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Molecular identification of six species of Calliphoridae (Diptera) with forensic interest in Bogotá, Colombia

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ABSTRACT

Taxonomic identification of the species involved in the processes of cadaveric decomposition is a fundamental procedure in forensic entomological analysis. Among the species involved in the processes of decay, those of the Calliphoridae family are particularly important because they come to the body in the early stages of decomposition. The aim of this research is to identify six species of Calliphoridae (Calliphora nigribasis, Calliphora vicina, Compsomyopsis verena, Sarconesiopsis magellanica, Chrysomia albiceps *and* Roraimomusca roraima) *with forensic interest found in Bogotá. For that, sequences of 599 bp from mitochondrial gene COII wereas obtained. The identification was made by analysis of genetic distances under Jukes-Cantor model. The results showed levels of interspecific distances greater than 3.7%, while intraspecifics levels does not exceed 2.3%. The genetic distances obtained were used to construct a phenogram under the Maximum Likelihood model and the topology of that tree agrees with the current taxonomic organization for the family Calliphoridae family.*

Key-Words: Forensic entomology; Barcode; mtDNA; COII.

INTRODUCTION

After obtaining the entomological evidence present in a corpse, taxonomic identification is the first step (Wells & Sperling, 2001; Yusseff, 2006; Saigusa *et al.,* 2009) and is considered the most important procedure performed in entomological forensic analysis (Wells *et al.,* 2001; Zehner *et al.,* 2004). The correct taxonomic identification makes possible to obtain biological and ecological data useful to determine the post-mortem interval (PMI). That is why forensic entomologists must ensure that each specimen found in a crime scene, either larva, pupa or adult, to be properly identified up to the species level.

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Within the entomofauna that participates in the processes of cadaverous putrefaction, the family Calliphoridae is of particular interest because their carrion habits makes it one of the main decomposers groups (Segura *et al.,* 2009). In Colombia, Amat *et al.,* 2008 recorded 12 genera and 29 species of blowflies (not including Mesembrinellidae). Of which at least 13 species represent medico legal importance (Pape *et al.,* 2004; Florez & Wolff, 2009) and the identification by morphological characters has had in recent years significant advances. Unfortunately, in many cases the morphological identification is very difficult or impossible to perform due to lack of experience in a given taxonomical group, or by the high degree of similarity between species of the same genus, or even by loss of morphological characters during procedures of collection, preservation and packaging the entomological samples. In such circumstances, the molecular identification is particularly important because it allows identifying species from fragments of adult individuals, immatures and even empty pupae (Benecke, 1998; Harvey *et al.,* 2003).

This methodological alternative is already being used in several countries from all continents (Cainé *et al.,* 2009; Meiklejohn *et al.,* 2011; Park *et al.,* 2009). In Latin America however, the progress of forensic entomology is slow and although we can highlight the achievements of countries like Brazil or Colombia (Buenaventura *et al.,* 2009; Carvalho & Mello-Patiu, 2008), the fact is that there is a lag generalized. In Colombia for example, only until 2011 Giraldo *et al.* (2011) published the first work that uses mitochondrial genes as potential use for the identification of species with forensic relevance.

Among the major genes used for identifying insects, mitochondrial genes are widely accepted. Of these, the Cytochrome Oxidase I (COI) is by far the most used gene (Hebert *et al.,* 2003; Tuccia *et al.,* 2016). However, authors like Wells *et al.,* (2007) and Whitworth *et al.* (2007) warn about possible errors and even the inability of the COI gene to identify species of forensic interest mainly from the family Calliphoridae. Under these circumstances, the same authors point out the need to evaluate mitochondrial regions differents from COI, like COII or ND5.

Given the importance of molecular studies for identification of forensic interest species and lack of studies of this kind in Colombia, the objective of this work is to determine the molecular variability of a segment of mitochondrial gene COII like an alternative to identify six species of the family Calliphoridae with forensic interest present in Bogotá, Colombia.

MATERIAL AND METHODS

Obtaining specimens

Individuals were obtained by modified trap of Ferreira (1978), baited with 200 g of beef liver and pork in fresh state. The traps were placed in two different locations within the urban area of Bogotá and were checked at intervals of three days during one month. The collected individuals were preserved in 70% alcohol at a temperature of 4°C until extraction process. Taxonomic identification by morphological characters was performed following the work of Amat *et al.* (2008) and Whitworth (2010).

DNA extraction

DNA extraction was performed from a similar protocol to that proposed by Castalanelli *et al.,* 2010, which is based on the alkaline hydrolysis of proteins. The procedure consists of introducing the complete specimen into a micro test tube with 100 mL of an alkaline solution and then heating for a period not exceeding 30 minutes at temperatures between 70°C and 98°C, depending on the size of the specimen. After this process, the alkaline reaction is stopped by the addition of a stabilizer tris-HCL solution. These products were verified by spectrophotometry and used to performing a PCR reaction.

PCR AMPLIFICATION AND SEQUENCING

Oligonucleotides primers (TCTTCCACGATCATGCACTT) and (GAGACCAGTACTTGCTTTCAGTCA) were designed to amplify a region of 656 bp of the mitochondrial gene COII. The PCR reaction was carried out under the following conditions: 1.5 uL of each primer (0.5 uM), 2.4 mL of dNTPs (0.8 mM), 2.1 mL of MgCl2 (3.5 mM), 0.2 mL of Taq polymerase (1U) 3 uL of stabilizer buffer (1x) and 4 uL of DNA adjusted to a final volume of 30 uL. The amplification program had an initial denaturation step at 98°C for three minutes, followed by 40 cycles of 96°C for 30 seconds, 54°C for one minute and 72°C for a minute. A final extension step was added for five minutes at 72°C. The PCR products were evaluated on agarose gels dyed with 1% ethidium bromide and subsequently sequenced with ABI PRISM BigDyeTM Terminator Cycle Sequencing Ready Reaction Kit version 3.1 (Applied Biosystems, Foster City, CA, USA).

TABLE 1: Information of species used in this study.

GenBank access number	Species name	Locality	Reference
AF686030	*Calliphora nigribasis*	Bogotá-Col	Newly sequenced
AF686031	*Calliphora nigribasis*	Bogotá-Col	Newly sequenced
AF686032	*Calliphora nigribasis*	Bogotá-Col	Newly sequenced
AF686033	*Calliphora nigribasis*	Bogotá-Col	Newly sequenced
AF686034	*Calliphora vicina*	Bogotá-Col	Newly sequenced
AF686035	*Calliphora vicina*	Bogotá-Col	Newly sequenced
JX913760.1	*Calliphora vicina*	Austrália	Nelson *et al.* 2012
AF686036	*Compsomyopsis verena*	Bogotá-Col	Newly sequenced
AF686037	*Compsomyopsis verena*	Bogotá-Col	Newly sequenced
AF686038	*Compsomyopsis verena*	Bogotá-Col	Newly sequenced
AF686039	*Compsomyopsis verena*	Bogotá-Col	Newly sequenced
AF295549.1	*Compsomyiopsis callipes*	USA	Wells & Sperling, 2001
AF686040	*Sarconesiopsis magellanica*	Bogotá-Col	Newly sequenced
AF686041	*Sarconesiopsis magellanica*	Bogotá-Col	Newly sequenced
AF686042	*Sarconesiopsis magellanica*	Bogotá-Col	Newly sequenced
AF686043	*Sarconesiopsis magellanica*	Bogotá-Col	Newly sequenced
AF686044	*Chrysomia albiceps*	Bogotá-Col	Newly sequenced
AF686045	*Chrysomia albiceps*	Bogotá-Col	Newly sequenced
JX913736.1	*Chrysomia albiceps*	Austrália	Nelson *et al.* 2012
AF686046	*Roraimomusca roraima*	Bogotá-Col	Newly sequenced
AF686047	*Roraimomusca roraima*	Bogotá-Col	Newly sequenced
JX913757.1	*Lucilia sericata*	Austrália	Nelson *et al.* 2012
AF295555.1	*Cochliomya macellaria*	USA	Wells & Sperling, 2001
DQ345118.1	*Calliphora nibribarbi*	China	Without reference

Sequence analysis

The sequences obtained were compared with those previously reported in the National Center for Biotechnology Information (NCBI) using the BLAST tool. Alignments were performed in Clustal W (Thompson *et al.,* 1994) and the analysis of intra and interspecific variability from Jukes-Cantor model (Jukes & Cantor, 1969) were made in MEGA 4.0 software (Tamura *et al.,* 2007). External sequences were included (Table 1) to construct a graphical representation of the levels of similarity from genetic distances, using the criterion of Maximum Likelihood (ML).

RESULTS

The species *Calliphora nigribasis* Macquart, *Calliphora vicina* Robineau-Desvoidy, *Compsomyopsis verena* (Walker), *Sarconesiopsis magellanica* (Le Guillou), *Chrysomia albiceps* (Wiedemann) and *Roraimomusca roraima* Townsend, are into the principal entomofauna with forensic interest present in Bogotá, Colombia (Camacho, 2005; Segura *et al.,* 2009). For these, the extraction protocol yielded good DNA quality with concentrations between 50 ng/uL to 200 ng/uL. That DNA was used to obtain 18 sequences available for identifying and differentiate these six species based in their genetics distances (Table 1).

TABLE 2: Nucleotide composition in percent

SPECIES	T	C	G	A
Calliphora nigribasis	38.06	14.02	13.52	34.39
Calliphora vicina	37.73	14.19	14.52	33.56
Compsomyopsis verena	38.23	14.86	15.36	31.55
Sarconesiopsis magellanica	40.40	13.19	13.19	33.22
Chrysomia albiceps	38.90	14.02	14.52	32.55
Roraimomusca roraima	37.40	14.86	14.19	33.56

Comparison of the sequences in BLAST tool allowed verify their similarity with previously reported sequences from COII gene in Calliphoridae. The segment has 599 base pairs and is located in the region 3,104-3,702 from complete mitochondrial genome of *Chrysomia albiceps* (JX913736.1). The nucleotide sequence composition present the normal pattern described for mitochondrial genes in insects (Junqueira *et al.,* 2004; Oliveira *et al.,* 2008), where thymine and adenine have over 70% of total base pairs (Table 2).

The distance matrix for COII gene (Table 3) reveals intraspecific variation ranging from 0.1% to 1.8%; while interspecific variation reach values rang-

TABLE 3: Intra and interspecific genetic distances. (Top right: absolute number of nucleotide differences. Bottom left: nucleotide divergence expressed in %). **GB:** represent the sequences obtained from GenBank database.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	*1. C. vicina*	—	0	69	69	69	69	58	60	32	30	30	33	74	72	67	68	67	68	53	53	51	44	27	11
2	*2. C. vicina*	0.0	—	69	69	69	69	58	60	32	30	30	33	74	72	67	68	67	68	53	53	51	44	27	11
3	*1. C. verena*	11.5	11.5	—	0	0	0	53	55	76	76	76	77	37	35	36	36	36	36	22	46	50	61	70	72
4	*2. C. verena*	11.5	11.5	0.0	—	0	0	53	55	76	76	76	77	37	35	36	36	36	36	22	46	50	61	70	72
5	*3. C. verena*	11.5	11.5	0.0	0.0	—	0	53	55	76	76	76	77	37	35	36	36	36	36	22	46	50	61	70	72
6	*4. C. verena*	11.5	11.5	0.0	0.0	0.0	—	53	55	76	76	76	77	37	35	36	36	36	36	22	46	50	61	70	72
7	*1. C. albiceps*	9.7	9.7	8.8	8.8	8.8	8.8	—	2	62	60	60	63	64	62	58	58	58	58	41	42	12	55	53	57
8	*2. C. albiceps*	10.0	10.0	9.1	9.1	9.1	9.1	0.3	—	64	62	62	65	61	64	60	60	60	60	43	44	14	57	55	59
9	*1. C. nigribasis*	5.3	5.3	12.6	12.6	12.6	12.6	10.3	10.6	—	4	4	4	85	83	79	80	79	80	61	57	57	52	25	35
10	*2. C. nigribasis*	5.0	5.0	12.6	12.6	12.6	12.6	10.0	10.3	0.6	—	0	8	83	81	77	78	77	78	61	55	55	52	23	35
11	*3. C. nigribasis*	5.0	5.0	12.6	12.6	12.6	12.6	10.0	10.3	0.6	0.0	—	8	83	81	77	78	77	78	61	55	55	52	23	35
12	*4. C. nigribasis*	5.5	5.5	12.8	12.8	12.8	12.8	10.5	10.8	0.6	1.3	1.3	—	86	84	80	81	80	81	62	58	58	53	26	36
13	*1. R. roraima*	12.3	12.3	6.1	6.1	6.1	6.1	10.6	11.2	14.1	13.8	13.8	14.3	—	2	25	25	25	25	37	57	63	69	71	77
14	*2. R. roraima*	12.0	12.0	5.8	5.8	5.8	5.8	10.3	10.6	13.8	13.5	13.5	14.0	0.3	—	23	23	23	23	35	55	61	67	71	77
15	*1. S. magellanica*	11.1	11.1	6.0	6.0	6.0	6.0	9.6	10.0	13.1	12.8	12.8	13.3	4.2	3.8	—	6	0	6	26	48	54	59	66	69
16	*2. S. magellanica*	11.3	11.3	6.0	6.0	6.0	6.0	9.6	10.0	13.3	13.0	13.0	13.5	4.2	3.8	1.0	—	6	0	26	48	54	60	67	70
17	*3. S. magellanica*	11.1	11.1	6.0	6.0	6.0	6.0	9.6	10.0	13.1	12.8	12.8	13.3	4.2	3.8	0.0	1.1	—	6	26	48	54	59	66	69
18	*4. S. magellanica*	11.3	11.3	6.0	6.0	6.0	6.0	9.6	10.0	13.3	13.0	13.0	13.5	4.2	3.8	1.0	1.1	0.0	—	26	48	54	60	67	70
19	*C. callipes(GB)*	8.8	8.8	3.7	3.7	3.7	3.7	6.8	7.1	10.1	10.1	10.1	10.3	6.1	5.8	4.3	4.3	4.3	4.3	—	26	35	42	50	52
20	*C. macellaria (GB)*	8.8	8.8	7.6	7.6	7.6	7.6	7.0	7.3	9.5	9.1	9.1	9.6	9.5	9.2	8.0	8.0	8.0	8.0	4.3	—	33	48	50	54
21	*C. albiceps(GB)*	8.5	8.5	8.3	8.3	8.3	8.3	2.0	2.3	9.5	9.1	9.1	9.6	10.5	10.2	9.0	9.0	9.0	9.0	5.8	5.5	—	48	46	50
22	*L. sericata (GB)*	7.3	7.3	10.1	10.1	10.1	10.1	9.1	9.5	8.6	8.6	8.6	8.8	11.5	11.2	9.8	10.0	9.8	10.0	7.0	8.0	8.0	—	41	41
23	*C. nigribarbi (GB)*	4.5	4.5	11.6	11.6	11.6	11.6	8.8	9.1	4.1	3.8	3.8	4.3	11.8	11.9	11.0	11.1	11.0	11.1	8.3	8.3	7.6	6.8	—	24
24	*C. vicina (GB)*	1.8	1.8	12.0	12.0	12.0	12.0	9.5	9.8	5.8	5.8	5.8	6.0	12.8	12.9	11.5	11.6	11.5	11.6	8.6	9.0	8.3	6.8	4.0	—

ing from 3.7% to 14.3%. From the fragments sequenced in this study, the higher intraspecific variability occurs in the sequences of *C. nigribasis* with 1.3%, representing a variation of eight nucleotides between 599 obtained. Unlike these, sequences obtained for *C. verena* and *C. vicina* present no nucleotide change. *Chrysomia albiceps* and *R. roraima* presents changes in two nucleotides, and *S. magellanica* have variability in six nucleotides.

Regarding interspecific relationships, the highest percentages of variation are present for the comparision of all species againts *R. roraima,* being the largest difference is between *R. roraima* and *C. nigribasis* with 14.3%. Conversely, the lower values occur between *C. vicina* and *C. nigribasis* with maximum percentages 5.3%, which means changes in 33 of the 599 nucleotides sequenced. The comparison of the sequences obtained with those reported in others study for the same gene, show intraspecific differences of 1.8% between the *C. vicina,* and 2.0% between the *C. albiceps.* Intraspecific variation between *C. verena* and *C. callipes* is 3.7% and between *C. nigribasis* and *C. nigribarbis* is 3.8% (Table 3).

The topological representation of genetic distance shows taxonomic relationships of the species compared in this study (Fig. 1). In a level of genus, *Compsomyiops* includes species *C. callipes* and *C. verena* in one group; *Calliphora* grouped species *C. vicina, C. nibribasis* and *C. nigribarbi,* while *Chrysomya* contains all three individuals of *C. albiceps. Roraimomusca, Sarconesiopsis* and *Cochliomya* are located in separated taxonomic groups.

DISCUSSION

In addition to the phylogenetic reconstruction, genetic distance analyses have become an important

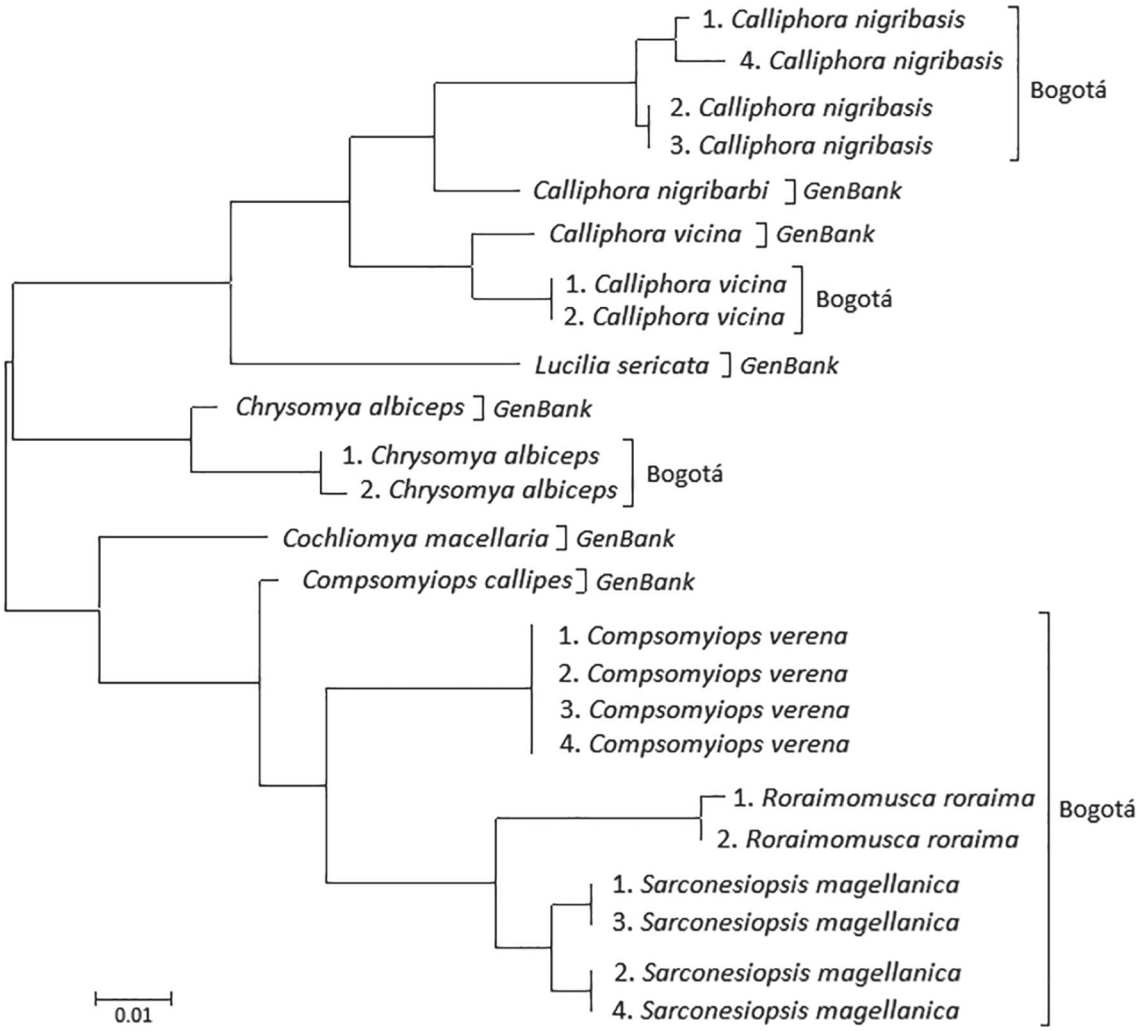

FIGURE 1: Phenogram distances created under the maximum likelihood model (ML).

mechanism for taxonomic identification when using molecular data (Desmyter & Gosselin, 2009). The Barcode initiative (Hebert *et al.,* 2003) focused the attention to use a segment of COI gene; however, in some cases – not only in forensic entomology –, this gene has shown some problems to identify species (Elias *et al.,* 2007; Wells & Stevens, 2008). These difficulties have made it necessary to use alternative genes in the procedures of identification and phylogeny. In the case of forensic entomology, the COII, ND1, ND5 and CYTB genes are being used (Alessandrini *et al.,* 2008; Wells & Stevens, 2008; Zehner *et al.,* 2004).

In this research, the use of mitochondrial gene COII fragment allowed taxonomically identification of six species from the family Calliphoridae forensic interest present in Bogotá. This fragment of mitochondrial genome can be used for identify not just adult stages but also larvae or pupae stages as proposed by Boehme *et al.,* 2010.

Hebert *et al.,* 2003 and Meiklejohn *et al.,* 2011 indicates that rates of genetic diversity of 3% are sufficient for identifying species using the COI gene, however Boehme *et al.,* 2012 and Nelson *et al.,* 2007 discovered lower interspecific variation than 3% for segments COI in species of *Lucilla* and *Chrysomya.* In this research, the values of diversity in gene COII are in all cases higher than 3% and reach peak levels of 14.3% with 86 nucleotide changes. These results could indicate the importance of COII gene for differentiating species of forensic interest that are closely related and which are difficult to identify using COI.

Intraspecific differences shown in Table 3, never exceed interspecific values. For *C. albiceps,* the comparison between the newly obtained and the previously sequenced GenBank shows the highest values of the entire table (2.3%). This variability may be indicative of the presence of different haplotypes within the same species that respond to the geographical distance between individuals of whom are from the sequences (Colombia and Austrália).

CONCLUSSION

Six species of the family Calliphoridae with forensic interest present in Bogotá were identified from a 599 bp fragment of the mitochondrial gene COII. For all the six species analyzed the percentages of intraspecific and interspecific variability show that this fragment of COII gene can be used as alternative for identifying species of Calliphoridae.

RESUMEN

La identificación taxonómica de las especies envueltas en los procesos de descomposición cadavérica es una etapa fundamental en los análisis de la entomología forense. Dentro de las especies que participan en los procesos de descomposición, aquellos que pertenecen a la familia Calliphoridae son de particular importancia por ser colonizadores durante los primeros estados de descomposición. El objetivo de este estudio es identificar seis especies de Calliphoridae (Calliphora nigribasis, Calliphora vicina, Compsomyopsis verena, Sarconesiopsis magellanica, Chrysomia albiceps *y* Roraimomusca roraima) *con interés forense presentes en Bogotá, Colombia. Por tanto fueron obtenidas secuencias de 599pb del gen mitocondrial COII. La identificación fue realizada a través del análisis de distancias genéticas con el modelo* Jukes-Cantor. *Los resultados muestran niveles de distancias interespecificas mayores de 3,7%, mientras que las intraespecificas no exceden 2,3%. Las distancias genéticas obtenidas fueron utilizadas para construir un fenograma con el modelo de* Maximum Likelihood *y la topología del árbol concuerda con la organización taxonómica aceptada para la familia Calliphoridae.*

Palabras-Clave: Entomología forense; Barcode; mtDNA; COII.

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REFERENCES

Alessandrini, F.; Mazzanti, M.; Onofri, V.; Turchi, C. & Tagliabracci, A. 2008. MtDNA analysis for genetic identification of forensically important insects. *Forensic Science International: Genetics, Supplement Series,* 1:584-585.

Amat, E.; Velez, M. & Wolff, M. 2008. Clave ilustrada para la identificación de los géneros y las especies de califóridos (Diptera: Calliphoridae) de Colombia. *Caldasia,* 30(1):231-244.

Benecke, M. 1998. Six forensic entomology cases: description and commentary. *Journal of Forensic Sciences,* 43:797-805; 43:1303.

Boehme, P.; Amendt, J. & Zehner, R. 2012. The use of COI barcodes for molecular identification of forensically important fly species in Germany. *Parasitology Research,* 110:2325-2332.

Boehme, P.; Amendt, J.; Disney, H. & Zehner, R. 2010. Molecular identification of carrion-breeding scuttle flies (Diptera: Phoridae) using COI barcodes. *International Journal of Legal Medicine,* 124(6):577-581.

Buenaventura, E.; Camacho, G.; García, A. & Wolff, M. 2009. Sarcophagidae (Diptera) de importancia forense en Colombia: claves taxonómicas, notas sobre su biología y distribución. *Revista Colombiana de Entomología,* 35(2):189-196.

Cainé, L.; Corte, F.; Saloña-Bordas, M.; Pancorbo, M.; Lima, G.; Magalhaes, T. & Pinheiro, F. 2009. DNA typing of Diptera collected from human corpses in Portugal. *Forensic Science International,* 184:21-23.

Camacho, G. 2005. Sucesion de la entomofauna cadaverica y ciclo vital de Calliphora vicina (Diptera: Calliphoridae) como primera especie colonizadora, utilizando cerdo blanco *(Sus scrofa)* en Bogotá. *Revista Colombiana de Entomología,* 31(2):189-197.

Carvalho, C. & Mello-Patiu, C. 2008. Key to the adults of the most common forensic species of Diptera in South America. *Revista Brasileira de Entomologia,* 52(3):390-406.

Castalanelli, M.; Severtson, D.; Brumley, C.; Szito, A.; Foottit, R.; Grimm, M.; Munyard, K. & Groth, D. 2010. A rapid non-destructive DNA extraction method for insects and other arthropods. *Journal of Asia-Pacific Entomology,* 13:243-248.

Desmyter, S. & Gosselin, M. 2009. COI sequence variability between Chrysomyinae of forensic interest. *Forensic Science International: Genetics,* 3(2):89-95.

Elias, M.; Hill, R.; Willmott, K.; Dasmahapatra, K.; Brower, A.; Mallet, J. & Jiggins, C. 2007. Limited performance of DNA barcoding in a diverse community of tropical butterflies. *Proceedings of the Royal Society of London B,* 274:2881-2889.

Ferreira, M.J.M. 1978. Sinantropia de dípteros muscóideos em Curitiba, Paraná. I. Calliphoridae. *Revista Brasileira de Biologia,* 38:445-454.

Florez, E. & Wolff, M. 2009. Descripción y clave de los estadios inmaduros de las principales especies de Calliphoridae (Diptera) de importancia forense en Colombia. *Neotropical Entomology,* 38(3):418-429.

Giraldo, P.; Uribe, S. & López, A. 2011. Análisis de secuencias de ADN mitocondrial (Cytb y ND1) en *Lucilia eximia* (Diptera: Calliphoridae). *Revista Colombiana de Entomología,* 37(2):273-278.

Harvey, M.; Dadour, I. & Gaudieri, S. 2003. Mitochondrial DNA Citochrome oxidase I gene: potential for distinction between immature stages of some forensically important fly species (Diptera) in western Austrália. *Forensic Science International,* 131:134-139.

Hebert, P.D.N.; Cywinska, A.; Ball, S.L. & de Waard, J.R. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London B,* 270:313-321.

Jukes, T.H. & Cantor, C.R. 1969. *Evolution of protein molecules.* (Munro H N, eel.) Mammalian protein metabolism. III. New York, Academic Press. p. 21-132.

Junqueira, A.; Lessinger, A.; Torres, T.; Rodrigues da Silva, F.; Vettore, A.; Arruda, P. & Azeredo, A. 2004. The mitochondrial genome of the blowfly Chrysomya chloropyga (Diptera: Calliphoridae). *Gene,* 339:7-15.

Meiklejohn, K.; Wallman, J. & Dowton, M. 2011. DNA-based identification of forensically important Australian Sarcophagidae (Diptera). *International Journal of Legal Medicine,* 121(1):27-32.

Nelson, L.; Wallmann, J. & Dowton, M. 2007. Using COI barcodes to identify forensically and medically important blowflies. *Medical and Veterinary Entomology,* 21:44-52.

Nelson, L.A.; Lambkin, C.L.; Batterham, P.; Wallman, J.F.; Dowton, M.; Whiting, M.F.; Yeates, D.K. & Cameron, S.L. 2012. Beyond barcoding: A mitochondrial genomics approach to molecular phylogenetics and diagnostics of blowflies (Diptera: Calliphoridae). *Gene,* 511(2):131-142.

Oliveira, M.; Barau, J.; Martins, A.; Feijão, P.; Coelho da Rosa, A.; Abreu, C.; Azeredo-Spin, A. & Lessinger, A. 2008. Structure and evolution of the mitochondrial genomes of *Haematobia irritans* and *Stomoxys calcitrans:* The Muscidae (Diptera: Calyptratae) perspective. *Molecular Phylogenetics and Evolution,* 48:850-857.

Pape, T.; Wolff, M. & Amat, E. 2004. Los califóridos, éstridos, rinofóridos y sarcofágidos (Diptera: Calliphoridae, Oestridae, Rhinophoridae, Sarcophagidae) de Colombia. *Biota Colombiana,* 5(2):201-208.

Park, S.; Zhang, Y.; Piao, H.; Ha Yu, D.; Jeong, H.; Young Yoo, G.; Tae-Ho, J.O. & Hwang, J. 2009. Sequences of the Cytochrome C Oxidase Subunit I (COI) gene are suitable for species identification of Korean Calliphorinae flies of forensic importance (Diptera: Calliphoridae). *Journal of Forensic Sciences,* 54(5):1131-1134.

Saigusa, K.; Matsumasa, M.; Yashima, Y.; Takamiya, M. & Aoki, Y. 2009. Practical applications of molecular biological species identification of forensically important flies. *Legal Medicine,* 11(Supplement 1):344-347.

Segura, N.; Usaquen, W.; Sanchez, M.; Chuaire, L. & Bello, F. 2009. Succession pattern of cadaverous entomofauna in a semi-rural area of Bogotá, Colombia. *Forensic Science international,* 187:66-72.

Tamura, K.; Dudley, J.; Nei, M. & Kumar, S. 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution,* 24:1596-1599.

Thompson, J.; Higgins, D. & Gibson, T. 1994. Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research,* 22:4673-4680.

Tuccia, F.; Giordani, G. & Vanin, S. 2016. A general review of the most commom COI primers for Calliphoridae identification in forensic entomology. *Forensic Science International: Genetics,* 24:e9-e11.

Wells, J. & Sperling, F. 2001. DNA-based identification of forensically important Chrisomyinae (Diptera: Calliphoridae). *Forensic Science International,* 120:110-115.

Wells, J. & Stevens, J. 2008. Application of DNA-Based Methods in Forensic Entomology. *Annual Review of Entomology,* 53:103-20.

Wells, J.; Pape, T. & Sperling, F. 2001. DNA-Based Identification and Molecular Systematics of Forensically Important Sarcophagidae (Diptera). *Journal of Forensic Science,* 46(5):1098-1102.

Wells, J.; Wall, R. & Stevens, J.R. 2007. Phylogenetic analysis of forensically important Lucilia flies based on cytochrome oxidase I sequence: a cautionary tale for forensic species determination. *International Journal of Legal Medicine,* 121:229-233.

Whitworth, T. 2010. Keys to the genera and species of blow flies (Diptera: Calliphoridae) of the West Indies and description of a new species of Lucilia Robineau-Desvoidy. *Zootaxa,* 2663:1-35.

Whitworth, T.; Dawson, R.; Magalon, H. & Baudry, E. 2007. DNA barcoding cannot reliably identify species of the blowfly genus Protocalliphora (Diptera: Calliphoridae). *Proceedings of the Royal Society of London B,* 274:1731-1739.

Yusseff, S. 2006. Entomología forense: los insectos en la escena del crimen. *Revista Luna Azul,* 23:1-10.

Zehner, R.; Amendt, J. & Shutt, S. 2004. Genetic identification of forensically important flesh flies (Diptera: Sarcophagidae). *Journal of Legal Medicine,* 118:245-247.

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