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mtDNA-based identification of *Lucilia cuprina* (Wiedemann) and *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) in the continental United States

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ABSTRACT

Existing data suggest that the forensically important dipteran species *Lucilia cuprina* (Wiedemann) and *Lucilia sericata* (Meigen) may be particularly difficult to discriminate using DNA sequence data. *L. cuprina* is paraphyletic with respect to *L. sericata* in mtDNA phylogenies, with some *L. cuprina* having mtDNA haplotypes that are very similar to those of *L. sericata*. We examine this problem by providing the first DNA data for *L. cuprina* from North America, including portions of both the mitochondrial COI gene and the nuclear 28S rRNA gene. With the new data, *L. cuprina* remains monophyletic for 28S but paraphyletic with respect to *L. sericata* for COI. However, we find that all flies that are identified as *L. cuprina* by morphology and have *L. sericata*-like mtDNA form a distinctly monophyletic mtDNA clade. This clade may possibly have originated by hybridization between *L. cuprina* and *L. sericata*, but its wide geographic distribution strongly suggests a singular origin as opposed to repeated incidents of hybridization. The phylogenetic results strongly support the hypothesis that *L. cuprina* and *L. sericata* can be discriminated using mtDNA sequence data. We find that a fragment of COI spanning approximately 1200 base pairs is sufficient to discriminate between the two species with greater than 95% bootstrap support.

1. Introduction

Lucilia cuprina (Wiedemann) and *L. sericata* (Meigen) are closely related species of blow fly that may be involved in medico-forensic investigations [1–3]. In forensic entomology, larval development may be used to infer time since oviposition and, potentially, the post-mortem interval [4]. Correct identification of the species to which larvae belong is critical, because even closely related species can have different developmental characteristics [5]. Larvae can be difficult or even impossible to identify to species by morphology alone, so an increasing amount of DNA sequence data has been obtained with the goal of using molecular phylogenetic methods for species identification of larvae [6]. However, the genealogical relationships between *L. cuprina* and *L. sericata* are sufficiently complex that Wells et al. [7] have suggested that mtDNA data alone may be insufficient for full species identification in the forensic setting. Their warning was based on two factors. First, the two species do not exhibit a pattern of reciprocal monophyly in mtDNA phylogenetic trees [7–11]. Instead, some *L. cuprina* haplotypes are much more closely related to haplotypes from *L. sericata* than they are to other *L. cuprina*. Second, there are reports that mtDNA haplotypes of a third species from a separate genus, *Hemipyrellia ligurriens* (Wiedemann) are phylogenetically intermixed with those of *L. cuprina* [7,12].

The status of *L. cuprina* and *L. sericata* as reciprocally monophyletic entities depends on whether one examines the nuclear or the mitochondrial genome [7–11]. Nuclear data, primarily from portions of the 28S ribosomal RNA genes, support reciprocal monophyly between the two species [10,11]. In contrast, mitochondrial data, primarily from the Cytochrome oxidase I (COI) and II (COII) genes, overwhelmingly support a pattern in which *L. cuprina* is split into two distinct clades that are paraphyletic with respect to *L. sericata* [7,10]. The separation of *L. cuprina* mtDNA into two clades may have a deeper biological origin, as there is a long-recognized division of *L. cuprina* into two morphological subspecies. One subspecies, *L. cuprina dorsalis* Robineau-Desvoidy, occurs in Africa and is the predominant form in much of Australia [13,14]. This geographic distribution corresponds to that of the mtDNA clade that is more distantly related to *L. sericata*. The other subspecies, *L. cuprina cuprina* (Wiedeman), is found in the Neotropics, Southern Nearctic, Hawaii, Southeast Asia, and some tropical regions of Australia [13,14]. The distribution of *L. c. cuprina* corresponds to the geographic origin of flies belonging to the group that has mtDNA haplotypes that are very similar to those of *L.* sericata. Recent data from South Africa [11] confound this simple picture, however, with the finding of *L. sericata*-like mtDNA in *L. cuprina* flies from within the geographic range of *L. c. dorsalis*.

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Paraphyly in the mitochondrial genome does not in itself preclude successful DNA-based species identification. Mitochondrial sequence data could still be used to identify *L. cuprina* and *L. sericata*, provided that the *L. sericata*-like mtDNA haplotypes found in *L. cuprina* are themselves reciprocally monophyletic with respect to *L. sericata*. While that appears to be the case with all data presented to date, no mtDNA sequences have yet been reported for *L. cuprina* from North America In this paper, we provide COI sequence data for *L. cuprina* from localities in Florida and California, along with several new U.S. samples of *L. sericata*. We use these data to test two hypotheses: that all *L. cuprina* flies from the range ascribed to *L. c. cuprina* form a monophyletic clade; and that there exists a sufficient sampling of mtDNA sequence data that will allow species-level discrimination between *L. cuprina* and *L. sericata* in the continental U.S.

Reports that haplotypes from *H. ligurriens* are intermixed with those of *L. c. cuprina* present another challenge to the utility of COI sequence data for forensic species identification. No species of *Hemipyrellia* occur in North America, so issues related to forensic species identification would be of concern only in parts of the world where *Hemipyrellia* occurs. With regard to those previous observations [7,12], we note a contrasting report [15] that included two species of *Hemipyrellia* (*H. fergusoni* and *H. ligurriens*) from Australia along with a number of *Lucilia* (including both *L. cuprina* and *L. sericata*) in a phylogenetic analysis. In that study, *Hemipyrellia* formed a clade that was distinctly separate from the entire, monophyletic genus *Lucilia* with >90% bootstrap support. In this paper, we do not develop any new data for *Hemipyrellia*, but we include, for the first time, all available *Hemipyrellia* data in a single analysis.

2. Materials and methods

2.1. Specimens

Flies were collected either by net or by attraction to a meat bait (aged chicken thigh and liver). Frozen specimens were returned to the lab for morphological identification (based on [16]) and molecular analysis. Three legs were removed from each specimen and transferred to 95% EtOH, and the remainder of each specimen was pinned and retained as a voucher. Vouchers are currently held by GAD, and will be deposited in an established natural history collection at the end of the larger studies we are currently conducting. Additional COI sequences from species in the Calliphoridae and 28S sequences from *L. cuprina* and *L. sericata* were obtained from Genbank. Tables 1(COI) and 2(28S) list the specimens used for this study. For *L. cuprina*, many of the specimens represented in Genbank, as well as those newly reported here, have not been identified to subspecies on the basis of morphological characters. We will refer to those *L. cuprina* that were collected in North America, Hawaii and Southeast Asia as being from the range ascribed to *L. cuprina cuprina*, and flies from other parts of the world as being from the range ascribed to *L. cuprina dorsalis*. For specimens

Table 1
Specimen locality data and references for COI sequences included in this study.

Species	Locality	Accession	Citation/voucher
Calliphora vicina Robinaeu-Desvoidy	Bristol University colony, UK	AJ417702	[10]
Chrysomya putoria (Wiedemann)	Sao Joao, Sao Paulo State, Brazil	NC_002697	[30]
Cochliomyia hominivorax (Coquerel)	Brazil	AF260826	[31]
Dyscritomyia fasciata (Grimshaw)	Kilaeua Iki, HI, USA	AY074902	[32]
Dyscritomyia lucilioides (Grimshaw)	Kilaeua Iki, HI, USA	AY074903	[32]
Hemipyrellia fergusoni Patton	NSW, Australia	AY842613	[6]
Hemipyrellia ligurriens (Wiedemann)	Kuranda, Qld, Australia	AY842614	[6]
Hemipyrellia ligurriens (Wiedemann)	Unknown	DQ345092	Zhu et al. Unpub.
Hemipyrellia ligurriens (Wiedemann)	Taipei, Taiwan	DQ453493	[7]
Hemipyrellia ligurriens (Wiedemann)	Chingmei, Taipei City, Taiwan	AY097334	[12]
Hemipyrellia pulchra (Wiedemann)	Unknown	DQ345091	Zhu et al. Unpub.
Lucilia adisoemartoi Kurahashi	Bobo, Tengah, Indonesia	AY074901	[32]
Lucilia ampullacea Villeneuve	Bristol, UK	DQ453487	[7]
Lucilia ampullacea Villeneuve	Montferrier-Sur-Lez, France	EU418575	[19]
Lucilia bazini Seguy	Mt. Chuyun, Kaohsiung County, Taiwan	AY346450	[12]
Lucilia caesar (Linnaeus)	Langford, Somerset, UK	AJ417703	[10]
Lucilia caesar (Linnaeus)	Bristol, UK	DQ453488	[7]
Lucilia cluvia (Walker)	New Orleans, LA, USA	DQ453490	[7]
Lucilia coeruleiviridis Macquart	West VA, USA	DQ453494	[7]
Lucilia coeruleiviridis Macquart	Greyton Beach State Park, FL, USA	GU002402	This study AB02
Lucilia coeruleiviridis Macquart	Niagra Falls State Park, NY, USA	GU002401	This study AV59
Lucilia coeruleiviridis Macquart	Hamlin Beach State Park, NY, USA	FJ650558	This study AU64
Lucilia cuprina Wiedemann	Perth, WA, Australia	AB112852	[20]
Lucilia cuprina Wiedemann	Perth, WA, Australia	AB112853	[20]
Lucilia cuprina Wiedemann	Perth, WA, Australia	AB112863	[20]
Lucilia cuprina Wiedemann	Honolulu, HI, USA	AJ417704	[10]
Lucilia cuprina Wiedemann	Waianae, HI, USA	AJ417705	[10]
Lucilia cuprina Wiedemann	Dorie, New Zealand	AJ417706	[10]
Lucilia cuprina Wiedemann	Perth, WA, Australia	AJ417707	[10]
Lucilia cuprina Wiedemann	Dakar, Senegal	AJ417708	[10]
Lucilia cuprina Wiedemann	Townsville, Qld, Australia	AJ417710	[10]
Lucilia cuprina Wiedemann	Tororo, Uganda	AJ417711	[10]
Lucilia cuprina Wiedemann	Chingmei, Taipei City, Taiwan	AY097335	[12]
Lucilia cuprina Wiedemann	Unknown	DQ345087	Zhu et al. unpub.
Lucilia cuprina Wiedemann	Honolulu, HI, USA	DQ453495	[7]
Lucilia cuprina Wiedemann	Honolulu, HI, USA	DQ453496	[7]
Lucilia cuprina Wiedemann	Gladstone, Tasmania, Australia	EU418576	[19]
Lucilia cuprina Wiedemann	Chiang Mai University lab colony, Thailand	EU418577	[19]
Lucilia cuprina Wiedemann	N30°16.413′/W082°47.069′, FL, USA	FJ650559	This study AA18
Lucilia cuprina Wiedemann	Panama City, FL, USA	FJ650560	This study AA40
Lucilia cuprina Wiedemann	N30°16.413′/W082°47.069′, FL, USA	FJ650546	This study AA21
Lucilia cuprina Wiedemann	Panama City, FL, USA	FJ650548	This study AA42
Lucilia cuprina Wiedemann	Artois, CA, USA	FJ650543	This study AE61
Lucilia cuprina Wiedemann	N30°16.413′/W082°47.069′, FL, USA	FJ650545	This study AA20
Lucilia cuprina Wiedemann	Jacksonville, FL, USA	FJ650544	This study AA05