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CARACTERIZACIÓN QUÍMICA Y RECONSTRUCCIÓN FILOGENÉTICA DE *DACTYLOPIUS* (HEMIPTERA: DACTYLOPIIDAE): BIODIVERSIDAD Y CONSERVACIÓN DE ESTE RECURSO GENÉTICO Y SUS ESPECIES HOSPEDERAS (OPUNTIOIDEAE: CACTACEAE) EN MÉXICO

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P R E S E N T A

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Dr. Isidro Ávila Martínez
Director General de Administración Escolar, UNAM
P r e s e n t e

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día 17 de agosto de **2009**, se aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** del (la) alumno (a) **CHAVEZ MORENO CARLA KARINA** con número de cuenta **96812956** con la tesis titulada: **"Caracterización química y reconstrucción filogenética de *Dactylopius* (Hemiptera: Dactylopiidae): Biodiversidad y conservación de este recurso genético y sus especies hospederas (Opuntioideae: Cactaceae) en México."**, realizada bajo la dirección del (la) **DR. ALBERTO TECANTE CORONEL:**

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Contenido

Resumen

México es una de las principales áreas de diversidad de cactáceas e insectos del mundo. Destacan, los insectos del género *Dactylopius* Costa (Hemiptera: Dactylopiidae), las cochinillas y sus plantas hospederas de la subfamilia Opuntioideae (Cactaceae), endémicos del continente Americano y que poseen un estrecha relación con la historia de los pueblos mexicanos. México cuenta con 83 a 104 de las cerca de 200 especies de opuntias que han sido reportadas en el mundo y aproximadamente la mitad son endémicas de este país. 20 especies de opuntias son cultivadas, poseen diferentes grados de domesticación, son usadas principalmente como alimento, ensilaje y en la fabricación de medicamentos; y tienen una amplia distribución en México, principalmente en las zonas áridas y semiáridas del país.

El género *Dactylopius* incluye nueve especies, cinco de las cuales ocurren de manera natural en México y sólo la especie domesticada *D. coccus*, es utilizada para la producción comercial de colorante. Son pocos los trabajos acerca de la distribución de *Dactylopius* en México y se enfocan principalmente en aspectos taxonómicos y etnobiológicos del insecto, la mayoría son catálogos o listas que incluyen una o varias especies. El número limitado de caracteres morfológicos de *Dactylopius* dificulta su reconocimiento y estudio. La información sobre *Dactylopius* y sus hospederos carece de descripciones específicas de su patrón de distribución, las características de sus hábitats y no consideran la identificación de los insectos usando caracteres químicos o moleculares. Por ello, la presente investigación doctoral plantea cuatro objetivos que que dieron lugar a las cuatro partes en que se divide este trabajo:

La primera parte tuvo como objetivo hacer una revisión histórica, cultural, geográfica, y ecológica de la información de los géneros *Opuntia* y *Dactylopius* y su distribución en México. Por ello, se documentó el uso de ambos géneros desde la época prehistórica, y el uso y cultivo de la cochinilla desde el siglo XI hasta la actualidad. Durante el periodo colonial y hasta mediado del siglo XIX, México fue el primer productor de grana cochinilla y su colorante. Actualmente, *Dactylopius* y *Opuntia* poseen una distribución mundial, siendo México el principal productor de nopal verdura y tuna de *Opuntia*. Sin embargo, el cultivo de la cochinilla es escaso y se conserva bajo el rescatado sistema tradicional indígena. Se reconoce que establecer en forma combinada estrategias *in situ* para la conservación, el reestablecimiento del uso y la producción de cochinilla, permitirían generar políticas de conservación para rescatar esta actividad.

En la segunda parte, el objetivo fue analizar el patrón de distribución del género *Dactylopius* y sus plantas hospederas, para evaluar la especificidad de esta asociación y describir las características de sus hábitats en México. Para esto, se recolectaron y georreferenciaron especímenes de insectos de 208 poblaciones de 120 localidades, de 14 estados de la República Mexicana de 2005 a 2007. Los datos de recolecta permitieron establecer las áreas de distribución y las características de los hábitats de *Dactylopius* y de sus hospederos de los géneros *Opuntia* Miller *sensu stricto*, *Nopalea* Salm-Dyck y *Cylindropuntia* (Engelm.) F.M. Knuth. Esta información se complementó con la revisión exhaustiva de 367 preparaciones de especímenes de *Dactylopius* de la Colección Nacional de Insectos de la Universidad Nacional Autónoma de México y 262 plantas de los herbarios Nacional de México (MEXU) y de Guadalajara (IBUG). Los insectos tienen una distribución continua y guardan correspondencia con sus hospederos. Se registraron las cinco especies de *Dactylopius,* principalmente en el altiplano central y norte y el sureste de México. Los insectos descritos en este trabajo sugieren que el número de especies de *Dactylopius* en México es superior a lo registrado hasta ahora y es posible que estén ocurriendo nuevos procesos de hibridación entre las especies nativas y las especies introducidas.

La tercera analiza el perfil metabólico del colorante de los insectos *Dactylopius* por cromatografía líquida de alta eficiencia (CLAE) para determinar si la composición del colorante permite diferenciar a las especies y si todas las especies de insectos son fuente potencial de colorante. Se analizaron los pigmentos de las cinco especies de *Dactylopius* provenientes de 35 poblaciones recolectadas en 13 estados de México y 2 de Argentina. Las cinco especies de *Dactylopius* analizadas fueron diferenciadas por sus perfiles metabólicos. La presencia de ácido carmínico como componente mayoritario en todos los *Dactylopius* hace de estos insectos una fuente potencial de este colorante.

La cuarta parte fue la reconstrucción filogenética de las cinco especies de *Dactylopius* mexicanos secuenciando sus genes mitocondrial 12S rRNA y nuclear 18S rRNA de los insectos y 16S rARN bacterial para identificar los endosimbioentes presentes en los insectos y generar información que apoye la identificación taxonómica de *Dactylopius*. Se obtuvo la secuenciación de las cinco especies de *Dactylopius* mexicanas, a partir de insectos recolectados en cinco localidades de cinco estados de México. La última parte del trabajo es la discusión de la integración de los resultados ecológicos, morfológicos, químicos y moleculares y su importancia en el estudio sistemático de *Dactylopius* y sus especies hospederas.

Abstract

Mexico is one of the world's main areas of diversity of cacti and their mutualist insects. In particular, the association between the species of the genus *Dactylopius* Costa (Hemiptera: Dactylopiidae) and their host plants of the subfamily Opuntioideae (Cactaceae), both endemic to the American Continent, have had a great significance to Mexican human cultures. A total of 200 *Opuntia* species have been worldwide reported, 83 to 104 ocurred in Mexico where nearly half of them are endemic. 20 *Opuntia* species have been recognized as cultivated, showing various degrees of domestication, mainly used as food, ensilage and medicine. The opuntioids have a large distribution in Mexico, mainly in the arid and semiarid zones of this country.

The genus *Dactylopius* includes nine species; five of them occur naturally in Mexico. *D. coccus* is the only domesticated species used for commercial colorant production. Few works deal with the distribution of *Dactylopius* in Mexico; they are mainly focused on taxonomic and ethno-biologic aspects of the insects. Most of the works are catalogs or listings reporting on one or several species of *Dactylopius,* which includes records of the five Mexican, reported insect species and their localities. *Dactylopius* possess very limited morphological characteristics, making their taxonomic identification difficult. Besides, information available on *Dactylopius* and their hosts lacks precise descriptions of their distribution pattern and characteristics of their habitats and does not considered chemical or molecular information for the insect species identification. Because of these, the present work stablished four objectives that are presented in the following four parts of this doctoral investigation:

The first chapter aims to review historical, cultural, geographical, and ecological information about the genera *Opuntia* and *Dactylopius* and their distribution in Mexico. To reach this objective, the information on their origin, diversity and distribution of *Dactylopius* and their hosts was exhaustivelly reviewed, as well as aspects of their conservation were discussed. *Opuntia* species were among the main components of human diet during pre-agricultural times. Cochineal was used and probably cultivated at least from the 11th century. During the colonial period, Mexico was the world's first producer of insects and dyes until the mid 19th century. Today, *Dactylopius* and *Opuntia* have worldwide distribution, Mexico is the main producer of *Opuntia* cladodes and prickly pear, but cochineal cultivation is marginal and only maintained in traditional indigenous systems. Strategies for *in situ* conservation combined with re-established use and cochineal production may enhance conservation policies.

The second chapter aims to analyze the distribution pattern of species of the genus *Dactylopius* in relation to the distribution of their corresponding host plants, in order to in order to evaluate the specificity of their association in the Mexican territory, as well as described the characteristics of their habits. To reach this objective, specimens of insects and their hosts were collected and georeferenced in 14 states of Mexico from 2005 to 2007. The distribution area, maps, and habitat characteristics of *Dactylopius*, *Opuntia* Miller *sensu stricto*, *Opuntia* (*Nopalea* Salm-Dyck) and *Cylindropuntia* (Engelm.) F.M. Knuth was determined on the basis of field collections. This information was complemented with that from exhaustive examination of 367 microscope slides of the CNI-IB-UNAM and 262 plants from the National Herbarium of Mexico (MEXU) and the herbarium of the University of Guadalajara (IBUG). It was found that the current distribution of the genus *Dactylopius* is continuous according to their hosts, broader than recognized hitherto. New georeferenced records of the five Mexican *Dactylopius* species are reported, mainly in the central and northern plateau and southeast of Mexico. These records suggest that the number of *Dactylopius* species in Mexico could be higher than that considered until now or that possible new processes of hybridization between native and introduced species may be occurring.

The third chapter aims to analyze the variations in the metabolic profiling of *Dactylopius* colorant content using high-performance liquid chromatography (HPLC) with a photodiode array detector, to establish the difference in the colorant composition according to the insect species, their geographical origin and hosts. For this, 35 populations, collected from 13 states of Mexico and 2 from Argentina were analysed. This analysis allowed each species to be identified on the basis of differences in their metabolic profiles. For all species, carminic acid was the major compound present in significant amounts, making all five species potential sources of colorant.

Finally, the forth chapter aims to make the phylogenetic analyses of the five Mexican *Dactylopius* species by the sequence of the 12S rRNA mithocondrial and 18S rRNA PCR nuclear amplified insect gene and the 16S rARN *Dactilopius* bacterial gene. The five *Dactylopius* Mexican species, recollected from five localities, in five states of this country was sequenced. The last part of this work was the discussion of the integration of ecological, chemical and phylogenetic anylisis and its importance in the systematic study of *Dactylopius* species and their host.

Introducción general

Los insectos del género *Dactylopius* Costa (Hemiptera: Dactylopiidae), conocidos como "cochinillas" y las cactáceas que son sus hospederas, de la subfamilia Opuntioideae (Cactaceae), son endémicos del continente Americano (Britton y Rose, 1963; Bravo-Hollis y Sánchez-Mejorada, 1978; Brummitt y Powell, 1992, Anderson, 2001). El aprovechamiento de la interacción entre insectos y sus plantas hospederas está estrechamente relacionado con la historia de los pueblos precolombinos de mesoamérica quienes incluían a las cactáceas como parte fundamental de su dieta desde hace 12,000 a 14,000 años (Smith, 1967; MacNeish, 1992) y a las cochinillas como fuente de colorante (Martín del Campo 1957; MacGregor, 1976; Piña, 1977; Anderson, 1981; Colunga *et al*., 1986; Bravo-Hollis y Scheinvar, 2002; Casas y Barbera, 2002; Reyes-Agüero *et al*., 2005a).

En México, la producción del colorante de la cochinilla data del siglo XI (Piña 1977) y existen documentos sobre este proceso (Cortés, 1981; Díaz del Castillo, 2005; Dahlgren 1990; Butler, 2006). Durante el periodo colonial, la producción de grana cochinilla llegó a representar grandes beneficios para los colonizadores españoles que usaban las especies de *Dactylopius* spp., lo que aumentaba sustancialmente la cantidad de insecto y colorante que producían (Humboldt, 2006). México llegó a ser el primer productor de grana cochinilla y su colorante durante la colonia y hasta mediados del siglo XIX (Dahlgren, 1990; Humboldt, 1966). Desafortunadamente, tras la guerra de independencia y la exitosa expansión del cultivo en otros países, México perdió esta posición (Thiéry de Menonville, 1787; Humboldt, 2006; Balaram-Tolat, 2002; Butler, 2006).

Actualmente, *Dactylopius* y sus hospederos han sido registrados en los cinco continentes (De Lotto, 1974), México es el principal productor de nopal verdura y tuna de *Opuntia*, pero la cría de cochinilla, bajo el rescatado sistema tradicional indígena, genera una escasa producción (Pérez-Sandi, 1999; Campos-Figueroa y Llanderal-Cázares, 2003). Hace falta desarrollar estrategias que permitan reestablecer esta actividad combinando políticas de conservación de estos recursos genéticos y el uso de tecnología sustentable para su aprovechamiento.

El género *Opuntia* incluye 250 especies de acuerdo con Britton y Rose (1963), 181 especies de acuerdo con Anderson (2001), mientras que Hunt y Taylor (2002) describen 195 especies, todas registradas como especies nativas del continente Americano. En México, se ha registrado un total de 104 especies de *Opuntia* de acuerdo con Bravo-Hollis y Sánchez Mejorada (1978), y 83 de

acuerdo con Guzmán *et al.* (2003), de las cuales cerca de la mitad son endémicas de este país (Britton y Rose 1963; Anderson 2001). Un total de 20 especies han sido reconocidas como cultivares (Reyes-Agüero *et al*., 2005a), poseen diferentes grados de domesticación y son usadas principalmente como alimento, ensilaje y en la fabricación de medicamentos (Frati *et al*., 1991, Fernández *et al*., 1992). En conjunto, las opuntias y sus cultivares han sido nombradas con más de 900 nombres (Britton y Rose 1963; Kiesling 1998; Anderson 2001; Casas y Barbera 2002; Griffith 2004; Reyes-Agüero *et al*. 2005a), que se asocia a la gran variación morfológica, fisiológica y genética (Colunga *et al*., 1986; Anderson 2001; Hunt y Taylor 2002; Reyes-Agüero *et al.* 2005c).

El género *Dactylopius* comprende nueve especies *D. ceylonicus* Green, *D. coccus* Costa, *D. confusus* Cockerell, *D. opuntiae* Cockerell y *D. tomentosus* Lamarck, registradas para América del Norte (Mann, 1969; De Lotto, 1974; MacGregor y Sampedro, 1984; Pérez-Guerra y Kosztarab, 1992; Portillo, 2005) y *D. austrinus* De Lotto, *D. ceylonicus*, *D. coccus*, *D. confusus*, *D. confertus* De Lotto, *D. salmianus* De Lotto, *D. tomentosus* y *D. zimmermanni* De Lotto, descritas para América del Sur (De Lotto 1974; Pérez-Guerra y Kosztarab 1992; Claps y de Haro 2001; Diodato *et al*. 2004; Portillo, 2005). Algunos autores también reconocen a la especie *D. bassi* (Ben-Dov y Marotta 2001) pero no se ha llegado a un consenso sobre ello.

Los insectos del género *Dactylopius* se alimentan exclusivamente de cactáceas. A nivel mundial se cuenta con registros de su asociación con especies de la subfamilia Opuntioideae, particularmente con los géneros *Cylindropuntia*, *Grusonia*, *Maihueniopsis*, *Opuntia* (que incluye a *Nopalea)*, *Tacinga*, *Tephrocactus* y *Tunilla*; y otros géneros como *Cereus*, *Cleistocactus*, *Denmoza*, *Echinopsis*, *Gymnocalycium*, *Harrisa*, *Maihuenia*, *Mammillaria*, *Pilosocereus* y *Selicereus.* Sin embargo, el 60% de los hospederos pertenecen al género *Opuntia* (Mann, 1969; De Lotto, 1974; MacGregor y Sampedro, 1984; Pérez-Guerra y Kosztarab, 1992; Portillo y Vigueras-Guzmán, 2003). En México el número de hospederos se restinge sustancialmente al género *Opuntia* (Mann, 1969; De Lotto, 1974; MacGregor y Sampedro, 1984; Pérez-Guerra y Kosztarab, 1992; Portillo y Vigueras-Guzmán, 2003). La condición sésil de las hembras adultas y la limitada movilidad de los machos adultos los hace totalmente dependientes de sus hospederos (Mann 1969; Gullan y Kosztarab 1997).

Hasta antes del presente trabajo, las especies de *Dactylopius* norteamericanos habían sido registradas en 24 especies y tres variedades de opuntioides hospederos, en 8 y 24 estados de

México, respectivamente (Mann, 1969; De Lotto, 1974; MacGregor y Sampedro, 1984; Pérez-Guerra y Kosztarab, 1992; Portillo y Arreola-Nava, 1994; González *et al*., 2001, Portillo, 2005). Muy pocos trabajos (De Lotto, 1974; Pérez-Guerra & Kosztarab, 1992) indican la distribución real de los insectos *Dactylopius* en México. La mayoría se enfocan principalmente en su taxonomía y etnobiología. Los registros de la distribución de estos insectos y sus hospederos, son en su mayoría catálogos o listas de registros de uno o varias especies de insectos y los estados donde se localizan (Pérez-Guerra & Kosztarab, 1992; Portillo & Zamarripa, 1992; Miller, 1996). Por ejemplo, Miller (1996) registró a *D. coccus* en Oaxaca, *D. confusus* en Durango, Guerrero, Jalisco, Morelos, Nuevo León, Puebla, Sonora, *D. opuntiae* en Baja California, Distrito Federal, Durango, Hidalgo, Estado de México, Michoacán, Morelos, Oaxaca y Tamaulipas, y *D. tomentosus* en Baja California, Chihuahua y Oaxaca. Pérez-Guerra y Kosztarab (1992) observó a *D. coccus, D. confusus* y *D. tomentosus* en Oaxaca sobre *O. tomentosa*, *O. pumila* y *O. acanthocarpa*, respectivamente.

El catálogo de coccidios mexicanos de la familia Dactylopiidae de MacGregor y Sampedro (1984) es la publicación más detallada del género, incluye registros de (1) *D. ceylonicus* sobre *O. ficus-indica* en Jalisco y sobre *Opuntia* sp. y *Nopalea* sp. en Hidalgo, México, Morelos, Oaxaca y Veracruz, (2) *D. coccus* sobre *O. ficus-indica* y *O. hyptiacantha*, y *Opuntia* sp. en Oaxaca y Puebla, (3) *D. confusus* sobre *Opuntia* sp., *Nopalea* sp. y *Cactus* sp. en Chihuahua, Distrito Federal, Guanajuato, Guerrero, Jalisco, Morelos, Oaxaca, Puebla y Tamaulipas, (4) *D. opuntiae* sobre *O. ficus-indica* y *O. tomentosa* en Oaxaca y sobre *Opuntia* sp., *Nopalea* sp. y *Cactus* sp. en Aguascalientes, Baja California, Chiapas, Distrito Federal, Durango, Guerrero, Hidalgo, Jalisco, México, Michoacán, Nayarit, Nuevo León, Oaxaca, Puebla, Querétaro, Veracruz y Zacatecas, y (5) *D. tomentosus* sober *O. megacantha* y *N. Karwinskiana* en Baja California, sobre *O. vulgaris* en Coahuila, y sobre *Opuntia* sp. y *Cactus* sp. en el Distrito Federal, Guanajuato y Nuevo León. Sin embargo, carece de datos precisos sobre los patrones de distribución y características de sus hábitats.

En cambio, la distribución de las plantas hopederas de los generos *Opuntia*, *Grusonia* y *Cylindropuntia* conocida hasta ahora es mucho mayor que la de los insectos y se ha descrito que su localización está comprendida principalmente en las zonas áridas y semiáridas de México (Britton y Rose, 1963; Bravo-Hollis y Sánchez-Mejorada, 1978; Anderson, 2001, Reyes-Agüero *et al*., 2005a).

Los insectos del género *Dactylopius* poseen un limitado número de caracteres morfológicos que dificulta su identificación y estudio. Miden entre 1 a 6 mm de largo, poseen un cuerpo rojo cubierto de una capa conspicua blanca, de secreción grasa (Mann, 1969; Pérez-Guerra y Kosztarab, 1992). El pigmento representa entre el 10 al 25% del peso seco de las hembras adultas y está compuesto de un colorante soluble en agua cuyo principal componente es el ácido carmínico ($C_{22}H_{21}O_{13}$) (Venkataraman y Rama Rao, 1972; Yamada *et al*., 1993). Estudios del ácido carmínico permiten suponer que potencialmente podría usarse como antibacteriano, antiviral, e insecticida (Palma de la Cruz, 2008; Food and Drug Administration, 2009). Actualmente, *D. coccus* es la única especie que se usa para la producción comercial de colorante, por presentar una mayor concentración y calidad de colorante (MacGregor, 1976). Las otras especies del género tienen limitaciones para usarse con este propósito, por su reducido tamaño y su menor concentración de colorante (MacGregor, 1976). Se necesita estudiar la composición de las especies silvestres para reestablecer su potencial como una fuente alternativa de colorante y sus derivados.

El perfil metabólico es el arreglo cuidadoso de la secuencia ordenada y organizada de la información de la distribución de los compuestos secundarios en diferentes especies o géneros de organismos; su aplicación ha resultado de gran utilidad para los taxónomos de plantas que realizan estudios sistemáticos (Gottlieb, 1982). El perfil metabólico de las plantas se utiliza como una herramienta para identificar plantas que contienen compuestos químicamente activos, con diferencias en la composición de su metabolitos secundarios; esto ha permitido diferenciar entre especies de plantas que contienen compuestos activos de utilidad, de aquellas que no los contienen, determinar su origen y reconocer su importancia como fuente de medicamentos (Cardoso-Taketa, *et al*., 2008).

Para determinar la composición de química de las especies de *Dactylopius* se han utilizado técnicas de cromatografía líquida de alta eficiencia (CLAE), combinadas con espectrometría de masas (EM) y resonancia magnética nuclear (RMN) para el análisis de colorantes naturales derivados de hidroxiantraquinonas (Venkataraman y Rama Rao, 1972). Ha resultado efectivo su empleo para determinar el origen, la composición y las características estructurales de sus componentes. Más aún, estas técnicas se han usado para identificar la presencia de ácido carmínico en alimentos (Yamada *et al.*, 1993; Lancaster y Laurence, 1996) y materiales textiles antiguos (Wouters, 1985; Wouters y Verheeken, 1989), para optimizar las condiciones de

extracción del pigmento insectos cochinilla (González *et al.*, 2002), y para determinar su calidad (Méndez *et al.*, 2004; Maier, 2004). También ha sido posible diferenciar muestras de la especie *D. coccus* de acuerdo con su origen geográfico, mediante la combinación técnicas estadísticas como el análisis de agrupamiento y el análisis de componentes principales al análisis de los datos cromatográficos (Méndez *et al.*, 2004). Sin embargo, hasta el momento no se ha estudiado la variación del perfil metabólico de las especies de género *Dactylopius*, en general, ni se han analizado las diferencias cuantitativas de la concentración de ácido carmínico entre las especies de insectos, ni su variación de acuerdo con su origen geográfico u hospedero, combinando datos obtenidos por CLAE y análisis multivariado.

El análisis molecular es una herramienta importante en el estudio sistemático de los insectos (Cook *et al*., 2002). En el caso del género *Dactylopius*, únicamente se ha publicado la filogénia para la superfamilia Coccoidea basada en la secuencia 18S rARN, en la que Dactylopiidae se localiza en un clado cercano a E1 que corresponde a Eriococcidae (Cook *et al*., 2002), pero no se ha estudiado la filogenia molecular de las cinco especies mexicanas del género *Dactylopius*. Además, existen dos publicaciones de análisis moleculares de las especies de *Dactylopius* que describen marcadores genéticos que permiten identificar poblaciones y migraciones del parásito del nopal *Dactylopius* sp. (García, *et al*., 2001), y han detectado el polimorfismos en el DNA usando RAPD-PCR y comparando las species *Dactylopius coccus* y *Dactylopius* spp. (García, *et al*., 2001), pero incluyen un reducido número de especies.

Adicionalmente, ha sido publicada una topología con caracteres morfológicos de *Dactylopius*, que incluye cinco species del género (Rodríguez *et al*., 2001); El dendograma presentado por Rodríguez *et al*. (2001) fue construido usando algunas de las características morfológicas de *Dactylopius* que describe De Lotto (1974). Sus resultados muestran que *D. confusus* y *D. opuntiae* forman un grupo cercano a *D. coccus* y *D. ceylonicus*; mientras que *D. austrinus* pertenece a otro grupo aparte de *D. confusus* y *D. opuntiae*, sin explicar la razón de estas agrupaciones. El escaso número de caractéres morfológicos de los insectos *Dactylopius* dificulta el estudio sistemático del género, por lo que algunos autores han manifestado la necesidad de realizar análisis moleculares de *Dactylopius* para comprender la taxonomía de estos insectos (Portillo y Vigueras, 2006).

Por otra parte, en el orden Hemiptera, se han observado insectos que albergan bacterias simbióticas en su aparato digestivo o en bacteriocitos que son células especializadas capaces de

alojan bacterias (Baumann, 2005; Moran, 2006). Las bacterias endosimbiontes proveen de nutrientes a estos insectos que están limitados al floema de la savia de las plantas, el cual generalmente es deficiente de aminoácidos esenciales y vitaminas (Baumann, 2005; Moran, 2006). Algunos endosimbiontes pueden sintetizar compuestos bioactivos que después pueden ser usados por los insectos como sustancias de defensa contra sus predadores, parásitos y microorganismos patógenos (Moran, 2006). Como los endosimbiontes se transmiten de manera vertical, sus secuencias de ADN pueden usarse para trazar la filogenia de los insectos (Baumann, 2005). Dentro de Coccoidea, se han registrado endosimbiontes en las familias Pseudococcidae (Thao *et al*., 2002), Diaspididae, y Margarodidae (Gruwell *et al*., 2007). La diversidad de endosimbiontes en *Dactylopius* spp. no ha sido reportada hasta el momento, excepto para la bacteria del género *Wolbachia* presente en huevecillos de *Dactylopius* sp. (Pankewitz *et al*., 2007).

Con base en lo precedente, los objetivos del presente trabajo fueron:

1. Hacer la revisión histórica, cultural, geográfica, y ecológica de la información de los géneros *Opuntia* y *Dactylopius* y su distribución en México.
2. Determinar el patrón de distribución de *Dactylopius* en México en relación con la distribución de sus hospederos *Opuntia*, *Grusonia* y *Cylindropuntia*, describiendo las características más importantes de sus hábitats (altitud, vegetación, tipo de suelo y clima).
3. Analizar la variación en el perfil metabólico del colorante de los dactilópidos por cromatografía líquida de alta eficiencia (CLAE), comparando las cinco especies de México con una especie sudamericana en común con México.
4. Hacer la reconstrucción filogenética de las cinco especies de *Dactylopius* presentes en México secuenciando los genes mitocondrial 12S rRNA y nuclear 18S rRNA de los insectos y secuenciar el gene rRNA de las muestras de *Dactylopius* 16S para determinar las bacterias simbiontes presentes en este género de insectos.

Las hipótesis que se buscaron probar fueron:

1. La distribución reconocida hasta ahora para *Dactylopius* no es representativa de este género y se plantea que debe ser mayor, en correspondencia con la también mayor distribución de sus plantas hospederas.

2. La variación del perfil metabólico de las especies de *Dactylopius* permite diferenciar las especies y está relacionada con el hospedero y el origen de los insectos, por lo tanto la variación química de la composición del colorante cambiará de acuerdo con la distribución de los insectos y sus hospederos, presentándose semejanza entre la especie simpátrica *D. ceylonicus* registrada para México y Argentina.
3. La secuenciación del gene nuclear ribosomal de bacterias endosimbiontes del género *Dactylopius* permite reconstruir la filogenia de estos insectos. Se propone que la construcción filogenética molecular de *Dactylopius* será similar a la propuesta con caracteres morfológicos.

Generalidades metodológicas.

Para lograr los objetivos y probar las hipótesis se revisaron 150 fuentes bibliográficas referentes al origen, la diversidad, la distribución, los análisis químicos y moleculares del género *Dactylopius* y la subfamilia Opuntioideae. Para describir los patrones de distribución de *Dactylopius* y sus hospederos se colectaron y georreferenciaron especímenes de insectos de 208 poblaciones, de 120 localidades de 14 estados de la República, de 2005 a 2007. El área de estudio se localizó ente 98 y 104º latitud norte y entre 18 y 23º longitud este, abarcando las entidades federativas de Aguascalientes, DF, Guanajuato, Hidalgo, Jalisco, Estado de México, Michoacán, Morelos, Oaxaca, Puebla, Querétaro, San Luis Potosí, Tlaxcala, Veracruz y Zacatecas. Se montaron 153 preparaciones microscópicas de *Dactylopius* utilizando la técnica de De Haro y Claps (1995) y se depositaron en la Sección Hemiptera de la Colección Nacional de Insectos Universidad Nacional Autónoma de México (CNI-IB-UNAM). 50 especies de opuntias se propagaron y depositaron en el Jardín Botánico del Centro de Investigaciones (CIEco-UNAM). Los datos de colecta permitieron establecer las áreas de distribución (mapas) y las características de los hábitats de *Dactylopius*, *Opuntia*, *Nopalea* y *Cylindropuntia*. Mediante el sistema de información geográfico ILWIS 3.3 se construyeron los mapas de localización geográfica de los sitios estudiadoss para las distintas fuentes de información y los registros de *Dactylopius* y sus hospederos. Esta información se complementó con la revisión exhaustiva de 367 preparaciones microscópicas de especímenes de *Dactylopius* de la Colección Nacional de Insectos Universidad Nacional Autónoma de México (CNI-IB-UNAM) y 262 plantas de los herbarios Nacional de México (MEXU) y de Guadalajara (IBUG).

Para determinar el perfil metabólico de los pigmentos de *Dactylopius* se usó cromatografía líquida de alta eficiencia con arreglo de fotodiodo (CLAE). Se analizaron las 5 especies de *Dactylopius* provenientes de 35 poblaciones recolectadas en 13 estados del país y 2 provincias de Argentina. El origen geográfico y los hospederos de todas las poblaciones de dactilópidos fueron analizadas combinando los análisis cuantitativos de los datos obtenidos por CLAE (componentes mayoritarios y minoritarios) con análisis anidado (AC) y de componentes principales (ACP).

El análisis filogenético de *Dactylopius* se hizo mediante la secuenciación de los genes amplificados por PCR 12S rRNA y 18S rRNA de las cinco especies de insectos presentes en México, recolectadas en 5 localidades de 5 estados del país. Se utilizó la secuenciación 16S rRNA de las muestras de *Dactylopius* para estudiar la presencia de bacterias endosimbiotes.

Esta tesis se integra con 5 capítulos. El primero es la revisión histórica, cultural, geográfica, y ecológica de la información de los géneros *Opuntia* y *Dactylopius* y su distribución en México. El segundo capítulo es el estudio de la distribución y hábitats de *Dactylopius* y sus hospederos de la subfamilia Opuntioideae en México. El tercer capítulo es el perfil metabólico de las especies de *Dactylopius*, origen geográfico y hospederos y el análisis multivariado de los datos CLAE. En el cuarto capítulo se describe la filogénia molecular del género *Dactylopius* y la identificación de sus bacterias simbióticas. Finalmente, el capítulo quinto es la discusión general y conclusión del presente trabajo. Se recapitulan los principales resultados obtenidos y se concluye integrando la información obtenida de *Dactylopius* y sus hospederos, dando una perpectiva de la aplicación de esta investigación doctoral.

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I. The *Opuntia* (Cactaceae) and *Dactylopius* (Hemiptera: Dactylopiidae) in Mexico: a historical perspective of use, interaction and distribution

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REVIEW PAPER

The *Opuntia* (Cactaceae) and *Dactylopius* (Hemiptera: Dactylopiidae) in Mexico: a historical perspective of use, interaction and distribution

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Abstract A historical perspective on the use and production of species of *Dactylopius* (Hemipetera: Dactylopiidae) and *Opuntia* (Cactaceae: Opuntioidae), information on their origin, diversity and distribution in Mexico are reviewed, and aspects of their conservation are discussed. The use and exploitation of both genera are part of Mexican cultures since prehistory. *Opuntia* species were among the main components of human diet during pre-agricultural times. Cochineal was used and probably cultivated at least from the Tenth century. During the colonial period, cochineal generated significant benefits to the Spaniard colonizers and Mexico was the world's first producer of insects and dyes until the mid Nineteenth century. Currently, Mexico is the main producer of *Opuntia* cladodes and prickly pear, but cochineal cultivation is marginal and only maintained in traditional indigenous systems. Mexico is one of the main areas of diversity of *Opuntia*, having 83–104 out of nearly 200 species worldwide. More than 50 species are used mainly as food, fodder and medicine and 20 species are cultivated with different degrees of domestication. The genus *Dactylopius* includes nine species, with five of them naturally occurring in Mexico. Only *D. coccus* has been cultivated and domesticated but other wild species have been used throughout history. Arid and semiarid areas of Mexico are among the most important reservoirs of biological diversity for both genera, particularly for *D. coccus*. Specific measures for protection of such biodiversity and generic resources are required. Strategies for in situ conservation combined with re-established use and cochineal production may enhance conservation policies.

Keywords Cactaceae · Conservation · *Dactylopius* · Hemiptera · Mexico · *Opuntia*

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Introduction

Mexico is one of the world's main areas of diversity of cacti and their mutualist insects (Britton and Rose 1963; Anderson 2001; Hunt and Taylor 2002). In particular, the association between species of the genus *Opuntia* and insects of the genus *Dactylopius* has had a great significance to Mexican human cultures. This article reviews historical, cultural, geographical and ecological information about the genera *Opuntia* and *Dactylopius* and their distribution in Mexico. The genus *Opuntia* (Cactaceae: Opuntioidae) includes 250 species according to Britton and Rose (1963), 181 according to Anderson (2001) and 195 according to Hunt and Taylor (2002), all of them recorded as naturally distributed in the American continent. A total of 104 *Opuntia* species, according to Bravo-Hollis and Sánchez-Mejorada (1978), and 83 according to Guzmán et al. (2003), have been reported for Mexico, where nearly half of the species of this genus are endemic (Britton and Rose 1963; Anderson 2001). A total of 20 species have been recognized as cultivated, showing various degrees of domestication, and traditional users have designated the different species and cultivars with more than 900 names (Britton and Rose 1963; Kiesling 1998; Anderson 2001; Casas and Barbera 2002; Griffith 2004; Reyes-Agüero et al. 2005a), associated with high morphological, physiological and genetic variation (Anderson 2001; Hunt and Taylor 2002; Reyes-Agüero et al. 2005a).

The genus *Dactylopius* includes a homologous group of phytophagous hemipterous insects of 1–6 mm in length with the adult males being smaller than the adult females. These insects are characterized by their white cotton-type waxy cover secretion and their colorful bodies, and commonly are called "cochineal insects" or "cochineal scale insects" (De Lotto 1974; Piña 1977; Claps and de Haro 2001). The family Dactilopiidae is unigeneric and includes nine species (Table 1): *Dactylopius ceylonicus*, *D. coccus*, *D. confusus*, *D. opuntiae*, *D. tomentosus*, *D. austrinus*, *D. confertus*, *D. salmianus* and *D. zimmermanni* (De Lotto 1974; Pérez-Guerra and Kosztarab 1992; Claps and de Haro 2001). Some authors also recognize the species *D. baziі* (Ben-Dov and Marotta 2001), but there is no consensus about it. The first five aforementioned species have been identified in Mexico (Table 1), with *D. coccus* being the only one cultivated and used for commercial purposes, because of the higher amount and quality of dye it contains (Piña 1977). However, the other species have also been used. It has been estimated that during the Colonial period between the Sixteenth and Nineteenth centuries nearly one-third of the cochineal produced derived from other wild species (Humboldt 1966). Even when their dye had inferior quality, their commercialization represented substantial incomes to the Spanish Empire (Thiéry de Menonville 1787; Humboldt 1966; Anderson 1981). Production and exploitation of *D. coccus* has been called "coccidoculture", i.e. cochineal cultivation (Piña 1977). The Dactylopiidae is characterized by feeding exclusively on cacti (Pérez-Guerra and Kosztarab 1992; Claps and de Haro 2001) with nearly 80 species of this plant group having been reported as hosts worldwide (Table 1). In Mexico, 22 cacti species, mainly belonging to the genus *Opuntia*, have been reported as hosts to *Dactylopius*. The sessile condition of the female insects and the limited mobility of the adult males make them totally dependent on their hosts (Mann 1969; Gullan and Kosztarab 1997).

Opuntia and *Dactylopius* occur naturally in the American continent with species native to both North and South America (Pérez-Guerra and Kosztarab 1992; Anderson 2001). On the basis of up-to-date evolution data, Hunt and Taylor (2002) consider that the early cactus family ancestor was most likely of South American origin. These authors propose that the initial cactus primary radiation region, i.e. center of origin, is most likely the mid-Andean region, presently northern Chile, Bolivia and Peru, where the opuntioid ancestors,

Table 1 Worldwide distribution of *Dactylopius* and their hosts

Species	Countries[a]	Hosts[b]/Mexican States[c]	Reference
D. ceylonicus Green 1896	2	30, 30iv, 32, 40, 54, 57, 63, 65, 69	Mann (1969)
	2	30ii	De Lotto (1974)
	2	30ii, 44, 65, 69, 78, 79	Pérez-Guerra and Kosztarab (1992)
	2	30, 30ii, 40, 44, 65, 69, 79	Claps and de Haro (2001)
	3, 28	54	De Lotto (1974)
	3, 27, 28	54	Pérez-Guerra and Kosztarab (1992)
	5, 18, 27	39	Idem
	6	30	De Lotto (1974)
	6, 24	30	Pérez-Guerra and Kosztarab (1992)
	7, 31	32, 54, 63	Mann (1969)
	7, 18, 19	72	Pérez-Guerra and Kosztarab (1992)
	18	70	Idem
	20	**44/30**, 45/**14**	Piña (1977)
	20	**44/14**, 72/**10**, **13**, **17**, **20**, **30**	MacGregor and SanPedro (1984)
	22	18	Pérez-Guerra and Kosztarab (1992)
	24	72	De Lotto (1974)
D. coccus Costa 1835	2	44	Claps and De Haro (2001)
	3	70	Mann (1969)
	9, 25	44	Idem
	9, 10, 11, 12, 14, 21, 26i–24iv, 28, 32	72	Pérez-Guerra and Kosztarab (1992)
	10, 29	(*)	Idem
	20	35/**20**, 44/**20**, 59/**20**	Piña (1977)
	20	**44/20**, 47/**20**, 28, 72/**10**, **13**, **14**, **20**, **30**	MacGregor and SanPedro (1984)

Table 1 continued

Species	Countries[a]	Hosts[b]/Mexican States[c]	Reference
	20	**70/20**, **72/20**	Pérez-Guerra and Kosztarab (1992)
	20	**70/14**	Miller (1996)
	21, 28, 32	72	Idem
	25, 28	72	De Lotto (1974), Mann (1969)
	28	35, 44, 66	Mann (1969)
D. confusus Cockerell 1893	1, (*)	44	Pérez-Guerra and Kosztarab (1992)
	2	1, 18, 25, 30iv, 32, 40, 54, 63, 69, 71, 76, 77	Mann (1969)
	3, 28	54, 67	De Lotto (1974), Mann (1969)
	4, 10, 24, 28	72	Pérez-Guerra and Kosztarab (1992)
	8, 10	60	Idem
	10	39, 42, 43, 46	Idem
	13	74	Idem
	19	28	Idem
	20	**8/20**, **9/20**, **10/20**, **20/20**	Mann (1969)
	20	**72/6**, **11**, **13**, **15**, **17**, **20**, **21**, **24**	MacGregor and SanPedro (1984)
	20	**61/20**, **72/20**	Pérez-Guerra and Kosztarab (1992)
	24	68	Idem
	28	54, 72	Idem
	30	6, 7, 8, 10, 11, 13, 19, 20, 33, 34, 37, 39, 57, 60, 62, 67	Mann (1969)
D. opuntiae Cockerell 1896	3	38, 39, 42, 53, 54, 58, 67	Mann (1969)
	3	66, 67, 70	De Lotto (1974)
	3	67	Pérez-Guerra and Kosztarab (1992)
	10	7, 50, 55, 80, 72i	Idem
	11	72	Idem
	2, 15, 17, 23, 27	72	De Lotto (1974)

Table 1 continued

Species	Countries[a]	Hosts[b]/Mexican States[c]	Reference
	14	39	Pérez-Guerra and Kosztarab (1992)
	16	72, 72i	Idem
	17, 19, 26, 26iii, 27, 28	72	Idem
	18	(*)	Miller (1996)
	19	70	Pérez-Guerra and Kosztarab (1992)
	20	**28/16, 20, 28,** 44/20, 64/12, **13,** 70/20, 72/1, **2, 3, 9, 10, 12, 13, 14, 15, 18, 20, 21**	MacGregor and SanPedro (1984)
	20	27/15, 35, 72, 80	Pérez-Guerra and Kosztarab (1992)
	20	8, 20, 43, 44, 45, 47, 49, 52, 64, 66, 70	Mann (1969)
	23	27	Pérez-Guerra and Kosztarab (1992)
	28	36, 52	Idem
	28	43cv, 52	De Lotto (1974)
	30	19, 20, 29, 31, 37, 43, 50, 51, 58	De Lotto (1974), Mann (1969)
D. tomentosus Lamark 1896	2	1, 69	Pérez-Guerra and Kosztarab (1992)
	3	8	De Lotto (1974), Pérez-Guerra and Kosztarab (1992)
	10	2, 3, 5, 8, 10, 14, 72, 80	Pérez-Guerra and Kosztarab (1992)
	15	7	De Lotto (1974)
	20	52/**2, 3,** 48/**20,** 72/**3, 7, 11, 15, 20, 30, 32**	MacGregor and SanPedro (1984)
	20	4/**2, 3,** 72, 80	Pérez-Guerra and Kosztarab (1992)
	28, 30	8, 12, 72	De Lotto (1974)
D. austrinus De Lotto (1974)	2, 3, 28	32	De Lotto (1974)
	2	30, 30iii, 56	Pérez-Guerra and Kosztarab (1992)
	2	30, 30i, 30ii, 30iii, 32, 56, 69	Claps and de Haro (2001)
	3, 28	32	Pérez-Guerra and Kosztarab (1992)
	28	30, 30ii, 30iii, 41, 69, 75	Idem

Table 1 continued

Species	Countries[a]	Hosts[b]/Mexican States[c]	Reference
D. confertus De Lotto (1974)	2	3	De Lotto (1974)
	2	3, 17	Pérez-Guerra and Kosztarab (1992)
	2	1, 2, 15, 16, 17, 21, 22, 73	Claps and de Haro (2001)
	10	69	Pérez-Guerra and Kosztarab (1992)
D. salmianae De Lotto (1974)	2	65	Claps and de Haro (2001), De Lotto (1974), Pérez-Guerra and Kosztarab (1992)
D. zimmermanni De Lotto (1974)	2	76	De Lotto (1974)
	2	1, 23, 24, 25, 26, 72	Claps and de Haro (2001)
	2	1, 23, 25, 72	Pérez-Guerra and Kosztarab (1992)

[a] 1 = Algeria, 2 = Argentina, 3 = Australia, 4 = Bahamas, 5 = Bangladesh, 6 = Bolivia, 7 = Brazil, 8 = Canada, 9 = Ecuador, 10 = England, 11 = Egypt, 12 = France, 13 = Greece, 14 = Haiti, 15 = India, 16 = Jamaica, 17 = Kenya, 18 = Madagascar, 19 = Mauritania, 20 = Mexico, 21 = Morocco, 22 = Nepal, 23 = Pakistan, 24 = Paraguay, 25 = Perú, 26 = Spain (i = Canary Islands, ii = Madeira, iii = Reunion, iv = Azores), 27 = Sri Lanka, 28 = South Africa, 29 = Turkey, 30 = United States, 31 = Uruguay, 32 = Venezuela and 33 = Zimbabwe

[b] 1 = *Cereus aethiops*, 2 = *Cleistocactus baumannii*, 3 = *Cleistocactus* sp., 4 = *Cylindropuntia acanthocarpa*, 5 = *C. bigelovii*, 6 = *C. echinocarpa*, 7 = *C. fulgida*, 8 = *C. imbricata*, 9 = *C. kleiniae*, 10 = *C. leptocaulis*, 11 = *C. spinosior*, 12 = *C. tunicata*, 13 = *C. whipplei*, 14 = *Cylindropuntia* sp., 15 = *Denmoza rhodacantha*, 16 = *Echinopsis candicans*, 17 = *E. leucantha*, 18 = *Echinopsis* sp., 19 = *Gruemia emoryi*, 20 = *G. grahamii*, 21 = *Gymnocalycium mesillei*, 22 = *Harrisia tortuosa*, 23 = *Malhuenia patagonica*, 24 = *Malheniopsis darwanii*, 25 = *M. ovata*, 26 = *Malheniopsis* sp., 27 = *Mammillaria elongata*, 28 = *Nopalea* sp., 29 = *Opuntia aciculata*, 30 = *O. anacantha* (i = var. *anacantha*, ii = var. *kiska-loro*, iii = var. *retrorsa*, iv = var. *utkilio*), 31 = *O. atrispina*, 32 = *O. aurantiaca*, 33 = *O. austrina*, 34 = *O. basilaris*, 35 = *O. cochenillifera*, 36 = *O. crystalenia*, 37 = *O. cymochila*, 38 = *O. dejecta*, 39 = *O. dillenii*, 40 = *O. discolor*, 41 = *O. elata*, 42 = *O. elatior*, 43 = *O. engelmannii*, 44 = *O. ficus-indica*, 45 = *O. fuliginosa*, 46 = *O. humifusa*, 47 = *O. hyptiacantha*, 48 = *O. karwinskiana*, 49 = *O. leucotricha*, 50 = *O. littoralis*, 51 = *O. macrocentra*, 52 = *O. megacantha*, 53 = *O. microdasys*, 54 = *O. monacantha*, 55 = x *O. occidentalis*, 56 = *O. pampeana*, 57 = *O. paraguayensis*, 58 = *O. phaeacantha*, 59 = *O. pilifera*, 60 = *O. polyacantha*, 61 = *O. pumila*, 62 = *O. pusilla*, 63 = *O. quimilo*, 64 = *O. robusta*, 65 = *O. salmiana*, 66 = *O. streptacantha*, 67 = *O. stricta*, 68 = *O. tehuiana*, 69 = *O. sulphurea*, 70 = *O. tomentosa*, 71 = *O. wentiana*, 72 = *Opuntia* spp. (i = *Platyopuntia* spp.), 73 = *Pilosocereus* sp., 74 = *Selicereus* sp., 75 = *Tacinga palmadora*, 76 = *Tephrocactus aoracanthus*, 77 = *T. weberi*, 78 = *Tunilla lingispina*, 79 = *T. soehrensii*, 80 = cactus; species and generic names based on Anderson (2001)

[c] Mexican State shown in Fig. 1

as well as other cactus ancestors diverged in their different lineages, thereby resulting in migration paths to North, East and South America. Molecular evidence indicates that two lineages, cylindrical-stemmed and flat-stemmed prickly pears, diverged in South America prior to their migration. Therefore, their present co-occurrence in many North-American desert and semi-desert habitats is the result of parallel migration as a xeric-adapted floristic cohort (Hunt and Taylor 2002). *Opuntia* have undergone an extensive evolutionary radiation (Anderson 2001) and have occupied the most widespread geographic region for any group within the Cactaceae (Hunt and Taylor 2002).

Expansion and uses of *Opuntia* culture and *Dactylopius* species

Human activities have been decisive in the expansion of both *Opuntia* and *Dactylopius*. At present, *Dactylopius* and *Opuntia* have worldwide distribution because humans have propagated species of these genera as part of intercultural and economic exchanges during the last 500 years. The history of use of *Opuntia* in Mexico started in pre-agricultural times, with the earliest occupations of the Mexican territory by humans, 12,000–14,000 years ago, when gathering included products of plant species such as *Opuntia*, *Prosopis* and *Agave* (Smith 1967; MacNeish 1992). The process of domestication has included at least 20 *Opuntia* species, with management and artificial selection mainly directed to optimize their use for their edible stems and fruits (Anderson 2001; Hunt and Taylor 2002; Reyes-Agüero et al. 2005c), but also the use and management of *Opuntia* has been directed to the cultivation of *Dactylopius* insects. The latter activity has been characterized by the stages described below.

Pre-Columbian period

Opuntia and *Dactylopius* were important elements of the household economy, political life and religious practices in the everyday life of the ancient communities in Mexico (Martín del Campo 1957; Humboldt 1966; Anderson 1981). The young cladodes or *nopalli* were consumed as food, whereas, the mature cladodes were used to propagate cochineal insects. The prickly pear fruit or *nochtli* was consumed fresh or fermented to prepare the prickly pear wine called *nochoctli* (Humboldt 1966; Anderson 1981; Dahlgren 1990). *Opuntia* also occupied an important place in the indigenous Mexican pharmacopoeia; according to several codices *Opuntia* plants were used to cure contusions and skin burns and immobilize broken bones, among other health problems (Gibson 1964; Humboldt 1966; Anderson 1981).

As revealed by codices and historical documents from the early years after the Spanish conquest, *Opuntia* cultivation had been an agricultural activity of great importance in Mexico for over 700 years ago. *Opuntia* species often were exhibited in the pre-Columbian botanical gardens or iconographically represented on building walls (Martín del Campo 1957; Humboldt 1966; Dahlgren 1990). Rearing of cochineal dates back to the Toltec period, which corresponds to the Tenth century (Humboldt 1966; Anderson 1981), and activities related to it were often illustrated in murals and the traditional Aztec paper called *amatl* (Martín del Campo 1957; Dahlgren 1990; Pérez-Sandi 2001a). The pre-Columbian Mexican people distinguished the fine cochineal (*nopalnochextli*) from wild cochineal species (*izquimilluhiqui*) and low-grade cochineal (*tulnochextli*; Humboldt 1966; Anderson 1981; Dahlgren 1990). They also processed cochineal (*nochetlaxcalli*), and both fresh

and processed cochineals along with the dye were sold at the local markets (Humboldt 1966; Anderson 1981). The dye, known as the "blood of prickly pears", was used by painters and writers to paint sculptures, wood, codices, murals, public and religious buildings, food, color ceremonial cakes or tortillas, but especially for dying cotton and feathers used for decorating clothes, as well as cosmetic materials used by military, religious and government leaders (Martín del Campo 1957; MacGregor 1976; Piña 1977; Anderson 1981; Butler 2006). In medicine, the insects of an unidentified species and their dye were used to cure stomach, head and heart diseases and were considered a good stimulating tonic, sudorific (inducing sweat), alexipharmic (antidotal), febrifuge (fever reducing), and collutory (mouthwash; MacGregor 1976; Anderson 1981; Butler 2006). The Mixtec, in the surroundings of the city of Oaxaca (Fig. 1) was the main area dedicated to cochineal production with the best quality dye and the greatest commercial distribution (Humboldt 1966; Anderson 1981). The dye from this region was used as part of the exchange system and given as tribute to the Aztec city of Mexico-Tenochtitlan (Headrick 2007). According to the Mendocino Codex, a total of 394 communities participated in the production of 4,404 kg tributed to Moctezuma's Empire (Martín del Campo 1957; Pérez-Sandi 2001a).

González et al. (2001) have suggested that Mesoamerican and Andean societies had commercial and cultural links through which the exchange and propagation of *Opuntia* and *D. coccus* could have occurred. Mexico and Peru shared the use of the dye from the Tenth to Twelfth centuries. In Mexico the Toltec and Teotihuacan cultures knew the dye and used

Fig. 1 States of the Mexican Republic: 1 = Aguascalientes, 2 = Baja California Norte, 3 = Baja California Sur, 4 = Campeche, 5 = Chiapas, 6 = Chihuahua, 7 = Coahuila, 8 = Colima, 9 = Durango, 10 = Estado de Mexico, 11 = Guanajuato, 12 = Guerrero, 13 = Hidalgo, 14 = Jalisco, 15 = Mexico City (Federal District), 16 = Michoacán, 17 = Morelos, 18 = Nayarit, 19 = Nuevo León, 20 = Oaxaca, 21 = Puebla, 22 = Querétaro, 23 = Quintana Roo, 24 = San Luis Potosí, 25 = Sinaloa, 26 = Sonora, 27 = Tabasco, 28 = Tamaulipas, 29 = Tlaxcala, 30 = Veracruz, 31 = Yucatán, 32 = Zacatecas

it in different applications, while in Peru it was used for coloring cloth in pre-Inca times (MacGregor 1976). Some authors have considered that since indigenous Andean people do not have native names (e.g. Quechua) for *Dactylopius* insects and their host species of *Opuntia* (Piña 1981), it is unlikely that cochineal was used in that region before the colonial period (Martín del Campo 1957, Piña 1981). However, chemical analyses have revealed that Andean textiles were mainly colored with the native South American species *D. ceylonicus* and *D. confusus*, although a small fraction also was colored with *D. coccus* (Wouters and Verhecken 1989), which supports the hypothesis of pre-Columbian interchange of materials and management techniques.

Conquest period

The first contact of Europeans with the genus *Opuntia* occurred in 1492 during the first voyage of Christopher Columbus, who took samples of these cacti to Lisbon in 1493 (Anderson 2001). In the Sixteenth century living specimens of *Opuntia* were exhibited in the main botanical gardens of Europe and cultivated in them for centuries (Humboldt 1966). In 1700, Tournefort named these cacti as *Opuntia* because of their similarity to spiny plants growing in the town of Opus, in Greece (Harris 1833). Since his arrival in Mexico in 1519 and until 1526, Hernán Cortés wrote a series of epistles to Charles V, in which Cortés describes the splendid treasures and natural wonders found when he discovered the "Western Indies", including cochineal and its dyestuff that were abundant in Mexico and could be of "a great profit for the royal purse" (Cortés 1962). In 1523 Cortés shipped several products to the king, among them cochineal that arrived for the first time in Europe (Butler 2006).

The clergy realized that in the New World cochineal dye was actively marketed among the different communities and the degree of domestication of these insects was comparable with the culture of silkworms or bees (Martín del Campo 1957; Dahlgren 1990). In Mexico as well as in Spain the religious groups and chroniclers started important efforts in documenting the indigenous activities (Cortés 1962; Díaz del Castillo 1963; Anderson 1981). Knowledge of the variety, production, cultivation, growth, pests and uses of *Opuntia*; as they drew in detail the activity of cochineal culture, natural enemies, the collection process and drying of the insect, selling of cochineal cakes in the markets, and the production of the final dye and its uses, were well documented (Díaz del Castillo 1963; Humboldt 1966; Anderson 1981; Dahlgren 1990). In order to prevent contraband and piracy, the majority of pre-Columbian writings and codices were faithfully copied by priests and then destroyed, and only some Masters of the Aztec schools called *Calmecac*, who kept the oral tradition alive, and the professional writers, called *tlahcuilos*, maintained valuable information on the traditional management and production processes (Martín del Campo 1957).

Colonial period

The Spaniards controlled and promoted "coccidoculture", an activity that opened the way to a prosperous industry during the colonial period since the sources of natural red coloring known at that time were scarce (Gibson 1964; Piña 1977). Other sources known were the mollusks of the genera *Purpura* and *Murex* from the Mediterranean sea, the lichen archil, orchil or orchilla (*Roccella canariensis*) from the coastal rocks of Spain and the Middle East (Piña 1981; Dahlgren 1990), and the insect species *Coccus ilicis* Linnaeus, 1758, now

Kermes ilicis (Kermesidae), from Greece, *C. polonicus*, now *Porphyrophora polonica* (Margarodidae), that grows on the roots of *Scleranthus* from the sandy lands of eastern Europe, and *C. cacti* Linnaeus, 1758, now *Protortonia cacti* (Homoptera-Coccoidea-Margarodidae), on *Opuntia dillenii* from India (Balaram-Tolat 2002; Butler 2006). Other colorings used by that time were "Armenian red" which was made from the insect *Porphyrophora hameli*, a parasite on the roots and stems of certain grasses in Armenia, Azerbaijan, Georgia, Turkey, and Iran, *Laccifer lacca*; an insect native to India and Southeast Asia (Butler 2006). However, none of these colorings seemed as attractive in quality and permanence as that from *D. coccus*; therefore, cochineal coloring rapidly displaced them (Piña 1977; Butler 2006). The Spanish authorities carefully recorded data on the production, transformation and exportation of the insect and its dye, and that information makes it possible to have a clear view of the productive regions, amount of insect produced, coloring obtained, volumes of export and economic benefit it represented for the Spanish Empire.

In 1525, cochineal was cultivated in Oaxaca, considered the area of origin of this activity, but rearing was also carried out in Puebla (Humboldt 1966; Piña 1977), Tlaxcala and Valley of Mexico (Gibson 1964). Cochineal produced in Oaxaca, quickly became an important export, with shipments first reaching Spain in 1526 (Meyer and Beezley 2000). Consumption and use of the dye extended to the Mayan region at the southeastern portion of the country (Cortés 1962; Humboldt 1966; Dahlgren 1990). Later on, cochineal cultivation was successfully established in the state of Tlaxcala (Fig. 1; Díaz del Castillo 1963), among other regions of Mexico. During the first century of the Colonial period, insect production was managed only by indigenous people and the production given as tribute to the conquerors (Gibson 1964); who established laws to prevent falsification and fraud of cochineal in 1592 and 1594 (Humboldt 1966). The cochineal exported from Mexico to Spain during the period from 1760 to 1850 averaged 700 tons per year (Fig. 2; Humboldt 1966, Dahlgren 1990). This level of production was achieved with *Opuntia* plantations constituted of 5,000–6,000 plants/ha (Humboldt 1966) in which 23,000–48,000 groups of

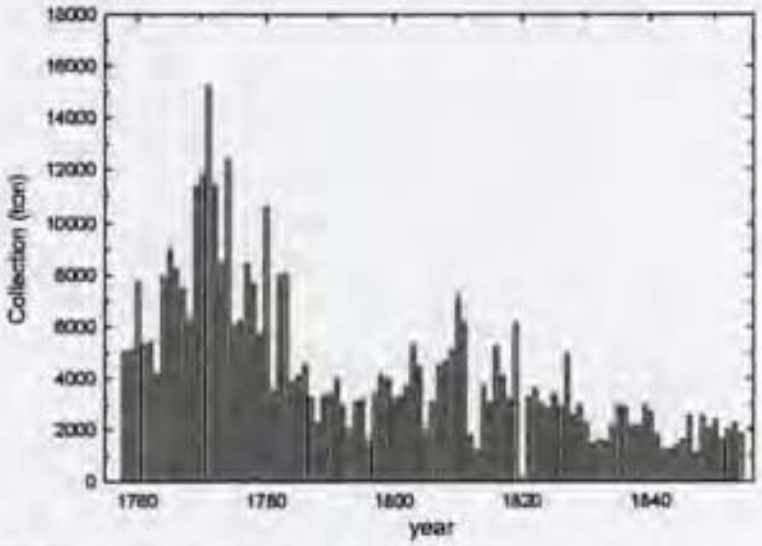

Fig. 2 Collection of grana cochineal in Oaxaca from 1758 to 1854

indigenous households worked in modules measuring 500–1,000 m^3 (Humboldt 1966; Portillo and Vigueras-Guzmán 2003). The total production included cochineal cultivated in these modules, cochineal collected from cultivated species of *Opuntia*, and cochineal insects collected from wild species of *Opuntia*, which grew spontaneously on the sides of the mountains and substantially increased the amount reported, and came to constitute one-third of the total production of Mexico (Thiéry de Menonville 1787; Humboldt 1966; Anderson 1981). The coloring obtained was highly valued and became the third greatest source of income for the Spanish crown in the New Spain, only after gold and silver (Humboldt 1966; Piña 1977; Dahlgren 1990). The grater part of the dye was exported to Spain (Gibson 1964); its destination was the European market (Meyer and Beezley 2000) through commercialization that involved a chain of Spanish merchants (Humboldt 1966). The successful production amount and quality of cochineal dye increased its demand worldwide both in the European and Asian markets, reaching China and Turkey with great acceptance (Anderson 1981). For this reason, cochineal cultivation was extended into the Spanish crown territories, from the New Spain to the New Galicia that corresponded to the territory extending from the southern U.S. and Mexico to Tucumán, Argentina (Humboldt 1966; MacGregor 1976). The expansion of *Opuntia* species and cochineal production continued during the Seventeenth and Eighteenth centuries; Flemish, Dutch, and England textile weavers constituted the major market for this dye (Meyer and Beezley 2000). The Spanish authorities controlled cochineal cultivation and concentrated its production in Oaxaca (Gibson 1964) to avoid internal and external access to this information, however, espionage could not be prevented (Piña 1981; Dahlgren 1990).

The French (Thiéry de Menonville 1787), British and The Netherlands Empires got the information on this activity and put into practice cochineal cultivation in their respective colonies in the Eighteenth century. From Mexico, both *Opuntia* and *Dactylopius* were brought to Saint-Domingue (later known as Haiti) in 1777 by the French botanist Nicolas Joseph Thiéry de Menonville, who established an experimental field on this island (Thiéry de Menonville 1787, Humboldt 1966, Butler 2006). The Englishmen introduced cochineal and its host plants into Australia from Brazil. Capitan Nelson carried off the insect from Rio de Janeiro in 1793, and culture lands were established in the environs of Calcutta, Chittagong, and Madras (Humboldt 1966). The main goal was to establish this activity and provide the dye to the textile industry of the army (MacGregor 1976; Piña 1981). From Australia, cochineal was re-introduced in India in 1795 (Balaram-Tolat 2002). In all cases, cochineal cultivation was unsuccessful and in Australia *Opuntia* became invasive.

In Mexico a combination of external and internal circumstances marked the end of the prosperous industry of cochineal cultivation. Among the internal conflicts, the Independence war was decisive, but also in a single night all the cacti (nopal) hosts were cut down and cochineal production centers closed by indigenous people incited by the constant abuses and frauds on behalf of merchants and middlemen (Humboldt 1966). The main external causes were the successful expansion of cochineal cultivation in other countries, conflicts in Europe and the synthesis of artificial colorings (Perkin1896). Mexico reduced its production (Fig. 2; Humboldt 1966), and no insect or dye was exported by the middle of the Nineteenth century (Dahlgren 1990).

Post-colonial period

After the Independence war finished, Mexico re-established some economic activities, but not cochineal production. Other countries and empires supplied the market that demanded

this dye. In 1825, in order to maintain its source of the coloring, Spain introduced the cultivation of *D. coccus* and its host plants into Cadiz and then into the archipelago of the Canary Islands (Piña 1980). Since 1876, the successful propagation of the plants and insects allowed this region to produce 3,178 ton per year (Piña 1980). After several attempts, other cases of successful adaptation occurred in Guatemala (Humboldt 1966) and Argentina (Claps and de Haro 2001), in South Africa in 1824 (Piña 1981), Peru in 1958, and Chile in 1988 (Pérez-Sandi 2001a). Additionally, in 1856 William Henry Perkin discovered in London the first synthetic dyestuff known as aniline purple, Tyrian purple, or mauve (Perkin 1896). The synthesis of this bluish substance, with excellent dyeing properties, was the beginning of a new industry and started the dominance of synthetic dyes that lasted almost a century (Harrow and Perkin 2009). During this time, the list of artificial colorings grew rapidly, because of its easy production and low cost, to later decrease constantly when it was discovered they were hazardous to human health. In this manner, in the 1960's the list of synthetic colorings decreased, allowing natural dye production to increase and among them the dye extracted from *D. coccus* and its derivatives (Duxbury 1990; Food and Drug Administration 2006).

Currently, cochineal cultivation is an activity of worldwide interest (MacGregor 1976; Portillo and Arreola-Nava 1994; González et al. 2002), with focus on its dye, including on carminic acid extraction and purification (Yamada et al. 1993). Since 2002, Peru (producing cochineal in an area of 70,000 ha), Chile (producing 5,000 ha), Bolivia (producing 1,000 ha), Spain (producing 300 ha), South Africa (producing 100 ha) and Mexico (producing 10 ha), have been the main producing countries of cochineal, whereas, Germany, The United States, France, England, Italy and Japan constitute the main market (Pérez-Sandi 2001a). Peru, the main producer, cultivates *Opuntia* and cochineal intensively across a broad area, with just two regions, Ayacucho and Apurimac, having 38,000 ha and 10,000–40,000 *Opuntia* plants/ha (Pérez-Sandi 2001a). This country produces 8,000 kg/ha of prickly pear and 700 kg/ha of cochineal, that represents 85% of the total amount of insects in the world, with a yield of up to 24% of carminic acid. The Peruvian cochineal producing regions are mainly located in arid lands.

Mexico is a country with a series of advantages which would allow an activity of this sort to be restarted. Arid and semi-arid zones comprise nearly half of the Mexican territory. It is the primary producer of *Opuntia* prickly pear and cladodes worldwide; *Opuntia* species adequate for the development of cochineal cultivation naturally occur in its territory, and this country has an ancient cultural history in the production of *Opuntia*, *Dactylopius* and its dye that currently occurs disseminated in indigenous villages. Nearly 13 species of *Opuntia* are used as fodder for cattle (nearly 130,000 ha cultivated for this purpose), 24 species are used for prickly pear production (nearly 170,000 ha cultivated for this purpose), the cladodes of nine species are consumed as vegetables (9,000 ha cultivated for this purpose), and 14 species are used for cochineal cultivation (10 ha cultivated for this purpose). In addition, *Opuntia* species are cultivated to produce raw material for medicinal preparations, cosmetic products, bio-gas, ethanol, adhesives for whitewashing and building of living fences, and barriers for protection against soil erosion (Casas and Barbera 2002; Campos-Figueroa and Llanderal-Cázares 2003).

Industrialization of *Opuntia* products is growing. There is a long list of food as well as cosmetic products prepared from young cladodes (Casas and Barbera 2002). The mature cladodes, with high fiber content, mucilage and pectin, are used in the development of food supplements and have been tested in the pharmaceutical industry for applications in the control of body weight, diabetes, hyperglycemia, glycemia, cholesterol and triglycerides levels in blood (Frati et al. 1991; Fernandez et al. 1992). The use of *Opuntia* for cochineal

cultivation in Mexico is currently reduced to small scale production in traditional indigenous villages, some few specialized producers in the states (Fig. 1) of Tlaxcala, Hidalgo, Mexico, Jalisco, Colima, Guerrero, Guanajuato, Michoacán, San Luis Potosí, and Yucatán, educational and research centers in Baja California, Chiapas, Chihuahua, Coahuila, Colima, Durango, Estado de Mexico, Guanajuato, Guerrero, Hidalgo, Jalisco, Michoacán, Morelos, Oaxaca, Puebla, Querétaro, San Luis Potosí, Tlaxcala, Zacatecas and Yucatan (Piña 1977; Pérez-Sandi 2001a; Campos-Figueroa and Llanderal-Cázares 2003; Flores-Hernández et al. 2006). They are mainly dedicated to improve production methods of *D. coccus* and its dye, and part of the production is destined for art, craft and research (Campos-Figueroa and Llanderal-Cázares 2003). Presently, in Mexico *D. coccus* is not reared intensively, however, the insect is found in more than half of the Mexican territory spread in southern, northern and central regions and there is a constant interchange between different states and in some cases with the Canary islands and Peru. Therefore, there is an important genetic diversity.

It is possible to re-establish cochineal production in Mexico, and it is useful for this purpose to generate information about the available resources, the geographical distribution and conservation status of the commercial and wild species of both *Opuntia* and *Dactylopius*. Such information is valuable from the perspective of use of genetic resources but also for designing strategies and policies of biodiversity conservation of the taxa involved.

Current distribution of species of the genera *Opuntia* and *Dactylopius*

The species of *Dactylopius* have been reported to occur in 33 countries worldwide (either naturally or as introductions), with more than a third of them occurring in North America (Table 1). Argentina reports the greatest number of species of *Dactylopius*, and host species for this genus (Claps and de Haro 2001). *D. opuntiae*, occurs in 16 countries, *D. coccus* in 15 countries and *D. tomentosus* in 7 countries, with these three found on five continents. *D. ceylonicus* occurs in 13 countries and it is known to be the most widely recorded species in South America, from where it is thought to originate (Claps and de Haro 2001); *D. ceylonicus* also has been established in Asia (Sri Lanka, from where its names derives) and in Africa. *D. confusus* occurs in 12 countries, being mainly abundant in North America and to a lesser extent also in the central and southern parts of the American Continent but also has been introduced to Africa and Australia. The rest of the species of *Dactylopius* occur in Argentina (De Lotto 1974). The genus *Dactylopius* is associated with the Cactaceae, particularly the subfamily Opuntioideae, with the genera *Cylindropuntia*, *Grusonia*, *Maihueniopsis*, *Opuntia* (that included *Nopalea*), *Tacinga*, *Tephrocactus* and *Tunilla*; other genera *Cereus*, *Cleistocactus*, *Denmoza*, *Echinopsis*, *Gymnocalycium*, *Harrisa*, *Maihuenia*, *Mammillaria*, *Pilosocereus* and *Selicereus*, but more than 60% of the hosts belong to the genus *Opuntia* (Table 1) with species and generic names given by Anderson (2001).

In Mexico, *Dactylopius* is distributed in 23 states (Fig. 1), with a greater distribution in the northern and central part of the country, mainly in dry and semi-dry areas (Fig. 3). The *Dactylopius* species most widely distributed are *D. opuntiae* in 19 states followed by *D. confusus* (13), *D. ceylonicus* (9) and *D. tomentosus* (7) (Table 1). Oaxaca is the only state of Mexico where the five species of *Dactylopius* co-inhabit with half of the host species of the genus *Opuntia* (Piña 1977), whereas, Jalisco has the greatest number of *Opuntia* (Fig. 3) and four species of *Dactylopius* (Portillo and Vigueras-Guzmán 2003;

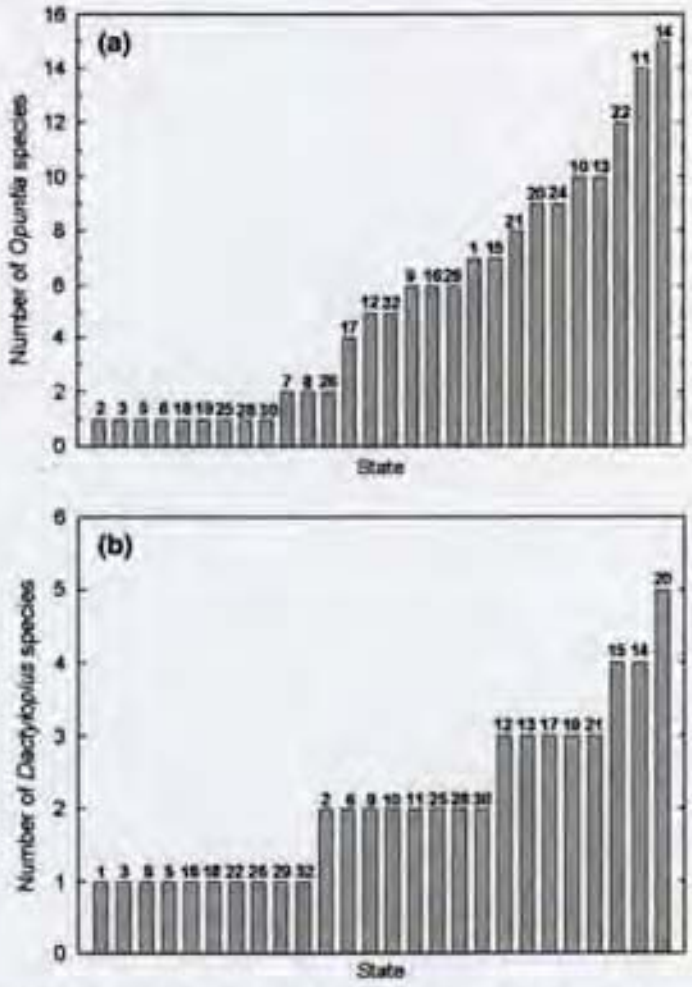

Fig. 3 Distribution of *Opuntia* species (**a**) and *Dactylopius* species (**b**) in Mexico. *Numbers* on *bars* indicate the state shown in Figure 1

Fig. 3). The species with the larger number of hosts are *D. opuntiae*, *D. coccus*, *D. confusus*, *D. tomentosus* and *D. ceylonicus* (Table 1). The range of elevations for growth of *Dactylopius* and *Opuntia* are 0–2,650 m and 0–2,850 m, respectively (Britton and Rose 1963; MacGregor and Sampedro 1984; Anderson 2001). The *Opuntia* species with the greatest distribution is *O. ficus-indica*. The majority of the species of *Opuntia*, as well as its variants and cultivars, are concentrated in the central and high plateau regions of Mexico (Fig. 3; Reyes-Agüero 2005b).

Perspective for use and biodiversity conservation in Mexico

Several attempts have been made in Mexico to re-establish cochineal cultivation through national, international, public and private programs (Pérez-Sandi 2001a), unfortunately

with unsuccessful results mainly due to a lack of economic integration and deficiencies in making use of native cultural elements and techniques adapted to local environments (Pérez-Sandi 2001a). It is necessary that the great producers of *Opuntia* cooperate to carry out this activity combining cladode, fruit, and insect production, dye and its derivates, which may represent great profits (Campos-Figueroa and Llanderal-Cázares 2003). All of the wild *Dactylopius* species must be considered as they are potential sources of a dye that historically have proved to be useful (Anderson 1981).

Opuntia can be highly adaptive to a wide range of ecological conditions and due to their success in vegetative propagation, have become some of the primary invasive species in many xeric or subtropical non-American habitats, e.g. Australia, South Africa (Hunt and Taylor 2002). The five species of *Dactylopius* have been used in Australia against *Opuntia* species, e.g. *D. tomentosus* was used in Australia to fight the *O. imbricata* species (Pérez-Sandi 2001a; Volchansky et al. 1999). For the same purpose, *D. opuntiae* was used to reduce populations of *O. stricta* and *O. ficus-indica* in South Africa (Volchansky et al. 1999).

The wild *Dactylopius* species have several biological advantages over *D. coccus*. For instance *D. opuntiae* has a shorter lifespan and reproductive cycles with a larger number of generations per year (Mann 1969) and is widely distributed with the greatest number of hosts. Wild species also have a repellent thick waxy cover that protects them against desiccation and rain (Flores-Hernández et al. 2006) and their dye is consumed by other insects and used as a defense mechanism (Mann 1969). All of the cochineal species have a high content of proteins and minerals, and the residuals from coloring extraction may be used to enrich food for avian species or to prepare fertilizers (Quijano and Vergara 2007).

The importance of conserving the *Dactylopius-Opuntia* association, makes it necessary to continue studying this biological system, which currently is seriously threatened by the accelerated degradation of the wild species that constitute the main source of raw material for research studies on the genetic improvement of domesticated species (Flores-Hernández et al. 2006) and for resource conservation programs and the improvement of land (Casas and Barbera 2002).

The South American cactus-feeding moth, *Cactoblastis cactorum* (Berg; Lepidoptera: Pyralidae; Zimmermann et al. 2001), is a serious threat to the high diversity of native *Opuntia* species in Mexico, both wild-growing and cultivated (Pérez-Sandi 2001b). The list includes 79 native *Opuntia* species (specifically platyopuntias), 51 of them endemic to this country; about six species under intensive cultivation and at least 18 wild-growing species are also actively used, mainly for fruit, fodder and, to a lesser extent, rearing of cochineal insects (Pérez-Sandi 2001b). All these species are more or less vulnerable to moth attack, and will be impossible to protect them once the moth has naturalized (Pérez-Sandi 2001b). *C. cactorum* has demonstrated to be an efficient biological agent for eradicating *Opuntia* species (Zimmermann and Granata 2002). The United States (Florida) and Cuba are regions from which possible invasions to Mexico could occur (Zimmermann et al. 2001). The threat represented by *C. cactorum* to existing agricultural resources and cactus biodiversity, with the concomitant implications for social and economic security, must be determined as well as its presence confirmed in Mexico in order to find out the type of control measure to be implemented (Pérez-Sandi 2001b). Pérez-Sandi (2001b) published a strategy for Mexico to prevent the damage caused to these resources by *C. cactorum*. The program involves mobilizing the Mexican government, several leading organizations, stakeholders and experts at national and international levels, with management by a national steering committee whose participation in a medium- to long-term plan would ensure the protection of the cactus pear industry and the native cactus flora.

Another problem is that the constant fragmentation promoted by the extraction and exploitation of wild species, including *Opuntia* (Anderson 2001), without restrictions is reducing the possibility of studying the interaction between *Dactylopius* and *Opuntia*. The Norm for "Environmental Protection of native species in Mexico of wild flora and fauna, risk categories and specifications for their inclusion or change, including lists of species at risk" considers five *Opuntia* species of a total of 284 cacti listed, i.e. less than 2%. The species of *Opuntia* are part of the special category and four of them are endemic: *Opuntia bravoana* (nopal from Bravo), *O. excelsa* (excelso nopal), *O. rosarica* (Cholla tasajo de Rosario) and *O. santamaria* (Cholla de Santa María), the fifth species is *O. polyacantha* var. *arenaria* (SeMARNat 1994). On the other hand, the insects *Dactylopius* are not even considered in this norm.

The importance of conserving this plant-insect association, makes it necessary to continue studying this biological system that constitutes a genetic resource (Hunt and Taylor 2002) which is currently seriously threatened by the accelerated degradation of the wild species, a main source of raw material for research studies on the genetic (Flores-Hernández et al. 2006) improvement of cultivated plants, in resource conservation programs and the improvement of land.

The history of use of *Opuntia* and *Dactylopius* shows that Mexico has a high and unique variety of species and infraspecific taxa of these genera, which therefore deserve particular attention for biodiversity conservation. Information on diversity of *Opuntia*, *Dactylopius* and on the antiquity of cochineal cultivation indicates that the Mexican territory is the main area of diversification of the genus *Opuntia* and probably the center of origin of *D. coccus*. Mexico may be the area with the highest genetic diversity of *D. coccus* and, therefore, the main reservoir of genetic resources for cochineal production and a priority area for its conservation in situ.

Conclusions

From pre-Columbian times to the Nineteenth century, Mexico was one of the world's main producers of cochineal and dye, but until recently the activity was almost extinct. The Mexican territory is an important reservoir of biological diversity of both *Opuntia* and *Dactylopius*, particularly of *D. coccus* the main species used for cochineal production, and their conservation could be benefited from systematic policies of use and protection. Reactivation of cochineal cultivation could be achieved based on the current wide production of *Opuntia* and the recovering of traditional knowledge and production techniques developed throughout the Mexican cultural history with assistance from current technology. Production of cochineal dye would add benefits to the current *Opuntia* cladode and fruit industry and may achieve high profits. The study of the species *Opuntia* and *Dactylopius* as genetic resources could serve for the development and interaction between the cultured and wild species of both genera. The ecological direction necessary for recovering cochineal cultivation in a sustainable manner and adapting it to the current needs of the different regions of Mexico must be seriously considered.

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II. Distribution and habitat in Mexico of *Dactylopius* (Hemiptera: Dactylopiidae) and their hosts of the subfamily Opuntioideae (Cactaceae)

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Distribution and Habitat in Mexico of *Dactylopius* (Hemiptera: Dactylopiidae) and Their Hosts of the Subfamily Opuntioideae (Cactaceae)

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ABSTRACT - The distribution pattern of species of the genus *Dactylopius* Costa in Mexico was analyzed in relation to the distribution of their host plants (subfamily Opuntioideae), to evaluate the specificity of the insect-host association. The distribution of *Dactylopius* currently recognized is narrower than that of its hosts and probably is not representative. Therefore, a broader distribution of the *Dactylopius* species in correspondence with those of their hosts was hypothesized. Insects and their hosts were collected and georeferenced in 14 states of Mexico from 2005 to 2007. The distribution areas, maps, and habitat characteristics of *Dactylopius*, *Opuntia* Miller *sensu stricto*, *Nopalea* Salm Dyck and *Cylindropuntia* (Engelm.) F.M. Knuth were determined on the basis of field collections. This information was complemented with information from the exhaustive examination of microscope slides from a local insect collection, plants from local herbaria, and literature reviews. It was found that the current distribution of the genus *Dactylopius* and its hosts included 20 and 25 states of Mexico, respectively, and that *Dactylopius* had a continuous distribution according to its hosts, broader than recognized hitherto. The new georeferenced records of the five Mexican *Dactylopius* species are reported. Insects with morphological characteristics of *D. confusus* combined with those of *D. salmianus* were identified, as well as insects with characteristics of *D. opuntiae* combined with those of *D. salmianus*. These records suggest that the number of local *Dactylopius* species could be higher than previously thought or that possible new processes of hybridization between native and introduced species may be occurring.

KEY WORDS: Biodiversity, cacti, cochineal insects.

Introduction

Insects of the genus *Dactylopius* Costa (Hemiptera: Dactylopiidae), the cochineals, and their cacti

hosts *Opuntia* Mill., *Nopalea* Salm-Dick, *Cylindropuntia* (Engelm.) F.M. Knuth and *Grusonia* Rchb. ex Britton and Rose, are endemic to the American Continent (Britton & Rose 1963, Bravo-Hollis & Sánchez-Mejorada 1978, Brummitt & Powell 1992, Anderson 2001). Interactions between these insects and cacti were known and profitably used for centuries by pre-Columbian Mesoamerican people for whom cacti were food and cochineals a source of dye (Martin del Campo 1957, MacGregor 1976, Piña 1977, Colunga *et al* 1986, Bravo-Hollis & Scheinvar 2002, Casas & Barbera 2002, Reyes-Agüero *et al* 2005, Chávez-Moreno *et al* 2009).The genus *Dactylopius* includes nine species. *Dactylopius coccus* Costa 1835, *D. ceylonicus* (Green) 1876, *D. confusus* (Cockerell) 1893, *D. opuntiae* (Cockerell) 1896 and *D. tomentosus* (Lamarck) 1801 have been reported for North America (Portillo 2005), whereas *D. tomentosus* (Lamarck) 1801, *D. coccus* Costa 1835, *D. ceylonicus* (Green) 1876, *D. confusus* (Cockerell) 1893, *D. austrinus* De Lotto 1974, *D. confertus* De Lotto 1974, *D. salmianus* De Lotto 1974, and *D. zimmermanni* De Lotto 1974 have been reported for South America (Diodato *et al* 2004, Portillo 2005). Before our study, the 5 North American species of *Dactylopius* and 24 species and three varieties of host opuntioids had been reported in 8 and 24 states of Mexico, respectively (Table 1).

Table 1. Distribution of the species of *Opuntia*, *Nopalea*, *Cylindropuntia* and *Grusonia* and records of *Dactylopius* on these species in Mexico.

Species[a]	States[b] [References[c]]	*Dactylopius*[d] States[b] [References[c]]
C. acanthocarpa Engelm. & Bigelow	26 [I, III]	E: 2,3[V]
C. imbricata Haworth	6[I]; 7[I, III]; 10[I]; 11[I]; 12[I]; 14[I]; 17[I]; 19[I]; 22[I, VII]; 24[I] and 32[I]	C:7[I]; D: 33[I]
C. kleiniae D.C.	13[I, III]; 14[I]; 24[I]; 28[I] and 32[I]	C: 7[I]
C. leptocaulis D.C.	14 [I]; 21[III] and 22[I]	C:7[I]
C. tunicata (Lehm.) F. M. Knuth.	7[I], 22[I] and center of Mexico [I, III]	C: 7[I]
G. grahamii Engelm.	6[I, III]	C: 7[I]
N. cochenillifera (L.) Salm-Dyck	15[V]; 20[I] and 22[VII]	B: 15[VI]; 20[III, VII]; 33[IX]; D: 9[V]; 15[VIII]
N. karwinskiana Salm-Dyck	8[I, III, VI]; 13[I, VI]; 15[I, V, VI]; 16[I, VI]; 18[I, VI]; 20[I, VI]; 25[I, VI] and 26[I, VI]	D: 15[VIII]; E: 20[III,IV]
O. amyclaea (cultivated)	Cultivated [I, III]	B: 33[IX]

O. atropes (Rose) Smith	1[VII]; 10[VII]; 11[I, III, VI]; 12[IV]; 13[I, VI]; 14[I, VII]; 15[V, VI]; 16[VI]; 17[I, VI]; 22[VII] and 24[VII]	B: 15[VI, VIII], 33[IX] D: 15[VIII]
O. crassa Haworth	9[I]; 11[III] and 12[IV]	B: 33[IX]
O. engelmannii (O. cantabrigensis*) Salm-Dyck ex Engelm	11[III]; 14[I, III, VI]; 15[V, VI]; 22[I, III, VI] and 24[I, III, VI]	D: 33[I]
O. ficus-indica (L.) Mill.	1[III]; 11[I]; 12[IV]; 15[V]; 20[I], 21[I, VII] and 22[VII]	A: 15[IV]; 30[III]; B: 15[VIII]; 20[III, IV]; 21[I]; 33[IX]; D: 15[VIII]; 20[IV]; 33[I,IV]
*O. vulgaris** Tenore	cultured [III]	D: 33[VIII]
O. fuliginosa Griffiths	8[I]; 12[IV]; 15[I, III, V]; 16[I] and 22[VII]	A: 15[III]; B: 33[IX]; C: 15[VIII]; D: 33[I]
O. hyptiacantha F.A.C. Weber	1[I, VI]; 11[VI]; 12[VI]; 14[VI]; 15[V, VI]; 20[III, VI]; 21[VI]; 22[VI, VII]; 24[VI]; 29[VI] and 32[I, VI]	B: 20[IV]; D: 33[I]
O. jaliscana Bravo	12[I, III, IV, VI]; 15[I, V, VI] and 16[I, III, VI]	B: 15[VIII]; 33[IX]; D: 15[VIII]

O. leucotricha D.C.	10[I, VI]; 11[III]; 12[I, VI]; 14[I, VI]; 15[V, VI]; 22[I, VI, VII]; 24[I, VI] and 32[I, VI]	D: 33[I]
O. megacantha Salm-Dyck	1[I, III, VI]; 12[I, IV, VI]; 15[V, VI]; 24[I, VI] and 32[I, VI]	B: 33[IX]; D: 15[VIII]; 33[I]; E 2,3[IV]
O. pilifera F.A.C. Weber	20[I, VI]; 21[I, III, VI] and 29[VI]	B: 20[III]; 33[IX]
O. pumila (Rose) Smiths	13[I]; 15[V]; 17[I, III]; 20[I]; 21[I] and 22[VII]	C: 20[V]
O. robusta Wendland	6[VI]; 9[VI] ; 10[VI]; 11[VI]; 12[I, III, IV, VI]; 13[VI]; 14[I]; 15[V, VI]; 16[I, VI]; 22[I, VI, VII]; 24[I, VI]; 26[VI] and 32[I, III, VI]	D: 14[IV]; 33[I]
O. streptacantha Lem.	1[I]; 9[VI]; 10[VI]; 11[VI]; 12[I, IV, VI]; 14[I, VI]; 15[V, VI]; 20[VI]; 21[VI]; 22[I, VI, VII]; 24[I, III, VI]; 29[VI] and 32[I, VI]	B: 33[IX]; D: 33[I]
O. tomentosa Salm-Dyck	9[III, VI]; 11[I, VI]; 12[IV, VI]; 13[VI]; 14[VI]; 15[V, VI]; 16[VI]; 17[VI]; 20[VI]; 21[VI]; 22[VI, VII] and 24[VI]	B: 20[IV,V,VIII]; 33[IX] D: 20[IV]; 33[I]

*O. hernandezii** D.C. (Bravo)	Cultured [III]	B: 20[VIII]; D: 20[IV]
*O. macdougaliana** (Rose) Bravo	20,21 [I]; 21 [III]	D: 33[I]
O. undulada Griffiths	1[I, III]; 12[IV] and 15[V]	B: 33[IX]

[a] Nomenclature based on Anderson (2001), * = synonyms; [b] 1 = Aguascalientes, 2 = Baja California Norte, 3 = Baja California Sur, 4 = Campeche, 5 = Chiapas, 6 = Chihuahua, 7 = Coahuila, 8 = Colima, 9 = Mexico City, 10 = Durango, 11 = Estado de México, 12 = Guanajuato, 13 = Guerrero, 14 = Hidalgo, 15 = Jalisco, 16 = Michoacán, 17 = Morelos, 18 = Nayarit, 19 = Nuevo León, 20 = Oaxaca, 21 = Puebla, 22 = Querétaro, 23 = Quintana Roo, 24 = San Luis Potosí, 25 = Sinaloa, 26 = Sonora, 27 = Tabasco, 28 = Tamaulipas, 29 = Tlaxcala, 30 = Veracruz, 31 = Yucatán, 32 = Zacatecas, 33 = unknown; [c] I = Bravo-Hollis & Sánchez-Mejorada (1978); II = Bravo-Hollis & Scheinvar (2002); III = Britton & Rose (1963); IV = Colunga *et al* (1986); V = González *et al* (2001); VI = Guzmán *et al* (2003); VII = Scheinvar (2004). [d] A = *Dactylopius ceylonicus*, B = *D. coccus*, C = *D. confusus*, D = *D. opuntiae* and E = *D. tomentosus*. [e] I = Mann (1969); II = De Lotto (1974); III = Piña (1977); IV = MacGregor & Sanpedro (1984); V = Pérez-Guerra & Kosztarab (1992); VI = Portillo & Zamarripa (1992); VII = Miller (1996); VIII = Portillo & Vigueras (2003a) and IX = Portillo & Vigueras (2003b).

Studies by De Lotto (1974) and Pérez-Guerra & Kosztarab (1992) describe the distribution of *Dactylopius* in Mexico, although they are mainly focused on taxonomic and ethno-biological aspects of the insects. Some reports (e. g. Pérez-Guerra & Kosztarab, 1992; Portillo & Zamarripa, 1992; Miller, 1996) are catalogs or check lists of *Dactylopius* species and the states where they are localized, with scarce data about the the features of their habitats. Miller (1996) reported *D. coccus* in Oaxaca, *D. confusus* in Durango, Guerrero, Jalisco, Morelos, Nuevo León, Puebla, Sonora, *D. opuntiae* in Baja California, Mexico City, Durango, Hidalgo, Estado de México, Michoacán, Morelos, Oaxaca and Tamaulipas, and *D. tomentosus* in Baja California, Chihuahua and Oaxaca. Pérez-Guerra & Kosztarab (1992) reported *D. coccus, D. confusus* and *D. tomentosus* in Oaxaca interacting with *O. tomentosa*, *O. pumila* and *O. acanthocarpa*, respectively. The most important, systematic and detailed report is the catalog of Mexican coccids of the family Dactylopiidae by MacGregor & Sampedro (1984), which includes records of (1) *D. ceylonicus* on *O. ficus-indica* in the state of Jalisco and on *Opuntia* sp. and *Nopalea* sp. in the states of Hidalgo, México, Morelos, Oaxaca and Veracruz, (2) *D. coccus* on *O. ficus-indica* and *O. hyptiacantha*, and *Opuntia* sp. in Oaxaca and Puebla, (3) *D. confusus* on *Opuntia* sp., *Nopalea* sp. and *Cactus* sp. in Chihuahua, Mexico City, Guanajuato, Guerrero, Jalisco, Morelos, Oaxaca, Puebla and Tamaulipas, (4) *D. opuntiae* on *O. ficus-indica* and *O. tomentosa* in Oaxaca and on *Opuntia* sp., *Nopalea* sp. and *Cactus* sp. in the states of Aguascalientes, Baja California, Chiapas, Mexico City, Durango, Guerrero, Hidalgo, Jalisco, México, Michoacán, Nayarit, Nuevo León, Oaxaca, Puebla, Querétaro, Veracruz and Zacatecas, and (5) *D. tomentosus* on *O. megacantha* and *N. Karwinskiana* in Baja California, on *O. vulgaris* in Coahuila, and on *Opuntia* sp. and *Cactus* sp. in Mexico City, Guanajuato and Nuevo León. Conversely, the distribution of host plants of the genera *Opuntia, Grusonia* and *Cylindropuntia* reported until now is wider than

that of the insects (Table 1). However, information available on *Dactylopius* and their hosts lacks precise descriptions of their distribution pattern and characteristics of their habitats.

This study aimed to determine the distribution pattern of *Dactylopius* in Mexico in relation to the distribution of *Opuntia*, *Grusonia* and *Cylindropuntia*, describing the main features of their habitats (altitude, vegetation, soil and climate). Our investigation was based on the hypothesis that the distribution of *Dactylopius* currently recognized is not representative and should be broader in correspondence with the distribution of their host plants.

Material and Methods

A database aimed at comparing the distribution areas of *Dactylopius* and their hosts was constructed based on: (1) an exhaustive literature review, (2) a meticulous examination of 262 specimens of opuntioids at MEXU and IBUG, and (3) the examination of 367 microscope slides of *Dactylopius* at CNI-IB-UNAM (Tables 1 and 2). A geographic information system was constructed through ILWIS 3.3 mapping the geographic location of the reported sites of *Dactylopius* and their hosts that we reviewed.

Sampling area. To identify the interacting species and gather information about their distribution, species of cochineals and their hosts were sampled in the area enclosed between 98 and 104º northern latitude and 18 and 23º western longitude, comprising the states of Aguascalientes, Mexico City, Guanajuato, Hidalgo, Jalisco, Estado de México, Michoacán, Morelos, Oaxaca, Puebla, Querétaro, San Luis Potosí, Tlaxcala, Veracruz and Zacatecas. This area was chosen because it was considered to be the main reservoir of species richness of host species of *Dactylopius.*

Table 2. Consulted records of herborized species of *Opuntia* hosts to *Dactylopius*. Altitude in meters above sea level.

Locality (States[a])	Altitude	Collector [b]	Collect No.[c]	Date	Reference
		O. atropes			
La mina (1)	1700	35	40007*	30/08/1983	MEXU-UNAM
Tonalapa (13)	1000	40	3017	11/12/1972	MEXU-UNAM
Acatic (15)	1600	25, 27	160	27/07/2002	IBUG-UDG
	1700	24	141574*	08/04/1990	IBUG-UDG
	1700	33, 58	6	04/04/1990	IBUG-UDG
Arandas (15)	2150	7, 38	*	02/04/1977	IBUG-UDG
	2150	42	24984*	22/03/1980	MEXU-UNAM
Atoyac (15)	1500	25, 54	66	01/05/1986	IBUG-UDG
	1500	25, 54	65	01/05/1986	IBUG-UDG
	1400	25, 54	69	02/05/1986	IBUG-UDG
Cerro gordo (15)	1400	14	1279*	20/04/1977	MEXU-UNAM
Cocula (15)	1400	6, 34	248	09/03/1986	IBUG-UDG
	1700	6, 34	261	09/03/1986	IBUG-UDG
	1600	6, 34	247	09/03/1986	MEXU-UNAM
	1400	6	257	09/03/2006	MEXU-UNAM
Cuquio (15)	1800	3	56989*	12/02/1986	IBUG-UDG

	1800	3	56989*	12/02/1986	IBUG-UDG
	1700	6, 64	1331	24/04/1993	IBUG-UDG
	1700	6, 64	1331	24/04/1993	IBUG-UDG
El Grullo (15)	900	6	1020	16/03/1984	IBUG-UDG
	900	6	1020	16/03/1984	IBUG-UDG
	900	6	1020	16/03/1984	IBUG-UDG
	900	6	48	16/03/1984	IBUG-UDG
Jocotepec (15)	1600	34, 51	47	01/02/1986	IBUG-UDG
	1600	34, 51	48	01/02/1986	IBUG-UDG
Juanacatlán (15)	1600	15	49692*	18/03/1985	IBUG-UDG
	1600	12	49680*	18/03/1985	IBUG-UDG
	2550	24	49683*	18/03/1985	MEXU-UNAM
La Manzanilla de la Paz (15)	2100	6	177	22/06/1985	IBUG-UDG
Lagos de Moreno (15)	2000	6, 54	1266	15/07/1991	IBUG-UDG
	2000	5,6	146835*	22/07/1997	IBUG-UDG
Mascota (15)	1300	49	39045*	13/04/1982	MEXU-UNAM
Nevado de Colima (15)	1500	60	40004*	06/10/1983	IBUG-UDG
Nevado de Colima (15)	1500	60	40005*	06/10/1983	IBUG-UDG
Ocotlán, Poncitlán (15)	2000	2	30124*	08/03/1981	MEXU-UNAM
Poncitlán (15)	1600	49	41663*	22/05/1984	MEXU-UNAM
Sn. Martín Hidalgo	1900	32	766	21/04/1990	IBUG-UDG

(15)					
Tamazula (15)	1700	21	30405*	22/03/1981	MEXU-UNAM
	1700	21	30402*	22/03/1981	MEXU-UNAM
Tecolotlán (15)	1200	16, 24	46373*	02/02/1985	MEXU-UNAM
Tenamaxtlan (15)	1500	6	46376*	03/02/1985	IBUG-UDG
Tlajomulco (15)	1600	11	30125*	01/03/1981	IBUG-UDG
Tolimán (15)	1200	6, 50	1181	27/05/1990	IBUG-UDG
Tuxpan (15)	1150	9	49693*	17/03/1985	IBUG-UDG
	1100	31	49689*	17/03/1985	MEXU-UNAM
Barranca Huentitán (15)	**	28	1499	13/06/1986	IBUG-UDG
Cuernavaca (17)	2350	63	2391	06/03/1970	MEXU-UNAM
		O. engelmannii			
La Paz (3)	50	5, 48	1134	14/03/1994	MEXU-UNAM
Moyahua (32)	1200	22	888	28/04/1996	MEXU-UNAM
		O. ficus-indica			
Zacatecas-Aguascalientes (1)	1400	34	6257	02/10/1983	MEXU-UNAM
Coyoacán (7)	**	61	6538	17/08/1999	MEXU-UNAM
El Cordonal (14)	300	36	964120*	18/08/1998	MEXU-UNAM
Ameca (15)	1300	19	1267	10/06/1976	MEXU-UNAM
Tala (15)	1400	4	49682*	18/03/1985	MEXU-UNAM

Zapopan (15)	2200	6	57004*	--/03/1986	MEXU-UNAM
Sn. Juan Mixtepec (20)	1600	37	OAX903	07/03/1997	MEXU-UNAM
Oyameles (21)	2850	20	3678	Undated	MEXU-UNAM
Cadereyta (22)	2100	47, 61	3678	02/07/1984	MEXU-UNAM
		O. fuliginosa			
Guadalajara-Nogales (15)	1200	49, 67	29451*	Undated	MEXU-UNAM
		O. hyptiacantha			
Ecatepec de Morelos (11)	2400	61	2167	30/04/1976	MEXU-UNAM
Texcoco (11)	2300	1, 61	1036	30/03/1973	MEXU-UNAM
		O. jaliscana			
José de García (1)	2700	34	6200	31/10/1983	IBUG-UDG
Penjamo (12)	1700	10	652	03/09/1995	IBUG-UDG
Atemajac de Brizuela (15)	2350	43, 50, 57	45	01/07/1989	IBUG-UDG
Atoyac (15)	1500	34, 54	70	02/05/1986	IBUG-UDG
Atoyac (15)	1400	34, 54	68	02/05/1986	IBUG-UDG
Sayula-Sn. Gabriel (15)	1400	6, 58	1347	05/05/1993	IBUG-UDG
Concepción Buenos	1900	**	1250	10/06/1976	IBUG-UDG
Aires (15)	2100	21, 39, 65	74	20/05/1990	IZTA
El Picacho (15)	2100	50, 52	2747	14/05/1992	IBUG-UDG

	2100	50, 52	2748	14/05/1992	IBUG-UDG
Encarnación Díaz (15)	2300	6, 34	239	27/04/1986	IBUG-UDG
Ixtlahuacan (15)	1300	6, 64	1334	24/04/1993	IBUG-UDG
Jala (15)	2000	6	1254	14/06/1991	IBUG-UDG
Jesús María (15)	2050	30	2286	02/02/1986	IBUG-UDG
Jocotepec (15)	2000	34, 50, 51	44	01/02/1986	IBUG-UDG
Lagos de Moreno (15)	2100	6, 34	390	04/07/1986	IBUG-UDG
	2100	6	390	04/07/1986	IBUG-UDG
	1600	6, 34	590	27/09/1986	IBUG-UDG
	2100	6, 34	396	05/06/1986	IBUG-UDG
	1900	6	69	12/10/1984	IBUG-UDG
Laguna de Sayula, Tapalpa (15)	2100	13, 66	566	27/10/1994	IBUG-UDG
Manzanilla de la Paz (15)	1850	6	171	22/06/1985	IBUG-UDG
Ocampo (15)	1200	6	53	14/07/1984	IBUG-UDG
Sn. Miguel el Alto (15)	1900	6	1054	10/09/1988	IBUG-UDG
Tepatitlán (15)	1800	6, 34	284	27/04/1986	IBUG-UDG
Venustiano Carranza (15)	1300	6	290	18/03/1986	IBUG-UDG
Zapopan (15)	1600	23	41417*	09/06/1984	IBUG-UDG

O. joconostle

Valle de Santiago (12)	(i)	18	61	03/06/1981	MEXU-UNAM
Corralejo, Penjamo (12)	1700	10	692568*	03/09/1995	MEXU-UNAM
Sn. Miguel Allende (12)	1900	10	940416*	04/06/1994	MEXU-UNAM
Alfajayucan (14)	1900	17, 61	2332	22/03/1979	MEXU-UNAM
Guadalcazar (24)	1600	26	763386*	15/03/1997	MEXU-UNAM
		O. megacantha			
Valle de Santiago (12)	1800	18	PC-28	15/06/1980	MEXU-UNAM
	1800	18	PC-18	13/06/1980	MEXU-UNAM
Tapalpa (15)	2100	62	381552*	30/09/1983	MEXU-UNAM
		O. robusta			
Sn. Miguel Allende (12)	1900	10, 45	584	30/07/1995	MEXU-UNAM
		O. streptacantha			
Pilotos (1)	2150	**	1263	11/02/1965	MEXU-UNAM
Jilotepec de Abasolo (11)	2450	8	73	04/10/1986	IZTA
	2450	8	72	04/10/1986	IZTA
Jocotitán (11)	3900	8	145	18/10/1987	IZTA
Sn. Luis de la Paz (12)	2000	10, 44	T-126	21/02/1994	MEXU-UNAM
Ocampo (12)	2200	6	54	14/07/1984	MEXU-UNAM

Valle de Santiago (12)	2000	18	PC100	08/06/1981	IZTA
Arandas (15)	1800	50, 52	152220*	14/05/1992	MEXU-UNAM
El Cuarenta (15)	1750	5, 6	1488	14/07/1997	MEXU-UNAM
	2150	5, 6	1486	21/07/1997	MEXU-UNAM
Encarnación Díaz (15)	2200	6, 34	289	27/04/1986	IBUG-UDG
Encarnación Díaz (15)	1750	29	1443	17/05/2001	MEXU-UNAM
Encarnación Díaz (15)	1950	6, 34	289	27/04/1986	MEXU-UNAM
Lagos de Moreno (15)	1900	16, 47	198	Undated	MEXU-UNAM
	2200	6	204	13/10/1984	MEXU-UNAM
	1800	6, 34	599	28/09/1986	MEXU-UNAM
	1900	6	821	11/06/1987	MEXU-UNAM
	2000	6	430	15/06/1986	MEXU-UNAM
	2000	6	592	27/09/1986	MEXU-UNAM
	2000	6	347	22/05/1997	MEXU-UNAM
	2000	5, 6	1491	22/07/1997	MEXU-UNAM
	2000	6	1484	21/07/1997	MEXU-UNAM
	2100	5, 6	1488	21/07/1997	MEXU-UNAM
	2000	6	821	11/06/1987	MEXU-UNAM
Los Alpes (15)	1900	6	204	21/09/1985	MEXU-UNAM
Ojuelos (15)	2200	6, 58	415	14/06/1986	MEXU-UNAM
	2200	6	200	22/09/1985	MEXU-UNAM
	2200	29	127	16/06/2000	MEXU-UNAM

	2200	27, 53, 56	132	16/06/2000	MEXU-UNAM
Picacho (15)	1900	50,52	2746	22/07/1992	MEXU-UNAM
Sn. Juan de los Lagos (15)	2200	25, 47, 68	1273	15/05/1977	MEXU-UNAM
	1750	59	1276	01/05/1977	MEXU-UNAM
Sn. Miguel el Alto (15)	2300	6	1053	10/09/1988	MEXU-UNAM
Villa de Hidalgo (15)	2300	6, 57	1385	17/11/1989	MEXU-UNAM
Tequisquiapan (22)	1900	46, 61	7867	19/03/1992	MEXU-UNAM
Corral de Palmas (24)	2300	**	18020	27/10/1983	MEXU-UNAM
Charoas (24)	2200	35	22179	08/12/1988	IZTA (100556)
El Alamillo (32)	1800	50, 57	75	12/08/1989	MEXU-UNAM
		O. tomentosa			
Ixtapalapa (9)	2300	61	1100A	17/05/1973	MEXU-UNAM
Metztitlán (14)	1900	26	1800	05/11/2000	MEXU-UNAM
Xaltocan (29)	2500	41, 61	2470	25/07/1980	MEXU-UNAM

[(a)] See Table 1; [(b)] 1 = Ahuatzin, 2 = Alatorre, 3 = Aldrete, 4 = Amezquita-Díaz, 5 = Arias, 6 = Arreola-Nava, 7 = Ascencio, 8 = Ávalos –Otento, 9 = Aviña, 10 = Bárcenas, 11 = Benítez-Rojo, 12 = Carrillo-Arellano, 13 = Carvajal, 14 = Castellanos, 15 = Cera-Iriba, 16 = Cervantes, 17 = Cintara, 18 = Colunga, 19 = Cuenca, 20 = Cházaro-Basañez, 21 = Chávez, 22 = Enríquez, 23 = Espinosa, 24 = Flores, 25 = García, 26 = Gómez, 27 = González, 28 = González-Álvarez, 29 = González-Durán, 30 = González-Villareal, 31 = Gudiño, 32 = Guerrero-Nuño, 33 = Gutiérrez, 34 = Guzmán, 35 = Hernández, 36 = Hidalgo, 37 = Hunn, 38 = Jiménez, 39 = Koch, 40 = Kruse, 41

= León, 42 = León-Villaseñor, 43-= López, 44 = Luna, 45 = Meade, 46 = Olalde, 47 = Orozco, 48 = Piña, 49 = Quintero, 50 = Ramírez, 51 = Reyna, 52 = Reynoso, 53 = Riojas, 54 = Rodríguez, 55 = Rodríguez-Macías, 56 = Rosas, 57 = Salcedo–Pérez, 58 = Sánchez, 59 = Sandoval, 60 = Santana-Michel, 61 = Scheinvar, 62 = Suárez Jaramillo, 63 = Vázquez, 64 = Vigueras, 65 = Villa, 66 = Villegas, 67 = Zavala, 68 = Zúñiga, i = unreadable herbarium label ** = Not indicated on the herbarium label; $^{(c)}$ = herbarium number.

Field collection of specimens. *Dactylopius* from 208 insect populations in 120 localities of 14 states of Mexico within the sampling area were collected from 2005 to 2007. The number of collected sample was variable, between 25 to 100 specimens, depending on the size of the population. Male and female insects at different stages of development were collected. In the plants where insects were present in different portions of the same host, insects were collected separately from each portion. Samples were preserved in 70% ethanol. Samples of the species *Dactylopius* and their hosts were collected from wild populations, production and research centers, and urban and rural zones. Cladodes of *Opuntia*, *Nopalea* and *Cylindropuntia* were collected in triplicate for propagation. Specimens of *Dactylopius* were vouchered in the Hemiptera collection of CNI-IB-UNAM. Host plants, *Opuntia*, *Opuntia* (*Nopalea*) and *Cylindropuntia* were vouchered in the area of desert-zone plants of the living collection of the Botanical Garden at the Centro de Investigaciones en Ecosistemas (CIEco-UNAM).

Identification of species of *Dactylopius*, *Opuntia, Nopalea,* and *Cylindropuntia*. *Dactylopius* specimens were identified using the taxonomic keys of De Lotto (1974), Pérez-Guerra & Kosztarab (1992) and de Haro & Claps (1995). The technique of de Haro & Claps (1995) was

used to prepare 153 microscope slides with four to eight insects per slide. Slides were observed under a light microscope (Olympus BX45, Olympus, Japan) coupled to a CDD camera (High Performance Pro-Series UTV 0.5 XC, model 1E08849, Japan) connected to a personal computer (Blue Code, Pentium IV). The captured images were analyzed with the program IPwin 32 (Image Pro version 4.5.1 XProf 22, 2000, for Windows 1998). The identity of *Opuntia*, *Opuntia* (*Nopalea*) and *Cylindropuntia* was corroborated by comparisons to the literature (Britton & Rose 1963, Bravo-Hollis & Sánchez-Mejorada 1978, González *et al* 2001) and to specimens from the herbaria MEXU and IBUG. Some specimens were assigned their common name due to their morphological complexity.

Environmental database. The database included the following fields: name of insect species, host (portion of the plant where the insect was found), place of collection, i.e. locality and state, geographic coordinates of localization, i.e. latitude, longitude and altitude, collection date and information reference. The data from our fieldwork, including new records, vegetation and soil types, were inserted in the previously generated database in boldface typeset. The keys and descriptions of Rzedowski (1978, 1992) were used to characterize weather of the studied area.

Results and Discussion

Dactylopius. The information shown in Tables 1 and 2 was used to draw the distribution maps of *Dactylopius* and their hosts of the genera *Opuntia*, *Nopalea*, *Cylindropuntia* and *Grusonia*, shown in Figs 1a and 1b. Specimens of the five *Dactylopius* species were collected; 175 of *D. ceylonicus*, 575 of *D. coccus*, 675 of *D. confusus*, 1200 of *D. opuntiae* and 200 of *D. tomentosus.* Fig 2 shows the distribution of collecting sites of *D. ceylonicus* (A), *D. coccus* (B), *D. confusus*

(C), *D. opuntiae* (D) and *D. tomentosus* (E) in the studied area. The field observations of this work are shown in Table 3.

Dactylopius ceylonicus. This species had been previously reported in six states of Mexico, on species of *Opuntia* and *Nopalea* (Tables 1, 3, Figs 1 and 2). Additionally, in this investigation *D. ceylonicus* was collected for the first time in Mexico City and Hidalgo on *O. ficus-indica* and *Cylindropuntia imbricata* (Haw.) F.M. Knuth, respectively. Like the rest of dactilopids, the cottony-white thin layer covering the insect's body characterizes this species. Insect specimens were collected during April to June on the top portion of their hosts, on ripe cladodes of *Opuntia* with more than three levels of cladodes and on the areoles of prickly pears, in living fences with scarce vegetation and regosol. During the rest of the year, *D. ceylonicus* was collected on root nodules of *Opuntia* sp., in wild populations where xerophilous thickets and arenosol predominate. The presence of the insect is scarce without perceptible damage to its host. As shown in Table 3, specimens of this species collected in this work were localized within the previously reported altitude range of 950 to 2650 m.

Dactylopius coccus. Its pulverulent white cover and a larger size than the rest of the species of the genus distinguish *Dactylopius coccus*. It had been reported in five states of Mexico on *Opuntia* and *Nopalea* species within the altitude range of 1250 to 2200 m, as illustrated in Figure 1a and Tables 1 and 3. In this study, *D. coccus* was collected in research and production centers: Tlapanochestli in Santa María Coyotepec, Oaxaca; Nopaltepec A.L.P.R. in Nopaltepec, Estado de México and Campo Carmín S.P.R. de R.L. in Tetecalita, Morelos, where the species *O. ficus-indica* is used as the main host for culturing and processing the insect. Additionally, species were

collected in localities close to those centers. The presence of *D. coccus* in wild localities of Estado de México, San Luis Potosí and Mexico City is reported here for the first time. The most frequent habitat of these cochineals was formed by intensive cultures of *O. ficus-indica*, on rain-watered lands with presence of nopale where the type of soils included vertisol, calcisol, xerosol, regosol, leptosol, and feozem, within the previously reported altitude range of 1654 to 2845, as shown in Table 3.

Dactylopius confusus. This species had been previously reported in 11 states of Mexico, mainly on species of *Opuntia*, *Cylindropuntia* and *Grusonia*, within the altitude range of 1100 to 2200 m (Fig 1a and Tables 1 and 3). In this work, the analysis of morphological characteristics of insects of the species *D. confusus*, resulted in two separate groups designated here as *D. confusus* and *D. confusus* biotype 1 whose description is as follows.

Dactylopius confusus. Insects with the typical morphology of this species were designated with this name (De Lotto, 1974; Pérez-Guerra y Kosztarab, 1992). They were collected in Mexico City, Hidalgo, Jalisco, Morelos and Puebla on *O. ficus-indica*; in Jalisco on different hosts and their presence in the states of Veracruz and Zacatecas is reported here for the first time as shown in Table 3. Our field observations show that *D. confusus* grows mainly on cladodes of tree and shrub cactus forms and on their prickly pear fruits in a predominantly desert habitat with scarce vegetation and arenosol, within the altitude range of 1200 to 2547, which is higher to the one previously reported shown in Table 3.

Dactylopius confusus biotype 1**.** Insects with the morphological characteristics diagnosed for *D. confusus* (De Lotto 1974, Pérez-Guerra & Kosztarab, 1992) combined with characteristics corresponding to the *D. salmianus* species were designated with this name. It is worth mentioning

that *D. salmianus* has been reported only for South America (De Lotto 1974, Pérez-Guerra & Kosztarab 1992, de Haro & Claps 1995), without reports of its presence in Mexico. Insects were collected in the states of Hidalgo, Morelos, San Luis Potosi, Puebla and Tlaxcala on different species of the genus *Opuntia* and one species of *Cylindropuntia*; additionally, *D. confusus* and *D. confusus* biotype 1 were collected in the states of Morelos cohabiting on the same host (Table 3). Our field observations and records show that these insects promote changes in the color of cladodes and fruits, and when the insects are closely gathered at the trunk-stem and stem-fruit joints, these parts are damaged and may detach from the main plant body. Our data also show that *D. confusus* biotype 1 develops mainly on the cladodes of ripe tree or bush plants and prickly pear fruits in the urban zones and production cultures of *Opuntia* and on rain-watered lands in wild habitats where xerophilous thickets growing on arenosol and calcisol predominate. The insects were localized within the altitude range of 1654 to 2773 m (Table 3).

***Dactylopius opuntiae*.** This species has the greatest number of records for the genus (Fig 1); it has been reported in 20 states of Mexico, on 17 species of Cactaceae, including the genera *Opuntia*, *Nopalea*, and *Cylindropuntia*, within an altitude range of 25 to 2678 m (see Tables 1 and 3). The analysis of the morphological characteristics of the insects of the species *D. opuntiae* resulted in two separate groups, designated here as *D. opuntiae* and *D. opuntiae* biotype 1, described as follows.

Dactylopius opuntiae. Insects with a typical morphology of this species (De Lotto 1974, Pérez-Guerra & Kosztarab 1992) were designated by this name. They were collected in Mexico City, Jalisco, Michoacán, Puebla, San Luis Potosi, Veracruz and Zacatecas on species and cultivars of *Opuntia*. Additionally, specimens and records of *D. opuntiae* in the states of Guanajuato and

Tlaxcala (Table 3) are reported here for the first time. Insects of this species develop on any portion of the plant, the cladodes, fruits, flower calyx and trunk, during any stage of host development. This is the more aggressive species of the genus; its development and invasive growth in the host plant promote changes in the color of the cladodes and fruits, the detaching of the cladodes and fruits when the insect grows on cladode-cladode, cladode-flower and cladode-fruit joints, and even death when the insects damage the trunk. The collected specimens were located in all types of vegetation, soil and climates already reported for this genus, within an altitude of 750 to 2845 m, which is higher than that reported by several sources, as shown in Table 3 and Fig 2.

Dactylopius opuntiae biotype 1. Insects with typical morphological characteristics of *D. opuntiae* (De Lotto 1974, Pérez-Guerra & Kosztarab, 1992), combined with characteristics corresponding to the species *D. salmianus,* were designated by this name. Unlike the species *D. confusus* and *D. opuntiae*, the species *D. salmianus* has a thinner and elongated body and the structures of the setae and pores are more elongated and more separated or dispersed over the insect body (De Lotto 1974, Pérez-Guerra & Kosztarab, 1992). This insect was collected in Guanajuato, Jalisco, San Luis Potosí and Tlaxcala on species of the genera *Opuntia* and *Cylindropuntia*. Additionally, *D. opuntiae* and *D. opuntiae* biotype 1 were collected in Mexico City and Michoacán cohabiting on the same host (see Table 3). It was noticed that *D. opuntiae* biotype 1 was less aggressive than *D. opuntiae*. It develops mainly on cladodes and fruits and the aerial parts of its hosts. *D. opuntiae* biotype 1 was found in urban zones and production cultures of *Opuntia*, on rain-watered lands, in wild habitats where xerophilous thickets and other cacti growing on arenosol predominate, within an altitude range of 1663 to 2773 m (Table 3).

***Dactylopius tomentosus*.** No records for this species exist at CNI-IB-UNAM. In the literature, its presence is reported in eight states of Mexico on cacti of the genera *Opuntia, Nopalea* and *Cylindropuntia,* within the altitude range of 0 to 2500 m (Fig 1a and Table 3). *Dactylopius tomentosus* was collected in the states of Guanajuato and Hidalgo on species of the genera *Opuntia* and *Cylindropuntia,* within the previously mentioned altitude range. The insects develop exclusively on the cladodes of their hosts and their tiny size makes them almost imperceptible. They do not damage or promote changes in the plant and develop in a desert habitat where xerophilous thickets predominate, on vertisol and arenosol, as shown in Table 3. The presence of spiders was frequently observed with this species.

***Opuntia, Nopalea* and *Cylindropuntia*.** Our fieldwork revealed the presence of *Dactylopius* only on the genera *Opuntia* and *Cylindropuntia*. As shown in Table 3, the species of hosts identified and recorded were: *Opuntia ficus-indica* (variants and cultivars), *O. streptacantha* Lem. and *O. streptacantha* ssp. *Aguirreana* Bravo, *O. robusta* (variants and cultivars), *O. tomentosa*, *O. albicarpa* (cultivar), *O. joconostle* Weber, *O. hyptiacantha*, *O. jaliscana*, *O. phaeacantha* Engelm. *O. megacantha, O. fuliginosa, O. spinulifera* Salm-Dick, *O. atropes, Cylindropuntia imbricata* and *C. tunicata* Link & Otto in Pfeiffer and in 20 cultivars. Insects were not found on some of the hosts previously reported in the literature (Tables 1 and 3). For instance, we collected the species *Nopalea cochenillifera, N. karwinskiana* and *N. auberi* Salm-Dick in Jalisco without observing the presence of the insects during our complete period of fieldwork. *Dactylopius* was

mostly found on tree and shrub cactus forms. The parts of the plant where the insect was localized were mainly the areoles of cladodes and fruits and the stem commissures, during the months of April to June (aerial cycle) and the rest of the year on root nodules (latency period). The insects were collected only in Mexico City and Estado de México, in their aerial cycle throughout the year on *O. ficus-indica*. Fifty-three species and varieties of opuntiods were vouchered in the living collection of the desert zone plants of the Botanical Garden at CIEco-UNAM.

Distribution of collected *Dactylopius, Opuntia, Nopalea* and *Cylindropuntia*. Our research shows that the five species of *Dactylopius* have a continuous distribution broader than previously reported (Figs 1, 2 and Tables 2, 3). The insects are localized in correspondence with their hosts in different ecosystems of the northern and central plateau, and southeastern regions of Mexico. Our fieldwork made it possible to recognize localities where one species of *Dactylopius* is present on one or different hosts or shares hosts with different species of *Dactylopius,* or where two, three and four species of insects coexist. It was also possible to obtain a larger number of records of populations with one or two species of insects. Localities with one species were recorded in production centers (*D. coccus*) and in the states of Veracruz (*D. confusus*), Aguascalientes, Michoacán (*D. opuntiae*) and Guanajuato (*D. opuntiae* biotype 1). Localities with two species included Jalisco, Puebla, Veracruz, Zacatecas (*D. confusus* and *D. opuntiae*), San Luis Potosí (*D. coccus* and *D. opuntiae)* and Tlaxcala (*D. confusus* biotype 1 and *D. opuntiae* biotype 1). Three species were localized in Guanajuato (*D. coccus, D. opuntiae* and *D. tomentosus*) and San Luis Potosí (*D. coccus, D. confusus* and *D. opuntiae*). Four species were localized in Hidalgo (*D. ceylonicus, D. confusus, D. tomentosus* and *D. coccus*) and Mexico City

(*D. ceylonicus, D. coccus, D. confusus* and *D. opuntiae* on *O. ficus-indica*) in Milpa Alta, the main production zone of nopal vegetables in Mexico. According to our registers, the distribution area of the host species *Opuntia, Nopalea* and *Cylindropuntia*, is broader than previously recorded for *Dactylopius*, which suggests that these insects can be found distributed over a large area, in correspondence with their hosts. *Opuntia ficus-indica* was the most common and most widely distributed host. The altitude range of *Dactylopius* and their hosts, considering the georeferenced data of reports and specimens presented in this work was 0 to 2845 m.

Vegetation, soil and weather. *Dactylopius* and their hosts develop on diverse types of vegetation (Table 3): xerophilous thickets, and tropical dry, tropical deciduous and coniferous forests, in which insects share habitats with columnar cacti (*Stenocereus* spp.), pirul (*Schinus molle*), huizache (*Acacia* spp.), izotes (*Yucca* spp.) and maguey (*Agave* spp.). They can also be found in pine-oak forests, natural grasslands with or without weed vegetation, living collections and intensive cultures of nopale in monocultures or in association with rain-watered lands, home gardens, orchards, ornamental plants, and fragmented and anthropogenic lands. Likewise, the types of soil included arenosol, vertisol, calcisol, xerosol, leptosol and foezem. The area of study included different climate types, which according to Köppen's climate classification are BSh, BSk, and BW for xerophilous thickets and natural grasslands, and Cfb, Cf, Cwa, Cwb and Cwc for tropical dry, tropical deciduous, temperate coniferous and pine-oak forests (Rzedowski, 1978)(Figure 1b). BSh, BSk, and BW correspond to arid to semiarid dry climates, where precipitation is less than the evapotranspiration potential, i.e., a hydric deficit, and annual

temperatures lie around 18 ºC. Cfb, Cf, Cwa, Cwb, and Cwc indicate warm and humid climates, where the average temperature is 10 °C in the warmest months, and between 0 to 18 °C in the coldest months (Rzedowski 1978).

Table 3. Database for the genus *Dactylopius*: hosts, georeferenced distribution, collector, vegetation type and soil. Data from the fieldwork of this investigation are shown in boldface typeset. Altitude in meters above sea level.

Host[a](Plant portion[b])	Locality[c]	Altitude	Collector[d]/Collect No.	Date	Reference[e]	Vegetation[f])	Soil[g]
			D. ceylonicus				
17i	Chapingo (11)	2334	6 / DTY-RMG 516	09/09/1964	II	*	*
17i	Teotihuacan (11)	2300	8, 23 / DTY-RMG 1155	23/11/1978	II	*	*
19	Tlapacoya (11)	1150	5 / DTY-RMG 1309	25/08/1981	II	*	*
17i	Acahuatlán (14)	1000	3, 8 / 1391	08/08/1961	II	*	*
17i	Actopan (14)	2000	14 / DTY-RMG 1087	14/04/1979	II	*	*
17	Actopan (14)	2000	12, 14/DTY-RMG 1187	14/04/1977	II	*	*
19	Meztitlán(14)	2000	3, 8 / 1390	05/11/1982	II	*	*
17i	Pachuquilla (14)	2400	11 / DTY-RMG 848	12/09/1970	II	*	*
17	Singuilucan (14)	2650	8 / DTY-RMG 1192	13/08/1979	II	*	*
6	Autlán Navarro (15)	950	15 / DTY-RMG 1041	02/07/1975	II	*	*
17i	Cuautla (17)	1300	6 / DTY-RMG 516	08/07/1961	II	*	*

Table 3. Database for the genus *Dactylopius*: hosts, georeferenced distribution, collector, vegetation type and soil. Data from the fieldwork of this investigation are shown in boldface typeset. Altitude in meters above sea level.

Host[a](Plant portion[b])	Locality[c]	Altitude	Collector[c]/Collect No.	Date	Reference[e]	Vegetation[f])	Soil[g]
			D. ceylonicus				
17i	Chapingo (11)	2334	6 / DTY-RMG 516	09/09/1964	II	•	•
17i	Teotihuacan (11)	2300	8, 23 / DTY-RMG 1155	23/11/1978	II	•	•
19	Tlapacoya (11)	1150	5 / DTY-RMG 1309	25/08/1981	II	•	•
17i	Acahuatlán (14)	1000	3, 8 / 1391	08/08/1961	II	•	•
17i	Actopan (14)	2000	14 / DTY-RMG 1087	14/04/1979	II	•	•
17	Actopan (14)	2000	12, 14/DTY-RMG 1187	14/04/1977	II	•	•
19	Meztitlán(14)	2000	3, 8 / 1390	05/11/1982	II	•	•
17i	Pachuquilla (14)	2400	11 / DTY-RMG 848	12/09/1970	II	•	•
17	Singuilucan (14)	2650	8 / DTY-RMG 1192	13/08/1979	II	•	•
6	Autlán Navarro (15)	950	15 / DTY-RMG 1041	02/07/1975	II	•	•
17i	Cuautla (17)	1300	6 / DTY-RMG 516	08/07/1961	II	•	•

19iii	Coixtlahuaca (20)	2100	8 / DTY-RMG 1021	18/07/1973	II	*	*
17i	Ejutla (20)	1450	8 / DTY-RMG 1034	09/02/1975	III	*	*
17i	Nochistlán (20)	2067	9 / DTY-RMG 602	27/05/1966	*	*	*
19	Chacaltianguis (30)	1100	10 / DTY-RMG 747	06/08/1961	II	*	*
6 (C)	Milpa Alta (9)	2459	DTY-ChM 110	28/06/2005	*	3	I
1 (C)	Tepeapulco (14)	2364	DTY-ChM 127	29/06/2005	*	3	III
			D. coceus				
17	Meztitlán (14)	2000	15/ DTY-RMG 1317	10/07/1981	II	*	*
6	Amatengo (20)	1400	8,23/DTY-RMG 1157	24/11/1978	II	*	*
19vi	Amatengo (20)	1400	8, 23 DTY-RMG 1157	24/11/1978	II	*	*
*	Amatengo (20)	1400	22/ RMG173	27/02/1952	II	*	*
17i	Coixtlahuaca (20)	2200	15 / DTY-RMG 1003	08/05/1974	II	*	*
19iv	Coixtlahuaca (20)	2100	22 / DTY-RMG 1016	17/07/1973	II	*	*
19ii	Coixtlahuaca (20)	2100	22 / DTY-RMG 1019	18/02/1975	II	*	*
17	Sn Antonio Abad (20)	2200	21 / DTY-RMG 1133	08/08/1978	II	*	*
19v	Tepelmeme (20)	2100	22 / DTY-RMG 1017	18/02/1975	II	*	*

*	Tunillo (20)	2100	22 / DTY-RMG 752	09/01/1961	II	*	*
8	Zona Mixteca (20)	1250	8 / DTY-RMG 1025	06/02/1975	V	*	*
17	Cacaloapan (21)	1892	16 / DTY-RMG 991	05/04/1973	*	*	*
19	Chacaltianguis (30)	1100	*		II	*	*
6 (C)	Ajusco (9)	2898	DTY-ChM 115	28/06/2005	*	5,12,15	I
15	IB- UNAM (9)	2309	DTY-ChM 117	28/06/2005	*	2	IV
6 (C)	Milpa Alta-Oaxtepec (9)	2457	DTY-ChM 109	28/06/2005	*	3	I , IV
6 (C)	Milpa Alta-Oaxtepec (9)	2166	DTY-ChM 113	28/06/2005	*	3	I , IV
6 (C)	Oaxtepec (9)	2323	DTY-ChM 114	28/06/2005	*	3	I
6 (C)	Nopaltepec (11)	2425	DTY-ChM 101	20/02/2007	*	3,12	I
6 (C)	Sn. Felipe (12)	1776	DTY-ChM 096	04/06/2005	*	1	III
6 (C)	Tetecalita (17)	1203	DTY-ChM 188	20/02/2007	*	3,12	I
6 (C)	Sta Ma Coyotepec (20)	1527	DTY-ChM 187	05/02/2005	*	3,12	I
6 (C)	IIZD (24)	1654	DTY-ChM 087	04/06/2005	*	9, 2	I, IV

13i (C)	IIZD (24)	1654	DTY-ChM 087	04/06/2005	•	9, 2	I, IV
16 (C,T)	IIZD (24)	1654	DTY-ChM 087	04/06/2005	•	9, 2	I, IV
18ii (C)	INIFAP (24)	1663	DTY-ChM 067	03/06/2005	•	2	I , III
			D. confusus				
17	cerro Chilicote (6)	1200	•	•	II	•	•
17	Distrito Federal (9)	2376	•	•	II	•	•
17i	Tacuba (9)	1700	8 / DTY-RMG 323	10/09/1953	II	•	•
17ii	Dolores Hidalgo (12)	1950	17 / DTY-RMG 554	04/10/1967	II	•	•
•	Río Papagayo (13)	100	•	•	II	•	•
17	Taxco (13)	1600	•	•	II	•	•
19	Ihuatlán (14)	2100	22 / DTY-RMG 1018	17/07/1973	II	•	•
•	Cuautla (17)	1300	•	•	II	•	•
•	Cuernavaca (17)	1450	•	•	II	•	•
19iii	Ihuatlán (20)	2100	22/ DTY-RMG 1015		II	•	•
•	Petlalcingo (20)	1367	•	•	II	•	•
17i	Tehuantepec (20)	1600	8 / DTY-RMG 1158	25/12/1978	II	•	•

17i	Tepelmeme (20)	2100	*	*	II	*	*
*	Cacaloapan (21)	1892	*	*	II	*	*
17i	Yautepec (21)	1200	*	*	II	*	*
17i	SLP-Matehuala (24)	1200	8 / DTY-RMG 1170	30/03/1979	*	*	*
6 (C,T)	Milpa Alta (9)	2469	DTY-ChM 108	28/06/2005	*	3	I
6 (C)	Cd Sahagún (14)	2457	DTY-ChM 128	29/06/2005	*	3	I , III
6 (C)	Tepeapulco (14)	2364	DTY-ChM 125	29/06/2005	*	8	III
6 (C,T)	Lagos de Moreno (15)	1803	DTY-ChM 150	09/04/2006	*	9	I , IV
7 (C,T)	Lagos de Moreno (15)	1689	1DTY-ChM 148	09/04/2006	*	9	I , IV
9 (C,T)	Lagos de Moreno (15)	1689	DTY-ChM 148	09/04/2006	*	9	I , IV
10 (C,T)	Lagos de Moreno (15)	1828	DTY-ChM 152	09/04/2006	*	9	I , IV
2 (C)	Ojuelos (15)	2167	DTY-ChM 166	10/04/2006	*	9,6,14,16	III
14 (C)	Ojuelos (15)	2166	DTY-ChM 167	10/04/2006	*	9	III
14 (C)	Ojuelos (15)	2167	DTY-ChM 168	10/04/2006	*	9	III
15 (C)	Ojuelos (15)	2166	DTY-ChM 169	10/04/2006	*	9	III
6 (C)	Oaxtepec (17)	2323	DTY-ChM 114	28/06/2005	*	5,12,15	I

6 (C)	Zayaleta (21)	2375	DTY-ChM 138	30/06/2005	*	3	I , III
17i (C)	Perote (30)	2367	DTY-ChM 142	30/06/2005	*	3	III
	Sn. Antonio Limón						
17i (C)	(30)	2547	DYTChM 138	30/06/2005	*	3	III, V
12 (C)	Noria de Ángeles (32)	2196	DTY-ChM 176	11/04/2006	*	4,10	III
15 (C)	Villa Hidalgo (32)	2196	DTY-ChM 179	11/04/2006	*	4,10	III
8 (C)	Villa Hidalgo (32)	2196	DTY-ChM 179	11/04/2006	*	4,10	III
			D. confusus Biotype 1				
6 (C)	Tepeapulco (14)	2364	DTY-ChM 127	29/06/2005	*	3	III
6 (C)	Oaxtepec (17)	2323	DTY-ChM 114	28/06/2005	*	5,12,15	I
19 vii (C)	Zayaleta (21)	2375	DTY-ChM 138	30/06/2005	*	7	III
6 (C)	IIZD (24)	1654	DTY-ChM 087	04/06/2005	*	9,2	I, IV
17i (C)	Huamantla (29)	2773	DTY-ChM 133	30/06/2005	*	3	I , III
			D. opuntiae				
17i	Aguascalientes (1)	1900	*	*	II	*	*
17i	La Paz (2)	25	13 / DTY-RMG 805	Undated	II	*	*

17	La Palma Dto. Sur (3)	250	•	•	II	•	•
17	Sn Cristóbal Casas (3)	350	18 / DTY-RMG 464	21/04/1954	II	•	•
17i	Sn Cristóbal Casas (3)	350	18 / DTY-RMG 464	21/04/1954	II	•	•
19	Distrito Federal (9)	2300	22 / DTY-RMG 159	14/02/1952	II	•	•
•	Contreras (9)	1650	•	•	II	•	•
19	Sn. Ángel (9)	2600	2,8 / DTY-RMG 50	28/02/1951	II	•	•
17i	Tlalpan (9)	2600	20 / DTY-RMG 1038	18/04/1975	II	•	•
17	Xochimilco (9)	2245	8 / DTY-RMG-438	24/04/1960	II	•	•
17i	Durango (10)	1900	•	•	II	•	•
•	Acolman (11)	2300	•	•	II	•	•
17	Chapingo (11)	2334	1 / DTY-RMG 461	03/06/1954	II	•	•
•	Otumba (11)	2400	•	•	II	•	•
19	Teotihuacan (11)	2300	22 / DTY-RMG 153	16/02/1952	II	•	•
•	Tepenahuac (11)	2424	•	•	II	•	•
•	Tepozotlán (11)	1600	•	•	II	•	•
19	Texcoco (11)	2300	8 / DTY-RMG 607	18/08/1966	II	•	•

17i	Tlalmanalco (11)	2400	*	*	II	*	*
17i	Toluca (11)	2678	13 / DTY-RMG 805	Undated	*	*	*
13	Dextiu (13)	2000	8,19 / DTY-RMG 1425	18/02/1983	II	*	*
19	Río Papagayo (13)	100	2 / DTY-RMG 165	20/02/1952	II	*	*
17i	Arenal (14)	2050	8,19 / DTY-RMG 1079	29/06/1977	II	*	*
13	Atocpan (14)	2000	8 , 19 / 1421	16/02/1983	IV	*	*
17	Atocpan (14)	2000	8, 19 / 1426	18/02/1983	IV	*	*
13	Atocpan (14)	2000	8,19 / DTY-RMG 1421	18/02/1983	II	*	*
17i	Meztitlán (14)	2000	8,3 / 1392	05/11/1982	II	*	*
17	Pachuca (14)	2419	8 / DTY-RMG 1124	17/02/1978	*	*	*
17i	Pachuca (14)	2000	8 / DTY-RMG 671	05/05/1967	IV	*	*
17i	Pachuca (14)	2000	8 / DTY-RMG 671	05/05/1967	II	*	*
17	Atenquique (15)	2000	8 / DTY-RMG 962	08/07/1972	IV	*	*
17i	Ocotlán (15)	2200	*	*	I	*	*
3	Morelia (16)	1900	*	*	II	*	*
17i	Acahuatlán (18)	1000	4 / DTY-RMG 169	29/02/1952	II	*	*

17i	Sabinas Hidalgo (19)	300	•	•	II	•	•
•	Amatengo (20)	1400	3, 8 / 1392	•	II	•	•
16	Coixtlahuaca (20)	2100	8, 23 / DTY-RMG 1156	24/02/1978	II	•	•
19	Coixtlahuaca (20)	2100	22 /DTY-RMG 1022	19/07/1973	II	•	•
19iii	Coixtlahuaca (20)	2100	22 / DTY-RMG 1015	17/06/1973	II	•	•
17i (C)	Ejutla (20)	1450	15 /DTY-RMG 1004	08/05/1974	II	•	•
16i	Ejutla (20)	1450	15 / DTY-RMG 1004	08/04/1974	II	•	•
17	Nochistlán (20)	2067	8 / DTY-RMG 1030	07/02/1975	II	•	•
17i	Oaxaca (20)	1600	7 / DTY-RMG 1024	02/11/1974	II	•	•
17	Sinaxtla (20)	2076	8 / DTY-RMG 1028	07/02/1975	•	•	•
17	Tehuantepec (20)	1600	8 / DTY-RMG 1031	08/02/1975	II	•	•
17ii	Tehuantepec(20)	1600	8 / DTY-RMG 1031	08/02/1975	II	•	•
3	Tunillo (20)	2100	8 / DTY-RMG 1032	08/02/1975	II	•	•
17	Calpan (21)	300	8 / DTY-RMG 736	20/08/1967	•	•	•
17	Xalitzintla (21)	2601	8 / DTY-RMG 684	28/02/1951	•	•	•
17	Xalitzintla (21)	2601	8 / DTY-RMG 684	17/06/1967	•	•	•

17i	Xalitzintla (21)	2601	8/ DTY-RMG 684	17/06/1967	*	*	*
3	Ciudad Victoria (28)	350	*	*	II	*	*
6 (C)	Aguascalientes (1)	2196	DTY-ChM 170	10/04/2006	*	3,12	I
8 (C)	Aguascalientes (1)	2196	DTY-ChM 172	10/04/2006	*	3,12	I
16 (C)	IB-UNAM (9)	2309	DTY-ChM 117	28/06/2005	*	2	IV
6 (C)	Milpa Alta (9)	2469	DTY-ChM 030	05/05/2005	*	3	I
6 (C)	Milpa Alta (9)	2845	DTY-ChM 115	28/05/2005	*	3	I, IV
17i (C)	Acolman (11)	2260	DTY-ChM 118	29/06/2005	*	3	I
6 (C)	Las Pirámides (11)	2304	DTY-ChM 124	29/06/2005	*	3	I
6 (C,T)	Las Pirámides (11)	2364	DTY-ChM 125	29/06/2005	*	3	I
15 (C,T)	Las Pirámides (11)	2439	DTY-ChM 119	29/06/2005	*	15	I
17i (C)	Las Pirámides (11)	2439	DTY-ChM 119	29/06/2005	*	15	I
6 (C,T)	Otumba (11)	2293	DTY-ChM 126	29/06/2005	*	3	III
15 (C)	Teotihuacan (11)	2300	DYTChM 121	29/06/2005	*	7,11,13	I
17i (C)	Teotihuacan (11)	2300	DTY-ChM 121	29/06/2005	*	7,11,13	I
6 (C)	San Felipe (12)	1776	DTY-ChM 095	04/06/2005	*	4	III

6 (C)	San Felipe (12)	1776	DTY-ChM 095	04/06/2005	*	1	III
6 (C)	San Felipe (12)	1776	DTY-ChM 096	04/06/2005	*	3	I
6 (C)	Silao (12)	1809	DTY-ChM 099	04/06/2005	*	15	I
6 (C)	Silao (12)	1809	DTY-ChM 100	05/06/2005	*	1	IV
9 (C,T)	Lagos de Moreno (15)	1924	DTY-ChM 153	09/04/2006	*	6	II, III
9 (C,T)	Lagos de Moreno (15)	1950	DTY-ChM 154	09/04/2006	*	6	II, III
11 (C,T)	Lagos de Moreno (15)	1950	DTY-ChM 155	09/04/2006	*	6	II, III
8 (C,T)	Ojuelos (15)	2196	DTY-ChM 157	10/04/2006	*	6,14,16	III, IV
10 (C,T)	Ojuelos (15)	2196	DTY-ChM 158	10/04/2006	*	6,14,16	III, IV
13 (C,T)	Ojuelos (15)	2196	DTY-ChM 159	10/04/2006	*	6,14,16	III, IV
13 (C,T)	Ojuelos (15)	2184	DTY-ChM 160	10/04/2006	*	6,14,16	III, IV
15 (C,T)	Ojuelos (15)	2196	DTY-ChM 157	10/04/2006	*	6,14,16	III, IV
17i (C,T)	Ojuelos (15)	2196	DTY-ChM 158	10/04/2006	*	6,14,16	III, IV
6 (C)	Morelia (16)	1964	DTY-ChM 105	27/06/2005	*	3	I
6 (C)	Morelia (16)	1968	DTY-ChM 106	27/06/2005	*	3	I
6 (C)	Las Casitas (21)	2369	DTY-ChM 135	30/06/2005	*	3	I, III

13i (T)	Las Casitas (21)	2369	DTY-ChM 135	30/06/2005	*	3	I, III
17i (C)	San Salvador (21)	750	DTY-ChM 147	30/06/2005	*	12	III
17i (C)	San Salvador (21)	750	DTY-ChM 148	30/06/2005	*	12,3	III
6 (C)	Tlacaloapan (21)	772	DTY-ChM 145	30/06/2005	*	3	II, III
13i (C)	Tlacaloapan (21)	772	DTY-ChM 146	30/06/2005	*	3	II
6 (C)	IIZD (24)	1654	DTY-ChM 087	04/06/2005	*	3	III
13i (C,F)	IIZD (24)	1654	DTY-ChM 087	04/06/2005	*	2	III
15 (C)	IIZD (24)	1654	DTY-ChM 087	04/06/2005	*	9,2	I, IV
15i (C)	IIZD (24)	1654	DTY-ChM 087	04/06/2005	*	2	III
13ii (C)	INIFAP (24)	1663	DTY-ChM 064	03/06/2005	*	2	I, III
13iii (C,F)	INIFAP (24)	1663	DTY-ChM 065	03/06/2005	*	2	I, III
18i (C)	INIFAP (24)	1663	DTY-ChM 066	03/06/2005	*	2	I, III
18iii (C)	INIFAP (24)	1663	DTY-ChM 068	03/06/2005	*	2	I, III
18iv (C)	INIFAP (24)	1663	DTY-ChM 069	03/06/2005	*	2	I, III
18v (C)	INIFAP (24)	1663	DTY-ChM 070	03/06/2005	*	2	I, III
18vi (C)	INIFAP (24)	1663	DTY-ChM 072	03/06/2005	*	2	I, III

18vi (C)	INIFAP (24)	1663	DTY-ChM 073	03/06/2005	*	2	I, III
18vii (F)	INIFAP (24)	1663	DTY-ChM 071	03/06/2005	*	2	I, III
18viii (C)	INIFAP (24)	1663	DTY-ChM 074	03/06/2005	*	2	I, III
18ix (C)	INIFAP (24)	1663	DTY-ChM 075	03/06/2005	*	2	I, III
18x (C,F)	INIFAP (24)	1663	DTY-ChM 076	03/06/2005	*	2	I, III
18xi (C)	INIFAP (24)	1663	DTY-ChM 077	03/06/2005	*	2	I, III
18xii (C)	INIFAP (24)	1663	DTY-ChM 078	03/06/2005	*	2	I, III
18xiii (C,T)	INIFAP (24)	1663	DTY-ChM 079	03/06/2005	*	2	I, III
18xiv (C,F)	INIFAP (24)	1663	DTY-ChM 080	03/06/2005	*	2	I, III
18xv (F)	INIFAP (24)	1663	DTY-ChM 081	03/06/2005	*	2	I, III
18xvi (C,F)	INIFAP (24)	1663	DTY-ChM 081	03/06/2005	*	2	I, III
8 (C)	Las tortugas (24)	1653	DTY-ChM 009	05/09/2005	*	9,2	III, IV
15 (C)	Las tortugas (24)	1653	DTY-ChM 009	05/09/2005	*	9,2	III, IV
6 (C,T)	Calpulalpan (29)	2488	DTY-ChM 131	29/06/2005	*	3	I, III
15 (C,T)	Calpulalpan (29)	2460	DTY-ChM 129	29/06/2005	*	3	I, III
16 (C,T)	Calpulalpan (29)	2587	DTY-ChM 130	29/06/2005	*	3	I, III

17i (C)	Perote (30)	2367	DTY-ChM 142	30/06/2005	*	3	III
17i (C)	Totalco (30)	2547	DTY-ChM 138	30/06/2005	*	7	II, III
10 (C)	Las Pilas (32)	2196	DTY-ChM 176	11/04/2006	*	16,10	III, IV
15 (C)	Las Pilas (32)	2196	DTY-ChM 176	11/04/2006	*	10,16	III, IV
15 (C)	Pánfilo Nateras (32)	2196	DTY-ChM 180	11/04/2006	*	4,6,10,16	I, II
12 (C)	Villa González (32)	2196	DTY-ChM 178	11/04/2006	*	4,6,10,16	I, II
17i (C)	Villa González (32)	2196	DTY-ChM 178	11/04/2006	*	4,6,10,16	I, II
12 (C)	Zacatecas (32)	2196	DTY-ChM 179	11/04/2006	*	10,16	III, IV
			D. opuntiae biotype 1				
6 (C,T)	Milpa Alta (9)	2469	DTY-ChM 107	28/06/2005	*	3	I
5 (C)	Sn Fco. Rincón (12)	1776	DTY-ChM 002	04/09/2005	*	4,6,7,10,16	III
17i (C)	Sn. Felipe (12)	2092	DTY-ChM 098	04/06/2005	*	3	I
6 (C)	Valle Santiago (12)	1732	DTY-ChM 062	03/06/2005	*	1	I
2 (C)	Ojuelos (15)	2167	DTY-ChM 168	10/04/2006	*	9	III
6 (C,T)	Morelia (16)	2368	DTY-ChM 028	05/02/2005	*	15	I
4i (C)	INIFAP (24)	1663	DTY-ChM 067	03/06/2005	*	2	I

4ii (F)	INIFAP (24)	1663	DTY-ChM 067	03/06/2005	•	2	I
6i (C)	INIFAP (24)	1663	DTY-ChM 067	03/06/2005	•	2	I
15ii (C)	INIFAP (24)	1663	DTY-ChM 067	03/06/2005	•	2	I
17i (C,T)	Calpulalpan (29)	2587	DTY-ChM 130	29/06/2005	•	3	I, III
6 (C)	Huamantla (29)	2773	DTY-ChM 133	30/06/2005	•	3	I, III
17i (C)	Huamantla (29)	2773	DTY-ChM 133	30/06/2005	•	3	I, III
			D. tomentosus				
11	Ensenada (3)	0	•	•	II	•	•
17i	Los Cabos (3)	100	•	•	II	•	•
17i	Torreón (7)	1300	•	•	IV	•	•
17i	Distrito Federal (9)	2374	•	•	II	•	•
17i	Silao (12)	1800	•	•	II	•	•
20	Cadereyta (19)	300	•	•	II	•	•
17i	Ihuatlán (20)	2100	•	•	II	•	•
19iii	Nochistlán (20)	2067	•	•	II	•	•
17i	Alvarado (30)	0	•	•	II	•	•

17i	Zacatecas (32)	2500	*	*	II	*	*
5 (C)	San Francisco (12)	1776	DTY-ChM 186	11/04/2006	*	4,6,7,10,16	III
2 (C)	Tulancingo (14)	2000	DTY-ChM 190	10/11/2007	*	9	II

[a] 1 = *C. imbricata*, 2 = *C. tunicata*, 3 = *Opuntia* (*Nopalea*), 4 = *O. albicarpa* (i = Blanca 23, ii = Reyna 155), 5 = *O. atropes*, 6 = *O. ficus-indica* (i = Liso 48), 7 = *O. fuliginosa*, 8 = *O. hyptiacantha*, 9 = *O. jaliscana*, 10 = *O. joconostle*, 11 = *O. megacantha*, 12 = *O. phaeacantha*, 13 = *O. robusta* (i = var. *larrey* Weber, ii = Camuesa, iii = Camuesa 58), 14 = *O. spinulifera*, 15 = *O. streptacantha* (i = var. *aguirreana*, ii = Charola), 16 = *O. tomentosa* (i = var. *O. hernandezii*), 17 = *Opuntia* (i = *Opuntia* spp., ii = *Opuntia* nopal), 18 = Variants (i = Amarilla chapeada, ii = Anaranjada montecillos, iii = Bola de maza 77, iv = Calabazona 50, v = Cascaron 75, vi =Chapeada 21, vii =Cristalina 138, viii = L Coloradito 49, ix = Line 116, x = Mexicana 53, xi = Mieluda 10, xii = Pachón blanco, xiii = Pepino 136, xiv = Redonda 61, xv = Sandio Ñ, xvi =Virginia 66), 19 = nopal (i = prickly pear, ii = Castilla, iii = yellow prickly pear, iv = artona prickly pear, v = white prickly pear, vi = red prickly pear, vii = duraznillo), 20 = Cactus and * = not specified; [b] C = cladode, F = fruit (prickly pear), T = trunk; [c] see Table 1; [d] 1 = Barnes, 2 = Barrera, 3 = Butze, 4 = Estrada-Leal, 5 = Gallardo, 6 = García-Mayoral, 7 = Gutiérrez, 8 = MacGregor, 9 = Majoral, 10 = Mendoza, 11 = Mitastein, 12 = Moreno, 13 = Ortega, 14 = Pino, 15 = Piña, 16 = Ramírez, 17 = Rangel, 18 = Robello, 19 = Sampedro, 20 = Scheinvar, 21 = Torres, 22 = Vázquez, 23 = Williams. Boldface typeset = collects presented in this document, collector Carla K. Chávez-Moreno; [e] Reference: I = González (2001); II = MacGregor & Sampedro (1984); III = Pérez Guerra & Kosztarab (1992); IV = Portillo & Vigueras-Guzmán (2003a); V = Portillo & Vigueras-Guzmán (2003b) & * = unpublished (CNI-IB.UNAM); [f] vegetation 1 = living fence , 2 = collections, 3 = commercial plantation, 4 = *Cylindropuntia* spp., 5 =

orchard, 6 = huizache (*Acacia* spp.), 7 = maguey (*Agave* spp.), 8 = weed vegetation, 9 = xerophitic scrubs; 10 = *Opuntia* spp.; 11 = columnar cacti (*Stenocereus* spp.), 12 = natural grassland; 13 = pine and oak forest, 14 = pirul (*Schinus molle*), 15 = ornamentation, 16 =izotes (*Yucca* spp.) and * = not specified; [g] I = feozems, II = vertisol, III = xerosol and arenosol, IV = regosol, V = calcisol, VI leptosol and * = others.


30°
25°
20°
15°
115°
110°
105
100°
95°
90°
N
400 km
D. ceylonicus
D. coccus
D. confusus
D. opuntiae
D. tomentosus

(a)

(b)

Figure 1 Records of distribution in Mexico of the genera *Dactylopius* (a) and *Opuntia* (b) compiled from Britton & Rose (1963), Bravo-Hollis & Sánchez-Mejorada (1978), González *et al* (2001), Guzmán *et al* (2003) and consulting the herbaria MEXU and IBUG. Insect registers from Mann (1969), De Lotto (1974), Piña (1977), MacGregor & Sampedro (1984), Pérez-Guerra & Kosztarab (1992), Portillo & Vigueras (2003a, 2003b) and CNI-IB-UNAM. Nomenclature based on Anderson (2001). Black figures correspond to known georeferenced coordinates; approximated localization data are shown in gray.

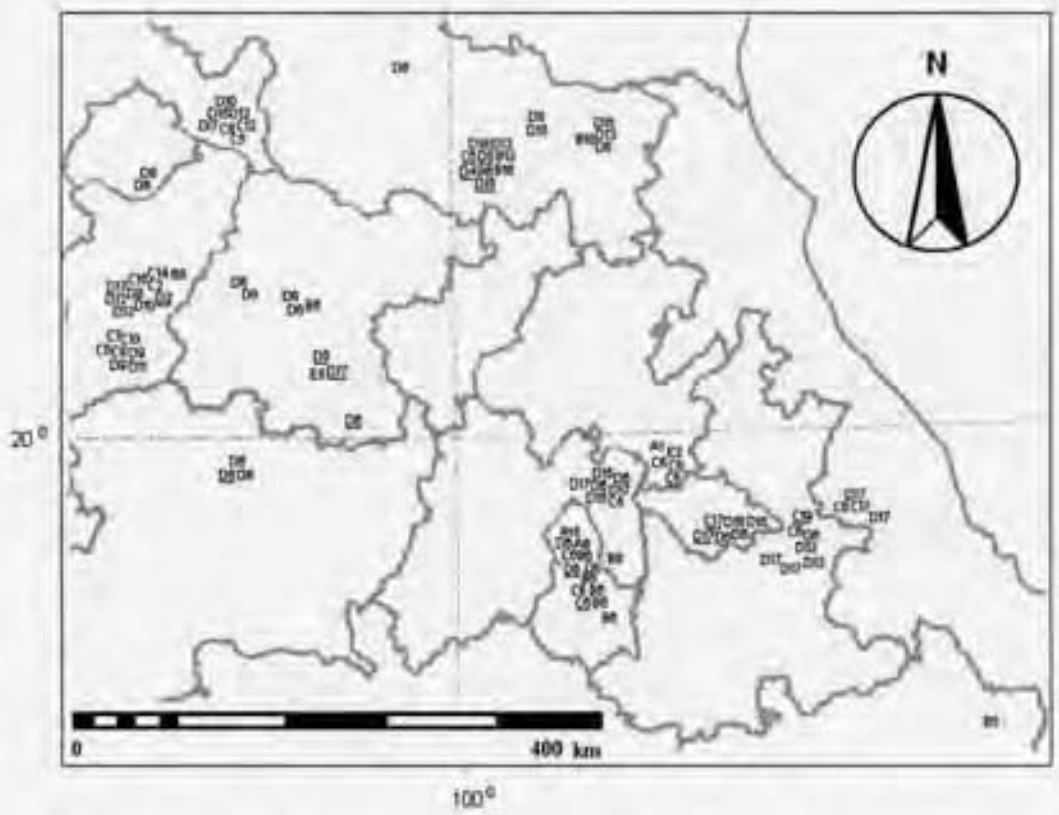

Figure 2. Distribution of collects of *Dactylopius* and their hosts *Opuntia*, *Nopalea* and *Cylindropuntia* within the studied area. Letters correspond to insects: A = *D. ceylonicus*, B = *D. coccus*, C = *D. confusus*, C = *D. confusus* biotype 1, D = *D. opuntiae*, D = *D. opuntiae* biotype 1 and E = *D. tomentosus*. Numbers correspond to hosts shown in Table 3.

Species associated with the genus *Dactylopius*. In this work, the recorded species of *Dactylopius* were found sharing hosts with ants (Hymenoptera: Formicidae), the lady beetles *Chilocorus* sp. and *Hyperaspis* sp. (Coleoptera: Coccinellidae), spiders (Araneae), the weaver worm *Laetilia coccidivora* (Lepidoptera: Pyralidae), the needle worm *Symherobius* sp. (Neuroptera: Hemerobidae) and other coleopters.

Conclusions

The distribution of *Dactylopius* within the studied area is continuous, maintains correspondence with the host plants and is broader than previously known. The studied area was located between 98 to 104º northern latitude and 18 to 23º eastern longitude, comprised the states of Aguascalientes, Mexico City, Guanajuato, Hidalgo, Jalisco, Estado de México, Michoacán, Morelos, Oaxaca, Puebla, Querétaro, San Luis Potosi, Tlaxcala, Veracruz and Zacatecas, and extended mainly from the northern and central plateau to the southeast of Mexico. These zones are characterized by xerophilous thickets, and temperate coniferous and pine-oak forests, with a wide variety of soils (arenosol, vertisol, calcisol, xerosol, regosol, leptosol and feozems), climates ranging from arid to semiarid dry to warm and humid, within the altitude range of 0 to 2845 m. This work provides new georeferenced records about the five species of *Dactylopius* and their hosts not reported previously, further describing the distribution areas of the insects. The presence of the species of *Dactylopius* with different morphological characteristics cohabiting in the same locality on different portions of the same host was reported here for the first time. Insects with morphological characteristics of *D. confusus* and *D. opuntiae* blended with characteristics of *D. salmianus*, named here as *D. confusus* biotype 1 and *D. opuntiae* biotype 1, respectively, were identified in this work for the first time. This suggests the presence of

new species not yet studied or the possibility of interspecific hybridization between the identified species.

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III. HPLC metabolic profiling of *Dactylopius* (Hemiptera: Dactylopiidae) species pigments by geographical origin and hosts using multivariate data analysis

Enviado a

Journal of Biochemical, Systematics and Ecology

HPLC metabolic profiling of *Dactylopius* (Hemiptera: Dactylopiidae) species pigments by geographical origin and hosts using multivariate data analysis

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Abstract

The genus *Dactylopius* includes the group of insects historically used in Mexico as a source of the natural red, the cochineal color. Five species of *Dactylopius* collected in thirteen states of Mexico and two provinces of Argentina were analyzed using high-performance liquid chromatography (HPLC) with a photodiode array detector. This analysis allowed each species to be identified on the basis of differences in their metabolic profiles. Cluster analysis (CA) and principal component analysis (PCA) differentiated the species by localities and host plant. Multivariate analyses techniques were complementary and confirmed the grouping of all analyzed *Dactylopius* samples. For all species, the carminic acid, identified by reference to a commercial sample, was the main compound occurring in significant amounts, making all five species potential sources of colorant. In addition, each species could be differentiated by the presence of other minor compounds.

Keywords: *Dactylopius*, cochineal, carminic acid, HPLC, cacti, *Opuntia*, multivariate analysis

1. Introduction

In the last three decades, worldwide demand for natural colorants has significantly increased. *Dactylopius* dye has been used for centuries in textiles, traditional medicine, and mural paintings and more recently in cosmetics, pharmaceuticals, and foods (MacGregor, 1976). The genus *Dactylopius* (Hemiptera: Dactylopiidae) includes nine species (De Lotto, 1974), five of them occurrings in Mexico: *D. ceylonicus* Green, *D. coccus* Costa, *D. confusus* Cockerell, *D. opuntiae* Cockerell, and *D. tomentosus* Lamarck (Mann, 1969; De Lotto, 1974; MacGregor and Sampedro, 1984; Pérez-Guerra and Kosztarab, 1992; Chávez-Moreno et al., 2009). The others have mainly occur in South America (De Lotto, 1974).

Dactylopius insects, "cochineals", are 1 to 6 mm in length, and their colorful red bodies are covered with a conspicuous white, waxy secretion (Mann, 1969). The insects feed exclusively on cacti, mainly of the *Opuntia* genus (Mann, 1969; De Lotto, 1974; MacGregor and Sampedro, 1984; Pérez-Guerra and Kosztarab, 1992; Portillo and Vigueras-Guzmán, 2003; Chávez-Moreno et al., 2009), and possess very limited morphological characteristics; wich makes their taxonomic identification difficult. Ten to twenty-five percent of the weight of dried female adults is a water-soluble colorant; mainly composed by the carminic acid ($C_{22}H_{21}O_{13}$) is the main component (Venkataraman and Rama Rao, 1972; Yamada et al., 1993). During the last decade, this coloring material has been studied for its role in the immune mechanism of *D. coccus* (Hernández-Hernández et al., 2003). Studies of this colorant could potentially be extended to antibacterial, antiviral,

and insecticidal applications (Palma de la Cruz, 2008; Food and Drug Administration, 2009). *D. coccus* is the only species used for commercial production (MacGregor, 1976), the use of other species for the same purpose being limited because of its wild condition and smaller size. Analysis of wild species is necessary to know whether they could serve as alternative or additional sources of the colorant and its derivatives.

High-performance liquid chromatography (HPLC), combined with mass spectrometry (MS) and nuclear magnetic resonance (NMR), has been used for the analysis of natural colorants derived from hydroxyanthraquinones (Venkataraman and Rama Rao, 1972) and has been effectively used to elucidate their origin, constituents, and structural characteristics. Further, it has been used to identify carminic acid in food (Yamada et al., 1993; Lancaster and Laurence, 1996) and antique textiles (Wouters, 1985; Wouters and Verheeken, 1989), to optimize conditions for the extraction of pigments in cochineal insects (González et al., 2002), and to determine their quality (Méndez et al., 2004; Maier, 2004). Also, it has been used to differentiate the geographical origin of the *D. coccus* species by combining statistical techniques such as cluster analysis and principal components analysis and applying these tools to HPLC data (Méndez et al., 2004).

The main goal of this investigation was to analyze variations in the HPLC metabolic profiling of *Dactylopius* colorant content, using multivariate data analysis techniques, for the five major, abundant Mexican species and then compare these results with those of one wild South American species common to Mexico.

2. Materials and methods

2.1 Insect and plant material

Adult female specimens were collected from 2005 to 2007 from different localities and hosts in Mexico and Argentina. The insects were identified by their morphological characteristics and then compared with Hemiptera voucher insects housed in the Colección Nacional de Insectos (National Collection of Insects) of the Instituto de Biología, Universidad Nacional Autónoma de México (CNI-IB-UNAM).Voucher specimens from the 2005-2007 collections were deposited under the code DTY-ChM-collection numbers at the CNI-IB-UNAM. The specimens, hosts, localities, and codes are shown in Table 1.

Table 1. Insect, plant material, collection localities and identification codes.

Specimen	Host	Locality	DTY-ChM-
D. coccus	*O. ficus-indica (L.) Mill.*	Oaxaca	101
D. ceylonicus	*O. ficus-indica* (L.) Mill.	Mexico City	110
	Opuntia spp.	Salta[(a)]	201
		Jujuy[(a)]	202
D. confusus	*O. ficus-indica* (L.) Mill.	Hidalgo	125, 128
		San Luis Potosí	087
	O. fuliginosa Griffiths	Jalisco	148a, 148b
	O. joconostle F.A.C. Weber	México City	152
	O. spinulifera Salm-Dyck		167, 168
	O. streptacantha Lem.		108a, 108b

	O. ficus-indica (L.) Mill.	Puebla	138a, 138b
	Opuntia spp.		138c
	Opuntia spp.	Veracruz	142, 143
D. opuntiae	*O. ficus-indica* (L.) Mill.	Aguascalientes	170
		Hidalgo	125
		Michoacán	106
		Tlaxcala	131
	O. ficus-indica (L.) Mill.	Mexico City	115a, 115b
	O. tomentosa Salm-Dyck	Estado de México	117
	O. ficus-indica (L.) Mill.	Guanajuato	095
	Opuntia spp.		098a, 098b
	O. ficus-indica (L.) Mill.	Jalisco	152
	O. hyptiacantha F.A.C. Weber		157
	O. jaliscana Bravo		153, 154
	O. megacantha Salm-Dyck		155
	O. robusta Wendland		159
	O. albicarpa Sheinvar	San Luis Potosí	067
	O. streptacantha		087
	Opuntia spp.		066
	O. phaeacantha Engelmann	Zacatecas	178
D. tomentosus	*Cylindropuntia tunicata* Haworth, Griffiths	Hidalgo	190

[(a)]Provinces of Argentina.

Dactylopius host plant materials were identified by comparing them with vouchers housed at MEXU Herbarium, UNAM and taxonomical information described in keys to the Opuntioideae subfamily (Anderson 2001). Insects were preserved in a methanol:water (7:3) solution until used, then cleaned by removing their external waxy secretion and dried.

2.2 Extraction

All samples were dried in an oven at 89 ^{o}C (Ohaus MB45, Pine Brook, NJ) before to extraction. Dry insects (0.125 g) were ground and mixed at room temperature with absolute methanol (2 mL). Fifty milliliters of 5% orthophosphoric acid was added to the extract solution (1.709 g), mixed in a sealed vessel and boiled for 30 min, then diluted to a volume of 200 mL with water and filtered through a nylon membrane (Waters, Milford MA, USA) 13 mm in diameter with a pore size of 0.45 µm (Méndez et al. 2004). All extractions were carried out in triplicate.

2.3 Generation of HPLC profiles

The instrumentation used for HPLC analysis consisted of a Perkin Elmer 250 multisolvent delivery system equipped with a dual-wavelength UV absorbance detector (Waters 2487) with a UV detection range of 250-500 nm. Control of the equipment, data acquisition, processing, and management of chromatographic information were performed by a PE Nelson model 220 interfase. Samples were analyzed in triplicate using a 300 mm × 5 mm i.d., 10 µm, 60 Å pore diameter Spherosorb RP C_{18} column (Waters, Milford MA, USA). A linear gradient for elution was performed as previously described (Méndez et al., 2004): water:methanol:5% orthophosporic acid (5:4:1) to methanol:5% orthophosphoric acid (9:1) over 30 min, using a flow rate of 0.5 mL/min and sample injection of 10 µL (concentration: 1 mg/10 mL). The amount of carminic acid (CAc) in each HPLC profile

was determined and quantified by reference to a calibration curve within the concentration range of 1 to 23 µg/mL. Carminic acid, flavokermesic acid (FkAc), and kermesic acid (KAc) peaks were identified by comparing their retention times (t_R) with previously reported values (Méndez et al., 2004).

2.4 Statistical analysis

Cluster analysis (CA) and principal component analysis (PCA) were carried out with the Statistica 6.0 software (StatSoft Inc., Tulsa, OK). Two CA analyses were carried out. The first technique was performed using the chromatographic profile of eluted compounds, i.e. peaks; each peak was treated as a chemical characteristic and its presence or absence, 0 or 1, respectively, was taken as a measure of difference or similarity among species (Méndez et al., 2004; Son et al., 2008; Cardoso-Taketa et al., 2008). The chromatographic profiles of all the species were compared and grouped by their similarities under the principle of parsimony (Méndez et al., 2004; Son et al., 2008). The second CA was performed by building a joining-tree diagram using the HPLC quantitative analysis data of the insect material. The Euclidean distance was used as a method to measure the simplest distance among clusters, and the clustering algorithm of the unweighted pair group method with arithmetic mean (UPGMA) was used to indicate the distance between groups. From this analysis, factors 1 and 2, PC1 and PC2, eigenvalues of the correlation matrix, direct and cumulative values, and factors of the coordinate variables were determined.

3. Results and discussion

3.1 HPLC metabolic profiling

Nineteen peaks were detected by HPLC, corresponding to CAc, FkAc, KAc, and sixteen unknowns (Table 2). The presence or absence of a given peak distinguished each species profile.

Table 2. HPLC profile (qualitative analysis), of five *Dactylopius* species (CAc = carminic acid, FkAc = flavokermesic acid, KAc = kermesic acid, 0 = absence, 1 = presence, bold = unique compound), 275nm.

Peak number	t_R (min)[a]	*D. ceylonicus*	*D. coccus*	*D. confusus*	*D. opuntiae*	*D. tomentosus*
1	0.678	1	1	1	1	1
CAc	1.84	1	1	1	1	1
3	3.29	1	0	1	1	0
4	4.58	0	0	1	1	0
5	5.36	0	1	1	1	0
6	6.05	0	0	**1**	0	0
7	8.31	0	0	0	**1**	0
8	9.73	0	0	0	**1**	0
9	10.6	0	0	0	**1**	0
10	12.5	1	0	1	1	0
11	13.4	0	0	0	**1**	0
12	14.3	0	1	1	1	0
FkAc	15.4	1	1	1	1	1
KAc	16.6	1	1	1	1	1
15	17.5	1	1	1	1	1
16	18.8	1	1	1	1	1
17	19.3	0	1	0	1	0
18	21.9	0	**1**	0	0	0
19	22.1	0	**1**	0	0	0

[a] t_R = retention time.

D. opuntiae had the most complex profile with sixteen peaks, four of which were unique: t_R of 8.31 min (peak 7), 9.73 min (peak 8), 10.6 min (peak 9), and 13.4 min (peak 11). *D. confusus* showed twelve peaks, one of them was unique with t_R of 6.05 min (peak 6). Eleven peaks were detected for *D. coccus*, two of them were unique with t_R of 21.9 min (peak 18) and 22.1 min (peak 19). Eight peaks were observed for *D. ceylonicus*, with no difference between the Mexican and the Argentinean specimens. Six peaks were recorded for *D. tomentosus*, all of which were common to the genus with t_R of 0.678 min (peak 1), 1.84 min (CAc), 15.4 min (FkAc), 16.6 min (KAc), 17.5 min (peak 15), and 18.8 min (peak 16).

Although CAc was the most abundant compound in *Dactylopius*, its concentration differed among the species, with *D. coccus* having the largest amount, followed by *D. tomentosus*, *D. opuntiae*, *D. ceylonicus*, and *D. confusus* (Table 3), indicating that all of them are potential sources of dye.

3.2 Multivariate data analysis

The dendogram shows the CA for the five analyzed *Dactylopius* species in Figure 1. Because of its taxonomic proximity with *Dactylopius* and its chromatographic profile containing flavokermesic and kermesic acids (Wouters, 1985), the species *Kermes vermilio* Planchon (Kermesidae) was included as the out-group, helping to establish the taxonomic evolutionary relationship between both genera.

In this study, the analysis assumed that the species with the more complex characteristic(s) were the more evolved species, and these differences establish or mark the distances among them. Careful arrangements in proper sequence and organization of the available information about the distribution of secondary compounds in different genera have been useful for plant taxonomists studying systematics (Gottlieb, 1982).

Variation of secondary plant constituents in major plant taxa takes place in small steps, which makes it possible to trace plant evolution while at the same time identifying the geographic origin of these taxa (Gottlieb and Kubitzki, 1983). For this study, the distance among the species can be established according to the observed differences in the chromatographic profiles, such as number and peak combinations, which are considered to be chemical characteristics.

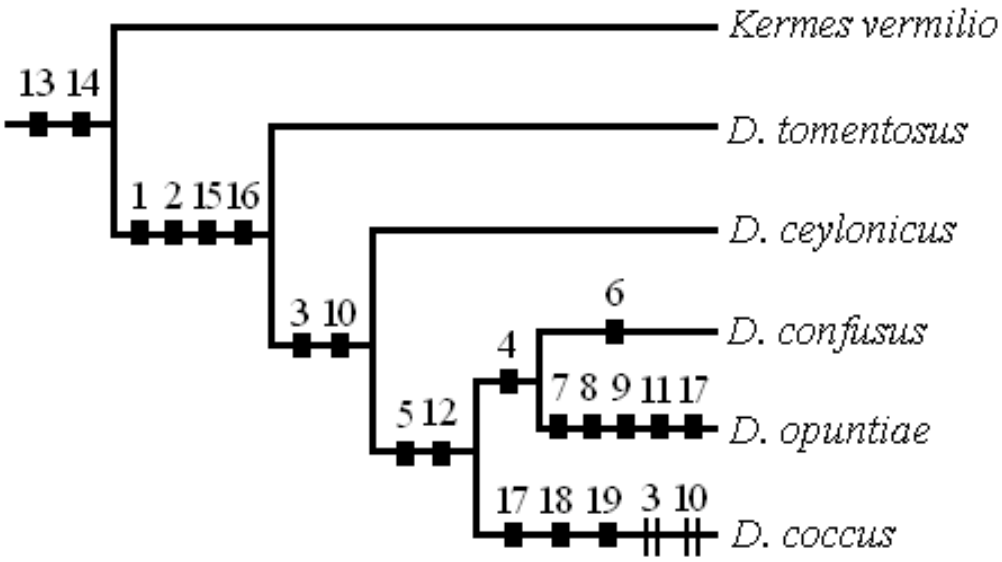


Figure 1. Dendogram (peaks, presence or absence) resulting from a cluster analysis using the principle of parsimony for *Dactylopius* species and *Kermes vermilio*. The numbers above the lines indicate the peaks of the chromatographic profile **(**Table 2**)**, squares and parallels represent the acquisition or lost of a peak in a species of a joined group.

In Figure 1, numbers above the lines correspond to the peaks shown in Table 2, which could indicate informative acquired (squares) or lost (parallels) chemical characteristics for the species of each joined group evolutionarily over time.

The most complex profile was found for *D. opuntiae*, followed by *D. confusus*; both exhibited 10 compounds in common. Next in complexity was *D. coccus*, differing in the presence of peaks 17, 18, and 19 and the absence of 3 and 10. *D. ceylonicus* had two peaks more than *D. tomentosus*. As expected, the out-group showed the least complexity (Figure 1). The second CA applied to the quantitative data analysis of the insect material (Table 3) resulted in the dendogram shown in Figure 2.

Table 3. Quantitative analyses. Absolute area ($*10^{-4}$) of the HPLC profile of commercial carminic acid and five *Dactylopius* species (0 = absence of a peak, CAc = carminic acid, KAc = kermesic acid), 275nm.

Peak number	Carminic acid	*D. ceylonicus*	*D. coccus*	*D. confusus*	*D. opuntiae*	*D. tomentosus*
Cac	9.83 ± 3.13	3.70 ± 0.241	8.60 ± 3.12	3.45 ± 0.91	4.70 ± 0.41	6.37 ± 0.280
3	1.83 ± 3.17	> 0 ± 0.002	0	0.50 ± 0.13	0.24 ± 0.028	0
4	0	0	0	0.30 ± 0.12	0.18 ± 0.31	0
5	0	0	1.08 ± 0.38	0.30 ± 0.12	0.12 ± 0.13	0
6	0	0	0	0.020 ± 0.023	0	0
9	0	0	0	0	> 0 ± 0.001	0
10	0.010 ± 0.016	0.13 ± 0.23	0	0.06 ± 0.05	> 0 ± 0.0002	0
12	0	0	2.39 ± 1.21	0.62 ± 0.34	> 0 ± 0.0003	0
Kac	0.83 ± 0.05	0.75 ± 0.17	0.03 ± 0.05	0.07 ± 0.13	0.59 ± 0.09	1.92 ± 0.286

The samples clustered in three separate regions. The first cluster includes cochineal samples collected in central and southeastern Mexico, which includes Mexico City, Tlaxcala, Veracruz, and the southern part of Hidalgo. These samples appear at the top of the dendogram and are differentiated by the presence of peak 3 and increased magnitudes of peaks 1, FkAc, and KAc (Figure 2). Appearing at the bottom of the dendogram, the second cluster consists of samples collected in the northern and central plateau areas of

Mexico, which includes the states of Zacatecas, San Luis Potosi, Jalisco, Aguascalientes, and the northern parts of Guanajuato and Hidalgo.

These samples are differentiated from the other groups by similarities in peaks 5, 10, and 11. In the middle of the dendogram, the third cluster is populated only by samples from northern Argentina, differentiated by peak 16, and from the Mexican *D. ceylonicus*, differentiated by peaks 10 and 15.

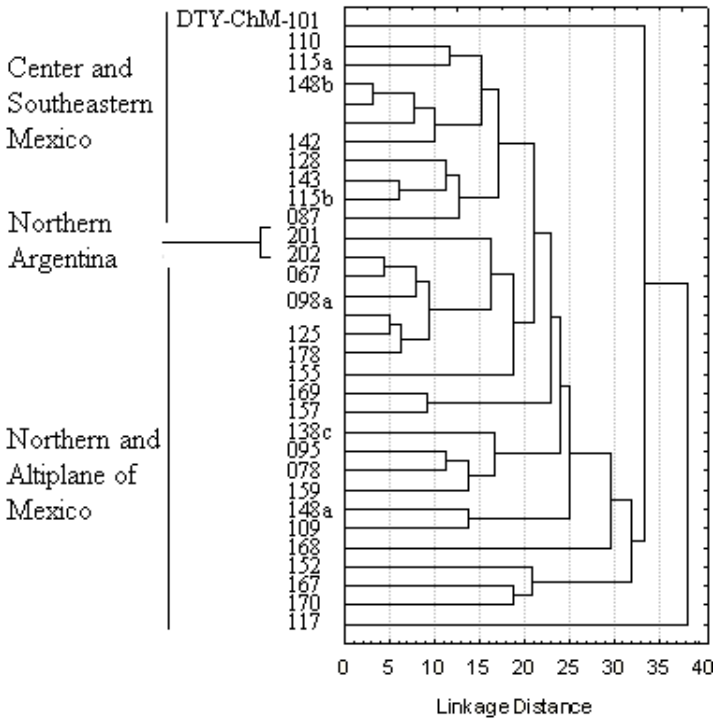
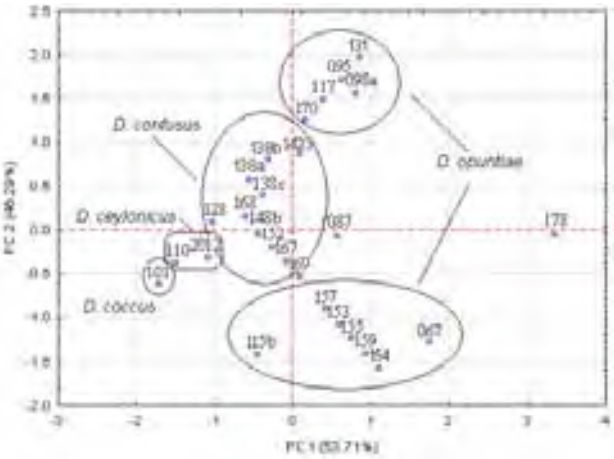
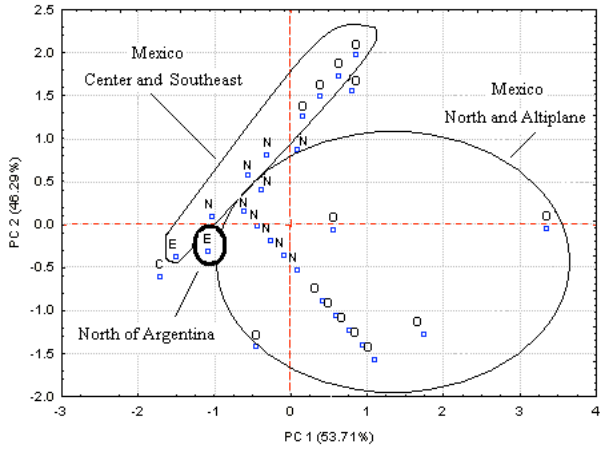


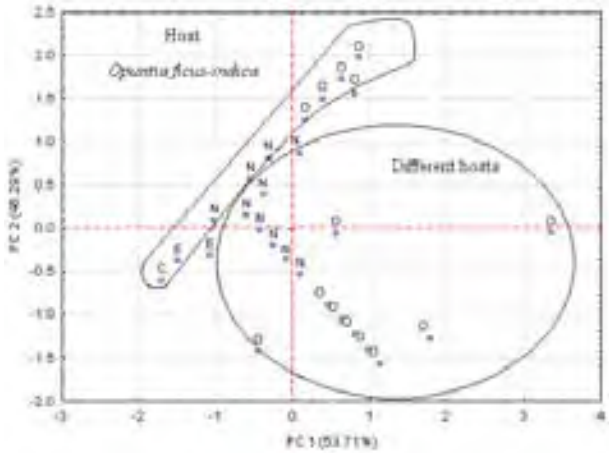
Figure 2. Dendogram with Euclidean distance as a measure of similarity using UPGMA algorithm for the HPLC quantitative analysis of cochineal samples (Table 1).



(a)



(b)



(c)

Figure 3. Distribution of the cochineal species **(**a**)**, from different localities **(**b**)** and on different host **(**c**)** on the plane formed by the first two principal components. *D. coccus* (C), *D. ceylonicus* (E), *D. confusus* (N) and *D. opuntiae* (O).

The notorious separation of one *D. opuntiae* species at the bottom of the dendogram and *D. coccus* at the top of the dendogram could be due to several factors. First, *D. opuntiae* (DTY-ChM-117) was collected in Mexico feeding on *O. tomentosa*, a plant species taxonomically distant from the other hosts. Second, *D. coccus* is the only domesticated species and was collected in Oaxaca, which is geographically distant from the other sampling regions.

Principal component analysis (PCA) was also employed to further support the results obtained by CA. The retention time for each peak in the HPLC profiles was considered, treating the host and the sample locality as the active variables. Thus, the first two principal components acquired were PC_1, peak 1, 18.1%, and PC_2, CAc, 15.8%, which represent 33.9% of the total variance, with seven eigenvalues higher than 1.0; accumulative eigenvalues 18.1%, 33.9%, 46.7%, 56.8%, 65.5%, 72.9%, 79.3%, and 85.3%, chosen on the basis of Kaiser's criterion. The second PCA considered only the retention time for each observed peak in the HPLC profiles of *Dactylopius* species as the active variable; the supplementary variables were host and locality.

This approach explained almost 98.7% of the total observed variance. The loading of PC_1 53.7% and PC_2 46.3% is shown in Figure 3, which correspond to eigenvalues higher than 1.0. The higher variance percentage was related to peak 1 (98.2%). The second PC is related to the areas of CAc (0.53%). The second loading of PC_1 and PC_2 separated the *Dactylopius* species into five groups (Figure 3a): *D. coccus* (C), *D. ceylonicus* (E), *D. confusus* (N), and two groups for *D. opuntiae* (O). *D. coccus* is separated as a group due to peaks 18 and 19. *D. ceylonicus* diverges from the genus because of peak 10 and a larger value of KAc, and is closer to *D. coccus* (DTY-ChM-101) because of similar amounts of peaks FkAc, KAc, 15, and 16. *D. confusus* forms a group due to peak 6, and is closer to *D.*

ceylonicus (DTY-ChM-110, 201, and 202) for peaks FkAc (smaller magnitude) and peak 15.

D. opuntiae, the group at the top (DTY-ChM-095, 098a, 117, 131 and 170) is separated from the group at the bottom (DTY-ChM-067, 115b, 153, 154, 155, 157, 159) because of peak 9, and the larger magnitude of peaks 11 and FkAc, and smaller magnitude of peak 17. In addition, the group at the top was collected mainly on *O. ficus-indica* and the species are taxonomically related (labelled as *Opuntia* sp.), while the group at the bottom was collected on different hosts; the result suggests that the variations in the metabolic profile are due in part to the insect host. The sample collected from Zacatecas (DTY-ChM-178) appears on the right side of the plot and it is separated from the two main *D. opuntiae* groups. The reason for this divergent behavior could be associated with peaks 3, 9, and 15; the differences are probably due to its host, *O. phaeacantha*, a shrub cactus taxonomically distant from the other hosts.

The loading samples grouped according to their geographical origin (Figure 3b**)** could clearly be divided into three groups: central and southeastern Mexico, the northern and central plateau areas of Mexico, and northern Argentina. The latter group also included the Mexican *D. ceylonicus* species. The three groups were separated by the presence of the same components as previously indicated in the CA.

Figure 3c shows another grouping considering the *Dactylopius* hosts. The samples collected on *Opuntia ficus-indica* shared peaks 3, 7, and 11 and all presented increased amounts of CAc compared to the other species. The rest of the species collected on different hosts could also be grouped. For example, *D. opuntiae* and *D. confusus* species from Jalisco collected on different species of *Opuntia* presented peak 12 in greater amounts than in the other species. *D. coccus* was separated again, due to a larger amount of CAc and

the presence of peaks 18 and 19, probably due to its domesticated condition. Patterns of classification of *Dactylopious* species in CA and PCA were consistent.

3.3 Mexican perspective on the use of Dactylopius

Based on their metabolic profiles, it was possible to identify the five analyzed species of *Dactylopius* using an HPLC instrument equipped with a photodiode array detector. This HPLC analysis could be performed as a fast and simple procedure and as a complementary tool for taxonomic differentiation among the *Dactylopius* species. CAc was the most abundant compound in all the analyzed species, making all of them potential sources of this colorant, in addition to the commercial species *D. coccus.* PCA was employed to further support the results obtained by CA, and the results from both techniques were complementary, confirming the grouping of all analyzed *Dactylopius* samples. This study provides an example of how the variation in constituents of cochineal color makes it possible to trace the geographic origin and host plants of *Dactylopius* species, and how it could also help to explain evolutionary divergence through the observed differences in their metabolic profiles.

Acknowledgments

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IV. Molecular phylogeny of the genus *Dactylopius* (Hemiptera: Dactylopiidae) and identification of the symbiotic bacteria

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Journal of Systematic Entomology

Molecular phylogeny of the genus *Dactylopius* (Hemiptera: Coccoidea: Dactylopiidae) and identification of the symbiotic bacteria

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Running title: Molecular phylogeny and symbionts of *Dactylopius*

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Abstract

Phylogenetic analyses from PCR amplified 12S rRNA and 18S rRNA gene sequences from cochineal insects of the genus *Dactylopius* present in Mexico, showed that *D. ceylonicus, D. confusus,* and *D. opuntiae* are closely related. *D. coccus* constitutes a separate clade, and *D. tomentosus* is the most distantly related. Bacterial 16S rRNA sequences from the sampled *Dactylopius* species revealed a common β-Proteobacteria, related to *Azoarcus,* also found in eggs and in bacteriocytes in *D. coccus*. Other bacterial sequences recovered from the samples were close to those from soil or plant associated bacteria, like *Massilia, Herbaspirillum*, *Acinetobacter*, *Mesorhizobium,* and *Sphingomonas*, suggesting a possible horizontal transmission from Cactaceae plants sap to *Dactylopius* spp. during feeding. This is the first molecular analysis of the five *Dactylopius* Mexican species and of their associated bacteria.

Introduction

Dactylopius Costa is a genus of insects commonly known as cochineals that belongs to the family Dactylopiidae (Signoret) from the super family Coccoidea (scale insects) within the Hemiptera. *Dactylopius* insects feed on Cactaceae plants from the genera *Opuntia* and *Nopalea* (Pérez-Guerra & Kosztarab, 1992), both of them, insects and their host plants, are considered native to the Americas (Pérez-Guerra & Kosztarab, 1992; Chávez-Moreno *et al*., 2009). Dactylopiidae has only one genus that includes nine described species (De Lotto, 1974; Pérez-Guerra & Kosztarab, 1992). Five of these species have been reported to be present in Mexico: *D. ceylonicus, D. confusus, D. opuntiae, D. coccus*, and *D. tomentosus* (Portillo & Vigueras, 2006; Chávez-Moreno *et al*., 2009). *Dactylopius* spp. are the natural

source of carminic acid, a red dye used in the production of cosmetics, drugs, food and textile. Carminic acid is used by the insects for protection against predators (Eisner *et al*., 1994). *Dactylopius* spp. have also been used as a biological control agent against invasive cactus (Moran & Zimmermann, 1984). *D. coccus* has been preferentially used for carminic acid extraction due to its pigment quality and higher acid content (Hernández-Hernández *et al*., 2005). There are reports of its use in America since the X century (Portillo, 2005; Chávez-Moreno *et al*., 2009). Currently, *D. coccus* is considered the only commercially important species and it has undergone a domestication process. This species is very vulnerable to natural enemies under wild conditions (Pérez-Guerra & Kosztarab, 1992).

Many insects harbor symbiotic bacteria in their guts or as endosymbionts inside specialized insect cells called bacteriocytes. Bacterial endosymbionts in the Hemiptera provide nutrients to insects with a limited diet such as phloem sap that is deficient in essential amino acids and vitamins (Baumann, 2005; Moran, 2006). Some endosymbionts also synthesize bioactive compounds that can be used by insect hosts as defense against predators, parasites and pathogenic microorganisms (Moran, 2006). As endosymbionts are vertically transmitted, their DNA sequences can be used to trace insect phylogenies (Baumann, 2005). Within the Coccoidea, endosymbionts have been found in the families Pseudococcidae (Thao *et al*., 2002), Diaspididae, and Margarodidae (Gruwell *et al*., 2007). The diversity of endosymbionts in *Dactylopius* spp. has not been reported, except for bacteria from the genus *Wolbachia* present in *Dactylopius* sp. eggs (Pankewitz *et al*., 2007).

The current identification and taxonomy of *Dactylopius* spp. has been based on morphological characters (De Lotto, 1974; Pérez-Guerra & Kosztarab, 1992), and Rodríguez *et al*. (2001) published a phylogeny of *Dactylopius* spp. on this basis. Until now there are no molecular phylogenies of the genus. There is only one phylogeny based on 18S

rRNA sequences of the Coccoidea, which places Dactylopiidae close to clade E1 from the Eriococcidae (Cook *et al*., 2002). The aims of this work were to sequence and analyze mitochondrial and nuclear ribosomal genes from *Dactylopius* spp. to assess the phylogenetic relationships between the five species present in Mexico, and to determine the symbiont bacteria species present in these insects.

Materials and methods

Insect sampling

Specimens from five different *Dactylopius* species were collected from different regions in Mexico. Insects were identified by morphological comparison with specimens from the Colección Nacional de Insectos of the Instituto de Biología, Universidad Nacional Autónoma de México (CNI-IB-UNAM) and the descriptions given by De Lotto (1974) and Pérez-Guerra & Kosztarab (1992). Specimens were collected from the following states: *D. coccus* from Oaxaca, *D. confusus* from Tlaxcala, *D. ceylonicus* from Mexico state, *D. opuntiae* from Michoacán and Querétaro and additionally we obtained *D. opuntiae* from Pernambuco state from Brazil; all of these were parasiting *Opuntia ficus-indica* (Linnaeus) Miller plants. *D. tomentosus* was collected from Hidalgo on *Cylindropuntia tunicata* (Lehmann) Knuth. *Parasaissetia* sp. (Hemiptera: Coccidae) insects were collected in Morelos state on *Jacaranda mimosifolia* (D. Don) plants and gene sequences derived from this Coccidae were considered as outgroup in the phylogenetic analyses.

DNA extraction, amplification and sequencing

Female specimens from each insect species, freshly collected or frozen (-20 ºC) were superficially cleaned removing the white wax, washed and vortexed several times with sterile distilled water. DNA was extracted from whole insects, individually in the case of *D. coccus* or from 2-4 specimens from the other species with DNeasy Blood and Tissue Kit (Qiagen). Two DNA samples were analyzed from each species. DNA was used as a template in PCR reactions using primers F-12S-2 y R-12S-2 for insect mitochondrial 12S rRNA gene (Thao *et al.*, 2004), primers 2880 (Tautz *et al*., 1988) and B- (von Dohlen & Moran, 1995) for insect nuclear 18S rRNA gene, and primers fD1 and rD1 for bacterial 16S rRNA gene (Weisburg *et al*., 1991). DNA from eggs and bacteriocytes from *D. coccus* was extracted after dissection and washing, and used in PCR reactions as described above. Primers that specifically amplify a fragment from the16S rRNA gene of END1 and closely related β-Proteobacteria were designed (Beta428F: 5'-GTGAATATCCGAAGCCGATGAC-3', Beta1205R: 5'-GGCTTGGCAACCCTCTGTACCG-3'). Primers to identify clone O1 and related α-Proteobacteria were also designed (Alpha141F: 5'-ACGGAAGAAAGTAGATATACGC-3' and Alpha944R: 5'-ACCTGTTATGCTCCAACTAAAT-3'). These were used in addition to fD1 and rD1 primers with DNA of *Dacytilopius* spp.

PCR protocols were performed as described (Weisburg *et al*., 1991; Thao *et al.*, 2004), except for the 18S rRNA gene that was amplified using the procedures described by Cook *et al*. (2002). The following protocol was used with Beta428F - Beta1205R and Alpha141F - Alpha944R primers: an initial denaturation at 94 ºC for 3 min, followed by 33 cycles of amplification (1 min at 94 ºC, 1 min at 57 ºC or 52 ºC (respectively), and 1 min at 72 ºC), and a final extension step of 5 min at 72 ºC. The amplified products were 1500 (fD1 and

rD1), 460 (F-12S-2 and R-12S-2), 620 (2880 and B-), 800 bp (Beta428F and Beta1205R), and 825 bp (Alpha141F and Alpha944R). PCR products were cloned and individual plasmid clones were sequenced in Macrogen Inc. (Korea).

Phylogenetic analyses

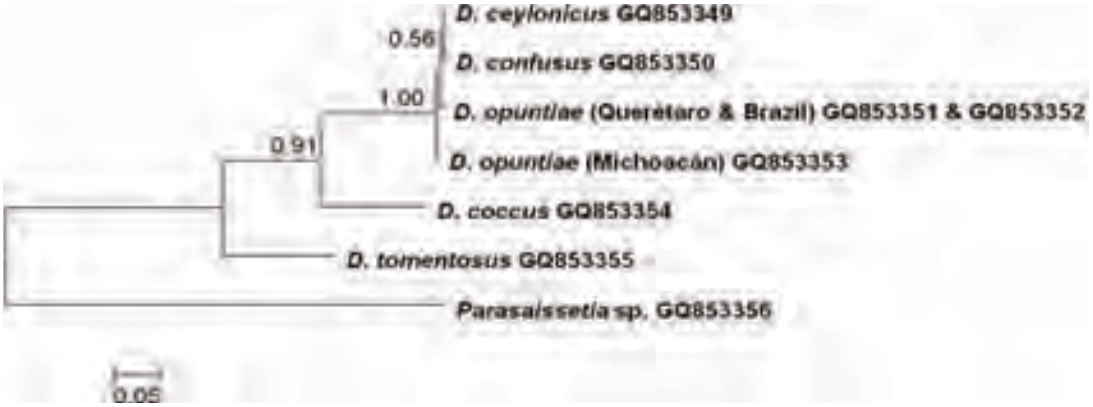
Nucleotide sequences were compared to the nr GenBank database, using Blastn and sequences from closely related organisms were retrieved. Sequence alignments were done with CLUSTAL W (Thompson *et al.,* 1994) and manually edited. Phylogenies were constructed with the Bayesian method using MrBayes (Huelsenbeck & Ronquist, 2001). The best model of sequence evolution for each gene was selected using the MrAIC Perl script written by J. A. A. Nylander (http://www.abc.se/~nylander/). In all the cases the selected model was GTR+I+G.

Results

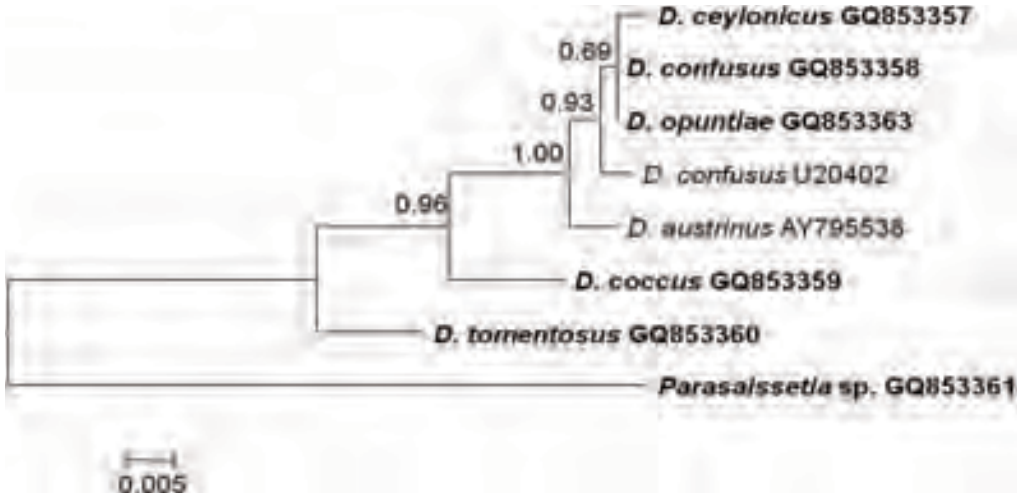
Phylogenetic analyses

Two to eight individuals were used from each species and sequences from three clones (from each marker) of the same species were analyzed. Sequences obtained from a single insect species were more than 99% identical, and only one clone from each species is shown. Phylogenetic trees obtained from the mitochondrial 12S rRNA and nuclear 18S rRNA genes are shown in Fig. 1 A and B. Trees were congruent and showed that *D. ceylonicus*, *D. confusus*, and *D. opuntiae* clustered together with an identity of 99%. 12S rRNA gene sequences from *D. opuntiae* from three different geographic regions were very similar (more than 98.9% of identity) and 18S rRNA gene sequences from *D. confusus* and from the three *D. opuntiae* were 100% identical. Two 18S rRNA gene sequences were

retrieved from NCBI GenBank corresponding to *D. confusus* (U20402, collected from Arizona [von Dohlen & Moran, 1995]) and *D. austrinus* (AY795538). The *D. confusus* sequence is similar to those reported here for *D. ceylonicus*, *D. confusus*, and *D. opuntiae* (99% of identity), and *D. austrinus* sequence was close to this cluster. *D. coccus* is separated and *D. tomentosus* is the most distantly related.



(A)



(B)

Fig. 1. Phylogenetic trees of 12S rRNA gene sequences (426 pb) (A), and 18S rRNA gene sequences (584 pb) (B), obtained from the different *Dactylopius* species. Sequences from this work are in bold. Accession numbers are shown. The tree was inferred with the

Bayesian method using MrBayes under model GTR+I+G. Numerical values at each node indicate posterior probabilities.

Identification of symbiotic bacteria

The analyses of 16S rRNA gene sequences indicated that different bacteria were found inside *Dactylopius*. The abundance of clones found in each species and the percentage of identity to the closest NCBI match is shown in Table 1. The 16S rRNA gene sequence from Candidatus *Sulcia muelleri* was used as an out group; this is an endosymbiotic flavobacteria of many species of the suborder Auchenorrhyncha of the Hemiptera (Moran *et al*., 2005).

Dactylopius species had different associated bacteria and the only one common in all of them was a β-Proteobacteria (named here as END1) that was highly conserved with almost identical 16S rRNA gene sequences. END1 was first identified with 16S rRNA universal primers (fD1 and rD1) in all the individuals collected from *D. opuntiae,* including the ones collected from Brazil, and in *D. coccus* and *D. tomentosus* (Table 1). Subsequently with primers specific to END1 it was also found in *D. ceylonicus* and *D. confusus* as well as in eggs and bacteriocytes of *D. coccus*. The sequence had high identity (95%) to a soil isolate (AB024934) which was erroneously assigned to *Sphingomonas* sp. A1 (α-Proteobacteria) when it is really a sequence from a β-Proteobacteria close to *Azoarcus* sp (Fig. 2).

Additionally in all the specimens collected from *D. opuntiae* 39% of the clones (O1) presented 88% identity to Candidatus *Hepatincola porcellionum*, the extracellular symbiont of the hepatopancreas of *Porcellio scaber* (common woodlouse, Crustacea: Isopoda) (Wang *et al*., 2004) (Fig. 2), which belongs to the order Rickettsiales from the α-Proteobacteria. Specific primers for O1 did not amplify DNA from other *Dactylopius* species.

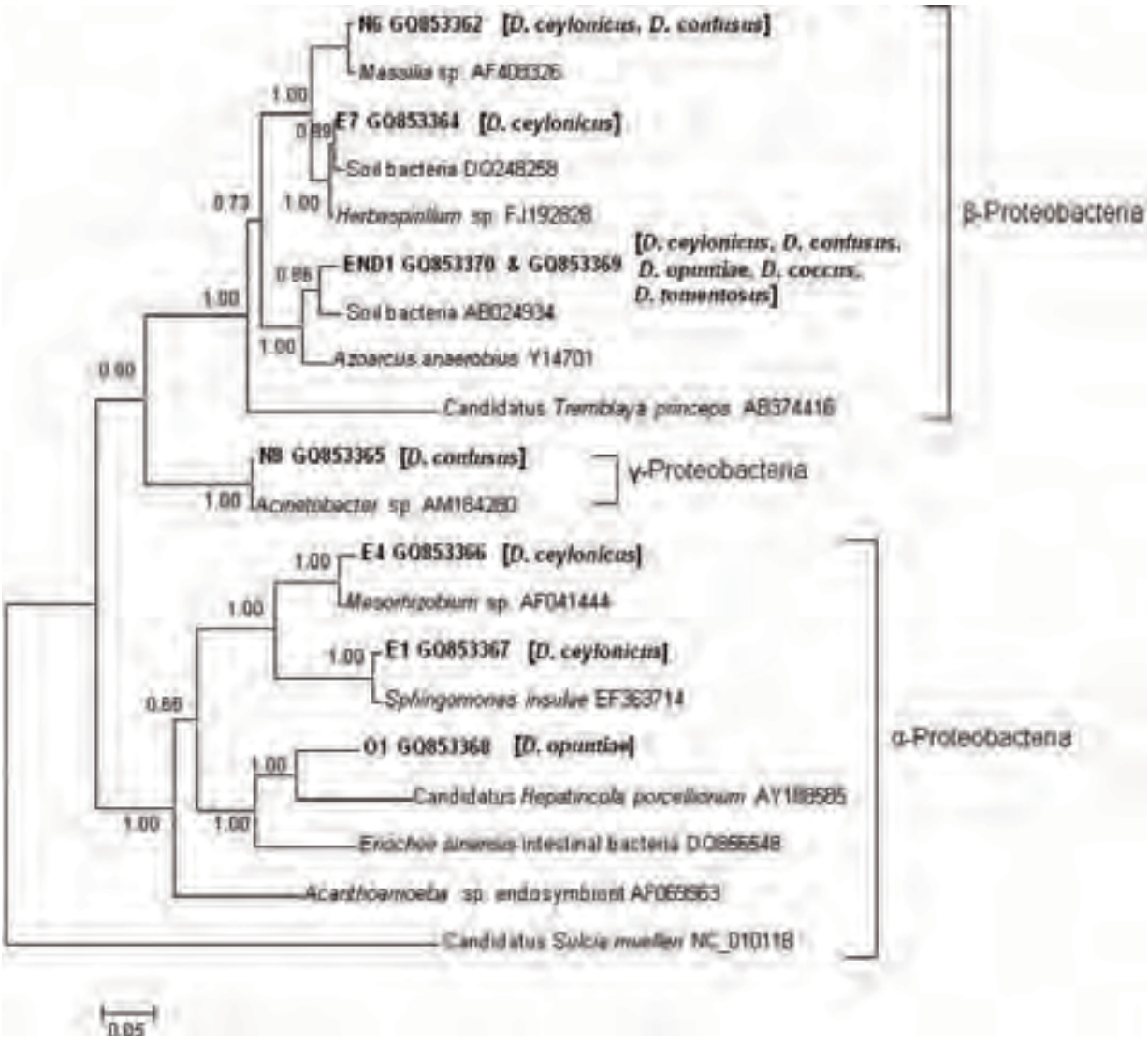


Fig. 2. Phylogenetic tree of 16S rRNA gene sequences of bacteria (1284 pb) obtained from the different *Dactylopius* species. Sequences from this work are in bold. Other sequences from closely related organisms were included. Accession numbers are shown. Host *Dactylopius* species are shown in brackets. The tree was inferred with the Bayesian method using MrBayes under model GTR+I+G. Numerical values at each node indicate posterior probabilities.

Other 16S rRNA gene clones were close to free living bacteria, like *Massilia* sp., *Herbaspirillum* sp., *Acinetobacter* sp., *Mesorhizobium* sp., and *Sphingomonas* sp. (Table 1 and Fig. 2), with 98, 96, 99, 97, and 98% identity respectively. These were obtained from *D. ceylonicus* and *D. confusus*.

Table 1. Assignment and abundance (percentage of clones) of bacteria in *Dactylopius* species using 16S rRNA gene sequences obtained with universal primers rD1 and fD1. The closest NCBI match is shown. Name of each clone is shown in parenthesis. 2-8 insect individuals were used.

	β-Proteobacteria			**α-Proteobacteria**			**γ-Proteo-bacteria**	
***Dactylopius* species**	**Soil bacteria (END1)**	***Massilia* sp. (N6)**	***Herbas-pirillum* sp. (E7)**	***Porcellio scaber* symbiont (O1)**	***Sphingo-monas insulae* (E1)**	***Meso-rhizobium* sp. (E4)**	***Acineto-bacter* sp. (N8)**	**Number of analyzed clones**
D. ceylonicus	0[a]	29	43	0	14	14	0	**7**
D. confusus	0[a]	88	0	0	0	0	12	**8**
D. opuntiae (Michoacán)	100	0	0	0	0	0	0	**2**
D. opuntiae (Querétaro)	63	0	0	37	0	0	0	**27**
D. opuntiae (Brazil)	43	0	0	57	0	0	0	**7**
D. coccus	100	0	0	0	0	0	0	**10**
D. tomentosus	100	0	0	0	0	0	0	**6**

[a] END1 symbiont was not found when universal 16S rRNA gene primers (rD1 and fD1) were used, but it was amplified by PCR when specific primers for β-Proteobacteria were used (Beta428F and Beta 1205R).

Discussion

Our phylogenetic results differ from those reported by Rodríguez *et al*. (2001) based mainly on morphological characters and using published information, where *D. confusus* and *D. opuntiae* constitute a clade related to *D. coccus* and *D. ceylonicus*; and *D. austrinus* constitute another clade separated from *D. confusus* and *D. opuntiae*. The need to establish molecular phylogenies for *Dactylopius* has been recognized in several papers as conflicting results were derived from morphological data (Portillo & Vigueras, 2006).

D. coccus was domesticated and selected for producing higher amounts of carminic acid. It has a larger body size and presents a cover of white powdery wax instead of a white cottony wax with long filaments (Pérez-Guerra & Kosztarab, 1992). The cottony wax cover protects the insects against desiccation and rain (Chávez-Moreno *et al*., 2009). Pérez-Guerra & Kosztarab (1992) considered the characteristic cover important for proposing *D. coccus* as the most primitive of the *Dactylopius* species; otherwise this character could be a consequence of domestication.

In accordance with our results, *D. tomentosus* has been reported as the most distant species of the genus, as it has unique biological and morphological characteristics that differ considerably from other *Dactylopius* species (Mathenge *et al*., 2009). *D. tomentosus* host range is restricted to the subgenus *Cylindropuntia*, its egg incubation period is longer (17 days instead of minutes or hours), eggs are held on a mesh of waxy threads and remain attached to the female during the incubation period (in other *Dactylopius* species eggs are not enclosed in a mesh and continue to hatch as more are laid), the size of female adults is smaller than in most of the other species (similar to *D. confusus*) (Mathenge *et al*., 2009), and its anal ring is obsolete (Pérez-Guerra & Kosztarab, 1992; Rodríguez *et al*., 2001).

We found a characteristic set of bacteria from each *Dactylopius* species. Of special interest is END1, a β-Proteobacteria found in all the *Dactylopius* species as well as in eggs and bacteriocytes of *D. coccus*. These facts suggest that END1 is vertical transmitted and may be a primary endosymbiont, and that it could have been acquired before the radiation of this genus. Its location should be subsequently confirmed by *in situ* hybridization. Within the Coccoidea in the Pseudococcidae family another β-Proteobacteria primary endosymbiont, Candidatus *Tremblaya princeps* has been reported (Thao *et al*., 2002) with a16S rRNA gene sequence 79% identical to that from END1 (see Fig. 2).

Clone O1 found in the specimens collected form *D. opuntiae* belongs to the order Rickettsiales. In this order many intracellular symbionts and pathogens of eukaryotes have been found (Weinert *et al*., 2009). A phylogenetic tree including the sequences from clone O1, the closest NCBI match sequences with 85 -88% of identity (Candidatus *Hepatincola porcellionum* AY1885, a gut bacterium from the Chinese mitten crab *Eriocheir sinensis* DQ856548, and an endosymbiont of *Acanthoamoeba* sp. AF069963, see Fig. 2), as well as those of the different clades within the Rickettsiales reported by Weinert *et al*. (2009), showed that the sequence from O1 and of the three related sequences did not group with any of the major clades of Rickettsia, meaning that they could represent new taxons within this group. In contrast to published data (Pankewitz *et al*., 2007), we did not find *Wolbachia* in *Dactylopius* spp.

The sequences from the clones close to free living bacteria (*Massilia* sp., *Herbaspirillum* sp., *Acinetobacter* sp., *Mesorhizobium* sp., and *Sphingomonas* sp.) belong to soil or to plant associated bacteria. The location of some of these bacteria could be the gut, in which case its origin could plausibly be the sap which serves as food for the insects. It would be of

ecological interest to explore this fact by analyzing the endophytic bacteria from the plants parasited by the insects. Their location inside the insect must be also determined.

Our description of *Dactylopius* spp. endosymbionts will be the base for studying its role in the development and physiology of the host. These bacteria could be implicated in providing amino acids, vitamins, antimicrobial compounds to the host, or degrading plant toxic compounds, as occurs in other sap feeding insects.

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V. Discusión General

Revisión histórica, cultural, geográfica, y ecológica de la información de los géneros *Opuntia* y *Dactylopius* y su distribución en México

La revisión de literatura muestra la estrecha relación entre las especies de los géneros *Opuntia* y *Dactylopius* con la historia de los pueblos precolombinos de mesoamérica (Martín del Campo 1957; Smith, 1967; MacGregor, 1976; Piña, 1977; Bravo-Hollis y Sánchez-Mejorada 1978; Anderson, 1981; Colunga *et al*., 1986; MacNeish, 1992; Bravo-Hollis y Scheinvar, 2002; Casas y Barbera, 2002; Reyes-Agüero *et al*., 2005a). Su trayectoria de uso y producción llevó a México a ser el principal productor de grana cochinilla y su colorante durante más de un siglo (Dahlgren, 1990; Humboldt, 1966) para después permanecer casi extinta después de la independencia (Butler, 2006). La reactivación del cultivo de la grana cochinilla en nuestro país requiere de la participación de los productores de *Opuntia*, el rescate del conocimiento tradicional y las técnicas de producción desarrolladas a través de la historia cultural de México combinadas con el uso de la tecnología actuales (Pérez-Sandi, 1999). Actualmente, *Opuntia* y *Dactylopius* se han propagado en todo el mundo y sus usos se han diversificado (Quijano y Vergara 2007, Palma de la Cruz 2008).

México es una importante reserva de la diversidad biológica de *Opuntia* y *Dactylopius*. Sin embargo, la Norma de Protección ambiental de especies nativas de flora y fauna silvestre de México (SeMARNat, 1994) sólo mencionan cinco especies de *Opuntia* de un total de 284 cactáceas que dicha norma considera. Las especies son: *O. bravoana* (nopal from Bravo), *O. excelsa* (excelso nopal), *O. rosarica* (Cholla tasajo de Rosario), *O. santamaria* (Cholla de Santa Maria) consideradas endémicas y *O. polyacantha* var. *arenaria* (SeMARNat, 1994). Por otra parte, los insectos *Dactylopius* no se mencionan en esta norma (SeMARNat, 1994).

El estudio de las especies de *Opuntia* y *Dactylopius* como recursos genéticos ayuda al conocimiento para el aprovechamiento, el desarrollo y la interacción entre las especies cultivadas y silvestres de ambos géneros. No debe omitirse la directriz ecológica que permitiría la restauración del cultivo de la cochinilla de una forma sustentable y el reconocimiento de las necesidades actuales de las distintas regiones de México para su implementación.

Distribución y hábitats de *Dactylopius* y sus hospederos de la subfamilia Opuntioideae
Distribución en México

La distribución de *Dactylopius* dentro del área de estudio es continua, más amplia que la reportada en la literatura hasta antes del presente estudio y guarda una correspondencia con sus hospederos. El área estudiada se localiza entre 98 a 104º latitud norte y los 18 a 23º longitud, abarcando los estados de Aguascalientes, Guanajuato, Hidalgo, Jalisco, Estado de México, Michoacán, Morelos, Oaxaca, Puebla, Querétaro, San Luis Potosí, Tlaxcala, Veracruz, Zacatecas y el Distrito Federal. Se extiende principalmente en las regiones del altiplano norte y central y sureste de México. Esta zona se caracteriza porque el tipo de vegetación predominante es matorral xerófilo, bosque templado de confieras y bosque de pino-encino; cuenta con una amplia variedad de suelos: arenosol, vertisol, calcisol, xerosol, regosol, leptosol y feozem, y climas seco árido y semiarido, templado y húmedo de acuerdo con Rzedowski (1978). El rango altitudinal del género fue entre los 0 y los 2845 m (sobre el nivel del mar). Este trabajo reporta nuevos registros georreferenciados de las cinco especies de *Dactylopius* y sus hospederos, evaluando el área de ditribución de los mismos.

D. ceylonicus. Previamente había sido registrada en 6 estados de México, sobre especies de *Opuntia* y *Nopalea*. En este estudio se registra por primera vez en el Distrito Federal y en el estado de Hidalgo sobre *O. ficus-indica* y *Cylindropuntia imbricata*. Esta especie desarrolla preferentemente en cladodios maduros de *Opuntia*, en las areolas de cladodios y tunas, en nopales que forman cercas vivas, con escasa vegetación y suelo tipo regosol. También se puede recolectar en los nódulos de las raíces de *Opuntia* sp., en poblaciones silvestres con vegetación de matorral xerófilo y suelo arenosol, altitud entre 950 y 2650 m.

D. coccus. Esta especie había sido registrada en cinco estados de México sobre *Opuntia* y *Nopalea* en un rango altitudinal entre 1250 y 2200. En este estudio fue recolectado en centros de producción de Oaxaca; Estado de México y Morelos sobre *O. ficus-indica*, principal hospedero para la producción de cochinilla. Se registra por primera vez *D. coccus* en poblaciones silvestre en los estados de México, San Luis Potosí y el Distrito Federal. Su hábitat más común es en los cultivos intensivos de *O. ficus-indica*, suelos drenados, suelo

vertisol, calcisol, xerosol, regosol, leptosol, y feozem, dentro del rango altitudinal registrado entre 1654 y 2845 m.

D. confusus. Esta especie había sido registrada en 11 estados de México, sobre especies de *Opuntia*, *Cylindropuntia* y *Grusonia*, entre 1100 y 2200 m. En este estudio los especímenes estudiados se dividieron en dos grupos, de acuerdo con sus características morfológicas, *D. confusus* y *D. confusus* biotipo 1. *D. confusus* son los insectos con morfología típica (De Lotto, 1974; Pérez-Guerra y Kosztarab, 1992). Recolectados en el Distrito Federal, Hidalgo, Jalisco, Morelos y Puebla sobre *O. ficus-indica*; en Jalisco sobre distintos hospederos y por primera vez en los estados de Veracruz y Zacatecas. Crece sobre los cladodios y frutos de cactáceas arbóreas, predominantemente en hábitat desértico con escasa vegetación y suelo arenosol, en un rango de altitud entre 1200 y 2547 m, superior al registrado para esta especie.

D. confusus biotipo 1. Insectos que combinan características morfológicas de *D. confusus* típico (De Lotto, 1974; Pérez-Guerra y Kosztarab, 1992) con la especie *D. salmianus*, especie únicamente registrada en Sudamérica (De Lotto, 1974; Pérez-Guerra y Kosztarab, 1992; de Haro y Claps, 1995) nunca antes descrita en México. Estos insectos fueron recolectados en los estados de Hidalgo, Morelos, San Luis Potosí, Puebla y Tlaxcala sobre distintas especies de *Opuntia* y una especie de *Cylindropuntia*. *D. confusus* y *D. confusus* biotipo 1 fueron recolectadas en el estado de Morelos sobre el mismo hospedero. *D. confusus* biotipo 1 promueve cambios de coloración de sus hospederos y puede dañar el tejido donde crece. Se desarrolla preferentemente en cladodios y tunas en zonas urbanas y cultivos de producción de *Opuntia* en tierras con suelos de temporal, en poblaciones silvestres donde predomina el matorral xerófilo y el suelo de tipo arenosol y calcisol, ramgo altitudinal entre los 1654 y 2773 m.

D. opuntiae es la especie con el mayor número de registros en la literatura, ha sido registrada en 20 estados de México, sobre 17 especies de cactáceas de los géneros *Opuntia*, *Nopalea* y *Cylindropuntia* dentro de un rango altitudinal entre 25 a 2678 m. En este estudio se recolectaron y separaron en dos grupos los especímenes de esta especie, *D. opuntiae* y *D. opuntiae* biotipo 1. *Dactylopius opuntiae,* corresponden a insectos con la morfología típica (De Lotto, 1974; Pérez-Guerra y Kosztarab, 1992). En este trabajo fueron recolectados en el Distrito Federal, Jalisco, Michoacán, Puebla, San Luis Potosí, Veracruz, y Zacatecas

sobre *Opuntia*; y por primera vez en Guanajuato y Tlaxcala. Se desarrolla en cualquier porción de las plantas y puede causar daño a sus hospederos e incluso la muerte. Es la especie más agresiva e invasora del género. Crece en un rango altitudinal entre 750 y 2845, superior al registrado hasta ahora.

D. opuntiae biotipo 1. Insectos que combinan características morfológicas de *D. opuntiae* típico (De Lotto, 1974; Pérez-Guerra y Kosztarab, 1992) con la especie *D. salmianus*, especie únicamente registrada en Sudamérica (De Lotto, 1974; Pérez-Guerra y Kosztarab, 1992; de Haro y Claps, 1995) nunca antes registrados en México. Fueron recolectados en los estados de Guanajuato, Jalisco, San Luis Potosí y Tlaxcala sobre *Opuntia* y *Cylindropuntia*; Además, *D. opuntiae* y *D. opuntiae* biotipo 1 fueron recolectadas cohabitando en el Distrito Federal y Michoacán sobre el mismo hospedero. *D. opuntiae* biotipo 1 es menos agresivo que con *D. opuntiae* con sus hospederos y se desarrolla sobre cladodios y frutos en zonas urbanas y cultivos de *Opuntia*, en tierras de temporal, donde predomina el matorral xerófilo y suelo arenosol, rango altitudinal entre 1663 y 2773 m.

D. tomentosus. No hay registros de esta especie en CNI-IB-UNAM. En la literatura se en ocho estados de México sobre *Opuntia*, *Nopalea* y *Cylindropuntia,* rango altitudinal entre 0 y 2500 m. En este estudio fue recolectada en los estados de Guanajuato e Hidalgo sobre especies de *Opuntia* y *Cylindropuntia*, dentro del rango altitudinal señalado. Se desarrolla exclusivamente sobre los cladodios y poseen un tamaño casi imperceptible. Esta especie no daña a sus hospederos ni promueve cambios en la planta. Se desarrolla en hábitat desértico donde predomina el matorral xerófilo, suelos vertisol y arenosol.

En este estudio las especies de hospederos identificados para *Dactylopius* fueron principalmente *Opuntia ficus-indica* (variantes y cultivares), *O. streptacantha*, O. *streptacantha* ssp. *aguirreana* Bravo, *O. robusta* (variantes y cultivares), *O. tomentosa*, *O. albicarpa* (cultivar), *O. joconostle*, *O. hyptiacantha*, *O. jaliscana*, *O. phaeacantha*., *O. megacantha*, *O. fuliginosa*, *O. spinulifera*, *O. atropes*, *Cylindropuntia imbricata* y *C. tunicata*, además de 20 cultivares.

La presencia de especies de *Dactylopius* con distintos caractéres morfológicos cohabitando en una misma localidad e incluso compartiendo distintas porciones de un mismo hospedero se da a conocer en este trabajo por primera vez. Asimismo, se presentan

por primera vez registros de insectos con características de *D. confusus* y *D. opuntiae* combinadas con características de *D. salmianus*, nombrados en este texto *D. confusus* biotipo 1 y *D. opuntiae* biotipo 1, respectivamente, lo que sugiere la presencia de nuevas especies aún no estudiadas o la posibilidad de hibridación interespecífica entre las especies identificadas. Los resultados mencionados ponen de manifiesto la necesidad de implementar técnicas de análisis de *Dactylopius* que permitan identificar las especies y establecer su estatus taxonómico, entre las técnicas documentadas para otras especies de insectos se reportan la caracterización química y el análisis molecular.

El perfil metabólico de las especies de *Dactylopius*, origen geográfico y hospederos y el análisis multivariado de los datos CLAE

El estudio de los perfiles metabólicos de *Dactylopius*, permitió identificar la composición de los pigmentos de cinco especies de insectos usando un equipo de cromatografía líquida de alta eficiencia (CLAE) equipado con un detector de arreglo de fotodiodo. Fue posible detectar 19 picos que correspondieron a: ácido carmínico (CAc), ácido flavokermésico (FkAc), y ácido kermésico (KAc), además de otros 16 picos no identificados. La presencia o ausencia de un determinado pico permitió distinguir el perfil de cada especie. *D. opuntiae* presentó el perfil más complejo con 16 picos, cuatro de ellos únicos con: t_R 8.31 min (pico 7), 9.73 min (pico 8), 10.6 min (pico 9), y 13.4 min (pico 11). *D. confusus* con 12 picos, uno de ellos único con t_R de 6.05 min (pico 6). Se detectaron 11 picos para *D. coccus*, dos de ellos únicos con t_R de 21.9 min (pico 18) y 22.1 min (pico 19). *D. ceylonicus*, con ocho picos sin encontrarse diferencias entre las especies mexicanas y argentinas. *D. tomentosus* con seis picos, todos ellos comunes al género y con t_R de 0.678 min (pico 1), 1.84 min (CAc), 15.4 min (FkAc), 16.6 min (KAc), 17.5 min (pico 15), y 18.8 min (pico 16). El análisis CLAE puede considerarse un procedimiento rápido y sencillo y es una herramienta complementaria en la diferenciación taxonómica de las especies de *Dactylopius*. El ácido carmínico fue el compuesto más abundante de todas las especies analizadas; su concentración es distinta entre las especies, siendo *D. coccus* la que tiene la mayor cantidad de este colorante, seguido de *D. tomentosus*, *D. opuntiae*, *D. ceylonicus*, y *D. confusus* lo que permite afirmar que las especies de *Dactylopius* analizadas representan una fuente potencial de colorante, alternativa a la especie comercial *D. coccus*.

En este estudio se asumió que la complejidad del perfil cromatográfico refleja el grado de evolución de las especies, considerándose de esta manera que la especie con el perfil más complejo era la más evolucionada, suposición que ha sido de gran utilidad en el estudio sistemático y taxónomico de plantas (Gottlieb, 1982 y 1983). Los picos compartidos entre especies permitieron hacer agrupaciones. A través de un análisis de agrupamiento (AC) se construyó el dendograma para las cinco especies de *Dactylopius* analizadas utilizando la especies *Kermes vermilio* Planchon (Kermesidae) como grupo externo. Este género presenta un perfil cromatográfico que contiene AFkc y KAc (Wouters, 1985 y 1989) lo que permitió plantear la posible relación taxonómica evolutiva entre ambos géneros.

El dendograma obtenido del CA es el análisis de los datos cuantitativos del material de insectos, muestra tres regiones separadas: la primera incluye las muestras de cochinilla recolectadas en la parte central y el sureste de México, que incluye a las entidades de Distrito Federal, Tlaxcala, Veracruz, y el sureste de Hidalgo agrupadas por la presencia del pico 3 y la magnitud de los picos 1, FkAc, y KAc. La segunda región está integrada por muestras recolectadas en el altiplano norte y central de México, en los estados de Zacatecas, San Luis Potosí, Jalisco, Aguascalientes, y la parte norte de Guanajuato e Hidalgo. Estas muestras se distinguen por los picos 5, 10, y 11. El tercer agrupamiento corresponde a *D. ceylonicus*, muestras recolectadas en el norte de Argentina, diferenciadas por el pico 16, y las muestras de México por sus picos 10 y 15.

También las muestras de insectos pudieron separarse de acuerdo con su hospedero. Las muestras recolectadas sobre *Opuntia ficus-indica* comparten los picos 3, 7, y 11 y la mayor cantidad de CAc comparado con las otras especies. Las especies recolectadas sobre distintos hospederos formaron un grupo distinto. Por ejemplo, las especies de *D. opuntiae* y *D. confusus* recolectadas en Jalisco sobre distintas especies de *Opuntia* presentaron el pico 12 en común y en cantidad superiores al de las otras especies. *D. coccus* se separó nuevamente por contener la mayor cantidad de CAc y por los picos 18 y 19, para esta especie se asume que sus diferencias en el perfil cromatográfico y su separación del resto de las especies es debida al proceso de domesticación y selección artificial . Los resultados CA y PCA son similares y no se contradicen.

Filogénia molecular del género *Dactylopius* e identificación de sus bacterias simbióticas

El análisis de 2 a 8 individuos de cada especie de *Dactylopius* permitió la secuenciación de tres clonas (para cada marcador) de la mismas especies. Las secuencias obtenidas a partir de un sólo insecto, de una sola especies fueron 99% idénticas por lo que se muestran los resultados de una clona para cada especie. Se obtuvieron los árboles filogenéticos a partir del análisis de genes mitocondrial 12S rRNA y nuclear 18S rRNA. Los árboles resultaron congruentes y mostraron que *D. ceylonicus*, *D. confusus*, y *D. opuntiae* constituyen un clado agrupado con una identidad del 99%. Este resultado hace suponer que se trata de la misma especie. El resultado del la secuencia del gene 12S rRNA de la especie *D. opuntiae* de tres regiones geográficas presenta una identidad del 98.9% que significa que son muestras muy cercanas o similares. Las secuencias del gene 18S rRNA de *D. confusus* y tres de *D. opuntiae* resultaron 100% idénticas. Dos de las secuencias del gene 18S rRNA fueron extraídas del NCBI GenBank y correspondieron alas muestras *D. confusus* (U20402, recolectadas en Arizona [von Dohlen & Moran, 1995]) y *D. austrinus* (AY795538) para incorporarlas al análisis. La secuencia de la muestra de *D. confusus* es similar a las reportadas aquí para *D. ceylonicus*, *D. confusus*, y *D. opuntiae* (99% de identidad), y la secuencia de la especie *D. austrinus* es cercana a este agrupamiento. *D. coccus* se encuentra separada y *D. tomentosus* resultó la especie más distante.

La comparación de la topografía obtenida en este trabajo por los resultados filogenéticos de *Dactylopius* con el dendograma presentado por Rodríguez *et al*. (2001), generado con el algunas de las características morfológicas del género (Portillo & Vigueras, 2006), no son similares. En este trabajo, *D. confusus* y *D. opuntiae* constituyen un clado relacionado con *D. coccus* y *D. ceylonicus*; y *D. austrinus* comprende otro clado separado de *D. confusus* y *D. opuntiae*. *D. coccus* contiene la mayor cantidad de colorante (ácido carmínico), posible resultado del proceso de domesticación de esta especie (Piña, 1977) y que se refleja en sus características morfológicas: es el insecto con la mayor talla, cubierto de una capa pulvurulenta y de cera seca, en vez de largos filamentos algodonosos como el resto de las especies del género (Pérez-Guerra & Kosztarab, 1992), cuya función principal es protegerle

contra la desecación y la lluvia (Chávez-Moreno *et al*., 2009). Pérez-Guerra & Kosztarab (1992).

D. tomentosus resultó la especie más distante suponiéndose igualmente que es reflejo de su características biológicas y morfológicas únicas y que difieren considerablemente de su género (Mathenge *et al*., 2009). *D. tomentosus* posee un rango de hospederos restringido al subgénero *Cylindropuntia*, sus huevecillos tienen un periodo de incubación más largo (17 días en vez de minutos u horas), sus huevecillos están envueltos en una masa de cera y permanecen junto a la hembra durante el periodo de incubación (en otras especies de *Dactylopius* los huevecillos están encerrados en una red continua, el tamaño de las hembras adultas es más pequeño que el resto de las especies (similar a *D. confusus*) (Mathenge *et al*., 2009), y su anillo anal es no funcional (Pérez-Guerra & Kosztarab, 1992; Rodríguez *et al*., 2001).

Se encontró un grupo de bacterias para cada especie de *Dactylopius*. De especial interés fue END1, una β-Proteobacteria encontrada en todas las especies de *Dactylopius* así como en sus huevecillos y los bacteriocitos de *D. coccus*. Este hecho sugiere que la END1 es de transmisión vertical y pudiera ser un endosimbionte primario, en cual pudo ser adquirido durante la radiación de este género. Su localización puedo confirmarse mediante hibridación *in situ*. Se ha escrito sobre la presencia de la β-Proteobacteria en la familia Pseudococcidae (Coccoidea), otro endosimbionte primario, Candidatus *Tremblaya princeps* ha sido reportada (Thao *et al*., 2002) con una secuencia genética de 16S rRNA 79% idéntica a END1.

La clona nombrada en este trabajo O1 encontrada en los especímenes de *D. opuntiae* pertenece al orden Rickettsiales, así como muchos simbiontes intracelulares y patógenos de eucariontes que han sido investigadas (Weinert *et al*., 2009). Se construyó un árbol filogenético incluyendo la secuencia de la clona O1, tel más cercano NCBI presenta un 85 - 88% de identidad (Candidatus *Hepatincola porcellionum* AY1885, bacteria originaria de China localizada en cangrejos *Eriocheir sinensis* DQ856548, y un endosimbionte de *Acanthoamoeba* sp. AF069963, así como los que corresponden a distintos agrupamientos de Rickettsiales descritos por Weinert *et al*. (2009), muestran que la secuencia a partir de O1 y las tres secuencias relacionadas no forman un grupo con agrupaciones superiores de Rickettsia, significando que pudieran representar taxones nuevos de este grupo. En

contraste con los datos publicados por Pankewitz *et al*., (2007), no se encontró *Wolbachia* en *Dactylopius* spp.

Las secuencias de las clonas de bacterias cercana a bacterias de vida libre (*Massilia* sp., *Herbaspirillum* sp., *Acinetobacter* sp., *Mesorhizobium* sp., y *Sphingomonas* sp.) son similares a las de suelos o plantas que albergan bacterias. La localización de algunas de estas bacterias es el intestino de los insectos, por lo que se supone que al estar localizadas en la savia de las plantas que sirve de alimento para los insectos. Resulta de interés ecológico explorar este hecho a través del análisis de las bacterias endofíticas de las plantas parasitadas por estos insectos. Debe especificarse la localización dentro del insecto.

La descripción de los endosimbiontes de *Dactylopius* spp. presentada en este trabajo sienta un precedente para conocer de la importancia de estas bacterias en la fisiología de sus hospederos. Estas bacterias podrían estar participando en la producción de aminoácidos, vitaminas, compuestos antimicrobianos para el hospedero, o en la degradación de degradación de compuestos tóxicos de la planta, como ocurre con las bacterias presentes en la savia de las que se alimentan otros insectos.

Conclusiones

De acuerdo a los resultados obtenidos los insectos *Dactylopius* guardan una estrecha relación con sus hospederos del género *Opuntia*. Esto se refleja en la correspondencia que tiene el patrón de distribución de *Dactylopius* y el patrón de distribución sus hospederos. Dentro de los resultados obtenidos en este trabajo cabe destacar que el uso combinado del análisis morfológico, químico y molecular brinda mayor aporte a la identificación taxonómica de *Dactylopius* y permite plantear hipótesis a cerca de la distribución, ecología y biología de estas especies. Por ejemplo la especie silvestre *D. opuntiae* no solamente es la más ampliamente distribuida a nivel mundial, y en México, posee el mayor número de hospederos, también, contiene el colorante con el mayor número de componentes. Esta información en conjunto, podría sugerir que *D. opuntiae* es la especie con la mayor adaptación a distintos hábitats y hospederos, lo que le ha permitido la colonización de diferentes lugares y plantas y su exitosa radiación. La complejidad de su pigmento tal vez

es parte de su mecanismo de defensa contra los enemigos naturales y posiblemente es lo que lee permite producir distintos grados de daño a sus hospederos.

En el extremo opuesto, podríamos colocar a *D. tomentosus,* es la especie con la menor distribución mundial y nacional, ha sido observada en el menor número de hospederos y es casi imperceptible en algunos casos. Su pigmento, contiene el menor número de componentes y son los compuestos comunes al género. Además, *D. tomentosus* en este estudio resultó la especie menos agresiva con sus hospederos. El análisis molecular de *D. tomentosus* también muestra una signitifcativa diferencia que la separa como una especie distante del resto del género.

Por su parte *D. confusus y D. opuntiae* cohabitan en el mismo hábitat e incluso comparten hospederos. En este estudio estas dos especies pudieron diferenciarse por caracteres morfológicos y por la composición de su colorante, pero la separación con caracteres moleculares no es tan evidente y esto se repite con *D. ceylonicus.*

Finalmente, la única especie domesticada *D. coccus,* debe su distribución al intenso proceso de domesticación que ha tenido durante los últimos 500 años. Gracias a la selección humana, *D. coccus,* está presnte en los cinco continentes, y ha sido llevada a gran parte de México. El perfil químico del colorante de *D. coccus* difiriere del resto del género por una mayor concetración de ácido carmínico, principalmente, pero este compuesto no es exclusivo de esta especies. Las otras cuatro especies de *Dactylopius* contienen cantidad significativas de este compuesto, lo que las hace fuentes potenciales de este colorante. En conjunto la información morfológica, química, molecular y ecológica resultó complementaria y su integración permitió conocer más de cada especie de *Dactylopius* y sus hospederos, así como de las características de esta asociación.

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