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Taxonomy and systematics

## Leaf gall diversity in the neotropical plant *Coccoloba barbadensis* (Polygonaceae) and its associated insect species richness

## Diversidad de agallas de hojas en la planta neotropical Coccoloba barbadensis (Polygonaceae) y riqueza de especies de sus insectos asociados

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## Abstract

Galls are an abnormal growth of plant tissue in response to the presence generally of an inducing insect, which ensures food and protection during specific periods of its life. Besides gall formers, a vast community of arthropods are associated with galls, including inquilines and parasitoids. Few studies have assessed the gall diversity and its associated insect community in Neotropical vascular plants. Here, we characterised the leaf gall diversity of *Coccoloba barbadensis* Jacq. (Polygonaceae) in a Mexican tropical dry forest, as well as their associated entomofauna based on morphology and DNA barcoding. Five different gall morphotypes were observed during both dry (April-June) and rainy (November) seasons. A total of 34 and 38 species of Diptera, Hymenoptera, Coleoptera, and Lepidoptera were delimited with the 2% divergence criterion and the GMYC model, respectively. Based on our rearing observations and literature, Cecidomyiidae (Diptera) species might induce all leaf gall morphotypes, whereas hymenopterans are represented by parasitoid and probably inquiline species of the families Braconidae, Eulophidae, Eulophidae, Platygastridae and Torymidae. Our results highlight the importance of performing integrative species delineation studies of arthropods present in galls to have an accurate knowledge of their diversity and trophic interactions.

Keywords: Trophic interactions; Host; Parasitoid; DNA barcode; Gall former

#### Resumen

Las agallas son un crecimiento anormal de tejido de plantas por la presencia de un insecto inductor que le asegura alimento y protección durante periodos específicos. Además de los formadores de agallas, una vasta comunidad de artrópodos está también asociada, incluidos inquilinos y parasitoides. Pocos estudios han evaluado la diversidad de agallas y su comunidad de insectos en plantas vasculares neotropicales. Aquí se caracteriza la diversidad de agallas foliares de *Coccoloba barbadensis* Jacq. (Polygonaceae) en un bosque seco tropical mexicano, así como su entomofauna asociada basada en morfología y el código de barras del DNA. Se observaron 5 morfotipos de agallas durante las temporadas seca (abril-junio) y lluviosa (noviembre). Se delimitó un total de 34 y 38 especies de Diptera, Hymenoptera, Coleoptera y Lepidoptera con el criterio de divergencia de 2% y el modelo GMYC, respectivamente. Según las observaciones y datos de literatura, especies de Cecidomyiidae (Diptera) inducen todos los morfotipos de agallas, y los himenópteros están representados por especies parasitoides y probablemente inquilinas de las familias Braconidae, Eulophidae, Eupelmidae, Platygastridae y Torymidae. Los resultados resaltan la importancia de estudios integradores para la delimitación de especies de artrópodos de agallas para tener conocimiento preciso de su diversidad e interacciones tróficas.

Palabras clave: Interacciones tróficas; Hospedero; Parasitoide; Código de barras del DNA; Formador de agallas

### Introduction

Ecological interactions among species form the basis of ecosystem functioning and underlie evolutionary and ecological principles of conservation biology (Clare et al., 2013). Three types of biological networks have been defined based on interactions and the types of organisms involved (Ings et al., 2009): traditional or antagonistic food webs (e.g., predators and prey/food webs), mutualistic networks (e.g., seed dispersal and pollination), and host-parasitoid networks. The study of these biological networks provides a whole ecosystem outline to examine the effects of biodiversity loss on communities and ecosystem functions (Ings et al., 2009).

Understanding the links of a network relies on the idea that descriptors of interaction structure are unbiased and accurate (Wirta et al., 2014). In practice, however, networks are difficult to create, especially using traditional methods. Taxonomic resolution and the methodology employed to delimit species are crucial to reconstruct interaction structure (Paine, 1980; Kaartinen & Roslin, 2011). If the links are poorly resolved and multiple taxa are inadvertently grouped within the nodes of a web, there is a risk of misunderstanding its composition and thus its functioning system (Kaartinen & Roslin, 2011; Wirta et al., 2014).

An astonishing number of arthropod taxa depend on plants as food resources or closely interact with them. Among these are species that form enclosed structures known as galls (Mani, 1964; Raman, 2011). These structures are defined as abnormal growth of tissues of host plants in response to the activity or presence of an inducing organism (Nieves-Aldrey, 1998; Price, 2005; Redfern et al., 2002). Galls can be found in several plant structures such as flowers, roots, fruits, leaves, thorns, or stems. Arthropods induce galls to ensure food resources and to protect themselves against predators or unfavourable environmental conditions during certain periods of their life cycle (Nieves-Aldrey, 1998; Raman & Withers, 2003).

Gall induction in insects mainly occurs in species of Hymenoptera, but also in species of Diptera, Hemiptera and Thysanoptera, and less frequently in Coleoptera and Lepidoptera (Raman, 2011). Besides gall formers, there is an intricate community of different insect species that also are associated with galls, including inquilines (i.e., species that develop within galls made by other insects and feed on plant tissue), parasitoids of gall formers and inquilines and hyperparasitoids (i.e., parasitoids of other parasitoid species) (Forbes et al., 2015). Despite the great ecological importance of galls in most terrestrial ecosystems due to the extraordinary arthropod diversity that they comprise, to date, most of this species diversity and the interactions that are involved are largely unknown, especially in tropical and subtropical regions.

In recent years, molecular techniques have provided detailed analyses of interaction reconstruction, allowing precise identification of members of natural communities and the structure of networks (Clare et al., 2013; Kaartinen et al., 2010; Wirta et al., 2014). The DNA barcoding locus, a fragment of the cytochrome oxidase I (COI) mitochondrial DNA gene, is the most employed genetic marker for species discrimination of closely related animal species (Hebert et al., 2003; Ratnasingham & Hebert, 2013). This marker is a valuable tool for the rapid identification of megadiverse, poorly known taxa (Hebert et al., 2003). Moreover, it allows the association of morphologically distinct semaphoronts (e.g., insect larvae and adults; Yeo et al., 2018) and sexes of the same species (e.g., Sheffield et al., 2009).

*Coccoloba barbadensis* is a widely distributed Neotropical vascular plant species that in Mexico occurs in tropical regions from central to southeast Mexico (Howard, 1959). In this study, the diversity of galls on the leaves of the vascular plant *C. barbadensis* Jacq. (Polygonaceae) in a Mexican tropical dry forest was characterised and their associated entomofauna assessed using both morphological and DNA barcoding data. We highlight the necessity to perform integrative species delimitation studies of arthropods present in galls, particularly in the tropics, to have a more accurate knowledge of their species richness.

#### Materials and methods

The study was conducted in the Biological Station of Chamela (EBCH), Jalisco, Mexico (19'29" N, 105'01" W; Noguera et al., 2002), owned by the Instituto de Biología, Universidad Nacional Autónoma de México. The Chamela region is mainly composed of tropical dry forest and is characterised by having 85% of the ~ 750 mm of yearly rain from July to November and a mean annual temperature of 24.9 °C (14.8-32 °C) (Méndez-Alonzo et al., 2013). Tropical dry forests frequently show extreme changes in the physiognomy and available resources during the rainy and dry seasons, therefore altering the composition and diversity of their fauna (Razo-González et al., 2014).

We carried out 2 collecting trips at the EBCH, one during the dry (from March to June 2013) and the other during the rainy season (November 2013). Fifteen trees belonging to *C. barbadensis* were located and marked, all of which were situated near seasonal streams. We collected 1-5 leaves with galls from different parts of the selected trees. By *in situ* photographs we documented the presence/absence of galls for each tree, as well as general leaf features such as colour and size. The main gall's features, including shape, size, colour, and pubescence, and the number of all collected galls present on the leaves were classified by morphotypes and recorded.

The levels of infestation, presence, and type of galls were weekly recorded, and the total number of galls per leaf was recorded. Galls were subsequently dissected or maintained in the laboratory to rear their insects. All reared insects were preserved in 96% ethanol and stored at -20  $^{\circ}$ C.

All collected insects were sorted out into adults, larvae, or pupae, and were counted and discriminated into morphospecies with a Zeiss<sup>™</sup> Stemi DV4 (Göttingen, Germany) stereomicroscope. Larvae and adults of Hymenoptera were identified to order and genus level, respectively, using the specialised literature. Larvae and adults of Diptera and larvae of Coleoptera and Lepidoptera could only be identified to family and order level, respectively.

### Gall abundance

The relationship between both the total number of collected galls and the average number of each gall morphotype concerning the time of collection during the dry season was evaluated using a simple linear regression with the statistical program R (R Core Team, 2013). Data of rainy season were not statistically analysed, since there were not enough samples to perform statistical tests. The insect family frequency throughout the sampled period was also analysed and then a canonical correspondence analysis to characterise the association between insect species and gall morphotypes with the program Statistica version 10 (StatSoft, Inc., 2011).

#### Molecular data

It is widely recognised that species misidentifications have negative consequences in ecological studies (Bortolus, 2008; Vink et al., 2012). Species delimitation and identification can be considerably improved using morphology-based taxonomy coupled with DNA sequence data (Dexter et al., 2010). Some representative specimens of most identified morphospecies were molecularly characterised generating for them DNA sequences of a fragment belonging to the barcoding locus COI mitochondrial gene (Hebert et al., 2003). This gene marker has been proved to be a generally reliable tool for the rapid delimitation of animal species, including insects (Hebert et al., 2003, 2004).

The genomic DNA from 1-6 specimens belonging to each discriminated insect morphotype was extracted. DNA extractions were conducted with the kit Tissue and tissue plus SV mini (Gene All<sup>®</sup>, Seoul, Korea), by placing each individual in 20  $\mu$ l of proteinase K and 200  $\mu$ l of TL Buffer, at 56 °C for 8 h. The larvae bodies were completely degraded after digestion, whereas the pupae exoskeletons and the adult individuals were subsequently washed with distilled water, placed back in 96% ethanol, and stored at -20 °C until they were mounted and labelled.

The COI fragment was amplified using the LCO1460/ HCO2198 primers (Folmer et al., 1994). The following PCR conditions were used: initial denaturation at 95 °C for 3 min, followed by 30 cycles of denaturation at 94 °C for 40 s, 40 s annealing at 45 °C, 40 s extension at 72 °C, and a final extension at 72 °C for 7 min. PCRs were prepared in a final volume of 15  $\mu$ l of reaction mixture, which included 1.5  $\mu$ l of 10X buffer, 0.75  $\mu$ l of MgCl 2 (50 mM), 0.3  $\mu$ l of dNTPs (10 mM), 0.24  $\mu$ l of each primer (10  $\mu$ M), 0.12  $\mu$ l of Taq Platinum polymerase (Invitrogen<sup>®</sup>, Carlsbad, CA, USA), 8.85  $\mu$ l of water and 3  $\mu$ l of DNA template. Unpurified PCR products were sent for DNA sequencing to the High-Throughput Genomics Unit of the University of Washington, Seattle, USA (http://www. htseq.org/). Sequences were edited and aligned manually based on their translated amino acids and compared individually with the sequences available in GenBank (http://www.ncbi.nlm.nih.gov/genbank) using the BLAST online program (Altschul et al., 1990; http://blast.ncbi. nlm.nih.gov/Blast.cgi).

DNA sequence-based species delimitation of dipterans and hymenopterans was carried out separately with the barcoding locus using 2 approaches, the General Mixed Yule Coalescence (GMYC) model (Pons et al., 2006) and the 2% genetic divergence criterion (Hebert et al., 2003).

The GMYC model requires an ultrametric tree, which was obtained with the program BEAST version 1.7.4 (Drummond et al., 2012), running the analysis for 10 million generations, sampling trees every 1,000 generations, using an uncorrelated lognormal clock and a coalescent tree prior. Only 1 partition was considered, which used the  $GTR+\Gamma+I$  evolutionary model. The duplicated haplotypes from the matrix were removed using the program Collapse 1.2 (Posada, 2004). The first 2,500 trees were eliminated as "burn-in" and the remaining trees were used to reconstruct a maximum clade credibility tree with the program TreeAnnotator version 1.7.4 (part of the BEAST 1.7.4 package). The GMYC model implemented in the SPLITS package (http://r-forge.r-project.org/projects/ splits/) was performed with the R program version 2.10.1 (R core Team. 2021).

For the 2% divergence criterion, the allocation of molecular taxonomic units (Hebert et al., 2003) was made depending on the percentage of similarity in the genetic distances of the analysed sequences. If the percentage was less than 2, those MOTUs were considered to belong to the same barcoding species. Uncorrected COI divergences were obtained with the program PAUP\* version 4.0 (Swofford, 2003). A Neighbor-Joining (NJ) distance tree for Diptera and Hymenoptera was reconstructed separately with the above program to visualise the genetic distances obtained. The trees obtained from 2 species delineation approaches were visualised with the program Figtree version 1.4.4 (Bouckaert et al., 2014).

## Results

## Description and abundance of galls

A total of 11,044 and 1,127 galls were dissected from 480 and 50 leaves obtained from the 15 trees that were sampled during the dry and rainy seasons, respectively. Five gall morphotypes from the leaves of *C. barbadensis* were identified (Fig. 1A-F): *1*) capsule-shaped (Fig. 1B),

green on both sides, glabrous, with a central inner elongated canal; 2) conical (Fig. 1C), pale green on both sides, ending on a sharp tip on the beam, glabrous, with an internal round chamber; 3) flattened (Fig. 1D), greenish-yellow on the beam and brown on the underside, glabrous, with a horizontal centrally elongated chamber; 4) spherical (Fig. 1E), glabrous, flat, indistinct and brown on the beam, brown and spherical on the underside, distributed irregularly along the leaf; and 5) rounded (Fig. 1F), brown on both sides, with abundant whitish pubescence. All gall morphotypes were recorded in both seasons except gall morphotype 5, which was not recorded during the rainy season.

A significant relationship ( $R^2 = 0.731$ , F = 21.796, p < 0.001) between the average number of total galls collected during the sampled weeks was observed, with the presence of galls in the leaves gradually increasing throughout the weeks during the dry season. The number of galls was relatively constant during the first 4 weeks (March 25-29 to April 22-23), but from the fifth week (April 29-30) it gradually increased (Fig. 2A).

Most galls collected during the dry season corresponded to gall morphotypes 1 and 4 (35% and 32%, respectively) and were present in 87% and 80% of the examined trees, respectively. Cecidomyiid exuviates adhered to the latter 2 gall morphotypes during the first 2 sampling weeks (25-26<sup>th</sup> of March - 1-2<sup>nd</sup> of April) and the last week of February, respectively. On the other hand, 13%, 18.5%, and 1.5% of the remaining galls belonged to gall morphotypes 2, 3, and 5 and occurred in 73%, 80%, and 13% of the trees, respectively. During the rainy season, 17.5%, 26.4%, 30.7%, and 25.4% of the collected galls corresponded to morphotype galls 1 to 4, respectively.

The average number of each gall morphotype varied over time during the dry season. There were significant differences between each type of gall ( $F_{3,128} = 8.616$ , p < 0.001) (Fig. 2B). The frequency of gall morphotype 1 gradually increased during the first weeks, though by the last week of April they were no longer registered. In contrast, gall morphotype 2 showed a lower abundance but its number was relatively constant throughout the sampling period, whereas gall morphotypes 3 and 4 had a low frequency but their number increased towards the first week of May. Gall morphotype 5 was only observed during the last 2 sampling weeks.

#### Integrative taxonomy

A total of 2,008 larvae and 356 adult insects were obtained from the dissected galls during both seasons. The morphospecies including larvae, pupae, and adults were first discriminated, for which were subsequently generated 230 COI sequences (151 sequences of Hymenoptera, 77 of Diptera, 1 of Coleoptera, and 1 of Lepidoptera (GenBank accession numbers in Appendix)



Figure 1. Leaf gall morphotypes found on *Coccoloba barbadensis* Jacq. (Polygonaceae). A) Leaves of *C. barbadensis*; B) capsule-shaped gall; C) conical gall; D) flattened gall; E) spherical gall; F) rounded gall.

resulting in 125 haplotypes. The number of species per family delimited by the 2 DNA sequence-based species delimitation approaches is provided in Table 1. The 2% COI divergence criterion and the GMYC model discriminated between 34 and 38 species, respectively. The NJ distance tree derived from the examined COI sequences of Hymenoptera and Diptera is shown in figures 3 and 4, respectively. There were 2 inconsistencies between both approaches. The 2% COI divergence criterion delimited 1 species of *Chrysonotomyia* (Entedoninae: Eulophidae) and 2 of *Teniupetiolus* (Eurytominae: Eurytomidae), whereas the GMYC model divided them into 3 and 2 species, respectively. DNA sequence data supported most of the delimited species using larvae and adults, except for the only species of *Torymus* Dalman (Hymenoptera: Torymidae), the 3 species of Cecidomyiidae (Diptera), and the single species of Lepidoptera and Coleoptera, for which we only generated sequences of larvae.

## Insect-gall association

Gall morphotype 3 had the highest insect species richness (20 species), which comprised all sampled families except the hymenopteran species of Braconidae (*Allorhogas coccolobae* Martínez & Zaldívar-Riverón) and Torymidae (*Torymus* sp.). This gall morphotype also had the highest number of eulophid species (8), from



Figure 2. A) Simple linear regression showing the average number of collected leaf galls on *C. barbadensis* during the time of collect in the dry season; B) graphic showing the average number of each leaf gall morphotype during the time of collect in the dry season.

which 5 belong to the subfamily Tetrastichinae and was only present in this gall morphotype. Gall morphotype 1 had 18 associated insect species, with Platygastridae and Eurytomidae (Hymenoptera) being the families with more species (5 and 4 species, respectively; Table 2). Gall morphotypes 2 and 4, on the other hand, registered 9 and 12 species, respectively, whereas gall morphotype 5 only had 4 species, 1 belonging to Eulohpidae (Hymenoptera), 1 to Platygastridae (Hymenoptera) and 2 to Cecidomyiidae (Diptera) (Table 2).

Larvae of Cecidomyiidae were highly abundant throughout the dry season. The abundance of larvae and

adults of the families Eurytomidae and Braconidae, on the other hand, considerably increased towards the sixth week of the dry season. In contrast, the presence of immature stages of Eulophidae was more frequent at the beginning of the dry season, though adults were also observed throughout this sampling period. Platygastridae was the hymenopteran family that was most frequently found in both larval and adult stages. Only immature individuals of Cecidomyiidae (Diptera) and Eulophidae (Hymenoptera) were collected during the rainy season.

A statistically significant difference ( $c^2 = 912,989$ , df = 102, p < 0.001) was observed between the 4 morphotypes of galls recorded during the dry season and their associated insect species. Fourteen out of the 37 insect species delimited with 2% barcoding were associated with a single gall morphotype. These included a species of Tenuipetiolus (Eurytomidae) in gall morphotype 1, 5 of Tetrastichinae (Eulophidae) in gall morphotypes 3, 1 species of Chrysonotomyia (Eulophidae) in gall morphotypes 1, 2 and 3, respectively, the single species of Eupelmidae and Torymidae in gall morphotypes 1 and 4, respectively, 2 and 1 species of Platygastridae in gall morphotypes 1 and 3, respectively, and 1 species of Cecidomyiidae in gall morphotype 3. Five delimited species of Cecidomyiidae were recorded in gall morphotypes 3 and 4, whereas 4, 3, and 2 were present in gall morphotypes 2, 1, and 5, respectively.

#### Discussion

A considerable leaf gall diversity in C. barbadensis is recorded here. Based on the gathered information, the inducers of these 5 types of leaf galls were species of Cecidomyiidae (Diptera) (see below). Other cecidomyiid galls that have been reported on species of Coccoloba include those found on stems of C. mosenii Lindl., on leaves of Coccoloba cf. warmingii Meisn., C. diversifolia Jacq., C. swartzii Kuntze, and C. uvifera Meins., and on inflorescences of C. alnifolia Casar (Mead, 1970; Maia et al., 2008; Ramos-Rodrigues et al., 2014). The number of different types of galls found in C. barbadensis (5 morphotypes) is higher than those reported for the above species ( $\leq 2$  morphotypes). However, based on their general appearance, the gall morphotypes 1 and 2 may be variants made by the same inducer species. Further rearing observations and molecular characterisation of gall inducers will help to confirm the actual gall diversity that is present on the leaves of this plant species.

There is considerable morphological diversity in the leaf gall morphotypes found on *C. barbadensis* (Fig. 1A-F). All these gall morphotypes were located on the leaf blade, with 4 of them being located on the beam and 1 on the



Figure 3. Neighbour Joining phenogram reconstructed for specimens of Cecidomyiidae that were reared from the 4 leaf gall morphotypes found in *C. barbadensis*. Bars refer to the 7 species of Cecidomyiidae that were delimited with the 2% barcoding approach.

underside. The considerable morphological diversity of these leaf galls can be attributed to several factors, among which include the high synthetic activity, rapid growth, structural and functional features, and characteristic morphogenetic patterns of leaf development (Mani, 1964). Further studies on these leaf galls are therefore necessary for a better understanding of the structural and histological differences among the leaf galls found on *C. barbadensis*.

#### *Insect species richness*

The high morphological diversity found on leaf galls favours the existence of a complex insect community (Mani, 1964); however, few studies have assessed in detail the insect community associated with leaf galls of Neotropical plant species (e.g., Maia, 2012). In this research, the extensive insect rearing, and examination of both molecular and morphological information helped



Figure 4. Neighbour Joining phenogram reconstructed for specimens of Hymenoptera that were reared from the 4 leaf gall morphotypes found in *C. barbadensis*. Bars refer to the 25 species of Hymenoptera that were delimited with the 2% barcoding approach.

to thoroughly characterise the insect species diversity found in the 5 types of galls present on leaves of C. *barbadensis*. Despite that, was not possible to confirm at this stage the biology of the reared taxa, and then their probable role is based on the relevant literature and the field observations.

Cecidomyiids, commonly known as gall midges, represent by far the group of insects with the most gallinducer species (Gagné & Jaschhof, 2021). Species of Cecidomyiidae are mainly gall-formers, though members of the tribe Cecidomyiini are known to have a wide range of biologies, including only simple and complex gall formers, free-living, mycophagous, inquiline phytophagous, predator species of mites, aphids, and coccids, as well as internal parasitoids of aphids and psyllids (Kim et al., 2014; Uechi et al., 2011). The field observations and the insect species diversity found in the 5 leaf gall morphotypes of *C. barbadensis* strongly suggest that they are induced by cecidomyiid species. Among this evidence was that most of the dissected galls had a single Table 1

Order/Family	Subfamily	Genus	2% DC	GMYC
Diptera				
Cecidomyiidae	-	-	7	7
Lepidoptera	-	-	1	-
Coleoptera	-	-	1	-
Hymenoptera				
Braconidae	Doryctinae	Allorhogas coccolobae	1	1
Eulophidae	Entedoninae	Chrysonotomyia spp.	6	8
	Tetrastichinae	Quadrastichus spp.	5	5
Eupelmidae		Brasema sp.	1	1
Eurytomidae	Eurytominae	Tenuipetiolus spp.	5	7
Platygastridae		Synopeas spp.	3	3
		Inostemma spp.	1	1
		Undetermined	2	2
Torymidae		Torymus sp.	1	1
Total			34	38

Number of insect species discriminated by DNA sequence-based species delineation approaches conducted in this study (2% COI divergence criterion; GMYC method).

#### Table 2

Insect species richness by order and family associated with 5 gall morphotypes found on leaves of *Coocoloba barbadensis* Jacq.

	Gall morphotypes (GM)					
Insect Order/ Family	GM 1	GM 2	GM 3	GM 4	GM 5	TOTAL
Coleoptera	-	-	1	-	-	1
Lepidoptera	-	-	1	-	-	1
Diptera						
Cecidomyiidae	3	4	5	5	2	19
Hymenoptera	15	5	13	7	2	42
Eurytomidae	4	2	3	3	-	12
Eulophidae	3	2	8	1	1	15
Eupelmidae	1	-	-	-	-	1
Braconidae	1	-	-	1	-	2
Platygastridae	5	1	2	2	1	11
Torymidae	1	-	-	-	-	1
Total	18	9	20	12	4	63

cecidomyiid larva. Moreover, several galls belonging to morphotype 2 had a cecidomyiid pupal exuviae hanging outside of a small opening, which is a common feature of many galls with former cecidomyiids. Since the 5 leaf gall morphotypes were formed by cecidomyiid species, the remaining species of this family delimited in the study probably are inquilines.

The cecidomyiid larvae and their emerging adults could not be identified at the genus level; however, a BLAST similarity search of the barcoding locus for the delimited cecidomyiid species suggests that they are closely related to species of the Cecidomyiini genera *Contarinia* Geer and *Macrodiplosis* Kieffer. The gall-inducing species of the genus *Contarinia* are cosmopolitan and can be either monophagous or polyphagous with a wide range of hosts (Uechi et al., 2011). Most species of this genus live gregariously in the floral parts of the plant or in the galls that they induce on the leaves (Gagné & Jaschoff, 2021). Species of *Macrodiplosis*, on the other hand, are mainly gall inducers on leaves of plant species of the genus *Quercus* (Kim et al., 2014).

Currently, 4 gall midge species are known to be associated with species of *Coccoloba*. The genus *Ctenodactylomyia* Felt (supertribe Cecidomyiidi, unplaced tribe) has 2 leaf gall inducer species on *C. diversifolia*  Jacq., *C. swartzii*, and *C. uvifera* L. from the Caribbean (Gagné & Jaschoff, 2021). Moreover, *Marilasioptera tripartite* Möhn and *Meunieriella magdalenae* Wünsch (supertribe Lasiopteridi, tribe Alycaulini) were described as inquilines on galls induced by other insects on species of *Coccoloba* from El Salvador and Colombia, respectively (Gagné & Jaschoff, 2021). The present study increases the number of gall midge species associated with *Coccoloba* to 11.

Our study also found a considerable number of hymenopteran species reared from the 5 examined leaf gall morphotypes, most of which probably are parasitoids of the cecidomyiid species. These parasitoid species belong to the wasp families Eulophidae, Eupelmidae, Platygastridae, and Torymidae, whereas the only reared braconid species probably are phytophagous inquiline. Some members of the Platygastridae are known to be koinobiont endoparasitoids of gallery cecidomyiid eggs, and they are known to be closely associated with the parts of the plant where the host gall is found (Masner & Huggert, 1989; Masner, 1993). Eulophidae are also mainly parasitoids of holometabolous insect larvae (though in some cases also of eggs, prepupae, and pupae) of Lepidoptera, Hymenoptera, Diptera, and Coleoptera (Graham, 1991); however, some species have been reported to be phytophagous or predators (Gibson, 1993). Finally, members of the eupelmid subfamily Eupelminae, from which the genus Brasema belongs, mainly are parasitoids of larval stages of various insect hosts (Gibson, 1993).

The wasp family Eurytomidae is represented by entomophagous species that parasitise larval or pupal stages of Coleoptera, Diptera, and Hymenoptera as solitary endoparasitic idiobionts, though some species can be phytophagous feeding on seeds, or inquilines feeding on both their host and gall tissue (Lotfalizadeh et al., 2007; Gates & Delvare, 2008). Species of *Tenuipetiolus* have been reported to be parasitoids of gall inducer insects, including cecidomyiids and cynipids (Gates & Hanson, 2006; Zhang et al., 2014). Similarly, several species of Torymidae are known to be ectoparasitoids of gallforming insects of the latter 2 families (Gibson, 1993).

The braconid species A. coccolobae (Doryctinae) was described a decade ago from the Chamela region in Jalisco, Mexico, based on specimens reared from leaf galls of C. barbadensis (Martínez & Zaldívar-Riverón, 2013), that correspond to the morphotype 2. Here, this braconid species was reared not only from the above leaf gall morphotype but also from morphotype 4. Several species of Allorhogas have been confirmed to feed on various plant families either by being gall formers or inquilines of galls made by other insect taxa (Centrella & Shaw, 2010; Chavarría et al., 2009; de Mâcedo et al., 1998; Zaldívar-Riverón et al., 2014). Since none of the Allorhogas species with recorded biology are known to be parasitoids, and due to the low abundance of this species compared with the cecidomyiid species that were reared from the same gall morphotypes, it is presumed that it could be a phytophagous inquiline.

The considerably high, mostly undescribed species diversity of cecidomyiid dipterans and parasitoid hymenopterans that we reared from the 5 leaf gall morphotypes of *C. barbadensis* remark the necessity to carry out more studies that focus on the species diversity of galls, particularly in tropical regions. Moreover, this study highlights the importance of performing integrative species delineation studies of insects present in galls to have a more accurate knowledge of their actual diversity and trophic interactions.

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Order (Insecta)	Family, Genus	Species	DNA voucher No.	Genbank accession No.
Diptera	Cecidomyiidae	sp.	CeciAN177_L4	PP659769
Diptera	Cecidomyiidae	sp.	CeciAN178_L4	PP659770
Diptera	Cecidomyiidae	sp.	CeciAN179_L4	PP659771
Diptera	Cecidomyiidae	sp.	CeciAN181_L4	PP659772
Diptera	Cecidomyiidae	sp.	CeciAN182_L4	PP659773
Diptera	Cecidomyiidae	sp.	CeciAN185_L3	PP659774
Diptera	Cecidomyiidae	sp.	CeciAN192_L4	PP659775
Diptera	Cecidomyiidae	sp.	CeciAN197_L4	PP659776
Diptera	Cecidomyiidae	sp.	CeciAN204_L4	PP659777
Diptera	Cecidomyiidae	sp.	CeciAN206_L4	PP659778
Diptera	Cecidomyiidae	sp.	CeciAN208_L4	PP659779
Diptera	Cecidomyiidae	sp.	CeciAN210_L4	PP659780
Diptera	Cecidomyiidae	sp.	CeciAN211_L4	PP659781
Diptera	Cecidomyiidae	sp.	CeciAN238_L4	PP659782
Diptera	Cecidomyiidae	sp.	CeciAN240_L4	PP659783
Diptera	Cecidomyiidae	sp.	CeciAN241_L4	PP659784
Diptera	Cecidomyiidae	sp.	CeciAN242_L4	PP659785
Diptera	Cecidomyiidae	sp.	CeciAN256_L2	PP659786
Diptera	Cecidomyiidae	sp.	CeciAN260_L4	PP659787
Diptera	Cecidomyiidae	sp.	CeciAN261_L4	PP659788
Diptera	Cecidomyiidae	sp.	CeciAN263_L4	PP659789
Diptera	Cecidomyiidae	sp.	CeciAN264_L4	PP659790
Diptera	Cecidomyiidae	sp.	CeciAN17_L4	PP659793
Diptera	Cecidomyiidae	sp.	CeciAN205_L4	PP659796
Diptera	Cecidomyiidae	sp.	CeciAN226_L4	PP659798
Diptera	Cecidomyiidae	sp.	CeciAN232_L2	PP659799
Diptera	Cecidomyiidae	sp.	CeciAN254_L2	PP659800
Diptera	Cecidomyiidae	sp.	CeciAN161_L3	PP659757
Diptera	Cecidomyiidae	sp.	CeciAN162_L3	PP659758
Diptera	Cecidomyiidae	sp.	CeciAN163_L3	PP659759
Diptera	Cecidomyiidae	sp.	CeciAN172_L3	PP659760
Diptera	Cecidomyiidae	sp.	CeciAN174_L2	PP659761
Diptera	Cecidomyiidae	sp.	CeciAN199_L4	PP659762
Diptera	Cecidomyiidae	sp.	CeciAN200_L1	PP659763
Diptera	Cecidomyiidae	sp.	CeciAN201_L5	PP659764
Diptera	Cecidomyiidae	sp.	CeciAN202_L4	PP659765
Diptera	Cecidomyiidae	sp.	CeciAN203_L2	PP659766
Diptera	Cecidomyiidae	sp.	CeciAN235_L2	PP659767

Appendix.	Continued.
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Order (Insecta)	Family, Genus	Species	DNA voucher No.	Genbank accession No.
Diptera	Cecidomyiidae	sp.	CeciAN255_L2	PP659768
Diptera	Cecidomyiidae	sp.	CeciAN23_L2	PP659794
Diptera	Cecidomyiidae	sp.	CeciAN193_L2	PP659795
Diptera	Cecidomyiidae	sp.	CeciAN291_A3	PP659802
Diptera	Cecidomyiidae	sp.	CeciAN292_A1	PP659803
Diptera	Cecidomyiidae	sp.	CeciAN257_L2	PP659804
Diptera	Cecidomyiidae	sp.	CeciAN259_L3	PP659805
Diptera	Cecidomyiidae	sp.	CeciAN262_L4	PP659806
Diptera	Cecidomyiidae	sp.	CeciAN207_L4	PP659797
Diptera	Cecidomyiidae	sp.	CeciAN290_A1	PP659801
Diptera	Cecidomyiidae	sp.	CeciAN160_L3	PP659807
Diptera	Cecidomyiidae	sp.	CeciAN02_L4	PP659731
Diptera	Cecidomyiidae	sp.	CeciAN05_L4	PP659732
Diptera	Cecidomyiidae	sp.	CeciAN159_L1	PP659733
Diptera	Cecidomyiidae	sp.	CeciAN165_L2	PP659734
Diptera	Cecidomyiidae	sp.	CeciAN166_L2	PP659735
Diptera	Cecidomyiidae	sp.	CeciAN167_L3	PP659736
Diptera	Cecidomyiidae	sp.	CeciAN168_L4	PP659737
Diptera	Cecidomyiidae	sp.	CeciAN169_L4	PP659738
Diptera	Cecidomyiidae	sp.	CeciAN171_L1	PP659739
Diptera	Cecidomyiidae	sp.	CeciAN173_L1	PP659740
Diptera	Cecidomyiidae	sp.	CeciAN176_L1	PP659741
Diptera	Cecidomyiidae	sp.	CeciAN180_L2	PP659742
Diptera	Cecidomyiidae	sp.	CeciAN183_L1	PP659743
Diptera	Cecidomyiidae	sp.	CeciAN186_L5	PP659744
Diptera	Cecidomyiidae	sp.	CeciAN188_L1	PP659745
Diptera	Cecidomyiidae	sp.	CeciAN191_L1	PP659747
Diptera	Cecidomyiidae	sp.	CeciAN231_L1	PP659750
Diptera	Cecidomyiidae	sp.	CeciAN233_L1	PP659751
Diptera	Cecidomyiidae	sp.	CeciAN234_L2	PP659752
Diptera	Cecidomyiidae	sp.	CeciAN237_L1	PP659753
Diptera	Cecidomyiidae	sp.	CeciAN245_L1	PP659755
Diptera	Cecidomyiidae	sp.	CeciAN267_L1	PP659756
Diptera	Cecidomyiidae	sp.	CeciAN06_A3	PP659791
Diptera	Cecidomyiidae	sp.	CeciAN209_L5	PP659749
Diptera	Cecidomyiidae	sp.	CeciAN43_A2	PP659792
Diptera	Cecidomyiidae	sp.	CeciAN189_L3	PP659746
Diptera	Cecidomyiidae	sp.	CeciAN194_L3	PP659748
Diptera	Cecidomyiidae	sp.	CeciAN239_L4	PP659754

Order (Insecta)	Family, Genus	Species	DNA voucher No.	Genbank accession No.
Hymenoptera	Platygaster	sp.	TeleAN08_L3	PP481223
Hymenoptera	Platygaster	sp.	TeleAN19_P3	PP481224
Hymenoptera	Platygaster	sp.	TeleAN46_A1	PP481225
Hymenoptera	Platygaster	sp.	TeleAN45_A3	PP481226
Hymenoptera	Platygaster	sp.	TeleAN50_A3	PP481227
Hymenoptera	Platygaster	sp.	TeleAN30_A3	PP481228
Hymenoptera	Platygaster	sp.	TeleAN132_A3	PP481229
Hymenoptera	Platygaster	sp.	TeleAN133_A1	PP481230
Hymenoptera	Platygaster	sp.	TeleAN134_A3	PP481231
Hymenoptera	Platygaster	sp.	TeleAN135_A1	PP481232
Hymenoptera	Platygaster	sp.	TeleAN137_A1	PP481233
Hymenoptera	Platygaster	sp.	TeleAN138_A3	PP481234
Hymenoptera	Platygaster	sp.	TeleAN141_A3	PP481235
Hymenoptera	Platygaster	sp.	TeleAN151_A3	PP481236
Hymenoptera	Platygaster	sp.	TeleAN152_A3	PP481237
Hymenoptera	Platygaster	sp.	TeleAN153_A3	PP481238
Hymenoptera	Platygaster	sp.	TeleAN154_A3	PP481239
Hymenoptera	Platygaster	sp.	TeleAN156_A3	PP481240
Hymenoptera	Platygaster	sp.	TeleAN215_L3	PP481241
Hymenoptera	Platygaster	sp.	TeleAN216_L3	PP481242
Hymenoptera	Platygaster	sp.	TeleAN217_L3	PP481243
Hymenoptera	Platygaster	sp.	TeleAN218_L3	PP481244
Hymenoptera	Platygaster	sp.	TeleAN224_L1	PP481245
Hymenoptera	Platygaster	sp.	TeleAN225_L1	PP481246
Hymenoptera	Platygaster	sp.	TeleAN275_L3	PP481247
Hymenoptera	Platygaster	sp.	TeleAN276_L3	PP481248
Hymenoptera	Platygaster	sp.	TeleAN281_A3	PP481249
Hymenoptera	Platygaster	sp.	TeleAN158_A1	PP481250
Hymenoptera	Platygaster	sp.	TeleAN110_A1	PP481251
Hymenoptera	Platygaster	sp.	TeleAN111_A3	PP481252
Hymenoptera	Synopeas	sp.	PlatyAN22_A1	PP481253
Hymenoptera	Synopeas	sp.	PlatyAN32_A1	PP481254
Hymenoptera	Synopeas	sp.	PlatyAN52_A1	PP481255
Hymenoptera	Synopeas	sp.	PlatyAN14_A1	PP481256
Hymenoptera	Synopeas	sp.	PlatyAN157_A1	PP481257
Hymenoptera	Synopeas	sp.	PlatyAN53_A1	PP481258
Hymenoptera	Synopeas	sp.	PlatyAN99_A1	PP481259
Hymenoptera	Synopeas	sp.	PlatyAN101_A1	PP481260
Hymenoptera	Synopeas	sp.	PlatyAN102_A1	PP481261

## Appendix. Continued.

Appendix.	Continued.
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Order (Insecta)	Family, Genus	Species	DNA voucher No.	Genbank accession No.
Hymenoptera	Synopeas	sp.	PlatyAN103_A1	PP481262
Hymenoptera	Synopeas	sp.	PlatyAN105_A1	PP481263
Hymenoptera	Synopeas	sp.	PlatyAN190_L2	PP481264
Hymenoptera	Synopeas	sp.	PlatyAN219_L1	PP481265
Hymenoptera	Inostemma	sp.	PlatyAN221_L4	PP481266
Hymenoptera	Synopeas	sp.	PlatyAN223_L2	PP481267
Hymenoptera	Synopeas	sp.	PlatyAN220_L5	PP481268
Hymenoptera	Inostemma	sp.	PlatyAN112_A4	PP481269
Hymenoptera	Synopeas	sp.	PlatyAN116_A1	PP481270
Hymenoptera	Synopeas	sp.	PlatyAN117_A1	PP481271
Hymenoptera	Synopeas	sp.	PlatyAN118_A1	PP481272
Hymenoptera	Inostemma	sp.	PlatyAN126_A1	PP481273
Hymenoptera	Synopeas	sp.	PlatyAN214_L1	PP481274
Hymenoptera	Platygaster	sp.	PlatyAN222_L3	PP481275
Hymenoptera	Synopeas	sp.	PlatyAN272_A1	PP481276
Hymenoptera	Synopeas	sp.	PlatyAN273_L	PP481277
Hymenoptera	Synopeas	sp.	PlatyAN274_L	PP481278
Hymenoptera	Synopeas	sp.	PlatyAN277_L5	PP481279
Hymenoptera	Synopeas	sp.	PlatyAN278_L2	PP481280
Hymenoptera	Synopeas	sp.	PlatyAN279_L1	PP481281
Hymenoptera	Tenuipetiolus	sp.	EurytAN96_A1	PP481282
Hymenoptera	Tenuipetiolus	sp.	EurytAN265_L3	PP481283
Hymenoptera	Chrysonotomyia	sp.	EntedAN01_P1	PP481284
Hymenoptera	Chrysonotomyia	sp.	EntedAN07_A1	PP481285
Hymenoptera	Chrysonotomyia	sp.	EntedAN29_A1	PP481286
Hymenoptera	Chrysonotomyia	sp.	EntedAN11_A1	PP481287
Hymenoptera	Chrysonotomyia	sp.	EntedAN31_A1	PP481288
Hymenoptera	Chrysonotomyia	sp.	EntedAN51_A1	PP481289
Hymenoptera	Chrysonotomyia	sp.	EntedAN59_P1	PP481290
Hymenoptera	Chrysonotomyia	sp.	EntedAN61_P1	PP481291
Hymenoptera	Chrysonotomyia	sp.	EntedAN67_P1	PP481292
Hymenoptera	Chrysonotomyia	sp.	EntedAN69_A1	PP481293
Hymenoptera	Chrysonotomyia	sp.	EntedAN70_A1	PP481294
Hymenoptera	Chrysonotomyia	sp.	EntedAN71_A1	PP481295
Hymenoptera	Chrysonotomyia	sp.	EntedAN72_A1	PP481296
Hymenoptera	Chrysonotomyia	sp.	EntedAN75_A1	PP481297
Hymenoptera	Chrysonotomyia	sp.	EntedAN94_A1	PP481298
Hymenoptera	Chrysonotomyia	sp.	EntedAN95_A1	PP481299
Hymenoptera	Chrysonotomyia	sp.	EntedAN25_A2	PP481300

Order (Insecta)	Family, Genus	Species	DNA voucher No.	Genbank accession No.
Hymenoptera	Chrysonotomyia	sp.	EntedAN64_P4	PP481301
Hymenoptera	Chrysonotomyia	sp.	EntedAN73_A3	PP481302
Hymenoptera	Chrysonotomyia	sp.	EntedAN142_A1	PP481303
Hymenoptera	Chrysonotomyia	sp.	EntedAN143_A1	PP481304
Hymenoptera	Chrysonotomyia	sp.	EntedAN144_A1	PP481305
Hymenoptera	Chrysonotomyia	sp.	EntedAN145_A1	PP481306
Hymenoptera	Chrysonotomyia	sp.	EntedAN146_A2	PP481307
Hymenoptera	Chrysonotomyia	sp.	EntedAN198_L3	PP481308
Hymenoptera	Chrysonotomyia	sp.	EntedAN236_L1	PP481309
Hymenoptera	Chrysonotomyia	sp.	EntedAN243_L1	PP481310
Hymenoptera	Chrysonotomyia	sp.	EntedAN244_L1	PP481311
Hymenoptera	Chrysonotomyia	sp.	EntedAN288_A2	PP481312
Hymenoptera	Chrysonotomyia	sp.	EntedAN289_A2	PP481313
Hymenoptera	Chrysonotomyia	sp.	EntedAN58_P1	PP481314
Hymenoptera	Chrysonotomyia	sp.	EntedAN55_A1	PP481315
Hymenoptera	Chrysonotomyia	sp.	EulopAN125_A5	PP481316
Hymenoptera	Tetrastichinae	sp.	TetrasAN84_A3	PP481317
Hymenoptera	Tetrastichinae	sp.	TetrasAN62_P3	PP481318
Hymenoptera	Tetrastichinae	sp.	TetrasAN63_P3	PP481319
Hymenoptera	Tetrastichinae	sp.	TetrasAN66_P3	PP481320
Hymenoptera	Tetrastichinae	sp.	TetrasAN83_A3	PP481321
Hymenoptera	Tetrastichinae	sp.	TetrasAN60_P3	PP481322
Hymenoptera	Tetrastichinae	sp.	TetrasAN269_L3	PP481323
Hymenoptera	Tetrastichinae	sp.	TetrasAN270_L3	PP481324
Hymenoptera	Tenuipetiolus	sp.	EurytAN77_A1	PP481325
Hymenoptera	Tenuipetiolus	sp.	EurytAN81_A3	PP481326
Hymenoptera	Tenuipetiolus	sp.	EurytAN93_A3	PP481327
Hymenoptera	Allorhogas	coccolobae	AllorAN18_L4	PP481328
Hymenoptera	Allorhogas	coccolobae	AllorAN28_A4	PP481329
Hymenoptera	Allorhogas	coccolobae	AllorAN35_A4	PP481330
Hymenoptera	Allorhogas	coccolobae	AllorAN48_A4	PP481331
Hymenoptera	Allorhogas	coccolobae	AllorAN56_A4	PP481332
Hymenoptera	Allorhogas	coccolobae	AllorAN107_A4	PP481333
Hymenoptera	Allorhogas	coccolobae	AllorAN108_A4	PP481334
Hymenoptera	Allorhogas	coccolobae	AllorAN109_A4	PP481335
Hymenoptera	Allorhogas	coccolobae	AllorAN121_A4	PP481336
Hymenoptera	Allorhogas	coccolobae	AllorAN122_A4	PP481337
Hymenoptera	Allorhogas	coccolobae	AllorAN195_L1	PP481338
Hymenoptera	Allorhogas	coccolobae	AllorAN248_L4	PP481339

Order (Insecta)	Family, Genus	Species	DNA voucher No.	Genbank accession No.
Hymenoptera	Allorhogas	coccolobae	AllorAN249_L4	PP481340
Hymenoptera	Tenuipetiolus	sp.	EurytAN212_L3	PP481341
Hymenoptera	Tenuipetiolus	sp.	EurytAN228_L3	PP481342
Hymenoptera	Tenuipetiolus	sp.	EurytAN246_L3	PP481343
Hymenoptera	Tenuipetiolus	sp.	EurytAN285_A3	PP481344
Hymenoptera	Tenuipetiolus	sp.	EurytAN286_A3	PP481345
Hymenoptera	Tenuipetiolus	sp.	EurytAN37_A1	PP481346
Hymenoptera	Tenuipetiolus	sp.	EurytAN147_A3	PP481347
Hymenoptera	Tenuipetiolus	sp.	EurytAN148_A4	PP481348
Hymenoptera	Tenuipetiolus	sp.	EurytAN149_A3	PP481349
Hymenoptera	Tenuipetiolus	sp.	EurytAN130_A1	PP481350
Hymenoptera	Tenuipetiolus	sp.	EurytAN87_A2	PP481351
Hymenoptera	Tenuipetiolus	sp.	EurytAN91_A4	PP481352
Hymenoptera	Tenuipetiolus	sp.	EurytAN80_A3	PP481353
Hymenoptera	Tenuipetiolus	sp.	EurytAN82_A4	PP481354
Hymenoptera	Tenuipetiolus	sp.	EurytAN90_A4	PP481355
Hymenoptera	Tenuipetiolus	sp.	EurytAN85_A1	PP481356
Hymenoptera	Torymus	sp.	EurytAN57_A4	PP481357
Hymenoptera	Tenuipetiolus	sp.	EurytAN164_L1	PP481358
Hymenoptera	Tenuipetiolus	sp.	EurytAN227_L1	PP481359
Hymenoptera	Tenuipetiolus	sp.	EurytAN230_L1	PP481360
Hymenoptera	Tenuipetiolus	sp.	EurytAN283_A2	PP481361
Hymenoptera	Tenuipetiolus	sp.	EurytAN33_A4	PP481362
Hymenoptera	Tenuipetiolus	sp.	EurytAN34_A1	PP481363
Hymenoptera	Tenuipetiolus	sp.	EurytAN15_L1	PP481364
Hymenoptera	Tenuipetiolus	sp.	EurytAN114_A3	PP481365
Hymenoptera	Tenuipetiolus	sp.	EurytAN79_A1	PP481366
Hymenoptera	Tenuipetiolus	sp.	EurytAN78_A1	PP481367
Hymenoptera	Tenuipetiolus	sp.	EurytAN24_L1	PP481368
Hymenoptera	Chrysonotomyia	sp.	EurytAN88_A3	PP481369
Hymenoptera	Brasema	sp.	ToryAN170_L1	PP481370
Hymenoptera	Brasema	sp.	ToryAN196_L1	PP481371
Hymenoptera	Torymus	sp.	EurytAN229_L4	PP481372
Hymenoptera	Torymus	sp.	EurytAN266_L4	PP481373
Coleoptera			AN184	PP897664
Lepidoptera			AN250 L3	PP897665

## Appendix. Continued.

#### References

- Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, 215, 403–410. https://doi.org/10.1016/ S0022-2836(05)80360-2
- Bortolus, A. (2008). Error cascades in the biological sciences: the unwanted consequences of using bad taxonomy in ecology. *AMBIO: A Journal of the Human Environment*, *37*, 114–118. https://doi.org/10.1579/0044-7447(2008)37[114:EC ITBS]2.0.CO:2
- Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C. H., Xie, D., Suchard, M. A., Rambaut, A., & Drummond, A. J. (2014). BEAST 2: A software platform for Bayesian evolutionary analysis. *Plos Computational Biology*, 10, e1003537. https://doi.org/10.1371/journal.pcbi.1003537
- Centrella, M. L., & Shaw, S. R. (2010). A new species of phytophagous braconid Allorhogas minimus (Hymenoptera: Braconidae: Doryctinae) reared from fruit galls on *Miconia longifolia* (Melastomataceae) in Costa Rica. *International Journal of Tropical Insect Science*, 30, 101–107. https://doi. org/10.1017/S1742758410000147
- Chavarría, L., Hanson, P., Marsh, P., & Shaw, S. (2009). A phytophagous braconid, *Allorhogas conostegia* sp. nov. (Hymenoptera: Braconidae), in the fruits of *Conostegia xalapensis* (Bonpl.) D. Don (Melastomataceae). *Journal* of Natural History, 43, 2677–2689. https://doi.org/10.1080/ 00222930903243996
- Clare, E. L., Schiestl, F. P., Leitch, A. R., & Chittka, L. (2013). The promise of genomics in the study of plant-pollinator interactions. *Genome Biology*, 14, 1–11. https://doi.org/10. 1186/gb-2013-14-6-207
- de Macêdo, M. V., & Monteiro, R. F. (1989). Seed predation by a braconid wasp, *Allorhogas* sp. (Hymenoptera). *Journal of the New York Entomological Society*, 97, 358–362.
- Dexter, K. G., Pennington, T. D., & Cunningham, C. W. (2010). Using DNA to assess errors in tropical tree identifications: How often are ecologists wrong and when does it matter? *Ecological Monographs*, 80, 267–286. https://doi. org/10.1890/09-0267.1
- Drummond, A. J., Suchard, M. A., Xie, D., & Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, 29, 1969–1973. https:// doi.org/10.1093/molbev/mss075
- Folmer O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3, 294–299.
- Forbes, A. A., Hall, M. C., Lund, J., Hood, G. R., Izen, R., Egan, S. P. et al. (2016). Parasitoids, hyperparasitoids, and inquilines associated with the sexual and asexual generations of the gall former, *Belonocnema treatae* (Hymenoptera: Cynipidae). *Annals of the Entomological Society of America*, 109, 49–63. https://doi.org/10.1093/ aesa/sav112

- Gagné, R. J., & Jaschhof, M. (2021). A catalog of the Cecidomyiidae (Diptera) of the World, 5<sup>th</sup> Edition. Available at: https://www.ars.usda.gov/ARSUserFiles/80420580/Gag ne Jaschhof 2021 World Cat 5th Ed.pdf
- Gates, M. W., & Hanson, P. E. (2006). Familia Eurytomidae, In P. E. Hanson, & I. D. Gauld (Eds.), Hymenoptera de la region tropical (pp. 380–387). Memoirs of the American Entomological Institute, 77, 1–994.
- Gates, M., & Delvare, G. (2008). A new species of *Eurytoma* (Hymenoptera: Eurytomidae) attacking *Quadrastichus* spp. (Hymenoptera: Eulophidae) galling *Erythrina* spp. (Fabaceae), with a summary of African *Eurytoma* biology and species checklist. *Zootaxa*, 1751, 1–24. https://doi. org/10.11646/zootaxa.1751.1.1
- Gibson, L. (1993). Superfamilies Mymarommatoidea and Chalcidoidea. In H. Goulet, & J. T. Huber (Eds.), *Hymenoptera* of the world: an identification guide to families (pp. 570–655). Research Branch, Agriculture, Canada, Publication 1894.
- Graham, M. W. R. V. (1991). A reclassification of the European Tetrastichinae (Hymenoptera: Eulophidae). Revision of the remaining genera. *Memoirs of the American Entomological Institute*, 49, 1–322.
- Hebert, P. D., Cywinska, A., Ball, S. L., & DeWaard, J R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 2070, 313–321. https://doi.org/10.1098/ rspb.2002.2218
- Hebert, P. D., Penton, E. H., Burns, J. M., Janzen, H. D., & Hallwachs, W. (2004). Ten species in one: DNA barcoding reveals cryptic species in the Neotropical skipper butterfly *Astrapes fulgerator. Proceeding of the National Academy* of Sciences, 101, 14812–14817. https://doi.org/10.1073/pnas. 0406166101
- Howard, R. A. (1959). Studies in the genus Coccoloba. VII A synopsis and key to species in Mexico and Central America. Journal of the Arnold Arboretum, 40, 176–203.
- Ings, T. C., Montoya, J. M., Bascompte, J., Blüthgen, N., Brown, L., Dormann, C. F. et al. (2009). Ecological networks-beyond food webs. *Journal of Animal Ecology*, 78, 253–269. https:// doi.org/10.1111/j.1365-2656.2008.01460.x
- Kaartinen, R., Stone, G. N., Hearn, J., Lohse, K., & Roslin, T. (2010). Revealing secret liaisons: DNA barcoding changes our understanding of food webs. *Ecological Entomology*, 35, 623–638. https://doi.org/10.1111/j.1365-2311.2010.01224.x
- Kaartinen, R., & Roslin, T. (2011). Shrinking by numbers: landscape context affects the species composition but not the quantitative structure of local food webs. *Journal of Animal Ecology*, 80, 622–631. https://doi.org/10.1111/ j.1365-2656.2011.01811.x
- Kim, W., Yukawa, J., Harris, K. M., Minami, T., Matsuo, K., & Skrzypczyńska, M. (2014). Description, host range, and distribution of a new *Macrodiplosis* species (Diptera: Cecidomyiidae) that induces leaf-margin fold galls on deciduous *Quercus* (Fagaceae) with comparative notes on

Palaearctic congeners. *Zootaxa*, *3821*, 222–238. https://doi. org/10.11646/zootaxa.3821.2.3

- Lotfalizadeh, H., Delvare, G., & Rasplus, J. Y. (2007). Phylogenetic analysis of Eurytominae (Chalcidoidea: Eurytomidae) based on morphological characters. *Zoological Journal of the Linnean Society*, *151*, 441–510. https://doi.org/10.1111/j.1096-3642.2007.00308.x
- Maia, V. C., Magenta, M. A. G., & Martins, S. E. (2008). Ocorrência e caracterização de galhas de insetos em áreas de restinga de Bertioga (São Paulo, Brasil). *Biota Neotropica*, 8, 167–197. https://doi.org/10.1590/S1676-06032 008000100020
- Maia, V. C. (2012). Richness of hymenopterous galls from South America. Papéis Avulsos de Zoologia, 52, 423–429. https:// doi.org/10.1590/S0031-10492012021500001
- Mani, M. S. (1964). Ecology of plant galls. The Hague: W. Junk.
- Martínez, J. J., & Zaldívar-Riverón, A. (2013). Seven new species of *Allorhogas* (Hymenoptera: Braconidae: Doryctinae) from Mexico. *Revista Mexicana de Biodiversidad*, 84, 117–139. https://doi.org/10.7550/rmb.31955
- Masner, L., & Huggert, L. (1989). World review and keys to genera of the subfamily Inostemmatinae with the reassignment of the taxa to the Platygastrinae and Sceliotrachelinae (Hymenoptera: Platygastridae). The Memoirs of the Entomological Society of Canada, 121, 3–216.
- Masner, L. (1993). Superfamily Platygastroidea. In H. Goulet, & J. T. Huber (Eds.), *Hymenoptera of the world: an identification guide to families* (pp. 558–565). Research Branch, Agriculture, Canada, Publication 1894.
- Mead, F. W. (1970). Ctenodactylomyia watsoni Felt, a gall midge of seagrape, Coccoloba uvifera L. Florida (Diptera: Cecidomyiidae). Entomology Circular, 97. Florida Department of Agriculture & Consumer Services Division of Plant Industry. Florida, USA.
- Méndez-Alonzo, R., Pineda-García, F., Paz, H., Rosell, J. A., & Olson, M. E. (2013). Leaf phenology is associated with soil water availability and xylem traits in a tropical dry forest. *Trees*, 27, 745–754. https://doi.org/10.1007/ s00468-012-0829-x
- Nieves-Aldrey, J. L. (1998). Insectos que inducen la formación de agallas en las plantas: Una fascinante interacción ecológica y evolutiva. *Boletín de la Sociedad Entomológica Aragonesa*, *23*, 3–12.
- Noguera, F. A., Vega-Rivera, J. H., García-Aldrete, A. N., Quesada-Avendaño, M. (Eds.). (2002). *Historia natural de Chamela*. Mexico City: Universidad Nacional Autónoma de México.
- Paine, R. T. (1980). Food webs: linkage, interaction strength, and community infrastructure. *Journal of Animal Ecology*, 49, 667–685. https://doi.org/10.2307/4220
- Pons, J., Barraclough, T. G., Gomez-Zurita, J., Cardoso, A., Duran, D. P., Hazell, S., Kamoun, S., Sumlin, W. D., & Vogler, A. P. (2006). Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology*, 55, 595-609. https://doi.org/10.1080/10635150600852011

- Posada, D. (2004). Collapse: describing haplotypes from sequence alignments. Vigo, Spain: University of Vigo. https://dposada.webs.uvigo.es/
- Price, P. W. (2005). Adaptive radiation of gall-inducing insects. *Basic and Applied Ecology*, 6, 413–421. https://doi. org/10.1016/j.baae.2005.07.002
- Prosser, S., Martínez-Arce, A., & Elías-Gutiérrez, M. (2013). A new set of primers for COI amplification from freshwater microcrustaceans. *Molecular Ecology Resources*, 13, 1151– 1155. https://doi.org/10.1111/1755-0998.12132
- R Core Team (2021). R: A language and environment for statistical computing. R Foundation for statistical computing, Vienna, Austria. http://r-forge.r-project.org/projects/splits/
- Raman, A., & Withers, T. M. (2003). Oviposition by introduced Ophelimus eucalypti (Hymenoptera: Eulophidae) and morphogenesis of female-induced galls on Eucalyptus saligna (Myrtaceae) in New Zealand. Bulletin of Entomological Research, 93, 55–63. https://doi.org/10.1079/BER2002217
- Raman, A. (2011). Morphogenesis of insect-induced plant galls facts and questions. *Flora-Morphology, Distribution, Functional Ecology of Plants, 206*, 517–533. https://doi. org/10.1016/j.flora.2010.08.004
- Ratnasingham, S., & Hebert, P. D. N. (2013). A DNA-based registry for all animal species: the barcode index number (BIN) system. *Plos One*, *8*, e66213. https://doi.org/10.1371/ journal.pone.0066213
- Razo-González, M., Castano-Meneses, G., Callejas-Chavero, A., Pérez-Velázquez, D., & Palacios-Vargas, J. G. (2014). Temporal variations of soil arthropods community structure in El Pedregal de San Ángel ecological reserve, Mexico City, Mexico. *Applied Soil Ecology*, 83, 88–94. https://doi. org/10.1016/j.apsoil.2014.02.007
- Redfern, M., Shirley, P., & Bloxham, M. (2002). British plant galls: identification of galls on plants and fungi. Shrewsbury, UK: FSC publications.
- Rodrigues, A. R., Maia, V. C., & Couri, M. S. (2014). Insect galls of restinga areas of Ilha da Marambaia, Rio de Janeiro, Brazil. *Revista Brasileira de Entomologia*, 58, 173–197. https://doi.org/10.1590/S0085-56262014000200010
- Sheffield, C. S., Hebert, P. D. N., Kevan, P. G., & Packer, L. (2009). DNA barcoding a regional bee (Hymenoptera: Apoidea) fauna and its potential for ecological studies. *Molecular Ecology Resources*, 9, 196–207. https://doi. org/10.1111/j.1755-0998.2009.02645.x
- Statsoft, Inc. (2011). STATISTICA (Data Analysis Software System, version 10. http://www.statsoft.com
- Swofford, D. L. (2003). PAUP\*. Phylogenetic Analysis using parsimony (\*and other methods). Version 4.0. Sinauer Associates, Sunderland, Massachusetts.
- Uechi, N., Yukawa, J., Tokuda, M., Ganaha-Kikumura, T., & Taniguchi, M. (2011). New information on host plants and distribution ranges of an invasive gall midge, *Contarinia maculipennis* (Diptera: Cecidomyiidae), and its congeners in Japan. *Applied Entomology and Zoology*, 46, 383–389. https://doi.org/10.1007/s13355-011-0050-1

- Vink, C. J., Paquin, P., & Cruickshank, R. H. (2012). Taxonomy and irreproducible biological science. *Bioscience*, 62, 451– 452. https://doi.org/10.1525/bio.2012.62.5.3
- Wirta, H. K., Hebert, P. D., Kaartinen, R., Prosser, S. W., Várkonyi, G., & Roslin, T. (2014). Complementary molecular information changes our perception of food web structure. *Proceedings of the National Academy of Sciences*, 111, 1885–1890. https://doi.org/10.1073/pnas.1316990111
- Yeo, D., Puniamoorthy, J., Ngiam, R. W. J., & Meier, R. (2018). Towards holomorphology in entomology: rapid and cost-effective adult–larva matching using NGS barcodes. *Systematic Entomology*, 43, 678–691. https://doi.org/10.1111/ syen.12296
- Zaldívar-Riverón, A., Martínez, J. J., Belokobylskij, S. A., Pedraza-Lara, C., shaw, S. R., Hanson, P. E., & Varela-Hernández, F. (2014). Systematics and evolution of gall formation in the plant-associated genera of the wasp subfamily Doryctinae (Hymenoptera: Braconidae). *Systematic Entomology*, *39*, 633–659. https://doi.org/10.1111/syen.12078
- Zhang, M. Y., Gates, M. W., & Shorthouse, J. D. (2014). Testing species limits of Eurytomidae (Hymenoptera) associated with galls induced by *Diplolepis* (Hymenoptera: Cynipidae) in Canada using an integrative approach. *The Canadian Entomologist*, 146, 321–334. https://doi.org/10.4039/tce. 2013.70