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Molecular systematics of the endemic Hawaiian blowfly genus Dyscritomyia Grimshaw, (Diptera: Calliphoridae)

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Abstract: The calliphorid genus Dyscritomyia is endemic to the Hawaiian Islands, and these flies provide another example of adaptive radiation within that archipelago. Dyscritomyia species have traditionally been considered members of the Tribe Luciliini, or greenbottle flies, although the exact systematic position of the genus has never been known. This is part of a larger problem in that the limits and systematic position of many calliphorid species are unclear, and the monophyly of the Calliphoridae itself is in dispute. As part of a larger investigation of calliphorid systematics, we performed a phylogenetic analysis of Dyscritomyia and members of the greenbottle genus Lucilia based on mitochondrial cytochrome oxidase (COI+II) sequence data. The resulting cladogram had ≥87% bootstrap support for all branches and it recovered the Lucilia species-groups that are supported by morphological criteria. Dyscritomyia was the sister lineage to Lucilia. Therefore it is unlikely that the Hawaiian genus evolved from any Lucilia species. Although these results are preliminary it appears that COI+II sequence data will provide a strong phylogenetic signal for any future investigation of Dyscritomyia and other greenbottle flies.

The Hawaiian Islands are arguably the most isolated landmass in the world. The chain of islands is located in the mid-Pacific, approximately 3820 km from the nearest continental land mass, 960 km from the nearest atoll formation (Johnson Island), and 3700 km to the nearest high oceanic islands (Marquesas). This isolation and limited numbers of introductions has resulted in rates of speciation and development of complex structures and habit unparalleled anywhere else in the world (Hardy 1981). Excellent examples of this may be seen in the families Drosophilidae (Diptera) and Carabidae (Coleoptera). While the levels of endemism among the Hawaiian Drosophilidae are well known (Hardy 1965), more recent work by Liebherr and Zimmerman (2000) has shown levels of carabid endemism ranging from 78% in the Bembidiini to 99% in the Psydrini. By contrast, the Calliphoridae in the Hawaiian Islands are more limited in scope, with 8 of the 9 genera represented having a cosmopolitan distribution and represented by few species. The remaining genus, Dyscritomyia Grimshaw, 1901, is exceptional in being endemic to the Hawaiian Islands and represented by 35 nominal species (Hardy 1981). At least one Dyscritomyia species is found on each of the major islands, with the majority of individuals found at elevations above 600 m. In a distinct departure from the pattern observed in the majority of Calliphoridae, each ovary is reduced to only 2 ovarioles and a single egg is produced at a time. This egg is retained in the uterus through the 2nd instar, with a late 2nd or early 3rd larval instar being produced by the female fly. The eggs are large, 2 mm or more in length, dependent on species, and contain enough yolk to provide nutrients for the 2 instars (Pollock 1974). There are no adaptations for adenotrophic viviparity and no milk glands have been observed (Pollock 1974). Among the Calliphoridae, macrolarviparity has arisen in 4 independent lines: Mesembinellinae

(Neotropical Region), Ameniinae and Phumosiinae (Oriental and Australasian Regions), and the Calliphoriinae (Dyscritomyia) (Pollock 1974).

In spite of the relatively large number of species and the abundance of individuals at elevations above 600 m, the biology of the Dyscritomyia species is relatively unknown. Adults have been reported from a variety of decomposing carrion, feces of pigs, rodents, humans and birds, dead arthropods and dead snails (Hardy 1981). There has been considerable speculation as to the habits of the larvae. Pollock (1974) speculated that the larvae were parasitic, based on modifications of the mouthhooks. Other workers indicate that the larvae may be scavengers on other invertebrates. Perkins (1913) in Fauna Hawaiiensis speculated that these species were scavengers on mollusks. Various individuals have reported rearing specimens of Dyscritomyia from specimens of the Hawaiian land snails in the genus Achatinella (Terry 1912, Swezey 1914). Larvae were also recovered from torn bodies of sphinx moths by Dr. S. Montgomery on Lanai (Hardy 1981). Based on these observations, snails appear to be logical hosts for these species with Lepidoptera larvae as the second choice.

As an example of adaptive radiation within the Hawaiian Islands that is independent of well-studied examples such as the Drosophilidae (Carson 1997, Remsen and DeSalle 1998), Dyscritomyia flies are a potential source of further insight into the biogeography of the archipelago. However, an understanding of the origin and speciation patterns of Dyscritomyia will not be possible until the phylogenetic relationships of these flies are better understood. The genus has been placed within the Tribe Luciliini (Hardy 1981) (Subfamily Lucilliinae of some authors, e.g., Rognes 1998), often referred to as the "greenbottle" flies. However the systematics of the Calliphoridae is extremely

problematic. Despite a considerable amount of taxonomic investigation the limits of many genera are uncertain and the evidence for the monophyly of the family is very weak (Rognes 1997, 1998). We believe that new data should be gathered for phylogenetic analyses of as many calliphorid species as is possible. Mitochondrial DNA (mtDNA) sequence data have been useful for resolving a variety of questions in insect systematics (Caterino et al. 2000). As part of an ongoing effort to resolve calliphorid relationships we made a preliminary investigation of the molecular systematics of the genus Dyscritomyia with particular reference to relationships with the major lineages within the genus Lucilia Robineau-Desvoidy.

Materials and Methods

Specimens and Published Sequences

The specimens newly sequenced for this study are listed in Table 1. The remains of each has been stored in 95% ethanol and deposited as a voucher in the entomology collection of the Bishop Museum, Honolulu.

In addition to the three Dyscritomyia species that were available to us, we were able to obtain DNA from two species from the fumicosta-group of the genus Lucilia (Table 1). Representatives of the other two Lucilia species-groups (Kurahashi 1966) were included in the form of published sequence data for Lucilia caesar (L.) (AJ417703) (Stevens et al. in press), Lucilia illustris (Meigen) (L14945) (cluvia-group), Lucilia (=Phaenicia) sericata (L14947, Sperling et al. 1994), and Lucilia (=Phaenicia) cuprina from the distinct and possibly hybrid Hawaiian population (AJ417704) and also from Australia (AJ417707) (Stevens et al. in press) (richardsi-group).

Also included were published sequences from the Calliphoriini (the sister group to the Luciliini) Eucalliphora latifrons (Hough) (GenBank accession number AF295557, Wells and Sperling 2001) and Calliphora vicina Robineau-Desvoidy (AJ417702, Stevens et al. in press), and members of another blowfly subfamily Chrysomyinae Chrysomya rufifacies (Macquart) (AF083658, Wells and Sperling 1999) and Phormia regina (Meigen) (AF295550, Wells and Sperling 2001).

DNA Methods

Genomic DNA from thoracic muscle of ethanol-preserved specimens or the entire thorax of each pinned specimen was extracted using QIAamp tissue columns (QIAGEN

INC., Valencia, CA) following the manufacturer's instructions.

The PCR amplification primers and conditions used are described in Wells and Sperling (2001). PCR product was cleaned using a QIAquick PCR Purification Kit (QIAGEN INC., Valencia, CA), and cycle sequencing product was cleaned using spin columns filled with Sephadex® G-50 beads (Sigma-Aldrich, Milwaukee, WI). Automated sequencing was conducted with an Applied Biosystems 310 genetic analyzer using the BigDye™ Terminator Cycle Sequencing Kit. The sequence was determined for both forward and reverse DNA strands.

Computer Analyses and Software

Sequences were confirmed and aligned manually using Sequence Navigator (Applied Biosystems, Foster City, CA). All other analyses were performed using the default parameters in PAUP 4.0b8 (Swofford 1998). E. latifrons, C. vicina, C. rufifacies, and P. regina were designated as outgroup taxa.

Results and Discussion

Sequence Data

Approximately 2.3 kilobases of sequence including the genes for cytochrome oxidase subunit one, transfer RNA leucine, and cytochrome oxidase subunit two (COI+II) was obtained for each of the specimens with one exception. The results for D. robusta included a 65 bp gap near the 5' end of the COI gene corresponding to base positions 1687-1751 in Drosophila yakuba (GenBank accession NC_001322, Clary and Wolstenholme 1985). This was because the primer combination TY-J-1460/C1-N-1840 failed to produce PCR product. The new sequences have been deposited in GenBank (see Table 1 for accession numbers).

The genetic distances as measured by percent divergence between the sequences we studied are shown in Table 2. Most notable is the very small divergence (0.3%) separating L. caesar and L. illustris. This is less than the amount of intraspecific divergence we have observed in the blowfly species P. regina (Wagner and Wells 2000), C. rufifacies, C. albiceps (Wells and Sperling 1999), and Cochliomyia macellaria (F.) (J.D.W. unpublished). However Wallman and Donnellan (2001) found that some closely related Australian Calliphora species could not be distinguished based on cytochrome oxidase sequence and at this point in time we do not have enough information to generalize about the expected amount of genetic distance between calliphorid sister species. The close similarity between the mtDNA of Hawaiian L. cuprina and L. sericata may be the result of a hybridization event (Stevens et al. in press).

One estimate of insect cytochrome oxidase sequence evolution is about 2.3% per million years (Brower 1994). The well understood geological history of the Hawaiian

Islands makes it possible to judge whether this evolutionary rate is applicable to Dyscritomyia, although until data from additional species are available we can reach only very tentative conclusions. For example if one accepts the rate in Brower (1994) then the 1.3% separating the D. fasciata haplotype from the D. lucilioides haplotype indicates that the two shared a common ancestor about 560,000 years ago. D. fasciata is found on all of the major islands while D. lucilioides is endemic to the big island of Hawaii (Hardy 1981). The island of Hawaii is estimated to be 700,000 years old (Howarth and Mull 1992). These data are consistent with D. lucilioides originating from D. fasciata not long after the latter could have colonized the newly available habitat on the island of Hawaii.

Phylogenetic Analysis

Maximum parsimony analysis produced a phylogeny with strong bootstrap support for all branches (Fig. 1). This hypothesis of relationships is in agreement with previous hypotheses based on morphological criteria, including the species-group divisions within the genus Lucilia (Kurahashi 1966). Dyscritomyia was monophyletic and found to be the sister group to Lucilia rather than being nested within that lineage. Therefore the Hawaiian genus does not appear to have descended from any Lucilia species although we believe it is properly placed within the tribe Luciliini.

Conclusions

Although the analysis of additional Dyscritomyia and other greenbottle species will be necessary before we can reconstruct the origin and diversification of this genus, the strong phylogenetic signal we observed in this preliminary study indicates that COI+II

sequence analysis is a valuable tool for phylogenetic analysis of these and other greenbottle flies.

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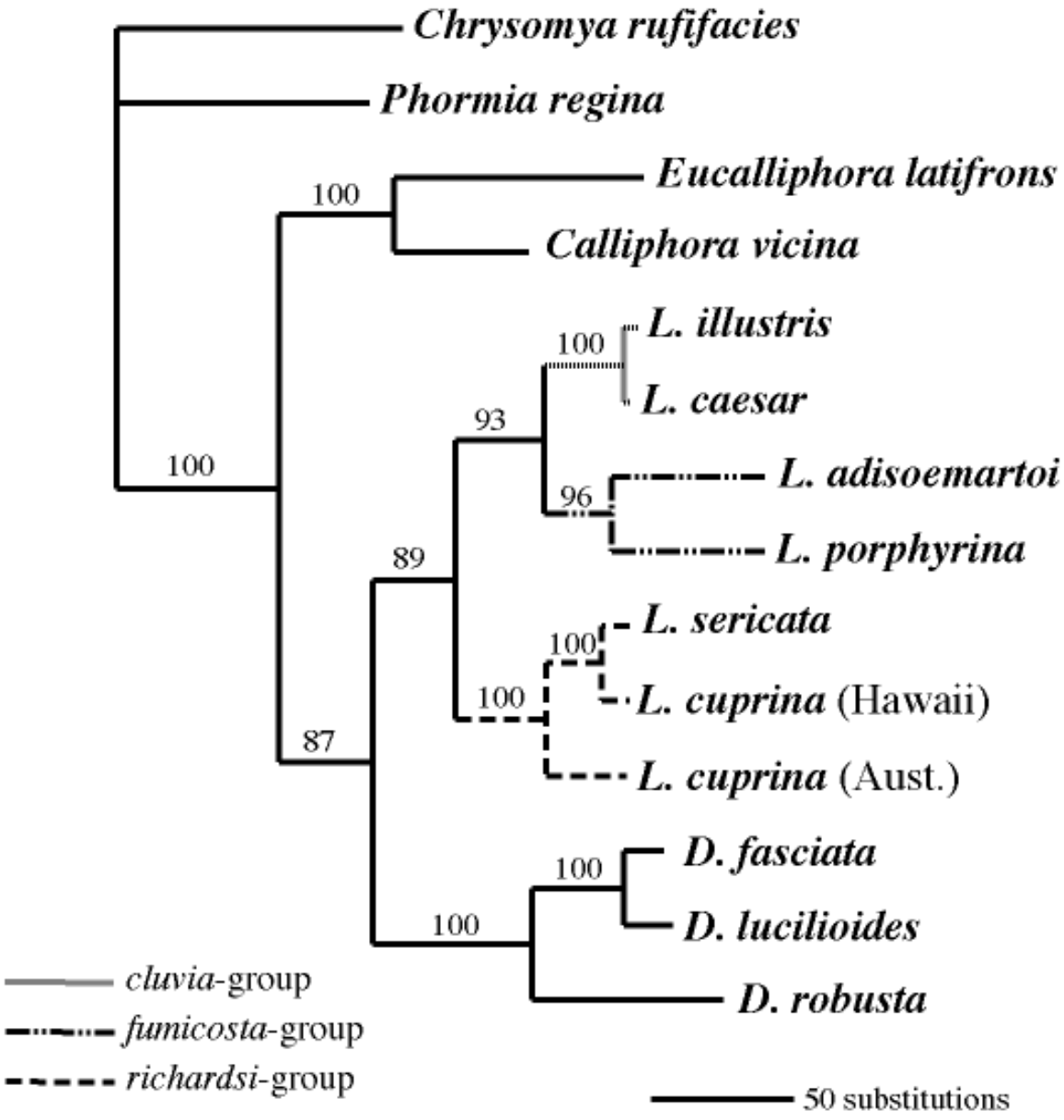
TABLE. 1. Specimens newly sequenced for this study. Additional published DNA sequences used for our analysis are described in the Materials and Methods.

SPECIES	GENBANK ACCESSION NO.	LOCATION	METHOD AND DURATION OF PRESERVATION
Lucilia adisoemartoi Kurahashi	AY74901	Bobo, Sulawesi, Tengah, Indonesia	pinned, 3 yr
Lucilia porphyrina (Walker)	AY074900	Torishima Is., Japan	pinned, 0.3 yr
Dyscritomyia fasciata (Grimshaw)	AY074902	Kilaeua Iki, Hawaii Is., Hawaii	95% ethanol, 1.25 yr
Dyscritomyia robusta (Grimshaw)	AY074898 & AY074899	S. Monjoney, Molokai Is., Hawaii	95% ethanol, 1.0 yr
Dyscritomyia lucilioides (Grimshaw)	AY074903	Kilaeua Iki, Hawaii Is., Hawaii	95% ethanol, 1.25 yr

Table 2. Percent sequence divergence (uncorrected "p" (Swofford 1998) X 100) among calliphorid mitochondrial DNA sequences analyzed in this study. See Table 1 and Methods and Materials for a more complete description of the specimens.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1. C. rufifacies	---												
2. P. regina	8.14	---											
3. E. latifrons	10.46	9.93	---										
4. C. vicina	9.55	8.59	5.84	---									
5. L. illustris	8.88	8.31	8.50	6.97	---								
6. L. sericata	9.36	8.40	8.54	7.10	5.05	---							
7. L. cuprina (Hawaii)	9.23	8.27	8.32	7.19	5.05	0.87	---						
8. L. cuprina (Aust.)	8.84	8.19	8.46	7.20	5.01	2.48	2.48	---					
9. L. caesar	8.88	8.49	8.45	7.02	0.31	5.00	4.83	5.05	---				
10. L. adesoemartoi	9.23	8.88	8.71	8.06	4.57	5.44	5.48	6.01	4.53	---			
11. L. porphyrina	9.88	9.31	8.84	7.89	4.74	5.74	5.74	6.53	4.61	4.61	---		
12. D. fasciata	9.62	9.84	9.50	8.50	7.05	7.09	6.96	6.97	7.09	7.75	8.27	---	
13. D. robusta	10.65	10.38	10.18	8.88	8.15	7.92	8.01	8.24	8.19	8.81	9.05	4.97	---
14. D. lucilioides	9.57	10.14	9.59	8.72	7.18	7.18	7.09	7.14	7.22	7.92	8.49	1.35	5.05

FIGURE 1. Single most parsimonious phylogeny (heuristic search with 1000 random stepwise additions) of calliphorid flies based on 2.3 kilobases of cytochrome oxidase sequence data. The tree is presented as a phylogram in which branch length corresponds to the amount of evolutionary change implied by this topology. Numbers indicate percent bootstrap support for a particular branch (1000 replications). The concordance between this phylogeny and the traditional species-groups of the genus *Lucilia* is indicated by the various types of dashed lines. See Table 1 and Materials and Methods for a more complete description of the specimens.


Chrysomya rufifacies
Phormia regina
Eucalliphora latifrons
Calliphora vicina
L. illustris
L. caesar
L. adisoemartoi
L. porphyrina
L. sericata
L. cuprina (Hawaii)
L. cuprina (Aust.)
D. fasciata
D. lucilioides
D. robusta
100
100
93
100
96
89
100
100
87
100
100
cluvia-group
fumicosta-group
richardsi-group
50 substitutions