

Molecular Identification of a Parasitic Fly (Diptera: Tachinidae) from the Introduced *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Brazil

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Abstract

The African cotton bollworm, *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) was detected in Brazil, triggering a major phytosanitary crisis during the 2012/2013 harvest. Despite its recent introduction, larvae were found being parasitized by indigenous dipterans with estimated parasitism rates even higher than 30% at some localities. Two of these flies from the municipality of Campo Verde, Central-East Mato Grosso, were sent to the State University of Campinas to be identified through mitochondrial DNA. Partial sequences of the mitochondrial cytochrome c oxidase subunit I gene were obtained and matched more than 99% with *Archytas marmoratus* Townsen (Diptera: Tachinidae) known sequences from BOLD database. The pairwise genetic distances (K2P and p-distance) between the two Brazilian specimens and the 14 other tachinid species were all higher than 4%. The findings of this study serve as first evidence that an indigenous species of tachinid exists in Brazil that may be a good biological agent for the exotic *H. armigera*.

Keywords: Archytas; Mitochondrial DNA; Biological control

Introduction

The African cotton bollworm, *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae), is the most widely distributed pest of the Heliothinae group [1]. It is a highly polyphagous pest of several economically significant crops. Worldwide, annual yield losses and control costs reach US\$ 5 billion [2]. In China, the estimated losses in cotton reached US\$ 1.3 billion in 1992 [3] and, in India, the losses with this pest alone exceeded US\$ 627 million in the late 1980s [2]. *H. armigera* was not present in the Americas, but it was detected in Brazil during the 2012/2013 growing season [4-6] and rapidly triggered a major phytosanitary crisis. In western Bahia, infestations of *H. armigera* larvae reduced crop yields up to 35%, the number of pesticide applications increased by more than 15% and the costs in cotton yields doubled (from US\$ 400 to US\$ 800 per hectare) [6]. In the State of Mato Grosso, one of the largest soybeans producer states in Brazil, extensive economic losses were also registered [4,7].

Inspectors from the Ministry of Agriculture, Livestock and Food Supply (Ministério da Agricultura, Pecuária e Abastecimento, abbreviated MAPA) performed intensive surveys for natural enemies between April and June 2013 at farms from the main municipalities from the State of Mato Grosso. More than 200 *Helicoverpa* larvae collected from millet, cotton and beans, were taken to laboratory and allowed to pupate individually in plastic card boards. However, instead of the moths, many dipterans emerged, revealing an estimated parasitism rate up to 38.8% and 37.5% (larvae from the municipalities of Querência and Nova Xavantina, respectively) [8]. Some of the specimens that emerged were sent for taxonomic identification at the

University of Brasília (UnB), but morphological keys gave as result the genus *Archytas*. Two specimens (Figure 1) that emerged from parasitized larvae collected from infested beans in the municipality of Campo Verde, Central-East Mato Grosso, were then sent to the State University of Campinas (UNICAMP), trying to obtain the species identification through mitochondrial DNA (mtDNA).

Materials and Methods

Total DNA was extracted from the entire body of the two dry pinned specimens using the phenol:chloroform method, adapted for microcentrifuge tubes [9], resuspended in 100 µL of TE buffer and stored at -20 °C. The partial sequences of the mitochondrial cytochrome c oxidase subunit I gene (COI) were amplified by PCR using the primers COIF and COIR [10].

The PCR reactions were conducted separately with 25 ng of total DNA, 2.5 mM MgCl₂, 0.25 mg/mL of BSA, 100 µM dNTPs, 0.5 mM of each primer, 1.5 U of TaqDNA polymerase (Fermentas International Inc., Burlington, Canada), and 10xTaqBuffer for a final reaction volume of 25 µL. Amplification was carried out on a GeneAmpPCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA), with the following conditions: an initial denaturation at 94°C for 3 min, followed by 35 cycles of 94°C for 30 s, 45°C for 30 s, 70°C for 1:30 s, and ended with a 7 min final extension at 70°C. After amplification, 2 µL aliquots were analyzed by 1% agarose gel electrophoresis in 1x TAE (40 mM Tris-acetate, 1 mM EDTA).

Amplicons were purified with the IllustraGFX kit (GE Healthcare, Bucks, UK) and sequenced bidirectionally by the ABI3730xl DNA Analyzer sequencer (Applied Biosystems, Foster City, CA), with the same primers used for the PCR reactions. The sequences were

assembled into a contig for each specimen by using the Geneious 6.0.6 Software (Biomatters Ltd., Auckland, New Zealand) and aligned using the multiple sequences alignment algorithm implemented in Clustal Ω [11].

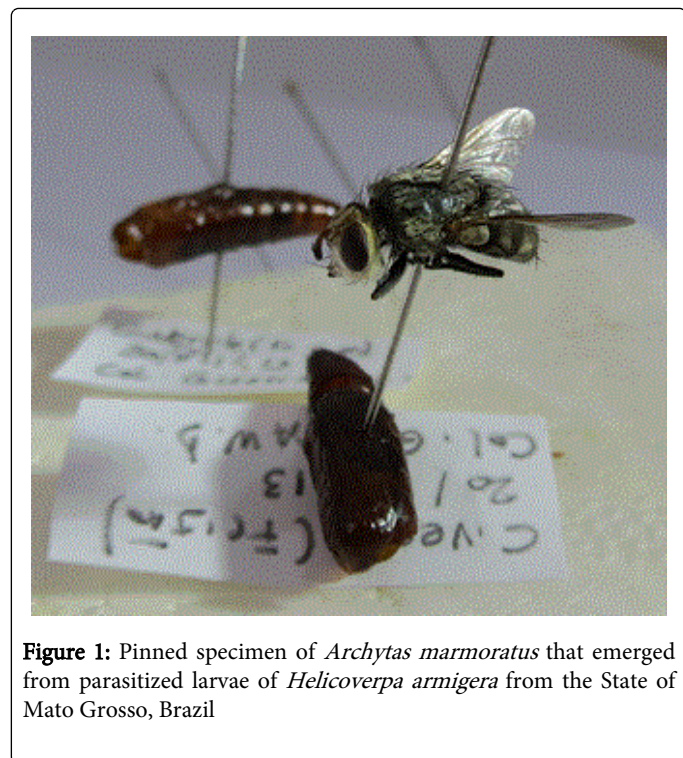


Figure 1: Pinned specimen of *Archytas marmoratus* that emerged from parasitized larvae of *Helicoverpa armigera* from the State of Mato Grosso, Brazil

matched 94-96% with *Archytas* spp. sequences from the GenBank but 99.3% with Costa Rican *Archytas marmoratus* Townsen (Diptera: Tachinidae) sequences from BOLD. According to BOLD [12], this identification is accurate unless there is a very closely allied congeneric species that has not yet been analyzed. Such cases, however, are rare. The sequences from the two Brazilian *A. marmoratus* were deposited in GenBank (KM099221, KM099222).

Partial sequences of the COI gene (a 480 bp fragment) of the two Brazilian *A. marmoratus* specimens were used for the estimation of interspecific genetic distances between them and the following 14 tachinids: *Lydella thompsoni* Herting (Diptera: Tachinidae) (AY649319), *Pseudoperichaeta nigrolineata* Walker (AY649320), *Chrysotachina* sp. (JQ575016), *Leskia* sp. (JQ574740), *Lespesia* sp. (JQ574724), *Lespesia postica* (JQ574654), *Winthemia* sp. (JQ575021), *Siphona* sp. (JQ574987), *Genea* sp. (JQ574980), *Phytomyptera* sp. (JQ576395), *Archytas* sp. (GU141891), *Archytas* sp. (JQ574993), *Archytas basifulvus* (BOLD: AAC5527), and *Archytas marmoratus* (BOLD: AAJ1909) (numbers in parentheses are the respective GenBank or BOLD Accession numbers). The species *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) (KF573406) and *Helicoverpa armigera* (GQ995232) were used as outgroups.

The pairwise genetic distances (K2P and p-distance) between the Brazilian specimens and the other species are presented in Table 1. The genetic distance between the two Brazilian *A. marmoratus* and the *A. marmoratus* from Costa Rica was only 1.1%, what suggests conspecificity when considering a 2-3% mtDNA COI sequence divergence as the threshold to discriminate the insect species [13,14]. On the other hand, the estimated divergence values between the Brazilian *A. marmoratus* and the other 14 tachinid species, *T. pretiosum* and *H. armigera* were all higher than 4% (Table 1).

Results

The two COI sequences obtained were blasted against GenBank database and checked in BOLD Identification System [12] and they

Species compared		Kimura two-parameters (K2P)	Uncorrected sequence divergences (p-distance)	
<i>Archytas marmoratus</i> from Brazil	vs	<i>Helicoverpa armigera</i> (GQ995232)*	24.4	18.4
		<i>Lydella thompsoni</i> (AY649319)	16.2	13.3
		<i>Pseudoperichaeta nigrolineata</i> (AY649320)	13.2	11.2
		<i>Trichogramma pretiosum</i> (KF573406)	37.4	24.9
		<i>Chrysotachina</i> sp. (JQ575016)	16.5	13.5
		<i>Leskia</i> sp. (JQ574740)	17.2	14.0
		<i>Lespesia</i> sp. (JQ574724)	14.4	12.1
		<i>Lespesia postica</i> (JQ574654)	13.8	11.6
		<i>Winthemia</i> sp. (JQ575021)	14.4	12.1
		<i>Siphona</i> sp. (JQ574987)	18.8	15.0
		<i>Genea</i> sp. (JQ574980)	14.4	12.1

	<i>Phytomyptera</i> sp. (JQ576395)	12.9	11.0
	<i>Archytas</i> sp. (GU141891)	7.5	6.8
	<i>Archytas</i> sp. (JQ574993)	6.9	6.3
	<i>Archytas basifulvus</i> (BOLD: AAC5527)	4.3	4.0
	<i>Archytas marmoratus</i> (BOLD: AAJ1909)	1.1	1.1

Table 1: Mitochondrial DNA COI pairwise genetic distances (%) for the *Archytas marmoratus* specimens collected in Brazil and other species, numbers in parentheses are the respective GenBank or BOLD Accession numbers.

A. marmoratus is a solitary larval-pupal parasitoid of several noctuid species and it is indigenous to the southern United States, Central America, and South America as far south as Chile [15,16]. In Brazil, there are a few reports of tachinids parasitizing native pests [17-19]. The females of *A. marmoratus* are efficient in finding host larvae even at low densities [20] because they can sense the kairomones associated with the frass of the host [21]. Earlier instars of the corn earworm, *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae), and the tobacco budworm, *Heliothis virescens* F. (Lepidoptera: Noctuidae), can be successfully parasitized by *A. marmoratus*, but the parasitoid survival is higher when the later instars are attacked [22]. The eggs laid by the females hatch immediately, releasing planidium-type maggots that penetrate the host larva and molt to second instar after host pupation. The maggots go under the pupa wing pads where they induce a secondary respiratory funnel and complete development, killing the host [22].

Previous studies have demonstrated that the mtDNA COI gene is very promising for the correct detection and discrimination of tachinid parasitoids, which are morphologically very similar [23,24]. Accurate identification of parasitoids is a critical step to the success of biological control programs both during the initial phases when candidate natural enemies are chosen and also during subsequent evaluations phases [25]. The findings of this study, therefore, serve as first evidence that an indigenous species of tachinid exists in Brazil that may be a good biological agent for the invasive *H. armigera*.

Many studies have suggested a role for *A. marmoratus* in Area-wide Integrated Pest Management (AW-IPM) through inundative releases, due to the high number of planidia produced by the females and technical feasibility of mass rearing, along with the efficiency of parasitism [20,26]. Gross [27] demonstrated that release rates of 370 to 860 female *A. marmoratus* per hectare could yield between 50 and 80% parasitism of late instar corn earworm larvae. After a pilot test, Proshold et al. [20] verified that 70% of the parasitized *H. zea* larvae collected from release fields was superparasitized. In the field tests performed by Carpenter & Proshold [28], nearly 23% of the corn earworm larvae were parasitized and 18% superparasitized, and the authors suggested that inundative releases would be most effective against the first generation to reduce the seasonal increase of *H. zea* populations.

The results of this study demonstrated that a molecular method was very useful for the accurate identification of an indigenous tachinid fly capable of parasitize field populations of the recently introduced *H. armigera* in Brazil. This data also shows the great potential of *A. marmoratus* as controlling agent for AW-IPM programs against this exotic pest in South America. Further investigations of this parasitoid

in Brazil must be conducted to determine its geographical distribution, relative abundance and genetic diversity.

References

- King ABS (1994) *Heliothis/Helicoverpa* (Lepidoptera: Noctuidae), pp. 39-106. In GA Matthews, JP Tunstall (eds.), *Insect Pests of Cotton*. CAB International, Wallingford.
- Lammers JW, Macleod A (2007) Report of a pest risk analysis: *Helicoverpa armigera* (Hubner, 1808).
- Sheng CF (1993) Outbreak of *Heliothis armigera* in North China: possible causes and control strategies, pp. 841-844. In *Proceedings, Beltwide Cotton Production Research Conferences*. National Cotton Council of America, Memphis, TN.
- Czepak C, Albernaz KC, Vivan LM, Guimarães HO, Carvalhais T (2013) First reported occurrence of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) in Brazil. *Pesq Agropec Trop* 43: 110-113.
- Tay WT, Soria MF, Walsh T, Thomazoni D, Silvie P, et al. (2013) A brave new world for an old world pest: *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Brazil. *PLoS ONE* 8: e80134 2013.
- Mastrangelo T, Paulo DF, Bergamo LW, Morais EGF, Silva M, et al. (2014) Detection and Genetic Diversity of a Heliothine Invader (Lepidoptera: Noctuidae) From North and Northeast of Brazil. *J Econ Entomol* 107: 970-980.
- Specht A, Sosa-Gómez DR, Paula-Moraes SV, Yano SAC (2013) Morphological and molecular identification of *Helicoverpa armigera* (Lepidoptera: Noctuidae) and expansion of its occurrence record in Brazil. *Pesq Agropec Bras* 48: 689-692.
- Guerra WD, Guerra ALLD, Ribas LN (2013) Ocorrência de *Archytas* (Diptera: Tachinidae) parasitando lagartas de *Helicoverpa armigera* (Lepidoptera: Noctuidae) em Mato Grosso. 9º Congresso Brasileiro de Algodão, 3-6 de setembro de 2013, Brasília-DF, Brazil.
- Lyra ML, Klaczko LB, Azeredo-Espin AML (2009) Complex pattern of genetic distribution in populations of the New World Screwworm fly revealed by mitochondrial DNA markers. *Med Vet Entomol* 23: 32-42.
- Li QQ, Li DY, Ye H, Liu XF, Shi W, et al. (2011) Using COI gene sequence to barcode two morphologically alike species: the cotton bollworm and the oriental tobacco budworm (Lepidoptera: Noctuidae). *Mol Biol Rep* 38: 5107-5113.
- Sievers F, Wilm A, Dineen DG, Gibson TJ, Karplus K, et al. (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol* 7: 1-6.
- (BOLD) The Barcode of Life Data System. 2007. Bold.v3. *Mol Ecol Notes* 7: 355-364.
- Avise JC (2000) *Phylogeography: the history and formation of species*. Harvard University Press, Cambridge, MA.
- Hebert PDN, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. *Proc R Soc B* 270: 313-322.
- Sabrosky CW (1955) The taxonomic status of the armyworm parasite known as *Archytas piliventris* (van der Wulp) (Diptera: Larvaevoridae). *Fla Entomol* 38: 77-83.

16. Arnaud PH Jr. (1978) A host-parasite catalog of North American Tachinid (Diptera). U.S.Dep.Agric. Misc.Publ., n.1319, 860 pp.
17. Guimarães JH (1961) Segunda contribuição ao conhecimento do gênero *Archytas* Jaennecke, 1867 (Diptera: Tachinidae). *Mem.Inst.Oswaldo Cruz* 59: 163-179.
18. Santos CH, Panizzi AR (1997) Tachinid parasites of adult *Megalotomus parvus* West. (Hemiptera: Alydidae). *An Soc Entomol Bras.* 26: 577-578.
19. Valicente FH, Barreto MR (1999) Levantamento dos Inimigos Naturais da Lagarta do Cartucho do Milho, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), na Região de Cascavel, PR. *An Soc Entomol Bras* 28: 333-337.
20. Proshold FI, Gross Jr HR, Carpenter JE (1997) Inundative release of *Archytas marmoratus* (Diptera: Tachinidae) against the corn earworm and fall armyworm (Lepidoptera: Noctuidae) in whorl-stage corn. *J Entomol Sci* 33: 241-255.
21. Nettles Jr WC, Burks ML (1975) A substance from *Heliothis virescens* larvae stimulating larviposition by females of the tachinid, *Archytas marmoratus*. *J Insect Physiol* 21: 965-978.
22. Hughes PS (1975) The biology of *Archytas marmoratus* (Townsend). *Ann Entomol Soc Am* 68: 759-767.
23. Agustí N, Bourguet D, Spataro T, Delos M, Eychenne N, et al. (2005) Detection, identification and geographical distribution of European corn borer larval parasitoids using molecular markers. *Mol Ecol* 14: 3267-74.
24. Smith MA, Woodley NE, Janzen DH, Hallwachs W, Hebert PDN (2006) DNA barcodes reveal cryptic host-specificity within the presumed polyphagous members of a genus of parasitoid flies (Diptera: Tachinidae). *PNAS* 103: 3657-3662.
25. Delluchi V, Rosen D, Schlinger EI (1976) Relationship of systematic to biological control, pp. 81-91. In Huffaker CB, Messenger PS (eds), *Theory and Practice of Biological Control*. Academic Press, New York.
26. Gross Jr HR (1994) Mass propagation of *Archytas marmoratus* (Diptera: Tachinidae). *Environ Entomol* 23: 183-189.
27. Gross Jr HR (1990) Field release and evaluation of *Archytas marmoratus* (Diptera: Tachinidae) against larvae of *Heliothis zea* (Lepidoptera: Noctuidae) in whorl stage corn. *Environ Entomol* 19:1122-1129.
28. Carpenter JE, Proshold FI (2000) Survival of *Archytas marmoratus* (Diptera: Tachinidae) from superparasitized corn earworm larvae (Lepidoptera: Noctuidae). *Environ Entomol* 29: 606-611.