PHYLOGEOGRAPHY OF THE WESTERN POPULATIONS OF *STYLODIPUS TELUM* (RODENTIA, DIPODIDAE) BASED ON MITOCHONDRIAL DNA

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Phylogeography of the Western Populations of *Stylodipus telum* (Rodentia, Dipodidae) based on Mitochondrial DNA. Rusin, M. — The phylogeographic analysis of *Stylodipus telum* was performed based on *cytochrome b* mitochondrial gene. Two westernmost subspecies, *S. t. falzfeini* and *S .t. turovi,* both formed independent monophyletic clades, but the genetic distance between them clearly indicates that neither should be treated as a separate species. Individuals from remote populations of *S. t. falzfeini* mix together and do not have a clear phylogeographic structure, which could indicate a recent panmixia within the subspecies. Populations in Kherson Region are under threat due to the ongoing Russian invasion.

K e y w o r d s: *cytb*, Lower Dnipro Sands, Tsimla Sands, Kherson Region, threatened populations.

Introduction

Thick-tailed three-toed jerboa *Stylopidus telum* (Lichtenstein, 1823) is one of the three species within the genus *Stylodipus* G. M. Allen, 1925 (Shenbrot et al., 1995). The range of *S. telum* is highly fragmented and consists of several isolates. The westernmost populations occupy a narrow strip within the Lower Dnipro Sands in Kherson Region, Ukraine. This region is inhabited by the largest subspecies *S. telum falzfeini* (Brauner, 1913). The second subspecies — *S. telum turovi* (Heptner, 1934) — lives in Ciscaucasia at the interfluve of the Don

and Volga rivers. One isolate of this taxon is found west of the Don River in Tsimla Sands (Rostov Region, Russia) and at least two isolates are known from the east of the Volga River near the towns of Astrakhan and Kamyshyn (Shenbrot et al., 1995). The nominative subspecies *S. telum telum* (Lichtenstein, 1823) occupies vast areas in Kazakhstan east of the Ural River (with small isolate in the Volga–Ural interfluve) up to the Irtysh River (Shenbrot et al., 1995). Three more subspecies are represented by the isolated populations: *S. telum amankaragai* (Selevin, 1934) in Naurzum (Northern Kazakhstan), *S. telum birulae (*Vinogradov, 1937) in Zaisan depression (Eastern Kazakhstan) and finally *S. telum nastjukovi* Shenbrot, 1991 is known from the desert of Kyzylkumy south of the Balkhash Lake (South-Eastern Kazakhstan). Two other species in the genus are *S. sungorus (*Sokolov and Shenbrot, 1987) from Dzungaria (China) and *S. andrewsi* G. Allen, 1925 from Mongolia (Shenbrot et al., 1995).

Stylodipus telum is found in a wide range of habitats. Both western subspecies are strongly associated with sandy steppes (Lower Dnipro Sands, Tsimla Sands etc.), whereas the eastern populations inhabit various types of deserts and semi-deserts (Shenbrot et al., 1995). Previous genetic studies of *Stylodipus* primarily focused on identification of the position of the genus within the superfamily Dipodoinae, but lacked a more detailed resolution at the species/subspecies level (Lebedev et al., 2013; Pisano et al., 2015). Here I present the first study of intraspecific variation of *S. telum* with a focus on the two western subspecies.

Methods

Eight new samples were collected for phylogeographic assessment of *Stylodipus telum* (table 1). Four samples originated from Solonoozernyi department of the Black Sea Reserve and two more samples from the Sagi Zakaznik close to the town of Oleshki. I was also able to obtain two samples of *S. telum turovi* from Tsimla Sands isolate on the western side of the Don River (fig. 1). I was searching for *S. telum turovi* in the main part of its range, including the type locality (town of Remontnoye in Rostov Region), but failed to find any extant population there. The only other sample available so far is a previously published sequence from the individual from Kalmykia (Achinery) (Lebedev et al., 2013; Pisano et al., 2015). That individual was captured in 2003, whereas during my field survey in 2016 I did not find any *S. telum* in Achinery.

Live trapping of *S. telum* was possible using the Tomahawk 201 live trap (Wisconsin, USA).

DNA isolation was performed following standard protocols in commercially available kits. For *cytochrome b* (*cytb*) mitochondrial gene, a standard set of primers (L7/H6) was used (Montgelard et al., 2002). Sequences were aligned manually in BioEdit (Hall, 1999).

All new sequences obtained in this study were deposited in the NCBI GenBank with accession numbers OP882098-OP882105.

I used Maximum Likelihood (ML) and Bayesian Inference (BI) methods for phylogenetic analysis. Substitution model was chosen in MEGA 11 (Stecher et al., 2020) using all available sites. The HKY+G model

Fig. 1. Sampling localities of *S. telum* used in the study. White — range of *S. telum falzfeini* (original unpublished data) and green — range of *S. telum turovi* (Shenbrot et al., 1995).

T a b l e 1 . Individual sampling data used in the study

Species/subspecies	Sample ID	Sampling data	GenBank Acc.N
S. telum falzfeini	RX1	Ukraine, Kherson Region, Oleshki, Sagi, N46.6078 E32.84181, 2017-05-17, col. M. Rusin & M. Ghazali. This study	OP882102
—	RX2	Ukraine, Kherson Region, Hola Prystan, Solonoozernyi, N46.4576 E31.9948, 2017-05-22, col. N. Nedyalkov & M. Rusin. This study	OP882103
—	RX3	Ukraine, Kherson Region, Oleshki, Sagi, N46.6079 E32.84181, 2017-05-17, col. M. Rusin & M. Ghazali. This study	OP882104
—	RX4	Ukraine, Kherson Region, Hola Prystan, Solonoozernyi, N46.4550 E32.0118, 2017-07-24, col. M. Rusin & M. Ghazali. This study	OP882105
—	X2	Ukraine, Kherson Region, Hola Prystan, Solonoozernyi, N46.4533 E32.0234, 2016-04-20, col. M. Rusin & V. Busel. This study	OP882100
—	X3	Ukraine, Kherson Region, Hola Prystan, Solonoozernyi, N46.4526 E32.0210, 2016-04-20, col. M. Rusin & V. Busel. This study	OP882101
S. telum turovi	X1	Russia, Volgograd Region, Tsimla Sands, N48.0208 E42.4121, 2016-05-10, col. M. Rusin & N. Nedyalkov. This study	OP882098
—	S202221	Russia, Volgograd Region, Tsimla Sands, N48.0205 E42.4246, 2019-07-28, col. A. Tikhonov & V. Sinyavskaya. This study	OP882099
—		Russia, Kalmykia, Chernye Zemli, col. L. Savinetskaya. Lebedev et al., 2013; Pisano et al., 2015	KM397197
S. telum telum		Kazakhstan, Kostanay province, Amangeldy District, near Urpek, N50.1 E65.3. Shenbrot et al., 2017	MF076855
S. sungorus		Pisano et al., 2015	KM397199
*S. telum (sungorus)**		China. Cheng et al., 2021	MN979747
*S. telum (sungorus)**		Luo et al., 2016	NC027692
S. andrewsi		China. Cheng et al., 2021	MN979746

*Originally attributed to S. telum, but reidentified as *S. sungorus*, see Discussion section.

had the lowest BIC score. ML has been run in MEGA11 with 1000 bootstrap pseudoreplications. BI was analyzed using MrBayes 3.2.7 (Ronquist & Huelsenbeck, 2003) on CIPRES Science Gateway (Miller et al., 2010) with the following set of parameters: 2 runs with 5 million generations each, sampling every 2000, burn in rate set at 250 trees for each run. Trees were checked for congruency in Tracer 1.7 (Rambaut et al., 2018) and then manually combined. Kimura two-parameter distance (K2p) between operation taxonomic units (OTUs) calculated in MEGA 11 (Tamura et al., 2021). Trees visualized in FigTree 1.4.4 (Rambaut, 2018) using midpoint root.

Results

In total, 14 sequences were used in the final analyzes: eight obtained in this study and six downloaded from the GenBank. The length of each sequence varied between 997 and 1140 bp. The nucleotide composition within *S. telum* was: T = 28 %, C = 31 %, A = 27 %, G = 14 %. 39 positions were variable, out of which 16 were parsimony-informative.

T a b l e 2. Population K2p distances of the *Stylodipus* OTUs. Bold font: intraspecific, regular font: interspecific distances

	falzfeini	*turovi*	*telum*	*sungorus*	*andrewsi*
falzfeini	0.005				
turovi	0.0114	**0.0054**			
telum	0.0195	0.0179	n/a		
sungorus	0.0488	0.0487	0.0487	**0.0046**	
andrewsi	0.1470	0.1438	0.1508	0.1478	n/a

N o t e. n/a — the intraspecific distance could not be computed since only a single specimen was analyzed in the OTU.

Both ML and BI analyzes resulted in similar topologies (fig. 2).

The most remote species in the genus, *S. andrewsi*, had a pronounced distance from all other OTUs in the genus of 14–15 % (table 2). The other currently recognized species, *S. sungorus*, barely meets the accepted species threshold for *cytb* in Rodentia (Baker & Bradley, 2006). The distances between the subspecies of *S. telum* were at least 3 times smaller compared to those between *S. telum* and *S. sungorus* and at least 14 times smaller compated to that between *S. andrewsi* and the rest of the taxa.

Discussion

The two subspecies *S. telum falzfeini* from the Lower Dnipro Sands and *S. telum turovi* from Tsimla Sands form a separate phylogroup each, yet the K2p distance between them is not high (app. 1.1 %). The individual from Kalmykia (KM397197), although belonging to *S. telum turovi*, grouped with *S. telum falzfeini*, but this association was weak and did not reach the significance level neither in ML, nor in BI phylogenetic reconstruction.
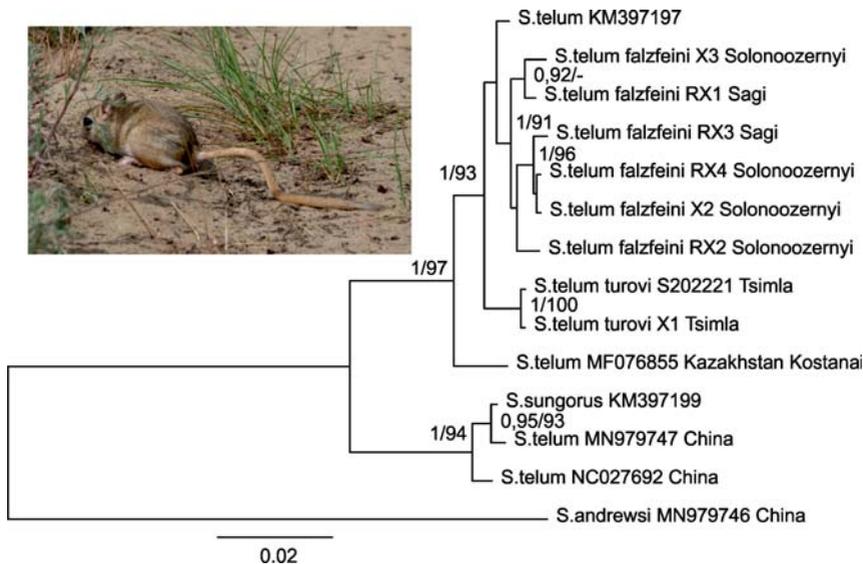
Fig. 2. Phylogeographic reconstruction of *S. telum* based on *cytb*. BI posterior probability / ML bootstrap support per 1000 replications is indicated at the nodes (only values over 70 % are shown). Photo: *S. telum falzfeini* from Sagi (Oleshki District) by M. Rusin, 2017-05-17.

Mitochondrial data supports the integrity of all three analyzed subspecies (*S. t. turovi*, *S. t. falzfeini* and nominative *S. t. telum* from Kazakhstan) into one species.

The Chinese specimens, originally published as *S. telum*, most likely represent *S. sungorus* (see fig. 2).

Using the available sampling and only a single mitochondrial marker, I could not detect any sound phylogeographic structure within *S. t. falzfeini*. I was able to sample only two populations — one originating from Solonoozernyi department of Black Sea Reserve, and the other from Sagi Zakaznik near the town of Oleshki. The distance between these two populations is app. 70 km. Surprisingly, two samples from Sagi locality did not form a sister pair, instead they shared many similarities with different animals from Solonoozernyi population.

Stylodipus telum is a protected species in Ukraine, but on a global level it is currently listed as Least Concern according to IUCN classification (Cassola, 2017). *Stylodipus telum turovi* is likely experiencing a population decline and has been recently included as the protected species into the Red Book of Rostov Region. Populations in Kalmykia also experienced a decline, but more detailed studies should be done there to map actual distribution of *S. telum* and draw evidence-based conclusions.

In Ukraine and Western Russia there is a significant population fragmentation as a result of massive destruction of habitats, with the most significant threats being the artificial forestation, development of settlements and the road network.

Moreover, the entire range of *S. t. falzfeini* was occupied by the Russian military in February 2022. Uncontrollable wild fires spread in those areas, which may potentially harm the jerboa populations. After the withdrawal from Kherson city in November 2022, the Russian military began to construct extensive 'defense lines', such as trenches and fortifications. At the time of writing this article, the exact configuration of defense lines remains unknown, but it is possible, that they will run across the Black Sea Reserve, Sagi Zakaznik and Oleshki Sands National Park — all of which are important localities for jerboas. Before the above areas are liberated, intensive fighting is likely to happen, drawing the small isolated populations towards the risk of extinction.

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