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MALARIA VECTORS ON BUKA AND BOUGAINVILLE ISLANDS, PAPUA NEW GUINEA

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ABSTRACT. Anophelines were sampled from 82 locations on Buka and Bougainville islands in Papua New Guinea by larval collections, carbon dioxide–baited mosquito traps, and human biting catches. *Anopheles farauti* s.s. was collected in larval surveys but infrequently in mosquito traps on both islands; on Buka Island this species was readily collected in human biting catches. *Anopheles farauti* 2 was commonly collected in larval surveys on both islands; however, it was not collected in either mosquito traps or human biting catches. *Anopheles punctulatus* was found only on Buka Island, where it was commonly collected as larvae, but rarely in human biting catches and mosquito traps. *Anopheles lungae* was collected as larvae from only 1 site on Bougainville. *Anopheles farauti* s.s. fed consistently throughout the night (1900–0600 h); small peaks at midnight and dawn were not statistically significant. Of 1,156 *An. farauti* s.s. specimens examined by enzyme-linked immunosorbent assay for malaria sporozoites, 20 were found to be positive; 12 were positive for *Plasmodium falciparum* and 8 were positive for *P. vivax* (247 variant = 5; 210 variant = 3). *Anopheles farauti* s.s. seems to be the major malaria vector on these islands, whereas *An. punctulatus* may play a minor role on Buka Island. *Anophele farauti* 2 is unlikely to be involved in malaria transmission on Buka or Bougainville islands.

KEY WORDS *Anopheles punctulatus* group, malaria, Bougainville, Papua New Guinea

INTRODUCTION

The islands of Buka and Bougainville are located in the southwestern Pacific at the northern end of the Solomon Island Archipelago, and make up the North Solomons Province of Papua New Guinea (PNG). Malaria occurs on both islands and dichlorodiphenyltrichloroethane (DDT) indoor residual spraying for vector control was implemented in 1961 and continued, at 2 spray rounds per year, until the early 1980s. Potential malaria vectors on the islands were identified as *Anopheles farauti* Laveran, *Anopheles punctulatus* Dönitz, and *Anopheles koliensis* Owen (Spencer 1961, 1971). These species belong to the *Anopheles punctulatus* group, which is now known to consist of 12 closely related species distributed throughout the southwestern Pacific and northern Australia (Foley et al. 1994; Cooper et al. 1996, 2002). Identification of the members of the group by morphological characteristics is unreliable. The *An. farauti* species are isomorphic and the other members are now known to be polymorphic for previously used diagnostic characters (Cooper et al. 2002). Because of this, over the last decade, various molecular-based techniques have been developed to accurately identify these species (Cooper et al. 1991, Beebe and Saul 1995). Although the original members of the *An. punctulatus* group were known to be major vectors in the region, the recent recognition of several cryptic species within the group has now resulted in uncertainty as to the actual role the different members play in transmission of malaria.

In the mid-1980s, the North Solomons Province attempted to secede from PNG. This resulted in a civil war that closed the province until 1998, when a peace agreement was signed and a multinational peace-keeping group (PKG) was installed while the political issues were resolved. Concern for the incidence of malaria in PKG personnel led to renewed interest in the malaria vectors of the province. The following report details surveys conducted to ascertain the species present and their role in malaria transmission.

MATERIALS AND METHODS

Survey area and timings: Collections of anopheline mosquitoes were made from Arawa, Tonu, and Buka areas of North Solomon Province, PNG (Fig. 1). Collections were made during the period February–March 1999.

Topography and climate of the region: Buka Island (area 850 km^2) is a raised coral reef composed of limestone overlaid with plastic clays. Most of the island (75%) is below 100 m; the highest point is 1,000 m. Much of the island has been cleared for copra plantations and native gardens. Bougainville Island (area 8,150 km^2) is made up of a series of volcanic peaks up to 2,600 m. More than one half of the island consists of hills and mountains. Coastal plains, made up of volcanic material distributed by rivers, terminate on the coast with swamps impounded by coastal sand dunes (Scott et al. 1967). The climate is continuous hot–wet with precipitation of 2,600–3,300 mm/year (median of 14 years of data); rainless periods on average do not exceed 2.5 days. The mean temperature is 27.3°C (24–30°C) and the average humidity is 80% (McAlpine et al. 1983).

Survey methods: Larval and human night biting collections were made in the Buka, Arawa, and Tonu areas, and carbon dioxide (CO_2)-baited mosquito trap (Rohe and Fall 1979) collections were

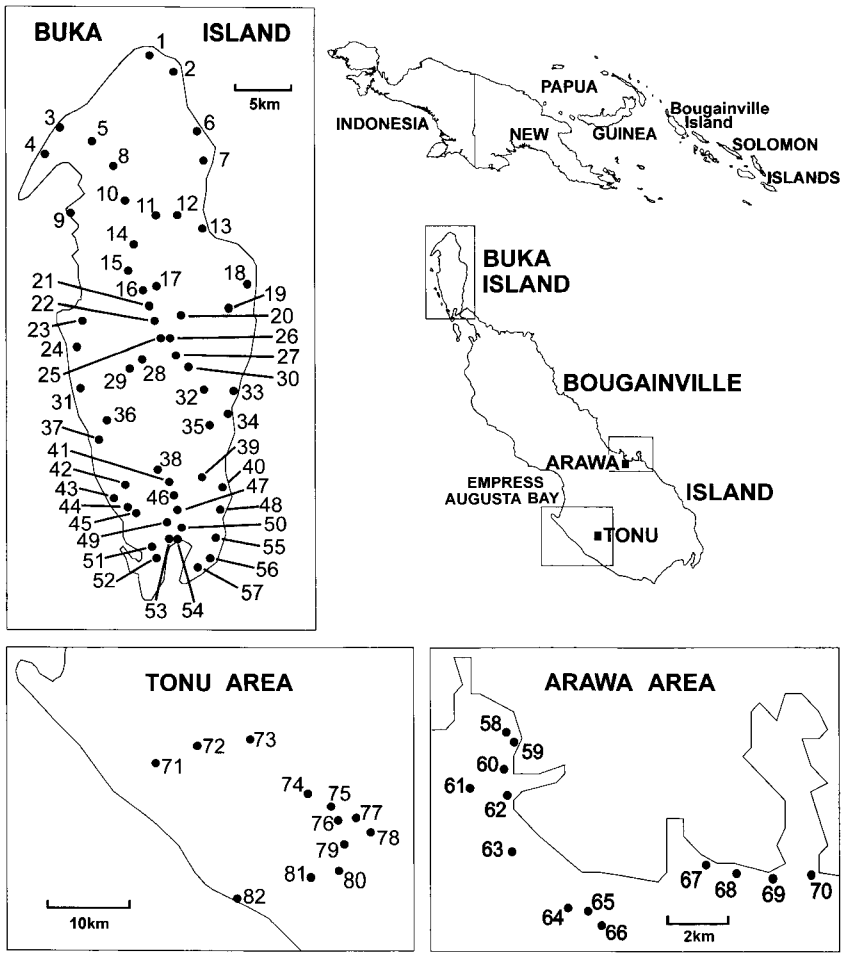

Fig. 1. Location of anopheline sites surveyed on Buka and Bougainville islands, Papua New Guinea (site numbers as in Table 1).

made in Buka and Arawa. Larval sites were classified as established or unestablished. Established sites varied in size from small temporary pools to permanent swamps; all had well-established flora and fauna and all contained some degree of organic matter and plant debris. Unestablished sites were all small temporary pools with little or no flora and fauna and no organic debris; wheel ruts (tire tracks) in unsealed roads were a common example of this type of site.

Anophelines collected as larvae were maintained in their site water, fed finely ground goldfish food, and reared through to adults. All adults were identified in the field by using *The Mosquitoes of the South Pacific (Diptera, Culicidae)* (Belkin 1962). Adults belonging to the *An. punctulatus* group then were preserved in liquid nitrogen, whereas specimens that were still larvae and pupae at the end of the study were preserved in 100% methanol. With this material, DNA was extracted from the abdomens by the Pat Roman method (Black and Munsterman 1996) and further identification was carried out by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis of the ribosomal DNA internal transcribed spacer 2 region (ITS2), by the method of Beebe and Saul (1995). Where numbers permitted, at least 5 specimens from each site were identified with PCR-RFLP analysis.

All specimens collected in traps and human biting were analyzed individually for the presence of human malaria sporozoites by the enzyme-linked immunosorbent assay (ELISA) procedure of Wirtz et al. (1987). The head and prothorax were used for this analysis; the remainder of the specimen was used for identification by PCR-RFLP analysis, as mentioned above.

Average hourly biting collections made over 5 nights (1900–0600 h) were compared and analyzed by a 1-way analysis of variance (ANOVA; SigmaStat for Windows, Jandel Scientific Software,

Table 1. Collection sites on Buka and Bougainville islands, indicating collection method, number and species collected, and larval site type (site numbers as in Fig. 1).

Site no.	Collection	Total collected	Species[1]			Established larval site	Unestablished larval site
			fs.s.	f2	p		
1	Larval	25	0	0	25		Drain
2	Larval	25	0	0	25		Drain
3	Larval	7	0	0	7		Wheel rut
4	Larval	6	0	6	0	Wheel rut	
5	Larval	9	0	5	0		Wheel rut
6	Larval	21	1	0	20		Wheel rut
7	Larval	32	0	0	32	Wheel rut	
8	Larval	9	0	5	0	Wheel rut	
9	Larval	15	2	3	0	Swamp	
10	Larval	16	0	5	0	Ground pool	
11	Larval	67	0	5	0	Wheel rut	
12	Larval	13	0	5	0		Wheel rut
13	Larval	70	0	0	70	Wheel rut	
14	Larval	33	0	5	14		Wheel rut
15	Larval	26	0	5	0	Swamp	
16	Larval	35	0	0	35		Drain
17	Larval	38	0	5	0	Swamp	
18	Larval	40	0	0	40	Wheel rut	
19	Larval	27	0	5	9		Wheel rut
20	Larval	6	0	0	6		Wheel rut
21	Larval	23	0	0	23	Wheel rut	
22	Larval	5	0	5	0	Swamp	
23	Larval	39	0	4	35	Wheel rut	
24	Larval	49	0	0	49	Drain	
25	Larval	55	0	0	55		Wheel rut
26	Larval	10	0	0	10	Creek	
27	Larval	26	0	0	26		Drain
27	Trap	0	—	—	—		
28	Larval	36	0	0	36	Wheel rut	
29	Larval	35	0	0	35	Wheel rut	
30	Larval	40	0	0	40		Wheel rut
30	Trap	0	—	—	—		
31	Larval	30	0	0	30		Drain
32	Larval	60	0	0	60	Wheel rut	
33	Larval	43	0	0	43	Wheel rut	
34	Larval	33	0	0	33		Wheel rut
35	Larval	56	0	0	56	Wheel rut	
36	Larval	15	0	3	12	Pig wallow	
37	Larval	87	0	0	87	Wheel rut	
38	Larval	10	0	5	0	Swamp	
38	Biting	4	1	0	3		
39	Larval	45	0	0	45	Wheel rut	
40	Larval	9	0	0	9		Wheel rut
41	Larval	32	0	0	32		Pig wallow
42	Larval	7	0	7	0	Ground pool	
43	Larval	59	0	5	0	Wheel rut	
44	Larval	20	0	4	16		Wheel rut
45	Larval	8	0	5	0	Ground pool	
46	Larval	20	0	0	20		Wheel rut
47	Larval	10	0	6	0	Wheel rut	
47	Biting	1	0	0	1		
48	Larval	26	0	0	26		Wheel rut
49	Larval	31	0	1	28	Wheel rut	
49	Trap	0	—	—	—		
50	Larval	6	0	6	0	Drain	
51	Larval	35	0	5	0	Swamp	
52	Larval	26	0	5	0	Swamp	
52	Biting	64	64	0	0		
52	Trap × 2	6	5	0	1		
53	Larval	11	0	0	11		Wheel rut
53	Trap	0	—	—	—		
54	Larval	16	0	5	11		Pig wallow
54	Trap	0	—	—	—		

Table 1. Continued.

Site no.	Collection	Total collected	Species[1]			Established larval site	Unestablished larval site
			fs.s.	f2	p		
55	Larval	47	0	3	17	Wheel rut	
55	Trap	0	—	—	—		
56	Larval	6	3	3	0	Drain	
56	Trap	0	—	—	—		
57	Larval	25	5	0	0	Swamp	
57	Biting × 5	1,500	80	0	0		
57	Trap × 4	27	27	0	0		
58	Larval	18	2	3	0	Ground pool	
58	Trap × 10	5	5	0	0		
59	Biting × 3	0	—	—	—		
60	Larval	6	0	5	0	Drain	
61	Larval	2	0	2	0	Drain	
62	Larval	5	0	5	0	Wheel rut	
63	Larval	10	0	5	0	Wheel rut	
64	Larval	15	0	5	0		Wheel rut
64	Trap × 3	0	—	—	—		
65	Larval	14	0	5	0	Ground pool	
65	Trap × 3	0	—	—	—		
66	Larval	5	0	5	0		Wheel rut
66	Trap × 2	0	—	—	—		
67	Larval	39	2	3	0	Drain	
68	Larval	5	3	2	0	Wheel rut	
69	Larval	3	1	2	0	Ground pool	
70	Larval	15	3	2	0	Ground pool	
71	Larval	8	0	5	0	Ground pool	
72	Larval	29	0	5	0	Wheel rut	
73	Larval	1	0	1	0	Wheel rut	
74	Larval	6	0	6	0	Drain	
75	Larval	13	0	5	0	Ground pool	
76	Larval	13	0	5	0		Wheel rut
77	Larval	17	0	5	0	Ground pool	
78	Larval	29	0	5	0		Wheel rut
78	Biting × 2	0	—	—	—		
79	Larval	1	0	1	0	Ground pool	
80	Larval	48	0	5	0		Wheel rut
81	Larval	2	0	2	0	Ground pool	
82	Larval	43	5	0	0	Ground pool	

[1] fs.s. = *Anopheles farauti* s.s.; f2 = *An. farauti* 2; p = *An. punctulatus*.

Table 2. *Anopheles farauti* s.s. collected human biting in 30-min catches and the number positive for malaria (*Plasmodium* spp.) sporozoites on Buka Island, Papua New Guinea, March 6–10, 1999.

Time of collection (h)	No. collected (mean ± SE; n = 5)	Number infected with sporozoites		
		P. falciparum	*P. vivax* 210	*P. vivax* 247
1900–2000	19.5 ± 8.7			2
2000–2100	26.5 ± 9.5	1	1	
2100–2200	23.3 ± 12.8	2		
2200–2300	36.5 ± 6.4	5		2
2300–2400	37.0 ± 13.9		2	
2400–0100	44.3 ± 9.8			
0100–0200	41.0 ± 5.1			
0200–0300	34.0 ± 13.1	4		
0300–0400	25.0 ± 5.0			
0400–0500	34.3 ± 12.3			
0500–0600	65.0 ± 29.5			

San Rafael, CA). The level of significance was set at $P < 0.05$.

RESULTS

Identification

Specimens identified as belonging to the *An. punctulatus* group were initially separated on the basis of proboscis morphology. Those that had an all black-scaled proboscis were considered to be *Anopheles farauti* s.l., and those with the apical one third to one half of the proboscis pale scaled were considered to be *An. punctulatus.* Further analysis with PCR-RFLP identified *An. farauti* s.s. and *An. farauti* 2 from within the *An. farauti* s.l. material and confirmed the morphological identification of *An. punctulatus.* Because these 3 species are very consistent with regards to proboscis coloration and in the absence of *An. farauti* 4 and *An. koliensis,* which are quite polymorphic for this character, reliance can be placed on identifying *An. punctulatus* by proboscis morphology in this region of PNG (Cooper et al. 2002).

Buka Island

A comprehensive larval survey based on 57 sites was conducted on Buka Island (Fig. 1 and Table 1). *Anopheles punctulatus* (37 sites) and *An. farauti* 2 (28 sites) were abundant and widespread throughout the island and *An. farauti* s.s. was found at 4 coastal locations. Both *An. farauti* s.s. and *An. farauti* 2 preferred established sites (26 established, 6 unestablished), whereas *An. punctulatus* inhabited both types of sites (18 established, 19 unestablished), although *An. punctulatus* was never found in large permanent swamps. Thirteen CO_2 trap collections were made at 5 coastal sites (sites 52, 53, 54, 55, and 57) and 4 inland sites (sites 27, 30, 47, and 49; Table 1). *Anopheles farauti* s.s. was collected from traps at sites 52 and 57 with 5 and 27 specimens collected, respectively, whereas 1 *An. punctulatus* was collected in a trap at site 52. At site 57, 5 human night biting catches were made (1900–0600 h with 30-min catches/h); only *An. farauti* s.s. was identified from these catches and for this species a biting rate of 58.3 bites/human/h was determined. A fairly consistent biting pattern throughout the night was noted; small peaks at midnight and a predawn peak at 0500–0600 h (Table 2) were not statistically significant (1-way ANOVA: F = 1.41, df = 10,22, $P = 0.24$). Biting collections (1900–2400 h) made at site 3 collected 1 *An. punctulatus*; and from site 13, 1 *An. farauti* s.s. and 3 *An. punctulatus* were collected; whereas 3 biting collections made at site 52 resulted in an average biting rate of 12.4 bites/human/h for *An. farauti* s.s.

Arawa area

Twelve larval sites were sampled in the Arawa area (Fig. 1 and Table 1) and all contained *An. farauti* 2, whereas 5 sites also contained *An. farauti* s.s. and 1 site (site 63) contained *Anopheles lungae* Belkin and Schlosser. All sites were small pools, with the majority well established (10 established, 2 unestablished). Three human biting catches were made (1800–2400 h) at site 59, with no anophelines collected. Eighteen CO_2 traps were set at sites 58, 64, 65, and 66, with 5 *An. farauti* s.s. being collected from traps set at site 58.

Tonu area

Twelve larval sites were samples in the Tonu area (Fig. 1 and Table 1). Of these, 11 contained *An. farauti* 2 and 1 coastal site contained *An. farauti* s.s. All sites were small pools, with 9 established and 3 unestablished. Two human biting collections (1900–2100 h) were made at site 78, with no anophelines collected.

Sporozoite infections

An ELISA, to detect the presence of sporozoite antigen, was carried out on all anophelines collected biting humans. Of a total of 1,157 *An. farauti* s.s. collected on Buka Island, 19 were found to be infected with human malaria sporozoites, and all were from site 57. Twelve specimens carried *Plasmodium falciparum* (Welch) and 7 carried *Plasmodium vivax* (Grassi and Feletti) (247 variant = 4; 210 variant = 3; Table 2). None of the 4 *An. punctulatus* collected biting humans were positive. Of the 5 *An. farauti* s.s. collected in traps at site 58 in the Arawa area on Bougainville Isand, one was positive for *P. vivax* (247 variant).

DISCUSSION

Previous anopheline surveys identified *An. farauti* s.l., *An. punctulatus,* and *An. koliensis* from Buka Island (Spencer 1961) and *An. farauti* s.l. and *An. lungae* from Bougainville Island (Spencer 1971). Perry (1950) indicated the presence of *An. punctulatus* from Bougainville Island (Perry infers the presence of this species on Bougainville in his Fig. 1; however, he makes no mention of it in the text under the section "Bougainville"). On Buka Island, the pre-DDT spray survey conducted by Spencer (1961) indicated that *An. koliensis* was uncommon; it was not found as larvae and adults were taken in small numbers at only 3 locations. A post-spray survey conducted in 1961 (after 2 spray rounds) failed to find this species and the results reported by us indicate that it no longer occurs on Buka Island. This species possibly has been eliminated by the DDT spraying. In other areas of New Guinea and the Solomon Islands, this species has

proven susceptible to this control measure (Taylor 1975a, Sweeney 1983), although it has reestablished itself by reinvasion from untreated areas, something that it has been unable to do on an isolated island like Buka.

Anopheles punctulatus was also absent in the postspray survey of Spencer (1961), although it appears to have recovered after the cessation of DDT spraying because it was common and wide spread throughout Buka Island at the time of our surveys. Perry (1950) reported this species from Empress Augusta Bay, adjacent to the Tonu area (Fig. 1); however, it was not found during this survey despite the presence of what appeared to be suitable breeding sites in the area. Spencer (1971) could not find this species in the Arawa area nor was it found during our survey. This is not the eastern limit of the range of this species, because it occurs on Guadalcanal in the Solomon Islands (Taylor 1975a, Beebe et al. 2000). The apparent absence of *An. punctulatus* from the Arawa and Tonu areas on Bougainville Island may be explained by soil type, because Horsfall and Porter (1946) believed that soil type is important in determining the presence and abundance of *An. punctulatus*. They noted that, in PNG, this species was dominant where clay soils were present that allowed the formation of small temporary unestablished larval sites. Laird (1946) and Peters (1965) also made note of the preference *An. punctulatus* has for these unestablished sites with a clay substrate. On Buka, where *An. punctulatus* was abundant, clay soils covered 75% of the island; however, on Bougainville, clays made up less than 1% of the soil (Scott et al. 1967). The absence of this soil type possibly has restricted the dispersal of *An. punctulatus* on Bougainville Island.

Two sibling species of the *An. farauti* complex, *An. farauti* s.s. and *An. farauti* 2, were identified on both Buka and Bougainville islands. *Anopheles farauti* 2 was not collected in CO_2 traps nor was it collected biting humans. Similar observations were made on populations of *An. farauti* 2 from Guadalcanal, in the Solomon Islands (Foley et al. 1994). However, in northern Australia and in Western Province, PNG, this species was collected biting humans and in CO_2 traps (Cooper et al. 1996, 1997). *Anopheles farauti* 2 has a wide distribution, occurring throughout northern Australia, PNG, and the Solomon Islands. Several rDNA ITS2 genotypes of this species have been identified with the Buka, Bougainville, and Solomon islands populations, all belonging to 1 genotype (Beebe and Cooper, unpublished data). This species apparently contains a geographically isolated genotype that is zoophilic in nature, and hence is not a vector of malaria on Buka and Bougainville islands. This conclusion is supported by the observations of Spencer (1971), who despite collecting high numbers of *An. farauti* larvae in the Arawa area collected few that were biting humans. This can now be explained by the fact that both species were collected together as larvae but only *An. farauti* s.s. was collected biting humans.

Anopheles punctulatus was rarely collected in CO_2 traps on Buka Island despite high larval densities. Similar observations have been made on the PNG mainland (Cooper et al. 2002) and on Guadalcanal in the Solomon Islands (Foley et al. 1994). This species also was poorly represented in the human biting collections made on Buka Island, a characteristic also noted by Belkin et al. (1945) on Guadalcanal. Standfast (1967) found that at inland locations, 36.5% of the biting population of *An. punctulatus* fed before midnight; however, at coastal locations, only 9.8% did so. Therefore, because the collections on Buka Island ended at midnight, the peak biting time of this species may have been missed. The biting collections made at site 57, which were conducted between 1900 and 0600 h, contained only *An. farauti* s.s.; however, this was in an area with no larval populations of *An. punctulatus*. Despite the late-night biting habits of this species in coastal areas, as shown by Standfast (1967), the paucity of biting adults in catches made between 1800 and 2400 h on Buka Island needs to be further studied, particularly in light of the high larval densities present at that time.

Various workers have found a shift in the night biting activity of coastal populations of *An. farauti* s.l. after years of DDT spraying (reviewed in Sweeney 1983). This shift resulted in a higher proportion of mosquitoes feeding earlier in the night. This change in behavior was quite pronounced with *An. farauti* (most likely *An. farauti* s.s.) in the Solomon Islands (Taylor 1975b). Because most Melanesians spend the early part of the night outside, this change in behavior allowed the continual transmission of malaria as early outdoor feeding mosquitoes avoided the DDT sprayed inside the houses. This type of feeding behavior would also limit the effectiveness of permethrin-impregnated bed-nets in controlling malaria. The night collections conducted here showed that the feeding behavior either was not effected by DDT or that if a shift to early night feeding did occur, then it has reverted since the cessation of DDT spraying. However, although only 26% of biting occurred from 1900 to 2300 h, this degree of early outdoor feeding might still be high enough to reduce the effectiveness of intervention measures such as permethrin-impregnated bed-nets, particularly because 68.4% (13/19) of the infected bites occurred during this feeding period.

The presence of *An. lungae* in the Arawa area also was recorded by Spencer (1971). This species is a member of the Lungae Complex, with a distribution restricted to the Solomon Islands, of which, geographically, Bougainville Island is a part. This species was absent from Buka Island and therefore Bougainville is probably the northern limit of this species.

Anopheles farauti s.l. has been incriminated as an important vector of malaria in PNG on numerous occasions (Heydon 1923, Spencer 1969, Burkot et al.

1988). However, this taxon is now known to contain 7 sibling species, 6 of which occur in PNG; the role that each of these species play in malaria transmission is not known. The presence of *P. falciparum* and *P. vivax* sporozoites in *An. farauti* s.s. found in this study is the 1st time this species has been implicated in the transmission of malaria in PNG.

On Buka and Bougainville islands, *An. farauti* s.s. seems to be the major malaria vector. On Buka, despite its restricted coastal distribution, *An. farauti* s.s. can occur in high densities, readily bites humans, and is capable of developing malaria parasites. *Anopheles punctulatus,* although abundant and wide spread on Buka, was not often found biting humans and despite its reputation as a major vector of malaria on mainland PNG, its role in malaria transmission on Buka Island may be secondary. *Anopheles farauti* 2 was abundant and widespread on both islands; however, as has been found on other islands of the Solomon Islands Archipelago, this species does not seem to bite humans and therefore is not a vector of malaria on Buka and Bougainville islands.

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