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Sequence analysis of the *cytb* gene of *Mesocestoides* Vaillant, 1863 tetrathyridia from small mammals of the Russian Far East

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ABSTRACT

This paper continues a series of articles concerning the molecular genetic analysis of widespread helminths – cestodes of the genus *Mesocestoides* Vaillant, 1863, parasitising (at the metacestode stage) small mammals. In this study, we examine the genetic diversity of *Mesocestoides* spp. from micromammals of the Russian Far East using the *cytb* gene as an example. Polymorphism of the nucleotide sequence of the *cytochrome b* gene and the amino acid sequence of the encoded polypeptide was detected for the first time in *Mesocestoides* spp. parasitising hosts of different genera and species inhabiting this region. Two species of *Mesocestoides* spp. were identified that were not related to the genetically confirmed species of the genus. One of them was found only in *Micromys minutus* (Pallas, 1771), obtained near Georgievka village in Khabarovsk Territory. The second species is represented by nine individuals from geographically distant locations, in which two genetic sublines and 11 nucleotide differences in the *cytb* gene sequence were found. Molecular diversity indices show a high level of polymorphism in the nucleotide sequence of this gene in the gene pool of the studied species of *Mesocestoides*. In addition, the presence of three isoforms of the cytochrome b polypeptide was established. Analysis of amino acid substitutions in these polypeptide isoforms and the polypeptide from the *M. minutus* sample also indicates that the latter belongs to a separate species.

Key words: *cytb* gene, genetic diversity, *Mesocestoides*, polypeptide, tetrathyridium

Анализ последовательности гена *cytb* тетратиридиев *Mesocestoides* Vaillant, 1863 от мелких млекопитающих Дальнего Востока России

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РЕЗЮМЕ

Данная работа продолжает серию статей, посвященных молекулярно-генетическому анализу широко распространенных гельминтов – цестод рода *Mesocestoides*, паразитирующих (на стадии метацестоды) у мелких млекопитающих. В этой работе мы изучаем генетическое разнообразие *Mesocestoides* spp. от микромаммалий Дальнего Востока России на примере гена *cytb*. Впервые у *Mesocestoides* spp., паразитирующих у хозяев разных родов и видов, обитающих в этом регионе, выявлен полиморфизм нуклеотид-

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ной последовательности гена цитохрома *b* и аминокислотной последовательности кодируемого полипептида. Были выявлены два вида *Mesocestoides* spp., не относящиеся к генетически подтвержденным видам рода. Один из них обнаружен только у *Micromys minutus* (Pallas, 1771), добытого в районе села Георгиевка Хабаровского края. Второй вид представлен девятью особями из географически далеких локаций, у которых обнаружено две генетические сублинии и 11 нуклеотидных различий в последовательности гена *cytb*. Индексы молекулярного разнообразия показывают высокий уровень полиморфизма нуклеотидной последовательности этого гена в генофонде изученного вида *Mesocestoides* sp. Кроме того, было установлено наличие трех изоформ полипептида цитохрома b. Анализ аминокислотных замен в этих изоформах полипептида и в полипептиде из образца от *M. minutus* также указывает на принадлежность последнего к отдельному виду.

Ключевые слова: ген *cytb*, генетическое разнообразие, *Mesocestoides*, полипептид, тетратириди

INTRODUCTION

This paper continues a series of articles concerning the molecular genetic analysis of tetrathyridia (metacestode stage) of the genus *Mesocestoides* Vaillant, 1863, parasitising small mammals of the Russian Far East (Pospekhova et al. 2018), as well as those of Alaska (Pospekhova et al. 2023, 2024). The medical and veterinary significance of this cestode genus has led to a large number of publications on the biology (life cycles, intermediate and definitive hosts together with host specificity and species identification) of *Mesocestoides* (Padgett and Boyce 2005; Literák et al. 2006; Hrčkova et al. 2011; Zaleśny and Hildebrand 2012; Skirnisson et al. 2016; Bajer et al. 2020). Most of these studies were carried out using molecular genetic methods and contain elements of phylogeography, molecular phylogeny and systematics. Phylogenetic analysis of the *18S rDNA* data recovered a monophyletic group composed of all samples of *Mesocestoides* spp. from dogs, coyotes and tetrathyridia representing laboratory isolates, while initial analysis of the *ITS 2* data resolved three clades within these *Mesocestoides* (Crosbie et al. 2000). Padgett et al. (2005) reported that clades A, B, and C of *Mesocestoides* are separate species and provided evidence that clade B and *Mesocestoides vogae* Etges, 1991 are conspecific. Many molecular genetic studies of *Mesocestoides* are the only way to identify phylogenetic relationships between parasites from different hosts and different geographic locations if the material used is embryonic or larval stages, which do not have clear diagnostic features (Pospekhova et al. 2018; Ulziijargal et al. 2020; Pospekhova et al. 2023, 2024).

The variability of the *cytb* nucleotide sequence is actively used in population genetics and molecular phylogeography of various species of animals, in particular parasitic flatworms (Yang et al. 2013; Zhong et al. 2014; Zhang et al. 2018; Kołodziej-Sobocińska et al. 2019; Alvi et al. 2023). However, the nucleotide sequence of the *cytb* gene in *Mesocestoides* remains poorly understood. In addition to the examined nucleotide sequences of *cytb* of *Mesocestoides* spp., the GenBank database contains three complete mitochondrial genomes of representatives of this genus. Two of them were obtained from laboratory cultures of tetrathyridia: *M. vogae* Etges, 1991 (Nakao, unpublished) and *M. corti* (Hoeppli, 1925) (Kikuchi et al., unpublished), which probably represent a single species (Etges 1991). The third, *Mesocestoides* sp., was isolated from tetrathyridia obtained from naturally infected *Neodon irene* (Thomas, 1911) (Wu et al. 2022).

We determined the sequences of the gene fragment and polypeptide of cytochrome b in *Mesocestoides* spp. tetrathyridia from six species of small mammals of the Russian Far East for a preliminary analysis of the relationships between the haplotypes of the studied *Mesocestoides* with each other, as well as with the data available in GenBank.

MATERIAL AND METHODS

The material was obtained as a result of theriological studies of rodents and shrews conducted by N.E. Dokuchaev in 2002–2019; the locations of micromammal capture are shown in Fig. 1 and Table 1. It should be noted that all studied species of shrews were infected with tetrathyridia. At the same time, there were exceptions among rodents: in voles of the genus *Alexandromys* Ognev, 1914 [*A. oeconomus* (Pallas, 1776) and *A. shantaricus* (Ognev, 1929)]

Fig. 1. Capture locations for intermediate hosts of *Mesocestoides*. The numbers correspond to the sample numbers from Table 1.

they were not found even in locations with high tetrathyridia infestation of other micromammals. The localisation of tetrathyridia is somewhat different in different hosts. According to our observations, in shrews they are more often localised in the liver and large lymphoid organs, while in rodents they are found in the body cavity.

Some *Mesocestoides* host names that we previously submitted to GenBank [*Myodes rutilus* (Pallas, 1779) and *M. rufocanus* (Sundevall, 1846)] do not comply with the current classification system (Kryštufek and Shenbrot 2022), so we use the names *Clethrionomys rutilus* and *Craseomys rufocanus* in the text, respectively.

Before DNA extraction from the alcohol-fixed material, tissue-localised tetrathyridia were freed from cysts and washed with alcohol of the same concentration (70 or 96%).

Isolation, purification of total DNA, amplification of nucleotide sequences, purification of polymerase chain reaction products, sequencing of the nucleotide sequence of the *cytb* gene, and mapping of this mtDNA fragment were performed using previously described methods (Pospekhova et al. 2018).

To determine the phylogenetic relationships of the *cytb* gene haplotypes of *Mesocestoides* spp. found in our research, all available information on the structure of the nucleotide sequence of this gene in *M. vogae* LC102498 (Nakao, unpublished), *M. corti* AP017667 (Kikuchi et al. unpublished) and *Mesocestoides* sp. NC_061204 (Wu et al. 2022) was retrieved from GenBank. The *cytb* gene sequence NC000928 of *Echinococcus multilocularis* (Nakao et al. 2002) was used as an outgroup. The abbreviation Mesctb was assigned to the *cytb* haplotypes found in the examined *Mesocestoides* spp.

Genetic data analysis was performed using the MEGA 10.0.2.74 (Tamura et al. 2013), ARLEQUIN 3.5 (Excoffier et al. 2005), and Network 4.5.1.0

Table 1. Samples of *Mesocestoides* tetrathyridia used in this work with host capture sites, sample and haplotype numbers, and GenBank numbers. Sample No. 11 (MG845686 from *Clethrionomys rutilus* from Magadan Province) was taken from our previous article (Pospekhova et al. 2018).

Sample number	Haplotypes_ haplogroups	Host species	Sample collection location	GenBank number
11	Mescytb**2**_1	*Clethrionomys rutilus* (Pallas, 1779)	Kulu settlement, Magadan Province	MG845686
12	Mescytb**3**_1	*Sorex isodon* Turov, 1924	Bolshoy Shantar Island, Khabarovsk Territory	MK904832
13	Mescytb**1**_1	*Craseomys rufocanus* (Sundevall, 1846)	Bolshoy Shantar Island, Khabarovsk Territory	MK904833
14	Mescytb**5**_1	*Craseomys rufocanus*	Kulu settlement, Magadan Province	MK904834
28	Mescytb**4**_2	*Craseomys rufocanus*	Umara River floodplain, Magadan Province	MK904835
47	Mescytb**8**	*Micromys minutus* (Pallas, 1771)	Georgievka village, Khabarovsk Territory	ON855353
48	Mescytb**4**_2	*Craseomys rufocanus*	Umara riwer floodplain, Magadan Province	OP221671
50	Mescytb**4**_2	*Sorex caecutiens* Laxmann, 1788	"Contact" station, Magadan Province	OP221672
52	Mescytb**6**_1	*Sorex caecutiens*	Yakutsk, Republic of Sakha (Yakutia)	OP221673
53	Mescytb**7**_2	*Sorex tundrensis* Merriam, 1900	Yakutsk, Republic of Sakha (Yakutia)	OP221674

(Bandelt et al. 1999) software packages. The sites of synonymous/non-synonymous nucleotide substitutions (ns) and amino acid sequences of the identified cytochrome b polypeptide (Cytb) isoforms were determined in the MEGA program using the mitochondrial genome code for flatworms. A phylogenetic tree was constructed for cluster analysis of haplotypes using the maximum likelihood (ML) method. The stability of branching nodes was assessed using the bootstrap method (500 iterations). Median haplotype networks were constructed using the Network 4.5.1.0 software package (Bandelt et al. 1999).

A one-letter notation of amino acids recommended by IUPAC was used in the study. The abbreviation Fmesctb was assigned to the isoforms of the cytochrome b polypeptide found in the examined *Mesocestoides* sp. To assess the adaptivity of changes in the *cytb* gene, the TreeSAAP software was used, which determines the selective effects during cladogenesis on 31 structural and biochemical physicochemical properties of amino acids using the z-test (Woolley et al. 2003). In the TreeSAAP analysis, an unrooted ML tree was used, constructed using the Hasegawa-Kishino-Yano (HKY) model with 1000 bootstrap iterations. In addition, the methods of Sneath, Bachinsky and Grantham (Butvilovsky et al. 2009) were used to evaluate the identified amino acid substitutions (aas) in the Cytb polypeptide sequence of *Mesocestoides* sp.

RESULTS AND DISCUSSION

Nucleotide sequence characteristics of *cytochrome b* gene haplotypes in *Mesocestoides* spp. samples

Previously, we (Pospekhova et al. 2018) determined the nucleotide sequence (GenBank No. MG845686) of the *cytb* gene of the *Mesocestoides* sp. tetrathyridia, which we compared with a similar locus of the mtDNA of *M. corti*, the only species of *Mesocestoides* for which the complete mitochondrial genome had been sequenced at that time (AP017667; Kikuchi et al. unpublished). This paper presents information on the nucleotide sequence of 1082 base pairs (bp) of the *cytb* gene in nine specimens of the *Mesocestoide*s spp. tetrathyridia, extracted from hosts of different genera and species, captured in different localities (Table 1). The determination of nucleotide and amino acid substitutions showed that haplotypes Mesctb1–Mesctb7 belong to one species of *Mesocestoides,* while Mesctb8 belongs to another. In total, Mesctb1–Mesctb7 and Mesctb8 differ by 155 ns and 37 aas. Haplotypes Mesctb1–Mesctb7 differ by 11 ns and form two haplogroups: Mesctb1–Mesctb3, Mesctb5, and Mesctb6 belong to the first, while Mesctb4 and Mesctb7 belong to the second (Fig. 2).

The first haplogroup includes five ns – four transitions in the third position of the codon: Mesctb3_1 (G309A); Mesctb2_1, Mesctb6_1 (C441T); Mesctb5_1 (T768C); Mesctb6_1 (T987C) and one transition

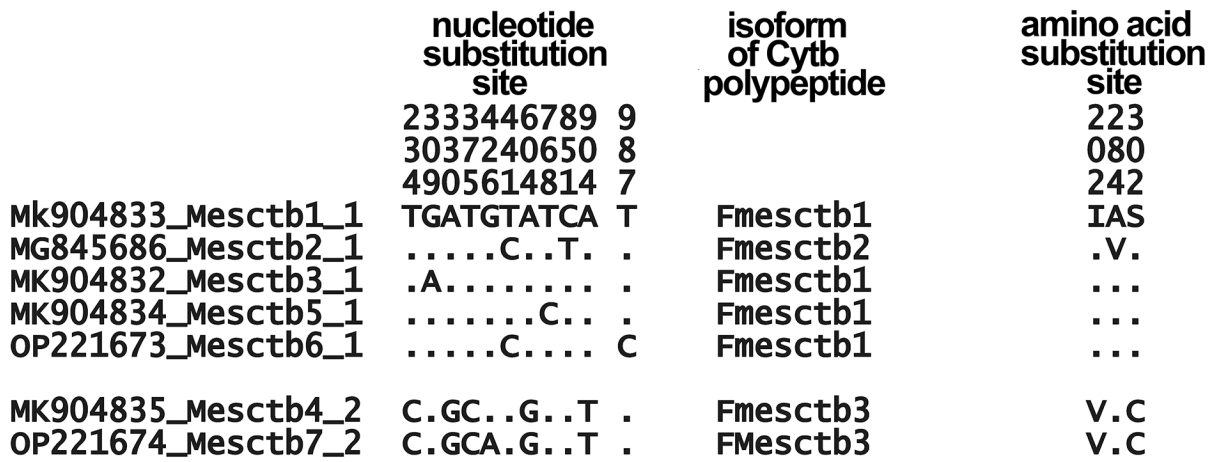

Fig. 2. Mesctb1–Mesctb7 haplotypes of the *cytb* gene of *Mesocestoides* sp. and their corresponding isoforms of cytochrome b polypeptide. Nucleotide substitutions are presented relative to the sequence of the Mesctb1 variant, amino acid substitutions relative to Fmesctb1. Substitution sites are shown from the beginning of the *cytochrome b* gene and polypeptide.

Table 2. Localisation of nucleotide substitutions in codons and indices of molecular diversity of nucleotide sequences of the *cytb* gene of Mesctb1–Mesctb7 *Mesocestoides* sp. haplotypes.

Haplogroup	N	n	K	Proportion of substitution						Molecular diversity indices		
				Transition			Transversion			$\pi \pm sd$	$h \pm sd$	$Pi \pm sd$
				Substitution position in codon								
				1	2	3	1	2	3			
1	5	5	5	–	0.2000	0.8000	–	–	–	0.0039±0.0027	1.0000±0.1265	4.2000±2.5039
2	4	1	2	–	–	1.00	–	–	–	0.0028±0.0022	0.5000±0.2652	3.0000±1.9640
Between 1 and 2	–	5	–	0.2000	–	0.6000	0.2000	–	–	–	–	–
General sample	10	11	7	0.0909	0.0909	0.7273	0.0909	–	–	0.0040±0.0025	0.9167±0.0920	4.2778±2.3380

Note: N – sample size; n – number of substitutions; k – number of haplotypes in the sample; π – nucleotide diversity; *h* – haplotype diversity; *Pi* – average number of pairwise differences between haplotypes; *sd* – standard deviation.

in the second position of the codon: Mesctb2_1 (C851T). Haplotypes of the second group differ by one transition in the third position of the codon G426A. The haplogroups differ from each other by five ns – a transition in the first (G604A), three transitions in the third (T234C, T375C, A330G) and a transversion in the first (T904A) position of the codon. The localisation and proportions of substitutions are shown in Table 2.

The proportion of variable sites in haplogroup 1 is 0.0046, in haplogroup 2 – 0.0009, between haplogroups – 0.0046 and in the total sample – 0.0102 of the total length of the examined nucleotide sequence of the *cytb* gene. The proportion of haplotypes Mesctb1_1–Mesctb3_1 and Mesctb5_1–Mesctb7_2 in the total sample is 0.1111, and that of Mesctb4_2 is 0.3333. The number of transitions is an order of magnitude greater than transversions, which corresponds to the published data (Nei 1987; Nei and Kumar 2000). The degeneracy of the genetic code determines the greatest variability of the third nucleotide in most codons in the translated regions of the gene (Zardoya and Meyer 1996).

The results of the 1:1:8 transition ratio in the total Mesctb1_1–Mesctb7_2 sample obtained in our study approximately correspond to the usual distribution of substitutions in triplets [2:1:9 in the first, second, and third positions, respectively (Hassanin et al. 1998)]. The transversion was found only in the first site of the triplet, which is probably due to the limited sample size. The calculated indices of molecular diversity, in particular, nucleotide diversity (π), haplotype diversity (*h*) and the average number of pairwise differences between haplotypes (*Pi*) (Table 2) indicate a high level of polymorphism of the *cytb* nucleotide sequence in the gene pool of the studied species (Mesctb1–Mesctb7) of *Mesocestoides*.

For comparison, the *cytb* nucleotide sequences of another cyclophyllid cestode, *Echinococcus granulosus* Batsch, 1786 from western China, demonstrate a haplotype diversity of 0.626 (*Mesocestoides* sp. in our study – 0.9167), and a nucleotide diversity of 0.001 (in *Mesocestoides* sp. – 0.004) (Zhong et al. 2014). In *Taenia multiceps* Leske, 1780, when analysing 12 unique haplotypes obtained from GenBank (JX546905–JX546916), the haplotype diversity, as expected, was equal to 1.000(±)0.034; the nucleotide diversity was 0.0033±0.002036 (Hao et al. unpublished).

Phylogenetic relationships of nucleotide sequences of *cytb* gene haplotypes in *Mesocestoides* spp. tetrathyridia

Fig. 3 shows the median network of the Mesctb1–Mesctb7, Mesctb8 haplotypes and the GenBank data on the *cytb* gene nucleotide sequence in the mtDNA of *M. vogae* (LC102498), *M. corti* (AP017667) and *Mesocestoides* sp. from *N. irene* (NC_061204), which is possibly a new species (Wu et al. 2022). The structure of the median network indicates that the *Mesocestoides* spp. Mesctb1–Mesctb7 and Mesctb8 we studied are not *M. vogae* (*M. corti*) or *Mesocestoides* sp. from *N. irene*. Haplotype Mesctb8 differs from Mesctb3 by 146 ns and nucleotide sequences of the synonymous species *M. vogae* and *M. corti* differ from Mesctb3 by 155 ns. The close level of genetic differences between the nucleotide sequences of the Mesctb1–Mesctb7 haplotypes on the one hand and Mesctb8, *M. vogae/M. corti* and *Mesocestoides* sp. NC061204 on the other hand (146, 155 and 180 ns, respectively) shows that the specimen of *Mesocestoides* sp. with the Mesctb8 haplotype belongs to a different species of the genus than the specimens of the Mesctb1–Mesctb7 variants.

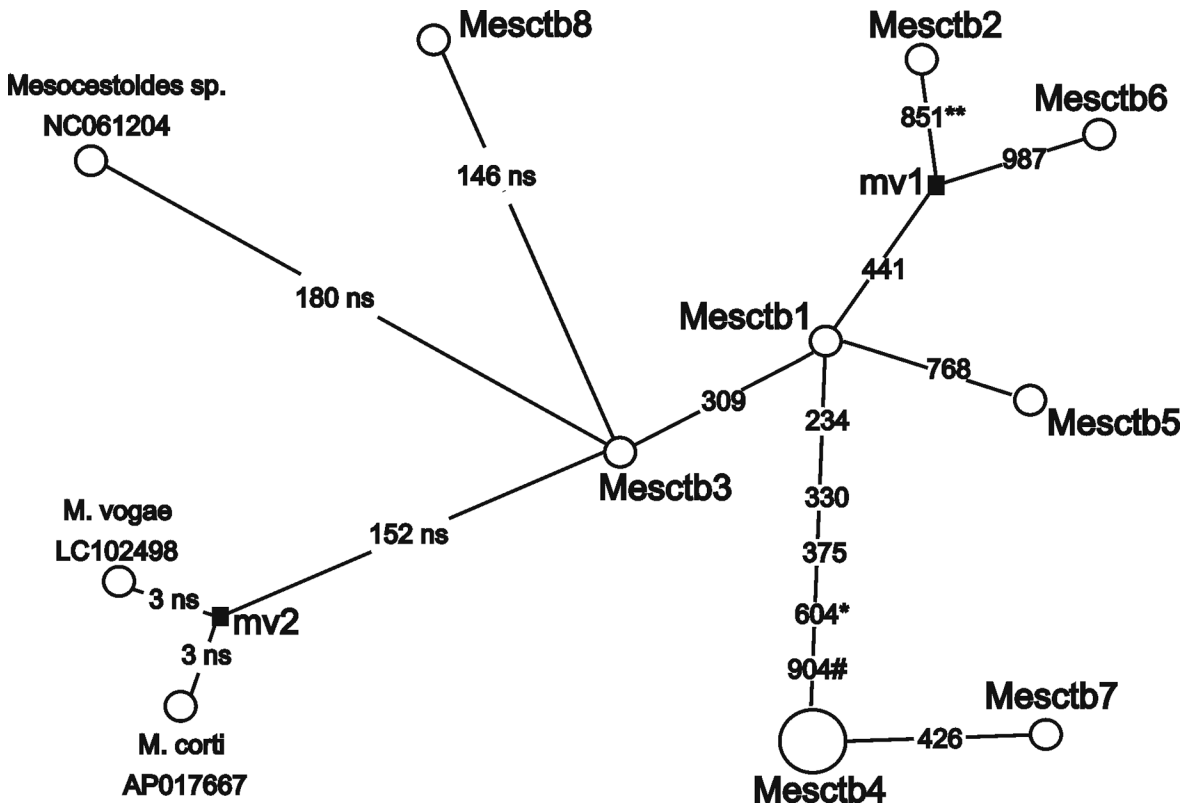

Fig. 3. Median network of the *cytb* gene of *Mesocestoides* spp. haplotypes Mesctb1–Mesctb7 and Mesctb8. *Mesocestoides* sp. NC061204, *M. vogae* and *M. corti* are reference specimens. The size of the circles is proportional to the frequency of the mtDNA variant. * – transition in the 1st codon position, ** – transition in the 2nd codon position, # – transversion in the 1st codon position. The numbers indicate mutation sites from the beginning of the *cytb* gene; mv – median vector.

The haplotype group of *Mesocestoides* sp. Mesctb1–Mesctb7 has a clear radial structure indicating its monophyletic origin. The ancestral haplotype is Mesctb1; the others originated from it through one (Mesctb3 and Mesctb5), two (Mesctb2 and Mesctb6), five (Mesctb4) and six (Mesctb7) mutations. The structure of the median network shows the presence of two subclusters of haplotypes similar in structure: the first includes Mesctb2 and Mesctb6, the second Mesctb4 and Mesctb7. Mesctb4 was found in three hosts inhabiting the territory of the Magadan Province: in two grey red-backed voles and in the Laxmann's shrew. Mesctb7, which is similar to Mesctb4 in nucleotide sequence, was found in the Laxmann's shrew caught in a geographically remote location in Yakutia.

The ML phylogenetic tree (Fig. 4) also indicates the presence of two subclades with a bootstrap support of 99%. Clade 1 contains the Mesctb2 and Mesctb6 subclades with a bootstrap support of 65%. The Mesctb2 haplotype from the first subclade was obtained from the same tetrathyridia sample that was used in the analysis of the nucleotide sequences of the *12S rRNA* gene of *Mesocestoides* spp. (Pospekhova et al. 2018, 2023). The corresponding sequence of the *12S rRNA* gene fragment is deposited in GenBank under the number MG873046. It should be noted that among the sequences of this gene from *Mesocestoides* spp. available in GenBank, there are samples that are completely identical to the fragment MG873046. These identical sequences were obtained from the eggs of *Mesocestoides* spp. from carnivorous mammals of Mongolia: AB787552 from *Canis lupus* Linnaeus, 1758, AB787554 from *Vulpes vulpes* (Linnaeus, 1758), and were initially assigned to the species *M. lineatus* (Goeze, 1782), and subsequently to *Mesocestoides* sp.-1 (Ulziijargal et al. 2020).

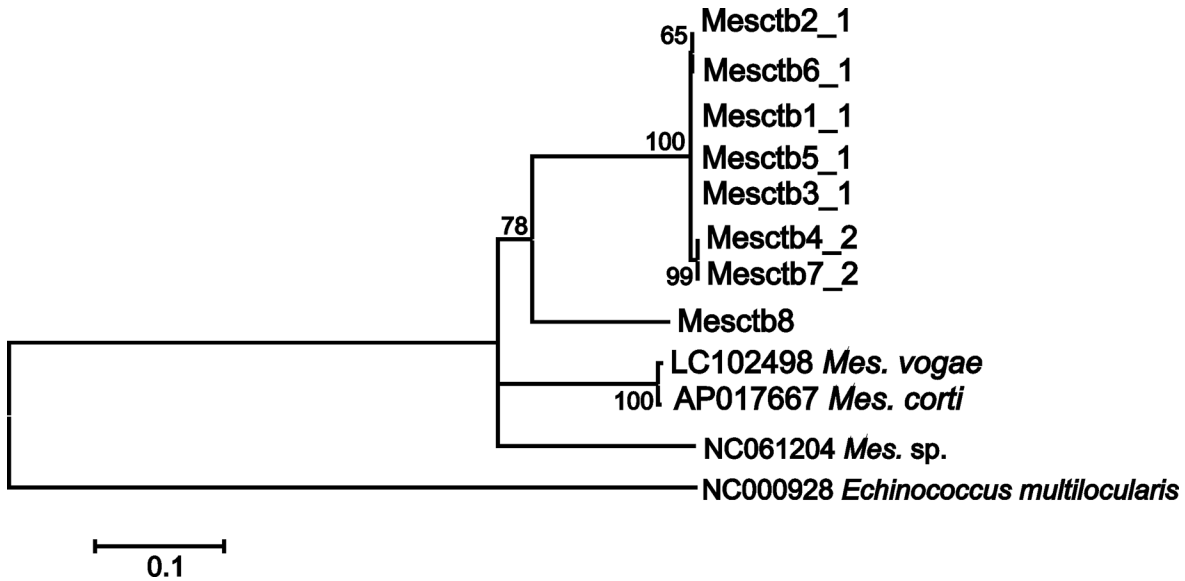

Fig. 4. ML phylogenetic tree constructed based on the variability of nucleotide sequences of the *Mesocestoides cytb* gene. Bootstrap indices (>50%) are indicated at branch nodes. The scale bar represents the number of nucleotide substitutions per site.

The nucleotide sequences of the second branch of haplotypes (Mesctb4 and Mesctb7) include two significant substitutions: transition and transversion in the first position of the codon (Fig. 3). Both of these substitutions lead to a change in the amino acid sequence of the Cytb polypeptide (Fig. 2). Based on the results of the phylogenetic analysis of the nucleotide sequences, it is possible to make an assumption about two genetic sublineages of the studied samples of *Mesocestoides* sp.

The arrangement of branches on the ML phylogenetic tree and the bootstrap support level also indicate species-level genetic differences between the nucleotide sequences of Mesctb1–Mesctb7 and Mesctb8. In addition, the presence of two large clusters should be noted. One includes all the haplotypes (Mesctb1–Mesctb8) identified by us in *Mesocestoides* spp., the other includes *M. vogae* and *M. corti*, and *Mesocestoides* sp. from *N. irene* is located separately. The results indicate the presence of genetically distinct groups of species in the genus *Mesocestoides*.

Of particular interest are tetrathyridia from micromammals from Georgievka village, Khabarovsk Territory. Our previous data (Pospekhova et al. 2023, 2024), as well as the present study, show that the nucleotide sequences of *12S rRNA*, *18S rRNA* and *cytb* gene fragments of *Mesocestoides* sp. samples from this locality form separate haplotypes, and sometimes haplogroups. All the obtained results indicate that tetrathyridia from Georgievka village belong to a separate species.

Characteristics of amino acid sequences of cytochrome b polypeptide isoforms in *Mesocestoides* sp. tetrathyridia

The Fmesctb1 isoform is encoded by Mesctb1 and Mesctb3–Mesctb6, Fmesctb2 by Mesctb2, and Fmesctb3 by Mesctb4 and Mesctb7 (Fig. 2). The transition C851T at the second codon position in the nucleotide sequence of Mesctb2 results in the amino acid substitution A284V and the isoform Fmesctb2. Two nucleotide substitutions lead to the formation of the polypeptide variant Fmesctb3: the transition G604A (V202I) at the first codon position and the transversion T904A (C302S). The data of the analysis on the significance of amino acid substitutions are presented in Table 3.

The T904A# transversion results in the amino acid substitution S302C. The results obtained by the Grantham and Bachinsky methods indicate its radical nature. The significance category calculated in the TreeSAAP analysis software package is 7 at $P < 0.01$. The T904A# nucleotide substitution has a clade-forming nature. It leads to the formation of a branch of the Mesctb4_2 and Mesctb7_2 haplotypes on the ML tree constructed for the TreeSAAP

Table 3. The nature of amino acid substitutions in the isoforms of the cytochrome b polypeptide Fmesctb2 and Fmesctb3, based on different methods (Butvilovsky et al. 2009).

Haplotype	Polypeptide isoform	Substitution		Coefficient		
		nucleotide	amino acid	Grantham >57.9 – K	Bachinsky >12.4 – K	Sneath >0.416 – K
Mesctb2_1	Fmesctb2	C851T**	A284V	68	17	0.675
Mesctb4_2, Mesctb7_2	Fmesctb3	T904A#	S302C	**45**	**4**	0.613
		G604A*	I202V	86	35	0.843

Note: K – conservative nature of the replacement; a radical substitution is highlighted in bold. * – transition in the 1st codon position, ** – transition in the 2nd codon position, # – transversion in the 1st codon position. Nucleotide substitutions are presented relative to the sequence of the Mesctb1 variant; amino acid substitutions are presented relative to Fmesctb1. Substitution sites are shown from the beginning of the *cytochrome b* gene and polypeptide.

analysis with a bootstrap support of 99%. With S302C, the average number of surrounding amino acid residues changes.

The conservative nature of the amino acid substitutions A284V and I202V, as determined by the Grantham, Bachinsky and Sneath methods, is not reliably confirmed by TreeSAAP analysis. Further studies on a larger range of samples are needed to clarify the selective significance of the amino acid substitutions S302C and I202V.

The proportion of identified polypeptide isoforms in the sample is: Fmesctb1 – 0.4444; Fmesctb2 – 0.1111; Fmesctb3 – 0.4444. The most represented isoforms, Fmesctb1 and Fmesctb3, were found in *Mesocestoides* sp. from hosts of different genera and species with different levels of metabolism and living in geographically distant regions. The Fmesctb1 variant was found in *Mesocestoides* sp. from *Sorex isodon* and *Craseomys rufocanus* (Bolshoy Shantar Island, Khabarovsk Territory), *C. rufocanus* (Kolyma River basin), and *Sorex caecutiens* (Yakutia). The Fmesctb3 isoform was found in *C. rufocanus* (the coast of the Sea of Okhotsk), *S. caecutiens* (Kolyma River basin), and *S. tundrensis* (Yakutia). The wide geographic distribution of the Fmesctb1 and Fmesctb3 isoforms might be explained by a variety of reasons and may indirectly indicate their high adaptive significance.

It should be noted that the polypeptide encoded by haplotype Mesctb8 differs from the Fmesctb1–Fmesctb3 isoforms by 37 amino acid substitutions, which also indicates that the samples from small mammals from the village of Georgievka belong to a separate species.

At present, only five species of the genus *Mesocestoides* have been confirmed by both morphological and molecular genetic data: *M. lineatus* (Goeze, 1782), *M. litteratus* (Batsch, 1786), *M. canislagopodis* (Rudolphi, 1810) (Krabbe, 1865), *M. corti* (Hoeppli, 1925) (= *M. vogae* Etges, 1991) and *M. melesi* Yanchev et Petrov, 1985 (Yanchev, 1986; Gubányi and Eszterbauer, 1998; Nickisch-Rosenegk et al. 1999; Padgett and Boyce 2005; Literák et al. 2006; Hrčkova et al. 2011; Skirnisson et al. 2016; Bajer et al. 2020).

Based on our observations and the published data, it can be assumed that several new species of *Mesocestoides* have now been identified that do not correspond to the genetically confirmed species of the genus (Pospekhova et al. 2018, 2023, 2024; Ulziijargal et al. 2020; Wu et al. 2022).

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