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No guts, no glory: Gut content metabarcoding unveils the diet of a flower-associated coastal sage scrub predator

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Citation: Masonick, P., M. Hernandez, and C. Weirauch. 2019. No guts, no glory: Gut content metabarcoding unveils the diet of a flower-associated coastal sage scrub predator. Ecosphere 10(5):e02712. 10.1002/ecs2.2712

Abstract. Invertebrate generalist predators are ubiquitous and play a major role in food-web dynamics. Molecular gut content analysis (MGCA) has become a popular means to assess prey ranges and specificity of cryptic arthropods in the absence of direct observation. While this approach has been widely used to study predation on economically important taxa (i.e., pests) in agroecosystems, it is less frequently used to study the broader trophic interactions involving generalist predators in natural communities such as the diverse and threatened coastal sage scrub communities of Southern California. Here, we employ DNA metabarcoding-based MGCA and survey the taxonomically and ecologically diverse prey range of *Phymata pacifica* Evans, a generalist flower-associated ambush bug (Hemiptera: Reduviidae). We detected predation on a wide array of taxa including beneficial pollinators, potential pests, and other predatory arthropods. The success of this study demonstrates the utility of MGCA in natural ecosystems and can serve as a model for future diet investigations into other cryptic and underrepresented communities.

Key words: biodiversity; blocking primers; DNA detectability half-life; *Eriogonum fasciculatum;* food webs; intraguild predation; natural enemies.

Received 24 January 2019; accepted 11 February 2019. Corresponding Editor: Karen A. Haubensak.

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INTRODUCTION

Predatory arthropods can have profound impacts on pollinator–plant communities as their presence may alter the behavior and abundance of other flower-visiting insects and indirectly affect plant fitness (Dukas 2005, Jones 2010, Wirsing et al. 2010, Huey and Nieh 2017). Generalist ambush predators can engage in a variety of trophic interactions, ranging from direct predation on both pests and beneficial organisms to complex trophic cascades through intraguild predation (Polis and McCormick 1987, Rosenheim et al. 1993, Finke and Denno 2004, Gagnon et al. 2011). A refined understanding of trophic interactions not only has implications for pest management, but also has implications for the conservation of biodiversity and endangered species (Polis and Holt 1992, Bampfylde and Lewis 2007, Hurd 2008, Gagnon et al. 2011, Chisholm et al. 2014). Fundamentally, it enables biologists to unravel the complex dynamics and functions of ecosystems (Agrawal 2000).

While much research has been devoted to natural enemies that specialize on pests (Sheehan 1986, Landis et al. 2000, Snyder and Ives 2003, Choate and Lundgren 2015, Morgan et al. 2017), trophic interactions involving generalist arthropod predators in natural systems remain vastly understudied. One such system is the coastal sage scrub (CSS) or soft chaparral of Southern California. With a great diversity of endemic flora and fauna, this habitat covers lower elevation portions of a complex Mediterranean-type scrub ecoregion that is part of one of Earth's biodiversity hotspots

(Cowling et al. 1996, Myers et al. 2000). Once widespread, CSS communities have become increasingly fragmented and altered through anthropogenic disturbance and the introduction of non-native species over the past two centuries (Westman 1981, Minnich and Dezzani 1998, Lambrinos 2000). As a result, many endemics of CSS have become rare and some even endangered such as the California gnatcatcher, *Polioptila californica californica* Brewster (McCormack and Maley 2015), and the Quino checkerspot butterfly, *Euphydryas editha editha* (Boisduval; Parmesan et al. 2015).

Coastal sage scrub and surrounding communities support a particularly high number of unique arthropods. Approximately 500 native bee species have been documented here (Michener 1979), many of which provide important services for natural, urban, and agricultural landscapes (Kremen et al. 2002*b*, Hernandez et al. 2009). Despite recent concerns surrounding the general decline of pollinators (Committee on the Status of Pollinators in North America 2007, Potts et al. 2010), little is known about the current status of native pollinator populations in CSS. The fitness of many flowering plants relies largely on interactions with pollinating insects; mutually, many insects require pollen and/or nectar from flowers for sustenance and development (Tepedino 1979, Kearns and Inouye 1997). Flowering plants which have evolved mutualistic relationships with specific pollinating insects are particularly vulnerable to environmental changes (Gilman et al. 2012), and a reduction in the services provided by a unique pollinator can negatively impact plant populations (Wilcock and Neiland 2002, Romero and Koricheva 2011). Predatory arthropods are also diverse and ubiquitous in CSS and influence ecosystem dynamics (Burger et al. 2001, 2003). Predation on pollinators may have strong indirect effects given that pollinators provide essential services for natural communities and help maintain healthy ecosystems (Klein et al. 2007). The occurrence of predators on flowers may reduce pollinator visitation, causing some pollinators to spend less time at or avoid certain flowers, or potentially diminish the numbers of pollinators that share mutualisms with rare native plants (Elliott and Elliott 1991, 1994, Reader et al. 2006, Romero et al. 2011, Tan et al. 2013).

California buckwheat, *Eriogonum fasciculatum* Bentham (Polygonaceae), is a dominant and widespread perennial of CSS that serves as an important resource for many arthropods including over 30 bee species (Kremen et al. 2002*a*, Montalvo and Beyers 2010). This plant is commonly, although patchily, frequented by *Phymata pacifica* Evans (Hemiptera: Reduviidae), an ambush bug native to CSS and a presumed generalist predator of other flower-associated arthropods. Like crab spiders (Thomisidae; Llandres and Rodríguez-Gironés 2011, Llandres et al. 2013, Huey and Nieh 2017) and weaver ants (Gonzálvez et al. 2013), ambush bugs can alter the foraging behavior of other flower visitors. For example, pollinators will spend significantly less time foraging on flowers harboring these ambush predators than on vacant flowers (Elliott and Elliott 1991, 1994). Given their flower-dwelling niche, a diverse range of prey taxa are available to ambush bugs in CSS. Despite this, the composition of their diet remains unclear.

Over the past two decades, novel methods to delineate food-web linkages have been devised that eliminate the need for direct observation or the visual inspection of gut or fecal matter (King et al. 2008, Pompanon et al. 2012, González-Chang et al. 2016, Birkhofer et al. 2017). Among the most commonly applied and successful approaches for gathering qualitative prey data is DNA-based MGCA; Šerić Jelaska et al. 2014, Rondoni et al. 2015, Roubinet et al. 2015, Schmidt et al. 2016, Curtsdotter et al. 2018, Eitzinger et al. 2018). This method provides a reliable means to examine the diets of small, cryptic arthropods that pre-orally digest their food such as spiders and true bugs (Heteroptera). To capture the wide taxonomic range of a generalist predator's diet, DNA metabarcoding can be used to accumulate large amounts of prey data (Ji et al. 2013, Brandon-Mong et al. 2015). In metabarcoding, large numbers of amplicon sequences are derived via high-throughput sequencing and compared to existing barcode databases for identification (Blaxter 2016). Of studies on terrestrial arthropods that have utilized MGCA, many have focused on one or several specific prey taxa (often pests) and relied on prey-specific primers to determine which natural enemies are consuming those taxa in agroecosystems (Harwood et al. 2007, Fournier et al. 2008, Juen et al. 2011, Szendrei et al. 2014, Gomez-Polo et al. 2015). A disproportionately small number of studies have attempted to assess the diet range of generalist predators in natural communities (Šerić Jelaska et al. 2014).

For this study, our attention focused on four main objectives: I. determine whether native bees constitute the main group of prey of ambush bugs in a CSS community; II. document the diet breadth of *P. pacifica* with respect to (1) the taxonomic diversity of prey (i.e., the number of different families, genera, and species consumed) and (2) the trophic category of prey (pollinators, herbivores, or entomophagous) found on the dominant host plant, *E. fasciculatum*, and search for any indication that certain arthropod groups also associated with California buckwheat are absent from their diet; III. estimate the detectability half-life of DNA recovered from the guts of *P. pacifica;* and IV. employ MGCA using DNA metabarcoding and test the effectiveness of a predator-specific blocking primer.

MATERIALS AND METHODS

Field sampling and specimen vouchering

We collected *Phymata pacifica* specimens from two field sites along Lytle Creek in San Bernardino National Forest over three visits during late June and early July of 2016. Each of the field sites was visited at least once in the morning (08:00–12:00 hours) and again at least once in the afternoon (12:00–16:00 hours). We exclusively sampled from *Eriogonum fasciculatum*. Anticipating taxonomic gaps among the barcode sequences available online for local fauna, we collected other buckwheat-associated arthropods for sequencing by means of beating, sweeping, and aerial netting on and around blooming flowers. Upon capture, *P. pacifica* specimens were immediately placed into separate vials containing 95% ethanol and cooled with dry ice. In the laboratory, all *P. pacifica* specimens were stored in a −80°C freezer to retard DNA degradation until they could be dissected. All dissected ambush bugs were given unique specimens identifier numbers and databased using the Plant Bug Planetary Biodiversity Inventory instance of the Arthropod Easy Capture Specimen Database (http://www.research.amnh.org/pbi/locality/index.php) and deposited in the Entomology Research Museum at the University of California, Riverside (UCR). Specimen information can be accessed through the Heteroptera Species Pages (http://research.amnh.org/pbi/heteropteraspeciespage). Non-reduviid buckwheat-associated arthropods were also mounted and identified to the lowest taxonomic level possible using reference specimens from UCR's Entomology Research Museum, online searches (BugGuide.net), taxonomic keys (Goulet and Huber 1993, Triplehorn and Johnson 2005, Lawrence et al. 2010), and advice from specialists of various groups. These specimens were also deposited in the Entomology Research Museum at UCR.

Gut and DNA extraction

Sterilized forceps were used to remove mid- and hindguts from 225 specimens, and each was placed into individual crosslinked 1.5-mL Eppendorf tubes and homogenized using sterile pestles. DNA extraction was conducted using a QIAGEN DNeasy Blood and Tissue Kit. DNA was also extracted from the legs of 60 non-reduviid buckwheat-associated arthropods to construct a de novo COI reference library (hereafter denoted as our "local database" or "LocalDB") for taxa for which COI barcoding sequences were unavailable from BOLD or GenBank (as of January 2019).

Primer design, PCR, and sequencing

Major challenges include finding a set of universal primers that can accommodate the entire, often unknown, prey range, and limiting the amplification of predator DNA so that signal from the prey is not overwhelmed. To address the first issue, we used a universal primer pair that amplifies a 313-bp sequence of the COI barcoding region: mlCOIintF (Leray et al. 2013) and HCO-2198 (Folmer et al. 1994). When used in tandem, these primers can amplify a wide range of metazoan taxa (Leray et al. 2013). To overcome the second challenge, predator-specific blocking primers were developed and added to the PCR cocktail to limit non-target DNA amplification. These oligonucleotides contain a C3 spacer at their 3′ end that inhibits polymerization during the elongation phase of PCR (Vestheim and Jarman 2008). The program PrimerMiner (Elbrecht et al. 2017) facilitated selection of these oligonucleotides over other possible primer pairs and design of a blocking primer for this study. In the process of developing a blocking primer, we downloaded all available Lepidoptera, Hymenoptera, and Diptera (known prey groups of *P. pacifica*) COI barcode sequences from NCBI and BOLD (available as of June 2016) and compared these sequences with that of *P. pacifica* to find a region suitable for a blocking primer. We designed a blocking primer (mlCOIintF-BLK-Phymata: 5′-TCCACCACTATC AAGAAATCTTGC/3SpC3/-3′) that contains a C3 spacer at its 3′ end to inhibit elongation of *P. pacifica* DNA. This oligonucleotide competes for binding sites with the mlCOIintF primer in its 5′ region and spans into a *Phymata*-specific region along its 3′ end. Since test PCR trials and Sanger sequencing demonstrated that the *P. pacifica*-specific blocking primer does limit the amplification of non-target host DNA (Appendix S1: Fig. S1), this oligonucleotide was used in conjunction with fusion primers that contain Illumina adaptor sequences at their 5′ ends and the universal primers mentioned above at their 3′ ends (see Appendix S1: Table S1 for primer information) during the initial round of PCR for metabarcoding.

To generate amplicons during the first round of PCR, we used a touchdown protocol with the following conditions: initial denaturation for 5 min at 95°C, followed by denaturation for 30 s at 95°C, and then annealing starting at 62°C for 30 s and decreasing by 1°C over 16 subsequent cycles until reaching a minimum annealing temperature of 46°C, with intervening extension phases run for 60 s at 68°C. Once an annealing temperature of 46°C was reached, we then continued with a 95°C–48°C–68°C regime for 24 cycles and ended with a final 7 min of extension phase at 68°.

The resulting products were run on a 2% agarose gel and then cleaned using solid-phase reversible immobilization with carboxylated Sera-Mag SpeedBeads (GE Healthcare UK Limited, Little Chalfont, Buckinghamshire, UK) in NaCl- and PEG-containing buffer (Rohland and Reich 2012) and indexed with dual index primers from NEBNext Multiplex Oligo kits (New England Biolabs, Ipswich, Massachusetts, USA). Normalization was carried out with Charm Biotech Just-a-Plate 96-well clean-up kits. A PureLink PCR Purification Kit was used to concentrate the final library after pooling and remove DNA fragments of less than 300 base pair in length. To confirm fragment size, the pooled samples were analyzed on a Bioanalyzer. The library was then sequenced on a single run of Illumina MiSeq v3 2 × 300 bp at the UCR Institute for Integrative Genome Biology.

Bioinformatics and prey identification

MiSeq reads were demultiplexed on UCR's Linux Cluster at the High-Performance Computing Center. Adaptor primers, barcodes, and low-quality ends were cut from reads using Trimmomatic v0.36 (Bolger et al. 2014). Paired-end output reads were then filtered, trimmed, dereplicated, and merged using the DADA2 package v1.6.0 (Callahan et al. 2016) in RStudio. Briefly, the DADA2 pipeline is designed to filter/denoise amplicon data and infer sequence variants by modeling and correcting errors present after Illumina sequencing. Following merging, chimeras were removed using the *removeBimeraDenovo()* function. We included a negative (blank) sample in our sequencing run and, after processing with DADA2, recovered no sequence variants from it. Because of this and the fact that we recovered many rare and unique amplicon sequence variants, we opted not to set a minimum abundance threshold.

All resulting output amplicon sequence variants were queried against the Barcode of Life Data System (BOLD) COI database (Ratnasingham and Hebert 2007). Sequences for which no close matches were found on BOLD (<95% identity) were then searched against both NCBI GenBank and the local database of buckwheat-associated arthropods with BLAST. To assign identifications to sequence variants, we used identity thresholds. Only sequences sharing 100% identity with BOLD/NCBI/LocalDB matches were classified as identified to species. Matches below this threshold were only identified to genus, family, or order level depending on confidence values estimated using a taxonomic classifier (see below). Generally, NCBI sequences that matched our queried sequences in combination with the smallest E values, greatest nucleotide percent identity, and longest query cover were used to make taxonomic designations.

To measure the confidence of these identifications, we then used the insect (informatic sequence classification trees) R package (Wilkinson et al. 2018), a tool designed to assign rank-based taxonomic identifications to amplicon sequence variants generated by DADA2. For classifying the sequence variants of this study, we used the trained COI classifier (i.e., classification tree) specific to mlCOIintF/jgHCO2198 (Leray et al. 2013) barcoding amplicons (classifier.rds v5

20181124) provided through the insect package. The *classify* function was set to a threshold value of 0.6 as many sequences (including those of *P. pacifica*) returned uninformative taxon identifications when run with the default threshold parameter of 0.9. In addition to outputting a taxon name and rank, the classify function also reports an Akaike weight value (i.e., confidence score ranging from 0 to 1) for each of the final taxon assignments and this was used to judge the accuracy of initial BOLD/NCBI/LocalDB-based identifications.

Following identification, prey taxa were assigned to one of five general trophic categories (or a combination of) based on their biology and affiliation with California buckwheat: pollinators, herbivores, parasitoids, predators, or other (e.g., scavengers or fungivores). Pollinators were categorized by taxa that typically only visit buckwheat to acquire nectar or pollen. Herbivores were classified as phytophagous arthropods that feed primarily on plant material other than nectar or pollen (but may also feed on nectar or pollen as adults). Any taxa that exhibit entomophagy during some stage of their life cycle were subsequently categorized as either predators or parasitoids. See Table 1 for a breakdown of trophic assignment per prey taxon.

DNA detectability half-life feeding trials

Adult *P. pacifica* were collected alive from our CSS field site along the North Fork of Lytle Creek in late June of 2017. Predators were housed in petri dishes with a photoperiod of 15:9 L:D and held at a constant temperature of 27°C and starved for seven days prior to beginning the feeding trial. Each *P. pacifica* was fed a single house fly (*Musca domestica* Linnaeus) and allowed to feed for one hour. Ambush bugs that failed to feed were dropped from the experiment. At $t = 0$ h, five unfed individuals were placed immediately into a −80°C freezer to serve as a negative control. Due to substantial mortality during the starvation period, only four *P. pacifica* were available for each time interval post-feeding: 0, 6, 12, 24, 48, 72, and 96 h. Only three fed *P. pacifica* were available for the remaining 120-hr time point. After death by freezing, specimens were placed into 100% EtOH and stored at −80°C until their mid- and hindguts could be extracted (as described previously). *Phymata pacifica*-specific primers were used to confirm the success of DNA extractions, and *M. domestica*-specific primers (MuscaF1: 5′-TGAATTAGGACACCCTGGTGCTCTA-3′ and MuscaR1_268: 5′-AGTTCAACCTGTTCCAGCTCCCTT-3′) were designed by comparing *Musca* COI sequences downloaded from GenBank to those of ambush bugs to test for the presence of prey DNA. The presence or absence of ~268-bp bands was verified with electrophoresis on a 1% agarose gel. The DNA detectability half-life and predicted 95% confidence intervals were determined using a linear regression model in RStudio (function *lm()*).

RESULTS AND DISCUSSION

Predation on flower visitors

Unlike many predatory arthropods that are limited by their size when hunting, ambush bugs can take prey of an extensive size range. This is reflected in our prey data and can be attributed to the fact that *Phymata* employ fast-acting paralytic venom while holding their prey in place with powerful raptorial forelegs (Walker et al. 2016). Identified prey taxa range in length from roughly 2 mm (e.g., *Orius tricolor* Fabricius (Anthocoridae)) to over 10 mm (e.g., *Apis mellifera* Linnaeus (Apidae)), roughly twice the length of *P. pacifica*. Like other ambush bugs, it is evident that *P. pacifica* is an opportunistic generalist predator and consumes a wide range of prey, as those analyzed fed on members of at roughly 46 families of arthropods spanning 10 orders (Fig. 1, Table 1).

Contrary to our expectations, of the resulting 280 total prey amplicon sequence variants obtained from the 225 gut samples sequenced, only a small proportion (41/280: ~15%) were identified as native bees. Regardless of this relatively low number, the ambush bugs examined fed on a broad diversity of Hymenoptera. Of the eight genera of Apoidea consumed, *Lasioglossum* Curtis (Halictidae; 31/280: ~11%) and non-native *A. mellifera* (14/280: ~5%) were recovered most frequently. Among the ~80 unique buckwheat-associated arthropod morphospecies collected from CSS, we obtained nine genera of native apoids (Appendix S1: Table S2). Of these, four were also sequenced from *P. pacifica* gut contents. Additionally, several amplicon sequence variants were identified to apoid genera not collected

Table 1. List of the 280 prey items sequenced from *Phymata pacifica* mid- and hindguts.

Phymata ID	Sex	USI ID (UCR_ENT #)	Prey order	Prey family	Prey genus species	Identity (%)	Det. by	“Insect” assignment	Assign. score	Diet
106(2/5)	F	00124726	**Ara**	**Thomisidae**	***Mecaphesa rothi***	100	BOLD	***Mecaphesa rothi***	1.00	Pred
218(1/3)	F	00124834	**Bla**	Ectobiidae	–	99	BOLD	**Blattodea**	0.71	Other
248(1/2)	M	00125639	Col	–	–	92	BOLD	–	–	Undet
108(2/2)	M	00124728	Col	Anobiidae	Anobiidae sp. - POL097	99	LocalDB	–	–	Herb
190(4/4)	F	00124806	Col	Anobiidae	Anobiidae sp. - POL097	99	LocalDB	–	–	Herb
002	F	00123462	**Col**	Anobiidae	Anobiidae sp. - POL097	99	LocalDB	Melyridae	1.00	Herb
097(1/2)	M	00124718	**Col**	Anobiidae	Anobiidae sp. - POL097	99	LocalDB	Melyridae	1.00	Herb
189(2/2)	M	00124805	**Col**	Anobiidae	Anobiidae sp. - POL097	99	LocalDB	Melyridae	1.00	Herb
266(2/2)	F	00125618	Col	Anthicidae	–	93	BOLD	–	–	Pred
143(1/2)	M	00124761	**Col**	**Chrysomelidae**	–	99	BOLD	Bruchinae	0.85	Herb
075(2/3)	M	00124696	Col	Chrysomelidae	–	89	BOLD	–	–	Herb
140(2/2)	F	00124758	Col	Chrysomelidae	–	89	BOLD	–	–	Herb
157(1/2)	M	00124774	Col	Chrysomelidae	*Zabrotes* sp. - POL102	100	LocalDB	–	–	Herb
164(3/6)	F	00124781	Col	Chrysomelidae	*Zabrotes* sp. - POL102	100	LocalDB	–	–	Herb
195(3/3)	F	00124811	Col	Chrysomelidae	*Zabrotes* sp. - POL102	100	LocalDB	–	–	Herb
214	M	00124830	Col	Chrysomelidae	*Zabrotes* sp. - POL102	100	LocalDB	–	–	Herb
227	M	00124843	Col	Chrysomelidae	*Zabrotes* sp. - POL102	100	LocalDB	–	–	Herb
249(2/2)	M	00125601	Col	Chrysomelidae	*Zabrotes* sp. - POL102	100	LocalDB	–	–	Herb
256(3/3)	M	00125608	Col	Chrysomelidae	*Zabrotes* sp. - POL102	100	LocalDB	–	–	Herb
257(2/2)	M	00125609	Col	Chrysomelidae	*Zabrotes* sp. - POL102	100	LocalDB	–	–	Herb
261(2/3)	F	00125613	Col	Chrysomelidae	*Zabrotes* sp. - POL102	100	LocalDB	–	–	Herb
274(3/3)	F	00125626	Col	Chrysomelidae	*Zabrotes* sp. - POL102	100	LocalDB	–	–	Herb
251	M	00125603	Col	Chrysomelidae	*Zabrotes* sp. - POL102	99	LocalDB	–	–	Herb
192	M	00124808	**Col**	Chrysomelidae	*Zabrotes* sp. - POL102	99	LocalDB	**Cucujiformia**	0.64	Herb
096(3/3)	F	00124717	**Col**	**Cleridae**	*Phyllobaeneus* sp. - POL044	99	LocalDB	**Cleridae**	1.00	Pred
190(1/4)	F	00124806	**Col**	**Cleridae**	*Phyllobaeneus* sp. - POL044	99	LocalDB	**Cleridae**	1.00	Pred
068(5/5)	F	00124689	Col	Dermestidae	–	98	BOLD	–	–	Other
169	M	00124785	Col	Melyridae	–	99	BOLD	–	–	Pred
145(1/5)	F	00124763	Col	Melyridae	–	98	BOLD	–	–	Pred
164(1/6)	F	00124781	Col	Melyridae	–	98	BOLD	–	–	Pred
225	M	00124841	Col	Melyridae	–	98	BOLD	–	–	Pred
261(3/3)	F	00125613	Col	Melyridae	–	98	BOLD	–	–	Pred
274(2/3)	F	00125626	Col	Melyridae	–	98	BOLD	–	–	Pred
223	M	00124839	Col	Melyridae	–	98	BOLD	–	–	Pred
155	F	00124772	**Col**	**Melyridae**	–	98	BOLD	**Melyridae**	1.00	Pred
269	M	00125621	**Col**	**Melyridae**	–	98	BOLD	**Melyridae**	1.00	Pred
157(2/2)	M	00124774	**Col**	**Melyridae**	–	97	BOLD	**Melyridae**	1.00	Pred
108(1/2)	M	00124728	**Col**	**Melyridae**	–	97	BOLD	**Melyridae**	1.00	Pred
215	M	00124831	**Col**	**Melyridae**	–	97	BOLD	Malachiinae	0.82	Pred
191(2/3)	F	00124807	**Col**	**Melyridae**	–	96	BOLD	Malachiinae	0.82	Pred
266(1/2)	F	00125618	**Col**	**Melyridae**	–	94	BOLD	Malachiinae	0.82	Pred
146(1/2)	M	00124764	Col	Melyridae	–	94	BOLD	–	–	Pred
003(1/2)	M	00108062	Col	Melyridae	–	93	BOLD	–	–	Pred
014(1/2)	N	00123465	Col	Melyridae	–	93	BOLD	–	–	Pred
134(6/6)	F	00124753	**Col**	**Melyridae**	*Attalus* sp. - POL052	97	LocalDB	**Malachiinae**	0.82	Pred
136(2/4)	F	00124755	**Col**	**Melyridae**	*Attalus* sp. - POL052	97	LocalDB	**Malachiinae**	0.82	Pred
208(2/3)	F	00124824	**Col**	**Melyridae**	*Attalus* sp. - POL052	96	LocalDB	**Malachiinae**	0.82	Pred
106(1/5)	F	00124726	**Col**	**Melyridae**	*Tanaops* sp. - POL103	100	LocalDB	**Malachiinae**	0.82	Pred
193	M	00124809	**Col**	**Melyridae**	*Tanaops* sp. - POL092	97	LocalDB	**Malachiinae**	0.82	Pred
099(2/2)	M	00124720	Col	Mordellidae	–	86	BOLD	–	–	Pol
145(5/5)	F	00124763	**Col**	**Mordellidae**	***Mordella*** *atrata*	100	BOLD	***Mordella***	1.00	Pol
071(1/2)	F	00124692	**Col**	**Mordellidae**	***Mordella* sp.**	99	BOLD	***Mordella***	1.00	Pol
084(2/2)	F	00124705	**Col**	**Mordellidae**	***Mordella* sp.**	99	BOLD	***Mordella***	1.00	Pol
100(1/3)	M	00124721	Dip	–	–	92	BOLD	–	–	Undet
129(5/5)	F	00124748	Dip	–	–	92	BOLD	–	–	Undet
160(3/4)	F	00124777	Dip	–	–	92	BOLD	–	–	Undet
019	M	00124640	Dip	–	–	91	BOLD	–	–	Undet
145(3/5)	F	00124763	Dip	–	–	91	BOLD	–	–	Undet
252	M	00125604	Dip	–	–	90	BOLD	–	–	Undet
208(3/3)	F	00124824	Dip	–	–	90	BOLD	–	–	Undet
110	M	00124730	Dip	–	–	90	BOLD	–	–	Undet
229(2/2)	M	00124845	Dip	–	–	87	BOLD	–	–	Undet

(Table 1. Continued.)

Phymata ID	Sex	USI ID (UCR_ENT #)	Prey order	Prey family	Prey genus species	Identity (%)	Det. by	"Insect" assignment	Assign. score	Diet
062(3/3)	F	00124683	Dip	–	–	87	BOLD	–	–	Undet
194(2/2)	F	00124810	Dip	–	–	86	BOLD	–	–	Undet
183(1/2)	F	00124799	Dip	–	–	86	BOLD	–	–	Undet
186(2/2)	F	00124802	Dip	–	–	86	BOLD	–	–	Undet
222(1/2)	M	00124838	Dip	–	–	86	BOLD	–	–	Undet
031(1/2)	F	00124652	Dip	–	–	86	NCBI	–	–	Undet
062(2/3)	F	00124683	Dip	Asilidae	*Atomosia* sp. - POL025	100	LocalDB	–	–	Pred
023	M	00124644	Dip	Bombyliidae	–	100	BOLD	–	–	Para/Pol
041(1/2)	F	00124662	Dip	Bombyliidae	–	100	BOLD	–	–	Para/Pol
059(1/2)	M	00124680	Dip	Bombyliidae	–	100	BOLD	–	–	Para/Pol
068(2/5)	F	00124689	Dip	Bombyliidae	–	100	BOLD	–	–	Para/Pol
106(5/5)	F	00124726	Dip	Bombyliidae	–	100	BOLD	–	–	Para/Pol
107(2/2)	F	00124727	Dip	Bombyliidae	–	100	BOLD	–	–	Para/Pol
126(3/3)	F	00124745	Dip	Bombyliidae	–	100	BOLD	–	–	Para/Pol
134(4/6)	F	00124753	Dip	Bombyliidae	–	100	BOLD	–	–	Para/Pol
142	F	00124760	Dip	Bombyliidae	–	100	BOLD	–	–	Para/Pol
148(1/2)	F	00124766	Dip	Bombyliidae	–	100	BOLD	–	–	Para/Pol
164(5/6)	F	00124781	Dip	Bombyliidae	–	100	BOLD	–	–	Para/Pol
229(1/2)	M	00124845	Dip	Bombyliidae	–	100	BOLD	–	–	Para/Pol
117	M	00124736	Dip	Bombyliidae	–	100	BOLD	–	–	Para/Pol
270(2/2)	F	00125622	Dip	Bombyliidae	–	100	BOLD	–	–	Para/Pol
064(2/2)	M	00124685	Dip	Bombyliidae	–	99	BOLD	–	–	Para/Pol
127	F	00124746	Dip	Bombyliidae	–	99	BOLD	–	–	Para/Pol
177	M	00124793	Dip	Bombyliidae	–	99	BOLD	–	–	Para/Pol
253	M	00125605	Dip	Bombyliidae	–	99	BOLD	–	–	Para/Pol
048(3/4)	M	00124669	Dip	Bombyliidae	–	99	BOLD	–	–	Para/Pol
201(2/2)	F	00124817	Dip	Bombyliidae	–	99	BOLD	–	–	Para/Pol
063(2/2)	F	00124684	Dip	Bombyliidae	–	98	BOLD	–	–	Para/Pol
120	F	00124739	Dip	Bombyliidae	–	98	BOLD	–	–	Para/Pol
206(3/3)	M	00124822	Dip	Bombyliidae	–	98	BOLD	–	–	Para/Pol
191(3/3)	F	00124807	Dip	Bombyliidae	–	98	BOLD	–	–	Para/Pol
260(1/2)	F	00125612	Dip	Bombyliidae	–	98	BOLD	–	–	Para/Pol
185	F	00124801	Dip	Bombyliidae	–	93	BOLD	–	–	Para/Pol
099(1/2)	M	00124720	Dip	Bombyliidae	–	88	BOLD	–	–	Para/Pol
005(1/2)	F	00123473	**Dip**	**Calliphoridae**	***Chrysomya*** *rufifacies*	100	BOLD	***Chrysomya***	1.00	Other
260(2/2)	F	00125612	Dip	Chloropidae	*Olcella* sp.	100	BOLD	–	–	Other
048(1/4)	M	00124669	**Dip**	Heleomyzidae	–	98	BOLD	**Schizophora**	0.94	Other
003(2/2)	M	00108062	**Dip**	Heleomyzidae	–	95	BOLD	**Schizophora**	0.94	Other
164(4/6)	F	00124781	**Dip**	Muscidae	*Coenosia pilosissima*	89	BOLD	**Brachycera**	1.00	Pred
068(4/5)	F	00124689	Dip	Phoridae	*Megaselia* sp.	100	BOLD	–	–	Other
256(1/3)	M	00125608	Dip	Phoridae	*Megaselia* sp.	100	BOLD	–	–	Other
097(2/2)	M	00124718	Dip	Sarcophagidae	*Hilarella hilarella*	100	BOLD	–	–	Other
062(1/3)	F	00124683	Dip	Sarcophagidae	*Hilarella hilarella*	100	BOLD	–	–	Other
275(2/2)	M	00125627	Dip	Scatopsidae	–	100	BOLD	–	–	Other
164(2/6)	F	00124781	**Dip**	**Sciaridae**	–	92	BOLD	**Sciaridae**	1.00	Other
053(2/2)	M	00124674	**Dip**	**Sciaridae**	***Scatopsciara atomaria***	100	BOLD	***Scatopsciara atomaria***	1.00	Other
112	M	00124732	**Dip**	Tabanidae	*Pegasomyia* sp.	95	NCBI	**Tabanoidea**	1.00	Pol
203	M	00124819	**Dip**	Tabanidae	*Pegasomyia* sp.	95	NCBI	**Tabanoidea**	1.00	Pol
176(2/2)	F	00124792	Dip	Tachinidae	–	86	BOLD	–	–	Para
160(4/4)	F	00124777	Dip	Tachinidae	nr *Paradidyma* sp.	95	BOLD	–	–	Para
030(1/2)	F	00124651	**Dip**	Tachinidae	*Chetogena parvipalpis*	100	BOLD	**Schizophora**	0.94	Para
136(1/4)	F	00124755	**Dip**	Tachinidae	*Chetogena parvipalpis*	100	BOLD	**Schizophora**	0.94	Para
195(1/3)	F	00124811	**Dip**	Tachinidae	*Chetogena parvipalpis*	100	BOLD	**Schizophora**	0.94	Para
201(1/2)	F	00124817	**Dip**	Tachinidae	*Chetogena parvipalpis*	100	BOLD	**Schizophora**	0.94	Para
206(1/3)	M	00124822	**Dip**	Tachinidae	*Chetogena parvipalpis*	100	BOLD	**Schizophora**	0.94	Para
264(1/2)	F	00125616	**Dip**	Tachinidae	*Chetogena parvipalpis*	100	BOLD	**Schizophora**	0.94	Para
265(2/4)	F	00125617	**Dip**	Tachinidae	*Chetogena parvipalpis*	100	BOLD	**Schizophora**	0.94	Para
274(1/3)	F	00125626	**Dip**	Tachinidae	*Chetogena parvipalpis*	100	BOLD	**Schizophora**	0.94	Para
275(1/2)	M	00125627	**Dip**	Tachinidae	*Chetogena parvipalpis*	100	BOLD	**Schizophora**	0.94	Para
095	M	00124716	Dip	Tachinidae	*Eucelatoria* sp.	100	BOLD	–	–	Para
129(1/5)	F	00124748	Dip	Tachinidae	*Eucelatoria* sp.	100	BOLD	–	–	Para
134(5/6)	F	00124753	Dip	Tachinidae	*Eucelatoria* sp.	100	BOLD	–	–	Para

(Table 1. Continued.)

Phymata ID	Sex	USI ID (UCR_ENT #)	Prey order	Prey family	Prey genus species	Identity (%)	Det. by	"Insect" assignment	Assign. score	Diet
136(3/4)	F	00124755	Dip	Tachinidae	*Eucelatoria* sp.	100	BOLD	–	–	Para
187(1/3)	F	00124803	Dip	Tachinidae	*Eucelatoria* sp.	100	BOLD	–	–	Para
220(1/2)	F	00124836	Dip	Tachinidae	*Eucelatoria* sp.	100	BOLD	–	–	Para
069(2/3)	F	00124690	**Dip**	Tachinidae	*Leucostoma aterrimum*	100	BOLD	**Calyptratae**	1.00	Para
270(1/2)	F	00125622	Dip	Tachinidae	*Peleteria* sp.	100	BOLD	–	–	Para
264(2/2)	F	00125616	**Hem**	**Anthocoridae**	*Orius* sp.	100	NCBI	**Anthocoridae**	1.00	Pred
005(2/2)	F	00123473	**Hem**	**Anthocoridae**	*Orius* sp.	99	NCBI	**Anthocoridae**	1.00	Pred
129(3/5)	F	00124748	**Hem**	**Anthocoridae**	*Orius* sp.	99	NCBI	**Anthocoridae**	1.00	Pred
130(1/2)	M	00124749	**Hem**	**Anthocoridae**	*Orius* sp.	99	NCBI	**Anthocoridae**	1.00	Pred
187(3/3)	F	00124803	**Hem**	**Anthocoridae**	*Orius* sp.	99	NCBI	**Anthocoridae**	1.00	Pred
198(2/2)	M	00124814	**Hem**	**Anthocoridae**	*Orius* sp.	99	NCBI	**Anthocoridae**	1.00	Pred
222(2/2)	M	00124838	**Hem**	**Anthocoridae**	*Orius* sp.	99	NCBI	**Anthocoridae**	1.00	Pred
262	F	00125614	**Hem**	**Anthocoridae**	*Orius* sp.	99	NCBI	**Anthocoridae**	1.00	Pred
265(4/4)	F	00125617	**Hem**	**Anthocoridae**	*Orius* sp.	99	NCBI	**Anthocoridae**	1.00	Pred
194(1/2)	F	00124810	**Hem**	**Geocoridae**	***Geocoris pallens***	100	BOLD	***Geocoris pallens***	1.00	Pred
195(2/3)	F	00124811	**Hem**	**Geocoridae**	***Geocoris pallens***	100	BOLD	***Geocoris pallens***	1.00	Pred
218(2/3)	F	00124834	**Hem**	**Geocoridae**	***Geocoris pallens***	100	BOLD	***Geocoris pallens***	1.00	Pred
249(1/2)	M	00125601	**Hem**	**Geocoridae**	***Geocoris pallens***	100	BOLD	***Geocoris pallens***	1.00	Pred
245(1/2)	M	00125636	**Hem**	**Miridae**	*Rhinacloa forticornis*	100	BOLD	**Phylini**	1.00	Herb
179	M	00124795	**Hem**	**Miridae**	***Rhinacloa forticornis***	100	BOLD	***Rhinacloa forticornis***	1.00	Herb
050(1/2)	M	00124671	**Hem**	**Miridae**	***Rhinacloa forticornis***	100	BOLD	***Rhinacloa forticornis***	1.00	Herb
134(3/6)	F	00124753	**Hem**	**Nabidae**	***Nabis*** **sp.**	100	BOLD	***Nabis*** *alternatus*	1.00	Pred
259	F	00125611	**Hym**	**Apidae**	*Apis mellifera*	100	BOLD	**Apidae**	1.00	Pol
006	F	00108716	**Hym**	**Apidae**	***Apis mellifera***	100	BOLD	***Apis mellifera***	1.00	Pol
007	M	00115003	**Hym**	**Apidae**	***Apis mellifera***	100	BOLD	***Apis mellifera***	1.00	Pol
036(2/3)	F	00124657	**Hym**	**Apidae**	***Apis mellifera***	100	BOLD	***Apis mellifera***	1.00	Pol
052(1/2)	F	00124673	**Hym**	**Apidae**	***Apis mellifera***	100	BOLD	***Apis mellifera***	1.00	Pol
075(3/3)	M	00124696	**Hym**	**Apidae**	***Apis mellifera***	100	BOLD	***Apis mellifera***	1.00	Pol
089	F	00124710	**Hym**	**Apidae**	***Apis mellifera***	100	BOLD	***Apis mellifera***	1.00	Pol
134(1/6)	F	00124753	**Hym**	**Apidae**	***Apis mellifera***	100	BOLD	***Apis mellifera***	1.00	Pol
161	F	00124778	**Hym**	**Apidae**	***Apis mellifera***	100	BOLD	***Apis mellifera***	1.00	Pol
176(1/2)	F	00124792	**Hym**	**Apidae**	***Apis mellifera***	100	BOLD	***Apis mellifera***	1.00	Pol
182(1/2)	F	00124798	**Hym**	**Apidae**	***Apis mellifera***	100	BOLD	***Apis mellifera***	1.00	Pol
202	F	00124818	**Hym**	**Apidae**	***Apis mellifera***	100	BOLD	***Apis mellifera***	1.00	Pol
272	F	00125624	**Hym**	**Apidae**	***Apis mellifera***	100	BOLD	***Apis mellifera***	1.00	Pol
273	F	00125625	**Hym**	**Apidae**	***Apis mellifera***	100	BOLD	***Apis mellifera***	1.00	Pol
257(1/2)	M	00125609	**Hym**	**Apidae**	***Ceratina*** *acantha*	100	BOLD	***Ceratina***	0.98	Pol
190(2/4)	F	00124806	**Hym**	**Braconidae**	*Agathis* sp.	99	BOLD	**Agathidinae**	1.00	Para
220(2/2)	F	00124836	**Hym**	**Braconidae**	***Cotesia*** **sp.**	99	BOLD	***Cotesia***	1.00	Para
245(2/2)	M	00125636	Hym	Braconidae	*Illidops* sp.	93	BOLD	–	–	Para
172	F	00124788	**Hym**	**Braconidae**	*Orgilus* sp.	100	BOLD	**Braconidae**	1.00	Para
133(2/2)	F	00124752	Hym	Chalcididae	–	91	BOLD	–	–	Para
036(3/3)	F	00124657	**Hym**	**Chalcidoidea**	–	90	NCBI	**Chalcidoidea**	0.85	Para
140(1/2)	F	00124758	**Hym**	**Colletidae**	***Colletes*** *slevini*	98	BOLD	***Colletes***	0.80	Pol
247	F	00125638	**Hym**	**Colletidae**	***Hylaeus*** **sp.**	100	BOLD	***Hylaeus*** *mesillae*	1.00	Pol
148(2/2)	F	00124766	**Hym**	**Eulophidae**	–	95	BOLD	Tetrastichinae	0.93	Para
256(2/3)	M	00125608	**Hym**	**Eulophidae**	–	94	BOLD	Tetrastichinae	0.93	Para
207	F	00124823	Hym	Halictidae	*Augochlorella pomoniella*	100	BOLD	–	–	Pol
183(2/2)	F	00124799	**Hym**	**Halictidae**	*Halictus* sp.	100	BOLD	**Halictini**	0.88	Pol
052(2/2)	F	00124673	**Hym**	**Halictidae**	*Halictus tripartitus*	97	BOLD	**Halictini**	0.88	Pol
261(1/3)	F	00125613	**Hym**	**Halictidae**	*Halictus tripartitus* - POL064	100	LocalDB	**Halictini**	0.88	Pol
145(2/5)	F	00124763	**Hym**	**Halictidae**	***Lasioglossum*** *argemonis*	100	BOLD	***Lasioglossum***	0.98	Pol
159	F	00124776	**Hym**	**Halictidae**	***Lasioglossum*** *argemonis*	100	BOLD	***Lasioglossum***	0.98	Pol
206(2/3)	M	00124822	**Hym**	**Halictidae**	***Lasioglossum*** *argemonis*	100	BOLD	***Lasioglossum***	0.98	Pol
248(2/2)	M	00125639	**Hym**	**Halictidae**	***Lasioglossum*** *nevadense*	100	BOLD	***Lasioglossum*** **(*Dialictus*)**	0.91	Pol
071(2/2)	F	00124692	**Hym**	**Halictidae**	***Lasioglossum punctatoventre***	100	BOLD	***Lasioglossum*** **(*Dialictus*)**	0.91	Pol
144(2/2)	F	00124762	**Hym**	**Halictidae**	***Lasioglossum*** *punctatoventre*	96	BOLD	***Lasioglossum***	0.98	Pol
050(2/2)	M	00124671	**Hym**	**Halictidae**	*Lasioglossum punctatoventre*	96	BOLD	**Halictinae**	1.00	Pol
014(2/2)	N	00123465	Hym	Halictidae	*Lasioglossum* sp.	100	BOLD	–	–	Pol

(Table 1. Continued.)

Phymata ID	Sex	USI ID (UCR_ENT #)	Prey order	Prey family	Prey genus species	Identity (%)	Det. by	"Insect" assignment	Assign. score	Diet
041(2/2)	F	00124662	Hym	Halictidae	*Lasioglossum* sp.	100	BOLD	–	–	Pol
044	F	00124665	Hym	Halictidae	*Lasioglossum* sp.	100	BOLD	–	–	Pol
075(1/3)	M	00124696	Hym	Halictidae	*Lasioglossum* sp.	100	BOLD	–	–	Pol
111(1/2)	M	00124731	Hym	Halictidae	*Lasioglossum* sp.	100	BOLD	–	–	Pol
116	F	00124735	Hym	Halictidae	*Lasioglossum* sp.	100	BOLD	–	–	Pol
129(4/5)	F	00124748	Hym	Halictidae	*Lasioglossum* sp.	100	BOLD	–	–	Pol
138	F	00124756	Hym	Halictidae	*Lasioglossum* sp.	100	BOLD	–	–	Pol
146(2/2)	M	00124764	Hym	Halictidae	*Lasioglossum* sp.	100	BOLD	–	–	Pol
187(2/3)	F	00124803	Hym	Halictidae	*Lasioglossum* sp.	100	BOLD	–	–	Pol
190(3/4)	F	00124806	Hym	Halictidae	*Lasioglossum* sp.	100	BOLD	–	–	Pol
218(3/3)	F	00124834	Hym	Halictidae	*Lasioglossum* sp.	100	BOLD	–	–	Pol
221	M	00124837	Hym	Halictidae	*Lasioglossum* sp.	100	BOLD	–	–	Pol
056(3/3)	F	00124677	**Hym**	**Halictidae**	***Lasioglossum* sp.**	100	BOLD	***Lasioglossum***	0.98	Pol
106(3/5)	F	00124726	**Hym**	**Halictidae**	***Lasioglossum* sp.**	100	BOLD	***Lasioglossum***	0.98	Pol
119(2/2)	F	00124738	**Hym**	**Halictidae**	***Lasioglossum* sp.**	100	BOLD	***Lasioglossum***	0.98	Pol
130(2/2)	M	00124749	**Hym**	**Halictidae**	***Lasioglossum* sp.**	100	BOLD	***Lasioglossum***	0.98	Pol
149	M	00124767	**Hym**	**Halictidae**	***Lasioglossum* sp.**	100	BOLD	***Lasioglossum***	0.98	Pol
059(2/2)	M	00124680	**Hym**	**Halictidae**	***Lasioglossum* sp.**	100	BOLD	***Lasioglossum* (*Dialictus*)**	0.91	Pol
060	M	00124681	**Hym**	**Halictidae**	***Lasioglossum* sp.**	97	BOLD	***Lasioglossum***	0.98	Pol
139(1/2)	M	00124757	**Hym**	**Halictidae**	***Lasioglossum* sp.**	97	BOLD	***Lasioglossum***	0.98	Pol
189(1/2)	M	00124805	**Hym**	**Halictidae**	***Lasioglossum* sp.**	97	BOLD	***Lasioglossum***	0.98	Pol
096(2/3)	F	00124717	**Hym**	**Halictidae**	***Lasioglossum* sp.**	97	BOLD	***Lasioglossum***	0.98	Pol
126(2/3)	F	00124745	**Hym**	**Halictidae**	***Lasioglossum* sp. - POL056**	100	LocalDB	***Lasioglossum***	0.98	Pol
168(2/2)	M	00124784	**Hym**	**Halictidae**	***Sphecodes* sp.**	100	BOLD	***Sphecodes***	1.00	Pol
145(4/5)	F	00124763	**Hym**	**Halictidae**	***Sphecodes* sp.**	100	BOLD	***Sphecodes***	1.00	Pol
268	F	00125620	**Hym**	**Megachilidae**	*Ashmeadiella cactorum basalis*	100	BOLD	*Hoplitis*	0.77	Pol
139(2/2)	M	00124757	**Hym**	Pteromalidae	–	100	BOLD	**Chalcidoidea**	0.85	Para
048(2/4)	M	00124669	Lep	Blastobasidae	*Holcocera* sp.	100	BOLD	–	–	Herb
067(3/3)	F	00124688	Lep	Blastobasidae	*Holcocera* sp.	100	BOLD	–	–	Herb
126(1/3)	F	00124745	**Lep**	Blastobasidae	*Holcocera* sp.	100	BOLD	**Ditrysia**	0.97	Herb
134(2/6)	F	00124753	**Lep**	Blastobasidae	*Holcocera* sp.	100	BOLD	**Ditrysia**	0.97	Herb
265(3/4)	F	00125617	Lep	Coleophoridae	*Coleophora* sp.	100	BOLD	–	–	Herb
100(3/3)	M	00124721	**Lep**	**Coleophoridae**	*Coleophora* sp.	100	BOLD	**Coleophoridae**	1.00	Herb
143(2/2)	M	00124761	**Lep**	**Coleophoridae**	*Coleophora* sp.	100	BOLD	**Coleophoridae**	1.00	Herb
208(1/3)	F	00124824	**Lep**	**Coleophoridae**	*Coleophora* sp.	100	BOLD	**Coleophoridae**	1.00	Herb
182(2/2)	F	00124798	Lep	Cosmopterigidae	*Anoncia* sp.	100	BOLD	–	–	Herb
076	M	00124697	**Lep**	Cosmopterigidae	*Anoncia* sp.	100	BOLD	**Ditrysia**	0.97	Herb
107(1/2)	F	00124727	**Lep**	Cosmopterigidae	*Anoncia* sp.	100	BOLD	**Ditrysia**	0.97	Herb
174	F	00124790	**Lep**	Crambidae	*Evergestis fuscistrigalis*	100	BOLD	**Ditrysia**	0.97	Herb
053(1/2)	M	00124674	**Lep**	Crambidae	*Evergestis* sp.	100	BOLD	**Ditrysia**	0.97	Herb
198(1/2)	M	00124814	Lep	Depressariidae	*Ethmia* sp.	100	BOLD	–	–	Herb
056(2/3)	F	00124677	**Lep**	Depressariidae	*Ethmia* sp.	100	BOLD	**Gelechioidea**	0.95	Herb
178	F	00124794	**Lep**	Gelechiidae	–	100	BOLD	**Ditrysia**	0.97	Herb
058	F	00124679	**Lep**	Gelechiidae	–	100	BOLD	Spilomelinae	0.94	Herb
081	F	00124702	**Lep**	Gelechiidae	*Aroga morenella*	99	BOLD	**Ditrysia**	0.97	Herb
067(2/3)	F	00124688	**Lep**	Gelechiidae	*Aroga morenella*	99	BOLD	**Ditrysia**	0.97	Herb
030(2/2)	F	00124651	**Lep**	Gelechiidae	*Aroga* sp.	100	BOLD	**Ditrysia**	0.97	Herb
096(1/3)	F	00124717	**Lep**	Gelechiidae	*Aroga* sp.	100	BOLD	**Ditrysia**	0.97	Herb
100(2/3)	M	00124721	**Lep**	Gelechiidae	*Aroga* sp.	100	BOLD	**Ditrysia**	0.97	Herb
103	M	00124723	**Lep**	Gelechiidae	*Aroga* sp.	100	BOLD	**Ditrysia**	0.97	Herb
104	M	00124724	**Lep**	Gelechiidae	*Aroga* sp.	100	BOLD	**Ditrysia**	0.97	Herb
111(2/2)	M	00124731	**Lep**	Gelechiidae	*Aroga* sp.	100	BOLD	**Ditrysia**	0.97	Herb
119(1/2)	F	00124738	**Lep**	Gelechiidae	*Aroga* sp.	100	BOLD	**Ditrysia**	0.97	Herb
141(1/2)	F	00124759	**Lep**	Gelechiidae	*Aroga* sp.	100	BOLD	**Ditrysia**	0.97	Herb
150	M	00124768	**Lep**	Gelechiidae	*Aroga* sp.	100	BOLD	**Ditrysia**	0.97	Herb
160(2/4)	F	00124777	**Lep**	Gelechiidae	*Aroga* sp.	100	BOLD	**Ditrysia**	0.97	Herb
168(1/2)	M	00124784	**Lep**	Gelechiidae	*Aroga* sp.	100	BOLD	**Ditrysia**	0.97	Herb
265(1/4)	F	00125617	**Lep**	Gelechiidae	*Aroga* sp.	100	BOLD	**Ditrysia**	0.97	Herb
048(4/4)	M	00124669	**Lep**	Gelechiidae	*Aroga* sp.	100	BOLD	**Ditrysia**	0.97	Herb
032	F	00124653	**Lep**	Gelechiidae	*Aroga* sp.	100	BOLD	**Ditrysia**	0.97	Herb
036(1/3)	F	00124657	**Lep**	Gelechiidae	*Aroga* sp.	100	BOLD	**Ditrysia**	0.97	Herb
040	M	00124661	**Lep**	Gelechiidae	*Aroga* sp.	100	BOLD	**Ditrysia**	0.97	Herb

(Table 1. Continued.)

Phymata ID	Sex	USI ID (UCR_ENT #)	Prey order	Prey family	Prey genus species	Identity (%)	Det. by	"Insect" assignment	Assign. score	Diet
055	M	00124676	**Lep**	Gelechiidae	*Aroga* sp.	100	BOLD	**Ditrysia**	0.97	Herb
056(1/3)	F	00124677	**Lep**	Gelechiidae	*Aroga* sp.	100	BOLD	**Ditrysia**	0.97	Herb
061	M	00124682	**Lep**	Gelechiidae	*Aroga* sp.	100	BOLD	**Ditrysia**	0.97	Herb
028	M	00124649	**Lep**	Gelechiidae	*Aroga* sp.	100	BOLD	**Ditrysia**	0.97	Herb
180	F	00124796	**Lep**	Gelechiidae	*Aroga* sp.	99	BOLD	**Ditrysia**	0.97	Herb
069(3/3)	F	00124690	**Lep**	Gelechiidae	*Aroga* sp.	98	BOLD	**Ditrysia**	0.97	Herb
031(2/2)	F	00124652	Lep	Geometridae	*Chlorochlamys appellaria*	100	BOLD	–	–	Herb
085	M	00124706	**Lep**	**Geometridae**	*Chlorochlamys appellaria*	100	BOLD	**Geometridae**	0.66	Herb
136(4/4)	F	00124755	**Lep**	**Geometridae**	*Chlorochlamys appellaria*	100	BOLD	**Geometridae**	0.66	Herb
113	M	00124733	**Lep**	**Geometridae**	*Chlorochlamys appellaria*	100	BOLD	**Geometridae**	0.66	Herb
054	M	00124675	**Lep**	**Geometridae**	*Cyclophora nanaria*	100	BOLD	**Geometridae**	0.66	Herb
068(3/5)	F	00124689	**Lep**	**Geometridae**	*Cyclophora nanaria*	100	BOLD	**Geometridae**	0.66	Herb
133(1/2)	F	00124752	**Lep**	**Geometridae**	*Digrammia aliceata*	98	BOLD	Ennominae	1.00	Herb
170	F	00124786	**Lep**	Geometridae	*Drepanulatrix* sp.	100	BOLD	**Obtectomera**	1.00	Herb
063(1/2)	F	00124684	**Lep**	**Geometridae**	*Idaea occidentaria*	99	BOLD	**Geometridae**	0.66	Herb
065	M	00124686	**Lep**	**Geometridae**	*Idaea occidentaria*	99	BOLD	**Geometridae**	0.66	Herb
015(1/2)	N	00123466	**Lep**	**Geometridae**	***Pero*** *occidentalis*	100	BOLD	***Pero***	1.00	Herb
082	M	00124703	**Lep**	**Geometridae**	***Sericosema wilsonensis***	100	BOLD	***Sericosema wilsonensis***	1.00	Herb
084(1/2)	F	00124705	**Lep**	**Geometridae**	***Sericosema wilsonensis***	100	BOLD	***Sericosema wilsonensis***	1.00	Herb
164(6/6)	F	00124781	**Lep**	**Lycaenidae**	***Aricia*** *lupini*	100	BOLD	***Aricia***	0.94	Herb/Pol
001	M	00114844	**Lep**	**Lycaenidae**	***Aricia*** *lupini monticola*	100	BOLD	***Aricia***	0.94	Herb/Pol
106(4/5)	F	00124726	**Lep**	**Lycaenidae**	*Euphilotes* sp.	100	BOLD	**Lycaenidae**	0.96	Herb/Pol
191(1/3)	F	00124807	**Lep**	Lycaenidae	*Leptotes marina*	100	BOLD	**Papilionoidea**	1.00	Herb/Pol
069(1/3)	F	00124690	**Lep**	Lycaenidae	*Satyrium saepium*	100	BOLD	**Obtectomera**	1.00	Herb/Pol
199	M	00124815	**Lep**	Lycaenidae	*Satyrium saepium*	100	BOLD	**Obtectomera**	1.00	Herb/Pol
213	F	00124829	**Lep**	Lycaenidae	*Satyrium saepium*	100	BOLD	**Papilionoidea**	1.00	Herb/Pol
068(1/5)	F	00124689	**Lep**	Lycaenidae	*Satyrium saepium*	100	BOLD	**Papilionoidea**	1.00	Herb/Pol
186(1/2)	F	00124802	**Lep**	**Noctuidae**	*Protorthodes alfkenii*	100	BOLD	**Noctuidae**	0.70	Herb
067(1/3)	F	00124688	**Lep**	**Noctuidae**	*Ulolonche dilecta*	100	BOLD	**Noctuidae**	0.70	Herb
129(2/5)	F	00124748	**Lep**	Pyralidae	*Arta epicoenalis*	100	BOLD	**Ditrysia**	0.97	Herb
160(1/4)	F	00124777	**Lep**	**Pyralidae**	***Ephestiodes gilvescentella***	100	BOLD	***Ephestiodes gilvescentella***	1.00	Herb
141(2/2)	F	00124759	**Lep**	**Pyralidae**	***Phycitodes reliquellum***	100	BOLD	***Phycitodes reliquella***	1.00	Herb
064(1/2)	M	00124685	**Neu**	**Chrysopidae**	***Chrysoperla*** *rufilabris*	100	BOLD	***Chrysoperla***	1.00	Pred
144(1/2)	F	00124762	**Neu**	**Chrysopidae**	***Chrysoperla*** *rufilabris*	100	BOLD	***Chrysoperla***	1.00	Pred
015(2/2)	N	00123466	**Thy**	**Thripidae**	***Frankliniella occidentalis***	100	BOLD	***Frankliniella occidentalis***	1.00	Herb

Notes: *Phymata pacifica* specimen identification numbers given. If multiple taxa were detected from a single ambush bug gut, the specimen number is listed with number of detected prey taxa in parentheses. Percent identity for matches found using searches against BOLD, GenBank, or our local buckwheat-associated arthropod barcoding dataset is listed, and the database used for taxonomic identification is given in the Det. by column (denoted as BOLD, NCBI, or LocalDB). Names in bold represent identifications supported by both database searches and the insect classifier. Prey unidentified at a particular taxonomic level or that were not assigned by the "Insect" classifier are denoted with an en dash.

Prey order abbreviations are Ara, Araneae; Bla, Blattodea; Col, Coleoptera; Dip, Diptera; Hem, Hemiptera; Hym, Hymenoptera; Lep, Lepidoptera; Neu, Neuroptera; Thy, Thysanoptera.

from the field: *Ashmeadiella* Cockerell, *Ceratina* Latreille, *Colletes* Latreille, and *Sphecodes* Latreille. A myriad of pollinators were found in high abundance on and around *E. fasciculatum* at the CSS field sites. To what degree predation impacts plant–pollinator relationships in CSS remains to be determined, as data on native bee populations in CSS are currently lacking.

Perhaps most surprising is the great diversity of entomophagous arthropods found as prey. Approximately 35% (99/280) of detected prey were classified as either parasitoids or predators. Multiple genera of tachinid flies and braconid wasps, several of which are common lepidopteran parasitoids such as *Agathis* Latreille and *Cotesia* Cameron (Whitfield 1995, Sharkey et al. 2006), fell victim to *P. pacifica*. Among entomophagous prey identified to at least genus, *Chetogena parvipalpis* (Wulp), a tachinid fly known to parasitize Hesperiidae, Pyralidae, and Gryllacrididae (Arnaud 1978), was recovered most often (9/280: ~3%). Several taxa of predatory

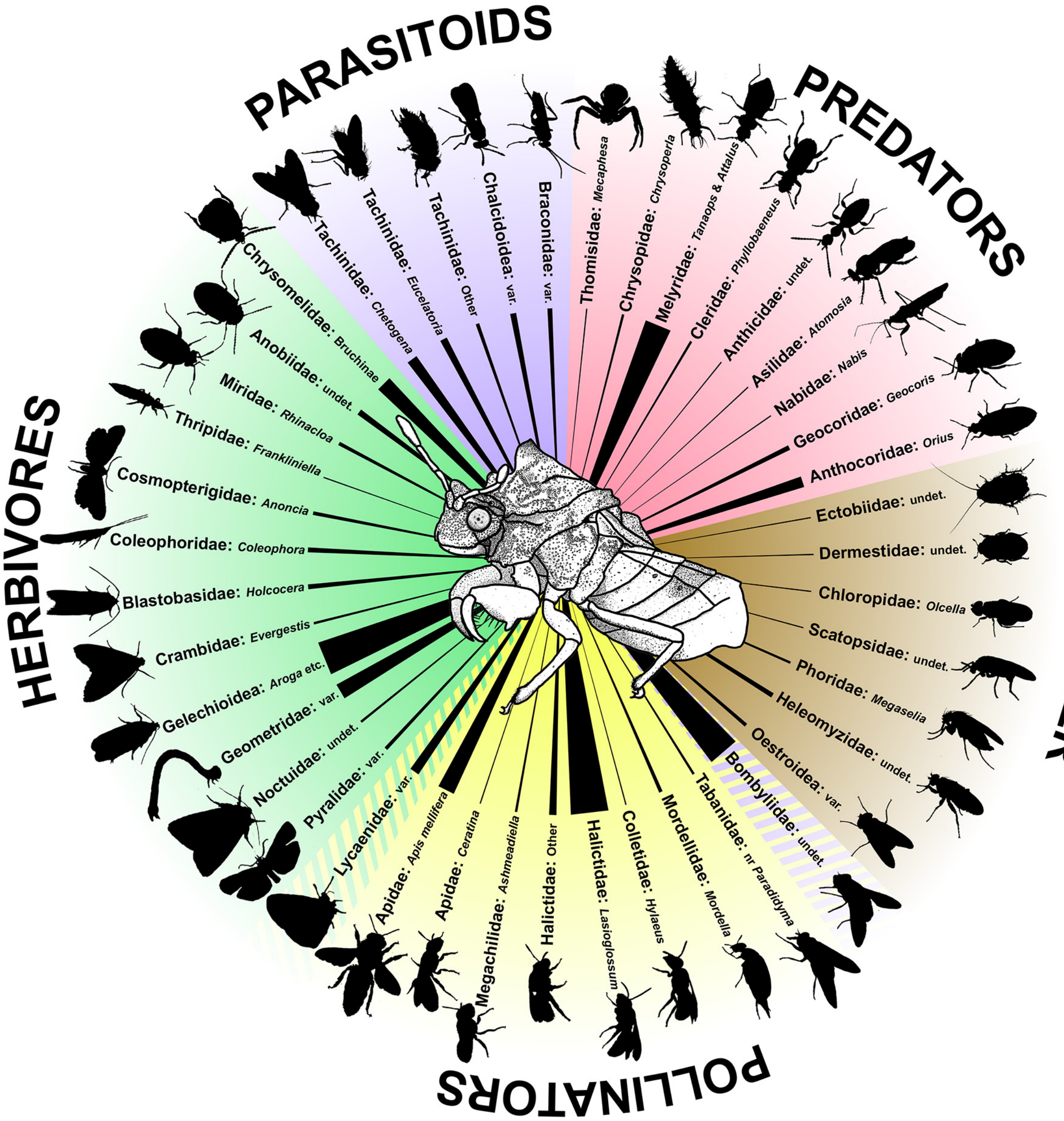

Fig. 1. Diversity of prey taxa identified from the guts of *Phymata pacifica*. Line thickness corresponds to the number of instances that a given taxon was detected from the 225 gut samples. Color shading represents the general trophic categories recognized in this study. Only taxa which matched with 90% or greater identity to the recovered amplicon sequence variants are displayed. Amplicon sequence variants which could not be identified below order level are not included.

heteropterans, including groups that are used in some systems as biological control agents such as *Nabis* Latreille (Cabello et al. 2009) and *Orius* Distant (Van De Veire and Degheele 1992), were also consumed by *P. pacifica*. Other examples of intraguild predation involved predation on *Mecaphesa* Simon, a genus of crab spiders that share a niche and are potentially direct competitors with *Phymata*; and predation on entomophagous Coleoptera such as Cleridae and Melyridae that visit blooming *E. fasciculatum* (Arnett et al. 2002). To our knowledge, intraguild predation between

Phymata and Thomisidae has never been formally documented until now.

Phytophagous insects also comprised a great proportion of the prey identified (91/280: ~33%). The diversity of lepidopteran prey at the generic level is unrivaled by other arthropod groups identified from gut contents. Sequence amplicon variants were matched to 10 families and ~22 genera of moths and butterflies. This diversity of lepidopteran prey is not surprising since *E. fasciculatum* serves as an important host resource for both immature and adult Gelechiidae (*Chionodes* Hübner), Lasiocampidae (*Gloveria* Packard), Geometridae (*Glaucina* Hulst, *Nemoria* Hübner, *Synchlora* Guenée), and Saturniidae (*Hemileuca* Walker) (Powell and Opler 2009). Gelechiid moths were one of the most frequently detected types of prey (26/280: ~9%). Other common non-lepidopteran herbivorous prey included potentially pestiferous chrysomelid beetles such as *Zabrotes* Horn (Meik and Dobie 1986) and plant bugs (Miridae; Culliney 2014).

Balduf (1941, 1942, 1943) conducted an observational study in a tallgrass prairie community in Illinois on the diet of a related ambush bug, *Phymata americana* Melin, and reported many of the same families of prey detected here. All six prey insect orders identified by Balduf were also recovered in this study on *P. pacifica* (Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, and Neuroptera).

Prey detectability half-life

Musca domestica DNA fragments of ~268 bp were successfully amplified from more than half of the fed *P. pacifica* for each post-feeding interval up until the 72 h mark (Appendix S1: Fig. S2). Based on the regression analysis, the DNA detectability half-life for prey in the guts of *P. pacifica* was estimated to be 90.6 h (intercept: 0.9781; slope: −0.005276). This detectability window is substantially longer than most insect predators for which half-lives have been estimated (Greenstone et al. 2014). Given their sit-and-wait predation strategy, ambush bugs, like many spiders, may have a lower metabolic rate and digest food slower than active-foraging predators (Anderson 1970, 1996, New 1975, Greenstone and Bennett 1980, Greenstone et al. 2007, 2014, Kobayashi et al. 2011, Virant-Doberlet et al. 2011). A long DNA detectability half-life could help explain why numerous ambush bugs from the MGCA survey simultaneously yielded DNA from two or more prey taxa (Table 1; 82/225: ~36%).

Heteropterans examined to date exhibit a wide spread of DNA detectability half-lives that range from less than a day to more than three days (Simmons et al. 2015), placing *P. pacifica* on the greater end of this spectrum. *Zelus renardii* (Kolenati), the only other assassin bug for which a detectability half-life has been estimated, also exhibits a rather long PCR prey detectability half-life time window of 51 h (Fournier et al. 2008). Our findings, as well as those from prior studies, are in line with the general notion that true bugs have relatively long detectability half-lives compared to other predatory insects such as beetles (Agustí et al. 2003, *Anthocoris*; Greenstone et al. 2007, *Podisus*; Hosseini et al. 2008, *Nabis*).

Efficacy of methods

In total, 280 different prey items were detected (Table 1). Of the 225 total *P. pacifica* gut samples sequenced, 203 remained after DADA2 filtering and denoising. Prey amplicon sequence variants were recovered from more than half of these (151 *P. pacifica* specimens). We also detected multiple prey items simultaneously from the guts of 82 different ambush bugs. Using a 95% identity threshold for sequences, 195 (~69%) of the 280 total prey items were identified to the generic or species level. Although the arthropod communities of CSS in Southern California have been relatively well sampled (Buffington and Redak 1998, Burger et al. 2003, Hung et al. 2015), it is clear that many taxa are yet to be COI-barcoded. The local database we compiled also facilitated identification; matches for 27 prey items (~10% of the 280 total) were obtained through BLAST searches against our COI database.

Approximately 80 morphospecies of buckwheat-associated arthropods were collected from CSS communities at our two field sites and were identified to the lowest taxonomic rank possible (Appendix S1: Table S2). We generated COI barcoding sequences for 51 of these specimens that did not have sequences available online (see Appendix S1: Table S2 for GenBank accession numbers). Among these, nine matched with 97% or greater identity to amplicon sequence variants recovered from the guts analyzed. Despite our efforts, sampling of non-*Phymata* arthropods was not comprehensive as we obtained prey sequences for numerous taxa that were not observed or collected in the field. This is likely a result of sampling time bias and/or ineffective collection methods (beating and sweeping vegetation and aerial netting).

The *P. pacifica*-specific blocking primer developed for this study appeared to greatly limit the amplification of host DNA as we witnessed a strong negative correlation between blocking primer concentration and the resulting visual signal from host DNA (Appendix S1: Fig. S1). This approach of coupling host-specific blocking primers with gut metabarcoding shows promise for use with predatory arthropods. Thus far, only a few molecular gut content analyses have been conducted on Reduviidae, the largest clade of non-holometabolous predators (~6800 spp.; Weirauch et al. 2014). While these studies have investigated the vertebrate host association of blood feeding kissing bugs (Reduviidae: Triatominae; Georgieva et al. 2017) and narrow diet range of termite assassin bugs (Reduviidae: Salyavatinae; Gordon and Weirauch 2016), this is the first study to evaluate the diet of a generalist assassin bug from a natural community using molecular gut analysis.

Limitations and solutions

Molecular gut content analysis can be limited or derailed by a host of issues. When conducting analyses that rely on universal primers, primer bias and taxonomic range are major concerns as they may fail to amplify certain taxonomic groups (Deagle et al. 2014, Sharma and Kobayashi 2014, Piñol et al. 2015). The universal primer set used here was highly effective and amplified DNA from 58 (~93.5%) of the 62 buckwheat-associated taxa for which PCR was attempted (Appendix S1: Table S2). We failed to amplify COI from two different hymenopterans, one heteropteran, and one coleopteran.

Our power to draw conclusions regarding trophic interactions ultimately hinges on the taxonomic breadth and reliability of databased sequences. Additional sampling of buckwheat-associated arthropods enabled us to identify some taxa for which limited sequence data are publicly available. However, even with additional sampling of flower-visiting taxa from CSS, we sequenced many amplicon variants that could not be classified below genus. It is clear that available COI sequence databases for CSS arthropods lack completeness, which is not surprising given the great biotic diversity associated with this community.

While naïvely reporting secondary predation (i.e., committing a false-positive error) is a potential problem when conducting MGCAs on predators that engage in intraguild predation (Sheppard et al. 2005, Hagler 2016), many of the prey items were identified from guts which bore DNA from only a single prey taxon (70/280: 25%) or multiple taxa of which none are considered to be entomophagous (24/280: ~8.6%). Ambush bugs, like all Hemiptera, possess piercing-sucking mouthparts and must extra-orally digest their food before siphoning it through a food canal formed by their maxillary stylets. Whether or not DNA from a previous meal in the prey's alimentary tract spills into the body cavity and is secondarily acquired by the true bug predator ultimately hinges on the time allowed for digestion and/or the ability of the stylet bundle to lacerate the gut (Cohen 1995).

Future directions

This study aimed to categorize ambush bug diet for only a short period in early summer and does not address the CSS community from a phenological prospective. The short timeframe allowed us to pool *P. pacifica* samples into one dataset and maximize sample size for the MGCA survey but inhibited us from comparing trophic interactions across an entire season. Future studies could potentially investigate temporal diet changes in generalist predators as different dominant plants come into bloom (e.g., California sage (*Artemisia californica* Lessing), chamise (*Adenostoma fasciculatum* Hook. & Arn.), or broomsage (*Lepidopartum squamatum* Gray)), as the temporal diversity of pollinators may vary (Hung et al. 2017).

In seeking to better understand natural systems, studies such as this provide useful data that can facilitate improved modeling of trophic networks. This study analyzes the diet of a single generalist from a community which supports a plethora of predatory arthropods, offering a unique perspective into trophic interactions in a diverse ecosystem. A diversity of predators were found hunting on buckwheat in relatively high abundance, including many crab, jumping, and lynx spiders (Thomisidae, Salticidae, and Oxyopidae, respectively) as well as other reduviids (*Apiomerus californicus* Berniker and Szerlip, *Zelus renardii* Kolenati, and *Zelus tetracanthus* Stål).

Phymata pacifica engage in an array of trophic interactions with pollinators, herbivores, and other entomophagous arthropods found in CSS communities in Southern California. While a wide diversity of hymenopteran pollinators were preyed upon, we detected DNA more frequently from non-pollinating taxa. We advocate that more studies make use of gut content metabarcoding to categorize trophic interactions between generalist predators and their prey in other complex and understudied natural systems. Since predation by generalists can have cascading effects across multiple trophic levels, it behooves us to discover and characterize their feeding habits.

ACKNOWLEDGMENTS

This study was funded by the Shipley-Skinner Reserve—Riverside County Endowment. The authors wish to thank the following people: Paul Rugman-Jones and Erin Rankin for advice on molecular protocols; Chrissy Dodge for aid with specimen collection; Erin Rankin and Jong Soon Lee for allowing us to run several test samples on a MiSeq lane; Alexander Knyshov and Eric Gordon for their kind assistance with script writing and execution of post-sequencing analyses; Austin Baker, Jacob Cecala, Alexander Knyshov, Adrian Mayor, Kyle Whorrall, and Doug Yanega for assistance with identification of non-reduviid buckwheat-associated arthropods; and finally Stephanie Castillo, Chrissy Dodge, John Heraty, Rochelle Hoey-Chamberlain, Alexander Knyshov, Carlos Rosas-Sanchez, and Samantha Smith for draft editing and their contribution of helpful comments.

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