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Geometric Morphometrics: a quantitative tool for modern taxonomists

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“If you are studying a vector, or any other kind of organism, first try to recognize it”

http://time.com/5144257/fewer-scientists-studying-insects-entomology/
Fewer Scientists Are Studying Insects. Here's Why That's So Dangerous

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Taxonomists: an endangered species...

The "**old guards**" of taxonomy have either retired or are retiring, many others have turned their backs on taxonomic research because of obvious and justifiable reasons, and unfortunately **new talents are nowhere in sight** mainly due to **lack of employability of taxonomists** (Jairajpuri, **1996**)

Melissa Mert. **2002** Taxonomy in Danger of Extinction. News from Science. http://www.sciencemag.org/news/2002/05/taxonomy-danger-extinction

"...Since 1992, f**unding for systematic biology** at major research institutions in the United Kingdom **has dropped** between 15% and 25% …"

Are We Losing the Science of Taxonomy?: As need grows, numbers and training are failing to keep up.

Lisa W. Drew

BioScience, Volume 61, Issue 12, 1 December **2011**, Pages 942–946,

"...During one recent visit to a **museum** that Mares will not name—"but it's one everyone on this planet has heard of," he says—he found that every **one of roughly 50 specimens**, representing seven species and three genera, was **mislabeled**."

The decline of Medical entomology...

About American and Canadian medical school curricula, a few years ago: **only 11 of 120 institutions include course content about arthropods**.

(**http://time.com/5144257/fewer-scientists-studying-insects-entomology/ Fewer Scientists Are Studying Insects. Here's Why That's So Dangerous**)

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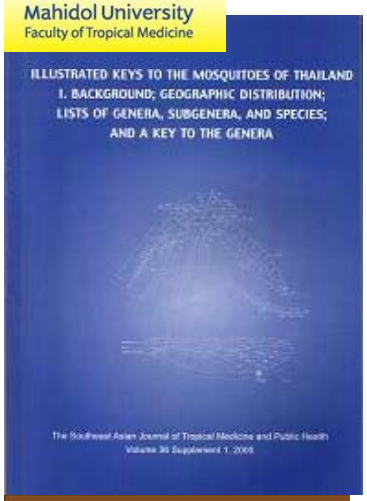
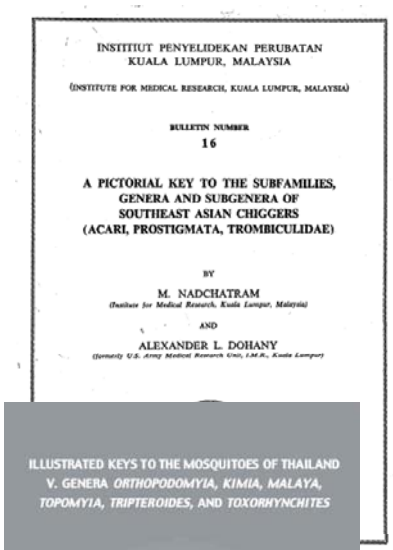
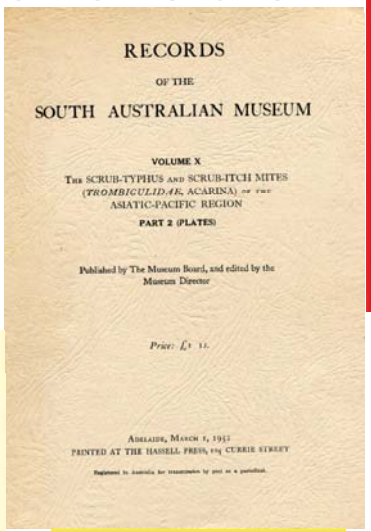
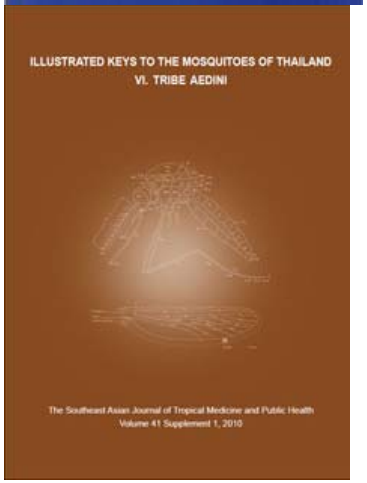
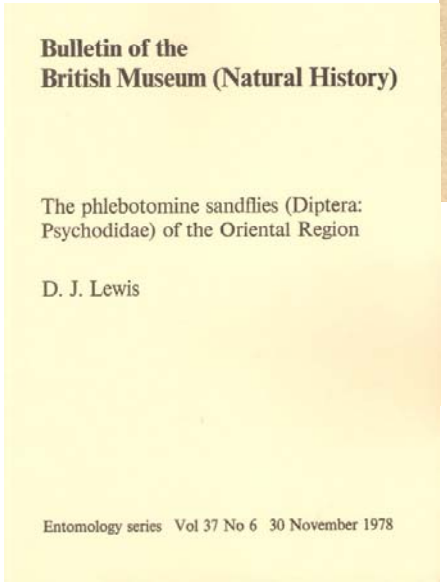
Taxonomist must to identify species correctly…..by available **keys**

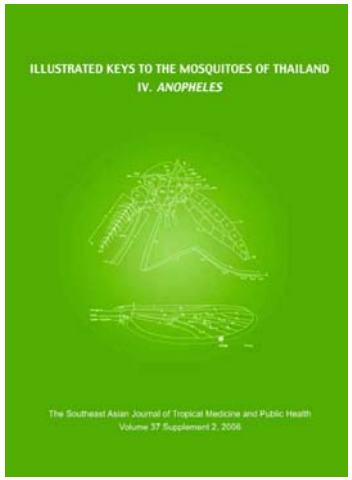












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Traditional taxonomy was based on qualitative morphological characters <- Human EYES

Example color, present/absent, etc

Some misidentification

-sibling species (species complex)

-specimens have lost some characters (used to identify species) during capture and manipulation of insects, it is hard to make the correct identification.

It was too subjective of an approach.

It cannot quantitative characters.

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Size and **shape !**

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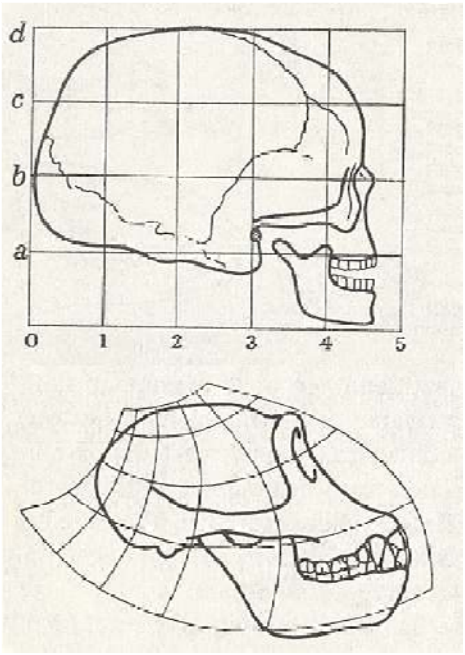
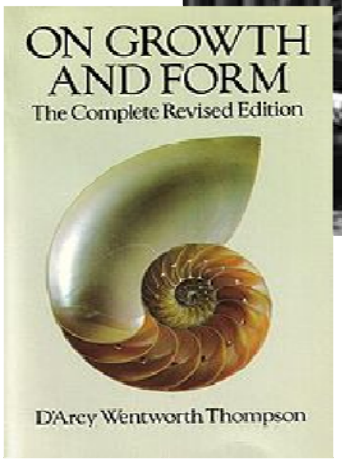
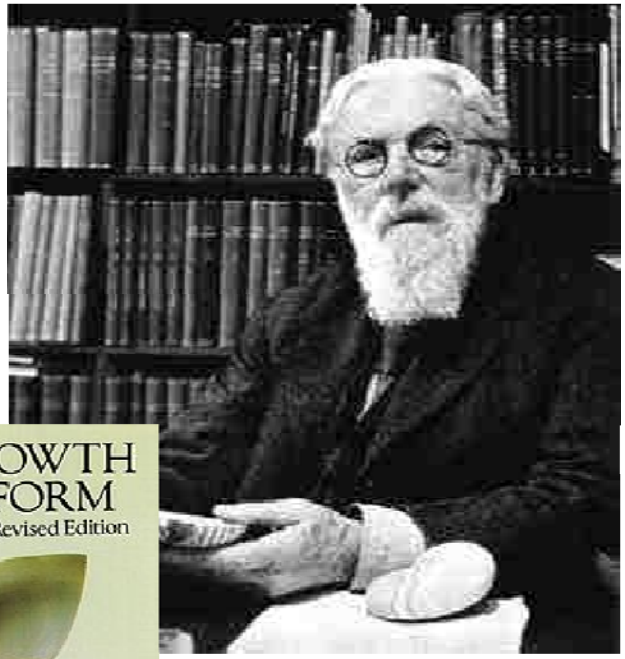


« landmark-based »





Born
2 May 1860
Edinburgh
Died 1948 (aged 88)
St Andrews
Occupation
Mathematical biologist

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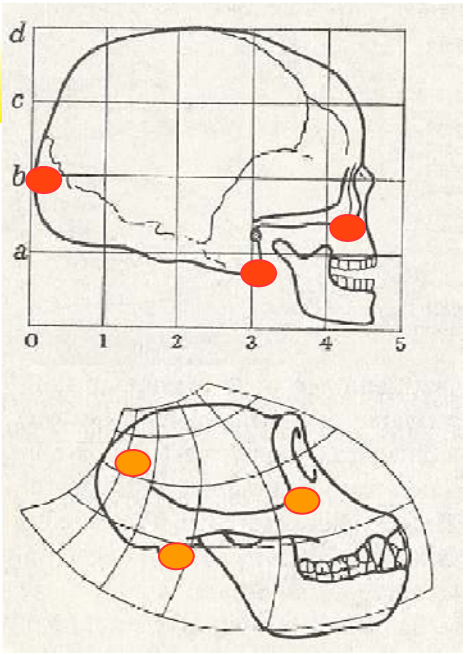

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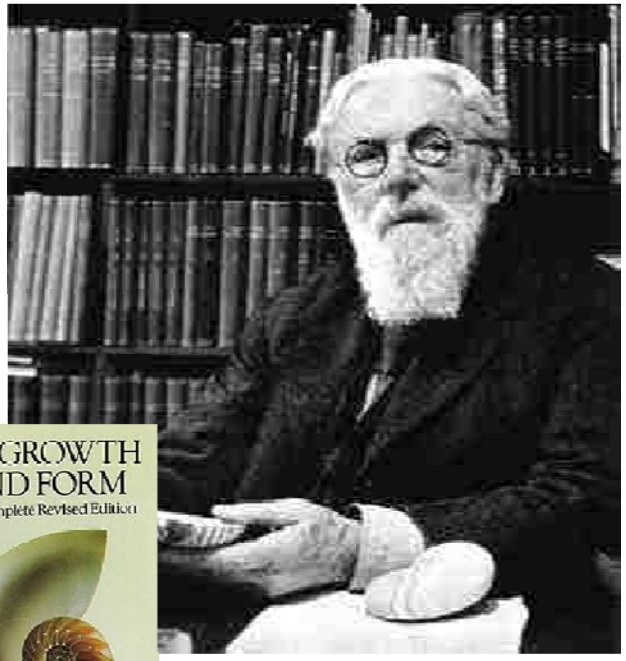

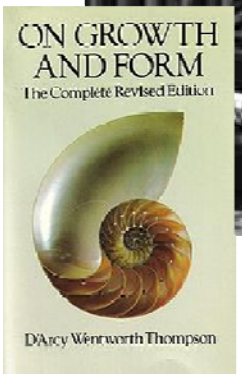

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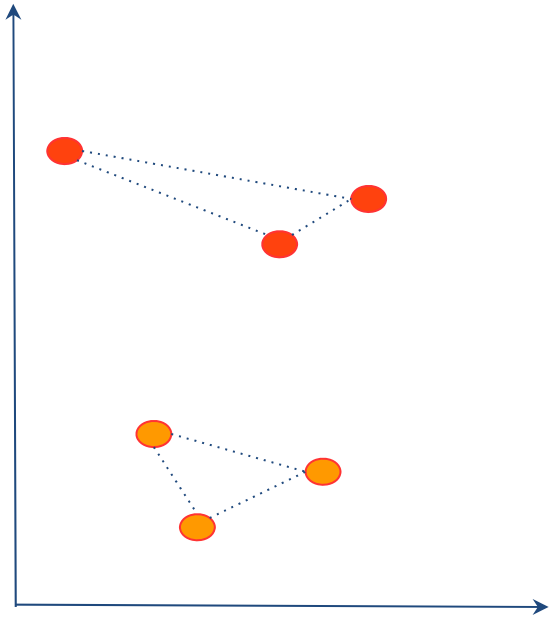
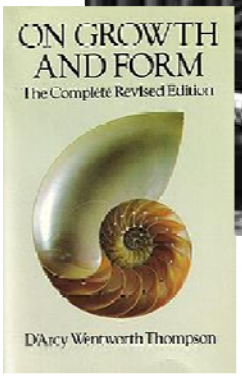
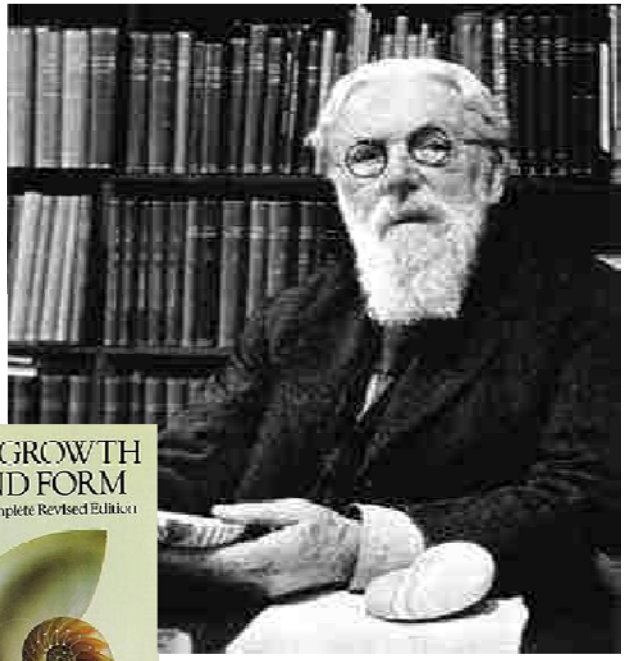


« **landmark-based** »





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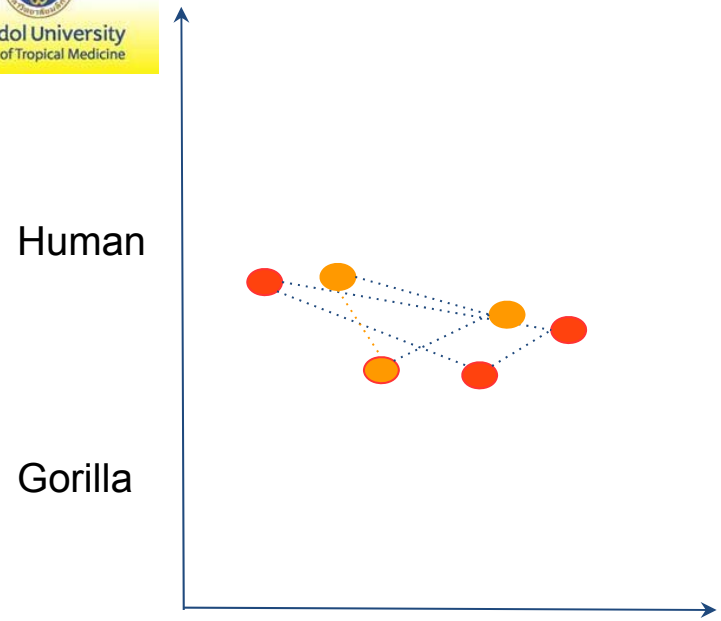

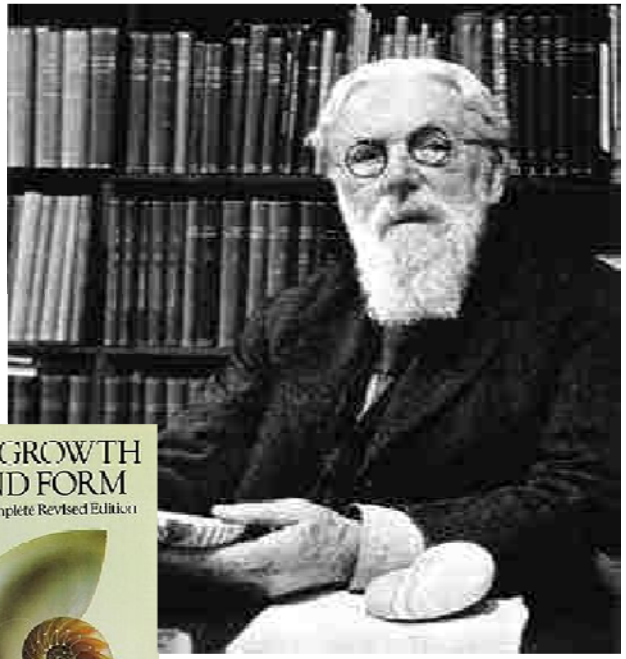

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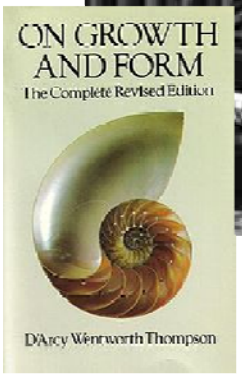

« landmark-based »

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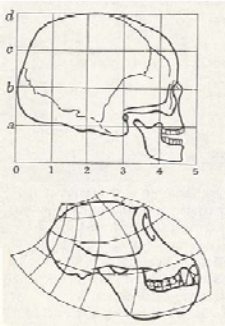

Kendall D. G. 1984. Shape manifolds, Procrustean metrics and complex projective spaces. Bulletin of the London Mathematical Society 16: 81-121

Bookstein F. L. 1984. A statistical method for biological shape comparisons. Journal of Theoretical Biology 107: 475–520.

Bookstein F. L. 1986. Size and shape spaces for landmark data in two dimensions (with discussion). Statistical Science 1: 181–242.

Rohlf J. F. 1986. Relationships among eigenshape analysis, Fourier analysis, and analysis of coordinates. Mathematical Geology 18: 845–654.

Rohlf J. F. 1999. Shape statistics: Procrustes superimposition and tangent spaces. Journal of Classification 16: 197–223.

Goodall C. R. 1991. Procrustes methods in the statistical analysis of shape (with discussion). Journal of the Royal Statistical Society, Series B 53: 285-339

Other important names in developing techniques and theory since 1990

Ian **Dryden**,
Kanti **Mardia**,
Dennis **Slice,**
Dean **Adams**,
… Etc.

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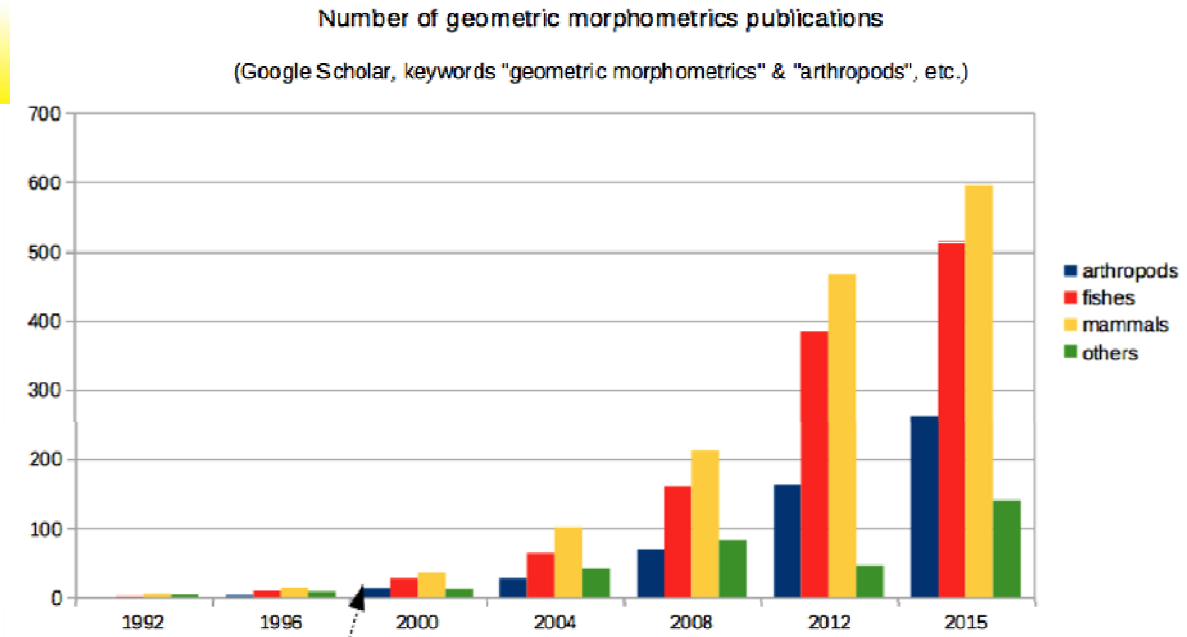
Size and **shape !**



A revolution morphometrics. Rohlf, Marcus
Trends Ecol Evol. **1993** 8(4):129-32. doi: 10.1016/0169-5347(93)90024-J..

Geometric Morphometrics: Ten Years of Progress Following the 'Revolution'
Adams, Rohlf, Slice. Ital. J. Zool., **2004** 71:5-16.

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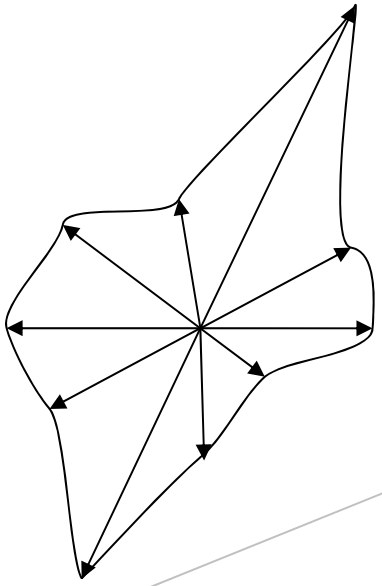
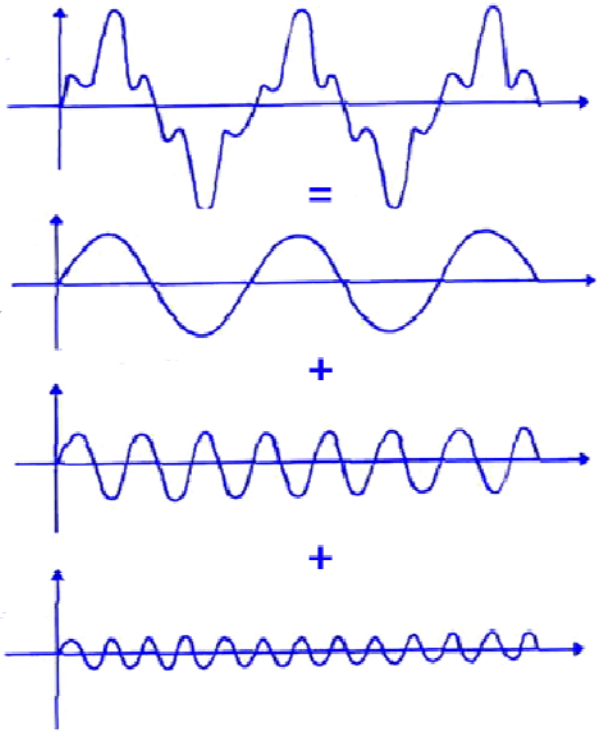


Outline-based



Harmonics

Jean Baptiste Joseph Fourier (1768 – 1830) was a French physicist and mathematician who is known for investigating the Fourier series and its application to problems of heat flow.

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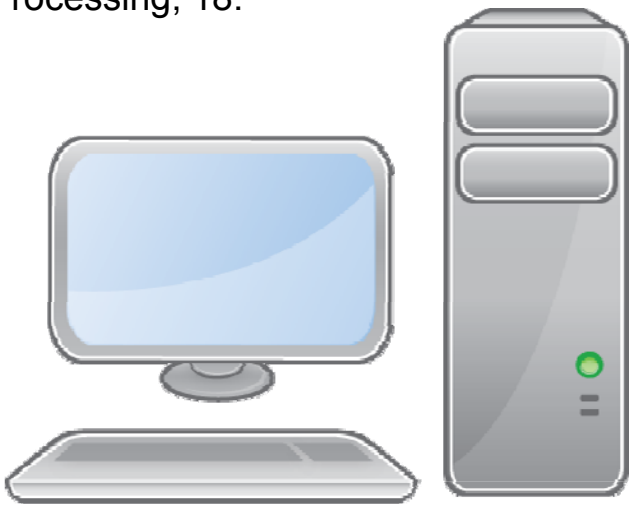
Outline-based

Kuhl FP and **Giardana CR**, **1982**. Elliptic Fourier features of closed contour. Computer Vision, Graphics and Image Processing, 18: 236-258.

Other names

Lestrel, 1987
Rohlf, 1990
Etc.



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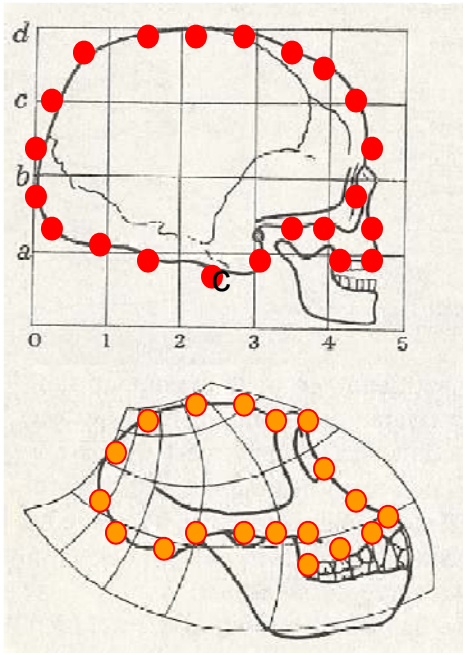

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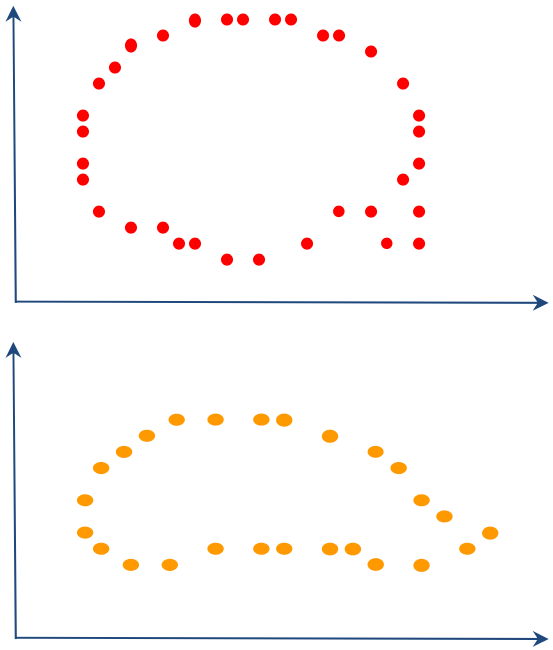

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SUMMARY

Traditional taxonomy was based on qualitative morphological characters <- Human EYES

It was too subjective of an approach.

Modern taxonomy makes use of quantitative morphological characters <- Computer EYES

- It removes the requirement to be an expert of the group of insects under study.
- It removes subjectivity of the taxonomist (89%, 95%, etc.).

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The new taxonomist is expected **to be able to use computerized methods** for taxonomic data collection, analyses and classification.

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Recent geometric morphometrics studies on Thailand fauna

Aedes mosquitoes in 2016

Sumruayphol S, Apiwathnasorn C, Ruangsittichai J, Sriwichai P, Attrapadung S, Samung Y, Dujardin JP*.

DNA barcoding and wing morphometrics to distinguish three *Aedes* vectors in Thailand. Acta Trop 2016 Jul;159: 1-10.

Stomoxys flies in 2016

Changbunjong T*, **Sumruayphol S**, Weluwanarak T, Ruangsittichai J, Dujardin JP. Landmark and outline-based geometric morphometrics analysis of three *Stomoxys* flies (Diptera: Muscidae). Folia parasitologica 2016 Oct;63: 037.

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Recent geometric morphometrics studies on Thailand fauna

Chigger mites in 2018

Sungvornyothin S, Kumlert R, Paris D, Prasartvit A, Sonthayanon P, Apiwathnasorn C, Morand S, Alexandr Stekolnikov, **Sumruayphol S***. Geometric morphometrics of the dorsal scutum for differentiation of Trombiculid mites within the genus Walchia (Acari: Trombiculidae), a vector of scrub typhus. Ticks and Tick-Borne Diseases. https://doi.org/10.1016/j.ttbdis.2018.11.013

Anopheles mosquitoes in 2018

Chaiphongpachara T, Sriwichai P, Samung Y, Ruangsittichai J, Morales Vargas RE, Dujardin JP, **Sumruayphol S***. *Anopheles maculatus* complex in Thailand: geometric morphometrics approach towards discrimination of sibling species. Under manuscript development. **Accepted** in Acta Tropica.

Fasciola spp. in 2018

Sumruayphol S, Siribat P, Dujardin JP, Dujardin S, Komalamisra K, Thaenkham U*. *Fasciola gigantica*, *F. hepatica* and *Fasciola* intermediate form: novel approaches to help morphological species discrimination. **Under Review** in Parasites and Vectors.

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Objective

- to investigate the power of geometric morphometrics (landmark and outline-based geometric morphometrics) to help morphological species determination of target organisms.

Acta Tropica 159 (2016) 1–10

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

DNA barcoding and wing morphometrics to distinguish three *Aedes* vectors in Thailand

Suchada Sumruayphol[a], Chamnarn Apiwathnasorn[a], Jiraporn Ruangsittichai[a], Patchara Sriwichai[a], Siriluck Attrapadung[a], Yudthana Samung[a], Jean-Pierre Dujardin[b,*]

[a] *Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand*
[b] *Unité Mixte de Recherche 177-Interactions Hôte-Vecteur-Parasite-Enrironnement dans les Maladies Tropicales Négligées dues aux Trypanosomatidés, Centre International de Recherches Agronomiques pour le Développement (CIRAD), Institut de Recherches pour le Développement (IRD), Campus international de Baillarguet, Montpellier, France*

ARTICLE INFO

Article history:
Received 7 July 2015

ABSTRACT

Aedes aegypti (Diptera: Culicidae) (L.), *Ae. albopictus* (Skuse), and *Ae. scutellaris* (Walker) are important mosquito vectors of dengue and chikungunya viruses. They are morphologically similar and sympatric

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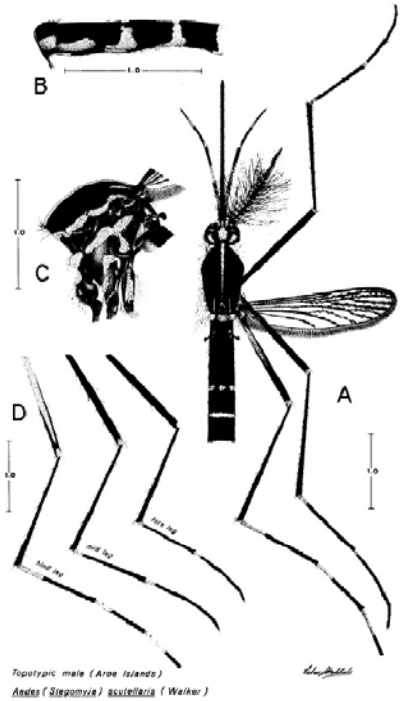

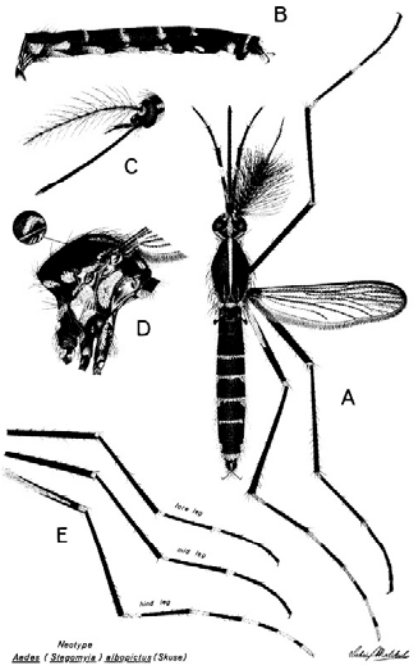

Huang, 1972

Ae. scutellaris ***Ae. albopictus***

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- **Mosquito collection**
- Mosquito larvae were collected in Chachoengsao Province (13°28'25.68"N 100°52'19.65"E) , Thailand in 2013.
- Collected larval mosquitoes were reared until adult emergence in the Department of Medical Entomology , Faculty of Tropical Medicine, Mahidol University.
- Adults were morphologically identified based on the Huang key (Huang, 1972).

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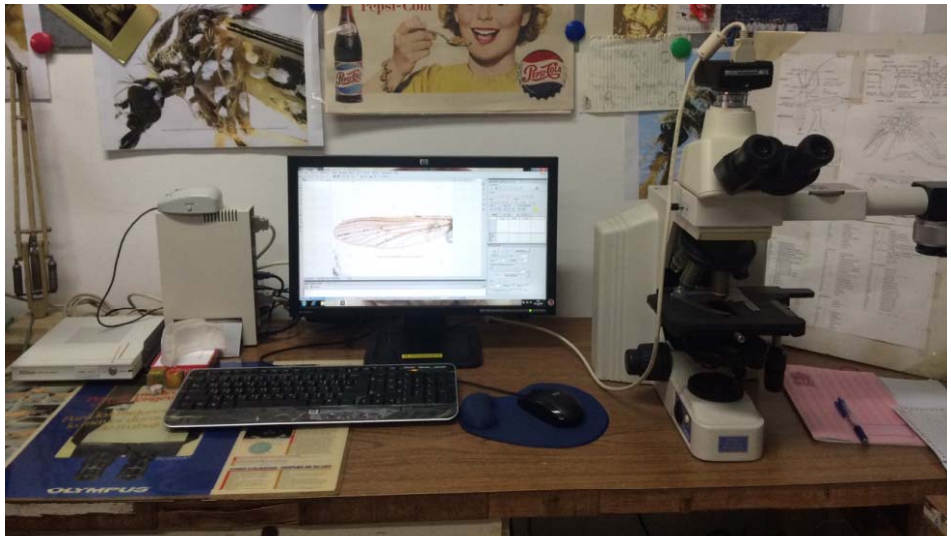
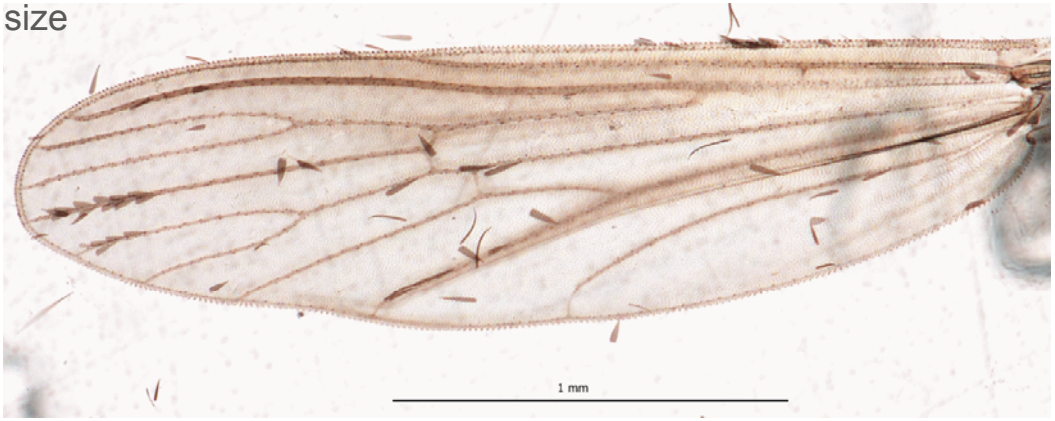


- **Mosquito preparation**
- Left wings of mosquitoes were dissected and mounted using the Hoyer medium on microscopic slides and photographed by Nikon DS-Ri1 SIGHT digital camera connected to stereo-microscope Nikon AZ 100M (Nikon Corp., Tokyo, Japan) with a size scale apparent on the picture.



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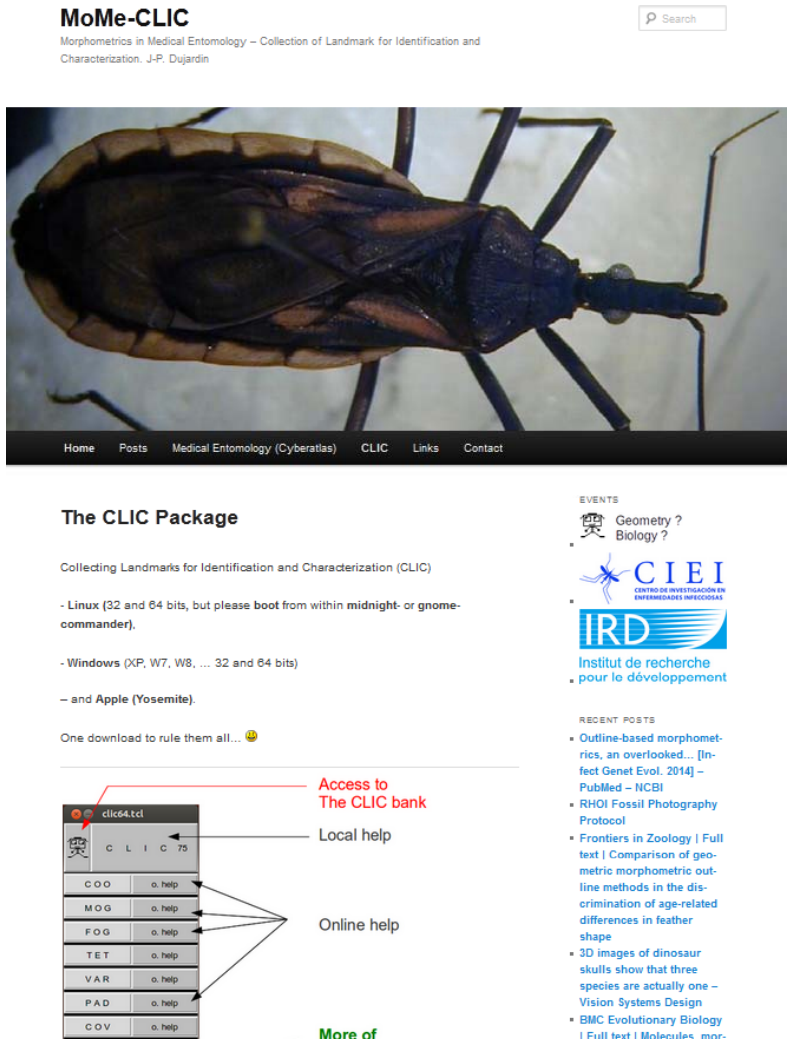

Landmark and outline-based geometric morphometric analysis

- The CLIC software (Dujardin and Slice, 2007; Dujardin et al., 2010), freely available at https://xyom-clic.eu, was used for the complete set of geometric and multivariate analyses on the morphometric data.

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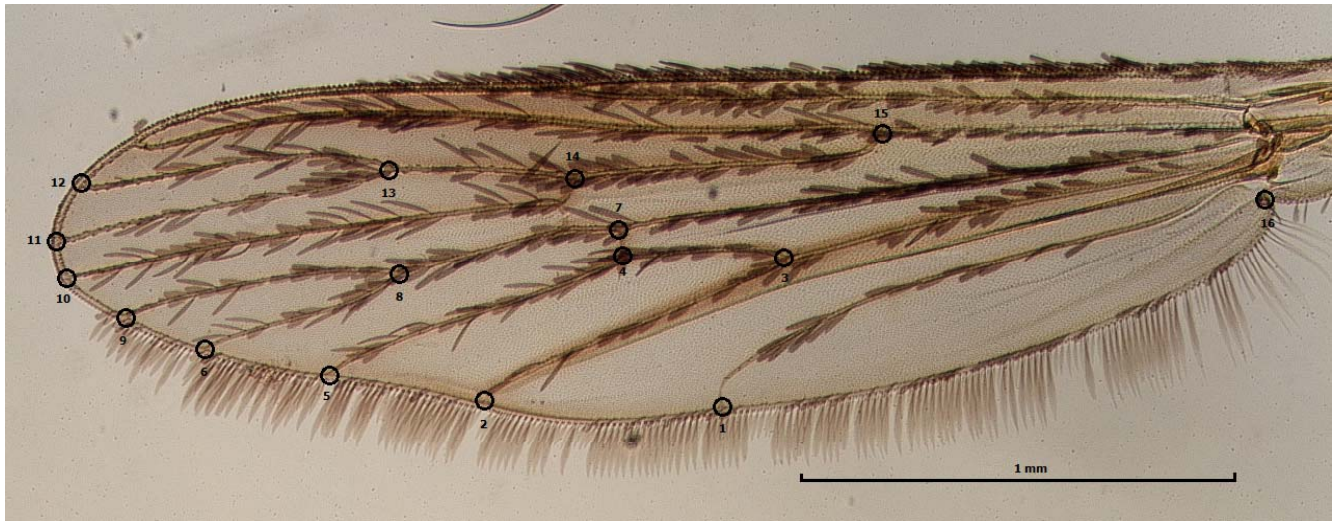
Landmark-based analysis



Position of 16 landmarks digitized on *Aedes* spp. wing for landmark-based geometric morphometrics

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Landmark-based geometric morphometric method

- Centroid size (CS) was computed for wing variables**.**
- Statistical significance for size variation was based on non-parametric, permutation tests (1000 cycles).

- The wing shape variables were computed as the principal components of the “partial warp” (PW) scores calculated after the Generalized Procrustes Analysis (GPA) (Rohlf & Slice, 1990) of raw coordinates.
- An UPGMA tree was built from Euclidean distances.
- Statistical significance of shape differences was tested using non-parametric (1000 permutations), of pairwise Mahalanobis distances.

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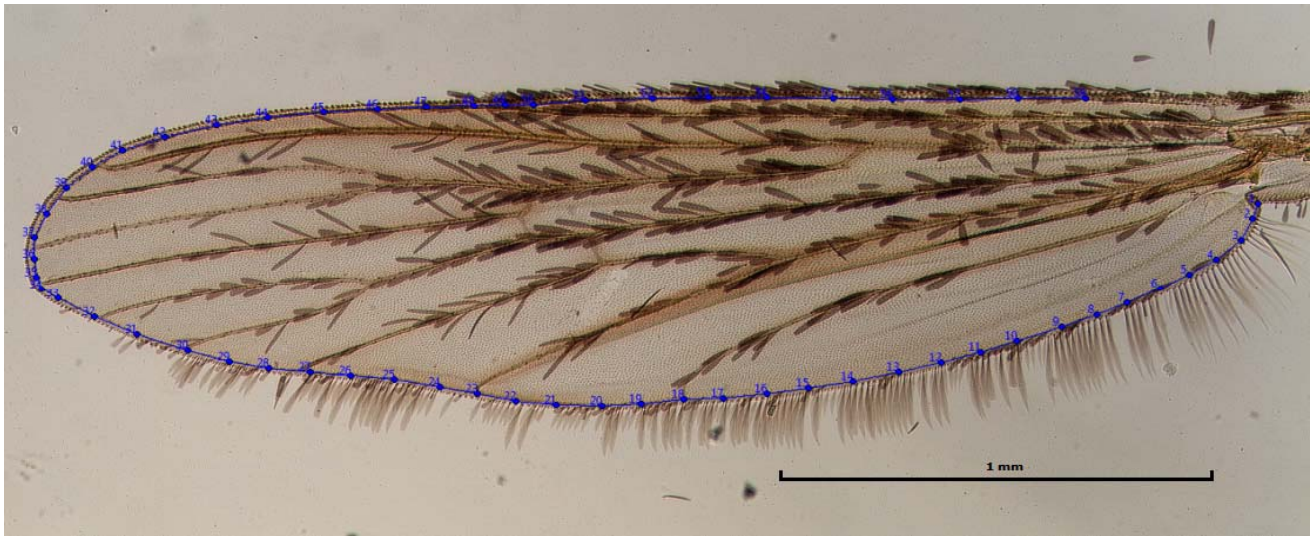
Outline-based analysis



Contour digitized on *Aedes* spp. wing for outline-based geometric morphometrics

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Outline-based geometric morphometric method

- Size was estimated as the perimeter of the contour and compared between species using non-parametric tests in the same way as for the centroid size.

- Shape variables, the Elliptic Fourier Analysis (EFA) approach (Kuhl & Giardana 1982).

- Statistical comparisons of shape between species used the same procedure as for the landmark-based approach.

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To determine individuals to their corresponding species

- Each individual was classified after a discriminant analysis of final shape variables.
- The discriminant analyses were illustrated by the factor maps.

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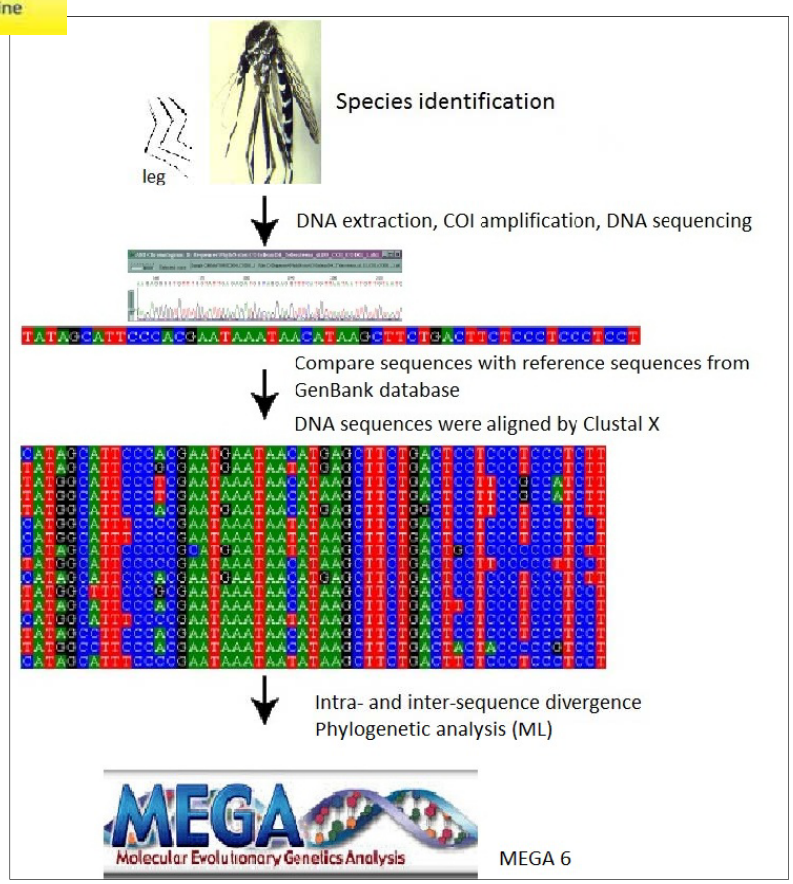

DNA barcoding analysis

-30 COI sequences were submitted to the GenBank database,

Ae. aegypti (KP843372- KP843381)

Ae. albopictus (KP843382-KP843391)

Ae. scutellaris (KP843392-KP843401)

-The DNA sequences were compared to sequences of *Ae. flavopictus*, *Ae. riversi* and *Ae. malayensis* and *Aedes* spp. available in GenBank.

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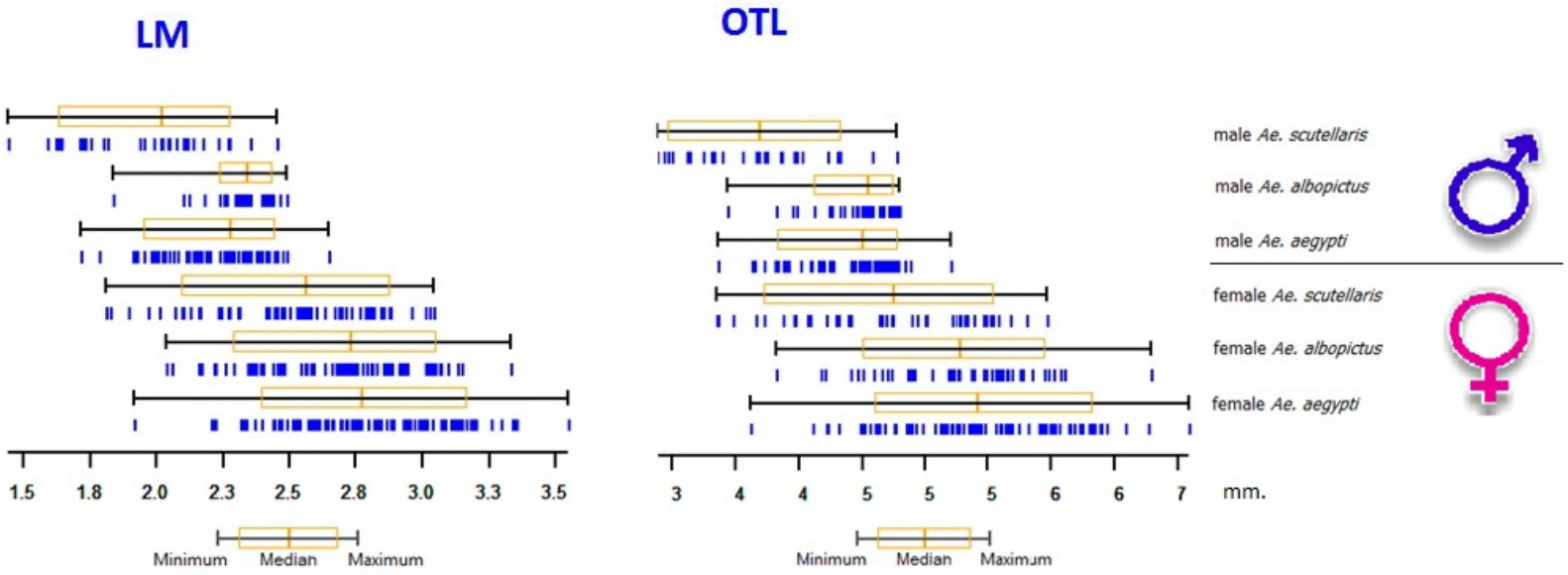
Geometric morphometrics

Wing size



Variation of wing sizes (mm.) of female and male *Ae. aegypti*, *Ae. albopictus* and *Ae. scutellaris* for landmark-based (LM) and outline-based (OTL) geometric morphometrics

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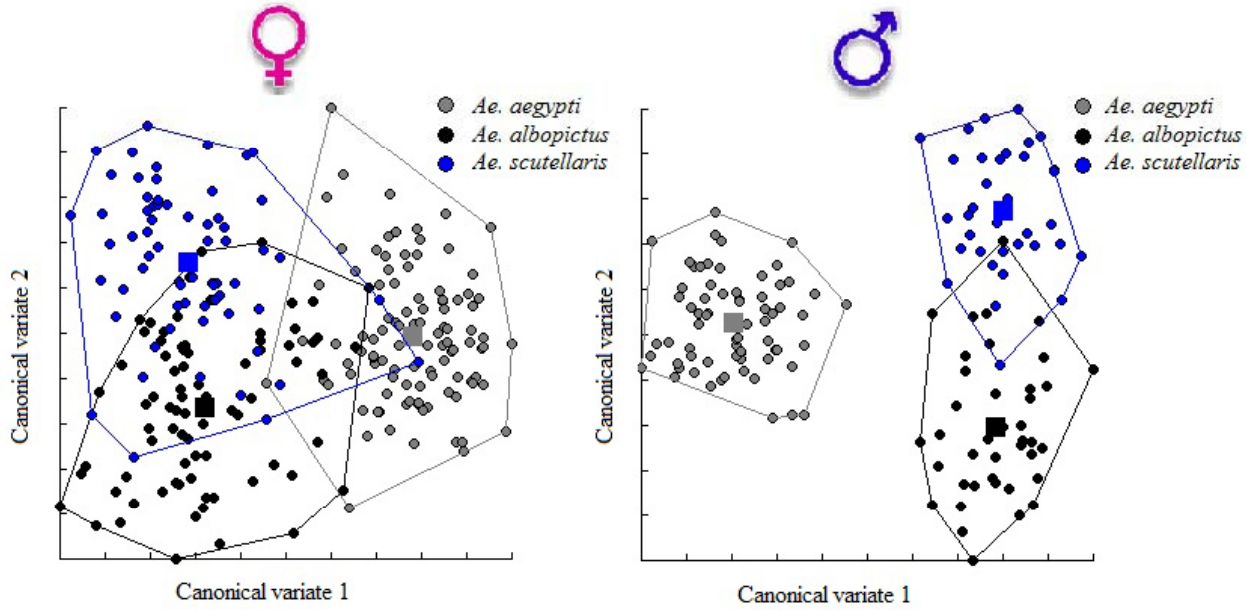
Landmark-Wing shape



Factor map of the two discriminant factors (or canonical vectors) derived from shape variables for **females** (left) and **males** (right) of *Ae. aegypti* (gray), *Ae. albopictus* (black) and *Ae. scutellaris* (blue).

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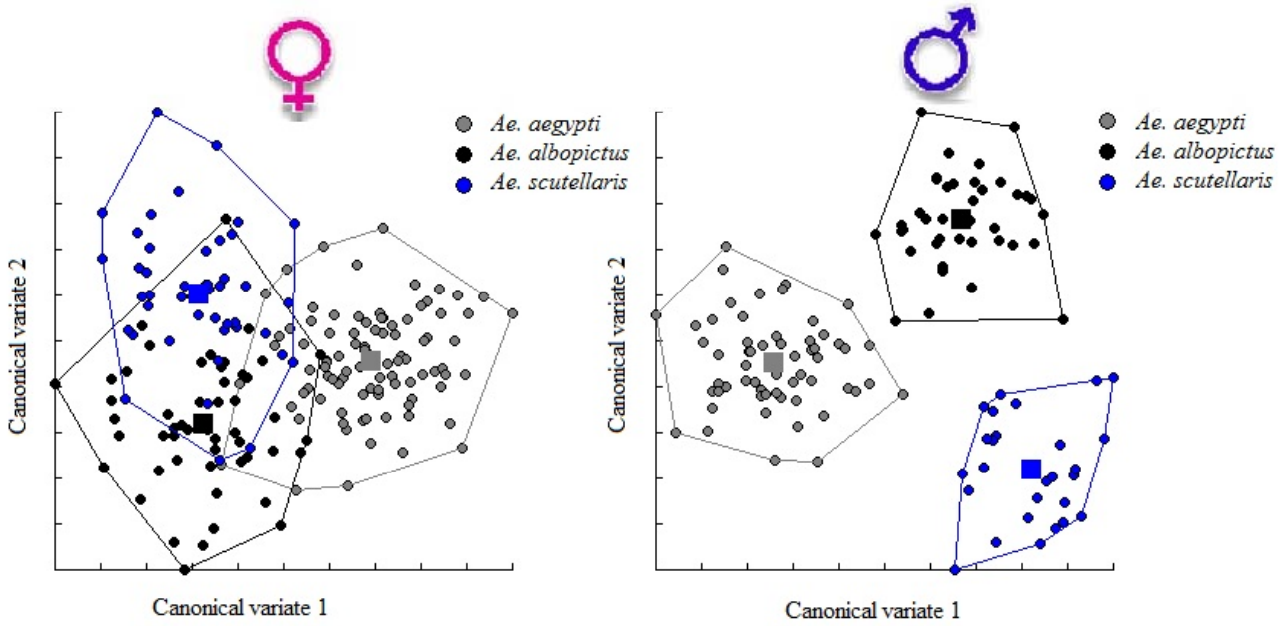
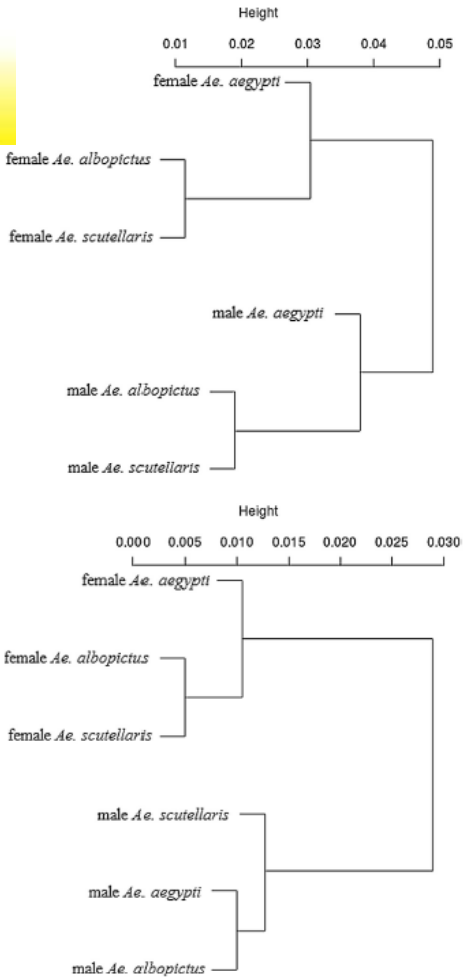
Outline-wing shape



OTL, Factor map of the two discriminant factors (or canonical vectors) derived from shape variables for female (left) and male (right) *Ae. aegypti* (grey), *Ae. albopictus* (black) and *Ae. scutellaris* (blue).



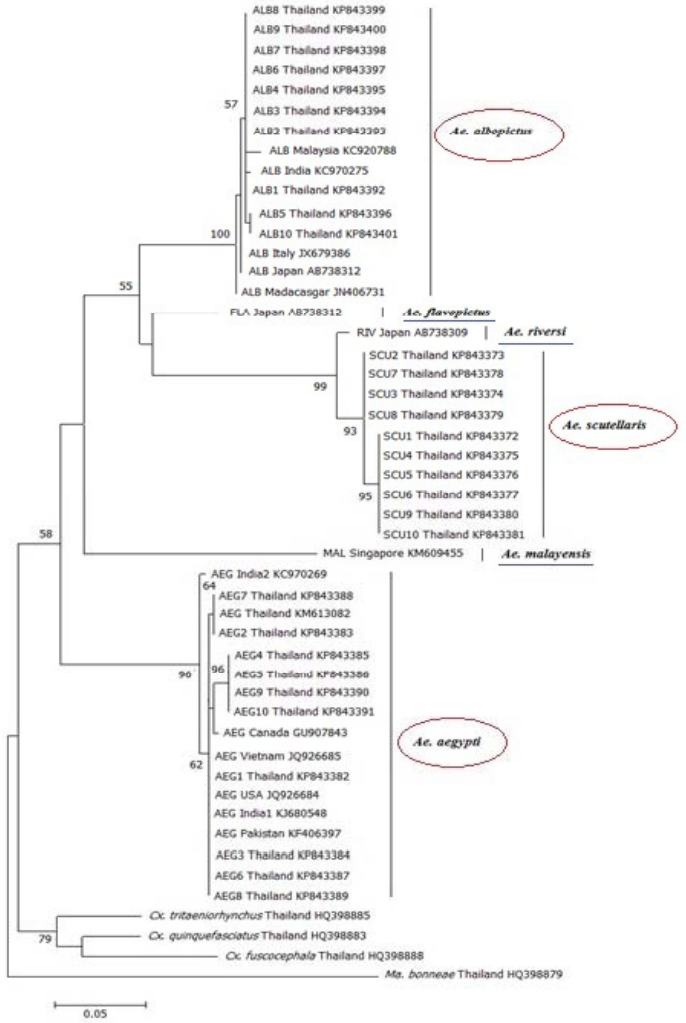


Fig. 7. UPGMA trees for shape based on landmark- (left) and outline-based (right) analyses of *Ae. aegypti*, *Ae. albopictus*, and *Ae. scutellaris*, including both sexes.

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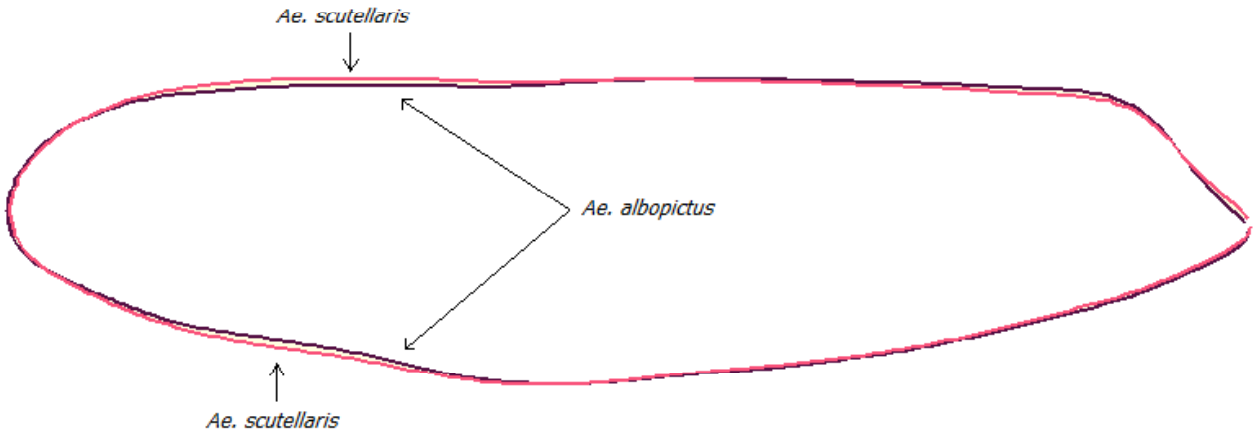

Superposition of the outlines of male *Ae. albopictus* (black) and *Ae. scutellaris* (red)

Table 5

Cross-validated classification for males and females of the three *Aedes* species based on landmark- and outline-based approaches.

Species	Landmark-based		Outline-based	
	female	male	female	male
Ae. aegypti	93 (97/103)	98 (68/69)	87 (81/93)	95 (57/60)
Ae. albopictus	65 (52/80)	82 (33/40)	61 (31/51)	90 (36/40)
Ae. scutellaris	73 (48/65)	91 (32/35)	71 (32/45)	96 (27/28)

Values represent percent of correctly assigned individuals, with corresponding details between brackets.

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Conclusion

- We showed that, for these 3 taxa, the outline-based approach was slightly more powerful than the landmark-based one.

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Institute of Parasitology, Biology Centre CAS
Folia Parasitologica 2016, 63: 037
doi: 10.14411/fp.2016.037

Folia Parasitologica
http://folia.paru.cas.cz

Research Article OPEN ACCESS

Landmark and outline-based geometric morphometrics analysis of three *Stomoxys* flies (Diptera: Muscidae)

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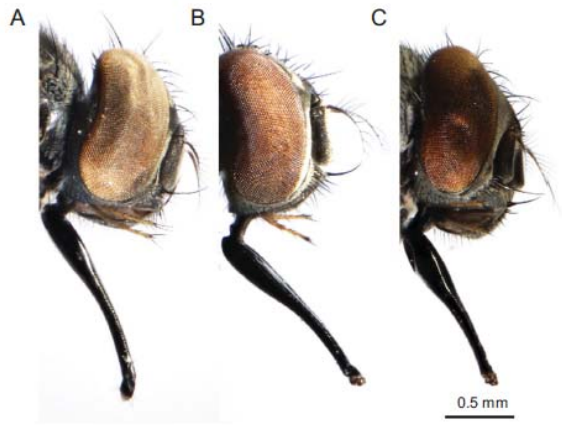

Fig. 1. Morphological characters of palpi used to separate *Stomoxys pullus* Austen, 1909 (**A**), *S. uruma* Shinonaga et Kano, 1966 (**B**) and *S. indicus* Picard, 1908 (**C**).

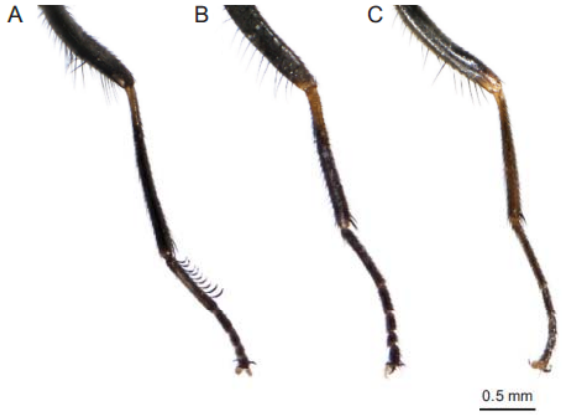

Fig. 2. Morphological characters of tibia and tarsus used to separate *Stomoxys pullus* Austen, 1909 (**A**), *S. uruma* Shinonaga et Kano, 1966 (**B**) and *S. indicus* Picard, 1908 (**C**).

Table 1. Number of flies of species *Stomoxys* Geoffroy, 1762 used for geometric morphometrics analysis.

Species (sex)	Number	
	Landmark-based method	Outline-based method
S. pullus Austen, 1909 (male)	35	34
S. pullus (female)	35	33
S. uruma Shinonaga et Kano, 1966 (male)	34	32
S. uruma (female)	34	31
S. indicus Picard, 1908 (male)	30	30
S. indicus (female)	30	30
Total	198	190

Table 2. Description of landmarks on wings of species of *Stomoxys* Geoffroy, 1762 (see Fig. 3).

Landmark	Description of the landmark
1	medial vein 3 and cubital vein 1
2	medial cross vein
3	midpoint branch of medial vein
4	radio-medial cross vein
5	distal end of medial vein 1 and 2
6	distal end of the radial vein 4 and 5
7	distal end of the radial vein 2 and 3
8	origin of radial vein 2 and 3
9	intersection of costa and radial vein 1
10	intersection of costa and subcosta

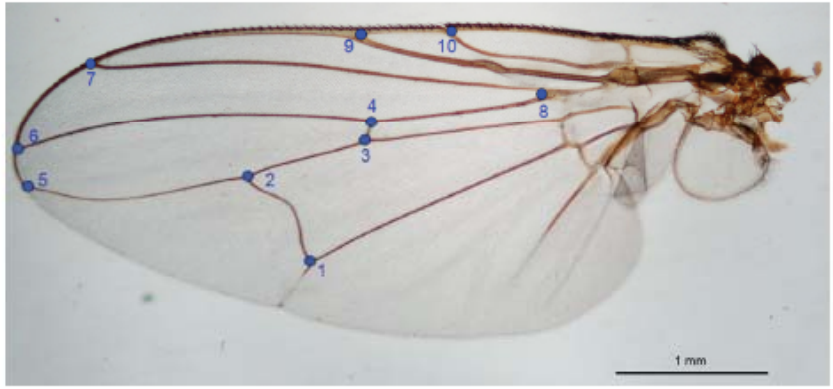

Fig. 3. Ten landmarks digitised on wings of species of *Stomoxys* Geoffroy, 1762 flies for landmark-based geometric morphometrics analysis (see Table 2 for description).

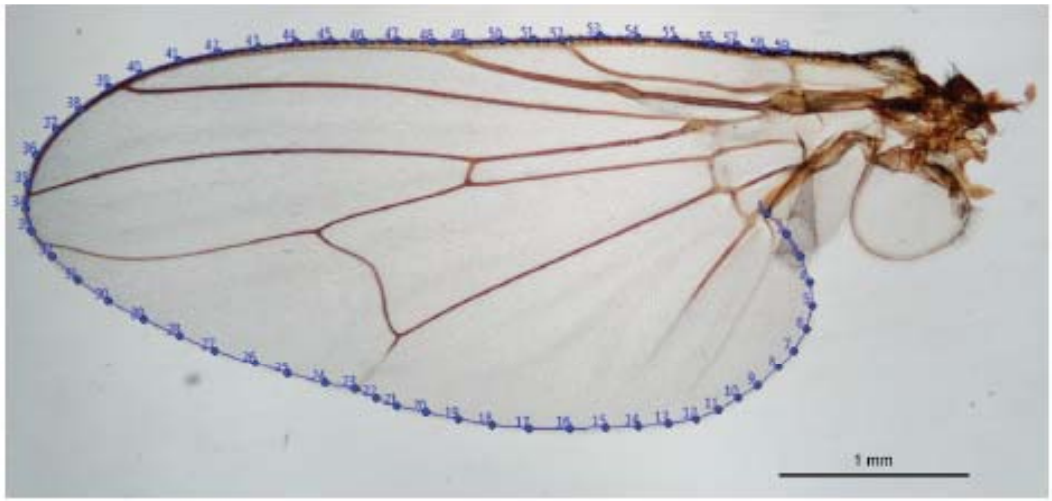

Fig. 4. Contour digitised on *Stomoxys* Geoffroy, 1762 flies wing for outline-based geometric morphometrics analysis. A short, artificial segment is computed by the digitising program to completely close the contour.

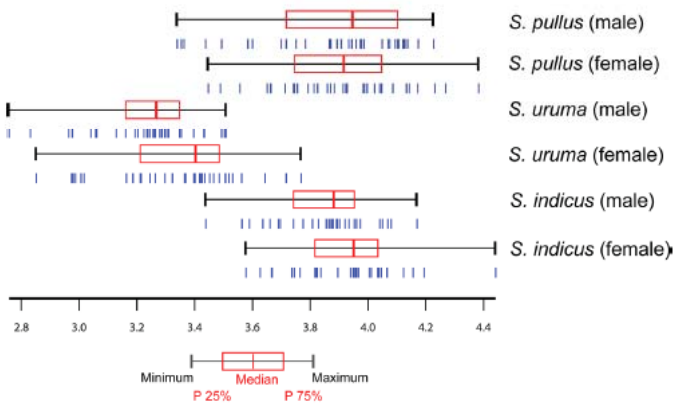

Fig. 5. Centroid size variation of the wings between species and sexes, shown as quartile boxes. Each box shows the group median separating the 25th and 75th quartiles. Vertical bars under the boxes represent the wing (units as mm).

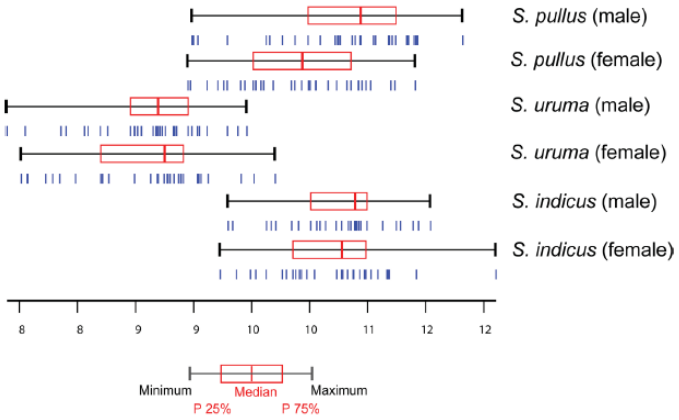

Fig. 8. Perimeter variation of the wings between species and sexes, shown as quartile boxes. Each box shows the group median separating the 25th and 75th quartiles. Vertical bars under the boxes represent the wing (units as mm).

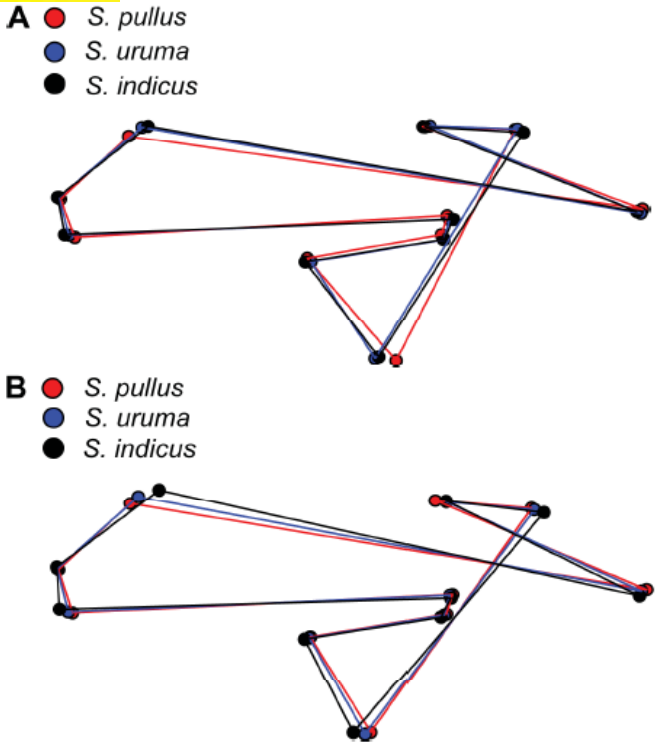

Fig. 6. Configurations of the ten anatomical landmarks connected by a straight line after procrustes superimposition of three species of *Stomoxys* Geoffroy, 1762, in males (**A**) and females (**B**).

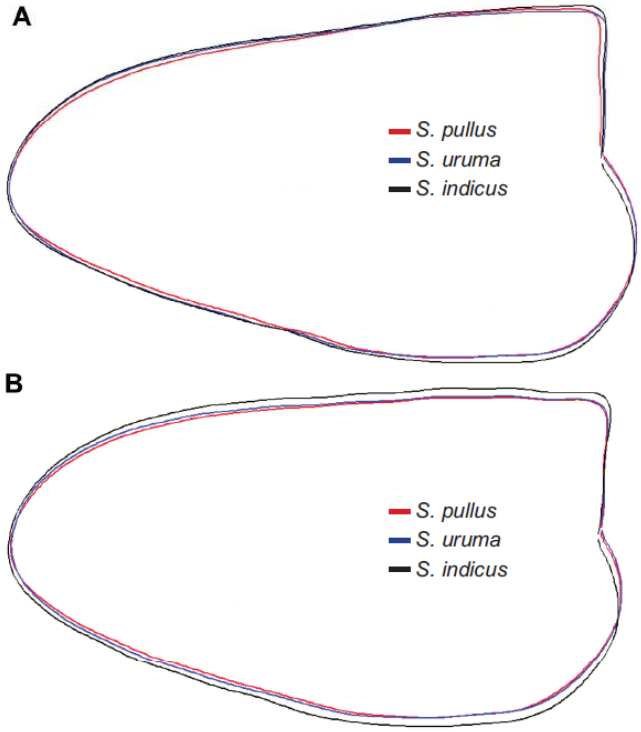

Fig. 9. Configurations of the outlines after Elliptic Fourier Analysis of *Stomoxys pullus* Austen, 1909, *S. uruma* Shinonaga et Kano, 1966 and *S. indicus* Picard, 1908, in males (**A**) and females (**B**). Areas outlined by different colours represent shape, not size.

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Landmark

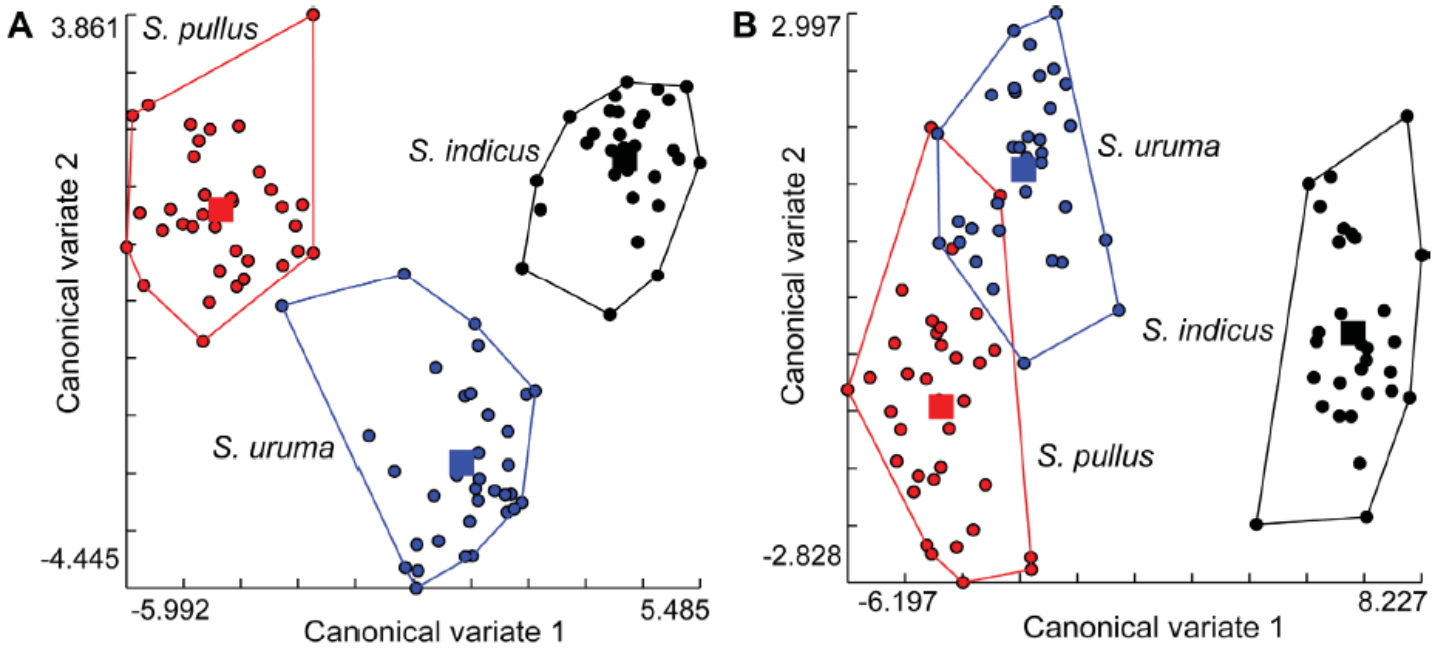

Fig. 7. Landmark-based discriminant analysis. Factor map of canonical variates resulting from comparison among the three species of *Stomoxys* Geoffroy, 1762, in males (**A**) and females (**B**).

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Outline

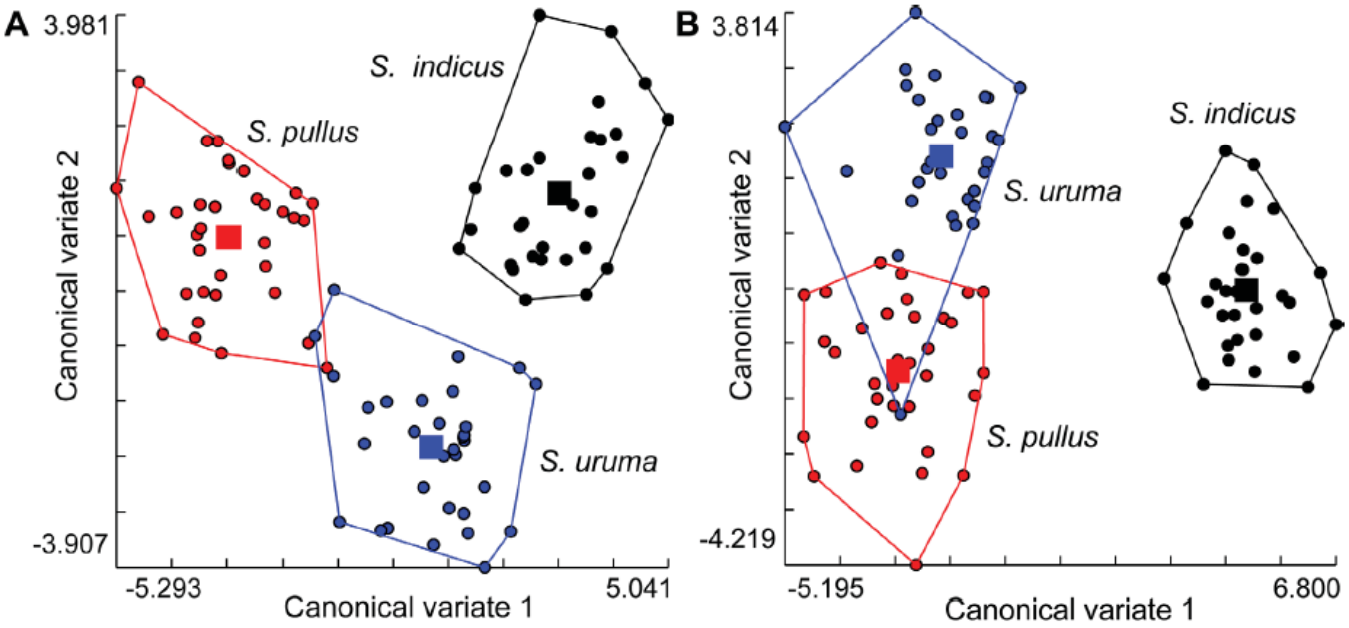

Fig. 10. Outline-based discriminant analysis. Factor map of canonical variates (i.e. discriminant factors) derived from the principal components of the Normalised Elliptic Fourier coefficients of three species of *Stomoxys* Geoffroy, 1762, in males (**A**) and females (**B**).

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In conclusion

Both landmark and outline-based geometric morphometrics of the wings proved to be a very useful tool to help in the morphological distinction of the vectors *S. pullus*, *S. uruma* and *S. indicus*.

Male was appropriate to species identification for these 3 *Stomoxys* species.

Ticks and Tick-borne Diseases xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

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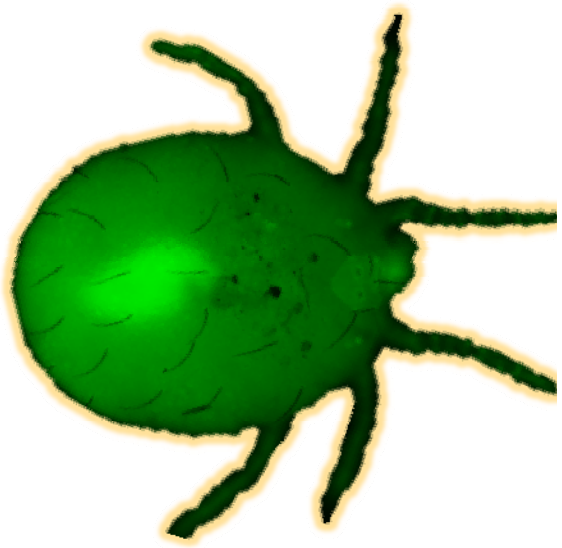

Original article

Geometric morphometrics of the scutum for differentiation of trombiculid mites within the genus *Walchia* (Acariformes: Prostigmata: Trombiculidae), a probable vector of scrub typhus

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ARTICLE INFO

Keywords:
Landmark-based geometric morphometrics
Outline-based geometric morphometrics
scutum
Walchia
Trombiculid mites
Chigger
Scrub typhus
Orientia tsutsugamushi

ABSTRACT

The vectors of scrub typhus are the larval stage of trombiculid mites, termed "chiggers". These vectors are very small – the larvae are approximately 0.2 mm in size - and therefore their morphological identification is difficult. Trombiculid mites are widely distributed across Asia and they can be identified at the genus level by the shape, size and setae/sensilla distribution of their dorsal chitin plate (scutum = shield), while morphological identification at the species level requires more mite characteristics.

We recently developed a methodology to ascertain paired matched genotype and morphotype of individual chiggers, based on autofluorescence and brightfield microscopy with subsequent molecular identification using the *COI* gene (approximately 640bp length). However, based on 20 chigger specimens characterised by paired genotypic and morphological data consisting of the four species [*Walchia ewingi* with 2 subspecies]: *Walchia*

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Chigger mites in rodent’s ear

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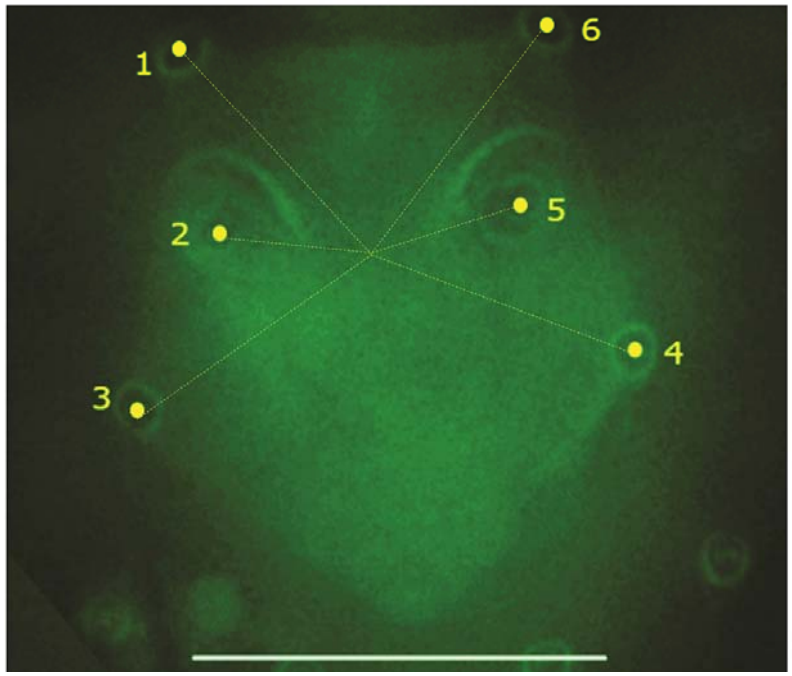
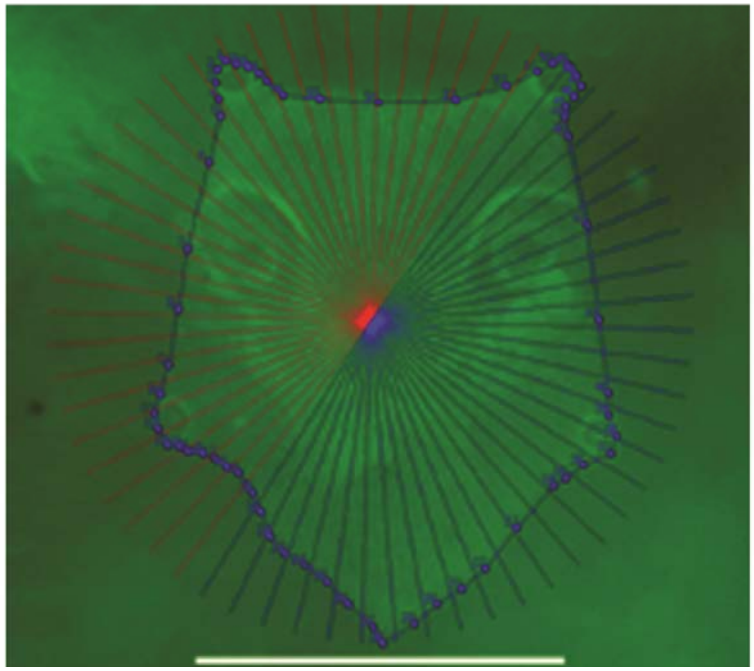
Scutum of chigger mites

A



landmark

B



outline

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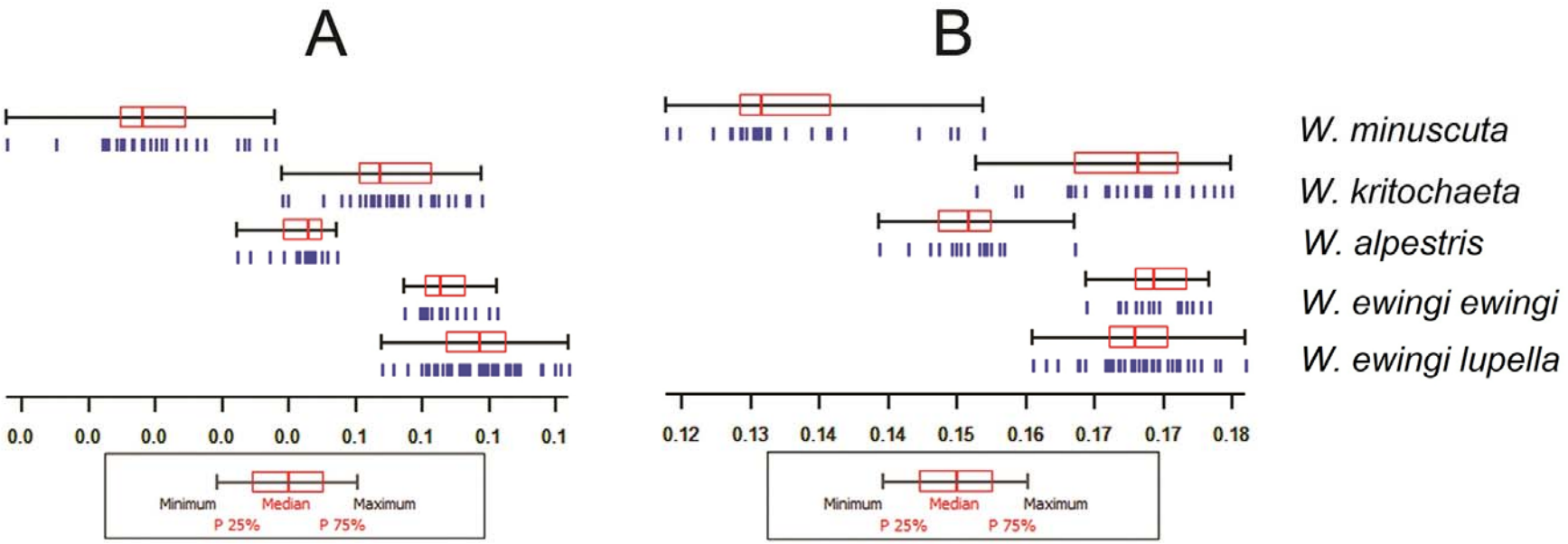

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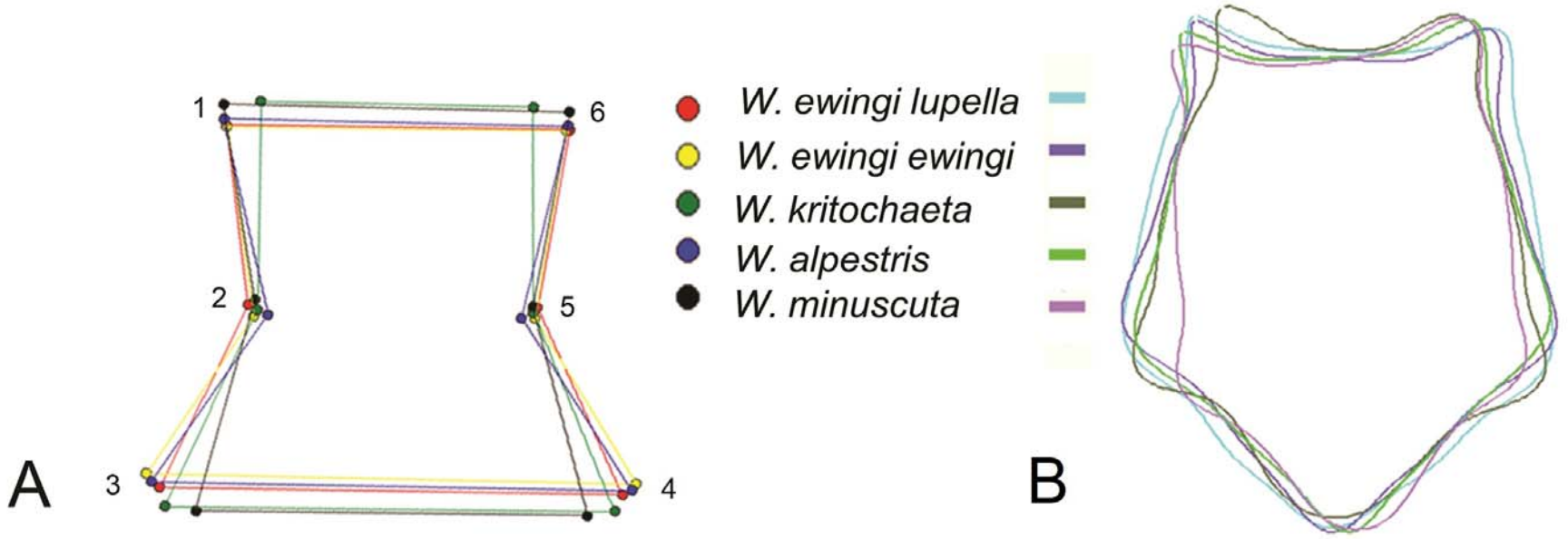

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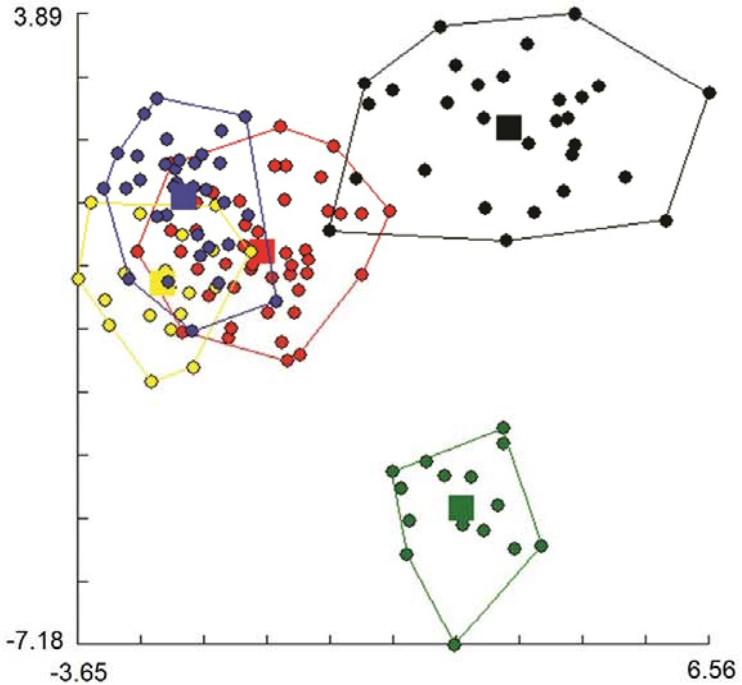

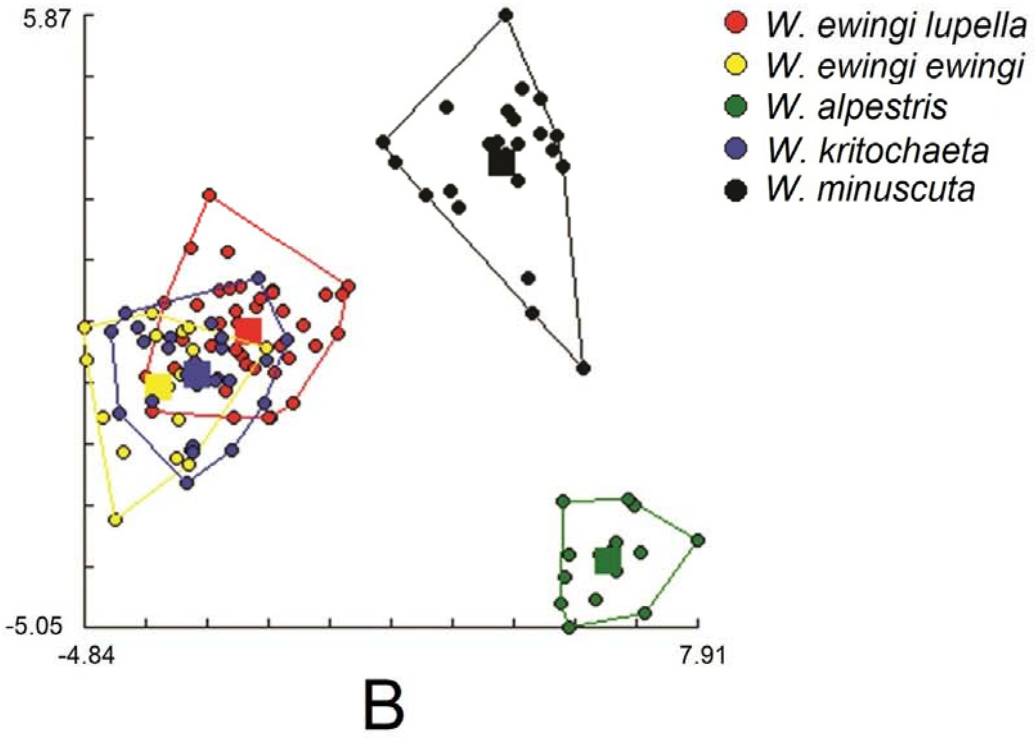

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Table 3. Cross-validated classification for scouting of 5 *Walchia* species based on a landmark –based and outline-based GM analyses.

Species	classification score (N)	
	landmark-based GM	outline-based GM
W. ewingi lupella	88% (40 / 45)	85% (35/41)
W. ewingi ewingi	88% (16 / 18)	43% (7/16)
W. alpestris	100% (15 / 15)	100% (15/15)
W. kritochaeta	84% (28 / 33)	69% (18/26)
W. minuscuta	93% (27 / 29)	91% (21/23)

Values represent percent of correctly assigned individuals with corresponding details

Cross-validated classification scores were different depending on species and digitizing techniques and landmark-based GM showed better scores than outline-based GM.

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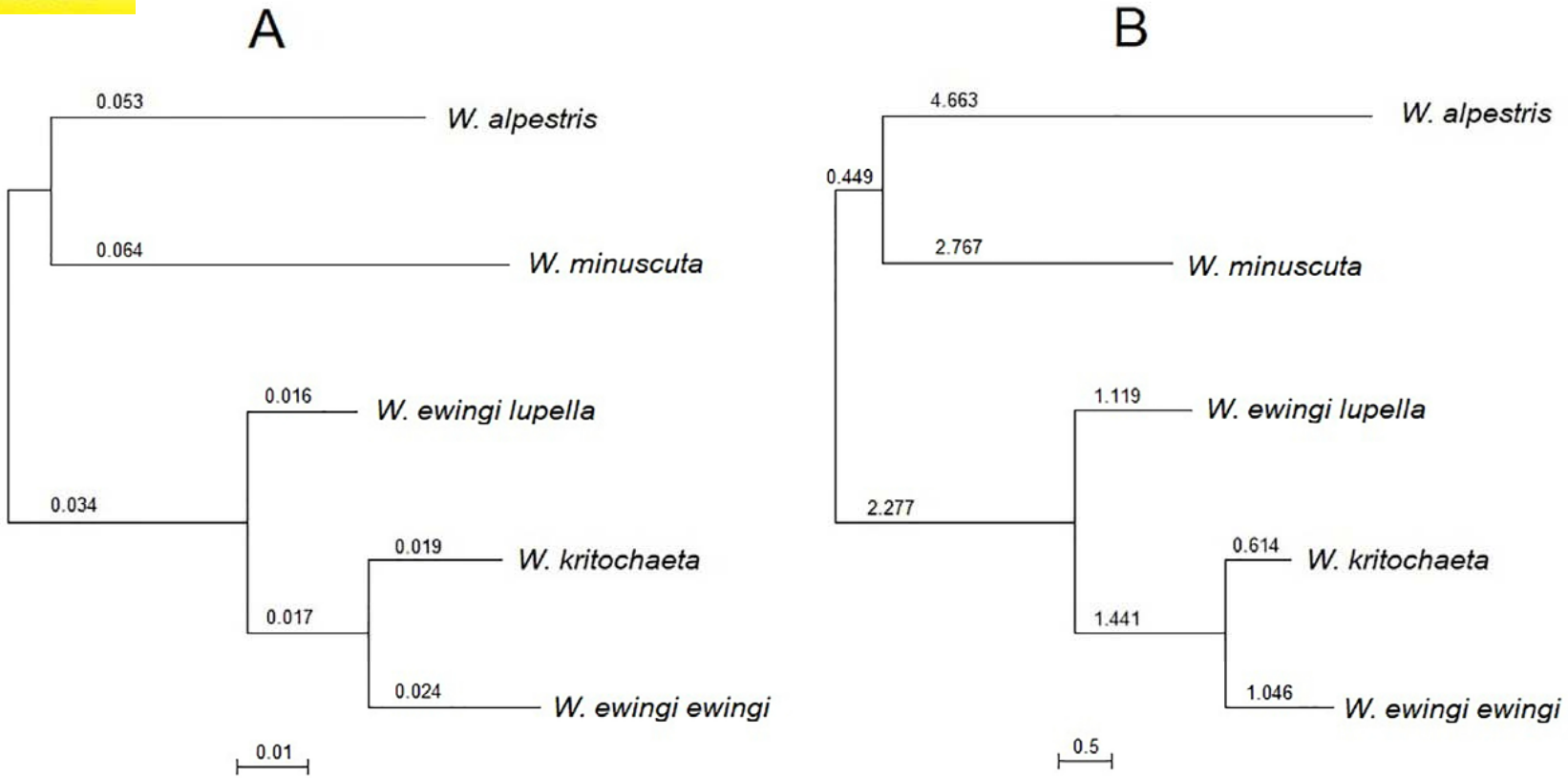

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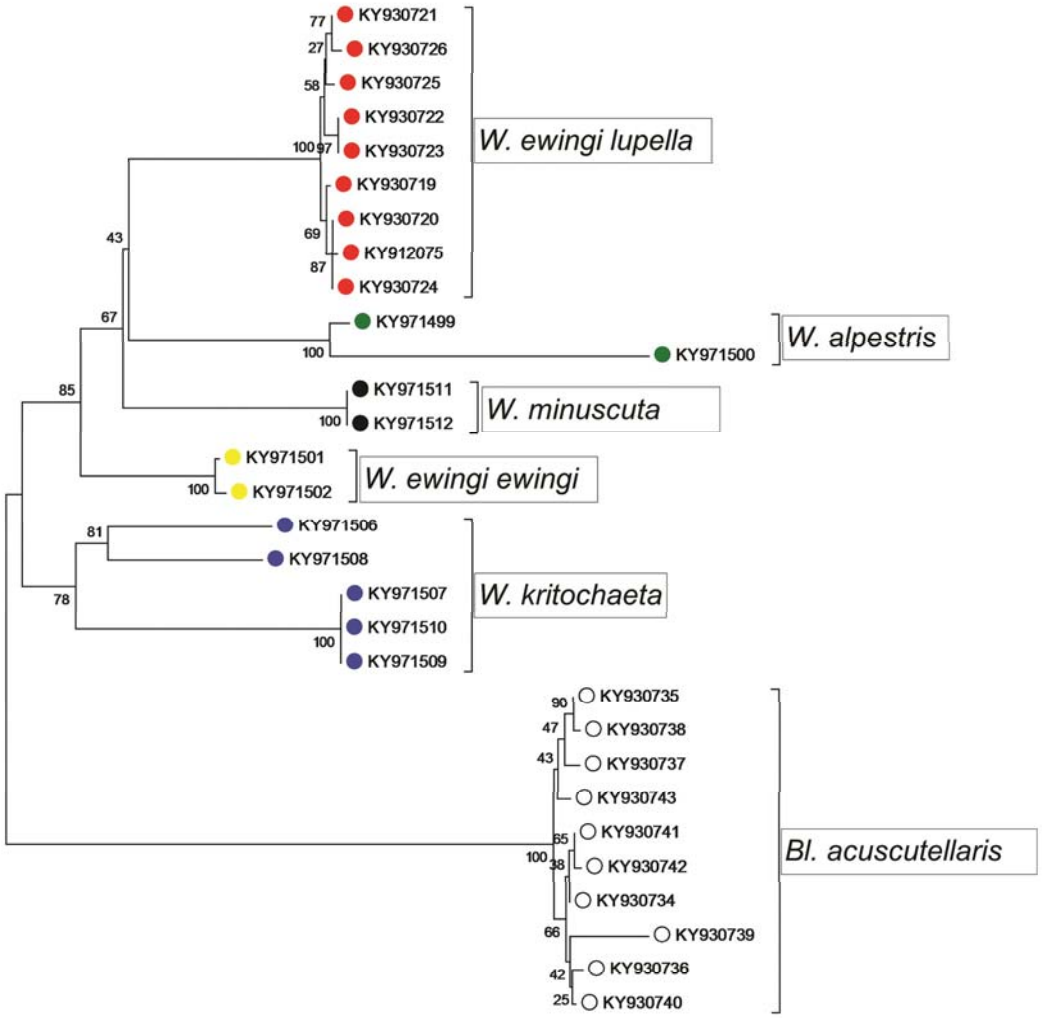

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We conclude that the morphologically closely-related trombiculid mite species can be further differentiated by their scutum features alone, using GM approaches.

This technique is a promising tool for the much-needed characterization studies of chiggers and needs evaluation using matched morphometric and genotyping data for other genera of trombiculids.

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Anopheles maculatus complex in Thailand: geometric morphometrics approach towards discrimination of sibling species

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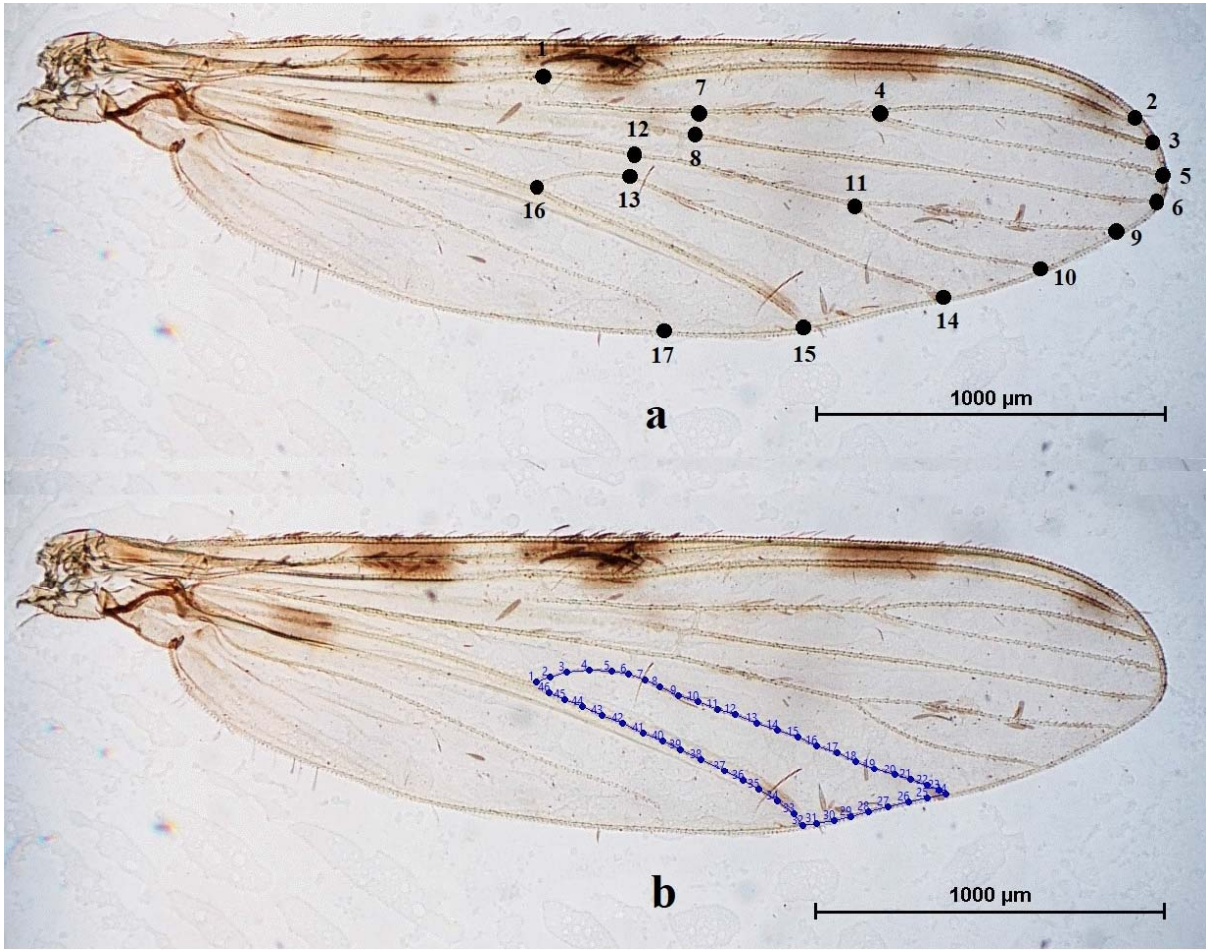

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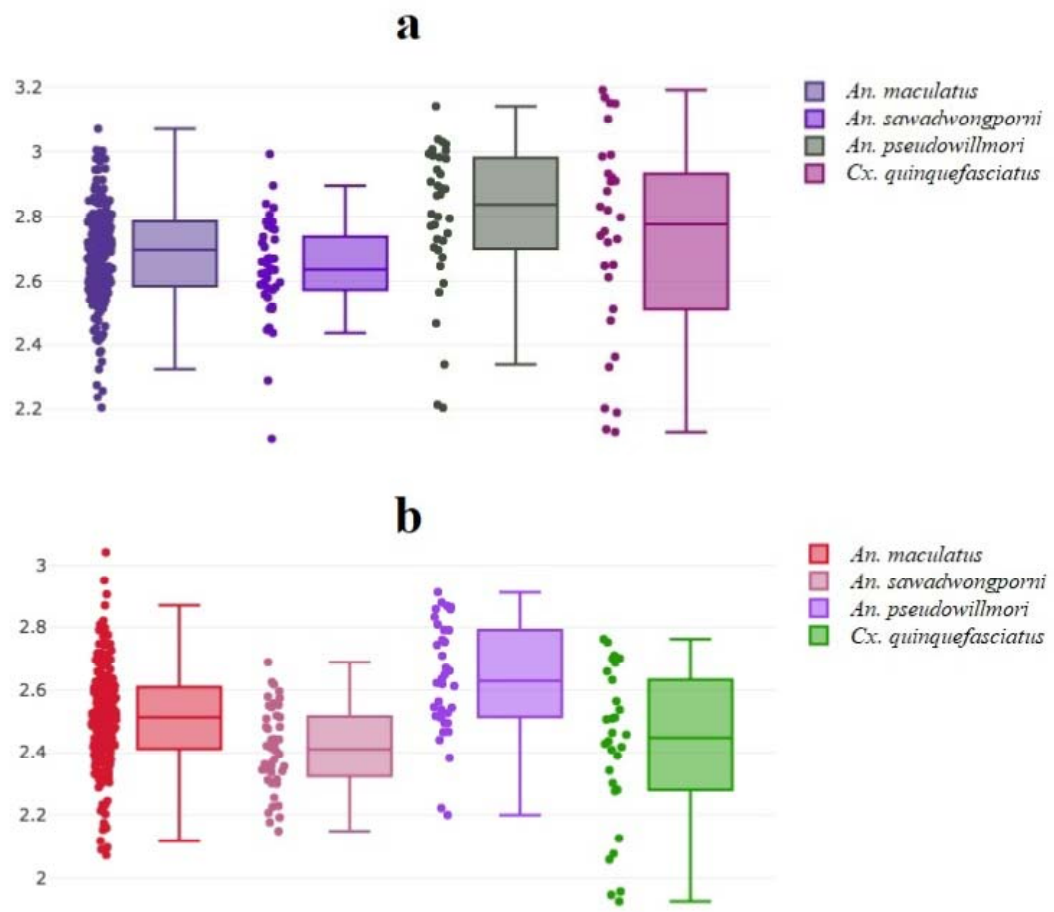

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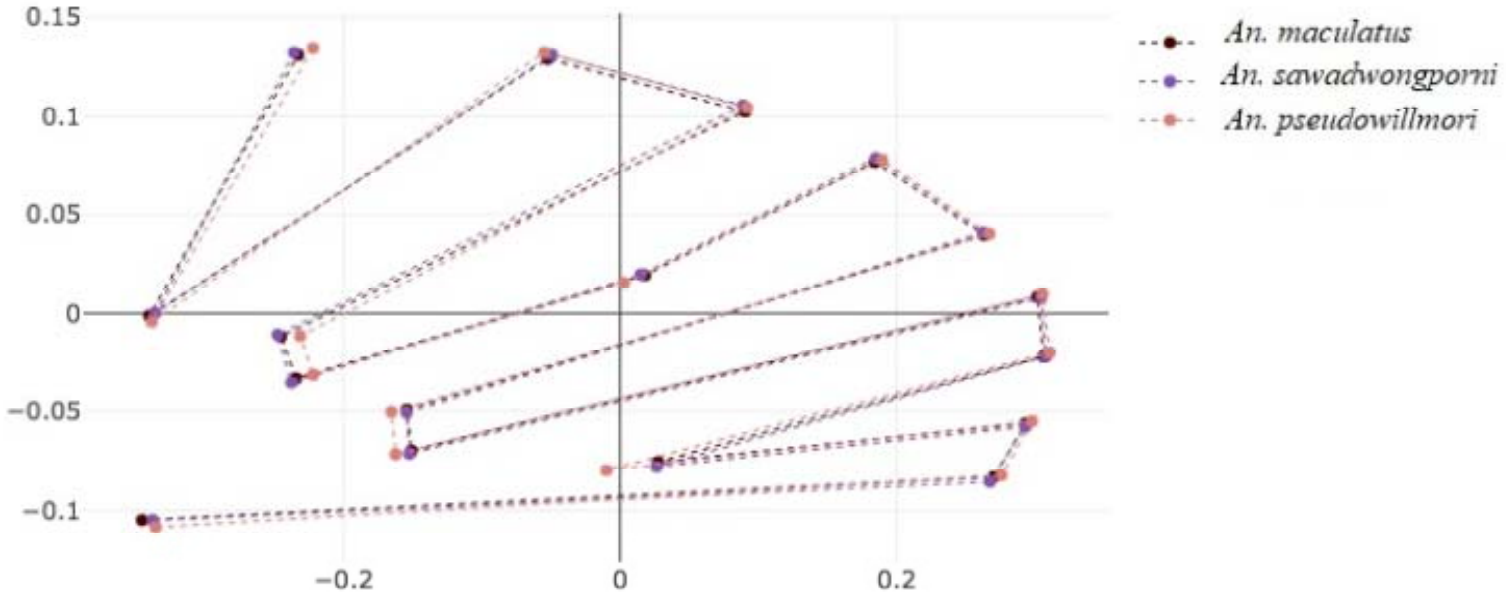

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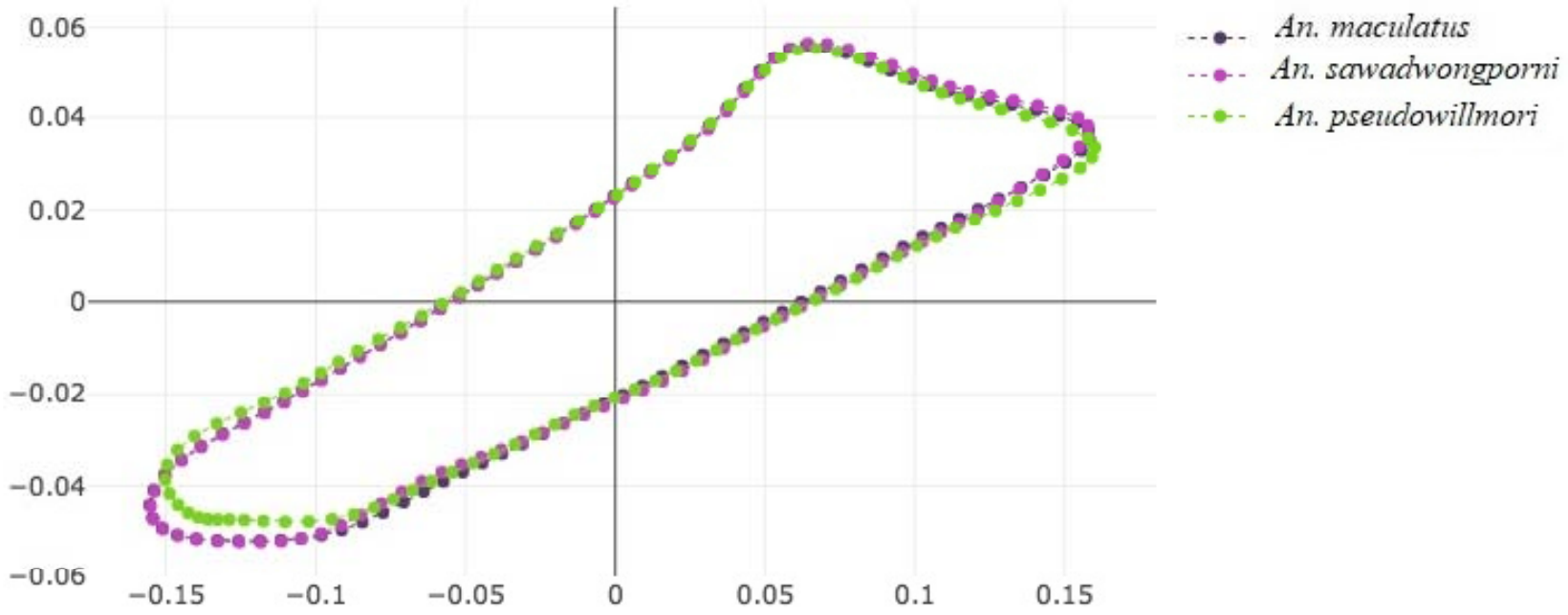

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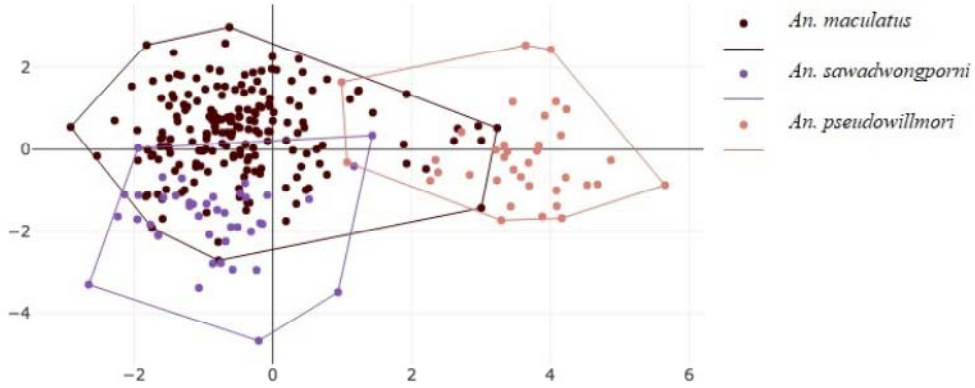
a



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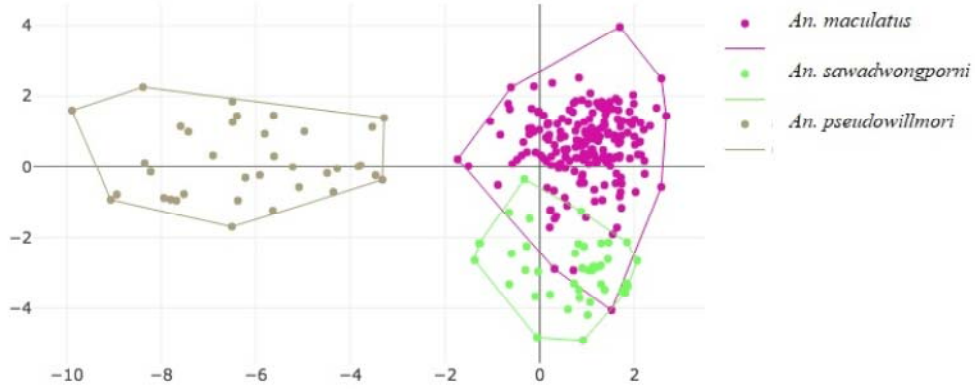

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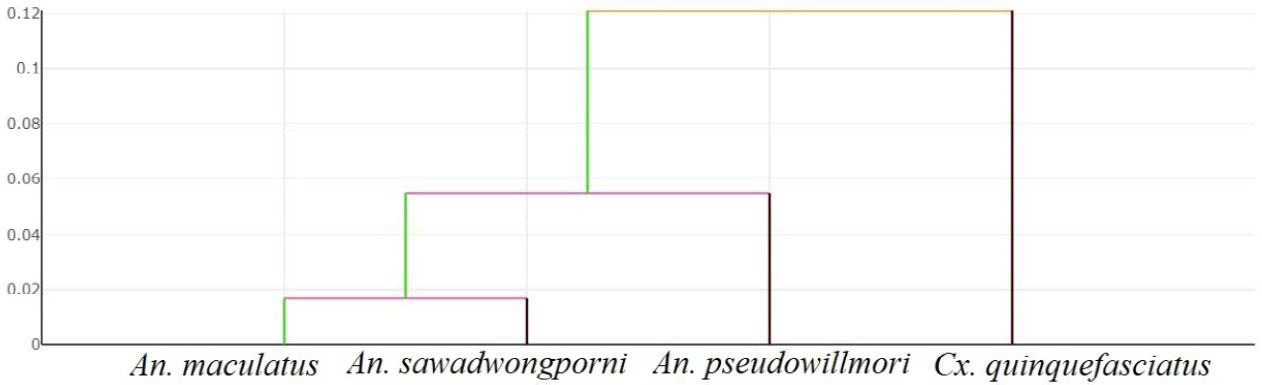

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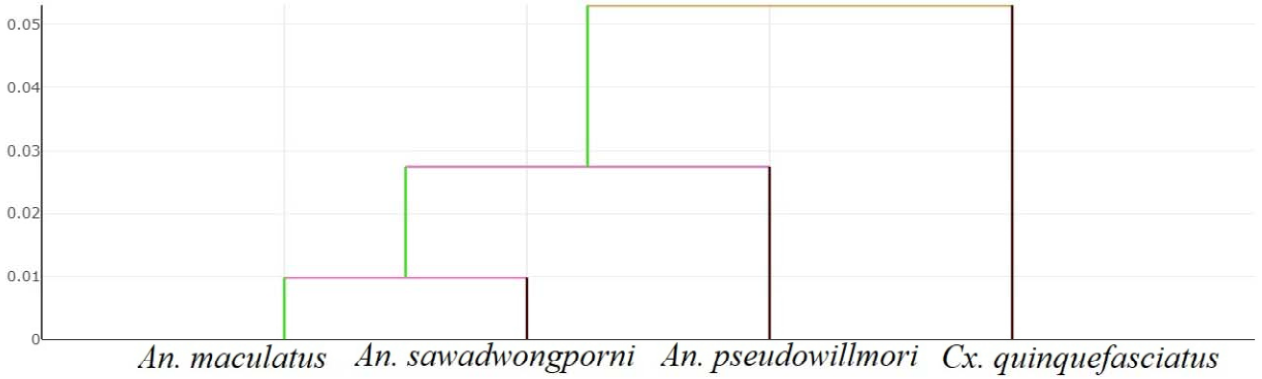

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Table 3

Validated reclassification scores of *Anopheles* species according to landmark-based and outline-based approaches, using *Cx. quinquefasciatus* as a putative outgroup.

Species	Percentage of reclassification scores (assigned/observed)	
	Landmark-based method	Outline-based method
An. maculatus	77% (147/191)	93% (186/200)
An. sawadwongporni	76% (32/42)	95% (41/43)
An. pseudowillmori	94% (34/36)	100% (36/36)
Cx. quinquefasciatus	100% (30/30)	100% (30/30)
Total	81% (243/299)	95% (293/309)

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Conclusions

-Outline-based method was the most appropriate technique for distinguishing between *An.maculatus* and *An. sawadwongporni*.

- In both approaches, *An. pseudowillmori* was separated from the other species with high confidence.

-Geometric morphometrics may provide an alternative and useful complement for discriminating members of the *An. maculatus* complex.

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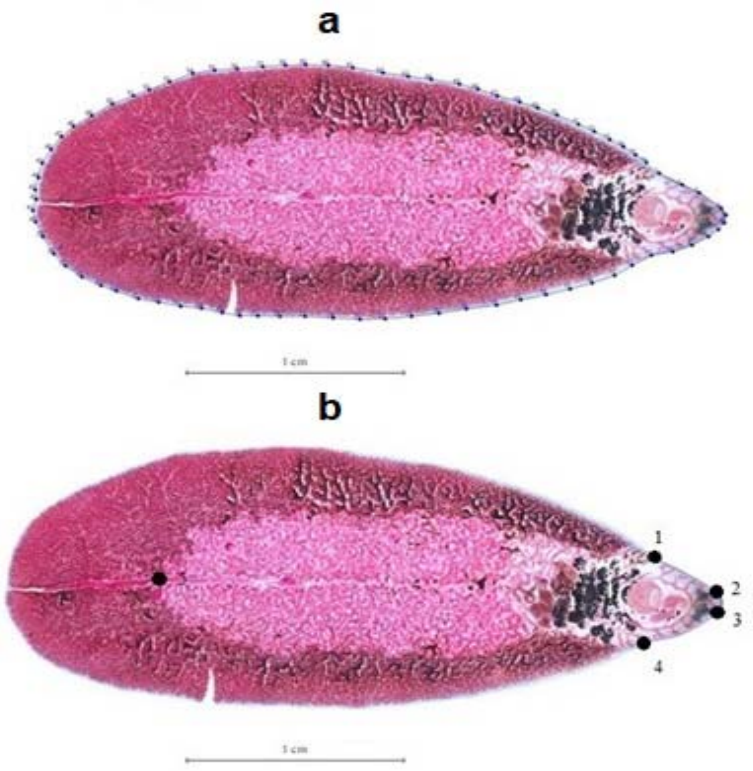

Sumruayphol S, Siribat P, Dujardin JP, Dujardin S, Komalamisra K, Thaenkham U*. *Fasciola gigantica*, *F. hepatica* and *Fasciola* intermediate form: novel approaches to help morphological species discrimination.

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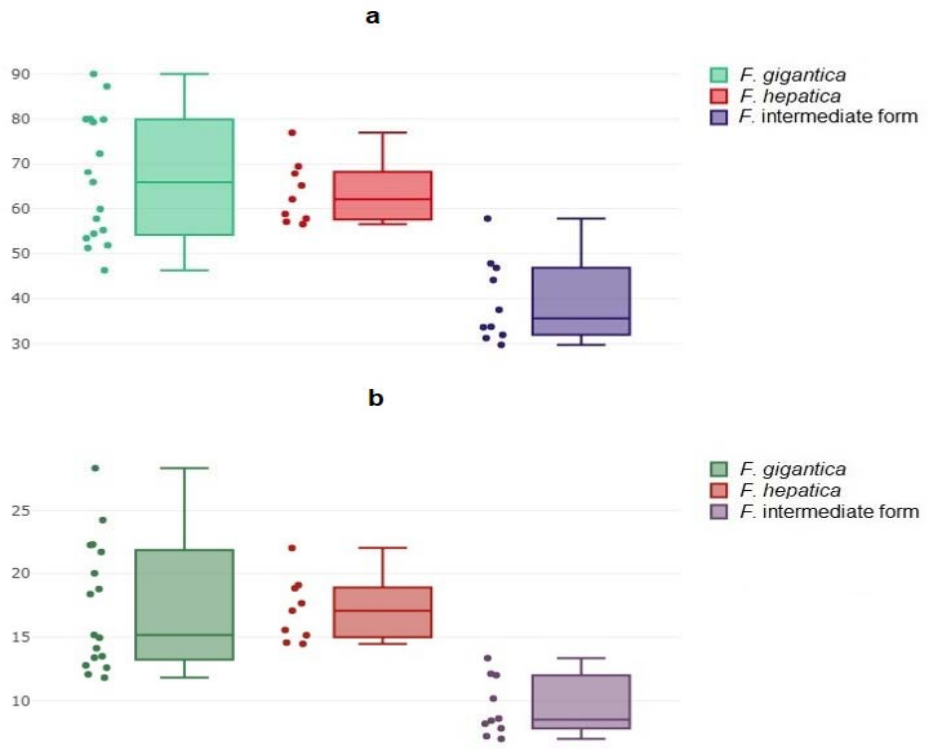

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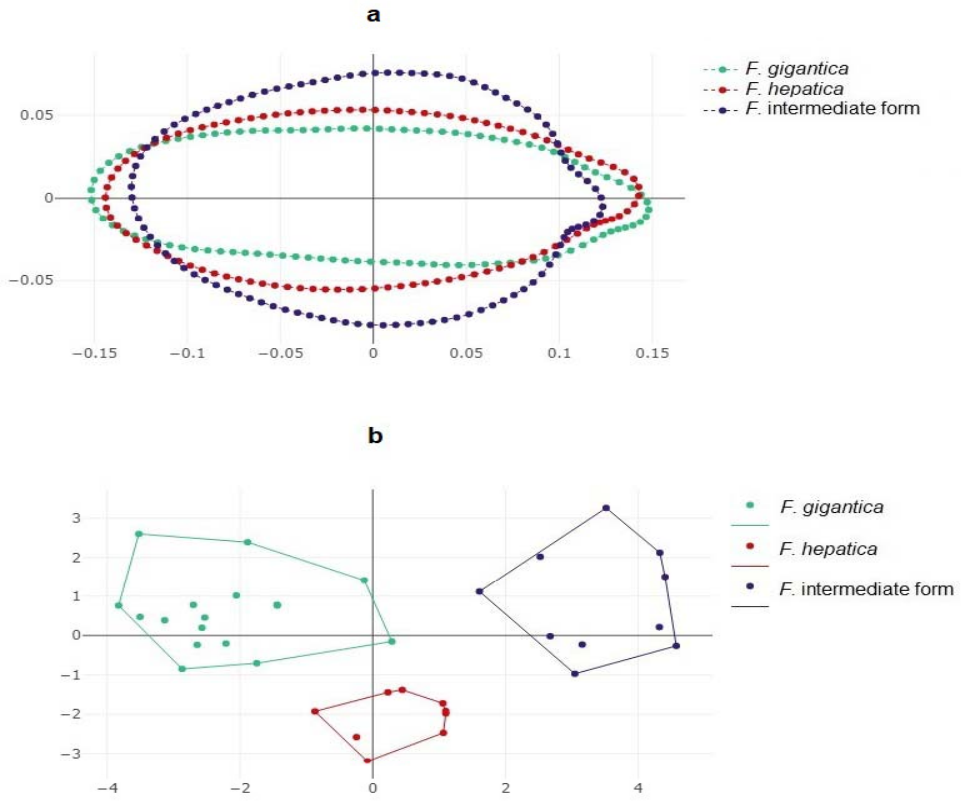

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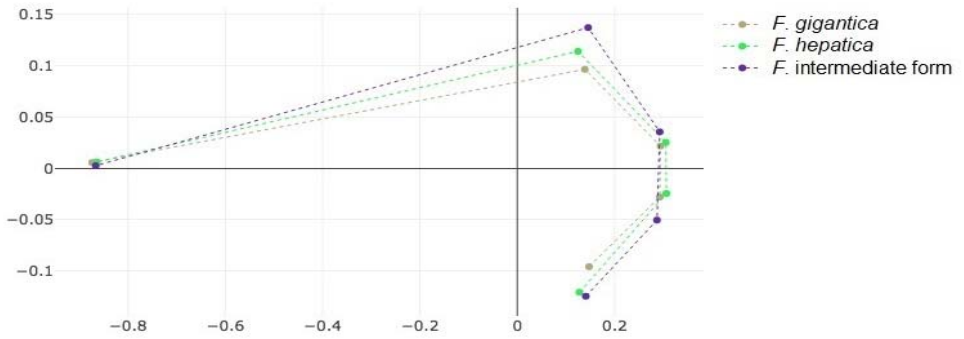

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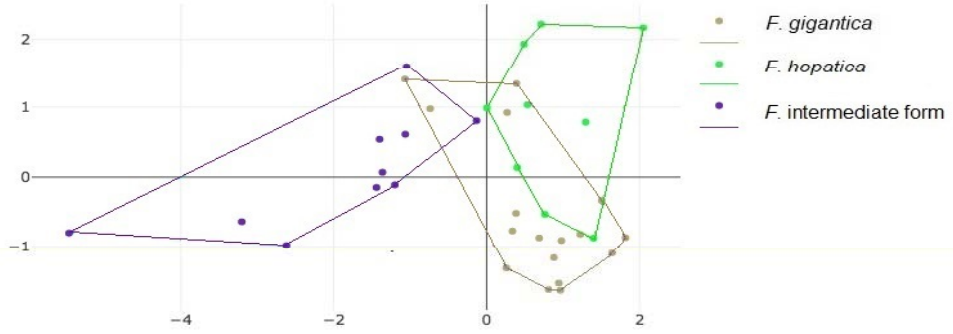

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Conclusions

The landmark-based approach produced unsatisfactory results probably because of the low number (5) of available, reliable landmarks.

Using the outline-based approach, our study could provide a first description of the shape changes between species, highlighting the more globulous shape of the “intermediate forms.

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Conclusions

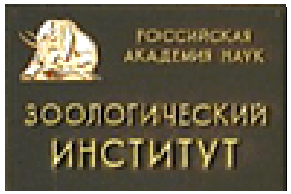
Geometric morphometrics approach provides a powerful tool to identify species .

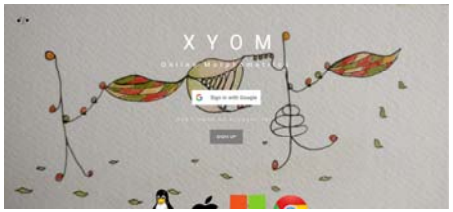
It represents a faster, low-cost but still informative approach.

It could become a routine complement to morphological studies for future epidemiological investigation of vectors in Thailand.

Acknowledgements

Prof. Jean-Pierre Dujardin
Assoc. Prof. Chamnarn Apiwathnasorn
Dr. Rawadee Kumlert
Prof. Danial H Paris
Prof. Serge Morand
Prof. Alexandr A Stekonikov
Mr. Tanawat Chaiphongpachara
Asst. Prof. Jiraporn Ruangsittichai
Asst. Prof. Patchara Sriwichai
Mr. Yudthana Samung
Asst. Prof. Ronald Enrique Morales Vargas
Mr. Sebastien Dujardin
Dr. Sungsit Sunvornyothin
Assoc. Prof. Urusa Thaenkham
Asst. Prof. Tanasak Changbunjong
Dr. Jetsumon Sattabongkot
Asst. Prof. Piengchan Sonthayanon





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Thank you for your attention