

Article

DNA Barcoding of Flesh Flies (Diptera: Sarcophagidae) in Jamaica: A Preliminary Assessment

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Abstract

The Caribbean is a recognized biodiversity hotspot; however, on islands such as Jamaica, many faunal groups of ecological and forensic importance, including flesh flies, remain poorly characterized, particularly at the genetic level. This study represents an initial step towards addressing that gap through DNA barcoding. Forty-four specimens from 16 species were analyzed through partial sequencing of the cytochrome c oxidase subunit I (COI) gene. Of the 44 sequences, 30% shared 97–100% similarity with reference sequences in GenBank and BOLD; however, taxonomic resolution varied between databases. The remaining sequences gave lower similarity matches (88–96%), with assignments restricted to the genus level or higher taxonomic ranks, or were discordant with morphology-based identifications. With one exception, pairwise intraspecific genetic distances were low (0.17%), whereas interspecific distances ranged from 5.68% to 12.83%. Neighbor-joining analysis showed that Jamaican taxa, including five endemic species and two new records, formed largely distinct monophyletic clades relative to sequences from North and South America, Europe and Oceania, with >70% bootstrap support. These findings demonstrate the utility of COI barcoding for species discrimination among Jamaican flesh flies and represent an important step towards establishing a regional DNA barcode library. Expanding Caribbean representation in global databases will be essential for improving the accuracy of molecular identification and resolving uncharacterized taxa.

Keywords: Caribbean; COI; DNA barcoding; integrative taxonomy; island

1. Introduction

The Sarcophagidae, or flesh flies, represent a highly diverse family comprising over 3000 species [1,2], divided into three subfamilies: Miltogramminae, Paramacronychiinae, and Sarcophaginae [3]. Of these, Sarcophaginae is the most species-rich, with approximately 2000 species [4]. Most species are found in the Neotropics, while only a few occur in subarctic regions [5]. Sarcophaginae inhabit nearly all terrestrial ecosystems and exhibit a wide range of feeding habits [1], particularly during the larval stages, which include feeding on live and dead vertebrates, invertebrates, and organic substrates such as feces [6–9]. In the Neotropics, necrophagous species play an important role in nutrient cycling as primary decomposers and are of ecological, medical, and forensic relevance [10,11]. However, their potential contribution to forensic investigations is limited by extreme morphological similarity between species [12].



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Adult flesh flies exhibit relatively uniform external morphology across species. They are generally medium to large in size (8–25 mm), although smaller species (3–8 mm) also occur. They are typically gray, with gray-black longitudinal stripes on the thorax, a checkerboard abdomen, and a strongly bristled body [3]. Species-level identification primarily relies on the morphology of male terminalia; females are difficult to identify, and immature stages are often unknown or lack species-specific diagnostic characters [13]. Increasingly, molecular markers are being used to complement morphological identification and provide species-level designations regardless of the insect's life stage or condition. They have become essential tools in biodiversity studies, identification, monitoring, and description. Various studies, for example, have identified forensically important flesh flies using nuclear internal transcribed spacer (ITS) regions and 28S rRNA as well as the mitochondrial genes NADH dehydrogenase subunit 5 and cytochrome c oxidase subunits I (COI) and II (COII) [12]. COI has proven particularly effective because of its maternal inheritance, high mutation rate, high copy number, and the absence of recombination and introns [14–17]. Moreover, the standard ~658 bp COI barcode region in *Sarcophaga* achieved species-level identification at rates of 98.2–99.3% [18]. Similar levels of identification have been reported in early COI DNA barcoding studies of Lepidoptera, where interspecific divergence values exceeding 3% corresponded with morphology-based identification [19]. COI barcoding is presently a widely adopted tool for Sarcophagidae identification in Europe, Asia, and North and South America [4,19,20].

However, the Sarcophagidae fauna of the Caribbean remains relatively understudied when compared to mainland South America. Islands such as Antigua and Barbados in the Lesser Antilles, the Cayman Islands in the western Caribbean, and Curaçao in the southern Caribbean off the coast of Venezuela have reported one species [11]. In contrast, Cuba in the Greater Antilles has recorded as many as 55 species, and the Bahamas in the Lucayan Archipelago recorded 43 species [11]. In Jamaica, also part of the Greater Antilles, Dodge [21] documented 21 genera and 39 species, including 15 endemic taxa. This checklist was later updated by Foote-Gordon et al. [11], increasing the total to 45 species. Although morphological keys for Neotropical species are well developed [22], both their application to geographically isolated island populations and validation using molecular approaches is limited. This study represents an initial effort to bridge the gap between morphological taxonomy and DNA barcoding for Sarcophagidae in Jamaica. Specifically, the study aims to (1) develop a preliminary COI reference dataset for Jamaican Sarcophagidae by linking DNA barcode sequences to morphologically identified specimens and (2) evaluate the concordance of species identification generated by GenBank and BOLD.

2. Materials and Methods

2.1. Sample Collection

Adult flies were collected from 17 localities across Jamaica between 2018 and 2024 using van Someren-Rydon (VSR) traps baited with decomposing pork or chicken [11]. Each specimen was assigned a unique identifier comprising the letters LF or ZG followed by a four-digit number. A total of 50 adult flies were selected for analysis. Morphological identification was performed using diagnostic keys listed in Foote Gordon et al. [11]. Specimens for molecular analysis were selected from individuals remaining after representative voucher specimens were deposited in museum collections [11] and were preserved in 95% alcohol for subsequent DNA extraction.

2.2. Sample Preparation and DNA Extraction

Three legs were removed from each fly and rinsed with ultra-pure water (MilliQ), followed by two washes in absolute ethanol to eliminate surface contaminants. The remaining

body parts were retained as voucher specimens and deposited in the Department of Life Sciences collection. Legs were then air-dried, transferred to sterile 1.5 mL tubes, and genomic DNA extracted using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. A negative control containing only reagents was included to monitor potential contamination during the extraction process. DNA concentration and purity were assessed using a NanoDrop ND-One spectrophotometer (Thermo Fisher Scientific™, Wilmington, NC, USA), and the extracts were stored at -20°C until further analysis.

2.3. PCR Amplification, Purification and Sequencing

The COI fragment was amplified using the universal primers LCO1490 and HCO2198 [23]. Each 20 μL PCR reaction contained 2 μL of DNA template, 7 μL of $1\times$ Biotechrabbit™ Lyo Hot Start PCR Master Mix, 8 μL of MilliQ water, 1 μL of $1\times$ BSA, and 0.5 μM in 1 μL of each primer (10 μM). The thermal cycling protocol started with denaturation at 95°C for 2 min, followed by 30 cycles of 95°C for 30 s, 50°C for 30 s, and 72°C for 1 min, and ended with a final extension at 72°C for 7 min. PCR amplification was verified by electrophoresis on 2% agarose gels in $0.5\times$ Tris-borate-EDTA (TBE) buffer. Following purification using the QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany), PCR products were submitted for bidirectional Sanger sequencing at the Centre for PanorOmic Sciences (CPOS), The University of Hong Kong.

2.4. Sequence Analysis for Species Identification

COI sequences were assembled, and primer sequences were trimmed in Geneious Prime® (2023 v.2.1). Sequence alignment was performed with ClustalW in MEGA 12. Nucleotide diversity, GC content, and the number of conserved, variable, and parsimony-informative sites were calculated using DnaSP v.5.10.01 [24]. Species identification was validated by sequence searches against the GenBank (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>, accessed on 23 April 2025) and BOLD database (<https://id.boldsystems.org>, accessed on 23 April 2025). A threshold of $\geq 97\%$ similarity ($\leq 3\%$ genetic distance) was used for species assignment [19]. Maximum intraspecific and minimum interspecific genetic distances were calculated using the Kimura two-parameter (K2P) distance model within the BOLD platform and available COI sequences from all species within the targeted genera and family. A barcode gap was considered when the maximum intraspecific distance was lower than the minimum interspecific distance. A neighbor-joining (NJ) tree was constructed in MEGA 12 using the K2P model with 1000 bootstrap replicates [25].

3. Results

3.1. Morphological Identification

Male sarcophagid flies used in this study were previously identified by Foote-Gordon et al. [11] using taxonomic keys (Table 1) [3,11,21]. The 42 males represented 18 species across 11 genera. Eight female flies collected concurrently could not be reliably identified morphologically.

3.2. Similarity-Based Identification

Amplification products and COI sequences were obtained with 44 of the 50 specimens (36 males and 8 females). Sequences ranged from 556 to 629 bp; most sequences were 585 bp in length. As expected for mitochondrial genes [26], the nucleotide composition was highly biased [26], with an average AT content of 66.2%. Among the aligned sequences, 432 sites were conserved, and 193 were variable, including 11 singleton variable sites and 182 parsimony-informative sites. All sequences have been submitted to GenBank under accession numbers PZ059504, PZ269060, PZ093051–PZ093092. Amplification was

not successful for six male specimens which were previously morphologically identified as *Oxysarcodexia bakeri* (Lopes, 1945), *Ravinia effrenata* (Walker, 1861), *Sarcophagrtiopsis farri* (Dodge, 1965), *Bahamiola orbitalis* (Dodge, 1965), *Blaesoxipha jamacoorum* (Dodge, 1965) and *Sarcodexiopsis welchi* (Hall, 1930) possibly due to PCR inhibition or DNA quality.

Sequence-based identifications obtained from searches against GenBank and BOLD for male and female specimens are listed in Tables 1 and 2, respectively. Of the 44 sequences analyzed, 13 (30%) showed 97–100% similarity to reference sequences. However, in some instances, the same sequences also returned equally high similarity to species that were inconsistent with the morphology-based identifications. The remaining sequences returned database assignments with lower similarity matches (88–96%).

Among male specimens, for example, three COI sequences (ZG2007, ZG2034, and ZG2043) from specimens morphologically identified as *Peckia* (*Sarcodexia*) *lambens* (Wiedemann, 1830) shared 100% similarity with *Sarcodexia lambens* sequences from Brazil and Ecuador in GenBank (Table 1). The same sequences also shared 100% similarity with *Oxysarcodexia xanthosoma* (Aldrich, 1916) sequences from Brazil and Ecuador, suggesting ambiguity in species-level assignments. In BOLD, these sequences returned only family-level matches (Sarcophagidae) from the USA and Trinidad and Tobago, the twin island state located in the Lesser Antilles, off the coast of Venezuela (Table 1). Similarly, one male specimen (LF1027) morphologically identified as *Helicobia morionella* (Aldrich, 1930) shared 100% similarity with sequences identified only to family level in both BOLD and GenBank. Four specimens morphologically identified as *Oxysarcodexia peltata* (Aldrich, 1916) showed 99% similarity to *Oxysarcodexia* sp. from Costa Rica and 97% similarity to Sarcophaginae or Sarcophagidae sequences from Ecuador.

Table 1. Morphological identification and sequence match percentages (GenBank and BOLD) for male Jamaican Sarcophagidae specimens.

Sample ID	GenBank Accession Number	Morphological Identification	Species Name in GenBank (Accession Number)	Species Name in BOLD (Accession Number)	GenBank Similarity (%)	BOLD Similarity (%)
LF001	PZ093051	<i>Peckia nicasia</i>	<i>Peckia abrupta</i> MW200984; Ecuador	Sarcophagidae sp. BOLD: AAA2280; Costa Rica	94.60	95
LF1018	PZ093052	<i>Peckia nicasia</i>	<i>Peckia abrupta</i> MW200984; Ecuador	Sarcophagidae sp. BOLD: AAA2280; Costa Rica	95	95
LF1019	PZ269060	<i>Tapacura mariarum</i>	<i>Sarcophaga langi</i> KU746601; Burundi	Sarcophagidae sp. BOLD: AFD5075; Suriname	91.82	94.23
LF1027	PZ093053	<i>Helicobia morionella</i>	Sarcophagidae sp. JF872339; USA	Sarcophagidae sp. BOLD: AAP2703; USA,	100	100
LF1036	PZ093054	<i>Oxysarcodexia corolla</i>	<i>Oxysarcodexia ventricosa</i> KJ208554; Canada	No result	93.37	No result
LF1041	PZ093055	<i>Peckia nicasia</i>	<i>Peckia abrupta</i> MW200984, Ecuador	Sarcophagidae sp. BOLD: AAA2280; Costa Rica	95	94.99
LF1044	PZ093056	<i>Sarcophagrtiopsis farri</i>	<i>Sarcophagrtiopsis</i> sp. MG967985; Costa Rica	Sarcophagidae sp. BOLD: AAP4729; USA	94.75	100

Table 1. Cont.

Sample ID	GenBank Accession Number	Morphological Identification	Species Name in GenBank (Accession Number)	Species Name in BOLD (Accession Number)	GenBank Similarity (%)	BOLD Similarity (%)
LF1048	PZ093057	<i>Peckia nicasia</i>	<i>Peckia abrupta</i> MW200984; Ecuador	Sarcophagidae sp. BOLD: AAA2280; Costa Rica	94.60	94.94
LF1068	PZ093058	<i>Titanogrypa fimbriata</i>	<i>Sarcophaga okaliana</i> JQ582026; Western Europe	Sarcophagidae sp. BOLD: AEQ2626; Mexico	88.62	94.54
LF1068(2)	PZ093059	<i>Titanogrypa fimbriata</i>	<i>Peckia aequata</i> MW200972; Ecuador	Sarcophagidae sp. BOLD: AEQ2626; Mexico	88.71	94.42
LF1069	PZ093060	<i>Boettcheria parkeri</i>	<i>Boettcheria</i> sp. KM945900; Canada	No result	92.85	No result
ZG2000	PZ093061	<i>Boettcheria parkeri</i>	<i>Boettcheria</i> sp. KM945900; Canada	No result	92.85	No result
ZG2001	PZ093062	<i>Bahamiola orbitalis</i>	<i>Sarcophaga vicina</i> KU746569, JQ582038; Croatia	No result	92.03	No result
ZG2002	PZ093063	<i>Oxysarcodexia amilleae</i>	<i>Oxysarcodexia parva</i> MK055777; Brazil	Sarcophagidae sp. BOLD: ABW8000; Argentina	94.74	94.90
ZG2003	PZ093064	<i>Oxysarcodexia corolla</i>	<i>Oxysarcodexia ventricosa</i> KJ208554; Canada	<i>Oxysarcodexia</i> sp. BOLD: ACP4874; Costa Rica	93.37	94.54
ZG2005	PZ093066	<i>Bahamiola orbitalis</i>	<i>Sarcophaga vicina</i> KU746569, JQ582038; Croatia	No result	92.03	No result
ZG2007	PZ093068	<i>Peckia lambens</i>	<i>Sarcodexia lambens</i> MW200981; Ecuador <i>Oxysarcodexia xanthosoma</i> KU315816; Brazil	Sarcophagidae sp. BOLD: AAA1909; Trinidad and Tobago	100	100
ZG2008	PZ093069	<i>Oxysarcodexia peltata</i>	Sarcophaginae sp. MW200654; Ecuador	<i>Oxysarcodexia</i> sp. BOLD: AGO1597; Costa Rica	97.13	99.36
ZG2009	PZ093070	<i>Peckia chrysostoma</i>	<i>Peckia chrysostoma</i> AF259515, KX054328; French Polynesia	<i>Peckia chrysostoma</i> BOLD: AAL3638; French Polynesia	96.10	96.23
ZG2010	PZ093071	<i>Oxysarcodexia peltata</i>	Sarcophaginae sp. MW200654; Ecuador	<i>Oxysarcodexia</i> sp. BOLD: AGO1597; Costa Rica	97.13	99.36
ZG2012	PZ093073	<i>Sarcophaghiopsis farri</i>	<i>Sarcophaghiopsis</i> sp. MG967985; Costa Rica	Sarcophagidae sp. BOLD: AAP4729; USA	94.75	100
ZG2015	PZ059504	<i>Oxysarcodexia peltata</i>	Sarcophagidae sp. MW200654; Ecuador	<i>Oxysarcodexia</i> sp. BOLD: AGO1597; Costa Rica	97.13	99.35

Table 1. Cont.

Sample ID	GenBank Accession Number	Morphological Identification	Species Name in GenBank (Accession Number)	Species Name in BOLD (Accession Number)	GenBank Similarity (%)	BOLD Similarity (%)
ZG2019	PZ093078	<i>Oxysarcodexia bakeri</i>	Sarcophagidae sp. JF871267; USA	Sarcophagidae sp. BOLD: AEZ5362; Mexico	92	99.02
ZG2020	PZ093079	<i>Oxysarcodexia bakeri</i>	Sarcophagidae sp. JF871267; USA	Sarcophagidae sp. BOLD: AEZ5362; Mexico	92	99.02
ZG2022	PZ093080	<i>Peckia chrysostoma</i>	<i>Peckia chrysostoma</i> AF259515, KX054328; French Polynesia	<i>Peckia chrysostoma</i> BOLD: AAL3638; French Polynesia	96.10	96.40
ZG2024	PZ093081	<i>Peckia nicasia</i>	<i>Peckia abrupta</i> MW200984; Ecuador	Sarcophagidae sp. BOLD: AAA2280; Costa Rica	94	94.99
ZG2026	PZ093082	<i>Peckia dominicana</i>	<i>Tricharaea</i> sp. OM557520; Argentina	<i>Peckia</i> sp. BOLD: ACY5042; Argentina	94.44	94.28
ZG2027	PZ093083	<i>Bahamiola orbitalis</i>	<i>Sarcophaga vicina</i> KU746569, JQ582038; Croatia	No result	92.03	No result
ZG2030	PZ093084	<i>Oxysarcodexia peltata</i>	Sarcophagidae sp. MW200654; Ecuador	<i>Oxysarcodexia</i> sp. BOLD: AGO1597; Costa Rica	97.10	99.35
ZG2031	PZ093085	<i>Blaesoxipha jamacoorum</i>	<i>Blaesoxipha eleodis</i> JQ806811; USA	No result	92.11	No result
ZG2034	PZ093086	<i>Peckia lambens</i>	<i>Sarcodexia lambens</i> MW200981; Ecuador <i>Oxysarcodexia xanthosoma</i> KU315816; Brazil	Sarcophagidae sp. BOLD: AAA1909; Trinidad and Tobago	100	100
ZG2035	PZ093087	<i>Oxysarcodexia amilleae</i>	Sarcophagidae sp. MG293037; Canada	Sarcophagidae sp. BOLD: ABW8000; Argentina	94.41	95.75
ZG2036	PZ093088	<i>Oxysarcodexia amilleae</i>	<i>Oxysarcodexia angrensis</i> MW200603; Ecuador	Sarcophagidae sp. BOLD: ABW8000; Argentina	94.47	94.96
ZG2039	PZ093089	<i>Oxysarcodexia amilleae</i>	<i>Oxysarcodexia angrensis</i> MW200603; Ecuador	Sarcophagidae sp. BOLD: ABW8000; Argentina	94.35	94.83
ZG2043	PZ093091	<i>Peckia lambens</i>	<i>Sarcodexia lambens</i> MW200981; Ecuador <i>Oxysarcodexia xanthosoma</i> KU315816; Brazil	Sarcophagidae sp. BOLD: AAA1909; Trinidad and Tobago	100	100
ZG2045	PZ093092	<i>Peckia buethni</i>	<i>Peckia</i> sp. MG967786; Dominican Republic	<i>Peckia</i> sp. BOLD: ADL008; Dominican Republic	96.26	96.26

For the remaining male specimens, the sequences (28/36; 78%) returned low-similarity matches (88–96%) and unresolved identifications at the genus or higher taxonomic levels or were discordant with the morphology-based identifications. For example, two male specimens of *Peckia chrysostoma* (Wiedemann, 1830) (ZG2009 and ZG2022) showed 96% similarity to reference sequences from French Polynesia in both databases. Similarly, *Peckia* sp. sequences from the Dominican Republic, another Caribbean country within the Greater Antilles, shared 96% similarity to the Jamaican endemic *Peckia (Euboettcheria) buethni* Dodge, 1965 (ZG2045) in both GenBank and BOLD. Other Jamaican endemics that returned with unresolved or discordant identifications included *Peckia (Euboettcheria) nicasia* (Lopes, 1941) (LF001, LF1018, LF1041, LF1048, ZG2024), *Oxysarcodexia corolla* Dodge, 1965 (LF1036, ZG2003), *Blaesoxipha (Kellymyia) jamacoorum* (Dodge, 1965) (ZG2031), and *Boettcheria parkeri* (Aldrich, 1916) (LF1069, ZG2000). Sequences from newly recorded Jamaican species, *Bahamiola orbitalis* (ZG2001, ZG2005, ZG2027), *Tapacura mariarum* Tibana and Lopes, 1985 (LF1019), and *Oxysarcodexia amilleae* Foote, Pape, and Buenaventura, 2024 (ZG2002, ZG2035, ZG2036, ZG2039), also returned database assignments inconsistent with morphology-based identifications (Table 1). Of note, BOLD returned no identification results for 19% (7/36) of the sequences (Table 1).

Species identification of female specimens that could not be resolved morphologically was not consistently achieved through GenBank and BOLD searches only and required comparison with COI sequences from morphologically identified Jamaican male specimens (Table 2). Five female sequences (ZG2004, ZG2006, ZG2014, ZG2016, and ZG2040) shared 99.84–100% identity with sequences from Jamaican male *Peckia (Sarcodexia) lambens* (ZG2007) and *P. lambens* reference sequences from Brazil and Ecuador. However, these sequences also shared 100% similarity with *O. xanthosoma* sequences from Brazil. Lower similarity matches (92–96%), assignments inconsistent with the corresponding male specimens or matches restricted to family level were obtained with the remaining three sequences: (1) ZG2018 matched sequences from male specimens identified as *P. chrysostoma* (ZG2009 and ZG2022), and shared 96.39% similarity, with GenBank and BOLD accessions from French Polynesia, (2) ZG2011 showed 98.71% and 92.06% similarity to *Sarcophagidae* sp. sequences from Mexico and the USA, respectively, and also shared high similarity to the male specimen ZG2019, morphologically identified as *O. bakeri*, and (3) ZG2017 was 95% similar to *P. abrupta* sequences from Ecuador and Costa Rica and was identical to sequences from male *P. nicasia* specimens (LF001 and LF1048).

Table 2. Molecular identification of female specimens using Jamaican male *Sarcophagidae* COI barcodes and reference sequences from GenBank and BOLD.

Sample ID	GenBank Accession Number	Species Name in GenBank (Accession Number)	Species Name in BOLD (Processed ID)	GenBank Similarity (%)	BOLD Similarity (%)	Match with Jamaican Male Species and K2P Distance		
ZG2004	PZ093065	<i>Sarcodexia lambens</i> MW200981 Ecuador; <i>Oxysarcodexia xanthosoma</i> KU315816, Brazil	<i>Sarcophagidae</i> sp. BOLD: AAA1909, Trinidad & Tobago	100	100	ZG2007	<i>Peckia lambens</i>	0.000
ZG2006	PZ093067	<i>Sarcodexia lambens</i> MW200981, Ecuador <i>Oxysarcodexia xanthosoma</i> KU315816, Brazil	<i>Sarcophagidae</i> sp. BOLD: AAA1909 Trinidad & Tobago	100	100	ZG2007	<i>Peckia lambens</i>	0.000

Table 2. Cont.

Sample ID	GenBank Accession Number	Species Name in GenBank (Accession Number)	Species Name in BOLD (Processed ID)	GenBank Similarity (%)	BOLD Similarity (%)	Match with Jamaican Male Species and K2P Distance		
ZG2011	PZ093072	Sarcophagidae sp. JF872101 JF871267, USA	Sarcophagidae sp. BOLD: AEZ5362, Mexico	92.06	98.71	ZG2019	<i>Oxysarcodexia bakeri</i>	0.000
ZG2014	PZ093074	<i>Sarcodexia lambens</i> MW200981, Ecuador MW200968, Brazil <i>Oxysarcodexia xanthosoma</i> KU315816, Brazil	Sarcophagidae sp. BOLD: AAA1909, Trinidad & Tobago	100	100	ZG2007	<i>Peckia lambens</i>	0.000
ZG2016	PZ093075	<i>Sarcodexia lambens</i> MW200981, Ecuador MW200968, Brazil <i>Oxysarcodexia xanthosoma</i> KU315816, Brazil	Sarcophagidae sp. BOLD: AAA1909, Trinidad & Tobago	99.84	99.84	ZG2007	<i>Peckia lambens</i>	0.002
ZG2017	PZ093076	<i>Peckia abrupta</i> MW200984 MW200908, Ecuador	Sarcophagidae sp. BOLD: AAA2280, Costa Rica	95	95	LF001 LF1048	<i>Peckia nicasia</i>	0.000
ZG2018	PZ093077	<i>Peckia chrysostoma</i> KX054328 <i>Peckia chrysostoma</i> AF259515, French Polynesia	<i>Peckia chrysostoma</i> BOLD: AAL3638, French Polynesia	96.39	96.39	ZG2009 ZG2022	<i>Peckia chrysostoma</i>	0.000 0.001
ZG2040	PZ093090	<i>Sarcodexia lambens</i> MW200981, Ecuador <i>Oxysarcodexia xanthosoma</i> KU315816, Brazil	Sarcophagidae sp. BOLD: AAA1909, Trinidad & Tobago	100	100	ZG2007	<i>Peckia lambens</i>	0.000

The genetic distances among COI sequences of 15 of the 16 species are summarized in Table 3. All species exhibited maximum intraspecific distances lower than 3%, with 67% (10/15) showing 0% intraspecific divergence. Interspecific distances ranged from 5.68% (*P. nicasia*) (Lopes, 1941) to 12.83% (*T. fimbriata*). In all cases, intraspecific distances were lower than interspecific distances. There was no overlap between maximum intraspecific and nearest-neighbor distances. The smallest gap of 5.68% occurred between *P. buethni* and *P. nicasia*, suggesting relatively recent divergence.

Table 3. Cluster analysis for 15 species based on divergence metrics from 43 COI sequences.

Species ¹	Maximum Intraspecific Distance (%)	Minimum Interspecific Distance (%)
<i>Bahamiola orbitalis</i>	0	9.49
<i>Blaesoxipha jamacoorum</i> *	0	11.1
<i>Boettcheria parkeri</i>	0	12.32

Table 3. Cont.

Species ¹	Maximum Intraspecific Distance (%)	Minimum Interspecific Distance (%)
<i>Helicobia morionella</i> *	0	10.37
<i>Oxysarcodexia bakeri</i>	0.16	10.02
<i>Oxysarcodexia corolla</i>	0.84	7.86
<i>Oxysarcodexia peltata</i>	0	7.86
<i>Peckia buethni</i> *	0	5.68
<i>Peckia chrysostoma</i>	0.16	9.43
<i>Peckia dominicana</i> *	0	9.46
<i>Peckia lambens</i>	0.17	9.81
<i>Peckia nicasia</i>	0	5.68
<i>Sarcofahrtiopsis farri</i>	0	11.65
<i>Tapacura mariarum</i> *	0	10.02
<i>Titanogrypa fimbriata</i>	0.16	12.83

¹ * represents species with $n = 1$; all other species were represented by 2–8 individuals.

3.3. Tree-Based Analysis

Neighbor-joining (NJ) analyses produced 15 well-supported, monophyletic clades, each corresponding to one of the morphologically identified species (Figure 1). The dataset comprised 35 male and 8 female Jamaican sarcophagid sequences along with 18 sequences retrieved from GenBank and the calliphorid species, *Chrysomya megacephala*, as the outgroup. The NJ tree clearly separated the outgroup from the sarcophagids, and all species clustered into distinct monophyletic groups supported with bootstrap values exceeding 70%, validating morphological identification, with the exception of *Oxysarcodexia* species at 67%. Multiple individuals of the same species clustered together (e.g., *Peckia* sp.), indicating low intraspecific divergence and genetic homogeneity across sampling locations in Jamaica. Additionally, the Jamaican sequences clustered with conspecific sequences from the USA, Canada, French Polynesia, Brazil, and Ecuador, further supporting species identification despite some sequence divergence. Of note, the *Peckia abrupta* from Ecuador and the Jamaican *P. nicasia*, which shared <97% sequence similarity, clustered together. The majority of Jamaican female specimens grouped with their respective male counterparts as well as sequences from other regions, even where sequence similarity was <97% (e.g., *P. chrysostoma*). ZG2004, which shared 100% similarity with *P. lambens* reference sequences in GenBank and was identical to sequences obtained from Jamaican male *P. lambens*, clustered with the Brazilian sequence annotated as *O. xanthosoma* and the other Jamaican male *P. lambens* sequences (97% bootstrap support).



Figure 1. Neighbor-joining tree based on COI sequences of sarcophagid species from Jamaica and North and South America, Europe and Oceania. Specimen labels include the GenBank accession number, species name (an indication of female specimens where applicable), specimen ID number, and collection location. Numbers on branches indicate the bootstrap support value (among 1000 replicates). Only support values above 50% are shown. KC966375: Calliphoridae is the outgroup to root the tree.

4. Discussion

DNA barcoding, particularly using COI sequences, has been widely established as a reliable method for identifying Diptera species across families such as Calliphoridae, Phoridae, Muscidae, and Sarcophagidae [25,27]. This approach is especially useful for resolving closely related or morphologically ambiguous taxa; however, its effectiveness is dependent on the availability of the barcodes in reference databases. Molecular repositories are often biased to temperate regions or well-studied fauna [28], and for many tropical Diptera, newly generated sequences frequently lack close matches (<90–95%). Consequently, identification declines markedly when a reference library is less than 50% complete for a region [29].

Jamaica has 46 documented species of flesh flies [11,30]. This study represents the first DNA barcode database for Sarcophagidae species in the country. Overall, species-level molecular identification was limited. Although thousands of database hits were retrieved for COI sequences from the 16 Jamaican species, only 30% (13/44) returned 97–100% similarity to reference sequences. Taxonomic resolution varied among databases, with assignments ranging from species-level to broader family or subfamily-level placements, and some high-similarity matches were ambiguous. For example, sequences from eight specimens of *Peckia* (*Sarcodexia*) *lambens* achieved species-level assignments based on 99–100% similarity to reference sequences, but the same sequences also returned equally high-similarity matches to *Oxysarcodexia* *xanthosoma* (KU315816). However, phylogenetic analysis supported the identification as *P. lambens*, since KU315816 clustered within a well-supported clade (97%) comprising other *P. lambens* reference sequences. In addition, there is no record of *O. xanthosoma* in Jamaica [11]. Together with the high proportion (70%) of sequences returning matches below the 97% identity threshold and unresolved or discordant species-level assignments, these results highlight gaps in Caribbean taxonomic representation in public databases and are consistent with previous reports of misidentified reference sequences in DNA barcode databases [31].

Furthermore, even when morphological identifications were concordant with sequence-based assignments, similarity values were sometimes below the 97% threshold for species-level identification. For example, specimens morphologically identified as *P. chrysostoma* (ZG2022, PZ093080) showed only 96.10–96.40% similarity to available *P. chrysostoma* reference sequences from GenBank and BOLD. This level of divergence (~4%) among conspecific sequences may reflect deep intraspecific structuring or incomplete reference sampling. Meiklejohn et al. [32] reported similar observations with *Sarcophaga* species in Australia. It was posited that flesh flies exhibit deep intraspecific clades, which sometimes exceed 4% divergence. Bergsten et al. [33] also found that divergence increases with geographic distance even within species, supporting the possibility that Caribbean populations of *P. chrysostoma* may represent a geographically structured lineage from mainland species. Similarly, Pinto et al. [34] reported approximately 90% matches between Neotropical Diptera and public databases despite morphological confirmation, suggesting that limited reference coverage or cryptic diversity may also contribute to observed divergence. This uncertainty is supported by the phylogenetic analysis, where Jamaican *P. chrysostoma* formed a sister clade to sequences from French Polynesia (bootstrap 99%). Collectively, the results indicate that species boundaries in this group cannot yet be fully resolved and highlight the need for expanded regional barcode sampling [29] and perhaps whole-genome sequencing approaches.

The utility of public databases was further constrained by incomplete taxonomic resolution within GenBank and BOLD. This was evident for three species, *O. peltata*, *H. morionella*, and *S. farri*, which were represented in the reference databases only at the genus or family level. The effective use of these databases depends not only on the availability of reference sequences but also on the accuracy of their taxonomic annotation. The sequences generated in this study (accession numbers PZ05904; PZ093069; PZ093084; PZ093053; PZ093073) now provide validated voucher-linked references. The clustering observed in the NJ analysis, with strong bootstrap support (99%), further supports the utility of these COI sequences for taxonomic identification of Jamaican flesh flies. This is an important contribution to the database because, according to Mello-Patiu and Pape [35], Sarcophagidae fauna are neglected globally, and disciplines such as forensic entomology require accurate identification. Additionally, COI sequences for five Jamaican endemic species (*B. jamacoorum*, *B. parkeri*, *O. corolla*, *P. buethni*, and *P. nicasia*), three new records (*P. dominicana*, *B. orbitalis*, and *T. mariarum*) and a newly described species (*O. amilleae*)

are provided. All taxa formed distinct, well-supported monophyletic clades in the NJ tree, confirming their morphological identification and genetic distinctiveness within the Jamaican fauna. Nonetheless, a relatively small number of individuals were sampled in this study. Because single DNA sequences may contain errors or may not capture intraspecific variation, generating multiple barcodes per species is essential to ensure the reference data are representative. Future work should therefore focus on increasing sample sizes, incorporating additional molecular markers such as mitochondrial COII and 16S rRNA, or nuclear ITS2 and 28S rRNA [36,37].

At the species level, strong barcode gaps were observed across all taxa. Intraspecific divergence was low ($<0.84\%$), while interspecific distances were substantially higher (mean $\sim 9.5\%$), with no overlap between these values. This is consistent with expected barcode separation in insects [19]. However, studies on Sarcophagidae suggest that the interspecific distance can be as low as 2% [18]. The smallest interspecific distance of 5.86% occurred between *P. buethni* and *P. nicasia*. This relationship will need to be confirmed, as only one sequence of *P. buethni* was used in the analysis. Notwithstanding, both species are classified under the subgenus *Euboettcheria* and are similar morphologically, as they possess a long plate phallic tube [38]. Sister grouping in the NJ tree suggests recent divergence. Given the overall limited number of available sequences, these values should be considered preliminary, as they may not fully capture the extent of genetic variation and may potentially inflate the observed barcode gap. Future studies should prioritize increasing the number of individuals sampled per species to better characterize intraspecific diversity and provide a more accurate assessment of genetic variation.

Finally, this study addressed the challenge of identifying female Sarcophagidae in Jamaica, as noted by Foote-Gordon et al. [11]. By using COI barcode sequences from morphologically identified Jamaican males, together with database comparisons and NJ analysis, most female specimens (5/8; 63%) were assigned to species-level identities. These results demonstrate the value of an integrative taxonomic approach combining a regional barcode reference library with traditional morphological expertise for resolving morphologically ambiguous taxa in Caribbean and Jamaican Sarcophagidae.

5. Conclusions

This study provides the first report of molecular data for species of Sarcophagidae in Jamaica. Twelve new COI barcodes for species of flesh flies in Jamaica were generated, adding to global data repositories. While these findings represent an initial step that warrants further validation, they open the door for the use of COI as a diagnostic tool for Jamaican flesh flies, serve as a confirmatory tool for existing records that lacked species identification, and underscore the need for a Caribbean region-based reference repository. Future efforts should prioritize expanding and curating regional barcode libraries as well as integrating multiple genetic markers along with morphological data to resolve taxonomic uncertainties and support both biodiversity and forensic applications in the Caribbean.

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