First Record of the Striped Mealybug, *Ferrisia virgata* and the Wax Scale *Ceroplastes* sp. on Coffee Plants in Saudi Arabia

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Abstract

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Coffee is one of the most important and promising agricultural commodities in Saudi Arabia and all over the world. This is the first report of the occurrence of *Ferrisia virgata* and *Ceroplastes* sp. associated with coffee in urban landscapes in Jazan region, Saudi Arabia during the 2022 fall season. Both insects were identified according to the morphological characteristics and amplification of 800 bp amplicon of 28s rRNA of the mealybug and the wax scale. The phylogenetic analysis of the amplified 28s sequences with sequences already available in GenBank confirmed the occurrence of such pests in Saudi Arabia on coffee cultivars, and it represents a potential threat to coffee production in Saudi Arabia and neighboring countries. Thus, quick management action is highly needed to prevent the expected spread of these insect pests.

Keywords: *Ferrisia virgata, Ceroplastes* sp., mealybug, wax scale, phylogenetic analysis.

Introduction

For decades, mealybugs (Hemiptera: Pseudococcidae) and wax scale (Hemiptera: Coccidae) have been considered as significant pests, with 2047 species of Pseudococcidae and 1232 species of Coccidae, that infest a variety of crops and ornamental plants worldwide (Miller *et al.,* 2002; Sutter *et al.,* 2021). Due to the significant direct yield loss of agricultural crops, and indirectly through the spread of viruses and reduction in yield quality, these sap-sucking insects have been the subject of extensive study for decades (Jahn *et al.,* 2003; Mahfoudhi *et al.,* 2009; Meyer *et al.,* 2008; Mibey, 1997; Nakaune *et al.,* 2008).

Investigations of the systematic or the population biology of mealybugs and wax scales as well as the control of these pests, represent a significant challenge due to the high degree of morphological resemblance between different species of these insects. Three areas in the Jazan region, produce the majority of Saudi's coffee that is marketed locally and exported to the neighboring countries. More than 79,000 thriving coffee plants on the steep mountainside and hillsides are planted in such areas, which is ideal for growing coffee because of the temperate environment with appropriate rainfall and high humidity. Saudi Arabia is home to several distinctive coffee varieties, such as Khawlani, Berri, Harari, and Bahri, imported some time ago from Brazil to Saudi Arabia via Turkey. In the Fall of 2022, infestation of mealybugs as well as wax scales were observed in several coffee plants in different farms of Jazan.

The purpose of this study is to determine the spread and to identify the mealybugs and wax scales that threaten coffee plants in Saudi Arabia.

Materials and Methods

Collecting insect specimens and DNA Extraction

A total of 13 adults (8 for mealybug and 5 for wax scale) were collected from coffee farms during the period October 2022 to February 2023. Specimens were stored in 100% ethanol and maintained at -20ºC until examination. All specimens were preserved in Pests and Plant Diseases Unit (PPDU), College of Agriculture & Food Sciences, King Faisal University, Saudi Arabia. Total DNA was extracted from collected specimens using Qiagen DNAeasy Blood & Tissue Kit (Qiagen, Germany) according to the manufacturer's instructions.

Molecular analysis of 28s ribosomal RNA (rRNA) together with morphological features were used for the identification of different specimens of the mealybug and wax scale collected specimens. The techniques outlined by Malausa *et al.* (2011) were followed to amplify the nuclear ribosomal DNA loci 28S-D2. The PCR was employed to amplify a specific fragment corresponding to the 28s-D2 region using the primer pairs 28S-D2_F (5'-AGAGAGAGTTCAAGAGTACGTG-3') and 28S-D2_R (5'-CTGGTTGATCCTGCCAGTAG-3'). The PCR products were sent to Macrogen for nucleotide sequencing.

Phylogenetic analysis

The amplicons were analyzed, and nucleotide BLASTn was run against the NCBI GenBank database to determine the evolutionary relatedness of the sequences obtained. Sequence alignment and building of the phylogenetic tree were performed using the Molecular Evolutionary Genetics

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Analysis (MEGAX) software. Using the Neighbor joining method, a phylogenetic tree based on the 28s rDNA was created.

Results and Discussion

The taxonomic identification of mealybugs and the wax scale pests is a significant obstacle for the development of effective integrated pest management practices. This study identified a single specimen of mealybug as *errisia virgata*, and an additional specimen of wax scale as *Ceroplastes* sp., both were collected from the twigs and apical shoots of infested coffee plants in the Jazan region during the period from October 2022 to February 2023. These pests *Ferrisia virgata* and *Ceroplastes* sp. were reported previously in Brazil (Williams *et al.,* 1992). Based on our knowledge, this is the first report of these two species in Jazan, Saudi Arabia. The nucleotide sequence identity between each segment of 28s-D2 and its potential genes identified in the public database varied from 99 to 100%. The developed phylogenetic trees (Figure 1) showed that *Ferrisia virgata* (AY179454.1) was grouped together with the newly collected mealybug species on coffee from Jazan (*Ferrisia virgata* _Jazan_2022).

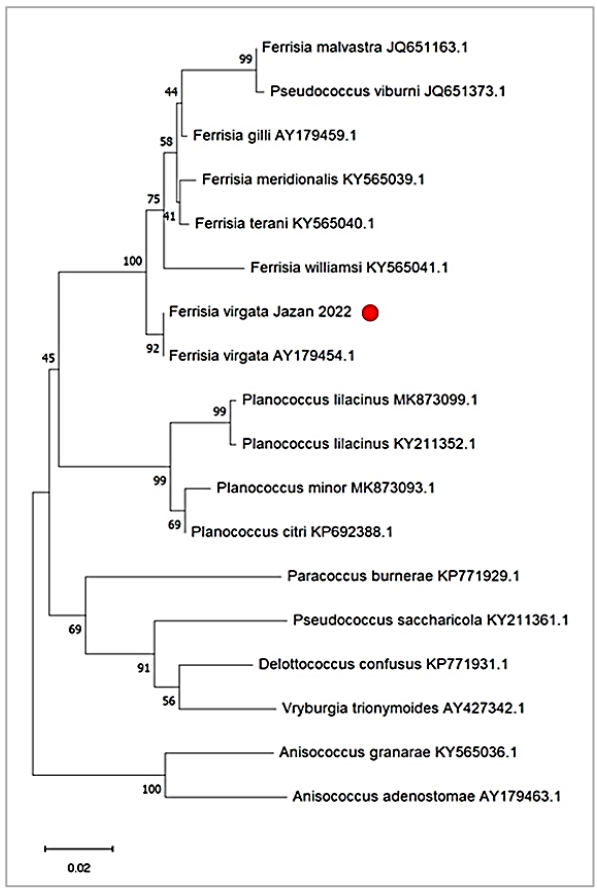

Figure 1. Phylogenetic analysis of *Ferrisia virgata*_Jazan_2022 compared with other sequences retrieved from GenBank.

The identification of individuals from Jazan on the basis of morphological traits as *Ferrisia virgata* was confirmed through 28S fragment amplification from genomic DNA, followed by direct sequencing and BLAST analysis. The resulting sequences were aligned to reference sequences from NCBI compared with publicly available data at the GenBank, with 100% similarity with *Ferrisia virgata* isolates from the United States (Accession numbers AY179454.1 and AY179465.1, and 99.68% similarity with another USA mealybug accession (AY179468.1). On the other hand, the analysis for wax scale insect sample showed a homology level of 99.42% with *Ceroplastes* sp. isolate from South Africa (Accession n. JQ651262.1), 98.98 % with *Ceroplastes destructor* (Accession n. JQ651205.1) from south Africa, and 91.37% with *Ceroplastes sinensis* (Accession n. KY085822.1) from Chile.

Phylogenetic analysis

A total of eighteen 28S sequences, one of which was sequenced in this study, were compared. Among them, *Ferrisia virgata* accessions collected from different countries, including its native location (USA). Based on to the phylogenetic analysis, the phylogenetic tree included two main distinct clades: one clade consisted of samples from Spain (KP771929 & KP771931), China (KY211361) and USA (AY427342), and another clade of grouped accessions from different *Ferrisia virgata* invaded areas (Figure 1). Notably, both clades shared a common ancestor derived from Brazil (Acc. No. AY179463) and the United States (KY565036). Interestingly, the sample of *Ferrisia virgata* we sequenced (*Ferrisia virgata* _Jazan_2022), clustered together with isolates coming from only three different world regions (Brazil, the United States and South Africa). Its area of origin is not known, even though it was described from Jamaica, but it may have been an introduction from elsewhere (Kaydan & Gullan, 2012).

Ferrisia virgata, commonly known as the striped mealybug, is a worldwide pest that infests a wide variety of host plants, including species from several important agricultural families, such as Anacardiaceae, Bromeliaceae, Rubiaceae, Cucurbitaceae, Rutaceae, Solanaceae and Myrtaceae (Ben-Dov, 2005). It attacks 207 genera in 78 families (Morales *et al.,* 2016), including *Plumeria rubra* L. However, *Coffee arabica* (arabica) appears to be a new host for the species.

Ferrisia virgata was most frequently observed to feed on leaves and reported as a vector for the viruses such as piper yellow mottle virus (PYMV) in India and Cacao swollen-shoot badnavirus (CSSV) in Africa (Bhat *et al.,* 2003). In addition, it has been suggested that it affects *C. papaya* in Micronesia (Nafus *et al.,* 1999). This species is widespread originally in Brazil (Anonymous, 2003). *Ferrisia virgata* is easily recognized by its distinctive appearance. Adult females are typically grayish in color, covered in white powdery wax, and have two dark stripes (or rows of spots) on their dorsal surfaces. Their bodies are also relatively elongated, tapering posteriorly, and ending in two long wax filaments (Figure 2).

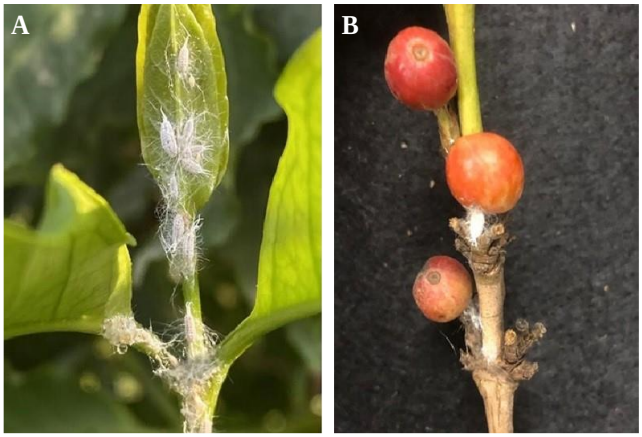

Figure 2. *Ferrisia virgata* adult females on twigs and leaves of coffee plants, A: and on coffee beans, B: in open fields of coffee plants.

In the same context, 18 wax scale 28S sequences were analyzed, one of which was sequenced in the current study. According to the phylogenetic analysis, the phylogenetic tree included two main distinct clades: one clade consisted of samples of *Parasaissetia nigra* from Chile (KY085827.1), *Parasaissetia nigra* from Egypt (LC440351), *Ceroplastes rusci* from China (MT317013) and *Ceroplastes rusci* from Egypt (LC440351.1). The other clade grouped accessions from different *Ceroplastes* sp. invaded areas (Figure 3). The sample of *Ceroplastes* sp. we sequenced (*Ceroplastes* sp._Jazan_2022) clustered together with isolates coming from only one region (South Africa) (JQ651262 & JQ651205.1). Hence, the exact species of *Ceroplastes* sp. could not to be recognized using 28s sequence.

The adult female of the genus members is known to have thick wax test and the presence of stigmatic setae in each cleft in groups of more than three (Figure 4). *Ceroplastes* species are present in all tropical and subtropical zoogeographic zones, with high diversity (Hodgson & Peronti, 2012).

There are 143 species in the genus *Ceroplastes* worldwide, some are significant pests in agriculture. Therefore, accurate identification of the species is necessary, as a first step in choosing the effective approach to manage that species. Additional research is needed on the food preferences of the species and the environmental factors that favor its multiplication and spread.

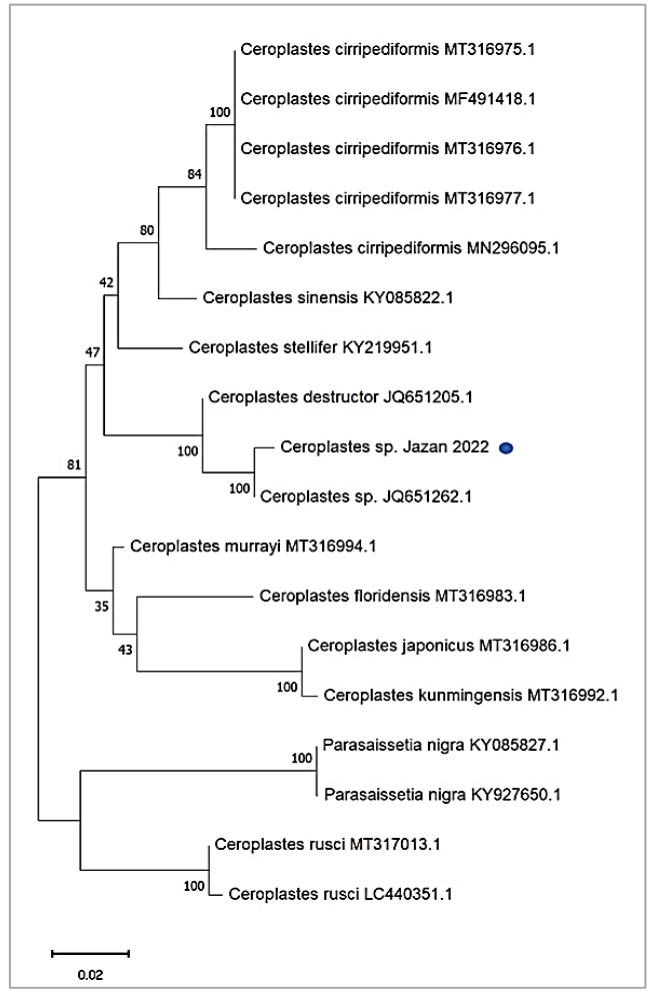

Figure 3. Phylogenetic analysis of wax scale *Ceroplastes* sp. compared with other sequences retrieved from GenBank.

Figure 4. Adult female coffee wax scale, *Ceroplastes* sp. on twig of coffee plant.

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الملخص

الهديب، خالد، وائل المنوفي وعائشة الغزواني. 2024. التسجيل الأول لحشرة البقّ الدقيقي (*Ferrisia virgata*) والحشرة القشرية *Ceroplastes* sp. على نباتات البنّ/القهوة في المملكة العربية السعودية. مجلة وقاية النبات العربية، 42(4): 456−460.
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يعدّ البنّ/القهوة من أهم السلع الزراعية التجارية الواعدة في المملكة العربية السعودية وتعدّ حشرة البق الدقيقي (Hemiptera: Pseudococcidae) والحشرات القشرية (Hemiptera: Coccidae) من الآفات ذات الخطورة العالية والتي يمكن أن تسبب أضراراً جسيمة للنباتات المزروعة. في هذه الدراسة، تمّ تسجيل أحد أنواع البق الدقيقي وكذلك الحشرة القشرية الشمعية للمرّة الأولى على نباتات البنّ/القهوة في منطقة جازان بالمملكة العربية السعودية خلال موسم الخريف لعام 2022. تمّ تصنيف حشرة البقّ الدقيقي بأنها النوع *Ferrisia virgata* والحشرة القشرية بأنها تتبع جنس *Ceroplastes* sp. وذلك طبقاً للفحص الظاهري وتحليل تفاعل البلمرة المتسلسل لحوالي 800 قاعدة نيتروجينية لجزء محدد من الحمض النووي الريبوزى S28 لكلٍ من حشرة البق الدقيقي والحشرة القشرية. كما أشار تحليل النشوء والتطور ما بين التتابعات النيكليوتيدية للحمض النووي الريبوزي من نوع S28 والتي تم الحصول عليها من بنك الجينات إلى احتمالية انتشار هذه الآفات بالمملكة العربية السعودية، ومن الممكن أن تكون قد وفدت من خارج السعودية مع الشتلات المستوردة من دول أخرى ومنها انتشرت هذه الآفات إلى أصناف البنّ/القهوة المختلفة. وقد تمثل هذه الآفات تهديداً محتملاً لإنتاج البنّ/القهوة في المملكة والبلدان المجاورة. لذلك فإن التدخل ببرامج المكافحة المتكاملة بات مطلوباً لمنع الانتشار المحتمل لتلك الآفات فى المملكة.

كلمات مفتاحية: *Ferrisia virgata*، *Ceroplastes* sp.، حشرة البق الدقيقي، الحشرة القشرية، شجرة القرابة.

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