

# Crossing, Cytogenetic and Molecular Evidence for Five Sibling Species within the Taxon *Anopheles barbirostris* (Diptera: Culicidae) in Thailand

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## Introduction and Objective

*Anopheles barbirostris* s.l. has been reported as a vector of *Plasmodium vivax*, *Brugia malayi* and *B. timori* in some Southeast Asian countries. Four karyotypic forms of *An. barbirostris* have been detected in Thailand. Although a difference in chromosomes is obvious in this species, little is known about their genetic proximity. Thus, this study searches for sibling species members in the taxon *An. barbirostris*.

## Methods

Crossing experiments, cytogenetic investigations, and comparative DNA sequencing of the internal transcribed spacer 2 (ITS2) and mitochondrial cytochrome *c* oxidase subunit I and II (COI and COII) were used in the recognition of sibling species members.

## Results

A total of 113 isolines of *An. barbirostris*, derived from human- and animal-biting females, showed branch summation in 2 groups of seta 2-VI pupal skins: 42 isolines were *An. barbirostris* (9-16 branches), and 71 were *An. campestris* (20-30 branches). Four karyotypic forms [Form A (X<sub>1</sub>, X<sub>2</sub>, Y<sub>1</sub>), B (X<sub>1</sub>, X<sub>2</sub>, X<sub>3</sub>, Y<sub>2</sub>), C (X<sub>2</sub>, Y<sub>3</sub>) and D (X<sub>2</sub>, Y<sub>4</sub>)] were obtained in *An. barbirostris*, and 3 karyotypic forms [Form B (X<sub>2</sub>, Y<sub>2</sub>), E (X<sub>1</sub>, X<sub>2</sub>, X<sub>3</sub>, Y<sub>5</sub>) and F (X<sub>2</sub>, X<sub>3</sub>, Y<sub>6</sub>)] were recovered in *An. campestris*. Based on the similarity of X<sub>2</sub>-chromosomes, the latter species was designated as *An. campestris*-like Form B, E and F. Results of non post-mating reproductive isolation among the crosses of *An. campestris*-like Form B, E and F indicated intraspecific karyotype variation. The low sequence divergence of the ITS2 of rDNA, and COI and COII of mtDNA among the forms, supported their intraspecific karyotype variation. Results of post-mating reproductive isolation among the crosses of *An. campestris*-like Form E and *An. barbirostris* Form A strains from Chiang Mai, Phetchaburi and Kanchanaburi supported the existence of 5 species within the taxon *An. barbirostris*, designated as *An. campestris*-like, *An. barbirostris* species A1, A2, A3, and A4, respectively. The large sequence divergence of ITS2, COI, and COII supported this evidence. Results of non-post mating reproductive isolation from the crosses of different karyotypic forms of species A1 (A, B, C, D) and A2 (A, B), suggested different karyotypic forms occurring in natural populations of species A1 and A2.

## Conclusion

Five sibling species were obtained within the taxon *An. barbirostris*, i.e., *An. campestris*-like and *An. barbirostris* species A1, A2, A3 and A4.

**Keywords:** *Anopheles barbirostris*, crossing experiments, cytogenetics, ITS2, COI and COII

## Selected References:

1. Saeung A, Baimai V, Otsuka Y, Rattanarithikul R, Somboon P, Junkum A, Tuetun B, Takaoka H, Choochote W. Molecular and cytogenetic evidence of three sibling species of the *Anopheles barbirostris* Form A (Diptera: Culicidae) in Thailand. Parasitol Res 2008; 102: 499-507.