The Culicidae (Diptera): a review of taxonomy, classification and phylogeny*

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Abstract

The taxonomy, classification and phylogeny of family Culicidae are reviewed. The application of explicit methods of phylogenetic analysis has revealed weaknesses in the traditional classification of mosquitoes, but little progress has been made to achieve a robust, stable classification that reflects evolutionary relationships. The current phenetic classification is discussed in view of phylogeny reconstructions based on cladistic analyses of morphological and molecular data. It is concluded that the generic and suprageneric relationships and the validity and monophyly of the generic and subgeneric groupings of Culicidae are in need of extensive reappraisal. If the classification is to reflect evolutionary history, changes to the nomenclature of mosquitoes are inevitable. There is strong morphological and molecular evidence that subfamily Anophelinae and tribes Aedini, Culicini and Sabethini of subfamily Culicinae are monophyletic, but the other taxonomic groupings are not demonstrably monophyletic or have not been subjected to phylogenetic analyses.

Key words: Culicidae, mosquitoes, taxonomy, classification, phylogeny, evolution, Anophelinae, Aedini, Culicini, Sabethini

Introduction

Mosquitoes, family Culicidae, comprise a monophyletic taxon (Wood & Borkent, 1989; Miller *et al.*, 1997; Harbach & Kitching, 1998) belonging to order Diptera. The family is a large and abundant group that occurs throughout temperate and tropical regions of the world, and well beyond the Arctic Circle. Mosquitoes are most diverse and least known in tropical forest environments. Some 3,490 species are currently formally recognized (Harbach & Howard, 2007), but some 3–5 times this number may exist if the known species, like many *Anopheles* mosquitoes (see below), prove to be isomorphic members of sibling species complexes.

Mosquitoes are slender, long-legged insects that are easily recognized by their long proboscis and the presence of scales on most parts of the body. Larvae are distinguished from other aquatic insects by the absence of legs, the presence of a distinct head bearing mouth brushes and antennae, a bulbous thorax that is wider than the head and abdomen, posterior anal papillae and either a pair of respiratory openings (subfamily Anophelinae) or an elongate siphon (subfamily Culicinae) borne near the end of the abdomen. Mosquitoes are usually, and most reliably, identified as mature (fourth-instar) larvae and adults. Males are especially needed to distinguish many species because the females of various generic-level taxa are remarkably similar in habitus. Whereas the taxonomy of numerous apparently closely related species is confounded by overt similarity, the limits of many supraspecific groups are clouded by morphological diversity. Some genera include diverse elements of indefinite affinities that will inevitably be recognized as separate monophyletic lines once they are thoroughly studied (Belkin, 1962; Judd, 1996; Harbach & Kitching, 1998; Reinert *et al.*, 2004, 2006).

The immature stages of mosquitoes occupy a spectrum of aquatic environments. They occur primarily in temporary or permanent bodies of ground water, but a large number of species occupy leaf axils, tree-holes, rock-holes, crab-holes, bamboo internodes, bromeliads and aroids, fruit shells and husks, fallen leaves and spathes, flower bracts, snail shells and pitcher plants. Some utilize artificial containers as well as the normal ground-water habitats. The majority of larvae feed on suspended particulate matter and microorganisms that they extract from the water with filamentous mouth brushes. Other species are obligatory or facultative predators that capture and feed largely on the immature stages of other mosquitoes by means of modified mouth brushes or grasping mandibles or maxillae. Some larvae resort to scavenging or cannibalism when food is scarce. The larvae of most mosquitoes obtain oxygen from the atmosphere by coming to the water surface. All species of *Mansonia* and *Coquillettidia* and some species of *Mimomyia* obtain oxygen from the air vessels of aquatic plants, which they pierce with a specialized siphon. *Aedeomyia* species apparently use their enlarged antennae for respiration. Some species have greatly enlarged anal papillae that are well supplied with tracheae, and these species seldom come to the surface and probably obtain dissolved oxygen from the water. In view of the ecological diversity of mosquitoes and the broad range of aquatic habitats they occupy, they can be used as environmental indicators on a variety of scales. As an example, the immature stages of numerous mosquito species inhabit a variety of tropical plants (phytotelmata), which makes them good indicators of the health of forests.

Since mosquitoes are delicate insects, they are always found where the air is relatively cool and the humidity is high. Many species live within a few meters of the ground whereas many sylvan species occur primarily in forest canopy. Vertical distribution is largely dependent on feeding preferences. All males and the females of many species feed exclusively on plant liquids, including nectar, honeydew, fruit juices and exudates. Females of numerous species feed on the blood of living animals, but some that are normally hematophagous may produce eggs without a blood meal. Warm-blooded vertebrates are a common source of blood for most species, but many species also attack cold-blooded animals such as snakes, turtles, toads, frogs and other insects, including nymphal cicadas, lepidopterous larvae and mantids. The time of flight and feeding activity is usually quite specific for most species. Some species are active at night (nocturnal) or twilight (crepuscular) whereas others are active during the daylight hours (diurnal). Despite our current knowledge of mosquito biology, practically nothing is known about the specific bionomics of most species.

Many species of mosquitoes are pests or vectors of pathogens that cause disease in humans and domesticated animals. The pathogens transmitted by mosquitoes include viruses (arboviruses), filaria1 worms (helminths) and protozoa. Fewer than 150 species, largely confined to genera *Anopheles*, *Aedes* (traditional broad sense) and *Culex*, are the indirect cause of more morbidity and mortality among humans than any other group of organisms. Despite their medical importance and long history of study, the taxonomy of mosquitoes is far from complete and the existing system of classification is not entirely natural (Belkin, 1962; Judd, 1996; Harbach & Kitching, 1998; Reinert *et al.*, 2004, 2006).

Taxonomic history and classification

The discovery at the turn of the nineteenth century that mosquitoes transmit malaria and yellow fever initiated an outburst of interest in the description and classification of these insects. The British Museum (Natural History) employed Fred. V. Theobald in 1899, and as a consequence many new generic names were introduced in an effort to classify numerous new species into seemingly natural groups. It eventually became apparent that Theobald's system of classification was neither practical nor natural. Consequently, during the two decades following the publication of Theobald's *Monograph of the Culicidae* in 1910, significant changes were made toward a much more conservative system of classification. Particularly noteworthy were the efforts of F.W. Edwards in Europe and Harrison G. Dyar in North America, whose work contributed most significantly to the acceptance of the broad genus-group concepts (Edwards, 1932) that provided the framework on which the "traditional" classification (Stone *et al.*, 1959; Belkin, 1962; Knight & Stone, 1977) of the twentieth century was built.

Edwards (1932) included dixid and chaoborid midges as subfamilies of Culicidae and regarded the "true mosquitoes" as members of a third subfamily, Culicinae. He recognized three tribes within Culicinae, i.e. Anophelini, Toxorhynchitini (as Megarhinini) and Culicini, and divided the last tribe into five groups, i.e. *Sabethes*, *Uranotaenia*, *Theobaldia*, *Aedes* and *Culex*. Stone (1957) removed Dixidae and Chaoboridae from Culicidae and restricted family Culicidae to the Culicinae of Edwards (1932). This brought about changes in subfamily and tribal designations that were adopted by Stone *et al.* (1959) in their world catalogue of mosquitoes. This classification recognized subfamilies Anophelinae, Culicinae and Toxorhynchitinae, and two tribes within Culicinae, the Culicini and Sabethini. Belkin (1962) disagreed with this change and retained Edwards' subfamily structure, but reorganized the classification of Culicinae ("true mosquitoes") to include 12 tribes instead of three. He retained Anophelini and Toxorhynchitini and recognized ten tribes in place of Edwards' Culicini. At least some authors (e.g. Belkin *et al.*, 1970) continued to treat dixids and chaoborids as subfamilies of Culicidae until Knight & Stone (1977) once again excluded them from the family. This action resulted in the recognition of three subfamilies, i.e. Anophelinae, Culicinae and Toxorhynchitinae, and the division of subfamily Culicinae into the 10 tribes established by Belkin (1962). Mattingly (1969, 1971, 1981), however, was unwilling to accept the division of Culicinae into 10 tribes and consequently followed Stone *et al.* (1959) in recognizing only two, i.e. Culicini and Sabethini. Service (1993) utilized Mattingly's tribal divisions as a matter of convenience, but for the most part mosquito taxonomists accepted all of the tribal groups introduced by Belkin, including Toxorhynchitini (Harbach & Kitching, 1998; Mitchell *et al.*, 2002).

Leaving aside the controversial proposals of Reinert *et al.* (2004, 2006) to divide tribe Aedini into 63 genera instead of 12, surprisingly few changes have been made in the recognition of mosquito genera since Edwards (1932). While the number of formally recognized species has more than doubled, having risen from 1400 to nearly 3500, and the number of subgenera increased from 89 to 145, the number of generally accepted genera has only increased from 30 to 44 (Table 1).

TABLE 1. Subfamilies, tribes, genera, subgenera, numbers of species and distribution of Culicidae. The list includes 44 genera, 145 subgenera and 3,490 species (with the exception of *Borichinda*, the classification of Aedini only includes generic-level taxa recognized prior to Reinert *et al*. 2004). Subspecies, varieties and *nomina dubia* are not included. In cases where a taxon is listed as residing "principally" within one or more zoogeographic regions, one or two species of that taxon may also occur in an adjoining region or regions.

Subfamily Tribe	Genera	Number of subgenera	Number of species	Distribution
Anophelinae	*Anopheles*	7	455	Cosmopolitan
	Bironella	3	8	Australasian
	Chagasia	–	4	Neotropical
Culicinae				
Aedeomyiini	*Aedeomyia*	2	6	Afrotropical, Australasian, Oriental, Neotropical
Aedini	*Aedes*	23	363	Old Word, Nearctic
	Armigeres	2	58	Australasian, Oriental
	Ayurakitia	–	2	Oriental
	Borichinda	–	1	Oriental
	Eretmapodites	–	48	Afrotropical
	Haemagogus	2	28	Principally Neotropical
	Heizmannia	2	39	Oriental
	Ochlerotatus	22	550	Cosmopolitan
	Opifex	–	1	New Zealand
	Psorophora	3	48	New World
	Udaya	–	3	Oriental
	Verrallina	3	95	Principally Australasian, Oriental
	Zeugnomyia	–	4	Oriental
Culicini	*Culex*	23	763	Cosmopolitan
	Deinocerites	–	18	Principally Neotropical
	Galindomyia	–	1	Neotropical
	Lutzia	3	7	Afrotropical, Australasian, Oriental, Neotropical, eastern Palaearctic
Culisetini	*Culiseta*	7	37	Old World, Nearctic
Ficalbiini	*Ficalbia*	–	8	Afrotropical, Oriental
	Mimomyia	3	44	Afrotropical, Australasian, Oriental
Hodgesiini	*Hodgesia*	–	11	Afrotropical, Australasian, Oriental
Mansoniini	*Coquillettidia*	3	57	Old World, Neotropical
	Mansonia	2	23	Old World, Neotropical
Orthopodomyiini	*Orthopodomyia*	–	38	Afrotropical, Nearctic, Neotropical, Oriental, Palaearctic
Sabethini	*Isostomyia*	–	4	Neotropical

TABLE 1 (continued)

Subfamily Tribe	Genera	Number of subgenera	Number of species	Distribution
	Johnbelkinia	–	3	Neotropical
	Kimia	–	5	Oriental
	Limatus	–	8	Neotropical
	Malaya	–	12	Afrotropical, Australasian, Oriental
	Maorigoeldia	–	1	New Zealand
	Onirion	–	7	Neotropical
	Runchomyia	2	7	Neotropical
	Sabethes	5	38	Neotropical
	Shannoniana	–	3	Neotropical
	Topomyia	2	54	Principally Oriental
	Trichoprosopon	–	13	Neotropical
	Tripteroides	5	122	Principally Australasian, Oriental
	Wyeomyia	15	140	Principally Neotropical
Toxorhynchitini	*Toxorhynchites*	4	88	Afrotropical, Australasian, Neotropical, eastern Palaearctic, Oriental
Uranotaeniini	*Uranotaenia*	2	265	Afrotropical, Australasian, Oriental, Neotropical

Taxonomic treatments and molecular systematics

Modern monographic treatments or revisions exist for a number of generic and subgeneric groups, but for the most part these types of studies are limited to subgenera and species groups of particular countries or regions. Significant gaps in taxonomic knowledge exist for most genera and subgenera, as indicated in Table 2. The following traditionally accepted genera have never been revised in their entirety, and there are many unresolved questions about the taxonomic status of many species and higher groupings: *Aedes*, *Armigeres*, *Coquillettidia*, *Culex*, *Mansonia*, *Mimomyia*, *Ochlerotatus*, *Psorophora*, *Topomyia*, *Tripteroides*, *Toxorhynchites*, *Uranotaenia* and *Wyeomyia*. These are large genera comprised of species that are extremely varied and difficult to identify because of overlapping suites of shared anatomical features. Many undescribed species of *Culex*, *Topomyia*, *Tripteroides*, *Uranotaenia* and *Wyeomyia* are known to exist in major museums of the world.

Major vector species are generally those most thoroughly described by conventional taxonomy. Because malaria is undoubtedly the most important mosquito-borne disease, the principal *Anopheles* vectors and their closest allies have received more attention than other groups. Anopheline taxonomy reached the pinnacle of classical morphological study with the application of genetic techniques during the latter part of the twentieth century. The result has been the discovery of cryptic species complexes. A highly desirable approach to the study of cryptic species is the integration of morphological taxonomy with molecular studies, which ensures that the results of DNA sequencing are properly connected into the existing systematics. The integration of these methods fosters the resolution of non-scientific but important questions of nomenclature and provides a testable means of delimiting and unambiguously identifying species for practical reasons—to learn about their

bionomics, distribution and relationships to disease transmission. This knowledge is essential for epidemiological studies, the design and implementation of appropriate vector control measures and the development of strategies for monitoring the spatial-temporal fluctuations of vector species that are needed to assess the potential risk of malaria outbreaks. In addition to *Anopheles*, cryptic species complexes are known among *Culex* (e.g. the Pipiens Complex) and *Sabethes* mosquitoes (P. Pedro, unpublished).

TABLE 2. Major works, revisions and monographic treatments of mosquitoes (post World War II).

Genus	Authorship (infrageneric group and/or geographic coverage)
Anopheles	Lane, 1953 (Neotropical Region); Mattingly & Knight, 1956 (Arabia); Cova-Garcia, 1961 (Venezuela); Belkin, 1962 (South Pacific); Grjebine, 1966 (Madagascar); Gillies & de Meillon, 1968 (Afrotropical Region); Reid, 1968 (Malaysia, Borneo); Belkin *et al.*, 1970 (Jamaica); Zavortink, 1970 (treehole species, New World); Zavortink, 1973 (subgenus *Kerteszia*); Gutsevich *et al.*, 1974 (former USSR); Harrison & Scanlon, 1975 (subgenus *Anopheles*, Thailand); Faran, 1980 (Albimanus Section, subgenus *Nyssorhynchus*); Tanaka *et al.*, 1979 (Japan); Wood *et al.*, 1979 (Canada); Harrison, 1980 (Myzomyia Series, subgenus *Cellia*, Thailand); Faran & Linthicum, 1981 (subgenus *Nyssorhynchus*, Amazonia); Rao, 1984 (India); Linthicum, 1988 (Argyritarsis Section, subgenus *Nyssorhynchus*); Das *et al.*, 1990 (keys, India); Nagpal & Sharma, 1995 (India); Lu Baolin *et al.*, 1997b (China).
Bironella	Tenorio, 1977 (revision).
Chagasia	Lane, 1953.
Aedeomyia	Tyson, 1970a (revision).
Aedes and *Ochlerotatus*	Barraud, 1934 (southern Asia); Edwards, 1941 (adults, Afrotropical Region); Hopkins, 1952 (larvae, Afrotropical Region); Lane, 1953 (Neotropical Region); Muspratt, 1956 (subgenus *Stegomyia*, South Africa); Belkin, 1962 (South Pacific); Belkin, 1968 (New Zealand); Berlin, 1969 (subgenus *Howardina*); Belkin *et al.*, 1970 (Jamaica); Reinert, 1970a,b, 1972, 1973a,b, 1974, 1976a,b, 1981, 1982, 1984, 1985, 1990b, 1993 (various subgenera, Oriental and Australasian Regions); Schick, 1970 (former Terrens Group in Neotropical Region, see Zavortink, 1972); Tyson, 1970b (subgenus *Mucidus*); McIntosh, 1971 (subgenus *Neomelaniconion*, southern Africa); Huang, 1972 (Scutellaris Group, Southeast Asia); Zavortink, 1972 (various subgenera, New World); Ribeiro & da Cunha Ramos, 1973 (Angola); Gutsevich *et al.*, 1974 (former USSR); McIntosh, 1975 (subgenus *Aedimorphus*, southern Africa); Arnell, 1976 (Scapularis Group, subgenus *Ochlerotatus*, New World); Abercrombie, 1977 (subgenus *Christophersiomyia*); Huang, 1977, 1979 (subgenus *Stegomyia*, Oriental Region); Tanaka *et al.*, 1979 (Japan); Wood *et al.*, 1979 (Canada); Reinert, 1987 (subgenus *Albuginosus*, Afrotropical Region); Huang, 1990 (Africanus Group, subgenus *Stegomyia*, Afrotropical Region); Lu Baolin *et al.*, 1997a (China); Reinert, 1999a (subgenus *Rusticoidus*); Reinert, 1999b (subgenus *Zavortinkius*); Reinert, 2000a (subgenus *Fredwardsius*); Reinert, 2003 (subgenus *Bruceharrisonius*); Huang, 2004 (subgenus *Stegomyia*, Afrotropical Region); Huang, 2005 (subgenus *Cornetius*); Reinert, 2006a (*Tewarius*); Reinert 2006b (*Mucidus*).
Armigeres	Belkin, 1962 (South Pacific); Steffan, 1968 (Papuan Subregion); Tanaka *et al.*, 1979 (Japan); Lu Baolin *et al.*, 1997a (China).
Ayurakitia	Reinert, 2000c.
Borichinda	Harbach *et al.*, 2007b.
Eretmapodites	Service, 1990.
Haemagogus	Arnell, 1973 (revision).
Heizmannia	Mattingly, 1970 (revision); Reinert, 1973c, 2000b (subgenus *Mattinglyia*).

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TABLE 2 (continued)

Genus	Authorship (infrageneric group and/or geographic coverage)
Opifex	Belkin, 1968.
Psorophora	Belkin *et al.*, 1970 (Jamaica).
Udaya	Reinert, 1990a.
Verrallina	Reinert, 1974, 1984, 1999c.
Zeugnomyia	Delfinado, 1966 (Philippines).
Culex	Barraud, 1934 (southern Asia); Edwards, 1941 (adults, Afrotropical Region); Hopkins, 1952 (larvae, Afrotropical Region); Lane, 1953 (Neotropical Region); Mattingly & Knight, 1956 (Arabia); Belkin, 1962 (South Pacific); Cova-Garcia *et al.*, 1966 (Venezuela); Delfinado, 1966 (Philippines); Bram, 1967a (Thailand); Bram, 1967b (subgenus *Culex*, New World); Belkin, 1968 (New Zealand); Belkin *et al.*, 1970 (Jamaica); Sirivanakarn, 1971, 1972, 1976, 1977 (various subgenera, Oriental Region); Valencia, 1973 (subgenus *Carrollia*); Gutsevich *et al.*, 1974 (former USSR); Tanaka *et al.*, 1979 (Japan); Wood *et al.*, 1979 (Canada); Berlin & Belkin, 1980 (various subgenera, Neotropical Region); Ribeiro & da Cunha Ramos, 1980 (Angola); Sirivanakarn, 1983 (subgenus *Melanoconion*); Harbach, 1988 (subgenus *Culex*, southwestern Asia and Egypt); Pecor *et al.*, 1992 (subgenus *Melanoconion*); Sallum & Forattini, 1996 (Spissipes Section, subgenus *Melanoconion*); Lu Baolin *et al.*, 1997a (China); Tanaka, 2004 (subgenera *Oculeomyia* and *Sirivanakarnius*).
Deinocerites	Adames, 1971 (revision).
Galindomyia	Stone & Barreto, 1969 (adult, Colombia); Adames & Galindo, 1975 (larva, Colombia).
Lutzia	Tanaka, 2003.
Culiseta	Belkin, 1962 (South Pacific); Belkin, 1968 (New Zealand); Dobrotworsky, 1971 (Southeast Asia); Gutsevich *et al.*, 1974 (former USSR); Tanaka *et al.*, 1979 (Japan); Wood *et al.*, 1979 (Canada); Maslov, 1989 (world); Service, 1990 (Afrotropical Region); Lu Baolin *et al.*, 1997a (China).
Ficalbia	Edwards, 1941 (adults, Afrotropical Region); Hopkins, 1952 (larvae, Afrotropical Region); Grjebine, 1986 (Madagascar); Service, 1990 (Afrotropical Region).
Mimomyia	Barraud, 1934 (southern Asia); Edwards, 1941 (adults, Afrotropical Region); Hopkins, 1952 (larvae, Afrotropical Region); Belkin, 1962 (South Pacific); Delfinado, 1966 (Philippines); Tanaka *et al.*, 1979 (Japan); Grjebine, 1986 (Madagascar); Service, 1990 (Afrotropical Region); Lu Baolin *et al.*, 1997a (China).
Hodgesia	Barraud, 1934 (southern Asia); Edwards, 1941 (adults, Afrotropical Region); Hopkins, 1952 (larvae, Afrotropical Region); Thurman, 1959 (Thailand); Belkin, 1962 (South Pacific); Service, 1990 (Afrotropical Region); Lu Baolin *et al.*, 1997a (China).
Coquillettidia	Lane, 1953 (subgenus *Rhynchotaenia*); Belkin, 1962 (South Pacific); Ronderos & Bachmann, 1963 (Neotropical Region); Cova-Garcia *et al.*, 1966 (Venezuela); Delfinado, 1966 (Philippines); Belkin, 1968 (New Zealand); Belkin *et al.*, 1970 (Jamaica); Tanaka *et al.*, 1979 (Japan); Service, 1990 (Afrotropical Region); Lu Baolin *et al.*, 1997a (China).
Mansonia	Lane, 1953 (Neotropical Region); Belkin, 1962 (South Pacific); Ronderos & Bachmann, 1963 (Neotropical Region); Cova-Garcia *et al.*, 1966 (Venezuela); Delfinado, 1966 (Philippines); Belkin *et al.*, 1970 (Jamaica); Tanaka *et al.*, 1979 (Japan); Service, 1990 (Afrotropical Region).
Orthopodomyia	Zavortink, 1968 (revision), 1971 (Southeast Asia).
Isostomyia	Lane, 1953.
Johnbelkinia	Zavortink, 1979 (revision).
Kimia	Harbach *et al.*, 2007a.

TABLE 2 (continued)

Genus	Authorship (infrageneric group and/or geographic coverage)
Limatus	Lane, 1953; Cova-Garcia *et al.*, 1966 (Venezuela); Belkin *et al.*, 1970 (Jamaica).
Malaya	Barraud, 1934 (as genus *Harpagomyia*, southern Asia); Thurman, 1959 (Thailand); Belkin, 1962 (South Pacific); Tanaka *et al.*, 1979 (Japan); Service, 1990 (Afrotropical Region); Lu Baolin *et al.*, 1997a (China).
Maorigoeldia	Belkin, 1968.
Onirion	Harbach & Peyton, 2000.
Runchomyia	Lane, 1953 (see Zavortink, 1979 for included species).
Sabethes	Lane, 1953 (in part, Neotropical Region); Cova-Garcia *et al.*, 1966 (Venezuela); Harbach & Peyton, 1991 (subgenus *Davismyia*); Harbach, 1991 (subgenus *Peytonulus*); Harbach, 1994a (subgenus *Sabethinus*).
Shannoniana	Lane, 1953 (see Zavortink, 1979 for included species).
Topomyia	Thurman, 1959 (Thailand); Tanaka *et al.*, 1979 (Japan); Lu Baolin *et al.*, 1997a (China).
Trichoprosopon	Lane, 1953 (see Zavortink, 1979 for included species).
Tripteroides	Barraud, 1934 (southern Asia); Thurman, 1959 (Thailand); Belkin, 1962 (South Pacific); Tanaka *et al.*, 1979 (Japan); Mattingly, 1981 (subgenera *Rachionotomyia* and *Tricholeptomyia*, Oriental Region); Lu Baolin *et al.*, 1997a (China).
Wyeomyia	Lane, 1953 (Neotropical Region); Correa & Ramalho, 1956 (subgenus *Phoniomyia*); Cova-Garcia *et al.*, 1966 (Venezuela); Belkin *et al.*, 1970 (Jamaica); Zavortink, 1985 (subgenus *Zinzala*); Harbach & Peyton, 1990 (subgenus *Caenomyiella*), 1992 (subgenus *Exallomyia*); Motta & Lourenço-de-Oliveira, 1995 (subgenus *Dendromyia*); Lourenço-de-Oliveira *et al.*, 1999 (subgenus *Prosopolepis*); Motta & Lourenço-de-Oliveira, 2005 (subgenus *Sphilonympha*).
Toxorhynchites	Barraud, 1934 (southern Asia); Edwards, 1941 (adults, Afrotropical Region); Hopkins, 1952 (larvae, Afrotropical Region); Lane, 1953 (Neotropical Region); Thurman, 1959 (Thailand); Belkin, 1962 (South Pacific); Cova-Garcia *et al.*, 1966 (Venezuela); Belkin *et al.*, 1970 (Jamaica); Tanaka *et al.*, 1979 (Japan); Steffan & Evenhuis, 1985 (subgenus *Toxorhynchites*); Evenhuis & Steffan, 1986 (Acaudatus Group, Oriental Region); Service, 1990 (Afrotropical Region); Ribeiro, 1991a (Brevipalpis Group, Afrotropical Region); Ribeiro, 1991b (subgenus *Afrorhynchus*); Lu Baolin *et al.*, 1997a (China).
Uranotaenia	Barraud, 1934 (southern Asia); Edwards, 1941 (adults, Afrotropical Region); Hopkins, 1952 (larvae, Afrotropical Region); Lane, 1953 (Neotropical Region); Galindo *et al.*, 1954 (Panama); Belkin, 1962 (South Pacific); Delfinado, 1966 (Philippines); Belkin *et al.*, 1970 (Jamaica); Peyton, 1977 (subgenus *Pseudoficalbia*, Southeast Asia); Tanaka *et al.*, 1979 (Japan); Service, 1990 (Afrotropical Region); da Cunha Ramos, 1993 (Afrotropical Region); Lu Baolin *et al.*, 1997a (China); da Cunha Ramos & Brunhes, 2004 (Madagascar).

Phylogeny and classification of Culicidae

The traditional classification of Culicidae is phenetic. Consequently, all levels of classification are arbitrary groupings based on the subjective interpretation of anatomical similarity. As will be seen below, the traditional acceptance of broad genus-group concepts has resulted in a classification of paraphyletic and polyphyletic taxa. It will also be seen that recent phylogenetic studies based on morphology and DNA sequence data have uncovered previously unrecognized relationships that suggest major changes in the classification of Culicidae are inevitable.

The first evolutionary tree of Culicidae (Fig. 1) was constructed by Ross (1951) based on an intuitive interpretation of comparative bionomics and morphological data. The tree reflected the traditional division of family Culicidae into three subfamilies: Anophelinae, the basal lineage; Toxorhynchitinae, the intermediate lineage; and Culicinae, the large derived lineage. The relationships illustrated by Ross (1951) are those more or less traditionally accepted and unchallenged by later workers until Harbach & Kitching (1998) (Fig. 2) examined the generic relationships and higher classification of the family based on cladistic analyses of morphological data. The implied weighting analysis of 73 characters from larvae, pupae and adults coded for the 38 genera of mosquitoes that were recognized at the time resulted in relationships and groupings that differed significantly from traditional hypotheses. The analysis supported the monophyly of subfamily Anophelinae and tribes Culicini and Sabethini. The Anophelinae formed the most basal clade of the family. Aedini was recovered as a paraphyletic assemblage with respect to Mansoniini, which itself was monophyletic. Aedini + Mansoniini formed a sister group to Culicini + Sabethini, both of which were found to be monophyletic. The boundaries and relationships of the other tribes were problematic. Relationships between genera of tribe Aedini were generally poorly resolved due to a significant amount of polymorphism, especially within the traditional broad concept of genus *Aedes*. The analyses did not support the recognition of a separate subfamily for genus *Toxorhynchites*, and the taxon was formally downgraded to tribal status within subfamily Culicinae. This action was consonant with the opinion of Belkin (1962) that recognition of *Toxorhynchites* as a separate subfamily exaggerated the differences between this genus and genera placed in subfamily Culicinae.
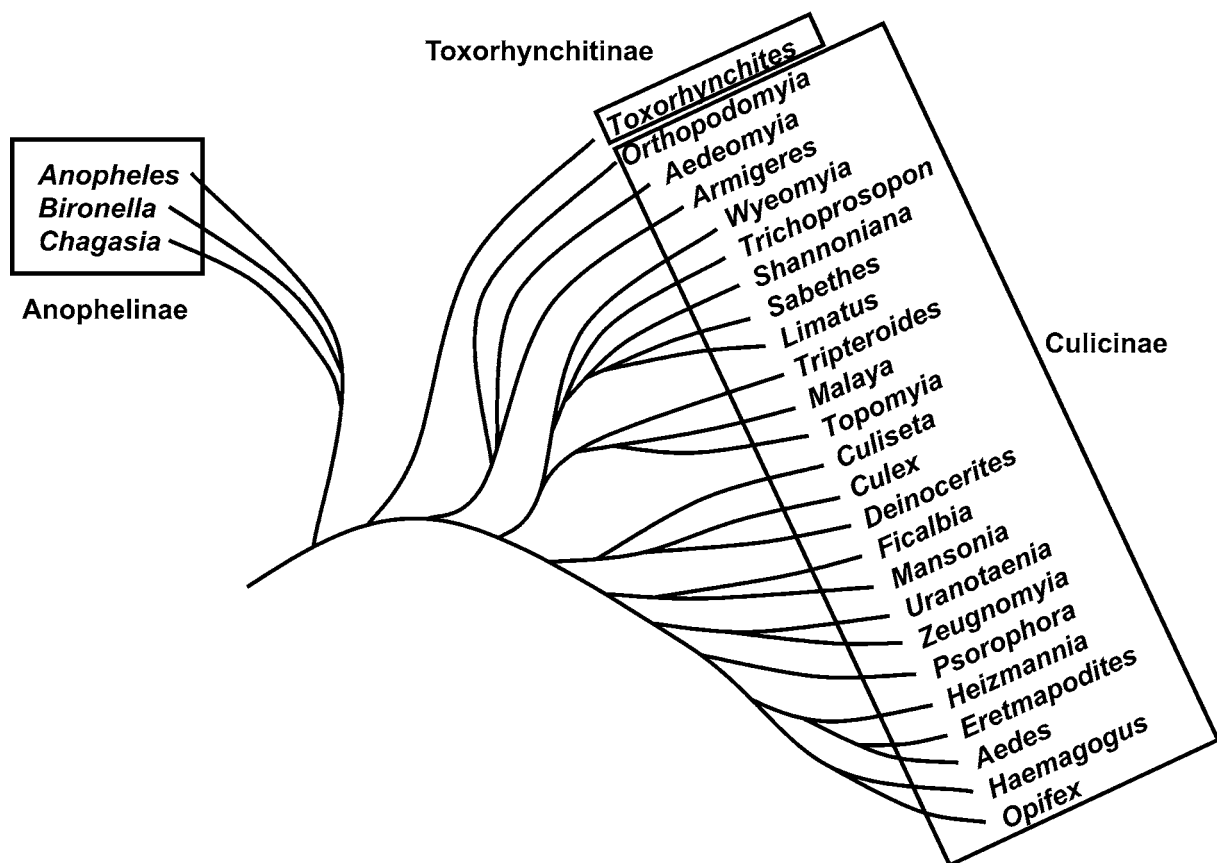

FIGURE 1. Intuitive phylogeny of mosquito genera (after Ross, 1951), based on "comparative bionomics and morphology", principally larvae.

There is little doubt that Culicidae is a monophyletic assemblage (Wood & Borkent, 1989; Miller *et al.*, 1997; Harbach & Kitching, 1998). Three anatomical features of adult mosquitoes included in the cladistic

analysis of Harbach & Kitching (1998) support the monophyly of the family: erect scales on the head (with numerous reductions and losses), mouthparts developed into a long proboscis (unique) and the presence of prealar setae (lost in *Hodgesia*, *Malaya* and *Sabethes*). The erect scales on the vertex and occiput of the head are expanded flattened setae (Harbach & Knight, 1980), a unique development of Culicidae that is reversed in *Opifex* and lost in *Hodgesia* and some *Wyeomyia*. The restricted distribution of erect scales on the occiput appears to be an apomorphic condition that evolved independently a number of times in the family. In addition to the elongate proboscis with its piercing stylets, Wood & Borkent (1989) listed the premandible of larvae as a synapomorphy for Culicidae. Harbach & Kitching (1998) did not include the premandible in their analysis because it is an entirely internal structure that has not been examined in most mosquito genera.
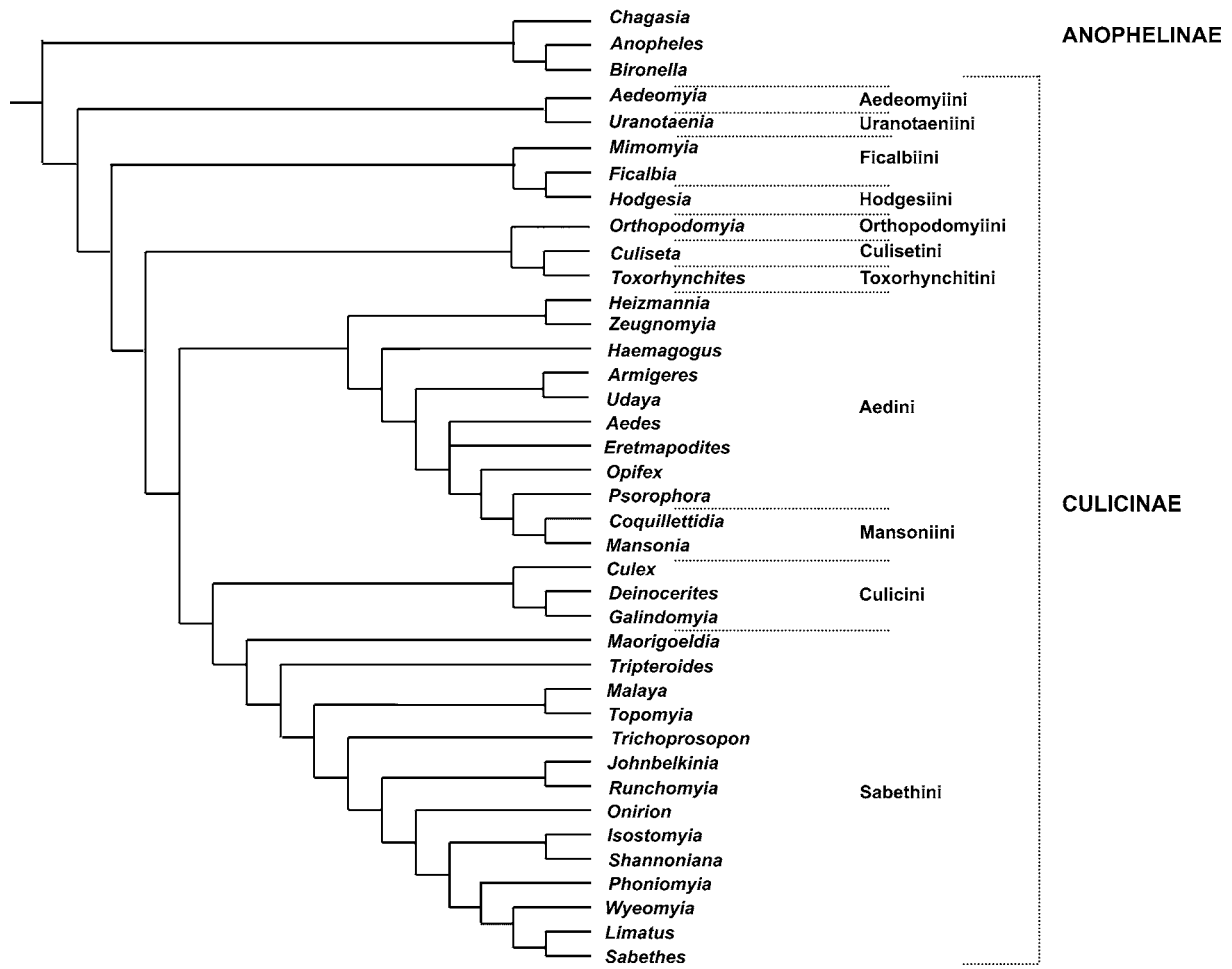

FIGURE 2. Phylogeny of mosquito genera based on morphology (after Harbach & Kitching, 1998): MPC from implied-weighting analysis (*K*=1) of 38 genera and 73 morphological characters.

When the intuitive phylogeny of Ross (1951) is reproduced as a cladogram (Fig. 3), it is evident that only three of his lineages are supported by the results of Harbach & Kitching (1998): (1) the monophyly and basal placement of Anophelinae, with *Chagasia* the sister group to the clade comprised of *Anopheles* + *Bironella*, (2) the monophyly of Sabethini and (3) the monophyly of Culicini. There is also agreement in the sister-group relationships of *Sabethes* + *Limatus* and *Malaya* + *Topomyia*. Other than these similarities, the evolutionary relationships hypothesized by Ross (1951) are very different than those based on explicit methodology.

Chromosomal karyotypes have been observed in more than 200 mosquito species representing approximately half of the traditionally recognized genera (White, 1980; Rao & Rai, 1987, 1990). With the exception

of *Chagasia bathana* (Dyar), which has three pairs of autosomes and a pair of sex chromosomes ($2n = 8$) (Kreutzer, 1978), the basic number of chromosomes in all species examined is $2n = 6$. Based on chromosome studies of Culicidae, Dixidae, Chaoboridae and Tipulidae, Rao & Rai (1987) concluded that mosquitoes evolved from a *Mochlonyx*-like chaoborid ancestor and subfamilies Anophelinae and Culicinae diverged from a common lineage. These authors considered the karyotype of *Chagasia*, which is similar to that of *Mochlonyx velutinus* (Ruthe) ($2n = 8$), to be primitive for Anophelinae before this notion was supported by the cladistic analyses mentioned above.
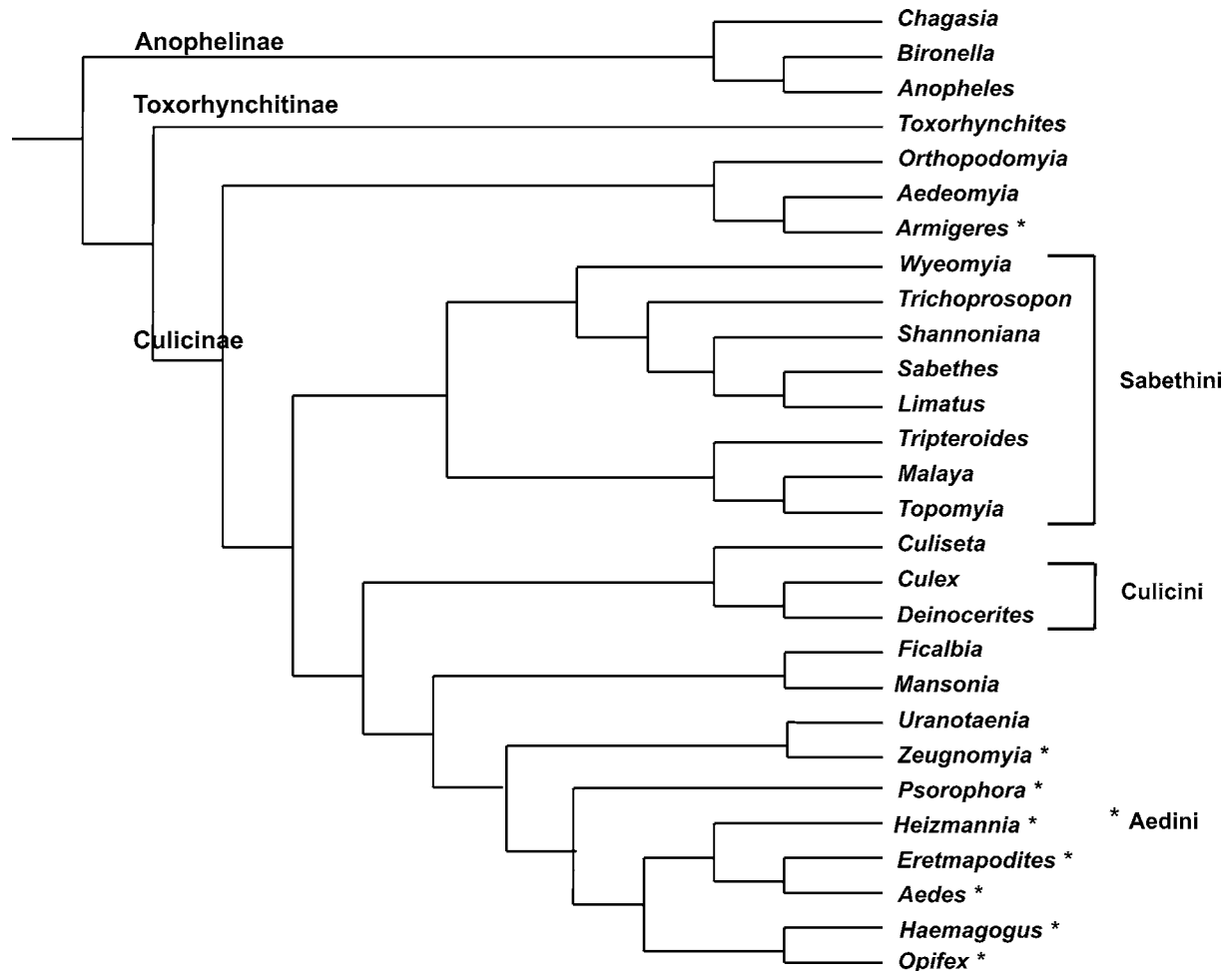

FIGURE 3. Intuitive phylogeny of mosquito genera (Ross, 1951) reproduced as a cladogram. Asterisks (*) indicate genera of tribe Aedini.

With the formal reduction of Toxorhynchitinae to tribal status within subfamily Culicinae, the classification of Culicidae before the end of the twentieth century included two subfamilies, 11 tribes and 38 genera. Subsequent division of *Aedes* into four genera, i.e. *Verrallina*, *Ayurakitia*, *Aedes* and *Ochlerotatus* (Reinert, 1999c, 2000c, 2000d, respectively) and the recent recognition of two additional genera, *Borichinda* (tribe Aedini) and *Kimia* (tribe Sabethini), resulted in the modified traditional classification indicated in Table 1. Based on the intuitive interpretation of morphology, this classification appears to reflect natural groupings, but is there any molecular evidence to support this?

Miller *et al*. (1997) examined the phylogenetic relationships of four mosquito species, one from each of genera *Aedes*, *Anopheles*, *Culex* and *Toxorhynchites*, using 18S and 5.8S rDNA sequences (Fig. 4). Their analysis placed *Anopheles* in a position basal to the other species. In a parsimony analysis (Fig. 4A), *Toxo-*

rhynchites was placed as the sister group of *Aedes* + *Culex*, whereas *Aedes* was the sister group of *Toxorhynchites* + *Culex* in a maximum likelihood tree (Fig. 4B). However, neither position was strongly supported by bootstrapping. The use of neighbour-joining procedures produced a trichotomy for the three species. Consequently, the results of these taxon-limited analyses neither support nor refute the traditional position of *Toxorhynchites* as the sister group of subfamily Culicinae (Ross, 1951).
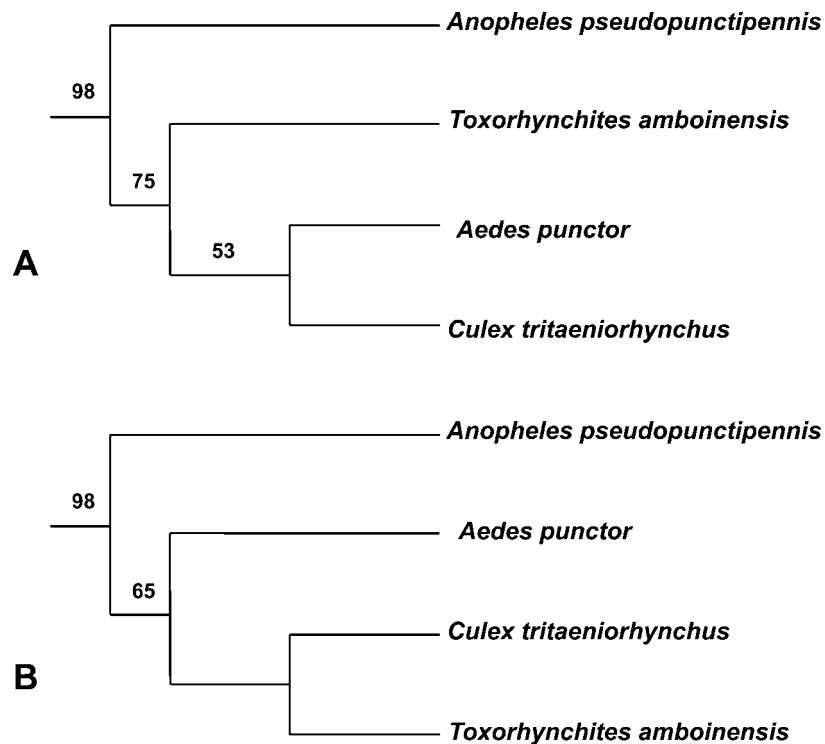

FIGURE 4. Phylogenetic relationships of four mosquito species based on 18S and 5.8S rDNA sequences (after Miller *et al.*, 1997): A, parsimony analysis tree; B, maximum likelihood tree. Bootstrap values >50% are shown.

Besansky & Fahey (1997) used the nuclear protein-coding *white* gene to examine the phylogenetic relationships of 13 species representing the three traditionally accepted subfamilies and nine genera (Fig. 5). Unweighted analysis of their data generated cladograms that disagreed with previous relationships based on the intuitive interpretation of morphology. The application of successive approximations character weighting (SACW) generated three trees that agreed with the traditional view that Anophelinae is basal to *Toxorhynchites* and the latter is basal to Culicinae. Other than recognizing the sister-group relationships of *Anopheles* + *Bironella* and *Culex* + *Deinocerites*, there is little agreement with the results of Harbach & Kitching (1998). Major disagreement involves the basal relationship of Sabethini to Aedini + Culicini derived from their analysis.

Isoe (2000) examined the phylogenetic relationships of 39 species representing 12 genera of mosquitoes based on vitellogenin gene sequences (Fig. 6). Maximum parsimony analyses showed that deeper and terminal relationships were significantly resolved when *Anopheles albimanus* Wiedemann was used to root the trees. Relationships inferred by maximum likelihood and distance methods were congruent with the most parsimonious trees, and trees resulting from the analyses were strongly supported. However, some of the nodes, especially those uniting the terminal taxa, were not strongly supported when the third codon position was excluded from the analysis, indicating that this position provides significant phylogenetically informative signal.
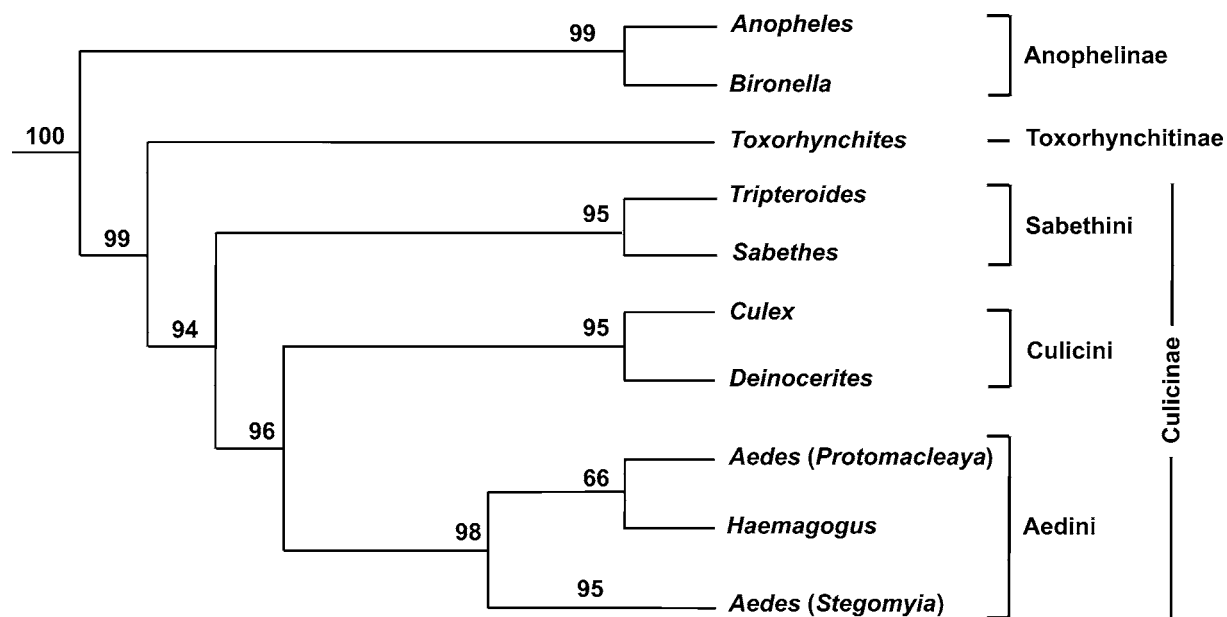
FIGURE 5. Phylogenetic relationships of nine genera (13 species) based on nuclear protein-coding *white* gene sequences (modified from Besansky & Fahey, 1997): SACW maximum parsimony tree with third codon positions excluded. Bootstrap values >50% are shown.
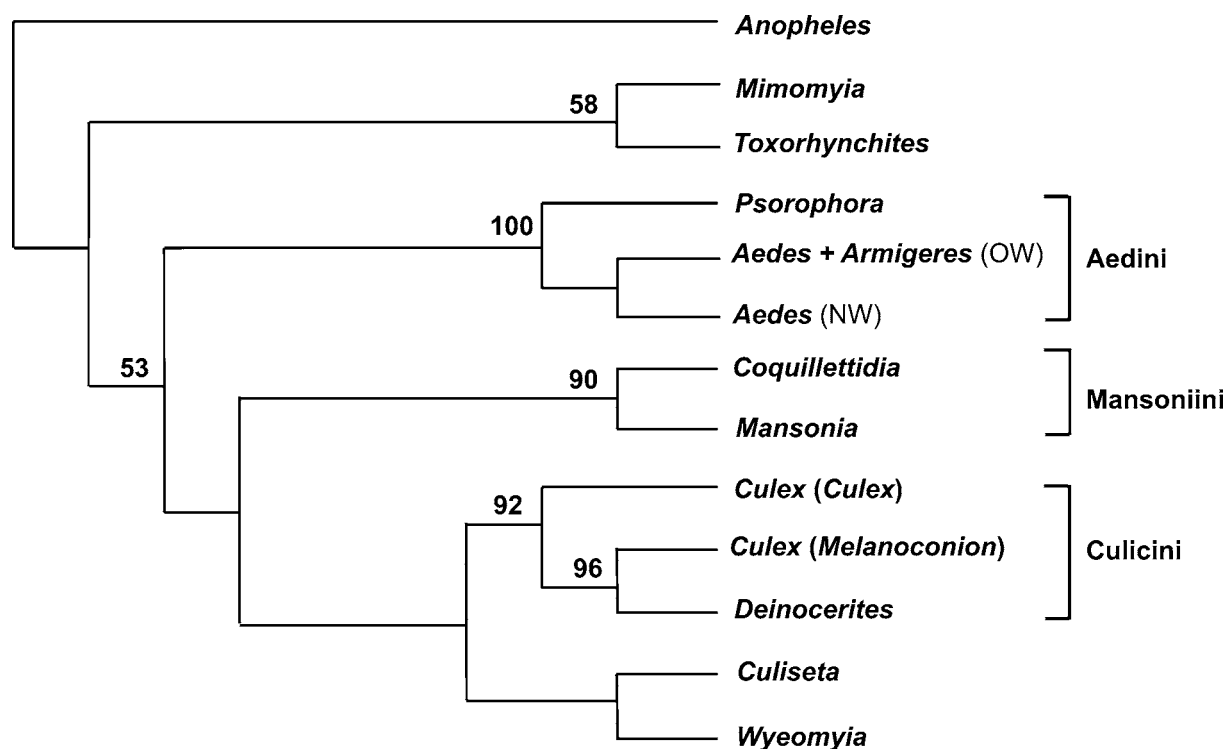

FIGURE 6. Phylogenetic relationships of 12 genera (39 species) based on vitellogenin gene sequences (modified from Isoe, 2000): maximum parsimony tree rooted on *Anopheles* with all codon positions equally weighted. Bootstrap values >50% are shown.

Maximum parsimony (MP) analyses of the vitellogenin gene sequences placed *Toxorhynchites* in a sister relationship with *Mimomyia*, which corroborates the inclusion of *Toxorhynchites* within subfamily Culicinae based on morphology (Harbach & Kitching, 1998) and mitochondrial cytochrome oxidase genes (Mitchell *et*

al., 2002). Likewise, the analyses of vitellogenin gene sequences supported the monophyly of tribes Aedini, Culicini and Mansoniini, but the relationships among the tribes were significantly different than those based on morphology. For example, whereas Aedini and Mansoniini were arrayed in a paraphyletic relationship in the preferred cladogram of Harbach & Kitching (1998), these groups are reciprocally monophyletic based on vitellogenin gene sequences. Whereas the MP analysis of vitellogenin data supported the monophyly of Aedini, it did not support the monophyly of genus *Aedes*. All analyses of vitellogenin nucleotide sequences consistently placed *Armigeres subalbatus* (Coquillett) in a clade with three species of subgenus *Stegomyia*, thus indicating that genus *Aedes* as traditionally defined is not a monophyletic taxon. Although species of *Armigeres* were not included in earlier molecular phylogenetic studies, this finding is consonant with the rDNA ITS2 data of Wesson *et al.* (1992) and the nuclear *white* gene data of Besansky & Fahey (1997) which suggest that the historical concept of *Aedes* is polyphyletic relative to *Haemagogus* (Fig. 5). Interestingly, the analyses of both morphological and vitellogenin gene sequence data placed *Deinocerites* in a derived position relative to *Culex*, but differ significantly in the placement of *Culiseta*. Whereas *Culiseta* was paired with *Toxorhynchites* in the implied weighting analysis (Fig. 2) of Harbach & Kitching (1998), it was placed in a sister-group relationship with *Wyeomyia* in the vitellogenin analysis of Isoe (2000). Alternatively, when all characters in the data set of Harbach & Kitching (1998) are equally weighted or analyzed using SACW, *Toxorhynchites* is recovered as the sister of Sabethini (Harbach & Kitching, 1998: Figs 13 and 14, respectively). Although a relationship with *Wyeomyia* may at first seem absurd, it is not inconsistent with the observation that *Toxorhynchites* shares morphological features with tribe Sabethini (Belkin, 1962).

The molecular phylogenies of Shepard *et al.* (2006) based on 18S rDNA sequences (Fig. 7) are consistent with the traditional recognition of three subfamilies of Culicidae. *Anopheles* was strongly supported as the sister to all other mosquitoes, and *Toxorhynchites* was recovered as a monophyletic sister group to genera traditionally placed in subfamily Culicinae. The deeper relationships of the culicine genera were not resolved satisfactorily, but *Aedes* (4 species) and *Ochlerotatus* (15 species) formed distinct clades in a strongly supported sister-group relationship.
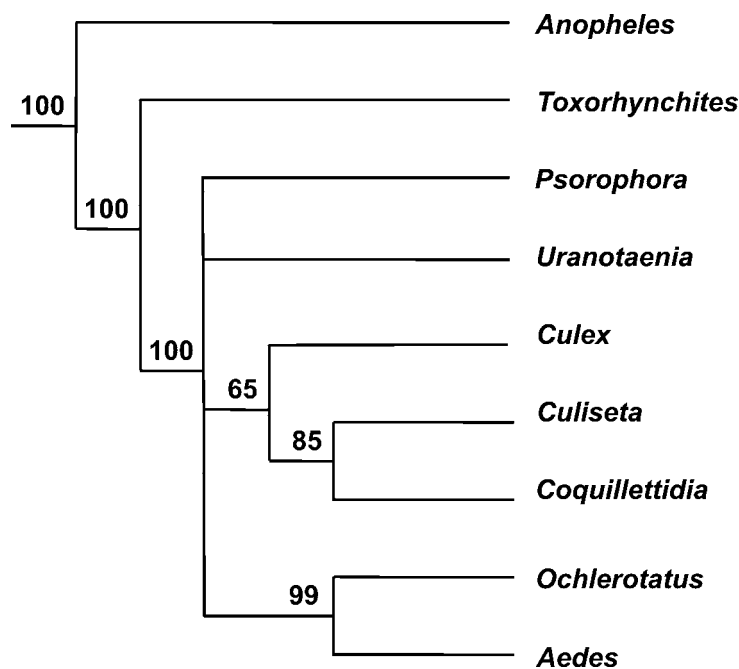

FIGURE 7. Relationships of 9 genera (39 species) based on 18S rDNA sequences (modified from Shepard *et al.*, 2006): maximum likelihood tree showing bootstrap values >50%.

With regard to the preceding, it is important to note that divergences in Culicidae are expected to be very deep in evolutionary time. Also, there is evidence of a rapid burst of radiation in some taxa. Both of these issues create problems for phylogeny reconstruction based on both morphology and DNA sequence data. If the situation is extreme, then it will not be possible to reconstruct the evolutionary history of mosquitoes. However, the initial results of an ongoing study that aims to assess the potential of several single-copy nuclear protein-coding genes, including CAD, for resolving deeper relationships among generic-level taxa (N. Besansky, K. Reidenbach, S. Cook, E. Holmes and R. Harbach) suggest that this is not beyond hope. The use of CAD to resolve the Mesozoic divergence of major clades within eremoneuran flies (Moulton & Wiegmann, 2004) is also encouraging.

Mosquito fossils and the antiquity of Culicidae

Fossil records can provide insights into anatomical diversification, historical biogeography and the antiquity of taxa, but they are too incomplete to document precisely the divergence and ages of taxonomic groups. In the case of mosquitoes, the fossil record is so poor that it is not possible to establish the actual ages of the family and extant taxa. Edwards (1923) surmised that "The origin and phylogenetic history of the Culicidae must go back to well into the Mesozoic Era; and, from the small size and fragile nature of the insects, it is probably too much to hope that we can ever obtain much direct palaeontological evidence on these matters".

Poinar *et al*. (2000) provided a list and critical evaluation of mosquito fossils. A number of extinct species have been assigned to Culicidae since the beginning of binomial nomenclature, but only 15 can be placed in the family with confidence (Poinar *et al*., 2000; Zavortink & Poinar, 2000; Borkent & Grimaldi, 2004). Thirteen of these species are from the Tertiary, including species of *Aedes*, *Culex*, *Mansonia* and two non-extant genera, and two are from the Cretaceous. The discovery of the two cretaceous species confirms Edwards' (1923) view that the evolution of Culicidae must extend into the Mesozoic. The oldest fossil, *Burmaculex antiquus* Borkent & Grimaldi, is embedded in Burmese amber from the mid-Cretaceous (90–100 Mya). This species bears several plesiomorphic features, including a short proboscis, which suggest it is a stem-group mosquito that is intermediate between extant mosquitoes and other midges. In fact, the phylogenetic analysis of morphological data conducted by Borkent & Grimaldi (2004) indicates that *Burmaculex* is the sister group of all other fossil and modern mosquitoes. The second oldest fossil, *Paleoculicis minutus* Poinar *et al*., is entombed in Canadian amber from the Late Cretaceous (76.5–79.5 Mya). Morphological features indicate that *Paleoculicis* shares a closer affinity with culicine than anopheline mosquitoes, which suggests that this ancestral lineage is younger than the lineage that gave rise to subfamily Anophelinae. *Anopheles* (*Nyssorhynchus*?) *dominicanus* Zavortink & Poinar and *An*. (?) *rottensis* Statz are the only fossil anopheline mosquitoes. The former is contained in Dominican amber from the mid-Tertiary (15–45 Mya) and the latter is from the Late Oligocene of Germany (approximately 25 Mya). If the Anophelinae are indeed basal to all other Culicidae, it would appear from available fossil evidence that extant groups of Culicidae may have evolved in the Cenozoic Era.

Considering the impact of mosquitoes on human health, it is not surprising that phylogenetic studies of these insects have only dealt with relationships within the four major groups that contain the majority of medically important species. These include subfamily Anophelinae and tribes Aedini, Culicini and Sabethini of subfamily Culicinae. As the following discussions will show, the phylogenies of these groups are far from being resolved and their classifications are not entirely natural.

Phylogeny and classification of Anophelinae

Subfamily Anophelinae includes 476 formally described species. Many genetic species of sibling species complexes await formal names. The traditional classification of the subfamily includes three genera: *Anopheles* (cosmopolitan), *Bironella* (Australasian) and *Chagasia* (Neotropical). Cladistic analyses of morphological data and DNA sequences of various ribosomal, mitochondrial and nuclear genes strongly support the monophyly of the subfamily and the placement of *Chagasia* in an ancestral relationship to all other anophelines (Harbach & Kitching, 1998, 2005; Besansky & Fahey, 1997; Foley *et al.*, 1998; Krzywinski *et al.*, 2001a,b; Sallum *et al.*, 2000, 2002).

The majority of anopheline species belong to genus *Anopheles*, which is subdivided into seven subgenera: *Anopheles s.s.* (cosmopolitan), *Baimaia* (Oriental), *Cellia* (Old World), *Kerteszia* (Neotropical), *Lophopodomyia* (Neotropical), *Nyssorhynchus* (Neotropical) and *Stethomyia* (Neotropical). The subgenera are defined primarily by the number and positions of specialized setae on the gonocoxites of the male genitalia (Christophers, 1915). Christophers (1915) proposed three generic subdivisions which Edwards (1921) and Root (1923) formally recognized as subgenera *Anopheles*, *Myzomyia* (= *Cellia*) and *Nyssorhynchus*. Edwards (1932) adopted this scheme of classification and added subgenus *Stethomyia*. This system followed Christophers (1924) in recognizing *Kerteszia* as an informal group within subgenus *Nyssorhynchus* whereas Dyar (1928) and later Komp (1937, 1942) treated it as a subgenus. Subgenus *Lophopodomyia* was proposed by Antunes (1937). Although several subgenera have been shifted in and out of synonymy, and subgenus *Baimaia* was recently proposed for *An. kyondawensis* Abraham in the Oriental Region (Harbach *et al.*, 2005), the basic concepts and limits of the subgenera have changed little since Edwards (1932).

The history and internal classification of genus *Anopheles* were reviewed in detail by Harbach (1994b, 2004). The three largest subgenera, i.e. *Anopheles*, *Cellia* and *Nyssorhynchus*, are divided into hierarchical systems of informal taxonomic categories that include Sections, Series, Groups, Subgroups and Complexes. Subgenus *Anopheles* is divided into two Sections based on the shape of the pupal trumpet. The Laticorn Section was created for species with a wide funnel-shaped trumpet having the longest axis transverse to the stem, and the Angusticorn Section for species with a semi-tubular trumpet having the longest axis vertical more or less in line with the stem. Subgenus *Nyssorhynchus* is divided into three Sections based on unique combinations of larval, pupal and adult characters (Peyton *et al.*, 1992). Subgenus *Cellia* and the Sections of subgenera *Anopheles* and *Nyssorhynchus* are divided into Series, the larger Series are divided into Species Groups, and some Groups are further divided into Subgroups and species Complexes. Most of the groupings at each level of classification are presumed to represent natural groups of species, thus implying phylogenetic relationships. As will be seen below, much additional basic taxonomic research is needed before the formal and informal taxa can be firmly established as monophyletic entities.

The phylogenetic studies of anopheline mosquitoes conducted to date are summarized in Table 3. In view of the impact of malaria on human health, it is not surprising that most of these studies have dealt with Species Groups, Subgroups and Complexes that include vectors of human malaria. Considering the taxonomic breadth of subfamily Anophelinae, it is obvious that the evolutionary relationships of malaria vectors and their closest allies have received more attention than other groups. Because a discussion of interspecific and species-group relationships is beyond the scope of the present review, no further consideration is given here to the majority of the studies listed in Table 3.

Sallum *et al.* (2000) performed the first phylogenetic analysis of subfamily Anophelinae (Fig. 8) based on 163 morphological characters in the larval, pupal and adult stages of 64 species representing the three genera and the six subgenera of genus *Anopheles* that were recognized at the time. The analysis supported the monophyly of the subfamily and the early divergence of *Chagasia* from other anophelines, and indicated that the traditional concept of genus *Anopheles* is paraphyletic because it excludes *Bironella*. Subgenera *Kerteszia*, *Nyssorhynchus*, *Cellia*, *Lophopodomyia* and *Stethomyia*, along with genus *Bironella*, were found to be mono-

TABLE 3. Phylogenetic studies of anopheline mosquitoes. None of the studies included all taxa that comprise the group investigated, but those marked with an asterisk (*) included the majority of species. Nucleotide sequences include *COI*, *COII*, *cyt b* and *ND4*, *ND5*, *ND6* from mitochondrial DNA (mtDNA); *D2*, *D3*, *18S* and *ITS2* from ribosomal DNA (rDNA); *G₆pd* and *white* from nuclear DNA.

Group	Data set	Authors
Anophelinae	Morphology	Harbach & Kitching (1998), Sallum *et al.* (2000), Harbach & Kitching (2005)
	DNA: *cyt b*, *ND5*, *D2* *ND5*, *D2*, *G₆pd*, *white* *COI*, *COII*, *D2*	Krzywinski *et al.* (2001a) Krzywinski *et al.* (2001b) Sallum *et al.* (2002)
Genus *Anopheles*	DNA: *18S*	Bargues *et al.* (2006)
Subgenus *Anopheles*	Morphology	Collucci & Sallum (2007)
	DNA: *COII*	Foley *et al.* (1998)
Anopheles Series		
Maculipennis Group	Chromosomes	White (1978)
	DNA: *ITS2*	Linton (2004)*, Gordeev *et al.* (2004), Djadid *et al.* (2007)
Maculipennis Subgroup	DNA: *ITS2*	Marinucci *et al.* (1999)
Freeborni and Quadri-maculatus Subgroups	DNA: *D2*	Porter & Collins (1996)
Myzorhynchus Series		
Barbirostris Complex	DNA: *ITS2*, *COI*, *COII*	Saeung *et al.* (2007)
Hyrcanus Group	DNA: *ITS2*	Ma & Qu (2002), Ma & Xu (2005), Hwang (2007)*
Subgenus *Cellia*	Chromosomes	Green (1982), Pape (1992)
	DNA: *COII*	Foley *et al.* (1998)
Myzomyia Series	Chromosomes	Green (1982), Pape (1992)
	DNA: *COII*, *D3*	Chen *et al.* (2003)
Funestus Group	DNA: *COI*, *D3*, *ITS2*	Garros *et al.* (2005a)*
	DNA: *COII*, *D3*	Garros *et al.* (2005b)*
Minimus Subgroup	DNA: *COII*, *D3*	Sharpe *et al.* (2000)
Minimus Complex	DNA: *D3*, *ITS2*	Sawabe *et al.* (2003)*
Neocellia Series	Chromosomes	Green *et al.* (1985)
Annularis Group	DNA: *D3*, *ITS2*	Alam *et al.* (2007)
Maculatus Group	DNA: *COII*, *D3*, *ITS2*	Ma *et al.* (2002, 2006), Walton *et al.* (2007)
Neomyzomyia Series		
Leucosphyrus Group	DNA: *COI*, *ND6*	Sallum *et al.* (2007)
Punctulatus Group	DNA: *ITS2*	Beebe *et al.* (1999)
Pyretophorus Series	Morphology	Anthony *et al.* (1999)*
	Chromosomes	Pape (1992)
	DNA: *COII*	Foley *et al.* (1998)

TABLE 3 (continue)

Group	Data set	Authors
Gambiae Complex	Chromosomes	Coluzzi *et al.* (1979)
	rDNA, mtDNA	Besansky *et al.* (1994)
	mtDNA	Caccone *et al.* (1996)
Subgenus *Kerteszia*	Morphology	Collucci & Sallum (2003)
Subgenus *Nyssorhynchus*	DNA: *ITS2*	Marrelli *et al.* (2005)
Albimanus Section	Morphology	Faran (1980)
Argyritarsis Section	Morphology	Linthicum (1988)
Albitarsis Complex	DNA: *ITS2*, *D2*, *COI*, *ND4*	Wilkerson *et al.* (2005)
	white	Brochero *et al.* (2007)

phyletic taxa dispersed among various Series and Species Groups of subgenus *Anopheles*. Despite the pairing of the Christya Series (arrow in Fig. 8) of subgenus *Anopheles* with *Kerteszia* + *Nyssorhynchus* and the sister-group relationship of this clade with *Cellia* + all other anophelines except *Chagasia*, it is surprising that Sallum *et al.* (2000) elected to retain *Kerteszia*, *Nyssorhynchus* and *Cellia* as subgenera and formally synonymize *Lophopodomyia*, *Stethomyia* and *Bironella* with subgenus *Anopheles*. A subsequent cladistic analysis of *Kerteszia* based on morphological data (Collucci & Sallum, 2003) provided further support for the monophyly of the subgenus and its sister relationship with *Nyssorhynchus*.
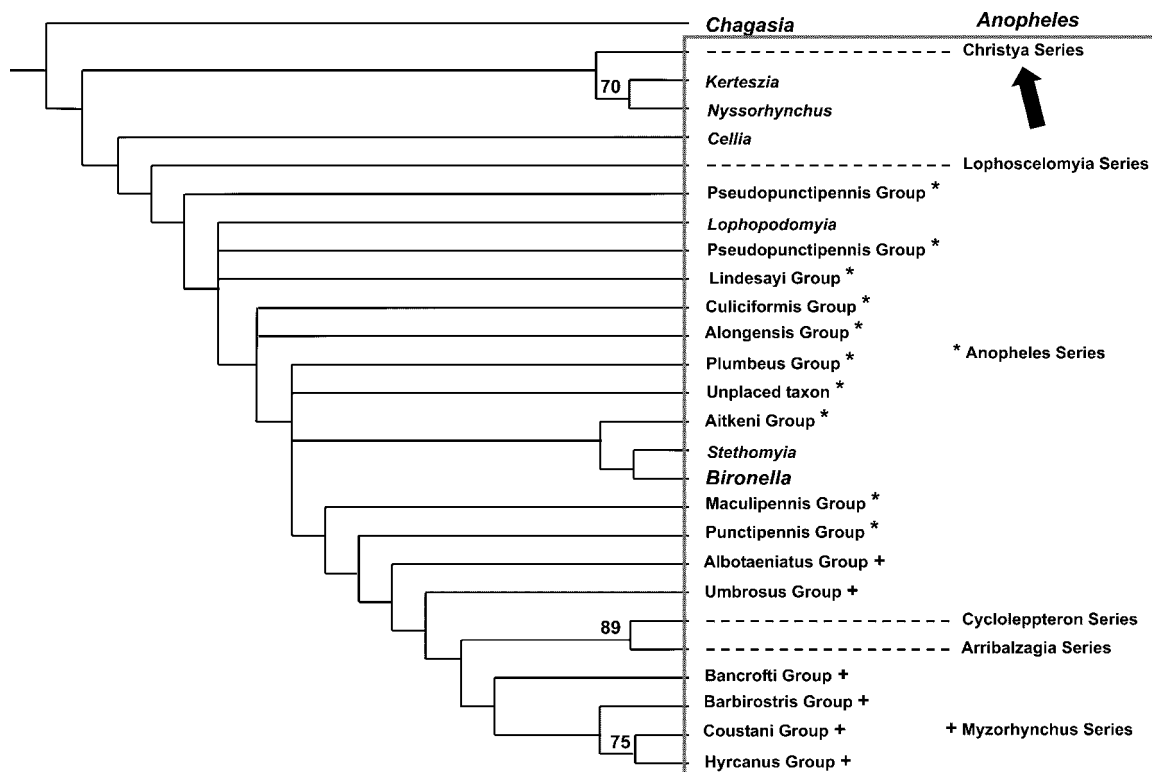

FIGURE 8. Phylogeny of Anophelinae based on morphology (modified from Sallum *et al.*, 2000): strict consensus of 144 MPCs from equal-weighting analysis of 60 anopheline species and 163 anatomical characters. Bootstrap values >50% are shown. Asterisks (*) indicate Species Groups of the *Anopheles* Series and plus symbols (+) indicate Species Groups of the *Myzorhynchus* Series. The authors elected to retain *Kerteszia*, *Nyssorhynchus* and *Cellia* as valid subgenera and formally synonymize *Lophopodomyia*, *Stethomyia* and *Bironella* with subgenus *Anopheles* although the position of the Christya Series (arrow) clearly indicates that all six of these groups fall within the nominotypical subgenus.

Sallum *et al*. (2002) conducted a molecular analysis of anopheline relationships based on ribosomal (18S, 28S) and mitochondrial (COI, COII) DNA sequences (Fig. 9). The results of this study cannot be compared directly to those of Sallum *et al*. (2000) because significantly fewer taxa were included in the analyses. Whereas Sallum *et al*. (2000) included 64 species of *Anopheles* in their analyses of morphological data, Sallum *et al*. (2002) only included 32 species in their molecular analyses. Six Sections/Series and 22 Species Groups represented in the former study were not represented in the latter. Subgenus *Anopheles* was particularly underrepresented: three Series and 10 Species Groups of subgenus *Anopheles* included in the analyses of morphological data were not represented in the analyses of molecular data. Nevertheless, the molecular data corroborate the basal placement of *Chagasia* within subfamily Anophelinae, the paraphyly of genus *Anopheles* relative to *Bironella* and the sister-group relationship of *Kerteszia* and *Nyssorhynchus*. Surprisingly, in the parsimony analysis (Fig. 9A) *Stethomyia* was recovered as the sister group of subgenus *Anopheles*, but in the maximum likelihood analysis (Fig. 9B) it formed a sister relationship with *Cellia*. Contrary to the results of Sallum *et al*. (2000), the analyses support the monophyly of subgenus *Anopheles* as well as the monophyly of the other subgenera and genus *Bironella*, which is reconstructed as the sister to *Lophopodomyia* rather than *Stethomyia* (cf. Sallum *et al*., 2000).
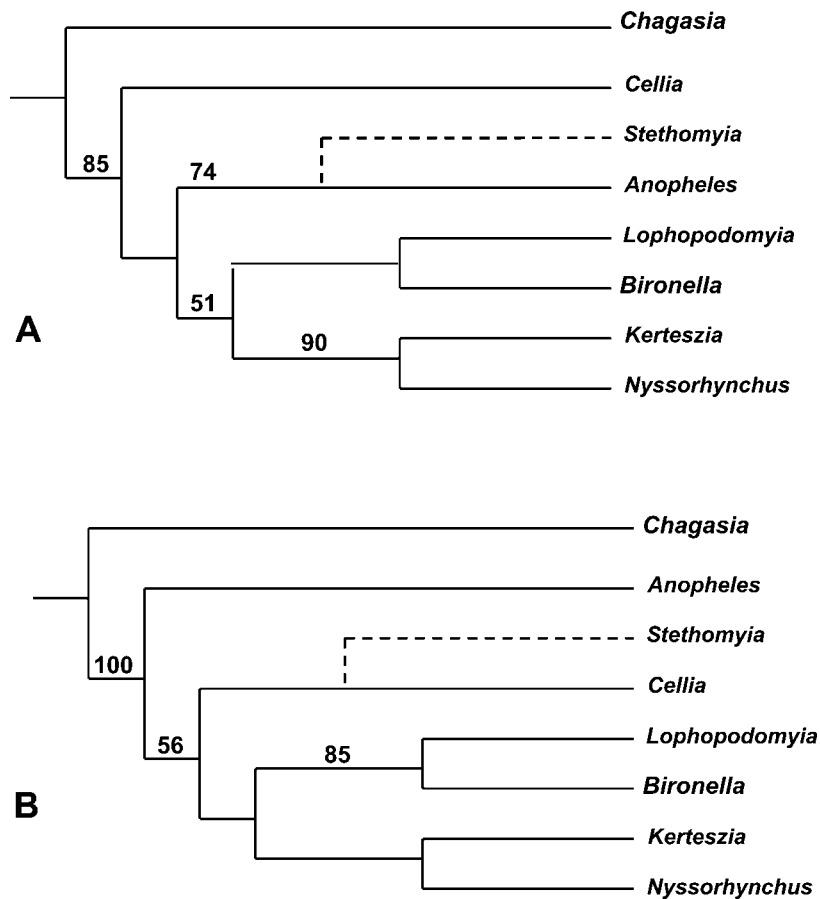

FIGURE 9. Phylogeny of Anophelinae (32 species) based on rDNA and mtDNA sequences (modified from Sallum *et al*., 2002): A, strict consensus of 22 MPTs from parsimony analysis using SACW of combined data; B, single tree from maximum likelihood analysis of combined data. Bootstrap values >50% are shown.

Harbach & Kitching (2005) expanded the phylogenetic analysis of Sallum *et al*. (2000) to include a member of the Stigmaticus Group and *An. kyondawensis* of subgenus *Anopheles*. In so doing, they found it necessary to reinterpret certain homologies, especially the homologies of the specialized setae of the male gonocoxites that diagnose the subgenera of genus *Anopheles* (see above), and revise the coding for a number

of morphological characters used by Sallum *et al.* (2000). The revised data set consisted of 167 characters for 66 species representing the three traditionally recognized genera and all informal Series and most Species Groups of subgenera *Anopheles*, *Cellia* and *Nyssorhynchus*. Parsimony analysis of the data set under implied weighting (IW) (Fig. 10) supported the monophyly of Anophelinae, the basal position of *Chagasia*, the monophyly of subgenera *Cellia*, *Kerteszia* and *Nyssorhynchus*, and the sister relationship of *Kerteszia* + *Nyssorhynchus*. Subgenus *Anopheles* was recovered as a polyphyletic lineage basal to a monophyletic clade consisting of *Kerteszia* + *Nyssorhynchus* and *Cellia* in a sister-group relationship. *Bironella*, *Lophopodomyia* and *Stethomyia* were firmly nested within subgenus *Anopheles*, which would be paraphyletic even if these taxa were subsumed within it. *Anopheles kyondawensis*, which was well supported as the sister of *Bironella* + all other *Anopheles*, was subsequently recognized as the monotypic member of a new subgenus named *Baimaia*. *Bironella* and *Stethomyia*, contrary to Sallum *et al.* (2000), were also strongly supported as monophyletic clades separate from subgenus *Anopheles*.
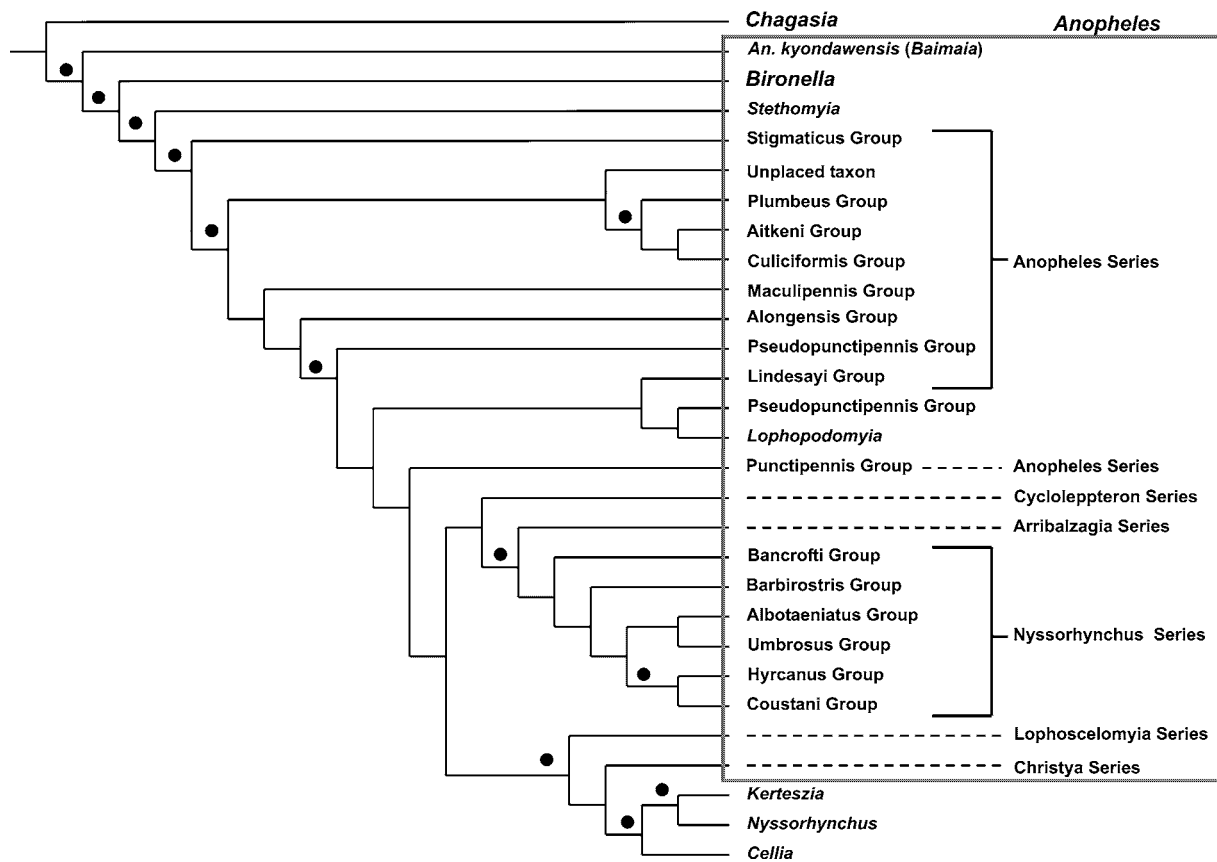

FIGURE 10. Phylogeny of Anophelinae based on morphology (modified from Harbach & Kitching, 2005): strict consensus tree from implied weighting analysis (*K*=1) of 66 anopheline species and 167 anatomical characters. Filled circles indicate Bremer support values greater than 0.8.

A recent analysis of subgenus *Anopheles* by Collucci & Sallum (2007) included 38 species representing the same Series (6) and Species Groups (15) of the subgenus that were included in the study of Sallum *et al.* (2000). The data were analyzed using SACW and IW. The strict consensus trees (Figs 11 and 12) that resulted from these analyses were identical except for the placement of *An. annulipalpis* Lynch Arribalzaga (Cycloleppteron Series), *An. pseudobarbirostris* Ludlow (Myzorhynchus Series), *An. implexus* (Theobald) (Christya Series) and the Arribalzagia Series. Most of the relationships between members of the subgenus were either moderately or poorly supported by both Bremer and bootstrap supports. The monophyly of the subgenus was poorly supported (= 50% bootstrap value) and based entirely on homoplastic characters. The Laticorn Section was recovered as a monophyletic clade in the IW analysis, suggesting that the laticorn devel-

opment of the pupal trumpet is a derived condition for subgenus *Anopheles*. In the SACW analyses, members of the group comprised a paraphyletic lineage relative to the Cycloleppteron Series. The Angusticorn Section was recovered as a polyphyletic assemblage in both the SACW and IW analyses. These results are contradicted by those of Sallum *et al.* (2000) and Harbach & Kitching (2005) who found that neither section is monophyletic. Below the section level of classification, only the Lophoscelomyia and Arribalzagia Series were recovered as monophyletic assemblages. The Myzorhynchus Series was paraphyletic relative to the Cycloleppteron, Christya and Arribalzagia Series, and the Anopheles Series was polyphyletic. Surprisingly, the two species of the Cycloleppteron Series included in the analyses were not grouped together, suggesting that the series is not monophyletic. In contrast, the Arribalzagia, Christya, Cycloleppteron, Lophoscelomyia and Myzorhynchus Series were recovered as monophyletic assemblages in the IW analysis of Harbach & Kitching (2005). Furthermore, with the removal of *An. kyondawensis* to subgenus *Baimaia*, the remaining species of the Anopheles Series included in their analysis also formed a monophyletic group. With the exception of the Pseudopunctipennis Group, all the species groups represented in the analysis of Collucci & Sallum (2007) (Aitkenii, Albotaeniatus, Culiciformis, Hyrcanus, Plumbeus, Umbrosus Groups) were recovered as monophyletic assemblages with moderate to strong bootstrap support. The Hyrcanus Group was paired with *An. coustani* Laveran, which corroborates the close relationship of the Hyrcanus and Coustani Groups hypothesized by Reid & Knight (1961), Harrison & Scanlon (1975) and Sallum *et al.* (2000).

Unfortunately, the analyses of Collucci & Sallum (2007) are biased by the selection of outgroup taxa whose interrelationships with the ingroup taxa were unresolved in previous studies. Thus, the results of their study cast doubt on their assertion that subgenus *Anopheles* is monophyletic (clade A in Figs 11 and 12). On closer examination, the results of Collucci & Sallum (2007) are not significantly different than those of Sallum *et al.* (2000) regarding the phylogenetic placement of *Bironella*, *Stethomyia* and *Lophopodomyia*. Strictly speaking, these three entities can be interpreted as basal taxa within subgenus *Anopheles* (clade B in Figs 11 and 12). Collucci & Sallum (2007) provided no objective evidence to suggest that these taxa should be excluded from the subgenus, and it is likely that they would have recovered a different set of relationships if they had included species of genus *Chagasia* (as outgroup), subgenus *Baimaia* and the Stigmaticus Group of subgenus *Anopheles* in their analyses.
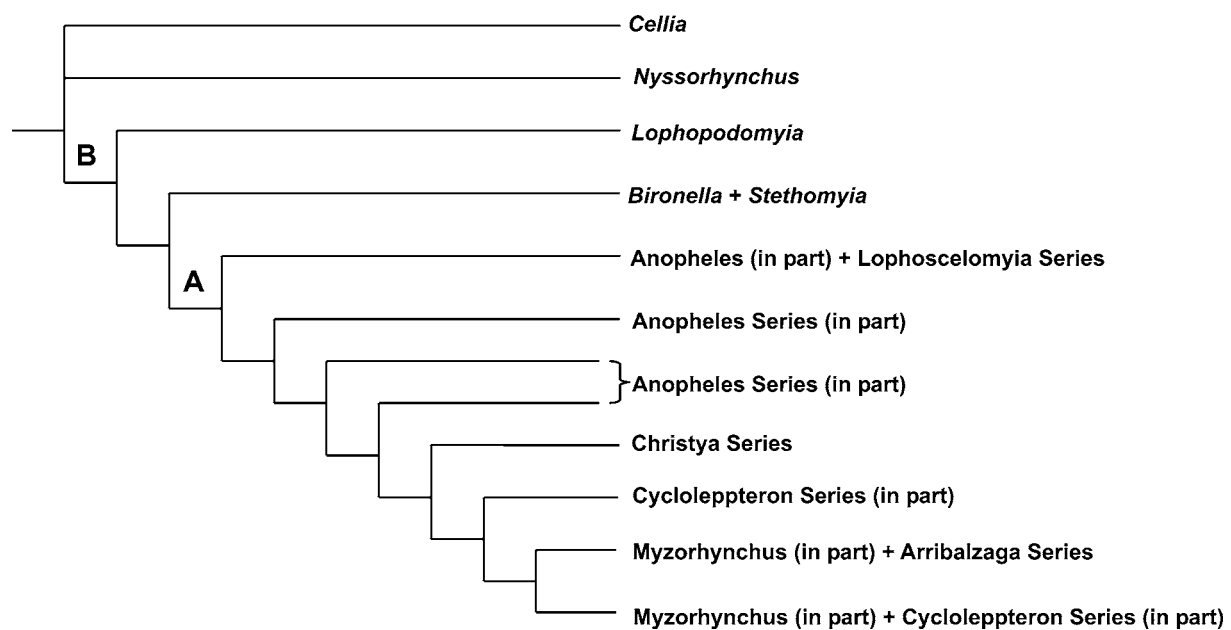

FIGURE 11. Phylogeny of subgenus *Anopheles* based on morphology (modified from Collucci & Sallum, 2007): strict consensus tree from parsimony analysis of 38 species and 101 anatomical characters using SACW. Clade A = subgenus *Anopheles* of the authors; clade B = subgenus *Anopheles* of Sallum *et al.* (2000).
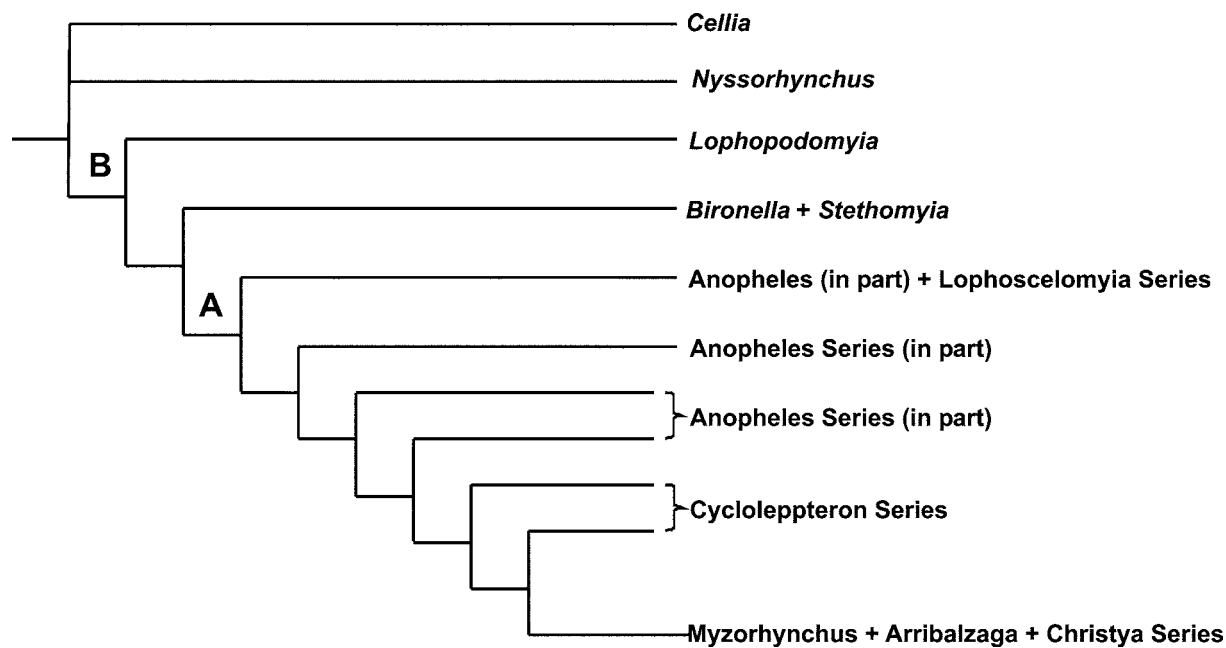
FIGURE 12. Phylogeny of subgenus *Anopheles* based on morphology (modified from Collucci & Sallum, 2007): strict consensus tree from implied-weighting analysis (*K*=1) of 38 species and 101 anatomical characters. Clade A = subgenus *Anopheles* of the authors; clade B = subgenus *Anopheles* of Sallum *et al.* (2000).

The studies of Sallum *et al.* (2000), Harbach & Kitching (2005) and Collucci & Sallum (2007) demonstrate the effects of homology interpretation, character coding and taxon sampling on the outcome of a phylogenetic reconstruction. Similarly, the choice of DNA fragments and interpretations of gene structure and homology (paralogy), as well as alignment and sequencing errors and choice of phylogenetic method, have major effects on sequence-based phylogenies. As pointed out by Stevens & Schofield (2003), genes are not organisms and so it is illogical to assume that genomic evolution is congruent with the evolution of the organisms. For these reasons, a reasonable course of action would be to recognize those groups as subgenera or genera whose monophyly is strongly supported by both morphological and molecular data in studies that are not limited by taxon sampling. At this point in time, the results of morphological and molecular studies strongly agree only on the recognition of four generic-level taxa within Anophelinae, i.e. *Chagasia*, *Kerteszia*, *Nyssorhynchus* and *Cellia*.

With regard to subgenus *Anopheles*, it should be noted that the vernacular names of the informal Series are derived from available formal names originally proposed for genera that are currently considered to be junior synonyms of *Anopheles*. In addition to the senior synonym (*Anopheles*), five of the 21 available junior synonyms are in use for Series-level categories of classification within the subgenus. Should the monophyly of the Arribalzagia, Christya, Cycloleppteron, Lophoscelomyia and Myzorhynchus Series be firmly established, these groups could legitimately be recognized as subgenera with these same names. Alternately, if *Bironella* proves to warrant generic status, as propounded by Krzywinski *et al.* (2001a,b) and Krzywinski & Besansky (2003), then the firmly established monophyletic lineages, most of which were originally proposed as genera, including *Cellia*, *Kerteszia* and *Nyssorhynchus*, may also require generic status. Based on the phylogeny of Harbach & Kitching (2005), if *Bironella* is recognized as a genus, then the only other taxon that needs to be raised to generic status is *Baimaia*. Everything else could be treated as subgenera in a slightly smaller genus *Anopheles*.

Phylogeny and classification of Aedini

Aedini is the largest tribe in family Culicidae with 1,240 currently recognized species. The traditional classification of Aedini is based on the concept of recognizing few genera and numerous subgenera (Edwards, 1932; Belkin, 1962). Belkin (1962) viewed the tribe as a natural group but noted that some members showed affinities with all other higher-level taxa of subfamily Culicinae. Species of the tribe are extremely varied, and many are difficult to identify to genus because of overlapping suites of shared anatomical features. Hence, combinations of characters are required to define the majority of the genera, subgenera and species. General features of the tribe include the presence of toothed ungues (tarsal claws) and a pointed abdomen in most females. Although toothed ungues are not universally present, they are not found in any other tribe of Culicinae. Larvae have relatively short, stout siphons with a single pair of seta 1-S (except for species of *Aedes s.s.* and *Ochlerotatus* subgenus *Rusticoidus*) inserted well above the base, usually beyond the middle of the siphon. A comb is always present on segment VIII and the ventral brush is usually represented by five or more pairs of setae. Based on the assumption that annectent forms may have been derived by hybrid origin, Belkin (1962) believed that Aedini differentiated in the Indomalayan area of the Old World where the majority of annectent forms presently exist.

The traditional classification of Aedini before the end of the twentieth century included nine genera and 50 subgenera (Knight & Stone, 1977; Knight, 1978; Ward, 1984, 1992). *Aedes* was by far the largest genus with approximately 1,000 species divided between 41 subgenera. Reclassification of genus *Aedes* began with the elevation of *Verrallina* and *Ayurakitia* to generic status (Reinert, 1999c, 2000c, respectively) and the subsequent separation of the remaining subgenera into genera *Aedes* and *Ochlerotatus* (Reinert, 2000d). Whereas the generic status of *Verrallina* and *Ayurakitia* was readily accepted, the elevation of *Ochlerotatus* to generic status was widely condemned by taxonomists and medical entomologists, particularly in North America. Critics suggested that this change should not have been made without strong corroborating evidence from other character systems, presumably principally molecular data. As the following discussion will show, analyses of molecular data are lending support to taxonomic changes based on morphology.

Anderson *et al.* (2001) examined the phylogenetic relationships of six mosquito species belonging to genera *Aedes* (3 species), *Armigeres* (1 species) and *Culex* (2 species) by comparing chromosomal rearrangements based on shared restriction fragment length polymorphisms. Phylogenetic analysis of eight informative chromosomal rearrangements generated a single most parsimonious tree (Fig. 13), which the authors rooted on *Armigeres subalbatus* because Ross (1951) considered *Armigeres* to be ancestral to *Aedes* and *Culex*. Genus *Aedes* was paraphyletic, with two species of *Stegomyia* [*Ae. aegypti* (Linnaeus) and *Ae. albopictus* (Skuse)] forming one clade and *Ae.* (*Protomacleaya*) *triseriatus* (Say) clustering with the two *Culex* species [*Cx. pipiens* Linnaeus and *Cx. tritaeniorhynchus* Giles]. The analysis of *white* gene sequences by Besansky & Fahey (1997) also indicated that *Aedes* was paraphyletic, with *Ae. triseriatus* clustered with *Haemagogus equinus* Theobald. In an earlier study, Wesson *et al.* (1992) found that analyses of ITS2 rDNA sequences also separated *Stegomyia* from *Haemagogus* and *Ae. triseriatus*. Overall, the analyses of Anderson *et al.* (2001), Besansky & Fahey (1997) and Wesson *et al.* (1992) lend support to the separation of *Aedes* and *Ochlerotatus* proposed by Reinert (2000d).

Kumar *et al.* (1998) investigated the relationships of 15 culicine species representing six genera, including nine species of *Aedes* and one each of *Armigeres* and *Haemagogus*. More recently, Cook *et al.* (2005) investigated the relationships among 20 species of *Aedes* representing six traditionally recognized subgenera, including *Aedes* (1 species), *Aedimorphus* (1), *Diceromyia* (2), *Halaedes* (3), *Ochlerotatus* (10) and *Stegomyia* (3), based on sequence data for the cytochrome oxidase c subunits I and II (COI, COII) of mtDNA. Finally, Shepard *et al.* (2006) employed the 18S subunit of rDNA to analyze the phylogenetic relationships of 39 North American species representing nine genera. The results of Kumar *et al.* (1998), in agreement with Wesson *et al.* (1992) and Besansky & Fahey (1997), indicated a paraphyletic Aedini, but these studies provided little

insight into the phylogeny and classification of the tribe because they included very few generic-level taxa and few species. The COI and COII phylogenies (Figs 14 and 15) of Cook *et al.* (2005) agreed with respect to species-level groupings, but because sequences for neither gene were available for all the species analyzed, it was "difficult to draw firm conclusions regarding the interspecies relationships". Support for supraspecific clades was either very low or meaningless in cases where higher-level taxa were represented by several specimens of a single species. In general, clade support, assessed by bootstrap proportions, was slightly higher for the COII phylogeny. Species of *Ochlerotatus* included in the analysis of the COI data (COII data were not available) were placed in a sister-group relationship to *Aedes* (*Aedes*) (represented by *Ae. cinereus* Meigen), but all of the internal branches were only weakly supported. Nevertheless, the results are not inconsistent with the recognition of *Aedes s.s.* and *Ochlerotatus* as separate genera. As noted previously, *Aedes* and *Ochlerotatus* formed distinct clades in a strongly supported sister-group relationship in the 18S rDNA phylogeny of Shepard *et al.* (2006) (Fig. 7), which also provides molecular support for the elevation of *Ochlerotatus* to generic rank by Reinert (2000d).
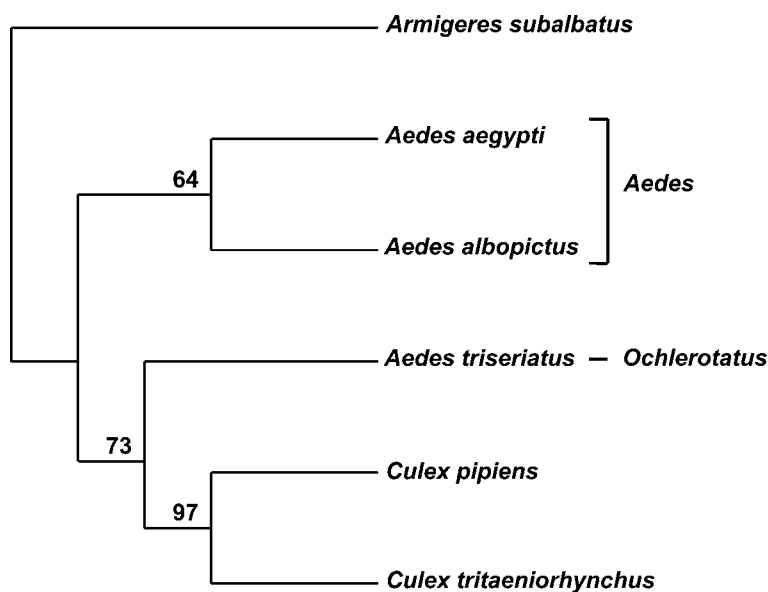

FIGURE 13. Phylogenetic relationships of six culicine species based on chromosomal rearrangements (after Anderson *et al.*, 2001): tree from parsimony analysis showing bootstrap values >50%.
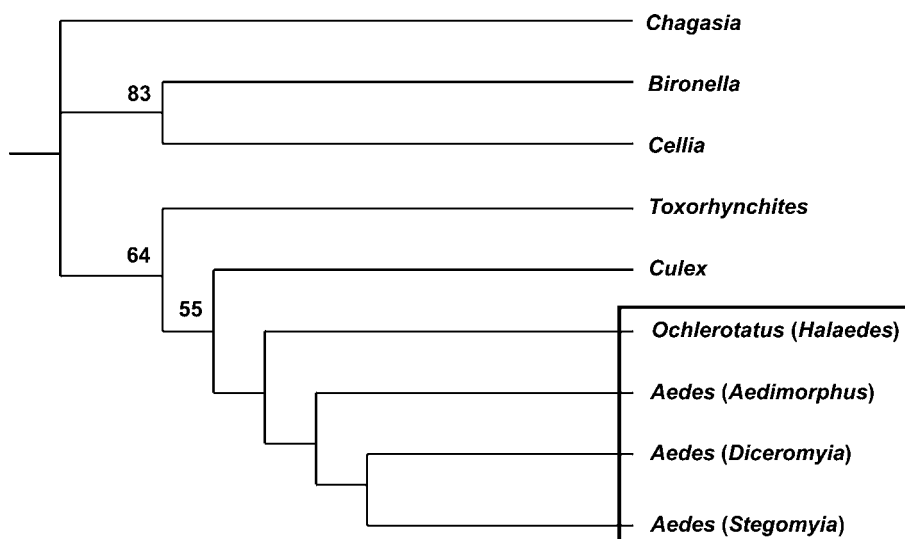

FIGURE 14. Relationships of five generic-level taxa (17 species) of Aedini based on COI sequences (modified from Cook *et al.*, 2005): maximum likelihood tree showing bootstrap values >50%.
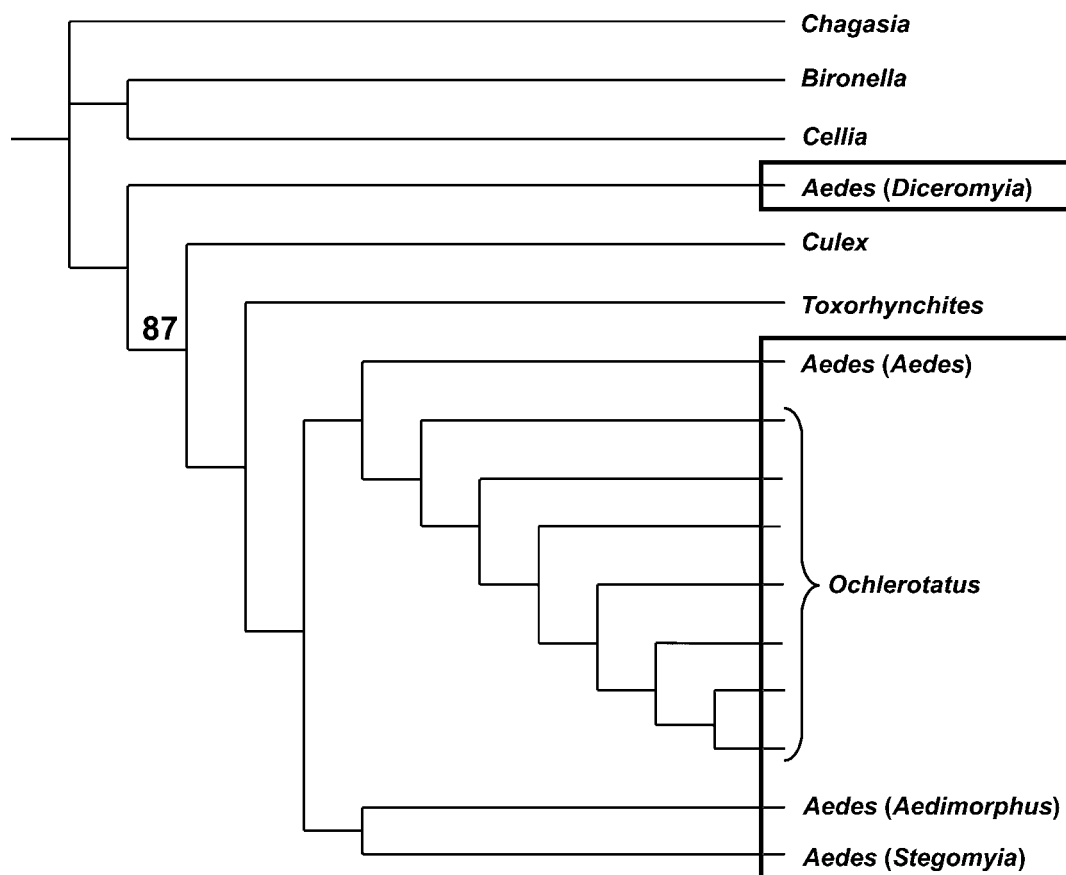
FIGURE 15. Relationships of four generic-level taxa (nine species) of Aedini based on COII sequences (modified from Cook *et al.*, 2005): maximum likelihood tree showing bootstrap values >50%.

Reinert *et al.* (2004) conducted a phylogenetic analysis of higher-level relationships within Aedini (Fig. 16) using 172 morphological characters from all life stages and 119 exemplar species representing the 12 genera and 56 subgenera that were recognized at the time. All of the genera except *Aedes* and *Ochlerotatus* were recovered as monophyletic in an implied-weighting analysis of the combined data. The tribe as a whole was portrayed as a polyphyletic assemblage of *Aedes* and *Ochlerotatus* within which the other 10 genera were embedded. *Ochlerotatus* formed a polyphyletic assemblage basal to *Aedes* that included *Haemagogus*, *Opifex* and *Psorophora*, and *Aedes* was polyphyletic relative to *Armigeres*, *Ayurakitia*, *Eretmapodites*, *Heizmannia*, *Udaya*, *Verrallina* and *Zeugnomyia*. With the exception of four subgenera, i.e. *Ae.* (*Aedimorphus*), *Oc.* (*Finlaya*), *Oc.* (*Ochlerotatus*) and *Oc.* (*Protomacleaya*), all subgenera with two or more species included in the analysis were recovered as monophyletic. Rather than leave the generic classification of Aedini in its traditional (polyphyletic) state, the authors decided that a reasonable course of action was to recognize "weighting independent" groups as genera, i.e. those that were common to the results of equal- and implied-weighting analyses of the data set. The strict consensus of these combined analyses (Fig. 17) resulted in a topology of 63 clades, 29 of which were comprised of between two and nine taxa, and 30 (including *Mansonia*) that fell within an unresolved basal polytomy. In addition to 12 formerly recognized genera, the authors proposed generic status for 32 subgenera of *Aedes* and *Ochlerotatus*, raised a clade consisting of the *Oc.* (*Finlaya*) *kochi* Group to generic rank as genus *Finlaya*, elevated *Downsiomyia* from synonymy with *Finlaya* as the generic name for the *Oc.* (*Fin*laya) *niveus* Group and described a new genus, *Tanakaius*, to accommodate the *Oc.* (*Finlaya*) *togoi* Group. Genus *Aedes* was restricted to the former subgenus *Aedes* and genus *Ochlerotatus* was limited to a clade consisting of the type species, six related species and members of subgenus *Rusticoidus*. These actions resulted in the recognition of 46 genera and left 496 species without generic placement.
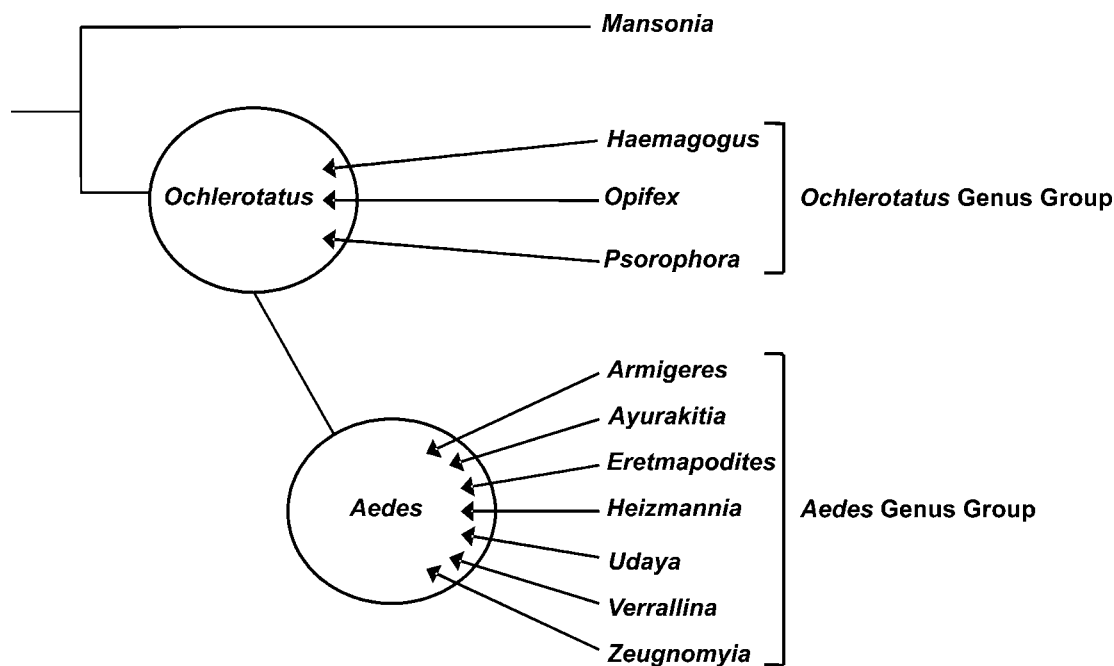
FIGURE 16. Diagrammatic and simplified phylogeny of Aedini based on morphology (modified from Reinert *et al.*, 2004): diagram of the strict consensus of eight MPCs from implied-weighting analysis (*K*=1) of 172 anatomical characters observed in 119 species of 12 genera and 56 subgenera. *Ochlerotatus* and *Aedes* are polyphyletic relative to other genera.

Reinert *et al.* (2006) attempted to resolve the relationships and generic placement of species and Species Groups of uncertain taxonomic position that were previously included in subgenus *Finlaya* of *Ochlerotatus*, and species previously removed from *Finlaya* by Zavortink (1972) and placed in subgenera *Protomacleaya* and *Ochlerotatus* of genus *Ochlerotatus*. The study employed 116 exemplar species and 232 anatomical characters from eggs, larvae, pupae, adults and immature habitat to explore relationships among Species Groups. The ingroup comprised 74 species, including 41 classified as *Finlaya*, 25 previously placed in *Finlaya* and eight related species, and the outgroup included non-aedine species and aedine species representing all generic-level taxa and major clades within the polyphyletic *Ochlerotatus* and *Aedes* Genus Groups of Reinert *et al.* (2004). The data were analyzed in a total-evidence approach using implied weighting. The strict consensus of four most parsimonious cladograms obtained from the analysis (Fig. 18) showed remarkable congruence with the results of Reinert *et al.* (2004) despite differences in the taxa and morphological characters included in the two studies. Based on character support and the principle of equivalent rank (*sensu* Hennig, 1966), Reinert *et al.* (2006) proposed generic status for 17 monophyletic clades, 11 of which required new names. As is usual with generic-level groups of Aedini, the 17 genera are polythetic taxa that are diagnosed by unique combinations of characters. These and the previous actions of these authors resulted in the recognition of 63 genera and now left 372 species without generic assignment. The authors recently completed a third study (Reinert *et al.*, in press) of taxa included in *Ochlerotatus* (*sensu* Reinert, 2000d) and are presently conducting a comprehensive analysis of tribe Aedini to further resolve the evolutionary relationships and classification of problematic taxa.

When the study of Reinert *et al.* (in press) is published, readers will notice that the authors did not compare their results with the results of earlier studies of the relationships and classification of North American *Ochlerotatus* based on intuitive and numerical (phenetic) analyses of morphological and enzyme electrophoretic data (Rohlf, 1963a,b, 1977; Lunt & Nielsen, 1971a,b; Steward, 1968; Schultz *et al.*, 1986; Grueber & Bradley, 1994). It is not possible here to contrast the earlier studies with the *Ochlerotatus* phylogeny of Rein-

ert *et al.* (in press), but it is worthwhile to point out that the results of the intuitive and numerical analyses show a high level of correlation with one another despite differences in the taxa, data and methodology used in the studies. It is unfortunate that the results of the numerical analyses have been shunted because they contain some useful information about the relationships and classification of Nearctic Aedini based on shared morphological features.
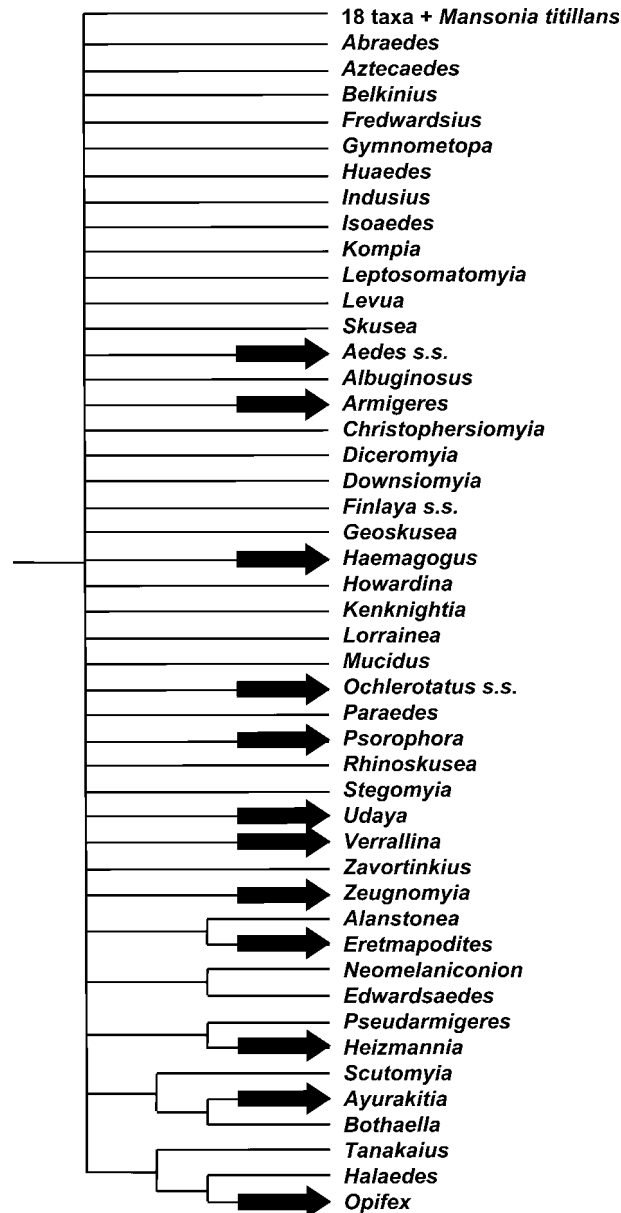

FIGURE 17. Phylogeny of Aedini based on morphology (modified from Reinert *et al.*, 2004): strict consensus of 97 MPCs from equal-weighting and eight MPCs from implied-weighting analyses of characters and taxa indicated in Fig. 16. Surviving monophyletic groups are weighting independent. The 12 previously accepted genera are indicated by arrows.

Rey *et al.* (2001) used a 763-bp segment of the mitochondrial COI gene to examine the phylogenetic relationships of 14 species traditionally placed in genus *Aedes* subgenera *Aedes* (1 species), *Aedimorphus* (1), *Ochlerotatus* (11) and *Stegomyia* (2). Parsimony and neighbour-joining analyses of the data rooted on *Anopheles claviger* (Meigen) produced identical trees with all branches supported by bootstrap values greater than 50%. Overall, the relationships inferred from their analyses, i.e. *Stegomyia* + (*Aedes* + *Ochlerotatus*), support the generic status of *Aedes*, *Ochlerotatus* and *Stegomyia* proposed by Reinert (2000d) and Reinert *et al.*

(2004). The relationships of the aedine taxa recovered by Isoe (2000) in his analyses of vitellogenin gene sequences (Fig. 19), although taxon limited, provide some support for the hypotheses of Reinert *et al.* (2004, 2006). First, the placement of *Armigeres subalbatus* among several *Aedes* species is consonant with the inclusion of *Armigeres* within the *Aedes* Genus Group, thus providing further evidence that genus *Aedes* (*sensu* Reinert, 2000d) is polyphyletic. Second, *Ochlerotatus* is a strongly supported monophyletic clade placed in a sister-group relationship with the *Aedes* Genus Group. And finally, the topology of the *Ochlerotatus* clade reflects the recognition of multiple generic-level taxa. The only disagreement appears to be the exclusion of *Psorophora* from the *Ochlerotatus* Genus Group.
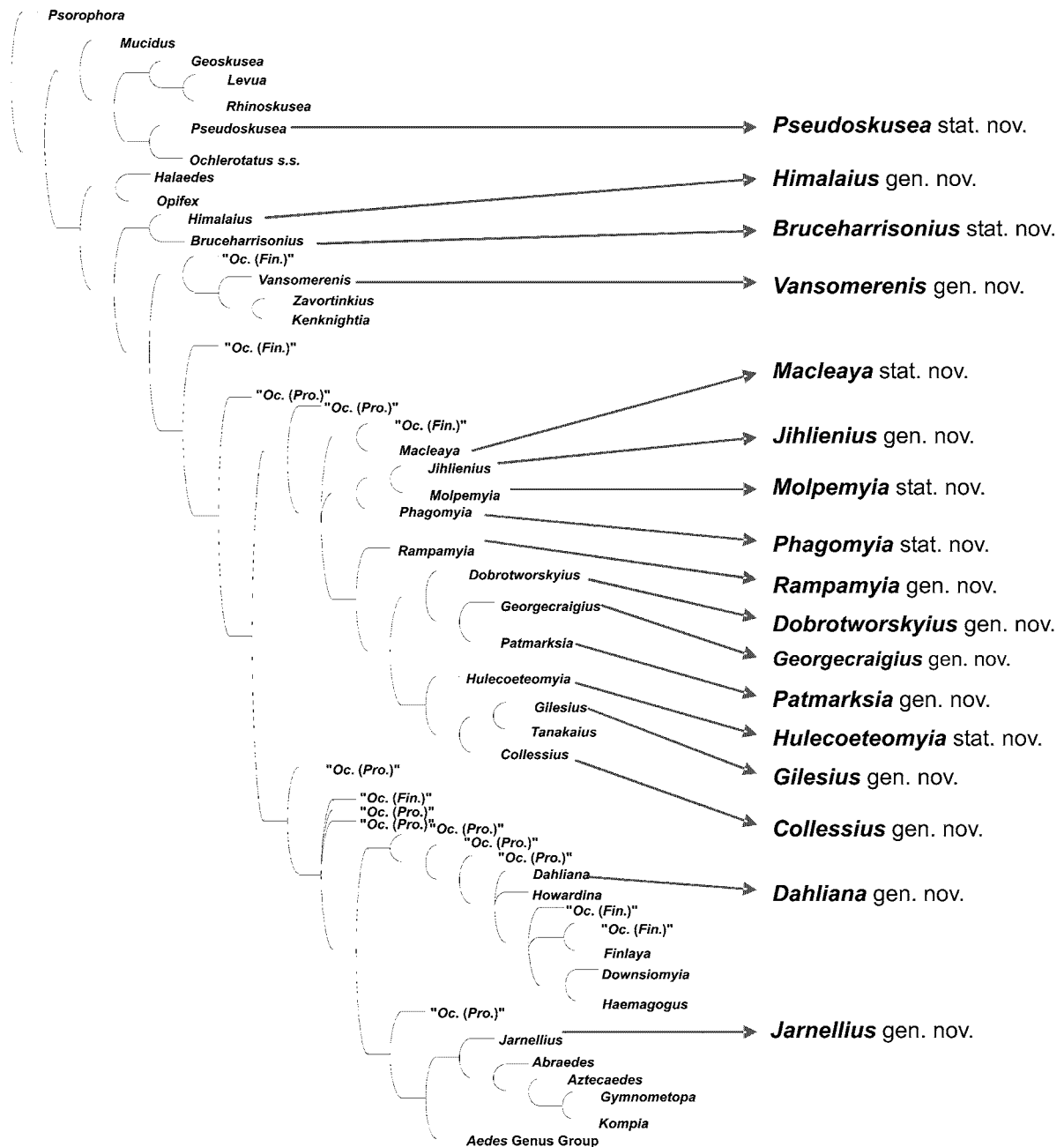

FIGURE 18. Phylogeny of *Finlaya* and allied taxa based on morphology (after Reinert *et al.*, 2006): strict consensus of four MPCs from implied-weighting analysis (*K*=1) of 116 species (74 ingroup species) and 232 anatomical characters.
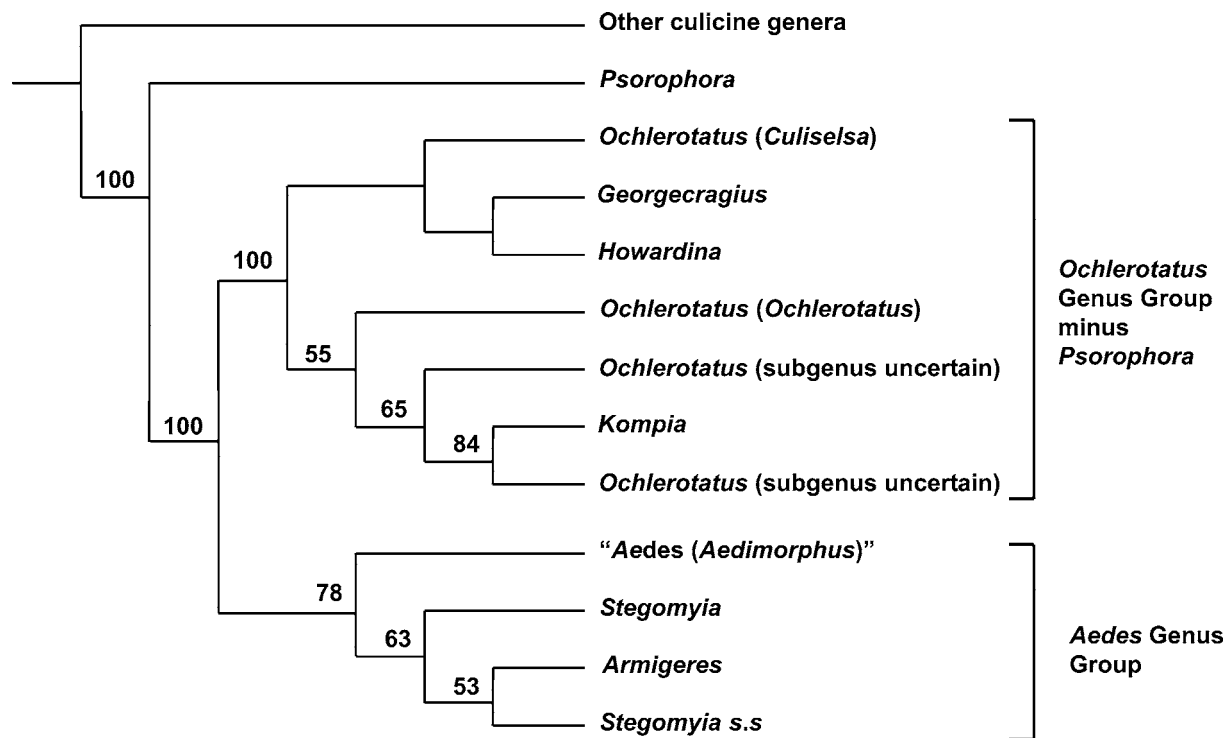
FIGURE 19. Phylogenetic relationships of some Aedini (20 species) based on vitellogenin gene sequences (modified from Isoe, 2000): maximum parsimony tree with all codon positions equally weighted. Bootstrap values >50% are shown. Generic-level names of Reinert *et al.* (2004, 2006) are substituted for species names of Isoe (2000).

Phylogeny and classification of Culicini

The monophyly of Culicini has never been disputed. The tribe includes 789 species classified in four genera: *Culex* (cosmopolitan), *Deinocerites* (Neotropical), *Galindomyia* (Neotropical) and *Lutzia* (absent from the western Palaearctic and Nearctic Regions). Belkin (1962) recognized two genera within the tribe, *Culex* and *Deinocerites*. Genus *Galindomyia* was described and assigned to the tribe by Stone & Barreto (1969). *Lutzia* was recently elevated from subgeneric rank within *Culex* (Tanaka, 2003). *Culex* is by far the largest genus with 763 species divided between 23 subgenera. In comparison, *Deinocerites* has 18 species, *Galindomyia* one species and *Lutzia* seven species. Like other large genera, *Culex* includes many polymorphic features and exceptional forms. Adames (1971) considered *Deinocerites* and *Galindomyia* as a monophyletic group based on shared characteristics of the antenna and male genitalia, and regarded this clade as the sister group of *Culex*. These relationships were supported in the generic analysis of Harbach & Kitching (1998).

Danilov (1989) regarded subgenera *Afroculex*, *Barraudius* and *Kitzmilleria* of the Afrotropical and Palaearctic Regions to be closely related based on shared anatomical features of the larvae and male genitalia. Among the other Old World subgenera, Sirivanakarn (1972) hypothesized a close relationship between *Eumelanomyia*, *Lophoceraomyia*, *Maillotia* and *Neoculex* based on similarities of the adults, and suggested that *Eumelanomyia* is an ancient derivative of the more primitive *Maillotia*. Belkin (1962) and Bram (1968) suggested a relationship between *Acalleomyia*, *Acallyntrum* and *Culiciomyia* based on similarities observed in larvae, and surmised that *Culiciomyia* is the more primitive group. Belkin (1962) indicated that the affinities of the subgenera were obscure, but surmised that *Lutzia* is an ancient derivative that appears to have a strong affinity with subgenus *Culex*.

It is generally assumed that the Melanoconion Group of subgenera, i.e. *Aedinus*, *Anoedioporpa*, *Belkinomyia*, *Carrollia*, *Melanoconion*, *Micraedes*, *Microculex* and *Tinolestes*, includes the most derived mosquitoes

of the genus (Belkin, 1968; Berlin, 1969; Adames & Galindo, 1973; Sirivanakarn, 1983). Sirivanakarn (1983) noted that *Melanoconion* shares several anatomical features with the "more primitive" and widespread subgenus *Neoculex*, suggesting that an offshoot of *Neoculex* may have given rise to the Melanoconion Group. Valencia (1973) suggested that *Carrollia* and the closely related *Deinocerites* and *Galindomyia* share a similar evolutionary history as derivatives from a primitive stock of subgenus *Melanoconion*.

Cladistic analyses tend to support traditional ideas about the relationships between the subgenera of genus *Culex*. The unpublished analysis (Fig. 20) of Mallampalli (1995) based on 67 morphological characters of larvae, pupae and adults showed that the Melanoconion Group appears to be the source of the New World radiation of *Culex* mosquitoes and indicated that many of the traditionally hypothesized relationships were accurate, with *Allimanta*, *Maillotia* and *Culex* appearing to be the most primitive taxa. Additionally, her analysis showed that genus *Culex* formed a paraphyletic clade relative to *Deinocerites* and *Galindomyia*, which were recovered as a sister pair in a derived relationship to all other Culicini, as indicated in the analysis of mosquito genera by Harbach & Kitching (1998).
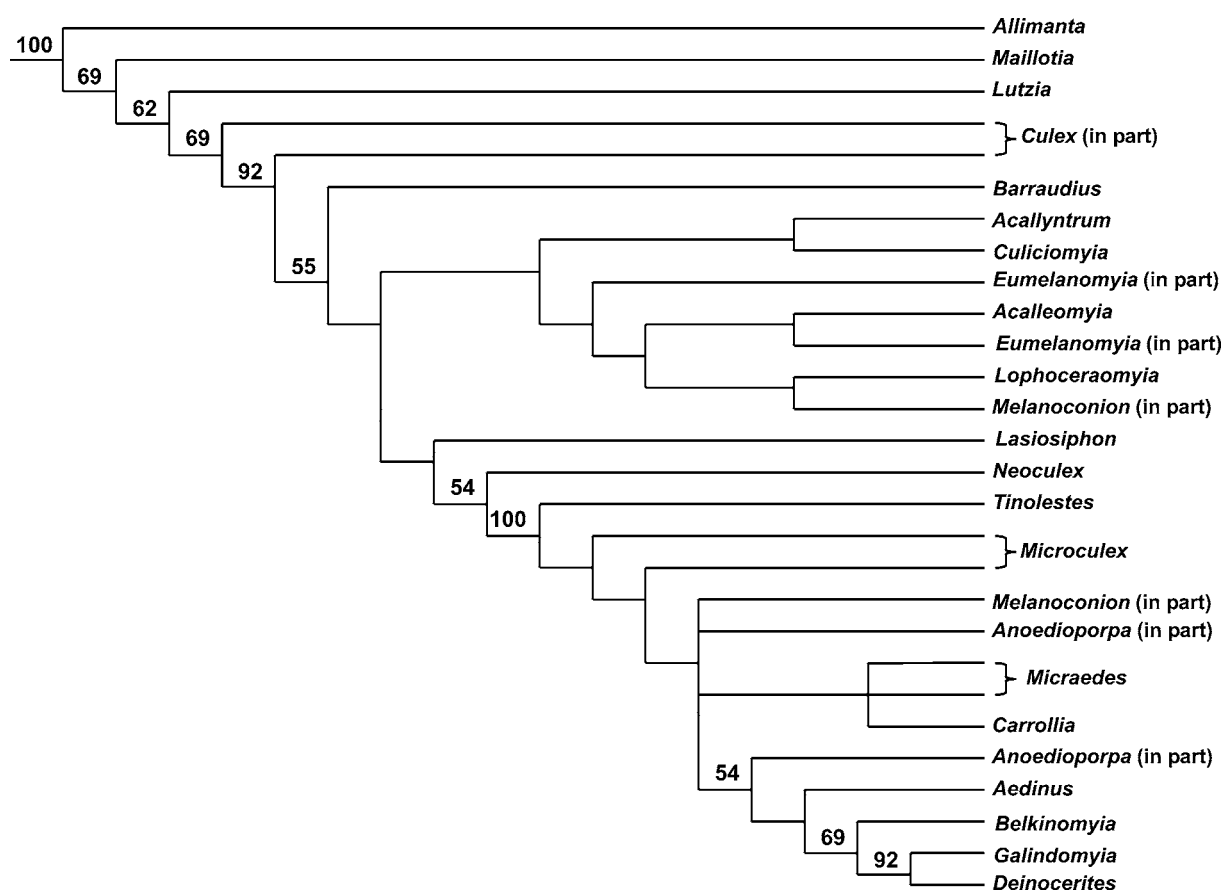

FIGURE 20. Phylogenetic relationships of Culicini based on morphology (modified from Mallampalli, 1995): strict consensus tree of seven MPCs from parsimony analysis of 43 species (40 Culicini) and 67 anatomical characters using SACW. Bootstrap values >50% are shown.

Miller *et al.* (1996) were the first investigators to examine relationships among *Culex* mosquitoes based on molecular data (Fig. 21). These authors used sequence divergence in the ITS1 and ITS2 regions of rDNA to infer relationships between 14 species representing four subgenera of *Culex*. Neighbour-joining analyses produced a tree (rooted on *Melanoconion*) that was consonant with Belkin's (1962) notion that *Melanoconion* bears a number of resemblances with *Neoculex*, and *Lutzia* shares an ancestry with subgenus *Culex*. The authors noted the monophyly of the four subgenera and discussed the evolutionary relationships of species of

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subgenus *Culex* included in the analysis, but did not notice that the Pipiens Group does not appear to be monophyletic. The results of the analysis suggest that the Old World Sitiens Group is more closely related to New World members of the Pipiens Group than to Old World members.
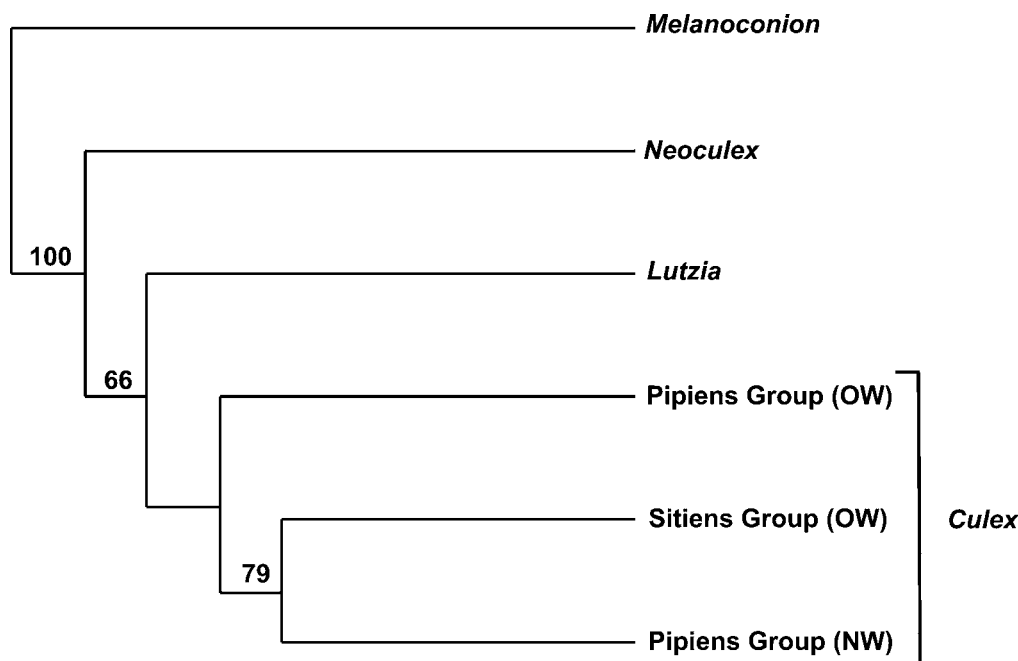

FIGURE 21. Phylogenetic relationship of four subgenera (14 species) of *Culex* based on ITS1 and ITS2 rDNA sequences (modified from Miller *et al.*, 1996): neighbour-joining tree rooted on *Melanoconion*. Bootstrap values >50% are shown.

Navarro & Liria (2000) conducted a parsimony analysis of 30 characters of larval mouthparts (mandibles and maxillae) to infer the phylogenetic relationships of *Deinocerites* and seven subgenera of *Culex*. The phylogeny (Fig. 22) indicated that genera *Lutzia* and *Culex* formed distinct monophyletic clades, with *Lutzia* being the more primitive of the two. Although only New World species were included in the analysis, members of the Melanoconion Group formed a distinct monophyletic clade in a sister-group relationship with subgenus *Culex*. It is interesting to note that this clade included *Deinocerites* in an unresolved polytomy with *Anoedioporpa*, *Melanoconion* and *Microculex* in a derived position relative to *Carrollia*.

A limited molecular study by Juthayothin (2004) using the COI protein coding gene showed poorly resolved differences between 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 data set making this conclusion unreliable.

St John (2007) explored the generic and subgeneric relationships within Culicini using morphological characters of larvae, pupae and adults. Sixty-four characters were scored for 41 exemplar species representing 26 generic-level taxa of Culicini, and three outgroup species of genera *Mansonia*, *Orthopodomyia* and *Maorigoeldia*. Characters from Mallampalli (1995) and Harbach & Kitching (1998) were included along with several novel characters. Characters were chosen to contain maximum phylogenetic information about subgeneric relationships within *Culex*. Characters that were internally polymorphic for subgenera were discarded. Maximum parsimony analysis of the data set under equal weighting yielded eight most parsimonious trees (MPTs). The strict consensus of these MPTs (Fig. 23) indicates that genus *Culex* is not monophyletic because genus *Deinocerites* is embedded within the clade that includes all subgenera of *Culex* (clade A in Fig. 23). *Deinocerites* is placed as the sister group to subgenus *Belkinomyia*, and genus *Lutzia* is placed in an ancestral relationship to genus *Culex*, which is polyphyletic relative to genus *Deinocerites*. Interestingly, the

clade that includes *Deinocerites* (clade B in Fig. 23) includes all subgenera of the New World Melanoconion Group except for *Melanoconion* itself. Most subgenera were recovered as monophyletic. Bremer supports were positive for all nodes, but they were often less than 3. Bootstrap supports for nodes were generally poor with the majority less than 50%. The monophyly of Culicini was supported with a bootstrap proportion of 55% and the majority of monophyletic subgenera represented by the type species and a related species had support values greater than 60%. Many characters in the MP analysis showed a high degree of homoplasy. Implied weighting and SACW search strategies both resulted in a single MPT. The tree topology obtained under SACW differed from the unweighted MPT consensus tree only in the monophyly of subgenus *Lophoceraomyia* and the Pipiens Group of subgenus *Culex*. SACW 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). Hence, implied-weighting analyses were conducted with the constant of concavity set at $K=$ 3, which was favored because it moderately down-weighted homoplastic characters while maintaining clades that were considered to be realistic. The IW tree (Fig. 24) differed in several ways from the unweighted tree. Clade B in the IW tree exclusively contains all New World *Culex,* including subgenus *Melanoconion*, which is placed basal to the other New World subgenera. Clade A shows a substantially different topology to the unweighted tree, with the Asian subgenera such as *Eumelanomyia*, *Culiciomyia* and *Acallyntrum* removed from a monophyletic clade and the loss of monophyly of subgenera *Culiciomyia* and *Neoculex*.
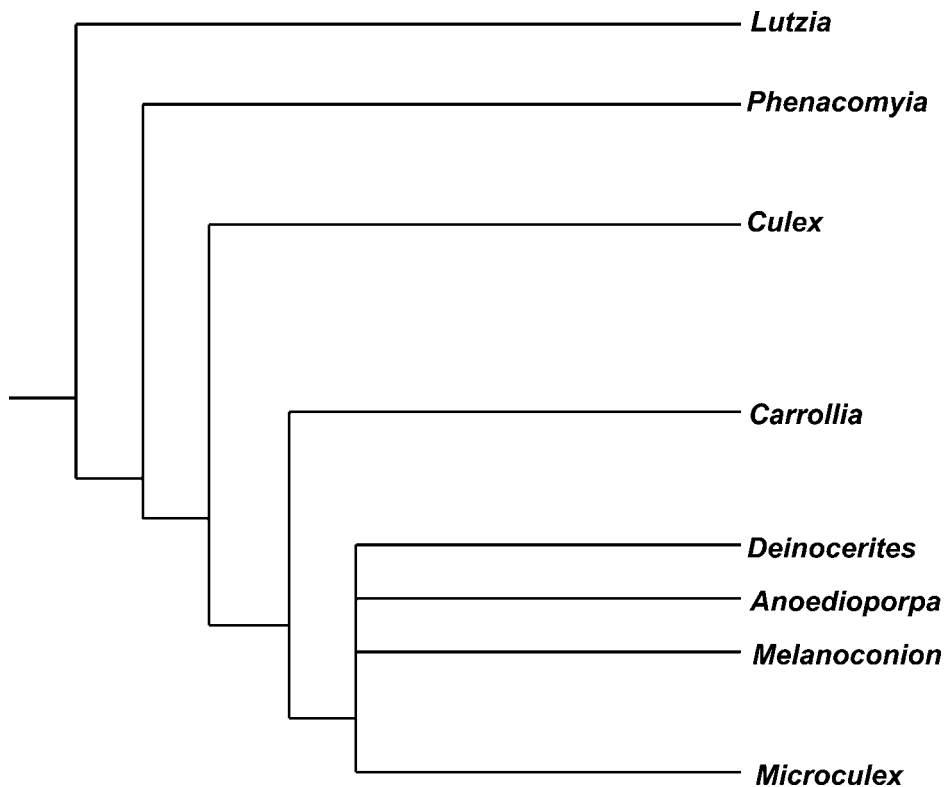
FIGURE 22. Phylogenetic relationships of some Culicini based on larval mouthpart morphology (modified from Navarro & Liria, 2000): strict consensus of two MPCs from parsimony analysis of 18 ingroup species and 30 characters of larval maxillae and mandibles.

Navarro & Liria (2000) formally reduced genus *Deinocerites* to subgeneric status in genus *Culex*, but this action has not been widely accepted because this group of species is diagnosed by many unique (autapomorphic) features in the immature and adult stages. Autapomorphic features are normally excluded from phylogenetic studies because they are uninformative in the context of cladistic analyses. This does not mean, however, that autapomorphies should be disregarded as denotations of taxonomic rank, providing that doing so does not conflict with the principle of recognizing only monophyletic groups in classifications. Autapomorphies are a

measure of (often rapid) divergence and adaptive radiation, which traditional taxonomists have intuitively taken into consideration when defining genera. In this case, an alternative action would be to retain genus *Deinocerites* and afford equivalent rank to the monophyletic subgenera of *Culex*. It should be borne in mind that the majority of taxa (14) currently regarded as subgenera of *Culex* were originally recognized as genera.

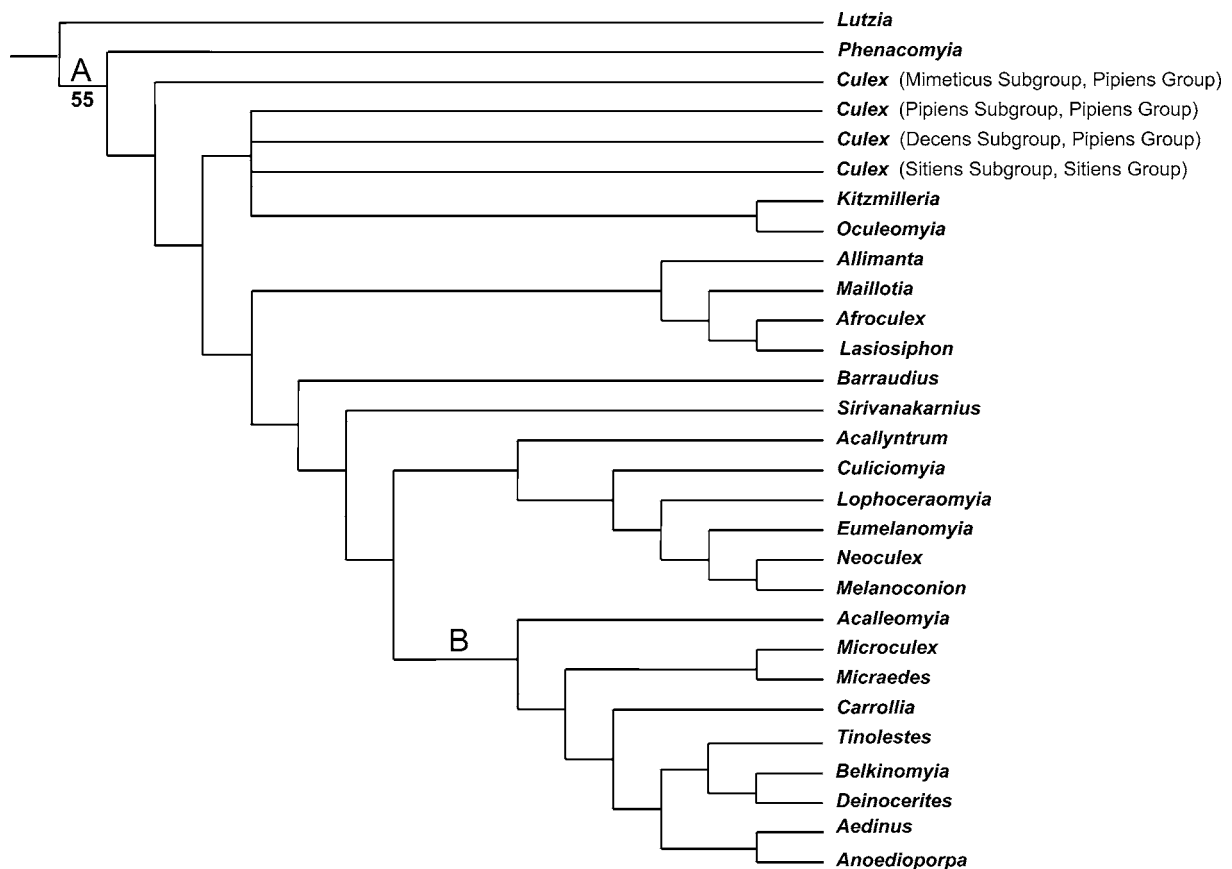**FIGURE 23.** Phylogenetic relationships of Culicini based on morphology (modified from St John, 2007): strict consensus of eight MPTs from maximum parsimony analysis of 43 ingroup species and 64 equally weighted anatomical characters. Clade A indicates that genus *Culex* is polyphyletic relative to genus *Deinocerites*. Clade B includes *Deinocerites* and all New World subgenera of *Culex* except Melanoconion.

Phylogeny and classification of Sabethini

Tribe Sabethini includes 417 species, which comprise 14 genera found mainly in the Neotropical, Oriental and Australasian Regions. Nine genera, including *Isostomyia*, *Johnbelkinia*, *Limatus*, *Onirion*, *Sabethes*, *Shannoniana*, *Runchomyia*, *Trichoprosopon* and *Wyeomyia*, are almost entirely restricted to the Neotropical Region. A few species of *Wyeomyia* occur in eastern North America. Only five genera are recognized in the Old World, principally in the Australasian and Oriental Regions. Members of genus *Malaya* occur in Africa as well as the Oriental and Australasian Regions, *Topomyia* is known from the Oriental Region and New Guinea, *Maorigoeldia* is restricted to New Zealand and *Tripteroides* occurs in the Oriental, Australasian and southern part of the Palaearctic.

Sabethine adults are difficult to characterize. Most species have the base of the hindcoxa in line with or slightly above the base of the mesomeron, the mesomeron is generally distinctly smaller than in non-sabethine mosquitoes, prespiracular setae or scales are virtually always present (absent in one species of *Malaya*; scales replace setae in *Limatus*), and the mesopostnotum usually has a cluster of setae or scales. The position of the

hindcoxa relative to the mesomeron is not constant among sabethines and is not unique to the tribe. It is below the base of the mesomeron in *Maorigoeldia*, some *Malaya* and some *Tripteroides*, and many species of the tribe Aedini, including *Armigeres*, *Udaya*, *Zeugnomyia* and certain *Aedes* and *Heizmannia*, resemble the majority of sabethines in having the base of the hindcoxa in line with the base of the mesomeron. Of the culicine genera, prespiracular setae are found elsewhere only in species of *Culiseta* and *Psorophora*. Mesopostnotal setae are absent in *Malaya*, *Maorigoeldia*, *Topomyia* and some *Tripteroides*, and are present in many species of tribes Aedini and Culicini. The reduction of the larval ventral brush (seta 4-X) to one or rarely two pairs of setae is more or less diagnostic for the tribe (the homology of these setae with the ventral brush of non-sabethines is questionable). The position of seta 3-C on the ventral side of the head and the lateral displacement of the anterior labropalatal setae appear to be diagnostic of sabethine larvae.
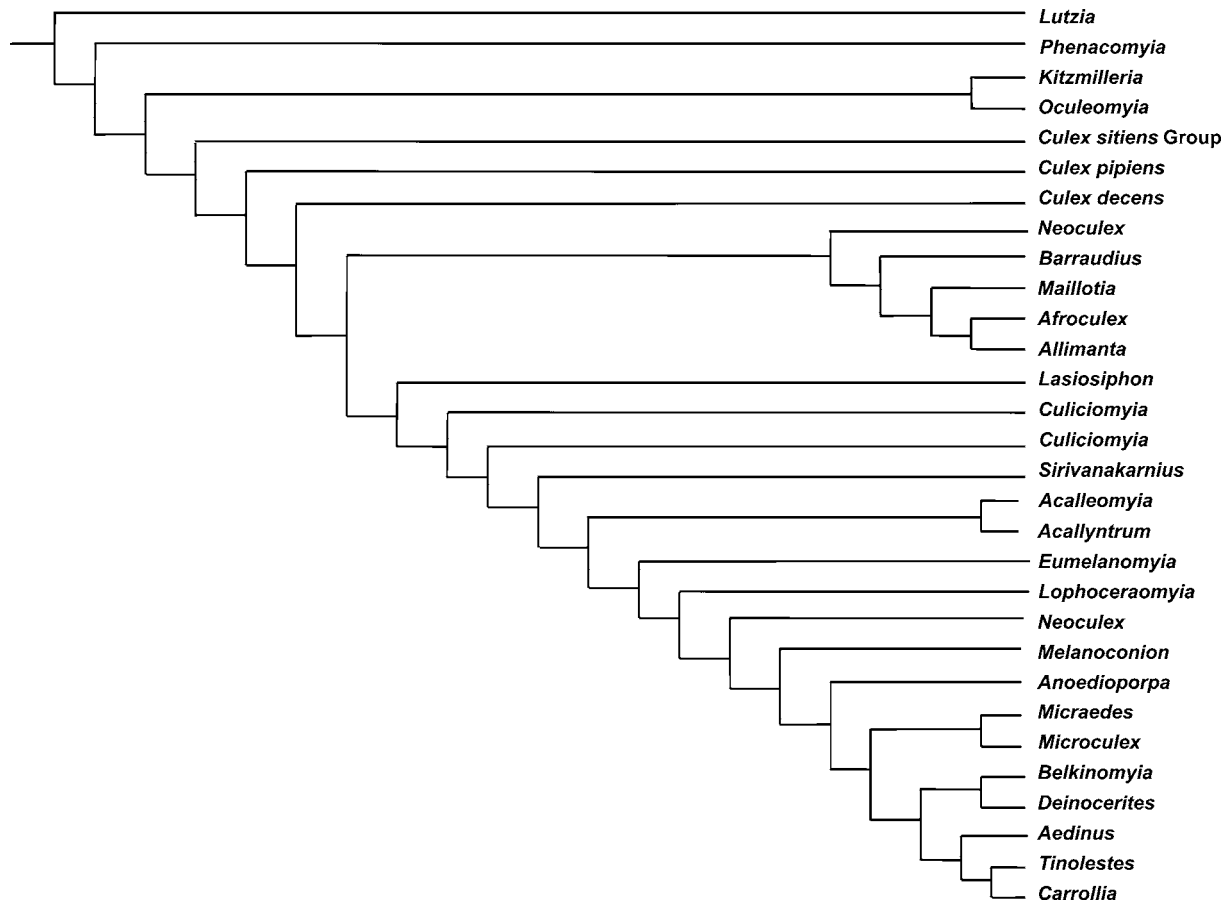

FIGURE 24. Phylogenetic relationships of Culicini based on morphology (modified from St John, 2007): single MPT from implied-weighting analysis (*K*=3) of 43 ingroup species and 64 characters. Compare clades A and B with clades A and B in Fig. 23. Clade B contains *Deinocerites* and all New World subgenera of *Culex*.

Judd (1996) conducted a parsimony analysis of 59 morphological characters of larvae, pupae and adults to examine the phylogenetic relationships of 37 sabethine species representing 13 genera and 19 subgenera that were recognized at the time. Her results (Fig. 25) confirmed the intuitive hypothesis that sabethine mosquitoes comprise a monophyletic group, and provided evidence that genera *Runchomyia*, *Tripteroides* and *Wyeomyia*, because it included *Phoniomyia*, were not monophyletic. The analysis also indicated that the Old World taxa comprise a paraphyletic assemblage relative to the monophyletic New World taxa.

Genus *Maorigoeldia* contains a single species found only in New Zealand. The species bears many peculiar characteristics that led Belkin (1962) to regard it as the most primitive sabethine. This view, however, is contradicted by the results of Judd's analysis which placed *Maorigoeldia* as the sister to the New World gen-

era. The cladistic analysis of mosquito genera conducted by Harbach & Kitching (1998) two years later (Fig. 26) corroborated the results of Judd (1996) in recognizing tribe Sabethini and the New World sabethines as monophyletic groups; however, their results contradicted those of Judd and agreed with the contention of Belkin (1962) by recovering *Maorigoeldia* as the most basal clade of the Sabethini.
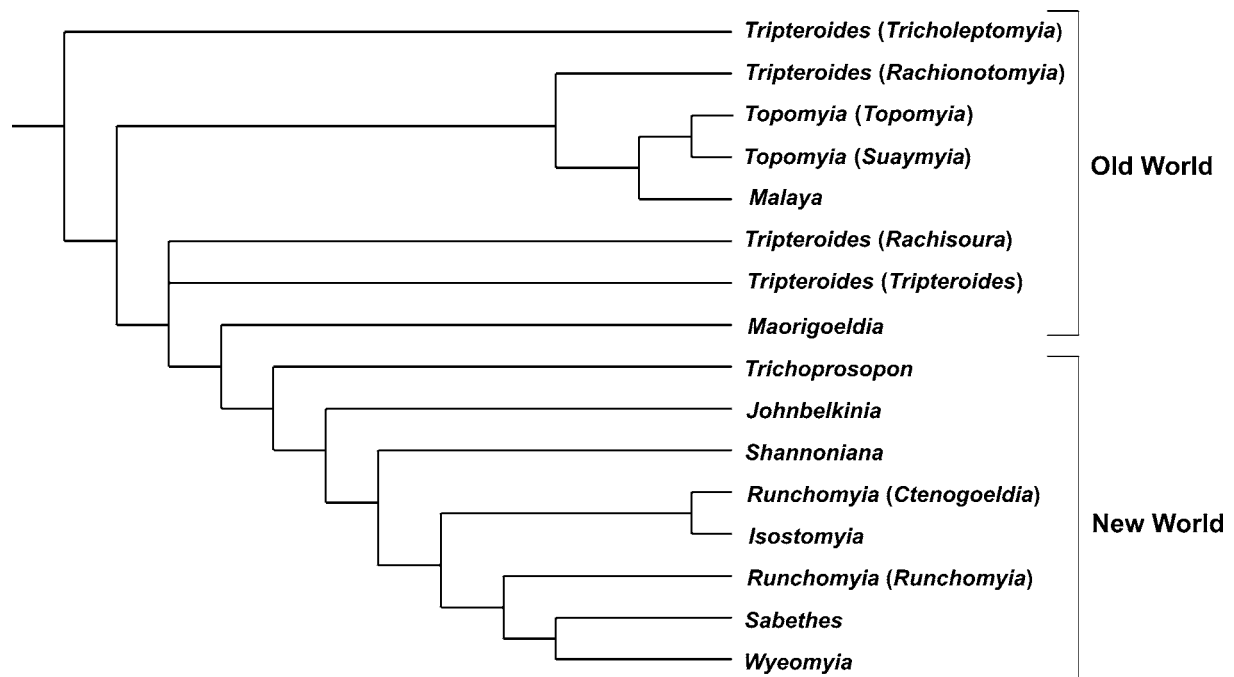
FIGURE 25. Phylogeny of Sabethini based on morphology (modified from Judd, 1996): strict consensus of three MPCs from parsimony analysis of 37 sabethine species and 59 anatomical characters.

Before Judd (1998) relegated genus *Phoniomyia* to subgeneric status within genus *Wyeomyia*, nine genera of sabethine mosquitoes were recognized in the New World. Based on taxonomic history and morphological associations, these genera formed two functional Groups (Fig. 26): the *Trichoprosopon* Group, which included *Isostomyia, Johnbelkinia, Runchomyia, Shannoniana* and *Trichoprosopon*; and the *Sabethes* Group, which included *Phoniomyia, Limatus, Sabethes* and *Wyeomyia*.

FIGURE 26. Phylogeny of Sabethini based on morphology (after Harbach & Kitching, 1998): relationships of 13 sabethine genera in MPC from implied-weighting analysis ($K=1$) of 38 genera and 73 anatomical characters.

Harbach & Peyton (2000) proposed genus *Onirion* for several distinct species previously included in genus *Wyeomyia*. When the new genus was included in the data set of Harbach & Kitching (1998) (Fig. 27), the relationships of the genera that comprise the Trichoprosopon and Sabethes Groups were altered. *Trichopros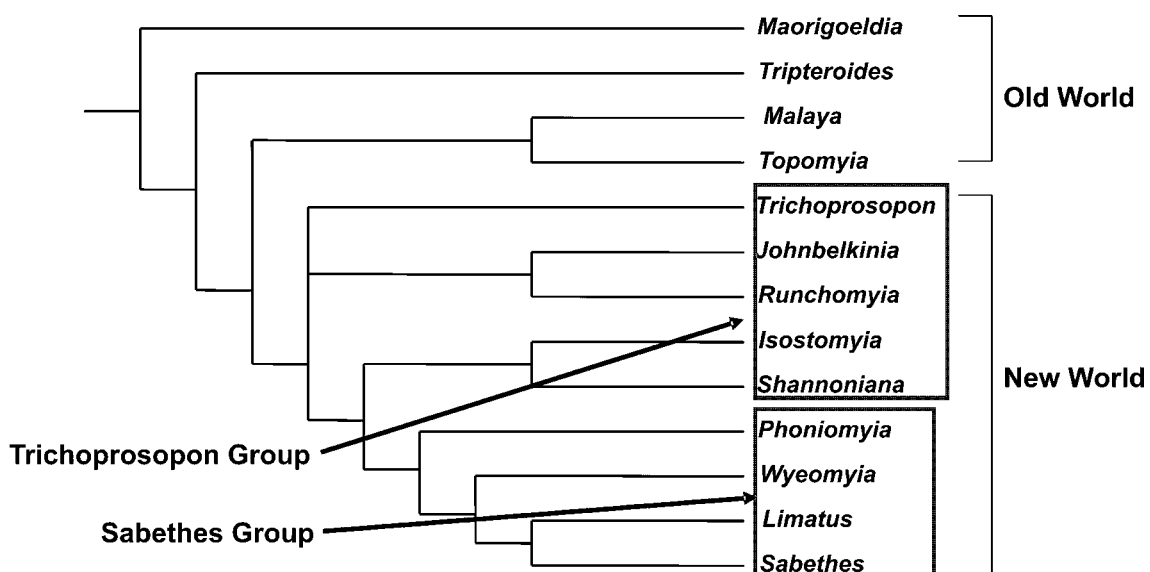opon* was placed as the sister group to *Johnbelkinia* + *Runchomyia*, and *Onirion* was placed in an unresolved relationship with *Isostomyia* + *Shannoniana* and the Sabethes Group. The sister-group relationship of *Trichoprosopon* + (*Johnbelkinia* + *Runchomyia*) and the polytomy including *Onirion* were each only supported by a single synapomorphy, but the characters appeared to provide fairly strong support for the relationships because there was little homoplasy in the distribution of the applicable character states.
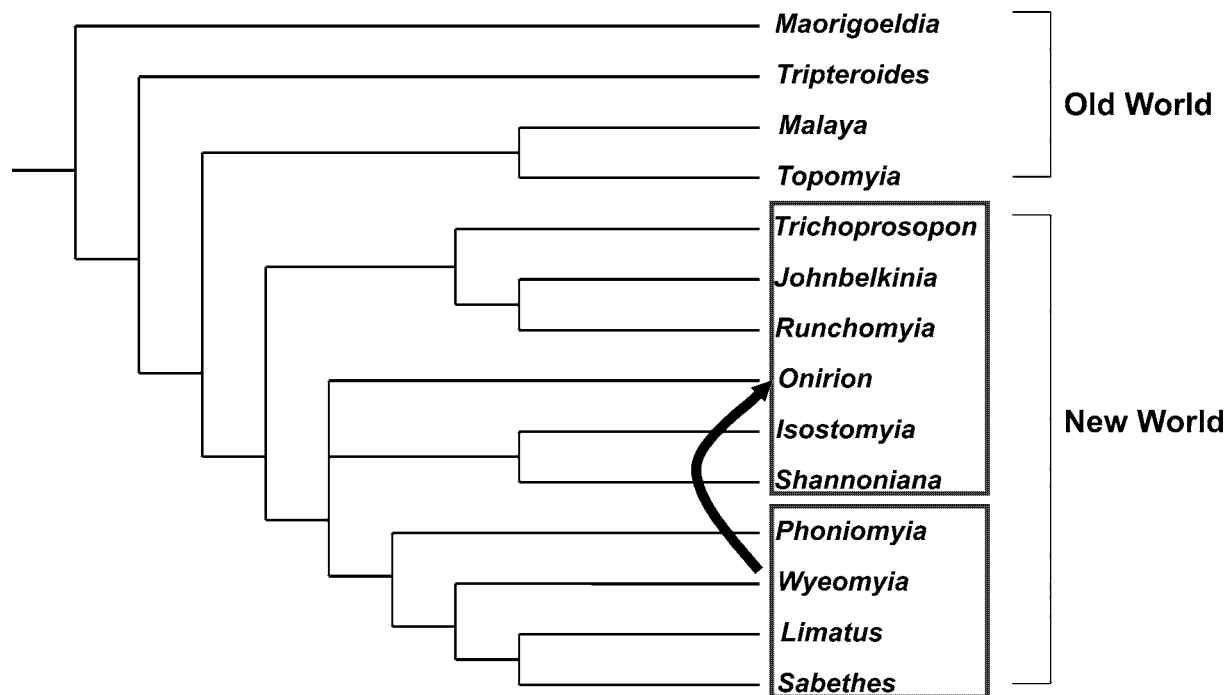

FIGURE 27. Phylogeny of Sabethini based on morphology (after Harbach & Peyton, 2000): relationships of sabethine genera when *Onirion* is included to the data set of Harbach & Kitching (1998) (cf. Fig. 26).

More recently, Harbach *et al.* (2007a) erected genus *Kimia* for several unique species previously included in genus *Topomyia*. When *Kimia* was included in the data set of Harbach & Peyton (2000), the analysis produced a very different pattern of relationships among sabethine genera (Fig. 28). Contrary to the earlier findings of Judd (1996), Harbach & Kitching (1998) and Harbach & Peyton (2000), the New World genera of Sabethini were not recovered as a monophyletic clade in a derived relationship to the Old World genera. The New World genus *Trichoprosopon* was paired with the Old World *Tripteroides* in a sister relationship with *Kimia*, and the Old World *Malaya*, which was sister to *Topomyia* in the previous analyses, was placed as sister to the New World *Limatus*. Although only weakly supported, the sister-group relationship between *Kimia* + (*Trichoprosopon* + *Tripteroides*) was based on a single unique character (absence of larval seta 8-M) that was not contradicted. Furthermore, a number of morphological characters not included in the analysis provided additional evidence for a relationship with *Trichoprosopon*. These findings open up questions about the phylogeography of tribe Sabethini.

When the terminal taxa represented by species in the analysis of Judd (1996) are replaced, where possible, with currently recognized generic and subgeneric entities, it is obvious that genus *Wyeomyia* is a polyphyletic assemblage (Fig. 29). Because *Phoniomyia* had generic status at the time, Judd (1996) stated that "As currently structured the genus [*Wyeomyia*] is paraphyletic and *Limatus* and *Phoniomyia* are firmly embedded within the assemblage of *Wyeomyia* taxa". Disregarding genus *Onirion*, which was not recognized at the time,

Judd's statement is obviously incorrect as *Wyeomyia* would only be paraphyletic (relative to *Phoniomyia*) in the absence of *Limatus*. In view of the topology of relationships portrayed in Fig. 29, it is surprising that Judd (1998) elected to exclude *Limatus* from the analyses which justified her decision to reduce *Phoniomyia* to subgeneric status within *Wyeomyia*. Based on the results of Judd (1996), it would be equally justifiable to reduce *Limatus* to a subgenus in *Wyeomyia*, an action which is unlikely to be acceptable. The adults of *Limatus* are unique in having a single claw on the hindleg, and differ from all other mosquitoes of tribe Sabethini in having scales on the prespiracular area instead of setae. They generally resemble *Sabethes* in overall ornamentation, but the scutum is distinctive in bearing a striking pattern of gold, blue and violet scales. The short and broad caudolateral slits of the occipital foramen and the absence of an apical tooth on the maxilla distinguish the larvae of *Limatus* from those of *Sabethes* and the majority of species currently assigned to genus *Wyeomyia*. As in the case of *Deinocerites*, a reasonable alternative to recognizing *Phoniomyia* as a subgenus would be to afford generic status to monophyletic taxa currently regarded as Species Groups and subgenera of genus *Wyeomyia*. In parallel with *Culex*, the majority of taxa currently recognized as subgenera of *Wyeomyia* were originally established as genera (see Fig. 29).
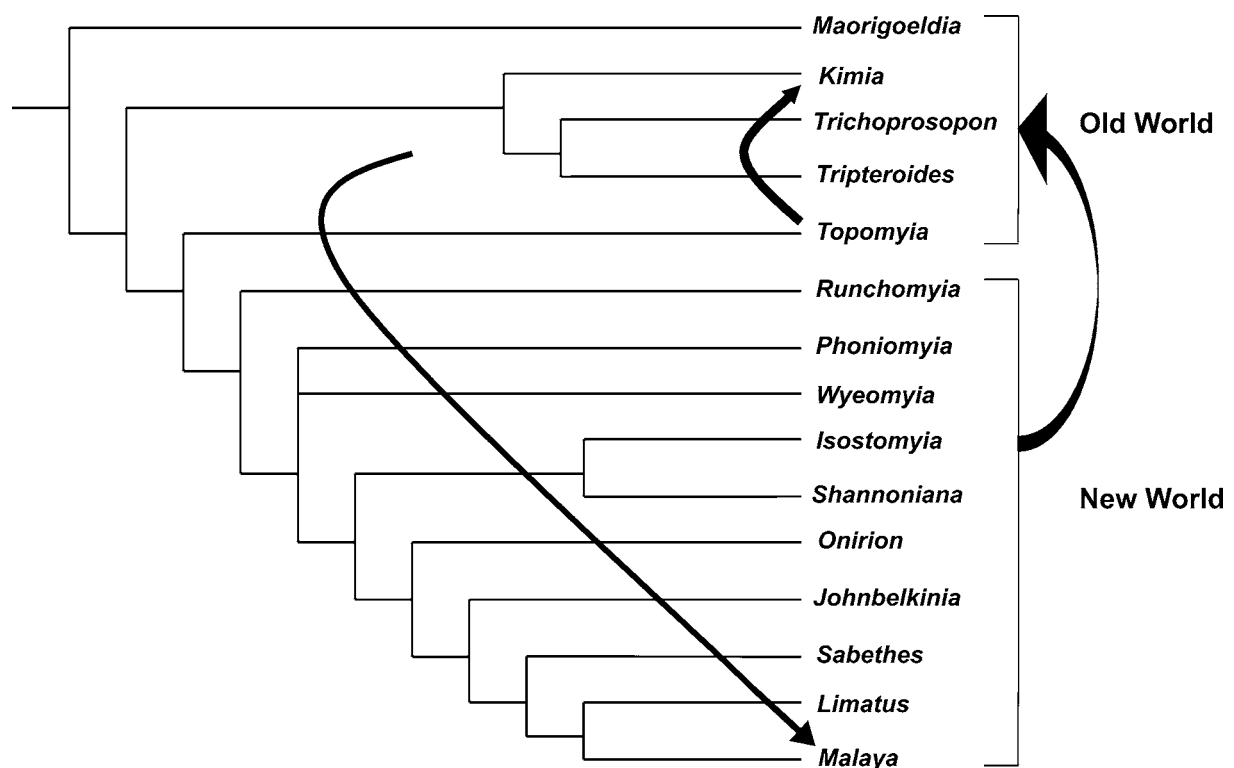
FIGURE 28. Phylogeny of Sabethini based on morphology (after Harbach *et al.*, 2007a): relationships of sabethine genera when *Kimia* is included to the data set of Harbach & Peyton (2000) (cf. Fig. 27).

While the present review was in press, a cladistic analysis of genus *Wyeomyia* based on morphological and allozyme data (Motta *et al.*, 2007) was published that provides further evidence that this taxon is not a monophyletic lineage because it includes genus *Onirion*. Contrary to the findings of Judd (1996), however, the results of the study show that *Limatus* and *Phoniomyia* are separate monophyletic lineages outside of *Wyeomyia*. But as the authors pointed out, it was not possible to test the sister-group relationship of subgenus *Hystatomyia* and *Phoniomyia* (Fig. 29) that led Judd (1998) to formally reduce the latter to subgeneric status in *Wyeomyia*. Consequently, the placement of *Phoniomyia* in a sister relationship to *Wyeomyia* (including *Onirion*) may be due to the exclusion of *Hystatomyia* from the data set. Likewise, although Motta *et al.* (2007) included allozyme data for *Onirion* [*Onirion personatum* (Lutz)], the placement of this taxon within *Wyeo-*

myia may have been influenced by the exclusion of the many morphological characters that distinguish the adults, larvae and pupae of *Onirion* from those of *Wyeomyia*.

Wy. clasoleuca / phroso*
Wy. (**Decamyia**)
Wy. occulta*
Wy. (**Dendromyia**)
Wy. melanocephala*
Onirion
Wy. (**Wyeomyia**) in part
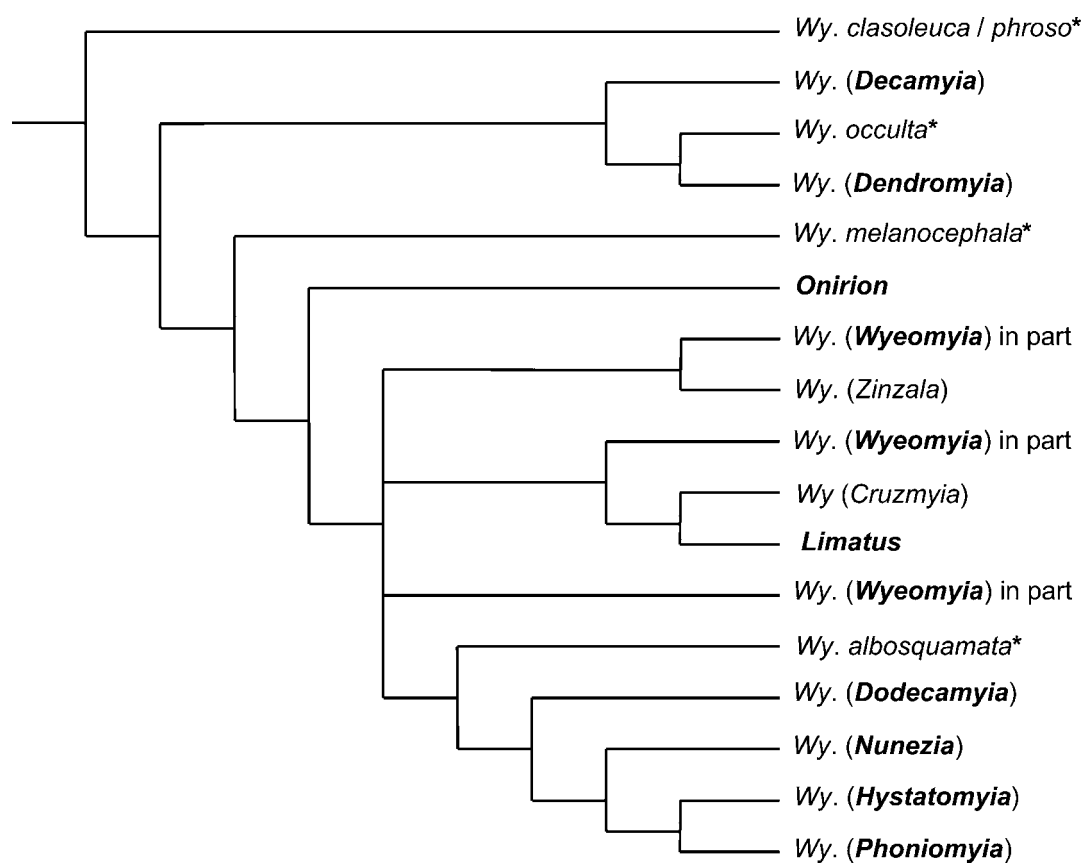*Wy.* (*Zinzala*)
Wy. (**Wyeomyia**) in part
Wy (*Cruzmyia*)
Limatus
Wy. (**Wyeomyia**) in part
Wy. albosquamata*
Wy. (**Dodecamyia**)
Wy. (**Nunezia**)
Wy. (**Hystatomyia**)
Wy. (**Phoniomyia**)

FIGURE 29. Phylogeny of *Wyeomyia* and related taxa based on morphology (modified from Judd, 1996): portion of strict consensus tree from parsimony analysis of 37 sabethine species and 59 anatomical characters (see Fig. 28). Currently recognized generic and subgeneric affiliations are used; species marked with an asterisk (*) are currently without subgeneric placement. Taxa in boldface were originally described as genera. Judd (1998) reduced *Phoniomyia* to subgeneric status in *Wyeomyia*.

What is known about mosquito phylogeny and classification?

Mosquitoes, because of their medical importance, are one of the most thoroughly studied groups of insects. Ironically, despite the significant amount of morphological and molecular work that has been done, little progress has been made toward an understanding of the evolutionary history of the family. As the synthesis of available information clearly shows, the absence of a comprehensive phylogeny for mosquitoes is a major obstacle to realizing a robust, stable classification of the family. At this point in time, what is known about the phylogeny of mosquitoes is succinctly summarized in the following statements:

- Family Culicidae is monophyletic but deeper relationships are largely unresolved.
- Subfamily Anophelinae is a monophyletic lineage basal to all other Culicidae.
- Genus *Chagasia* is a monophyletic lineage basal to other Anophelinae.
- Genus *Anopheles* is not demonstrably monophyletic with regard to genus *Bironella*.
- Subgenera *Kerteszia*, *Nyssorhynchus* and *Cellia* are monophyletic, and *Kerteszia* and *Nyssorhynchus* are sister taxa.
- Subfamily Culicinae is not demonstrably monophyletic in relation to genus *Toxorhynchites*.

- Tribes Aedini, Culicini and Sabethini are monophyletic.
- Genera *Aedes* and *Ochlerotatus* as traditionally defined are polyphyletic.
- Genus *Culex* is not demonstrably monophyletic with regard to *Deinocerites*.
- Genus *Wyeomyia* is polyphyletic relative to *Onirion* and ?*Phoniomyia*.
- The monophyly of the other tribes, genera, subgenera and Species Groups of subfamily Culicinae has not been tested, and their phyletic relationships are uncertain.

Concluding statement

The application of explicit methods of phylogenetic analysis is revealing weaknesses in the traditional classification of mosquitoes, but there is strength in intuitive interpretation because the explicit methodology often confirms the monophyly of mosquito taxonomic groups that are diagnosed by unique combinations of characters. The principal problem, then, is not in recognizing monophyletic groups, but in deciding which taxonomic ranks should be assigned to such taxa once their phylogenetic relationships have been established. Just as philosophical differences about generic boundaries contributed to the instability of mosquito classification, the ranking of natural groups is likely to be a contentious issue among mosquito workers. However, it must be borne in mind that acceptance of broad genus-group concepts for convenience is phylogenetically insupportable, and in cases where a taxon is found to be paraphyletic or polyphyletic, it is generally justifiable to reclassify the group to ensure that taxonomic ranking reflects monophyly. On that basis, current data suggest that many of the subgenera and Species Groups of genus *Anopheles* and tribes Aedini, Culicini and Sabethini should be raised to generic level. It is noteworthy that most subgenera of these taxa were originally described as genera.

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