1 Article

2 **Insights on the assembly rules of a continent-wide multilayer network**

3 Marco A. R. Mello^{1§}, Gabriel M. Felix², Rafael B. P. Pinheiro³, Renata L. Muylaert⁴, Cullen
4 Geiselman⁵, Sharlene E. Santana⁶, Marco Tschapka^{7,8}, Nastaran Lotfi⁹, Francisco A.
5 Rodrigues^{9,10,11} & Richard D. Stevens¹²

6 ¹Department of Ecology, Institute of Biosciences, University of São Paulo (USP), Rua do Matão
7 321/Travessa 14, 05508-090 São Paulo, SP, Brazil. E-mail: marmello@usp.br. [§]Corresponding
8 author.

9 ²Graduate School in Ecology, State University of Campinas (UNICAMP), Campinas, Brazil.

10 ³Graduate School in Ecology, Conservation, and Wildlife Management, Federal University of
11 Minas Gerais (UFMG), Belo Horizonte, Brazil.

12 ⁴Graduate School in Ecology and Biodiversity, São Paulo State University (UNESP), Rio Claro,
13 Brazil.

14 ⁵Bat Conservation International, Austin, Texas, USA.

15 ⁶Department of Biology and Burke Museum of Natural History and Culture, University of
16 Washington, Seattle, USA.

17 ⁷Institute of Evolutionary Ecology and Conservation Genomics, Ulm University, Ulm, Germany.

18 ⁸Smithsonian Tropical Research Institute, Balboa Ancon, Panama, Panama.

19 ⁹Department of Applied Mathematics and Statistics, University of São Paulo, São Carlos, Brazil.

20 ¹⁰Mathematics Institute, University of Warwick, Gibbet Hill Road, Coventry CV4 7AL, UK.

21 ¹¹Centre for Complexity Science, University of Warwick, Coventry CV4 7AL, UK.

22 ¹²Department of Natural Resources Management and Museum of Texas Tech University,
23 Lubbock, USA.

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Introductory paragraph

How are ecological systems assembled? Here, we aim to contribute to answering this question by harnessing the framework of a novel integrative hypothesis. We shed light on the assembly rules of a multilayer network formed by frugivory and nectarivory interactions between bats and plants in the Neotropics. Our results suggest that, at a large scale, phylogenetic trade-offs separate species into different layers and modules. At an intermediate scale, the modules are also shaped by geographic trade-offs. And at a small scale, the network shifts to a nested structure within its modules, probably as a consequence of resource breadth processes. Finally, once the topology of the network is shaped, morphological traits related to consuming fruits or nectar determine which species are central or peripheral. Our results help understand how different processes contribute to the assemblage of ecological systems at different scales, resulting in a compound topology.

Introduction

Since Darwin's "tangled bank" metaphor¹, one of the most important quests in ecology has been to unveil the assembly rules of ecological systems². Different study models have been used in an attempt to generate unifying principles, from sets of species (i.e., communities³) to systems formed by species interactions (i.e., networks⁴). Knowing those rules is crucial for understanding the architecture of biodiversity⁵, restoring degraded environments⁶, and controlling emerging diseases⁷, among other applications. However, identifying those rules remains one of the main unsolved challenges in ecology⁸.

Major advances in network science have shed light on some assembly rules that govern interaction systems^{9–11}. These breakthroughs permitted 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 should generate those patterns (niche or neutral). 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 geographic and phylogenetic scales¹⁵.

A novel conceptual framework, termed “the integrative hypothesis of specialization” (IHS¹⁶), proposes that a balance between trade-offs¹⁷ at larger scales and resource breadth processes¹⁸ at smaller scales shapes host-parasite networks. The IHS, in its updated form¹⁹, is based on premises that can be extrapolated from parasites to consumers in general: (i) types of resources differ in their ability to be exploited by consumers; (ii) resources are more different from one another at larger than smaller scales; and (iii) an adaptation to exploit a resource helps exploit similar resources but becomes a maladaptation to exploit dissimilar resources.

Using the framework from the IHS and new models of 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 in the entire Neotropical region. From the IHS, we deduced that different processes should shape the bat-plant network at different scales. If this is true, firstly, there should be strong phylogenetic and geographic trade-offs 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 trade-offs

should lead to strongly separated layers (large scale) and modules (intermediate scale). However, within the modules (small scale) resource breadth processes should lead to a nested structure, resulting in a compound topology: a modular network with modules internally nested. Secondly, considering that some bat species are able to feed both on fruits and nectar²², different organismal traits related to those diets^{22,23} should thus determine the relative importance of different bat species for the structure of each layer and for bridging layers.

Our results support the IHS as a good model to explain the topology of interaction networks. They also provide the first evidence of a compound topology in multilayer networks, with different processes operating at different scales.

Results

The Neotropical bat-plant multilayer network analyzed here (Fig. 1a) 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 (i.e., 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 10 bat species and 8 plant species.

As predicted, the studied network showed a compound topology (Table 1, Fig.1b). The modularity score for the whole multilayer structure ($M = 0.53$, $Z_{\text{free}} = 49.18$, $P < 0.001$) was much higher than expected by the free null model, which does not consider the network's modular structure (see Methods for explanations of the null models). The same was observed for the frugivory ($M = 0.48$, $Z_{\text{free}} = 44.44$, $P < 0.001$) and nectarivory layers, respectively, using the free

null model ($M = 0.63$, $Z_{\text{free}} = 24.94$, $P < 0.001$). In contrast, the entire multilayer structure was slightly nested ($\text{NODF} = 0.18$, $Z_{\text{free}} = 4.72$, $P_{\text{free}} < 0.001$), as well as the frugivory ($\text{NODF} = 0.29$, $Z_{\text{free}} = 7.12$, $P_{\text{free}} < 0.001$) and nectarivory layers ($\text{NODF} = 0.16$, $Z_{\text{free}} = 2.39$, $P_{\text{free}} < 0.013$). In other words, the studied multilayer network is both modular and nested at the same time, but the modular structure is stronger than the nested structure at larger scales.

Corroborating this result, nestedness between species of different modules (NODF_{DM}) was lower than expected by the free null model in the nectarivory layer and the multilayer network but, interestingly, equal to the expected value in the frugivory layer. This result suggests that the modules impose greater constraints to nectarivory than to frugivory interactions. Furthermore, nestedness in general (NODF), between species of the same module (NODF_{SM}), and between species of different modules (NODF_{DM}) was higher than expected considering the modular structure of the multilayer network and its layers. The exception was the nectarivory layer, in which species of different modules (NODF_{DM}) show higher nestedness than expected given the modules.

Geographic co-occurrence and phylogeny of bat species were also important predictors of the network's compound structure. Most bat species analyzed have small geographic ranges, while a few are broadly distributed. The species with the smallest range was *Lonchophylla bokermanni* (23,309 km²), whereas the species with the largest range was *Sturnira lilium* (17,327,789 km²). Mantel tests found no correlation between the geographic co-occurrence and phylogenetic distances of bat species, which means that these bat clades are distributed in the Neotropical Region independently of their evolutionary origin (Figure 2a). Though we found a strong phylogenetic signal in the modules of the network (intermediate scale) we did not find such signal in the interactions within the modules (small scale). Nevertheless, the contrary was

true for the geographic signal: it is strong at the scale of within-module interactions, but very weak in the modules (Figure 2a). We found these same general trends (Figure 2b-c) when we used partial Mantel tests to discount for mutual effects between the structuring factors and the scales.

There was dependence between modules and interaction types ($\chi^2 = 554.33$, $N = 12$, $P < 0.001$), which means that some modules are formed mainly by nectarivorous bats and others by frugivorous bats. Additionally, we detected a phylogenetic signal in layer composition (Figure 2d), where some bat clades are preferentially nectarivorous while others are preferentially frugivorous, which corroborates the structuring power of phylogeny at a large scale. Then, because of the dependence between layers and modules, we tested and confirmed that the phylogenetic signal in the modules remains even when discounting the correlation with the layers (Figure 2d). We conclude that phylogeny structures the layers of the network (large scale) and the modules inside each layer (intermediate scale), and geographic co-occurrence structures the interactions within each module (small scale). Finally, there was no phylogenetic signal in bridge species, which make both interactions of frugivory and nectarivory ($r = 0.04$, $Z = 0.75$, $P = 0.21$).

Few centrality metrics presented significant correlations with one another, whereas most were only weakly correlated or not correlated (Supplementary Results 1). Centrality varied largely among all species. It varied also between layers in the case of bridge species (Figure 3). For these bridge species, there was no relationship between degree, betweenness centrality, closeness, or eigenvector centrality across layers (all $P > 0.05$, Table 2, Supplementary Results 2). 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 2,

Supplementary Results 2). In the nectarivory layer, none of the centrality metrics explained the probability of a species being a bridge between layers.

Geographic range size did not affect the centrality of bat species. Among the morphological attributes, body size and bite force were the most important predictors of species' centrality. For the frugivory layer, the latent variable analysis ($N = 16$, $df = 29$) indicated that eigenvector decreased with body size (coefficient = -0.524 , $P = 0.003$), increased with bite force (coefficient = 1.585 , $P < 0.001$), and was not explained by the other latent and indicator variables (Fig.4a). For the nectarivory layer ($N = 15$, $df = 29$), eigenvector 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.4b). For dual interactions, the model could not be calculated due to the small number of observations. Finally, considering the entire multilayer structure and a multilayer version of centrality ($N = 18$, $df = 29$), eigenvector increased with bite force (coefficient = 0.517 , $P = 0.013$), and was not explained by the other variables (Fig.4c).

Discussion

Our analysis of a continent-wide multilayer interaction network shows that, in order to build a complex ecological system, a combination of processes operating at different scales is needed. This finding supports the integrative hypothesis of specialization (IHS^{16,19}), which we here extend from parasite-host to plant-animal interactions.

Firstly, at large and intermediate scales, phylogenetic and geographic trade-offs generate a multilayered and modular structure. After the influence of those trade-offs, at a small scale, the modules of the network are internally nested and shaped first by geographic trade-offs. For

sympatric species, this nested structure is probably a result of resource breadth processes¹⁸, neutral processes related to differences in abundance²⁴, or universal processes observed in different kinds of complex networks such as preferential attachment²⁵. Scale-dependence has been pointed out as a critical issue in biodiversity research²⁶ and here we show that the same is true for species interactions. Secondly, after the network is shaped, biological traits determine how important each species is for the structure of each layer of the network. Those traits determine also which species bridge the layers by being frugivorous and nectarivorous at the same time.

Organismal attributes, 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 resources²⁷. Bite force is a whole-organism performance trait that is tightly linked with the physical demands imposed by diet²⁸. Specialized neotropical frugivores have evolved foreshortened rostra and large jaw adductors, which allows these species to have exceptionally forceful bites for their size and consume fruit across a broader hardness spectrum than species that have weaker bite forces^{23,29,30}. Conversely, an elevated bite force is not a feeding performance requirement for nectarivores, to whom an elongated skull and thus weaker bite forces, and a larger body size may be an advantageous trait for accessing a broader array of flower sizes and types³¹.

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 by evidence from other recent studies^{14,15,32}, which highlight that modularity and nestedness are states along a continuum³. Ecologists foresaw this continuum for interaction networks in the past¹⁴, and it seems applicable to other types of

ecological systems, such as communities and metacommunities^{3,33}. The IHS provides us with a mechanistic model that predicts this compound topology^{16,19}. In addition, the evidence provided here also corroborates the importance of biological traits to the hierarchy of centrality in interaction networks^{34,35}.

In conclusion, we found evidence that to build a continent-wide, hyper-diverse interaction network, we need different processes operating at different scales. Our findings integrate different debates in the ecological and parasitological literatures, and may also help understand the emergence of hierarchical structures in other complex systems, such as social and economic networks³⁶.

Methods

Data set

The data set used in the present study came from the Bat-Plant Interaction Database³⁷, which was partially published in a book on seed dispersal by bats³⁸, and was later updated and used in other studies on ecological networks³⁴. 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 different papers. The list of data sources is presented in Supplementary Table 1.

Network building

The original studies from which we sourced the bat-plant interaction data used a myriad of methods, ranging from mist-netting to roost inspection and direct observation. In addition, these studies varied in their focus, from single bat species or plant families, to whole bat-plant

ensembles at a local scale³⁹. Therefore, we decided to use binary data (i.e., presence or 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 scales. Furthermore, binary data are more adequate to assess fundamental ecological niches^{40,41}, which is the case of our study. The multilayer network was compiled at the scale of the whole Neotropical Region. Henceforth, its nodes represent interactions across the entire geographic range of species of bats and plants, and not just single local populations. Its binary links (edges) 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, whether an interaction of frugivory or nectarivory between them had been recorded in the wild. Several species make links of both types, and thus belong to both layers of the network. We call these “bridge species”. Furthermore, a few bat and plant species were connected to one another in both layers, making what we call here “dual links”. In other words, some bat species are both seed dispersers and pollinators of the same plant species. Consequently, the multilayer network contained two types of links: frugivory and nectarivory. Those link types were modeled as interconnected layers in the format of an edge list (Supplementary Data 1). See also Supplementary Methods 1, where we explain how the multilayer structure was modeled. Full Latin names of bats and plants are presented in Supplementary Data 1. Network science terms used here are explained in detail in Supplementary Table 2.

Compound topology

Compound topology analysis

To test whether each layer and the aggregated network were formed by internally nested modules (compound topology, *sensu*¹⁴), we used a recently proposed protocol¹⁹, which is based on the following steps.

Step 1, find the best partition of a network and compare its modularity score to that expected by a given null model of interest⁴². Step 2, compute the nestedness (NODF) 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 their values expected both in the absence (free null model) and in the presence (restricted null model) of the modular structure.

In a modular network, NODF_{SM} should be higher than expected when interactions are reshuffled regardless of the modular structure, *i.e.*, 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 connectance⁴³. 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 (*i.e.*, keeps the observed connectance values within and between modules in the null matrices).

Null models

On the one hand, the free null model produces null matrices of the same size, connectance, and species relative degrees. On the other hand, besides size, connectance, and relative degrees, the restricted null model also conserves the modular structure of the original

matrix when generating the null matrices. This is made by weighting the *a priori* probability of interaction among species C_i and resource R_j (P_{ij}) by the connectance of the matrix sub-area to which the cell M_{ij} belongs¹⁹.

For each layer, and the aggregated network, we generated 1,000 random matrices using the free null model and another 1,000 matrices using the restricted null model. 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 real network. Finally, a Z-score was calculated as $Z = [Value_{obs} - \text{mean}(Value_{sim})] / \sigma(Value_{sim})$, where $Value_{obs}$ is the observed value of the metric and $Value_{sim}$ represents the values of the metric in the randomized matrices. Observed and expected modularity values were also compared using Z-scores, but only for the free null model, as it does not make sense to compare observed and expected modularities with 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 of the phylogeny of bats at different scales of the multilayer network. In this analysis, we used only the bat species that belong to the main component of the network, whose distribution data were available in the IUCN red list global assessment (65 bat species). First, we computed five pairwise distance matrices for bat species: phylogenetic, geographic, interactions, modules, and layers.

To generate the phylogenetic distance matrix, we used the branch lengths in the most up-to-date, species-level phylogeny of phyllostomids⁴⁴ (for 8 bat species not presented in the

phylogeny we used an alternative approach, see Supplementary Methods 2). For the 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 the signals, we performed a combination of Mantel and partial Mantel tests, and used the Z-Score as a measure of effect size (observed correlation minus the average correlation in randomized matrices, divided by the standard deviation). 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 a phylogenetic signal in bridge species.

Centrality and biological traits

We assessed the relative importance of each bat species to the structure of each layer or the entire multilayer network through the centrality metrics degree, closeness centrality, betweenness centrality, complementary specialization, within-module degree, participation coefficient, and eigenvector centrality. For details on their definition and calculation, see Supplementary Methods 1.

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. All models were checked for over- and sub-dispersion, and then tested 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 generalized linear models (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 two first sets of statistical tests were conducted in R, using the package *lme4*⁴⁵ (see Supplementary Results 2).

To test the relationship between 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. Santana from published studies^{23,29,46}. This dataset spans a large variety of morphometric and feeding performance traits, which were collected from wild animals and museum specimens using standardized methods²⁹. 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 (e.g., frugivory or nectarivory), as they cannot rely on specialized diets all over their distribution^{47,48}. Animals with larger body size are expected to have broader diets, as they have higher energy requirements than small-bodied animals^{34,49}. Frugivorous bats are expected to bite more forcefully than nectarivorous bats, considering differences in hardness between solid and liquid diets²⁹. Skull morphology is another important trait related to diet in bats, as frugivorous species tend to have shorter and broader skulls than nectarivorous species²².

As there should be complex direct and indirect paths of influence among body size, dietary morphology and performance, geographic range size, and centrality, we used a latent variable analysis (LaVaAn) to disentangle these relationships. In all models, the response

variable, eigenvector centrality (eg.), was determined by three latent variables: body size (Siz), bite force (Bit), and skull morphology (Skl), and one single indicator: geographic range size (rng). The latent variable body size was composed of the exogenous variables body mass (Mss) and forearm length (Frr). The latent variable Bit was composed of the exogenous variables length of maxillary toothrow (LMT), breadth across upper molars (BUM), and maximum bite force (MBF). The latent variable Skl was composed of the exogenous 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 (i.e., the same bat and plant species connected to one another in both layers), and one for the entire multilayer.

As not all bat species participate in both layers of the network, the sample size (N) of each test was smaller than the number of bat species analyzed in the present study. All statistical tests related to this prediction were carried out in R, using the package *lavaan*⁵⁰ (significance level $\alpha = 0.05$ for all tests).

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Author contributions

MARM conceived the project. The first version of the working question, hypothesis, and predictions was conceived by MARM together with RBPP and GMFF, and all authors contributed to improving the central argument of the study. CG and MT acquired the literature data and field data used to build the dataset of bat-plant interactions. SES reconstructed the bat phylogeny. SES and RDS built the dataset on bat morphology and performance. FAR and NL developed the new multilayer version of the centrality metrics. MARM, RLM, RBPP, GMFF, FAR, and NL performed tasks related to data analysis and coding in R and Python. The first draft of the manuscript was written by MARM, RBPP, GMFF, and RLM, and all authors contributed to editing the text.

360   **Competing financial interests**

361   None to declare.

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363   **Literature cited**

- 364   1.   Darwin, C. R. *The origin of species by means of natural selection or the preservation of*
365   *favoured races in the struggle for life*. (Bantam Classics, 1858).
- 366   2.   Thompson, J. N. *et al.* Frontiers of ecology. *Bioscience* **51**, 15–24 (2001).
- 367   3.   Latombe, G., Hui, C. & McGeoch, M. A. Beyond the continuum: a multi-dimensional
368   phase space for neutral–niche community assembly. *Proc. R. Soc. B Biol. Sci.* **282**,
369   20152417 (2015).
- 370   4.   Guimarães, P. R. & Deyn, G. B. De. Ecological networks: assembly and consequences.
371   *Oikos* **125**, 443–445 (2016).
- 372   5.   Estes, J. A. *et al.* Trophic downgrading of planet Earth. *Science* (80-.). **333**, 301–306
373   (2011).
- 374   6.   Peters, V. E. *et al.* Using plant–animal interactions to inform tree selection in tree-based
375   agroecosystems for enhanced biodiversity. *Bioscience* **66**, 1046–1056 (2016).
- 376   7.   Luis, A. D. *et al.* Network analysis of host–virus communities in bats and rodents reveals
377   determinants of cross-species transmission. *Ecol. Lett.* **18**, 1153–1162 (2015).
- 378   8.   Sutherland, W. J. *et al.* Identification of 100 fundamental ecological questions. *J. Ecol.*
379   **101**, 58–67 (2013).
- 380   9.   Guimarães, P. R., Pires, M. M., Jordano, P., Bascompte, J. & Thompson, J. N. Indirect
381   effects drive coevolution in mutualistic networks. *Nature* **550**, 511–514 (2017).
- 382   10.   Dormann, C. F., Fründ, J. & Schaefer, H. M. Identifying causes of patterns in ecological
383   networks: opportunities and limitations. *Annu. Rev. Ecol. Evol. Syst.* **48**, 559–584 (2017).
- 384   11.   Delmas, E. *et al.* Analysing ecological networks of species interactions. *Biol. Rev.* (2018).
385   doi:10.1111/brv.12433
- 386   12.   Thebault, E. & Fontaine, C. Stability of ecological communities and the architecture of
387   mutualistic and trophic networks. *Science* (80-.). **329**, 853–856 (2010).
- 388   13.   Fortuna, M. A. *et al.* Nestedness versus modularity in ecological networks: two sides of
389   the same coin? *J. Anim. Ecol.* **79**, 811–817 (2010).

- 390 14. Lewinsohn, T. M., Inácio Prado, P., Jordano, P., Bascompte, J. & M. Olesen, J. Structure
391 in plant-animal interaction assemblages. *Oikos* **113**, 174–184 (2006).
- 392 15. Flores, C. O., Valverde, S. & Weitz, J. S. Multi-scale structure and geographic drivers of
393 cross-infection within marine bacteria and phages. *ISME J.* **7**, 520–532 (2013).
- 394 16. Pinheiro, R. B. P. *et al.* Trade-offs and resource breadth processes as drivers of
395 performance and specificity in a host–parasite system: a new integrative hypothesis. *Int. J.*
396 *Parasitol.* **46**, 115–121 (2016).
- 397 17. Futuyma, D. J. & Moreno, G. The evolution of ecological specialization. *Annu. Rev. Ecol.*
398 *Syst.* **19**, 207–233 (1988).
- 399 18. Brown, J. H. On the relationship between abundance and distribution of species. *Am. Nat.*
400 **124**, 255–279 (1984).
- 401 19. Felix, G. M., Pinheiro, R. B. P., Poulin, R., Krasnov, B. R. & Mello, M. A. R. The
402 compound topology of a continent-wide interaction network explained by an integrative
403 hypothesis of specialization. *bioRxiv preprint*, (2017).
- 404 20. Boccaletti, S. *et al.* The structure and dynamics of multilayer networks. *Phys. Rep.* **544**, 1–
405 122 (2014).
- 406 21. Fleming, T. H. & Kress, W. J. *The ornaments of life: coevolution and conservation in the*
407 *tropics*. (University of Chicago Press, 2013).
- 408 22. Dumont, E. R. *et al.* Morphological innovation, diversification and invasion of a new
409 adaptive zone. *Proc. R. Soc. B Biol. Sci.* **279**, 1797–1805 (2012).
- 410 23. Dumont, E. R. *et al.* Built to bite: cranial design and function in the wrinkle-faced bat. *J.*
411 *Zool.* **279**, 329–337 (2009).
- 412 24. Coelho, M. T. P., Rodrigues, J. F. M. & Rangel, T. F. Neutral biogeography of
413 phylogenetically structured interaction networks. *Ecography (Cop.)*. (2017).
414 doi:10.1111/ecog.02780
- 415 25. Barabasi, A.-L. & Albert, R. Emergence of scaling in random networks. *Science (80-.).*
416 **286**, 509–512 (1999).
- 417 26. Chase, J. M. *et al.* Embracing scale-dependence to achieve a deeper understanding of
418 biodiversity and its change across communities. *Ecol. Lett.* (2018). doi:10.1111/ele.13151
- 419 27. Stevan J. Arnold. Morphology, performance and fitness. *Am. Zool.* **23**, 347–361 (1983).
- 420 28. Anderson, R. A., Mcbrayer, L. D. & Herrel, A. Bite force in vertebrates: opportunities and
421 caveats for use of a nonpareil whole-animal performance measure. *Biol. J. Linn. Soc.* **93**,
422 709–720 (2008).
- 423 29. Santana, S. E., Dumont, E. R. & Davis, J. L. Mechanics of bite force production and its
424 relationship to diet in bats. *Funct. Ecol.* **24**, 776–784 (2010).

- 425 30. Villalobos-Chaves, D., Padilla-Alvarez, S. & Rodrguez-Herrera, B. Seed predation by the
426 wrinkle-faced bat *Centurio senex*: a new case of this unusual feeding strategy in
427 Chiroptera. *J. Mammal.* **97**, 726–733 (2016).
- 428 31. Gonzalez-Terrazas, T. P., Medellin, R. A., Knorrschild, M. & Tschapka, M.
429 Morphological specialization influences nectar extraction efficiency of sympatric nectar-
430 feeding bats. *J. Exp. Biol.* **215**, 3989–3996 (2012).
- 431 32. Bezerra, E. L. S., Machado, I. C. & Mello, M. A. R. Pollination networks of oil-flowers: a
432 tiny world within the smallest of all worlds. *J. Anim. Ecol.* **78**, 1096–1101 (2009).
- 433 33. Vellend, M. *The theory of ecological communities (MPB-57)*. (Princeton University Press,
434 2016).
- 435 34. Mello, M. A. R. *et al.* Keystone species in seed dispersal networks are mainly determined
436 by dietary specialization. *Oikos* **124**, 1031–1039 (2015).
- 437 35. Minoarivelo, H. O. & Hui, C. Trait-mediated interaction leads to structural emergence in
438 mutualistic networks. *Evol. Ecol.* **30**, 105–121 (2016).
- 439 36. Borge-Holthoefer, J., Banos, R. A., Gracia-Lzaro, C. & Moreno, Y. Emergence of
440 consensus as a modular-to-nested transition in communication dynamics. *Sci. Rep.* **7**,
441 41673 (2017).
- 442 37. Geiselman, C. K., Mori, S. A. & Blanchard, F. Database of neotropical bat/plant
443 interactions. (2002). Available at: <http://www.batplant.org>.
- 444 38. Lobova, T. A., Geiselman, C. K. & Mori, S. A. *Seed dispersal by bats in the Neotropics*.
445 (New York Botanical Garden Press, 2009).
- 446 39. Fauth, J. E. *et al.* Simplifying the jargon of community ecology: a conceptual approach.
447 *Am. Nat.* **147**, 282–286 (1996).
- 448 40. Frund, J., McCann, K. S. & Williams, N. M. Sampling bias is a challenge for quantifying
449 specialization and network structure: lessons from a quantitative niche model. *Oikos* **125**,
450 502–513 (2016).
- 451 41. Jordano, P. Sampling networks of ecological interactions. *Funct. Ecol.* **30**, 1883–1893
452 (2016).
- 453 42. Gotelli, N. J. & Graves, G. R. *Null models in ecology*. (Smithsonian Institution Press,
454 1996).
- 455 43. Ulrich, W., Almeida-Neto, M. & Gotelli, N. J. A consumer’s guide to nestedness analysis.
456 *Oikos* **118**, 3–17 (2009).
- 457 44. Dvalos, L. M., Cirranello, A. L., Geisler, J. H. & Simmons, N. B. Understanding
458 phylogenetic incongruence: lessons from phyllostomid bats. *Biol. Rev.* **87**, 991–1024
459 (2012).
- 460 45. Bates, D., Machler, M., Bolker, B. & Walker, S. Fitting Linear Mixed-Effects Models

- using lme4. *Drug Inf. J.* **35**, 1215–1225 (2014).
46. Santana, S. E. Quantifying the effect of gape and morphology on bite force: biomechanical modelling and in?vivo measurements in bats. *Funct. Ecol.* **30**, 557–565 (2016).
47. Brown, J. H., Stevens, G. C. & Kaufman, D. M. The geographic range: size, shape, boundaries, and internal structure. *Annu. Rev. Ecol. Syst.* **27**, 597–623 (1996).
48. MacArthur, R. H. *Geographical ecology: patterns in the distribution of species*. (Princeton University Press, 1972).
49. Woodward, G. *et al.* Body size in ecological networks. *Trends Ecol. Evol.* **20**, 402–409 (2005).
50. Rosseel, Y. lavaan: An R package for structural equation modeling. *J. Stat. Softw.* **48**, 1–36 (2012).

475 Figures

476

477 **Figure 1.** The multilayer bat-plant network, built for the entire Neotropical Region based on
478 interactions of frugivory and nectarivory recorded in the wild, showed a strong separation
479 between interaction types (layers) and guilds (modules). **A.** Multilayer graph; the layers represent
480 interactions of frugivory (blue), nectarivory (orange), and dual interactions (purple, i.e.,
481 interactions of both types between the same bat and plant species). Bat species are represented as
482 squares, plant species as circles, and interactions as lines. Node colors represent modules
483 detected in the network using the DIRT_LPAwb+ algorithm. **B.** Multilayer matrix; bat species
484 are represented in the rows, plant species in the columns, and filled cells represent interactions
485 (same colors as in the graph); boxes represent the modules found. See the full-sized graph with
486 species labels in Supplementary Data 1.

Figure 2. Phylogenetic (Phy) and geographic (Geo) signals at different scales of the multilayer network: interactions (Int) (small scale), modules (Mod) (intermediate scale), and layers (large scale). **A.** Results of Mantel tests for all the correlations between bat distances in phylogeny, geographic co-occurrence, interactions, and modules. **B.** We used partial Mantel tests to discount the mutual effects between phylogeny and geographic co-occurrence; therefore, when testing geographic signals at each scale, we conditioned the correlation on the phylogenetic distance, and vice-versa. **C.** We used partial Mantel tests but conditioning the correlations with distances in one scale on the distances in the other scale. **D.** We used a Mantel test to assess a phylogenetic signal in the layers of the network and then used a partial Mantel test to test the phylogenetic signal in the modules accounting for the distance between layers. Arrows in black represent significant correlations and in gray, non-significant correlations. Arrow width scaled by Z-scores. In partial Mantel tests, the crossed circle with a letter inside indicates on which distances the correlation tested (arrow) was conditioned (geographic – G, phylogenetic – P, modules – M, or interactions – I).

502

503 **Figure 3.** The centrality metrics varied largely in the same species between layers of the network
504 (frugivory and nectarivory). Each axis of each spider chart represents a centrality metric calculated,
505 and its original range of variation. Different bat species are represented by different colors. Only
506 the most central species that occurred in both layers are presented here. Species codes were made
507 using the first three letters of the genus and the first three letters of the epithet (e.g., Carper =
508 *Carollia perspicillata*). See binomial nomenclature in Supplementary Data 1.

Figure 4. The eigenvector centrality (Eigen) of a bat species was determined by a combination of biological traits (indicators) related to morphology (the latent variables: Skull, Bite, Size) and geographic range size (Range). A bat species was more central in the frugivory layer, when it had a strong bite force (Bite) and small body (Size). In the nectarivory layer, larger bats (Size) with weak bite force (Bite) were the most central. In the complete multilayer structure, only bite force (Bite) was positively related to centrality. Numbers on the lines are the standardized coefficients of each path, and line thickness was drawn proportionally to this coefficient only for the latent variables (Skull, Bite, Size) and single indicator variable (Range). Significance was estimated only for those main variables. See full indicator names in Supplementary Results 1

519 Tables

520 Table 1. The multilayer network presented a compound topology, with a modular structure that
 521 comprises internally nested modules. Scores of modularity (M) and nestedness (NODF) for the
 522 entire multilayer matrix and its layers, including NODF scores calculated between species of the
 523 same module (sm) and of different modules (dm). The scores were calculated for the studied
 524 matrix (Obs), and also randomized according to the free and restricted null models. P-values (P)
 525 were estimated based on a Monte Carlo procedures run for each null model (1,000 iterations),
 526 which lead to expected scores (E) and Z-scores (Z). The free null model randomizes the entire
 527 matrix, whereas the restricted null model conserves its modular structure. We did not run the
 528 fixed null model for modularity. All scores were standardized varying from 0 to 1. Significance
 529 level (α): 0.05.

	Obs	E _{free}	Z _{free}	P _{free}	E _{rest}	Z _{rest}	P _{rest}
Frugivory layer							
Mod	0.48	0.35	44.45	0.001	NA	NA	NA
NODF	0.29	0.22	7.00	0.001	0.23	6.44	0.001
NODF _{sm}	0.60	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							
Mod	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	0.999	0.09	-0.56	0.710
Multilayer							
Mod	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	0.40	8.85	0.001
NODF _{dm}	0.13	0.15	-2.23	0.994	0.11	3.58	0.001

530

Table 2: 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 (i.e., making interactions on both the frugivory and the nectarivory layers). Relationships between centrality metrics calculated in different layers of the network using GLMs. Significant P-values are highlighted in boldface. Significance of the models of the set 1 was estimated using F tests, while for the sets 2 and 3 we used χ^2 tests.

Model	df	deviance	F	P
1. Centralities vs. 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 vs. frugivory				
ndeg ~ bridge	54	12.607		0.000
bet ~ bridge	54	16.125		0.000
clo ~ bridge	54	1.119		0.290
eig ~ bridge	54	14.940		0.000
3. Bridge species vs. 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

Legend: ndeg = normalized degree, bet = betweenness, clo = closeness, eig = eigenvector, frug = frugivory layer, nect = nectarivory layer. Significance level (α): 0.05.

540   **Supplementary information**

541   Supplementary Data 1. Dataset used to build the multilayer network, including an R code for
542   drawing it. Available on GitHub: <https://github.com/marmello77/mello-et al-2018-SD1>.

543   Supplementary Table 1. References used to build our dataset on bat-plant interactions in the
544   Neotropics.

545   Supplementary Table 2. A small dictionary of network science.

546   Supplementary Methods 1. Details on the calculation of centrality and the definition of the
547   multilayer structure and the calculation of multilayer versions of the main centrality metrics.

548   Supplementary Methods 2. Phylogenetic and geographic signals.

549   Supplementary Results 1. Correlograms of centrality for each layer of the network (A: frugivory,
550   B: nectarivory, and C: dual) and for the multilayer network (D).

551   Supplementary Results 2. Correlations between centrality metrics between layers. Trend lines are
552   presented only for statistically significant relationships. (a) Correlations between three centrality
553   metrics between layers for bat species that make interactions of frugivory and nectarivory. (b)
554   Relationship between the centrality of bat species in the frugivory layer and the probability of
555   being a bridge species (i.e., making dual links with the same plant species). (c) Relationship
556   between the centrality of bat species in the nectarivory layer and the probability of being a bridge
557   species (i.e., making dual links with the same plant species).

558