“ยุง” พาหะนำโรคร้าย

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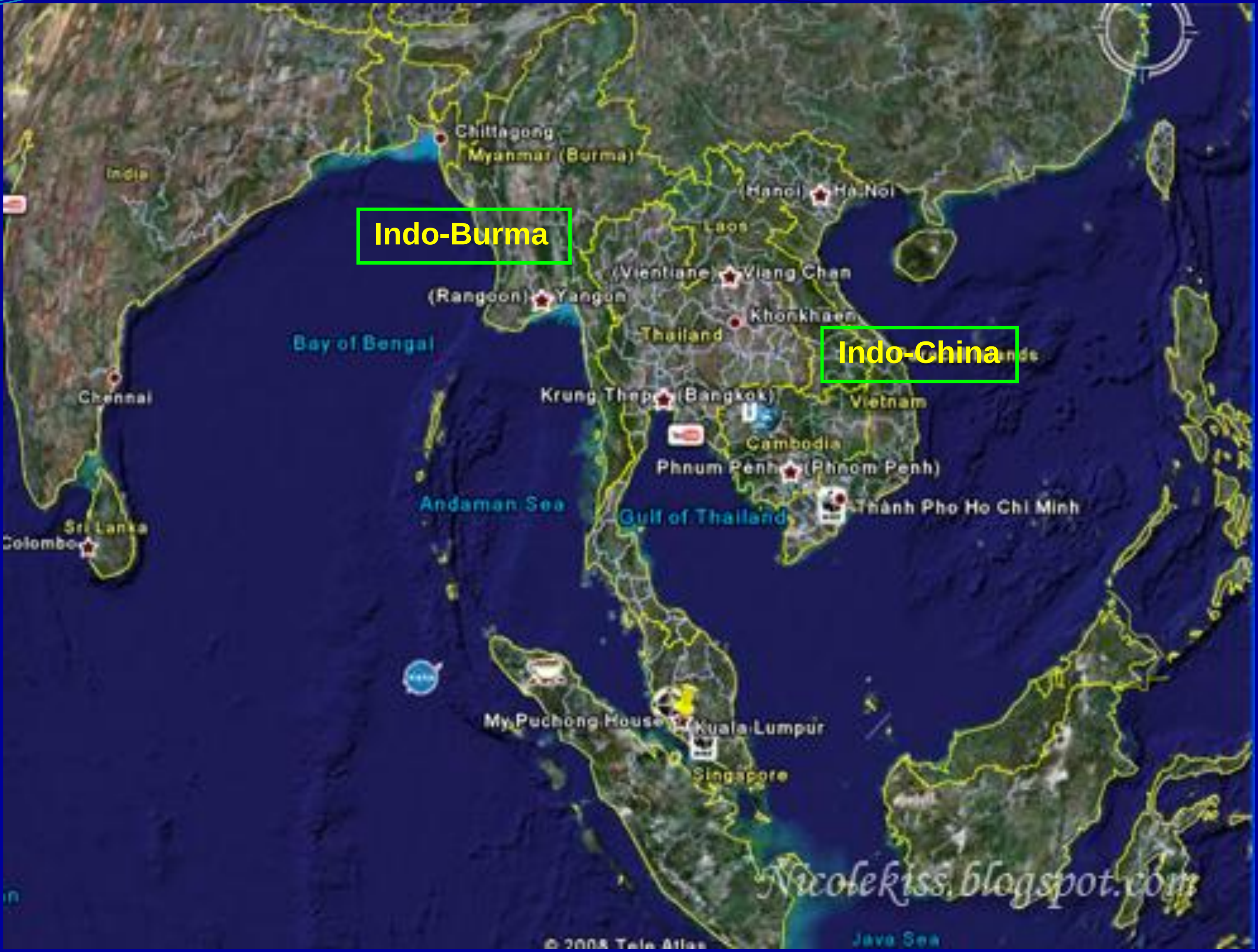
มหาวิทยาลัยเชียงใหม่



Indo-Burma
Indo-China
Chittagong
Myanmar (Burma)
India
(Hanoi) Ha Noi
Laos
(Vientiane) Viang Chan
(Rangoon) Yangon
Khonkhaen
Thailand
Bay of Bengal
Chennai
Krung Thep (Bangkok)
Vietnam
Cambodia
Phnum Penh (Phnom Penh)
Andaman Sea
Gulf of Thailand
Thanh Pho Ho Chi Minh
Sri Lanka
Colombo
My Puchong House
Kuala Lumpur
Singapore
Nicolekiss.blogspot.com
Java Sea
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“ยุงร้ายกว่าเสือ”

“ขโมยชุมกว่ายุง”

ปทานุกรมฉบับหลวงกล่าวถึงยุงไว้ว่า

“ยุงที่ปากน้ำเจ้าพระยามีชุกชุมมากจนพวกฝรั่งที่เข้ามาทางนั้นเรียกเขตนั้นว่า

“THE GREAT MUSKITO GATE” (ประตูยุงอันยิ่งใหญ่)

Culex sitiens

Anopheles epiroticus (*sundaicus* A)

วิสุทธิ์ ใบไม้, 2545
Scanlon *et al*, 1968
Rattanarithikul *et al*, 2005 & 2006
Prummongkol *et al*, 2009

<u>ยุงในประเทศไทย</u>

ยุงก้นปล่อง (*Anopheles*)

ยุงลายบ้าน (*Stegomyia aegypti*)

ยุงลายสวน (*Stegomyia albopicta*)

ยุง (ลาย) เสือ (*Coquillettidia & Mansonia*)

ยุงรำคาญ (*Culex quinquefasciatus*)

ยุงนาข้าว (*Culex tritaeniorhynchus*, etc.)

ยุงป่าชายเลน (*Culex sitiens*, etc.)

ยุงชายฝั่งทะเล- ยุงลาย (*Tanakaius togoi*)

- ยุงก้นปล่อง (*Anopheles epiroticus ~ sundaicus* A)

ยุงแม่ไก่ (*Armigeres subalbatus*)

ยุงยักษ์ (*Toxorhynchites*)

Mosquitoes

Throughout the world ~ 4,300 species
~ 42 genera

Man-biting & disease transmission ~ 10 genera
~ 156 species

Malaria: *Anopheles* ~ 70 species

Filaria: *Anopheles* ~ 43 species
Aedes ~ 5 species
Downsiomyia ~ 2 species
Ochlerotatus ~ 4 species
Tanakaius ~ 1 species
Culex ~ 5 species
Mansonia ~ 3 species
Coquillettidia ~ 1 species

Mosquitoes (cont.)

Dengue hemorrhagic fever: *Stegomyia* 2 species

Chikungunya: *Stegomyia* 2 species

Japanese encephalitis: *Culex* 6 species
: *Anopheles* 6 species,
3 species group

West Nile Virus: *Aedes* ~ 3 species
Anopheles ~ 3 species
Coquilletidia ~ 1 species
Culex ~ 6 species
Culiseta ~ 1 species
Ochlerotatus ~ 13 species
Orthopodomyia ~ 1 species
Psorophora ~ 2 species

Gould *et al*, 1974
Burke and Leake, 1988
van den Hurk *et al*, 2009
Thammapalo, 2010
Manguin *et al*, 2010
Rattanarithikul *et al*, 2005, 2006 & 2010

Mosquito-borne diseases in Thailand

Malaria

Dengue

Chikungunya

Filariasis

Japanese encephalitis

West Nile Virus ..?

Malaria

Plasmodium vivax (52.70%)
P. falciparum (46.74%)
} 0.51%

P. malariae (0.04%)

Primary (principal & regional) vectors
(infective stage found in transmitted organ)

Anopheles dirus (*dirus* A), *An. baimaii* (*dirus* D)

An. minimus (*minimus* A)

An. maculatus (species B)

Secondary (local) vectors
(infective stage found in transmitted organ)

An. aconitus

An. pseudowillmori (*maculatus* species I)

An. epiroticus (*sundaicus* A)

Gould *et al*, 1967; Scanlon *et al,* 1968; Harrison, 1980; Rosenberg *et al,* 1990; Green *et al,* 1991; Rattanarithikul *et al,* 1996; Subbarao, 1998; Linton *et al,* 2005; Sallum *et al,* 2005a & b

Malaria (cont.)

Suspected vectors
(developing stage found in organ)

An. annularis
An. barbirostris
An. campestris
An. karwari
An. kochi
An. nigerrimus
An. nivipes
An. peditaeniatus
An. philippinensis
An. sawadwongporni (*maculatus* species A)
An. sinensis
An. tessellatus
An. vagus

Baker *et al,* 1987; Harbach *et al,* 1987; Gingrich *et al,* 1990; Frances *et al,* 1996; Rattanarithikul *et al,* 1996

<u>Malaria (cont.)</u>

<u>Potential vectors</u>
(high susceptibility to malarial parasites in the laboratory)

An. cracens **(*dirus* B)**

An. harrisoni **(*minimus* C)**

An. campestris**-like (Forms B & E)**

Sallum and Peyton, 2005
Junkum *et al*, 2005
Thongsahuan *et al*, 2011

Malaria (cont.)

P. ovale curtisi (classic type)
P. ovale wallikeri (variant type)
} 0.01%

Anopheles spp ..?

P. knowlesi ~ 10 cases

An. latens (*leucosphyrus* A): Malaysia

An. cracens: Malaysia

An. dirus: Vietnam

Indra *et al*, 2008
Nakazawa *et al*, 2009
Putaporntip *et al*, 2009
Indra, 2010

Filariae

Nocturnally subperiodic *Wucheria bancrofti* (rural type: Thailand strain)

Primary vectors

Downsiomyia harinasutai, *Stegomyia desmotes*,

St. annandalei, *St. gardneri imitator,*

Mansonia dives

Harinasuta *et al*, 1970
Gould *et al*, 1982
Division of Filariasis, 1998

Potential vectors

An. dirus, An. maculatus, An. mimimus,

An. nigerrimus, *An. philippinensis*,

An. sinensis, An. subpictus malayansis,

An. vagus, Do. albolateralis, Cx. quinquefasciatus,

Cx. sitiens, St. desmotes,Tanakaius togoi

Iyengar, 1953
Harinasuta *et al*, 1971
Choochote *et al*, 1987
Chaithong *et al*, 1991
Jitpakdi *et al*, 1998
Choochote *et al*, 2001
Division of Filariasis, 2002
Junkum *et al*, 2003
Pothikasikorn *et al*, 2008

Filariae (Cont.)

Nocturnally periodic *W. bancrofti* (urban type: Myanmar strain)

Primary vector

Cx. quinquefasciatus — Meillon *et al*, 1967

Potential vectors (Thailand)

Cx. quinquefasciatus

Ta. togoi — Jitpakdi *et al*, 1998; Triteeraprapab *et al*, 2002; Jumkum *et al*, 2003

Filariae (cont.)

Nocturnally subperiodic *Brugia malayi*

Primary vectors

Ma. uniformis (0pen swamp)
Ma. bonnae (swamp-forest)

Secondary vectors

Ma. dives, *Ma. indiana*, *Ma. annulata, Ma. annulifera*

Suspected vectors

An. barbirostris/campestris

Potential vectors

An. barbirostris

An. peditaeniatus

Diurnally subperiodic *B. malayi*

Primary vectors

Coquillettidia crassipes

Iyengar, 1953
Choochote *et al*, 1984
Guptavanij *et al*, 1971
Division of Filariasis, 1995
Choochote *et al*, unpublished data

Zoonotic filarial infection

B. pahangi

Primary vectors

Armigeres subalbatus

Potential vectors

Ma. uniformis, Ta. togoi

Sucharit, 1988
Edeson *et al*, 1960
Choochote *et al*, 1986

Dirofilaria immitis

Primary vectors

St. aegypti*, *St. albopicta,
Cx. quinquefasciatus, Ma. uniformis

Suspected vectors

Cx. gelidus, Cx. tritaeniorhynchus

Potential vectors

***Neomelaniconion lineatopennis*,**
Ta. togoi, An. barbirostris/campestris

Rattanachanpichai *et al*, 1989
Tippawangkosol *et al*, 1998
Choochote *et al*, 1986 &1992

D. repens

Possible vector

St. albopicta

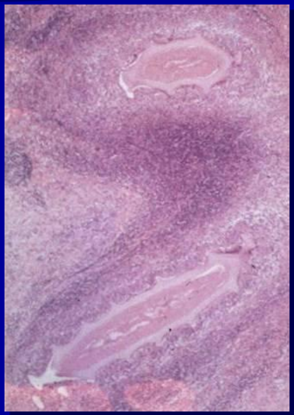
Jariya andSucharit, 1983
Cancrini *et al*, 1995 & 2003

Zoonotic filarial infection (cont.)

Japan

O. dewittei japonica

Onchocerca nodule



Adult worm: x-section

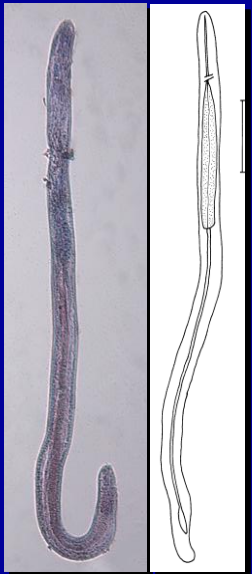
Thailand

- **86 black-fly species**
- **29 new records**
- **57 new species [31]**
- **5 man-biting species**
- **2 natural vectors**

S. nigrogilvum

S. nodosum

Dermatitis



Infective larva

Takaoka *et al*, 2001 & 2003; Fukuda *et al*, 2003; Choochote *et al*, 2005; Ishii *et al*, 2008

Dengue hemorrhagic fever

Primary vectors

St. aegypti, St. albopicta

Gould *et al*, 1968
Runick, 1978
Self, 1979
Monath, 1994

Transovarian transmission & Sexual transmission → **Polygamous behavior**

Rosen *et al*, 1983 & 1987
Tu *et al*,1998
Choochote *et al*, 2001b

Potential vectors

Do. albolateralis

Choochote *et al*, 2001a

Dengue hemorrhagic fever (cont.)

POLYGAMY: THE POSSIBLY SIGNIFICANT BEHAVIOR OF *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* IN RELATION TO THE EFFICIENT TRANSMISSION OF DENGUE VIRUS

Table 1
Mating ability of one *Ae. aegypti* male with 10, 20 and 30 females during co-habitation in a 30 cm^3 cage.

Duration of co-habitation (Day)	No. females that succeeded in insemination at		
	1 : 10	1 : 20[a]	1 : 30
1	0	4	0
2	2	8	11
3	5	5	14
4	7	7	7
5	7	8	7

Table 2
Mating ability of one male *Ae. aegypti* and male *Ae. albopictus* with 20 females during co-habitation in a 30 cm^3 cage.

Duration of co-habitation (Day)[a]	No. females that succeeded in inseminating		Mean ± SD No. females mated by one male (range)		*t*, p
	Ae. aegypti	*Ae. albopictus*	*Ae. aegypti*	*Ae. albopictus*	
1	22	4	1.10 ± 1.33 (0-4)	0.20 ± 0.70 (0-3)	2.68, =0.01
2	82	34	4.10 ± 1.41 (1-8)	1.70 ± 0.80 (0-3)	6.62, <0.001
3	108	47	5.40 ± 1.10 (4-8)	2.35 ± 0.88 (1-4)	9.73, <0.001
4	102	46	5.10 ± 1.71 (2-8)	2.30 ± 1.13 (0-4)	6.10, <0.001
5	103	47	5.15 ± 1.69 (3-9)	2.35 ± 1.04 (1-4)	6.30, <0.001

[a]Twenty experiments for each duration.

Choochote *et al*, 2001b

Chikungunya

Vectors

St. aegypti, St. albopicta

Ross, 1956
McIntosh and Gear, 1981
Gratz, 2004
Vazeille *et al,* 2007
Reiskind *et al,* 2008

Transovarian transmission & Sexual transmission..? → **Polygamous behavior**

Choochote *et al*, 2001b
Thavara *et al*, 2009
Dubrulle *et al*, 2009

Japaneses encephalitis

Primary vectors

Cx. tritaeniorhynchus

Secondary vectors

***Cx. fuscocephala*, *Cx. gelidus*,**

Cx. pseudovishui*, *Cx. vishui

Possible vectors

***An. annularis*; *An. peditaeniatus*; *An. sinensis*; *An. subpictus*;**

***An. tessellatus*; *An. vagus*; various members of the Barbirostris,**

Hyrcanus, Umbrosus Groups; *Cx. quinquefasciatus*;

Cx. bitaeniorhynchus*; *Cx. infula

Gould *et al*, 1962 & 1974
Thao *et al*, 1974
Gingrich *et al*, 1992
Rattanarithikul *et al*, 2005 & 2006

West Nile Virus

Primary vectors

Culex quinquefasciatus*, *Cx. pipiens*, *Cx. restuan

Suspected vectors

***St. albopicta*, *Ae. cinereus*, *Aedimorphus vexans*, *Anopheles barberi*,**

***An. punctipennis*, *An. quadrimaculatus*, *Coquilletidia perturbaur*,**

***Cx. nigripalpus*, *Cx. salinarius*, *Cx. vishnui*, *Culiseta melanura*,**

***Ochlerotatus atlanticus*, *Oc. atropalpers*, *Oc. canadensis*, *Oc. cantator*,**

***Oc. japoricus*, *Oc. sollicitaus*, *Oc. taenirynchus*, *Oc. tormentor*, *Oc. trivittatus*,**

Orthopodomyia signifera*, *Psorophora columbiae*, *Ps. fero

Potential vectors

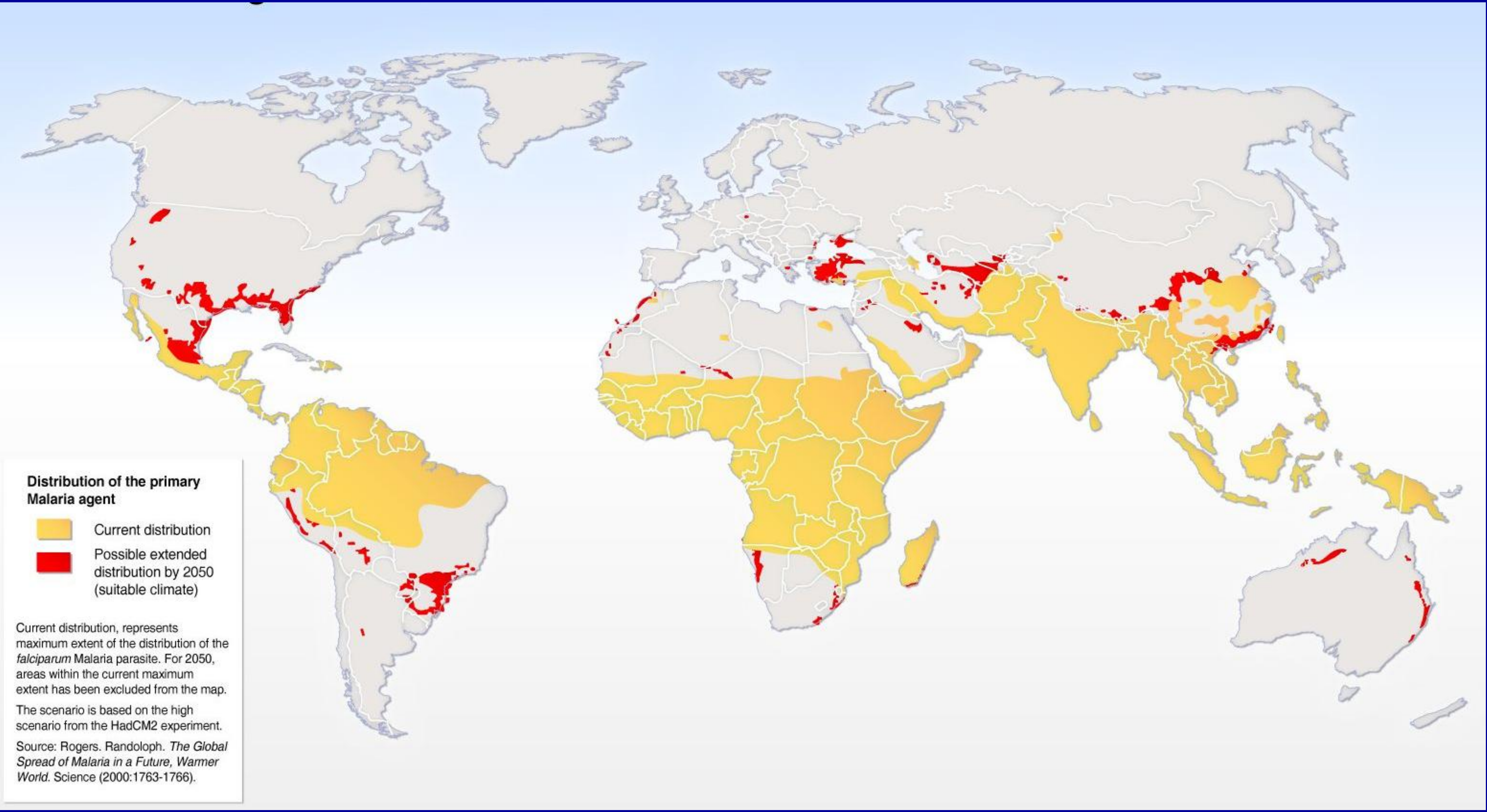
Cx. tritaeniorynchus*, *Cx. pseudovishui*, *Cx. univittatus

Possible vectors (Thailand ~ 6 species)

***St. albopicta*, *Am. vexans*, *Cx. quinquefasciatus*,**
Cx. tritaeniorynchus*, *Cx. vishnui*, *Cx. pseudovishui

Likal *et al,* 1997; Turell *et al*, 2001; Thammapalo, 2010; Rattanarithikul *et al*, 2005, 2006 & 2010

Will global warming spread mosquito-borne diseases ..?

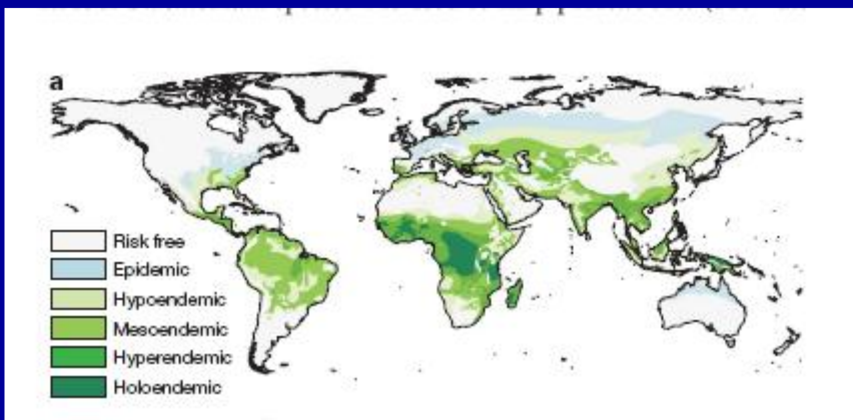


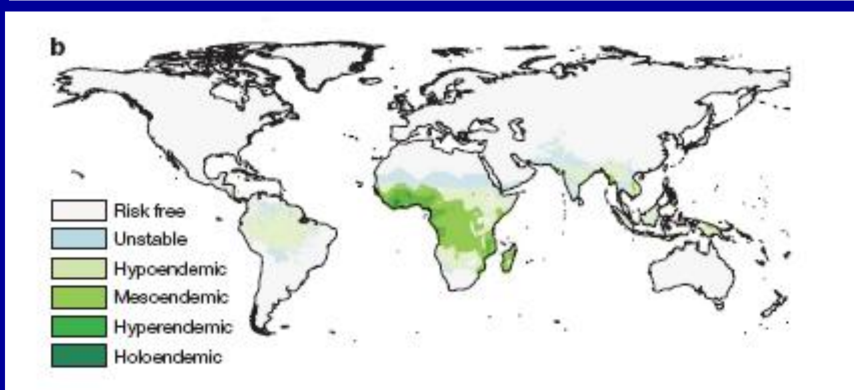
nature Vol 465 | 20 May 2010 | doi:10.1038/nature09098

LETTERS

Climate change and the global malaria recession

Peter W. Gething[1], David L. Smith[2,3], Anand P. Patil[1], Andrew J. Tatem[2,4], Robert W. Snow[5,6] & Simon I. Hay[1]





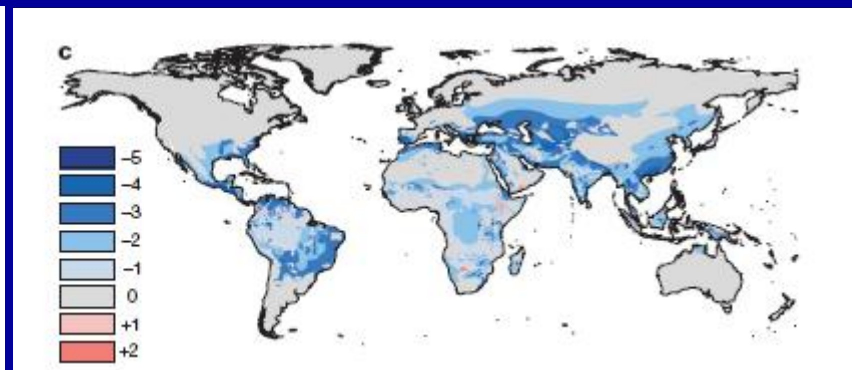


Figure 1 | Changing global malaria endemicity since 1900. a, Pre-intervention endemicity (approximately 1900) as defined in ref. 13. **b**, Contemporary endemicity for 2007 based on a recent global project to define the limits and intensity of current *P. falciparum* transmission[8]. **c**, Change in endemicity class between 1900 and 2007. Negative values denote a reduction in endemicity, positive values an increase.

Extent of malaria decreases during global warming (1900-2007)

Malaria in Africa likely to decline with global warming

- Malaria is the main public health problem in Burundi
- 2 million clinical cases and more than 15,000 deaths each year
- Statistician and epidemiologist from Australia and Burundi
  - monthly rainfall, temperature, humidity data,
    monthly malaria morbidity data from Burundi for 1996-2007
  - data on monthly malaria morbidity for each province of Burundi
  - Bayesian Generalized Additive Model (GAM)

↓

Although malaria transmission is positively associated with minimum temperature & maximum humidity, increasing temperature in Burundi will not result in increasing malaria transmission

Nkurunziza and Pilz, 2011
Science daily, 2011

The effect of extrinsic incubation temperature on development of Dengue serotype 2 and 4 viruses in *Aedes aegypti*

Extrinsic incubation period (EIP)

26-28^{o}C = 9 days
30^{o}C = 5 days

Rohani *et al*, 2009

Chikungunya virus & *Aedes* mosquitoes: Saliva is infectious as soon as 2 days after oral infection

Dubrulle *et al*, 2009

Global warming altering genes of mosquito

Wyoemyia smithii
(plant pitcher mosquito)

" Evolution is happening & it is happening very fast"
Dr. Willium Bradshaw, University of Oregon

The insects' life cycle is controlled by a genetic switch linked to the length of day, or photoperiod

⬇

Delay dormancy: the insects are now entering their pupae 8-10 days later than did in the 1970s

http://news.bbc.co.uk/2/hi/science/nature/1639284.stm

Biological evolution of mosquito

Speciation ~ species A --> A_1

--> A_2

--> A_3

--> …

SPECIES COMPLEX

sibling species members

วิสุทธิ์ ใบไม้ 2545, 2548 & 2553

Evolutionary Processes

MUTATION

- **Gene** ~ base substitution; changes in DNA content
- **Chromosome** ~ sequence rearrangements; changes in heterochromatin

↓

NEW GENETIC RESOURCES

↓

GENE RECOMBINATION

(through sexual reproduction and crossing over (diplotene → diakinesis)

meiosis II

↓

GENETIC VARIATION → GENETIC DIVERSITY

↓

Speciation → SPECIES DIVERSITY

↑

MICROENVIRONMENTAL VARIATION → ECOLOGICAL DIVERSITY

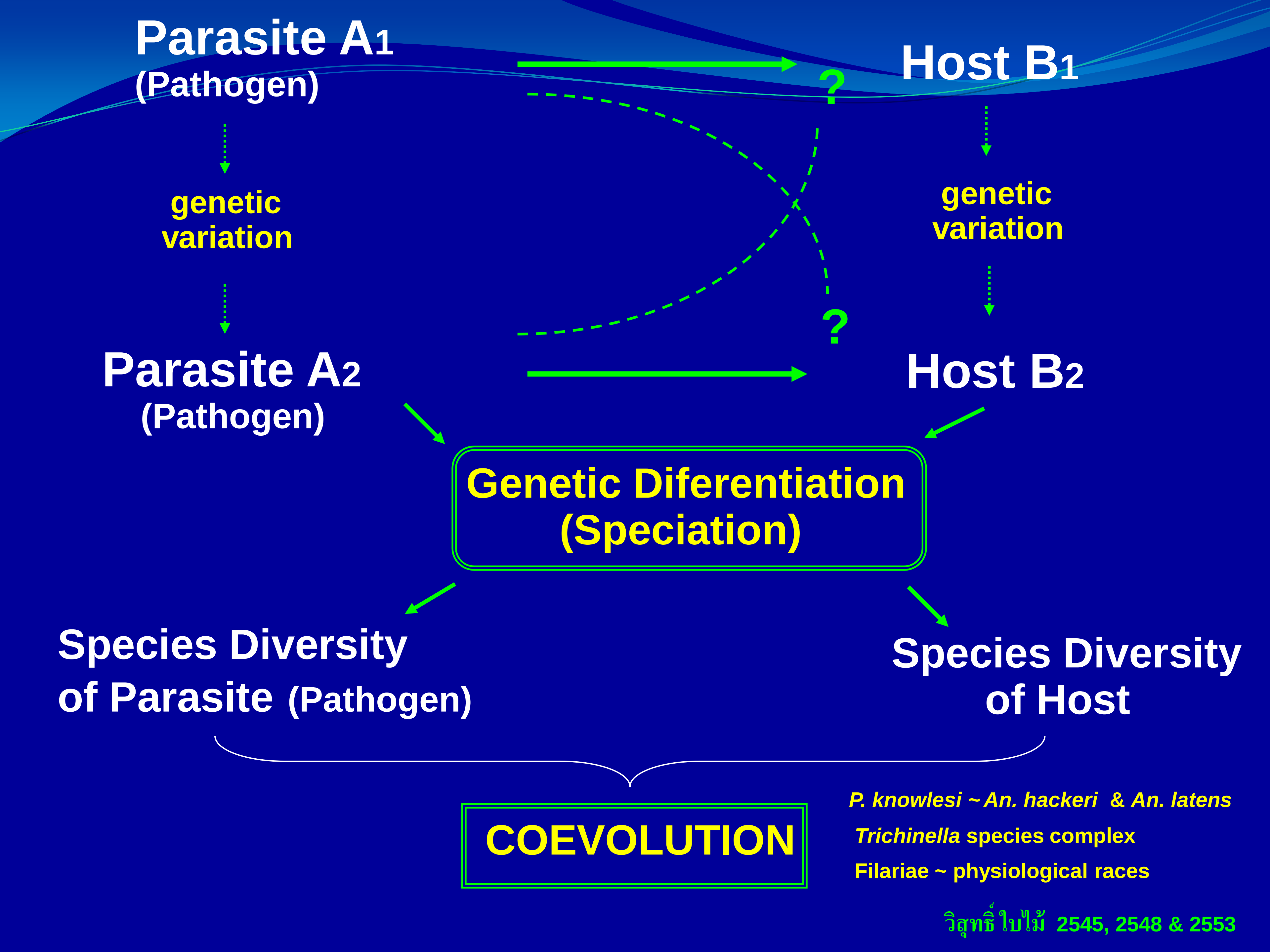
(microhabitat)

↑

Global warming

BIODIVERSITY

วิสุทธิ์ ใบไม้ 2545, 2548 & 2553


Parasite A1
(Pathogen)
Host B1
?
genetic variation
genetic variation
?
Parasite A2
(Pathogen)
Host B2
Genetic Diferentiation
(Speciation)
Species Diversity
of Parasite (Pathogen)
Species Diversity
of Host
COEVOLUTION
P. knowlesi ~ An. hackeri & An. latens
Trichinella species complex
Filariae ~ physiological races
วิสุทธิ์ ใบไม้ 2545, 2548 & 2553

Anopheles

Throughout the world ~ 30 species: exhibit species complexes

~ 145 sibling species members

Characteristics of sibling species members

morphology ~ morphologically identical (isomorphic)

~ minimal morphological distinction

~ cryptic species identification

genetic isolation at pre- and/or post-mating barriers

In Thailand: *Anopheles* mosquitoes ~ 72 species

~ 21 sibling species members

Subbarao, 1998
Rattanarithikul *et al*, 2006
Saeung *et al*, 2007 & 2008
WHO, 2008
Suwannamit *et al*, 2009

Significance of sibling species members

❖ **Distinct distribution**

Anopheles dirus complex

An. dirus **(species A) ~ throughout Thailand, except southern region**
An. cracens **(species B) ~ southern Thailand & peninsular Malaysia**
An. scanloni **(species C) ~ Kanchanaburi province & southern Thailand**
An. baimaii **(species D) ~ Thai-Myanmar border**

Baimai *et al*, 1988
Baimai, 1989

An. minimus complex

An. ninimus **~ throughout Thailand**
An. harrisoni **~ rather confined to Kanchanaburi province**

Green *et al*, 1990

An. barbirostris complex

***An. campestris*-like** **~ widely distributed throughout Thailand- plane areas**
An. barbirostris **species A1 ~ widely distributed throughout Thailand**
An. barbirostris **species A2 ~ north, northeast & central Thailand**
An. barbirostris **species A3 ~ confined to Kanchanaburi province**
An. barbirostris **species A4 ~ confined to Chiang Mai province**

} **foot-hill areas**

Saeung *et al*, 2007 & 2008
Thongsahuan *et al*, 2009
Suwannamit *et al*, 2009

Significance of sibling species members (cont.)

❖ **Distinct behavior ~ anthropophilic, zoophilic, nocturnal biting activity**

An. barbirostris **complex**

***An. campestris*-like ~ anthropophilic**
An. barbirostris **species A1, A2, A3, A4 ~ zoophilic**

Saeung *et al*, 2007 & 2008
Thongsahuan *et al*, 2009

An. dirus **complex**

An. dirus **~ 0900-1200 pm**
An. cracens **~ 0600 -1000 pm**
An. scanloni **~ 0600-0900 pm**
An. baimaii **~ 0200-0500 am**

Baimai *et al*, 1988

❖ **Distinct vector potential ~ vector & non-vector**

An. barbirostris* complex~ *P. vivax

- ***An. barbirostris* species A4 ~ refractory vector**
- ***An. barbirostris* species A1, A2 & A3 ~ low potential vectors**
- ***An. campestris*-like ~ high potential vector**

An. culicifacies* complex ~ *P. vivax

- ***An. culicifacies* species A & C ~ susceptible vectors**
- ***An. culicifacies* species B ~ refractory vector**

❖ **Different degrees of development for insecticide resistance**

***An. culicifacies* complex**

- ***An. culicifacies* species A & B ~ sympatric association**
  ~ species A more susceptible to DDT than species B
- ***An. culicifacies* species B & C ~ sympatric association**
  ~ species C developed resistance to malathion at a faster rate than species B

❖ **Complication of identifying target vectors**

❖ **Potentially misleading methods of control**

Subbarao *et al*, 1988
Raghavendra *et al*, 1991 & 1992
Subbarao, 1998
WHO, 2008
Thongsahuan *et al*, 2011

Population-genetic study of *Anopheles* vectors
(pre- and/or post-mating barriers)

Thailand ~ 72 *Anopheles* spp.

***Anopheles* species complexes**

- ***Anopheles annularis* complex (p)**
- ***An. culicifacies* complex (p)**
- ***An. dirus* complex (m & c) [5]**
- ***An. leucosphyrus* complex (m & c) [1]**
- ***An. maculatus* complex (p & i) [7]**
- ***An. minimus* complex (i) [2]**
- ***An. philippinensis-nivipes* complex (c & p)**
- ***An. sundaicus* complex (m, p & i) [1]**

p ~ fixed paracentric inversion of polytene chromosome
m ~ metaphase karyotype
i ~ isoenzyme divergence
c ~ crossing experiment

Baimai *et al*, 1987, 1993, 1996a & b
Green *et al*, 1985, 1990, 1992a & b
Sukowati and Baimai, 1996
Sukowati *et al*, 1999
Subbarao, 1998

Flow chart for rapid systematic procedure

Human-baited and/or animal-baited traps ~ different microhabitat

↓

Wild-caught fully engorged females ~ species identification

↓

Individually deposited eggs ~ isoline colony

↓ → Molecular investigation of feral females by PCR (rDNA: ITS2; mtDNA: COI, COII; etc.)

F_1-progeny

↓

- Morphometric & morphological studies of eggs, larvae, pupal skins & adults
- Karyotype identification of 4th-instar larvae and/or newly emerged adults
- Molecular confirmation by PCR- (rDNA: ITS2; mtDNA: COI, COII; etc.)
- Crossing experiment
- Polytene chromosome investigation of 4th-instar larvae

Saeung *et al*, 2007

By the application of this systematic procedure

Sibling species

An. barbirostris complex

(*An. barbirostris* A1, A2, A3, A4, *An. campestris*-like)

Saeung *et al,* 2007, 2008
Suwannamit *et al,* 2009

Subspecies (cytological races)

An. vagus Form A & B

An. pullus Form A & B (*An. yatsushiroensis*)

An. aconitus Form B & C

An. sinensis Form A & B

An. campestris-like Form B, E & F

An. peditaeniatus Form B, C, D & E

Choochote *et al,* 2002
Park *et al,* 2003
Junkum *et al,* 2005
Park *et al,* 2008
Thongsahuan *et al,* 2009
Choochote *et al,* 2011

Population-genetic study of

Anopheles barbirostris complex

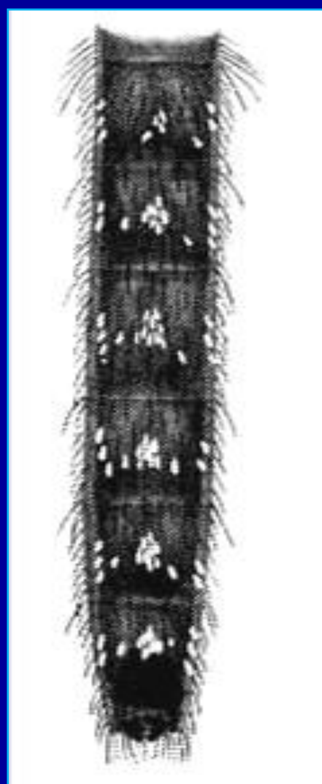
(Diptera: Culicidae) in Thailand

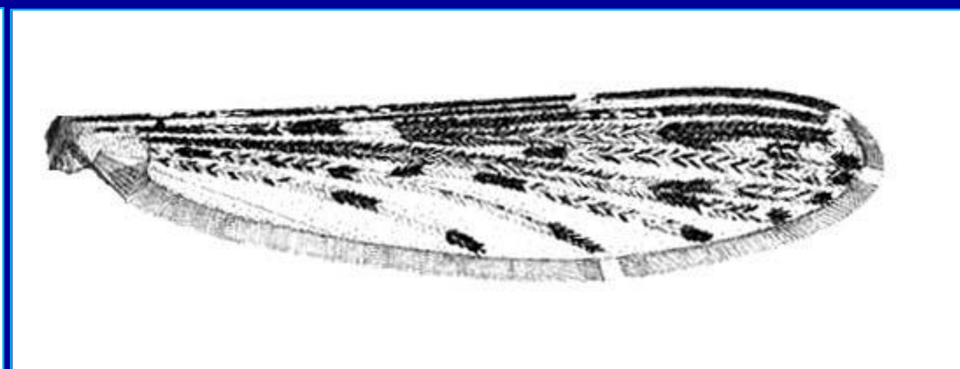
Morphology

Adult: morphologically cryptic

Two types of *An. barbirostris* were recognized in Malaysia

- **The light-wing type, with a few pale sternal scales**

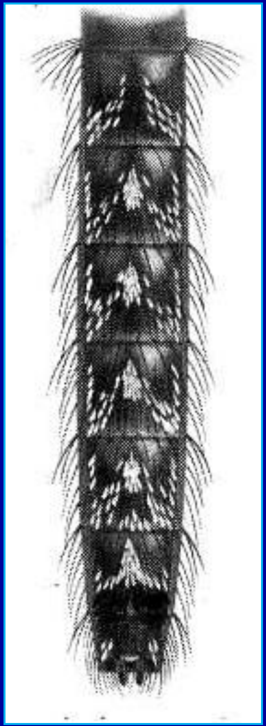




An. barbirostris

Reid, 1942

- The dark-winged type, with an abundance of pale sternal scales
- Morphological difference, behavioral trait, vector capabilities, quite distinct from *An. barbirostris*



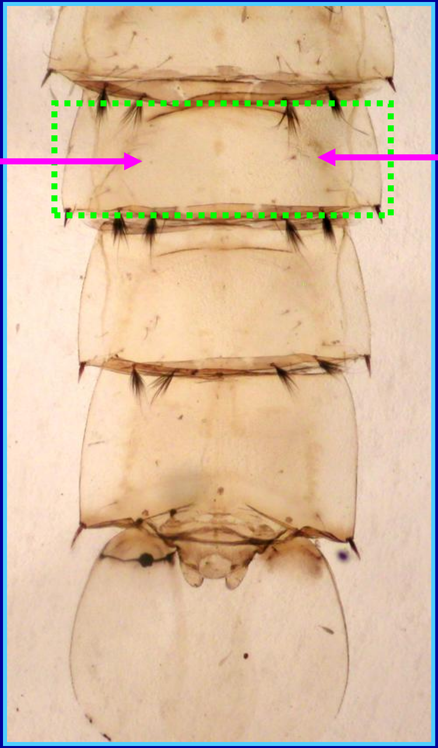
An. campestris

Reid, 1942

Morphology

***An. barbirostris/An. campestris*: seta 2-VI pupal skin (95-97% level)**

An. barbirostris
(6-18 branches)

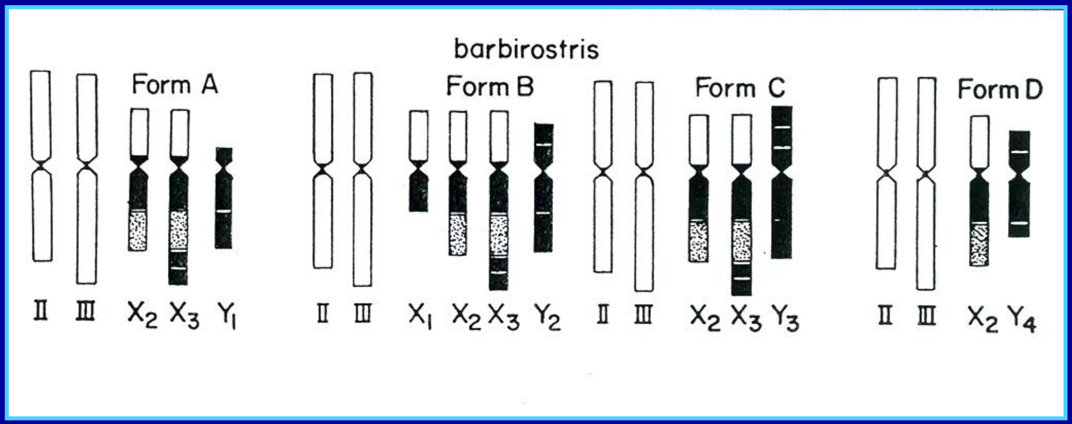


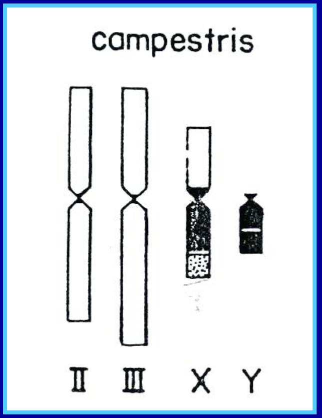
An. campestris
(17-58 branches)

Harrison and Scanlon, 1975

Cytology

Diagrammatic representation of metaphase karyotypes of *An. barbirostris* & *An. campestris*





***An. barbirostris*: Form A, Form B & Form C**

(sympatric and/or allopatric populations in Thailand)

: Form D (only in Java, Indonesia)

***An. campestris*: unique karyotype**

Baimai *et al*, 1995

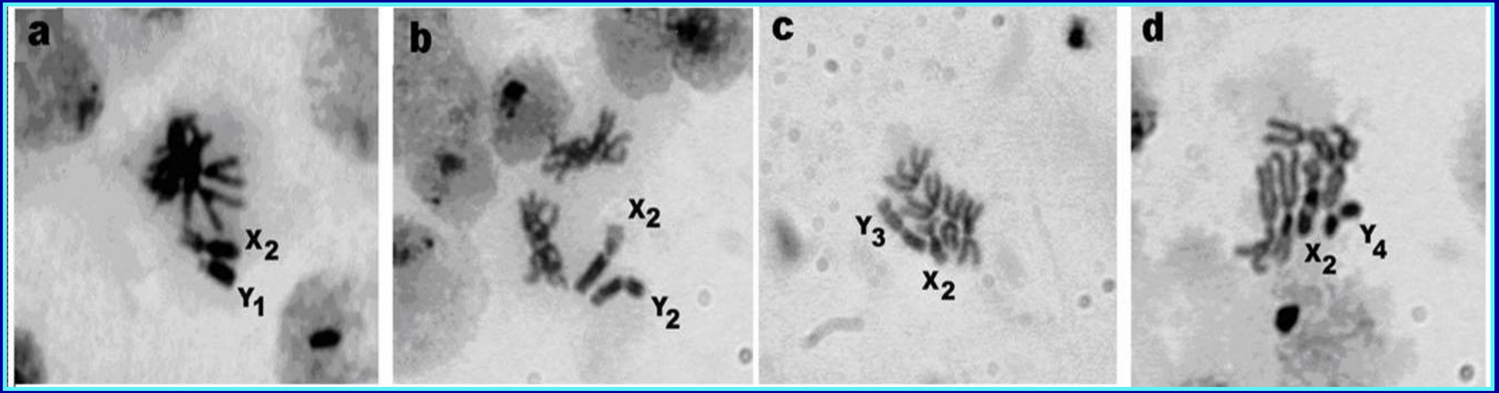
Total isolines : 133

<u>Morphology</u> : 2 groups of seta 2-VI pupal skins

: 42 isolines ~ *An. barbirostris* (9-16 branches)

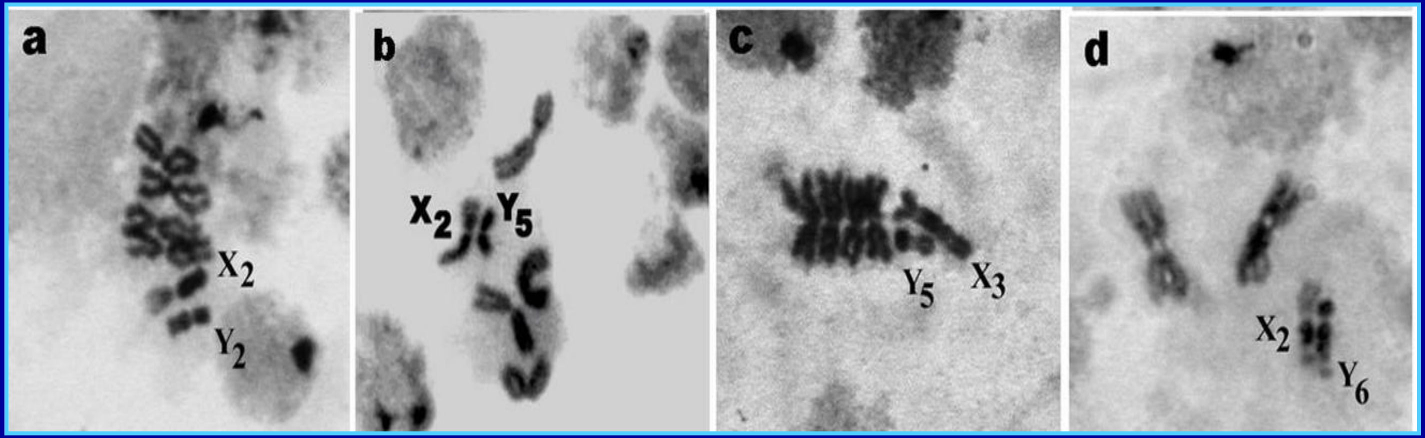
: 71 isolines ~ *An. campestris* (20-30 branches)

<u>Cytology</u> : *An. barbirostris* ~ 4 karyotypic forms



Form A **Form B** **Form C** **Form D**

Cytology: *An. campestris* ~ 3 karyotypic forms



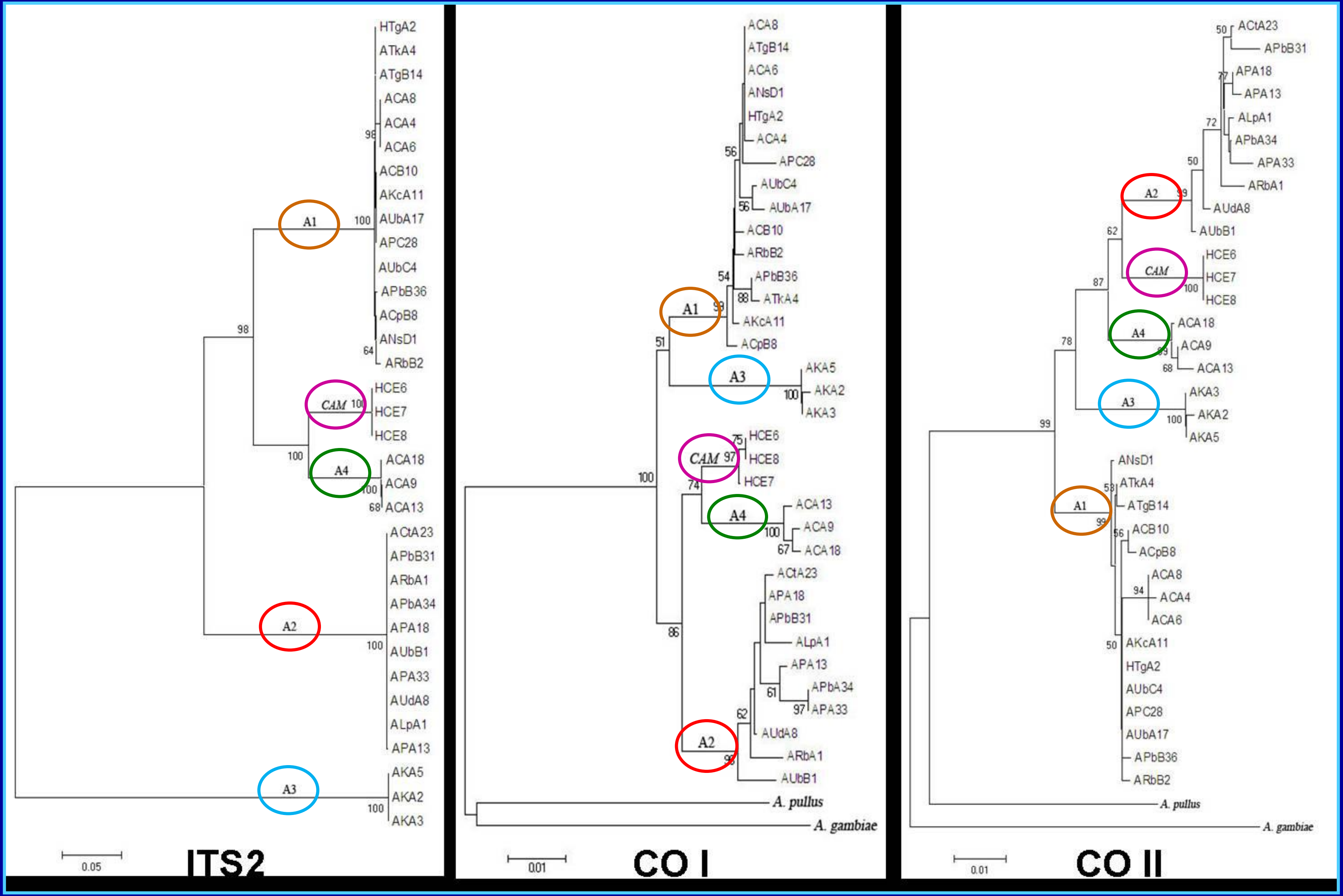
Form B   Form E   Form E   Form F

similarity of X_2-chromosome to *An. barbirostris*

↓

designated as *An. campestris*-like Form B, E & F

DNA analyses: 5 different groups ~ 4 groups of *An. barbirostris* ~ 1 group of *An. campestris*-like



Crossing experiments

Large sequence divergence: genetic distance (0.02-0.62)
(ITS2, COI & COII)

Group A1 (1,861 bp): Form A ~ Chiang Mai (iACA6)

Group A2 (1,717 bp): Form A ~ Phetchaburi (iAPA13)

Group A3 (1,070 bp): Form A ~ Kanchanaburi (iAKA5)

Group A4 (1,676 bp): Form A ~ Chiang Mai (iACA18)

CAM **(1,651 bp): Form E ~ Chiang Mai (iHCE6)**

A1 X A2, A1 X A3, A1 X A4, A1 X *CAM*

A2 X A3, A2 X A4, A2 X *CAM*

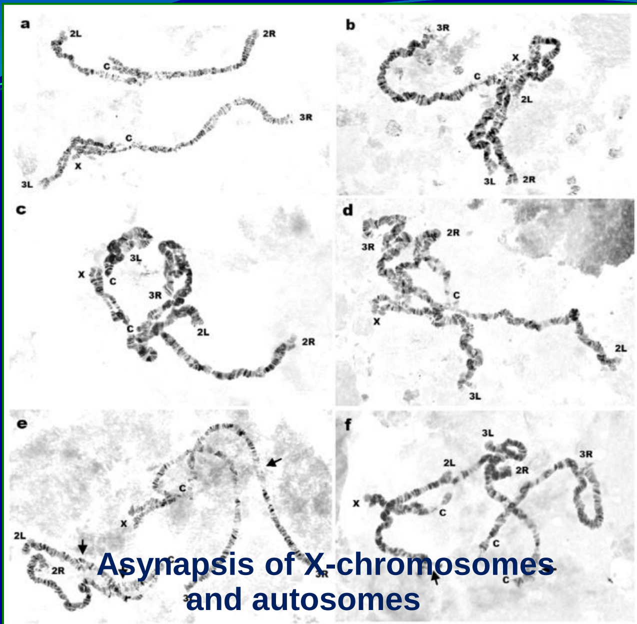
A3 X A4, A3 X *CAM*

A4 X *CAM*

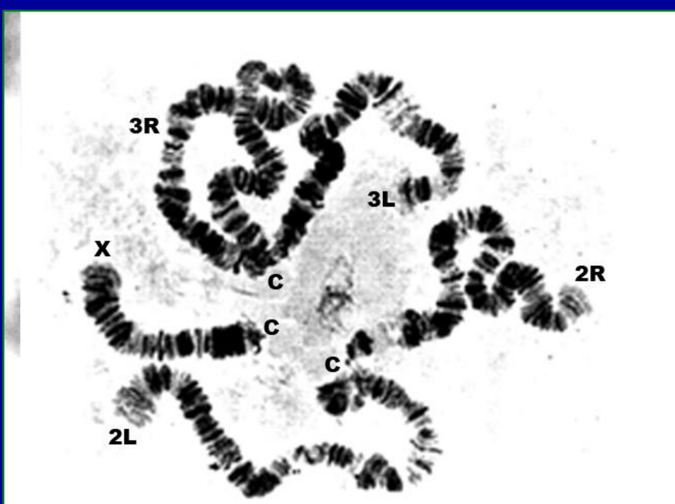
⬇

Post-mating reproductive isolation/ genetic incompatibility
(inviable F_1-progenies)

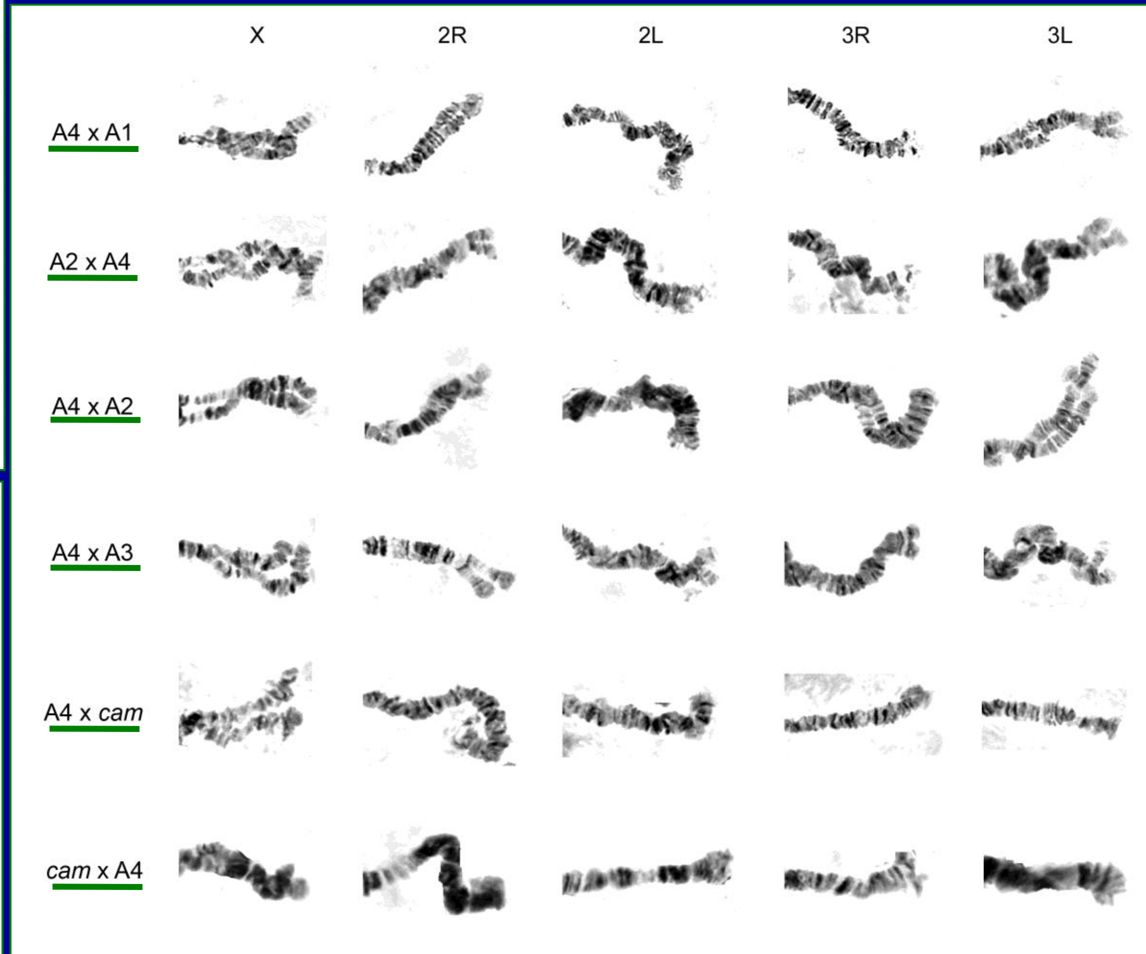
Salivary gland polytene chromosome of the F_1-hybrid larvae



Asynapsis of X-chromosomes and autosomes

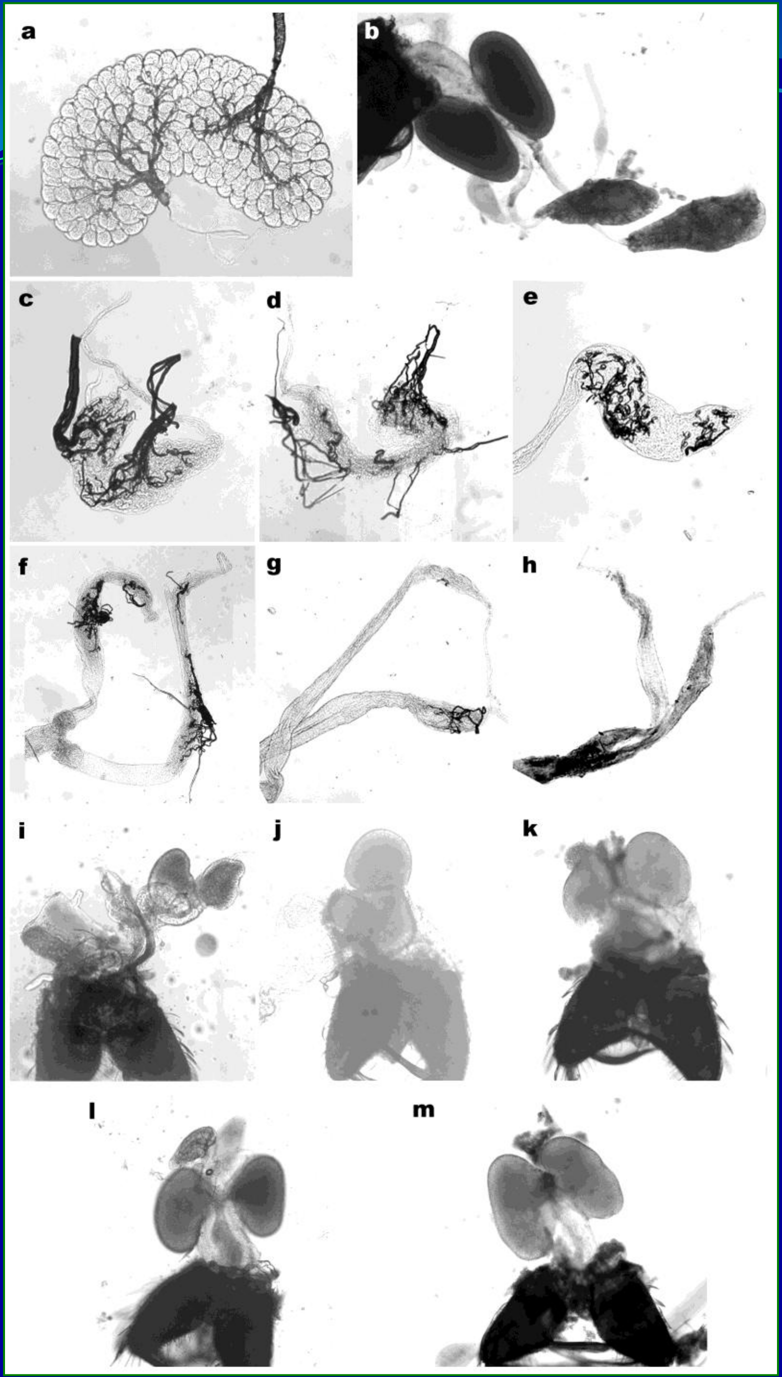


Complete synapsis in all arms



Asynapsis at the free ends

An. barbirostris **species A1 x A4: homosequential banding pattern**



Normal reproductive systems

(a) Ovarian follicles ~ *An. barbirostris* species A4

(b) Accessory glands & testes ~ *An. campestris*-like Form E

Atrophied ovarian follicles of F_1-hybrids

(c) A4 x A1

(d) A2 x A4

(e) A4 x A2

(f) A4 x A3

(g) A4 x *An. campestris*-like Form E

(h) *An. campestris*-like Form E x A4

Atrophied accessory glands& testes of F_1-hybrids

(i) A4 x A1

(j) A2 x A4

(k) A4 x A3

(l) A4 x *An. campestris*-like Form E

(m) *An. campestris*-like Form E x A4

Conclusion

Based on comparative morphology, cytogenetics, molecular analysis & crossing experiment

⇩

5 sibling species were discovered in the taxon *An. barbirostris*

***An. barbirostris* species A1:** widely distributed throughout Thailand

species A2: distributed and occurred in sympatry with species A1 in some populations in north, northeast and central Thailand

species A3: confined to Kanchanaburi

species A4: confined to Chiang Mai

} Foot-hill areas

***An. campestris*-like:** widely distributed throughout Thailand

} Plane areas

Malaria susceptibility test

P. vivax

Refractory vector ~ 0% oocyst & sporozoite rates
[*An. barbirostris* species A4]

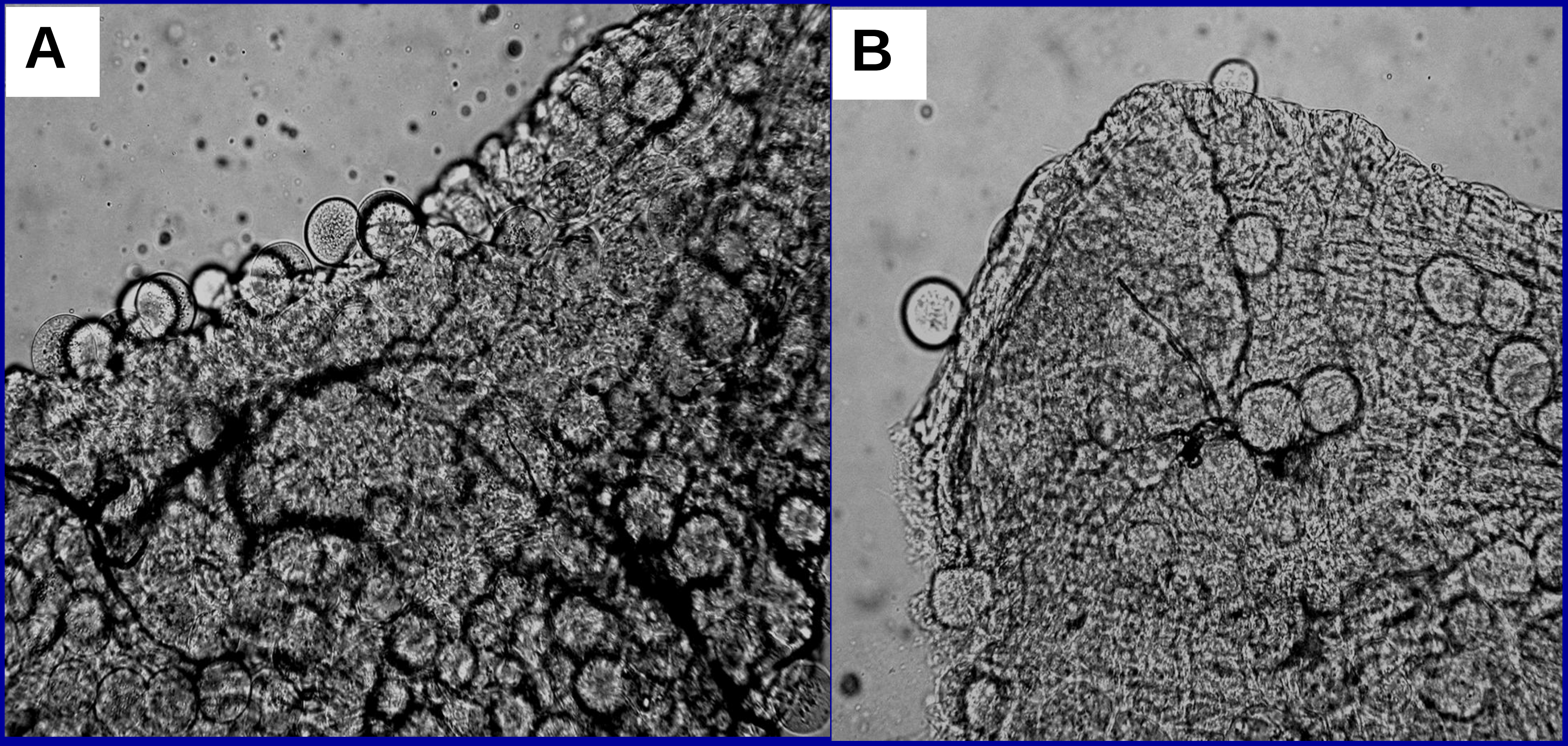
Low potential vectors ~ 40-60% oocyst rates
~ 6.67-11.76% sporozoite rates
[*An. barbirostris* species A1, A2, A3]

High potential vectors ~ 100% oocyst rates
~ 64.29-66.67% sporozoite rates
[*An. campestris*-like Form B & E]

Control vectors ~ 76.92-100% oocyst rates
~ 85.71-92.31% sporozoite rates
[*An. cracens*]

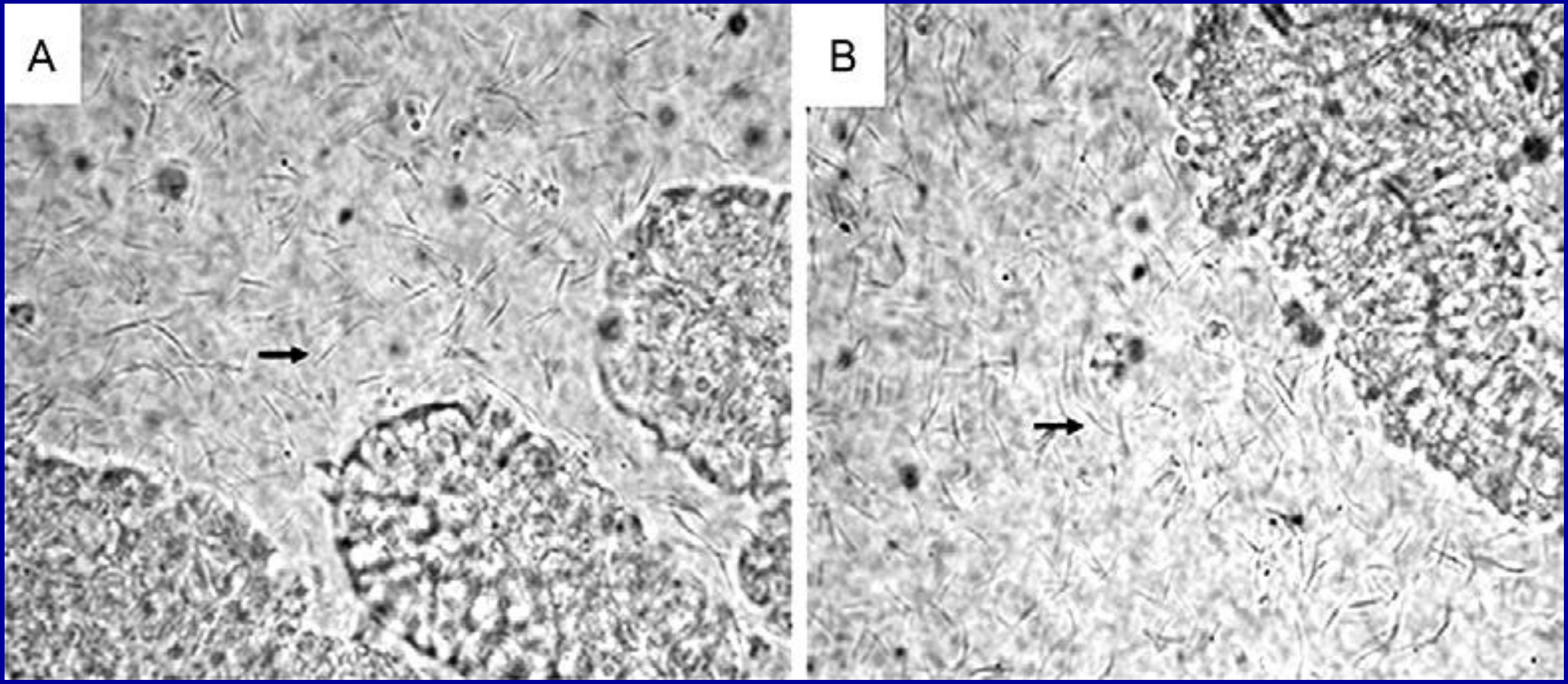
Target vector for control ~ *An. campestris*-like

Thongsahuan *et al*, 2011



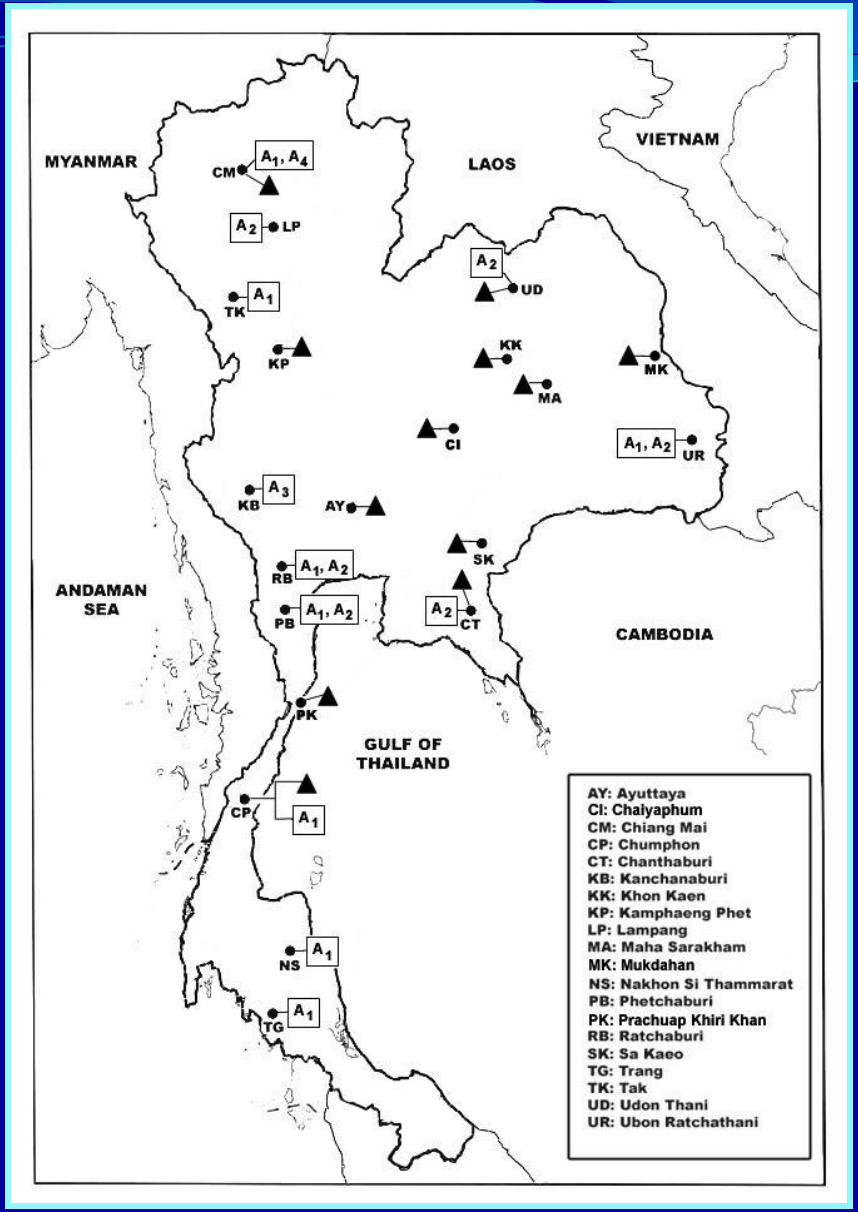
Oocysts of *Plasmodium vivax* recovering from the midgut of
(A) *Anopheles cracens*
(B) *Anopheles campestris*-like Form E (Chiang Mai)
on day eight after infection

Thongsahuan *et al*, 2011



Free flow regular spindle-shaped sporozoites of *Plasmodium vivax* from the squashed salivary glands (small arrow) of
(A) *Anopheles cracens*
(B) *Anopheles campestris*-like Form E (Chiang Mai) on day 14 after infection

Thongsahuan *et al*, 2011



Map of Thailand showing distribution of *An. campestris*-like (▲) and *An. barbirostris* species A_1, A_2, A_3, A_4 (□)

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ขอบคุณมากครับ