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Escuela de Ciencias Biológicas e Ingeniería

TÍTULO: Insects (Diptera and Coleoptera) Associated with Three Human Cadavers and a Preliminary Checklist of Diptera Around the *Servicio Nacional de Medicina Legal y Ciencias Forenses* in Quito, Ecuador

Trabajo de integración curricular presentado como requisito para la obtención del título de Bióloga

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Urcuquí, junio 2020

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Sofia Amanda Abad Sojos

Abstract

Forensic entomology is the study of arthropods that colonize decomposing cadavers to help clarify the circumstances behind the death of individuals within the setting of a criminal, legal investigation. In this study, we report on the conditions, characteristics, and the entomofauna (orders Diptera and Coleptera) found on three human cadavers at the *Servicio Nacional de Medicina Legal y Ciencias Forenses* (SNMLCF) in Quito, Ecuador from August to December 2019. Five insect species of forensic relevance were identified on the human corpses: *Sarconesiopsis magellanica*, *Chrysomya albiceps, Compsomyiops verena* (Calliphoridae), *Peckia* sp. (Sarcophagidae), and *Oxelytrum discicolle* (Silphidae). Simultaneously, we sampled for insects of the order Diptera of forensic relevance around the SNMLCF using six modified Van Someren-Rydon bait traps. A total of 3,473 adult flies were collected that belonged to eight families (Calliphoridae, Sarcophagidae, Fanniidae, Muscidae, Lonchaeidae, Phoridae, Tachinidae, and Drosophilidae) and twenty-three different species. Interestingly, the most frequent fly species belonged to the family Calliphoridae and were the following: *Sarconesiopsis magellanica* (54.19%), *Chrysomya albiceps* (18.31%), *Lucilia sericata* (10.39%), and *Compsomyiops verena* (5.12%). The forensic entomological analysis is largely neglected in Ecuador; therefore, this study involving the collection of insects from human cadavers is pioneering for this country. Accordingly, the results presented here constitute a framework that may pave the way for further applications of forensic entomology in the near future in Ecuador.

Keywords:

Forensic Entomology; Human Cadaver; Diptera; Insects; Forensic Medicine; Ecuador

Resumen

La entomología forense es el estudio del patrón de invasión y sucesión de artrópodos que colonizan cadáveres en descomposición para aclarar las circunstancias detrás de la muerte de individuos en el marco de una investigación penal y legal. En este estudio, describimos las condiciones, características y entomofauna (Órdenes Diptera y Coleptera) encontradas en tres cadáveres humanos que fueron llevados al *Servicio Nacional de Medicina Legal y Ciencias Forenses* (SNMLCF) en Quito, Ecuador de agosto a diciembre de 2019. Cinco especies de insectos de relevancia forense fueron identificados en los cadáveres humanos: *Sarconesiopsis magellanica, Chrysomya albiceps, Compsomyiops verena* (Calliphoridae), *Peckia* sp. (Sarcophagidae) y *Oxelytrum discicolle* (Silphidae). Simultáneamente, muestreamos insectos del orden Diptera de relevancia forense alrededor del SNMLCF utilizando seis trampas Van Someren-Rydon con cebo modificadas. Se recolectaron un total de 3.473 moscas adultas que pertenecían a ocho familias (Calliphoridae, Sarcophagidae, Fanniidae, Muscidae, Lonchaeidae, Phoridae, Tachinidae y Drosophilidae) y veintitrés especies diferentes. Curiosamente, las especies de moscas más frecuentes pertenecían a la familia Calliphoridae y fueron las siguientes: *Sarconesiopsis magellanica* (54.19%), *Chrysomya albiceps* (18.31%), *Lucilia sericata* (10.39%) y *Compsomyiops verena* (5.12%). El análisis entomológico forense es descuidado en gran medida en Ecuador, por lo que este estudio que involucra la recolección de insectos de cadáveres humanos es pionero en este país. En consecuencia, los resultados presentados por este estudio constituyen un marco de referencia que puede aportar en un futuro cercano para la aplicación de la entomología forense en Ecuador.

Palabras clave:

Entomología forense; Cadáveres humanos; Diptera; Insectos; Medicina Forense; Ecuador

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1. Introduction

Medico-legal forensic entomology is the study of the invasion and succession patterns of arthropods that colonize decomposing cadavers. This field contributes to elucidating circumstances of criminal deaths usually associated with violent crimes in legal and criminal investigations (Cherix et al., 2012). Insects can provide information that cannot be obtained by traditional forensic methods (Wolff et al., 2001). Thus, the knowledge of the ecological behavior, distribution, and biology of insects can provide valuable information about where, when, how, and under what specific conditions the crime occurred (Amendt et al., 2007).

Decomposition stages are characterized by the physicochemical changes that the corpse undergoes through time. These stages are divide into fresh, bloated, decay (active/advanced), and dry (skeletonized; Carter et al., 2007). Depending on their ecological preferences, insects will be attracted to different stages of decomposition of a cadaver. Necrophagous species are normally attracted to specific olfactory cues; for this reason, there is a predictable chronological sequence of insect species at a cadaver, called insect succession pattern (Anderson, 2009).

Forensic entomologists commonly focus on two important groups of insects: flies (order Diptera) and beetles (order Coleoptera). Calliphoridae is the most prominent family of flies at fresh cadavers; hence, they dominate the first days and weeks of cadaver decomposition. Necrophagous dipterans can detect the location of an odor source with great spatial precision and deposit their eggs on a cadaver within minutes or hours from death. The larvae, called maggots, emerge from the eggs and feed on the decomposing tissues, particularly their protein content (Campobasso et al., 2001). Other groups of Diptera are present at later stages depending on the degree of decomposition of the cadaver. Coleopterans, on the other hand, have preferences for dry tissues; hence, this order appears at the latter stages of decomposition (Anderson, 2009; Amendt et al., 2011).

There are three main applications of forensic entomology. First, an estimation of the post-mortem interval (PMI), which refers to the time between the death and the discovery of the cadaver. PMI is normally determined based on known natural processes that a corpse goes through during decomposition such as rigor mortis (cadaver stiffness) and livor mortis (pooling of blood), although the efficacy of this forensic diagnosis is limited to the first 72 hours after death

(Campobasso et al., 2001). Forensic Entomology overcomes this drawback of the traditional PMI. To perform a PMI calculation, it is necessary to identify taxonomically the species on the cadaver, reconstruct the crime scene's temperatures and other ambient conditions, model the speed of larval development, and thus estimate the age of the insect larvae (Richards et al., 2009). Second, forensic entomology allows determining whether the deceased person ingested drugs or narcotics through an "entomotoxicological" analysis. In case that soft tissues and organs are no longer present, maggots and pupae become the key evidence to estimate PMI and perform toxicological analyses. Through toxicological analyses of maggots found in a cadaver, it has been possible to detect traces of drugs such as paracetamol, tricyclic antidepressants, benzodiazepines, amphetamines, steroids, cocaine, morphine, among many others. Nevertheless, these substances can only be detected in maggots when the rate of absorption exceeds the rate of the chemical's metabolization (Introna et al., 2001; Byrd & Peace, 2012; Amendt et al., 2011). Finally, forensic entomology determines whether a cadaver has been transported from one location to somewhere else. If a corpse contains insects that do not belong to the fauna of a specific geographic area, it is possible to conclude that the corpse has been moved. Insects provide pivotal information about where the crime occurred, although it is only possible if there is previous knowledge of insect diversity in a particular area (Wolff et al., 2001).

The application of forensic entomology is gaining acceptance in many Latin American countries. Recently, scientists in Argentina, Venezuela, Brazil, Colombia, Chile, and Peru have been conducting and recording entomological surveys, and preliminary studies on ecological successions of insects of forensic relevance. To elaborate checklists of necrophagous insects, they generally have used pigs, rats, chickens, and fish as baits or experimental animal models. All these experiments have proven the feasibility of using animal tissues to attract a wide diversity of insects of forensic significance (Oliveira et al, 2016; Carvalho et al., 2000; Velásquez, 2008). This scientific research offers excellent potential to contribute to the resolution of legal proceedings. It is therefore advisable to implement new procedures from forensic entomology, that complements current legal techniques and allows more precise investigation (Wolff et al., 2001).

1.1. Problem statement

The topic of forensic entomology in Ecuador was introduced in 2013 when the entomologist Álvaro Barragán taught a forensic entomology course to 88 members of the *Fiscalía General del*

Estado (public prosecutors' office) of Ecuador, intending to introduce this science in medico-legal investigations (Barragán & Moreno, 2015). In 2016, the *Servicio Nacional de Medicina Legal y Ciencias Forenses* (SNMLCF) was created in Quito to provide standardized technical and scientific services. However, a forensic entomology section has not yet been considered in the organigram of this forensic institute. García-Ruilova & Donoso (2015) conducted polls with legal health professionals, which suggested that 34 cases from January to July 2014 could have greatly benefitted from forensic entomology. Unfortunately, so far there have been no attempts to implement forensic entomology in Ecuador. Perhaps, a first step to change this situation would be to obtain baseline information on the insects of forensic relevance associated with carrion in different habitats, along altitudinal gradients, and during different seasons of the year. Currently, Ecuador has a limited number of catalogs listing the most important groups of insects with forensic interest (Aguirre, 2014; Salazar & Donoso, 2015). It is necessary to obtain information on the distribution, ecology, life cycles, and behavior to apply forensic entomology in the future and solve criminal cases in Ecuador (Salazar & Donoso, 2015; Moreno & Barragán, 2015). Preliminary forensic entomology checklists will undoubtedly help to establish the baseline of scientific information required for future applications of this science in Ecuador (Velásquez, 2008). This preliminary study represents a first step in the establishment of a database for medico-legal purposes in Ecuador

1.2. Objectives

General objectives:

- Characterize the insect communities present on three cadavers that were autopsied at SNMLCF in Quito, Ecuador.
- Elaborate a preliminary checklist of dipterans of forensic relevance around the SNMLCF facilities.

Specific Objectives:

- Deduce the forensic conditions under which the cadavers colonized by insects were autopsied at the SNMLCF.
- Identify taxonomically the insects of forensic relevance to the lowest possible category using dichotomous keys.
- Determine the abundance and frequency of each family and species collected around the SNMLCF facilities.

2. Methodology

2.1. Sampling area and ethical statement

The present study was conducted at the facilities of the SNMLCF in Quito, Ecuador. Here, autopsies are carried out to identify a victim and/or determine the cause of death. The SNMLCF is located in a highly-populated neighborhood next to the *Rumipamba Archeological Park* on the highly busy Mariana de Jesús Avenue (0°10'54.83"S; 78°30'14.41"W, 2939 m.a.s.l.; Fig. 1). This study was approved by the SNMLCF through Memorandum number SNMLCF-CTSML-2019-3025-M and by the *Ministerio del Ambiente Ecuador* under authorization number 015-2019-IC-FAU-DPAP-MA (See Appendices A & B). The author declares not to have had any access to the cadavers and any information regarding their identity.

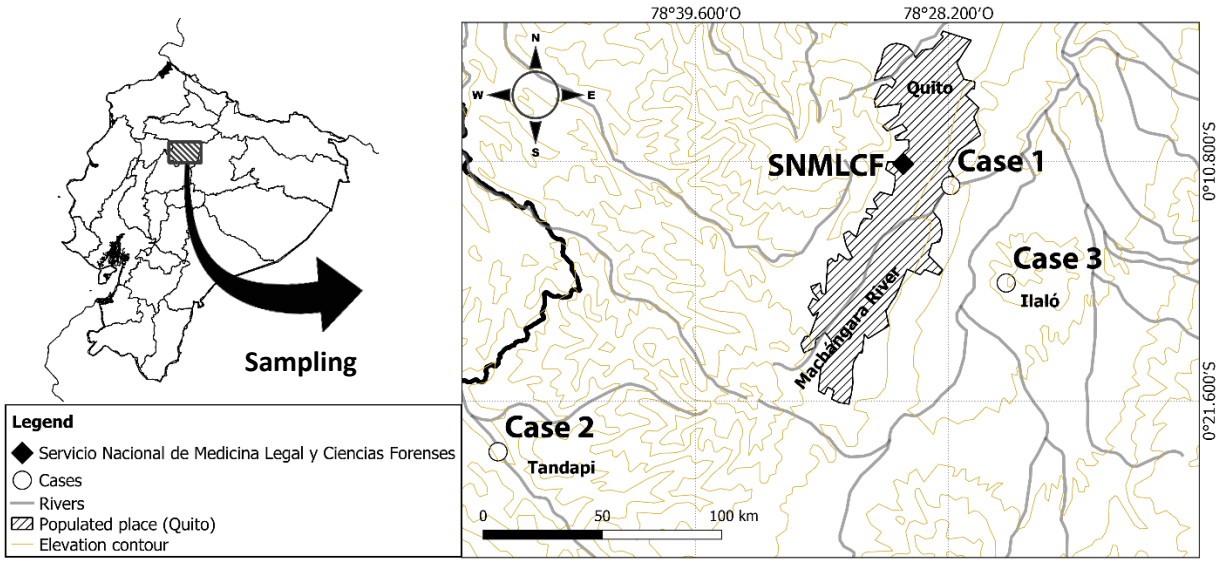

Fig. 1. Location of the *Servicio Nacional de Medicina Legal y Ciencias Forenses* (sampling area) in Quito, Ecuador, and the location of the three corpses whose insect communities were studied (made with QGIS 3.10.6 software).

2.2. Insects sampling drew from cadavers and identification

The sampling of insects drawn from cadavers took place from August 2019 to December 2019. To guarantee the anonymity of each case, each corpse was assigned a new number different from the codes used at SNMLCF. Data recorded for each case were estimated age, sex, the location where the corpse was discovered, cause of death, degree of decomposition, estimated PMI, level of colonization, and distribution of insects. Forensic anthropologists and medical doctors of the SNMLCF were responsible for reporting and removing insects from cadavers before performing the autopsy. Entomological evidence was taken from corpses as soon as they arrived at the

SNMLCF. Insects were carefully removed using entomological forceps and placed into 100 mL containers with a small piece of raw meat. Containers were then taken to the Entomology Laboratory of the *Pontificia Universidad Católica del Ecuador* (PUCE), where the containers with maggots were transferred to a climate chamber with the following settings until the emergence of adults: constant temperature of 25°C temperature, 70% relative humidity, and a 12-hour light-dark cycle. Beetles and adult flies were captured, killed with ethyl acetate, and stored until their entomological classification. Several entomological keys were used for the identification of the insects (Amat et al., 2008; Brown et al., 2010; Carvalho & Mello-Patiu, 2008; Amat-García & Valcárcel, 2014). Since the level of colonization was difficult to quantify, we followed the criteria set forth by Oliveira & Vasconcelos (2010), in which the level of colonization of each corpse is visually classified into low (up to 500 maggots), intermediate (between 501 to 1000 maggots) and high (over 1000 maggots).

2.3. Diptera traps and sampling around SNMLCF facilities

Six Van Someren-Rydon traps, modified specially to collect flies, were set up around SNMLCF in the following arrangement. Trap 1 was located at 10 meters from the autopsy room (0°10'54.84"S, 78°30'15.14"W), trap 2 above a water reserve tank (0°10'54.48"S, 78°30'15.00"W), trap 3 above the garbage storage (0°10'54.29"S, 78°30'14.85"W), traps 4 and 5 in the courtyard of the SNMLCF (0°10'53.73"S, 78°30'14.33"W and 0°10'53.88"S, 78°30'13.93"W), and trap 6 on the roof of the forensic anthropology building (0°10'53.99"S, 78°30'13.69"W; see Appendix C). A data logger was located in the courtyard of the SNMLCF, which recorded an average temperature of 15.6 °C and the relative humidity of 66.7% throughout the sampling period.

The bait for each trap consisted of two chicken heads that attract flies ready for oviposition and 200 ml of processed fish and chicken viscera that have been decomposing for 4 days before being used in the traps. New bait was placed in all the traps to start with each collection effort. Adult dipterans were collected from traps after 48 hours of bait exposure to the environment. Adult flies were killed using ethyl acetate and then stored in a 75% ethanol solution. Eggs and maggots obtained from chicken heads were reared in the laboratory only for the two firsts weeks of the study to check the effectiveness of the traps. Diptera sampling was performed in the period from September 11th to October 19th of 2019 with a total of 11 collection events. The frequencies of each family and species were calculated following the formula F [%] = (abundance per family or

species ÷ total number of individuals) x100. A species accumulation curve was calculated using diversity statistics in EstimateS 8.2.0 software (Colwell, 2006). Diversity indexes to compare the species diversity among traps were not calculated due to short distances of 15 m or less between traps. For this reason, the traps cannot be treated as independent samples. Traps are pseudoreplicates and must be considered part of the same sample unit representing the same community of flies.

3. Results

3.1. Insects found on human cadavers

During the five months of study, 832 cadavers were delivered to SNMLCF to perform autopsies, only three of which have been colonized by necrophagous insects. In other words, 99.64% of the cadavers that arrived at SNMLCF were at a fresh stage of decomposition with no entomologic evidence. This percentage of corpses belonged mostly to victims of violent deaths, and a few to people who died by natural causes, in hospitals, traffic accidents, among others. The remaining 0.36% of the cadavers were colonized by insects. The three corpses were male and aged 35, 30, and 23, respectively. They were found in rural sectors at different distances from the SNMLCF (Fig. 1). The decay and dry stages of decomposition of the cadavers were not uniform throughout the body (Table 1). The law does not allow the SNMLCF to reveal the exact coordinates of the sites where the corpses were found in order to maintain the anonymity of the cases. For this reason, only approximate locations are provided in Table 1 and Figure 1.

Table 1
Conditions and characteristics of the corpses colonized by insects examined at SNMLCF, Quito, Ecuador. PMI is the post-mortem interval.

Case	Estimated age	Sector of the corpse discovery	Cause of death	Stage of decomposition	PMI (days)
Case 1	35	*Machángara* river, at the height of *Guápulo*	Possible fall and drowning in the river	Head uniformly at the dry stage, body at the decay stage	34
Case 2	30	The ravine in the sector *Cara del Diablo, Tandapi,*	Undetermined	Partially at the decay and dry stages	7
Case 3	23	*San Pedro del Tingo*, the cross of the hill of *Ilaló*	Homicide - Stabbing	Partially at the decay stage	5

The three cases showed from low to intermediate levels of colonization by insects, which were mainly located in the thorax, head, and at natural orifices of the body. Insects of both orders, Coleoptera and Diptera were found on the cadavers. Coleopterans were represented by only one species from the family Silphidae, *Oxelytrum discicolle* (Brullé, 1840). On the other hand, four species from two different families of Diptera were identified. The species belonging to the Calliphoridae were *Sarconesiopsis magellanica* (Le Guillou, 1842), *Chrysomya albiceps* (Wiedemann, 1819), and *Compsomyiops verena* (Walker, 1849). The only species of Sarcophagidae was *Peckia* sp. (Robineau-Desvoidy, 1830; Table 2).

Table 2
Insect evidence: levels of colonization of low (up to 500 maggots) and intermediate (between 501 and 1000), distribution of insects along with the cadaver, order, family, species, and the number of specimens (N) found in each case.

Case	Level of colonization	Distribution along with the cadaver	Order	Family	Species	N
Case 1	Low	Coleoptera concentrated on the head and thorax	Coleoptera	Silphidae	*O. discicolle*	2
		Diptera maggots concentrated on the thorax	Diptera	Sarcophagidae	*Peckia* sp.	6
Case 2	Low	Coleoptera concentrated on the thorax	Coleoptera	Silphidae	*O. discicolle*	3
Case 3	Intermediate	Internal organs of the thorax, especially around stabbing wounds; concentrated on neck, head and body orifices (anus and mouth)	Diptera	Calliphoridae	*S. magellanica*	34
					C. albiceps	2
					C. verena	8
				Sarcophagidae	*Peckia* sp.	4

3.2. Sampling of Diptera around the SNMLCF facilities

A total of 3,473 dipteran adults belonging to 8 families and 23 different species were collected around the SNMLCF facilities during the study. The family with the highest number of species was Calliphoridae (7 species), followed by Sarcophagidae (6 species), Fanniidae (3 species) and Muscidae (3 species); the families with the lowest numbers of species were Lonchaeidae, Drosophilidae, Phoridae, and Tachinidae (1 species each). In terms of the abundances and frequencies of fly families collected, Calliphoridae was the most abundant family (abundance 3,142; frequency 90.5%) followed by Sarcophagidae (115; 3.3%), Muscidae (95; 2.7%), Fanniidae (52; 1.5%), Lonchaeidae (33; 0.9%), Drosophilidae (26; 0.7%), Phoridae (9; 0.3%), and

Tachinidae (1; <0.05%; Tables 3 & 4). The forensic relevance of each family is also shown in Table 3.

Table 3
Family of insects collected around the SNMLCF, Quito, Ecuador: number of species collected for each family, abundance per family, frequency [%] = (Abundance/3473) x 100, and reported forensic importance.

Family	Number of species	Abundance per family	Frequency [%]	Forensic importance
Calliphoridae	7	3,142	90.469	X[1]
Sarcophagidae	6	115	3.311	X[1]
Muscidae	3	95	2.735	X[1]
Fanniidae	3	52	1.497	X[2]
Lonchaeidae	1	33	0.950	-
Phoridae	1	9	0.259	X[3]
Tachinidae	1	1	0.029	-
Drosophilidae	1	26	0.749	X[4]
Total	23	3,473	100	

[1]Byrd & Castner (2001)
[2]Grisales et al., (2016); Carvalho & Mello-Patiu (2008)
[3]Bugelli et al. (2015); Zuha et al., (2016)
[4]Tomberlin & Benbow (2015); Disney (2008)

The most abundant and frequent species were *Sarconesiopsis magellanica* (abundance 1882, frequency 54.19%), *Chrysomya albiceps* (636, 18.31%), *Lucilia sericata* (361, 10.39%), and *Compsomyiops verena* (181, 5.12%), all of them belong to the family Calliphoridae (Table 4). The remainder of the species have abundances lower than 100 specimens and therefore frequencies below 3%. Diversity and abundance of fly species throughout the sampling period were highest in traps numbers 4, 5, and 6 with 595, 1099, and 1187 flies, respectively. The species and families collected from cadavers are also shown in Table 4.

Table 4

Species collected throughout the study, site of collection (see text for the site of each trap), abundance per species, frequency, and presence or absence of these species in cadavers

Family	Species and references	Site of collection						Abundance per species	Frequency [%]	Presence in cadavers
		T 1	T 2	T 3	T 4	T 5	T 6			
Calliphoridae	*Sarconesiopsis magellanica* (Le Guillou, 1842)	231	77	60	279	404	831	1882	54.189	X
	Compsomyiops verena (Walker, 1849)	4	7	6	34	97	33	181	5.212	X
	Chrysomya albiceps (Wiedemann, 1819)	15	37	33	126	351	74	636	18.313	X
	Chrysomya rufifacies (Macquart, 1843)	-	6	5	11	10	7	39	1.122	-
	Lucilia sericata (Meigen, 1826)	13	20	31	71	118	108	361	10.394	-
	Calliphora vicina (Robineau-Desvoidy, 1851)	1	3	-	3	8	4	19	0.547	-
	Calliphora nigribasis (Macquart, 1851)	2	3	4	6	7	2	24	0.691	-
Sarcophagidae	*Peckia* sp. (Robineau-Desvoidy, 1830)	-	-	-	6	32	11	49	1.410	X
	Boettcheria sp. (Parker, 1914)	-	-	-	1	-	1	2	0.058	-
	Ravinia rufipes (Robineau-Desvoidy, 1863)	-	-	5	8	13	4	30	0.864	-
	Ravinia sp1 (Robineau-Desvoidy, 1863)	-	-	-	6	4	-	10	0.288	-
	Ravinia sp2 (Robineau-Desvoidy, 1863)	1	-	1	2	1	2	7	0.202	-
	Dexosarcophaga sp. (Townsend, 1917)	-	-	-	1	3	13	17	0.489	-
Fanniidae	*Fannia obscurinervis* (Stein, 1990)	3	1	-	-	3	15	22	0.633	-
	Fannia trimaculata (Stein, 1898)	-	1	-	2	2	10	15	0.432	-
	Fannia pusio (Wiedemann, 1830)	-	1	1	4	5	4	15	0.432	-
Muscidae	*Sarcopromusca pruna* (Shannon & Del Ponte, 1926)	1	-	1	2	4	4	12	0.346	-
	Musca domestica (Linnaeus, 1758)	4	2	7	21	29	15	78	2.246	-
	Muscidae 1 (Not identified)	-	-	-	2	-	3	5	0.144	-
Lonchaeidae	*Dasiops* sp. (Rondani, 1856)	-	-	-	6	7	20	33	0.950	-
Phoridae	Phoridae 1 (Not identified)	2	-	-	2	-	5	9	0.259	-
Tachinidae	Tachinidae 1 (Not identified)	-	-	-	-	1	-	1	0.029	-
Drosophilidae	Drosophilidae 1 (Not identified)	1	2	-	2	-	21	26	0.748	-
Silphidae	*Oxelytrum discicolle* (Brullé, 1840)	-	-	-	-	-	-	-	-	X
TOTAL		278	160	154	595	1099	1187	3473	100	

3.2.1. Diptera oviposition and traps efficacy around the SNMLCF facilities

During the first weeks of the experiment, the eggs deposited by flies on the bait traps were reared until emerging into adults. These adults and the adults collected from traps belonged to the same species which meant that the traps were effective to not let escape any flies. For this reason, we concluded that we had not lost any species due to escape from the traps and discontinued rearing flies from eggs and maggots. The species accumulation curve is a representation of species richness based on a cumulative number of individual samples. In this case, the accumulation curve (Fig. 2) indicates that the study area was well sampled because the curve reaches a horizontal asymptote over which no more Diptera species were detected. In other words, our sampling effort was sufficient to sample the species richness around the SNMLCF facilities, namely 23 species. This curve was calculated using the diversity statistics option on the EstimateS 8.2.0 software (Colwell, 2006; see Appendix D).

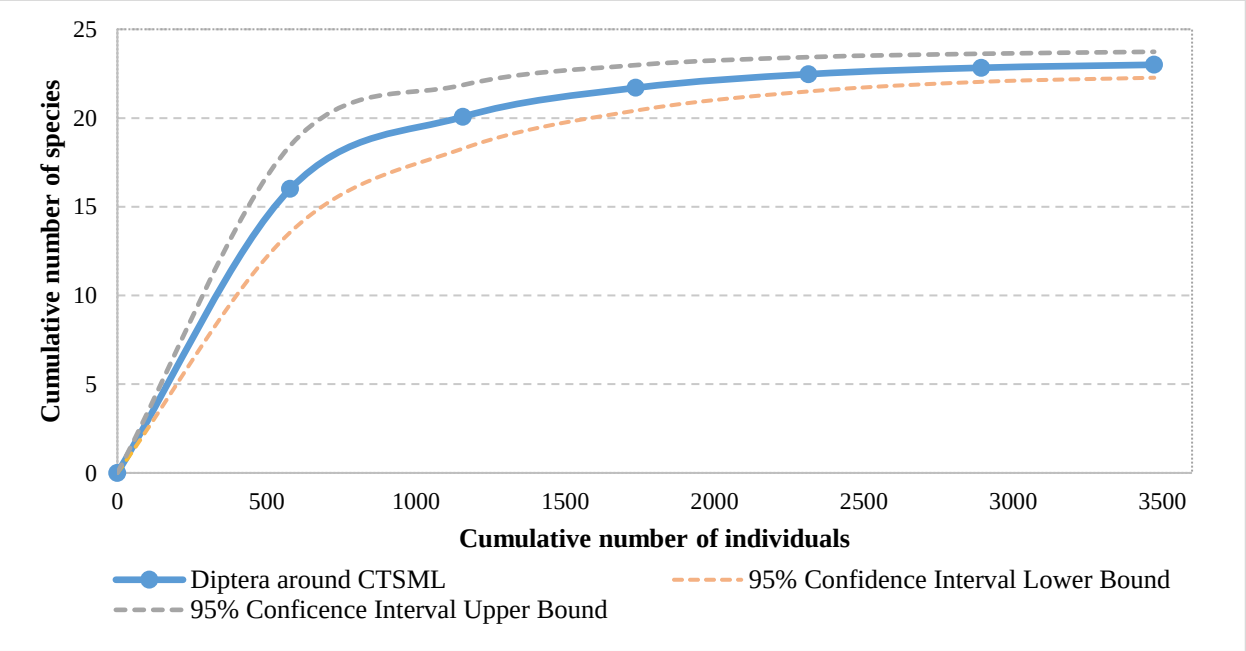

Fig. 2. Species accumulation curve for dipterans of forensic relevance collected around SNMLCF facilities.

4. Discussion

4.1. Sampling of insects found in human cadavers

Five species of forensic relevance are reported here for the first time for Ecuador directly from human cadavers (*Sarconesiopsis magellanica, Compsomyiops verena, Chrysomya albiceps, Peckia* sp., and *Oxelytrum discicolle*). There are no previous studies on insects associated with human cadavers in this country. The presence of these species had thus far only known from baited traps (Aguirre, 2014; Salazar & Donoso, 2015).

Compared to studies from other South American countries, only *Chrysomya albiceps* and *Oxelytrum discicolle* coincide with those reported in human cadavers of Brazil and Colombia (Carvalho et al., 2000; Oliveira & Vasconcelos, 2010; Barreto et al., 2002). Geographic and climatic parameters that shape the flies' distribution and population dynamics may explain the different patterns in species richness and abundances found in human cadavers in Ecuador compared to those of other South American countries.

In cases 1 and 2, the cadavers were partially decaying and showed dry stages of decomposition, which explains the presence of *Oxelytrum discicolle* (order Coleoptera), a species with a preference for dry tissues found in later stages of decomposition (Anderson, 2009; Amendt et al., 2011). A higher number of maggots were found on the corpse from case 3 (only at 5-day postmortem, the "freshest" cadaver); both inside natural orifices and in wounds as blood attract flies (Campobasso et al., 2001). The insect community collected around SNMLCF was more diverse than the ones found on corpses. This is probably due to the limited number of corpses colonized by insects that arrived at SNMLCF. Besides, entomological evidence around and inside the corpse may have been lost during the transport of the cadavers.

Interestingly, Dipteran species collected from bodies coincided with those captured by bait traps around the SNMLCF, the cadavers of cases 2 and 3 were found and colonized by insects at a distance greater than 50 km from the SNMLCF (Fig.1). Normally, the different environmental parameters such as altitude, temperature, and humidity affect the presence of different Diptera specimens. Due to the restriction of the SNMLCF to publish the precise coordinates where the cadavers were found, it is not possible to compare the exact

environmental parameters that justify the presence of the same insects at different places and distances.

4.2. Sampling of Diptera collected around SNMLCF

Bait traps and animal carcasses are widely used in most Latin American studies due to their operational ease and few ethical concerns. Those studies have found similar trends in species collected by traps and insects found on animal corpses (Oliveira & Vasconcelos, 2010). The same trend is seen in this study because all dipteran species found on cadavers were also present in bait traps. However, field surveys using bait traps to collect insects of forensic interest may not reflect the species richness and the abundance that would truly be found on a real decomposing cadaver.

According to the literature, six dipteran families (Calliphoridae, Sarcophagidae, Muscidae, Fanniidae, Phoridae, and Drosophilidae) of the eight families collected in this study are relevant for applications of forensic entomology (Byrd & Castner, 2001; Grisales et al., 2016; Carvalho & Mello-Patiu, 2008; Bugelli et al., 2015; Zuha et al., 2016; Tomberlin & Benbow, 2015; Disney, 2008; Table 3). The most abundant species of the study were *Sarconesiopsis magellanica*, *Chrysomya albiceps*, *Lucilia sericata*, and *Compsomyiops verena*. All of these are well known forensic indicators that reveal important information to investigate the causes or circumstances of death (Florez & Wolff, 2009).

In contrast, we discovered the presence of Lonchaeidae and Tachinidae families on bait traps. These families are not considered to be of forensic importance, and their abundance was the lowest of any group in the study (Table 3). The species *Dasiops* sp. of the family Lonchaeidae usually develops under the bark of dead or dying trees. The one specimen of tachinid found in traps might have been attracted to the maggots as the family Tachinidae are parasitoids; therefore, the larval stage normally develops inside the bodies of another arthropod, although rarely in those of other dipterans (Brown et al., 2010).

Musca domestica is a synanthropic species; maggots and adults have a feeding and breeding preference for manure, excrement, fermented vegetable and fruit matter, garbage, and occasionally cadavers. The family Drosophilidae is known to feed on microorganisms that decompose organic material, but the subfamily Steganinae is capable to feed on animal carrion and might be parasitic (Máca & Otranto 2014). Although both species collected do

not have a preference for decomposing cadavers, they are considered relevant for forensic entomology. *Musca domestica* has been used in the past to estimate PMI and for entomotoxicological analyses (Al-Qahtni et al., 2019; Al-Qahtni et al., 2020; Introna et al., 2001; Amendt et al., 2010)

The six traps were placed around the SNMLCF with a distance of less than 15 meters between them due to the small extension of the facilities, and to prevent the traps from interfering with the work of the forensic scientists. Traps 1, 2, and 3 were located at 10 m from the autopsy room, above a water reserve tank and above garbage storage, respectively, all surrounded by concrete walls without the presence of any natural elements. A total of 592 dipterans were collected in these traps throughout the study (Table 4). In contrast, traps 4, 5, and 6 were located near or in open spaces within the courtyard of the SNMLCF surrounded by few trees and grassy areas. In these traps, a total of 2,881 dipterans were collected. The first three traps captured 16 of the 23 species collected throughout the study. The remaining seven species were not present, and most of them have low abundances and frequencies in the study. Four of these belong to family Sarcophagidae (*Peckia* sp., abundance 49; *Boettcheria* sp., 2; *Ravinia* sp. 1, 10; *Dexosarcophaga* sp., 13), the other one is the morphotype Muscidae 1 (abundance 5), and the last two species are not of forensic interest, including *Dasiops* sp. (abundance 33) and Tachinidae species 1 (abundance 1). It is difficult to explain why these dipterans were not present in traps 1, 2, and 3, but it is likely that the location of these traps, being surrounded by concrete walls without vegetation, did not attract these insects to the traps.

In Ecuador, previous studies of forensic entomology have been carried out using bait traps (Aguirre, 2014; Salazar & Donoso, 2015; Torres, 2016; Blacio, 2018; Hidalgo, 2015; Arbeláez & Narváez, 2019). All of these studies were performed in localities with different geographic and climatic parameters. For this reason, each previous study determined a wide variety of patterns in species richness, with more than 20 different dipteran species of forensic relevance that were not found around the sampling area of the present study. Previous studies were performed in different provinces of Ecuador, but most of them belong to Pichincha province. Contrasting the present study with the previous ones performed in Ecuador, there is a notorious difference between the species communities at each location (Table 5).

Comparing the current study to those performed in Pichincha, three species are shared: *Sarconesiopsis magellanica, Chrysomya albiceps,* and *Lucilia sericata,* being the most abundant in this study. The species, *Ravinia rufipes, Ravinia* sp., *Dexosarcophaga* sp., *Fannia obscurinervis, Fannia trimaculata,* and *Fannia pusio* were not recorded previously in bait traps. Thus, this study reports the presence of these species of forensic importance for the first time in Quito, Pichincha, Ecuador. Contrasting dipteran communities found of Pichincha with those of distant provinces of Ecuador like Orellana, Pastaza, and Guayas, *Chrysomya albiceps* is the only species shared with those localities. *Chrysomya albiceps* is a very important forensic indicator, its presence has been reported in subtropical and template habitats, and their maggots are predators of other dipteran maggots (Faria et al., 1999). *Sarconesiopsis magellanica,* on the other hand, is limited to Pichincha province, but more studies are needed to confirm this hypothesis. All of the species recorded in the present study had already been reported from different localities in other South American countries, but none of those sites shared the same dipteran community composition (Durango & Ramírez-Mora, 2019; Oliveira et al, 2016; Carvalho & Mello-Patiu, 2008; Florez & Wolff, 2009; Buenaventura et al., 2009).

Table 5. Comparison between Diptera collected and identified in the present study and previous studies of forensic entomology performed in Ecuador using bait traps

Author reference		Aguirre (2014)	Salazar & Donoso (2015)	Torres (2016)	Blacio (2018)	Hidalgo (2015)	Arbeláez & Narváez (2019)
Province of Ecuador		Pichincha	Pichincha	Pichincha	Pichincha	Orellana and Pastaza	Guayas
Diptera collected in this study							
Calliphoridae	*S. magellanica*	X	X	X	X	-	-
	C. verena	-	-	X	-	-	-
	C. albiceps	X	X	X	X	X	X
	C. rufifacies	X	-	-	-	-	-
	L. sericata	X	X	X	X	-	X
	C. vicina	-	X	X	-	-	-
	C. nigribasis	-	X	X	X	-	-
Sarcophagidae	*Peckia* sp.	X	-	-	X	X	X
	Boettcheria sp.	-	-	-	X	-	-
	Ravinia sp.	-	-	-	-	-	-
	Dexosarcophaga sp	-	-	-	-	-	-
Fanniidae	*F. obscurinervis*	-	-	-	-	-	-
	F. trimaculata	-	-	-	-	-	-
	F. pusio	-	-	-	-	-	-

Muscidae	*S. pruna*	X	-	-	-	-	-
	M. domestica	X	X	-	-	-	X
Phoridae		X	-	-	-	-	-
Drosophilidae		-	-	-	-	-	-

The application of forensic entomology is gaining acceptance in many Latin American countries and it offers an excellent potential to contribute to legal proceedings. Bait traps are an efficient sampling tool to provide preliminary checklists of Diptera, which serve as a baseline for future investigations and help consolidate a framework for future applications of forensic entomology in Ecuador. This small country contains many ecosystems with a great variety of habitats and types of vegetation. For this reason, it is necessary to perform more field surveys, and to study patterns of ecological succession in different habitats, along altitudinal gradients, during different seasons of the year, and maintaining a precise and accurate record of temperature and humidity among other intrinsic and extrinsic factors.

5. Conclusion

Forensic entomology provides important tools to clarify criminal cases. Unfortunately, in Ecuador, there is a dearth in knowledge in this science, caused by a lack of interest to apply this field of study by the medico-legal institutes. To help fill in this gap in knowledge, we provide a checklist of insects of forensic relevance found on both human cadavers and in six bait traps located around the SNMLCF in Quito, Ecuador. More than 3,473 insects belonging to the orders Diptera (the great majority) and Coleoptera were collected and identified taxonomically. It is necessary to continue performing field surveys at other localities of the country to promote the future implementations of this science to solving crimes in Ecuador.

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Appendices

Appendix A

Research permit issued by the *Servicio Nacional de Medicina Legal y Ciencias Forenses* of Quito, Ecuador

SERVICIO NACIONAL DE MEDICINA LEGAL Y CIENCIAS FORENSES

Toda una Vida

EL GOBIERNO DE TODOS

Memorando Nro. SNMLCF-CTSML-2019-3025-M

Quito, D.M., 01 de agosto de 2019

PARA: Sra. Dra. Valeria del Rosario Yánez Serrano
Coordinadora General Administrativa Financiera

ASUNTO: Solicitud de investigación para realización de tesis sobre entomología forense

De mi consideración:

BASE LEGAL:

CONSTITUCIÓN DE LA REPUBLICA DEL ECUADOR
Art. 195, "*la Fiscalía dirigirá de oficio o a petición de parte, la investigación pre-procesal y procesal penal...la Fiscalía organizará y dirigirá un sistema especializado integral de investigación, de medicina legal y ciencias forenses*".

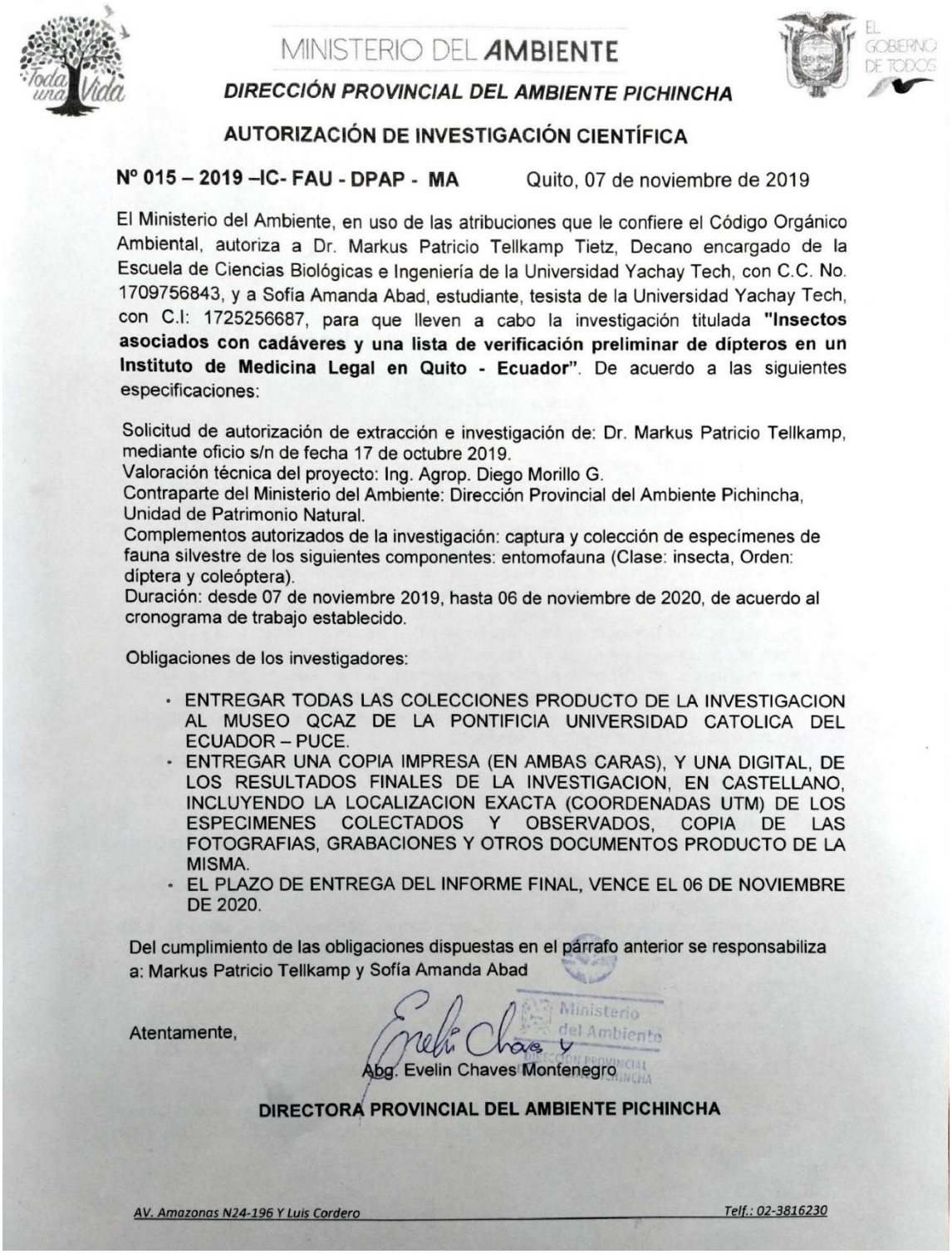
CÓDIGO ORGÁNICO INTEGRAL PENAL
Art 442.- "*La Fiscalía dirige la investigación pre-procesal y procesal penal e interviene hasta la finalización del proceso".*
Art. 448.- "*En materia pre-procesal y procesal penal, la Fiscalía organizara y dirigirá el Sistema Especializado Integral de Investigación de Medicina Legal y Ciencias Forenses que presentara servicios especializados de apoyo técnico y científico a la administración de justicia. El Sistema contara con el apoyo del organismo especializado de la Policía Nacional y personal civil de Investigación, quienes llevaran a cabo las diligencias necesarias para cumplir los fines previstos en este código, ejecutaran sus tareas bajo la dirección de la Fiscalía y dependerán administrativamente del ministerio del ramo".*
Art. 456.- Cadena de custodia.- "*Se aplicará cadena de custodia a los elementos físicos o contenido digital materia de prueba, para garantizar su autenticidad, acreditando su identidad y estado original; las condiciones, las personas que intervienen en la recolección, envío, manejo, análisis y conservación de estos elementos y se incluirán los cambios hechos en ellos por cada custodio. La cadena inicia en el lugar donde se obtiene, encuentra o recauda el elemento de prueba y finaliza por orden de la autoridad competente. Son responsables de su aplicación, el personal del Sistema especializado integral de investigación, de medicina legal y ciencias forenses, el personal competente en materia de tránsito y todos los servidores públicos y particulares que tengan relación con estos elementos, incluyendo el personal de servicios de salud que tengan contacto con elementos físico que puedan ser de utilidad en la investigación*".

CÓDIGO ORGÁNICO DE LAS ENTIDADES DE SEGURIDAD CIUDADANA Y ORDEN PÚBLICO – COESCOP
Art. 144: "*El Servicio Nacional de Medicina Legal y Ciencias Forenses – SNMLCF es la entidad operativa responsable de la gestión de la investigación técnica y científica en materia de medicina legal y ciencias forenses*."

Por su parte, el art. 146 del citado cuerpo legal, al tratar sobre la naturaleza jurídica del Servicio Nacional indica: "(...). *Prestará apoyo técnico y científico a los órganos de la administración de justicia. Estará adscrito al ministerio rector de orden público, protección interna y seguridad ciudadana. Tendrá personalidad jurídica y autonomía administrativa, financiera y de gestión. En materia pre-procesal y procesal penal actuará bajo dirección de la Fiscalía General del Estado*."

Conforme lo establece el "**ESTATUTO ORGÁNICO DE GESTIÓN ORGANIZACIONAL POR PROCESOS DEL SERVICIO NACIONAL DE MEDICINA LEGAL Y CIENCIAS FORENSES**" emitido mediante Resolución No. SNMLCF-DG-2017-09 y publicado en el Registro Oficial Edición Especial 1013 de 03-may.-2017, dentro de la misión de la Coordinación Técnica de Servicios de

Appendix B
Scientific research authorization issued by the *Ministerio del Ambiente* Ecuador



MINISTERIO DEL AMBIENTE

EL GOBIERNO DE TODOS

DIRECCIÓN PROVINCIAL DEL AMBIENTE PICHINCHA

AUTORIZACIÓN DE INVESTIGACIÓN CIENTÍFICA

Nº 015 – 2019 –IC- FAU - DPAP - MA Quito, 07 de noviembre de 2019

El Ministerio del Ambiente, en uso de las atribuciones que le confiere el Código Orgánico Ambiental, autoriza a Dr. Markus Patricio Tellkamp Tietz, Decano encargado de la Escuela de Ciencias Biológicas e Ingeniería de la Universidad Yachay Tech, con C.C. No. 1709756843, y a Sofía Amanda Abad, estudiante, tesista de la Universidad Yachay Tech, con C.I: 1725256687, para que lleven a cabo la investigación titulada **"Insectos asociados con cadáveres y una lista de verificación preliminar de dípteros en un Instituto de Medicina Legal en Quito - Ecuador"**. De acuerdo a las siguientes especificaciones:

Solicitud de autorización de extracción e investigación de: Dr. Markus Patricio Tellkamp, mediante oficio s/n de fecha 17 de octubre 2019.
Valoración técnica del proyecto: Ing. Agrop. Diego Morillo G.
Contraparte del Ministerio del Ambiente: Dirección Provincial del Ambiente Pichincha, Unidad de Patrimonio Natural.
Complementos autorizados de la investigación: captura y colección de especímenes de fauna silvestre de los siguientes componentes: entomofauna (Clase: insecta, Orden: díptera y coleóptera).
Duración: desde 07 de noviembre 2019, hasta 06 de noviembre de 2020, de acuerdo al cronograma de trabajo establecido.

Obligaciones de los investigadores:

- ENTREGAR TODAS LAS COLECCIONES PRODUCTO DE LA INVESTIGACION AL MUSEO QCAZ DE LA PONTIFICIA UNIVERSIDAD CATOLICA DEL ECUADOR – PUCE.
- ENTREGAR UNA COPIA IMPRESA (EN AMBAS CARAS), Y UNA DIGITAL, DE LOS RESULTADOS FINALES DE LA INVESTIGACION, EN CASTELLANO, INCLUYENDO LA LOCALIZACION EXACTA (COORDENADAS UTM) DE LOS ESPECIMENES COLECTADOS Y OBSERVADOS, COPIA DE LAS FOTOGRAFIAS, GRABACIONES Y OTROS DOCUMENTOS PRODUCTO DE LA MISMA.
- EL PLAZO DE ENTREGA DEL INFORME FINAL, VENCE EL 06 DE NOVIEMBRE DE 2020.

Del cumplimiento de las obligaciones dispuestas en el párrafo anterior se responsabiliza a: Markus Patricio Tellkamp y Sofía Amanda Abad

Atentamente,

Ministerio del Ambiente DIRECCIÓN PROVINCIAL PICHINCHA

Abg. Evelin Chaves Montenegro

DIRECTORA PROVINCIAL DEL AMBIENTE PICHINCHA

AV. Amazonas N24-196 Y Luis Cordero *Telf.: 02-3816230*

Appendix C

Six Van Someren-Rydon traps located around the SNMLCF. **A**. Trap 1 was located at 10 meters from the autopsy room (0°10'54.84"S; 78°30'15.14"W). **B.** Trap 2 was located above a water reserve tank (0°10'54.48"S; 78°30'15.00"W). **C.** Trap 3 was located above the garbage storage (0°10'54.29"S; 78°30'14.85"W). **D.** Traps 4 and 5 in the courtyard of the SNMLCF (0°10'53.73"S; 78°30'14.33"W), (0°10'53.88"S; 78°30'13.93"W). **E.** Trap 6 in the roof of the forensic anthropology area

Appendix D

Statistic calculation of rarefraction curve using the diversity statistics option on the EstimateS 8.2.0 software (Colwell, 2006). Plot made with excel.

Samples	Individuals (Computed)	Species Richness (Computed)	95% Confidence interval lower bound	95% Confidence interval upper bound
0	0	0	0	0
1	578.83	16	13.56	18.44
2	1157.67	20.07	18.28	21.86
3	1736.5	21.7	20.42	22.98
4	2315.33	22.47	21.5	23.43
5	2894.17	22.83	22.04	23.62
6	3473	23	22.27	23.73