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Phylogeny of fourteen *Culex* mosquito species, including the *Culex pipiens* complex, inferred from the internal transcribed spacers of ribosomal DNA

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Abstract

Ribosomal DNA sequence divergence in the internal transcribed spacer regions (ITS-1 and ITS-2) was examined for fourteen species and four subgenera (sixty-two clones) in the mosquito genus *Culex* (Diptera: Culicidae). A neighbour-joining tree produced with Kimura 2-parameter distances showed that each of the four subgenera was monophyletic at confidence probabilities of 70–99%. *Culex (Lutzia)* formed the sister group of *Cx. (Culex)*. Two major clades, a *Cx. pipiens* complex–*Cx. torrentium* assemblage and a *Cx. restuans*–*Cx. salinarius*–*Cx. erythrothorax* assemblage, formed monophyletic groups. *Cx. torrentium* was closely related to members of the *Cx. pipiens* complex. Phylogenetic analysis of ITS-1 and ITS-2 sequences from members of the *Cx. pipiens* complex separated populations from northern latitudes and southern latitudes, but did not support the traditional taxa as monophyletic units.

Keywords: *Culex*, *Culex pipiens* complex, rDNA.

Introduction

Although mosquitoes (Diptera: Culicidae) are one of the best-studied insect families, comprehensive, evolutionary-based phylogenies are lacking. The preponderance of mosquito research is applied, and has centred on the identification, biology, and control of species that transmit pathogens to humans and to domestic animals. *Culex*, with over 560 species described, is one of the largest genera in the Culicidae and, in a taxonomic sense, one of the least known (Edwards, 1932, 1941; Belkin, 1962). Members of this extensive genus are important vectors of pathogens, notably human filariasis and many arthropod-borne viruses (arboviruses) including Japanese encephalitis, Murray Valley encephalitis and St Louis encephalitis viruses (W.H.O., 1992; Karabatsos, 1985). Identification of field-collected adult specimens for study and pathogen isolation is often difficult or impossible because adult specimens frequently lack salient characters of scale ornamentation, and the females of certain species are remarkably similar.

The well-studied *Cx. pipiens* complex is an assemblage of closely related taxa with a worldwide distribution. The complex demonstrates an array of behavioural, morphological and physiological characters that vary clinally from temperate to tropical regions. The difficult systematics of this group are reflected in the thirteen different names that have been applied to *Cx. pipiens* complex members (Barr, 1975). There are two major taxa in the complex, *Cx. pipiens* and *Cx. quinquefasciatus*. *Cx. pipiens* occupies temperate regions and has a Holoarctic distribution, whereas *Cx. quinquefasciatus* is found in subtropical and tropical areas as well as in temperate regions during the summer months. Because *Cx. quinquefasciatus* is unable to diapause, it is eliminated from temperate regions during the winter. Where their ranges overlap (36–39°N latitude in the USA), hybrid or intermediate populations are found (Barr, 1957). The taxon *Cx. pipiens pallens* from Japan is thought by some workers to be of hybrid origin (Barr, 1975); however, Japanese workers treat *pallens* as a subspecies of *Cx. pipiens* (Tanaka *et al.*, 1979). Other features that differ between these taxa include autogeny (producing eggs without a bloodmeal), host preferences, and mating behaviour (Barr, 1982). The only reliable morphological character for discriminating *Cx. pipiens* from *Cx. quinquefasciatus* is based on quantitative differences in phallosome structure in the male genitalia expressed as the DV/D ratio (see

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