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► To cite this version:

Julia Marion Schmack, Gavin Lear, Carmen Astudillo-garcia, Stéphane Boyer, Darren Ward, et al.. DNA metabarcoding of prey reveals spatial, temporal and diet partitioning of an island ecosystem by four invasive wasps. *Journal of Applied Ecology*, 2021, 58 (6), pp.1199-1211. 10.1111/1365-2664.13856 . hal-03408083

HAL Id: hal-03408083

<https://univ-tours.hal.science/hal-03408083v1>

Submitted on 28 Oct 2021

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1 **DNA metabarcoding of prey reveals spatial, temporal, and diet partitioning of an island**
2 **ecosystem by four invasive wasps**

3

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17

18 **Abstract**

19 1. Invasive alien species can cause detrimental changes in native ecosystems, but our
20 understanding of the interactions between multiple exotic species is limited. To evaluate the
21 joint effect of multiple sympatric invaders on an ecosystem, we must first understand how
22 they interact with each other.

23 2. Here, we quantified the spatial distribution, dietary composition and overlap of four
24 invasive generalist vespid species (two *Vespula* and two *Polistes*) that co-occur on Ahuahu

25 off the north-east coast of New Zealand. We used DNA metabarcoding of larval faecal
26 material to identify prey species, and mapped the locations of nests.

27 3. We observed clear spatial partitioning, with different wasp species aggregating in different
28 habitats. Diet differed both among wasp species (diet partitioning) and among seasons
29 (temporal partitioning). There was stronger competition (highest diet overlap) between *V.*
30 *germanica* and *V. vulgaris* than between other wasp species, but partitioning resources
31 minimised spatial and diet overlap between the four species.

32 4. Additionally, we obtained high-resolution diet inventories that help assess the combined
33 ecological impact of invasion by multiple wasps. These species consumed mostly native and
34 endemic invertebrates from multiple trophic levels, but particularly herbivores (Lepidoptera).
35 We provide direct evidence that in combination they exploited a wide range of endemic
36 invertebrates.

37 5. *Synthesis and applications.* Our study demonstrates resource partitioning among four
38 invasive wasps, facilitating coexistence of these generalist invaders. We conclude that
39 coexistence probably amplifies their ecological impact. Quantifying how multiple invaders
40 exploit and partition resources provides insights into how communities assemble and helps to
41 assess the cumulative effects of multiple invaders on the recipient community.

42

43 **Keywords**

44 *Vespula*, *Polistes*, diet overlap, invasive species, environmental DNA, Lepidoptera, resource
45 competition, niche partitioning

46

47

48 **Introduction**

49 A fundamental question in invasion ecology is how multiple invaders interact in native
50 ecosystems. When an invader colonises a novel habitat, one of the key factors for its success
51 is access to resources (Tilman, 2004; Stachowicz & Byrnes, 2006). Accordingly, the presence
52 of multiple invaders can result in competition between them. An invader may avoid this
53 competition by shifting its ecological niche (e.g. foraging behaviour, habitat use, or activity
54 patterns), thereby partitioning resources to minimise overlap with its sympatric counterpart
55 (Schoener, 1974; Shea & Chesson, 2002). In the absence of niche shifting, either or both
56 species may suffer deleterious effects (e.g. competitive exclusion, Gause, 1934; Griffen &
57 Delaney, 2007). Understanding the degree of interspecific competition and the mechanisms
58 that partition resources will provide insights into how communities assemble. It may also
59 help invasion ecologists to predict which invaders are likely to co-exist, and to assess the
60 cumulative effects of multiple invaders on the receiving community.

61 Invasive species can minimise competition with each other across many dimensions.
62 Competing species can coexist in one region by utilizing different habitats (*spatial*
63 *partitioning*, Chesson, 2000; Raby, Johnson, Kessel, Stewart, & Fisk, 2019). Species may
64 also avoid competition by exploiting different food resources (*diet partitioning*, Schoener,
65 1974; Takahashi et al., 2020). Finally, should species rely on the same resource – be it space,
66 food, or both – coexistence can be facilitated by exploiting this resource at different times
67 (*temporal partitioning*, Chesson, 2000; Valeix, Chamaillé-Jammes, & Fritz, 2007; Mori,
68 Ferretti, & Fattorini, 2019). Many studies have investigated resource partitioning between
69 invaders and their native counterparts to estimate ecological impacts of invasion (e.g. Junker,
70 Bleil, Daehler, & Blüthgen, 2010; Coccia et al., 2016; Culbertson & Herrmann, 2019).
71 However, comparatively few have asked how multiple invaders partition resources among

72 themselves (e.g. Boyer & Rivault, 2006; Freed & Leisnham, 2014), despite the potentially
73 important role of resource competition in determining coexistence.

74 Darwin (1859) proposed that close relatives should compete more intensely with one
75 another than with distant relatives (*competition-relatedness hypothesis, CRH*), as they are
76 more ecologically similar regarding habitat use, food resources and predators. Darwin's
77 hypothesis could be applied to biological invasions in two ways. Firstly, phylogenetic
78 relatedness between invasive species and native species may predict the strength of
79 competitive interactions and subsequently the ecological impact of invasion. However, recent
80 studies are mixed in their support for this (Venail et al., 2014; Alexandrou et al., 2015; Tan,
81 Pu, Ryberg, & Jiang, 2015). Secondly, relatedness among multiple invaders should predict
82 the extent to which they compete with each other and thereby the probability of coexistence.
83 According to the CRH, competition should be strongest between closely related invaders,
84 resulting in either the displacement of the inferior competitor or niche partitioning. The
85 present study will help inform the debate about Darwin's hypothesis in regards to invader-
86 invader relatedness.

87 Four invasive vespid species, *Vespula vulgaris*, *V. germanica*, *Polistes chinensis*
88 *antennalis*, and *P. humilis*, have established on New Zealand's 'mainland' and many of the
89 surrounding offshore islands (Beggs et al., 2011; Schmack et al., 2019; Schmack, Schleuning,
90 Ward, & Beggs, 2020). The specific invasion history of vespid wasps on New Zealand's
91 offshore islands is unknown, but all four species are found on many islands (Schmack et al.,
92 2020) and they have been widespread throughout New Zealand for many decades
93 (Clapperton, Moller, & Sandlant, 1989). They outcompete the native community for
94 resources and prey heavily on native taxa (Beggs & Wardle, 2006; Beggs et al., 2011; Ward
95 & Ramón-Laca, 2013). *Vespula* and *Polistes* exploit carbohydrate resources such as
96 honeydew produced by scale insects (Beggs, 2001) and prey on invertebrates, with

97 Lepidoptera constituting one of their main protein resources (Beggs et al., 2011). Vespid
98 invaders are likely to compete strongly with each other because they have similar resource
99 requirements; demonstrated by competition between *Vespula vulgaris* and *V. germanica* for
100 honeydew resources in southern beech (*Fuscospora* spp.) forests (Harris, 1991; Harris,
101 Moller, & Winterbourn, 1994). However, little is known of the interactions in other habitats
102 or between other invasive vespid species introduced to New Zealand.

103 The co-occurrence of four invasive wasp species on a small offshore island provides a
104 unique opportunity to investigate resource partitioning among multiple closely related
105 invaders. Because small islands are typically species depauperate and host less complex
106 communities than mainland habitats of equivalent size (MacArthur & Wilson, 1963) they
107 offer model ecosystems for studying invasion ecology against a less complicated ecological
108 background (Wardle, 2002). By assessing the distribution of nests and diet of wasp species,
109 we examined whether these four generalist invaders partition resources.

110 We evaluated the interactions of four sympatric wasp species on an offshore island to
111 address the following research questions: (a) do the four species spatially partition the island?
112 (b) do they partition food resources? To do this, we systematically mapped wasp nests in
113 seven different habitats on the island, and we collected larval faecal samples from the nests,
114 utilising next generation sequencing (NGS) of DNA to identify diet composition. Given New
115 Zealand's Lepidopteran fauna is characterised by an exceptionally high rate of endemism
116 (Hoare, 2010) and predation by invasive wasps represents a threat to indigenous biodiversity,
117 we additionally asked which invasive wasp species consumed the most endemic Lepidoptera.
118 Overall, this approach produces results at a high taxonomic resolution that can be utilised by
119 conservation practitioners to assess the ecological impacts of invasive species.

120

121 **Materials and methods**

122 *Study site and habitat types*

123 We collected *Polistes* and *Vespula* nests on Ahuahu (Great Mercury Island), an island off
124 north-eastern New Zealand (Fig. 1). We classified seven habitat types on Ahuahu based on
125 vegetation cover using the Land Cover Data Base v. 4.1 (Land Resource Information
126 Systems) coastal grassland; *Kanuka/Manuka* (*Kunzea ericoides*; *Leptospermum scoparium*)
127 forest; exotic pine *Pinus radiata* plantation with native undergrowth; native restorations;
128 residential gardens; clear-cutting; and mature native forest (dominated by Pohutukawa
129 *Metrosideros excelsa*, Kohekohe *Dysoxylum spectabile*, Puriri *Vitex lucens*) (Wright, 1976).
130 We used ArcGIS 10.5.1 (Environmental Systems Research Institute (ESRI) 2011) to
131 calculate the cumulative area covered by these habitat types (1830 ha) which comprised 98%
132 of the total island area (1860 ha). The remaining areas were sand and gravel.

133

134 *Nest sampling*

135 Two researchers searched 10 m x 500 m strip plots (0.5 ha) for nests during the peak of the
136 wasp season (March 2019). We covered approximately 70% of the island, allocating our
137 search effort proportional to the size of each habitat (Table 1) and avoiding inaccessible or
138 unsafe areas. Our search effort totalled 52 hours over nine days between 9 am and 7 pm when
139 foraging wasps were most active. We visually located nests by following the flight paths of
140 foraging wasps. GPS coordinates and habitat of each nest site were recorded. We collected 64
141 active nests (Table 1). *Polistes* nests were cut from branches or scraped off rocks and placed
142 in sealable plastic bags. *Vespula* nests were poisoned using a permethrin-based product 6 - 24
143 hours before being dug out of the ground and placed in sealable plastic boxes. All nests were
144 transported in chilly bins and stored at -20°C within 5 hours.

145

146 *DNA extractions*

147 Of the nests collected for the first part of this study, 51 were used to identify prey species that

148 adult wasps have fed their young over an entire wasp season. We collected three faecal
149 samples deposited at the base of a cell from each nest using tweezers, which were flame
150 sterilized before and after the collection of each faecal pellet to avoid cross-contamination.
151 Samples were collected from three nest layers containing different batches of brood
152 (Spradbery, 1973) to explore seasonal (early, mid, late) differences in prey composition (see
153 Supplementary Text S1, Fig. S1). One small nest of *P. chinensis* contained faecal matter
154 sufficient for one sample only. Samples were placed directly in microcentrifuge tubes and
155 disrupted for DNA extraction using a DNeasy Power Soil DNA extraction kit (Qiagen,
156 Germany) following manufacturers instructions, except for the bead-beating step where we
157 used a TissueLyser (Qiagen, Germany) at 30,000 rpm for 1 min. The DNA was extracted
158 following manufacturers procedures. DNA concentrations in the resulting eluates were
159 measured by fluorometry using a Qubit® with the dsDNA HS Assay Kit (Life Technologies,
160 Carlsbad, CA).

161

162 *DNA amplification and sequencing*

163 For DNA amplification, primers targeting a short (313 bp) gene region of the mitochondrial
164 gene COI were used (namely m1COIintF: 5'-TCGTCGGCAGCGTCAGATGTGTAT
165 AAGAGACAGGGWACWGGWTGAACWGTWTAYCCYCC-3'; Leray et al., 2013) and
166 HCO2198: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAAGAGACAGTAAACTTCAGG
167 GTGACCAAAAATCA-3'; Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 2013). This primer
168 pair performs well across a range of metazoan samples (Leray et al., 2013), and has
169 successfully been used for faecal samples of *Polistes* (Lefort et al., 2020). Primers include the
170 Illumina adapter sequences (underlined) required for downstream sequencing. The following
171 PCR conditions were applied to our DNA templates: 12.5 µL KAPA HiFi Hotstart ReadyMix
172 (Kapa Biosystems, catalog number KK2602), 0.75 µL of each primer at 10 µM (m1COIintF

173 and HCO2198), 3.5 μ L fecal DNA extraction and 7.5 μ L PCR water to fill the reaction up to
174 25 μ L. Negative controls containing DNA-free water instead of the DNA template were
175 included in all PCR runs. PCR cycling conditions were 95 $^{\circ}$ C for 3 min followed by 14
176 cycles of 95 $^{\circ}$ C for 10 s, 62 $^{\circ}$ C (- 1 $^{\circ}$ C per cycle) for 30 s and 72 $^{\circ}$ C for 60 s. We then
177 conducted 25 cycles of 95 $^{\circ}$ C for 10 s, 48 $^{\circ}$ C for 30 s and 72 $^{\circ}$ C for 60 s and a final elongation
178 of 7 minutes at 72 $^{\circ}$ C. PCR products were electrophoresed in 1 % agarose gel stained with
179 SYBR Safe (Thermo Fisher, US) and visualized by UV light. According to standard protocol
180 (Support Illumina 2016), PCR products were purified using ZR-96 DNA Cleanup kits (Zymo
181 Research). DNA concentrations in the resulting eluates were measured by fluorometry using
182 a Qubit $^{\circ}$ with the dsDNA HS Assay Kit (Life Technologies, Carlsbad, CA) and standardised
183 to 2 μ M before being submitted to New Zealand Genomics Ltd. (Auckland, New Zealand)
184 for sequencing on an Illumina MiSeq instrument using 2-by-300-bp chemistry. Prior to
185 sequencing, a unique combination of Nextera XT dual indices (Illumina Inc., USA) was
186 attached to the DNA to allow multiplex sequencing; two sequencing runs were performed
187 with 151 samples multiplexed per sequencing run.

188

189 *Bioinformatics*

190 We used USEARCH v 10 (Edgar, 2010) for quality filtering of DNA sequences. Primer
191 sequences were removed by trimming 24 bp of each read and paired end reads were merged.
192 Merged reads were filtered using an expected error threshold of one and replicates removed
193 to identify unique sequences. Quality filtered reads were clustered at 100% identity to
194 generate zero-radius operational taxonomic units (zOTUs) using the UNOISE algorithm in
195 USEARCH. zOTUs were then compared to the Barcode of Life Database (BOLD). Only
196 zOTUs for which read relative abundance within a sample was higher than 0.5% of the total
197 reads per sample were retained to limit false positives. We used a conservative approach and

198 kept only zOTUs identified at species level above the percentage identity of 98 %. We
199 checked suggested matches of Lepidoptera with a taxonomic index (Hoare, 2010). zOTUs
200 identified as terrestrial and aquatic invertebrates or vertebrates were used for further analysis,
201 while reads corresponding to fungi and plant DNA as well as *Vespula* and *Polistes* DNA
202 were removed to avoid contamination. We assigned prey as native, endemic, or exotic using
203 the New Zealand Organisms Register (www.nzor.org.nz).

204

205 *Statistical analysis*

206 All analyses were performed in RStudio; the main packages used were *vegan* (Oksanen et al.,
207 2019), *mvabund* (Wang, Naumann, Wright, & Warton, 2012), *EcoSimR* (Gotelli & Ellison,
208 2013) and *spaa* (Zhang & Zhang, 2013). To answer whether wasp species partition spatially,
209 we quantified whether the nests of a given species were more aggregated than expected by
210 chance alone using Stone and Roberts' (1990) c-score (see Text S2).

211 The model-based approach *mvabund* uses simultaneous generalized linear models
212 (ManyGLM; Wang et al., 2012), which specify the mean-variance relationship instead of
213 relying on the assumptions of a distance-based approach. We used multivariate generalised
214 linear models with binomial distribution to investigate differences in prey composition
215 among wasp species, seasonal samples and seasonal samples of a given wasp species.
216 Likelihood ratio tests (LRT) and resampled p-values were used to test effects of wasp species
217 and seasonality on diet composition In two different input data: a zOTU table containing
218 presence/absence of all prey species and a zOTU table containing presence/absence of
219 Lepidopteran prey species.

220 We quantified dietary niche width of wasps using Levin's index (Levin, 1968) and
221 calculated trophic niche overlap between all pairs of wasp species (0 = no overlap, 1 =
222 complete overlap) using the Pianka index (Pianka, 1973). We visualized diet overlap with

223 network diagrams generated using the *bipartite* package (v.2.15, Dormann, Gruber, & Fründ,
224 2008). We calculated mean prey richness (number of species per sample) for each wasp
225 species and compared it among wasp species and nest layers, using a non-parametric
226 Kruskal-Wallis test and the non-parametric Dunn's test (p -values adjusted with the Holm
227 method) to determine significant differences between means.

228 When quantifying the ecological impact of wasps on the endemic community, we
229 intentionally did not account for differences in sample size. Instead, we counted all endemic
230 taxa identified as prey of the four wasp species.

231

232 **Results**

233 Spatial partitioning

234 We observed clear spatial partitioning among all four species of invasive wasps (Fig. 1, Table
235 1). Nests of all four species were significantly more aggregated than if they were randomly
236 distributed across habitats as expected under null simulation (*P. chinensis*, $c_{observed} = 0.476$,
237 $c_{simulated} = [0.824, 0.877]$, $p = 0.001$; *P. humilis*, $c_{observed} = 0.429$, $c_{simulated} = [0.713, 0.964]$, $p =$
238 0.001 ; *V. germanica*, $c_{observed} = 0$, $c_{simulated} = [0.667, 1]$, $p = 0.02$; *V. vulgaris*, $c_{observed} = 0.533$,
239 $c_{simulated} = [0.783, 0.9]$, $p = 0.001$). For *V. vulgaris* nests ($n=16$), 50% of nests occurred in
240 *Kanuka/Manuka* forest and 50% occurred in pine plantation. In contrast, *V. germanica* nests
241 ($n=3$) were only found in coastal grassland. Both *P. humilis* and *P. chinensis* nests were
242 exclusively found in open habitats. However, while *P. chinensis* occurred in four types of
243 open habitat ($n=25$ in coastal grassland, $n=1$ in residential gardens, $n=9$ in native restorations,
244 $n=3$ in clear cutting), *P. humilis* inhabited solely grassy habitats ($n=5$ in coastal grassland,
245 $n=2$ in residential gardens). No nests of any species were found in mature native forest.

246

247 *DNA sequencing and identified prey*

248 DNA was successfully amplified and sequenced from 148 (n=67 for *P. chinensis*, n=24 for *P.*
249 *humilis*, n=9 for *V. germanica*, n=48 for *V. vulgaris*) of the 151 faecal samples (three samples
250 per nest, except for one small nest where faecal matter was sufficient for one sample only).
251 We obtained an average of 4703 DNA reads (\pm 497 SE) per sample. Out of 1,729 zOTUs,
252 262 (15%) could be assigned to species level using the Barcode of Life Database (BOLD);
253 85% yielded no match and were discarded before analysis. Seven zOTUs were assigned to
254 the four predators (n = 2 *P. chinensis*, n = 2 *P. humilis*, n = 2 *V. vulgaris*, n = 1 *V.*
255 *germanica*), and were discarded prior to analysis to account for potential cross-
256 contamination. We identified 105 species belonging to 16 orders of animals (Table 2).
257 Individual faecal samples contained between one and six prey orders and between one and 17
258 prey species (Table S1, for a list of prey species per wasp species). Out of the 105 identified
259 prey species, 59 (i.e. 56%) occurred exclusively in the diet of different wasp species and only
260 10 were shared among all four wasp species. Lepidoptera, Diptera and Hymenoptera were
261 important prey orders consumed by all four wasp species (Fig. 3). Of the 105 prey species
262 identified, 44 (42%) were endemic, 18 (17%) were native and 41 (39%) were exotic. For two
263 prey species, their status in New Zealand could not be identified (*Amblyseius sp. JCS08*,
264 *Spilomelinae gen. spiloBioLep01*). Lepidoptera comprised the majority of identifications (46
265 taxa) and occurred in 95% of all samples (Table 2). Of the 45 Lepidopteran species
266 identified, 18 were endemic species, 12 were native, 14 exotic and one was of unknown
267 status (*Spilomelinae gen. spiloBioLep01*).

268 Rarefaction curves (Fig. 2) with the samples collected for this study did not reach
269 an asymptote, suggesting that more prey species could have been identified with more
270 samples. *V. germanica* had particularly few samples (n=3 nests equivalent to 9 samples).
271 Nevertheless, we are confident that although diets may be incomplete, the main prey taxa

272 were detected. Furthermore, indices used to compare diets among wasps (i.e. species
273 richness, niche width) are calculated taking sample size into account.

274

275 *Diet and temporal partitioning*

276 Diet composition (at the zOTU level) differed significantly between wasp species and
277 seasonal samples (Table 3, Fig. 3). The diet of each wasp species varied across seasonal
278 samples, indicating a change in prey species detected as the season progressed. Lepidoptera
279 diet composition (at the zOTU level) differed significantly between wasp species and
280 seasonal samples (Table 3, Fig. 3). The Lepidopteran diet of each wasp species varied across
281 seasonal samples, indicating a change in Lepidopteran prey species detected as the season
282 progressed.

283

284 *Comparison of diet composition*

285 Average prey richness per sample varied significantly among wasp species ($Chi = 39$, $p =$
286 0.001) (Table 4, Fig. 4). *P. chinensis* fed on fewer species than *V. germanica* ($z = -3.09$, $p =$
287 0.005) and *V. vulgaris* ($z = -5.96$, $p < 0.001$). *P. humilis* fed on fewer species than *V. vulgaris*
288 ($z = -2.80$, $p = 0.01$). Niche width (Table 4) and overlap varied among wasp species (Fig. 5).
289 *V. germanica* had the widest and *V. vulgaris* the narrowest trophic niche. Pianka index for
290 trophic niche overlap was highest between *V. germanica* and *V. vulgaris* (0.68). It was lowest
291 between *P. chinensis* and *V. vulgaris* (0.27) as well as between *P. humilis* and *V. vulgaris*
292 (0.27). Trophic niche overlap was 0.33 between *P. chinensis* and *P. humilis*, 0.45 between *P.*
293 *chinensis* and *V. germanica*, and 0.3 between *P. humilis* and *V. germanica*.

294 Lepidoptera were an important part of the diet of all wasp species, although they
295 consumed different families and species (Fig. 3, Fig. 5). While *P. humilis* and *P. chinensis*
296 preyed predominantly on Geometridae (80% and 46%, respectively), *V. germanica* and *V.*

297 *vulgaris* consumed mainly Tortricidae (37% and 57%, respectively) (Fig. S2). Lepidopteran
298 prey richness per sample differed significantly among wasp species ($Chi = 18$, $p = 0.001$), as
299 did niche width (Table 4) and overlap of Lepidopteran diet. *P. chinensis* fed on fewer
300 Lepidopteran species than *V. vulgaris* ($z = -4$, $p < 0.001$) and *P. humilis* ($z = -3$, $p < 0.05$).
301 This trend was not significant in *V. germanica* ($z = -1$, $p > 0.05$). *P. humilis* had the widest
302 and *V. vulgaris* the narrowest Lepidopteran trophic niche.

303 *V. vulgaris* consumed the most endemic species overall ($n=36$) and the most
304 endemic Lepidoptera species ($n=16$). In contrast, *V. germanica* fed on 12 endemic species, of
305 which five were endemic Lepidoptera. *P. humilis* and *P. chinensis* consumed 10 and 15
306 endemic species respectively, of which eight and seven were endemic Lepidoptera (Fig. 6).

307

308 **Discussion**

309 We provide evidence of spatial, trophic and temporal partitioning among four
310 invasive social wasps co-occurring on Ahuahu, New Zealand. Using DNA metabarcoding,
311 we identified 105 prey species in the seasonal diet of the four wasp species. This highlights
312 the large number of species, particularly Lepidoptera, that are potentially impacted when
313 generalist introduced predators co-occur. Although we could not assign the majority of
314 zOTUs to species level, we did reveal trophic interactions with soft-bodied species that would
315 have been impossible to detect using morphological techniques. For example, we recorded
316 the basket-cocoon parasitoid *Meteorus pulchricornis* and their Lepidopteran hosts (Berry &
317 Walker, 2004) in the same samples for all four wasp species. It is likely that wasps preyed on
318 Lepidoptera larvae parasitised by *M. pulchricornis* rather than directly preying on the
319 parasitoid. Our interpretation of wasp diet is a conservative description because of the limited
320 availability of reference sequences for New Zealand, particularly for endemic species. This
321 improves the robustness of our ecological conclusions (Calderón-Sanou et al., 2019)

322 although we note that differences in barcoding efforts for taxonomic groups may have led to
323 over- or underrepresentation of some groups.

324

325 *Spatial partitioning*

326 Wasps partitioned Ahuahu spatially as different wasp species aggregated in different habitats.
327 Only two out of seven habitat types were occupied by more than one wasp species. Maximum
328 overlap occurred in grassland habitat, where *P. chinensis*, *P. humilis* and *V. germanica* were
329 present. Mature native forest was not utilised by any species, but we did find *V. vulgaris* nests
330 in both the more open exotic pine plantation and in native *Kanuka/Manuka* forest that had an
331 abundant honeydew resource produced by the endemic scale insect *Coelostomidia wairoensis*
332 (Gardner-Gee & Beggs, 2012). This is consistent with prior research suggesting that dense
333 canopy cover without honeydew resources inhibits wasp invasion on islands (Schmack et al.,
334 2020). *P. chinensis* was the only species found in native restorations and clear cutting, which
335 are human-modified habitats characterized by young native vegetation and recent succession
336 of exotic weeds, respectively. *P. humilis* nests were less abundant than *P. chinensis* nests and
337 restricted to grassy habitat.

338 Spatial partitioning may result from intense competition between closely related
339 species. *V. germanica* was only found in coastal grassland. It is probably displaced from
340 *Kanuka/Manuka* forest by *V. vulgaris* competing for honeydew. Competitive exclusion in this
341 habitat is in line with earlier studies from New Zealand's honeydew-producing southern
342 beech (*Fuscospora* spp.) forests, which demonstrated that *V. vulgaris* outcompetes *V.*
343 *germanica* for honeydew (Harris, 1991; Harris et al., 1994). The co-existence of both *Vespula*
344 species at broader spatial scales suggests alterations to other aspects of their niche, preventing
345 total displacement (Crowder & Snyder, 2010).

346

347 *Diet and temporal partitioning*

348 There was also evidence of both diet and temporal partitioning amongst the four wasp
349 species. Seasonal changes in diet composition are likely due to seasonal differences in prey
350 availability. For example, the second most consumed species in our study was Plantain moth
351 *Scopula rubraria*, which are most abundant from summer to mid-autumn (Gerard, Philip,
352 Ferguson, & Eden, 2017). We recorded twice as many occurrences of *S. rubraria* in late
353 season samples compared to earlier in the season. We have interpreted a difference between
354 nest layers/circles as a seasonal difference in diet. However, an alternative explanation is that
355 differences between layer/circle may instead reflect differences in DNA degradation over
356 time. Further research is needed to resolve this, but we have no reason to expect that DNA
357 degradation would vary within taxa (such as Lepidoptera). Trophic partitioning has been
358 demonstrated in a range of closely related sympatric species. For example, partitioning of
359 food resources allowed invasive round gobies *Apollonia melanostoma* and bighead gobies
360 *Neogobius kessleri* to coexist in the Danube river (Borza, Erős, & Oertel, 2009). Similarly,
361 diet partitioning occurred in four sympatric Gomphocerinae grasshopper species in subalpine
362 grassland in the French Alps (Ibanez et al., 2013). Future assessments on whether an
363 introduced species will establish should consider the potential for resource partitioning with
364 other species.

365 *Polistes* are known to feed predominantly on Lepidoptera (Kasper et al., 2004; Todd
366 et al., 2015; Ward & Ramon-Laca, 2013), while *Vespula* also consume other invertebrates
367 such as Diptera and Araneae (Harris & Oliver, 1993; Kasper et al., 2004). Our research
368 confirms this, but we also show that the four wasp species partition within Lepidoptera; while
369 both *Polistes* mainly fed on Geometridae, *V. germanica* and *V. vulgaris* mostly consumed
370 Tortricidae. This contrasts with *Polistes* in New Zealand kiwifruit orchards, which collected
371 more Noctuidae rather than Geometridae (Todd et al., 2015). We suggest that the diet of

372 generalist Vespids reflects differences in prey availability in different habitats and seasons,
373 although foraging preferences likely play a role as well.

374 Prey richness and niche width differed considerably among wasp species. *V.*
375 *germanica* had the most species rich diet and the widest trophic niche, while both *Polistes*
376 species had a relatively narrow niche. Likewise, *V. germanica* in Australia had a wide niche
377 that entirely included the comparatively narrow niche of *P. humilis* (Kasper et al., 2004).
378 Theoretically, the species with an included niche must be a superior competitor for the shared
379 resource on a per capita basis to coexist with its more generalist counterpart (Chase, 1996;
380 Guevara & Avile's, 2011). Our findings are consistent with theoretical predictions about
381 included niche and may at least partly explain why *V. germanica*, *P. humilis* and *P. chinensis*
382 can coexist in the same habitat. Alternatively, our results could demonstrate that instead of
383 being the stronger competitor, *P. humilis* avoids competition with *V. germanica* by separating
384 spatially.

385 We observed the highest diet overlap between *V. vulgaris* and *V. germanica*,
386 indicating stronger competition between these closely related species than between *Vespula*
387 and *Polistes*. This supports Darwin's (1859) *competition-relatedness hypothesis*. In contrast,
388 we found *P. chinensis* shared more prey species with *V. vulgaris* than with its congeneric *P.*
389 *humilis*. In fact, other studies also show that phylogenetic relatedness does not always predict
390 the nature and strength of competition among species (Venail et al., 2014; Alexandrou et al.,
391 2015; Lyu, Liu, Venail, & Zhou, 2017), but rather that species interactions and resource
392 overlap shape the assembly and evolution of communities (Wilcox, Schwartz, & Lowe,
393 2018).

394

395 *Ecological impact*

396 Coexistence of multiple generalist invaders is likely to intensify their ecological effect
397 (Crowder & Snyder, 2010). The four wasp species in our study fed on a broad range of both
398 herbivores (e.g. Lepidoptera) and predators (e.g. Araneae), potentially affecting the native
399 community on multiple trophic levels. Furthermore, the majority of prey species were
400 endemic or native to New Zealand. By partitioning resources, the four species in combination
401 would impact a wide range of endemic species in the ecosystem. *Vespula vulgaris* are known
402 to cause ecological damage to New Zealand's native ecosystems (Beggs et al., 2011),
403 particularly to the Lepidopteran fauna (Beggs & Rees, 1999). We conclude that coexistence
404 of *V. vulgaris* and three other vespid wasps probably amplifies their ecological impact
405 because they partition resources spatially and trophically.

406

407 *Conclusion*

408 DNA metabarcoding of faecal material revealed novel insights into the nature and strength of
409 trophic interactions among four sympatric social wasp species. We conclude that these
410 invaders were avoiding competition with each other by shifting their ecological niche,
411 partitioning resources to minimise overlap with their counterparts. There was stronger
412 competition (highest diet overlap) between *V. germanica* and *V. vulgaris* than between other
413 wasp species. Indeed, in one habitat we found evidence of competitive exclusion between the
414 two *Vespula* species, probably because of competition for honeydew resources. In contrast, *P.*
415 *chinensis* shared more species with *V. vulgaris* than with its congener *P. humilis*. We
416 therefore provide further evidence that competitive interactions and resource overlap rather
417 than taxonomic relatedness shape the structuring of ecological communities. We suggest
418 coexistence of these generalist invaders on Ahuahu intensified their ecological effect,
419 particularly on endemic Lepidoptera that comprised a large part of the diet of all wasp
420 species. By providing a better understanding of resource partitioning by these four generalist

421 species, we have gained an improved assessment of the total ecological impact when there
422 are multiple invaders.

423

424 **Acknowledgements**

425 Thanks to the owners and caretakers of Ahuahu (Great Mercury Island) for supporting this
426 research, Matthew Biddick for field assistance, Syrie Hermans and Michael Hoggard for
427 support with laboratory and data analysis, Robert Hoare for his invaluable expertise
428 identifying Lepidoptera, and Bryce McQuillan and Olivier Ball for photos. This study was
429 funded by MBIE-SSIF (Biota Portfolio, Landcare Research), the Te Hauturu Supporters
430 Trust, the University of Auckland and the Biological Heritage National Science Challenge.

431 **Authors' Contributions**

432 JMS and JRB designed the study. JMS collected wasp nests, performed laboratory analysis,
433 analysed the data, prepared the figures and led the writing. SB provided the method. GL and
434 CAG supported laboratory analysis. All authors contributed critically to the drafts and gave
435 final approval for publication.

436 **Data Availability Statement**

437 Data available via the Dryad Digital Repository <https://doi:10.5061/dryad.r4xgxd2bq>
438 (Schmack et al., 2021). .

439

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441

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633 Table 1. Number of wasp nests of *P. chinensis*, *P. humilis*, *V. germanica*, and *V. vulgaris* collected from seven habitat types on Ahuahu off the
634 east coast of New Zealand. For each habitat type, we give its size, the proportion of the total island area it covers, the search effort spent finding
635 nests and its proportion of the total search effort for the whole island.

Habitat type	Size		Search effort		Number of nests per species			
	[ha]	[%]	[h]	[%]	<i>P. chinensis</i>	<i>P. humilis</i>	<i>V. germanica</i>	<i>V. vulgaris</i>
Coastal grassland	683	37.3	15	28.9	26	6	3	0
Residential garden	2	0.1	2	3.9	1	2	0	0
Clear cutting	5	0.3	2	3.9	3	0	0	0
Native restorations	38	2.1	4	7.7	7	0	0	0
<i>Kanuka/Manuka</i> forest	297	16.2	9	17.3	0	0	0	8
Pine plantation	772	42.2	18	34.6	0	0	0	8
Mature native forest	29	1.6	2	3.9	0	0	0	0

636 Table 2. Numbers and percentages of prey identified from 148 wasp faecal samples of four invasive vespid wasps co-occurring on Ahuahu off
 637 the east coast of New Zealand: *Polistes chinensis* (Pc), *P. humilis* (Ph), *Vespula germanica* (Vg) and *V. vulgaris* (Vv). The most common taxa
 638 for each order and the number of samples it was identified from is given, along with its status in New Zealand.

Order (# species)	Percentage of total diet				Number of prey taxa				Most common species (# samples it was recorded in)	NZ Status
	Pc	Ph	Vg	Vv	Pc	Ph	Vg	Vv		
Number of nests	24	8	3	16	24	8	3	16		
Number of samples	70	24	9	48	70	24	9	48		
Insecta										
Lepidoptera (45)	77	87.5	46.9	62	27	19	13	31	<i>Ctenopseustis obliquana</i> (72)	endemic
Hymenoptera (6)	2.8	7.2	3.1	5.2	5	3	2	6	<i>Meteorus pulchricornis</i> (22)	exotic
Diptera (13)	3.2	2	22.4	5.2	3	2	8	10	<i>Oxysarcodexia varia</i> (21)	exotic
Hemiptera (8)	7.3	0	9.2	11.5	6	0	2	4	<i>Kikihia ochrina</i> (43)	endemic
Orthoptera (3)	0	0	8.2	0.2	0	0	2	1	<i>Hemiandrus pallitarsis</i> (6)	endemic
Coleoptera (2)	0.6	0	0	0.2	1	0	0	1	<i>Eucolaspis sp. PRCD2015c</i> (2)	endemic
Neuroptera (2)	0	0	0	2.5	0	0	0	2	<i>Wesmaelius subnebulosus</i> (6)	exotic
Psocodea (2)	1.3	0	1	0	2	0	1	0	<i>Liposcelis paeta</i> (4)	exotic
Annelida										
Haplotaxida (1)	0.3	0	0	0.2	1	0	0	1	<i>Enchytraeus buchholzi</i> (2)	exotic
Mollusc										
Stylommatophora (1)	0	0	0	0.2	0	0	0	1	<i>Athoracophorus bitentaculatus</i> (1)	endemic
Octopoda (1)	0	0	1	0	0	0	1	0	<i>Argonauta nodosa</i> (1)	native

Arachnida

Araneae (12)	1.6	0	5.1	12.6	2	0	3	12	<i>Eriophora pustulosa</i> (28)	native
Mesostigmata (2)	0	2	0	0	0	2	0	0	<i>Amblyseius sp. JCS08</i> (2)	exotic
Opliliones (1)	0.3	0	0	0	1	0	0	0	<i>Phalangium opilio</i> (1)	exotic

Vertebrata

Rodentia (1)	5.7	1.3	0	0.2	1	1	0	1	<i>Mus musculus</i> (21)	exotic
Charadriiformes (1)	0	0	3.1	0	0	0	1	0	<i>Chroicocephalus novaehollandiae</i> (3)	native

639 Table 3. Analysis of deviance tables for differences in diet among four invasive wasp species
 640 (*P. chinensis*, *P. humilis*, *V. germanica* and *V. vulgaris*); seasonal samples across all wasp
 641 species and seasonal samples of each wasp species are given. Differences were calculated
 642 among all prey and within the main prey order Lepidoptera.

	Prey composition		Lepidopteran prey composition	
	LRT	p	LRT	p
Wasp species	1508.5	0.001***	1084.9	0.001***
<i>P. humilis</i>	125.41	0.001***	89.88	0.001***
<i>V. germanica</i>	189.03	0.001***	75.96	0.001***
<i>V. vulgaris</i>	306.43	0.001***	219.79	0.001***
Time of season	424.0	0.002**	224.6	0.039*
<i>Mid-season</i>	75.71	0.006**	43.81	0.002**
<i>Late season</i>	97.29	0.002**	69.81	0.001***
Wasp species x Time of season	224.1	0.003**	164.9	0.001***
<i>P. humilis x mid season</i>	41.93	0.007**	32.97	0.003**
<i>V. germanica x mid season</i>	28.90	0.006**	13.45	0.032*
<i>V. vulgaris x mid season</i>	31.82	0.008**	22.07	0.005**
<i>P. humilis x late season</i>	26.17	0.004**	21.97	0.003**
<i>V. germanica x late season</i>	26.47	0.003**	9.00	0.127
<i>V. vulgaris x late season</i>	29.91	0.008**	19.21	0.003**

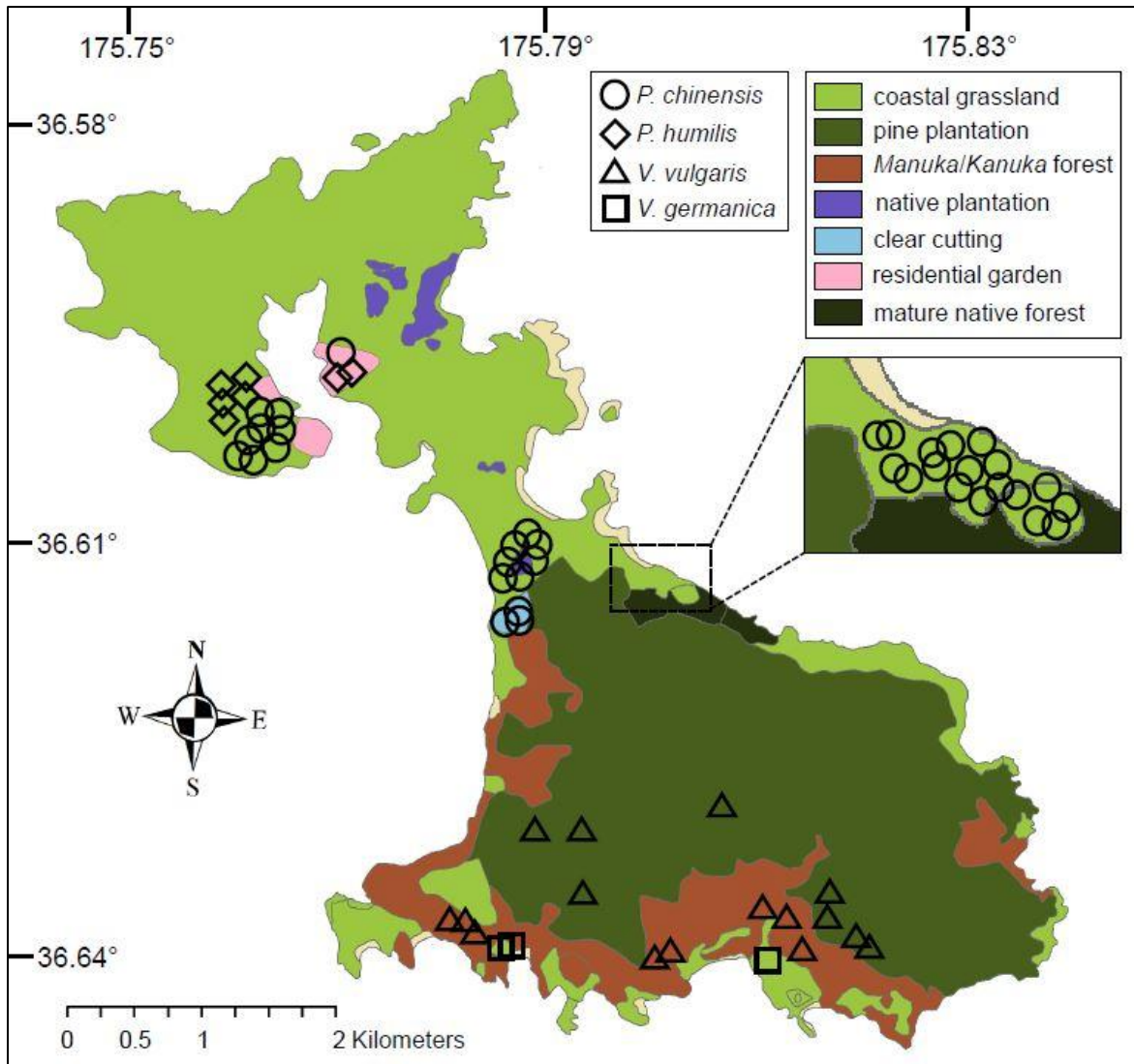
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644

645 Table 4. Diet indices of *P. chinensis*, *P. humilis*, *V. germanica* and *V. vulgaris* based on DNA
 646 metabarcoding of faecal samples collected from nests on Ahuahu, New Zealand. Given are
 647 the mean prey species richness and Levin's index of trophic niche width. This was calculated
 648 for all prey taxa and for only Lepidopteran prey taxa.

Species	Prey richness (\pm SE)	Niche width	Lepidopteran prey richness (\pm SE)	Lepidopteran niche width
<i>P. chinensis</i>	4.73 \pm 0.05	9.54	3.64 \pm 0.04	6.02
<i>P. humilis</i>	6.33 \pm 0.16	11.13	5.54 \pm 0.15	8.48
<i>V. germanica</i>	10.89 \pm 0.76	16.56	5.11 \pm 0.38	5.94
<i>V. vulgaris</i>	9.27 \pm 0.08	9.32	5.75 \pm 0.06	3.53

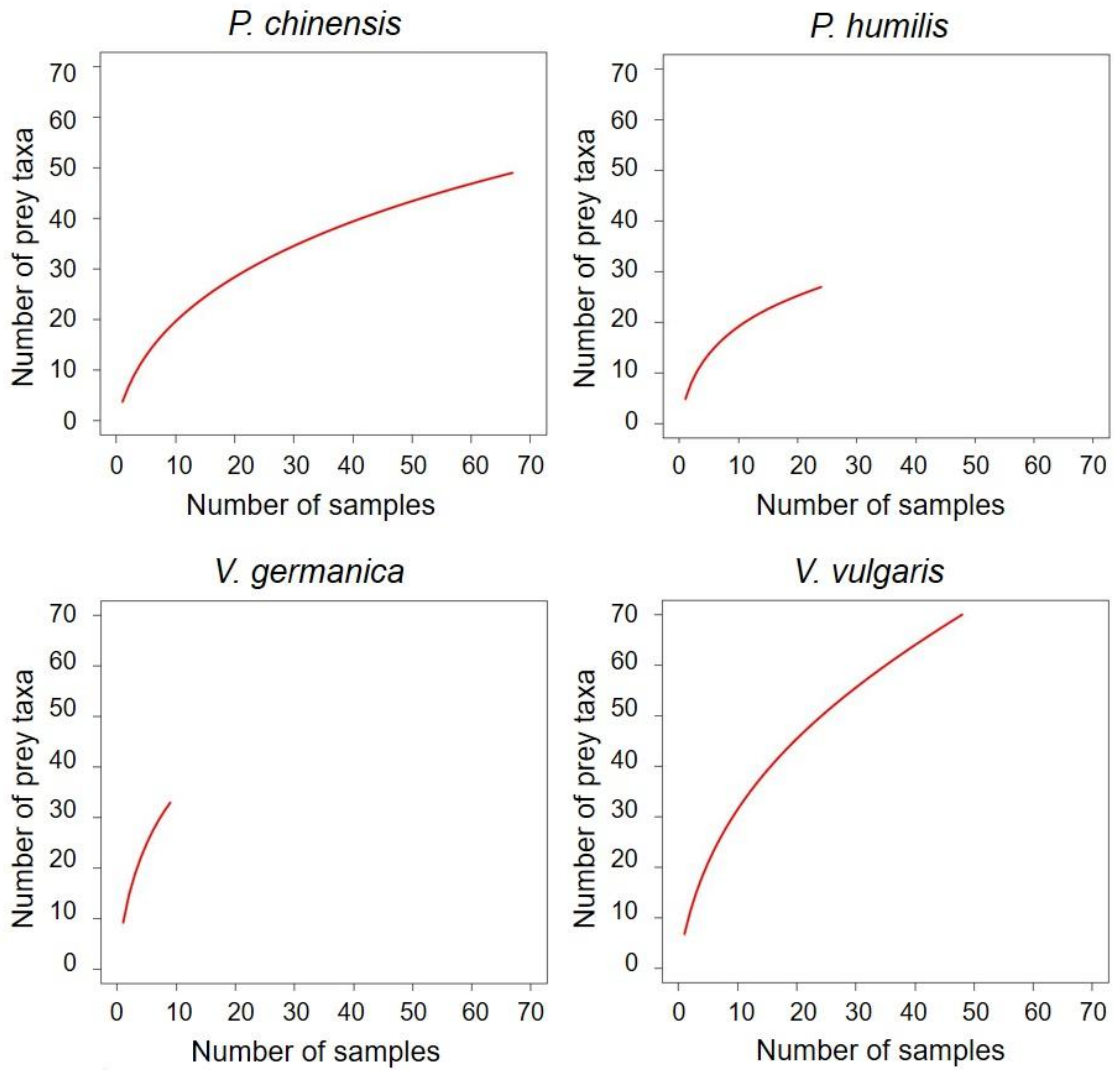
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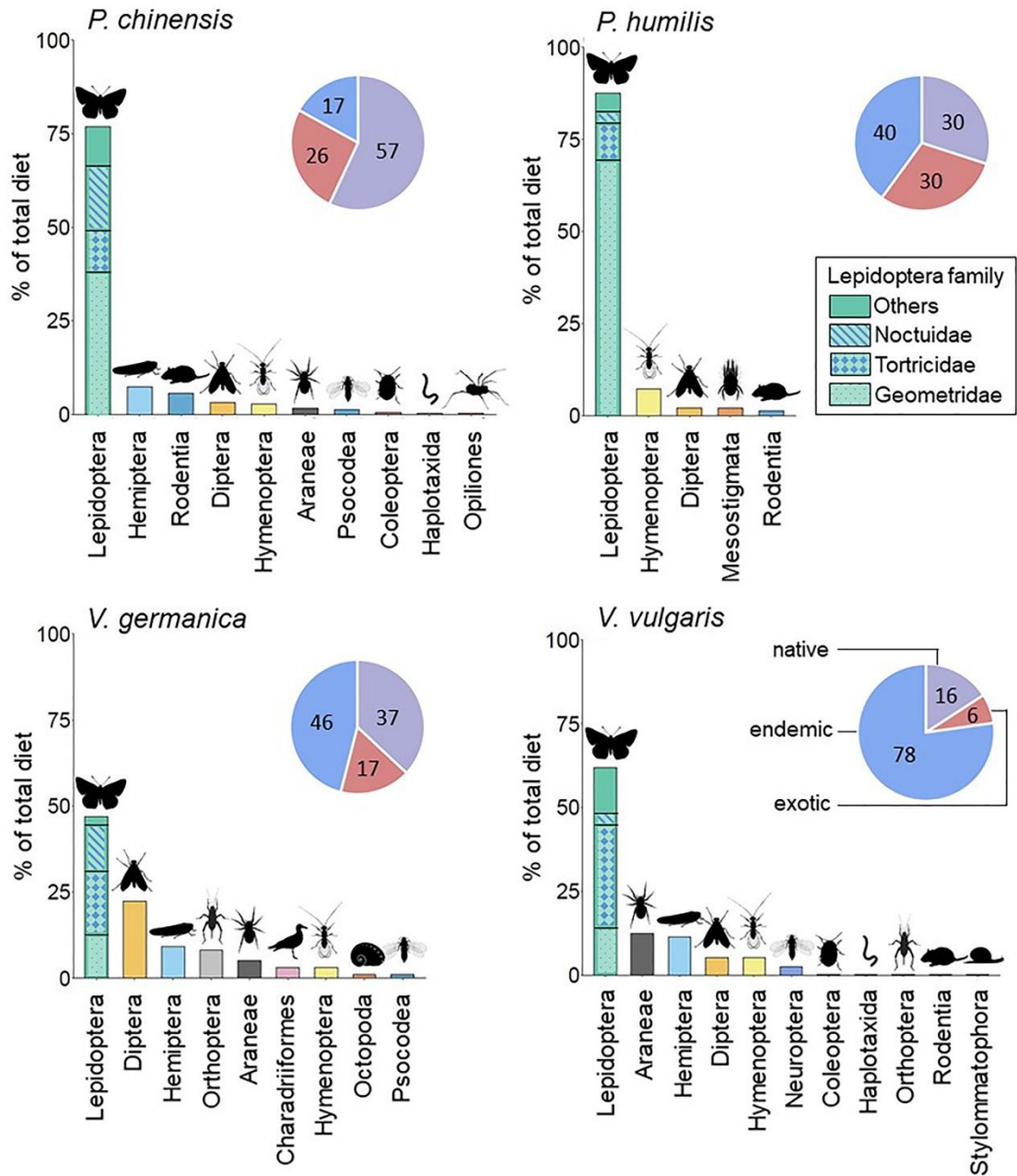
651 Figure 1. Location of nests of four invasive wasp species (*Polistes chinensis*, *P. humilis*,
 652 *Vespula vulgaris* and *V. germanica*) systematically searched for on Ahuahu, located 9 km off
 653 the north-east coast of New Zealand. The pale cream colour denotes sand/gravel, a habitat
 654 that was not included in this study.

655

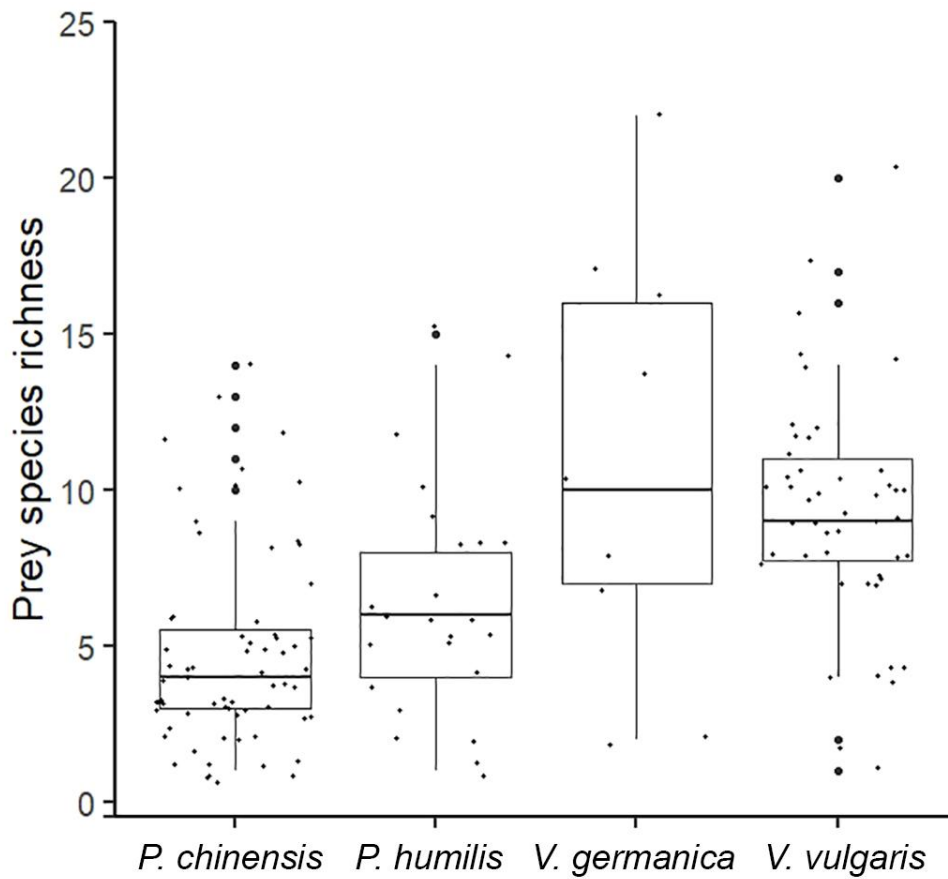


656

657 Figure 2. Rarefaction curves of prey taxa identified in faecal samples collected from vespid
 658 nests on Ahuahu, New Zealand.



659 Figure 3. Diet composition (% prey taxa detected) of four invasive vespid species inhabiting
 660 Ahuahu, New Zealand. The Lepidopteran families detected are also shown. Pie charts show
 661 the percentages of endemic, native and exotic Lepidoptera taxa.
 662



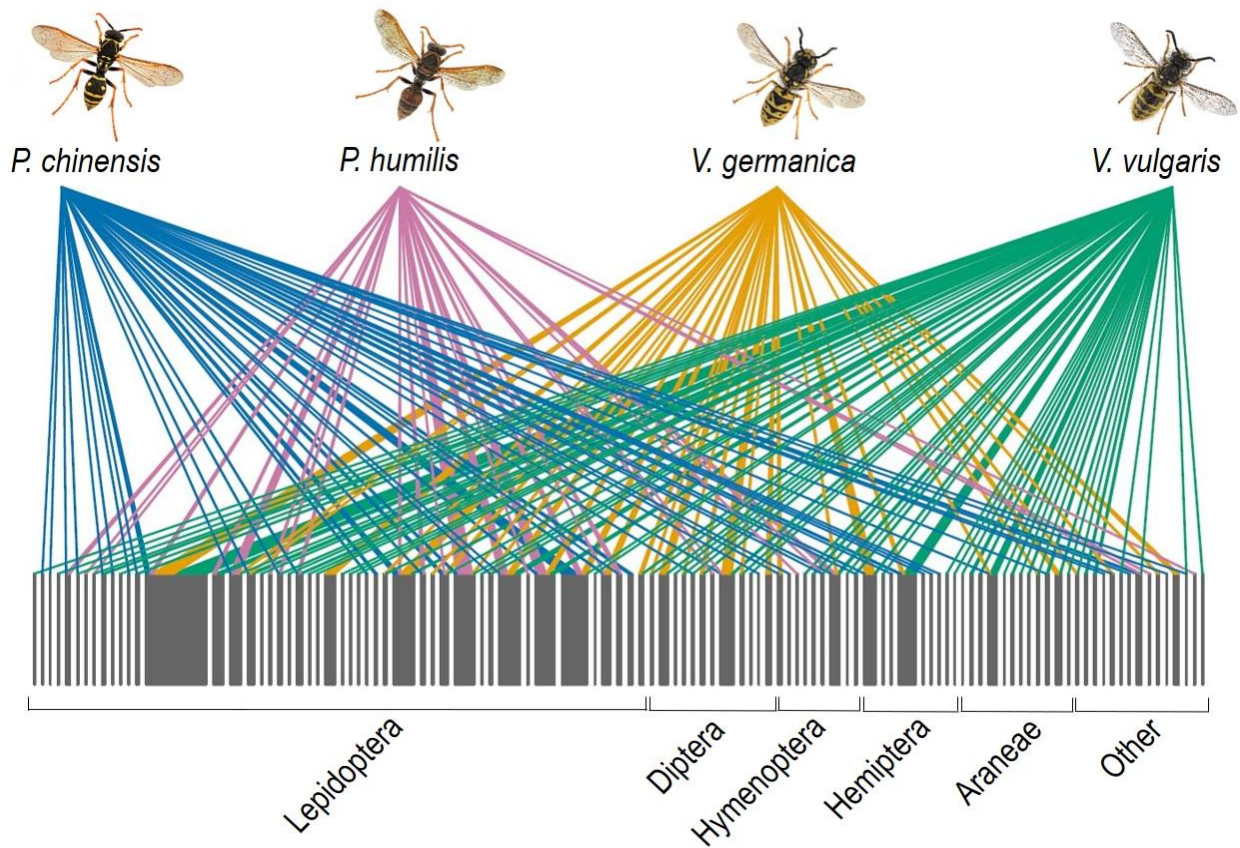
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664 Figure 4. Boxplot of mean prey richness per sample identified in faecal samples from *P.*

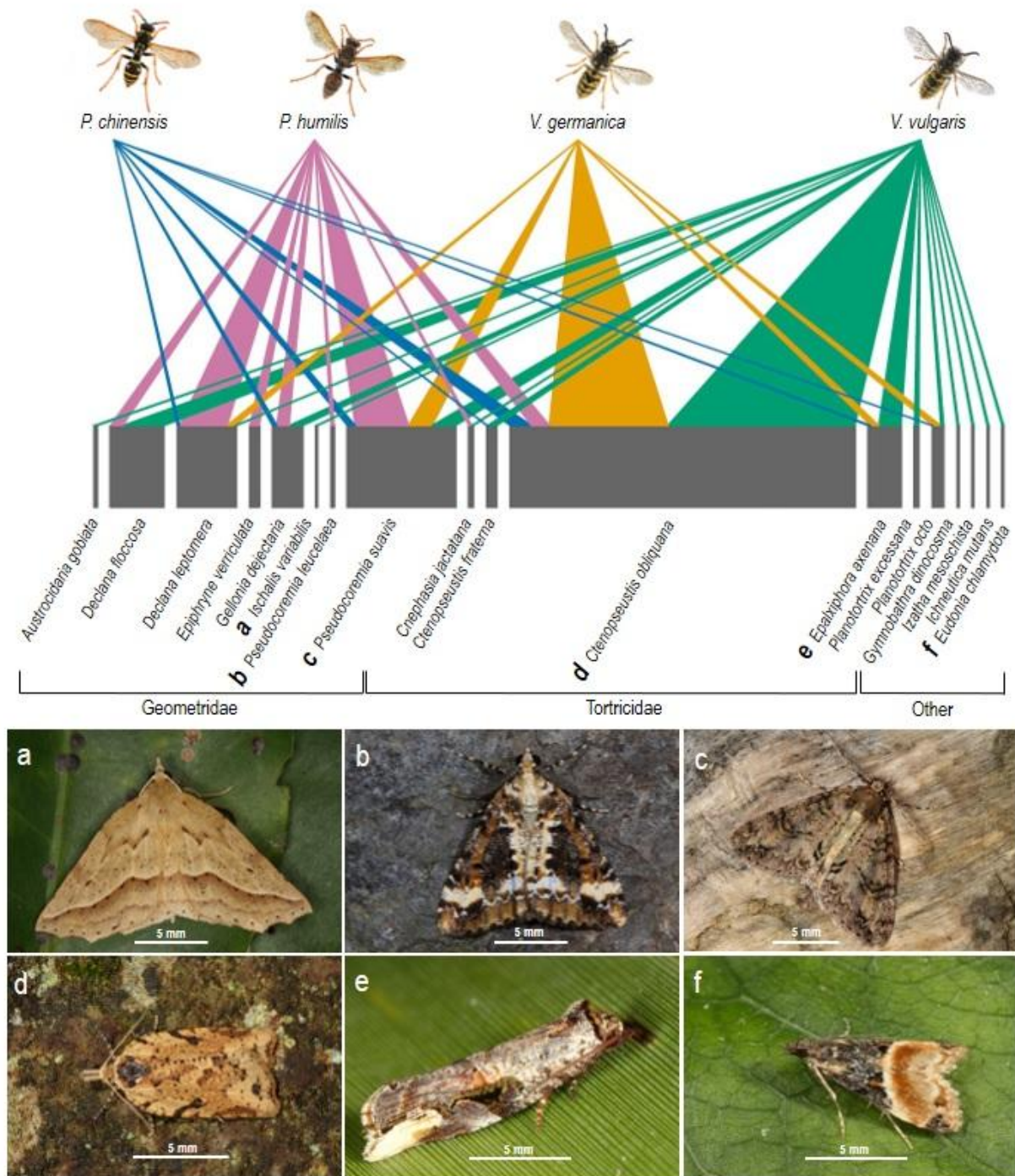
665 *chinensis* (n=70), *P. humilis* (n=24), *V. germanica* (n=9) and *V. vulgaris* (n=48). Presented

666 are 25th and 75th percentiles (lower and upper box boundaries), median (line inside box) and

667 10th and 90th percentiles (lower and upper error lines).



668 Figure 5. Network diagram showing the diet overlap between four invasive wasp species
 669 coexisting on Ahuahu. Presented are the nature (denoted by grey bars) and strength (denoted
 670 by thickness) of predation on 105 prey species identified from faecal samples. Note that
 671 strength of predation is proportional to sample size. Photos of wasps courtesy of Bryce
 672 McQuillan.



673 Figure 6. Network diagram showing the predation of endemic Lepidopteran species by four
 674 invasive wasp species coexisting on Ahuahu. Presented are the nature (denoted by grey bars)
 675 and strength (denoted by bar thickness) of predation on 18 endemic Lepidoptera identified
 676 from faecal samples. Note that strength of predation is proportional to sample size. Photos of
 677 wasps and Lepidoptera courtesy of Bryce McQuillan and Olivier Ball, respectively.