

MOLECULAR (DNA) IDENTIFICATION OF THE MAIN PEST OF ALMOND ALMOND WASP (EURYTOMA SAMSONOVI) AND DATA SUBMISSION TO NCBI DATABASE

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The DNA sequences obtained from species belonging to the genus *Eurytoma* through sequencing, as well as nucleotide sequences of related species retrieved from the National Center for Biotechnology Information (NCBI) database (<https://www.ncbi.nlm.nih.gov>), were utilized in this study. These sequences were manually edited using the Genius Prime software, and consensus sequences were calculated with the MEGA X computational program (Figure 1).

The preliminary data obtained from this program, together with additional sequences retrieved from the GenBank database, were aligned using the MAFFT v.7 online tool and the Clustal Omega 1.2.2 software with standard parameters, and subsequently edited in Genius Prime. The nucleotide sequences belonging to the COI region were analyzed through the construction of a Maximum Likelihood (ML) phylogenetic tree, performed with 1000 bootstrap replications using the RAxML 1.6.12 version with ultrafast bootstrap approximation, and further analyses were conducted in PAUP* version 3.3 (Swofford D.L.).

The nucleotide sequence of the COI region belonging to *Adelphocorisella lespedezae* (KY367057) was included as an outgroup to facilitate the construction of consensus trees. The resulting phylogenetic tree was analyzed and edited using the iTOL v6.6 software.

The molecular genetic classification of *Eurytoma samsonovi* Vassiliyev, a species belonging to the genus *Eurytoma* Illiger, 1807, was performed. Accurate species identification is considered crucial for conducting ecological and evolutionary studies. Morphology-based keys often fail to provide reliable identification (Toft, S., 1983; Grosjean, S., Thomas, M., Glaw, F., Vences, M., 2006).

In recent years, alongside morphological, morphometric, and other traditional approaches, molecular-genetic studies have increasingly been employed to facilitate species identification. One of the pioneering works in this field was conducted by Hebert et al. (2002), who introduced the concept of DNA barcoding based on nucleotide sequences of the COI region of genomic DNA, enabling reliable species identification.

For members of this family, preliminary studies utilizing mitochondrial DNA COI sequences for species identification were carried out by Damgaard (2008) and Memon et al. (2006). Subsequent research by Jung et al. (2011) and Park et al. (2011) confirmed that DNA barcoding represents an effective method for accurate species determination.



Figure 1. Sample collection for the molecular (DNA) identification of the almond wasp (*Eurytoma samsonovi*).

Hebert (2003) proposed that, for species identification, nucleotide divergence within the COI region of mitochondrial DNA should not exceed 3% at the intraspecific level (R.A.Jumaev).

Table 1.

Information on species (mtDNA, COI) obtained from the International Center for Biotechnology Information (GenBank).

№	Species	Accession Number	Country of Study
1.	<i>Eurytoma samsonovi</i>	HC 037042	USA
2.	<i>Eurydema gebleri</i>	NC 027489	USA
3.	<i>Eurydema oleracea</i>	NC 044764	USA
4.	<i>Eurydema qinlingensis</i>	NC_044765	USA
5.	<i>Eysarcoris ventralis</i>	MT165688	China
6.	<i>Bagrada hilaris</i>	KU525197	USA
7.	<i>Bagrada abeillei</i>	PP736568	Uzbekistan
8.	<i>Bagrada kaufmanni</i>	PP726658	Uzbekistan
9.	<i>Dolycoris penicillatus</i>	OQ909523	China
10.	<i>Chlorochroa pinicola</i>	PP724395	Uzbekistan
11.	<i>Chlorochroa pinicola</i>	MZ660163	Sweden
12.	<i>Chlorochroa juniperina</i>	MZ660781	Sweden
13.	<i>Chlorochroa ligata</i>	KR581752	Canada
14.	<i>Chlorochroa ligata</i>	KR039777	Canada
15.	<i>Chlorochroa uhleri</i>	KR032364	Canada
16.	<i>Chlorochroa uhleri</i>	MF679641	USA
17.	<i>Chlorochroa persimilis</i>	GU692433	Canada
18.	<i>Chlorochroa persimilis</i>	HM374605	Canada
19.	<i>Chlorochroa sayi</i>	MF679624	USA
20.	<i>Chlorochroa sayi</i>	MF679623	USA
21.	<i>Nezara viridula</i>	KY843444	Canada
22.	<i>Chlorochroa rossiana</i>	KU874601	USA

Table 2.

DNA extraction of *Eurytoma samsonovi* and identification of nucleotide differences among closely related species.

	10	20	30	40	50	60	70	80
								
<i>E_samsonovi_Uz</i>	TCGAATAAAT AATATAAGTT TTTGATTTTT							
	AATTCCTAGA TTAATGTTAT TAATTTTATAG GATGTTTATT							
	GGTAATGGAA							
<i>E_rhois_EF185154</i>	A..A.....CA.. A..A.....A....G.							
	90	100	110	120	130	140	150	160
								
<i>E_samsonovi_Uz</i>	CTGGAAGTGG ATGAACTGTT TATCCTCCTT							
	TATCCGGAAA TTTATCTCAT GGGGGTCCTT CAGTTGATTT							
	ATCAATTTTT							
<i>E_rhois_EF185154</i>	...G..... ..A..G ..C.....A.T.....G..A.A..... ..T....							
	170	180	190	200	210	220	230	240
								
<i>E_samsonovi_Uz</i>	TCTTTACATG TAGCAGGAGT AAGTTCTATT							
	ATAGGTTCAA TTAATTTTAT TTCTACGATT ATAAAGATAA							
	AAGTTTATAA							
<i>E_rhois_EF185154</i>	G....T..T..G.. T....A... ..A..G.. .C.....A..A.....T...A.							
	250	260	270	280	290	300	310	320
								
<i>E_samsonovi_Uz</i>	AATGGAGTTA ATTCCATTAT TTGCTTGAGC							
	AATATTATTG ACAGCTATTT TATTACTTTT ATCTTTACCT							
	GTTTTCAGCAG							
<i>E_rhois_EF185154</i>	...T..A.....T.....G..A ...A.....T.A..G.....T.							
	330	340	350	360	370	380	390	400
								
<i>E_samsonovi_Uz</i>	GTGCTATTAC TATACTATTA TTTGATCGAA							
	ATTTAAATAC TTCTTTTTTTT GATCCTGCTG GAGGGGGTGA							
	TCCAATTTTA							
<i>E_rhois_EF185154</i>	.A..... ..G..T... ..T.A.....G.. ...T...							
	410	420	430	440	450	460	470	480
								
<i>E_samsonovi_Uz</i>	TATCAACATT TATTTTGATT TTTTGGACAT							
	CCAGAGGTTT ATATTTTAAT TTTACCAGGA TTTGGTTTAA							
	TTTCTCATAT							
	490	500	510	520	530	540	550	560
								
<i>E_samsonovi_Uz</i>	AATTTGTAAT GAAAGAATAA AAAAAGAAAC							
	TTTGGAGCT ATAGGAATAA TTTATGCAAT AATTTCTATT							
	GGTTTATTGG							

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E_rhois_EF185154 ...GG .....G.. .....G... ..G.....A... ..A..G....
570      580      590      600      610      620      630      640
      ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
E_samsonovi_Uz  GATTATTGT TTGAGCTCAT CATATATTTA
CTGTAGGTAT AGATGTAGAT ACTCGTGCAT ATTTTACATC
                TGCAACAATA
E_rhois_EF185154 ..... G..... ..A....A.. G.....A..T.....T.....T...
650      660      670      680      690      700
      ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....
E_samsonovi_Uz  ATTATTGCAG TTCCTACAGG GATTAAAATT
TTTAGATGAT TAGCTTCTAT GAATGGTATA AAAA
E_rhois_EF185154..... A..... A.....

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In addition to species identification, nucleotide sequences of the COI region of DNA can also serve to investigate phylogenetic relationships (Hua et al., 2008; Li et al., 2005).

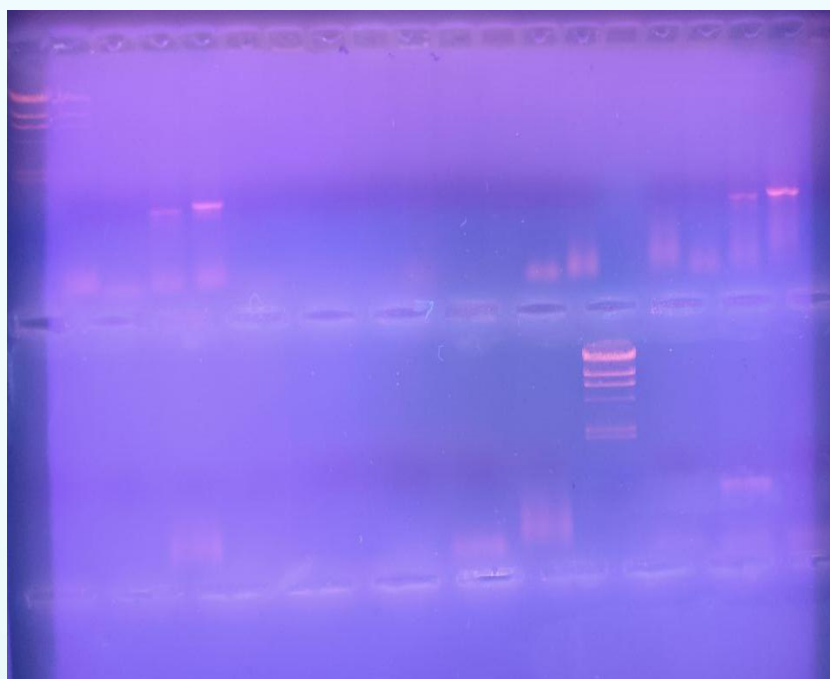


Figure 2. Electrophoresis process for molecular (DNA) identification of almond wasp (*Eurytoma samsonovi*) specimens.

In this study, molecular genetic analysis was conducted on *Eurytoma samsonovi* Vassiliyev, a species belonging to the genus *Eurytoma* Illiger, 1807, collected from the Fergana Valley regions of our Republic. Based on the results of molecular-genetic investigations (sequence chromatograms) of *Eurytoma* species, the mitochondrial DNA COI region of *E. samsonovi* was found to consist of 704 base pairs. For comparative analysis, sequences of *E. rhois* (accession number:

EF577427) were obtained from the International Bioinformatics Center (<https://blast.ncbi.nlm.nih.gov>).

According to bioinformatic analysis, *E. samsonovi* exhibited 69 nucleotide differences when compared with *E. rhois* sequences retrieved from GenBank (accession number: KU525197). (R.A.Jumaev).

Eurytoma samsonovi isolate es11 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial

GenBank: PQ038756.1

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LOCUS	PQ038756	704 bp	DNA	linear	INV 21-JUL-2024
DEFINITION	Eurytoma samsonovi isolate es11 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial.				
ACCESSION	PQ038756				
VERSION	PQ038756.1				
KEYWORDS	.				
SOURCE	mitochondrion Eurytoma samsonovi				
ORGANISM	Eurytoma samsonovi				
	Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Hymenoptera; Apocrita; Proctotrupomorpha; Chalcidoidea; Eurytomidae; Eurytominae; Eurytoma.				
REFERENCE	1 (bases 1 to 704)				
AUTHORS	Hasanov,A.M., Jumayev,R.A., Kuchboyev,A.E. and Amirov,O.				
TITLE	Molecular genetic analysis of Eurytoma samsonovi species				
JOURNAL	Unpublished				
REFERENCE	2 (bases 1 to 704)				
AUTHORS	Hasanov,A.M., Jumayev,R.A., Kuchboyev,A.E. and Amirov,O.				
TITLE	Direct Submission				
JOURNAL	Submitted (16-JUL-2024) Plant Protection and Quarantine, Tashkent State Agrarian University, 2 University Street, Tashkent, Tashkent 100140, Uzbekistan				

Figure 3. Information on the deposition of *Eurytoma samsonovi* into the International Insect GenBank (NCBI) (July 21, 2024).

([https://www.ncbi.nlm.nih.gov/nucleotide/PQ038756.1?report=genbank&log\\$=nucldtop&blast_rank=1&RID=F9ZJ26AYE013](https://www.ncbi.nlm.nih.gov/nucleotide/PQ038756.1?report=genbank&log$=nucldtop&blast_rank=1&RID=F9ZJ26AYE013)).

As shown above, nucleotide differences were identified between *Eurytoma samsonovi* and *Eurytoma rhois* (GenBank accession number: KU525197). The comparative analysis revealed the following substitutions(R.A.Jumaev):

At positions 28, 58, 73, 97, 109, 139, 145, 187, 196, 214, 298, 322, 376, 466, 547, 553, 592, 598, and 616, *E. samsonovi* contained thymine (T), whereas *E. rhois* contained adenine (A). At positions 46, 61, 64, 217, 233, 247, 280, 284, 436, 661, and 691, *E. samsonovi* contained guanine (G), while *E. rhois* contained adenine (A). At positions 57, 103, and 202, *E. samsonovi* contained thymine (T), whereas *E. rhois* contained cytosine (C). At positions 79, 85, 166, 178, 199, 268, 307, 334, 489, 508, 517, 523, 556, and 601, *E. samsonovi* contained adenine (A), while *E. rhois* contained guanine (G). At positions 100, 136, 388, 490, and 571, *E. samsonovi* contained thymine (T), whereas *E. rhois* contained guanine (G). At positions 115 and 296, *E. samsonovi* contained cytosine (C), while *E. rhois* contained thymine (T). At positions 154, 172, 175, 181, 256, 319, 337, 349, 394,

427, 619, 628, and 637, *E. samsonovi* contained adenine (A), whereas *E. rhois* contained thymine (T). At positions 226 and 244, *E. samsonovi* contained guanine (G), while *E. rhois* contained thymine (T). These findings highlight significant nucleotide substitutions between the two species, supporting the molecular distinction of *E. samsonovi* within the genus *Eurytoma*. In the present study, bioinformatic analysis was performed on the mitochondrial DNA COI region sequences of *Eurytoma samsonovi* and *Eurytoma rhois* (GenBank accession number: KU525197). The comparison revealed 69 nucleotide differences between the two species, corresponding to a divergence of 9.8%.

For the first time, the species *Eurytoma samsonovi* has been deposited in the National Center for Biotechnology Information (NCBI) database, and an accession number was assigned (*Eurytoma samsonovi* – accession number: PP726658).

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