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Spatial distribution of Culicidae (Diptera) larvae, and its implications for Public Health, in five areas of the Atlantic Forest biome, State of São Paulo, Brazil

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ABSTRACT

In view of the adaptive ability of mosquitoes and their role in the transport of infective agents, entomological surveys undertaken in transitional environments are very important for the determination of the risk they represent for Public Health. Among the principal vectors of the infectious agents involved in the occurrence of important arboviruses, such as dengue, for example, are the Culicidae-insects capable of installing themselves in the urban nuclei, which exist within areas containing vestigial forests. This present study conducted a survey of mosquito species by means of traps to catch their larvae installed in five rural areas within the Atlantic Forest domain and containing its vestigial vegetation in the municipality of Santa Bárbara D'Oeste, São Paulo, Brazil. A total of 13,241 larvae belonging to six mosquito species were collected on 920 occasions (32.52% of positive collections). *Aedes albopictus* (64.23%) and *Aedes aegypti* (32.75%) were the most frequent, followed by *Culex quinquefasciatus* (1.32%), *Aedes fluviatilis* (1.04%), *Culex* Complex Coronator (0.40%) and *Toxorhynchites theobaldi* (0.22%). Three areas were analyzed by means of Simpson's diversity index and the spatial analysis showed that the sites with the greatest abundance of *Ae. aegypti* presented lower diversity values and were associated with more highly consolidated urban nuclei. The vector of dengue, chikungunya and zika has great infesting ability in urban areas, which means that the early implementation of entomological surveillance and control activities in specific areas – such as transitional ones – is highly important.

Introduction

Some mosquito species transmit pathogens and contribute to the dissemination of disease around the World (Marcondes and Ximenes, 2015). In Brazil, the control of diseases such as dengue, chikungunya and zika, all of them arboviruses transmitted by the same common vector, stands out as one of the greatest investments in the Public Health field in the country, given the seriousness and the impact of these diseases on society (Câmara et al., 2007; Brazil, 2015). This makes the surveillance of Culicidae vector species dispersal and dynamics in both endemic and non-endemic areas extremely important.

The Culicidae family is subdivided into Anophelinae and Culicinae. This latter group is the larger, consisting of more than 3500 species, 108 genera and 11 tribes. Among the Culicidae are species whose representatives are of great epidemiological importance (Consoli and Lourenço-de-Oliveira, 1994; Harbach, 2016).

Ecological dynamic and the relations established between mosquito species are thus seen to be important for medical entomology, especially under conditions of transition between urban and rural environments. In these localities, interspecific competition and environmental factors may alter the dominance of one species over others (Juliano et al., 2002), creating conditions appropriate for the transmission of pathogens and consequently, for the occurrence of disease outbreaks and epidemics in the human population established in these areas.

These transitional environments, as one is not here dealing with consolidated urban areas, may not be benefited by vector control activities in the same way as when these are carried out on

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the outskirts of cities. Within this context, entomological surveillance is of the greatest importance – and among the most efficient and sensitive methods for monitoring mosquitoes are ovitraps and larvitraps, both of which are widely used (Braga and Valle, 2007; Brazil, 2009; Silva et al., 2009; SUCEN, 2010), being of extremely simple application for use in the daily routine of entomological surveillance.

Entomological surveillance is therefore an important tool in the accompaniment of the mosquito populations found in rural areas, seeing that it assists in expanding knowledge of the patterns of occupation and permits the development of strategies of control addressed to the specific species. Further, the interpolation of the mosquito diversity presented on the maps produced permits the differentiation of the diversity existing in the different regions of each of the areas and the estimation of the values in the areas in which collections have not been undertaken.

Our study seeks to present the Culicidae fauna found in different rural areas of the municipality of Santa Bárbara d'Oeste, as also the composition of the species in these places by mean of the use of larvitraps. The places selected are the largest population nuclei found outside the urban center, and raise the possibility of these environments' presenting cases of diseases which normally circulate in the urban areas and whose etiological agents are mosquito-borne. Under these conditions, it is imperative to undertake a more rigorous analysis of control actions, which invariably require adaptations so that they may be carried out in transitional environments – as is the case of the rural areas. There also emerges the further possibility of the dispersal of pathogens with the participation of mosquitoes, involving both forested environments and urban areas. This is an important condition, seeing that there is considerable movement of people in these places which often contain specific structures such as schools, churches and health posts, among others, known to be centers for social gatherings.

Material and methods

This present study was undertaken in the municipality of Santa Bárbara d'Oeste, situated in the interior of São Paulo state (Fig. 1), at 22.75° S and 49.38° W, with an average altitude of 560 m. The total area of the municipality is of approximately 271 km^2, with a population estimated at about 190 thousand inhabitants, 98% of whom are concentrated in the urban area and just 2% of whom live in the rural zone (IBGE, 2013). The municipality is situated among the foothills of the Depressão Periférica Paulista, possessing relief of gently undulating, medium-sized hills. The surviving vegetation of the area belongs to a zone of transition between the Atlantic Forest and Savannah biomes. In accordance with the classification proposed by Koeppen, Santa Bárbara d'Oeste possesses a climate defined as Cwa, characterized as tropical highland with summer rainfall and dry winters. The average temperature of the hottest month is above 22 °C and that of the coldest month 12 °C. The average annual rainfall is of 1466 mm (IBGE, 2013; CEPAGRI, 2014).

The samples were collected by means of larvitraps during the period from February 2013 to March 2014, inclusive, giving a total of 14 months of collections. Five rural areas of the municipality were selected (Fig. 1), namely the suburbs of Cruzeiro do Sul, Vale das Cigarras, Santa Alice, Santo Antônio do Sapezeiro and Glebas Califórnia. The traps were distributed in the suburbs cited, using as criterion a ratio of one trap per 90 inhabitants. These places were selected because they are the largest residential nuclei outside the urban perimeter of the municipality. It is important to add that each of these areas presents its own specific characteristics in terms of scenery structure, being more or less close to forest fragments and presenting differences as to the proximity of rivers, the size of the resident population and the urban structures installed (schools, churches, health posts).

The total number of traps in each suburb was thus as follows: Cruzeiro do Sul – 13; Vale das Cigarras – 12; Santa Alice – 9; Santo Antônio do Sapezeiro and Glebas Califórnia – 2 each. The blocks were selected and the houses drawn by lot visited to request permission for the installation of the traps, thus giving a total of thirty-eight larvitraps, which were inspected weekly throughout the study period.

The larvitraps, consisting of tires cut transversely, were fixed in the peridomicile of the sites selected. After its installation, each trap received approximately 1 L of water and remained in the same place for the subsequent weekly inspections. During the inspections, the most highly developed larvae were collected and the liquid from the trap was filtered for the collection of the remaining larvae which were, later, transferred to plastic recipients. The larvae were individually identified in the laboratory with the help of a microscope and the use of dichotomic identification keys (Consoli and Lourenço-de-Oliveira, 1994; Forattini, 2002).

The results were stored in a spatial data bank which permitted the verification of the spatial distribution of the Culicidae species in the rural area of Santa Bárbara d'Oeste studied. However, samples from Santo Antônio do Sapezeiro and Glebas Califórnia were omitted from this analysis as they did not present significant sampling units.

Spatial analysis of Culicidae distribution

The geo-referenced sites of the traps were transformed into a spatial information plane in a Geographic Information System (ArcView™) (Esri™, 1996), into which were entered data on the positivity of the mosquito species. The connection between the geographical characteristics and the table of attributes is guaranteed by the geo-relational model, that is to say, one single identifier undertakes the connection between them, maintaining a correspondence between the space and the register of attributes. The table of attributes was provided with the occurrences of the mosquito species classified in hierarchies in the following two forms: total number of species and number of co-habitations. This procedure permits the compiling of six theme maps, on which the places with the greatest mosquito frequency were identified.

Spatial analysis of Culicidae diversity

To undertake this spatial analysis, the diversity indices of Shannon and Simpson were first estimated for each of the traps distributed in the Cruzeiro do Sul district of the city of Santa Bárbara d'Oeste. The procedures employed to calculate these indices are described in Magurran (1988).

After estimating these parameters, an analysis was undertaken on the basis of the collection of semi-variograms of the species diversity, because this is the first and most important phase seeing that this type of approach analyzes the spatial dependence of the values of a variable $V(x)$, separated by a vector Δh.

Andriotti (2004) defined the semi-variogram as the variance of the error committed by the estimation of an unknown contentin $(x+h)$ with the help of a particular point in (x), that is to say, semi-variograms express the spatial distribution of a continuous variable, showing its zone of influence and anisotropic aspects, implying a preferential direction for the spatial distribution of the variable (Pfeiffer et al., 2008) and the presence of anomalies caused by sampling error or random components.

Semi-variograms of the diversity of mosquito species were estimated in order to obtain a mathematical model for mounting the variability of all the data. The variogram is an important tool which demonstrates the degree of spatial dependence of the sample and

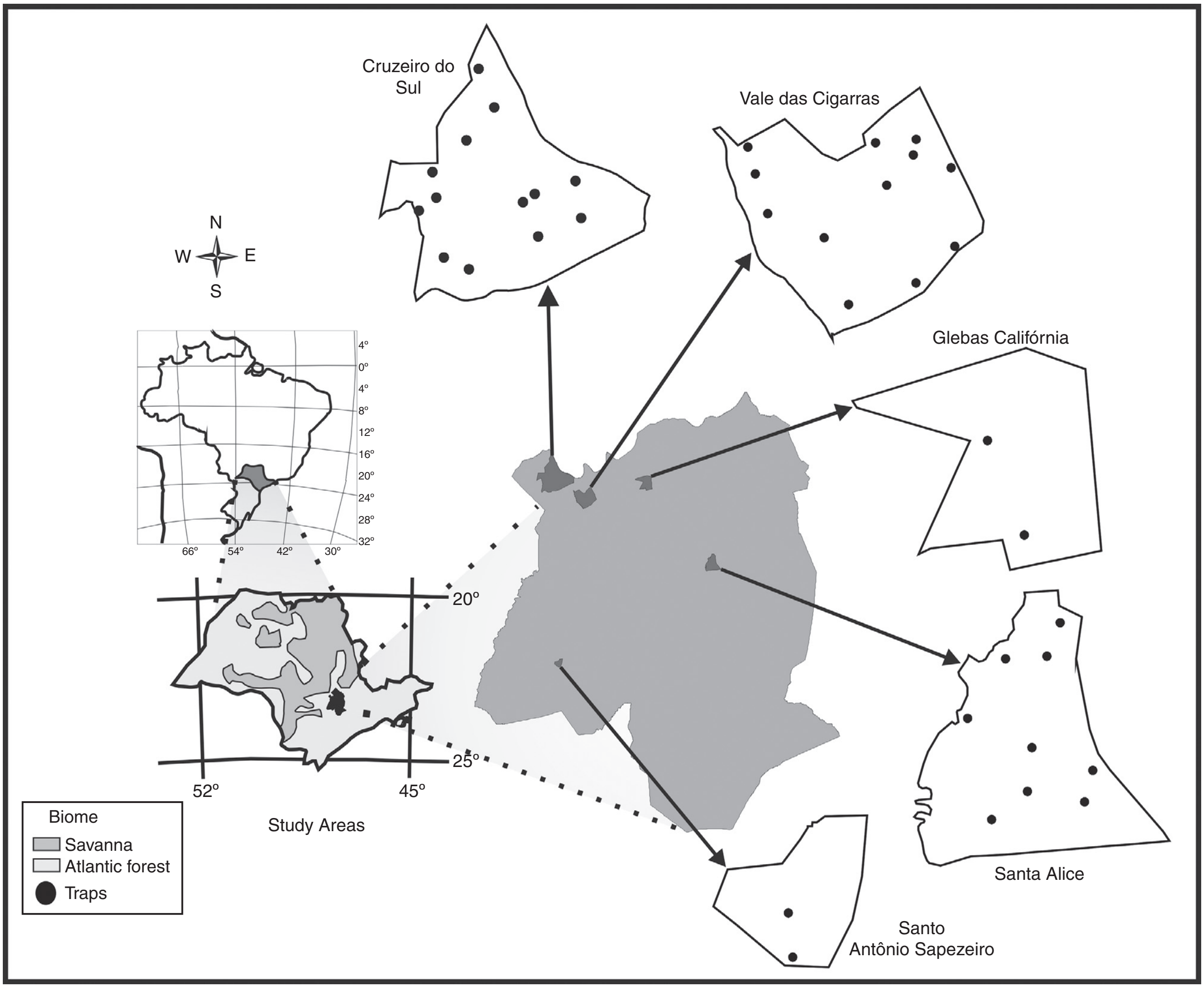

Fig. 1. Localization of the study areas in the Atlantic Forest biome, São Paulo state – Brazil.

its specific support. The relation is expressed on two axes, *x* which expresses the vector of the distance and *y* which is denominated variance or covariance. In this present study, the semi-variograms assist in the verification of the existence of the lack of spatial correlation and contribute to the validation of the experimental model of collection, in view of the fact that the sites were chosen randomly and the finding of the larvae and the species was fortuitous (Isaaks and Srivastava, 1989).

Ordinary kriging was, therefore, adopted as the method of interpolation for the compiling of maps of mosquito diversity. This procedure was undertaken on surfer software (Golden Software, 1995) and resulted in the construction of four quantitative isopletic maps. This map consists of lines that link sites of diversity distribution showing the spatial distribution and abundance of the Culicidae species.

Results

During the period of the study, Culicidae larvae were collected on 920 occasions (32.52% of positive collections), 719 without the larvae of other species and 201 jointly with others (there being more than one species present in the trap at the moment of collection). Taking all the collections made into consideration, a total of 13,241 larvae were identified in the laboratory, belonging to six different species: *Aedes* (*Stegomyia*) *albopictus* ‘Skuse, 1894 (1895):20 (F*; *Culex*)’, *Aedes* (*Stegomyia*) *aegypti* ‘Linnaeus, 1762:470 (A; *Culex*)’, *Aedes* (*Georgecraigius*) *fluviatilis* ‘Lutz, 1904:72 (A; as *Culex*)’, *Toxorhynchites* (*Lynchiella*) *theobaldi* ‘Dyar and Knab, 1906c:246 (M.F; *Megarhinus*)’, *Culex* Coronator Complex and *Culex* (*Culex*) *quinquefasciatus* ‘Say, 1823:10 (A; as sp.)’. The species and the percentages of each of them, whether found in isolation or jointly with others, are presented in Tables 1 and 2.

The occurrences when there were more than one species present were dominated by *Ae. albopictus* and *Ae. aegypti*, in absolute values, which were present in 77.61% of the catches. This kind of occurrence was distributed by area as follows: 106 in Cruzeiro do Sul, 46 in Vale das Cigarras, 38 in Santa Alice, 9 in Glebas Califórnia and one in Santo Antônio do Sapezeiro (Table 2). As has already been cited, the species *Ae. albopictus* and *Ae. aegypti* presented the greatest absolute values in terms of co-habitation, though a proportional analysis – that is to say, the number of occurrences of the species in cohabitation in relation to the total number of occurrences of that same species in the area – demonstrated that *Ae. albopictus* and *Ae. fluviatilis* were the species which presented the highest proportional values, being, in Cruzeiro do Sul 6.8% and in Vale das Cigarras 11.3%. Thus also *Tx. theobaldi* presented high proportional values, cohabiting 42.8% of the times in which the species appeared in Vale das Cigarras, in 50% in Santa Alice and in 100% of the occurrences in Glebas Califórnia.

Table 1
Species of Culicidae collected in larvitraps in the rural areas of the municipality of Santa Bárbara d'Oeste from February 2013 to March 2014.

Species	Cruzeiro do Sul		Vale das Cigarras		Santa Alice		Sto. A. Sapezeiro		Glebas Califórnia		All five areas	
	N Larvae	% Larvae	*N* Larvae	% Larvae	*N* Larvae	% Larvae	*N* Larvae	% Larvae	*N* Larvae	% Larvae	*N* total	% Total
Ae. aegypti	1945	42.01%	479	14.63%	1787	48.53%	56	26.67%	70	4.84%	4337	32.75%
Ae. albopictus	2533	54.72%	2676	81.73%	1865	50.65%	118	56.19%	1313	90.8%	8505	64.23%
Ae. fluviatilis	44	0.95%	53	1.62%	28	0.76%	14	6.67%	–	–	139	1.05%
Cx. quinquefasciatus	56	1.21%	37	1.13%	–	–	22	10.48%	61	4.22%	176	1.33%
Cx. Coronator Complex	39	0.84%	15	0.46%	–	–	–	–	–	–	54	0.41%
Toxorrynchites theobaldi	12	0.26%	14	0.43%	2	0.05%	–	–	2	0.14%	30	0.23%
Total	4629	100%	3274	100%	3682	100%	210	100%	1446	100%	13,241	100%

N, represents the number of larvae found in the collections.

Ae. albopictus was the species most frequently found during the study and represented 95.5% of the occasions when it was found jointly with other species and 67.68% when caught in isolation (with just one species present during the collection). In general, taking both situations into consideration, the larvae of this species accounted for 64.23% of the total found.

For *Ae. aegypti*, the second most abundant and frequent in this study, its distribution by area was patchy. The highest values obtained, in terms of the proportion of larvae of this species found in relation to the total number, were observed in Cruzeiro do Sul and Santa Alice (Table 1). The occasions on which two or more species were found together were also more frequent in those two districts (Table 2).

The other species of Culicidae presented a low frequency in the areas studied. *Culex quinquefasciatus* was the third most abundant, followed by *Ae. fluviatilis*, *Cx.* Coronator Complex and *Tx. theobaldi*. The spatial distribution of Culicidae species in the districts of Cruzeiro do Sul, Vale das Cigarras and Santa Alice is given in Figs. 2–4. The small number of samples from the areas of Santo Antônio do Sapezeiro and Glebas Califórnia made it impossible to map the distribution of the species there and these areas have not therefore been included in the graphic representations of the calculations of diversity.

The variographic analysis of Simpson's and Shannon's diversity indices may be seen in Fig. 5. Shannon's index shows a very clearly fragmented effect which reveals a lack of spatial correlation with the variable studied, whereas for Simpson's index this effect is smaller. These results show that the kriging method of interpolation may only be applied to Simpson's diversity index.

On the basis of these findings, the experimental semi-variograms of Shannon's diversity index for the Cruzeiro, Vale das Cigarras and Santa Alice rural districts were rejected and replaced by the experimental semi-variograms of Simpson's diversity index for the same localities. The appropriate experimental semi-variograms were taken into consideration and the adjustment to the theoretical model undertaken. The results of the adjustment (Fig. 6) show that the most appropriate model is the linear one. In these models the inclinations take on a value which approximates to 6.5 and attain their highest level (C) at 3.086, 2.729 and 0.0032, the maximum distance within the mosquito is spatially autocorrelated are 730 m, 64 m and 620 m respectively, for the rural districts of Cruzeiro do Sul, Santa Alice and Vale das Cigarras. This corresponds in practice to a distance at which the 95% threshold was attained.

After the development of the experimental semi-variogram, the estimation of the spatial distribution of Simpson's diversity index (Fig. 7) for the three suburbs was undertaken. This figure shows that the central and northwestern areas of the Santa Alice rural district present the greatest mosquito diversity (0.96). The southern part of this rural district presents the smallest diversity (0.42).

The diversity map for the rural district of Cruzeiro do Sul showed that the diversity is greater (between 0.78 and 0.86) in its western and southeastern parts. In the rest of this rural district Simpson's diversity attains its smallest magnitude (0.42).

Finally, in the Vale das Cigarras rural district, the diversity is greatest in the southern part (0.86), though it is more evenly distributed than in the other rural districts. In this area one finds the regions with the least diversity situated in the northeastern and western parts.

Table 2
Species of Culicidae collected in larvitraps together with other species (cohabitation) in the rural areas of the municipality of Santa Bárbara d'Oeste from February 2013 to March 2014.

Species collected together	Cruzeiro do Sul		Vale das Cigarras		Santa Alice		Sto. A. Sapezeiro		Glebas Califórnia		All five areas	
	N	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%
Ae. aegypti X *Ae. albopictus*	95	89.62%	22	47.82%	34	89.47%	1	100%	4	44.45%	156	77.61%
Ae. albopictus X *Ae. fluviatilis*	3	2.83%	6	13.04%	–	–	–	–	–	–	9	4.47%
Cx. quinquefasciatus X *Ae. albopictus*	1	0.94%	1	2.17%	–	–	–	–	2	22.23%	4	1.99%
Cx. Coronator Complex *X Ae. aegypti*	1	0.94%	–	–	–	–	–	–	–	–	1	0.49%
Cx. Coronator Complex X *Ae. fluviatilis*	–	–	1	2.17%	–	–	–	–	–	–	1	0.49%
Ae. albopictus X *Toxorrynchites theobaldi*	2	1.88%	6	13.04%	–	–	–	–	2	22.23%	10	4.97%
Cx. quinquefasciatus X *Ae. aegypti*	–	–	2	4.34%	–	–	–	–	–	–	2	0.99%
Ae. aegypti X Toxorrynchites theobaldi	–	–	–	–	1	2.63%	–	–	–	–	1	0.49%
Cx. Coronator Complex X *Ae. albopictus*	1	0.94%	2	4.34%	–	–	–	–	–	–	3	1.49%
Ae. aegypti X *Ae. albopictus* X *Toxorrynchites theobaldi*	2	1.88%	1	2.17%	–	–	–	–	–	–	3	1.49%
Ae. aegypti X *Ae. albopictus* X *Ae. fluviatilis*	1	0.94%	6	13.04%	3	7.89%	–	–	–	–	10	4.97%
Cx. quinquefasciatus X *Ae. albopictus* X *Ae. aegypti*	–	–	–	–	–	–	–	–	1	11.12%	1	0.49%
Total	106	100%	47	100%	38	100%	1	100%	9	100%	201	100%

N, represents the number of collections made in which different species were found together.

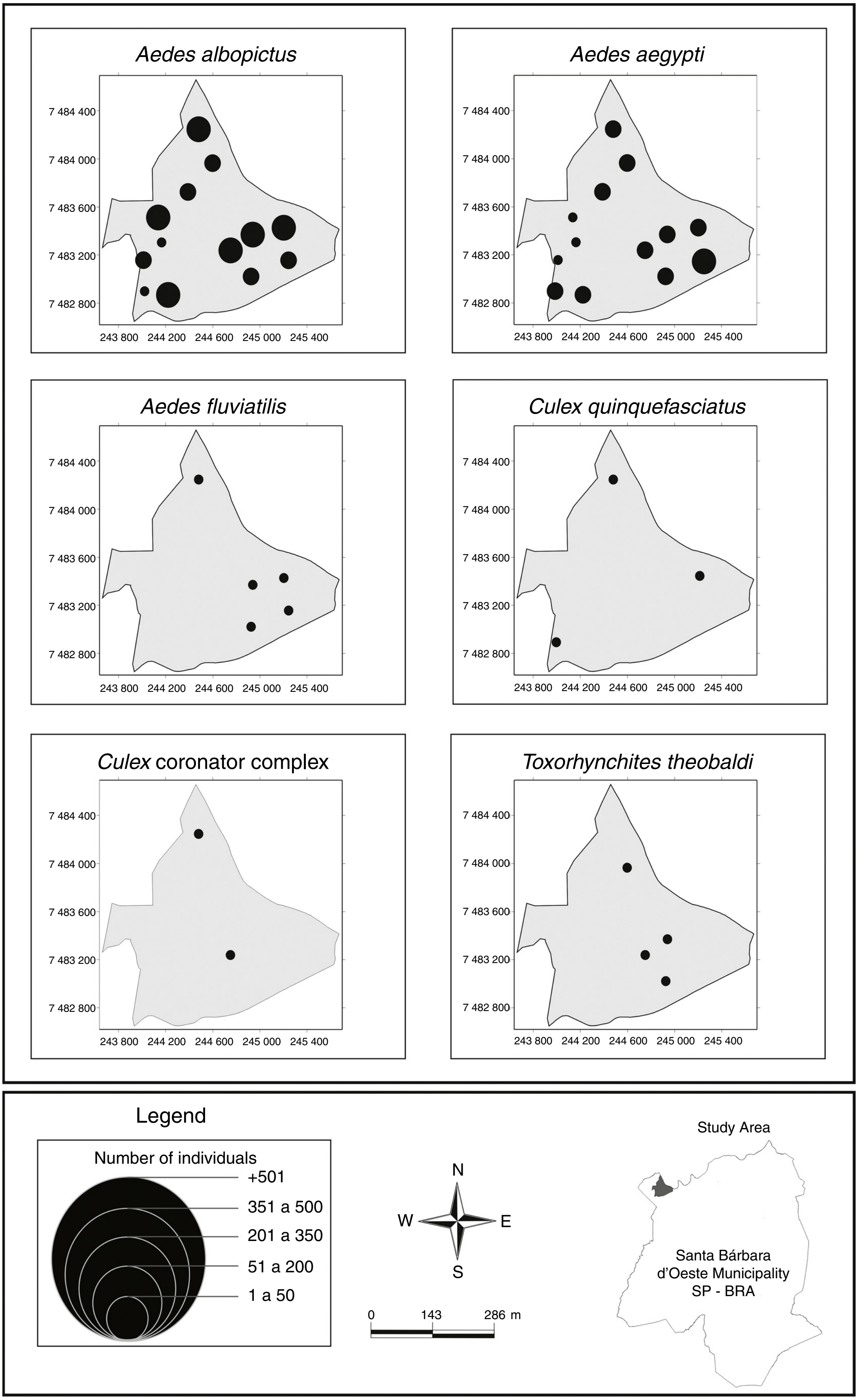

Fig. 2. Spatial distribution of the species found in the Cruzeiro do Sul rural district.

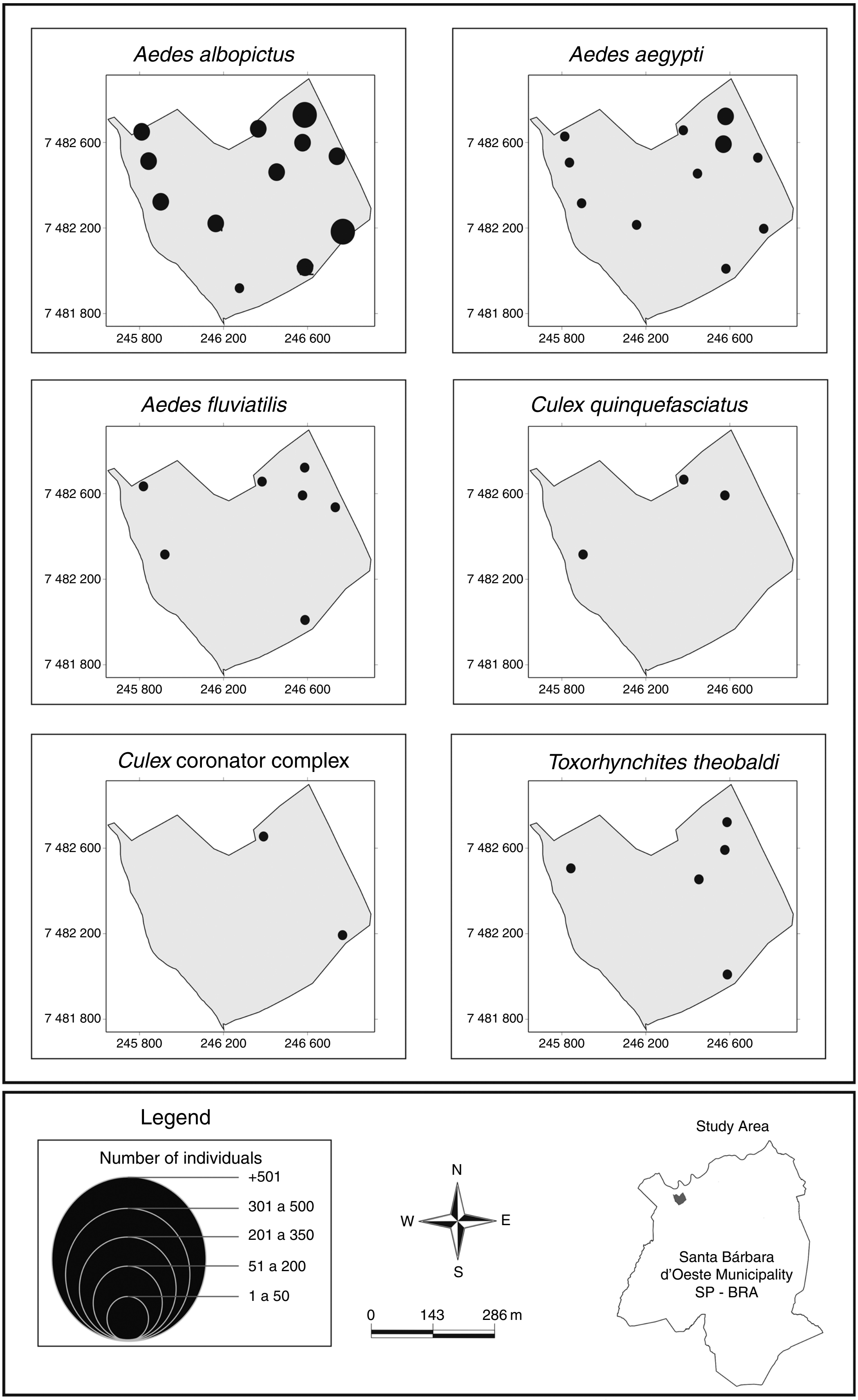

Fig. 3. Spatial distribution of the species found in the Vale das Cigarras rural districts.

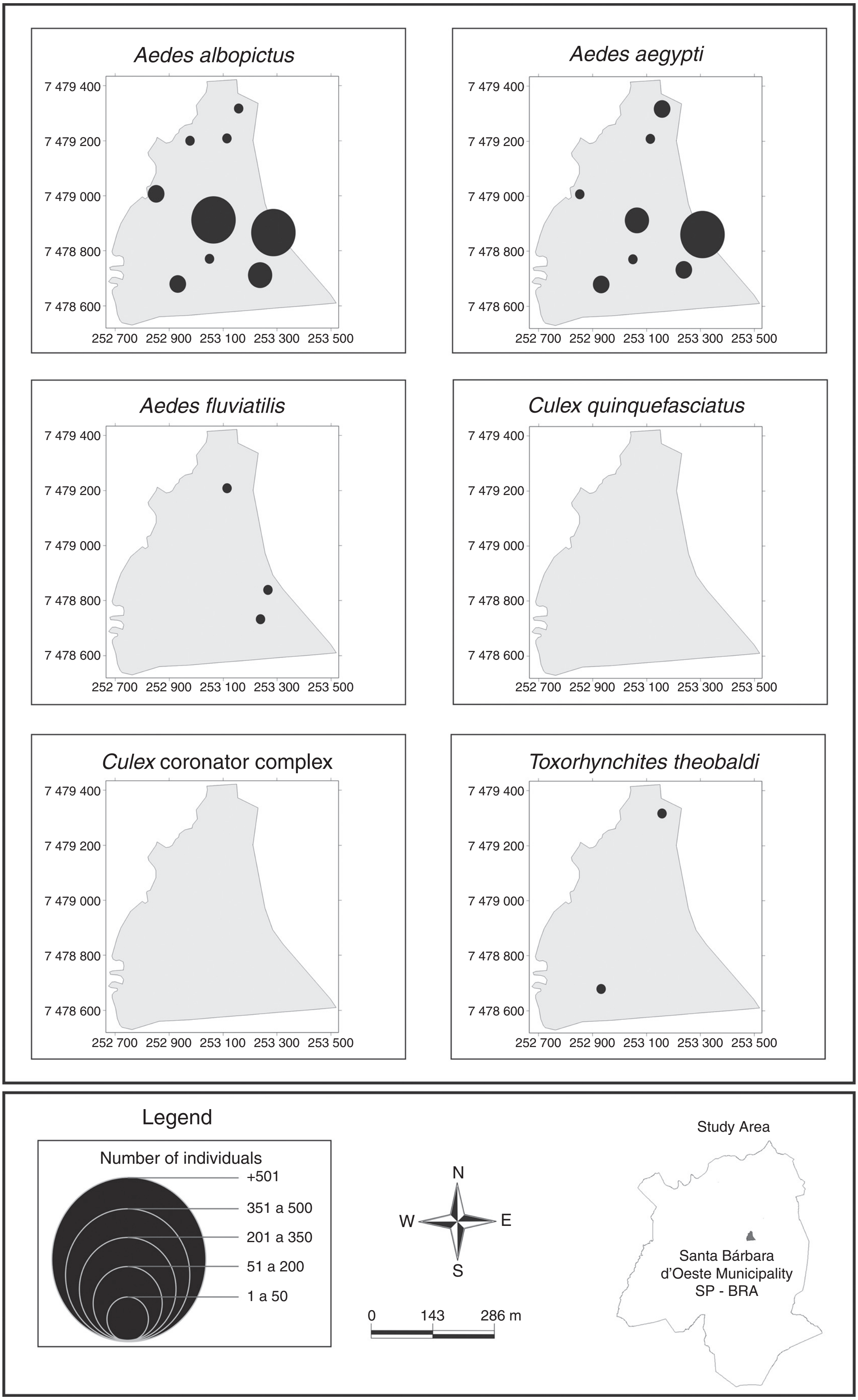

Fig. 4. Spatial distribution of the species found in the Santa Alice rural districts.

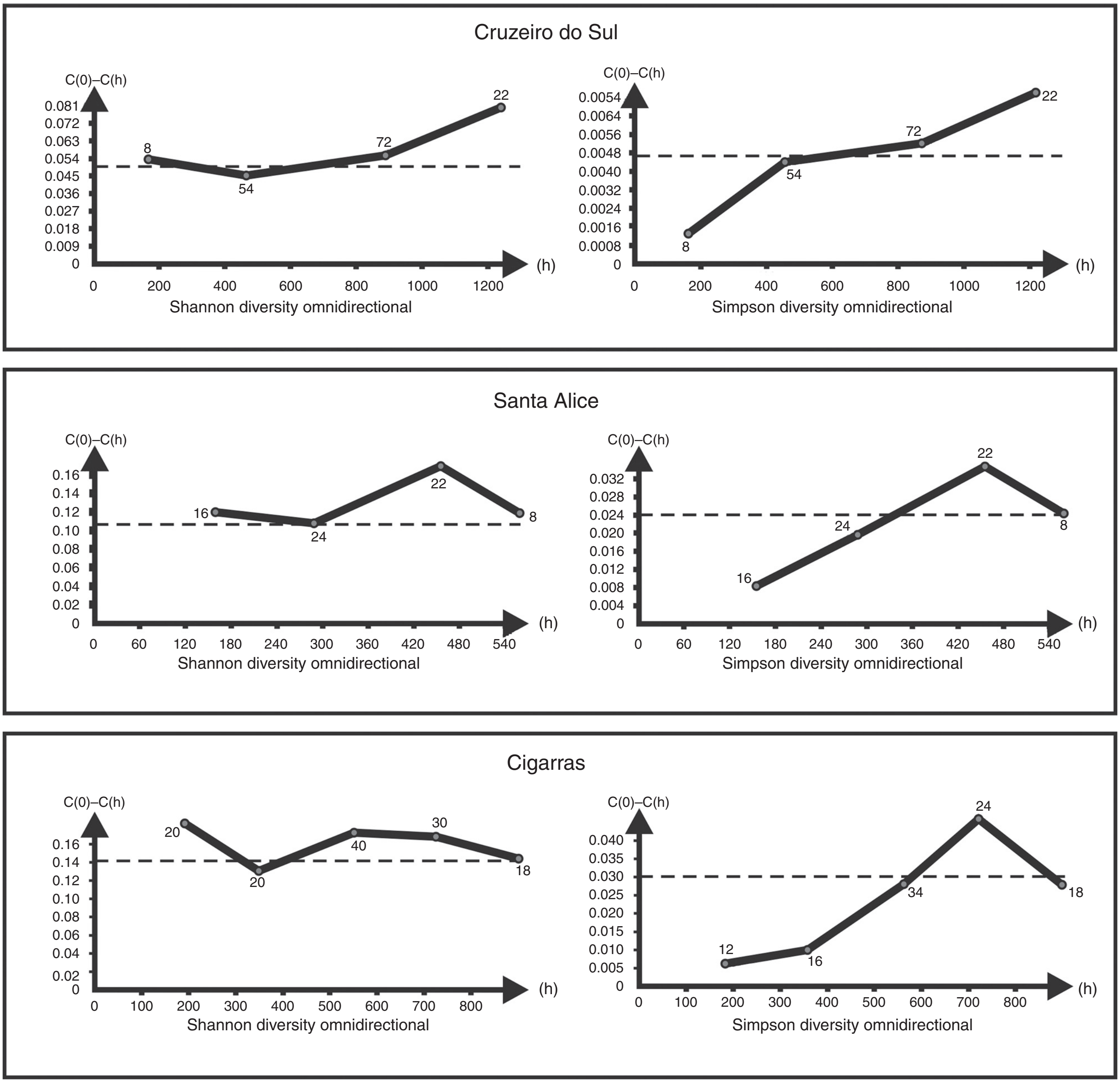

Fig. 5. Semi-variograms of Shannon and Simpson diversity indices.

Discussion

Larvitraps employed in this present study resulted in the collection of a total of 13,241 larvae of six distinct Culicidae species. Despite their simplicity, larvitraps can give satisfactory results, permitting the monitoring of species the habits of development of whose immature stages involve the use of both natural and artificial breeding places (SUCEN, 2001; Brazil, 2002; Gama et al., 2007; Alarcón-Elbal et al., 2014; Monteiro et al., 2014a,b), as they also demonstrate the ecological plasticity of the species found and their potential for adaptation to human dwellings (Lopes, 1997), making use of artificial breeding places for the development of their immature forms. Both breeding places and traps made from tires have already demonstrated their efficiency in surveys undertaken near urban areas or even in degraded forest areas (Lopes et al., 1993), which have shown themselves to be similar to those investigated in this present study.

The distribution of *Ae. albopictus* in the various areas of collection was seen to be uneven. It was the most abundant species, attaining the greatest value (90% frequency) of all the larvae sampled in Glebas Califórnia. Specifically in the area of Cruzeiro do Sul, Piovezan et al. (2013) had already observed the distribution of this species, and the comparison of these two studies, undertaken in the same area with an interval of two years between them, showed a reduction in the percentages of the occurrence of *Ae. albopictus* and an increase observed for *Ae. aegypti*. Although, in Brazil, *Ae. albopictus* has not been incriminated in the transmission of the Dengue virus, it possesses the proven competence of transmitting several arboviruses such as Dengue and Chikungunya (Gratz, 2004; Marcondes and Ximenes, 2015). Further, as regards dengue, this vector's ability has been proved by the epidemics that have occurred in Japan, Indonesia, the Seychelles, Thailand and Malaysia (Gratz, 2004), in Hawaii (Effler et al., 2005), Gabon (Paupy et al., 2010) and in Europe (Field et al., 2010; La Ruche et al., 2010). The Chikungunya virus has also been transmitted by *Ae. albopictus*, as

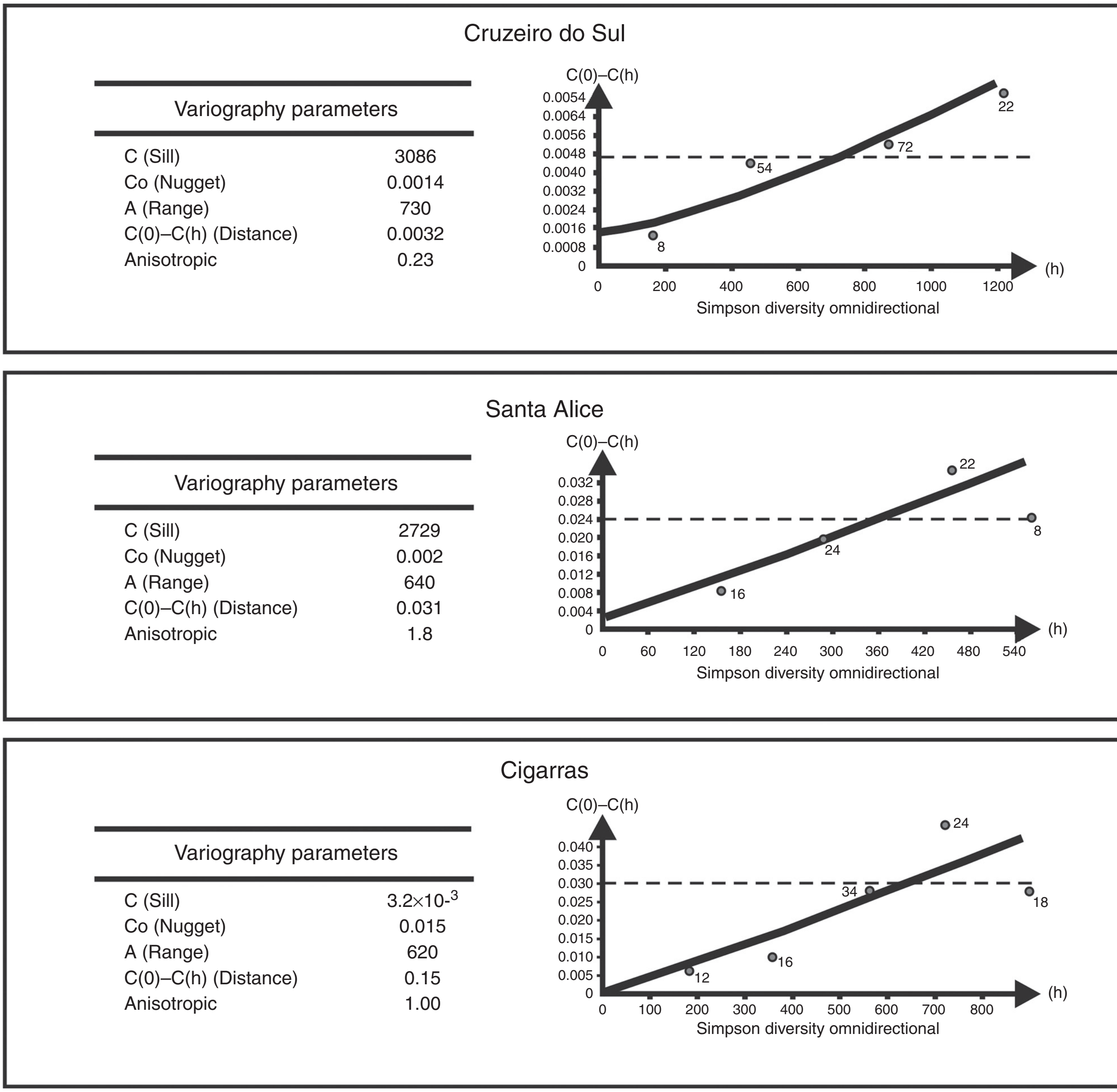

Fig. 6. Adjusted Semi-variograms of the Simpson diversity index.

has recently been reported in Europe, Africa and the Indian Ocean (Reiter et al., 2006; Bonilauri et al., 2008; Pagès et al., 2009; Paupy et al., 2010).

Aedes aegypti was the second most frequent species overall, presenting patterns of occurrence which varied between 5% in Glebas Califórnia and 49% in Santa Alice. This species presents a high degree of anthropophily and has spread into urban areas by making use of artificial breeding places as sites for reproduction (Consoli and Lourenço-de-Oliveira, 1994). On the other hand, its presence in relatively well vegetated areas is due to its ecological plasticity in the colonization of habitats, and its ability to develop in natural breeding places such as those provided by bromeliads (Souza-Santos, 1999; Forattini and Marques, 2000; Varejão et al., 2005; Gonçalves and Messias, 2008). As has been stated above, significant changes in its abundance were observed in the Cruzeiro do Sul area as compared with the results previously obtained by Piovezan et al. (2013). This species increased from 8.1% of the samples obtained in that first study to the 42% observed in this one, thus presenting an increase in the species' frequency and indicating a possible replacement of *Ae. albopictus*.

The interaction between *Ae. aegypti* and *Ae. albopictus* may be influenced by environmental factors. In situations of co-habitation, the characteristics of the scenery and environmental conditions may be determining factors in the success or failure of the species. Places with accentuated rural characteristics and the presence of vegetation favor the displacement and exclusion of *Ae. aegypti* by *Ae. albopictus*, while in urban environments or those subject to great anthropic influence, *Ae. aegypti* persists or dominates over *Ae. albopictus* (Lounibos et al., 2010). In situations in which the quantity of food available is reduced or even slightly limited, the interspecific larval competition is the main factor determining the abundance of these two mosquito species and it is *Ae. albopictus* which, under these circumstances, has the advantage over *Ae. aegypti* (Braks et al., 2004). However, under environmental conditions such as the low relative humidity and high temperatures which prevail in drier periods, the competition between the two species may be reduced

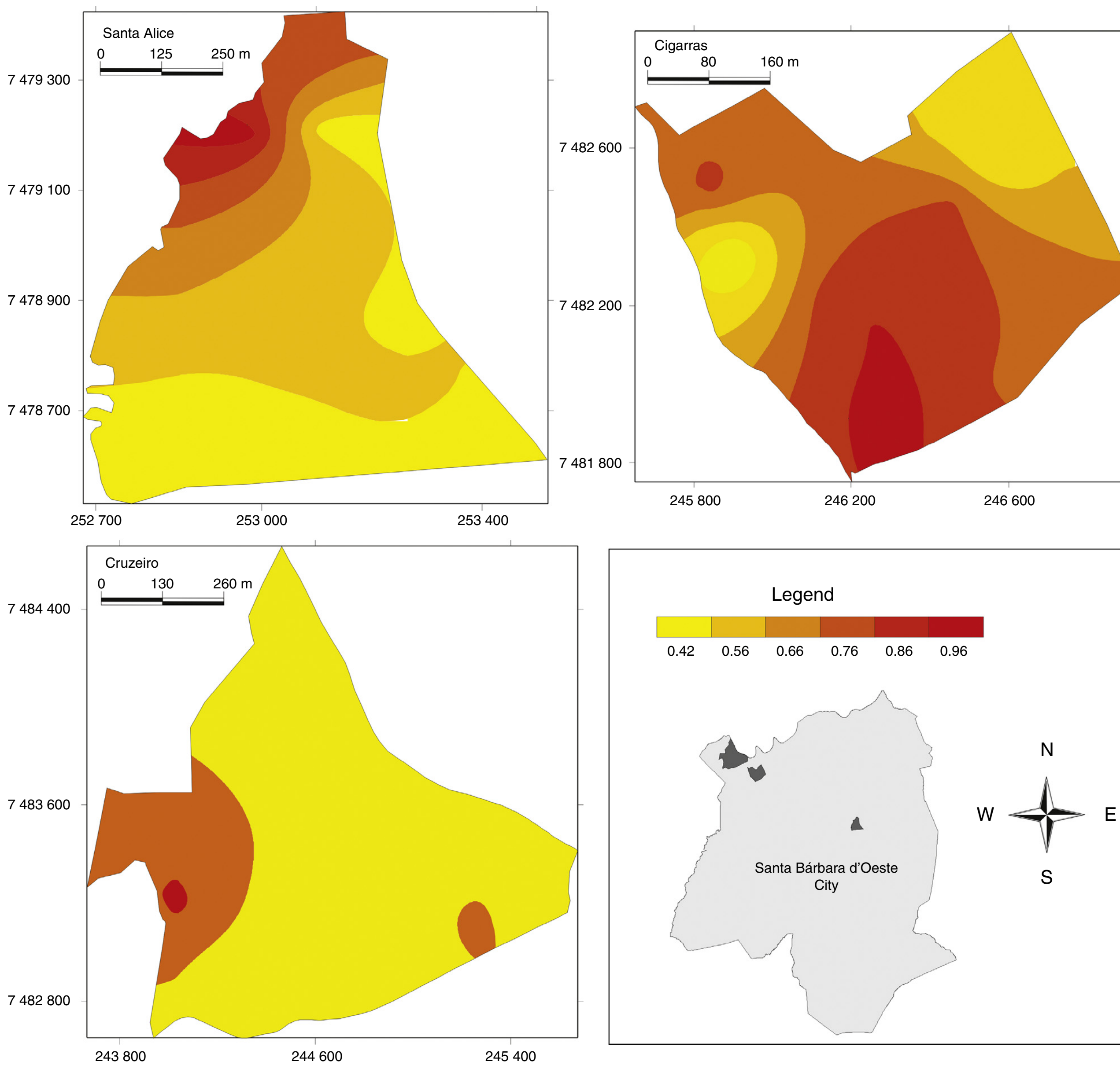

Fig. 7. Simpson diversity index maps.

seeing that *Ae. albopictus* is more sensitive to these variations, both in the egg and in the adult phases (Juliano et al., 2002).

Ae. aegypti and *Ae. albopictus* were also those which were present in greater numbers in situations of cohabitation, on a total of 156 occasions. In Cruzeiro do Sul, of the total of their joint occurrences in the area, 89.62% were of these two species. The method of collection employing larvitraps is widely used in monitoring research projects related to these two species, which explains the greater frequency with which these taxa were found in this study. The analysis of the figures generated shows that the spatial dispersion of *Ae. albopictus* has always been more closely associated with areas close to denser vegetation cover or bodies of water.

Further, relating to the occurrences in co-habitation, in relative terms, that is to say, comparing the number of occasions on which the species appears in co-habitation with the number of total occurrences of the species in a particular area, *Ae. albopictus* and *Ae. fluviatilis* presented higher values. The stratification in the foraging of the species might be one of the factors which influence coexistence in environments such as the breeding places, beyond which one should take into consideration that there exist various ecological relationships which present a dependence on environmental factors, density of existing populations, presence of predatory species, or even the kind of organic material used to feed the larvae (Juliano, 2009).

For *Ae. aegypti*, the places in which the species had its densest distribution were closer to the more urban stretches of the areas concerned, demonstrating the need to accompany the density of this mosquito species in transitional areas with rural characteristics but close to urban nuclei – whether existing or in process of development. Its epidemiological role in the transmission of pathogens on several of the continents (Consoli and Lourenço-de-Oliveira, 1994) causes great problems within the field of Public Health and considerable economic loss in terms of the attendance to and treatment of patients (Kouri et al., 1981). The present Zika virus epidemic in Brazil and its consequences constitute a factor of the greatest importance which has to be taken into consideration especially in environments where the mosquito control services may not be receiving the same attention as is dedicated to those in urban areas.

The vector control activities foreseen for urban areas are not normally extended to cover transitional areas such as, for example, rural areas, as the presence of *Ae. aegypti* in these latter is less frequent. One should also bear in mind the difference between the breeding places often available, which are distinct as between urban areas and rural environments. The analysis of the scenery also takes important differences into consideration, there being important differences such as the greater density of trees in rural areas, which makes control by nebulization, for example, difficult, and the environmental impact on wild arthropods which are not the target of control programs and are frequently found in transitional environments.

The increase in the abundance of *Ae. aegypti* in Cruzeiro do Sul, evident when the present study is compared with that of Piovezan et al. (2013), may be related to the fact that this species' eggs are more resistant to desiccation and to long periods of drought than are those of *Ae. albopictus* (Juliano et al., 2002). This hypothesis is based on the fact that this region faced, in the years 2013 and 2014, the period in which this present study was carried out, the longest dry period in all of recent history, with a volume of rainfall below the average of the last 50 years (Coelho et al., 2015, 2016), which would give a certain advantage to *Ae. aegypti*, to the detriment of *Ae. albopictus*.

Ae. aegypti has dominated urban environments and demonstrated great adaptability in its use of breeding places (Forattini, 1992; Consoli and Lourenço-de-Oliveira, 1994; Barata et al., 2001). The control of this vector depends on financial investment for the control of dengue – which exceeds R$ 1 bi (US$ 308 m) annually in Brazil alone (Brazil, 2015). Beyond its role in the dissemination of dengue in this country, this vector has recently been involved in the spread of the Zika virus which, despite its relatively mild symptomatology, can, exceptionally, cause serious alterations in the nervous system of fetuses in the process of growth (Brazil, 2016; CDC, 2016; Mlakar et al., 2016; Rasmussen et al., 2016). Thus, in the light of the various impacts which this vector has been making on human health, the accompaniment of its infestation in new areas, as has been happening in Cruzeiro do Sul, provides relevant information for the strategic and specific vector control actions which are needed to protect the existing human populations.

The species which presented the lower frequencies in the collections made were distributed randomly throughout the surveys undertaken, not all of them occurring in particular situations or areas. The least frequent species was *Tx. theobaldi* which, in the majority of observations, was found to be present together with *Ae. albopictus*, a result to be expected seeing that the species is recognized as an aggressive predator of the immature forms of other mosquito species, its cohabitation having already been recorded in other studies (Lopes et al., 1993; Lopes, 1997).

Ae. fluviatilis and *Cx. quinquefasciatus* are species which occurred in low frequency in the present study, by virtue of the methodology employed, but whose anthropophilic habits have already been described and which frequent the environment of the residences, making use of the traps placed there for their egg-laying (Consoli and Lourenço-de-Oliveira, 1994; Forattini, 2002). *Ae. fluviatilis* possesses the ability to infect with the Yellow Fever virus in the laboratory and *Cx. quinquefasciatus*, as well as being the principal vector of *Wuchereria bancrofti* on the American continent, has also been incriminated in the transmission of arboviruses (Consoli and Lourenço-de-Oliveira, 1994; Forattini, 2002), which demonstrates the importance of the monitoring of these species.

Representatives of the *Culex* Complex Coronator group accounted for 0.40% of the total larvae collected. This group is widely distributed throughout the Americas and uses breeding places, both natural and artificial, whether with clean or polluted water (Forattini, 2002). The *Culex coronator* 'Dyar and Knab, 1906' species occurs in urban environments (Silvério et al., 2007) and is related to the transmission of St. Louis encephalitis (Fernandez et al., 2000).

It was only possible to undertake the analysis of diversity in the areas of Cruzeiro do Sul, Vale das Cigarras and Santa Alice by virtue of the number of traps existing there for the application of this method. In these places, by means of the maps generated, it was found that in the regions within each of the areas where *Ae. aegypti* presented greater abundance the diversity values were lower. The proximity of more highly vegetated areas increased the Simpson's diversity values obtained, where as *Ae. aegypti* presents greater occurrence in particular regions within the areas studied here, closer to urban nuclei and their access roads. The residents' vehicle traffic may offer conditions favorable to this greater abundance of the species as it offers passive transport for the species which also profits from the ecological advantages which the environment (Juliano et al., 2002) – or even the human being himself – provides (Lounibos et al., 2010) to expand its distribution in these areas.

In Cruzeiro do Sul, the greater diversity values observed were found in the west of the area, which corroborates the results set out in an earlier study of the locality (Piovezan et al., 2013). The accompaniment of the mosquito populations is a fundamental necessity for the establishment of strategic measures which will minimize the risks run by the human population. Differential control and prevention activities should certainly be carried out in those localities which retain their relationship with the natural environment but which also, at the same time, possess some urban structure. These locations not are neither exclusively urban or rural, and the vector control in these areas should be differentiated from that which is often presented in the manuals.

It should be noted that the work of vector control to be undertaken in the rural areas calls for adaptation, seeing that there exist critical differences in terms of scenery, of available breeding places, of the ecological relationships established in these environments and of the environmental and anthropic conditions proper to these areas. Certainly in these areas, entomological and epidemiological surveillance activities are the basic tools for the minimization of epidemic risks. The monitoring of information on the diseases and vectors which may be found in these areas can help on both work fronts: the first permitting that local authorities work with publicity and pedagogical concepts, expanding the knowledge of what needs to be done by the local resident population itself to reduce risk and, secondly, providing elements for control actions in the areas of greater priority or risk.

Within the field of the discussion of the control of *Ae. aegypti*, in the face of the new risks of the transmission of arboviruses today both in Brazil and in the World as a whole, it is imperative that the methods applied in this fight should be revised and reassessed and revised. The long battle waged on this mosquito vector has achieved some successes, but as a rule it has been lost and this has had a negative impact on society. This article presents some considerations as to what can be done in transitional environments, which are new frontiers of occupation for *Ae. aegypti*, but which also present the presence of species such as *Ae. albopictus*, which has increased the probability of its infection and dissemination of arboviruses under conditions of competition (Juliano, 2009).

In urban environments, control methods are greatly influenced by the behavior of the human population. It is commonly found that 35% or more of residences are not visited during mosquito control operations by reason of the absence of the resident on the occasion of the visit. The efficiency of control operations calls for the adoption of means which will minimize these problems and which will inform the population that if they are carried out they will help the public authorities in their control measures. We understand that our studies should now be directed to the quantification and qualification of the effort and investment made by public authorities with a view to the control of *Ae. aegypti*. This new research should present operational and financial information which will contribute in such a way as to enable the manager to choose the method most appropriate to his financial condition, as well as satisfying those technical concepts whose efficacy in control have been proven.

Conflicts of interest

The authors declare no conflicts of interest.

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