

## ARTICLE

# Hunting habits die hard: Conserved prey preferences in army ants across two distant neotropical rainforests

Philipp O. Hoenle<sup>1,2</sup>  | Christoph Merkel<sup>1</sup> | David A. Donoso<sup>1,3</sup>  |  
Adriana A. Argoti<sup>4,5</sup> | Nico Blüthgen<sup>1</sup>  | Christoph von Beeren<sup>1</sup> 

<sup>1</sup>Department of Biology, Technical University of Darmstadt, Darmstadt, Germany

<sup>2</sup>Biology Centre of the Czech Academy of Sciences, Institute of Entomology, České Budějovice, Czech Republic

<sup>3</sup>Grupo de Investigación en Ecología y Evolución en los Trópicos - EETrop - Universidad de las Américas, Quito, Ecuador

<sup>4</sup>Escuela de Ciencias Biológicas, Pontificia Universidad Católica del Ecuador, Quito, Ecuador

<sup>5</sup>Fundación Reserva Tesoro Escondido, Hacienda San Miguel, Ecuador

**Correspondence**

Philipp O. Hoenle  
Email: [philipp.hoenle92@gmail.com](mailto:philipp.hoenle92@gmail.com)

**Funding information**

Deutsche Forschungsgemeinschaft, Grant/Award Numbers: BE5177/4-1, BE5177/4-2; Studienstiftung des Deutschen Volkes; DFG-funded Research Unit REASSEMBLY, Grant/Award Number: FOR5207

**Handling Editor:** Uffe N. Nielsen

**Abstract**

Army ants are widely recognized as keystone species in neotropical rainforests due to their role as important arthropod predators. Their large-scale raids involve thousands of workers scouring the forest floor in pursuit of prey, primarily capturing other invertebrates. Up to 20 species of army ants coexist in a rainforest, and dietary niche differentiation has been proposed as a mechanism to alleviate competition among them. Based on only a handful of study sites, however, our understanding of the precise dietary preferences and the extent of niche differentiation remains notably limited. In this study, we aimed to expand our knowledge of army ant communities by resolving an Ecuadorian predation network consisting of 244 prey species and 13 army ant species representing the five known neotropical army ant genera: *Cheliomyrmex*, *Eciton*, *Labidus*, *Neivamyrmex*, and *Nomamyrmex*. We collected 2156 prey items from 180 army ant raids/emigrations, and of these, we identified 1945 prey items to the family level, 1313 to the genus level, and 664 to the species level based on morphological identifications and DNA barcodes. Prey consisted primarily of other ants (1843 prey items; 153 ant species), to the largest part ant brood ( $N = 1726$ ). Hence, most army ant species chiefly plundered the nests of other ants, while the three swarm raiding species, that is, *Lab. praedator*, *Lab. spininodis*, and *Ec. burchellii*, exhibited a relatively high proportion of non-ant invertebrate prey in their diet. The predation network showed a high degree of specialization ( $H_2' = 0.65$ ), characterized by little dietary niche overlap among sympatric species. We compared the Ecuadorian network with one previously studied in Costa Rica and found that, despite the large geographic distance, prey preferences remained remarkably similar. We discovered species-specific preferences for captured ant genera and species, despite some species turnover in both army ants and prey. Additionally, army ants also exhibited consistent spatiotemporal raiding preferences across study sites. In conclusion, predation preferences within army ant communities

Philipp O. Hoenle and Christoph Merkel contributed equally to this study.

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2024 The Authors. *Ecosphere* published by Wiley Periodicals LLC on behalf of The Ecological Society of America.

exhibited consistency in multiple niche dimensions across two distant neotropical rainforests, suggesting a notable level of predictability within army ant predation networks.

#### KEYWORDS

army ant, Canandé, Costa Rica, *Eciton*, Ecuador, food web, predator–prey network

## INTRODUCTION

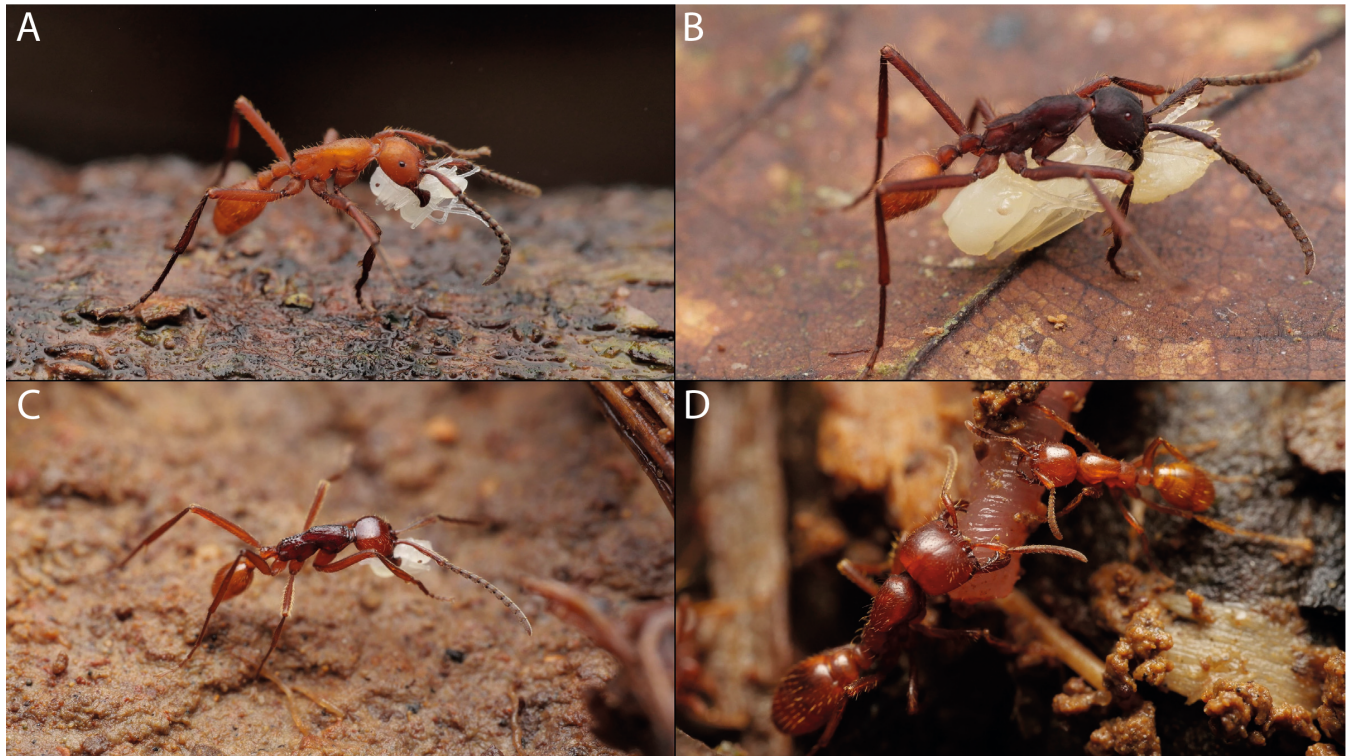
Apex predators such as lions or jaguars are recognized for their crucial role in preserving ecosystem functioning, sometimes even promoting local biodiversity by controlling prey populations (Ripple et al., 2014; Sergio et al., 2008). Their presence can have cascading effects throughout the food web, possibly facilitating a more resilient and sustainable ecosystem (Heithaus et al., 2012; Myers et al., 2007; Sergio et al., 2008). Not surprisingly then, the predatory behavior of large notorious vertebrate predators has been extensively studied across geographic areas (Duffy, 2002; Ripple et al., 2014; Sergio et al., 2008). This stands in stark contrast to numerous significant insect predators, such as army ants. These infamous mass raiding ants are often considered keystone species in neotropical rainforests (Kronauer, 2020; Pérez-Espona, 2021; Rettenmeyer et al., 1983; von Beeren et al., 2021, 2023). They play a crucial role as invertebrate predators, making them central components of neotropical food webs (Gotwald, 1995; Hoenle et al., 2019; Kaspari & O'Donnell, 2003; Kronauer, 2020; Powell, 2011; Rettenmeyer et al., 1983). During raids, thousands of workers leave the nest in search of living prey and return with a significant number of prey items (Powell, 2011), likely affecting the accumulation of biomass at lower trophic levels (Kaspari & O'Donnell, 2003; Rettenmeyer et al., 1983).

Despite their ecological relevance, the basic biology of army ants is still poorly understood (Kronauer, 2020). Army ant species richness in neotropical rainforests is relatively high (O'Donnell et al., 2007), with up to 20 species co-occurring at a single site (Kaspari et al., 2011; Rettenmeyer et al., 1983). These species vary in worker size, colony size, raiding strategies (e.g., column vs. swarm raiders or aboveground vs. belowground), and various other aspects related to prey capture (e.g., the specialized mandibular shape of *Cheliomyrmex* army ants; see Figure 1; Borowiec, 2016; Gotwald, 1995; Kronauer, 2020; Rettenmeyer, 1963; Schneirla, 1971). Such differing traits might facilitate niche differentiation in predation behavior and thus reduce competition among sympatric species, as suggested by general ecological theory (Mittelbach, 2012). However, dietary niches of most species remain unknown,

partly, because fractionalized prey and brood of other ants (Figure 1A–C)—the latter being a major prey component of most army ants (Kronauer, 2020; Rettenmeyer et al., 1983)—are difficult to identify (Hashimoto & Yamane, 2014; Hirosawa et al., 2000; Hoenle et al., 2019; Powell & Franks, 2006; Rettenmeyer et al., 1983). Consequently, the knowledge of army ant prey niches remains vague, mostly covering information at the level of the prey genus, or even at a coarser taxonomic resolution (Hashimoto & Yamane, 2014; Hirosawa et al., 2000; Powell & Franks, 2006; Rettenmeyer et al., 1983). Nonetheless, these studies clearly pointed toward dietary niche differentiation, as sympatric army ant species showed preferences for different prey ant genera (Hashimoto & Yamane, 2014; Hirosawa et al., 2000; Powell & Franks, 2006; Rettenmeyer et al., 1983).

Recently, Hoenle et al. (2019) resolved a Costa Rican army ant predation network at the level of prey species. By comparing DNA barcodes of prey items with a reference database of neotropical ants, the study overcame the impediments of identifying fractionalized and immature prey (Hoenle et al., 2019). The results largely confirmed previous work in that neotropical army ants were primarily specialized predators of other ants, with a strong generic prey specialization in most army ant species (Hoenle et al., 2019). Overall, the predation network, consisting of 11 army ant species and 129 prey species, showed a high degree of prey specialization and a clear signal of prey niche differentiation (Hoenle et al., 2019). The army ants' complementary diets provided evidence in line with a fundamental concept in ecological theory, which suggests that competing species sharing the same habitat can coexist by utilizing different food sources or foraging strategies, thus reducing interference (Mittelbach, 2012). Differentiation in further niche dimensions, namely, temporal and spatial raiding patterns, were found among the co-occurring army ant species, likely relaxing competition even further (Hoenle et al., 2019).

Numerous species of army ants are widely distributed across tropical America (Guénard et al., 2017; Watkins II, 1976; Winston et al., 2016; [www.antmaps.org](http://www.antmaps.org)). This fact, together with expected changes in community composition of potential prey, provides the opportunity to



**FIGURE 1** Army ant workers with prey. (A) *Eciton hamatum* worker carrying two prey pupae of *Pheidole* ants. Army ants often carry several small prey items at once. (B) *Eciton vagans* worker with a prey pupa of the ant genus *Odontomachus*. Note that *Odontomachus* pupae usually have cocoons, but army ants seem to unpack them, possibly facilitating efficient transportation (Hoenle et al., 2019). (C) *Neivamyrmex gibbatus* worker carrying a small ant prey pupa. (D) Two *Cheliomyrmex andicola* workers attack an earthworm. Their unusually sickle-shaped mandibles are armed with elongated, spine-like teeth, apparently enabling them to dismember large, soft-bodied prey. Photo credit: P. O. Hoenle.

study the flexibility of army ant predation networks in distant communities. Existing research has focused on examining neotropical army ant prey interactions on a local scale (e.g., Hoenle et al., 2019; Powell, 2011; Vieira & Höfer, 1994), leaving a knowledge gap regarding the broader implications across different geographic scales. Hoenle et al. (2019) compared the prey preferences of *Eciton* army ants in Costa Rica with those found in Panama (Powell & Franks, 2006), suggesting a consistent, species-specific preference for particular prey ant genera across these regions. However, research on army ant predation has largely taken place in Central America (Hoenle et al., 2019; Powell, 2011; Rettenmeyer et al., 1983), with little understanding of the flexibility of predation networks on a larger geographical scale.

The present work had two main objectives: first, to resolve an Ecuadorian army ant predation network at the species level, and second, to compare this network with the one previously studied in Costa Rica (Hoenle et al., 2019). It is important to note that the two study sites belong to an interconnected tropical rainforest system that extends from Central America to the western part of northern South America in Colombia and Ecuador (González-Maya et al., 2012; Sarkar et al., 2009). The

Costa Rican study site is part of the Isthmian–Atlantic moist forest biome, while the Ecuadorian site is part of the Chocó-Darién moist forest biome. Despite the substantial straight-line distance of approximately 1220 km between the study sites, we expected to discover a notable overlap in army ant and prey species, particularly as intensive faunal exchange led to the homogenization of many animal groups once the land bridge between Central America and South America, the Isthmus of Panama, had closed (O’Dea et al., 2016).

In army ants, phylogenomic and population genomic data, combined with species distribution patterns, have yielded compelling evidence that several lineages of army ants dispersed from South America to Central America and underwent speciation there before the complete closure of the Isthmus of Panama (Winston et al., 2016). Although many of these lineages are currently classified as subspecies, at least some of them most likely represent distinct species (Winston et al., 2016). Notably, in three instances, sister species pairs (two distinct genetic lineages of each *Ec. burchellii*, *Ec. mexicanum*, and *Ec. vagans*) occur in secondary contact zones in Central America where the respective sister species exhibit parapatric distributions without showing any discernible evidence of gene



flow (Winston et al., 2016). This contact zone stretches along an 800-km area along the Chorotega volcanic front, extending from Costa Rica into Nicaragua and Panama (Winston et al., 2016). While three of these army ant species occur west of the Chorotega block extending into northern Central America, the distribution of their corresponding sister species east of the Chorotega block stretches down to South America through the Chocó-Darién moist forest biome present in Panama, Colombia, and Ecuador (Winston et al., 2016; see also Appendix S1). Our study site in Costa Rica, that is, La Selva Biological Station, is situated to the east of the volcanic barrier, and therefore, we expected that the distribution of the studied army ant species extends southward into South America. Based on these biogeographic considerations, our expectation was to observe a considerable species overlap between the two sites, encompassing both army ants and their prey. This anticipation holds, despite the Chocó being recognized for some notable degree of endemism (Cucalón et al., 2022; Pérez-Escobar et al., 2019; Ruiz-Guerra et al., 2007), including remarkable species of ants (e.g., Booher & Hoenle, 2021; Hoenle et al., 2020).

We had two primary expectations regarding army ant predation networks. Firstly, we anticipated detecting a high degree of predation specificity and pronounced dietary niche differentiation at the Ecuadorian study site, similar to our previous findings in Costa Rica. Secondly, due to the high degree of prey specialization detected in Costa Rica (Hoenle et al., 2019), we expected to find consistent species-specific dietary preferences as well as similar spatiotemporal activity patterns of army ants across the two studied geographic regions.

## METHODS

### Data deposition, collection protocol, and research permit

A file containing information on prey collection, prey identification, prey life stage, GenBank accession numbers, network metrics, and on BOLD Systems BIN numbers has been deposited in the open-access repository Dryad under the name “Dryad file 1” (von Beeren, 2023). Images and DNA barcodes of army ants and their prey have been deposited at BOLD Systems (Hoenle et al., 2023).

The study was conducted at the Río Canandé and the associated Tesoro Escondido reserves in Ecuador (0.5263° N, 79.2129° W), a research area consisting of a mosaic of primary and secondary forests, agricultural land, pastures, and monocultural cocoa plantations (Hoenle et al., 2022). The study took place during the rainier months from April to June 2018 and February to May 2019, as well as during the less rainy months from

September to October 2021 (Gale & Barfod, 1999; Morelos-Juárez et al., 2015). No measurements of temperature and precipitation were yet available for the study site, but annual average precipitation was estimated to be 4000–5000 mm at Quindindé, a city with the closest weather station (Gale & Barfod, 1999). We estimated annual temperature to be about 22–24°C based on data retrieved from climate databases for two nearby locations: Santo Domingo de los Tsachilas and Luis Vargas Torres (<https://en.climate-data.org/>). Our previous study on Costa Rican army ant predation at La Selva Biological Station took place during the less rainy months from February to April 2017 (annual average precipitation: 4.260 mm; annual average temperature: 26.3°C; Fernandez-Bou et al., 2019; Jiménez-Rodríguez et al., 2020). Importantly, for a site comparison of prey preferences, both study sites are evergreen lowland tropical rainforests that exhibit modest temperature and rainfall seasonality (Costa Rica: Fernandez-Bou et al., 2019; Jiménez-Rodríguez et al., 2020; McDade et al., 1994; Tozetto et al., 2023; Ecuador: Freile & Vázquez, 2005; Gale & Barfod, 1999; Morelos-Juárez et al., 2015). Therefore, we do not anticipate the season playing a dominant role in army ant prey choice, especially considering that ants, as a primary prey resource, generally exhibit high nest site fidelity.

We searched for army ant raiding trails and colony emigrations by walking the trails at the reserve during daytime (7:00 a.m.–6:00 p.m.) and nighttime (8:00 p.m.–5:00 a.m.; Dryad file 1—Specimen information; von Beeren, 2023). Our search covered an area of approximately 7 × 8 km. Upon detection of a raiding or an emigration column, we aimed to collect the first 5–20 army ant workers carrying prey items into a 1.5-mL collection vial filled with absolute ethanol.

Overall, we collected 2156 prey items. Per raid/emigration, we collected between 1 and 49 prey items (mean ± SE: 11.97 ± 5.77 prey items per raid/emigration; median = 11; number of raids/emigrations = 180; Table 1; Dryad file 1—Specimen information; von Beeren, 2023). We sometimes collected prey from swarm fronts in the swarm raiding species *Ec. burchellii*, *Lab. praedator*, and *Lab. spininodis*. Similar to the Costa Rican study (Hoenle et al., 2019), we omitted raids of the same species at the same day within a radius of approximately 50 m of the last sampling spot to minimize resampling of the same colony on the same day.

Collection, exportation, and genetic permits were issued by the Ministerio del Ambiente, Agua y Transición Ecológica (Contrato Marco: MAE-DNB-CM-2017-0068, export and genetics: 41-2018-EXP-CM-FAU-DNB/MA and 144-2019-EXP-CM-FAU-DNB/MA; Contrato Marco: MAE-DNB-CM-2019-0115, export and genetics: 007-2022-EXP-CM-FAU-DBI/MAAE).



**TABLE 1** Summary of the sample sizes of the Ecuadorian/Costa Rican study.

Army ant species	Raids	Emigrations	Prey	Prey identified to species
<i>Cheliomyrmex andicola</i>	1/-	0/-	1/-	0/-
<i>Ecton burchellii</i>	37/37	1/4	385/467	160 (4)/167 (4)
<i>Ecton dulcium</i>	-/14	-/2	-/162	-/151 (9)
<i>Ecton hamatum</i>	43/20	2/6	699/530	123 (3)/83 (3)
<i>Ecton lucanoides</i>	15/5	0/4	151/126	53 (4)/52 (6)
<i>Ecton mexicanum</i>	5/36	1/3	60/436	29 (5)/233 (6)
<i>Ecton vagans</i>	20/49	1/1	279/782	103 (5)/238 (5)
<i>Labidus praedator</i>	11/-	0/-	97/-	44 (4)/-
<i>Labidus spininodis</i>	17/-	0/-	156/-	69 (5)/-
<i>Neivamyrmex asper</i>	3/2	0/0	41/22	9 (3)/5 (3)
<i>Neivamyrmex curvnotus</i>	5/-	0/-	56/-	21 (4)/-
<i>Neivamyrmex gibbatus</i>	15/20	0/2	194/310	42 (3)/101 (5)
<i>Neivamyrmex pilosus</i>	-/22	-/0	-/268	-/47 (2)
<i>Nomamyrmex esenbeckii</i>	3/10	0/0	26/149	9 (3)/22 (2)
<i>Nomamyrmex hartigii</i>	1/1	0/0	11/10	2 (NA)/4 (NA)

Note: The number of raids and emigrations from which we collected prey is provided for each army ant species for the Ecuadorian army ants and, separated by a slash, for the Costa Rican ones (Hoenle et al., 2019). Note that we added prey cache collections (accumulations of prey items in raiding columns) from Hoenle et al. (2019) as raid collections in the present work. Furthermore, information is given on the total number of collected prey items, as well as the subset of these prey items we identified to species level. Numbers in parentheses give the mean number of identified prey items per collection event. A short dash indicates a species was not detected at this site.

Abbreviation: NA, not available as no means can be calculated.

## Vouchered material—Specimen images, DNA barcodes, and DNA extracts

We positioned all prey items from each collection event on either white or black paper to capture an overview photograph using a Nikon D5300 or a Panasonic G91 camera. From this point on, each prey item received a unique identification code (e.g., EB102\_P003; EB = *Ec. burchellii*; 102 = collection ID; P003 = prey item number three). Prey overview images of each collection event are provided as Appendix S2. Additionally, we uploaded an image of each prey item to its respective record in BOLD System (totaling 2156 images). Specimen vouchers and DNA extracts were deposited at the TU Darmstadt Insect Collection. We will update the information in BOLD Systems in case of depository changes.

## Classification and morphological identification of army ant prey

Army ant prey contained eggs, larvae, pupae, and adults of other ants, as well as immature and adult bodies and,

in particular, body fragments of other invertebrates (Appendix S2). To categorize prey items, we initially distinguished ants from other invertebrates (later referred to as non-ant prey). Eggs were not assigned to any taxonomic level, except for five eggs that we barcoded for genetic identification.

For each collection event (raid or emigration), we aimed to barcode at least one specimen of each species. For this, we classified the prey per raid/emigration into morphologically distinct groups (morpho-clusters), which represented groups of specimens with similar appearances (mean  $\pm$  SD =  $5 \pm 3$  morpho-clusters per raid/emigration; median = 4; range = 1–14;  $N = 180$  raids/emigrations). Initially, for adult specimens and hemimetabolous insects, we attempted identification to higher taxonomic levels such as order or family. In the case of ants, we have often succeeded in identifying the genus, and in some instances, even the species, primarily using two species keys for neotropical ants (Fernández et al., 2019; Longino, 2010). Noteworthy, prey collection of the same raid/emigration often contained various body parts possibly deriving from the same prey specimen, such as single legs or dismembered body parts (e.g., raid NO100 in Appendix S2).

## Molecular protocol and genetic prey identification

The primary components of army ant prey were ant brood and arthropod fragments, and thus, our main method for species identification relied heavily on DNA barcoding. We selected at least one specimen from each morpho-cluster per collection event for DNA barcoding (mean  $\pm$  SD =  $7 \pm 3$  prey items per raid/emigration; median = 7). In total, we attempted to acquire DNA barcodes for 1219 prey items out of a total of 2156 collected prey items.

DNA of prey items was extracted in 96-well plates using the Qiagen DNeasy 96 Blood & Tissue Kit (Hilden, Germany). We applied the standard protocol except for the homogenization of material, which we skipped to keep specimens as morphological vouchers. However, some larval and pupal specimens lost their shape after the protein lysis step, hampering future morphological work. Voucher images may serve as a reference in these cases (Appendix S2).

For molecular species identification, we amplified the classical animal DNA barcode, a fragment of the mitochondrial *cytochrome oxidase I* (*COI*) gene by standard polymerase chain reaction (PCR). PCRs were set up as described previously (von Beeren et al., 2016). We used several published primers, mostly relying on the universal DNA barcode primer combinations LCO1490/HCO2198 (Folmer et al., 1994) and LepF1/LepR1 (Hebert et al., 2004; a full primer list is given in Appendix S3: Table S1). Successful DNA amplification was verified via positive signals in a standard gel electrophoresis. PCR products were sent to Macrogen Inc. Europe (Amsterdam) for purification and Sanger sequencing. Amplicons were always sequenced in forward and reverse directions. If low-quality reads were encountered, we repeated the PCR steps using alternative primer combinations to enhance sequence quality.

In comparison with our previous study (Hoenle et al., 2019), the success rate of DNA barcoding in the current work was significantly lower. Of those specimens used for DNA extractions, we successfully acquired *COI* barcodes for 52% of the Ecuadorian samples, whereas in Costa Rica, this success rate was 86%. There were two primary reasons for this discrepancy. First, we refrained from reattempting to amplify *COI* in specimens if a successful barcode had already been generated for specimens of the same morpho-cluster within a raid/emigration. Second, we employed fewer primer combinations and made fewer efforts to reattempt amplification due to limited resources, including time and labor constraints.

All subsequent sequence analyses were performed using Geneious Prime 2023.1.2 software (<https://www.geneious.com>). This included tasks such as assembling forward and reverse sequences and trimming sequences. The final consensus sequences were aligned using the MUSCLE algorithm (Edgar, 2004). We performed several quality checks with consensus sequences, including the search for stop codons or shifts in the reading frame due to gaps or extra base pairs. Further, we compared barcoding identifications with prey images to detect apparent errors in genetic specimen identifications, which might have occurred due to contamination or pipetting errors.

The remaining DNA barcodes were uploaded to BOLD Systems for further analysis. To genetically identify the ant prey specimens, we compared the acquired DNA barcodes with a curated reference database of neotropical ants in BOLD Systems (reference database: 8335 records, 843 BINs; data manager: DD). BOLD Systems designates “Barcode Index Numbers” (BINs), unique alphanumeric codes to define distinct genetic clusters in the entire BOLD Systems database (Ratnasingham & Hebert, 2013). We used species names when a sequence was clustered within an existing BIN with a species name. If a species identification failed, we used genus names of the closest neighbor in a neighbor-joining (NJ) tree, which encompassed all records of the reference database (Appendix S4). NJ trees were generated in BOLD Systems based on Kimura two-parameter distances (Appendix S4; see also Appendix S5 for RAxML trees). We incorporated the BIN number in species naming as alternate for the species epithet (e.g., *Camponotus* ACH1136).

Invertebrate specimens other than ants were identified by a DNA barcode comparison with the entire BOLD Systems database (including early-release records). As for ants, we applied species names when a sequence was clustered within an existing BIN. If a species identification failed, we used genus names when a sequence match was between  $\geq 95\%$  and  $< 99\%$  and family names when sequences matched between  $\geq 90\%$  and  $< 95\%$ . In cases where a sequence match was  $\geq 80\%$  and  $< 90\%$ , we adopted the order name. Between  $< 80\%$  and  $\geq 70\%$  sequence similarity, we adopted the class name and lower than 70% the phylum name. The taxon name was then accompanied by the sample’s BIN number (e.g., Blaberidae AER0783). However, some sequences were not clustered into a BIN, because BOLD Systems will not create a new BIN for sequences of  $\geq 300$  bp and  $< 500$  bp. Sequences of  $< 300$  bp are not considered at all in the BIN analyses (Ratnasingham & Hebert, 2013). We added a unique identifier to the name for those sequences without a BIN (e.g., Hemiptera CM13).

## Interaction specificity, prey diversity, and degree of prey differentiation

To assess the level of prey diversity, predation specificity, and the degree of prey differentiation within the Ecuadorian army ant community, we utilized a range of standardized metrics from ecological network analyses (Blüthgen, 2010; Ings et al., 2009; Ivens et al., 2016; Vázquez et al., 2009). These standardized metrics allowed for an unbiased comparison between the Ecuadorian and the Costa Rican predation network (e.g., Blüthgen, 2010; Ivens et al., 2016). To enable cross-site comparisons, we conducted analyses focusing solely on ant species as prey, because our prior Costa Rican study lacked species-level identification of non-ant prey (Hoenle et al., 2019).

We defined an “interaction” as the presence of a particular prey species during a raid or emigration event of a particular army ant species. The link strength summarizes these interactions between predators and prey and thus represents the number of times prey species were present in distinct, spatially and temporally independent collection events (army ant raids/emigrations). As an illustration, when we consider the trap-jaw ant species *Odontomachus meinerti*, we identified eight prey items in three separate raids of *Ec. vagans*, resulting in a link strength of three between these two species (Dryad file 1—Specimen information; von Beeren, 2023). We visualized army ant predation networks using the command `plotweb()` of the R package “bipartite” (Dormann et al., 2009; R version 4.3.1; RStudio version: 2023.09.0).

The degree of interaction specificity at the network level was described using the two-dimensional Shannon entropy ( $H_2'$ ), and at the species level, the Kullback–Leibler distance ( $d'$ ) (Blüthgen et al., 2006). Both metrics are normalized relative to the maximum and minimum possible values. Consequently, they can range from zero (indicating the lowest level of specificity) to one (indicating the highest level of specificity; Blüthgen et al., 2006). To assess whether the Ecuadorian predation network differs from a purely random network, we conducted a test by comparing  $H_2'$  with 1000 null models created from random networks with the same size and total number of interactions (Blüthgen et al., 2006; Patefield, 1981).

For each army ant species, we counted the number of prey species ( $S_{\text{obs}}$ ) and calculated the effective Shannon diversity of prey ( $e^{H_{\text{obs}}}$ ; Jost, 2006). To enable unbiased species comparisons among army ant species with varying sample sizes, we also determined the rarefied prey species number ( $S_{\text{rare}}$ ) and rarefied Shannon diversity ( $e^{H_{\text{rare}}}$ ) using 100 permutations for 27 prey interactions. This represented the minimum number of interactions for an army ant species with more than 10 collection events, as observed in *Neiv. gibbatus*. Army ants with

fewer than 10 collection events were excluded from this particular analysis (see Table 1).

To explore dietary niche differentiation in army ant species, we used a network modularity analysis with the quantitative modularity metric  $Q$  (Dormann & Strauss, 2014). This metric quantifies how species interactions within a network are organized into distinct modules. These modules are characterized by a high density of interactions within the module and minimal interactions between modules, providing insights into the degree of prey niche differentiation. Like  $H_2'$  and  $d'$  values,  $Q$  is normalized and ranges from 0 (random network configuration) to 1 (perfectly modular network). We compared  $Q$  against 1000 randomized null models (Schleuning et al., 2014), as previously described for  $H_2'$ .

It is crucial to also acknowledge the limitations of our network approach. We managed to acquire DNA barcodes for only every third prey item collected (see [Results](#)), inevitably leading to the omission of certain species links in our network analysis. For example, there is a possibility that barcoding consistently failed for specific species. It is important to recognize that interaction networks inherently have missing links between species (Dormann & Blüthgen, 2017; Hoenle et al., 2019; Sorensen et al., 2011). Despite these limitations, we remain confident that the sample size used in this study, coupled with the standardized sampling design employed, has yielded robust findings concerning interaction specificity and niche differentiation within the Ecuadorian predation network (see Hoenle et al., 2019 for a test on the stability of network specificity values with decreased sample sizes).

## Spatiotemporal raiding preferences of army ant species

To evaluate the spatial raiding preferences of army ant species, we gathered information on the nesting habits of their prey ants from the literature. We differentiated between arboreal-nesting species and those nesting on or in the ground, primarily using resources such as Longino (2010), AntWeb, AntWiki, and own observations at the study site (see Dryad file 1—Specimen information; von Beeren, 2023). We categorized prey species nesting in leaf litter and soil as “ground nesters,” while “arboreal nesters” encompassed all species nesting from lower arboreal zones to the canopy. Prey species with nesting habits spanning both categories, and those with unclear nesting habits, were excluded from this analysis (see Dryad file 1—Specimen information; von Beeren, 2023). We used a contingency matrix that summarized the number of ground-nesting and arboreal-nesting prey species in raids/emigrations of the different army ant



species (Dryad file 1—Network matrices; von Beeren, 2023). We then assessed differences in spatial raiding preferences among army ant species by comparing the  $H_2'$  values with null models, as described earlier. We compared the spatial raiding preferences between the two study sites by conducting a Spearman rank correlation based on the proportion of raids on ground-nesting species. Note that the statistical power was comparatively low in this comparison ( $N = 9$  army ant species per site).

Likewise, we analyzed the temporal raiding preferences of army ants. A raid was classified as “diurnal” if it occurred during daylight hours (7:00 am–6:00 pm), while raids that took place during darkness (8:00 pm–5:00 am) were labeled as “nocturnal.” We assessed differences in raid activity patterns (excluding emigration data) of the army ant community by comparing  $H_2'$  against null models as described above. This analysis relied on a contingency table that captured the frequency of detecting raids during both nocturnal and diurnal periods for each army ant species (Dryad file 1—Specimen information; von Beeren, 2023). As described above for spatial raiding preferences, we compared the temporal raiding preferences between the two study sites by conducting a Spearman rank correlation based on the proportion of diurnal raids. When examining and interpreting the temporal raiding preferences, it is essential for the reader to notice that our collection efforts were skewed, with a greater number of diurnal raids relative to nocturnal raids being collected in Ecuador (119 diurnal raids vs. 57 nocturnal raids) compared with Costa Rica (56 diurnal raids vs. 159 nocturnal raids).

## Comparison of army ant prey preferences between study sites

To compare prey preferences between Costa Rica and Ecuador, a robust species identification of the army ants was crucial but presented some challenges. Importantly, a phylogenomic and population genomic study provided strong evidence that several *Eciton* species represent species complexes (Winston et al., 2016), that is, two or more species are currently taxonomically described as a single species, sometimes as distinct subspecies (e.g., *Ec. burchellii foreli* and *Ec. burchellii parvispinum*). We provide detailed information on army ant identification in Appendix S1, which includes a discussion on biogeographic distribution and morphological characters and a comparison of 155 DNA barcodes of army ant specimens from the two study sites with a reference database of neotropical ants of the subfamily Dorylinae. In short, we are confident that we compared the same species between the two study sites for the following species: *Ec. burchellii*

(subspecies *foreli*), *Ec. vagans*, *E. lucanoides*, *Ec. hamatum*, and *Nom. hartigii* (see detailed discussion in Appendix S1). Some uncertainties remain regarding the species status across study sites for the following species considered in the prey analysis: *Ec. mexicanum*, *Neiv. asper*, and *Neiv. gibbatus* (see detailed discussion in Appendix S1). In *Nom. esenbeckii*, it appears that we have studied two distinct subspecies between the study sites: *Nom. esenbeckii wilsoni* at La Selva Biological Station and *No. esenbeckii crassicornis* at the Reserva Río Canandé in Ecuador (Appendix S1: Figure S1). The question of whether these subspecies are geographic variants of the same species or represent distinct species remains uncertain and warrants further in-depth investigation. Solving the uncertainties in resolving army ant species boundaries in a taxonomic revision is beyond the goals of the present work. Here, we identified the army ants to the species level for cross-community-level comparisons, neglecting subspecies affiliations (Appendix S1). However, readers should be aware that it is possible that some species denoted here under the same name might later turn out to be two or more distinct species.

A primary goal of the present work was to compare the prey spectra of army ant species between the Ecuadorian and Costa Rican predation networks. For this, we first examined the preferences for ant genera, thus accounting for community differences in prey species composition between the two study sites. We generated species-pair bipartite network graphs that illustrated the prey preferences of each army ant species at both study sites. Subsequently, we computed  $H_2'$  values and compared these with null models, as detailed earlier. In cases where prey preferences are highly similar between study sites, networks should not deviate significantly from random network models, and  $H_2'$  values should approach values near zero.

We also examined compositional prey preferences between regions at the community level using the software PRIMER 7 (PRIMER-e; version 7.0.23). To do this, we constructed a contingency matrix that separated different army ant species for each region and recorded their link strengths with prey ant genera. To focus on compositional rather than quantitative differences, we standardized link strengths by setting the maximum prey link value for each army ant per study site to 100% and square-root-transformed the data. We calculated a resemblance matrix using Bray–Curtis similarity and visualized similarities between species in a non-multidimensional scaling plot (NMDS plot). We added vectors showing how individual prey genera contributed to the separation of data in the NMDS plot, with their length indicating the strength of this contribution (as determined by a Pearson correlation; see Anderson et al., 2019).

We employed a permutational analysis of variance (PERMANOVA) to assess overall differences in compositional prey preferences of army ants between the two communities, with “study site” (either Ecuador or Costa Rica) as the fixed factor. To account for possible dispersion effects that would affect the interpretation of a significant PERMANOVA test, we also computed a permutational multivariate analysis of dispersion (e.g., Brückner & Heethoff, 2017). This test showed a nonsignificant result (PERMDISP;  $F = 0.017$ ;  $p = 0.919$ ), indicating that data dispersion did not differ between the two study sites.

## RESULTS

### Army ant community composition

We found 13 army ant species at the Reserva Río Canandé in Ecuador (Table 1). Of these, the following nine species were also detected at the Costa Rican study site: *Ec. burchellii*, *Ec. hamatum* (Figure 1A), *Ec. lucanoides*, *Ec. mexicanum*, *Ec. vagans* (Figure 1B), *Neiv. asper*, *Neiv. gibbatus* (Figure 1C), *Noma. esenbeckii*, and *Noma. hartigii* (Hoenle et al., 2019; Table 1). *Cheliomyrmex andicola* (Figure 1D), the two *Labidus* species as well as *Neiv. curvinotus* were exclusively detected at Canandé, while we encountered *Ec. dulcium* and *Neiv. pilosus* exclusively at La Selva Biological Station in Costa Rica (Hoenle et al., 2019; Table 1).

Among the nine species found at both study sites, prey collection of four species involved a minimum of 15 or more raids/emigrations (Table 1), enabling a robust comparison of prey preferences across regions. However, for the following species, we encountered 10 or fewer raids/emigrations in one of the two study sites, which somewhat limited our ability to conduct a comprehensive cross-community comparison for these species: *Ec. lucanoides*, *Ec. mexicanum*, *Neiv. asper*, and the two *Nomamyrmex* species (Table 1).

### Prey composition

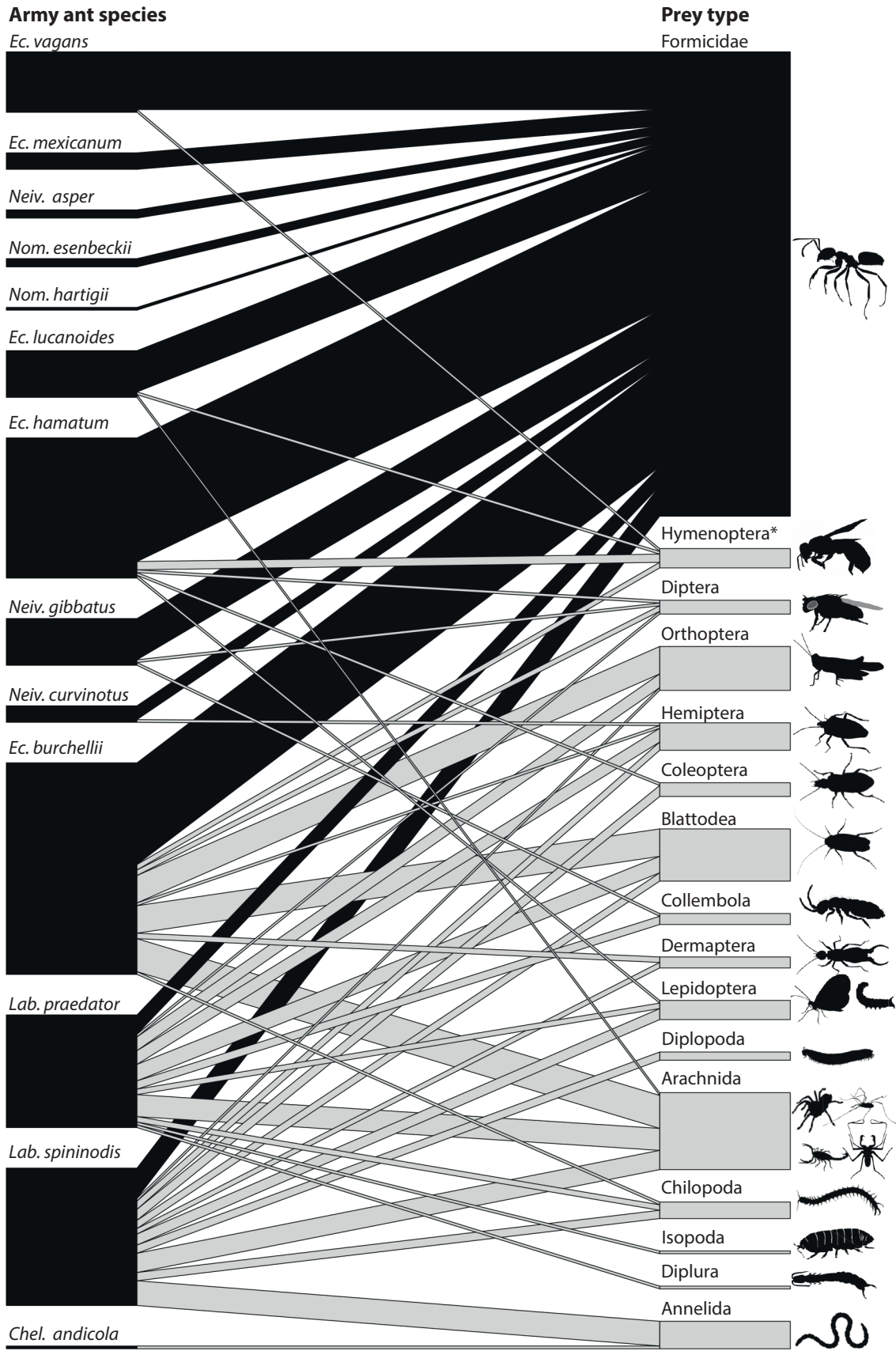
We collected a total of 2156 prey items from 175 army ant raids and 5 army ant emigrations (Table 1). Most prey were other ants ( $N = 1843$ ), to the largest part ant immatures (117 adults, 1141 pupae, 583 larvae, and 2 eggs). The two *Labidus* species as well as *Ec. burchellii* showed the highest proportion of invertebrates other than ants in their prey spectra such as spiders, true bugs, cockroaches, annelids, and orthopterans (Figure 2; Dryad file 1—Specimen information; von Beeren, 2023). *Labidus praedator* showed the highest proportion of

non-ant prey (70 non-ant prey items vs. 27 ant prey items), followed by *Lab. spininodis* (78 non-ant prey items vs. 76 ant prey items), and *Ec. burchellii* (98 non-ant prey items vs. 280 ant prey items; Figures 2 and 3; Dryad file 1—Specimen information; von Beeren, 2023). Other army ant species had either no non-ant prey or only a minimal proportion of it (Figure 2). Noteworthy, we found a single raid of the rarely observed army ant *Chel. andicola* on an earthworm (Lumbricidae; Figure 1D; see also O'Donnell et al., 2005).

### Prey identification

In total, we identified 244 prey species, of which 153 species were other ants (Figure 3; Dryad file 1—Specimen information; von Beeren, 2023). We classified 2118 prey items to the phylum level, 2065 to the class level, 2041 to the order level, 1945 to the family level, 1313 to the genus level, and 664 to the species level (Dryad file 1—Specimen information; von Beeren, 2023). Of those identified to the species level, 639 prey items were identified based on DNA barcode similarity to reference barcodes (Appendices S4 and S5). Additionally, we identified 24 adult ant specimens of the following species solely based on morphology: *Camponotus sericeiventris*, *Crematogaster nigropilosa*, *Ectatomma goninion*, *Ectatomma ruidum* species complex, *Odontomachus bauri*, *Odontomachus chelifer*, *Pachycondyla harpax*, and *Pachycondyla impressa* (Dryad file 1—Specimen information; von Beeren, 2023).

COI barcodes were recovered from 639 prey items, while either PCR or sequencing failed for 580 specimens (GenBank accession numbers are provided in Dryad file 1—Specimen information; von Beeren, 2023). Following sequence trimming, we acquired high-quality reads with variable fragment sizes, ranging from 163 to 666 bp (mean:  $621 \pm 84$  bp, median: 660 bp). Among these barcodes, 39 derived from adult ants, 483 from ant brood, and 117 from non-ant prey (Dryad file 1—Specimen information; von Beeren, 2023). No stop codons, unusual amino acid substitutions, or internal sequence deletions were detected, except for four sequences from *Apterostigma* ants, where a 3-bp deletion was observed. This deletion did not disrupt the reading frame nor introduce an upstream stop codon. Prior studies have reported 3-bp deletions in *Apterostigma* ants, suggesting that the target region was amplified (Hanisch et al., 2017; Hoenle et al., 2019; Sosa-Calvo et al., 2017). BOLD's clustering algorithm grouped 611 prey barcodes into 248 BINs (Dryad file 1—Specimen information; von Beeren, 2023). In 25 cases, we assigned a single species name to distinct BINs, because genetic clusters of what we consider to be likely the same species contained several BINs



**FIGURE 2** Legend on next page.



(e.g., *Camponotus excisus* and *Camp. textor* in Appendix S4; see Dryad file 1—BIN numbers; von Beeren, 2023). Due to sequence lengths of <500 bp, no BINs were assigned to 28 prey barcodes, which we identified via sequence similarities in genetic trees instead (Dryad file 1—Specimen information; von Beeren, 2023; Appendix S4, Appendix S5: Figures S1 and S2).

## Interaction specificity, prey diversity, and degree of prey differentiation

We assessed the army ants' prey specificity at the network and at the species level. The Ecuadorian predation network showed a high degree of specificity ( $H_2' = 0.65$ ; Figure 3) and differed significantly from random network models ( $p < 0.001$ ). Focusing solely on ant prey across the two study sites, the networks showed similarly high levels of specificity (Ecuador:  $H_2' = 0.63$ ; Costa Rica:  $H_2' = 0.66$ ), and both networks differed from random network models ( $p < 0.001$ ). Network-level specificity of ant prey networks decreased with coarser taxonomic classification ( $H_2'$  at the ant prey genus level in Ecuador/Costa Rica: =0.43/0.55;  $H_2'$  at the ant prey subfamily level in Ecuador/Costa Rica:  $H_2' = 0.24/0.39$ ; Appendix S6: Figure S1), yet these networks still differed from random network models ( $p < 0.001$ ).

When considering the entire predation network including non-ant prey, prey specificity varied among Ecuadorian army ants, but it was relatively high in all species (range:  $d' = 0.60$ – $1.00$ ; Table 2). The maximum  $d'$  values of 1.00 for the two *Nomamyrmex* species were obtained based on their unique prey species within the predation network. However, the scarcity of available prey samples for both species (Table 1) hinders the ability to draw any conclusions regarding their true level of specialization. The greatest diversity of prey, as measured by rarefied Shannon diversity, was found among the three army ant species known for conducting swarm raids and having a relatively high portion of non-ant prey in their diet: *Ec. burchellii* ( $e^H_{\text{rare}}$ : mean  $\pm$  SD:  $22 \pm 3$  prey species; Table 3), *Lab. praedator* ( $e^H_{\text{rare}}$ : mean  $\pm$  SD:  $25 \pm 2$  prey species), and *Lab. spininodis* ( $e^H_{\text{rare}}$ : mean  $\pm$  SD:  $25 \pm 2$  prey species; Table 3). The lowest prey diversity was found in *Ec. vagans* ( $e^H_{\text{rare}}$ : mean  $\pm$  SD:  $14 \pm 2$  prey species; Table 3).

Species-level specificities of army ant species were surprisingly similar between the Ecuadorian and the Costa Rican study sites (Table 2), and the  $d'$  values between overlapping army ant species across the two regions were even strongly correlated (Spearman rank test,  $\rho = 0.819$ ,  $S = 21.76$ ,  $p = 0.007$ ,  $N = 9$  species per site). Further, prey diversity ( $e^H_{\text{rare}}$ ) reached similar levels for each army ant across the two study sites (range  $e^H_{\text{rare}} = 10$ – $14$  prey species per 20 interactions; Table 3), but no correlation was detected in species-specific  $e^H_{\text{rare}}$  between sites (Spearman rank test,  $\rho = -0.471$ ,  $S = 29.43$ ,  $p = 0.423$ ,  $N = 5$  species per site).

The overlap in prey spectra among Ecuadorian army ant species was limited as indicated by a high network modularity (entire network:  $Q = 0.70$ ; only ants as prey:  $Q = 0.66$ ; test against null models for both networks:  $p < 0.001$ ). Each army ant species formed an idiosyncratic module within the Ecuadorian predator–prey network (Appendix S6: Figure S2). Such pronounced dietary niche differentiation had previously also been observed in the predation network of Costa Rican army ants (only ants as prey:  $Q = 0.60$ ; Hoelen et al., 2019).

## Spatiotemporal raiding preferences

The Ecuadorian army ant prey strata network differed significantly from random networks ( $p < 0.001$ ; Figure 4A), indicating that army ant species exhibited different hunting ground preferences. Preferences for ground-nesting ant prey were detected in all species of the genera *Nomamyrmex*, *Neivamyrmex*, and *Labidus* as well as in *Ec. mexicanum* and *Ec. vagans*, while *Ec. hamatum* and *Ec. burchellii* preferred to raid arboreal-nesting ant prey (Figure 4A). *Eciton lucanoides* showed no clear raiding preference for any stratum. When visually examining network graphs, it becomes evident that the stratum preferences of species remained relatively stable across both studied communities. This is also supported by a significant correlation between spatial raiding preferences of army ants at the two study sites (Spearman rank test,  $\rho = 0.803$ ,  $S = 23.59$ ,  $p = 0.009$ ,  $N = 9$  species per site; Figure 4A,B).

Ecuadorian army ant species also had distinct temporal raiding activities, as indicated by the raiding activity network differing significantly from random network models ( $p < 0.001$ ; Figure 4C). *Eciton hamatum*, *Ec.*

**FIGURE 2** Ecuadorian army ant predation network at a coarse taxonomic level. Network visualization of predator–prey interactions between 13 army ant species and various types of prey ( $N = 2096$  prey specimens) at a coarse taxonomic level. Army ant species are represented by black rectangles, ant prey by dark gray ones, and non-ant prey by light gray ones. Connecting lines between them (network links) indicate observed instances of predation, with the thickness of lines proportional to the frequency of prey taxa being encountered during distinct army ant raids or emigrations. \*Note that ants are excluded from the category Hymenoptera to better visualize their prevalence as army ant prey. *Chel.*, *Cheliomyrmex*; *Ec.*, *Eciton*; *Lab.*, *Labidus*; *Neiv.*, *Neivamyrmex*; *Nom.*, *Nomamyrmex*.



**TABLE 2** Species-level prey specificities of army ants.

Species	$d'_{\text{entire network}}$	$d'_{\text{ants EC}}$	$d'_{\text{ants CR}}$
<i>Eciton burchellii</i>	0.79	0.78	0.76
<i>Eciton dulcium</i>	-	-	0.60
<i>Eciton hamatum</i>	0.84	0.83	0.78
<i>Eciton lucanoides</i>	0.64	0.62	0.67
<i>Eciton mexicanum</i>	0.60	0.60	0.61
<i>Eciton vagans</i>	0.67	0.66	0.58
<i>Labidus praedator</i>	0.82	0.66	-
<i>Labidus spininodis</i>	0.84	0.70	-
<i>Neivamyrmex asper</i>	0.60	0.60	0.57
<i>Neivamyrmex curvinothus</i>	0.85	0.84	-
<i>Neivamyrmex gibbatus</i>	0.67	0.66	0.62
<i>Neivamyrmex pilosus</i>	-	-	0.97
<i>Nomamyrmex esenbeckii</i>	1.00	1.00	0.70
<i>Nomamyrmex hartigii</i>	1.00	1.00	0.80

Note: Calculations are either based on the entire army ant species – prey species predation network ( $d'_{\text{entire network}}$ ) or on the network that exclusively summarizes predation of army ant species on other ant species ( $d'_{\text{ants}}$ ), making it comparable with the Costa Rican study where non-ant prey remained unidentified (Hoenle et al., 2019). Specificity is given as standardized Kullback–Leibler distance ( $d'$ ), which can range from values of zero (lowest level of prey specificity) to one (highest level of prey specificity). Note that *Cheliomyrmex andicola* is not included in the table as its single prey item could not be identified to species level. A short dash indicates a species was not detected at this site.

Abbreviations: CR, Costa Rica; EC, Ecuador.

*lucanoides*, *Ec. burchellii*, and *Lab. praedator* showed preferences for diurnal raiding activity, *Ec. vagans* and *Lab. spininodis* showed no clear activity preferences, and all remaining species showed a preference for nocturnal raiding activity (Figure 4C). The activity patterns of those army ants occurring at both study sites overall resembled each other (Figure 4C,D), which is also supported by a significant correlation between site-specific temporal raiding preferences of army ant species (Spearman rank test,  $\rho = 0.75$ ,  $S = 30.00$ ,  $p = 0.020$ ,  $N = 9$  species per site). Pronounced differences between study sites were detected in two army ant species: *Ec. vagans*, which showed a clear preference for nocturnal raids in Costa Rica but not so in Ecuador (Figure 4C,D); *Ec. lucanoides*, which exhibited a clear preference for

diurnal raids in Ecuador but lacked a clear preference in Costa Rica (Figure 4C,D).

### Comparison of army ant prey preferences between study sites

When considering prey at the ant genus level, prey preferences of army ants did not differ between the two study sites (PERMANOVA; pseudo- $F = 0.881$ ;  $p = 0.574$ ; unique permutations = 998; Figure 5). When conducting pairwise comparisons of the same army ant species between the two neotropical study sites, it became further evident that their dietary preferences regarding typical ant genera showed substantial similarity (Figure 6A–I). This similarity is reflected in the relatively low network specificity values observed in most of the comparisons ( $H_2'$ : mean = 0.30; median = 0.25; range = 0.10–0.65; Figure 6A–H). Nevertheless, six out of eight species-pair networks deviated from purely random network models (range of  $p$ -values = 0.483–0.001; Figure 6A–H). If the prey preferences had been identical between study sites, we would have anticipated obtaining non-significant results. In other words, while there was considerable overlap, their prey spectra were not identical. This observation is further underscored when examining the combined compositional prey preferences of the two army ant communities. Specifically, the species *Ec. burchellii*, *Ec. hamatum*, *Ec. lucanoides*, *Ec. mexicanum*, *Ec. vagans*, and *Neiv. gibbatus* exhibited similar preferences for particular prey ant genera across the two study sites. This similarity is apparent through the close spatial proximity of species pairs from both study sites on the NMDS plot (Figure 5). Genera which predominantly contributed to dietary niche differentiation among army ant species at different regions were *Acromyrmex*, *Anochetus*, *Apterostigma*, *Camponotus*, *Crematogaster*, *Cyphomyrmex*, *Neoponera*, *Nylanderia*, *Odontomachus*, *Pachycondyla*, and *Solenopsis* (Figure 5).

When considering prey at the species level, we found 27 ant species as prey at both study sites, while 102 ant species were exclusively found in Costa Rica and 126 ant species exclusively in Ecuador. Those species occurring at both study sites were often raided by the same army ant species (Appendix S6: Figure S3). A network subset containing all army ant species and all prey species found at both sites

**FIGURE 3** Ecuadorian army ant predation network at the species level. Network visualization of predator–prey interactions between 12 army ant species and 244 prey species. Each species is represented by a rectangle, which are dark gray for prey ant species, light gray for non-ant prey, and colored according to genus affiliation in army ant species (*Eciton*, blue/purple shading; *Labidus*, brown shading; *Neivamyrmex*, green shading; *Nomamyrmex*, red shading). Observed predation is indicated by connecting lines (network links), with line width being proportional to the number of times prey species were detected in distinct army ant raids/emigrations. Note that *Cheliomyrmex andicola* is not included in the network as we were unable to identify its only prey, an earthworm, to species level. *Ec.*, *Eciton*; *Lab.*, *Labidus*; *Neiv.*, *Neivamyrmex*; *Nom.*, *Nomamyrmex*.



**TABLE 3** Army ant prey diversity.

Army ant species	$S_{\text{obs}}$ (EC/CR)	$S_{\text{rare}}$ (EC/CR)	$e^H_{\text{obs}}$ (EC/CR)	$e^H_{\text{rare}}$ (EC/CR)
<i>Ecton burchellii</i>	77 (48/29)	24 ± 3 (13/12)	60 (37/17)	22 ± 3 (12/10)
<i>Ecton dulcium</i>	- (-/7)	- (-/6)	- (-/4)	- (-/4)
<i>Ecton hamatum</i>	38 (32/17)	21 ± 3 (12/11)	30 (25/13)	19 ± 3 (11/10)
<i>Ecton lucanoides</i>	24 (22/20)	20 ± 1 (12/15)	21 (19/18)	18 ± 1 (11/14)
<i>Ecton mexicanum</i>	11 (11/33)	-	10 (10/20)	-
<i>Ecton vagans</i>	25 (25/39)	17 ± 2 (11/14)	19 (19/25)	14 ± 2 (10/12)
<i>Labidus praedator</i>	37 (13/-)	26 ± 2 (13/-)	35 (12/-)	25 ± 2 (12/-)
<i>Labidus spininodis</i>	48 (18/-)	26 ± 2 (13/-)	44 (17/-)	25 ± 2 (12/-)
<i>Neivamyrmex asper</i>	4 (4/3)	-	4 (4/3)	-
<i>Neivamyrmex curvinothus</i>	6 (5/-)	-	5 (4/-)	-
<i>Neivamyrmex gibbatus</i>	20 (19/27)	20 ± 0 (12/14)	17 (16/21)	17 ± 0 (11/12)
<i>Neivamyrmex pilosus</i>	- (-/11)	- (-/9)	- (-/7)	- (-/6)
<i>Nomamyrmex esenbeckii</i>	2 (2/7)	-	2 (2/6)	-
<i>Nomamyrmex hartigii</i>	1 (1/2)	-	1 (1/2)	-

Note: Species-level prey diversity is given as the total number of prey species detected per army ant species ( $S_{\text{obs}}$ ) and as effective Shannon diversity ( $e^H_{\text{obs}}$ ). Rarefaction was only applied to those army ant species with more than 10 collection events (see Table 1 and Hoenle et al., 2019). We computed the rarefied prey diversity of species counts ( $S_{\text{rare}}$ ) and the rarefied exponential Shannon diversity ( $e^H_{\text{rare}}$ ) for the minimum number of prey interactions, which was found in *Neiv. gibbatus* with 27 interactions. Calculations are based on the entire army ant prey food network, including non-ant prey. To compare prey diversity values with the Costa Rican study (Hoenle et al., 2019), we additionally computed the rarefied prey diversity for 20 interactions per army ant species for the ant prey network (excluding other taxa). Numbers in parentheses present these latter calculations for the Ecuadorian and the Costa Rican study sites (Ecuador/Costa Rica). Note that *Cheliomyrmex andicola* is not included in the table as its single prey item could not be identified to species level. A short dash indicates that a species was not detected at this site. Abbreviations: CR, Costa Rica; EC, Ecuador; obs, observed; rare, rarefied.

showed a moderate level of modularity ( $Q = 0.49$ ), which was statistically significant when compared with null models ( $p < 0.001$ ). Moreover, in most cases, the same army ant species but from different sites tended to cluster together within the same module (Appendix S6: Figure S3).

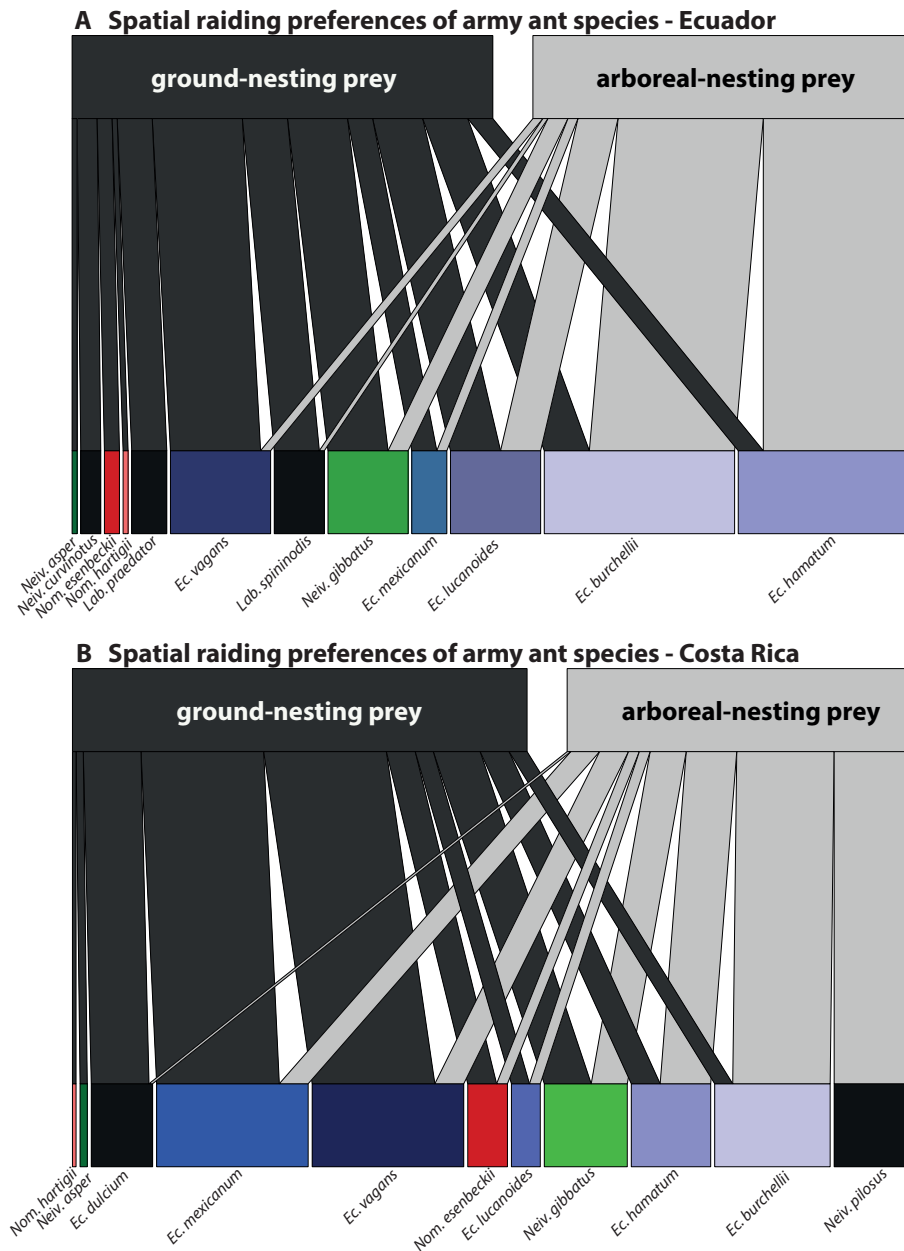
## DISCUSSION

We uncovered a comprehensive species-level predation network of an Ecuadorian army ant community and compared it with a previously studied Costa Rican network (Hoenle et al., 2019). Our central finding is a remarkably consistent nature of army ant predation networks across two distant neotropical rainforests separated by more than 1000 km. Army ants in both communities are characterized by high prey specificity, consistent prey preferences, and differentiation in additional niche dimensions, that is, temporal activity patterns and stratum raiding preferences. Such pronounced multidimensional niche differentiation patterns most likely contribute to a coexistence of up to 20 sympatric species in many tropical rainforests (Kaspari et al., 2011; Rettenmeyer et al., 1983).

The prey preferences of most army ants for specific ant genera, and in many cases, even for particular ant species, remained remarkably consistent. For example,

in both communities *Ec. burchellii* showed a strong preference for *Camponotus* carpenter ants and additionally preyed upon ants of the genera *Nylanderia*, *Pheidole*, and *Neoponera*. In accordance with this, *Camponotus* ants were also the most prevalent ant prey of *Ec. burchellii* at Barro Colorado Island in Panama (Powell & Franks, 2006). When comparing the prey spectra across these three neotropical study sites, consistent prey preferences further existed for *Ec. vagans* preying on *Pheidole* and *Odontomachus*, *Ec. hamatum* on *Acromyrmex*, and *Ec. mexicanum* on *Ectatomma*. Certainly, nuanced predation differences also existed between studies, which can partly be attributed to variations in local ant compositions among study sites. For instance, the common leaf-litter-inhabiting ant *Aphaenogaster araneoides* (McGlynn et al., 2003, 2004) was frequently found as prey in Costa Rica but was absent in Ecuador as it has a strictly Central American distribution (Longino & Cover, 2004; [www.antmaps.org](http://www.antmaps.org)). Taking into account such differences in local ant communities, however, the army ants' preferences for specific ant genera matched surprisingly well among studies (Gotwald, 1995; Hoenle et al., 2019; Powell & Franks, 2006; Rettenmeyer et al., 1983; this study), emphasizing relatively hard-wired dietary preferences of army ant species.

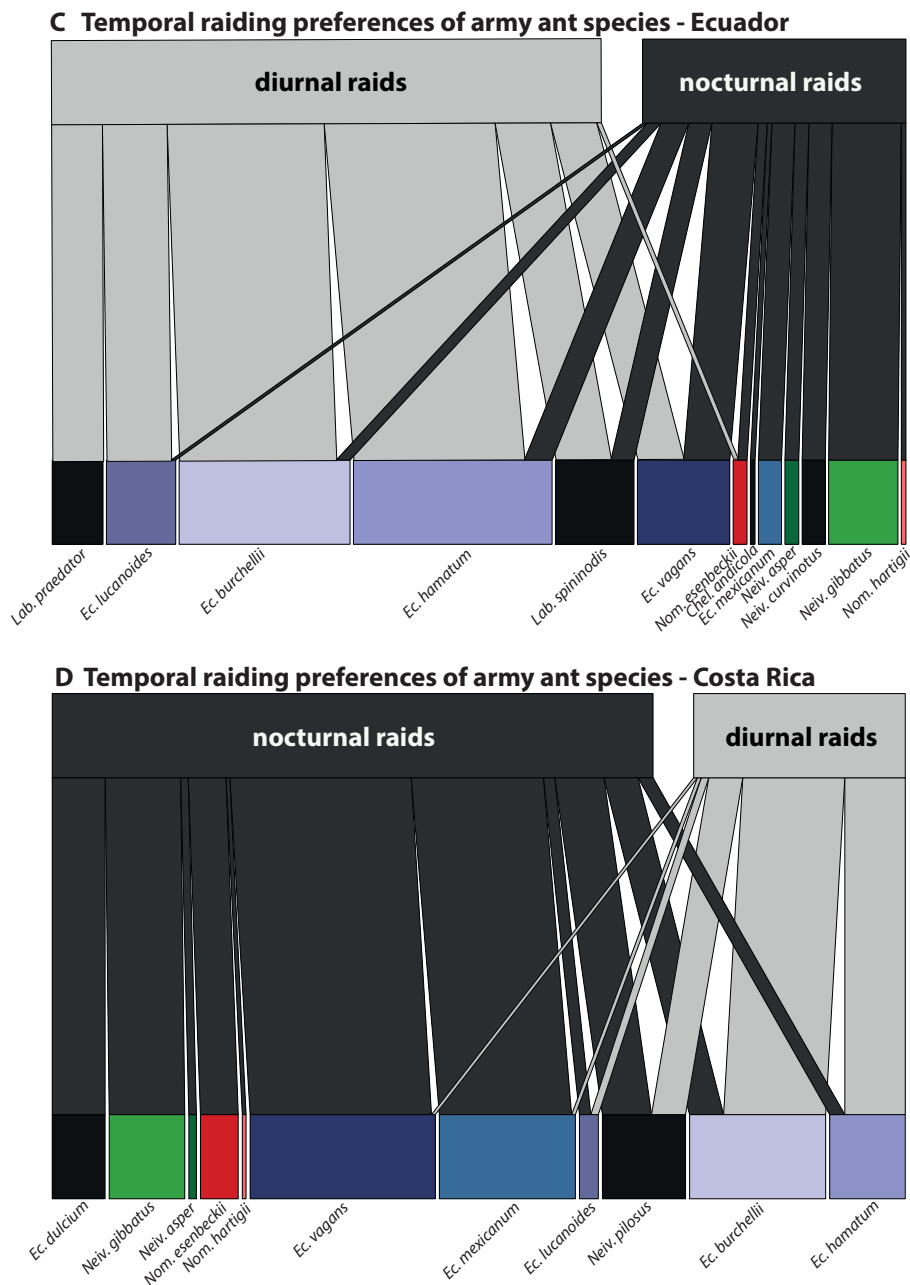
Contrastingly, Rettenmeyer et al. (1983) described pronounced predation differences in the army ant



**FIGURE 4** Spatiotemporal raiding activity of army ants at both study sites. Network graphs depicting raiding preferences in stratum and temporal raiding preferences of the Ecuadorian (A, C) and the Costa Rican (B, D; data taken from Hoenle et al., 2019) army ant communities. Army ant species found in both communities are depicted as colored rectangles (using the color code from Figure 3), while army ant species exclusive to one community are represented by black rectangles. Width of links represents (A, B) the number of times ground-nesting or arboreal-nesting prey species were detected in distinct army ant raids/emigrations, (C, D) or the number of diurnal versus nocturnal raiding events. *Chel.*, *Cheliomyrmex*; *Ec.*, *Eciton*; *Lab.*, *Labidus*; *Neiv.*, *Neivamyrmex*; *Nom.*, *Nomamyrmex*.

*Ec. hamatum* between a Panamanian study site and one on the edge of the Amazon basin in Ecuador, east of the Andes. Recent phylogenomic data, however, indicated that two genetically divergent clades, possibly distinct species, fall under the name *Ec. hamatum* (Winston et al., 2016). The Amazonian *Ec. hamatum* might thus represent a distinct species to that studied in Costa Rica, Panama, and the Reserva Río Canandé

in Ecuador (see discussion in Appendix S1). Such uncertainties in species classifications certainly hamper across-community comparisons. In the present comparative work, we found high DNA barcode similarities in many army ant species from the Reserva Río Canandé in Ecuador to the species collected in Central America, overall supporting our biogeographic expectations that we, in most cases, compared the same army ant (sub)

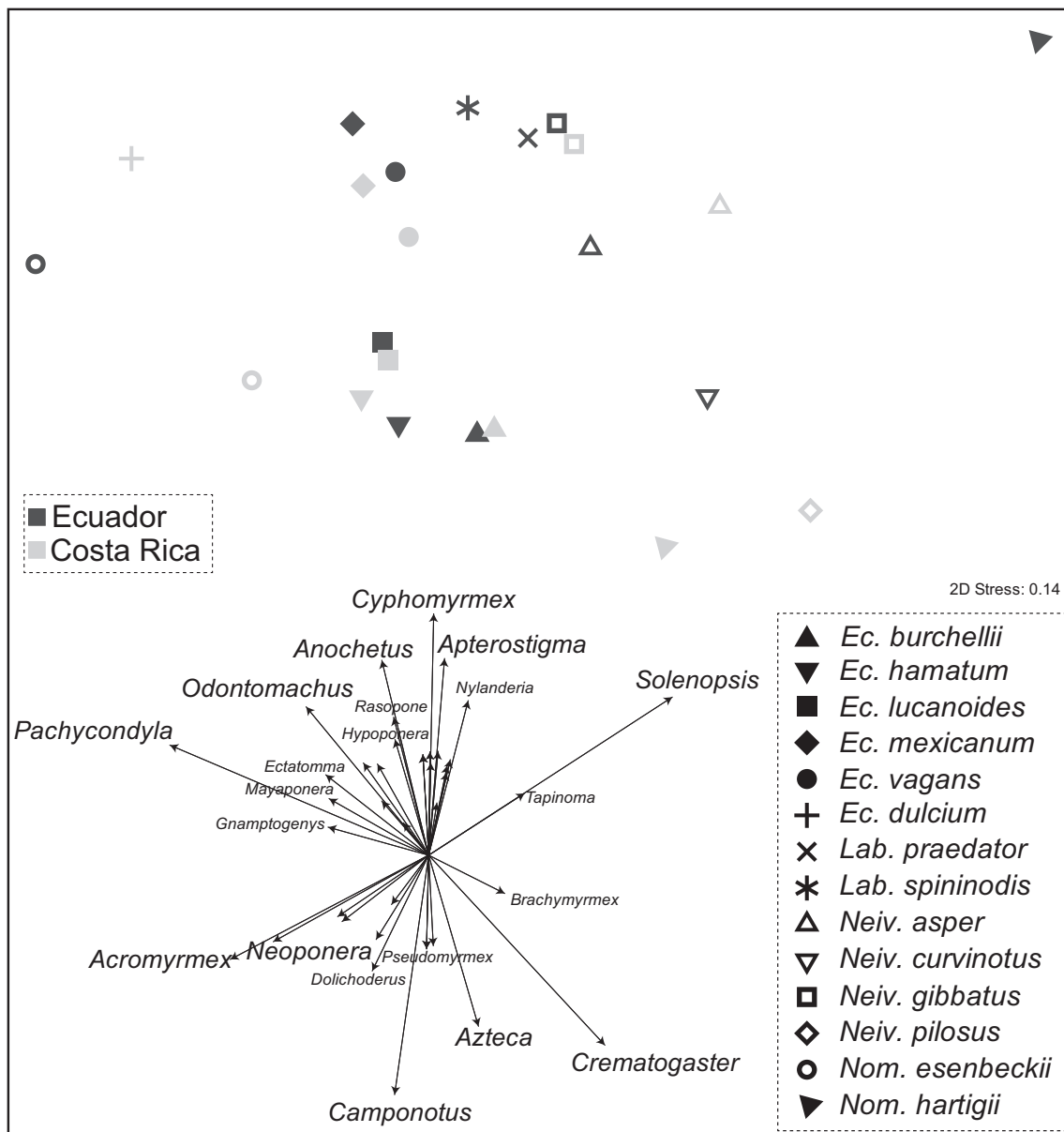


**FIGURE 4** (Continued)

species (or genetic lineages; Winston et al., 2016) across distant geographic regions (Appendix S1). As expected, we discovered similar dietary preferences across the two examined predation networks for most army ants, including *Ec. hamatum*, with one noteworthy exception in the latter species: *Azteca* ants served as a significant portion of *Ec. hamatum*'s prey in Ecuador, with 23 recorded raids/emigrations. In contrast, this prey genus was not at all observed as part of *Ec. hamatum*'s diet in Costa Rica (Hoenle et al., 2019). Instead, the army ant *Neiv. pilosus*, a common species at La Selva Biological Station, showed a preference for *Azteca* ants (Hoenle et al., 2019), an army

ant species that we did not detect at the Ecuadorian study site. *Crematogaster* is another prey genus preferred by *Neiv. pilosus* in Costa Rica (Hoenle et al., 2019), while this ant genus was primarily raided by *Neiv. curvinotus* in Ecuador. It is tempting to speculate that, although the predation preferences exhibit a relatively high level of rigidity, they seem to possess a certain degree of flexibility. In other words, the absence of an army ant species in a given community could potentially be compensated for by other army ant species. Such functional compensation effects have been observed in various ecological interactions (Mittelbach, 2012), such as scavenging and predation





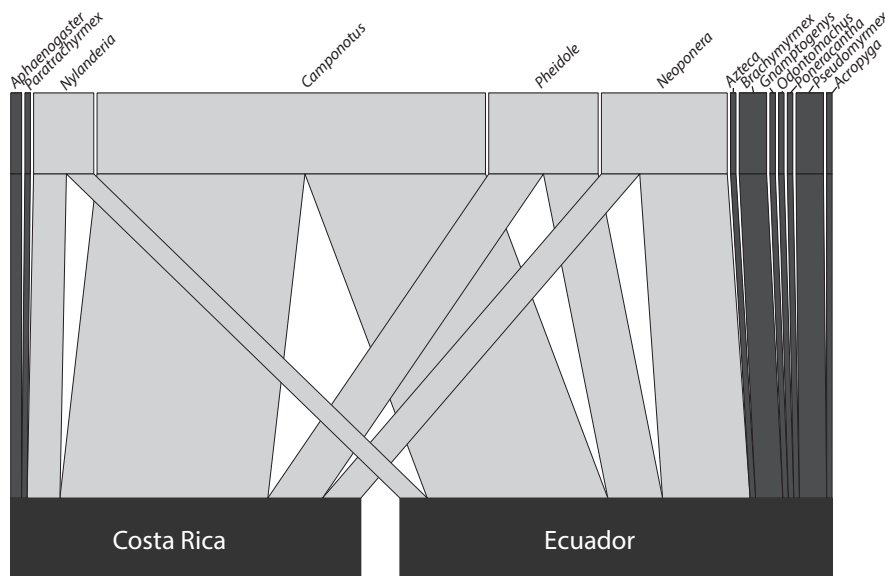
**FIGURE 5** Army ant predation across two neotropical regions. Non-multidimensional scaling (NMDS) plot visualizes compositional differences in ant predation at the generic level between army ant species of the two study sites (Reserva Río Canandé, Ecuador; La Selva Biological Station, Costa Rica). Vectors show how individual prey genera contribute to the separation of data, with their length indicating the strength of this contribution (as determined by Pearson correlations). The origin of arrows is not centered in the plot and only genera names with relatively long vectors are labeled for better visibility. “Stress” is a quality measure of the NMDS. The resemblance matrix is based on Bray–Curtis similarities.

in vertebrate communities (Huijbers et al., 2016; Olds et al., 2018; Touchton & Smith, 2011). These community-level predation differences further suggest that army ant specialization may not solely result from inherent traits among army ant species (e.g., body size constraints; Hashimoto & Yamane, 2014; Hoenle et al., 2019; Rettenmeyer et al., 1983) but could potentially be influenced by competitive effects in army ant communities. However, setting up manipulative field experiments, such as exclusion experiments, is challenging in army

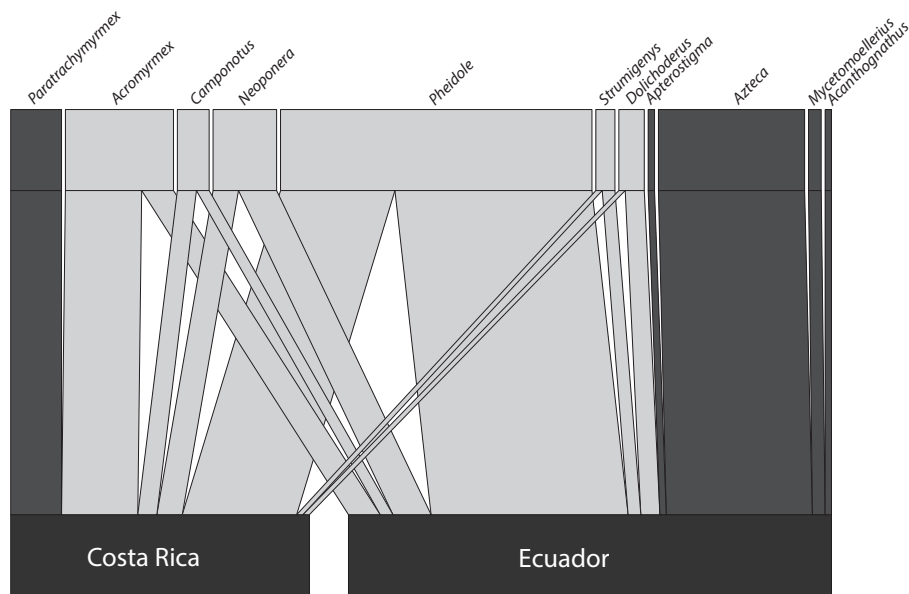
ants, and with the scarcity of comparative datasets on the dietary habits of army ants across geographic regions, this subject remains purely speculative.

Despite the similar nature of the two distant army ant predation networks, one prominent difference was the vastly different proportion of non-ant invertebrate prey (Ecuador: 12% non-ant prey; Costa Rica: 2% non-ant prey; Hoenle et al., 2019). This difference can be primarily linked to the integration of two swarm raiding *Labidus* species in Ecuador, whereas no *Labidus* raids

**A** *Ec. burchellii* ( $H_2' = 0.10$ ;  $p = 0.014$ ;  $N = 143$ )



**B** *Ec. hamatum* ( $H_2' = 0.29$ ;  $p = 0.001$ ;  $N = 123$ )

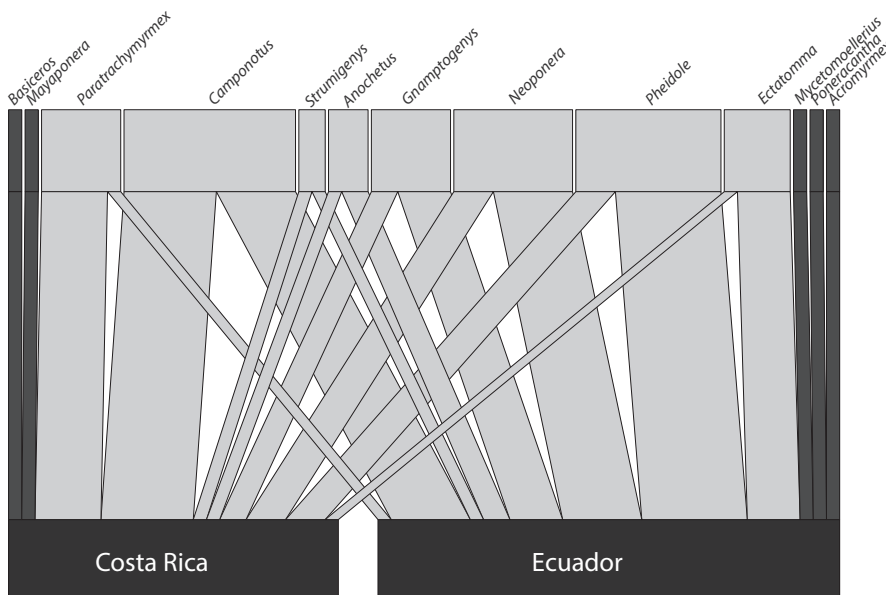


**FIGURE 6** Pairwise comparisons of army ant prey preferences between study sites. Bipartite network graphs illustrating prey preferences of army species (A–I) between the two studied neotropical predation networks (Reserva Río Canandé, Ecuador; La Selva Biological Station, Costa Rica). Considered is only ant prey, which is resolved to the genus level. Prey genera present in both communities are depicted as light gray rectangles, while prey genera that represent exclusive prey of a given army ant species in one of the communities are represented by dark gray rectangles. Widths of links represent the number of times a given prey genus was found in distinct army ant raids/emigrations. Network-level specificity values ( $H_2'$ ),  $p$ -values of tests against null models, and the sum of link strengths (the number of times prey genera were present in distinct army ant raids/emigrations) is given in parentheses. With three raids being detected in total, the sample size in *Noma. hartigii* (I) was too low to perform a meaningful statistical test. *Ec.*, *Eciton*; Ecu., Ecuador; *Lab.*, *Labidus*; NA, not available; *Neiv.*, *Neivamyrmex*; *Nom.*, *Nomamyrmex*.

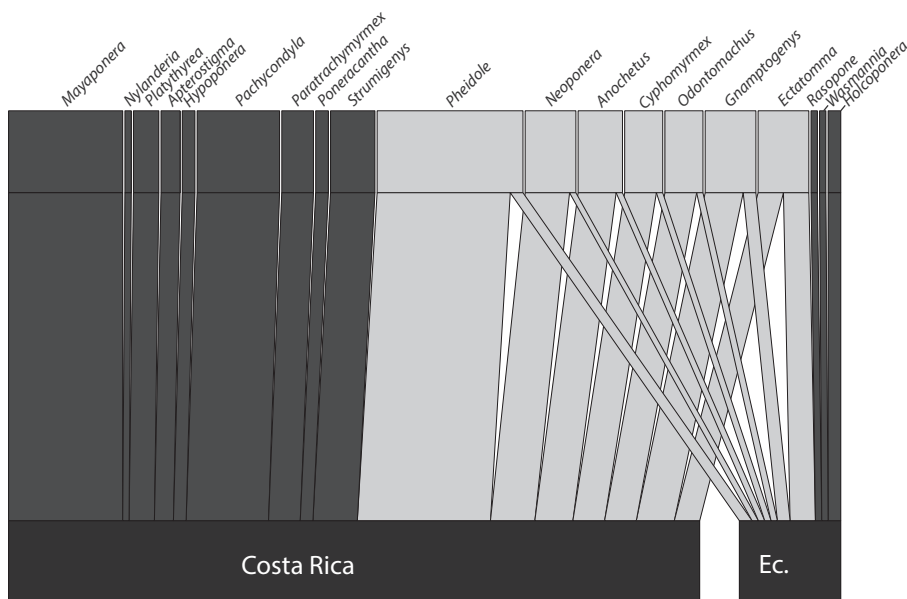
were found in Costa Rica (Hoenle et al., 2019), despite the known presence of two species at La Selva Biological Station (Longino, 2010; Longino et al., 2002). These army ants are known for their diverse prey spectrum, which

even includes occasional use of plant parts such as fruits and seeds (Kronauer, 2020; Powell, 2009). We detected 28 *Labidus* raids in the present study, confirming a diet with a high proportion of non-ant prey. *Labidus* swarm

**C** *Ec. lucanoides* ( $H_2' = 0.11$ ;  $p = 0.483$ ;  $N = 60$ )



**D** *Ec. mexicanum* ( $H_2' = 0.37$ ;  $p = 0.003$ ;  $N = 125$ )

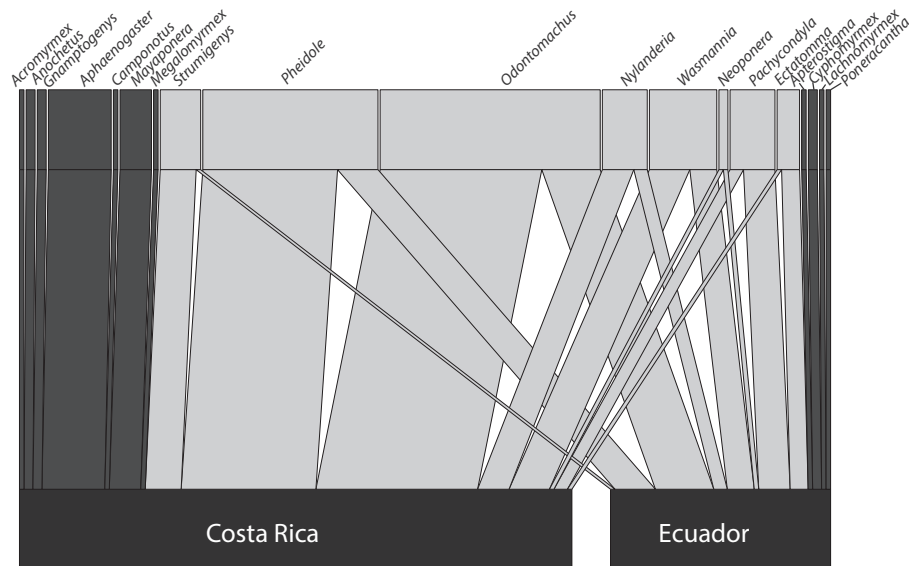


**FIGURE 6** (Continued)

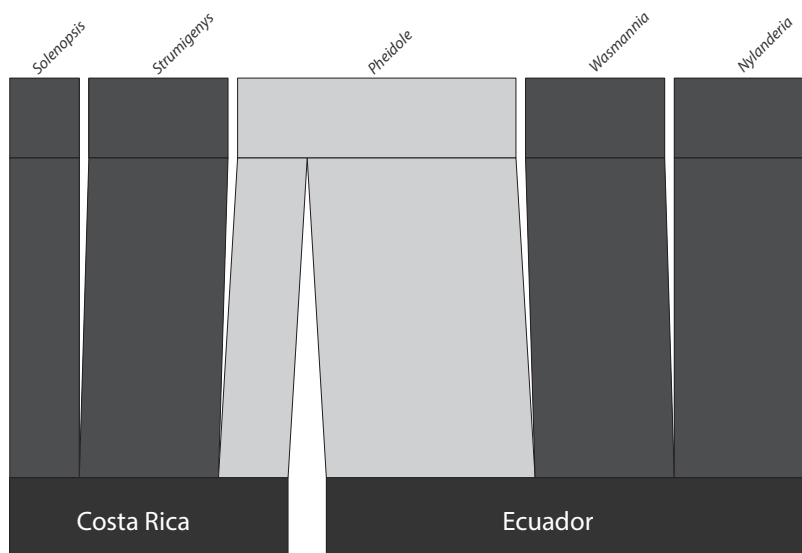
raiders likely play an important ecological role as arthropod predators in tropical rainforests as shown by a significant reduction in the abundance of leaf-litter arthropods after *Lab. praedator* raids passing through (Kaspari et al., 2011). In contrast to the occasional aboveground swarms detected in *Lab. praedator* and *Lab. spininodis*, *Lab. coecus* maintains a predominantly underground lifestyle making it infeasible to collect sample prey from their raids, and thus, it is not included in either of the predation networks (even though it is widely distributed in the neotropical realm including both study sites). This

raises an important point: the presented army ant predation networks do not accurately measure army ant density or activity, nor do they encompass all army ant species at a given site, as they solely focus on aboveground army ant activity. Information on abundance, activity, and dietary preferences of strictly subterranean army ants is limited, and verifying their presence best requires underground baiting (Kronauer, 2020; O'Donnell et al., 2007). For instance, only two recorded instances of prey observations have existed for the species *Chel. andicola*: one instance of predation on a snake carcass and

**E** *Ec. vagans* ( $H_2' = 0.21$ ;  $p = 0.001$ ;  $N = 172$ )



**F** *Neiv. asper* ( $H_2' = 0.65$ ;  $p = 0.049$ ;  $N = 11$ )



**FIGURE 6** (Continued)

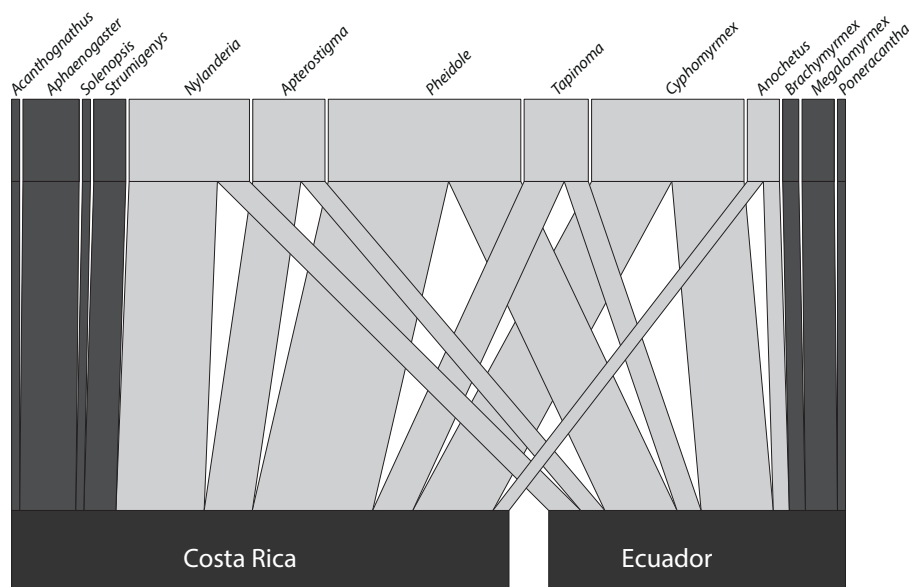
another on an earthworm (O'Donnell et al., 2005), with the latter being the only prey item identified for this species in the present work.

Our study included three species of swarm raiding army ants, which were *Lab. predator*, *Lab. spininodis*, and *Ec. burchellii*. Swarm raiding army ants usually overwhelm a diverse spectrum of leaf-litter invertebrates (Gotwald, 1995; Kronauer, 2020; Rettenmeyer et al., 1983; Vieira & Höfer, 1994), and as expected, their prey encompassed a wide taxonomic range, including ants, wasps, flies, grasshoppers, katydids, cockroaches, true bugs, spiders, and even annelids. Noteworthy, we discovered pronounced regional differences in the ratio of ant prey to non-ant prey in the

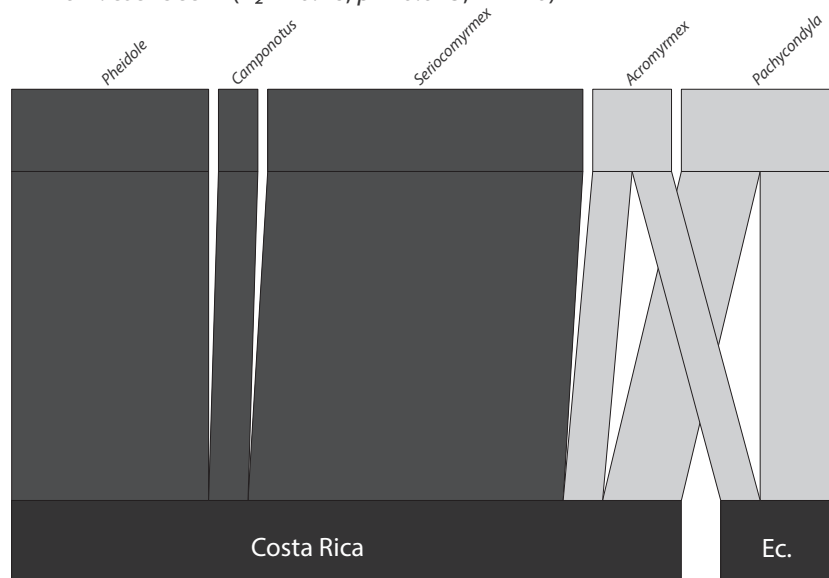
diet of *Ec. burchellii* (Ecuador: 66% ant prey; Costa Rica: 93% ant prey). Further studies reported 50% ant prey in Panama (Franks, 1982) and 24% ant prey in Brazil (Vieira & Höfer, 1994). Determining the origins of these differences remains challenging, as they may arise from variations in sampling designs, regional prey abundance, or local army ant prey preferences. However, such problems in community comparisons are mostly limited to army ants raiding in swarms and should not distract from the overall consistent nature of army ant predation across geographic locations. For instance, in the swarm raider *Eciton burchellii*, various studies have concurred that ants, especially *Camponotus* brood,



**G** *Neiv. gibbatus* ( $H_2' = 0.19$ ;  $p = 0.008$ ;  $N = 99$ )



**H** *Nom. esenbeckii* ( $H_2' = 0.46$ ;  $p = 0.075$ ;  $N = 20$ )



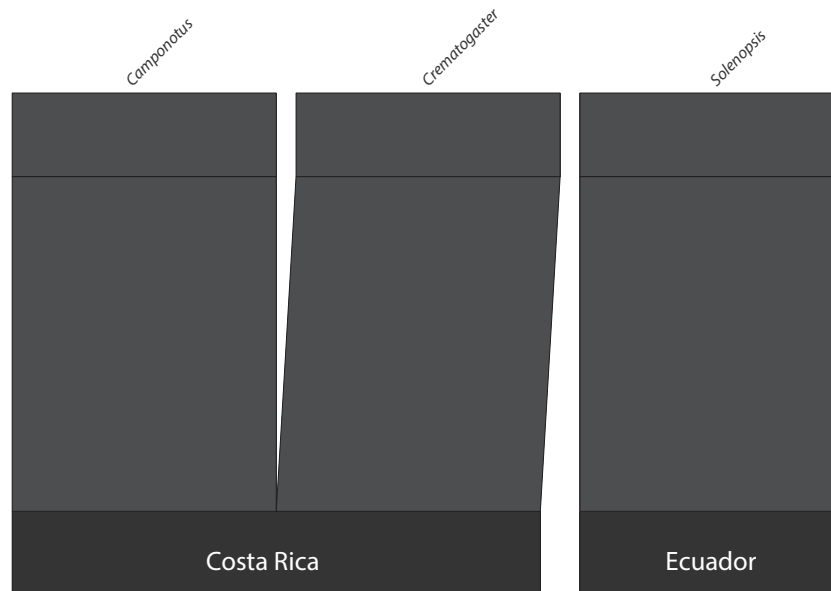
**FIGURE 6** (Continued)

serve as a primary food source, often accompanied by various other arthropods (Hoenle et al., 2019; Powell & Franks, 2006; this study).

Lastly, Ecuadorian army ants exhibited niche differentiation not just in their dietary preferences, but also in their spatiotemporal raiding patterns, likely to reduce interspecific competition (Hashimoto & Yamane, 2014; Hoenle et al., 2019; O'Donnell et al., 2009, 2020; Powell & Baker, 2008; Rettenmeyer et al., 1983). For example, *Ec. burchellii* and *Ec. hamatum* consistently conducted diurnal raids in arboreal zones, targeting their specific prey genera. However, a notable deviation was found in

the temporal raiding behaviors of *Ec. vagans*. In Costa Rica and Venezuela, the raids of *Ec. vagans* were primarily confined to the nighttime (Hoenle et al., 2019; O'Donnell et al., 2020). Contrarily, our observations in Ecuador did not reveal any such diel specialization. Despite a daytime collection bias in the present study, it is evident that this bias alone cannot fully explain the observed deviation, especially considering that such pronounced site deviations were only detected in *Ec. vagans* and *Ec. lucanoides*. This finding, akin to the nuanced differences in prey preferences, accentuates a certain level of behavioral flexibility, as similarly demonstrated in

I *Nom. hartigii* ( $H_2' = NA; N = 3$ )



**FIGURE 6** (Continued)

prior studies comparing army ant activity across different regions (O'Donnell et al., 2009, 2020). Nevertheless, spatial and temporal predation preferences remained remarkably consistent overall across various geographic regions, thereby reinforcing the notion of relatively stable army ant raiding behaviors.

## CONCLUSION

Comparing two well-resolved predation networks, we showed that most neotropical army ants are specialized predators with consistent dietary and spatiotemporal raiding preferences across distant geographic regions. However, our understanding of army ant predation preferences derives from a handful of examined research sites, and we were here only able to compare two geographically distant communities in more detail, thus restricting our capacity to formulate far-reaching conclusions. Furthermore, many aspects of army ant predation remain unstudied, such as whether the local abundance of different ant species correlates with the capture rates by army ant species. We hope that our research will inspire future in-depth community-level investigations into the predatory behavior of these keystone species across larger geographical regions.

## ACKNOWLEDGMENTS

We thank the Fundación Jocotoco (particularly Martin Schaefer) and Fundación Tesoro Escondido (particularly Citlalli Morelos-Juarez) for logistic support and

permission to do research on their reserves. We thank Maria Jose Endarra for her support with permits. Further, we thank Citlalli Morelos-Juárez, Adrian Brückner, and Karsten Mody for their kind support during fieldwork. We further thank Martin Schaefer, María-José Endara, Juan Guevara, Constance Tremlett, Sebastián Escobar, Julieta Muñoz, and Karin Römer for project coordination and administration. Thanks also goes to Leonardo Tozetto for help with the morphological identification of *Neivamyrmex* species and Hendrik Thomas for help in curating the army ant collection. Lastly, Christoph von Beeren is going to leave academia and would like to take this opportunity to thank all colleagues, collaborators, and reviewers, particularly his four main hosts, Rüdiger Wehner, Volker Witte, Daniel Kronauer, and Nico Blüthgen, for their time and efforts in improving his scientific work. Philipp O. Hoenle was supported by a scholarship from the German National Academic Foundation, and Christoph von Beeren by the German Research Foundation (DFG: BE5177/4-1 and BE5177/4-2). This work was additionally supported by the DFG-funded Research Unit REASSEMBLY (FOR5207; CM: BL960/12-1). Open Access funding enabled and organized by Projekt DEAL.

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

Sequences are deposited in GenBank and the BOLD Systems database (Army ant records: [dx.doi.org/10.5883/](https://dx.doi.org/10.5883/)

DS-NEOARMY; prey records: [dx.doi.org/10.5883/DS-PREYARMY](https://doi.org/10.5883/DS-PREYARMY)). GenBank accession numbers are listed in the supplemental file deposited at Dryad: <https://doi.org/10.5061/dryad.zcrjdfnkc> (von Beeren, 2023). Voucher specimens and DNA extracts are deposited at the TU Darmstadt Insect Collection. Specimen images have been uploaded to BOLD Systems.

## ORCID

Philipp O. Hoenle  <https://orcid.org/0000-0001-8160-8859>

David A. Donoso  <https://orcid.org/0000-0002-3408-1457>

Nico Blüthgen  <https://orcid.org/0000-0001-6349-4528>

Christoph von Beeren  <https://orcid.org/0000-0002-0072-5795>

## REFERENCES

- Anderson, M., R. N. Gorley, and K. P. Clarke. 2019. *Permanova+ for Primer: Guide to Software and Statistical Methods*. Auckland: PRIMER-e.
- Blüthgen, N. 2010. "Why Network Analysis Is Often Disconnected from Community Ecology: A Critique and an Ecologist's Guide." *Basic and Applied Ecology* 11: 185–195. <https://doi.org/10.1016/j.baae.2010.01.001>.
- Blüthgen, N., F. Menzel, and N. Blüthgen. 2006. "Measuring Specialization in Species Interaction Networks." *BMC Ecology* 6: 9. <https://doi.org/10.1186/1472-6785-6-9>.
- Booher, D. B., and P. O. Hoenle. 2021. "A New Species Group of *Strumigenys* (Hymenoptera, Formicidae) from Ecuador, with a Description of Its Mandible Morphology." *ZooKeys* 1036: 1–19. <https://doi.org/10.3897/zookeys.1036.62034>.
- Borowiec, M. L. 2016. "Generic Revision of the Ant Subfamily Dorylinae (Hymenoptera, Formicidae)." *Zookeys* 608: 1–280. <https://doi.org/10.3897/zookeys.608.9427>.
- Brückner, A., and M. Heethoff. 2017. "A Chemo-Ecologists' Practical Guide to Compositional Data Analysis." *Chemoecology* 27: 33–46. <https://doi.org/10.1007/s00049-016-0227-8>.
- Cucalón, R. V., J. Valdiviezo-Rivera, P. Jiménez-Prado, R. Navarrete-Amaya, V. R. Shervette, A. Torres-Noboa, N. Wierzal, et al. 2022. "Phylogeography of the Chocó Endemic Rainbow Characin (Teleostei: Rhoadsia)." *Ichthyology & Herpetology* 110: 138–155.
- Dormann, C. F., and N. Blüthgen. 2017. "Food Webs Versus Interaction Networks: Principles, Pitfalls and Perspectives." In *Adaptive Food Webs – Stability and Transitions of Real and Model Ecosystems*, Vol. 3, edited by J. Moore, K. McCann, P. Ruiter, and V. Wolters, 1–11. Cambridge: Cambridge University Press. <https://doi.org/10.1017/9781316871867.003>.
- Dormann, C. F., and R. Strauss. 2014. "A Method for Detecting Modules in Quantitative Bipartite Networks." *Methods in Ecology and Evolution* 5: 90–98. <https://doi.org/10.1111/2041-210X.12139>.
- Dormann, C. F. J., N. B. Fründ, and B. Gruber. 2009. "Indices, Graphs and Null Models: Analyzing Bipartite Ecological Networks." *The Open Ecology Journal* 2: 7–24.
- Duffy, J. E. 2002. "Biodiversity and Ecosystem Function: The Consumer Connection." *Oikos* 99: 201–219. <https://doi.org/10.1034/j.1600-0706.2002.990201.x>.
- Edgar, R. C. 2004. "MUSCLE: Multiple Sequence Alignment with High Accuracy and High Throughput." *Nucleic Acids Research* 32: 1792–97. <https://doi.org/10.1093/nar/gkh340>.
- Fernández, F., R. J. Guerrero, and T. Delsinne. 2019. *Hormigas de Colombia*. Colombia: Univ. Nacional de Colombia. <https://doi.org/10.21676/9789587837650>.
- Fernandez-Bou, A. S., D. Dierick, A. C. Swanson, M. F. Allen, A. G. F. Alvarado, A. Artavia-León, O. Carrasquillo-Quintana, et al. 2019. "The Role of the Ecosystem Engineer, the Leaf-Cutter Ant *Atta cephalotes*, on Soil CO<sub>2</sub> Dynamics in a Wet Tropical Rainforest." *Journal of Geophysical Research: Biogeosciences* 124: 260–273. <https://doi.org/10.1029/2018JG004723>.
- Folmer, O., M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek. 1994. "DNA Primers for Amplification of Mitochondrial *Cytochrome c Oxidase Subunit I* from Diverse Metazoan Invertebrates." *Molecular Marine Biology and Biotechnology* 3: 294–99.
- Franks, N. 1982. "Ecology and Population Regulation in the Army Ant *Eciton burchelli*." In *The Ecology of a Tropical Forest Seasonal Rhythms and Long-Term Changes*, edited by E. G. Leigh, Jr., A. S. Rand, and D. M. Windsor, 389–398. Washington DC: Smithsonian Institution Press.
- Freile, J., and M. Vázquez. 2005. "Los bosques del suroccidente de la provincia de Esmeraldas: una visión general." In *Biodiversidad en el suroccidente de la provincia de Esmeraldas – Un reporte de las evaluaciones ecológicas y socioeconómicas rápidas*, edited by M. Vázquez, J. Freile, and L. Suarez, 5–9. Quito: EcoCiencia y MAE Seco.
- Gale, N., and A. S. Barfod. 1999. "Canopy Tree Mode of Death in a Western Ecuadorian Rain Forest." *Journal of Tropical Ecology* 15: 415–436.
- González-Maya, J. F., L. R. Viquez, A. Pineda-Guerrero, M. Vela-Vargas, I. Cruz-Lizano, A. Hoepker, M. Calvo, M. González, and D. A. Zárrate-Charry. 2012. "Connecting Two Continents: Species Richness, Functional Traits and Extinction Risk in the Panamanian Isthmus-Choco Continuum." *Revista Biodiversidad Neotropical* 2: 5–14.
- Gotwald, W. H., Jr. 1995. *Army Ants: The Biology of Social Predation*. Ithaca, NY: Comstock Publishing Associates.
- Guénard, B., M. D. Weiser, K. Gomez, N. Narula, and E. P. Economo. 2017. "The Global Ant Biodiversity Informatics (GABI) Database: Synthesizing Data on the Geographic Distribution of Ant Species (Hymenoptera: Formicidae)." *Myrmecological News* 24: 83–89. [https://doi.org/10.25849/myrmecol.news\\_024:083](https://doi.org/10.25849/myrmecol.news_024:083).
- Hanisch, P. E., P. D. Lavinia, A. V. Suarez, D. A. Lijtmaer, M. Leponce, C. I. Paris, and P. L. Tubaro. 2017. "Mind the Gap! Integrating Taxonomic Approaches to Assess Ant Diversity at the Southern Extreme of the Atlantic Forest." *Ecology and Evolution* 7: 10451–66. <https://doi.org/10.1002/ece3.3549>.
- Hashimoto, Y., and S. Yamane. 2014. "Comparison of Foraging Habits between Four Sympatric Army Ant Species of the Genus *Aenictus* in Sarawak, Borneo." *Asian Myrmecology* 6: 95–104.
- Hebert, P. D. N., E. H. Penton, J. M. Burns, D. H. Janzen, and W. Hallwachs. 2004. "Ten Species in One: DNA Barcoding Reveals Cryptic Species in the Neotropical Skipper Butterfly *Astraptes fulgerator*." *Proceedings of the National Academy of*

- Sciences of the United States of America* 101: 14812–17. <https://doi.org/10.1073/pnas.0406166101>.
- Heithaus, M. R., A. J. Wirsing, and L. M. Dill. 2012. “The Ecological Importance of Intact Top-Predator Populations: A Synthesis of 15 Years of Research in a Seagrass Ecosystem.” *Marine and Freshwater Research* 63: 1039–50. <https://doi.org/10.1071/MF12024>.
- Hirosawa, H., S. Higashi, and M. Mohamed. 2000. “Food Habits of *Aenictus* Army Ants and their Effects on the Ant Community in a Rain Forest of Borneo.” *Insectes Sociaux* 47: 42–49. <https://doi.org/10.1007/s000400050007>.
- Hoehnle, P. O., N. Blüthgen, A. Brückner, D. J. C. Kronauer, B. Fiala, D. A. Donoso, M. A. Smith, B. Ospina Jara, and C. von Beeren. 2019. “Species-Level Predation Network Uncovers High Prey Specificity in a Neotropical Army Ant Community.” *Molecular Ecology* 28: 2423–40. <https://doi.org/10.5061/dryad.s6743c5>.
- Hoehnle, P. O., D. A. Donoso, A. Argoti, M. Staab, C. von Beeren, and N. Blüthgen. 2022. “Rapid Ant Community Reassembly in a Neotropical Forest: Recovery Dynamics and Land-Use Legacy.” *Ecological Applications* 32: e2559. <https://doi.org/10.1002/eap.2559>.
- Hoehnle, P. O., J. E. Lattke, D. A. Donoso, C. von Beeren, M. Heethoff, S. Schmelzle, A. Argoti, L. Camacho, B. Ströbel, and N. Blüthgen. 2020. “*Odontomachus davidsoni* sp. nov. (Hymenoptera, Formicidae), a New Conspicuous Trap-Jaw Ant from Ecuador.” *ZooKeys* 948: 75–105. [10.3897/zookeys.948.48701](https://doi.org/10.3897/zookeys.948.48701).
- Hoehnle, P. O., C. Merkel, D. Donoso, A. Argoti, N. Blüthgen, and C. von Beeren. 2023. “Hunting Habits Die Hard: Conserved Prey Preferences in Neotropical Army Ants across Distant Neotropical Rainforests [Datasets].” BOLD Systems. Army Ant Records. <https://doi.org/10.5883/DS-PREYARMY>.
- Huijbers, C. M., T. A. Schlacher, R. R. McVeigh, D. S. Schoeman, A. D. Olds, M. B. Brown, K. B. Ekanayake, M. A. Weston, and R. M. Connolly. 2016. “Functional Replacement across Species Pools of Vertebrate Scavengers Separated at a Continental Scale Maintains an Ecosystem Function.” *Functional Ecology* 30: 998–1005. <https://doi.org/10.1111/1365-2435.12577>.
- Ings, T. C., J. M. Montoya, J. Bascompte, N. Blüthgen, L. Brown, C. F. Dormann, F. Edwards, et al. 2009. “Review: Ecological Networks – Beyond Food Webs.” *Journal of Animal Ecology* 78: 253–269. <https://doi.org/10.1111/j.1365-2656.2008.01460.x>.
- Ivens, A. B. F., C. von Beeren, N. Blüthgen, and D. J. C. Kronauer. 2016. “Studying the Complex Communities of Ants and Their Symbionts Using Ecological Network Analysis.” *Annual Review of Entomology* 61: 353–371. <https://doi.org/10.1146/annurev-ento-010715-023719>.
- Jiménez-Rodríguez, C. D., M. Coenders-Gerrits, J. Wenninger, A. Gonzalez-Angarita, and H. Savenije. 2020. “Contribution of Understorey Evaporation in a Tropical Wet Forest during the Dry Season.” *Hydrology and Earth System Sciences* 24: 2179–2206.
- Jost, L. 2006. “Entropy and Diversity.” *Oikos* 113: 363–375. <https://doi.org/10.1111/j.2006.0030-1299.14714.x>.
- Kaspari, M., and S. O’Donnell. 2003. “High Rates of Army Ant Raids in the Neotropics and Implications for Ant Colony and Community Structure.” *Evolutionary Ecology Research* 5: 933–39.
- Kaspari, M., S. Powell, J. Lattke, and S. O’Donnell. 2011. “Predation and Patchiness in the Tropical Litter: Do Swarm-Raiding Army Ants Skim the Cream or Drain the Bottle?” *Journal of Animal Ecology* 80: 818–823. <https://doi.org/10.1111/j.1365-2656.2011.01826.x>.
- Kronauer, D. J. C. 2020. *Army Ants: Nature’s Ultimate Social Hunters*. Cambridge, MA: Harvard University Press.
- Longino, J. 2010. “Ants of Costa Rica.” <http://academic.evergreen.edu/projects/ants/GENERA/eciton/specieslist.html>.
- Longino, J., J. Coddington, and R. Colwell. 2002. “The Ant Fauna of a Tropical Rain Forest: Estimating Species Richness Three Different Ways.” *Ecology* 83: 689–702. <https://doi.org/10.2307/3071874>.
- Longino, J., and S. Cover. 2004. “A Revision of the *Aphaenogaster phalangium* Complex (Hymenoptera: Formicidae: Myrmicinae).” *Zootaxa* 655: 1–12. <https://doi.org/10.11646/zootaxa.655.1.1>.
- McDade, L. A., K. S. Bawa, H. A. Hespenheide, and G. S. Hartshorn. 1994. *La Selva, Ecology and Natural History of a Neotropical Rainforest*. Chicago, IL: University of Chicago Press.
- McGlynn, T. P., R. A. Carr, J. H. Carson, and J. Buma. 2004. “Frequent Nest Relocation in the Ant *Aphaenogaster araneoides*: Resources, Competition, and Natural Enemies.” *Oikos* 106: 611–621. <https://doi.org/10.1111/j.0030-1299.2004.13135.x>.
- McGlynn, T. P., M. D. Shotell, and M. S. Kelly. 2003. “Responding to a Variable Environment: Home Range, Foraging Behavior, and Nest Relocation in the Costa Rican Rainforest Ant *Aphaenogaster araneoides*.” *Journal of Insect Behavior* 16: 687–701. <https://doi.org/10.1023/B:JOIR.0000007704.22329.a5>.
- Mittelbach, G. G. 2012. *Community Ecology*. Sunderland, MA: Sinauer Associates, Inc.
- Morelos-Juárez, C., A. Tapia, G. Conde, and M. Peck. 2015. “Diet of the Critically Endangered Brown-Headed Spider Monkey (*Ateles fusciceps fusciceps*) in the Ecuadorian Chocó: Conflict between Primates and Loggers over Fruiting Tree Species.” *PeerJ PrePrints* 3: e1574v1. <https://doi.org/10.7287/peerj.preprints.1574v1>.
- Myers, R. A., J. K. Baum, T. D. Shepherd, S. P. Powers, and C. H. Peterson. 2007. “Cascading Effects of the Loss of Apex Predatory Sharks from a Coastal Ocean.” *Science* 315: 1846–50. <https://doi.org/10.1126/science.1138657>.
- O’Dea, A., H. A. Lessios, A. G. Coates, R. I. Eytan, S. A. Restrepo-Moreno, A. L. Cione, L. S. Collins, A. de Queiroz, D. W. Farris, and R. D. Norris. 2016. “Formation of the Isthmus of Panama.” *Science Advances* 2: e1600883. <https://doi.org/10.1126/sciadv.1600883>.
- O’Donnell, S., M. Kaspari, and J. Lattke. 2005. “Extraordinary Predation by the Neotropical Army Ant *Cheliomyrmex andicola*: Implications for the Evolution of the Army Ant Syndrome.” *Biotropica* 37: 706–9. <https://doi.org/10.1111/j.1744-7429.2005.00091.x>.
- O’Donnell, S., J. Lattke, S. Powell, and M. Kaspari. 2007. “Army Ants in Four Forests: Geographic Variation in Raid Rates and Species Composition.” *The Journal of Animal Ecology* 76: 580–89. <https://doi.org/10.1111/j.1365-2656.2007.01221.x>.
- O’Donnell, S., J. Lattke, S. Powell, and M. Kaspari. 2009. “Species and Site Differences in Neotropical Army Ant Emigration Behaviour.” *Ecological Entomology* 34: 476–482. <https://doi.org/10.1111/j.1365-2311.2008.01074.x>.



- O'Donnell, S., J. Lattke, S. Powell, and M. Kaspari. 2020. "Diurnal and Nocturnal Foraging Specialisation in Neotropical Army Ants." *Ecological Entomology* 46: 352–59. <https://doi.org/10.1111/een.12969>.
- Olds, A. D., B. A. Frohloff, B. L. Gilby, R. M. Connolly, N. A. Yabsley, P. S. Maxwell, C. J. Henderson, and T. A. Schlacher. 2018. "Urbanisation Supplements Ecosystem Functioning in Disturbed Estuaries." *Ecography* 41: 2104–13. <https://doi.org/10.1111/ecog.03551>.
- Patefield, W. M. 1981. "Algorithm AS 159: An Efficient Method of Generating Random  $R \times C$  Tables with Given Row and Column Totals." *Journal of the Royal Statistical Society. Series C (Applied Statistics)* 30: 91–97. <https://doi.org/10.2307/2346669>.
- Pérez-Escobar, O. A., E. Lucas, C. Jaramillo, A. Monro, S. K. Morris, D. Bogarín, D. Greer, S. Dodsworth, J. Aguilar-Cano, and A. Sanchez Meseguer. 2019. "The Origin and Diversification of the Hyperdiverse Flora in the Chocó Biogeographic Region." *Frontiers in Plant Science* 10: 1328.
- Pérez-Espona, S. 2021. "Eciton Army Ants – Umbrella Species for Conservation in Neotropical Forests." *Diversity* 13: 136. <https://doi.org/10.3390/d13030136>.
- Powell, S. 2009. "An Army Ant that Likes a Bit of Fruit." Blog Post: Myrmecos Blog. <https://myrmecos.wordpress.com/2009/03/13/an-army-ant-that-likes-a-bit-of-fruit/>.
- Powell, S. 2011. "How Much Do Army Ants Eat? On the Prey Intake of a Neotropical Top-Predator." *Insectes Sociaux* 58: 317–324. <https://doi.org/10.1007/s00040-011-0152-3>.
- Powell, S., and B. Baker. 2008. "Os grandes predadores dos neotrópicos: comportamento, dieta e impacto das formigas de correição (Ecitoninae)." In *Insetos sociais da biologia à aplicação*, edited by E. Ferreira Vilela, I. A. dos Santos, J. E. Serrão, J. H. Schoederer, J. Lino-Neto, and L. A. D. O. Campos, 18–37. Viçosa: Universidade Federal de Viçosa.
- Powell, S., and N. R. Franks. 2006. "Ecology and the Evolution of Worker Morphological Diversity: A Comparative Analysis with *Eciton* Army Ants." *Functional Ecology* 20: 1105–14. <https://doi.org/10.1111/j.1365-2435.2006.01184.x>.
- Ratnasingham, S., and P. D. Hebert. 2013. "A DNA-Based Registry for All Animal Species: The Barcode Index Number (BIN) System." *PLoS One* 8: e66213. <https://doi.org/10.1371/journal.pone.0066213>.
- Rettenmeyer, C. W. 1963. "Behavioral Studies of Army Ants." *The University of Kansas Science Bulletin* 44: 281–465.
- Rettenmeyer, C. W., R. Chadab-Crepet, M. G. Naumann, and L. Morales. 1983. "Comparative Foraging by Neotropical Army Ants." In *Social Insect in the Tropics: Proceedings of the International Symposium Organized by the International Union for the Study of Social Insects and the Sociedad Mexicana de Entomología, Cocoyoc, Morelos, Mexico*, edited by P. Jaisson, 59–73. Paris: Université Paris-Nord.
- Ripple, W. J., J. A. Estes, R. L. Beschta, C. C. Wilmers, E. G. Ritchie, M. Hebblewhite, J. Berger, B. Elmhagen, M. Letnic, and M. P. Nelson. 2014. "Status and Ecological Effects of the world's Largest Carnivores." *Science* 343: 1241484. <https://doi.org/10.1126/science.1241484>.
- Ruiz-Guerra, C., R. Johnston-González, Y. Cifuentes-Sarmiento, F. A. Estela, L. F. Castillo, C. E. Hernández, and L. G. Naranjo. 2007. "Noteworthy Bird Records from the Southern Chocó of Colombia." *Bulletin-British Ornithologists Club* 127: 283.
- Sarkar, S., V. Sánchez-Cordero, M. C. Londoño, and T. Fuller. 2009. "Systematic Conservation Assessment for the Mesoamerica, Chocó, and Tropical Andes Biodiversity Hotspots: A Preliminary Analysis." *Biodiversity and Conservation* 18: 1793–1828. <https://doi.org/10.1007/s10531-008-9559-1>.
- Schleuning, M., L. Ingmann, R. Strauss, S. A. Fritz, B. Dalsgaard, D. M. Dehling, M. Plein, et al. 2014. "Ecological, Historical and Evolutionary Determinants of Modularity in Weighted Seed-Dispersal Networks." *Ecology Letters* 17: 454–463. <https://doi.org/10.1111/ele.12245>.
- Schneirla, T. C. 1971. *Army Ants: A Study in Social Organization*. San Francisco, CA: W. H. Freeman & Co.
- Sergio, F., T. Caro, D. Brown, B. Clucas, J. Hunter, J. Ketchum, K. McHugh, and F. Hiraldo. 2008. "Top Predators as Conservation Tools: Ecological Rationale, Assumptions, and Efficacy." *Annual Review of Ecology, Evolution, and Systematics* 39: 1–19. <https://doi.org/10.1146/annurev.ecolsys.39.110707.173545>.
- Sorensen, P. B., C. F. Damgaard, B. Strandberg, Y. L. Dupont, M. B. Pedersen, L. G. Carvalheiro, J. C. Biesmeijer, J. M. Olsen, M. Hagen, and S. G. Potts. 2011. "A Method for Under-Sampled Ecological Network Data Analysis: Plant-Pollination as Case Study." *Journal of Pollination Ecology* 6: 20120101. [https://doi.org/10.26786/1920-7603\(2011\)18](https://doi.org/10.26786/1920-7603(2011)18).
- Sosa-Calvo, J., A. Ješovnik, C. T. Lopes, A. Rodrigues, C. Rabeling, M. Bacci, H. L. Vasconcelos, and T. R. Schultz. 2017. "Biology of the Relict Fungus-Farming Ant *Apterostigma megacephala* Lattke, Including Descriptions of the Male, Gyne, and Larva." *Insectes Sociaux* 64: 329–346. <https://doi.org/10.1007/s00040-017-0550-2>.
- Touchton, J. M., and J. N. M. Smith. 2011. "Species Loss, Delayed Numerical Responses, and Functional Compensation in an Antbird Guild." *Ecology* 92: 1126–36. <https://doi.org/10.1890/10-1458.1>.
- Tozetto, L., D. L. Forrister, M. Duval, T. Hays, N. C. Garwood, R. V. Castro, J. E. Lattke, S. Sendoya, and J. T. Longino. 2023. "Army Ant Males Lose Seasonality at a Site on the Equator." *Biotropica* 55: 382–395. <https://doi.org/10.1111/btp.13192>.
- Vázquez, D. P., N. Blüthgen, L. Cagnolo, and N. P. Chacoff. 2009. "Uniting Pattern and Process in Plant–Animal Mutualistic Networks: A Review." *Annals of Botany* 103: 1445–57. [10.1093/aob/abp057](https://doi.org/10.1093/aob/abp057).
- Vieira, R. S., and H. Höfer. 1994. "Prey Spectrum of Two Army Ant Species in Central Amazonia, with Special Attention on their Effect on Spider Populations." *Andrias* 13: 189–198.
- von Beeren, C. 2023. "Hunting Habits Die Hard: Conserved Prey Preferences in Neotropical Army Ants across Distant Neotropical Rainforests [Dataset]." Dryad. <https://doi.org/10.5061/dryad.zcrjdfnkc>.
- von Beeren, C., N. Blüthgen, P. O. Hoenle, S. Pohl, A. Brückner, A. K. Tishechkin, M. Maruyama, et al. 2021. "A Remarkable Legion of Guests: Diversity and Host Specificity of Army Ant Symbionts." *Molecular Ecology* 30: 5229–46. <https://doi.org/10.1111/mec.16101>.
- von Beeren, C., M. Maruyama, and D. J. C. Kronauer. 2016. "Cryptic Diversity, High Host Specificity and Reproductive Synchronization in Army Ant-Associated *Vatesus* Beetles." *Molecular Ecology* 25: 990–1005. <https://doi.org/10.1111/mec.13500>.
- von Beeren, C., S. Pohl, M. Fikáček, S. Kleinfelder, A. K. Tishechkin, S. Yamamoto, M. Chani-Posse, et al. 2023. "Army Ant Middens – Home and Nursery of a Diverse Beetle Fauna." *Ecology and Evolution* 13: e10451. <https://doi.org/10.1002/ece3.10451>.

- Watkins, J. F., II. 1976. *The Identification and Distribution of New World Army Ants (Dorylinae: Formicidae)*. Waco, TX: The Markham Press Fund of Baylor University Press.
- Winston, M. E., D. J. C. Kronauer, and C. S. Moreau. 2016. "Early and Dynamic Colonization of Central America Drives Speciation in Neotropical Army Ants." *Molecular Ecology* 26: 859–870. <https://doi.org/10.1111/mec.13846>.

### SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Hoenle, Philipp O., Christoph Merkel, David A. Donoso, Adriana A. Argoti, Nico Blüthgen, and Christoph von Beeren. 2024. "Hunting Habits Die Hard: Conserved Prey Preferences in Army Ants across Two Distant Neotropical Rainforests." *Ecosphere* 15(4): e4812. <https://doi.org/10.1002/ecs2.4812>