Pictures

and

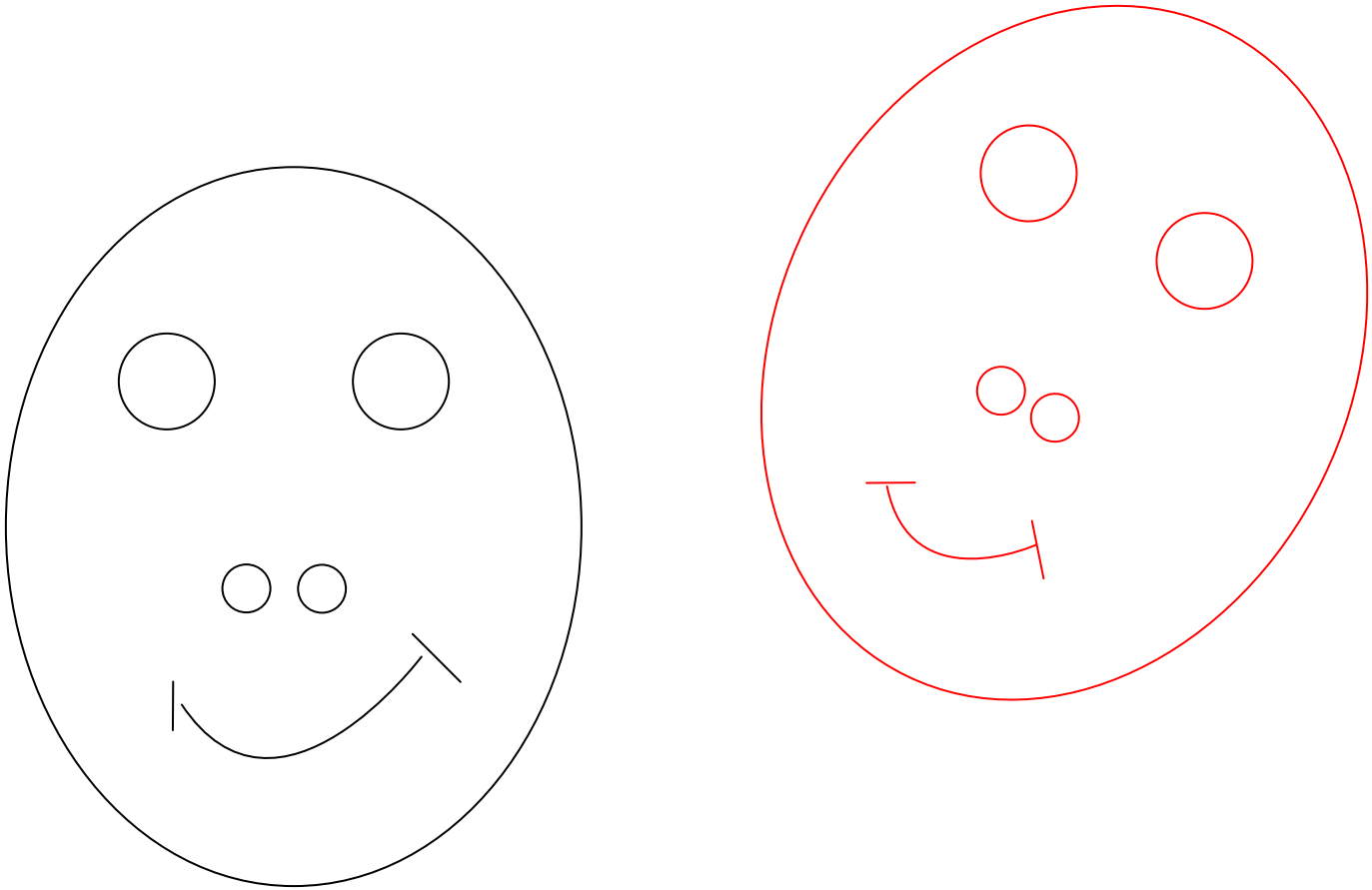
anatomical landmarks

to identify isomorphic species:

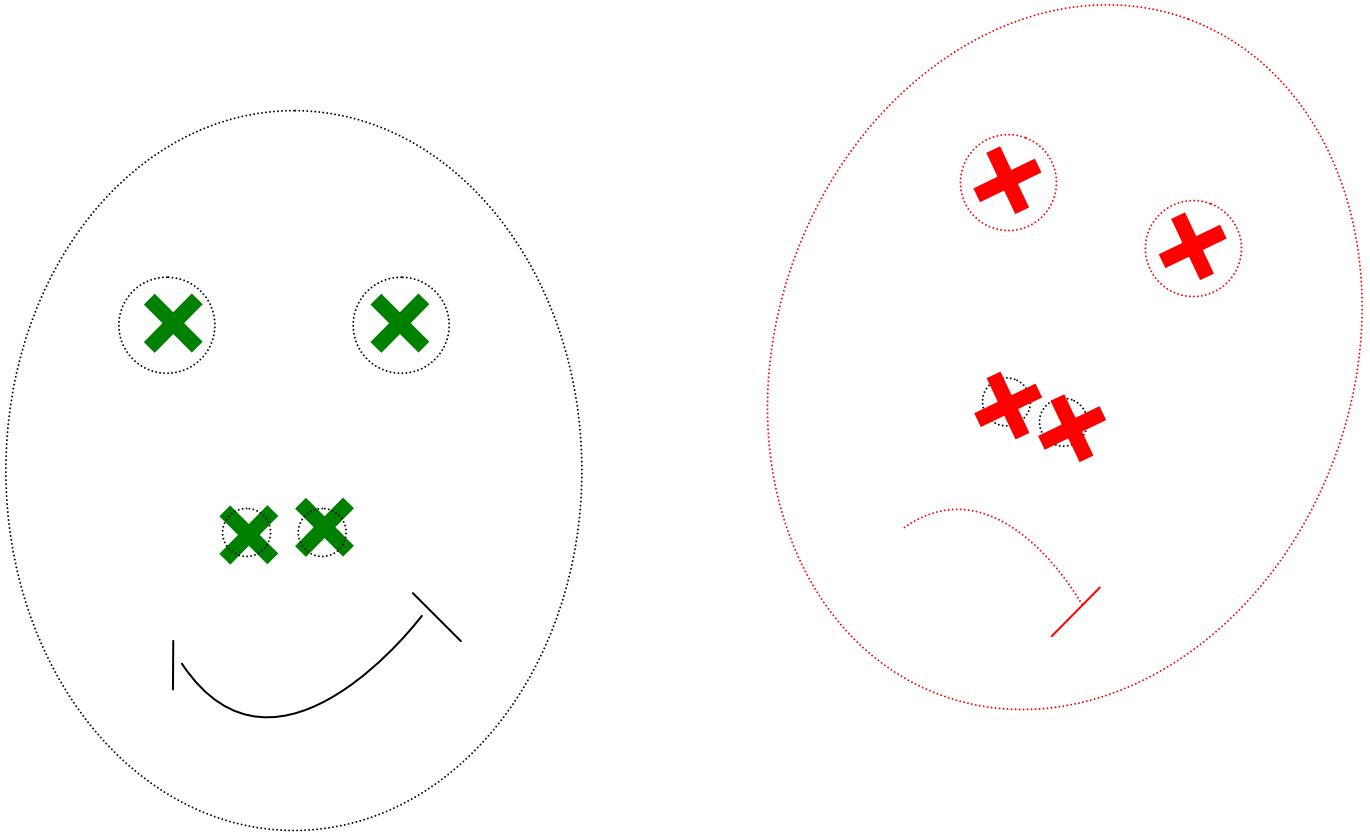
a new tool for entomological surveillance.

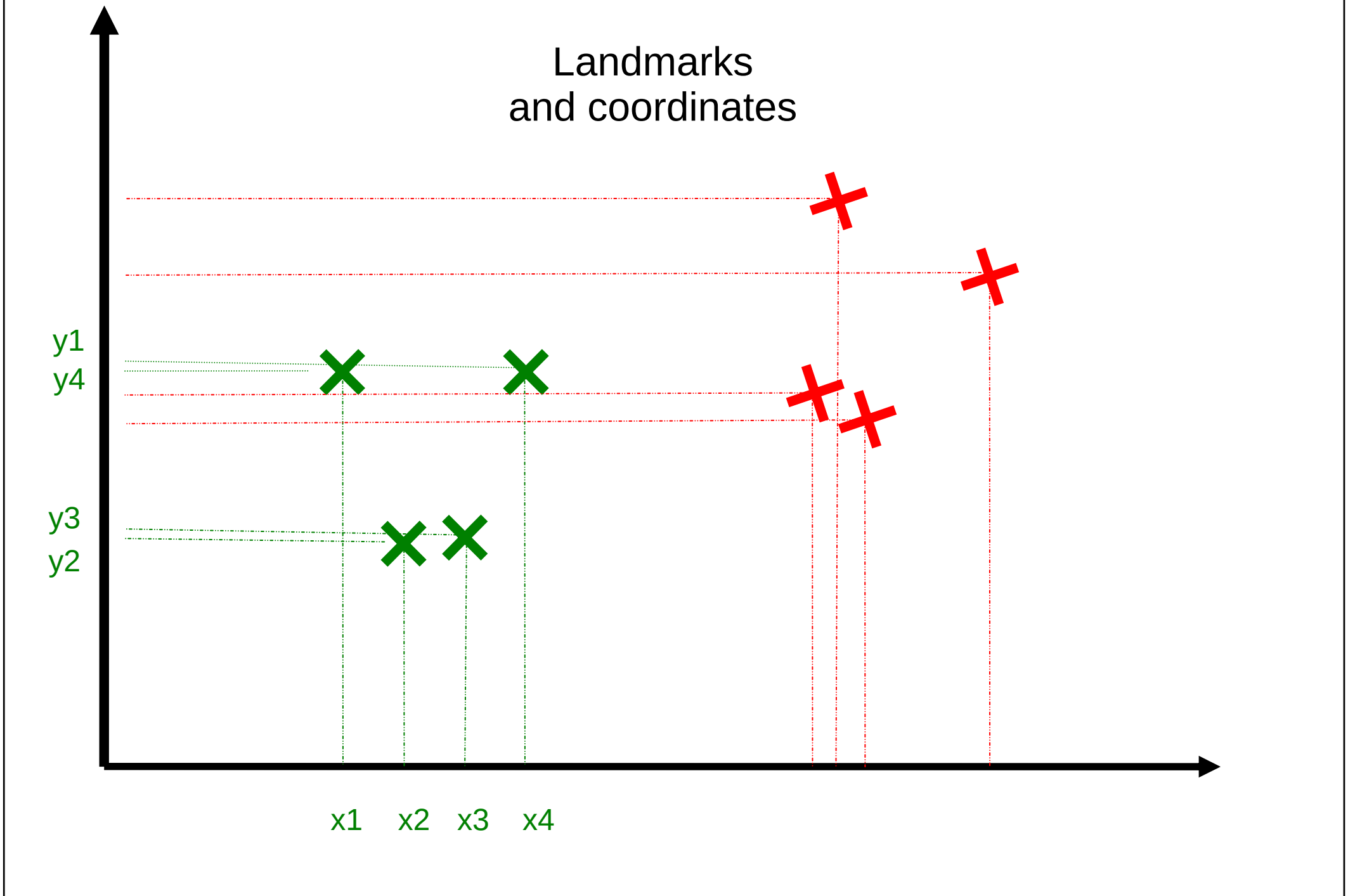
Jean-Pierre Dujardin
(IRD, France at CVVD, Mahidol University (Bangkok)

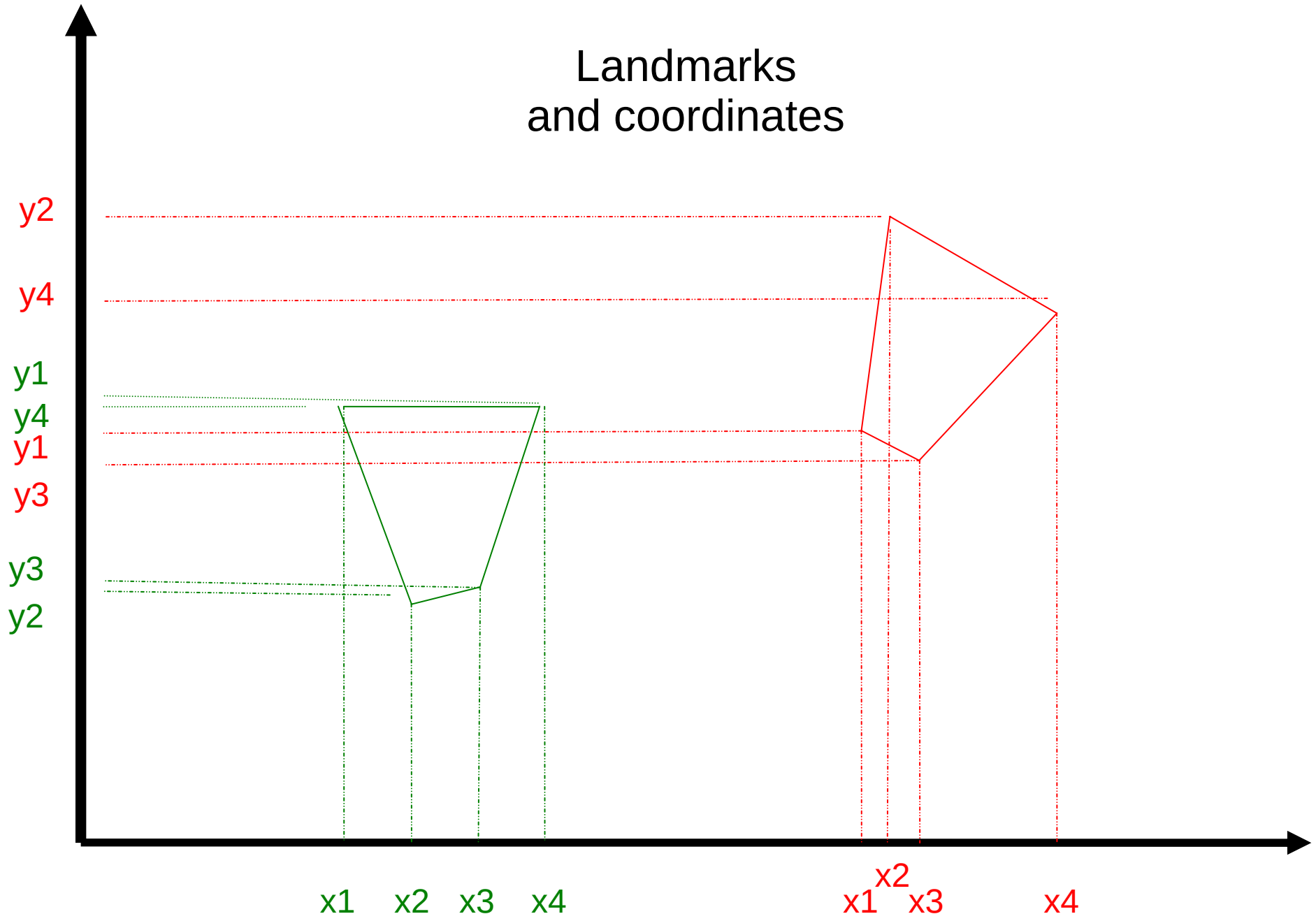
Pictures

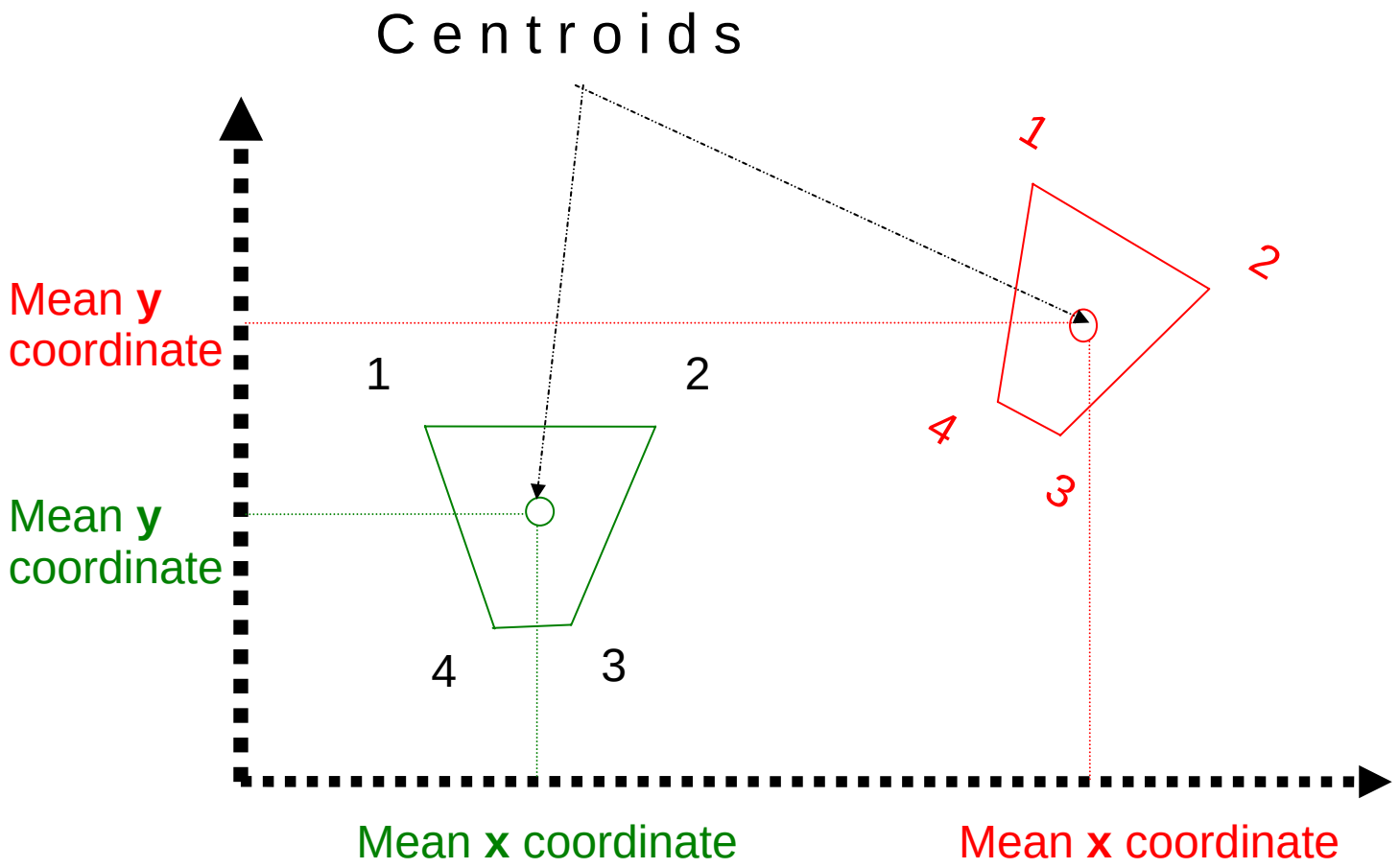


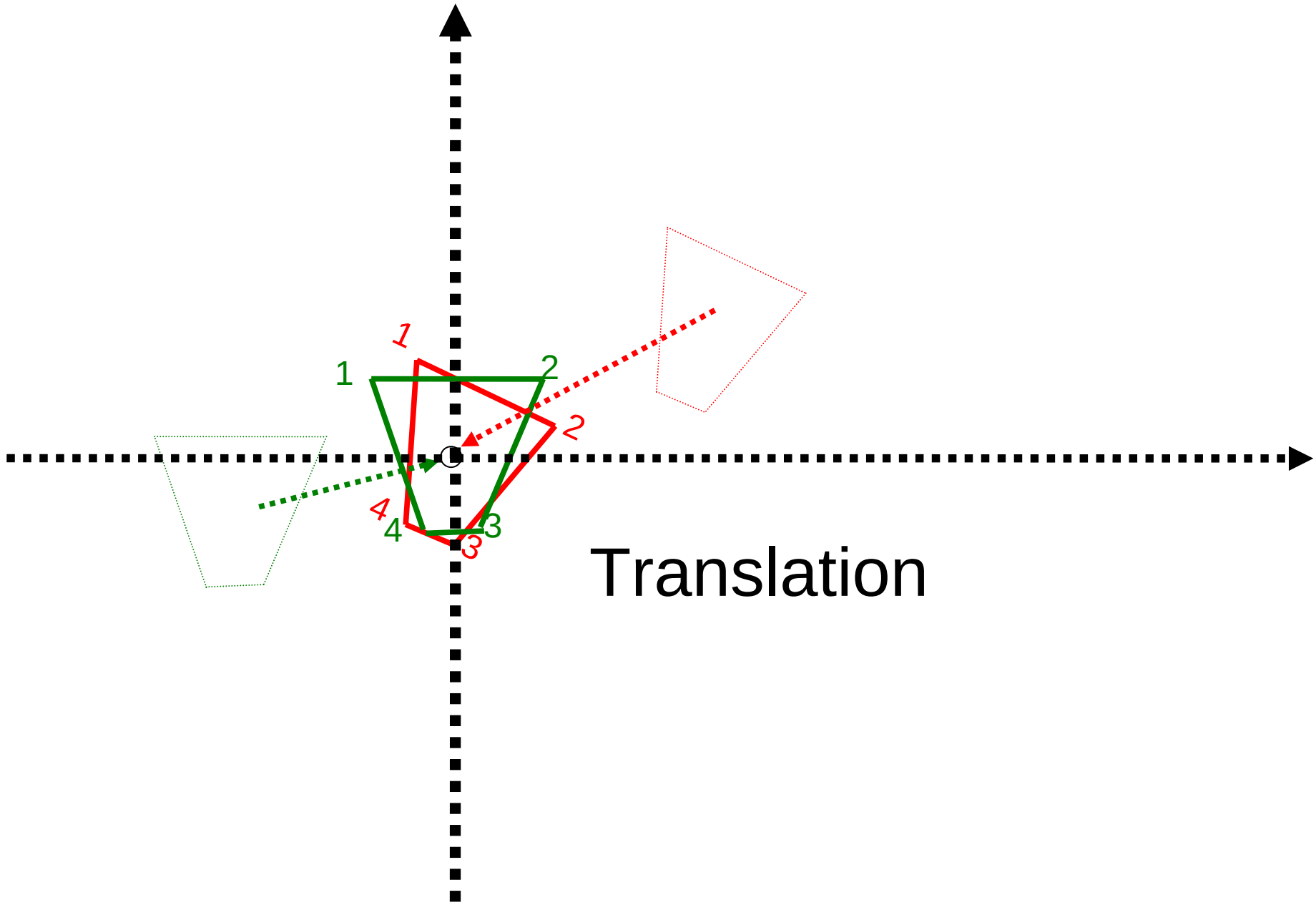
Pictures and anatomical landmarks

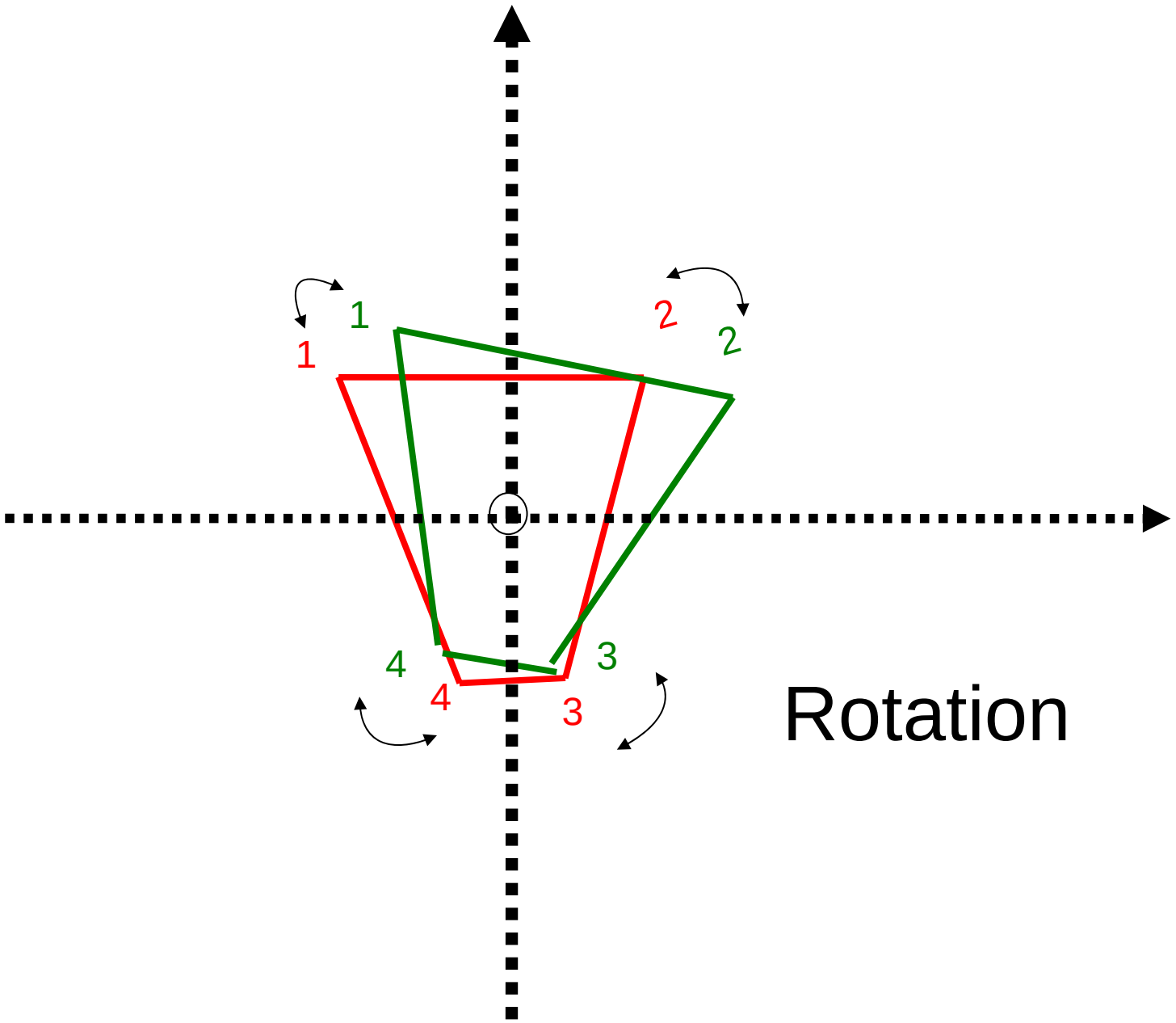



Landmarks
and coordinates
y1
y4
y3
y2
x1
x2
x3
x4


Landmarks
and coordinates
y2
y4
y1
y4
y1
y3
y3
y2
x1
x2
x3
x4
x2
x1
x3
x4


Centroids
Mean y coordinate
Mean y coordinate
1
2
4
3
1
2
4
3
Mean x coordinate
Mean x coordinate

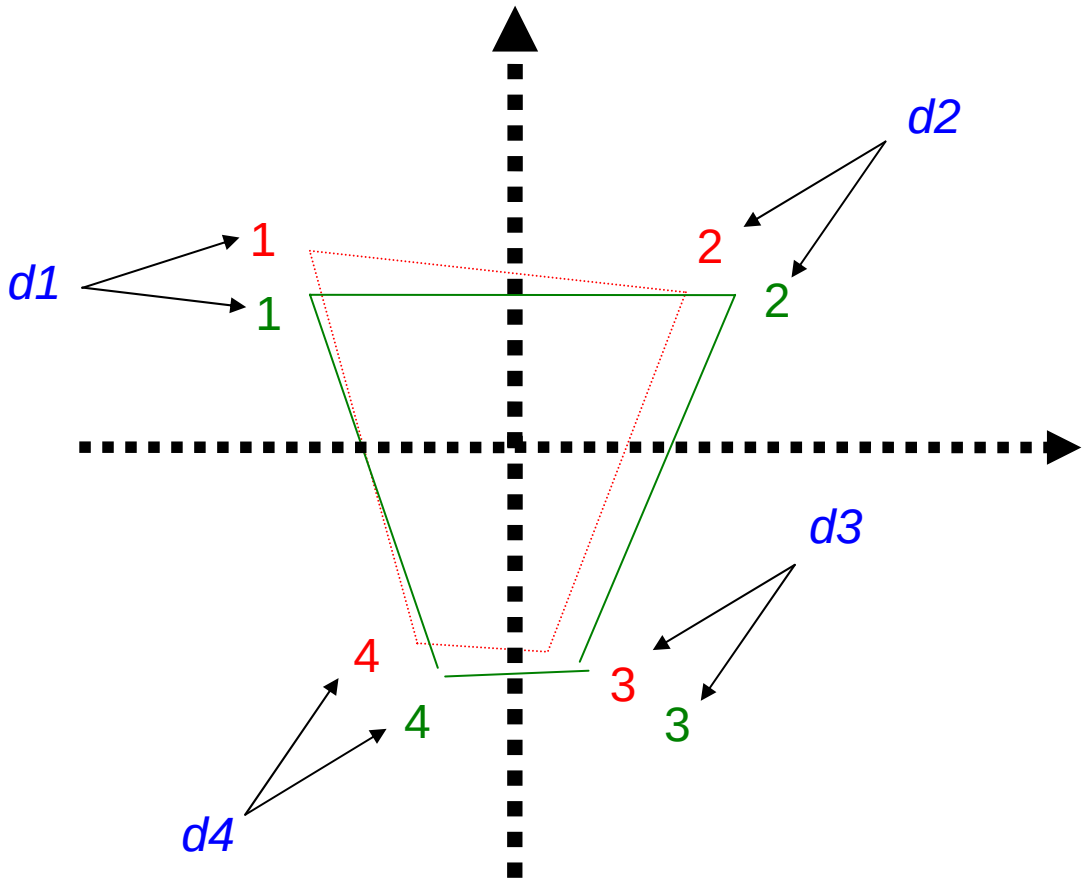

1
2
3
4
1
2
3
4
Translation



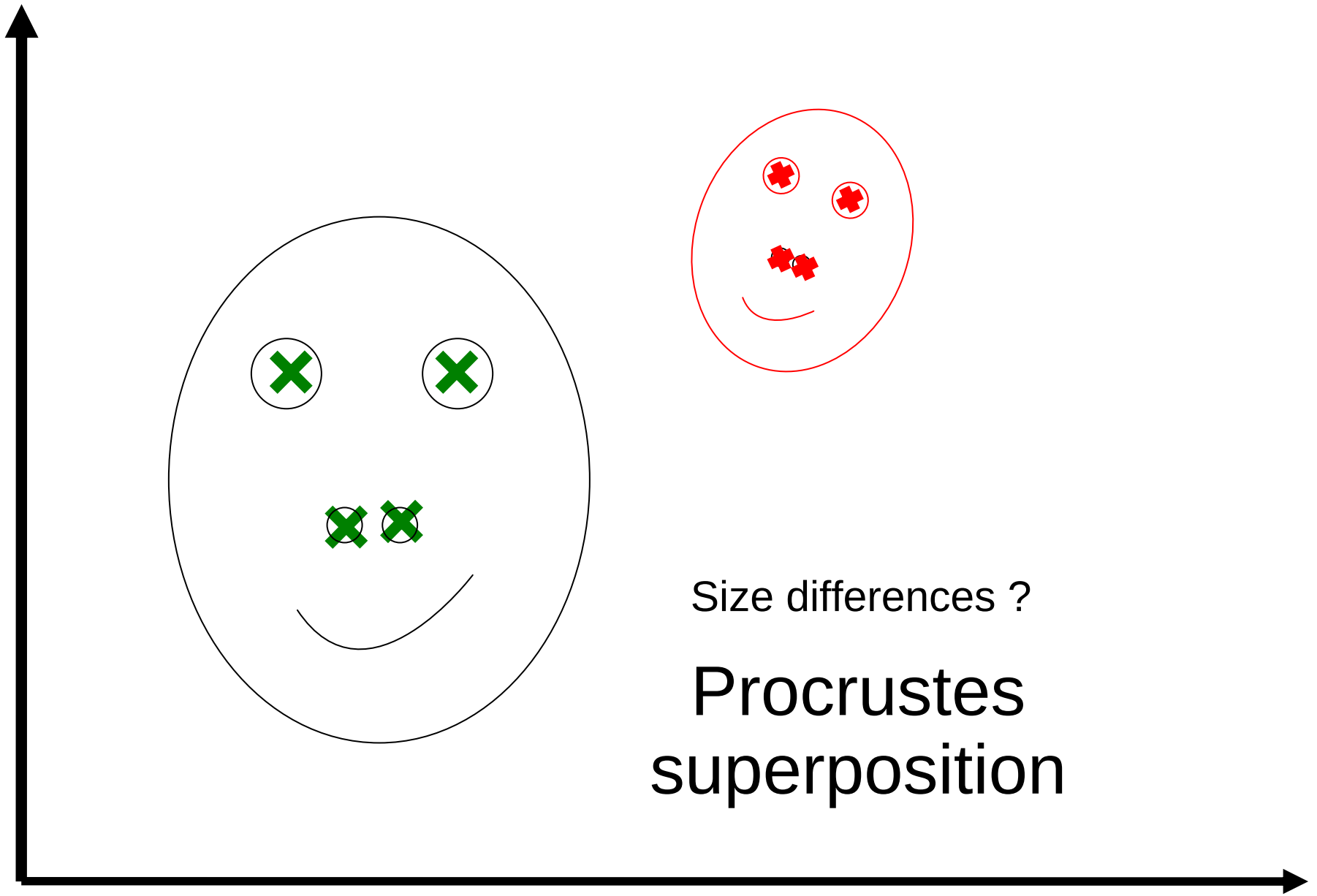
Rotation

Procrustes distance between two forms

D = *d1* + *d2* + *d3* + *d4*

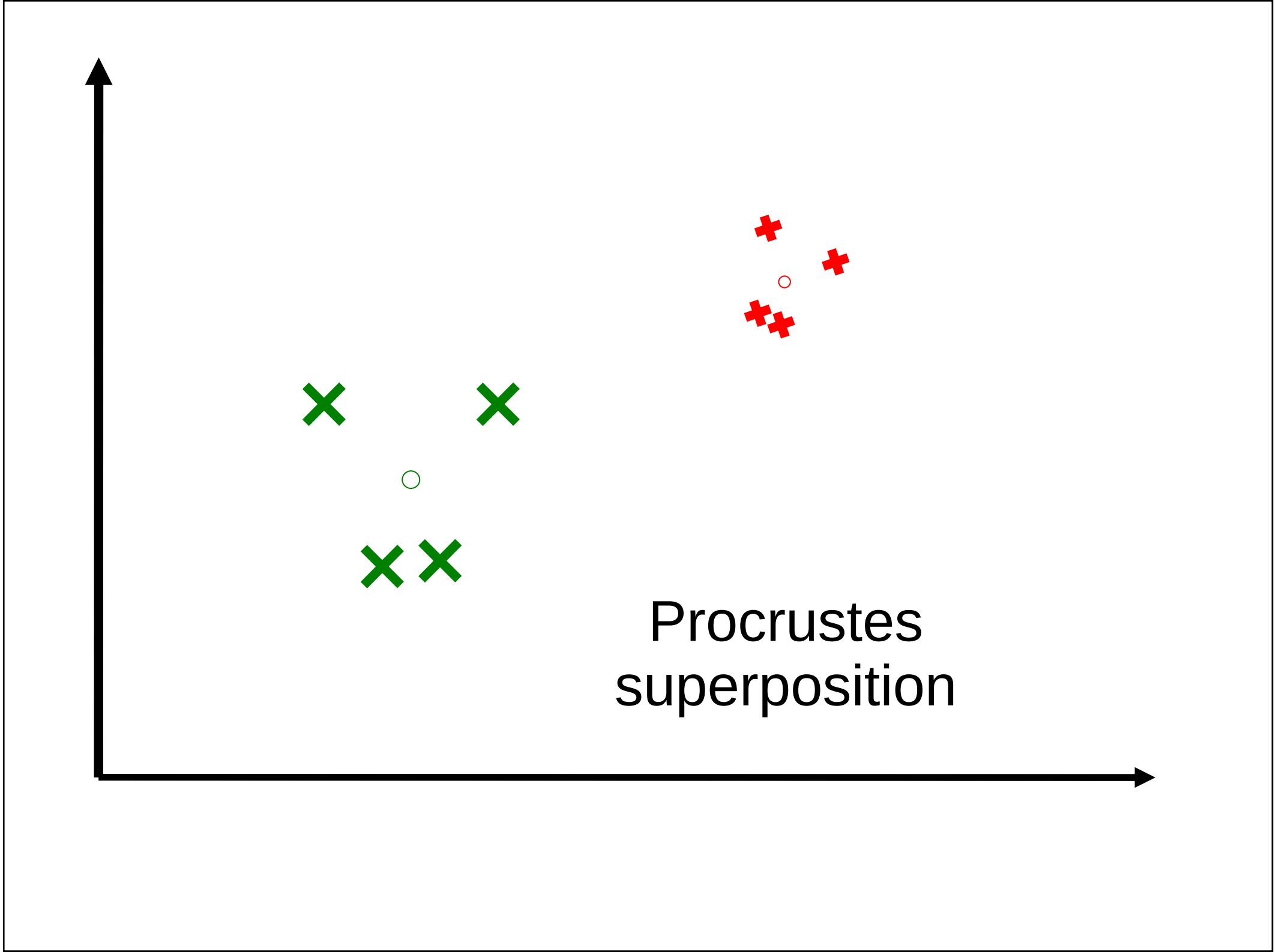


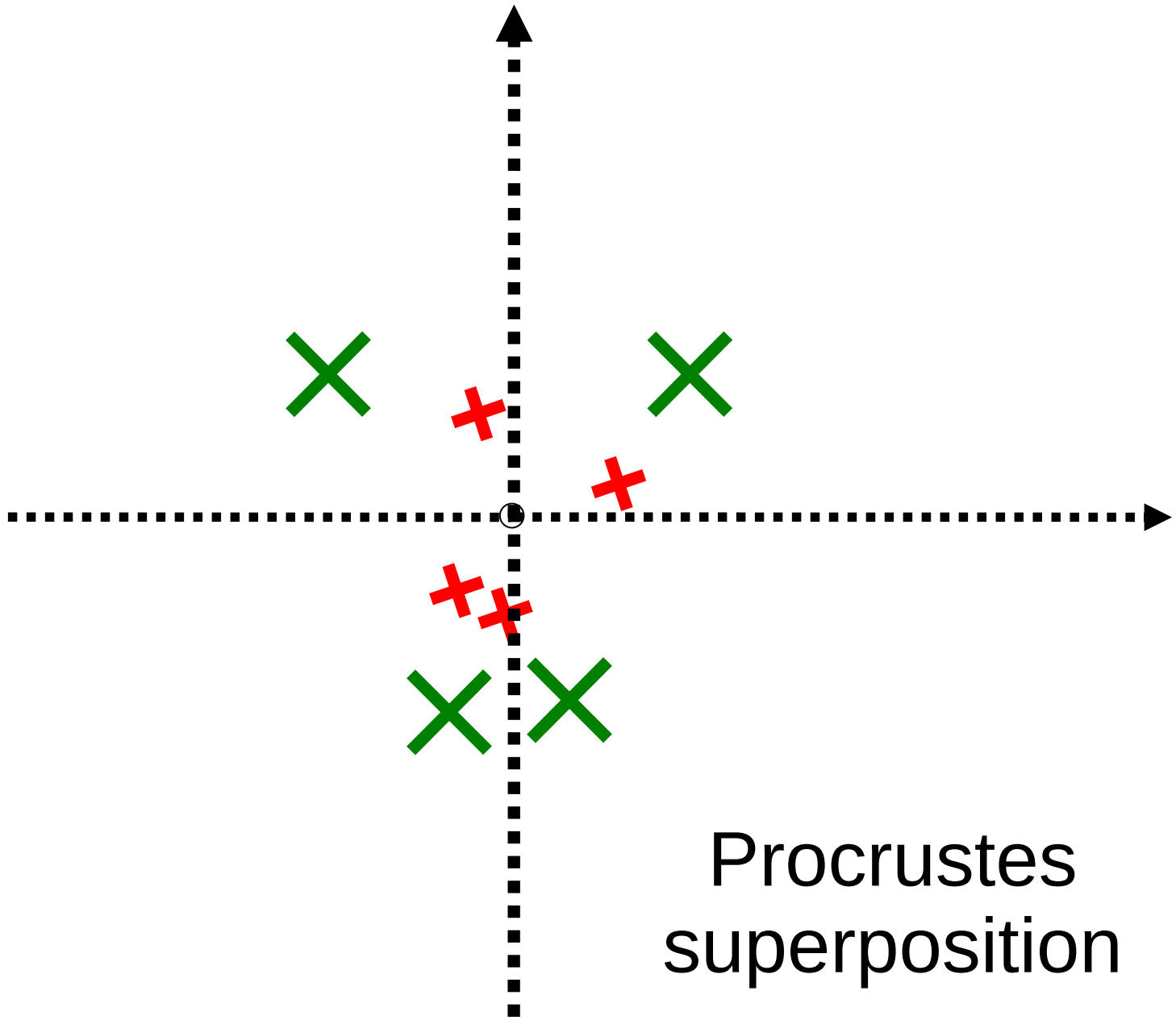
Procrustes superposition



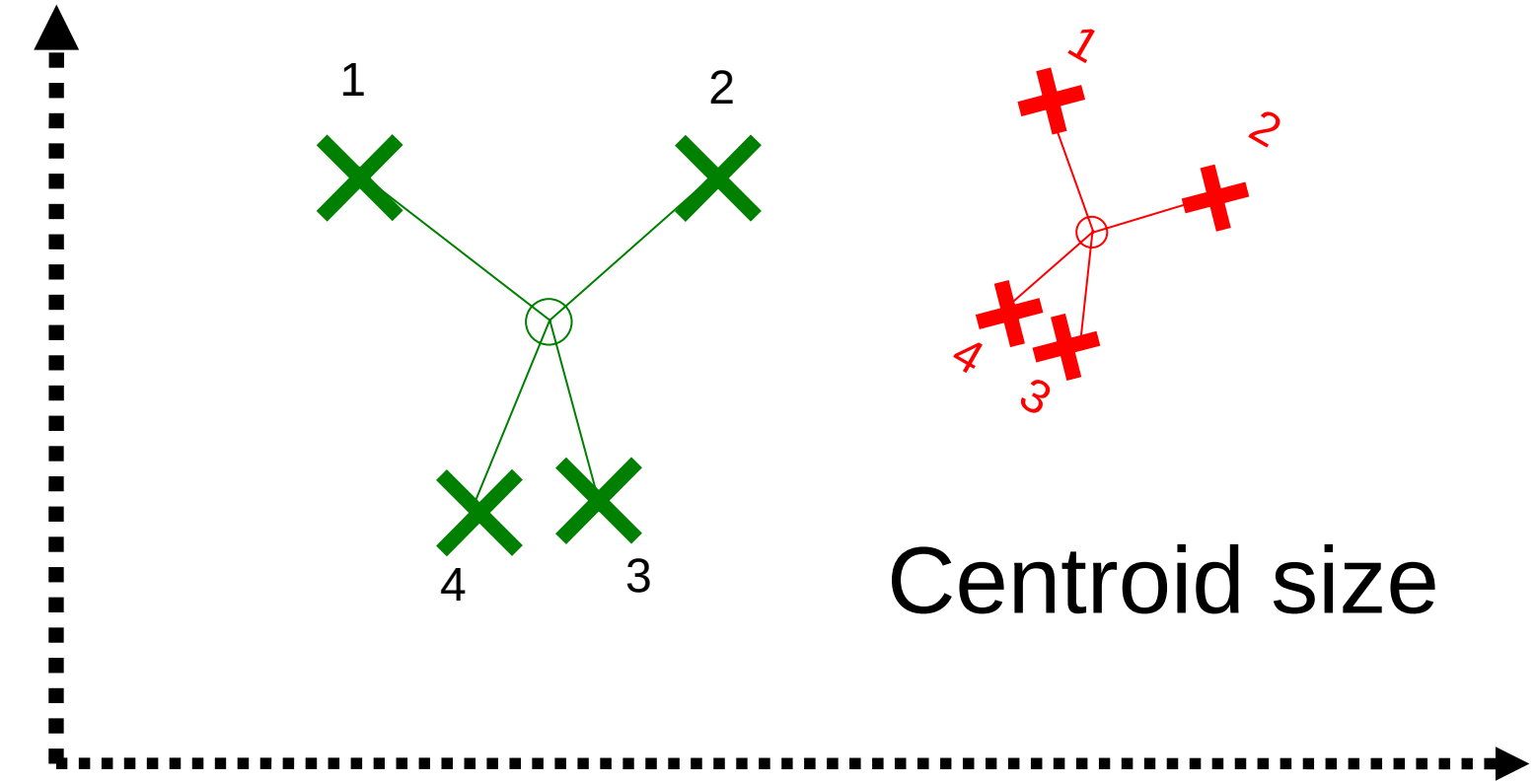
Size differences ?

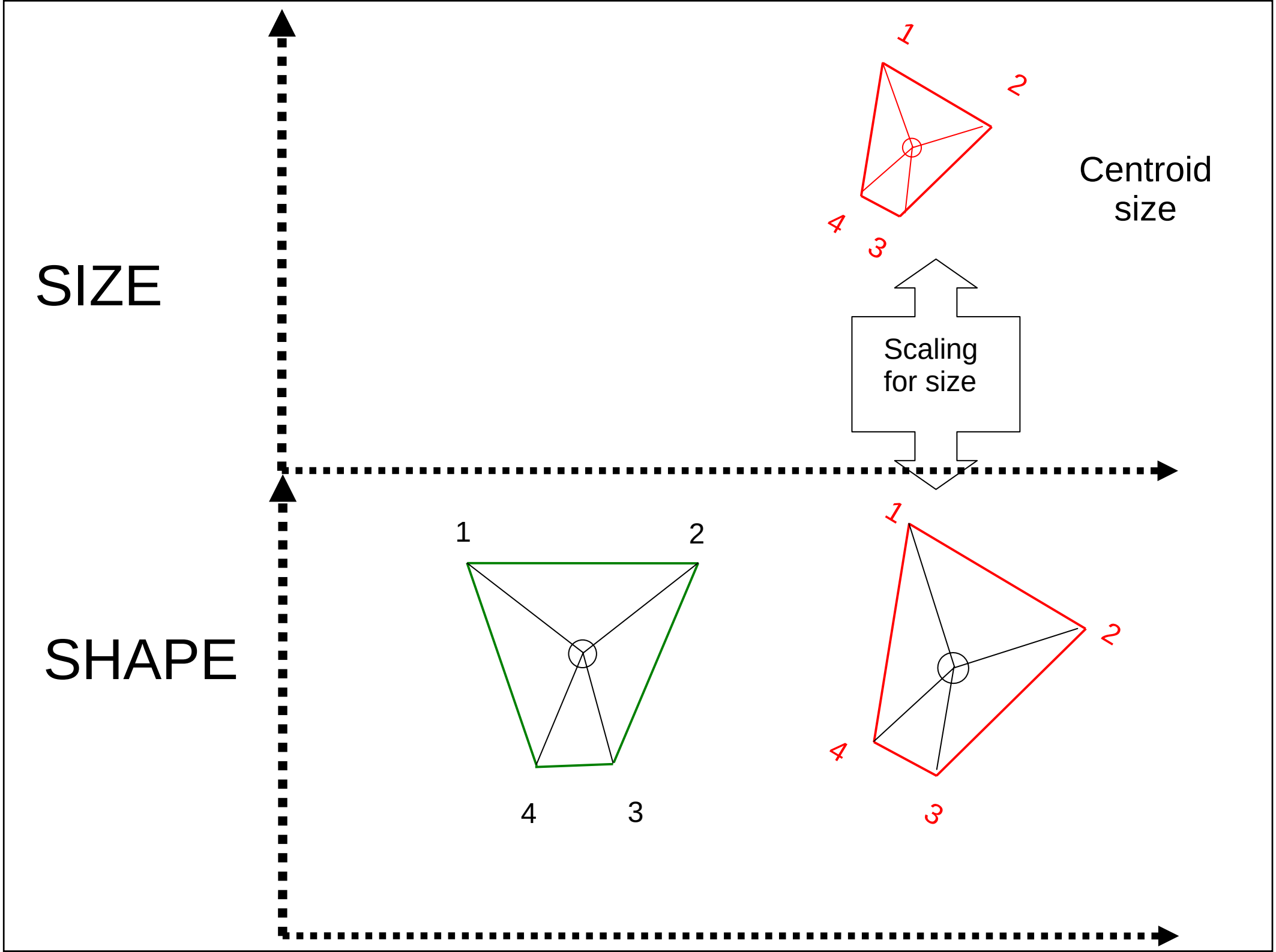
Procrustes superposition


Procrustes
superposition

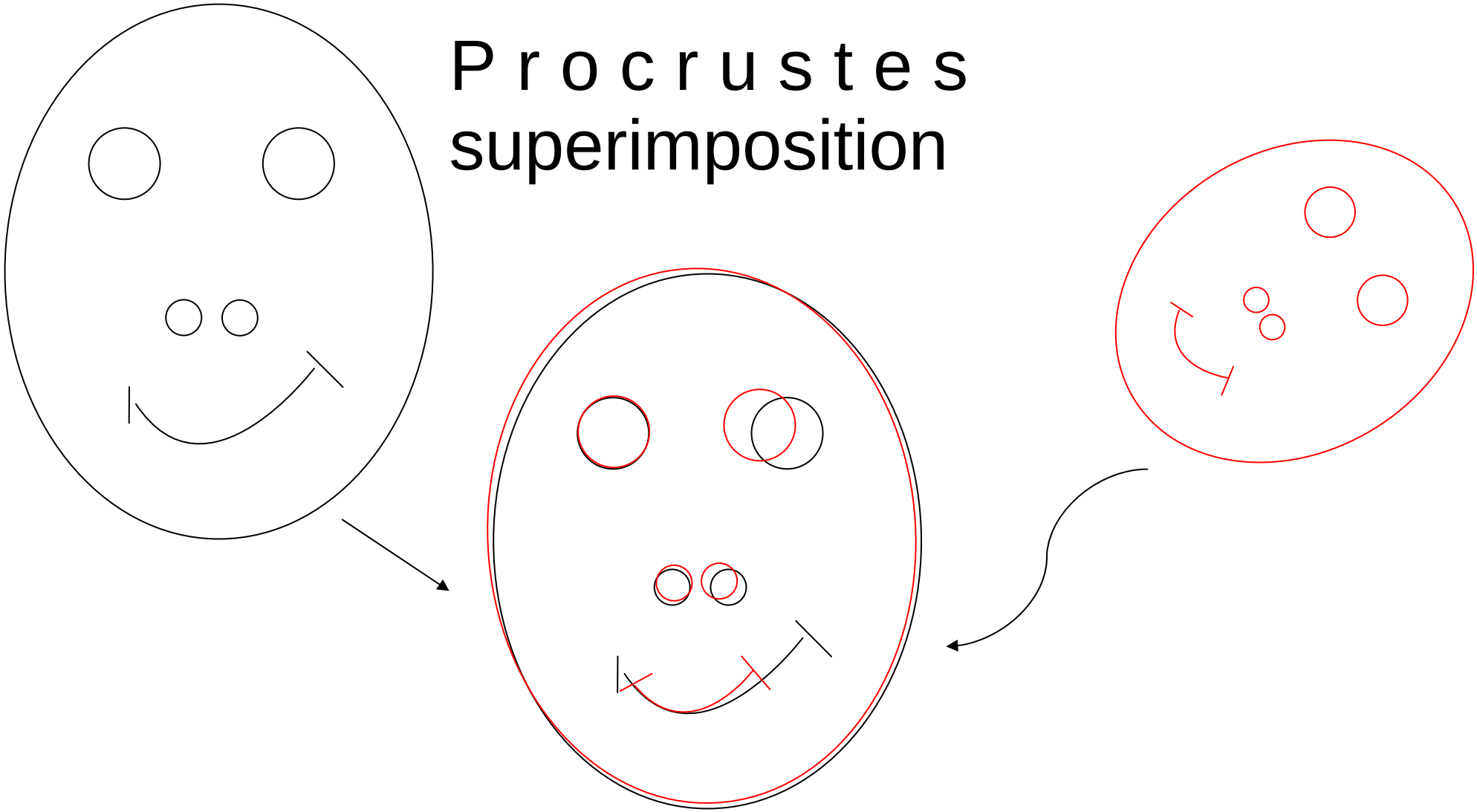


Procrustes
superposition


1
2
4
3
1
2
4
3
Centroid size


SIZE
1
2
4
3
Centroid
size
Scaling
for size
SHAPE
1
2
4
3
1
2
4
3

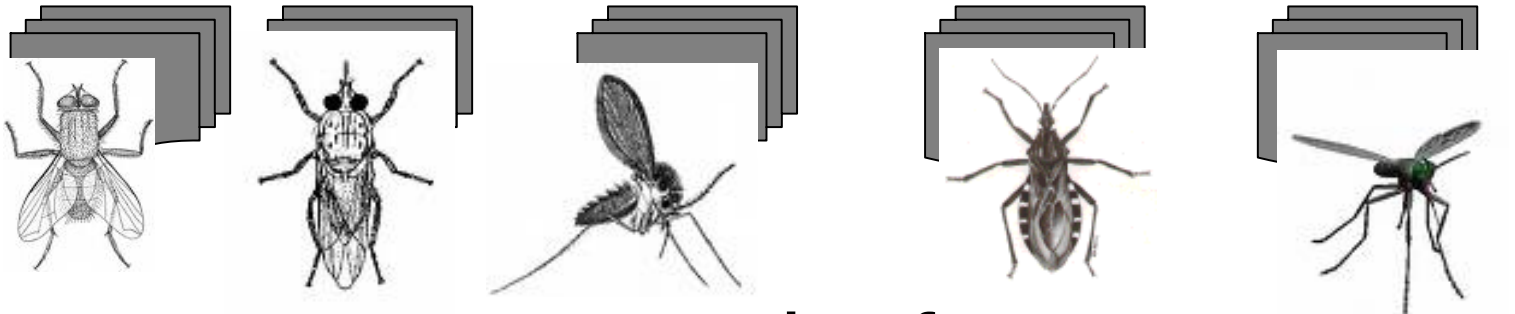
Procrustes superimposition



Procrustes distance

A single specimen

PROCRUSTES
Superposition

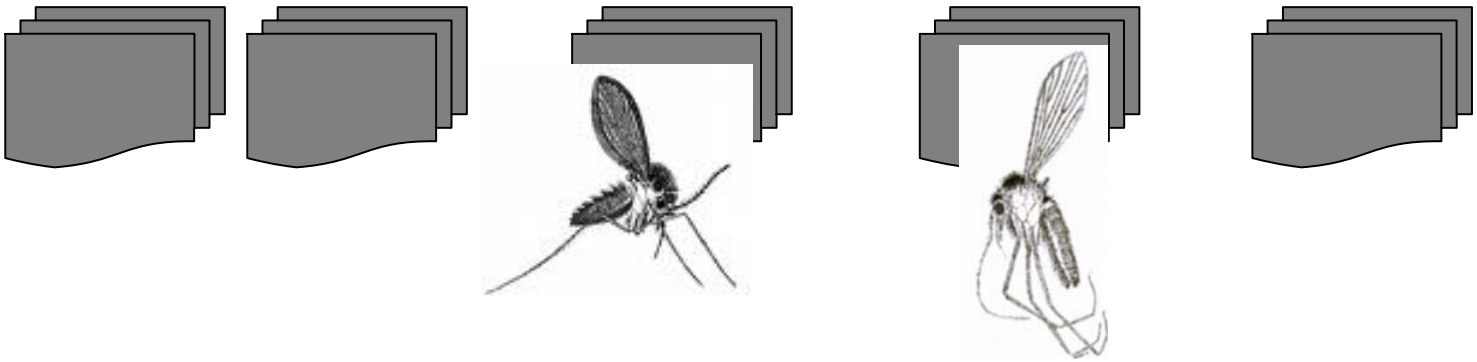


Bank of images

(p i c t u r e s data base)

A single specimen

PROCRUSTES
Superposition



Is the shape of the wing

able to distinguish

cryptic species ?

Xpdf: MOC_longipalpis_11v98n8.pdf

Mem Inst Oswaldo Cruz, Rio de Janeiro, Vol. 96(8): 1089-1094, November 2001 1089

Wing Geometry as a Tool for Studying the *Lutzomyia longipalpis* (Diptera: Psychodidae) Complex

J De la Riva/+, F Le Pont*, V Ali, A Matias, S Mollinedo, JP Dujardin**

INLASA, CP M-10019, Rafael Zubieta 1889, La Paz, Bolivia *UMR IRD-CNRS 9926, Montpellier, France **UMSA, La Paz, Bolivia

Toro Toro (T) and Yungas (Y) have been described as genetically well differentiated populations of the Lutzomyia longipalpis *(Lutz & Neiva, 1912) complex in Bolivia. Here we use geometric morphometrics to compare samples from these populations and new populations (Bolivia and Nicaragua), representing distant geographical origins, qualitative morphological variation ("one-spot" or "two-spots" phenotypes), ecologically distinct traits (peridomestic and silvatic populations), and possibly different epidemiological roles (transmitting or nor transmitting* Leishmania chagasi). *The Nicaragua (N) (Somotillo) sample was "one-spot" phenotype and a possible peridomestic vector. The Bolivian sample of the Y was also "one-spot" phenotype and a demonstrated peridomestic vector of visceral leishmaniasis (VL). The three remaining samples were silvatic, "two-spots" phenotypes. Two of them (Uyuni and T) were collected in the highlands of Bolivian where VL never has been reported. The last one (Robore, R) came from the lowlands of Bolivia, where human cases of VL are sporadically reported. The decomposition of metric variation into size and shape by geometric morphometric techniques suggests the existence of two groups (N/Y/R, and U/T). Several arguments indicate that such subdivision of* Lu. longipalpis *could correspond to different evolutionary units.*

Key words: *Lutzomyia longipalpis* - geometric morphometrics - landmarks - centroid size - shape components - *Leishmania chagasi* - visceral leishmaniasis

A high variability in *Lutzomyia longipalpis* (Lutz & Neiva 1912) (Diptera: Psychodidae: Phlebotominae), vector of *Leishmania chagasi*, has been described at morphological and evolutionary levels. Morphologically, the most significant observation is the existence of "one-spot" and "two-spots" phenotypes (Mangabeira 1969). This male trait corresponds to the occurrence of sexual pheromone glands (Lane & Ward 1984). Evolutionarily *Lu. longipalpis* has been characterized as a complex of species. This was deduced from the existence of different sexual pheromones, not necessarily corresponding to the number of abdominal spots (Lane et al. 1985), and from hybridization and isoenzyme comparisons (Lanzaro et al. 1993), and recently, the detection in Venezuela of two cryptic species occurring in sympatry (Lampo et al. 1999). In samples from Brazil, two isoenzyme comparisons suggested that *Lu. longipalpis* is a single, but genetically heterogeneous, polymorphic species (Mukhopadhyay et al. 1998, Mutebi et al. 1999, Azevedo et al. 2000).

In Bolivia the "one-spot" phenotype of *Lu. longipalpis*, vector of canine and human visceral leishmaniasis, is currently found in domestic and peridomestic environment of the Yungas, Y (Department of La Paz) (Le Pont & Desjeux 1985). The "two-spots" phenotypes are described at the entrance of caves (Chiflonkaka, Humajalanta) at Toro

Page 1 of 6 125% Quit

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Infection, Genetics and Evolution 56 (2002) 1–8

Infection, Genetics and Evolution

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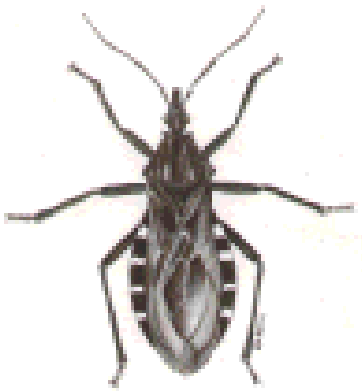
Wing shape divergence between *Rhoduius prolixus* from Cojedes (Venezuela) and *Rhoduius robustus* from Mérida (Venezuela)

J. Villegas[a], M.D. Feliciangeli[b], J.P. Dujardin[c,*]

[a] *Escuela de Malariologia y Saneamiento Ambiental "Dr. Arnoldo Gabaldon" (EMSA), Maracay, Venezuela*
[b] *Universidad de Carabobo, BIOMED, Núcleo Aragua/EMSA, Maracay, Venezuela*
[c] *Institut de Recherches pour le Développement (IRD), Unité Mixte de Recherche (UMR) Centre National de Recherche Scientifique (CNRS), Institut de Recherches pour le Développement (IRD) CNRS-IRD 9926, CDC, 4770 Buford Hwy, 30341 Chamblee, GA, USA*

Page 1 of 8 125% Quit



Anopheles dirus complex

Polytene chromosome characteristics
(Baimai et al. 1988.; Poopittayasataporn and Baimai, 1995.)

RFLP
(Yasothornsrikul, Panyim, and Rosenberg, 1988.),

isoenzymes
(Green et al.,1992),

DNA hybridization method
(Audtho et al., 1995)

PCR related techniques
(Xu et al., 1998.; Walton et al., 1999.; Manguin et al., 2002.).

CRYPTIC GEOMETRIC PATTERNS SEPARATE CRYPTIC SPECIES

Sangvorn Kitthawee[1] Siriporn Phasomkusolsil [2] Jean-Pierre Dujardin

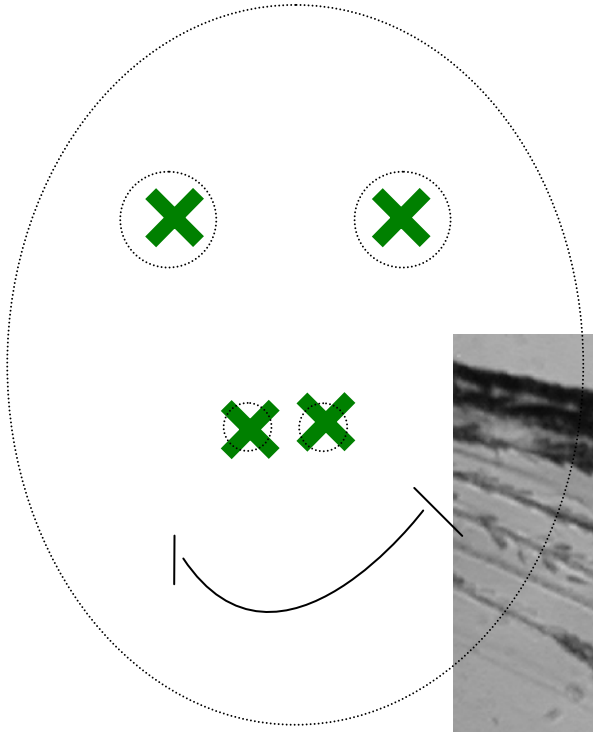
Origin of the material:
Armed Forces Research Institute of Medical Sciences (**AFRIMS**) laboratory, Bangkok.

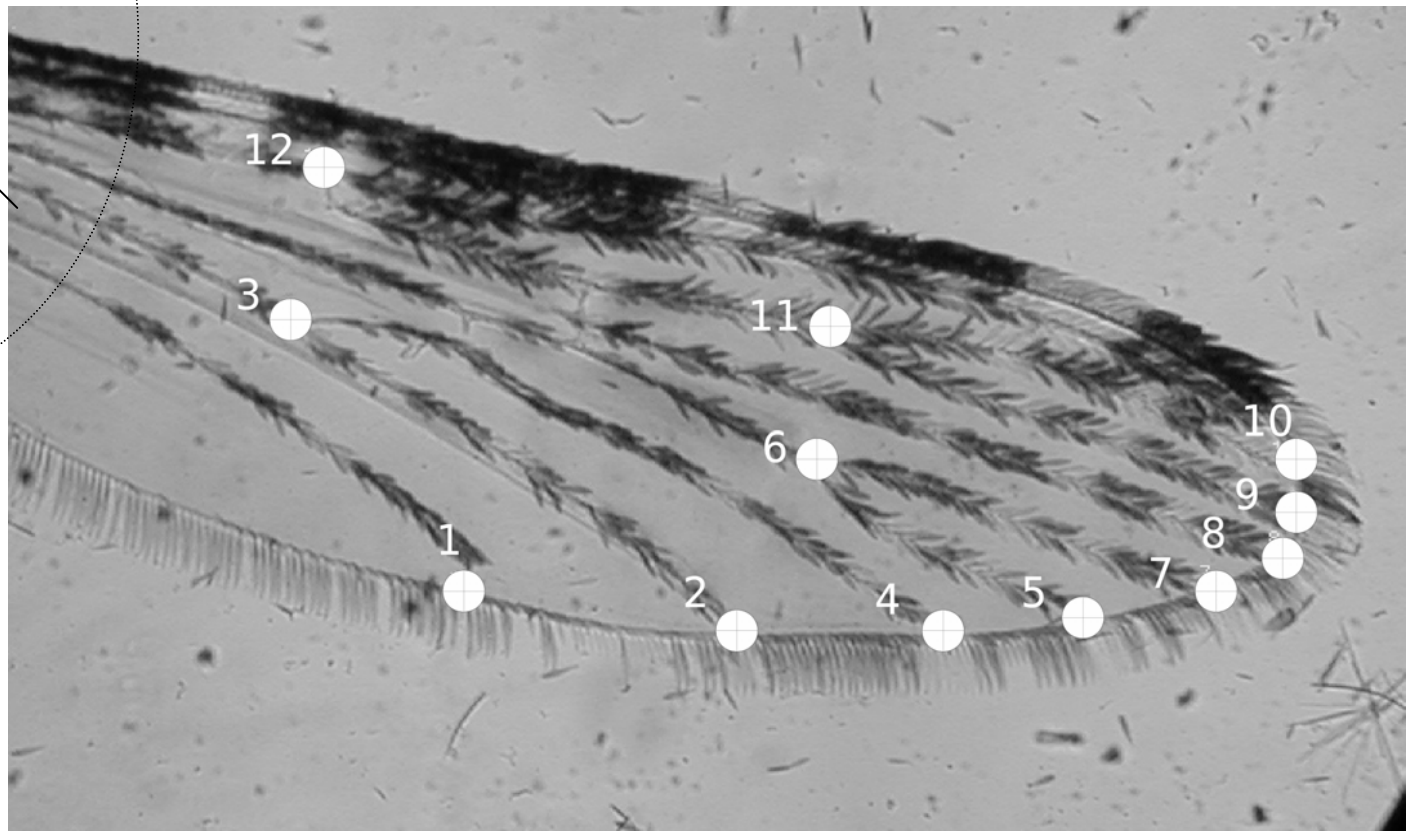
An. dirus A was collected from **Chantaburi Province** and established in **1983.**
An. dirus B (***An. cracens***) was collected from **Malaysia** and established in **1978**.

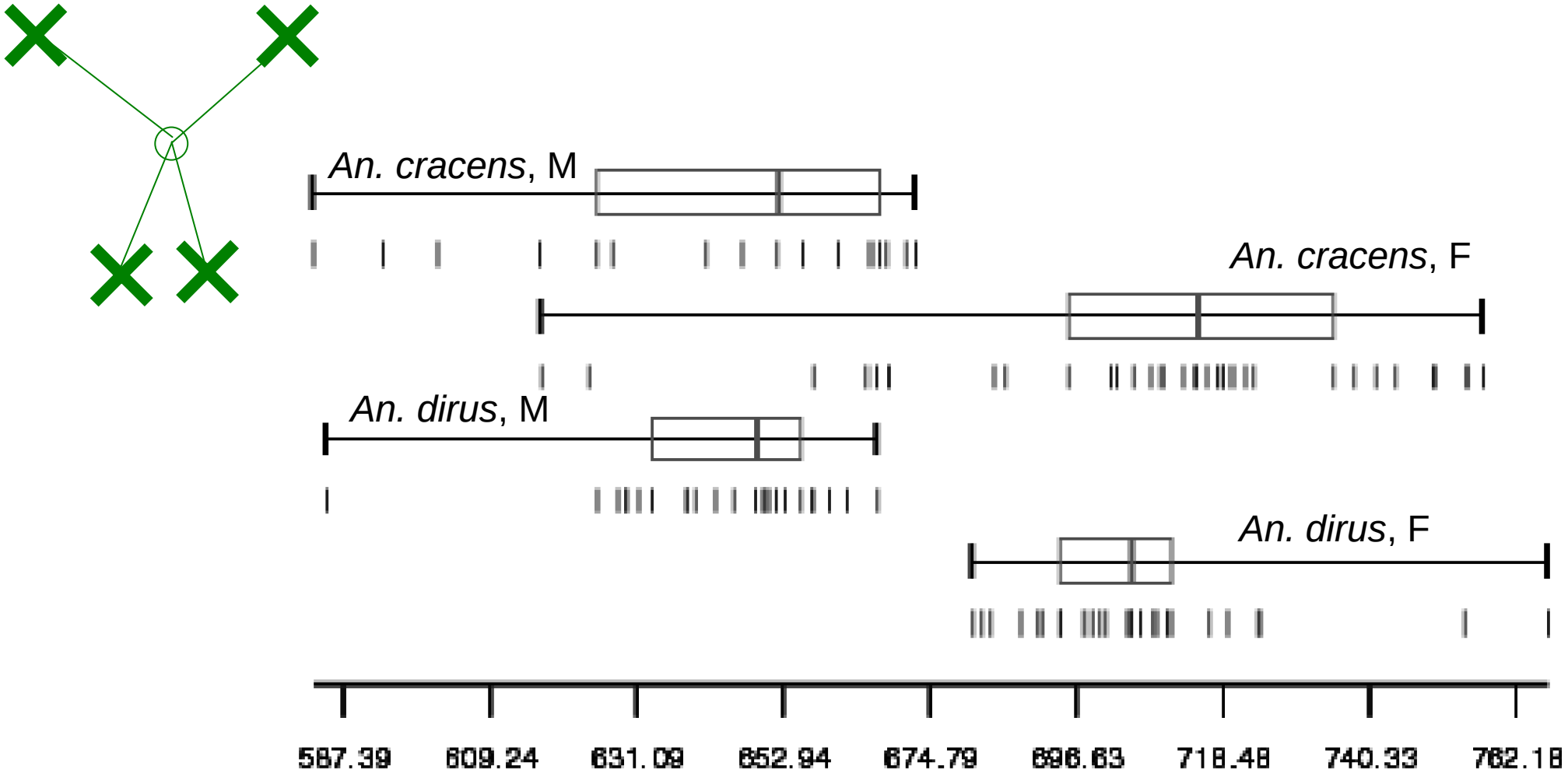
These two lines are regularly monitored (every 6 months) by molecular techniques at the AFRIMS laboratories.

CRYPTIC GEOMETRIC PATTERNS SEPARATE CRYPTIC SPECIES

Sangvorn Kitthawee[1] Siriporn Phasomkusolsil [2] Jean-Pierre Dujardin





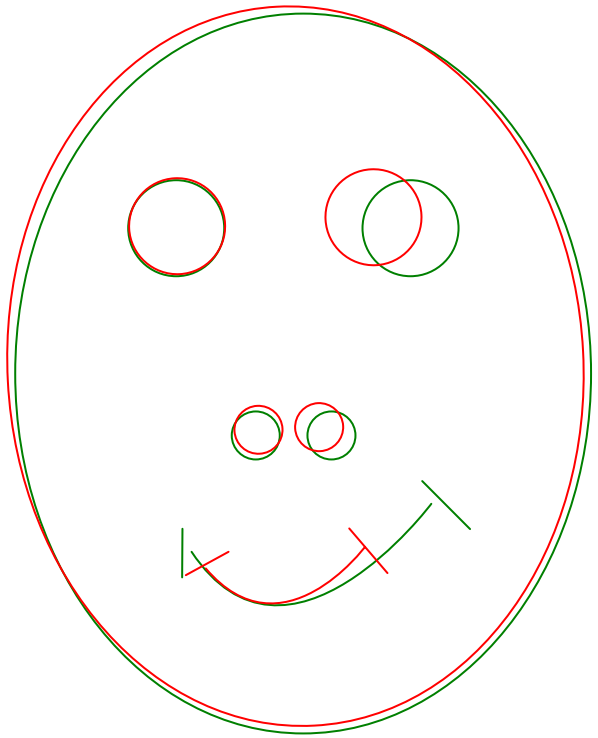


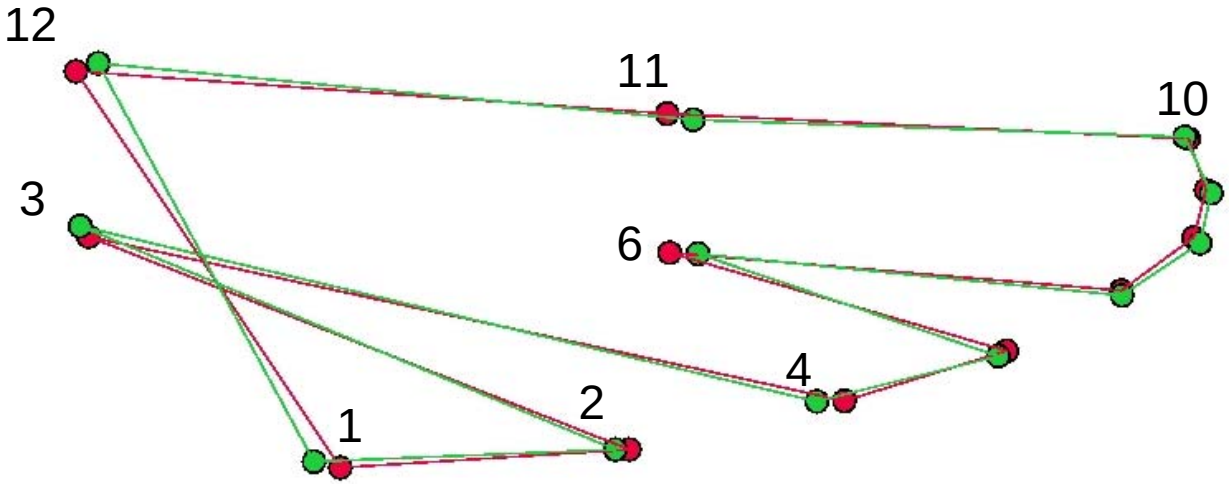
Centroid size variation (in pixels) presented as quantile plots for *A. dirus* (species A) males and females (bottom) and *A. cracens* (species B) males and females (top). Males are smaller.

?

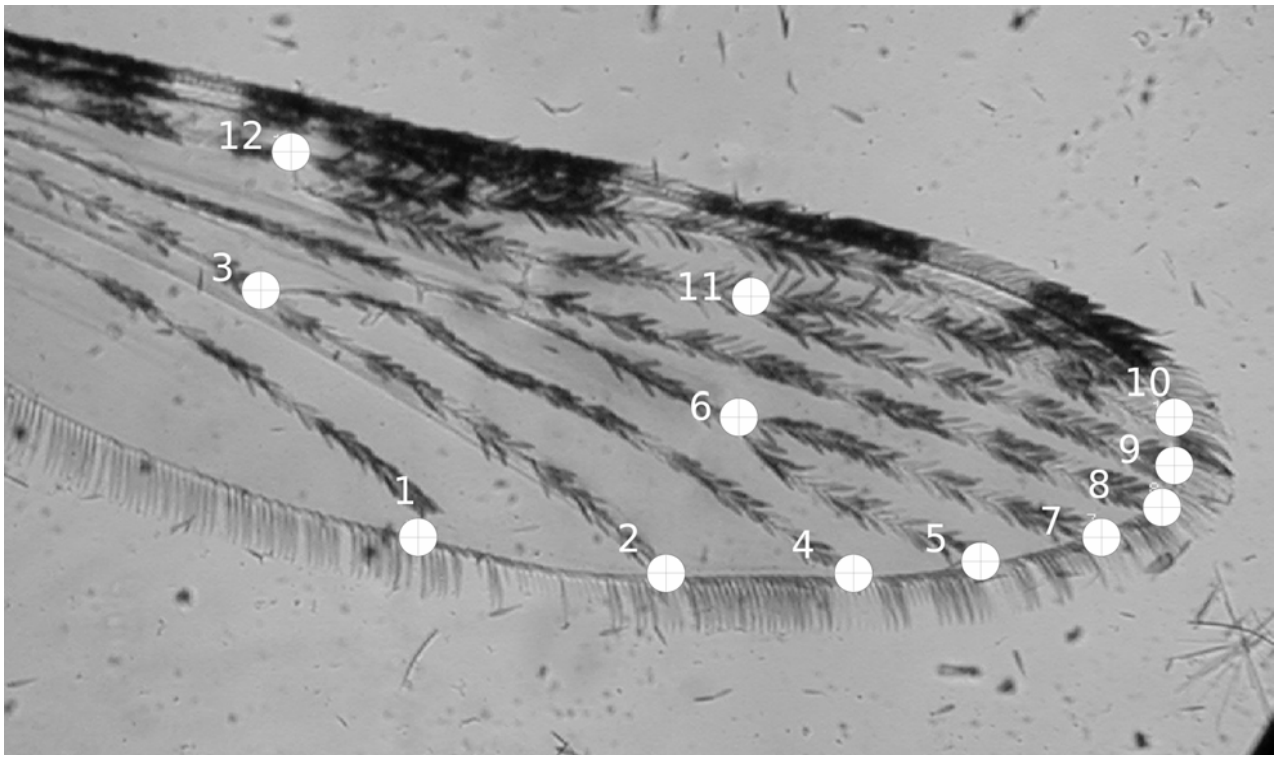
A ?

B ?



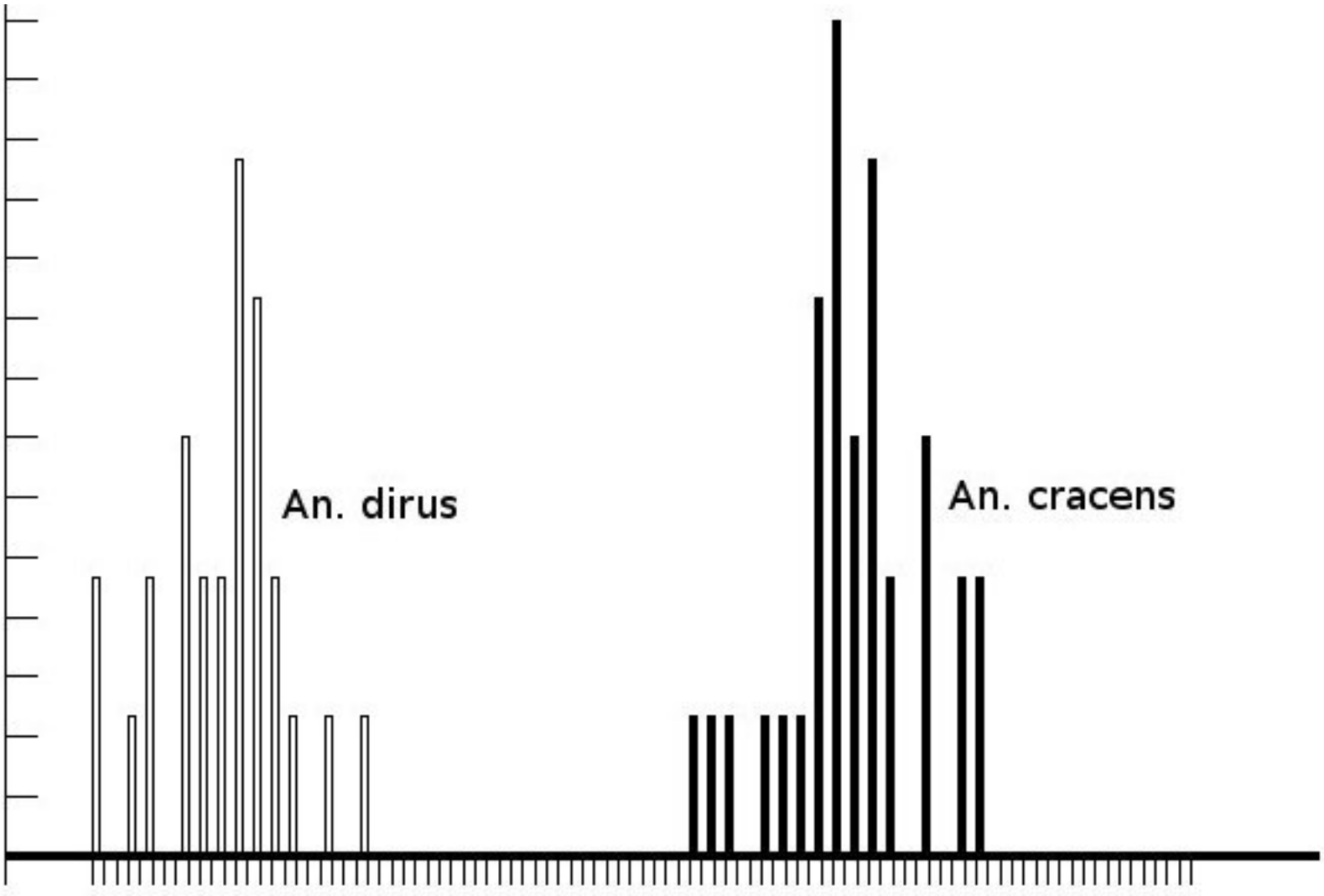


A
B



CRYPTIC GEOMETRIC PATTERNS SEPARATE CRYPTIC SPECIES

Sangvorn Kitthawee[1] Siriporn Phasomkusolsil [2] Jean-Pierre Dujardin

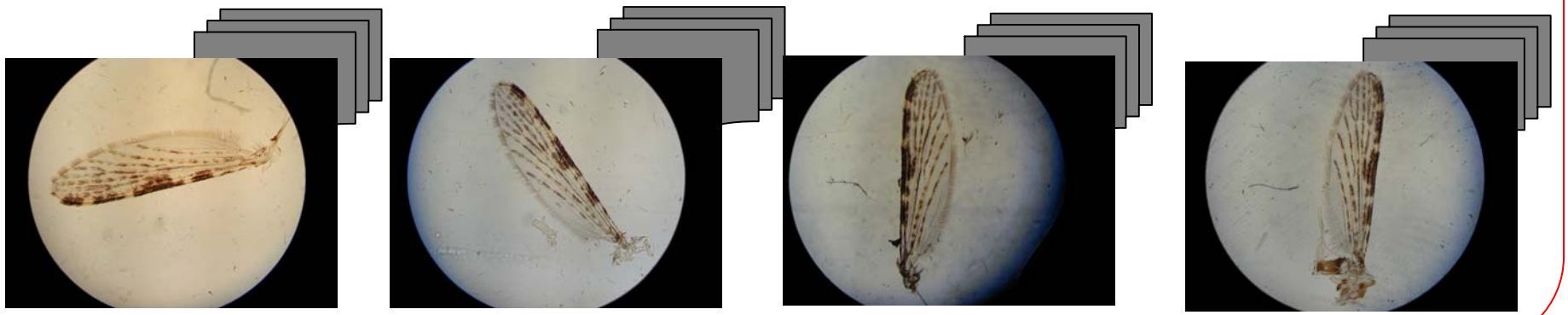


``WING PRINT"

PROCRUSTES
Superposition

The reference PICTURES must be checked by available genetic techniques.

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



Bank of images and metric recognition of sibling species

Advantages ?

Cheap

Fast

Habitat

GPS

Morfología

Óptica

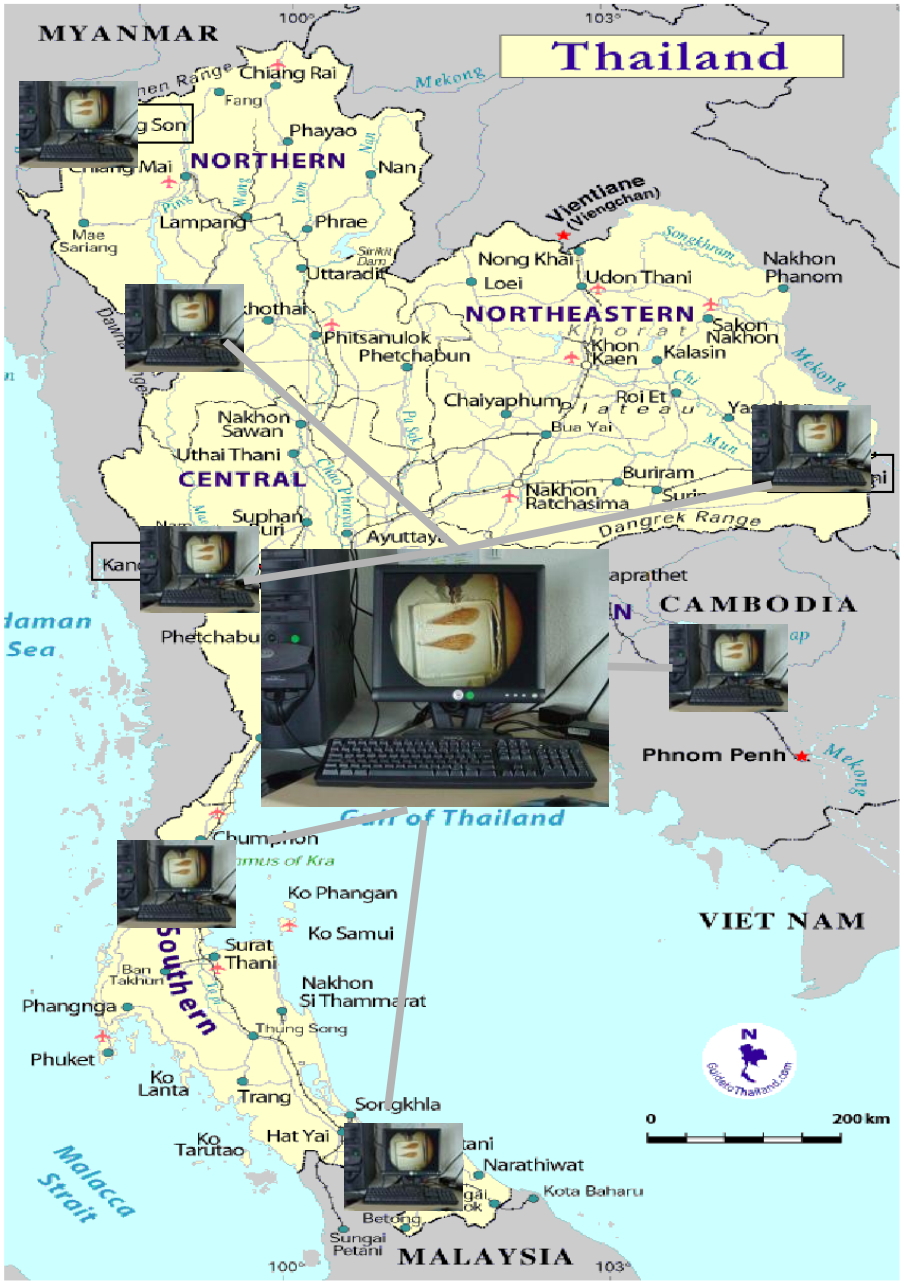
Entomological surveillance

Bank of images and metric recognition of sibling species

Advantages ?

Cheap

Fast


Thailand
MYANMAR
CAMBODIA
VIET NAM
MALAYSIA
Gulf of Thailand
Andaman Sea
Malacca Strait
Mekong
NORTHERN
NORTHEASTERN
CENTRAL
Southern
Chiang Rai
Phayao
Nan
Lampang
Phrae
Uttaradit
Phitsanulok
Phetchabun
Nong Khai
Loei
Udon Thani
Nakhon Phanom
Sakon Nakhon
Khon Kaen
Kalasin
Roi Et
Chaiyaphum
Bua Yai
Buriram
Nakhon Ratchasima
Surin
Dangrek Range
Vientiane (Viengchan)
Nakhon Sawan
Uthai Thani
Suphan Buri
Ayuttaya
Phetchabun
Ko Phangan
Ko Samui
Surat Thani
Nakhon Si Thammarat
Phangnga
Phuket
Ko Lanta
Trang
Songkhla
Hat Yai
Ko Tarutao
Narathiwat
Kota Baharu
Sungai Petani
Phnom Penh
0
200 km
100°
103°

Bank of images and metric recognition of sibling species

Limits ?

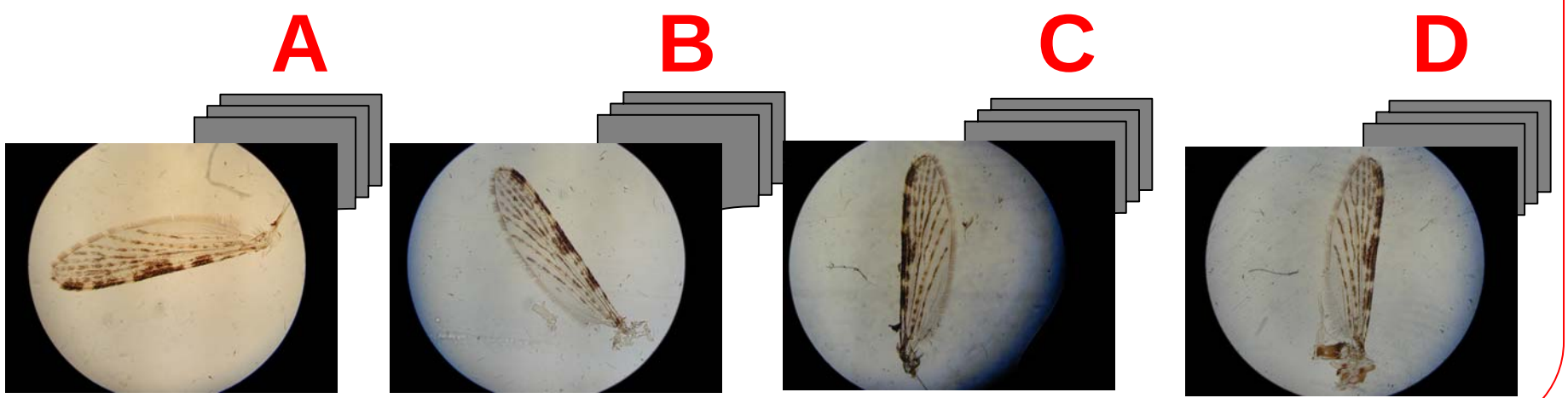
Cannot be used to decide
whether a population or an individual
is a new species

It is an application tool,
*dedicated to **species distinction**,*
*and depending on a reference **bank of images***
(data base of species).

Bank of images and metric recognition of sibling species

Limits ?

The reference PICTURES must be checked by available genetic techniques.



Possible problems

- Laboratory versus natural populations ?

- Interfering variation (season, geography, etc.) ?

- Stability of shape on large time scale ?

- Limits of classification techniques (probabilities, smallest distances, K nearest neighbors (KNN), artificial neural network (ANN), etc.)

- It has never been done !

Acknowledgments

People

Prof. Sangvorn Kitthawee,
Prof. Siriporn Phasomkusolsil
Prof. Rampa Rattanaritikul
Prof. Pattamaporn Kittayapong

Institutions

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CVVD, Mahidol university,
Faculty of Tropical Medicine, Mahidol university,
IRD, France