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MTCO1 ANALYSIS OF MIRIDS- *ADELPHOCORIS LINEOLATUS* AND *A SETICORNIS*

Hudoiberdieva O Marifat[1], Mirzaeva S Gulnara[1]*, Kholmatov R Baxtiyor[2], Musaev M Dilshod[2] and Amirov O Oybek[2]

[1]Research Institute of Plant Protection, Tashkent City Babur 1-Berk Street, House 17
[2]Institute of Zoology Academy of Sciences the Republic of Uzbekistan, Tashkent city, Yunusabad district, Bagishamol Str., 232 b, 100053
*Email: mirzayeva.gulnora@mail.ru (corresponding author): ORCID ID 0009-0006-3520-6174

ABSTRACT

This article presents the results of study on the species *Adelphocoris lineolatus* (Goeze, 1778) and *A. seticornis* (Fabricius, 1775) belonging to the genus *Adelphocoris* (Reuter, 1896) collected from the Tashkent region and Fergana Valley of our Republic during 2019-2023. The mtCO1 region of the mitochondrial DNA of these species were analyzed and the phylogenetic relationships of the representatives of this genus were studied.

Key words: Heteroptera, Miridae, *Adelphocoris*, genus, DNA, mtCO1, PCR, phylogeny, *lineolatus*, *seticornis*, Uzbekistan, Tashkent

The family Miridae (Heteroptera: Miridae) is one of the largest families of insects, with 11,000 species belonging to 1,200 and (Cassis, Schuh, 2012; Jung and Lee, 2012). Although representatives of this family feed on the sap of cultivated plants and cause economic damage (Cassis, Schuh, 2012; Lu et al., 2010), there are also species that kill pests and actively participate in biological control (Cassis, Schuh, 2012). There are 52 species belonging to the genus *Adelphocoris* (Reuter, 1896) (https://www.gbif.org/ru), and two species have been recorded in Uzbekistan, Tashkent (Musaev, 2020); *A. lineolatus* is the main pest of clover, widespread in the Palearctic (Benedek et al., 1970) causing a reduction of the yield from 65% to 90% (Yakhontov, 1966). Members of the Miridae family are morphologically diverse and species identification is difficult; and hence mtCO1 analyses are important methods (Avise, 2009; Hebert et al., 2003; Jinbo et al., 2011; Simon et al., 2006). The purpose of this study is the molecular genetic identification of *A. lineolatus* (Goeze, 1778) and *A. seticornis* (Fabricius, 1775).

MATERIALS AND METHODS

Specimens were collected from various agrocenoses of Tashkent region and Fergana valley of Uzbekistan Republic during 2019-2023. The taxonomic status of these along with distribution was carried out using the relevant scientific sources of Kirichenko (1951), Kerzhner (1962), Schuh (1995), Linnavuori (1998) and Esenbekova (2015). A variety of traps and general entomological techniques were used to collect (Golub, 2012). An entomological trap with a diameter of 38 cm was used to collect. Collections were studied in the laboratory, species were determined, and their photographs were taken. A BPM-350P microscope and a Canon EOS 80D camera were used. Also, the method of DNA separation using the Diatom DNA Prep kit was used to extract DNA from the collected samples (http://www.galartdiag.ru). Nucleotides of the COI region of the mitochondrial DNA (mDNA) of various insect families (Insecta: Hemiptera) were isolated using LepF1 forward (att caa cca atc ata aag ata ttgg) and LepR1 reverse (taa act tct gga tgt cca aaa aat ca) primers widely used in molecular taxonomy (Foottit, 2008). In the preparation of master-mix for PCR, water (distilled) 7.1 µl, 10x PCR buffer 1 µl, dNTP 0.2 µl, primers 0.5 µl, Taq-polymerase 0.2 µl=10 µl were prepared. Polymerase chain reaction from isolated DNA samples was performed using an automatic programmable amplifier (PR-96E) in the following mode. Amplification of DNA fragments was carried out in a thermocycler for 35 steps. PCR was carried out according to the following scheme: step 1 - denaturation of DNA at 95°C for 2 min, step 2 - denaturation of DNA at 93°C for 20 sec, step 3 - binding of primers to DNA at 52°C for 45 sec, step 4 – elongation at 72°C for 2 min, step 5 – chain elongation at 72°C for 10 min. The process from the second to the fourth step was repeated up to 35 times in a loop form. The presence of DNA in PCR products was determined by electrophoresis in a 1.0% agarose gel with a voltage of 120 V. DNA amplification and DNA extraction from the gel were performed using a reagent kit manufactured by Silex M (Moscow, Russia) following the manufacturer's instructions.

DNA sequencing was performed using the ABI PRISM® BigDye™ Terminator v 3.1 reagent kit, and reaction products were sequenced at GATC Biotech AG. Analysis of the obtained nucleotide sequence was carried out using Bioedit, Clustal W and DNAstarTM computer programs. Nucleotide sequences obtained were compared with those from International Center for Biotechnology Information database (https://www.ncbi.nlm.nih.gov/) to construct a phylogenetic tree (Table 1). Nucleotide sequences were manually edited using Genius prime software, consensus sequences were calculated using Mega X computing software. Primer data from this program and complementary sequences from the GenBank database were aligned using MAFFT v.7 online software using default settings and Clustal Omega 1.2.2 software and edited with Genius prime software. Nucleotide sequences belonging to the obtained COI domain were identified by ultrafast bootstrapping maximum (maximum likelihood-ML) phylogenetic tree with 1000 iterations in IQ-TREE version 1.6.12, and analyzes were performed in CIPRES Science Gateway V 3.3. The nucleotide sequence of the COI domain of *Adelphocorisella lespedezae* (accession number KY367057) was included as an outgroup to facilitate the production of consensus trees. The resulting phylogenetic tree was analyzed and edited in iTOL v6.6 software.

RESULTS AND DISCUSSION

A comparative analysis of the morphology of male and female of *A. lineolatus* and *A. seticornis* was conducted. Molecular and genetic studies were carried out in order to make the morphological data more accurate. To conduct molecular-genetic studies, the leg part of male individuals was used in molecular-genetic analysis. Based on the results of molecular genetic research (sequence chromatography) on the species of the genus *Adelphocoris*, nucleotides with a length of 658 base pairs were extracted from the COI region of the mRNA of *A. lineolatus* and *A. seticornis*, and *A. lineolatus* (Accession number: KY840413) and *A. seticornis* (Accession number: MZ 610619) from the genebank database were used to compare these species (Fig. 1). When comparing the *A. lineolatus* sample and *A. lineolatus* (Accession number: KY840413) obtained from the genebank, there are 3 nucleotides, and differences between the nucleotides of *A. seticornis* and *A. seticornis* (Accession number: MZ 610619) obtained from the genebank not identified. There are 26 differences between the nucleotides of *A. lineolatus* and *A. seticornis*, belonging to the genus *Adelphocoris*, and these differences are at nucleotides 70, 145, 178, 220, 397, 428, 614, 654 (cytosine in *A. lineolatus*, and thymine in *A. seticornis*), in nucleotides 100, 566, 569 (adenine in *A. lineolatus*, and thymine in *A. seticornis*), in nucleotides 118, 223,358, 474, 529, 530, 539, 554 (thymine in *A. lineolatus*, cytosine in *A. seticornis*), at nucleotides 127, 274 (cytosine in *A. lineolatus*, and adenine in *A. seticornis*), at nucleotide 379 (adenine in *A. lineolatus*, and cytosine in *A. seticornis*), at nucleotides 400, 472, 620 (adenine in *A. lineolatus* and guanine in *A. seticornis*) and 629 nucleotides (guanine in *A. lineolatus* and adenine in *A. seticornis*) were found to be exchanged.

Table 1. Information from the international genebank database

No.	Species names	Date deposited in GenBank	Country of research	Record number in GenBank
1.	*A. fasciaticollis*	21.02.2018	China	KY430611
2.	*A. fasciaticollis*	13.01.2018	China	KY430602
3.	*A. lineolatus*	28.05.2017	Pakistan	KY838906
4.	*A. lineolatus*	23.06.2019	China	KY840413
5.	*A. quadripunctatus*	21.09.2014	Germany	KM021535
6.	*A. quadripunctatus*	10.11.2021	Finland	MZ608071
7.	*A. nigritylus*	30.06.2020	China	MF285435
8.	*A. detritus*	21.09.2014	Germany	KM022753
9.	*A. reichelii*	21.09.2014	Germany	KM022302
10.	*A. reichelii*	21.09.2014	Germany	KM021671
11.	*A. suturalis*	25.10.2016	China	KU234538
12.	*A. suturalis*	20.05.2015	China	KJ020288
13.	*A. seticornis*	21.09.2014	Germany	KM021574
14.	*A. seticornis*	21.09.2014	Germany	KM022015
15.	*A. seticornis*	29.07.2018	China	MZ610619
16.	*A. rapidus*	19.09.2014	Canada	GU692418
17.	*Adelphocorisella lespedezae*	13.11.2018	South Korea	KY367057

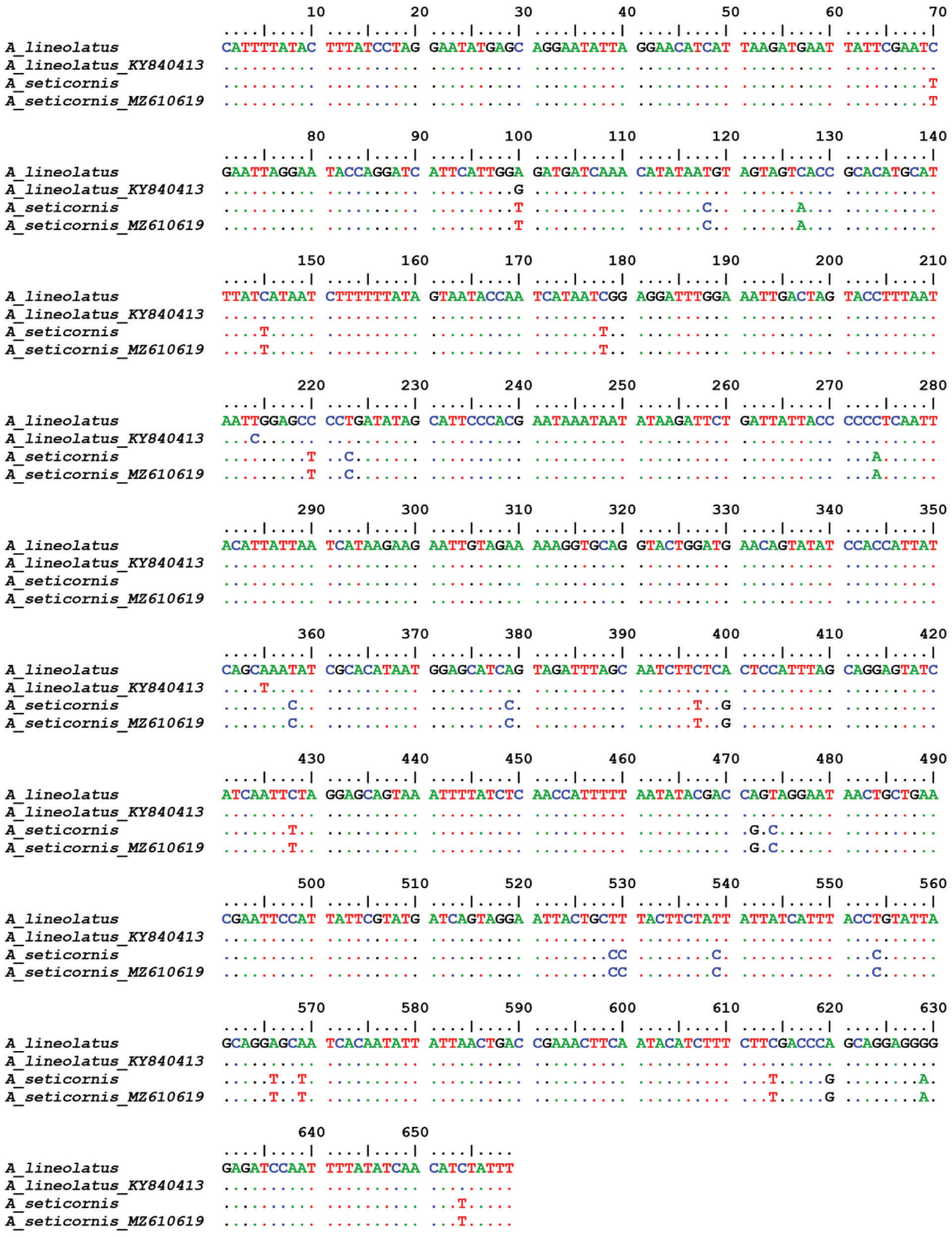

Fig. 1. mtCO1 region of mRNA of *Adelphocoris* spp. viz., *A. lineolatus* and *A. seticornis* (from 5' to 3'- terminal end direction, identical nucleotide bases marked with dots)

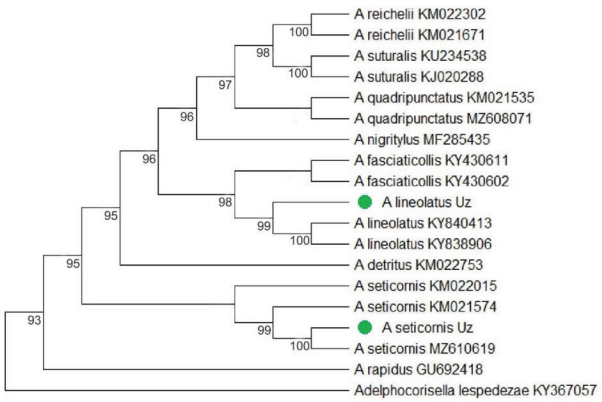

Fig. 2. A phylogenetic tree of mtCO1 region of the genus *Adelphocoris*

Phylogenetic analysis was carried out using the Maximum Likelihood method and the Tamura 3 model (Tamura K. 1992). This analysis includes 658 pairs of nucleotide sequences belonging to 14 species. Evolutionary analyzes were analyzed in Mega 11 software (Tamura K., 2021) (Fig. 2). According to the results of the study, according to the comparative analysis of the nucleotide sequences belonging to the COI domain and the nucleotide sequences obtained from the GenBank database, it was found that the species belonging to the genus *Adelphocoris* are united into 7 monophyletic groups. The first monophyletic group belonging to the genus *Adelphocoris*, species *A. reichelii* and *A. suturalis* produced a bootstrap loading value of 98–100%. The second monophyletic group, the species *A. quadripunctatus*, produced a bootstrap loading value of 97% compared to the representatives of the genus *Adelphocoris*. The third monophyletic group contained the species *A. nigritylus*, which clustered with a bootstrap loading value of 96% relative to the other species. The fourth monophyletic group, *A. fasciaticollis* and *A. lineolatus*, combined to form a bootstrap loading value of 96% relative to the other species, and 98-100% between the two species. The fifth monophyletic group, *A. detritus* species, combined to form a bootstrap support of 95%. In the sixth monophyletic group, *A. seticornis* species combined with 95% and 99-100% bootstrap support compared to other species, and in the seventh monophyletic group, *A. rapidus* species combined with 95% bootstrap support.

AUTHOR CONTRIBUTION STATEMENT

Hudoiberdieva O Marifat planned and designed this study. Mirzaeva S Gulnara performed molecular diagnosis. MHR analyzed data. Kholmatov R Baxtiyor revised the draft. Musaev M Dilshod and Amirov O Oybek drafted and revised original manuscript.

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CONFLICT OF INTEREST

No conflict of interest.

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