# Speciation and Distribution of the Members of the *Anopheles punctulatus* (Diptera: Culicidae) Group in Papua New Guinea

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Source: Journal of Medical Entomology, 39(1):16-27. 2002.
Published By: Entomological Society of America
DOI: http://dx.doi.org/10.1603/0022-2585-39.1.16
URL: http://www.bioone.org/doi/full/10.1603/0022-2585-39.1.16

ARTICLE

# Speciation and Distribution of the Members of the *Anopheles punctulatus* (Diptera: Culicidae) Group in Papua New Guinea

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J. Med. Entomol. 39(1): 16–27 (2002)

**ABSTRACT** Mosquito collections were made throughout the mainland of Papua New Guinea to identify the members of the *Anopheles punctulatus* group present and to determine their distribution. Identification was made using morphology, DNA hybridization, and polymerase chain reaction (PCR)-RFLP analysis. Nine members of the group were identified: *An. farauti* s.s. Laveran, *An. farauti* 2, *An. koliensis* Owen, and *An. punctulatus* Dönitz, were common and widespread; *An. farauti* 4 was restricted to the north of the central ranges where it was common; *An. farauti* 6 was found only in the highlands above 1,000 m; and *An. farauti* 3, *An.* sp. near *punctulatus* and *An. clowi* Rozeboom & Knight were uncommon and had restricted distributions. Identification of *An. koliensis* and *An. punctulatus* using proboscis morphology was found to be unreliable wherever *An. farauti* 4 occurred. The distribution and dispersal of the members of the *An. punctulatus* group is discussed in regard to climate, larval habitats, distance from the coast, elevation, and proximity to human habitation.

**KEY WORDS** *Anopheles punctulatus* group, speciation, distribution, Papua New Guinea

MEMBERS OF THE *Anopheles punctulatus* group are major vectors of malaria and Bancroftian filariasis in the southwest Pacific. The group as originally described consisted of four closely related species, *Anopheles punctulatus* Dönitz, *Anopheles farauti* Laveran, *Anopheles koliensis* Owen and *Anopheles clowi* Rozeboom & Knight (Rozeboom and Knight 1946). *Anopheles clowi* has a restricted distribution, however the other three species are believed to be wide spread throughout Irian Jaya, Papua New Guinea, and the Solomon Islands (van den Assam and van Dijk 1958; Rozeboom and Knight 1946; Belkin 1962, pp. 46–59; Spencer et al.1974; Taylor 1975). The standard method for identifying *An. punctulatus*, *An. koliensis* and *An. farauti* has been by proboscis morphology and to a lesser extent the presence or absence of an accessory sector pale area on the costa (Rozeboom and Knight 1946, Belkin 1962, Bryan 1974). These characteristics are easy to apply in the field and are still widely used to identify mosquitoes in epidemiological studies and in the evaluation of control strategies (Bockarie et al. 1996, Attenborough et al. 1997, Hii et al.1997)

Cross-mating experiments and allozyme analysis have now revealed a number of cryptic species within the *An. punctulatus* group, *An. farauti* 2 (Bryan 1970), *An. farauti* 3 (Mahon and Miethke 1982), *An. farauti* 4–6 (Foley et al.1993), *An. farauti* 7 (Foley et al. 1994), and *An.* species near *punctulatus* (Foley et al.1995). There are now seven members of the group that are isomorphic (*An. farauti* 1–7 all with a black scaled labium), and there is evidence that other species within the group may be polymorphic for proboscis morphology. Woodhill, in 1946, and before cryptic species were suspected, reported problems with identification based on proboscis morphology and further evidence for this was found by Foley et al. (1993) and Cooper et al. (1997). The importance of accurate vector identification in malaria studies has led to the development of DNA hybridization techniques (Cooper et al. 1991; Beebe et al. 1994, 1996) and polymerase chain reaction-restriction fragment-length polymorphism analysis (PCR-RFLP) (Beebe and Saul 1995) for identifying the members of this group.

In Papua New Guinea (PNG) the following members of the *An. punctulatus* group have been identified: *An. punctulatus*, *An. koliensis*, *An. farauti* s.s. (formerly *An. farauti* 1), and *An. farauti* 4–6 (Bryan 1974, Charlwood et al. 1985, Foley et al. 1993). These studies however were very limited in scope. The only comprehensive distribution study reported to date was that conducted in Western Province and which identified *An. farauti* 2 and 3 as occurring in PNG and recorded the presence of a new species *An.* sp. near *punctulatus* (Cooper et al. 1997). Until the species present in PNG are known and their distribution determined for the whole country, it will be difficult to assess the relative importance of the various species as vectors of malaria. This information is needed to provide a rational basis for the application of control measures.

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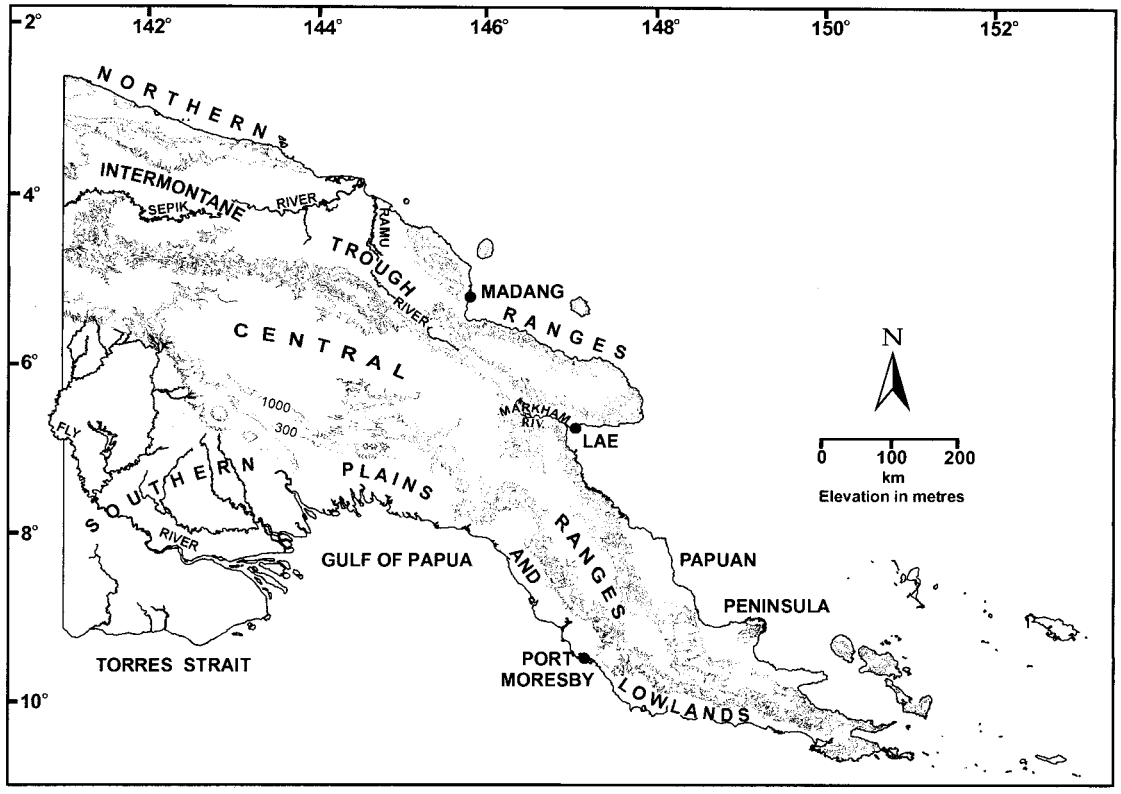


**Fig. 1.** Map of Papua New Guinea showing main geographical features.

This study reports on a series of surveys made of the mainland of PNG to determine the members of the *An. punctulatus* group present and their distribution. Identification of field-collected material was made by standard proboscis morphology and compared with DNA hybridization and PCR-RFLP analysis. This work includes and completes the earlier survey of Cooper et al. (1997).

## Materials and Methods

**Geography and Climate.** Papua New Guinea forms the eastern half of the island of New Guinea and lies between the latitudes of 2° and 11° south of the equator (Fig. 1). The mainland of Papua New Guinea is ≈420,000 km$^2$ and is made up of four major land forms: (1) the southern plains and lowlands, covering over 100,000 km$^2$ in the west but rapidly narrowing to the east where mountains run close to the sea; (2) the central ranges running down the middle of the island varying in width from 50 to 200 km and made up of steep, rugged mountains (with peaks >4,000 m), interspersed with upland valleys and intramontane plains; (3) an intermontane trough forming the Sepik, Ramu, and Markham river valleys; and (4) the northern ranges, a series of discontinuous coastal ranges separated from the central cordillera by the Sepik-Ramu-Markham trough (Löffler 1985) (Fig. 1).

The climate is hot wet with no marked dry season. Most of the country receives 2,000–4,000 mm of rain per annum and in only a few areas—south of the Fly River and along the coastline near Port Moresby—is the rainfall below 2,000 mm p.a. and the climate more monsoonal with a marked dry season. Except for these low rainfall areas the country rarely experiences rainless periods of more than 4 d duration. In coastal and inland lowland (<500 m) areas the mean temperature is 26°C (maximum 31°, minimum 22°C), whereas in the highland regions (500–2,000 m) the mean temperature is 20°C (maximum 23°C, minimum 14°C) (McAlpine et al. 1983). Much of the country is covered with either lowland or mountain rainforest, this gives way to open forest and grassland in low rainfall areas where there is a marked dry season. The major river systems, particularly the Fly, Sepik, and Ramu Rivers, have associated grass and wooded swamps and flood plains, which are extensive in their middle reaches (Paijmans 1985).

**Survey Methods.** Collections of mosquitoes were made for 1 mo of seven successive years (1992–1998), during the April–June period. Each year a section of the island was systematically surveyed using two helicopters (Bell Jet Ranger 204) and four-wheel drive vehicles using the available road network. The region to be surveyed was divided up into areas of ≈3,000 km$^2$ with each area being covered over 2 d by three aerial-

and one road-based collecting team. Many of the collections were made in remote localities far from roads and major population centers. A systematic survey of this nature was only possible with the use of helicopters.

Mosquitoes were collected as larvae from as many different bodies of water as possible. These collections were made using standard larval dippers with ≈30 min being spent at each site, less if larval densities were high. Larvae were fed powdered goldfish food and reared to adults in their site water. Adult mosquitoes were collected using carbon dioxide-baited encephalitis vector surveillance light traps (Rohe and Fall 1979). Traps were set near human habitation (villages, missions, schools, and government stations) and in uninhabited areas (at least 1 km from a potential human blood source) and were set in the evening and run over night. A limited number of human-bait collections were also performed between 2000 and 0100 hours. All collection localities were recorded on 1:100,000 scale maps from which latitude and longitude coordinates were determined within 100 m accuracy and entered into a spatial database (TNTmips, MicroImages, Lincoln, NE). Elevation of localities and their distance from the coast were determined directly from the maps. Proximity to human habitation was recorded at the time of collection.

An association between the presence of a species and human habitation was tested by a Fisher exact test (Jandel Scientific 1995) on $2 \times 2$ tables, with $H_o$: there being no association between the presence of a particular species and human habitation.

**Mosquito Identification.** All adults were killed by freezing and were examined microscopically in the field to identify members of the *An. punctulatus* group. Specimens with an all black scaled labium were recorded as *An. farauti* s.l., those with extensive white scaling on the apical half of the labium as *An. punctulatus* and those with a patch of white scales on the ventral surface of the apical half of the labium as *An. koliensis.* Following this preliminary identification the specimens were placed in vials according to species, site number, and date, then stored in liquid nitrogen. Larvae that could not be reared to adults were preserved in absolute ethanol.

A sample of each species, from each site, was further analyzed using DNA probes or PCR-RFLP. DNA hybridization was carried out on squash blots of abdomens using $^{32}$P-labeled species-specific DNA probes and the methods of Cooper et al. (1991) with slight modifications by Cooper (1998). The number of specimens processed was increased by probing up to 50 filters (100 specimens/filter) at one time in 240 ml of hybridization buffer; the filters were stripped using boiling 1% SDS before reprobing. For PCR-RFLP analysis DNA was extracted from the abdomen using the method of Pat Roman (Black and Munsterman 1996). With this method the final 100% ethanol wash was omitted and the DNA pellet resuspended in 50 $\mu$l of TE plus RNase (60 U/ml) made up fresh for each set of extractions. Further, the DNA was incubated at 65°C for 60 min or left at 4°C overnight before ITS2 amplification. Modification to the PCR method of Beebe and Saul (1995) included the use of Ampli taq Gold polymerase for a hot start and the following cycling regime: initial heating to 92°C for 10 min, then 35 cycles of 94°C for 0.5 min, 48°C for 1 min, and 72°C for 1 min, with minimal transition times. The product was cut with the restriction enzyme *Msp*I, run out on a 3% agarous gel, stained with ethidium bromide and visualized under a UV transilluminator (312 nm). The head and thorax of each specimen was relabeled and stored at −70°C for sporozoite ELISA analysis and further genetic studies.

## Results and Discussion

**Species Identification—Molecular Based Techniques.** Anophelines were collected from 794 sites throughout the mainland of PNG with 735 of these sites positive for members of the *An. punctulatus* group (Fig. 2). Using DNA probes and PCR-RFLP the following species were identified: *An. farauti* s.s., *An. farauti* 2, *An. farauti* 3, *An. farauti* 4, *An. farauti* 6, *An. koliensis*, *An. punctulatus*, *An.* sp. near *punctulatus*, and *An. clowi* (Table 1).

DNA hybridization analysis was quick and had the benefits of handling large numbers of specimens. With the preliminary identification of some species based on proboscis morphology helped with the selection of DNA probes. It also proved beneficial to separate coastal and inland *An. farauti* s.l. and to probe all coastal specimens for *An. farauti* s.s. However, with at least nine possible members of the group present in PNG frequent reprobing of the filters was required, this damaged the template DNA and added to the workload. Cross reactivity between some species, possibly due to technique (inadequate washing or stripping of the filters), was also a problem with some filters. The *An. farauti* 2 probe, which was originally derived from specimens collected in northern Queensland (Cooper et al. 1991), failed to hybridize with DNA extracted from *An. farauti* 2 collected in northern PNG. It has been noted that these populations of *An. farauti* 2 represent a different rDNA genotype than those from which the probe was made (N.W.B. and R.D.C., unpublished data). This highlights the need to test probes across the full geographic range of the various species to include all intraspecific variation.

PCR-RFLP analysis was the least problematic, most reliable, and most specific of the identification methods. It was more labor intensive than DNA hybridization because DNA had to be extracted before amplification; however, the purified DNA was then available for further molecular-based studies (Beebe et al. 2000a, Cooper et al. 2000).

**Mosquito Identification—Morphology.** Early workers had to rely on microscopic identification, based on proboscis morphology, as the only means of identifying the members of this group of mosquitoes before the development of molecular based techniques. Morphological characters were simple to use and are still the primary method for field identification of the

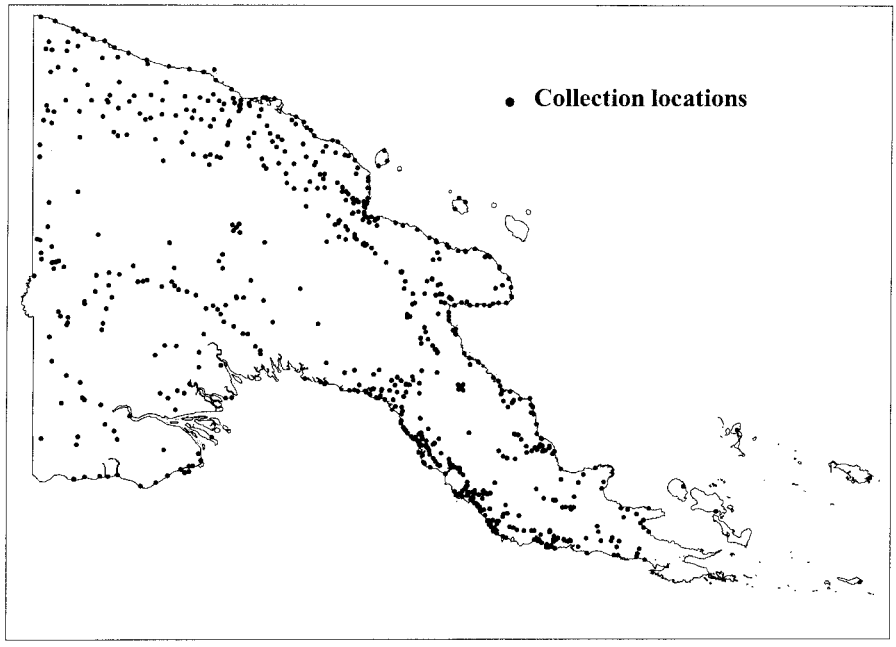


**Fig. 2.** Map of Papua New Guinea showing all collection localities.

members of the *An. punctulatus* group in Papua New Guinea. Anomalies associated with proboscis morphology were first recorded by Woodhill (1946). He collected adult females with koliensis-like proboscis near Lae and reared their progeny through another generation; some of the offspring were found to have either *An. punctulatus* or *An. farauti* proboscis types. We made similar observations with specimens collected in the Madang region. The progeny of 17 *An. koliensis*, identified by DNA hybridization analysis, were scored for proboscis type. As in the Woodhill study, specimens with all three proboscis types were found in the progeny. In only three of the 17 families did all progeny have a koliensis-like proboscis. Some individuals of 10 families had farauti-like proboscis; two families had some individuals with punctulatus-like proboscis; and a further two families had some individuals with farauti-like proboscis and some other specimens with punctulatus-like proboscis.

**Table 1. Number and type of collections made of members of the *An. punctulatus* group in Papua New Guinea**

| Species | Type and No. of Collections | | | |
|---|---|---|---|---|
| | Sites | Larval | Trap | Biting |
| *An. farauti* s.s. (fl) | 239 | 65 | 174 | 7 |
| *An. farauti* 2 (f2) | 324 | 179 | 152 | 0 |
| *An. farauti* 3 (f3) | 13 | 5 | 8 | 0 |
| *An. farauti* 4 (f4) | 43 | 8 | 36 | 4 |
| *An. farauti* 6 (f6) | 8 | 8 | 1 | 0 |
| *An. punctulatus* (p1) | 211 | 193 | 18 | 4 |
| *An. sp. nr. punctulatus* (p2) | 10 | 4 | 7 | 0 |
| *An. koliensis* (k) | 246 | 73 | 192 | 11 |
| *An. clowi* (c) | 1 | 1 | 0 | 0 |

Using specimens collected throughout their range in PNG a comparison of identification by proboscis morphology and DNA based techniques was made for the five most common species (Table 2). More than 99% of *An. farauti* s.s. and *An. farauti* 2 had the typical all black scaled proboscis, and >97% of *An. punctulatus* showed the characteristic white scaling on the proboscis. However, *An. farauti* 4 showed considerable polymorphism with regards to this characteristic, and with *An. koliensis* there was also some degree of polymorphism. Foley et al. (1993) noted similar variability with smaller numbers of specimens collected from fewer sites. Based on our comprehensive collections, more than half of the *An. farauti* 4 specimens would be identified microscopically as *An. koliensis*, with another 15% identified as *An. punctulatus.* Clearly, this species is not isomorphic, with *An. farauti* s.s. having, more often than not, white scaling on the proboscis. These findings are supported by a recent phylogenetic analysis based on the 18S ribosomal subunit (Beebe et al. 2000c). These workers showed that *An. farauti* 4 was more closely related to those species with white scaling on the proboscis and was included in the Punctulatus clade rather than the Farauti clade, which contained the species with an all black proboscis. We also found *An. koliensis* to be polymorphic, 15% of specimens of this species would be wrongly identified by microscopic examination as either *An. farauti* or *An. punctulatus.*

Thus, there are serious difficulties in microscopic identification of these two species. This problem is exacerbated by the fact that *An. farauti* 4, *An. koliensis*, and *An. punctulatus* occur sympatrically over a large

**Table 2. Proboscis morphology of 5 members of the *An. punctulatus* group collected in Papua New Guinea and identified using DNA hybridisation and PCR-RFLP analysis**

| | *An. farauti* s.s. ($n$ = 1,131) | | | *An. farauti* 2 ($n$ = 1,050) | | | *An. farauti* 4 ($n$ = 842) | | | *An. koliensis* ($n$ = 1,223) | | | *An. punctulatus* ($n$ = 676) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Proboscis | f | k | p | f | k | p | f | k | p | f | k | p | f | k | p |
| Number | 1,128 | 0 | 3 | 1,048 | 1 | 1 | 235 | 472 | 135 | 151 | 1,035 | 37 | 4 | 16 | 656 |
| % | 99.7 | 0 | 0.3 | 99.8 | 0.1 | 0.1 | 28.0 | 56.0 | 16.0 | 12.3 | 84.7 | 3.0 | 0.6 | 2.4 | 97.0 |

f, farauti type (all blacked scaled labium); k, koliensis type (white patch of scales of varying size on the ventral surface towards the apex); p, punctulatus type (apical one-half of labium white scaled).

area on the northern side of the central ranges. In this region, extreme caution should be exercised in using proboscis morphology, and identification of field material should be confirmed by DNA hybridization or PCR-RFLP analysis.

**Larval Habitats.** Table 3 lists the bodies of water found to be larval sites of the *An. punctulatus* group throughout PNG. The majority of these sites could be described as small (≈20 cm deep, ≈5 $m^2$ in area) semipermanent pools (depending on prevailing rainfall) with well-established populations of flora and fauna and containing decaying plant matter. These included a variety of sites formed naturally or as result of human activity (Table 3). There were fewer, larger, and more permanent sites, such as swamps, fish ponds, and ponded creeks, but in these sites the larvae were usually found around the shallow edges where marginal and emergent vegetation as well as floating debris were present. A quite different type of site was that commonly used by *An. punctulatus* (93/193) and occasionally by *An. farauti* s.s. (3/65), *An. farauti* 2 (14/179), and *An. koliensis* (5/73). These were unestablished sites with little or no flora, fauna, or organic debris. They appeared most often as ruts in roads and walking tracks (105/118), but occasionally as natural ground pools (13/118). These sites were shallow (<10 cm) and small (surface area usually <2 $m^2$) and thus could be quite temporary, their persistence though would be difficult to assess because their existence depended on local rainfall patterns. For example, in April 1995, small road ruts around Madang were observed supporting large numbers of *An. punctulatus.* Because of regular rainfall these "temporary" sites persisted for at least 5 wk and produced up to four generations of mosquitoes.

**Table 3. Types of breeding sites used by members of the *An. punctulatus* group in Papua New Guinea**

| Type of site | No. located | Species | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | f1 | f2 | f3 | f4 | f6 | p1 | p2 | k | c |
| Unestablished site[a] | 118 | 3 | 14 | 0 | 0 | 0 | 93 | 0 | 5 | 0 |
| Ground pool | 117 | 19 | 78 | 3 | 2 | 2 | 35 | 3 | 17 | 0 |
| Wheel track | 45 | 9 | 21 | 0 | 1 | 0 | 19 | 0 | 15 | 1 |
| Drain | 42 | 6 | 19 | 0 | 3 | 3 | 20 | 0 | 18 | 0 |
| Water ponded by beach | 23 | 18 | 6 | 0 | 0 | 0 | 2 | 0 | 3 | 0 |
| Swamp | 17 | 3 | 8 | 0 | 0 | 1 | 2 | 0 | 2 | 0 |
| Pig wallow | 15 | 1 | 6 | 0 | 2 | 0 | 12 | 0 | 4 | 0 |
| Edge of creek/river | 15 | 2 | 11 | 0 | 0 | 0 | 1 | 0 | 2 | 0 |
| Water ponded by road | 7 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Well | 7 | 4 | 3 | 1 | 0 | 0 | 3 | 0 | 3 | 0 |
| Fish pond | 7 | 0 | 3 | 1 | 0 | 2 | 1 | 0 | 1 | 0 |
| Foot/hoof print | 6 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 3 | 0 |
| Flooded rubbish pit | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Rock pool | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| Total | 421 | 65 | 179 | 5 | 8 | 8 | 193 | 4 | 73 | 1 |
| Human made | | 23 | 69 | 2 | 7 | 5 | 148 | 0 | 48 | 1 |
| Natural | | 42 | 110 | 3 | 1 | 3 | 45 | 4 | 25 | 0 |

Abbreviations as in Table 1.

[a] Unestablished sites: small, shallow with little or no established flora, fauna or debris, as discussed in text.

**Distribution.** *Anopheles farauti* s.s. was collected from 239 localities (Fig. 3; Table 1), it was found along the entire coastline of the country and was the dominant coastal anopheline. In northern Australia, *An. farauti* s.s. was also found to be a coastal species (Cooper et al. 1996, 1997). In our surveys, >50% of collections were made <1 km from coast and >75% of collections were made within 10 km of the coast (Table 4). However, inland populations of this species were also encountered, 42 sites were >10 km from the sea, of which there were two >100 km inland and three at altitudes of over 300 m, the highest being 920 m (Tables 4 and 5). Several genotypes have been identified in this taxon (Beebe et al. 2000a) and there is evidence that these inland populations represent a distinct genotype (N.W.B. and R.D.C., unpublished data). Larvae of *An. farauti* s.s. can tolerate saline conditions (Sweeney 1987, Cooper et al. 1996) but brackish water is not obligatory for oviposition and this species is often found in fresh water. Typical breeding sites are swamps and pools formed by water courses (creeks and streams) that, because of coastal sand ridges, have become blocked or have restricted access to the sea, their salinity varying, depending on tides, storms, and rainfall. These types of sites are very common along the coastline of PNG. Accordingly, the majority of breeding sites used are natural (42/65); however, larvae were also found in human created sites in association with *An. farauti* 2, *An. punctulatus*, and *An. koliensis* (Tables 3 and 6). Although this species could be found far from human habitation and natural sites were most often used for oviposition there was a positive correlation between the occurrence of is species and human habitation (Table 7). This is possibly due to coastal villages being located, out of necessity, close to a source of fresh water which can act as potential breeding site of this species.

*Anopheles farauti* 2 was collected from 324 localities (Fig. 4; Table 1) primarily from the south side of the central ranges where it was the most common and widely distributed member of the *An. punctulatus* group. North of the ranges it was also widespread but

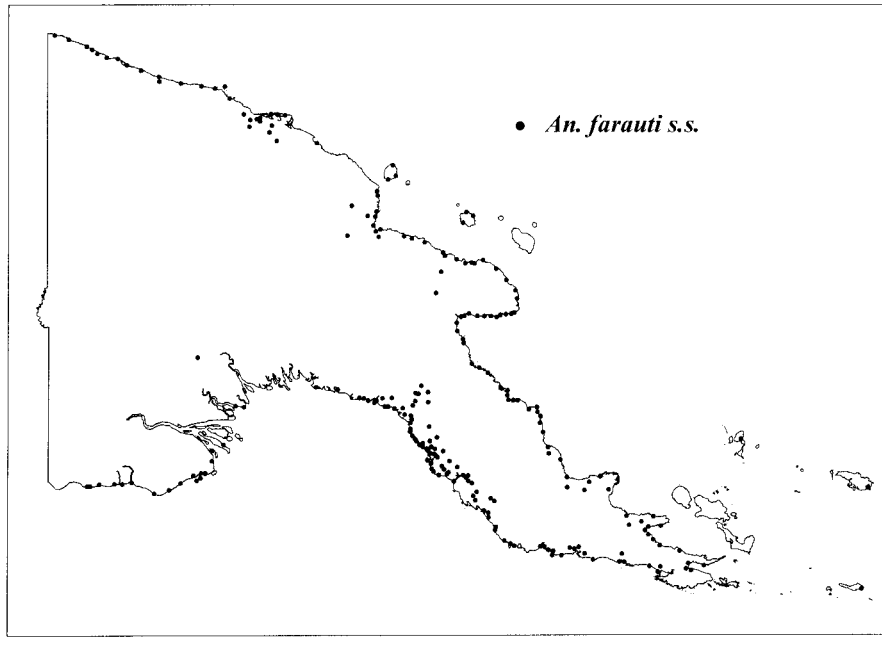


**Fig. 3.** Map of Papua New Guinea showing localities positive for *Anopheles farauti* s.s.

less common possibly due to competition with *An. koliensis* and *An. farauti* 4. Predominantly a species of inland, lowland river valleys, *An. farauti* 2 was also commonly found on the coast and on a number of small off-shore islands. It was collected in the highlands where 35 sites were over 300 m; three of which were over 1,500 m, with the highest being 1,740 m (Table 5). The most frequented breeding sites were small ground pools and pools formed in creek and riverbeds (Table 3). The occurrence of *Anopheles farauti* 2 had a positive correlation with human habitation (Table 7) and this species readily used artificial breeding sites such as drains and wheel ruts (Table 3). The larvae of *An. farauti* 2 were found in association with all the other members of the *An. punctulatus* group except *An. clowi* (Table 6).

*Anopheles farauti* 3 was collected from only 13 localities (Fig. 5; Table 1) and was uncommon in PNG with a limited distribution restricted to the southern plains of the Fly River region. Larvae were found in well-established water bodies such as the edges of fish-ponds, wells, and natural pools. This species appears to have adapted to the drier monsoon climate of northern Australia and was the most common member of the *An. punctulatus* group found in the Northern Territory, Australia (Cooper et al. 1996). In PNG it was found in the drier (<2,000 mm p.a.) parts of the south plains, a region influenced by the same monsoon climate as northern Australia. This species appears not to have dispersed widely into the continuous hot wet climate regions of PNG.

**Table 4. Number of collections made of each member of the *An. punctulatus* group and their proximity to the coast**

| Species | Distance from the coast, km | | | |
|---|---|---|---|---|
| | 0–<1 | 1–<10 | 10–<100 | >100 |
| *An. farauti* s.s. | 136 | 61 | 40 | 2 |
| *An. farauti* 2 | 40 | 78 | 152 | 54 |
| *An. farauti* 3 | 0 | 1 | 5 | 7 |
| *An. farauti* 4 | 3 | 3 | 34 | 3 |
| *An. farauti* 6 | 0 | 0 | 0 | 8 |
| *An. punctulatus* | 24 | 39 | 138 | 10 |
| *An.* sp. nr. *punctulatus* | 0 | 0 | 0 | 10 |
| *An. koliensis* | 39 | 35 | 151 | 21 |
| *An. clowi* | 0 | 1 | 0 | 0 |

*Anopheles farauti* 4 was recorded from 43 localities (Fig. 5; Table 1) distributed throughout the inland lowland river valleys north of the central ranges, in most cases below 300 m. It was only occasionally found near the coast. South of the central ranges it was recorded from one isolated location on the Papuan Peninsula. An erroneous record of this species was made from the northern Fly River area (Cooper et al. 1997), but further analysis has shown that this misidentification was the result of cross reactivity with the *An. farauti* 2 DNA probe. In the Sepik, Ramu, and Markham River valleys this species was quite abundant being the dominant anopheline at a number of localities. It had a positive association with humans (Table 7), and in villages around Lae in 1996 it was the major biting anopheline making up >90% of the catch. However, in the Madang area, although commonly collected in encephalitis vector surveillance light traps, it was not readily collected on human baits. *Anopheles farauti* 4 used artificial sites for oviposition (Table 3) and was commonly found in association with *An. punctulatus* and *An. koliensis* larvae (Table 6).

**Table 5. Number of collections made of each member of the *An. punctulatus* group and their elevation above sea level**

| Species | Altitude in meters (highest) | | | | |
|---|---|---|---|---|---|
| | 0–<150 | 150–<300 | 300–<1,000 | 1,000–<1,500 | >1,500 |
| *An. farauti* s.s. | 233 | 3 | 3 (920) | 0 | 0 |
| *An. farauti* 2 | 276 | 13 | 27 | 5 | 3 (1,740) |
| *An. farauti* 3 | 13 (100) | 0 | 0 | 0 | 0 |
| *An. farauti* 4 | 32 | 10 | 1 (320) | 0 | 0 |
| *An. farauti* 6 | 0 | 0 | 0 | 1 | 7 (2,000) |
| *An. punctulatus* | 135 | 20 | 43 | 10 | 3 (1,760) |
| *An.* sp. nr. *punctulatus* | 5 | 4 | 1 (640) | 0 | 0 |
| *An. koliensis* | 219 | 12 | 14 | 0 | 1 (1,600) |
| *An. clowi* | 0 | 0 | 1 (480) | 0 | 0 |

*Anopheles farauti* 6 was collected from eight localities (Fig. 5; Table 1) all restricted to highland river valleys and intramontane plains. All locations were above 1,000 m, the majority (seven sites) were over 1,500 m and the highest was 2,000 m (Table 5). In this restricted habitat it was quite common. It was the most abundant anopheline in the Baliem Valley, an extensive intramontane plain above 1,500 m, situated in the central ranges in Irian Jaya 250 km to the west of the PNG border (R.D.C., unpublished data). This species is noticeably larger than all the other *An. farauti* species and is likely to be the large *An. farauti* recorded from highlands by Lee (1946).

*Anopheles punctulatus* was recorded from 211 localities (Fig. 6; Table 1) and was common and widespread throughout PNG. Only in the southern plains was its occurrence sparse and in the drier, monsoon climate affected area south of the Fly River it was not recorded. It has a distribution similar to that of *An. farauti* 2 in that it frequents inland, lowland river valleys. However, *An. punctulatus* was also common on the coast and in the highlands with 56 sites above 300 m; three above 1,500 m, the highest being 1,760 m (Table 5). This species was rarely collected in $CO_2$ baited traps and then only in small numbers (<5/trap). However, it was readily collected as larvae, mainly from unestablished, temporary sites such as road ruts and wheel tracks. The ability to use these sites has extended the distribution and prevalence of this species, particularly over the last 20 yr where mineral and oil exploration and to a greater extent logging activities have created an extensive network of poorly drained, unsealed vehicle tracks. By adapting to these temporary sites it has exploited a niche where there is little competition from other anophelines as other anophelines rarely use this type of site (Table 3). Charlwood et al. (1986) noted that *An. punctulatus*, in the Madang area, used these sites to the exclusion of all other species and that *An. farauti* s.l. and *An. koliensis* larvae when artificially introduced into these sites failed to develop. Our result support the near exclusive use of these unestablished sites by *An. punctulatus;* however, mixed larval populations of this species and *An farauti* s.s., *An. farauti* 2, and *An. koliensis* were found. Natural sites were mainly ground pools in the sand or gravel of creek and riverbeds and occasionally rock pools (Table 3). Despite its ability to readily use artificial sites for oviposition, *An. punctulatus* did not have a significant association with human habitation (Table 7). This, though, is probably due to a bias in the collecting methods used in these surveys. This species is not readily attracted to $CO_2$ baited traps which were frequently set in villages, but it was readily collected as larvae in sites along roads and tracks far from human habitations.

*Anopheles* species near *punctulatus* was recorded from only10 localities (Fig. 5; Table 1) and was uncommon and had a limited distribution; being found at several scattered locations in lowland river valleys north and south of the central ranges. Most localities were in isolated and uninhabited areas from which larvae were collected in rock or ground pools in riverbeds in association with the larvae of *An. farauti* 2 and *An. punctulatus.*

*Anopheles koliensis* was collected from 246 localities throughout PNG (Fig. 7; Table 1). It was well distributed and common in the Sepik, Ramu, and Markham River valleys on the north side of the central ranges. Fewer localities were found on the southern side of the central ranges though it was common east of the

**Table 6. Larval associaitons for the members of the *An. punctulatus* group collected in Papua New Guinea**

| Species | No. of larval sites for each species | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | f1 | f2 | f3 | f4 | f6 | p1 | p2 | k | c |
| *An. farauti* s.s. (f1) | 65 | 30 | 0 | 1 | 0 | 11 | 0 | 16 | 0 |
| *An. farauti* 2 (f2) | | 179 | 5 | 1 | 2 | 51 | 2 | 35 | 0 |
| *An. farauti* 3 (f3) | | | 5 | 0 | 0 | 2 | 0 | 2 | 0 |
| *An. farauti* 4 (f4) | | | | 8 | 0 | 5 | 0 | 3 | 0 |
| *An. farauti* 6 (f6) | | | | | 8 | 0 | 0 | 0 | 0 |
| *An. punctulatus* (p1) | | | | | | 193 | 1 | 33 | 1 |
| *An.* sp. nr. *punctulatus* (p2) | | | | | | | 4 | 0 | 0 |
| *An. koliensis* (k) | | | | | | | | 73 | 1 |
| *An. clowi* (c) | | | | | | | | | 1 |

**Table 7. Association between human habitation and the presence or absence of members of the *An. punctulatus* group in Papua New Guinea**

| Species | Association with humans | | |
|---|---|---|---|
| | Uninhabited sites (260) | Inhabited sites (534) | Fisher exact test $P$ = |
| *An. farauti* s.s. | 58 | 181 | 0.001[a] |
| *An. farauti* 2 | 144 | 180 | 0.001[a] |
| *An. farauti* 3 | 7 | 6 | 0.093 |
| *An. farauti* 4 | 2 | 41 | 0.001[a] |
| *An. farauti* 6 | 1 | 7 | 0.188 |
| *An. punctulatus* | 60 | 151 | 0.070 |
| *An.* sp. nr. *punctulatus* | 1 | 9 | 0.110 |
| *An. koliensis* | 34 | 212 | 0.001[a] |
| *An. clowi* | 1 | 0 | — |

[a] $P < 0.05$ $H_o$ rejected, an association does exists between these species and human habitation.

Papuan Gulf. In the southern plains of the Fly River its distribution was sparse and like *An. punctulatus* was not found south of the Fly River. It most frequently inhabited lowland river valleys below 300 m and, although it was recorded at 1,600 m, it was uncommon in the highlands. It was also readily found along the coast. Ground pools and pools in watercourses were common natural breeding sites. This species readily used artificial sites such as well-established wheel ruts and drains around human habitations (Table 3). In these larval habitats it was associated with all of the other members of the *An. punctulatus* group with the exception of *An. farauti* 6 (Table 6). This species had a positive correlation with human habitation (Table 7).

*Anopheles clowi* was collected as larvae from one location (Fig. 5) in PNG. This species has only been recorded previously from Jayapura in Irian Jaya, 600 km to the west of our collection site (Rozeboom and Knight 1946). It has a proboscis morphology similar to *An. koliensis* and it may have been collected from other locations in PNG in the past and misidentified. However, with PCR analysis this species has its own distinct RFLP (Cooper et al. 2000) and in this study was not recognized from any other locations. Therefore it is likely that *An.* clowi is a relict species with a sparse and limited distribution.

Foley et al. (1993) collected *Anopheles farauti* 5 from one locality in the highlands of PNG and identified it by allozyme analysis. This species was not collected in any of the surveys reported on here. The PCR-RFLP analysis of this species (Beebe and Saul 1995) indicated a very similar fragment pattern to that displayed by *Anopheles subpictus*, a coastal species in PNG (R.D.C., unpublished data).

**Dispersal.** In the Australian region, New Guinea is regarded as one of the epicentres for insect speciation and radiation. This is most likely from ancestors of Oriental stocks, although some original Gondwanic elements may have survived the drying of Australia in the uplifted central ranges and more recently there is the possible penetration of drought adapted species from northern Australia (Marks 1960, Taylor 1972). The climate throughout much of PNG is quite uniform being continuously hot wet and most members of the *An. punctulatus* group have evolved in and adapted to this climate.

Dispersal of members of the *An. punctulatus* group in PNG may have been aided directly by human agen-

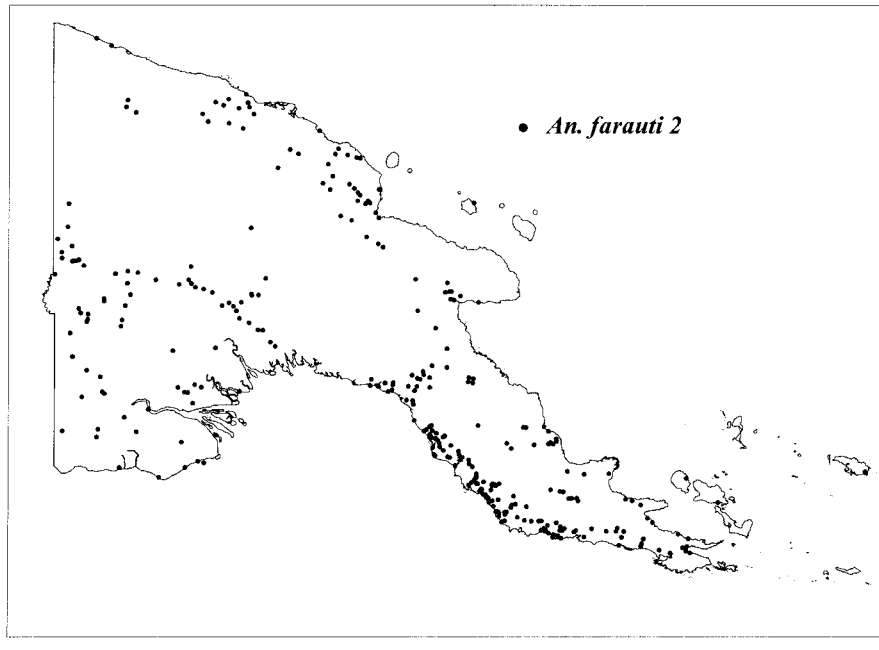


**Fig. 4.** Map of Papua New Guinea showing localities positive for *Anopheles farauti* 2.

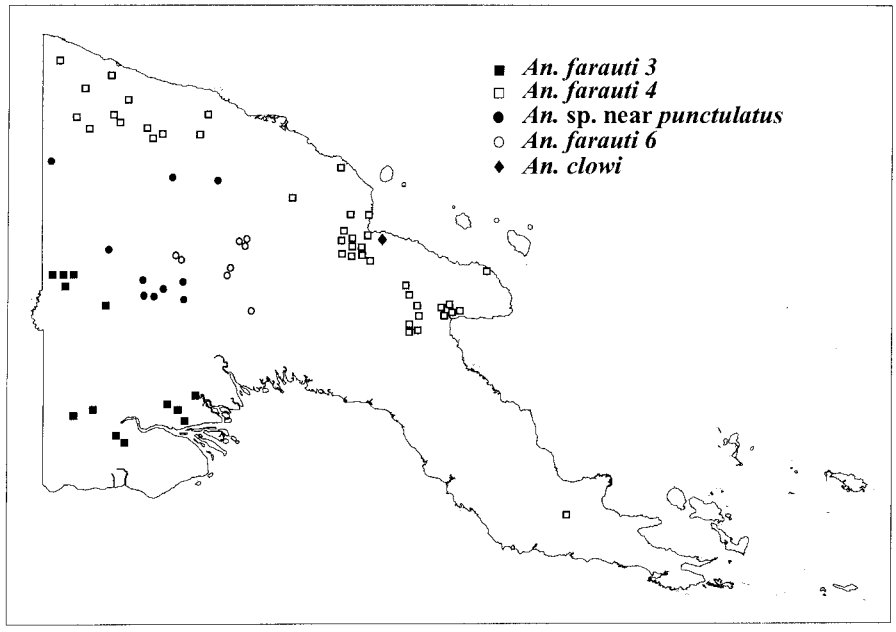


**Fig. 5.** Map of Papua New Guinea showing localities positive for *Anopheles farauti* 3, *An. farauti* 4, *An. farauti* 6, *An.* sp. near *punctulatus* and *An. clowi.*

cies such as aircraft or boats. However, dispersal is more likely due to appetential flight of the adult females responding to cues in seeking a blood meal, a resting site and an oviposition site (Belkin 1962, Service 1997). Thus, for dispersal the availability of suitable breeding sites is essential. Members of the *An. punctulatus* group do not appear to have become very specialized in this area. Ground pools, natural or human made, are the most common sites used by all species and these types of sites are ubiquitous in PNG. Another prerequisite for dispersal is access to a suitable blood source. As all members of the *An. punctulatus* group have been found far from human habitation it appears that none have a high degree of

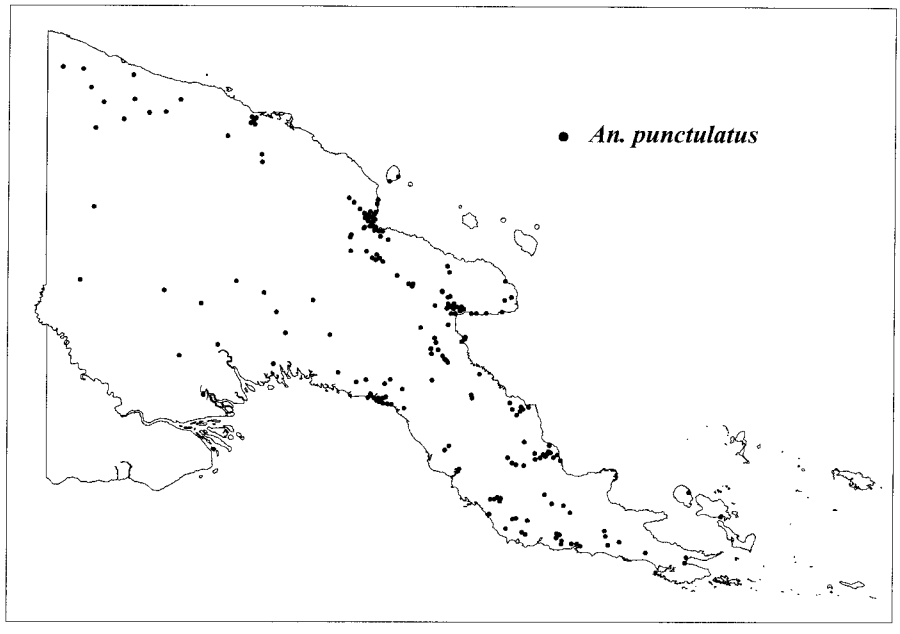


**Fig. 6.** Map of Papua New Guinea showing localities positive for *Anopheles punctulatus.*

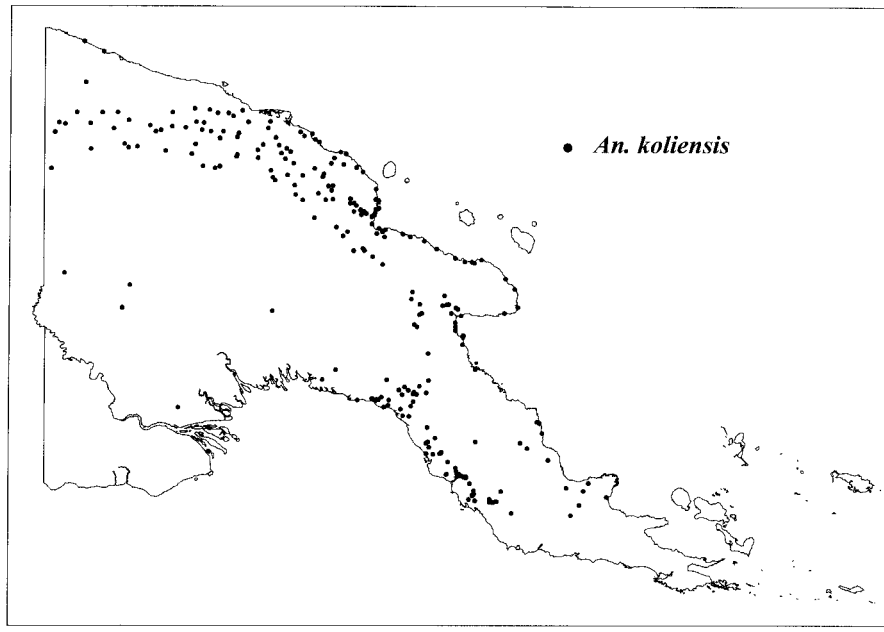


**Fig. 7.** Map of Papua New Guinea showing localities positive for *Anopheles koliensis.*

anthropophilism. Charlwood et al. (1985, 1986) working in the Madang area noted that *An. farauti* s.l. and *An. koliensis* could be quite zoophilic, depending on the availability of alternative blood sources such as pig and dog. Thus, limits of human population are not likely to restrict the distribution of these species. These factors would imply that all members of the group should be widespread throughout PNG. This though is not the case as some members of the group have restricted distributions and in some areas are uncommon indicating ecological or physical barriers exist which effect their dispersal and successful colonization.

Two obvious barriers to dispersal are, first, the elevational climate change caused by the central ranges. Only *An. farauti* 6 has become fully adapted to this cooler environment. *Anopheles punctulatus* has penetrated this region to some extent and *An. farauti* 2 and *An. koliensis* only sufficiently to allow dispersal from the north to south lowlands, most likely through highland valleys. *Anopheles farauti* 4 is a lowland species of northern PNG that finds the central ranges a barrier to dispersal into the south. Except for an isolated population on the Papuan Peninsula, the distribution of this species ends just south of Lae. At this point the mountains of the central ranges come right to the coast and for over 150 km there is no coastal plain but only small embayments.

The second barrier is that caused by the interface of the continuous hot wet climate, which influences most of PNG, and the drier seasonal monsoon climate that occurs in the southern plains south of the Fly River. This interface is a major discontinuity for many insect species and very few can penetrate and adapt to the drier regions in the south (Taylor 1972). However, *An. farauti* 2 and particularly *An. farauti* 3 have achieved this. Both species appear to have centers of origin in the southern lowlands of PNG, where they are genetically most complex (N.W.B. and R.D.C., unpublished data). However, *An. farauti* 3 is now better represented, in terms of numbers and range, in northern Australian than in PNG (Cooper et al. 1996). *Anopheles farauti* 2 has also successfully colonized the drier open forests and woodlands of Cape York Peninsula and to a lesser extent the Northern Territory (Cooper et al. 1996). This species appears to be the most adaptable and, with regards to dispersal, the most successful member of the *An. punctulatus* group having a wide range both within and outside PNG (Cooper et al. 1996, Beebe et al. 2000b). Our study implies that *An. punctulatus* and *An. koliensis* cannot cross this climate interface because neither were well represented in the southern plains and neither were found south of the Fly River, nor have they been found in northern Australia.

*Anopheles farauti* s.s. had the widest distribution of any member of the *An. punctulatus* group in the southwest Pacific (Foley et al. 1994, Cooper et al. 1996). This is due to its adaptation to a coastal habitat, which because of its high degree of uniformity allows for favorable dispersal. Service (1997) indicated that exposure to wind was another important factor favoring the dispersal of coastal mosquito species. Belkin (1962) believes that larval tolerance to brackish water could reduce competition for a newly arrived immigrant and help with colonization. Despite the presence of inland populations, *An. farauti* s.s. is a coastally adapted species and barriers must exist to its large scale dispersal inland. However, it appears that the absence of brackish water away from the coast is not

a barrier, as this species will readily oviposit in fresh water.

The breeding habitat data collected in our study suggests that the dispersal and colonization of members of the *An. punctulatus* group has benefited from the activities and movements of humans. In the early 1940s the activities associated with World War II would have created major opportunities for dispersal and more recently mineral exploration and logging activities, which are expanding throughout PNG, will affect the dispersal of these species.

The distribution of the members of the *An. punctulatus* group has been well documented for northern Australia (Sweeney et al. 1990; Cooper et al. 1995, 1996). There is limited information on the speciation and distribution of the group in the Solomon Islands and Vanuatu in the east (Foley et al.1994, Beebe et al. 2000b) and, to the west, further works need to done in Irian Jaya and the Moluccas. With regards PNG, our study has made a significant contribution to increasing the knowledge and understanding of the distribution of this group of medically important mosquitoes.

## Acknowledgments

The authors thank F. Torova (PNG Defense Force Health Services) and the members of his staff who supported this study; S. Popat (PNGDF Health Services) and A. Campbell (ADF Health Services) for excellent technical assistance; the pilots and crew of 162 Reconnaissance Squadron and 173 Surveillance Squadron, Australian Army Aviation Corps who supported the study and K. Barrington (Operations Officer, Australian Defense Staff, PNG) for excellent in-country logistical support. This article is published with the approval of the Director General of the Health Services Branch, Australian Defense Force.

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*Received for publication 22 November 2000; accepted 13 March 2001.*