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Identification of *Megaselia scalaris* (Loew, 1866) (Diptera: Phoridae) in mummified human body from Itacambira (MG), Brazil, using scanning electron microscopy and cuticular hydrocarbons

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ABSTRACT

Insect puparia were found adhered to the ribs and other tissues in the abdominal cavity of a natural male mummy found in Itacambira (State of Minas Gerais, Brazil) dating to the Colonial Period. They were collected for identification by scanning electron microscopy, and for comparison of several morphological features with those described in the literature. The puparia were found open and dorsoventrally flattened, making it difficult to visualize the dorsal projections. The tegument is covered by tapered spines and contains rows of small tubercles on the dorsal and lateral regions of the puparium. The posterior spiracle consists of four parallel openings arranged in pairs. These results are indicative that the specimens belong to the species *Megaselia scalaris* (Loew, 1866) (Diptera: Phoridae). Additionally, cuticular hydrocarbons of the puparia were analysed by gas chromatography coupled to mass spectrometry and compared with the profile of *M. scalaris* reared in the laboratory.

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Introduction

The use of insects in the reconstruction of cultural as well as taphonomic information, useful to the interpretation of archaeological contexts, is already widespread in Europe and the Mediterranean (Buckland and Coope 1991; Panagiotakopulu 2000). The field of forensic entomology can assist in understanding the various mortuary rituals, conditions and practices (Huchet et al. 2009) and also in clearing

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of parasitoses and mode of disease transmission between humans in ancient cultures (Rick et al. 2002; Reinhard and Buikstra 2003; Raoult et al. 2006).

The identification of insects in archaeological investigations can often be hampered and limited by poor preservation of specimens found, or, as often happens, by finding only parts of insects (Isidro et al. 2006; Couri et al. 2008, 2009). In this case, scanning electron microscopy (SEM) and chemotaxonomy by analysis of cuticular hydrocarbons (CHCs) can be additional tools to classical taxonomy for the identification of insects.

According to Greenberg and Singh (1995), morphological variations between populations of the same species may occur. Collections in various regions are needed in order to observe the greatest morphological diversity in each species. There are no identification keys for immature insect stages in Brazil to provide diagnostic morphological characters. For the last few years, Mendonça and collaborators (2008, 2010, 2012a, 2012b, 2013, 2014a, 2014b) and Carriço et al. (2015) have been establishing a database for Diptera (Calliphoridae, Sarcophagidae and Muscidae) using SEM. This technique brings new contributions to the identification of morphological features that cannot be observed by light microscopy.

The external surface of insects is covered by a species-specific layer of cuticular lipids, which are often composed of a complex mixture of CHCs. These compounds limit water loss and act as pheromones, providing recognition signals between individuals. Gas chromatography coupled to mass spectrometry (GC-MS) is a technique that has great accuracy in the separation, identification and quantification of chemicals (Collins et al. 1997). The commonly found CHCs in insects are *n*-alkanes, alkenes and methyl-branched alkanes (Blomquist et al. 1987; Lockey 1991; Gibbs 2002; Howard and Blomquist 2005). The extracted compounds from the cuticle may vary qualitatively and quantitatively in some species according to sex, population and stage of development (Lockey 1991). Several studies have been published in recent years demonstrating that CHCs can be used to resolve taxonomic diversity of isomorphic species (Lusebrink et al. 2007; Calderón-Fernández et al. 2012) and to determine variability according to sex and age in the genus *Drosophila* (Vaničková et al. 2012). In cases where the insect DNA is degraded, the analysis of CHCs is a very effective and fast (Ye et al. 2007) alternative tool, because they are stable molecules. Several authors have used cuticular hydrocarbons for species differentiation in insects, such as parasitic wasps (Bernier et al. 1998), sand flies (Bejarano et al. 2003), *Anopheles* (Anyanwu et al. 2001), Culicidae (Horne and Priestman 2002), triatomines (Calderón-Fernández et al. 2012), termites (Jenkins et al. 2000), ants (Van Wilgenburg et al. 2011) and mites (Estrada-Peña et al. 1994), and also for the identification of eggs, larvae and pupae of dipterous flies (Greenberg and Singh 1995; Sukontason et al. 2006; Thyssen and Linhares 2007; Ye et al. 2007; Braga et al. 2013).

Materials and methods

Several puparia were found adhered to the ribs and other tissues in the abdominal cavity of a naturally mummified male corpse. That corpse was exhumed from a Colonial cemetery at the village of Itacambira, State of Minas Gerais, Brazil. After exhumation, many decades ago, this and other human remains remained stored inside the church's basement. Together with two other mummies found in the same site, the corpse of the

present study was received by Dr Adauto Araujo (*in memoriam*) and Dr Luiz Fernando Ferreira for scientific studies. Today these mummies are part of a collection of palaeoparasitological interest (Coleção de Coprólitos e Materiais de Interesse Paleoparasitológico Luiz Fernando Ferreira) in the Laboratório de Paleoparasitologia, Escola Nacional de Saúde Pública Sérgio Arouca (ENSP), from the Oswaldo Cruz Foundation (Fiocruz), Rio de Janeiro, Brazil. The puparia were collected for identification at the Laboratório de Entomologia Médica e Forense, Instituto Oswaldo Cruz, Fiocruz, Brazil.

Scanning electron microscopy

The specimens were prepared for SEM according to the methodology used by Mendonça et al. (2012a, 2012b). The samples were analysed at the Plataforma de Microscopia Rudolf Barth of the Oswaldo Cruz Institute/FIOCRUZ. The observed morphological features were compared to the existing literature.

Cuticular hydrocarbon extraction and GC-MS analysis

The puparia that were not used for SEM were divided into three groups of 30, due to their very small size, for extraction of CHCs following the methodology described in Braga et al. (2013). Puparia of *Megaselia scalaris* (Loew, 1866) (Diptera: Phoridae) reared in the laboratory were used as control group.

Aliquots (1 µl) were analysed by GC-MS using the splitless injection mode onto a capillary DB-5 column (30-m length, 0.25-mm diameter, 0.25-µm thick film). The initial temperature of the oven was 150°C and this was increased to a final temperature of 320° C, at a 5°C/min ramp. The temperatures of the injector and the MS detector were 290°C and 325°C, respectively. Analyses were performed on a Thermo-Finnigan Trace GC with Polaris Q Mass Spectrometer (GC-MS) in the Proteomics Center of Nevada, UNR, Reno, NV, USA. Helium was the carrier gas. The GC-MS yielded qualitative results and was used to identify components. CHCs with carbon-chain lengths of 21 carbons or more were selected to be analysed because these are the ones usually present in insects. The analyses were made in triplicate for each species. CHCs were identified according to the Electron Impact mass spectra as described previously (Blomquist et al. 1987).

Results and discussion

The first step for the identification of the puparia found in the abdominal cavity of a natural male mummy from Itacambira (State of Minas Gerais, Brazil) (Figure 1) was made using SEM. The puparia were open and flattened dorsoventrally, making it difficult to visualize the dorsal projections. Tapered spines cover the tegument that also contains rows of small tubercles on the dorsal and lateral regions of the case. The posterior spiracle consists of four parallel openings arranged in pairs (Figures 2 and 3). These results corroborate the information described by Sukontason et al. (2006) and confirmed the specimens as belonging to the species *M. scalaris*.

The next step was the biochemical analysis of the CHC profiles of the puparial cases. These were compared with those of puparia obtained from *M. scalaris* reared in the

Figure 1. Male mummy from Itacambira, Minas Gerais, Brazil. (A) Opening of the abdominal cavity of the mummy; (B) puparia of *Megaselia scalaris* (arrow) adhered to a rib (photographs by S. Novo).

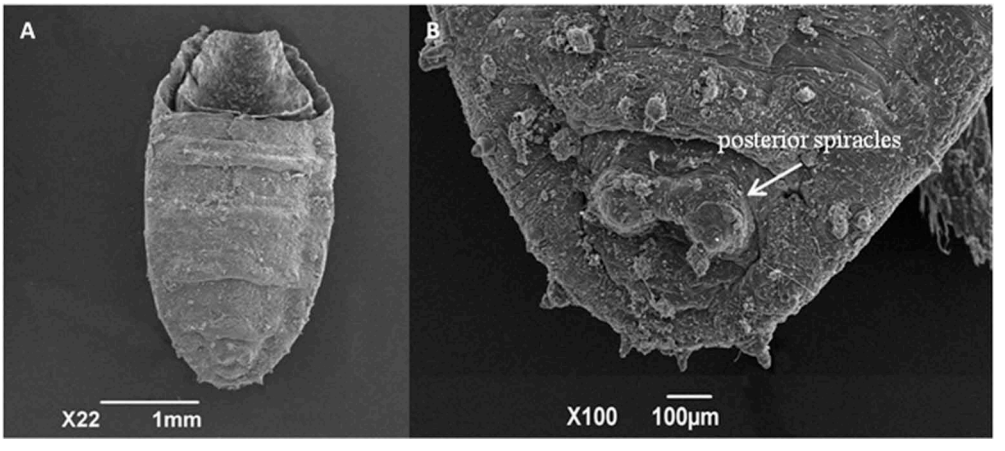

Figure 2. Scanning electron microscopy of the puparium of *Megaselia scalaris* from a male mummy from Itacambira, Minas Gerais, Brazil. (A) Overview (dorsal) of the puparium of *Megaselia scalaris* with the opening for adult emergence; (B) posterior spiracles (one pair), located at the posterior end of the puparium.

laboratory. GC-MS of the hexane extract of the puparia that were present inside the mummy's abdomen show high amounts of hydrocarbons that do not appear to be insect derived, but contaminants accumulated over centuries. Selective ion monitoring at a mass-to-charge ratio (m/z) of 365–366 demonstrates a peak in the mummy's puparium hydrocarbons with the same retention time as 3-methylheptacosane in hydrocarbons from current-day insects. The m/z at 365 is a major fragment ion of 3-methylheptacosane (M-29 fragment) (data not shown). Martin and co-workers (2009) analysed

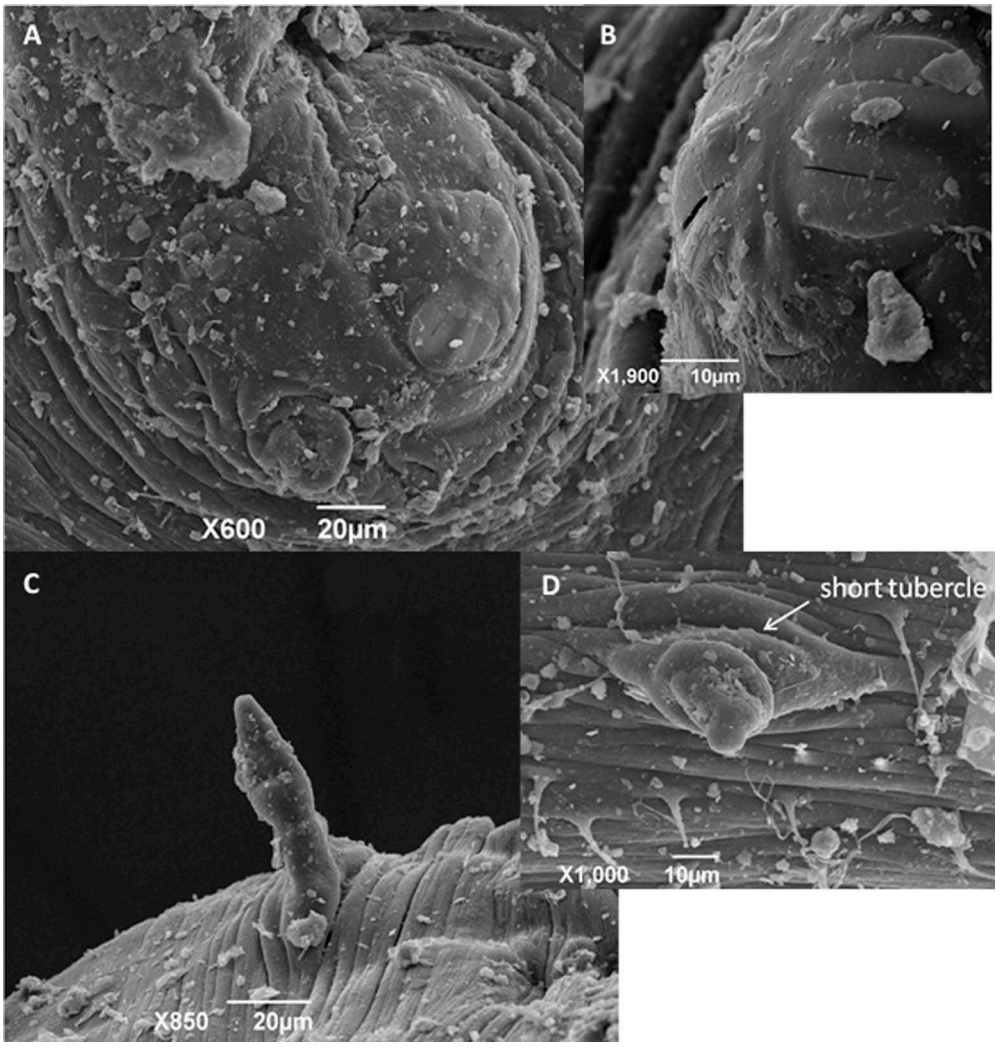

Figure 3. Scanning electron microscopy of the puparium of *Megaselia scalaris* from a male mummy from Itacambira, Minas Gerais, Brazil: (A, B) Detailed view of the posterior spiracle, containing four openings arranged in parallel; (C, D) detail of the small tubercles located on the dorsal surface of the puparium.

the long-term stability of hornet CHCs. These authors compared the hydrocarbon profiles of three groups: fresh, 1-year-old and 20-year-old museum specimens. They reported that the species-specific profiles were qualitatively stable in the 20-year-old specimens. In the case of the hydrocarbons from the puparia found inside the mummy, due to the mummy's age these insects must have been more than 100 years old and this may explain the poor CHC profile.

Therefore, while it appears that the mummy's puparia are too old to identify species using CHC profiles, we can provide strong evidence that at least one hydrocarbon, 3-methylpentacosane, which was present on modern puparial cases, was also present on those obtained from the mummy. Although CHCs are not useful for insects that are

more than 100 years old, SEM is a reliable tool for the identification of insect species when these are well preserved even if they are very old specimens. We are certain that the use of several tools, such as the ones in this study, in addition to classic taxonomy is valuable for the correct identification of insects in several fields, including archaeological, forensic and taxonomic investigations.

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Geolocation Information

17°03'53.70"S, 43°18'35.80"W

Disclosure statement

No potential conflict of interest was reported by the authors.

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