

## Molecular identification of *Dermatobia hominis* (Diptera: Oestridae): a neglected agent causing myiasis in Mexico

Yokomi N. Lozano-Sardaneta<sup>1</sup>, Sokani Sánchez-Montes<sup>1,2</sup>, Edith Fernández-Figueroa<sup>1,3</sup>, Claudia Rangel-Escareño<sup>3,4</sup>, Ingeborg Becker<sup>1</sup>

### ABSTRACT

Myiasis represents a group of neglected tropical diseases caused by the infestation of vertebrate tissues by dipterous larvae. We herein report an imported case of furuncular myiasis caused by *Dermatobia hominis* in Mexico City. The species was confirmed by DNA sequencing and phylogenetic reconstruction analysis.

**KEYWORDS:** Myiasis. Bot fly. Colmoyote. *Cuterebrinae*. *Cytochrome oxidase I (cox1)*. *Dermatobia hominis*.

### INTRODUCTION

Myiasis (Greek μυῖα [myia] = fly) is defined as the infestation of tissues of terrestrial vertebrates species by several dipterous larvae of the genus *Chrysomya*, *Cochliomyia*, *Cordylobia*, *Cuterebra*, *Dermatobia*, *Lucilia*, *Oestrus* and *Sarcophaga*<sup>1,2</sup>. Due to its host dependency, these larvae are divided into obligatory parasites that grow in live tissue or in dead tissue, and the facultative parasites that grow on decomposing organic matter<sup>3-5</sup>. Myiasis can be present in several parts of the host body<sup>6</sup>, and the clinical manifestations include a broad range of signs depending on the infestation site<sup>1,4,7</sup>.

In the Americas, the most common species causing myiasis is the “human bot fly” *Dermatobia hominis* (Linnaeus, 1781), that produces a furuncular myiasis which can penetrate the host’s skin and develop in the subcutaneous tissue during most of its lifetime<sup>2,3,7,8</sup>. This dipteran exhibits a wide distribution ranging from Mexico to Northern Argentina<sup>3,4,6,7</sup>.

In Mexico, autochthonous cases have been documented in endemic areas in the States of Campeche, Chiapas, Quintana Roo, Tabasco and Yucatan where it is known as *colmoyote* or *moyocuitl*. Furthermore, imported cases have been reported in Mexico City<sup>5-7,9</sup>.

In the last years, human cases increased in non-endemic areas as a consequence of enhanced ecotourism, during which infestations are common<sup>7</sup>. Fortunately, the treatment of myiasis is curative, yet the prognosis depends on the degree of damage inflicted upon the tissue<sup>10</sup>.

A major problem in non-endemic areas is the, misidentification of *D. hominis*, since it is especially difficult to diagnose and therefore to treat<sup>7,9</sup>. In this study, we report the molecular identification of an imported case of furuncular myiasis caused by *Dermatobia hominis* in Mexico City.

<sup>1</sup>Universidad Nacional Autónoma de México, Facultad de Medicina, División de Investigación, Centro de Medicina Tropical, Ciudad de México, México

<sup>2</sup>Universidad Veracruzana, Facultad de Ciencias Biológicas y Agropecuarias, Veracruz, México

<sup>3</sup>National Institute of Genomic Medicine, Department of Population Genomics, Computational and Integrative Genomics, Ciudad de México, México

<sup>4</sup>Tecnologico de Monterrey, Basic Sciences, Queretaro, México

**Correspondence to:** Yokomi N. Lozano-Sardaneta  
Hospital General de México, Dr. Balmis 148, Col. Doctores, 06726 CDMX, México  
Tel: +52 55 5623 2674

**E-mail:** [nisei\\_sardaneta@ciencias.unam.mx](mailto:nisei_sardaneta@ciencias.unam.mx)

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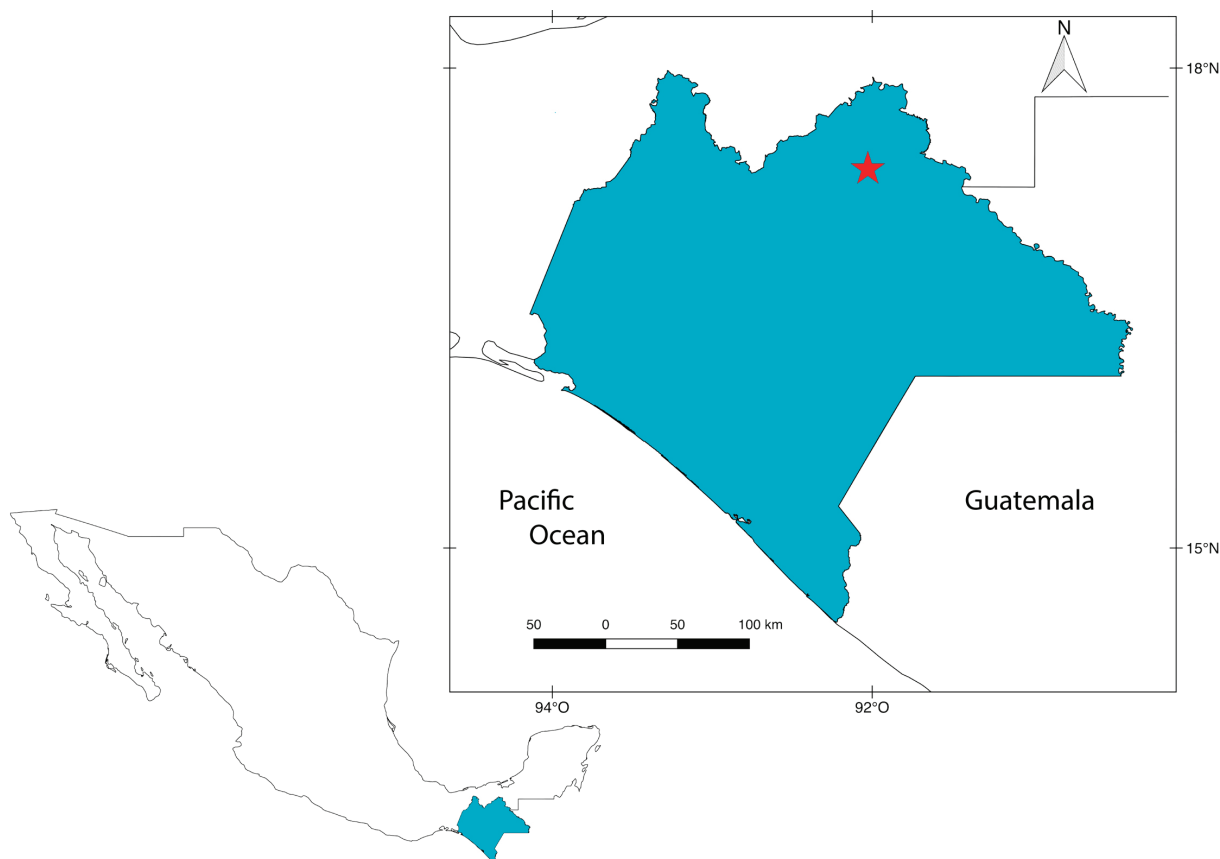
## CASE DESCRIPTION

On February 02, 2019, a 53 year-old female resident of Mexico City was referred to the Centro de Medicina Tropical, due to a painful mass in the scalp above the right ear. During the medical appointment, she referred having made several trips to the Southeastern States of Chiapas and Campeche (on January 2019), during the previous four weeks. She reported activities in the wilderness, particularly overnight in a jungle region in the State of Chiapas (Figure 1). She referred having been bitten by multiple insects, particularly mosquitoes, during her stay in Chiapas. Two weeks after returning to Mexico City, she detected an indurated mass in the scalp above the right ear, which increased in size in the following days. Additionally, she reported a localized pain and a sensation of fluctuation in the affected area. At the time of the consultation, the patient brought a maggot inside a bottle with ethanol that had been manually removed hours earlier. The maggot was cylindrical shaped with a little spine over its body of light brown colour, the size was of 12 mm length per 5 mm wide, approximately. The lesion showed no evidence of bacterial infection but was disinfected anyway. The patient was asked to return a week later for the clinical

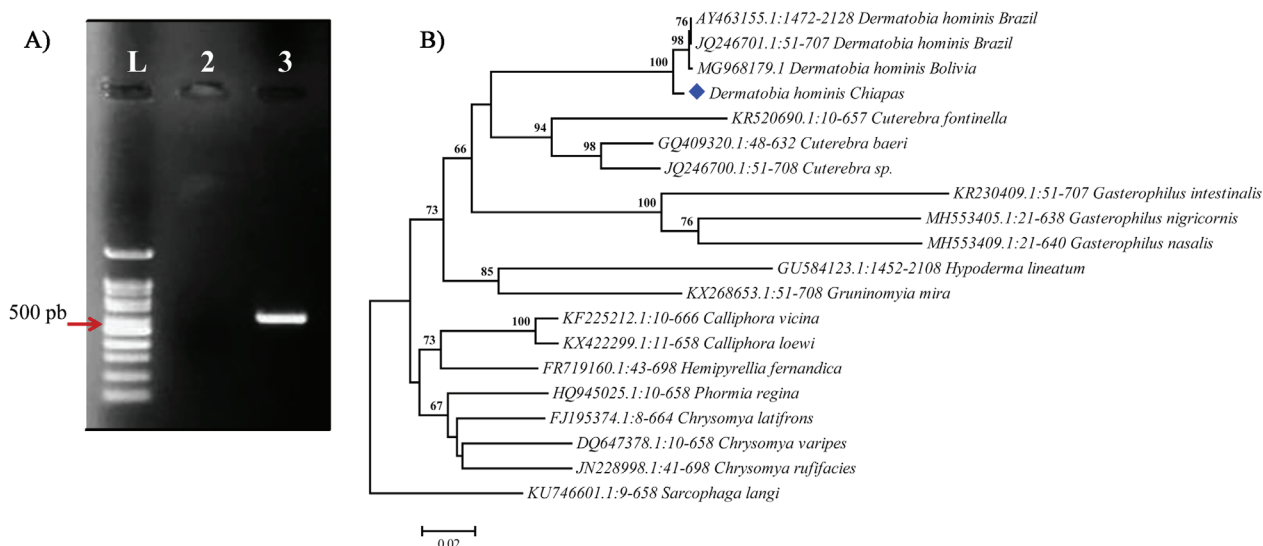
follow up, and as she had a normal healing process, she was dismissed.

## Molecular identification

For the molecular identification of the extracted maggot, firstly it was cleaned with chlorine 10% during 30 s and thereafter washed with nuclease free water. The genomic DNA was extracted using 500 µL of a 10% solution of the resin Chelex-100 (Bio-Rad, Hercules, CA, US)<sup>11</sup>. For the DNA barcoding of the specimen, we amplified a fragment of around 700 bp from the mitochondrial Cytochrome Oxidase subunit I (*cox1*) gene, using the primers LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCO2198 (5' TAA ACT TCA GGG TGA CCA AAA AAT CA-3')<sup>12</sup>. The reactions were conducted under the following conditions: initial denaturation at 94 °C for 10 min, followed by 35 cycles at 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 45 s and a final extension at 72 °C for 5 min. The reaction mixture was prepared in a final volume of 25 µL, with 12.5 µL of the 2 X GoTaq Green Master Mix (Promega Corporation, Madison, WI, USA), 100 ng of each oligonucleotide, 6.5 µL of nuclease-free water and 200-500 ng of DNA template. We only included



**Figure 1** - Map of the Mexican territory, highlighting the jungle region with a red star in the State of Chiapas.



**Figure 2** - Molecular identification of *Dermatobia irritans*: A) *cox1*. PCR product of the bot fly line 3 (4  $\mu$ L), Nuclease-free water as the negative control (line 2). L=Ladder 100 pb; B) Neighbor-joining tree with Cytochrome Oxidase I (*cox1*) sequences of the fly species causing myiasis. Numbers on the branches indicate the bootstrap values and the blue diamond is the sequence obtained in this study.

ultrapure water instead of DNA in the template control. The amplification products were analysed by electrophoresis in 2% agarose gels stained with Smartglow (Benchmark Scientific, Sayreville, USA) (Figure 2A).

The PCR product was purified using the Agencourt AMPure XP kit (Beckman Coulter, Brea, CA, USA), and the amplicons were sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher, Waltham, MA, USA). The sample was purified by the BigDye XTerminator prior to loading on the ABI 3730xL DNA analyzer (Applied Biosystems, Thermo Fisher, Waltham, MA, USA). Sequencing was carried out at the Sequencing Unit of the Instituto Nacional de Medicina Genomica.

The electropherograms were visualized and edited in Chromas (version 2.6.6, Technelysium, South Brisbane, Australia). The generated sequence was compared with those available at the NCBI data base using the BLASTn (Basic Local Alignment Search Tool)<sup>13</sup> as a preliminary confirmation of the identity of the specimen. The DNA sequences were aligned using the ClustalW (MEGA, version 6.0, University Park, PA, USA). Genetic pairwise distances (*p*-distances) were estimated using the Maximum Composite Likelihood substitution model and a Neighbor-Joining (NJ) tree was built using the MEGA software (version 6.0, University Park, PA, USA). The obtained sequence was deposited in GenBank under the accession N<sup>o</sup> MT159662.

The NJ analysis showed that the sequences of *Dermatobia hominis* from the present study clustered with a high bootstrap value of 100% with other sequences of the same species from Brazil (AY463155.1; JQ246701.1) and Bolivia (MG968179.1). The analysed sequences

of *Dermatobia* showed genetic differences, especially, when comparing the sequences obtained in this study of 0.011-0.010% with the South American specimens, while the genetic distance between the South American specimens was 0.000 to 0.002%, probably due their geographical locations (Figure 2B).

## DISCUSSION

In the last years, human cases of cutaneous myiasis have become more frequent, and are considered one of the most common dermatological conditions in tropical areas. This has become an increasing problem for travellers after visiting endemic regions in Central and South America<sup>1,7</sup>. In general, myiasis studies are mainly focused on the morphological taxonomic identification of the larvae, yet few molecular studies have been conducted for the identification and assess the genetic variation of *Dermatobia hominis*. The use of the molecular taxonomic marker such as *cox1* gene has proven to be useful for a rapid and efficient identification of this bot fly<sup>1,9</sup>. In this study, we registered an imported case of myiasis in Mexico City from a patient infected in Chiapas, a coastal State which has been historically recognized as an endemic area of the parasite<sup>7</sup>. Even though there is a previous study on the use the *cox1* gene for the identification of this species in Mexico, the amplified segment of the earlier study does not correspond to the same region that we amplified, and this is the reason not to include those sequences in the present analysis<sup>7</sup>. It is interesting that the obtained sequences were not identical to the sequences of South America (Figure 2B). This is in

accordance with previous studies, in which Mexican *D. hominis* species were shown to differ from the specimens of other countries. The differences are likely to be related to the geographic area in which they were isolated<sup>9,14</sup>. It is therefore, warranted to carry out more studies to identify the genetic diversity of *D. hominis* species in endemic areas for human myiasis in Mexico. This would enable to evaluate critical elements for its prevention, such as the degree of mobilization of the parasite population from other endemic regions of Central America, and its degree of expansion in the Mexican territory.

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## AUTHORS' CONTRIBUTIONS

YNLS performed the PCR, analyses of sequences, writing and reviewing; SSM and IB were responsible for the clinical attention to patients; EFF and CRE performed the purification of amplicons, and sequencing assays; SSM, EFF, CRE and IB contributed with critical comments. All the authors participated during the discussion and writing of the manuscript and approved its final version.

## ETHICAL GUIDELINES

The current report was approved by the Ethics and Research Committee of the Medical Faculty of the *Universidad Nacional Autónoma de México* (UNAM, FMED/CI/JMO/129/2017). The patient read, accepted, and signed the informed consent form for the publication of the case.

## CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

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