






Insights into the assembly rules of a continent-wide multilayer network

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How are ecological systems assembled? Identifying common structural patterns within complex networks of interacting species has been a major challenge in ecology, but researchers have focused primarily on single interaction types aggregating in space or time. Here, we shed light on the assembly rules of a multilayer network formed by frugivory and nectarivory interactions between bats and plants in the Neotropics. By harnessing a conceptual framework known as the integrative hypothesis of specialization, our results suggest that phylogenetic constraints separate species into different layers and shape the network's modules. Then, the network shifts to a nested structure within its modules where interactions are mainly structured by geographic co-occurrence. Finally, organismal traits related to consuming fruits or nectar determine which bat species are central or peripheral to the network. Our results provide insights into how different processes contribute to the assemblage of ecological systems at different levels of organization, resulting in a compound network topology.

One of the most important quests in ecology has been to unveil the assembly rules of ecological systems¹. Different study models have been used in an attempt to generate unifying principles, from sets of species (that is, communities²) to systems formed by species interactions (that is, networks³). Knowing those rules is crucial not only for a basic understanding of the architecture of biodiversity⁴, but also for restoring degraded environments⁵ and controlling emerging diseases⁶, among other applications. However, identifying those underlying rules remains one of the main unsolved challenges in ecology⁷.

Major advances in network science have shed light on some assembly rules that govern interaction systems^{8–10}. These breakthroughs allowed the ecological and evolutionary analysis of monolayer networks formed by a single interaction type. Since then, there has been much debate concerning the prevalent topology among interaction networks (nested or modular) and which processes generate those patterns—niche or neutral^{9,11}. Early evidence suggested that antagonistic networks should be predominantly modular while mutualistic networks should be nested¹². However, recent studies suggest that those topological archetypes are not exclusive to particular interaction types¹³, may occur in combination¹⁴ and depend on spatial and phylogenetic scales¹⁵.

A conceptual framework, termed ‘the integrative hypothesis of specialization’ (IHS¹⁶), proposes that host–parasite networks are shaped by a combination of ecological and evolutionary constraints (that is, trade-offs¹⁷) at larger scales (network and layers), and resource breadth processes¹⁸ at smaller scales (modules). The IHS, in its updated form¹⁹, is based on premises that can be extrapolated from parasites to consumers in general: (1) types of resources

differ in their potential to be used by consumers; (2) dissimilarity in use potential is organized into hierarchical clusters among resource types; and (3) an adaptation to use a particular resource supports the use of other similar resources but becomes a maladaptation to using dissimilar resources.

Using the frameworks of the IHS and multilayer networks²⁰, here we aimed to understand the assembly rules of a system formed by bats and plants that interact with one another through frugivory and nectarivory across the entire Neotropics. From the IHS, we deduced that different processes should shape the bat–plant network at different scales (that is, network, layers and modules). If this is true, first there should be strong phylogenetic and geographic constraints in the network studied, as it contains two interaction types and high phylogenetic diversity (one large bat family and several plant families²¹), distributed over an entire biogeographic region. These constraints should lead to strongly separated layers and modules. However, resource breadth and other processes should lead to a nested structure within modules, resulting in a compound topology—a modular network with internally nested modules. Second, considering that some bat species are able to feed on both fruits and nectar²², different organismal traits related to those diets^{22,23} should determine the relative importance of different bat species to the structure of each layer, and to bridging layers. Bats connecting the layers by consuming both fruits and nectar are named here ‘bridge species’ (that is, ‘state nodes’^{20,24}). Similarly, when the same bat and plant species are linked by both kinds of interaction, we named it a ‘dual interaction’ (that is, ‘multilink’^{20,24}).

Our results support the IHS as a good model for explaining the topology of interaction networks. They also provide evidence of

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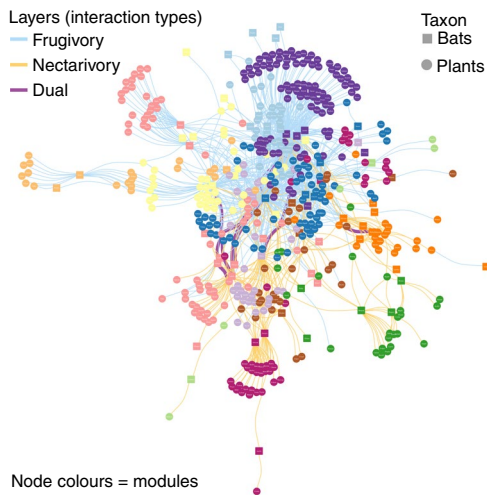


Fig. 1 | The bat-plant multilayer network. By compiling bat-plant interactions (lines) across the Neotropics, we found a compound topology with a strong separation between interaction types (layers) and guilds (modules). The layers represent interactions of frugivory, nectarivory and dual interactions. Modules were detected using the LPA. A high-resolution version with visible node codes is provided in Supplementary Data 1.

a compound topology in multilayer networks, with different processes operating at different network scales.

Results

The Neotropical bat-plant multilayer network analysed here (Fig. 1) is hyper-diverse and massive. It is composed of 439 plant species, 73 bat species, 911 links of frugivory, 301 links of nectarivory and 18 dual links (that is, links of both frugivory and nectarivory between the same bat and plant species). The frugivory layer contains 307 plant species and 56 bat species, while the nectarivory layer contains 139 plant species and 39 bat species. The 18 dual links were made between ten bat species and eight plant species.

As predicted, the main component of the aggregated network (that is, with all layers collapsed into one) showed a compound topology (Table 1 and Fig. 2). The modularity score for the entire structure (modularity (M) = 0.53, Z -score $Z_{free} = 49.18$, $P < 0.001$) was much higher than expected by the free null model (which shuffles the links in the network without considering module membership). The same was observed for the frugivory ($M = 0.48$, $Z_{free} = 44.45$, $P < 0.001$) and nectarivory ($M = 0.63$, $Z_{free} = 24.95$, $P < 0.001$) layers, using the free null model. In contrast, the entire network was slightly nested (nestedness (NODF) = 0.18, $Z_{free} = 4.72$, $P_{free} < 0.001$), as were the frugivory (NODF = 0.29, $Z_{free} = 7.12$, $P_{free} < 0.001$) and the nectarivory layers (NODF = 0.16, $Z_{free} = 2.39$, $P_{free} < 0.013$).

In addition, nestedness between species of the same module (NODF_{sm}) was much higher than nestedness between species of different modules (NODF_{dm}) in the aggregated network (NODF_{sm} = 0.55, NODF_{dm} = 0.13), as well as in the frugivory (NODF_{sm} = 0.60, NODF_{dm} = 0.23) and nectarivory (NODF_{sm} = 0.55, NODF_{dm} = 0.09) layers. In addition, NODF_{sm} was higher than expected by the free null model in all cases while NODF_{dm} was lower than expected in the aggregated network and the nectarivory layer, and equal to expected in the frugivory layer (Table 1). Finally, except for NODF_{dm} in the frugivory layer, nestedness was higher than expected in all other cases when compared to the restricted null model (which shuffles the links considering their module membership) (Table 1). Thus, the studied system is modular at the scales of the entire network and in each layer, and nested at the scale of modules.

Table 1 | The compound topology

	Obs	E_{free}	Z_{free}	P_{free}	E_{rest}	Z_{rest}	P_{rest}
Aggregated							
M	0.53	0.38	49.18	<0.001	NA	NA	NA
NODF	0.18	0.15	4.73	<0.001	0.15	6.14	<0.001
NODF _{sm}	0.55	0.14	53.32	<0.001	0.4	8.85	<0.001
NODF _{dm}	0.13	0.15	-2.23	0.006	0.11	3.58	<0.001
Frugivory layer							
M	0.48	0.35	44.45	<0.001	NA	NA	NA
NODF	0.29	0.22	7	<0.001	0.23	6.44	0.001
NODF _{sm}	0.6	0.19	34.69	<0.001	0.43	8.37	0.001
NODF _{dm}	0.23	0.22	0.92	0.179	0.19	4.04	0.002
Nectarivory layer							
M	0.63	0.47	24.95	0.001	NA	NA	NA
NODF	0.16	0.13	2.39	0.013	0.13	3.02	0.003
NODF _{sm}	0.55	0.13	37.41	<0.001	0.35	8.41	<0.001
NODF _{dm}	0.09	0.13	-4.82	<0.001	0.09	-0.56	0.29

The aggregated network has a modular structure with internally nested modules. We present the scores of modularity (M) and nestedness (NODF) for the aggregated network and its layers, including NODF scores calculated between species of the same module (c_{sm}) and of different modules (c_{dm}). These scores were calculated for the studied matrix (Obs), and also for matrices randomized using the free (free) and restricted (rest) null models. P values (P) were estimated using a Monte Carlo procedure run for each null model (1,000 iterations), which led to expected scores (E) and Z -scores (Z). The free null model randomizes the entire matrix without restriction, whereas the restricted null model considers the modular structure when randomizing the links. The fixed null model was not used for modularity, as it was designed for assessment of nestedness assuming a modular structure. All scores were standardized from 0 to 1. Significance level $\alpha = 0.05$; significant P values are in bold. NA, not applicable.

Phylogeny and geographic co-occurrence of bat species were also important predictors of the network's compound structure. Most bat species analysed have small geographic ranges, while a few are broadly distributed. The species with the smallest range was *Lonchophylla bokermanni* (23,309 km²), whereas that with the largest range was *Sturnira lilium* (17,327,789 km²). Mantel tests (Table 2) detected no correlation between the geographic co-occurrence and phylogenetic distances of bat species ($r = -0.01$, $P = 0.56$), which means that these bat clades are distributed in the Neotropical region independently of their evolutionary origin. Although we found a phylogenetic signal in the modules of the network ($r = 0.11$, $P < 0.001$), we did not find such a signal in the interactions ($r = 0.1$, $P = 0.07$). The geographic signal was stronger in the interactions ($r = 0.33$, $P < 0.001$) than in the modules ($r = 0.05$, $P = 0.03$). We found these same general trends when we used partial Mantel tests to discount for mutual effects between phylogeny and geography at different network scales (Table 2).

The interactions created a signal in the modules ($r = 0.25$, $P < 0.001$), indicating that some modules are formed mainly by nectarivorous bats and others by frugivorous bats. Additionally, we detected a phylogenetic signal in layer composition ($r = 0.12$, $P < 0.01$) where some bat clades are mainly nectarivorous while others are preferentially frugivorous. The phylogenetic signal remains in the modules even when correlation with the layers is discounted ($r = 0.1$, $P < 0.001$). Within the modules, geographic co-occurrence structures the interactions ($r = 0.33$, $P < 0.001$).

Few centrality metrics presented significant correlations with one another, whereas most were only weakly correlated or not correlated at all (Supplementary Results 1: Supplementary Figs. 10–13). Centrality varied greatly among all species and between layers in bridge species (Fig. 3), for which there was no relationship between degree, betweenness centrality, closeness or eigenvector centrality across layers (all $P > 0.05$; Table 3 and Supplementary Results 2:

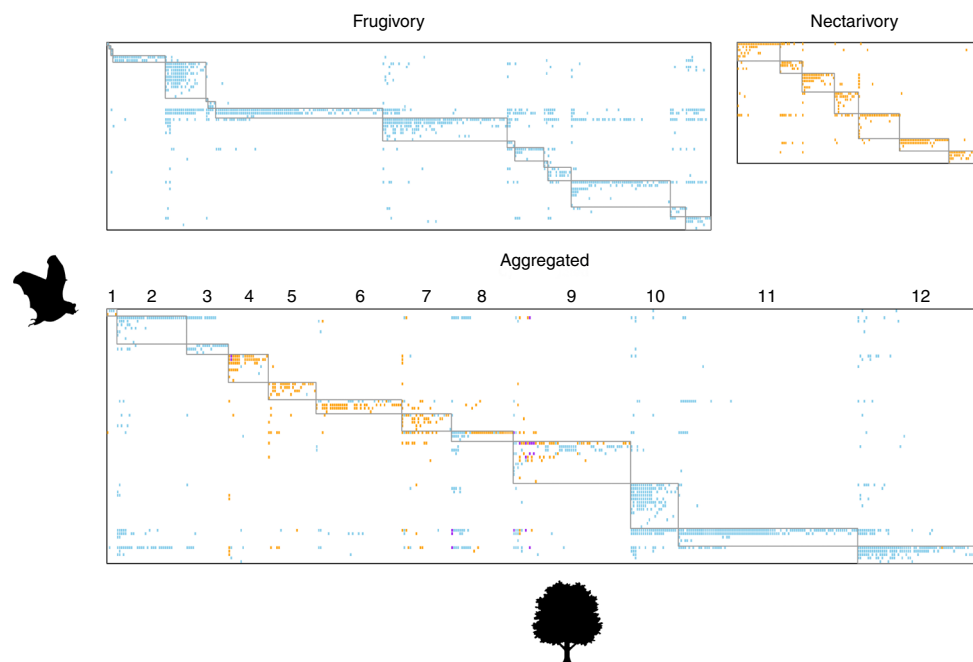


Fig. 2 | Matrices evidencing compound topology. Incidence matrices of the bat–plant network: frugivory layer, nectarivory layer and aggregated (that is, both layers collapsed into one). Bat species are represented in the rows, plant species in the columns. Coloured cells represent interactions of frugivory (blue), nectarivory (orange) or dual interactions (purple). Boxes and numbers represent the modules found in the main component of the network and each layer using the LPA. Their overall structure is modular and the modules are internally nested. Credit: bat and plant cartoons drawn by Nelson Vega.

Table 2 | Phylogenetic and geographic signals

Tested correlation	Controlling for	<i>r</i>	Z-score	<i>P</i>
Geography versus interactions	None	0.33	5.66	<0.001
Geography versus modules	None	0.05	2.05	0.03
Geography versus phylogeny	None	−0.01	−0.16	0.56
Interactions versus modules	None	0.25	10.89	<0.001
Phylogeny versus interactions	None	0.1	1.49	0.07
Phylogeny versus layers	None	0.12	2.42	<0.01
Phylogeny versus modules	None	0.11	4.71	<0.001
Phylogeny versus interactions	Geography	0.11	1.65	0.04
Phylogeny versus modules	Geography	0.11	4.73	<0.001
Geography versus modules	Interactions	−0.04	−1.65	0.96
Phylogeny versus modules	Interactions	0.09	3.78	<0.001
Phylogeny versus modules	Layers	0.1	4.24	<0.001
Geography versus interactions	Modules	0.33	5.65	<0.001
Phylogeny versus interactions	Modules	0.07	1.09	0.14
Geography versus interactions	Phylogeny	0.34	5.72	<0.001
Geography versus modules	Phylogeny	0.05	2.14	0.02

Results of Mantel and partial Mantel tests used to detect phylogenetic and geographic signals in the layers, modules and within-module interactions of the multilayer network. We report the Mantel correlation coefficient (*r*), the Z-score of the comparison between the observed correlation and that of the null model, and the associated *P* value. Significant *P* values are in bold.

Supplementary Fig. 15A). However, bat species with larger degree, betweenness centrality and eigenvector centrality in the frugivory layer had higher probabilities of being bridge species (all $P < 0.05$; Table 3 and Supplementary Results 2: Supplementary Fig. 15B). In the nectarivory layer, none of the centrality metrics explained the probability of a bat species being a bridge between layers (Supplementary Results 2: Supplementary Fig. 15C).

Geographic range size did not affect the centrality of bat species. Among the organismal and performance attributes, body size and bite force were the most important predictors of eigenvector centrality (see detailed results in Supplementary Results 2: Supplementary Table 1). For the frugivory layer, the latent variable analysis ($n = 16$, d.f. = 29) indicated that eigenvector centrality decreased with body size (coefficient = -0.524 , $P = 0.003$), increased with bite force

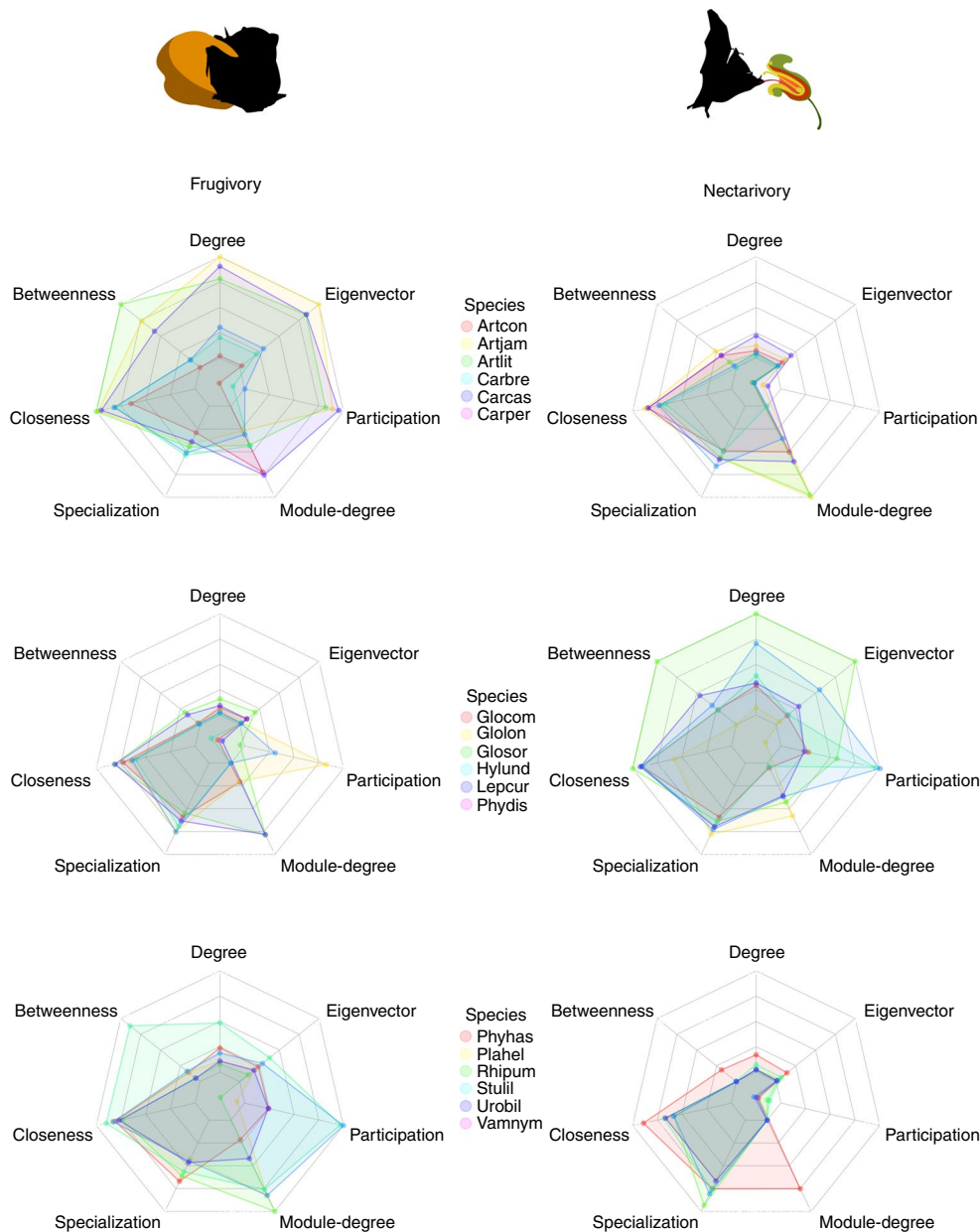


Fig. 3 | Centrality of bat species across layers. Scores of different centrality metrics varied greatly in each bat species between layers of the network (frugivory and nectarivory). Each axis of each spider chart represents the standardized magnitude of a centrality metric. Different bat species are represented by different colours. Only the six most central species that occurred in both layers are presented here. Species codes were created using the first three letters of the genus and epithet (for example, Carper = *Carollia perspicillata*). See full Latin names in Supplementary Data 1. Credit: bat and plant cartoons drawn by Nelson Vega.

(coefficient = 1.585, $P < 0.001$) and was not explained by the other latent and indicator variables (Fig. 4). For the nectarivory layer ($n = 15$, d.f. = 29), eigenvector centrality increased with body size (coefficient = 1.268, $P < 0.001$), decreased with bite force (coefficient = -1.841, $P < 0.001$) and was not explained by the other variables (Fig. 4). For dual interactions, the model could not be calculated due to the small number of observations. Finally, when considering the original structure of the multilayer network ($n = 18$, d.f. = 29), eigenvector centrality increased with bite force (coefficient = 0.517, $P = 0.013$) and was not explained by the other variables (Fig. 4).

Discussion

Our analysis of a continent-wide multilayer interaction network shows that a combination of processes operating at different scales

(network, layers and modules) explains the assembly of the system. This finding supports the IHS^{16,25}, which we here extend from parasite–host to plant–animal interactions.

A network analysis spanning an entire biogeographic region is not an easy task. It was made possible only due to the efforts of hundreds of scientists who collected data on bat–plant interactions over six decades in the Neotropics. Compiling these studies into a single dataset required certain decisions due to differences in data collection approaches (see Methods). Mainly, we converted data from frequency-weighted to binary (that is, presence/absence). Metrics calculated for the same network may diverge in their binary and weighted versions²⁶. Nevertheless, we agree with the view that binary data are adequate to study species interactions with a focus on fundamental niches, as in this case, whereas

Table 3 | Bridge species living between layers

Model	d.f.	Deviance	F	P
(1) Centralities versus layers				
ndeg.frug ~ ndeg.nect	20	0.027	0.269	0.610
bet.frug ~ bet.nect	20	0.003	0.033	0.857
clo.frug ~ clo.nect	20	0.002	2.208	0.153
eig.frug ~ eig.nect	20	0.018	4.870	0.832
(2) Bridge species versus frugivory				
ndeg ~ bridge	54	12.607		<0.001
bet ~ bridge	54	16.125		<0.001
clo ~ bridge	54	1.119		0.290
eig ~ bridge	54	14.940		<0.001
(3) Bridge species versus nectarivory				
ndeg ~ bridge	41	0.073		0.787
bet ~ bridge	41	0.858		0.354
clo ~ bridge	41	1.759		0.185
eig ~ bridge	41	0.002		0.963

ndeg, normalized degree; bet, betweenness; clo, closeness; eig, eigenvector; frug, frugivory layer; nect, nectarivory layer. Significance level $\alpha=0.05$. The centrality of a bat species on one layer of the network did not predict its centrality on the other layer. However, the higher the centrality of a bat species in the frugivory layer, the higher its probability of being a bridge species (that is, making interactions on both the frugivory and nectarivory layers). Relationships between centrality metrics were calculated for different layers of the network using GLMs. Significance of the models of set 1 was estimated using *F*-tests, while for sets 2 and 3 we used χ^2 tests. See also the plots in Supplementary Results 2: Supplementary Fig. 15. Significant *P* values are in bold.

frequency-weighted data would be best suited for study of local realized niches²⁷. With this in mind, we interpret our results from the perspective of entire bat species (that is, the consumers) in our system, and not local bat populations.

The bat–plant multilayer network is assembled by different processes that operate at different scales. First, phylogenetic and geographic constraints generate the layers and modules of the multilayer network. After those constraints play their role, the modules of the network become internally nested and are shaped by geographic co-occurrence. For sympatric species, this nested structure is probably a result of resource breadth¹⁸, neutral²⁸ or universal processes observed in different kinds of complex networks such as preferential attachment²⁹. Scale dependence has been pointed out as a critical issue in biodiversity research³⁰, and here we show that the same is true for species interactions. Second, organismal traits determine the importance of each species for the structure of each network layer and module. In the frugivory layer, these traits also determine which species bridge the layers by feeding on both fruits and nectar.

Organismal traits, such as body size and bite force, predict eigenvector centrality in a manner that is consistent with predictions from ecomorphological theory; species with greater performance are expected to have access to a broader array of ecological resources in the main layer to which they belong³¹. Bite force is a whole-organism performance trait that is tightly linked with the physical demands imposed by diet³². Specialized neotropical frugivores have evolved foreshortened rostra and large jaw adductors, which allows these species to have exceptionally forceful bites for their size and to consume fruits across a broader hardness spectrum than species with weaker bite force^{23,33,34}. Conversely, an elevated bite force is not a feeding performance requirement for nectarivores, for whom a long tongue stored within an elongated rostrum, and a larger body size, may be advantageous traits for accessing a broader array of flower sizes and types³⁵. The elongated rostrum

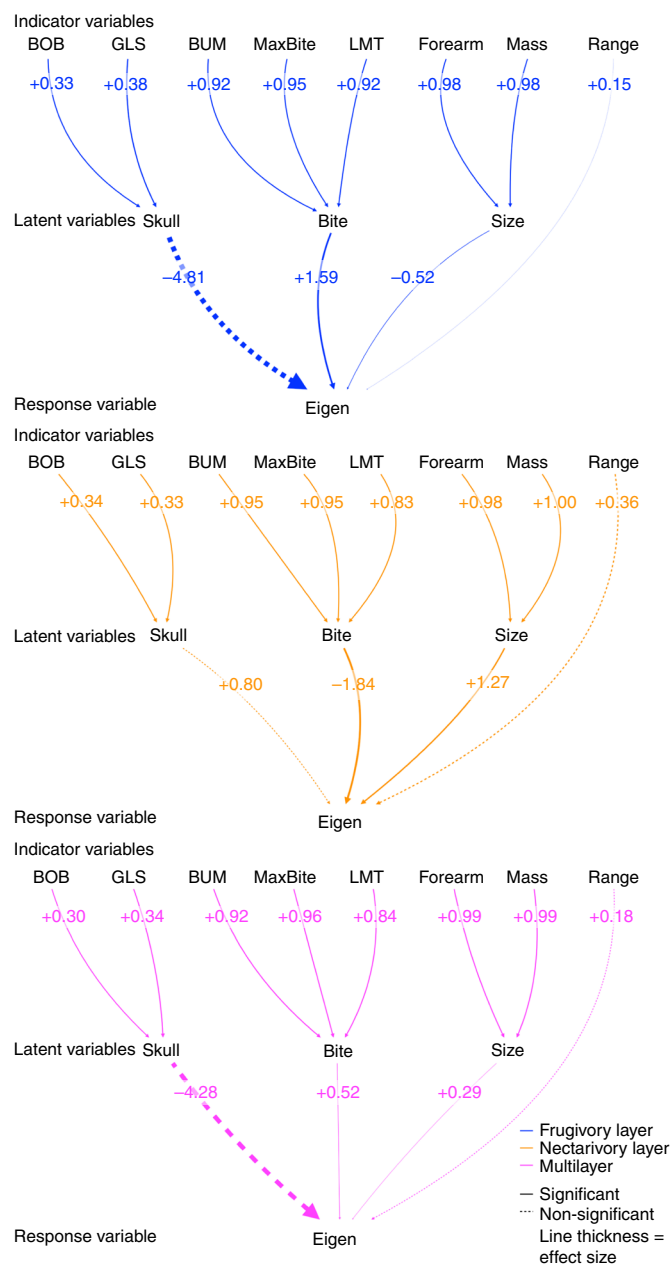


Fig. 4 | Influence of organismal traits on centrality. The relative importance of a bat species in regard to the structure of the network (measured as its eigenvector centrality, Eigen) was indirectly determined by a combination of organismal traits (indicator variables). Those traits were used to calculate the latent variables: skull morphology (Skull), bite force (Bite) and body size (Size). Those latent variables, together with a single indicator variable (geographic range size, Range), directly determined the eigenvector centrality. In other words, a bat species was more important to the structure of the frugivory layer (blue lines) when it had a strong bite force and a small body size. In the nectarivory layer (orange lines), larger bats with weak bite force were the most important. Finally, in the multilayer structure (purple lines), the most important roles were played by bats that bite more forcefully. Numbers on lines represent effect sizes (standardized path coefficients calculated in the latent variable analysis), and line thickness is drawn proportionally to effect size. Significance was estimated only for the main variables (Skull, Bite, Size and Range). Indicator variable names: BOB, breadth of braincase; GLS, greatest length of skull; BUM, breadth across upper molars; MaxBite, maximum bite force; LMT, length of maxillary toothrow; Forearm, forearm length; Mass, body mass.

results in relatively weaker bite forces and is mainly a consequence of tongue elongation, as it provides the storage space for the tongue.

Our results suggest that the dilemma of identifying the predominant topology among interaction networks (nested or modular) creates a false dichotomy. This interpretation is supported not only by our results, but also by evidence from other recent studies^{14,15,36} and challenges the traditional perspective that considers modularity and nestedness states along a continuum¹³. Ecologists foresaw these topologies for interaction networks in the past¹⁴, and it seems applicable to other types of ecological systems, such as communities and metacommunities^{2,37}. The IHS provides us with a mechanistic model that predicts this compound topology^{16,25}. In addition, the evidence obtained here also corroborates the importance of organismal traits, such as body size and feeding performance, to the hierarchy of centrality in interaction networks^{38,39}.

In conclusion, we found evidence that in a continent-wide, hyper-diverse, multilayer interaction network different processes operate at different network scales, and that organismal traits influence the roles played by different consumer species. Our findings integrate different debates from the ecological and parasitological literatures, and may also provide a means to understand the emergence of hierarchical structures in other complex systems, such as social and economic networks⁴⁰.

Methods

Dataset. The dataset used in the present study is from the Bat–Plant Interaction Database⁴¹ (recently expanded to Bat Eco-Interactions Database), which was partially published in a book on seed dispersal by bats⁴² and was later updated and used in other studies on ecological networks³⁸. In the present study, we added new data on bat–flower interactions collected by the authors in Mexico, Costa Rica and French Guiana, which were published in several papers. The list of data sources, together with a map of the study sites, is presented in Supplementary Data Sources 1.

Network building. The original studies from which we sourced the bat–plant interaction data used a variety of methods, ranging from mist-netting to roost inspection and direct observation. In addition, these studies varied in their foci, from single bat species or plant families to whole bat–plant ensembles in one location. Therefore, we decided to use binary data (that is, presence/absence of interactions) to build the multilayer network, as it would be very complicated to integrate and standardize frequency data from different methods collected at different taxonomic levels. Furthermore, binary data are more adequate in the assessment of fundamental ecological niches^{27,43}, which is the topic of our study. The multilayer network was compiled for the entire Neotropical region. Henceforth, its links (edges) represent interactions across the entire geographic range of species of bats and plants (that is, the nodes or vertices), and not just single local populations. These links thus represent dimensions of the fundamental niches of those species, and not their local realized niches.

On each layer of the network, a bat species and a plant species were connected to each other by a link if an interaction of frugivory or nectarivory between them had been recorded in the wild (intra-layer link), and they were also connected to their counterparts on the other layer (interlayer link). All bat and plant species were represented in both layers, even when the bat species fed on only one kind of food. Several bat species consume fruits and nectar, and thus belong to both layers of the network. We call these bridge species (or state nodes^{30,24}). Furthermore, a few bat and plant species were connected to one another in both layers, representing what we call dual interactions (or multilinks^{30,24}). In other words, those bat species are potentially both seed dispersers and pollinators of the same plant species^{44,45}. Thus, the multilayer network fits the specific category of a ‘multiplex network’ (node-aligned, equally sized and diagonally coupled^{24,46}). This multilayer network contains two types of interaction (frugivory and nectarivory) and two types of link (intra- and interlayer).

We modelled interaction types as interconnected layers in the format of an edge list (Supplementary Methods 1; see also the edge list in Supplementary Data 1). For the analyses of topology and phylogenetic and geographic signals, we used the aggregated version of the network which we created by collapsing the layers into a single one. In this analysis, we also assessed the layers separately. We used the original multilayer structure for the centrality analysis. Full Latin names of bats and plants are presented in Supplementary Data 1. Network science terms used here are explained in detail in Supplementary Glossary 1.

Compound topology. *Compound topology analysis.* To test whether each layer and the aggregated network were formed by internally nested modules (a compound topology⁴⁴), we used a recently proposed protocol²⁵ based on the steps below. We performed all analyses only for the main component of the network (that is, the

largest connected subset), as the other isolated components were too small to assure sufficient statistical power.

Step 1, find the best partition of a network and its modularity score using the package bipartite⁴⁷ for R⁴⁸ and the label propagation algorithm (LPA)⁴⁹, then compare the value to that expected by a given null model of interest⁵⁰.

Step 2, determine the nestedness (NODF value for of the entire network and disentangle it into two components: nestedness between pairs of species of the same module (NODF_{sm}) and nestedness between pairs of species of different modules (NODF_{dm}).

Step 3, compare the observed values of NODF_{sm} and NODF_{dm} to those expected in both the absence (free null model, see explanation in the next section) and presence (restricted null model²⁵) of the modular structure.

In a modular network, NODF_{sm} should be higher than expected by chance when interactions are reshuffled, regardless of the modular structure—that is, following the free null model. The reason is that connectance of areas within the modules of the null matrices will be smaller than that of the real matrix, and NODF increases monotonically with connectance⁵¹. Therefore, to test whether interactions are more nested than expected given the modular structure, we compared the observed NODF_{sm} and NODF_{dm} to the values expected by a null model that conserves the modular structure (that is, keeps the observed connectance values within and between modules in the null matrices).

Null models. The free null model produces null matrices of the same size, connectance and species-relative degrees, and follows the same algorithm as the vaznull model⁵² implemented in the package bipartite for R⁴⁷. In addition to size, connectance and relative degrees, the restricted null model also conserves the modular structure of the original matrix when generating the null matrices, following the same algorithm proposed in a previous study²⁵. This is done by weighting the a priori probability of interaction among consumer C_i and resource R_j (P_{ij}) by the connectance of the module to which the cell M_{ij} belongs.

For each layer and the aggregated network, we generated 1,000 random matrices using the free null model and 1,000 matrices using the restricted null model, to estimate significance using Monte Carlo procedures. Next, for each random matrix, we computed its overall NODF and decomposed it into NODF_{sm} and NODF_{dm} using the observed partitions of their corresponding observed network. Finally, we calculated a Z-score as $Z = [\text{Value}_{\text{obs}} - \text{mean}(\text{Value}_{\text{sim}})] / \sigma(\text{Value}_{\text{sim}})$, where $\text{Value}_{\text{obs}}$ is the observed value of the metric and $\text{Value}_{\text{sim}}$ represents the values of the metric in the randomized matrices. We also compared observed and expected modularity values using Z-scores, but only for the free null model as it does not make sense to compare observed and expected modularities to a null model that fixes the modules.

Geographic and phylogenetic signals. We used a combination of analyses to detect the signals of the geographic distribution and phylogeny of bats at different scales of the multilayer network (that is, network, layers and modules). In this analysis, we used only bat species belonging to the main component of the network and whose distribution data were available in the International Union for Conservation of Nature (IUCN) Red List global assessment (65 bat species, <http://www.iucnredlist.org>). First, we computed five pairwise distance matrices for bat species: phylogenetic, geographic, interactions, modules and layers.

To generate the phylogenetic distance matrix, we used branch lengths from the most up-to-date, species-level phylogeny of phyllostomids⁵³ (for eight bat species not presented in the phylogeny, we averaged the distances of species in the corresponding genus; see Supplementary Methods 2). For pairwise geographic distances, we used a measure of the overlap in the distribution of bat species recovered from IUCN databases. Interaction, module and layer pairwise distances were calculated based on Jaccard index (for details, see Supplementary Methods 2).

To test for the geographic and phylogenetic signals, we performed a combination of Mantel and partial Mantel tests. We also used the Z-score as a measure of effect size (observed correlation minus the average correlation in randomized matrices, divided by s.d.). We tested the dependence between modules and layers of the network using a chi-squared test of independence. Lastly, we used a Mantel test to test for phylogenetic signal in bridge species.

Centrality and organismal traits. We assessed the relative importance of each bat species to the structure of each layer and for the entire network through a set of centrality metrics commonly used in the ecological literature^{38,54,55}. We calculated degree, complementary specialization, within-module degree and participation coefficient for each layer separately, considering their bipartite structure. We calculated closeness centrality, betweenness centrality and eigenvector centrality for the unipartite projections (of the bat nodes) of each layer and also for the original multilayer structure. For details on their definition and calculation, see Supplementary Methods 1. As most of these metrics are strongly correlated with one another, we selected four metrics that represent the key aspects of relative structural importance for further analysis: degree, betweenness centrality, closeness and eigenvector centrality.

Using generalized linear models (GLMs) based on a quasi-Poisson distribution of errors, we tested whether the centralities of bat species in the frugivory

and the nectarivory layers were correlated with one another. We checked all models for over- and underdispersion, and then tested them with an analysis of variance (ANOVA).

To test for a correlation between centrality indices of bat species in each layer (frugivory and nectarivory) and the probability of a bat being a bridge species between the layers, we also used GLMs. Since the response variable was binary (bridge species: yes or no), we used a binomial distribution of errors in those GLMs. We checked all models for overdispersion, and then tested them with a chi-squared test. These first two sets of statistical tests were conducted in R using the package lme4 (ref. ⁵⁶) (see Supplementary Results 2).

To test the relationships among body size, skull morphology, feeding performance, geographic range size and centrality, we used a dataset on morphometric and performance traits of phyllostomid bats for the whole Neotropics, compiled by R. Stevens and S.E. Santana from published studies^{23,33,57}. This dataset spans a large variety of morphometric and feeding performance traits, which were collected from wild animals and museum specimens using standardized methods³³. As many of these are strongly correlated with one another, we relied on previous studies to select traits considered most relevant to feeding function in the context of frugivory and nectarivory (see Supplementary Results 1).

In relation to organismal traits, species with larger geographic range size are expected to have broader diets within their trophic niches (for example, frugivory or nectarivory), as they cannot rely on specialized diets all over their distribution^{58,59}. Bats with larger body size can be expected to have broader diets, as they may cover larger distances and thus can access resources from a wider range of habitats^{38,60}. Skull morphology is another important trait related to diet in bats, as frugivorous species tend to have shorter and broader skulls than nectarivorous species²². Frugivorous bats are expected to bite more forcefully than nectarivorous bats, considering the differences in hardness between solid and liquid diets³¹.

As there should be complex direct and indirect paths of influence among body size, feeding morphology and performance, geographic range size and centrality, we used a latent variable analysis (LaVaAn) to summarize and assess these relationships. As the response variable, we chose eigenvector centrality (Eigen) because this metric synthesizes all others as it operationalizes relative importance as the number of links made by a node and the distribution of those links among modules. In all models, the response variable (Eigen) was determined by three latent variables: body size (Size), bite force (Bite) and skull morphology (Skull)—one single indicator variable, geographic range size (Range). The latent variable body size was composed of the indicator variables body mass (Mass) and forearm length (Forearm). The latent variable Bite was composed of the indicator variables length of maxillary toothrow (LMT), breadth across upper molars (BUM) and maximum bite force (MaxBite). The latent variable Skull was composed of the indicator variables breadth of braincase (BOB) and greatest length of skull (GLS). We built four similar models: one for the frugivory layer, one for the nectarivory layer, one for dual interactions and one for the original multilayer network.

Not all bat species participate in all layers of the network. In addition, we did not have morphological data for all bat species. Therefore, the sample size (n) of each model was smaller than the number of bat species that participate in each layer of the network. All statistical tests related to this prediction were carried out in R, using the package lavaan⁶¹ (significance level $\alpha = 0.05$ for all tests).

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Raw network data are freely available on GitHub via Zenodo: <https://doi.org/10.5281/zenodo.1487572>.

Code availability

Visualization codes are freely available on GitHub via Zenodo: <https://doi.org/10.5281/zenodo.1487572>.

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Author contributions

M.A.R.M. conceived the project. The first version of the working question, hypothesis and predictions was created by M.A.R.M. together with R.B.P.P. and G.M.F., and all authors contributed to improving the logical argument of the study. C.G. and M.T. acquired the literature data and field data used to build the dataset of bat–plant interactions, and M.A.R.M. updated the dataset. S.E.S. reconstructed the bat phylogeny. S.E.S. and R.D.S. built the dataset on bat morphology and performance. F.A.R. and N.L. developed the new multilayer version of the centrality metrics. M.A.R.M., R.L.M., R.B.P.P., G.M.F., F.A.R. and N.L. performed tasks related to data analysis and coding in R and Python. The first draft of the manuscript was written by M.A.R.M., R.B.P.P., G.M.F. and R.L.M., and all authors contributed to editing the text.

Competing interests

The authors declare no competing interests.

Additional information

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Data collection

Interaction data came from a literature database that was first compiled by C. Geiselman and then updated by M.A.R. Mello (correspondent author). Morphometric data were compiled from the literature and lab measurements by S. Santana and R. Stevens.

Data analysis

All network and statistical analyses were run in R version 3.6.0 (2019-04-26) -- "Planting of a Tree". Custom algorithms used in our study have already been previously published in previous papers (<https://doi.org/10.1101/236687> and <https://doi.org/10.1101/362871>).

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Study description	Our study was carried out using a database on bat-plant interactions in the Neotropics, as well as a database on bat morphometrics. We worked under the framework of the integrative hypothesis of specialization and tested: (i) the topology of the multilayer network, (ii) the relationship between centrality metrics between layers, and (iii) organismal traits as predictors of centrality.
Research sample	Interactions of frugivory and nectarivory between bats (Phyllostomidae) and plants of several families in the Neotropics. Data compiled from 365 papers and dissertations.
Sampling strategy	We included in our database only information from primary sources, including studies published in peer-reviewed journals and dissertations (MSc and PhD).
Data collection	Data collected from raw tables published in several studies.
Timing and spatial scale	The sources included in our database cover the entire Neotropical Region and span from 1950 to 2006.
Data exclusions	We started by working with all data. After the analysis of compound topology, we retained only the giant component of the network for further analysis.
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