TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2020) 44: 449-455

doi:10.3906/vet-1911-82

First molecular characterization of the facultative myiasis agent *Eristalis tenax* in Turkey using mitochondrial cytochrome c oxidase subunit I DNA barcoding sequences

Mübeccel OKUR*, Alparslan YILDIRIM, Abdullah İNCİ
Department of Parasitology, Faculty of Veterinary Medicine, Erciyes University, Kayseri, Turkey

Received: 25.11.2019 • **Accepted/Published Online:** 22.02.2020 • **Final Version:** 06.04.2020

Abstract: This report is the first molecular characterization of the facultative myiasis agent *Eristalis tenax* collected from cattle barns in Kayseri province of Central Anatolia, Turkey. A total of 10 adult fly specimens were included in the study. All flies were identified as female adults of *E. tenax* by morphologic characteristics under a stereomicroscope. The barcode region of the mitochondrial cytochrome oxidase 1 (CO1) gene was amplified with PCR from the genomic DNA extracts of *E. tenax* specimens for molecular identification and phylogenetic analyses. There was no polymorphism among the CO1 sequences of all isolates leading to the detection of a single haplotype for *E. tenax*. Haplotype and nucleotide diversities in the CO1 data set comprised of the sequence of the detected haplotype and the published sequences of *E. tenax* from different countries in GenBank were determined as 0.775 and 0.002, respectively. The newly characterized haplotype in this study clustered with the haplotypes from Australia and Canada in a monophyletic clade and exhibited a 100.0% identity to the Australian *E. tenax* isolate (JN991985). Interspecific genetic differences between *E. tenax* and other *Eristalis* species were in the range of 5.7% to 7.9%.

Key words: *Eristalis tenax*, facultative myiasis, DNA barcoding, molecular characterization, Turkey

1. Introduction

The flies in the family Syrphidae (Diptera: Lower Cyclorrhapha), one of the most speciose of dipteran families, are known as flower flies or hoverflies, and this family includes more than 5000 described species [1]. *Eristalis tenax* Linnaeus, 1758 in the family Syrphidae, is known as a drone fly and may offer potential as a managed pollinator of field crops. This fly has a worldwide distribution including Europe [2], China [3], Japan [4], the Indian subcontinent, and throughout the New World [5–7]. The adults of *E. tenax* are strong fliers, have fairly uniform flower-feeding habits, and their size, shape, and color pattern are similar to honey and bumblebees [8–10]. The larvae of *E. tenax*, also known as rat-tailed maggots, are aquatic and mainly found in wetlands contaminated with livestock manure and wastewater processing facilities [11–13].

Despite their important role in pollination for many flowering plants, *E. tenax* have medical and veterinary importance due to the vector potential of adults, immature stages for mycobacteria [14,15], and causing facultative myiasis by early-stage maggots in humans and livestock [16–21]. The presence and distribution of *E. tenax* have been documented from several regions of Turkey, including the Kayseri region [22–25]. In addition, Yalçınkaya [26] and Mumcuoğlu et al. [18] reported nasal and urinary myiasis cases, respectively, in humans caused by the rat-tailed maggots of *E. tenax*. Dik et al. [27] also described a case of traumatic myiasis in a dog from the Central Anatolia Region.

DNA barcoding is a powerful and useful approach for the accurate identification of numerous organisms, including several insect species, especially those that have similar morphology. The mitochondrial cytochrome-c oxidase subunit 1 gene (CO1) is commonly used for species delimitation and exploring the cryptic diversity in closely related species due to its phylogenetic signal with high resolution [28,29]. The barcode region of 658 bp of the 5′ end of CO1 [30] is the primarily targeted gene fragment in DNA barcoding of several kinds of vertebrates and invertebrates, including some hoverfly species [11,31–34]. However, there has been no study in Turkey to explore the genetic characterization and diversity of Syrphidae species, including *E. tenax*.

The objective of this study was to provide the first outputs on the molecular characterization of *E. tenax* distributed in the Central Anatolia Region of Turkey and to reveal the genetic diversity of the lineages by comparing

* Correspondence: m.okurr@hotmail.com

the available CO1 sequences of *E. tenax* from several countries in GenBank.

2. Materials and methods

2.1. Study area and collection of flies

A total of 10 adult flies were collected from cattle barns by using insect nets in a village in Kayseri province in May 2018. The fly specimens were put into sterile tubes with 70% ethanol and then transferred to the laboratory. The identification of fly specimens was done under a stereomicroscope (SZX16, Olympus, Japan) by following the keys provided by Hippa et al. [35] and images were recorded.

2.2. Genomic DNA isolation and amplification of CO1 gene

Genomic DNA (gDNA) was extracted from the tissue sections of each individual specimen via GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, USA). Prior to gDNA isolation, the tissue pieces were homogenized with the TissueLyser LT (Qiagen, UK). All gDNA isolates were stored at –20 °C until PCR analyses. The concentration of gDNA isolates was determined by Qubit Fluorometric Quantitation (Thermo Fisher Scientific, USA) prior to PCR amplification.

A partial 709 bp CO1 gene from the individual specimens was amplified using universal primers described by Folmer et al. [30]. Amplifications were performed using C1000 Touch™ Thermal Cycler (Bio-Rad, USA) using conditions reported by Folmer et al. [30] and adjusting the annealing temperature to 50 °C. The success of PCR amplification was checked by loading 10 mL of PCR products on 1.5% w/v agarose gel containing 10 µL/mL ethidium bromide/TAE buffer. Amplicons on the agarose gel were visualized in the Fusion FX Gel Documentation System (Vilber Lourmat, France).

2.3. Sequence analysis and phylogenetic relationships

PCR products were purified from agarose gel using a commercial kit (Roche, Mannheim, Germany). Purified amplicons were sequenced bidirectionally with amplification primers on the Sanger Sequencing Platform (Macrogen, Netherlands). Primer sequences in the obtained chromatograms were trimmed prior to analyses. The final forward and reverse sequences were assembled in Geneious Prime 2019.2.1 (https://www.geneious.com) to create a single consensus sequence with the De Novo Assemble tool.

Finalized consensus sequences were compared in the NCBI database by using BLASTn searchers. A CO1 data set comprised of 32 sequences in total was constituted with retrieved genetically related sequences from different countries in GenBank for phylogenetic analyses. MUSCLE plugin in Geneious Prime was utilized for multiple alignments of the sequences [36]. DNA polymorphism and haplotype analyses were performed with DnaSp v.5.1 [37]. The calculation of intraspecific and interspecific differences was evaluated with MEGA 7 [38] via the Kimura two-parameter (K2P) model [39].

Maximum Likelihood (ML) estimation was used to infer phylogenetic relationships. GTR+G was determined as the most appropriate DNA substitution model for ML analyses using the software jModeltest v.0.1.1 [40]. ML analyses were performed in PhyML software [41]. The robustness of the ML tree was estimated using a bootstrap analysis of 1000 replications.

3. Results

3.1. Morphologic identification

The collected flies that were close to both honey and bumble bees in appearance were identified as the females of *E. tenax* according to the described keys (Figure 1). Briefly, the black medial strip of the face was broad, more than one-third the width of the face. The arista of the flies was short-haired, almost bare, eyes with a dorsoventral band of generally denser and darker hairs than on other parts. Katepimeron was hairy, front and mid tarsi were black, prostigma was short, and abdomen was broad and shining.

3.2. Sequence characterization and phylogenetic relationships

Successful amplification of the barcode region of CO1 mt-DNA was obtained for all 10 isolates in PCR analyses (Figure 2). After trimming the primer sequences from the reads, the consensus sequences of the barcoding region (658 bp) from all isolates were successfully obtained with high-quality chromatogram scores. All sequences were designated as forming functional mitochondrial products due to the absence of insertions, deletions, or stop codons. The BLASTn analysis of the obtained sequences confirmed the morphological identifications as the specimens belonging to *E. tenax*. No polymorphic sites were designated among the CO1 sequences of all *E. tenax* isolates, resulting in the detection of a single haplotype (EtenaxTR1). The CO1 sequence of EtenaxTR1 was submitted to GenBank with accession MN565029. The base composition of the barcode CO1 region of the obtained haplotype was highly AT biased with a mean GC content of 31.2%.

A total of 111 polymorphic sites, of which 79 were parsimony informative, were determined within the entire CO1 data set, including the sequences of *E. tenax*, *E. cryptarum*, *E. dimidiate*, *E. fraterculus*, *E. hirta*, *E. interrupta*, *E. rupium*, and *E. stipator*. Five totally different haplotypes were determined among the CO1 sequences of *E. tenax*. Mean haplotype and nucleotide diversities for

E. tenax were 0.775 and 0.002, respectively. Intraspecific nucleotide differences for *E. tenax* were determined as 0.2 ± 0.1%. Interspecific differences among the *Eristalis* species in the CO1 data set are presented in Table 1. *E. tenax* CO1 sequences were closer to *E. hirta*, while the highest distance was found with *E. stipator*.

The ML tree with bootstrap support (ML) values is given in Figure 3. All sequences were classified within

Figure 1. Adult female *E. tenax* collected from Central Anatolia, Turkey. Lateral view (left): Hairy katepimeron, black front and mid tarsi, broad and shinning abdomen, prostigma was short and abdomen was broad and shining. Dorsal view (right): Short-haired arista, black and broad medial strip of the face.

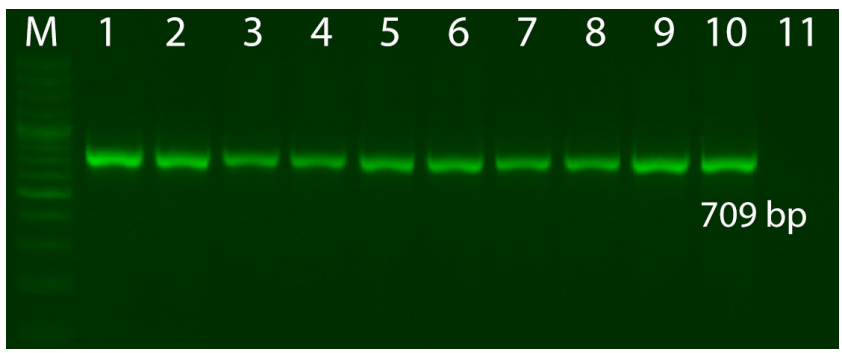

Figure 2. Amplification products of the barcode CO1 region from the examined *E. tenax* isolates. M: Marker (100 bp), 1–10: gDNA from *E. tenax* isolates, 11: No DNA.

Table 1. Interspecific genetic distances (below the diagonal) ± standard deviations (above the diagonal) for *Eristalis* species in CO1 data set.

		1	2	3	4	5	6	7	8
1	*E. tenax*		0.010	0.010	0.010	0.011	0.011	0.012	0.011
2	*E. hirta*	0.057		0.001	0.008	0.008	0.010	0.010	0.012
3	*E. rupium*	0.059	0.002		0.009	0.008	0.010	0.010	0.012
4	*E. interrupta*	0.064	0.044	0.045		0.009	0.010	0.011	0.012
5	*E. cryptarum*	0.071	0.048	0.050	0.057		0.010	0.011	0.012
6	*E. fraterculus*	0.068	0.058	0.060	0.059	0.062		0.011	0.011
7	*E. dimidiata*	0.075	0.060	0.062	0.067	0.068	0.067		0.012
8	*E. stipator*	0.079	0.077	0.079	0.072	0.078	0.070	0.080	

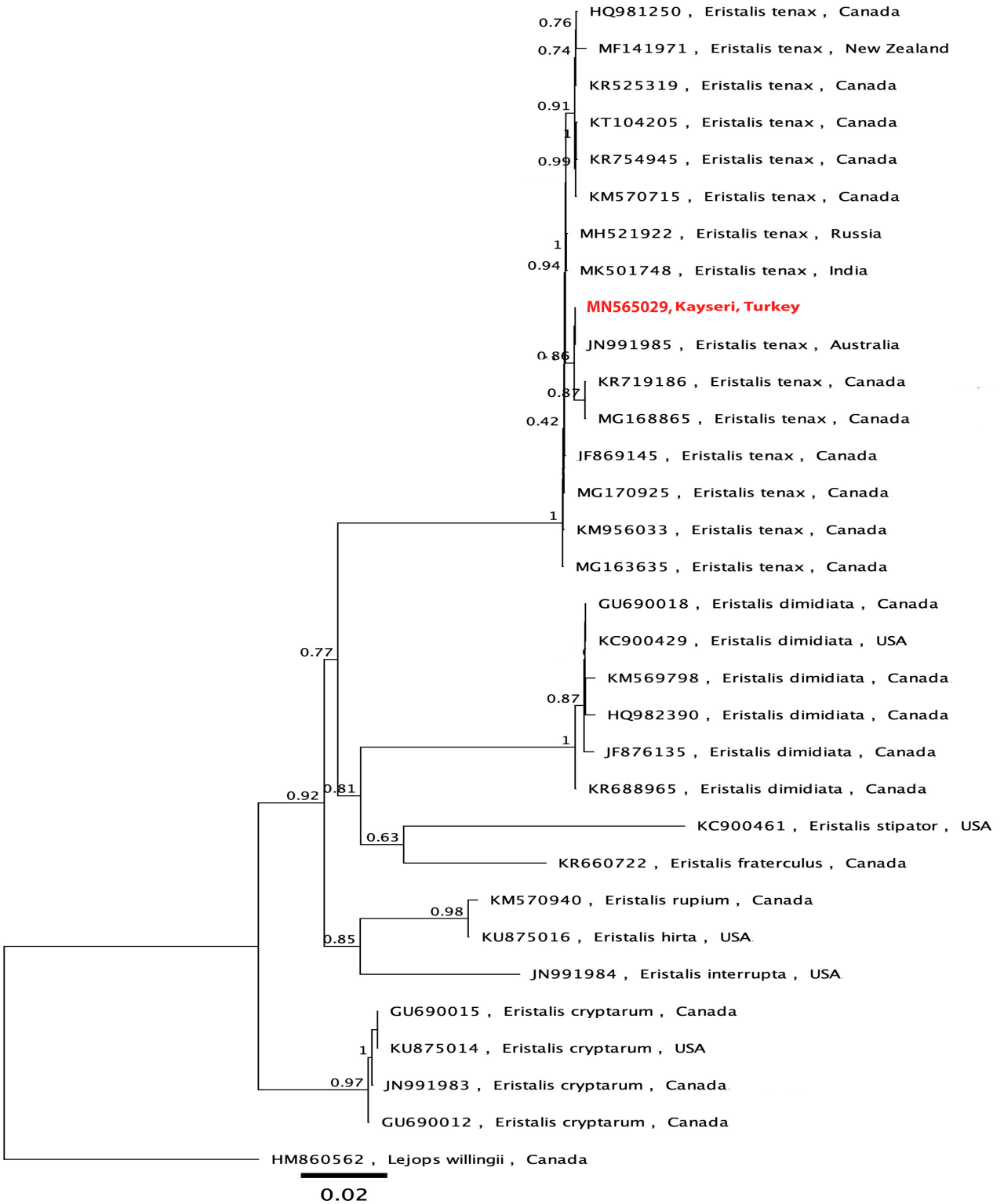

Figure 3. Phylogenetic analysis of *E. tenax* and other species of *Eristalis* genus. The obtained haplotype is shown in bold red character. Bootstrap values (1000 replicates) are shown at the nodes. *Lejops willingii* CO1 sequence was used as the outgroup. The scale bar represents a 0.02% divergence.

species-based clades with the support of bootstrap values over 97.0%. The newly identified EtenaxTR1 haplotype clustered in a monophyletic clade (Figure 3) with published haplotypes from Australia and Canada with high bootstrap value (86.0%). The EtenaxTR1 was found wholly identical to the isolate reported from Australia (JN991985) and also showed a high identity rate of 99.8% to the isolates reported from Canada (KR719186, MG168865).

4. Discussion

Due to their heterogeneous feature and global distribution, the flies in family Syrphidae constitute an important and diverse taxon for ecological and biogeographical studies [42]. Although the prevalence of *E. tenax* in several regions of Turkey has been reported [22–25], no data is available for the genetic composition and diversity of these flies prevalent throughout Turkey. We determined that many active flies were present in the cattle barns during visits for screening hypodermosis in cattle. It was thought that those flies could belong to the *Hypoderma* species because many cattle harbored typical subcutaneous nodules that contained warbles under the skin in the dorsal and lumbar regions. Laboratory examination and later molecular identification revealed that all flies belonged to *E. tenax*. As indicated by several researchers [43,44], the environment polluted with livestock manure probably presented suitable conditions for the development of *E. tenax* in the region.

Genotyping and phylogenetic characterization of *E. tenax* populations circulating in the Kayseri province of Central Anatolia Region were utilized by using CO1 DNA barcode sequences in this study. This standardized short gene region from mitochondrial DNA is generally considered as a reliable, cost-effective, and easy molecular identification tool, with wide use throughout the metazoan taxa [45,46]. CO1 barcoding has also been used for species delimitation in various genera in Syrphidae, including *Cheilosia vernalis* complex [47], the genus *Merodon* [48], *Chrysotoxum* [49], *Platycheirus* [50], *Eumerus* [42], and the Afrotropical species [51]. In accordance with those studies [42,50,51], the barcode CO1 region successfully delimitated the species of *Eristalis* in separate monophyletic clusters with an overall interspecific genetic distance of 6.8%. However, haplotype and nucleotide diversities within *E. tenax* were relatively lower than some other members of the Syrphidae, such as the species of *Eumerus* [42] and *Platycheirus* [50]. The low level of genetic diversity in *E. tenax* among different geographical areas might suggest a lower impact of geographical distance over the gene flow among *E. tenax* populations. Wholly identical genetic structure of the isolates from this study and from Australia (JN991985), which are very far from each other, could also support this inference. However, further large-scale sampling from different regions is needed to explore the accurate genetic diversity of *E. tenax*.

In conclusion, this study provides the first genetic characterization of *E. tenax* populations in Kayseri province of Turkey and contributes knowledge to molecular biology and genetic diversity of flies belonging to Syrphidae. Considering the importance of drone fly *E. tenax*, further studies are needed to clarify the genetic diversity and epidemiology of these flies in Turkey.

References

1. Thompson FC, Rotheray G. Family Syrphidae. In: Papp L, Darvas B (editors). Manual of Palaearctic Diptera. 3rd ed. Budapest, Hungary: Science Herald; 1998. pp. 81-139.
2. Francuski L, Djurakic M, Ludoški J, Milankov V. Landscape genetics and spatial pattern of phenotypic variation of *Eristalis tenax* across Europe. Journal of Zoological Systematics and Evolutionary Research 2013; 51 (3): 227-238. doi: 10.1111/jzs.12017
3. Guo Y, Zhang X, Shao Y, Li J. Evaluation of diversity and abundance of pollinating insects on oilseed rape in major planting area of China. International Journal of Agricultural Policy and Research 2017; 5 (6): 117-124. doi: 10.15739/IJAPR.17.013
4. Buckton GB. The Natural History of *Eristalis tenax* or the Drone-fly. London, UK: Macmillan; 1895.
5. Mehrabi R, Ssymank A. Species composition and flower visiting by Syrphidae (Diptera) in north-eastern Iran. Zoology in the Middle East 2008; 45 (1): 73-78. doi: 10.1080/09397140.2008.10638309
6. Patnaik H, Satapathy C, Panda N. Prevalence, species richness and diversity of flower visiting insects at Bhubaneswar (Odisha). Journal of Plant Protection and Environment 2012; 9 (1): 1-10.
7. Howlett BG, Gee M. The potential management of the drone fly (*Eristalis tenax*) as a crop pollinator in New Zealand. New Zealand Plant Protection 2019; 72: 221-230. doi: 10.30843/nzpp.2019.72.304
8. Golding Y, Edmunds M. Behavioural mimicry of honeybees (*Apis mellifera*) by droneflies (Diptera: Syrphidae: *Eristalis* spp.). Proceedings of the Royal Society B. Biological sciences 2000; 267 (1446): 903-909. doi: 10.1098/rspb.2000.1088
9. Golding YC, Ennos AR, Edmunds M. Similarity in flight behaviour between the honeybee *Apis mellifera* (Hymenoptera: Apidae) and its presumed mimic, the dronefly *Eristalis tenax* (Diptera: Syrphidae). The Journal of Experimental Biology 2001; 204: 139-145.

10. Howarth B, Edmunds M, Gilbert F. Does the abundance of hoverfly (Syrphidae) mimics depend on the numbers of their hymenopteran models? Evolution 2004; 58 (2): 367-375. doi: 10.1554/02-491

11. Pérez-Bañón C, Rojo S, Ståhls G, Marcos-García M. Taxonomy of European *Eristalinus* (Diptera: Syrphidae) based on larval morphology and molecular data. European Journal of Entomology 2003; 100 (3): 417-428. doi: 10.14411/eje.2003.064

12. Riedel L, Lunau K. Limitations of the innate proboscis reflex in *Eristalis tenax* L. (Diptera: Syrphidae) by the spatial resolution of the compound eye. Entomologie Heute 2015; 27: 45-54.

13. Gerhardt RR, Hribar LJ. Flies (Diptera). In: Mullen G, Durden L (editors). Medical and Veterinary Entomology. 3rd ed. London, UK: Academic Press; 2019. pp. 171-188.

14. Fischer OA, Matlova L, Dvorska L, Svastova P, Bartos M et al. Potential risk of *Mycobacterium avium* subspecies paratuberculosis spread by syrphid flies in infected cattle farms. Medical and Veterinary Entomology 2005; 19 (4): 360-366. doi: 10.1111/j.1365-2915.2005.00585.x

15. Francuski L, Matić I, Ludošk, J, Milankov V. Temporal patterns of genetic and phenotypic variation in the epidemiologically important drone fly, *Eristalis tenax*. Medical and Veterinary Entomology 2011; 25 (2): 135-147. doi: 10.1111/j.1365-2915.2011.00956.x

16. Aguilera A, Cid A, Regueiro B, Prieto J, Noya M. Intestinal myiasis caused by *Eristalis tenax*. Journal of Clinical Microbiology 1999; 37 (9): 3082.

17. Catts EP, Mullen GR. Myiasis (Muscoidea and Oestroidea). In: Mullen G, Durden L (editors). Medical and Veterinary Entomology. USA: Academic Press; 2002. pp. 317-348.

18. Mumcuoglu I, Akarsu GA, Balaban N, Keles I. *Eristalis tenax* as a cause of urinary myiasis. Scandinavian Journal of Infectious Diseases 2005; 37 (11-12): 942-943. doi: 10.1080/00365540510043275

19. González M, Comte G, Monárdez J, Díaz de Valdés M, Matamala I. Miasis genital accidental por *Eristalis tenax*. Revista Chilena de Infectologia 2009; 26 (3): 270-272 (in Spanish with an abstract in English). doi: 10.4067/S0716-10182009000400012

20. Desoubeaux G, Gaillard J, Borée-Moreau D, Bailly É, Andres CR et al. Gastrointestinal symptoms resembling ulcerative proctitis caused by larvae of the drone fly *Eristalis tenax*. Pathogens and Global Health 2014; 108 (3): 158-163. doi: 10.1179/2047773214Y.0000000135

21. Salimi M, Edalat H, Jourabchi A, Oshaghi M. First report of human nasal myiasis caused by *Eristalis tenax* in Iran (Diptera: Syrphidae). Iranian Journal of Arthropod-Borne Diseases 2010; 4 (1): 77-80.

22. Hayat R. Fauna of Syrphidae (Diptera) in the vicinity of Erzurum (I), Syrphinae. Turkish Journal of Entomology 1990; 14: 173-182.

23. Candemir D, Kara K. Syrphidae (diptera) fauna in Tokat provinces (Turkey). Turkish Journal of Entomology 2003; 27: 95-105.

24. Sarıbıyık S. Contributions to the Syrphidae fauna of Turkey (Diptera: Syrphidae). Entomological News 2008; 119: 501-509. doi: 10.3157/0013-872X-119.5.501

25. Gözüacık C, Özgen I. Contribution to the syrphidae (diptera) fauna in the cereal fields of souteasthern Anatolia, Turkey. Munis Entomology & Zoology Journal 2018; 13: 522-526.

26. Yalcinkaya F. A case of nasal myiasis caused by the larvae of the drone fly. Turkish Bulletin of Hygiene and Experimental Biology 1976; 36 (1): 40-44.

27. Dik B, Uslu U, Isık N. Myiasis in animals and human beings in Turkey. Journal of the Faculty of Veterinary Medicine, University of Kafkas 2012; 18 (1): 37-42. doi: 10.9775/kvfd.2011.4654

28. Hebert PD, Cywinska A, Ball SL, Dewaard JR. Biological identifications through DNA barcodes. Proceedings of the Royal Society B. Biological sciences 2003; 270 (1512): 313-321. doi: 10.1098/rspb.2002.2218

29. Hebert PD, deWaard JR, Landry JF. DNA barcodes for 1/1000 of the animal kingdom. Biology Letters 2010; 6: 359-362. https://doi.org/10.1098/rsbl.2009.0848

30. Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 1994; 3 (5): 294-299.

31. Stahls G, Vujic A, Perez-Banon C, Radenkovic S, Rojo S et al. COI barcodes for identification of Merodon hoverflies (Diptera, Syrphidae) of Lesvos Island, Greece. Molecular Ecology Resources 2009; 9 (6): 1431-1438. doi: 10.1111/j.1755-0998.2009.02592.x

32. Gibson JF, Kelso S, Jackson MD, Kits JH, Miranda GFG et al. Diptera-specific polymerase chain reaction amplification primers of use in molecular phylogenetic research. Annals of the Entomological Society of America 2011; 104: 976-997. doi: 10.1603/an10153

33. Marcos-García MÁ, Vujić A, Ricarte A, Ståhls G. Towards an integrated taxonomy of the *Merodon equestris* species complex (Diptera: Syrphidae) including description of a new species, with additional data on Iberian Merodon. The Canadian Entomologist 2011; 143: 332-348. doi: 10.4039/n11-013

34. Radenković S, Vujić A, Ståhls G, Pérez-Bañón C, Rojo S et al. Three new cryptic species of the genus Merodon Meigen (Diptera: Syrphidae) from the island of Lesvos (Greece). Zootaxa 2011; 2735 (1): 35-56. doi: 10.11646/zootaxa.2735.1.5

35. Hippa H, Nielsen TR, van Steenis J. The West Palaearctic species of the genus *Eristalis* Latreille (Diptera, Syrphidae). Norwegian Journal of Entomology 2001; 48: 289-289.

36. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 2004; 32 (5): 1792-1797. doi: 10.1093/nar/gkh340

37. Librado P, Rozas J. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 2009; 25 (11): 1451-1452. doi: 10.1093/bioinformatics/btp187

38. Kumar S, Stecher G, Tamura K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 2016; 33 (7): 1870-1874. doi: 10.1093/molbev/msw054

39. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 1980; 16 (2): 111-120. doi: 10.1007/bf01731581

40. Posada D. jModelTest: phylogenetic model averaging. Molecular Biology and Evolution 2008; 25 (7): 1253-1256. doi: 10.1093/molbev/msn083

41. Guindon S, Gascuel O. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology 2003; 52 (5): 696-704. doi: 10.1080/10635150390235520

42. Chroni A, Djan M, Vidakovic DO, Petanidou T, Vujic A. Molecular species delimitation in the genus *Eumerus* (Diptera: Syrphidae). Bulletin of Entomological Research 2017; 107 (1): 126-138. doi: 10.1017/s0007485316000729

43. Pérez-Bañón C, Hurtado P, García-Gras E, Rojo S. SEM studies on immature stages of the drone flies (diptera, syrphidae): *Eristalis similis* (Fallen, 1817) and *Eristalis tenax* (Linnaeus, 1758). Microscopy Research and Technique 2013; 76 (8): 853-861. doi: 10.1002/jemt.22239

44. Campoy A, Pérez-Bañón C, Nielsen TR, Rojo S. Micromorphology of egg and larva of *Eristalis fratercula*, with an updated key of *Eristalis* species with known third instar larvae (Diptera: Syrphidae). Acta Entomologica Musei Nationalis Pragae 2017; 57 (1): 215-227. doi: 10.1515/aemnp-2017-0070

45. Hebert PD, Penton EH, Burns JM, Janzen DH, Hallwachs W. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly Astraptes fulgerator. Proceedings of the National Academy of Sciences of the United States of America 2004; 101 (41): 14812-14817. doi: 10.1073/pnas.0406166101

46. Hebert PD, Stoeckle MY, Zemlak TS, Francis CM. Identification of birds through DNA barcodes. PLoS Biology 2004; 2 (10) :e312. doi: 10.1371/journal.pbio.0020312

47. Ståhls G, Vujić A, Milankov V. *Cheilosia vernalis* (Diptera, Syrphidae) complex: molecular and morphological variability. Annales Zoologici Fennici 2008; 45 (2): 149-159. doi: 10.5735/086.045.0206

48. Milankov V, Ludoški J, Francuski L, Ståhls G, Vujić A. Genetic and phenotypic diversity patterns in *Merodon albifrons* Meigen, 1822 (Diptera: Syrphidae): evidence of intraspecific spatial and temporal structuring. Biological Journal of the Linnean Society 2013; 110 (2): 257-280. doi: 10.1111/bij.12127

49. Nedeljković Z, Ačanski J, Vujić A, Obreht D, Dan M et al. Taxonomy of *Chrysotoxum festivum* Linnaeus, 1758 (Diptera: Syrphidae) – an integrative approach. Zoological Journal of the Linnean Society 2013; 169 (1): 84-102. doi: 10.1111/zoj.12052

50. Young AD, Marshall SA, Skevington JH. Revision of *Platycheirus* Lepeletier and Serville (Diptera: Syrphidae) in the Nearctic north of Mexico. Zootaxa 2016; 4082 (1): 1-317. doi: 10.11646/zootaxa.4082.1.1

51. Jordaens K, Goergen G, Virgilio M, Backeljau T, Vokaer A et al. DNA barcoding to improve the taxonomy of the afrotropical hoverflies (Insecta: Diptera: Syrphidae). PLoS One 2015; 10 (10): e0140264. doi: 10.1371/journal.pone.0140264