Bacterial communities associated with *Megalopyge opercularis* (Smith) (Lepidoptera: Megalopygidae): exploring poisonous lepidopterans

*Mayra A. Gómez-Govea[1], María de Lourdes Ramírez-Ahuja[1], Luz M. Castellanos-López[2], Irene Ruvalcaba-Ortega[3], Luisa M. Reyes-Cortes[4], Gerardo de Jesús Trujillo-Rodríguez[1], Olga Karina Villanueva-Segura[1], Margarita Martínez-Fierro[5], Ivan Delgado-Enciso[6], Gustavo Ponce-García[7], Ramón Gerardo Rodríguez-Garza[2], Adriana E. Flores-Suárez[7], and Iram P. Rodríguez-Sánchez[1,]**

Abstract

Megalopyge opercularis (Smith) (Lepidoptera: Megalopyridae) is a nocturnal moth of medical importance because it causes adverse immunological reactions in humans. In this study, we determined the microbiota composition of *M. opercularis* at the larval (caterpillars) and adult (moths) stages by next-generation sequencing. DNA was extracted from the caterpillars and moths, and the 16S rRNA prokaryote gene was then amplified and sequenced with next-generation sequencing to assess bacterial richness. Comparison of the microbiota of the caterpillars and adults revealed variation in species composition and diversity. The microbiota of the caterpillars of *M. opercularis* was composed of 259 species, dominated by the families Geodermatophilaceae (12%), Propionibacteriaceae (10.41%), Clostridiaceae (9.63%), and Nitriliruptoraceae (7.72%). In the adult moths, we found 138 species, and the most abundant families were Nostocaceae (24%) and Methylobacteriaceae (21%). Species richness in *M. opercularis* was higher in the caterpillars compared to adults. We determined that only some groups of bacteria could persist from 1 stage to another. The results obtained are essential to know about the ecology of *M. opercularis* and contribute to our understanding of the impact that microorganisms have on the physiological mechanisms of poisonous lepidopterans.

Key Words: moth; caterpillar; gut; symbionts; microbiome; poisonous lepidopterans

Resumen

Megalopyge opercularis (Smith) (Lepidoptera: Megalopyridae) es una especie de polilla nocturna perteneciente a la familia Megalopyridae de importancia médica debido a las reacciones inmunológicas causadas en humanos por sustancias urticantes ubicadas en sus estructuras espinosas. En este estudio, determinamos el microbioma de *M. opercularis* en su estado larval y en su estado adulto. Se extrajo el ADN de larvas y adultos y se amplificó el gen rRNA 16S mediante tecnologías de secuenciación de nueva generación. Las comparaciones del microbioma larval contra el microbioma adulto revelaron variación tanto en la composición como en la diversidad de especie. El microbioma larval de *M. opercularis* está compuesto de 259 especies, dominado por las familias Geodermatophilaceae (12%), Propionibacteriaceae (10.41%), Clostridiaceae (9.63%), y Nitriliruptoraceae (7.72%). En el microbioma adulto encontramos 138 espcies, las familias más abundantes fueron Nostocaceae (24%) y Methylobacteriaceae (21%). La riqueza de especies encontradas en *M. opercularis* fue mayor en las orugas en comparación con los adultos. Determinamos que solo algunos grupos de bacterias podían persistir de una etapa a otra. Los resultados obtenidos son importantes para conocer la ecología de esta polilla y contribuyen a comprender el impacto que tienen los microorganismos en los mecanismos fisiológicos de los lepidópteros venenosos.

Palabras Clave: polilla; oruga; intestino; simbiontes; microbioma; lepidópteros venenosos

[1]Universidad Autónoma de Nuevo León, Facultad de Ciencias Biológicas, Laboratorio de Fisiología Molecular y Estructural, San Nicolás de Los Garza, Nuevo León, C.P. 66450, Mexico; E-mail: mayragee@gmail.com (M. A. G.-G.), lulu.ahuja@hotmail.com (M. L. R.-A.), entogerry36@gmail.com (G. J. T.-R.), kary_trujillo@hotmail.com (O. K. V.-S.), iramrodriguez@gmail.com (I. P. R.-S.)
[2]Universidad Autónoma de Nuevo León, Facultad de Ciencias Biológicas, Departamento de Genética, San Nicolás de Los Garza, Nuevo León, C.P. 66450, Mexico; E-mail: luzmaria0105@gmail.com (L. M. C.-L.), ramon.rodriguezgrz@uanl.edu.mx (R. G. R.-G.)
[3]Universidad Autónoma de Nuevo León, Facultad de Ciencias Biológicas, Laboratorio de Biología de la Conservación y Desarrollo Sostenible, San Nicolás de Los Garza, Nuevo León, C.P. 66450, Mexico; E-mail: i.ruvalcaba.o@gmail.com (I. R.-O.)
[4]Desarrollos Biomédicos y Biotecnológicos de México (DeBBIOM) S.A. de C.V. Belisario Domínguez 2303 Col. Obispado, Monterrey, Nuevo León, Mexico; E-mail: lreyes@novogen.mx (L. M. R.-C.)
[5]Universidad Autónoma de Zacatecas, Laboratorio de Medicina Molecular, Unidad Académica de Medicina Humana, Zacatecas, Zacatecas, C.P. 98160, Mexico; E-mail: margarita.mtz.fierro@gmail.com (M. M.-F.)
[6]Universidad de Colima, Facultad de Medicina, Colima, Colima, C.P. 28040, Mexico; E-mail: ivancoliman@hotmail.com (I. D.-E.)
[7]Universidad Autónoma de Nuevo León, Facultad de Ciencias Biológicas, Laboratorio de Entomología Médica, San Nicolás de Los Garza, Nuevo León, C.P. 66450, Mexico; E-mail: gponcealfa@gmail.com (G. P.-G.), adrflores@gmail.com (A. E. F.-S.)
*Corresponding author; E-mail: iramrodriguez@gmail.com

The order Lepidoptera is one of the most diverse groups of the class Insecta worldwide (Villas-Boas et al. 2018). There are more than 165,000 species and more than 80 families of caterpillars. Approximately 12 families can cause serious human injuries (Villas-Boas et al. 2018) and some are potentially devastating threats to crops and forests (Diaz 2005). One of these families includes insects secreting poisonous substances (Herzig 2019). Caterpillars are endowed with protective spines or hairs that serve as a defense mechanism against its natural predators. These poisonous hairs or spines contain toxins that are passively secreted into the victim upon touch (Severs & Elston 2003; Hossler 2010; Villas-Boas et al. 2018). *Megalopyge opercularis* (Smith) (Lepidoptera: Megalopyridae), or southern flannel moth, is a nocturnal moth that is predominant in the tropical regions of the Americas (Díaz 2005; Avilán et al. 2010). Its distribution includes the south-central and southeastern US from Maryland to Mexico (Fig. 1). In Mexico, studies report this species in Nuevo Leon, Hidalgo, Morelos, Michoacan, Puebla, Oaxaca, Quintana Roo, Merida, Tabasco, Nayarit, San Luis Potosi, Jalisco, and Tamaulipas (Hossler 2010; Arquieta & Martínez 2014; Llorente-Bousquets et al. 2014). The toxic effects caused by *M. opercularis* caterpillar are related to specialized poisonous hairs or spines, which also are referred to as toxic, urticating, or nettling hairs (Foot 1922; Cramér 1946; Avilán et al. 2010). Several studies have shown that these toxins are thermolabile proteins, some possessing enzymatic and proteolytic activities, formic acid, and substances similar to histamine (Severs & Elston 2003; Eagleman 2008).

Insects possess bacterial symbionts that are important for their ecology and evolution (Fromont et al. 2017; Smith et al. 2017). These bacteria participate in the development process, acquisition of nutrients, digestion, immunity, vectorial competition, susceptibility to pathogens, and speciation (Bouchon et al. 2016; Chen et al. 2016; Jehmlich et al. 2016). The microbiota varies according to the host insect's diet, habitat, lifestyle, and stage of development (Hossler 2010; Yun et al. 2014). Imbalance in the microbiota could have consequences such as infections (Dillon & Dillon 2004), impaired detoxification of pesticides (Kikuchi et al. 2012; van den Bosch & Welte 2017; Xia et al. 2017), and disruption of essential physiological functions (Xia et al. 2017). Some studies have evaluated the role of symbiotic microorganisms in caterpillars and moths. For example, in the diamondback moth (*Plutella xylostella* [L.]; Lepidoptera: Plutellidae), bacterial species such as *Enterobacter cloacae* (Jordan), *Enterobacter asburiae* (Jordan) (both Enterobacterales: Enterobacteriaceae), and *Carnobacterium maltaromaticum* Collins (Lactobacillales: Carnobacteriaceae) were found to be involved in processes such as decomposition of plant cell walls, detoxification of phenolic compounds, and amino acid synthesis (Xia et al. 2017). In *Galleria mellonella* (L.) (Lepidoptera: Pyralidae), bacteria such as *Enterococcus mundtii* Collins (Lactobacillales: Enterococcaceae), *Staphylococcus* Rosenbach (Bacillales: Staphylococcaceae), and *Serratia* Bizio (Enterobacterales: Yersiniaceae) were detected. It was observed that in adult hosts, these bacteria involve fitness cost and, hence, the lepidopterans die early (Johnston & Rolff 2015). Other studies have reported that *E. mundtii* can survive, propagate, and persist in the digestive tract of *Spodoptera littoralis* across its life cycle in 2 consecutive generations (Teh et al. 2016). The study of *M. opercularis* in the gut microbiota is essential for a better understanding of its interactions and ecological role in the environment. Thus, in this study, we assessed and compared the composition of the gut microbiota in caterpillars and moths of *M. opercularis*. Knowledge of the abundance and role of essential host bacteria during metamorphosis provides a basis for studying physiological mechanisms in Lepidoptera, which also is highly important from several perspectives linked to medicine, agriculture, and ecology.

Fig. 1. Records of *Megalopyge opecularis* in North America.

Materials and Methods

COLLECTION SITES

The caterpillars were collected from the leaves of *Quercus virginiana* Mill. (Fagaceae) in Jardines Floridos, municipality of Pesqueria, Nuevo León, Mexico (25.709111°N, 100.104555°W) during 2018. Thirty caterpillars in the last larval instar were selected; 15 caterpillars then were deposited in sterile plastic 15 mL Eppendorf® tubes with sterile tweezers (15) (Corning, Tewksbury, Massachusetts, USA) for immediate extraction of DNA, whereas 15 caterpillars were allowed to continue through pupation. These specimens were reared in a cleaned and disinfected room at 25 ± 2 ℃ at 65 to 85% relative humidity on branches of *Q. virginiana* to simulate native growth conditions until their emergence so as to obtain the adult sample and subsequently extract DNA.

GUT SAMPLE COLLECTION

Whole gut extraction was performed according to Liu et al. (2018) with some modifications. Before dissection, caterpillars and adults were sterilized with 100% ethanol for 3 min and rinsed with sterile deionized water 3 times. Whole guts of 15 caterpillars and 15 adults were dissected on a plate that contained 2 mL of sterile phosphate-buffered solution (10 mmol per L, pH 7.4; Ambion, ThermoFisher Scientific, Madison, Wisconsin, USA) using a pair of sterilized entomological forceps (flame-sterilized) under a stereomicroscope (Leica MZ16,

1.6×, Leica Microsystems, Wetzlar, Germany). Guts were deposited in plastic 1.5 mL Eppendorf® tubes with DNA shield and stored at −70 °C for DNA extraction and sequencing.

MICROBIOTA ASSESSMENT

DNA Extraction

Frozen gut of caterpillars and moths was homogenized independently in conical tubes with distilled water and centrifuged at 4500 rpm. DNA then was extracted using the QIAamp DNA Microbiome kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Nucleic acids were quantified for each sample using the Qubit® 2.0 (ThermoFisher Scientific, Wilmington, Delaware, USA) fluorometer and the Qubit dsDNA HS Assay reagent (ThermoFisher Scientific) according to the manufacturer's instructions. Samples were stored at −80 °C until use.

rRNA Amplification and Sequencing by Ion Torrent

The ribosomal subunit 16S hypervariable region was amplified from 5 ng of microbial DNA using the Ion 16S Metagenomics kit (ThermoFisher Scientific) in 2 PCR steps with the set of primers of the V3–V4 region of the 16S rRNA gene. Amplicons were purified using Agencourt AMPure XP magnetic beads (Beckman Coulter, Inc., Atlanta, Georgia, USA) and were later quantified with the Agilent High Sensitivity DNA kit (Agilent Technologies, Santa Clara, California, USA) in an Agilent 2100 Bioanalyzer. Fifty ng of mixture and amplicons were used to develop the DNA libraries using the Ion Plus Fragment Library kit (ThermoFisher Scientific) instructions. DNA libraries were purified using Agencourt AMPure XP magnetic beads (Beckman Coulter) and were later quantified with the Agilent High Sensitivity DNA kit (Agilent Technologies) in an Agilent 2100 Bioanalyzer. Each sample was adjusted to a concentration of 26 pM and subjected to polymerase chain reaction in an emulsion with the Ion PGM Hi-Q OT2 kit (ThermoFisher Scientific) in a OneTouch 2 system (Life Technologies, Grand Island, New York, USA) with subsequent enrichment with the Dynabeads MyOne Streptavidin C1 Beads kit in an Ion OneTouch ES (Life Technologies). The sequencing of each library was performed with an Ion PGM system using the Ion PGM Hi-Q Sequencing kit (ThermoFisher Scientific) in a 314 V2 chip. Base calling was performed with Torrent Suite version 4.4.2 (Life Technologies) and FastQC version 3.4.1.1 (Life Technologies) to generate FastQ files for each sample.

DATA ANALYSIS

The sequences obtained were exported to Ion Reporter version 5.2 (ThermoFisher Scientific). Grouping and taxonomic assignments to operational taxonomic units were determined using the cured databases MicroSEQ 16S Reference Library v2013.1 and Greengenes v13.5 (Life Technologies). Operational taxonomic unit relative abundance (%) was computed and compared between caterpillar and moth adult stages at different taxonomic ranks. For the taxonomic assignment, sequences were grouped according to sequence similarity. Group membership was determined according to a threshold on sequence similarity, for instance 97%. Low proportions in samples were removed (< 1%).

COMMUNITY PARAMETERS

Richness

We calculated Chao2 estimator of species richness (Chao 1984, 1987) in EstimateS 9.1.0 (Colwell 2013), which is recommended for small samples (Colwell & Coddington 1994; Unterseher et al. 2008). These values were used as reference of expected microbiota richness to estimate sample coverage (S obs per S Chao2).

Diversity

We estimated and compared microbiota diversity ordering profiles for each developmental stage (larvae and adult) obtaining a continuum of diversity values that gives the highest to the least weight to rare species and that better represents the complexity of this parameter for a community (Chao et al. 2014). We used the exponential of the Rényi's (1961) diversity ordering family, also known as Hill's (1973) diversity. We particularly estimated 3 values (Hill's numbers), where $\alpha = 0, 1, 2$, corresponding to S (number of species per operational taxonomic units), $e^{H'}$ (exponential of Shannon's index), and 1/D (reciprocal of the Simpson's index), respectively. We also obtained 95% confidence intervals from bootstrapping procedure (2,000 replicates). Diversity values $e^{H'}$ and 1/D were compared between stages with Hutcheson's *t*-tests (1970). All analyses were performed in PAST 7.0 (Hammer et al. 2001).

Similarity

We estimated Chao's Jaccard Abundance-based similarity index (Chao et al. 2005) and its 95% confidence intervals from bootstrapping procedure (10,000 replicates) between both developmental stages and for each taxonomic rank (species to class). This index was chosen because it is recommended for samples with a high number of rare species and for under-sampled conditions (Chao et al. 2005). All analyses were performed in EstimateS 9.1.0 (Colwell 2013).

Results

In this study, gut microbiota of 2 stages of *M. opercularis* (caterpillar and adult moth) were evaluated. The most abundant phylum in adult moths was Proteobacteria (54.6%) followed by Cyanobacteria (24.9%), while Actinobacteria (32.3%), Proteobacteria (31.3%), and Firmicutes (24.5%) accounted for 88.1% of the bacterial community in caterpillars. In adults, phylum Firmicutes represented only 7.1% of the bacterial community, whereas it was one of the most representative phylum in caterpillars (Fig. 2).

Seven classes were detected in both stages (Actinobacteria, Alphaproteobacteria, Bacilli, Gamaproteobacteria, Clostridia, Nostocophycideae, and Betaproteobacteria). The most abundant class in caterpillars was Alphaproteobacteria, with a proportion of 32.2%, whereas in adults it was 48.5%. The class Nostocophycideae represented only 10.5% in caterpillars and showed a clear increase in adults (24.1%). There was no difference in the proportion of the class Actinobacteria in the 2 stages (caterpillars 12.1% and adults 12.8%), unlike the class Bacilli which showed a decrease from caterpillars (12.3%) to adults (4.9%). Moreover, the Gammaproteobacteria accounted for 24.3% in caterpillars compared to 4.3% in adults. Clostridia showed similar percentages in caterpillars (2.5%) and in adults (2.1%). The class Betaproteobacteria showed 4.3% in caterpillars and 2.5% in adults. The other classes represented the minority, < 1%.

At the order level, Actinomycetales showed the highest proportion (32%) in caterpillars and Rhizobiales (30%) in adults. The orders found in adults were Nostocales (24.1%), Actinomycetales (12.5%), Rhodobacterales (7.7%), Caulobacterales (5.1%), Bacillales (3.7%), Sphingomonadales (3.1%), Pseudomonadales (3.0%), Rhodospirillales (2.6%), Clostrodiales (2.1%), Burkholderiales (1.8%), and Lactobacillales (1.2%). Clostridiales (12.3%), Rhodobacterales (11.4%), Nostocales (10.5%), Bacillales (9.5%), Rhizobiales (5.7%), Rhodospirillales (4.1%), Lactobacillales (2.6%), Pseudomonadales (2.6%), Sphingomonadales

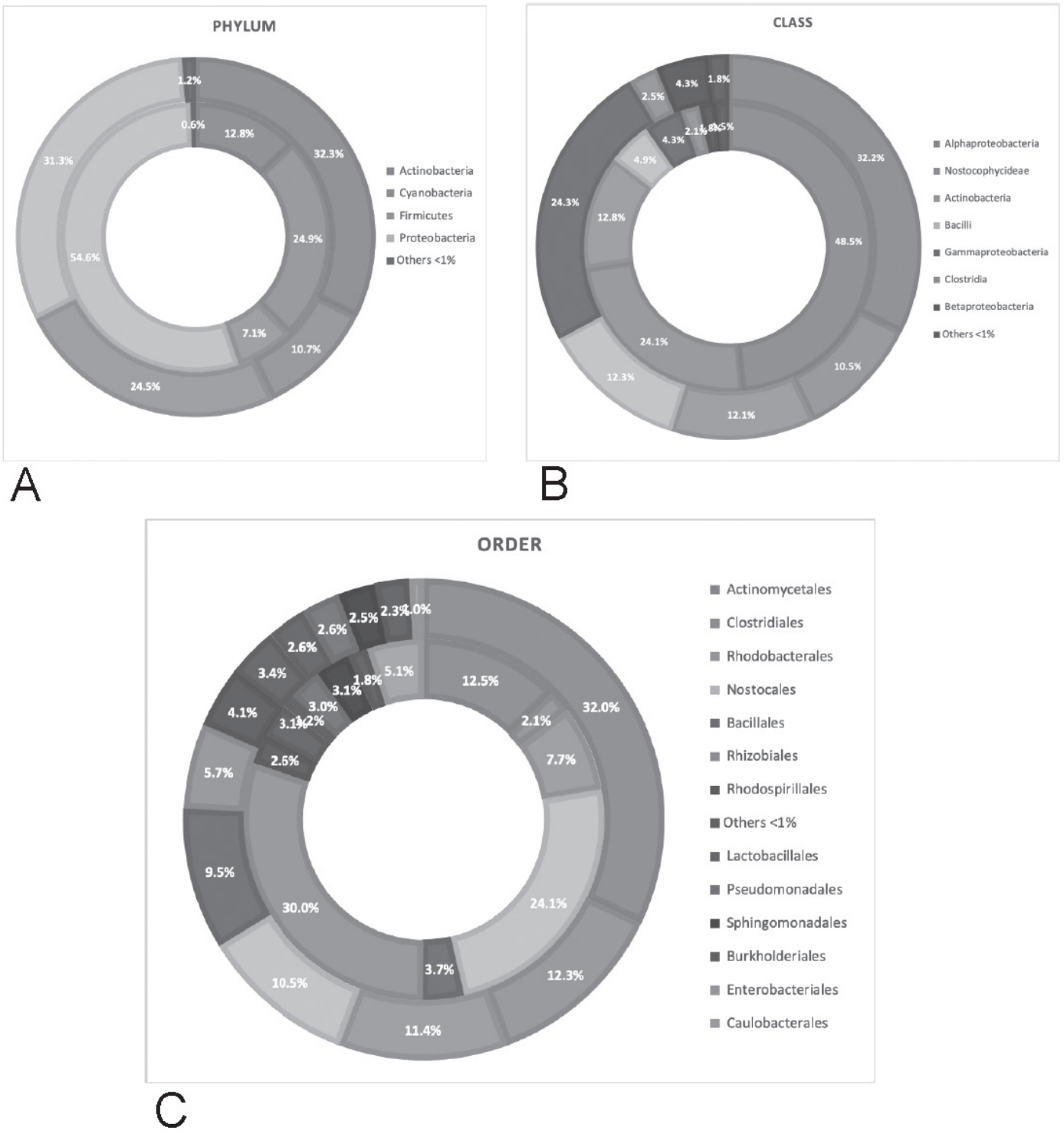

Fig. 2. Dominant bacterial community found in *Megalopyge opercularis*. *Inner circle corresponds to percentage of bacterial community in moths. *Outer circle corresponds to percentage of bacterial community in caterpillars.

(2.5%), Burkholderiales (2.3%), and Enterobacteriales (1%) were the orders found in caterpillars. Thirteen orders were found in both stages with a percentage < 1% (Fig. 3).

Our analysis showed that 31.8% of the families in caterpillars were in low proportion (< 1%). The other 67.6% of families in caterpillars included Geodermatophilaceae, Propionibacteriaceae, Clostridiaceae, Nitriliruptoraceae, Micrococcaceae, Dermatophilaceae, Planococcaceae, Corynebacteriaceae, Rhodobacteraceae, Intrasporangiaceae, Comamonadaceae, Rhizobiaceae, and Streptococcaceae (Fig. 3).

Most of the gut bacterial families detected in adults belonged to the phylum Cyanobacteria (Nostocaceae 24%) and Proteobacteria (Methylobacteriaceae 21%). Only 16.5% of the families showed < 1% abundance. Bacteria belonging to the Rhodobacteraceae, Geodermatophilaceae, Caulobacteraceae, Rhizobiaceae, Sphingomonadaceae,

Pseudomonadaceae, Xanthobacteraceae, Comamonadaceae, Micrococcaceae, Propionibacteriaceae, and Peptostreptococcaceae represented 56% of families in adult moths (Fig. 3).

The genus *Methylobacterium* (Hyphomicrobiales: Methylobacteriaceae) (20.92%) was the most abundant in adults compared with caterpillars, in which it was present at less than 1%. The species detected for this genus were *Methylobacterium adhaesivum* Gallego, *Methylobacterium gregans* Kato, *Methylobacterium hispanicum* Gallego, *Methylobacterium populi* (Van Aken) Green & Ardley, *Methylobacterium radiotolerans* (Ito & Iizuka) Green & Bousfield, *Methylobacterium rhodesianum* (Green) Green & Ardley, and *Methylobacterium thiocyanatum* (Wood) Green & Ardley. Similar profiles were observed in *Blastococcus* (Geodermatophilales: Geodermatophilaceae), which showed 3.28% in adults, but < 1% in caterpillars. The species found belonging to this genus were *Blastococcus aggregatus* Ahrens & Moll, *Blastococcus endophyticus* Zhu, and *Blastococcus saxobsidens* Urzi. *Paracoccus* (Rhodobacterales: Rhodobacteraceae) was the most abundant genus in caterpillars (10.6%), where the species *Paracoccus denitrificans* Davis, *Paracoccus sphaerophysae* Deng, and *Paracoccus tibetensis* Zhu were detected. Most of the genera in both stages showed an abundance < 1% (Fig. 4). A total of 259 species were found in caterpillars (146 in percentages > 1%), and in the same way, 138 species were identified in adults (68 in percentages > 1%). Some of the species identified were present in both stages (*P. tibetensis*, *Cutibacterium acnes* Scholz & Kilian, and *Pseudomonas lini* Delorme) (Table 1).

Chao2 estimator predicted 597 (95% CI = 517–712) operational taxonomic units indicating that our results represent only 55.6% of the predicted richness (95% CI = 46.7–64.2%) for the bacterial community of this species at both developmental stages. Diversity profiles showed that caterpillar bacterial community was significantly higher than moth's in all diversity orders (Fig. 5; Table 2). At the species level, there is a probability of 63% those 2 bacteria belong to a shared species between both developmental stages. This probability increased to 99% at the order and higher taxonomic levels (Table 3).

Discussion

Insects are the most diverse and abundant animal clade, in numbers of species globally, in ecological habits, and in biomass (Basset et al. 2012; Engel & Moran 2013). Little information exists about microbial profiles of poisonous lepidopterans, although comprising the second order with major impact on ecosystems. Thus, in this study we determined the microbiota of caterpillars and adults of *M. opercularis*. The phylum Proteobacteria is one of the bacteria most detected in the gut of lepidopterans (Paniagua Voirol et al. 2018). This phylum participates in many essential functions such as digestion, nutrient assimilation, the

Fig. 3. Dominant bacterial families found in *Megalopyge opercularis*: (A) corresponds to percentage of bacterial community in caterpillars; (B) corresponds to percentage of bacterial community in moths.

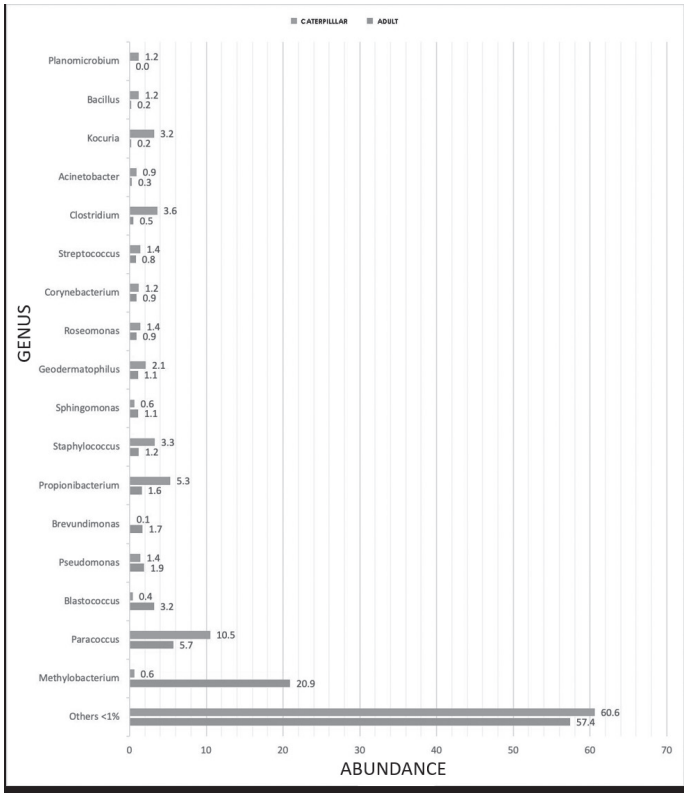

Fig. 4. Percentage of bacterial community at genus level in *Megalopyge opercularis*. Orange bars correspond to caterpillars and blue bars correspond to moths.

Table 1. Species detected in the bacterial community of *Megalopyge opercularis (*caterpillars and adult in percentages >1%).

Order	Family	Genus	Species	Larvae	Adult
Bacillales	Bacillaceae	*Bacillus*	*B. decisifrondis*		
			B. kribbensis		
			B. nealsonii		
	Planococcaceae	*Planomicrobium*	*P. flavidum*		
			P. okeanokoites		
	Staphylococcaceae	*Staphylococcus*	*S. hominis*		
			S.passteuri		
			S. saccharolyticus		
			S. warneri		
			S. xylosus		
Geodermatophilales	Geodermatophilaceae	*Blastococcus*	*B. aggregatus*		
			B. endophyticus		
			B. saxobsidens		
Caulobacterales	Caulobacteraceae	*Brevundimonas*	*B. diminuta*		
Propionibacteriales	Propionibacteriaceae	*Cutibacterium*	*C. acnes*		
			C. granulosum		
Clostridiales	Peptostreptococcaceae	*Clostridium*	*C. botulinum*		
			C. butycium		
			C. disporicum		
			C. grantii		
			C. haemolyticum		
			C. novyi		
			C. perfringens		
			C. bartletti		
			C. ghonii		
			C. hiranonis		
Geodermatophilales	Geodermatophilaceae	*Geodermatophilus*	*G. arenarius*		
			G. obscurus		
			G. siccatus		
Micrococales	Micrococcaceae	*Kocuria*	*K. flava*		
			K. marina		
			K. palustris		
			K. rhizophila		
			K. rosea		
			K. turfanensis		
Hyphomicrobiales	Methylobacteriaceae	*Methylobaceterium*	*M. adhaesivum*		
			M. gregans		
			M. hispanicum		
			M. populi		
			M. radiotolerans		
			M. rhodesianum		
			M. thiocyanatum		
Rhodobacterales	Rhodobacteraceae	*Paracoccus*	*P. denitrificans*		
			P. sphaerophysae		
			P. tibetensis		
			P. yeei		
Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*P. bauzanensis*		
			P. chlororaphis		
			P. formosensis		
			P. lini		
			P. veronii		
			P. pseudoalcaligenes		
Rhodospirillales	Acetobacteraceae	*Roseomonas*	*R. pecuniae*		
Lactobacillales	Streptococcaceae	*Streptococcus*	*S. cristatus*		
			S. infantis		
			S. pseudopneumoniae		
			S. salivarius		

Table 1. (Continued) Species detected in the bacterial community of *Megalopyge opercularis (*caterpillars and adult in percentages >1%).

Order	Family	Genus	Species	Larvae	Adult
			S. sanguinis		
			S. sinensis		
			S. thermophilus		

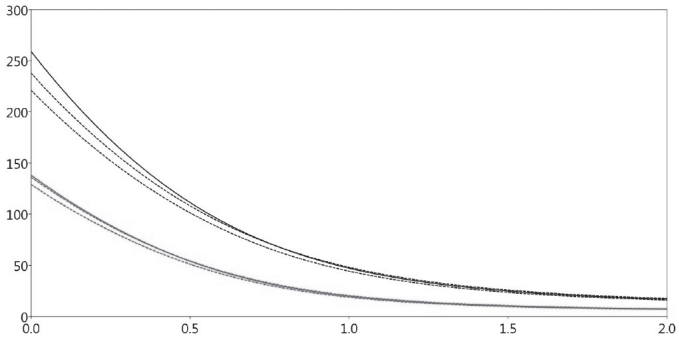

Fig. 5. Diversity order profile (Rényi 1961, Hill 1973) and 95% confidence intervals (dotted) for each developmental stage: larvae (caterpillar, black) and adult (moth, red).

use of certain photochemical substances, the synthesis of amino acids and vitamins, and the detoxification of insecticides in several insect species. The presence of this phylum has been observed in moths such as cabbage white, *Pieris rapae* (L.) (Lepidoptera: Pieridae) (Robinson et al. 2010), and some phylogenetically related to *M. opercularis* such as *Lymantria dispar* (L.) (Lepidoptera: Erebidae) (Broderick et al. 2004), *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) (Xiang et al. 2006), and *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) (Chen et al. 2016), where it is common to find it in lepidopterans. It is known that Actinobacteria are found in many different habitats including human and animal environments, and also in insects such as ants, bees, and wasps (Montoya et al. 2017; Ramirez-Ahuja et al. 2019). The role of Actinobacteria in insects is to contribute as facultative symbionts in nutrient supplementation such as vitamin B (Salem et al. 2013). Our results showed a large reduction in the proportion of this phylum from caterpillars to adults, where this change was due to the nutritional requirements during the larval stage compared with the adult stage (Salem et al. 2013). Our results are similar to other reports in *S. littoralis*; it is known that caterpillars harbor a bacterial community which is principally composed of Firmicutes, and after metamorphosis they undergo a remarkable change in the composition of the bacterial community. Likewise, moths exhibit a large decrease in Firmicutes abundance and an increased abundance of Proteobacteria (Chen et al. 2016). Another phylum found in larvae was Cyanobacteria. This phylum is another dominant phylum described in the gut microbiota among species of lepidopterans such as *Mythimna separata* Walker (Lepidoptera: Noctuidae) (He et al. 2013) and *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) (Gao et al. 2019) and other insects such as carabid beetles. This phylum has been described as playing a role in the consumption of insect prey and seeds (Lundgren & Lehman 2010).

Table 2. Hill's numbers (N_0 [*S*], N_1[$e^{H'}$], and N_2[*1/D*] mean values (95% confidence interval) for adult and larvae. Different letters indicate significant difference ($P < 0.05$).

Microbiota diversity	Adult (moth)	Larvae (caterpillar)
S	138	259
$e^{H'}$	19.7 (18.7–20.3)[a]	47.1 (44.3–48.0)[b]
1/D	7.2 (6.9–7.5)[a]	16.8 (15.9–17.6)[b]

Table 3. Shared and unique operational taxonomic units, and Chao-Jaccard index of microbiota similarity (95% confidence interval) between larvae (caterpillar) and adult stage of *Megalopyge opercularis.*

Taxonomic rank	Larvae	Adult	Shared	Chao-Jaccard index mean
Phylum	9	7	5	0.99 (0.83–1.00)
Class	25	16	14	0.99 (0.83–1.00)
Order	47	32	27	0.99 (0.83–1.00)
Family	105	66	55	0.94 (0.78–1.00)
Genus	153	89	47	0.43 (0.27–0.59)
Species	259	138	66	0.63 (0.47–0.79)

Lepidopterans are an order of insects that undergo a holometabolous metamorphosis, in which their body is entirely re-shaped. During metamorphosis, lepidopterans are accompanied by morphological rearrangements and changes in diet, having a direct impact on the composition of the gut microbiota. Also, certain taxonomic groups persist throughout the entire life cycle and others accompany them just in specific stages (Teh et al. 2016; Paniagua Voirol et al. 2018). Hence, our results showed that some groups of bacteria persisted from larval to adult stage, such as *P. tibetensis*, *C. acnes*, and *P. lini*.

Several studies have shown that members of the order Actinomycetales are implicated in metabolic activities and nutrient acquisition (Gomes et al. 2018). The order Actinomycetales was found in 32.8% of caterpillars of *M. opercularis*. It is known that the midguts of lepidopteran larvae show extreme alkalinity (pH 11–12), and digestive enzymes are adapted to the alkaline conditions (Appel & Martin 1990; Harrison 2001). In addition, *M. opercularis* is a large caterpillar that takes between 5 and 6 molts that require high consumption and protein synthesis, as well as essential fatty acids to perform their metamorphosis (Cookman et al. 1984). The presence of this group at this stage could be to acquire the energy necessary to complete its life cycle prior to metamorphosis. However, a large reduction in this order was found in the adult stage of *M. opercularis* (12.5%). This could be associated with changes in diet from caterpillar to adults and the decrease in metabolic activity after metamorphosis.

In contrast, Nostocales showed a remarkable increase in adults (24.1%) compared with caterpillars. Nostocales is a symbiont taxon involved in nitrogen fixation (Futuyma & Antonovics 1992) and has been associated with the production of indole metabolites and siderophores with antagonistic activity against pathogenic bacteria and fungi (Maxwell et al. 1994; Dillon & Dillon 2004; Indiragandhi et al. 2007). The adult stage feeds on nectar, where most of the time dietary nitrogen is particularly limiting. Therefore, the symbiotic association with this group of bacteria is beneficial by fixing nitrogen and converting it into physiological nitrogen compounds (Garrido-Oter et al. 2018). Here, we observed that Rhizobiales underwent a 30% increase in moths versus 5.7% in caterpillars, which agrees with previous studies (Indiragandhi et al. 2007).

The genus *Methylobacterium* was detected in high proportion in moths. Some of the detected species belonging to this genus have been

associated with the environment in soil, water, and leaf surfaces. Also, it has been isolated from human bodily fluids and as an opportunistic pathogen such as *M. thiocyanatum* (Lai et al. 2011), *M. gregans* (Kato et al. 2008), and *M. populi* (Van Aken et al. 2004).

In caterpillars, the results revealed that the most abundant genus was *Paracoccus* (10.6%). The species *Paracoccus yeei* Daneshvar (Rhodobacterales: Rhodobacteraceae) found in this study is an environmental bacterium that is suspected to be responsible for ocular infections and peritonitis in humans (Courjaret et al. 2014; Sastre et al. 2016). Another genus found in this study was *Cutinobacterium*, which has been reported previously in other insects. In addition, *C. acnes* Scholz & Kilian (Propionibacteriales: Propionibacteriaceae) has been found in the dipteran *Anopheles gambiae* Giles (Diptera: Culicidae) (Wang et al. 2011), species of *Lutzomyia* França (Diptera: Psychodidae) (Sant'Anna et al. 2012), and wasp parasitoids (Ramirez-Ahuja et al. 2019) as a component of the natural microbiota. The role of this microorganism in insect physiology correlates with the production of antibiotic barriers, the delivery of nutrients, and nitrogen fixation (Zucchi et al. 2012). Also, species of *Bacillus* Cohn (Bacillales: Bacillaceae), *Pseudomonas* Migula (Pseudomonadales: Pseudomonadaceae), *Kocuria* Stackebrandt (Micrococcales: Micrococcaceae), *Staphylococcus* Rosenbach (Bacillales: Staphylococcaceae), *Streptococcus* Rosenbach (Lactobacillales: Streptococcaceae), and *Clostridium* Prazmowski (Clostridiales: Clostridiaceae) were found. These kinds of bacteria are considered ubiquitous as they are common in soil, plants, and water, and are found commonly in insect microbiota (Orberá et al. 2005; Skarin & Segerman 2014; Redford et al. 2017; Toda et al. 2017).

Bacteria inhabit bodies of insects establishing different levels of mutualistic relationships. Only a limited number of them have a known function in insects. Even though some are pathogens in humans, many of them participate in essential functions within the insect. Less than 0.1% of the recognized lepidopteran species have been screened for bacterial associates, which means that our knowledge of bacterial associates in Lepidoptera is still limited.

It is well known that gut bacterial communities depend on the host, the diet, and the host plant, and it is not surprising that gut bacterial communities differ considerably between caterpillars and adult moths (Staudacher et al. 2016; Xia et al. 2017). This may be due to the physiological changes through their life cycle. Compared with other insects, lepidopterans harbor a wide diversity of microbial communities (Paniagua Voirol et al. 2018). The complex anatomy of the intestine of lepidopterans determines the establishment of a bacterial community in high densities, but at the same time the lack of compartments impedes the persistence of some groups of bacteria (Staudacher et al. 2016). In our study, we observed that both stages had a complex bacterial community in which only some groups of bacteria could persist from 1 stage to another. Also, we observed that the species richness found in *M. opercularis* was higher in the caterpillars compared to the adults. The bacteria found have important niches, so it is interesting to elucidate their function with complementary investigations with other phylogenetically related species.

Acknowledgments

The authors are grateful to Sergio Lozano-Rodriguez for the critical review of the manuscript, and A. Leyva provided English editing of the manuscript. All authors contributed equally to this work.

References Cited

Appel HM, Martin MM. 1990. Gut redox conditions in herbivorous lepidopteran larvae. Journal of Chemical Ecology 16: 3277–3290.

Arquieta SC, Martínez HQ. 2014. Orugas urticantes (Insecta: Lepidoptera) de importancia médica en el estado de Nuevo León, México. Artrópodos y Salud 1: 45–51.

Avilán L, Guerrero B, Álvarez E, Rodríguez-Acosta A. 2010. Description of envenomation by the "gusano-pollo" caterpillar (*Megalopyge opercularis*) in Venezuela. Investigación Clínica 51: 127–132.

Basset Y, Cizek L, Cuénoud P, Didham RK, Guilhaumon F, Missa O, Novotny V, Ødegaard F, Roslin T, Schmidl J, Tishechkin AK. 2012. Arthropod diversity in a tropical forest. Science 338: 1481–1484.

Bouchon D, Zimmer M, Dittmer J. 2016. The terrestrial isopod microbiome: an all-in-one toolbox for animal-microbe interactions of ecological relevance. Frontiers in Microbiology 7: 1472. doi.org/10.3389/fmicb.2016.01472

Broderick NA, Raffa KF, Goodman RM, Handelsman J. 2004. Census of the bacterial community of the gypsy moth larval midgut by using culturing and culture-independent methods. Applied and Environmental Microbiology 70: 293–300.

Chao A. 1984. Nonparametric estimation of the number of classes in a population. Scandinavian Journal of Statistics 11: 265–270.

Chao A. 1987. Estimating the population size for capture-recapture data with unequal catchability. Biometrics 43: 783–791.

Chao A, Chazdon RL, Colwell RK, Shen TJ. 2005. A new statistical approach for assessing similarity of species composition with incidence and abundance data. Ecology Letters 8: 148–159.

Chao A, Chiu CH, Jost L. 2014. Unifying species diversity, phylogenetic diversity, functional diversity, and related similarity and differentiation measures through Hill numbers. Annual Review of Ecology, Evolution, and Systematics 45: 297–324.

Chen B, Teh BS, Sun C, Hu S, Lu X, Boland W, Shao Y. 2016. Biodiversity and activity of the gut microbiota across the life history of the insect herbivore *Spodoptera littoralis*. Scientific Reports 6: 29505. doi.org/10.1038/srep29505

Colwell RK. 2013. EstimateS 9.1.0. Statistical estimation of species richness and shared species from samples. Version 9.1. University of Connecticut, Storrs, Connecticut, USA.

Colwell RK, Coddington JA. 1994. Estimating terrestrial biodiversity through extrapolation. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences 345: 101–118.

Cookman JE, Angelo MJ, Slansky F, Nation JL. 1984. Lipid content and fatty acid composition of larvae and adults of the velvetbean caterpillar, *Anticarsia gemmatalis*, as affected by larval diet. Journal of Insect Physiology 305: 523–527.

Courjaret JC, Drancourt M, Hoffart L. 2014. *Paracoccus yeei* keratitis in a contact lens wearer. Eye Contact Lens 40: e21–e22.

Cramér H. 1946. Mathematical Methods of Statistics. Princeton University Press, Princeton, New Jersey.

Diaz JH. 2005. The evolving global epidemiology, syndromic classification, management, and prevention of caterpillar envenoming. American Journal of Tropical Medicine and Hygiene 72: 347–357.

Dillon RJ, Dillon VM. 2004. The gut bacteria of insects: nonpathogenic interactions. Annual Reviews in Entomology 49: 71–92.

Douglas AE. 1992. Symbiosis in evolution, pp. 354–382 *In* Futuyma D, Antonovics J [Eds.], Oxford Surveys in Evolutionary Biology, Vol. 8. Oxford University Press, New York, USA.

Eagleman DM. 2008. Envenomation by the asp caterpillar (*Megalopyge opercularis*). Clinical Toxicology 46: 201–205.

Engel P, Moran NA. 2013. The gut microbiota of insects – diversity in structure and function. FEMS Microbiology reviews 37: 699–735.

Foot NC. 1922. Pathology of the dermatitis caused by *Megalopyge opercularis*, a Texan caterpillar. The Journal of Experimental Medicine 35: 737–753.

Fromont C, Riegler M, Cook JM. 2017. Relative abundance and strain diversity in the bacterial endosymbiont community of a sap-feeding insect across its native and introduced geographic range. Microbial Ecology 74: 722–734.

Gao X, Li W, Luo J, Zhang L, Ji J, Zhu X, Wang L, Zhang S, Cui J. 2019. Biodiversity of the microbiota in *Spodoptera exigua* (Lepidoptera: Noctuidae). Journal of Applied Microbiology 126: 1199–1208.

Garrido-Oter R, Nakano RT, Dombrowski N, Ma KW, McHardy AC, Schulze-Lefert P. 2018. Modular traits of the Rhizobiales root microbiota and their evolutionary relationship with symbiotic *Rhizobia*. Cell Host and Microbe 24: 155–167.

Gomes ED, Dias LR, Rita de Cassia M. 2018. Actinomycetes bioactive compounds: biological control of fungi and phytopathogenic insect. African Journal of Biotechnology 17: 552–559.

Hammer Ø, Harper DA, Ryan PD. 2001. PAST: paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4: 1–9.

Harrison JF. 2001. Insect acid-base physiology. Annual Review of Entomology 46: 221–250.

He C, Nan, X, Zhang Z, Li M. 2013. Composition and diversity analysis of the gut bacterial community of the Oriental armyworm, *Mythimna separata*, determined by culture-independent and culture-dependent techniques. Journal of Insect Science 13: 165. doi: 10.1673/031.013.16501

Herzig V. 2019. Arthropod assassins: crawling biochemists with diverse toxin pharmacopeias. Toxicon 158: 33–37.

Hill MO. 1973. Diversity and evenness: a unifying notation and its consequences. Ecology 54: 427–431.

Hossler EW. 2010. Caterpillars and moths. Part II. Dermatologic manifestations of encounters with Lepidoptera. Journal of the American Academy of Dermatology 62: 29–30.

Hutcheson K. 1970. A test for comparing diversities based on the Shannon formula. Journal of Theoretical Biology 29: 151–154.

Indiragandhi P, Anandham R, Madhaiyan M, Poonguzhali S, Kim GH, Saravanan VS. 2007. Cultivable bacteria associated with larval gut of prothiofos-resistant, -susceptible, and field-caught populations of diamondback moth-*Plutella xylostella* and their potential for antagonism towards entomopathogenic fungi and host insect nutrition. Journal of Applied Microbiology 103: 2664–2674.

Jehmlich N, Müller M, Meyer S, Tischer A, Potthast K, Michalzik B, von Bergen M. 2016. Proteome data on the microbial microbiome of grasshopper feces. Data in Brief 9: 1147–1154.

Johnston PR, Rolff J. 2015. Host and symbiont jointly control gut microbiota during complete metamorphosis. PLoS Pathogens 11: e1005246. doi: 10.1371/journal.ppat.1005246

Kato Y, Asahara M, Goto K, Kasai H, Yokota A. 2008. *Methylobacterium persicinum* sp. nov., *Methylobacterium komagatae* sp. nov., *Methylobacterium brachiatum* sp. nov., *Methylobacterium tardum* sp. nov. and *Methylobacterium gregans* sp. nov., isolated from freshwater. International Journal of Systematic and Evolutionary Microbiology 58: 1134–1141.

Kikuchi Y, Hayatsu M, Hosokawa T, Nagayama A, Tago K, Fukatsu T. 2012. Symbiont-mediated insecticide resistance. Proceedings of the National Academy of Sciences 109: 8618–8622.

Lai CC, Cheng A, Liu WL, Tan CK, Huang YT, Chung KP, Hsueh PR. 2011. Infections caused by unusual *Methylobacterium* species. Journal of Clinical Microbiology 49: 3329–3331.

Liu S-H, Chen Y, Li W, Tang G-H, Yang Y, Jiang H-B, Dou W, Wang J-J. 2018. Diversity of bacterial communities in the intestinal tracts of two geographically distant populations of *Bactrocera dorsalis* (Diptera: Tephritidae). Journal of Economic Entomology 111: 2861–2868.

Llorente-Bousquets J, Vargas-Fernández I, Luis-Martínez A, Trujano-Ortega M, Hernández-Mejía BC, Warren AD. 2014. Biodiversidad de Lepidoptera en México. Revista Mexicana de Biodiversidad 85: 353–371.

Lundgren JG, Lehman RM. 2010. Bacterial gut symbionts contribute to seed digestion in an omnivorous beetle. PLoS One 5: e10831. doi: 10.1371/journal.pone.0010831

Maxwell PW, John GC, Webster M, Dunphy GB. 1994. Stability and activities of antibiotics produced during infection of the insect *Galleria mellonella* by two isolates of *Xenorhabdus nematophilus*. Applied and Environmental Microbiology 60: 715–721.

Montoya LM, Triana OJ, Alzate F, Moreno XC, Cadavido GE. 2017. 16S rRNA gene amplicon sequencing reveals dominance of *Actinobacteria* in *Rhodnius pallescens* compared to *Triatoma maculata* midgut microbiota in natural populations of vector insects from Colombia. Acta Tropica 178: 327–332.

Orberá T, Pérez I, Ferrer D, Cortés N, González Z. 2005. Aislamiento de cepas del genero *Bacillus* sp. con potencialidades para la bioprotección y la estimulación del crecimiento vegetal. Revista Cubana de Química 17: 189–195.

Paniagua Voirol LR, Frago E, Kaltenpoth M, Hilker M, Fatouros NE. 2018. Bacterial symbionts in Lepidoptera: their diversity, transmission, and impact on the host. Frontiers in Microbiology 9: 556. doi: 10.3389/fmicb.2018.00556

Ramírez-Ahuja ML, Gómez-Govea MA, Lugo-Trampe A, Borrego-Soto G, Delgado-Enciso I, Ponce-Garcia G, Martínez-Fierro ML, Ramírez-Valles EG, Treviño V, Flores-Suarez AE, Rodríguez-Sanchez IP. 2019. Microbiota of *Telenomus tridentatus* (Platygastroidea: Scelionidae): an unwanted parasitoid. Journal of Applied Entomology 143: 834–841.

Redford T, Cubberley JC, Hengeveld P, Zabek E, Britton AP. 2017. Myocardial necrosis associated with *Clostridium novyi* infection in a bighorn sheep (*Ovis canadensis*). Journal of Wildlife Diseases 53: 695–698.

Rényi A. 1961. On measures of entropy and information, pp. 547–561 *In* Neyman J [Ed.], Proceedings of the Fourth Berkeley Symposium on Mathematical Statistics and Probability, Volume 1: Contributions to the Theory of Statistics. University of California Press, Berkeley, California, USA.

Robinson CJ, Schloss P, Ramos Y, Raffa K, Handelsman J. 2010. Robustness of the bacterial community in the cabbage white butterfly caterpillar midgut. Microbial Ecology 59: 199–211.

Salem H, Kreutzer E, Sudakaran S, Kaltenpoth M. 2013. Actinobacteria as essential symbionts in firebugs and cotton stainers (Hemiptera, Pyrrhocoridae). Environmental Microbiology 15: 1956–1968.

Sant'Anna MR, Darby AC, Brazil RP, Montoya-Lerma J, Dillon VM, Bates PA, Dillon RJ. 2012. Investigation of the bacterial communities associated with females of *Lutzomyia* sand fly species from South America. PLoS One 7: e42531. doi: 10.1371/journal.pone.0042531

Sastre A, González-Arregoces J, Romainoik I, Mariño S, Lucas C, Monfá E, Stefan G, De Leon B, Prieto M. 2016. *Paracoccus yeei* peritonitis in dialysis. Nefrología 36: 445–446.

Severs GA, Elston DM. 2003. What's eating you? *Megalopyge opercularis*. Cutis 71: 445–448.

Skarin H, Segerman B. 2014. Plasmidome interchange between *Clostridium botulinum*, *Clostridium novyi* and *Clostridium haemolyticum* converts strains of independent lineages into distinctly different pathogens. PLoS One 9: 107777. doi: 10.1371/journal.pone.0107777

Smith CC, Srygley RB, Healy F, Swaminath K, Mueller UG. 2017. Spatial structure of the Mormon cricket gut microbiome and its predicted contribution to nutrition and immune function. Frontiers in Microbiology 8: 801. doi: 10.3389/fmicb.2017.00801

Staudacher H, Kaltenpoth M, Breeuwer JA, Menken SB, Heckel DG, Groot AT. 2016. Variability of bacterial communities in the moth *Heliothis virescens* indicates transient association with the host. PLoS One 11: e0154514. doi: 10.1371/journal.pone.0154514

Teh BS, Apel J, Shao Y, Boland W. 2016. Colonization of the intestinal tract of the polyphagous pest *Spodoptera littoralis* with the GFP-tagged indigenous gut bacterium *Enterococcus mundtii*. Frontiers in Microbiology 7: 928. doi: 10.3389/fmicb.2016.00928

Toda H, Koyanagi T, Enomoto T, Itoh N. 2017. Characterization of two cryptic plasmids from *Kocuria palustris* IPUFS-1 and construction of novel *Escherichia coli*-Kocuria shuttle vector for biocatalysis. Journal of Bioscience and Bioengineering 124: 255–262.

Unterseher M, Schnittler M, Dormann C, Sickert A. 2008. Application of species richness estimators for the assessment of fungal diversity. FEMS Microbiology Letters 282: 205–213.

Van Aken B, Peres CM, Doty SL, Yoon JM, Schnoor JL. 2004. *Methylobacterium populi* sp. nov., a novel aerobic, pink-pigmented, facultatively methylotrophic, methane-utilizing bacterium isolated from poplar trees (*Populus deltoides* × nigra DN34). International Journal of Systematic and Evolutionary Microbiology 54: 1191–1196.

van den Bosch TJM, Welte CU. 2017. Detoxifying symbionts in agriculturally important pest insects. Microbial Biotechnology 10: 531–540.

Villas-Boas IM, Alvarez-Flores MP, Chudzinski-Tavassi AM, Tambourgi DV. 2018. Envenomation by caterpillars, pp. 429–449 *In* Gopalakrishnakone P, Faiz S, Gnanathasan C, Habib A, Fernando R, Yang CC [Eds.], Clinical Toxinology. Springer, Dordrecht, Netherlands.

Wang Y, Gilbreath III TM, Kukutla P, Yan G, Xu J. 2011. Dynamic gut microbiome across life history of the malaria mosquito *Anopheles gambiae* in Kenya. PLoS One 6: e24767. doi.org/10.1371/journal.pone.0024767

Xia X, Gurr GM, Vasseur L, Zheng D, Zhong H, Qin B, Lin H, Wang Y, Song F, Li Y, Lin H, You M. 2017. Metagenomic sequencing of diamondback moth gut microbiome unveils key holobiont adaptations for herbivory. Frontiers in Microbiology 8: 663. doi.org/10.3389/fmicb.2017.00663

Xiang H, Wei GF, Jia S, Huang J, Miao XX, Zhou Z, Zhao LP, Huang YP. 2006. Microbial communities in the larval midgut of laboratory and field populations of cotton bollworm (*Helicoverpa armigera*). Canadian Journal of Microbiology 52: 1085–1092.

Yun JH, Roh SW, Whon TW, Jung MJ, Kim MS, Park DS, Yoon C, Nam YD, Kim YJ, Choi JH, Kim JY, Shin NR, Kim SH, Lee WJ, Bae JW. 2014. Insect gut bacterial diversity determined by environmental habitat, diet, developmental stage, and phylogeny of host. Applied and Environmental Microbiology 17: 5254–5264.

Zucchi TD, Prado SS, Consoli FL. 2012. The gastric caeca of pentatomids as a house for actinomycetes. BMC Microbiology 12: 101. doi: 10.1186/1471-2180-12-101