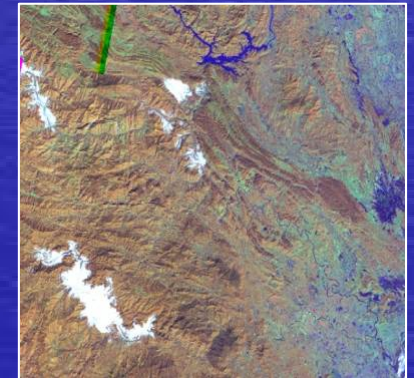


From molecular to spatial approaches for the characterization of malaria vectors in Southeast Asia



Sylvie MANGUIN, PhD
Research Professor
IRD, UMR-MD3
Faculty of Pharmacy (UMI)
34093 Montpellier. FRANCE
sylvie.manguin@ird.fr



Vector Control

- To implement efficient vector control programs, characterization of the vectors is necessary as for:
 - Identification of the species,
 - Estimation of the vectorial capacity (pathogen detection),
 - Resistance/susceptibility to insecticides,
 - Spatial characterization of habitats, densities and distribution

Species identification

An efficient vector control must target the correct vector species

- Morphology is still the method of reference for sorting out specimens at the genus or subgenus or complex level
- However, for species complex, molecular assays (PCR) are the only reliable methods for identifying *Anopheles* at the species level

Why molecular approaches for identifying mosquito species?

- Most of the malaria vectors belong to species complexes in which **sibling species** are **morphologically indistinguishable** and may present **different vectorial capacities** and **trophic behaviors**
- Precise identification will allow to **target the correct vector species** in control programs, and help to define the **appropriate way to control** them in relation to their specific behavior

High biodiversity in the Oriental Region

- Numerous *Anopheles* species per site
- Each vector belongs to a species complex such as:
 - *An. barbirostris*
 - *An. culicifacies*
 - *An. dirus*
 - *An. fluviatilis*
 - *An. leucosphyrus*
 - *An. philippinensis*
 - *An. maculatus*
 - *An. minimus*
 - *An. subpictus*
 - *An. sundaicus*

Main malaria vectors in Southeast Asia

- *Anopheles dirus*, *An. minimus* and *An. maculatus*: predominant malaria vectors in hilly and forested regions

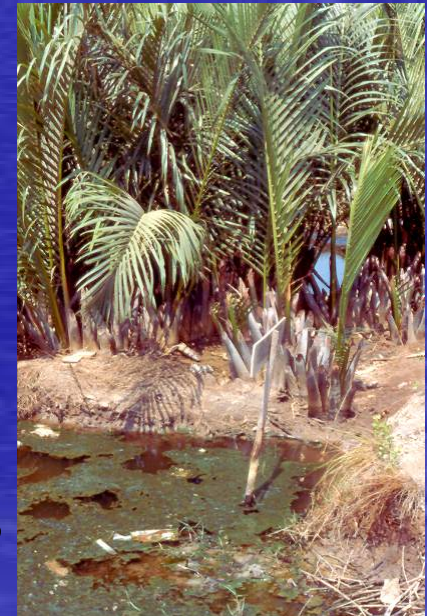
An. dirus



An. minimus



Vectors of both
P. falciparum
and *P. vivax*



- *An. sundaicus*: main vector along coastal areas

Anopheles dirus complex: one of the world's most efficient vectors

➤ Main vectors of *P. f.* and *P. v.* in deep forest

➤ Highly anthropophilic and exophilic (difficult to control)

➤ Larval habitats in temporary pools of dense forests, stagnant water, artificial containers

➤ Complex of 7 species with different roles in malaria transmission (from highly efficient vector to non-vector)

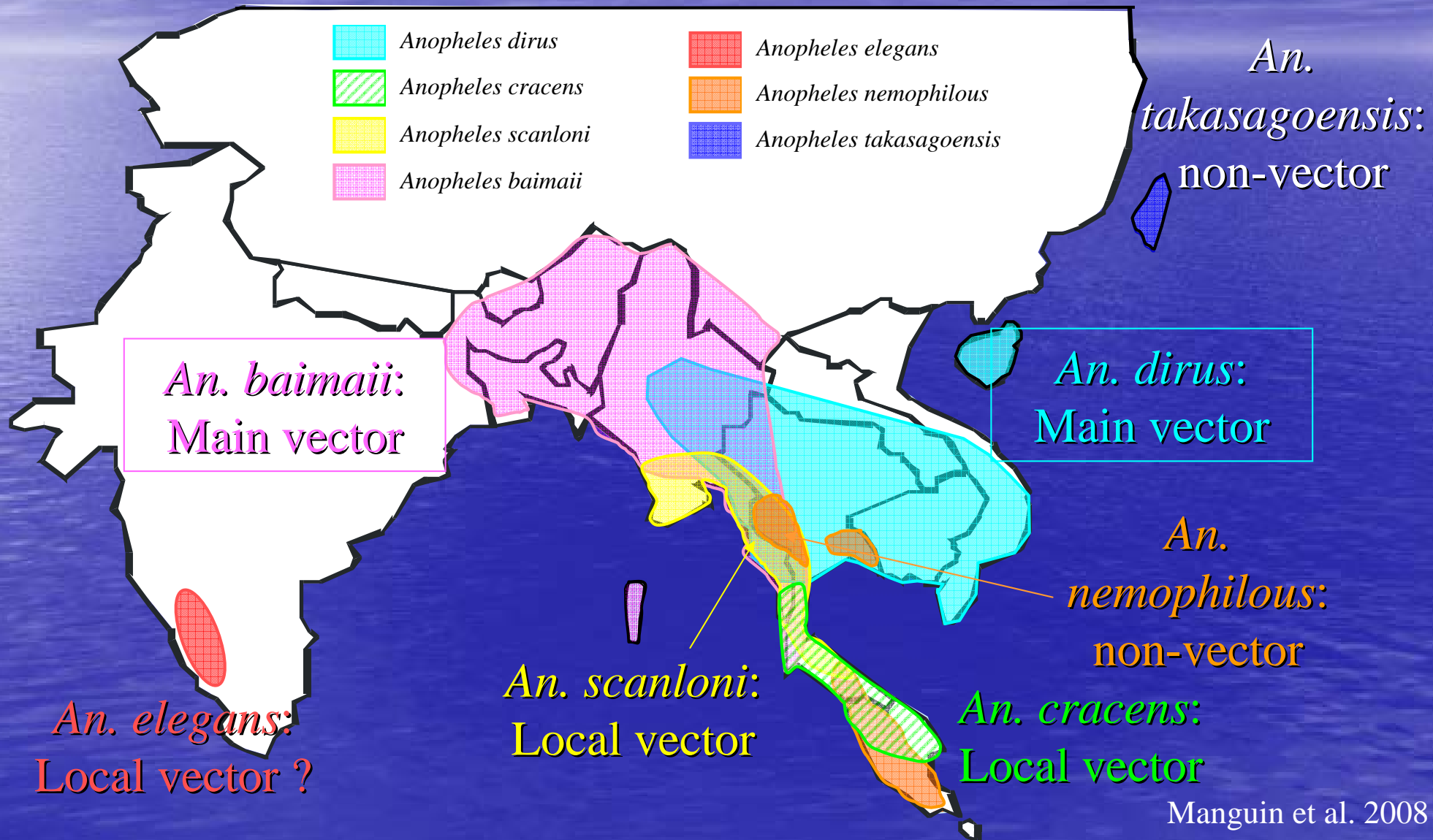


Hevea plantation in Ratanakiri, Cambodia



Artificial container, Khanh Hoa, Vietnam

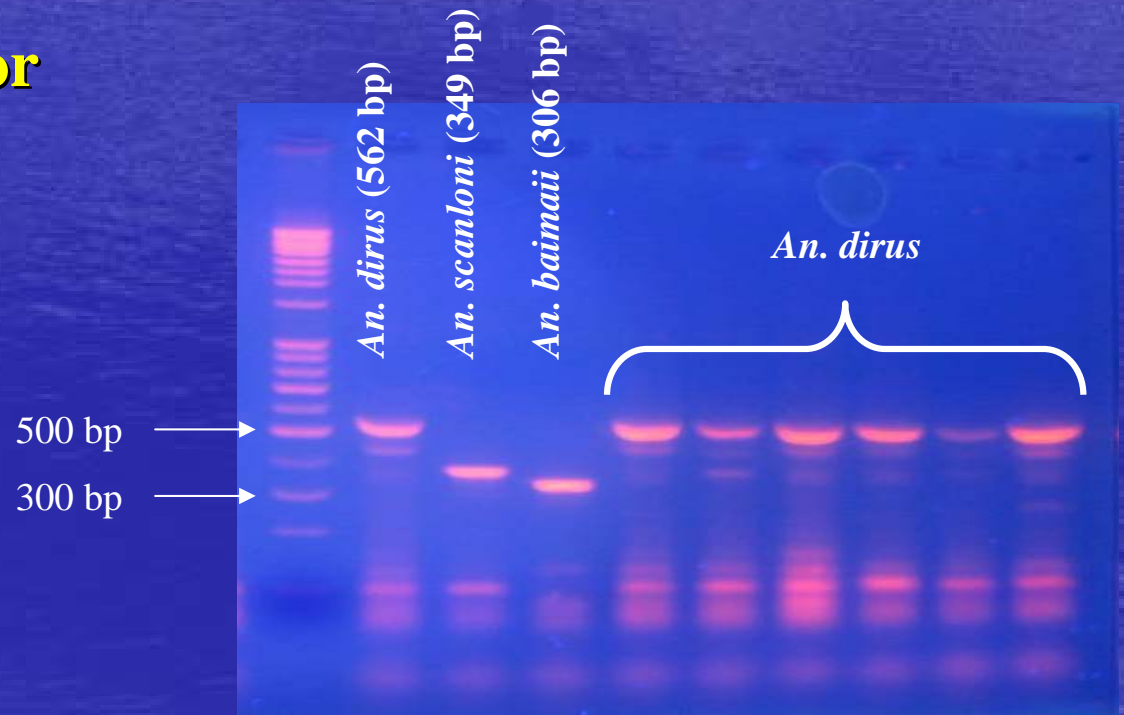
Distribution of the 7 species of the *An. dirus* complex



Dirus species identification of samples from Thailand

Molecular assay:

AS-PCR on the ITS2 sequence for
the identification of 5 species
within the *An. dirus* complex

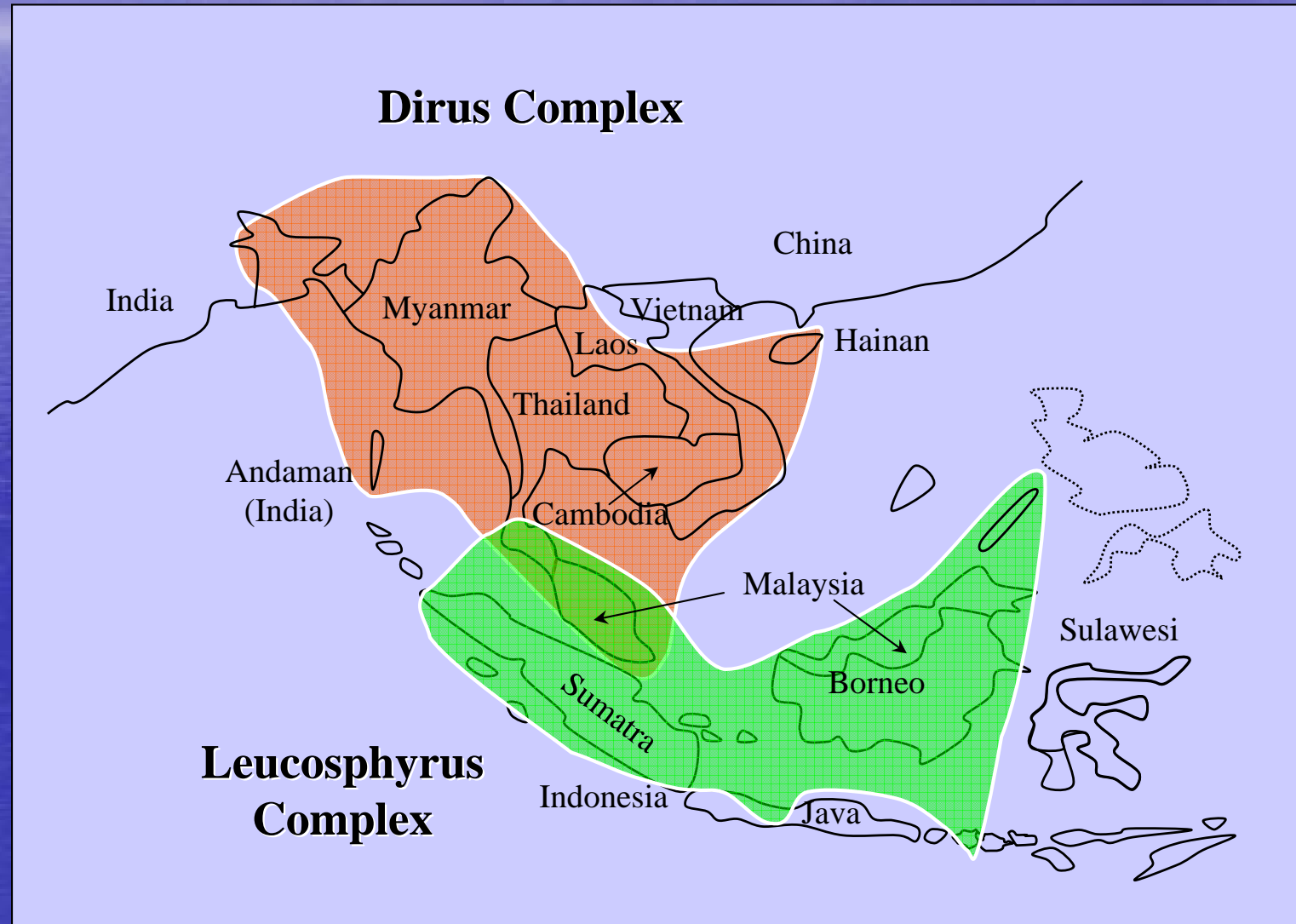


Walton et al. 1999

Distribution of the *Anopheles dirus* complex (orange) and the *An. leucosphyrus* complex (green)

These 2 complexes are closely related:

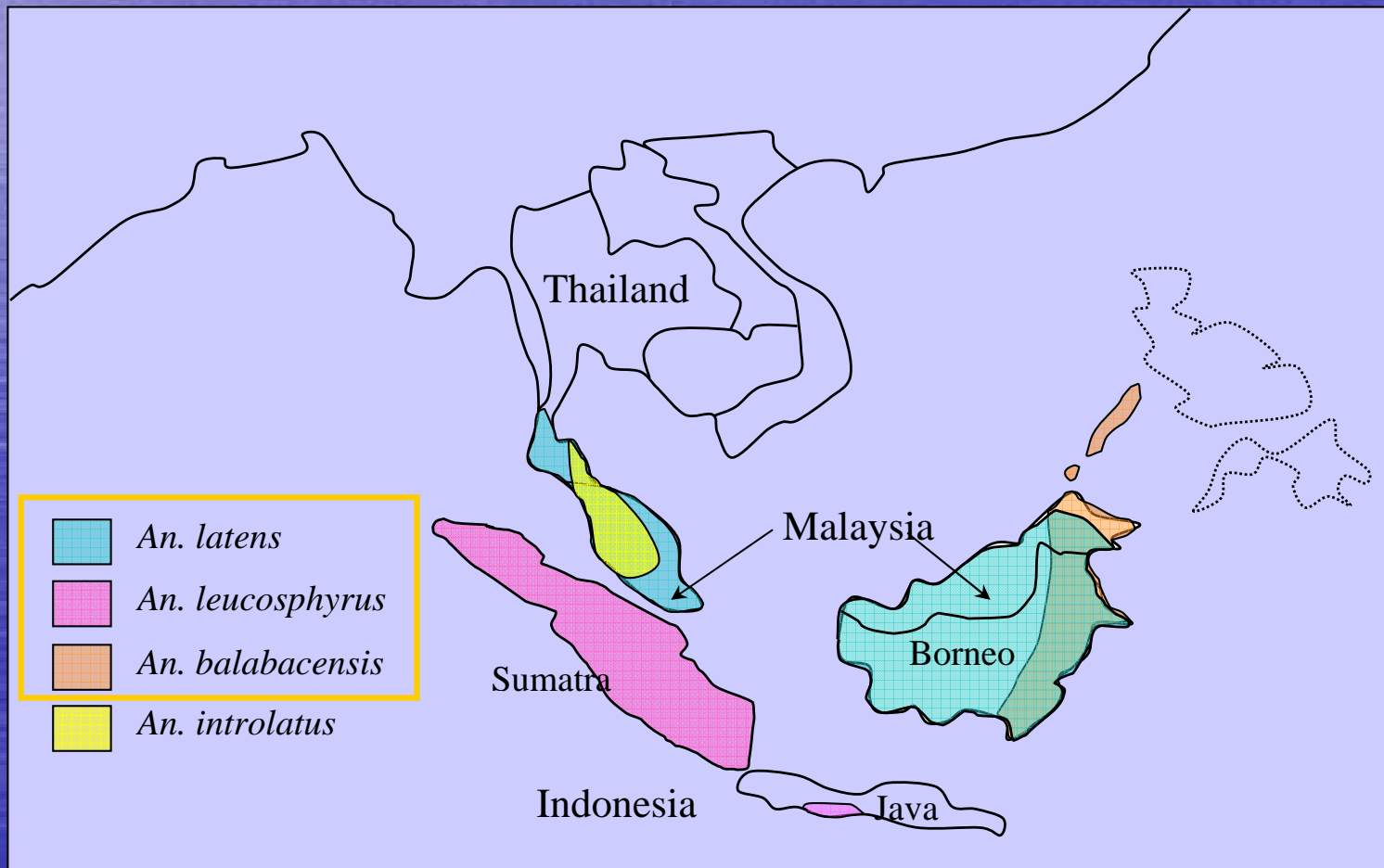
➤ misidentifications possible among species of both complexes



Distribution of the 4 species of the Leucosphyrus complex

No PCR available!!

Major vectors of forest malaria



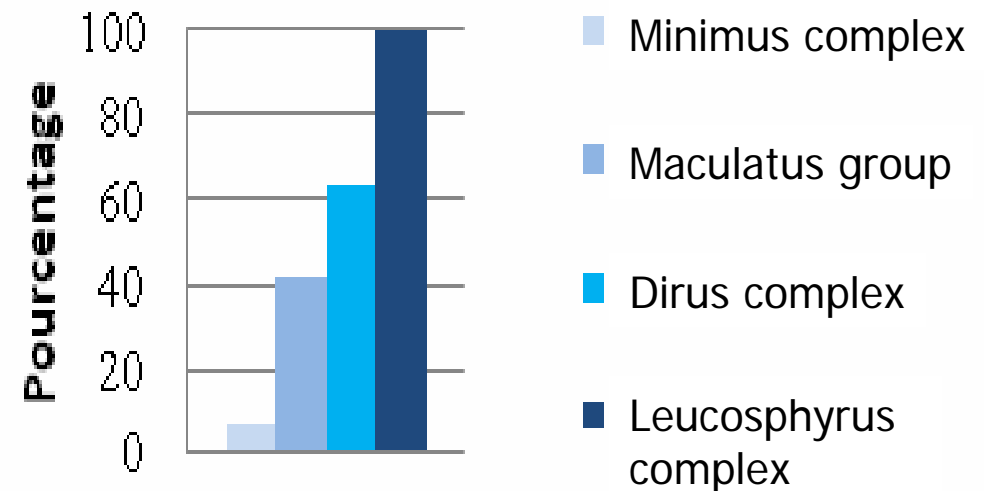
Frequency of morphological misidentifications at the species level

(On-going Franco-Thai research project)

**On 497 *Anopheles* specimens: 75
misidentifications (15% errors)**

**Frequency of misidentifications
depend on the complex (or group):**

- **7.3% for the Minimus complex**
- **41.5% for the Maculatus group**
- **63.2% for the Dirus complex**
- **100% for the Leucosphyrus complex**



Comparison morphological and molecular
identifications

**Molecular assays necessary for
reliable species identifications**

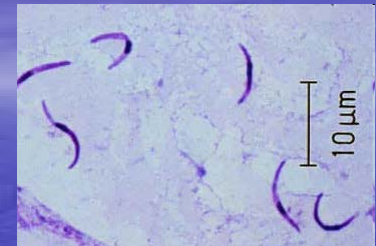
Vector Control

- To implement efficient vector control programs, characterization of the vectors is necessary as for:
 - Identification of the species,
 - Estimation of the vectorial capacity (pathogen detection),
 - Resistance/susceptibility to insecticides,
 - Spatial characterization of habitats, densities and distribution

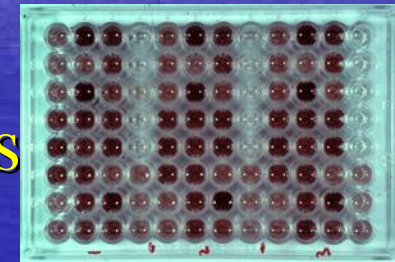
Plasmodium detection assays

Several methods available:

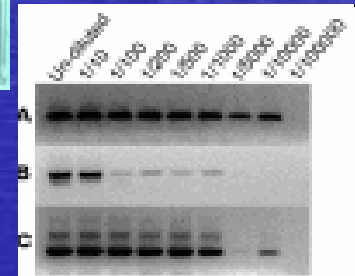
– Microscopic examination: search for oocysts and sporozoites (WHO gold standard method)



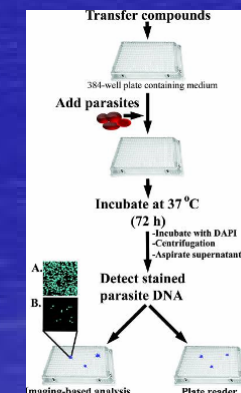
– ELISA* test: detection of circumsporozoites
(Wirtz et al. 1985) * ELISA: enzyme-linked-immunosorbent assay



– Nested PCR: standard *18S rRNA* PCR using specific primers (Singh et al. 1999), conventional PCR, qPCR



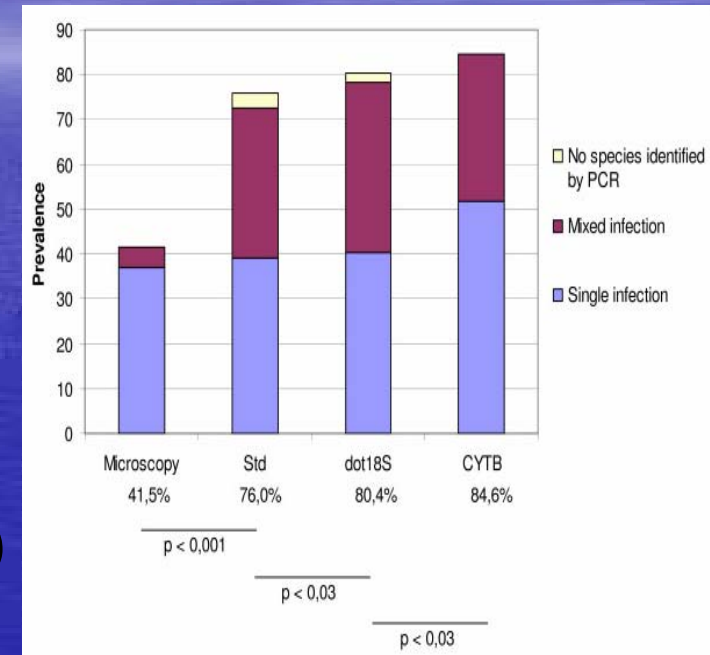
– High-throughput molecular detection: approach based on Dot 18S (18S rRNA) or Cyt-B (mtDNA)
(Steenkeste et al. 2009)



Molecular detection tools

❖ Advantages:

- High sensitivity: detect up to 8 fold *Plasmodium* spp. infections than microscopy
- High rate of mixed infections detected (>1/3)
- Automation of the process and objective reading of results by appropriate apparatus
- Adapted to large-scale epidemiologic studies



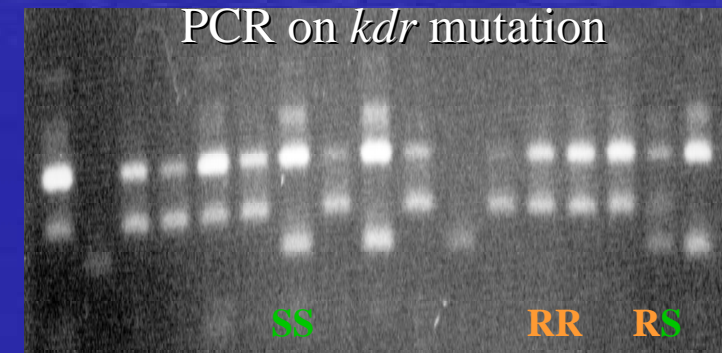
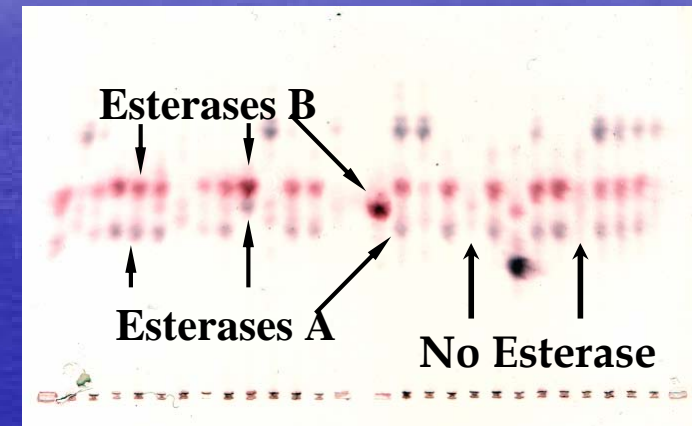
(Steenkeste et al. 2009)

Vector Control

- To implement efficient vector control programs, characterization of the vectors is necessary as for:
 - Identification of the species,
 - Estimation of the vectorial capacity (pathogen detection),
 - Resistance/susceptibility to insecticides,
 - Spatial characterization of habitats, densities and distribution

Techniques to detect insecticide resistance?

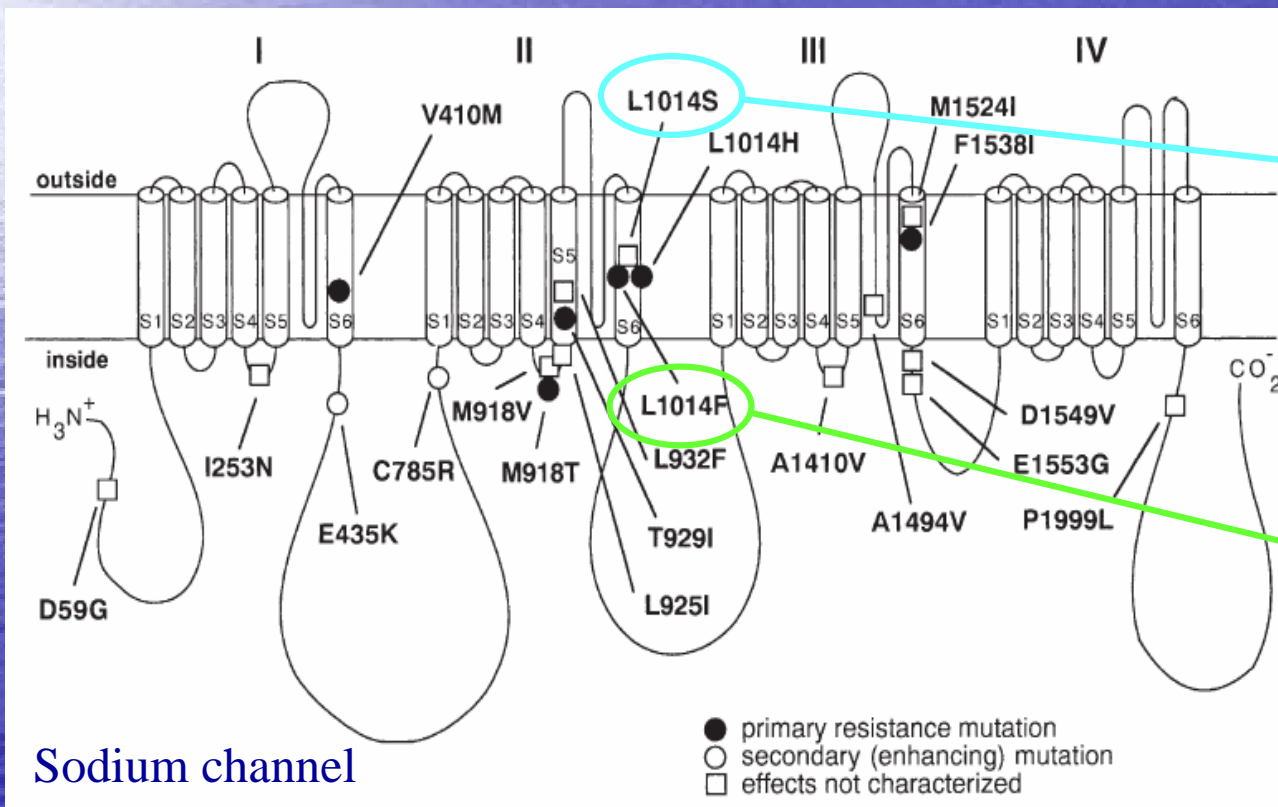
- Standard WHO bioassays
- Biochemical or enzymatic tests: metabolic mechanism alterations (oxidases, esterase, GST)*
- PCR assays: Target site modifications: (**kdr**, **Ace.1**, **Rdl**)*



**kdr*: knockdown resistance; *ACE*: Acetylcholine esterase; *Rdl*: Dieldrin resistance; *GST*: Glutathione S-Transferase

*Kdr** mutations confer cross-resistance to pyrethroids and DDT

* knockdown resistance



Sodium channel

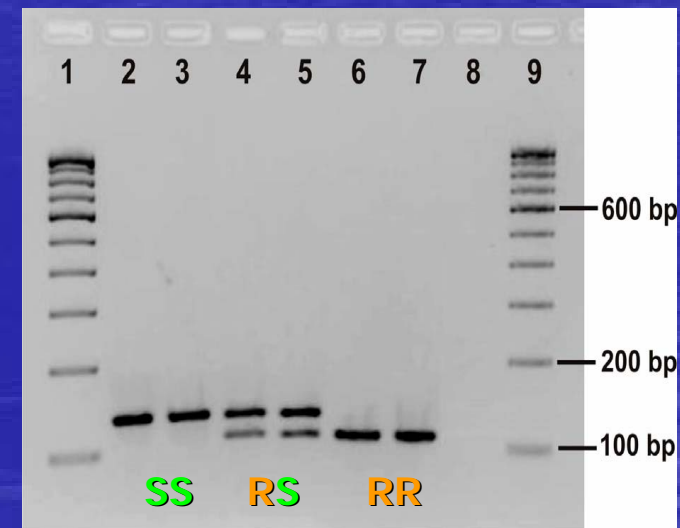
- East African *kdr* mutation (L1014S)
 - *An. gambiae* (S-form)
 - *An. arabiensis*
 - *An. culicifacies* (V1010L)
- West African *kdr* mutation (L1014F)
 - *An. gambiae*
 - *An. arabiensis*
 - *An. culicifacies*
 - *An. stephensi*

Soderlund & Knipple 2003; Gayathri & Murthy 2006; Singh et al 2010

Status of insecticide resistance in *Anopheles* species from Asia

- *Anopheles dirus* and *An. minimus*: susceptible to pyrethroids
- *An. epiroticus*: highly resistant to pyrethroids in the Mekong delta (Vietnam)
- *An. culicifacies* B and C, and *An. stephensi*: resistance (*kdr*) in India

PCR assay for *kdr* detection (L1014S mutation) in *An. culicifacies* B and C from India



Singh OP et al. 2010

Vector Control

- To implement efficient vector control programs, characterization of the vectors is necessary as for:
 - Identification of the species,
 - Estimation of the vectorial capacity (pathogen detection),
 - Resistance/susceptibility to insecticides,
 - Spatial characterization of habitats, densities and distribution

Spatial tools for the surveillance of malaria vectors

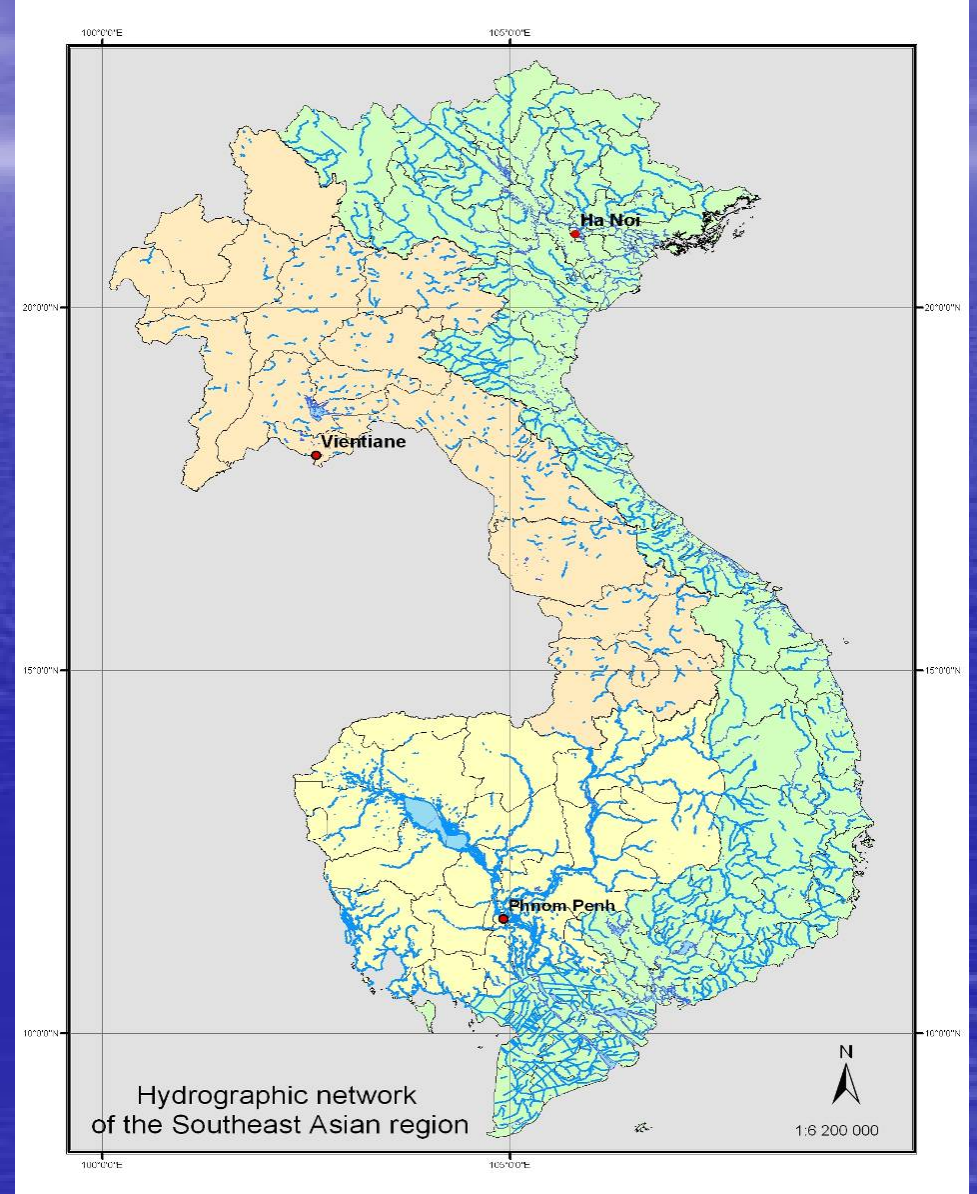
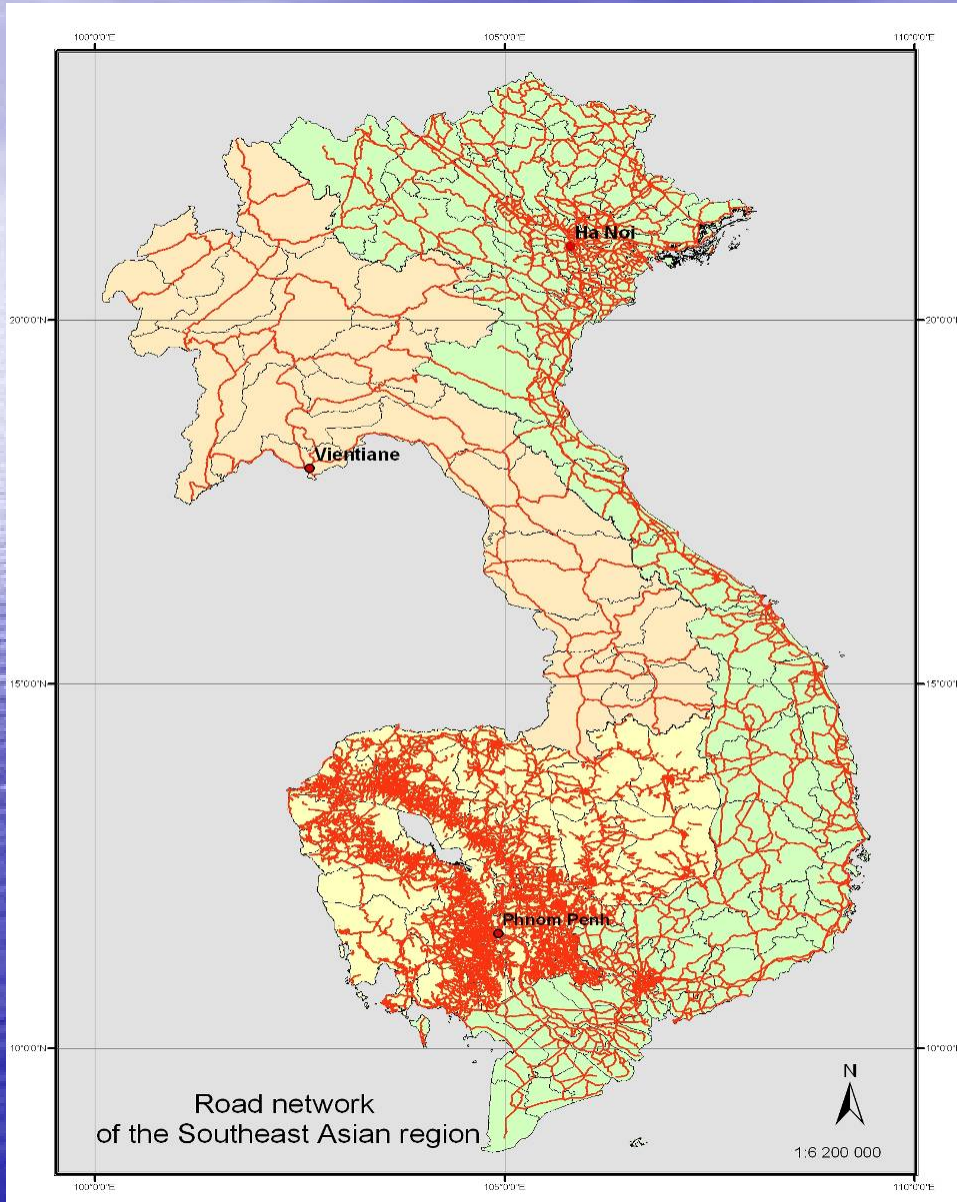
- Localization of vector-borne diseases: largely determined by **environmental factors**
- Some factors (altitude, temperature, rainfall, vegetation) can be estimated or quantified from **satellite image data**
- Geo-referenced data of interest (entomology, parasitology, epidemiology, environment, etc.) are integrated into a geographic information system (GIS)

Principal Objectives

Help for field managers to take decision through:

- Surveillance and prediction of the spatio-temporal dynamic of vector populations
- Distribution maps of endemic zones
- Spatial analysis provide information on the determinants of malaria transmission risks
- Possibilities of anticipation and improvement of vector control strategies

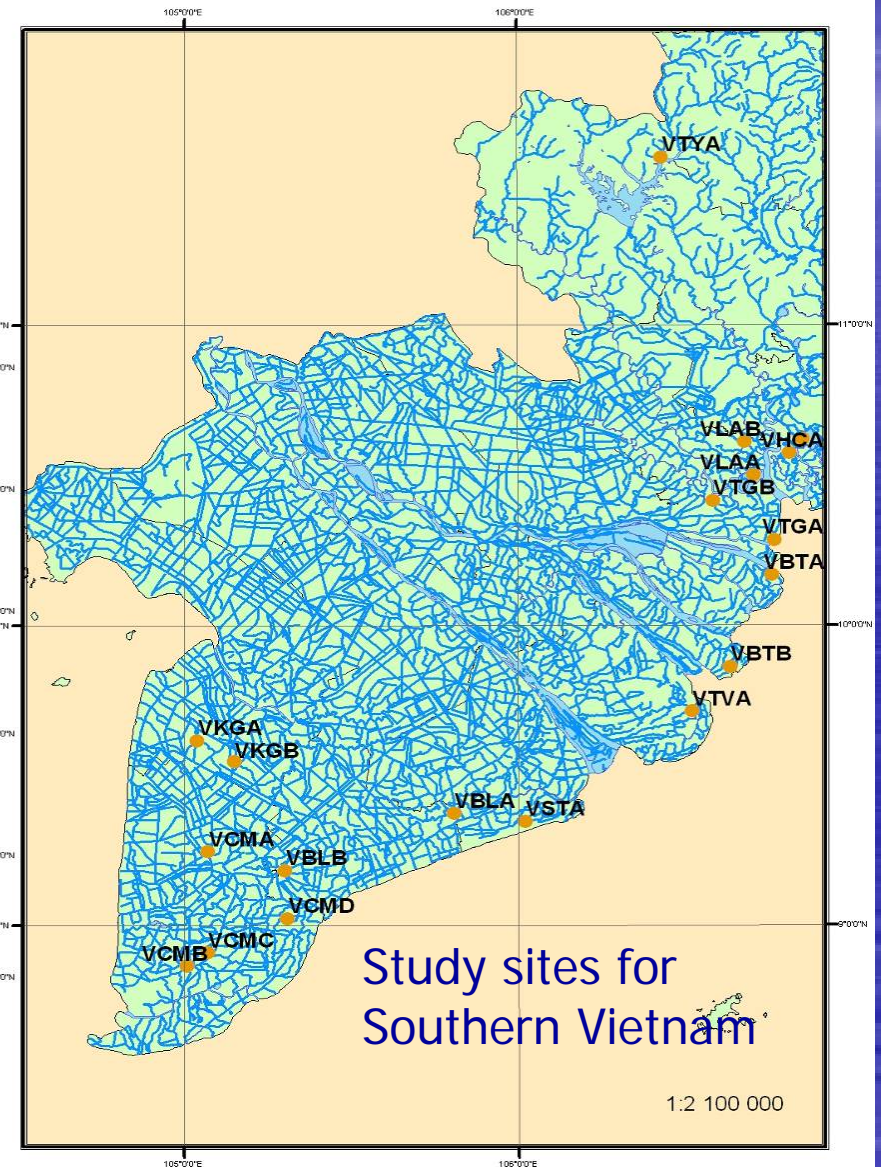
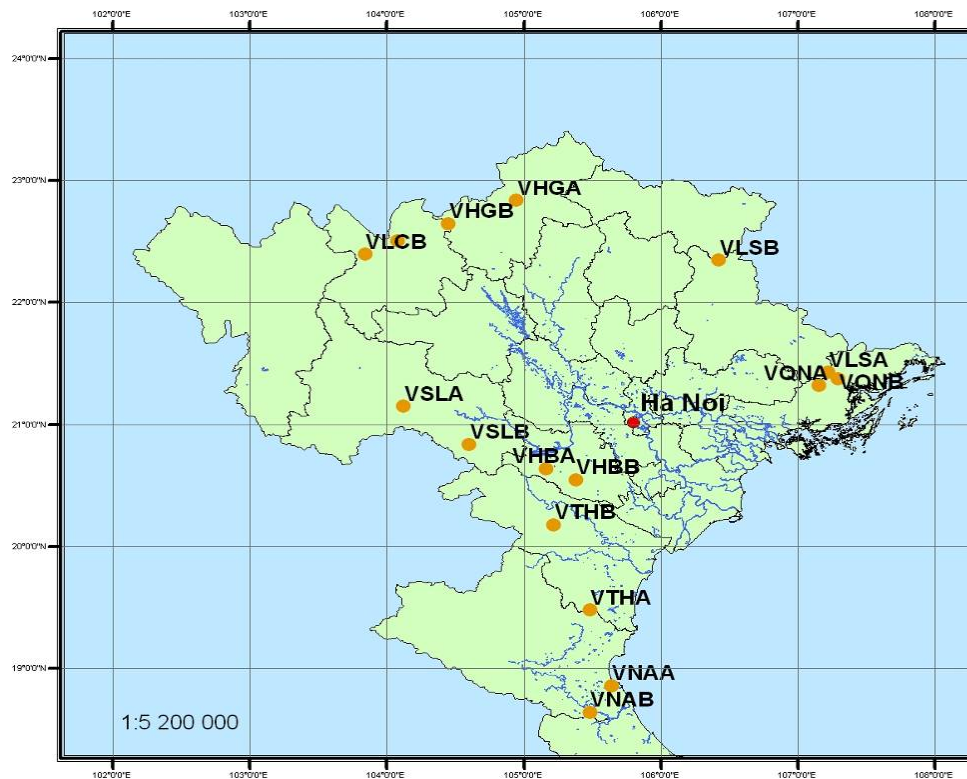
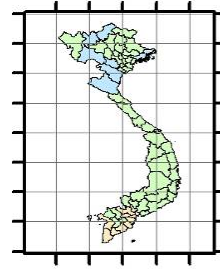
GIS with geographical regional background (Malvecasia project)



Study sites in northern and southern Vietnam

Scale: 1/500.000

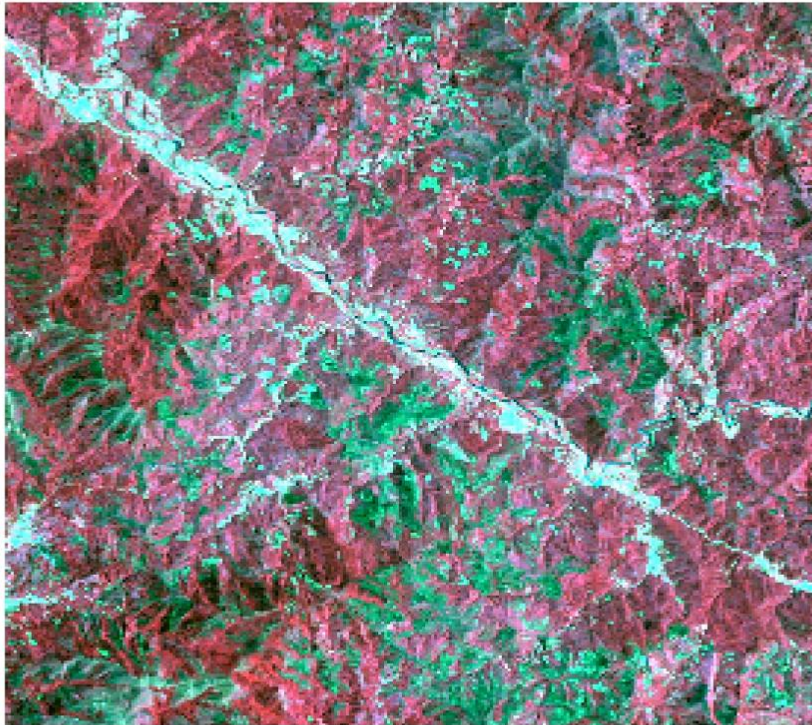
Study sites for
Northern Vietnam



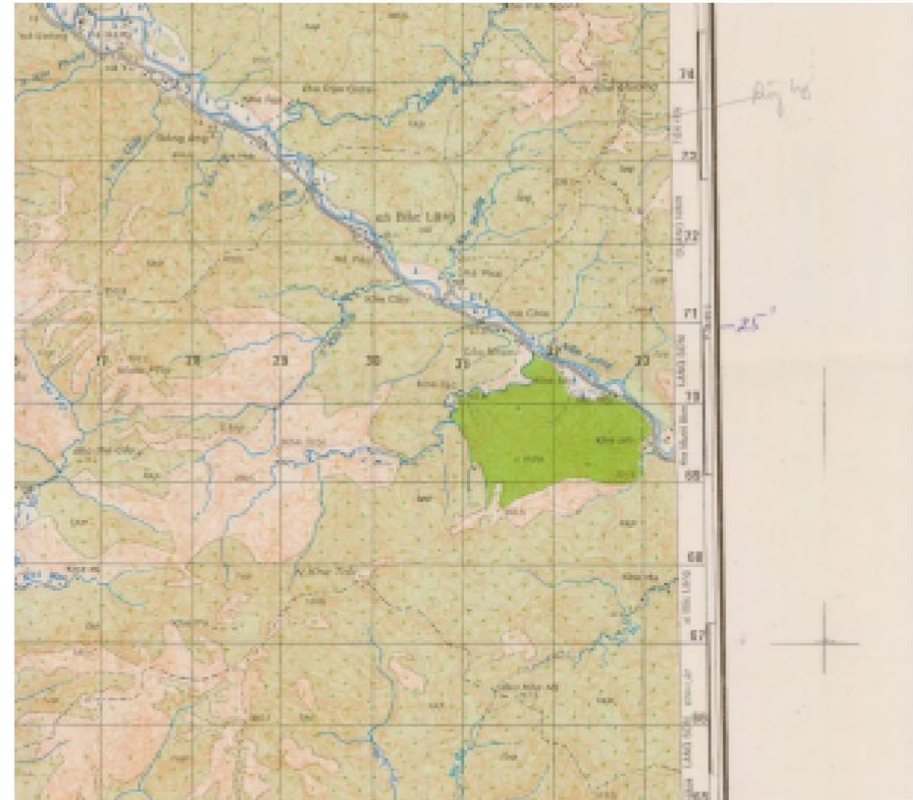
Study sites for
Southern Vietnam

Use of Remote sensing

SPOT satellite image (2004)

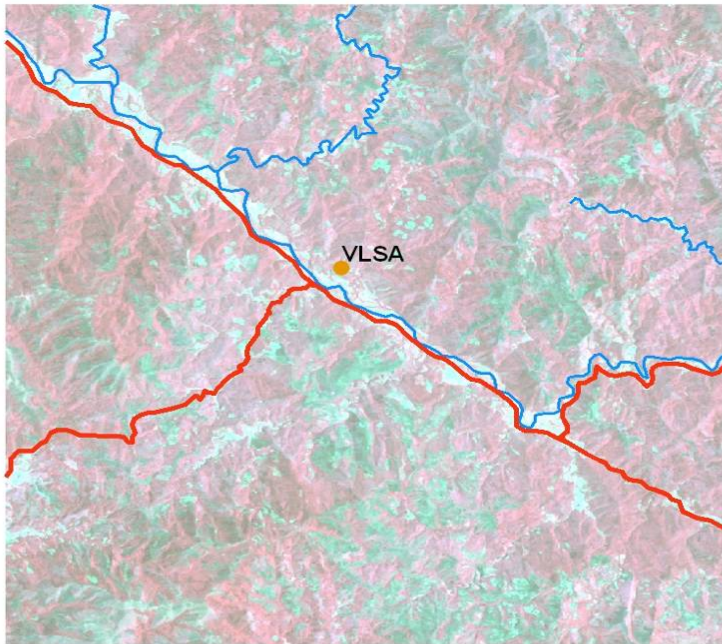


Updated land cover information

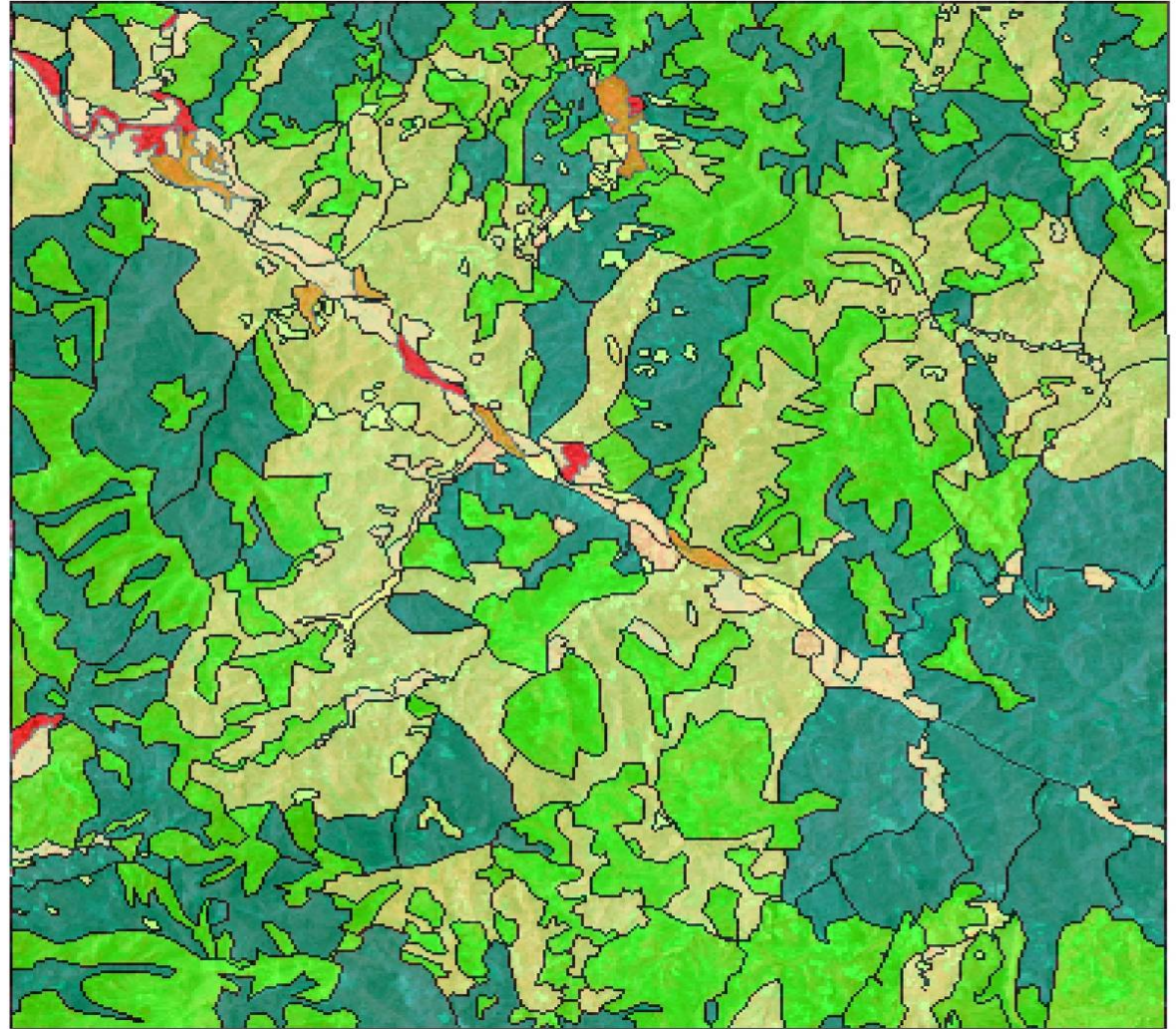


Topographic map (quite old)

Satellite image used for developing a land use layer



Legend	
category	
agricultural mosaic	orange
crops	light orange
high density forest	dark green
low density forest	light green
regrowth forest	bright green
rice field	yellow
rural habitat and gardens	red



Khoi village, Hoa Binh Province, VHBA



Na Bo village, Son La Province, VSLB



Ground proof

Na Bo village, Son La Province, VSLB

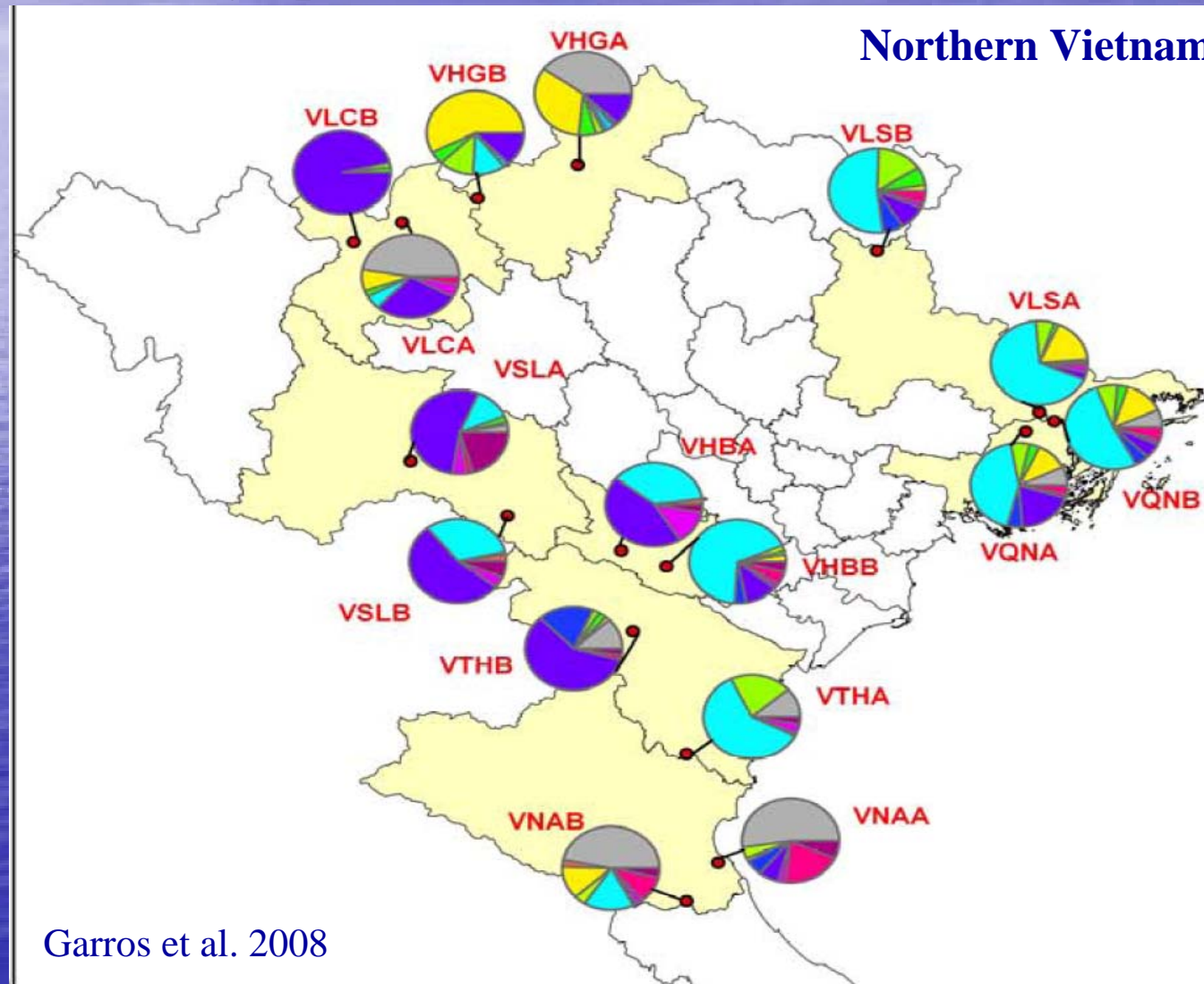


Tao Tuong village, Ha Giang Province, VHGB



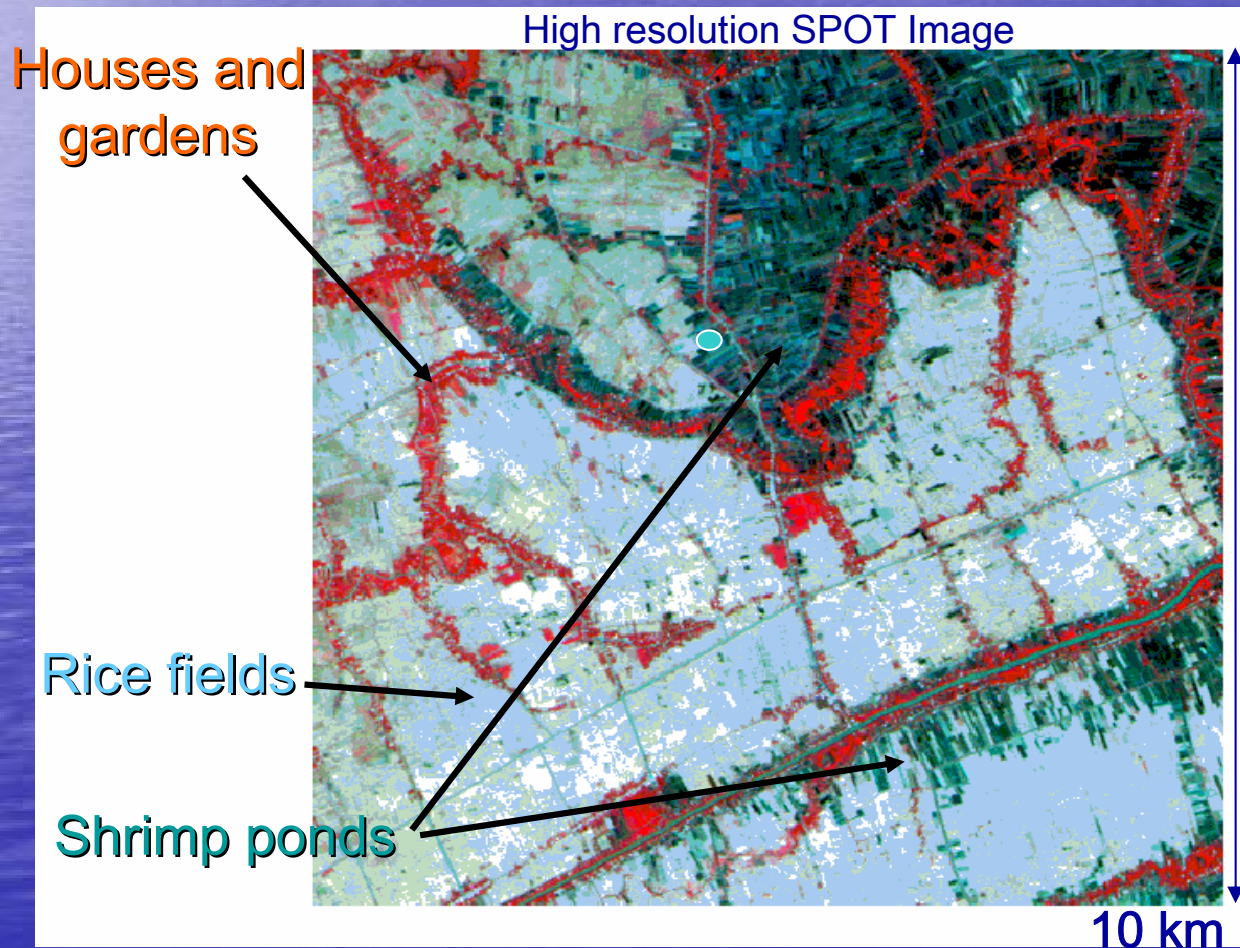
Anopheles species association

Anopheles density/species/site



Ecological associations

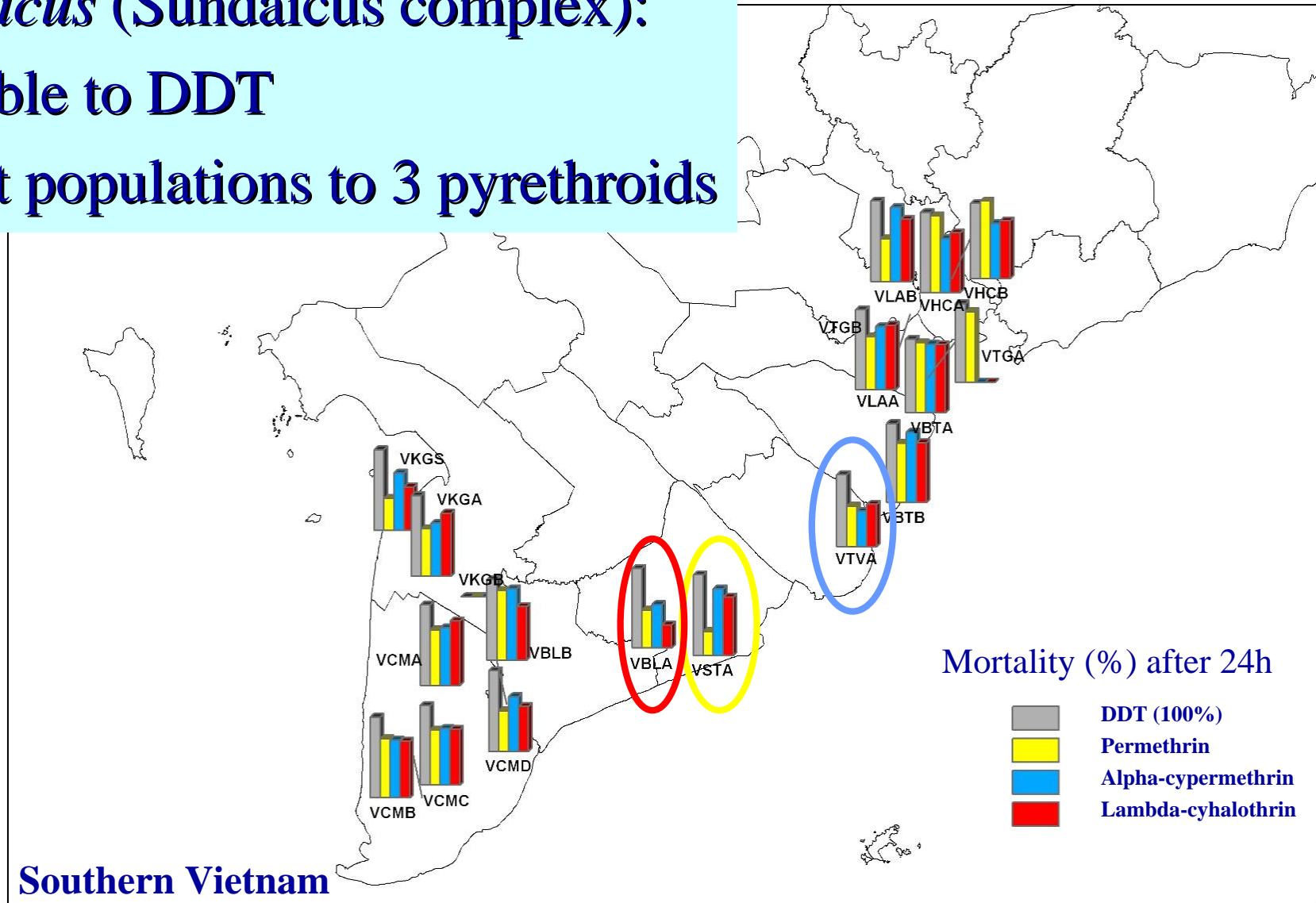
High correlation between the main malaria vector, *An. epiroticus* (Sundaicus complex), and shrimp farm basins



Insecticide resistance

An. epiroticus (Sundaicus complex):

- Susceptible to DDT
- Resistant populations to 3 pyrethroids



Conclusions

- Molecular tools are the most reliable ways to identify sibling species within *Anopheles* complex: the first step towards the characterization of malaria vectors and targeted vector control program
- Vectorial capacities and resistance to insecticides: important parameters to evaluate and to monitor on a regular basis in malaria endemic foci
- Spatial approaches: efficient and powerful indicators that help program managers to implement the appropriate control strategy and develop an early warning of the potential for malaria epidemics



Acknowledgements



- Collaboration through the Franco-Thai research projects (2007-10) with Prof. T. Chareonviriyaphap (Entomology Research Lab, KU), funding from the French Ministry of Foreign Affairs, Kasetsart University and Thai Research Funds.
- Collaboration through the “Malvecasia” research project (2002-06) P.I.: Prof. M. Coosemans (Institute of Tropical Medicine, Antwerp, Belgium) and teams from SE Asia (4) and Europe (4), funding from the European Commission.



Thank you
for your
Attention!

