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Identification of Morphologically Similar Species of Necrophagous Flies (Diptera: Calliphoridae) in Oklahoma: Reliability and Application of Techniques in a Forensic Setting

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Identification of Morphologically Similar Species of Necrophagous Flies (Diptera: Calliphoridae) in Oklahoma:

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APPROVED FOR THE FORENSIC SCIENCE INSTITUTE

2013

By _____

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ABSTRACT

The development of taxonomic keys for carrion-associating blow flies (Diptera: Calliphoridae) has greatly enhanced the field of forensic entomology by facilitating identification of species often associated with crime scenes. Keys for morphological identification of blow flies have been developed and refined by Whitworth (2006) and Marshall, et al. (2011). Research involving habitat preferences, ovipositional behavior, developmental rates, and succession to decaying matter has proved vital for the estimation of a post mortem interval (PMI) for crime scene investigators. Within the state of Oklahoma, there is suspected habitat overlap and migration of Calliphoridae species, stemming from varying environmental conditions and resource availability. This study assessed the relationship between morphological and genetic identification of three blowfly species (*Lucilia cuprina*, *Lucilia sericata,* and *Lucilia mexicana*) sampled from eight different locations within Oklahoma and one island location off of the coast of New Hampshire. A 308 basepair amplicon within the cytochrome oxidase I gene of mitochondrial DNA was obtained for twenty-four specimens. An additional genomic location was targeted to support the robustness of laboratory analyses. A 330 basepair amplicon within the 28S large subunit of ribosomal DNA was obtained for thirty-five specimens. Molecular phylogenetic results were compared to morphological identifications in order to ascertain the reliability of the respective laboratory techniques. Morphological and genetic identification techniques confirmed the previously undocumented presence of *L. mexicana* within Oklahoma. COI data was unreliable for distinguishing between morphologically similar *Lucilia* species; however 28S phylogenetic assessments were successful in defining most Calliphoridae species. Results serve as a template for future ecological and forensic research.

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1. INTRODUCTION

In the realm of forensic science and forensic entomology, insects, specifically blow flies (Diptera: Calliphoridae) play a vital role. These highly mobile insects are attracted to remains immediately after death, and have been observed and documented to arrive within minutes after expiration (Anderson, 2001; Haskell, et al., 1997). The presence or absence of blow fly evidence can signify whether a body has been moved or manipulated, as well as give an indication of the time since first oviposition, which is often close to the time of death (Hall, 2001). In this latter estimation, flies of the family Calliphoridae are utilized, as they are regularly the first to colonize a fresh corpse, followed by a variety of other necrophagous arthropods (Amendt, et al., 2004; Anderson, 2001).

1.1. Historical Application of Blow Fly Evidence

Since the earliest uses of insects as forensic indicators, the progression of forensic entomology research has experienced periods of scientific growth and stagnation. In the thirteenth century, the Chinese lawyer and death investigator Sung Tz'u documented the ability carrion-associating Calliphoridae have in detecting trace amounts of blood and tissue (Benecke, 2001; McKnight, 1981). He described a village stabbing, after which all suspected workers were instructed to place their sickles in the sun. After a short time, flies were attracted to traces of blood on only one sickle, and confronted with this, the owner confessed to the murder (McKnight, 1981). For centuries following this, little progress was achieved for the field of forensic entomology. It was not until the renaissance movement that a propagation of research was seen again. The theory of spontaneous generation was not disproven until the 18th century, with scientists formerly believing that maggots would miraculously appear on corpses and carrion (Benecke, 2001). This lack in knowledge of general insect ecology and application to

forensics was not remedied until the 1800s, when scientists and criminal investigators alike realized the value of insect evidence. Jean Pierre Megnin was one of the first entomologists to observe that different insect species colonize corpses at different stages of decomposition (Megnin, 1894). Over the next 150 years, research into insect colonization and succession on decomposing remains continued, as well as studies into spatial and temporal variability of flies, which proved useful for death investigators as well as served to broaden the knowledge of insect distribution (Benecke, 2001; Cruickshank & Wall, 2002; Goff, 2000; Payne 1965; Reed, 1958; Richards & Goff, 1997). In the last seventy years, research has also studied preferred habitats, development rates, and succession patterns of carrion-associating Diptera in various parts of the world (Giao & Godoy, 2006; Grassberger, 2001; Hwang & Turner, 2005). Studies carried out in the United States have been focused in the eastern and western thirds of the nation, as well as the islands of Hawaii, but little information exists in regard to Oklahoma blow fly populations (DeBry, Timm, Dahlem, & Stamper, 2010; Goff, 1991; Introna, Suman, & Smialek, 1991; Richards & Goff, 1997). Oklahoma's central location within the North American continent, diverse weather patterns, and wide range of habitat types make it an optimal location for studying overlapping or converging necrophagous Diptera species (Oklahoma Climatological Survey, 2013; Woods, et al., 2005).

1.2. Factors Influencing Decomposition and Diptera Colonization

When a person expires the remains, or corpse, transitions through multiple stages of decomposition known as fresh, bloated, decay, and dry (or skeletonization) (Forbes & Dadour, 2010). As a corpse decomposes, the odors released, in addition to the state of the body, attract a variety of insects. The first colonizers to a corpse are typically flies of the family Calliphoridae, commonly known as blowflies or blue- and green-bottle flies, which are succeeded by other

Dipteran and Coleopteran (beetle) families (Byrd & Castner, 2009; Payne, 1965; Reed, 1958). An example of carrion transitioning through the fresh and bloated stages of decomposition (optimal for Diptera colonization) is given in Figure 1. Members of the Calliphoridae family are dispersed worldwide and are most active in warmer months. Female blow flies oviposit on a corpse or carrion, and the hatching larvae begin to feed upon the decaying flesh. These maggots, along with active adult flies above and around the body, can be collected and utilized for species identification. The identification of Diptera species allows forensic entomologists to determine a time since initial colonization, which is then applied to estimate the postmortem interval (PMI).

Forensic entomology greatly aids investigators in determining postmortem intervals (PMI), or time since death (Goff, 1993; Greenberg, 1991). Specimens collected at crime scenes also serve to associate suspects to victims or crime scenes, indicate if remains have been moved since death, or hold important toxicological information in cases of poisoning or drug overdose (Goff, 1993; Goff, 1994; Hall, 1990; Lord, 1990; Smith, 1986). Central to the reliability and validity of forensic entomology is the ability to accurately calculate a PMI based on development rates of various researched insects. These rates may vary dramatically across species, and are influenced by environmental factors such as temperature or scavengers (Byrd & Castner, 2009). Adult diptera, larvae, and eggs are typically collected at a crime scene, either by investigators or entomologists. Once in the lab, the eggs and early instar larvae, which are much more difficult to

Figure 1: Example of decomposing carrion (*Sus scrofa domestica*). This pig was transitioning from the fresh to bloated stage, exhibiting a distended abdomen and discoloration of the skin becoming apparent. Large concentrations of necrophagous Diptera are observed on the head.

distinguish morphology, are placed in rearing chambers and allowed to reach the adult stage (Byrd & Tomberlin, 2009). Additionally, different species of blow flies can have different developmental rates, which may be influenced by weather patterns. Wind speed, rainfall, and temperature, are a few of the most prominent climatological factors influencing colonization, decomposition, and development in and around a corpse (Anderson, 2001, Introna, et al., 1991). A hot, humid climate (similar to summers experienced in Oklahoma) greatly increases the feeding, breeding, and development of Diptera (Grassberger & Frank, 2004; Smith, 1986). Human or animal remains may be buried, submerged, or wrapped by their killer to conceal their existence, thereby creating a barrier for blow flies and other decomposers and postponing decomposition (Goff, 1991; Rodriguez & Bass, 1983; Rodriguez & Bass, 1985). Also, some species may only be found in particular locations around the globe, while others exhibit extensive habitat overlap. To make the field of forensic entomology even more arduous, many blowfly species share both habitat overlap and morphological characteristics, making accurate species identification problematic (Giao & Godoy, 2007; Hwang & Turner, 2005; Malgorn & Coquoz, 1999; Tourle, et al., 2009).

1.3. Classical Dipteran Identification

Much debate has arisen in the quest to categorize and develop accurate and usable taxonomic keys for the identification to species of forensically important necrophagous flies (Hall, 1948; Stevens & Wall, 2001; Whitworth, 2006). Entomologists must often use a microscope in order to identify minute, variable, and often subtle morphological features of maggots and adult flies. Within the Calliphoridae family, subfamily Luciliinae, the three species *Lucilia sericata, Lucilia cuprina,* and *Lucilia mexicana* share similar morphological characteristics. Both *L. sericata* and *L. cuprina* have known habitat overlap (Whitworth, 2006)

and it is suspected that *L. mexicana's* habitat has spread into parts of Oklahoma. This habitat overlap between morphologically similar species presents a possibly of species misidentification by forensic entomologists. Incorrect identification of morphologically similar species has the potential to adversely affect calculation of species-specific developmental rates, thereby potentially contributing to inaccurate PMI determinations (Anderson, 2000; Byrd & Castner, 2009; Goff, 1993; Grassberger & Reiter, 2001; Sperling, Anderson, & Hickey, 1994).

The objective of this study was to collect and identify populations of Oklahoma blow flies via separate morphological and genetic-based protocols and to assess if these techniques were consistent in species identification. Diptera population data were collected, as well as development of a DNA-based identification methodology. This DNA-based methodology allowed for differentiation between closely related Calliphoridae species in areas of spatial and temporal confluence. Three related blowfly species of the genus *Lucilia* (Diptera: Calliphoridae), *L. sericata, L. mexicana,* and *L. cuprina,* which are commonly found in Oklahoma, were targeted for this study. These three species share morphologically defining characteristics as well as presumed habitat overlap. A geographically distinct population from an island off the coast of the northeastern United States was also collected for morphological and genetic comparison.

2. SURVEY AND MORPHOLOGICAL IDENTIFICATION OF COLLECTED BLOWFLY POPULATIONS

2.1. Introduction

2.1.1. Initial Observations of Blowfly Research

Insects, particularly necrophagous Diptera, are attracted to a corpse or carrion almost immediately after death (Anderson, 2001; Haskell et al., 1997). They are drawn by the odors produced during decomposition, but may also locate decaying matter through visual indicators such as color, and movement of other insects, either on or around the decomposition site (Anderson, 2001; Fisher, et al., 1998; Wall & Fisher, 2001). Research using animal carcasses has illustrated the diversity in species composition across regional and ecological habitats (Anderson, 2001; Introna, et al., 1991; Richards & Goff, 1997), however, when applying this information to crime scenes and forensic entomology, caution must be taken in determining post mortem intervals. Data collected in one region may not be applicable to the location of a crime scene. Research has revealed that the ecology of an area or amount of sun exposure on a corpse can influence certain necrophagous species to arrive sooner or later than expected (Hwang & Turner, 2005; Shean, et al., 1993; Smith, 1986). As well, some blow fly species are common in both rural and urban environments, while others may be specific to a certain locale (Catts & Haskell, 1990). Some rural species have been found in urban locations (and vice versa), possibly indicating that a corpse has been moved (Anderson, 2001; Grassberger & Frank, 2004; Hwang & Turner, 2005).

In Oklahoma, summer temperatures average over 90ºF for 60-65 days (with approximately 15 days over 100ºF) with prevailing winds from the south for most of the year (Oklahoma Climatological Survey, 2013). The unique climate conditions produced from this

weather affect Diptera colonization on carrion. Higher temperatures increase the rate of decomposition and larval development, and dispersal of carrion odours are greatly facilitated on breezy days (Anderson, 2001). However, extreme temperatures cause evaporation, thus dessicating carrion and reducing oviposition. As well, excessive winds limit Diptera flight and olfactory detection of remains, therein delaying colonization (Wall & Fisher, 2001). These climatological variables, in conjunction with Oklahoma's diverse habitats, may lead to significant variation in necrophagous Diptera populations found throughout the state.

Some research has addressed variables such as seasonality of Calliphoridae flies, while other studies have served as population surveys of possibly invasive or migrating species (Harvey, et al., 2008; Hwang & Turner, 2005). Entomologists such as Goff (1991) and Rodriguez and Bass (1985) have manipulated corpses by covering with carpet, partially burying, and other techniques, such as placement in the sun versus the shade and exposing bodies to chemical elements such as insect repellent. These studies served to verify correct colonization, succession, and developmental rates of various species of necrophagous Diptera and have been used to determine accurate PMI estimates. However, this spatial data is limited to specific locations, and cannot be applied universally to blow fly populations, even if they are relatively close in proximity.

2.1.2. National and Statewide Ecoregion Diversity

The United States Environmental Protection Agency and the Department of Agriculture's Forest Service provide a descriptive hierarchy of ecosystems present in the United States (Bailey, 1995). These defined ecoregions illustrate the diversity of ecosystems surveyed across the nation. Four eco-levels are distinguished based on specific environmental factors such as temperature, precipitation, vegetation/landcover, terrain features, or elevation (see Appendix A for maps and

descriptions). These ecoregions are highly useful for scientists attempting to target their research to specific climatological or terrain environments.

The state of Oklahoma is home to its own diversity of ecoregions, as shown in Figure 2. Oklahoma's location in the south central United States/central North America lends to the unique convergence of many distinct habitat and terrain types. It is one of only four states with more than ten ecoregions, the most per square mile (Woods, et al., 2005). Oklahoma's location between the western Rocky Mountains and the eastern Appalachians as well as proximity to the Gulf of Mexico gives the state an extremely varied and often tumultuous climate. These important environmental and climatological factors play a significant role in the flora and fauna of the state. The Oklahoma Forestry Service has adapted the EPA's published ecoregions and defined them based on terrain and sub-climates (Woods et al., 2005). Appendix B describes each region's unique characteristics and its geographic location within the state.

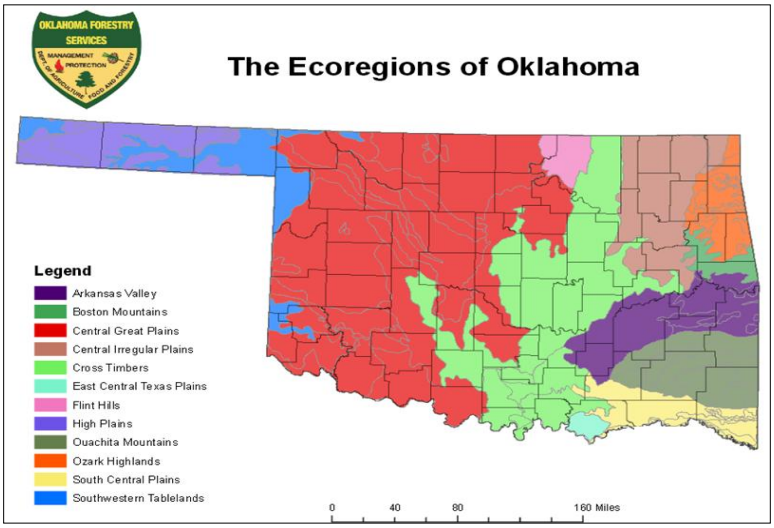

Figure 2: Ecoregions of Oklahoma. Adapted by the Oklahoma Forestry Services from Woods et al., 2005. See Appendix B for detailed descriptions of each region.

Ecological and topographical factors play a role in insect migration to new resources and geographic expansion of species. As cited in chaper one, research regarding carrion-associating Dipteran populations has been conducted in Europe, Asia, Canada, and across the northeastern United States. However studies are lacking which document blow fly prevalence and distribution within Oklahoma. Blow flies of subfamily Luciliinae share taxonomically defining morphological characteristics, which are difficult to visualize

macroscopically. The species *Lucilia cuprina, Lucilia sericata*, and *Lucilia mexicana* exhibit habitat overlap in the United States. *Lucilia mexicana's* distribution was once limited to the Southwestern U.S. and Mexico, but has been documented in Texas, with the possibility that it has migrated into Oklahoma (Figure 3). Typically, blowflies will habitat an area with a radius of one half mile from their breeding location (Mayer & Atzeni, 1993; Stafford, 2008), but as competition for resources increases and/or the availability of mates decreases, pressure to migrate to more suitable habitat increases (Schoof & Siverly, 1954). In regard to isolated island populations, studies limit Dipteran flight distances to approximately two miles, with maximum reports of three miles when accessible landing areas are not available (MacLeod & Donnelly, 1963).

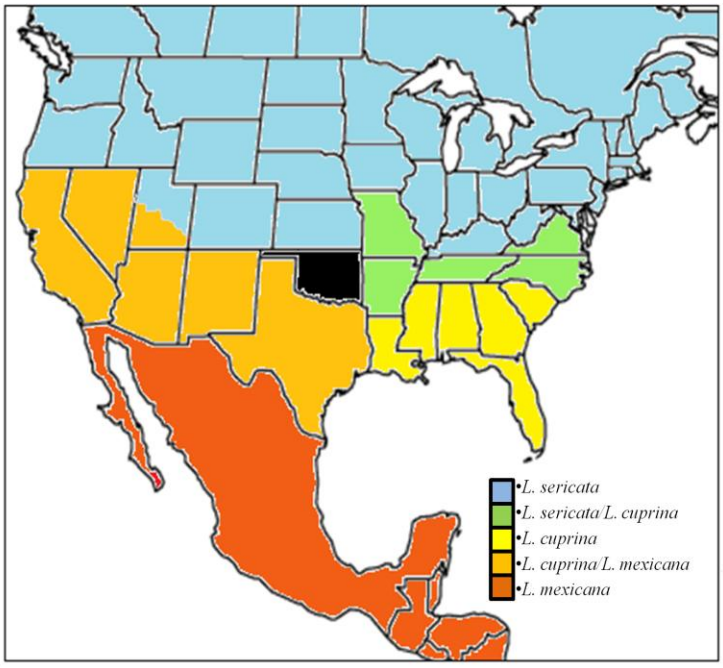

Figure 3: Approximate habitat distribution of three *Lucilia* species (Whitworth, 2006). Oklahoma (black) exhibits suspected habitat overlap of all three species.

2.1.3. Identification of Diptera Species

This overlapping of forensically relevant Calliphoridae species leaves entomologists with the arduous task of correctly identifying, via minute morphological traits, extremely similar species. In 1948, researcher David Hall pioneered the most descriptive taxonomic key of the time to identify blowflies commonly found in North America. His detailed descriptions of morphological features of regionally selected species relied heavily on proportional measurements derived from sampling approximately five to ten specimens per species

(Whitworth, 2006). This limited measurement data cannot be applied comprehensively to all North American diptera species, leaving researchers to rely heavily on indiscriminate morphological features. Since first being published, Hall's research and nomenclature for many species of Calliphoridae has been modified and redefined (Table 1). Current taxonomic keys continue to rely heavily on extremely fine features which are not always easily discernable.

Table 1: Changes to Calliphoridae Nomenclature

Hall (1948)	**Whitworth (2006)**
Phaenicia cluvia	*Lucilia cluvia*
Phaenicia caeruleiviridis	*Lucilia coeruleiviridis*
Phaenicia pallescens	*Lucilia cuprina*
Bufolucilia elongata	*Lucilia elongata*
Phaenicia exima	*Lucilia eximia*
Lucilia illustris	*Lucilia illustris*
Francilia alaskensis	*Lucilia magnicornis*
Phaenicia mexicana	*Lucilia mexicana*
Phaenicia sericata	*Lucilia sericata*
Bufolucilia silvarum	*Lucilia silvarum*

Accurate species identification is crucial to forensic investigators when attempting to determine PMI or relocation of remains (Amendt, et al., 2004; Giao & Godoy, 2007; Greenberg, 1991). Closely related, morphologically indistinct Calliphoridae species can have differing developmental rates and habitat preferences (Anderson, 2000; Hall, 1990; Smith, 1986). All Diptera progress through four growth stages (egg, larva, pupa, and adult) and forensic identifications are frequently delayed because larval Diptera collected from a corpse must be reared to adulthood (Byrd & Tomberlin, 2009; Wallman & Donnellan, 2001). Physical damage to specimens during collection at crime scenes also impedes definitive identification. Knowledge of preferred habitats has aided forensic entomologists in determining movement of remains or in linking a suspect to a victim (Anderson, 2001; Goff, 1991; Greenberg, 1991; Lord, 1990; Rodriguez, & Bass, 1985; Shean, et al., 1993). Research has described geographical and seasonal data for select species, such as rural or urban environment preferences or species prevalence in the summer versus winter (Payne, 1965; Goff, 1993; Grassberger & Frank, 2004; Schoof & Siverly, 1954). However, information currently available has limited application to crimes

committed in Oklahoma, as reference data acquired for one location may not always be applicable in another.

2.1.4. Research Objectives

The specific objectives for this research were to: (1) sample various locations across Oklahoma to establish a reference collection for morphological and subsequent molecular research, (2) identify forensically relevant Oklahoma Diptera using taxonomic morphological features, and (3) document the presence or absence of *L. mexicana* migration into Oklahoma. The following general hypotheses were tested:

H_1 = Use of classical morphological techniques will definitively identify and verify the presence of *Lucilia mexicana* in blowfly populations collected from various locations in Oklahoma.

H_0 = Use of classical morphological techniques will definitively identify and verify the absence of *Lucilia mexicana* in blowfly populations collected from various locations in Oklahoma.

Morphological identification of collected specimens focused on the subtle similarities and differences between three Calliphoridae species (*L. sericata, L. cuprina,* and *L. mexicana*). These identifications were based on taxonomically defined morphological characteristics, namely differences in thorax and abdomen coloration, location and presence or absence of setae, and basicosta pigmentation.

2.2. Materials and Methods

2.2.1. Sampling locations

Specimen collection was conducted in the spring, summer, and fall months during the 2010, 2011, and 2012. Each site was sampled at least once for a minimum of three hours. Time

spent at each sampling location varied based on temperature, wind conditions, and fly abundance. Sampling was typically conducted on sunny or partly sunny days, when little precipitation was forecast. Sampling locations were chosen based on diversity of ecological habitat and spatial distance from other collection locations (Figure 4; Table 2). Collection sites were either in public state parks, with permission of appropriate wildlife officials, or on private land with owner permission. Specimen collection permits were obtained through the Oklahoma Wildlife Department Conservation.

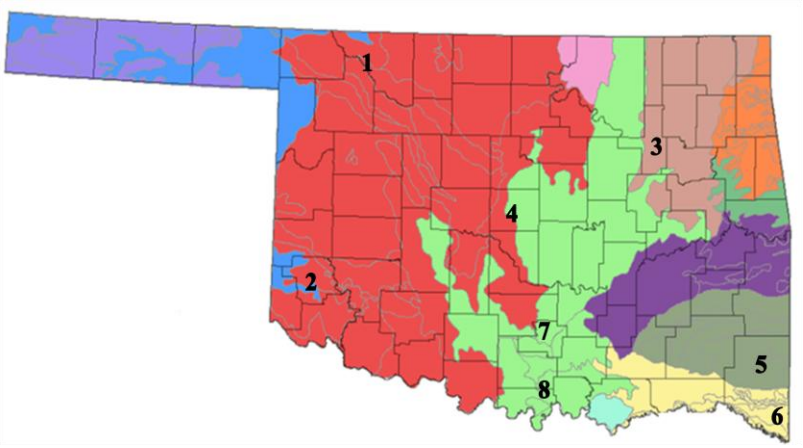

Figure 4: Oklahoma Diptera Sampling Locations. Eight locations were sampled over three summers, with habitat diversity and spatial distance factors in site selection (adapted from Woods, et al., 2005).

Table 2: Oklahoma Diptera Sampling Site Descriptions

Site	Description	Ecoregion	Sampling area conditions
1	Selman Living Laboratory, a learning facility owned and operated by the University of Central Oklahoma	Central Great Plains	Rugged, rural prairie land with low brush and grasses
2	Quartz Mountain State Park surrounding Lake Altus	Central Great Plains	Rural lakeside recreational area
3	Bixby, OK	Central Irregular Plains	Surburban wooded area with livestock within 1/4 mile radius
4	Edmond, OK	Cross Timbers	Maintained backyard in an urban residential neighborhood
5	Broken Bow State Park	Ouachita Mountains	Heavily wooded rural lakeside recreational area
6	Red Slough Wildlife Management Area	South Central Plains	Marsh and wetlands (most areas in extreme drought)
7	Chickasaw National Recreation Area	Cross Timbers	State highway alongside rural pasture and farmland
8	Lake Murray State Park	Cross Timbers	Rural area with brush and trees, in addition to sandy lake shores

A geographically distinct Diptera population was sampled on Appledore Island in the Gulf of Maine, six statute miles off the coast of New Hampshire (Figure 5). Sampling was conducted in coordination with and under permission of the Shoals Marine Lab, a collaborative project between Cornell University and the University of New Hampshire. The 38 hectare island was the largest of the nine islands in the Isle of Shoals, and represented a near-pristine coastal environment, with robust vegetation and seagull populations. The island is a distinct, geographically isolated area with a presumed predominantly holarctic Calliphoridae species complex (Whitworth, 2006). Approximate geodesic ("as the fly travels") distances between sampling sites are listed in Table 3.

Figure 5: Location of Appledore Island, ME, in the Isle of Shoals. Inset: Enlarged view of the island, with sampling location (star) (Google Maps, 2013).

Table 3: Approximate Geodesic Distances (mi.) Between Sampling Locations

Site	(1) SLL	(2) Quartz Mtns.	(3) Bixby	(4) Edmond	(5) Broken Bow	(6) Red Slough	(7) Chickasaw Natl. Rec.	(8) Lake Murray	Appledore Island, ME
1									
2	127								
3	210	208							
4	138	117	92						
5	318	280	151	199					
6	330	287	170	212	21				
7	192	132	121	82	149	155			
8	219	142	149	113	140	154	30		
Appledore	1556	1626	1420	1509	1424	1435	1530	1551	

2.2.2. Trapping and collection techniques

The commercial nuisance fly trap branded Rid-Max® was utilized for trapping blowflies. Per the manufacturer's description, "The Rid-Max® Fly Trap is placed over suitable bait or fly

attractant. Flies are attracted to the bait placed under the trap. Insects which are lured to the bait instinctively travel upward and into the coned area. They then crawl through the opening at the top of the cone, and are trapped in the "holding area" of the trap" (Figure 6) (Rid-Max® Products, 2011).

Rid-Max® traps were baited with commercially available beef liver, or if encountered while sampling, roadside fauna. Beef liver was thawed from frozen and left at room temperature for 12-24 hours before placement under traps, in order to allow for the meat's decomposition process to begin, simulating the decay encountered at crime scenes. To prolong research sampling time, bait was rehydrated when temperatures, wind or other environmental factors rapidly increased desiccation of the meat. This use of moist, decaying bait preferentially attracts the targeted species of necrophagous Calliphoridae species (Introna & Campobasso, 2000). Multiple traps were placed at each location to facilitate the collection of ample quantities of target species. Captured fly specimens were transported to the laboratory and euthanized while still in the collapsible mesh traps via freezing. Collected flies were then transferred to a 70% ethanol solution if curation of specimens was not immediate; this had no effect on later DNA analyses.

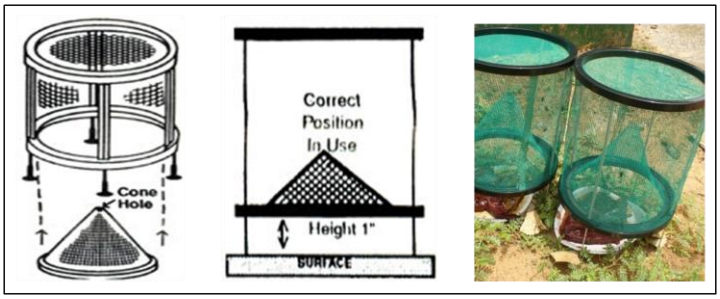

Figure 6: Rid-Max® Trap Configuration (RidMax® Products, 2011). Left to right: trap assembly; placement over bait; use in this research project.

2.2.3. Curation and identification of collected blowflies

Specimens were individually pinned and prepared by accepted entomological curation methods (National Park Service, 2006), then subjected to classical morphological taxonomic identification. All suitable specimens collected during the course of the study were identified to

family, with all Calliphoridae then identified to genus and species. Taxonomic hierarchy for *Lucilia* species is described in Table 4. All collected specimens were stored in unit trays within insect drawers, which were then housed in entomology cabinets at the UCO Natural History Museum.

Table 4: Taxonomic Hierarchy of Lucilia species.

Rank	Term
Kingdom	Animalia
Phylum	Arthropoda
Subphylum	Hexapoda
Class	Insecta
Subclass	Ptyergota (winged insects)
Infraclass	Neoptera (modern wing-folding insects)
Order	Diptera
Suborder	Brachycera
Infraorder	Muscomorpha
Family	Calliphoridae (blow flies)
Subfamily	Luciliinae
Genus	Lucilia
Species	*sericata, cuprina, mexicana, et al.*

2.3. Results

2.3.1. Identification of collected reference samples

During the summer/fall of 2010, 2011, and 2012, fifteen collection attempts were made among the eight sites sampled across Oklahoma. Sampling dates, locations, and specimens collected are listed in Appendix C. Arial netting techniques were employed when traps failed to attract sufficient number of flies. Some locations were sampled on multiple occasions when previous collection attempts yielded few or no specimens. Initial morphological examination separated the Calliphoridae family from other families via identification of a meron with a distinct row of setae, an absent or weak subscutellum, setose (or plumose) aristae, and a shining metallic thorax and/or abdomen (Figure 7). From these morphological features, flies of the subfamily Luciliinae were identified first by having the basal section of the stem vein (circled in

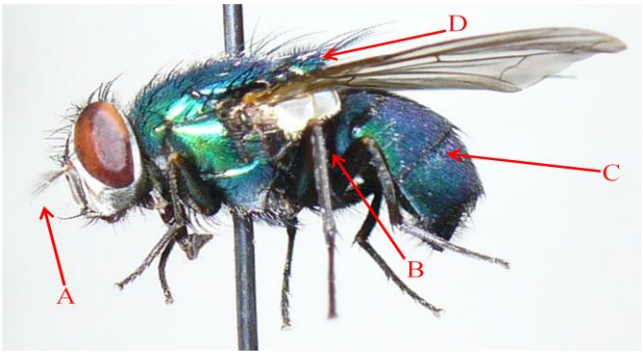

Figure 7: Defining morphological features of family Calliphoridae. A-plumose aristae; B-row of bristles on meron; C-shining metallic abdomen; D-subscutellum weak or absent.

Figure 8) bare above, their characteristic shining green or copper thorax and abdomens (Figures 7 and 8), as well as a bare lower calypter (circled in Figure 9).

Figure 8: Basal section of Luciliinae stem vein. Note the absence of setae (circled).

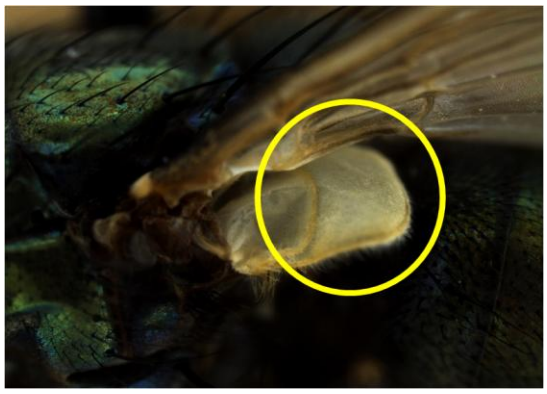

Figure 9: Lower calypter of Luciliinae. Note the bare appearance and lack of fine hairs (circled).

The subfamily Luciliinae includes one genus, *Lucilia*, with eleven species in North America. *Lucilia sericata, Lucilia cuprina,* and *Lucilia mexicana* specimens were differentiated using morphological features defined by Whitworth (2006). Orange palps were easily observed in all three species, as well as the yellow to orange basicosta of *L. sericata* and *L. cuprina*, and the brown basicosta of *L. mexicana.* More difficult features to observe were the postsutural acrostichal setae, located on the dorsal side of the thorax (Figure 10) and the central occipital setae above the eyes (Figure 11).

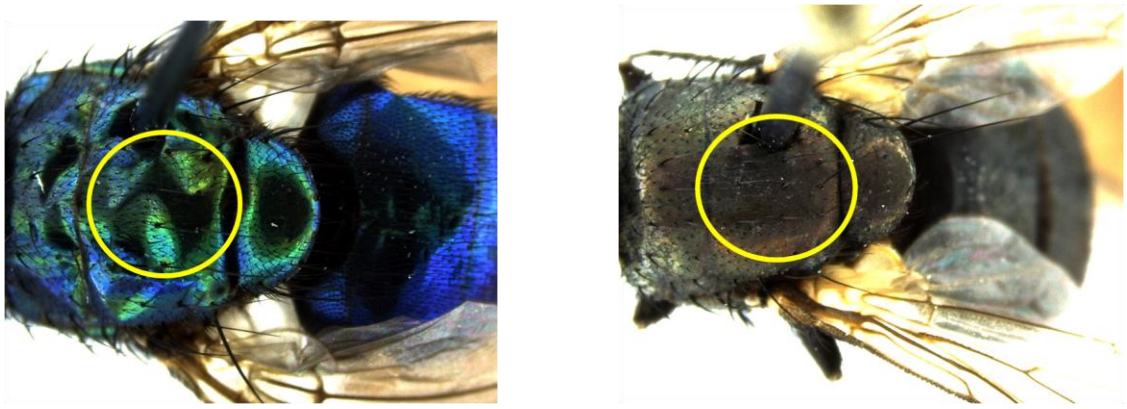

Figure 10: Distinguishing between *Lucilia* species. Presence of two postsutural acrostichal setae (left, L. mexicana) or three postsutural acrostichal setae (right, L. sericata or L. cuprina.

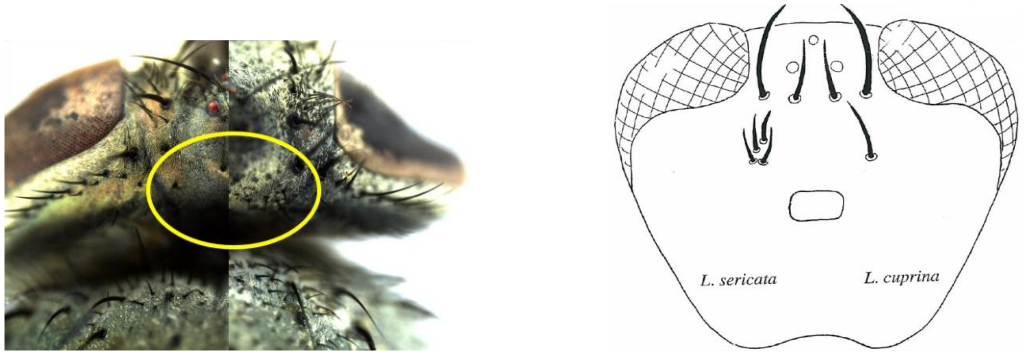

Figure 11: Photo left: Central occipital setae of *Lucilia cuprina* (left) and *Lucilia sericata* (right). Diagram right: central occipital area with placement and number of setae for each species (Whitworth, 2006).

2.3.2. Blowfly abundance and distribution in Oklahoma

From all sampling locations, 585 Diptera specimens were obtained from 10 of 15 collection attempts. Trap disruption by animals, unfavorable trapping locations, and extreme weather conditions negatively affected sampling yields, leading to few or no specimens collected at multiple locations. Trapped specimens were identified as being members of either family Calliphoridae or Sarcophagidae. Specimens identified as belonging to the taxonomic grouping of Muscidae, Anthomyidae, or Scathophagidae were excluded from further analysis as these are not considered flies of forensic importance. Together, two subfamilies of Calliphoridae (Luciliinae and Chrysomyinae) comprised eighty percent of total specimens collected in Oklahoma. One specimen was not suitable for identification (NSID) due to a lack of distinguishing morphological features. NSID specimens were excluded from further analyses.

Within the Calliphoridae subfamilies, 296 specimens were taxonomically identified to subfamily Chrysomyinae and 172 to the subfamily Luciliinae. Three species of subfamily Chrysomyinae (and number of specimens collected) were identified: *Chrysomya megacephala* (1), *Cochliomyia macellaria* (248), and *Phormia regina* (47). The subfamily Luciliinae includes only one genus, *Lucilia*. Of the blowflies collected, 112 were *Lucilia cuprina*, 38 *Lucilia sericata*, 21 *Lucilia mexicana*, and 1 *Lucilia coeruleiviridis*. Figure 12 illustrates the relative

abundance of Calliphoridae collected. The proportion of each family or species of fly collected in regard to location is described in Figure 13. Chrysomyinae was the most prominent subfamily collected in most locations; therefore, sampling time was often extended until members of Luciliinae were visible in the traps. After sampling was completed, *Lucilia* species accounted for less than one-third of the total number of Diptera collected in Oklahoma (Figure 14). Oklahoma regional blowfly populations were described in Figure 15. Location 8 (Lake Murray) contained no data from sampling attempts yielding zero Diptera collected. Family Sarcophagidae was present in every sampling, and in higher prevalence in southeastern Oklahoma. A significant proportion of subfamily Chrysomyinae was collected in five sampling locations, primarily from eastern and southern collections. Subfamily Luciliinae was also collected in five locations, with the highest proportion found in southwestern and

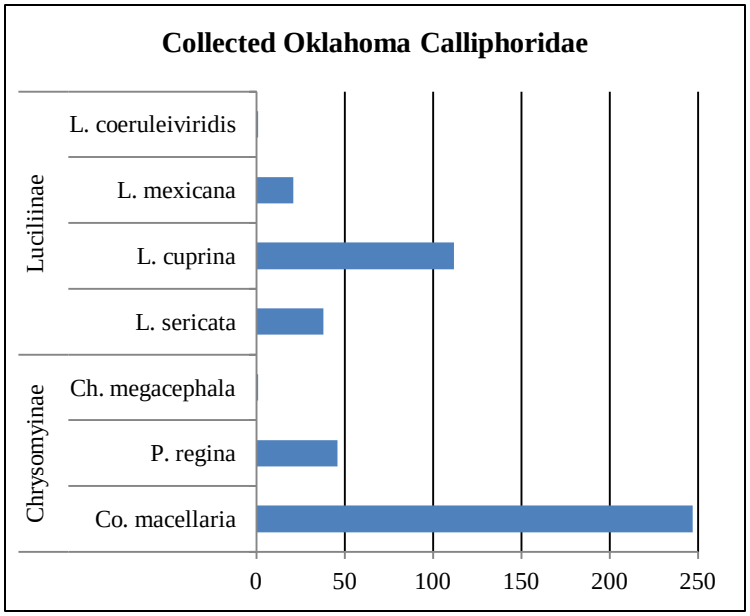

Figure 12: Abundance of blowflies collected in Oklahoma. Chrysomyinae (n=296) and Luciliinae (n=172) subfamilies were identified to species.

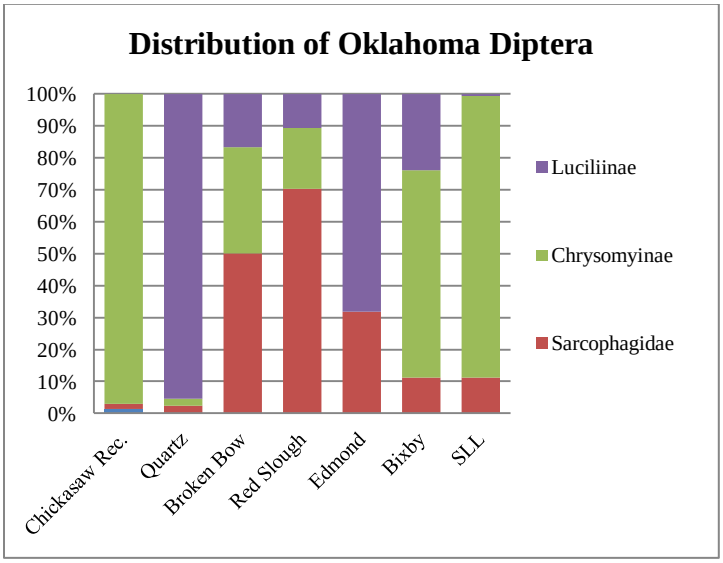

Figure 13: Diptera families of subfamilies collected in Oklahoma. The proportion collected respective to sampling location represents the percent abundance for carrion-associating Diptera.

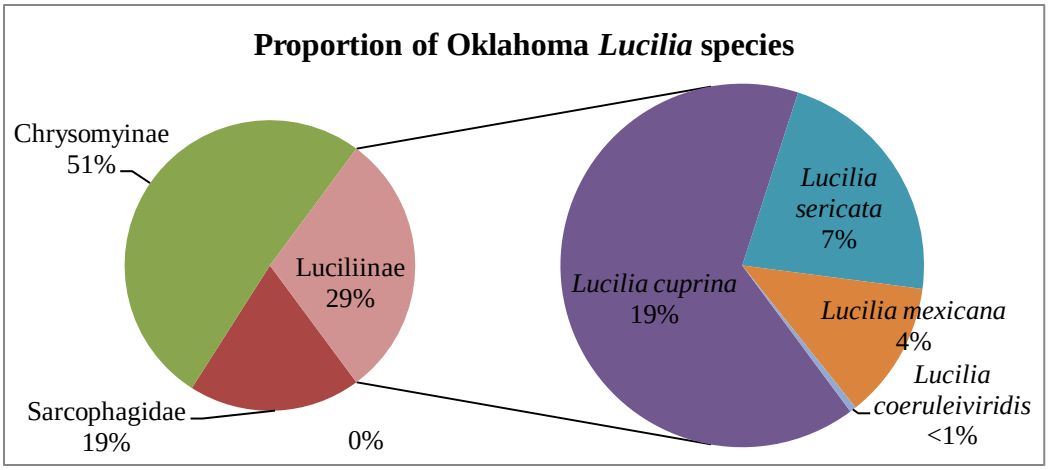

Figure 14: Proportion of Oklahoma *Lucilia* species collected from Oklahoma sites. Members of the Luciliinae subfamily represented less than one-third of the necrophagous Diptera sampled across Oklahoma.

central Oklahoma. Eastern Oklahoma sampling locations possessed higher concentrations of forested habitat than western locations, with eastern collection sites demonstrating a more cosmopolitan distribution of carrion-associating Diptera species.

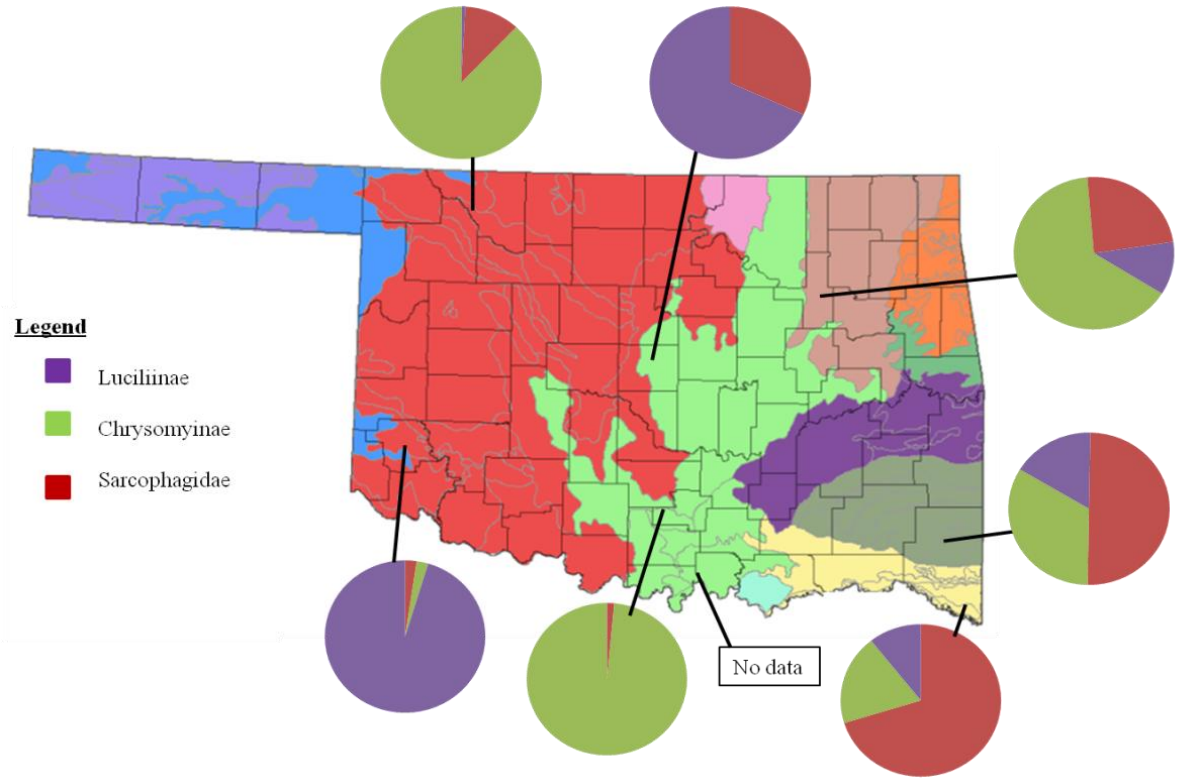

Figure 15: Proportion of blowflies collected with respect to Oklahoma sampling location. *Lucilia* populations were in greater proportions in the central and western sampling sites.

2.3.3. Appledore Island Diptera reference samples

Two hundred sixty-three Diptera were collected on Appledore Island (NH) over the span of two consecutive days in 2011 (Appendix D). Materials and methods were replicated from research conducted in Oklahoma. Specimens were placed in 2mL tubes containing 70% ethanol for shipment, however upon arrival in Oklahoma, many tubes were found to have opened in transit and samples degraded. In total, 28 of 263 samples were not suitable for identification. The remaining 235 samples were all identified as Calliphoridae. Within this family, 94% of the collected flies were identified to subfamily Chrysomyinae: species *Phormia regina*, which is largely cosmopolitan in distribution; and *Protophormia terraenovae*, a species found throughout Canada and the northern United States. The remaining 6% of Calliphoridae were all in the genus *Lucilia*: *Lucilia illustris*, *Lucilia silvarum*, and *Lucilia sericata* (Figure 16).

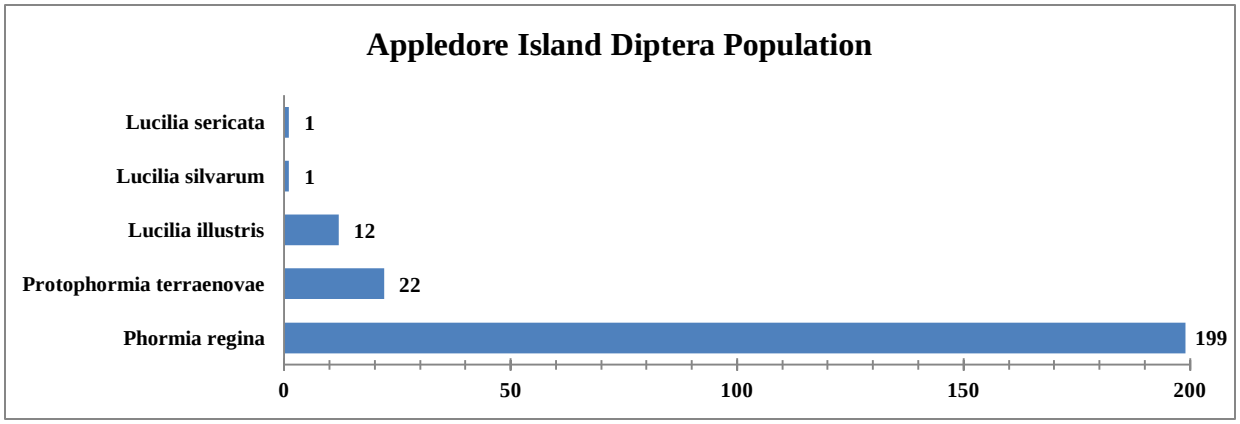

Figure 16: Blowfly species collected (n=235) on Appledore Island, ME. *Lucilia* species were found to be in a significant minority, with data not including 28 damaged samples lacking features suitable for identification.

2.4. Discussion

2.4.1. Utility of classical morphological taxonomic identification

In the process of morphologically identifying each fly sample, specific characteristics were essential to accurate identification. In samples where a morphologically distinguishing feature was damaged or presumed missing, identification was stopped at the last distinct taxonomic feature observed. On the majority of Oklahoma specimens, the postsutural acrostichal

setae were easily counted as either two or three, differentiating between *L. sericata/L. cuprina* and *L. mexicana.* Difficulty arose when attempting to distinguish between *Lucilia sericata* and *Lucilia cuprina*, as the central occipital setae were located in an area that was challenging to view properly. Extensive manipulation and angling of the fly specimen was necessary in order to achieve a focused microscopic view. Multiple specimens displayed a "pore" or follicular hole that may have at one time had a setae attached, but if it was not present at the time of examination, then it was not a definitive indicator of species.

Abdomen coloration was also not a reliable indicator of Oklahoma *Lucilia* species. Some *Lucilia cuprina* possessed single setae in the central occipital area, but did not display a coppery abdomen color. On many *Lucilia sericata,* coloration was not a reliable feature for identification. Often a specimen would have multiple central occipital setae in conjunction with a coppery abdomen. Initial preservation in 70% ethanol may have discolored or faded the flies (National Park Service, 1999). The disagreement between central occipital setae number and abdomen coloration demonstrates the potential for errors inherent in classical morphological identification. This relative phenotypic variation could indicate hybridization between Oklahoma *L. cuprina* and *L. sericata* blowflies (Nelson, et al., 2012). Blowfly hybridization is rarely encountered in the wild, but has been observed in laboratory populations. Viable hybrid offspring were only produced between *L. cuprina* male/*L. sericata* female crosses (Ullyet, 1945; Waterhouse & Paramonov, 1950). In the present study, abdomen coloration of *L. sericata* and *L. cuprina* was not a reliable indicator of species. Body size of adult specimens was not a definitive taxonomic characteristic, as *Lucilia* species exhibit sexual dimorphism between males and females.

2.4.2. Confirmation of hypothesis H_1: classical morphological techniques identified *Lucilia mexicana* in Oklahoma

This study confirmed the hypothesis that *Lucilia mexicana* has migrated from the southwestern United States into Oklahoma, and identified its presence as far north as Oklahoma County (location 4) and as far east as McCurtain County (location 6). Previously, *L. mexicana* was observed in highest concentrations in rural locations, with its abundance in urban locations similarly great (Brundage, Bros, & Honda, 2011). Location 4, a suburban backyard, more closely represented an urban habitat while Location 6, a wildlife refuge area, was supremely rural. This sampling data confirmed the fairly cosmopolitan habitat preferences of *L. mexicana* in Oklahoma, and should serve as a template for future studies on *L. mexicana* prevalence and distribution.

2.4.3. Spatial and temporal distribution of Oklahoma Diptera populations

Within the state of Oklahoma, most carrion-associating flies exhibit seasonal behavior, with peaks of activity during spring and summer months of the year, and within that time, the warmer parts of the day (Brundage, et al., 2011). This was typically observed during research sampling. Oklahoma experiences short, mild, often wet winters, with warmer temperatures stretching from early March to late November (Oklahoma Climatological Survey, 2013). These conditions promoted a long breeding season, therein greatly influencing blowfly abundance. However, from 2010 to 2012, most of the state of Oklahoma experienced excessive heat, lack of precipitation, and typical strong plains winds which contributed to severe drought (Figure 17). This restricted water and resource availability to farmers, livestock, and wildlife, as well as Diptera populations. Additionally, decomposing remains (including bait used in this study) progressed through the stages of decomposition to desiccation much more rapidly. High temperatures and arid weather conditions influenced the relative abundance of specific blow fly

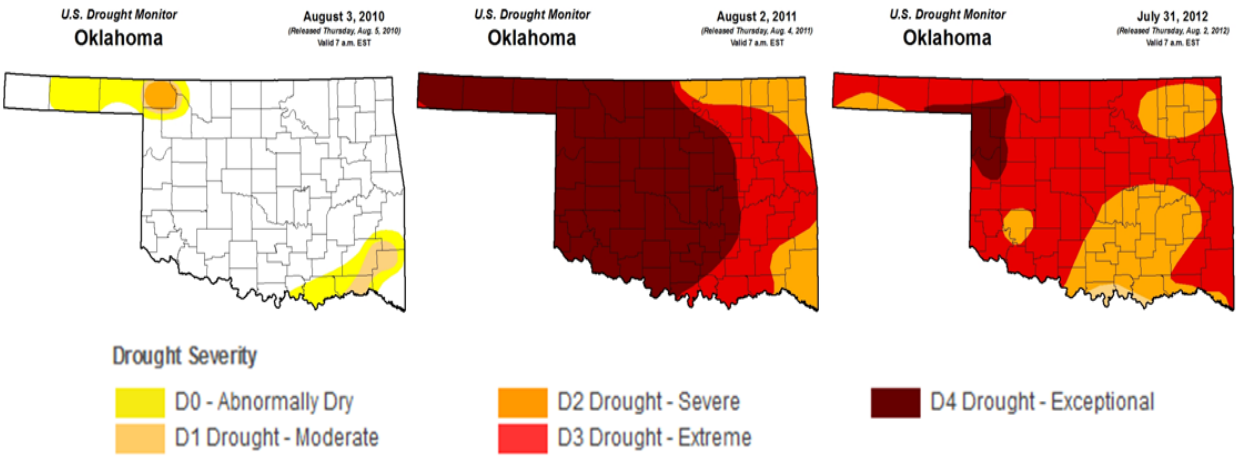

Figure 17: U.S. Drought Monitor maps of Oklahoma for three consecutive summers depicting severity of drought conditions across the state for selected dates. From left to right: August 3, 2010; August 2, 2011; and July 31, 2012 (National Drought Mitigation Center, 2013).

species and led to significant variation in families and species collected in each location (Figure 18).

Between 2010 and 2011, the Sarcophagidae and *Lucilia* populations dropped below ten percent of the entire total of carrion associating species collected. Members of Chrysomyinae were the dominant subset of Diptera populations collected for 2011, but then dropped to nearly zero in 2012. The extreme climate conditions likely contributed to a lack of optimal feeding and breeding sites, in conjunction with an increase in resource competition. Resurgence in *L. sericata* and *L. mexicana* blowflies was seen in 2012, possibly due to reduced competition with other carrion-associating species.

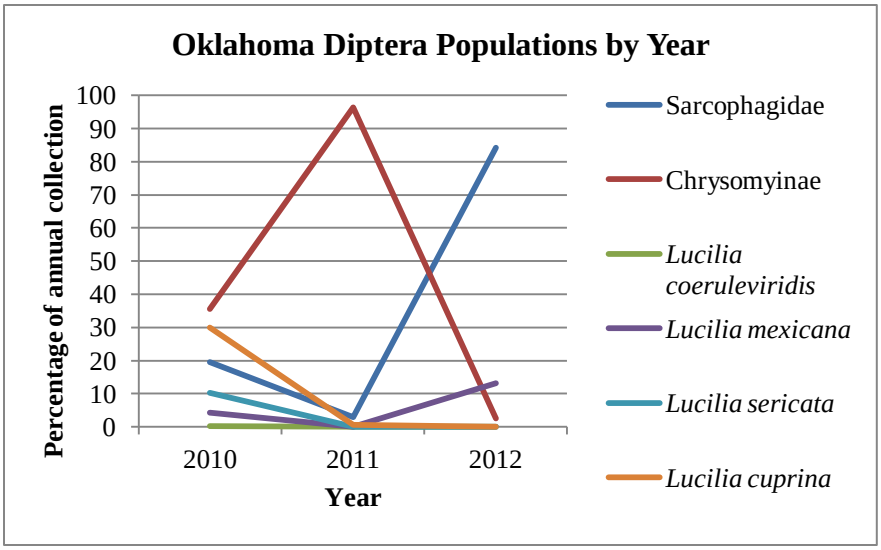

Figure 18: Oklahoma Diptera population concentration based on annual percentage proportions of blowfly.

In Oklahoma, habitat overlap was observed among members of Sarcophagidae, Luciliinae, and Chrysomyinae. In two sampling locations (1 and 7) where carrion was utilized as trap bait, a significantly higher proportion of Chrysomyinae were collected, followed by a smaller number of Sarcophagidae. This suggests Chrysomyinae and Sarcophagidae flies are in higher abundance than Luciliinae in rural locations. A large proportion of *Lucilia* cuprina and *L. sericata*, which are more often found in urban locales, were collected from rural north- and southwestern Oklahoma. *L. mexicana* displayed more cosmopolitan habitat preferences. Its presence in both urban and rural locations substantiates previous research (Brundage, et al., 2011).

2.4.3. Appledore Island blowfly populations

Identification of blowfly specimens collected on Appledore Island illustrated the prevalence of Chrysomyinae species on the island, followed by some Luciliinae. The majority of *Lucilia* species identified were of distinctly northern species (*Lucilia silvarum* and *Lucilia illustris*), with one *Lucilia sericata* collected. *Lucilia sericata* has been documented in the northern latitudes of the U.S. and Canada, but taxonomic keys describe it being more prevalent than encountered in this research (Marshall, et al., 2011). The island's geographically distance location offshore may have isolated this population. Competition for habitat and resources against other well adapted *Lucilia* sampled may have a direct affect on Luciliinae proportions on the island. However, had morphological damage to other specimens during shipment not occurred, a higher number of *Lucilia* (more specifically *Lucilia sericata*) may have been present.

2.4.4. Implications for forensic entomology

Forensic entomologists and researchers lacking proper training and knowledge of specific, often minute morphological features, or who rely too heavily on coloration or

inconsistent measurements, may subjectively misidentify a specimen. This is potentially detrimental to investigations and an entomologist's reputation. Estimation of post mortem intervals may be affected as well. *L. sericata, L. cuprina* and *L. mexicana* have similar developmental rates, and accurate identification is essential for PMI calculations (see Appendix E). Fundamental to identification of any forensically relevant insect is understanding each species encountered, its habitat, and any possible migration or overlap into another morphologically similar species' habitat. This research showed the presence of habitat overlap in selected locations between *Lucilia* species, and confirmed the research hypothesis of the presence of *L. mexicana* within Oklahoma. Further research should focus on documenting the area of *L. mexicana's* habitat across the state. For crime scene and investigative applications, development rates of *L. mexicana* should be assessed in regard to Oklahoma's climate typical.

Taxonomic keys continue to be the principle method for identification of forensically relevant blowflies because of their universal availability and production of a rapid identification. However, they should be used with caution when attempting to identify similar species with known or presumed habitat overlap. As well, morphological variation observed within and between species is significant enough to necessitate a more robust and definitive identification technique. The following chapter describes the use of DNA-based classification techniques as they were applied to blowflies collected and morphologically identified herein.

3. GENETIC IDENTIFICATION OF BLOW FLIES: RELIABILITY AND EFFICACY OF PROPOSED TECHNIQUES

3.1. Limitations of Classical Morphological Diptera Identification

Often forensic entomological specimens are of poor quality, fragmented, or inadequately preserved, leading to inconclusive or incorrect morphological assessment (Goff, 1993). Additionally, specimens collected at crime scenes are frequently in the larval stage, with multiple Diptera species colonizing the same corpse (Hwang & Turner, 2005). These immature blowfly specimens are easily recovered from decaying tissue, but are extremely difficult to identify morphologically (Amendt, et al., 2004). In addition, rearing larval Diptera to adulthood is a time-consuming process, ranging from a few days to weeks (Anderson, 2000; Grassberger & Reiter, 2001). Correct species identification is central to accurately determining the PMI for a crime (Byrd & Castner, 2009). When the identity of entomological evidence cannot be ascertained via classical morphological taxonomy, alternative, reliable methods of identification must be employed.

3.1.1. Previous Diptera Genetic Research

Modern molecular biology technology, including advancements in insect DNA isolation and analysis, has allowed scientists to sequence entire genomes and clearly differentiate between morphologically similar species (Harvey et al., 2008; Malgorn & Coquoz, 1999; Nelson, Wallman, & Dowton, 2007; Stevens & Wall, 2001; Wells & Sperling, 2001). DNA-based identification techniques continue to offer rapid and accurate genetic assessments, and have demonstrated the ability to produce satisfactory results with limited amounts of specimen material (Sunnucks & Hales, 1996; Trewick, 2000), including fragmented larvae and pupae (Benecke, 1998; Malgorn & Coquoz, 1999).

3.1.1.1. Random amplified polymorphic DNA typing

A variety of regions have been investigated for their utility in genetically identifying Diptera. Benecke (1998) used random amplified polymorphic DNA (RAPD) typing to differentiate larvae and pupae, while Stevens & Wall (1996) conducted a worldwide comparison of *Lucilia cuprina* and *L. sericata* populations. Advantages to RAPD analysis were a low chance of sample contamination and quick processing, but Benecke's study only determined exclusions, or non-matches, of specimens. Stevens and Wall definitively identified species and sub-species, but supported their RAPD analysis with sequencing an additional mitochondrial gene. Confirming specific species using RAPD typing is not typically successful or reliable, because the possibility of finding the same randomly amplified pattern, as well as the potential for inconsistency between laboratories (amplifying different RAPD products with the same primer sets) exists (Hadrys, et al., 1992; Schierwater & Ender, 1993).

3.1.1.2. PCR-restriction fragment length polymorphism analysis

Another rapid analysis technique was polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), in which a region of DNA was amplified with primers, and then digested with appropriate restriction enzymes (Rassmussen, 2012). Studies targeting a 2,300 base pair fragment containing 129 different restriction enzymes have been successful in identifying particular *Lucilia* species (Sperling, et al., 1994). However, because of the size of the fragment analyzed this method was considered time-consuming, expensive, and unreliable. Comparison reproducibility studies demonstrated that while some Diptera species could be identified, if the restriction enzyme was unable to splice DNA, or cut it in an incorrect location, identification was impossible (Schroeder, et al., 2003). Their research was not able to differentiate blowfly specimens sampled in Germany from United States specimens analyzed

used in Sperling, et al. (1994). Also, this approach has been proven unsuitable for high-throughput analyses (Rassmussen, 2012).

3.1.1.3. 28S large subunit of nuclear ribosomal DNA

The 28S large subunit of nuclear ribosomal DNA (Figure 19) has historically been a heavily sequenced genetic location for the identification of evolutionary relationships (Gillespie, et al., 2006). The 28S region within rDNA is identified by divergent domains, or "D" regions separated by conserved areas, as depicted in Figure 20. The D regions are separated by conserved sequences, and exhibit a higher rate of insertion and deletion, thereby making them an appropriate choice to study phylogenetic similarities and differences among both older (higher) evolutionary relationships and those more closely related species (Gibson, et al., 2011). Studies have shown the efficacy of sequencing both the D1 and D2 region for resolving members of closely related species (Baldwin, 1992; Larson, 1991; Stevens & Wall, 2001; Verma & Serajuddin, 2012). Unlike mitochondrial markers, which alone cannot distinguish species hybridization, ribosomal markers, like the 28S D1 or D2 region, have proven effective (Sonnenberg, et al., 2007). Still, mitochondrial analyses continue to be one of the foremost options for researchers investigating the phylogenetic identities of forensically important Diptera, warranting consideration herein (DeBry, et al., 2013; Guo, et al., 2011;

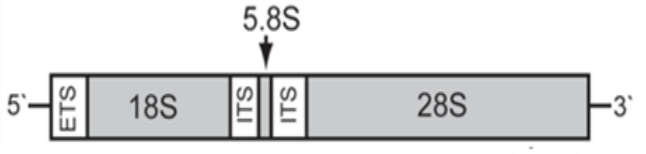

Figure 19: Location of 28S large subunit of nuclear ribosomal DNA. Located downstream of internal transcribed spacers I and II is the nuclear encoded 28S region (adapted from Gillespie et al., 2006).

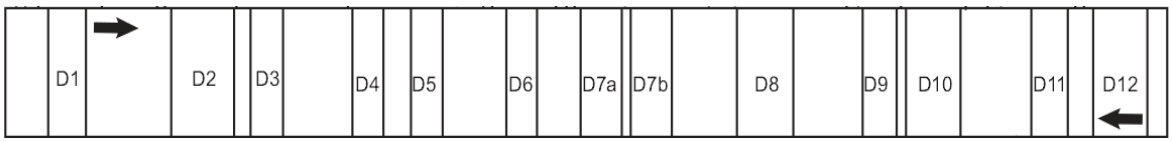

Figure 20: Divergent "D" Domains of the 28S gene. The 28S gene is numbered in the 5' to 3' direction of DNA and separated by conserved regions. A portion of the D2 region was selected for this research project (adapted from Gibson, et al., 2011).

Harvey, et al., 2008; Lundt, et al., 1996; Malgorn & Coquoz, 1999; Preativatanyou, et al., 2010; Sonet, et al., 2012; Sperling, et al., 1994; Stevens & Wall, 1997; Wells, et al., 2007).

3.1.1.4. Cytochrome oxidase subunits I and II.

Predominantly, research has investigated the utility of the cytochrome oxidase subunits I and II for species identification (Lunt, et al., 1996; Malgorn & Coquoz, 1999; Sperling, et al. 1994; Wallman & Donnellan, 2001; Wells, et al., 2001; Wells & Sperling, 2001). Located within the mitochondrial genome, these two regions are often chosen for genetic research due to their easy isolation, high copy number, and conserved function across diverse phylum (Schroeder, et al., 2003). Figure 21 shows the approximate location within the mitochondrial genome and relative sizes (base pairs) for the COI and COII genes. Researchers have worked with large and small DNA sequences in order to successfully identify forensically-relevant species: *Phormia regina, Lucilia sericata,* and *Lucilia illustris* (Sperling et al., 1994); *Calliphora vicina, Lucilia ampullacea, L. caesar, L. illustris, and L. sericata* (Malgorn & Coquoz, 1999); *Lucilia cuprina* and *L. sericata* (Stevens, et al., 2002); and *Lucilia coeruleiviridis* and *L. mexicana* (DeBry, et al., 2013).

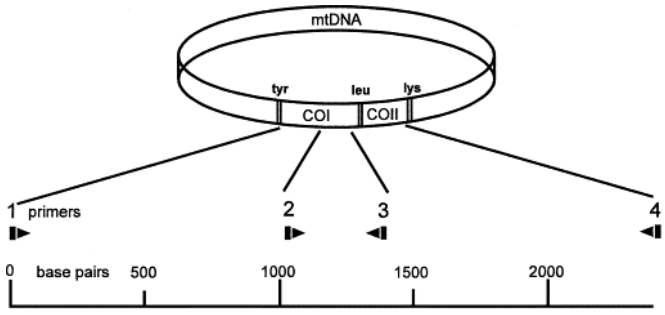

Figure 21: Location of COI and COII genes within mitochondrial DNA. Relative size (base pairs) shown along bottom. The gene for leucine (leu) separates the two subunits (adapted from Sperling et al., 1994).

3.1.1.5. Challenges to cytochrome oxidase genetic analysis and identification.

In its infancy, genetic identification of forensically-important blowflies aimed to simply identify a specimen to a genus and potentially species. As the quantity of published data increased, so too did the realization that both spatial variability and habitat diversity of Dipteran species played a key role in population dynamics. This variability included between carrion-

associating families and subfamilies, as well as within geographically distinct species. Genetic assessments of Diptera in geographically isolated (e.g. islands) or spatial and temporally distant locales (e.g. China versus South Africa) have alluded to the existence of various clades or sub-species (Guo, et al., 2011; Stevens, et al., 2002; Tourle, et al., 2009). In a study of forensically-relevant Diptera from numerous countries around the globe, Harvey, et al. (2008) found that a large, 1167bp COI sequence was not sufficient for separating the morphologically similar *Chrysomyia saffranea* and *Ch. megacephala*, nor *Calliphora stygia* and *C. albifrontalis*. In addition, the sequencing of such a large portion of the COI gene would require multiple smaller fragments to be amplified, sequenced, and then aligned, a time- and resource-consuming process.

The extensive use and proliferation of data from mtDNA studies has left some researchers to conclude that quantity does not equal quality, and that the utility of DNA-based identification has actually declined. Wells, et al. (2007) criticized past studies, citing poor experimental design, failed ability for replication, and inadequately small sample sizes (for phylogenetic comparisons). Their work recommends the utility of performing additional site sequencing to COI analyses. Since then, many researchers have combined COI analyses with an additional genetic region, such as 16s rDNA (Guo, et al., 2011), COII (Preativatanyou, et al., 2010), internal transcribed spacer II (Sonet, et al., 2012), or 28S rDNA (DeBry, et al., 2010; Tourle, et al., 2009). DeBry, et al., (2010). COI genetic identification supplemented previous research defining the sub-species of *Lucilia cuprina cuprina* from *Lucilia cuprina dorsalis*, but was facilitated by the addition of 28S rDNA sequencing (Stevens, et al., 2002). This data supported earlier predictions of two separate *L. cuprina* clades (Waterhouse & Paramonov, 1950). Morphological identifications performed and reported in chapter two of this thesis were

ambiguous for some *L. cuprina* and *L. sericata*, warranting further investigation of the possibility for intermediate or sub-species in Oklahoma.

Stevens, et al. conducted a study in Hawaii which demonstrated that *Lucilia cuprina* and *Lucilia sericata* had the identical COI lineage, but produced separate branches when 28S rDNA was genotyped. The Hawaiian *L. cuprina* was also determined to be genetically distinct from mainland *L. cuprina*. Researchers concluded the genetic data identified not two, but three species: *L. sericata,* Hawaiian *L. cuprina* (as they are confined to an isolated geographic area), and all other *L. cuprina* (Stevens, et al., 2002). Subsequent studies demonstrated that the closely related species, *Lucilia illustris* and *Lucilia caesar* could not be distinguished using shorter sequences (approximately 200bp in length) within the COI region, and some *L. cuprina* were assigned to incorrect lineages. In summation, researchers have concluded that based on analysis of the COI region, the entire *Lucilia* genus shares a more recent common ancestor with each other than with any other lineage on a phylogenetic tree. This lack of reciprocal monophyly was determined to be common for *Lucilia*, and challenging for resolving an unknown specimen to species level (Wells, et al., 2007).

3.1.2. Analysis of Genetic Variations and Distances

When two taxa share a more recent common ancestor with each other than with any other taxon members, while remaining in the same monophyletic group or clade, they are said to exhibit reciprocal monophyly (Zhu, et al., 2011). Genetic variants of single Diptera species are expected to group together when analyzed phylogenetically (Stevens & Wall, 1997). The utilization of geographic phylogenetic analysis aids in defining the "branches" of a phylogenetic tree, therein showing the degrees of relatedness or non-relatedness similar species may exhibit genetically. Morphologically similar species (i.e. *L. cuprina* and *L. sericata*) are predicted to

cluster closer together than with other *Lucilia* when defined via DNA-based methodology. It is optimal to have monophyletic or reciprocally monophyletic grouping when defining taxonomic phylogenetic trees. Previously, it was determined that DNA-based identification of closely related species was likely unreliable unless a condition of reciprocal monophyly was to exist for that species (Funk & Omland, 2003; Palumbi, et al., 2001). Hence, within the genus *Lucilia*, more discretion must be used when utilizing a COI-specific, DNA-based approach to identify species, whether through increased sample size, analyzation of longer fragments, or in combination with other evolutionary markers from mitochondrial or nuclear ribosomal genes.

Probems with analyzing phylogenies based on genetic markers arise when Diptera populations are undersampled, or genetic sequences are too short, causing intra- and interspecific variation data to overlap, therein reducing phylogenetic confidence (Harvey, et al., 2008). This has also occurred within some genera in isolated geographic regions. These geographic clades exhibit a higher degree of intraspecific variation, or variation within the species. This variation suggests some species may be more closely related genetically than was previously believed.

3.1.3. Potential for *Lucilia* species hybridization.

A handful of researchers proposed the possibility of hybridization between similar species of *Lucilia* (Sonnenberg, et al., 2007; Stevens, et al., 2002; Stevens & Wall, 1996). Besides the previously mentioned Hawaiian clade, *Lucilia cuprina* has subsequently been classified into two subspecies, *L. c. dorsalis*, commonly found in the New World, Asia, Indonesia, and Oceania, and *L. c. cuprina*, found in Afrotropical and Australasian regions (Harvey, et al., 2008). As early as 1950 researchers Waterhouse and Paramonov concluded, by means of morphological identification alone, the existence of these two Lucilia subspecies. In laboratory settings, both *L. c. cuprina* and *L. c. dorsalis* have been documented to interbreed,

with evidence that this has already occurred in areas of Australia (Norris, 1990). Research attempting to cross *Lucilia sericata* and *Lucilia cuprina* species, while successful, only produces viable offspring between male *L. cuprina* and female *L. sericata* (Ullyet, 1945; Waterhouse & Paramonov, 1950). This information furthers the prospect that Oklahoma *Lucilia* populations could exhibit hybridization.

3.1.4. Relevance of DNA-based Methods to Proposed Research

In areas of spatial confluence, genetic assessment of blowfly species have application to identify forensically important blowflies in specific geographic regions, such as Oklahoma, where populations of closely related species display variable morphological characteristics as well as habitat overlap. Morphological identification data described in Chapter 2 established the presence of *Lucilia mexicana* within Oklahoma, as well as the nearly indistinguishable *Lucilia cuprina* and *Lucilia sericata* species. The possibility of hybridization between the two latter blowfly populations has been enhanced by the fact that Oklahoma's central location serves as a bridge between converging geographic regions (as seen in Figure 3). As well, the spatially isolated Appledore Island sampling could have the potential to harbor separate Lucilia species clade populations. By utilizing a DNA-based identification approach, genetic markers from these species were sequenced and phylogenetically evaluated.

3.1.5. Research Objectives

The research objectives for this portion of the study were to (1) develop an effective and reliable laboratory protocol for sequencing the variable region of the COI mitochondrial and 28S rDNA genes, (2) investigate previous morphological identification of *L. mexicana* collected in Oklahoma, and (3) describe the amount of genetic variation observed in *Lucilia* blowflies

collected in Oklahoma and on Appledore Island. To accomplish these objectives, the following hypotheses were tested:

H_1 = Within sampled populations of carrion-associating Diptera in Oklahoma, the presence of *Lucilia mexicana* will be confirmed via genetic identifications.

H_0 = Within sampled populations of carrion-associating Diptera in Oklahoma, the presence of *Lucilia mexicana* will *not* be confirmed via genetic identifications.

Presence or absence of genetic variation followed the hypotheses:

H_1 = Genetic variation observed in COI sequence data will be significantly higher than genetic variation observed in 28S sequence data obtained from populations of *Lucilia spp.* collected in Oklahoma and on Appledore Island.

H_0 = Genetic variation observed in COI sequence data will *not* be significantly higher than genetic variation observed in 28S sequence data obtained from populations of *Lucilia spp.* collected in Oklahoma and on Appledore Island.

3.2. Materials and Methods

3.2.1. DNA sequencing of COI mtDNA and 28S rDNA in *Lucilia spp*.

In order to definitively classify collected *Lucilia* species, portions of the genetically distinct COI and 28S genes were analyzed by DNA sequencing. Novel primer pairs were designed for both regions using the online resources provided by the National Center for Biotechnology Information (NCBI) and corroborated with Primer3 software (Rozen & Sklatsky, 1998). Prior to ordering, all primers were systematically tested using NCBI's Basic Local Alignment Tool (BLAST) (Lobo, 2008) to confirm they would not bind to and amplify mammalian DNA. A 469 base pair portion of the COI gene was selected with the primer set: COI469F 5'- TTGGWCACCCTGAAGTTTA-3' and COI469R 5'-ATCCWGTAAATAAT

GGG-3'. Additionally, a 330 base pair fragment of the D2 region of 28s rDNA was targeted with the primer set: 28S330F 5'-GGTTAAGCCCGATGAACCTG-3' and 28S330R 5'-ACTCCTTG GTCCGTGTTTCA-3'. All oligonucleotide primers were ordered from Integrated DNA Technologies. All laboratory analyses were conducted in the Department of Biology at the University of Central Oklahoma.

When available, all six legs from an individual fly were removed, homogenized, and subjected to DNA extraction. DNA was extracted from each specimen using the DNeasy® Blood & Tissue kit (Qiagen, Venlo, Limburg). Protocol modifications included allowing the samples to lyse approximately 18-24 hours to facilitate the breaking down and lysis of the chitinous exoskeleton. Quantitation of extracted DNA was determined using a Thermo Scientific NanoDrop 2000, and blanked with elution buffer provided in the DNeasy® Blood & Tissue kit between readings. Following quantification, buffer optimization of PCR amplification reactions was performed using the FailSafe™ PCR PreMix Selection kit (Epicentre Technologies, Madison, Wisconsin). Reaction conditions for optimize were as follows: 2-5µL DNA template, 1.2µL each primer (10µM), 5u/µL Taq DNA polymerase (Promega, Madison, Wisconsin) 3.5µL FailSafe PreMix Buffer (100mM Tris-HCl, 100mM KCl, 400µM of each dNTP, 3-7mM $MgCl_2$, and 0-8X FailSafe PCR Enhancer), and deionized sterile water to achieve a final reaction volume of 20µL. Amplification of targeted COI and 28s regions utilized the Polymerase Chain Reaction (Mullis, 1990), with COI conditions: initial denaturation at 96ºC for 3min, 35 cycles at 94ºC for 30s, 42ºC for 30s, 72ºC for 59s, with a final extension of 72ºC for 7min; and 28s conditions: initial denaturation at 95ºC for 3min, 35 cycles at 94ºC for 30s, 55ºC for 30s, 72ºC for 59s, and a final extension of 72ºC for 7min.

Amplified genetic products were separated by electrophoresis in 2.0% Tris-borate/ETDA (TBE) agarose gels, stained with ethidium bromide and visualized under UV-illumination. Positive gel products were purified with the MinElute PCR Purification Kit (Qiagen, Venlo, Limburg), with a protocol modification of adding 30uL for final elution of DNA instead of 10uL to maximize DNA yield. Purified products were then run according to BigDye v3.1 (Applied Biosystems, 2004) protocols, and clean-up of excess dye-labeled dNTPs and other low molecular weight materials was achieved using Performa® DTR Gel Filtration Cartridges (EdgeBio, Gaithersburg, Maryland). Sanger sequencing was performed in two directions via capillary electrophoresis carried out on an Applied Biosystems® 3130 Genetic Analyzer (2007) with complementary software. DNA amplification and sequencing methods were replicated three times for each sample. Sequenced COI and 28S amplicons are listed in Appendix F and G, respectively.

3.2.2. Genetic data collection and subsequent analysis.

Assembled sequences were aligned with the software program MEGA 5.2 using ClustalW at default setting (Tamura, et al., 2011). Final sequence adjustments were made manually based on electropherogram data. Phylogenetic analyses employed the maximum composite likelihood method (Tamura, et al., 2004), which was able to show pairwise distances using the Tamura-Nei (1993) distance correction. Phylogenetic bootstrapping was calculated using 1,000 pseudoreplicates.

3.2.3. Choice of phylogenetic outgroup.

For decades, taxonomic definitions within the Calliphoridae family have been subjected to revisions, culminating in the morphological keys utilized for this study (Hall, 1948; Marshall et al., 2011; Whitworth, 2006). A large proportion of diptera collected in Oklahoma and on

Appledore Island were identified to the sister subfamily Chrysomyinae. It is generally well accepted today that the subfamilies Luciliinae and Chrysomyinae are sufficiently distinct for phylogenetic purposes (Rognes, 1997). Hence, the Chrysomyinae blowfly *Cochliomyia macellaria*, identified in both Oklahoma and Appledore Island collections, was used as a phylogenetic outgroup for this study.

3.3. Results

3.3.1. Cytochrome oxidase I (COI) data analysis.

Primers designed to amplify a 308bp portion of the COI gene were successful for 24 of 26 individuals analyzed. Two *L. cuprina*, four *L. sericata*, and eighteen *L. mexicana* specimens was sequenced, however the COI data obtained was shorter than anticipated. Agarose gel visualization of the COI PCR product confirmed that the expected 469bp amplicon was amplified, meaning that the reduction in size occurred during the sequencing step. Subsequent to sequencing, the fragment produced was 308bp in length. This was concluded to have been caused by incomplete removal of excess unincorporated dye labeled terminators during the purification process, thus causing dye blobs (Applied Biosystems, 2009). The presence of these dye blobs in the first 120 bases hindered accurate reading of base pair calls, leading to rejection of approximately eighty base pairs in the beginning of forward and reverse fragment sequences.

Nine potential haplogroups were identified from the COI data. *L. sericata* and *L. cuprina* each had one consensus haplogroup, respectively, while *L. mexicana* exhibited seven distinct haplogroups. Genetic distances were calculated in order to observe any patterns of variability (Table 1). A divergence of 0.003 (0.3%) was observed between *L. sericata* and *L. cuprina* samples, and a range of 0.0-0.027 (0.0-2.7%) intraspecific divergence was between *L. sericata/L. cuprina* and *L. mexicana* samples. Within the *L. mexicana* haplogroups, the range of interspecific

Table 5: COI Pairwise Distances

			L. mexicana Haplogroups						
	L. sericata	*L. cuprina*	HG1	HG2	HG3	HG4	HG5	HG6	HG7
L. sericata	0.000								
L. cuprina	0.003								
L. mexicana HG1	0.003	0.007							
L. mexicana HG2	0.003	0.007	0.007						
L. mexicana HG3	0.010	0.013	0.013	0.013					
L. mexicana HG4	0.023	0.027	0.027	0.020	0.013				
L. mexicana HG5	0.003	0.007	0.007	0.007	0.007	0.020			
L. mexicana HG6	0.003	0.007	0.007	0.007	0.007	0.020	0.007		
L. mexicana HG7	0.000	0.003	0.003	0.003	0.010	0.023	0.003	0.003	

Intragenic and intraspecific COI variation among Lucilia species sequenced. Pairwise distances calculated represent the variation in base pair substitutions per site.

variation was 0.003-0.023 (0.3-2.3%). The overall mean evolutionary divergence for all COI sequences was calculated to be 1.0%, or 0.010 base substitutions per site, averaging across all sequences. This revealed lower variation within the COI genetic sequence marker.

Phylogenetic analysis of the same 308bp region of the COI gene was performed with GenBank reference sequences to show the expected grouping of Lucilia species (Figure 22). Expected phylogenetic separation among the species and subfamilies was observed for these reference sequences. Figure 23 displays the actual genetic phylogenetic analysis of Oklahoma *Lucilia* species obtained in this study. The shortened fragments were not able to discriminate between *L. mexicana* specimens, nor were *L. sericata* or *L. cuprina* able to be segregated

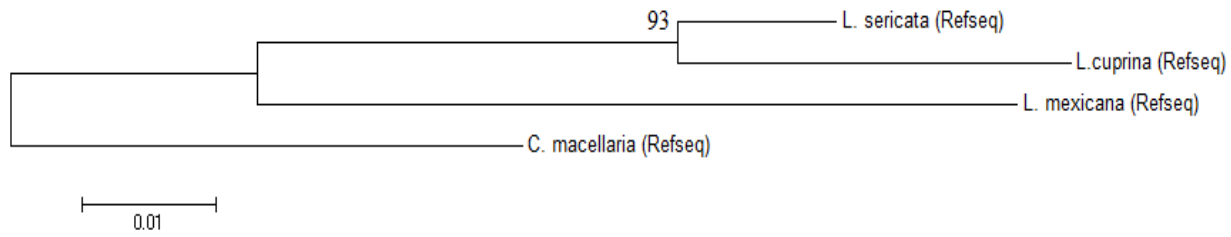

Figure 22: Expected phylogenetic grouping of *Lucilia* species utilizing the 308bp fragment from GenBank reference sequences (Refseq). Bootstrap confidence values are listed.

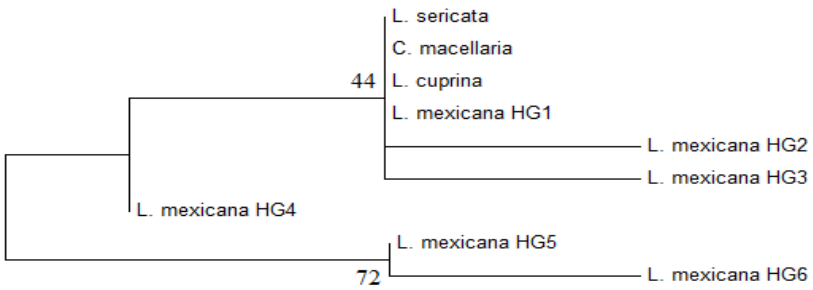

Figure 23: Phylogenetic separation of analyzed Oklahoma *Lucilia* specimens. The 308bp fragment was not sufficient for discriminating among selected Oklahoma species.

phylogenetically. Due to the poor phylogenetic data, genetic analyses were not performed on the Appledore Island specimens.

3.4.2. 28S nuclear ribosomal DNA data analysis.

Primers designed to amplify a 330bp region of 28S rDNA succeeded in producing the entire expected target sequence. Thirty-five of thirty-six Oklahoma samples were successfully sequenced, as well as eight of nine samples from Appledore Island. From the Oklahoma samples, 11 *Lucilia cuprina*, 8 *Lucilia sericata*, and 16 *Lucilia mexicana* specimens were sequenced, and of the Appledore Island population, 7 *Lucilia illustris* and 1 *Lucilia silvarum* were successfully sequenced using DNA-based techniques. From the sequence data, five distinct haplogroups were resolved; hence intraspecific distances were zero for each species. Within the *Lucilia* genus, pairwise variation was established (Table 6) and the overall mean evolutionary divergence for all 28S sequences was calculated at 2.8%, or 0.028 base substitutions per site, averaging across all

Table 6: 28S Pairwaise Distances

	L. sericata	L. cuprina	L. mexicana	L. illustris	L. silvarum
L. sericata					
L. cuprina	0.000				
L. mexicana	0.035	0.035			
L. illustris (Appledore Is.)	0.041	0.041	0.006		
L. silvarum (Appledore Is.)	0.019	0.019	0.041	0.041	

Interspecific variation among 28S genetic sequences for Oklahoma and Appledore Island *Lucilia spp.* collected.

sequences. This value indicated greater interspecific genetic conservation for 28S than COI genes.

A phylogenetic tree was produced from the 330bp 28S amplicon, with most species clearly defined from one another. *Lucilia* species collectively were monophyletic, however the genetic relationship between *Lucilia cuprina* and *L. sericata* was not separated completely (Figure 24).

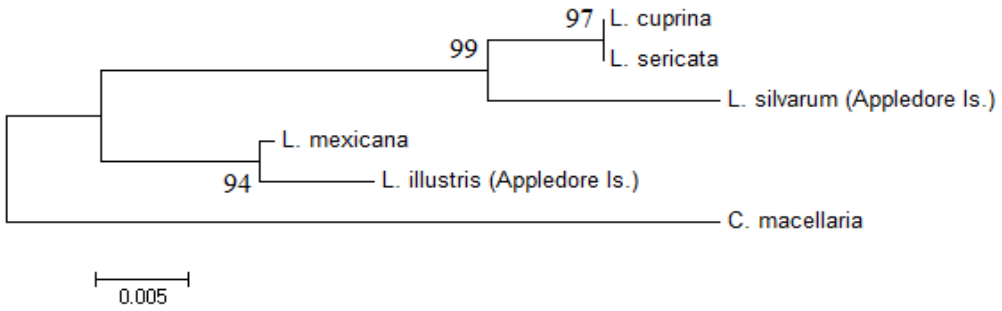

Figure 24: Phylogenetic analysis of the 28S rDNA genetic target sequence for Oklahoma and Appledore Island *Lucilia* collected. The closely related *L. cuprina* and *L. sericata* species could not be distinguished. Bootstrap values are listed

When GenBank reference sequences were included for phylogenetic analyses, *Lucilia sericata* samples sequenced grouped with *Lucilia cuprina* (Figure 25). This lack of reciprocal monophyly between the two species is consistent with previous studies (Harvey, et al., 2008;

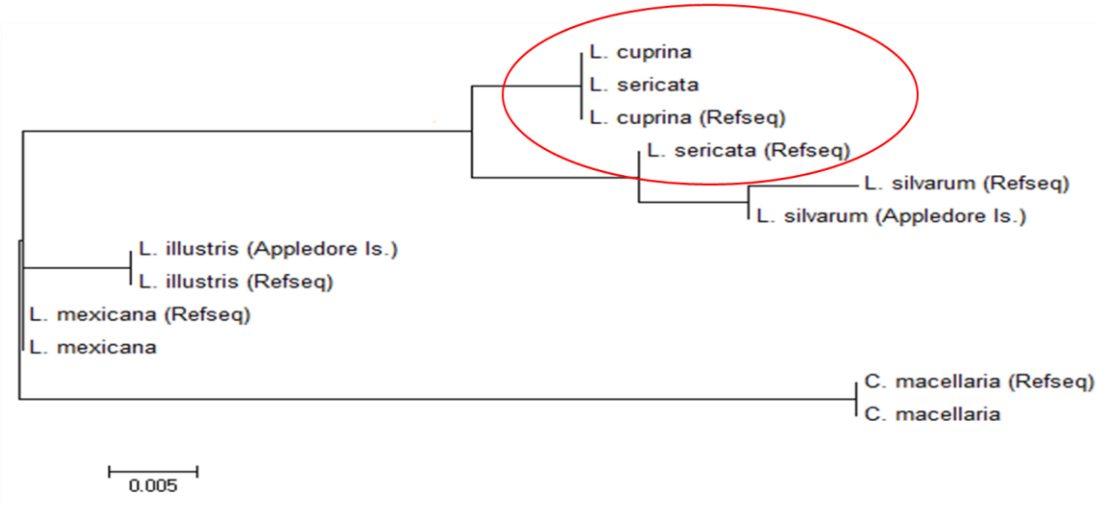

Figure 25: Comparison of 28S phylogenetic data with GenBank reference sequences (Refseq). A subset of the 28S phylogenetic tree (circled in red) demonstrates the grouping of Oklahoma *L. sericata* with Oklahoma and Refseq *L. cuprina*. *L. mexicana* collected in Oklahoma was correctly identified when compared to the Refseq.

Stevens et al., 2002; Stevens & Wall, 1996; Wells, et al., 2007).

3.4. Discussion

This study produced a genetic-based phylogenetic analysis of forensically-relevant blowfly species found in Oklahoma and on Appledore Island, ME. The efficacy of a 308bp length amplicon of the cytochrome oxidase I gene within mitochondrial DNA, and a 330bp length amplicon of the D2 region with 28S nuclear ribosomal DNA were tested. It was anticipated that the application of DNA-based species identification techniques developed in this study would provide an easier and more accurate means of differentiation between morphologically similar blowfly (Diptera: Calliphoridae) species.

3.4.1. Genetic identification of Calliphoridae subfamilies.

At a minimum, the ability to distinctly identify the Calliphoridae subfamilies Chrysomyinae and Luciliinae was successful with the 28S evolutionary marker chosen in this study (Figure 25). The support for this subfamily relationship grouping has previously been supported by molecular genetics research (Harvey, et al., 2008; Wallman, et al., 2005). These results are applicable for forensically relevant entomological evidence analysis where small quantities or poorly preserved insect specimens are recovered from crime scenes or off of suspects or victims. Known habitat preferences of Chrysomyinae and Luciliinae can further facilitate assessment of whether a corpse may have been moved from the initial death site to a different geographically distinct location (Goff, 1993; Lord, 1990).

While GenBank reference sequences correctly defined the outgroup *Cochliomyia macellaria* (subfamily Chrysomyinae) as distinct from Luciliinae species (Figure 22), use of COI genetic data obtained from Oklahoma specimens was insufficient in resolving species within these two subfamilies (Figure 23). Inadequate amplification of the targeted genetic sequence

produced indistinct phylogenies for both subfamilies. Variation in the fragments produced calls into question the reliability of this location for use in subfamily identifications.

3.4.2. First objective confirmed hypothesis H_1: *L. mexicana* blowflies are present in Oklahoma.

In chapter two of this thesis, classical morphological identification techniques taxonomically identified twenty-one Oklahoma blowfly specimens as *Lucilia mexicana*. Pairwise interspecific variation calculated for COI genetic fragments was variable between *L. mexicana* sequences (Table 5). From the eighteen *L. mexicana* samples subjected to molecular genetic sequencing, seven different haplogroups were identified. This COI phylogenetic data was not sufficient for clearly defining these as being reciprocally monophyletic to one another (Figure 23). This phylogenetic data neither confirms nor rejects the hypothesis of *L. mexicana's* presence in Oklahoma, but instead demonstrates the limitations of the COI genetic fragment sequenced.

Alternatively, the 28S target sequence was successful in confirming the distinct presence of *L. mexicana* collected in Oklahoma (Figure 24). This genetic sequence was appropriate for clearing identifying all other *Lucilia* species tested, establishing the utility of newly designed primers as well as laboratory protocols developed for defining members of the Calliphoridae family. The relatively short (330bp) 28S fragment sequence can be used singularly as a reliable dataset for species identification, without the necessity of analyzing an additional genetic location (e.g. COI). This information is especially significant for forensic investigations, as the genetic product produced from 28S analysis of small amounts of blowfly evidence can be used to quickly and accurately identify a particular specimen to species.

Previously the habitat range of *Lucilia mexicana* was known to stretch from California east to Texas (Whitworth, 2006). Genetic sequencing of this species predominantly researched

mitochondrial variations, and defined populations in California, New Mexico, and Texas (DeBry, et al., 2013; Stevens, 2003). The present study genetically identified via 28S nuclear ribosomal sequencing the presence of multiple *L. mexicana* specimens in Oklahoma.

3.4.3. Second objective confirmed hypothesis H_1: COI genetic variation was significantly higher than that observed in 28S data sets.

Among blowflies sampled from locations in Oklahoma and on Appledore Island, COI mtDNA variation was observed to be higher than that of 28S nuclear rDNA (Tables 5 and 6). Genetic differences among *L. mexicana* specimens were excessively variable (up to 2.3%), with sequences characterizing multiple paraphyletic *L. mexicana* haplogroups (Figure 23). This data suggests the ineffectiveness and unreliability of the genetic fragment amplified. Interspecific differences were low between *L. sericata* and *L. cuprina* (0.03%), nonetheless these two closely related species could not be distinguished monophyletically. Similar results have been encountered by other researchers studying COI fragments (DeBry et al., 2013; Harvey et al., 2008; Stevens & Wall, 1996, 1997; Stevens et al., 2002; Tourle et al., 2009; Wells et al., 2007). Funk & Omland (2003) stress the likelihood and reputation of mitochondrial DNA phylogenies to display paraphyly between closely related species. Only when a large (1000bp or more) COI segment is sequenced have results unambiguously separated *L. sericata* from *L. cuprina*, and the subspecies *L. cuprina cuprina* from *L. cuprina dorsalis* (DeBry et al., 2010; Harvey et al., 2008; Lunt et al., 1996; Stevens, 2003; Stevens et al., 2002; Wells & Sperling 2001).

By investigating the hypothesized value of 28S rDNA genetics, members of subfamily Luciliinae were accurately separated. Interspecific pairwise distances were low among nearly all *Lucilia* (Table 5), and the phylogenetic tree produced distinguished the monophyletic Luciliinae species from members of Chrysomyinae (Figure 24). However, a lack of reciprocal monophyly

existed between *L. sericata* and *L. cuprina* when compared with references sequences obtained from GenBank (Figure 25). 28S genetic research by Stevens & Wall (2001) supports the concept that this smaller region is sufficient for phylogenetic blowfly identification, with globally sampled *L. sericata* specimens branching separately from other *Lucilia* as well as members of subfamily Calliphorinae. Their study did not include *Lucilia cuprina*, leading to the assumption that Oklahoma *L. sericata* may in fact be *L. cuprina*.

3.4.4. Potential for hybridization in Oklahoma *Lucilia* populations.

As was hypothesized, the presence of *L. mexicana* was confirmed for populations of carrion-associating blowflies in Oklahoma. It was unexpected that *L. cuprina* and *L. sericata* would be genetically indistinguishable. Analysis of a fragment of COI mtDNA was insufficient for phylogenetic identification of forensically relevant species sampled in Oklahoma. Conversely, 28S genetic data established the potential presence of a separate *L. cuprina* clade within Oklahoma, one that is genetically indistinct, but morphologically identical to *L. sericata*. This suggests that hybridization has occurred among populations of *L. cuprina/L. sericata* in Oklahoma. A re-evaluation of 28S genetic data did not reveal hybridization at any locations within the sequences produced. It is expected that two peaks would be present at a single location (see Figure 26 as an example).

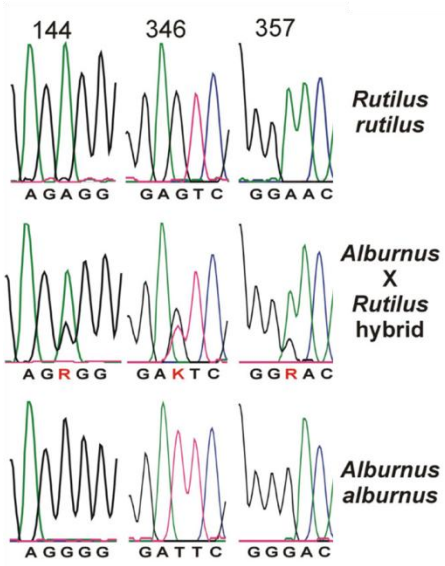

Figure 26: Example of suspected hybridization in fishes. Numbers represent sequence location where a double peak was observed, indicating an apparent hybrid (adapted from Sonnenberg, et al., 2007).

An alternative explanation was described by Tourle, et al. (2009), in which the ambiguous *L. sericata* 28S sequence, considered as a separate *L. cuprina* haplogroup, could actually represent a nuclear pseudogene

(a copied region of noncoding mitochondrial DNA found within the nuclear genome). The researchers describe the possibility of this occurring if universal primers (such as those often used for COI) co-amplified a nuclear pseudogene. It is unexpected that this has occurred during the research herein, since the 28S gene is located within nuclear DNA. Additionally, sequence analysis with MEGA automatically tests for the existence of stop codons, which could indicate the presence of a nuclear pseudogene (Buhay, 2009).

3.4.5. Value of DNA-based identification of forensic entomological samples.

The utility of definitive genetic identification of questioned samples is only worthwhile if police laboratories can afford to process them. Human allelic sequencing has become a streamlined process during criminal investigations, with companies designing reliable high-throughput DNA systems, therein lowering costs and reducing sample analysis time. Analysis of forensically relevant entomological specimens has been accomplished using similar techniques on the same machine technologies (as evidence by cited research and this study). Sequencing entire genes is undoubtedly a costly enterprise, ranging from $300-700 per gene sequenced (Nelson, et al., 2012). The use of DNA barcoding, an alternative genetic identification technique that sequences smaller, previously defined locations (such as the first 500bp of the COI gene), has been estimated to cost $5-10 per sample (Cameron, et al., 2006). These researchers noted that the scientific community has yet to decide on the non-monetary advantages of DNA barcoding, with disagreements on gene choice, reliability of genetic information produced (i.e. COI discrepancies), and the fact that identification of wild species in their native habitat is not feasible without first trapping them (i.e. birds). Meanwhile, morphological identification remains the fastest (but not necessarily reliable) technique for forensic species identification.

The greatest hurdle the forensic entomologist or investigator faces in the use of DNA-based identifications is the lack of laboratory and quality control standards. Guidelines have been established for human identity testing that describe proper protocols for evidence collection and storage, laboratory methodology and technology validation, chain of custody documentation, calculation of error rates, et cetera (National Research Council, 1996). There is no consensus on methodology for testing forensically relevant entomological evidence, and analysts (trained entomologist or not) must develop a "best practices" approach to insect identification.

4. CONCLUSION

For selected populations of Oklahoma and Appledore Island blowflies, this research demonstrated the ambiguities associated with both morphological and molecular DNA identification. Morphological identification of forensically relevant Calliphoridae is notoriously difficult, especially between closely related species that display similar taxonomically defining characteristics and known habitat overlap. Use of published taxonomic keys (Whitworth, 2006) provided quick and accurate identification of carrion-associating Diptera subfamilies. Complications arose when attempting to classify *L. sericata* and *L. cuprina* because minute setae were difficult to distinguish, and suggested taxonomic measurements were inconsistent for application to this research. The subjectivity associated with visualization of morphological characteristics holds great potential for species misidentification.

Habitat preferences of sampled blowfly populations reconfirmed the prevalence and diversity of necrophagous Diptera in Oklahoma and on Appledore Island, ME. In the western half of Oklahoma sampling locations, the prevalence of Chrysomyinae and Luciliinae species was skewed toward one or the other (i.e. neither was proportionally sampled at a given location). The eastern sampling locations showed a more cosmopolitan distribution between subfamilies collected. Most significantly, the presence of *L. mexicana* was confirmed in multiple Oklahoma locations. To date, the presence and prevalence of *Lucilia* populations in varying ecoregions of Oklahoma and on Appledore Island have not been documented. The data acquired from this research are a significant addition to the field of forensic entomology.

This study also evaluated the COI (mtDNA) and 28S (rDNA) regions as potential markers for identification of Lucilia species. Novel, Diptera-specific primer sets were developed for amplification of targeted COI and 28S fragments. DNA-based assessment of the COI genetic

fragment proved ineffective, producing a shorter than expected sequence with reduced phylogenetic resolution. It was concluded that this location was not optimal for distinguishing Calliphoridae species, nor was it reliable in separating subfamilies. As cited previously, sequencing large (>1000bp) fragments or entire genes has produced robust identification results, but would be costly and time-consuming, since multiple genetic fragments would have to be sequenced in tandem and then aligned for consensus.

Data obtained from sequencing a 330bp section of 28S rDNA did confirm morphological identifications made in chapter two. Subsequent robust phylogenetic analyses were successful in classifying Calliphoridae subfamilies, as well as separating Luciliinae species. An exception to this was the lack of delineation between *L. sericata* and *L. cuprina* species. The indistinctiveness of key taxonomic features used in morphological identification was analogous to the genetic data produced. This method for DNA-based identification further confirms the close genetic relationship of *Lucilia cuprina* and *Lucilia sericata*. Short of reassessing morphological features used to taxonomically separate these two species, the prospect of *L. sericata/L. cuprina* hybridization in Oklahoma may prove relevant for future research. Although the possibility of morphological misidentification cannot be excluded, this research shows some specimens have the phenotype of one species (*L. sericata*) but a genotype for another (*L. cuprina*). Additional sampling across all Oklahoma ecoregions (in conjunction with complementary ecological data) may establish the extent of encountered phenotypic variation between *L. sericata* and *L. cuprina*, as well as improve known habitat distribution of migrating *L. mexicana*. This information can be an influential factor in determining PMI for forensic investigations.

In the realm of forensic entomology, court cases are often delayed by the time-consuming process of entomological identification and verification of evidence specimens. Molecular

analysis rapidly increases production and validation of results. However, speed and reliability of techniques and the cost of reagents, equipment and personnel are important factors for forensic laboratories to consider. The lab procedures developed in this study simplify specimen identification processes, minimize the need for advanced taxonomic training, and enhance the accuracy and reliability of blowfly species identification for forensic investigators. Overall, the results produced herein effectively strengthen the field of forensic molecular entomology.

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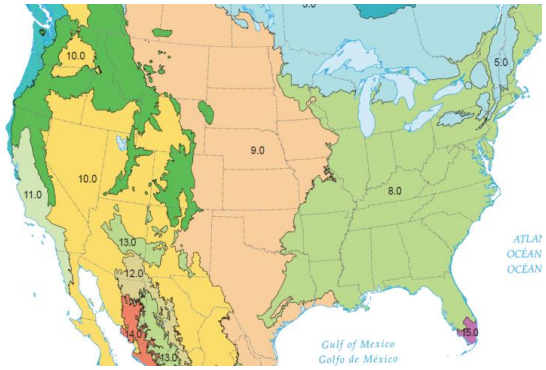
Appendix A: United States Ecoregions

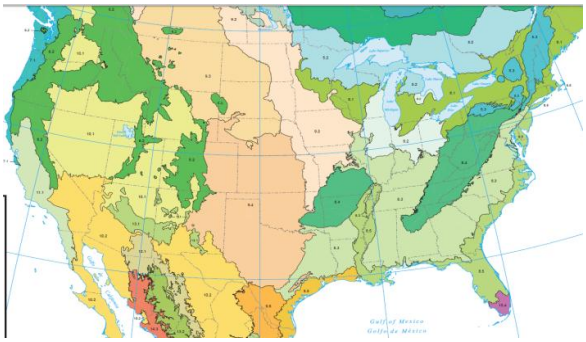
Level I

Ecoregions are defined by precipitation amounts and temperatures. Numbers correspond to specific regions: (5.0) Northern Forests; (8.0) Eastern Temperate Forests; (9.0) Great Plains; (10.0) North American Deserts; (11.0) Mediterranean California; (12.0) Southern Semi-arid Highlands; (13.0) Temperate Sierras; (15.0) Tropical Wetland Forests.



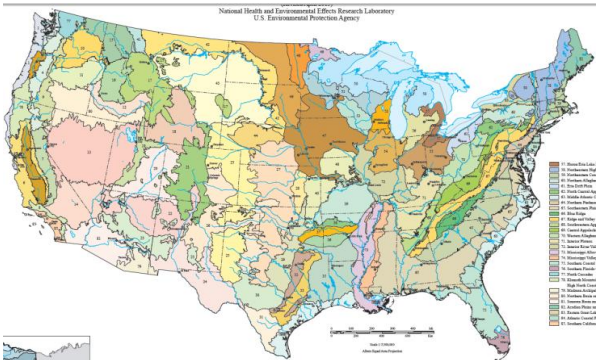
Level I

United States ecoregions from Level I are further divided into subregions. These are also based on precipitation levels and patterns and temperature differences, but on a microclimate scale.



Level III

From each Level II subregion, vegetation present or other natural landcovers are assessed and serve as differentiating factors for this third level of ecoregion distinction.



Level IV

This level is defined by terrain features within the above Level III regions. Mountainous areas are also defined by their ecological zones relative to elevation.

(USEPA, 2005)

Appendix B. Ecoregions of Oklahoma	
Region	**Location and Characteristics**
Arkansas Valley	East central Oklahoma; home to hardwood forested valleys and rugged ridges.
Boston Mountains	Along the southern border of the Arkansas Valley region; heavily forested with red oak, white oak, and hickory trees with a sandstone and shale composition.
Central Great Plains	Much of the western half of the state; home to flat farm and ranchland.
Central Irregular Plains	Irregular composition of forests and grassland along with varied land use, from farms and ranches to urban cities to mining.
Cross Timbers	Also known as the Central Oklahoma/Texas Plains; transitional area between farmland to the west and forests to the east.
East Central Texas Plains	Originally covered by post oak and other vegetation, but most of the region has been converted into rangeland for livestock.
Flint Hills	Home to the Tallgrass Prairie; with a prevalence of limestone and shale hills.
High Plains	In the Western panhandle of the state; are higher in elevation and more arid than the Central Great Plains and are comprised predominately of various types of grassland.
Ouachita Mountains	Eastern Oklahoma; home to pine-filled ridges with commercial logging the primary land use.
Ozark Highlands	Northeastern Oklahoma; heavily forested with small areas of pasture and crop land.
South Central Plains	Characterized as the “piney woods”, with the majority of the area covered by pine forests.
Southwestern Tablelands	Areas of western Oklahoma; uniquely elevated and comprised of both sub-humid and semiarid grasslands.

Appendix C: Oklahoma Diptera Reference Sample

Specimen #	Collection Date	Sampling Location	Family	Subfamily	Genus	Species
1	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
2	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
3	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
4	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
5	8-Jul-10	1	Sarcophagidae			
6	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
7	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
8	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
9	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
10	8-Jul-10	1	Calliphoridae	Chrysomyinae	Phormia	regina
11	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
12	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
13	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
14	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
15	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
16	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
17	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
18	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
19	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
20	8-Jul-10	1	Sarcophagidae			
21	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
22	8-Jul-10	1	Sarcophagidae			
23	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
24	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
25	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
26	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
27	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
28	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
29	8-Jul-10	1	NSID			
30	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
31	8-Jul-10	1	Sarcophagidae			
32	8-Jul-10	1	Calliphoridae	Chrysomyinae	Phormia	regina
33	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
34	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
35	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
36	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
37	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
38	8-Jul-10	1	Sarcophagidae			
39	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria

40	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
41	8-Jul-10	1	Calliphoridae	Chrysomyinae	Phormia	regina
42	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
43	8-Jul-10	1	Calliphoridae	Chrysomyinae	Phormia	regina
44	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
45	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
46	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
47	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
48	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
49	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
50	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
51	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
52	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
53	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
54	8-Jul-10	1	Calliphoridae	Chrysomyinae	Phormia	regina
55	8-Jul-10	1	Calliphoridae	Chrysomyinae	Phormia	regina
56	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
57	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
58	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
59	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
60	8-Jul-10	1	Sarcophagidae			
61	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
62	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
63	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
64	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
65	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
66	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
67	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
68	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
69	8-Jul-10	1	Sarcophagidae			
70	8-Jul-10	1	Sarcophagidae			
71	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
72	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
73	8-Jul-10	1	Calliphoridae	Chrysomyinae	Phormia	regina
74	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
75	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
76	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
77	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
78	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
79	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
80	8-Jul-10	1	Sarcophagidae			

81	8-Jul-10	1	Sarcophagidae			
82	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
83	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
84	8-Jul-10	1	Sarcophagidae			
85	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
86	8-Jul-10	1	Sarcophagidae			
87	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
88	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
89	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
90	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
91	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
92	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
93	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
94	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
95	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
96	8-Jul-10	1	Calliphoridae	Chrysomyinae	Phormia	regina
97	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
98	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
99	8-Jul-10	1	Calliphoridae	Chrysomyinae	Phormia	regina
100	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
101	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
102	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
103	8-Jul-10	1	Calliphoridae	Luciliinae	Lucilia	sericata
104	8-Jul-10	1	Calliphoridae	Chrysomyinae	Chrysomya	megacephala
105	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
106	8-Jul-10	1	Sarcophagidae			
107	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
108	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
109	8-Jul-10	1	Calliphoridae	Chrysomyinae	Phormia	regina
110	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
111	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
112	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
113	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
114	8-Jul-10	1	Calliphoridae	Chrysomyinae	Phormia	regina
115	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
116	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
117	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
118	8-Jul-10	1	Sarcophagidae			
119	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
120	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
121	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria

122	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
123	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
124	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
125	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
126	8-Jul-10	1	Sarcophagidae			
127	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
128	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
129	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
130	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
131	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
132	8-Jul-10	1	Calliphoridae	Chrysomyinae	Phormia	regina
133	8-Jul-10	1	Sarcophagidae			
134	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
135	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
136	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
137	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
138	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
139	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
140	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
141	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
142	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
143	8-Jul-10	1	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
144	15-Jul-10	2	Sarcophagidae			
145	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
146	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
147	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
148	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
149	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
150	15-Jul-10	2	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
151	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
152	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
153	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
154	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
155	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
156	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
157	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
158	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
159	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
160	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
161	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
162	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina

163	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
164	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
165	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
166	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
167	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
168	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
169	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
170	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
171	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
172	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
173	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
174	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
175	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
176	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
177	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
178	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
179	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
180	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
181	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
182	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
183	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
184	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
185	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
186	15-Jul-10	2	Calliphoridae	Luciliinae	Lucilia	cuprina
187	20-Jul-10	3	Sarcophagidae			
188	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	cuprina
189	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	cuprina
190	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	cuprina
191	20-Jul-10	3	Sarcophagidae			
192	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	cuprina
193	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	cuprina
194	20-Jul-10	3	Sarcophagidae			
195	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	cuprina
196	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	cuprina
197	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	cuprina
198	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	sericata
199	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	cuprina
200	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	sericata
201	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	cuprina
202	20-Jul-10	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
203	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	cuprina

204	20-Jul-10	3	Sarcophagidae			
205	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	sericata
206	20-Jul-10	3	Sarcophagidae			
207	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	cuprina
208	20-Jul-10	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
209	20-Jul-10	3	Sarcophagidae			
210	20-Jul-10	3	Sarcophagidae			
211	20-Jul-10	3	Sarcophagidae			
212	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	sericata
213	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	cuprina
214	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	sericata
215	20-Jul-10	3	Sarcophagidae			
216	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	cuprina
217	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	sericata
218	20-Jul-10	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
219	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	cuprina
220	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	cuprina
221	20-Jul-10	3	Sarcophagidae			
222	20-Jul-10	3	Sarcophagidae			
223	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	cuprina
224	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	cuprina
225	20-Jul-10	3	Sarcophagidae			
226	20-Jul-10	3	Sarcophagidae			
227	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	cuprina
228	20-Jul-10	3	Sarcophagidae			
229	20-Jul-10	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
230	20-Jul-10	3	Sarcophagidae			
231	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	cuprina
232	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	cuprina
233	20-Jul-10	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
234	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	cuprina
235	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	sericata
236	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	sericata
237	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	sericata
238	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	sericata
239	20-Jul-10	3	Sarcophagidae			
240	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	sericata
241	20-Jul-10	3	Calliphoridae	Luciliinae	Lucilia	cuprina
242	4-Sep-10	5	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
243	4-Sep-10	5	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
244	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina

245	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	mexicana
246	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
247	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
248	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	mexicana
249	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	mexicana
250	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
251	5-Sep-10	4	Sarcophagidae			
252	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	mexicana
253	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
254	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
255	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	coeruleiviridis
256	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
257	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	mexicana
258	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
259	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
260	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
261	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
262	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
263	5-Sep-10	4	Sarcophagidae			
264	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
265	5-Sep-10	4	Sarcophagidae			
266	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
267	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
268	5-Sep-10	4	Sarcophagidae			
269	5-Sep-10	4	Sarcophagidae			
270	5-Sep-10	4	Sarcophagidae			
271	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
272	5-Sep-10	4	Sarcophagidae			
273	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
274	5-Sep-10	4	Sarcophagidae			
275	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	mexicana
276	5-Sep-10	4	Sarcophagidae			
277	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	mexicana
278	5-Sep-10	4	Sarcophagidae			
279	5-Sep-10	4	Sarcophagidae			
280	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	mexicana
281	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
282	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	sericata
283	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
284	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	sericata
285	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina

286	5-Sep-10	4	Sarcophagidae			
287	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
288	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	sericata
289	5-Sep-10	4	Sarcophagidae			
290	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
291	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
292	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	sericata
293	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
294	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
295	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
296	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	mexicana
297	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
298	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
299	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
300	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
301	5-Sep-10	4	Sarcophagidae			
302	5-Sep-10	4	Sarcophagidae			
303	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	mexicana
304	5-Sep-10	4	Sarcophagidae			
305	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
306	5-Sep-10	4	Sarcophagidae			
307	5-Sep-10	4	Sarcophagidae			
308	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
309	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	sericata
310	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
311	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	sericata
312	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
313	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	sericata
314	5-Sep-10	4	Sarcophagidae			
315	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
316	5-Sep-10	4	Sarcophagidae			
317	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	sericata
318	5-Sep-10	4	Sarcophagidae			
319	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	sericata
320	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	sericata
321	5-Sep-10	4	Sarcophagidae			
322	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	mexicana
323	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	sericata
324	5-Sep-10	4	Sarcophagidae			
325	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
326	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	sericata

327	5-Sep-10	4	Sarcophagidae			
328	5-Sep-10	4	Sarcophagidae			
329	5-Sep-10	4	Sarcophagidae			
330	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
331	5-Sep-10	4	Sarcophagidae			
332	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	sericata
333	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
334	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	sericata
335	5-Sep-10	4	Sarcophagidae			
336	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
337	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
338	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
339	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
340	5-Sep-10	4	Sarcophagidae			
341	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	sericata
342	5-Sep-10	4	Sarcophagidae			
343	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	sericata
344	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
345	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
346	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	sericata
347	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	sericata
348	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	sericata
349	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	sericata
350	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	mexicana
351	5-Sep-10	4	Sarcophagidae			
352	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	mexicana
353	5-Sep-10	4	Sarcophagidae			
354	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
355	5-Sep-10	4	Sarcophagidae			
356	5-Sep-10	4	Sarcophagidae			
357	5-Sep-10	4	Sarcophagidae			
358	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	sericata
359	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	sericata
360	5-Sep-10	4	Sarcophagidae			
361	5-Sep-10	4	Sarcophagidae			
362	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	sericata
363	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	mexicana
364	5-Sep-10	4	Sarcophagidae			
365	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	mexicana
366	5-Sep-10	4	Sarcophagidae			
367	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina

368	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	mexicana
369	5-Sep-10	4	Sarcophagidae			
370	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	sericata
371	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	sericata
372	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	sericata
373	5-Sep-10	4	Sarcophagidae			
374	5-Sep-10	4	Calliphoridae	Luciliinae	Lucilia	cuprina
375	5-Sep-10	4	Sarcophagidae			
376	7-Jul-11	5	Calliphoridae	Luciliinae	Lucilia	cuprina
377	7-Jul-11	5	Sarcophagidae			
378	7-Jul-11	5	Sarcophagidae			
379	7-Jul-11	5	Sarcophagidae			
380	13-Aug-11	6	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
381	13-Aug-11	6	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
382	13-Aug-11	6	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
383	13-Aug-11	6	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
384	13-Aug-11	6	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
385	13-Aug-11	6	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
386	13-Aug-11	6	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
387	13-Aug-11	6	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
388	13-Aug-11	6	Sarcophagidae			
389	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
390	2-Oct-11	3	Calliphoridae	Chrysomyinae	Phormia	regina
391	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
392	2-Oct-11	3	Calliphoridae	Chrysomyinae	Phormia	regina
393	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
394	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
395	2-Oct-11	3	Calliphoridae	Chrysomyinae	Phormia	regina
396	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
397	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
398	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
399	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
400	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
401	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
402	2-Oct-11	3	Calliphoridae	Chrysomyinae	Phormia	regina
403	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
404	2-Oct-11	3	Calliphoridae	Chrysomyinae	Phormia	regina

405	2-Oct-11	3	Calliphoridae	Chrysomyinae	Phormia	regina
406	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
407	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
408	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
409	2-Oct-11	3	Calliphoridae	Chrysomyinae	Phormia	regina
410	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
411	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
412	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
413	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
414	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
415	2-Oct-11	3	Calliphoridae	Chrysomyinae	Phormia	regina
416	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
417	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
418	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
419	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
420	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
421	2-Oct-11	3	Calliphoridae	Chrysomyinae	Phormia	regina
422	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
423	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
424	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
425	2-Oct-11	3	Calliphoridae	Chrysomyinae	Phormia	regina
426	2-Oct-11	3	Muscidae, Anthomyidae, Scathophagidae*			
427	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
428	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
429	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
430	2-Oct-11	3	Calliphoridae	Chrysomyinae	Phormia	regina
431	2-Oct-11	3	Calliphoridae	Chrysomyinae	Phormia	regina
432	2-Oct-11	3	Calliphoridae	Chrysomyinae	Phormia	regina
433	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
434	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
435	2-Oct-11	3	Calliphoridae	Chrysomyinae	Phormia	regina
436	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
437	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
438	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
439	2-Oct-11	3	Calliphoridae	Chrysomyinae	Phormia	regina
440	2-Oct-11	3	Calliphoridae	Chrysomyinae	Phormia	regina
441	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
442	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
443	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
444	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria

445	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
446	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
447	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
448	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
449	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
450	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
451	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
452	2-Oct-11	3	Calliphoridae	Chrysomyinae	Phormia	regina
453	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
454	2-Oct-11	3	Calliphoridae	Chrysomyinae	Phormia	regina
455	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
456	2-Oct-11	3	Calliphoridae	Chrysomyinae	Phormia	regina
457	2-Oct-11	3	Calliphoridae	Chrysomyinae	Phormia	regina
458	2-Oct-11	3	Muscidae, Anthomyidae, Scathophagidae*			
459	2-Oct-11	3	Calliphoridae	Chrysomyinae	Phormia	regina
460	2-Oct-11	3	Calliphoridae	Chrysomyinae	Phormia	regina
461	2-Oct-11	3	Muscidae, Anthomyidae, Scathophagidae*			
462	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
463	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
464	2-Oct-11	3	Calliphoridae	Chrysomyinae	Phormia	regina
465	2-Oct-11	3	Calliphoridae	Chrysomyinae	Phormia	regina
466	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
467	2-Oct-11	3	Calliphoridae	Chrysomyinae	Phormia	regina
468	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
469	2-Oct-11	3	Muscidae, Anthomyidae, Scathophagidae*			
470	2-Oct-11	3	Calliphoridae	Chrysomyinae	Phormia	regina
471	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
472	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
473	2-Oct-11	3	Calliphoridae	Chrysomyinae	Phormia	regina
474	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
475	2-Oct-11	3	Calliphoridae	Chrysomyinae	Phormia	regina
476	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
477	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
478	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
479	2-Oct-11	3	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
480	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
481	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
482	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria

483	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
484	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
485	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
486	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
487	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
488	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
489	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
490	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
491	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
492	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
493	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
494	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
495	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
496	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
497	16-Oct-11	7	Calliphoridae	Chrysomyinae	Phormia	regina
498	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
499	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
500	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
501	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
502	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
503	16-Oct-11	7	Calliphoridae	Chrysomyinae	Phormia	regina
504	16-Oct-11	7	Calliphoridae	Chrysomyinae	Phormia	regina
505	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
506	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
507	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
508	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
509	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
510	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
511	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
512	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
513	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
514	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
515	16-Oct-11	7	Calliphoridae	Chrysomyinae	Phormia	regina
516	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
517	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
518	16-Oct-11	7	Calliphoridae	Chrysomyinae	Phormia	regina
519	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
520	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
521	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
522	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
523	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria

524	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
525	16-Oct-11	7	Calliphoridae	Chrysomyinae	Phormia	regina
526	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
527	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
528	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
529	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
530	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
531	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
532	16-Oct-11	7	Sarcophagidae			
533	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
534	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
535	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
536	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
537	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
538	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
539	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
540	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
541	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
542	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
543	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
544	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
545	16-Oct-11	7	Calliphoridae	Chrysomyinae	Phormia	regina
546	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
547	16-Oct-11	7	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
548	30-Jun-12	6	Sarcophagidae			
549	30-Jun-12	6	Sarcophagidae			
550	30-Jun-12	6	Sarcophagidae			
551	30-Jun-12	6	Sarcophagidae			
552	30-Jun-12	6	Sarcophagidae			
553	30-Jun-12	6	Sarcophagidae			
554	30-Jun-12	6	Sarcophagidae			
555	30-Jun-12	6	Sarcophagidae			
556	30-Jun-12	6	Sarcophagidae			
557	30-Jun-12	6	Sarcophagidae			
558	30-Jun-12	6	Sarcophagidae			
559	30-Jun-12	6	Sarcophagidae			
560	30-Jun-12	6	Sarcophagidae			
561	30-Jun-12	6	Sarcophagidae			
562	30-Jun-12	6	Sarcophagidae			
563	30-Jun-12	6	Sarcophagidae			
564	30-Jun-12	6	Sarcophagidae			

565	30-Jun-12	6	Sarcophagidae			
566	30-Jun-12	6	Sarcophagidae			
567	30-Jun-12	6	Sarcophagidae			
568	30-Jun-12	6	Calliphoridae	Chrysomyinae	Cochliomyia	macellaria
569	30-Jun-12	6	Sarcophagidae			
570	30-Jun-12	6	Sarcophagidae			
571	30-Jun-12	6	Calliphoridae	Luciliinae	Lucilia	mexicana
572	30-Jun-12	6	Calliphoridae	Luciliinae	Lucilia	mexicana
573	30-Jun-12	6	Sarcophagidae			
574	30-Jun-12	6	Sarcophagidae			
575	30-Jun-12	6	Sarcophagidae			
576	30-Jun-12	6	Calliphoridae	Luciliinae	Lucilia	mexicana
577	30-Jun-12	6	Calliphoridae	Luciliinae	Lucilia	mexicana
578	30-Jun-12	6	Sarcophagidae			
579	30-Jun-12	6	Sarcophagidae			
580	30-Jun-12	6	Sarcophagidae			
581	30-Jun-12	6	Sarcophagidae			
582	30-Jun-12	6	Sarcophagidae			
583	30-Jun-12	6	Sarcophagidae			
584	30-Jun-12	6	Sarcophagidae			
585	30-Jun-12	6	Calliphoridae	Luciliinae	Lucilia	mexicana

NSID = Not suitable for identification. * = Diptera families of no forensic importance. Five additional collection days are not listed, as they did not yield any trapped specimens. These were: 23-Jun-10, site 3; 1-Jul-10, site 4; 19-Sep-11, site 8; 7-Jul-12, site 8; 1-Sep-12, site 2.

Appendix E: Estimated Development Rates for Selected Species of Forensically Important Diptera

	Life Stage			
	Egg	**Larvae (L1-L3)**	**Pupae**	**Total**
L. sericata	**18-24hr**	**3-10d**	**6-14d**	**2-3wks**
L. cuprina	**8-24hr**	**3-5d**	**10-20d**	**2-3.5wks**
L. mexicana	**8-16hr**	**3-8d**	**6-12d**	**1.5-3wks**

Adapted from Anderson, 2000; Grassberger & Reiter, 2001.

Appendix D: Appledore Island Diptera Reference Sample

Specimen #	Collection Date	Sampling Location	Family	Subfamily	Genus	Species
SML 1	25-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 2	25-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Protophormia	terraenovae
SML 3	25-Jun-11	Appledore Island, ME	NSID			
SML 4	25-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 5	25-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 6	25-Jun-11	Appledore Island, ME	NSID			
SML 7	25-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 8	25-Jun-11	Appledore Island, ME	NSID			
SML 9	25-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 10	25-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 11	25-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 12	25-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Protophormia	terraenovae
SML 13	25-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 14	25-Jun-11	Appledore Island, ME	NSID			
SML 15	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 16	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 17	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 18	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 19	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 20	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 21	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 22	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 23	26-Jun-11	Appledore Island, ME	Calliphoridae	Luciliinae	Lucilia	illustris
SML 24	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 25	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 26	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 27	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 28	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 29	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 30	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 31	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 32	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Protophormia	terraenovae
SML 33	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 34	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 35	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 36	26-Jun-11	Appledore Island, ME	Calliphoridae	Luciliinae	Lucilia	illustris
SML 37	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 38	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 39	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina

SML 40	26-Jun-11	Appledore Island, ME	NSID			
SML 41	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 42	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Protophormia	terraenovae
SML 43	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 44	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 45	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 46	26-Jun-11	Appledore Island, ME	Calliphoridae	Luciliinae	Lucilia	illustris
SML 47	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 48	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 49	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 50	26-Jun-11	Appledore Island, ME	Calliphoridae	Luciliinae	Lucilia	illustris
SML 51	26-Jun-11	Appledore Island, ME	Calliphoridae	Luciliinae	Lucilia	illustris
SML 52	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 53	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 54	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Protophormia	terraenovae
SML 55	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 56	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Protophormia	terraenovae
SML 57	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 58	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 59	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 60	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 61	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 62	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 63	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 64	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 65	26-Jun-11	Appledore Island, ME	NSID			
SML 66	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Protophormia	terraenovae
SML 67	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 68	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 69	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 70	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 71	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 72	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 73	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 74	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 75	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 76	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 77	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 78	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 79	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 80	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina

SML 81	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 82	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 83	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 84	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 85	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 86	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 87	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 88	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 89	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 90	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 91	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 92	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 93	26-Jun-11	Appledore Island, ME	NSID			
SML 94	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Protophormia	terraenovae
SML 95	26-Jun-11	Appledore Island, ME	Calliphoridae	Luciliinae	Lucilia	illustris
SML 96	26-Jun-11	Appledore Island, ME	NSID			
SML 97	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 98	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 99	26-Jun-11	Appledore Island, ME	Calliphoridae	Luciliinae	Lucilia	illustris
SML 100	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 101	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 102	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 103	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 104	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 105	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 106	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 107	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 108	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 109	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 110	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 111	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 112	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 113	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 114	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 115	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 116	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 117	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 118	26-Jun-11	Appledore Island, ME	NSID			
SML 119	26-Jun-11	Appledore Island, ME	NSID			
SML 120	26-Jun-11	Appledore Island, ME	NSID			
SML 121	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina

SML 122	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 123	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 124	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 125	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 126	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 127	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 128	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 129	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 130	26-Jun-11	Appledore Island, ME	NSID			
SML 131	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 132	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 133	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 134	26-Jun-11	Appledore Island, ME	NSID			
SML 135	26-Jun-11	Appledore Island, ME	NSID			
SML 136	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 137	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 138	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 139	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 140	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 141	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 142	26-Jun-11	Appledore Island, ME	Calliphoridae	Luciliinae	Lucilia	illustris
SML 143	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 144	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 145	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Protophormia	terraenovae
SML 146	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 147	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 148	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 149	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Protophormia	terraenovae
SML 150	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 151	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 152	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 153	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 154	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 155	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 156	26-Jun-11	Appledore Island, ME	NSID			
SML 157	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 158	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 159	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Protophormia	terraenovae
SML 160	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Protophormia	terraenovae
SML 161	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 162	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina

SML 163	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 164	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 165	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 166	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 167	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 168	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 169	26-Jun-11	Appledore Island, ME	NSID			
SML 170	26-Jun-11	Appledore Island, ME	NSID			
SML 171	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 172	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Protophormia	terraenovae
SML 173	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Protophormia	terraenovae
SML 174	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 175	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 176	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 177	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 178	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 179	26-Jun-11	Appledore Island, ME	Calliphoridae	Luciliinae	Lucilia	illustris
SML 180	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 181	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 182	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 183	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Protophormia	terraenovae
SML 184	26-Jun-11	Appledore Island, ME	Calliphoridae	Luciliinae	Lucilia	illustris
SML 185	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 186	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 187	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Protophormia	terraenovae
SML 188	26-Jun-11	Appledore Island, ME	Calliphoridae	Luciliinae	Lucilia	illustris
SML 189	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 190	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 191	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 192	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 193	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 194	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 195	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 196	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Protophormia	terraenovae
SML 197	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 198	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 199	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 200	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Protophormia	terraenovae
SML 201	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 202	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 203	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina

SML 204	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Protophormia	terraenovae
SML 205	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 206	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 207	26-Jun-11	Appledore Island, ME	Calliphoridae	Luciliinae	Lucilia	illustris
SML 208	26-Jun-11	Appledore Island, ME	Calliphoridae	Luciliinae	Lucilia	silvarum
SML 209	26-Jun-11	Appledore Island, ME	NSID			
SML 210	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 211	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 212	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 213	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 214	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 215	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Protophormia	terraenovae
SML 216	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 217	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 218	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 219	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 220	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 221	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 222	26-Jun-11	Appledore Island, ME	Calliphoridae	Luciliinae	Lucilia	sericata
SML 223	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 224	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 225	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 226	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 227	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 228	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 229	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 230	26-Jun-11	Appledore Island, ME	NSID			
SML 231	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 232	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 233	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 234	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 235	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 236	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 237	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 238	26-Jun-11	Appledore Island, ME	NSID			
SML 239	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Protophormia	terraenovae
SML 240	26-Jun-11	Appledore Island, ME	NSID			
SML 241	26-Jun-11	Appledore Island, ME	NSID			
SML 242	26-Jun-11	Appledore Island, ME	NSID			
SML 243	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 244	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina

SML 245	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 246	26-Jun-11	Appledore Island, ME	NSID			
SML 247	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 248	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 249	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 250	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 251	26-Jun-11	Appledore Island, ME	NSID			
SML 252	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 253	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 254	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 255	26-Jun-11	Appledore Island, ME	NSID			
SML 256	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 257	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 258	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 259	26-Jun-11	Appledore Island, ME	NSID			
SML 260	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 261	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Protophormia	terraenovae
SML 262	26-Jun-11	Appledore Island, ME	Calliphoridae	Chrysomyinae	Phormia	regina
SML 263	26-Jun-11	Appledore Island, ME	NSID			

SML = Shoals Marine Laboratory; NSID = Not suitable for identification.

Appendix F: COI Sequenced Amplicons for Selected Calliphoridae Species

C. macellaria
ATTTATGCCATATTAGCTATTGGATTATTAGGATTTATTGTTTGAGCTCACCATATATTTACTGTAGGGA
TAGACGTTGATACTCGAGCTTACTTTACTTCAGCCACAATGATTATTGCTGTACCCACAGGAATTAAAA
TTTTTAGTTGATTAGCAACACTTTATGGAACTCAATTAAATTATTCCCCAGCTACTTTATGAGCCTTAG
GATTTGTATTTTTATTTACAGTAGGGGGATTAACAGGAGTAGTTTTAGCTAACTCTTCTGTTGATATTAT
TTTACATGACACATACTATGTAGTAGCTCA

L. sericata
ATTTATGCCATATTAGCTATTGGATTATTAGGATTTATTGTTTGAGCTCACCATATATTTACTGTAGGGA
TAGACGTTGATACTCGAGCTTACTTTACTTCAGCCACAATGATTATTGCTGTACCCACAGGAATTAAAA
TTTTTAGTTGATTAGCAACACTTTATGGAACTCAATTAAATTATTCCCCAGCTACTTTATGAGCCTTAG
GATTTGTATTTTTATTTACAGTAGGGGGATTAACAGGAGTAGTTTTAGCTAACTCTTCTGTTGATATTAT
TTTACATGACACATACTATGTAGTAGCTCA

L. cuprina
ATTTAAGCCATATTAGCTATTGGATTATTAGGATTTATTGTTTGAGCTCACCATATATTTACTGTAGGG
ATAGACGTTGATACTCGAGCTTACTTTACTTCAGCCACAATGATTATTGCTGTACCCACAGGAATTAAA
ATTTTTAGTTGATTAGCAACACTTTATGGAACTCAATTAAATTATTCCCCAGCTACTTTATGAGCCTTA
GGATTTGTATTTTTATTTACAGTAGGGGGATTAACAGGAGTAGTTTTAGCTAACTCTTCTGTTGATATT
ATTTTACATGACACATACTATGTAGTAGCTCA

L. mexicana HG1
ATTTATGCCATATTAGCTATTGGATTATTAGGATTTATTGTTTGAGCTCACCATATATTTACTGTAGGGA
TAGACGTTGATACTCGAGCTTAGTTTACTTCAGCCACAATGATTATTGCTGTACCCACAGGAATTAAAA
TTTTTAGTTGATTAGCAACACTTTATGGAACTCAATTAAATTATTCCCCAGCTACTTTATGAGCCTTAG
GATTTGTATTTTTATTTACAGTAGGGGGATTAACAGGAGTAGTTTTAGCTAACTCTTCTGTTGATATTAT
TTTACATGACACATACTATGTAGTAGCTCA

L. mexicana HG2
ATTTATGCCATATTAGCTATTGGATTATTAGGATTTATTGTTTGAGCTCACCATATATTTACTGTAGGGA
TAGACGTTGATACTCGAGCTTACTTTACTTCAGCCACAATGATTATTGCTGTGCCCACAGGAATTAAAA
TTTTTAGTTGATTAGCAACACTTTATGGAACTCAATTAAATTATTCCCCAGCTACTTTATGAGCCTTAG
GATTTGTATTTTTATTTACAGTAGGGGGATTAACAGGAGTAGTTTTAGCTAACTCTTCTGTTGATATTAT
TTTACATGACACATACTATGTAGTAGCTCA

L. mexicana HG3
ATTTATGCCATATTAGCTGTGGGATTATTAGGATTTATTGTTTGAGCTCACCATATATTTACTGTAGGG
ATAGACGGTGATACTCGAGCTTACTTTACTTCAGCCACAATGATTATTGCTGTACCCACAGGAATTAAA
ATTTTTAGTTGATTAGCAACACTTTATGGAACTCAATTAAATTATTCCCCAGCTACTTTATGAGCCTTA
GGATTTGTATTTTTATTTACAGTAGGGGGATTAACAGGAGTAGTTTTAGCTAACTCTTCTGTTGATATT
ATTTTACATGACACATACTATGTAGTAGCTCA

L. mexicana HG4
ATTTATGCCATATTAGCTGTGGGATTATTAGGATTTATTGTTTGAGCTCACCATGTATTTACTGTAGGG
ATAGACGGGGATACTCGGGCTTACTTTACTTCAGCCACAATGATTATTGCTGTGCCCACAGGAATTAA
AATTTTTAGTTGATTAGCAACACTTTATGGAACTCAATTAAATTATTCCCCAGCTACTTTATGAGCCTT
AGGATTTGTATTTTTATTTACAGTAGGGGGATTAACAGGAGTAGTTTTAGCTAACTCTTCTGTTGATAT
TATTTTACATGACACATACTATGTAGTAGCTCA

L. mexicana HG5
ATTTATGCCATATTAGCTATGGGATTATTAGGATTTATTGTTTGAGCTCACCATATATTTACTGTAGGG
ATAGACGTTGATACTCGAGCTTACTTTACTTCAGCCACAATGATTATTGCTGTACCCACAGGAATTAAA
ATTTTTAGTTGATTAGCAACACTTTATGGAACTCAATTAAATTATTCCCCAGCTACTTTATGAGCCTTA

GGATTTGTATTTTTATTTACAGTAGGGGGATTAACAGGAGTAGTTTTAGCTAACTCTTCTGTTGATATT
ATTTTACATGACACATACTATGTAGTAGCTCA

L. mexicana HG6
ATTTATGCCATATTAGCTGTTGGATTATTAGGATTTATTGTTTGAGCTCACCATATATTTACTGTAGGGA
TAGACGTTGATACTCGAGCTTACTTTACTTCAGCCACAATGATTATTGCTGTACCCACAGGAATTAAAA
TTTTTAGTTGATTAGCAACACTTTATGGAACTCAATTAAATTATTCCCCAGCTACTTTATGAGCCTTAG
GATTTGTATTTTTATTTACAGTAGGGGGATTAACAGGAGTAGTTTTAGCTAACTCTTCTGTTGATATTAT
TTTACATGACACATACTATGTAGTAGCTCA

L. mexicana HG7
ATTTATGCCATATTAGCTATTGGATTATTAGGATTTATTGTTTGAGCTCACCATATATTTACTGTAGGGA
TAGACGTTGATACTCGAGCTTACTTTACTTCAGCCACAATGATTATTGCTGTACCCACAGGAATTAAAA
TTTTTAGTTGATTAGCAACACTTTATGGAACTCAATTAAATTATTCCCCAGCTACTTTATGAGCCTTAG
GATTTGTATTTTTATTTACAGTAGGGGGATTAACAGGAGTAGTTTTAGCTAACTCTTCTGTTGATATTAT
TTTACATGACACATACTATGTAGTAGCTCA

Appendix G: 28S Sequenced Amplicons for Selected Calliphoridae Species

C. macellaria
AATATCCATTATAGAAAATTCATCATTATGATTTTAATATTTATAATATTATAATAATGGTGTGCATTTT
TTCTATAAAGGACATTGTAATCTATTAACATCAATATATTTATCAAAAGATCAATTGCAAAAGTTTATT
CAAATTATTTTGCTTGCAATTTAACATAGAATAAATGCTTTTGATTTGATAAAGTGTTGATAGATTTAT
TATATATAGTGCTTGAATATTTATATTCTATAATAGCATATTAATCATTGATTTTTATGTTTATTATATG
CACTTATATGATTAACAATGCGAAAGATTCAGGATACCTTCGGGACCCGTCT

L. sericata
AATATCCATTATGGAAAATTCATCATTATGATTTTAATATTTGTAATATTATAATAATGGTGTGCATTTT
TTCTATAAAGGACATTGTAATCTATTAACTTTAATATATTTATCATAAGATCATTAGATTATGTTTATTC
AAATTATTTTGCTTGCAATTTAATATCGAATAAATTCTTTTGATTTGATAAAGTGTTGATAGATTTATTA
TATACAGTGCTTAAATATTTATATTTTATAATATCATATTAATCAATGATTTTTATGTTCATTATATGCA
CTTGTATGATTAACAATGCGAAAGATTCAGGATACCTTCGGGACCCGTCT

L. cuprina
AATATCCATTATGGAAAATTCATCATTATGATTTTAATATTTGTAATATTATAATAATGGTGTGCATTTT
TTCTATAAAGGACATTGTAATCTATTAACTTTAATATATTTATCATAAGATCATTAGATTATGTTTATTC
AAATTATTTTGCTTGCAATTTAATATCGAATAAATTCTTTTGATTTGATAAAGTGTTGATAGATTTATTA
TATACAGTGCTTAAATATTTATATTTTATAATATCATATTAATCAATGATTTTTATGTTCATTATATGCA
CTTGTATGATTAACAATGCGAAAGATTCAGGATACCTTCGGGACCCGTCT

L. mexicana
AATATCCATTATGGAAAATTCATCATTATGATTTTAATATTTATAATATTATAATAATGGTGTGCATTTT
TTCTATAAAGGACATTGTAATCTATTAACATAAATTAATTTATCATAAGATCATTTGCGTAAGTTTATT
CAAATTATTTTGCTTGCAATTTAATATCGAATAAATGCTTTTGATTTGATAAAGTGTTGATAGATTTATT
ATATACAGTGCTTAAATATTTATATTTTATAATAGCATATTAATCAATGATTTTTATGTTCATTATATGC
ACTTGTATGATTAACAATGCGAAAGATTCAGGATACCTTCGGGACCCGTCT

L. illustris (Appledore Island)
AATATCCGTTATGGAAAATTCATCATTATGATTTTAATATTTATAATATTATAATAATGGTGTGCATTTT
TTCTATAAAGGACATTGTAATCTATTAACATAAATTAATTTATCATAAGATCATTTGCGTAAGTTTATT
CAAATTATTTTGCTTGCATTTTAATATCGAATAAATGCTTTTGATTTGATAAAGTGTTGATAGATTTATT
ATATACAGTGCTTAAATATTTATATTTTATAATAGCATATTAATCAATGATTTTTATGTTCATTATATGC
ACTTGTATGATTAACAATGCGAAAGATTCAGGATACCTTCGGGACCCGTCT

L. silvarum (Appledore Island)
AATATCCGTTATGGAAAATTCATCATTATGATTTTAATATTAATAATATTATAATAATGGTGTGCATTT
TTTCTATAAAGGACATTGTAATCTATTAACATTCATATTTATCATAAGATCATTAGATTATGTTTATTCA
AATTATTTTGCTTGCAATTTAATATCGAATAAATTCTTTTGATTTGATAAAGCGTTGATAGATTTATTAT
ATACAGTGCTTAAATATTTATATTTTATAATATCATATTAATCAATGATTTTTATGTTCATTATATGCAC
TTGTATGATTAACAATGCGAAAGATTCAGGATACCTTCGGGACCCGTCT