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First Molecular Data for the Genus *Kovalius* (Opiliones: Sclerosomatidae: Leiobuninae) and their Phylogenetic Relationships

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Abstract: *Kovalius* (Opiliones: Sclerosomatidae: Leiobuninae) is a small genus of harvestmen described by Tchemeris, 2023. The first description of *Kovalius logunovi* was made from Russia and it was subsequently recorded from Türkiye on the basis of the morphological data. The use of morphological data alone for the identification of taxa and determination of their relationships may lead to limitations and difficulties in some cases. As a result, the use of molecular data in addition to the morphological data in the identification of new taxa and determination of phylogenetic relationships increases the reliability of studies. In this study, the mitochondrial 16S rRNA gene region of the *Kovalius logunovi* species was examined for the first time and a sequence of approximately 408 bp was obtained. Based on these data, the phylogenetic relationships of the species with similar species were revealed.

Keywords: 16S rRNA, *Kovalius logunovi*, phylogenetic tree, Türkiye.

Kovalius (Opiliones: Sclerosomatidae: Leiobuninae) Cinsinin İlk Moleküler Verileri ve Filogenetik İlişkileri

Öz: *Kovalius* (Opiliones: Sclerosomatidae: Leiobuninae) Tchemeris tarafından 2023 yılında tanımlanan küçük bir otbiçen cinsidir. *Kovalius logunovi*'nin ilk tanımı Rusya'dan yapılmış ve daha sonra morfolojik verilere dayanarak Türkiye'den kaydedilmiştir. Taksonların tanımlanması ve ilişkilerinin belirlenmesi için sadece morfolojik verilerin kullanılması bazı durumlarda sınırlamalara ve zorluklara yol açabilir. Sonuç olarak, yeni taksonların tanımlanmasında ve filogenetik ilişkilerin belirlenmesinde morfolojik verilere ek olarak moleküler verilerin de kullanılması çalışmaların güvenilirliğini artırmaktadır. Bu çalışmada *Kovalius logunovi* türünün mitokondriyal 16S rRNA gen bölgesi ilk kez çalışılmış ve yaklaşık 408 bp'lik bir dizi elde edilmiştir. Bu verilere dayanarak türün benzer türlerle olan filogenetik ilişkileri ortaya konmuştur.

Anahtar kelimeler: 16S rRNA, *Kovalius logunovi*, filogenetik ağaç, Türkiye.

1. Introduction

The genus *Kovalius* Tchemeris, 2023 is monotypic genus of the family Sclerosomatidae Simon, 1879 subfamily Leiobuninae Banks, 1893 that is distributed in Russia and Türkiye (Tchemeris, 2023; Kurt, 2024). It has been recognized that only one species (*Kovalius logunovi* Tchemeris, 2023) of the genus *Kovalius* is extant.

The genus *Kovalius* was described for the first time from the Sokolova cave in the NW Caucasus, Russia. In 2024, the genus was recorded from Türkiye for the first time. Furthermore, the genitalia of female individuals were also described for the first time. Both studies were on the basis of morphological data and no data was available on the molecular characteristics of the genus (Tchemeris, 2023; Kurt, 2024).

Mitochondrial DNA has become the most widely used molecular marker in animal systematics in recent years, playing an important role in the revolution in molecular systematics. Fragments of mtDNA markers, so-called DNA barcodes, have been developed to facilitate species identification and accelerate DNA-based taxonomy. For mitochondrial (mt) DNA analysis, conserved genes such as 16S rRNA, Cyt b or cytochrome oxidase subunit I (COI) are usually used. The 16S rRNA gene is approximately 1500 base pairs (bp) in length, although this can vary between 500 bp, 800 bp, and 1500 bp depending on the primer combinations utilized. It is frequently utilized as a DNA barcode region in various taxonomic groups, including gastropods, hydrozoans, amphibians, and Pholcid spiders (Wang et al., 2018; Chan et al., 2022).

In this study, the molecular characteristics of the genus *Kovalius* are presented for the first time. It also reveals the phylogenetic relationships of the species with other members of similar species based on 16S rRNA gene regions.

2. Material and Method

2.1. Material examined and morphological analyses

In this study, harvestmen samples were collected by hand and forceps from the Kürtün district of Gümüşhane province, Turkey in 2020 and stored in 70% ethyl alcohol. Samples were identified by Dr. Kemal KURT (Gümüşhane University, Türkiye) based on morphological data. A detailed description of the species is given in Tchemeris (2023) and Kurt (2024).

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2.2. Molecular analysis

Samples were washed with distilled water, dried and the body and legs were crushed. DNA was extracted according to the manufacturer's instructions using the GeneAll Exgene Tissue Kit (Korea). The 16S rRNA gene was amplified using the primers 16Sa 5′-CGCCTGTTTATCAAAAACAT-3′ (Xiong & Kocher, 1991); 16Sb 5′-CTCCGGTTTGAACTCAGATCA-3′ (Edgecombe et al., 2000).

The polymerase chain reaction was conducted in a complete volume of 20 µl, comprising: 3 µl of DNA template, 8 µl of master mix (2x) (master mix: 10X buffer, 2.5 mM dNTP, 25 mM $MgCl_2$, Taq polymerase), 1 µl of all primer and 7 µl of sterile destilled water.

The amplification conditions comprised an initial denaturation step for a duration of 5 minutes at a temperature of 95°C. This was followed by 40 cycles of denaturation for 30 seconds at 95°C, annealing for 30 seconds at a temperature of 49-50°C, elongation for 30 seconds at 72°C and a final extension step at 72°C for a duration of 5 minutes. These amplification conditions were implemented as outlined in the GeneAll protocol (Seoul, Korea). The PCR products obtained were evaluated by gel electrophoresis in 1% agarose and DNA was extracted in pure form using a gel extraction kit (GeneAll Gel SV).

2.3. Phylogenetic analysis

Nucleotide sequences were edited using the software CodonCode Aligner ver.5.0.2 (CodonCode Corporation, Dedham, MA). The data were queried through the GenBank database using the Basic Local Alignment Search Tool (BLAST) algorithm (Altschul et al., 1990). GenBank accession numbers for sequences used in the present study are provided in Table 1. Phylogenetic analyses were performed using neighbor-joining (NJ) and Bayesian inference (BI) methods (Figs. 1-2). To construct a neighbor-joining (NJ) tree using the P-distance model MEGA 12 version was used for analyses on 1000 bootstrap replicates (Saitou & Nei, 1987; Nei & Kumar, 2000; Kumar et al., 2024).

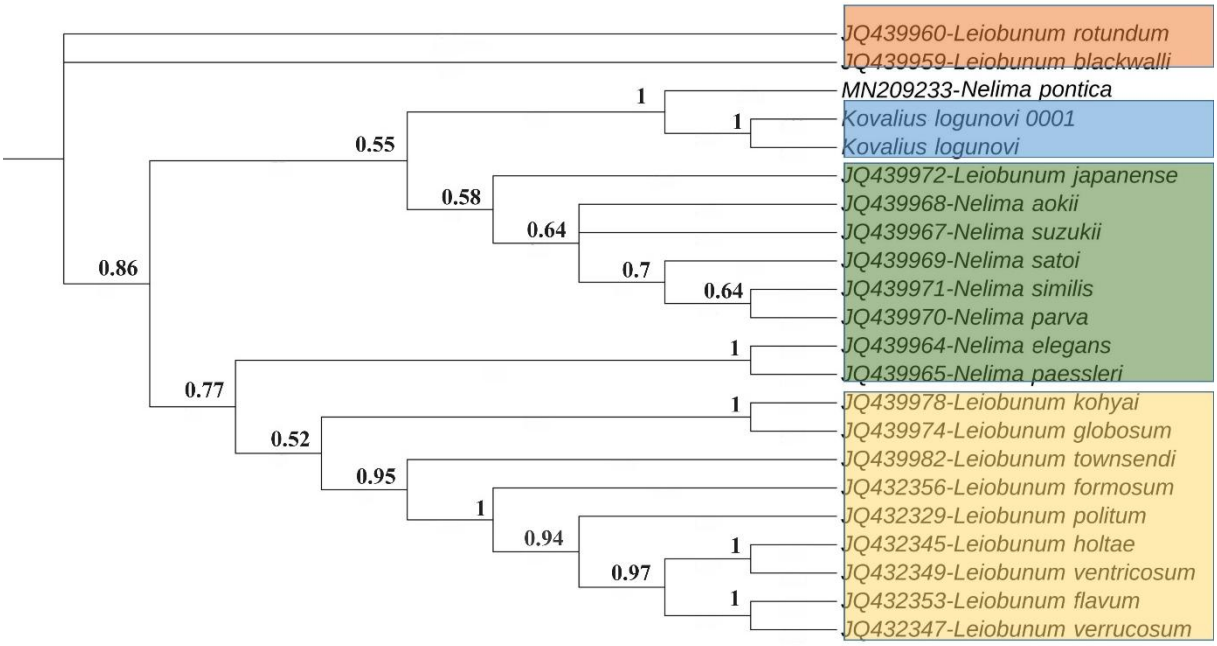

Figure 1. A phylogenetic tree was inferred through Bayesian analysis (BI) based on the sequences of the 16S rRNA gene region.

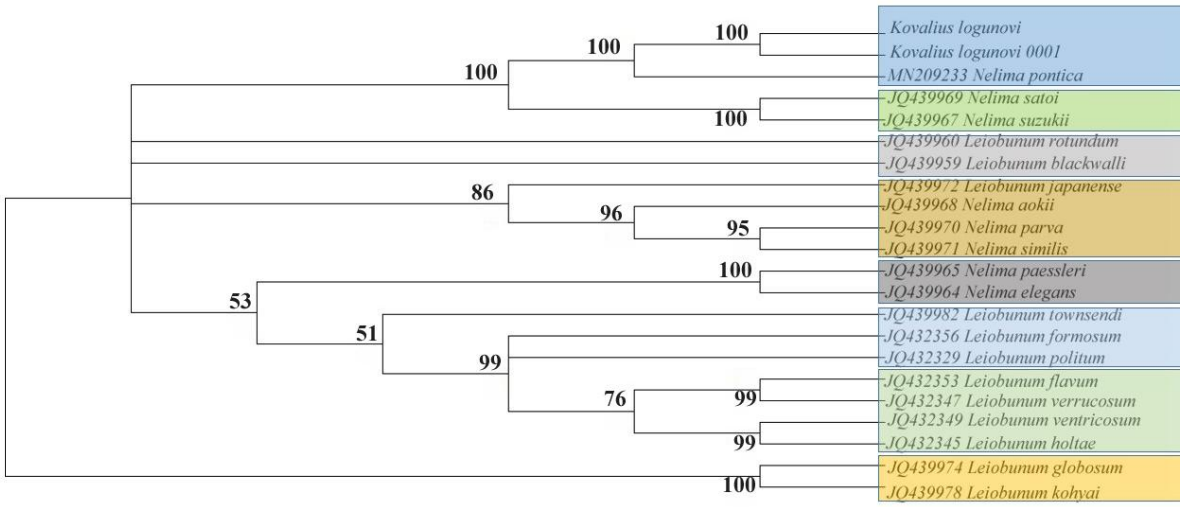

Figure 2. A phylogenetic tree was inferred through Neighbour-joining (NJ) based on the sequences of the 16S rRNA gene region.

Analyses were performed on NGPhylogeny.frserver using Multiple Alignment (Clustal Omega), Alignment Curation (Noisy); Tree Inference (Mr Bayes) (Lemoine et al., 2019). The sequences were aligned with Clustal Omega alignment tool (Sievers et al., 2014); then, sequences were alignment curated with Noisy (commandline: cutoff=0.80, distance=HAMMING, missing=N, nogap=0, noconstant=0, ordering=nnet, shuffles=1000, smooth=1, seqtype=D) (Dress et al., 2008). We performed phylogenetic reconstruction for concatenated 16S rRNA alignment using Bayesian inference (BI) analyses. The BI analysis was run in MrBayes ver. 3.2.7 (Huelsenbeck & Ronquist, 2001).

In the BI analysis, the following settings were used: (Nst =6; Rates= Equal; Setting number of generations to 100000; sample frequency to 500; check-pointing frequency to 100000; burnin fraction to 0.25; number of chains=4; number of runs=1). The best-fit evolutionary model (GTR+G+I) selected according to the Akaike information criterion AIC (Akaike, 1998) in the MEGA 12 version. The analyses were performed using NGPhylogeny.frserver (Lemoine et al., 2019). Phylogenetic trees were visualized using ITOL v5 (Letunic & Bork, 2021). Mean genetic distances (p-distances) between sequences were determined with MEGA 12 version (Kumar et al., 2024).

3. Results and Discussion

A fragment of 408 bp from the 16S rRNA gene was sequenced. The mean frequencies for Adenine (A), Thymine (T), Cytosine (C), and Guanine (G) were 37, 32, 20, and 11%, respectively. The mean nucleotide frequencies were higher and more significant in the A + T ratio than in the C + G ratio (Table 2).

Table 1. GenBank sequence accession numbers of the species used in this study.

Species name	Sequence accession numbers	References
Leiobunum blackwalli	JQ439959	Hedin et al., 2012
Leiobunum flavum	JQ432353	Burns et al., 2012
Leiobunum formosum	JQ432356	Burns et al., 2012
Leiobunum globosum	JQ439974	Hedin et al., 2012
Leiobunum holtae	JQ432345	Burns et al., 2012
Leiobunum japanense	JQ439972	Hedin et al., 2012
Leiobunum kohyai	JQ439978	Hedin et al., 2012
Leiobunum politum	JQ432329	Burns et al., 2012
Leiobunum rotundum	JQ439960	Hedin et al., 2012
Leiobunum townsendi	JQ439982	Hedin et al., 2012
Leiobunum ventricosum	JQ432349	Burns et al., 2012
Leiobunum verrucosum	JQ432347	Burns et al., 2012
Nelima aokii	JQ439968	Hedin et al., 2012
Nelima elegans	JQ439964	Hedin et al., 2012
Nelima paessleri	JQ439965	Hedin et al., 2012
Nelima parva	JQ439970	Hedin et al., 2012
Nelima pontica	MN209233	Doğan & Kurt, 2019
Nelima satoi	JQ439969	Hedin et al., 2012
Nelima similis	JQ439971	Hedin et al., 2012
Nelima suzukii	JQ439967	Hedin et al., 2012

Table 2. Length of base pairs and nucleotide frequencies of the region of the 16S rRNA gene

	T(U)	C	A	G	Base pair length
Kovalius logunovi 0001	38	10	32	20	408
Kovalius logunovi	38	10	32	20	408
Nelima pontica	40	8	32	20	333
Leiobunum blackwalli	33	17	41	8	413
Leiobunum flavum	31	22	36	11	412
Leiobunum formosum	30	22	36	11	411
Leiobunum verrucosum	31	22	36	11	412
Leiobunum japanense	30	21	39	9	412
Leiobunum politum	28	24	35	13	412
Leiobunum ventricosum	29	22	36	12	412
Leiobunum holtae	30	22	36	12	412
Nelima parva	30	21	40	9	416
Leiobunum globosum	28	23	38	10	409
Leiobunum kohyai	28	24	38	10	411
Leiobunum townsendi	33	19	38	10	409
Leiobunum rotundum	35	17	39	9	410
Nelima similis	30	20	40	10	414
Nelima satoi	33	20	39	8	402
Nelima aokii	31	19	41	9	415
Nelima suzukii	32	21	38	9	400
Nelima paessleri	31	22	38	10	413
Nelima elegans	31	22	38	9	412
Avg.	32	20	37	11	389

P-distance, used in phylogenetic tree construction, expresses the ratio of nucleotide differences between sequences. It helps to determine evolutionary relationships in phylogenetic trees and shows the relationship of species based on genetic similarity. While genetic distances between closely related species are small, large genetic differences occur at high genetic distances (Kaleshkumar et al., 2015; Alyamani, 2024). P-distances vary from 0.01% to 0.71% between the opilionid species used in the study. The distance value was 0.36% overall. Genetic distances ranged from 0.01 to 0.70% between *Kovalius logunovi* species and other species. The lowest genetic distance to species of *Kovalius logunovi* was found between species of *Nelima pontica* (0.22) while the highest genetic distance was found between species of *Leiobunum politum* (0.70). The minimum genetic distance is between *K. lounovi* and *N. pontica*, indicating that they have a close relationship (Table 3).

The genus *Kovalius* Tchemeris, 2023 is morphologically similar to the genus *Nelima* Roewer, 1910. However, it is morphologically distinguished from *Nelima* by the structure of the penis, ovipositor, and the prominent apophysis of patellae and tibiae on pediplap. The genus *Nelima* Roewer, 1910 is comprised of two species, *N. pontica* Charitonov, 1941 and *N. doriae* (Canestrini, 1871), within the geographical areas of Türkiye and the NW Caucasus (Kurt, 2014; Tchemeris, 2023). The database at the National Centre for Biotechnology (NCBI) contains data on the 16S rRNA gene region of the *N. pontica* species. However, no such data is available for the *N. doriae* species.

Table 3. Genetic distances (p-distance) based on partial 16S rRNA sequences.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Kovalius logunovi 0001																						
Kovalius logunovi	0.01																					
Nelima pontica	0.22	0.23																				
Leiobunum blackwalli	0.66	0.66	0.67																			
Leiobunum flavum	0.69	0.69	0.69	0.18																		
Leiobunum formosum	0.68	0.68	0.67	0.19	0.09																	
Leiobunum verrucosum	0.69	0.69	0.69	0.18	0.01	0.10																
Leiobunum japanense	0.66	0.67	0.68	0.19	0.21	0.21	0.20															
Leiobunum politum	0.70	0.70	0.70	0.21	0.09	0.11	0.10	0.22														
Leiobunum ventricosum	0.68	0.69	0.69	0.20	0.05	0.09	0.06	0.21	0.11													
Leiobunum holtae	0.68	0.69	0.69	0.20	0.06	0.09	0.06	0.21	0.11	0.00												
Nelima parva	0.67	0.68	0.68	0.18	0.20	0.21	0.20	0.16	0.21	0.20	0.20											
Leiobunum globosum	0.69	0.69	0.71	0.21	0.22	0.21	0.22	0.21	0.21	0.20	0.20	0.22										
Leiobunum kohyai	0.68	0.68	0.69	0.22	0.21	0.20	0.21	0.21	0.21	0.20	0.20	0.22	0.05									
Leiobunum townsendi	0.68	0.69	0.69	0.17	0.15	0.14	0.15	0.20	0.16	0.16	0.16	0.19	0.22	0.21								
Leiobunum rotundum	0.68	0.68	0.66	0.16	0.21	0.20	0.21	0.22	0.23	0.22	0.21	0.23	0.23	0.23	0.19							
Nelima similis	0.67	0.67	0.68	0.17	0.21	0.20	0.20	0.15	0.22	0.20	0.20	0.08	0.22	0.22	0.20	0.21						
Nelima satoi	0.59	0.59	0.61	0.62	0.61	0.63	0.61	0.61	0.62	0.61	0.61	0.63	0.63	0.63	0.61	0.61	0.63					
Nelima aokii	0.67	0.67	0.67	0.17	0.18	0.17	0.19	0.15	0.19	0.19	0.19	0.10	0.21	0.22	0.17	0.21	0.11	0.62				
Nelima suzukii	0.59	0.59	0.58	0.62	0.61	0.63	0.61	0.62	0.63	0.63	0.63	0.62	0.65	0.65	0.61	0.59	0.62	0.26	0.62			
Nelima paessleri	0.68	0.68	0.69	0.19	0.16	0.16	0.16	0.18	0.17	0.17	0.17	0.18	0.19	0.20	0.16	0.21	0.18	0.62	0.17	0.61		
Nelima elegans	0.68	0.68	0.69	0.19	0.16	0.16	0.16	0.19	0.18	0.17	0.17	0.19	0.20	0.20	0.17	0.20	0.19	0.62	0.18	0.61	0.04	

The p-distances and high bootstrap values obtained in this study indicate a stronger similarity between the genus *Kovalius* and the genus *Nelima* than between the other genera (Figs. 1-2). This finding provides further support for the molecular and morphological data and thus contributes to our understanding of taxonomic relationships within the indicated group.

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Conflict of interest: The authors declare that there is no conflict of interest.

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References

Akaike, H. (1998) Information theory and an extension of the maximum likelihood principle. *In*: Parzen, E., Tanabe, K. &Kitagawa, G. (Eds.), *Selected Papers of Hirotugu Akaike*. Springer, New York, New York, pp. 199–213.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., & Lipman, D.J. (1990). Basic local alignment search tool. *Journal of molecular biology*, 215(3), 403-410.

Alyamani, N.M. (2024). Mitochondrial 16S rRNA gene as a molecular marker in the phylogenetic relationships of some Rabbitfishes species (Siganidae: Perciformes). *Open Veterinary Journal*, 14(8), 1936.

Burns, M., Hedin, M., & Shultz, J.W. (2012). Molecular phylogeny of the leiobunine harvestmen of eastern North America (Opiliones: Sclerosomatidae: Leiobuninae). *Molecular Phylogenetics and Evolution*, 63(2), 291-298.

Chan, K.O., Hertwig, S.T., Neokleous, D.N., Flury, J.M., & Brown, R.M. (2022). Widely used, short 16S rRNA mitochondrial gene fragments yield poor and erratic results in phylogenetic estimation and species delimitation of amphibians. *BMC Ecology and Evolution*, 22(1), 37.

Doğan, N.Y., & Kurt, P. (2019). DNA barcoding and phylogenetic analysis of *Nelima pontica* Charitonov, 1941 (Opiliones: Sclerosomatidae) based on mitochondrial COI and 16S rRNA genes. *Acta Biologica Turcica*, 33(1), 8-11.

Dress, A.W., Flamm, C., Fritzsch, G., Grünewald, S., Kruspe, M., Prohaska, S.J., & Stadler, P.F. (2008). Noisy: identification of problematic columns in multiple sequence alignments. *Algorithms for Molecular Biology*, 3, 1-10.

Edgecombe, G.D., Wilson, G.D.F., Colgan, D.J., Gray, M.R., & Cassis, G. (2000). Arthropodcladistics: combined analysis of histone H3 and U2 snRNA sequences andmorphology. *Cladistics*, 16, 155-203.

Hedin, M., Tsurusaki, N., Macías-Ordóñez, R., & Shultz, J.W. (2012). Molecular systematics of sclerosomatid harvestmen (Opiliones, Phalangioidea, Sclerosomatidae): geography is better than taxonomy in predicting phylogeny. *Molecular Phylogenetics and Evolution*, 62(1), 224-236.

Huelsenbeck, J.P., & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17(8), 754-755.

Kaleshkumar, K., Rajaram, R., Vinothkumar, S., Ramalingam, V., & Meetei, K.B. (2015). Note DNA barcoding of selected species of pufferfishes (Order: Tetraodontiformes) of Puducherry coastal waters along south-east coast of India. *Indian Journal of Fisheries,* 62(2), 98–103.

Kumar, S., Stecher, G., Suleski, M., Sanderford, M., Sharma, S., & Tamura, K. (2024). MEGA12: Molecular Evolutionary Genetic Analysis version 12 for adaptive and green computing. *Molecular Biology and Evolution*, 41(12), msae263.

Kurt, K. (2014). Updated checklist of harvestmen (Arachnida: Opiliones) in Turkey. *Archives of Biological Sciences*, 66(4), 1617-1631.

Kurt, K. (2024). A New Harvestman Genus Record for Turkey: *Kovalius* Tchemeris, 2023 (Opiliones Sclerosomatidae, Leiobuninae). *Entomological News*, 131(4), 186-192.

Lemoine, F., Correia, D., Lefort, V., Doppelt-Azeroual, O., Mareuil, F., Cohen-Boulakia, S., & Gascuel, O. (2019). NGPhylogeny. fr: new generation phylogenetic services for non-specialists. *Nucleic acids research*, 47(W1), W260-W265.

Letunic, I., & Bork, P. (2021). Interactive Tree of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Research,* 49(W1), W293–W296.

Nei, M., & Kumar, S. (2000). *Molecular evolution and phylogenetics*. Oxford university press.

Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular biology and evolution*, 4(4), 406-425.

Sievers, F., Wilm, A., Dineen, D., Gibson, T. J., Karplus, K., Li, W., ..., & Higgins, D. G. (2011). Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Molecular systems biology*, 7(1), 539.

Tchemeris, A.N. (2023). *Kovalius* - a new genus of cave-dwelling harvestmen from the Caucasus (Opiliones: Sclerosomatidae: Leiobuninae). *Zootaxa*, 5227(4), 486-494.

Wang, Z.L., Yang, X.Q., Wang, T.Z., & Yu, X. (2018). Assessing the effectiveness of mitochondrial COI and 16S rRNA genes for DNA barcoding of farmland spiders in China. *Mitochondrial DNA Part A*, 29(5), 695-702.

Xiong, B., & Kocher, T. D. (1991). Comparison of mitochondrial DNA sequences of seven morphospecies of black flies (Diptera: Simuliidae). *Genome*, 34(2), 306-311.