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Research Article

Contribution to the lady beetle fauna of the Yucatan Peninsula and integrative taxonomy for species delimitation

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Lady beetles (Coleoptera: Coccinellidae) are among the most familiar insects; many species are of economic importance, but their diversity in the tropics is poorly known. We aimed to contribute to the knowledge of the lady beetle fauna of the Yucatan Peninsula, particularly for Quintana Roo state. We used an integrative approach for species identification, comparing classical morphological identifications and quick automated methods for species delimitation using DNA barcode sequences. Through a literature review and a survey of lady beetles in gardens in Quintana Roo, we further provide an updated list of the species found on the Yucatan Peninsula. Out of the 40 species delimited in our study, 34 are new reports for the peninsula, and 36 are new for Quintana Roo state. Overall, 62 species of lady beetles are now recorded for the entire region, including three exotics: the invasive *Harmonia axyridis*, *Chilocorus nigrita*, and *Delphastus catalinae*. Our study also contributed to public reference libraries with 110 barcode sequences for the tropics belonging to 34 delineated species. We showed that cytochrome oxidase 1 (COI) sequences can be useful for lady beetle species delimitation and that the Automatic Barcode Gap Discovery algorithm (ABGD) was the best method, complementing the number of initially delineated morphospecies. The Barcode Index Number (BIN) approach overestimated seven putative species due to the splitting of conspecifics, while the ABGD method suggested two additional MOTUs at a prior intraspecific distance of 0.059. Combined molecular and morphological data in our study revealed one additional putative species of *Diomus*, initially considered a tentative colour variation. Our study exemplifies how molecular methods paired with classical taxonomy can efficiently assist in delineating species when descriptions and identification keys are unavailable and highlights the possible great richness of coccinellid species awaiting exploration and description on the Yucatan Peninsula.

Key words: ABGD method, barcode index number, Coccinellidae, *Diomus*, DNA barcodes, Mexico, tropics

Introduction

Lady beetles (Coleoptera: Coccinellidae) are among the most charismatic and familiar insects. There are approximately 6000 species in 370 genera distributed worldwide (Ślipiński et al., 2011). As a group, Coccinellidae exhibits remarkable morphological and biological diversity in all life stages. It is also a family of economic importance, as many species are considered beneficial due to their predatory behaviour and their ability to reduce other insect populations (Dixon et al., 1997; Hagen, 1962; Iperti, 1999). Other species are serious agricultural pests in tropical and subtropical countries (Obrycki & Kring, 1998), and a few more are widespread invasive species (e.g., *Harmonia axyridis*; Roy et al., 2016).

Coccinellids have long attracted much taxonomic attention; however, there is no consensus on the phylogenetic relationships at the subfamily and tribe levels or on the evolutionary history of this ecologically important and species-rich beetle lineage (Che et al., 2021; Kovář, 1996; Seago et al., 2011; N. Song et al., 2020). Furthermore, several biogeographic regions and biotopes have been under-sampled, mainly in the tropics. It has been acknowledged that only a fraction of the global species diversity on Earth is presently known (Scheffers et al., 2012; Stork, 2018), and major declines in insect diversity and biomass have prompted concerns about species becoming extinct without being documented (Costello et al., 2013).

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As in most parts of the neotropical region, the lady beetle fauna of Mexico is poorly known. Mexico is the fifth most megadiverse country in the world (Llorente-Bousquets & Ocegueda, 2008). Here, the two major biogeographic regions of the continent meet, the Nearctic and Neotropics (Wallace, 1876), coinciding with a diverse variety of ecosystems, geographic relief, and climatic conditions (Espinosa Organista et al., 2008). In addition, Mexico forms part of the Mesoamerican corridor, which makes it susceptible to invasions of exotic insect species from both sides, north and south (Williams et al., 2013).

The first revisionary studies that included lady beetles collected in Mexico date back to the 19th century (e.g., Crotch, 1874; Mulsant, 1850). The most recent revision and identification key for the Coccinellidae of North America, including northern Mexico, was provided by Gordon (1985), who mentioned a few Mexican species. Recently, several partial listings of lady beetles in some Mexican states were published (e.g., Burgos Solorio & Trejo-Loyo, 2001; Flores-Mejía & Salas Araiza, 2004; López Piña & Ponce-Saavedra, 2017; Marín-Jarillo & Bujanos-Muñiz, 2008; Ruíz Cancino & Coronado Blanco, 2002); however, listings of lady beetle species for the state of Quintana Roo and for the entire Yucatan Peninsula are lacking.

Most lady beetles have characteristic colour patterns, but variability within species is common (Honěk, 1996). This phenotypic variability in lady beetles is not well understood, but it can be influenced by genetics, geographic location, season of the year, or temperature during preimaginal development (Honek et al., 2020; Koch, 2003; Marin et al., 2010), which makes their identification based on morphology challenging. Identification using colour patterns in particular often leads to errors (Marin et al., 2010), and the use of identification keys demands a certain level of expertise (Hebert et al., 2003) and often requires dissecting genitalia (e.g., Ramos et al., 2020; Vandenberg & Hanson, 2019). The use of male genitalia for species identification, however, is not informative when tackling singletons.

Complementary methods such as molecular analysis in an integrative approach can hence assist in species delimitation in regions with limited knowledge of lady beetle fauna and a lack of taxonomic identification keys. DNA barcodes have proven to be helpful in distinguishing and identifying species of lady beetles and for phylogenetic reconstructions (e.g., Greenstone et al., 2011; Halim et al., 2017; Huang et al., 2020; Poolprasert et al., 2019; Poorani et al., 2015; Rodríguez-Vélez, Gallou, et al., 2019; Z. L. Wang et al., 2019). Nevertheless, DNA barcoding has limitations when attempting to resolve species assignments in recently divergent sibling species (Greenstone et al., 2011). Furthermore, to date, there is a low number of public reference sequences for lady beetles available for comparison (Rodríguez-Vélez, Gallou, et al., 2019; Sloggett & Honěk, 2012). Moreover, these are mainly from temperate regions. Increasing the number of reference sequences for this group is therefore needed, particularly in the tropics.

Despite these constraints, molecular species delimitation based on barcodes can be useful in the assignment of specimens to operational taxonomical units (OTUs) (Ratnasingham & Hebert, 2013). The putative species delineated by such methods can therefore be useful in ecological studies of groups that lack morphological descriptions and can help to create a clearer picture of the actual biodiversity. Species discovery and species delimitation through quick and reliable methods, paired with classical taxonomic knowledge, are therefore central to the conservation of biodiversity (Rannala & Yang, 2020).

The present study aims to (1) contribute to the knowledge of lady beetle fauna on the Yucatan Peninsula, particularly for Quintana Roo state; (2) contribute to the number of barcode sequences available for species of lady beetles in the tropics; (3) contrast morphological delineation with molecular methods such as Barcode Index Number (BIN) and Automatic Barcode Gap Discovery (ABGD) for lady beetle species delimitation; and (4) provide an updated list of lady beetle species, both native and exotic, reported on the Yucatan Peninsula through a literature review.

Materials and methods

Lady beetle survey

As a part of a larger independent study, we collected lady beetles in private gardens in the coastal city of Chetumal (Quintana Roo) and in rural zones around the city in the south-eastern part of the Yucatan Peninsula, Mexico (Catzim et al., unpubl. data; Fig. 1). Briefly, the surveys were carried out monthly from January 2018 to February 2019 through visual surveys, yellow sticky traps and yellow pan traps. Eggs or larvae collected during the visual surveys were provided with aphids and plant material and reared to the adult stage. All adults and larvae that did not survive were preserved in alcohol for their identification. Field sampling complied with the current laws of Mexico and was carried out under permit number FAUT-0277 issued by the Secretaría de Medio Ambiente y Recursos Naturales, México.

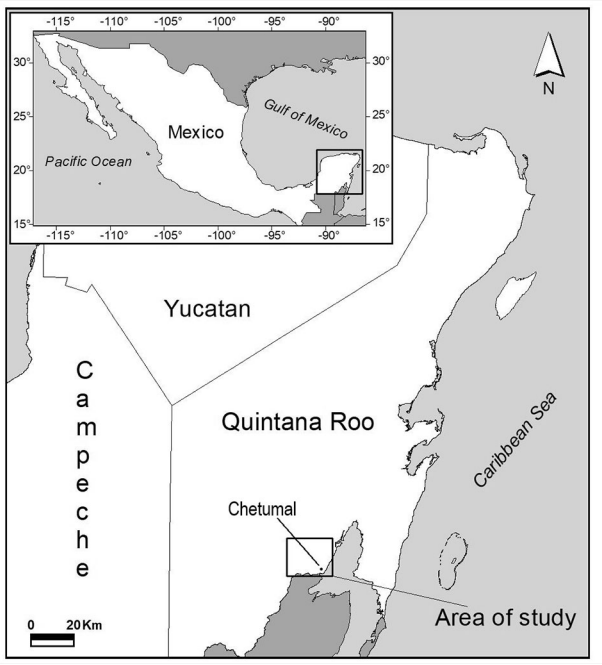

Fig. 1. Map of the area of study in south-eastern Mexico.

Lady beetle identification and molecular data generation

We sorted lady beetles into morphospecies based on morphological features according to Gordon (1985), Rodríguez-Vélez (2018), and Nestor-Arriola and Toledo-Hernández (2019). Individuals of Coccinellidae can be recognized by their characteristic round to oval and convex shape and the presence of postcoxal lines on the 1st abdominal sternum (Gordon, 1985). Some tribes can be readily distinguished by characteristic features of the maxillary palpus, the form of the clypeus (Gordon, 1985; Vandenberg, 2002), the form and number of antennomeres in the club, and the number of tarsomeres (Vandenberg & Hanson, 2019). In some groups, details of the abdominal postcoxal line can be used for generic discrimination (Gordon, 1985; Vandenberg & Hanson, 2019). Based on variations in colour patterns (position and shape of maculations on pronotum and elytra, colour of legs, etc.), we also separated some specimens into putative morphospecies, particularly for specimens from the genus *Diomus* that did not match species previously described from North America and because there is no available key for Mexican lady beetles.

For molecular analysis of lady beetles, we selected a maximum of five specimens from each morphospecies; some morphospecies were represented by fewer individuals and, in some cases, by a single specimen. Almost all specimens included in our analysis were adults, except for two larvae. For DNA extraction of the selected specimens, we used a standard glass fibre method that is similar to high-performance commercial kits (Ivanova et al., 2006). We extracted DNA from the entire body of small lady beetles measuring less than 2 mm, while for specimens between 2 and 3 mm, we used two legs, and for larger specimens (> 4 mm), we used one leg. DNA was then amplified by PCR using Zooplankton primers (ZplankF1_t1 and ZplankR1_t1) (see Montes-Ortiz & Elías-Gutiérrez, 2018 for details). The results were visualized on 2% agarose gels (E-Gel 96, Invitrogen™), and positive PCR products were sent for sequencing at Eurofins Genomics, LLC, Kentucky, USA.

Sequences were edited using CodonCode v. 3.0.1 (CodonCode Corporation, Dedham, MA, USA). We prepared a dataset under the name DS-QROOCOCC (doi: dx.doi.org/10.5883/DS-QROOCOCC) with all specimens and sequence information generated in this study in the Barcode of Life (BOLD, boldsystems.org). Sequences were uploaded to GenBank (www.ncbi.nlm.nih.gov). GenBank accession numbers are given in the supplemental material (Table S1). Voucher specimens that could be preserved were deposited in the Arthropoda collection (ECO-CH-AR) at El Colegio de la Frontera Sur, Chetumal, Quintana Roo, Mexico (vouchers C2411–C2559).

Delimitation methods

For molecular species delimitation, we used the Barcode Index Number (BIN) (Ratnasingham & Hebert, 2013) and the Automatic Barcode Gap Discovery (ABGD) algorithm (Puillandre et al., 2012) as a first approach. These two methods use information from a single locus, the cytochrome oxidase 1 (COI) mitochondrial gene, and apply clustering algorithms to delineate species into molecular operational taxonomic units (MOTUs).

For the BIN method, we uploaded all barcode sequences obtained from the lady beetles surveyed in Quintana Roo to the BOLD platform (https://www.boldsystems.org/). The BIN system retrieves, stores, and indexes MOTUs produced through the Refined Single Linkage algorithm (RESL) and assigns each MOTU a unique alphanumeric code (Ratnasingham & Hebert, 2013).

The ABGD method (Puillandre et al., 2012) was performed through the online portal (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html). We first downloaded all records that had sequences from our BOLD data project and realigned sequences using MUSCLE with default settings in Mega 7 software. For the ABGD analysis, we used default settings for intraspecific divergence ($p$min = 0.001, $p$max = 0.100), steps (10), and

Nb bins (20); we used a relative gap width (X) = 1 and selected the Kimura-2-parameter (K2P) distance model. This method detects the presence of a gap between the distribution of intraspecific and interspecific divergence followed by an initial partition and recursive partitions that are used to separate the data into candidate species based on the different values of *P* (Puillandre et al., 2012).

Lady beetles of the Yucatan Peninsula checklist

We searched online for all papers and graduate theses that included taxonomic treatments, surveys, and coincidental reports of species of lady beetles in Mexico using coccinellid, Coccinellidae, lady beetle, and ladybird beetle, as well as Yucatan Peninsula, Quintana Roo, Campeche, taxonomic/systematic revision, and checklist as search terms in Google Scholar. We also searched for records of introduced species of lady beetles as part of biological control programmes in the country. The literature cited in previously identified publications was also scrutinized for relevant information. We constructed a list of all species reported in one of the three states on the Yucatan Peninsula (Campeche, Quintana Roo, and Yucatan) and included updated names for all species and their synonyms based on the studies of Crotch (1874), Gordon (1985), Gorham (1891, 1892, 1894, 1897) and Nestor-Arriola and Toledo-Hernández (2019). Revisionary studies and theses were thoroughly reviewed to check for inconsistencies. Only primary records were included. We also added to the list all species of lady beetles collected in our survey in gardens that were identified or delineated.

Results

Morphological species delimitation

A total of 980 lady beetles were collected in private gardens in the southern part of Quintana Roo, which were sorted into 39 morphospecies (Table 1); some of these specimens were assigned tentative colour variations of a particular morphospecies (*Diomus* sp. 2 A, 2 B, 2 C, 2 D, Fig. 2). Based on morphological traits, 16 could be identified at the species level, 20 at the genus level, and three could not be identified and were only assigned to the subfamily level. We found specimens from the two subfamilies of lady beetles according to Bouchard et al. (2011). In the subfamily Microweiseinae, we found three morphospecies from the tribe Serangiini, and within the subfamily Coccinellinae, we found 35 morphospecies, which were distributed among 10 tribes, also recognized by Bouchard et al. (2011) (Azyini, Brachiacanthini, Chilocorini, Chnoodini, Coccinellini, Diomini, Hyperaspidini, Scymnillini, Scymnini, and Stethorini).

DNA amplification, barcodes, and molecular species delimitation

We extracted and amplified the COI gene from 139 specimens and obtained successful sequences from 125 of them (90% success, indicating good performance of the Zplank primers, initially developed for planktonic crustaceans). Sequence base pair lengths ranged from 525–659 bp, and barcode compliance standards were met by 95 records (>500 bp, <1% ambiguous bases) (Table S1). No stop codons or failed sequences were found. We included only 110 sequences in the analysis to compare species delimitation methods, since 13 sequences were of poor quality and were obtained from failed trace files (both forward and reverse). Two more were contaminations. The 110 sequences belonged to 33 of the 39 species delineated through morphology. The sequences not included in the analyses pertained to six morphospecies.

Barcode index number (BIN) system. The BIN method sorted the 110 sequences into 41 MOTUs, and 40 were assigned barcode index numbers (BINs); only the sequence of *Zagloba hystrix* B did not meet the barcode standard requirements for BIN assignment (Table 1). Twenty-seven of the 33 (82%) morphologically delineated species were accurately delineated by the BIN system. The additional putative species suggested by this method resulted from splitting five morphospecies, mainly represented by singletons (Table 1, Fig. 3).

Seven of the 16 lady beetles identified to the species level through morphology had concordant BINs in BOLD and confirmed our identifications (*Chilocorus cacti*, *C. nigrita*, *Cycloneda sanguinea sanguinea*, *Delphastus catalinae*, *Diomus roseicollis*, *Olla v-nigrum*, and *Psyllobora vigintimaculata,* Table 1). The sequences of our morphospecies *Nephaspis* sp. 1 matched sequences of the species identified as *Nephaspis indus* in BOLD (98% similarity, Fig. S1C). In contrast, the sequences of the specimens identified morphologically as *Nephus* (*Scymnobius*) *flavifrons* did not match sequences of this species in BOLD and are thus reported as *Nephus* (*Scymnobius*) sp. 5 (Table 1).

Automatic barcode gap discovery (ABGD)

ABGD detected the presence of a barcode gap (distance = 0.080). The initial partition of sequences was stable and yielded 35 groups (putative species) at different

Table 1. Summarized comparison of lady beetle species delineation through BIN and ABGD methods based on COI sequences.

Morphology ID	Species Code[§]	Number of Sequences	BINs	ABGD Group $P = 0.059$
Azya orbigera orbigera	sp. 1	5	AEE7664	16
Brachiacantha bistripustulata	sp. 2	1	AEE2422	8
		1	AEE2423	
Brachiacantha sp. 1	sp. 3	1	AEE4037	27
Chilocorus cacti **	sp. 4	3	AAN6019	20
Chilocorus nigrita **	sp. 5	2	ABX2096	2
Cycloneda sanguinea sanguinea **	sp. 6 Adult	4	AAN6221	15
	sp. 6 Larva	1	AAN6125	
Delphastus catalinae **	sp. 7	2	AAE7462	10
Delphastus pusillus	sp. 8	2	ADB5988	9
Delphastus sp. 1	sp. 9	1	AEE9389	25
Diomus roseicollis **	sp. 10	3	ACJ2867	4
Diomus sp. 1	sp. 11	5	AEE3128	5
Diomus sp. 2[a]	sp. 12 A	5	AEE0829	13
	sp. 12 B	4	AEE0829	
	sp. 12 C	5	AEE0829	
	sp. 12 D	**1**	**AEE8233**	**14**
		1	AEE6698	
Diomus sp. 3	sp. 13	–	–	–
Diomus sp. 4	sp. 14	1	AEE4124	32
Diomus sp. 5	sp. 15	1	ADB2460	33
Diomus sp. 6	sp. 16	1	AEC5539	30
Diomus sp. 7	sp. 17	4	AEE6697	23
Exochomus insatiabilis	sp. 18	3	AEE8414	6
Exochomus sp. 1	sp. 19	1	AEE5320	28
Exoplectra sp. 1	sp. 20	–	–	–
Hyperaspis globula	sp. 21	2	AEE5172	3
Nephaspis sp. 1 (*Nephaspis indus*)**	sp. 22	5	ACO6393	17
Nephaspis sp. 2	sp. 23	1	AEE7118	31
Nephaspis sp. 3	sp. 24	1	AEE5624	35
Nephus (*Scymnobius*) sp. 1	sp. 25	2	AEE6725	19
Nephus (*Scymnobius*) sp. 2	sp. 26	2	AEE2657	18
Nephus (*Scymnobius*) sp. 3	sp. 27	–	–	–
Nephus (*Scymnobius*) sp. 4	sp. 28	–	–	–
Nephus (*Scymnobius*) sp. 5	sp. 29	3	AEE2656	12
Olla v-nigrum **	sp. 30	1	AAH3312	29
Psyllobora vigintimaculata **	sp. 31	3	ABX0675	24
Scymnus (Pullus) sp. 1	sp. 32	1	AEE7429	34
Scymnus (*Pullus*) *pulvinatus*	sp. 33	4	AAM7642	7
Stethorus punctum picipes	sp. 34	2	AEE2433	1
		1	AEE2629	
		2	AEE9089	
Zagloba hystrix	sp. 35 A	1	AEE1887	22
	sp. 35 B	1	NA	21
Zagloba satana	sp. 36	–	–	–
Unidentified sp. 1	sp. 37	–	–	–
Unidentified sp. 2	sp. 38 A	4	AEE5331	11
	sp. 38 B	1	AEE5332	
Unidentified sp. 3	sp. 39	1	AEE5014	26

§Morphospecies numbers followed by a letter represent tentative colour variations.
**Morphospecies identified to species level through morphology that had matching BINs in the BOLD System Database.
– no DNA was obtained, or sequence derived from failed DNA trace files.
[a]Tentative colour variation of *Diomus* sp. 2 which was later considered another species after molecular analysis, is highlighted in bold.

prior intraspecific divergence values ($P = 0.0215$, $P = 0.0359$, $P = 0.059$), while the recursive partition fluctuated between 48 and 35 putative species (Fig. S2). Both the initial and recursive partition reached consensus at a prior intraspecific distance $P = 0.059$ (Fig. S2) and yielded 35 groups, which was the closest and

Fig. 2. Tentative colour variations in *Diomus* sp. 2 identified through morphology (A–D).

congruent approximation to morphological delineation (Table 1). Sequences of 31 morphologically identified species (94%) were clustered accordingly at $P = 0.059$, and two morphospecies were split into two groups by this method. *Zagloba hystrix* A was separated from *Zagloba hystrix* B, which were hypothesized to be colour variations of the same species; the neighbour-joining tree obtained through the ABGD algorithm indicated a 13% genetic distance between sequences of these specimens (Fig. 3, Table S2). The other putative species suggested by the ABGD algorithm concerned *Diomus* morphospecies 2 D, which we assumed to be one of four tentative colour variations of *Diomus* sp. 2 but that the ABGD method recovered as a different group. The sequences of *Diomus* sp. 2 D had a 10% genetic distance from the other colour morphs (2 A, 2B, and 2 C), which were all clustered in the same group (Fig. 3, Table S2). All *Diomus* species were clustered in the same clade (Fig. 4), except for *Diomus* sp. 1 which was grouped with *Hyperaspis globula*. The neighbour-joining tree consistently retrieved morphospecies within the same tribe. For example, *Chilocorus cacti*, *C. nigrita*, and *Exochomus* spp. were grouped in the same clade (Chilocorini, Fig. 5) and species in the tribe Coccinellini, the true ladybird beetles, such as *Olla v-nigrum*, *Psyllobora vigintimaculata* and *Cycloneda sanguinea sanguinea*, were in a separate clade (Fig. 6).

Lady beetles of the Yucatan Peninsula

In total, we found 43 studies that mentioned species of lady beetles collected in Mexico, including published articles, reports, taxonomic treatments, and three theses. Of these, only 19 sources mentioned species found on the Yucatan Peninsula. After considering synonyms, a total of 28 species were found reported on the Yucatan Peninsula in the literature, two of which were exotics (Table 2). Of the 28 species, seven were reported in the state of Campeche, eight in Quintana Roo state and 23 in Yucatan state.

Discussion

Identification of lady beetle species can be a difficult task, particularly for very small-bodied specimens, species showing colour polymorphism, or species having similar elytral shapes and colour patterns, such as those in the *Scymnus*, *Nephus*, and *Diomus* genera (Seago et al., 2011; Vandenberg & Hanson, 2019, see Fig. S1). Combining detailed studies of morphology with molecular analysis has been useful in disentangling hyperdiverse and taxonomically difficult taxa (e.g., Tyagi et al., 2019; Zhou et al., 2019), and the suitability of this approach has been demonstrated for coccinellids (Huang et al., 2020). In the present study, we were able to delineate lady beetle species from the southern part of

Fig. 3. Discrepancies between the ABGD and BIN analyses based on DNA barcodes of lady beetles from gardens in Quintana Roo, Mexico. (A) Simplified neighbour-joining tree obtained through ABGD based on the K2P distance model. (B) Neighbour-joining tree generated with the Taxon ID function in the BOLD platform (https://www.boldsystems.org/). BIN splits highlighted in bold.

the Yucatan Peninsula through a combined approach using morphological features and barcode sequences. The BIN and ABGD delimitation methods revealed 40 and 35 molecular operational taxonomic units (MOTUs) for 33 lady beetle morphospecies, respectively. As several studies have indicated, molecular species delimitation algorithms frequently generate a larger number of MOTUs compared with a morphology-based concept (e.g., Huang et al., 2020; Zhou et al., 2019). Studies with a diversity of taxa have also found that the ABGD algorithm is more conservative than the BIN algorithm (e.g., Lin et al., 2015; Pentinsaari et al., 2017; C. Song et al., 2018), probably due to the low intracluster distance threshold (2.2%) at the initial clustering step of

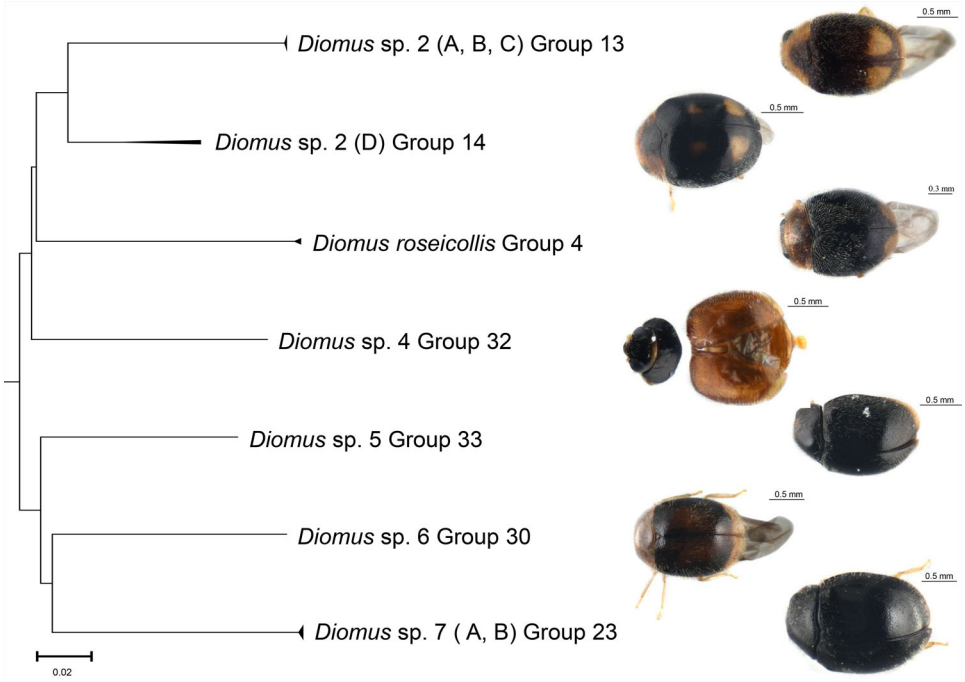

Fig. 4. Representative illustrations of species in the *Diomus* genus (Diomini). COI sequences clustered through ABGD based on the K2P distance model.

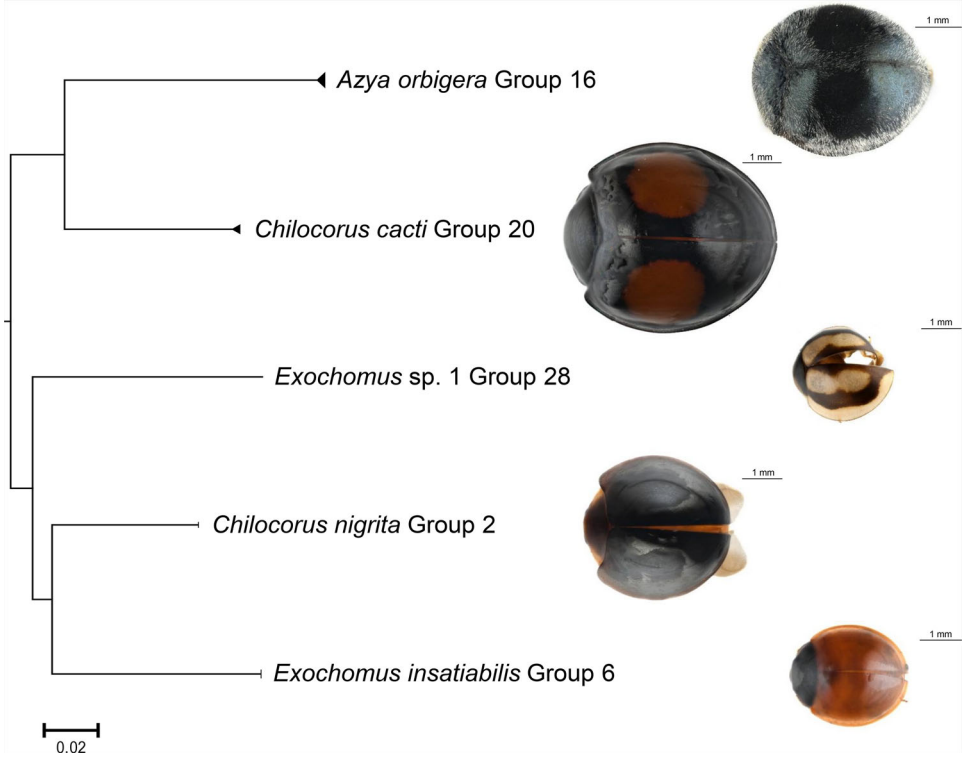

Fig. 5. Representative illustrations of species in the Chilocorini tribe. COI sequences clustered through ABGD based on the K2P distance model.

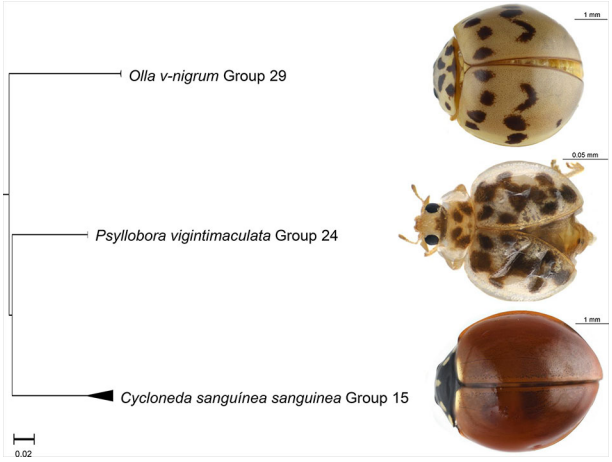

Fig. 6. Representative illustrations of species in the Coccinellini tribe. COI sequences clustered through ABGD based on the K2P distance model.

RESL in the BIN method (Ratnasingham & Hebert, 2013). In accordance, we observed an overestimation of lady beetle species by using the BIN algorithm and a closer match to our morphological identification when employing the ABGD algorithm. Similar observations were made by Huang et al. (2020), who evaluated the potential of four species delimitation methods using a large set of coccinellid sequences, mainly from temperate regions, and concluded that the ABGD method was the most accurate and efficient approach among those tested. These authors further suggested 3% as a suitable genetic distance threshold to delimit species of Coccinellidae using DNA barcodes. In contrast, we detected a closer and more congruent match (94%) to our morphological identification with a prior intraspecific distance of 0.059.

In our analyses, both BIN and ABGD methods suggested *Diomus* sp. 2 D as a distinct MOTU and not a colour variation of this morphospecies as was first considered based on external morphology. *Diomus* Mulsant, 1850 is a cosmopolitan genus of minute pubescent lady beetles. It is considered one of the most species-rich genera in Coccinellidae (Pang & Slipinski, 2009), with the greatest diversity in the tropics and subtropics (Ramos et al., 2020; Vandenberg & Hanson, 2019). Despite the dense pilosity and small size of specimens that tends to hinder observation of cuticular features (Vandenberg & Hanson, 2019), we were able to separate *Diomus* specimens into eight morphospecies and further noted four colour morphs among *Diomus* sp. 2 (Fig. 2; morphs 2 A, 2 B, 2 C, and 2 D). The neighbour-joining tree generated from the ABGD algorithm (Figs 3 and 4) and BIN results (Table S2) indicated more than 10% genetic distance between sequences of *Diomus* sp. 2 D and sequences from the other three morphs. According to Huang et al. (2020) and Wang et al. (2019), the minimum interspecific distance observed in lady beetles is 10% (range 10 – 29.1%). *Diomus* sp. 2 D differs from other morphs by having a slightly more rounded body shape and by the apex of elytra lacking a yellow border (Fig. 2). Differences in morphology and consistency of delimitation patterns across the two molecular approaches used in this study strongly support *Diomus* sp. 2 D as a valid putative species (*Diomus* sp. 8 in Table 2). It should also be noted that species of *Diomus* were classified into three distinct clusters in our neighbour-joining tree, with *Diomus* sp. 1 clearly separated from the rest of the morphospecies in this genus and on a distinct branch (Fig. 3). *Diomus* taxonomic placement has been historically problematic and was initially described as a subgenus of *Scymnus* (Mulsant, 1850). Recently, Vandenberg and Hanson (2019) reviewed the taxonomic history of this genus and concluded that *Diomus* is possibly polyphyletic. Our findings seem to support this hypothesis, but further research is needed.

Similarly, the assumed morphological variations of *Zagloba hystrix* A and *Z. hystrix* B were proposed as different MOTUs by both BIN and ABGD approaches (Table 1, Fig. 3). RESL in the BIN method did assign separate MOTUs for both morphs, but the sequence of *Z. hystrix* B did not meet barcode standards and was not assigned a BIN. Morphologically, *Z. hystrix* A and B share diagnostic characteristics of the species: both have incomplete postcoxal lines and coarse punctures within the arc of the postcoxal line, which separates *Z. hystrix* from *Z. satana*, the other species of *Zagloba* described by Gordon (1985) for North America. *Zagloba hystrix* B has a darker colouration ventrally and is slightly more pubescent than *Z. hystrix* A, but we could not find any other morphological difference between the specimens of these two morphs. Since ABGD clustering was based on a single sequence and there were no evident morphological differences, there is no support to consider these morphs as separate putative species at this time. Further sampling and taxonomic research are required to clarify the taxonomic status of *Z. hystrix* B.

Discrepancies between the BIN and ABGD methods resulted from splits of conspecific specimens into two or three BINs (in the species *Brachiacantha bistripustulata*, *Cycloneda sanguinea sanguinea*, *Diomus* sp. 2, *Stethorus punctum picipes*, and unidentified sp. 2; Fig. 3, Table 1). Some of these BIN splits were represented by singletons, typically with low sequence divergence (Table S2), and no obvious differences between specimens. Morphological species with BIN splits have been observed in Lepidoptera (Janzen et al., 2017; Ortiz et al., 2017), Orthoptera (Zhou et al., 2019), Araneae

Table 2. Updated list of lady beetle species (Coleoptera: Coccinellidae) recorded on the Yucatan Peninsula, Mexico.

Tribe	Species	Location	References
Subfamily Microweiseinae			
Serangiini	***Delphastus* sp.**	Yucatán	Lozano-Contreras and Jasso-Argumedo (2012)
	***Delphastus* sp. 1**	Quintana Roo	This study
	***Delphastus catalinae* (Horn, 1895)** **	Quintana Roo	This study
	***Delphastus pusillus* (LeConte, 1852)**	Quintana Roo	This study
Subfamily Coccinellinae			
Azyini	***Azya orbigera orbigera* Mulsant, 1850**	Quintana Roo	This study
	Azya orbigera	Yucatán	Lozano-Contreras and Jasso-Argumedo (2012)
Brachiacanthini	***Brachiacantha* sp. 1**	Quintana Roo	This study
	***Brachiacantha quadrillum* LeConte, 1858**	Quintana Roo & Yucatán	Nestor-Arriola and Toledo-Hernández (2019)
	***Brachiacantha bistripustulata* (Fabricius, 1801)**	Quintana Roo	This study
	Brachyacantha erythrocephala	Yucatán	Gorham (1894)
	***Brachiacantha dentipes* (Fabricius, 1801)**		
	Brachyacantha dentipes	Yucatán	Gorham (1894)
	***Brachiacantha erythrura* Mulsant, 1850**		
	Brachyacantha erythrura	Yucatán	Gorham (1894)
	***Brachiacantha subfasciata* Mulsant, 1850**	Campeche & Yucatán	Nestor-Arriola and Toledo-Hernández (2019)
Chilocorini	***Chilocorus cacti* (Linnaeus, 1767)**	Campeche	Gorham (1892)
		Quintana Roo	Catzim (2015); Juarez Monroy (1986); Machkour-M'Rabet et al. (2015); Rodríguez-Vélez, Sarmiento-Cordero, et al. (2019); This study
		Yucatán	Gorham (1892); Juarez Monroy (1986); Nestor Arriola (2011); Lozano-Contreras and Jasso-Argumedo (2012)
	Chilocorus sp.	Quintana Roo	Catzim (2015)
	***Chilocorus nigrita* (Fabricius, 1798)** **	Quintana Roo	Rodríguez-Vélez, Sarmiento-Cordero, et al. (2019); This study
	***Exochomus childreni childreni* Mulsant, 1850**		
	Exochomus childreni	Campeche	Crotch (1874)
	***Exochomus insatiabilis* Rodríguez-Vélez, 2018**	Yucatán	Rodríguez-Vélez (2018)
		Quintana Roo	This study
	***Exochomus* sp. 1**	Quintana Roo	This study
	***Arawana* sp.**	Yucatán	Lozano-Contreras and Jasso-Argumedo (2012)
Chnoodini	***Dioria sordida* Mulsant, 1850**	Yucatán	Gorham (1897)
	***Exoplectra* sp. 1**	Quintana Roo	This study
Coccinellini	***Cycloneda retrospiciens* Crotch, 1874**	Quintana Roo	Juarez Monroy (1986)
	***Cycloneda sanguinea sanguinea* (Linnaeus, 1763)**	Quintana Roo	This study

(*continued*)

Table 2. Continued.

Tribe	Species	Location	References
	Cycloneda sanguinea	Quintana Roo	Catzim (2015)
		Campeche	Juarez Monroy (1986)
		Yucatán	Lozano-Contreras and Jasso-Argumedo (2012)
	***Neoharmonia venusta venusta* (Melsheimer, 1847)**		
	Coccinella venusta	Yucatán	Crotch (1874); Gorham (1891)
	***Neohalyzia perroudi* Mulsant, 1850**	Yucatán	Gorham (1892); Juarez Monroy (1986)
	***Harmonia axyridis* (Pallas, 1773)** **	Campeche, Quintana Roo & Yucatán	Munguía (2002); López-Arroyo et al. (2003); López-Arroyo et al. (2008)
	***Olla v-nigrum* (Mulsant 1866)**	Quintana Roo	Catzim (2015); This study
		Yucatán	Lozano-Contreras and Jasso-Argumedo (2012)
	Cycloneda abdominalis	Yucatán	Gorham (1892)
	Olla abdominalis	Campeche	Juarez Monroy (1986)
	***Psyllobora vigintimaculata* (Say, 1824)**	Quintana Roo	This study
Cryptognathini	***Cryptognatha flaviceps* Crotch, 1874**	Yucatán	Crotch (1874); Gorham (1894)
Diomini	***Diomus roseicollis* (Mulsant, 1853)**	Quintana Roo	This study
	***Diomus* sp. 1**	Quintana Roo	This study
	***Diomus* sp. 2**	Quintana Roo	This study
	***Diomus* sp. 3**	Quintana Roo	This study
	***Diomus* sp. 4**	Quintana Roo	This study
	***Diomus* sp. 5**	Quintana Roo	This study
	***Diomus* sp. 6**	Quintana Roo	This study
	***Diomus* sp. 7**	Quintana Roo	This study
	***Diomus* sp. 8**	Quintana Roo	This study
Epilachnini	***Epilachna borealis* (Fabricius, 1775)**	Yucatán	Gorham (1898)
Hyperaspidini	***Hyperaspis globula* Casey, 1899**	Quintana Roo	This study
	***Hyperaspis sexverrucata* (Fabricius, 1801)**	Quintana Roo	Juarez Monroy (1986)
	***Hyperaspis subsignata* Crotch, 1874** (An unrecognized species; see Gordon, 1985)	Campeche	Crotch (1874); Gorham (1894)
Scymnillini	***Zagloba* sp.**	Yucatán	Lozano-Contreras and Jasso-Argumedo (2012)
	***Zagloba hystrix* Casey, 1899**	Quintana Roo	This study
	***Zagloba satana* Gordon, 1985**	Quintana Roo	This study
Scymnini	***Scymnus atomus* Mulsant, 1850**	Yucatán	Mulsant (1850); Crotch (1874); Gorham (1897)
	***Scymnus bilucernarius* Mulsant, 1850**	Yucatán	Mulsant (1850); Crotch (1874); Gorham (1897)
	***Scymnus pilatii* Mulsant, 1850**	Yucatán	Mulsant (1850); Crotch (1874); Gorham (1897)
	***Scymnus* (*Pullus*) sp. 1**	Quintana Roo	This study
	***Scymnus* (*Pullus*) *pulvinatus* Wingo, 1952**	Quintana Roo	This study
	***Scymnus theyls* Mulsant, 1853**	Yucatán	Crotch (1874); Gorham (1897)
	Scymnus (*Pullus*) *theyls*	Yucatán	Mulsant (1853)
	***Nephaspis indus* Gordon, 1996**	Quintana Roo	This study
	***Nephaspis* sp. 2**	Quintana Roo	This study

(*continued*)

Table 2. Continued.

Tribe	Species	Location	References
	***Nephaspis* sp. 3**	Quintana Roo	This study
	***Nephus* (*Scymnobius*) sp. 1**	Quintana Roo	This study
	***Nephus* (*Scymnobius*) sp. 2**	Quintana Roo	This study
	***Nephus* (*Scymnobius*) sp. 3**	Quintana Roo	This study
	***Nephus* (*Scymnobius*) sp. 4**	Quintana Roo	This study
	***Nephus* (*Scymnobius*) sp. 5**	Quintana Roo	This study
Stethorini	***Stethorus punctum picipes* Casey, 1899**	Quintana Roo	This study
	***Parastethorus histrio* (Chazeau, 1974)**		
	Stethorus (*Parastethorus*) *histrio*	Yucatán	Gordon and Chapin (1983)
	Unidentified sp. 1	Quintana Roo	This study
	Unidentified sp. 2	Quintana Roo	This study
	Unidentified sp. 3	Quintana Roo	This study

Subfamily and tribe classification follows Bouchard et al. (2011); genus placement follows Gordon (1985). Valid species names are in bold.
**exotic species to Mexico.

(Blagoev et al., 2015) and Coleoptera (Mitchell et al., 2020), including Coccinellidae (Huang et al., 2020). Sometimes these BIN splits can indicate cryptic species complexes (Blagoev et al., 2015; Janzen et al., 2017; Ortiz et al., 2017) or may represent different haplotypes. Increased sampling and further analysis are necessary to determine the taxonomic importance of these BIN splits.

Following our combined approach to delineate the coccinellid species surveyed in our study, we considered a total of 40 lady beetle species. Molecular data confirmed 33 morphologically identified species, some of them awaiting description, and revealed one additional putative species. Six species were only identified through morphology because we did not obtain DNA from the specimens, or the sequences were of poor quality.

As our literature review revealed, the Mexican lady beetle fauna has been poorly explored, considering the large size of Mexican territory; much of the important contributions of this group of species date back to the 19th century, and certain regions have remained undersampled. The states within the Yucatan Peninsula, for example, lacked formal listings of their lady beetle fauna, contrary to other Mexican states such as Morelos (Burgos Solorio & Trejo-Loyo, 2001), Guanajuato (Flores-Mejía & Salas Araiza, 2004; Marín-Jarillo & Bujanos-Muñiz, 2008), Michoacán (López Piña & Ponce-Saavedra, 2017), Tamaulipas and Nuevo León (Ruíz Cancino & Coronado Blanco, 2002). Prior to this study, only 28 species of lady beetles had been reported on the Yucatan Peninsula, mainly as incidental records.

Among the species formerly recorded, two are exotic: *Chilocorus nigrita* native to India (Rodríguez-Vélez, Sarmiento-Cordero, et al., 2019; Thomas & Blanchard, 2013) and *Harmonia axyridis* native to Asia (López-Arroyo et al., 2003, 2008; Munguía, 2002). In contrast to *H. axyridis*, which was released in the area as part of a biological control programme against the aphid *Toxoptera citricida* (López-Arroyo et al., 2003, 2008; Munguía, 2002), *C. nigrita* seems to have been accidentally introduced to Mexico. This species has been detected in earlier years in nearby countries such as the USA and Dominican Republic in the Caribbean (Thomas & Blanchard, 2013).

As part of our survey in gardens, we recorded exotic *C. nigrita* and another exotic species, *Delphastus catalinae*, which represents a new record for the Yucatan Peninsula. *Delphastus catalinae* is native to Colombia and was apparently imported into Florida under the name *Delphastus pusillus* and is now distributed throughout Mexico (Hoelmer & Pickett, 2003). Apart from the records of these exotic species, we further contributed 33 new records of seemingly native lady beetle species for the Yucatan Peninsula. Most of them lacked external similarity to previously described species in Gordon's (1985) treatise, which represents the nearest species descriptions that can be used as a reference; most of them could only be identified to the genus level and are probably new species awaiting taxonomic description.

Overall, six species of lady beetles identified in our survey in gardens were already documented on the Yucatan Peninsula; hence, 34 lady beetle species contributed herein are new records. These new records, together with the 28 species previously reported on the peninsula, amount to 62 species of lady beetles (Table 2). Our findings represent a more than 50% increase in the number of lady beetle species for the region and a more than 80% increase for the state of Quintana Roo. Although this study was limited to urban and peri-urban gardens, of the 34 species sequenced, only eight had been previously barcoded. Our study highlights the

likely great diversity of lady beetles in this region and provides the basis for further systematic study and taxonomic investigation of the coccinellid fauna of the Yucatan Peninsula.

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No potential conflict of interest was reported by the author(s).

Supplemental material

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Data accessibility statement

The dataset that supports the findings of this study is available in BOLD (https://www.boldsystems.org/). doi: dx.doi.org/10.5883/DS-QROOCOCC.

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