

Integrative taxonomy reveals the first record of the genus *Carinostoma* Kratochvíl, 1958 (Opiliones: Nemastomatidae) in Turkey

Первая находка сенокосцев рода *Carinostoma* Kratochvíl, 1958 (Opiliones: Nemastomatidae) в Турции, обнаруженная с помощью интегративного подхода в таксономии

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КЛЮЧЕВЫЕ СЛОВА: 28S rRNA, *Carinostoma carinatum*, сенокосец, филогенетический анализ, Türkiye.

ABSTRACT. Based on both morphological and molecular data, the genus *Carinostoma* Kratochvíl, 1958 is recorded for the first time from Turkey. Detailed illustrations of the dorsal body structure, chelicera, and pedipalp are provided. Additionally, an 835 bp fragment of the 28S rRNA gene was sequenced. The systematic position of the species and its genus within the family Nemastomatidae was determined based on Maximum Likelihood (ML) and Bayesian (BEAST) analyses.

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РЕЗЮМЕ. На основании морфологических и молекулярных данных, род сенокосцев *Carinostoma* Kratochvíl, 1958 впервые отмечен в фауне Турции. Детально проиллюстрированы структура тела с дорсальной стороны, хелицеры и педипальпы. Дополнительно, проведен сиквенс фрагментов 835 бп и гена 28S rRNA. При помощи методов максимального правдоподобия (ML) и Байесова (BEAST) определено систематическое положение видов и самого рода в семействе Nemastomatidae.

Introduction

The genus *Carinostoma* belongs to the family Nemastomatidae (Simon, 1872) and was established by Kratochvíl in 1958. This genus is widely distributed across Europe and currently includes three recognized species: *C. carinatum* (Roewer, 1914), *C. elegans* (Sørensen, 1894), and *C. ornatum* (Hadži, 1940). However, neither the genus nor its species have been recorded in Turkey to date [Bayram *et al.*, 2010; Kurt *et al.*, 2010; Kurt, 2014; Kury *et al.*, 2023, 2024].

Carinostoma carinatum was first described by Roewer in 1914, from Bosnia and Herzegovina (Jablanica). Martens [1978] provided detailed information on the species' morphological characteristics and genital structures. Subsequently, the nuclear 28S rRNA and mitochondrial cytochrome b (cytb) gene regions of specimens collected from Italy were studied, and the resulting data were uploaded to the NCBI GenBank database [Schönhofer, Martens, 2010].

This study aims to document the first record of the genus *Carinostoma* and the species *C. carinatum* from Turkey, and to confirm its identity based on both morphological characteristics and molecular data from the 28S rRNA gene region. Furthermore, this record extends the known distribution range of *C. carinatum* and contributes to the biodiversity knowledge of the region.

Material and methods

Sample collection

The specimens used in this study were collected by hand and using forceps in Sinop province of Turkey in 2014. The samples examined were preserved in 70% ethanol and were kept in the collection of the Arachnological Laboratory of Şiran Vocational School, Gümüşhane University (GUSAL), Gümüşhane, Turkey. Photographs were taken with an Olympus SZ61 stereomicroscope equipped with an Olympus LC20 digital camera. The images were then combined using Combine ZM soft ware and edited with Adobe Photoshop CS3.

DNA extraction

The samples stored in 70% ethanol were first removed and air-dried to allow the alcohol to evaporate completely. They were then washed with distilled water and dried again. Due to the small size of the samples, the entire bodies and legs were used for DNA extraction. DNA extraction was performed using the GeneAll, Exgene™ Animal Tissue kit according to manufacturer's protocols (GeneAll, Biotechnology Co., Ltd., Seoul, Korea).

Table 1. List of GenBank accession numbers for harvestmen species included in this study.
Таблица 1. Номера Генбанка для исследованных в данной работе видов сенокосцев.

Taxon name	Accession numbers	References
<i>Carinostoma carinatum</i> (Turkey)	OM049505.2	In this study
<i>Carinostoma carinatum</i>	GQ466280.1	Schönhofer, Martens, 2010
<i>Carinostoma elegans</i>	GQ466281.1	Schönhofer, Martens, 2010
<i>Histicostoma dentipalpe</i>	GQ466291.1	Schönhofer, Martens, 2010
<i>Histicostoma argenteolunulatum</i>	GQ466290.1	Schönhofer, Martens, 2010
<i>Paranemastoma silli</i>	GQ466304.1	Schönhofer, Martens, 2010
<i>Paranemastoma</i> sp.	GQ466305.1	Schönhofer, Martens, 2010
<i>Paranemastoma quadripunctatum</i>	GQ466303.1	Schönhofer, Martens, 2010
<i>Pyza bosnica</i>	GQ466312.1	Schönhofer, Martens, 2010
<i>Mediostoma</i> sp.	GQ466297.1	Schönhofer, Martens, 2010
<i>Mediostoma vitynae</i>	GQ466296.1	Schönhofer, Martens, 2010
<i>Nemastomella dubia</i>	GQ912774.1	Giribet <i>et al.</i> , 2010
<i>Nemastoma bimaculatum</i>	GQ466300.1	Schönhofer, Martens, 2010
<i>Nemastoma lugubre</i>	GQ466301.1	Schönhofer, Martens, 2010
<i>Nemastoma bidentatum</i>	GQ466299.1	Schönhofer, Martens, 2010
<i>Nemastoma hankiewiczii</i>	GQ466302.1	Schönhofer, Martens, 2010
<i>Dendrolasma parvulum</i>	GQ912771.1	Giribet <i>et al.</i> , 2010
<i>Dendrolasma parvulum</i>	EF108578.2	Boyer <i>et al.</i> , 2007
<i>Ortholasma</i> sp.	KF181758.1	Richart, Hedin, 2013
<i>Nemastoma hankiewiczii</i>	GQ912772.1	Giribet <i>et al.</i> , 2010
<i>Mitostoma chrysomelas</i>	GQ466298.1	Schönhofer, Martens, 2010
<i>Trogulus tricarinatus</i> (outgroup)	FJ373264.1	Schönhofer, Martens, 2008

PCR amplification and DNA sequencing

Polymerase chain reaction (PCR) was performed using specific primers to amplify the target 28S rRNA region. The following primers were used to amplify the 28S rRNA gene region: ZX1 (5'-ACCCGCTGAATTTAAGCATATAT-3') and ZR2 (5'-GCTATCCTGAGGGAAACTTCGG-3') [Mallatt, Sullivan, 1998]. The PCR reaction was performed in a total volume of 20 µL and contained 2 µL of template DNA, 10 µL of 2× master mix (GeneAll, Seoul, Korea), 7 µL of sterile distilled water, and 0.5 µL of each primer. The amplification conditions included an initial denaturation step at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 51 °C for 30 s, and extension at 72 °C for 30 s. A final extension was performed at 72 °C for five min. The PCR amplicons were visualized on a 1% agarose gel stained with ethidium bromide. Successfully amplified products were purified using a WizPure™ DNA Gel Extraction Kit and were then subjected to nucleotide sequencing. Subsequently, DNA sequencing was performed using the Sanger sequencing method in both directions.

Molecular phylogenetic analyses

The 28S rRNA gene sequences resulting from this study were edited using BioEdit v7.2.5 [Hall, 1999] software. These sequences were then uploaded to the NCBI (National Center for Biotechnology Information) GenBank database, and BLAST (Basic Local Alignment Search Tool) searches were performed to identify the most closely related species. Reference sequences from similar species were downloaded from the NCBI database for use in phylogenetic analyses. Data and accession numbers of

similar species were obtained from NCBI and are listed in Table 1. Alignment of sequences was done using MUSCLE algorithm [Edgar, 2004]. The most appropriate evolutionary model was determined using the Akaike Information Criterion [Akaike, 1974] in MEGA 12 [Kumar *et al.*, 2024], and the GTR+G+I model was selected. Maximum likelihood analyses were conducted in MEGA 12 with 1,000 bootstrap replicates. Uncorrected pair-wise sequence divergence was calculated among 28S rRNA genes using the MEGA 12 software [Kumar *et al.*, 2024].

Bayesian phylogenetic analyses were performed in BEAST X v10.5.0 [Suchard *et al.*, 2018]. The input XML file was prepared in BEAUti v10.5.0 using the GTR+G+I substitution model. We selected an uncorrelated lognormal relaxed clock model [Drummond *et al.*, 2006] and used a Yule process as the tree prior [Yule, 1925; Gernhard, 2008]. The MCMC analysis was run for 10 million generations, sampling every 1,000 generations. A maximum clade credibility (MCC) tree was generated using TreeAnnotator, discarding the first 10% of trees as burn-in. The resulting phylogenetic tree was visualized in iTOL [Letunic, Bork, 2021].

Results

Genus *Carinostoma* Kratochvíl, 1958
Carinostoma carinatum (Roewer, 1914)
Figs 1, 2; Tables 1, 2.

Material examined. 1 ♀, 3 juveniles (GUSAL), Turkey, Sinop Province, Sarıkum District (42°00'46.8"N, 34°53'59.1"E), 80 m a.s.l., 4 May 2014, leg. H. Koç.

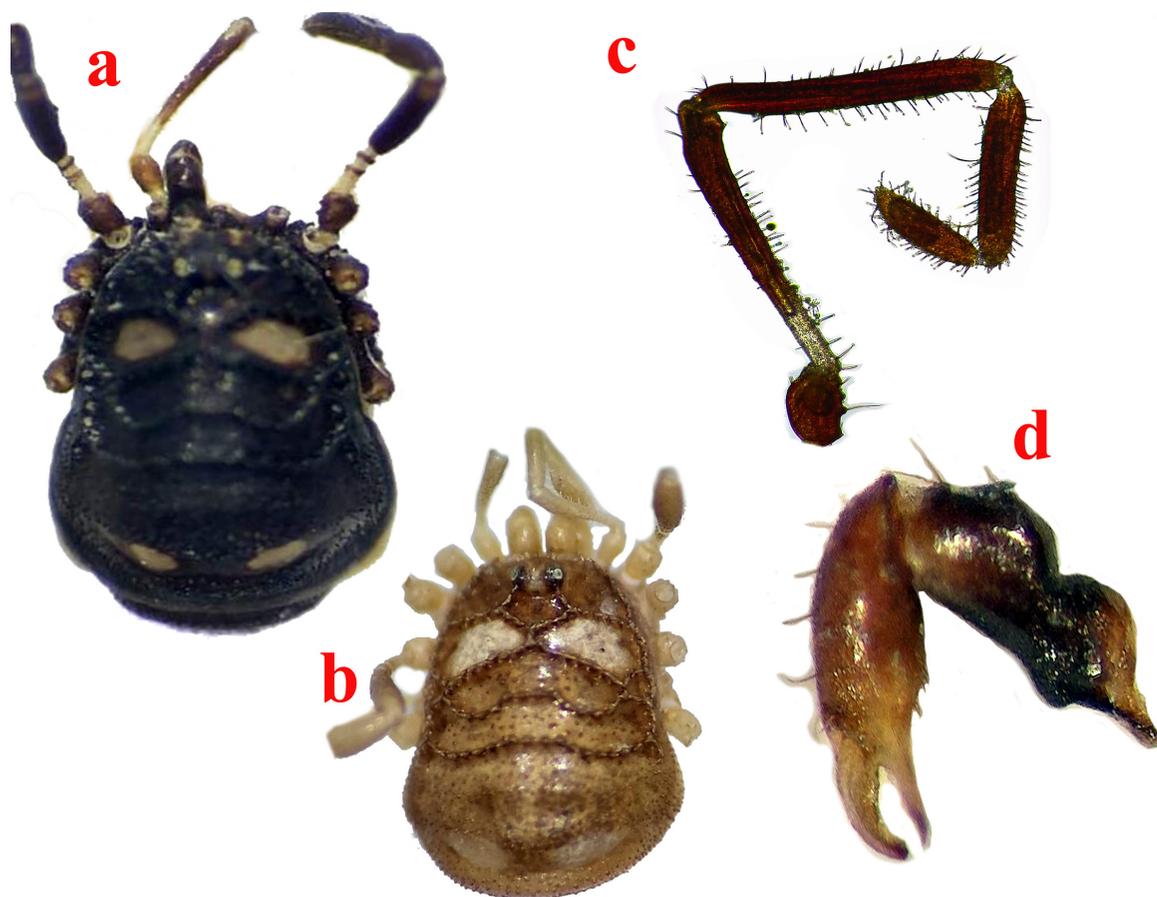


Fig. 1. *Carinostoma carinatum*: a — body, dorsal view (female); b — idem (juvenile); c — pedipalp, lateral view, d — chelicera, lateral view.
Рис. 1. *Carinostoma carinatum*: a — тело, дорсально (самка); b — то же (ювениль); c — педипальпа, латерально, d — хелицера, латерально.

DESCRIPTION. FEMALE. Body small, oval-shaped body is black and measures 1.8 mm long by 1.2 mm wide. Two large, silver-colored spots are located on both sides of the prosoma, behind the eye tubercle. Three rows of bridge-like spines are behind these spots. Two smaller silver spots are present on the posterior of the abdomen (Fig. 1A–B). Chelicerae normal shape, without apophysis, covered with sparsely setae (Fig. 1D). Pedipalp covered with setae (Fig. 1C). For a more detailed description, see Roewer [1914, 1923] and Martens [1978].

DISTRIBUTION. Austria, Bosnia-Herzegovina, Croatia, Italy, Montenegro, North Macedonia, Serbia, Slovenia [Šestáková, Mihál, 2014; Raspotnig *et al.*, 2014; Kury *et al.*, 2024].

MOLECULAR REMARKS. A sequence of 835 bp was obtained from the 28S rRNA gene region of the species *Carinostoma carinatum*. This sequence was uploaded to the NCBI GenBank database and assigned the accession number OM049505.2. According to the BLAST analysis, the sequence exhibited 99.27% similarity (E-value: 0.0) to previously recorded specimens from Italy. This high degree of genetic similarity strongly confirms the species identity of the analyzed specimen, supporting its assignment to the same species. The molecular data are consistent with the morphological findings and together validate the first record of this species from Turkey.

The results showed that the interspecies genetic distances based on the 28S rRNA gene ranged from 0.04 to 15.1%. The greatest interspecies genetic distance was observed between *Mitostoma chrysomelas* and *Dendrolasma parvulum* (15.1%),

while the smallest was between *Carinostoma carinatum* (Turkey) and *C. carinatum* (Italy) (0.04%). Additionally, based on uncorrected p-genetic distance, *Carinostoma carinatum* was found to be closest to *C. elegans*, while *Mitostoma chrysomelas* exhibited the greatest genetic difference (Table 2).

Schönhofer & Martens [2010] molecular phylogenetic assessment of eight Nemastomatidae genera provided evidence for a division of the family into two main clades. The phylogenetic position of the genus *Mitostoma* within the family was found to be controversial. Based on both morphological characteristics (particularly the structure of the penis) and molecular data, it was suggested that this genus could potentially be evaluated at the subfamilial level. On the other hand, the genera *Carinostoma*, *Histicostoma* ve *Paranemastoma* were grouped within the same clade, supported by both morphological characters (penis morphology and the structure of the cheliceral apophysis) and molecular evidence. Additionally, the genera *Mediostoma*, *Nemastoma*, *Nemastomella* ve *Pyza* were classified within a second clade.

In our study, molecular data based on the 28S rRNA gene region revealed that the Nemastomatidae family is divided into three major clades. The genus *Mitostoma* is positioned as a sister taxon to all other genera; *Carinostoma*, *Histicostoma* ve *Paranemastoma* are grouped within the same main clade, while *Mediostoma*, *Nemastoma*, *Nemastomella* ve *Pyza* form a separate second clade. Furthermore, *Ortholasma* ve *Dendrolasma* are clearly placed in a third distinct clade in the resulting phylogenetic tree (Fig. 2).

Table 2. Uncorrected genetic distances between analysed Nematostomatidae species based on 28S rRNA sequences. Таблица 2. Нескорректированные генетические дистанции между изученными видами сенюковцев семейства Nematostomatidae на основе сиквенсов 28S rRNA.

Taxon name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
<i>Carinostoma carinatum</i> (Turkey)																					
<i>Carinostoma carinatum</i>	0.004																				
<i>Carinostoma elegans</i>	0.014	0.011																			
<i>Histicostoma dentipalpe</i>	0.025	0.021	0.029																		
<i>Histicostoma argenteolunulatum</i>	0.028	0.024	0.029	0.007																	
<i>Paranemastoma silli</i>	0.045	0.041	0.046	0.033	0.033																
<i>Paranemastoma</i> sp.	0.049	0.045	0.047	0.042	0.038	0.020															
<i>Paranemastoma quadripunctatum</i>	0.046	0.042	0.047	0.034	0.034	0.008	0.022														
<i>Pyza bosnica</i>	0.053	0.049	0.049	0.045	0.046	0.051	0.053	0.053													
<i>Mediostoma</i> sp.	0.063	0.059	0.060	0.053	0.053	0.060	0.063	0.059	0.037												
<i>Mediostoma vitynae</i>	0.067	0.063	0.064	0.055	0.054	0.063	0.066	0.062	0.038	0.022											
<i>Nemastomella dubia</i>	0.078	0.074	0.076	0.068	0.064	0.070	0.063	0.070	0.055	0.053	0.057										
<i>Nemastoma bimaculatum</i>	0.072	0.068	0.070	0.063	0.063	0.066	0.064	0.064	0.038	0.053	0.054	0.058									
<i>Nemastoma lugubre</i>	0.076	0.072	0.074	0.067	0.064	0.071	0.070	0.067	0.047	0.066	0.059	0.066	0.025								
<i>Nemastoma bidentatum</i>	0.079	0.075	0.076	0.071	0.070	0.074	0.076	0.070	0.042	0.059	0.058	0.070	0.049	0.046							
<i>Nemastoma hankiewiczii</i>	0.072	0.068	0.071	0.062	0.060	0.072	0.071	0.074	0.049	0.057	0.060	0.037	0.059	0.066	0.072						
<i>Dendrolasma parvulum</i>	0.099	0.097	0.099	0.101	0.101	0.095	0.096	0.099	0.104	0.108	0.108	0.114	0.118	0.118	0.116	0.121					
<i>Dendrolasma parvulum</i>	0.099	0.097	0.099	0.101	0.101	0.095	0.096	0.099	0.104	0.108	0.108	0.114	0.118	0.118	0.116	0.121	0.000				
<i>Ortholasma</i> sp.	0.109	0.108	0.112	0.109	0.112	0.105	0.108	0.105	0.105	0.116	0.120	0.124	0.117	0.122	0.120	0.118	0.074	0.074			
<i>Nemastoma hankiewiczii</i>	0.074	0.070	0.072	0.063	0.059	0.068	0.068	0.070	0.049	0.051	0.057	0.032	0.058	0.063	0.066	0.018	0.118	0.118	0.117		
<i>Mitostoma chrysomelas</i>	0.114	0.112	0.117	0.104	0.104	0.104	0.108	0.112	0.112	0.118	0.118	0.117	0.116	0.113	0.126	0.114	0.151	0.151	0.147	0.113	
<i>Trogulus tricarlinatus</i> (outgroup)	0.124	0.120	0.124	0.114	0.117	0.126	0.126	0.127	0.121	0.118	0.118	0.130	0.131	0.131	0.135	0.124	0.122	0.122	0.138	0.129	0.164

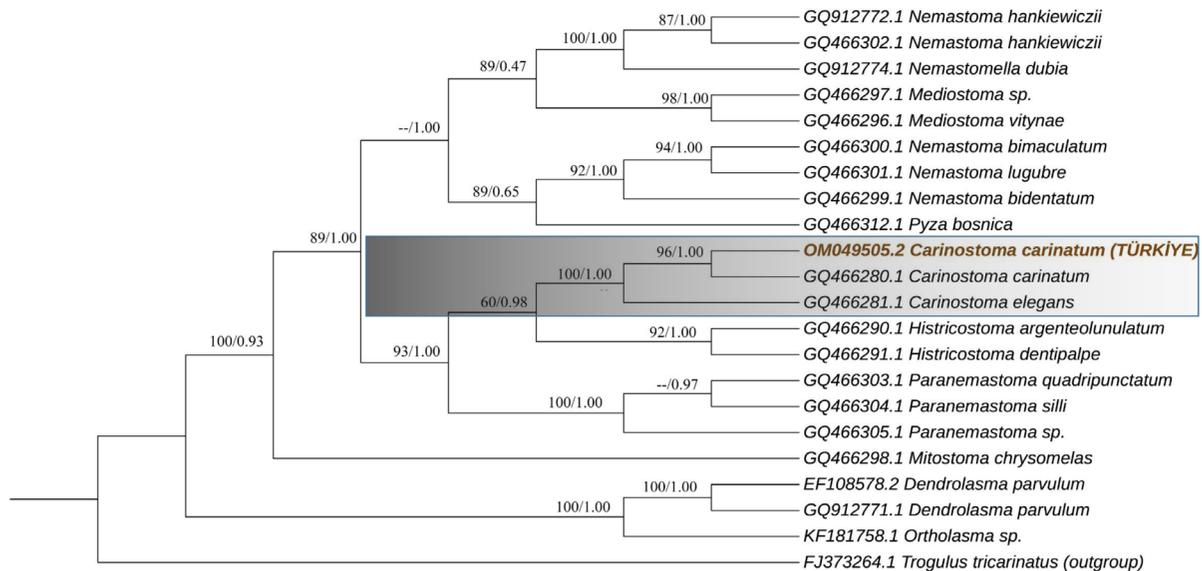


Fig. 2. Maximum Likelihood (ML) and Bayesian (BEAST) phylogenetic tree of the 28S rRNA dataset (835 bp, GTR+G+I). Numbers at nodes indicate ML bootstrap and Bayesian posterior probability values, respectively.

Рис. 2. Филогенетические деревья, построенные методами максимального правдоподобия (ML) и Байесовым (BEAST) по данным 28S rRNA (835 бп, GTR+G+I). Номера в узлах указывают бутстрепы ML и оценки апостериорной вероятности по Байесову методу, соответственно.

This study presents the first confirmed record of *Carinostoma carinatum* from Turkey, contributing to the documentation of the country's opilionid diversity and zoogeographic distribution of the species. The combined use and corroboration of morphological and molecular data strengthens the reliability of this new record and highlights the need for further integrative taxonomic research on Turkish opilionids, particularly at the molecular level, where data are still scarce.

Compliance with ethical standards

CONFLICT OF INTEREST: The authors declare that they have no conflict of interest.

Ethical approval: No ethical issues were raised during our research.

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References

- Akaike H. 1974. A new look at the statistical model identification // IEEE Transactions on Automatic Control. Vol.19. No.6. P.716–723.
- Bayram A., Çorak İ., Danişman T., Sancak Z., Yiğit N. 2010. Checklist of the harvestmen of Turkey (Arachnida: Opiliones) // Munis Entomology & Zoology. Vol.5. No.2. P.563–585.
- Boyer S.L., Clouse R.M., Benavides L.R., Sharma P., Schwendinger P.J., Karunarathna I., Giribet G. 2007. Biogeography of the world: a case study from cyphophthalmid Opiliones, a globally distributed group of arachnids // Journal of Biogeography. Vol.34. No.12. P.2070–2085.
- Drummond A.J., Ho S.Y.W., Phillips M.J., Rambaut A. 2006. Relaxed phylogenetics and dating with confidence // PLoS biology. Vol.4. No.5. Art.e88.
- Edgar R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput // Nucleic Acids Research. Vol.32. No.5. P.1792–1797.
- Gernhard T. 2008. The conditioned reconstructed process // Journal of theoretical biology. Vol.253. No.4. P.769–778.
- Giribet G., Vogt L., Gonzalez A.P., Sharma P., Kury A.B. 2010. A multilocus approach to harvestman (Arachnida: Opiliones) phylogeny with emphasis on biogeography and the systematics of Laniatores // Cladistics. Vol.26. No.4. P.408–437.
- Hall T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for window 95/98/NT // Nucleic Acids Symposium Series. Vol.41. P.95–98.
- Kratochvíl J. 1958. Höhlenweberknechte Bulgariens (Palpatores–Nemastomatidae) // Acta Academiae Scientiarum Českoslovenicae Basis Brunensis. Vol.30. P.523–576.
- 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. Vol.41. No.12. Art.msae263.
- Kurt K. 2014. Updated checklist of harvestmen (Arachnida: Opiliones) in Turkey // Archives of biological sciences. Vol.66. No.4. P.1617–1631.
- Kurt K., Erman Ö.K., Demir H., Seyyar, O. 2010. The Turkish harvestmen (Opiliones) with zoogeographical remarks // Serket. Vol.12. No.2. P.33–44.
- Kury A.B., Kury I.S., De Oliveira A.B.R. 2024. Checklists of extant harvestman (Arachnida: Opiliones) species for all the countries of the World // Zootaxa. Vol.5515. No.1. P.1–162.
- Kury A.B., Mendes A.C., Cardoso L., Kury M.S., Granado A.de A. Giribet G., Cruz-López J.A., Longhorn S.J., Medrano M., Oliveira A.B.R., Kury I.S., Souza-Kury M.A. 2023. World Catalogue of Opiliones. WCO-Lite. Version 2.5.1. Available from: <https://wco-lite.com/> (accessed 17 September 2024).
- Letunic I., Bork P. 2021. Interactive Tree of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation // Nucleic Acids Research. Vol.49. P.293–296.
- Mallatt J., Sullivan J. 1998. 28S and 18S rDNA sequences support the monophyly of lampreys and haghfishes // Molecular Biology and Evolution. Vol.15. No.12. P.1706–1718.
- Martens J. 1978. [Spinnentiere, Arachnida: Weberknechte, Opiliones]. F. Senglaub, H.J. Hannemann, H. Schumann (Hrsg.). Die Tierwelt Deutschlands. Lfg.64. Jena: Gustav Fischer. 464 S.
- Rasputnig G., Schaider M., Stabentheiner E., Leis H.J., Karaman I. 2014. On the enigmatic scent glands of dyspnoan harvestmen (Arachnida,

- Opiliones): first evidence for the production of volatile secretions // *Chemoecology*. Vol.24. No.2. P.43–55.
- Richart C.H., Hedin M. 2013. Three new species in the harvestmen genus *Acuclavella* (Opiliones, Dyspnoi, Ischyropsalidoidea), including description of male *Acuclavella* quattuor Shear, 1986 // *ZooKeys*. Vol.311. P.19–68.
- Roewer C.F. 1914. Die Familien der Ischyropsalidae und Nematomatidae der Opiliones = Palpatores // *Arch. Naturg.* Bd.80A. P.99–169.
- Roewer C.F. 1923. Die Weberknechte der Erde. Systematische Bearbeitung der bisher bekannten Opiliones. Jena: Gustav Fischer. 1116 S.
- Schönhofer A.L., Martens J. 2008. Revision of the genus *Trogulus* Latreille: the *Trogulus coriziformis* species-group of the western Mediterranean (Opiliones: Trogulidae) // *Invertebrate Systematics*. Vol.22. No.5. P.523–554.
- Schönhofer A.L., Martens J. 2010. Hidden Mediterranean diversity: Assessing species taxa by molecular phylogeny within the opilionid family Trogulidae (Arachnida, Opiliones) // *Molecular Phylogenetics and Evolution*. Vol.54. No.1. P.59–75.
- Šestáková A., Mihál I. 2014. *Carinostoma elegans* new to the Slovakian harvestmen fauna (Opiliones, Dyspnoi, Nematomatidae) // *Arachnologische Mitteilungen*. Vol.48. P.16–23.
- Suchard M.A., Lemey P., Baele G., Ayres D.L., Drummond A.J., Rambaut A. 2018. Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus evolution*, 4(1): vey016.
- Yule G.U. 1925. II.—A mathematical theory of evolution, based on the conclusions of Dr. J.C. Willis, F.R.S // *Philosophical transactions of the Royal Society of London. Series B*. Vol.213. P.21–87.

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