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Classification of the genus *Lucilia* (Diptera: Calliphoridae): a preliminary parsimony analysis

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A parsimony analysis was performed for 25 species of the genus *Lucilia* (Diptera: Calliphoridae) using the phylogenetic analysis package PAUP, based on 14 of the morphological characters most commonly used for *Lucilia* species identification. Species descriptions were derived primarily from those given by Aubertin (1933). Parsimony analysis, using equally weighted characters, produced 45 trees and the strict consensus tree derived from these identified three groupings to be present in all 45 trees. The first group was composed of two uniquely North American species *Lucilia caeruleiviridis* and *Lucilia cluvia*. The second group contained *Lucilia ampullacea*, *Lucilia caesar* and *Lucilia illustris*. The third group, contained the six species previously described by some authors as the sub-genus *Phaenicia*: *Lucilia cuprina*, *Lucilia pilosiventris*, *Lucilia regalis*, *Lucilia richardsi*, *Lucilia sericata* and *Lucilia thatuna*, but also contained *Lucilia bufonivora* and *Lucilia silvarum*. Using a majority rule consensus tree the 12 unresolved species grouped together in 98% of the trees. The results are discussed in terms of the classification of the genus and the evolution of the myiasis habit. However, the lack of resolution observed in this study indicates the limitations of the current data set and suggests that more detailed studies using a greater number of characters are needed to uncover the evolutionary pathways which have given rise to the diversity of this genus.

KEYWORDS: Blowfly, *Lucilia*, *Phaenicia*, taxonomy, parsimony analysis.

Introduction

The genus *Lucilia* (Diptera: Calliphoridae) is a small, relatively homogeneous group of metallic, green-blue, calliphorid flies commonly known as greenbottles. The genus was first established by Robineau-Desvoidy in 1830 and included 37 species, most of which are no longer recognized. Over the next hundred years the term *Lucilia* was used by various authors to include a variety of Calliphoridae until the genus become more clearly defined as reliable diagnostic characters became known (Shannon, 1924; Aubertin, 1933).

The type for the genus is *Lucilia caesar* (L.) and the genus is characterized by a bare stem-vein, setae on a considerable part of the underside of the third wing vein, bare squamae, presence of parasquamal and tympanic tufts of hair and the presence of three pairs of postsutural dorso-central bristles (Aubertin, 1933). All the species in this genus bear a very close resemblance to each other and for many species females are almost indistinguishable. Species identification is most reliably based on the shape of the male genitalia.

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The larvae of most species of the genus are saprophages, living in animal carcasses and proteinaceous waste materials. Many have become strongly associated with humans and livestock husbandry and a small number of species, in particular *Lucilia sericata* (Mg.) and *Lucilia cuprina* (Wied.) and to a lesser extent *L. caesar* and *Lucilia illustris* (Mg.) have also evolved a specialised facultative ectoparasitic lifestyle, causing cutaneous myiasis of mammals, largely affecting sheep, although they may also strike a range of other wild and domestic animals and humans (Hall and Wall, 1995). Another species, *Lucilia bufonivora* Mon., is a specialized agent of myiasis in toads (Zumpt, 1965).

The *Lucilia* appear to have been originally and predominantly palaearctic in distribution (Aubertin, 1933). However, as humans and domestic livestock began to move worldwide with increasing facility, a number of species appear to have been carried to new habitats where they began to diverge genetically (Stevens and Wall, unpublished). In some cases, for species of veterinary importance such as *L. cuprina*, a relatively precise chronology can be constructed for these introductions (Norris, 1990). By the early 20th century *Lucilia* had been described in North and South America (Shannon, 1926), South Africa, Australia and other regions of the Pacific (Zumpt, 1965; Norris, 1990) and at least 27 good species of *Lucilia* were recognised worldwide by Aubertin (1933).

Groups of species of *Lucilia* have been treated as sub-genera, or genera within the tribe Luciliini by some authors (Shannon, 1926; Townsend, 1935). For example, Malloch (1926) erected the sub-genus *Phaenicia* for species with a pale basicostal scale and three postsutural acrostichal bristles. This group, as originally defined, would include six species: *L. cuprina*, *Lucilia pilosiventris* Kram., *Lucilia regalis* (Mg.), *Lucilia richardsi* Coll., *L. sericata* and *Lucilia thatuna* Snn. However, subdivision of the genus was rejected by Aubertin (1933). She argued that it was of no taxonomic value since although some species may clearly group together, doing so leaves an unsatisfactory heterogeneous residue of species collected under the term *Lucilia sens. str.* While this argument was accepted by most taxonomists (Zumpt, 1965), in North America, Townsend (1935) followed by Hall (1948), subsequently restored *Phaenicia* as a genus or sub-genus and this convention has been maintained in North America.

Here we use a cladistic parsimony analysis to investigate phylogenetic relationships among *Lucilia* species, using presence or absence data based on the morphological characters most commonly used for *Lucilia* identification. The significance of the results for *Lucilia* taxonomy is considered.

Methods

To try to ensure uniformity, species descriptions were derived primarily from those given by Aubertin (1933) and the characters used in the analysis were those given comparably for all, or almost all, species. Where details of only one or two characters were missing from Aubertin's description, where possible, information was obtained from the inspection of museum specimens. In one instance, where data could not be obtained for a specific character for one species, a missing value was entered in the analysis. Where information on several characters was missing, as was the case for *Lucilia pionia* (Walk.) and *Lucilia alaskensis* (Snn.), these species were not included in the analysis. The calliphorid, *Calliphora vicina* (L.) was also included in the analysis as an outgroup.

Parsimony analysis was performed using the package PAUP version 3.1.1. (Swofford, 1993). The number of species (25) necessitated the use of a heuristic

search strategy to find the most parsimonious trees (MPTs). The default options of PAUP were used: 10 random addition sequences, TBR branch swapping, and zero length branches collapsed. In total 14 morphological characters, coded as 17 binary factors, were used in the parsimony analysis. These were:

1 Colour of the basicostal scale (0 = black/brown, 1 = white/cream).
2 Number of postsutural acrostichal bristles (0 = two pairs, 1 = three pairs).
3 Eye separation in the male (0 = distance of greater than the width of the third antennal segment, 1 = less than the width of the third antennal segment).
4 Number of anterio-dorsal bristles on the mid tibia (0 = one, 1 = two).
5 Colour of the palpi (0 = yellow/orange, 1 = black/brown).
6 Subcostal sclerite (0 = bristles absent, 1 = bristles present).
7 Colour of the squamae (0 = uniform white/cream, 1 = partially or totally brown).
8 Wings (00 = hyaline, 01 = lightly infuscated, 11 heavily infuscated).
9 Eye separation in the female (0 = distance of greater than one quarter of the width of the head, 1 = less than one quarter of the width of the head).
10 Colour of the antennae (0 = uniformly dark, 1 = non-uniform).
11 Male hypopygium (00 = inconspicuous, 01 = conspicuous, 11 = highly conspicuous).

Table 1. Presence or absence data for fourteen characters for 25 species of the genus *Lucilia* as used in the parsimony analysis; *Calliphora vicina* is included as an outgroup.

	Character number													
Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Lucilia ampullacea	0	0	1	0	0	1	0	00	0	0	00	0	01	0
Lucilia bufonivora	0	0	0	0	1	0	0	00	0	0	01	0	11	0
Lucilia caeruleiviridis	1	0	1	0	0	0	0	00	1	1	00	0	00	0
Lucilia caesar	0	0	1	0	0	1	0	00	0	0	11	0	01	0
Lucilia cluvia	1	0	0	0	0	0	0	00	1	0	00	0	00	0
Lucilia cuprina	1	1	0	0	0	0	0	00	0	0	01	0	11	0
Lucilia eximia	0	0	1	0	0	0	1	00	0	1	00	0	00	0
Lucilia fumicosta	0	0	1	1	0	1	1	01	0	1	00	0	00	0
Lucilia graphita	0	1	1	0	0	1	1	01	0	1	00	1	01	0
Lucilia ibis	0	0	1	0	0	0	1	01	1	1	00	1	11	0
Lucilia illustris	0	0	1	0	0	1	0	00	0	0	01	0	11	0
Lucilia infernalis	0	1	1	0	0	1	1	11	0	1	00	1	01	0
Lucilia mexicana	0	0	1	0	0	0	1	01	0	1	00	0	11	0
Lucilia ochricornis	0	0	1	0	0	0	1	01	0	0	00	1	??	0
Lucilia papuensis	0	0	1	1	0	1	1	01	0	1	00	0	11	0
Lucilia porphyrina	0	0	1	0	0	1	1	01	1	0	00	1	00	0
Lucilia purpurascens	0	0	1	0	0	0	1	01	1	1	00	1	00	0
Lucilia pilosiventris	1	1	0	1	1	0	0	00	0	0	01	0	11	0
Lucilia regalis	1	1	0	1	1	0	0	00	0	0	01	0	11	0
Lucilia rica	0	0	1	0	0	0	1	00	0	0	00	1	00	0
Lucilia richardsi	1	1	0	1	1	0	0	00	0	0	00	0	11	0
Lucilia sericata	1	1	0	0	0	0	0	00	0	0	00	0	11	0
Lucilia silvarum	0	1	0	0	1	0	0	00	0	0	01	0	11	0
Lucilia sinensis	0	0	1	0	0	0	1	01	0	1	00	1	11	0
Lucilia thatuna	1	1	1	0	0	0	0	00	1	0	00	0	11	0
Calliphora vicina	1	1	1	1	0	0	1	00	0	0	11	1	01	1

12 Colour of abdomen and thorax (0 = predominantly brassy green/green, 1= predominantly purple/blue/black).
13 Colour of the legs (00 = dark brown, 01 = brown/black, 11 = black).
14 Lower squamal lobe (0 = setae absent, 1 = setae present).

All character state changes were equally weighted. This approach was adopted as only three characters (8, 11 and 13) were coded for three states; of these three, two (8 and 11) had third states (11) which were present in only one species (Table 1). Hence, scale-weighting of such characters would in practice have served only to down-weight the predominant changes between states 00 and 01 in relation to the other characters used in the analysis.

Results

Parsimony analysis, using equally weighted characters, produced 45 MPTs of length 43. The consistency index for these MPTs was 0·372 and the retention index was 0·713.

The strict consensus tree derived from this analysis (Fig. 1) identified only three groupings to be present in all 45 trees. The first of the groups identified is composed of

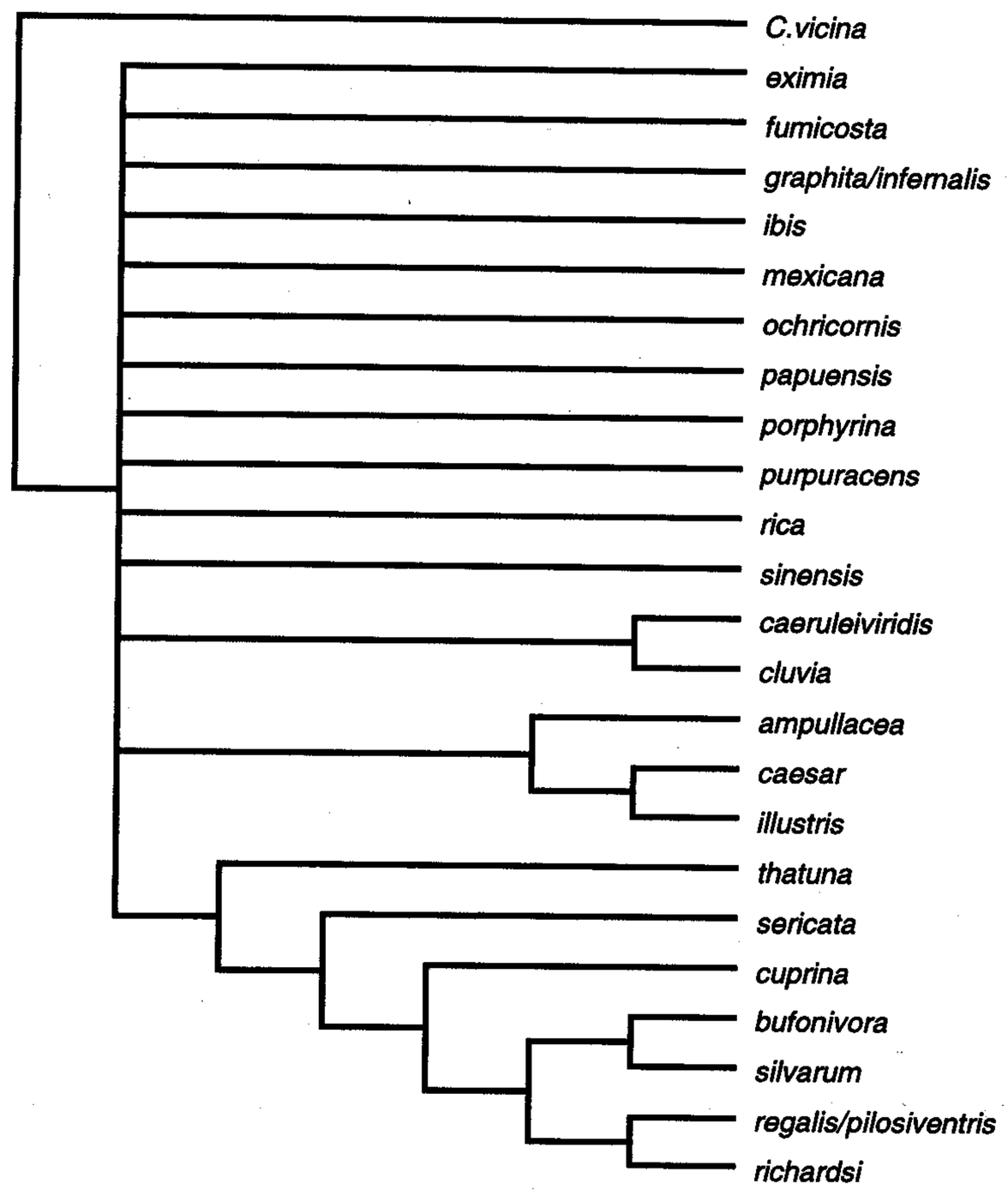

FIG. 1. Strict consensus tree for 25 species of *Lucilia* derived from the 45 most parsimonious trees calculated from the data in Table 1; outgroup = *Calliphora vicina*.

two uniquely North American species *Lucilia caeruleiviridis* Macq. and *Lucilia cluvia* (Walk.). The second group contains three species: *Lucilia ampullacea* Vill., *L. caesar* and *L. illustris*. The third group, contains the six species previously proposed as composing the sub-genus *Phaenicia*, but also contains *L. bufonivora* and *Lucilia silvarum* (Mg.). *Lucilia* species which are known to act as agents of livestock myiasis, *L. cuprina*, *L. sericata*, *L. caesar* and *L. illustris* occur in two of the three principal groups identified.

The species *Lucilia graphita* Snn. and *Lucilia infernalis* (Vill.) differ from each other by the single character of wing infuscation (character 8). For *L. infernalis* the wings are heavily infuscated and its character state 11 is unique among those species included in this analysis. Consequently, the evolutionary significance of this unique character state, in terms of providing information on the relationships of *L. infernalis* to other species in parsimony analyses is unknown; *L. graphita* and *L. infernalis* were thus treated as identical for the purposes of analysis. Relationships between the

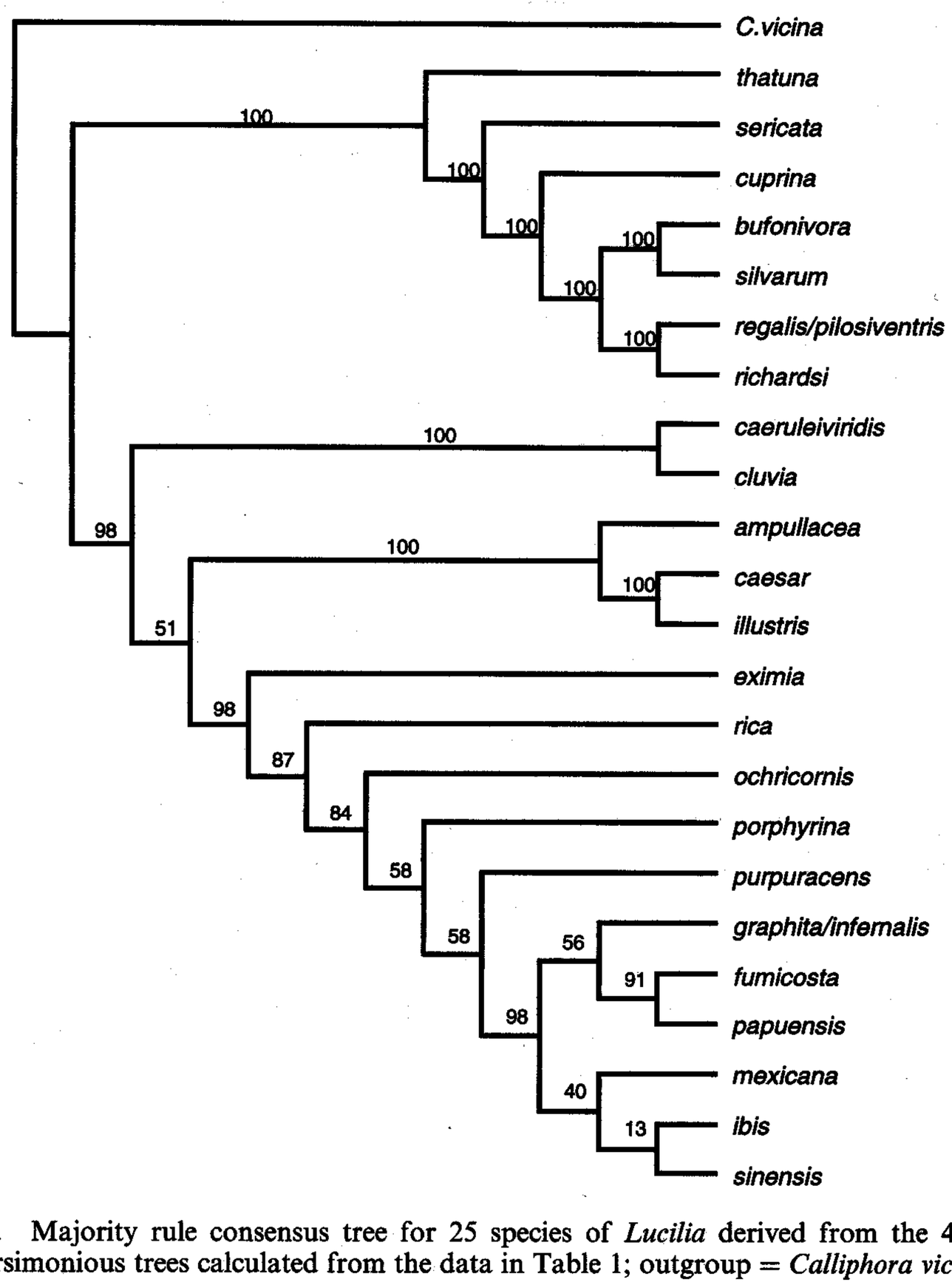

FIG. 2. Majority rule consensus tree for 25 species of *Lucilia* derived from the 45 most parsimonious trees calculated from the data in Table 1; outgroup = *Calliphora vicina*.

remaining species included in this analysis are unresolved in the strict consensus tree (Fig. 1).

To further investigate the MPTs the majority rule consensus method was used. This technique groups species based on the number of times they cluster together in all MPTs (Fig. 2); the percentage of times that a cluster appears can be taken as a rough measure of relative support. Clusters in the majority rule tree which occur in <100% of MPTs are obviously less robust than those identified by the strict consensus method but, nevertheless, can provide a useful insight into underlying relationships in unresolved areas of a strict consensus tree. From this analysis, as would be expected, the three principal groups emerged again as in Fig. 1 but, additionally, the 12 unresolved species from the strict consensus tree were shown to group together in 98% of trees. The species *Lucilia graphita* Snn. from the Hawaiian Is., *L. infernalis* from eastern Africa, *Lucilia papuensis* Macq. and *Lucilia fumicosta* Mall. from the western Pacific, *Lucilia mexicana* Macq. from North, Central and South America, *Lucilia ibis* Snn. from Peru and *L. sinensis* Aubertin from China, also appear to cluster in 98% of the trees.

Discussion

Three categorical species clusters emerged from the parsimony analysis, accounting for 13 of the 25 species of *Lucilia* included. Clearly, this analysis lends only partial support for the continued description of *Phaenicia* as a separate sub-genus. While the six species, *L. cuprina*, *L. pilosiventris*, *L. regalis*, *L. richardsi*, *L. sericata* and *L. thatuna*, grouped on the basis of their pale basicostal scale and three postsutural acrostichal bristles (Malloch, 1926), did cluster together, both consensus trees also included *L. silvarum* and *L. bufonivora* within the group, despite the presence of a black basicostal scale in both of these species and only two pairs of postsutural acrostichal bristles in the latter species. The inclusion of *L. silvarum* and *L. bufonivora* nested within this grouping implies that *Phaenicia* is paraphyletic, and highlights further the taxonomic inadequacies of the putative generic or sub-generic status of *Phaenicia*.

Lucilia silvarum is a generalised saprophage, while *L. bufonivora* is a specialist agent of toad myiasis (Zumpt, 1965). As can be seen from the present study, both are morphologically similar and it has been proposed that *L. bufonivora* may have only recently diverged from *L. silvarum* through its highly specialised choice of host. Aubertin (1933) suggested that a North American species, described as *Lucilia elongata* Shannon, which is also a specialist agent of toad myiasis, was a synonym of *L. bufonivora*. However, Hall (1948) disagreed, maintaining the separate identity of *L. elongata* in North America. To further complicate matters, *L. silvarum* and *L. bufonivora*, were themselves given generic status as *Bufolucilia* by Townsend (1935) and this generic ranking has been perpetuated by some authors, including the description of *Bufolucilia elongata* in North America occupying an equivalent niche to the palaearctic *L. bufonivora* (e.g. Hall and Townsend, 1977). Although this study supports the hypothesis that *L. bufonivora* and *L. silvarum* are closely related, the separation of *Bufolucilia* as even a sub-genus suffers from the same problem as for *Phaenicia*, i.e. it leaves the other species in a heterogeneous and paraphyletic group.

A similar debate has taken place over the description of the myiasis specialist, *L. cuprina*, in North America. Two distinct strains of *L. cuprina* are thought to exist worldwide, *L. cuprina cuprina* and *L. cuprina dorsalis* (Norris, 1990). However, it

has been suggested that since ovine myiasis is largely unknown in North America, the *L. cuprina* found there may be a separate species, described as *Phaenicia pallescens* (e.g., Hall and Townsend, 1977). It is the view of the present authors that North American *L. cuprina* will probably prove to be *L. cuprina cuprina*, however, this debate as with the debate about the identity of *Lucilia elongata*, indicates the need for further, possibly molecular, comparative analyses particularly for studies at the sub-specific level.

If *Phaenicia* is to be treated as a sub-genus then evidently so too should the three species *L. ampullacea*, *L. caesar* and *L. illustris* and the two North American species with a pale basicostal scale and two pairs of postsutural acrostichal bristles: *L. caeruleiviridis* and *L. cluvia* which cluster together strongly. However, this again leaves Aubertin's original objection to sub-grouping within the genus, i.e. that a heterogeneous residue of species is left unaccounted for.

As noted, species implicated as agents of livestock myiasis, namely *L. cuprina*, *L. sericata*, *L. caesar* and *L. illustris* (Hall and Wall, 1995), occur in two of the three principal groupings. The prevalence of *L. caesar* and *L. illustris* in livestock myiasis appears to be greater in the more northerly parts of Europe (MacLeod, 1943; Brinkmann, 1976) whereas, in general, *L. sericata* is found in cool temperate and *L. cuprina* in warm temperate and sub-tropical habitats worldwide; however, as stated earlier, this latter species only strikes sheep in certain parts of its geographical range, such as Australia and South Africa. This would suggest that the myiasis habit may have evolved independently more than once in groups of species adapted to climatically different habitats. This conclusion is supported by Erzinclioglu (1989) who suggests that for many species of Calliphoridae, the myiasis habit arose in endemic species after the arrival of humans and domestic animals. Indeed, parsimony analysis supports such a hypothesis for the *Lucilia*, the consensus trees presented requiring the habit to have arisen three times given the relationship of *L. cuprina* to *L. sericata* described. Alternatively, a second apparently less parsimonious but still plausible explanation is that the habit evolved once in a common ancestor of these two groups, and was subsequently modified (e.g. *L. bufonivora*) or lost (e.g. *L. richardsi*) in certain members of these two clusters. Why this should have occurred or why a greater number of the very closely related species of *Lucilia* are not also found in livestock myiases remain questions of interest.

Within the three tightly clustered groups identified by parsimony analysis, all species except *L. cluvia*, *L. caeruleiviridis*, *L. thatuna* and *L. cuprina* are thought to have been endemic palaearctic species. *Lucilia cluvia*, *L. caeruleiviridis* and *L. thatuna* are known only from North America and, as mentioned previously, *L. cuprina* is now distributed worldwide. The original distribution of *L. cuprina* is unknown but may have been either East African or Oriental. The unresolved relationships between the remaining species may be due to the relatively disparate origins of these representatives of the genus as it spread worldwide from its palaearctic origins possibly in association with humans and domestic livestock. Alternatively, such a result may reflect the unsuitability of the characters scored for elucidating relationships among these less well studied species; indeed, the virtually identical classification of *L. graphita* and *L. infernalis*, which originate from Hawaii and East Africa respectively, highlights the degree of homoplasy within the data. It may be that re-examination of specimens will yield additional characters which will improve the resolution of subsequent analyses; molecular (e.g., Simon *et al.*, 1994) and/or behavioural (e.g., Basibuyuk and Quicke, 1994) characters may also have an important role to play.

This limited study has shown that the *Lucilia* blowflies are a group of considerable interest for studies of diversity, speciation and the underlying population genetics. However, lack of resolution indicates the limitations of the current data set due, in part, to the high taxa (25) to character (17) ratio which does not allow detailed, robust inferences to be drawn. Further studies using a greater number of characters need to be undertaken to attempt to uncover the evolutionary pathways which have given rise to the diversity of this genus.

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