

This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>



Contents lists available at ScienceDirect

Infection, Genetics and Evolution

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

Wing shape of dengue vectors from around the world.

A. Henry^{a,b}, P. Thongsripong^{a,b}, I. Fonseca-Gonzalez^c, N. Jaramillo-Ocampo^c, Jean-Pierre Dujardin^{a,d,*}^a Center of Excellence for Vectors and Vector-borne Diseases (CVVD), Faculty of Sciences, University of Mahidol, Thailand^b Asia-Pacific Institute of Tropical Medicine and Infectious Diseases, University of Hawaii at Manoa, Honolulu, United States^c Grupo Chagas, Instituto de Biología, Universidad de Antioquia, A.A 1226 Medellín, Colombia^d UMR IRD-CNRS 2724, Agropolis, 911, BP 64501, 34394, Montpellier, France

ARTICLE INFO

Article history:

Received 11 June 2009

Received in revised form 16 November 2009

Accepted 5 December 2009

Available online 22 December 2009

Keywords:

*Aedes aegypti**Ae. albopictus*

Colombia

Thailand

Florida

Hawaii

Morphometrics

ABSTRACT

Wing shape is increasingly utilized in species identification and characterization. For dengue vectors *Aedes aegypti* and *Aedes albopictus*, it could be used as a complement for ensuring accurate diagnostic of damaged specimens. However, the impact of world migration on wing shape is unknown. Has the spread of these invasive species increased shape variation to the extent of producing interspecific overlapping? To answer this question, the geometric patterns of wing venation in *Ae. aegypti* and *Ae. albopictus* were compared between natural populations from the Pacific Islands, North and South America and South East Asia. The geometry of 178 female and 174 male wings were described at 13 anatomical landmarks, and processed according to Procrustes superposition, partial warps and subsequent multivariate analyses. The variation of shape did not produce significant interspecific overlapping. Regardless of geographic origin, *Ae. aegypti* was recognized as *Ae. aegypti* and *Ae. albopictus* as *Ae. albopictus*. Some significant geographic differentiation was observed in Colombia for *Ae. aegypti* and in Thailand for *Ae. albopictus*. Globally, the morphology of these mosquitoes, for both size and shape, appeared well preserved. Strong canalizing mechanisms could account for the observed patterns of relatively uniform morphology, which could also be attributed to sporadic, recurrent mixing of populations, thwarting phenotypic drift.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Aedes aegypti (Linnaeus) (Diptera: Culicidae) and *Ae. albopictus* (Skuse) (Diptera: Culicidae) are two of the most important mosquito vectors of human diseases. *Ae. aegypti* is the main vector of urban yellow fever, a disease that during the eighteenth and nineteenth centuries caused devastating epidemics in tropical America, Northern American coastal cities, and Europe (Staples and Monath, 2008). *Ae. aegypti* and *Ae. albopictus* are the primary and secondary vectors, respectively, of dengue virus (DF/DHF), possibly the most important arbovirus of the twenty first century (Gubler, 2002). They also transmit the chikungunya virus, a virus that originated from Africa, is re-emerging in India and Asia and has recently caused outbreaks in southern Europe, and Italy (Chastel, 2005; Josseran et al., 2006; Rezza et al., 2007).

Both mosquito vectors have undergone transcontinental migration (Gubler, 1997) and were likely exposed to large variations in environment. The species are morphologically close, and their respective adaptation to new continents could have

affected their morphological distinction. In this study we explore empirically the shape canalization in *Ae. aegypti* and *Ae. albopictus* using geometric morphometrics on wing venation: were the canalization mechanisms strong enough to oppose external forces?

The anthropogenic worldwide migration of *Ae. aegypti* began a few centuries ago, while the spread of *Ae. albopictus* initiated within the last few decades. The domestic *Ae. aegypti* most likely differentiated from the darker sylvan form found in African tropical forests and after adapting to peridomestic life it migrated to the New World and to the South East Asian continents. It is believed to have reached the New World from West Africa aboard slave ships, starting in the seventeenth century (Gubler, 1997). Efforts to eliminate *Ae. aegypti* from the Americas to stop yellow fever started in 1915 by the International Health Commission of the Rockefeller foundation and then later in the 1940s by the Pan American Health Organization (PAHO). By the mid 1960s *Ae. aegypti* was eliminated from most countries in South America. The program was dismantled in the early 1970s before achieving complete eradication; Suriname, the Guyanas, Venezuela, the Southern USA, and some Caribbean Islands were still infested with *Ae. aegypti* (Lourenço-de-Oliveira et al., 2004). It has been suggested that all of Colombia was eradicated except for the city of Cucuta, close to the Venezuelan border (Groot, 1980). At the end of the decade many countries were reinfested and within a few years began to

* Corresponding author at: Centre of Excellence for Vectors and Vector Borne Diseases (CVVD), Faculty of Science, Mahidol University, Rama VI Rd., Bangkok 10400, Thailand. Tel.: +66 2 201 5276; fax: +66 2 354 7161.

E-mail addresses: dujardinbe@gmail.com, grskt@mahidol.ac.th (J.-P. Dujardin).

Table 1
Geographic origin and date of capture of *Aedes aegypti* and *Ae. albopictus*.

Species	F/M	Geographic location	Time of collection	Life stages captured
<i>Ae. aegypti</i>	30/32	Belen, Comuneros, C(C)	2008	Juveniles
	30/20	Clear Water, F(US)	2007–2008	Adults
	30/30	Nakhon Nayok, NN(T)	2008	Adults
<i>Ae. albopictus</i>	30/26	Pinellas, F(US)	2005–2008	Adults
	9/14	Vero Beach, F(US)	2009	Juveniles
	19/22	Honolulu, H(US)	2009	Adults
	30/30	Nakhon Nayok, NN(T)	2008	Adults

“juveniles” = collected as larvae and reared in breeding containers, “adults” = collected using traps with various attractants (see Section 2). F(US) = Florida (United States), H(US) = Hawaii (United States), NN(T) = Province of Nakhon Nayok (Thailand), C(C) = Municipality Cucuta (Colombia). F, females; M, males.

experience epidemic DF followed by the emergence of DHF (Gubler, 2005). Phylogenetic studies suggest that *Ae. aegypti* populations within South America are made up of multiple reintroductions and populations that survived the initial eradication programs (Mousson et al., 2005; Bracco et al., 2007).

Aedes aegypti was probably introduced into coastal cities of South East Asia from East Africa around the nineteenth century via the shipping industry but not until World War II did it establish itself and spread to interior cities (Gubler, 1997; Failloux et al., 2002). Genetic analysis using mitochondrial genes revealed two distinct clusters in South East Asia, one cluster included *Ae. aegypti* strains from French Polynesia, Guinea, and Brazil, the other cluster contained strains from Martinique, Europa Island, and Northeast Amazonia (Mousson et al., 2005). A study looking at mitochondrial genetic diversity of *Ae. aegypti* within Thailand found that the most common haplotype is also present but rare in Mexico (Bosio et al., 2005).

Aedes albopictus is said to be native to South East Asia and only within the last three decades did it start its global spread. At least 28 countries have imported *Ae. albopictus*, largely due to the international trade of used tires (Benedict et al., 2007). It was imported to Hawaii in the early 1900s and it was first introduced into the Continental United States in the 1980s, where it has become widely dispersed (Reiter and Darsie, 1984; Effler et al., 2005). Global tire trade statistics and allozyme analysis revealed the origin of the continental US introduction was Japan (Hawley et al., 1987; Kambhampati et al., 1991). Mousson et al. (2005) analyzed the mitochondrial genes of *Ae. albopictus* from 13 different geographic locations revealing a generally low variability, which they attributed to the recent expansion of the species. Specimens from island populations such as Hawaii showed the highest rates of sequence evolution (Mousson et al., 2005).

It would be expected that processes such as different migration routes, passive dispersion at regional and continental scales, and repeated vector control efforts that disrupt populations, would create founder effects inducing genetic and subsequent morphological variation (Jirakanjanakit et al., 2008). Also one can anticipate differences in habitat environment for introduced populations would lead to local adaptation and morphological change (Jirakanjanakit et al., 2007; Caro-Riaño et al., 2009). Our study investigated the impact these processes had on metric properties.

2. Materials and methods

2.1. Insects

A total of 352 mosquitoes (178 females and 174 males) were analyzed from 4 geographical locations: Nakhon Nayok province of Thailand, Cucuta municipality of Colombia, and Florida and Hawaii in the United States (Table 1). Thailand mosquitoes were collected as adults during 2008 from the Muang district of Nakhon Nayok

province, central Thailand. The BG sentinel trap, which uses a combination of lactic acid, ammonia, and fatty acids as attractants, and the Mosquito Magnet Liberty Plus, which utilizes CO₂ as an attractant, were used. Mosquitoes from Hawaii were collected in Honolulu as adults in 2009, using similar traps as in Thailand. Adult mosquitoes from Florida were collected in Pinellas county between 2005 and 2008 using a John Hock miniature CDC light trap baited with CO₂. The trap ran in 24-h intervals with the light turned off during the day. The Colombia specimens were collected during the end of 2008 from multiple breeding sites in Belen and Comuneros, two different locations in the Cucuta municipality. They were collected as pupae and 3rd and 4th instar larvae, reared to adults under laboratory conditions, and the progeny of multiple broods (F1) was used in the experiment. A subset of specimens from Florida were collected in Vero Beach in 2009 as pupae and 3rd and 4th instar larvae. They were obtained from multiple breeding habitats at distances between 1 to 100m apart and reared to adults (F0) in breeding containers at room temperature.

2.2. Data collection

Wings were digitized at 13 landmarks (LM), all of them of “type I” (venation intersections, see Bookstein, 1991) (Fig. 1). Other possible type I LM were not considered because they were less consistently visible. To avoid possible optical distortion at the periphery of optical lens, each wing was located at the center of the visual field. Different optical tools were used but each picture was calibrated to permit accurate size comparisons. The measurement error was estimated comparing two sets of digitization. It was computed as 1-R, with R the repeatability index as described by Arnqvist and M?artensson (1998), i.e. a Model II oneway ANOVA on repeated measures, where R is provided by the ratio of the

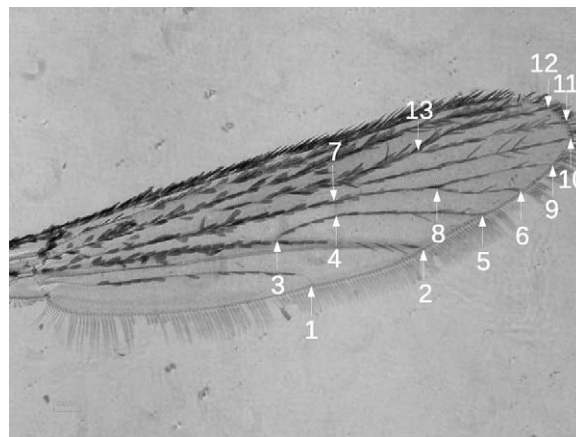


Fig. 1. Wing and landmarks positions. Arrows indicate the position of the landmark. Numbers refer to the order of collection, and are reproduced in Fig. 3. Scale equals .1 mm.

between-individual variance and the total variance. A sample of images was digitized twice by each author and the respective repeatabilities were scored. The coordinates from the author producing the highest repeatability were used for the present analyses, though each author performed the analyses and obtained similar results.

2.3. Size variable

For comparing overall wing size among different populations, we used the isometric estimator known as centroid size (CS) derived from coordinates data. It is defined as the square root of the sum of the squared distances between the center of the configuration of landmarks and each separate landmark (Bookstein, 1991). The centroid sizes of different species and sexes were compared by non-parametric analyses based on permutations (1000 runs), allowing the comparison of both means and variances of size (Caro-Riaño et al., 2009). To determine significance, the Bonferroni correction was applied for $P \leq .05$.

2.4. Shape variables

Shape variables were obtained through the Generalized Procrustes Analysis (GPA) superimposition algorithm and the subsequent projection of the Procrustes residuals into an Euclidean space (Rohlf, 1999). Of these projections, both non-uniform (“partial warps”, strictly speaking) and uniform components (Rohlf, 1990) were used as shape variables. The uniform component describes global variation such as stretching and compression, and the non-uniform component corresponds to local variation (Zelditch et al., 2004). These two components describe the differences in shape as deviations from an average configuration of landmarks. Their principal components are called “relative warps” (RW). To highlight areas of the wing that possibly diverge between species, the average residual coordinates after GPA were shown for males and for females. To illustrate morphological divergence among populations and species, a tree for each sex was built based on the Procrustes distances. Since the latter are not Euclidean distances, the *neighbor* tree building algorithm (Saitou and Nei, 1987) was preferred to the UPGMA method.

2.5. Allometry

The scaling for centroid size does not remove the allometric changes of size and their influence on shape. To evaluate the allometric content of shape, we used a multivariate regression test computed with size as the independent variable and shape as the dependent variable; statistical significance was assessed by non-parametric method (Good, 2000). The common allometric model was verified through a MANCOVA of shape versus size, groups and the corresponding interaction. Where no statistical significance for the interaction was found (Wilks test), allometry-free shape variation was predicted in each group, and the morphological distances computed again (as Euclidean distances between predicted RW).

2.6. Classification based on shape

Each wing (individual) was reclassified according to its similarity to the average shape of each group. This was performed using Mahalanobis distances as an estimation of metric distance. As a validating procedure, distances were computed on discriminant axes estimated without the wing to be classified. Each wing to be classified was then introduced as supplementary data (validated classification). Classification rates were computed on

the total sample according to species (“interspecies accuracy rate”, IAR), to both species and geography (“total accuracy rate”, TAR) and within species according to geographic origin (“geographic accuracy rate”, GAR). These scores were computed separately for males and females.

2.7. Software

Data collection, analyzes and graphical output were performed using the various modules (COO, TET, MOG, VAR, PAD and COV) of specialized software developed by Dujardin J.P. and freely available at <http://www.mpl.ird.fr/morphometrics>. Trees were constructed using the *neighbor* module of the PHYLIP packages (by J. Felsenstein).

3. Results

3.1. Morphological examination

A total of 352 specimens were studied (178 females and 174 males, see Table 1). No apparent external attribute or color pattern could distinguish the *Ae. aegypti* or *Ae. albopictus* specimens from the different geographic locations: Cucuta (Colombia), Nakon Nayok (Thailand), Pinellas and Vero Beach, Florida (US) and Honolulu, Hawaii (US).

3.2. Repeatability (R)

The comparison of two repeated sets of measurements for the same images (30 *Ae. aegypti* and 30 *Ae. albopictus*) showed fairly good agreement for the centroid size ($R = .999$ for males and $.998$ for females). The average repeatability for each landmark, represented by x, y coordinates, was $.94, .90$ for males and $.96, .92$ for females.

3.2.1. Size

There was no significant difference in size or variance of size between the two species in Thailand and Florida (Fig. 2). Although there was no significant size difference among *Ae. aegypti* males, the Florida (US) *Ae. aegypti* females were significantly larger than the specimens from Cucuta (Colombia) and Nakon Nayok (Thailand). In *Ae. albopictus*, male and female specimens from Nakon Nayok (Thailand) were significantly smaller, while the US samples were similar in size. Sexual size dimorphism was significant for both species at each location, but more prominent in the two samples collected as juveniles: the Florida subset of *Ae. albopictus* (9 females and 14 males) and the Cucuta *Ae. aegypti*. Most of the 14 sample sets exhibited comparable ranges in size except for the significantly less variable Florida *Ae. albopictus* that were collected as juveniles. The ranges in size were statistically similar between sexes in both species except for the *Ae. albopictus* from Hawaii and adults collected from Florida where females showed significantly larger variances.

3.3. Shape

The residual coordinates of *Ae. aegypti* and *Ae. albopictus* were superimposed onto the common consensus residual coordinates, displaying obvious differences in wing venation (see the Procrustes superimposition Fig. 3). The superimposition was performed on the total sample, and separately in Thailand and in Florida, with the same results. The interspecific variations were more apparent in males than females, though both exhibited the same type of divergence at each of the landmarks. The Procrustes superimposition suggested that the posterior border and the center of the wing could be more important in distinguishing the two species (Fig. 3).

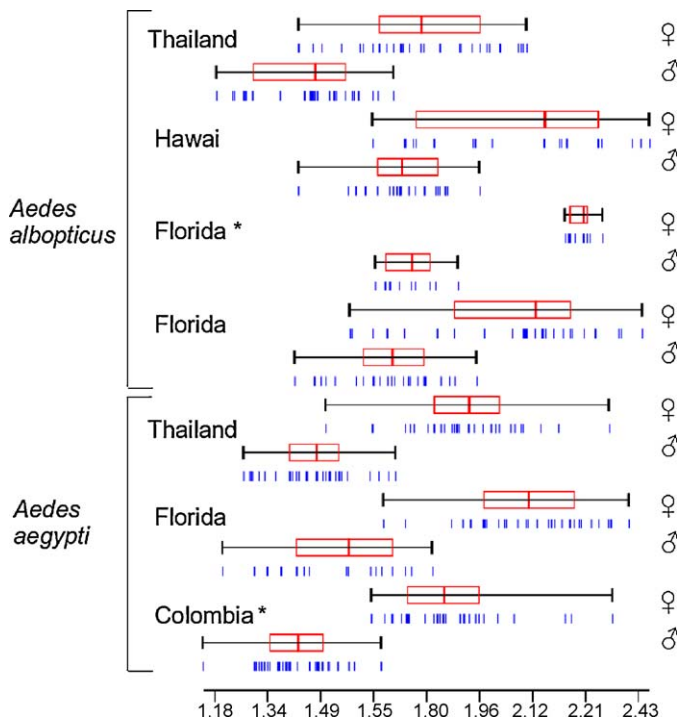


Fig. 2. Centroid size by geographic location for female and male specimens. Centroid size variation among species, geographic locations and sexes, presented as quantile plots. Vertical lines under the quantiles are individuals. Each box shows the median as a line across the middle and the quartiles (25th and 75th percentiles) as its ends. Units are pixels converted to millimeters. ♀, females; ♂, males. *, collected as juveniles (see Table 1).

The average Procrustes distance among *Ae. aegypti* populations from different continents was $.018 \pm .004$, which was not statistically higher than the average distance within Thailand (Jirakanjanakit et al., unpublished data). Such comparison could not be verified for *Ae. albopictus*.

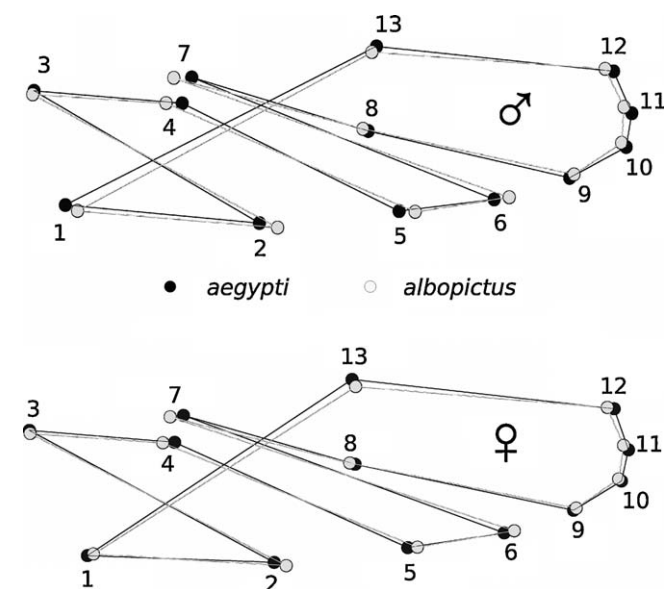


Fig. 3. Average residual coordinates for *Aedes aegypti* (black) and *Ae. albopictus* (gray). Coordinates obtained after translating, scaling and rotating the initial configurations, and then averaged by species. Landmarks are numbered as in Fig. 1 and connected to display the wing geometry. Their positions are relative to the ones of the consensus configuration (not shown). The patterns observed here for the total sample were visually identical to the patterns produced when comparing species coming from the same geographic location.

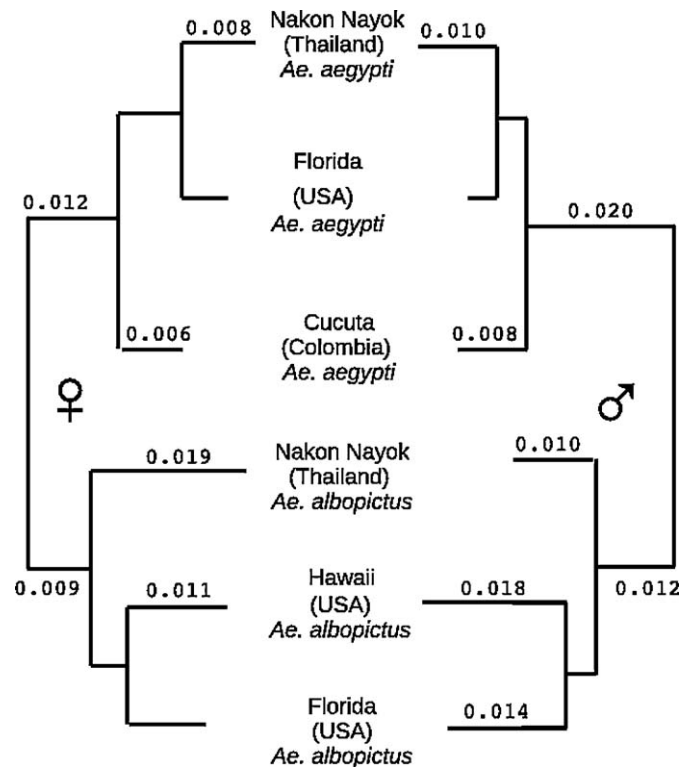


Fig. 4. Phenetic tree using Procrustes distances. The main subdivision is between species (*Ae. aegypti* above and *Ae. albopictus* below). Trees corresponding to males (right) and females (left) are displayed to highlight the global agreement of topologies according to sex. Values are the patristic distances derived from Procrustes distances. Algorithm for tree building was the *neighbor* algorithm as implemented in the PHYLIP package (J. Felsenstein), with the “no outgroup” option.

Phenetic analysis of both males and females using Procrustes distances resulted in trees with similar configurations (Fig. 4). Two clusters were seen representing the two species, and within each cluster the geographic areas were distinguishable. *Aedes aegypti* from Thailand and Florida grouped together and Colombia was an external population. *Aedes albopictus* from Hawaii and Florida formed a group and Thailand was separate. According to Procrustes patristic distances, *Ae. albopictus* was more differentiated than *Ae. aegypti* and the distance between species was larger in males (Fig. 4), but females had greater geographic differentiation.

3.4. Size and shape

In each species and sex, there was a significant amount of allometric residues still present in shape variables ($P < .0001$). The hypothesis of a common allometric model could not be rejected within each species, except for male *Ae. albopictus* ($P < .0025$). The complete removing of allometric residue in the *Ae. aegypti* samples and in the female *Ae. albopictus* did not modify the relative positions as observed in the trees (details not shown).

3.4.1. Classification

The IAR scores, before and after validation, were very high. The classification of the total sample using the Mahalanobis distances misclassified only 4 specimens (3 females and 1 male) out of 352, resulting in a 99% IAR (Tables 2 and 3). Upon validation, the IAR lowered to 97% in both sexes. In the two locations where both species were sampled the validated IAR combining the two sexes was 99% (Nakhon Nayok) and 94% (Florida). When considering both species and geographic location, the TAR were lower, ranging

Table 2

Females classification table.

	Female <i>Ae. albopictus</i>			Female <i>Ae. aegypti</i>		
	F(US)	H(US)	NN(T)	C(C)	F(US)	NN(T)
Female <i>Ae. albopictus</i>						
F(US)	36/27	0/2	2/2	0/0	1/1	0/0
H(US)	0/4	19/16	0/1	0/0	0/0	0/0
NN(T)	2/6	0/1	28/26	0/0	0/0	0/0
Female <i>Ae. aegypti</i>						
C(C)	0/1	0/0	0/0	27/23	0/1	1/1
F(US)	1/1	0/0	0/0	2/3	25/21	1/5
NN(T)	0/0	0/0	1/1	1/4	4/6	28/24
Total	39	19	30	30	30	30
TAR	.92/.69	1/.84	.93/.87	.90/.70	.83/.77	.93/.80
IAR	.97/.95	1/1	1/.97	1/1	.97/.97	1/1

Total (TAR) and interspecies (IAR) accuracy rates. Classification scores based on Mahalanobis distances. The accuracy rates are summarized as IAR and TAR. IAR (“interspecies accuracy rate”) gives the percentage of specimens correctly assigned to species, either *Ae. aegypti* or *Ae. albopictus*, while TAR (“total accuracy rate”) refers to correct species and geographic location assignment. Two values are separated by a slash: left values refer to correct assignments after reclassification, right values are obtained after validation (see Section 2). F(US) = Florida (United States), H(US) = Hawaii (United States), NN(T) = Nakhon Nayok (Thailand), C(C) = Cucuta (Colombia).

Table 3

Males classification table.

	Male <i>Ae. albopictus</i>			Male <i>Ae. aegypti</i>		
	F(US)	H(US)	NN(T)	C(C)	F(US)	NN(T)
Male <i>Ae. albopictus</i>						
F(US)	32/26	2/5	1/4	0/0	0/1	0/0
H(US)	6/8	18/14	3/4	0/0	0/0	0/0
NN(T)	1/4	2/2	26/22	0/0	0/2	0/0
Male <i>Ae. aegypti</i>						
C(C)	0/0	0/1	0/0	30/26	1/2	1/3
F(US)	1/2	0/0	0/0	1/2	18/11	3/4
NN(T)	0/0	0/0	0/0	1/4	1/4	26/23
Total	40	22	30	32	20	30
TAR	.80/.65	82/.64	.87/.73	94/.81	.90/.55	.87/.77
IAR	.98/.95	1/.95	1/1	1/1	1/.85	1/1

Total (TAR) and interspecies (IAR) accuracy rates. Classification scores based on Mahalanobis distances. The accuracy rates are summarized as IAR and TAR. IAR (“interspecies accuracy rate”) gives the percentage of specimens correctly assigned to species, either *Ae. aegypti* or *Ae. albopictus*, while TAR (“total accuracy rate”) refers to correct species and geographic location assignment. Two values are separated by a slash: left values refer to correct assignments after reclassification, right values are obtained after validation (see Section 2). F(US) = Florida (United States), H(US) = Hawaii (United States), NN(T) = Nakhon Nayok (Thailand), C(C) = Cucuta (Colombia).

from 80 to 100% before validation, and from 55% to 87% after validation. Geographic origin was determined, resulting in GAR ranging from 77% to 97% before validation, and from 50% to 80% after validation (Table 4). In both species, GAR was 67% on average and slightly higher in females (72%) than in males (64%) (from

Table 4). The highest GAR were obtained for Cucuta (Colombia) *Ae. aegypti* in both sexes. In the *Ae. albopictus* samples, Thailand had relatively high GAR in both sexes. Hawaii, which had the smallest sample sizes, showed very different GAR according to sex: among the highest in females and the lowest in males.

Table 4

Geographic accuracy rate (GAR) within species.

	Female <i>Ae. aegypti</i>			Male <i>Ae. aegypti</i>		
	C(C)	F(US)	NN(T)	C(C)	F(US)	NN(T)
C(C)	28/24	0/2	0/0	30/24	1/4	0/5
F(US)	2/2	25/20	2/10	0/3	18/10	5/5
NN(T)	0/4	5/8	28/20	2/5	1/6	25/20
Total	30	30	30	32	20	30
GAR	.93/.80	.83/.67	.93/.67	.94/.75	90/.50	.83/.67
	Female <i>Ae. albopictus</i>			Male <i>Ae. albopictus</i>		
	F(US)	H(US)	NN(T)	F(US)	H(US)	NN(T)
F(US)	36/26	1/2	0/6	36/27	2/7	0/2
H(US)	0/4	18/15	1/3	4/10	17/11	2/6
NN(T)	3/9	0/2	29/21	0/3	3/4	28/22
Total	39	19	30	40	22	30
GAR	.92/.67	.95/.79	.97/.70	.90/.68	.77/.50	93/.73

Classification scores within species according to geography, based on Mahalanobis distances. Two values separated by a slash: left values refer to correct assignments after reclassification, right values are obtained after validation (see Section 2). F(US) = Florida (United States), H(US) = Hawaii (United States), NN(T) = Nakhon Nayok (Thailand), C(C) = Cucuta (Colombia).

4. Discussion

Wing venation in mosquito genera like *Culex*, *Anopheles* and *Aedes* show the same venation patterns. Theoretically, bio-mechanical constraints impose an optimal arrangement of wing parts and produce convergent shapes among close genera and species. Therefore few differences should be exhibited between species, especially in very closely related species as are *Ae. albopictus* and *Ae. aegypti* (Reinert et al., 2004).

However, in mosquitoes and other insects, geometric morphometric analysis of the wing venation has proved to be useful in distinguishing sibling species (Matias et al., 2001; De la Riva et al., 2001; Villegas et al., 2002), geographic or ecotopic conspecific populations (Dujardin et al., 2003; Broide et al., 2004; Bustamante et al., 2004; Camara et al., 2006; Bouyer et al., 2007; Feliciangeli et al., 2007; Aytekin et al., 2007) and laboratory lines (Jirakanjanakit and Dujardin, 2005; Aytekin et al., 2009). It has even been shown powerful enough to allow identification at the individual level (Dujardin et al., 2007; Jirakanjanakit et al., 2008).

These subtle but consistent differences of the wing geometry between species, even cryptic species, could be partly attributed to their different interaction with the environment. We thus could expect some interference between interspecific and interregional differences, which has been observed to some extent (Dujardin and Le Pont, 2000; Gumiel et al., 2003; Dujardin et al., 2003). The present study demonstrates that in *Ae. aegypti* and *Ae. albopictus*, the specific patterns of the wing remains unchanged despite drastic founder effects, transcontinental migration, and differences in environment. It also shows that among four distant geographic areas, some local differentiation took place within *Ae. aegypti* and *Ae. albopictus* but was not sufficient to interfere with species identification.

Different sampling strategies could impact relative size variation in mean, variance or sexual dimorphism, as seen in the Florida subset of *Ae. albopictus* that were collected as juveniles (Fig. 3). Although size is generally considered as a labile trait (West-Eberhard, 1989), it did not significantly differ between species and tended to be relatively unaffected by the wide geographic sampling. In *Ae. aegypti*, Florida females were significantly larger and in *Ae. albopictus*, both sexes from Thailand were significantly smaller. These specimens were collected as adults, which reduces the likelihood that size variation was a simple reflection of breeding habitats conditions.

Different sampling strategies could also impact relative shape variation. We believe the use of both field-caught and F1 specimens within our collection of various species from different continents was unlikely to produce erratic changes in the tree topology. This is supported by the generally high levels of shape heritability. Various studies examining cross-environment heritability in Diptera produced high and stable heritability of wing shape, reaching 60% and more (Bitner-Mathé and Klaczko, 1999; Roff and Mousseau, 1987; Gilchrist and Partridge, 2001; Hoffman and Shirriffs, 2002), and recent quantitative genetic studies provided evidence for strong genetic determinism (Iriarte et al., 2003; Breuker et al., 2006; Patterson and Klingenberg, 2007).

The superimposed residual coordinates for the two species showed obvious differences in wing venation (Fig. 2), and these interspecific differences were visually the same in Thailand and in Florida. The sexes consistently diverged similarly at each of the landmarks, supporting the conclusion that there are evolutionary differences in wing shape between the two species.

The phenetic analysis for each sex resulted in similar relationships between species and geographic locations (Fig. 4). The branching patterns observed in both sexes of *Ae. aegypti* did not accord with geographic distances: Florida was closer to Thailand than to Colombia. Size variation could not explain such relation-

ships since according to size (Fig. 2), Florida would have constituted an external group. The use of F1 specimens which has the potential to induce lower variability could have caused Colombia to become an external group. This probability is lowered by the fact that the F1 specimens came from multiple broods. The shape differentiation of the Colombia *Ae. aegypti* from Cucuta might also be related to the history of *Ae. aegypti* conquest of South America: a first wave during the slave trade a few centuries ago (Gubler, 1997), a second wave after incomplete eradication in the 1960s (Lourenço-de-Oliveira et al., 2004). In this hypothesis, the peculiarity of wing shape in Cucuta would be inherited from the first wave of invasion. This idea is supported by historical records suggesting that *Ae. aegypti* from Cucuta was never completely eradicated due to its proximity to Venezuela (Groot, 1980). In both sexes the *Ae. albopictus* from Thailand was distant from the Hawaii-Florida pair. The phenetic structuring could not be related to the significantly smaller size of Thailand specimens (Fig. 2), since the complete removal of allometric effects in females did not change the respective positions of the three localities. It might be related to the difference expected between native and founder populations. *Aedes albopictus* is believed to originate from South East Asia (Hawley, 1988), thus both Hawaii and Florida samples would correspond to nonindigenous populations. It might also reflect a higher frequency of domestic and commercial travel between Florida and Hawaii than either with Thailand.

Aedes aegypti began its worldwide invasion centuries earlier than *Ae. albopictus*. For this reason, more differentiation was expected among the *Ae. aegypti* samples, but the opposite was observed (Fig. 4). Either gene flow was maintained more efficiently for *Ae. aegypti*, or the ability of *Ae. albopictus* to maintain its flexibility in habitats and hosts (Rodhain and Rosen, 1997) makes it prone to faster morphological change.

Ecological forces may also have influenced the hierarchy of the tree. We believe these influences were not as important since size, commonly considered as very responsive to ecological changes, was similar among most samples (Fig. 2). This suggests that within our data set ecological conditions or their effects were similar between areas. In addition, there was no evidence to support an influence of size variation on the tree classification: in the samples where size difference existed, it was removed with no effect on tree topology.

The high interspecies accuracy rate after validation (IAR = 97%) suggests that the two species could maintain a specific wing shape, not significantly influenced by genetic bottlenecks or by the environment. They confirm that the wing geometry can be a helpful tool for species recognition of insects (Dujardin, 2008).

The corresponding lower geographic accuracy rates (GAR = 67%) and the comparable Procrustes distances among and within countries, as seen with *Ae. aegypti*, indicate poor to moderate differentiation, suggesting a relatively robust morphology of the wing in each species and in each sex.

We believe at least two factors could account for the present patterns of relative morphological uniformity: canalizing mechanisms and persistent gene flow. The wing of *Aedes* sp. is an important organ, not only for flying but also for sexual signaling (Cator et al., 2009). As a consequence, strong canalizing mechanisms are expected to be at work. It is also possible that continual migrations may counteract local drift processes. Thus, the morphological steadiness observed here could also be attributed to sporadic, recurrent mixing of populations, thwarting phenotypic drift.

The present *Aedes* material composed of *Ae. aegypti* and *Ae. albopictus* from around the world provided sufficient material to explore the possible interference between interspecific and geographic differences of metric properties. In spite of likely founder effects and ecological variations, the morphology of these mosquitoes, for both size and shape, appeared well preserved.

Acknowledgments

To Jason Stuck (Pinellas County Public Works Highway, US) for providing Florida adult mosquitoes samples, and to Georges O'Meara (Florida Medical Entomology Lab, US). To Lincoln Suesdek (Instituto Butantan, Brazil) and Darcy Welch (Jules Stein Institute, US) for critical relecture. To P. Kittayapong (CVVD, Mahidol Univ, Thailand) for helping this investigation. This study has been supported by IRD (Institut de Recherche pour le Développement), Colciencias Colombia (project 1115-343-19131, contract 360-2006) and the University of Hawaii, Integrated Graduate Research Training Grant funded by National Science Foundation.

References

- Arnqvist, G., Mårtensson, T., 1998. Measurement error in geometric morphometrics: empirical strategies to assess and reduce its impact on measure of shape. *Acta Zoologica Academiae Scientiarum Hungaricae* 44 (1/2), 73–96.
- Aytekin, A.M., Alten, B., Caglar, S., Ozbel, Y., Kaynas, S., Simsek, F.M., Kasap, O.E., Belen, A., 2007. Phenotypic variation among local populations of phlebotomine sand flies (Diptera: Psychodidae) in southern Turkey. *Journal of Vector Ecology* 32 (2), 226–234.
- Aytekin, S., Aytekin, A.M., Alten, B., 2009. Effect of different larval rearing temperatures on the productivity (Ro) and morphology of the malaria vector *Anopheles superpictus* Grassi (Diptera: Culicidae) using geometric morphometrics. *Journal of Vector Ecology* 43 (1), 32–42.
- Benedict, M.Q., Levine, R.S., Hawley, W.A., Lounibos, L.P., 2007. Spread of the tiger: global risk of invasion by the mosquito *Aedes albopictus*. *Vector Borne Zoonotic Diseases* 7 (1), 76–85.
- Bitner-Mathé, B.C., Klaczko, L.B., 1999. Size and shape heritability in natural populations of *Drosophila mediopunctata*: temporal and microgeographical variation. *Genetica* 105, 35–42.
- Bookstein, F.L., 1991. Morphometric tools for landmark data, Geometry and Biology. Cambridge University Press, NY.
- Bosio, C.F., Harrington, L.C., Jones, J.W., Sithiprasasna, R., Norris, D.E., Scott, T.W., 2005. Genetic structure of *Aedes aegypti* populations in Thailand using mitochondrial DNA. *American Journal of Tropical Medicine and Hygiene* 72 (4), 434–442.
- Bouyer, J., Ravel, S., Dujardin, J., de Meeus, T., Vial, L., Thevenon, S., Guerrini, L., Sidibe, I., Solano, P., 2007. Population structuring of *Glossina palpalis gambiense* (Diptera: Glossinidae) according to landscape fragmentation in the Mouhoun river, Burkina Faso. *Journal of Medical Entomology* 44 (5), 788–795.
- Bracco, J.E., Capurro, M.L., Lourenço-de, R., Sallum, M.A.M., 2007. Genetic variability of *Aedes aegypti* in the Americas using a mitochondrial gene: evidence of multiple introductions. *Memorias de Instituto Oswaldo Cruz* 102 (5), 573–580.
- Breuker, C., Patterson, J., Klingenberg, C., 2006. A single basis for developmental buffering of *Drosophila* wing shape. *PLoS ONE* 1 (1), e7.
- Broide, J.S., Dujardin, J.P., Kitron, U., Gurtler, R.E., 2004. Spatial structuring of *Triatoma infestans* (Hemiptera: Reduviidae) populations from northwestern Argentina using wing geometric morphometry. *Journal of Medical Entomology* 41 (4), 643–649.
- Bustamante, D.M., Monroy, C., Menes, M., Rodas, A., Salazar-Schettino, P.M., Rojas, G., Pinto, N., Guhl, F., Dujardin, J.P., 2004. Metric variation among geographic populations of the Chagas vector *Triatoma dimidiata* (Hemiptera: Reduviidae: Triatominae) and related species. *Journal of Medical Entomology* 41 (3), 296–301.
- Camara, M., Caro-Riño, H., Ravel, S., Dujardin, J., Hervouet, J., de Meeus, T., Kagbadouno, M.S., Bouyer, J., Solano, P., 2006. Genetic and morphometric evidence for isolation of a tsetse (Diptera: Glossinidae) population (Loos islands, Guinea). *Journal of Medical Entomology* 43 (5), 853–860.
- Caro-Riño, H., Jaramillo, N., Dujardin, J.P., 2009. Growth changes in *Rhodnius pallescens* under simulated domestic and sylvatic conditions. *Infection, Genetics and Evolution* 9 (2), 162–168.
- Cator, L.J., Arthur, B.J., Harrington, L.C., Hoy, R.R., 2009. Harmonic convergence in the love songs of the dengue vector mosquito. *Science* 323 (5917), 1077–1079.
- Chastel, C., 2005. Chikungunya virus: its recent spread to the southern Indian Ocean and Reunion Island (2005–2006). *Bulletin Academic of National Medicine* 189 (8), 1827–1835.
- De la Riva, J., Le Pont, F., Ali, V., Matias, R., Mollinedo, S., Dujardin, J.P., 2001. Wing geometry as a tool for studying the *Lutzomyia longipalpis* (Diptera: Psychodidae) complex. *Memorias do Instituto Oswaldo Cruz* 96 (8), 1089–1094.
- Dujardin, J.P., 2008. Morphometrics applied to medical entomology. *Infection, Genetics and Evolution* 8, 875–890.
- Dujardin, J.P., Beard, C.B., Ryckman, R., 2007. The relevance of wing geometry in entomological surveillance of Triatominae, vectors of Chagas disease. *Infection, Genetics and Evolution* 7 (2), 161–167.
- Dujardin, J.P., Le Pont, F., 2000. Morphometrics of a neotropical sandfly subspecies, *Lutzomyia carrerai thula*. *Comptes-rendus de l'Académie des Sciences Série III Sciences de la Vie—Life Sciences* 323 (3), 273–279.
- Dujardin, J.P., Le Pont, F., Baylac, M., 2003. Geographic versus interspecific differentiation of sandflies: a landmark data analysis. *Bulletin of Entomological Research* 93, 87–90.
- Effler, P.V., Pang, L., Kitsutani, P., Vorndam, V., Nakata, M., Ayers, T., Elm, J., Tom, T., Reiter, P., Rigau-Perez, J.G., Hayes, J.M., Mills, K., Napier, M., Clark, G.G., Gubler, D.J., 2005. Dengue fever, Hawaii, 2001–2002. *Emerging Infectious Diseases* 11 (5), 742–749.
- Failloux, A.B., Vazeille, M., Rodhain, F., 2002. Geographic genetic variation in populations of the dengue virus vector *Aedes aegypti*. *Journal of Molecular Evolution* 55 (6), 653–663.
- Feliciangeli, M., Sanchez-Martin, M., Marrero, R., Davies, C., Dujardin, J., 2007. Morphometric evidence for a possible role of *Rhodnius prolixus* from palm trees in house re-infestation in the State of Barinas (Venezuela). *Acta Tropica* 101, 169–177.
- Gilchrist, A.S., Partridge, L., 2001. The contrasting genetic architecture of wing size and shape in *Drosophila melanogaster*. *Heredity* 86, 144–152.
- Good, P., 2000. Permutation Tests: A Practical Guide to Resampling Methods for Testing Hypotheses. Springer, New York.
- Groot, H., 1980. The reinvansion of colombia by *Aedes aegypti*: aspects to remember. *American Journal of Tropical Medicine and Hygiene* 29 (3), 330.
- Gubler, D.J., 1997. Dengue and dengue hemorrhagic fever; its history and resurgence as a global public health problem. In: Gubler, D.J., Kuno, G. (Eds.), *Dengue and Dengue Hemorrhagic Fever*. CAB International Press, pp. 1–22.
- Gubler, D.J., 2002. Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. *Trends in Microbiology* 10 (2), 100–103.
- Gubler, D.J., 2005. The emergence of epidemic dengue fever and dengue hemorrhagic fever in the Americas: a case of failed public health policy. *Revista Panameana de Salud publica* 17 (4), 221–224.
- Gumiel, M., Catala, S., Noireau, F., de Arias, A.R., Garcia, A., Dujardin, J.P., 2003. Wing geometry in *Triatoma infestans* (Klug) and *T. melanosome* Martinez, Olmedo and Carcavallo (Hemiptera: Reduviidae). *Systematic Entomology* 28 (2), 173–179.
- Hawley, W.A., 1988. The biology of *Aedes albopictus*. *Journal of American Mosquito Control Association* 4 (Suppl.), 1–40.
- Hawley, W.A., Reiter, P., Copeland, R.S., Pumpuni, C.B., Craig, G.B., 1987. *Aedes albopictus* in North America: probable introduction in used tires from northern Asia. *Science* 236 (4805), 1114–1116.
- Hoffman, A.A., Shirriffs, J., 2002. Geographic variation for wing shape in *Drosophila serrata*. *Evolution* 56, 1068–1073.
- Iriarte, F.P.W., Céspedes, W.C., Santos, M., 2003. Quantitative genetic analysis of wing form and bilateral asymmetry in isochromosomal lines of *Drosophila subobscura* using Procrustes methods. *Journal of Genetics* 82, 95–113.
- Jirakanjanakit, N., Dujardin, J., 2005. Discrimination of *Aedes aegypti* (Diptera: Culicidae) laboratory lines based on wing geometry. *The Southeast Asian Journal of Tropical Medicine and Public Health* 36 (4), 1–4.
- Jirakanjanakit, N., Leemingsawat, S., Dujardin, J.P., 2008. The geometry of the wing of *Aedes (Stegomyia) aegypti* in isofemale lines through successive generations. *Infection Genetics and Evolution* 8, 414–421.
- Jirakanjanakit, N., Leemingsawat, S., Thongrunkiat, S., Apiwathnasorn, C., Singhanon, S., Bellec, C., Dujardin, J.P., 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.
- Josseran, L., Paquet, C., Zehgnoun, A., Caillere, N., Tertre, A.L., Solet, J.L., Ledrans, M., 2006. Chikungunya disease outbreak, Reunion Island. *Emerging Infectious Diseases* 12 (12), 1994–1995.
- Kambhampati, S., Black, W., Rai, K.S., 1991. Geographic origin of the US and Brazilian *Aedes albopictus* inferred from allozyme analysis. *Heredity* 67 (1), 85–93.
- Lourenço-de-Oliveira, R., Vazeille, M., de Filippis, A.M.B., Failloux, A.B., 2004. *Aedes aegypti* in Brazil: genetically differentiated populations with high susceptibility to dengue and yellow fever viruses. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 98 (1), 43–54.
- Matias, A., De la Riva, J.X., Torrez, M., Dujardin, J.P., 2001. *Rhodnius robustus* in Bolivia identified by its wings. *Memorias do Instituto Oswaldo Cruz* 96 (7), 947–950.
- Mousson, L., Dauga, C., Garrigues, T., Schaffner, F., Vazeille, M., Failloux, A.B., 2005. Phylogeography of *Aedes (Stegomyia) aegypti* (L.) and *Aedes (Stegomyia) albopictus* (Skuse) (Diptera: Culicidae) based on mitochondrial DNA variations. *Genetical Research* 86 (1), 1.
- Patterson, J., Klingenberg, C., 2007. Developmental buffering: how many genes? *Evolution and Development* 9 (6), 525–526.
- Reinert, J.F., Harbach, R.E., Kitching, I.J., 2004. Phylogeny and classification of *Aedini* (Diptera: Culicidae), based on morphological characters of all life stages. *Zoological Journal of the Linnean Society* 142, 289–368.
- Reiter, P., Darsie, R.F., 1984. *Aedes albopictus* in Memphis, Tennessee (USA): an achievement of modern transportation? *Mosquito News* 44, 396–399.
- Rezza, G., Nicoletti, L., Angelini, R., Romi, R., Finarelli, A.C., Panning, M., Cordioli, P., Fortuna, C., Boros, S., Magurano, F., Silvi, G., Angelini, P., Dottori, M., Ciufolini, M.G., Majori, G.C., Cassone, A., 2007. Infection with chikungunya virus in Italy: an outbreak in a temperate region. *The Lancet* 370 (9602), 1840–1846.
- Rodhain, F., Rosen, L., 1997. Mosquito vectors and dengue virus–vector relationships. In: Gubler, D.J., Kuno, G. (Eds.), *Dengue and Dengue Hemorrhagic Fever*. CAB International Press, pp. 45–60.
- Roff, D.A., Mousseau, T.A., 1987. Quantitative genetics and fitness: lessons from *Drosophila*. *Heredity* 58, 103–118.
- Rohlf, F.J., 1990. Rotational fit (Procrustes) methods. In: Rohlf, F., Bookstein, F. (Eds.), *Proceedings of the Michigan Morphometrics Workshop. Special Publication Number 2*. The University of Michigan Museum of Zoology, Ann Arbor, MI, pp. 380. University of Michigan Museums, Ann Arbor, pp. 227–236.

- Rohlf, F.J., 1999. Shape statistics: Procrustes superimpositions and tangent spaces. *Journal of Classification* 16, 197–223.
- Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4 (4), 406–425.
- Staples, J.E., Monath, T.P., 2008. Yellow Fever: 100 years of discovery. *JAMA* 300 (8), 960–962.
- Villegas, J., Feliciangeli, M., Dujardin, J., 2002. Wing shape divergence between *Rhodnius prolixus* from Cojedes (Venezuela) and *R. robustus* from Mérida (Venezuela). *Infection, Genetics and Evolution* 2, 121–128.
- West-Eberhard, M., 1989. Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics* 20, 249–278.
- Zelditch, M.L., Swiderski, D.L., Sheets, H.D., Fink, W.L., 2004. *Geometric Morphometrics for Biologists: A Primer*. Elsevier, Academic Press, New York.