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Molecular Food Web of Lepidopteran Hosts and Their Parasitoids in a Tropical Secondary Forest in Thailand

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ABSTRACT. – A semi-quantitative molecular food web was constructed for caterpillars (Lepidoptera) and juvenile insect parasitoids dissected from them, using the barcoding region of the mitochondrial cytochrome oxidase *c* subunit 1 gene. Sample collection was carried out in a secondary forest in Saraburi province of Thailand. A total of 5,673 caterpillars were collected between November 2015 and November 2016. Overall, 6%. of these were found to be parasitised. DNA barcoding revealed 118 provisional host species and 119 species of parasitoids. The most abundant caterpillar hosts were *Haritalodes derogate* (Lepidoptera, Crambidae) and the most abundant parasitoid was a tachinid fly, *Peribaea* sp. (Diptera, Tachinidae). Apparently, specialist parasitoids constituted 80% of the species, however, this seems likely to be an overestimate because of the large number of singletons. Compared with a number of other comparable food web studies the connectedness of our web was extremely low whereas generality, vulnerability and linkage density were very high. This might reflect the heterogeneous nature of the recently disturbed study area. Understanding the relationship and the unique attributes of secondary forests is essential for enhancing biodiversity and maintaining ecosystem stability in these fragile environments.

KEYWORDS: DNA barcoding, Braconidae, Ichneumonidae, Tachinidae, dissection, quantitative food web

INTRODUCTION

Thailand is a biodiverse country and much of its insect fauna is poorly known. Whilst for charismatic species such as butterflies there is a complete checklist (currently with a little more than 1,300 species) (Beaver et al., 2002). For the moths, the situation is very different. Whilst 4,684 species are currently identified in the Moths of Thailand Project (iNaturalist, 2025), the actual number is likely two to three times higher. The situation is even worse for parasitoids of lepidopteran species with the great majority undescribed and virtually nothing known of their host relationships (Quicke et al., 2023).

Understanding host-parasitoid interactions, and, indeed, all ecological interactions, requires accurate identification (Agusti et al., 2005; Hrček and Godfray, 2015). Information on host-parasitoid relationships is still limited, especially for the tropics. Compared to the better-studied and less diverse temperate regions, the biology of the great majority of tropical Lepidoptera and parasitoid insects is still largely unknown (Novotny et al., 2004). Identification to the species level of many adults is challenging, and for caterpillars, identification beyond family level is essentially impossible for 99% of species (Gilligan et al., 2019). The traditional method of studying host-parasitoid interactions is rearing wild caught hosts until either adult butterflies/moths or parasitoids emerge. Several factors make such a rearing program particularly difficult in the tropics. There is difficulty in obtaining sufficient food plant for the caterpillars, especially if they are collected at an early stage or if rearing must take place far from the sampling site. Additionally, the plants may be scarce, difficult to locate, or challenging to identify. The high temperatures and humidity in the tropics can lead to premature host mortality, and this may not be random with respect to the parasitism status which would be especially important if one of the aims of a study is to try to quantify parasitism rates (Day, 1994).

Some successful caterpillar rearing programmes exist, notably the large-scale initiative by Dan Janzen and Winnie Hallwachs in the Área de Conservación Guanacaste, Costa Rica. This long-term project required significant funding for infrastructure, operations, and staff (Janzen et al., 2005; Janzen and Hallwachs, 2011). However, most researchers lack the resources for such extensive efforts. The cost of rearing must account for challenges such as food plant availability, host mortality, and logistical constraints.

When it comes to the taxonomy of the hosts and parasitoids in such a biodiverse tropical country, much needs to be based on molecular recognition of taxa. Modern molecular approaches are now allowing all the material (hosts and parasitoids) to be DNA barcoded and this has revealed a great diversity including large numbers of cryptic species complexes as well as the inadequacy of available identification keys. Beyond identifying cryptic diversity, barcoding can also clarify species boundaries, sometimes showing that taxa previously considered distinct are actually conspecific. These advancements highlight the power of molecular approaches in improving species identification and understanding biodiversity (Smith et al., 2006, 2007, 2008). This has led to a minimalist approach to make names available for the large numbers of undescribed tropical parasitoid wasp species (Sharkey et al., 2021a). Whilst this has been strongly criticized by many taxonomists (e.g., Zamani et al., 2021; Meier et al., 2021; Fernandez-Triana, 2022), the proportions of tropical parasitoid wasps and flies that are currently lacking scientific names, the large numbers of cryptic species complexes, and the lack of species-level identification keys to most groups underscore the need for molecular methods (Sharkey et al., 2021b).

Gariepy et al. (2008) directly compared three methods—rearing, dissection, and molecular techniques (multiplex PCR)—to assess parasitism levels and parasitoid species composition. Their findings showed that while rearing is commonly used, it resulted in over 30% mortality, limiting the accuracy of host-parasitoid association studies. In contrast, both dissection and molecular techniques provided more reliable results by identifying parasitoids even in cases where hosts did not survive to adulthood. Similarly, Hrček et al. (2011) demonstrated the benefits of integrating DNA barcoding with rearing to overcome the identification challenges posed by tropical caterpillars and their diverse parasitoid fauna in a food web study in Papua New Guinea. Their approach improved detection by sequencing host tissue remnants, parasitoid larvae, and non-surviving hosts, ensuring more comprehensive identification. These studies collectively highlight the limitations of traditional rearing and underscore the necessity of molecular approaches for accurately resolving host-parasitoid interactions, particularly in diverse tropical ecosystems.

Molecular methods can detect hymenopteran parasitoids within host insects with high specificity and sensitivity (Greenstone, 2006; Sow et al., 2019) and can greatly increase the number of trophic links detected (Wirta et al., 2014). Insect DNA barcoding based on a 658 base pair sequence of the mitochondrial *COI* gene (Hebert et al., 2004), offers a fast and accurate method for species identification. Food webs are complex networks of interactions, and understanding their structure and diversity can be challenging, especially when studying parasitoid species involved in biological control. Smith et al. (2011) used DNA barcoding to study the diversity and connectance of the food web, focusing on the frequency of polyphagous (generalist) versus oligophagous (specialist) species in different forest habitats of spruce budworm, *Choristoneura fumiferana* (Clemens, 1865). Their findings demonstrate how DNA barcoding can be an effective tool for accurately identifying parasitoid species and assessing their role in biological control, especially for managing future budworm outbreaks. Kitson et al. (2019) used nested tagging DNA metabarcoding for determining host-parasitoid interactions and parasitism rates in the larval stage of biological control parasitoids in the new environment by next next-generation generation sequencing. This methodology effectively resolved ecological networks of host-parasitoid relationships. However, in a large community such as tropical forest, relationships among parasitoids and their caterpillar hosts are very complex and difficult to establish.

To understand the complete set of interactions between hosts and parasitoids in a community, various levels of sophistication can be used depending on the data available. At the simplest level, relationships can be expressed as observed associations between species forming a connectance food web, where each link represents an interaction without quantifying its strength (Paine, 1980). However, a more detailed approach can be used if the relative abundance of parasitoids per host species can be quantified. In this case, the food web becomes semi-quantitative: instead of just indicating the presence of interactions, it incorporates the relative numbers of parasitoids per host, providing a more nuanced view of community structure. This semi-quantitative food web helps reveal the intensity of parasitism across different host species, allowing for a deeper understanding of ecological relationships (Paine, 1980; Godfray, 1994; Stireman, 2016; Heijboer et al., 2017). In this respect, tropical secondary forests are well studied, even though they exhibit both high abundances and species diversity of parasitoids and their lepidopteran hosts (Horstmann et al., 2005; Sääksjärvi et al., 2006; Tylianakis et al., 2006; Maeto et al., 2009; Butcher et al., 2012; Ek-Amnuay, 2012; Rodríguez and Medianero, 2022). We therefore might expect tropical secondary forests to display highly complex of host-parasitoid relationships.

In this study, the relationships between the lepidopteran hosts and their parasitoids were explored using both the dissecting method to find parasitoid and DNA barcoding methods to identify both caterpillars and parasitoids. These samples were collected from a poorly studied tropical secondary forest in South East Asia. In this study, we hypothesize that DNA barcoding methods can resolve complex host-parasitoid relationships in poorly studied tropical regions, where traditional identification methods may be limited. We also propose that while molecular techniques offer significant advantages, the accuracy of species identification could be affected by potential issues with collecting methods. By using DNA barcoding alongside traditional dissection methods, we aim to better understand the host-parasitoid interactions and address the challenges posed by cryptic species and gaps in identification keys in tropical ecosystems. However, the study also emphasizes the challenges of collecting methods, which can influence the accuracy

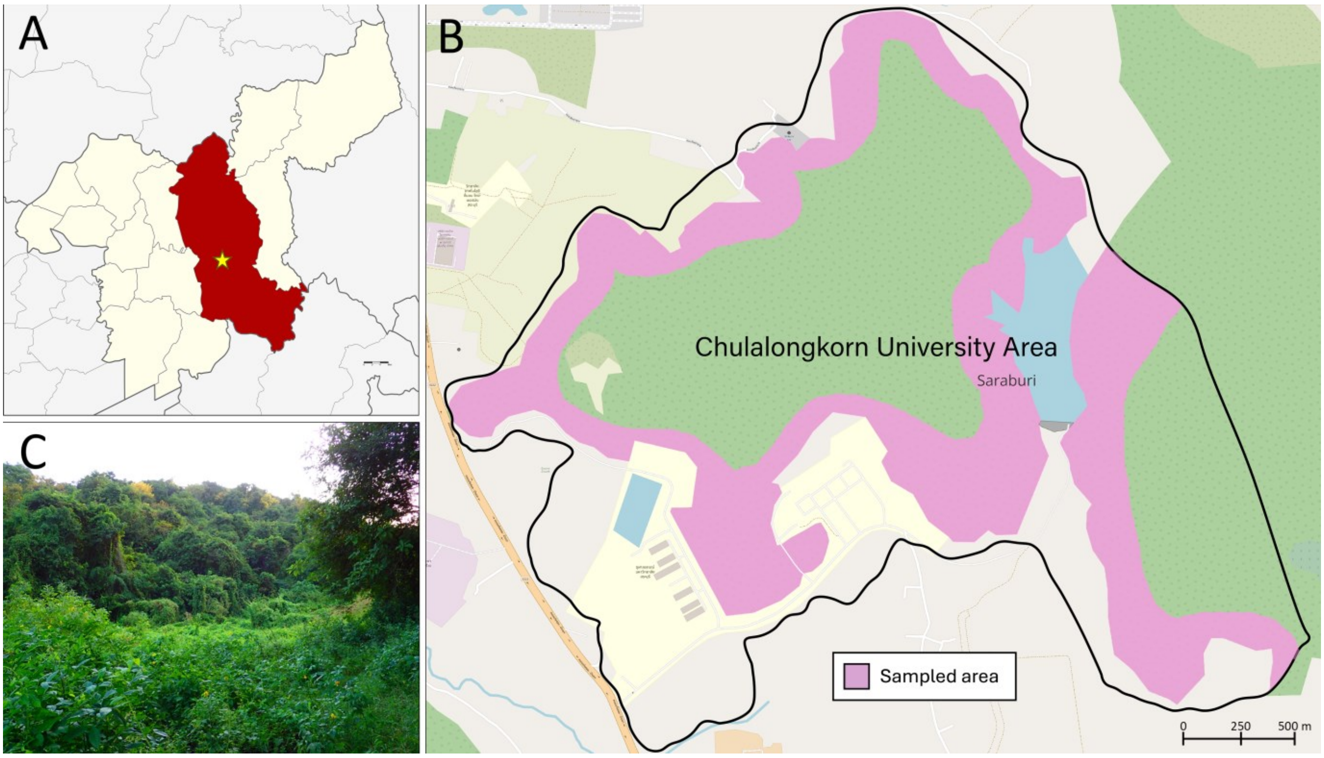

FIGURE 1. Map of the study area. A, map of Saraburi Province, Kaeng Khoi District is located at the star symbol. B, map of the Chulalongkorn University Area at Kaeng Khoi showing in pink the area that was sampled. C, view of typical deciduous forest at the study area.

of species identification. Despite these challenges, molecular techniques, especially DNA barcoding, provide significant advantages for studying ecological interactions and improving biodiversity understanding in complex ecosystems.

This study aims to explore the complex interactions between lepidopteran hosts and their parasitoids in a poorly studied tropical secondary forest in Southeast Asia. By employing both traditional dissection methods and DNA barcoding, we seek to overcome the challenges posed by the lack of comprehensive identification keys and the high cryptic diversity of species in tropical ecosystems. We hypothesize that DNA barcoding will enhance the accuracy of species identification, providing deeper insights into the host-parasitoid relationships and offering a more reliable approach for studying food webs in these underexplored regions. Furthermore, this study will examine the potential advantages and limitations of molecular methods in resolving ecological interactions in tropical forests. Finally, our results recovered several new host-parasitoid associations, some involving pest species, suggesting that secondary habitats, that were once farmed, might be a useful source of potential new biological control agents.

MATERIALS AND METHODS

Study area and sample collection

Fieldwork was conducted in a tropical secondary forest habitat at Chulalongkorn University Area, Kaeng Khoi District, Saraburi Province, Thailand, (15.52° N, 101° E) (Fig. 1A, B). The site covers an area of more than 500 Ha, ranges from 40 to 150 m above mean sea level, and consists of a mix of 80% deciduous and dry dipterocarp forests (Fig. 1C) and 20% successional grassland. Land surrounding the area is used for various agricultural practices.

Caterpillars were collected every two weeks during 34 site visits carried out over 13 months (November 2015 – November 2016). Each day, two randomly selected plots were sampled. Insect collecting took place from 06.00 to 09.00 in one of the sites and from 16.00 to 18.00 in the other. Caterpillars were located by visual inspection of the vegetation and using a standard beating tray and stick to dislodge them from low shhrub and tree branches up to a height of approximately 2 m above the ground. They were immediately to 95% ethanol and stored at 5°C until dissection.

Dissection for parasitoids

Caterpillars (before dissection) and parasitoid larvae (found during caterpillar dissection) were photographed using Olympus Stylus (TG–2 Tough) camera. Prior to dissection, caterpillars were placed in distilled water for 30–60 minutes to rehydrate them and soften their tissues. When a larva was found to be parasitised, the egg or larva(e) of the parasitoid were removed and a tissue sample of the caterpillar was placed separately in 96-well PCR plates. All images were uploaded to BOLD (Barcode of Life Data System, http://boldsystems.org, accessed on 15 January 2025) along with full collecting data for each specimen. Solitary parasitoids (one parasitoid larva per one host) and Gregarious parasitoids (more than one parasitoid larva per one host) were counted as a single parasitism event for any particular caterpillar. Parasitism rate was evaluated per month and between host families.

Identification levels

DNA sequencing was carried out according to standard protocols (Ivanova et al., 2006; deWaard et al., 2008; Hebert et al., 2013). Putative species were separated by their Barcode Index Numbers (BINs) (Ratnasingham and Hebert, 2013) and Automatic Barcode Gap Discovery (ABGD) (Puillandre et al., 2012). Family, subfamily, genus and, in some cases, species level identifications were made using BLAST searches (National Center for Biotechnology Information, 2023) and the BOLD ID engine. Identifications were considered virtually certain if percentage sequence identity in BOLD was 100% to a taxon at whatever level of accuracy. When percentage identity was lower than 90% but at least 99% of BLAST closest matches belonged to a taxon, that was also taken to provide a working identification.

Food web construction

Data analyses were performed using the R environment for statistical computing (R Development Core Team, 2017). A semi-quantitative food web was constructed using the R package Bipartite package and networkD3 (Dormann et al., 2009) and illustrated. Moreover, in order to provide an overall view that included all detected interactions, a graphical representation of the cumulative food web was drawn, using the same package (Hirao and Murakami, 2007) (see Supplementary material 1).

Food web statistics were calculated using the R package Bipartite (Dormann et al., 2009). Abbreviations: P = number of parasitoid species, H_p = number of parasitised host (caterpillar) species, and L = number of realised links. The following metrics are presented (Bersier et al., 2002; Lewis et al., 2002; Dormann et al., 2009; Gilbert, 2009): 1, connectance (C) (Interaction density across all potential species), i.e., $2L/(P + H_p)^2$ or, for smaller webs, $2L//(P + H_p)/(P + H_p\text{-}1)$; 2, realised connectance (RC) (Interaction density among only observed species), i.e., the realised proportion of possible links which is $L\ /(P \times H_p)$; 3, linkage density (LD), the average number of links per species, i.e., $L/(H_p+P)$; 4, number of compartments (NC); 5, generality (polyphagy) (G), i.e., mean number of host links per parasitoid, i.e. L/P; 6, vulnerability (V), i.e., mean number of parasitoids per host; 7, interactions evenness, a measure of the uniformity of energy flows along different pathways (IE). Results are compared with those of other published caterpillar parasitoid food webs.

RESULTS

A total of 5,673 caterpillars were collected. The highest number of caterpillars (837 individuals) were collected on 25–26 May 2016, while the lowest number (48 individuals) were collected at 18 – 19 March 2016. Caterpillar abundance peaked in late May and declined gradually thereafter (Fig. 2). Parasitism rate fluctuated markedly and apart from its lowest value corresponding to the onset of peak host abundance, showed no obvious relationship with host density.

Barcoding analyses and molecular identification of the specimens

Approximately 85% of specimens were successfully barcoded but success was higher for hosts (95%; n=457) than for parasitoids (73%; n=347). Barcoding BINS based on a minimum of 558 bps suggests that the sample comprises 116 Lepidoptera host species representing 93 genera in 24 families, and 119 parasitoid species collectively, representing 33 genera in 6 families (Table 1).

The most frequently represented host (23 samples) was identified as the as the amaltas leaf stitcher, *Copamyntis obliquifasciella* (Hampson, 1896) (Pyralidae) (Fig. 3A). The most abundant parasitoid (25 samples) detected was a tachinid fly, Tachinidae sp.1 (Fig. 3H). By using BLAST, the BOLD ID system identification at least to the family level was achieved for all but 22 of the sequences representing unknown Lepidoptera. For the caterpillars, confident species level identification was possible for 180 sequences representing 31 species. However, confident species level identification was only possible one tachinid, *Exorista xanthaspis* (Wiedemann, 1830) and two braconid species (the microgastrine *Microplitis prodeniae* (Rao & Kurian, 1950) and the agathidine *Zosteragathis contrasta* (Achterberg & Long, 2010)). Based on their DNA sequences, the hosts spanned

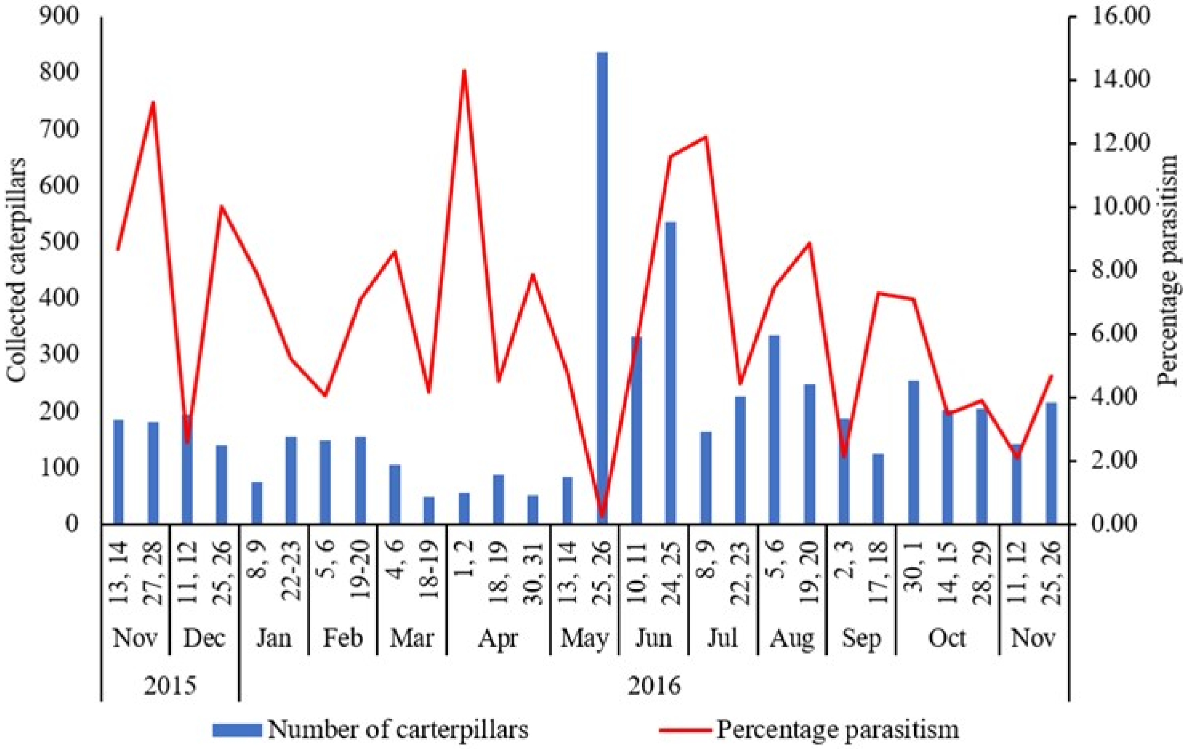

FIGURE 2. Numbers of collected caterpillars and parasitism rate through the study period.

several families of 'macrolepidoptera' (Erebidae, Euteliidae, Geometridae, Hesperiidae, Limacodidae, Lycaenidae, Noctuidae, Nolidae, Nymphalidae, Papilionidae, Pieridae, Sphingidae, Uraniidae) and 'microlepidoptera' (Crambidae, Gelechiidae, Gracillariidae, Hyblaeidae, Immidae, Pterophoridae, Pyralidae, Thyrididae, Tortricidae). The parasitoid groups detected were the Braconidae, Ichneumonidae, Chalcidoidea, Bethylidae and Eulophidae, in the Hymenoptera, and Tachinidae in the Diptera (see Tables S-1 and S-2 in Supplementary material 2).

Relationships between host and parasitoids

338 parasitised caterpillars revealed the interaction between caterpillars and their parasitoids, some of caterpillars being attacked by more than one parasitoid species. The caterpillar in Immidae sp.1 was parasitised with the highest number (8) of parasitoid species. The parasitoid in Tachinidae sp.1 was detected in seven species of caterpillars (7). The most frequently recorded association (13) was between the polyphagous pest erebid, *Orvasca subnotata* (Fig. 3C) and the microgastrine braconid *Glyptapanteles* sp.3 (Fig. 3D). From the molecular food web, 91 of the parasitoid species were recorded from only a single hosts species and might be specialists, whilst 26 species were recorded from more than one host species might be generalists.

One hundred sixty-four different host-parasitoid associations were recorded between 103 host species and 117 parasitoid species (Fig. 4) (see D3 JavaScript version in Supplementary material 3). Most parasitoids interacted with just one host; those with a wider host range generally showed strongly skewed interactions to one particular host.

Food web metrics from this study are compared with other published examples in Table 2.

DISCUSSIONS

Our data provide the first molecular food web for a community of lepidopteran hosts and their parasitoids conducted in South East Asian. DNA barcoding enabled identification of all except twenty-two sequences of the caterpillars to family level and more than 93% to genus with reasonable confidence based on both GenBank and BOLD databases. Of the 235 putative molecular species of both caterpillar hosts and their parasitoids identified based on barcode BINs, 50 sequences had close matches at the species level, with sequence divergences from reference sequences ≤ 2%. However, further perusal of these excluded some as being unlikely to be correct. Reasons for such exclusions were mostly biogeographic, such as closest match species having no previous records from South East Asia. For example, the Erebid moth, *Pericyma mendax* (Walker, 1858), matched 100% similarity by

12 individuals, is distributed in the south of the Sahara in Africa (Hampson, 1918).

Given the close proximity of the study site to agricultural areas and the nature of the habitats with occasional legacy trees such as mango (*Mangifera indica*), it is not surprising that some of the hosts were known agroforestry pest species of various levels of importance such as *Archips machlopis* (Meyrick, 1912), *Bastilla amygdalis* (Moore, [1885]), *Orgyia postica* (Walker, 1855) and *Spirama retorta* (Clerck, 1764).

TABLE 1. Host/parasitoid associations based on barcodes in this study and previous reported larval stage parasitoid records for that host. (numbers of individual host caterpillars in parentheses).

Host	Parasitoids detected in this study	Previously recorded larval stage parasitoid	Reference
Crambidae			
Crambidae sp.1	Cheloninae sp.6 (1)	-	-
Ecpyrrhorrhoe damastesalis	Microgastrinae sp.23 (1)	-	-
Haritalodes derogata	*Apanteles* sp.2 (7)	*Apanteles angaleti* Muesebeck,1956	Gahramanova et al. (2020)
	Cheloninae sp.1 (3)	*Apanteles bredoi* de Saeger,1941	Kumar et al. (2013)
	Cheloninae sp.5 (3)	*Apanteles diparopsidis* Lyle,1927	Odebiyi (1982)
	Chelonus sp.1 (1)	*Apanteles goron* Nixon,1965	Odebiyi (1982)
	Campopleginae sp.1 (4)	*Apanteles lanassa* Nixon,1965	Odebiyi (1982)
		Apanteles langenburgensis SzÈpligeti,1911	Odebiyi (1982)
		Apanteles opacus Ashmead,1905	Odebiyi (1982)
		Apanteles sagax Wilkinson,1929	Odebiyi (1982)
		Apanteles significans Walker,1860	Odebiyi (1982)
		Apanteles stantoni Ashmead,1904	Odebiyi (1982)
		Apanteles syleptae FerriËre,1925	Odebiyi (1982)
		Apanteles xanthostigma Haliday,1834	Odebiyi (1982)
		Enicospilus dolosus Tosquinet, 1896	Odebiyi (1982)
Herpetogramma platycapna	*Chelonus* sp.1 (1)	*Alabagrus texanus* Cresson, 1872	Ghazali et al. (2014)
	Dolichogenidea sp.4 (4)	*Anicetus* sp.	Ghazali et al. (2014)
Herpetogramma stultalis	*Apanteles* sp.3 (1)	*Apanteles hemara* Nixon,1965	Fernandez-Triana et al. (2017)
	Chelonus sp.1 (1)	*Apanteles opacus* Ashmead, 1905	Fernandez-Triana et al. (2017)
		Cotesia opacus Ashmead, 1905	Barrion & Litsinger (1994)
Hodebertia testalis	*Apanteles* sp.2 (1)	-	-
Hyalobathra brevialis	*Apanteles* sp.4 (6)	-	-
Notarcha aurolinealis	*Apanteles* sp.2 (3)	-	-
Notarcha obrinusalis	*Schoenlandella* sp.1 (2)	-	-
Parotis marinata	*Dolichogenidea* sp.8 (2)	-	-
Pycnarmon cribrata	*Apanteles* sp.2 (1)	*Diolcogaster* sp.	Huddleston & Walker (1994)
Spilomelinae sp.2	*Pseudoperichaeta nigrolineata* (4)	-	-
Synclera sp.1	Microgastrinae sp.24 (2)	-	-
Erebidae			
Asota caricae	*Dolichogenidea* sp.5 (3)	-	-
Avitta ophiusalis	*Diolcogaster* sp.2 (1)	-	-
Avitta sp.1	*Diolcogaster* sp.2 (1)	-	-
Bastilla amygdalis	Eulophinae sp.9 (1)	-	-
Boletobiinae sp.1	*Dolichogenidea* sp.3 (3)	-	-
	Microgastrinae sp.4 (7)		
Boletobiinae sp.2	Microgastrinae sp.4 (1)	-	-
Dinumma sp.1	Eulophinae sp.5 (8)	-	-
	Eulophinae sp.8 (1)		
	Eulophinae sp.10 (1)		
	Eulophinae sp.11 (2)		
Eilema sp.1	*Aleiodes contemptus* (1)	-	-
Episparina tortuosalis	*Parapanteles* sp.1 (1)	-	-
Erebinae sp.1	*Dolichogenidea* sp.5 (1)	-	-
	Microgastrinae sp.1 (1)		
Herminiinae sp.1	*Cotesia melanoscelus* (1)	-	-
	Glyptapanteles sp.8 (1)		
	Eulophinae sp.6 (1)		
	Exoristinae sp.8 (1)		
Herminiinae sp.2	*Snellenius maculipennis* (1)	-	-
Olene sp.1	*Cotesia melanoscelus* (1)	-	-
	Diolcogaster sp.1 (1)		
Orgyia postica	*Cotesia melanoscelus*	*Telenomus euproctidis* Wilcox, 1920	Pardede (1986)

TABLE 1. (Continue).

Host	Parasitoids detected in this study	Previously recorded larval stage parasitoid	Reference
Orvasca subnotata	*Glyptapanteles* sp.3 (13) Microgastrinae sp.8 (1),	-	-
Pericyma mendax	*Glyptapanteles* sp.5 (1) Tachinidae sp.1 (9) *Wilkinsonellus* sp.1 (3)	-	-
Progonia sp.1	Tachinidae sp.3 (1)	-	-
Rhesala sp.1	*Apanteles* sp.5 (3) *Dolichogenidea* sp.2 (2)	-	-
Rhesalides sp.1	*Dolichogenidea* sp.2 (2) *Zosteragathis contrasta* (2) Chalcidinae sp.1 (1)	-	-
Spirama helicina	Eulophinae sp.5 (4) Eulophinae sp.8 (9) Eulophinae sp.10 (3) Tachinidae sp.1 (2)	*Blepharella* sp. *Carcelia* sp. *Exorista* sp.	Sajap et al. (1997) Sajap et al. (1997) Sajap et al. (1997)
Spirama retorta	Eulophinae sp.5 (1)	*Blepharella* sp. *Carcelia* sp. *Exorista* sp.	Sajap et al. (1997) Sajap et al. (1997) Sajap et al. (1997)
Euteliidae			
Marathyssa sp.1	*Dolichogenidea* sp.1 (2)	-	-
Gelechiidae			
Anarsia sp.1	Orgilinae sp.1 (1)	-	-
Geometridae			
Ascotis sp.1	*Leptophion* sp.1 (1)	-	-
Biston suppressaria	Eulophinae sp.4 (1)	*Apanteles* sp. *Apanteles taprobanae* Cameron, 1897	Danthanarayana & Kathiravetpillai (1969) Chen & Kunshan (1987)
Chiasmia nora	*Dolichogenidea cerialis* (1) Eulophinae sp.2 (2)	-	-
Chiasmia sp.1	Cheloninae sp.5 (2) Cheloninae sp.3 (1) *Chelonus* sp.2 (1) *Dolichogenidea cerialis* (1) Exoristinae sp.1 (2) Tachinidae sp.1 (7)	-	-
Chiasmia sp.2	*Cotesia* sp.4 (1) Tachinidae sp.1 (1)	-	-
Chiasmia sp.3	*Glyptapanteles* sp.4 (1) Tachinidae sp.1 (3)	-	-
Cleora repulsaria	*Glyptapanteles* sp.1	-	-
Ectropis sp.1	*Dolichogenidea cerialis* (1)	-	-
Ennominae sp.1	*Diolcogaster* sp.1 (1)	-	-
Geometrinae sp.1	Microgastrinae sp.13 (1)	-	-
Hyperythra sp.1	Campopleginae sp.3 (1)	-	-
Hyposidra talaca	*Cotesia* sp.6 (1) *Dolichogenidea cerialis* (1) Microgastrinae sp.9 Tachinidae sp.4	*Cotesia* spp. *Cotesia ruficrus* Haliday, 1834	Das et al. (2010) Sarkar et al. (2020)
Larentiinae sp.1	Microgastrinae sp.5 (1)	-	-
Macaria abydata	Tachinidae sp.1 (1)	*Meteorus laphygmae* Viereck, 1913	Méndez-Abarca et al. (2012)
Pelagodes sp.1	Microgastrinae sp.14 (1)	-	-
Petelia medardaria	*Dolichogenidea cerialis* (1)	-	-
Petelia sp.1	*Dolichogenidea cerialis* (1)	-	-
Scopula sp.1	*Cotesia* sp.5 (1)	-	-

TABLE 1. (Continue).

Host	Parasitoids detected in this study	Previously recorded larval stage parasitoid	Reference
Gracilariidae			
Gracillariidae sp.1	Microgastrinae sp.12 (1)	-	-
Hesperiidae			
Hasora chromus	Microgastrinae sp.3 (1)	*Brachymeria lasus* Walker, 1841	Gupta et al. (2016)
		Crinibracon chromusae Gupta & van Achterberg, 2016	Gupta et al. (2016)
Hyblaeidae			
Hyblaea puera	*Drino solennis* (2)	*Sympiesis* sp.	Sudheendrakumar (2002)
		Sarcophaga sp.	Roychoudhury (2016)
		Stictopisthus sp.	Roychoudhury (2016)
		Elasmus sp.	Roychoudhury (2016)
		Elachertus sp.	Roychoudhury (2016)
		Exorista sp.	Roychoudhury (2016)
		Peribaea sp.	Roychoudhury (2016)
		Phanerotoma sp.	Roychoudhury (2016)
		Xanthopimpla sp.	Roychoudhury (2016)
		Brachymeria lugubris Walker, 1871	Roychoudhury (2016)
		Tetrastichus howardi Olliff, 1893	Roychoudhury (2016)
		Theronia maskeliyae Cameron, 1905	Roychoudhury (2016)
		Palexorista solennis Walker, 1858	Roychoudhury (2016)
		Apanteles ruidus Wilkinson, 1928	Sudheendrakumar (2002)
		Echthromorpha agrestoria notulatoria Fabricius, 1804	Roychoudhury (2016)
		Sympiesis hyblaeae Surekha, 1996	Roychoudhury (2016)
		Brachymeria lasus Walker, 1841	Roychoudhury (2016)
		Palexorista solennis Walker, 1858	Sudheendrakumar (2002)
		Trichogramma chilonis Ishii, 1941	Roychoudhury (2016)
Immidae			
Immidae sp.1	Microgastrinae sp.17 (1)	-	-
	Rogadinae sp.1 (1)		
	Glyptapanteles sp.7 (1)		
	Microgastrinae sp.10 (7)		
	Eulophinae sp.11 (1)		
	Exoristinae sp.5 (1)		
	Exoristinae sp.7 (1)		
	Tachinidae sp.4 (7)		
Immidae sp.2	Rogadinae sp.1 (1)	-	-
Limacodidae			
Limacodidae sp.1	Microgastrinae sp.11 (1)	-	-
Lycaenidae			
Arhopala pseudocentaurus	Microgastrinae sp.22 (1)	-	-
Castalius rosimon	Microgastrinae sp.15 (2)	dipteran parasitoids	Harinath et al. (2012)
	Paradrino sp.1 (1)		
Noctuidae			
Acontia sp.1	*Glyptapanteles* sp.5 (3)	-	-
Amyna octo	*Diolcogaster* sp.4 (1)	*Euplectrus euplexiae* Rohwer,1921	Meena et al. (2016)
	Lespesia sp.1 (1)	*Schoenlandella testacea* Kriechbaumer, 1894	Meena et al. (2016)
Arsacia rectalis	Cheloninae sp.4 (4)	-	-
	Dolichogenidea sp.10 (2)		
Chasmina sp.1	*Glyptapanteles* sp.7 (1)	-	-
	Euplectrus euplexiae (1)		
	Tachinidae sp.4 (1)		
Chrysodeixis eriosoma	*Diolcogaster* sp.3 (2)	*Microplitis* sp.	Perera et al. (2000)
	Euplectrus euplexiae (1)	*Ctenochares bicolorus* Linnaeus, 1767	Perera et al. (2000)
		Echthromorpha intricatoria Fabricius, 1804	Perera et al. (2000)
		Eulophus pennicornis Nees, 1834	Perera et al. (2000)
		Copidosoma floridanum Ashmead, 1900	Valentine & Walker (1991)
		Cotesia kazak Telenga, 1949	Valentine & Walker (1991)
		Cotesia marginiventris Cresson, 1865	Valentine & Walker (1991)
		Cotesia ruficrus Haliday, 1834	Valentine & Walker (1991)
		Cotesia plutellae Kurdjumov, 1912	Valentine & Walker (1991)
		Meteorus pulchricornis Wesmael, 1835	Berry & Walker (2004)
		Meteorus gyrator Thunberg, 1922	Perera et al. (2000)
		Trichogramma evanescens Westwood, 1833	Perera et al. (2000)

TABLE 1. (Continue).

Host	Parasitoids detected in this study	Previously recorded larval stage parasitoid	Reference
Hypospila bolinoides	*Blepharella* sp.1 (1)	-	-
Mocis trifasciata	*Microplitis* sp.1 (1)	*Apantales* sp.	Stanaway et al. (2001)
Noctuidae sp.1	Tachinidae sp.1 (1)	-	-
Noctuidae sp.2	*Diolcogaster* sp.1 (2) *Glyptapanteles* sp.8 (2)	-	-
Noctuidae sp.3	*Enicospilus* sp.1 (1)	-	-
Noctuidae sp.4	Microgastrinae sp.2 (2)	-	-
Noctuidae sp.5	Eulophinae sp.5 (3) Eulophinae sp.8 (1)	-	-
Noctuidae sp.6	*Glyapanteles* sp.2 (1)	-	-
Noctuidae sp.7	*Glyptapanteles* sp.6 (1)	-	-
Penicillaria jocosatrix	Eulophinae sp.7 (1)	*Aleiodes circumscriptus* Nees, 1834	Nafus (1991)
		Blepharella lateralis Macquart, 1851	Nafus (1991)
		Euplectrus parvulus Ferrière, 1941	Nafus (1991)
		Trichogramma platneri Nagarkatti, 1975	Nafus (1991)
Plecoptera quaesita	Eulophinae sp.3 (1) *Glyptapanteles* sp.8 (1)	-	-
Spodoptera litura	*Exorista xanthaspis* (1)	*Actia nigritula* Mall, 1939	Punia et al. (2021)
	Microplitis prodeniae (1)	*Apanteles colemani* Viereck, 1912	Ranga Rao et al. (1993)
	Exoristinae sp.6 (1)	*Apanteles prodeniae* Viereck, 1912	Yan et al. (2018)
	Tachinidae sp.2 (5)	*Brachymeria lasus* Walker, 1841	Ranga Rao et al. (1993)
		Bracon brevicornis Wesmael, 1838	Ranga Rao et al. (1993)
		Charops bicolor Szépligeti,1906	Ranga Rao et al. (1993)
		Charops obtusus Morley,1913	Ranga Rao et al. (1993)
		Chelonus helipae Gupta, 1971	Ranga Rao et al. (1993)
		Chelonus blackburni Cameron, 1886	Ranga Rao et al. (1993)
		Chelonus carbonator Marshall, 1885	Ranga Rao et al. (1993)
		Chelonus formosanus Sonan, 1932	Ranga Rao et al. (1993)
		Cotesia chilonis Munakata, 1912	Ranga Rao et al. (1993)
		Cotesia marginiventris Cresson 1865	Ranga Rao et al. (1993)
		Cotesia kazak Telenga 1949	Ranga Rao et al. (1993)
		Cotesia plutellae Kurdjumov, 1912	Ranga Rao et al. (1993)
		Cotesia ruficrus Haliday, 1834	Ranga Rao et al. (1993)
		Eriborus argenteopilosus Cameron, 1907	Ranga Rao et al. (1993)
		Euplectrus gopimohani Mani, 1941	Ranga Rao et al. (1993)
		Fannia leucosticta Meigen,1838	Ranga Rao et al. (1993)
		Habrobracon hebetor Say, 1836	Ranga Rao et al. (1993)
		Hyposoter didymator Thunberg,1822	Ranga Rao et al. (1993)
		Litomastix maculata Ishii, 1928	Ranga Rao et al. (1993)
		Microplitis demolitor Wilkinson, 1934	Ranga Rao et al. (1993)
		Microplitis pallidipes Szepligeti, 1902	Ranga Rao et al. (1993)
		Microplitis prodeniae Rao & Kurian, 1950	Ranga Rao et al. (1993)
		Microplitis tuberculifer Wesmael, 1837	Ranga Rao et al. (1993)
		Netelia ferruginea Cameron, 1899	Ranga Rao et al. (1993)
		Netelia producta Brullé,1846	Ranga Rao et al. (1993)
		Parasarcophaga misera Walker, 1849	Ranga Rao et al. (1993)
		Sarcophaga albiceps Meigen,1826	Ranga Rao et al. (1993)
		Sarcophaga dux Thomson, 1869	Ranga Rao et al. (1993)
		Snellenius manilae Ashmead, 1904	Ranga Rao et al. (1993)
		Telenomus dignus Gahan, 1925	Ranga Rao et al. (1993)
		Telenomus nawai Ashmead, 1904	Ranga Rao et al. (1993)
		Telenomus remus Nixon, 1937	Ranga Rao et al. (1993)
		Temelucha biguttula Matsumura,1912	Ranga Rao et al. (1993)
		Tetrastichus ayyari Rohwer, 1921	Ranga Rao et al. (1993)
		Trichogramma australicum Girault, 1912	Ranga Rao et al. (1993)
		Trichogramma chilonis Ishii, 1941	Ranga Rao et al. (1993)
		Trichogramma dendrolimi Matsumura, 1926	Ranga Rao et al. (1993)
		Trichogramma japonicum Ashmead, 1904	Ranga Rao et al. (1993)
		Trichospilus pupivora Ferrière,1930	Ranga Rao et al. (1993)
		Zele chlorophthalmus Spinola,1808	Ranga Rao et al. (1993)
Xanthodes sp.1	*Diolcogaster* sp.3 (1)	-	-
Nolidae			
Selepa sp.1	*Dolichogenidea* sp.6 (5) *Parapanteles* sp.2 (1)	-	-
Westermanniinae sp.1	Cheloninae sp.3 (1)	-	-
Nymphalidae			
Danaus chrysippus	*Sturmia* sp.1 (1)	*Sturmia convergens* Wiedemann, 1824	Gupta et al. (2016)
	Exoristinae sp.4 (1)	*Cotesia danaisae* Hedqvist, 1965	Gupta et al. (2016)
		Apanteles chrysippi Viereck, 1911	Gupta et al. (2016)
		Charops sp.	Edmunds (1976)

TABLE 1. (Continue).

Host	Parasitoids detected in this study	Previously recorded larval stage parasitoid	Reference
Papilionidae			
Papilio clytia	Exoristinae sp.2 (1)	-	-
Pieridae			
Eurema hecabe	*Cotesia* sp.1 (4)	*Nasonia vitripennis* Walker, 1836	Narita et al. (2007)
	Campopleginae sp.2 (1)	*Asobara tabida* Nees, 1834	
		Exorista sp.	Khan & Sahito (2012)
		Senometopia sp.	Narita et al. (2007)
		Microplitis sp.	Vavre et al. (1999)
		Cotesia sp.	Narita et al. (2007)
			Heath et al. (1999)
Pterophoridae			
Sphenarches anisodactylus	*Cotesia* sp.2 (1)	*Cotesia* sp.	Matthews (2008)
Pyralidae			
Copamyntis obliquifasciella	Microgastrinae sp.20 (11)	*Phanerotoma* sp.	*Shivakumara et al. (2023)*
	Cheloninae sp.2 (12)		*Shivakumara et al. (2023)*
		Apanteles taragamae Viereck, 1912	
Phycita sp.1	Microgastrinae sp.18 (3)	-	-
Phycitinae sp.2	Cheloninae sp.2 (1)	-	-
Ptyobathra hypolepidota	Microgastrinae sp.16 (1)	-	-
Thylacoptila paurosema	Cheloninae sp.3 (3)	-	-
	Exoristinae sp.3 (2)		
Sphingidae			
Macroglossum belis	Microgastrinae sp.7 (1)	*Senometopia ridibunda* Walker, 1860	Shima & Tachi (2023)
Tortricidae			
Archips machlopis	Microgastrinae sp.21 (1)	-	-
Tortricinae sp.1	Microgastrinae sp.21 (1)	-	-
Uraniidae			
Orudiza sp.1	*Choeras* sp.1 (1)	-	-

Sequencing success rate

The lower barcoding success rate for parasitoids compared with caterpillars that we encountered almost certainly reflects the small physical size of the samples, sometimes single parasitoid egg. This difficulty can easily be overcome using more sensitive DNA analysis protocols such as next generation sequencing (Shokralla et al., 2014). The latter would also allow early and small parasitoid stages to be sequenced from even very small host larvae and hyperparasitoids at various life stages. Several groups of parasitoid Hymenoptera are ovo-larval parasitoids and other koinobiont species attack freshly-eclosed first instar host lavae. These molecular methods can differentiate species based on their DNA sequences, providing a more accurate assessment of complex host-parasitoid-hyperparasitoid interactions (Ashfaq et al., 2017). More sensitive DNA sequencing protocols will therefore open up the opportunity to discover these trophic links.

Abundance of lepidopteran hosts and physical factors

The abundance of caterpillars through the sampling year was undoubtedly affected by an El Niño event which led to drought conditions and a consequent reduction in available fresh foodplants (Ashton et al., 2024). The greatest number of parasitism events detected involved the amaltas leaf stitcher, *Copamyntis obliquifasciella* (Hampson, 1896) a widely distributed pyralid moth whose caterpillars feed on is a severe pest on *Cassia fistula* L. (Caesalpiniaceae) (Bajwa, 1998), commonly known as Indian Laburnum. This leguminous tree is native to the Indian subcontinent and is prevalent in deciduous and mixed forests across the region (Kushwaha and Agrawal, 2012). *C. fistula* is also an important medicinal plant, widely used in traditional healing practices such as Ayurveda and Oriental medicine (Shivakumara et al., 2023).

Parasitism rate

From November 2015 to November 2016, the highest parasitism rate (14%) occurred in early April (early rainy season) while early December had the lowest parasitism rate (~0.2%) but also low during the late dry season (both late March and late May 2016). The observed parasitism rate found in this study was a little higher than commonly stated rate of 10% (Feener and Brown, 1997; Godfray, 1994) but there is was high variation throughout the year.

Assessing parasitism rate by caterpillar dissection has its drawbacks because parasitoid eggs and early instar parasitoid larvae may be overlooked (Symondson and Hemingway, 1997), whereas parasitoids that attack pupal stages will be missed. However, a previous study by Smith et al. (2008) found no difference between the parasitism rate estimated from the dissection method and from rearing method.

FIGURE 3. Representative pairs of parasitised caterpillars and the parasitoid larvae recovered from them by dissection. A, The most frequency DNA identified caterpillar, *Copamyntis obliquifasciella* (Pyralidae) (SID: SRKk 131036, PID: BBTH936-17); B, braconid wasp larva, Cheloninae sp.2 (SID: SRKk 191029, PID: BBTH1072-17); C, the most frequency found pest species, *Orvasca subnotata* (Erebidae) (SID: SRKk 101010, PID: BBTH907-17); D, braconid wasp larva *Glyptapanteles* sp.3 (Microgastrinae) (SID: SRKk 051010, PID: BBTH287-16); E, cotton leaf roller caterpillar, *Haritalodes derogata* (Crambidae) (SID: SRKk 071026, PID: BBTH226-16); F, ichneumonid wasp larva, Campopleginae sp.1 (SID: SRKk 041012, PID: BBTH332-16); G, caterpillar with 100% similarity to the Afrotropical *Acacia*-feeding brown shades moth, *Pericyma mendax* (Erebidae) (SID: SRKk 081056, PID: BBTH251-16); H, the most frequency DNA identified parasitoid, Tachinidae sp.1 larva (SID: SRKk 231041, PID: BBTH1290-17); I, caterpillar of *Rhesalides* sp.1 (Erebidae) (SID: SRKk091046, PID: BBTH267-16); J, chalcidoid wasp larva, Chalcidinae sp.1 (Chalcididae) (SID: SRKk032035, PID: BBTH057-16). Abbreviations: PID, = BOLD process ID; SID = BOLD sample ID.

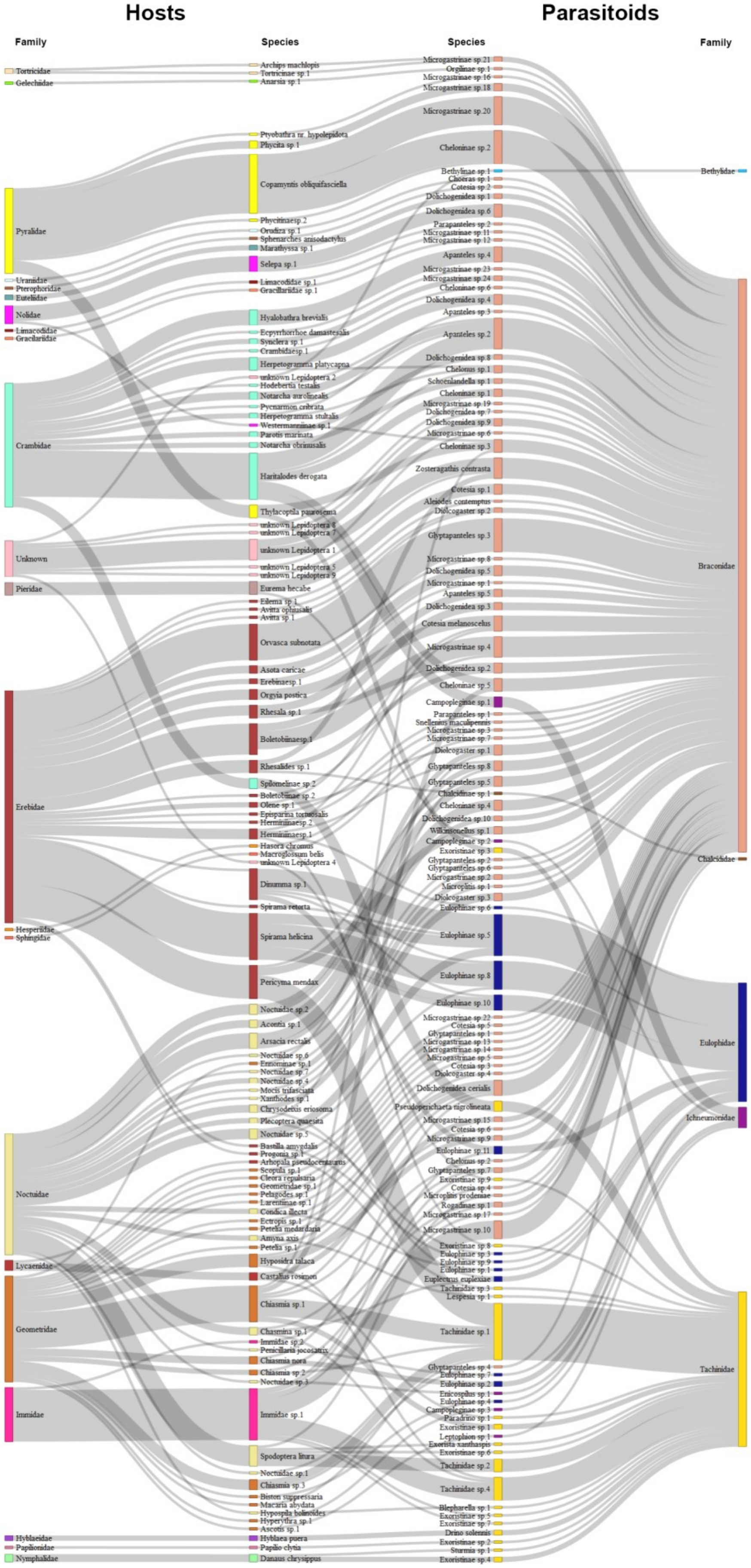

FIGURE 4. Molecular food web shows relationships of trophic links between lepidopteran host (left) and their parasitoids (right), based on results of caterpillar dissections. (See D3 JavaScript version in Supplementary material 3)

Barcoding analyses and molecular identification of the specimens

At present, both GenBank and BOLD databases still only provide sparse coverage when it comes to the diverse tropics and many of the specimens that have been sequenced have yet to be identified to species, or in some cases even to genus, by experts. However, a significant advantage of sequence-based identification is the ability to continuously expand the reference library. Anything that remains unidentified at the present time may be identified in the future as more sequences from previously identified specimens become available. This approach also offers provisional identification for species that are not yet represented in the library. The BOLD database (Ratnasingham and Hebert, 2007) serves as an efficient platform and repository for this sequence library, encompassing comprehensive data about the samples and includes an identification engine and now, obligately, images of every specimen.

Food web structure

From the molecular food web, 90 apparently specialist and 26 generalist (polyphagous) parasitoid species were found although our sample size is limited. Smith et al. (2006, 2007) similarly found that over 87% of tachinids were specialists. Fernandez-Triana et al. (2020) similarly found that Microgastrinae is a highly diverse subfamily of specialist parasitoids, with genera such as *Cotesia* and *Glyptapanteles* exhibiting strong host specificity, primarily targeting Lepidoptera species.

In many host-parasitoid food web studies, researchers have traditionally compared specific parameters across various systems, as exemplified by works (e.g., Van Veen et al., 2008; Macfadyen et al., 2009; Murakami et al., 2008; Gagic et al., 2012; Valladares et al., 2012). The research presented here explores a food web of host-parasitoid relationships in a secondary forest environment, utilising DNA barcoding to provide identifications of juvenile stages that otherwise, could only be identified through rearing.

One interesting finding was a number of parasitised caterpillars yielded barcodes 100% identical to the erebid moth *Pericyma mendax* the first record from Thailand, which otherwise had long been recorded only from mainland Africa, Madagascar and Reunion, although recently it was discovered In the Karnataka district of India (Ravindrakumar, 2021) and from Turkey (Kocasaraç et al., 2023), both based on morphology. Whether this species has recently started to expand its range or whether it has gone unnoticed (It is fairly dull looking moth.) in these newly reported regionsa, is unknown, perhaps both.

Kocasaraç, N., Çalışkan, S.S., and Özdemir, M. 2023. The Macroheterocera Fauna of the Sündiken Mountains (Türkiye: Eskişehir). Journal of Bartin Faculty of Forestry, 25(1): 137 – 152. 10.24011/barofd.1183114

In Table 2 we compare our food web statistics with those reported for a number of broadly comparable host-parasitoid studies including exophytic as well as leaf-mining caterpillars, but also parasitoids of cavity-nesting solitary Hymenoptera, gall forming insects and armoured scale insects. Several of the statistics calculated from the present data are towards or beyond the extremes of those reported elsewhere. For example, overall connectance (*C*) was by far the lowest, and the realised connectance (*RC*) the second lowest of all the studies summarised in Table 2. These low values may reflect enduring effects of past disturbances and the consequent spatial heterogeneity, despite the ecosystem's substantial recovery. However, small sample size relative to the number of host species might also mean that we failed to detect some links. A similar result might be expected if parasitoids tend to stay within localised areas or microhabitats, targeting only a subset of potential hosts rather than moving freely throughout the study area (Lewis et al., 2002). Murakami et al. (2008) found that separation of host plants on a scale of 1 – 3 m, had little effect on food web metrics. Several of the studies summarised in Table 2 combined data from multiple sites, which one might expect to reduce connectance and increase number of compartments because many species may occur at only a subset of localities. However, it is not really possible from the available data to say whether this is the case.

The relatively high generality (*G*), linkage density (*LD*), and vulnerability (*V*) found suggests that, despite prior disturbances, the ecosystem has successfully sustained or gained a diverse array of species interactions which, highlights the potential resilience and adaptability within secondary forest environments. However, understanding the factors contributing to vulnerability in a recovering ecosystem such as this, is crucial for well-informed conservation efforts. The high linkage density identified may play a pivotal role in ecosystem recovery by enhancing nutrient cycling and energy flow efficiency, potentially aiding in the restoration of ecological functions. The high level of generality (polyphagy) contributes to the high linkage density is an aspect of complexity and increased complexity may be associated with heightened vulnerability and biodiversity loss (Gilbert, 2009; Calizza et al., 2015, 2017). Recognising the interplay between ecological recovery and the specific characteristics of the secondary forest is crucial for promoting biodiversity and ensuring ecosystem stability in these valuable yet delicate settings.

TABLE 2. Host-parasitoid food web statistics for secondary forest of Thailand (this study, top row) compared with those from other published parasitoid studies.

Food web system	*C*	*RC*	*NC*	*G*	*V*	*LD*	*IE*	Reference
Caterpillar parasitoids, tropical secondary forest, Thailand	0.0067	0.013	61	1.985	2.394	2.19	0.542	present study
Leaf-miner parasitoids, moist tropical forest, Belize	0.013	0.047	8	2.333	-	1.463	-	Lewis et al. (2002)
Leaf-miner (Gracillariidae) parasitoids, deciduous forest, Japan[1]	0.0468	0.139	-	2.203	-	1.733	-	Hirao and Murakami (2007)
All leaf miners and exposed caterpillars, oak plantation low density, Japan	0.088[1]	0.191	-	1.035	1.581	1.308	-	Murakami et al. (2008)
All leaf miners and exposed caterpillars, oak plantation high density, Japan	0.087[1]	0.196	-	1.031	1.913	1.472	-	Murakami et al. (2008)
Predominantly primary, caterpillar parasitoids, deciduous forest understory, Czech Republic*	0.162	0.034	-	1.59	-	0.957	-	Šigut et al. (2018)
Caterpillar parasitoids, tropical forest, Papua New Guinea, site A	-	0.051	13	2.124	2.802	2.463	0.518	M. Libra & J. Hrcek, pers comm.
Caterpillar parasitoids, tropical forest, Papua New Guinea, site B	-	0.045	6	3.273	5.259	4.266	0.585	"
Caterpillar parasitoids, tropical forest, Papua New Guinea, site C	-	0.045	12	1.723	3.877	2.800	0.733	"
Caterpillar parasitoids, tropical forest, Papua New Guinea, site D	-	0.026	24	1.888	2.975	2.431	0.450	"
Caterpillar parasitoids, tropical forest, Papua New Guinea, site E	-	0.025	28	1.886	2.142	2.013	0.457	"
Caterpillar parasitoids, tropical forest, Papua New Guinea, site F	-	0.049	11	1.977	5.271	3.624	0.528	"
Caterpillar parasitoids, tropical forest, Papua New Guinea, site G	-	0.038	16	2.031	3.123	2.577	0.502	"
Caterpillar parasitoids, tropical forest, Papua New Guinea, site H	-	0.051	16	1.593	2.089	1.841	0.489	"
Caterpillar parasitoids, tropical forest, Papua New Guinea, site I	-	0.044	12	2.590	4.828	3.709	0.556	"
Caterpillar parasitoids, forests without gypsy moth outbreak in 2006, Canada	0.011	0.071	9	1.416	-	0.911	-	Timms et al. (2012)
Caterpillars on *Piper* spp (Piperaceae), 3 sites, S. Mexico[1]	0.033	0.068	11	1.095	-	0.622	-	Campos-Moreno et al. (2021)
Caterpillar parasitoids, 3 techniques combined, arctic Greenland[1]	0.1	0.205	-	2.864	-	1.75	-	Wirta et al. (2014)
Tachinidae on caterpillars, mostly cloud forest, Ecuador	-	0.033	-	4.09	1.94	3.02	-	Stireman et al. (2017)
Leaf-mining sawfly parasitoids, Austria, Switzerland, and Germany	0.059	0.142	-	3.54	-	2.488	-	Leppänen et al. (2013) [1, 4]
Seed and fruit feeding insects, lowland rain forest, Panama	-	0.006	85	1.845	2.234	-	-	Basset et al. (2021)
Seed and fruit feeding insects, lowland rain forest, Thailand	-	0.021	20	6.835	4.695	-	-	Basset et al. (2021)
Seed and fruit feeding insects, lowland rain forest, Papua New Guinea	-	0.022	9	5.151	6.050	-	-	Basset et al. (2021)
Cavity-nesting Hymenoptera, rainforest, understory, low elevation Australia[3]	-	0.154	-	-	-	2.618	0.855	Morris et al. (2015)
Cavity-nesting Hymenoptera, Brazil	-	0.34	1	1	1.65	1.31	0.45	Nether et al. (2019)
Galler parasitoids, understory, Panama	0.054[1]	0.138	6	3.691	-	0.91[1]	-	Paniagua et al. (2009)
Armoured scale parasitoids, many sites, China	-	0.04	25	1.7	-	1.92	-	Qin et al. (2018)
Grass-feeding chalcidoids, UK[1,2]	0.046	0.109	3	1.44	-	1.44	-	Dawah et al. (1995)

C = connectance, RC = realised connectance, *NC* = number of compartments, *G* = generality, *V* = vulnerability, *LD* = linkage density *(L/S)*, and *IE*=interaction evenness.

[1] calculated from numbers or figures in the reference.

[2] includes obligate and facultatively primary parasitoids.

[3] weighted RC, weighted LD, weighted IE.

[4] based on data in Pschorn-Walcher and Altenhofer (1989).

TABLE 3. Records of Lepidoptera species identified in this study acting as pests in various agroforestry areas in other countries and food plants on which the outbreaks affected.

Lepidoptera species	Food plant	Country(-ies)	Reference
Archips machlopis	garlic (*Allium sativum*)	India	Soumia et al. (2019)
–	stylo (*Stylosanthes guianensis*)	India	Bhumannavar et al. (1991)
–	sweet orange (*Citrus sinensis*)	Bangladesh	Sarkar et al. (2023)
–	tea (*Camellia sinensis*)	China	Sarkar et al. (2023)
Arhopala pseudocentaurus	kindal (*Terminalia paniculata*)	India	Davidson et al. (1896)
–	kusum (*Schleichera oleosa*)	India, Sri Lanka	Wynter-Blyth (1957)
Bastilla amygdalis	black-honey shrub (*Phyllanthus reticulatus*)	India	Robinson et al. (2010)
–	Indian gooseberry (*Phyllanthus emblica*)	India	Robinson et al. (2010)
Copamyntis obliquifasciella	emperor's candlesticks (*Senna alata*)	Pakistan	Chaudhry et al. (1970)
–	Indian laburnum (*Cassia fistula*)	Pakistan	Chaudhry et al. (1970)
–	katari (*Xylosma longifolium*)	Pakistan	Chaudhry et al. (1970)
Episparina tortuosalis	champak (*Michelia champaca*)	India	Robinson et al. (2010)
–	Indian mahogany (*Chukrasia tabularis*)	India	Robinson et al. (2010)
Macaria abydata	giant sensitive plant (*Mimosa invisa*)	Samoa	Ferguson (2008)
–	Jerusalem thorn (*Parkinsonia aculeata*)	Mexico	Ferguson (2008)
–	jumbie bean (*Leucaena latisiliqua*)	Hawaii	Ferguson (2008)
–	rambutan (*Nephelium litchi*)	Hawaii	Ferguson (2008)
–	soybean (*Glycine max*)	Brasil	Ferguson (2008)
–	sweet acacia (*Vachellia farnesiana*)	U.S.A.	Ferguson (2008)
Notarcha obrinusalis	corn (*Zea mays*)	D.R. Congo, Zambia	Goff (2019)
Orgyia postica	cocoa (*Theobroma cacao*)	Myanmar, Borneo, Java, New Guinea, and Taiwan	Browne (1968)
–	rubber (*Hevea brasiliensis*)	Sri Lanka	Browne (1968)
–	soybean (*Glycine max*)	India	Browne (1968)
–	tea (*Camellia sinensis*)	India, Sri Lanka, Myanmar and New Guinea	Browne (1968)
Orvasca subnotata	bilimbi (*Averrhoa bilimbi*)	India, Indonesia	Ahmed et al. (2021)
–	mango (*Mangifera indica*)	Vietnam	Oanh and Duc (2020)
Spirama retorta	black wattle (*Acacia mangium*)	Malaysia	Sajap et al. (1997)
–	Moluccan albizia (*Falcataria falcata*)	India	Sambath et al. (2012)
–	northern black wattle (*Acacia auriculiformis*)	Malaysia	Sajap et al. (1997)
–	northern wattle (*Acacia crassicarpa*)	Malaysia	Sajap et al. (1997)
–	oil cake tree (*Albizia amara*)	India	Sambath et al. (2012)
–	pink silk tree (*Albizia julibrissin*)	Korea	Kim and Lee (1986)

Secondary forest as a potential source of biological control agents

Ten of the host species (*Archips machlopis* (Meyrick, 1912), *Arhopala pseudocentaurus* (Doubleday, 1847), *Bastilla amygdalis* (Moore, [1885]), *Copamyntis obliquifasciella* (Hampson, 1896), *Episparina tortuosalis* (Moore, 1867), *Macaria abydata* (Guenée, 1858), *Notarcha obrinusalis* (Walker, 1859), *Orgyia postica* (Walker, 1855), *Orvasca subnotata* (Walker, 1865) and *Spirama retorta* (Clerck, 1764)) that were found to have been parasitised are known pests that have outbreaks in many agricultural areas around the world, including Thailand (CABI, 2023). Table 3 provides details of known food plant species of the caterpillar species included in the present samples as well as references to their pest status. Whether these were in our study area as a result of past cultivation there, had spread in from surrounding agricultural areas, or naturally occur there is uncertain. Some of the species that originated from S.E. Asia may fall into the latter category. In the case of several of these pest species our findings provide the first known parasitoid associations (Table 1), which suggests the possibility that regenerating secondary habitat, perhaps especially in proximity to farmed areas, may provide a source of potential biocontrol agents and/or act as a reservoir for these and other beneficial insects.

Supplementary materials

- Supplementary material 1: R code used to construct food webs.
- Supplementary material 2: Full list of parasitised hosts and their parasitoids with details of collection data, and molecular identifications using both BOLD and GenBank identification engines.
- Supplementary material 3: D3 JavaScript Sankey diagram of molecular food web between lepidopteran host and their parasitoids.

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