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ECOLOGICAL DISTRIBUTION OF MOSQUITO LARVAE OF THE *ANOPHELES PUNCTULATUS* GROUP ON NIOLAM (LIHIR) ISLAND, PAPUA NEW GUINEA

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ABSTRACT. We surveyed the larval habitats of members of the *Anopheles punctulatus* group of mosquitoes on Niolam (Lihir) Island, Papua New Guinea. Identification of this group was undertaken by polymerase chain reaction–restriction fragment length polymorphism analysis of the amplified internal transcribed spacer unit 2 of rDNA, because morphologic separation of member species is unreliable. The most widespread malaria vector species and their most common larval habitats were identified to aid source-reduction programs for malaria control. The most ubiquitous species was *An. punctulatus,* followed by *An. farauti* no. 2. then *An. farauti* s.s. *Anopheles punctulatus* has increased relative to *An. farauti* s.l. since the start of development projects on Lihir Island. The most common larval habitats were shallow temporary pools with clay substrate and with plants or floatage. These habitats, mostly encountered alongside poorly drained roads, may be increased by development projects.

KEY WORDS Lihir Island, *Anopheles punctulatus* group, malaria, larval habitat, internal transcribed spacer unit 2

INTRODUCTION

The major vectors of malaria in the southwest Pacific are the members of the *Anopheles punctulatus* group of mosquitoes. A number of species with overlapping morphology within this group have been discovered (Bryan 1973; Mahon and Meithke 1982; Foley et al. 1993, 1994, 1995). In Papua New Guinea (PNG) these species are *An. farauti* Laveran sensu stricto (formerly also known as *An. farauti* no. 1; Foley et al. 1994); *An. farauti* no. 2, no. 3, no. 4, no. 5, and no. 6; *An. koliensis* Owen; *An. punctulatus* Dönitz; and *An.* sp. near *punctulatus.*

Species can be identified by allozymes (Foley and Bryan 1993), genomic DNA probes (Beebe et al. 1994), and a polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) procedure using the internal transcribed spacer unit 2 (ITS-2) of rDNA (Beebe and Saul 1995). Because morphologic identification is unreliable (Foley et al. 1993), earlier species data will have to be reinterpreted if confusion with the newly identified species is likely to occur.

Accurate species-specific biological data are important. For instance, information on larval biology is necessary to target larval control measures effectively. However, very few studies have been undertaken in PNG using valid identification procedures. Exceptions are the detailed study by Cooper et al. (1997) of the distribution of the sibling species in the Western Province and the work by Foley et al. (1993), which gives some distribution and biological data.

Niolam (Lihir) Island in PNG (Fig. 1) is the site of a large gold mine (Lihir Management Company [LMC]) and as such is a model for types of problems associated with human changes to a tropical environment. A survey on Niolam Island in May 1993 revealed a malaria parasite rate of 55%, predominantly *Plasmodium falciparum* Welch (90%), and a *Wuchereria bancrofti* (Cobbold) microfilaria parasite rate of 14% (Anonymous 1993). Entomological surveys were conducted on Niolam Island during 1993 and 1994, with an emphasis on communities in the northeastern sector in the vicinity of a proposed mine and infrastructure development, and those in the swampy but (then) relatively isolated, southwestern section of the island. Bockarie et al. (1993, 1994) and Hii et al. (1994) concluded there was intense, year-round transmission of malaria on Niolam Island, with *Anopheles farauti* s.l. the most important vector of malaria and filariasis. *Anopheles punctulatus* was recorded in small numbers at 2 locations. Bockarie et al. (1994) predicted that *An. punctulatus* might increase when road building and construction commenced.

We present the results of a distribution study of the *An. punctulatus* group from Niolam Island identified by PCR-RFLP of ITS-2. We were interested in recording changes in the species composition and distribution of the *An. punctulatus* group after development. We analyzed the association of the different species with larval habitats, including those resulting from development projects, to aid source-reduction programs aimed at malaria control.

MATERIALS AND METHODS

Study site: Niolam Island, in New Ireland Province, is about 75 km in circumference, occupies a land area of about 240 km^2, and is the largest of 4 inhabited islands comprising the Lihir Group (Fig. 1). The island is of volcanic origin with complex geology and rugged topography. Almost all human

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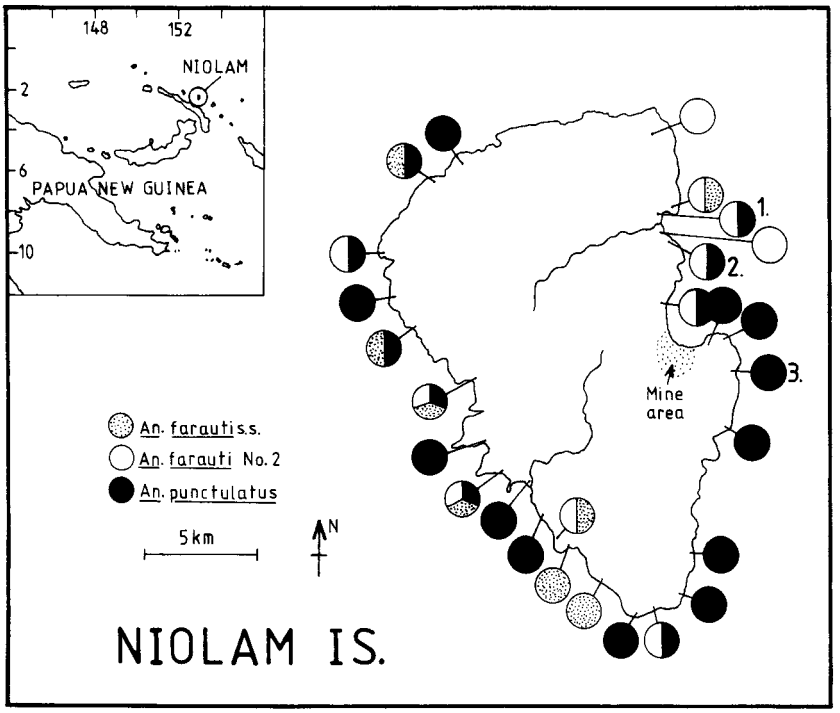

Fig. 1. Niolam Island showing geographical distribution of larval sample sites and which anopheline species were present at each. Larval sites where human-baited collections were undertaken are numbered.

habitation is confined to a series of small villages evenly distributed on a narrow coastal strip composed of Quaternary limestone with occasional overlying alluvial and volcanic elements. An unsealed road that extended along the north and eastern coast was upgraded and extended in 1995, linking villages around the island.

Since 1995, the LMC gold mine, port, processing plant and housing, and an upgraded airport and public commercial center have been constructed in the northeastern sector. Royalties from the mine are being used to construct modern housing for local inhabitants throughout the island group.

Mosquito collections and identification: As many different sites throughout the island as possible were sampled. However, the mine processing and residential and commercial areas (which were negative for larvae) were not included in this study because they were subject to insecticidal fogging. A minimum of 10 standard dips were taken from each site unless larvae were not found, in which case sampling effort was increased to minimize false negatives. Clusters of 10 or more dips were taken from larger pools to include all microhabitats. The location of a subset of 48 sites, being those from which adult identifications were obtained, is shown in Fig. 1. These sites were all within 1 km (0.6 mi) of the coast. Adults were collected by human-baited catches (see Fig. 1) at Londolovit Crossing (site 1) between 2100 and 2300 h on February 19, 1998, Londolovit Village (site 2) between 2115 and 2315 h on February 24, 1998, and Put Put 2 (site 3) between 1900 and 2330 h on February 21, 1998. Adult anophelines reared from larvae were identified with the morphologic keys of Lee et al. (1987). Specimens were stored in 100% ethanol and identified to species by the PCR-RFLP technique of Beebe and Saul (1995). Briefly, 1 leg or piece of a larva was ground in 15 μl of PCR mix and the ITS-2 region of rDNA was amplified. The PCR product was then digested with the restriction endonuclease *Msp* I and the resulting pattern of bands were compared with known patterns.

Larval habitat classification: Larval habitats were classified to generate field information that could be used in the monitoring and suppression of malaria vectors. Our categories are adapted from other schemes for classifying mosquito larval habitats, most notably those of Hopkins (1936) and Laird (1988). Laird (1988) concluded that past efforts to classify larval habitats were not universally applicable and it is impossible to categorize every situation uniquely. To minimize this problem some sites were scored for more than 1 category. Unless noted under habitat condition, sites were exposed to sunlight.

RESULTS

Of 94 sites sampled, 17 (18.1%) were negative for larvae. Negative sites included 1 affected by

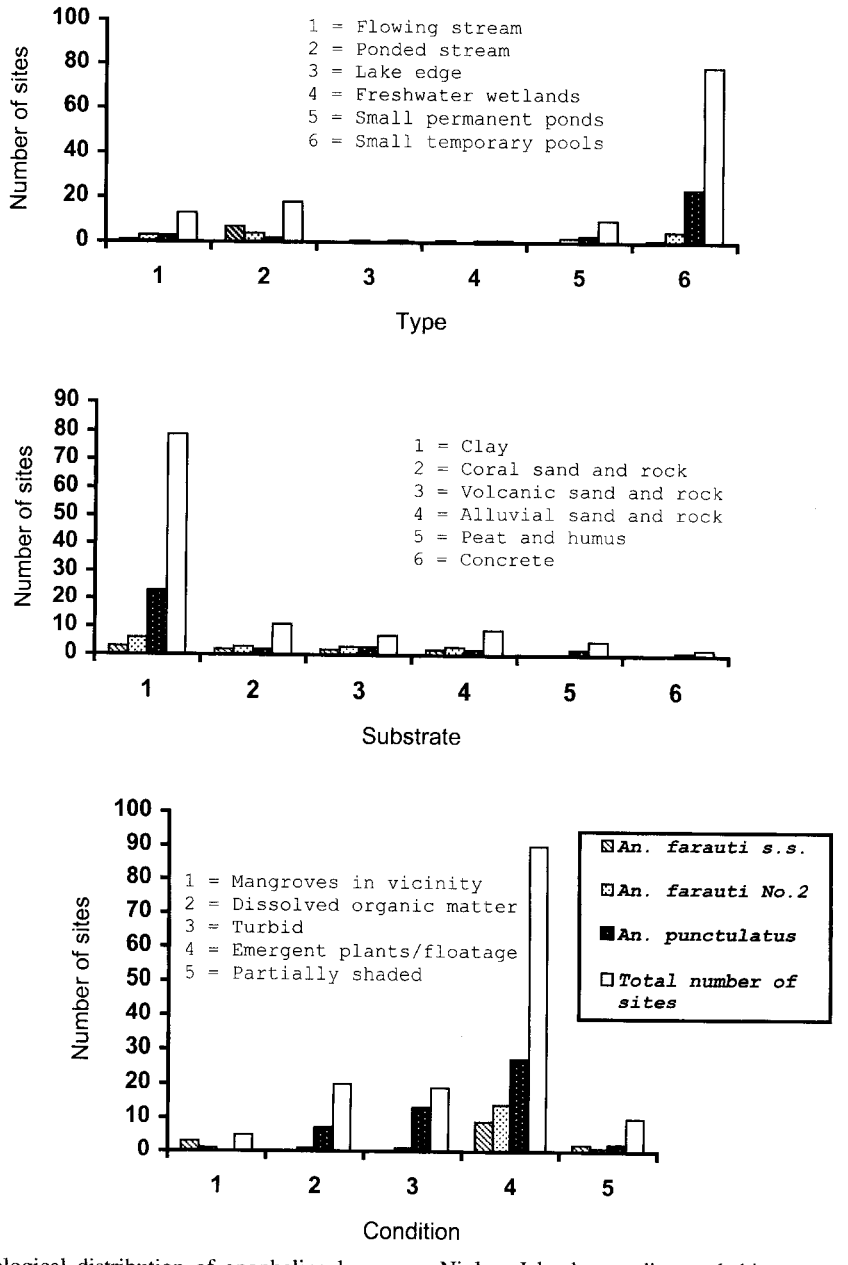

Fig. 2. Ecological distribution of anopheline larvae on Niolam Island according to habitat type, substrate, and condition.

detergent, 3 only recently filled with water, 8 in flowing stream or river mouth habitats, and 3 subject to disturbance by vehicle traffic.

We experienced problems with PCR amplification of late instar larval DNA, some adult DNA was degraded, and larvae from some sites died while in the field. Of the 77 positive sites, 29 did not yield a PCR identification (however, 6 of these corresponded morphologically to *An. punctulatus*). A total of 163 anophelines from 48 sites positive for larvae and 21 from human-baited catches were identified (see Fig. 1). Of the anophelines from larval sites, 57.7% were *An. punctulatus* (from 33 sites), 25.2% were *An. farauti* no. 2 (from 15 sites), and 17.2% were *An. farauti* s.s. (from 9 sites). Only 8 sites had more than 1 species with 1 site having all 3 species. The characteristics of all sites and those where larvae were identified to species are presented in Fig. 2. Only *An. farauti* s.s. and *An. punctulatus* were taken in the night-biting collections despite *Anopheles farauti* no. 2 being collected as larvae at sites 1 and 2.

DISCUSSION

Analysis of the data reveals a major change in relative abundance and distribution of the principal malaria vectors on Niolam Island. Bockarie et al. (1993, 1994) reported that *Anopheles farauti* s.l. was ubiquitous and predominant and *An. punctulatus* was present only in isolated, small populations; *An. punctulatus* is now widespread and abundant, in agreement with the prediction of Bockarie et al. (1994).

The larval habitats of the sibling species of the *An. punctulatus* group are not well known. Larvae of *An. farauti* s.s. and *An. farauti* no. 2 have been found in a variety of natural, animal-made, and human-made sites including ground pools, swamps, hoofprints, roadside ditches, concrete drains, wheel ruts, borrow pits, and pig wallows (Sweeney et al. 1990, Cooper et al. 1995). *Anopheles farauti* no. 2 was also noted in pools within creek beds (Sweeney et al. 1990; Cooper et al. 1997). Larvae of *An. punctulatus* have been found in the Western Province of PNG in rockpools with no vegetation and in pools associated with roads (Cooper et al. 1997). Previous studies of morphologically identified *An. punctulatus* typically found larvae in human-made temporary puddles and shallow pools, without vegetation, either clear or muddy but not foul (Lee et al. 1987).

Understanding where larvae occur enables us to identify sites for larval control or prevent larval sites arising from human activity. Our data should reflect the prevalence of different habitat types available for anopheline larval development on Niolam Island. Sites negative for larvae include a variety of characteristics (such as pollution) already recognized as unsuitable for the larval development of the *An. punctulatus* group (Lee et al. 1987). However, overall the most common negative sites were similar to the most common positive sites. Sites positive for larvae not identified to species also had similar habitat characteristics to the subsample identified to species.

The most common sites in this study were small temporary ponds and the rarest were the more permanent ecotypes of lake edges and wetlands. The most prevalent species, *An. punctulatus,* was mostly recorded from shallow ponds and ephemeral puddles. The rapid, synchronous development of *An. punctulatus* and their ability to survive in damp mud for up to 2 days (see Lee et al. 1987) enables this species to exploit such habitats. *Anopheles farauti* s.s. was mostly recorded in the more permanent ponded streams. *Anopheles farauti* no. 2 was recorded from both temporary pools and the more permanent stream habitats. Clay substrates were by far the most numerous of habitat substrates for *An. punctulatus* and to a less obvious extent for *An. farauti* s.s. and *An. farauti* no. 2.

Larvae of all species were most frequently associated with plants or floatage, in agreement with the findings of Sweeney et al. (1990) and Cooper et al. (1995), who noted an association of *An. farauti* s.s. and *An. farauti* no. 2 larvae with emergent reeds and grasses, algal mats, or floating plant debris. We also found *An. punctulatus* associated with dissolved organic matter and suspended mud. Proximity to mangroves was the 2nd most important indicator for *An. farauti* s.s., in contrast to *An. punctulatus,* which was not recorded from this habitat type. This is in agreement with observations of a coastal and sometimes brackish water larval habitat for *An. farauti* s.s. (Foley et al. 1993, 1994; Cooper et al. 1995, 1997) and the absence of any association between morphologically identified *An. punctulatus* and brackish water (Lee et al. 1987).

Anopheles farauti no. 2 has not been observed biting humans on Niolam Island, despite larvae of this species being recorded at 2 of the human-baited catch sites (sites 1 and 2; Fig. 1). Although more adult collections are needed, this observation parallels our experience in the Solomon Islands and Irian Jaya (West Papua) where *Anopheles farauti* no. 2 has been collected as larvae but not from humans (Foley et al. 1994; Ebsworth, unpublished data). However, this species has been taken during human-baited collections in Australia (Sweeney et al. 1990; Bryan and Foley, unpublished data), suggesting that feeding preference may be polymorphic in this species.

Overall, the most common larval habitats are shallow temporary ponds with clay substrate and with plants or floatage. Larval control measures have been implemented to target these habitat types, which are most commonly represented by pools and puddles formed at the edges of poorly drained sections of roads on the island.

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