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MOLECULAR GENETIC ANALYSIS OF SPECIES BELONGING TO THE GENUS PODALONIA FERNALD, 1927 IN UZBEKISTAN

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Abstract

In this article, the nucleotide sequence of mitochondrial DNA belonging to the COI region of the species *Podalonia affinis* (Kirby, 1798), *P. ebenina* (Spinola, 1839) and *P. hirsuta* (Scopoli, 1763), belonging to the genus *Podalonia* Fernald, 1927 registered in the Fergana Valley, was studied in a comparative plan. Based on the information obtained as a result of molecular genetic studies, phylogenetic relationships have been identified. *P. ebenina* species was first placed at the National Center for Biotechnology Information.

Keywords

Family, genus, species, mtDNA, phylogeny.

Introduction

Representatives of the Sphecidae family are one of the largest families of the Hymenoptera order, and in nature they control the number of various insects. The genus *Podalonia* Fernald, 1927 includes 60 known species and 8 subspecies in the world at present (Pulawski 2015). 33 species and 5 subspecies occur in the Palearctic Region, 21 species in Nearctic, 7 species and 2 subspecies in Afrotropical, 2 species in Oriental, 8 species and 1 subspecies in Australo-Papuan, 5 species in both Palearctic and Australo-Papuan, 7 species in both Nearctic and Neotropical, 2 species in Palearctic, Afrotropical and Australo-Papuan, and 1 species in Palearctic, Afrotropical, Australo-Papuan and Oriental Regions (Li et al. 1995; Li and Yang, 2005; Pulawski 2015).

Materials and methods

To collect entomological samples in this study during 2019-2023, mountainous, high-mountainous, hilly and flat areas were selected in the Ferghana Valley (Fig. 1).

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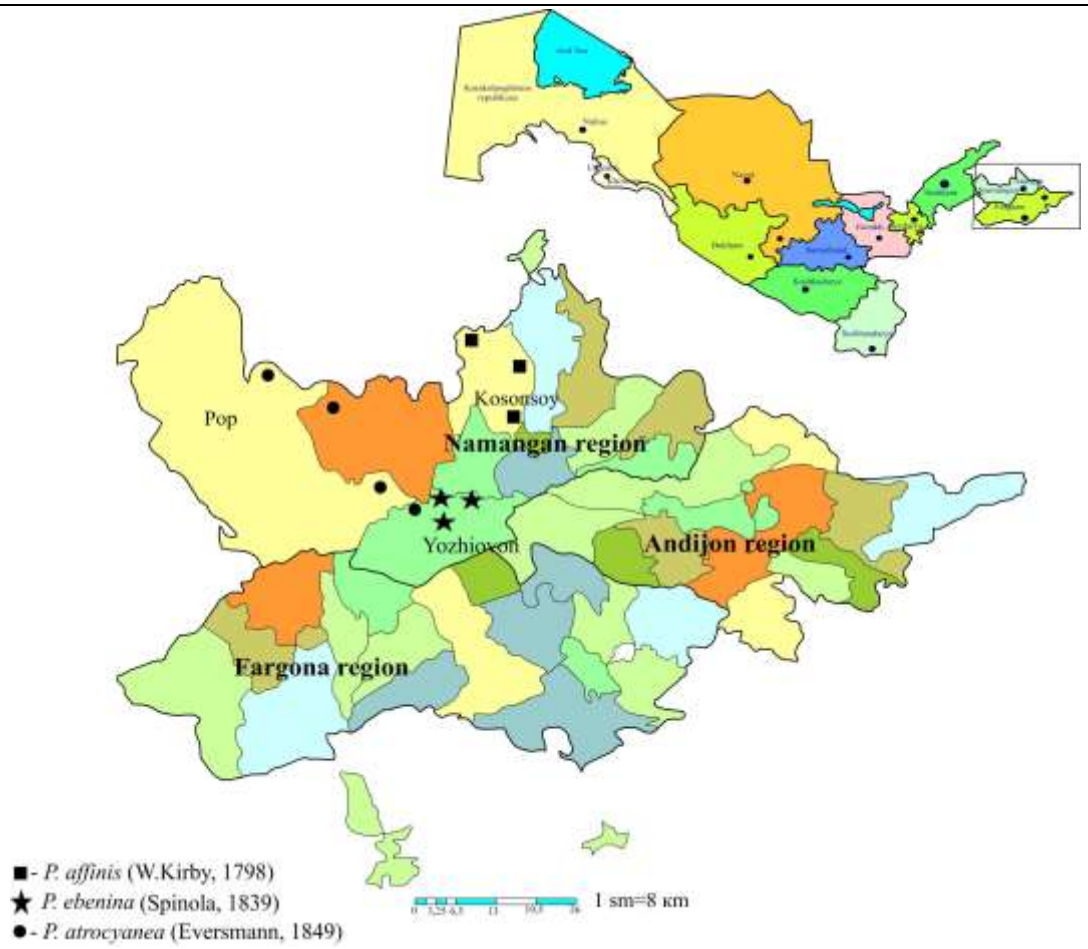

Figure 1. Distribution of species of the genus *Podolonia* in the Fergana Valley (2019-2023).

Podalonia Fernald, 1927 was collected using Malese, Merike and Barber soil traps while collecting bees. The Malese trap used three N-shaped interlocking plates and covers flush with the soil surface. In addition, entaamological nets of various sizes were used (Pravdin 1978). An entomological grid is carried out over the surface of lawns, young shrubs and trees with a whip-like movement (with a quantitative score of 50 or 100) (Vinokurov 2015). The collected entomological material was stored in 70% ethanol solution.

To describe the species, the generally accepted literature of Bohart and Menke (1976) on morphology and the guides of species Dollfuss (2010, 2013) were used.

To isolate DNA from leg samples of males of the genus *Podalonia* stored in a 70% alcohol solution, they were removed, placed on dry paper and kept at room temperature for 10-15 minutes until the alcohol evaporated.

To isolate the DNA of wasps of the genus *Podalonia*, we used (2 ml 0.5 M Tris/HCl, pH 8.5, 10 ml 2 M KCl solution, 500 µl 1 M $MgCl_2$, 2 ml NP40 and 27

sucrose solution). 200 ml of distilled and autoclaved water) and 2 ml of proteinase 10 mg/ml K. Legs of *P. affinis and P. atrocyanea* bees placed in Eppendorf tubes were incubated at 65°C for 1-2 hours. After incubation, solutions of the extraction solution (lysis buffer + protease K) were added and kept at 92°C for 10 minutes. The isolated DNA samples were then stored (-20°C) (Issa 2008).

Polymerase chain reaction from the isolated DNA samples was carried out using an automatic programmable cycler (PR-96E).

Nucleotides of COI fragments of mitochondrial DNA (mDNA) of representatives of the genus Podalonia were isolated using primers LEP-F-forward, 5-ATTCAACCAATCATAAAGATAT-3, and LEP-R-reverse 5-TAAACTTCTGGATGTCCAAAAA-3, widely used in molecular taxonomy (Paul 2004; Mardanova 2023).

When preparing the Master-mix for PCR, water (distilled) was prepared - 7.1 µl, 10x PCR buffer - 1 µl, dNTP - 0.2 µl, Each primer - 0.25 µl, Taq polymerase - 0.2 µl = 10 µl (Ikromov 2023; Mardanova 2023).

Amplification of DNA fragments was carried out in a thermal cycler for 35 cycles. PCR was carried out according to the following scheme: 1st stage - DNA denaturation at 95°C for 2 minutes, 2 stage - DNA denaturation at 93°C for 20 seconds, 3 stage - binding of primers to DNA at 52°C for 45 seconds, 4 - stage - elongation at 72°C for 2 minutes, 5 - stage - elongation of the chain at 72°C for 10 minutes. From the second to the fourth stage, the process was repeated up to 35 times in a cyclic form (Ibrokhimov 2023, Mardanova 2023).

To determine the length of fragments, bacteriophage DNA hydrolyzed with PstI endonuclease and a special marker Ladder 3-1 from firm "Axigen" were used as a marker.

To build a phylogenetic tree, we used nucleotide sequences of species of the genus *Podalonia* Fernald, 1927, obtained as a result of sequencing, and DNA sequences obtained from the International Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov) and these sequences were edited manually using Genius Prime software and consensus sequences were calculated using Mega X calculation software. Primer data from this program and additional 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.

Nucleotide sequences belonging to the resulting mtDNA domain were determined using IQ-TREE version 1.6.12 by ultra-fast bootstrap with a maximum likelihood-ML phylogenetic tree with 1000 iterations, and analyzes were performed in CIPRES Science Gateway V 3.3. Nucleotide sequences belonging to the COI

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domain of the species Ammophila heydeni (OP601358) were included as an outgroup to facilitate the creation of consensus trees. The resulting phylogenetic tree was analyzed and edited in the iTOL v6.6 program.

Results and discussions

According to the results of entomological studies conducted in different geographical regions of the Fergana Valley, the species *Podalonia affinis* (Kirby, 1798), belonging to the genus *Podalonia* Fernald, 1927, was recorded in the Kosonsoy district of the Namangan region, and the species *Podalonia ebenina* (Spinola, 1839) was recorded in Yazyavan district of Ferghana region and the species *Podalonia hirsuta* (Scopoli, 1763) was recorded in Pop, Chust districts of Namangan region and Yazyavan district of Fergana region (Fig. 2).

Figure 2. Morphological view of the species of the genus *Podalonia:*

1 - *Podalonia affinis* (Kirby, 1798); 2 - *Podalonia ebenina* (Spinola, 1839); 3 - *Podalonia hirsuta* (Scopoli, 1763). Note: a - general view, b - head, c - stomach, d - chest.

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Results of molecular genetic studies. For molecular genetic studies, DNA was isolated from the legs and whiskers of *P. affinis, P. ebenina,* and *P. hirsuta* males fixed in 70% ethanol solution.

The results of the molecular genetic studies performed show that nucleotides with 658 base pairs belonging to the mtDNA COI region of *P. affinis, P. ebenina*, and *P. hirsuta* species have been isolated.

To compare these species, *P. affinis* (accession number: JF927346), *P. hirsuta* (Accession number: MZ627508) were obtained from the National Center for Biotechnology Information (https://blast.ncbi.nlm.nih.gov) (Table 1).

Table 1

Comparison of the nucleotide sequence of the mtDNA COI region of species of the genus Podalonia, (%)

№	Species names	*P_hirsuta*_Uz	*P_affinis*_Uz	*P_ebenina*_Uz	*P_hirsuta*_ 7508	*P_affinis*_ 346
1	*P_hirsuta*_Uz	-	14,08	13,08	0	14,08
2	*P_affinis*_Uz	88	-	11,9	14	0
3	*P_ebenina*_Uz	82	75	-	13,09	12
4	*P_hirsuta*_ MZ627508	0	88	82	-	14,08
5	*P_affinis*_JF927346	88	0	75	88	-

As can be seen from the table above, there is a difference of 88 nucleotides between *P. hirsuta* and *P. affinis_Uz* and *P. affinis* (Accession number: JF927346), which is 14.08%, and *P. hirsuta* species, and *P. ebenina_Uz*, 82 nucleotide differences were found, 13.09%, and no differences were found between the nucleotides of *P. hirsuta* and *P. hirsuta* species (Accession number: MZ627508). There are 75 nucleotide differences between *P. affinis_Uz* and *P. ebenina_Uz,* 11.9%, and 88 nucleotide differences between *P. hirsuta* (stock no: MZ627508) and 14.08%, *P. affinis* (stock no: JF927346) and differences between nucleotides were not found. Between *P. ebenina_Uz* and *P. hirsuta* (stock no: MZ627508) 82 nucleotide differences were found, and this was 13.9%, and 88 nucleotide differences were found between *P. affinis* (stock no: JF927346) and this was 14.08%.

The mtDNA COI domain nucleotides of *P. affinis, P. ebenina,* and *P. hirsuta*, studied from a molecular genetic point of view, are located at the National Center

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for Biotechnology Information (https://blast.ncbi.nlm.nih.gov) and registration certificates have been obtained. Numbers (*P_affinis*_OR345346, *P_ebenina*_OR345060, *P_hirsuta*_OR342224).

Phylogenetic connections. Based on the results of a molecular genetic study and analysis of nucleotide sequences obtained from the National Center for International Biotechnology Information, it was found that species belonging to the genus *Podalonia* are grouped into 7 clades (monophyletic groups) (Table 2, Fig. 3).

Table 2

Species from the National Center for Biotechnology Information

№	Species of the genus *Podalonia*	COXI
	Podalonia hirsuta	HQ563082
	P. hirsuta	MH610509
	P. tydei	MH610077
	P. tydei	MH609526
	P. affinis	MH611172
	P. fera	MH610997
	P. fera	MH610746
	P. minax	MH608402
	P. minax	MH610742
	P. mexicana	HQ567685
	P. mexicana	HM423044
	P. robusta	KM556156
	P. robusta	KM568255
	Ammophila heydeni	OP601358

The first monophyletic group, *P. affinis* species belonging to the genus *Podalonia*, gave a bootstrap value of 99/100%. The second monophyletic group, *P. minax*, gave a bootstrap loading value of 97% compared to *P. affinis*. However, *P. minax* species showed a 100% bootstrap load value from a phylogenetic point of view, and the next third monophyletic group, *P. fera* species, combined to form a 100% bootstrap load value.

The fourth monophyletic group included *P. ebenina* species, which together gave a bootstrap load value of 94/97% in relation to the main link.

The fifth monophyletic group combined *P. mexicana* and *P. robusta* into the genus *Podalonia* with 98/100% bootstrap support.

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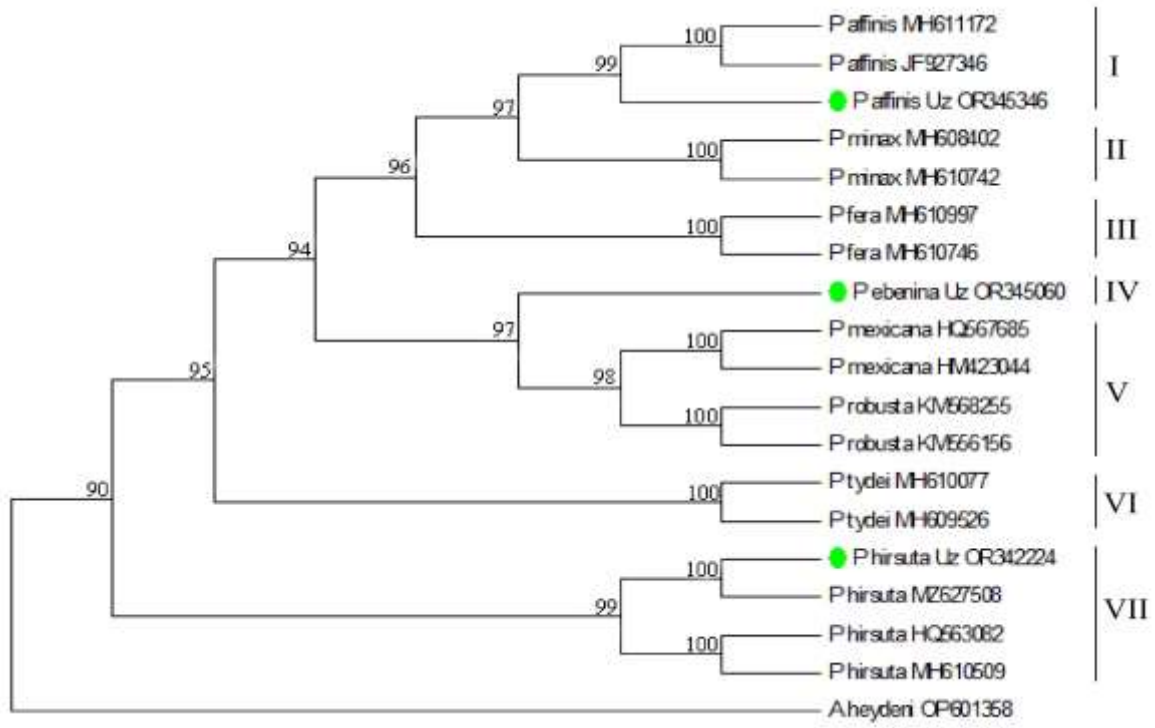

Figure 3. Phylogenetic tree of species belonging to the genus *Podalonia.*

The sixth monophyletic group combined *P. tydei* species with 95/100% bootstrap support compared to the main joint.

A seventh monophyletic group, *P. hirsuta* species, was split into two subgroups and these groups combined to form a 99/100% bootstrap support.

In conclusion, according to the results of entomological studies conducted in different geographical areas of the Ferghana Valley, the species *P. affinis, P. ebenina* and *P. hirsuta* belonging to the genus *Podalonia* Fernald, 1927 were registered. As a result of molecular genetic studies, the difference between the nucleotides of *P. hirsuta* species and *P. affinis* species is 14,08%, with *P. ebenina* species 13,09%, *P. affinis* species and *P. ebenina* species have differences in nucleotides by 11,9%. A phylogenetic tree between 8 species of the genus *Podalonia* yielded 7 monophyletic groups giving initial bootstrap loading values of 90-100%. *P. ebenina* listed for the first time by the National Center for Biotechnology Information.

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