

## OPEN ACCESS



The Journal of Threatened Taxa is dedicated to building evidence for conservation globally by publishing peer-reviewed articles online every month at a reasonably rapid rate at [www.threatenedtaxa.org](http://www.threatenedtaxa.org). All articles published in JoTT are registered under [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/) unless otherwise mentioned. JoTT allows unrestricted use of articles in any medium, reproduction, and distribution by providing adequate credit to the authors and the source of publication.



## Journal of Threatened Taxa

Building evidence for conservation globally

[www.threatenedtaxa.org](http://www.threatenedtaxa.org)

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

### COMMUNICATION

#### PUPAL SHAPE AND SIZE DIMORPHISM IN *Aedes albopictus* (Skuse, 1894) (Diptera: Culicidae)

Elvira Sánchez, Daniel Castillo & Jonathan Liria

26 June 2017 | Vol. 9 | No. 6 | Pp. 10314–10319  
10.11609/jott.3059.9.6.10314-10319



For Focus, Scope, Aims, Policies and Guidelines visit [http://threatenedtaxa.org/About\\_JoTT](http://threatenedtaxa.org/About_JoTT)  
For Article Submission Guidelines visit [http://threatenedtaxa.org/Submission\\_Guidelines](http://threatenedtaxa.org/Submission_Guidelines)  
For Policies against Scientific Misconduct visit [http://threatenedtaxa.org/JoTT\\_Policy\\_against\\_Scientific\\_Misconduct](http://threatenedtaxa.org/JoTT_Policy_against_Scientific_Misconduct)  
For reprints contact <[info@threatenedtaxa.org](mailto:info@threatenedtaxa.org)>

Partner



Publisher/Host





## PUPAL SHAPE AND SIZE DIMORPHISM IN *Aedes albopictus* (SKUSE, 1894) (DIPTERA: CULICIDAE)

Elvira Sánchez<sup>1,2</sup>, Daniel Castillo<sup>3</sup> & Jonathan Liria<sup>1,4</sup>

ISSN 0974-7907 (Online)  
ISSN 0974-7893 (Print)

### OPEN ACCESS



<sup>1</sup>Centro de Estudios en Zoología Aplicada, Laboratorio Museo de Zoología, Departamento de Biología, Facultad Experimental de Ciencias y Tecnología, Universidad de Carabobo, Valencia 2005, Carabobo, Venezuela

<sup>2</sup>Departamento de Biología de Organismos, División de Ciencias Biológicas, Universidad Simón Bolívar, Miranda, Valle de Sartenejas 89000, Venezuela

<sup>3</sup>Departamento de Biología, Facultad Experimental de Ciencias y Tecnología, Universidad de Carabobo, Valencia 2005, Carabobo, Venezuela

<sup>4</sup>Universidad Regional Amazónica IKIAM, 7 Km vía Muyuna 150150, Napo, Ecuador

<sup>1</sup>easanchez@uc.edu.ve, <sup>3</sup>alex\_15\_1@hotmail.com, <sup>4</sup>jonathan.liria@ikiam.edu.ec (corresponding author)

**Abstract:** *Aedes albopictus* (Skuse, 1894) is a culicid mosquito associated with the transmission of pathogens causative for dengue, yellow fever, chikungunya, Mayaro and other diseases. Several recent studies have proposed that demographic surveys of dengue vector pupae are more useful than traditional larval indices for estimating populations. Geometric morphometrics is a tool for describing phenotypic variation that has been validated for characterizing sexual dimorphism. We undertook to apply this method to describe sexual and morphological dimorphism in *A. albopictus* pupae. Two-dimensional co-ordinates were digitalized from 60 specimens in two stages using 10 landmarks in pupae and 14 in wings. Configuration matrices were aligned by generalized procrustes analysis to extract matrix configurations and centroid size (CS). A discriminant analysis (DA) was used to test group (female or male) membership significance, and non-parametric ANOVA was used for CS differences. We found significant differences (Kruskal-Wallis  $P < 0.01$ ) among pupal cephalothorax CS and adult wings; female pupae and adults were larger than males. The DA for cephalothorax and wing specimens showed significant differences (Hotelling  $P < 0.0001$ ) between females and males. Through cross-validation, females and males were correctly classified with greater than 90% accuracy using the conformation characteristics described. Our study is the first description of phenotypic variation of pupal shape and size in *A. albopictus* laboratory colonies, and the results can be used as an additional tool in dengue entomological survey programs. More studies are necessary to confirm the variation between natural and laboratory populations.

**Keywords:** Aedini, Geometric morphometrics, immature, *Stegomyia*.

**DOI:** <http://doi.org/10.11609/jott.3059.9.6.10314-10319> | **ZooBank:** urn:lsid:zoobank.org:pub:499DF0AB-C6BF-4122-8D6E-B31BA9199331

**Editor:** David M. Claborn, Missouri State University, Springfield, USA.

**Date of publication:** 26 June 2017 (online & print)

**Manuscript details:** Ms # 3059 | Received 21 September 2016 | Final received 30 May 2017 | Finally accepted 09 June 2017

**Citation:** Sánchez, E., D. Castillo & J. Liria (2017). Pupal shape and size dimorphism in *Aedes albopictus* (Skuse, 1894) (Diptera: Culicidae). *Journal of Threatened Taxa* 9(6): 10314–10319; <http://doi.org/10.11609/jott.3059.9.6.10314-10319>

**Copyright:** © Sánchez et al. 2017. Creative Commons Attribution 4.0 International License. JoTT allows unrestricted use of this article in any medium, reproduction and distribution by providing adequate credit to the authors and the source of publication.

**Funding:** Facultad Experimental de Ciencias y Tecnología (FACYT), Laboratorio Museo de Zoología, Universidad Regional Amazónica IKIAM.

**Competing interests:** The authors declare no competing interests.

**Author Details:** Mrs. Elvira Sánchez is a graduate of the BS Biology program of the Biology Department, University of Carabobo. Actually is a Lecturer-Researcher of Biology Department, University of Carabobo. Her research interest is on mosquitoes ecology and taxonomy. Mr. Daniel Castillo is a graduate of the BS Biology program of the Biology Department, University of Carabobo. Dr. Jonathan Liria is a Lecturer-Researcher at the Ikiam University. His research interests are medical entomology, geometric morphometrics, and Culicidae systematics and biogeography.

**Author Contribution:** ES conducted the geometric morphometrics analysis, and wrote the first manuscript draft. She also served as Daniel Castillo's thesis adviser. DC conducted lab experiments (immature rearing) and landmarks data acquisition as part of his undergraduate thesis. JL conducted additional geometric morphometrics analysis and statistical tests, also wrote the final manuscript (including figures).

For **Spanish Abstract** see end of this article.

**Acknowledgements:** We thank Franger García (Departamento de Biología, Universidad de Carabobo), the subject editor and anonymous reviewers for suggestions to improve the manuscript. This research is part of the collaborative investigation "Geometric morphometrics in insects of medical and forensic importance" being implemented by the Universidad de Carabobo and the Universidad Regional Amazónica Ikiam.



## INTRODUCTION

*Aedes (Stegomyia) albopictus* (Skuse, 1894) or “the Asian Tiger Mosquito” is a culicid associated with the transmission of several pathogens (Benedict et al. 2007). The vector competence and capacity of this species vary with locality. *A. albopictus* can serve as a primary or secondary vector of the four dengue virus serotypes (Massad et al. 2001; Hernandez et al. 2015) and can transmit several other arboviruses including the causative agents of west Nile encephalitis, yellow fever, chikungunya, Ross River and Mayaro (Bueno & Jiménez 2012; Muñoz & Navarro 2012; Rúa-Uribe et al. 2012). The species range includes Europe, Africa and the Americas. Introductions to new geographic regions are caused by importation of tires containing immature stages, especially the desiccation-resistant eggs (Reiter 1998; Gratz 2004).

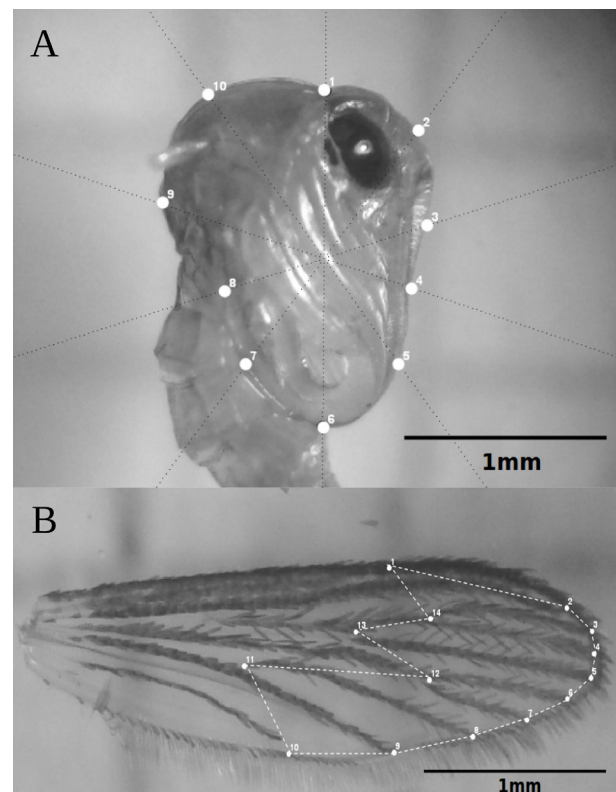
Adult female mosquitoes are medically important because they are hematophagous. They are distinguished from males by antenna morphology (dense, plumose in males and sparse in female) and male exposed reproductive structures (gonostylus and gonocoxite). Dengue entomological surveys are traditionally focused on the determination of culicid larval indices (house, container and Breteau) for estimating adult populations (Focks 2003). Several recent studies have proposed that pupal demographic surveys are better for this purpose, because pupal productivity in an area is associated with the number of females emerging daily under steady-state conditions (Barrera et al. 2006; Barrera 2009). Harrison (2005) stated that taxonomic identification of culicid pupae is commonly ignored in mosquito surveillance programs except to alert the participants that a major hatch is imminent. A more reliable pupal survey could be accomplished by determining the male/female ratio, which would require the ability to distinguish the sex of pupae. Geometric morphometrics is a tool for describing the phenotypic variation in organisms (Rohlf & Marcus 1993; Adams et al. 2004), and it has recently been shown useful for characterizing sexual dimorphism in mosquito wings (Devicari et al. 2011; Virginio et al. 2015). In this study we set out to describe shape and size dimorphism in *A. albopictus* pupae to facilitate dengue vector surveys.

## MATERIAL AND METHODS

### Specimens source and data acquisition

*A. albopictus* specimens were obtained from

collections in the Universidad de Carabobo campus (Hernandez et al. 2015) and maintained in the laboratory. Each specimen was individually reared from fourth instar larva to adult; pupae were photographed by transferring individual specimens to a petri dish and then returning them to a vial so that after emergence their sex could be determined via female and male morphological characters. Pupal cephalothorax images were retained from 30 females and 30 males, from which right wings were dissected and slide-mounted. For each pupal specimen a photo was created of 10 equidistant fans with MakeFan software (Sheets 2010). Ten landmarks were digitized with TPSDig (Rohlf 2008) in order to describe the cephalothoracic curve (Fig. 1A). Finally, each wing was digitalized using 14 landmarks (Fig. 1B), all type I and II following Bookstein (1991). The following landmarks were used: subcostal end (LM1), radial 1 end (LM2), radial 2 end (LM3), radial 3 end (LM4), radial 4+5 end (LM5), medial 1 end (LM6), medial 2 end (LM7), medial 3+4 end (LM8), cubital end (LM9), anal end (LM10), intersection between cubital and M3+4 (LM11), intersection between M1+2 and M3+4



**Figure 1. A - Cephalothorax of *Aedes albopictus* pupa showing the landmarks (1-10) disposition; landmarks 2-5 and 7-10 were treated as sliding; B - Right wing *A. albopictus* showing the landmarks (1-14) disposition. The polygon enclosed by the points conform the configurations analyzed.**

(LM12), intersection between radio-sectorial and radial 3 (LM13), and intersection between radial 2 and radial 3 (LM14).

### Geometric morphometric analysis

From each configuration matrix (pupal and adult) the coordinates were aligned by generalized procrustes analysis using tpsRelw software (Rohlf 2016) for extracting the matrix configurations and centroid size (CS). In particular, the pupal coordinates were analyzed with sliding semilandmarks. The semilandmarks were thus allowed to slide along their curve or surface in order to remove the effects of the arbitrary spacing by “optimizing” the position of the semilandmarks with respect to the average shape of the entire sample (Gunz & Mitteroecker 2013). Later, each stage aligned matrix was performed in MorphoJ software (Klingenberg 2011). A discriminant analysis (DA) was used to identify group (female or male) membership significance with Hotelling’s test (Webster & Sheets 2010). Finally, CS dimorphism differences were analyzed with PAST software (Hammer & Harper 2011) by means of the non-parametric Kruskal-Wallis test ( $P < 0.05$ ) with Bonferroni correction.

## RESULTS

### Centroid size

We found significant differences ( $H = 7.400$ ,  $df = 1$ ,  $P < 0.01$ ) between sexes for pupal cephalothorax measurements (Fig. 2). Female specimens were larger (mean  $3.498\text{mm} \pm \text{SD } 0.106$ ) than males ( $3.088\text{mm} \pm 0.600$ ). The female wing size (Fig. 2) was significantly ( $H = 29.39$ ,  $df = 1$ ,  $P < 0.001$ ) larger ( $4.105\text{mm} \pm 0.080$ ) than males ( $3.406\text{mm} \pm 0.054$ ).

### Shape differences

The DA histogram for cephalothorax specimens showed (Fig. 3A) significant size differences between females and males (Procrustes distance: 0.022; Mahalanobis distance: 2.841; T-square: 121.10,  $P < 0.0001$ ). In the cross-validation DA, the females were 90% and males 93.3% correctly classified into a posteriori groups (sex). Figure 3B shows the thin-plate spline deformation grid between females and males; the LM8 (located in the mesothoracic wing) displaced posteriorly to the first abdominal segment, and LM9 (located over the postscutal area) displace anteriorly to the mesothoracic wing. Figure 4A shows the DA histograms for wing measurements, where the

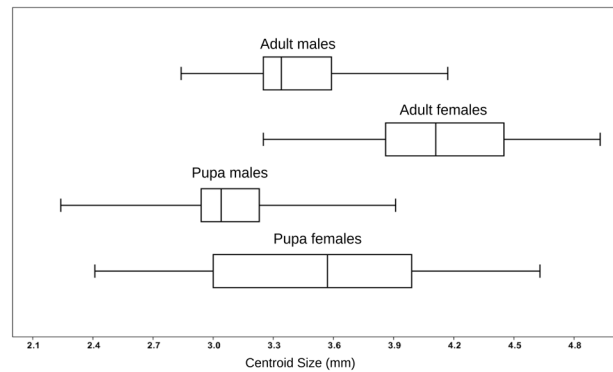


Figure 2. Box-plot diagram showing the centroid size sex differences between adult and pupa of *Aedes albopictus*.

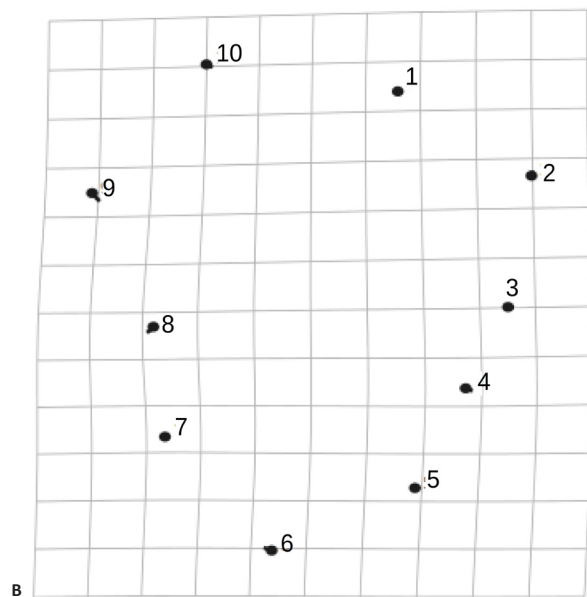
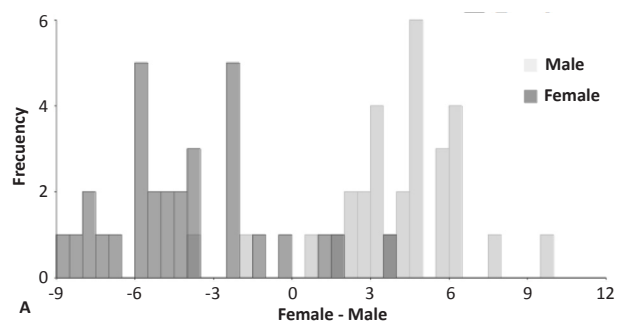
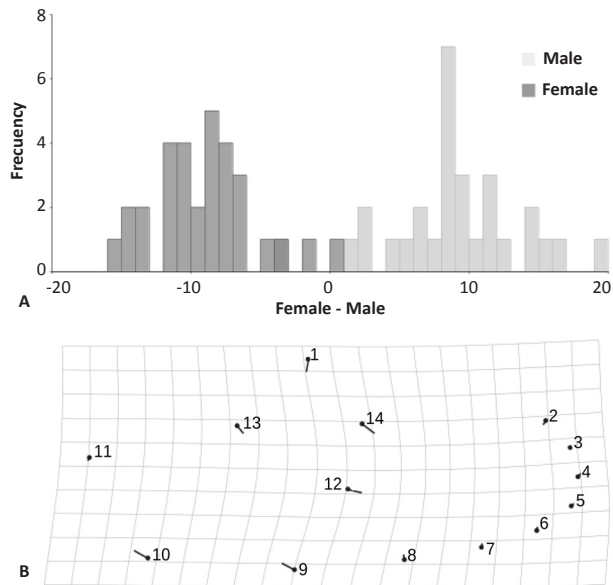


Figure 3. Discriminant analysis for 60 *Aedes albopictus* pupae specimens: A - histogram with the values of the discriminant scores for groups (females and males) and B - the thin-plate spline deformation grid with the differences between females and males.

configuration for females and males differs significantly (Procrustes distance: 0.053; Mahalanobis distance: 4.239; T-square: 269.34,  $P < 0.0001$ ). In the cross-



**Figure 4. Discriminant analysis for 60 *Aedes albopictus* wing specimens: A - histogram with the values of the discriminant scores for groups (females and males), and B - the thin-plate spline deformation grid with the differences between females and males.**

validation DA, the females were 96.7% and males 96.5% correctly classified. Figure 4B shows the thin-plate spline deformation grid between female and male wings: LM9 and LM10 displace to the wing basis, LM12, LM13 and LM14 displace to the wing apex and LM1 displaces to the posterior wing margin.

## DISCUSSION

### Sexual size dimorphism

Since pupae do not feed, the larval period critically influences pupal size and development (Castro et al. 1994; Consoli & de Oliveira 1994; Couret et al. 2014). Pupal size has been reported to be useful as a sex indicator (Cantrell 1939; Hayes 1953; Moorefield 1953), although this has been challenged for species where male/female size ranges overlap (Penn 1949; Vargas 1968). Recently, Mikery-Pacheco et al. (2015) used cephalothorax and abdomen lengths to perform a sieving device for sexing *A. albopictus* males and females. They considered pupae morphometrics sufficient to support such a device with good separation efficiency, although smaller females can pass through the sieve. In our investigation the relationship between size and dimorphism was established by means of the isometric or centroid size, an estimator used in geometric morphometrics for calculating the global dimensions of a biological structure. The isometric size

can express variations in longitudinal, lateral or oblique measurements (Bookstein 1991; Dujardin 2008). In medical and forensic entomology, the isometric size has been used for size estimation (Belen et al. 2004; Calle et al. 2008; Vásquez & Liria 2012; Nuñez-Rodríguez & Liria 2017).

The CS analysis indicates that female pupae are larger than males, consistent with evidence of adult sexual dimorphism in *A. albopictus* and other mosquitoes (Devicari et al. 2011; Oliveira et al. 2012; Virginio et al. 2015). Lounibos (1994), however, considered adult size to be a direct effect of feed and density conditions during larval instars. In adults, larger females are related to life history components. Haramis (1985) and Nasci & Mitchell (1994) reported positive relationships between vector size and longevity that are associated with vector capacity. Armbruster & Hutchinson (2002) found that wing length and pupal mass were reliable indicators of fertility in *A. albopictus* and *A. (Finlaya) geniculatus* (Oliver, 1791) females. In contrast, male size is mainly related to survival and spermatic capacity (Xue et al. 2010).

### Sexual shape dimorphism

Moorefield (1951) and Vargas (1968) observed sexual dimorphism in size and shape of the ninth pupal abdominal segment (genital segment) in various mosquito species; Moorefield (1951) considered the genital segment variations to be useful taxonomic characters for species recognition. Harrison (2005) described morphological characters for separating *A. albopictus* pupae from others species inhabiting artificial containers. The characteristics used were paddle shape, seta 1-P length, and the presence of a thick fringe of long filamentous spicules on the lateral and mesal edges of the paddles.

In relation to wing shape, our results are similar to those of Devicari et al. (2011) and Virginio et al. (2015) who observed wing shape dimorphism in *A. albopictus* and other culicid species. Several studies indicate that mosquito wing conformation has a polygenic basis, being weakly influenced by heritable epigenetic factors (Jirakanjanakit et al. 2007; Dujardin 2008; Morales-Vargas et al. 2010). The observed sexual shape dimorphism in *A. albopictus* corroborates studies suggesting that wing conformation is sex-specific (Devicari et al. 2011; Virginio et al. 2015), and that differences can be attributed to various genes that participate in the expression of wing shape waving additive and/or differential effects on the phenotype (Carreira et al. 2011).

To our knowledge this is the first description of

phenotypic variation in pupal shape and size in *A. albopictus* laboratory colonies. The results may be useful for creating new tools for dengue entomological surveys once potential variations between natural and laboratory populations have been characterized, along with the effects of variations in larval density.

## REFERENCES

- Adams, D.C., J. Rolhf & D.E. Slice (2004). Geometric morphometrics: Ten years of progress following the 'revolution'. *Italian Journal of Zoology* 71: 5–16; <http://doi.org/10.1080/11250000409356545>
- Armbruster, P. & R. Hutchinson (2002). Pupal Mass and Wing Length as Indicators of Fecundity in *Aedes albopictus* and *Aedes geniculatus* (Diptera: Culicidae). *Journal of Medical Entomology* 39(4): 699–704; <http://doi.org/10.1603/0022-2585-39.4.699>
- Barrera, R. (2009). Simplified Pupal Surveys of *Aedes aegypti* (L.) for Entomologic Surveillance and Dengue Control. *American Journal of Tropical Medicine and Hygiene* 81(1): 100–107.
- Barrera, R., M. Amador & G.C. Clark (2006). Use of the pupal survey technique for measuring *Aedes aegypti* (Diptera: Culicidae) productivity in Puerto Rico. *American Journal of Tropical Medicine and Hygiene* 74(2): 290–302.
- Belen, A., B. Alten & A.M. Aytakin (2004). Altitudinal variation in morphometric and molecular characteristics of *Phlebotomus papatasi* populations. *Medical and Veterinary Entomology* 18: 343–350; <http://doi.org/10.1111/j.0269-283X.2004.00514.x>
- Benedict, M.Q., R.S. Levine, W.A. Hawley & L.P. Lounibos (2007). Spread of the tiger: Global risk of invasion by the mosquito *Aedes albopictus*. *Vector-borne and Zoonotic Diseases* 7: 76–85; <http://doi.org/10.1089/vbz.2006.0562>
- Bookstein, F.L. (1991). *Morphometric Tools for Landmark Data: Geometry and Biology*. EEUU, Cambridge.
- Bueno, R. & R. Jiménez (2012). Implicaciones sanitarias del establecimiento y expansión en España del mosquito *Aedes albopictus*. *Revista Española de Salud Pública* 86: 319–330.
- Calle, D.A., M. Quiñónez, H. Erazo & N. Jaramillo (2008). Discriminación por morfometría geométrica de once especies de *Anopheles* (*Nyssorhynchus*) presentes en Colombia. *Biomedica* 28: 371–385.
- Cantrell, W. (1939). Relation of size to sex in pupae of *Aedes aegypti* (L.), *A. triseriatus* (Say) and *A. vexans* (Meig.). *Journal of Parasitology* 24(4): 448–449.
- Carreira, V., I. Soto, J. Mensch & J. Fanara (2011). Genetic basis of wing morphogenesis in *Drosophila*: sexual dimorphism and non-allometric effects of shape variation. *BMC Developmental Biology* 11: 32; <http://doi.org/10.1186/1471-213X-11-32>
- Castro, A., S. Davidson, C. Zevedo, M. Bicudo de Paula & R. Marques (1994). Duration of larval and pupal development stages of *Aedes albopictus* in natural and artificial containers. *Revista de Saúde Pública* 29(1): 15–19.
- Consoli, A.G. & R.L. de Oliveira (1994). *Principais Mosquitos de Importância Sanitária no Brasil*. Fiocruz, Rio de Janeiro, 228pp.
- Couret, J., E. Doton & M.Q. Benedict (2014). Temperature, larval diet, and density effects on development rate and survival of *Aedes aegypti* (Diptera: Culicidae). *PLoS One* 9(2): e87468; <http://doi.org/10.1371/journal.pone.0087468>
- Devicari, M., A.R. Lopes & L. Suesdek (2011). Wing sexual dimorphism in *Aedes scapularis* (Diptera: Culicidae). *Biota Neotropica* 11(2): 165–169; <http://doi.org/10.1590/S1676-06032011000200016>
- Dujardin, J.P. (2008). Morphometrics applied to medical entomology. *Infection, Genetics and Evolution* 8: 875–890; <http://doi.org/10.1016/j.meegid.2008.07.011>
- Focks, D.A. (2003). *A Review of Entomological Sampling Methods and Indicators for Dengue Vectors*. Gainsville, World Health Organization, 40pp.
- Gratz, N.G. (2004). Critical review of the vector status of *Aedes albopictus*. *Medical and Veterinary Entomology* 18: 215–227.
- Gunz, P. & P. Mitteroecker (2013). Semilandmarks: a method for quantifying curves and surfaces. *Italian Journal of Zoology* 24: 103–109.
- Hammer, Ø. & D.A.T. Harper (2011). PAST: Palaeontological Statistics, versión 2.10. Available in: <http://folk.uio.no/ohammer/past>.
- Haramis, L.D. (1985). Larval nutrition, adult body size, and the biology of *Aedes triseriatus*, pp. 431–437. In: Lounibos, L.P., J.R. Rey, J.H. Frank (eds.). *Ecology of Mosquitoes: Proceedings of a Workshop*. Florida Medical Entomology Laboratory, Vero Beach, FL.
- Harrison, B. (2005). Easily seen characters to identify the pupa of *Aedes albopictus* in the united states. *Journal of the American Mosquito Control Association* 21(4): 451–454.
- Hayes, R.O. (1953). Studies on the artificial insemination of the mosquito *Aedes aegypti* (L.). *Mosquito News* 13(2): 145–152.
- Hernandez, M., M. Piña, A. Soto-Vivas, M.A. Rangel & J. Liria (2015). Primer registro de *Aedes albopictus* (Skuse, 1894) (Diptera: Culicidae) en el Estado Carabobo, Venezuela. *Salus* 19: 39–41.
- Jirakanjanakit, N., W.A.T. Leemingsa, S. Thongrunkiat, S. Apiwathnasorn, C. Singhaniyom, C. Bellec & J.P. Dujardin (2007). Influence of larval density or food variation on the geometry of the wing of *Aedes* (*Stegomyia*) *aegypti*. *Tropical Medicine and International Health* 12(11): 1354–1360.
- Klingenberg, C.P. (2011). MorphoJ: an integrated software package for geometric morphometrics. *Molecular Ecology Resources* 11: 353–357; <http://doi.org/10.1111/j.1755-0998.2010.02924.x>
- Lounibos, L.P. (1994). Geographical and developmental components of adult size of neotropical *Anopheles* (*Nyssorhynchus*). *Ecological Entomology* 19: 138–146.
- Massad, E., F.A.B. Coutinho, M.N. Burattini & L.F. Lopez (2001). The risk of yellow fever in a dengue-infested area. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 9: 370–374.
- Mikery-Pacheco, O., K. Serrano-Domínguez, P. Marcellin-Chong & D. Sánchez-Guillén (2015). Efficiency of the separation of *Aedes* (*Stegomyia*) *albopictus* (Diptera: Culicidae) male and female pupae using a sieving device. *Acta Zoológica Mexicana* 31(1): 113–115.
- Moorefield, H.M. (1951). Sexual dimorphism in mosquito pupae. *Mosquito News* 11: 175–177.
- Morales-Vargas, E.R., P. Ya-Umpham, N. Phumala-Morales, N. Komalamisra & J.P. Dujardin (2010). Climate associated size and shape changes in *Aedes aegypti* (Diptera: Culicidae) populations from Thailand. *Infection, Genetics and Evolution* 10: 580–585; <http://doi.org/10.1016/j.meegid.2010.01.004>
- Muñoz, M. & J.C. Navarro (2012). Virus Mayaro: un arbovirus reemergente en Venezuela y Latinoamérica. *Biomedica* 32: 286–302; <http://doi.org/10.7705/biomedica.v32i2.647>
- Nasci, R.S. & C.J. Mitchell (1994). Larval diet, adult size, and susceptibility of *Aedes aegypti* (Diptera: Culicidae) to infection with Ross River virus. *Journal of Medical Entomology* 31: 123–126.
- Nuñez-Rodríguez, J.A. & J. Liria (2017). Geometric morphometrics sexual dimorphism in three forensically-important species of Blow Fly (Diptera: Calliphoridae). *Life: The Excitement of Biology* 4(4): 272–284; [http://doi.org/10.9784/LEB4\(4\)NunezRodriguez.01](http://doi.org/10.9784/LEB4(4)NunezRodriguez.01)
- Oliveira, P., E. Carvalho & L. Suesdek (2012). Temporal variation of wing geometry in *Aedes albopictus*. *Memórias do Instituto Oswaldo Cruz* 107(8): 1030–1034; <http://doi.org/10.1590/S0074-02762012000800011>
- Penn, G.H. (1949). The pupae of mosquitoes of New Guinea. *Pacific Science* 3(1): 1–85.
- Reiter, P. (1998). *Aedes albopictus* and the world trade in used tires, 1988–1995: the shape of things to come? *Journal of the American Mosquito Control Association* 14: 83–94.
- Rohlf, J. (2008). tpsDig, Program for Digitalizing morphologic landmark and outlines for Geometric Morphometric Analyses, ver. 2.11. Department of Ecology and Evolution, State University of New York at Stony Brook. Available from: <http://life.bio.sunysb.edu/morph/index.html>

- Rohlf, J. (2016). tpsRelw, Program provides a low dimensional approximation (via a principal components analysis) to the tangent space approximation of shape space, ver. 1.62. Department of Ecology and Evolution, State University of New York, Stony Brook. Available from: <http://life.bio.sunysb.edu/morph/index.html>
- Rohlf, J. & L. Marcus (1993). A revolution in morphometrics. *Trends in Ecology and Evolution* 8: 129–132; [http://doi.org/10.1016/0169-5347\(93\)90024-J](http://doi.org/10.1016/0169-5347(93)90024-J)
- Rúa-Uribe, G., C. Suárez-Acosta & R. Rojo (2012). Implicaciones epidemiológicas de *Aedes albopictus* (Skuse) en Colombia. *Revista Facultad Nacional de Salud Pública* 30(3): 328–337.
- Sheets, H.D. (2010). MakeFan, a tool for drawing alignment “fans” at equal angular spacing. Available from: <http://www3.canisius.edu/~sheets/imp7.htm>
- Vargas, M. (1968). Sexual dimorphism of larvae and pupa of *Aedes aegypti* (Linn.). *Mosquito News* 28(3): 374–379.
- Vásquez, M. & J. Liria (2012). Morfometría geométrica alar para la identificación de *Chrysomya albiceps* sy *C. megacephala* (Diptera: Calliphoridae) de Venezuela. *Revista de Biología Tropical* 60(3): 1-10; <http://doi.org/10.15517/rbt.v60i3.1776>
- Virginio, F., P. Oliveira & L. Suesdek (2015). Wing sexual dimorphism of pathogen-vector culicids. *Parasite and Vectors* 8(1): 159–167; <http://doi.org/10.1186/s13071-015-0769-6>
- Webster, M. & H.D. Sheets (2010). A practical introduction to landmark-based geometric morphometrics. pp. 163–188. In: Alroy, J. & G. Hunt (eds.). *Quantitative Methods in Paleobiology*. Paleontological Society Papers. Volume 16.
- Xue, R.D., D.R. Barnard & G.C. Muller (2010). Effects of body size and nutritional regimen on survival in adult *Aedes albopictus* (Diptera: Culicidae). *Journal of Medical Entomology* 47(5): 778–782; <http://doi.org/10.1603/ME09222>

**Resumen:** *Aedes albopictus* (Skuse, 1894) es un culicido asociado con la transmisión de patógenos causantes de dengue, fiebre amarilla, chikungunya, Mayaro, y otras enfermedades. Ciertas investigaciones sugieren la importancia de los estudios demográficos en pupas de vectores de dengue, respecto a la estimación poblacional basada en índices larvarios. La morfometría geométrica es una herramienta que describe la variabilidad fenotípica relacionada con la caracterización del dimorfismo sexual. Nosotros utilizamos este método para describir el dimorfismo sexual en pupas de *A. albopictus*. Se digitalizaron coordenadas 2D en 60 especímenes en dos fases: 10 hitos en pupa y 14 en alas. Las matrices de configuraciones fueron alineadas mediante análisis generalizado de procrustes para extraer matrices de conformación y tamaño centroide (TC). Se utilizó el Análisis Discriminante (AD) para evaluar la significancia en la membresía de grupos (hembras o machos), y una prueba no-paramétrica para el TC. Se encontraron diferencias significativas (Kruskal-Wallis  $P < 0.01$ ) entre el TC de pupas y alas; las hembras fueron más grandes que los machos. El AD para los especímenes de pupas y alas mostraron diferencias (Hotelling  $P < 0.0001$ ) entre sexos. En la reclasificación, las hembras y machos fueron correctamente asignados en un 90% con base en la conformación. Este estudio es el primero que describe la variación fenotípica en la conformación de pupas y adultos en *A. albopictus* mantenidos en laboratorio, y los resultados podían ser usados como herramientas adicionales en programas de vigilancia entomológica. Son necesarios investigaciones adicionales para confirmar la variación entre poblaciones naturales y de laboratorio.





OPEN ACCESS



The Journal of Threatened Taxa is dedicated to building evidence for conservation globally by publishing peer-reviewed articles online every month at a reasonably rapid rate at [www.threatenedtaxa.org](http://www.threatenedtaxa.org). All articles published in JoTT are registered under [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/) unless otherwise mentioned. JoTT allows unrestricted use of articles in any medium, reproduction, and distribution by providing adequate credit to the authors and the source of publication.

ISSN 0974-7907 (Online); ISSN 0974-7893 (Print)

June 2017 | Vol. 9 | No. 6 | Pages: 10249–10368

Date of Publication: 26 June 2017 (Online & Print)

DOI: 10.11609/jott.2017.9.6.10249-10368

[www.threatenedtaxa.org](http://www.threatenedtaxa.org)

#### Articles

**Co-occurrence patterns of fish communities in littorals of three floodplain lakes of the Orinoco River, Venezuela**

-- Gabriela E. Echevarría & Nirson González, Pp. 10249–10260

**Genetic diversity of the Green Turtle (Testudines: Cheloniidae: *Chelonia mydas* (Linnaeus, 1758)) population nesting at Kosgoda Rookery, Sri Lanka**

-- E.M.L. Ekanayake, T. Kapurusinghe, M.M. Saman, D.S. Rathnakumara, P. Samaraweera & R.S. Rajakaruna, Pp. 10261–10268

**Identity of *Sphaerotheca pluvialis* (Jerdon, 1853) and other available names among the burrowing frogs (Anura: Dicroglossidae) of South Asia**

-- Neelesh Dahanukar, Shauri Sulakhe & Anand Padhye, Pp. 10269–10285

***Sphaerotheca pashchima*, a new species of burrowing frog (Anura: Dicroglossidae) from western India**

-- Anand Padhye, Neelesh Dahanukar, Shauri Sulakhe, Nikhil Dandekar, Sunil Limaye & Kirti Jamdade, Pp. 10286–10296

**Population status and species diversity of wetland birds in the Rapti and Narayani rivers and associated wetlands of Chitwan National Park, Nepal**

-- Bed Bahadur Khadka, Paras Mani Acharya & Sunil Lal Rajbhandari, Pp. 10297–10306

#### Communications

**Wildlife hunting by indigenous people in a Philippine protected area: a perspective from Mt. Apo National Park, Mindanao Island**

-- Krizler Cejuela Tanalgo, Pp. 10307–10313

**Pupal shape and size dimorphism in *Aedes albopictus* (Skuse, 1894) (Diptera: Culicidae)**

-- Elvira Sánchez, Daniel Castillo & Jonathan Liria, Pp. 10314–10319

#### Short Communications

**Occurrence and conservation of the Indian Leopard (Mammalia: Carnivora: Felidae: *Panthera pardus*) in Cox's Bazar District of Bangladesh**

-- M. Tarik Kabir, M. Farid Ahsan & Ayesha Khaton, Pp. 10320–10324

**A checklist of the avian fauna of Chittagong University campus, Bangladesh**

-- M. Tarik Kabir, M. Farid Ahsan, M. Mizanur Rahman & M. Manirul Islam, Pp. 10325–10333

**Diversity and new records of intertidal hermit crabs of the genus *Clibanarius* (Crustacea: Decapoda: Diogenidae) from Gujarat coast off the northern Arabian Sea, with two new records for the mainland Indian coastline**

-- Pradip Kachhiya, Jatin Raval, Pares Poriya & Rahul Kundu, Pp. 10334–10339

#### Notes

**Four species of Commelinaceae, as additions to Andhra Pradesh, India**

-- S. Salamma, M. Chennakesavulu Naik, M. Anil Kumar, A. Sreenath & B. Ravi Prasad Rao, Pp. 10340–10344

**Trematode infestation in coral colonies at Poshitra Reef, Gulf of Kachchh Marine National Park, Gujarat, India**

-- D. Adhavan, R. Chandran, S. Tikadar & K. Sivakumar, Pp. 10345–10346

**First report of *Mantibaria mantis* (Dodd) (Hymenoptera: Scelionidae: Scelioninae) from India and additional descriptors for the species**

-- Kamalanathan Veenakumari & Prashanth Mohanraj, Pp. 10347–10350

**A new record of *Tenodera fasciata* (Olivier, 1792) (Insecta: Mantodea: Mantidae: Mantinae) for western India**

-- Gopal Ambrushi Raut & Sunil Madhukar Gaikwad, Pp. 10351–10354

**First records of butterflies *Anthene emolus emolus* (Godart, [1924]) (Lepidoptera: Lycaenidae: Polyommatainae) and *Gandaca harina assamica* Moore, [1906] (Lepidoptera: Pieridae: Coliadinae) from Kumaon, Uttarakhand, India**

-- Sanjay Sondhi, Pp. 10355–10357

**A new locality record of the rare Anomalous Nawab *Polyura agrarius* (Swinhoe, 1887) (Lepidoptera: Nymphalidae: Charaxinae) from central India**

-- Deepika Mehra, Jagatjot Singh Flora & Vivek Sharma, Pp. 10358–10360

**Taxonomic note about Willow Ermine Moth *Yponomeuta rorrellus* Hübner (Lepidoptera: Yponomeutidae) from Ladakh division of Jammu & Kashmir, India**

-- Mudasir Ahmad Dar, Shahid Ali Akbar & Govindasamy Mahendiran, Pp. 10361–10364

**First record of hagfish (Cyclostomata: Myxinidae) in Indian waters**

-- B. Fernholm, A. Biju Kumar & Michael Norén, Pp. 10365–10368

**zoo!**  
Z Ü R I C H

**WILD  
ZOO**  
Threatened Taxa