


# Intra- and interspecific variation in trophic ecology of ‘predatory’ ants in the subfamily Ponerinae

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**Abstract.** 1. The characterisation of energy flow through communities is a primary goal of ecology. Furthermore, predator–prey interactions can influence both species abundance and community composition. The ant subfamily Ponerinae includes many predatory species that range from generalist insectivores to highly specialised hunters that target a single prey type. Given their high diversity and ubiquity in tropical ecosystems, measuring intra- and interspecific variation in their trophic ecology is essential for understanding the role of ants as predators of insect communities.

2. The stable isotopic composition of nitrogen of 22 species from the ant subfamily Ponerinae was measured, relative to plants and other predatory and herbivorous insects at two Atlantic Forest sites in Argentina. The study tested the general assumption that ponerine ants are all predatory, and examined intra- and interspecific variation in trophic ecology relative to habitat, body size and cytochrome *c* oxidase subunit 1 sequences (DNA barcoding).

3. Stable isotope analysis revealed that most ponerines occupy high trophic levels (primary and secondary predators), but some species overlapped with known insect herbivores. Species residing at low trophic levels were primarily arboreal and may rely heavily on nectar or other plant-based resources in their diet. In addition, larger species tend to occupy lower trophic positions than smaller species.

4. Although some of the species were divided into two or more genetic clusters by DNA barcoding analysis, these clusters did not correspond to intraspecific variation in trophic position; therefore, colony dietary flexibility most probably explains species that inhabit more than one trophic level.

**Key words.** Atlantic Forest, body size, generalist predators, trophic position.

## Introduction

Estimating an organism’s trophic position provides a mechanism to identify subsets of species within communities that acquire energy in similar ways, facilitating the study of food webs in species-rich ecosystems. Typically, an ecosystem contains no more than four or five trophic levels (Pimm & Lawton, 1977), including primary producers, herbivores, primary carnivores and secondary carnivores. Predators can be vital for community stability via ‘top-down’ effects by controlling herbivore

abundance, preventing them from overexploiting the vegetation (Hairston *et al.*, 1960; Terborgh *et al.*, 2006). However, determining a predator’s specific trophic position can be difficult due to intraspecific variation in diet and prey selectivity. Accurate information on diet is essential for studies of trophic ecology as the effects of specialist and generalist predators on community structure may be different; specialist predators may limit populations of particular prey species, while generalist predators are more likely to be ecosystem stabilisers, by consuming the most abundant prey (Paine, 1966).

Diet often has a strong phylogenetic signal due to behavioural, physiological and morphological constraints (Bersier & Kehrli, 2008). Subsequently, taxonomic information can be used to

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estimate an organism's position in a food web. For example, we can reliably assume that spiders are predators (but see Nyffeler *et al.*, 2016), although the degree to which they are specialists (e.g. predators of other spiders or specific arthropods) can still make determining their exact trophic level difficult. However, niche conservatism is far from ubiquitous and even individual species can exhibit considerable variation in trophic position (Meehan *et al.*, 2009; Xiaoqiong *et al.*, 2010; Stuble *et al.*, 2013; Roeder & Kaspari, 2017; Hoenle *et al.*, 2019). This variation can result from ontogeny (e.g. size), experience, sex, environmental variation, or intra- and interspecific competition (Bolnick *et al.*, 2002; Grant & Grant, 2006; Villamarín *et al.*, 2018). Life-history traits also inform foraging ecology. Body size, for example, is correlated with trophic position in many taxa, with larger species feeding at lower trophic levels (e.g. herbivores), on average, than smaller species (Case, 1979; Price & Hopkins, 2015). However, excluding herbivores can reverse this pattern as predators tend to eat organisms with smaller body sizes, especially when considering intraguild predation (Cohen *et al.*, 1993; Layman *et al.*, 2005).

Ants are key components of food webs, especially in tropical ecosystems, due to their diversity and abundance (Fittkau & Klinge, 1973). For example, surveys of an Atlantic Forest site in Argentina revealed over 220 species, with a single common ant, *Dinoponera australis* (Ponerinae), reaching an estimated biomass of 2.5 kg ha<sup>-1</sup> (Tillberg *et al.*, 2014; Hanisch *et al.*, 2018). However, without careful knowledge of their feeding ecology, ants can be difficult to place in food webs due to their wide dietary range, including arthropods, fungus, seeds, and other plant material (Feldhaar *et al.*, 2010). This limitation has been addressed by estimating trophic positions with stable isotope analyses. As many metabolic processes discriminate between lighter and heavier isotopes, consumers are enriched in heavy nitrogen [i.e. they have a higher <sup>15</sup>N/<sup>14</sup>N ( $\delta^{15}$ N)] relative to their prey (Deniro & Epstein, 1981; Post, 2002). Additionally, if prey items differ isotopically, the variance of stable isotopes (in particular  $\delta^{15}$ N) can provide information about the range of prey species consumed (Bearshop *et al.*, 2004). The use of stable isotope analysis has revolutionised ant trophic ecology, both uncovering hidden intraspecific variation (Tillberg *et al.*, 2007; Wilder *et al.*, 2011; Roeder & Kaspari, 2017) and revealing their trophic breadth in communities where different species range from feeding on plants to acting as top predators in the same ecosystems (e.g. Blüthgen *et al.*, 2003; Fiedler *et al.*, 2007; Tillberg *et al.*, 2007; Russell *et al.*, 2009; Pfeiffer *et al.*, 2014).

Some of the most conspicuous ants in the tropics are from the subfamily Ponerinae; ponerines are often large and predatory, and include specialised hunters on specific taxa such as termites or millipedes (Brandão *et al.*, 1991; Leal & Oliveira, 1995; Schmidt & Shattuck, 2014). However, diet and natural history information remains unknown for most ponerines, and especially for genera with cryptic habits, making generalisations about this important group difficult. All ponerines have a sting to help them subdue prey. However, many utilise liquid food sources, and several species are known to collect nectar, seeds, and fruits (Evans & Leston, 1971; Christianini *et al.*, 2007; Ávila Núñez *et al.*, 2011). Additionally,

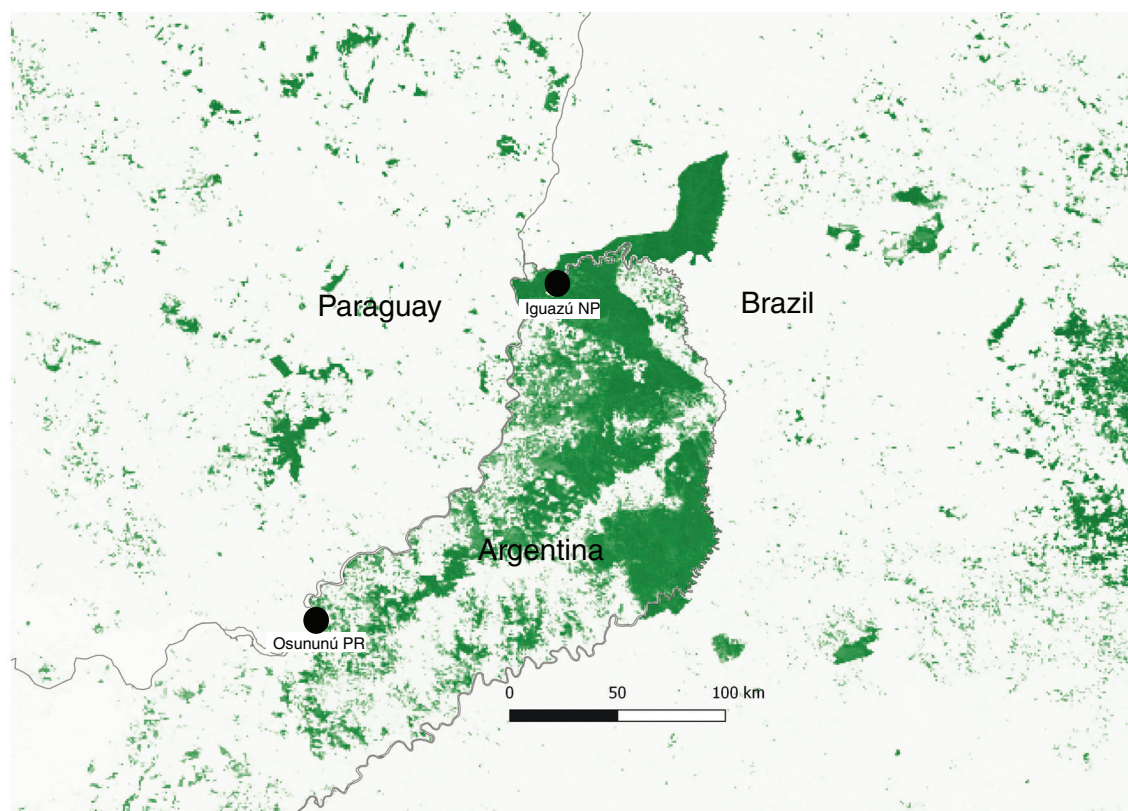
Bottcher *et al.* (2014) found a positive correlation between larva weight and the consumption of lipid-rich seed arils in the trap jaw ant *Odontomachus chelifer*, suggesting that the ingestion of plant material could be important for nutrition in this species despite its hunting-specialised mandibles. Furthermore, ponerines vary considerably in body size, with the subfamily housing genera that include some of the world's largest (e.g. *Dinoponera*) and smallest (e.g. *Hypoponera*) ant species. Body size can influence prey selection and the microhabitat used by ants (Farji-Brener *et al.*, 2004; Wills *et al.*, 2018), and therefore influence access to food sources (Farji-Brener *et al.*, 2004). For example, in tropical forests the canopy is protein-limited relative to litter microhabitats (Yanoviak & Kaspari, 2000).

In this study, we used stable isotope analysis to test three hypotheses relating to ponerine ants in invertebrate tropical food webs at two Atlantic Forest sites: (i) that all ponerine species are primarily predatory; (ii) that body size and habitat influence a species' trophic position, specifically predicting either that body size will be positively correlated with trophic position if they are all primarily predatory, or that body size will be negatively correlated with trophic position if they include species that are predominantly herbivorous (e.g. dependent on plant-based resources) in addition to predatory; (iii) that species exhibiting high variation in trophic position reflected the presence of cryptic species. This was determined by using cytochrome *c* oxidase subunit 1 (COI) sequences in combination with traditional morphological approaches.

## Materials and methods

### Study site

Sampling was conducted in two protected areas of the Atlantic Forest in Misiones Province, Argentina: Iguazú National Park (INP; S25.68015°, W54.454192°) and Osununú Private Reserve (OPR; S27.279167°, W55.578056°) (Fig. 1). These sites consist of semi-deciduous subtropical forest, with mean monthly temperatures ranging from 15 °C (June–August) to 26 °C (December–February), annual rainfall of between 1800 and 2000 mm, and humidity between 70% and 90%. In summer (December–March) of 2015 and 2016, we collected ants in INP and OPR in three types of habitats (leaf litter, soil surface and arboreal). We sampled directly from colonies when possible to collect multiple individuals of the same colony for each replicate. If the colony was not located, individuals collected at the same spot were placed into a Petri dish and were assumed to belong to the same nest if they did not display any aggressive interactions. We also collected arthropods of known trophic position (e.g. herbivores, predators), plant material and suspected prey items at the same locations where ants were collected (Fourcassie & Oliveira, 2002). In total, 268 samples were collected in INP and 163 in OPR. The surveyed areas were c. 0.5 km<sup>2</sup> for OPR and c. 0.70 km<sup>2</sup> for INP. All samples were stored in 96% alcohol and maintained at –20 °C until processing (average 3 months after collection, range 1–8 months).



**Fig. 1.** The study was conducted in two protected areas of the Atlantic Forest in Misiones Province, Argentina: Iguazú National Park and Osunúní Private Reserve (black dots). [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].

### Specimen identification

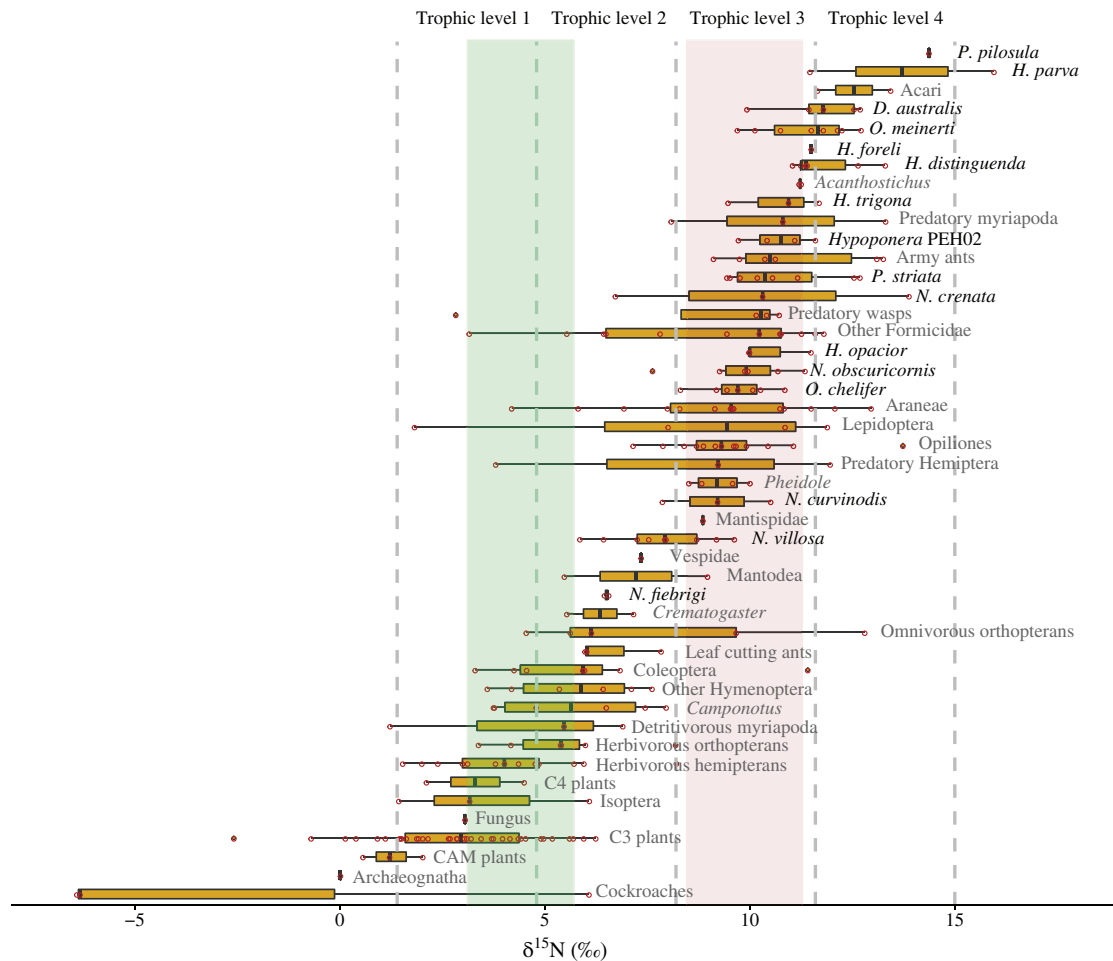
Ants were identified to species using available keys (Brown, 1975; Wild, 2005; Jiménez *et al.*, 2008; MacKay & Mackay, 2010; Dash, 2011; Lenhart *et al.*, 2013; Fernandes *et al.*, 2014), and other taxa to the lowest taxonomic level possible. To improve taxonomic information for all ponerines and select insects, we sequenced a 658-bp fragment near the 5' end of the COI gene following standard protocols developed for DNA barcoding (see Hanisch *et al.*, 2017). All trace files, collection data, taxonomic information, and images were uploaded to the online Barcode of Life Data Systems (BOLD, [www.boldsystems.org](http://www.boldsystems.org); Ratnasingham & Hebert, 2007) where they are publicly available in the dataset DS-PONERECO. All voucher specimens are deposited in the Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia'.

### Stable isotope analysis

To obtain a more accurate estimate of  $\delta^{15}\text{N}$  (Tillberg *et al.*, 2006), gasters were removed for ponerines, but kept for other ants so they would experience the same treatment as prey items and  $\delta^{15}\text{N}$  reference samples. Although including the gaster may influence  $\delta^{15}\text{N}$  values, this is not always the case (Tillberg *et al.*, 2006). Moreover, when ponerines prey on other ants,

they eat the gaster (PEH, pers. obs.), and hence we did not remove them as they are included as part of their diet. All samples were dried at 60 °C and homogenised using a mortar and pestle (with the help of liquid nitrogen if needed). After drying, all samples were weighed to c. 2.00 µg (invertebrates) or c. 5.00 µg (plants) on an electronic microbalance (Mettler Toledo XP6, Columbus, Ohio) and placed in tin capsules. Two technical replicates were prepared for analysis if enough sample mass was available. Analysis of nitrogen isotopic composition was performed at the University of Illinois at Urbana-Champaign using an IsoPrime 100 continuous-flow isotope ratio mass spectrometer (IsoPrime, Cheadle Hulme, U.K.) interfaced to an Elementar vario MICRO cube elemental analyser (Elementar, Hanau, Germany). Bovine liver NIST standard reference material 1577c was calibrated against USGS40 and USGS41 reference materials for  $\delta^{15}\text{N}$  and then used as the working standard. Stable isotope abundance is expressed using the  $\delta$  notation, with  $\delta^{15}\text{N}$  [‰] =  $(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}} \times 1000$ .  $R_{\text{sample}}$  and  $R_{\text{standard}}$  represent the  $^{15}\text{N}/^{14}\text{N}$  ratios of the sample and the international reference standard (air), respectively. For ponerines, an average of four colonies and three individuals per colony were sampled for each species at each site (Table S1).

Thirty-seven samples with high variability between technical replicates (SD > 0.5 or a coefficient of variance > 1.0) were excluded from the analysis. We defined trophic levels (TLs) with



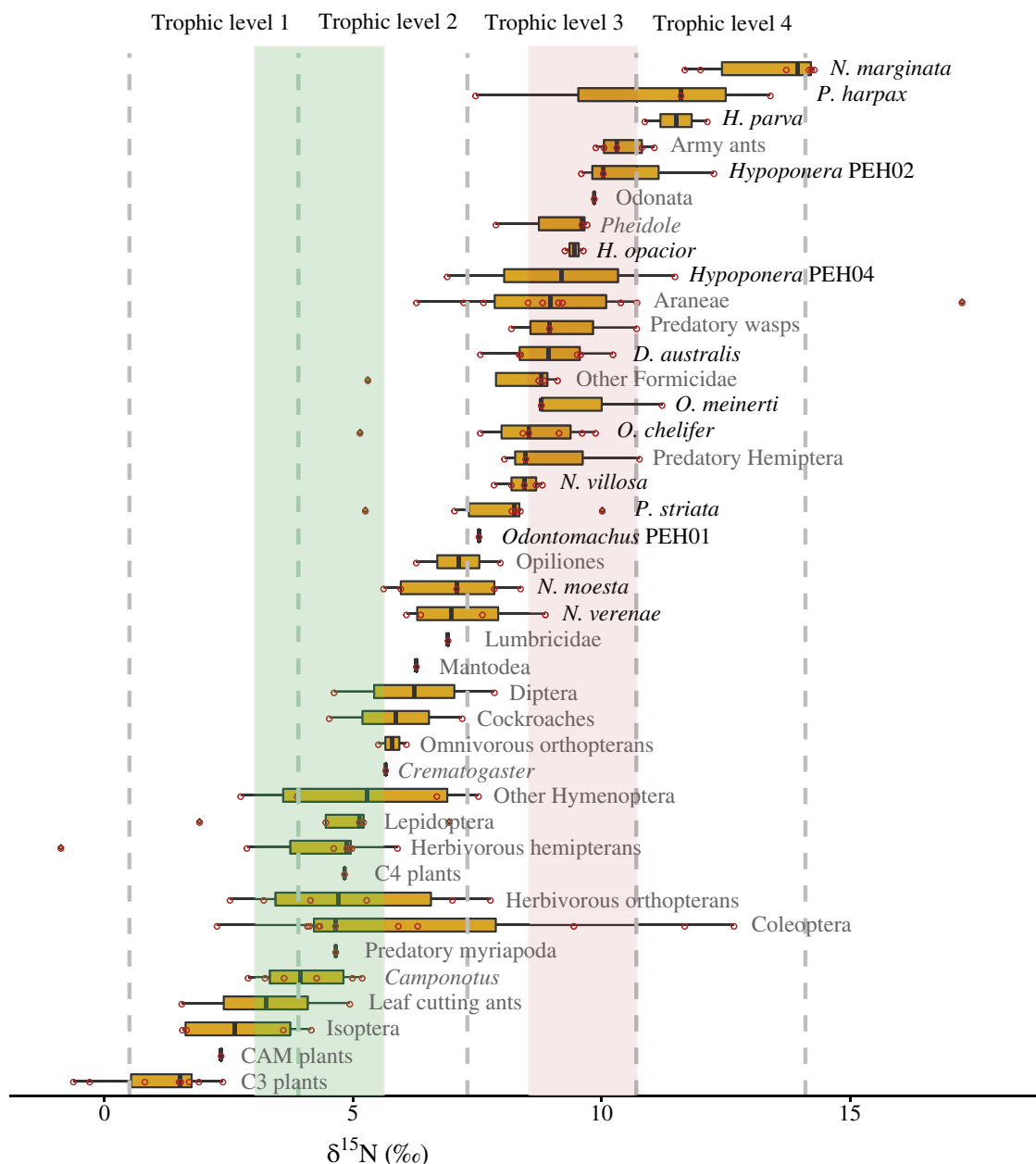
**Fig. 2.** Isotopic composition ( $\delta^{15}\text{N}$ ) of Ponerinae (in black) and plants and other arthropods (in grey) for Iguazú National Park. Green and red represent the 25th and 75th percentiles for trophic levels 2 and 3, respectively, measured from known herbivores and predators. Dashed lines represent trophic levels separated by 3.4‰. Red circles represent individual sample values. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].

two different approaches: first, based on  $\delta^{15}\text{N}$  data for primary producers (plants, TL = 1), primary consumers (orthopteran and hemipteran herbivores, TL = 2), and secondary consumers (spiders and army ants, TL = 3). We used these specific groups because we were able to determine their diet with confidence using natural history information. We then compared  $\delta^{15}\text{N}$  measurements of ants with these values. Second, we also assumed that trophic levels are separated by an average difference of 3.4‰  $\delta^{15}\text{N}$  due to fractionation (Deniro & Epstein, 1981; Cabana & Rasmussen, 1994; Post, 2002). In this case, as a baseline, we used the 25th percentile of the  $\delta^{15}\text{N}$  signature of the plants. Finally, we calculated the relative trophic position as in Tillberg *et al.* (2007) using a trophic step of 3.4‰ and the  $\delta^{15}\text{N}$  specific values of known herbivores and predators for each locality.

To assess the association between habitat and diet, a general linear mixed-effects model (LMM) with a normal error distribution was fitted using function 'glms' of the package NLME (Pinheiro *et al.*, 2018).  $\delta^{15}\text{N}$  was included as the response variable, while 'habitat' (leaf litter, soil surface and arboreal)

and 'locality' (INP, OPR) were included as fixed factors, and 'species' as a random factor. Model assumptions, normality and homoscedasticity of residuals were graphically checked. As homoscedasticity was not accomplished, the model was fitted by the addition of the VarIdent variance structure to 'species'. Additionally, a compound symmetry structure was included to account for the non-independence of repeated measurements of the same species. *Posthoc* comparison analysis was done with the 'glht' function from the MULTCOMP package (Hothorn *et al.*, 2008) in R to investigate which habitats differed from each other. Finally, because body size is strongly correlated with head length (Kaspari & Weiser, 1999), we used this measurement to compare trophic position with body size, by averaging the head length value of three individuals per species. The relative trophic position was averaged for each species and the association between these variables was investigated through Spearman correlation test. All analyses were carried out in R v.3.5.2 (R Development Core Team, 2016).





**Fig. 3.** Isotopic composition ( $\delta^{15}\text{N}$ ) of Ponerinae (in black) and plants and other arthropods (in grey) for Osunú Private Reserve. Green and red represent the 25th and 75th percentiles for trophic levels 2 and 3, respectively, measured from known herbivores and predators. Dashed lines represent trophic levels separated by 3.4‰. Red circles represent individual sample values. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].

### Sequence analysis

Generated sequences were compared with DNA barcodes on BOLD using barcode index numbers (BINs), which are unique alphanumeric codes automatically generated by BOLD. In short, each sequence uploaded to BOLD is automatically assigned a BIN based on a specific algorithm (refined single linkage, or RESL), or alternatively a new BIN is created if the specimen's sequence is different enough from

all BINs in the database (Ratnasingham & Hebert, 2013). Different BINs within a single species could indicate the presence of cryptic species and are particularly useful for understudied groups or biodiversity assessments (Ratnasingham & Hebert, 2013; Hanisch *et al.*, 2017). We then generated a neighbour-joining tree with software MEGA7 using Kimura two-parameter substitution model and pairwise deletion. Node support was computed with 1000 bootstrap pseudoreplicates.

## Results

### Trophic position

A total of 243 samples from INP and 151 samples from ONP were used for analyses. Plant samples (TL1) at INP had a range of  $\delta^{15}\text{N}$  values from  $-2.58$  to  $6.24\text{‰}$  (mean  $\pm$  SD,  $2.77 \pm 1.9\text{‰}$ ,  $n = 42$ ) and at OPR from  $-0.62$  to  $4.82\text{‰}$  ( $1.60 \pm 1.52\text{‰}$ ,  $n = 10$ ). Primary consumers (TL2) were very similar between INP and OPR (mean  $\pm$  SD, range: INP,  $\delta^{15}\text{N} = 4.53 \pm 1.83\text{‰}$ ,  $1.5$ – $8.21\text{‰}$ ,  $n = 19$ ; OPR,  $\delta^{15}\text{N} = 4.39 \pm 2.18\text{‰}$ ,  $-0.87$ – $7.76\text{‰}$ ,  $n = 13$ ), as were the secondary consumers (TL3; INP,  $\delta^{15}\text{N} = 9.76 \pm 2.36\text{‰}$ ,  $4.19$ – $13.25\text{‰}$ ,  $n = 20$ ; OPR,  $\delta^{15}\text{N} = 9.81 \pm 2.49\text{‰}$ ,  $7.62$ – $17.25\text{‰}$ ,  $n = 15$ ). Assuming a  $3.4\text{‰}$  separation per TL, we obtained similar results (TL1 = INP,  $1.47$ – $4.87\text{‰}$ ; OPR,  $0.53$ – $3.93\text{‰}$ ; TL2 = INP,  $4.87$ – $8.27\text{‰}$ ; OPR,  $3.93$ – $7.33\text{‰}$ ; TL3 = INP,  $8.27$ – $11.67\text{‰}$ ; OPR,  $7.33$ – $10.73\text{‰}$ ; Figs 2, 3). Information for each specimen is summarised in Table S2.

### Ant community

Ant samples at INP had a range of  $\delta^{15}\text{N}$  values from  $3.15$  to  $15.9\text{‰}$  ( $n = 108$ ) and at OPR from  $1.55$  to  $14.27\text{‰}$  ( $n = 77$ ). Lowest trophic positions were occupied by leaf-cutting ants in the genera *Atta* and *Acromyrmex*, the arboreal nesting *Procrystocerus* and *Cephalotes*, and the genera *Camponotus* and *Crematogaster*, which also include many arboreal species. At higher trophic levels, ants were represented by army ants (genera *Eciton* and *Labidus*) and genera from the subfamilies Ectatomminae and Ponerinae. The little fire ant, *Wasmannia auropunctata*, a generalist worldwide invasive species, was also predatory in this habitat within its native range (Table 1).

### Subfamily Ponerinae

$\delta^{15}\text{N}$  values of ant species from the subfamily Ponerinae ranged from  $5.8$  to  $15.9\text{‰}$  (INP) and  $5.1$  to  $14.2\text{‰}$  (OPR), forming a gradient of nearly  $10\text{‰}$  or roughly three trophic positions. Most species were located at TL3 and TL4 (primary and secondary predators), but a few species appeared more reliant on plant-based resources. The arboreal *Neoponera* had the lowest  $^{15}\text{N}$  enrichment: in INP, *N. fiebrigi* had the lowest  $\delta^{15}\text{N}$  value ( $6.50 \pm 0.07\text{‰}$ ) followed by *N. villosa* ( $8.03 \pm 1.02$ ). Additionally, in OPR, *N. veranae* ( $7.22 \pm 1.28\text{‰}$ ) and *N. moesta* ( $6.97 \pm 1.18\text{‰}$ ) had similar values. The remaining species were primarily predatory around TL3 and TL4 (Figs 2, 3). The top Ponerinae predators were *H. parva* and *Platythyrea pilosula* in INP and *N. marginata* and *Pachycondyla harpax* in OPR. Some species also exhibited high variation in  $\delta^{15}\text{N}$  values. For example, *Neoponera crenata* in INP ( $10.30 \pm 3.58\text{‰}$ ) and *P. harpax* in OPR ( $10.81 \pm 3.04\text{‰}$ ) (Figs 2, 3).

Arboreal species differed in their  $\delta^{15}\text{N}$  values compared with species living in the litter or soil surface (LMM,  $F = 42$ ,  $P < 0.001$ ; Fig. 4). This pattern was driven primarily by arboreal *Neoponera*, which tended to have the lowest  $\delta^{15}\text{N}$  values (Fig. 4). Relative trophic position also varied with body size;

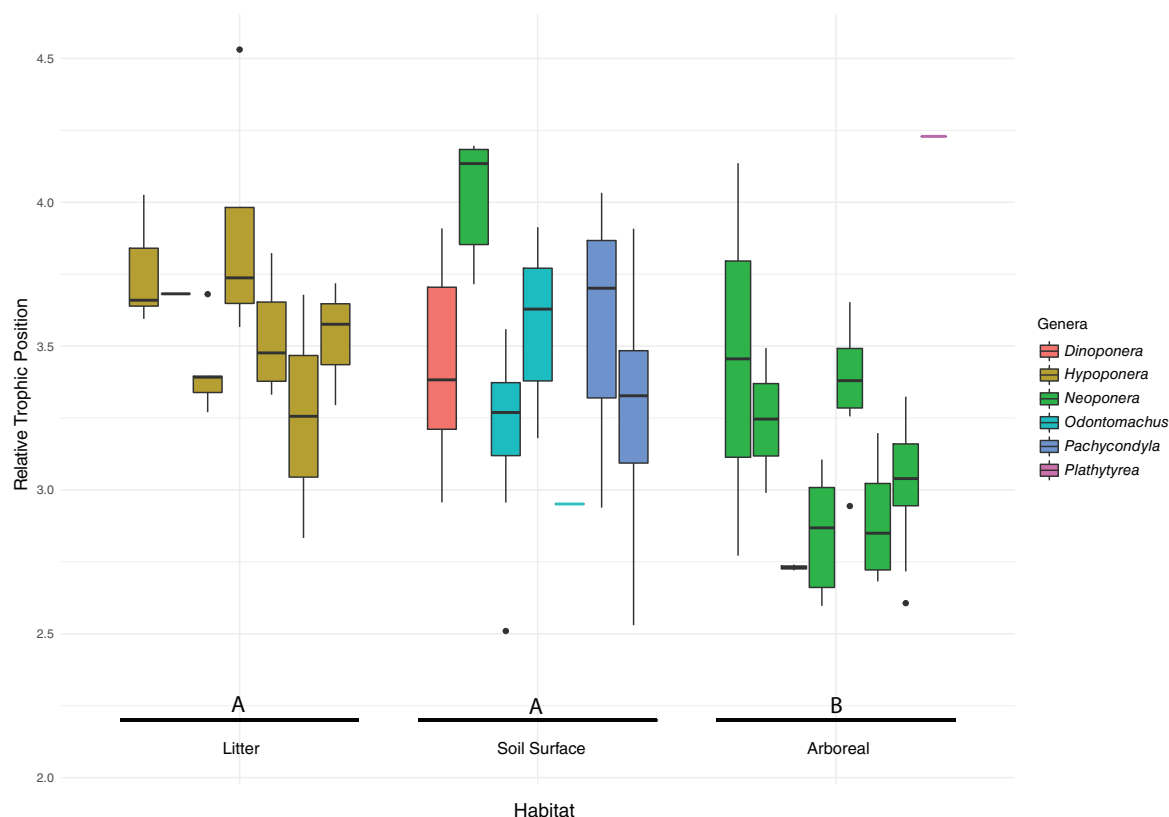
**Table 1.** Relative trophic position of non-ponerine ants.

	Relative TP (average) $\pm$ SD
<i>Gnamptogenys striatula</i>	3.74
<i>Ectatomma edentatum</i>	3.70
<i>Eciton vagans</i>	$3.67 \pm 0.29$
<i>Gnamptogenys tryangularis</i>	3.64
<i>Acanthostichus quadratus</i>	$3.63 \pm 0.01$
<i>Labidus praedator</i>	$3.55 \pm 0.31$
<i>Linepithema</i> (queens)	3.54
<i>Wasmannia rochai</i>	3.54
<i>Nylanderia</i> sp.	3.54
<i>Labidus coecus</i>	$3.51 \pm 0.05$
<i>Nylanderia fulva</i>	3.44
<i>Pheidole rugatula</i>	3.35
<i>Pheidole dinophila</i>	$3.30 \pm 0.09$
<i>Solenopsis richteri</i>	3.29
<i>Brachymyrmex termitophilus</i>	3.24
<i>Neivamyrmex</i> (males)	3.23
<i>Apterostigma</i> sp. (pilosum group)	3.19
<i>Wasmannia auropunctata</i>	3.17
<i>Pheidole fimbriata</i>	3.11
<i>Pheidole</i> PEH06	3.01
<i>Heteroponera dolo</i>	2.98
<i>Camponotus lespesii</i>	2.91
<i>Crematogaster</i> cl. <i>rochai</i>	2.86
<i>Acromyrmex subterraneus</i>	$2.72 \pm 0.36$
<i>Pseudomyrmex gracilis</i>	2.73
<i>Dolichoderus bispinosus</i>	2.72
<i>Acromyrmex laticeps</i>	2.63
<i>Camponotus rufipes</i>	$2.61 \pm 0.57$
<i>Camponotus sericeiventris</i>	$2.61 \pm 0.23$
<i>Crematogaster obscurata</i>	2.60
<i>Pogonomyrmex naegelli</i>	2.55
<i>Crematogaster montezumia</i>	2.55
<i>Cephalotes clypeatus</i>	2.54
<i>Camponotus renggeri</i>	2.52
<i>Camponotus fuscocinctus</i>	$2.35 \pm 0.18$
<i>Camponotus punctulatus</i>	2.35
<i>Atta sexdens</i>	$2.24 \pm 0.06$
<i>Camponotus</i> PEH02	2.21
<i>Camponotus crassus</i>	2.16
<i>Camponotus cingulatus</i>	2.09
<i>Procrystocerus hylaeus</i>	2.09

bigger species tended to obtain energy from a lower trophic position than smaller ones (Spearman correlation,  $\rho = -0.43$ ,  $P = 0.042$ ; Fig. 5).

### DNA barcoding

After discarding sequences  $< 500$  pb, we obtained a total of 114 COI sequences (90% of the ponerine samples with  $\delta^{15}\text{N}$  signatures). Overall, the 22 species were split into 31 BINs. Six species split into two or three BINs: *D. australis* (3), *N. crenata* (2), *Neoponera obscuricornis* (2), *Odontomachus meinerti* (3), *Pachycondyla striata* (3), and *Pachycondyla harpax* (2). We examined whether variation in trophic position within the same species corresponded to the presence of genetic diversity (e.g.



**Fig. 4.** Relative trophic position of the 22 studied species separated by habitat and genera. Habitats that share letters at their bases are not statistically distinguishable from one another based on a Tukey test at the  $P = 0.05$  level. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

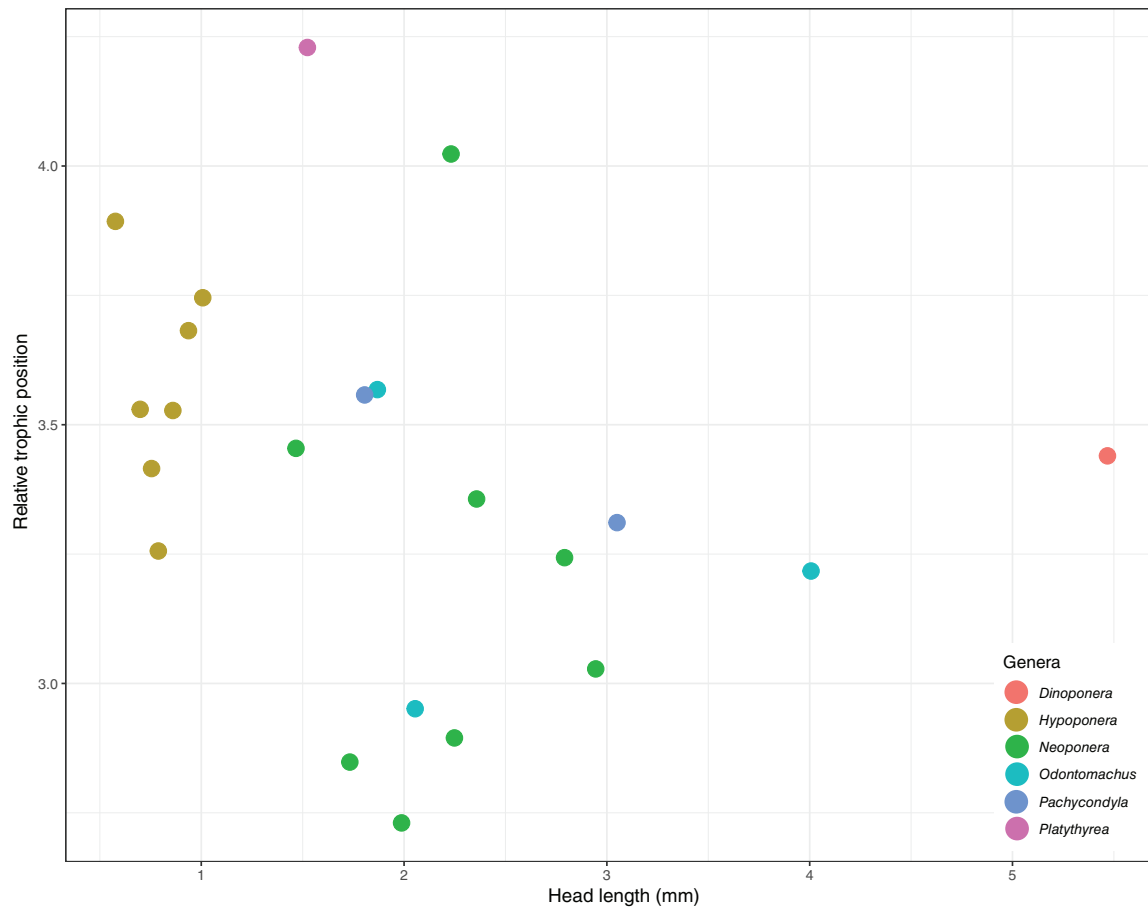
the possibility of cryptic species). However, we did not find any correspondence between BINs and relative trophic position. Instead, workers from different BINs within the same species had the same trophic position (*D. australis* and *O. meinerti*), or colonies with different trophic position cluster in the same BIN (*N. crenata*, *N. obscuricornis*, *P. striata* and *P. harpax*). Some species also had colonies with similar relative trophic positions cluster in the same BIN (*N. fiebrigi*, *H. cf. opacior*, *H. trigona* and *Hypoponera* PEH02) while others had individuals from the same BIN reside in two or three different trophic positions (*N. moesta*, *N. verenae*, *N. curvinodis*, *N. villosa*, *O. chelifera*, *Hypoponera* PEH04, *H. parva* and *H. distinguenda*) (Fig. 6).

## Discussion

We examined the trophic ecology of arthropod communities at two Atlantic Forest sites with the specific goal of elucidating the placement of ants from the subfamily Ponerinae in these food webs. This was accomplished by using the  $\delta^{15}\text{N}$  signature of known herbivores and predators and assuming an average trophic level separation of 3.4‰ (Post, 2002). Twenty-two ponerine species were included in the trophic position analysis, representing c. 60% of their diversity for the region (Hanisch *et al.*, 2015, 2017). As predicted, most ponerines were estimated to be predatory, with  $\delta^{15}\text{N}$  values placing them at TL3 or TL4.

In addition, the  $\delta^{15}\text{N}$  values for *N. marginata*, *P. pilosula* and *H. parva* place them among the top predators of the arthropod food webs at these sites. However, our results revealed that four ponerine species (*N. fiebrigi*, *N. villosa*, *N. verenae* and *N. moesta*) feed at TL2, suggesting they are more dependent on plant-based resources at these sites. We also found a negative relationship between body size and trophic position, a contrast with the positive relationship predicted for primarily predatory taxa. Nonetheless, this pattern is consistent with findings from other taxonomic groups where taxa range from herbivorous to predatory. Finally, our data revealed considerable intraspecific variation in trophic position for a few species; however, this variation was not correlated with an increase in genetic diversity within these taxa as predicted by the presence of cryptic species.

Ants occupied all trophic levels from herbivores to secondary predators (Figs 2,3; Table 1). The position of genera like *Pogonomyrmex* and *Cephalotes* (subfamily Myrmicinae) were not surprising given their known dependence on plants for food. Our results also confirmed previous records of *Camponotus renggeri* and *Camponotus rufipes* feeding mainly on extrafloral nectaries and honeydew-producing insects (Ronque *et al.*, 2018). We found that *Pheidole dinophila* occupied the same trophic level as its host *D. australis* (Table 1). This species lives inside *D. australis* colonies (Wilson, 2003) and has never been seen foraging outside the nest entrance (PEH, pers. obs.), suggesting



**Fig. 5.** Relationship between average relative trophic position and head length (mm) for each species. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].

that *P. dinophila* probably feeds on food brought by *D. australis* foragers.

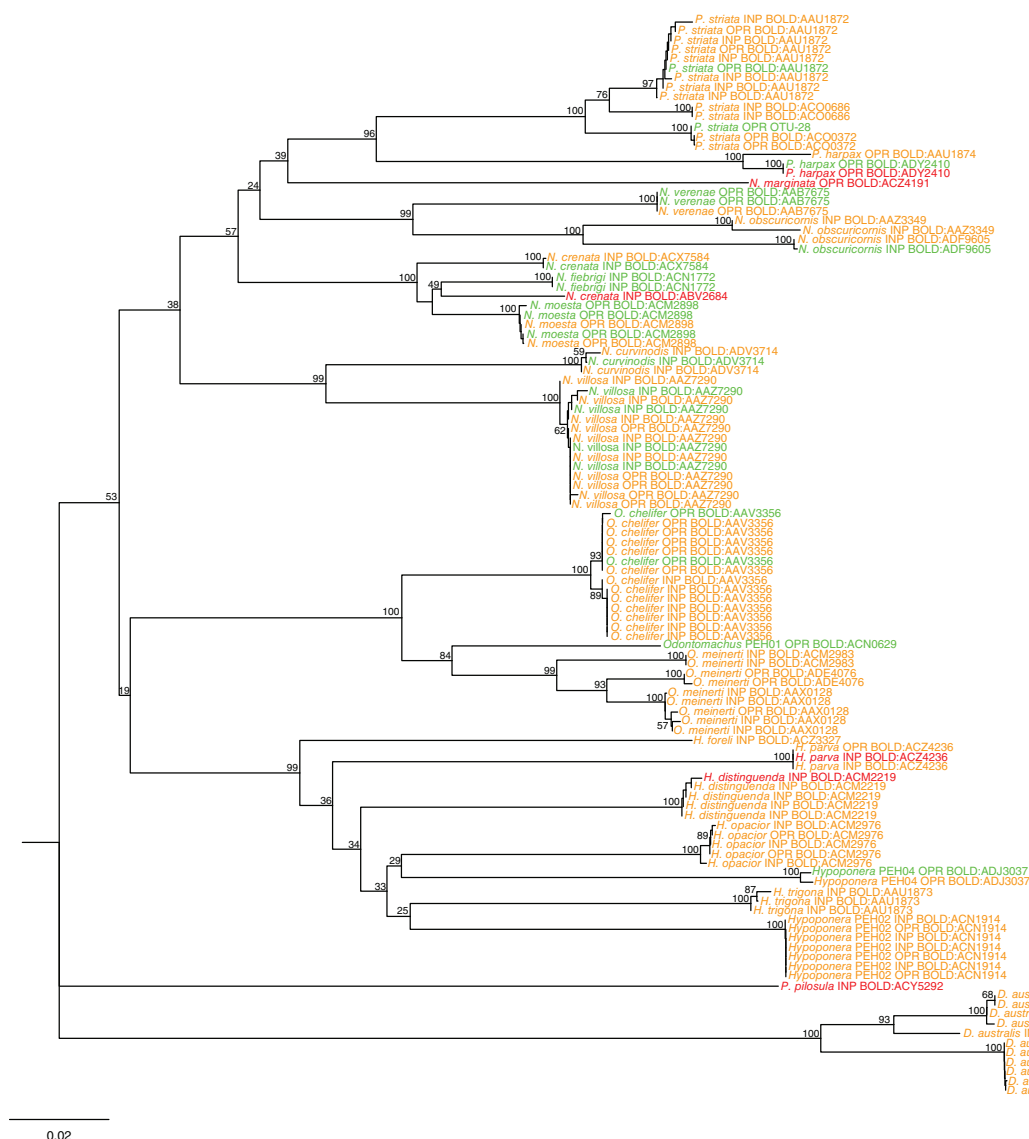
Due to their nomadic colonies and mass raids behaviour, army ants might be the most studied predatory insects of the invertebrate community (e.g. Franks & Bossert, 1983; Kaspari & O'Donnell, 2003; Powell, 2011). Moreover, many species are specialist predators of other ants and almost 50% of *Eciton vagans* raids target ponerine colonies (Hoenle *et al.*, 2019). The current study only included a few of the army ant species that occur at our sites (*E. vagans*, *Labidus praedator*, *Labidus coecus* and *Neivamyrmex* sp.) (Hanisch *et al.*, 2015). Nonetheless army ants occupied TL3 and TL4 (Figs 2, 3; Table 1) and have a  $\delta^{15}\text{N}$  signature range of 9.1–13‰ (INP) and 9.7–11‰ (OPR). In comparison, ponerine  $\delta^{15}\text{N}$  signatures were broader [5.8–15.9‰ (INP) and 5.1–14.2‰ (OPR)] and occupied three trophic levels. The ecological impact of army ants is expected to be significant, e.g. the mean daily prey intake of one colony has been calculated as 38.2 g of dry weight (Powell, 2011).

The volume and identity of prey taken by the ponerines in this study are largely unknown. However, their ecological impact could be high, as some species are locally abundant (Hanisch *et al.*, 2018). Interestingly, some of the species with highest  $\delta^{15}\text{N}$  signature may represent specialist predators. For

example, *N. marginata* are specialists feeding only on the termite *Neocapritermes opacus* (Leal & Oliveira, 1995). In the case of *P. pilosula*, termites and other food items were offered to foraging workers on the trees in an attempt to localise the entrance colony; none of the food items were accepted, and captured individuals died shortly afterwards, suggesting that this species might have a specialised diet (*sensu* Jacquemin *et al.*, 2014). Another ponerine with highly specialised foraging, the millipede specialist *Thaumatomyrmex mutilatus*, also occurs at our site, but unfortunately we did not find workers to include in this analysis. Future studies examining the isotopic composition and relative trophic position of additional dietary specialists will continue to add to our understanding of how ants help to structure invertebrate food webs.

We found that the relative trophic position of ponerine ants varied among habitats. While we found no differences in average trophic position of species from the leaf litter or the soil surface, arboreal species tended to have lower  $\delta^{15}\text{N}$  values (Figs 2–4). This pattern was primarily driven by the genus *Neoponera* and suggests it may incorporate more plant-based resources into their diet (e.g. nectar or honeydew). This is in agreement with the suggestion that arboreal habitats are protein-limited in the tropics (Yanoviak & Kaspari, 2000). It also indicates





**Fig. 6.** Neighbour-joining (NJ) tree of 114 cytochrome *c* oxidase subunit 1 (COI) sequences corresponding to voucher specimens from stable isotope study computed with a Kimura two-parameter substitution model. All samples correspond to different colonies. Numbers on the nodes are NJ bootstrap support values based on 1000 pseudoreplicates. Species identified by taxonomy, locality and barcode index number (BIN) code are shown. Colours correspond to relative trophic level of the samples. Herbivore (level 1), green; primary predators (level 2), yellow; secondary predators (level 3), red. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].

that the *Neoponera* genus could be phylogenetically constrained towards a herbivorous diet. Moreover, one *N. villosa* colony was collected on a *Cecropia* tree, a plant known for its mutualistic association with other ant species, providing them with housing and food resources (Berg *et al.*, 2005). However, the remaining colonies were found in other unidentified tree species, and hence this association is not as exclusive as seen in other *Neoponera* species, such as *N. luteola* (Davidson & Fisher, 1991).

We also found considerable variation in the range of  $\delta^{15}\text{N}$  values of the plant samples between two sites which affected primary consumers' isotopic composition (TL 2), but these differences did not persist for TL3 (Figs 2,3). This variability is

common in plants and may result from variation in precipitation, nitrogen fixation, mycorrhizae or microbial processes between sites (Handley & Raven, 1992; Austin & Vitousek, 1998; Evans, 2001).

Bigger ponerine ants tended to occupy lower trophic positions than smaller ones. This pattern may hold for ants generally, as many large species (e.g. from the genera *Camponotus*, *Atta*, *Pogonomyrmex*, *Paraponera*) often feed on plant-based resources. A notable outlier in this study was *D. australis*, which is the largest ant in the community and had a trophic position between TL3 and TL4. A possible explanation for this pattern is that bigger species require more resources, and more resources

can be found in lower trophic levels (Pimm & Lawton, 1977). The biggest ants in this study (*D. australis* and *P. striata*) are also two of the most abundant ant species in INP (Hanisch *et al.*, 2018). As generalist predators, these two species play an important role in their ecosystem – probably via top-down effects, by consuming the most abundant prey and preventing single species from monopolising resources, which leads to a higher local species diversity (Paine, 1966). Furthermore, their large size may allow them to take advantage of prey that are not usually part of invertebrate food webs, such as small vertebrates. For example, *D. australis* has been observed preying on small frogs and even a hatchling of the bird *Nyctidromus albigollis* (Moresco, 2018). This extreme diet breadth may also contribute to the large size and abundance of many army ant species in tropical communities.

Diet frequently varies among individuals within the same species (Tillberg & Breed, 2004; Roeder & Kaspari, 2017). For example, Tillberg and Breed (2004) found intraspecific variation in  $\delta^{15}\text{N}$  signatures of 2.38‰ in *Paraponera clavata*. This variation could be related to colony age, developmental stage, or the proximity of resources (Tillberg & Breed, 2004; Tillberg *et al.*, 2007; Smith & Suarez, 2010; Smith *et al.*, 2011). Alternatively, diet differences may be due to ecological variation between morphologically similar cryptic species. Many genera in the subfamily Ponerinae are suspected to include unresolved species complexes (Lucas *et al.*, 2002; Wild, 2002; Delabie *et al.*, 2008; Ferreira *et al.*, 2010). We used DNA barcoding to look for evidence of cryptic diversity that could explain high intraspecific variation of  $\delta^{15}\text{N}$ . However, we found no correspondence between different BINs and trophic position within the same species (Fig. 6). This suggests that in species occupying several trophic levels (e.g. *N. crenata* and *P. harpax*), individual colonies may specialise in specific resources based on their availability or colony demography (Tillberg & Breed, 2004).

Our study examined the ecology of the predatory ant subfamily Ponerinae, confirming that most are generalist predators. However, both microhabitat in the forest and body size predicted variation in relative trophic position; arboreal taxa and species with larger workers appeared to depend more on plant-based resources in this tropical ecosystem. Possible future research could focus on the study of the ecological impact of this group and the combination of DNA barcode and prey remains, in order to investigate in more detail the diet of these organisms, especially in those species with cryptic habits.

## Acknowledgements

The authors thank the Administración de Parques Nacionales de Argentina, Fundación Temaikén and Ministerio de Ecología y Recursos Naturales Renovables y Turismo de la provincia de Misiones. Special thanks to Facundo Tejeda Cajas, Guillermo Gil, Marcelo Cavicchia, Verónica Bernava, Dalma Raymundo and Paola Favre for administration guidance and support. Collection permit numbers were NEA 353 Rnv1 and Rnv2. We also thank Cristian Grismado, Abel Pérez González, Diego Carpintero, Fabián Gatti, Sam W. Heads and Braun Holger for help in identifying non-ant material. The authors are grateful to two

anonymous reviewers for their insightful and helpful comments on an earlier version of this manuscript. PEH gives special thanks to Joshua C. Gibson and Janice Jayes for the enormous help received during her stay at the University of Illinois at Urbana-Champaign and to Carolina I. Paris for help during the collection of the samples in OPR. We also thank Jonathan Treffkorn and Chad Tillberg for assisting with the stable isotope analyses. The authors declare there are no conflicts of interest.

## Supporting Information

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

**Table S1.** For stable isotope analysis, colony and number of individuals are provided for each sample.

**Table S2.** Details of the samples used for stable isotope analysis. Voucher specimens for some samples were included for DNA barcode analysis to improve their taxonomical identification; for these samples, the associated sample ID is shown.

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Accepted 30 September 2019

First published online 1 November 2019

Associate Editor: Simon Robson