Journal of the American Mosquito Control Association, 13(1):5–12, 1997

ANOPHELINE MOSQUITOES OF THE WESTERN PROVINCE OF PAPUA NEW GUINEA

R. D. COOPER,[1] D. G. E. WATERSON,[2] M. KUPO,[3] D. H. FOLEY,[4]
N. W. BEEBE[5] and A. W. SWEENEY[1]

ABSTRACT. A survey of the *Anopheles* species of Western Province, Papua New Guinea, was made in April–May 1992. A total of 6,427 specimens was collected from 74 sites within the province using carbon dioxide-baited light traps and larval sampling. Eleven species were identified using morphological characteristics, allozyme analysis, and species-specific DNA probes. These were, in order of prevalence: *Anopheles farauti* 2 (51 sites), *An. bancroftii* (17 sites), *An. farauti s. s.* (16 sites), *An. longirostris* (9 sites), *An. farauti* 3 (7 sites), *An. punctulatus* (4 sites), *An. koliensis* (4 sites), *Anopheles* sp. near *punctulatus* (4 sites), *An. meraukensis* (4 sites), *An. farauti* 4 (3 sites), and *An. novaguinensis* (2 sites). Members of the *An. farauti* complex made up 93.3% of the specimens collected with *An. farauti* 2 being the most abundant and widespread species inland and *An. farauti s. s.* the dominant species on the coast. The abundance and distribution of the species are discussed.

INTRODUCTION

The anophelines of the southwest Pacific region have been well reviewed in the annotated bibliography of Lee et al. (1987). The major vectors of malaria in this region are the members of the *Anopheles punctulatus* group: *Anopheles farauti* Laveran, *Anopheles punctulatus* Dönitz, and *Anopheles koliensis* Owen (Peters and Standfast 1960). The 3 species occur in Irian Jaya, Papua New Guinea (PNG), and the Solomon Islands, with the range of *An. farauti* extending to the Moluccas in the west, Vanuatu in the east, and northern Australia in the south.

Members of the *An. punctulatus* group were originally identified on morphological characteristics (Rozeboom and Knight 1946). However cross-mating experiments and allozyme analysis have revealed 7 isomorphic species of *An. farauti* (1–7) and a new species near *An. punctulatus* within this group (Bryan 1973; Mahon and Miethke 1982; Foley et al. 1993, 1994, 1995).

Anopheles farauti sensu stricto (*s.s.*) (= *An. farauti* 1; Hii et al. 1993) has been recorded from northern Australia, PNG, the Solomon Islands, and Vanuatu; *An. farauti* 2 from northern Australia and the Solomon Islands; *An. farauti* 3 from northern Australia; *An. farauti* 4, 5, and 6 from PNG; and *An. farauti* 7 from the Solomon Islands (Bryan 1973; Sweeney et al. 1990; Foley et al. 1991, 1993, 1994; Cooper et al. 1995). However, the distribution of these isomorphic species, and of the other members of the group, within these countries is not known in any detail.

In PNG the existence of species complexes makes epidemiological studies on malaria difficult, as simple microscopy may no longer be reliable for identifying the vectors. Correct identification is essential as differences in the biology and behavior of the various species will determine their ability to transmit disease and the control measures that may be implemented against them.

The techniques of cross mating, polytene chromosome mapping, and allozyme electrophoresis all have limitations associated with their use, particularly where large numbers of field specimens must be handled (Cooper et al. 1991). The use of species-specific DNA probes, developed for the members of the *An. punctulatus* group (Cooper et al. 1991), have overcome some of these limitations. However, even with this method field-collected specimens must be protected from excessive DNA degradation.

As yet none of the above techniques have been simplified for field use. The problem of identifying which species occur in a particular area could be overcome if the distribution of each species was known. It is likely that the different species will have well-defined and distinct distributions due to environmental requirements, geographical barriers, and interspecific competition. For example, *An. farauti s. s.* has a strong affinity for brackish water conditions and its range in Australia and in other countries where it has been found is restricted to the coast and areas of tidal influence (Cooper et al. 1995; Foley et al. 1993, 1994). The compilation of distribution maps for the different species may overcome the need for continually screening large numbers of specimens collected from study sites using techniques not yet available in the country. It may also extend the usefulness of morphological markers as a means of identification provided there is little overlap in the distribution of the isomorphic species.

[1] Australian Army Malaria Institute, Milpo Enoggera, Queensland 4052, Australia.

[2] Forestry Research and Development Division, State Forests of NSW, P. O. Box 100 Beecroft, New South Wales 2119, Australia.

[3] Health Services, HQPNG Defense Forces, Murray Barracks Free Bag, Boroko, Papua New Guinea.

[4] Tropical Health Program and Department of Entomology, University of Queenland, Brisbane, Queensland 4072, Australia.

[5] Tropical Health Program, Queensland Institute of Medical Research, Brisbane, Queensland, Australia.

This paper reports on a survey of the anopheline mosquitoes of Western Province, PNG; a region where malaria is hyperendemic (Peters 1957) but where little is known about the anopheline fauna.

MATERIALS AND METHODS

Survey area: Western Province (WP) lies on a large alluvial plain that continues westward throughout the southern region of Irian Jaya (for political and topographic features see Fig. 1). The province covers an area of 98,000 km^2 and is sparsely populated (<5 persons per km^2) with the majority of people living in isolated villages. The province is largely undeveloped, but increased mining and logging activities over the last decade have begun to open up the region. There are few roads in the province and the movement of people is largely by boat and foot tracks.

The climate above the 3,000 mm isohyet is hot–wet with little seasonality, whereas below this zone the climate is monsoonal hot–wet with a distinct wet season occurring from January to April (McAlpine et al. 1983).

The predominant vegetation is lowland rain forest, although south of the Fly River savanna woodland with grass and wooded swamps around the major water courses predominates. This latter flora has strong floristic and structural affinities with that of Cape York Peninsula in northern Australia (Paijmans 1976).

Survey methods: The survey was conducted in April–May 1992 using helicopters (Bell 206 Jet Ranger). The versatility of these aircraft enabled sampling from all parts of the province. Mosquitoes were collected as adults using carbon dioxide-baited encephalitis vector surveillance (EVS) light traps, or as larvae, which were reared to adults. All specimens were identified in the field using the morphological keys of Lee and Woodhill (1944) and then stored in liquid nitrogen. Material belonging to the *An. punctulatus* group was later subjected to allozyme analysis (Foley and Bryan 1993) and hybridization experiments on squash blots using ^{32}P-labelled species-specific DNA probes (Cooper et al. 1991). Using DNA probes, 5–20 specimens of *An. farauti sensu latu* (*s.l.*) were examined from each site, where numbers permitted; a total of 505 specimens from the *An. farauti* complex were typed.

RESULTS

Mosquitoes were collected from 89 sites throughout WP. Seventy-four of these sites were positive for anophelines (Fig. 1) and 6,427 specimens were collected. From this material 11 species were identified, *Anopheles bancroftii* Giles, *An. longirostris* Brug, *An. meraukensis* Venhuis, *An. novaguinensis* Venhuis, and from within the *An. punctulatus* group: *An. farauti s.s.*, *An. farauti* 2, *An. farauti* 3, *An. farauti* 4, *An. koliensis*, *An. punctulatus*, and *Anopheles* sp. near *punctulatus*. Figures 2–4 show the distribution of these species and Tables 1 and 2 show their prevalence.

Members of the *An. farauti* complex made up 94% of all anophelines collected, with *An. farauti* 2 being the most abundant and widespread species inland (Fig. 2). This species was also common along the coast (within 1 km of the sea), although the dominant species in this area was *An. farauti s.s.* (Fig. 3).

Anopheles sp. near *punctulatus* was morphologically similar to *An. punctulatus*, but in some specimens the white scaling on the proboscis was reduced and patchy. This species was collected as adults from 4 locations in the northeast of the province (Fig. 3). Larval collections were also made at sites 55 and 56 and material from these sites was used in the identification of the species (Foley et al. 1995). These 2 sites were 240–300 m above sea level in a 100-m-deep ravine formed by the East Branch Rentoul River. At site 55 larvae were abundant in recently formed pools left by receding flood waters. The pools ranged from less than 1 to 2 m^2 and contained clean, clear water on a mud/silt substrate with peripheral and emergent vegetation. Larvae of *An. farauti* 2 were also collected from this site. Site 56 consisted of small rock pools of clean, clear water less than 1 m^2, located on extensive rock platforms that formed the bank of the river. The larger pools appeared to be permanent and were up to 0.6 m deep; the pools contained little or no vegetation. At this site the larvae of *Anopheles* sp. near *punctulatus* were found in association with *An. punctulatus* larvae. Voucher specimens of *An.* sp. near *punctulatus* will be lodged with the Australian National Insect Collection.

More than 600 specimens of the *An. punctulatus* group were identified using DNA probes. The majority of this material was collected from traps that were set overnight. Microscopic examination indicated that a number of these specimens were badly damaged and unsuitable for morphological identification. However, the majority (>90%) had sufficient useable DNA to enable them to be identified using DNA probes.

DISCUSSION

Few studies have been made of the anopheline fauna of WP and these studies predate the recognition of the isomorphic species of *An. farauti*. In villages along the Fly River south of Kiunga, Peters (1957) found *An. farauti s.l.* and *An. koliensis* to be the dominant species and *An. punctulatus* to be scarce. *Anopheles bancroftii*, *An. koliensis*, *An. punctulatus*, and *An. farauti s.l.* have been recorded from villages around Kiunga, the latter 2 species being uncommon (Schuurkamp 1993). A 2-year (1978–80) study by the Entomology Section of the PNG Health Department's Malaria Control Branch recorded *An. farauti s.l.* as the dominant species in

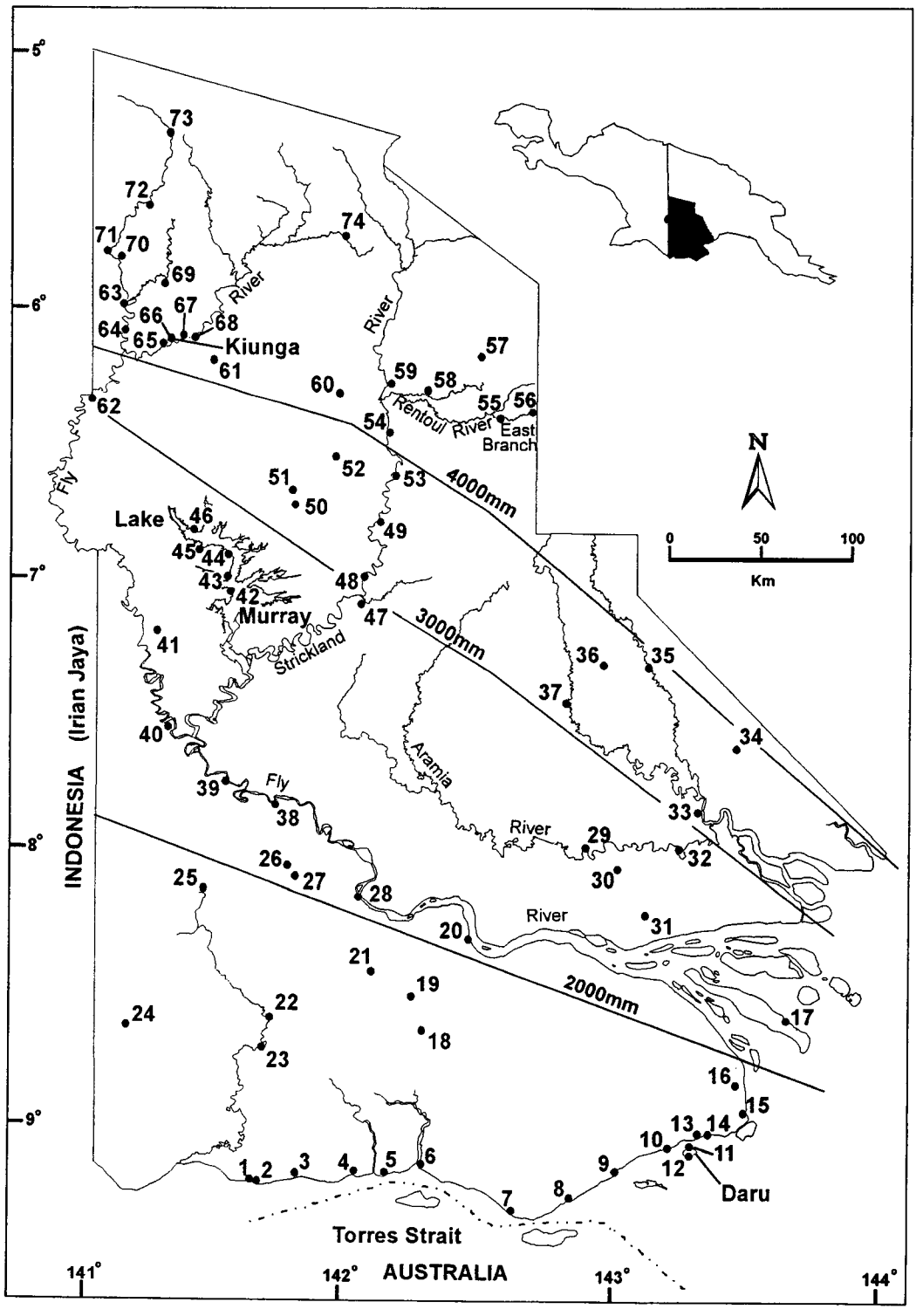

Fig. 1. Map of Western Province indicating the collection sites and political and topographic features mentioned in the text.

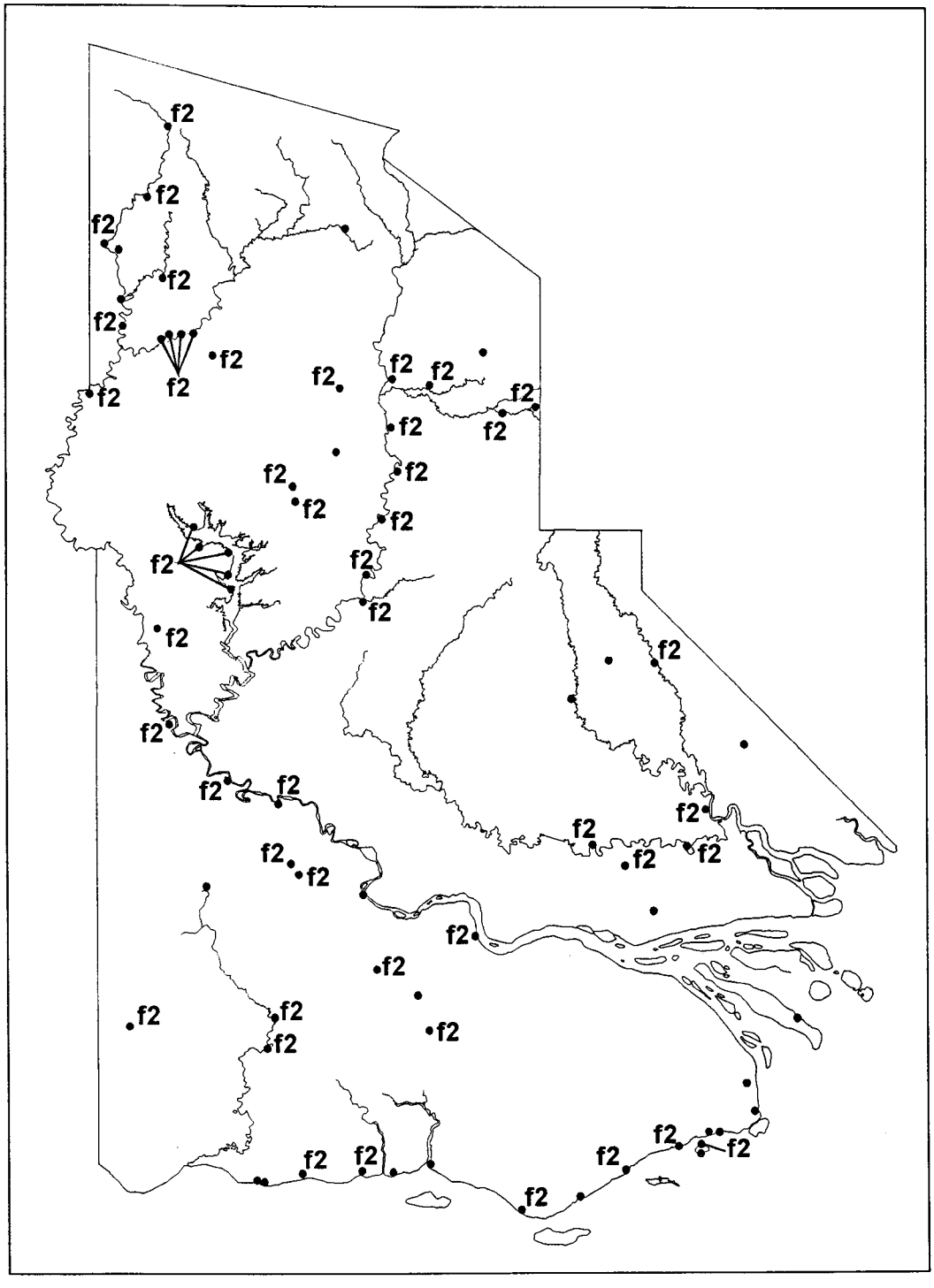

Fig. 2. Map of Western Province showing the distribution of *Anopheles farauti* 2 (f2).

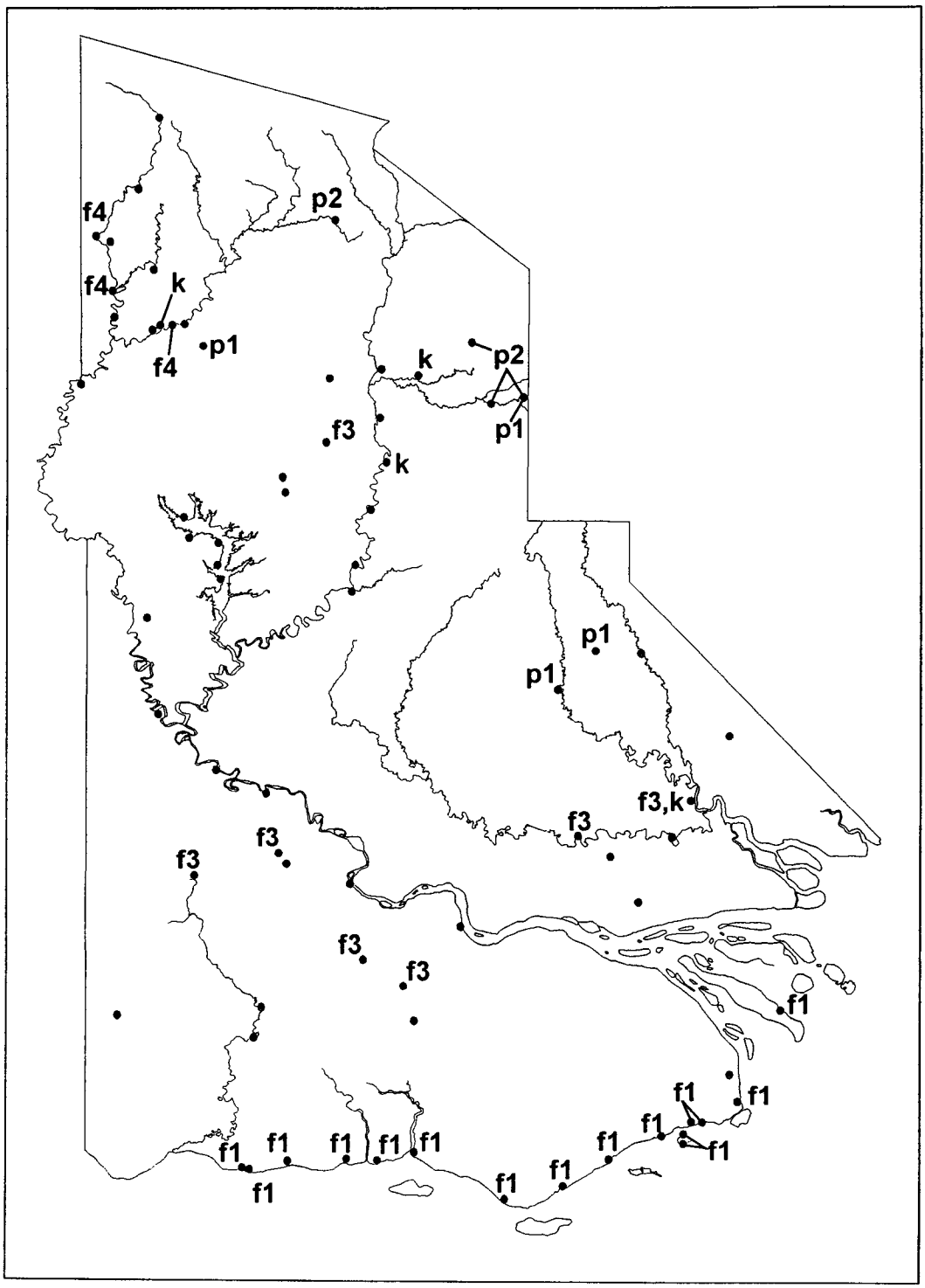

Fig. 3. Map of Western Province showing the distribution of *Anopheles farauti s. s.* (f1), *An. farauti* 3 (f3), *An. farauti* 4 (f4), *An. koliensis* (k), *An. punctulatus* (p1), and *Anopheles* sp. near *punctulatus* (p2).

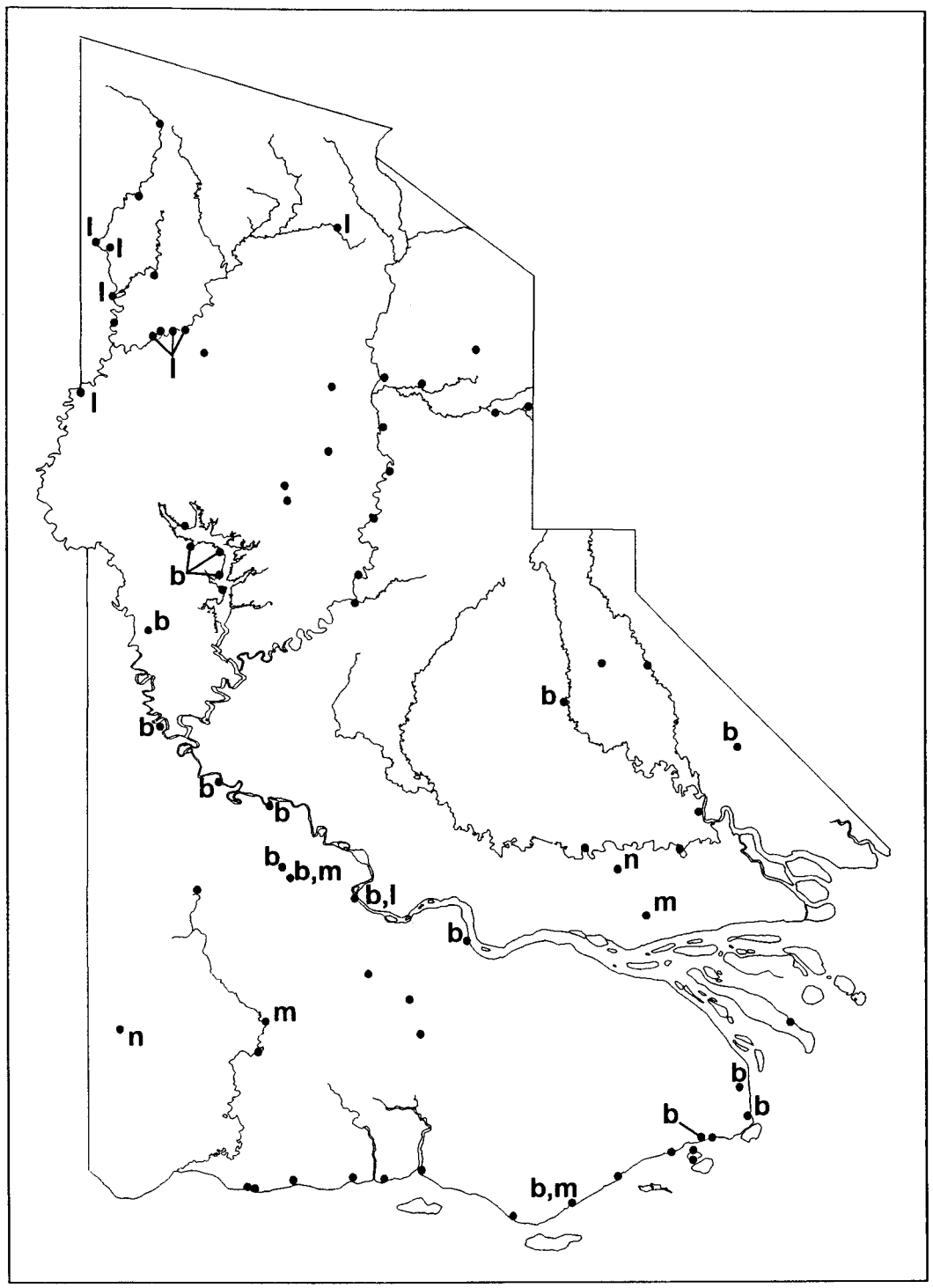

Fig. 4. Map of Western Province showing the distribution of *Anopheles bancroftii* (b), *An. longirostris* (l), *An. meraukensis* (m), and *An. novaguinensis* (n).

Table 1. Prevalence of anopheline species (in the genus *Anopheles*) collected in Western Province, Papua New Guinea, April–May 1992.

Species	No. of sites	No. collected	% of total collected
An. bancroftii	17	155	2.4
An. longirostris	9	36	0.6
An. meraukensis	4	18	0.3
An. novaguinensis	2	8	0.2
An. punctulatus	4	51	0.8
An. sp. near *punctulatus*	4	100	1.5
An. koliensis	4	10	0.2
An. farauti complex	64	6,049	94.0
Total		6,427	100

Table 2. Prevalence of species within the *Anopheles farauti* complex collected in Western Province, Papua New Guinea, April–May 1996.

Members of the *An. farauti* complex[1]	No. of sites	No. identified	% of total identified
An. farauti s.s.	16	164	32.5
An. farauti 2	51	308	61.0
An. farauti 3	7	25	5.0
An. farauti 4	3	8	1.5
Total		505	100

[1] Based on 505 specimens identified using DNA probes.

villages in the Daru coastal area, whereas *An. punctulatus* was uncommon and *An. koliensis* not recorded (Barker-Hudson 1981). These findings are similar to those of this survey; however, although *An. koliensis* was found in the Kiunga area it was not the dominant species there or in any other part of the province.

In southern Irian Jaya, van den Assem and van Dijk (1958) found *An. farauti s.l.* to be widespread and common, whereas *An. koliensis* and *An. punctulatus* were uncommon and had limited distributions, as was found in WP. *Anopheles bancroftii* and *An. longirostris* were widespread, but not abundant, whereas *An. meraukensis* and *An. novaguinensis* were uncommon and had very restricted distributions, as was also found in this survey.

The presence of *An. farauti s.s.* along the WP coastline is not surprising as it is common along the coastline of northern Australia and throughout the Torres Strait Islands, one of which lies within 3 km of WP (Sweeney et al. 1990, Foley et al. 1991). *Anopheles farauti s. s.* has also been found in other coastal areas of PNG and the Solomon Islands (Foley et al. 1993, 1994). This species can utilize brackish water for larval habitats (Cooper et al. 1996), an adaptation that would facilitate its spread along coastlines and throughout island groups; hence, it is likely to be the common coastal anopheline around the island of New Guinea and other islands of the southwest Pacific.

The Torres Strait represents a region of climatic and environmental interchange between the wet tropical rainforests of WP in the north and the dry savanna woodlands of Cape York Peninsula in the south (Taylor 1972). Of the species identified on both sides of the Strait only *An. farauti* 2 and *An. bancroftii* have successfully adapted to the extremes of the 2 regions. *Anopheles longirostris* and *An. farauti* 4 were uncommon or absent in the drier savanna woodlands of the southern areas of WP and northern Australia, whereas *An. farauti* 3, *An. meraukensis*, and *An. novaguinensis*, which are common and widespread on Cape York Peninsula (Cooper et al. 1996), do not appear to have become well established in the wetter, rainforest areas of northern WP.

Anopheles punctulatus appeared to be uncommon in WP (Fig. 3). This species can rapidly colonize areas where the forest has recently been cleared and vehicles have been used. In this survey large numbers of *An. punctulatus* larvae were recorded from sites made by heavy vehicle traffic at a recently established refugee camp (site 61) and at logging camps and roads (sites 36 and 37). It is possible that this species may become more abundant as further development within the province disturbs the natural environment.

Anopheles farauti s. l. is one of the major vectors of malaria in PNG; however, the vectorial status of each of the 6 isomorphic species now recorded there is not known. Charlwood et al. (1985) implicated *An. farauti s.s.* as a vector in the coastal villages around Madang; in the present survey *An. farauti s.s.* was found to be the dominant coastal species and it is possible that it may also be a vector in this region. In WP, *An. farauti* 2 was found on the coast and was the most abundant species inland. Lake Murray (Fig. 1) is an area of intense malaria transmission (Peters 1957). As *An. farauti* 2 was the dominant species in this region (Fig. 1, sites 41–46), it is possible that this species may play a role in malaria transmission here and in other areas of WP.

The suggestion that *An. farauti s.s.* and *An. farauti* 2 are vectors is based on their relative abundance, and although this is a basic characteristic of an efficient vector (Mackerras 1947), other factors such as longevity, host preference, and susceptibility to infection are possibly more important (Burkot 1988) and would need to be assessed before the vectorial status of these species can be determined.

The aim of this study was to identify and map the distribution of the *Anopheles* species in Western Province so that future workers will have a knowledge of the anopheline fauna of the area and thus an understanding of which species might be implicated in disease transmission. Identification techniques using DNA-based technology are not suitable for field work in developing countries, and the use of morphological characteristics must still be considered. On the coast of WP the use of mor-

phological markers may be limited, as *An farauti s.s.* and *An. farauti* 2 occurred sympatrically here. Inland, individuals with an all-black proboscis would most likely be *An. farauti* 2 and the morphological markers for *An. punctulatus* and *An. koliensis* may still prove to be useful; however, this will depend upon the relative abundance of each species, as found in this survey, remaining constant throughout the year.

ACKNOWLEDGMENTS

This paper is published with the approval of the Director General of Army Health Services. We thank WO1 Dan Whelan, 11 Field Ambulance, Royal Australian Army Medical Corps and SGT Tony Tabatuna, Health Services, Papua New Guinea Defence Force (PNGDF) for technical support; the members of 162 Reconnaissance Squadron, Australian Army Aviation Corps, who enabled the collections to be made; and the Commander, PNGDF, for use of the Kiunga Garrison. We are grateful to J. H. Bryan and S. P. Frances for their critical review of the manuscript.

REFERENCES CITED

Barker-Hudson, P. 1981. A report on entomological baseline data collected on malaria vectors along the Daru coastline of the Western Province, Papua New Guinea. Malaria Control Programme, Papua New Guinea Department of Health, Waigani, Papua New Guinea.

Bryan, J. H. 1973. Studies on the *Anopheles punctulatus* complex. 1. Identification by proboscis morphological criteria and by cross-mating experiments. Trans. R. Soc. Trop. Med. Hyg. 67:64–69.

Burkot, T. R. 1988. Non-random host selection by anopheline mosquitoes. Parasitol. Today 4:156–162.

Charlwood, J. D., H. Dagoro and R. Paru. 1985. Blood-feeding and resting behaviour in the *Anopheles punctulatus* Dönitz complex (Diptera: Culicidae) from coastal Papua New Guinea. Bull. Entomol. Res. 75:463–475.

Cooper, L., R. D. Cooper and T. R. Burkot. 1991. The *Anopheles punctulatus* complex: DNA probes for identifying the Australian species using isotopic, chromogenic and chemiluminescence detection systems. Exp. Parasitol. 73:27–35.

Cooper, R. D., S. P. Frances and A. W. Sweeney. 1995. Distribution of members of the *Anopheles farauti* complex in the Northern Territory of Australia. J. Am. Mosq. Control Assoc. 11:66–71.

Cooper, R. D., S. P. Frances, D. G. E. Waterson, R. G. Piper and A. W. Sweeney. 1996. Distribution of anopheline mosquitoes in northern Australia. J. Am. Mosq. Control Assoc. 12:000–000.

Foley, D. H. and J. H. Bryan. 1993. Electrophoretic keys to identify members of the *Anopheles punctulatus* complex of vector mosquitoes in Papua New Guinea. Med. Vet. Entomol. 7:49–53.

Foley, D. H., R. D. Cooper and J. H. Bryan. 1995. A new species within the *Anopheles punctulatus* complex in Western Province, Papua New Guinea. J. Am. Mosq. Control Assoc. 11:122–127.

Foley, D. H., S. R. Meek and J. H. Bryan. 1994. The *Anopheles punctulatus* group of mosquitoes in the Solomon Islands and Vanuatu surveyed by allozyme electrophoresis. Med. Vet. Entomol. 8:340–350.

Foley, D. H., J. H. Bryan, D. Booth and C. G. Freebairn. 1991. *Anopheles farauti* Laveran *sensu lato* and *Anopheles hilli* Woodhill and Lee (Diptera: Culicidae) in the Torres Strait, Australia. J. Aust. Entomol. Soc. 30:307–312.

Foley, D. H., R. Paru, H. Dagoro and J. H. Bryan. 1993. Allozyme analysis reveals six species within the *Anopheles punctulatus* complex of mosquitoes in Papua New Guinea. Med. Vet. Entomol. 7:37–48.

Hii, J. L. K., L. Kanai, A. Foligela, S. K. P. Kan, T. R. Burkot and R. A. Wirtz. 1993. Impact of permethrin-impregnated mosquito nets compared with DDT house-spraying against malaria transmission by *Anopheles farauti* and *An. punctulatus* in the Solomon Islands. Med. Vet. Entomol. 7:333–338.

Lee, D. J. and A. R. Woodhill. 1944. The anopheline mosquitoes of the Australasian Region. Monograph no. 2, a publication of the University of Sydney. Australasian Medical Publishing Company Limited, Glebe, New South Wales, Australia.

Lee, D. J., M. M. Hicks, M. Griffiths, M. L. Debenham, J. H. Bryan, R. C. Russell, M. Geary and E. N. Marks. 1987. The Culicidae of the Australasian Region, Volume 5. Australian Government Publishing Service, Canberra, Australia.

Mackerras, I. M. 1947. The Australasian anophelines as vectors of malaria. Med. J. Aust. 1:1–8.

Mahon, R. J. and P. M. Miethke. 1982. *Anopheles farauti* no. 3, a hitherto unrecognised biological species of mosquito within the taxon *A. farauti* Laveran (Diptera: Culicidae). Trans. R. Soc. Trop. Med. Hyg. 76:8–12.

McAlpine, J. R., G. Keig and R. Fall. 1983. Climate of Papua New Guinea. Australian National University Press, Canberra, Australia.

Paijmans, K. 1976. New Guinea vegetation. Commonwealth Scientific and Industrial Research Organisation in association with the Australian National University Press, Canberra, Australia.

Peters, W. 1957. A malaria survey in the Western District of Papua. Papua New Guinea Med. J. 2:26–38.

Peters, W. and H. A. Standfast. 1960. Studies on the epidemiology of malaria in New Guinea. 1. Holoendemic malaria—the entomological picture. Trans. R. Soc. Trop. Med. Hyg. 54:249–254.

Rozeboom, L. E. and K. L. Knight. 1946. The Punctulatus complex of mosquitoes (Diptera: Culicidae). J. Parasitol. 32:95–131.

Schuurkamp, G. J. T. 1993. The epidemology of malaria and filariasis in the Ok Tedi region of Western Province, Papua New Guinea. Ok Tedi Public Health, Ok Tedi Mining Limited, Papua New Guinea.

Sweeney, A. W., R. D. Cooper and S. P. Frances. 1990. Distribution of the sibling species of *Anopheles farauti* in the Cape York Peninsula, northern Queensland, Australia. J. Am. Mosq. Control Assoc. 6:425–429.

Taylor, R. W. 1972. Biogeography of insects of New Guinea and Cape York Peninsula, pp. 213–230. *In:* D. Walker (ed.). Bridge and barrier: the natural and cultural history of Torres Strait. Research School of Pacific Studies Publication BG/3. Australian National University, Canberra, Australia.

van den Assem, J. and W.J.O.M. van Dijk. 1958. Distribution of anopheline mosquitoes in Netherlands New-Guinea. Trop. Geogr. Med. 10:249–255.