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Journal of Threatened Taxa

10.11609/jott.2025.17.5.26939-27034
www.threatenedtaxa.org

26 May 2025 (Online & Print)
17(5): 26939-27034
ISSN 0974-7907 (Online)
ISSN 0974-7893 (Print)

ISSN 0974-7907 (Online); ISSN 0974-7893 (Print)

Publisher
Wildlife Information Liaison Development Society
www.wild.zooreach.org

Host
Zoo Outreach Organization
www.zooreach.org

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Cover: A digital art of water birds of Noyyal River and its wetlands in Coimbatore District by Megha A. Kashyap.

Journal of Threatened Taxa | www.threatenedtaxa.org | 26 May 2025 | 17(5): 26939–26950

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

https://doi.org/10.11609/jott.9638.17.5.26939-26950

#9638 | Received 21 January 2025 | Final received 02 April 2025 | Finally accepted 17 April 2025

ARTICLE

A review of Tsimlyansk Birch Mouse *Sicista cimlanica* (Mammalia: Rodentia: Sminthidae): distribution, phylogeography, and conservation

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Abstract: All known localities of the Tsimlyansk Birch Mouse are summarized. The area of occupancy of the species is estimated as 123,000 km^2, whereas the extent of occurrence is estimated as 4,000 km^2. The species is proposed as 'Near Threatened' according to IUCN Red List categories and criteria. Analyses of the full mitochondrial cytochrome b gene sequences from four distinct populations indicate that all *Sicista cimlanica* individuals form a monophyletic clade. Having a limited distribution of the Middle Don area in western Russia and eastern Ukraine, this species has an exceptionally high haplotype diversity (h = 0.98), though the nucleotide diversity is considerably low (π = 0.009).

Keywords: Birch mice, cytochrome b, diversity, Don River, genetic diversity, genetic diversity, PCR protocol, pitfall trap.

Editor: Giovanni Amori, Italian National Research Council, Rome, Italy. **Date of publication:** 26 May 2025 (online & print)

Citation: Rusin, M. (2025). A review of Tsimlyansk Birch Mouse *Sicista cimlanica* (Mammalia: Rodentia: Sminthidae): distribution, phylogeography, and conservation. *Journal of Threatened Taxa* 17(5): 26939–26950. https://doi.org/10.11609/jott.9638.17.5.26939-26950

Funding: No direct funding was obtained for this research.

Competing interests: The author declares no competing interests.

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Acknowledgements: Animal live trapping was permitted by the appropriate legislation of the Rostov Nature Reserve. Special thanks go to V. Matrosova and A. Tikhonov. Acknowledgements extend to Abi Gazzard (IUCN SSC SMSG, UK) for English proofreading and commenting on the manuscript.

INTRODUCTION

Birch mice *Sicista* are characterized by high karyologic and genetic variability within the genus but limited differences in morphology. The Steppe Birch Mouse *Sicista subtilis* species group is one of the best studied examples within *Sicista*. Unlike all other species of the genus, which tend to occupy mesophyte tall grasslands, *S. subtilis* s.l. is adapted to arid and semi-arid environments (Lebedev et al. 2019). The western edge of its range begins from the Pannonian Plain, extending to Siberia in the east.

Originally, most of the currently recognized forms of Steppe birch mice were described as separate species. However, when Ognev (1948) united all of them under one species – *Sicista subtilis* (Pallas, 1773) – his perspective remained dominant for a long time until the 1980s, when cytogenetic studies revealed substantial differences in chromosomes for different populations (Sokolov et al. 1986). The latter authors divided all Steppe birch mice into two species: *S. subtilis* and *S. severtzovi* Ognev, 1935. This taxonomy was accepted by subsequent researchers (Shenbrot et al. 1995). According to Shenbrot et al. (1995), the nominative form *S. subtilis* was distributed from Hungary to Kazakhstan and Siberia, thus covering most of the species group's range, excluding only the area of Middle Don River. The second species – Severtsov's Birch Mouse *S. severtovi* – was believed to occur in the basin of the Don River.

Subsequently, it was found that birch mice from different populations in the Don Basin were characterized by polymorphism both in chromosome numbers (2n) and their fundamental numbers (nFa) (Kovalskaya et al. 2011). It was revealed that *S. severtzovi* from the type locality (Voronezh Region, east of Don River) were different from all other Middle Don populations. Therefore, it was suggested to treat all those forms, previously attributed to *S. severtzovi*, as two undescribed species: S. sp.1 and S. sp.2 (Kovalskaya et al. 2011). It remains unclear why the authors did not include *S. severtzovi cimlanica* Kovalskaya et al. (2000) in their review.

The first genetic studies of the *S. subtilis* species group (Cserkész et al. 2016) used mtDNA cytb and nDNA IRBP genes and included five populations from the Middle Don area (out of 12 studied populations). It was discovered that all birch mice from the Middle Don area formed one clade and were sister to the nominative form (*S. subtilis subtilis*) from the left bank of the Volga River. Thus, it was concluded that birch mice from Middle Don should be attributed to one taxon: *S. subtilis severtzovi*. This assumption was premature since there were no sampled animals from the type locality of *S. severtzovi*.

The next work (Lebedev et al. 2020) was based on mtDNA cytb and COI markers from 28 populations of *S. subtilis* species group, with eight populations from Middle Don area. It was shown that Middle Don Birch Mice were not conspecific with specimens of *S. severtzovi* from the type locality, thus reinforcing the results of cytogenetic studies (Kovalskaya et al. 2011). Authors concluded that the only available name for birch mice from Middle Don was *Sicista cimlanica* Kovalskaya et al., 2000 (Lebedev et al. 2020). This species includes chromosomal forms 'cimlanica', 'S.sp.1' and 'S.sp.2'. The putative range of this species lies within western Russia and eastern Ukraine. Thus far, only a few populations have been genotyped, with the number of animals used for these studies varying from one to three. Nonetheless, this taxonomy has been accepted and *S. cimlanica* is now included as a valid species in the American Society of Mammologists (ASM) Mammal Diversity Database.

In the present study, original data are combined with available material to shed light on within-species polymorphism in *S. cimlanica*, to describe the most accurate species distribution and discuss conservation outputs.

MATERIALS AND METHODS

Animal sampling

Nine birch mice were captured using pitfall traps during field surveys conducted in 2016 and 2019 in the western part of the Tsimla Sands. Pitfalls were set for 1–2 nights in the psammophyte steppe. The animals were examined, and small tissue samples were taken for DNA testing. After that, the mice were released back into the wild. The tissue samples were kept in ethanol. Details on all specimens used in the study are provided in Table 1.

DNA isolation, PCR, and sequencing

Genomic DNA from ethanol-preserved tissues was extracted using a Diatom DNA Prep100 kit (Isogen Laboratory) according to the manufacturer's instructions. To extract full mitochondrial cytochrome b (cytb) genes, a set of universal primers L7/H6 (Montgelard et al. 2002) was used. A polymerase chain reaction (PCR) was conducted in a volume of 25 ml using the Taq 5X Master Mix (New England Biolabs); the reaction mixture contained 5 pM of each primer, 0.1–0.2 mkg of DNA, and ddH2O to the final volume.

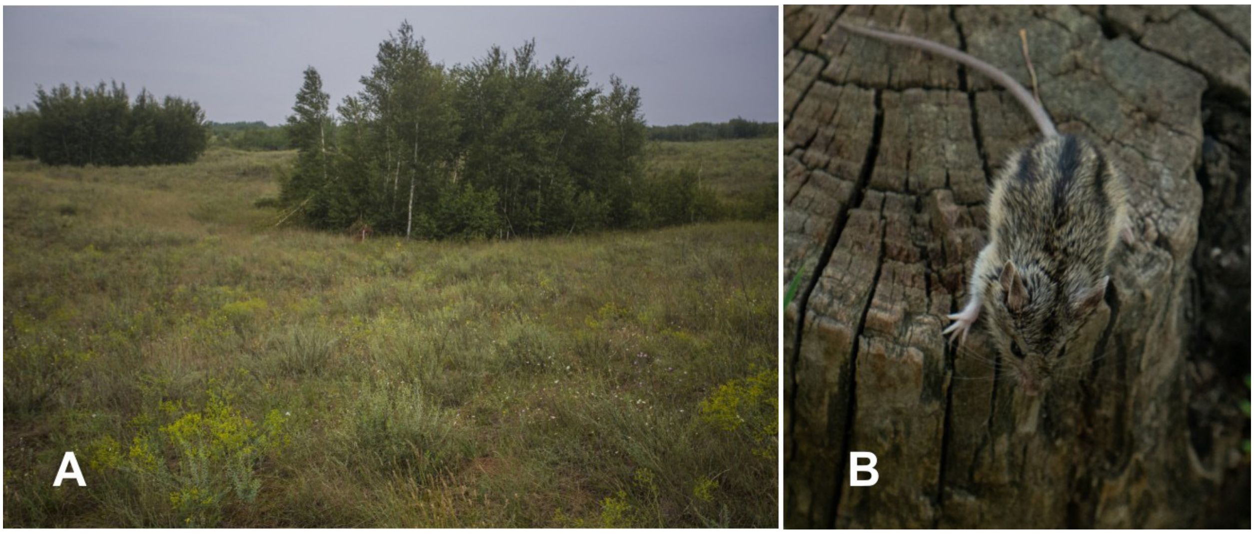

Image 1. A—Typical habitat of *Sicista cimlanica*. © Alexey Tikhonov | B—*Sicista cimlanica* from Tsimla Sands. © Mikhail Rusin.

The PCR protocol for all samples was an initial denaturation step at 95°C for 1 min, then 35 cycles of 95°C for 20 s, 55°C for 20 s, and 72°C for 20 s, with a final extension of 72°C for 5 mins. PCR products were visualized using UV light in 1.5% agarose gel stained with ethidium bromide, cut off, and purified using a GeneJET Gel Extraction kit (ThermoFisher Scientific) according to the manufacturer's instructions.

The nucleotide sequence of gene cytb was determined using an ABI PRISM 3500xL automatic sequencer with the BigDye Terminator Chemistry v. 3.1 (Applied Biosystems) and each of the pair of external primers. The resulting nucleotide sequences were manually aligned with the SeqMan (Lasergene) and BioEdit v 7.0.4.1 (Hall 1999) software.

Phylogenetic analyses

Total alignment contained 21 sequences (17 *S. cimlanica* and four outgroups). Sequence MK259967 was not included in the analyses as it was found to be another isolate from the same specimen as MK758100 (Vladimir Lebedev pers. comm. 2021).

Nine sequences generated in this study were deposited in GenBank (Acc. No.: MT295493–MT295501). MEGA X software (Kumar et al. 2018) was used for sequence analysis and distance estimation. The within- and between-group genetic differences were estimated according to the Kimura two-parameter model (K2p) calculated in MEGA X. Haplotype diversity (h) and nucleotide diversity (π) were calculated in DnaSP v.5.10.01.

The substitution model was chosen in MEGA X, and HKY+G (ncat = 5) had the lowest Bayesian information criterion (BIC) score. The maximum likelihood (ML) tree was constructed in MEGA X. Node support values were estimated according to bootstrapping (1,000 replicates). A Bayesian inference (BI) of phylogeny tree was constructed in MrBayes 3.2.7 (Ronquist & Huelsenbeck 2003) and run on the CIPRES gateway (Miller et al. 2010). The following parameters were used: two runs of five million generations, with four chains, sample frequency set at every 2,000 generations. Runs were checked for convergence and effective sample size in Tracer 1.7.1 (Rambaut et al. 2018), and the burn-in rate was set at 300 trees. Both runs were combined manually and annotated with TreeAnnotator 1.10.4 (Suchard et al. 2018).

For analysis of haplotypes, the sequence data were slightly shortened. After trimming the unequal conservative flanks, all the sequences had the same length of 1,122 bp (positions from 14 to 1,135 bp in the alignment), excluding the only sequence MK758099 from GenBank, which had a length of 1,095 bp (positions 26–1,120 bp in alignment). A haplotype network was constructed using Network v. 10.0.0.0 software (Fluxus Technology Ltd).

RESULTS

Genetic structure and diversity

The mitochondrial DNA cytb gene (1,095–1,140 b.p.) from 17 *S. cimlanica*, belonging to four populations, was analysed. Overall, 94 sites (approximately 8% of the full fragment length) were variable, and 64 of them were parsimony-informative. The mean nucleotide composition was 27.7% (A), 32.9% (T), 13.1% (G), and 26.3% (C).

An unusually high variability in the structure of this marker both over the species range (17 individuals, 15 haplotypes, π = 0.00875 ± 0.00098 SD, h = 0.978 ± 0.031 SD), and in the type locality (11 individuals, 9 haplotypes, π = 0.009528 ± 0.00091 SD, h = 0.945 ± 0.066 SD) was registered (Table 2). The level of intraspecific variability was approximately 0.9%. There were no shared haplotypes among the four genotyped populations.

The total sample set of haplotypes was found to be distributed among four weakly differentiated haplogroups in accordance with the geographical location of the samples. The haplogroup "Tsimla Sands" represents the type locality, where most of the cytb variants are registered (nine haplotypes). It formed a star-like pattern, which may indicate a recent population expansion. The central haplotype (cim4) is probably ancestral. The three other haplogroups are less studied and therefore fewer haplotypes are known: "Serafimovich" (two haplotypes) from the northern part of the Archedin-Don Sands, "Yamskaya Steppe" (three haplotypes) from Belgorod Region, and "Lugansk" (one haplotype) from Triokhizbenka Sands in Ukraine (Figure 1).

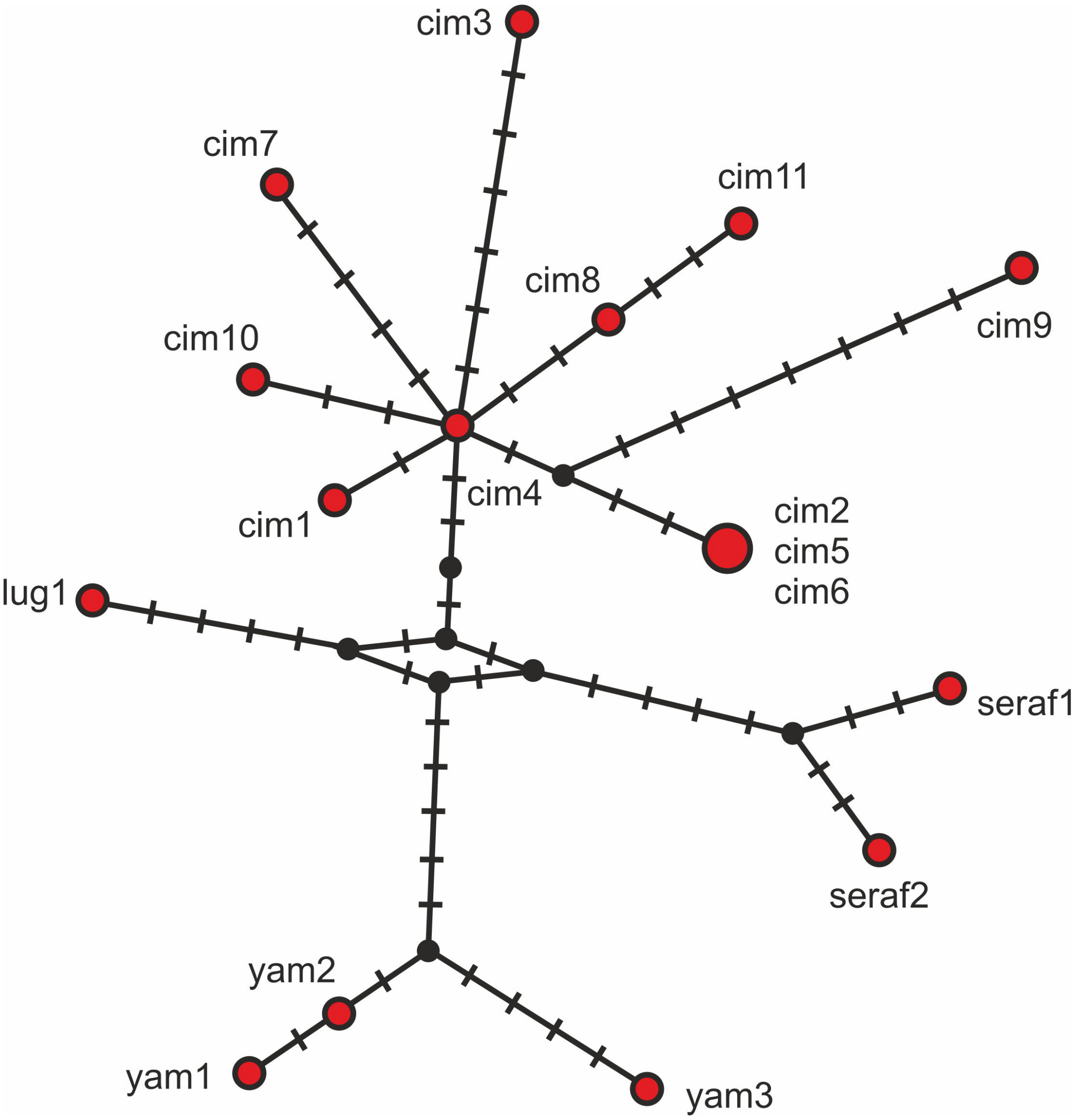

Figure 1. Unrooted haplotype network of absolute distances between mtDNA haplotypes of the cytb gene. Each circle represents a unique haplotype, its size proportional to the haplotype frequency. Black circles represent hypothetical haplotypes. Lines connecting each haplotype represent a single nucleotide substitution, and the hatch marks along those branches represent additional substitutions. Haplotypes with more than one branch connecting them to other haplotypes represent alternative pathways of equal likelihood. Sample names are given in Table 1.

Table 1. A compiled list of verifiable capture localities of birch mice in the middle Don area (both original and previously published data). Designations: ID on Figures — the encoding of the sample names used for illustration | N0 & E0 — coordinates not specified | Karyotype = unknown — local karyotype has not been studied. ZMMU—Zoological Museum of Moscow State University, Moscow, Russia | ZMKNU—Zoological Museum of Kyiv National University | NMNHU—National Museum of Natural History, Kyiv, Ukraine | HNHM—Hungarian Natural History Museum, Budapest, Hungary.

Species	Population	Sampling data	ID on Fig.	GenBank Acc.No.	References
Sicista cimlanica	Tsimla sands, Rostov and Volgograd regions, Russia 2n = 22, NF = 35–36	Eight specimens: holotype S-165916 ZMMU, paratypes S-165917, S-165919, S-165920, S-165923–S-165926, viii.1996; 2 specimens (S-165921 – S-165922, viii.1997; 1 specimen S-195918, 22.vi.1996; 1 specimen S-165927, 24.viii.1996; Rostov part of Tsimla sands, col. G. Tikhonova, N? E?			Kovalskaya et al. 2000
		One specimen: ZMMU S-178462; 2002; col. Yu. Kovalskaya, N? E?			
		Two specimens: NMNHU 10994–10995, col. S. Zolotukhina, 17.vi.1986 and 29.v.1986			Shevchenko & Zolotukhina 2005
		Two specimens: 14–15.viii.2002; ZMMU S-173549, S-173550; col. I. Tikhonov; 48.15N 42.85E	cim11	MK758100	Lebedev et al. 2020
		Two specimens: col. T. Cserkész, M. Rusin, D. Czaban & G. Sramko; 12.vi.2014, 47.818N, 42.663E*	cim10	KP715870	Cserkész et al. 2016
		vouch. ZMMU S-197469; col. M. Rusin & N. Nedyalkov; 11.v.2016, 48.020N 42.413E	cim1	MT295493	this study
		vouch. ZMMU S-197468; col. M. Rusin & N. Nedyalkov; 11.v.2016, 48.022N 42.410E	cim2	MT295494	this study
		released; col. A. Korneev, A. Tikhonov, V. Kilyakova; 28.vii.2019, 47.934N 42.451E	cim3	MT295495	this study
		released; col. A. Korneev, A. Tikhonov, V. Kilyakova; 28.vii.2019, 47.935N 42.446E	cim4	MT295496	this study
		released; col. A. Korneev, A. Tikhonov, V. Kilyakova; 27.vii.2019, 47.935N 42.450E	cim5	MT295497	this study
		released; col. A. Korneev, A. Tikhonov, V. Kilyakova; 28.vii.2019, 47.935N 42.445E	cim6	MT295498	this study
		vouch. ZMMU S-202215; col. A. Korneev, A. Tikhonov, V. Kilyakova; 27.vii.2019, 47.884N 42.480E	cim7	MT295499	this study
		released; col. A. Korneev, A. Tikhonov, V. Kilyakova; 27.vii.2019, 47.933N 42.450E	cim8	MT295500	this study
		vouch. ZMMU S-202214; col. A. Korneev, A. Tikhonov, V. Kilyakova; 26.vii.2019, 47.886N 42.478E	cim9	MT295501	this study
	Alekseevskie Sands, Volgograd Region, Russia, 2n = 26, NF = 46	One specimen: ZMMU S-183022, col. A. Surov, G. Tikhonova, I. Tikhonov, 28.viii.1999, 50.2N 42.3E			Kovalskaya et al. 2011
	Medveditza riv. right bank, Volgograd Region, Russia, 2n = 26, NF = 46	Three specimens: 49.65N 42.62E			Kovalskaya et al. 2000 Kovalskaya et al. 2011
	Medveditza riv. left bank, Volgograd Region, Russia, 2n = 22, NF = 41	One specimen: 49.94N 43.22E			Kovalskaya et al. 2011
	Archedinskie Sands (north), Volgograd Region, Russia, 2n = 23, NF = 44	One specimen: 49.65N 42.72E	seraf2	MK758099	Kovalskaya et al. 2011 Lebedev et al. 2020
		One specimen taken to HNHM (vauch. publicly not available), 5.vi.2013, col. T. Cserkész, M. Rusin, D. Czaban & G. Sramko, 49.65N 42.72E	seraf1	KP715865	Cserkész et al. 2016
	Archedinskie sands (south), Volgograd Region, Russia, 2n = 24, NF = 46	Two specimens: 49.24N 44.82E			Kovalskaya et al. 2011
	Ilovlya, Volgograd Region, Russia, 2n = 24, NF = 46	One specimen: 49.25N 44.12E			Kovalskaya et al. 2011
	Yamskaya steppe, Belgorod Region, Russia, 2n = 21–22, NF = 29–31	At least seven specimens: one stored in ZMMU S-178461, col. Yu. Kovalskaya, 2002	yam2 yam3	MK758095 MK758096	Kovalskaya et al. 2011 Lebedev et al. 2020
		Three specimens: col. T. Cserkész, M. Rusin, D. Czaban & G. Sramko, 13–14.vi.2013, 51.187N 37.637E*	yam1	KP715869	Cserkész et al. 2016
	Aidar river, Belgorod Region, Russia, 2n = 16–18, NF = 28	Two specimens stored in ZMMU S-177985 and S-178332, 2001, col. Yu. Kovalskaya, 49.89N 38.89E			Kovalskaya et al. 2011
		One specimen, 49.89N 38.89E			Oparin et al. 2001

Species	Population	Sampling data	ID on Fig.	GenBank Acc.No.	References
	Oskol River, Belgorod Region (probably same as Yamskaya Steppe?)	Three specimens: ZMMU S-174761–174763, col. Yu. Kovalskaya, 2001, N? E?			
	Stenki Izgorya, Novooskolskiy District, Belgorod Region, Russia, 2n = 22, NF = 30	Three specimens: 50.69N 37.85E			Kovalskaya et al. 2011
	Krasnogorovka, Voronezh Region, Russia, 2n = 18, NF = 28	One specimen: viii–ix.1996, 49.97N 40.8E			Kovalskaya et al. 2000
	Barkalovka, Kursk Region, Russia, 2n = 19–20, NF = 29–30	Two specimens: 51.558N 37.645E			Baskevich et al. 2011
	Bukreevy Barmy, Kursk Region, 2n = 19–20, NF = 28–29	Three specimens: 51.503N 37.347E			Baskevich et al. 2011
	Streletzkaya steppe, Kursk Region, Russia, 2n = 18–20, NF = 28–30	Six specimens: 51.58N 36.12E			Sokolov et al. 1986
	Triokhizbenka Sands, Lugansk Region, Ukraine, karyotype = unknown	Five specimens: 1 specimen taken to HNHM (vauch. publicly not available), col. T. Cserkész, M. Rusin, D. Czaban & G. Sramko, 1–2.vi.2013, 48.793N 38.956E	lug1	KP715864	Cserkész et al. 2016
		Two specimens: col. V. Timoshenkov 26.iv.2012, 1.x.2012, 48.774N 38.948E			Timoshenkov 2018
	Streltsovskaya steppe, Lugansk region, Ukraine, 2n = 17, NF = unknown	One specimen: col. A. Kondratenko, 1998, 49.29N 40.08E			Zagorodniuk & Kondratenko 2000
		NMNHU 13985, col. A. Kondratenko, 18.vii.1988 NMNHU 14389, 19.v.1991, col. A. Kondratenko NMNHU 14390, col. V. Timoshenkov & A. Kondratenko, 21.v.1991 NMNHU 14391, col. V. Timoshenkov & A. Kondratenko, 18.vii.1988 NMNHU 2700, col. G. Modin, 8.v.1951			Shevchenko & Zolotukhina 2005
		ZMKNU 3415, col. G. Modin, 6.vii.1956			
Sicista severtzovi	Kamennaya Steppe, Voronezh Region, Russia, karyotype = unknown	Holotype ZMMU S-26104, col. S. Obolenskiy, 22.vii.1921, 51.04N 40.72E			
	Krasnoe, Novohkoperskiy district, Voronezh Region, Russia, 2n = 26, NF = 48	Five specimens: ZMMU S-182605-182609, col. Yu. Kovalskaya, 16–17.v.2007, 51.15N 41.47E		MK758097, MK758098	Kovalskaya et al. 2011 Lebedev et al. 2020
Sicista subtilis	Grachi, Yenotaevskiy District, Astrakhan Region, Russia, karyotype = unknown	Two specimens: ZMMU S-197171–197172, col. G. Ryurikov & N. Poplavskaya, 11.vii.2016, 47.827N 46.234E	astrakhan	KY967417	Rusin et al. 2018
	Ilovlya, Volgograd Region, Russia, Karyotype = unknown	Five specimens: One taken to HNHM (vauch. publicly not available), col. T. Cserkész, M. Rusin, D. Czaban & G. Sramko, 7.vi.2013, 49.23N 44.12E		KP715866	Cserkész et al. 2016
	Kalach Sands, Volgograd Region, Russia, 2n = 24, NF = 44	One specimen, viii–ix.1996, 48.77N 43.51E** One specimen, col. V. Stakheev, 29.iv.2021 48.840N 43.615E, skull transferred to ZMMU			Kovalskaya et al. 2000 V. Stakheev, pers. comm. 2021
	Kamyshin, Volgograd Region, Russia, 2n = 24, NF = 41	One specimen, date not specified, 49.92N 45.23E			Kovalskaya et al. 2011
		One specimen taken to HNHM (vauch. publicly not available), col. T. Cserkész, M. Rusin, D. Czaban & G. Sramko, 8.vi.2013, 49.92N 45.23E			Cserkész et al. 2016
	Manych, Rostov Region, Russia, karyotype = unknown	Three specimen: ZMMU S-197470–197472, col. M. Rusin & N. Nedyalkov, 18–19.v.2016, 46.94N 43.02E		MK758101 MK758102	Lebedev et al. 2020
	Tuva, Russia, karyotype = unknown	One specimen: ZMMU S-188542; col. A. Surov, 10.viii.2010, 50.57N 95.06E	tuva	KY967415	Rusin et al. 2018
Sicista lorigera	Khomutovskaya steppe, Donetsk Region, Ukraine, 2n = 26, NF = 48	Two specimens, 47.29N 38.18E			Sokolov et al. 1986
	Borisovka, Ostrasyevy Yary, Belgorod Region, Russia, 2n = 26, NF = 48	Six specimens: col. T. Cserkész, M. Rusin, D. Czaban & G. Sramko, 12.vi.2013, 50.560N 36.058E		KP715877	Cserkész et al. 2016

Species	Population	Sampling data	ID on Fig.	GenBank Acc.No.	References
	Provalskaya Steppe, Lugansk Region, Ukraine, 2n = 26, NF = unknown	One specimen, col. A. Kondratenko, 1999, 48.15N 39.89E			Zagorodniuk &Kondratenko 2000
		Eight specimens: NMNHU 13994, col. A. Kondratenko, 25.v.1997 NMNHU 11322–11324, col. V. Marochkina & V. Timoshenkov, 7.v.1997 NMNHU 11396, col. V. Timoshenkov, 9.v.1988 NMNHU 11986–11988, col. A. Kondratenko, 13.viii.1998			Shevchenko & Zolotukhina 2005
Sicista sp.	Taganrog, Rostov Region, Russia, Karyotype = unknown	One specimen: NMNHU 14033, col. G. Guliy, 18.xi.1994, 47.3N 38.9E			Shevchenko & Zolotukhina 2005
	Artemovsk (Bakhmut), Donetsk Region, Ukraine, Karyotype = unknown	Four specimens: NMNHU 12373 14. ix.1960, col. R. Skobichevskiy; NMNHU 9987–9989, col. S. Valkh, 14.vi.1928, 11.v.1928 and 20.v.1929, 48.6N 38.0E			Shevchenko & Zolotukhina 2005
	Novo-Vodolazhskiy District, Kharkiv Region, Ukraine, Karyotype = unknown	One specimen: NMNHU 10699, col. Rudinskiy, 21.iv.1934, 49.6N 35.9E			Shevchenko & Zolotukhina 2005
	Malinovka, Kharkiv Region, Ukraine, Karyotype = unknown	One specimen: NMNHU 9990, col. N. Yumatov, 30.vii.1947, 47.8N 36.7E			Shevchenko & Zolotukhina 2005

Note: * in the original publication (Cserkész et al. 2016), incorrect coordinates were given; here, corrected data are provided; ** we believe that in the original publication (Kovalskaya et al. 2011), coordinates are given with error, therefore we put coordinates which better suit the text description of the locality.

Table 2. Characteristics of cytb gene sequences of *Sicista cimlanica* populations.

Population	*N*	N_{hapl}	N_{uniq}	*π* (*SD*)	*h* (*SD*)	Tajima's *D*, *P*	Fu's *Fs*, *P*
Tsimla sands (type locality)	11	9	8	0.00528 (0.00091)	0.945 (0.066)	-1.26741, N/s	-2.262, N/s
The other four populations	6	6	6	0.00938 (0.00147)	1.000 (0.096)	-0.14620, N/s	-0.917, N.s.
Total	**17**	**15**	**14**	**0.00875 (0.00098)**	**0.978 (0.031)**	**-1.35731,** N/s	**-4.924,** N/s

N—the number of assayed animals | N_{hapl}—number of found haplotypes | N_{uniq}—number of unique haplotypes | *π*—nucleotide diversity (averaged over loci) | *h*—haplotype diversity | *SD*—standard deviation | tests of selective neutrality: Tajima's *D* and Fu's Fs; N/s—Not significant, P >0.10.

DISCUSSION

Species distribution and phylogeography

The Tsimlyansk Birch Mouse represents a species with a restricted distribution area, though the exact limits of its range are not yet fully understood. Birch mice, in general, tend to have fragmented distributions occupying narrow species-specific landscapes. While most *Sicista* dwell in mesophyte habitats, *S. subtilis* s.l. is unique in its adaptations to dry environments (Lebedev et al. 2019). Tsimlyansk Birch Mice are no exception within the *S. subtilis* group and are found mostly in dry steppe grasslands. The majority of the known populations occur in psammophyte (sandy) steppes.

These sandy areas were formed as a result of flooding by melting glaciers during the Pleistocene. At least four layers of deposits were created, corresponding to glacier maximums in the Pleistocene: Don 650 kya, Oka 450 kya, Moscow 150 kya and Valdai 20 kya (Brylev 2008). Valdai (last glacial maximum) deposits are the least represented, probably because this glaciation was the weakest in Eastern Europe and had little effect on the region (Brylev 2008). The regular flooding of large areas could have affected both past and present distribution, with regular isolations and local extinctions. The sandy areas form narrow clusters of optimal habitats for *S. cimlanica*, but often they are detached from each other by tributary rivers. Some populations known to exist in other landscapes (from Belgorod and north of Lugansk Regions) are associated with drier vegetation. Nonetheless, the large transformation of natural habitats within most parts of the range of *S. cimlanica* have likely led to increased and widespread isolation and, to the best of our knowledge, this species rapidly declines in human-transformed habitats (e.g., where there is agriculture, settlements, artificial tree-plantations, etc.),

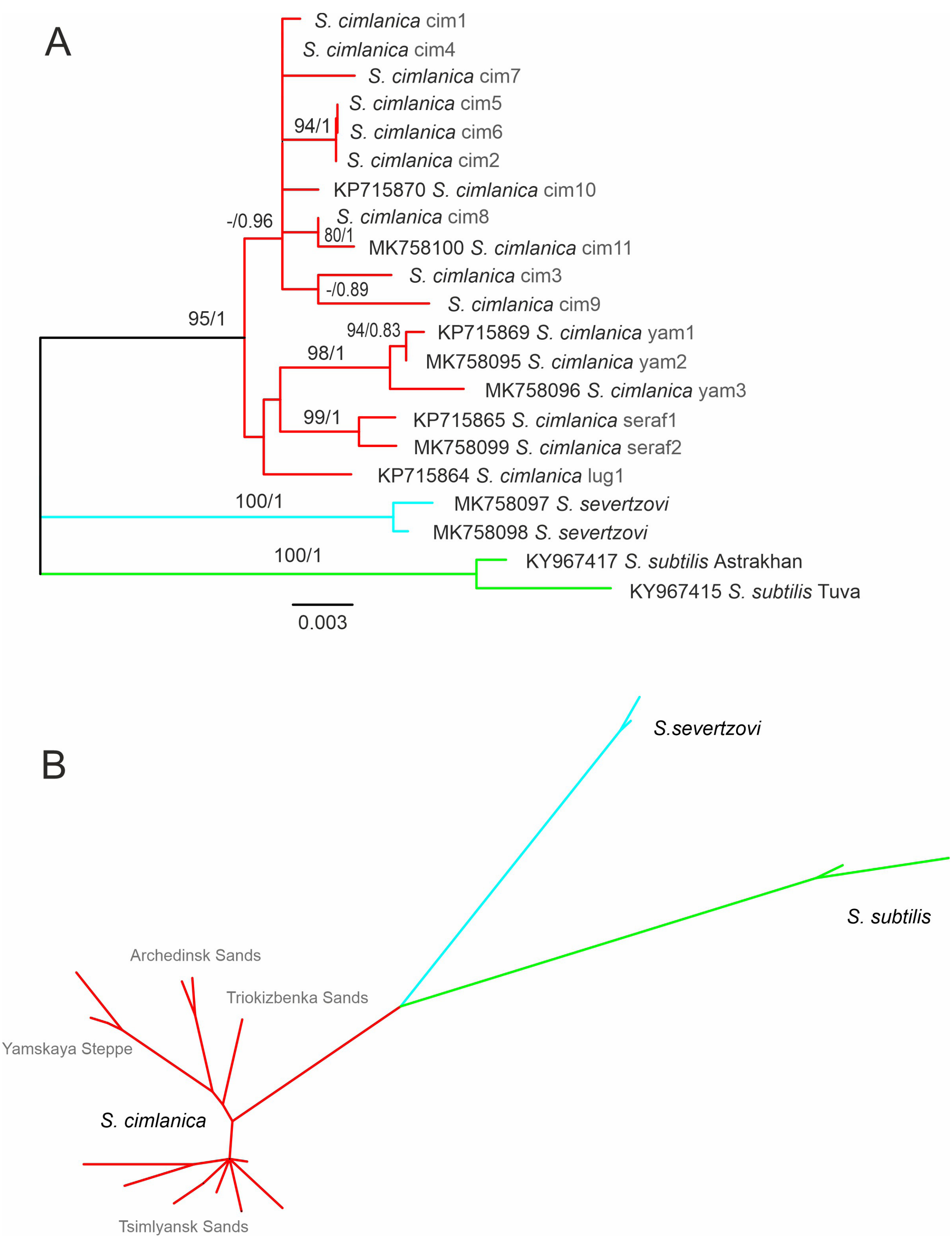

Figure 2. Inter and intraspecific relationships of *Sicista cimlanica* with closest OTUs reconstructed from the cytb gene: A—Unrooted maximum likelihood tree. Values at nodes represent ML bootstrap support from 1,000 replications / Bayesian posterior probabilities. Bootstrap support is only shown for the values exceeding 70%. Sample names are given in Table 1 | B— Radial layout indicating main lineages.

Figure 3. Putative species range of *S. cimlanica* and sampled localities (original data, GenBank sequences and literature data). Sample names correspond to Table 1. Larger red circles represent genotyped populations of *S. cimlanica*, smaller circles — population not genotyped. Dots within populations indicate karyotyped populations. Red star — type locality of *S. cimlanica*. White squares indicate verified populations of *S. lorigera*. Green diamonds — verified populations of *S. subtilis*. Cyan star — *S. severtzovi*.

eventually completely disappearing.

The natural range of *S. cimlanica* may be best described as lying between the Don and Seversky Donets Rivers (Figure 4). All verifiable localities of this species are summarized in Table 1. The southern distribution of this species is most likely limited by the Don River. Consensus on the western border is still lacking. Earlier, it was suggested that the distribution of *S. severtzovi* s.l. expands as far west as the Kyiv Region (Shenbrot et al. 1995). Conversely, further studies have not supported this hypothesis: few known localities from the Lugansk and Belgorod Regions suggest that the border is close to the Seversky Donets River (but does not necessarily follow it). *S. cimlanica* is found only from the left bank of the Seversky Donets (Zagorodnyuk & Kondartenko 2000; Kovalskaya et al. 2011; Cserkész et al. 2016), while on the right bank, only *S. lorigera* is known (Kovalskaya et al. 2011; Lebedev et al. 2020; Zagorodnyuk & Kondartenko 2000).

The northern border remains unstudied, though it is known to occur in the Kursk Region (Sokolov et al. 1986; Baskevich et al. 2011). The eastern border most likely follows the right bank of the Don River in the Voronezh Region. Further to the south in Volgograd Region, *S. cimlanica* crosses the Don River and can be found on Archedinsk and Alekseevski Sands (Kovalskaya et al. 2011; Cserkész et al. 2016). How far they infiltrate the left bank of the Don River remains unknown. In Ilovlya Sands, Kovalskaya et al. (2011) reported karyotypes that are now attributed to *S. cimlanica* ('S.sp 1'), while Cserkész et al. (2016), based on cytb sequences, identified animals from this locality as *S. subtilis* s.str. These conflicting results could relate to either sympatry or even hybridization (either recent or ancient) of two species in Ilovlya Sands, though more sampling in that region is needed to determine the true nature of this case.

There are several old records of birch mice that cannot be unambiguously attributed to any species at the current stage of knowledge. They are labeled as *Sicista* sp. in Table 1. Yet, it is rather likely that the population from Malinovka (Kharkiv Region) belonged to *S. cimlanica*, while populations from Taganrog, Bakhmut and Novo-Vodolazhsk most likely belonged to *S. lorigera*. This assumption requires further testing based on genetic or karyological markers.

Due to limited sampling, the phylogeographic structure within the above- described species range cannot be explained thoroughly. At present, the birch mice from Tsimla Sands, Yamskaya Steppe, Trokhizbenka Sands, and Archedinsk Sands were assumed each form their own branch, though none could be named as an ancestral population, as the number of substitutions from each branch to the potential ancestor is equal (Figure 1). Further sampling covering all known populations as well as searching for new populations, especially at the central part of the species range, could answer the questions concerning the phylogeography of *S. cimlanica*.

Genetic diversity in the type locality

Since almost all examined individuals had unique haplotypes (Figure 3), the haplotype diversity (0.98 ± 0.03) was close to its maximum value ≈ of 1. In contrast, the average nucleotide variability for the studied mtDNA region was not high (0.9 ± 0.01%).

To reconstruct the processes of the modern species, range formation, it is necessary to collect more data for testing the hypothesis of sudden population expansion and computer modeling of historical demography processes.

Implications for conservation

The conservation of genetically complex groups of mammals with narrow distributions requires more sophisticated approaches (Csorba et al. 2015). Conservation efforts should focus on below-species level for effectively preserving the breadth of persisting genetic diversity (Garner et al. 2005). Species groups of so-called ‘microspecies’ often suffer from their wider-species concepts, as one superspecies normally has a wide distribution range with multiple populations. This can result in recognising such superspecies as facing relatively low levels of risk, thus resulting in listings of ‘Least Concern’ on the IUCN Red List. Each microspecies within these groups often has a different conservation status and can be much more threatened (Csorba et al. 2015). *S. cimlanica* is an example of such ‘microspecies’ requiring a specialized approach for its conservation.

Despite intensive studies of cytogenetic aspects, *S. cimlanica* remains one of the most poorly-documented taxa in Europe. Only approximately 14–15 populations have been recorded (Table 1) in the past 30 years. Most of these populations are strongly isolated from one another, lying within protected areas such as Tsimlyansk Reserve, Yamskaya Steppe Reserve, Triokhizbenka Sands Reserve, and Central-Chernozem Reserve. In the Streltsovskaya Steppe (Lugansk Region, Ukraine), the Tsimlyansk Birch Mouse was last recorded in 1999 (Zagorodniuk & Kondratenko 2000), and has not been found since, despite intensive small mammal surveys and birch mice monitoring (Mikhail Rusin’s original data 2018). This may indicate that small, isolated populations are under threat of extinction even within protected areas. Relatively large populations of *S. cimlanica* are recorded only from Tsimlyansk and Archedinsk sands in the Rostov and Volgograd regions of Russia.

Grasslands – such as those within which this species is found – are among the most transformed ecosystems on earth, though one receiving the poorest level of conservation attention (Hoekstra et al. 2005; Carbutt et al. 2017). Nevertheless, grasslands represent some of the largest biodiversity hotspots on the planet (Habel et al. 2013). High diversity of the genus *Sicista* within limited grassland areas (i.e., the Eastern European Steppe, and especially the Middle Don area) is not surprising if compared to the diversity of plants and other taxa in the same region. Since the *S. subtilis* species group is highly associated with the threatened steppe biome, there is an argument that all members of the group require conservation focus. Until recently, *S. cimlanica* was omitted as a separate species for conservation work and it wasn’t until 2021 that it was included in the Red Book of Ukraine (Decree of Ministry of Ecology and Natural Resources of Ukraine № 29 from 19.01.2021), where it is listed as an Endangered species with a single active locality in Ukraine. However, most of its range lies in Russia, where it has no official protection or conservation status yet.

On a range-wide level, the IUCN Red List criteria can be applied to determine a conservation status for the Tsimlyansk Birch Mouse. The putative extent of occurrence – defined by the IUCN (2012) as the area contained within the shortest continuous imaginary boundary which can be drawn to encompass areas in which the taxon occurs – of the Tsimlyansk Birch Mouse is approximately 123,000 km^2. The area of occupancy – a metric representing the area of suitable habitat occupied by the taxon – of known populations is unlikely to exceed 4,000 km. Although this species has a somewhat limited and isolated distribution, the measures of its extent of occurrence and area of occupancy are outside of the thresholds that must be met for consideration as threatened under criterion B of the IUCN Red List (IUCN 2012). Following Red List terminology, the species does meet two of three required conditions of criterion B: (1) its range is severely fragmented and (2) there has been an observed reduction in the number of populations potentially relating to the transformation of steppe habitat. Therefore, on a global scale, this species can be considered Near Threatened, with a high risk of becoming Vulnerable in the future. This implication has already been adopted in the IUCN Red List (Rusin 2024a).

All known populations would benefit from the conservation of habitats. Thus far, there are no data on how management for birch mice in isolated populations could assist their survival. In any case, the example of Streltsovskaya Steppe, where *S. cimlanica* likely has gone extinct, raises questions regarding the survival of such small, isolated populations. Moreover, the species has suffered from the war in Ukraine, as some habitats (such as in Triokhizbenka Sands) were turned into battlefields, resulting in extensive habitat degradation and loss. The current population status of birch mice in the war-torn regions is unknown.

The sibling species, *S. severtzovi* s. str., is also on a steep path of decline. Decades of intensive surveys discovered only a single small population (Yulia Kovalskaya, pers. comm. 2016). This population, described in Kovalskaya et al. (2011), was checked in 2014, and no birch mice were recorded, with part of the habitat having been destroyed for pig farm construction (T. Cserkész, M. Rusin, D. Csaban, and G. Sramkó, unpublished data). Following IUCN Red List criteria (IUCN 2012), *S. severtzovi* should fall within the Critically Endangered category under criterion B (Rusin 2024b). There are no populations in captivity of this species, which means there is already a high risk of *S. severtzovi* going, or having gone, Extinct. Conservation and research actions for this species are clearly urgently needed.

In summary, a middle Don area is a region of high genetic variability for birch mice with two local endemics — *S. cimlanica* and *S. severtzovi*. Hypothetically, both species evolved in the region during the middle Pleistocene as a result of isolation during various glaciation maximums. Both species remain poorly known, with few active populations known. Conservationists and zoologists should be encouraged to conduct extensive surveys of both *S. cimlanica* and *S. severtzovi*.

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Journal of Threatened Taxa is indexed/abstracted in Bibliography of Systematic Mycology, Biological Abstracts, BIOSIS Previews, CAB Abstracts, EBSCO, Google Scholar, Index Copernicus, Index Fungorum, JournalSeek, National Academy of Agricultural Sciences, NewJour, OCLC WorldCat, SCOPUS, Stanford University Libraries, Virtual Library of Biology, Zoological Records.

NAAS rating (India) 5.64

Print copies of the Journal are available at cost. Write to:
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OPEN ACCESS

www.threatenedtaxa.org

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

May 2025 | Vol. 17 | No. 5 | Pages: 26939–27034
Date of Publication: 26 May 2025 (Online & Print)
DOI: 10.11609/jott.2025.17.5.26939-27034

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