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MOLECULAR ANALYSIS OF *ANAPULVINARIA PISTACIAE* (BODENHEIMER)

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ABSTRACT

This study is on the molecular evolutionary genetic analysis of *Anapulvinaria pistaciae* (Bodenheimer), which is a serious pest of pista. This study explored its ribosomal DNA from area 5.8S, and it now listed with the National Biotechnology Information Center (NCBI, GenBank- entry number OR074914.1). The codons involved in the coding of amino acid codons are distinguished. Tajima's relative velocity test was used. A dendrogram of *A. pistaciae* has been developed using the maximum likelihood method.

Key words: *Anapulvinaria pistaciae,* ITS, RSCU, codon, *Pistacia vera,* 5.8S rDNA, Uzbekistan, Tajima's test, phylogenetic tree, pest, coccidae, Hemiptera, dendrogram, 5.8^{s}

Pistachios are one of the plants widely used in national economy, industry and medicine (Khojimatov et al., 2009). *Pistacia vera* (L.) is a xerophytic deciduous tree that grows in arid regions of Central and Western Asia, including Iran and Afghanistan (Benny et al., 2022). Among the insects that cause serious damage to the pistachio, coccids are important. One such species is the *Anapulvinaria pistaciae* (Bodenheimer), which is characterized by yellowing of the leaves of plants, poor quality of the fruit, or spilling due to the plant's continuous absorption of tissue fluid (Zokirov et al., 2021). This pest is distributed in Syria, Tajikistan, Turkey, Ukraine, Uzbekistan, Kyrgyzstan, Azerbaijan, Turkmenistan, Iraq, Iran, Greece, Cyprus, Armenia, Afghanistan, Israel (GBIF) (https://www.gbif.org/species/7980945). Information about this pest can be found in many previous studies (Yaman, 1970; Santas, 1985; Mehrnejad, 2001; Yanik et al., 2001; Moghaddam, 2013; Ben-Dov, 2012; Korghond et al., 2018; Bolu et al., 2003). In Central Asia, this is known from few studies (Arkhangelskaya, 1937; Abdrashitova et al., 2005; Zokirov, 1972; Sobirov et al., 2018). These studies provide its distribution, morphological, biological and ecological characteristics. Its morphology and cytogenetics have been studied (Gavrilov-Zimin, 2011). However, its ribasomal DNA has not been studied in the 5.8 s domain; however, mtCo1 of the population from Iran is available (LC785427 and LC785426). This study found a phylogenetic inference of the ITSI region between species by identifying *A. pistaciae* closely related species in nucleotides of the rDNA 5.8 s domain.

MATERIALS AND METHODS

Specimen collection was done in 2021-2023 from pistachios of Andijan region, established in Hojaabad and Andijan districts. The GPS coordinates and collection details are as given below-

Date	Region	District	GPS coordinates		Collector
25.03.2022	Andijan	Bogishamol	40°43'08.9"N	72°26'09.8"E	X.R. Kahhorova
6.04.2022	Andijan	Bogishamol	40°43'09.4"N	72°26'09.6"E	X.R. Kahhorova
3.07.2022	Andijan	Bogishamol	40°43'09.8"N	72°26'07.8"E	X.R. Kahhorova
15.04.2023	Andijan	Bogishamol	40°43'08.4"N	72°26'07.5"E	X.R. Kahhorova
13.06.2022	Andijan	Bogishamol	40°43'08.7"N	72°26'08.2"E	O.T. Sobirov; X.R. Kahhorova
6.04.2022	Andijan	Bogishamol	40°43'06.2"N	72°26'13.0"E	X.R. Kahhorova
13.06.2022	Andijan	Bogishamol	40°43'11.4"N	72°26'07.6"E	O.T. Sobirov; X.R. Kahhorova
6.04.2022	Andijan	Bogishamol	40°43'09.9"N	72°26'09.0"E	X.R. Kahhorova
11.04.2023	Hojaobod	Imamota	40°32'40.2"N	72°36'31.0"E	O.T. Sobirov
18.04.2023	Hojaobod	Imamota	40°32'37.8"N	72°36'30.8"E	O.T. Sobirov

These samples were fixed in 70% ethanol in 10 ml via (Abdrashitova, 2005; Borhsenius, 1950); in 2023, females were obtained from the biennial branches of the plant *Pistacia vera* from Andijan district, Bogishamol Park (40°43'09.4"N 72°26'09.6"E, 630 masl) for the molecular analysis. These were brought to the laboratory at the Andijan State University, Department of Zoology and Biochemistry and examined under binocular microscope (B-380 ALS, Italy). These were brought to room temperature for 10-15 min until the alcohol evaporated over the dry stump. QIAamp DNA Mini Kit (QIAGEN, Germany) reagents were used in genomic DNA separation. Ribosomal DNA ITS 1-5,8 s-ITS 2 domain-specific nucleotide-reading prymers, widely used in molecular-genetic identification, were used in conducting PCR (Joyce et al., 1994). In PCR, water was prepared from 16.1 ml, 10x pcr buffer 2 ml, dNTP 0.4 ml, each primer (TW81 (5'-GTTTCCGTAGGTGAACCTGC-3') and reverse primer AB28 (5'-ATATGCTTAAGTTCAGGGT-3') 2 ml, Taq polymerase 0.4 ml with total of 20 ml. The PCR was carried out using a programmable automatic chain reaction amplifier (Touchgene Gradient, UK) (PCR). PCR was carried out according to the following scheme: stage 1- DNA denaturation at 95°C for 3 min, stage 2- DNA denaturation at 93°C for 20 sec, stage 3- adhesion of primers in DNA at 55°C for 30 sec, stage 4- elongation at 72°C for 2 min, stage 5- elongation of the chain at 72°C for 10 min. From the second to the fourth stage, the process was repeated up to 35 times in a cycle (Ibrokhimov 2023, Mardanova 2023).

To determine the presence of DNA, electrophoresis was performed at 100V in a 2% agarose gel; after 40-45 min, the gel was examined and photographed in the transilluminator. To purify the DNA, the desired DNA fragments were excised from the gel with a scalpel and placed in a 1.5 ml eppendorph tube. When extracting DNA from the gel, a set of reagents manufactured by "Sileks M" (Moscow, Russia) was used in accordance with the manufacturer's instructions. The DNA content of the purified PCR products was measured and sent to sequencing. Sequencing was carried out at the center of the Central Collective Use Center "Genome" ("Gentotex", Moscow). Analysis was carried out following- K Zakirov et al. (https://www.ncbi.nlm.nih.gov/nuccore/OR074914.1, NCBI, GenBank- OR074914.1), as well as the 17 species closely related to it, used sequences of ribosomal DNA 5.8 s nucleotides. ITS plot sequences of 735 nucleotide pairs were obtained, as well as ITS plot sequences from NCBI. The selected sequences were compared using Multiple Sequence Alignment by MAFFT (https://www.genome.jp/tools-bin/mafft). Based on the data obtained by comparison (a.pistaciae. ph) using IQ-TREE web server (http://iqtree.cibiv.univie.ac.at/), a phylogenetic tree was constructed on maximum likelihood. The phylogenetic tree was visualized (https://itol.embl.de/login.cgi) through iTOL web. Evolutionary analysis was conducted in the MEGA 11 Program (Tamura et al., 2021).

RESULTS AND DISCUSSION

In phylogenetic analysis of *A. pistaciae,* ITS (Internal transcribed spacer) sequences of 735 nucleotide pairs were compared using the nucleotide sequence comparison (nucleotide BLAST) section of the NCBI (Fig. 1). The comparison used the following parameters: database-Standard databases (nr etc.), nucleotide collection (nr/ nt), and highly similar sequences (megablast). According to the result obtained, no nucleotide sequences belonging to similar organisms have been found in high resolution with the *Anapulvinaria pistaciae* type ITS plot sequences. The highest similarity was observed in the ITS plot sequences of *Ceroplastes ceriferus* (access num: JF719820.1). The matching rate of nucleotide sequences that were similar (constant identity) was 91.42%, the total ITS plot coverage rate (query coverage) was 31%, and the total number of similar nucleotides (total score) was 315. These are select *Acutaspis albopicta* isolate

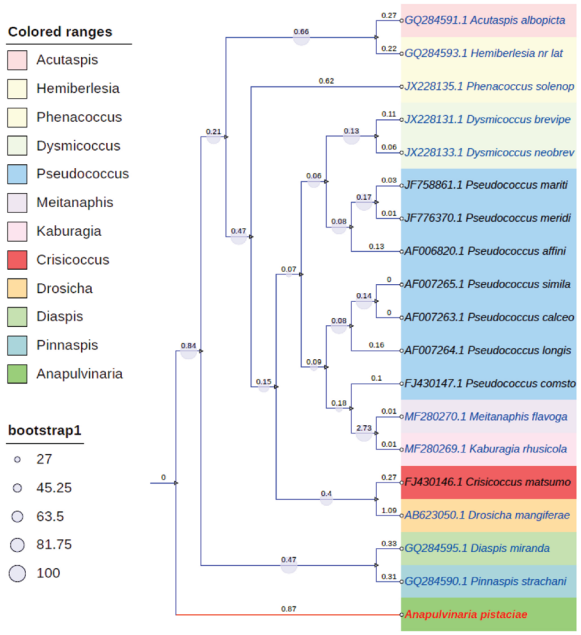

Fig. 1. Dendrogram of *A. pistaciae*'s 5.8 s rDNA sequence (United States, China, Chile, New Zealand, Korea, Pakistan and Uzbekistan)

(access num: GQ284591.1) Pi - 97.18%, Qc -24%, Ts-298 nucleotides (Table 1). Thus, *A. pistaciae* is a single monophyletic group and present material is completely different. RSCU (Sharp et.al 1986) when measured this codon was 0.83. In encoding 64 aminocysts, terminator codons are encoded 4.8 RSCU-1.16 nucleotides when UAA (*) is dated 6.2 RSCU-1.49 UAG (*), UAA-49.83% of stop codons, UAA-11.37%, UAG-38.8%. The least dated is codon GUC (V) 12.7, while the least dated is UUA (l) 1.4 UAG (*) 1.4 RSCU da UUA (L) 0.38 at RSCU. The most common to determine the bias of codon use are these RSCU values, which are greater than 1.5: CUC, UCG, CCG, ACG, GCG. Showing opposite properties (RSCU values less than 0.5), ACU, AGU, AGG they can be called a less common codon (Fig. 2).

The RSCU value is commonly used as a measure of codon usage bias. The general rscu values are to some extent, the use of codon can reflect features, but it is about using the codon of an individual sequence for a given genome, and it can be seen that there is a large difference in RSCU values (Gun et al., 2018). Using Tajima's relative velocity test, C (GQ284591 *Acutaspis albopicta*) was used as an outgroup between a (OR074914 *Anapulvinaria pistaciae*) and B (GQ284593

Table 1. NCBI GenBark accessions

S.No.	Country	Date	Entry numbers	Name of species	Author
1	USA	10-Aug-2009	GQ284591	*Acutaspis albopicta*	Rugman-Jones, P.F., Morse, J.G. va Stouthamer, R.
2	USA	10-Aug-2009	GQ284593	*Hemiberlesia nr lat*	Rugman-Jones, P.F., Morse, J.G. va Stouthamer, R
3	China	30-Jun-2013	JX228135	*Phenacoccus solenopsis*	He, Y.-B.
4	China	30-Jun-2013	JX228131	*Dysmicoccus brevipes*	He, Y.-B.
5	China	30-Jun-2013	JX228133	*Dysmicoccus neobrevipes*	He,Y.-B.
6	Chile	24-May-2011	JF758861	*Pseudococcus maritimus*	Aguirre, C.
7	Chile	07-Oct-2011	JF776370	*Pseudococcus meridionalis*	Correa, M., Aguirre, C., Germain, J.-F., Hinrichsen, P., Zaviezo, T., Malausa, T. and Prado, E.
8	New Zealand	05-Jan-1999	AF006820	*Pseudococcus affinis*	Beuning, L.L., Wu, E. and Murphy, P.
9	New Zealand	15-Jan-1999	AF007265	*Pseudococcus similans*	Beuning, L.L., Wu, E. and Murphy, P.
10	New Zealand	15-Jan-1999	AF007264	*Pseudococcus longispinus*	Beuning, L.L., Wu, E., Murphy, P., Charles, J. va Morris, B.A.M.
11	Korea	01-Nov-2009	FJ430147	*Pseudococcus comstocki*	Park, D.-S. and Oh, H.-W.
12	China	26-Apr-2019	MF280270	*Meitanaphis flavogallis*	Ren, Z., von Dohlen, C.D., Harris, A.J., Dikow, R.B., Su, X. and Wen, J.
13	China	26-Apr-2019	MF280269	*Kaburagia rhusicola rhusicola*	Ren, Z., von Dohlen,C.D., Harris, A.J., Dikow, R.B., Su, X. and Wen, J.
14	Korea	01-Nov-2009	FJ430146	*Crisicoccus matsumotoi*	Park, D.-S. and Oh, H.-W.
15	Pakiston	05-Oct-2011	AB623050	*Drosicha mangiferae*	Ashfaq, M., Ara, J. and Mansoor, S.
16	USA	10-Aug-2009	GQ284595	*Diaspis miranda*	Rugman-Jones, P.F., Morse, J.G. and Stouthamer, R
17	USA	10-Aug-2009	GQ284590	*Pinnaspis strachani*	Rugman-Jones, P.F., Morse, J.G. and Stouthamer, R
18	Uzbekistan	07-Jun-2023	OR074914	*Anapulvinaria pistaciae*	Zokirov, KX, Xusanov, AK, Kahhorova , XR, Isoqov, IB, va Xafiziddinov, M

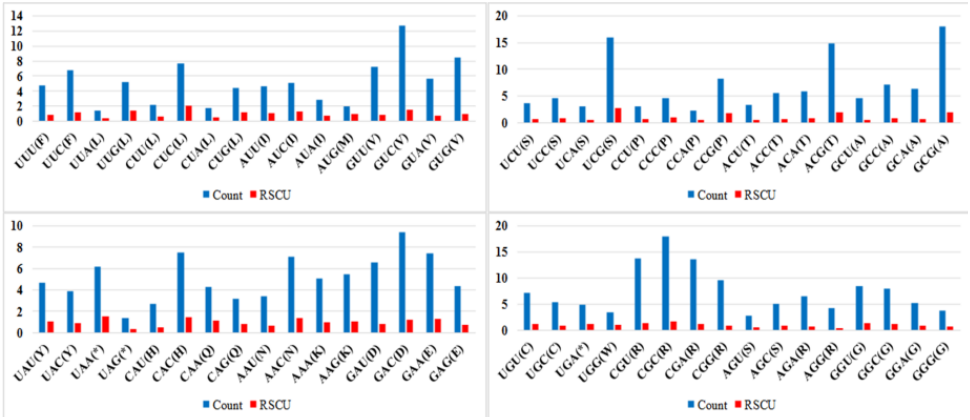

Fig. 2. Codons- encoding amino acids of *A. pistaciae* (396 codons, UUU (F) s 4.8)

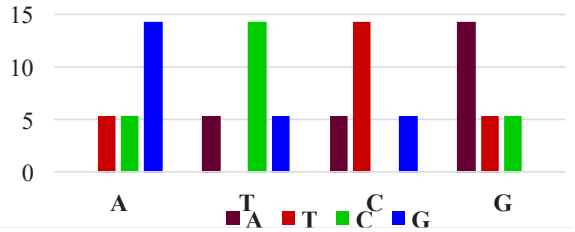

Fig. 3. Maximum likelihood of transition/ transversion

Hemiberlesia NR lataniae). The $\chi 2$ test statistic was 1.80 (with a freedom rate of p = 0.17971-1). This analysis involved a sequence of three nucleotides. Positions of codons included: 1st+2nd+3rd+noncoding. All slots containing gaps and missing information were deleted (complete deletion option). There were a total of 177 positions according to the final dataset (Tamura et al., 2021). The predicted transition/ transversion (R) is 1.33. Kimura (1980) evaluated substitution patterns and rates on the 2-parameter model (+G) (Kimura, 1980). A discrete gamma distribution has been used to model evolutionary rate differences between sites (category 16, [+G], parameter = 0.8630). Nucleotide frequencies are a = 25.00%, T/u = 25.00%, C = 25.00% and G = 25.00%. To estimate ML values, the tree topology is automatically calculated. The maximum logorifm value probability was -19330,876. This analysis included 19 nucleotide sequences, and positions of codons 1st+2nd+3rd+ noncoding. The last set given contained 2,039 positions (Fig. 3). Tajima's 3 sequence tests-counts revealed the following configurations- same sites in all three sequences 172; divergent sites 0; unique differences in sequence 4; in all three sequences 0; B unique differences in sequence 1.

AUTHOR CONTRIBUTION STATEMENT

Sobirov O planned and designed this study. Kakhkhorova R Kholidakhon performed molecular diagnosis. MHR analyzed data. Zokirov K revised the draft. Kakhorov R Dilshod, Isakov I and Amirov O Oybek drafted and revised original manuscript.

CONFLICT OF INTEREST

No conflict of interest.

REFERENCES

Abdrashitova N I, Gabrid N V. 2005. Methodological manual for collecting, studying and identifying coccids and aphids of trees and shrubs of Kyrgyzstan. - Bishkek, 82 p.

Abu-Yaman A. 1970. The pistachio cushion scale, Anapulvinaria pistaciae Boden., and its control in Iraq. Zeitschrift fur Angewandte Entomologie 66: 242-247.

Arkhangelskaya A D. 1937. Coccids of Central Asia. - Tashkent: Publishing House of the Committee of Sciences of the Uzbek SSR, 158 p.

Atlas of habitats and resources of medicinal plants. 1983. Moscow: GUGK, 340 p.

Ben-Dov Y. 2012. The scale insects (Hemiptera: Coccoidea) of Israel—checklist, host plants, zoogeographical considerations and annotations on species. Israeli Journal of Entomology 41(42): 21-48.

Bolu H, Uygun N. 2003. Determination of Coccoidea species, their distribution, infestations and natural enemies in Southeastern Anatolia Region. Bitki koruma bülteni 43(1-4): 111-123.

Borkhsenius N C. 1950. Collection and study of worms and shields to help those working on protective forest strips. Moscow: Publishing House of the USSR Academy of Sciences, 152 p.

Gavrilov-Zimin I A. 2011. New cytogenetic data for some Palaearctic species of scale insects (Homoptera, Coccinea) with karyosystematic notes. Comp Cytogenet 5(5): 375-90.

Gun L, Yumiao R, Haixian P, Liang Z. 2018. Comprehensive analysis and comparison on the codon usage pattern of whole Mycobacterium tuberculosis Coding Genome from different area. Biomed Res Int. 2018 May 8; 2018:3574976.

Hendricks L, Goris A, Nefs J, Per Van de J, Hennebert G, De Wachter R. 1989. The Nucleotide-sequence of the small ribosomal-subunit RNA of the Yeast Candida-albicans and the evolutionary position of the fungi among the Eukaryotes. Systematic and Applied Microbiology 12(1989): 223-229.

Hendriks L, De Baere R, Van de Peer Y, Neefs J, Goris A, De Wachter R. 1991. The evolutionary position of the rhodophyte Porphyra umbilicalis and the basidiomycete Leucosporidium scottii among other eukaryotes as deduced from complete sequences of small ribosomal subunit RNA. J Mol Evol. 1991 Feb; 32(2): 167-77.

Ibrokhimov A, Kuchboev A, Amirov O, Kakhorov B, Ayubov M. 2023. Identification of nematodes of the genus Teladorsagia, parasites of ruminants, using species-specific markers based on ITS2 rDNA. E3S Web Conference, 421: 4-14.

Issa M R, Figueiredo V L, De Jong D, Sakamoto C H, Simões Z L. 2013. Rapid method for DNA extraction from the honey bee Apis mellifera and the parasitic bee mite Varroa destructor using lysis buffer and proteinase K. Genet Mol Res. 12(4): 4846-54.

Joyce S A, Reed A P, Driver F, Curran J. 1994. Application of polymerase chain reaction (PCR) methods for the identification of entomopathogenic nematodes. p. 178-187.

Jubina B, Antonio G, Francesco P M, Bipin B, Federico M, Tiziano C, Annalisa M. 2022. Transcriptomic analysis of Pistacia vera (L.) fruits makes it possible to identify genes and a hormone-dependent gene associated with the fall of inflorescence buds. Multi-genomic approaches and computational biology on fruit trees. Genes 13(1): 60.

Khodzhimatov K Kh, Khodzhimatov O K, Sobirov U A. 2009. Collection of rules for the use of medicinal, food and industrial plant objects. New age generation, Tashkent: 171.

Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal Mol Evol. 16(2): 111-20.

Mardanova G, Khurramov A, Kuchboev A, Amirov O, Lebedeva N. 2023. Morphological and molecular identification of Anopheles mosquitoes (Diptera: Culicidae) in Surkhandarya region, Uzbekistan. Acta Biologica Sibirica 9: 729-745.

Mehrnejad M R. 2001. The current status of pistachio pests in Iran. Cahiers Options Mediterranéennes, 56(1): 315-322.

Moghaddam M. 2013. An annotated checklist of the scale insects of Iran (Hemiptera, Sternorrhyncha, Coccoidea) with new records and distribution data. ZooKeys.

Paul M S, Therese M F T, Krzysztof R M. 1986. Codon usage in yeast: cluster analysis clearly differentiates highly and lowly expressed genes. Nucleic Acids Research 149(13): 5125-5143; 14: 5125-5143.

Santas L A. 1985. Anapulvinaria pistaciae (Bod.), a pistachio tree scale pest producing honeydew foraged by bees in Greece. Entomologia Hellenica 3, 29-33.

Sharp P M, Tuohy T M, Mosurski K R. 1986. Codon usage in yeast: cluster analysis clearly differentiates highly and lowly expressed genes. Nucleic Acids Research 14: 5125-5143.

Sobirov O T, Khusanov A K, Zokirov K Z, Abdullaev I I. 2018. Features of biology and courtship ecology of the pistachio weevil (Homoptera, Coccinea: Anapulvinaria pistaciae Boden.) in the conditions of Eastern Fergana. [Xiva] 15-18.

Tavakkoli Korghond Ch R, Lotfalizadeh H. 2018. Pistachio mealybug, Anapulvinaria pistaciae (Bodenheimer) (Hem.: Coccidae) new host record for Coccophagus piceaeErdos (Hem: Chalcidoidea, Aphelinidae). Journal of Plant Protection 32(2): 59.

Tajima F. 1993. Simple methods for testing the molecular evolutionary clock hypothesis. Genetics 135(2): 599-607.

Tamura K, Stecher G, Kumar S. 2021. MEGA 11. Molecular evolutionary genetic analysis, version 11. Molecular Biology and Evolution.

Yanik E, Yücel A. 2001. The pistachio (P. vera L.) pests, their population development and damage state in Sanliurfa province. 56: 301-309.

Zokirov K. 1972. Fauna and biology of mealybugs and scale insects (Homoptera, Coccoidea) and their entomophages of cultivated and wild fruit plants of the Fergana Valley: Diss. Cand. Biol. Sci. – Tashkent, 1972. – 194 p.

Zokirov K, Sobirov O T. 2021. Fauna, morphology and ecological characteristics of coccids (Homoptera: Coccinea) of the Fergana Valley. Monograph T.: pp. 58-62

https://www.gbif.org/species/7980945

https://earth.google.com

https://www.genome.jp/tools-bin/mafft

http://iqtree.cibiv.univie.ac.at/

https://itol.embl.de/login.cgi

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