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Unravelling ant-symbiont network topology across Europe

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ABSTRACT

Long-term associations between different species are key drivers in community composition in all ecosystems. Understanding the ecological and evolutionary drivers of these symbiotic associations is challenging because of the diversity of species and interaction types hosted in natural ecological networks. Here, we compiled the most complete database on natural ant-symbiont networks in Europe to identify the drivers of bipartite network topology. These ant-symbiont networks host an unrivalled diversity of symbiotic associations across the entire mutualism-antagonism continuum, of which the most diverse types of symbionts are (1) trophobionts: mutualistic aphids and scale insects (2) myrmecophiles: commensalistic and parasitic arthropods, and (3) social parasites: parasitic ant species. These diverse ant-symbiont networks provide a unique opportunity to tease apart ecological and evolutionary drivers. To do so, we dissected network topology and asked what determines host specificity and which host factors drive symbiont species richness and facilitate host switching for the different types of symbionts.

We found an unexpectedly high number of 701 obligate symbionts associated with European ants. Symbiont type explained host specificity and the average relatedness of the targeted host species. Social parasites were associated with few, but phylogenetically highly related hosts, whereas trophobionts and myrmecophiles interacted with a higher number of hosts across a wider taxonomic distribution. Colony size, host range and habitat type predicted total symbiont richness, where ants hosts with larger colony size or larger distribution range contained more symbiont species. However, we found that different sets of host factors affected diversity in the different types of symbionts. Ecological factors, such as colony size, host range and niche width predominantly drive myrmecophile species richness, whereas evolutionary factors, such as host phylogeny and biogeography, mainly determine richness of mutualistic trophobionts and social parasites. Lastly, we found that hosts with a common

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biogeographic history support a more similar community of symbionts. Phylogenetic related hosts also shared more trophobionts and social parasites, but not myrmecophiles. Taken together, these results suggest that ecological and evolutionary processes drive host specificity and symbiont richness in large-scale ant-symbiont networks, but these drivers may shift in importance depending on the type of symbiosis. Our findings highlight the potential of well-characterized bipartite networks composed of different types of symbioses to identify candidate processes driving community composition.

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I. INTRODUCTION

Long-term associations between different species, known as symbioses, are crucial components of community assembly in all ecosystems. These intricate associations display a high diversity, ranging from mutually beneficial partnerships to parasitic interactions, where one species exploits another (Paracer & Ahmadjian, 2000). Interactions among species are central to ecological and evolutionary dynamics in assemblages of species that belong to different guilds and trophic levels. They are as such essential elements of the entangled bank, Darwin's metaphor for the complexity and connectedness of natural systems (Darwin, 1859), and give rise to important stabilising feedbacks that eventually maintain diversity and ecosystem functioning (Thrall *et al.*, 2007; Bastolla *et al.*, 2009). To date, these insights are merely derived from theory and empiricism focusing on either antagonistic competition and predator-prey interactions (e.g. Hairston, Smith, & Slobodkin, 1960; Pimm, 1979; Tilman, 1982) or more recently mutualistic interactions (Bascompte, Jordano, & Olesen, 2006; Bascompte & Jordano, 2007).

Communities contain a wide variety of interactions, rendering the ecological network very complex (Newman, 2003). One important feature of complex systems is the presence of emergent properties that result from interactions among the specific components of the system (Solé & Bascompte, 2006). Typically, the emergent properties result from the complex interaction between the different network components across time and space and are difficult to predict from the specific (isolated) pairwise interactions (Vázquez, Chacoff, & Cagnolo, 2009). Generally speaking, modular networks that are characterised by a high degree distribution and connectance tend to be more robust to species loss, and are less affected by disturbance (Solé & Bascompte, 2006; Olesen *et al.*, 2007). While theoretical progress has been made (Solé & Montoya, 2001), the field is suffering from a lack of comprehensive and manageable empirical systems.

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The representation of communities in networks constitutes a holistic approach, but the study and interpretation of the drivers and consequences of the topology will largely depend on the considered scale, both in terms of spatiotemporal and phylogenetic dimensions (Delmas *et al.*, 2019). While network studies at local scales will be informative on the local community assembly processes, those reconstructed at regional or global scales will allow inference of macro-ecological and evolutionary processes by making abstraction of the formerly described local drivers (Trøjelsgaard & Olesen, 2013).

In contrast to trophic networks, bipartite host-symbiont networks contain different kinds of links, with interactions between hosts and their symbionts ranging from antagonistic to mutualistic (Ings *et al.*, 2009). Examples of such networks are for instance plant-mycorrhiza and host-microbiome associations. The complexity of these networks is enormous, and their description is merely based on one interaction type, either antagonistic or mutualistic, although theory predicts that the diversity of interaction types may be essential for community stability (Fontaine *et al*., 2011;Mougi & Kondoh, 2012). The topology of bipartite host-symbiont networks can be dissected by adopting two different perspectives, i.e. that of each of the individual sets of species (hosts and symbionts). Asking what factors cause a given topology is equivalent to asking, for each of the sets, what explains the number of links per species and the specificity of these links, i.e. how links are distributed among species from the focal set. An example of this approach is found in studies on predator-prey networks, where average web vulnerability (i.e., the average number of predators per prey) and generality (i.e., the average number of prey eaten per predator) link the specificity of the two interacting species sets (Schoener, 1989).

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A determining feature of the ecology of symbionts is host specificity which can be quantified in host-symbiont networks by the number of links departing from a symbiont node. Yet, a measure of host specificity should ideally consider the relatedness of the targeted host species as well (Poulin & Mouillot, 2003). Generalist symbionts target multiple, unrelated host species and are thought to gain relatively low benefits in any host. Specialist symbionts, in turn, engage with one or only a few related species, but achieve high benefits with their hosts relative to the generalists due to advanced morphological, physiological and behavioural adaptations (Bronstein, Alarcón, & Geber, 2006; Thrall *et al.*, 2007). Their strong specialization, however, is offset by lower population densities and higher extinction risks due to the lower availability of hosts. Several studies in host-symbiont systems clearly found that host specificity is tightly linked with fundamental ecological processes and evolutionary history. Typically, host specificity is different among groups of the symbiont community as for example demonstrated in parasites of primates (Pedersen *et al.*, 2005) and in parasitic mites on mussels (Edwards & Malcolm, 2016). A study on moths and plants indicated that host specificity can be dependent on the type of symbiotic interaction with pollinating moths being more specific than the parasitic leaf-feeding relatives (Kawakita *et al.*, 2010).

From the perspective of the host, it is fundamental to understand the ecological, evolutionary and environmental drivers that promote the number of associated symbionts, i.e. the number of links departing from a host node to symbiont nodes. Studies on other host-symbiont systems reported multiple host variables which correlate with parasite species richness (PSR). Generally, the makeup of symbiont communities is orchestrated by both ecological and evolutionary host factors. The driving ecological factors are analogous to the ones that favour species richness on islands (cf. theory of island biogeography, MacArthur & Wilson, 1967) as host species can be regarded as biological islands (Kuris, Blaustein, & Alio, 1980). Symbiont richness is therefore expected to increase with the number and variety of microhabitats offered by the host, host longevity, host range, and the interaction probability

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with other hosts (Kamiya *et al.*, 2014; Stephens *et al.*, 2016). Evolutionary processes may affect symbiont species richness in different ways. Related hosts often show traits that are correlated throughout evolution (phylogenetic correlation) which lead to similar values in species richness. However, related host species may have diverged with time, whether or not in a common spatial evolutionary ancestry (biogeography), but may still attract a similar fauna of symbionts as unrelated host species with a similar ecology (Poulin, 1995).

Another pattern that emerges in host-symbiont networks is the sharing/transmission of symbiont species across host species. The degree of symbiont sharing is vital as symbiont transmissions can connect eco-evolutionary dynamics across hosts as a result from rapid symbiont spread in host populations (e.g. Jaenike *et al.*, 2010; Himler *et al.*, 2011 in endosymbionts). While little is known about the proximate mechanisms by which single symbionts switch between hosts, we can anticipate that host species with similar ecological niches and/or a shared evolutionary history tend to have similar symbiont communities. The pervasive effect of phylogenetic relatedness on symbiont sharing has for example been demonstrated in bat parasites (Luis *et al.*, 2015) and in plant-mycorrhiza (Veresoglou & Rillig, 2014).

Ant-symbiont networks are ideally suited to study which factors drive bipartite network topology (Ivens *et al.,* 2016). The diversity of symbiotic associations found in ants (Kistner, 1982; Hölldobler & Wilson, 1990; Rettenmeyer *et al.*, 2010; Parmentier, in press) is thought to be promoted by their omnipresence in terrestrial ecosystems, their stable and climate-controlled nest fortresses and the high number of available resources in the nest (Kronauer & Pierce, 2011). Ants interact with different types of symbionts spanning the entire parasitism-mutualism gradient. They include parasitic ants, different groups of arthropods living in the nests, mutualistic aphids, nematodes, plants, bacteria and fungi. Therefore, they are ideal systems to study different interaction types in a single framework (Fontaine *et al*., 2011).

Ant-symbiont networks that have been recently studied, deal with local interaction networks and mostly focus on one kind of symbiotic interaction in isolation, such as mutualistic plant-

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ant networks (Guimaraes *et al.*, 2006; Blüthgen *et al.*, 2007; Dáttilo, Guimarães, & Izzo, 2013; Cagnolo & Tavella, 2015), mutualistic aphid-ant networks (Ivens *et al.*, 2018) or parasite-ant networks (Elizalde *et al.*, 2018). A notable exception is the global meta-analysis on different types of ant-symbiont interactions by Glasier, Poore, & Eldridge (2018). Unfortunately, this study only included a limited set of interaction types and pooled interactions of well-studied bioregions with those of very poorly studied regions.

Here, we ask what factors explain the topology of ant-symbiont networks across Europe. We firstly provide a quantitative and systematic meta-analysis on the diversity of European ant-symbiont interactions. By adopting the symbiont perspective, we test the hypothesis that the type of symbiosis explains the number and identity of their host species (host specificity). More specifically, we expect that parasitic ants are more specific than the other types of symbionts. In addition, we predict that different groups of symbiotic arthropods have highly variable host specificity. Secondly, we follow a trait-based host perspective to identify the major drivers that promote the diversity of ant-symbiont interactions and facilitate symbiont sharing. We test the hypothesis that the number of symbionts with which an ant species interacts and the number of symbionts it shares with other ant hosts depends on ecological factors (6 factors, including colony size, nest type, distribution, habitat, degree of sympatry, worker size) and evolutionary drivers (2 factors: phylogeny, biogeography) associated with the host species.

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II. MATERIAL AND METHODS

(1) Ant symbionts

Symbionts are species that engage in a close association with a host species on which they may have beneficial, neutral or adverse effects. We limited our analyses to Europe (excluding the Canary Islands and Madeira), as ant-symbiont interactions in other continents are extremely fragmentary studied and poorly understood. Moreover a myriad of unknown symbionts are awaiting discovery and description in these continents (Parmentier, in press). By contrast, a firm body of knowledge on the distribution and diversity of ant symbionts in Europe has been recorded and has steadily grown from a long tradition of studying ant symbionts since the end of the 19th century (Wasmann, 1894; Janet, 1897). Depending on the intimacy of the relationship between ants and symbionts, we can distinguish obligate and facultative interactions. An obligate interaction occurs when a symbiont permanently lives inside or near an ant nest. Obligate symbionts completely depend on ants and cannot be found without them. Facultative myrmecophiles may associate with ants, but regularly (or mostly) occur without ants. In this study, we only focused on obligate symbionts.

We categorized five types of symbionts: (1) **Trophobionts** include aphids and scale insects that provide sugary honeydew in exchange of protection and hygienic services. These mutualistic arthropods are mostly found extranidally, except for aphids living on the roots of plants inside ant nests. (2) **Myrmecophiles ss** are a diverse group of arthropods that mostly live inside ant nests. Representatives of this group are distributed across many arthropod orders, but beetles and mites are the most diverse (Hölldobler & Wilson, 1990; Kronauer & Pierce, 2011). The life strategies of these organisms range from commensalism to specialized parasitism. (3) **Social parasites** encompass a group of ants that parasitize other ant species. There exist various types of social parasitism (see overview in Buschinger, 2009). In xenobiosis, cleptoparasitic ants construct a nest inside other ant nests, but raise their own brood. In temporary parasitism, a parasitic queen usurps a host colony and exploits

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the host work force to establish her own colony. Parasite workers will gradually substitute the host worker force. In dulosis (permanent parasitism with slavery), a parasite colony is established as in temporary parasitism. But here, the workers of the parasitic species will raid pupae of other ant species. Workers which will emerge from these pupae will do most of the tasks in the colony. In inquilinism (permanent parasitism without slavery), parasitic queens permanently exploit a host colony and no worker force is produced. The parasitic queen invests all her energy in producing sexuals. (4) ectoparasitic **Fungi**, (5) endoparasitic **Nematoda**. Plants engaging in mutualistic relationships (e.g. myrmecochory) were not included in our analyses. Contrary to the tropics, ant-plant relationships tend to be loose in Europe and are at best facultative.

(2) Ant-symbiont dataset compilation

We compiled all documented ant-symbiont interactions in Europe. The database of ant-symbiont interactions was assembled from 253 published references, including faunistic notes, research articles, reviews and books. In a first round of searches, we scanned reference works (e.g. Wasmann, 1894; Donisthorpe, 1927; Evans & Till, 1966; Uppstrom, 2010; Tykarski, 2017; Molero-Baltanás *et al*. 2017) for associations between ant hosts and symbionts. Next we searched for ant-symbionts interactions via Google Scholar™ using the terms: “myrmecophile” or “ant associate” or “inquiline” or “ant guest” or “ant symbiont”. We also found host-symbiont interactions within references that were cited in the retrieved publications. In a second phase, each symbiont occurring in Europe was then searched by its Latin binomial name and its common taxonomic synonyms combined with a search string with the names of all ant genera (*N* = *56*, AntWiki 2019) found in Europe (for example “Maculinea alcon” AND Acropyga OR Anochetus OR Aphaenogaster OR Bothriomyrmex OR Camponotus OR …) using Google Scholar. We chose Google Scholar over ISI Web of Science, as the latter does not retrieve faunistic notes or other types of grey literature. We omitted symbionts from our dataset when it was doubtful whether they are obligately

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267 associated with ants or not. Ultimately, we obtained a binary host-symbiont matrix filled with
268 interactions (1s) and non-interactions (0s) between ants (columns) and symbionts (rows).
269 Note that we included some references on ant-trophobiont interactions reported in the non-
270 European part of Russia (e.g. Novgorodova, 2005) to ramp up the relative modest number of
271 reported interactions in this type of association. The reported ants and trophobionts in these
272 references have a widespread Palearctic distribution and they are expected to interact in
273 Europe as well.

274

275 **(3) Host specificity in different symbiont types**

276 We compared the number of host species across different types of host symbionts.
277 Symbionts with only hosts identified at the genus level were not included in all following
278 analyses. Symbionts are unevenly studied, which may result in better studied symbionts
279 having a higher number of observed host species. To account for differences in sampling
280 effort, we therefore first regressed the total number of host species against the (ln+1)-
281 transformed number of google scholar hits for the binomial species name (and commonly
282 used synonyms) of the symbionts. The residuals of this regression were then compared
283 across symbiont groups using a GLM, followed by Tukey post-hoc tests in R 3.5.2.

284 In a second analysis, we wanted to test whether the relatedness of targeted host species
285 varies among different symbiont groups. For each symbiont, we estimated the average
286 taxonomic distance between the different hosts by using the specificity index, S_{TD} proposed
287 by Poulin & Mouillot (2003). Host ant species (all ants belong to the family Formicidae) were
288 classified following the Linnaean classification in subfamilies, tribes, genera and species
289 groups/subgenera. The taxonomic distance between two hosts is then defined as the number
290 of hierarchical steps that are needed to reach a common node in the taxonomic tree. The
291 taxonomic distance between two species of the same subgenus/species group equals to 1,
292 the distance between two species of the same genus, but from a different subgenus/species

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group equals to 2. A maximum taxonomic distance of 5 is reached between two ant host species from different subfamilies. S_{TD} was estimated by averaging the taxonomic distance across all pairs of host species. We modelled the specificity index against symbiont type with a GLM followed by Tukey post-hoc tests. Symbiont species with a single reported host species were omitted from this analysis, as no specificity index can be estimated for these species (a total of 382 symbionts were retained in this analysis). In parallel, we reran this analysis with a phylogenetic instead of a taxonomic distance matrix. The phylogenetic distance matrix was based on the phylogenetic tree of European ants by Arnan *et al.* (2017). Distances between species were estimated by node count (number of nodes along the branches of the tree from one species to another) and were retrieved using Mesquite v.3.5. Phylogenetic distances are more accurate than taxonomic distances to assess relatedness, but unfortunately we do not possess phylogenetic information at the species level for all ants in our dataset (phylogeny of 96 out of 177 ant species was known). We decided to drop the 81 ant species without phylogenetic information and the corresponding interactions with their symbionts for subsequent analyses. We believe that this is acceptable as these species only cover 13.5% of the interactions in our host-symbiont dataset. We also omitted the exotic ant species *Linepithema humile*, consequently we reduced our dataset to 95 ant species and 602 symbionts. In addition, symbionts which interacted with only one ant species were omitted, as no specificity index can be calculated for these species. Ultimately, we retained 349 symbiont species in this analysis.

(4) Predictors of symbiont diversity in European ants

A central question in this study is why some ant species host more symbionts than other ant species. Therefore, we first compiled for the European ant species several predictors, based on Arnan *et al.* (2017), Boulay *et al.* (2017), Seifert (2007) and AntWiki (2019) reflecting differences in host functional niche. Predictors of symbiont diversity were extended traits of the ant species and colony, including colony size (number of workers), average worker size

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(mm), nest type (levels: arboricolous, diverse, soil and organic mound) and phylogeny as a proxy for trait similarity, and ecological factors of the ant species, including habitat (levels: eurytope, introduced exote, open, open/sylvicolous and sylvicolous), distribution range, degree of sympatry and biogeographic region (levels: Atlantic, Boreomountain, Continental, Mediterranean, wide-ranging). Ants were assigned to the biogeographical region where they were found proportionally the most in sampled biogeographical regions based on the observations of Arnan *et al.* (2017). If the proportional occurrence in the most preferred region was less than double of the proportional occurrence in another region, the ant species was grouped under the "wide-ranging" category. We also estimated the distribution range (the number of countries where the host species has been reported, based on records on AntWiki, 2019), the degree of sympatry (number of ant species with symbionts which share at least one country in the distribution range, based on AntWiki, 2019) and the number of hits for their binomial name (and common synonyms) on google scholar as a proxy for sample effort for every ant species. Next we correlated total symbiont diversity with the host predictors described above, while correcting for the phylogenetic relatedness of the different ant species. The phylogenetic relatedness of host species should be accounted as closely related host species cannot be treated as independent observations. For that reason, we modelled a phylogenetic generalized least squares regression (PGLS) with total number of symbionts per ant species as dependent variable. A PGLS model incorporates a phylogenetic variance-covariance matrix in its error structure. We used the variance-covariance matrix based on the pairwise node counts retrieved from the phylogenetic tree of European ants by Arnan *et al.* (2017). The phylogenetic relatedness of 96 out of the 177 ant species found in our dataset was determined in this tree. We decided to drop the 81 ant species without phylogenetic information and the corresponding interactions with their symbionts for subsequent analyses. We believe that this is acceptable as these species only cover 13.5% of the interactions in our host-symbiont dataset. We also omitted the exotic ant species *Linepithema humile*, consequently we retained a dataset with 95 ant species and 602 symbionts. The phylogenetic covariance matrix was multiplied by Pagel's λ, a widely

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used parameter that scales and corrects for the expected degree of phylogenetic covariance (Pagel, 1999). This multiplier spans from 0, which corresponds with the complete absence of phylogenetic signal in the residuals of the model (the model is then similar to a regular GLM with an ordinary least squares error structure) to 1, when the covariance of the model residuals follows a Brownian motion model of evolution (Pagel, 1999; Freckleton, Harvey, & Pagel, 2002). The λ parameter characterizing the phylogenetic signal was estimated through maximum likelihood estimation within the PGLS model. We analysed this model using the *pgls* function embedded in the R 3.5.1-package 'caper'. We transformed the variables to meet the normality assumptions of the residuals. Number of symbionts was fourth root transformed, the predictors colony size, degree of sympatry and google scholar hits ln-transformed, and the distribution range was square root transformed. Finally, all continuous predictors were scaled to unit variance.

In addition to this analysis on the drivers of total symbiont diversity, we ran similar PGLS models with subsets of symbiont species richness as dependent variables (overall number of myrmecophiles, trophobionts, social parasites, and the diversity of the largest groups of myrmecophiles, i.e. beetles and mites, separately) and predictors of the subset of ant species that engage with these symbionts as predictors. Identical transformations of predictors and subsets of symbiont richness were applied as in the analysis on total symbiont richness described above. Diversity of social parasites and fungi were not regressed against ant predictors in separate PGLS models as the number of host ants are relatively low in these groups.

Models were ranked per analysis with the *dredge* function in 'MuMIn' R-package according to their AICc-value (corrected Akaike Information Criterion). We retained the best models identified with Δ AICc < 2. Significance levels of the predictors of the retained models were assessed using Likelihood ratio tests.

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(5) Predictors of symbiont sharing in European ants

Understanding the factors that facilitate or constrain the transmission of a symbiont from one host to another, is pivotal in the study of host-symbiont networks. Here we assessed which drivers promote the sharing of symbionts in ants. Predictors were similar to the previous analysis and encompassed extended traits of the ant species and colony, including colony size, worker size, nest type (levels: arboricolous, diverse, soil and organic mound) and phylogeny as a proxy for trait similarity; and ecological factors of the ant species, including habitat (levels: eurytope, introduced exote, open, open/sylvicolous and sylvicolous), distribution range, biogeographic region (levels: Atlantic, Boreomountain, Continental, Mediterranean, wide-ranging) and degree of sympatry. We used multiple regression on distance matrices (MRM), an extension of partial Mantel analysis, to test the association between different distance matrices (Lichstein, 2007). The dissimilarity symbiont matrix contained the pairwise Jaccard distances between each pair of host ants based on the presence-absence data of the symbionts they supported. This matrix was regressed against multiple distance matrices giving dissimilarities in the aforementioned predictors. Worker size distance was the absolute difference for this trait between every pair of ant species. The pairwise differences in colony size were ln-transformed. The sympatry matrix listed the ln-transformed number of countries in the distribution range that are shared between each pair of ant species. Entries in the distance matrices of habitat, nest type and biogeographic region were coded 0 when the pair of ants occupy the same habitat, nest type or biogeographic region, respectively, and 1 when the pair of ants show differences in these variables. The phylogenetic distances were the pairwise node counts. Again, we focused our analysis on the subset of 96 ants for which the phylogenetic relationship was resolved by Arnan *et al.* (2017). We also included a matrix of sampling effort in which we pairwise multiplied the (ln+1)-transformed hits on google scholar of one ant species with the (ln+1)-transformed hits on google scholar of another ant species. All matrices were standardized between 0 and 1 and Multiple Regression On Distance Matrices (MRM)-analyses were conducted in the R

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401 package ‘ecodist’ using the MRM function. Significance of the predictor matrices was tested
402 using 9999 permutations. We removed non-significant predictors, and reran the MRM
403 analysis until all predictors were significant (Martiny *et al.*, 2011). The relative importance of
404 the significant predictor matrices was calculated with the lmg-metric, which uses unweighted
405 averages of sequential R^2 of different orderings of the model predictors. The calculation and
406 visualization of the lmg-metrics was conducted with the R-package ‘relaimpo’.

407 We conducted similar MRM-analyses on subsets of the symbiont community, where the
408 response variable was the dissimilarity (pairwise Jaccard indices) in the set of
409 myrmecophiles, trophobionts, social parasites, myrmecophilous beetles and myrmecophilous
410 mites of the host ants, respectively.

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III. RESULTS

(1) Ant-symbiont networks display a diversity of species interactions

We identified 701 obligate ant symbionts interacting with 177 ant species in Europe (Appendix S1). The references we used to compile the host-symbiont interaction matrix are listed per symbiont species in Appendix S2. Myrmecophiles (*N* = 537) outnumbered the four other groups of ant symbionts (*N* = 71, *N* = 69, *N* = 13 and *N* = 11 for respectively social parasites, trophobionts, ectoparasites (all fungi) and endoparasites (all nematodes in our analysis). Within the group of myrmecophiles, beetles and mites were the most species-rich groups (Fig. 1). The hosts of 73 symbionts were not identified at the species level in the literature record. The distribution of the number of host species per symbiont was right-skewed (mean = 3.58, median = 2). The highest frequency (39%) of symbionts interacted with 1 host species and a maximum number of 34 host species was documented in the myrmecophilous silverfish *Proatelurina pseudolepisma*.

European ant genera contained a highly variable number of species (proportional to the genus font size in Fig. 2, Fig. S1), ranging from 1 to 82 species, in the genera that interact with symbionts. Generally, the species-rich ant genera, such as the Formicinae genera *Lasius*, *Formica* and *Camponotus* and the *Myrmicinae* genera *Messor* and *Myrmica* attracted a higher diversity of all five types of symbionts (Pearson's product-moment correlation: $r = 0.60$, $P < 0.001$, $df = 28$, Fig. S1). A notable exception was the species-richest European ant genus *Temnothorax*, which supported a relatively moderate number of symbionts. Myrmecophiles were the dominant group in most ant genera (mean 63.7% of the total symbiont diversity). Trophobionts were generally the second most diverse group (mean 14.9%), but were absent or nearly absent in some genera such as *Monomorium*, *Aphaenogaster*, *Leptothorax, Messor* and *Cataglyphis*. Social parasites slightly contributed to total symbiont diversity in most ant genera (mean 8.9%), but were very diverse in the ant genera *Temnothorax*, *Tertramorium* and *Leptothorax*. Fungi (mean: 11.7%) and nematodes

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437 (mean: 0.9%) are also small symbiont groups. Interestingly, *Myrmica* is targeted by a
438 relatively high number of ectoparasitic fungi. Ant genera shared many symbionts (74.2% on
439 average) with other genera, belonging to the same or different ant subfamilies (connecting
440 lines in Fig. 2). *Temnothorax*, *Leptothorax* and *Messor* are characterized by a relatively high
441 number of unique symbionts (cf. relative large inner circles in Fig. 2).

442

443 **(2) Host specificity in different symbiont types**

444 After controlling for sampling effort, host range of the symbiont groups was significantly
445 different (GLM, df = 25, P < 0.001, Fig. 3). Social parasites targeted the lowest number of
446 host species, whereas myrmecophilous Thysanura, Collembola and Isopoda are associated
447 with the highest number of ant hosts. Trophobionts also interacted with a relatively high
448 number of ant hosts (Fig. 3). Host range of symbiont groups without controlling for sampling
449 effort can be found in Fig. S2.

450 The average taxonomic distance between host species targeted by a symbiont was
451 significantly different among the symbiont groups (GLM, F = 9.4, df = 14 ,P < 0.001, Fig. 4).
452 Likewise, we found significant differences in average phylogenetic distance between host
453 species across the symbiont groups (GLM, F = 9.4, df = 14, P < 0.001, Fig. S3). Patterns in
454 the average host phylogenetic distance of the different symbiont groups were closely
455 matching those of the average host taxonomic distance. The host species of social parasites
456 were very closely related to each other (Fig. 4 and S3). Fungi also exploited related host
457 species. Trophobionts, ectoparasitic nematodes and myrmecophiles interacted with hosts
458 that are much more unrelated. The group of myrmecophiles was heterogeneous and when
459 we subdivided it into different subgroups, we obtained an entire gradient in average host
460 taxonomic/phylogenetic distance (Fig. 4 and S3).

461

462 **(3) Predictors of symbiont diversity in European ants**

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463 The number of symbionts is highly variable in ant species. Here we report the host drivers
464 that affect total symbiont richness and diversity of subsets of ant symbionts (myrmecophiles,
465 trophobionts, social parasites, myrmecophilous mites and myrmecophilous beetles). Total
466 symbiont diversity was clearly positively correlated with colony size. This factor was highly
467 significant (PGLS, $P < 0.001$) in the 4 top-ranking models (Table 1, Fig. 5). Habitat and
468 distribution range of the host were also incorporated in most of the top-ranking models. In
469 these models, symbiont richness increased with the host distribution range and was highest
470 in eurytopic habitats (PGLS, $P < 0.001$). As expected, sample effort has a major effect on the
471 reported total symbiont diversity and the five subsets of symbiont diversity. Symbiont
472 interactions were the highest in ants that are intensively studied. We controlled for sample
473 effort by including the proxy (ln+1)-transformed google scholar hits in our models.
474 Myrmecophile richness was also positively affected by colony size (PGLS, $P < 0.001$ in the 5
475 top-ranking models, Table 1, Fig. 5), distribution and eurytopic habitat. The diversity of
476 myrmecophilous beetles was positively correlated with colony size and distribution range of
477 the host (PGLS, P-values < 0.001). Myrmecophilous mite diversity was also positively
478 correlated with colony size and distribution range (PGLS, P-values < 0.001, Table 1) in all
479 retained models. Trophobiont diversity was mainly driven by sampling effort (PGLS, $P <$
480 0.001, Fig. 5, Table 1). Trophobiont diversity was consistently highest in the Atlantic region
481 and in ant species with small workers. There were no predictors consistently present in the
482 top-ranking models explaining social parasite species richness (Table 1).

483 We did not find a phylogenetic signal in the predictors of the PGLS models (Δ AICc < 2) with
484 total symbiont, total myrmecophile, myrmecophilous beetle and myrmecophilous mite
485 diversity as dependent variables. The residuals of these models showed no phylogenetic
486 covariance (Pagel's λ lambda was 0, Table 1). In contrast, phylogenetic relatedness of the
487 hosts explained additional variation in the social parasite models (Pagel's λ ranged between
488 0.74 and 0.96) and to a lesser degree in the trophobiont models (Pagel $\lambda = 0.30$-0.42) (Table
489 1).

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490

491 **(4) Predictors of symbiont sharing in European ants**

492 Here we examined whether the sharing of symbionts is affected by the similarity in particular
493 traits and ecological drivers of the host species. The maximum amount of variation explained
494 by our predictor matrices was in the subset of trophobionts (R^2 = 37.1%, Fig. 6). MRM
495 analyses aiming to explain similarities in particular groups of symbionts explained more
496 variation (R^2 ranging from 14.7% to 37.1%) than those focusing on similarities in the total
497 symbiont community (R^2 = 9.8%). Sample effort positively affected similarities in symbiont
498 communities including all symbionts (MRM, proportional contribution to the total R^2: lmg =
499 0.11, P < 0.001). Well-studied ant pairs also shared more trophobionts, social parasites,
500 myrmecophiles, myrmecophilous beetles and mites (MRM-analyses, lmg ranging from 0.18 -
501 0.58, all P-values < 0.01, Fig. 6). The most important predictors of similarity (dissimilarity) in
502 ant symbiont communities in the European ant dataset were phylogenetic relatedness of the
503 ant hosts (1-distance) (MRM, lmg = 0.40, P = 0.001) and similarity (1-dissimilarity) in
504 biogeographic region (MRM, lmg = 0.35, P = 0.001) (Fig. 6). Similarities in worker size and
505 colony size also facilitated the sharing of symbionts (MRM, lmg = 0.08, P = 0.01 and lmg =
506 0.05, P = 0.02, respectively, Fig. 6). Trophobiont sharing was also positively correlated with
507 phylogenetic relatedness and similarity in biogeographic regions of the ant hosts (MRM, lmg
508 = 0.26, P < 0.001 and lmg = 0.16, P < 0.001, resp.). The similarities in social parasite
509 communities was largely explained by phylogenetic relatedness (MRM, lmg = 0.53, P <
510 0.001). Similarities in biogeography and colony size explained additional variation in the
511 sharing of social parasites (MRM, lmg = 0.20, P < 0.001 and lmg = 0.05, P < 0.05).
512 Interestingly, phylogenetic relatedness of the hosts did not promote the sharing of
513 myrmecophiles and myrmecophilous mites. Host relatedness had only a minor effect on
514 myrmecophilous beetle sharing (MRM, lmg = 0.02, P = 0.03). The assemblage of
515 myrmecophiles, myrmecophilous beetles and mites was similar in pairs of ants occupying
516 identical biogeographic regions (MRM, lmg = 0.57, P < 0.001 and lmg = 0.38, P < 0.001 and

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517 lmg = 0.16, P = 0.02, resp.) and having a large overlap in distribution (degree of sympatry)

518 (MRM, lmg = 0.13, P = 0.04 and lmg = 0.24, P = 0.005 and lmg = 0.28, P = 0.002, resp.)

519 (Fig. 6).

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IV. DISCUSSION

Understanding the patterns of community assembly has been a central topic in ecology. Community structuring and the underlying species interactions have been increasingly been approached using network analysis. Yet, studies that describe and aim to understand the topology of large-scale ecological networks consisting of different interaction types are limited. We here provide a complete tally of the distribution of ant symbiont groups over European ants and compare host specificity, symbiont richness, host switching and its drivers for five different ant symbiont groups.

(1) Characterization of the European ant-symbiont network

It is widely acknowledged that the group of obligate ant symbionts is hyperdiverse (Wasmann, 1894; Kistner, 1979, 1982; Hölldobler & Wilson, 1990; Rettenmeyer *et al.*, 2010), although exact species numbers at a regional scale are lacking. Rough estimates of the global diversity of parasites living in ant nests reach 10.000 to 20.000 species (Thomas, Schönrogge, & Elmes, 2005), which is higher than mammal and bird diversity. We here identified 701 obligate symbionts distributed over 177 ant species in Europe. The majority of the symbionts were classified as myrmecophiles, which are commensalistic to parasitic arthropods mostly living inside the nest of ants (Kronauer & Pierce, 2011; Parmentier *et al.*, 2016; Parmentier, in press). The large diversity of this group is fuelled by the species-rich myrmecophilous beetle and mite communities. In other regions, beetles and mites outnumber other arthropod groups as well (Kistner, 1982; Hölldobler & Wilson, 1990). Social parasites and mutualistic trophobionts are medium-sized groups; parasitic nematodes and fungi are relatively species-poor, but definitely understudied. Mutualistic ant symbionts are thus clearly overshadowed by the diversity of commensalistic and parasitic ant symbionts in Europe. Speciose ant genera and subfamilies generally supported higher numbers of ant symbionts. Host-symbiont networks are characterized by an asymmetrical organization of

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546 interactions with host-specific symbionts and symbionts that interact with multiple host taxa
547 (Guimaraes *et al.*, 2006). Overall, a large proportion of the symbionts were shared among
548 heterogeneric ant species (Fig. 2). Some ant genera interacted with a relatively low number
549 of symbionts, but most of their symbionts were not found in association with other ant
550 genera. The highly specific group of social parasites was much more represented in the
551 symbiont community of these hosts. In addition, the group of trophobionts is diverse in some
552 ant genera, but it is marginal or even absent in others (Fig. 2). The uneven distribution of the
553 five types of symbionts among the ant genera suggest that some ant lineages are more
554 predisposed to associate with particular types of symbionts. Ant-host associations are thus
555 shaped by deep evolutionary processes as determined by biotic and environmental drivers of
556 speciation and extinction (Aguilée *et al.*, 2018).
557

558 **(2) Host specificity in different symbiont types**

559 Host specificity is a key feature of host-symbiont networks, and is moulded by the ecological
560 and evolutionary interactions between host and symbiont (Poulin & Mouillot, 2003). Patterns
561 in host specificity have been studied in a wide range of host-symbiont systems. Generally,
562 parasites are thought to have a tendency to evolve to extreme host specialization as they
563 need complex adaptations to by-pass host defence (Kawakita *et al.*, 2010). The drivers
564 favouring host specificity in mutualist partners are far less understood and both low and high
565 degrees of specificity are widespread (e.g. plant-seed dispersers and fig-fig wasps,
566 respectively). Ant symbioses are ideal to unravel patterns in host specificity. They occupy the
567 complete mutualism-parasitism continuum and allow comparison of host specificity in
568 different types of symbionts. We here demonstrate that average host range in European ant
569 symbionts was much broader than previously assessed in a study on host specificity of
570 myrmecophiles at a global scale (Glasier *et al.*, 2018). The authors of this study state that
571 obligate ant symbionts occurred on average with ca. 1.20 host species. We, however, found
572 that European symbionts were reported with three times this number of host species (3.57)

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on average. The extreme low number of the detected hosts in the study of Glasier *et al.* (2018) is the result of their searching method. They overlooked data hidden in faunistic notes, grey literature and books, which report the majority of the interactions between ants and their symbionts. Moreover, the symbiont fauna, let alone the range of their interactions, is poorly documented outside Europe, which makes hard predictions unreliable (Parmentier, in press). Ant symbionts were extremely variable in ant host range. After controlling for sample effort, social parasites clearly targeted the lowest number of host species, which is in line with the expectations as they are the most specialized group of parasites (Buschinger, 2009). The commensalistic myrmecophilous group of silverfish and the mutualistic trophobionts engaged symbioses with the highest number of ant species. Apart from the quantity of the hosts, the relatedness of the host species is a vital aspect of host specificity. It is well described that social parasites colonize nests of related hosts (Buschinger, 2009). However, this has never been compared with other type of symbiont groups. We showed that the hosts of social parasites were clearly the most related of all symbiont types. The hosts of myrmecophiles groups showed moderate to poor relatedness on average. Trophobionts and nematodes associated with distantly related ant species.

There is a large body of literature that explains the constraints of host switching in social insect symbionts. Generally, it is thought that host-specific myrmecophiles and social parasites rely on chemical deception, by mimicking the colony recognition cues or some key pheromones (overview in Parmentier, Dekoninck, & Wenseleers, 2017). They are completely integrated in the host colony and are treated as a true colony member. Because of the strict mimicking of the host's unique chemical profile, they are not able to colonize unrelated host species. Generalist myrmecophiles are typically poorly integrated in the colony, but host switching is more common in this group. It is facilitated by the use of general defensive chemicals, chemical insignificance or behavioural strategies (Stoeffler, Tolasch, & Steidle, 2011; Parmentier *et al*. 2017, 2018). The processes which make aphids attractive for one host, but not for another are hitherto unexplored. Likewise, the factors that facilitate or constrain host switching in parasitic nematodes and fungi are unknown.

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(3) Predictors of symbiont diversity in European ants

Associations between ant hosts and their symbionts are not random and structured according to both ecological and evolutionary factors that act at different spatiotemporal scales. From the perspective of ant symbionts, ant nests can be conceptualized as islands or local patches connected through dispersal. In analogy with McArthur & Wilson's island theory and Leibold's metacommunity framework, larger ant nests interact with more symbiont species. Ant nest size was repeatedly hypothesized as an important driver of ant symbiont diversity (Hughes, Pierce, & Boomsma, 2008; Kronauer & Pierce, 2011), but here formally tested for the first time. Previous studies across very different host-symbiont systems (e.g. ectoparasites of fishes in Guégan *et al.*, 1992, parasites of hoofed mammals in Ezenwa *et al.*, 2006, parasites of carnivores Lindenfors *et al.*, 2007, feather mites of finches Villa *et al.*, 2013) identified host body size as one of the key factors in determining symbiont species richness (Kamiya *et al.*, 2014). This positive association results from the fact that larger hosts provide more niches and are less ephemeral (Lindenfors *et al.*, 2007). Analogously, larger ant nests provide more space to allow larger population sizes and thereby reducing the extinction risk of symbionts. In addition, larger nests contain a higher diversity of microhabitats, including refuge areas that eventually facilitate species coexistence (Barabás, D'Andrea, & Stump, 2018). Larger ant nests are also expected to be more persistent (Kaspari & Vargo, 1995). Colony size is thus a strong local driver of symbiont richness and holding for myrmecophiles in particular. Myrmecophile diversity is additionally strongly determined by more regional ecological factors like range size and niche width of the host ants, so factors related to their general abundance and distribution. In that respect, eurytopic ants, such as *Lasius niger* and *Myrmica rubra* that can live in a wide variety of habitats including urban regions, hosted more symbionts. Both the effect of distribution and habitat reflect that more symbionts occur in widely distributed ant species with high densities. Symbionts associated with widely distributed ants are less prone to extinction as predicted by

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life history theory and metacommunity ecology (Nosil, 2002; Leibold *et al.*, 2004). Host density has widely been demonstrated as another key factor for explaining parasite species richness (Lindenfors *et al.*, 2007). Together with local colony sizes, sampling effects appear highly relevant, hence more neutral drivers, of their diversity among different hosts. Interestingly, trophobiotic and more specialised parasitic interactions as seen in the group of social parasites are more determined by evolutionary drivers. Indeed, affinity with their hosts is not only shaped by biogeography, hence a common spatial evolutionary ancestry but also by phylogeny. The effect of phylogeny is echoed in the high Pagel's lambda values of the corresponding PGLS models, implying that much of the residual variation in trophobiont and especially social parasite richness could be explained by the phylogeny of the hosts. The strong phylogenetic driver for social parasite richness is in line with our previous results that social parasites mainly target closely related ant species (referred to as Emery's rule, Buschinger, 2009) belonging to a small number of ant genera. Symbiont network structure thus shifts from more neutral ecological drivers related to regional species abundance to co-evolutionary drivers related to ancestry. The uniqueness and tightness of species interaction are known to be both a driver and consequence of co-evolutionary dynamics. Interestingly, we here show these evolutionary drivers to overrule any ecological one in the most antagonistic interactions, hence demonstrating the integrated nature of symbiont network formation according to the prevailing interaction strengths. Ant-symbiont networks are quite unique in the sense that the host associated network that is studied is covering a wide array of interactions, from putatively mutualists to strictly antagonistic. Studies on specialised host-parasite networks equally point at the dominance of evolutionary drivers (phylogeny and biogeography) of these associations (Feliu *et al.*, 1997; Rosas-Valdez & de León, 2011), while less obligatory animal parasitic (Nunn *et al.*, 2003; Ezenwa *et al.*, 2006; Lindenfors *et al.*, 2007; Nava & Guglielmone, 2013) or plant mutualistic interactions (Sanders, 2003; Wagner, Mendieta-Leiva, & Zotz, 2015) show more ecological factors related to distribution and abundance patterns that enhance contact and hence transmission of their diversity and host association patterns.

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656

657 **(4) Predictors of symbiont sharing in European ants**

658 We hypothesized that the shared evolutionary history of related ant species would promote
659 the sharing of similar symbiont communities. The positive correlation between phylogenetic
660 relatedness of the hosts and symbiont sharing was demonstrated in previous studies on
661 orchid mycorrhiza (Jacquemyn *et al.*, 2011) and bat viruses (Luis *et al.*, 2015), but no such
662 relationship was found in arbuscular mycorrhiza (Veresoglou & Rillig, 2014) and primate
663 parasites (Cooper *et al.*, 2012). Consistent with our prediction, we found that the main factor
664 that promoted symbiont sharing in European ants was the relatedness of the hosts. It
665 indicates that many symbionts pass more easily to related host species. As related ant
666 species employ nearly identical defence structures (nestmate recognition cues, physiological
667 and behavioural defence), it enables symbionts, especially specialized parasites, to by-pass
668 the host defence systems of related hosts. Another key factor that may facilitate the cross-
669 species transmission of symbionts is the overlap in geographical distribution of the hosts (cf.
670 bat viruses in Luis *et al.*, 2015). We showed that ant species living in the same
671 biogeographical region possessed more similar symbiont communities. This suggest that
672 both the spatial overlap and similarity in climatic conditions facilitate the sharing of
673 symbionts. Sampling effort also considerably explained the sharing of symbionts. More
674 shared symbionts were reported in well-studied pairs of species. Focusing on the different
675 subsets of ant symbionts, we found that the sharing of both trophobionts and social parasites
676 between host ant species was mainly determined by the same factors, i.e. sample effort,
677 biogeography and phylogenetic relatedness, that drive the sharing of the symbiont
678 community as a whole. However, phylogenetic relatedness was much more important in
679 explaining the sharing of social parasites. This effect is directly linked to the very low
680 taxonomic/phylogenetic distance between their hosts. Myrmecophiles as a whole and the
681 myrmecophilous beetle and mite communities were more similar in sympatric ant species
682 and ant species residing in the same biogeographical region. Interestingly, similarity in these

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symbiont communities was not positively correlated with host relatedness (cf. Cooper *et al.*, 2012; Veresoglou & Rillig, 2014). The host range of myrmecophilous mites is clearly understudied, which is visible in the relative importance of sample effort in the degree of mite sharing.

V. OUTSTANDING QUESTIONS

- Merging different interaction types into one ecological network framework is a key challenge in ecology (Fontaine *et al*., 2011). Diverse host-symbiont communities provide an opportunity to test the relative contributions of ecology and evolution to network assembly. For example, our study on ant-symbiont networks reveal the different role of ecological and evolutionary processes depending on the type of symbiosis. Our insights may provide a basis for theory development and across-ecosystem comparisons (e.g., against plant and coral-based networks) and synthesis.
- We lack theory on how the architecture and the interaction signs and sizes jointly affect the stability and productivity of these diverse networks, much in contrast to trophic or mutualistic networks. The relative ease with which one can manipulate ant-symbiont communities makes them suited as empirical systems to test theory.
- Host-symbiont networks offer an opportunity to understand both ecological and evolutionary processes behind community assembly, from meso- to macro-ecological scale (see Vellend, 2016). More specifically, as hosts occur spatially structured at these scales, it remains an open question how these assembly processes are determined by ecological and evolutionary dispersal limitation. One key-question here is whether and how symbionts are dispersing: to what degree is horizontal transfer and subsequent symbiont sharing across hosts a facilitator of symbiont community assembly, and to which degree is an obligatory co-dispersal established across the antagonism-mutualism gradient of host-symbiont networks. Mutualistic plant mycorrhizal fungi and plant diaspores are for instance passively co-dispersed by birds

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709 (Correia *et al.*, 2019). Are alike active processes equally prevalent in ant-symbiont
710 interactions, for instance by symbionts transported by their host during colony
711 relocation (Parmentier, 2019)?

712 - Different host species can coexist locally. In addition to stabilizing and equalizing
713 mechanisms such as competition and niche differentiation, symbiont interactions may
714 directly mediate coexistence of host species.

715 - Insights from this review are restricted and applicable to networks as characterised at
716 the species-level, thereby neglecting any intraspecific variation. Following the
717 relevance of ecological and evolutionary determinants, the question remains open to
718 which degree co-evolutionary dynamics between hosts and their symbiont community
719 occur. As strong selection may act on ant symbionts to bypass host colony defence,
720 cryptic speciation in ant symbionts is expected to be high (Schonrogge *et al.*, 2002;
721 Zagaja & Staniec, 2015; von Beeren, Maruyama, & Kronauer, 2015). Symbiont
722 populations may be adapted to an individual host population as was demonstrated in
723 the ant-parasitic syrphid fly *Microdon* and the butterfly *Maculinea* (Elmes *et al.*, 1999;
724 Tartally *et al.*, 2019). Ultimately, population divergence may result in cryptic symbiont
725 species each targeting a different host species.

726 - At a higher phylogenetic level, other Hymenopteran and insect lineages (Isoptera)
727 provide similar niches to nest symbionts. Our review learned that none of the listed
728 ant symbionts are shared with wasps, solitary and eusocial bees and termites (note
729 that the latter two groups are poorly represented in Europe). Apparently only
730 facultative symbionts (e.g., *Porcellio scaber* in wasp and bee nests for instance) are
731 shared, but more study is needed to understand the drivers of host-symbiont
732 divergence at these deep phylogenetic levels.

733

734

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VI. CONCLUSIONS

(1) Ant-symbiont networks are particularly interesting to study large-scales patterns and drivers in host-symbiont network topology and symbiont richness as they are extremely diverse and cover the entire mutualism-antagonism continuum. We assembled a complete network of ant-symbiont interactions in Europe and comparatively studied the drivers of host specificity, symbiont richness and symbiont sharing in the different interaction sub-networks.

(2) We identified 701 obligate ant macrosymbionts which we categorized in five types: (1) trophobionts: mutualistic aphids and scale insects (*N* = 69) (2) myrmecophiles: commensalistic and parasitic arthropods (*N* = 537), (3) social parasites: parasitic ant species (*N* = 71), (4) ectoparasitic fungi (*N* = 13), and (5) endoparasitic nematodes (*N* = 11).

(3) The different types of ant symbionts significantly varied in host specificity. Apart from quantitative differences in host range, we also found clear differences in the average taxonomic/phylogenetic relatedness of the targeted host species for the different types of ant symbionts. The species-richest and best studied ant genera generally supported the largest number of symbionts, but the different types of symbionts were unevenly distributed across the ant genera.

(4) We revealed that the ecological and evolutionary factors which drive symbiont species richness may shift depending on the type of symbiosis. Myrmecophile species richness is mainly determined by ecological drivers, such as colony size, host range and niche width of the host. In contrast, species richness of trophobionts and social parasites is driven by evolutionary factors, such as host phylogeny and host biogeography.

(5) Ants living in the same biogeographic region shared more symbionts. The sharing of trophobionts and social parasites, in particular, was also strongly facilitated in phylogenetic related hosts.

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VII. SUPPORTING INFORMATION

Appendix S1. Host-symbiont matrix listing the associations between ants and ant-symbionts in Europe.

Appendix S2. List of literature used to reconstruct the host-symbiont matrix in Appendix S1.

Fig. S1. Correlation between the number of described European species in an ant genus and the number of supported symbionts.

Fig. S2. Number of host species per symbiont type**.** Box plots displaying the variation in the number of targeted host species within and across different types of ant symbionts. The width of the boxes is proportional to the square root of reported species in each symbiont group. The different myrmecophilous arthropod orders are displayed in grey.

Fig. S3. Phylogenetic distance ± SE, based on the phylogenetic tree of Arnan *et al.* (2017), of targeted host species for different types of ant symbionts. The different myrmecophilous arthropod orders are displayed in grey. Letter codes refer to Tukey post-hoc test. Species with no letters in common are significant different $P < 0.05$.

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FIGURE LEGENDS

FIGURE 1. Distribution of different types of ant symbionts. Total number of symbionts *N* = 701, number of symbionts per type given in brackets.

FIGURE 2. Ant symbiont network displaying the proportional distribution of symbionts across the European ant genera. A multilevel pie chart is given for each ant genus. The size of the outer pie chart corresponds to the total number of associated symbionts. The size of the inner pie chart is related to the number of symbionts that are not shared with other ant genera (unique symbionts). The proportional distribution of the five types of ant symbionts is given for all associated symbionts (colour segments in outer pie charts) and for the symbionts that are not shared with other genera (colour slices in inner pie charts). The relative proportion of unique symbionts can be deduced by the relative size of the inner circle to the outer circle. The genera are organized in four groups, corresponding to the ant subfamily to which they belong. The genera are connected with lines, of which the width is directly proportional to the number of shared symbionts. The font size of a genus is proportional to its number of described species in Europe.

FIGURE 3. Number of host species per symbiont type, controlled for sampling effort ± SE**.** The different myrmecophilous arthropod orders are displayed in grey. Letter codes refer to Tukey post-hoc test. Species with no letters in common are significant different $P < 0.05$.

FIGURE 4. Taxonomic distance ± SE of targeted host species for different types of ant symbionts. The different myrmecophilous arthropod orders are displayed in grey. Letter codes refer to Tukey post-hoc test. Species with no letters in common are significant different $P < 0.05$.

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FIGURE 5. Ranking of the predictors from the six PGLS models by the corrected Akaike information criterion (ΔAICc). The change in AICc when adding or removing a variable from the most optimal model is compared. Predictors included in the most optimal model are removed (ΔAICc positive), whereas those not included are added (ΔAICc negative) to the best model (lowest AICc). The ranking is given for the six PGLS analyses, i.e. with dependent variable the number of symbionts (best model: ~ sample effort + colony size + distribution + habitat), trophobionts (best model: ~ sample effort + biogeographic region + worker size + sympatric ants), social parasites (best model: ~sympatric ants), myrmecophiles (best model: ~ sample effort + colony size + distribution + habitat), myrmecophilous beetles (Coleoptera) (best model: ~ colony size + sample effort + distribution range) and myrmecophilous mites (Acari) (best model: ~ colony size + habitat + distribution range + sample effort), respectively. Note that mites and beetles are nested within the group of myrmecophiles and myrmecophiles, trophobionts and social parasites are three subsets of all ant-associated symbionts.

FIGURE 6. Relative importance of the significant predictor matrices explaining the dissimilarity in symbiont communities across different ant species. Rankings are given for predictors explaining overall dissimilarity (1-similarity) in symbiont composition, and for dissimilarity in subsets of symbiont composition: trophobionts, social parasites, myrmecophiles, myrmecophilous beetles and mites, respectively. Note that beetles and mites are nested within the group of myrmecophiles, and myrmecophiles, trophobionts and social parasites are three subsets of all ant-associated symbionts. The allocated contribution (sequential R^2) of the different distance matrices (indicated with Δ) or other predictor matrices (sample effort and 1-sympatry) to the explained variation of the MRM models is estimated with the lmg-metric. All predictor matrices are positively correlated with dissimilarity in symbiont composition. Significance levels of the predictors were tested with a permutation test (n = 9999, $P < 0.001$: ***, $P < 0.01$: **, $P < 0.05$: *, $P < 0.10$: ˙).

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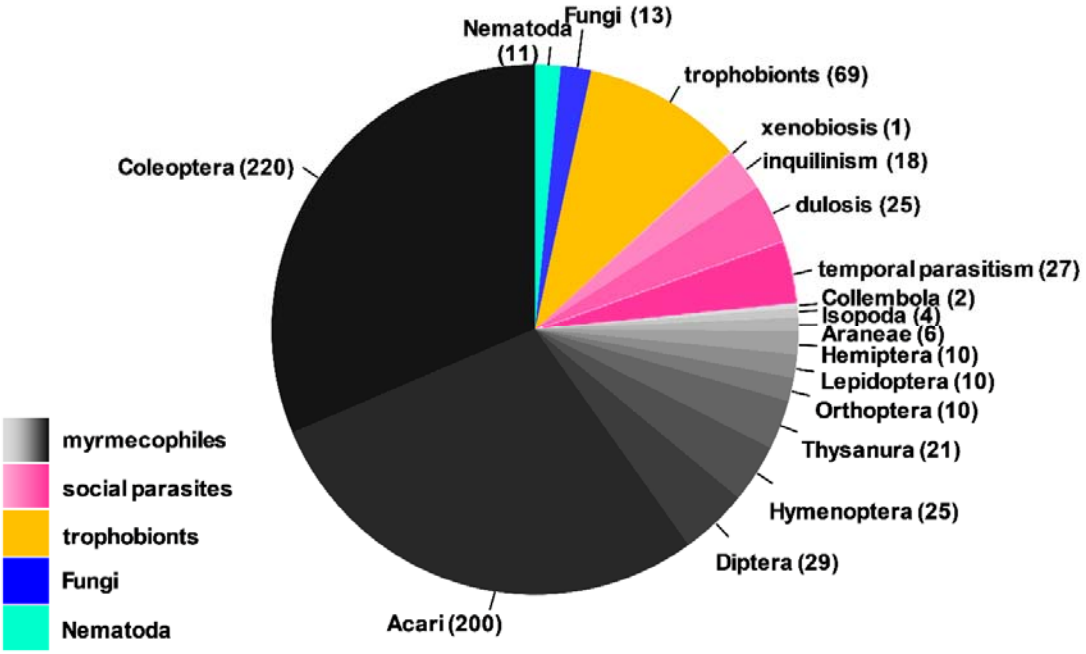
1093 # FIGURES



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1095 Fig. 1

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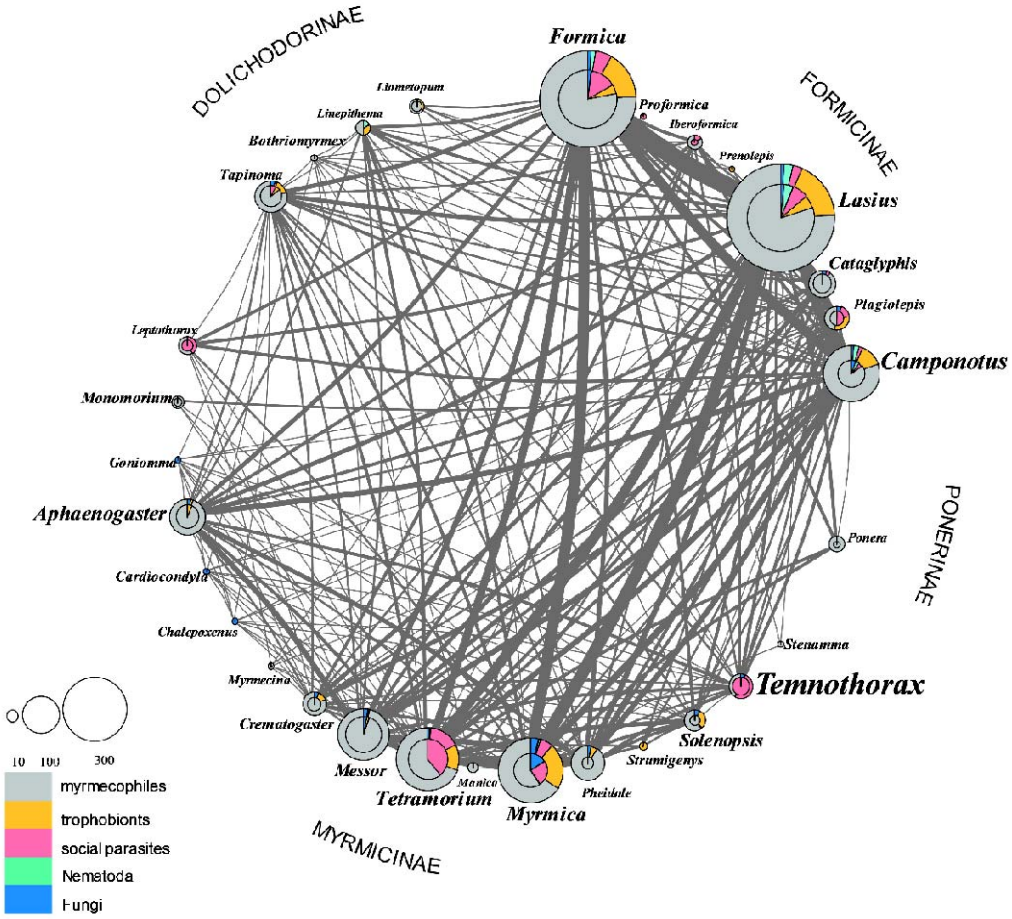

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1097 Fig.2

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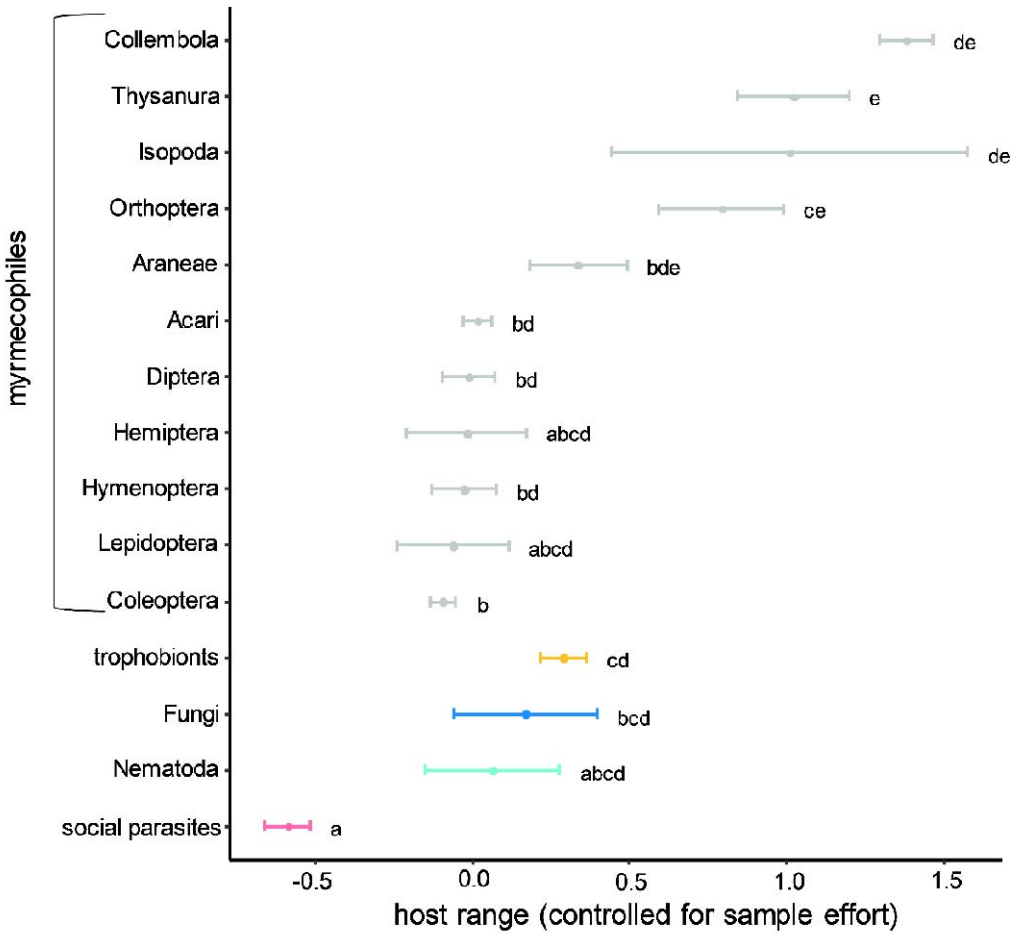

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1099 Fig. 3

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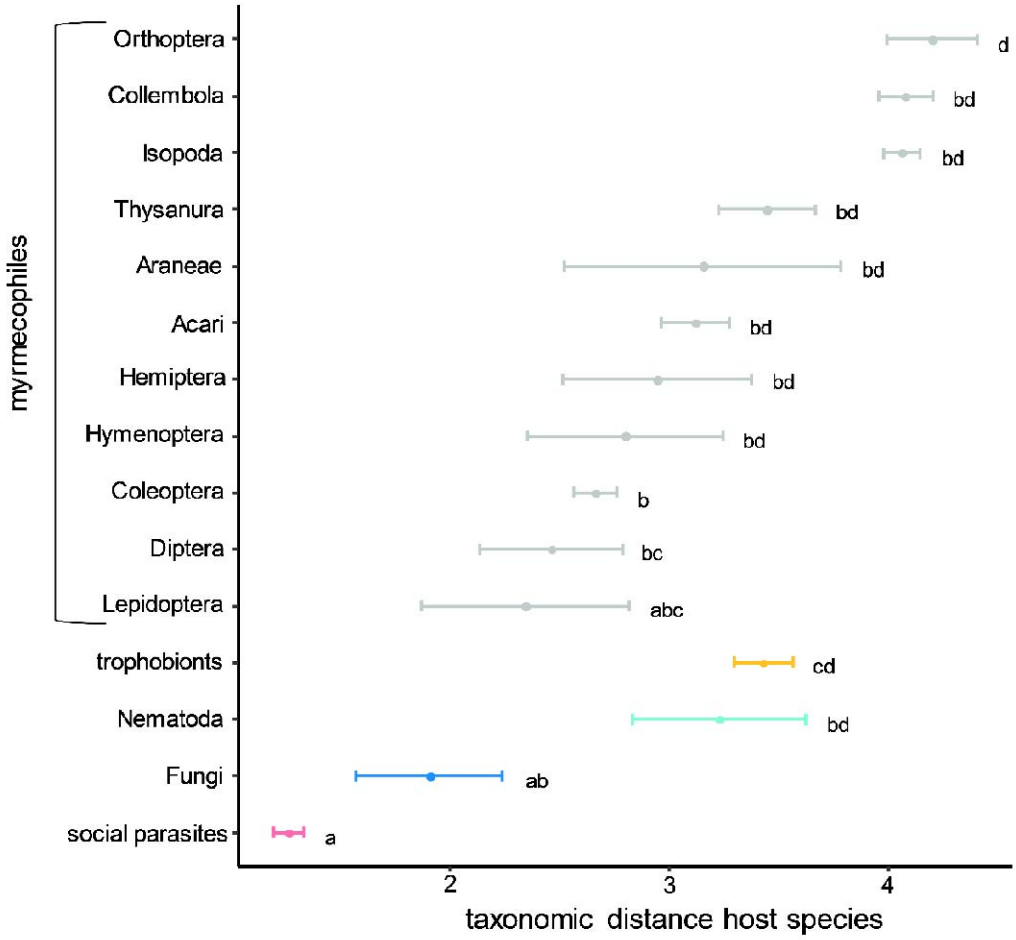

1103

1104 Fig. 4

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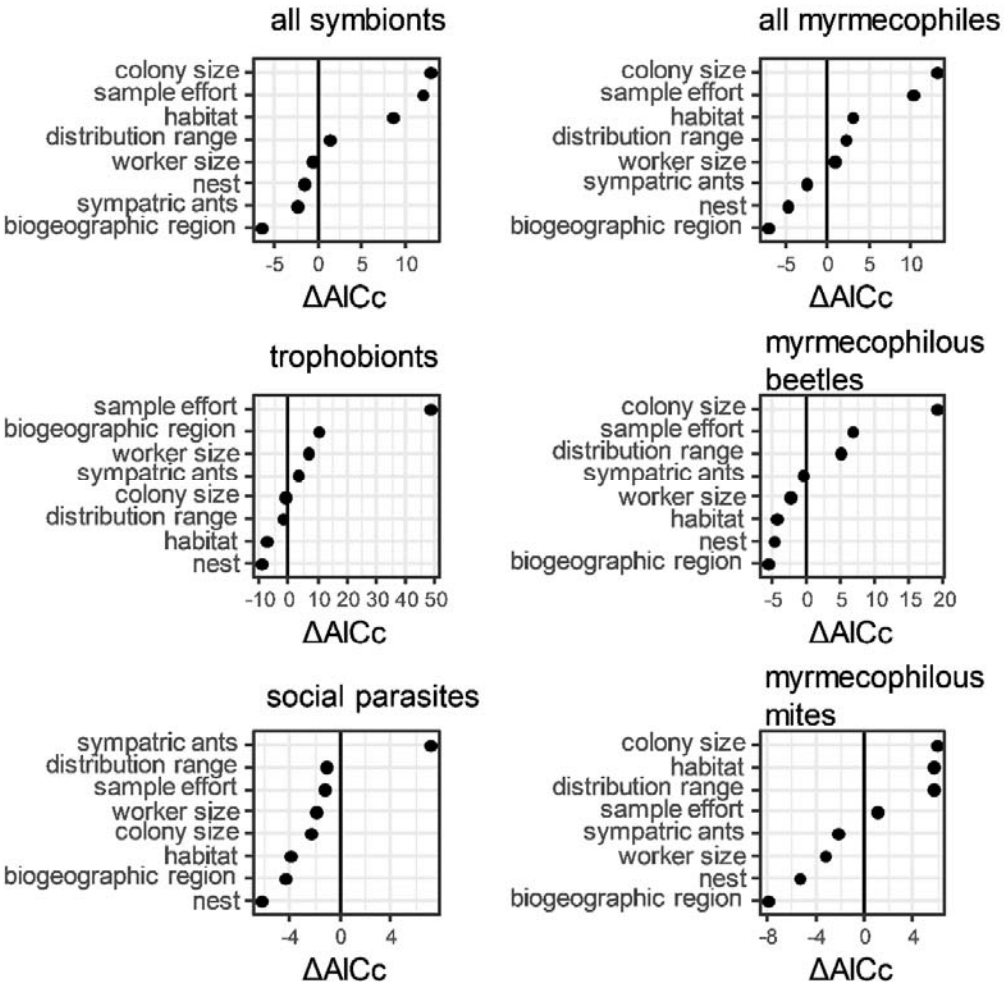

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1107 Fig. 5

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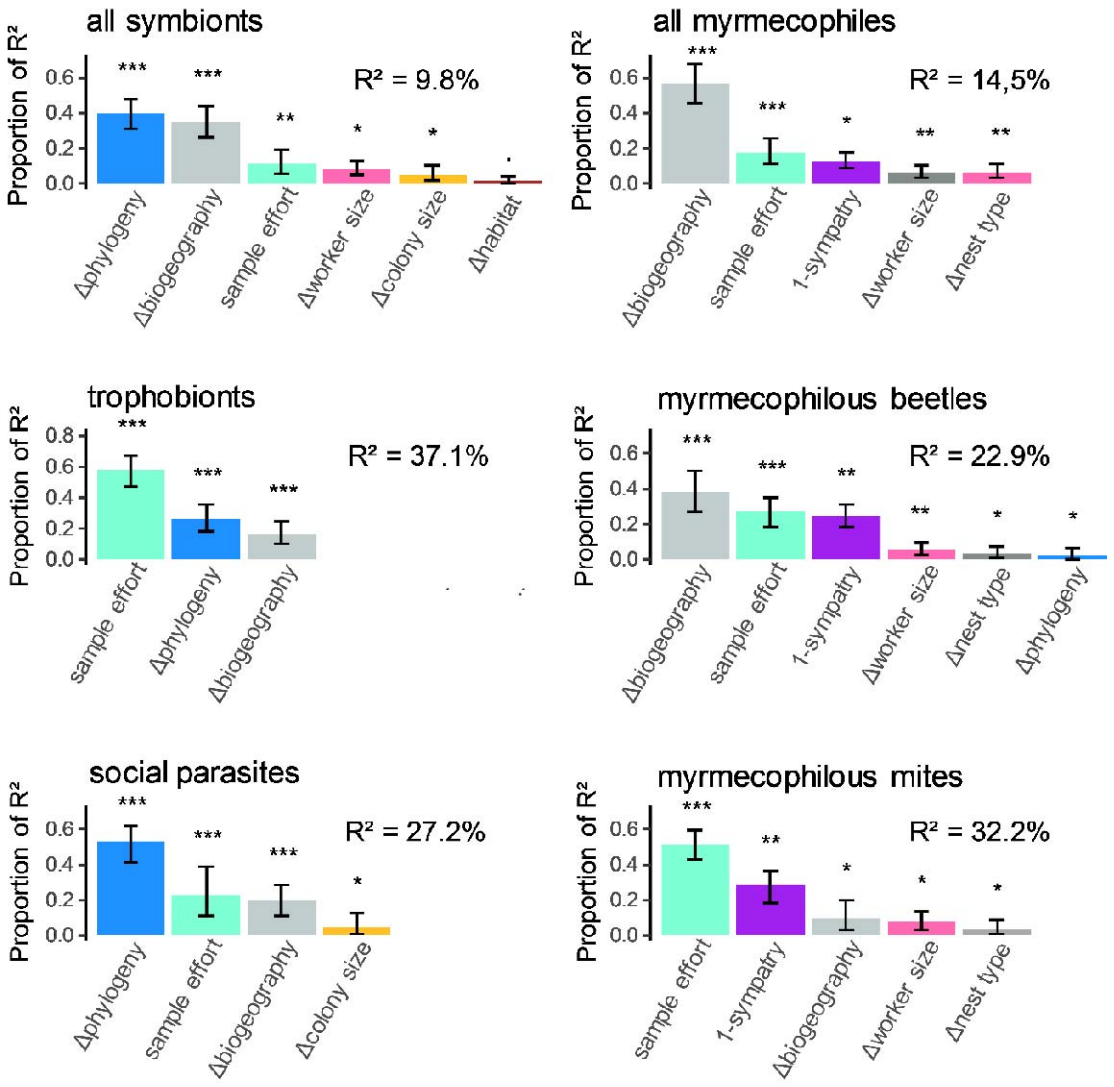

Fig. 6

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TABLES

Table 1. Estimates for the predictors of the top-ranked PGLS-analyses (ΔAICc < 2). The subset of best models is given for the analyses with total number of (1) symbionts, (2) myrmecophiles, (3) myrmecophilous beetles, (4) myrmecophilous mites, (5) trophobionts and (6) social parasites as dependent variable. Significant estimates indicated in bold (P < 0.001: ***, P < 0.01: **, P < 0.05: *, P < 0.10: ·).

	(Intercept)	colony size	sample effort	biogeography	distribution	habitat	sympatric ants	nest type	worker size	df	AICc	weight	lambda
all symbionts													
	2.41	**0.25*** **	**0.25*** **		**0.11*** **	**+*** **				7	88.7	0.37	0
	2.46	**0.24*** **	**0.23*** **		**0.13*** **	**+*** **			0.05	8	89.2	0.28	0
	2.40	**0.24*** **	**0.34*** **			**+*** **				6	90.0	0.19	0
	2.51	**0.22*** **	**0.23*** **		**0.14*** **	**+*** **		+		10	90.2	0.17	0
myrmecophiles													
	2.27	**0.21*** **	**0.24*** **		**0.13*** **	**+*** **			0.08·	8	69.2	0.33	0
	2.20	**0.21*** **	**0.26*** **		**0.10*** **	**+*** **				7	70.0	0.21	0
	1.76	**0.20*** **	**0.38*** **							3	70.6	0.16	0
	2.20	**0.20*** **	**0.33*** **			**+*** **				6	70.6	0.16	0
	1.76	**0.21*** **	**0.32*** **		**0.08*** **					4	70.9	0.14	0
myrmecophilous beetles													
	1.53	**0.22*** **	**0.17** **		**0.16*** **					4	25.5	0.58	0
	1.53	**0.21*** **	**0.17** **		**0.22*** **		-0.08			5	26.1	0.42	0
myrmecophilous mites													
	2.00	**0.14*** **	0.13·		**0.15*** **	**+*** **				7	19.0	0.42	0
	1.42	**0.13*** **	**0.25*** **		**0.26*** **		**-0.15***	**+***		8	19.5	0.34	0
	2.11	**0.18*** **			**0.17*** **	**+*** **				6	20.1	0.25	0
trophobionts													
	1.70		**0.33*** **	**+*** **			-0.08·		**-0.13** **	8	-5.2	0.36	0.35
	1.71		**0.34*** **	**+*** **	**-0.12** **				**-0.15** **	8	-4.8	0.30	0.42
	1.61	**0.05** **	**0.32*** **	**+*** **			-0.07		**-0.14** **	9	-4.0	0.20	0.30
	1.70		**0.34*** **	**+*** **	**-0.07** **		-0.05		**-0.14** **	9	-3.2	0.14	0.38
social parasites													
	1.11						**0.09** **			2	-14.25	0.25	0.79
	1.14				**0.10** **					2	-13.43	0.17	0.96
	1.12				**0.05** **		0.05			3	-13.18	0.15	0.87
	1.09						**0.08** **		-0.05	3	-13.15	0.15	0.74
	1.10		**0.05** **				0.06			3	-13.05	0.14	0.86
	1.09		**0.10** **							2	-13.04	0.14	0.96