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First record of *Dactylopius confusus* (Cockerell, 1929) (Hemiptera: Coccomorpha: Dactylopiidae) in Michoacan, Mexico

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Abstract. In April 2017, several scale insect specimens in the family Dactylopiidae were collected from infested cladodes of wild *Opuntia tomentosa* Salm-Dyck, 1822 (Cactaceae) in the outskirts of Morelia City, Michoacan, Mexico. The specimens were identified as *Dactylopius confusus* (Cockerell, 1929) (Hemiptera: Coccomorpha: Dactylopiidae). In this article, the presence of *D. confusus* in Michoacan state, Mexico, is recorded for the first time. A subsequent finding of *D. confusus* on *Opuntia ficus-indica* (L.) Mill., (1768) (Cactaceae) in October 2019 was confirmed with 18S rDNA and 28S rDNA.

Key words. Cochineal insect, *Opuntia tomentosa*, *Opuntia ficus-indica*, 18S rDNA and 28S rDNA.

Introduction

The family Dactylopiidae is a group of phytophagous scale insects specialized in feeding on Cactaceae (Ferris 1955; Mann 1969). Members of this family all belong to the genus *Dactylopius* Costa. The genus initially consisted of nine species worldwide (De Lotto 1974; Perez-Guerra and Kosztarab 1992; De Haro and Claps 1995). However, recently *Coccus bassi* Targioni Tozzetti, 1867 was transferred into *Dactylopius* (Ben-Dov and Marotta 2001) and *Dactylopius gracilipilus* Van Dam and May, 2012 was described as a new species (Van Dam and May 2012). The economic importance of the Dactylopiidae lies in the fact that all species produce carminic acid, the basis for the historically significant carmine dyes (De Lotto 1974; Perez-Guerra and Kosztarab 1992). Additionally, some species have been successfully used as biological control agents against invasive Cactaceae in several parts of the world (Moran and Zimmermann 1985).

In Mexico, six species in the genus *Dactylopius* have been recorded: *D. bassi* (Ben-Dov and Marotta 2001), *D. ceylonicus* (Green, 1896) (MacGregor and Sampedro 1983; Portillo and Vigueras 2006; Chávez-Moreno et al. 2010; Chávez-Moreno et al. 2011; Chávez-Moreno et al. 2013), *D. coccus* Costa, 1829 (MacGregor and Sampedro 1983; Perez-Guerra and Kosztarab 1992; Miller 1996; Portillo and Vigueras 2006; Chávez-Moreno et al. 2010; Chávez-Moreno et al. 2011; Chávez-Moreno et al. 2013), *D. confusus* (Cockerell, 1929) (MacGregor and Sampedro 1983; Perez-Guerra and Kosztarab 1992; Gill 1993; Miller 1996; Portillo and Vigueras 2006; Chávez-Moreno et al. 2010; Chávez-Moreno et al. 2011; Chávez-Moreno et al. 2013), *D. opuntiae* (Cockerell, 1896) (Mann 1969; MacGregor and Sampedro 1983; Gill 1993; Miller 1996; Portillo and Vigueras 2006; Chávez-Moreno et al. 2010; Chávez-Moreno et al. 2011; Chávez-Moreno et al. 2013), and *D. tomentosus* (Lamarck, 1801) (MacGregor and Sampedro 1983; Perez-Guerra and Kosztarab 1992; Gill 1993; Miller 1996; Chávez-Moreno et al. 2010, 2011, 2013).

So far, *D. confusus* has been recorded in 17 Mexican states (Table 1). In 2011, specimens appearing to possess morphological characters typical for both *D. confusus* and *D. salmianus* De Lotto, 1974 were found in Hidalgo, Morelos, Puebla, San Luis Potosí and Tlaxcala (Chávez Moreno et al. 2011). The taxonomic status of this population still has to be evaluated. In the current work, the presence of *D. confusus* in Michoacan state, Mexico, is recorded for the first time from slide-mounted specimens of two populations collected in 2017 and 2019 using morphological keys (Perez-Guerra and Kosztarab 1992; Claps 2010). In addition, in October 2019, DNA of female specimens from the second population was extracted. High-quality sequences from nuclear DNA (18S rDNA and 28S rDNA) were used to confirm morphological identification.

Materials and Methods

In April 2017, nymphs and adults of the genus *Dactylopius* (Fig. 1A) were collected from infested cladodes of wild *Opuntia tomentosa* Salm-Dyck (Cactaceae) plants (Fig. 1B, 1C) in the outskirts of Morelia City, Michoacan, Mexico, in a suburban site with the following coordinates: 19°39′50.5″N, 101°13′08.2″W, and 1920 m. The insects were preserved in 70% ethanol; subsequently, several adult females were prepared and slide-mounted for species identification according to Kosztarab (1963) and De Haro and Claps (1995). Specimens recorded in this work were deposited in the CIIDIR-IPN, Unidad Michoacán, Mexico, in the Colección de Insectos Escama del Colegio de Postgraduados, Campus Montecillo, Texcoco, Edo. de México, Mexico and in the Colección Nacional de Insectos, del Instituto de Biología, UNAM, Ciudad de México, Mexico.

Table 1. Mexican states where *Dactylopius confusus* has been recorded.

State	Reference
Baja California	Ferris 1955.
Chihuahua	MacGregor and Sampredo 1983; Perez-Guerra and Kosztarab 1992.
Distrito Federal (Ciudad de México)	MacGregor and Sampredo 1983; Chávez-Moreno et al. 2010; Chávez Moreno et al. 2011; Chávez Moreno et al. 2013.
Durango	Miller 1996.
Guanajuato	MacGregor and Sampredo 1983.
Guerrero	MacGregor and Sampredo 1983; Perez-Guerra and Kosztarab 1992; Miller 1996.
Hidalgo	Chávez-Moreno et al. 2010; Chávez Moreno et al. 2011; Chávez Moreno et al. 2013.
Jalisco	MacGregor and Sampredo 1983; Perez-Guerra and Kosztarab 1992; Miller 1996; Portillo and Vigueras 2006; Chávez-Moreno et al. 2010; Chávez Moreno et al. 2011; Chávez Moreno et al. 2013.
Morelos	MacGregor and Sampredo 1983; Perez-Guerra and Kosztarab 1992; Miller 1996; Chávez-Moreno et al. 2010; Chávez Moreno et al. 2011; Chávez Moreno et al. 2013.
Nuevo León	Perez-Guerra and Kosztarab 1992; Miller 1996.
Oaxaca	MacGregor and Sampredo 1983; Perez-Guerra and Kosztarab 1992.
Puebla	MacGregor and Sampredo 1983; Perez-Guerra and Kosztarab 1992; Miller 1996; Chávez-Moreno et al. 2010; Chávez Moreno et al. 2011; Chávez Moreno et al. 2013.
San Luis Potosí	Portillo and Vigueras 2006; Chávez-Moreno et al. 2010; Chávez Moreno et al. 2011; Chávez Moreno et al. 2013.
Sonora	Perez-Guerra and Kosztarab 1992; Miller 1996.
Tamaulipas	MacGregor and Sampredo 1983.
Veracruz	Chávez-Moreno et al. 2010; Chávez Moreno et al. 2011; Chávez Moreno et al. 2013.
Zacatecas	Chávez-Moreno et al. 2010; Chávez Moreno et al. 2011; Chávez Moreno et al. 2013.

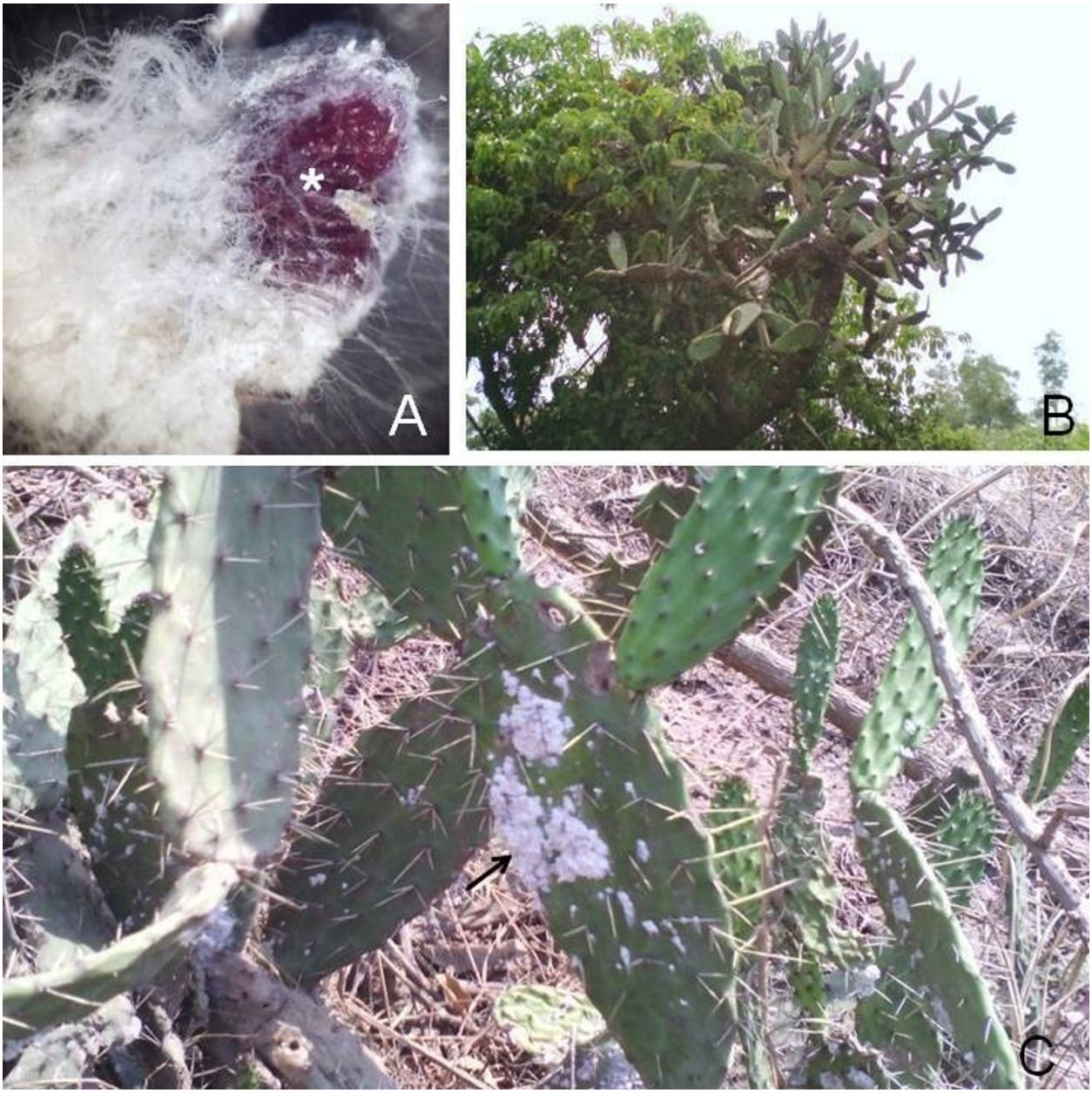

Figure 1. *Dactylopius confusus* on *Opuntia tomentosa*. **A)** Adult female of *D. confusus* (asterisk) covered with white cottony wax. **B)** Specimen of *O. tomentosa*. **C)** Cladodes of *O. tomentosa* infested with colonies of *D. confusus* (arrow).

A second population of the genus *Dactylopius* was collected in October 2019 from infested cladodes of wild *Opuntia ficus-indica* (L.) Mill. (Cactaceae) in Tenencia Morelos, Morelia, Michoacan, Mexico, coordinates: 19°39′063″N, 101°13′52.3″W, and 1950 m. Specimens were collected in 100% ethanol and some were slide-mounted and deposited in the CIIDIR-IPN, Unidad Michoacan, Mexico, and in the insect collection of LSUA, USA. DNA of female specimens of this population was extracted using a commercial kit (E.Z.N.A. 2019). PCR was run with the following primers: For 18S rDNA, "2880" (F), 5′-CTGGTTGATCCTGCCAGTAG-3′ (Tautz et al. 1988), "B-"(R), 5′-CCGCGGCTGCTGGCACCAG-3′ (Von Dohlen and Moran 1995); for 28S rDNA, "s3660" (F), 5′-GAGAGTTMAASAGTACGTGAAAC-3′ (Sethusa et al. 2014), "28b" (R), 5′-TCGGAAGGAACCAGCTACTA-3′ (Sethusa et al. 2014). Conditions for PCR were as follows: Step 1: 1 min at 94°C, step 2: 40 s at 94°C, step 3: 2 min at 53°C, step 4: 1 min at 68°C.

Steps 2–4 were repeated five times. Step 5: 30 s at 94°C, step 6: 40 s at 58°C, step 7: 1 min at 68°C. Steps 5–7 were repeated 36 times. Step 8: 5 min at 68°C, step 9: ∞ at 4°C. After confirmation of successful amplification on 1.5% agarose gels and cleanup of PCR products (Thermo Scientific 2019), PCR products were sent to a commercial laboratory for Sanger sequencing. Returned sequences were visualized in 4Peaks (Griekspoor and Groothuis 2015) and the largest possible sequences chosen by visual inspection for comparisons to known sequences in NCBI's BLASTn tool used with standard settings.

Results and Discussion

Female specimens of both populations were identified as *D. confusus* (Cockerell) using the keys of Perez-Guerra and Kosztarab (1992) and Claps (2010) (Fig. 2A). The differential diagnostic features identifying females of *D. confusus* reported in this work are: (1) truncate conical dorsal setae increasing in size toward abdomen (Fig. 2B, 2C), but in *D. ceylonicus* and *D. opuntiae* numerous similarly sized truncate setae evenly distributed on dorsum (Perez-Guerra and Kosztarab 1992); (2) anal ring present in *D. confusus* (Fig. 2B), but not in *D. tomentosus* (Perez-Guerra and Kosztarab 1992; Claps 2010); (3) clusters of quinquelocular wide-rimmed pores associated with ducts in *D. confusus* (Fig. 2D), but without ducts in *D. coccus* and *D. salmianus* (Perez-Guerra and Kosztarab 1992; Claps 2010); (4) spiracular opening with dentate margin in *D. confusus* (Fig. 2E), but with smooth margin in *D. zimmermanni* (Perez-Guerra and Kosztarab 1992; Claps 2010).

However, species identification in the genus *Dactylopius* is by no means a clear-cut affair, and hybrids may occur (Chávez Moreno et al. 2011). In order to confirm morphological identifications, DNA was extracted from specimens collected in 2019. Nuclear ribosomal DNA (both 18S rDNA and 28S rDNA) was amplified in high quality resulting in percent identities close to 100% with query covers of 99-100% using NCBI's BLASTn tool with standard settings. NCBI GenBank accessions for *D. confusus*: SUB6600587 18s_Dactylopius MN723892 and SUB6601590 28s_Dactylopius MN727057. The top hits were Ramírez-Puebla et al. (2010), acc. No. GQ853358.1, 99.83 percent identity for 18S rDNA, and Vea and Grimaldi (2016), acc. No. KT199061.1, 99.55 percent identity for 28S rDNA. Other species of *Dactylopius* also found with high max. score and query covers were *D. ceylonicus* and *D. austrinus* for 18S rDNA with percent identities between 99.50 (*D. ceylonicus*, Acc. No. GQ853357.1) and 98.98 (*D. austrinus*, Acc. No. AY795538.1), and *D.* sp. for 28S rDNA with 99.85 percent identity (acc. No. JQ651352.1). These two nuclear genes are highly conserved. However, because the greatest similarities between our sequences and the database sequences were found for *D. confusus*, we used this finding as an additional line of support for the morphological identification of *D. confusus*. In addition, we also amplified mt-DNA. While we could identify the genus *Dactylopius* with 12S and CO1 mt-DNA, low query coverage and 80 - 90 percent identities for both genes were not sufficient for species identifications.

The collection of *D. confusus* represents the first record of this species in the state of Michoacan, Mexico. Hitherto, only *D. opuntiae* has been recorded in Michoacan (MacGregor and Sampredo 1983; Perez-Guerra and Kosztarab 1992; Miller 1996; Chávez-Moreno et al. 2011; Chávez-Moreno et al. 2013).

In Mexico, species of cactus that can serve as hosts for the genus *Dactylopius* are numerous and widespread. It has been suggested that many more populations of different species of *Dactylopius* should be found in several Mexican states where no systematic collecting has taken place (Portillo and Vigueras 2006; Chávez-Moreno et al. 2011). More research is necessary to gain a better understanding of the distribution and abundance of different species of dactylopiids in Mexico.

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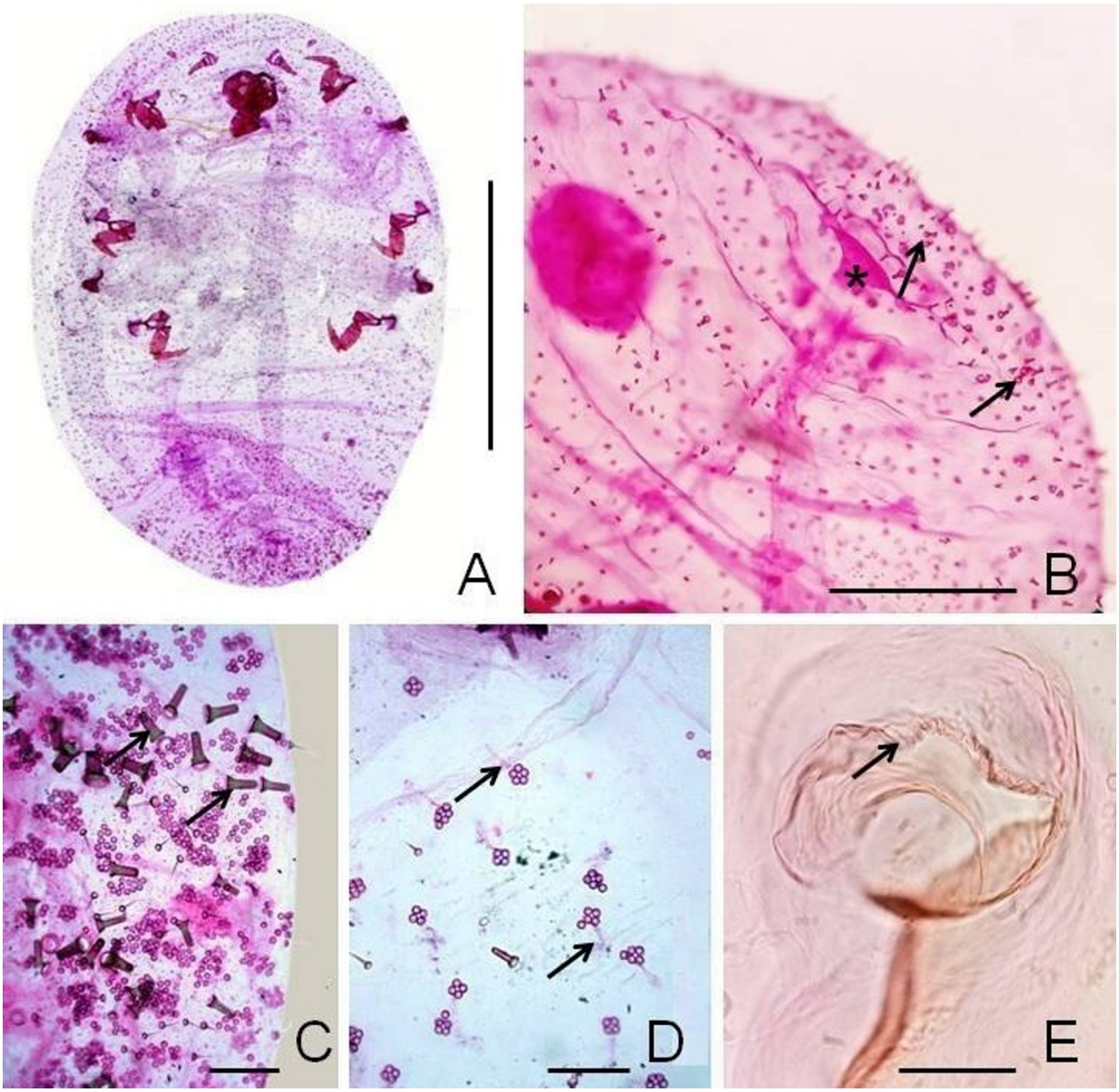

Figure 2. Slide-mounted adult females of *Dactylopius confusus*. **A)** Whole body. **B)** Posterior abdominal segments with modified setae increasing in size (arrows); anal ring (asterisk). **C)** Anal area with large modified setae (arrows). **D)** Quinquelocular pores with ducts (arrows). **E)** Spiracular opening with dentate margin (arrow). Scales: A= 2 mm. B= 0.5 mm. C, D, E= 50 µm.

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