

## Invited Review

# Distribution and evolution of the *Anopheles punctulatus* group (Diptera: Culicidae) in Australia and Papua New Guinea

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## Abstract

The members of the *Anopheles punctulatus* group are major vectors of malaria and Bancroftian filariasis in the southwest Pacific region. The group is comprised of 12 cryptic species that require DNA-based tools for species identification. From 1984 to 1998 surveys were carried out in northern Australia, Papua New Guinea and on islands in the southwest Pacific to determine the distribution of the *A. punctulatus* group. The results of these surveys have now been completed and have generated distribution data from more than 1500 localities through this region. Within this region several climatic and geographical barriers were identified that restricted species distribution and gene flow between geographic populations. This information was further assessed in light of a molecular phylogeny derived from the *ssrDNA* (18S). Subsequently, hypotheses have been generated on the evolution and distribution of the group so that future field and laboratory studies may be approached more systematically. This study suggested that the ability for widespread dispersal was found to have appeared independently in species that show niche-specific habitat preference (*Anopheles farauti* s.s. and *A. punctulatus*) and conversely in species that showed diversity in their larval habitat (*Anopheles farauti* 2). Adaptation to the monsoonal climate of northern Australia and southwest Papua New Guinea was found to have appeared independently in *A. farauti* s.s., *A. farauti* 2 and *Anopheles farauti* 3. Shared or synapomorphic characters were identified as saltwater tolerance (*A. farauti* s.s. and *Anopheles farauti* 7) and elevational affinities above 1500 m (*Anopheles farauti* 5, *Anopheles farauti* 6 and *A. farauti* 2). © 2002 Australian Society for Parasitology Inc. Published by Elsevier Science Ltd. All rights reserved.

**Keywords:** Malaria vector; Sibling species; Biogeography

## 1. Introduction

Anophelines have been intensively studied throughout the world due to their role as vectors of malaria, a disease that presents one of the most serious health problems for countries in the developing world. Studies of these vectors have identified sibling or cryptic species in many of the most important malaria vector taxa (Baimai et al., 1984). These cryptic species defy identification using traditional morphological characters (Harbach, 1994). While recognition of these species has resolved certain unexplained issues regarding malaria transmission (Paterson, 1963), it has also created the problem of how to accurately identify the large numbers of field collected specimens required for epidemiological studies and for evaluating control methods.

Recent advances in DNA-based technology have aided fieldworkers by providing reasonably simple and accurate

identification techniques (Beebe and Cooper, 2000). These techniques have utilised species-specific sequences. Such sequences are also amenable to phylogenetic analysis which may shed light on the nature of evolutionary forces effecting the mosquito populations or species and may possibly assist in understanding the phylogenetic relationships among closely related mosquitoes as well as the evolutionary dynamics of disease transmission. Such information coupled with comprehensive distribution records may also allow analysis of the biogeography of these species.

In the Australian region, the islands of New Guinea, the Bismarck Archipelago, the Solomons and Vanuatu are highly malarious. The disease was also present in northern Australia at least until 1962 when the last outbreak was recorded. Although now malaria free, several hundred cases come into Australia each year and, as competent vectors still occur here, there is always concern that further outbreaks of the disease will occur.

In New Guinea several anopheline species have been incriminated as vectors of malaria and Bancroftian filariasis

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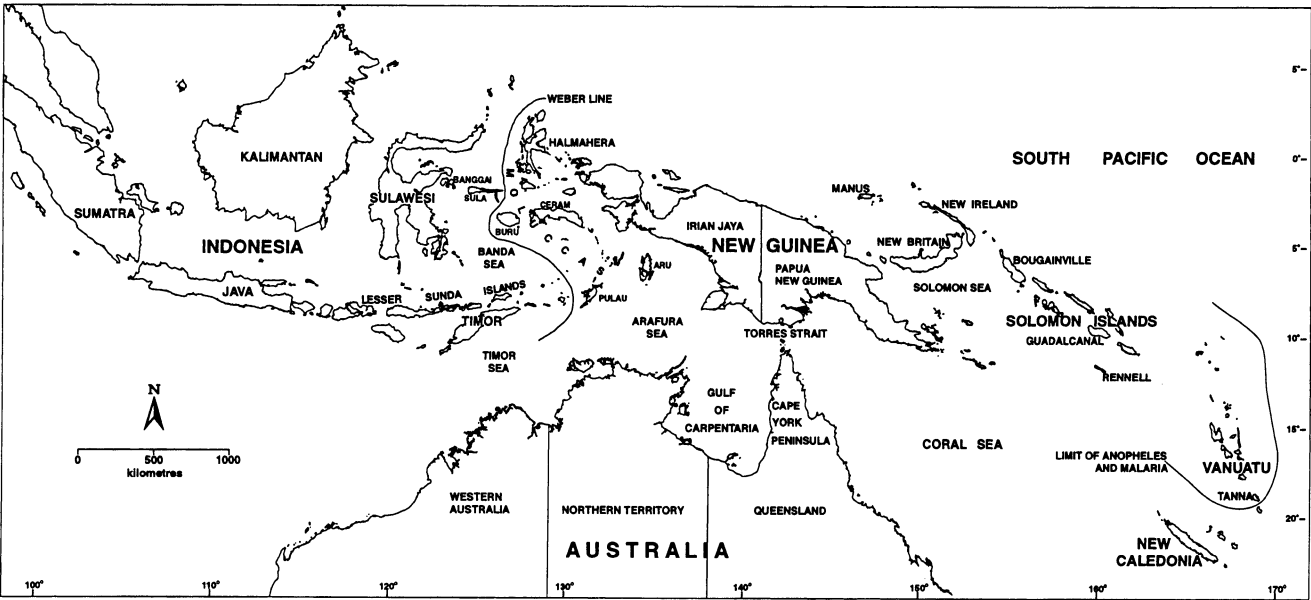


Fig. 1. Map of the Australasian region. The *Anopheles punctulatus* group exists from the Moluccas in the west (approximately at the Weber line) to Vanuatu in the east and south into northern Australia.

(Hii et al., 2000; Bryan, 1986). However, the major vectors belong to the *A. punctulatus* group (Burkot et al., 1988b; Bockarie et al., 1996). The group originally consisted of *Anopheles farauti*, *Anopheles koliensis* and *Anopheles punctulatus*. These could be identified by proboscis morphology with *A. farauti* having an all black scaled labium, *A. koliensis* a ventral patch of white-scales on the apical half of the labium and *A. punctulatus* the apical half of the labium almost entirely white scaled (Rozeboom and Knight, 1946). This group is now known to consist of 12 closely related cryptic species that are either isomorphic or polymorphic for previously used morphological characters (Foley et al., 1993; Cooper et al., 2002).

The members of the group are believed to occur from the Moluccas, east through New Guinea, the Bismarck Archipelago, and the Solomon Islands to Vanuatu and southward into northern Australia (Fig. 1). However, only in northern Australia and Papua New Guinea is the distribution of the various members of the group well understood. In this area, surveys conducted over the last 15 years have collected mosquitoes from over 1500 sites producing a comprehensive set of distribution records as well as information on the ecology and biology of these species (Sweeney et al., 1990; Cooper et al., 1995, 1996, 1997, 2002). This distribution data has been summarised in Table 1, with biogeographic regions identified in northern Australia and Papua New

Table 1  
Geographic distribution summary for members of the *Anopheles punctulatus* group<sup>a</sup>

Species	(1) Northern Australia, monsoonal		(2) South-western PNG, monsoonal		(3) Southern PNG, southern plains, hot/wet		(4) Highland regions PNG (>1500 m), mild/wet	(5) Northern PNG Sepik/Ramu plains, hot/wet		(6) Guadalcanal Island, hot/wet	
	Coastal	Inland	Coastal	Inland	Coastal	Inland		Coastal	Inland	Coastal	Inland
<i>A. farauti</i> s.s.	+++		+++		+++	+	+	+++		+++	
<i>A. farauti</i> 7										+++	+++
<i>A. farauti</i> 2	++	++	++	+++	+++	+++	++	+	++	+++	+++
<i>A. farauti</i> 6							++				
<i>A. farauti</i> 5							+				
<i>A. farauti</i> 3	++	+++		++							
<i>A. koliensis</i>					++	++	+	+++	++	+ ?	+ ?
<i>A. farauti</i> 4								++	++		
<i>A. punctulatus</i>						+++	+	++	+++	+	++
<i>A. sp. nr punctulatus</i>						++			++		
<i>A. clowi</i> .									+		

<sup>a</sup> PNG, Papua New Guinea; +++, Extensive distribution; ++, limited distribution; +, sparse distribution; +?, doubtful distribution.

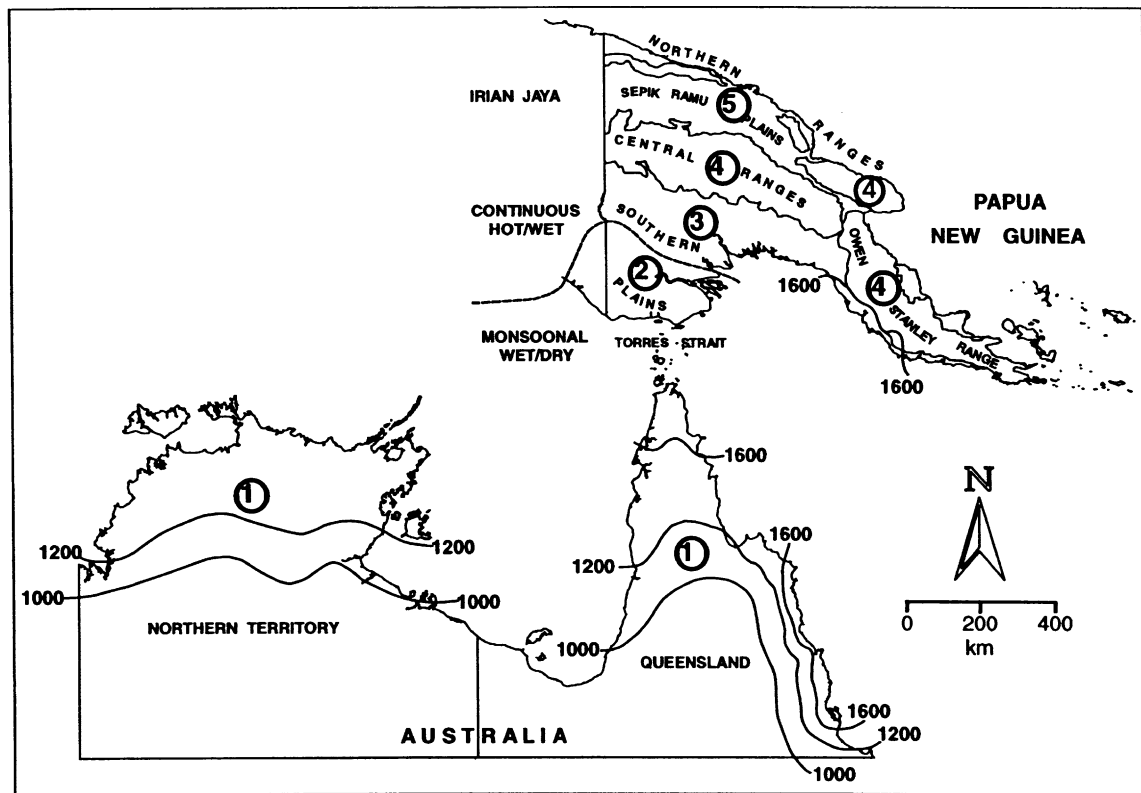


Fig. 2. Map of northern Australia and Papua New Guinea showing major geographic and climatic barriers. Numbers indicate the different biogeographic regions in Table 1: region 1 is the monsoonal open savannah region of northern Australia, region 2 is the open savannah plains of the Western Province of Papua New Guinea, region 3 is the continually wet lowland and foothill regions south of the central ranges in Papua New Guinea, regions 4 are the highland mountain and valley regions above 1500 m in Papua New Guinea, region 5 is the lowland river valleys and floodplains of the Sepik and Ramu rivers and region 6 is the Guadalcanal Island in the Solomon Islands (~1000 km east of Papua New Guinea). Yearly rainfall isohyets are shown as mm per annum.

Guinea corresponding to regions in Fig. 2. These regions were identified by natural barriers: a sea gap (Torres Strait) separates regions 1 and 2, climate disjunction (monsoonal/continual wet) separates regions 2 and 3, elevation separates region 4, while the central ranges are a barrier between regions 3 and 5. Collections have also been made from Manus Island (10 sites), Buka and Bougainville islands (81 sites) and Guadalcanal in the Solomon Islands (55 sites) (Beebe et al., 2000a; Cooper and Frances, 2002). As there are no reliable morphological markers to separate the members of this group, large-scale mosquito surveys were only possible after the development of a number of molecular-based techniques that allowed the identification of the species (Cooper et al., 1991; Beebe et al., 1994, 1996; Beebe and Saul, 1995).

Alongside species identification, regions of the rDNA gene family have been used for molecular phylogenetic studies on the group and many specimens collected in these studies have now been subjected to intraspecific molecular characterisation. This review will investigate these phylogenetic relationships in regards to the distribution, dispersal and biology of the *A. punctulatus* group in northern Australia and Papua New Guinea. Subsequently we will develop hypotheses about the speciation and relationships

of members in this group through which further field and laboratory studies can be more systematically approached.

## 2. Biogeography of the southwest Pacific region

### 2.1. History of the region

Within the Australian region the island of New Guinea is believed to be a major evolutionary centre of insect fauna originating from the Orient (Gressitt, 1961). New Guinea is a composite land mass; the area south of the central ranges is part of the Gondwanan Australian craton, while the northern half is made up of rifted and drifted Gondwanan microcontinents and volcanic island arcs produced at the Australian and Pacific plate interface (Pigram and Davis, 1987; Michaux, 1989, 1994). The accretion of these terranes took place during the Oligocene and Miocene (30–5 million years ago (mya)) (Michaux, 1994). By the end of the Miocene, about 5 mya, the present land mass of New Guinea had been created, though much of the lowlands were submerged (Dow, 1977), not fully emerging until some time in the late Pliocene (3.4–1.6 mya). The uplifting forces generated by the collision of the Australian and Pacific

plates led to the formation of the central ranges, a process which continued until the Holocene ( $<0.01$  mya) (Kikkawa et al., 1981).

The possible dispersal of culicid fauna east into the Australian region may have occurred during the early Pliocene (5–3.4 mya) when the Indo-Malayan Archipelago was in place and an easterly spread of Oriental fauna possible. However, at this time such movement would not have been easy, the archipelago acting as a filter bridge for dispersing fauna. A more likely time for this event is the Pleistocene (1.6–0.01 mya), during the great glaciation periods when sea levels were 120–180 m below their current levels. During these periods of glaciations, which could last for several thousand years, vast areas of the Sunda shelf (joining the Malaysian and Indonesian archipelagos) and Sahul shelf (joining Australia and New Guinea) were exposed facilitating the movement of fauna down the Indo-Malayan Archipelago (Kikkawa et al., 1981). As part of the Sahul shelf a continuous land mass existed between New Guinea and northern Australia. The present coastlines of these two land masses was formed approximately 15 000–8000 years ago with the sinking of the Gulf of Carpentaria and the formation of the Arafura Sea due to flooding caused by sea level rises associated with the thawing of the last glaciation period (Nix and Kalma, 1972). Land bridging the Torres Strait, which had been joined to New Guinea during the Pleistocene, was last flooded 8000–6000 years ago (Kikkawa et al., 1981).

In the geological events of the Miocene and Pliocene, with the formation of the Indo-Malayan Archipelago, the islands of the southwest Pacific, and the glaciations of the Pleistocene, with its climate and sea level changes, are believed to be responsible for considerable speciation events in the region (Belkin, 1962; Cranston and Naumann, 1991). It is possible that the ancestors of the *A. punctulatus* group dispersed from New Guinea, colonising any areas suitable for their survival, with the limits of this dispersal governed by geological barriers and unfavourable habitat or climatic conditions. This dispersal was accompanied by speciation caused by either dispersal or vicariance events that occurred in the region during the late Pliocene and Pleistocene epochs.

The anopheline fauna of the region is comprised of about 49 species, representing the two subgenera: *Anopheles* and *Cellia*. The fauna is highly endemic; the larger land masses of Australia and particularly New Guinea have acted as centres of speciation and dispersal with the fauna decreasing eastwards throughout the smaller island groups of the southwest Pacific. Westwards there has been very little penetration into the Indo-Malayan Archipelago. The age of the *A. punctulatus* group and its ancestors is not known. However the group appears to be monophyletic and at some point in time the ancestors of the group appeared in the Australian region, either as part of the original endemic Australian Gondwanan fauna or by easterly dispersal down the Indo-Malayan Archipelago. Much of the culicid fauna of the

region – or at least of the Papuan subregion – appears to be of Oriental origin (Iyengar, 1960). However, Belkin (1962) believed that the composition of the mosquito fauna of the South Pacific cannot be explained simply by easterly invasion from the Indo-Malayan subregion and that some elements must have a long history in the region to account for their dispersal over vast ocean barriers. The members of the *A. punctulatus* group belong to the Neomyzomyia Series of the *Cellia* subgenus which is predominantly Afro-tropical/Oriental in origin (Lee et al., 1987; Harbach, 1994), so invasion from the west seems a likely scenario. However, this will only be confirmed after further molecular-based comparative studies involving Oriental species from the Indo-Malayan Archipelago.

### 3. Dispersal and biogeographic barriers in the southwest Pacific

Concepts and mechanisms associated with mosquito dispersal have been reviewed by Service (1993, 1997) and, with regards to island dispersal in the South Pacific, by Belkin (1962). Natural long distance dispersal is believed to be primarily by wind action, though rafting and carriage by birds and animals have also been considered. Human movement has no doubt aided long distance dispersal, with sea, air and rail transportation responsible for the spread of a number of now cosmopolitan mosquito species over the last 200–300 years. Long-distance travel is extremely precarious for mosquito species: they have no choice in their destination and their chance of survival when they arrive is a major issue. Belkin (1962) lists a number of attributes that may aid the long distance dispersal of mosquito species across ocean gaps, suggesting that salt-water tolerance or non-specialisation with regards to breeding sites and adult host preference would be advantageous.

Short-distance dispersal is the most likely method by which a population of mosquitoes will spread throughout a region that can support their survival. Active, goal-orientated flight occurs for the following reasons: to seek a mate, to feed (on blood or nectar), to rest (for egg development) and to locate a suitable breeding site (Service, 1997). Pursuant on these it is conceivable that mosquitoes can fly several kilometres during one gonotrophic cycle. However, if all the species' needs are met in the immediate vicinity, it is unlikely the mosquitoes will leave the area. Catalysts that will change this situation and initiate dispersal probably include crowding and intra- and inter-specific competition.

In some regards, the members of the *A. punctulatus* group appear to be quite unspecialised in their behaviour. Most species will, for example, utilise a wide range of breeding sites (Cooper et al., 2002) and are not particular in their choice of a host (Charlwood et al., 1985; Burkot et al., 1988a), two behavioural aspects considered important with regards to dispersal (Belkin, 1962). One would expect the members to be widespread throughout Papua New

Guinea and northern Australia. However, this is not the case and barriers exist that not only limit the distribution of the various species but also play a role in isolating intraspecific genotypes for a number of the species. At least two types of barrier exist within the range of the members of the *A. punctulatus* group: climatic and geographical.

### 3.1. Climatic barriers

During the Miocene, the climate of northern Australia was hot/wet and the region was covered by extensive rain-forest. However, in the late Miocene and throughout the Pliocene, northern Australia became drier and cooler and there was a marked degree of seasonality in the rainfall (Cranston and Naumann, 1991). As a result there is now a major climate disjunction between northern Australia and New Guinea (Fig. 2) (Paijmans et al., 1971). The climate of northern Australia is monsoonal, cooler and drier with distinct seasons of wet and dry. During the wet season (December to April) 90% of the rain occurs, while rainless periods in the dry season can be measured in weeks and temperatures and humidity are lower. In New Guinea, the climate is continuous hot/wet, rainfall is > 2500 mm per annum with little seasonality, rainless periods on average rarely exceed 4.5 days, lowland temperatures vary little from 26 °C and humidity is > 80% throughout the year (McAlpine et al., 1983). The region of disjunction between these two climate types occurs approximately at the Fly River in south-western Papua New Guinea (Fig. 2).

In northern Australia there is a second climatic barrier which is the result of increasing dryness to the south. The rainfall in the northern parts of the Northern Territory and Cape York Peninsula is 1500–1600 mm per annum. The rainfall declines to the south with the region south of the Gulf of Carpentaria receiving less than 1000 mm per annum. Thus two areas of reasonably high rainfall – one in the north of the Northern Territory, the other on Cape York Peninsula – are separated by a semi-arid zone of rainfall below 1000 mm per annum.

A third climatic barrier occurs in the central ranges and Owen Stanley Range of Papua New Guinea. These mountains rise to over 4000 m above sea level, and in these highlands altitude moderates temperature, though the rainfall does not greatly change. Above 1000 m above sea level the climate is described as warm and wet with average temperatures of 15–18 °C (McAlpine et al., 1983).

### 3.2. Geographical barriers

Three geographical barriers exist in the region (Fig. 2). Firstly, the Torres Strait existed as a land bridge between northern Australia and Papua New Guinea during the glaciation periods of the Pleistocene. This connection was broken most recently 8000–6000 years ago following a rise in sea levels after the last great glaciation period, this flooding leaving a series of small islands scattered across a water gap of 150 km between the two land masses (Nix and

Kalma, 1972). Although *A. farauti* s.s., *A. farauti* 2 and *A. farauti* 3 occur on Cape York Peninsula and in southwest Papua New Guinea (on both sides of the strait), only *A. farauti* s.s. has been found commonly throughout the islands within the strait (Foley et al., 1991).

Secondly, the central ranges and Owen Stanley Range of the Papua New Guinea highlands form a central cordillera up to 200 km wide, with peaks over 4000 m above sea level (Löffler, 1982). Interspersed with these mountainous land-forms are numerous broad upland valleys rising more than 1500 m above sea level. In eastern Papua New Guinea, along the Papuan Peninsula, the mountains of the Owen Stanley Range run to the coast, fragmenting the coastal lowlands into a series of embayments on both the northern and southern sides of the peninsula (Löffler, 1982). At their northern limit these mountains extend to the coastline for over 150 km excluding any form of coastal plain. Among members of the *A. punctulatus* group only *A. farauti* 6 is found commonly above 1500 m with most species predominantly belonging to lowland habitats (Cooper et al., 2002). These ranges must form a sizeable barrier to the movement of coastal and lowland adapted species.

The third geographical barrier is that formed by the sea gaps between the various island arcs of the Bismarck Archipelago, the Solomon Islands and Vanuatu. These islands are made up of microcontinents and terranes, rifted from the Gondwanan Australian craton and island arcs, uplifted by the sea floor spreading of seas basins. These terranes moved into their present position, along stress lines and faults, during the late miocene/pliocene 11–3 mya (Michaux, 1989, 1994). Thus, island positions may not always have been as they are today. The close proximity of Vanuatu and Fiji 5 mya would, for example, have allowed the eastward dispersal of fauna into Fiji, although these two island groups have since become well separated by sea floor spreading of the North Fijian Basin (Burrett et al., 1991). Belkin (1962) believes that the dispersal of a number of relict mosquito species in the South Pacific would have been influenced by the movement of these island terranes. However, with the south Pacific islands in their present position, recent easterly dispersals would have to contend with ocean barriers of up to 300 km.

## 4. Molecular phylogeny of the *A. punctulatus* group

Reconstruction of the phylogenetic relationships between species is important in order to help understand their evolution. To achieve this, comparable and informative homologous characters are subjected to assumption-based mathematical structures which determine levels of genetic division operating within and between taxa to produce a phylogenetic tree. The resulting tree is an estimate or hypothesis of the evolution of only those homologous characters and not the whole organism. The tree branches connect branch points to the terminal taxa. Branch points

represent speciation events or hypothetical ancestors and the lines connecting two speciation events represent at least one ancestral species. If the tree is rooted by an outgroup (taxa assumed to be phylogenetically outside the ingroup), then character change direction can be assigned to the ingroup giving the tree direction and allowing the branch lengths on the tree to represent evolutionary time.

In the past phylogenies have been constructed based extensively on morphological and chromosomal characters; recently, however, with the advent of molecular-based technology, DNA sequences are now commonly used. Molecular characters should give a better estimate of phylogeny as the data is derived directly from the genome and the number of characters are limited only by the size of the DNA sequence (Cranston et al., 1991).

There are currently three published molecular phylogenetic reconstructions for the *A. punctulatus* group. These reconstructions have used either the mitochondrial cytochrome oxidase subunit II (COII) gene (Foley et al., 1998); the ribosomal DNA (rDNA) internal transcribed spacer 2 (ITS2) region (Beebe et al., 1999); or the rDNA small subunit (ssrDNA or 18S) as shown in Fig. 3 (Beebe et al., 2000b; Cooper et al., 2000). In all three studies the *A. punctulatus* group appears monophyletic, dividing the members into two major clades. The first – the Farauti

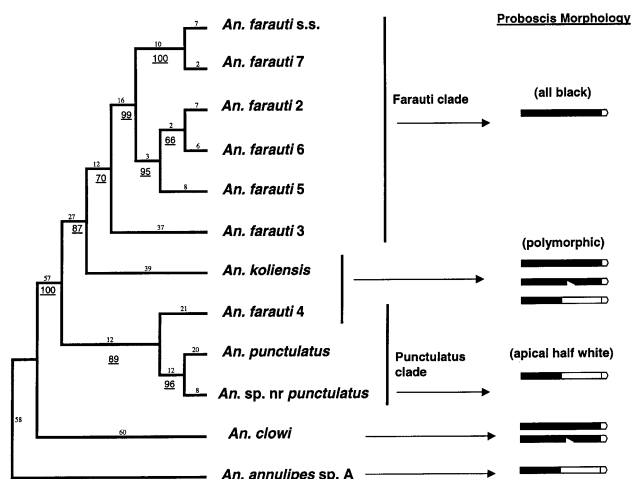


Fig. 3. Single most parsimonious tree generated from the structural alignment of the nuclear ssrDNA from members in the *Anopheles punctulatus* group (GenBank accession numbers AF121053–63 and AF178683). Maximum parsimony analysis was used with default settings (PAUP 3.1.1.; Swofford, 1993). *Anopheles annulipes* sp. A from the *A. annulipes* group was used as the outgroup and gaps were treated as missing data. A transition: transversion ratio of 1:1 identified by Beebe et al. (2000b) was used in the analysis and sampling error was assessed using 1000 bootstrap replicates (underlined values). The resulting fully resolved tree had a length of 424, a consistency index of 0.849, rescaled consistency index of 0.645 and retention index of 0.759. Numbers above the line represent branch lengths. Proboscis morphologies identified from field collections are displayed on the right and suggest there is some concordance with the phylogeny. Species displaying an all-black proboscis group together and species showing apical half to be white also group together. Additionally, the presence of proboscis polymorphism appears to be an ancestral characteristic that has been lost in the descendant species.

clade, where all members displayed an all-black scaled proboscis – contained *A. farauti* s.s. and *A. farauti* 7 grouping together forming a sister clade with *A. farauti* 2, 6 and 5. The second – the Punctulatus clade where the apical half of the proboscis has some white scaling – has *A. farauti* 4 grouped with *A. punctulatus* and *A. sp. nr. punctulatus*. Both the ITS2 and ssrDNA regions placed *A. koliensis* within or basal to the Farauti clade. The inability to align the ITS2 region to a suitably homologous outgroup resulted in an unresolved unrooted tree in which the branches containing *A. farauti* 2, 5 and 6 collapsed to form a polytomy. The mitochondrial COII region differed slightly in topology to the ssrDNA analysis, positioning *A. koliensis* basal to both the Farauti and Punctulatus clades and *A. sp. nr. punctulatus* basal to *A. farauti* 4 and *A. punctulatus*. However, branch supports for these positions were not strong. Nevertheless, the nuclear ITS2 and ssrDNA gene and the COII gene each identified a Punctulatus and Farauti clade and placed *A. farauti* 4 paraphyletic to the Farauti clade and in the Punctulatus clade.

The phylogeny of the *A. punctulatus* group illustrated in Fig. 3 is that from Cooper et al. (2000), which includes the recently rediscovered *Anopheles clowi* and was based on structural ssrDNA sequence alignments of 11 members of the *A. punctulatus* group. *Anopheles annulipes* was used as the outgroup (AF121053–63 and AF178683). Both the *A. annulipes* complex and the *A. punctulatus* group are endemic to the Australian Region, both belong to the Neomyzomyia series and are closely related morphologically and cytogenetically (Booth et al., 1987; Harbach, 1994).

Proboscis morphology, initially used to separate *A. farauti*, *A. koliensis* and *A. punctulatus* (Rozeboom and Knight, 1946), is now problematic with several species having an all-black scaled proboscis, while *A. koliensis* and *A. farauti* 4 display polymorphic proboscis morphologies with examples of the Farauti, Punctulatus and Koliensis proboscis types being found in field collected material (Cooper et al., 2002). These characteristics, however, are supported to some degree by phylogeny based on molecular characters (Fig. 3). *Anopheles farauti* s.s., 2, 3, 5, 6, and 7 consistently show an all-black proboscis and form a monophyletic group, while *A. punctulatus*, *A. sp. nr. punctulatus* and *A. farauti* 4, which frequently have some white scaling on the proboscis, form a monophyletic group. Like *A. clowi*, both *A. koliensis* and *A. farauti* 4 appear early in the tree and display proboscis polymorphisms, which appear to have been lost in the descendant species to result in either all-black proboscis species or half-black, half-white proboscis species.

Species divergence times for members of the *A. punctulatus* group have been estimated using the sequence variation and the transversion frequency of the COII gene (Foley et al., 1998). These estimates were based on studies from other insects, resulting in assumptions of evolutionary rates being 2.3% sequence divergence/million years (my) (Brower, 1994) and 0.13% and 0.3% transversion diver-

gence/my (Beckenbach et al., 1993). These assumptions were slightly different to those used by Sharpe et al. (2000), who used 2.0%/my for the COII gene-based on *Drosophila* for the *A. minimus* group members (DeSalle et al., 1987). However, estimates vary considerably between the different assumptions. For example the closely related species *A. farauti* s.s. and *A. farauti* 7 (1.7% sequence divergence) were estimated to have diverged either 0.74 mya or 1.92 mya, while more distant members *A. farauti* s.s. and *A. punctulatus* (7.72% divergence) produced an estimate of 3.36 mya or 34.6 mya, a 10-fold difference. If it is assumed that the *A. punctulatus* group evolved in the northern Australian/New Guinea area in the late Pliocene/Pleistocene epochs (3.4–0.01 mya) then the upper time estimates of the above divergent events are unlikely. The use of a molecular clock assumes a neutral rate of evolution and that the rate of a nucleotide substitution is the same across evolutionary time and independent of important factors such as population size, the magnitude of the selective advantage associated to the mutations and speciation events resulting from the vagaries of dispersal or vicariance occurrences. Thus the assumptions for a molecular clock are at odds with the process of natural selection.

## 5. Distribution of the *A. punctulatus* group members in the southwest Pacific

A summary of the geographic distribution of the members of the *A. punctulatus* group is shown in Table 1 and refers to biogeographic regions in Fig. 2.

### 5.1. The *Farauti* clade

Within the *Farauti* clade, *A. farauti* s.s. and *A. farauti* 7 are sister taxa as are *A. farauti* 2 and *A. farauti* 6: *A. farauti* 5 is basal to these latter two species. *Anopheles farauti* 3 is the most distant and basal to all the *Farauti* clade members. *Anopheles farauti* s.s. is the dominant coastal species of the group with more than 75% of all sites for this species in northern Australia and Papua New Guinea occurring within 1 km of the coast (Cooper et al., 2002). This species is saltwater tolerant (Sweeney, 1987) and utilises brackish water as breeding sites – an adaptation that, according to Belkin (1962) and Service (1997), would promote coastal dispersion. As a result this species has possibly the widest range of all the members of the *A. punctulatus* group, occurring from Vanuatu in the east to New Guinea and northern Australia in the west. *Anopheles farauti* s.l. has been recorded from Baggai Island in the Moluccas, the most westerly location for any member of the *A. punctulatus* group (Lee et al., 1987). Though the species status of these specimens has not been determined, they might have been *A. farauti* s.s. The long distance dispersal of this species across several sea gaps > 200 km was probably aided by humans as the larvae have been found breeding in brackish water in native canoes (Daggy, 1945; Belkin et

al., 1945). The barriers to species dispersal and gene flow that exist in the region (as discussed in Section 3) have not had a major effect in limiting the distribution of this species. Only in northern Australia is aridity (<1000 mm per annum) a barrier to its existence in the gulf region and to its further southern dispersal. However, there is evidence to suggest that the barriers that do exist have restricted gene flow in the past, resulting in the appearance of several different rDNA genotypes within this species (Beebe et al., 2000c; Beebe and Cooper, unpublished data). The Northern Territory and Queensland populations, separated by the arid zone of the gulf region, represent two distinct genotypes. The Torres Strait, however, is no barrier to this saltwater-tolerant species and the same genotype is found on both sides of the Strait. The monsoonal and continuous hot/wet climatic disjunction in southwest Papua New Guinea and the mountains of the central ranges and Owen Stanley Range are also barriers to gene flow within this taxon. Another, as yet undetermined, barrier also exists to the inland dispersal of this species. Although predominantly coastal, *A. farauti* s.s. will breed in fresh water and a few inland populations have been found (Cooper et al., 2002). These populations appear to represent a distinct rDNA genotype and may be evolving independently after having crossed a barrier still effective against most coastal populations of *A. farauti* s.s.

Analysis of the genotypes of this species may shed light on its dispersal across the South Pacific. Populations from Guadalcanal are a distinct genotype and are genetically close to the genotype in Queensland, whereas the Vanuatu genotype is genetically closer to the Northern Territory genotype (Beebe et al., 2000c). The reasons behind this distribution of genotypes is difficult to explain; however, they may revolve around the presence of *A. farauti* 7, which appears to exist only on Guadalcanal. *Anopheles farauti* s.s. and *A. farauti* 7 are both able to breed in brackish water (Foley and Bryan, 2000); this appears to be a synapomorphic feature of these species. However, unlike *A. farauti* s.s., which readily bites humans and is thus a potential malaria vector, *A. farauti* 7 appears to be purely zoophytic. These two species are very closely related sister taxa within the *Farauti* clade. They display little divergence and show only seven character changes on the ssrDNA phylogenetic tree (Fig. 3). When the ITS2 rDNA sequence of *A. farauti* 7 (AF033218) was compared to the 13 *A. farauti* s.s. ITS2 genotype sequences from northern Australia, Papua New Guinea, Guadalcanal and Vanuatu (AF104314–AF104326), the *A. farauti* 7 sequence was found to be most closely related to the Vanuatu genotype (AF404315), followed by the Australian Northern Territory genotypes (AF104316 and AF104314) and, then the Guadalcanal/Queensland genotypes (AF104317, AF104318). Thus *A. farauti* 7 appears genetically closer to the *A. farauti* s.s. population from Vanuatu than to the *A. farauti* s.s. population from Guadalcanal. This suggests the possibility of two radiations of *A. farauti* s.s. into the southwest Pacific with

the first reaching Guadalcanal and then Vanuatu and the second only reaching Guadalcanal. The populations of the first radiation may have changed under the conditions present on Guadalcanal at the time, developing prezygotic mating barriers to the second radiation of *A. farauti* s.s. and leading to the evolution of *A. farauti* 7.

Belkin (1962) considers several dispersal patterns for the Culicidae of the South Pacific. Of interest is his dispersal pattern 5 which links northern Australia with Vanuatu and, following this, another dispersal event (pattern 6) linking northern Queensland with the Solomon Islands. He notes that these dispersal patterns are likely to be far more complex than he depicts and that it is possible that pattern 5 may have allowed movement into the Solomon Islands, accounting for the two dispersal events indicated above for *A. farauti* s.s. However, the concept of the dispersal of *A. farauti* s.s. and the evolution of *A. farauti* 7 is based on fairly limited knowledge. More detailed studies on the distribution of these two species and their genotypes throughout the Solomon Islands and Vanuatu would be needed to verify this hypothesis.

*Anopheles farauti* 2 is possibly the most successful member of the *A. punctulatus* group with regards to dispersal. It has a wide distribution comparable with that of *A. farauti* s.s. It is common throughout northern Australia and Papua New Guinea, being found on small coastal islands as well as inland to 1700 m above sea level (Cooper et al., 2002). Although it has not been found in Vanuatu, it occurs on Buka and Bougainville islands and in the Solomon Islands (Beebe et al., 2000a; Cooper and Frances, 2002). In its habits it is the most non-specific of the group adapting to a wide range of breeding sites and hosts. This species comprises several distinct rDNA genotypes (ITS1-RFLP profiles), the distribution of which indicate that the Torres Strait and the central ranges in Papua New Guinea are the major barriers to gene flow (Beebe and Cooper, unpublished data).

The trees from the SSU and the COII alignments both place *A. farauti* 5 basal to *A. farauti* 2 and 6. All three species display little divergence and share a common ancestor that probably originated in the upland river valleys of Papua New Guinea between 1000 and 2000 m above sea level. The position of *A. farauti* 2 in the tree suggests that its highland affinities combined with its capacity to utilize a variety of larval habitats has enabled it to disperse both north and south of the central ranges and then throughout the southwest Pacific region. Little is known about *A. farauti* 5, which has only been recorded from only one location, but as with *A. farauti* 2 and 6 it has highland affinities – the type locality being the central ranges at 1600 m above sea level (Foley et al., 1993).

*Anopheles farauti* 6 is possibly the most specialised member of the group, being the only species to become fully adapted to the cooler, high-altitude regions of the New Guinea central ranges. In Papua New Guinea all collections of this species were at > 1500 m above sea

level and in Irian Jaya it was the most abundant and widespread anopheline species in the massive Baliem Valley at 1700 m above sea level (Cooper, 1998; Cooper et al., 2002). *Anopheles farauti* 6 is noticeably larger than any of the other members of the *A. punctulatus* group, a possible result of its high altitude adaptation.

*Anopheles farauti* 3 is the most basal member of the Farauti clade and shows considerable genetic divergence from all other members. This species is quite unique within the *A. punctulatus* group in that it is the only member whose distribution is confined to the monsoonal climate of northern Australia (Cooper et al., 1996). This species may have occupied the vast continental shelf that connected northern Australia and New Guinea throughout much of the Pliocene and Pleistocene. Adapting to the slow drying and cooling of the region, *A. farauti* 3 has flourished in the monsoonal climate of northern Australia following its separation from New Guinea 15 000–8000 years ago (Kikkawa et al., 1981). This species is now common in northern Australia: it is the most abundant member of the group in the Northern Territory and is common in northern Queensland. In Papua New Guinea it is uncommon and only occurs in the southwest where the monsoonal climate prevails. The formation of the Torres Strait, as recently as 6000 years ago, now presents a barrier to gene flow within this species and distinct genotypes now occur, one in southwest Papua New Guinea (north of the Strait) and the other to the south in northern Australia (Beebe and Cooper, unpublished data).

## 5.2. The Punctulatus clade

*Anopheles farauti* 4, *A. punctulatus* and *A. sp. nr. punctulatus* make up the Punctulatus clade, all members of which are capable of displaying some white scaling on the apical region of the proboscis. Of this clade, *A. punctulatus* and *A. sp. nr. punctulatus* appear to be the most closely related. The two species share a common habitat in inland river valleys, however whereas *A. sp. nr. punctulatus* is uncommon and possibly a relict species with a limited distribution, *A. punctulatus* has dispersed widely throughout the lowlands, both inland and coastal and has spread, to some extent, up into the highlands. *Anopheles punctulatus* was described as a weed species by Charlwood et al. (1986). It is capable of rapid invasion into new areas, colonising small transient bodies of water immediately they appear. As a result, it can avoid competition from other mosquito species and can take advantage of areas disturbed by human activities such as logging and mining – activities that have aided its local dispersal. To utilise these types of sites, larval development is relatively short and highly synchronised, and the eggs can resist desiccation for periods of several days (Horsfall and Porter, 1946; Charlwood et al., 1986). *Anopheles punctulatus* is a fairly specialised mosquito with regard to its breeding habits, but this specialisation appears to have benefited the dispersal of the species (Belkin, 1962). Populations from Irian Jaya, Papua New Guinea and the Solomon



Islands all generate the same rDNA ITS1-RFLP profile (Beebe and Cooper, unpublished data), and Foley et al. (1998) found *A. punctulatus* displayed the least intraspecific mitochondrial COII sequence variation throughout its distribution (0.81%,  $n = 10$ ) when compared with *A. farauti* s.s. (1.27%,  $n = 3$ ) and *A. farauti* 2 (1.42%,  $n = 6$ ). This decreased genetic variability in *A. punctulatus* could imply a recent dispersal with new populations having insufficient time to evolve distinct genotypes. Alternatively, it is possible that as it only invades areas to which it is fully adapted, thus avoiding any major external selection pressure. This may explain the absence of *A. punctulatus* from the monsoonal areas of northern Australia where transient small pools would not be available for several months during the long dry season. It appears that the mountains of New Guinea are not a barrier to the dispersal of this species as it has been collected as high as 1700 m above sea level, though it is not common in the highlands (Cooper et al., 2002).

*Anopheles farauti* 4 is an early diverging lineage in the *Punctulatus* clade. This species is the most polymorphic of the members of the *A. punctulatus* group with regards to proboscis morphology and is capable of displaying all three proboscis types (Cooper et al., 2002). This species has a distribution restricted to the inland, lowland areas (<300 m above sea level) north of the central ranges in Papua New Guinea (region 5, see Fig. 2). It is reasonably common in this region; however, one population has been collected on the Papuan peninsula. The central ranges appear to be the major barrier to the dispersal of this species as it has been unable to disperse successfully into the southern lowlands of Papua New Guinea.

### 5.3. *Anopheles koliensis*

*Anopheles koliensis* appears as an early diverging lineage in the *Farauti* clade and shows considerable divergence from all other members of the group. Like *A. farauti* 4, it is quite polymorphic with regard to proboscis morphology, but not to the same degree. *Anopheles koliensis* is common and widespread north of the central ranges in the inland river valleys (region 3). South of the ranges its distribution is restricted to region 6 and, like *A. punctulatus*, it appears unable to penetrate the monsoonal region in the southwest of Papua New Guinea. Found mostly below 300 m above sea level, it is uncommon above 1000 m but was collected at one site at 1600 m above sea level (Cooper et al., 2002). It has been recorded from Buka Island (Spencer, 1961) and several islands in the Solomons (Owen, 1945; Taylor, 1975) and so is capable of crossing sizable ocean gaps. However, on Buka and Guadalcanal, at least, its foothold seems to have been tenuous as it appears to have been eliminated by the use of indoor DDT residual spraying for malaria control, something which did not occur with *A. farauti* s.s. and *A. punctulatus* (Spencer, 1961; Beebe et al., 2000a). The effective control of *A. koliensis* by DDT indoor residual

spraying (Sweeney, 1983) is believed to be a result of this species' evolution as a highly anthropophilic species (Belkin et al., 1945). Thus the size of human populations may limit the numbers and distribution of this species.

### 5.4. *Anopheles clowi*

*Anopheles clowi* was the earliest diverging lineage species in the *A. punctulatus* group. It appears to be a relict species having been collected from only two locations in the last 50 years, one in northeast Papua New Guinea, the other 600 km to the west in Irian Jaya (Cooper et al., 2000). Its position in the phylogeny is interesting as this species shows a substantial genetic divergence from the rest of the group and – if not forced into the ingroup by *A. annulipes* – it would group more closely with *A. annulipes* species A, which is regarded as its sister taxon (Booth et al., 1987).

## 6. Conclusions

The distribution of the members of the *A. punctulatus* group appears to be defined by clear barriers such as climate, elevation and sea gaps, although these were not barriers to all species. Adaptation to the monsoonal climate of northern Australia and southwest Papua New Guinea was found to have appeared independently in *A. farauti* s.s., *A. farauti* 2 and *A. farauti* 3. Also, the climate disjunction in southwest Papua New Guinea (regions 1–3) appears to be an impenetrable barrier to *A. farauti* 3, *A. koliensis* and *A. punctulatus*, but not to *A. farauti* s.s. or *A. farauti* 2. Populations of *A. farauti* s.s. in this monsoonal region have developed into detectable rDNA genotypes suggesting that climate is a barrier to the gene flow of this species. This was not so obvious with *A. farauti* 2 as the Torres Strait appears to be the major barrier to gene flow between regions 1–3 rather than the climate disjunction in southwest Papua New Guinea. However, this will require further investigation. Interestingly, there appears to be no such sea barrier between northern Papua New Guinea and the Solomon Islands as the same rDNA genotype extends from the north-west Papua New Guinea into Bougainville and Guadalcanal. This could suggest a recent dispersal of this species east from Papua New Guinea.

Species' ability to show widespread dispersal was found to have appeared independently and includes those species that utilise niche-specific habitat (such as *A. farauti* s.s. and *A. punctulatus*) and, conversely, in species that show larval site versatility (*A. farauti* 2). Belkin (1962) notes that utilisation of a highly specialised and stable habitat is favourable for dispersal. In this regard the ability of *A. farauti* s.s. to adapt to brackish coastal breeding sites, which are common throughout the southwest Pacific region may have facilitated its wide dispersal. The niche-specific dispersal success of *A. punctulatus* appears to come from its ability to utilise transient larval habitats which are ubiquitous throughout the continually wet highland and lowland regions of Papua New

Guinea and into the Solomon Islands. However, this has not occurred with the closely related *A. sp. nr. punctulatus*, which appears to use similar larval habitats (Cooper et al., 2002). Interestingly, throughout its distribution, *A. punctulatus* did not reveal intra-specific ITS1 sequence polymorphism when assessed at the PCR-restriction fragment length polymorphism level. Perhaps this species has recently dispersed and has had little time to evolve specific genotypes, or conversely, perhaps the niche this species has adapted to places little selection pressure on it, resulting in genetic stability across the species. Comparisons using other markers such as the mitochondria or microsatellites would be required to assess these hypotheses.

The coastal affinities of *A. farauti* s.s. have facilitated its wide distribution and ocean gaps do not appear to present a barrier to this species. However, these features alone may not explain their dispersal, as *A. farauti* 7 is also saltwater tolerant – a synapomorphic feature of these two species – but appears not to have dispersed beyond Guadalcanal. Further surveys are required on the other islands to confirm this. The inability of both *A. farauti* 7 and *A. sp. nr. punctulatus* to successfully disperse outwards may have more to do with dispersal behaviour, competition or host preference selection.

The second group in the Farauti clade, *A. farauti* 2, 6 and 5, also displays a synapomorphic feature of affinities to the highlands, either only existing above 1500 m above sea level (*A. farauti* 6 and 5) or having populations at or above this altitude (*A. farauti* 2). It appears that the ancestor for these species emerged from the highlands and that the *A. farauti* 2, being highly adaptable with regards to larval breeding sites, has moved into the lowlands on both sides of the central ranges, successfully dispersing throughout the southwest Pacific region. *Anopheles farauti* 2, like *A. farauti* s.s., is well dispersed throughout southwest Pacific but, unlike the latter species, it has achieved this by being non-specialised and fits Belkin's category of a generalist breeder capable of utilising a wide variety of breeding sites (Belkin, 1962).

It is likely that the ancestors of the *A. punctulatus* group dispersed down the Indo-Malayan Archipelago during either the Pliocene (5–1.6 mya) or the Pleistocene (1.6–0.01 mya) epochs. As most of Papua New Guinea was submerged up until 3 mya, this dispersal appears more likely to have occurred during the Pleistocene. This would mean that the species divergence times based on the mitochondrial COII gene of Foley et al. (1998) overestimate the age of this group. Belkin (1962) notes that the mosquitoes of the south Pacific must be of some antiquity to allow time for their spread across large seas gaps. It is unlikely, however, that *A. farauti* s.s. was present in Vanuatu prior to 5–3 mya as it would have dispersed into Fiji which at the time was very close (Burrett et al., 1991).

Up until 15 000 years ago, northern Australia was joined to Papua New Guinea along the Sahul shelf, permitting gene flow between these two continents. Approximately 8000–

6000 years ago these land masses were fully separated by the flooding of the Torres Strait, preventing gene flow between northern Australia and Papua New Guinea as demonstrated by distinctive genotypes within *A. farauti* 2 and 3. It also appears that a population of *A. farauti* s.s. was isolated on the coast of Australia's Northern Territory with the flooding of the Gulf of Carpentaria. Of interest would be the comparison of *A. farauti* s.s. and *A. farauti* 2 genotypes between the Northern Territory populations and those from the southern coast of Irian Jaya to the northwest. If these species once occupied the exposed continental shelf that existed between northern Australia and New Guinea during the last great glaciation period (15 000 years ago), then it could be predicted that the same genotypes for the two species would be found in both areas.

A series of vicariance events have now resulted in populations within *A. farauti* s.s., 2 and 3 that are now distinct rDNA genotypes (Beebe et al., 2000c; Beebe and Cooper, unpublished data). How much more time would it take for these populations to develop either prezygotic or postzygotic mating barriers? Further investigation is now required, but it is difficult to believe that the period would be measured in millions of years.

Though the distribution of the members of the *A. punctulatus* group is now well known for northern Australia and Papua New Guinea, very little is known about their distribution throughout the south Pacific islands in the east and Irian Jaya and the Moluccas in the west. Species within the *A. punctulatus* group exhibit distinct distributions that appear to be governed by climatic and geographical barriers, and these barriers appear to be further affecting each species at the intraspecific level, leading to geographically specific genotypes. When the full distribution of the group is known, both for the different species and their genotypes, then a more complete understanding of the dispersal and speciation of these medically important mosquitoes will be possible.

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