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Functional trait mismatch between native and introduced bee pollinators servicing a global fruit crop

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Abstract

Background Understanding connections between biodiversity and ecosystem services can be enhanced by shifting focus from species richness to functional trait-based approaches, that when paired with comparative phylogenetic methods can provide even deeper insights. We investigated the functional ecology and phylogenetic diversity of pollination services provided by hymenopteran insects visiting apple flowers in orchards surrounded by either 'natural' or 'disturbed' landscapes in New South Wales, Australia. We assessed whether morphological and behavioural traits (hairiness, body size, glossa length, pollen load purity, and probability of loose pollen) exhibited non-random phylogenetic patterns. Then, explored whether bees, the primary pollinators in this system, filled unique or overlapping functional entities (FEs). For each landscape, we calculated phylogenetic diversity and used FEs to assess functional richness, evenness, and diversion.

Results A phylogenomic matrix based on ultraconserved elements (UCEs; 1,382,620 bp from 1,969 loci) was used to infer a fully-resolved and well-supported maximum likelihood phylogeny for 48 hymenopteran morphospecies. There was no significant difference in species richness between landscape categories. Pollinator communities at natural sites had higher phylogenetic complexity (X = 2.37) and functional divergence ($\bar{x} = 0.74 \pm 0.02$ s.e.) than disturbed sites (X = 1.65 and $\bar{x} = 0.6 \pm 0.01$ s.e.). Hairiness showed significant phylogenetic clustering (K = 0.94), whereas body size, glossa length, and loose pollen showed weaker non-random phylogenetic patterns (K between 0.3–0.5). Pollen load purity showed no association with phylogeny. The assemblage of 17 bee morphospecies comprised nine FEs: eight FEs consisted of native bees with three containing 65% of all native bee taxa. The introduced honey bee (*Apis mellifera*) occupied a unique FE, likely due to its different evolutionary history. Both landscape types supported six FEs each with three overlapping: two native bee FEs and the honey bee FE.

Conclusions Bee hairiness was the only functional trait to exhibit demonstrable phylogenetic signal. Despite differences in species richness, and functional and phylogenetic diversity between orchard landscape types, both maintained equal bee FE numbers. While no native bee taxon was analogous to the honey bee FE, four native bee FEs shared the same hairiness level as honey bees. Health threats to honey bee populations in Australia will likely disrupt pollination services to apple, and other pollination-dependent food crops, given the low level of functional redundancy within the investigated pollinator assemblages.

Keywords Functional agroecology, Ecological service, Pollination, Functional-trait analysis, Functional entities, Community phylogenomics, Ultraconserved genome elements, Bees, Apple

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Background

Biodiversity studies often measure the number of species present within a given area, otherwise known as species richness (e.g., [1–3]). While species richness data may sometimes be straightforward to collect and can provide informative outcomes, these data may not provide general insights into specific functions that individual taxa play within an ecosystem [4]. In the context of habitat restoration, for example, ensuring the presence of specific functional niches can assist with ecosystem rehabilitation, whereas restoration activities solely focused on improving species richness metrics may not achieve a functional outcome for ecosystem improvement [5]. Because a higher functioning ecosystem is typically associated with increased biodiversity [4–9], viewing biodiversity as a collection of functional traits [10], may be more informative than considering species richness alone. A functional trait can be any morphological, biochemical, physiological, structural, or behavioural trait expressed by an individual organism, species, clade, or community that is relevant to the response of such organisms to the environment or their effects on ecosystem functioning [11–14]. While the choice of functional traits should be biologically informed [15], comparative phylogenetic methods can be used to quantitatively assess functional diversity. Closely related taxa often share traits via common ancestry and will provide phylogenetic signal (as defined by Blomberg et al. [16]) within ecological community contexts.

These metrics can be deployed in a wide range of scenarios, incorporating the scope and contribution of the focal organismal traits to characterize communities, understand the consequences of physical disturbances (e.g., fire [17, 18]) and biotic change (e.g., species losses or turnover) [4, 10, 19–23], or evaluate ecosystem services [23]. Pollination is a key ecosystem service [24] that facilitates plant reproduction and diversity [25, 26] and is required for the production of many fruits and vegetables critical to the human food supply [27]. Most animal-mediated pollination services are provided by insects and are typically best provided by a community of diverse pollinators [4, 28], with the exception of extreme plant-pollinator specializations [29]. Increased pollinator diversity generally results in positive pollination outcomes including improved fruit and seed set [30–35], higher fruit quality [36–38], and a decreased dependence on managed pollinators in agricultural settings [39–41]. Hence, an accurate understanding of pollinator functional diversity is important for improving and maintaining ecosystem services to food crops.

The presence of multiple species within a community that are able to perform the same pollination function are often perceived as insurance that some degree of service-resilience will be maintained following ecological disturbance and community member disruption – however, such null concepts of functional redundancy may not empirically hold true [42] and are difficult to quantify [43]. To ensure functional diversity indices are calculated correctly and to avoid over-estimating richness, an assemblage can be converted from species-level units of diversity to functional units of diversity, or functional entities (FEs; [44]). If multiple taxa share the same traits (i.e., occupy the same trait space), they are grouped into the same functional entity (FE), indicating a degree of functional redundancy across these taxa. A taxon with a unique trait space represents its own FE. By translating a community of species into FEs, we can evaluate functional diversity using the number of functional units present within the community, providing a more accurate representation of functional diversity. As FEs may represent more than one species, this approach to evaluating diversity is less sensitive to species-level turnover within a community but may be more useful when approaching questions on ecosystem functioning.

In this study we test concepts of functional diversity in pollination service using a comparative phylogenetic approach. Advances in parallel sequencing platforms and targeted enrichment of ultra-conserved element (UCE) regions of the genome (e.g., [45–50]) are consistently yielding well-resolved phylogenetic relationships across a range of arthropod taxa [46, 51, 52], especially Hymenoptera, (wasps: [53, 54]; ants: [47, 55, 56]; bees: [48, 57–60]). The UCE loci and their faster-evolving flanking regions provide phylogenetic signals at both deep and shallow nodes in the phylogeny [45, 61] and the standardized capture of thousands of UCEs, using taxon-specific probe sets, avoids loci-bias in phylogenetic inference [46, 61, 62]. Using UCE phylogenomic methods it is possible to quickly and affordably generate species-level community phylogenies that have very limited phylogenetic uncertainty. These methods can also be used to extract mitochondrial barcode sequences that can aid in species identification and redundancy, which is helpful for studying diverse insect groups like Hymenoptera, especially in taxonomically poorly known areas like Australia.

From a quantitative perspective, agricultural crops provide a standardized functional niche [63] to assess functional diversity. Apples are the 10th most valuable crop grown worldwide (US$67 billion industry [64]) which requires cross pollination for successful fruit set [65]. Fruit production improves with increasing wild bee diversity and abundance [66–68], most commonly provided by bees from the genera *Andrena, Apis, Bombus, Lasioglossum* and *Osmia* [69, 70]. Understanding the functional diversity of pollination services is therefore important for the future security of this industry,

particularly in Australia where honey bee populations now face increased health stressors from the recent *Varroa* mite incursion [71].

Our approach was to examine hymenopteran communities visiting cultivated apple in two geographic regions of New South Wales (Australia) with contrasting surrounding landscapes: natural bushland *versus* disturbed agriculturally-intensive landscapes. We conducted a functional trait analysis on all hymenopteran visitors paired with an evaluation of phylogenetic diversity inferred from a molecular DNA matrix comprised of UCE regions. We use these data to answer the following questions: (1) How do the hymenopteran communities visiting apple differ in phylogenetic diversity and functional composition between regions of contrasting landscapes? (2) Do any of the functional traits in the insect assemblage exhibit evidence of phylogenetic signal? (3) Is there functional trait overlap between native and non-native managed pollinators?

Results

Hymenopteran assemblage

In total, 48 morphospecies were identified from 675 hymenopteran specimens collected from orchards across both habitat types: natural landscapes-28 morphospecies, $n=448$ specimens, $\bar{x}=8.29$ (± 1.91 s.e.) morphospecies per farm per year; disturbed landscapes—33 morphospecies, n = 227 specimens, $\bar{x}=10.2$ (± 1.74 s.e.) morphospecies per farm per year (Fig. 1; Table 1). We found no difference in species richness across landscape types (Mann–Whitney U test: U = 12.5, $p=0.46$) (Fig. 2a).

Orchards surrounded by natural landscape included taxa from the bee families Apidae and Halictidae ($n=6$ and 14 morphospecies, respectively), ants (Formicidae, $n=4$ morphospecies), and wasp families Bethylidae and Thynnidae ($n=1$ morphospecies of each family) as well as 2 unidentified wasp morphospecies (detailed in Table 1). The dominant species in the natural landscape-type were the eusocial honey (*Apis mellifera*) and stingless bees (*Tetragonula carbonaria*) followed by the facultatively eusocial *Exoneura robusta* (Fig. 1, Table 1).

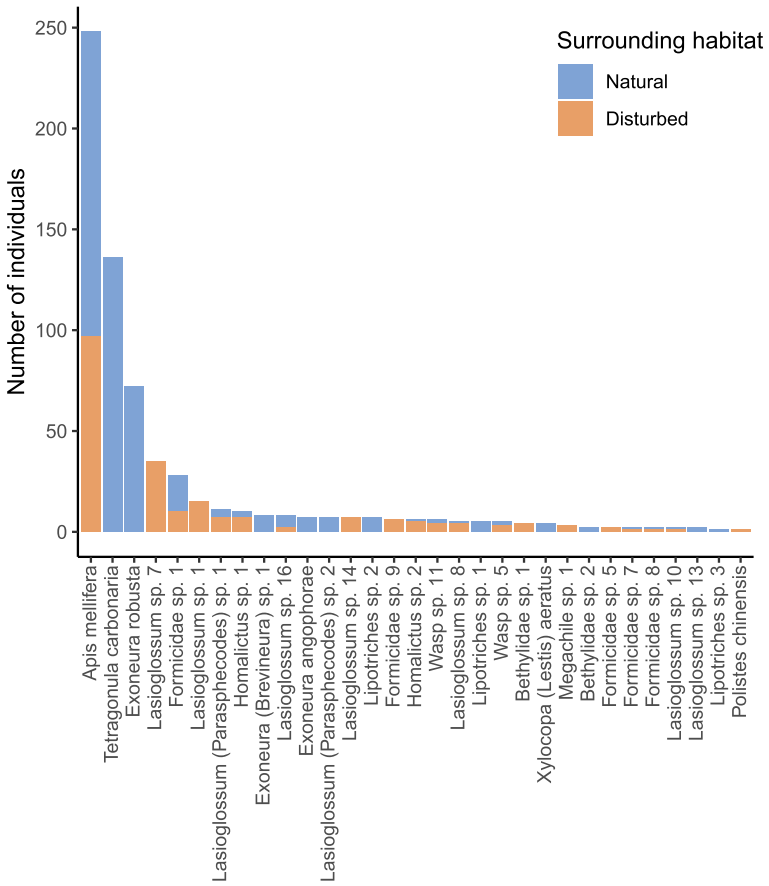

Fig. 1 Morphospecies frequency plot in alternate landscapes. Bars represent abundance of hymenopteran morphospecies collected on Pink lady apple flowers in natural and disturbed landscapes (pooled for 2018 and 2019 -more details in Table 1)

Table 1 Collected specimens summary. Hymenopteran specimens collected directly from Pink Lady apple flowers in orchards surrounded by natural and disturbed landscapes per year

			Natural		Disturbed	
Superfamily	**Family**	**Morphospecies**	**2018**	**2019**	**2018**	**2019**
Apocrita		Wasp sp. 2			1	
		Wasp sp. 3			1	
		Wasp sp. 4			1	
		Wasp sp. 5	2		2	1
		Wasp sp. 7				1
		Wasp sp. 8				1
		Wasp sp. 9				1
		Wasp sp. 10				
		Wasp sp. 11	1	1		3
		Wasp sp. 14			1	
Aculeata	Vespidae	Polistes chinensis				1
	Thynnidae	Thynnidae sp. 1	1			
		Thynnidae sp. 6			1	
	Bethylidae	Bethylidae sp. 1			3	1
		Bethylidae sp. 2		2		
	Mutilidae	Mutilidae sp. 1			1	
	Scoliidae	Laevicampsomeris sp. 1				1
	Formicidae	Formicidae sp. 1	12	6	8	2
		Formicidae sp. 5			2	
		Formicidae sp. 7	1		1	
		Formicidae sp. 8		2	1	
		Formicidae sp. 9			6	
		Formicidae sp. 10	1			

Table 1 (continued)

Superfamily	Family	Morphospecies	Natural		Disturbed	
			2018	2019	2018	2019
Apoidea (Anthophila)	Halictidae	Homalictus sp. 1	3		7	
		Homalictus sp. 2		1		5
		Lasioglossum (Chialictus) sp. 1	1			
		Lasioglossum (Parasphecodes) sp. 1	3	1	7	
		Lasioglossum (Parasphecodes) sp. 2	3	4		
		Lasioglossum sp. 1			4	11
		Lasioglossum callomelittum sp. 1			1	
		Lasioglossum sp. 3	1			
		Lasioglossum sp. 4	1			
		Lasioglossum sp. 6			1	
		Lasioglossum sp. 7			15	20
		Lasioglossum sp. 8		1	3	1
		Lasioglossum sp. 10		1	1	
		Lasioglossum sp. 13	2			
		Lasioglossum sp. 14			6	1
		Lasioglossum sp. 16		6	2	
		Lipotriches sp. 1	4	1		
		Lipotriches sp. 2	7			
		Lipotriches sp. 3		1		
	Apidae	Apis mellifera	60	91	46	51
		Exoneura (Brevineura) sp. 1	8			
		Exoneura (Brevineura) sp. 2				1
		Exoneura angophorae	4	3		
		Exoneura robusta	34	38		
		Tetragonula carbonaria	61	75		
		Xylocopa (Lestis) aerata	3	1		
	Megachilidae	Megachilidae sp. 1			1	2

Whereas orchards surrounded by disturbed landscape were composed of bees from the families Apidae, Halictidae, and Megachilidae ($n=2$, 11, and 1 morphospecies respectively), ants (Formicidae, $n=5$ morphospecies), wasps in the families Scoliidae, Mutilidae, Bethylidae, Thynnidae, and Vespidae (each family with $n=1$ morphospecies) and 9 unidentified wasp morphospecies (detailed in Table 1). The most common apple flower visitor in disturbed landscape-type were the honey bee (*Apis mellifera*) then *Lasioglossum* spp. (Fig. 1, Table 1).

DNA matrices & phylogenetic trees

We recovered a UCE data matrix consisting of 1,382,620 DNA nucleotide base pairs from 1,969 loci for 93 morphospecies, comprised of 962,386 (69.6%) parsimony informative sites, 284,750 (20.6%) invariant sites, and 1,153,481 distinct site patterns. From these, a maximum-likelihood tree was inferred to assess

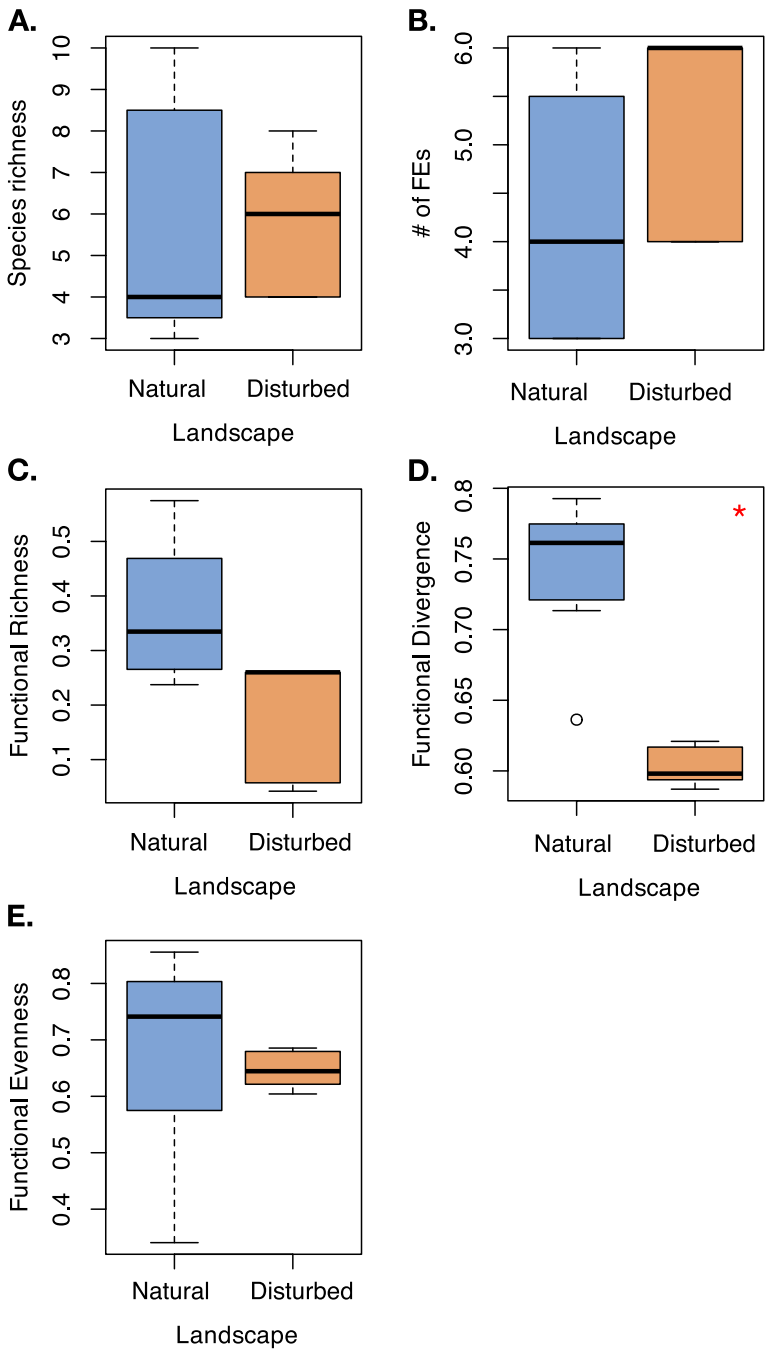

Fig. 2 Diversity measurements by landscape type. Boxplots of species richness (A), the number of FEs (B), functional richness (C), functional divergence (D), and functional evenness (E) across landscape types (natural vs. disturbed), both years pooled. Significant differences across regions are denoted with a red asterisk (*) in the upper right corner of the plot

morpho-species identifications (Fig. S1): log-likelihood tree value: -23,531,100.0713; AIC score: 47,062,584.1425; AICc score: 47,062,584.1961; BIC score: 47,064,914.9248; tree length: 8.3029; sum of internal branches: 4.0525 (48.81% of tree length).

COI sequences extracted from the phylogenomic data matched species-level sequences in BOLD and GenBank for 41 of our specimens (out of 43)—based on BOLD 'Top Hit' (95% or higher) and GenBank 'Best Match' (max. score of 600 or more) (accessed March 23, 2023; summarized in Table S1). The best matches for two specimens

were to species not present in Australia so these were not considered to be valid species confirmations.

For our working data set, duplicate specimens of each morphospecies were excluded (pruned from the 93-taxon tree), resulting in a final, 48-taxon alignment containing 879,842 (63.6%) parsimony informative sites, 316,129 (22.9%) invariant sites, and 1,093,197 distinct site patterns (1,382,620 DNA nucleotide base pairs from 1,969 loci). The refined matrix consisted of taxa from 12 hymenopteran families with the number of terminal taxa indicated in parentheses, including: Wasps—Ichneumonidae (1), Braconidae (1), Vespidae (1), Mutillidae (1), Scoliidae (1), Bethylidae (2), Thynnidae (3); Ants—Formicidae (6); and Bees—long-tongued Megachilidae (1), Apidae (7), and short-tongued Colletidae (2) and Halictidae (22).

The maximum-likelihood best tree for this reduced taxon set is presented in Fig. 3 (log-likelihood tree value: -19,014,661.2537; AIC score: 38,029,528.5075; AICc score: 38,029,528.5230; BIC score: 38,030,778.8750; tree length: 7.7814; sum of internal branches: 2.1529, 27.67% of tree length). All nodes showed SH-aLRT branch support values > 90 and most showed high bootstrap support values except for internal nodes for the clade containing *Lasioglossum* morphospecies ((10.1, (14, 16)), (8, 12)). We constructed a consensus tree from 1,000 bootstrap trees (log-likelihood tree value: -19,014,661.25387; Figure S2). The branching topology

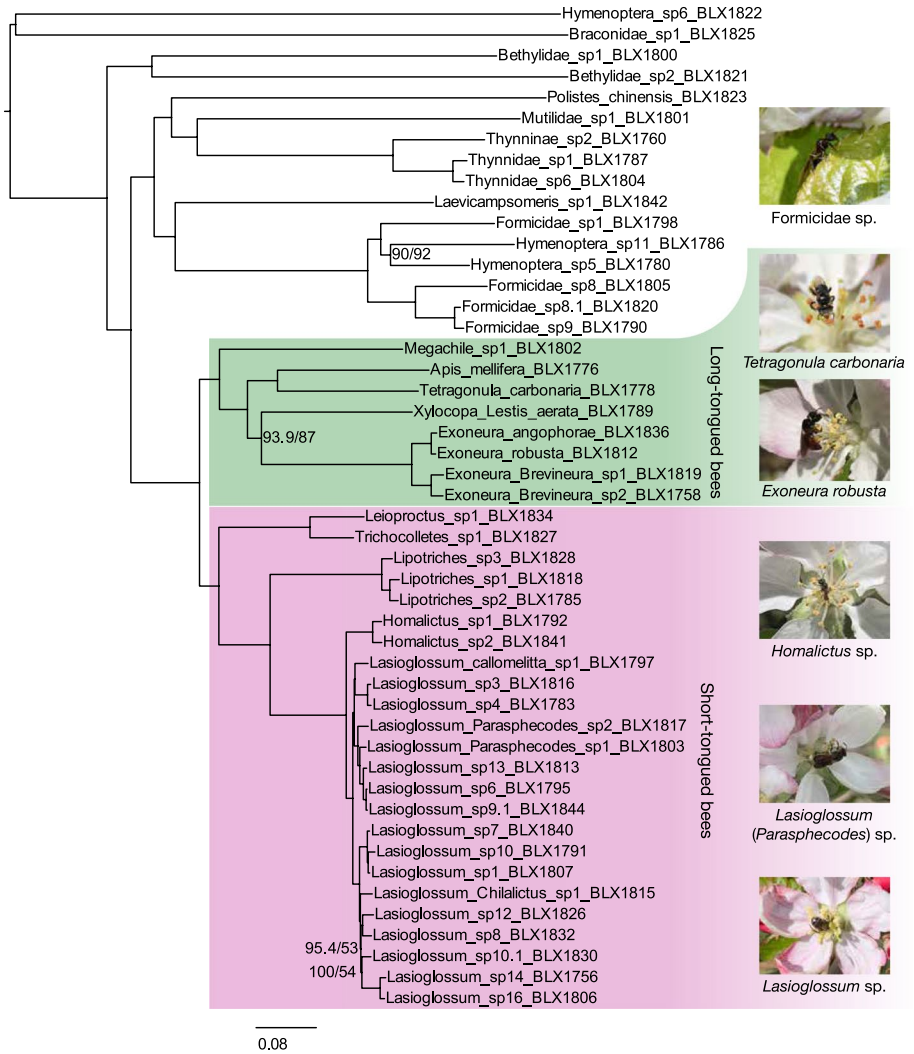

Fig. 3 The maximum-likelihood tree of 48 unique morphospecies. Tree node support values (provided when <100) are SH-like approximate likelihood ratio test scores followed by ultrafast bootstrap support values. Branch lengths are representative of the evolutionary distance between nodes. Terminal branch morphospecies are annotated by short-tongue (Halictidae and Colletidae) and long-tongue bee families (Apidae and Megachilidae). All photos taken by O. Bernauer

of the best tree and the consensus tree were identical (Robinson-Foulds distance = 0), with broadly equivalent bootstrap support for nodes, as detailed above (see Figure S2).

Functional trait categorization

To map functional trait data onto phylogenies (Fig. 4), data were categorized into five or fewer categories (detailed in Table 2), spanning the range of each trait (summarized in Table S2).

Phylogenetic signal of traits

We used Blomberg's K [16] values to investigate whether functional traits exhibited evidence for phylogenetic signal, categorized as follows: phylogenetic clustering (trait values more similar than expected by phylogenetic relatedness alone); non-random distributions (trait values are distributed non-randomly, but not based on phylogenetic relatedness), or no evidence for clustering (trait values distributed randomly). When the K value is significant, the trait displays significant non-random clustering while the K value itself determines whether these trait distributions are the result of phylogenetic clustering (K is above one) or not (K is below one) [16].

Four of the five evaluated traits showed significant non-random distributions and one exhibited phylogenetic clustering as assessed by Blomberg's K (Fig. 4). Hairiness (K = 0.94, $p = 0.001$), with a high K value (approaching 1 or higher), showed phylogenetic clustering. Meanwhile, probability of loose body pollen (K = 0.31, $p = 0.022$), glossa length (K = 0.30, $p = 0.007$), and body size (K = 0.49, $p = 0.001$), also showed significant non-random distributions, but since K values were lower than one, these are not indicative of phylogenetic clustering. Finally, pollen load purity (K = 0.10, $p = 0.368$) did not deviate from a random pattern.

To explore whether (a) the assortment of non-bees (which essentially act as outgroup taxa) or (b) the numerical bias towards bees in our data set was influencing trends in trait clustering, we re-calculated K values for subsets of bee and non-bee taxa independently for hairiness, ITD, and probability of loose body pollen as data for both bee and non-bee taxa were present for these traits. Data for glossa length and pollen load purity were only available for bees. For hairiness, bees continued to show a significant non-random distribution (K = 0.623, $p = 0.002$), but the relatively low K value (< 1) suggests no phylogenetic signal; there was no significant result among non-bees (K = 0.96, $p = 0.609$). Body size (ITD) for bees showed significant evidence for a non-random distribution that approaches a K value of 1 (K = 0.88, $p = 0.002$), but no significant outcome for non-bees (K = 0.88, $p = 0.942$). Probability of carrying loose pollen on the body was again significant for bees but with no phylogenetic signal (K = 0.175, $p = 0.045$) and no significance for non-bees (K = 0.81, $p = 0.879$).

Diversity and function

The phylogenetic diversity of hymenopteran specimens across all farms and landscapes (pooled) was 5.86. When considered independently, orchards surrounded by natural landscape exhibited relatively higher phylogenetic diversity (X = 2.37) than orchards surrounded by disturbed habitat (X = 1.65).

To better understand whether there is functional redundancy or trait-overlap within our studied assemblage, we categorized all bee morphospecies, the most important pollinators in this system [69, 72, 73], into functional entities (FEs) with each FE representing a unique functional unit. The 17 bee taxa in our assemblage resulted in nine FEs (Table 3). Six taxa represent unique FEs (FE 4—9) while the remaining 11 make up 3 FEs (FE 1—3). For orchards surrounded by natural habitat, the minimum number of FEs documented per farm in a single year was 3, the maximum was 6, and the average across farms in both years was 4.3 (± 0.52 s.e.) (summarized in Table S3). In orchards surrounded by disturbed habitat, there was a minimum of 4 bee FEs per farm per year, a maximum of 6, and a mean of 5.2 (± 0.49 s.e.) FEs per farm (summarized in Table S3). Between landscapes, only three FEs overlapped: FE1, FE 2, and FE 4; FE 1 and FE 2 consist of four taxa each, making up almost half of the entire assemblage; FE 4 represents honey bees (*Apis mellifera*). The mean number of FEs per farm did not differ between landscapes (Mann–Whitney U test: U = 10, $p = 0.23$, Fig. 2b).

Next, we used FEs to evaluate three functional diversity measures: functional richness, functional

(See figure on next page.)

Fig. 4 Pruned phylogenetic trees with functional traits mapped onto terminal nodes. Trees were obtained by pruning taxa from the ML tree in Fig. 3. Terminal node circles are coloured from highest value (darkest tone) to lowest value (lightest tone), whereby darkest circles indicate: (A) hairiest taxa; (B) highest level of pollen purity; (C) highest probability of dry pollen present on insect's bodies; (D) the longest glossa; and (E) the largest body size. Actual values are summarized in Table S2. Asterisks indicate traits with significant non-random distributions ($p < 0.05$) and the non-random distribution in hairiness is a result of phylogenetic clustering (K > 1)

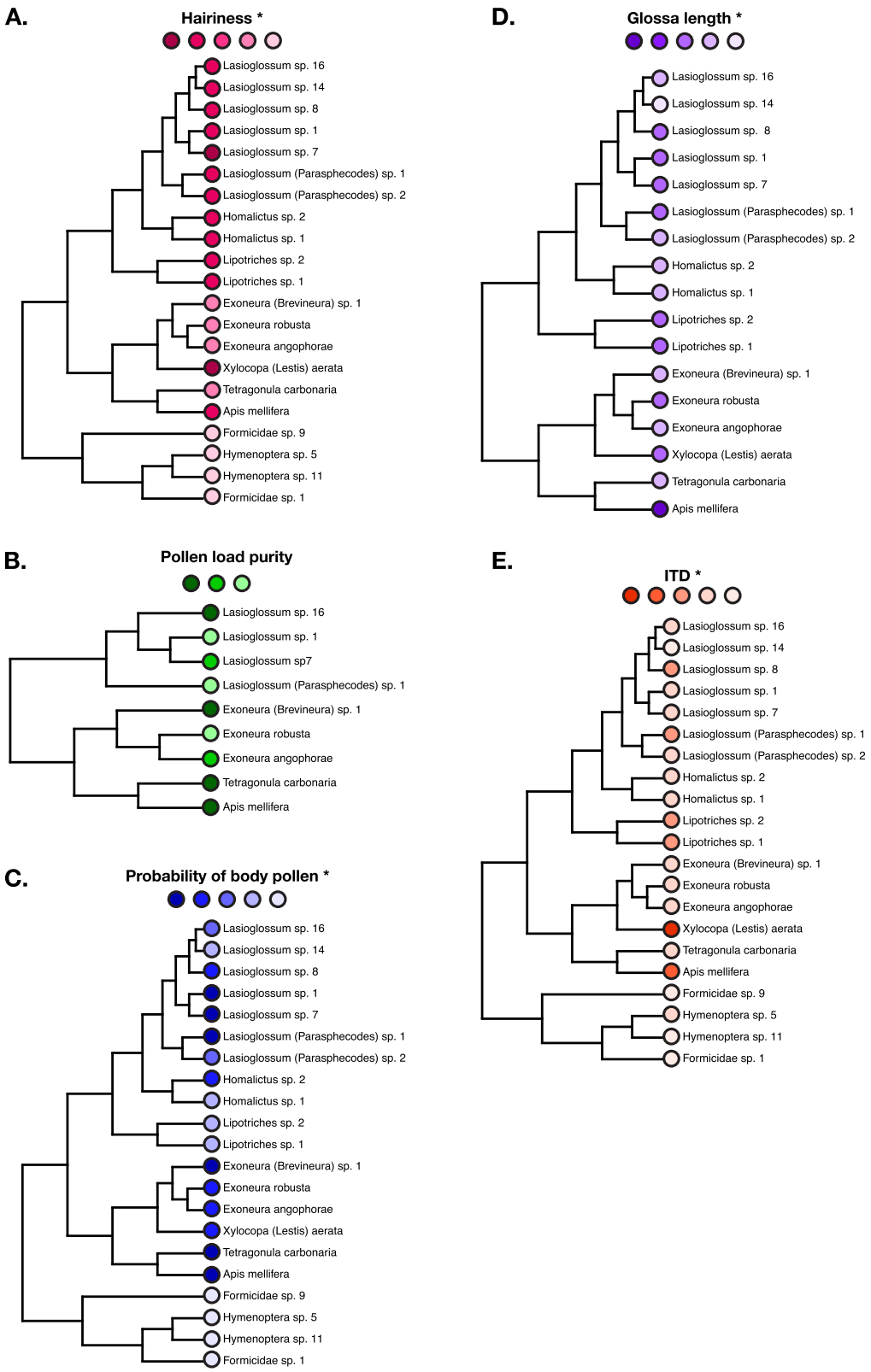

Fig. 4 (See legend on previous page.)

Table 2 Trait bins and summary statistics. Details on trait groups used to map trait data onto phylogenies along with the number of taxa included in each phylogeny, the minimum and maximum values and associated taxon name for each trait, along with mean trait values. Additional details on trait values by taxon can be found in Table S2

Trait	Bins	# of morphospp.	Min	Max	Mean value ($\bar{x} \pm$ s.e.)
Hairiness (Fig. 2a)	0–0.19, 0.2–0.39, 0.4–0.59, 0.6–0.79, 0.8–1	21	0, Formicidae sp. 1	1 to 0.92, *X.* (*Lestis*) *aerata* (Smith)	0.53 ± 0.06
Pollen load purity (Fig. 2b)	0.7–0.79, 0.8–0.89, 0.9–1	9	0.712 in *Exoneura robusta* (Cockerell)	0.992, *Lasioglossum* sp. 16	0.85 ± 0.04
Probability of loose body pollen (Fig. 2c)	0–0.19, 0.2–0.39, 0.4–0.59, 0.6–0.79, 0.8–1	21	(0): Formicidae sp. 1, Formicidae sp. 9, and Hymenoptera sp. 11	0.981, *Tetragonula carbonaria* (Smith)	0.53 ± 0.07
Glossa length (Fig. 2d)	0–0.99, 1–1.99, 2–2.99, 3–3.99, 4–4.99 mm	17	0.89 mm in *Lasioglossum* sp. 14	4.95 mm, *Apis mellifera* (L.)	1.97 mm ± 0.23
Body size (Fig. 2e)	0–0.99, 1–1.99, 2–2.99, 3–3.99, ≥ 4 mm	21	0.556 mm in Formicidae sp. 1	5.5 mm, *X.* (*Lestis*) *aerata*	1.71 mm ± 0.24

Table 3 Bee functional entities. FEs were determined using three functional traits (ITD (a measure of body size), glossa length, and hairiness index (adapted from [74]) using the package mFD (44). In the landscape column, N indicates a species present in orchards surrounded by natural landscape while D indicates species present in orchards surrounded by disturbed habitat. Relative abundance data are derived from Table 1, with rare taxa representing < 5% of collected specimens, common 5–15%, and abundant > 15%. Trait states are derived from the categories outlined for each trait in Table 2 by converting each trait category into a corresponding integer (i.e., the lowest trait category = 1, the highest = 5).Trait states that are filled in grey indicate unique traits (traits possessed by a single bee FE)

Functional Entity	# of taxa	Taxon ID	Relative abundance	Landscape	Trait		
					ITD	Glossa	Hairiness Index
FE 1	4	*Homalictus* sp. 1 *Homalictus* sp. 2 *Lasioglossum* (*Parasphecodes*) sp. 2 *Lasioglossum* sp. 16	rare	N, D N, D N N, D	2	2	4
FE 2	4	*Lasioglossum* (*Parasphecodes*) sp. 1 *Lasioglossum* sp. 8 *Lipotriches* sp. 1 *Lipotriches* sp. 2	rare	N, D N, D N N	3	3	4
FE 3	3	*Exoneura angophorae* *Exoneura* (*Brevineura*) sp. 1 *Tetragonula carbonaria*	abundant	N N N	2	2	2
FE 4	1	*Apis mellifera*	abundant	N, D	4	5	4
FE 5	1	*Exoneura robusta*	common	N	2	3	2
FE 6	1	*Lasioglossum* sp. 1	rare	D	2	3	4
FE 7	1	*Lasioglossum* sp. 14	rare	D	1	1	4
FE 8	1	*Lasioglossum* sp. 7	common	D	2	3	5
FE 9	1	*Xylocopa* (*Lestis*) *aerata*	rare	N	5	3	5

divergence, and functional evenness. In orchards surrounded by natural habitat, the assemblage of bee visitors had a significantly higher functional divergence value than orchards surrounded by disturbed habitat (divergence: natural: $\bar{x} = 0.74 \pm 0.02$; disturbed: $\bar{x} = 0.6 \pm 0.01$; Mann–Whitney U test: $U = 35$, $p = 0.003$; Fig. 2 c and d, respectively). When we compare functional richness and evenness between landscapes,

we find no significant differences (richness: natural: $\bar{x} = 0.37 \pm 0.05$; disturbed: $\bar{x} = 0.18 \pm 0.05$; Mann–Whitney U test: U = 29, $p = 0.07$; evenness: natural: $\bar{x} = 0.67 \pm 0.07$; disturbed: $\bar{x} = 0.65 \pm 0.02$; Mann–Whitney U test: U = 23, $p = 0.43$; Fig. 2e).

Discussion

A fully-resolved and well-supported phylogenetic tree was generated from UCE loci for an assemblage of 48 hymenopteran taxa visiting apple flowers grown in natural and disturbed landscapes in Australia (Fig. 3). We used this tree to examine the phylogenetic and functional diversity of the focal assemblage. Phylogenetic clustering analyses (Blomberg's K) identified bee body size, tongue length, and propensity to carry loose pollen as functional traits that exhibit non-random (and non-phylogenetic) trait distributions (Fig. 4). Meanwhile, body hairiness demonstrated evidence for phylogenetic clustering, possibly driven by the inclusion of non-bee taxa. Orchards surrounded by natural landscapes had higher phylogenetic and functional diversity, though functional richness (functional space occupied by the species in an assemblage) and functional evenness (functional trait redundancy within an assemblage) did not differ between landscape types (Fig. 2). Although there were numerically more bee morphospecies in orchards surrounded by natural habitat, both landscape types contained six distinctive FEs, three of which, including the FE represented by honey bees, were present in both landscapes. Exclusive examination of bees as FEs revealed that none of the native bee FEs matched the functional space of honey bees - which would not have been evident from investigation of species richness alone (Fig. 2). However, four native bee FEs have equivalent levels of hairiness to honey bees. Our results demonstrate a lack of trait overlap between native and introduced honey bees (the primary apple pollinators in both landscapes [69, 72, 73]), suggesting changes to the pollinator community have the potential to disrupt ecosystem services in this functional niche.

Despite differences in bee assemblages between landscapes at the morphospecies level, both had an equal number of FEs and previous studies show that each assemblage produced equivalent fruit set [73]. Functional diversity has long been linked with ecosystem functioning (reviewed by: [5, 9]), including pollination services in agroecosystems [4, 6–8]. However, our findings contradict this general pattern as bees visiting apple in the disturbed landscape had lower functional divergence but did not result in lower pollination service (as measured by fruit yield [73]). Among orchards surrounded by natural habitat, honey bees were the dominant visitors, but stingless bees (included in FE 3) were also abundant in large numbers, greater than any native bee visitor to flowers in the orchards in disturbed landscapes (Table 3) [69, 72, 73]. Therefore, the presence of abundant stingless bees resulted in two dominant pollinators in natural landscapes (*cf.* overwhelmingly honey bees in disturbed landscapes), which may contribute to these differences in functional divergence between land-use types. As non-bee pollinators contribute less than 4% of pollination services to apple in either land-use type [69, 73], we can rule out these insects being responsible for additional pollination resulting in the equivalent fruit set between landscapes. However, the lack of a difference in fruit set across land-use types could be masked by the low threshold for fruit set preferred by apple growers (2–5%, [70]). When fruit set is too high, growers must thin their crop (chemically or mechanically) to maximize the number of large fruits they can produce while ensuring their trees yield fruit each year [75]. Therefore, the threshold of functional diversity required to produce apples may be below what either landscape experienced. Our experimental design contrasts two landscape extremes, however, critical functional diversity thresholds for apple production may be clarified by incorporating more field sites of intermediate or mixed landscapes, resulting in a gradient between the two extremes (e.g., [23]).

In both landscape contexts, no native bees shared the same functional space as honey bees. This finding would not have been apparent through species richness investigations alone (Fig. 2), emphasising the added value of functional diversity analyses in pollination ecology. However, if we examine each trait independently, there are other FEs in both landscapes that contain the same level of hairiness as honey bees (FEs 1, 2, 6, and 7, which include halictid bees from the genera *Lasioglossum* and *Lipotriches*). The bees that share hairiness levels with honey bees are present within the orchards in either landscape but are far less abundant (at least by an order of magnitude) than honey bees (Fig. 1, Table 1; [69, 72, 73]). For both body size and glossa length, no native bee FEs shared this trait value with honey bees. Across all FEs, there are three traits each with five possible trait states, resulting in 15 potential trait states overall. When we consider each trait state, there are five instances of distinct trait states (i.e., trait values that do not overlap with another taxon): body size and glossa length in FE 4 (honey bees) and FE 7 (*Lasioglossum* sp. 14), and body size in FE 9 (*Xylocopa* (*Lestis*) *aerata*) (Table 3). Three possible trait states were not present in this study and the remaining seven overlapped in multiple FEs or by FEs consisting of multiple taxa (e.g., body size in FE 2, Table 3), suggesting a degree of functional trait redundancy within the assemblage, although these bees were often relatively rare within the orchards (based on visitation rate - see [73]).

Because honey bees fill a distinct functional space (FE 4) and are the numerically dominant visitor to apple in both landscapes [69, 72, 73], changes to honey bee populations are likely to result in pollination deficits for apple [76]. In Australia, apple growers in some agroecosystems rely heavily on dense feral honey bee colonies (as demonstrated for nearby regions of NSW in [77]) for pollination services. Disruptions to honey bee populations are of great contemporary importance following the recent incursion of *Varroa* mites into Australia [71] and the poor response of honey bee populations to *Varroa* in New Zealand [78]. Mitigation tools to preserve apple pollination services in response to honey bee declines will likely depend on landscape context. As orchards surrounded by natural habitat had a large abundance of stingless bees [69, 72, 73], loss of honey bees may be buffered by these native bees. In contrast, orchards surrounded by disturbed landscape may need to invest more in managed honey bee hives to sustain apple pollination. While other apple growing regions may be able to rely on wild native pollinators to provide pollination services if honey bee populations are absent or reduced, the diversity of wild bees visiting apple in Australia is relatively low [69, 72, 73] in comparison with Holarctic agricultural areas (i.e., New England, USA [23, 69, 70, 79]). Considering that the evolutionary origin of apple trees is in the Palearctic [80], the importance of honey bees for this crop in Australia is perhaps not surprising [69].

Regarding the relationships between FEs and traits, a potential limitation of this study is that FEs were evaluated using only three traits. For a trait to be included in the FE analysis, trait data needed to be available for all taxa, limiting the possible traits used to evaluate FEs in this study to three. However, the three included traits, body size, glossa length, and hairiness, are critical traits for pollination. For plants that require cross-pollination for successful fruit set as apples do [65], pollinators need to visit several plants across an orchard to obtain pollinizer pollen. Therefore, bees with a greater body size, which is positively correlated with flight distance [81], may be superior pollinators in cross-pollinated crops. The length of a bee's glossa dictates which flowers are accessible for nectar [82] and flower handling while foraging (unless they behaviourally adapt by robbing nectar [83]). Visitors to apple flowers can either forage for nectar from the top down, increasing their chances for stigmal contact and ultimately pollination [73], or from the side, effectively robbing nectar [72, 83]. Bees with shorter glossa may be forced to forage from the top down to collect nectar, while a longer glossa may permit bees to forage from the side more easily. Hairiness assists pollen collection and transfer [14] and ultimately dictates a pollinator's effectiveness [84]. To be a pollinator, a visitor must be able to transfer pollen from one flower to the next, and having a relatively hairy body will facilitate pollen transfer. Given the importance of these three functional traits to pollination broadly and as they relate to apple pollination more specifically, we feel confident that our functional diversity analyses are based on key pollination traits and serve as a useful proxy for pollination services in this functional niche.

When we examined trait distributions across the hymenopteran phylogeny using Blomberg's K, we found that most traits (except pollen load purity) exhibited non-random distributions (Fig. 4). Given that closely related organisms are likely to share similar phenotypes (e.g., allodapine bees [85]) and the examined community utilized a shared resource (apple flowers), we expected that all five examined traits might demonstrate phylogenetic signal. While pollen load purity did not show evidence for clustering, this may not be surprising as this trait contained the smallest data set for our assemblage (9 morphospecies cf. 17 or more for all other traits). Hairiness was non-randomly distributed and showed evidence for a phylogenetic clustering, suggesting that closely related taxa share similar levels of hairiness. When we investigated if specific taxa (i.e., bees or non-bees) were driving the phylogenetic signal in hairiness, we discovered that this was driven by the presence of non-bees in this phylogenetic tree. When we evaluated only bees, we found evidence for a non-random trait distribution, but not phylogenetic clustering. Bees have evolved into two clades: long- and short-tongued bees based on the length and morphology of their labial palps [86], which is correlated with glossa length [87]. While glossa length in this study showed evidence for non-random clustering, this result was not attributed to any phylogenetic signal — however increased taxon sampling within the clades under investigation may reveal a stronger phylogenetic association. Because we know that (a) closely related taxa are likely to share traits [18], as seen here with hairiness, and (b) closely related taxa are likely to be lost from community assemblages at similar rates [23]; it is evident that understanding the distribution of traits across a community can provide clarity on how ecosystem services like pollination might change in response to community disturbance and the potential loss of functional traits.

Our study paired functional diversity analyses with phylogenetic diversity using a tree inferred from UCE loci. We added phylogenetic diversity to our understanding of the assemblages and matched up phylogenetic data with functional trait data using Blomberg's K to understand how traits were distributed across the assemblage. Combining functional and phylogenetic data allows for deeper insights into community dynamics. For example,

a recent study in New York (USA) apple orchards found that with increasing agricultural intensity, bee communities decreased in species richness and this loss in richness could be predicted based on phylogenetic relatedness [23]. Basing conservation and agricultural management decisions on quantitative functional diversity metrics (approximated using phylogenetic diversity), rather than by species richness alone as most studies do (e.g., [1–3]), provides a more informed way to ensure ecosystem services are sustainable. Multi-faceted approaches to assessing diversity, as outlined in this study, allow for a deeper understanding of how community diversity relates to ecosystem function.

Conclusions

This research provides measures of functional and phylogenetic diversity derived from the behavioural and morphological phenotypes of insects that visit apple flowers. The use of advanced phylogenomic methods improves comparative knowledge of pollination services generally, and specifically builds upon antecedent research on the two focal Australian agroecological landscapes of our study system [69, 72, 73, 88]. While both natural and disturbed landscapes support the same number of bee FEs, we discovered that orchards surrounded by natural habitat supported a more functionally and phylogenetically diverse community of hymenopteran visitors than disturbed orchards surrounded by intensive agriculture. This pattern disagrees with some previous findings on diversity and ecosystem functioning, though the low pollination threshold of apple may be obscuring differences in pollination services between landscapes. Many native Australian bee taxa visit apple flowers, though there is little functional trait overlap between these native bees and the non-native honey bees. This lack of functional overlap between honey and native bee pollinators further emphasizes Australia's extreme dependence upon honey bees for apple production, as a corollary of biogeographic history [69]. This dependency is a considerable problem given the recent invasion of *Varroa* to Australia [71].

Methods

Field sites

Insects were collected from orchards that grow Pink Lady cultivar apples [89], situated in two landscape contexts: natural and disturbed. Orchards surrounded by natural landscape (n = 4 farms) were located near Bilpin, New South Wales, Australia (-33.503, 150.533, 600 m elevation) and surrounded by National Park lands (Blue Mountains National Park to the south and Wollemi National Park to the north). Orchards surrounded by disturbed landscapes ($n = 3$ farms) were located near Orange, New South Wales, Australia (-33.283, 149.100, 900 m elevation), surrounded by intensive agricultural lands producing a variety of fruit crops. Field site meteorological data are detailed in Bernauer et al. [72, 73].

Collection and identification

Insects were collected directly from apple flowers between 6:00 and 18:00 when the temperature was at or above 16 °C during September and October of 2018 and 2019. A variety of insects visit apple in these orchards, however, bees are the predominant and most efficacious pollinators [69, 72, 73] and in this study, we only focus on the phylogenetics of hymenopteran visitors. Bees were identified to genus using taxonomic keys [86, 90–94] and other hymenopterans were identified to family [95] per [73]; more detailed identifications are not currently possible given the lack of up-to-date species-level keys for Australia [96]. Duplicate specimens were sequenced where possible (Table S1) and voucher specimens were lodged at the Australian National Insect Collection, CSIRO Black Mountain, Australian Capital Territory, Australia (Accession 32–163230 to 32–163322).

DNA extraction

DNA was extracted from tissue derived from either dry-pinned or ethanol-preserved specimens, predominantly using legs, or the whole body for smaller organisms (detailed in Table S1). Tissues were digested in a Proteinase-K buffer solution and incubated overnight at 55 °C. DNA was then extracted using Zymo Quick-DNA Miniprep Plus Kits (Zymo Research, Irvine, CA, USA), according to manufacturer protocols, with the modifications denoted in Branstetter et al. [48]. DNA concentrations were assessed using a Qubit 3.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) and DNA quality was assessed via TapeStation (Agilent, Santa Clara, CA, USA).

Phylogenomic approach

Established UCE phylogenomic methods were used to generate sequence data [45, 47] with custom-designed RNA probes synthesized by Arbor Biosciences (MyBaits—Ann Arbor, MI, USA). A bee-ant probe set [23] was used for bees, and a more generalized hymenopteran probe set [47] was used on all other specimens that collectively target and enrich 2,545 UCE loci.

DNA library preparation, hybrid-capture enrichment, and high-throughput sequencing

Raw DNA samples were sheared to a fragment size of ~ 400–600 bp using a Qsonica sonicator (Q800R3; Qsonica, Newton, CT, USA). All samples were sonicated once for 60 s at 25% maximum amplitude and using a 10-s on–off pulse. Illumina libraries were generated using

KAPA HyperPrep kits (Roche Sequencing, Pleasanton, CA, USA) and custom dual-indexing adapters [97]. Fragmented DNA was purified and concentrated using an in-house SPRI-bead solution [98]. Once the final bead cleaning was complete, sample DNA concentration was measured using the Qubit fluorometer and then pooled into 12 groups containing 8–10 samples of equimolar concentrations.

Hybrid-capture in-solution enrichment of pooled samples followed standard protocols from Arbor Biosciences (MyBaits v4 chemistry 2018) and a modified custom protocol developed by Blumenstiel et al. [99]. Post enrichment, each pool was quantified using qPCR and combined into a single final pool containing enriched products for 93 insect specimens. This final pool was sent to Novogene Inc. (Sacramento, CA, USA) for single-lane multiplexed sequencing using Illumina HiSeq X.

Bioinformatic quality control, alignment, and identification

Raw sequence reads were demultiplexed using BBTools (Bushnell) and the reads were then cleaned, trimmed, and assembled using the wrapper package Phyluce v1.7 [100] within the Conda environment [101]. Raw reads were trimmed using Trimmomatic [102] within Illumiprocessor v2.0 [103]. Quality-controlled reads were then de novo assembled using Spades [104]. Contigs matching UCE loci were identified using the principle Hymenoptera probe set (v2; [47]) and extracted using the program LastZ v1.0 [105] with minimum-identity and minimum-coverage settings of 75 and 70, respectively, to optimize UCE recovery. Isolated target contigs were then aligned using MAFFT v7.130b [106] with default FFT-NS-i algorithm settings. Alignments were subsequently trimmed using Gblocks [107], with reduced stringency parameters (b1 = 0.5, b2 = 0.5, b3 = 12, b4 = 7). We then filtered alignments to include only loci which had data available for at least 90% of taxa.

The raw, assembled contigs for each insect specimen were searched for the COI barcode region [108] using a PHYLUCE script (match_contigs_to_barcodes) and a bait sequence (*Osmia lignaria*, Accession #RRMFE3077-15) downloaded from the BOLD database [109]. This was done to provide independent molecular identifications against reference material on publicly available genetic databases. Species-level identifications were made, where possible, when the top hit in BOLD (> 95% match) [109] and the best match in GenBank (Max. Score > 600) [110] agreed.

Phylogenetic inference

Aligned UCE loci were used to infer phylogenetic trees using a combination of parsimony and maximum-likelihood procedures in IQ-TREE v2 [111]. An objective modelling approach was undertaken [112] that made the fewest assumptions about the data by applying a general time-reversible (GTR + F + I + G) model for DNA base substitution rates [113]. Nucleotide base frequencies were empirically derived from the alignment and rate heterogeneity across sites was modelled using a discrete Gamma shape parameter [114], allowing for a proportion of invariable sites [115]. An ultrafast bootstrapping approach (1,000 replicates) was used to estimate branch support [116] as well as a single branch test (1,000 replicates) applying an approximate likelihood ratio test [117]. A rooted best maximum-likelihood tree and consensus tree (derived from 1000 bootstrap trees) were produced using FigTree v1.4.4 [118].

Functional traits

To evaluate functional diversity, two behavioural and three morphometric traits relevant for pollination were selected (Table 4). Functional trait data were collected for insect morphospecies with more than five observations. To obtain functional trait data, body size, measured as intertegular distance (ITD), glossa (tongue) length, and hairiness were measured ($n = 5$ specimens per morphospecies) in this study, and behavioural data obtained from Bernauer et al. [73]. Measurements for ITD and glossa length were obtained from photographs taken with a Leica EZ4 W microscope camera and measured using

Table 4 Functional trait descriptions. Both morphometric and behavioural traits were used to assess functional diversity, each trait is described here along with details of where data was obtained are also included here

Trait type	Trait name	Description	Data source
Morphometric	Body size	Inter tegular distance (ITD) is correlated with body mass and flight distance in bees [81]	this study
	Glossa length	Tongue length affects nectar accessibility [82]	this study
	Hairiness Index	Hairiness facilitates pollen collection and transfer, and increased hairiness can be linked to pollinator effectiveness [14]. Hairiness was measured by modifying the index developed by Woodcock et al. [74] to include a 13th hairiness location – the top of the thorax	this study
Behavioural	Pollen load purity	Proportion of pollen carried by insects that was apple pollen	[73]
	Loose pollen on body	Proportion of insects which carried body pollen when visiting apple flowers	[73]

ImageJ [119]. ITD was measured as the distance between the middle or widest part of the tegulae and glossa length was measured from the tip of the glossa to the end of the prementum (per [87]). Hairiness data were obtained by modifying hairiness indices created by Woodcock et al. [74] to include 13 hairiness locations (12 from Woodcock et al. [74] + dorsal side of thorax) to evaluate three hairiness levels (coarse setae or very short hairs, short and dense hairs, long and dense hairs, see [74] for more detail). The hairiness index was standardized so that it ranged from zero to one.

Statistical analysis

Statistical analyses were undertaken in R v4.0.3 [120] and Python 2 [121]. Means were compared using Mann–Whitney U tests and are reported with standard errors (s.e.).

Phylogenetic diversity

Phylogenetic diversity was assessed for the entire community of hymenopterans and for each landscape type using Faith's Phylogenetic Diversity (*pd* function in package "picante"; [122]). Phylogenies were plotted using the packages "phytools" [123] and "ape" [124]; the function *chronos* in "ape" was used to create ultrametric phylogenies.

Functional diversity

Functional diversity analyses were conducted using only bee taxa as these are the most important apple pollinators in our study orchards [69, 72, 73] and because we had the most complete trait data for these taxa. We used the package "mFD" [44] to determine if each bee taxon filled its own functional space, or whether multiple taxa were, from a functional perspective, occupying the same space (i.e., were functionally redundant), grouping taxa into functional entities (FEs). To calculate FEs, all traits must have data available for all taxa [44], therefore, we limited the traits used in this analysis to ITD (body size), glossa length, and hairiness as these data were available for all bee taxa in our study. FEs can only be calculated when traits are categorical or integers, so our traits were binned into categories as outlined in Table 3 and then converted to integers (i.e., the lowest trait category becomes an integer value of 1, the highest 3 or 5, depending on the number of trait categories, see Fig. 4 and Table 3).

Once FE's were calculated, we used "mFD" [44] to evaluate three measures of functional diversity. Functional richness is defined as the proportion of the functional space filled by the species in the focal assemblage. Functional divergence is defined as the proportion of the abundance supported by the species with the most extreme functional traits. Functional evenness is the regularity of abundance distribution in the functional space using the minimum spanning tree linking all species present in the assemblage. These functional diversity measures are biomass-weighted, meaning abundance is accounted for.

Trait clustering

To evaluate the statistical significance of non-random signals present in trait data, and to determine if signals are the result of phylogenetic clustering, Blomberg's K was calculated [16]. A significant K value, regardless of the actual value of K, indicates a non-random distribution. Meanwhile, a K value below one corresponds to random or convergent patterns of trait evolution, while a K value at or above one implies some degree of phylogenetic conservatism. A phylogenetic signal occurs when closely related species share more similar trait values than two species drawn at random [125]. In contrast, phylogenetic conservatism occurs when closely related species are more similar (i.e., share more of the same traits) than would be predicted by phylogenetic relationships alone [126]. Here we calculate Blomberg's K to look for evidence of phylogenetic patterns across our functional traits by quantifying the amount of phylogenetic signal across traits and trees [16].

Abbreviations

FE	Functional entity
ITD	Inter-tegular distance
ML	Maximum Likelihood
UCE	Ultra-conserved element

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12862-024-02293-4.

Supplementary Material 1.

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Authors' contributions

Olivia M Bernauer, Simon M Tierney, and James M Cook conceived and designed the study. Olivia M Bernauer and Simon M Tierney collected the data, Michael G Branstetter performed the UCE sequencing work, and Olivia M Bernauer, Simon M Tierney, and Michael G Branstetter analysed the data. The first draft of the manuscript was written by Olivia M Bernauer and all authors contributed feedback on subsequent drafts of the manuscript. All authors read and approve of the final manuscript.

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Availability of data and materials

The datasets generated and analysed in this study are available through DRYAD (https://doi.org/10.5061/dryad.ffbg79d1g). The raw DNA sequence data are also available at the NCBI Sequence Read Archive (BioProject # PRJNA1129209).

Declarations

Ethics approval and consent to participate

All insects collected for this study were done so on private land with permission of the landholders. Insects were collected directly from flowers into small plastic tubes and were stored on ice before transport to the lab where they were frozen ask quickly as possible to minimize suffering.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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