

New Record of *Pentalonia nigronervosa* Coquerel, 1859 (Hemiptera, Aphididae) in Turkey

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ABSTRACT

The banana aphid, *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae), is a major pest of banana, *Musa* spp., (Zingiberales) has worldwide distribution. This study was conducted to detect aphid species in the banana production regions of Adana, Antalya, and Mersin in Turkey. Aphid samples have been collected in total of thirty-seven locations. All the samples were identified as *P. nigronervosa*. Later on, molecular characterization has been done based on COI and it was recognized that the Turkish population was close to Florida and some Hawaiian genotypes. *Pentalonia nigronervosa* was recorded for the first time in Turkey in this study. Sustainable nature-friendly management tactics for controlling *P. nigronervosa* which poses less health threat to human should be developed.

Key words: Banana aphid, *Musa* spp, COI, mtDNA, Turkey, vector.

INTRODUCTION

The banana aphid, *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae), is one of the most important pests of banana (Mathers, Mugford, Hogenhout, & Tripathi 2020). The pest was also recorded on *Heliconia* spp., *Caladium* spp., *Alpinia* spp., and *Dieffenbachia* spp., *Colocasia esculenta* (Blackman & Eastop, 1984; Waterhouse, 1987). The banana aphid was described on the island of Madagascar in the 19th century (Coquerel, 1859). *Pentalonia nigronervosa* causes damage by sucking on the phloem of banana plants grown in tropical and subtropical areas and greenhouses in the world (Blackman & Eastop, 1984; Robson, Wright, & Almeida, 2007). High aphid population density may suppress the growth of young plants or cause die back of the plants, whereas direct damage to mature plants is often negligible (Mathers et al, 2020).

Pentalonia nigronervosa feeds on the stem, late leaves, and tips of the growth point of the banana, and produces honeydew causing fumagin over time (Waterhouse & Norris, 1987). The pest also forms big colonies on 7-8 cm below the soil surface in colonies are feeding leaf sheaths of the banana plant and the stem. Feeding damage of *P. nigronervosa* on the banana plant (*Musa* spp.) causes the formation of stunting, spotting on the fruit, fumagin, as well as transmission of Banana Bunchy Top Virus (BBTV) as a vector (Drew, Moisaner, & Smith, 1989).

Pentalonia nigronervosa usually has a viviparous life cycle (Bhanotar & Ghosh, 1969); it goes through four nymph periods before it becomes mature. The pest, which can reproduce 30 generations in a year under favorable conditions, has a high reproductive capacity. The nymph development stage was detected as 9-15 days, whereas longevity is lasting between 9.9 and 12.5 days, and a female gives 3-20 offspring under laboratory conditions (Rajan, 1981; Viswanathan & Regupathy, 1992). Lomerio & Calilung (1993) reported the life cycle of *P. nigronervosa* on a few hosts in greenhouses as 6-21 to 20-28 days. Moreover, the pest has annually four generations in Guangdong (China), and the population density is high in April and September - October (Yang, 1989). Alate forms migrate from plant to plant within the same orchards or to different locations in the ecological area and reach high populations in the growing plant (Waterhouse & Norris, 1987).

Due to the agricultural subventions and high profits, the production of bananas under the greenhouse has started to increase rapidly in Adana, Mersin, and Alanya provinces in the Mediterranean region, The banana production and its profit reaching Anamur's level, the production area where bananas have been grown for a long time. While the production of domestic bananas in Turkey was around 50 thousand tons in 2000, it has now increased to 728 thousand tons (TUİK, 2020). Increasing banana production brought plant protection problems. Seedlings of new varities may imported to meet the increasing demand in production areas, prepare the basis for bringing new pests and diseases.

Here it is reported that *Pentalonia nigronervosa*, a native species of tropical rainforest climate zones, was diagnosed for the first time by morphological and molecular methods on banana plants grown area in Turkey. This report includes new information on the host range and distribution of the species in Turkey.

MATERIAL AND METHOD

Sample collection

Wingled and wingless adult aphids were collected from commercial banana production areas which are coastal Mediterranean regions from Adana (n:2), Hatay (n:3), and Mersin (n:32) provinces in Turkey during spring and autumn in 2020 (Table 1). The aphids were collected from infested banana plants with a small brush and preserved in 70 percent ethyl alcohol in the Eppendorph tubes and taken to the Republic of Turkey Ministry of Agriculture and Forestry Biological Control Research Institute for examination.

Table 1. The list of banana aphid specimens examined in the study from Adana, Mersin, and Hatay provinces of Türkiye, including collection date, location, coordinate, size of the field or greenhouse, and variety.

No	Location	Date	Size (decare)	Variety	Coordinate	
					Latitude (E)	Longitude (N)
1*	Erdemli /Mersin	18.03.2020	6	Grand Naine	36°33'59"	34°14'08"
2	Arsuz/ Hatay	12.06.2020	22	Grand Naine	36°27'06"	35°57'43"
3	Anamur/ Mersin	03.04.2020	23	Şimşek	36°03'25"	32°48'57"
4	Anamur/ Mersin	03.04.2020	24	Grand Naine	36°03'25"	32°48'57"
5	Anamur/ Mersin	03.04.2020	25	Şimşek	36°03'25"	32°48'57"
6	Anamur/ Mersin	03.04.2020	26	Grand Naine	36°03'26"	32°48'59"
7	Anamur/ Mersin	03.04.2020	27	Grand Naine	36°03'26"	32°48'59"
8	Anamur/ Mersin	03.04.2020	28	Bodur Azman	36°03'42"	32°48'49"
9	Anamur/ Mersin	03.04.2020	29	Bodur Azman	36°03'42"	32°48'49"
10	Anamur/ Mersin	21.04.2020	30	Şimşek	36°03'19"	32°47'37"
11	Anamur/ Mersin	21.04.2020	38	Azman	36°03'42"	32°50'36"
12	Anamur/ Mersin	21.04.2020	39	Bodur Azman	36°02'37"	32°49'28"
13	Bozyazı/ Mersin	21.04.2020	40	Azman	36°07'20"	32°57'48"
14	Bozyazı/ Mersin	21.04.2020	41	Azman	36°07'19"	32°57'38"
15	Bozyazı/ Mersin	21.04.2020	42	Azman	36°07'19"	32°57'50"
16	Bozyazı/ Mersin	21.04.2020	43	Azman	36°07'16"	32°57'45"
17*	Bozyazı/ Mersin	06.05.2020	44	Azman	36°07'13"	32°57'47"
18*	Bozyazı/ Mersin	06.05.2020	45	Grand Naine	36°07'00"	32°57'14"
19	Bozyazı/ Mersin	06.05.2020	46	Şimşek	36°07'01"	32°57'16"
20	Bozyazı/ Mersin	06.05.2020	47	Bodur Azman	36°07'12"	32°56'59"
21*	Bozyazı/ Mersin	06.05.2020	53	Grand Naine	36°07'53"	32°56'54"
22	Bozyazı/ Mersin	06.05.2020	54	Bodur Azman	36°06'57"	32°57'41"

Table 1. Continued.

No	Location	Date	Size (decare)	Variety	Coordinate	
					Latitude (E)	Longitude (N)
23	Bozyazı/ Mersin	06.05.2020	55	Grand Naine	36°06'57"	32°57'40"
24	Bozyazı/ Mersin	06.05.2020	56	Azman	36°06'57"	32°57'39"
25	Bozyazı/ Mersin	06.05.2020	57	Şimşek	36°06'58"	32°57'39"
26	Bozyazı/ Mersin	06.05.2020	58	Şimşek	36°06'45"	32°57'14"
27	Bozyazı/ Mersin	06.05.2020	59	Bodur Azman	36°06'15"	32°56'54"
28*	Arsuz/ Hatay	18.05.2020	60	Azman	36°32.0400	36°6.1930
29	Arsuz/ Hatay	18.05.2020	25	No Data	36°26.7810	35°57.9100
30*	Yumurtalık/Adana	05.06.2020	30	No Data	36°27.1020	35°57.7130
31	Yumurtalık/Adana	05.06.2020	25	No Data	36°56.2200	35°4.8680
32	Silifke/ Mersin	18.03.2020	20	No Data	36°20.8590	34°14'55"
33*	Taşucu/ Mersin	18.03.2020	20	No Data	36°20.7650	34°15'00"
34	Silifke/ Mersin	18.03.2020		Grand Naine	36°22'28"	34°01'53"
35	Silifke/ Mersin	18.03.2020	50	Grand Naine	36°22'08"	34°03'15"
36	Silifke/ Mersin	18.03.2020	150	Grand Naine	36°20'43"	34°03'03"
37	Silifke/ Mersin	18.03.2020	10	Grand Naine	36°22'17"	34°01'48"

*the sample used for molecular studies.

Morphological identification

The Slide mounting technique was applied to winged and wingless adult aphids mainly based on the method of Hille Ris Lambers, (1950). The specimens were studied using a LEICA DM LB2 compound light microscope for identification. The species determination by an expert was done using Heie (1995), Blackman & Eastop (1994, 2000, 2006, 2020). The specimen vouchers are preserved in Nazife Tuatay Plant Protection and Insect Museum in Ankara.

Molecular identification

DNA isolation and PCR reaction

The genomic DNA of *P. nigronervosa* collected from nine locations (n = 18) was isolated according to DNAeasy Tissue Kit (QIAGEN, Hilden, Germany), following the manufacturer's recommendations. DNA quality and quantity were measured with a microplate reader (Multiscan GO, Thermo) and kept at -20 °C until PCR reaction. LCO1490 (GGTCAACAAATCATAAAGATATTGG) and HC02198 (TAAACTTCAGGGTGA CCAAAAAATCA) primer pairs used for PCR applications (Lourenço et al, 2006). PCR reaction was prepared as 50 µl final volumes with 5 µl Taq buffer (10X), 2,5 mM MgCl₂, 250 µM dNTPs, 1 µM primer, 0,5 U Taq, 1 µl DNA.

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Thermocycler (Applied Biosystems (Veriti)) condition was adjusted to 5 min. at 94°C for pre-denaturation, 1 min. at 94°C for denaturation, 1 min. at 50°C for annealing, 1 min. at 72°C for the extension as 35 cycles and 7 min. at 72°C for the last extension. The PCR reactions were verified using Agarose (1%) gel and gel imagen system. The Sanger sequencing was applied to the PCR products by Molgentek®, Istanbul, Turkey.

Bioinformatic analyses

The sequences of *P. nigronervosa* were verified by comparing with other reference genes in NCBI (The National Center for Biotechnology Information (<http://www.ncbi.nlm.gov/BLAST>)) Gene bank. The sequences translated to Fasta format were aligned at the Mega 7 program (Kumar, Stecher, & Tamura, 2016). The correction of sequences was done on the Finch TV program. The maximum likelihood Method with 2000 bootstrap value was used for the phylogenetic relationship between them. *Aphis gossypii* Glover was selected as the outgroup.

RESULTS

Description

Pentalonia nigronervosa is small, red-brown to almost black in the nymphal period, and completely black during adulthood. The height of the adults varies between 1.1-1.8 mm. Their bodies are oval to cylindrical and slightly swollen. The species has a pair of corniculus on both sides of the body towards the end of the abdomen. It has both winged and wingless forms. The body of wingless viviparous females is 1.1 to 1.8 mm in length, the head is pale or black. The first segment of the antennae (Scapus), the length of the body, is black and the remaining part is pale. The corniculus is black-pale colored. Legs are black-pale band-shaped. In winged viviparous females, the body is 1.1-1.8 mm in length, the head and thorax are black and the abdomen is pale-black. The eyes are blackish. The wing veins are broadly lined with brown pigments that accentuate the unusual venation. (Blackman & Eastop, 1984) (Figs. 1, 2).

Distribution

The species is widespread in all tropical and subtropical parts of the world (EUROPE, Azores, ASIA, Bonin Islands, Ceylon, China, Christmas Island, Cocos-Keeling, India, Indonesia, Israel, Jordan, Malaysia, Nepal, Bangladesh, Philippine Islands, Ryukyu Islands, Taiwan, AFRICA, Angola, Burundi, Cameroun, Canary Islands, Cano, Verde Islands, Congo, Dahomey, Egypt, Gambia, Ghana, Guinea, Ivory Coast, Kenya, Madagascar, Madeira, Malawi, Mali, Mauritania, Mauritius, Niger, Nigeria, Portuguese, Guinea, Réunion, Rhodesia, Rwanda, St. Helena, Senegal, Sierra Leone, Somalia, South Africa, Tanzania, Togo, Uganda, Upper Volta, AUSTRALASIA and PACIFIC ISLANDS, Australia, Caroline Islands, Cook Islands, Fiji, Gilbert and Ellice Islands, Hawaii, Mariana Islands, Marshall Islands, New Hebrides, Papua & New Guinea, Samoa, Tokelau, Tonga, Wallis, Irian Jaya, NORTH AMERICA, Mexico, U.S.A., CENTRAL AMERICA and WEST INDIES, Costa Rica, Guatemala, Honduras,

Nicaragua, Panama Canal Zone, West Indies, SOUTH AMERICA, Brazil, Colombia, French Guiana, Guyana, Surinam, Venezuela.) (CABI, 2021).



Fig. 1. Wingless (a) and winged (b) *Pentalonia nigronervosa* in Mersin, Turkey.



Fig. 2. Damage of *Pentalonia nigronervosa* on the banana plants (a, b: damage on banana shoots, c: backward death, d: severely damage).

Molecular identification

The research was carried out to reveal aphid species from banana greenhouses in the Mediterranean Region of Turkey. For this purpose, 18 specimens from ten aphid populations were analyzed by molecular method, and 700-bp product COI regions were sequenced. *Aphis gossypii* as an out-group was separated from the other specimens. The reference genes were compared to have an idea about the origin of *P. nigronervosa* in Turkey.

According to molecular studies, aphid specimens from Turkish banana plantations were identified as *P. nigronervosa*. The research is the first record of *P. nigronervosa* in Turkey. *Pentalonia nigronervosa* specimens in the phylogenetic tree were separated into three groups. Indian specimens were identical with 96 Bootstrap values and distinguished from the others. The other cluster had Turkish specimens and was distinguished with a 97 Bootstrap value. Turkish specimens are close to Florida and

some Hawaii specimens. The last group which has Kenya and some Hawaii specimens are separated from the other two with lower Bootstrap values.

DISCUSSION

Climatic conditions are the key factor for plant distribution. because of anthropologic activities climate-changing is observed all over the world although it has adverse effects on plants and animals in some cases. Opposite this, it provides new opportunities for cultured plants like the banana, which is a tropical plant. It has been brought from Egypt and cultured in Turkey since the beginning of the 1900s. Until the last two decades, its distribution was limited, but now it has dispersed to the entire Mediterranean region of Turkey. So Changing climatic conditions and accordingly changing plant patterns create favorable conditions for new plant and insect species to settle in a region. Banana aphid, *P. nigronervosa*, has a widespread distribution in tropic and subtropics regions of the world with more than 120 locations (CABI, 2021). Until the last 10 years, banana production was limited in Anamur district of Mersin and some districts of Antalya which have a microclimatic conditions to grow bananas in Turkey. Increasing temperatures, decreasing number of frosty nights, and subventions for production in greenhouse resulted in the spreading of banana production throughout the coast of the Mediterranean Region of Turkey with exponential growth. *Pentalonia nigronervosa* possibly entered Turkey as a result of this uncontrolled growth. Banana production regions have been exposed to *P. nigronervosa* probably due to the import of propagation material or materials imported for breeding programs and spread to all banana production areas. Though *Aphis gossypii*, *Aphis fabae*, and *Myzus persicae* were listed as an aphid on bananas (Uygun, Ulusoy, Karaca, & Satar, 2010), *Pentalonia nigronervosa* was found to be the only common aphid species on bananas in the entire region. The use of the conventional growing method for settling a new banana plantation causes contamination of the pest. Contaminated banana seedlings have growing problems and result in quality and quantity problems (Fig. 2). Moreover, our observation shows that the stem apex and flowering organs of mature plants encounter similar problems. Therefore, the use of material from tissue culture is an important method for preventing the spreading of the pest in controlled greenhouse conditions.

Pentalonia nigronervosa was first described in Isle de Bourbon of Madagascar and now had worldwide distribution in all banana-growing areas (Robson et al, 2007). Molecular studies revealed that *P. nigronervosa* clustered three different branches and one has all Turkish specimens in the world (Fig. 3). Turkish specimens have one haplotype which indicates that the aphid probably spread from one resource. Anholocyclic development of the aphid in the region contributed to fast-spreading and having one haplotype. However, the research of Galambao, (2011) in Hawaii islands refused that parthenogenetic production in aphids caused population-based differentiation. The researcher detected genetic differences not only among islands, and different host plants but also in banana populations that have more than ten genotypes. *Pentalonia nigronervosa* was listed on spinach in Hawaii by Holdaway,

(1944). Long-term adaptation of *P. nigronervosa* in Hawaii islands could be the reason for genetically differentiated populations. However, the species was first recorded with this study and possibly had only several months period in Turkey.

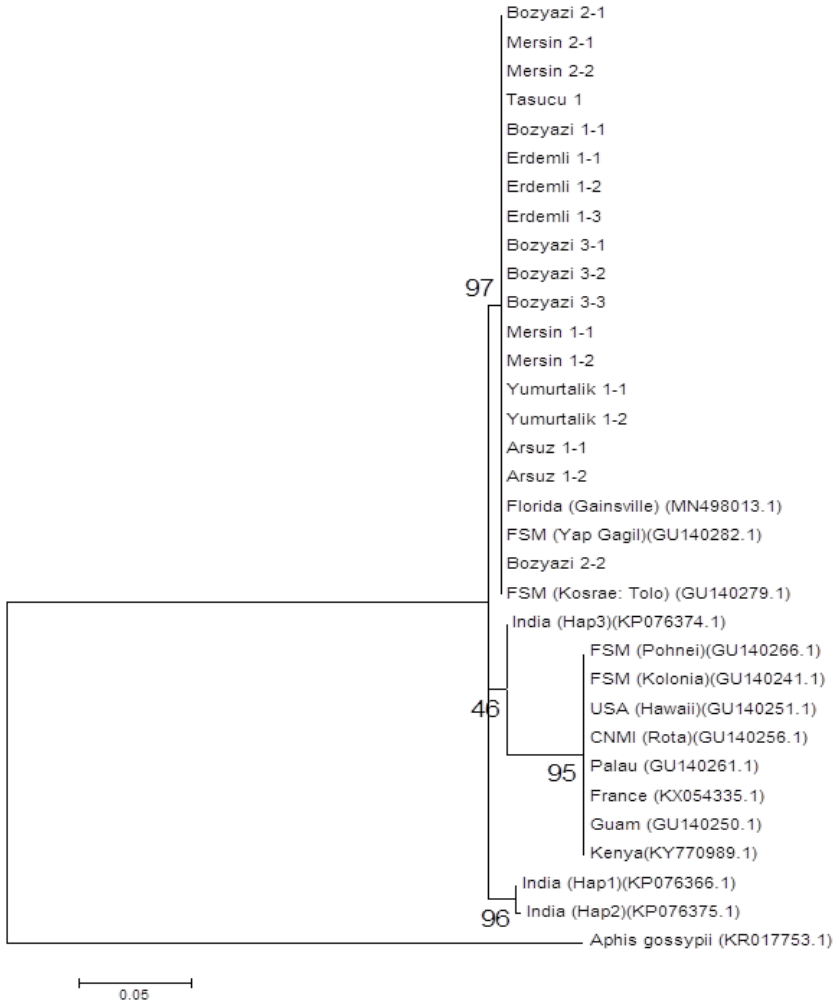


Fig. 3. Dendrogram constructed by Maximum-Likelihood method from the COI sequences of *Pentalonia nigronervosa*.

Although, all the collected samples from the different locations were cultured in the laboratory condition no biological control agents detected. *Aphidius colemani* Viereck and *Lysiphlebus testaceipes* Cresson (Hymenoptera: Braconidae) have the potential to control *P. nigronervosa* (Völkl et al, 1990), and these species were active in the region (Satar et al, 2014; 2021). But none of them recorded in *P. nigronervosa* in the region.

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The study is the first record for the determination of *Pentalonia nigronervosa* in Turkey. During the survey period of the study, any biological control agents from cultured samples in the laboratory condition were not determined in the region. Meanwhile, no product for pest control has not been registered for banana plantations yet. *Colocasia esculenta* being planted in Bozyazı (Mersin), known as taro, is also a minor host for this aphid (Suparman, Gunawan, Pujiastuti, & Cameron, 2017; Şahin & Erbilin, 2019). While applying control practices for the banana aphid, alternative hosts should not be ignored. Biological, chemical, and cultural control methods should be integrated under the IPM strategy and applied without harming the natural balance.

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