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Nestedness patterns of sand fly (Diptera: Psychodidae) species in a neotropical semi-arid environment

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ABSTRACT

A common pattern in neotropical *Leishmania* spp. transmission is the co-occurrence of several sand fly (SF) species at endemic foci. We collected 13 SF spp. by direct aspiration in natural resting places (NRP) and 10 SF spp. with Shannon traps (ST), totaling 15 spp. with both methods, at 6 locations within a semi-arid region with endemic visceral leishmaniasis transmission in Falcón State, Northwestern Venezuela. We used null model testing of species co-occurrence and nestedness metrics estimated with our field data to ask whether SF species composition was segregated/aggregated, and if aggregated whether there was nestedness, i.e., whether species composition across sampling locations could be described by ordered subsets of species from the most species rich location in a landscape. Results showed that SF species were aggregated ($P < 0.05$), i.e., most species were present in species rich locations. Similarly, SF species were significantly nested ($P < 0.05$). Differences in pairwise Sørensen and Simpson indices, estimated with the ST data and the combined ST and NRP data, were positively associated with the distance between sampling locations, suggesting that species nestedness might be partially shaped by dispersal limitation. Our data showed that three species of medical importance were common across the sampling locations: *Lutzomyia gomezi*, *Lutzomyia panamensis* and *Lutzomyia evansi*, suporting that vector species do not turnover in the studied setting.

1. Introduction

Sand flies are a group of medical and veterinary important hematophagous insects responsible for the transmission of several protozoa and viruses (Maroli et al., 2013). The most notorious group of parasites transmitted by sand flies, from a medical perspective, are parasitic protozoa of the genus *Leishmania* spp. (Kinetoplastida: Trypanosomatidae), which cause leishmaniasis in humans and whose clinical forms range from cutaneous to visceral (Alvar et al., 2012). From a veterinary perspective sand flies are important vectors of vesicular stomatitis virus (Killmaster et al., 2010). A unique characteristic of sand flies in the neotropics is the co-occurrence of several species at endemic leishmaniasis transmission foci, where even several medically important species, i.e., with proven vectorial competence and capacity, often co-occur (Feliciangeli, 1987; Ferro et al., 1995; Jimenez et al., 2000), a pattern also observed in foci where vesicular stomatitis virus affects domestic animals (Herrero et al., 1994). Nevertheless, little research has been done to study the structure of sand fly communities regarding their β-diversity patterns, i.e., the change in species composition across any environmental gradient (Baselga, 2010), a topic that has become increasingly studied in other vectors, mainly mosquitoes (Chaves et al., 2011; Hoshi et al., 2014; Laporta et al., 2013). Especially, knowledge about β-diversity patterns can be useful to predict species likely to become vectors, given that some species might have similar ecological patterns to those that are currently recognized as dominant vectors (Levins et al., 1994), the need for heterogeneous control strategies for dealing with different vector species in an endemic area (Chaves et al., 2013), or whether the co-occurrence of vector species with species without medical importance can be an indicator of the likelihood of disease transmission (Chaves et al., 2011; Laporta et al., 2013).

Null model tests of species co-occurrence and nestedness are ecological tools that have become increasingly useful to study β-diversity patterns. The underlying idea of these methods is to estimate metrics measuring co-occurrence and/or nestedness using field data and compare this result with distributions of

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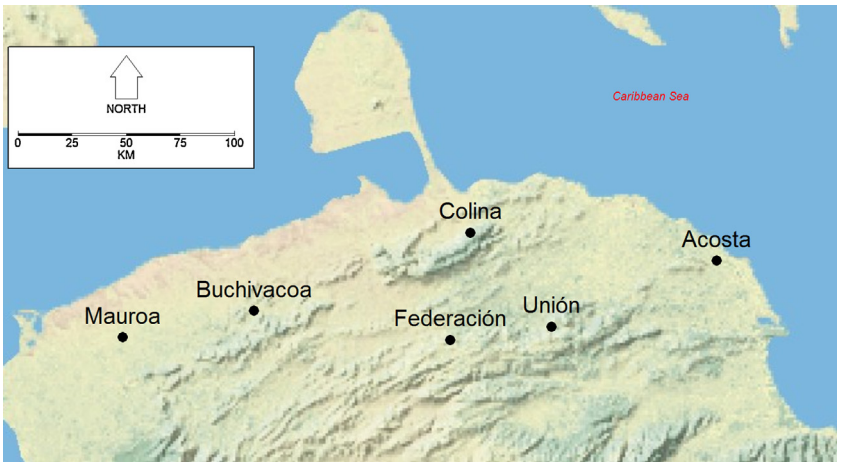

Fig. 1. Study locations. Each dot shows the sampling locations. Distances ranged from 46 km (Unión and Federación) to 272 km (Mauroa and Acosta).

the same metric generated by simulations fulfilling certain constrains/assumptions (Gotelli, 2000; Ulrich and Gotelli, 2007). For example, a study using null model tests of co-occurrence showed that SF species composition was segregated across an altitudinal gradient and by ecosystems (Chaves and Añez, 2004). A similar segregation pattern was observed when studying sand flies at their resting habitats in a forest, a pattern that became random when looking at agricultural landscapes, where vector species became more abundant (Chaves, 2011). Nevertheless, one pattern that has not been studied is what happens in SF communities that are sampled in locations embedded in relatively homogenous landscapes. The western focus of visceral leishmaniasis in Venezuela (Añez et al., 2012) occurs across a semi-arid region where there is little diversity in the natural ecosystem, characterized by a low diversity of plant and animal species (Ewel and Madriz, 1968). An epidemiological study in this region showed that slightly over 5% of the individuals in *Lutzomyia longipalpis* and *Lutzomyia evansi* populations were infected with *Leishmania infantum*, the etiologic agent of visceral leishmaniasis in Venezuela (Añez et al., 2012). Nevertheless, the understanding of SF β-diversity patterns in this endemic region is poor. Here, we use null models to ask whether sand fly vector species composition was segregated/aggregated, and if aggregated whether there was nestedness across locations from a semi-arid environment with endemic visceral leishmaniasis transmission in Falcón state, northwestern Venezuela. We found that species were both aggregated and nested, following null model tests ($P < 0.05$). Nestedness was both due to partial species turnover across sampled locations and to sampled locations having subsets of species from the more species rich sites. Species composition differences increased with distance, suggesting that species turnover might be partially shaped by dispersal limitation. Only one species without medical importance, *Lutzomyia venezuelensis*, was found across most of our study sites. By contrast, we found that three species of medical importance were common across the sampling locations: *Lu. gomezi*, *Lu. panamensis* and *Lu. evansi* which supports the idea that vector species do not turnover in the studied setting, and further supports that *L. evansi* could be a dominant vector of visceral leishmaniasis in the studied area.

2. Materials and methods

2.1. Data collection

Sand flies were collected by direct aspiration from natural resting places (NRP) and with Shannon illuminated traps (ST) at 6 localities (Fig. 1) in Northwestern Venezuela (10°18′08″–11°50′46″N and 68°14′28″–71°18′21″W) where visceral leishmaniasis is endemic (Añez et al., 2012). Both NRP and ST are standard methods for sand fly sampling (Alexander, 2000). We chose NRP and ST in order to compare species richness with data from sampling techniques targeting "active" (ST) and "resting" (NRP) sand flies. All our study locations are within a semi-arid environment characterized by a scarce annual rainfall, around 400 mm in total per year, with an average relative humidity (RH) 65% and an average temperature between 28 and 29 °C with minimal seasonal variability (Chaves and Vivas, 1972; Ewel and Madriz, 1968). We chose 6 sampling locations that were representative of the variability that mountain ranges (Fig. 1) create in an otherwise relatively homogenous semi-arid tropical environment (Chaves and Vivas, 1972). We also chose 6 locations because this a suitable number of locations for biogeographical comparison (MacArthur, 1984) and large enough for the proper estimation of regional species richness (Chao et al., 2009).

At each locality the sand fly sampling protocol was as follows: (i) NRP was conducted on the buttress of *Ceiba* spp. trees inside a 100 m perimeter from three peridomiciliary goat pens. Goat pens were between 3 and 10 km apart. We chose the buttress of *Ceiba* spp. trees because they are preferred resting places for phlebotomine sand flies (Christensen and de Vasquez, 1982; Rutledge and Mosser, 1972) and ubiquitous in the studied environment. Aspirations were carried out by two people between 7 and 9 h and 16–8 h (ii) in each of the three goat pens a ST was used to collect sand flies. The ST consisted of a 2 m × 2 m white linen and a 120 W light. Sampling was performed by two collectors between 19 and 21 h. NRP at each site was performed on two different days per month, one day sampling in the morning and afternoon, the other day only sampling in the afternoon. ST sampling was performed twice at each sampling site, on the same month that NRP sampling was performed. Sampling of the sites occurred between July 2009 and August 2012, in a temporally sparse manner, i.e., due to logistic constrains not all of the six locations were sampled at once. However, all locations had a homogenous ST sampling effort.

Collected sand flies were killed by freezing recently collected samples at −20 °C for 10 min, and subsequently preserved in 70% ethanol until used for identification. For each location and collection method we summarized sand fly abundance by species. We identified sand flies using the male genitalia and female spermathecae as taxonomic characters following Young and Duncan (1994) and used the classification system of Lewis et al. (1977) over competing ones, given its economy of genera (Vences et al., 2013) and also to ease comparison with previous studies on sand flies from the Neotropics. Sand fly voucher specimens are available at Centro

de Investigaciones Parasitológicas "J.F. Torrealba", Universidad de Los Andes, Mérida, Venezuela.

2.2. Statistical analysis

We estimated the total number of species based on sampled species abundance by each collection method using the Chao2 estimator (Chao et al., 2005). This was done in order to ensure that we performed an appropriate sampling of the sand fly metacommunity species richness, i.e., that the number of species we collected with each method was representative of species richness in the region comprised by our six sampling locations. For robustness, we also estimated species richness with species accumulation curves by rarefaction (Colwell and Coddington, 1994), which are expected to flatten when most species have been sampled with a given technique.

We then compiled data on the presence/absence of sand fly species at each site and proceeded with the estimation of C-score (Stone and Roberts, 1990) for data obtained with each collection method, as well as, with a combined dataset based on collections from both NRP and ST. The C-score is a metric used to investigate whether species aggregate or segregate across habitats, i.e., sampling locations in our study. Species aggregation indicates that most species tend to be concentrated in at least a sampling location, while species segregation means that species do not frequently co-occur across a set of sampling sites (Gotelli, 2000). Briefly, the inference for aggregation (or segregation) is based on whether an estimated C-score is below (or above) the distribution of simulated C-scores (Stone and Roberts, 1992).

For datasets that showed aggregation, we further investigated whether species were nested across sites, i.e., whether species composition changed in a fashion where some species were widespread while, nevertheless, species richness varied across sampling locations (Ulrich and Gotelli, 2007). For this end we estimated the nestedness metric based on overlap and decreasing fills (NODF) proposed by Almeida-Neto et al. (2008), which determines whether there is nestedness (NODF-Global), and which can also quantify whether nestedness is due to the partial segregation of less frequent species from the most frequent (NODF-species), often referred as partial species turnover (Baselga, 2010), and whether sampling locations progressively decrease species richness when compared to more species rich locations (NODF-Locations). The NODF metrics inference for nestedness is based on whether estimates from the field data are significantly above the distribution of the simulations (Ulrich and Gotelli, 2007).

We tested C-scores and NODF metrics employing null model tests (Gotelli, 2000; Ulrich and Gotelli, 2007). We simulated matrices assuming the number of times a species appeared across the sampling locations was constant, but the probability of sampling a species was the same across sites, and we only considered the presence/absence of species (not their abundance) when implementing the simulations, in order to make sound comparisons between the three datasets. Repeating the simulations 10,000 times we built a distribution for each index that was then compared to the estimate from the original datasets.

Given that our study locations were separated in space we further inquired to what extent dispersal limitation might have played a role on the species richness patterns that we observed. We therefore employed the multi-site Sørensen species dissimilarity index derived by Baselga (2010) which is expected to be positively associated with the distance between sampling locations when dispersal limitation plays a role on shaping diversity differences across sites. The multi-site Sørensen species dissimilarity index has the advantage of being furtherly decomposed into the Simpson index which is expected to increase with geographical isolation when there is a species turnover across localities, and the nestedness-resultant index which is expected to increase with distance if locations are nested in a manner where species richness progressively decreases. We then estimated the association between index dissimilarity and geographical distance using the Pearson correlation (Chaves et al., 2011). For statistical inference we performed a 999 randomizations Mantel test, in order to account for the lack of independence in our data (Chaves, 2010).

The null-model simulations were performed using the program co-occurrence described by Ulrich et al. (2009). All other analyses were performed using the package "vegan" in the statistical language R, version 3.1.0.

3. Results

Combining results from the two collection methods we found a total of 15 species (Table 1). Three species of medical importance were found in at least five of the six sampled localities: *Lutzomyia evansi*, *Lu. panamemsis* and *Lu. gomezi*, the first one a vector of visceral *Leishmania* spp. parasites (Feliciangeli et al., 1999), the second and third vectors of cutaneous *Leishmania* spp. parasites (Calzada et al., 2013; Christensen et al., 1983). No sand fly species was found in all the sites. Of the 15 species 13 were collected by NRP and 10 by ST. The most species rich site was Colina with 13 species, followed by Unión with 10. Two species: *Lutzomyia ovallesi* and *Lutzomyia migonei* were only sampled with ST, while *Lutzomyia walkeri*, *Lutzomyia micropyga*, *Lutzomyia pilosa*, *Lutzomyia venezuelensis* and *Lutzomyia punctigeniculata* were only sampled by NRP. The remaining eight species were sampled with both collection methods (Table 1).

A total 1675 sand flies were caught by NRP (Table 1). The most abundant species was *L. evansi* with 644 individuals. The location where most sandflies were collected was Buchivacoa. The Chao2 ± S.E. was 19.00 ± 6.48 species, which indicates that the 13 species we collected, which is within the 95% CI of the estimate, are an exhaustive sample of the number of species that could be found using NRP as a collection method in our study setting, a result confirmed by the species accumulation rarefaction curve (Fig. 2A).

A total 2243 sand flies were caught with ST (Table 1). The most abundant species was *L. evansi*, which accounted for nearly half of the samples with 1044 individuals. The location where most sandflies were collected was Colina. The Chao2 ± S.E. was 12.66 ± 3.49 species, which indicates that the 10 species we collected are an exhaustive sample of the number of species that could be found using ST as a collection method in our study setting, a result also observed in the species accumulation rarefaction curve (Fig. 2B).

Results for the C-score analysis are presented in Table 2. They show that in all cases the estimated C-scores were significantly smaller ($P < 0.05$) than the simulations, indicating that species were aggregated.

Since all three datasets showed aggregated patterns of sand fly species co-occurrence, we proceeded with the nestedness analysis for each dataset. Results for the NODF metrics are presented in Table 2. All NODF-Global metrics were significantly larger than expected by random ($P < 0.05$), indicating that sand fly communities were nested independently of the collection method. Similarly all NODF-Location metrics were significantly larger than expected by random ($P < 0.05$). This result indicates that there was a significant progressive nestedness between species rich and poor sites, as suggested by Table 1. Similarly, the NODF-Species was significantly larger than expected by random ($P < 0.05$), a result supporting some degree of species turnover. This last result is further illustrated by a cluster analysis of the Sørensen dissimilarities when employing results from both collection methods (Fig. 3), which shows that Acosta had the poorest sand fly fauna, and the clustering of Unión

Table 1
Species abundance by location and sampling method. ST indicates Shannon trap and NRP indicates direct aspiration of natural resting place and Both indicates absence(0)/presence (1) by either NRP or ST.

Location	Colina			Unión			Mauroa			Buchivacoa			Federación			Acosta		
Sampling method/species	ST	NRP	Both	ST	NRP	Both	ST	NRP	Both	ST	NRP	Both	ST	NRP	Both	ST	NRP	Both
Lutzomyia flaviscutellata (Mangabeira)	0	53	1	12	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Lu. walkeri (Newstead)	0	33	1	0	6	1	0	0	0	0	0	0	0	0	0	0	0	0
Lu. gomezi (Nitzulescu)	177	52	1	0	7	1	18	13	1	11	0	1	7	0	1	0	0	0
Lu. longipalpis (Lutz & Neiva)	41	73	1	0	0	0	0	0	0	267	339	1	0	0	0	0	0	0
Lu. atroclavata (Knab)	8	54	1	14	53	1	0	25	1	0	0	0	0	0	0	0	44	1
Lu. micropyga (Mangabeira)	0	0	0	0	7	1	0	0	0	0	0	0	0	0	0	0	0	0
Lu. pilosa (Damasceno & Causey)	0	45	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lu. trinidadensis (Newstead)	12	10	1	0	0	0	0	25	1	0	0	0	0	0	0	0	0	0
Lu. venezuelensis (Floch & Abonnenc)	0	39	1	0	7	1	0	14	1	0	11	1	0	25	1	0	0	0
Lu. migonei (Franca)	38	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lu. hernandezi (Ortiz)	0	0	0	10	8	1	0	0	0	0	0	0	0	0	0	0	0	0
Lu. evansi (Nuñez-Tovar)	376	27	1	66	9	1	0	0	0	412	608	1	100	0	1	90	0	1
Lu. ovallesi (Ortiz)	65	0	1	52	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Lu. punctigeniculata (Floch & Abonnenc)	0	49	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lu. panamensis (Shannon)	201	39	1	89	0	1	0	0	0	0	0	0	63	0	1	114	0	1
Total	918	474	13	243	97	10	18	77	4	690	958	4	170	25	4	204	44	3

Table 2
C-score and NODF estimates for sand fly species sampled by direct aspiration at natural resting places (NRP), Shannon traps (ST) and by the combination of both methods (Both).

Metric	Sampling method	Estimated	Simulation mean ± SD	95% CI
C-score	NRP	0.40*	1.42 ± 0.17	(1.04, 1.68)
	ST	0.73*	1.39 ± 0.23	(0.87, 1.76)
	Both	0.45*	1.36 ± 0.15	(1.02, 1.60)
NODF-Global	NRP	69.23*	41.23 ± 4.30	(33.80, 50.41)
	ST	65.69*	46.48 ± 5.68	(36.39, 58.36)
	Both	69.49*	49.45 ± 3.56	(43.06, 57.11)
NODF-Locations	NRP	76.43*	40.58 ± 7.78	(23.89, 55.48)
	ST	67.78*	46.67 ± 9.56	(26.67, 64.78)
	Both	64.22*	51.00 ± 8.06	(31.43, 65.23)
NODF-Species	NRP	67.84*	41.35 ± 4.10	(33.97, 50.53)
	ST	65.00*	46.42 ± 5.30	(36.67, 58.33)
	Both	70.24*	49.22 ± 3.26	(43.33, 56.19)

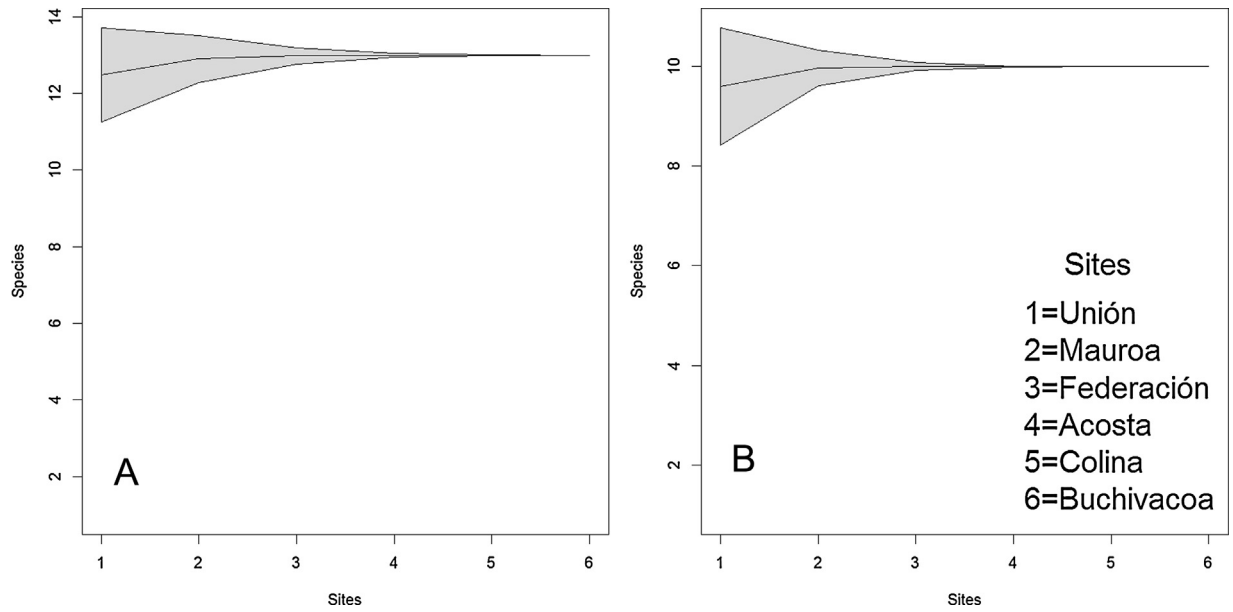

Fig. 2. Rarefaction curves for sand fly species. Species were sampled by (A) Direct Aspiration at Natural Resting Sites (B) Shannon traps. The black line indicates the estimate number of species for rarefaction and the gray polygon represents the 95% confidence limits of the estimate.

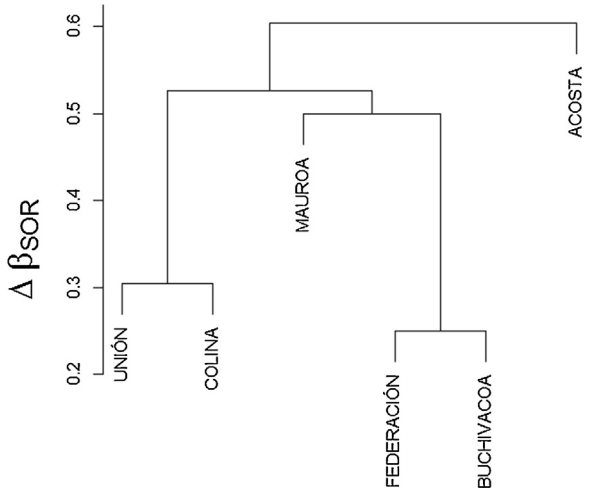

Fig. 3. Cluster of species similarities between sampling locations. This agglomerative cluster is based on the Sørensen pair-wise dissimilarity, the agglomerative coefficient was 0.31 and the axis indicates dissimilarity, i.e., farther away locations across the axis shared less species.

and Colina and of Federación and Buchivacoa, supports a partial degree of species turnover.

Tests for the impact of dispersal limitation on the nestedness/turnover of species (Fig. 4) suggested that species dissimilarities in the sand fly fauna collected with both NRP and ST (Fig. 4A, $P < 0.05$) and with STs (Fig. 4D, $P < 0.05$) had patterns influenced by the distance between sampling locations, and that species turnover was likely influenced by dispersal limitation (Fig. 4B and E, $P < 0.05$).

4. Discussion

Results from the Chao2 estimates using the NRP and ST datasets support a thorough sampling of the sand fly meta-community in the studied region, since predictions of 15 to 16 species in the region fits the 15 species we found when combining both methods. In that sense, we can affirm that our analysis is based on a high quality dataset, with data systematically collected and using standard sampling methods (Alexander, 2000). The fact that SF species were aggregated, independently of the sampling methodology, suggests that sites harboring the largest number of species might have more diverse habitats that supports a larger diversity of SF species (Stone and Roberts, 1992), a possibility re-inforced by the systematic nature of the sampling.

SF species were nested across the sampling locations, independently of the sampling methodology, and with a pattern of progressive species richness decrease from the most species rich site, i.e., Colina (13 spp.), to the site with least species, i.e., Acosta (3 spp.). This pattern might reflect the diversity associated with geographical differences (MacArthur, 1984) in our study site. Colina and Unión, the most species rich sites (Fig. 1) in our study region lie in the piedmont of the San Luis mountain range (Chaves and Vivas, 1972). By contrast, the other four sites tend to be in flat areas next to the San Luis mountain range (Fig. 1), where ecosystem bio-

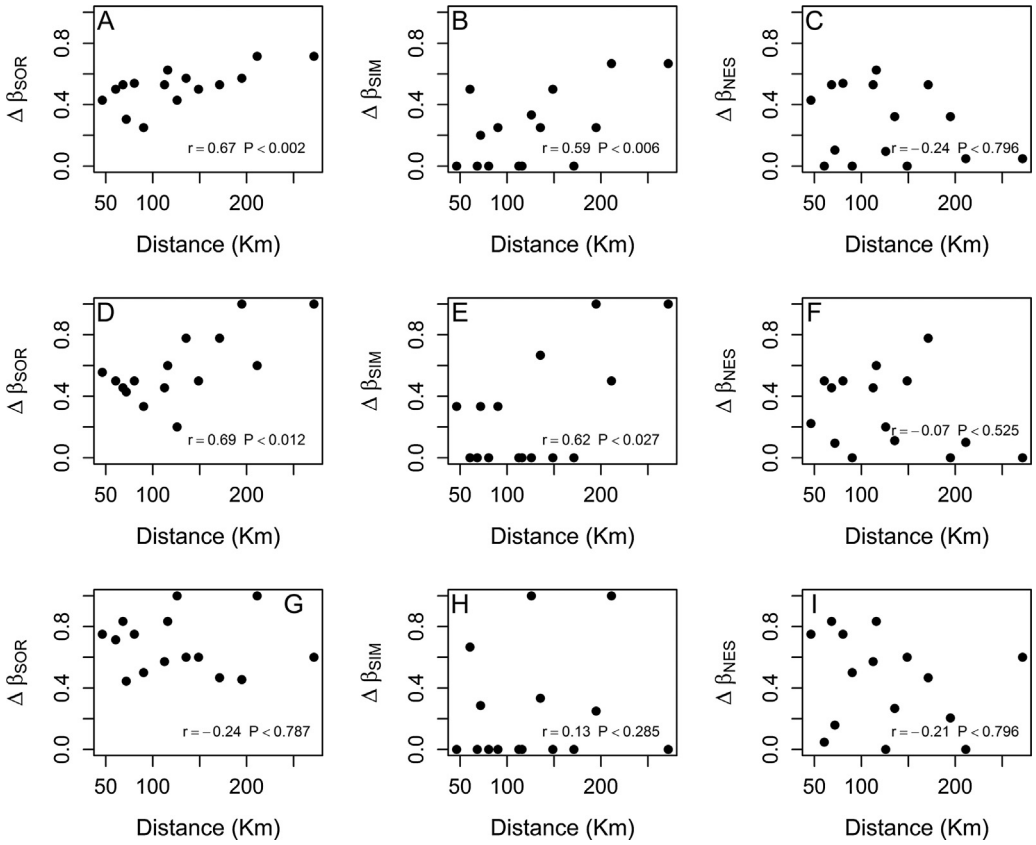

Fig. 4. β-Diversity patterns for sand fly species by collection method. Results for sand flies collected by direct aspiration at natural resting places: (**A**) Sørensen β-diversity dissimilarity, (**B**) Simpson β-diversity dissimilarity and (**C**) nestedness resultant β-diversity dissimilarity as function of geographic distance. Results for sand flies species collected with Shannon traps: (**D**) Sørensen β-diversity dissimilarity, (**E**) Simpson β-diversity dissimilarity and (**F**) nestedness resultant β-diversity dissimilarity as function of geographic distance. Results for sand fly species collected with both Shannon traps and by direct aspiration at natural resting places: (**G**) Sørensen β-diversity dissimilarity, (**H**) Simpson β-diversity dissimilarity and (**I**) nestedness resultant β-diversity dissimilarity as function of geographic distance. Inside the panels the Pearson's correlation coefficient (r) is indicated and its significance (P) was obtained with a Mantel test, given constraints on the independence of observations.

diversity is more restricted (Ewel and Madriz, 1968). This fact could also explain why the clustering of species by sites when considering data from all methods did not correspond with the geographical distance between the samples sites.

The partial turnover of species detected by the NODF-Species is related to rare species that were only found in the most species rich sites, specifically *Lu. pilosa* and *Lu. puntigeniculata* were only collected in Colina and *Lu. micropyga* and *Lu. hernandezi* which were only collected at Unión. Interestingly, three species of medical importance were common across the sampling locations: *Lu. gomezi* (Saldaña et al., 2013), *Lu. panamensis* (Christensen et al., 1983), and *Lu. evansi* (Travi et al., 1996). *Lu. ovallesi*, a vector of *Leishmania* spp. parasites causative of cutaneous leishmaniasis (Feliciangeli et al., 1988) and *Lu. longipalpis*, a vector of *L. infantum* the etiologic agent of visceral leishmaniasis (Young and Duncan, 1994) were only presented in the most species rich sites, suggesting that in the studied area vector species did not turnover. Only one species without medical importance, *Lu. venezuelensis*, was common across most sampling sites, suggesting nestedness in the community was mainly driven by medically important species.

The impact that a dispersal limitation (Baselga, 2010) might have on species turnover, requires further study. Although, the existence of species turnover in the meta-community of sandflies across the studied sites is a robust result, given similar inferences from the three datasets we analyzed, the significant impact of distance on SF species dissimilarity sampled with ST might reflect the fact that ST attract active sand flies, as opposed to NRP which samples resting SF species. This result also highlights trade-offs of different SF collection methods (Alexander, 2000), while ST is easy to standardize, it might miss some species, and as we implemented NRP, we could not standardize the number of treebuttress that we sampled. Thus, while concerns about an adequate sampling of SF species richness is not an issue when using several collection methods, problems might arise by the lack of consensus in results for other ecological analysis. A possible solution in our study setting will be sampling with an additional method that collects active SF species, for example CDC or similar light traps (Calzada et al., 2013; Rutledge et al., 1976, 1975).

Finally, the next step from this study will be to test how robust are inferences about co-occurrence and nestedness exclusively based on adult data when compared with results from sand flies sampled at the larval stage (Rutledge and Mosser, 1972), a task that is becoming feasible given advances in traps designed to sample immature sand flies (Casanova, 2001). This step is fundamental, since only a detailed sampling including several collection techniques can give a complete idea of species richness in a vector meta-community (Hoshi et al., 2014). Similarly, understanding the potential association between species composition nestedness and phylogenetic relationships of sand flies, (Vamosi et al., 2009) placing special attention to the context where vector species tend to be more widespread than species without medical and/or veterinary importance.

Conflict of interest

No competing interests have been declared by all authors.

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