Initial Evidence of *Aedes Albopictus* (Diptera: Culicidae) Domiciliation in Havana City, Cuba 1995-2018.

María del Carmen Marquetti Fernández (marquetti@ipk.sld.cu)
Instituto de Medicina Tropical Pedro Kouri

Magaly Castillo
Centro Provincial de Higiene, Microbiología y Epidemiología

Iris Peraza
Centro Provincial de Higiene, Microbiología y Epidemiología

Maricely Milian
Centro Provincial de Higiene, Microbiología y Epidemiología

Roberto Molina
Centro Provincial de Higiene, Microbiología y Epidemiología

Maureen Leyva
Instituto de Medicina Tropical Pedro Kouri

Juan A. Bisset
Instituto de Medicina Tropical Pedro Kouri

Veerle Vanlerberghe
Institute of Tropical Medicine Belgium

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Abstract

Background. Various arboviruses are transmitted to humans by mosquitoes, particularly Aedes *aegypti* and *Aedes albopictus,* two invasive and frequently sympatric species. The objective of this study was to evaluate the behavior of *Ae. albopictus* with regards to houses and association with other mosquitoes in Havana province.

Methods. All deposits containing water in the houses and vacant lots of urban and peri urban municipalities of the province Havana were sampled during two periods: the first 1995 – 1999 and the second 2010-2018.

Results. Patterns in the presence of Ae*. albopictus* in the study area were observed: the persistent absence of *Ae. albopictus* in one municipality; in two municipalities, starting from an absence in the first period to a rapid dispersion in the following period; and a sustained decrease in the dispersion of *Ae. albopictus* in two other municipalities. The association of *Ae. albopictus* with other mosquitoes smaller in the peripheral municipalities, although being the ones with the greatest presence of *Ae. albopictus*. However overall, we found an increase in this association when comparing the period 2010-2018 with the first period since its introduction in Havana. Inside the houses, *Ae albopictus* was present in 8% (2016) to 21.5% (2013) with an average of 15%, which evidences an initial domiciliation of the species.

Conclusions. The results obtained in this work show an initiation of domiciliation of *Ae albopictus* in the urban area of Havana province. This is important to alert the National Control Program to strengthen the entomological monitoring of *Ae. albopictus*, and not only *Ae. aegypti*. The follow up of this domiciliation is important to guide control efforts, knowing its role as a vector of different arboviruses.

Background

The different arboviruses, dengue, chikungunya, zika and yellow fever are primarily transmitted to humans by mosquitoes, particularly *Aedes aegypti* (Linnaeus, 1762) and *Aedes albopictus* (Skuse, 1894), two invasive and frequently sympatric species. *Ae albopictus* is often considered as a secondary vector, especially for dengue, based on its lower capacity to transmit pathogens to humans, but its potential to sustain transmission and provoke outbreaks is real [1–2].

Ae. albopictus presence was registered for the first time in Cuba in 1995, in La Lisa municipality in Havana province [3]. It gradually spread, so that nowadays it is distributed throughout the entire Cuban territory. The latest scientific report of this species informed, indeed, about its presence up to the province of Santiago de Cuba located in the eastern side of the country [4].

There are few studies on the ecology of *Ae. albopictus* in Cuba, among them the one that reports on the preferred breeding sites [5] which showed that larvitrap was the most positive container followed by cans, with a preference for breeding outside the houses [6]. On the other hand, this species was also found breeding in used car tires, mainly in the rainy season, pointing out an allopatric distribution with respect

to *Ae. aegypti* [7]. In Pinar del Río province situated in the western of Cuba, the gradual dispersion of *Ae. albopictus* occurred between 2003 and 2008 with preference to breed in larvitraps, cans and water storage containers [8].

In Havana, *Ae. albopictus* abundance is mainly present in the peripheral municipalities where there is abundant vegetation, and not in the center of town, where there is even a municipality that never reported the presence of this species up to now [6–7, 9]. The above indicates that in Cuba, as in other parts of the world, *Ae. albopictus* is typically more common in areas with presence of vegetation or in transitory environments with relatively low presence of vegetation and is coexisting with *Ae. aegypti* [10–12].

Several studies indicate that *Ae. albopictus* and *Ae. aegypti* can share the same habitat [6, 7]. In some settings, such as parts of Southeast Asia, it was hypothesized that *Ae. aegypti* has completely replaced *Ae. albopictus* [13]. Conversely, the observations on the dispersion of *Ae. albopictus* in the southern coastal states of the United States indicates that its expansion seems to occur at the expense of *Ae. aegypti*; and it has been observed that *Ae. albopictus* introduction has been accompanied by a drastic and rapid decline in *Ae. aegypti* populations [14–16].

Coexistence between *Ae. albopictus, Ae. aegypti* and *Culex quinquefasciatus* has been demonstrated in a study conducted in Havana about the inter-specific relationship between *Aedes spp*. and others culicids. This study showed evident displacement of *Aedes mediovittatus* by *Ae. albopictus* in the breeding sites [17].

Despite several probable explanations, both phenomena (coexistence and displacement) of competitive substitutions of *Aedes spp*. in several parts of the world still represent an ecological enigma. This knowledge about abundance and inter specific relationship between these species is essential, to be able to predict the role of each in the transmission of arboviruses. In this study we want to evaluate the behavior of *Ae. albopictus* with regards to houses and association with other culicids in Havana province. It's an important piece of evidence to understand the epidemiology and transmission dynamics of the arboviruses.

Methods

Study area

Havana is located in the 22°58', 23°10' north latitude and 82°30', 82°06 west longitudes, and belongs to the western region of Cuba. The hydrography is composed by the Almendares, Martín Pérez, Quibú, Cojímar and Bacuranao rivers, among others aquifer reservoirs. The climate of the city is tropical, as in the rest of the island, and there are two seasons: rainy season between May and October and dry season between November and April, although it is possible that rains occur during the dry season due to the presence of cold fronts coming from the north. Havana province is administratively divided into 15 municipalities which are: Plaza de la Revolución , Habana Vieja, Centro Habana, Diez de Octubre, Cerro, Arroyo Naranjo, Boyeros, Playa, Marianao, La Lisa, Guanabacoa, Regla, Habana del Este, San Miguel del

Padrón and Cotorro (**Fig. 1**). The city has a population of 2.1 million inhabitants (National Statistics Office of Cuba, 2011; Official Census, 2012).

Municipalities Classification

According the urbanization characteristics and the geographical location in the province, the municipalities were categorized in three groups: the peri-urban group, which are the peripheral or peri-urban municipalities Guanabacoa, Arroyo Naranjo, Boyeros and Cotorro characterized by having borders with other provinces and a concentrated urbanization in one specific area of the municipality: a mixed group, which are the municipalities Playa, La Lisa and Habana del Este which have borders with other provinces but with a scattered urbanization throughout the municipality; and an urban group , which are the municipalities of San Miguel del Padrón, Regla, Plaza de la Revolución, Marianao, Habana Vieja, 10 de Octubre, Centro Habana and Cerro which have a high population concentration in their territory and are concentrated in the center and north (sea-side) of the province surrounded by the municipalities of the other two groups. From now on, we refer to these three groups as 'urbanization groups'.

Entomological sampling

The routine *Aedes* control program in Havana province inspects all houses and all vacant lots twice a month and every month, in 1995-1999 and 2010-2018 respectively. During these surveys, all water containing deposits are inspected for the presence of immature stages of the *Aedes* mosquito. Of every container positive for *Aedes* larvae, stages are collected and transported to the laboratory for species identification (dissociating *Culex, Ae. albopictus, Ae. aegypti*). For this study, we retrieved the data from all houses and vacant lots that were surveyed in all municipalities of Havana province during two periods: the first period corresponded to 1995 – 1999 and the second to 2010-2018. The personnel who carried out the sampling were from the *Ae. aegypti* and *Ae. albopictus* control program established in Cuba. House-level sampling methodology used was as recommended by the program [18-19] and consisted in starting at the opening point of the house-block and continuing so that each house on your right is visited; upon arriving at the house, once the permit for sampling has been granted, starting by the yard to continue later with the rooms, always right-side and from the entry forward, in case one cannot inspect a room, as well as some closed housing, this is duly noted and inspection is done on another day. The data is collected in the established form for the program.All types of deposits were reviewed (water storage recipients such as low tanks, buckets, elevated tanks, cisterns among others; small miscellaneous artificial deposits such as cans, knobs, bottles, animal drinkers, plastic cups; used car tires; drains and sewage pits, water registers; natural breeding such as trees hole, puddles, coconut shells). In Cuba, larvitraps [20] are used for surveillance of *Ae. aegypti* and *Ae. albopictus* in all municipalities, except during periods of intensive chemical treatment carried out in reaction to increasing *Ae. aegypti* infestation.

Mosquito samples identification

A sample was taken from each container being positive for mosquito larvae. This larvae sample is placed in a vial with a label containing the necessary information about its location, and is subsequently identified, using morphological keys for culicids [21-22]. These samples are first identified in the laboratories at the municipal level and subsequently sent to the provincial laboratory for quality control of the morphological identification of mosquito species. Specimens collected and identified remain in this laboratory. *Ae. albopictus* presence outside and inside the house was only recorded in the period 2013 – 2018. The location of the containers with *Ae. albopictus* were known to be outside the houses and hence this information was not reported in the first period 1995-1999. During 2010-2013 no data are available on *Ae. albopictus*. **Statistical analysis**The number of breeding sites where *Ae. albopictus* was detected was mapped over the entire province and over the two study periods. Of all *Ae. albopictus* breeding sites per study period, the relative importance per urbanization group was calculated. A descriptive analysis over time was done of the *Ae. albopictus* breeding sites on co-existence or not with other species, including the most important species with whom it is associated. To evaluate the determinants of the presence of *Ae. albopictus* breeding sites, we constructed a generalized linear random effect regression model with a negative binomial link function. This model considered the nature of the data (repeated measurements in municipalities). We evaluated the effect of belonging to one of the two periods and belonging to a certain urbanization group on the yearly municipal number of *Ae. albopictus* breeding sites. For the periods separately, we estimated also the association between number of *Ae. albopictus* breeding sites and the year (as covariate) and urbanization group (as factor). IBM SPSS statistics version 25 was used.

Results

The number of *Ae. albopictus* breeding sites per municipality and per year are shown in Fig. 2, where an increase in the number of *Ae. albopictus* positive municipalities and the number of *Ae. albopictus* sites over the years can be observed. Over the first five year after its introduction, being 1995, on the island, *Ae. albopictus* was present in 10 of the 15 municipalities of the province of Havana. The yearly number of *Ae. albopictus* breeding sites is shown in Table 1. In this first period 1995–1999, besides the geographical expansion, we see also a statistical significant increase in the number of *Ae. albopictus* breeding sites over the years (adjusted OR of 2.05, with 95% CI (1.54–2.73) and depending on urbanization group: in comparison to the urban group, the mixed group had twice the risk (adjusted OR of 2.12, 95% CI (0.83–5.41)), and the peri-urban group a risk of five (adjusted OR of 5.65, 95% CI (1.92–16.64)). In the second period 2010–2018 the presence of *Ae. albopictus* increased up to 14 of the 15 municipalities, but the presence of breeding sites did not depend on the years (adjusted OR of 0.98, 95% CI (0.88–1.08)), but were more importantly associated with the urbanization groups: in comparison to the urban group, the mixed group had almost four times higher risk of infestation (adjusted OR of 3.74, 95% CI (1.15–12.20), and the peri-urban group a risk of thirteen (adjusted OR of 13.63, 95% CI (4.69–39.57)). This mosquito was not reported during the two study periods in the municipality ‘Centro Habana.

Table 1
Total breeding sites with Ae. albopictus presence sampled in Havana province, in two period 1995–1999 and 2010–2018.

Years	Total of breeding sites
1995	93
1996	260
1997	203
1998	984
1999	1699
2010	3194
2011	3134
2012	3466
2013	1993
2014	1912
2015	2032
2016	3332
2017	3599
2018	2821

Proportional, the peri-urban group takes a bigger proportion of *Ae. albopictus* breeding sites in period two in comparison to period 1. In both other groups, there is a proportional decline over (Figure. 3).

When looking over the two study periods, the peri-urban municipalities are indeed clearly more infested than the urban ones, and there is also a clear increase in the second period in comparison to the first period (Table 2).

Table 2
Factors associated with Ae. albopictus breeding sites, Havana province, study periods 1995–1999 and 2010–2018.

Factors	Adjusted OR (95% CI)
Mixed	3.18 (1.24–8.13)
Peri-urban	10.32 (4.17–25.56)
Study Periods	
Period 1 (reference)	1
Period 2	3.08 (2.02–4.70)

The *Ae. albopictus* breeding sites can be divided into sites with exclusive *Ae albopictus* and sites where a mix of culicids can be found. In (Fig. 4), one can observe the heterogeneity of this proportion over municipalities in the second study period. In the multivariable model, we see a statistically significant association between exclusive *Ae. albopictus* breeding sites and the second period (adjusted OR 1.64, 95%CI (1.09–2.46)) and with the mixed urbanization group (adjusted OR 2.28, 95% CI (1.43–3.63).

In the mixed breeding sites, we see an increased association of *Ae. albopictus* with *Ae. aegypti* in the second period accompanied by a decrease in the association with *Ae. mediovittatus*. (Fig. 5) The association with *Cx. quinquefasicatus* and the group of other species did not show considerable changes. This increase of association with *Ae. aegypti* is a general phenomenon and has an increasing trend over the years (Fig. 6). *Culex nigripalpus*, *Ochlerottatus scapularis*, *Psorophora confinnis*, *Culex corniger* and *Anopheles albimanus* were the species found associated with *Ae. albopictus* in the category of other associations.

The number of *Ae. albopictus* breeding sites inspected during 2013–2018 were categorized to be inside or outside the house/premise. There is an important proportion, ranging from 8–21.5%, of *Ae. albopictus* breeding sites that can be found inside the houses, while this was not observed in the first period, where only outside sites were recorded (Fig. 7). The outside/inside proportion remains relatively stable over the second study period, which evidences an initial stable domiciliation of the species.

Discussion

This study represents an analysis of *Ae. albopictus* behavior from its first registration in Havana, 1995, up to 2018. It highlights results on its dispersion and initial evidence of their domiciliation in the last years. There is a sustained increase in the number of Ae albopictus breeding sites shared / mixed with *Ae. aegypti*, known dominant specie in the urban ecosystem in Havana province [10].

A limitation of this study is that surveillance data from the routine *Ae. aegypti* and *Ae. albopictus* Control Program were used. This program carried out control measures such as the use of Temephos (Abate) and

Bactivec® (*Bacillus thuringiensis* H-14) permanently in water containers detected by vector operators who perform the inspection in homes, workplaces and vacant lots. In addition, the inspection frequency is generally monthly but sometimes the frequency is increased up to 11-days surveys in case of high *Ae. aegypti* infestation or epidemiological alerts. These variations are the same for all municipalities; hence no differential impact is expected.

Aedes albopictus expanded geographically from 2010 to 2018 to 14 of the 15 municipalities that the province of Havana possesses, which shows, in addition to its introduction, its dispersal power. It should be noted that in the first years of its introduction (1995–1997) an attempt was made to eliminate *Ae. albopictus* mainly through chemical measures, but the objective was, obviously, not achieved. This could be due to various factors such as those of an operational nature in the control carried out (being more targeted to indoor spaces, where the main *Ae. aegypti* breeding sites are localized) or due to ignorance about *Ae. albopictus* insecticide, widely used in national control actions, susceptibility and / or resistance status [17]. On the other hand, different authors argue that the control of *Ae. albopictus*, in particular, is excessively difficult by living often far from the dwellings which implies a more extensive coverage needed than for *Ae. aegypti* [23].

The scanty presence of *Ae. albopictus* in the municipalities Habana Vieja and Diez de Octubre and their absence during the period studied in Centro Habana can be explained by the lack of areas with abundant vegetation in these urban municipalities. The presence of vegetation is important for this mosquito, since it has been shown that it is rarely found in rural areas where vegetation is eliminated [24].

It is known that *Ae. aegypti* is abundant in urban areas and *Ae. albopictus* in rural areas and that both can coexist in semi-urban areas [7], breeding mainly in artificial and natural breeding sites. They can even be found in slightly polluted waters [25, 26] which explains the observed association with *Cx. quinquefasciatus* mosquito, which prefer these type of breeding sites, but can also be found together with *Aedes* in used car tires [6] [26–28].

Aedes mediovittatus the other species with one of the highest percentages of association is present mainly in the Caribbean area with preference for rearing in tree holes [22] [29]. During the intensive phase with insecticides that took place in Cuba during 1981, the presence of *Ae. aegypti* was drastically reduce in the urban ecosystem which favored an increase in the presence of *Ae. mediovittatus* until the introduction of *Ae. albopictus* [4] [10] where this species has since been subjected to strong pressure and where in recent years it has been displaced by *Ae. albopictus* [17].

No similar patterns were found regarding the number of exclusive *Ae. albopictus* breeding sites in the province, mostly found in the peri-urban municipalities (Boyeros, Cotorro, Guanabacoa and Arroyo Naranjo) followed by Habana del Este and La Lisa belonging to mixed urbanization group, while the urban municipalities presented the highest number of mixed *Ae. albopictus* breeding sites with other culicids.

Studies conducted with *Ae. aegypti* and *Ae. albopictus* showed that the last one has a greater longevity in adult phase at different temperatures and humidity, which determined the influence of these climatic factors on the geographical distribution of these species [30]. These parameters that favor the occurrence of microclimates in certain areas of the province of Havana mainly in peri-urban areas with greater vegetation and in vegetative patches within the center of the province guarantee the appropriate conditions for a greater presence of *Ae. albopictus* in certain areas and favors the coexistence of both species.

Coexistence between *Ae. aegypti* and *Ae. albopictus* could also be favored under the Cuban conditions of strong insecticide pressure, to which the *Ae. aegypti* populations are subjected from 1981 up to today in Havana [10] [31]. The finding of *Ae. albopictus* in breeding sites inside the houses, although in an incipient but stable way, indicate towards an initiation of a domiciliation of the species.

It is known that there is hybridization between both species where the male of *Ae. albopictus* mates with the female *Ae. aegypti* resulting in a descendant of permanent sterilization in females resulting from this crossing and influencing the displacement of one species by another. [32, 33], However, a 15-year study in Rio de Janeiro, Brazil [34] suggested that the two species can achieve relative coexistence in the absence of strong competitive displacement by *Ae. albopictus* males for mating with *Ae. aegypti* females suggesting the low potential of hybridization and the lack of displacement between both species for these reasons [11]. On the other hand, another Brazilian study showed that even with high seasonal densities the effects of inter specific competition with *Ae. aegypti* was not enough to displace *Ae. albopictus* [12].

In Cuba, there is only evidence of hybridization between *Ae. aegypti* and *Ae. albopictus* at the laboratory level [35]. At the field level, it was determined that until 2012 only one coexistence between both species could be demonstrated [17], a result that we continue to support in this study until 2018. We do not doubt that the Cuban populations of these species exhibit the same behavior, as observed in Brazil in terms of hybridization, although in the Cuban situation there is an important difference of an influencing condition, namely the maintained chemical control with the use of Temephos in the water-holding containers in the houses. This last indication whose objective is to control *Ae. aegypti* populations in Havana until now has not avoided that this species continuing to be the dominant species in the urban ecosystem of this province in Cuba.

[35].

Conclusions

The results obtained in this work show, however, an initiation of domiciliation of *Ae albopictus* in the province of Havana endorsed by the increase in breeding sites shared with *Ae. aegypti* over time and by the relatively stable trend of the indoor presence of this mosquito.

These findings together with results obtained by other authors about the ability of *Ae. albopictus* gravid females to disperse at least 800 meters [36–38], in addition to easily moving from areas with abundant vegetation to the urban area [38] and the finding of this specie in urban area [38, 39] we consider it important to alert the National Control Program to strengthen the entomological monitoring of *Ae. albopictus*. The follow up of this domiciliation is important to guide control efforts, knowing its role as a vector of different arbovirosis [40].

Study Limitations

The work was carried out with the analysis of the data provided by the *Aedes aegypti* and *Aedes albopicts* Control Program implemented in Cuba at the municipal level of each province. This program carried out control measures such as the use of Temephos (Abate) and Bactivec® (*Bacillus thuringiensis* H-14) permanently in water containers detected by vector operators who perform the inspection in homes, workplaces and vacant lots. In addition, the inspection frequency is generally monthly but sometimes with a high *Ae. aegypti* infestation and the presence of dengue cases in the population, the inspection in the province is reduced every 11 days. These variations in the frequency of sampling could in certain years influence in the number of samples.Despite these limitations; the authors consider it interesting to show these results of the program that allows us to evaluate *Ae. albopictus* behavior from its introduction in Cuba until the current days.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

This study was approved by ethical review committee of the Institute of Tropical Medicine, La Habana, Cuba and the ethical standards of the National Control Program of *Aedes aegypti* and *Aedes albopictus* established in Cuba. All the authors agree with the article publications.

Availability of data and materials

The data sets supporting the conclusions of this article are included within the article. Raw data are available from the corresponding author on request.

Competing interests

The authors declare that they have not competing of interests.

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Authors' contributions

MCM, VV and MC conceived and designed the study. MCM, MC, IP, MM, RM, ML JAB, identified the mosquitoes and collected data. MCM, VV, JAB and ML analyzed the data. VV. carried out the statistical analysis. MCM and VV wrote the manuscript. All authors read and approved the final manuscript.

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Authors' information (optional)

[1] Vector Control Department**,** Institute of Tropical Medicine Pedro Kourí (IPK) Havana,

Cuba.

[2] Entomology laboratory**,** Provincial Center of Hygiene Epidemiology and Microbiology, Havana, Cuba[3] Institute of Tropical Medicine, Antwerp, Belgium.

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Figures

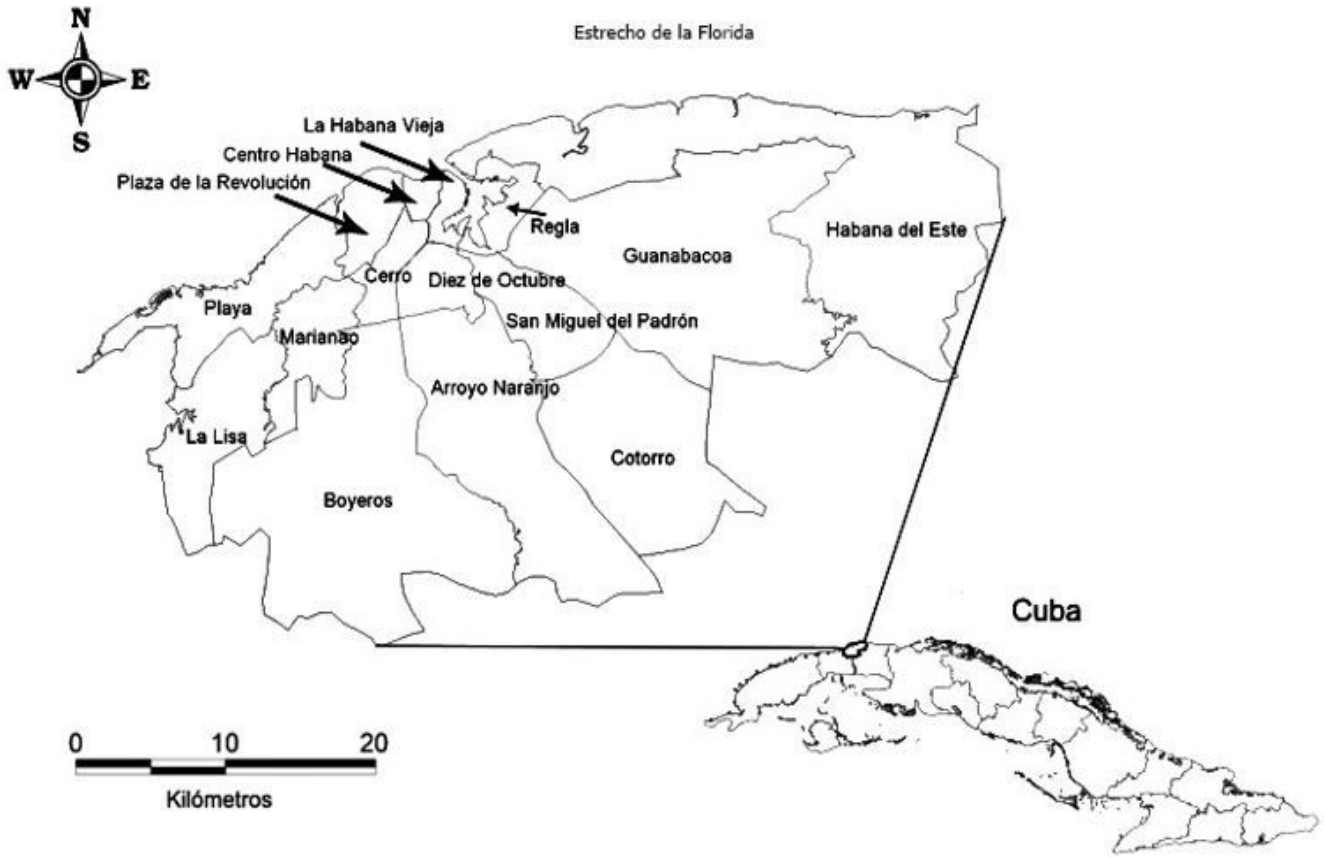

Figure 1

Municipalities' distribution in Havana province, Cuba.

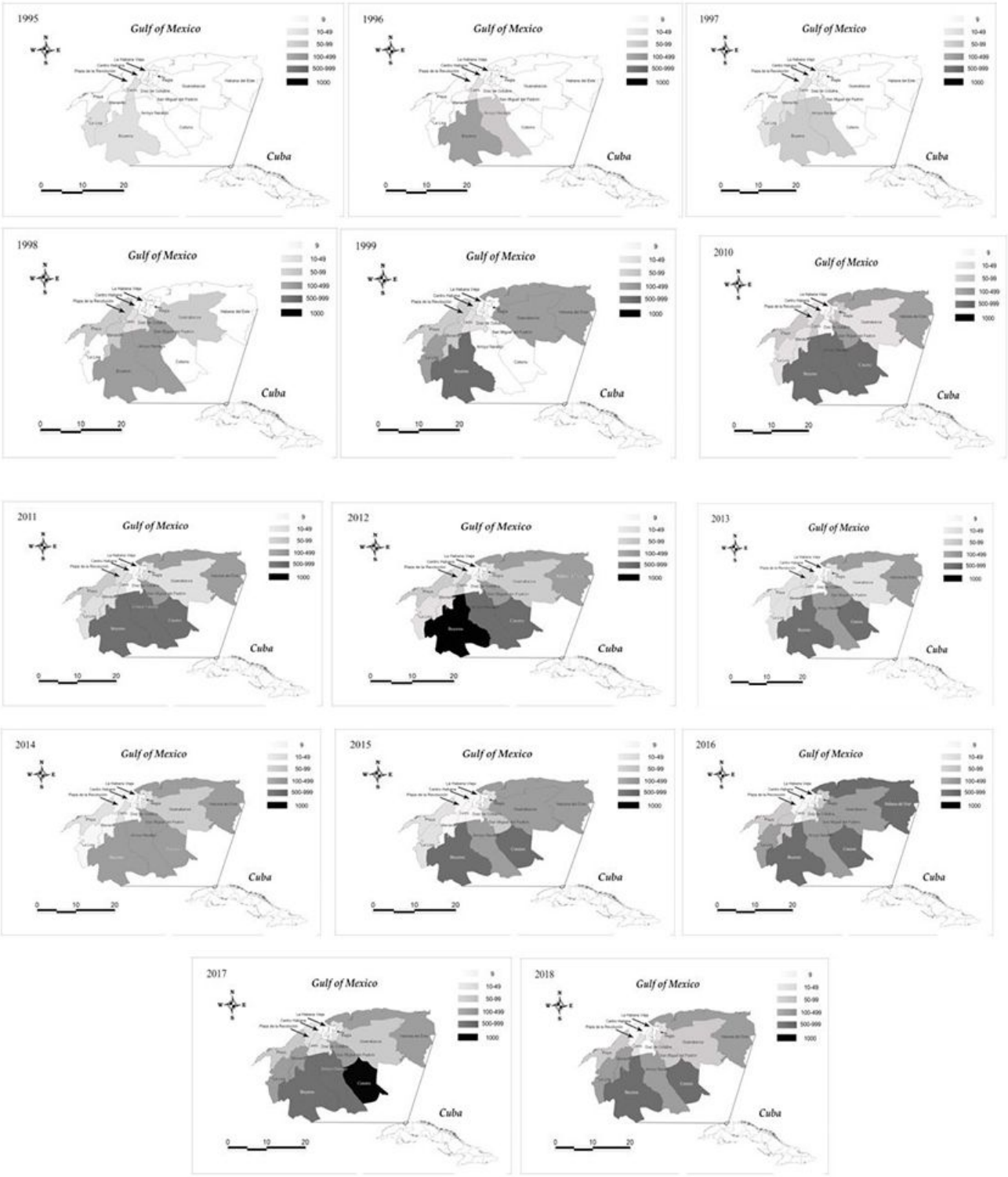

Figure 2

Yearly distribution of the number of Ae. albopictus breeding sites in the fifteen municipalities of the province of Havana in the two study periods 1995-1999; 2010-2018.

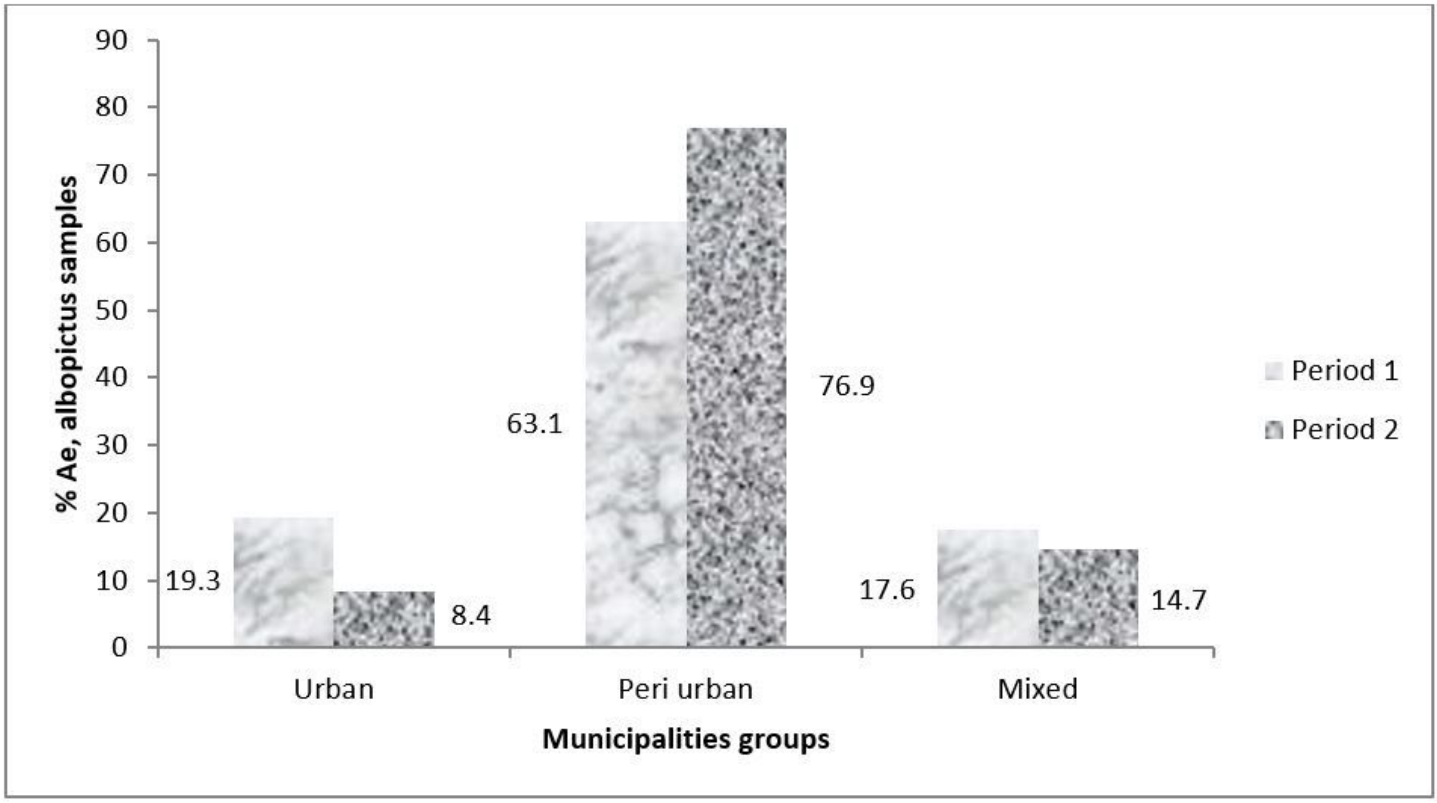

Figure 3

Yearly proportional distribution of Ae. albopictus breeding sites in the three urbanization groups, Havana province, study periods 1995-1999 and 2010-2018.

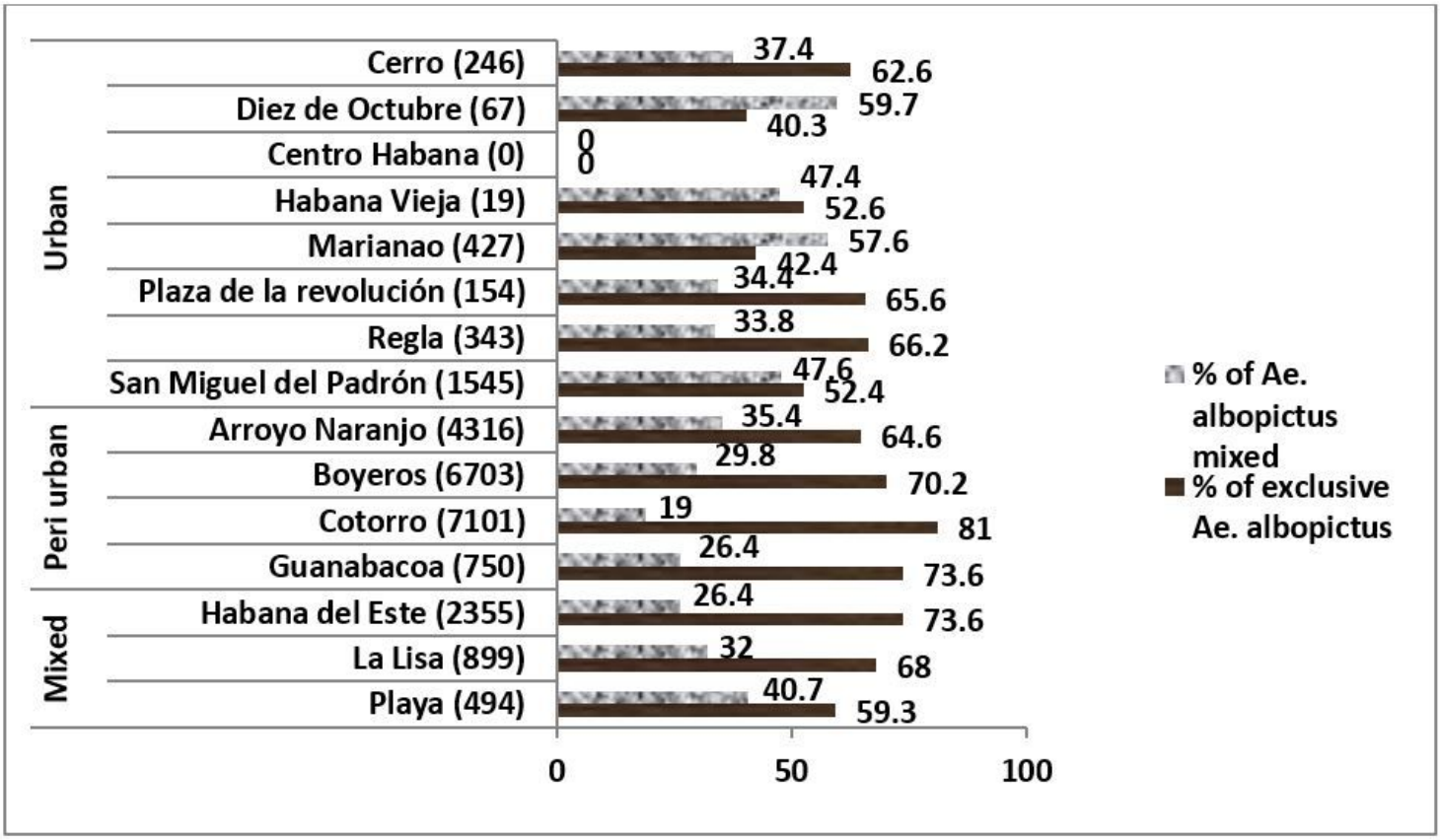

Figure 4

Percentage of exclusive and mixed, with other culicids, Ae. albopictus breeding sites by municipality and urbanization group, Havana province, 2010-2018.

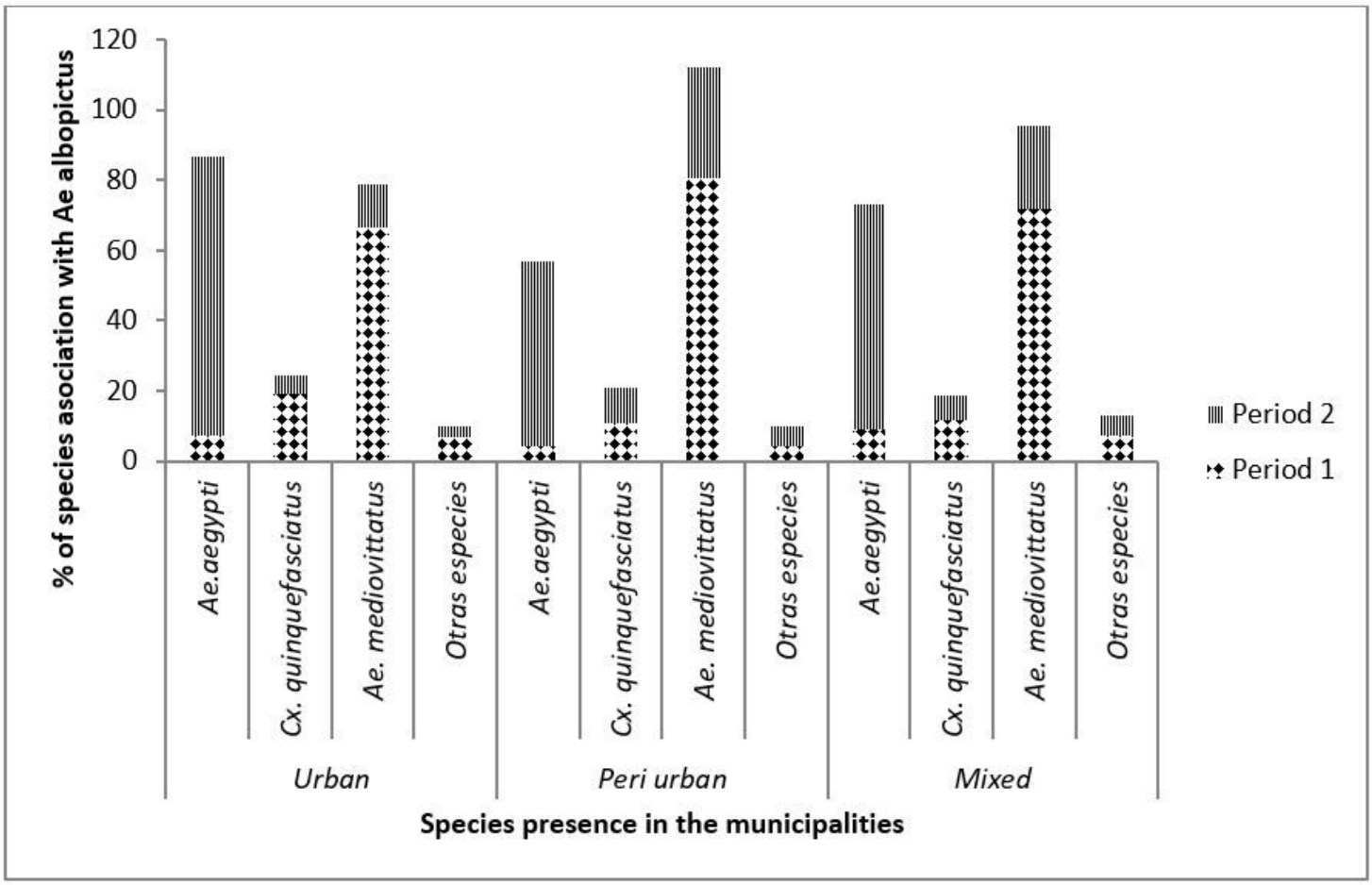

Figure 5

Species association in the mixed Ae. albopictus breeding sites per urbanization group, Havana province, study period 1995-1999 and 2010-2018

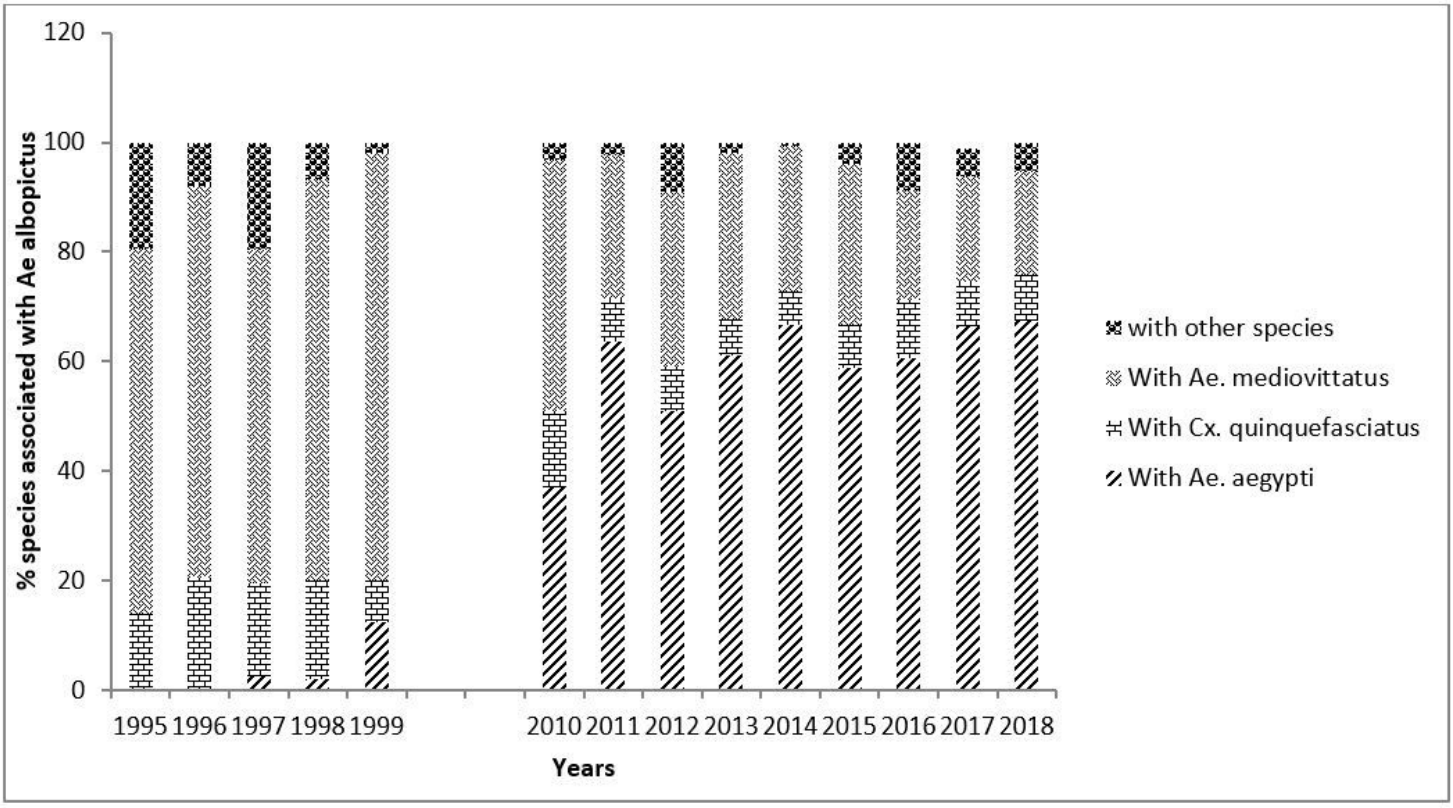

Figure 6

Species association in the mixed Ae. albopictus breeding sites per year, Havana province, study period 1995-1999 and 2010-2018

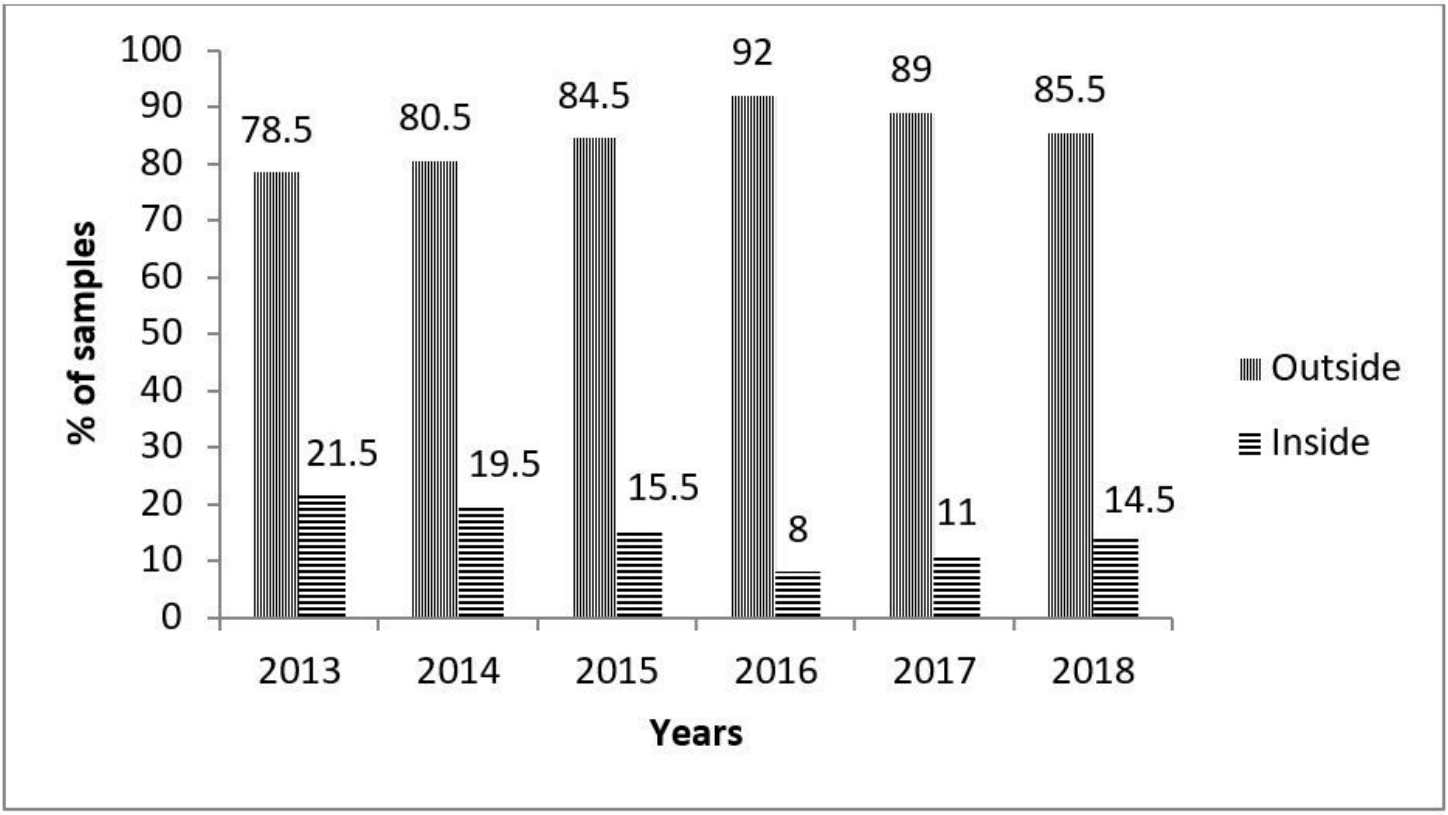

Figure 7

Proportion of Ae. albopictus breeding sites outside and inside the premises, Havana province, 2013-2018.