

SHORT REPORT

Prevalence of Sibling Species Complex of *Anopheles minimus* in Pyin Oo Lwin Township, Mandalay Region and Kamamaung Township, Kayin State

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The *Anopheles* mosquitoes breeding sites are very greatly depending on the climatic conditions and availability of water. In Myanmar, *An. minimus* and *An. dirus* are considered to be one of the most efficient malaria vectors. Previously, *An. minimus* was found in foothill and forest fringe but now found in rice field in plain areas. *An. dirus* is bred in deep forest areas but now their larvae were found in water wells in Mon State and Thaninthayi Region,¹ because of the certain environmental changes like deforestation and vegetation clearance for crop plantations. All these main vectors are species complex and morphologically indistinguishable, known as sibling species. Salivary gland polytene chromosome can be dissected from *An. dirus* only, although ovarian nurse cell chromosome can be dissected from other Anophelines species in different areas. About 23 *Anopheles* taxa have been identified so far as species complex.²

The study assessed the prevalence of sibling species complex of *An. minimus* using ovarian nurse cell polytene chromosome and determined the potential vector using ELISA methods in Pyin Oo Lwin Township, Mandalay Region and Kamamaung Township, Kayin State from October 2014 to September, 2015.

Anopheles mosquitoes were collected by animal bait K-net, human bait indoor and outdoor and light trap collection. Blood fed *An. minimus* were separated out by the help

of sucking tube to paper cup with glucose for ovary development. Humidity and temperature were maintained by covering with water soak towel (27°C, RH 90%). Christophe stage ovary specimens were dissected and preserved in Cornoy's fixative in screw type bottle. Samples were stored in 4°C in refrigerator. Preserved ovaries of *An. minimus* were processed in 50% propionic acid and were stained with 2% lacto-acetoorcein stain according to Green and Hunt³ for making polytene chromosome preparations. The complements of chromosome of individual mosquitoes were examined under 40x and 100x magnification lens of compound light Olympus microscope. Species diagnostic inversions were used for identification of the members of *An. minimus* complex. Head and thorax of mosquitoes were dissected to find out *Plasmodium* sporozoites in salivary gland followed by ELISA test for conforming potential vectors.

A total of 1153 *Anopheles* mosquitoes comprising 6 mosquito species were collected from Phonedun Village, Pyin Oo Lwin Township. Among them, the highest number (553) of *An. minimus* were collected. *Anopheles* mosquitoes 953 (524 from Katinehtit and 429 from Kinetaw) comprising 9 species were collected from Kamamaung Township. Among them,

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93 and 92 *An. minimus* were collected from Katinehtit and Kinetaw villages, respectively. All collected *An. minimus* were observed sibling species A in both areas. The ovarian nurse cell's polytene chromosomes of *An. minimus* exist as 5 arms. They were the telocentric X chromosome, the sub-metacentric chromosome 2 and the metacentric chromosome 3. The X chromosome is easily recognized by its length and shuttle-shaped zone 6. The most important characteristics are that each autosome arm has one to three big puffs.

Table 1. Identification of species complex of *An. minimus* in two different townships using ovarian nurse cell polytene chromosome

Collected mosquitoes	P		K		Total mosquitoes (sporozoite positive rate, Spp A) (%)
	Phonedun village	Katinehtit village	Kinetaw village		
<i>An. minimus</i> sporozoite	0/553 (0)	1/93 (1.075)	0/92 (0)	738 (0.136)	

P=Pyin Oo Lwin Township, Mandalay Region

K=Kamamaung Township, Kayin State

Spp=Species

Among the 46 zones, 7A, B and 19C in 2R which is the longest arm in the complement, 28A and 20A, B in 2L, 30A, B and 37D in 3R and 46D and 38A, B in 3L are considered as characteristic zones. The ovarian nurse cell polytene chromosome of *An. minimus* collected from Pyin Oo Lwin and Kamamaung townships were found species complex A. Higher numbers of *An. minimus* were observed in animal bait collection than human bait in both

townships. A two-year study in west Thailand (Kanchanaburi Province) found that higher number of 3,808(81.8%) *An. minimus* species C and A were captured on cattle collection.⁴ *Plasmodium falciparum* sporozoite protein was found in one *An. minimus* A in Kamamaung Township (0.54%, 1/185), and it was not found in Pyin Oo Lwin Township (0/553) by circumsporozoite ELISA test.

In the present study, sibling species A of *An. minimus* complex was found in all studied areas in Myanmar although sporozoite positivity rate was high in Kayin State. Therefore, *An. minimus* A is a main vector of malaria in Kayin State areas.

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