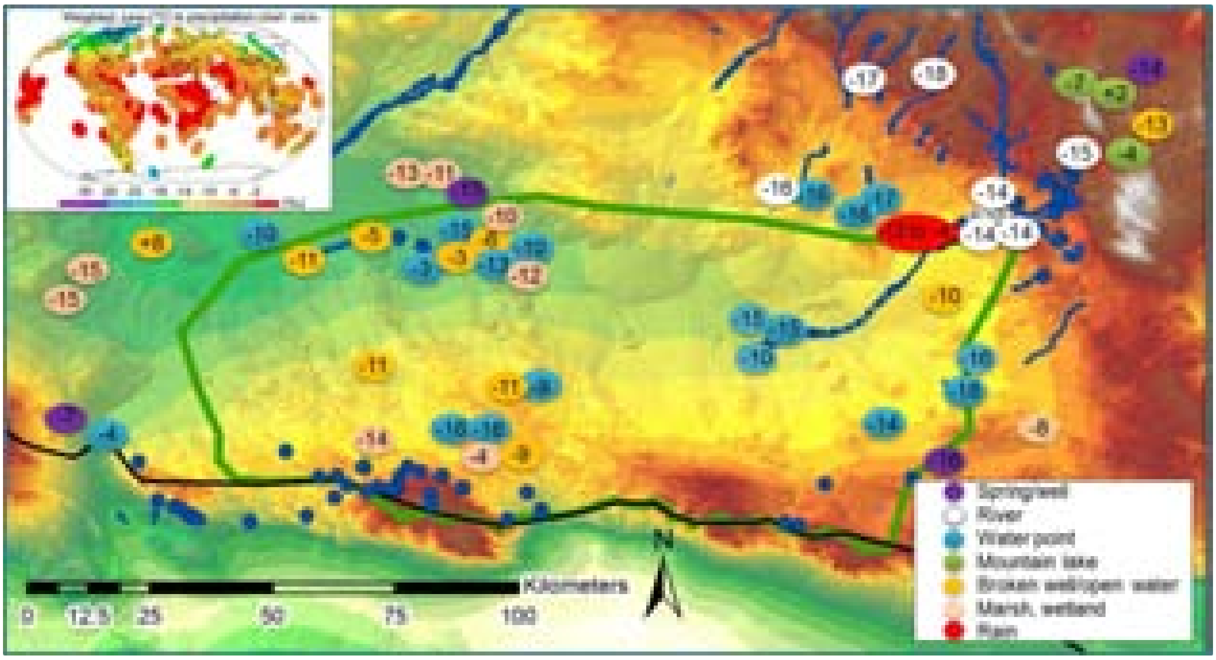


Figure 3: $\delta^{18}O$ values (in ‰) of different water samples in the Gobi study area (June, July, 2012). Green line denotes the boundary of the Great Gobi Strictly Protected Area, small blue circles denote springs (Burnik Sturm et al., 2012).

$\delta^{18}O$ values between -18.2 and -13.7 ‰ of water points, marked as springs, wells and mountain rivers (with the exception of spring water point 16 with $\delta^{18}O$ of -7.2 ‰) suggest that these water points are being fed by groundwater and/or precipitation.

Table 4: $\delta^{18}O$ values (‰) of water samples from different water points.

Lab. No.	#	Id	Date	XCO	YCO	Real name	Comments	Pictures	Sampling bottle	$\delta^{18}O$ (‰)	std
12-0674	1	1	17.06.2012	92.52825	45.26294	new water point	depression with 3 holes, digging by khulan	5832-5836	Water 5	-9.52	0.01
12-0675	2		20.06.2012	92.39906	45.61166	Well	Spring camp with well	6126-6127	Well	-15.30	0.07
12-0676	3	9	20.06.2012	92.49603	45.48013	Takhi Us 2	Takhi us water point, one of three, shallow pools	6139-6145	Takhi us 2	-12.87	0.02
12-0677	4		17.06.2012	92.51429	45.48184	Takhi Us 1	Takhi us water point, marsh and pool, Takhi re-introduction site	5760-5773	Takhi us - Ranger camp	-12.34	0.03
12-0678	5	10	17.06.2012	92.54503	45.13986	Khairchin Khukhin Tunge	Yellow Achnterum, old broken well, open water	5805-5809	Water 1	-5.62	0.06
12-0679	6		17.06.2012	92.45261	45.13961	Argali Shand	Camp location and wetland	5810-5812	Argal Shan	-4.34	0.06
12-0680	7	12	17.06.2012	92.51457	45.25644	Takhiin Tsavchaa	broken well with water & some pools	5823-5831	Water 4	-11.24	0.03
12-0681	8	45	20.06.2012	92.29084	45.65011	Tooroin Tugul	wetland with swampy areas at many places	6090-6117	Water 45	-13.42	0.04
12-0682	9		20.06.2012	92.31266	45.65646	Uzuur Us	wetland with swampy areas at many places	6118-6124	Water 45B	-11.35	0.01
12-0683	10	Tsaagan us	19.06.2012	91.83917	45.53840	Tsagaan Us	main water point of large wetland, small pools, near point 62	6073-6074	Tsagaan us	-9.92	0.00
12-0684	11	64	19.06.2012	91.56396	45.52187	Baruun Khuurai	standing	6056-6061	Point 64	8.34	0.03
12-0685	12	66	19.06.2012	91.33363	45.41427	Damjig	large wetland	6042-6050	Point 66	-15.16	0.00
12-0686	13	68	19.06.2012	91.40449	45.46449	no name	large wetland with flow	6051-6055	Point 68	-14.78	0.02
12-0687	14	75	19.06.2012	91.97418	45.49406	Khoolin Dund Us	river bed with pools - camp on 3 night	6075-6086	Point 76	-11.2	0.04
12-0688	15	78	20.06.2012	92.13435	45.53263	no name	river bed with pools	no pictures, but like Point 76	Water 78	-5.19	0.01
12-0689	16	86	18.06.2012	91.35791	45.18326	Gakhai Bulag	small spring in mountains	6005-6015	Point 86	-7.16	0.01
12-0690	17	Gashurn us 2	18.06.2012	92.15154	45.15259	Gashuun Us 2	salt marsh with fresh pools "little Chonin us"	5927-5957	Gashurn us 2	-13.93	0.00
12-0691	18	Water 3	17.06.2012	92.41294	45.18167	Zadgai	Water point with camp	5813-5822	Water 3	-16.14	0.02
12-0692	19	Water 6	18.06.2012	92.15012	45.29674	Surhaitin Dund Us	Water hole	5898-5912	Water 6	-10.65	0.03
12-0693	20	Gashurn us 3	20.06.2012	92.41369	45.52901	Gashuun Us 3	wetland with small pools	6128-6132	Gashurn us 3	-10.24	0.01
12-0694	21	Bosgo us	20.06.2012	92.36254	45.50712	Bosgin Us	Spring with riverbed and several pools, fresh takhi tracks nearby	6138	Bosgo us	-6.48	0.04
12-0695	22	Shanaga	20.06.2012	92.32127	45.49047	Shanagin Us	Waterpoint	6139-6140	Shanaga	-2.97	0.04
12-0696	23	Uvchoo river	20.06.2012	93.27103	45.62225	Uvchoo river	River		Uvchoo (Knie)	-15.92	0.08
12-0697	24	Shirin us	16.06.2012	93.44181	45.58680	Shirin us	Waterpoint		Shirin us	-15.92	0.06
12-0698	25	Gun Tamga	12.06.2012	93.68456	45.25739	Gun Tamga	Waterpoint		Gun Tamga	-17.64	0.01
12-0699	26	Toodog	11.06.2012	93.47908	45.20050	Toodog	Waterpoint		Toodog	-13.97	0.03
12-0700	27	Bij River	12.06.2012	93.67251	45.54700	Bij River	River		Bij River - Ranger Camp	-14.32	0.06

Table 5: $\delta^{18}O$ values (‰) of water samples from different water points (continuation).

Lab. No.	#	Id	Date	XCO	YCO	Real name	Comments	Pictures	Sampling bottle	$\delta^{18}O$ (‰)	std
12-0701	28	Rain puddle	12.06.2012	93.62820	45.43390	Rain	Rain puddle		1 Rain puddle on road	-9.78	0.05
12-0702	29	Chonin us	22.06.2012	93.15847	45.32514	Chonin Us	Water point - downriver from spring		Chonin us (West)	-10.52	0.07
12-0703	30	Chonin us	22.06.2012	93.22495	45.35328	Chonin Us	Water point		Chonin us 1	-15.44	0.05
12-0704	31	Gashun	22.06.2012	93.71385	45.31341	Gashun Us 1	Water point		Gashun 1	-16.52	0.05
12-0705	32	Mount1	26.06.2012	93.44210	45.83409		Upper Uvchoo River		Mount1	-16.80	0.09
12-0706	33	Plot 14	26.06.2012	93.63226	45.83689		Little spring / river		Plot 14	-18.22	0.02
12-0707	34	Mount 3	27.06.2012	94.02000	45.81199		Dammed lake on pass - snow/rainwater?	6427-6430	Mount 3	-6.69	0.00
12-0708	35	Hagin Noor	27.06.2012	94.06293	45.79938	Hagin Noor	Large mountain lake - snow/rainwater?	6445-6459	Hagin Nor	2.39	0.01
12-0709	36	Kharch Tolgoi	27.06.2012	94.16200	45.83591	Kharch Tolgoi	Spring	6461-6462	Kharch Tolgoi	-14.04	0.03
12-0710	37	Mount 5	27.06.2012	94.17175	45.72777		dry riverbed with pools - people dig for water here, pool may have been rain or river water		Mount 5	-13.24	0.02
12-0711	38	Dirchin Noor	27.06.2012	94.13357	45.69802	Dirchin Noor	Mountain lake - snow/rainwater & springs?	6470-6474	Dirchin noor	-3.80	0.03
12-0712	39	Upper Bij	27.06.2012	93.98796	45.68054	Upper Bij	Upper Bij river, in mountains before dam	6488	Upper Bij	-14.67	0.02
12-0713	40	W83	02.07.2012	93.89219	45.18604		water		W83	-8.26	0.00
12-0714	41	Khairchen Bulag	02.07.2012	93.63847	45.12986	Khairchen Bulag	spring with little river		Khairchen Bulag	-15.74	0.04
12-0832	42		24.07.2012	93.44181	45.58680	Shirin us	water point - bi-monthly sampling		Shirin us 2012.07.24	-16.66	0.04
12-0833	43		24.07.2012	92.41294	45.18167	Zadgai us	water point - bi-monthly sampling		Zadgai us 2012.07.24	-16.28	0.04
12-0834	44		27.07.2012	93.22495	45.35328	Chonin Us	water point - bi-monthly sampling		Chonin us Naiman 2012.07.27	-15.09	0.02
12-0835	45		24.07.2012	92.36254	45.50712	Bosgin Us	water point - bi-monthly sampling		Bosgo us 2012.07.24	-3.23	0.00
12-0836	46		24.07.2012	92.41369	45.52901	Gashun-W	water point - bi-monthly sampling		Gashuun-W 2012.07.24	-15.05	0.05
12-0837	47		24.07.2012	92.51429	45.48184	Takhi Us	water point - bi-monthly sampling		Takhi us 2012.07.24	-10.43	0.01
12-0838	48		24.07.2012	93.27103	45.62225	Ovchuu River	water point - bi-monthly sampling		Ovchuu 2012.07.24	-15.89	0.01
12-0839	49		18.07.2012	91.44225	45.16854	Rain water in salty depression	water point - bi-monthly sampling		N 45,16854° E91,44225° I. 18/7/2012	-3.95	0.09

3. Future work

In the next months, our main focus will be on:

- H isotope analysis of water samples,
- CNS and H isotope analysis of plant samples,
- continuation with the preparation of equid tail hair samples and subsequent CNS and H isotope analysis,
- analysis of the results obtained and their interpretation.

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