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Malaria vectors of Papua New Guinea

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ABSTRACT

Understanding malaria transmission in Papua New Guinea (PNG) requires exact knowledge of which *Anopheles* species are transmitting malaria and is complicated by the cryptic species status of many of these mosquitoes. To identify the malaria vectors in PNG we studied *Anopheles* specimens from 232 collection localities around human habitation throughout PNG (using CO_2 baited light traps and human bait collections). A total of 22,970 mosquitoes were individually assessed using a *Plasmodium* sporozoite enzyme-linked immunosorbent assay to identify *Plasmodium falciparum*, *Plasmodium vivax* and *Plasmodium malariae* circumsporozoite proteins. All mosquitoes were identified to species by morphology and/or PCR. Based on distribution, abundance and their ability to develop sporozoites, we identified five species as major vectors of malaria in PNG. These included: *Anopheles farauti*, *Anopheles hinesorum* (incriminated here, to our knowledge, for the first time), *Anopheles farauti* 4, *Anopheles koliensis* and *Anopheles punctulatus*. *Anopheles longirostris* and *Anopheles bancroftii* were also incriminated in this study. Surprisingly, *An. longirostris* showed a high incidence of infections in some areas. A newly identified taxon within the Punctulatus Group, tentatively called *An. farauti* 8, was also found positive for circumsporozoite protein. These latter three species, together with *Anopheles karwari* and *Anopheles subpictus*, incriminated in other studies, appear to be only minor vectors, while *Anopheles farauti* 6 appears to be the major vector in the highland river valleys (>1500 m above sea level). The nine remaining *Anopheles* species found in PNG have been little studied and their bionomics are unknown; most appear to be uncommon with limited distribution and their possible role in malaria transmission has yet to be determined.

1. Introduction

The vectorial status of an anopheline species, with regards to malaria transmission, can be defined by its distribution, abundance, host preference, ability to develop the parasite, association with humans and longevity (Mackerras, 1947; Pampana, 1969). Crucial to studies of these parameters is the accurate identification of species involved. The recent advent of molecular biology and PCR has enabled species identification issues associated with isomorphic species complexes to be resolved and now entomological parameters can be reliably ascribed to accurately identified specimens.

In Papua New Guinea (PNG) the members of the Punctulatus Group, which originally included *Anopheles farauti* sensu latu, *Anopheles koliensis* and *Anopheles punctulatus*, have until recently been considered the major vectors of malaria and were regularly found infected with sporozoites (Peters and Standfast, 1960; Burkot et al., 1987). With the application of allozyme electrophoresis, DNA hybridisation and PCR identification techniques this group, which includes the Farauti Complex, is now known to consist of 11 species in PNG (Foley et al., 1993; Cooper et al., 2002; Bower et al., 2008). Of these *An. punctulatus*, *An. koliensis*, *An. farauti*, *An. farauti* 4 have been incriminated as vectors (Cooper and Frances, 2002; Benet et al., 2004) based on spatially limited studies from one or two localities. Outside of the Punctulatus Group four species have been incriminated: *Anopheles karwari*, *Anopheles subpictus*, *Anopheles bancroftii* and *Anopheles longirostris* (Afifi et al., 1980; Hii et al., 2000). Thus of the 20 species currently recognised in PNG, eight have been found to harbour sporozoites and the other 12 have been little studied or received scant attention either because they are uncommon species with limited distributions or they appear to have little or no association with humans.

Much of the recent work on the identification of malaria vectors in PNG has been restricted to the Maprik and Madang regions on the north coast of PNG (Afifi et al., 1980; Burkot et al., 1987; Hii et al., 2000; Benet et al., 2004). During the period 1992–2005 field surveys were conducted to identify the anophelines and to map

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their distribution throughout PNG (Cooper and Frances, 2002; Cooper et al., 2002, 2006). These surveys resulted in collections being made from 793 localities across PNG and constitute the most extensive and comprehensive distribution study on anophelines in that country. Using species-specific genomic DNA probes (Cooper et al., 1991; Beebe et al., 1994, 1996) and a complementary PCR diagnostic technique (Beebe and Saul, 1995) these surveys have now established the range of the anopheline fauna throughout PNG.

In the incrimination of vector species and for other entomological studies the presence of malaria sporozoites in anophelines was previously determined by dissecting out the salivary glands, rupturing under a cover slip and examining for sporozoites under a microscope. This method has a number of disadvantages; firstly it is very time consuming and laborious and where there is low malaria transmission it can be a fruitless task; secondly, with this method there is no way of determining the species of plasmodia. In the 1980s a number of ELISAs were developed based on species-specific monoclonal antibodies (Burkot et al., 1984; Wirtz et al., 1985). High sensitivity and specificity, the potential for high throughput and the ability to identify the species of plasmodia has resulted in ELISA being the standard for sporozoite detection and has been used routinely in malaria studies for the past 20 years. The technique does though have some limitations. Despite the sensitivity of the monoclonals selected false negatives can occur where sporozoite loads are low (<100/gland) (Wirtz et al., 1987, 1991). Additionally, false positives can occur where the ELISA detects sporozoite antigen (circumsporozoite protein) in mature oocysts and circulating sporozoites not in the salivary glands. The possible implications this issue may have in determining sporozoite rates has been quantified and discussed by Beier and Koros (1991). In an attempt to overcome this problem ELISAs are often carried out using only the head and thorax of the specimen; however the only way of being certain to avoid this problem altogether is to dissect out the glands and assay those separately, which is counter productive. This issue may not be that important as in the normal life cycle of the parasite sporozoites from ruptured oocysts can be expected to end up in the salivary glands in the majority of infections.

The specimens collected from the extensive surveys of Cooper et al. (2002, 2006) were processed by ELISA to incriminate the vector species across their entire range. The results of this study are reported here.

2. Materials and methods

Surveys were made throughout the main island of PNG, the climate and topography of which is described in Cooper et al. (2002). From the original 793 collection sites (Cooper et al., 2002, 2006) only those where adult collections were made within 500 m of human habitation were used in this study. This represented 232 trap nights using CO_2 baited light traps (Rohe and Fall, 1979) and by 26 human landing collections (in most cases from 1900 to 2200 h) as described in Cooper et al. (2002, 2006). The paucity of human landing catches was due to the fact that the priority was to sample anophelines from as wide an area as possible. This could only be done using traps; with this method several villages could be sampled in 1 night by one person as opposed to night landing catches where only one village could be sampled each night and as the method is exhausting it cannot be performed regularly.

In the field, specimens were initially identified by morphology and then preserved either in liquid nitrogen or desiccated on silica gel. In the laboratory the head and thorax were removed from all specimens belonging to the Punctulatus Group and this material was assayed for circumsporozoite protein; the abdomens were used for species identification. This was done using PCR-restriction fragment length polymorphism (PCR-RFLP) analysis of the internal transcribed spacer region 2 (ITS2) of the ribosomal DNA using the methods and primers of Beebe and Saul (1995), while *Msp*1 digestion of the ITS1 resolved *An. farauti* 8 from *An. farauti*.

In this study ELISAs were performed using species-specific monoclonal antibodies to detect circumsporozoite (CS) protein of *Plasmodium falciparum*, *Plasmodium vivax* 210 (and 247 variant) and *Plasmodium malariae*. The monoclonal antibodies and associated reagents were obtained from the Centers for Disease Control and Prevention (MS F42, Atlanta, GA 30341-3717, USA) and the methods used were those provided with the ELISA reagents and developed by Dr. Robert Wirtz (Centers for Disease Control and Prevention). CS protein-positive specimens were scored as those with absorption values greater than twice the mean ($n = 5$) negative control value (Beier et al., 1988). With species belonging to the Punctulatus Group only the head and thorax were processed for CS protein; with other species the whole mosquito was used.

3. Results

Collections were made from 258 locations throughout PNG (Fig. 1). From these, 22,970 specimens were collected and processed by ELISA for sporozoites. Eleven species were identified from this material including 9962 *An. farauti*, 1189 *An. hinesorum* (formerly *An. farauti* 2), 3 *An. torresiensis* (formerly *An. farauti* 3), 1535 *An. farauti* 4, 245 *An. punctulatus* and 8600 *An. koliensis*; all specimens were individually identified by PCR. Additionally, 793 *An. longirostris*, 476 *An. bancroftii*, 116 *An. subpictus* and 13 *An. karwari* were identified by morphology. All species were found positive for sporozoites except *An. torresiensis*, *An. subpictus* and *An. karwari* (Table 1). Fig. 1 indicates the location of sites where mosquitoes were found positive for malaria sporozoites. The numbers listed match those in Table 1 for the *Anopheles* species involved. In Fig. 1 the species of plasmodia is indicated for each positive site.

Based on the number of larval sites located for *An. punctulatus* it would appear that this species is abundant throughout its range (Cooper et al., 2002). However only low numbers of adults were attracted to CO_2 baited light traps and thus in this study this species is under-reported. All other species collected in this study appear to be readily collected in CO_2 baited traps based on the number of larval sites located.

An additional species within the Farauti Complex has recently been recognised and tentatively named *An. farauti* 8 (Bower et al., 2008). This species displays the same ITS2 PCR-RFLP as *An. farauti*, but occurs inland while *An. farauti* is restricted to the coast. The two species can be separated using the ITS1 region and the digest enzyme Msp1. Two specimens of *An. farauti* s.l. positive for *P. falciparum* sporozoites were collected >50 km inland and upon re-examination were found to be *An. farauti* 8.

From a number of collection sites there was a high incidence of infections reported in *An. longirostris*. This species is considered a minor vector at best, to confirm that the results were correct and not due to contamination all samples were rerun with the same results being recorded.

O.D.s, which can reflect the relative amounts of CS protein and thus sporozoite numbers, for the three *Plasmodium* species are recorded for each species of *Anopheles*. This data is shown in Table 2.

4. Discussion

Historically *An. farauti* s.l., *An. koliensis* and *An. punctulatus* (the original members of the Punctulatus Group) have been considered the major vectors of malaria in PNG due to their wide distribution, ability to occur in high densities, association with humans and

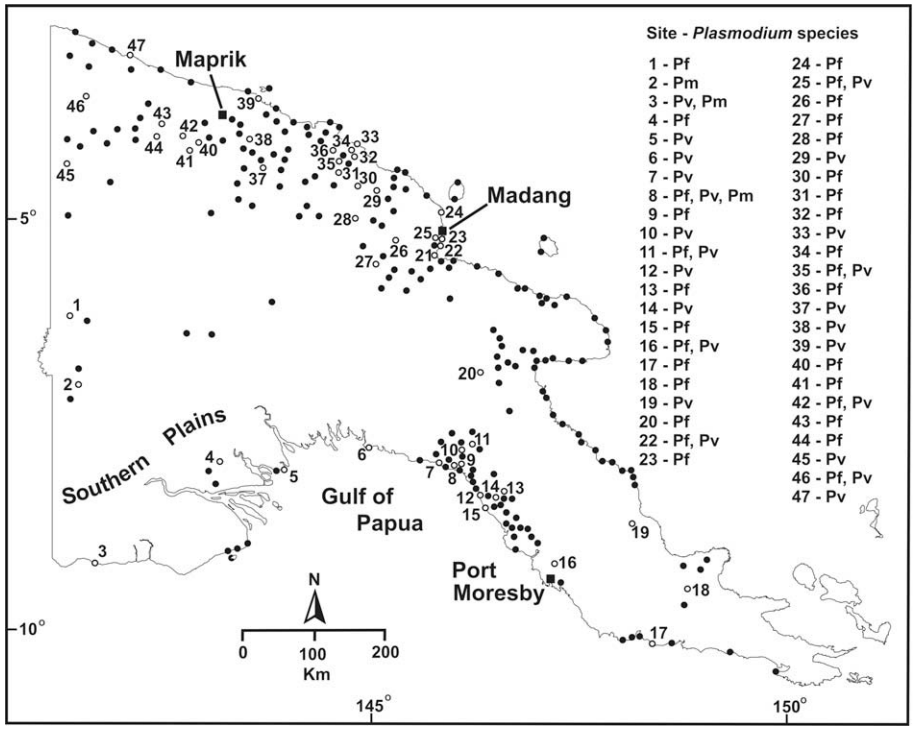

Fig. 1. Map of Papua New Guinea indicating sites where anophelines were collected (closed circles) and sites where anophelines positive for circumsporozoite protein were found (open circles). The list indicates where the species of plasmodia were found: *Plasmodium falciparum* (Pf), *Plasmodium vivax* (Pv) and *Plasmodium malaria* (Pm). The numbers associated with open circles correspond to those in Table 1.

Table 1
Anopheles species collected in Papua New Guinea and processed by ELISA for malaria circumsporozoite (CS) protein of *Plasmodium falciparum* (Pf), *Plasmodium vivax* (Pv) and *Plasmodium malariae* (Pm).

Species	No. assayed	No. positive for CS protein				Total	%	No. sites sampled	Site no. from Fig. 1
		Pf	Pv (210/247)	Pm	Pf and Pv (247)				
Anopheles farauti	9692	11	23 (2/21)[a]	7	0	41	0.40	110	3, 5, 6, 8, 9, 12, 15, 17, 33, 39, 47
Anopheles hinesorum	1189	6	3 (1/2)	1	0	10	0.84	61	1, 2, 4, 10, 14, 20, 25, 26
Anopheles torresiensis	3	0	0	0	0	0	0	1	
An. farauti 4	1535	12	3 (2/1)	0	0	15	0.98	46	27, 40, 41, 42
An. farauti 8	308	2	0	0	0	2	0.65	6	11, 13
Anopheles punctulatus	245	0	2 (2/0)	0	1	3	1.22	10	19, 46
Anopheles koliensis	8600	13	23 (5/18)[a]	2	3	41	0.46	123	7, 10, 11, 16, 18, 21, 28, 29, 30, 31, 32, 35, 36, 37, 38, 45
Anopheles longirostris	793	61[a]	1 (0/1)	0	0	62	7.82	21	1, 21, 22, 23, 24, 25, 44, 43
Anopheles bancroftii	476	1	0	0	0	1	0.21	8	34
Anopheles subpictus	116	0	0	0	0	0	0	9	
Anopheles karwari	13	0	0	0	0	0	0	2	
Total	22,970	106	55 (11/43)	10	4	175	0.75		

[a] Significantly higher value observed $P < 0.005$.

their ability to develop human malaria parasites. Accordingly, these species have received the most attention when implementing and evaluating control strategies.

However issues with the reliability of the morphological characters used to separate these three species and the recognition of additional isomorphic species within this group questions the vectorial status of these species. To overcome these problems in this study all specimens belonging to the Punctulatus Group were individually identified either by DNA hybridisation or by PCR. As a consequence *An. punctulatus* and *An. koliensis* were confirmed as major vectors of malaria in PNG due to their ability to develop the parasite, their wide-spread distribution and their abundance.

Within the Farauti Complex seven species are now recognised as occurring in PNG (Cooper et al., 2002; Bower et al., 2008). Within this complex three species have now emerged which are of considerable importance with regards to malaria transmission – *Anopheles farauti*, *An. hinesorum* and *An. farauti* 4. *Anopheles farauti* was found here to be the major coastal vector throughout PNG with sporozoite-positive specimens being found around the entire coastline of PNG (Table 1 and Fig. 1). Incrimination of this species supports earlier findings of Cooper and Frances (2002) from Buka and Bougainville Islands and Benet et al. (2004) from Madang. *Anopheles hinesorum* is widely distributed throughout PNG (Cooper et al., 2002) and here for the first time, to our knowledge, has been incriminated as a vector in a number of locations throughout its range (Table 1 and Fig. 1). *Anopheles farauti* 4 is common throughout the northern part of the country, it has recently been incriminated as a vector in the Madang region (Benet et al., 2004), and here we confirm the vectorial status of this species in other parts of its range (Fig. 1, sites 27, 40, 41 and 42). *Anopheles farauti* 4 presents a particular problem where its distribution overlaps with *An. hinesorum* and *An. koliensis* as it shares morphological markers with both of these species, markers that were

Table 2
Optical densities (absorbance at 414 nm) of *Plasmodium falciparum* (Pf), *Plasmodium vivax* (Pv) and *Plasmodium malariae* (Pm) circumsporozoite protein in *Anopheles* collected from Papua New Guinea.

Anopheles species	Parasite species	*n*	Median	Geometric mean	Range	25th percentile	75th percentile
Anopheles farauti	Pf	11	0.901	0.883	0.486–1.698	0.611	1.118
	Pv	23	0.661	0.632	0.116–1.289	0.549	0.848
	Pm	7	0.867	0.841	0.220–1.986	0.639	1.705
Anopheles hinesorum	Pf	6	0.303	0.339	0.216–0.675	0.250	0.463
	Pv	3	0.656	0.599	0.392–0.836	–	–
	Pm	1	0.222	–	–	–	–
An. farauti 4	Pf	12	0.381	0.330	0.153–0.610	0.263	0.438
	Pv	3	0.337	0.325	0.191–0.516	–	–
An. farauti 8	Pf	2	1.336	–	–	–	–
Anopheles punctulatus	Pf	1	0.556	–	–	–	–
	Pv	3	0.933	0.932	0.921–0.945	–	–
Anopheles koliensis	Pf	16	0.472	0.610	0.275–2.150	0.347	1.060
	Pv	25	0.655	0.697	0.230–2.053	0.441	1.127
	Pm	2	1.330	1.325	1.191–1.475	–	–
Anopheles longirostris	Pf	61	0.710	0.744	0.205–2.426	0.425	1.586
	Pv	1	0.628	–	–	–	–
Anopheles bancroftii	Pf	1	0.386	–	–	–	–
All species	Pf	110	0.610	0.677	0.205–2.426	0.391	1.127
All species	Pv	58	0.678	0.719	0.230–2.053	0.535	0.931
All species	Pm	10	0.950	0.785	0.182–1.986	0.534	1.533

previously considered diagnostically important and relied upon for field identification of these species (Cooper et al., 2002).

A fourth species, *An. farauti* 8, has recently been recognised within the Farauti Complex (Bower et al., 2008), in the present study it was also found to be a vector of malaria parasites. This species appears to have a limited distribution occurring inland on the eastern edge of the Gulf of Papua (Fig. 1, sites 11 and 13) however within this range it was common and it may play an important role in malaria transmission locally within this region.

Four species outside of the Punctulatus Group have also been incriminated as malaria vectors in PNG: *An. subpictus*, *An. karwari*, *An. bancroftii* and *An. longirostris*.

Anopheles longirostris has been little studied; a preference for a particular breeding site – pools in jungle or dense vegetation, appears to limit its association with humans, resulting in mixed reports of its affinity for human blood. Metselaar (1955), van den Assem and van Dijk (1958), Peters and Christian (1960) and Cooper et al. (2006) reported it biting humans while Lee and Woodhill (1944) and Charlwood et al. (1985) indicated that it was primarily zoophilic. Mackerras and Roberts (1947) found that *An. longirostris* were reluctant to feed on malaria-infected patients and of those that fed, none developed *P. vivax* although one developed *P. falciparum*. Burkot et al. (1988), working in the Madang area, processed 3000 *An. longirostris* for CS protein and found none positive, although CS protein was regularly found in specimens of *An. farauti* s.l., *An. koliensis* and *An. punctulatus* collected at the same time and from the same area. *Anopheles longirostris* was reported as sporozoite-positive by van den Assem and van Dijk (1958) with no further reports until the early 1990s when Hii et al. (2000) found five positive specimens in a sample of 2265 collected over a 3 year period in the Maprik area. In the present study *An. longirostris* positive for sporozoite protein were recorded from eight of the 20 collection sites; five closely located sites in the Madang area (sites 21–25) had very high numbers of infected specimens – site 21: five of 82, site 22: eight of 111, site 23: eight of 164, site 24: 37 of 197 and site 25: four of 146. Of these there was a significantly higher than expected number of infections with *P. falciparum* (61) than with *P. vivax* (1) ($\chi^2 = 13.3$ $P < 0.005$). At three sites *An. longirostris* was the dominant species collected while at the other two sites (21 and 25) appreciable numbers of other species were collected and infections were also found in five of 312 *An. koliensis* at site 21 and three of 94 *An. hinesorum* at site 25. Collections at sites 22 and 23 were made by human landing catches while those from sites 21, 24 and 25 were by CO_2 baited light traps; all collections were made at the end of the wet season in May 1995. Malaria transmission is intense in the Madang area with parasites rates of 37.5–42.5% for people of all ages and 53.6–56.7% in the 1–9 year age group, *Plasmodium falciparum* makes up 70–76% of infections and a high proportion of cases are asymptomatic (Cattani et al., 1986). Such transmission parameters may support the high incidence of sporozoite-positive mosquitoes, but why such high infection levels should occur in a rather obscure species such as *An. longirostris* and not in one of the main vector species which also can occur in the area is unclear. It is possibly that climatic factors at the time of these surveys created favourable breeding conditions for *An. longirostris* at the expense of other anopheline species. Somboon et al. (1993) reported a factor in the blood of some bovine (25%, 4/16 tested) and pigs (8.3%, 1/12 tested) that cross reacted with the *P. falciparum* monoclonal antibody resulting in false positives. Cattle are rare in the Madang area although pigs were quite common around the villages where the *An. longirostris* collections were made. However as none of the collections were made directly off pigs but were made either by human landing catches or by CO_2 baited light traps which attract unfed, host seeking mosquitoes, it is unlikely that the phenomenon reported by Somboon et al. (1993) could be responsible for the high incidence of infections recorded here. The findings here indicate that under certain climatic conditions *An. longirostris* may be an important local vector.

Anopheles bancroftii has a wide distribution throughout PNG but does not appear to be abundant anywhere within its range; it appears to be an indifferent feeder on humans and has rarely been collected biting humans in large numbers. It has been incriminated as a vector of malaria parasites by de Rook in 1929 (Lee et al., 1987), Metselaar (1957) in Papua Province on Indonesian side of New Guinea and in PNG by Hii et al. (2000) where over a 3 year collecting period in the Maprik area five sporozoite-positive specimens were identified in a collection of 642. In our study one specimen was found positive from 476 specimens collected from eight sites throughout PNG.

Anopheles subpictus is a Southeast Asian immigrant, it has been collected from a number of locations in PNG (Cooper et al., 2006) but is only common along the coast from Port Moresby west around the Gulf of Papua, and from within this region it has been incriminated as a vector on two occasions (Bang et al., 1947; Afifi et al., 1980). From collections made in the Madang area 754 *An. subpictus* were dissected for sporozoites but all were negative although in the same collections sporozoite-positive specimens of *An. farauti* s.l., *An. koliensis* and *An. punctulatus* were found (Afifi et al., 1980). *Anopheles subpictus* is a species complex consisting of four species (Suguna et al., 1994) one of which is a recognised vector of malaria in parts of coastal Southeast Asia. The specific identity of the PNG material in relation to other members of the complex is unknown. In this study only a small number were collected for analysis and none were found positive for CS protein.

Anopheles karwari, like *An. subpictus*, is a Southeast Asian introduction, and its present distribution is restricted to the north of PNG. Metselaar (1955) first incriminated it as a vector in Papua Province, Indonesia where he observed a sporozoite rate of 3.1%. In PNG it was first recorded as a vector of malaria by Afifi et al. (1980) but at the time was misidentified as *An. subpictus*. Hii et al. (2000) found it common in the Maprik area and recorded 14 sporozoite-positive specimens in a collection of 13,134 anophelines made by human landing and light trap catches over a 3 year period. Very few specimens were collected in this present survey and none were positive for sporozoites.

Anopheles subpictus, *An. karwari*, *An. bancroftii* and *An. longirostris* because of their loose association with humans, their limited and patchy distribution and their normally low abundance can only be considered as minor vectors of malaria. They may at times have some local importance under the right climatic and breeding conditions.

Of the remaining 10 *Anopheles* species found in PNG very little is known about their bionomics and their potential to transmit malaria. Species such as *An. clowi*, *An. papuensis* and *An. farauti* 5 appear to be quite rare with limited distributions and are unlikely to be involved in malaria transmission. *Anopheles* species near *punctulatus* is an uncommon species that so far has only been found in a few remote areas of PNG (Cooper et al., 2002). *Anopheles annulipes* sensu latu, both the coastal and highlands populations, appear to be zoophilic species; throughout their range they are readily collected as larvae but have never been collected biting humans – no specimens have been found with sporozoites (Peters and Standfast 1960; Cooper et al., 2006). *Anopheles torresiensis*, *An. meraukensis*, *An. novaguinensis* and *An. hilli* are all restricted to the southern plains of Western Province, a vast, sparsely populated region where few malaria vector studies have been performed. None of these species have been studied in any detail but owing to the fact that they appear to be uncommon with limited distribution their role, if any, in malaria transmission would be at a local level.

Anopheles farauti 6, the large farauti of Lee (1946), is found exclusively in the highland river valleys of New Guinea (>1500 m above sea level) (Cooper et al., 2002). It was studied by Peters and Christian (1960) who noted that it was the most common *Anopheles* species biting humans in the Waghi Valley where they recorded a sporozoite rate of up to 2.2% in this species. *Anopheles farauti* 6, confirmed by PCR-RFLP, was the most common anopheline biting humans in the Baliem Valley, a similar highland valley in Papua Province, Indonesia (Cooper et al., 2002). Malaria is unstable in these highland river valleys with low levels of transmission and periodic epidemics (Bangs and Subianto, 1999; Mueller et al., 2005). *Anopheles farauti* 6 is the only member of the Farauti Complex commonly found at these altitudes and is most likely an important malaria vector within its restricted range.

In PNG, *P. falciparum* and *P. vivax* are the two most common species and are wide-spread throughout the country; *P. malariae* is uncommon and has a patchy distribution; *P. ovale* is rare. Various studies, based on blood stage parasites, have determined the species composition as *P. falciparum*: 70%, *P. vivax*: 25% and *P. malariae*: 5% in the Madang area (Cattani et al., 1986), and *P. falciparum*: 59%, *P. vivax*: 29%, and *P. malariae*: 12% in the Maprik area (Mehlotra et al., 2000). In this study the sporozoite species composition was *P. falciparum*: 62%, *P. vivax*: 32% and *P. malariae*: 6% for the whole country. Since the late 1970s there has been a shift from *P. vivax* to *P. falciparum* predominance (Cattani et al., 1983; Cattani et al., 1986; Desowitz and Spark, 1987). This is thought to be due to the cessation, in the early 1980s, of the country's malaria control program and the appearance, in 1975, of resistance in *P. falciparum* to the antimalarial drug chloroquine (Grimmond et al., 1976). Continuing use of this compound over the next 15 years further suppressed *P. vivax* but not *P. falciparum*, until resistance in *P. vivax* appeared in 1989 (Rieckmann et al., 1989). In this study a predominance of *P. falciparum* infections was demonstrated in all anopheline species except for *An. farauti* and *An. koliensis* where there was a significantly high incidence of *P. vivax* infections (*An. farauti*: $\chi^2 = 21.8$ $P < 0.005$, *An. koliensis*: $\chi^2 = 18.8$ $P < 0.005$). This may be due to the shift to *P. falciparum* dominance not occurring in all parts of the country and in some areas *P. vivax* may still be more prevalent. This is possible as the drivers of this shift, DDT indoor residual spraying and chloroquine treatment, were never applied uniformly throughout the country. Additionally *P. falciparum*-infected mosquitoes appeared to be more common inland, the median distance from the coast for *P. falciparum*-infected mosquitoes was 32 km ($n = 27$, 0–330 km) while for *P. vivax* infected mosquitoes was 17 km ($n = 28$, 0–150 km) although there was no statistical difference ($P = 0.325$) in the distribution of *P. falciparum* (median 32, interquartile range 8.0–55.0) and *P. vivax* (median 17 interquartile range 0.0–57.0). *Anopheles farauti* is a coastal species usually found <1 km (Cooper et al., 2002) from the coast; only five *P. falciparum* sites were <1 km from the coast while 12 *P. vivax* sites occurred in this range. This however does not explain the high incidence in *An. koliensis* which is predominantly an inland species (Cooper et al., 2002).

The detection of *P. malariae* sporozoites is indicative of a long-lived mosquito as the extrinsic phase is >16 days for this species (Garnham, 1966). The high incidence of this parasite in *An. farauti* is biased by the fact that five of the seven collections occurred in Kerema (Fig. 1 site 8) which was sampled several times over a period of 5 years: 1994–1998. This location appears to be a foci for this species of plasmodia. Exceptional longevity must also be a feature of the vector, most likely *An. farauti* 6, responsible for the malaria epidemics that occur in the highlands of PNG, as the low temperatures of this region (mean 18.3 °C, range 13–23.7 °C) would require >20 days for the development of *P. vivax* (Pampana, 1969).

ELISA generated absorbance values can be indicative of the sporozoite numbers in the mosquito or in the salivary glands, if only the head and thorax are assayed. Using standard curves relating O.D. to sporozoite numbers Burkot et al. (1987, 1988) determined that there was no difference in the density of *P. falciparum* sporozoites in *An. punctulatus*, *An. koliensis* and *An. farauti* s.l. However for *P. vivax*, *An. punctulatus* had significantly more sporozoites than either *An. koliensis* and *An. farauti* s.l. In this study the absorbance values (Table 2) indicate that both *An. farauti* and *An. longirostris* were capable of developing more *P. falciparum* sporozoites than *An. koliensis*, *An. hinesorum* and *An. farauti* 4 which all had similar abilities to develop sporozoites for *P. falciparum*; however values for *An. longirostris* may be elevated by the fact that whole mosquitoes were used in the assay. *Anopheles punctulatus* appeared to be more efficient at developing *P. vivax* sporozoites than *An. farauti*, *An. hinesorum* and *An. koliensis* which appeared equally efficient.

Table 3
Vectorial status of the currently known *Anopheles* species in Papua New Guinea. Where known the species of plasmodia is indicated: *Plasmodium falciparum* (Pf), *Plasmodium vivax* (Pv) and *Plasmodium malariae* (Pm).

Species	Vectorial status	Distribution/abundance	Comments species of *Plasmodium* identified	Reference
Anopheles koliensis	Major	Widespread/common	Established vector three genotypes possible vectorial differences, Pf, Pv and Pm	Hii et al. (2000), Benet et al. (2004), this paper
Anopheles punctulatus	Major	Widespread/common	Established vector, Pf and Pv	Hii et al. (2000), Benet et al. (2004), this paper
Anopheles farauti	Major	Widespread/common coastally	Established vector, Pf, Pv and Pm	Cooper and Frances (2002), Benet et al. (2004), this paper
Anopheles hinesorum	Major	Widespread/common	Several genotypes, zoophilic on Buka and Bougainville Is. PNG, Pf, Pv and Pm	Cooper and Frances (2002), this paper
An. farauti 4	Major	Limited/common	Common throughout northern PNG only, Pf and Pv	Benet et al. (2004), this paper
Anopheles longirostris	Minor	Wide spread/uncommon	Possible species complex, Pf and Pv	Hii et al. (2000), this paper
An. farauti 8	Minor	Limited/uncommon	Recently discovered, species not studied, Pf	Bower et al. (2008), this paper
Anopheles bancroftii	Minor	Widespread/uncommon	Possible species complex, with varying host preferences, Pf, Pv and Pm	Lee et al. (1987), Hii et al. (2000), this paper
An. farauti 6	Minor	Limited/common in the highlands (>1500 m)	The large farauti of Lee (1946) incriminated on circumstantial evidence	Peters and Christian (1960)
Anopheles subpictus sensu latu	Minor	Limited/uncommon	Species complex – PNG species unknown	Bang et al. (1947), Afifi et al. (1980)
Anopheles karwari	Minor	Limited/uncommon	Previously misidentified as An. subpictus, Pf, Pv and Pm	Metselaar (1955), Afifi et al. (1980), Hii et al. (2000)
Anopheles meraukensis	None	Limited/uncommon	Species not studied – potential unknown Experimentally infected in Australia	Mackerras and Roberts (1947)
Anopheles novaguinensis	None	Limited/uncommon	Species not studied – potential unknown	
Anopheles torresiensis	None	Limited/uncommon	Species not studied – potential unknown	
An. species near punctulatus	None	Limited/uncommon	Species not studied – potential unknown	
Anopheles hilli	None	Limited/uncommon	Vector in Cairns, Australia; in PNG species not studied – potential unknown	Roberts and O'Sullivan (1948)
Anopheles annulipes sensu latu	None	Limited/uncommon	Two genotypes in PNG both zoophilic experimentally infected in Australia	Mackerras and Roberts (1947)
An. farauti 5	None	Limited/rare	Species not studied	
Anopheles clowi	None	Limited/rare	Species not studied	
Anopheles papuensis	None	Limited/rare	Species not studied	

However, for *An. hinesorum* and *An. punctulatus* and for a number of the other species assayed the numbers were small and comparisons should be viewed with caution. Burkot et al. (1988) also noted that for *An. punctulatus, An. farauti* s.l. *and An. koliensis*, *P. falciparum* sporozoites densities were about 10 times higher than *P. vivax* sporozoites densities. In this study, with all vector species combined and based on standard curves provided with the ELISA methods, *P. vivax* sporozoite densities were five times higher than those for *P. falciparum*.

This study highlights the lack of knowledge with regards to the bionomics of the anophelines of PNG and their role in malaria transmission. Table 3 provides an overview of the currently recognised species and their vectorial status which, for some species, is based on very limited information. As indicated a number of species – *An. torresiensis*, *An. meraukensis*, *An. novaguinensis*, *An.* species near *punctulatus* and *An. hilli* – have not been too adequately studied. Their limited distribution is partly responsible for this; however even in their restricted areas they may have the potential to occur in large numbers and may play an important, if local, role in malaria transmission. The findings in this study with regards to *An. longirostris* highlight the role that even a rather uncommon and obscure species can play in malaria transmission.

Further, the use of PCR is now the standard for verifying species identification and thus the species role in malaria transmission, but this technology is also raising new issues. *Anopheles farauti* 8, a recently discovered species based on molecular evidence, is here incriminated as a vector of malaria in PNG but as yet nothing is known of its behaviour or its distribution in PNG. The *An. koliensis* taxon is now known to contain three independently evolving rDNA genotypes suggesting the presence of three cryptic species (Benet et al., 2004; N.W. Beebe, unpublished data) which differ in their biting behaviour, a character important in monitoring control strategies. *Anopheles longirostris* and *An. bancroftii* also appear to be complexes of independently evolving rDNA genotypes, indicating the presence of several cryptic species (Beebe et al., 2001; N.W. Beebe, unpublished data). *Anopheles hinesorum*, incriminated for the first time, to our knowledge, in this study, is a complex of several rDNA genotypes, the members of two of these genotypes appear not to feed on humans (Cooper and Frances, 2002; N.W. Beebe, unpublished data). Until we fully understand the bionomics of the extant and newly emerging species and genotypes we will not have a complete understanding of how malaria is transmitted in PNG nor how it can be controlled or eliminated.

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