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What shapes galling insect-parasitoid interaction networks on closely related host plants? --Manuscript Draft--

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Corresponding Author:	Carolina Prauchner, M.D. Universidade Federal do Rio Grande do Sul Porto Alegre, Rio Grande do Sul BRAZIL
Order of Authors:	Carolina Prauchner, MSc Milton Mendonça Jr.
Response to Reviewers:	<p>Dear Editor:</p> <p>Thank you for the opportunity to once again provide a revised version of our manuscript on galler and parasitoid interaction networks on two phylogenetically close host plants. We especially thank the long time allowed until we returned this latest version. We also thank the reviewers for their work on trying to improve our manuscript further by exerting careful criticism. Please find below a list of the reviewer suggestions and our answers to them point by point, which also resulted in changes to the text, figures and tables. We hope to have produced a better manuscript overall and that it would now be deemed worthy of publication in Oecologia.</p> <p>Reviewer #3:</p> <p>Regarding the call by the reviewer to use classical literature, we added two historical references, as suggested. However, our aim was not to ignore the original sources, just reveal there are more recent ones. For example, Carneiro et al. (2009) may be from a regional journal but it constitutes one of the most recent and authoritative sources on demonstrating galls to be specific reviewing current data. This was actually an article developed precisely to justify repeated criticism received by articles “assuming” specificity for gallers, and we use it thus to fulfill its original purpose.</p> <p>We would like to point out that Table 1 already provides different morphological characteristics of the galls involved in our study. These basic characteristics do not change between host plants in any obvious way, we are sorry if we failed to make that more obvious. Our detection of galls in the field was not hampered by anything even close to this sort of problem, galls were clearly differentiated in spite of their host species and this was actually what lead us to propose this as a research project. We do not have quantitative data to directly prove this for all galls and plants, but we have size measurements for a few galls (n = 67; 34 galls on <i>M. glomerata</i> and 33 on <i>M. laevigata</i>) of one type (green spherical leaf/shoot gall induced by <i>Liodiplosis spherica</i>) to compare between the two hosts; this data was to be used on a different small project and is a sample of the same data we used in the analysis for our manuscript, that is, these are galls actually used to construct our networks. They are not statistically different in size (height: $t = 0.8577$; $P = 0.394$; diameter: $t = 1.654$; $P = 0.103$) as can be seen in the figure provided below as well. We present average height and diameter for galls on either <i>Mikania glomerata</i> or <i>Mikania laevigata</i>; error bars are 95% confidence intervals. We unfortunately do not have pictures of such galls on each plant and right now due to covid-19 restrictions we are unable to go out in the field to produce them.</p> <p>Considering the explanation the reviewer would like to have seen in the manuscript regarding gall structure, for example, we did not test this because of a low sample size, in the sense that there are not enough kinds of thicker vs. thin galls to compare, as well as hairy vs glabrous, larger vs. smaller galls, etc. We now included a few suggestions in the text comparing galls in terms of which structural aspect appears to affect</p>

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Differences in diversity indexes between the two years of sampling reveal, however, that slightly distinct sample regimes apparently had no significant implication for galling insect diversity (Simpson index_(1-D): 1st year = 0.7176; 2nd year = 0.7688; Shannon_index (H'): 1st year = 1.424; 2nd year = 1.548; Equitability (J): 1st year = 0.7316; 2nd year = 0.7956). An individual-based rarefaction for galling insect species also shows no differences in diversity between the two years of sampling (below, blue lines are 95% confidence intervals). Parasitism rates were equivalent between years, as well (1st year = 14.18%; 2nd year = 16.70%), and well within naturally expected variation. If the reviewer/editor believes this to be worth appearing explicitly in a final version, we could certainly arrange a further Electronic Supplemental Material unit with these data as presented here.

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Funding Information:	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Finance Code 001)	Ms. Carolina Prauchner
	Conselho Nacional de Desenvolvimento Científico e Tecnológico (309616/2015-8)	Dr. Milton Mendonça Jr.
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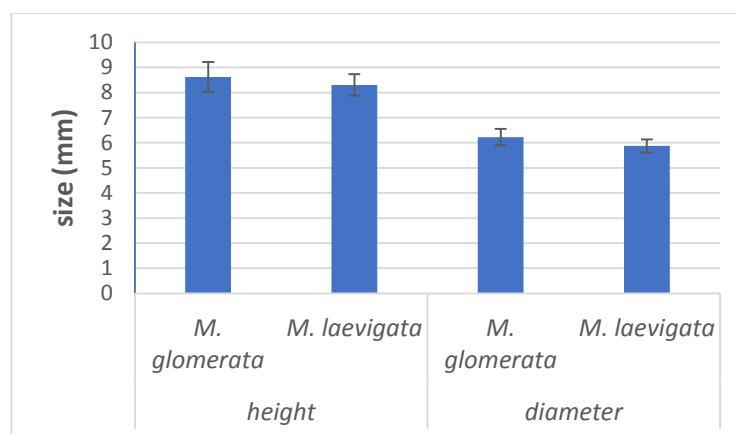
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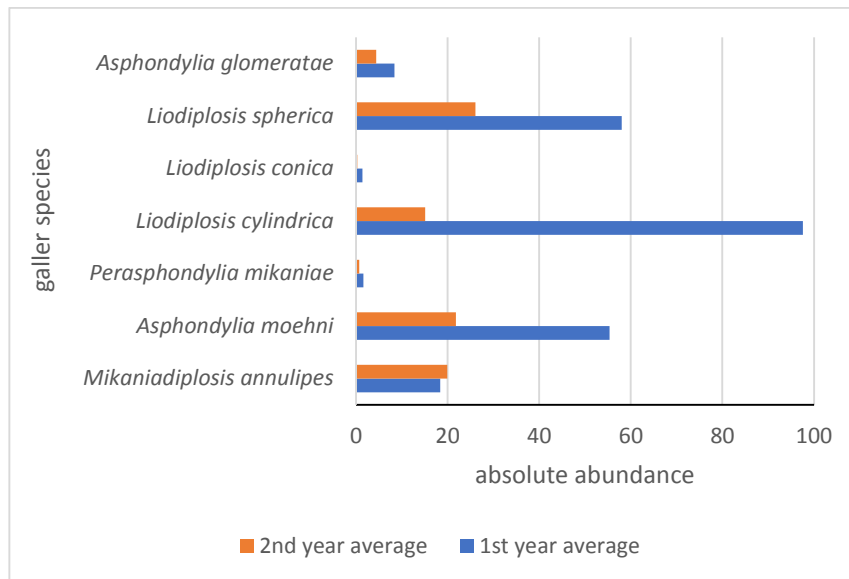
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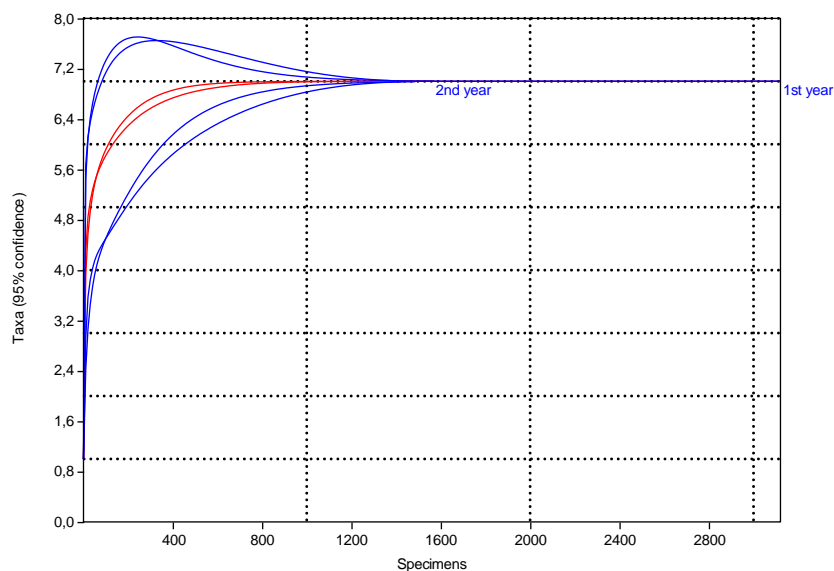
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Carolina Prauchner¹ & Milton de Souza Mendonça, Jr.^{2*}

¹ Graduate Program in Ecology (PPG-Ecologia), Biosciences Institute, Federal University of Rio Grande do Sul (UFRGS), Av. Bento Gonçalves 9500, CEP 91501-970, Porto Alegre, RS, Brasil; carol_prauchner@hotmail.com (correspondence author)

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Author Contributions: CP and MMJ conceived and designed the observations/experiments. CP performed the observations/experiments and analysed the data. CP and MMJ wrote the manuscript.

ABSTRACT

Ecological networks describe community structure by focusing on how nodes are connected by links (interactions). Related species have higher probabilities of sharing links if interactions depend on phylogeny and/or species are functionally similar; however, if and how this modularity escalates to higher trophic levels is still an open question. The aim of this work is to characterize the structure and topology of a bipartite interaction network of galling insects and associated parasitoids on a system with two closely related and recently diverged host plants, where host-tracking could be underway. Our hypothesis is that gall structure, galler taxonomy and host plant identity could contribute to network modularity; we generated networks by organising nodes according to each of these factors. Samples occurred between May/2015 and June/2017 on forest areas in Porto Alegre, southern Brazil. Overall, we found 4629 galls of three monophagous and four oligophagous galling species; 664 parasitoid individuals were obtained, of 37 morphospecies. All networks formed were significantly modular, independently of node clustering factor, and for three out of four analysed factors (galling insect species, galling species genus and interaction of galling and host plant species) modularity levels were at least intermediate (at about 0.4). This is the first time a quantitative galler-parasitoid network is organized comparing monophagous and oligophagous gallers. The relative parasitoid specificity and consequent higher modularity of this network contrasts with what is known for other systems with gall-inducers. We suggest the delayed host-tracking hypothesis may benefit from a tritrophic view, considering also top-down effects.

Key words: *Mikania glomerata*, *Mikania laevigata*, Cecidomyiidae, Oligophagy, Monophagy

INTRODUCTION

Biodiversity, more than a collection of individual species, is a combination of the diverse biological entities and the various processes that occur between them (Jordano 2016). Ecological interactions are among such processes responsible for the dynamics of biological communities, determining the exchange of matter, energy and information between species (Berlow et al. 2009). Ecological interaction networks represent the structure of biological communities by focusing on how network nodes (functional or taxonomic groupings of organisms) are connected by ecological links (interactions) (Bascompte 2007). These communities may be structured according to various types of external and internal factors, and the changes in network structure in response to environmental impacts can tell us a great deal about the how ecological and evolutionary processes organise communities (Tylianakis et al. 2008). For example, the topology of some interaction networks is often modular, with species sets interacting more intensively with each other than with other species in the network, forming strong interacting subsets. Many trophic networks assume this topology, explained at least in part by coevolved interactions between predator-prey species sets, in which preferences and specialization preclude greater network connectivity (Lewinsohn et al. 2006).

Galls are a specific growth response of plant tissues, induced by a range of organisms, many of them insects (Mani 1964, Stone and Schonrogge 2003). They serve insects as a source of food (through nutritive tissue), shelter from the weather, and protection from natural enemies such as predators and parasitoids (Price et al. 1987). Because they represent an extended phenotype of the insect galler, functional gall characteristics (Mendonça et al. 2014) may affect galler performance and its relationship with natural enemies. One example is the limit to interactions between gallers and certain parasitoids (their most common natural enemies, Toma and Mendonça 2014), given by structural factors such as gall shape and size,

the microenvironment they occupy (galled host plant organ) and their distribution in time and space. Given the relative ease of working with galls and parasitoids because of the ability to sample galls individually, food webs based on these systems have been researched in the past (e.g. Askew 1961, Schönrogge and Crawley 2000), but there are still only a few examples of interaction networks built around this ecological relationship using recent, more analytical network approaches (e.g. Araújo and Kollár 2019), especially on faunas outside the Holarctic region (e.g. Paniagua et al. 2009, Tylianakis et al. 2007).

A focal aspect of the parasitoid-prey exploitation strategy is the parasitoid ability to search the environment for prey (Godfray 1994). Among the main sources of clues for locating prey in the habitat is the galler host plant (through volatile substances). Because galling insects are usually species-specific (Carneiro et al. 2009), this effect is difficult to disentangle: either galls on the same plant can be compared in terms of their parasitoid load, or else galls on different plants. The parasitoid must also have a minimum discerning ability to interact with galls of different shapes and sizes. The oviposition process also involves, besides recognition, the ability to overcome any defenses when ovipositing in this structure (Stone & Schönrogge 2003). The host specificity of parasitoids in gall-parasitoid systems is still an open question, due to the lack of knowledge except for some better-known systems (oak galls, where parasitoids are not very specific, Hayward & Stone 2005), with few Neotropical examples. A higher degree of parasitoid specificity could lead to interaction networks with greater modularity, and may be caused either by specificity in host plant search, micro-habitat (plant organ) or gall structure (Luz et al. 2021). Network roles played by galler and parasitoid species regarding the modules are also of interest to show how these communities are structured. These can range from peripheral nodes (most links established within a module) to connector hub nodes (a hub with many links to most modules) by

comparing within-module connectivity (z) and between-module connectivity (c) for each species (Guimerá and Amaral 2005, adapted by Olesen et al. 2007).

The aim of this work is to test how gall structure, galler taxonomy and host plant identity affect parasitoid interaction with galling insects, using a system with monophagous and oligophagous gallers for comparison. For this purpose, four interaction networks were generated based on parasitoid morphospecies against ‘host nodes’ which were organised by four distinct factors, namely: 1) galling insect species (which also equates to gall structure), 2) galling insect genus (galler taxonomy), 3) host plant species and 4) both galling species and host plant. The modularity of these networks should be higher than expected by chance if the factor in question is important in structuring this network. Thus, our expectation is to find high network modularity for each of the factors analysed here. However, because some of the galls belonging to the same genus are morphologically dissimilar and host plants have a close evolutionary proximity, we expect to find different degrees of modularity according to each of the described factors, with gall species (and structure) having a stronger effect, followed by gall genus and host plant effects, respectively. We also expect simpler galls to act as network hubs (e.g. by being attacked by a higher diversity of parasitoids) while testing for the gall structural factor and to have more species as hubs and connectors when organising nodes by gall genus and host plant effects, given their modularities are expected to be lower.

MATERIAL & METHODS

Study System

Mikania glomerata and *Mikania laevigata* (Asteraceae) are climbing vines common in subtropical forests of southern Brazil. These species have medicinal use with identical effects and are confused because of their similar morphology (Napimoga and Yatsuda, 2010). Molecular phylogeny separates these species, though, despite the evolutionarily closer

proximity between them than to any other sampled species of the genus, with an estimated separation time of about 500,000 years only (Godoy et al. 2017).

The literature reveals galls in at least ten *Mikania* species (Online Resource 1), with all gallers belonging to the Cecidomyiidae family (Maia et al. 2008, Maia et al. 2014a, Maia et al. 2014b, Mendonça et al. 2014, Diaz et al. 2015, Coelho et al. 2013). Gagné et al. (2001) described eight species of insect galls in *M. glomerata*, with this plant considered a super-host. The structure of these galls varies greatly according to the galling species, the organ where the induction occurs and the host plant, with galls being uni or multilocular, found on leaves or stems and being either simpler or more complex in structure (Figure 1). Even species that belong to the same genus presents significant morphological differences, like galls induced by *Asphondylia glomeratae* (green midvein gall, Fig. 1C) and *A. moehni* (brown stem gall, Fig. 1E). For *M. laevigata*, there are records of three gall morphotypes: two on leaves and one on stems (Mendonça et al. 2014). These morphotypes are similar to three gall morphotypes in *M. glomerata*, but there is no published identification work on these species to confirm they are the same as described by Gagné et al. (2001). During our samples, five gall morphotypes were found on *M. laevigata* that are similar to the ones described on *M. glomerata* and to the ones recorded by Mendonça et al. (2014). In the laboratory, after a morphological analysis of the adults and pupae of the insects obtained from these galls based on the characters used by Gagné et al. (2001), we concluded that the gall species found in *M. laevigata* are the same as the ones described for *M. glomerata*, therefore oligophagous gallers.

Sampling

Samples were taken from natural areas within the city of Porto Alegre, Rio Grande do Sul State, southern Brazil; this area is characterised by a chain of granitic hills not higher than 300 m a.s.l. covered with a forest-savannah mosaic. On these dense submontane ombrophilous forests we can find species typical of the Atlantic forest biome and also from

the hydrographic basins of Paraná and Uruguay rivers (Müller et al. 2012). In the savannah areas there are predominantly herbaceous species of Poaceae and Asteraceae (Overbeck et al. 2006).

The first samples were taken between May-2015 and Jan-2016, in two areas at Morro Santana Wildlife Refuge (coordinates 30.050° S, 51.116° W), in Porto Alegre, Rio Grande do Sul State, southern Brazil. Two 200 m-long transects were used, one inside the forest and one on the forest edge with a grassland area (because of the slightly distinct plant abundances between habitats for the *Mikania* species, which are nevertheless syntopic). Transects were monthly traversed by two people in search of galls in either *M. glomerata* or *M. laevigata*. More samples were carried out between Dec-2016 and Jun-2017 in three areas located in Porto Alegre: the same Morro Santana Wildlife Refuge, plus Morro do Osso Natural Park (30.119° S, 51.237° W) and São Pedro Wildlife Refuge (30.172° S, 51.110° W). Each area was sampled once a month by two people along 100 m-long trails, with a maximum of one hour in the forest and one hour on the forest edge. Although they appear slightly different, these two sampling designs resulted in the same amount and diversity of galls; furthermore, sampling sites are not too far apart from each other (the three sites forming a nearly equilateral triangle with no more than 15 km on each side), and we thus considered them equivalent and analysed data by pooling them together.

All galls found in each host plant were taken to the laboratory. They were then counted, separated by morphotype (which corresponds to galling insect species) and stored in sealed plastic bags until adult emergence (either gallers or parasitoids). After emergence, parasitoids were stored in 70% GL ethanol and identified to family level with the help of a specific parasitoid identification key (Costa and Berti-Filho 2010). We used standard taxonomic wasp characteristics for morphotyping (head, thoracic and abdominal shape, colours and indument; wing venation and shape; antennae and tarsus segmentation and indument, etc). All insects

are deposited in the reference collection of the Interaction Ecology Lab (Department of Ecology, Biosciences Institute, Federal University of Rio Grande do Sul).

Data analysis

Different galler-parasitoid interaction networks were created in the *R* 3.4.1 environment (R Development Team, 2017) using the *Igraph* and *Bipartite* packages. Parasitoids were always separated in morphospecies, but galls were grouped according to different factors of interest, and the modularity of each network formed was calculated and its significance evaluated. The factors considered were: 1) Gall Insect Species (GIS), each galler species represents a distinct node in the network, regardless of the host plant they were found on, 2) Gall Insect Genus (GIG), all galler species of the same genus gathered to form a single node, 3) Host Plant Species (HPS), all galls found in a host plant species gathered in a single node, 4) Gall Insect and Host Plant Interaction (GPI), i.e. individuals of one gall species found in a specific host plant species are considered different from individuals of the same species found in another host plant, belonging to two distinct nodes in the network (check Table 1 for a list of nodes on each of the four networks). We employ this method hoping that by reorganising the network with different nodes, the effect of each of the factors could be revealed, for example, if the gall insect genus identity has no effect on parasitoids, a network with the lower trophic level clustered according to gall genus should not be significantly modular.

To analyse the influence of each of these factors on the structure of the four networks formed, modularities were calculated for each network and then compared with null models (random networks) using the QuanBiMo algorithm (10,000 randomizations, swap permutation method used: maintains same marginal totals and values of observed network connectivity, creating a more constricted network and possible forbidden links are kept

forbidden, for more details please see Beckett 2016) implemented through the *Bipartite* package.

In addition, we used the *Netcarto R* package, to calculate the function category of each node for both trophic levels, for all networks analysed, using the parameters defined by Gumierà and Amaral (2005, modified by Olsen et al. 2007). These authors defined seven possible roles for nodes in networks according to the between-module connectivity (or standardised connection value, called c) and within-module degrees (or participation value, called z) and suggest critical values of c and z of 0.625 and 2.5, respectively. Nodes with a c value > 0.625 are considered connectors and nodes with a z value ≥ 2.5 are considered hubs. Both categories link different modules within the network and are supposedly important for network coherence.

RESULTS

A total of 4629 galls were found in the two sampled plant species (*M. laevigata* and *M. glomerata*), belonging to seven galling insect species, all in the Cecidomyiidae family (Table 2, Figure 1). *Liodiplosis cylindrica* (cylindrical leaf gall) was the most abundant species with 1514 galls collected (32.7% of the total), even though it was found in only one of the host plant species (*M. glomerata*), being thus monophagous. It was followed by *Liodiplosis spherica* (spherical leaf gall, 1263 galls, 27.3% of the total, first record for this species attacking *M. laevigata*), *Asphondylia moehni* (ovoid shoot gall, 1139 galls, 24.6%), *Mikaniadiplosis annulipes* (fusiform shoot gall, 471 galls, 10.2%, first record for this species attacking *M. laevigata*) and *Asphondylia glomeratae* (leaf vein gall, 189, 4.1 %), all oligophagous, that is, found in the two host plant species sampled. The least abundant gall species are *Perasphondylia mikaniae* (bud gall, 30 galls, 0.6%) and *Liodiplosis conica* (conical leaf gall, 23 galls, 0.5%). The first was found mostly in *M. glomerata* and the second mostly in *M. laevigata*, with only one gall of each found in the other plant species, and no

insects emerging from these singletons. For this reason, these species were considered here as monophagous, even though *L. conica* is described originally as a galling species of *M. glomerata*. Thus, of the seven galling insect species, three are considered monophagous and four oligophagous.

For parasitoid insects, 664 individuals were found and classified into 37 morphospecies, belonging to 11 families of Hymenoptera and one of Diptera (Online Resource 2). The most abundant family was Eulophidae, with 403 individuals distributed in 9 morphospecies, followed by Platygasteridae with 68 individuals of a single morphospecies. The least abundant families were Encyrtidae, with two individuals, each of a different morphospecies, and Figitidae and Mymaridae, with one individual each.

Comparisons between observed network modularities (Figure 2) and randomised network modularities reveal significant differences in all cases. Each network presented a division of modules as follows. The network formed by the interactions between all the parasitoid morphospecies and the galling insects found (GIS factor), without taking into account the host plant in which this interaction occurred, has low connectivity ($C = 0.274$) and intermediate but highly significant modularity ($Q = 0.446$; $p < 0.001$). Four distinct modules formed: 1) spherical and cylindrical leaf galls; 2) ovoid shoot gall and conical leaf gall; 3) bud gall; 4) fusiform shoot gall and leaf vein gall. For the GPI factor there were four modules, where practically all oligophagous species remained together, regardless of the host plant they were on ($Q = 0.447$; $p < 0.001$); these modules are almost the same as the ones formed on the GIS network with only one exception, individuals of the fusiform shoot gall species (*M. annulipes*) found in *M. laevigata* were included in a module along with the cylindrical leaf gall and spherical leaf gall species. For the GIG factor network there was one distinct module for each of the four genera ($Q = 0.425$; $p < 0.001$). For the HPS factor, two modules were formed, one for each of the plant species ($Q = 0.249$, $p = 0.046$).

In the node function analysis, five of the seven possible roles were found: ultra-peripheral nodes (all their links within their module), peripheral nodes (most link within their module), connector nodes (with many links to other modules), provincial hub nodes (hub with majority of links within their module) and connector hub nodes (hub with many links to most of other modules). These analyses (Figure 3) revealed that parasitoids under the GPI factor presented the largest number of morphospecies considered as network connectors (20), a diverse group belonging to ten distinct families of Hymenoptera and Diptera. In networks grouped by GIS, GIG and HPS, the number of connectors decreased, and for the latter one there are only three hub connectors. Only the Platygasteridae morphospecies remained a connector in all networks, being a hub connector for two of them. On the other hand, one morphospecies of Eulophidae was considered peripheral, provincial hub and hub connector and one morphospecies of Diptera was considered ultra-peripheral, peripheral and a connector depending on the network in question (Online Resource 3). All galls are classified as peripheral in the networks, no matter which clustering factor was used to create the nodes, except in the GPI-factor network, where the node formed by the leaf vein gall (*A. glomeratae*) on *M. glomerata* establishes a connector position (Online Resource 4).

DISCUSSION

This analysis of modularity with different grouping factors for the lower trophic level nodes showed that there are apparent barriers or preferences for parasitoid attack on gallers in this system. Only a few of the possible interactions were found, creating a modular topological structure with few species connecting the modules to each other and thus representing potentially important hubs. Stronger connectors were mostly parasitoids, almost never gallers, not even those with simple galls, unlike what we predicted. Barriers to or preferences for certain interactions appear to be responses to multiple concomitant factors,

whether plant species, galler identity or attacked gall structure, confirming the hypotheses proposed in this study in terms of expected modularity of the networks.

Some of the most abundant parasitoid morphospecies were found on both host plant species, but showed a preference for one of them, like Eulophidae_sp. *d*: out of 217 individuals, 176 (81,1%) were found on *M. laevigata* galls and 41 (18,8%) on *M. glomerata* ones. This wasp species also showed a strong preference for attacking the stem galler species (*A. moehni*), with 213 (91,2%) emerging from these galls, and only four individuals from fusiform galls of *M. annulipes*. Another interesting case is Eulophidae_sp. *c*, found only in galls of *M. annulipes* and *A. moehni* induced on *M. glomerata*, even though both gall species were abundantly found on either host plant, showing a clear host plant species preference over galler species preference. This host plant effect was expected to work strongly in this system, since it is well known that parasitoids use plant olfactory clues to find the plant hosts of their galling prey (Stone and Schonrögge 2003). This host effect has already been found for oak gall parasitoids (Askew et al. 2013), perhaps the best studied galler-parasitoid system.

However, looking closely at the network formed when galler species and host plants are used as clustering effects (galler-plant interaction, GPI), we see that for the oligophagous galler species their two nodes (one for each plant species) were placed on the same module regardless of the plant they were found on, the only exception being the fusiform galler *M. annulipes*. For this species only, galls found on *M. glomerata* shared a module with the cylindrical (*L. cylindrica*) and spherical (*L. spherical*) galls, while those found on *M. laevigata* were placed in a module with vein galls (*A. glomerataeae*). This may reflect cases such as, for example, out of 68 individuals of Platygastriidae sp. *a*, 18 emerging from galls of *M. annulipes* on *M. glomerata*, but none from the same galls on *M. laevigata*. This could appear to be another host plant effect such as reported above, but 25 individuals of Platygastriidae sp. *a* emerged from other galls on *M. laevigata* (a reason why this parasitoid

was a hub connector in both GIG and HPS networks). A similar pattern characterised two other parasitoids, Pteromalidae sp. *d* and Eulophidae sp. *h*. We believe in this case galler preference for the host also had a play in this result, since out of 471 fusiform *M. annulipes* galls found, a large majority (366 galls, 77.7%) were found on *M. glomerata*. Thus, modularity is a network structure helping us reveal a series of particular galler-parasitoid relationships (sometimes through host plant identity too) and their effects on the community.

Gall structure is known to act as a strong barrier to parasitoid attack. For oak galls, despite parasitoid polyphagy, there is evidence that parasitoid community structure is defined by various attributes of galls such as hardness, hairiness and adhesion (some galls secrete sticky substances)(Bailey et al. 2009). Luz et al. (2020) also found clear evidence that a model considering morphological coupling between Cecidomyiidae galls wall thickness and parasitoid ovipositor length was the best one to explain interaction network structure on the host plant *Guapira opposita*. Waring and Price (1989), however, found no evidence that *Asphondylia* spp. gall attributes, such as thickness, surface texture and galled organ, affected either parasitism rate or parasitoid richness on creosote bush – although no network analysis was available at the time to help disentangle community-wide effects.

Analysing the modules found on the network formed by the galler species (independent of the host plant they were found on), we notice that gall morphology does not apparently have as strong an effect on parasitoid preferences as we expected. We can see this for example by comparing two of the modules, one formed by the gallers *A. moehni* (shoot gall) and *L. conica* (conical gall) and the other one formed by *L. cylindrical* and *L. spherica*. In the first module, shoot galls have very hard walls and several galls can be packed close together leading to potentially increased protection against parasitoid attack, whilst the conical differs in all these respects. In the second module, gall wall thickness is the main apparent difference, with spherical galls thick, possibly meaning a barrier for smaller

parasitoids or those with smaller ovipositors, but cylindrical galls much less so. However, this was apparently not the case since all the individuals of both parasitoids species of the Aphelinidae family, formed by small bodied species, emerged from spherical galls. The parasitoids seem to be responding more to the host plant organ in this network, since one module is formed by galls found mainly on stems (shoot and conical galls), another by galls mainly on leaves (cylindrical and spherical, but one also on buds, *P. mikaniae* bud gall) and one with galls found on leaf veins and petioles (*M. annulipes* and *A. glomeratae*).

Effects of gall traits have been detected previously in the form of parasitoid responses to gall structure, phenology and host organ attacked (Bailey et al. 2009). Although these responses are present for oak gall parasitoids, oak galls still seem to share natural enemies generating the expectation of complex dynamics such as apparent competition (Hayward and Stone 2005); because parasitoids are not so widely shared among gallers in the *Mikania* spp. system, we can expect not only lower apparent competition strengths but also differing selection pressures among galling species. Finally, although the literature mentions galler phylogeny as important for gall structure (Stone and Cook 1998) and thus indirectly for protection against natural enemies such as parasitoids, it is scant on disentangling the effects of galler taxonomy (and supposedly phylogeny) from gall structure on parasitoid preference as is done here.

The network organised by galler genus (GIG) had the low parasitoid sharing among modules, as can be seen from the function category analysis that classified one species as hub connector (Platygastridae sp. *a*), one as provincial hub (Eulophidae sp. *d*) and only three as connectors (Figitidae sp. *a*, Diptera sp. *c* and Diptera sp. *d*). All the other species were considered peripheral, with most links occurring only inside the module they were assigned to. Two of the networks clustered differently had stronger intermodule linkage and would show 30-50% of parasitoid species as not peripheral (connector species: GPI = 20; GIS = 12),

whilst for the galler genus and host plant clustered networks this amounted to only 13.5 and 8.1% (respectively). This result indicates that galler taxonomy (and thus probably phylogeny) and host plant identity are somehow stronger determinants for the parasitoids in terms of attacked hosts than gall structure. However, in this network we also found a small, very well defined module formed by the galler *P. mikaniae* (bud gall) and the parasitoid morphospecies Eurytomidae sp. *b*. All 40 individuals of this wasp emerged from these bud galls, as well as a single individual of each of three other parasitoids species (Eulophidae sp. *f*, Torymidae sp. *a* and Eupelmidae sp. *a*). This galler is monophagous, found only on *M. glomerata*, and has the only multilocular and only bud gall found for this system. It may be due to these unique characteristics that such a separate community structure is formed.

Comparing these results with other studies involving gall-parasitoid interaction networks, such as Paniagua et al. (2009), Tylianakis et al. (2007) and Luz et al. (2021), we found an indication that the pattern found here, with high network modularity and low connectivity, is typical of gall-parasitoid interaction networks, but the first two previous works have involved broader systems, with all gall species in different plants of a site. However, Luz et al. (2021) also found large parasitoid specificity in a system with a single host plant, parasitoids averaging only 1.1 connections/species in the network. Oak gall parasitoid species seem to exhibit polyphagy when viewed at a large geographical scale (Western Europe, Askew et al. 2013). Although we cannot rule out the possibility of galler parasitoids found in *Mikania* spp. attacking other galls locally on other plants, at least among the sampled galls they were only monophagous and oligophagous. Thus, high modularity levels even in such specific, focussed networks, may indicate the absence of a scale effect (Barabási and Bonabeau 2003) in gall-parasitoid networks, an open theme that may indicate potential stability for these networks (Montoya et al. 2006).

This is the first time a gall-parasitoid interaction network is organized focally around oligophagous galls. Since only a small percentage of galls are capable of gall induction in commonly related plant species (Carneiro et al. 2019), these systems are poorly explored. In these situations, a probable recent evolutionary divergence between plant species (Godoy et al. 2017) may be allowing this galls to escape monophagy, but it is expected that a gradual consolidation of specific plant divergence will also lead to evolutionary divergences of the galls of this system, i.e. co-cladogenesis (however little evidence there is as yet of this phenomenon for galls and plants, e.g. Stireman et al. 2012). The natural enemy fauna represented by the parasitoids having already consolidated responses to these differences between plants and between galls (demonstrated by the modules in the different networks), with few exceptions, as cited before, may mean that a top-down selection pressure is already in place, perhaps helping accelerate galler cladogenesis. The idea that speciation of the lower trophic level has a cascade effect onto the higher trophic level, with a possible delay in diversification time for the latter (the *delayed host-tracking hypothesis*, Hayward and Stone 2006) may benefit from also considering such top-down effects from an even higher trophic level. The fact that gall species did not evolutionarily diverged after host plant divergence could lead us to exclude the idea that evolutionary "host-tracking" can be explained by the "contemporary 'host-tracking' hypothesis" (Nicholls et al. 2010) as well, and in this case neither of these two hypotheses seems to adequately explain the evolutionary scenario potentially found in this system of galler parasitoids on *Mikania* spp.

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Table 1. Lower trophic level nodes employed in galler-parasitoid interaction network organisation to test for effect of each analysed factor (see text for acronyms) on modularity and species network role. **GPI** - grouping by both gall morphotype and host plant (codes as in Table 1, followed by **g** for *Mikania glomerata*, and by **l** for *Mikania laevigata*); **GIS** - grouping by gall morphotype only (gall structural effect test, codes as in Table 1); **GIG** - grouping by galler genus (galler phylogeny test); and **HPS** - grouping by host plant (host effect test).

<i>Species</i>		<i>Factor analysed</i>		
Galling species	GPI	GIS	GIG	HPS
<i>Asphondylia glomeratae</i>	Vei_l Vei_g	Vei	<i>Asphondylia</i>	
<i>Asphondylia moehni</i>	Sho_l Sho_g	Sho		
<i>Liodiplosis conica</i>	Con_l	Con		
<i>Liodiplosis cylindrica</i>	Cyl_g	Cyl	<i>Liodiplosis</i>	
<i>Liodiplosis spherica</i>	Sph_l Sph_g	Sph		
<i>Mikaniadiplosis annulipes</i>	Fus_l Fus_g	Fus	<i>Mikaniadiplosis</i>	
<i>Perasphondylia mikaniae</i>	Bud_g	Bud	<i>Perasphondylia</i>	
Plant species				
<i>Mikania glomerata</i> (g)				<i>glomerata</i>
<i>Mikania laevigata</i> (l)				<i>laevigata</i>

489 **Table 2.** Number of galls found per galling species (Cecidomyiidae) per host plant (*Mikania*
490 *glomerata* and *Mikania laevigata*), with observed galler niche amplitude (mono:
491 monophagous, oligo: oligophagous), basic gall morphological attributes (morphotype: shape,
492 colour) and morphotype codes (see Fig. 2), during samples from 2015 to 2017 in hill forests
493 of Porto Alegre, Brazil.

Galling species	<i>Mikania glomerata</i>	<i>Mikania laevigata</i>	Total	Niche amplitude	Galled plant organ	Gall morphotype	Morphotype code
<i>Liodiplosis cylindrical</i>	1541	0	1541	mono	leaf, shoot	cylindrical, green/red	cyl
<i>Liodiplosis spherical</i>	553	670	1223	oligo	leaf, shoot	spherical, green	sph
<i>Asphondylia moehni</i>	490	623	1113	oligo	shoot	ovoid, green/brown	sho
<i>Mikaniadiplosis annulipes</i>	461	136	497	oligo	petiole, shoot	fusiform, green	fus
<i>Asphondylia glomeratae</i>	145	43	189	oligo	leaf vein, petiole	fusiform, green	vei
<i>Perasphondylia mikaniae</i>	39	1	33	mono	bud	rosette, green	bud
<i>Liodiplosis conica</i>	1	22	23	mono	leaf, shoot	conic, green	con
Total	3223	1495	4718				

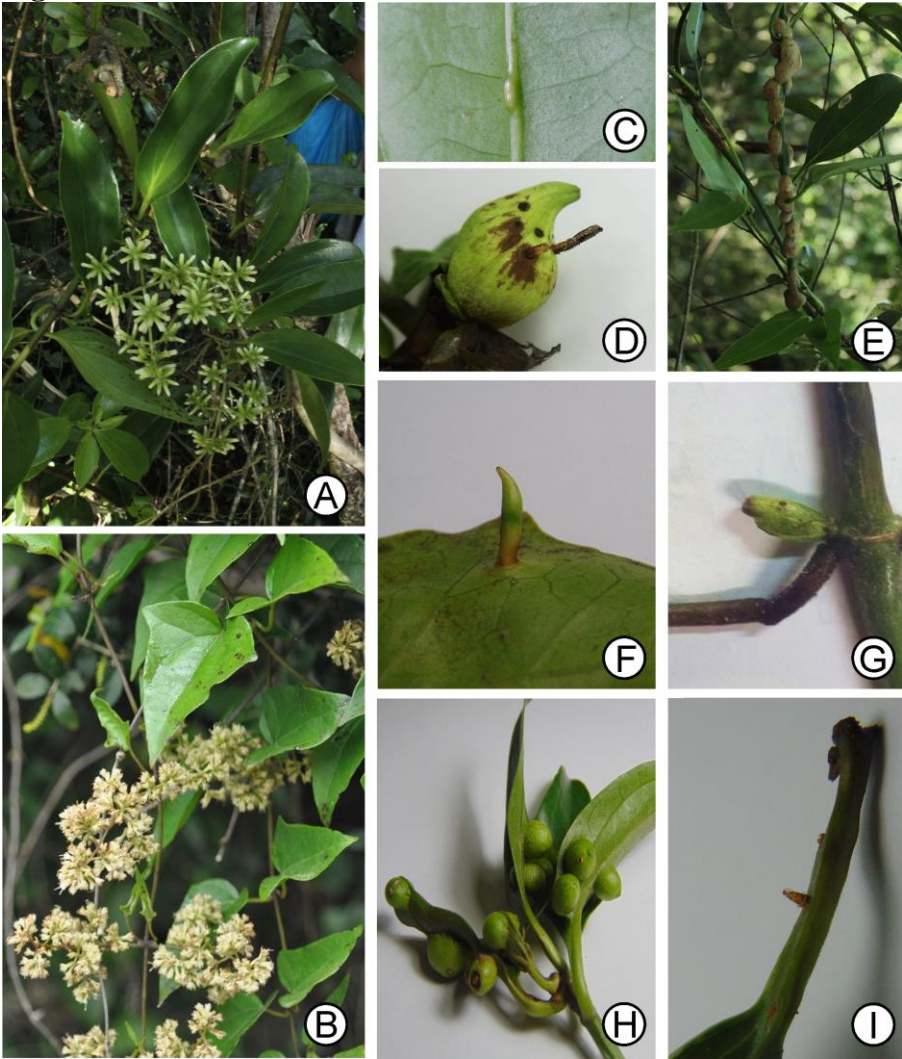
Figure legends

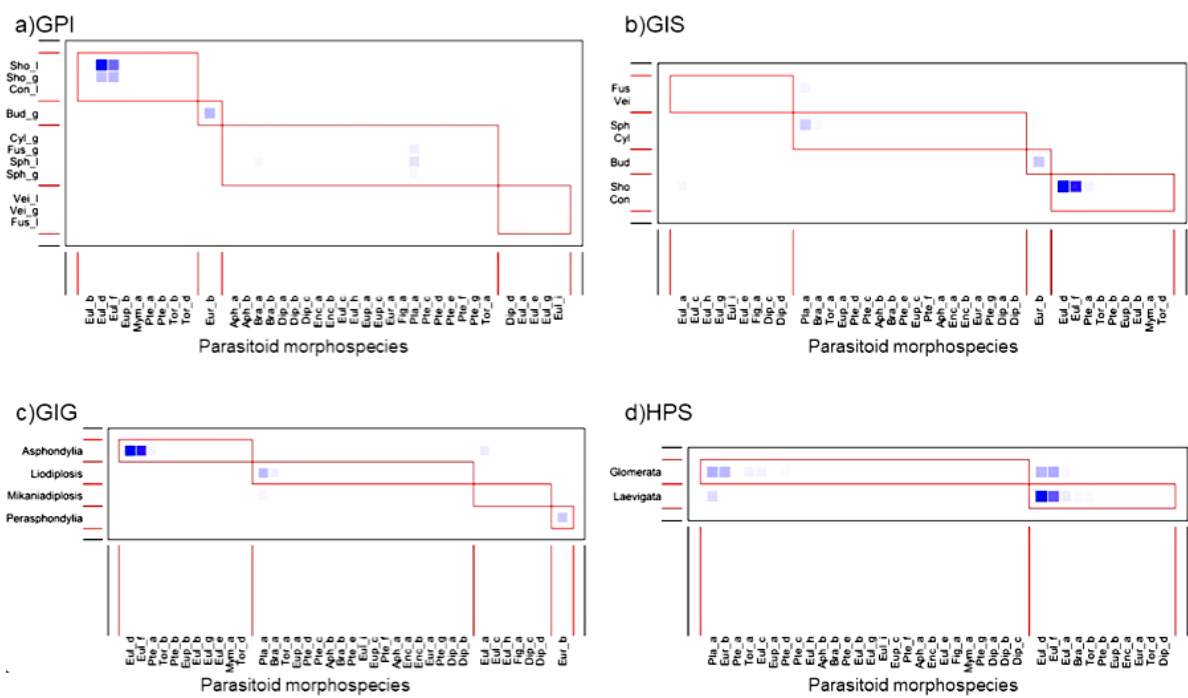
Figure 1. Insect (Cecidomyiidae) galls found on A) *Mikania laevigata* and B) *Mikania glomerata* (Asteraceae), in samples between 2015 and 2017 in hill forests of Porto Alegre, Brazil. C) *Asphondylia glomeratae*; D) *Perasphondylia mikaniae* – only found on *M. glomerata*; E) *Asphondylia moehni*; F) *Liodiplosis cylindrica* – only found on *M. glomerata*; G) *Liodiplosis conica* – only found on *M. laevigata*; H) *Liodiplosis spherical*; J) *Mikaniadiplosis annulipes*.

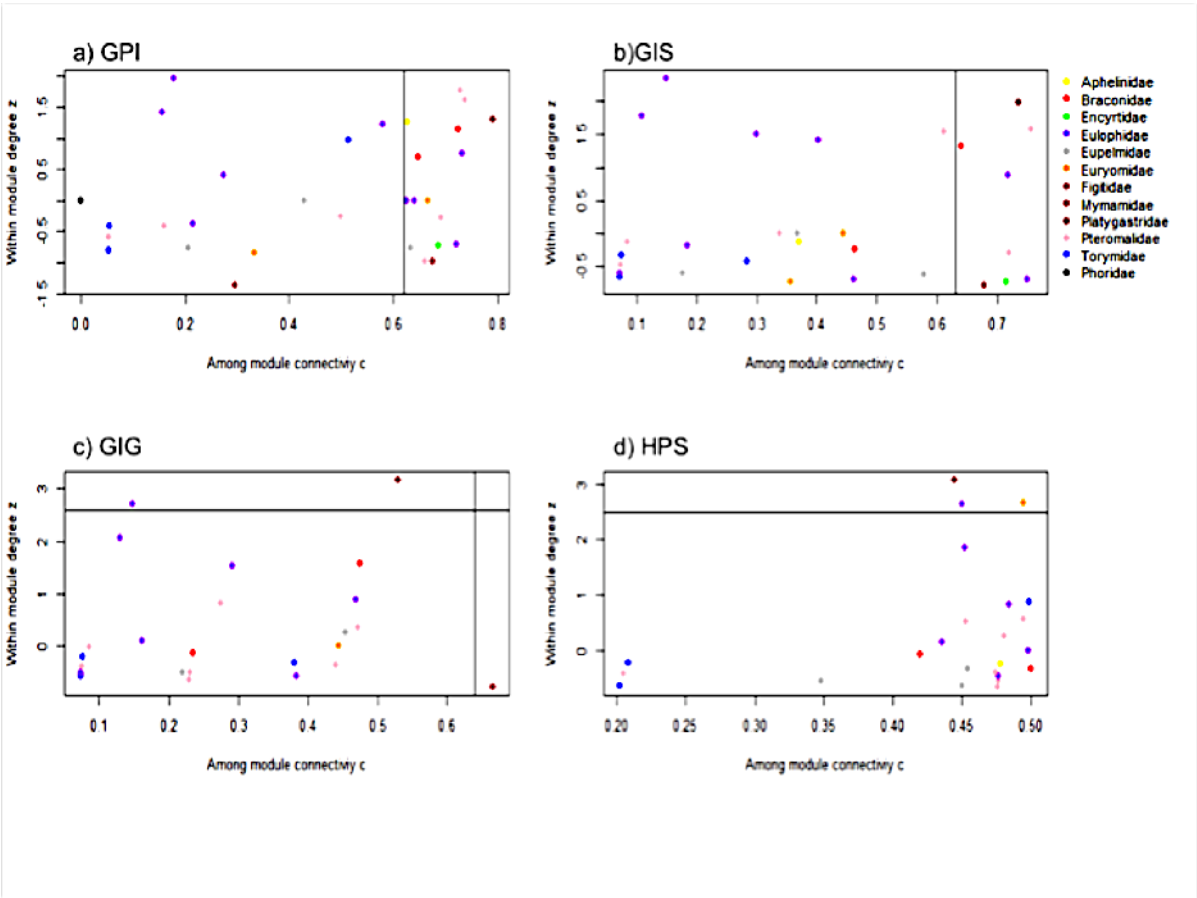
Figure 2. Galler-parasitoid ecological interaction networks showing significant modularity for Cecidomyiidae gallers on *Mikania* spp. organised by grouping lower trophic level nodes (rows) according to four factors being tested (see text). Upper trophic level on columns, with parasitoid morphospecies codes (see Online Resource 2). **a)** grouping by both gall morphotype and host plant (GPI, codes as in Table 1, followed by **g** for *Mikania glomerata*, and by **l** for *Mikania laevigata*); $Q = 0.447$; **b)** grouping by gall morphotype only (GIS, gall structural effect test)(codes as in Table 1); $Q = 0.446$; **c)** grouping by galler genus (GIG, galler phylogeny test); $Q = 0.425$ and **d)** grouping by host plant (HPS, host effect test)(**glo** for *Mikania glomerata* and **lae** for *Mikania laevigata*) $Q = 0.249$, sampled between 2015 and 2017 in hill forests of Porto Alegre, Brazil.

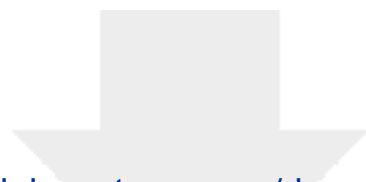
Figure 3. Network and module connecting roles for parasitoid morphospecies in modular ecological networks of Cecidomyiidae gallers on *Mikania* spp. (vertical axis: within module degree, horizontal axis: among module connectivity, calculated with Netcarto R package). Colors indicate the parasitoid family. The four networks are organised grouping lower trophic level nodes (gallers) according to four factors being tested (for details see text): a) grouping by both gall morphotype and host plant (GPI); b) grouping by gall morphotype only (GIS, gall

519 structural effect test); c) grouping by galler genus (GIG, galler phylogeny test) and d)
520 grouping by host plant (HPS, host effect test). Species to the right of the black vertical line in
521 **a**, **b** and **c** are considered connectors, the others peripheral, species above the black horizontal
522 line in **c** and **d** are hubs, the others non-hubs. All samples between 2015 and 2017 in hill
523 forests of Porto Alegre, Brazil.





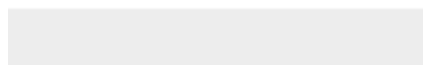
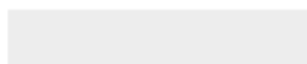


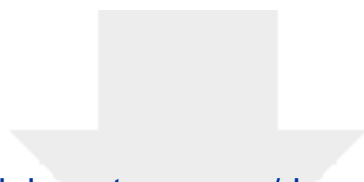


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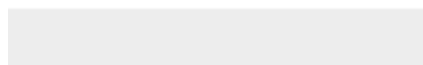
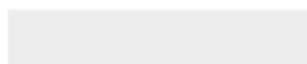
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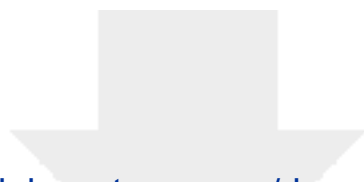




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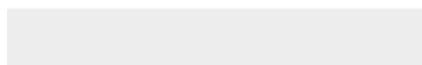
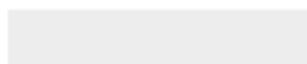
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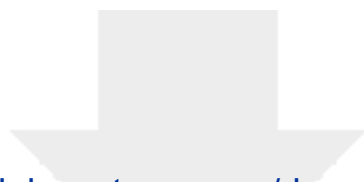




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