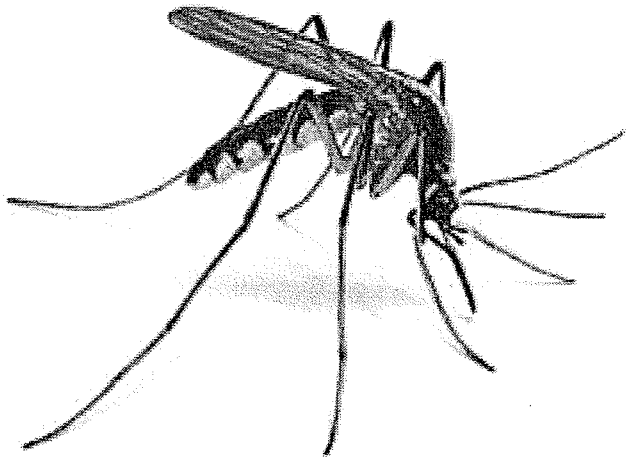
Phylogeny of the Genus *Culex* (Diptera: Culicidae)



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A Thesis submitted in partial fulfilment of the requirements for the degree of Master of Research of Imperial College London

Abstract:

Maximum parsimony, under varying weighting methods and Bayesian analyses of sixty-four morphological characters are used in the phylogenetic analysis of the genus *Culex* (Diptera: Culicidae). Three major groupings of subgenera within genus *Culex* are identified that agree well with traditional understandings of the affinities within the genus. The results show that the genus *Culex* is not monophyletic, with the Culicini genus *Deinocerites* found to be a derived member of the *Melanoconion* group of subgenera. On the basis of these results we suggest that a reclassification of the genus *Culex* is needed.

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1. Introduction:

The mosquitoes, family Culicidae, are among the most studied of the insect groups due to their role as primary vectors for numerous human pathogens, such as malaria, dengue fever and the West Nile virus. Due to their medical significance many phylogenies of the Culicidae have been published based on the use of morphological and molecular characters (Reinhart, 2004; Foley, 2007). Mosquitoes are holometabolous insects having four distinct life stages: egg, larva, pupa and adult. All life stages can provide characters for phylogenetic analyses resulting in larger morphological datasets.

The Culicidae comprise two subfamilies: Anophilinae and Culicinae, with the Culicinae comprising the majority of extant mosquitoes with about 3,000 species. The modified traditional higher level classification of the Culicidae is shown in **Table 1**. *Culex* is the second largest genus within the family, with over 700 described species which are currently sub-divided into 24 subgenera (See **Table 2** for a list). While no members of the genus have been identified as vectors for the malaria plasmodium, numerous species within the genus are known to transmit viral diseases such as the West Nile virus and Japanese encephalitis in the Old and New Worlds and pose a great risk in the spread of diseases such as Rift Valley fever into Europe and North America (Nasci, 2001; Fonseca, 2004; Meegan, 1980). Despite the medical importance of this genus no major phylogenetic study of these mosquitoes has been published and the relationships within *Culex* remain poorly understood.

Culex is placed within the tribe Culicini along with the genera *Deinocerites*, *Galindomyia* and *Lutzia*. The tribe is monophyletic, united by clear synapomorphies such as the presence of well-developed pulvilli, prealar setae and the length of their mouthparts (Harbach & Kitching, 1998).

Subfamily	Tribe	Genus			
Anophelinae		*Anopheles*	*Culicinae (cont.)*		
		Bironella		*Orthopodomyiini*	*Orthopodomyia*
		Chagasia			
				Sabethini	*Isostomyia*
	Aedeomyiini	*Aedes*			*Johnbelkinia*
		Armigeres			*Limatus*
		Ayurakitia			*Malaya*
		Eretmapodites			*Maorigoeldia*
		Haemagogus			*Onirion*
		Heizmannia			*Runchomyia*
		Opifex			*Sabethes*
		Psorophora			*Shannoniana*
		Tanakaius			*Topomyia*
		Udaya			*Trichoprosopon*
		Verrallina			*Tripteroides*
		Zeugnomyia			*Wyeomyia*
Culicinae	*Culicini*	*Culex*		*Toxorhynchitini*	*Toxorhynchites*
		Deinocerites			
		Galindomyia			
		Lutzia		*Uranotaeniini*	*Uranotaenia*
	Culisetini	*Culiseta*			
	Ficalbiini	*Ficalbia*			
		Mimomyia			
	Hodgesiini	*Hodgesia*			
	Mansoniini	*Coquillettidia*			
		Mansonia			

Table 1: Higher level classification of the family Culicidae

Synapomorphies that supported the monophyly of *Culex* were initially only identified by a few authors such as Belkin (1962) and Edwards (1941). The internal relationships of *Culex* have been speculated on by numerous authors but these have not been confirmed by any sort of phylogenetic analysis. Despite this, various affinities between the subgenera in *Culex* have emerged based on loose morphological similarities.

The most primitive subgeneric groupings appear to be *Barraudius*, *Afroculex* and *Kitzmilleria*, all distributed exclusively in Africa. Danilov (1989) assumed these to be closely related based on synapomorphies seen in the larvae and the male genitalia. Among the other Old World subgenera, Sirivanikarn (1972) hypothesised a close relationship between *Eumelanomyia*, *Lophoceraomyia*, *Maillotia* and *Neoculex* based on similarities in the adult morphology, suggesting that *Eumelanomyia* was an ancient derivative of the more primitive *Maillotia*. A further grouping of Asian subgenera was proposed by Belkin (1962) and Bram (1968) based on the similarities in the larvae of *Culiciomyia*, *Acalleomyia* and *Acallyntrum*, with *Culiciomyia* placed as the most primitive of the three. Similarities between *Culiciomyia*, *Acalleomyia* and

Acallyntrum and subgenera *Culex* and *Lutzia* (which has since been raised to generic rank) were also indicated, placing them among the most primitive members of the genus (Belkin, 1962).

It is assumed that the most derived group of species are those in, and associated with, the *Melanoconion* group of subgenera. These include *Melanoconion*, *Micraedes*, *Tinolestes*, *Aedinus*, *Anoedioporpa*, *Microculex*, *Belkinomyia* and *Carrollia*, which are found only in the New World, generally in the tropics of South America (Belkin, 1968; Berlin, 1969; Adames & Galindo, 1973). Sirivanikarn (1983) suggested that the *Melanoconion* group and related subgenera form a single major monophyletic clade exclusive to the New World. The origins of the *Melanoconion* group were discussed by Sirivanikarn (1983), who noted that *Melanoconion* shares several morphological features with the "more primitive" subgenus *Neoculex*, which has worldwide distribution, suggesting that an offshoot of *Neoculex* may have given rise to the *Melanoconion* group. The *Melanoconion* group may also have given rise to the more derived genera of the tribe Culicini: Valencia (1973) suggested that *Carrollia* and the closely related genera *Deinocerites* and *Galindomyia* shared a similar evolutionary history, both deriving from primitive stock of subgenus *Melanoconion*.

More recent and generally unpublished work has lent support to the traditional ideas about the relationships between the subgenera of *Culex*. Mallampalli (1995), using an analysis of 67 morphological characters, showed that the *Melanoconion* group does indeed seem to be the source of the New World radiation of *Culex* mosquitoes and indicated that many of the traditionally understood groupings were accurate, with *Allimanta*, *Maillotia* and *Culex* appearing as the most primitive taxa. Additionally Mallampalli (1995) showed that the genus *Culex* did not form a monophylectic clade, with *Deinocerites* and *Galindomyia* retrieved as a derived clade sister to *Belkinomyia*. The morphological phylogeny of Navarro and Liria (2000) based solely on larval mouthparts also showed the New World *Culex* as a distinct clade (although not originating from *Melanoconion*), but again with *Deinocerites* placed with the new world subgenera of *Culex*. Additionally their study indicated that the genera *Lutzia* and *Culex* formed distinct monophylectic clades, with *Lutzia* being the more primitive of the two.

A limited molecular study by Juthayothin (2004) using the COI protein coding gene showed poorly resolved differences between the subgenera *Culiciomyia*, *Eumelanomyia* and *Culex*. Based on this single molecular marker, *Eumelanomyia* seemed to be the most primitive of the three subgenera. However substantial homoplasy was detected in the dataset making this conclusion unreliable. A further molecular phylogeny based on the vitellogenin gene agrees with the conclusions from morphology in placing the genus *Deinocerites* within *Culex* (Isoe, 2000).

It is clear that the internal relationships of the genus *Culex* have not yet been adequately resolved. The limited studies conducted to date disagree on the placement of taxa and the monophyly of the genus. The present study was undertaken to investigate the phylogeny of the genus *Culex* based on pupal, larval and adult morphological characters to determine the evolutionary relationships of this important group of mosquitoes.

2. Materials and Methodology:

Morphological data were collected from adult, pupal and 4th-instar larval stages. Specimens of pinned adults and immature stages on slides were obtained from the collections of the Natural History Museum and the National Museum of Natural History, Smithsonian Institution. All subgenera of genus *Culex* were sampled for this study, with two species chosen as representatives for each subgenus where possible. A list of the species examined is shown in **Table 2**. In all cases the type species was used as one of the species. Male genitalia were dissected for comparative study. Genitalia were cleared in 5% NaOH at 40°C for 2h, neutralised in cellusolve, dissected in clove oil and mounted in Euparal on slides. Morphological terminology follows Harbach & Knight (1980). Terminology for the opisthophallus of the male genitalia is taken from Belkin (1968).

2.1. Outgroup Selection:

The primary purpose of this study was to investigate the internal phylogeny of the genus *Culex*. However taxa from two other Culicini genera, *Lutzia* and *Deinocerites*, were chosen to resolve the position and monophyly of *Culex* within the Culicini. Tribe Culicini was assumed to be monophylectic based on the findings of Harbach & Kitching (1998). Three additional taxa from tribes closely related to the Culicini were chosen to root the cladograms: *Mansonioides africana*, *Orthopodomyia anopheloides* and *Maorigoeldia argyropus*.

2.2. Characters and Coding Protocol:

Forty-four taxa were examined (using multiple specimens and supplemented with literature descriptions) resulting in 64 independent characters. Applicable characters from Mallampalli (1995) and Harbach & Kitching (1998) were included in this study along with several novel characters. Characters were chosen to contain maximum phylogenetic information about subgeneric relationships. Characters that were internally polymorphic for subgenera were discarded. Missing data were coded as '?' when specimens were unavailable or damaged; instances where the homology was not evident were coded as '-'. Multistate characters were coded as unordered. A single instance of polymorphism for *Maorigoeldia argyropus* was coded as polymorphic. Character statements refer to females except where otherwise noted. All characters

and their states are listed in **Appendix 1**, and are illustrated in **Figures 1** and **2**. The total data matrix is shown in **Appendix 2**.

Genus	Subgenus	Subgenus	Species	Larva	Pupa	Male	Female
Culicini	*Culex*	*Acalleomyia*	*obscurus*	x	x	x	x
		Acallyntrum	*axillicola*	x	x	x	x
			perkinsi	x	x	x	x
		Aedinus	*amazonensis*	x	x	x	x
		Afroculex	*lineata*	-	-	x	x
		Allimanta	*tramazayguesi*	x	x	x	x
		Anoedioporpa	*conservator*	x	x	x	x
			originator	x	x	x	x
		Barraudius	*modestus*	x	x	x	x
			pusillus	x	x	x	x
		Belkinomyia	*eldridgei*	x	x	x	x
		Carrollia	*Iridescens*	x	x	x	x
			infoliatus	x	x	x	x
		Culex	*decens*	x	x	x	x
			pipiens	x	x	x	x
			mimeticus	x	x	x	x
			sitiens	x	x	x	x
		Culiciomyia	*fragilis*	x	x	x	x
			nebulosus	x	x	x	x
		Eumelanomyia	*brevipalpis*	x	x	x	x
			inconspicuosus	x	x	x	x
		Kitzmilleria	*moucheti*	x	x	x	x
		Lasiosiphon	*adairi*	x	x	x	x
		Lophoceraomyia	*cinctellus*	x	x	x	x
			uniformis	x	x	x	x
		Maillotia	*arbieeni*	x	x	x	x
			hortensis	x	x	x	x
		Melanoconion	*atratus*	x	x	x	x
			erraticus	x	x	x	x
		Micraedes	*antillummagnorum*	x	x	x	x
			bisulcatus	x	x	x	x
		Microculex	*davisi*	x	x	x	x
			imitator	x	x	x	x
		Neoculex	*pseudomelanoconia*	x	x	x	x
			territans	x	x	x	x
		Oculeomyia	*bitaeniorhynchus*	x	x	x	x
		Phenacomyia	*corniger*	x	x	x	x
		Sirivanakarnius	*boninensis*	x	x	x	x
		Tinolestes	*latisquama*	x	x	x	x
	Deinocerites		*cancer*	x	x	x	x
	Lutzia	*Metalutzia*	*fuscanus*	x	x	x	x
Mansoniini		*Mansonioides*	*africana*	x	x	x	x
Orthopodomyiini	*Orthopodomyia*		*anopheloides*	x	x	x	x
Sabethini	*Maorigoeldia*		*argyropus*	x	x	x	x

Table 2: Species and lifestages used in the phylogenetic analysis of the genus *Culex*.

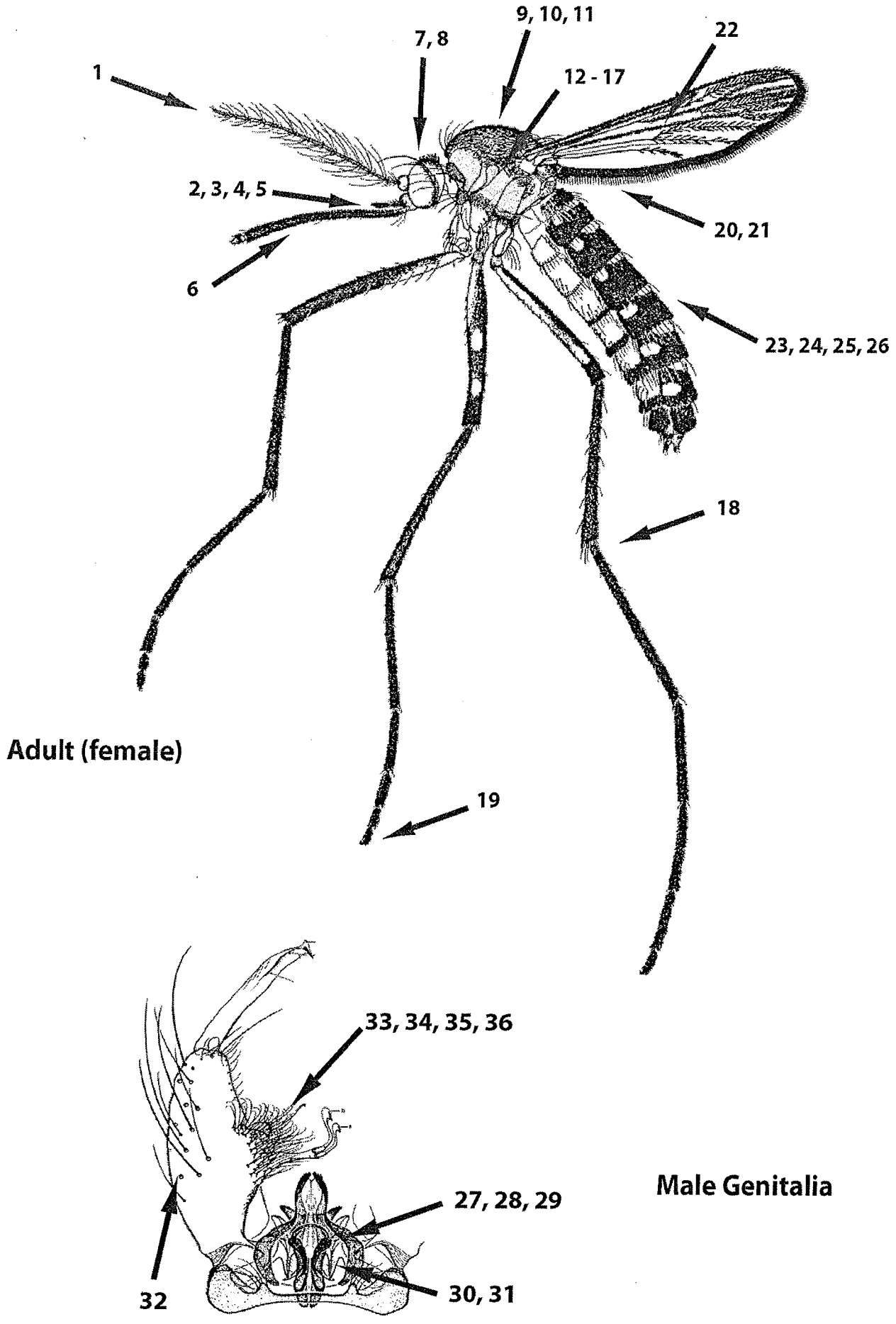

Figure 1: Morphological characters of adults and male genitalia used in this study. Numbers refer to coded characters listed in Appendix 1. Illustrations from Berlin & Belkin (1980)

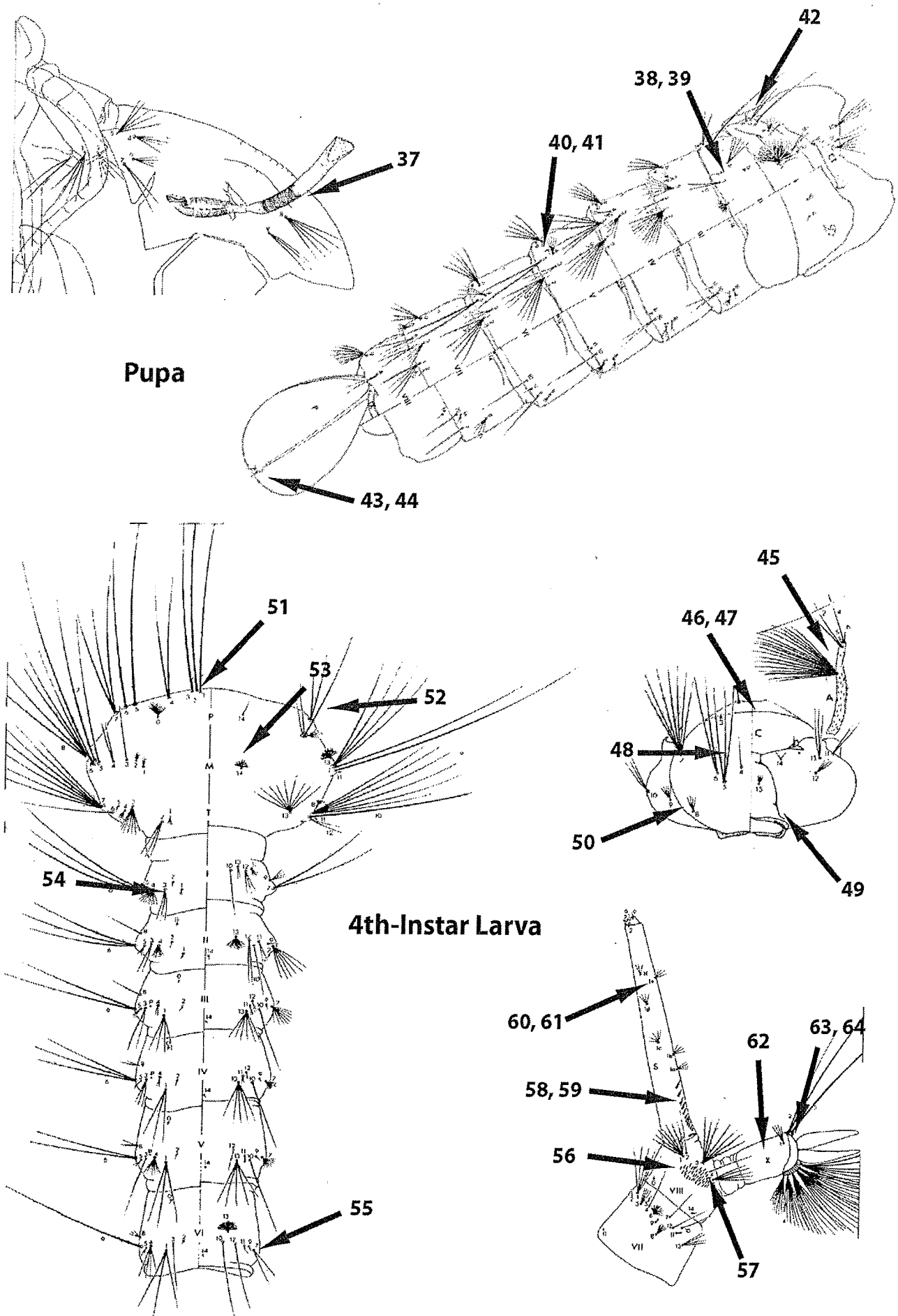

Figure 2: Morphological characters of pupae and larvae used in this study. Numbers refer to coded characters listed in Appendix 1. Illustrations from Harbach (1988)

2.3. Phylogenetic Analysis:

Phylogenetic analyses of the data were conducted using PAUP 4.0b (Swofford, 2003). Initial analysis of the dataset was preformed employing a heuristic search of 10,000 replicates, holding 10 trees at each step, using a tree bisection-reconnection (TBR) branch swapping algorithm, holding 10 trees at each step and treating all characters with equal weighting. Additional heuristic searches, of 10,000 replicates, using two methods of *a posteriori* weighting: successive approximations character weighting (SACW), where weights are adjusted according to the rescaled consistency index of the most parsimonious tree (MPT), and implied weighting using a concavity constants (K) of 1-3 to down-weight the homoplastic characters. Bootstrap values were calculated with 1000 replicates of 1000 random replications in PAUP. Total and partitioned Bremer support indices were calculated using PAUP and Treerot (Sorenson, 1999). Bayesian analyses were conducted with MrBayes vers. 3.1.2, simultaneously sampling four Markov chains (three cold and one hot) over 10 million generations (Ronquist & Huelsenbeck, 2003). The chains were sampled every 100^{th} generation with a burnin of 25,000 samples discarded. The likelihood model employed was the Mk model for morphological data used by Lewis (2001).

3. Results:

Maximum parsimony (MP) analysis of the 64 characters under equal weighting yielded 8 most parsimonious trees (MPT) (TL= 348, CI = 0.20, RI = 0.58), the consensus tree is shown in **Figure 3**. Total Bremer support (BS) for the consensus tree was 81, with a total support index (ti, ratio of total BS to the TL) of 0.24. Partitioned Bremer total support values for the 4 sets of characters are summarised in **Table 3**. The negative Bremer support values indicate that the male genitalia and larval datasets were not congruent with the adult and pupal datasets. This conclusion was supported by the partition homogeneity test which gave a P value of 0.02 (Farris et al., 1994; Swofford, 2003). This indicates that there is significant conflict in the datasets used, that does not support a congruent phylogenetic history (Johnson et al., 2001).

Character Partition	Partitioned BS values	Partitioned support index (ti)
Adult	114	0.33
Male genitalia	-35	-0.10
Pupa	30	0.10
Larva	-28	-0.10
Combined Partitions	81	0.24

Table 3: Partitioned Bremer support values and support indices for the 4 character partitions

The consensus tree (**Figure 3**) indicates that *Culex* is not monophyletic, with the genus *Deinocerites* placed as a derived sister group to the subgenus *Belkinomyia*. All of the subgenera of *Culex* are enclosed within Clade B, with the genus *Lutzia* paraphyletic with respect to *Culex*. Clade A contains a distinct grouping of taxa exclusive to Africa. Clade C contains only subgenera present in the New World and represents the majority of the *Melanoconion* group, though not including *Melanoconion* itself. Most subgenera are retrieved as monophyletic. Bremer supports were positive for all nodes, however they were often < 3. Bootstrap supports for nodes on the tree were generally poor with the majority <50%. The ingroup was supported at the 55% level and the majority of monophylectic subgenera had support values >60%. Many characters in the MP analysis showed a high degree of homoplasy, notably characters 17, 24, 25, 48 and 58 with high HI and low rescaled CI.

The implied weighting and SACW search strategies both resulted in a single MPT. The SACW tree (TL= 37.5, CI = 0.29, RI = 0.67) is shown in **Figure 4**. The implied weighting tree (K= 3, TL =354, CI = 0.20, RI = 0.57) is shown in **Figure 5**. The tree topology obtained under SACW differs from the unweighted MPT consensus tree only in the monophyly of subgenus *Lophoceraomyia* and the Pipiens Group of subgenus *Culex*. SACW weighting is extremely vulnerable to errors introduced in the initial weighting step, which can occur when heuristic searches fail to find all of the MPTs (Farris, 1969; Goloboff, 1993).

The value of the concavity constant (K) used in implied weighting analysis is arbitrary (Goloboff, 1995). Hence we conducted searches with values of K ranging from 1-3. K values of ≤ 2 resulted in the strong down-weighting of over 60% of the characters, a situation that we considered to have resulted in the exclusion of too much information. The intermediate value of K= 3 was favoured as it places moderate down weighting on homoplastic characters, while maintaining clades we considered to be realistic. The implied weighting tree (**Figure 5**) with K=3 differs in several ways from the unweighted tree. Clade E in the K=3 tree exclusively contains all New World *Culex,* including the subgenus *Melanoconion*, which is placed basal to the other New World subgenera. Clade D shows a substantially different topology to the unweighted tree, with the Asian subgenera such as *Eumelanomyia*, *Culiciomyia* and *Acallyntrum* removed from a monophylectic clade and the loss of monophyly of the subgenera *Culiciomyia* and *Neoculex*.

The Bayesian analysis supported a tree (**Figure 6**) with low resolution compared to the parsimony analysis. This was due to the poor convergence of the Markov chains (even when the MPT was used as a guide tree), which may have been caused by the poor information content of the characters used or inadequacy of the Mk likelihood model. Although many higher level relationships are not resolved, clades G and F, which are supported with reasonable posterior probabilities, are similar in composition to clades A and C in the unweighted consensus tree containing the subgenera exclusive to the Old and New World, respectively. The Bayesian tree also agrees with the parsimony analysis in supporting the monophyly of the majority of subgenera and the placement of *Lutzia* and *Deinocerites* as external and internal to the *Culex* clade respectively, with a high posterior probability of 0.86.

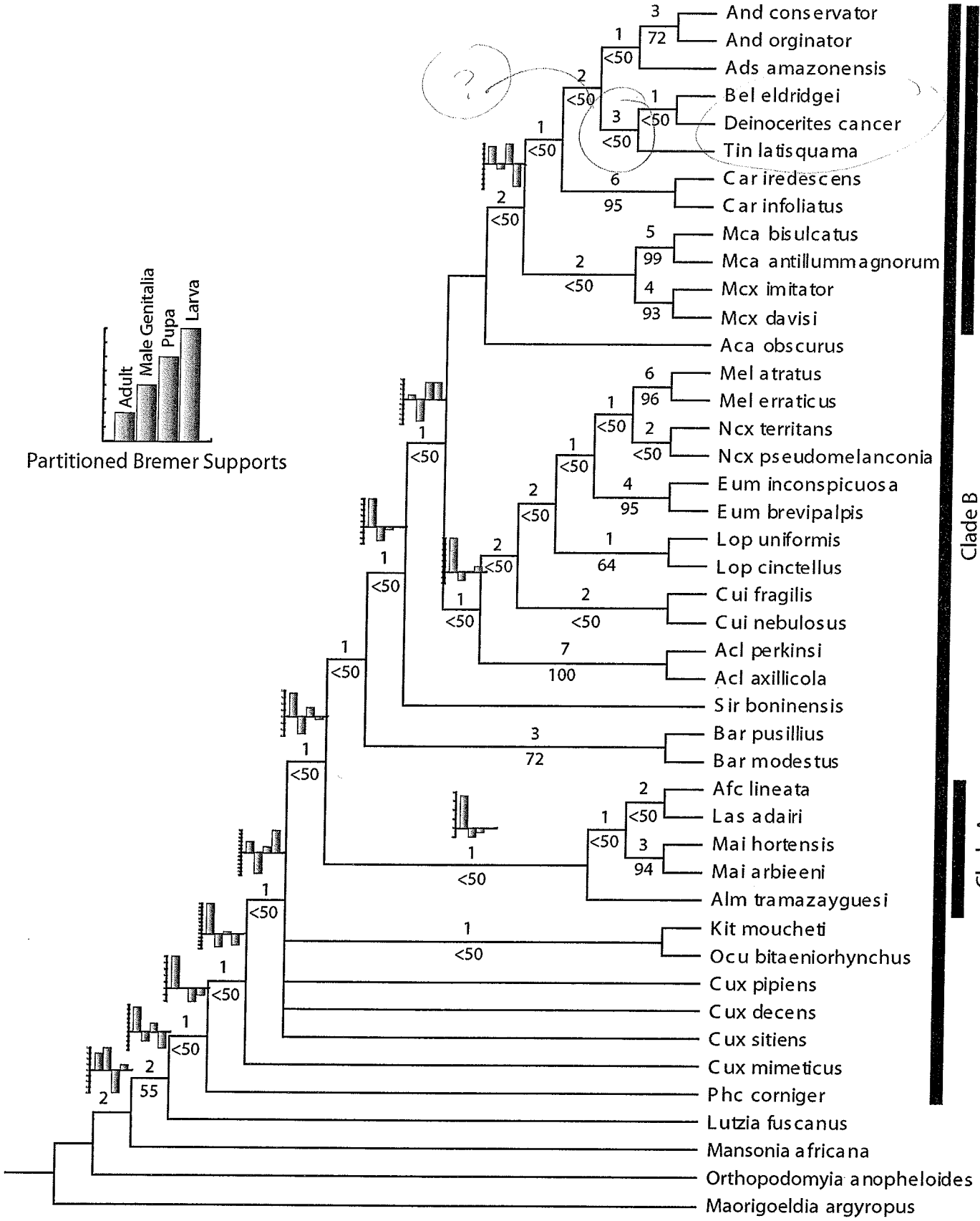

Figure 3: Most parsimonious tree generated from analysis of 64 morphological characters for 43 taxa (Length= 348, CI = 0.20, RI = 0.58). Three-letter abbreviations for subgenera are used. Bootstrap values plotted below branches with Bremer support values plotted above. Partitioned Bremer supports are plotted for some key nodes.

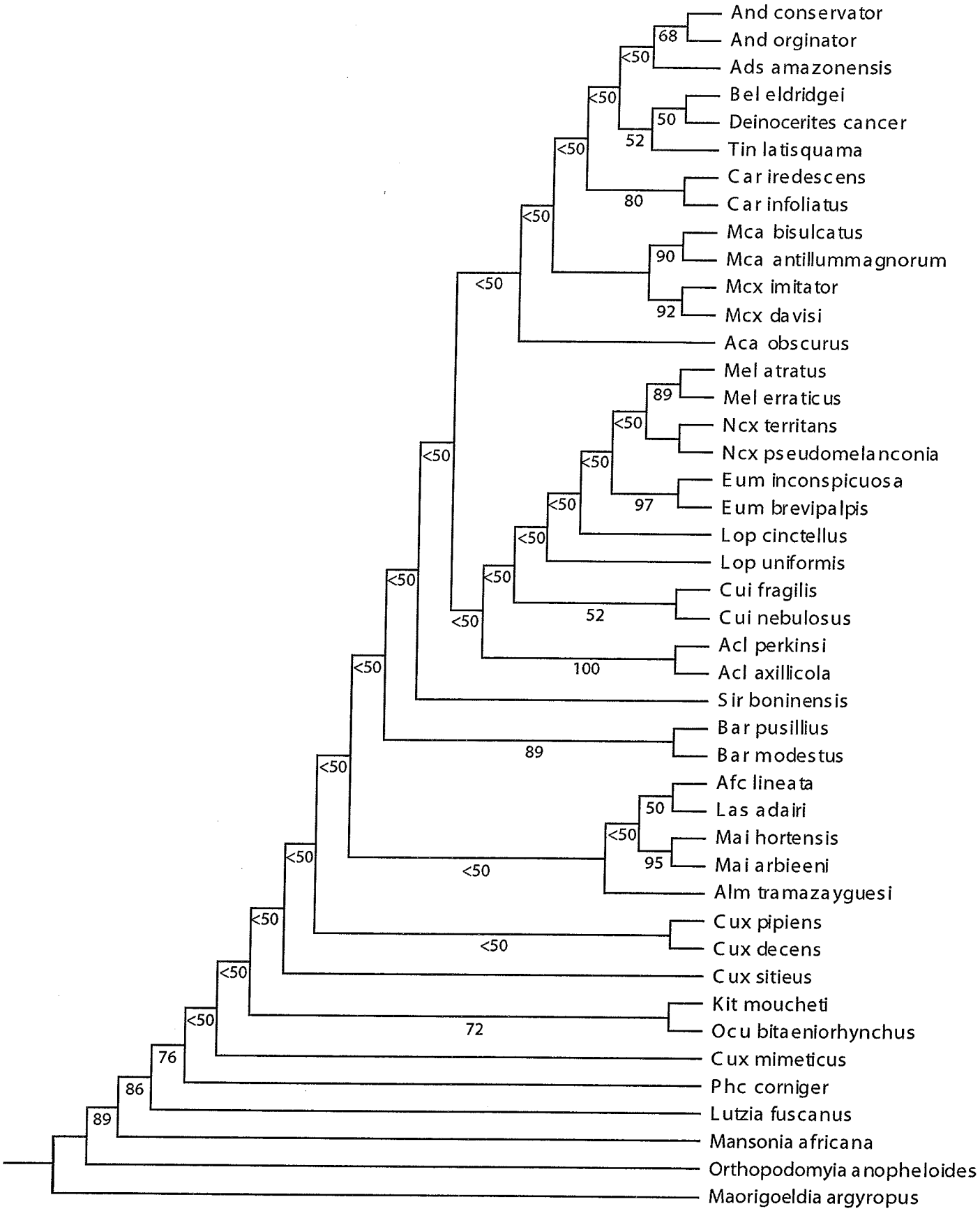

Figure 4: Single MPT from parsimony analysis with successive approximations character weighting. Bootstrap support values are placed beneath branches. Three-letter abbreviations for subgenera are used.

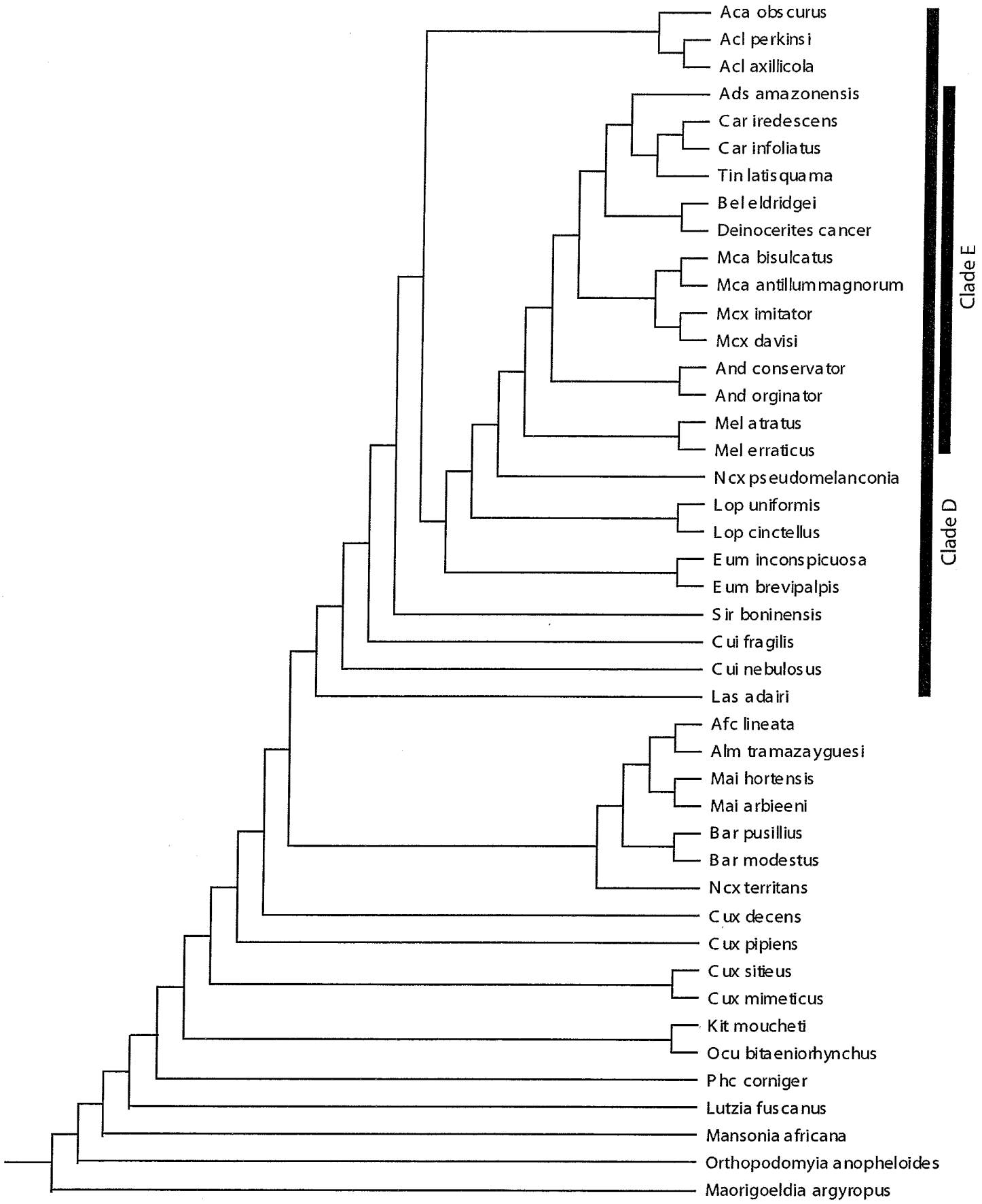

Figure 5: Most parsimonious tree generated from analysis of 64 morphological characters for 43 taxa under implied weighting of K= 3 (TL =354, CI = 0.20, RI = 0.57). Bootstrap support values are placed under branches. Three-letter abbreviations for subgenera are used.

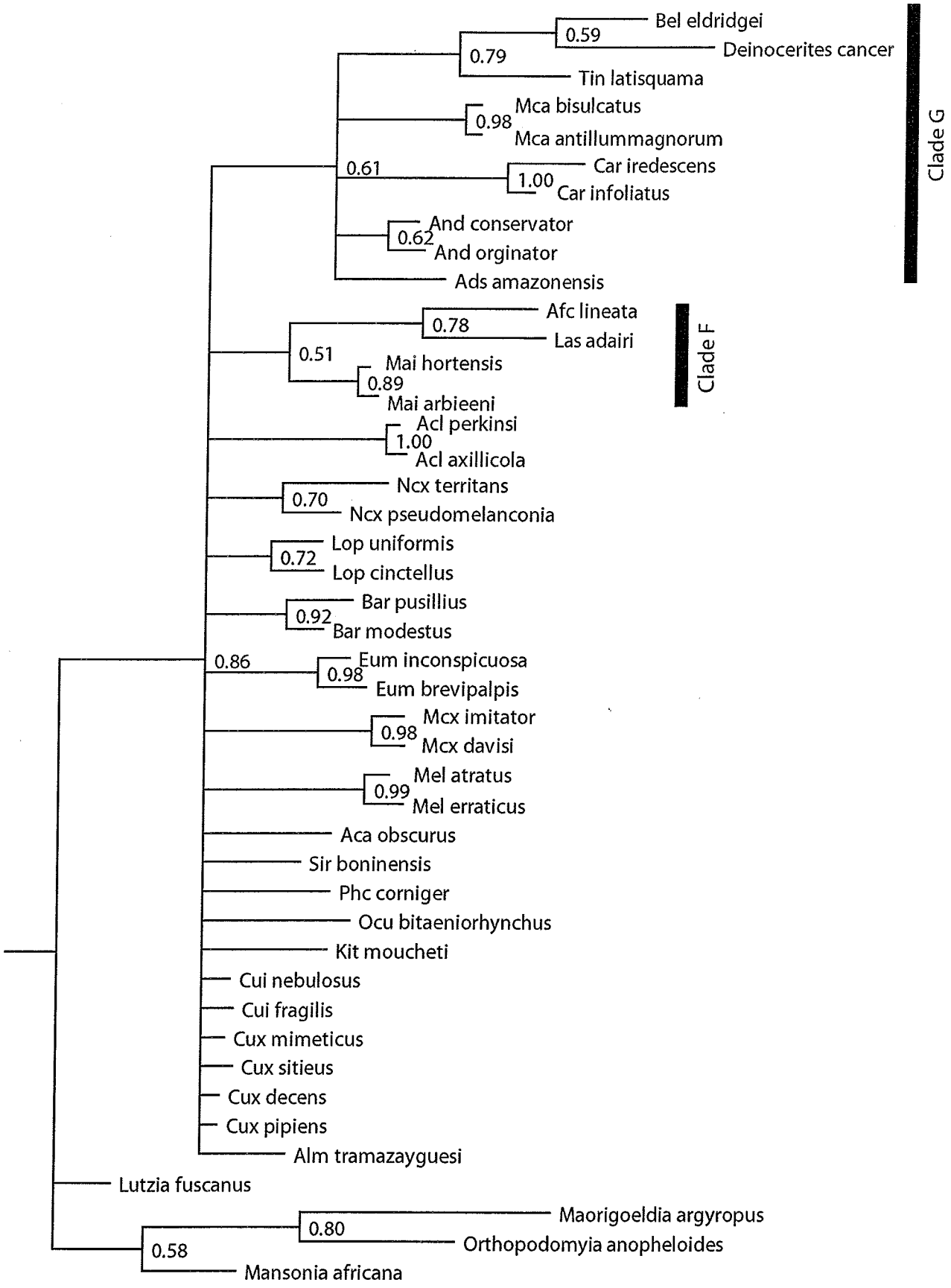

Figure 6: Tree from Bayesian analysis. Branches are annotated with Bayesian posterior probabilities. Three-letter abbreviations for subgenera are used.

4. Discussion:

4.1. Analysis of the Data and Concurrence of Results from Different Partitions:

Previous unpublished phylogenetic analyses of *Culex* suffered from substantial homoplasy in both morphological and molecular data (Mallampalli, 1995; Juthayothin, 2004). This was also the case in our unweighted MP analysis; where substantial homoplasy leads to low support values for many of the clades identified. Most of the homoplasy in the data seems to arise from the incongruence of the characters from the different data partitions, with both the Bremer support and partition homogeneity tests indicating different signals in the adult/pupal and larval partitions. These findings conflict with those of Judd (1998) who showed that life stage concordance was high for the Sabethin mosquitoes, but agrees with the recent phylogeny of the Aedine mosquitoes by Reinert et al. (2004) that showed conflicting phylogenetic signal between larval and adult characters.

4.2. The Culicini:

This study suggests that the genus *Culex* does not form a monophylectic clade within the tribe Culicini because the genus *Deinocerites* is placed as a derived member of the *Melanoconion* group, sister to subgenus *Belkinomyia*. Both the MP and Bayesian analysis agree on this conclusion, with the node receiving good support from the Bayesian posterior probabilities and the Bremer support. This conclusion agrees with Mallampalli's (1995) findings that placed *Deinocerites* and the closely related genus *Galindomyia* within the *Culex* clade, and also confirms Valencia's (1973) hypothesis of the close relationship of *Deinocerites* to the *Melanoconion* group. The latter hypothesis is also supported by the sister-group relationship based on Isoe's (2000) analysis of vitellogenin gene sequence data. The genus *Lutzia* is supported in all analyses as basal and paraphyletic to the *Culex* clade, agreeing with both Mallampalli (1995) and Navarro (2000). This result is also supported by the many unique synapomorphies seen in *Lutzia* that differentiate it from other Culicini (Belkin, 1962; Tanaka, 2003).

4.3. The Genus *Culex*:

The equivalent support values for the different trees make it impossible to decide on a preferred phylogeny. However similar topologies within genus *Culex* are supported by both the unweighted and weighted MP analyses and show relationships congruent with many of the traditional ideas about genus *Culex*.

All of the analyses place *Phenacomyia* and the closely related *Culex* as the most basal subgenera within the genus, a relationship which has been previously suggested (Navarro, 2000). The basal placement of *Kitzmilleria* and *Oculeomyia* as sister taxa finds strong support in our analyses, it being the first time this relationship has been proposed, though their affinity to subgenus *Culex* has been previously suggested, with the species of *Oculeomyia* originally traditionally placed in the subgenus *Culex* (Danilov, 1989; Tanaka, 2004; Knight & Stone, 1977).

The MP analyses support three distinct groupings within *Culex*. The largest of these places the South American taxa as a monophylectic clade, which is also supported by the Bayesian analysis. The basal placement of *Melanoconion* within this clade is only supported by the implied weighting analysis. The varying placement of *Melanoconion* in this study is caused by its affinity with *Neoculex*, which results in MPTs whereby *Melanoconion* is placed in the clade containing the Asian subgenera. This clade of South American taxa undoubtedly represents the traditional *Melanoconion* group (even if *Melanoconion* not placed in the clade by all analyses), which has always been one of the most distinct groups within genus *Culex* (Belkin, 1962; Sirivanikarn, 1983). *Melanoconion* is the largest and most diverse of the South American subgenera and the basal placement in the implied weighting analysis supports the idea that this subgenus may have been the source of the Neotropical radiation of genus *Culex*, as suggested by Sirivanikarn (1983).

The second major grouping to appear in the MP analyses is that containing *Acallyntrum*, *Culiciomyia*, *Lophoceraomyia*, *Eumelanomyia* and *Neoculex*. These are subgenera that occur primarily in the Oriental region. Our analyses indicate that *Acallyntrum* and *Culiciomyia* are the most primitive members of this group, with *Neoculex* being the most derived. In the unweighted and SACW trees this group forms a monophylectic clade sister to the "*Melanoconion* clade". Monophyly is lost in

the implied weighting tree and the group is placed basal to the "*Melanoconion* clade". Based on the support values from the analyses the correct relationship between the Asian and South American groups cannot be adequately resolved with the dataset.

In our phylogenies the other distinct group to appear is that containing subgenera *Afroculex*, *Lasiosiphon* and *Maillotia,* and *Barraudius* in the implied weighting analysis. All of these subgenera are distributed primarily on the African continent. The close relationship of these subgenera was first suggested by Danilov (1989), and is supported in all our analyses, though in the implied weighting analysis *Afroculex* forms a paraphyletic clade. The placement of *Barraudius* varies with the weighting scheme used, but it seems to have affinity with the Asian subgenera of *Culex*. All our analyses support a close relationship between this African group and subgenus *Allimanta,* which is only known to occur in Argentina. This makes *Allimanta* the only South American subgenus not originating within the "*Melanoconion* clade", a result also found by Mallampalli (1995).

This study supports the monophyly of the majority of subgenera within genus *Culex*. Notably the subgenus *Culex* is not monophyletic, 4 taxa included in the analysis form an unresolved polytomy in the unweighted MPT (**Figure 3**). Under the traditional groupings *Cx. pipiens* and *Cx. decens* fall within the Pipiens Group with *Cx. sitiens* and *Cx. mimeticus* in the Sitiens Group. *A posteriori* weighting results in the Sitiens (in the K=3) and Mimeticus subgroups (in the SACW tree) being retrieved, but never with strong support. This lack of resolution may indicate that the grouping within the subgenus *Culex*, which is the most diverse and most widely distributed of all the subgenera, does not represent monophyly (Harbach, 1988).

4.4. Implications for the Classification of *Culex*:

Many of the subgenera of *Culex* were originally described as genera and later moved to subgeneric level by Edwards (1932). Major ambiguity exists about the criteria for supraspecific taxonomic ranking, though the single most important is undoubtedly monophyly (Hennig, 1966; Mayr, 1999). This study strongly supports the conclusion that *Culex* is not monophyletic; because the genus *Deinocerites* is placed as a sister taxon to the more derived members of the *Melanoconion* group. In cases such as this, where a taxon is found to be paraphyletic, it is generally justifiable to reclassify the

group to ensure that the taxonomic ranking reflects monophyly (Zakharov, 2007). On that basis this study would support many of the subgenera of *Culex* being raised to generic level.

5. Conclusion:

This study represents the most comprehensive phylogenetic analysis of the genus *Culex* to date. Our results highlight the continuing problem of identifying informative characters for constructing a phylogeny of *Culex* based on morphology. A substantial degree of homoplasy exists in our dataset, much of this arising from the character conflict between life stages. Despite this our results support several clear groupings within the genus *Culex*, the most distinct being the Neotropical "*Melanoconion* clade". The monophyly of genus *Culex* within the tribe Culicini is not supported and on that basis this study shows the need for a reclassification of the genus, to better reflect the phylogeny of the group.

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Appendix 1: Characters used in the phylogenetic analysis of Culex

Lifestage	Character Number	Character	Character States
Adult	**1**	Antenna, length of penultimate flagellomere:	same as proximal flagellomeres (0); greater than proximal flagellomeres (1).
	2	Maxilla, palpomeres (males):	five(0); four(1); three, 4th vestigial or absent(2)
	3	Maxilla, palpomeres segments 3-5 (males):	with few setae(0); with numerous setae(1)
	4	Maxilla: proboscis ratio:	<0.7(0); 0.7-1.0(1); >1.0(2)
	5	Maxillary palpus, white scales, on dorsal surface:	absent(0); present(1)
	6	Proboscis, with pale ring:	absent(0); present(1)
	7	Vertex, broad scales on central part of orbital line:	absent(0); present(1)
	8	Vertex, anterior dorsocentral setae:	few(0); numerous(1)
	9	Scutum, dorsocentral setae: :	absent(0); present(1)
	10	Scutum, acrostichal setae:	absent(0); present(1)
	11	Scutum, acrostichal setae:	incomplete(0); complete line(1)
	12	Upper proepisternal scales:	absent(0); present(1)
	13	Upper mesokatepisternal scales:	absent(0); present(1)
	14	Prealar knob, scales:	absent(0); present(1)
	15	Postspiracular scales:	absent(0); present(1)
	16	Upper mespimeral scales:	absent(0); present(1)
	17	Lower mespimeral setae:	absent(0); present(1)
	18	Hindtibia, length relative to tarsomere	shorter(0); longer(1)
	19	Pulvilli:	absent(0); present(1)
	20	Dorsal tertiary fringe scales (DTFS) on proximal half of wing:	absent(0); present(1)
	21	Dorsal tertiary fringe scales (DTFS) on proximal half of wing (males):	absent(0); present(1)
	22	Anal vein (1A), relative to junction of crossvein mcu and vein M3+4:	terminates proximal to junction of mcu and M3+4 (0); ends distal to junction (1)
	23	Terga, apicolateral pale patches:	absent(0); present(1)
	24	Terga, basolateral pale patches:	absent(0); present(1)
	25	Terga, basal pale bands:	absent(0); present(1)
	26	Terga, apical pale bands:	absent(0); present(1)
Male Genitalia	**27**	Opisthophallus:	absent(0); present(1)
	28	Opisthophallus, development:	well developed, sclerotized (0); poorly developed, membranous (1)
	29	Aedeagal sclerites:	fused(0); not-fused(1)
	30	Phallosome, denticles on inner side:	absent(0); present(1)
	31	Paraproct, basal lateral arm:	absent(0); present(1)

	32	Gonocoxite, scales:	absent(0); present(1)
	33	Gonocoxite, subapical lobe:	absent(0); present(1)
	34	Gonocoxite, position of subapical lobe:	proximal to mid-length (0); distal to mid-length but not near apex (1); subapical (2)
	35	Subapical lobe of gonocoxite, development:	Short arm like lobe(0); long(1)
	36	Subapical lobe of gonocoxite, development:	undivided (0); bilobed (1)
Pupa	**37**	Trumpet, meatal cleft:	absent(0); present(1)
	38	Seta 1-II:	closer to each other then to setae 3-II(0); closer to setae 3-II then to each other(1)
	39	Position of setae 2-II relative to setae 3-II:	closer to each other then to seta 3 (0); closer to setae 3 than to each other (1)
	40	Seta 5-V, development:	single or double(0); multiple branched(1)
	41	Seta 5-V, length:	shorter then tergite(0); longer than tergite(1)
	42	Seta 6-I, length:	longer than seta 7(0); equal to or shorter than seta 7 (1)
	43	Seta 1-P:	absent(0); present(1)
	44	Seta 2-P:	absent(0); present(1)
Larva	**45**	Seta 1-A, position:	insertion near apex of antenna(0); insertion near mid-length of antenna (1)
	46	Median labral plate:	fused to dorsal apotome (0); distinct from dorsal apotome (1)
	47	Seta 3-C:	absent(0); present(1)
	48	Seta 4-C, position:	placed closer to setae 5-C then to each other(0); placed closer to each other then setae 5-C(1)
	49	Hypostomal suture:	does not extend caudad of posterior tentorial pit (PTP) (0); extends caudad of PTP (1)
	50	Seta 9-C, position:	anterior to seta 8 (0); posterior to seta 8(1); on same level as seta 8 (2)
	51	Length of setae 3-P relative to setae 1-P and 2-P:	shorter(0); equal or longer(1)
	52	Seta 8-P, development:	single(0); double(1); multiple branched(2)
	53	Seta 14-M:	weakly developed(0); strongly developed(1)

54	Setae 1,2,3-I, relative positions:	points of insertions more or less form an equilateral triangle (0); points of insertions form a narrow scalene triangle, almost inserted in a longitudinal line (1)
55	Seta 7-VI, development:	single(0); multiple branched(1)
56	Comb scales:	less than 10(0); more than 10(1)
57	Seta 4-VIII, position:	insertion in line with middle of segment X (0); insertion between base of siphon and dorsal margin of segment X (1)
58	Pecten:	absent(0); present(1)
59	Pecten spines, development:	lacking denticles (0); with one or more stout denticles (1); with slender, hair-like denticles (2)
60	Siphon, dorsolateral setae:	absent(0); present(1)
61	Siphon, arrangement of ventrolateral setae:	in continuous single row (0); single row with one or more setae laterally displaced(1)
62	Saddle:	incomplete(0); complete(1)
63	Segment X, spicules on posterior area:	absent(0); present(1)
64	Saddle, spicules on posterior margin:	absent(0); present(1)

Appendix 2: Data matrix for forty-three species and sixty-four morphological chracters used in the analysis.

Genus/subgenus	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64
Acalleomyia	A. obscurus	1	2	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	1	0	1	1	0	1	0	0	0	0	1	0	0	1	0	0	1	2	1	0	0	1	0	0	1	1	1	1	1	1	1	1	1	?	0	0	0	0	1	1	1	1	1	0	0	0	0	1
Acallyntrum	A. perkinsi	1	0	1	2	0	0	0	1	1	1	1	1	0	0	0	0	0	0	1	1	1	1	0	0	0	1	0	0	1	0	0	1	0	0	1	1	1	0	0	1	0	0	1	0	1	0	1	0	1	1	0	0	0	2	0	1	1	1	0	1	1	0	0	0	1	0
	A. axillicola	1	0	1	2	0	0	0	1	1	1	1	1	0	0	0	0	0	0	1	1	1	1	0	0	0	1	0	0	1	0	0	1	0	0	1	1	1	0	0	1	0	0	1	0	1	0	1	0	1	1	0	0	0	2	0	1	1	1	0	1	1	0	0	0	1	0
Aedinus	A. amazonensis	1	1	0	0	0	0	1	1	1	0	-	0	0	0	0	0	1	0	1	1	0	1	0	0	0	0	1	0	1	0	0	1	1	0	1	0	0	1	0	1	0	0	1	0	1	0	1	0	1	1	0	0	0	2	0	?	1	1	0	1	0	0	0	0	1	0
Afroculex	A. lineata	0	0	0	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	1	0	0	0	1	0	1	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1	1	1	1	1	1	0	1	0	0
Allimanta	A. tramazayguesi	1	0	0	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	1	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Anoedioporpa	A. conservator	1	1	0	0	0	0	1	0	1	0	-	0	0	0	0	0	0	0	1	1	1	0	1	0	0	0	0	1	0	1	0	0	0	0	1	0	0	1	0	1	1	0	1	1	0	1	0	1	0	1	1	0	1	0	1	1	1	1	0	1	1	?	1
	A. orginator	1	1	0	0	0	0	1	0	1	0	-	0	0	0	0	0	0	1	?	1	0	0	1	0	0	0	0	1	1	1	0	0	0	1	1	1	1	0	0	0	0	1	1	0	0	0	1	0	1	1	0	0	1	0	0	1	1	1	1	2	1	1	1	0	1
Barraudius	B. pusillius	1	0	0	2	0	0	1	1	1	1	0	1	1	1	0	1	1	1	1	1	0	1	0	1	0	0	1	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	1	1	0	0	0	1	0	1	1	1	0	1	0	0	1	1	1	1	1	1	1	0	1	1
Belkinomyia	B. eldridgei	?	2	0	0	0	0	0	1	1	0	-	0	0	0	0	0	0	0	1	1	1	?	0	0	0	0	0	1	1	1	1	0	0	1	0	1	0	1	0	0	1	1	0	1	0	1	0	0	0	2	1	1	0	0	1	1	0	1	1	0	0	1	0	1
Carrollia	C. iredescens	1	0	0	1	0	0	1	0	0	0	-	0	0	0	0	0	0	1	0	1	1	0	1	0	1	1	1	0	0	1	1	0	1	1	1	0	1	0	1	0	1	0	0	1	1	0	1	0	0	1	0	0	0	0	0	0	1	1	0	1	0	0	1	1	0	1
	C. infoliatus	1	0	0	1	0	0	1	0	0	0	-	0	0	0	0	0	0	1	0	1	1	0	1	0	1	1	1	0	0	1	1	0	1	1	0	1	1	0	1	1	1	0	1	1	1	1	0	0	0	1	1	0	2	1	0	1	1	1	1	2	0	1	1	0	1
Culex	C. pipiens	1	0	1	2	0	0	0		1	1	1	1	1	0	0	1	1	1	1	1	0	1	0	0	1	1	1	0	0	0	1	0	1	0	0	1	0	0	1	1	0	0	1	0	0	0	1	1	0	2	0	0	1	1	1	1	2	0	1	1	0	1
(pipiens group)	C. decens	1	0	1	2	0	0	0	0	1	1	1	1	1	0	0	1	1	1	1	1	0	1	0	1	1	1	0	1	0	0	0	1	0	1	1	1	0	0	1	0	0	1	0	1	1	0	1	1	0	1	1	1	1	0	0	0	1	0	1	1	0	1	1	0	0
Culex	C. sitiens	1	0	1	2	0	1	0	1	1	1	1	1	1	1	0	1	0	1	1	1	1	0	1	0	1	1	1	0	1	0	0	0	1	0	1	1	1	0	0	1	0	0	1	0	1	1	0	1	1	0	1	0	0	1	0	0	0	1	0	1	1	0	1	1	0	1
(sitiens group)	C.mimeticus	1	0	1	2	0	1	0	1	1	1	1	1	1	1	0	0	1	0	1	1	1	0	0	0	1	1	0	1	0	0	0	1	0	1	1	1	0	0	1	0	0	1	0	1	1	0	1	1	0	1	0	1	1	0	0	0	1	0	1	1	0	1	1	0	1
Culiciomyia	C. fragilis	1	0	1	2	0	0	1	0	1	0	-	0	0	0	0	0	1	1	1	1	0	1	0	0	0	0	1	0	0	0	1	0	1	1	1	0	0	1	0	0	1	0	1	1	0	1	1	0	1	1	1	2	0	0	0	1	1	1	1	0	1	1	0	0
	C. nebulosus	1	0	1	2	0	0	1	0	1	0	-	0	1	0	0	0	1	1	1	1	0	1	0	0	0	0	1	0	0	1	1	0	1	1	1	0	0	1	0	0	1	0	1	1	0	1	1	1	1	0	0	0	0	0	0	1	1	1	1	0	0	1	1	0
Eumelanomyia	E. inconspicuosa	0	0	0	0	0	0	1	0	1	1	1	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	1	0	0	1	0	0	1	0	1	1	1	0	0	1	0	0	1	0	1	0	1	1	1	0	1	?	0	1	1	1	1	?	0	2	0	0	0	1	1	1	1	0	1	1	1	1
	E. brevipalpis	0	0	0	1	0	0	1	0	1	1	0	0	0	0	0	0	0	0	1	1	?	0	1	0	0	0	0	1	0	0	1	0	0	1	1	1	0	1	0	0	1	0	0	0	1	0	1	1	1	1	0	0	0	0	0	1	1	0	1	2	0	0	1	0	1
Kitzmilleria	K. moucheti	1	0	1	2	0	0	0	1	1	1	1	1	1	0	0	1	1	1	1	1	0	1	0	1	1	0	1	0	0	0	1	0	0	1	1	1	0	1	0	0	1	0	0	0	1	0	1	1	1	1	0	0	0	0	1	1	1	0	1	2	0	0	1	0	1
Lasiosiphon	L. adairi	1	?	0	1	?	0	?	1	1	1	1	0	1	0	0	1	1	1	1	0	0	1	1	0	0	1	1	0	0	0	1	0	1	0	1	0	0	0	0	1	0	1	0	0	?	?	1	1	1	?	1	1	1	0	0	0	?	0	0	0	-	0	1	0	0	0
Lophoceramyia	L. uniformis	1	0	1	2	0	0	1	0	1	0	-	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	1	0	1	0	1	0	0	1	0	0	1	0	0	0	0	1	1	1	1	1	0	0	0	?	?	1	0	1	1	0	0	1	0	1
	L. cinctellus	1	0	1	2	0	0	1	0	1	0	-	0	0	0	0	0	0	1	1	1	0	0	1	0	1	0	0	1	0	0	0	0	0	1	1	1	0	0	0	0	1	0	0	1	1	0	1	1	0	1	0	0	2	0	0	1	1	1	1	0	1	1	1	1
Maillotia	M.hortensis	1	0	0	2	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	1	1	1	0	0	1	1	0	1	1	0	1	0	1	0	0	1	0	0	1	1	1	1	1	0	1	1	1	1
	M.arbieeni	1	0	0	2	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	1	1	1	0	0	1	0	1	1	0	1	0	0	1	0	1	0	0	0	0	1	0	1	1	0	1	1	1	1
Melanoconion	M. atratus	1	0	1	2	0	0	0	0	1	0	-	0	0	0	0	0	1	1	1	1	1	1	0	1	0	0	1	1	1	0	0	1	1	1	1	1	1	0	0	1	0	0	1	0	1	1	0	1	0	0	1	0	1	0	0	0	0	1	0	1	1	1	1	1	1
	M.erraticus	1	0	1	2	0	0	0	0	1	0	-	0	1	0	0	0	1	1	1	1	1	1	0	1	1	0	1	1	1	0	0	1	1	1	1	1	?	?	?	?	?	?	?	?	0	1	0	1	1	0	0	0	1	0	0	0	1	1	1	2	1	0	1	0	1
Micraedes	M. bisulcatus	1	1	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	1	1	1	1	0	1	0	0	1	1	1	0	0	0	1	1	1	1	0	1	0	0	1	1	1	0	1	1	0	1	1	0	0	2	1	0	1	1	1	1	1	0	1	1	0	1
	M. antillummagnorum	1	1	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	1	1	1	1	0	1	0	0	1	1	1	0	0	0	1	1	1	1	0	1	0	0	2	1	0	1	1	1	1	1	1	0	1	1	0	2	1	0	1	1	1	1	1	0	1	1	0
Microculex	M. imitator	1	0	1	2	0	0	1	1	1	1	1	1	1	0	0	0	1	0	1	1	0	1	0	1	1	0	1	1	1	0	0	0	1	1	1	1	?	?	?	?	?	?	?	?	0	1	0	1	1	0	1	0	1	1	0	0	2	1	0	1	1	1	1	1	1	0
	M. davisi	1	0	1	2	0	0	1	1	1	1	1	0	1	0	0	0	1	0	1	1	0	1	0	1	0	0	1	1	1	0	0	0	1	1	1	1	0	1	0	0	1	0	0	1	0	1	0	1	1	?	0	?	1	0	?	1	0	1	0	0	1	1	0	0
Neoculex	N. territans	1	0	1	2	0	0	1	0	1	0	-	1	1	1	1	1	0	1	1	1	0	1	0	1	0	0	0	1	1	0	0	0	0	0	1	1	1	1	?	?	?	?	?	?	?	?	0	1	1	0	1	0	0	1	0	?	1	0	1	0	0	1	1	1	0
	N. pseudomelanconia	1	0	1	2	0	0	1	0	1	0	-	0	1	0	0	0	0	0	1	1	0	1	1	0	0	1	1	0	0	0	0	0	0	1	1	1	1	1	0	1	0	0	1	1	0	1	0	0	1	0	0	1	1	0	1	1	0	0	1	1	1
Oeuleomyia	O.bitaeniorhynchus	1	0	1	2	0	1	0	1	1	0	-	1	1	0	0	0	0	0	1	1	0	0	0	1	0	1	1	0	0	0	1	0	1	1	1	0	0	1	0	1	0	0	1	1	1	0	1	1	1	1	1	0	0	0	0	0	1	1	0	1	1	0	0	1	0	1
Tinolestes	T. latisquama	0	0	0	0	0	0	0	1	0	0	-	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	1	1	1	0	0	1	1	2	1	1	0	1	0	0	1	0	1	1	0	1	0	0	1	1	1	1	1	1	0	0	0	0	1	1	0	0	1	0	0
Phenacomyia	P. corniger	1	0	1	2	0	1	0	1	1	1	0	1	1	0	0	0	1	0	1	1	0	1	0	1	1	0	1	0	0	1	1	0	1	1	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0	1	0	1	0	1	1	1	1	1	0	1	1	1	0	1
Sirivanakarnius	S.boninensis	1	0	0	1	0	0	0	0	1	1	1	0	0	0	0	0	0	0	1	1	1	1	1	0	1	0	0	1	0	0	1	1	0	1	1	1	0	0	1	0	0	0	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Lutzia	L.fuscanus	1	0	1	2	0	1	0	1	1	0	-	1	1	1	0	1	1	1	1	1	0	0	1	0	0	0	1	0	0	0	1	0	1	0	1	1	1	0	0	1	1	0	1	1	1	0	1	1	0	1	1	1	0	2	0	1	1	0	0	1	1	1	1	0	1	1	0	1
Deinocerites	D.cancer	0	2	0	0	0	0	0	0	1	0	-	0	1	0	0	0	1	1	1	1	1	0	0	0	0	0	1	1	1	?	0	0	1	0	0	0	0	0	1	0	1	0	1	0	1	1	1	0	1	0	1	0	0	0	0	0	1	1	1	1	1	0	0	1	0	1
Maorigoeldia	M.argyropus	1	2	0	0	1	0	1	0	1	1	1	1	1	0	0	1	0	?	0	1	1	1	1	0	0	0	1	0	0	0	0	1	0	-	-	-	1	1	1	0	1	?	0	0	1	1	1	0	1	1	0	2	0	1	0	1	1	1	1	1	1	1	0	?	1
Orthopodomyia	O.anopheloides	1	0	0	1	1	1	0	0	1	1	1	1	1	0	0	1	0	0	0	1	0	0	1	1	1	0	?	?	1	0	0	1	0	-	-	-	0	1	0	1	0	0	1	0	1	1	1	0	1	1	1	2	0	1	1	1	1	0	-	0	0	1	0	0
Mansoniodes	M.africana	1	0	1	2	0	1	0	1	1	1	0	1	1	0	0	1	1	1	0	1	0	0	1	0	0	0	?	?	0	0	0	0	0	-	-	-	0	1	1	0	0	1	0	0	1	1	1	0	1	0	0	0	0	?	?	-	1	0	-	0	0	1	0	0