

Myiasis caused by *Dermatobia hominis* in Mexico: morphological and molecular identification using the cytochrome oxidase I gene

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ABSTRACT

Myiasis caused by *Dermatobia hominis*, the human botfly, is frequent in the Americas, however, scarce morphological and molecular information exist regarding this dipteran. We describe three cases in urban areas of Mexico where *D. hominis* is not endemic. Morphological and genetic identification were performed using the cytochrome oxidase I as a molecular marker. The mitochondrial cytochrome oxidase I gene is useful for inferring the genetic divergence of *D. hominis*.

KEYWORDS: *Dermatobia hominis*. Myiasis. *Cytochrome oxidase I (coxI)*. Mitochondrial genes. Molecular markers.

INTRODUCTION

In the Americas, myiasis is caused by different genera of dipterans, such as *Cordylobia*, *Chrysomya*, *Cuterebra* and *Oestrus*. Larvae of these dipterans feed on the host's living or dead tissues, body fluids, or ingested food can cause a broad range of symptoms depending on the infestation site and the relationship between the larvae and the host¹⁻⁵. The human botfly, *Dermatobia hominis* (Linnaeus, 1781), causes obligatory myiasis and this parasite depends on the host to complete its life cycle. Although furuncular myiasis is a common disease caused by this fly, vaginal, palpebral, ocular, rhinal and cerebral myiasis can be fatal^{5,6}. Cases in people traveling to developing countries and cases in non-endemic countries are becoming more common^{4,5}. *D. hominis* is distributed from Mexico to Paraguay and in Northeast Argentina^{1,3}. In Mexico, endemic areas include territories in the Southern region, specifically Yucatan and Quintana Roo States, where several cases have been documented^{7,8}. However, in regions in which *D. hominis* is uncommon, infestations can be misidentified and incorrectly treated⁴. Here, we reported three cases: one imported case from Brazil and two autochthonous cases of myiasis caused by *D. hominis* in Mexico. In addition, morphological and molecular identification was performed using the cytochrome oxidase I gene.

CASE DESCRIPTION

A 50-year-old male resident of Quintana Roo, Mexico, with a personal history of type 2 diabetes controlled by metformin presented with dermatitis located in the lateral and posterior region of the left heel characterized by two furuncular lesions

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(Figures 1A and 1B), with a two-month history of pain, paresthesia and bloody serous secretion. He had returned from a trip to the Amazon region (Brazil) where he had been bitten by different insects. Several oral antibiotics were administered to the patient without improvement. Under anesthesia, the lesion was irrigated with saline solution, and two maggots that measuring 17 mm wide and 21 mm long with a diameter of approximately 5 mm were observed (Figure 1D). They showed growth and movement. According to the records, the clinical diagnosis was myiasis.

A 40-year-old female living in Mexico City, without significant pathological findings related to the studied condition, showed a dermatosis (Figure 1C) characterized by an indurated erythematous-colored plaque with a central bloody serous crust on the right leg after a trip to the State of Campeche. The patient had minor pain and did not report a significant pathological history. The same medical staff member extracted a maggot measuring 20 mm long (Figures 1E and F) by using mechanical pressure and presented it for medical examination. Again, the diagnosis was myiasis. Subsequently, a scab and a residual

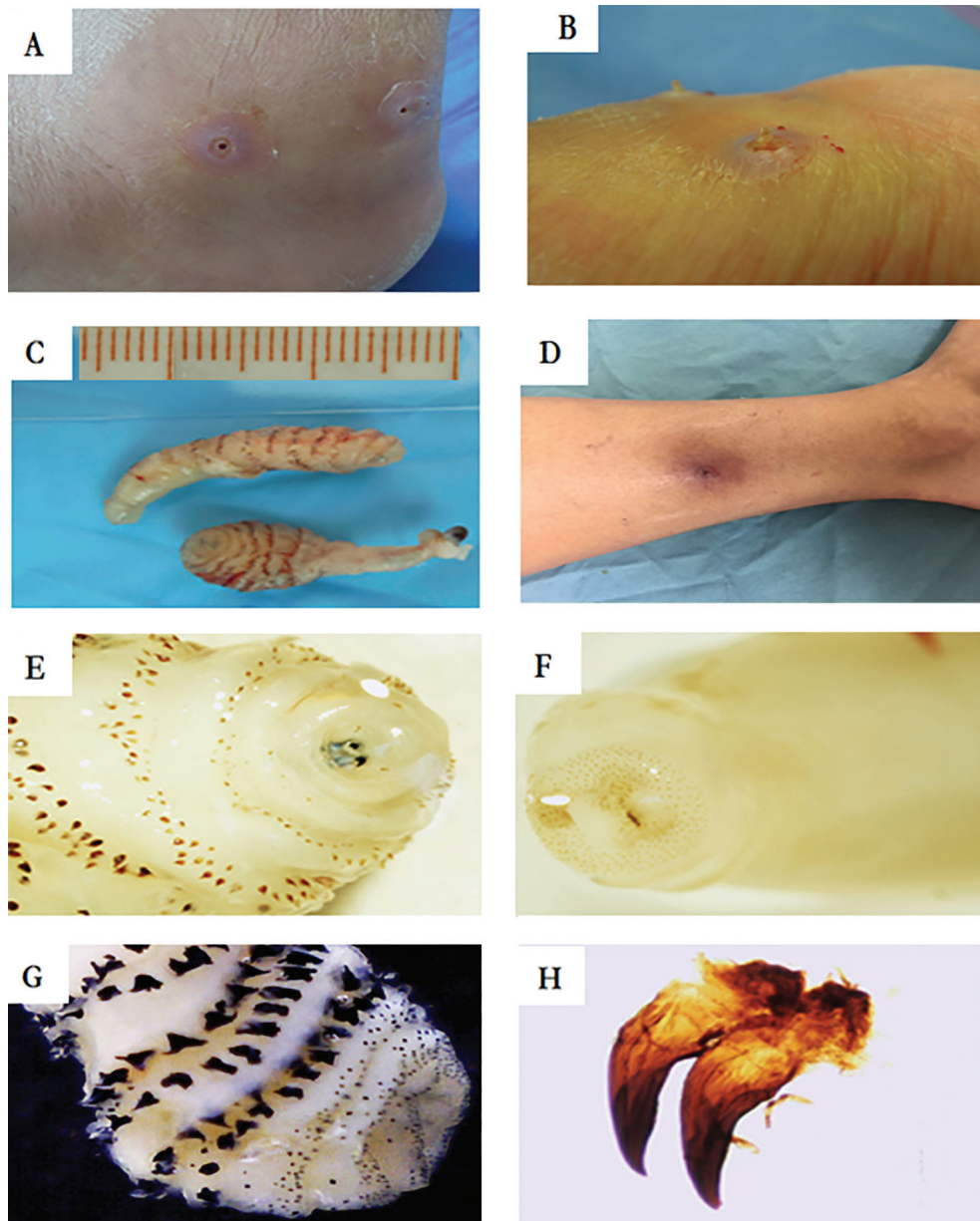


Figure 1 - A) Furuncular lesions in the lateral region of the left heel (case 1); B) Residual injuries caused by maggots of *D. hominis* after anesthetic infusion of the lesion (case 1); C) Maggots from ulcers (case 1); D) Left leg injury (case 2); E) Anteroposterior section of some of the larvae analyzed; F) Posterior section of some of the larvae analyzed; G) Presence of spicules in the larvae analyzed; H) Larval maxillar hook.

hyperchromic scar formed in the area, and the patient healed without specific treatment.

A 44-year-old male resident of Mexico City, with no significant personal history of disease, presented to the dermatologist with two furuncular lesions located on the lower back region that developed approximately one month after a trip to Chiapas State. The patient indicated inconspicuous pain. After a medical examination and oral treatment with clindamycin without improvement, surgery was indicated and removed two maggots (Figure 1G), under anesthesia, that were 9 mm wide and 12 mm long and approximately 4 mm in diameter. The clinical diagnosis was myiasis. Subsequently, a scab and a residual hyperchromic scar formed in the area, and the wound healed without specific treatment.

Morphological and molecular cytochrome oxidase I identification

Larvae extracted from the patients were morphologically studied and identified according to Villalobos *et al.*⁴. The maggots were cylindrical shaped and yellowish-white colored, with a size of 9 to 21 mm in length and approximately 5 to 7 mm in wide. Backward projecting spines that encircled the thorax and cephalic region were observed (Figures 1D, 1E, 1F and 1G). The maggots were identified as *D. hominis*; two of them were first instars and three were second instars. In the anterior portion where the oral opening is located, two oral or maxillary hooks were present; they were strongly sclerosed and had dark pigments (Figure 1H). The posterior portion of the maggot was fixed, and the presence of two respiratory stigmas or respiratory spiracles were observed, each of them presenting three eyelets or openings with spiracles and trabeculae. The peritreme was not evident. Behind the stigmas, in the inner portion of the exoskeleton, two scaffold-like structures were present. These structures were part of the tracheal system of the maggot.

Genomic DNA was extracted from the larvae using the quick-DNA extraction kit (ZYMO Research Corporation, CA, USA). A novel primer set, TdCO1int 5'-CTTCATTCTTTGACCCAGCAGGWGG-3' and TdCO1r 5'-TGAGTAT GARTGTTCTGCWGGNGG-3', was used to amplify approximately 857 base pair (bp) from the cytochrome oxidase I (coxI) gene. PCR cycling conditions were, as follows: initial denaturation at 95 °C for 10 min; followed by 38 cycles of denaturation at 95 °C for 30 s, annealing at 56 °C for 30 s and extension at 72 °C for 30 s ending with a final extension at 72 °C for 7 min.

The amplified sequences were submitted to the GenBank database (MK593540-2). Multiple alignments were

performed using the MEGA software version 7.0⁹. The best-fit model of nucleotide substitution was determined using the Akaike information criterion in the Modeltest version 3.7; the GTR+G+I model was used¹⁰. Phylogenetic reconstruction was performed according to Villalobos *et al.*⁴.

The phylogenetic inference showed a clear separation of different species by clades with high values of posterior probability support. The sequences of *D. hominis* obtained in the present study (MK593540-2) were clustered with other sequences belonging to the same species (AY463155, AY507157 and NC 006378), and grouped near the clade of species belonging to the genera *Cuterebra*. In particular, all the individuals of *Dermatobia* showed genetic differences that suggested a possible association with their geographical locations (Figure 2).

DISCUSSION

Human myiasis caused by *D. hominis* is endemic from the Southern region of Mexico to Paraguay and Northeast Argentina. However, cases have already been reported in nonindigenous people who traveled to endemic zones¹¹. Although Mexico has autochthonous cases, imported cases have also been documented¹². Cases of myiasis acquired in South America and other regions in Mexico were reported here. Regarding the two cases acquire in Mexico, both were diagnosed in regions of the country where it is uncommon to find reports of myiasis. For this reason, it was difficult to properly identify the maggots. Additionally, maggots were morphologically identified. The spine structures developed over 30 to 60 days depending on the host infection time^{12,13}. The presence of distinctive spines on the terminal body segments of the larvae was useful to identify this species. The final characterization showed maggots in the second and third instars that are commonly found in infected people due to the timeline of infection. A detailed morphological identification of the maggots has been described.

Although the gold standard for the identification of *D. hominis* is the morphological analysis, the inclusion of molecular techniques is relevant because these tools have high sensitivity and specificity for the diagnosis of several parasitic infections. In addition, some molecular markers support new taxonomic classifications and are useful in assessing the genetic variation of pathogens. In particular, the cytochrome oxidase I gene has shown to be a sensitive molecular marker to discriminate different species and genera of insects, dividing them into specific clades and identifying high genetic diversity. In the case of *D. hominis*, there are few sequences deposited in GenBank, and amplification of the barcoding region is troublesome¹⁴. The primers selected in this study contained only 88 bp of

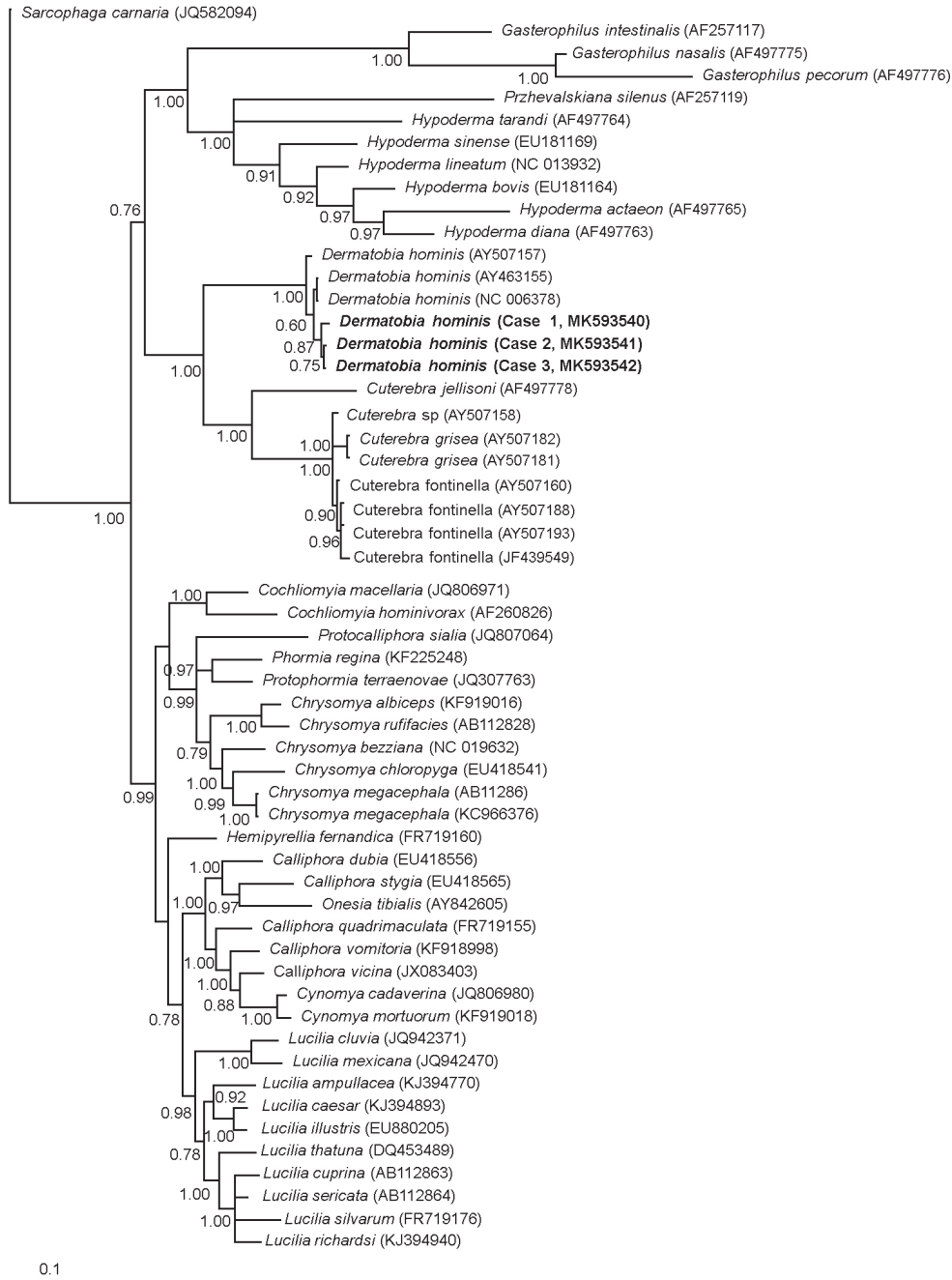


Figure 2 - The Bayesian phylogenetic tree constructed with cytochrome oxidase I (coxI) sequences from Dipteran species causing myiasis in the world. Numbers on branches indicate posterior probability values and different shades of gray indicate the taxonomic family to which each species belongs. Data sequences were obtained from the GenBank database. The samples highlighted in bold represent the sequences obtained in present study.

barcoding, however, our goal was to cover the major gene fragment and compare this fragment to the homologous region of *Dermatotbia* with the highest number of sequences available on GenBank. However, more detailed studies to identify *D. hominis* variations should be conducted. Finally, tourists traveling to endemic areas of myiasis should be instructed to use repellent and clothing that covers all skin exposed to insect bites.

CONCLUSIONS

Although *Dermatotbia hominis* is uncommon in Mexico City, we identified three cases. Mitochondrial markers such as coxI can differentiate the species and genera. This classification has been troublesome with other markers, such as ITS¹⁵.

AUTHORS' CONTRIBUTIONS

FM-H and GV performed the PCR, purification of amplicons, and sequencing assays. MEV-M, DA-S, and DP-R were responsible for clinical selection and attention to patients. NR and RA were responsible for the morphological identification of maggots. MR-V, MP, and FM-H contributed with critical comments. All authors participated during the discussion and writing of the manuscript and approved its final version.

CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

ETHICS APPROVAL

Written informed consent was obtained from each participant; all procedures were in accordance with the Regulations of the General Health Law in the Field of Health Research in Mexico: NOM-012-SSA3-2012, Title II, Chapter II, General Dispositions.

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