Chapter

Genetic Delimitation of Fall Armyworm Parasitoids Isolated in Maize in Durango, Mexico

María Berenice González-Maldonado, Miguel Mauricio Correa-Ramírez and Mónica Yazmín Flores-Villegas

Abstract

The fall armyworm *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) is the main pest that attacks maize crops in Durango, Mexico. For its biological control, it is desired to use the parasitoids of the Braconidae family; however, its identification is quite complex due to the lack of taxonomic keys that describe the complete morphological characters or are well-defined. It is necessary to study their genetic characters to estimate the variation within populations and species. For this, DNA extraction and amplification by PCR were carried out, as well as the sequencing of a fragment of subunit I of the cytochrome c oxidase (COI) gene. In *Chelonus* sp., morphological variability was observed between *Ch. insularis* and *Ch. sonorensis*, their genetic distances were conspecific, indicating that they probably belong to the same lineage. In *Meteorus*, taxonomically two species were found that had not been reported for Durango: *M. laphygmae* and *M. arizonensis*; however, the genetic distance between these and the species reported in the Genbank® could indicate that it is a single species. These results showed the high morphological and genetic variability in these braconids, probably due to evolutionary and climatic changes.

Keywords: parasitoids, Braconidae, genetic diversity, maize, Mexico

1. Introduction

In Mexico, maize is one of the main agricultural crops. Every year, there is a complex of pests, where Lepidoptera, such as the fall armyworm FAW, stand out. *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) is polyphagous to the American continent, where it causes economic losses up to 60% of total yield [1, 2]; among the damages, it causes are loss of photosynthetic area, structural damage to the whorl, direct damage to the grain, and low yields [3]; the pest is controlled with two or three applications of chemical insecticides because it is the most difficult pest to attack, according to an interview conducted by the Dow AgroSciences® company with producers in all regions of the country [4].

In Mexico, despite the importance of growing corn, an integrated pest management program IPM is not used, even though scientists have contributed information in this regard and even less have farmers implemented these techniques together or separately, in mostly only chemical control is used [2, 4].

Failing that, it is currently desired to implement the biological control of this pest due to the environmental benefits that could be achieved with the use of entomopathogenic agents (fungi, bacteria and viruses), some of which are mixed with bioremedial agents [2, 5–7] and natural enemies, where parasitoids stand out, which can be released in a massive way, if their habits, biological cycles and hosts are known, one of the families that has the most potential for the sustainable control of this pest is the parasitoids of the family Braconidae (Hymenoptera: Ichneumonoidea), which represents the parasitoids with the highest taxonomic richness, abundance and distribution in Mexico, after Ichneumonidae [8].

Worldwide, 45 subfamilies, 1103 genera, and 21,221 valid species of Braconidae are recognized [9]. In Mexico, although important attempts have been made to classify and describe species of this family in recent years, only 36 subfamilies, 319 genera, 707 determined species, and 845 morphospecies have been recorded [10].

In Mexico, the Braconidae family is very diverse and abundant in all terrestrial ecosystems; however, of 21,221 species recorded in the world, only 707 species are known. In Mexico, the study of the Braconidae family is extremely important; after Ichneumonidae, it is one of the main families of parasitoids used in the biological control of insects considered pests; they have a great taxonomic richness, and they are regulatory agents of various groups of phytophagous insects: being indicators of the presence or absence of these populations, parasitoids can be used as bioindicator organisms, to monitor changes in an ecosystem affected by anthropogenic activities; in addition, their study helps in understanding the evolution of parasitoid-host interactions, as well as from symbiosis with viruses, and they can be massively released in agriculture and forest environments [11–13].

In Durango, Mexico, for the fall armyworm, the egg, larva, and pupa parasitoids that attack it have been identified; in addition, both taxonomic and genetic studies have been carried out, especially with the Braconidae family, which allows us to know about its diversity and provides a tool to be able to implement biological control measures.

2. The cultivation of maize in Mexico

Maize (*Zea mays*) is native to Mexico, and from the evidence found in Tehuacan, Puebla, it is known that its cultivation began seven thousand years ago. Its domestication allowed the nomadic groups to become sedentary, thus becoming the livelihood of the Mesoamerican peoples.

In Mexico, corn is part of the daily diet, it is the crop with the greatest presence in the country, and it constitutes an input for livestock and for obtaining numerous industrial products, therefore, from the food, economic, political, and social, it is the most important agricultural crop [14].

Mexico is the center of origin of maize. Here, most likely, the greatest diversity of maize in the world is concentrated and here its wild relatives, the teocintles, and another set of related grasses, species of the genus *Tripsacum* (maicillos) have evolved and live [15].

Its production is divided into white and yellow maize; white corn is mainly for human consumption, while yellow corn production is for industry or the manufacture of balanced feed for livestock production.

Corn is the most widely produced maize in the world [4, 16]; the most important countries in terms of planting area in the 2021 agricultural cycle were: China, with an area of 42 million hectares and a production of 273 million tons, followed by the United States and Brazil, with an area of planting of 34.43 and 20.8 million ha and production of 382.6 and 118 million tons, respectively; Mexico ranked sixth in terms of planting area with 7.3 million ha and eighth in terms of production with 28.00 million ha. tons [17]; in Mexico, the main maize-producing states are: Sinaloa, Jalisco, State of Mexico, Guanajuato, and Michoacan [18].

2.1 Main maize pests in Mexico

The primary pests that attack maize are fall armyworm *Spodoptera frugiperda* (FAW), corn earworm *Heliothis zea* (Boddie), blind hen *Phyllophaga* sp., thrips *Frankliniella* sp./*Thrips tabaci* Lindeman [4], maize leafhopper *Dalbulus maidis* (Delong & Wolcott), corn weevil *Geraeus senilis, and* Gyllenhal and *Nicentrites testaceipes* (Champion). The genera and species that appear depend on the region, climatic conditions, and planting season (spring–summer) (winter–spring). Although generally, pests are specific to their host.

2.2 Fall armyworm

The fall armyworm (FAW) (*Spodoptera frugiperda*) has been a consistently important insect pest for several crop species, especially maize, in America for centuries. FAW prefers maize, but it is also common on sorghum and rice and is sporadically important on a vast array of additional crops and plants, including cotton and vegetables [19].

FAW has high fecundity, can rapidly develop resistance to insecticides, and has the capacity to migrate long distances, characteristics which have allowed it to rapidly disperse and establish in different regions (America, Australia, Africa, Asia, E.U. Oceania, Nepal, over 70 countries) [20–22].

2.3 Biological control

Biological control is a component of an integrated pest management strategy. It is defined as the reduction of pest populations by natural enemies, using natural enemies such as parasitoids, predators, pathogens, antagonists, or competitors to suppress pest populations [19].

Biological weed control includes insects and pathogens. Biological control agents for plant diseases are often referred to as antagonists. Parasitoids are species whose immature stage develops on or within a single insect host, ultimately killing the host. Many species of wasps and some flies are parasitoids. Pathogens are disease-causing organisms, including bacteria, fungi, and viruses. They kill or debilitate their host and are relatively specific to certain insect groups [23].

Parasite. It is an organism that lives at the expense of another organism.

Parasitoid. The insect in its immature stage acts as a parasite; when they are adults, they usually fly; parasitoids can kill their host in this case the armyworm. Parasitoids are natural enemies, which are widely used in biological control programs because when an arthropod is parasitized, the female parasitoid inserts its eggs with the help of an ovipositor inside the body of the host or attaches them outside of it, and instead of as long as the pest insect (in this case) continues to develop, it dies and the parasitoid(s) (Diptera and/or Hymenoptera) emerge from

its body. The main types of insects that act as parasitoids are wasps, flies, some beetles, mantis flies, and twisted-wing parasites [24].

2.4 Diversity of fall armyworm parasitoids

From ten years to date in Durango, Mexico, studies have been carried out that have made it possible to know the taxonomic diversity of parasitoids of FAW of the families: Ichneumonidae [(*Pristomerus spinator* Fabricius, *Campoletis sonorensis* Cameron) [25], Encyrtidae (*Euplectrus plathypenae* Howard), Tachinidae [(*Lespesia aletiae* Riley, *L. archippivora* Riley, *Winthemia deilephilae* Osten Sacken, y *Archytas marmoratus* Townsend) [26], Trichogrammatidae [(*Trichogramma pretiosum* Riley y *Trichogramma exiguum* Pinto y Platner) and Scelionidae (*Telenomus remus* Nixon) [27], and of the family Braconidae subfamily Homolobinae [(*Homolobus truncator* Say) [28], from this same family [*Ch. insularis*, *Ch. sonorensis*, *Microchelonus cautus* [29], *M. laphygmae* y *M. arizonensis* [30] the genetic part has also been studied [31, 32].

In the Mexican Republic, the Braconidae family has been studied, even so, there are states where the species are still unknown. **Table 1** shows their distribution in the country.

Parasitoid (genus, species)	State (Mexico)	Autors
Ch. insulares = (Ch. texanus)	Mexico	[33]
	Michoacan	[34]
	Chiapas	[35]
	Chihuahua	[36]
	Veracruz	[34, 37]
	Guanajuato	[38]
	Nayarit	[39]
	Sinaloa	[40]
	Sonora	[41]
	Oaxaca	[42]
	Durango	[43]
	Sinaloa	[44]
Ch. sonorensis	Michoacan	[45]
	Sinaloa	[40]
M. cautus = (Microchelonus cautus)	Sonora	[41]
	Mexico	[33]
	Michaoacan	[45]
	Chiapas	[35]
	Veracruz	[34]
	Nayarit	[39]
	Durango	[43]
Chelonus sp.	Sonora	[41]
	Oaxaca	[42]

Parasitoid (genus, species)	State (Mexico)	Autors
M. arizonensis	Chihuahua	[36]
	Nayarit	[39]
M laphygmae	Michoacan	[45]
	Veracruz	[34, 37]
	Nayarit	[39]
	Sinaloa	[44]
Meteorus sp.	Sinaloa	[40]
	Sonora	[41]

Table 1.
Diversity of parasitoids of the Braconidae family in Mexico.

2.5 Braconidae family

2.5.1 Meteorinae Subfamily

The genus *Meteorus* Haliday (Braconidae: Euphorinae, Meteorini) has 326 globally recorded species from the Nearctic, Neotropical, Palearctic, Oriental, Afrotropical, and Oceanic regions [36, 46]. *Meteorus* is a cosmopolitan genus of koinobiont endoparasitoids of Coleoptera and Lepidoptera [47, 48]; *Meteorus* is a paraphyletic group and its rearrangement into several monophyletic genera is pending [47]. The mature larvae of some species spin a cocoon suspended by a thread, and it is from this habit that the name of the genus is derived [36].

2.5.2 Cheloninae Subfamily

Cheloninae Förster is a moderately large subfamily within the family Braconidae. The subfamily comprises more than 1500 described species in the world. Members of this subfamily are present in almost all geographic regions [29, 49].

2.6 Mexico distribution

2.6.1 Chelonus

In 1995, for Guanajuato (state in the center of the country) the genera *Ascogaster*, *Chelonus,* and *Phanerotoma* of the subfamily Cheloninae were reported. Over the years, studies on the taxonomy of this genus have increased and more is known about its diversity.

For Mexico, it has been reported to *Chelonus busckiella* Viereck, 1912; *Ch. davinervis* Cameron, 1904; *Ch. insulares* Cresson, 1865; *Ch. mexicanus* Brètes, 1927; *Ch. quadrimaculatus* Cameron, 1887; *Ch. sericeus* Say, 1824, *Ch. sonorensis* Cameron, 1887; *Microhelonus blackburni* Cameron, 1886; *Microchelonus cautus* (Cresson, 1872), *M. heliopae* Gupta, 1955; *M. pectinophorae* Cushman, 1931 y *M. phrhorimaeae* Gahan, 1917 [9].

In the state of Durango, Mexico, it has been reported to *Chelonus insulares*, *Ch. sonorensis* y *Ch. cautus* (= *Microchelonus*); however, based on the coloration patterns in the metasoma (irregular spots), eight morphotypes were found that did not match

the taxonomic keys of [50–52]; therefore, its molecular identification was necessary; in this regard, [53] identified seven species of fall armyworm parasitoids, including *Ch. insularis* (isolates from Colima, Jalisco), *Ch. cautus* (Colima, Puebla, Nayarit) and *M. laphygmae* (Puebla, Colima)., using polymerase chain reaction amplification and restriction enzyme digestion, this enables the precise determination of the species of those parasitoids larvae that are usually not morphologically identifiable, where they appeared equal size amplification of the cytochrome c oxidase subunit 1 fragment was obtained for all seven species. It is also recommended to carry out genitalia or morphometry studies.

2.6.2 Meteorus

In 1990, in Tamaulipas and Nuevo Leon, Mexico, it was reported t*o Meteorus* prob. *laphygmae*, *M.* prob. *versicolor,* and four more species of *Meteorus*, unknown up to that time [48]. Other species of *Meteorus* have been reported over time, but there are few studies regarding their genomic sequences.

In Durango, Mexico, the genus *Meteorus* is mostly distributed in Santiago, Papasquiaro, and Durango, probably due to variations in climate and altitude (**Figure 1**). It belongs to the region of Las Quebradas and the other municipalities to the region of valleys and smooths.

From 2012 to date, studies have been carried out in various locations in municipalities located in the center and north of the state of Durango, which has allowed us to know the diversity of fall armyworm parasitoids. **Figure 1** shows the sampled municipalities.

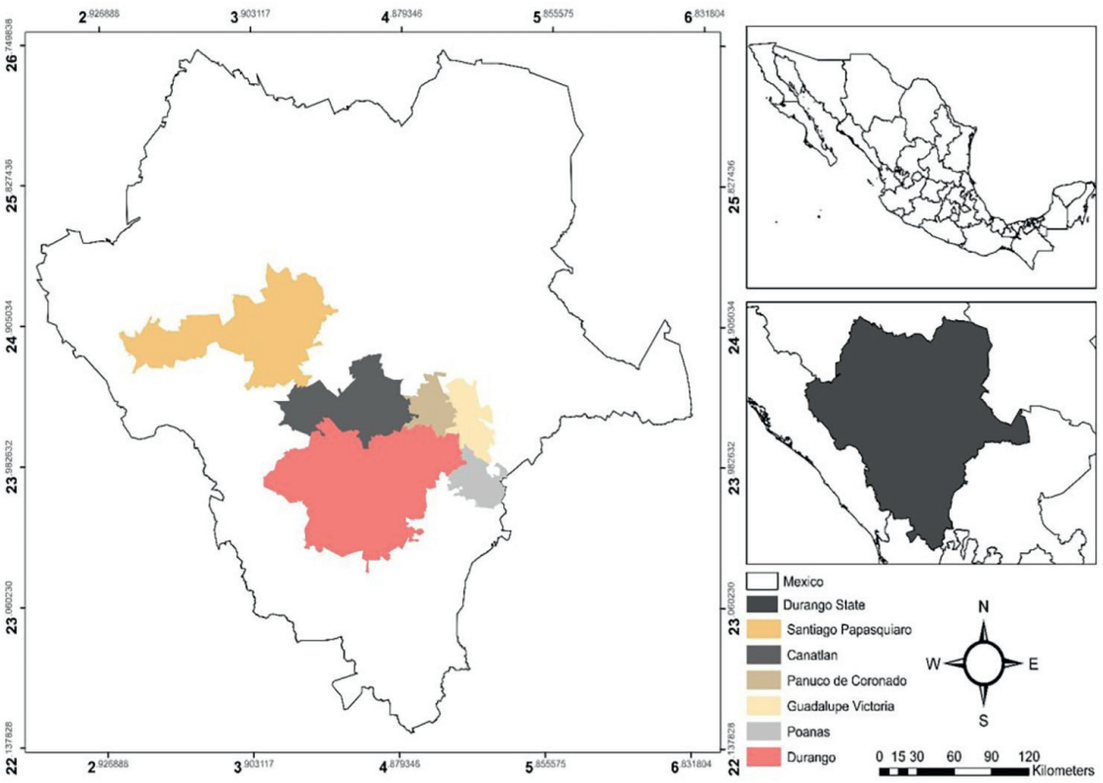

Figure 1.
Distribution of the Braconidae subfamily in Durango, Mexico.

Ch. insularis is the parasitoid that is mostly distributed in Durango and Mexico [54]; however, in the last two years, in Durango *Meteorus* sp., it is the parasitoid that presents greater capacities to be massively reproduced in the laboratory due to its development on an artificial diet (data not yet published).

2.7 Morphological delimitation

2.7.1 Chelonus

Specimens with morphological characters to belong to this genus were separated using the taxonomic keys of [50]. Species identification was carried out by PhD. Alejandro Gonzalez-Hernandez, through the comparison of the preserved material with reference specimens from the Collection of Entomophagous Beneficial Insects of the Facultad de Ciencias Biologicas de la Universidad Autonoma de Nuevo Leon, Mexico.

2.7.2 Meteorus

The obtained parasitoids were labeled and preserved in 70% alcohol. The Meteorinae (Euphorinae) material was studied at the Insect Museum (MI-FA) of the Universidad Autonoma de Tamaulipas, where it was mounted and labeled using the EntoPrint program with the respective collection data. For the determination of the subfamily and genus, we used the keys of [50] while for the determination of the species the keys of [55, 56]. For Durango, Dgo., Mexico, it has been reported to *Meteorus arizonensis* Muesebeck (Hymenoptera: Braconidae) y *Meteorus laphygmae* Viereck (Hymenoptera: Braconidae); however, their morphological characters do not coincide 100% with the taxonomic keys because they present color patterns in the mesosome that could indicate that they are other species. In this regard, [57] pointed out upon the unreliable color variability in identifying species. In fact, the color pattern is a variable that might be affected by environmental conditions [58].

The color patterns in *M. arizonensis* and *M. laphygmae* should not be considered as distinctive to identify a species; in *Meteorus,* there were nine morphotypes or different color patterns in the mesosome of the specimens of this species, even so, genetically they all belong to the same species; however, in this regard, [59] indicate that this property (melanism) increases the flight activity of wasps at low ambient temperatures of *M. pulchicornis* (Wesmael); they subjected this parasitoid (coconuts) to different temperatures (15, 20, 25 and 30°C); it was observed that at the lowest temperatures, the body of the parasitoid darkened more, which could indicate that the color change in some morphological characters of the parasitoids is due to the change in their body temperature and the environment in which they develop and not that they are different species. Similar situation with *Chelonus*, where coloration patterns in the metasome indicate that *Ch. insularis* and *Ch. sonorensis* belong to the same species [29, 32].

2.8 Genetic delimitation

2.8.1 DNA extraction

Twenty-seven individuals belonging to *Meteorus laphygmae* with five individuals, followed by *M. arizonensis* with four specimens, *Chelonus insularis* with 14 specimens,

Ch. sonorensis with two specimens, and *Ch. cautus* with three specimens, separated according to their morphological characteristics, were used.

Total genomic DNA was isolated per individual using the Promega DNA extraction kit, following the manufacturer's instructions with some modifications. Briefly, the digestion time was modified taking a total of 16 h at 56°C in a dry bath with continuous shaking. The next step was cleaning the aqueous phase with salt precipitation of detergent, proteins, and lipids followed by an organic solvent cleanup (adding 350 μl of chloroform-isoamyl alcohol 24:1), mixed it by inversion for 20 s each sample and centrifuge it by five minutes to recover the aqueous phase in a new tube (1.5 ml).

The DNA precipitation was reached by adding 1.5 volumes of cool isopropanol, followed by storing samples at −20°C for 12–16 h. Samples were cleaned with cool ethanol 80% two times. After washing samples with ethanol, those were dried and hydrated with 60 μl of milli-Q water.

From the isolated DNA, a fragment of the mitochondrial cytochrome c oxidase I (COI) gene was amplified in the individuals of the species analyzed using oligonucleotides HCO-2198 (5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′) (forward) and LCO-1490 (5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′) (reverse) reported by [60]. PCR conditions were 1 min 30 s at 94°C, denaturation 35 cycles at 94°C (1 min), alignment at 50°C (1 min), extension at 72°C for 1 min, and a final extension step at 72°C (15 min) in a thermal cycler (Model 9600, Labnet International, Edison, NJ) using 50–150 ng of DNA, 0.40 pmol of each oligonucleotides, 2.5 mM of MgCl 2, 0.2 mM of each dNTPs (Promega, Madison, WI), 1× of polymerase chain reaction buffer, and 1 unit of Taq polymerase (Promega) in a final volume of 50 μl [53, 61].

Amplified PCR products in 1% agarose gels stained with ethidium bromide were visualized by electrophoresis and observed at 430 nm in a UV transilluminator. The double-chain products were purified using a Wizard SV Gel and PCR Clean-up purification system. The amplified products were sequenced on a Genetic Analyzer Applied Biosystems 310 using the method of big dye terminator (Applied Biosystems Inc., Foster City, CA). The sequence files were edited and aligned using Chromas Pro ver. 2.1.10.1.

The sequences were translated into proteins to confirm the identity of the fragments [62]. Multiple alignments used Clustal X [63] with gap opening costs = 50, gap extension = 6.6, divergent delay of sequences = 30%, and DNA transition weight = 0.5 [64]. Genetic diversity in the species was measured as haplotype diversity (h), number of private haplotypes (P), and nucleotide diversity (p) analyzed using Arlequin version 3.5.1.21 and DnaSP version 5.1 [65]. Arlequin version 3.5.1.21 [66] was used for analysis of molecular variance (AMOVA) of population structure. Genetic differences between individuals were analyzed. Sum of the squares of deviation (SSD) and index of Harpending's-Raggedness [67] were calculated to evaluate the fit of the observed data using a model of sudden demographic expansion or a model of geographic range expansion. The mismatch distribution was compared with expected distributions by models of sudden population expansion [68] and spatial expansion [69, 70].

To reconstruct phylogenetic relationships, we used Bayesian inferences. We used *Campoletis sonorensis* Cameron, *C. flavicincta* (Ashmead), and *Homolobus truncator* Say as outgroups to polarize the characters within samples of *Meteorus* spp. and *Chelonus* spp.

Bayesian analysis used the GTR model with invariant rate heterogeneity. A posterior probability analysis [71] was performed using the program MrBayes version 3.0b4 [72]. Bayesian posterior probability calculations were implemented in a range of ten million generations, sampling every 1000 generations, and discarding the first

1000 trees sampled (as burn-in). Support for nodes was determined by posterior probabilities [72, 73]. For Bayesian analysis, three independent runs were conducted to get an impression of the robustness of the phylogenetic reconstruction.

The amplified fragments were between 695 and 710 bp, the sequences were editing and adjusting them to 650 bp. Regarding the AMOVA, the maximum distribution of variance was observed when two groups were formed: group 1: *Ch. insularis* + *Ch. sonorensis* and group 2: *Ch. cautus*; this indicates that the groups are different from each other (FSC = 0.02289, p = 0.011 at 95% confidence), there is no difference within them (FST = 0.97679, p = 0.43 at 95% confidence) (**Figures 2** and **3**), these were compared with group 3: *Ch. insularis* from different states of Mexico, the key assigned in the GeneBank® is indicated [53].

The unpaired distribution of DNA (Mismatch distribution) showed two peaks that reinforce the existence of two groups corresponding to group 1 and group 2, and each of them presented a sudden population increase.

2.8.2 Phylogeny

The median-joining networks of *Meteorus* and *Chelonus* haplotypes did not reveal divergent clusters of haplotypes by phenotype or color. Rather, the COI networks are star-shaped [74], whereas the network shows no structure, indicating that the phylogenetic information given by these sequences is adequate for phylogenetic inference. The trees generated by the Bayesian analyses are mostly unresolved within analyzed species, and the clusters that are formed may contain sequences from different localities and different morphospecies.

The phylogenetic affinities showed that the analyzed specimens of *Ch. insulares* and *Ch. sonorensis* are in a single group, where both are mixed (without forming different groups); in turn, this group is separated from another group of sequences belonging to *Ch. insularis* from other parts of Mexico. In the case of *Ch. cautus*, it is observed that the sequences obtained in this work tend to form a single group that is separated from

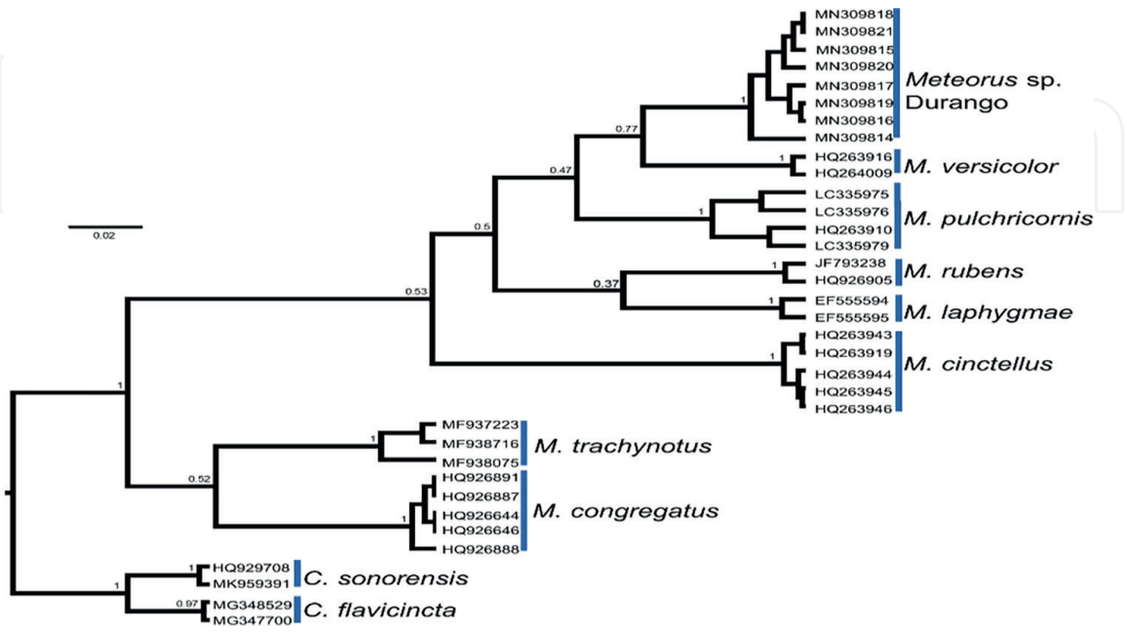

Figure 2.
Phylogenetic tree showing the groups of Meteorus sp. de Durango, compared to other species.

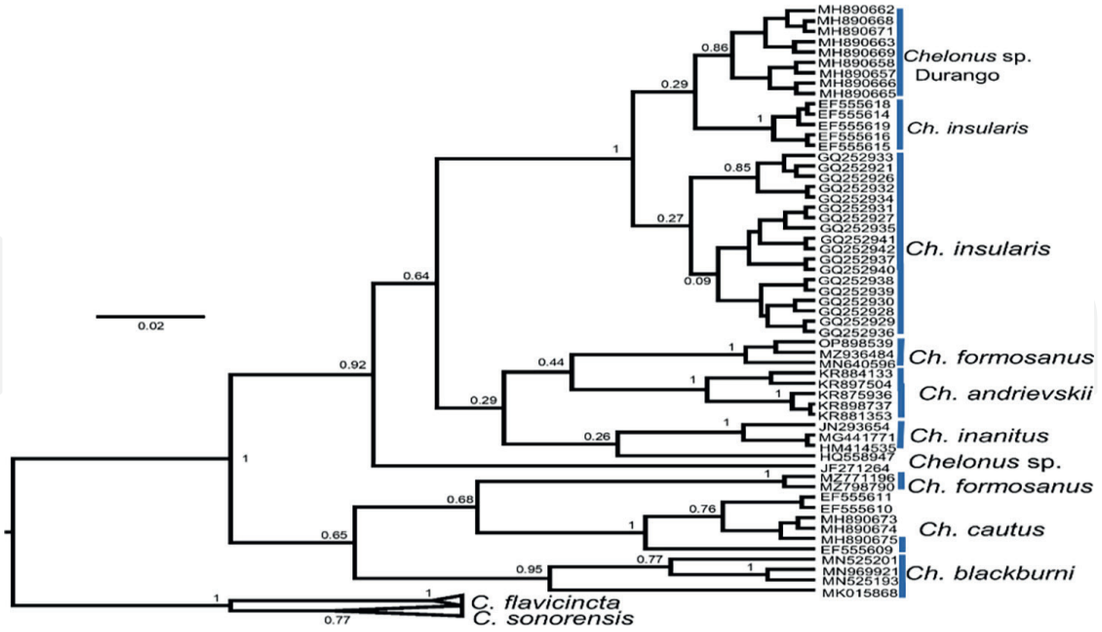

Figure 3.
Phylogenetic tree showing the groups of Chelonus sp. de Durango, compared to other species.

Chelonus insularis + *Ch. sonorensis;* it is reinforced by genetic distances between the two morphospecies of *Chelonus* (G. D. = 0.005) that occur in Durango, Mexico suggests that there is a reproductive isolation among populations that occurs in central and southern parts of Mexico contrasted with species from Durango. In the case of *M. laphygmae* and *M. arizonensis,* the phylogenetic affinities are like that described earlier, it means that the two morphospecies of *Meteorus* from Durango form a single genetic group or *M. laphygmae* + *M. arizonensis* represents a single species with a genetic distance closed to zero (G. D. = 000001) and the genetic distances with other valid species of the same genus are up to 5%, as shown in **Table 2** (G. D. higher than 0.050).

3. Discussion

Mitochondrial DNA fragment (COI) presented a high amount of genetic diversity. The high genetic diversity found is unlikely to be due to sequencing error or artifact because sequences were run in both directions, and we sequenced five samples to verify consistency of the results. We did not detect heterozygote base calling (double peaks in any sequence direction) in COI fragment. Furthermore, the COI data set was checked by amino acid translation, and we found no stop codons within sequences. Notwithstanding these high levels of variation, we were unable to detect any structure in the data. We expected that the pattern of genetic variation would reflect that of morphological variation shown us. We found no relationship between morphological variation and genetic variation.

4. Conclusions

This study has allowed to know the species of parasitoids of the family. Braconidae of fall armyworm, an important pest of maize in Durango, which

Species A	Species B	Gen. Dist.
Meteorus sp *Durango*	*M. versicolor*	0.058
Meteorus sp Durango	*M. rubens*	0.103
Meteorus sp Durango	*M. laphygmae*	0.118
Meteorus sp Durango	*M. pulchricornis*	0.112
Meteorus sp Durango	*M. cinctellus*	0.146
Meteorus sp. Durango	*M. sp.congregatus*	0.184
Meteorus sp. Durango	*M. trachynotus*	0.264
Meteorus sp. Durango	*Campoletis sonorensis*	0.284
Meteorus sp. Durango	*Campoletis flavicincta*	0.276
Chelonus sp. Durango	*Ch. blackburni*	0.109
Chelonus sp. Durango	*Ch. inanitus*	0.128
Chelonus sp. Durango	*Ch.andrievskii*	0.133
Chelonus sp. Durango	*Ch. formosanus*	0.134
Chelonus sp. Durango	*Chelonus* sp.	0.135
Chelonus sp. Durango	*Ch. cautus* Dgo.	0.160
Chelonus sp. Durango	*Ch. cautus*	0.164
Chelonus sp. Durango	*Campoletis sonorensis*	0.319
Chelonus sp. Durango	*Campoletis flavicincta*	0.318

Table 2.
Genetic distances between valid species of braconid wasps versus species that occurs in Durango, Mexico. Gen. Dist., Genetic distances calculated by DNA mutation model of Kimura 2 Parameters.

presented genetic variability, taxonomically their characteristics coincide with the diagnoses established for hundreds of years; however, genetically not. The study does not contemplate its redescription, but it provides important aspects of its genetic characterization, mainly of the genera *Chelonus* and *Meteorus*; these parasitoids contribute to the biodiversity of hymenopteran parasitoids of this pest in the corn region of Durango, which can also be candidates within the biological control of the pest within a context of sustainability and good agricultural practices, contributing to the environment. The delimitation of parasitoid species using taxonomic tools combined with the use of a molecular characterization allowed to clarify taxonomic hypotheses and doubts.

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