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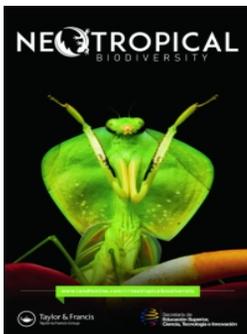
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First records of Diptera associated with human corpses in Ecuador

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ABSTRACT

Flies in the order Diptera are of forensic value because many species leave tractable evidence while harvesting nutrients from decomposing corpses. From December 2015 to January 2017, 41 fly specimens were collected in human bodies at crime scenes and autopsies across the south of Ecuador. Six species, e.g., *Chrysomya albiceps* (Wiedemann 1819), *Chrysomya mega-cephala* (Fabricius, 1794), *Synthesiomyia nudiseta* (Wulp, 1883), *Lucilia purpurascens* (Walker, 1836), *Hemilucilia segmentaria* (Fabricius, 1805), and *Stomoxys calcitrans* (Linneo, 1758) were identified to species level using morphological (dichotomous keys) and molecular (mitochondrial COI barcodes) techniques. One additional specimen remains unidentified to species level, but COI barcodes assigned it to the genus *Paralucilia*. These first taxonomically curated records of flies in real cases constitute a tangible groundwork for the development of forensic entomology in Ecuador.

Resumen. Primeros registros de Diptera asociados a cadáveres humanos para el Ecuador.

Moscas en el Orden Diptera tienen alto valor forense porque un gran número de especies dejan evidencia tractable mientras toman nutrientes de cadáveres en descomposición. Entre diciembre de 2015 y enero de 2017, se colectaron en humanos 41 especímenes de dípteros en varias escenas del crimen y autopsias en varias localidades del sur del Ecuador. Seis especies, e.g., *Chrysomya albiceps* (Wiedemann 1819), *Chrysomya megacephala* (Fabricius, 1794), *Synthesiomyia nudiseta* (Wulp, 1883), *Lucilia purpurascens* (Walker, 1836), *Hemilucilia segmentaria* (Fabricius, 1805), y *Stomoxys calcitrans* (Linneo, 1758) fueron identificadas a nivel de especie usando técnicas morfológicas (claves dicotómicas) y moleculares (códigos de barras basados en el gen mitocondrial COI). Un espécimen adicional no fue identificado a nivel de especie, pero los códigos de barras de COI lo incluyen dentro del género *Paralucilia*. Estos primeros registros, curados taxonómicamente, de moscas en casos reales constituyen una base concreta para el desarrollo de la entomología forense en el Ecuador.

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Introduction

Criminal investigation benefits the administration of penal justice by supplying technical and scientific support needed by prosecutors [1,2]. Traditional methods that use physical phenomena such as algor, livor, and rigor mortis to estimate the post-mortem interval (PMI), are not accurate once the corpse shows autolysis or putrefaction [3]. However, decomposition of organic material (animals and humans) is a process of constant physical, chemical, and biological changes that provide a microhabitat with suitable protection, humidity, temperature, and highly nutritious resources for different animal taxa, including insects [4–7]. Hence, forensic (or medicolegal) entomologists (FE) study the contribution of insects to crime scene investigation (CSI) [2]. Information provided by insects that colonize corpses is thus a valid strategy for criminal investigation when

calculating PMIs [2]. But, the application of forensic entomology principles is impeded by correct taxonomic identification of fly species found in CSIs, which is especially true in megadiverse countries in tropical regions across the globe.

The scientific foundation towards reliable application of forensic entomology was established during the first half of the twentieth century by taxonomists interested in insects of forensic importance [2,4]. It was quickly determined that species rich and abundant insect orders Diptera, Coleoptera, and Hymenoptera modify the fate of animal carcasses, [8,9]. From a forensic point of view, Diptera is the most important insect order during the colonization of a corpse. Early research found that many Diptera species present predictable and well-known successional stages [10]. Diptera species richness is high, as it comprises near 161,000 described species worldwide, with

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approximately 31,000 species in the Neotropics [11,12]. Specifically, specimens of the dipteran families Calliphoridae and Muscidae occur in all stages of carcass decomposition and serve as tools for medical forensic studies. For example, studying the development cycle of *Chrysomya* and *Cochliomyia* flies have proved helpful to scientists when determining a body's decomposition state [13].

New molecular techniques that use short and standard regions of DNA (barcodes) have been designed to help in the identification of species, and thus can assist FE by facilitating the identification of insects in immature stages [14], fragmented individuals, empty pupae [15], and/or when scientists need to distinguish among morphologically similar, or cryptic, species [16,17]. DNA barcoding requires comparison of DNA sequences obtained from biological samples collected as part of a CSI, with a reference library of well-identified biological species [18]. DNA barcoding is also an efficient tool that provides researchers with information of the geographical spread of forensically important species. The main DNA sequence used as a barcode in the animal kingdom is a short region of 648 bp of the cytochrome c oxidase I gene (COI), and it has been shown that over 95% of species from different animal groups can be identified using COI [19]. For example, a study using a 545 bp subset of the COI mitochondrial gene was successful as a molecular identification tool for forensic Calliphoridae flies in Colombia [20].

In Ecuador, studies of insects associated with corpses are scarce [1,5–7,21–23]. However, a total of 581 invertebrate specimens with forensic value are known for the country; these specimens belong to 99 species, 62 genera, 18 families, five orders, and three classes [23]. Most of these specimens belong to two families of the order Diptera, Calliphoridae and Muscidae. The main Diptera genera with forensic value occurring in Ecuador are *Calliphora*, *Chrysomya*, *Cynomya*, and *Lucilia*. To date, no insect species has been reported in human cadavers in Ecuador [23]. In collaboration with Ecuadorian law enforcement agencies, we had access to dipteran specimens found in cadavers from different CSI in Loja province (southern Ecuador). In this study, we present the results of this collaboration and report the first in-situ records for seven dipteran species with forensic value. Our final goal was to set key facts in the study of the biology and ecology of dipterans with forensic value in Ecuador.

Methods

Description of cases

The observation of insect infestation took place in six medical-legal autopsies in Loja province, southern Ecuador, between December 2015 and January 2017,

at the Centro de Investigación de Ciencias Forenses at Loja (CICF Loja) (Table 2). In the first real case (EFLoja-1) a cadaver was found outdoors on an irregular surface (i.e., cliff), at an approximate environmental temperature of 28–32°C. The body was found completely dressed; part of the corpse was already skeletonized and covered by cadaveric fauna (i.e., many dipteran larvae and some adult individuals) around the whole thorax. After removal, the body was transferred to the CICF Loja where it was kept refrigerated at 3–5°C for 8 hours.

The body of the EFLoja-2 case was found in a drain that was exposed periodically to waste water from a house. There was no light source, with ambient temperature between 24 and 26°C. The body was dressed, covered with water up to its knees, it showed skeletonization in the head, neck, forearms, hands, and feet; it was covered by many dipteran larvae and pupa along the whole body. It was also transferred to CICF Loja and stored at 3–5°C, but for a shorter period (around 1 hour).

EFLoja-3 was found in a drain near the road, at ambient temperature between 32 and 37°C. It was covered by many dipteran larvae and eggs in the corporal orifices. After removal, in the same way as in the cases mentioned above, the body was kept refrigerated (3–5°C) for 3 hours.

EFLoja-4 occurred in the municipal cemetery, during an exhumation performed by a medical examiner. Many dipteran larvae and pupa covered the remains. Ambient temperature was between 18 and 20°C; the collected specimens were kept at the same temperature for about 4 hours.

EFLoja-5 was found around sector of rubble. Only were found totally calcined remains which were covered with few dipteran larvae. Ambient temperature was between 15 and 17°C. After lifting the cadaver, the body was kept in refrigeration for approximately 45 min before was transferred to the CICF Loja.

Finally, in EFLoja-6 the corpse was seen next to the road, with daily solar exposure and ambient temperature between 20 and 25°C. Part of the cadaver was emphysematous and part calcined covered by abundant cadaveric fauna. After removal, the body was transferred to the CICF Loja and kept in refrigeration (3–5°C) for about 1 hour.

Collection of flies

Various individuals were collected from the six bodies (larvae in different stages of development, and adults). Collected individuals, adults and larvae, were identified by morphological means based on diagnostic characters for each species using identification keys for calyptrate flies [24–27]. To improve morphological identification, 17 collected larvae were boiled and

Table 1. Results of the BIN analysis for each species.

Species	BIN	Mean Intra-Sp	Max Intra-Sp
<i>Chrysomya albiceps</i> (Wiedemann 1819)	ABX6432	0.08	0.31
<i>Chrysomya megacephala</i> (Fabricius, 1794)	AAA5667	0	0
<i>Synthesiomyia nudiseta</i> (Wulp, 1883)	AAH9692	0.16	0.31
<i>Hemilucilia segmentaria</i> (Fabricius, 1805)	ACA1446	0	0
<i>Lucilia purpurascens</i> (Walker, 1836)	ACS3321	0.31	0.31
<i>Stomoxys calcitrans</i> (Linneo, 1758)	AAA3181	NA	0
<i>Paralucilia</i> sp.	ACA1399	0.81	0.81

Table 2. Localities and fly species.

Locality code	Location	Species	Position of corpse	Phase of descomp.
EFLoja-1	Zapotillo	<i>C. albiceps</i> , <i>C. megacephala</i>	Semi-fowler	Skeletonized
EFLoja-2	Loja	<i>C. albiceps</i> , <i>S. nudiseta</i>	Fetal	Putrefaction-Skeletonization
EFLoja-3	Sozoranga	<i>L. purpurascens</i> , <i>H. segmentaria</i>	Dorsal decubitus	Emphysematous
EFLoja-4	Alamor	<i>L. purpurascens</i>	Bone	Skeletonization
EFLoja-5	Taquil-Chuquirib.	<i>S. calcitrans</i> , <i>Paralucilia</i> sp	Bone	Skeletonization
EFLoja-6	Catamayo	<i>Paralucilia</i> sp.	Lateral decubitus	Emphysematous, Skeletonization

then preserved in 85% alcohol to keep them from becoming brownish and contracted.

DNA extraction of 24 specimens, that were preserved directly in 85% alcohol to preserve their DNA, was performed using primers LF1 and LR1 and following the standard protocols of the Biodiversity Institute of Ontario at the Guelph University [28,29]. We identified our specimens using molecular means by analyzing the Barcode Index Number (BIN). BINs are clusters of OTUs generated by a RESL algorithm and are automatically generated by the Barcode of Life Data (BOLD) System (www.barcodinglife.org) [30,31]. Specimens in a BIN are within a 2.18% similarity landscape, we considered the same species to all individuals belonging to the same BIN code [32]. Additional information (locality, elevation, region, coordinates, collector, and collecting date) and sequences are available in the BOLD Datasets DS-FFLIESRC (dx.doi.org/10.5883/DS-FFLIESRC) as part of the International Barcode of Life project (iBOL; www.ibol.org).

Results

Morphological character analysis

The 17 specimens identified with dichotomous keys belong to three species: *Chrysomya albiceps* collected in both localities EFLoja-1 and EFLoja-2, *Chrysomya megacephala* collected in locality EFLoja-1 only, and *Synthesiomyia nudiseta* collected in locality EFLoja-2. *Chrysomya albiceps* was identified in an immature stage using its posterior spiracles that have an incomplete peritreme, highly pigmented, with an almost inconspicuous button. Additionally, the majority of its segments have tubercles with apical spines; Florez and Wolff [24] stated that this character is specific to this species.

Adult individuals showed some representative characters such as the radial vein exposed on the ventral side

of the wing, the feathery antennal arista, the metallic thorax, mesonotum lacking longitudinal stripes, and the abdominal tergites with transverse black stripes in the posterior margin. Furthermore, the presence of an anterior thoracic spiracle, the white-colored inferior calyptra, entirely black antennae and genas, eyes with equal facets, characters shared with *C. putoria*. However *C. albiceps* was identified by the lack of stigmatic setae and 4 to 6 propleural setae [25,26].

Chrysomya megacephala (Fabricius, 1794), was identified with adult specimens only, this species shares some characters with *C. albiceps* such as the radial vein exposed in the ventral side of the wing, the feathery antennal arista, the metallic thorax, and the abdominal tergites with transverse black stripes. *Chrysomya megacephala* diagnostic characters listed in [25] are: the presence of an anterior thoracic spiracle, dark brown inferior calyptra, and reddish antenna and gena. Additionally, males have eyes with upper facets enlarged in comparison to the facets in the lower area [25,26]. *Synthesiomyia nudiseta* (Wulp, 1883) was identified based on individuals in immature stages. They have 1 to 7 abdominal segments with spineless posterior stripes; also they have round posterior spiracles with respiratory slits frequently S-shaped, darkly pigmented, arranged in a way that they do not surround the spiracle scar. Additionally, they show a small anal plate, triangular in ventral view; extra-anal and post-anal papillae on anal division well developed, and subanal papillae covered by spines; symmetric mouth hooks; well-developed anterior rods and oral bars; and dental sclerites joined ventrally. All of these diagnostic characters have been mentioned by [27].

Molecular analyses

We were able to amplify COI sequences for 100% of the 24 specimens sent to Canada. Our BIN analysis

suggests with high certainty that the specimens belonged to seven species: *Chrysomya albiceps*, *Chrysomya megacephala*, *Synthesiomyia nudiseta*, *Lucilia purpurascens*, *Hemilucilia segmentaria*, *Stomoxys calcitrans*, and *Paralucilia* sp. (Table 1).

Discussion

We provide the first evidence of the presence of six identified, and one non-identified, fly species on human corpses in Ecuador. *Chrysomya albiceps* is a species introduced to the New World from northern Africa [32–34]. This species may have severe impact in the abundance of endemic diptera on corpses because *C. albiceps* in immature stages (larva II) is a voracious predator and adopts an aggressive behavior resulting in alteration of the composition of dipteran species that undergo development in corpses [35]. Guimarães *et al.* [32] documented that approximately three decades ago this species was introduced to America through Brazil and Costa Rica and then it established its territory throughout the base of eastern Andes in Venezuela, along Brazil, Colombia, Uruguay and mid Bolivia and Peru, eastern Paraguay, northern Argentina and Ecuador [12,33,36]. It is distributed generally between 340 and 2,800 meters of elevation, between plains and the Andean region [37]. In Ecuador, it has been reported in Galapagos, Loja, Orellana, and Pichincha [23].

Chrysomya megacephala is one of the species with a great dispersion ability, its individuals can travel several kilometers in 24 hours, and their flight capability does not change as the average ambient temperature drops in temperate areas [38]. It has established itself along the Americas since its introduction in 1975 from Brazil towards the North [26,38,39]. *C. megacephala*'s great dispersion potential could become a hazard for native species because it carries pathogenic microorganisms such as viruses, bacteria, protozoan cysts, and helminth eggs [40]; besides *C. megacephala* can compete with native species for resources, and can even feed on larvae of other species [36]. Preying on fly larva by *C. megacephala* may be an additional aspect to consider in crime scene investigation, since it could affect the development and life cycle of colonizing flies, and thus it can directly affect the PMI [41].

Synthesiomyia nudiseta is widely distributed across the globe, and recorded in countries including Costa Rica, India, Malaysia, Thailand and United States [42,43] and throughout other tropical and subtropical regions of the World, with average annual temperatures of 20° C [44]. This species is considered synanthropic since its adults are attracted to environments influenced by human activities. It is known that *S. nudiseta* is one of the first species to colonize corpses found in urban buildings but generally in natural environments it arrives second to flies in the family Calliphoridae [44].

Lucilia purpurascens is characterized by maintaining a wide distribution in the Andes mountain range, from the eastern slopes in Argentina, Bolivia and Peru to approximately 2500 m in altitude; however, this species also was registered in our country at up to 2600 m in altitude [45]. It is considered a hemisynanthropic species since it lives in rural ecosystems, adapting to the slightly anthropogenic environmental conditions where there are already some changes made by humans; this species is somewhat generalist in terms of feeding, because it has usually been found feeding on fish, liver, cow feces and flowers, so it can be considered an indicator species of rural environments contributing significantly in forensic analysis for determination of the PMI or transfer of corpses [45,46].

Hemilucilia segmentaria is an endemic species of Central and South America (Dear 1985), reported in forested areas [47,48] where it has a necrophagous diet, feeding on decomposing bodies. *H. segmentaria* prefer humid habitats and warm temperatures [47,49]. *H. segmentaria*'s life cycle, from egg to adult, lasts an average of 310 hours [49]. This species is usually found at the crime scene and together with *C. albiceps* are used for more precise determination of the PMI [49].

Stomoxys calcitrans or "stable fly" is known by its economic importance because adult individuals act as biological and mechanical vectors of many diseases, such as anemia in cattle (reducing weight and blood in animals), diseases of nematodes, trypanosomiasis, and equine infectious anemia (producing growth retardation and low production) [50]. Its high presence is due to the amount of waste produced in homes and industries. Additionally, this species is resistant to common insecticides, which allows the species to be present in high numbers. Individuals of *S. calcitrans* in immature larvae stages grow in moist organic matter; while pupae prefer dry areas [51]. This species has been reported in forensic cases where it is considered a primary degrader in the chromatic phase of decomposing corpses [52]. *Paralucilia* is a genus of the family Calliphoridae represented mainly by necrophagous species important for the decomposition phases of organic matter [53].

Finally, the identification of fly species from real cases presented in this work contributes greatly to the development of forensic entomology in Ecuador, as it allows us to start building ecological and physiological information on them, as well as the human remains where they are found. Building this body of scientific literature is important for starting using local insect as legal evidence in Ecuador. Equally important, this study contributes to the BOLD COI database, increasing the number of molecular records for our country, available to

the wider scientific and medico-legal community in Ecuador.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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