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A global study of forensically significant calliphorids: Implications for identification

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Abstract

A proliferation of molecular studies of the forensically significant Calliphoridae in the last decade has seen molecule-based identification of immature and damaged specimens become a routine complement to traditional morphological identification as a preliminary to the accurate estimation of post-mortem intervals (PMI), which depends on the use of species-specific developmental data. Published molecular studies have tended to focus on generating data for geographically localised communities of species of importance, which has limited the consideration of intraspecific variation in species of global distribution. This study used phylogenetic analysis to assess the species status of 27 forensically important calliphorid species based on 1167 base pairs of the COI gene of 119 specimens from 22 countries, and confirmed the utility of the COI gene in identifying most species. The species *Lucilia cuprina*, *Chrysomya megacephala*, *Ch. saffranea*, *Ch. albifrontalis* and *Calliphora stygia* were unable to be monophyletically resolved based on these data. Identification of phylogenetically young species will require a faster-evolving molecular marker, but most species could be unambiguously characterised by sampling relatively few conspecific individuals if they were from distant localities. Intraspecific geographical variation was observed within *Ch. rufifacies* and *L. cuprina*, and is discussed with reference to unrecognised species.

Keywords: Calliphoridae; Forensic entomology; Blowflies; COI; Cox1; Intraspecific variation

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1. Introduction

The advent of DNA-based identification techniques for use in forensic entomology in 1994 [1] saw the beginning of a proliferation of molecular studies into the forensically important Calliphoridae. The use of DNA to characterise morphologically indistinguishable immature calliphorids was recognised as a valuable molecular tool with enormous practical utility. Numerous studies have since addressed the DNA-based identification of calliphorids [2–6]. A variety of regions of DNA have been suggested for study including the nuclear internal transcribed spacers (ITS) [7], mitochondrial rRNA genes [8] and the mitochondrial control region [8]. The majority of molecular studies, however, have used the cytochrome oxidase I (COI or cox1) encoding region of mitochondrial DNA (mtDNA) [1,2,4–6,9].

The COI gene holds enormous utility for species identification. Lying within the mitochondrial genome, it has the advantages of easy isolation, higher copy number than its nuclear counterparts, and conserved sequence and structure across taxa. COI has been well studied in the Insecta [10], with its utility for distinction between closely related species of Diptera demonstrated by the large number of COI studies of species complexes in the Culicidae (e.g. [11]).

Calliphorid molecular taxonomic studies have focused largely on sequencing of the COI gene and have illustrated the ability to successfully distinguish between a wide variety of forensically important species based largely on monophyly [1,2,4–6,9]. The main limitation to the use of COI sequence data has been the inability to distinguish between some closely related species of the genus *Calliphora*, generally due to incidences of para- or polyphyly. Wallman et al. [4,12] found

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Table 1
Individuals used in this study, listed with locality of origin and GenBank accession number, and publication data where identified from another publication[a]

Species	Locality	Accession no.	Source
Chrysomya saffranea	Broome, Australia	EU418533	New sequence
	Broome, Australia	EU418534	New sequence
	Brisbane, Australia	AB112841	[6]
Chrysomya megacephala	Sydney, Australia	EU418535	New sequence
	Perth, Australia	AB112846	[6]
	Perth, Australia	AB112847	[6]
	Pretoria, South Africa	AB112848	[6]
	Kitwe, Zambia	AB112861	[6]
	Kitwe, Zambia	AB112856	[6]
	KwaZulu-Natal, South Africa	AB112830	[6]
	Hawaii, United States	EU418536	New sequence
	Papua New Guinea	AF295551	[32]
	Kuala Lumpur, Malaysia	EU418537	New sequence
	Malaysia	AY909052	NCBI submission
	Malaysia	AY909053	NCBI Submission
Chrysomya pinguis	Hsintien, Taipei County, Taiwan	AY092759	[33]
Chrysomya bezziana	Bogor, Indonesia	AF295548	[32]
Chrysomya inclinata	KwaZulu-Natal, South Africa	AB112857	[6]
Chrysomya chloropyga	Graaf-Reinet, South Africa	EU418540	New sequence
	Graaf-Reinet, South Africa	EU418541	New sequence
	Pretoria, South Africa	EU418538	New sequence
	KwaZulu-Natal, South Africa	EU418539	New sequence
Chrysomya putoria	Kitwe, Zambia	AB112831	[6]
	Kitwe, Zambia	AB112860	[6]
	Snake Island, Botswana	AB112835	[6]
	Snake Island, Botswana	AB112855	[6]
	Sao Joao da Boa Vista, Brazil	EU418542	New sequence
	near Chilbre, Panama	AF295554	[32]
Chrysomya marginalis	Pretoria, South Africa	AB112838	[6]
	Pretoria, South Africa	AB112832	[6]
	Karoo, South Africa	AB112866	[6]
	Karoo, South Africa	AB112862	[6]
	Karoo, South Africa	EU418543	New sequence
	KwaZulu-Natal, South Africa	AB112837	[6]
	KwaZulu-Natal, South Africa	AB112834	[6]
Chrysomya varipes	Gladstone, Australia	EU418544	New sequence
	Sydney, Australia	EU418545	New sequence
	Adelaide, Australia	AF295556	[32]
	Perth, Australia	AB112868	[6]
	Perth, Australia	AB112869	[6]
	Perth, Australia	AB112867	[6]
Chrysomya norrisi	Wau, Papua New Guinea	AF295552	[32]
Chrysomya rufifacies	Perth, Australia	EU418546	New sequence
	Perth, Australia	AB112828	[6]
	Perth, Australia	AB112845	[6]
	Campbell Town, Tasmania	EU418547	New sequence
	Florida, USA	AF083658	[32]
	Knoxville, USA	EU418548	New sequence
	Oahu, Hawaii, USA	EU418549	New sequence
	Chingmei, Taipei City, Taiwan	AY092760	[33]
	Malaysia	AY909055	NCBI submission
	Malaysia	AY909054	NCBI submission
Chrysomya albiceps	Alexandria, Egypt	AF083657	[32]
	Pretoria, South Africa	AB112840	[6]
	Pretoria, South Africa	AB112839	[6]
	KwaZulu-Natal, South Africa	AB112836	[6]
	KwaZulu-Natal, South Africa	AB112842	[6]
	Deka, Zimbabwe	AB112849	[6]
	Deka, Zimbabwe	AB112858	[6]
	Manzini, Swaziland	AB112865	[6]
	Manzini, Swaziland	AB112854	[6]
	Manzini, Swaziland	AB112851	[6]
Cochliomyia hominivorax	Alfenas, Brazil	EU418550	New sequence
Cochliomyia macellaria	Salvador, Brazil	EU418551	New sequence