MOLECULAR ECOLOGY

Supplemental Information S4

Species-level predation network uncovers high prey specificity in a Neotropical army ant community

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Content:

Figure S1. Sampling effect in army ant-prey network. Influence of rarefaction of sampling effort on calculations of (A) network-level specialization H_2' and (B) modularity metric Q. The number of interactions has been reduced by randomly deleting one interaction per army ant species per deletion step. Black dot shows H_2' and Q of the original army ant-prey matrix, red dots the effect of down-sampling (50 randomizations per step).

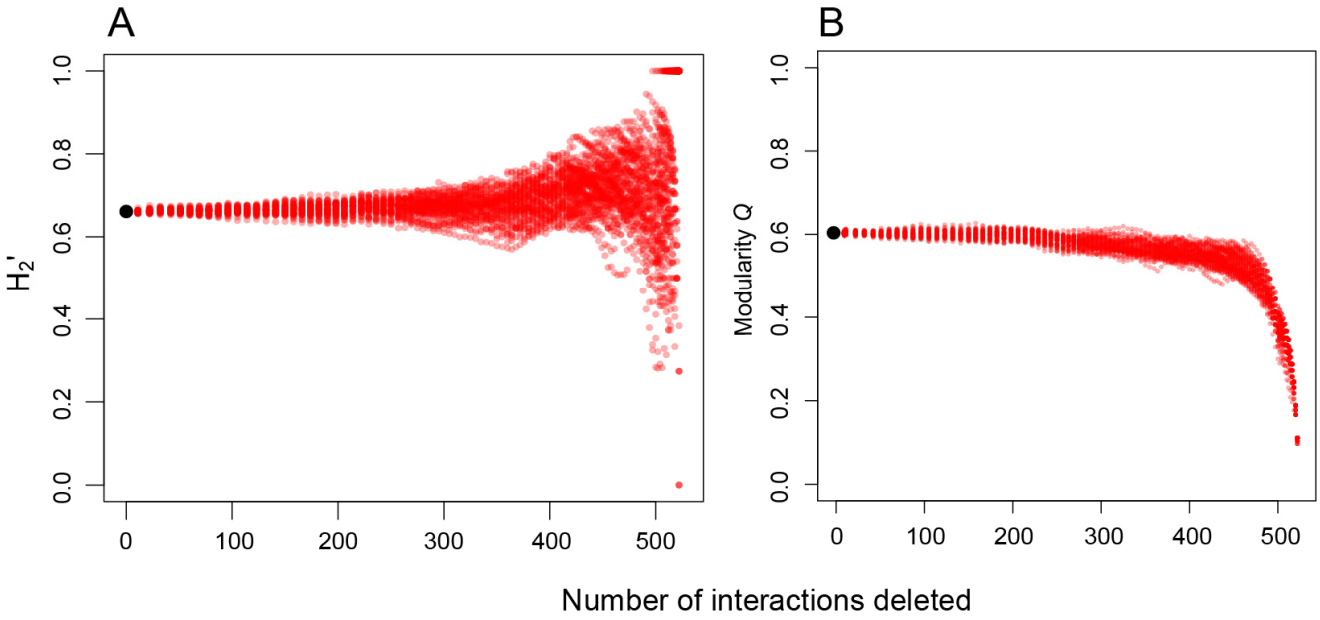

Figure S2. Collection events per time of the day. Histogram showing the number of collection events per hour of the day. Details about the time of the day for each collection event and species is given in Supporting information S2.

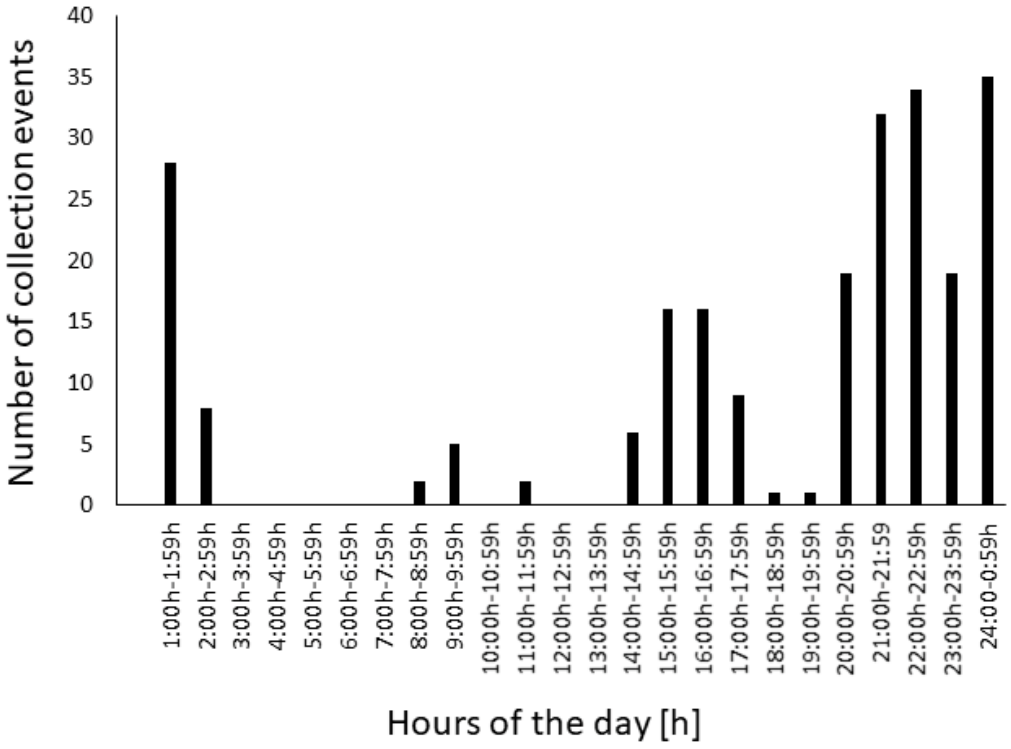

Figure S3. Prey composition. Composition of developmental stages of ant prey items. Different shades of gray depict adult, egg, larva and pupal stages. Total number of ant prey items per species is shown in parentheses.

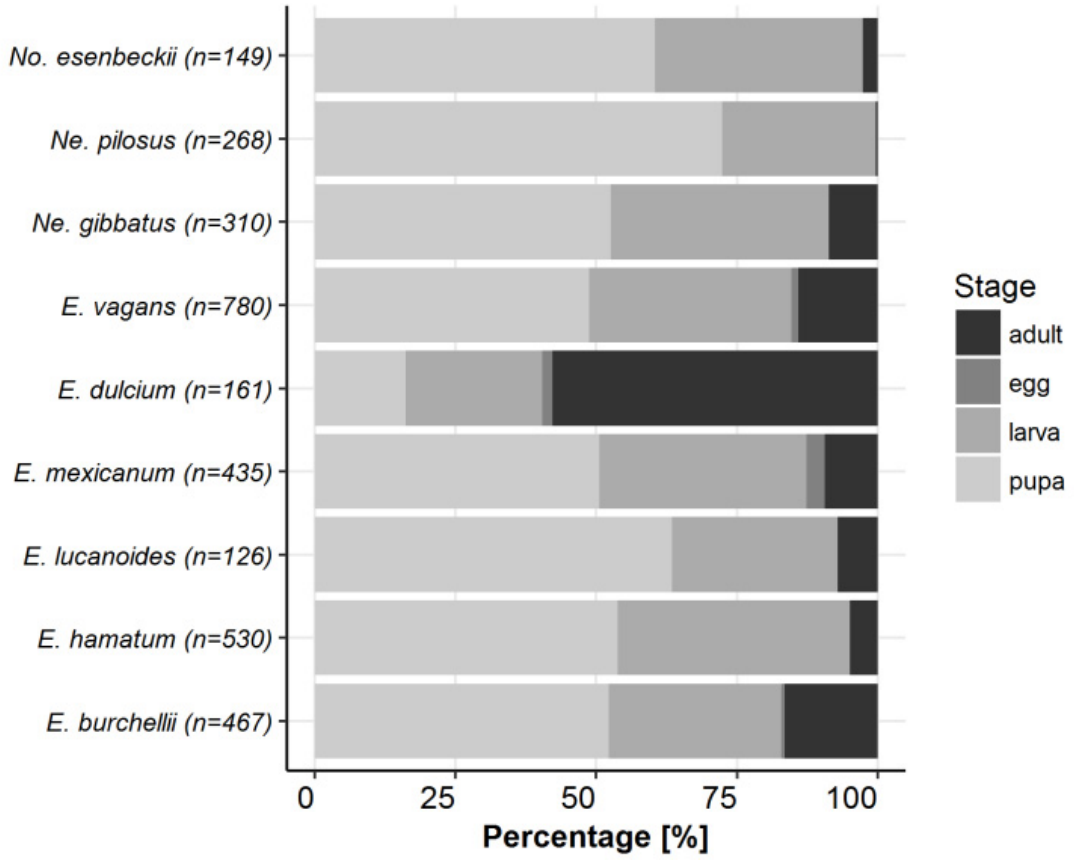

Figure S4. Distribution of *COI* barcode length fragments in base pairs.

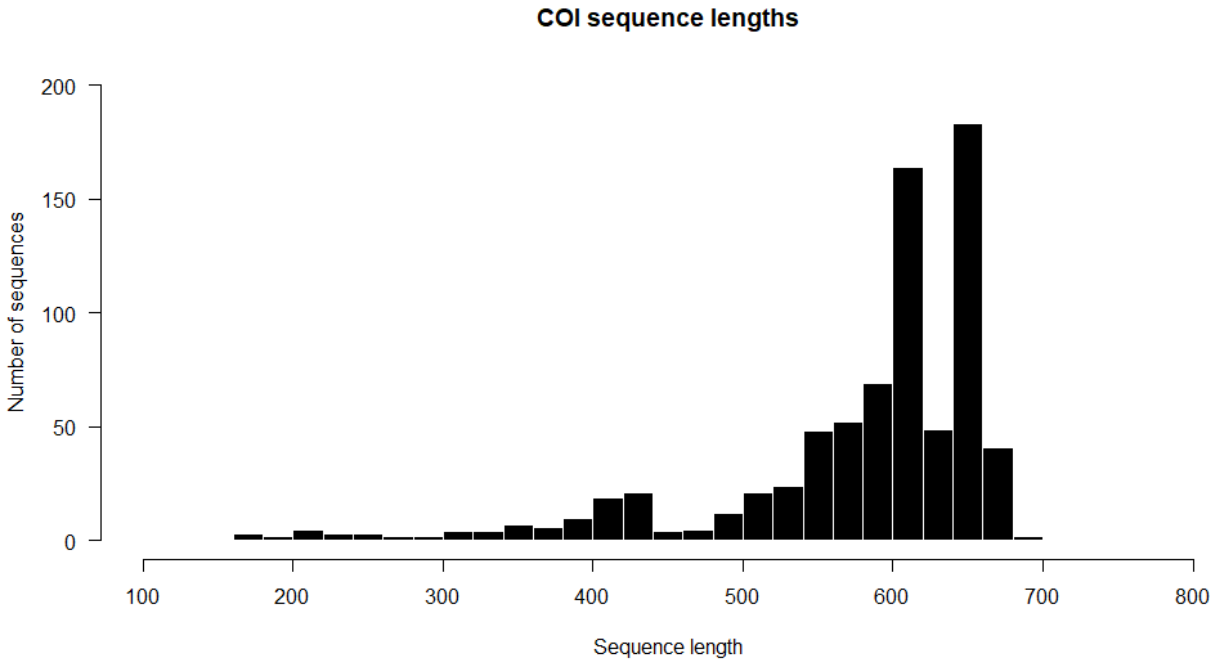

Figure S3. Possible intraguild predation in *Neivamyrmex*. We observed a case of possible intraguild predation at La Selva during the night of the 6th of April 2017 at 01:00h. *Neivamyrmex* cf. *iridescens* was swarming, seemingly uncoordinated, over a larger area. Workers carried their brood around and placed many of them in open brood caches. It first looked like an emigration to us, but less coordinated. Then we noted a second army ant species at the scene, *Neivamyrmex* cf. *swainsonii*. Smaller fights between both species were observed, and several *Ne.* cf. *swainsonii* workers carried away brood of *Ne.* cf. *iridescens*. Because we only observed this once, we cannot conclude with certainty that intraguild predation is a common behavior in this species. More detailed observations of the food habits in both species, *Neivamyrmex* cf. *swainsonii* and other *Neivamyrmex* species are urgently needed. Left: *Neivamyrmex* cf. *iridescens* with brood pile. Right: Fight of *Ne.* cf. *swainsonii* worker with *Ne.* cf. *iridescens* worker.

Table S1. *COI* forward and reverse primer combinations used in this study. Primer combinations with successful *COI* amplification are given for each ant genus. Primer combinations with most reliable *COI* amplification are marked with an asterisk and highlighted in bold. Annealing temperatures varied between 45°C and 55°C.

Ant genus	Primer combinations (Forward primer /reverse primer)
Acanthognathus	LCO1490 / HCO2198
Acromyrmex	**LCO1532 / HCO2198*,** LCO1532 / HCO1938, LCO1532 / AcromyR1
Anochetus	dgLCO1490 / CrematoR1, **LCO1490 / HCO2198*,** LCO1490 / dgHCO2198, LepF1 / LepR1, MLepF1 / LepR1
Aphaenogaster	LCO1490 / HCO2198
Apterostigma	dgLCO1490 / CrematoR1, dgLCO1490 / dgHCO2198, **LCO1490 / HCO2198*,** LepF1 / LepR1
Azteca	dgLCO1490 / AcromyR1, dgLCO1490 / CrematoR1, **LCO1490 / HCO2198***
Basiceros	LepF1 / LepR1
Camponotus	CampoF2 / CampoR2, **CampoF2 / dgHCO2198*,** CampoF2 / HCO2198, dgLCO1490 / AcromyR1, dgLCO1490 / CrematoR1, dgLCO1490 / dgHCO2198, **LCO1490 / HCO2198*,** LCO1490 / dgHCO2198, MLepF1 / LepR1
Crematogaster	**dgLCO1490 / CrematoR1***, dgLCO1490 / LepR1, LCO1490 / CrematoR1, LepF1 / CrematoR1, LCO1490 / HCO2198, LepF1 / LepR1
Cyphomyrmex	**dgLCO1490 / CrematoR1***, **LCO1490 / HCO2198***, LepF1 / LepR1
Dolichoderus	LCO1490 / HCO2198
Ectatomma	**LCO1490 / HCO2198***, LepF1 / LepR1, MLepF1 / LepR1
Gnamptogenys	dgLCO1490 / CrematoR1, LCO1490 / dgHCO2198, **LCO1490 / HCO2198***, LepF1 / LepR1,
Hypoponera	LCO1490 / HCO2198
Mayaponera	dgLCO1490 / CrematoR1, **LCO1490 / HCO2198***, LepF1 / LepR1
Megalomyrmex	LCO1490 / HCO2198
Neoponera	CampoF2 / dgHCO2198, dgLCO1490 / CrematoR1, LCO1490 / dgHCO2198, **LCO1490 / HCO2198***, LepF1 / LepR1, MLepF1 / LepR1
Nylanderia	CampoF2 / dgHCO2198, **dgLCO1490 / CrematoR1***, **LCO1490 / HCO2198***, LepF1 / LepR1
Odontomachus	CampoF2 / dgHCO2198, dgLCO1490 / CrematoR1, LCO1490 / dgHCO2198, **LCO1490 / HCO2198***, LepF1 / LepR1
Pachycondyla	dgLCO1490 / CrematoR1, **LCO1490 / dgHCO2198***, **LCO1490 / HCO2198***, LepF1 / LepR1, MLepF1 / LepR1,
Pheidole	dgLCO1490 / AcromyR1, dgLCO1490 / CrematoR1, **LCO1490 / HCO2198***, LCO1490 / dgHCO2198, LepF1 / LepR1, MLepF1 / LepR1, MLepF1 / dgHCO2198
Platythyrea	**LCO1490 / HCO2198***, LepF1 / LepR1, MLepF1 / LepR1
Sericomyrmex	LepF1 / LepR1, **MLepF1 / LepR1***
Solenopsis	dgLCO1490 / CrematoR1, LCO1490 / HCO2198
Strumigenys	**dgLCO1490 / CrematoR1***, MLepF1 / LepR1, LCO1490 / dgHCO2198, **LCO1490 / HCO2198***
Tapinoma	LCO1490 / HCO2198
Trachymyrmex	dgLCO1490 / CrematoR1, LCO1490 / dgHCO2198, **LCO1490 / HCO2198***, MLepF1 / LepR1
Wasmannia	dgLCO1490 / CrematoR1, **LCO1490 / HCO2198***

Table S2. *COI* primers used in this study.

Primer name	Forward/ reverse primer	Sequence (5' –3')	Source
AcromyR1	Reverse	CTCCGGCAAGAACGRGRAGGGAAAGRA	This study
CampoF2	Forward	TTTGCAATYTGATCWGGWATAATTGGATC	This study
CampoR2	Reverse	CCYCCYCCBGAWGGRTCRAARAA	This study
CrematoR1	Reverse	GGRTCTCCYCCTCCDGMDGGRTC	This study
dgLCO1490	Forward	GGTCAACAAATCATAAAGAYATYGG	[1]
dgHCO2198	Reverse	TAAACTTCAGGGTGACCAAARAAYCA	[1]
LCO1490	Forward	GGTCAACAAATCATAAAGATATTGG	[2]
LCO1532	Forward	TCAGGTATAGTAGGATCTGCCA	C. Rabeling
HCO1938	Reverse	GCACCAAGAATGGATGATATACCTGC	C. Rabeling
HCO2198	Reverse	TAAACTTCAGGGTGACCAAAAAATCA	[2]
LepF1	Forward	ATTCAACCAATCATAAAGATATTGG	[3, 4]
LepR1	Reverse	TAAACTTCTGGATGTCCAAAAAATCA	[3, 4]
MLepF1	Forward	GCTTTCCCACGAATAAATAATA	[4]

References

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2. Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. DNA primers for amplification of mitochondrial *cytochrome c oxidase subunit I* from diverse metazoan invertebrates. *Mol Marine Biol Biotechnol*. 1994;3:294–9.

3. Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *PNAS*. 2004;101:14812–7.

4. Hajibabaei M, Janzen DH, Burns JM, Hallwachs W, Hebert PDN. DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings of the National Academy of Sciences of the United States of America*. 2006;103:968–971.

Table S3. DNA barcode identification of non-ant prey. Non-ant barcodes were compared to the reference library of the 'Barcode of Life Data' System (BOLD) in September 2018. GenBank accession numbers of non-ant barcodes are given in Supporting information S2. We added several non-ant prey items that we obtained from a preliminary study about army ant diets at the same collection site (all sample IDs starting with the code 'cvb'; for details see Supporting information S2).

Sample ID	Best hit in BOLD	Order	Family	Similarity to reference [%]	GenBank/BOLD number	Collected from…
PH_EB58_ca_l_02	*Metapolybia cingulata*	Hymenoptera	Vespidae	92.59	GU596904	*Eciton burchellii*
PH_EB58_undet_np_03	*Synoeca septentrionalis*	Hymenoptera	Vespidae	92.76	Early release	*Eciton burchellii*
PH_EB64_nonant_l_05	*Scolopocryptops mexicanus*	Scolopendromorpha	Scolopocryptopidae	84.56	JX422679	*Eciton burchellii*
cvb777prey022	*Digiteps indicus*	Scolopendromorpha	Scolopendridae	84.26	JX531857	*Eciton burchellii*
cvb777prey006	*Rhiginia cinctiventris*	Hemiptera	Reduviidae	99.37	CNCHB1508	*Eciton burchellii*
cvb777prey015	*Labiduridae*	Dermaptera	Labiduridae	87.93	Private	*Eciton burchellii*
cvb709prey003	Blattodea	Blattodea	n.a.	83.75	Private	*Eciton burchellii*
cvb709prey001	*Eublaberus posticus*	Blattodea	Blaberidae	91.39	MF136388	*Eciton burchellii*
cvb708prey002	Ectobiidae	Blattodea	Ectobiidae	99.66	Private	*Eciton burchellii*
cvb777prey013	Scaraberiidae	Coleoptera	Scaraberiidae	90.10	Private	*Eciton burchellii*
cvb777prey014	Carabidae	Coleoptera	Carabidae	100.00	Private	*Eciton burchellii*
cvb777prey004	Fulgoridae	Hemiptera	Fulgoridae	100.00	Private	*Eciton burchellii*
cvb595prey002	*Panchlora* sp. CC-2017	Blattodea	Blaberidae	98.31	KY741983	*Eciton dulcium*
PH_EH60_undet_l_02	Phoridae	Diptera	Phoridae	98.63	Early release	*Eciton hamatum*
cvb011prey004	*Ugyops*	Hemiptera	Delphacidae	79.47	Early release	*Eciton hamatum*
cvb400prey001	*Protopolybia*	Hymenoptera	Vespidae	89.09	AY918914	*Eciton hamatum*
PH_EL34m_ca_l_01	*Synoeca septentrionalis*	Hymenoptera	Vespidae	93.37	Early release	*Eciton lucanoides*
PH_EM49_ec_l_03	*Metapolybia cingulata*	Hymenoptera	Vespidae	92.79	GU596904	*Eciton mexicanum*