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# The Diversity, Costs, and Benefits of Shelters Built by Lepidopteran Caterpillars in a Costa Rican Dry Forest

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The Diversity, Costs, and Benefits of Shelters Built by Lepidopteran Caterpillars in a Costa Rican  
Dry Forest

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2017

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## ABSTRACT

Thousands of Lepidoptera species build shelters as caterpillars using plant material and their own silk. Although these caterpillars and their shelters are recognized as playing important ecological roles, the structural diversity of shelters and the costs and benefits of different shelters to their builders are still poorly understood. In this dissertation, I use natural history observations, observational and manipulative field projects, and molecular and phylogenetic tools to investigate these questions for a diverse and abundant shelter-building caterpillar community within the dry forest of Palo Verde National Park, Costa Rica. In Chapter 1, I develop a system for categorizing and describing the structural diversity of caterpillar shelters and apply it to the 95 shelter-building species I encountered during five field seasons. When analyzed this way, it becomes apparent that certain shelter types and traits are more common in this community than others, and that some shelters are associated with particular lepidopteran families. In Chapter 2, I describe the unique shelter-building behavior of a caterpillar species (*Aristotelia corallina*: Gelechiidae) living on ant-defended acacias. I also use caterpillar shelters preserved on herbarium specimens to propose host plant and geographic ranges for members of the *A. corallina* species complex. Caterpillar shelters are frequently preserved in herbarium collections and represent an under-used resource for identifying plant-insect interactions. In Chapter 3, I disentangle the effects of shelter shape and caterpillar identity on predation and parasitism by placing *Urbanus dorantes* and *Urbanus proteus* caterpillars (Hesperiidae) in both species' shelters. This experiment demonstrates that shelter shape has a significant effect on predation, and possibly parasitism, independent of caterpillar species identity. In Chapter 4, I explore shelter traits, predation, and parasitism in a phylogenetic context for a subset of species. I demonstrate that phylogeny significantly predicts shelter traits, that both phylogeny and shelter traits significantly affect predation, and that parasitism is negatively correlated with predation. This provides strong support for the hypothesis that parasitoids target caterpillar species which are less likely to be killed by predators. Overall, this work demonstrates that caterpillar shelters can provide important insights into taxonomic and phylogenetic relationships, ecological interactions, and evolutionary pressures.

## GENERAL ACKNOWLEDGEMENTS

In addition to the specific contributions acknowledged in each chapter, I would like to thank Robert Marquis and the Students and Teachers As Research Scientists program for ultimately starting me on this road in 2006. Many thanks are also due to the staff and researchers of Palo Verde Biological Station and the Missouri Botanical Garden for offering welcoming and interesting places to work, and in the case of Palo Verde, live. Thanks to my committee and fellow graduate students for asking questions, offering advice, and occasionally translating things into Spanish. And thanks to my friends and family for their support, interest, and a willingness to act as a knowledgeable lay audience.

## CHAPTER 1: A framework for describing the structural diversity of caterpillar shelters and an analysis of the shelters built by a tropical dry forest caterpillar community

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### ABSTRACT

Larvae from at least 26 families of Lepidoptera build enclosed, stationary caterpillar shelters that vary dramatically in size, shape, construction materials, openness, and frass accumulation. Although these shelters are taxonomically, ecologically, and phylogenetically informative, they have not been described or classified in a uniform fashion. Here, a descriptive framework is presented as well as recommendations for accurately observing and describing caterpillar shelters. The descriptive framework is applied to the shelter-building caterpillar community of a tropical dry forest and compared to what is known about other communities of shelter-building caterpillars.

### INTRODUCTION

For the purposes of this paper, a caterpillar shelter is a stationary enclosed structure built by one or more larval Lepidoptera (caterpillars) and inhabited during some or all of a caterpillar's development. This definition is modeled on the definition of "leaf shelter" given by Lill and Marquis (2007). Caterpillar shelters can range from large silk shelters built by tens or hundreds of caterpillars to miniature leaf shelters built by individual first-instar caterpillars. At the same time, caterpillars create many other structures which do not meet this definition, including leaf mines, excavated tunnels (e.g., *Virachola isocrates*, Wynter-Blyth [1957]), frass chains (e.g., *Memphis* spp. [Nymphalidae], DeVries [1987], *Eunica bechina* [Nymphalidae], Freitas and Oliveira [1996]), and portable cases (Packard 1887). Cocoons that are spun for the protection of the pupa are not considered to be caterpillar shelters, although it should be noted that many shelter-building species also use shelters to protect their pupae. Many other insects, including sawflies, beetles, and thrips, also construct larval shelters similar to those built by caterpillars (Wagner and Raffa 1993, Marquis and Lill 2006).

Shelter-building caterpillar species come from at least 26 families of Lepidoptera, including such diverse and disparate taxa as the Tortricidae, Gelechiidae, Hesperidae, and Pyralidae (Jones 1999: 24 families; this study: 2 additional families). Shelters are highly distinctive, and their builders can frequently be identified to genus or species based on the shelter alone, even when the caterpillar is absent or lacks other identifying characters. In addition to this diversity, shelter-building caterpillars are also abundant in many ecosystems, especially forests, where they may be the most numerous group of insect herbivores (Lill and Marquis 2007). There is increasing interest in understanding the effects of caterpillar shelters on both shelter-builders themselves and arthropods that secondarily inhabit caterpillar shelters (Lill and Marquis 2003, Connahs et al. 2011, Diniz et al. 2012, Hreck et al. 2013, Vieira and Romero 2013, Baer and Marquis 2014, Covarrubias-Camarillo et al. 2016, Velasque and Del-Claro 2016). To successfully identify the

shelter characteristics affecting occupants and compare shelters in different systems, a uniform vocabulary is needed.

Despite interest in the natural history of caterpillar shelters since at least the late 1800s (Packard 1877, Scudder 1889) there is no widespread framework for describing and categorizing caterpillar shelters. Many natural history observers simply report that a caterpillar species builds shelters, with no descriptive information given. This is unfortunate because precise descriptions are needed to document shelter characteristics. The vast majority of caterpillar shelters are nearly impossible to preserve as three-dimensional specimens<sup>1</sup>, and are equally difficult to represent with a single image. While electronic storage and dissemination of multiple color photographs can reduce this problem, such storage requires either a researcher-curated online database or access to the electronic materials of one or more scientific journals. Illustrations can be highly effective, but require drawing skill and may be time-consuming to prepare.

When shelter descriptions are given, their level of detail and vocabulary vary dramatically. Many descriptions merely assign the shelter to a general category, such as a fold, roll, web, or tent. However, depending on the researcher, a caterpillar ‘tent’ might be a large silk structure constructed by gregarious caterpillars (e.g., Fitzgerald 1995) or a relatively small leaf and silk structure constructed by a single caterpillar (e.g., Moss 1949, Lind et al. 2001). Since many authors do not define their shelter categories, what a particular author means by ‘tent’ or other terms must be inferred from illustrations or descriptions of shelter-building behavior. If these are not available, comparing shelter categories across the literature is difficult, if not impossible.

Here I propose a system for characterizing caterpillar shelters, based on the different construction materials and five basic shelter types that can be built from them in various combinations. I also describe several additional shelter characteristics that aid in identification and likely have biological significance. Examples are given primarily from the tropical dry forest shelter-building caterpillar community at Palo Verde Biological Station (Palo Verde National Park, Guanacaste, Costa Rica), but shelters from other communities are referenced to illustrate additional conditions.

## METHODS

Shelter-building caterpillars were collected at Palo Verde Biological Station (10° 21' N, 85° 21' W, elevation approximately 0-200 m asl) within Palo Verde National Park, Guanacaste, Costa Rica. Palo Verde consists of secondary tropical dry forest surrounding the wetlands of the Rio Tempisque. Caterpillar shelters were sought primarily along roads, human- and animal-made trails, and natural edges, as these areas had the most accessible foliage from ground level to ~3 m. Caterpillars and shelters were collected from late May to early August 2013-2017, during what is normally the first part of the wet season. However, El Niño events in 2014-2015 severely decreased May-August rainfall, and phenological variation in these years may have allowed sampling of some caterpillar species more usually associated with the end of the dry season or beginning of the wet season (April-mid May).

Collected shelters were photographed from multiple angles, and caterpillars were photographed if they could be safely removed from their shelters. The caterpillar and shelter were placed in a

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<sup>1</sup> A surprising number of caterpillar shelters are preserved as two-dimensional herbarium specimens (for an example, see Chapter 2), but looking at such preserved shelters is rather like interpreting fossils.

plastic bag. To maintain the foliage, stems were placed in individual florists' tubes. Fresh foliage was added as needed. As this rearing system sometimes resulted in leaves overlapping or bending in unnatural ways, some reared individuals produced unusual shelters that were never observed in the field. These artefactual shelters were not analyzed, but are included in the complete descriptions of species' shelters (Appendix). Caterpillars were checked daily for new shelters, ecdysis, pupation, eclosion, and parasitoid emergence. New shelters and caterpillar instars were photographed. Old shelters were collected once they were no longer in use. Shelters' maximum length, width, and height were measured to the nearest millimeter. Shelters were then carefully opened using fine-tipped forceps and described. To image shelter interiors, opened shelters were placed underneath a sheet of clear plastic and scanned with a handheld document scanner (VuPoint Solutions MAGIC WAND scanner, Los Angeles, CA, USA).

In the field, caterpillars were generally assigned to morphospecies based on host plant and shelter appearance. Lepidoptera were identified using a combination of regional morphological resources (Janzen and Hallwachs 2009) and DNA barcoding (using the same methods described in Chapter 2). During rearing and identification, some apparently distinct morphospecies (usually on different host plants) were found to belong to the same species, while other morphospecies were found to consist of multiple species. Some novel shelters were collected without caterpillar inhabitants and could not be attributed to a species, but were sufficiently different from other shelters that they were assumed to represent previously uncollected shelter-building caterpillars.

Representative specimens of host plants were photographed, collected, and pressed for herbarium specimens. Host plants were identified using the Missouri Botanical Garden herbarium (MO). One set of specimens was deposited at the Missouri Botanical Garden and a second set at the Herbario Ulises Chavarría at Palo Verde Biological Station.

Statistical analyses of shelter characteristics were performed in R using  $\chi^2$  tests and logistic regression (R Core Team 2016). While there is clearly phylogenetic signal associated with caterpillar shelters, a complete phylogeny was not and cannot be constructed for the entire community, as some shelters lacked caterpillars and attempts to isolate and sequence DNA from some species represented by only a few specimens were unsuccessful (but see Chapter 4 for a phylogenetic analysis of some species' shelters).

## RESULTS

The caterpillar shelter characterization system

### *Shelter-building materials*

All caterpillar shelters are constructed using at least two materials: caterpillar silk and a solid substrate. While caterpillar silk is less well-studied than spider silk, like spider silk, it is an aqueous solution of amino acids that solidifies instantly when exposed to air (Iizuka 1966, Work 1985). Caterpillars stretch strands of silk between attachment points, and as a result of the tension generated during drying, stretched silk generates the force needed to construct and maintain shelters. There is some evidence that species that build different shelters also secrete silk with different contractile properties (Fitzgerald et al. 1991). If the silk is attached to relatively immovable points, such as twigs, then the strand is under tension and can support weight. If at least one of the attachment points is movable, then the points are pulled closer together. This allows caterpillars to pull the solid components of their shelters together.

Shelter-building caterpillars apply their silk to substrates in several distinctive ways that serve different functions. It is unknown whether these silks have different chemical compositions, as is the case with different types of spider silk (Vollrath and Knight 2001, Sponner et al. 2007), or if their differences result solely from how they are applied. When many single strands of silk are anchored to different points on two substrates, webbing is created. The solidity of webbing depends on the number of silk strands and how they are organized. Relatively few strands running in all directions result in light fluffy webbing, while many strands running across the same two-dimensional plane can create solid, clothlike webbing (“sheet webbing”). The other common silk type is a guy line, which is a single line of silk created by attaching several parallel strands of silk to the same two attachment points. The resulting guy line is very strong, and it is often much easier to remove one end from an attachment point than it is to break the guy line in the middle. Many caterpillars build their shelters by pulling substrates together with guy lines.

There are also more specialized uses for silk in shelter-building. One is resting silk, which the caterpillar applies to a small area of substrate, usually within the shelter. This flat mat of silk apparently provides the caterpillar with a good surface for crochet attachment (Greeney and Jones 2003). Another special case is edging silk, which some caterpillars apply parallel to the leaf edge inside a shelter like a line of glue. Such edging silk effectively seals the shelter: trying to pull edging silk apart generally tears the leaf rather than the silk. Silk is also used to create internal structures within the shelter, sometimes in combination with other materials. As such structures, as well as resting silk and edging silk, all occur within established shelters, very little is known about how exactly the silk is applied in these cases.

By far the most common substrate for shelter-building is plant material. This plant material is usually living leaves<sup>2</sup>, although dead leaves, stems, and reproductive parts may be used as well. All shelters found at Palo Verde were built directly on their host plants, although there are caterpillar species that build shelters on the ground (e.g., *Trapezites* spp. [Hesperiidae], Fisher [1978], Atkins [1999]). In addition to these large components, various types of debris may also be incorporated, including shaved trichomes (Crambidae sp. 1), the caterpillar’s frass (feces) (Crambidae sp. 1, *Latalva pseudosmithii*, Elachistidae sp. 1), and occasionally pieces of bark (*Spindasis lohita* [Lycaenidae], Corbet and Pendlebury [1992]) or soil (*Trapezites sciron sciron* [Hesperiidae], Williams et al. [1992]). These materials may be added to a shelter’s exterior or used to build internal structures.

### *Shelter types*

Silk and substrate components can be combined in various ways to create different types of shelters. Individuals of many shelter-building species can change the type of shelter they construct, either due to caterpillar ontogeny (e.g., Lind et al. 2001, Greeney and Jones 2003), differences in host plant species, individual plant architecture and phenology (Greeney and Sheldon 2008, Greeney 2009), or all three. Here, five types of shelters are proposed based on shelter characteristics and the steps required to construct them: webs, leaf ties, leaf folds, leaf rolls, and cut-and-fold shelters (Fig. 1).

**Webs.** Shelters built of silk webbing attached to leaves or other substrates. The webbing may form either the exterior of the shelter by covering twigs or relatively immovable leaves (e.g., Pyralidae sp. 1, Unknown sp. 1, Elachistidae sp. 1, *Conchylodes salamialis*), or the interior of the shelter

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<sup>2</sup> Many plants have compound leaves, and depending on shelter type and size, shelters on these plants may involve one leaflet, multiple leaflets, or multiple compound leaves. Here, I will use “leaves” throughout, unless it is necessary to distinguish between leaves and leaflets.

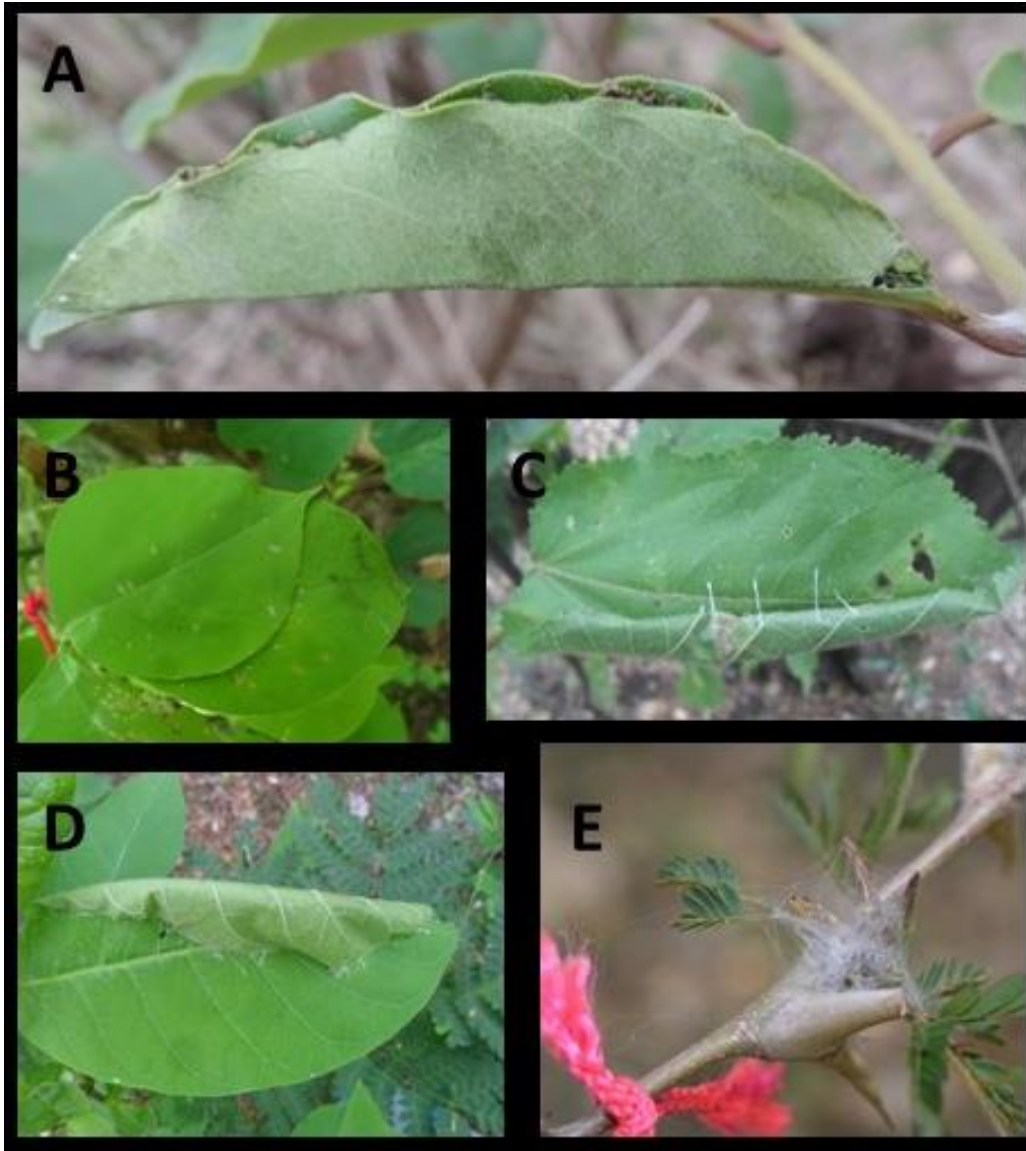


by pulling leaves into a mass (e.g., *Megalota* sp.). In at least one case, whether the webbing forms the exterior or interior of the shelter depends on the host plant used. When *Pococera sabbasa* caterpillars build webs on the stiff leaves of *Parkinsonia aculeata*, the webbing surrounds the leaves, but when they build webs on much more flexible leaves of *Caesalpinia eriostachys*, the webbing pulls the leaves into a mass. The resulting shelters are different enough that they were originally considered to represent different morphospecies (Fig. 2A-B). Many webs include internal tunnels, either built solely from silk or from silk and frass. No pads of resting silk have been observed in webs, presumably because the caterpillars can hook their crochets onto the webbing at any point.

**Leaf ties.** Two or more leaves tied together with guy lines, often forming flat sandwich-like structures. The leaves may be naturally overlapping or pulled into place by the caterpillar. Additional leaves can be added to the tie if more food is needed, although plant architecture limits the number of leaves that can be incorporated into a single tie. Various leaf-tying caterpillar species occupy resting pads or small tunnels, folds, or enclosures within the leaf tie when not feeding. These internal structures are built with silk, sometimes in combination with other materials.

**Leaf folds.** Leaves entirely or partially folded using guy lines. Leaves are often folded lengthwise, presumably to avoid needing to bend the midvein, although some caterpillar species can and do fold the distal portion of a leaf back on itself (e.g., *Eulepte concordalis*, Fig. 2C). Leaf folds may incorporate resting or edging silk, but usually do not contain internal structures. To demonstrate the amount of variation possible within a shelter type, several other leaf folds are shown in Fig. 2E-G.

Caterpillars have been observed to use at least three approaches to folding leaves. When the caterpillar is relatively long compared to the leaf's width (at least one-third of the leaf's width), the caterpillar strings guy lines from one edge of the leaf to the opposite side, effectively pulling the edge of the leaf over itself. The other strategies have been observed when caterpillars are significantly shorter than the width of the targeted leaf. In the second strategy, the caterpillar chews most of the way through the petiole, reinforces the connection with silk, and waits for the leaf to wilt (*Urbanus dorantes*, this study; *Neptis hylas* [Nymphalidae], Wynter-Blyth [1957]; *Urbanus esmeraldus*, Moraes et al. [2012]; *Lepidomys* sp. and *Tosale* sp. [Crambidae], Abarca et al. [2014]). The caterpillar may further secure a small fold with silk guy lines, but the leaf folding is accomplished by leaf dehydration, not silk tension. The final strategy has only been demonstrated by one Palo Verde species. *Massepha grammalis* caterpillars, which are short compared to *Maranta arundinacea* leaves, nonetheless manage to fold entire leaves in half by starting in the middle. They string relatively short guy lines from one side of the midvein to the other, bringing those central portions of leaf together. The caterpillars then repeat this process with successive sections of leaf until the entire leaf has been folded in half.



**Figure 1.** Types of caterpillar shelters: A. Cut-and-fold shelter (*Crambidae* sp. 1). B. Leaf tie (Unknown sp. 2). C. Leaf roll (*Crambidae* sp. 2). D. Leaf fold (*Diaphania* sp.). E. Web (*Aristotelia corallina*).

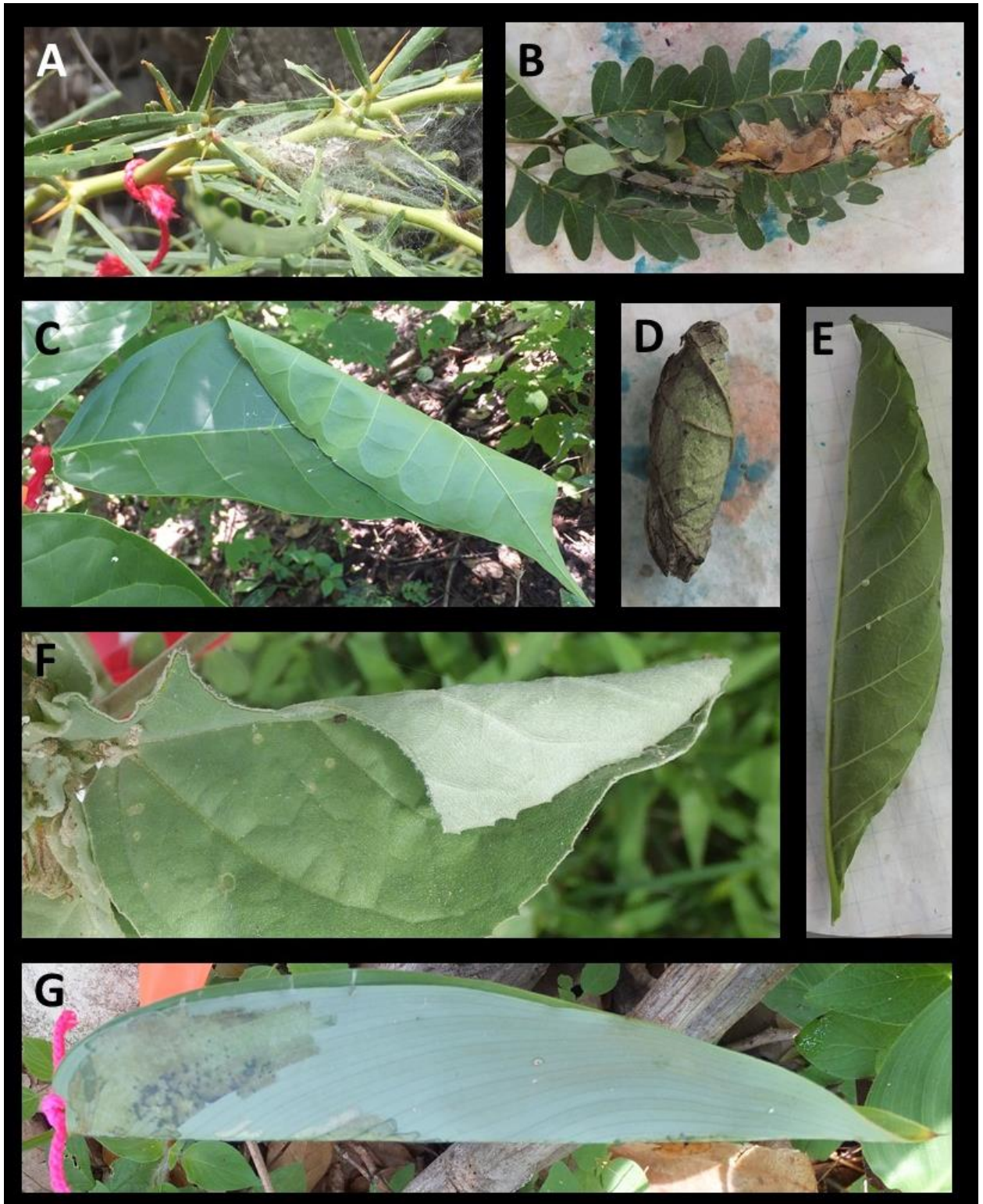
**Leaf rolls.** Leaves rolled in on themselves multiple times with guy lines to produce tubes with at least partially multi-layered walls. Like folds, rolls are often made parallel to the midvein, although some species do roll leaves from the apex of the leaf (Unknown sp. 19; five species in Fitzgerald et al. [1991]). The first stage of constructing a leaf roll is to construct a leaf fold. Observations of leaf-rolling and studies of opened leaf rolls suggest that all the Palo Verde leaf rollers make the initial fold by directly pulling the edge of the leaf over the leaf surface. Next, the caterpillar must exit the fold and use successive sets of guy lines to continue rolling the leaf around the initial fold. Most leaf rolls have two or three sets of guy lines between the outermost guy lines and the initial fold. After completing the initial fold, caterpillars will not continue rolling unless it is dark outside the shelter. In the field, this likely means that all rolls are finished at night, but leaf-rolling does not appear to be under circadian control, as putting caterpillars in a dark box is sufficient stimulus for them to complete their rolls. Other than the rolled leaf itself, no

internal structures have been observed in leaf rolls, although one species (Crambidae sp. 2) sometimes uses edging silk to seal the ends of the initial fold.

***Cut-and-fold shelters.*** Shelters in which a flap is cut from the leaf and folded using guy lines. The flap may be folded toward either the rest of the leaf or a second flap cut by the caterpillar. This shelter type is very commonly built by hesperiids and, at least in that family, there is evidence that the caterpillar cuts a stereotyped flap using body-based measurements (Weiss et al. 2003). The flap may be modified to create a conical shape or left flat, and different species cut rectangular, triangular, and even circular flaps (for more details of hesperiid cut-and-fold shelters, see Greeney and Jones [2003] and Greeney [2009]). Cut-and-fold shelters are also built by caterpillars in other families, and are frequently built as pupal shelters, even by species that construct no other cut-and-fold shelters. Two species (*Conchylodes salamisalis* and *C. plantinalis*) build a cut-and-fold pupal shelter and then cut through the tissue connecting the shelter to the rest of the leaf, resulting in a sealed capsule (Fig. 2D). Unfortunately, the mechanics of this process have not been observed.

***Other shelter types.*** Given the wide diversity of shelter-building caterpillars and the continuing accumulation of new natural history information, shelters that do not neatly fit these categories may be encountered. At least two species of lycaenids live within developing flowers that they hold closed with silk (*Jamides caeruleus*, Seufert and Fiedler [1996]; *Michaelus ira*, Bächtold and Alves-Silva [2013]). Although neither shelter is described in detail, they may result from tying the petals together with guy lines. If this is the case, they would be quite similar to leaf ties, with the only difference being that they are built out of leaf analogs (petals) rather than leaves.

More difficult to classify are the shelters built by two species of Mimallonidae. While earlier brief descriptions of mimallonid caterpillars suggested that they build two types of structures, leaf ties and portable cases (Forbes 1923, Stehr 1987), more recent descriptions present more detailed information. The early shelters of *Lacosoma arizonicum* (St. Laurent et al. 2017) and *L. chiridota* (Wagner 2005) are frass-covered networks of silk that appear to be made of intersecting guy lines rather than webbing, and are not leaf ties as previously interpreted. If such shelters are also constructed differently than webs, as seems likely, then an additional shelter category may need to be added to webs, leaf ties, leaf folds, leaf rolls, and cut-and-fold shelters.



**Figure 2.** A. *Pococera sabbasa* web on *Parkinsonia aculeata*. B. *P. sabbasa* web on *Caesalpinia eriostachys*. C. *Eulepte concordalis* open, frass-filled leaf fold. D. Cut-and-fold pupal shelter of *Conchylodes salamisalis*. E. *Diaphania* sp. closed, frass-filled leaf fold. F. *Anaea aidea* open, frass-free leaf fold. G. *Massepha grammalis* closed, frass-accumulating leaf fold.



### *Additional shelter characteristics*

Two additional shelter characteristics, openness and frass accumulation, have already been shown to affect shelter function in some instances (Weiss 2003, Moraes et al. 2012, Sendoya and Oliveira 2017). Openness is likely to affect a shelter's ability to protect a caterpillar from a range of abiotic and biotic effects, such as precipitation, temperature, or humidity on the one hand and predation or parasitism on the other. Different species' shelters can range from being quite open (e.g., *Psara obscuralis*, Crambidae species complex 1) to completely sealed (e.g., *Diaphania* sp.). Of course, openness and accessibility also depend on the size or type of objects entering a shelter. In this analysis, I consider a shelter to be "open" if it has openings larger than 1-2 millimeters wide and tall. If one wished to quantify a shelter's openness to natural enemies more explicitly, one could measure the height and width of the largest opening to estimate an upper limit for natural enemies that can enter the shelter. Similarly, one could also consider a shelter's permeability to abiotic factors, such as precipitation, humidity, or wind. As permeability to abiotic factors is also likely to be affected by factors such as leaf thickness, it is best assessed by directly measuring abiotic conditions inside and outside the shelter. It is quite possible that a shelter will differ in its openness and its permeability to abiotic factors, or even in its permeability to different abiotic factors.

Shelter-building caterpillars also differ greatly in the extent to which they allow their frass to accumulate in their shelters. Frass accumulation may be undesirable because it provides chemical cues for natural enemies, serves as a substrate for pathogens, or simply takes up too much space in a shelter (Weiss 2003). However, some frass volatiles can also deter oviposition by conspecific or heterospecific competitors (Renwick and Radke 1980, Jones et al. 1988, Anderson et al. 1993) or defend against predators (Gómez et al. 1999, Vencl et al. 1999). Many species remove all frass from their shelters, whether by pushing frass out of the shelter (e.g., Crambidae species complex 1), defecating over the edge of the shelter, or using ballistic defecation (Weiss 2003). Other species allow frass to accumulate in their shelters; in some cases, this frass is used as an additional building material, but in others, it is simply scattered around the shelter or pulled by gravity to the lowest part of the shelter. There can be some variation in frass accumulation among shelters built by a species. When rating shelters at a species level, I consider a species' shelters to be frass-free when most of the shelters contain no frass and the remainder only contain a few frass pellets each. Any species that actively incorporates frass into its shelters or consistently leaves frass in most of its shelters is considered to accumulate frass. Openness can frequently affect frass accumulation, as it is easier to remove frass from a more open shelter and unless a shelter is sealed, at least some frass usually falls out of the shelter (pers. obs.). However, some caterpillars seal their shelters except for a small hole that they exit through to feed and defecate (*Morrisonia confusa* [Noctuidae] and late-instar *Lacosoma chirodota* [Mimallonidae], R. J. Marquis, pers. comm.), and some open shelters still accumulate noticeable amounts of frass (e.g., Pyralidae sp. 1, Unknown sp. 4, *Conchylodes salamisalis*).

### The Palo Verde shelter-building caterpillar community

At least 95 shelter-building caterpillar species from at least ten families were found. These families were the Crambidae, Depressariidae, Elachistidae, Gelechiidae, Hesperidae, Hyblaeidae,

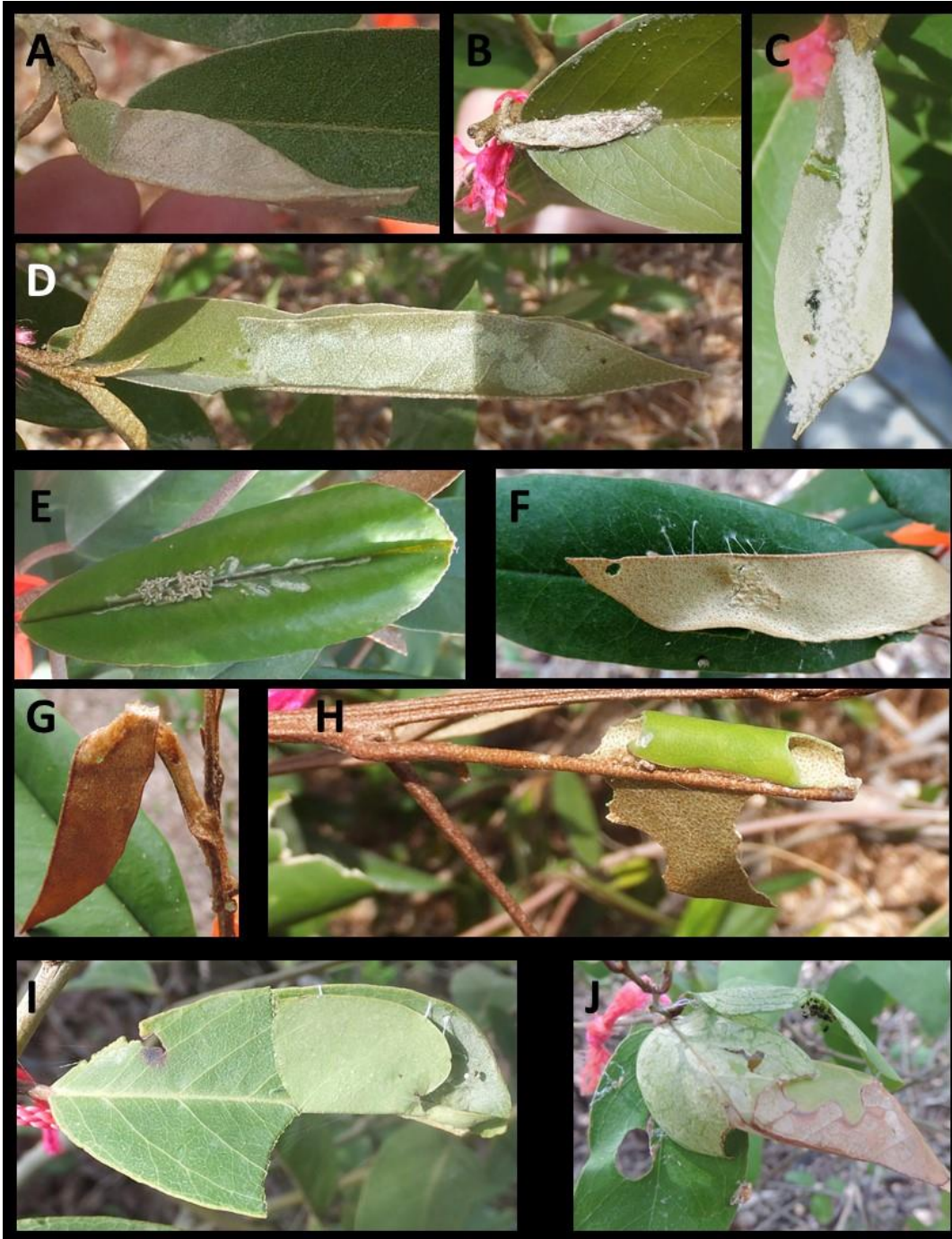
Noctuidae, Nymphalidae, Pyralidae, and Tortricidae. This number includes seven shelter morphotypes that were collected without caterpillars and cannot be identified. Additionally, Mueller and Dearing (1994) reported the presence of *Parapoynx rugosalis* (Pyralidae), which builds shelters on waterlilies and was not sampled during the current study. As I encountered the shelters of at least 95 species and new shelters continue to be found relatively easily (at least seven new species were found during 2017, even though there was no active search effort for new species), it is likely that many more shelter-builders remain to be found at Palo Verde.

The characteristics of the shelters built by these caterpillars are summarized in Tables 1-3 and described at greater length in the Appendix. Two of the 95 species were only represented by pupal shelters, and 32 other species were not sufficiently represented to determine whether they build different shelters throughout their development. Several general patterns can be seen in the community. First, the most common shelter types are webs and leaf ties, while leaf rolls are the least common (Table 1). However, when all shelters built throughout development (including pupal shelters) are considered, cut-and-fold shelters become as common as webs (Table 1). Second, 26 of the 61 species that were sampled throughout development demonstrate substantial flexibility in shelter construction depending on factors such as caterpillar age, plant species identity, leaf age and position, and, in some cases, interactions between these factors (Table 2). Twenty-three of these species built ontogenetically variable shelters. Seven of the twenty-six variable species were reared from multiple host plant species, and of these species, three species built different shelters on different host plants. Eleven species also built different shelter types depending on leaf maturity and/or architecture. One species, Crambidae sp. 1, varied in all three ways and built four shelter types depending on context: webs, leaf folds, leaf ties, and cut-and-fold shelters (Fig. 3).

**Table 1.** Shelter type frequencies for the Palo Verde community.

Shelter Type	Caterpillar shelters only	All observed shelters (including unique pupal shelters)
Cut-and-fold shelter	28	39
Leaf fold	23	23
Leaf roll	9	9
Leaf tie	39	39
Web	31	31

There are also patterns in if and how species transition between different shelter types. Caterpillars that begin by building cut-and-fold shelters transition to building leaf folds or leaf ties midway through their development, and most simply pupate in the final shelter rather than building a shelter specifically for pupation. Caterpillars that begin with leaf folds are somewhat more variable, with 9 of 15 species transitioning to a different shelter type midway through. Additionally, two species that normally fold leaves for their first shelters (*Diaphania* sp. and *Eulepte concordalis*) will occasionally build other types of first shelters if they are favored by leaf architecture, before switching to leaf folds. However, caterpillars that begin by building leaf ties, leaf rolls or webs do not switch to another shelter type midway through development, although some do build different pupal shelters.



**Figure 3.** Crambidae sp. 1 shelter variability. On *Quadrella indica*: A. Closed, frass-filled leaf fold on an unexpanded leaf, B. Closed, frass-filled leaf tie between an unexpanded leaf and a mature leaf, C. Closed, frass-and trichome-covered web on an expanding leaf, and D. Closed, frass-filled cut-and-fold shelter on a recently expanded leaf. On *Quadrella odoratissima*: E. Closed, frass-covered web on an expanding leaf, F. Closed, frass-filled leaf tie between an unexpanded leaf and a mature leaf, G. Closed, frass-filled leaf fold on an unexpanded leaf, H. Open, frass-filled cut-and-fold shelter on a mature leaf. On *Capparis flexuosa*: I. Closed, frass-filled cut-and-fold shelter on a mature leaf. J. Closed, frass-covered web on mature leaves.

Third, although not all caterpillars have been identified, there are some noteworthy associations between shelter types and taxonomic groups. Almost all the cut-and-fold shelters built as initial shelters are built by hesperiids, with the others built by several species of Crambidae. Leaf rolls also appear to be associated with crambids, as four of the five identified leaf rollers belong to that family. By contrast, webs were constructed by caterpillars from a wide range of families: Crambidae, Elachistidae, Gelechiidae, Hyblaeidae, Pyralidae, and Tortricidae. There is also variation in the number of shelter types that different families construct. The Crambidae are particularly noteworthy, as all five basic shelter types are built by different members of the family, and many species build multiple shelter types. At the same time, the closely related Pyralidae only build two shelter types (webs and leaf folds) at Palo Verde. Similarly, while the shelters of different hesperiid species differ in shape and construction, nearly all the species found in this study at Palo Verde follow the same basic trajectory of building cut-and-fold shelters before switching to leaf folds and/or leaf ties. The two clear exceptions to this rule are *Urbanus dorantes* and Hesperidae sp. 7, which typically build leaf folds from the beginning of their development.

Data on shelter openness and frass accumulation were analyzed for the most common shelters of 87 species. Although many species built multiple shelter types, only two species built shelters that varied in shelter openness (Crambidae sp. 1 and *Diaphania* sp.) and only one in frass accumulation (*Psara obscuralis*). These two shelter characteristics were not evenly distributed ( $\chi^2 = 16.001$ ,  $df = 1$ ,  $p < 0.0001$ ), with both open, frass-free shelters and closed frass-accumulating shelters more common than expected under a null model. The relationships between shelter type and both shelter openness and frass accumulation were tested using logistic regression. Shelters that were rolls, ties, or webs were all significantly more likely to accumulate frass than cut-and-fold shelters (factor  $p$  values 0.003, 0.011, and 0.002, respectively), while folds may be more likely to accumulate frass than cut-and-fold shelters ( $p = 0.050$ ). There was no relationship between shelter type and shelter openness.

## DISCUSSION

Studies of caterpillar or herbivorous insect communities have often included shelter-building caterpillars (Gentry and Dyer 2002, Connahs et al. 2011, Diniz et al. 2012, Hreck et al. 2013), but such studies have considered the ecology of shelter-building caterpillars without considering the nature of the shelters themselves. This first comparison of shelters within a caterpillar community offers several insights into how shelters are distributed throughout the community and raises further questions about how shelters are distributed across other communities and across the lepidopteran phylogeny.

First, the Palo Verde community sampled to date consists of numerous and diverse shelter-building caterpillars and shelters occurring on a wide variety of host plants (67 plant species in 31 families). At least preliminarily, the shelter-building caterpillars appear to be relatively specialized, with most species only found on one or two plant species, and even the most generalized species (*Pococera sabbasa*) feeds only on five species of caesalpinoid legumes (*sensu* LPWG 2017). However, all five types of shelters were built by multiple caterpillar families on the leaves of multiple plant families. This suggests that although individual shelter-building caterpillar species may have limited host ranges (perhaps due to factors such as host chemistry), different shelter types are adaptable to wide ranges of leaf characteristics.

One of the most striking results is the amount of context-specific variation in shelter-building



some species display. This context consists of at least three axes: caterpillar age, host plant species, and leaf variation. Interestingly, all the species which build different shelters depending on context appear to be hesperiids and crambids. The most common variation appears to be due to caterpillar age, although that may also be because that was the best-sampled of the three axes. Caterpillar age correlates with caterpillar size, and size likely drives most of the ontogenetic shelter changes. Crambids and hesperiids are the largest caterpillars in this study, with maximum lengths of three to four centimeters. Early instar caterpillars may be too short or too weak to manipulate entire leaves and therefore build cut-and-fold shelters, while later instars can manipulate entire leaves and may even be too large to build an effective shelter from a single leaf (Lind et al. 2001). Shelter variation due to host species and leaf variation were less commonly observed, although the number of species that could be examined for these types of variation was also smaller. Shelter differences between host species likely reflect consistent differences in leaf traits (e.g., size or flexibility) and their effects on shelter-building, while the effects of leaf phenology or position are more likely to be contingent. For example, while Greeney and Jones (2003) initially distinguished between two-leaf ties (“pockets”) and multi-leaf ties, Greeney (2009) merged these categories after observing that the number of leaves included in the shelter depended on the number of leaves contacted by the caterpillar during construction and the amount of leaf area needed to conceal it. Similarly, in many of the variable Palo Verde species, whether a leaf tie or a leaf fold is built appears to depend primarily on whether a second leaf is close enough to easily build a tie. Regardless, these results suggest that a complete understanding of a species’ shelter-building will often require shelters built by many caterpillars representing different instars for each known host species.

Second, while many shelter-building caterpillars have been found at Palo Verde, sampling to date has been somewhat limited both ecologically and phenologically. In addition to poorly sampled habitats with distinct plant assemblages (the wetlands, karst limestone outcrops, etc.), shelters are easier to detect on broad leaves, so shelter-builders feeding on grasses and sedges are underrepresented. All the grass-feeding species detected in this study were found on *Megathyrsus maximus* or *Lasiacis ruscifolia*, both species with wide blades (3-4 cm maximum width). The study has also been phenologically limited to the first part of the wet season. While I have visited Palo Verde during the dry season (January 2012), the only shelter-building caterpillars I looked for were hesperiids, which were not present. However, the phenological variation caused by the 2014-2015 drought suggests that additional shelter-building species might be detected at other times of year. Crambidae species complex 1 caterpillars were collected in late May and early June during the drought, but by that time in wet years they had already completed their development, suggesting that they are present as caterpillars only at the very beginning of the wet season. There may also be a few species that are active during parts of the dry season, as Elachistidae sp. 1 was found exclusively on *Bonellia nervosa*, a plant which normally bears leaves in the dry season and sheds them during the wet season (Chaves and Avalos 2008). *Bonellia* leaf phenology is controlled by light availability, which remained high during the drought, so the leaves, and presumably the caterpillars, persisted longer than normal. These observations suggest that if sampling were continued and extended, many more species of shelter-building caterpillars might be found at Palo Verde.

Third, while the diversity of shelters and shelter-building caterpillars at Palo Verde cannot be directly compared to other communities, some studies offer insights. The nearly forty-year-old caterpillar inventory in Area de Conservación Guanacaste (ACG), only approximately 60 km away from Palo Verde, is the obvious point of comparison for the Palo Verde shelter-building community. The publicly available data and publications from ACG are excellent resources for alpha taxonomy and species interactions, and include some information on shelter-building caterpillars as a whole (e.g. Janzen and Hallwachs 2009, Janzen et al. 2011), but they rarely

provide any shelter information for specific species. At least sixty-three percent ( $N = 3142$  species) of ACG caterpillar species are concealed feeders, although this estimate includes leaf miners and case builders as well as shelter builders (Janzen 1988). The most complete information available for ACG shelter-builders is a summary of the area's hesperiid caterpillar fauna. The ACG inventory has identified approximately 450 HesperIIDae species (Janzen et al. 2011), while this study encountered 25-27 hesperiids. All of Palo Verde hesperiids that have been identified to species also occur in ACG. Perhaps the most informative comparison is the observation that five Palo Verde non-hesperiid shelter-builders' DNA barcodes do not match barcodes from ACG (or anywhere else), indicating that either additional shelter-builders remain to be sampled at ACG or that some species occur at Palo Verde but not ACG, despite ACG's much larger size. The second possibility is supported by the fact that some of these caterpillars' host plants have not been sampled by the ACG project (Janzen and Hallwachs 2009).

Some studies from farther afield also offer insights on shelter-builders and their roles in the caterpillar community as a whole. A fifteen-year caterpillar project in the Brazilian cerrado found 338 species of shelter-building caterpillars (60% of all species) (Diniz et al. 2012). While there are no published data on the individual species or shelters found, shelter-building caterpillars were found year-round, and 60-80% of individuals collected each month were shelter-building caterpillars. A group in Papua New Guinea (Hreck et al. 2013) that reared 266 caterpillar species from over 39,000 individuals found that 58% of species and 84% of the individuals were "semi-concealed feeders" (both shelter-builders and portable case-makers). A brief caterpillar diversity field project at PVBS (Huval et al. 2014) found 34 species of free-feeding caterpillars (compared to 18 species of shelter-building caterpillars), but found nearly equal numbers of both caterpillar types.

The one community for which I have some detailed shelter information is the shelter-building caterpillar community of eastern Missouri forests (R. J. Marquis, pers. comm.). This community differs significantly from the Palo Verde community in at least three ways. First, there appear to be some differences in the number of species building certain types of shelters: in Missouri, leaf ties are relatively rare and occur mainly on one group of host plants (*Quercus*), while leaf rolls are more common than leaf ties and occur on a wider variety of plants. Second, there are some differences in the representation of shelter-building taxa: while many of the Palo Verde shelter-building species are pyralids and crambids, most of the Missouri forest shelter-builders are microlepidopterans. This may limit some types of shelter construction; for instance, all known Missouri leaf rollers only use expanding leaves, while the Palo Verde leaf rollers (including at least four crambids) can all use fully expanded leaves. Additionally, only a few hesperiids are present in the Missouri forest community, as most Missouri species utilize grasses or sedges. Third, while Palo Verde caterpillar shelters are occupied almost exclusively by their builders, caterpillar shelters in Missouri are commonly occupied by a wide range of arthropods, including herbivores, predators, and detritivores (Lill and Marquis 2003, Marquis and Lill 2006). Numerous dissimilarities between Palo Verde and eastern Missouri might result in different shelter-building caterpillar communities, and while it is too early to guess at their underlying causes, the existence of these differences is intriguing.

## CONCLUSIONS AND RECOMMENDATIONS

Using a systematic framework to assess the shelters built by a community of shelter-building caterpillars reveals several intriguing evolutionary and ecological patterns worthy of further investigation. First, while both general shelter-building and specific shelter types reoccur

throughout much of the lepidopteran phylogeny, shelter-building clades vary in their number of shelter types, from all five to only one or two. Studying a family that produces all five shelter types, such as the Crambidae, in further detail offers the chance to learn more about evolutionary transitions between different shelter types. Second, caterpillar size relative to leaf size affects how caterpillars build their shelters and what types of shelters they build. Many of the ontogenetic changes in shelter type occur when caterpillars become large enough to directly manipulate entire leaves, and caterpillars of different sizes relative to their leaves employ different strategies for constructing similar shelters. Given the importance of these phylogenetic and host plant contexts, shelter-building communities in different ecosystems likely vary dramatically in their shelters and in how those shelters affect the community as a whole.

To successfully investigate these patterns, more information about caterpillar shelters is needed. This information can be most effectively collected by rearing shelter-building caterpillars and documenting their shelters throughout development. Such rearing should occur in the most natural conditions possible, either protected on the host plant or on a spray of foliage maintained in water (or florist's foam, Abarca et al. [2014]) at a natural angle. Rearing conditions that crowd leaves together or close to the edges of the container will result in artefactual shelters, particularly from larger caterpillars. Given the frequency with which shelters vary according to caterpillar age, host plant species, and leaf-specific factors, observing many caterpillars under different conditions is highly recommended. Shelters should be documented both pictorially and with written descriptions. While images rarely capture all the relevant details of a shelter, it can also be difficult to describe a shelter in enough detail that it can be easily recognized from a description alone. Shelter descriptions should include shelter type, silk usage, any internal structures, and information about openness and frass accumulation. Any additional details, particularly observations about feeding or construction behavior, should also be included, as they provide context for the shelter. Complete shelter descriptions will be of use not only for caterpillar identification, but for better understanding their ecological and evolutionary roles.

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**Table 2.** Shelter traits for Palo Verde shelter-building caterpillars that build variable shelters, as well the causes of shelter variability.

For the identifications, “Unidentifiable” means that I was unable to preserve a caterpillar or adult associated with the shelter. “Undescribed” means that a species matches an established DNA barcode, but that the specimens corresponding to that barcode have not been described or given a single working name.

For full host plant names, see Appendix.

Shelter types: CF = cut-and fold, F = leaf fold, R = leaf roll, T = leaf tie, W = web

Unless otherwise specified, shelter openness and frass accumulation remain consistent throughout the different shelter types built by a species.

Reference Name	Family	Additional Identification Information	Ontogenetic Shelter Variation?	Host Species Variation? (# of spp.)	Leaf Variation? (# of caterpillars, if < 10)	Shelter Types	Shelters Open?	Shelters Accumulate Frass?	Pupal Shelters
<i>Achalarus toxeus</i>	Hesperiidae	<i>Achalarus toxeus</i>	Y	N (1)	N	CF => T	Y	N	T
<i>Antigonus erosus</i>	Hesperiidae	<i>Antigonus erosus</i>	Y	N (1)	Y	CF => F or T	Y	N	T
<i>Astrartes</i> sp. or spp.	Hesperiidae	<i>Astrartes</i> sp. or spp.	Y	N (1)	N (4)	CF => T	Y	N	T
<i>Calpodetes ethlius</i>	Hesperiidae	<i>Calpodetes ethlius</i>	Y	Y (2)	N	<i>Maranta</i> : CF => F <i>Thalia</i> : CF	Y	N	F
<i>Cephise aelius</i>	Hesperiidae	<i>Cephise aelius</i>	Y?	N (1)	Y? (4)	CF => T	Y	N	.
<i>Chioides catillus</i>	Hesperiidae	<i>Chioides catillus</i>	Y	N (1)	Y (4)	CF => F or T	Y	N	F or T
Crambidae sp. 1	Tentatively Crambidae	Not previously barcoded; 93% match to a crambid	Y	Y (3)	Y	<i>C. flexuosa</i> : CF (and 1 W) (mature leaves) <i>Q. indica</i> : F, T, or W (young leaves) => CF (mature leaves) <i>Q. odoratissima</i> : F, T, or W (young leaves) => CF or W	N, except for CF shelters on <i>Q. odoratissima</i>	Y	.
Crambidae sp. 3	Crambidae	Undescribed	Y	N (1)	N (4)	CF => W	Y	Y	.
<i>Desmia</i> sp.	Crambidae	<i>Desmia</i> sp.	Y	N (1)	N (1)	F => T	N	Y	T
<i>Desmia ufeus</i>	Crambidae	<i>Desmia ufeus</i>	Y	N (1)	N (1)	F => CF	Y	Y	CF

Reference Name	Family	Additional Identification Information	Ontogenetic Shelter Variation?	Host Species Variation? (# of spp.)	Leaf Variation? (# of caterpillars, if < 10)	Shelter Types	Shelters Open?	Shelters Accumulate Frass?	Pupal Shelters
<i>Diaphania</i> sp.	Crambidae	<i>Diaphania</i> Dapkey02	Y	N (1)	N	(occasionally W =>) F	W: Y F: N	Y	F
<i>Dichogama colotha</i>	Crambidae	<i>Dichogama colotha</i>	Y	N (3)	N	CF => T	N	Y	T
<i>Dichogama redentbacheri</i>	Crambidae	<i>Dichogama redentbacheri</i>	Y	N (1)	Y	CF => F or T	N	Y	.
<i>Epargyreus</i> sp.	Hesperiidae	<i>Epargyreus</i> sp.	Probably Y	N (1)	N (2)	(based on congeneric observations: CF =>) T	Y	N	.
<i>Eulepte concordalis</i>	Crambidae	<i>Eulepte concordalis</i>	Y	N (1)	Y	(if possible T =>) F	Y	Y	CF
Hesperiidae sp. 3	Hesperiidae	Unidentified	Y	N (1)	N (2)	CF => T	Y	N	.
Hesperiidae sp. 4	Hesperiidae	Unidentified	Y	N (1)	N (2)	CF => F	Y	N	.
<i>Latalvalva pseudosmithii</i>	Crambidae	<i>Latalvalva pseudosmithii</i>	N	Y (2)	N	<i>C. flexuosa</i> : W <i>Q. indica</i> : CF	N	Y	W
<i>Nyctelius nyctelius</i>	Hesperiidae	<i>Nyctelius nyctelius</i>	Y	N (1)	N (6)	CF => F	Y	N	.
<i>Psara obscuralis</i>	Crambidae	<i>Psara obscuralis</i>	N	N (1)	Y	F or T (or less commonly, W)	All: Y	F and T: N W: Y	.
<i>Rhinthon cubana</i>	Hesperiidae	<i>Rhinthon cubana</i> (DHJ02)	Probably Y	N (1)	N (1)	CF => ?	Y	N	.
<i>Rhinthon osca</i>	Hesperiidae	<i>Rhinthon osca</i>	Y	N (1)	N (2)	CF => F	Y	N	CF
<i>Syllepte amando</i>	Crambidae	<i>Syllepte amando</i>	N	N (1)	Y (1)	F or T	Y	N	.
<i>Timochares trifasciata</i>	Hesperiidae	<i>Timochares trifasciata</i>	Y	N (1)	Y	CF => F or T	Y	N	.
<i>Urbanus dorantes</i>	Hesperiidae	<i>Urbanus dorantes</i>	Y	N (3)	Y	F on vertical leaves or CF => F or T	Y	N	F or T
<i>Urbanus proteus</i>	Hesperiidae	<i>Urbanus proteus</i>	Y	N (4)	Y	CF => F or T	Y	N	F or T
<i>Xenophanes tryxus</i>	Hesperiidae	<i>Xenophanes tryxus</i>	Y	N (3)	N	CF => F	Y	N	CF or F

**Table 3.** Shelter traits for Palo Verde shelter-building caterpillars that build only one type of larval shelter.

For the identifications, “Unidentifiable” means that I was unable to preserve a caterpillar or adult associated with the shelter. “Undescribed” means that a species matches an established DNA barcode, but that the specimens corresponding to that barcode have not been described or given a single working name.

For Multiple Shelter Types, “UNK” means that whether the species builds multiple shelter types is unknown, because not all instars were observed.

Shelter types: CF = cut-and fold, F = leaf fold, R = leaf roll, T = leaf tie, W = web

Reference Name	Family	Additional Identification Information	Multiple Shelter Types?	Shelter Type	Shelter Open?	Shelter Accumulates Frass?	Pupal Shelters
<i>Amorbia concavana</i>	Tortricidae	<i>Amorbia concavana</i>	UNK	UNK	UNK	UNK	F
<i>Anaea aidea</i>	Nymphalidae	<i>Anaea aidea</i>	N	F	Y	N	N
<i>Aristotelia corallina</i>	Gelechiidae	Member of the <i>Aristotelia corallina</i> species complex	N	W	N	N	Domatium (if not available, pupates in W)
<i>Aristotelia</i> sp.	Gelechiidae	<i>Aristotelia</i> BioLep345	N	W	Y	N	.
<i>Conchylodes plantinalis</i>	Crambidae	<i>Conchylodes plantinalis</i>	N	N	W	Y	CF
<i>Conchylodes salamisalis</i>	Crambidae	<i>Conchylodes salamisalis</i>	N	W	Y	Y	CF
Crambidae sp. 2	Crambidae	<i>Phaedropsis</i> Janzen10	N	R	Y	Y	CF
Crambidae sp. 4	Crambidae	97% match to <i>Conchylodes salamisalis</i>	UNK	W	Y	Y	.
Crambidae species complex 1a	Crambidae	<i>Chilochromopsis sceletogramma</i>	N	W	Y	N	W
Crambidae species complex 1b	Crambidae	<i>Pilocrocis calamistis</i>	N	W	Y	N	W
Crambidae species complex 1c	Crambidae	<i>Syllepte belialis</i>	N	W	Y	N	W
Elachistidae sp. 1	Elachistidae	elachJanzen01 Janzen737	N	W	N	Y	.
<i>Ethmia catapeltica</i>	Depressariidae	<i>Ethmia catapeltica</i>	N	W	Y	N	W
Gelechiidae sp. 1	Tentatively Gelechiidae	Undescribed	N	W	N	Y	.

Reference Name	Family	Additional Identification Information	Multiple Shelter Types?	Shelter Type	Shelter Open?	Shelter Accumulates Frass?	Pupal Shelters
Gelechiidae sp. 2	Gelechiidae	Undescribed	N	R	Y	N	.
Gelechiidae sp. 3	Gelechiidae	Unidentified	N	T	N	Y	.
Hesperiidae sp. 1	Hesperiidae	unidentified Hesperinae	UNK	CF or F	Y	N	
Hesperiidae sp. 2	Hesperiidae	unidentified Hesperinae	UNK	CF	Y	N	.
Hesperiidae sp. 5	Hesperiidae	Unidentified	UNK	T	UNK	Y	.
Hesperiidae sp. 6	Hesperiidae	Unidentified	UNK	CF	Y	N	.
Hesperiidae sp. 7	Hesperiidae	Unidentified	UNK	F	Y	N	F
<i>Hyblaea puera</i>	Hyblacidae	<i>Hyblaea puera</i>	Y	W	Y	Y	CF
<i>Lerema liris</i>	Hesperiidae	<i>Lerema liris</i>	UNK	CF	Y	N	.
<i>Massepha grammalis</i>	Crambidae	<i>Massepha grammalis</i> DHJ02	N	F	N	Y	F or CF
<i>Megalota</i> sp.	Tortricidae	<i>Megalota</i> sp.	N	W	N	Y	.
Noctuidae sp. 1	Noctuidae	Not previously barcoded, 93% match to a noctuid	N	T	Y	N	.
<i>Palpusia</i> sp.	Crambidae	<i>Palpusia</i> sp. Solis25	N	R	N	Y	CF
<i>Platynota subargentea</i>	Tortricidae	<i>Platynota subargentea</i>	N	W	Y	Y	W
<i>Pococera sabbasa</i>	Pyralidae	<i>Pococera sabbasa</i> Janzen02	N	W	N	Y	.
Pyralidae sp. 1	Pyralidae	epipaJanzen01 Janzen15DHJ02	N	W	Y	Y	.
Pyralidae sp. 2	Pyralidae	chryJanzen01 Janzen347	N	F	Y	N	F
Pyralidae sp. 3	Pyralidae	phyBioLep01 BioLep774	N	W	Y	Y	.
<i>Spathilepia clonius</i>	Hesperiidae	<i>Spathilepia clonius</i>	UNK	CF	Y	N	.
<i>Stenoma</i> sp.	Depressariidae	<i>Stenoma</i> BioLep60	UNK	W	Y	Y	W
<i>Syllepis hortalist</i>	Crambidae	<i>Syllepis hortalist</i> DHJ01	N	R	Y	Y	R
<i>Symphysa lepidaria</i>	Crambidae	<i>Symphysa lepidaria</i>	N	W	Y	N	.
Unidentifiable sp. 1	Unidentifiable	Unidentifiable	UNK	F	N	Y	.

Reference Name	Family	Additional Identification Information	Multiple Shelter Types?	Shelter Type	Shelter Open?	Shelter Accumulates Frass?	Pupal Shelters
Unidentifiable sp. 2	Unidentifiable	Unidentifiable	UNK	F	Y	N	.
Unidentifiable sp. 3	Unidentifiable	Unidentifiable	UNK	W	Y	Y	.
Unidentifiable sp. 4	Unidentified	Unidentified	N	W	Y	N	.
Unidentifiable sp. 5	Unidentifiable	Unidentifiable	UNK	W	N	Y	.
Unidentifiable sp. 6	Unidentifiable	Unidentifiable	UNK	W	Y	N	.
Unidentifiable sp. 7	Unidentifiable	Unidentifiable	UNK	W	Y	N	.
Unknown sp. 1	Gelechiidae/Elachistidae	Undescribed	N	W	N	N	W
Unknown sp. 10	Unidentified	Unidentified	N	F	Y	N	.
Unknown sp. 11	Unidentified	Unidentified	UNK	W	N	Y	.
Unknown sp. 12	Unidentified	Unidentified	UNK	F	N	Y	.
Unknown sp. 13	Unidentified	Unidentified	UNK	W	Y	Y	.
Unknown sp. 14	Unidentified	Unidentified	UNK	W	Y	Y	.
Unknown sp. 15	Unidentified	Unidentified	UNK	T	Y	Y	.
Unknown sp. 16	Unidentified	Unidentified	N	F	Y	N	CF
Unknown sp. 17	Unidentified	Unidentified	UNK	W	Y	N	.
Unknown sp. 18	Unidentified	Unidentified	UNK	W	Y	Y	.
Unknown sp. 19	Unidentified	Unidentified	N	R	Y	Y	CF
Unknown sp. 2	Unidentified	Unidentified	N	T	Y	Y	.
Unknown sp. 20	Unidentified	Unidentified	N	W	.	.	.
Unknown sp. 21	Unidentified	Unidentified	UNK	R	Y	UNK	.
Unknown sp. 22	Unidentified	Unidentified	UNK	R	Y	Y	.
Unknown sp. 23	Unidentified	Unidentified	UNK	R	UNK	UNK	.
Unknown sp. 24	Unidentified	Unidentified	UNK	R	UNK	UNK	.
Unknown sp. 25	Unidentified	Unidentified	UNK	W	Y	UNK	.
Unknown sp. 3	Unidentified	Unidentified	UNK	F	Y	N	UNK



Reference Name	Family	Additional Identification Information	Multiple Shelter Types?	Shelter Type	Shelter Open?	Shelter Accumulates Frass?	Pupal Shelters
Unknown sp. 4	Unidentified	Unidentified	UNK	W	Y	Y	.
Unknown sp. 5	Unidentified	Unidentified	N	F	Y	Y	CF
Unknown sp. 6	Unidentified	Unidentified	UNK	F	Y	Y	.
Unknown sp. 7	Unidentified	Unidentified	UNK	UNK	UNK	UNK	F
Unknown sp. 8	Unidentified	Unidentified	UNK	W	N	Y	.
Unknown sp. 9	Unidentified	Unidentified	N	T	Y	Y	.
<i>Urbanus esta</i>	Hesperiidae	<i>Urbanus esta</i>	UNK	CF	Y	N	.
<i>Urbanus evona</i>	Hesperiidae	<i>Urbanus evona</i>	UNK	CF	Y	N	.

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## CHAPTER 2: Shelter building and extrafloral nectar exploitation by a member of the *Aristotelia corallina* species complex (Gelechiidae) on Costa Rican acacias

As accepted by *The Journal of the Lepidopterists' Society*

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### ABSTRACT

A new member of the unresolved *Aristotelia corallina* Walsingham species complex (Gelechiidae) from a Costa Rican dry forest (Parque Nacional Palo Verde) is reported. Its larval behavior and possible relationships to the rest of the complex are described and analyzed. Field and laboratory observations revealed that these caterpillars consumed the extrafloral nectar, Beltian bodies, and young foliage of two acacia species (*Vachellia collinsii* and *Vachellia farnesiana*; Fabaceae: Mimosoideae). The caterpillars were observed building individual silk webs surrounding a single stem node's thorns and leaf rachises. If open domatia are included in shelters, the caterpillars reside in the domatia between feeding bouts and during pupation. These caterpillars are the only non-myrmecophilous caterpillars known to regularly exploit a host plant's ant rewards. DNA barcoding of the Costa Rican specimens showed that they are significantly different from the United States members of *A. corallina* (12-13% difference in *cox1* sequence). An examination of host plant herbarium material for caterpillar shelters demonstrated likely differences in host plant use, with webs consistent with *A. corallina* only found on specimens of United States *Chamaecrista nictitans* (Fabaceae: Caesalpiniodeae), but not *Vachellia farnesiana*. By contrast, webs were found on *V. collinsii*, *V. cornigera*, and *V. farnesiana* from Central America (including Mexico), but not on Central American *C. nictitans*. This ecological evidence suggests that the Costa Rican populations of *A. corallina* may be more closely related to the Mexican populations than to the US populations.

Additional keywords: natural history; DNA barcoding; host plant; behavior

### INTRODUCTION

The species *Aristotelia corallina* (Gelechiidae) was first described by Walsingham in 1909 from three specimens collected in Guerrero, Mexico. Since then, adults have been reported from the southern United States (Kimball 1965, Hebbner et al. 2003, Moth Photographers Group. 2016), and Puerto Rico (Moth Photographers Group 2016), and caterpillars have been found in Florida (Kimball 1965) and Mexico (Janzen 1967). Unfortunately, *Aristotelia corallina* has never been given a modern taxonomic treatment and recent DNA barcoding work has identified at least three distinct clades within the United States and Costa Rican specimens identified as *A. corallina* (Barcode of Life Data System [Ratnasingham & Hebert 2007], unpublished data), suggesting it is an unresolved species complex.

Little is known of *A. corallina* biology or ecology beyond a few host plant records. There are two records of caterpillars feeding on *Chamaecrista nictitans* (L.) Moench (Fabaceae: Caesalpiniodeae; Kimball 1965, Hebbner et al. 2003) in Florida and one record on *Vachellia cornigera* (L.) Seigler & Ebinger (Fabaceae: Mimosoideae; Janzen 1967) in Mexico. There is also a host plant database record for *V. farnesiana* (L.) Wight & Arn., but the original source has been elusive (Robinson et al. 2017). Here, another cryptic species within the *A. corallina* complex is reported, including description of its exploitation of acacias' ant adaptations and its shelter-building behavior, and comparison to the other members of the *A. corallina* species complex.

To better understand these caterpillars, field and laboratory observations of over 40 individuals were collected, including video recordings of their shelter-building efforts. These observations were then supplemented with traditional natural history records, molecular data, and herbarium documentation of plant-insect interactions to understand how these caterpillars compare to the other members of the *Aristotelia corallina* species complex.

## METHODS

**Field and laboratory observations.** The caterpillars were first found in May 2015 in the tropical dry forest at Palo Verde Biological Station (Parque Nacional Palo Verde, Guanacaste, Costa Rica). The caterpillars occurred on both *Vachellia collinsii* (Saff.) Seigler & Ebinger and *V. farnesiana* (L.) Wight & Arn. In May-August 2015 and 2016, caterpillar shelters were found, marked, and checked each morning for up to seven days. Caterpillars and their shelters were then collected and reared in the lab for adults and parasitoids. Each acacia stem was placed in a water-filled florist's tube and kept in a plastic bag, while caterpillars were checked daily. The length, width, and height of shelters were also measured.

**Shelter-building.** To observe shelter construction, a freshly cut *Vachellia collinsii* stem with expanding foliage was placed in a water-filled florist's tube, inside a beaker. A caterpillar collected from *V. collinsii* was removed from its shelter, placed on the new foliage, and its behavior was recorded with a digital camera (Nikon D3200, Tokyo, Japan). For ease of handling and observation, all but one of the caterpillars used were in the final instar, but the shelters they built did not appear structurally different from the shelters of younger caterpillars.

**Specimens.** Adults and various immature stages were preserved. One or two legs were removed from each adult specimen and stored in microcentrifuge tubes for DNA samples before adults were pinned, spread, and dried in a drying oven. Immatures and exuviae were preserved in 95% ethanol. In 2017, caterpillar head capsules were examined to determine if the ingestion of nectar was associated with any unique mouthpart morphology. Voucher specimens will be deposited at the Smithsonian National Museum of Natural History (Washington D.C., USA) and the Museo Nacional de Costa Rica (San José, Costa Rica). One immature specimen is in the collection of Steven Passoa (USDA-APHIS-PPQ).

**Identification.** Upon return from the field in 2015, specimens were identified using both morphological and molecular methods. A series of adults were sent to a gelechiid specialist (Sangmi Lee, Arizona State University) and she dissected the male genitalia. To extract DNA, tissue samples were frozen with liquid nitrogen and ground with plastic pestles before using Macherey-Nagel Tissue kits (Macherey-Nagel GmbH & Co. KG, Düren, Germany). The mitochondrial cytochrome c oxidase subunit 1 (*cox1*) barcoding region was amplified using the

LCO1490 and HCO2198 primers designed by Folmer et al. (1994). Reaction conditions followed Levin and Parker (2013), except that 3 ul of template DNA were used for each 25 uL reaction because initial DNA concentrations were relatively low (~1-10 ng/ul). Amplicon length and concentration were checked using gel electrophoresis and UV spectrophotometry, respectively. Amplicons were then cleaned using a mixture of exonuclease I and antarctic phosphatase (#M0289S and #M0293S, New England Bio Labs Inc., Ipswich, MA), diluted to the appropriate concentrations, and sent to an outside company for sequencing (Beckmann Coulter Genomics in 2015). Sequences were then aligned using Geneious (Geneious 10, Biomatters Ltd.) and GenBank was searched for matching sequences using BLAST.

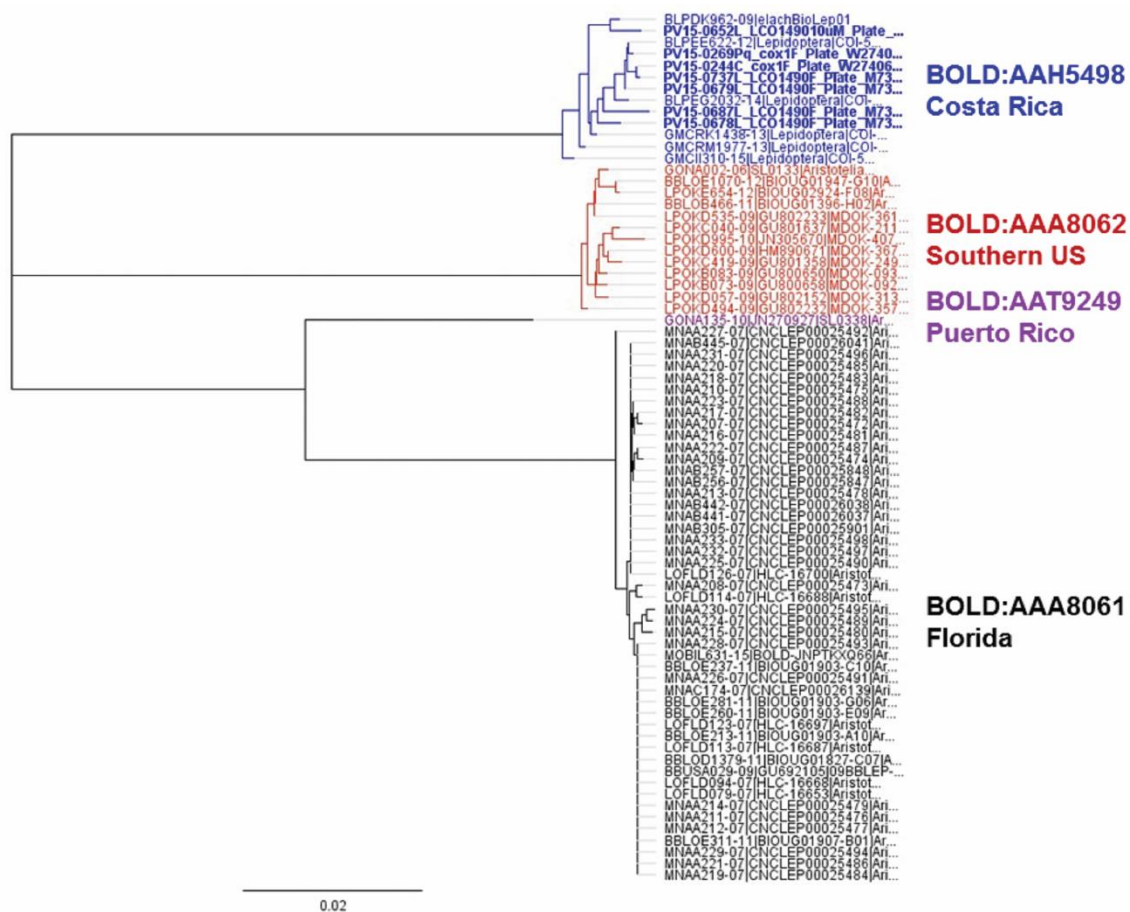
**Herbarium search.** To obtain more information on *A. corallina* host plants, plant specimens from the Missouri Botanical Garden herbarium (MO) were examined. Caterpillar shelters can be inadvertently preserved on herbarium specimens, particularly when many leaves are included on a herbarium sheet (i.e., the plant and/or the leaves are small). However, collectors may deliberately avoid damaged plants or collect specimens when caterpillar shelters are rare. For instance, if the caterpillars are most abundant when host plants are sterile, their shelters are unlikely to be sampled by collectors seeking fertile specimens. Shelters can also be damaged, discarded, or hidden during collection and mounting. Therefore, a major caveat of this method is that an absence of caterpillars or caterpillar shelters is not definitive proof that a plant species is not a host plant.

Accordingly, the United States, Central American (Mexico-Costa Rica), and Caribbean material of *Chamaecrista nictitans* and *Vachellia farnesiana* was examined, as well as the Central American material for *V. collinsii* and *V. cornigera*. Possible signs of *A. corallina* presence included silk webs or their remains on stems, EFNs, and/or domatia, small frass pellets and/or plant damage, and caterpillars or caterpillar exuviae of appropriate size and appearance. Specimens with possible *A. corallina* signs were photographed and annotated in the TROPICOS database (MBG 2017; see Table 1 for a full list of the specimens).

## RESULTS

**Identification.** Based on the genitalia dissection, the adults were identified as *Aristotelia corallina* Walsingham, 1909 (S. Lee, pers. comm.). However, DNA barcoding shows that this identification is incomplete (Fig. 1). The BLAST search matched the DNA barcodes of the new specimens to adult moths collected in Costa Rica's Area de Conservación Guanacaste and sequenced by Janzen and colleagues (<1% difference). These moths were only identified as barcode index number (BIN) BOLD:AAH5498 in the Barcode of Life Data System (BOLD). When these Costa Rican barcodes were compared to BOLD barcodes from 63 North American *A. corallina* specimens, they differed by 12-13%. Two sequences are generally considered to represent different species if they differ by more than 2% (Ratnasingham & Hebert 2007). Moreover, the barcodes in BOLD identified as *A. corallina* form three BINs (BOLD:AAA8061, BOLD:AAA8062, and BOLD:AAT9249) that correspond to their collection locations (Florida, the southern US, and Puerto Rico, respectively). Unfortunately, the type specimen of *A. corallina* originated from Guerrero, Mexico, and no Mexican specimens have been barcoded. As a result, it is currently impossible to determine with available barcodes which, if any, of these cryptic species is the true *A. corallina*.

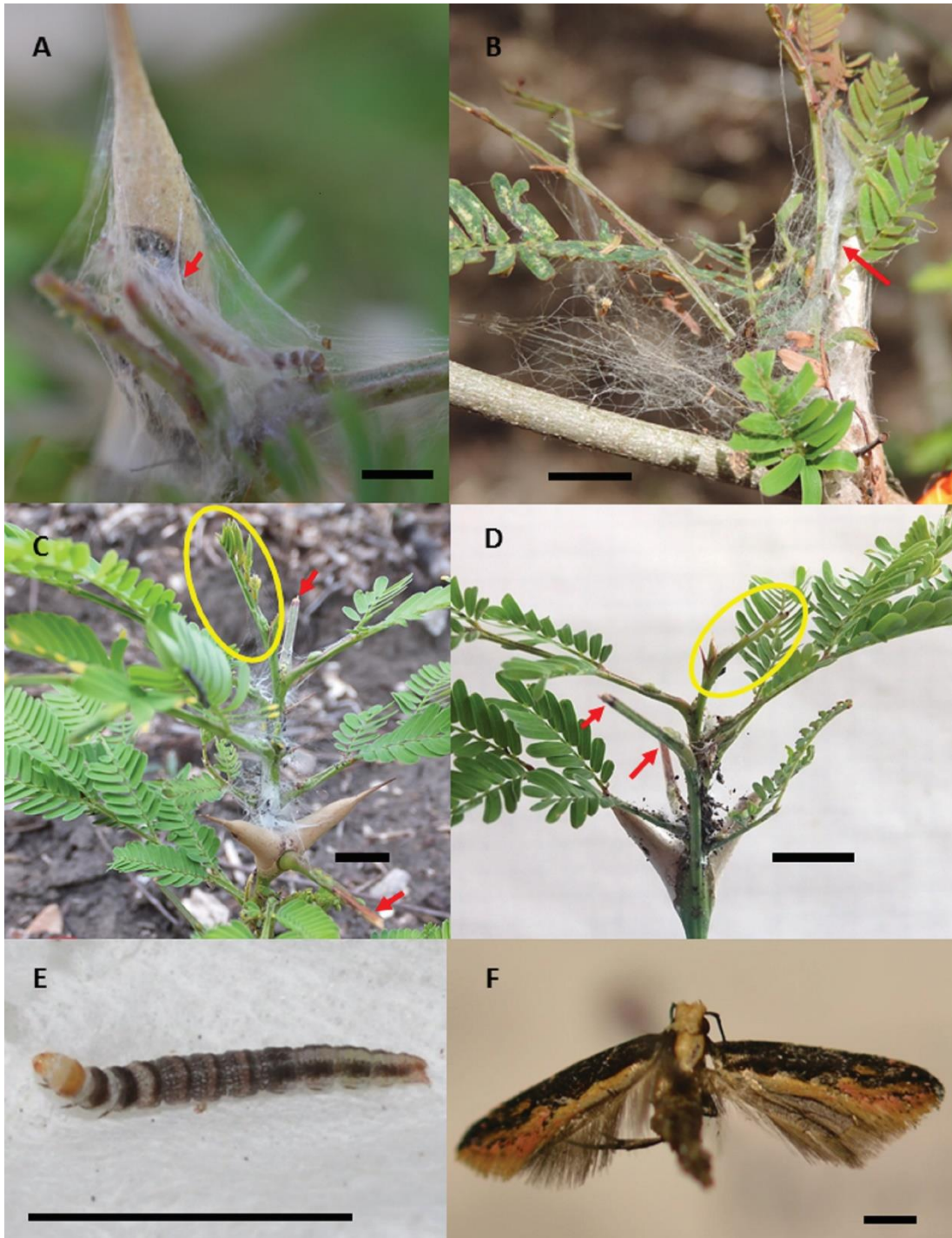




**Figure 1.** A neighbor-joining tree of *cox1* barcode sequences for members of the *Aristotelia corallina* species complex. The different colors indicate different BINs and the bolded specimen names indicate barcodes from this study. Branch lengths are proportional to sequence differences.

**Specimens and morphology.** The caterpillar (Fig. 2e). Unknown number of instars; the final instar is ~15 mm long. *Head:* Light brown, with a lighter band across the middle. The front of the hypopharyngeal complex is dark brown. Examination of a slide-mounted head capsule revealed normal mouthparts with sharp mandibles probably used for scraping (S. Passoa, pers. comm.). *Thorax:* Coloration of alternating dark maroon and white rings. *Abdomen:* Anterior portion is dark maroon with three thin dark rings. Posterior portion has broad horizontal maroon and white stripes.

The adult (Fig. 2f). *Head:* Light yellow, with dark grey antennae. *Thorax:* Same color as the head, with yellow- and grey-striped legs and dark grey scales over the base of the wings. Forewing length ~5 mm. Forewing elongate and narrowly triangular, apex slightly curved and fringed, and margin straight. Dorsum ground color dark grey with light yellow shading to pink along the anal margin of the wing. Distal third of forewing speckled with yellow and pink spots. Ventrums dark grey. Hindwing triangular with a fringe approximately as broad as the rest of the hindwing. Dorsum greyish brown. Ventrums dark grey. *Abdomen:* Dark grey.



**Figure 2.** **a.** Costa Rican *Aristotelia corallina* shelter, including the silk tunnel (red arrow) connecting the domatium entrance to the EFN and the caterpillar drinking EFN nectar. Scale bar = 1 mm. **b.** A shelter with an extended silk tunnel built on *Vachellia farnesiana*, which does not have domatia. Scale bar = 10 mm. **c and d.** The same shelter photographed five days apart, showing caterpillar damage to an expanding leaf (yellow circle) and past damage to now-mature leaves (red arrows). Note that the shelter was rotated 180° from its original orientation in d. Scale bar in both photos = 10 mm. **e.** Costa Rican *A. corallina* caterpillar. Scale bar = 5 mm. **f.** Adult. Scale bar = 1 mm.

**Field and laboratory observations.** During the 2015 and 2016 field seasons, it became apparent that the caterpillars exploited the acacias' ant adaptations in a variety of ways. Each caterpillar occupied its own shelter (Fig. 2a), a small web (mean dimensions 25 x 15 x 15 mm,  $N = 37$ ) surrounding a stem node. A web covered all expanding leaves at that node, but only reached the rachises of mature leaves. Caterpillars were frequently seen consuming nectar from the extrafloral nectaries (EFNs, Fig. 2a), but were not seen eating the foliage itself. However, shelter photographs taken days apart showed that young caterpillars were also consuming expanding foliage and Beltian bodies (Figs. 2c and 2d, Video 1). Specifically, pinnae and associated Beltian bodies (nutrient-rich bodies on the tips of expanding pinnules, Belt 1874) were stripped from leaf rachises, or rachises were chewed off just above the EFNs. Less commonly, the shoot tip itself was chewed, or the expanding foliage was intact but yellowed and wilted. Chewing damage was only associated with early instars: in cases when a caterpillar built a second shelter, expanding foliage in or near that shelter remained intact and developed normally.

The caterpillars also exploited the acacia domatia. When opened domatia were available, the caterpillars took refuge in them and often incorporated the entrance holes into their shelter architecture (Fig. 2a). Before pupation, caterpillars entered domatia, sealed the entrance holes with silk, and pupated inside (Video 2). In some cases, caterpillars travelled one or two nodes away from their shelters to find an open domatium for pupation. Occasionally, no domatium was available, either because none had been opened by ants or because the caterpillar was on *V. farnesiana*, which only produces small defensive thorns (Zamora 2010). In these cases, the caterpillar pupated within its web.

The caterpillars' relationship with the ants themselves appears to be more complex. Caterpillars were only found on plants unoccupied by aggressive acacia ants (at Palo Verde, these are primarily *Pseudomyrmex flavicornis* F. Smith and *P. spinicola* Emery [Hymenoptera: Formicidae]). While non-specialist ants visited many of the host plants, they did not interact with the caterpillars. As the ants sometimes walked over the webs or fed at an EFN used by a caterpillar, it seems likely that the ants were exposed to caterpillar cues but did not respond to them. By contrast, Janzen (1967) reported that when a Mexican *Aristotelia corallina* caterpillar shelter was discovered by specialist acacia ants (*P. ferrugineus* F. Smith), the shelter was quickly disassembled and the caterpillar killed.

Observations during laboratory rearing provided further evidence that late instar caterpillars were not feeding directly on acacia foliage. Although many caterpillars pupated within a few days of collection, several caterpillars were reared for long periods (7-18 days) before successfully pupating. During rearing, no loss of leaf area was observed, no fresh foliage was added, and very little frass was produced by the caterpillars.

**Shelter-building.** Four successful shelter-building observations revealed a consistent pattern (Video 3 shows an example). The caterpillar began by positioning itself on the domatium or stem and repeatedly stringing single strands of silk between anchor points (the stem, the rachis, and the thorns) by moving its head and thorax back and forth while its abdomen remained anchored to the substrate. Most strands were placed between adjacent anchors, forming a rough diamond, but some silk was placed along the diagonals. As building continued, strands were also run from anchor points to existing silk strands. Once there were enough intersecting strands to bear the caterpillar's weight, the larva shifted to hanging upside-down on the silk. The caterpillar then built up both the exterior and the interior of the web. These reinforcements led to the caterpillar hanging from a relatively solid silk platform. At this point (~20-30 min), the basic structure of the

web was established, and the caterpillar rested for several hours. The shelter was then completed during one or more additional building bouts (these occurred overnight, so the exact timing is unknown), with the caterpillar extending the outer webbing to the EFNs and completely enclosing the silk platform to form a tunnel.

Depending on foliage architecture, there was some variation in shelter construction. If there was an entry hole on the upper surfaces of the domatium, one end of the silk tunnel connected to the hole (Fig. 2a). If there was no conveniently located entry hole, the silk tunnel itself was used as a retreat. This was always true for shelters built on *V. farnesiana*, as this acacia species does not produce domatia. Instead, these shelters had a long silk tunnel running along the stem (Fig. 2b).

Unlike the other caterpillar species observed at Palo Verde, these caterpillars do not usually build additional shelters or significantly expand the original one. Only four unmanipulated caterpillars out of 33 built a second shelter and no unmanipulated caterpillar built more than two. This also supports the hypothesis that older caterpillars feed on a renewable resource (EFN).

Parasitism status may affect shelter-building behavior. During one shelter-building observation, the caterpillar built a simple web in the foliage, rather than the complex node-centered shelter described above. Two days later, a parasitoid larva emerged from the caterpillar and pupated in the web.

**Herbarium search.** Evidence suggestive of *A. corallina* was found on 2 of 33 United States *C. nictitans* specimens, but no webs were found on any of the 82 Central American *C. nictitans* specimens. Only one specimen of Caribbean (Puerto Rican) *C. nictitans* was present in the herbarium. None of the 12 United States *V. farnesiana* specimens showed any evidence of *A. corallina*. *A. corallina* evidence was most abundant on Central American *Vachellia farnesiana* specimens, with 14 of 221 specimens showing webs. One Mexican specimen even had a clearly preserved *A. corallina* caterpillar in a flattened web (Fig. 3). Eight of 35 Caribbean *V. farnesiana* specimens, all from Puerto Rico, had possible *A. corallina* webs. Five of 239 Central American *V. collinsii* specimens had webs, as did four of 110 Central American *V. cornigera* specimens. For a full list of specimens with apparent *A. corallina* webs and caterpillars, see Table 1.



**Figure 3.** *Aristotelia corallina* caterpillar and web on a herbarium specimen of *Vachellia farnesiana* (MO accession # 3118531, Esteban #8). The minor intervals on the ruler are millimeters.



**Table 1.** MO herbarium specimens bearing *A. corallina* material.

Specimen (MO Accession #)	Plant species	Locality
1779399	<i>Chamaecrista nictitans</i>	Mississippi, USA
3301402	<i>Chamaecrista nictitans</i>	North Carolina, USA
4216684	<i>Vachellia collinsii</i>	Cortes, Honduras
6610946	<i>Vachellia collinsii</i>	Guanacaste, Costa Rica
2998513	<i>Vachellia collinsii</i>	Matagalpa, Nicaragua
3136502	<i>Vachellia collinsii</i>	Leon, Nicaragua
3519751	<i>Vachellia collinsii</i>	Boaco, Nicaragua
2266399	<i>Vachellia cornigera</i>	Oaxaca, Mexico
4355797	<i>Vachellia cornigera</i>	Guerrero, Mexico
2922862	<i>Vachellia cornigera</i>	Veracruz, Mexico
6182893	<i>Vachellia cornigera</i>	Guanacaste, Costa Rica
3118531	<i>Vachellia farnesiana</i>	Morelos, Mexico
4216698	<i>Vachellia farnesiana</i>	Campeche, Mexico
4216698	<i>Vachellia farnesiana</i>	Campeche, Mexico
5337028	<i>Vachellia farnesiana</i>	Puerto Rico, USA
5337015	<i>Vachellia farnesiana</i>	Puerto Rico, USA
5337018	<i>Vachellia farnesiana</i>	Puerto Rico, USA
5337029	<i>Vachellia farnesiana</i>	Puerto Rico, USA
5337031	<i>Vachellia farnesiana</i>	Puerto Rico, USA
5290697	<i>Vachellia farnesiana</i>	Puerto Rico, USA
5337004	<i>Vachellia farnesiana</i>	Puerto Rico, USA
5337012	<i>Vachellia farnesiana</i>	Puerto Rico, USA
5616395	<i>Vachellia farnesiana</i>	Guanajuato, Mexico
5616394	<i>Vachellia farnesiana</i>	Guanajuato, Mexico
1991068	<i>Vachellia farnesiana</i>	Sinaloa, Mexico
4355764	<i>Vachellia farnesiana</i>	Fco. Morazan, Honduras
2635459	<i>Vachellia farnesiana</i>	Tamaulipas, Mexico
6291715	<i>Vachellia farnesiana</i>	Guatemala
1842789	<i>Vachellia farnesiana</i>	Yucatan, Mexico
3517336	<i>Vachellia farnesiana</i>	Esteli, Nicaragua
3518055	<i>Vachellia farnesiana</i>	Matagalpa, Nicaragua
3519568	<i>Vachellia farnesiana</i>	Leon, Nicaragua
3151495	<i>Vachellia farnesiana</i>	Matagalpa, Nicaragua

## DISCUSSION

During my field research in a Costa Rican tropical dry forest, a species of shelter-building gelechiid caterpillar was observed to exploit the domatia, EFN nectar and Beltian bodies acacias provide for their ant defenders. The caterpillars also build their silk webs by repetitively stringing silk strands between anchor points, but this apparently simple method is flexible enough to accommodate variations in plant architecture, such as the proximity of a domatium hole or EFN. The study species was identified morphologically as *Aristotelia corallina* Walsingham and genetically as a fourth member of the cryptic species complex currently represented by this name (BOLD:AAH5498). A search of known host plant species in the Missouri Botanical Garden

herbarium revealed geographic variation in host plant use, as well as additional locations where *A. corallina* likely occurs.

Costa Rican BOLD:AAH5498 *Aristotelia corallina* caterpillars are not the only caterpillars to consume EFN nectar, but they differ in two respects from previously described nectar-drinking caterpillars. First, all other known nectar-drinking caterpillars have close relationships with ants, that is, they are myrmecophilous. Many myrmecophilous riodinid caterpillars consume EFN nectar to supplement other food sources, including leaf tissue (DeVries and Baker 1989, DeVries 1997), floral parts (DeVries et al. 1992, Wagner & del Rio 1997), or membracids and/or their honeydew secretions (DeVries et al. 1992, DeVries 2000). Although several Australian lycaenid caterpillars have been observed drinking EFN nectar (N. E. Pierce, pers. comm.), I have been unable to find any published observations. Past reviews have cited three articles (Horvitz & Schemske 1984, Maschwitz et al. 1984, Pierce & Elgar 1985), but while they report lycaenid caterpillars near EFNs or food bodies, they do not contain direct observations of caterpillars consuming these resources. Belcher et al. (1983) reported that *Heliothis virescens* Fabricius (Lepidoptera: Noctuidae) caterpillars preferentially consumed the tissue of cotton EFNs, but found that the caterpillars were not attracted to the nectar itself. I have not found any reports of non-myrmecophilous caterpillars consistently consuming EFN nectar. This may simply mean that caterpillars that successfully consume EFN nectar and avoid ants are small and inconspicuous, or it may mean that the strategy is truly rare.

Second, while the caterpillars do feed on emerging foliage when young, they can successfully pupate after consuming EFN nectar for over two weeks, and the late instars may consume only nectar and Beltian bodies. This raises the question of how the caterpillars can fulfill all their dietary needs. While EFN nectar, especially from obligate myrmecophytes, contains relatively high concentrations of free amino acids in addition to sugars, the caterpillars must still successfully digest these nutrients (González-Teuber & Heil 2009, Shenoy et al. 2012). Research on specialist acacia ants and acacia rewards has shown that acacias generally “lock” their rewards by packaging nutrients in forms that require specific enzymes to digest them (Heil 2015). In the case of EFN nectar, acacias can make it unappealing to generalists by secreting EFN nectar with unusual sugar compositions (Heil et al. 2005). Beltian bodies are highly nutritious, but acacias also lock them by including enzymes that inactivate generalists’ digestive enzymes (Orona-Tamayo et al. 2013). This suggests that these Costa Rican BOLD:AAH5498 caterpillars must have one or more specific adaptations for feeding on acacia rewards, particularly if they successfully digest Beltian bodies.

The Costa Rican BOLD:AAH5498 caterpillars’ natural history is broadly similar to what is known for the Mexican *A. corallina* caterpillars. Janzen (1967) reported *A. corallina* caterpillars in Veracruz and Oaxaca, Mexico. These caterpillars spun webs on *Vachellia cornigera* shoots and emerged from them at night to feed on the shoot tips, often damaging them and preventing or slowing plant growth. This behavior seems consistent with the damage caused by the early instar Costa Rican BOLD:AAH5498 caterpillars, and nocturnal feeding would explain why folivory was never directly observed. No mention was made of caterpillars feeding at EFNs or consuming Beltian bodies, but since the EFNs would have been inside the shelters, visits to them may have been missed. Janzen’s surveys of other local mimosoid legumes, which included *V. macracantha* (Humb. & Bonpl. Ex Willd.) Seigler & Ebinger and *V. chiapensis* (Saff.) Seigler & Ebinger but not *V. collinsii* or *V. farnesiana*, did not find *A. corallina* caterpillars on any species but *V.*

*cornigera*. *V. cornigera* occurs at Palo Verde, but no *A. corallina* caterpillars were found on them, possibly because the plants were always occupied by acacia ants.

Herbarium specimens of *Vachellia farnesiana*, *V. collinsii*, and *V. cornigera* from Central America suggest that *A. corallina* caterpillars can be found throughout Central American dry forests, with webs found on plants collected in Mexico, Guatemala, Honduras, Nicaragua, and Costa Rica. One such specimen of *V. cornigera* (Janzen #1980) was collected in 1964 from Oaxaca, Mexico, apparently during the research described in Janzen (1967). Another *V. cornigera* specimen (Gentry #32254) had silk in a domatium entrance hole, suggesting that the Mexican caterpillars may also retreat into domatia. While many of the shelters have been somewhat distorted by pressing and mounting, and some may have been old when collected, they appear very similar to the shelters observed and collected at Palo Verde. These specimens suggest that there may be one cryptic species, or perhaps multiple species with similar larval behavior, throughout Central America.

The observed differences in the *A. corallina* complex appear to correspond to different geographic regions. For instance, information on the *A. corallina* complex in the continental United States presents a much different picture than that of the Central American *A. corallina*. First, while the non-myrmecophilic acacia *Vachellia farnesiana* is patchily distributed in the southern United States (USDA 2016), *A. corallina* adults have been reported in areas where there are no acacias, such as Tennessee and Oklahoma (Moth Photographers Group 2016). These moths likely belong to the ‘southern US’ BIN (BOLD:AAA8062). In Florida, this clade is joined by another (BOLD:AAA8061). Interestingly, Kimball (1965) reported two wing color forms for Florida *A. corallina* and suggested that these might represent two species. This would seem to fit the molecular data, although the barcoded specimens have not yet been reviewed to identify any morphological differences (J.-F. Landry, pers. comm.) and the different colors may not be species-specific.

Second, the only published host plant record for United States *Aristotelia corallina* is *Chamaecrista nictitans* in Florida (Kimball 1965, Heppner et al. 2003). No information about the reared individuals is given, so it is not possible to determine whether these records represent BOLD:AAA8061 or BOLD:AAA8062. *C. nictitans* produces EFN nectar, but it only has facultative relationships with ants and does not produce Beltian bodies (Ruhren & Handel 1999, Ruhren 2003). *C. nictitans* also belongs to a different subfamily than the acacias, the Caesalpinioideae. *C. nictitans* occurs throughout much of North and Central America and could be a host plant for the *A. corallina* complex outside of Florida. The herbarium search revealed possible *A. corallina* webs on *C. nictitans* specimens from North Carolina and Mississippi, suggesting that this may be the case. No webs were found on US *V. farnesiana*, although the sample size was small ( $N = 12$ ).

*Aristotelia corallina* have also been identified in Puerto Rico. One Puerto Rican specimen was included in the BOLD barcode data, and it was placed in its own BIN (BOLD:AAT9249). While there are no published Puerto Rican host records, both *V. farnesiana* and *C. nictitans* are native to the island. Several *A. corallina*-type webs were found on herbarium specimens of Puerto Rican *V. farnesiana*, suggesting that this acacia may be *A. corallina*’s Puerto Rican host. There was only one Puerto Rican specimen of *C. nictitans*, so its potential as a Puerto Rican host plant could not be assessed. The herbarium specimens from the rest of the Caribbean were far too few (2 *V. farnesiana*; 0 *C. nictitans*) to give any insight into whether *A. corallina* might occur on other islands.

## CONCLUSION

*Aristotelia corallina* Walsingham is a currently unresolved species complex, containing at least four distinct clades. Complex members from different locations apparently feed on different host plants with different levels of ant association, ranging from the facultative *C. nictitans* to the obligate myrmecophytes *V. collinsii* and *V. cornigera*. Additional research is needed to compare the morphologies and DNA barcodes of all four groups to those of *A. corallina* from the type locality of Guerrero, Mexico. If the type specimen corresponds to any of the currently identified clades, it is most likely the Costa Rican one (BOLD:AAH5498), as both the Costa Rican and eastern Mexican caterpillars exploit obligate myrmecophytes by consuming young foliage and building shelters that provide access to both EFNs and domatia. Inspection of herbarium specimens also shows that there are likely populations of *A. corallina* throughout Central America. Whether these caterpillars belong to identified cryptic species remains to be determined. It may be possible to barcode caterpillars preserved in herbaria, although projects that have used herbarium insect material have required large sample sizes to successfully generate sequences (e.g., Lees et al. 2011). However, this report shows significant ecological differentiation between the Costa Rican clade and the two clades found in the United States, as the Costa Rican BOLD:AAH5498 caterpillars exploit several aspects of an elaborate ant-plant relationship, including Beltian bodies and extrafloral nectar. Due to these feeding and developmental behaviors, these caterpillars appear to be particularly reliant on ant-adapted plants. To my knowledge, this is the first reported instance of a non-myrmecophilous caterpillar exploiting these resources.

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### CHAPTER 3: Trading spaces: changing shelters changes predation of *Urbanus dorantes* and *U. proteus* (Hesperiidae)

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#### ABSTRACT

Many caterpillar species build diverse shelters that protect them from natural enemies, but whether shelter differences change that protection is unknown, in part because it can be difficult to disentangle a caterpillar species' shelter from the rest of its phenotype. To address this question, we performed an experiment in which two closely related caterpillar species (*Urbanus dorantes* and *U. proteus*, Hesperiidae) were placed into the shelters of both species and predation and parasitism were measured during two different developmental periods (early and mid-instar caterpillars). Predation was intense, with 0-48% of caterpillars surviving depending on treatment. Shelter identity had a significant effect on predation, with caterpillars in *U. proteus* shelters experiencing higher predation than those in *U. dorantes* shelters. Further analysis found that this was linked to shriveled-leaf shelters built only by *U. dorantes*: caterpillars in other shelters built by either species had a 94% higher risk of predation than caterpillars in shriveled leaf folds. Caterpillar age also had a significant effect on predation, but caterpillar species did not. This experiment directly demonstrates that shelter shape affects predation and that the effect is independent of the occupant's identity.

#### INTRODUCTION

Leaf shelters are built by a diverse assemblage of arthropods, including at least 24 families of Lepidoptera (Jones 1999). The extent of shelter-building varies dramatically among lepidopteran families, with some families having only a few shelter-building species (e.g., Papilionidae) while shelter-building is nearly universal in others (e.g., Hesperiidae). Shelter-building by caterpillars occurs in biomes ranging from tropical forests and savannas to temperate and boreal forests (Lill and Marquis 2007). Depending on the question of interest, caterpillar shelters can be interpreted using different biological concepts, including extended phenotypes, ecosystem engineering and niche construction. Extended phenotypes are non-morphological traits that are still genetically encoded and heritable (Dawkins 1982). While the genetics of lepidopteran shelter-building are unknown, the fact that newly hatched conspecific caterpillars can build highly stereotyped shelters indicates that caterpillar shelter-building behaviors are highly heritable. The heritability of extended phenotype construction has also been estimated in other organisms (Weis and Abrahamson 1986, Aasen 2009, Weber et al. 2013). Ecosystem engineering considers how an organism's environmental modifications affect other community members (Jones et al. 1997), and many studies have addressed caterpillar shelters in this context (e.g., Cappuccino 1993, Lill and Marquis 2004, Wang et al. 2012, Velasque and Del-Claro 2016). The concept of niche construction incorporates aspects of both extended phenotypes and ecosystem engineering, but it also specifies that an organism's heritable constructed niche creates unique environmental conditions that can exert selective pressures on the organism or other members of the community (Odling-Smee et al. 2003). As we are interested in the short-term effects of caterpillar shelters on shelter-building caterpillars, we will primarily consider different caterpillar shelters as extended phenotypes with different costs and benefits to their occupants.

Building shelters is a costly strategy for caterpillars. Many shelters require at least 20 or 30 minutes to build, and require ongoing maintenance. Building shelters uses amino-acid-rich silk and often requires the caterpillar to manipulate leaves that are larger than its own body (Berenbaum et al. 1993). For instance, many skipper caterpillars construct cut-and-fold shelters, in which they measure and cut a piece of leaf before using silk to fold it over the leaf like an awning (Fig. 1D and E), fold single leaves in half, or pull together multiple leaves (Greeney and Jones 2003). Many caterpillar species must also construct new shelters once they have outgrown the shelters or consumed all the nearby leaf tissue, and seeking sites for new shelters and building them can be dangerous (Loeffler 1996). There is also some evidence (Jones 1999, Lill et al. 2007) that forcing caterpillars to constantly build new shelters is sufficiently energetically taxing to increase mortality (but see Loeffler [1996] for a counter-example).

Balancing these costs, one of the most frequently suggested benefits of shelters is that they protect caterpillars from natural enemies. At least 18 studies found that a variety of caterpillar shelters provided protection against predators (Lill and Marquis 2007, Covarrubias-Camarillo et al. 2016, Velasque and Del-Claro 2016). Shelters can protect caterpillars from predators by making caterpillars less accessible or less apparent. Some shelters are nearly or completely sealed, while others may be too narrow or densely packed with silk for any but very small predators to enter. Shelters can also make caterpillars less apparent to predators by visually concealing them (Jones et al. 2002, Weiss et al. 2004) or decreasing the chance that predators walking on the leaf will encounter the caterpillars (Sendoya and Oliveira 2017). Although these caterpillars may still be accessible to predators, it is a moot point if predators do not notice them.

Shelters do not protect caterpillars from all natural enemies, however. Some visual predators, including wasps and biologists, can learn to use shelters as search cues (Weiss et al. 2004). Furthermore, many studies of parasitism in caterpillar assemblages have found that shelter-building caterpillars experience equal or greater parasitism than free-feeding caterpillars (Gentry and Dyer 2002, Connahs et al. 2011, Hreck et al. 2013, although see Diniz et al. [2012]). It has been proposed that high parasitism is a direct consequence of low predation, as parasitoids would be expected to prefer caterpillars that are less likely to experience predation while hosting parasitoid larvae (Gentry and Dyer 2002, Murphy et al. 2014). This hypothesis, called the parasitoid enemy-free space hypothesis (Murphy et al. 2014), is a specific case of the enemy-free space (EFS) hypothesis of Jefferies and Lawton (1984). The latter proposed that organisms will prefer environments (habitats, hosts, etc.) that decrease their chances of being harmed by one or more natural enemies. In the case of caterpillar parasitoids, the hypothesis suggests that groups that differ in anti-predator defenses will show an inverse relationship between the predation and parasitism experienced by each group. Thus, caterpillars that experience the lowest predation are predicted to experience the highest parasitism, and vice versa. In the case of shelter-building caterpillars, it is reasonable to predict that caterpillars whose shelters provide greater protection against predators would suffer higher levels of parasitism, just as Murphy and colleagues have found that caterpillars with better morphological defenses against predation suffer higher levels of parasitism.

Our current understanding of shelter costs and benefits is based on either natural or experimental comparisons between sheltered and exposed caterpillars. Numerous experimenters have investigated the effects of shelters on predation, abiotic conditions, and other factors by removing shelter-building caterpillars from their shelters (e.g., Hunter and Willmer 1989, Mueller and Dearing 1994, Jones et al. 2002) or by creating artificial shelters (e.g. Lill and Marquis 2004, Wang et al. 2012). These experiments comparing sheltered and exposed shelter-building caterpillars effectively test whether shelters provide particular benefits, but switching caterpillars into other shelters can allow researchers to address the questions that naturally arise after a shelter

function is demonstrated. For instance, if a sheltered/exposed comparison shows that a shelter protects caterpillars from predation, a shelter-switching experiment can demonstrate which shelter characteristics affect predation and how much of a change in those characteristics is needed to alter predation. Shelter-switching also disentangles the effect of a caterpillar species' extended phenotype (the shelter) from other phenotypic effects (e.g., a caterpillar's morphology, physiology, chemical defenses, and/or behavioral responses to natural enemies). Free-feeding caterpillar species vary in their susceptibility to natural enemy attack as do shelter-building species (e.g., Gentry and Dyer 2002). The question for which we currently have no answer is how shelter shape affects natural enemy attack independent of the shelter inhabitant's identity. Separating caterpillars from their shelters can also test for traits and behaviors specifically suited to that species' particular shelters, which can offer insights into the pressures and specificity of niche construction (Odling-Smee et al. 2003). While there are logistical limitations to shelter-switching (e.g., the shelter-building species must share host plant species), shelter-switching offers the potential to better understand shelter functions for shelter-building caterpillars.

At Palo Verde National Park in 2013, two skipper butterfly caterpillar species (Hesperiidae), *Urbanus dorantes* and *U. proteus*, varied greatly in parasitism levels (C. S. Baer, unpub. data). These data showed that 65% of *U. dorantes* caterpillars were parasitized, while only 4% of *U. proteus* caterpillars were parasitized ( $p = 0.0008$ ). This difference is especially striking because the species occur on the same host plants in the same habitat, raising the question of what differences in caterpillar defense or quality cause such a difference in parasitism. One difference between the species that might alter their vulnerability to predators and their appeal to parasitoids is shelter shape. It is also possible that unobserved intrinsic differences between the caterpillars themselves could drive the difference in parasitism. This was an ideal system in which to test both the effect of shelter shape on shelter function and the parasitoid EFS hypothesis using shelter-switching.

Accordingly, we performed a field experiment in which *U. dorantes* and *U. proteus* caterpillars were switched into one another's shelters. We also measured shelter shape in order to link any shelter identity effects on natural enemies to differences in shelter characteristics. We hypothesized that the two caterpillar species' shelters vary in their effectiveness against predators and that parasitoids would seek enemy-free space in shelters that are better defended against predators. Based on the preliminary data showing higher parasitism of *U. dorantes* caterpillars, we predicted that both *U. dorantes* and *U. proteus* caterpillars in *U. dorantes* shelters would experience higher parasitism compared to caterpillars in *U. proteus* shelters. Due to this predicted tradeoff between predation and parasitism, we also predicted that the two shelters would provide equivalent EFS to the caterpillars inhabiting them (resulting in similar overall caterpillar survival). Finally, because the early instars of *U. dorantes* and *U. proteus* build different types of shelters while the mid-instar caterpillars build more similar shelters, we predicted that differences in predation and parasitism would be more apparent in early instar caterpillars.

## METHODS

### *Study site and organisms*

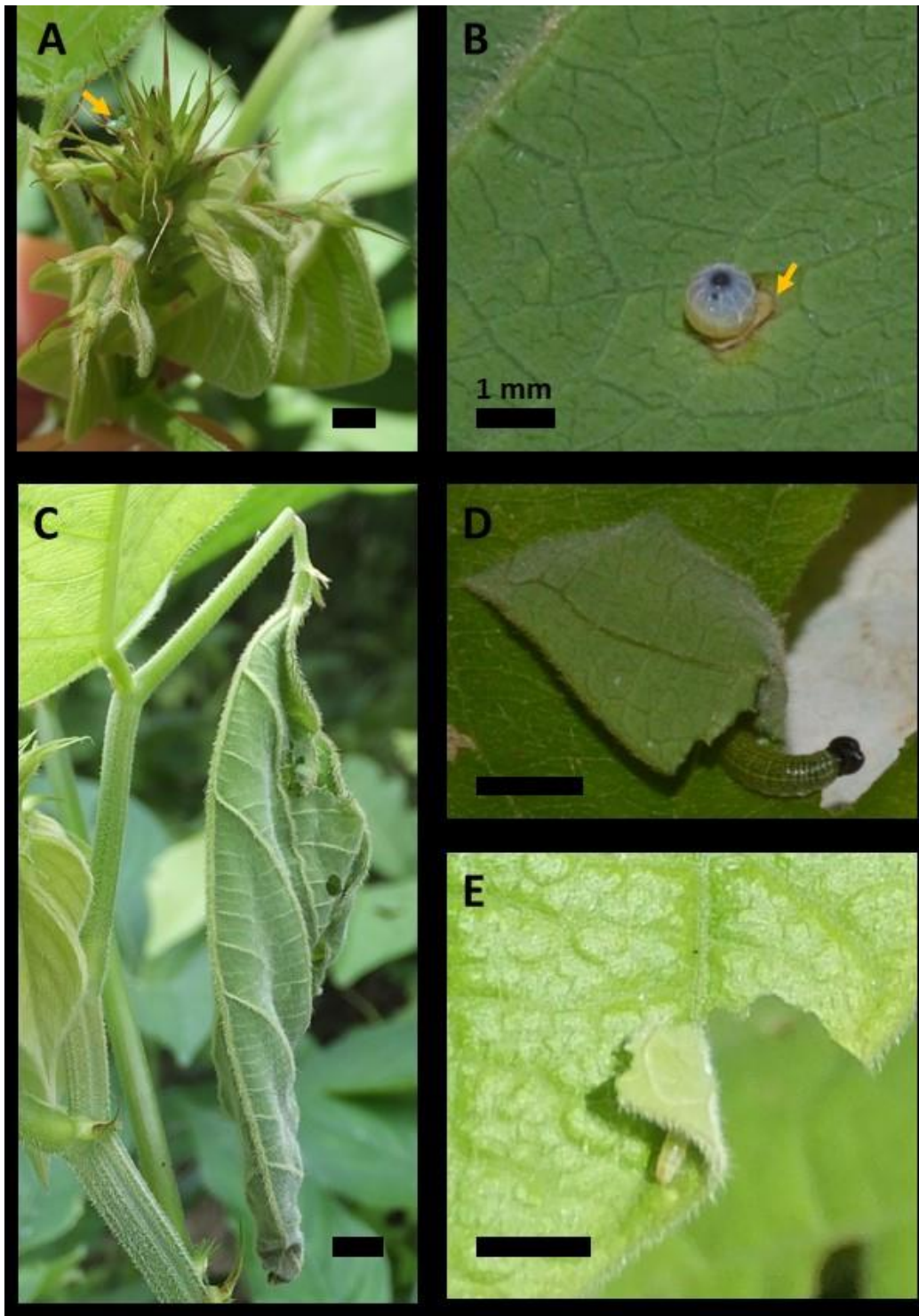
The research was conducted at Palo Verde Biological Station (10° 21' N, 85° 21' W, elevation approximately 0-100 m asl) within Palo Verde National Park, Guanacaste, Costa Rica. The Palo Verde station property consists of secondary tropical dry forest surrounding the wetlands of the Rio Tempisque.

The two species used were *Urbanus dorantes* and *U. proteus* (Hesperiidae). Although *U. dorantes* and *U. proteus* are in different clades of the polyphyletic *Urbanus* genus (Pfeiler et al. 2016), their larval ecology is quite similar. Both species have five larval instars before pupation and both feed on Fabaceae. At Palo Verde, both species feed on several species, including *Desmodium glabrum*, the host plant used in this experiment. *D. glabrum* is an annual herb that can reach up to 2.5 m. It has trifoliolate leaves whose leaflets vary in size from 2 to 9 cm long and 1.5 to 5 cm wide (Zamora 2010). It grows in relatively sunny and disturbed areas at Palo Verde, including road- and trailsides. Plants often grow in patches within touching distance of one another.

While early instar *U. dorantes* and *U. proteus* caterpillars cannot be visually distinguished in the field, they can be identified by their eggs and egg shells (Fig. 1A and 1B). *U. dorantes* eggs are pearlescent light green spheres and are usually found on the petioles, stipules, or upper leaf surfaces of *D. glabrum*. *U. proteus* eggs are larger, yellow, slightly pointed, and are found on the undersides of leaves. Furthermore, *U. proteus* eggs kill the leaf tissue they adhere to, creating brown patches on leaves. The eggshells of both species generally adhere to the plant for several days after hatching, and are rarely completely consumed by the caterpillars. Even if the eggshells do not persist, the brown patches caused by *U. proteus* eggs allow caterpillar identification. The two species also target different leaf developmental stages. *U. dorantes* females prefer to lay eggs on expanding leaves, particularly ones that are still vertical, while *U. proteus* females prefer fully expanded leaves.

The caterpillars also differ in their shelter-building techniques. First instar *U. dorantes* caterpillars on vertical leaflets will remain on the leaflets for one to two days without any shelter before partially cutting the petiolule and reinforcing it with silk (Fig. 1C). This causes the leaflet to wither and fold up around the caterpillar, which then feeds inside the leaflet. *U. dorantes* caterpillars on horizontal leaflets will cut a rectangular or triangular flap and use silk to fold it over the leaf, forming a flat awning (Fig. 1E). The caterpillar then rests on the underside of the awning or the side of the shelter and feeds outside on the leaflet. This style of shelter building continues until the caterpillar becomes large enough to manipulate whole leaflets (usually the fourth instar). Once caterpillars can manipulate whole leaflets, shelter construction becomes less stereotyped and more dependent on plant architecture. Depending on leaflet size and position, a caterpillar might fold an entire leaflet in half, tie together two leaflets that already overlap, or pull non-overlapping leaflets together.

By contrast, *U. proteus* caterpillars build their first shelters shortly after hatching. They cut a small flap from the edge of the leaflet and use silk to fold it into a cone-shaped awning over the leaf (Greeney and Sheldon 2008) (Fig. 1D). *U. proteus* caterpillars rest on the shelter ceiling and feed on the leaf outside the shelter. *U. proteus* caterpillars also continue building these cut-and-fold shelters until the fourth instar, when they switch to manipulating whole leaflets. Depending on plant architecture, fourth and fifth instar *U. proteus* caterpillars, like *U. dorantes*, will fold entire leaflets, tie two overlapping leaflets together, or pull non-overlapping leaflets together.



**Figure 1.** Life history of *Urbanus dorantes* and *U. proteus*. Unless otherwise specified, scale bars are 5 mm. **A.** *Urbanus dorantes* eggs, indicated by the arrow, on a stipule. **B.** A *U. proteus* egg beginning to hatch. Leaf discoloration around the egg base is indicated by the arrow. Scale bar = 1 mm. **C.** Second instar *U. dorantes* shriveled leaf fold created by cutting through the leaf petiolule. **D.** Second instar *U. proteus* shelter and caterpillar. **E.** A second instar *U. dorantes* shelter created by cutting and folding an expanded leaflet.



### *Shelter-switching*

Caterpillars were found by systematically searching *D. glabrum* plants in an area approximately 1.5-2 km west of Palo Verde Biological Station. If eggs were found, the plant was marked and checked daily for hatched caterpillars. When caterpillars were found, their size, species, and the presence of any shelters were recorded. If the caterpillar had not built a shelter, it was marked and checked daily. Caterpillars were assigned to one of the two shelter treatments: *U. dorantes* shelters or *U. proteus* shelters. Caterpillars were then switched into an appropriate shelter built by a caterpillar of the same age. No caterpillars were returned to shelters they had built.

Before beginning the shelter-switching process, a sheet of white packing foam (~1 m<sup>2</sup>) was spread below the plant to catch the caterpillar if it fell. A slit in the foam allowed the sheet to be folded around the plant's main stem. Each shelter was opened using fine-tipped forceps. The caterpillar was coaxed onto a slip of paper and transferred to a 0.7 mL microcentrifuge tube. If possible, an egg shell was collected from the plant as an identifying voucher. After the shelter was vacant, any large silk strands were removed. Once the destination shelter was also vacant, the caterpillar was removed from the microcentrifuge tube using a narrow (~1 mm) grass stem and placed in the shelter. The shelter was closed and often monitored for 10-30 minutes to confirm that the caterpillar remained in the shelter.

During the first generation (June 2016), early instar caterpillars were switched when they were still in their first shelters (late first instar or early second instar). However, nearly all caterpillars in all treatments during the first generation were lost to predation, often within 24 hours of the switch. Only fifteen of 65 early-instar caterpillars could be retrieved to determine parasitism status.

During the second generation (July-August 2016), the experiment was modified due to this heavy predation pressure. Eggs and first-instar caterpillars of both species were collected and reared in the lab to ensure appropriate sample sizes. Once caterpillars reached the third instar, they were returned to the field and allowed to build shelters. To ensure successful establishment, caterpillars were protected with organza bags until they had built shelters or until the following day (a few caterpillars transitioned to the fourth instar during this period). Then caterpillars were switched as described above, although slight modifications were needed to handle these caterpillars' greater size and weight. 1.7 mL microcentrifuge tubes and wider plant stems were used to hold and transfer the caterpillars, and shelters were lightly wrapped with organza to hold them in position, as the heavier caterpillars often caused the shelter awnings to fall open. This wrapping did not prevent predators or parasitoids from accessing the caterpillars, as several caterpillars were killed in wrapped shelters. The wrapping was removed the following day.

All caterpillars were checked daily for survival and new shelters. Caterpillars that built new shelters were switched into appropriate shelters built by other experimental caterpillars or non-focal individuals. In other words, if a *U. dorantes* caterpillar assigned to the *U. proteus* shelter treatment built a new shelter, it would be switched into an appropriately sized *U. proteus* shelter. Caterpillars were generally collected at death or after six days in the field, whichever came first. However, because the mid-instar caterpillar experiment was conducted at the very end of the field season, eight caterpillars had to be collected before six days had passed. All were *U. proteus* caterpillars; five were placed in *U. proteus* shelters and three in *U. dorantes* shelters. The shelters that caterpillars were switched into were either collected with their occupants or after two days of being unoccupied. Shelters that did not receive new caterpillars after their original caterpillars were switched into other shelters ("unoccupied shelters") were also collected.



After collection, live caterpillars were reared until either adult butterflies or parasitoid larvae emerged. Parasitoids were then reared and preserved in 95% ethanol, as were dead caterpillars and any shed skins and head capsules (exuviae). Adult butterflies were released after emergence. All dead caterpillars and pupae were dissected to check for parasitoids that died before emerging from their hosts. All emerged parasitoids and parasitoid larvae found were identified using DNA barcoding as described in Chapter 2, and caterpillars were DNA barcoded to confirm their identities.

### *Statistical analysis*

Due to small sample sizes, parasitism was first analyzed using a Fisher's exact test to test for a difference in parasitism between cohorts, and then exact hypergeometric tests for each instar, as the data were in the form of a 2 x 2 x 2 table (caterpillar species x shelter x parasitism; Agresti 1992). The hypergea package (Boenn 2016) for R was used (R Core Team 2016). Additionally, the data were coded in two different ways to account for caterpillars removed by predators. In the first, conservative analysis, caterpillars were only included in the analysis if they had been retrieved from the field (either alive or dead). However, we observed that parasitism often caused caterpillars to fast and seal their shelters with silk, which suggested that caterpillars captured by predators were likely to be unparasitized. Since we recovered and dissected 20 caterpillars killed by predators that were all unparasitized, we also performed an analysis that treated all unrecovered caterpillars as unparasitized.

Predation of all 143 caterpillars was analyzed using Cox proportional hazards in R (survival package, Therneau 2016). In this analysis, predation events were coded as failures and survival time was calculated from the date of the initial switch to the date when the caterpillar died or disappeared. If caterpillars were successfully recaptured, data were right-censored on that date. Eight caterpillars were lost in the field for reasons other than predation. In five cases, *U. dorantes* shelters, with their silk tethers weakened by the experimental manipulations, fell off the plant and could not be located. In three other cases, vertebrate herbivores (most likely horses associated with the park ranger station) consumed the plants. In these non-predation cases, the data were right-censored on the date the caterpillar and shelter were last seen. The significance of a variable was determined by performing a log-likelihood ratio test on the full model and a submodel omitting the variable of interest. This is the recommended method for model comparison, as an alternative approach, Analysis of Deviance, is highly dependent on variable order and the assumptions of Type III error tests do not fit Cox proportional hazards analysis (Therneau 2017). We assumed that any differences observed between the experiments with the first and second generations of caterpillars were due to instar and not cohort.

To assess the effect of shelters on overall caterpillar survival, a Cox proportional hazards analysis was performed in which both predation and parasitoid oviposition were coded as failures ( $N = 143$ ). The dates of parasitoid oviposition events were approximated using observations of encounters with adult parasitoids, parasitoid-induced changes in behavior, and the emergence of parasitoids from hosts. Data were right-censored as described above.

Analyses were also performed to assess the effects of shelter type and shape on predation. To compare the *U. dorantes* shriveled leaf folds to the other shelter types, another Cox proportional hazards analysis was performed with Shriveled Shelter, Caterpillar Species, and Instar as explanatory variables. To test whether *U. dorantes* and *U. proteus* cut-and-fold shelters differed in shape, intact shelters built during the experiment (including unoccupied shelters) were analyzed. Each shelter was traced with a permanent marker, opened, flattened, and scanned with a 30 mm x 30 mm reference square using a handheld document scanner (VuPoint Solutions MAGIC WAND

scanner, Los Angeles, CA, USA). The complete outline of each flattened shelter was then digitally traced and filled in, as there was not enough contrast between the outlined shelter and the rest of the leaf for an image processing program to automatically detect the shelter. The program SHAPE (version 1.3, Iwata and Ukai 2002) was used to convert the shelter shapes into normalized elliptical Fourier descriptors, and those data were then analyzed using the Momocs package in R (Bonhomme et al. 2014). Principal Component Analysis (PCA) and Linear Discriminant Analysis were used to compare and contrast the shapes of *U. dorantes* and *U. proteus* shelters and the shapes of predated and unpredated shelters.

## RESULTS

### *Parasitism*

Only ten parasitism events were observed in the 57 recovered caterpillars (Table 1), with no significant differences between instars, shelters, or caterpillar species detected by the Fisher's exact test and hypergeometric tests. When all 143 caterpillars were analyzed, with unrecovered caterpillars classified as unparasitized caterpillars, there was no change in the results (Table 1). It is worth noting that eight of the ten parasitism events occurred in the mid-instar cohort and seven of the ten parasitized caterpillars were *U. dorantes*. Additionally, three of the four parasitoid species have only been reared from *U. dorantes*.

**Table 1.** Parasitism of experimental caterpillars

# parasitized Total		Early-instar caterpillars		Mid-instar caterpillars	
		<i>U. dorantes</i> Shelter	<i>U. proteus</i> Shelter	<i>U. dorantes</i> Shelter	<i>U. proteus</i> Shelter
Recovered caterpillars only	<i>U. dorantes</i> Caterpillar	1/4	0/2	2/8	3/11
	<i>U. proteus</i> Caterpillar	0/5	1/7	2/7	1/13
All caterpillars	<i>U. dorantes</i> Caterpillar	1/19	0/12	2/19	3/20
	<i>U. proteus</i> Caterpillar	0/10	1/18	2/19	1/20

Several approaches by parasitoid wasps were observed in the field, some on experimental caterpillars and some on other *U. dorantes* and *U. proteus* caterpillars in the same area. When a parasitoid attempted to oviposit on mid-instar caterpillars outside of their shelters (two *U. dorantes* and one *U. proteus*), each caterpillar responded by jerking its head towards the wasp, and one *U. dorantes* caterpillar also directed some sort of secretion or regurgitant at the parasitoid. Other unsheltered caterpillars dropped from their plants when investigated by a parasitoid. By contrast, two parasitoids were observed investigating caterpillars inside their shelters. These parasitoids seemed to have less difficulty approaching the caterpillars, possibly because a parasitoid walking on the shelter was less apparent to the caterpillars than a flying one. The confines of the shelters may have also prevented the caterpillars from repelling the parasitoids. Oviposition was not observed, but both these caterpillars were found to be parasitized.

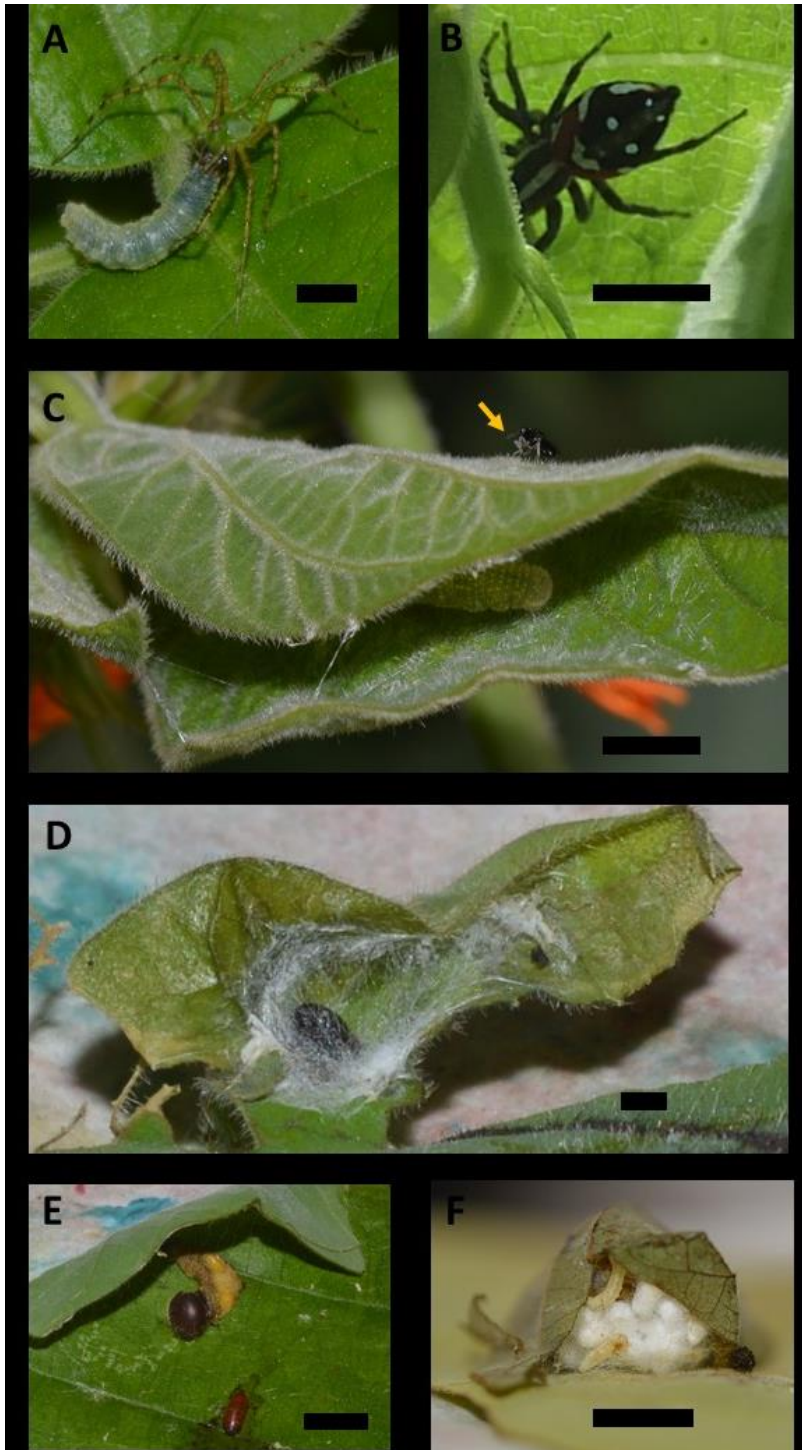
The most common parasitoid type emerged from five caterpillars during the second or third instar (Fig. 2C and D) after inducing the host to seal itself in its shelter for two or three days. DNA barcoding indicates that these parasitoids are in the Eulophidae (Hymenoptera) and that there are actually two species, which each appear to specialize on one of the caterpillar species, regardless of whether caterpillars were placed in conspecific or heterospecific shelters. (See the Supplemental Information for additional details.)

The other four parasitism events (all in *U. dorantes*) were caused by two other parasitoid species. Two mid-instar *U. dorantes* caterpillars each produced a single brown parasitoid pupa. Each pupa was slightly pointed at one end and had visible segments (Fig. 2E), and both morphology and DNA barcoding indicate that they are a species of tachinid fly. These are the only tachinid parasitoids reared from *Urbanus* caterpillars during four field seasons. The remaining two parasitism events were by a braconid wasp that is a common parasitoid of *Urbanus dorantes* caterpillars at Palo Verde (Fig. 2F). DNA barcoding matched them to a parasitoid reared at Area Conservación de Guanacaste, which has the working name *Apanteles* sp. Rodriguez24 (BOLD:ACF3142).

### Predation

Predation was intense, with 0-48% of caterpillars surviving until recollection, depending on treatment, caterpillar species, and cohort (Fig. 3A-B). The full Cox proportional hazards model for predation was highly significant (likelihood ratio test:  $\chi^2 = 20.36$ ,  $df = 7$ ,  $p = 0.005$ ), and the submodel comparisons showed that all three variables (Shelter Identity, Caterpillar Species, and Instar) were significant (Table 2). Both being in a *U. proteus* shelter and being a *U. proteus* caterpillar increased the risk of predation by 279% and 104%, respectively, while being a mid-instar caterpillar increased the risk of death by 13%. The additive submodel (Shelter + Species + Cohort) was significantly different from the full model ( $p = 0.011$ ), although omitting any single two-way interaction did not result in significant differences from the full model. However, when only a single interaction was included, only the Shelter x Species interaction resulted in a submodel that was indistinguishable from the full model ( $p = 0.19$ ). The Shelter x Species interaction predicts that an *U. proteus* caterpillar in an *U. proteus* shelter has a 79% lower risk of predation than would be expected based on the main effects alone.

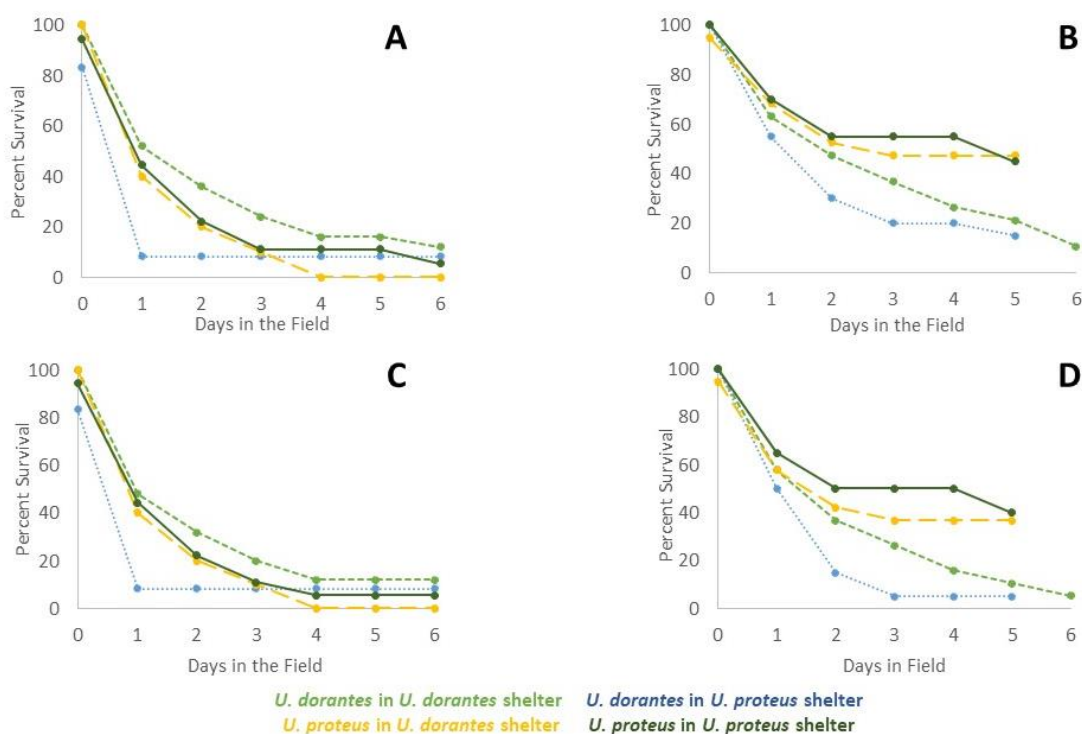
As previously stated, caterpillars were considered to have been predated when 1) predators were observed holding and/or eating caterpillars, 2) dead caterpillars were found with feeding damage, or 3) when caterpillars could not be found on their host plants or neighboring plants. During the experiment, predation was directly observed 10% of the time, inferred from dead caterpillars 12% of the time, and inferred from missing caterpillars the remaining 78% of the time. All predators observed holding experimental caterpillars were spiders. All but one were green lynx spiders (*Peucetia viridans*: Family Oxyopidae, Fig. 2A). That remaining spider was identified as *Nycerella delecta* (Salticidae, Fig. 2B, G. B. Edwards, pers. comm.). Additionally, one third instar *U. proteus* caterpillar was killed by a vespid wasp during the field reintroduction phase of the experiment. The wasp located and attacked the caterpillar before it could be protected with an organza bag. The caterpillar had been on the plant for less than one minute and had not begun feeding, suggesting that the wasp located the caterpillar visually. Killed caterpillars were frequently found near *P. viridans*, including one case in which very small ants were found feeding on a dead mid-instar caterpillar, which was likely killed by the spider (C. S. Baer, pers. obs.). The only other potential predator observed during the study was an orthopteran (Suborder Ensifera) found near a chewed caterpillar corpse.



**Figure 2.** Predators and parasitoids of *Urbanus dorantes* and *U. proteus*. Unless otherwise specified, scale bars are 5 mm. **A.** Green lynx spider (*Peuceetia viridans*) carrying a *U. dorantes* caterpillar. **B.** *Nycerella delecta*, the other observed spider predator. **C.** A eulophid parasitoid adult (indicated by the arrow) on a *U. dorantes* shelter with a *U. dorantes* caterpillar inside. **D.** Opened *U. proteus* shelter showing a eulophid pupa behind silk used to seal the shelter. Scale bar = 1 mm. **E.** Tachinid pupa and *U. dorantes* host. **F.** *Apanteles* Rodriguez24 larvae and cocoons inside a *U. dorantes* shelter.

**Table 2.** Cox proportional hazards model for predation only. The  $p$  value indicates the additional explanatory effect of adding the variable in that row to the indicated submodel. Caterpillar Species and Shelter Identity both use *U. dorantes* as the reference. Instar uses the early-instar caterpillars as the reference

Variable	Coefficient	SE of coefficient	Submodel	Log-likelihood of submodel	Likelihood ratio $\chi^2$	Degrees of freedom	$p$
Shelter Identity	1.332	0.402	Species + Instar + Species x Instar	-431.222	10.42	3	<b>0.019</b>
Caterpillar Species	0.711	0.423	Shelter + Instar + Shelter x Instar	-431.331	10.64	3	<b>0.014</b>
Instar	0.124	0.366	Shelter + Species + Shelter x Species	-433.566	15.11	3	<b>0.002</b>
All interactions			Shelter + Species + Instar	-431.57	11.12	3	<b>0.011</b>
Shelter x Species	-1.556	0.586	Shelter + Species + Instar + Shelter x Instar + Species x Instar	-429.51	7.00	5	0.221
Shelter x Instar	-1.165	0.542	Shelter + Species + Instar + Shelter x Species + Species x Instar	-428.25	4.48	5	0.482
Species x Instar	-1.050	0.588	Shelter + Species + Instar + Shelter x Species + Shelter x Instar	-427.60	3.17	5	0.674
Shelter x Instar AND Species x Instar			Shelter + Species + Instar + Shelter x Species	-429.07	6.12	4	0.190
Shelter x Species AND Species x Instar			Shelter + Species + Instar + Shelter x Instar	-429.93	7.84	4	0.098
Shelter x Species AND Shelter x Instar			Shelter + Species + Instar + Species x Instar	-430.48	8.93	4	0.063
Shelter x Species x Instar	1.167	0.8214	Shelter + Species + Instar + Shelter x Species + Shelter x Instar + Species x Instar	-427.01	1.99	6	0.921
Full Model				-426.011	20.36	7	<0.005



**Figure 3.** Survival of caterpillars after placement in shelters. Note that “Day 0”, etc. do not correspond to the same dates for all caterpillars. **A.** Early-instar caterpillars surviving predation. **B.** Mid-instar caterpillars surviving predation. **C.** Early-instar caterpillars surviving both predation and parasitism. **D.** Mid-instar caterpillars surviving both predation and parasitism.

#### *Predation and parasitism combined*

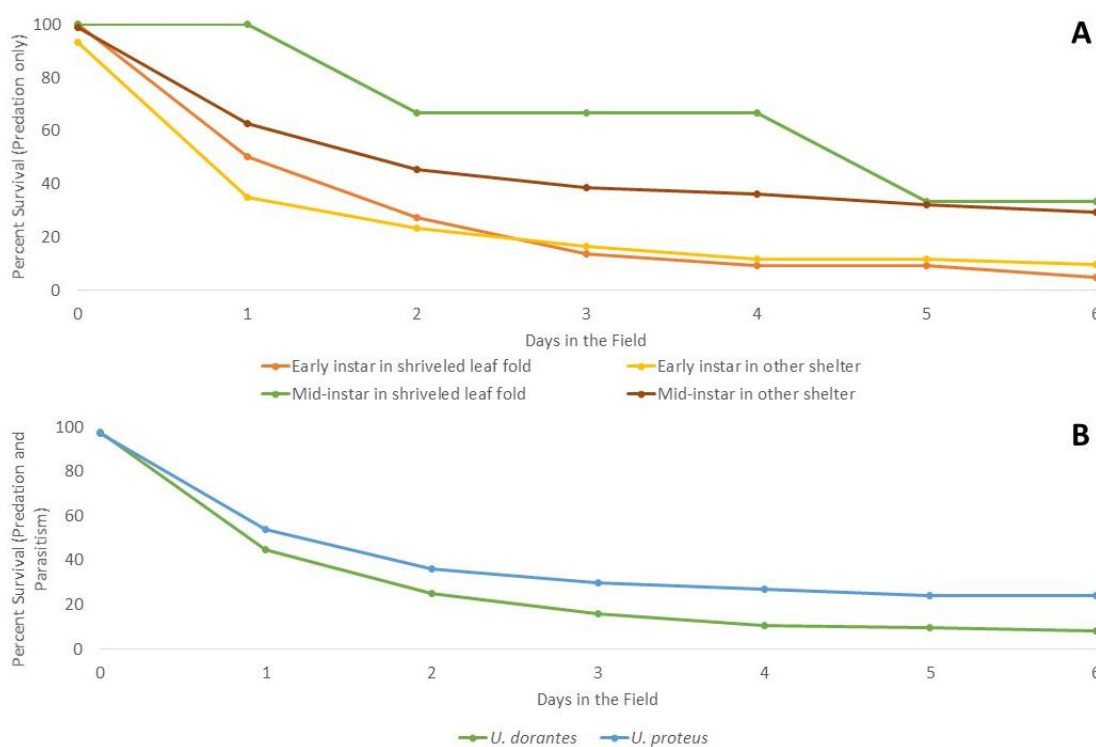
When predation and parasitism were combined, caterpillar survival dropped to 0-40%, again depending on treatment, species, and instar (Fig. 2C-D). The full Cox proportional hazards model was again highly significant (likelihood ratio test:  $\chi^2 = 19.5$ ,  $df = 7$ ,  $p = 0.007$ ), with similar significant effects of Shelter Identity, Caterpillar Species, and Instar (Table 3). Occupying a *U. proteus* shelter increased a caterpillar’s risk of death by 237%, while being a *U. proteus* caterpillar increased the risk by 81%, and being a mid-instar caterpillar increased the risk of death by 25%. Again, while the additive model was significantly different from the model ( $p = 0.006$ ), no single interaction made a significant difference when omitted. However, when only a single interaction was included, Shelter x Species provided the only submodel that was indistinguishable from the full model ( $p = 0.197$ ). In this case, the interaction suggests that a *U. proteus* caterpillar in a conspecific shelter has a 74% lower risk of death than would be expected based on the main effects alone.

**Table 3.** Cox proportional hazards for predation and parasitism (caterpillar survival). The  $p$  value indicates the additional explanatory effect of adding the variable in that row to the indicated submodel. Caterpillar Species and Shelter Identity both use *U. dorantes* as the reference. Instar uses the early-instar caterpillars as the reference.

Variable	Coefficient	SE of coefficient	Submodel	Log-likelihood of submodel	Likelihood ratio $\chi^2$	Degrees of freedom	$p$
Shelter Identity	1.214	0.396	Species + Instar + Species x Instar	-465.16	10.64	3	<b>0.014</b>
Caterpillar Species	0.593	0.418	Shelter + Instar + Shelter x Instar	-466.48	13.28	3	<b>0.004</b>
Instar	0.225	0.349	Shelter + Species + Shelter x Species	-465.02	10.36	3	<b>0.016</b>
All interactions			Shelter + Species + Instar	-466.06	12.44	3	<b>0.006</b>
Shelter x Species	-1.360	0.577	Shelter + Species + Instar + Shelter x Instar + Species x Instar	-463.52	7.35	5	0.196
Shelter x Instar	-0.868	0.520	Shelter + Species + Instar + Shelter x Species + Species x Instar	-461.34	3.00	5	0.700
Species x Instar	-0.833	0.563	Shelter + Species + Instar + Shelter x Species + Shelter x Instar	-461.04	2.40	5	0.792
Shelter x Instar AND Species x Instar			Shelter + Species + Instar + Shelter x Species	-462.62	5.55	4	0.236
Shelter x Species AND Species x Instar			Shelter + Species + Instar + Shelter x Instar	-464.22	8.76	4	0.067
Shelter x Species AND Shelter x Instar			Shelter + Species + Instar + Species x Instar	-464.43	9.17	4	0.057
Shelter x Species x Instar	0.555	0.789	Shelter + Species + Instar + Shelter x Species + Shelter x Instar + Species + Instar	-460.09	0.49	6	0.992
Full Model				-459.843	19.5	7	0.007

### Shelter type and shape mechanisms for predation

When the *Urbanus dorantes* shelters used in the experiment were sorted by shelter type, 34% (25 of 73) were found to be shriveled leaf folds, with 22 of the 25 built by early-instar caterpillars. The Cox proportional hazards analysis comparing predation in shriveled leaf folds to all other shelters was highly significant (Fig. 4A, likelihood ratio test:  $\chi^2 = 18.02$ ,  $df = 7$ ,  $p = 0.012$ ). When submodels omitting variables were compared to the full model, significant effects were shown for Shriveled Shelter ( $\chi^2 = 8.081$ ,  $df = 3$ ,  $p = 0.044$ ) and Instar ( $\chi^2 = 11.664$ ,  $df = 3$ ,  $p = 0.008$ ), but Caterpillar Species was not significant ( $\chi^2 = 7.407$ ,  $df = 3$ ,  $p = 0.060$ ). Accordingly, Caterpillar Species was discarded, and the results of the Shriveled Shelter x Instar model are shown in Table 4. Compared to early-instar caterpillars in shriveled leaf folds, early-instar caterpillars in other shelters had a 94% higher risk of predation, and mid-instar caterpillars in shriveled leaf folds had a 71% higher risk of predation than early instar caterpillars in shriveled leaf folds. The interaction between Shriveled Shelter and Instar was not significant.



**Figure 4.** Survival of caterpillars for shelter type analysis. Note that “Day 0”, etc. do not correspond to the same dates for all caterpillars. **A.** Caterpillars surviving predation. **B.** Caterpillars surviving predation and parasitism.

The caterpillar survival data (for both predation and parasitism) were also reanalyzed to compare the shriveled leaf folds to the other shelters. The full Cox proportional hazards model was significant (Fig. 4B, log-likelihood ratio test:  $\chi^2 = 14.74$ ,  $df = 7$ ,  $p = 0.039$ ), but interestingly, the only variable that had a significant effect on caterpillar survival was Caterpillar Species ( $\chi^2 = 8.65$ ,  $df = 3$ ,  $p = 0.034$ ; Shriveled Shelter:  $\chi^2 = 5.88$ ,  $df = 3$ ,  $p = 0.117$ ; Instar:  $\chi^2 = 6.91$ ,  $df = 3$ ,  $p = 0.075$ ). *U. proteus* caterpillars were 35% less likely to be killed by either a predator or parasitoid than *U. dorantes* caterpillars.



**Table 4.** Cox proportional hazards for predation, comparing *U. dorantes* shriveled leaf folds to other shelters. The *p* value indicates the additional explanatory effect of adding the variable in that row to the indicated submodel. Shriveled Shelter uses shriveled leaf folds as the reference. Instar uses the early-instar caterpillars as the reference.

Variable	Coefficient	SE of coefficient	Submodel	Log-likelihood of submodel	Likelihood ratio $\chi^2$	Degrees of freedom	<i>p</i>
Shriveled Shelter	0.664	0.323	Instar	-433.203	4.64	1	<b>0.031</b>
Instar	0.537	1.535	Shriveled Shelter	-435.868	9.97	1	<b>0.002</b>
Shriveled Shelter x Instar	-0.625	0.791	Shriveled Shelter + Instar	-431.158	0.55	2	0.760
Full Model				-430.884	10.62	3	<b>0.014</b>

A set of 123 cut-and-fold *Urbanus dorantes* (N = 33) and *U. proteus* (N = 90) shelters were analyzed for shape differences. The PCA and LDA found substantial overlap between the two species' shelters, with the more numerous *U. proteus* shelters occupying a wider area of principle component space. Accordingly, when shelter identities were predicted by LDA, only 2 *U. dorantes* shelters (6%) were correctly identified, while 93% of *U. proteus* shelters were correctly identified. The shelter fate analysis used the same data set, which contained 56 failed (predated), 32 successful (unpredated), and 35 unoccupied shelters. It produced similar results, with failed, successful, and unoccupied shelters overlapping widely. When shelter fates were predicted by LDA, 66% of failed shelters, 13% of successful shelters, and 26% of unoccupied shelters were correctly identified. Most of the incorrectly identified shelters were successful and unoccupied shelters incorrectly labeled as failed shelters.

## DISCUSSION

This experiment demonstrated that a difference between caterpillar shelters, specifically between *Urbanus dorantes* shriveled leaf folds and the other shelters built by *U. dorantes* and *U. proteus*, results in a substantial reduction in attacks by natural enemies. While the effect of shelters on parasitoid attacks was unclear due to the low level of parasitism, shelter type had a significant effect on predation. Furthermore, this shelter effect on predation is independent of the identity of the caterpillar inhabiting the shelter. As predation in this community is intense and more common than parasitism, our data suggest that a shelter's effectiveness against predators contributes to which caterpillars survive to metamorphose into adults.

### *The role of shelters in creating EFS for parasitoids*

The possibility of parasitoids seeking EFS in shelter-building caterpillars has been repeatedly suggested to explain the apparent contradiction of shelter-building caterpillars experiencing equal or greater parasitism compared to free-living caterpillars (Gentry and Dyer 2002, Connahs et al. 2011, Hreck et al. 2013). According to the parasitoid EFS hypothesis, parasitoids will more frequently attack caterpillars that experience lower predation (Murphy et al. 2014). Due to the

small number of parasitism events detected in this study, our parasitism analyses had limited power and we were unable to identify any statistically significant differences in parasitism between shelters or caterpillar species. However, other data from monitoring *U. dorantes* and *U. proteus* caterpillars from 2014 to 2016 confirm the initial 2013 observation that *U. dorantes* caterpillars are more heavily parasitized than *U. proteus* caterpillars (C. S. Baer, unpub. data). That previous result is consistent with this study's observation that more of the parasitized caterpillars (7 of 10) were *U. dorantes* caterpillars, but the extent to which parasitoids may directly respond to different caterpillar species' shelters remains unclear.

Our experiment clearly showed that the type of caterpillar shelter affects predation. The shriveled leaf folds built by some *U. dorantes* caterpillars provide greater protection from predators than the cut-and-fold shelters of either caterpillar species. Shriveled leaf folds likely decrease predation by making the caterpillars both less accessible, as only small predators can fit into the shriveled leaf fold, and less exposed, because the shelters are large enough for caterpillars to feed inside them.

Aside from the high predation numbers themselves, there is some additional indirect evidence that parasitoids in this system are experiencing pressures due to host caterpillar predation. First, encounters with parasitoids and predators can occur in close succession. We could not determine the outcomes of half of the observed parasitoid approaches (two of four approaches) because the caterpillars disappeared within a day or two. The two recovered caterpillars were both parasitized. This suggests that some hosts will be predated soon after oviposition unless either the caterpillar's defenses or immediate intervention by the parasitoid prevent it. Second, different parasitoid species vary in how rapidly they alter host behavior. Two of the three species that parasitize *U. dorantes* allow the host to continue feeding and growing for a time, while the only parasitoid species that attacks *U. proteus* almost immediately induces the caterpillar to seal its shelter. It appears that this strategy allows the parasitoid to avoid the faster predation observed in *U. proteus* shelters. While these observations are based on very small samples, they illustrate the need for more study of how different parasitoids avoid host caterpillar predation.

#### *The role of shelters in creating EFS for caterpillars*

To create EFS for caterpillars, shelters must provide protection from predators and/or parasitoids, and shelters that result in higher caterpillar survival provide more EFS. Several studies of shelter-building caterpillars have demonstrated that they are quickly killed by predators when they are exposed on leaves (Jones et al. 2002, Covarrubias-Camarillo et al. 2016, Velasque and Del-Claro 2016). Our experiment took the next step by demonstrating that different shelters provide different amounts of predator-free space, as shown by the lower predation of caterpillars in *U. dorantes* shelters, specifically *U. dorantes* shriveled leaf folds. However, when the total EFS for caterpillars, including parasitoid-free space, is considered, the advantage of the *U. dorantes* shriveled leaf folds appears to be lost: they do not provide significantly more protection than the other shelters. This corresponds with our observation that caterpillars were unable to defend themselves against parasitoids when inside their shelters.

This experiment also demonstrates that immediately collecting caterpillars for parasitoid rearing only captures a small fraction of the third trophic level's impacts on caterpillars because predation can be more frequent and preempts parasitism. In this case, both *U. dorantes* and *U. proteus* spend approximately three weeks as caterpillars, and the two instar treatments used roughly correspond to the first and second weeks of development. Caterpillars in this study experienced at least a daily predation rate of 10.6%. While this daily rate may seem high, it is actually very similar to the average of 10.5% per day for externally feeding larvae estimated in a meta-analysis

(Rommel et al. 2011). Under a daily predation rate of 10.6%, only 21% of caterpillars would still survive after two weeks of growth. Regardless of predation intensity during the final week of caterpillar development, or the number of parasitized survivors, the number of caterpillars killed by parasitoids in this community pales beside the number killed by predators.

### *The broader context of animal shelters*

While this is the first study to test the effect of caterpillar shelter characteristics on shelter function, the connection between shelter shape and function has been studied in other animals. For instance, Weis and Abrahamson (1986) demonstrated that gall size affected predation and parasitism of the gall-maker, with different natural enemies targeting different-sized galls. However, the effects of shelter characteristics have been best-studied in bird nests. The qualitative and quantitative characteristics that determine nest success have been widely investigated using both comparative and manipulative methods, such as nest boxes. Although breeding birds have not been directly switched into other species' nests, supplying birds with different types of nest boxes also disentangles the characteristics of a shelter and the organism occupying it.

Many studies have found that differences in nest type affect various components of nest preference and nest success, including predation and nest parasitism (but see Takahasi et al. [2013] for a counter-example). These studies have compared open and closed nests (Takahasi et al. 2013), open and cavity nests (Lack 1954, Nice 1957, Ricklefs 1969, Martin and Li 1992, Purcell and Verner 1999), natural nests and nest boxes (Purcell et al. 1997, Evans et al. 2012, Libois et al. 2012, Brazill-Boast et al. 2013), and different types of nest boxes (Evans et al. 2012, Kaliński et al. 2014). However, attempts to identify variables within a nest type that explain predation or nest success are rarer, and have been less successful. Of ten studies that tested for such within-type variation, four could not identify a nest variable that predicted predation (Purcell et al. 1997, Purcell and Verner 1999, Takahasi et al. 2013, Lambrechts et al. 2016), while the other six studies (Møller 1990, Alabrudzińska et al. 2003, Antonov 2004, Biancucci and Martin 2010, Kaliński et al. 2014, Wysocki et al. 2015) found that nest size was positively correlated with predation. Antonov (2004) also found that nest wall density was negatively correlated with predation. Our results are consistent with this pattern, as we found that the more enclosed shelters in our study (the shriveled leaf folds) experienced less predation, but we were also unable to identify shape differences between predated and unpredated shelters of the same type (i.e., between predated and unpredated cut-and-fold shelters).

### *Shelter-switching as a method*

To our knowledge, this is the first field experiment to place shelter-builders into different naturally constructed shelters. The species used in this study did not react strongly to being placed in either con- or heterospecific shelters. In both cases, when placed inside a new shelter, the most common responses were to either rest inside the shelter or to secure the shelter with new silk. Occasionally, a caterpillar would immediately leave its new shelter, but when the caterpillar was returned to the shelter, it would remain there. When web-building *Depressaria pastinacella* caterpillars (Oecophoridae) were presented with vacant shelters in the laboratory, they chose to occupy them 88% of the time, rather than construct new ones, likely because silk is a costly resource (Berenbaum et al. 1993). Although *Urbanus* caterpillar shelters do not require as much silk as webs, any time that *Urbanus* caterpillars spend exposed during the day is dangerous, as shown by our own observation. As long as shelter-building caterpillar species generally remain in vacant shelters they are placed in, they are potential candidates for shelter-switching experiments.

This experiment demonstrated both that differences in shelter shape can affect predation and that

switching caterpillars into different shelters can be an effective method for testing the relationship between shelter shape and function. Future experiments should explore the relative effects of shelter and caterpillar identity on predation and parasitism both within and between the various families of shelter-building caterpillars. Such switches could determine whether shelters are generally these caterpillars' main defenses, or whether intrinsic caterpillar traits, such as morphology (Janzen et al. 2010), are also important in some cases. Larger shelter-switching experiments, or experiments in systems with higher parasitism, are also needed to assess whether shelter shape can have a direct effect on parasitism.

Shelter switching could also be used to assess the effect of shelter shape on other shelter functions, such as abiotic amelioration. While it can be difficult to directly measure shelters' environmental conditions due to their small size, shelter-switching experiments could be used to assess shelters' relative abiotic conditions. For instance, some shelter-building caterpillars are already known to require very high relative humidity (Willmer 1980, Hunter and Willmer 1989), so such humidity-sensitive species could be used to test the relative humidity of other shelters. Regardless of the function of interest, host plant compatibility will be necessary for all shelter-switching experiments. Accordingly, the best systems for these experiments will be diverse shelter-building assemblages on a single host plant species (for instance, many species of *Inga*, *Piper*, and *Quercus*). Such shelter-switching experiments will provide a better understanding of how different shelters influence the predation, parasitism, and abiotic conditions of their inhabitants.

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## SUPPLEMENTAL INFORMATION

### *Parasitoid species and life histories*

Eulophid spp. 1 and 2:

Eulophid sp. 1 was only reared from *U. dorantes* caterpillars and Eulophid sp. 2 was only reared from *U. proteus* caterpillars. There is a 4.4% difference in *cox1* between the two parasitoid species.

One parasitized *U. proteus* caterpillar was from the early-instar cohort; the other caterpillars were from the mid-instar cohort. Because the mid-instar cohort caterpillars were reared in the lab, the parasitoids must have oviposited on the caterpillars after they were returned to the field. The *U. proteus*-feeding species was also reared from second instar *U. proteus* caterpillars not included in the experiment, providing further evidence that these parasitism events occurred during the experiment.

Both parasitoid species triggered similar behaviors in their hosts. A parasitized caterpillar would seal its shelter with silk and remain inside without feeding for two to three days. After that time, the parasitoids would emerge, consuming nearly the entire caterpillar in the process. The number of emerging larvae depending on the size of the caterpillar: one to two emerged from nonfocal second instar caterpillars, but as many as nine larvae emerged from mid-instar caterpillars. The larvae pupated within the shelter and did not spin cocoons.



Tachinid sp. 1:

The parasitoid emerged at the end of the host's third instar in one case and during the fourth instar in the other. Neither parasitoid pupa eclosed before the end of the field season. DNA barcoding indicates that they belong to the same species and are tachinid flies, but the barcodes are not close matches to any other tachinids in GenBank.

*Apanteles* sp. Rodriguez24 (Braconidae; BOLD:ACF3142)

The parasitoid larvae (usually 10-20) emerge in the caterpillar's final instar and spin small cottony cocoons on and around the caterpillar's corpse (Fig. 3F). They have only been reared from *U. dorantes* caterpillars.

## CHAPTER 4: Complex interactions between shelter traits, predation, and parasitism in a caterpillar community

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### ABSTRACT

Predation and parasitism are known to be affected by caterpillar shelters in general, and are widely believed to be negatively correlated between shelter-building and free-feeding species. However, the specific effects of particular shelters on predation and parasitism are unknown and such a negative correlation between predation and parasitism has not been directly demonstrated. To understand these relationships, shelter traits, predation, and parasitism were measured simultaneously for 24 caterpillar morphospecies in a tropical dry forest and analyzed in a phylogenetic context. Shelter type, shelter openness, and whether shelters accumulated frass were found to have different amounts of phylogenetic signal. In turn, all three traits affected the frequency with which caterpillar species experienced predation. Predation was elevated in three shelter types (leaf folds, leaf rolls, and leaf ties) compared to cut-and-fold shelters and webs, while different combinations of shelter openness and frass accumulation resulted in different levels of predation, with closed frass-free shelters having the lowest predation and closed frass-filled shelters having the highest. Parasitism, however, was not affected by shelter traits, but did show a strong negative correlation with predation. These results provide the first confirmation of a trade-off between predation and parasitism and demonstrate that different shelters result in different amounts of predation. This is also the first study to show that these defensive shelter traits vary phylogenetically. As shelter-building caterpillars are frequently diverse and abundant components of many communities, these interactions between shelters, predation, and parasitism have the potential to affect a wide range of other organisms.

### INTRODUCTION

Many studies of caterpillars have proposed and tested morphological, chemical, and behavioral traits that may defend caterpillars from predators and parasitoids (e.g., Stamp 1982a, Dyer and Floyd 1993, Dyer 1997, Gentry and Dyer 2002, Smilianich et al. 2009, Murphy et al. 2010, McClure and Despland 2011, Murphy et al. 2014, Sugiura and Yamazaki 2014, Lichter-Marck et al. 2015, Sendoya and Oliveira 2017; recently reviewed in Greeney et al. [2012] and Zvereva and Kozlov [2016]). Generally, these studies have investigated morphological or chemical defenses, which have been broadly demonstrated to decrease the success of predators and/or generalist parasitoids, but are less effective against specialists. A few studies have considered other potential defenses, including immunological (Smilianich et al. 2009) or behavioral (Gentry and Dyer 2002, Sendoya and Oliveira 2017) defenses, but these have been less well-studied. One such behavioral defense is shelter-building by caterpillars, which is relatively poorly understood. Although tens of thousands of caterpillar species from at least 26 families build shelters (Chapter 1), most studies of caterpillar defenses have focused on free-feeding caterpillars, and when shelter-building caterpillars have been included, they have been compared as a group to free-feeding caterpillars (Gentry and Dyer 2002, Connahs et al. 2011, Diniz et al. 2012, Hreck et al. 2013). These studies, as well as several experiments in which shelter-building caterpillars have been exposed to natural enemies without their shelters (Jones et al. 2002, Covarrubias-Camarillo

et al. 2016, Velasque and Del-Claro 2016, Sendoya and Oliveira 2017), demonstrate that caterpillar shelters in general can affect a caterpillar's chances of predation and parasitism.

However, whether different shelters provide different defenses and whether specific shelter traits affect predation or parasitism both remain unknown. Given the structural diversity of caterpillar shelters, which vary in type, openness, and frass accumulation, among other traits (Chapter 1), it is unlikely that all shelters or all shelter traits are equally effective against all predators and parasitoids. In fact, there may be a trade-off between defending against predators and defending against parasitoids. The comparisons between shelter-building and free-feeding caterpillars suggest that shelters increase parasitism (Gentry and Dyer 2002, Connahs et al. 2011, Hreck et al. 2013, but see Diniz et al. 2012). These investigators have proposed that this is because shelter-building caterpillars experience less predation than free-feeding caterpillars and are thus safer hosts for parasitoids. If this is the case, shelter-building caterpillars would be a prime example of the enemy-free space hypothesis applied to parasitoids (Jeffries and Lawton 1984, Murphy et al. 2014). While the parasitoid enemy-free space hypothesis has primarily been invoked when comparing shelter-builders to free-feeders, it can apply to any situation in which there is differential predation. If the hypothesis is correct, and if shelter differences affect predation, then those shelter-building species with higher predation should experience lower parasitism.

To answer these questions about the relationships between shelter traits, predation, and parasitism, we measured these variables in a community of shelter-building caterpillars. We hypothesized that shelter type, shelter openness, and the level of frass accumulation would contribute to the likelihood of predation. We predicted that shelter types would vary in the frequency of predation, although we did not have an *a priori* prediction about which shelter types (webs, leaf folds, leaf rolls, leaf ties, or cut-and-fold shelters) would be associated with higher predation. We did predict that predation would be higher in open shelters than closed shelters, due to their greater accessibility, and that open shelters with frass would have the highest predation, as frass has been shown to attract predators (Weiss 2003, Moraes et al. 2012). In accordance with the parasitoid enemy-free space hypothesis, we predicted that parasitism and predation would be negatively correlated, and that shelter factors associated with low predation would be associated with high parasitism. We constructed a phylogeny of the caterpillar species included to account for the relatedness of the different species. Caterpillar shelters and their potential effects on predation and parasitism were all analyzed within that phylogenetic context.

## METHODS

### *Study site and organisms*

The research was conducted in Palo Verde National Park, Guanacaste, Costa Rica, in the area around Palo Verde Biological Station (PVBS) (10° 21' N, 85° 21' W, elevation approximately 0-200 m asl). PVBS is surrounded by secondary tropical dry forest and is adjacent to a large seasonal wetland. Fieldwork was performed from late May to early August 2014-2016, during what is normally the first part of the wet season (May-November).

At least 20 individuals per morphospecies (mean  $\pm$  SE: 39.8  $\pm$  6.1) were collected for 24 morphospecies of shelter-building caterpillars (Table 1). One morphospecies (Crambidae species complex 1) consists of three species, but as their caterpillars and shelters are indistinguishable and they are more closely related to each other than to any of the other species, they are analyzed as a species complex here.

**Table 1.** Shelter-building morphospecies used in this study. “Undescribed” means that a species matches an established DNA barcode, but that the specimens corresponding to that barcode have not been described or given a single working name.

Morphospecies Reference Name	Family	Species	Host Plant Family
<i>Calpododes ethlius</i>	Hesperiidae	<i>Calpododes ethlius</i>	Marantaceae
<i>Urbanus dorantes</i>	Hesperiidae	<i>Urbanus dorantes</i>	Fabaceae
<i>Urbanus proteus</i>	Hesperiidae	<i>Urbanus proteus</i>	Fabaceae
<i>Psara obscuralis</i>	Crambidae	<i>Psara obscuralis</i>	Petiveriaceae
<i>Lativalva pseudosmithii</i>	Crambidae	<i>Lativalva pseudosmithii</i>	Capparaceae
Crambidae sp. 1	tentatively Crambidae	Not previously barcoded	Capparaceae
Gelechiidae sp. 1	Gelechiidae	Undescribed	Fabaceae
Pyralidae sp. 1	Pyralidae	epipaJanzen01 Janzen15DHJ02	Polygonaceae
Diaphania sp.	Crambidae	<i>Diaphania</i> Dapkey02	Apocynaceae
Gelechiidae sp. 2	Gelechiidae	Undescribed	Combretaceae
<i>Massepha grammalis</i> DHJ02	Crambidae	<i>Massepha grammalis</i> DHJ02	Marantaceae
<i>Eulepte concordalis</i>	Crambidae	<i>Eulepte concordalis</i>	Bignoniaceae
Crambidae sp. 2	tentatively Crambidae	<i>Phaedropsis</i> Janzen10	Malvaceae
<i>Antigonus erosus</i>	Hesperiidae	<i>Antigonus erosus</i>	Malvaceae
Pyralidae sp. 2	tentatively Pyralidae	chryJanzen01 Janzen347	Solanaceae
Unknown sp. 1	Gelechiidae/Elachistidae	Undescribed	Polygonaceae
<i>Anaea aidea</i>	Nymphalidae	<i>Anaea aidea</i>	Euphorbiaceae
Unknown sp. 2	Unidentified	Not previously barcoded	Capparaceae
<i>Dichogama colotha</i>	Crambidae	<i>Dichogama colotha</i>	Capparaceae
Elachistidae sp. 1	Elachistidae	elachJanzen01 Janzen737	Primulaceae
<i>Pococera sabbasa</i>	Pyralidae	<i>Pococera sabbasa</i> Janzen02	Fabaceae
<i>Aristotelia corallina</i>	Gelechiidae	<i>Aristotelia corallina</i> complex	Fabaceae
Crambidae species complex 1	Crambidae	<i>Syllepte belialis</i> <i>Chilochromopsis sceletogramma</i> <i>Pilocrocis calamistis</i>	Salicaceae
Gelechiidae sp. 3	Gelechiidae	Unidentified	Fabaceae

### Field methods

We searched for caterpillar shelters primarily along roads, human- and animal-made trails, and natural edges, as these areas had the most accessible foliage. We visually scanned foliage from ground level to ~ 3 m for shelters and feeding damage. Caterpillar shelters found in the field were checked for inhabitants by back-lighting them with a hand light. If the shelter was inhabited, the plant and shelter were marked and photographed. The shelter dimensions were measured to the nearest millimeter, the plant’s height was estimated, and the length of the caterpillar was also measured. Each plant was then checked for other shelter-building caterpillars. Caterpillars were collected when we estimated that they were at least half-grown based on their size. The original size cut-off used was 15 mm, as this corresponded to third instar caterpillars for common Palo

Verde hesperiids (e.g., *Calpododes ethlius*, *Urbanus dorantes*, and *U. proteus*). However, as many microlepidopteran caterpillars never reached 15 mm or reached it much later in development, those species were collected when they reached 10 mm in length. If a caterpillar was estimated to be less than half-grown, we checked it each day until it either reached that size or had been monitored for six days, at which time it was collected.

At each daily check, we located and measured the caterpillar and documented any new shelters built by the caterpillar. If we could not find a caterpillar in its shelter, we searched the plant and any touching conspecific plants for the caterpillar. If at least two days passed without locating the caterpillar, the caterpillar's disappearance was recorded as occurring the day after it was last seen and all shelters were collected. As shelter-building caterpillars spend nearly all their time in or near their shelters, the disappearance of a caterpillar was assumed to represent a predation event. Any other evidence of predation was recorded, including damage to the shelter, a predator attacking a caterpillar, or a predator in or near the shelter. If a dead caterpillar was found in a shelter or there was evidence of parasitoid emergence from a caterpillar, the shelter and its contents were immediately collected.

Surviving caterpillars were reared in an ambient air laboratory at PVBS inside their shelters in plastic bags. To maintain the foliage, stems were placed in individual florists' tubes filled with water. Fresh foliage was added as needed and caterpillars were checked daily for new shelters, ecdysis, pupation, eclosion, and parasitoid emergence. Old shelters were collected and measured once they were no longer in use.

#### *Shelter characteristics*

Shelters were photographed from multiple angles after collection or when newly built in the lab. Shelters vacated by caterpillars were opened, placed underneath a sheet of plastic and scanned with a handheld document scanner. In addition to this documentation, several shelter characteristics were recorded for each shelter: shelter type (web, leaf tie, leaf fold, leaf roll, or cut-and-fold shelter), whether the shelter was open or closed, and whether frass accumulated in the shelter. These traits were assessed based on the criteria described in Chapter 1.

#### *Laboratory methods*

All dead caterpillars and pupae were dissected for parasitoid larvae that had not emerged. Caterpillars and adults were identified using *cox1* DNA barcoding (methods as in Chapter 2). Two nuclear genes, *EFL- $\alpha$*  and *wg*, were also amplified using primers from Cho et al. (1995) and Brower and LaSalle (1998) and modified PCR methodologies based on those of Wahlberg and West Wheat (2008) (Tables 2 and 3). The amplicons were purified using the same methods as the *cox1* amplicons, and were sent to Eurofins Genomics (Louisville, KY, USA) for sequencing with 10  $\mu$ M solutions of the PCR primers as sequencing primers.

**Table 2.** PCR cocktail for *EF1- $\alpha$*  and *wg* reactions.

Reagent	Volume per reaction ( $\mu$ l)
5x Taq buffer	4.0
10 mM dNTPs	0.4
25 mM MgCl <sub>2</sub>	2.0
10 $\mu$ M Forward primer	1.0
10 $\mu$ M Reverse primer	1.0
10 mg/mL BSA	1.6
5 units/ $\mu$ l Taq (Promega)	0.1
Template DNA	5
Autoclaved doubly-distilled H <sub>2</sub> O	4.9
TOTAL VOLUME	20

**Table 3.** PCR programs for *EF1- $\alpha$*  and *wg* reactions.

PCR Program Parameter	<i>EF1-<math>\alpha</math></i> reactions	<i>wg</i> reactions
Initial denaturation	95°C for 7 min	95°C for 7 min
Cycle denaturation	95°C for 30 s	95°C for 30 s
Cycle annealing	57°C for 30 s	50°C for 30 s
Cycle extension	72°C for 2 min	72°C for 2 min
Number of cycles	40	40
Final extension	72°C for 10 min	72°C for 30 min
Final soak	10°C	10°C

### *Phylogenetic reconstruction and statistics*

The gene sequences for the Palo Verde species were combined with *cox1*, *EF1- $\alpha$*  and *wg* sequences from the 2010 phylogeny by Mutanen and colleagues. Each gene was aligned individually in AliView (version 1.18.1, Larsson 2014) using MUSCLE (Edgar 2004) and then checked by eye. Individual gene trees were then generated using RAxML (Stamatakis 2014) through the CIPRES Science Gateway (Miller et al. 2010), using *Micropterix calthella* (Micropterigidae) as an outgroup. After the gene trees were examined, the genes were concatenated in Mesquite (version 3.31, Maddison and Maddison 2017). A species tree was generated in RAxML using the partitioned three-gene matrix and an initial tree constraining the families supported by the full eight-gene Mutanen et al. (2010) tree to monophyly. For Palo Verde taxa, this was done if the morphospecies had been identified as a described species belonging to that family, but not if the species had only been tentatively identified as belonging to that family. When the resulting tree was examined, we found that the Mutanen et al. taxa near the focal taxa did not provide additional information about how the focal taxa were related to each other. Accordingly, the Mutanen taxa were removed except for *M. calthella* and *Agathiphaga queenslandensis* (Agathiphagidae), which were kept as outgroups. A smaller tree was then generated using this partitioned data set, again constrained so that shelter-building morphospecies which had been identified to species in two families supported by the full Mutanen et al. (2010) phylogeny (Crambidae and Hesperidae) would be monophyletic. This final tree was made

ultrametric in R (R Core Team 2016) using the ‘ape’ package (Paradis et al. 2004).

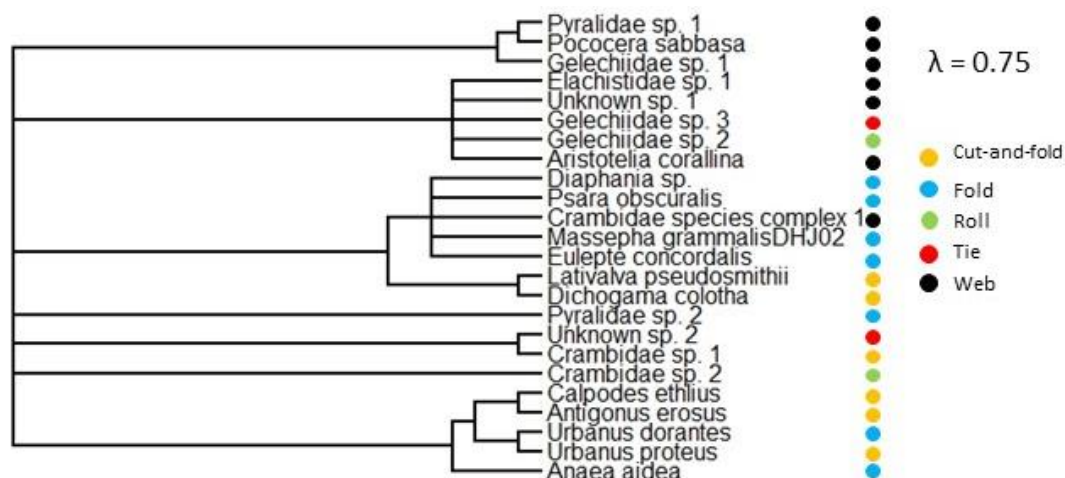
All phylogenetic least squares (PGLS) analyses were performed in R using ‘ape’. Because PGLS requires variables to be analyzed at the species level, a species’ parasitism was expressed as the number of parasitized and unparasitized caterpillars. Predation was expressed similarly, as the number of caterpillar-days during which caterpillars were and were not predated. When the numbers of parasitism and predation events were divided by their respective sample sizes (total caterpillars or caterpillar-days), the proportion of parasitized caterpillars and the daily predation rate were given. For shelter traits, shelter type was treated as an unranked categorical variable, while openness and frass accumulation were binomial variables. Some focal species build different shelters depending on circumstances (see Chapter 1); in these cases, shelter traits were drawn from the most common shelter built while caterpillars were exposed in the field.

The relationships between the different shelter traits and the effect of phylogeny were analyzed by modeling each trait as an independent variable predicted by the other two. In each case, Pagel’s lambda was calculated to measure phylogenetic signal. Potential interactions between shelter type and the other variables could not be analyzed as some combinations of shelter type and openness or shelter type and frass accumulation were not present in the data set. Parasitism and predation were modeled separately using the three shelter traits as independent variables and Pagel’s lambda was calculated in each case. The correlation between parasitism and predation was also calculated.

## RESULTS

### *Shelter traits*

Shelter type cannot be predicted based on shelter openness or frass accumulation, but shelter type does show a strong phylogenetic signal (Pagel’s  $\lambda = 0.75$ , Fig. 1). Shelter openness can be predicted by shelter type (leaf rolls are more likely to be open than other shelter types,  $p = 0.044$ ) and frass accumulation (frass-filled shelters are less likely to be open,  $p = 0.012$ ), although shelter openness also displays some phylogenetic signal (Pagel’s  $\lambda = 0.45$ ). Frass accumulation appears to be driven primarily by phylogeny (Pagel’s  $\lambda = 1.04$ ), although shelter type also has an effect: webs and leaf rolls are less likely to accumulate frass than the three other shelter types ( $p = 0.032$  and  $0.045$ , respectively).

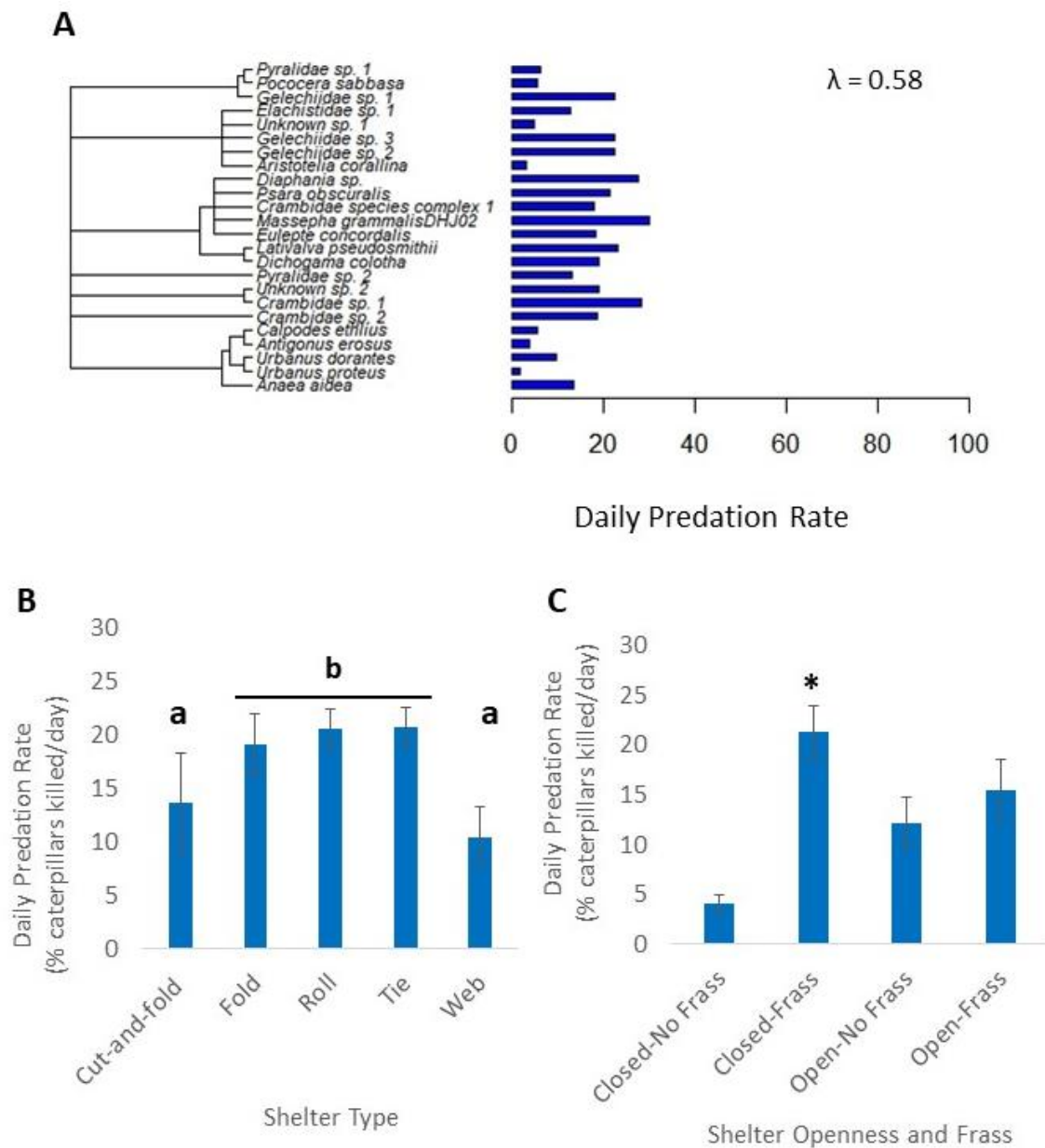


**Figure 1.** Focal species' shelter types mapped onto the phylogeny.

### *Predation*

Species' daily predation rates ranged from 1.67% to 29.85% (Fig. 2A). There was some phylogenetic signal for predation (Pagel's  $\lambda = 0.58$ ), and shelter type, as well as the interaction between shelter openness and frass accumulation, were significant predictors of the remaining variation in predation. Of the five shelter types (Fig. 2B), leaf folds, rolls, and ties all had significantly higher predation than cut-and-fold shelters ( $p = 0.002$ ,  $0.020$ , and  $0.001$ , respectively), while webs had predation levels similar to cut-and-fold shelters ( $p = 0.079$ ). While neither shelter openness nor frass accumulation alone had a significant effect on predation, this appears to be because they negatively interact with each other (Fig. 2C).



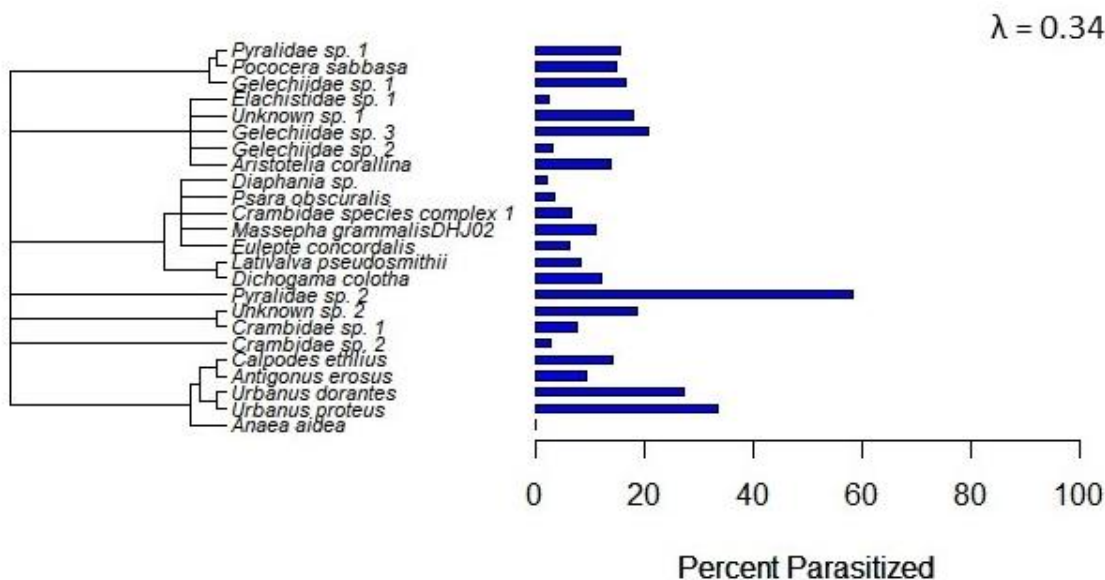


**Figure 2.** A. Daily predation rates mapped onto the phylogeny. Pagel's lambda indicates the strength of the phylogenetic signal. B. Daily predation rates by shelter type. Error bars = standard errors. Different letters indicate shelter types with significantly different predation. C. Daily predation rates by shelter openness and frass accumulation. Error bars = standard errors. The asterisk indicates a significant difference in predation compared to Closed-No Frass shelters.

### Parasitism

Parasitism for the different species ranged from 0% to 58% (Fig. 3A), with some phylogenetic signal (Pagel's  $\lambda = 0.34$ ). None of the shelter traits had a significant effect on parasitism. However, parasitism was negatively correlated with predation (Fig. 3B,  $r = -0.476$ ,  $p = 0.042$ ). The proportion of caterpillars that survived both predators and parasitoids was generally 50-90%

(Supplemental Figure 1), although these numbers should not be considered to represent the number of caterpillars surviving to adulthood in the field.



**Figure 3.** Percentages of parasitized caterpillars mapped onto the phylogeny. Pagel's lambda indicates the strength of the phylogenetic signal.

## DISCUSSION

Caterpillar shelters vary greatly in their size, shape, amount of silk, openness, and frass accumulation, even in this relatively small sample of 24 shelter-building morphospecies from a single site. These differences in shelter traits have a phylogenetic component, and shelter traits vary in their phylogenetic signal and relationships with other shelter traits, ranging from traits that are primarily determined by other traits (e.g., shelter openness) to traits that are very phylogenetically conserved (e.g., frass accumulation). These shelter differences have consequences for predation, with different shelters resulting in different amounts of predation. In turn, the results suggest that shelter traits also have consequences for parasitism because parasitoids avoid caterpillar species that are likely to be killed by predators.

### *The phylogenetics of shelter-building*

Caterpillar shelters have been suggested to be phylogenetically informative within families (Greeney and Jones 2003), and it has long been recognized that different lepidopteran families vary in the shelters they build (Stehr 1987, Jones 1999). However, to our knowledge, caterpillar shelters have not been previously mapped onto a phylogeny. Although this phylogeny is incompletely resolved and includes only a subset of the Palo Verde shelter-building community, it still raises several interesting points. First, webs are built by members of each of the three clades corresponding to the Crambidae, Gelechioidea, and Pyralidae. The only large clade in this tree that does not include at least one web-builder is the Hesperidae + Nymphalidae clade, but some nymphalid caterpillars build webs (Wynter-Blyth 1957, Stamp 1982b, DeVries 1987). This suggests that web-building is, if not the basal shelter type, the most phylogenetically widespread.

Second, this analysis suggests some intrafamilial patterns worthy of further study. In one case, the two crambid species that build cut-and-fold shelters form a clade of their own within the family, and it would be interesting to test whether other crambid species building cut-and-fold shelters also belong to that clade. In another, the two species of the polyphyletic *Urbanus* genus (Hesperiidae; Pfeiler et al. 2016) included in this analysis build different types of primary shelters, and analyzing the shelter traits of other species in that group may be informative. Mapping shelter types onto more complete phylogenies will certainly give further insights into the evolution of shelter-building.

Also of interest is the substantial variation in phylogenetic signal for different shelter traits. Shelter type showed strong a phylogenetic signal, while shelter openness showed less signal, and frass accumulation appears to be almost entirely predicted by phylogeny. One reason for frass accumulation's strong phylogenetic signal may be that frass-free shelters are frequently the result of morphological structures that allow caterpillars to excrete frass at high velocities. While these anal structures are not limited to shelter-building caterpillars, members of the Gelechiidae, Hesperiidae, and Nymphalidae are known to possess them (Weiss 2003), and eight of the eleven focal species that build frass-free shelters belong to these families. By contrast, shelter type and openness are traits that are behaviorally controlled, and may also depend on host plant characteristics. This may explain why these traits display less phylogenetic signal than frass accumulation.

#### *Predation and shelters*

This study demonstrates that certain shelter traits, such as shelter type, can affect predation, even when considered across a wide range of distantly related shelter-building species. In this community, leaf folds, rolls, and ties all experience higher predation than cut-and-fold shelters and webs. Further study is needed to determine whether this holds true in other shelter-building communities and why these shelter types are more vulnerable at Palo Verde. The effect of shelter openness and frass accumulation on predation also shows that shelter traits can have interacting effects on predation. Contrary to our prediction, the highest predation occurred in closed shelters that accumulate frass, while the lowest predation occurred in closed, frass-free shelters. In open shelters however, frass accumulation did not strongly affect predation. Frass has been shown to attract predators to open shelters (Weiss 2003, Moraes et al. 2012), but no behavioral experiments have been performed with predators and closed shelters. As many closed shelters are closed using frass in combination with silk, it may be that large amounts of frass volatiles are released at the shelter edges and the decreased accessibility of the shelter does not compensate for its increased apparency. Given the lack of damage to these shelters, it appears that predators may attack when the caterpillars leave the shelter to feed, perform maintenance, or build a new shelter.

More information about the predators of these shelter-building caterpillars may be necessary to understand how shelter traits affect predation. During the study, the most commonly observed predators of shelter-building caterpillars were actively hunting spiders (particularly the green lynx spider, *Peucea viridans*: Family Oxyopidae) and predatory wasps. Although ants and predatory hemipterans are known to attack free-feeding caterpillars at Palo Verde (Dyer 1997), they were rarely observed attacking or feeding on shelter-building caterpillars in this study. Ants in a Brazilian cerrado community rarely attacked caterpillars inside their shelters (Sendoya and Oliveira 2017), so ants may be more important predators of free-feeding caterpillars. Additionally, three caterpillar species in this study (Gelechiidae sp. 1, *Pococera sabbasa*, *Aristotelia corallina*) feed on plants with extra-floral nectaries, but ant activity on their specific host plants was intermittent or nonexistent. This pattern may reflect either rapid predation of eggs or newly hatched (unsheltered) caterpillars on plants with better ant attendance or lepidopteran

avoidance of such plants. Ovipositing females of both myrmecophilous (Pierce and Elgar 1985) and non-myrmecophilous species (Sendoya et al. 2009) can use ant presence and identity to assess the suitability of individual plants for oviposition. Mantids and orthopterans were also occasionally observed in or near the shelters of vanished caterpillars, and may have caused those disappearances, but they were never caught in the act of predation. All these observations suggest that the vast majority of predators were invertebrates. Only one probable case of vertebrate predation was observed, in which large holes were torn in a web to reach the caterpillar's resting site. Vertebrate bite damage to leaf shelters as described by Tvardikova and Novotny (2012) was never observed. These results are consistent with several artificial caterpillar experiments that found that most predation attempts were made by invertebrates in lowland tropical forests (Loiselle and Farji-Brener 2002, Richards and Coley 2007, Tvardikova and Novotny 2012, Sam et al. 2014, Roslin et al. 2017, Solis-Gabriel et al. 2017).

Natural caterpillar predation rates have rarely been measured directly in the field, as monitoring the survival of freely moving caterpillars is challenging. Accordingly, much of what is known about the frequency of caterpillar predation comes from experiments with artificial caterpillars such as those mentioned above, and studies that assess specific predators' caterpillar preferences (e.g., Janzen 1987, Dyer and Floyd 1993, Dyer 1997, Murphy et al. 2010, Sendoya and Oliveira 2017). Directly measuring the predation of caterpillars in the field is facilitated by shelter-builders, because a shelter both serves as a record of the caterpillar's presence and the caterpillar's center of activity. The usefulness of shelter-building caterpillars for studying predation and other causes of mortality can be seen in the literature: those studies that have tracked predation in the field or constructed life tables have commonly used caterpillars that live in shelters or other refuges, although this is rarely highlighted (e.g., Morris and Miller 1954, Gibb 1958, Korzytkowski and Ruiz 1979, Vargas and Nishida 1980, Crawford and Jennings 1989, Okeyo-Owuor and Oloo 1991, Caldas 1992, Gomes-Filho 2003). While tracking a shelter-building caterpillar from day to day is easier than tracking a free-feeding one, some shelters can be more difficult to monitor than others. If a shelter is difficult to see into, then it is difficult to determine whether a shelter is occupied or not. This was particularly true of leaf-rolling species in this study (*Gelechiidae* sp. 2 and *Crambidae* sp. 2), whose continued presence often had to be assessed using feeding damage or changes to the shelter structure, rather than direct observation of the caterpillar. The disappearance of some caterpillars may also be more complicated than simple predation, as caterpillars can fall off their plants while seeking new shelter sites (Loeffler 1996, Sliwinski and Sigmon 2013). Although these caterpillars are not removed from the plant by predators, it is likely that they do not return to the plant because they are killed by terrestrial predators (Sliwinski and Sigmon 2013). Despite these complications, shelter-building caterpillars offer an excellent opportunity to directly measure predation in the field. These measurements can be used to understand predation at a community level, as well as to assess how common predation estimates (artificial caterpillars or predator attack trials) compare to natural predation rates.

#### *Parasitism, shelters, and predation*

There was no evidence that shelter traits influenced parasitism, which contradicted our prediction that the same shelter traits would affect both predation and parasitism. Despite this, there was a strong negative correlation between parasitism and predation, which was influenced by shelter traits. There are at least three possible explanations for this pattern, although two seem unlikely. First, the correlation between parasitism and predation could be non-causal and parasitism could be driven by another caterpillar trait that is negatively correlated with predation. Caterpillar immune responses to implanted objects, for instance, have been shown to be better predictors of parasitism in La Selva caterpillars than the factors previously identified in that community by Gentry and Dyer (2002) (Smilanich et al. 2009). If parasitism at Palo Verde is primarily driven by

caterpillars' immunological defenses, parasitoids would still be seeking enemy-free space, but the "enemy" would be the caterpillar's immune system rather than predators. However, any relationship between caterpillar species' immune responses and predation has not been investigated, and there is no particular expectation that they would be negatively correlated.

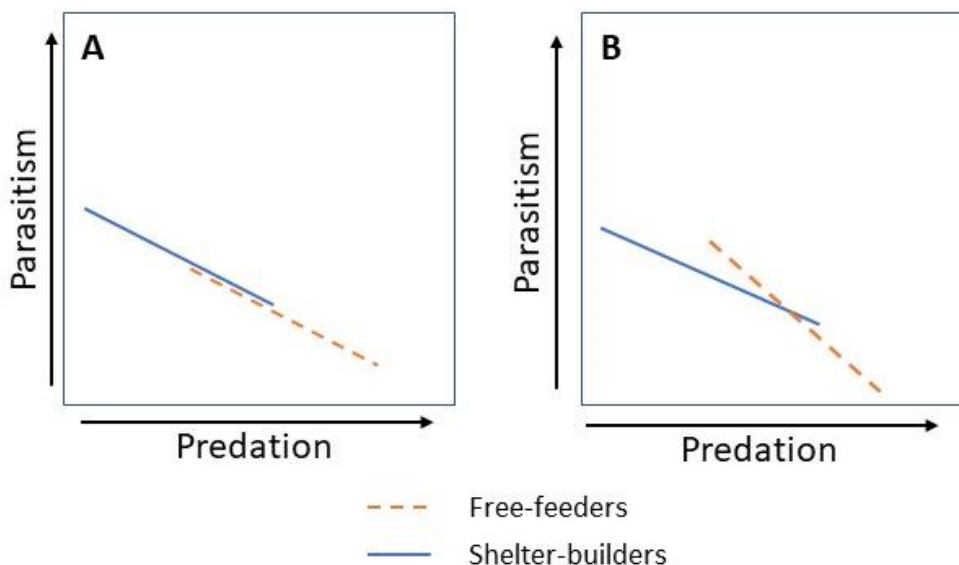
Second, parasitism could be correlated with predation due to non-shelter traits that influence both but in opposite directions, such as chemical or morphological defenses, which have been shown to influence parasitism and predation in free-feeding caterpillars (Dyer and Floyd 1993, Gentry and Dyer 2002, Murphy et al. 2010, Murphy et al. 2014). Although all the caterpillar species used in this study appeared glabrous and lacking in warning coloration to the human eye, no systematic observations of predators or parasitoids interacting with caterpillars have been made. These caterpillars also feed on host plants from fourteen different families (Table 1) with diverse secondary chemistries. Whether these compounds influence predation or parasitism is unknown.

Third, shelter traits may only indirectly influence parasitism through their effects on predation. That additional variation may obscure those shelter effects in the current analysis, which has both relatively few parasitism events and a relatively small number of species. Currently, potential indirect effects cannot be analyzed with path analysis because the sample size is too small (as the unit of replication is the morphospecies). Future research could address these three alternative hypotheses by collecting data on the immunological, morphological and chemical acceptability of more caterpillar species and analyzing the interactions between those traits, predation, and parasitism.

Regardless of the underlying reasons for the negative correlation between predation and parasitism in this community, this study provides strong support for the parasitoid enemy-free space hypothesis (Murphy et al. 2014), which proposes that parasitoids prefer hosts in which they are less likely to be killed by predators. To our knowledge, this study is the first to simultaneously quantify both predation and parasitism for a set of caterpillar species whose different traits create a gradient of defenses, although other studies have shown that defense gradients affect either parasitism or predation, often in contrasting directions (e.g., Dyer and Floyd 1993, Gentry and Dyer 2002, Murphy et al. 2010, Murphy et al. 2014). As the parasitism of shelter-building caterpillars is apparently linked to predation, this result suggests that the parasitoid enemy-free space hypothesis can answer one of the major questions raised by defense studies comparing shelter-building and free-feeding caterpillars: why are these groups parasitized at different frequencies?

Nearly all these comparisons have found that shelter-building caterpillars are more heavily parasitized than free-feeding caterpillars (Gentry and Dyer 2002, Connahs et al. 2011, Hreck et al. 2013). Accordingly, the hypothesis that this is due to parasitoids seeking predator-free space in shelter-building caterpillars has frequently been proposed, although the actual predation experienced by those sheltered and free-feeding caterpillars remains unknown. Similarly, the relative predation rates for these two groups are unknown in a cerrado community in which shelter-building caterpillars are less parasitized than exposed caterpillars (Diniz et al. 2012). These apparently contradictory results could be consistent with each other and the enemy-free space hypothesis if predation is higher for shelter-building caterpillars than free-feeding ones in the cerrado but not for the other communities that have been studied. In addition to the current study, this potential relationship is also somewhat supported by the work of Tvardikova and Novotny (2012), which was performed in the same area as the Hreck et al. (2013) study and found that predation was lower for clay caterpillars in leaf shelters. Further tests of the parasitoid enemy-free space hypothesis in these and other caterpillar communities could be performed by assessing predation and parasitism not just for shelter-builders and free-feeders in general but for

a gradient of individual species. This would allow investigators to test whether shelter-building and free-feeding caterpillars simply occupy somewhat different ranges of the same parasitism-predation relationship or if shelter-builders and free-feeders exhibit different relationships between parasitism and predation (Fig. 4).



**Figure 4.** Two possible parasitism-predation relationships for free-feeding and shelter-building caterpillars. A. The two groups experience the same negative correlation for parasitism and predation. B. The parasitism-predation correlations are different.

More broadly, caterpillar shelters can be conceptualized as constructed niches, heritable environmental modifications that in turn create unique selection pressures for the constructing organism (Odling-Smee et al. 2003). Under the framework of niche construction, caterpillar shelters can be compared to a wide range of defensive structures, from nests and burrows (e.g., Rand and Host 1942, Ricklefs 1969, Martin and Li 1992, Weber et al. 2013) to external shells (e.g., Vermeij 1977, Vermeij 1983, Boulding 1984) and galls (Weis and Abrahamson 1986). Unlike many of these structures, however, there does not yet appear to be a clear relationship between the complexity of caterpillar shelters and their effectiveness against predation or parasitism. For instance, leaf rolls are more structurally complex than leaf folds, but both experience similar (and relatively high) predation. This may change as caterpillar shelters' effects on predation and parasitism become more widely measured and sample sizes increase, but it may also reflect the unique challenges of defending against predators and parasitoids. Compared to pathogens and parasites, which reproduce within their hosts and have opportunities to be successfully transmitted to new hosts before (or even during) host predation, parasitoids are under intense selection to choose hosts that will not be predated before the parasitoid has completed its development (Lafferty and Kuris 2002). Gall-inducing insects are also targeted by predators and parasitoids, and Weis and Abrahamson (1986) found that these two guilds exerted opposing pressures on gall-makers, with the overall direction of selection shifting depending on whether predators or parasitoids were more abundant at a particular time and place. Shelter-building caterpillars are probably conducting a similar balancing act between defending themselves against predators and parasitoids.

## CONCLUSIONS

Simultaneously quantifying predation, parasitism, and potential defensive traits for multiple prey species can offer significant insights about a community. In this case, it showed that predation can be predicted by shelter traits and that it is negatively correlated with parasitism across a wide range of species. These results suggest that a caterpillar community's shelters determine which species will be more heavily preyed upon, and perhaps which predators will commonly feed on shelter-building caterpillars. As shelter-building caterpillars are often the most abundant caterpillars (Lill and Marquis 2007, Diniz et al. 2012, Hreck et al. 2013), and potentially the most abundant insect herbivores (Lill and Marquis 2007) in communities, shelter effects on predation may have wide-ranging effects on a community as a whole. Meanwhile, the negative relationship between predation and parasitism provides support for the parasitoid enemy-free space hypothesis. Such a relationship between predation and parasitism not only has the potential to explain many of the patterns found in caterpillar communities, but can also inform management decisions, including whether non-native or pest caterpillars (many of which are shelter-builders, Lill and Marquis [2007]) would be more vulnerable to predators or parasitoids. Different caterpillar shelters are not the same when it comes to providing protection from predators and parasitoids, and much can be learned by studying those differences.

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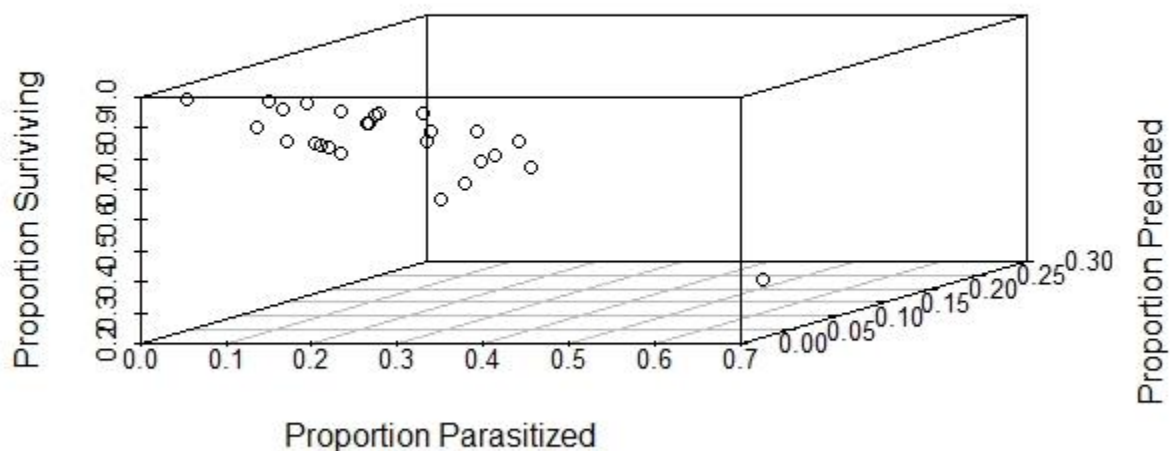
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#### SUPPLEMENTAL INFORMATION

**Supplemental Figure 1.** Three-dimensional scatterplot of caterpillar survival, predation, and parasitism.



## APPENDIX: PALO VERDE CATERPILLARS AND THEIR SHELTERS

### NOTES ON TERMINOLOGY

Silk and shelter types are defined in Chapter 1.

“Abaxial” and “adaxial” refer to the bottom and top surfaces of a leaf, respectively. In the context of shelter-building, if a leaf is folded abaxially, it means that the bottom surfaces of the leaf are folded together (below the plane of the leaf) and the top surface of the leaf faces outward.

Feeding types:

Scraping = Removing only part of the leaf cross-section; at least one layer of epidermis remains intact.

Cutting = Cutting off pieces of the entire leaf cross-section.

Skeletonization = Feeding (often cutting) that avoids some or all leaf veins.

For skipper shelters, I have also indicated the shelter type according to the revised classification of Greeney (2009).

### SHELTER DESCRIPTIONS

*Achalarus toxeus*, (field and lab code = C73) on *Pithecellobium lanceolatum*:

Early shelters are cut-and-fold skipper shelters with a long narrow hinge and a peaked more-or-less triangular awning (Type 5). The awning is secured over the leaf with guy lines along with a silk band at the base of the hinge. More silk was used to fold one side of the awning and create the peak and there is resting silk inside the awning. Feeding is by cutting outside the shelter and there is no frass inside the shelter.

Later shelters are ties with guy lines and resting silk (Type 2). Feeding is by cutting outside and inward from the edges, and these shelters are also open and frass-free. Pupation occurs in the final leaf tie, which is not sealed.

*Amorbia concavana*, (field and lab code = C28) on *Malvaviscus arboreus*:

Small sample (1): PV14-0403

Only a pupal shelter for this species was found. It was an open frass-free leaf fold with no signs of feeding.

*Anaea aidea*, (field and lab code = C22) on *Croton argenteus* and *C. nivieus* [1]:

Ab- and adaxial leaf folds, with a silk resting pad and thin guy lines. Feeding by cutting inward from shelter margins, resulting in parabolic curves at either end of the shelter (PV14-0210 (10096)), and, later, feeding outside the shelter. Shelters remain clear of frass. Leaves can be eaten completely except for the major veins. After exiting a shelter on a mostly-eaten leaf, the caterpillar cut the leaf vein that was the only point connecting the shelter to the rest of the leaf (PV14-0210-2 (10557)).

Pupae generally attach to the undersides of leaves and stems outside of the final shelters.

*Anaea* spp. are described as building frass chains in early instars and only building shelters in later instars (DeVries 1987); only ultimate, penultimate and third-to-last instars were found in shelters at Palo Verde. No younger caterpillars were directly observed, but shelters built by third-to-last instar caterpillars sometimes had old frass chains on the same leaf.

See Chapter 1, Figure 2F for shelter photo.

***Antigonus erosus*** on *Guazuma ulmifolia*:

Early shelters: Roughly trapezoidal adaxial cut-and-fold shelters (Type 5). Feeding by cutting outside the shelter and often on the flap itself, creating ragged channels inward from the flap margins.

Later shelters: Leaf ties and folds, depending on plant architecture, with feeding by cutting inward from the shelter edges and/or outside the shelter (Types 1 and 2). All shelters are open and kept clear of frass. Pupation occurs inside the last, unsealed shelter.

***Aristotelia corallina***, member of the *Aristotelia corallina* species complex (field and lab code = C44) on *Vachellia collinsii* and *Vachellia farnesiana*:

See Chapter 2 for shelter descriptions and photos.

***Aristotelia* sp.**, *Aristotelia* BioLep345 (field and lab code = C44a) on *Vachellia collinsii*:  
Small sample (6): PV15-0128, -0197, -0220, -0223, -0233, -0478

Open webs, usually between stems, domatia, inflorescences, and/or fruits, but occasionally on leaves. Little to no frass accumulation, feeding by cutting.

***Astraptus* sp. or spp.**, (field and lab code = C74) on *Cassia grandis*:  
Small sample (4): PV17-0171, -1066, -1067, -1284

These caterpillars belong to the *A. fulgurator* species complex proposed by Hebert et al. (2004). There has been ongoing discussion regarding how many of the ten species proposed on the basis of DNA barcoding, caterpillar appearance, and host plant use should be considered “good” species (Brower 2006, Brower 2010, Pfeiler et al. 2016). These caterpillars displayed three different coloration patterns but built similar shelters on the same host plant species. As they have not yet been barcoded, I have conservatively grouped them together.

Early shelters are cut-and-fold skipper shelters with a long narrow hinge and a steeply peaked triangular awning (Type 5). They are secured with guy lines at the tip of the awning and the peak is created by folding one edge of the awning in half. The awning has resting silk inside and the shelter is open and free of frass. Feeding by cutting occurs outside the shelter.

Later shelters are loose ties secured with guy lines and lined with resting silk (Type 2). Again, they are free of frass and feeding by cutting occurs outside the shelter. In the lab, the caterpillars generally pupated in loose ties made between the bag and one or more leaves, but they presumably pupate in a final leaf tie in the field.

***Calpodus ethlius*** on *Maranta arundinacea* and *Thalia genticulata*:

Early shelters are ab- or adaxial cut-and-fold shelters with roughly rectangular flaps (Type 5). Feeding by cutting on shelter margins and outside the shelter.

Later shelters are leaf folds (Type 1), usually on the adaxial side of the leaf. Feeding by cutting occurs primarily on shelter margins, although since the folds include the majority of the leaf, in practice the caterpillar consumes all but a caterpillar-sized fold and the thickest part of the midvein. Occasionally, lab rearing results in folds on the plastic bag and occasional leaf ties. Pupation occurs in last shelter, which is not sealed, but is coated with a white waxy secretion (previously described, Moore [1928]).

No shelters from *Thalia* were collected, but the leaves are larger and thicker than *Maranta* leaves, and it appears that the caterpillars on *Thalia* continue building cut-and-fold shelters throughout development.



**Figure 1.** A-B. *Achalarus toxeus* shelter and caterpillar; C. *Amorbia concavana* shelter; D. *Anaea aidea* caterpillar; E-F. *Antigonus erosus* shelter and caterpillar; G. *Aristotelia* sp. caterpillar in shelter; H-L. *Astraptes* sp. or spp. caterpillars (H, K, L) and shelters (I, J); M-N. *Calpodetes ethlius* shelter and fifth instar caterpillar.



***Cephise aelius***, (field and lab code = C31) on *Combretum farinosum*:

Small sample (1): PV14-0229

Early shelters: Adaxial cut-and-fold shelter with a roughly rectangular flap attached to the rest of the leaf by a narrow bridge. Feeding by cutting outside of shelter.

Later shelters are leaf ties, possibly influenced by in-lab leaf “architecture” artefacts, as leaves almost never overlap naturally.

***Chiodes catillus*** on *Desmodium infractum*:

Small sample (4): PV13-0061, -0062, -0132, -0197

Early shelters are cut-and-fold, with a rectangular or triangular broad flap pulled almost all the way down to the leaf to make a nearly tubular shelter (Type 5). The sides of the flap are made slightly peaked using additional silk guy lines. The presence/absence of resting silk was not recorded. Shelters are open and clear of frass. Feeding by cutting outside the shelter.

Later shelters are leaf folds or ties, depending on plant architecture (Types 1 and 2). Shelters are open and frass-free, and resting silk is present on the ceiling of the shelter. Feeding occurs outside and along the shelter edges.

***Conchylodes plantinalis***, (field and lab code = C55a) on *Cordia dentata*:

Webs similar to those of *C. salamisalis*, but the silk is less dense and the shelters are usually abaxial. Feeding is by cutting outside of the shelter and frass does not accumulate in the shelter. The pupal shelter is also a cut-and-fold shelter which is cut off to form a capsule.

***Conchylodes salamisalis***, (field and lab code = C55) on *Cordia dentata*:

Adaxial webs with sheet webbing that often cause some folding of the leaf, although the margin never touches the surface. Feeding is by scraping in or near the shelter. Some frass accumulates in and on the shelter, but not all of it. The pupal shelter is a cut-and-fold shelter which is completely excised from the leaf and sealed to form an ovoid capsule.

**Crambidae sp. 1**, a previously unbarcoded species (field and lab code = C2/C26crypto) on *Capparis flexuosa* [18], *Quadrella indica* [11], and *Q. odoratissima* [18]:

On *C. flexuosa*, caterpillars build cut-and-fold shelters involving progressively larger flaps on one or both sides of the midvein. A single flap is folded flat across the adaxial surface or two flaps are folded together above the adaxial surface. The flap is roughly rectangular to semicircular, depending on the fraction of the leaf involved, and the flap is usually lightly scored parallel to the midvein to create a sharp crease. In addition to guy lines securing the flap(s), there is often light webbing around the edges and outside of the shelter, and frass is packed around the edges of the flap. Feeding by scraping inside and outside the shelter. In one case (PV15-0413), a closed frass-covered web was built between leaves.

On *Q. indica*, early shelters are usually built on a young leaf. This can either be a fold that prevents the leaf from opening and expanding (e.g., PV15-0082), a web holding trichomes over the midvein of an expanding leaf (e.g., PV15-0154), or a tie between the young leaf and a more mature one (e.g., PV15-0148). In all cases, feeding by cutting occurs on the young leaf, and in ties, the more mature leaf may be fed on by scraping. Later shelters are ab- or adaxial cut-and-

fold shelters similar to those built on *C. flexuosa*.

On *Q. odoratissima*, early shelters include folds, frass-covered webs, or ties on young leaves similar to those described on *Q. indica*, and in all cases, feeding by scraping occurs in or immediately outside the shelter. Later shelters can be cut-and-fold shelters, but are often dense webs, either between two leaves or between the curled abaxial edge of the leaf and the midvein and fed by scraping and cutting.

See Chapter 1, Figure 3 for shelter photos.

**Crambidae sp. 2**, DNA barcode matches two specimens with different names, (field and lab code C11) on *Guazuma ulmifolia*:

Ab- or adaxial leaf rolls, with two to three series of guy lines, involving part the leaf for earlier instars the entire leaf for later ones. The distal end of the inner roll may be sealed with edging silk. Frass collects in the roll. Feeding is by scraping and/or cutting. Caterpillars begin cutting from the innermost roll layer (the leaf margin) and work outward, sometimes until there is only a single leaf layer remaining. In those cases, the old silk attachment points usually do not remain. Portions of the outer layer will often be skeletonized.

Shelters are too thick to effectively backlight and caterpillars cannot always be seen by looking lengthwise through the roll. Darkened or browned patches on the outside of a roll usually mean it is old and no longer occupied. Old shelters also often have a series of circular holes chewed through their walls, like finger holes on a wind instrument. The maker of these holes is unknown, but it is unlikely to be the caterpillar.

Pupation normally occurs in a sealed cut-and-fold shelter constructed with guy lines, edging silk, and lined with silk. In at least one case, the caterpillar pupated in a similarly prepared fold of a plastic bag.

See Chapter 1, Figure 1C for shelter photo.

**Crambidae sp. 3**, a 95% match to *Dichogama colotha* (field and lab code = C26crypto) on *Quadrella odoratissima*:

Small sample (4): PV15-0302, PV15-0432, PV15-0435, PV15-0451

Early shelters are cut-and-fold with exterior webbing and frass around the edges of the shelter. Flap roughly rectangular, scored parallel to the midvein and folded adaxially. Feeding by cutting outside the shelter. One individual's shelter (PV15-0432) suggests that the cut-and-fold shelter may be preceded by a frass-covered adaxial fold, but that individual was also parasitized.

Later shelters are dense webs holding two or more leaves together, with feeding by cutting outside the shelter.

**Crambidae sp. 4**, previously unbarcoded species, 97% barcode match to *Conchylodes salamisalis* (field and lab code = C49) on *Simarouba amara*:

Small sample (4): PV15-0618, -0619, PV16-0555, -0556

Very large (~45 cm x 30 cm x 5 cm) open gregarious webs involving multiple compound leaves. Some frass accumulation, feeding by cutting. No pupae were ever reared.



**Figure 2.** A-B. *Cephise aelius* shelter and caterpillar; C-D. *Chiodes catillus* shelter and caterpillar; E. *Conchylodes plantinalis* shelter; F-G. *Co. salamisalis* shelter and caterpillar; H. Crambidae sp. 1 caterpillar; I. Crambidae sp. 2 caterpillar; J. Crambidae sp. 3, K-L. Crambidae sp. 4 shelter and caterpillar.

**Crambidae species complex 1**, *Chilochromopsis sceletogramma*, *Syllepte belialis*, and *Pilocrocis calamistis* (field codes = C46, C48) on *Casearia corymbosa* and *C. tremula*:

C46 and C48 were originally separated based on host plant species (C46 on *C. tremula*, C48 on *C. corymbosa*), but this complex consists of at least three species that have readily distinguishable adults but indistinguishable caterpillars and shelters. Host plant data from Palo Verde and ACG confirms that *S. belialis* uses both *C. corymbosa* and *C. tremula*, but as the other two species are less common and many caterpillars disappeared from shelters before collection, it is difficult to determine their host ranges. *Ch. sceletogramma* was found on *C. corymbosa* at Palo Verde, and at ACG on *C. corymbosa* and a third species of *Casearia* (Janzen and Hallwachs 2009). The small number of reared *P. calamistis* at both Palo Verde (2) and ACG (9) all came from *C. corymbosa*.

Shelters are lightly silked webs on clusters of leaves. If the leaves are flexible enough, the webbing may result in leaf margins being somewhat folded, but this is very inconsistent and does not involve the use of guy lines. Feeding is very haphazard, occurring inside and outside the web, and caterpillars build multiple webs. Some caterpillars appear to share webs, although I cannot rule out the possibility that they simply built individual webs so close together that they appear to be a contiguous web.

Pupation occurs in a silk sac constructed within the final web (in the lab, this is frequently on the bag).

This complex is the only example of shelter-building species severely defoliating trees at Palo Verde. Caterpillar presence also appears to be tightly constrained by plant phenology: in both 2015 and 2016, caterpillars were most abundant immediately before and during flowering, and were rare afterwards. Plants flushed new crops of leaves after being nearly defoliated.

***Desmia* sp.**, (field and lab code = C18) on *Psychotria carthagenensis*:  
Small sample (1): PV14-0184

Initial leaf fold on distal-most leaf with successive layers of proximal leaves tied around it. Frass accumulates in the shelter, which is tightly closed by guy lines. Feeding by cutting within the shelter.

The caterpillar pupated within the shelter.

***Desmia ufeus***, (field and lab code = C36) on *Cissus microcarpa*:  
Small sample (1): PV14-0255

Leaf fold, with feeding by cutting outside shelter. Open, with frass accumulation. Later shelters open, frass-free cut-and-fold shelters, with rectangular or triangular flaps folded over the adaxial surface.

Pupation occurs in a sealed cut-and-fold shelter.

***Diaphania* sp.**, *Diaphania* Dapkey02 (field and lab code = C5) on *Stemmadenia pubescens*:

The first shelter is either a surface leaf web or a fold using a very small emerging leaf. (*S. pubescens* continues producing new leaves throughout the field season.) The surface webs are small, open, and accumulate frass. Later shelters are ab- or adaxial folds involving a large portion or the entirety of larger leaves. Folds are pulled together with guy lines and sealed from

the inside with edging silk. In whole-leaf shelters particularly, frass collects in a large bolus at the base of the leaf. It is unclear whether this is the result of gravity or caterpillar effort. All instars feed by scraping and frequently trench veins to release latex.

In the lab, prepupal caterpillars frequently build a plastic bag fold using guy lines and pupate within it, even when a leaf is available. It is unclear what substrate(s) or behaviors this would correspond to in the field.

See Chapter 1, Figures 1D and 2E for shelters.

***Dichogama colotha***, (field and lab code = C26) on *Capparis flexuosa* [2], *Quadrella indica* [36], and *Q. odoratissima* [9]:

Early shelters: Flaps cut on either side of the midvein are brought together, almost always under the leaf. On tougher leaves (e.g. *C. flexuosa*), the flap may be scored once parallel to the midvein to aid in folding. Feeding occurs by scraping on the flaps, and sometimes outside the shelter. Frass is used to seal the shelter edges.

Later shelters: Leaves are tied together using guy lines and secured with frass at the leaf edges, although other openings remain in the shelter. An early shelter may be opened and incorporated in the tie. Feeding switches to cutting in from the shelter edges.

Pupation occurs within the last shelter (or, in lab, on the bag), within a silk sac. The shelter itself may or may not be sealed.

One caterpillar on *Q. odoratissima* (PV14-0473) cut a single flap and folded it adaxially over the leaf.

***Dichogama redentbacheri***, on *Capparidastrum frondosum*:

Earlier shelters cut-and-fold similar to *Dichogama colotha*—one flap folded over the body of the leaf or two flaps folded together with guy lines. At least some of the time, shelter edges were blocked with frass and silk. Feeding by scraping inside the shelter.

Later shelters folds or ties, depending on plant architecture. Feeding by scraping inside and/or cutting outside.

***Elachistidae* sp. 1**, elachJanzen01 Janzen737 (field and lab code = C33) on *Bonellia nervosa*:

Leaf web enclosing multiple leaves and the intervening stem, although the caterpillar seems to move from the first leaf to the second leaf and so on. Feeding by scraping on the adaxial surface. Frass is used to build loose tunnels on the leaves and along the branch, and the caterpillars often rest in the tunnels.

***Epargyreus* sp.**, (field and lab code = C67) on *Gliracidia sepium*:  
Small sample (2): PV16-0481, -0482

Only later-instar shelters collected: leaf ties with guy lines and resting silk (Type 2). Feeding by cutting at shelter edges and outside.

Records of other *Epargyreus* spp. (e.g., Lind et al. 2001) show that they build cut-and-fold

shelters in their early instars.

*Ethmia catapeltica*, (field and lab code = C13) on *Cordia collococca*:  
Small sample (1): PV14-0316

An open web drawing the leaf together and containing an interior silk tunnel. Feeding by scraping and cutting just outside the web. No frass accumulation.

Pupation occurs within a denser silk web or sac within the original shelter.





**Figure 3.** A. Crambidae sp. complex 1 shelter and caterpillar; B-C. *Desmia* sp. shelter and caterpillar; D. *Desmia ufeus* shelter; E. *Diaphania* sp. caterpillar; F. *Dichogama colotha* shelter; G. *Dic. redentbacheri* shelter; H-I. Elachistidae sp. 1 shelter and caterpillar; J-K. *Epargyreus* sp. shelter and caterpillar; L. *Ethmia catapeltica* shelter.

***Eulepte concordalis***, (field and lab code = C10) on *Tabebuia rosea*:

Depending on plant architecture and caterpillar size, either leaf folds or ties occur, with younger caterpillars building leaf ties if possible. Leaf folds are almost always adaxial and start with folding the point of the leaf back over the distal portion of the leaf. Feeding by scraping. Shelters are not completely sealed, but have a few narrow openings, and frass accumulates in the shelter. Early shelters are usually communal, and younger conspecifics frequently appear in shelters previously occupied by a single caterpillar. In some cases, this is likely due to new clutches being laid on shelters, but cases of caterpillars migrating into nearby shelters have also been observed.

Pupation occurs in a final sealed cut-and-fold shelter, which is often built within the last leaf fold or tie.

See Chapter 1, Figure 2C for shelter photo.

July 3, 2017: A mid-instar (21 mm) caterpillar was observed to produce two types of silk: the standard smooth guy line silk, produced when the head moves horizontally back and forth, and “beaded” silk. This silk had small regularly spaced bumps, and was produced when the caterpillar moved its head in a zigzag pattern. The beaded strands were not layered on each other to produce guy lines.

**Gelechiidae sp. 1**, DNA barcode BOLD:AAH5091 (field and lab code = C3) *Pithecellobium lanceolatum*:

Paired leaflets held together by dense webbing perpendicular to the leaf surfaces. The caterpillar rests in a silk tunnel within the wider web and feeds by scraping. Frass accumulates in the web.

**Gelechiidae sp. 2**, DNA barcode BOLD:AAA0176 (field and lab code = C7, C14, C17) on *Combretum farinosum*:

Leaf roll secured by guy lines. Very little frass accumulates in the shelter. Feeding by cutting starting with the interior edge of the roll. Shelters are too thick to backlight, but the caterpillar can usually be seen by looking lengthwise through the roll.

**Gelechiidae sp. 3**, a previously unbarcoded species, 92% barcode match to a gelechiid (field and lab code = C53) on *Caesalpinia eriostachys*:

Shelters are ties involving two to five leaflets, pulled together with guy lines. An oval area is sealed with silk and the caterpillar scrapes it clean before either incorporating new leaflets or building a new shelter. Frass remains in the shelter. Pupal shelters are constructed in the same way, although there is no feeding within.

**Hesperiidae sp. 1**, an unidentified Hesperinae (field and lab code = Hesp2) on *Megathyrus maximus*:

Small sample (1): PV13-0063

A fold or cut-and-fold shelter with a long rectangular flap forming a nearly tubular shelter secured by guy lines and containing resting silk (Type 1 or Type 5). The shelter is open-ended and frass-free. With only one sample, it is difficult to tell if the shelter was a fold eaten inward from the edges or a cut-and-fold shelter with feeding outside the shelter.



**Hesperiidae sp. 2**, an unidentified Hesperinae (field and lab code = Hesp3) on *Megathyrsus maximus*:

Small sample (1): PV13-0078

A cut-and-fold shelter skipper shelter with a long narrow rectangular flap forming a flat shelter, secured by guy lines (Type 5). Resting silk presence/absence was not recorded. Shelter is open-ended and frass-free. Feeding by cutting outside the shelter, between the shelter and the base of the leaf, so that the shelter is connected to the leaf base only by ~30 mm of exposed midvein.

**Hesperiidae sp. 3**, unidentified species (field and lab code = C60) on *Hyraea reclinata*:

Small sample (2): PV16-0392, -0393

Cut-and-fold skipper shelter (Type 5) with resting silk inside the awning. A major vein is used as the hinge and continues down the center of the awning, creating a slight curve, although the awning is not peaked. Instead of being held to the leaf by one or two thick guy lines like a *U. proteus* shelter, the awning is secured with many fine silk guy lines. Feeding is by cutting, apparently starting at the tip of the leaf and working back to the shelter.

Later shelters can be leaf ties, with feeding starting at the leaf edges and working into the shelter.

**Hesperiidae sp. 4**, unidentified species (field and lab code = C66) on *Lonchocarpus parviflorus*:

Small sample (2): PV16-0243, -0255

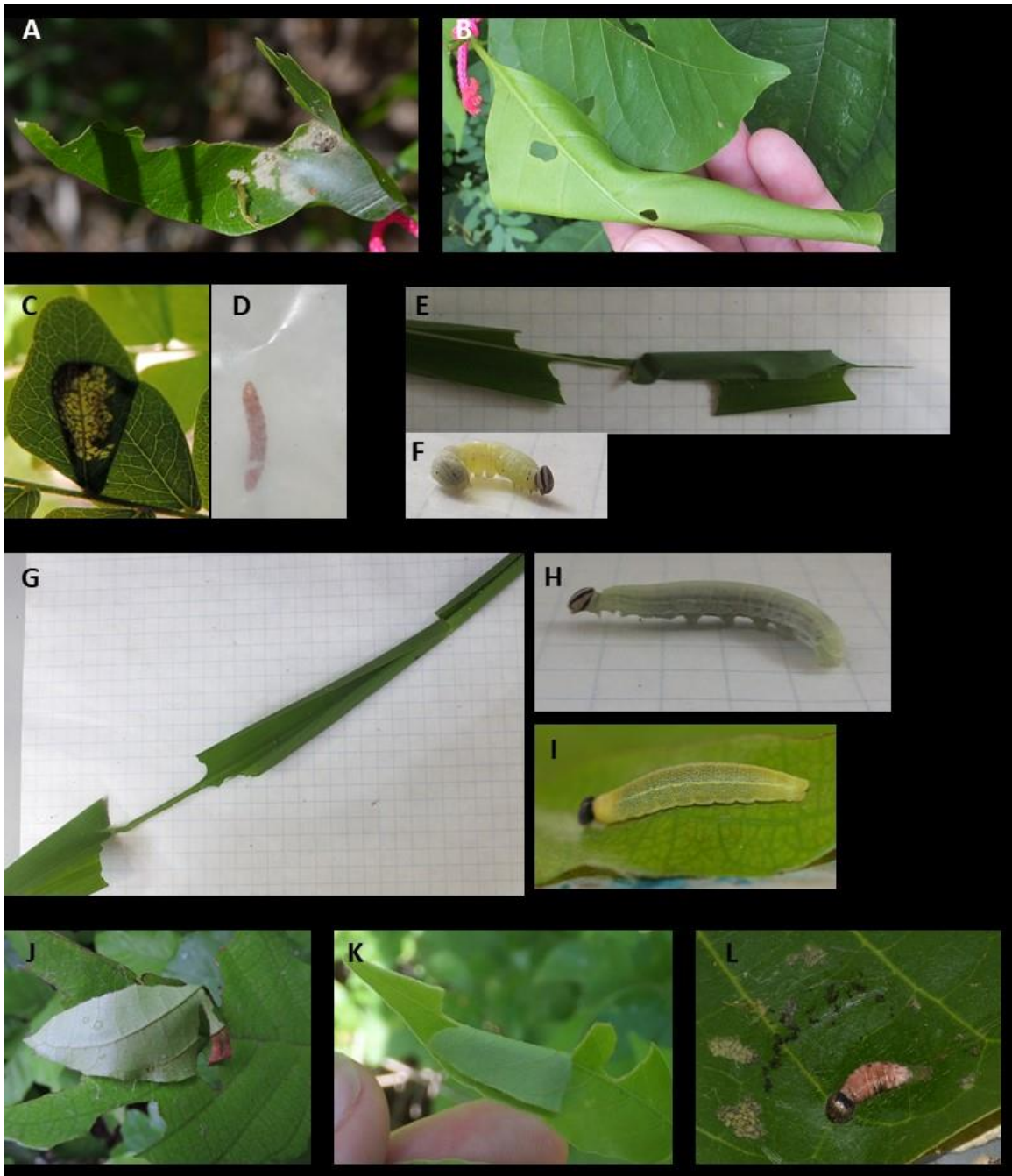
Cut-and-fold skipper shelter; a nearly rectangular flat awning with a wide hinge (Type 5). The awning is secured with guy lines and has resting silk inside. Feeding by cutting outside the shelter.

Later shelters are likely leaf folds (Type 1), although the only record for this (PV16-0255) was also parasitized.

**Hesperiidae sp. 5**, unidentified species (field and lab code = C76) on *Tabebuia rosea*:

Small sample (1): PV17-0279

Found on a recently fallen leaf in what appeared to be half of a leaf tie with resting silk and some frass accumulation on one side. Feeding damage appeared limited to spots of scraping damage around the silk. However, this individual was parasitized, so how similar this is to a normal shelter is unknown.



**Figure 4.** A. Gelechiidae sp. 1 shelter; B. Gelechiidae sp. 2; C-D. Gelechiidae sp. 3 shelter and caterpillar; E-F. Hesperidae sp. 1 shelter and caterpillar; G-H. Hesperidae sp. 2 shelter and caterpillar; I-J. Hesperidae sp. 3 caterpillar and shelter; K. Hesperidae sp. 4 shelter; L. Hesperidae sp. 5 caterpillar.

**Hesperiidae sp. 6**, possibly *Celaenorrhinus* (field and lab code = C81) on *Dicliptera sexangularis*:

Small sample (4): PV13-0186, -0194, PV17-0071, -0841

Cut-and-fold skipper shelters with short narrow hinges and wide, slightly arched triangular awnings (Type 5). The hinge always includes a major vein, and this seems to be responsible for the curvature. The awnings are secured with guy lines and have resting silk inside. The shelters are open and frass-free.

All caterpillars died during rearing, so no later shelters were observed.

**Hesperiidae sp. 7**, possibly *Cobalus fidicula* (field and lab code = C83) on *Acrocomia aculeata*:

Small sample (2): PV17-1291, -1292

Adaxial folds of individual sections of palm leaf (Type 1). The fold is secured with guy lines and lined with resting silk and a white waxy secretion. The shelter is open at the ends and frass-free. Feeding begins at the apex of the leaf and works down, often leaving part of the midvein.

**Hyblaea puera**, (field and lab code = C47) on *Tabebuia ochracea*:

Small sample (4): PV15-0334, -0335, -0336, -0337

Shelters are webs that pull the leaf up around them, with feeding by cutting and scraping. The pupal shelter is a cut-and-fold shelter secured with guy lines.

**Lativalva pseudosmithii** (field and lab code = C2) on *Capparis flexuosa* [10], *Quadrella indica* [25], and *Q. odoratissima* [1]:

Shelters may be either webs on or between leaves or cut-and-fold shelters. Webs on single leaves are essentially large frass tunnels (e.g., PV14-0007) and feeding by scraping occurs within and just outside the tunnel. If the web is between two leaves, both are fed on, and additional webbing secures frass around the edges. The cut-and-fold shelters are generally abaxial, with flaps on both sides of the midvein drawn down and edged with frass. Flaps are lightly scored parallel to the midvein at least once, but often twice, resulting in creases on the flaps and a somewhat inflated cross-section. Feeding by scraping occurs within the shelter.

Which type of shelter is built appears to have more to do with host plant characteristics than caterpillar age, as caterpillars on *Q. indica* generally built cut-and-fold shelters and caterpillars on *C. flexuosa* generally built webs. The host plant characteristics involved likely include leaf overlap and flexibility.

In both cases, pupation occurs in a sealed frass-covered silk sac within the larger shelter.

**Lerema liris** (field and lab code = Hesp4) on *Megathyrsus maximus*:

Small sample (2): PV13-0140, -0146

Early shelter is a cut-and-fold skipper shelter with either two long narrow rectangular flaps cut on either side of the midvein near the tip of the leaf or one long narrow flap folded over on a broader part of the leaf (Type 5). Shelters are open-ended and free of frass. Feeding by cutting outside the shelter.

**Massepha grammalis**, *Massepha grammalis*DHJ02 (field and lab code = C9) on *Maranta*

*arundinacea:*

Communal leaf fold or tie (plant architecture does not permit ties in the field, but it is sometimes seen in the lab). Feeding by scraping. Pupation occurs in the communal shelter or in an individual sealed cut-and-fold shelter within the communal shelter.

See Chapter 1, Figure 2G for shelter photo.

***Megalota* sp.**, (field and lab code = C54) on *Caesalpinia eriostachys*:  
Small sample (1): PV15-0686

Multilayered messy web, may have more dead leaflets in it than *Pococera sabbasa* webs, otherwise they're indistinguishable.



**Figure 5.** A-B. Hesperidae sp. 6 shelter and caterpillar; C-D. Hesperidae sp. 7 shelter and caterpillar; E-F. *Hyblaea puera* shelter and caterpillar; G-H. *Latalvalva pseudosmithii* shelters; I. *Lerema liris* shelter; K. *Megalota* sp. shelter.

**Noctuidae sp. 1**, a previously unbarcoded species, 93% barcode match to a noctuid (field and lab code = C50) on *Ziziphus guatemalensis*:

Leaf ties with the tying guy lines placed within the shelters, rather than at the edges as is more common. Sometimes a pleat is also formed on the underside of the top leaf using additional guy lines. The shelter is open at the edges, with little to no frass accumulation. The caterpillar feeds first by scraping and then by cutting at leaf edges near the shelter.

***Nyctelius nyctelius*** (field and lab code = Hesp5) on *Megathyrus maximus*:

Small sample (1-6): PV13-0174, possibly also PV13-0055, -0058, -0059, -0060, -0150

Early shelter is a cut-and-fold skipper shelter with a long narrow rectangular flap (Type 5). Shelter open-ended and free of frass. Feeding by cutting outside the shelter.

Later shelters are folds using the entire width of the leaf with feeding outside and inward from the edges (Type 1). Ties are also built in the lab, but whether the appropriate plant architecture occurs in the field is unknown.

***Palpusia sp.***, *Palpusia Solis25* (field and lab code = C42) on *Guttarda macrosperma*:

Small sample (4): PV14-0516, PV15-0440, PV15-0443, PV15-0444

Abaxial leaf rolls parallel to the midvein involving one (or in one instance, two) leaves. The ends of the roll are pinched shut with guy lines and frass accumulates within the shelter. Feeding by cutting starting on the inner leaf margin. Pupal shelter is an abaxial cut-and-fold shelter with a semicircular flap.

***Platynota subargentea***, (field and lab code = C30) on *Albizia niopoides*:

Small sample (10): PV14-0223, -0240, -0241, -0461, -0462, -0463, -0464, -0467, -0471, -0608

Leaf web pulling multiple bipinnate leaflets together into a mostly single-layered open web with frass accumulation. Feeding by cutting.

Pupation within the web.

***Pococera sabbasa***, *Pococera sabbasa*Janzen02 (field and lab codes = C35, C45) on *Caesalpinia eriostachys*, *Vachellia collinsii*, *Pithecelobium lanceolatum*, *Mimosa pigra*, *Parkinsonia aculeata*:

Usually gregarious leaf webs incorporating multiple layers of leaves and including some frass. Often the shelter is dense enough that backlighting is ineffective. However, on *Parkinsonia*, the stiff narrow rachis and minute leaflets result in the silk surrounding the leaves rather than vice versa. The caterpillars frequently continue incorporating new foliage into the web rather than starting a new shelter. Feeding is mostly by scraping, but may include some cutting. The wide range of caterpillar ages found in individual shelters suggests that multiple ovipositions occur.

See Chapter 1, Figure 2A-B for shelter photos.

***Psara obscuralis*** (field and lab code = C1) on *Petiveria alliacea*:

Some caterpillars build open webs that cause a leaf to fold around them. They feed inward from the shelter margins, and some frass accumulates in the shelter.

Most caterpillars build open ab- and adaxial leaf folds, with occasional leaf ties, depending on plant architecture. These are built with guy lines, but sometimes include webbing as well, which is rather unusual. Feeding is by cutting both inside and outside the shelter, and frass does not accumulate in the folds and ties. Pupation occurs in a silk sac, which in the lab was always located between two sections of plastic bag. What type of shelter this corresponds to in the field is unclear.

**Pyralidae sp. 1**, epipaJanzen01 Janzen15DHJ02 (field and lab code = C4) on *Coccoloba caracasana* and *C. guanacastensis*:

A web covering all or part of one or more leaves, depending on their size and architecture. The initial web is usually more or less parallel to the leaf blade. While the webbing is not sheet webbing or impenetrable, it is dense enough that some frass is usually caught in it. Early instars scrape the leaf, later ones cut the leaf, sometimes with some skeletonization. Shelters are almost always communal and are often shared by caterpillars from multiple clutches. Solitary caterpillars are generally large and usually appear to have dispersed from a nearby communal web.

**Pyralidae sp. 2**, chryJanzen01 Janzen347 (field and lab code = C19) on *Solanum hirtum*:

Small adaxial and abaxial leaf folds, either bringing the edge of the leaf over or pleating the interior of the leaf. Silk is generally limited to a few thin guy lines, and the shelter is open and free of frass. In some cases, the petiole of the leaf is partially cut and secured with silk. Feeding by scraping, but the remaining surface is more fragile than other scraped leaves. This may be due to the leaf structure, or the caterpillar may feed more extensively than other species. Pupation occurs in the final leaf fold, which remains open.





**Figure 6.** A-B. Noctuidae sp. 1 shelter and caterpillar; C. *Platynota subargentea* shelter; D. *Pococera sabbasa* caterpillar; E-F. *Nyctelius nyctelius* shelter and caterpillar; G. *Palpusia* sp. shelter; H-I. *Psara obscuralis* caterpillar and shelter; J. Pyralidae sp. 1 shelter; K. Pyralidae sp. 2 shelter.



**Pyralidae sp. 3**, phyBioLep01 BioLep774 (field and lab code = C52) on *Malpighia stevensii*:

Open cobwebby webs on clusters of leaves and stems, with frass accumulation. Feeding by scraping and cutting. The caterpillars often rest on the stems and the younger (green) and older (grayish-brown) ones generally closely match the stem they choose in color and diameter.

***Rhinthon cubana***, *Rhinthon cubana*DHJ02 (field and lab code = Hesp1) on *Maranta arundinacea*:

Small sample (1): PV13-0018

Early shelter was a cut-and-fold skipper shelter with a flat, approximately triangular flap produced with a single cut (Type 4). Caterpillar was parasitized and did not produce any other shelters.

***Rhinthon osca*** (field and lab code = C25) on *Maranta arundinacea*:

Small sample (2): PV14-0407, PV14-0633

Early shelter similar to *R. cubana* (Type 4). Later shelters are either larger, more rectangular cut-and-fold shelters or leaf folds (Type 4 or 5 and Type 1). Secured with guy lines and including resting silk, these shelters are open and frass-free. Feeding is by cutting outside and inward from the shelter edges.

The final shelter is a cut-and-fold shelter which is also sealed with edging silk.

***Spathilepia clonius*** on *Pachyrhizus erosus*:

Small sample (3): PV13-0143, -0144, -0145

Adaxial cut-and-fold skipper shelters with short wide hinges and relatively broad, slightly peaked awnings (Type 5). The awnings are secured to the leaf with guy lines and the peak is created by folding one edge of the awning in half. The presence or absence of resting silk was not recorded. Feeding by cutting occurred outside the shelters, and the shelters were free of frass.

***Stenoma* sp.**, *Stenoma* BioLep60 (field and lab code = C20) on *Guazuma ulmifolia*:

Small sample (1): PV14-0192

A closed web built between two leaves. A rough tube of frass was built on the bottom leaf.

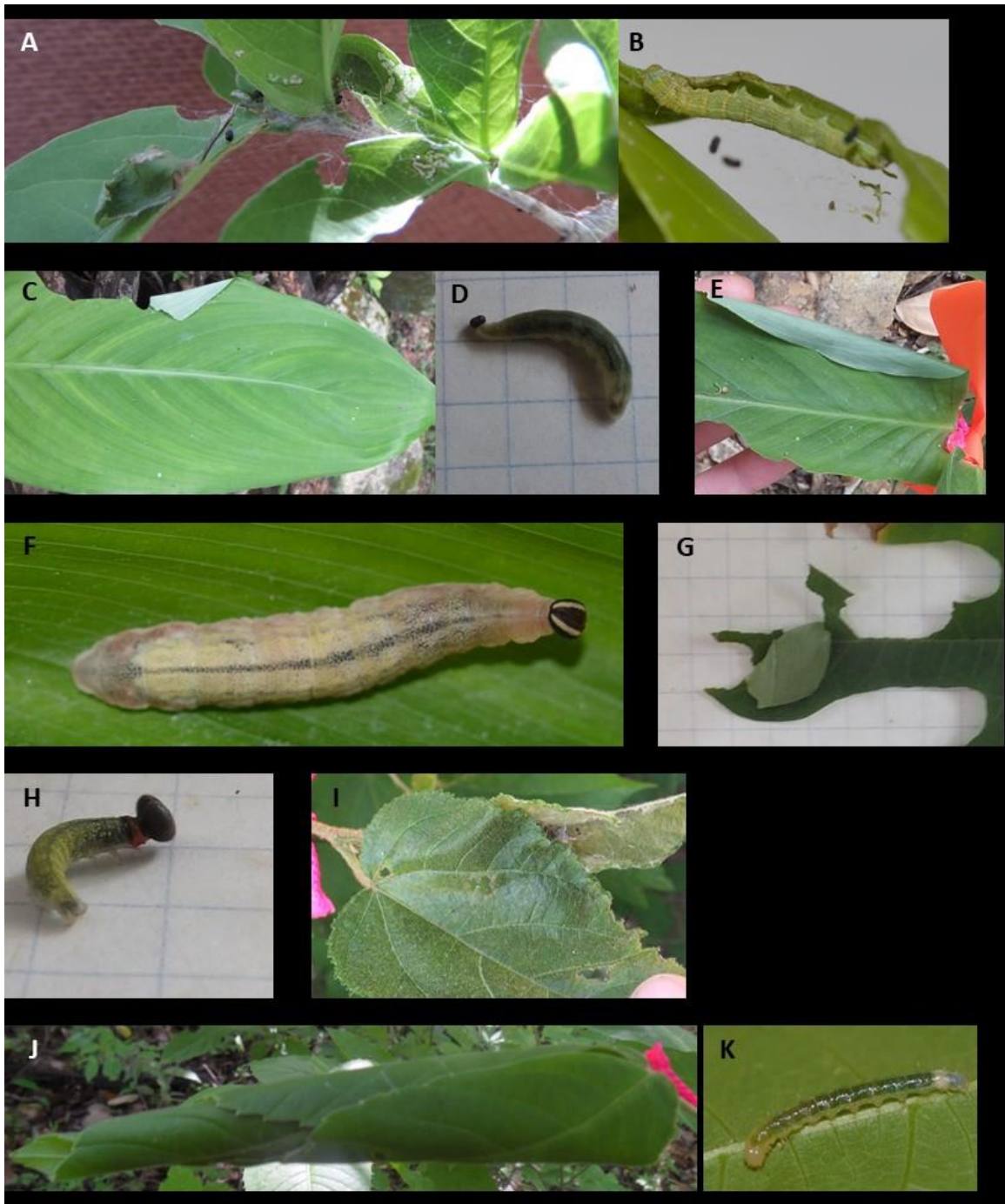
Pupation occurred in a silk sac between a leaf and the bag, and presumably would normally occur between two leaves.

***Syllepis hortalist***, *Syllepis hortalist*DHJ01 (field and lab code = C34) on *Thouinia serrata*:

Small sample (10): PV14-0427, -0445, -0446, -0447, -0449, -0450, -0451, -0465, -0487, -0509

Leaf roll. The (center) leaflet of a three-leaflet leaf is rolled into a tube with walls one to two layers thick. Sometimes, the side leaflets are wrapped around this tube with guy lines, forming a shelter with walls two to three layers thick. The rolls are open at the top end only and accumulate frass. Feeding by cutting, starting from the margins inside the roll.

Pupation occurs in the roll and occasionally on the plastic bag.



**Figure 7.** A-B. Pyralidae sp. 3 shelter and caterpillar; C-D. *Rhinthon cubana* shelter and caterpillar; E-F. *R. osca* shelter and caterpillar; G-H. *Spathilepia clonius* shelter and caterpillar; I. *Stenoma* sp. shelter; J-K. *Syllepis hortalist* shelter and caterpillar.

***Syllepte amando***, (field and lab code = C32) on *Petiveria alliacea*:  
Small sample (1): PV14-0057

Shelters are open frass-free folds and ties held together by a mixture of guy lines and webbing. Feeding is by cutting outside and inward from the edges of the shelter.

Pupation occurred in a silk sac between the sides of the plastic bag.

***Symphysa lepidaria***, (field and lab code = C15) on *Crateva palmeri*:  
Small sample (4): PV14-0348, -0355, -0356, -0459

Faint webbing connecting the three leaflets of a leaf. Feeding by cutting within the web, which is open and frass-free. Free pupation (1) or pupation within a silk sac attached to the bag.

***Timochares trifasciata*** (field and lab code = P0086 caterpillar) on an unidentified Malpighiaceae:

Earlier shelters cut-and-fold skipper shelters with tall narrow hinges and flat, acutely triangular awnings (Type 5). A guy line secures the point of the awning to the leaf, and resting silk is on the awning ceiling. Feeding occurs by cutting outside the shelter, which is both open and frass-free.

Later shelters are folds or ties with guy lines and resting silk on one or both inner surfaces (Types 1 and 2). Shelters are open and frass-free. Feeding is outside and inward from the edges.

The only observed pupal shelter was a fold built on the plastic bag.

**Unidentifiable sp. 1**, (field code = C6) on *Casearia corymbosa*:  
Small sample (5): PV14-0023, -0024, -0025, -0026, -0027

Sealed leaf folds with feeding by scraping. Frass accumulation visible in photos of older folds. The caterpillars completely defoliated the sapling host plant before disappearing.

**Unidentifiable sp. 2**, (field code = C8) on *Adenocalymna inundatum*:  
Small sample (1): PV14-0054

Leaf fold secured by guy lines. Feeding by cutting, with some skeletonization (major veins left).

**Unidentifiable sp. 3**, (field and lab code = C27) on *Cordia collococca*:  
Small sample (3): PV14-0216, -0217, -0219

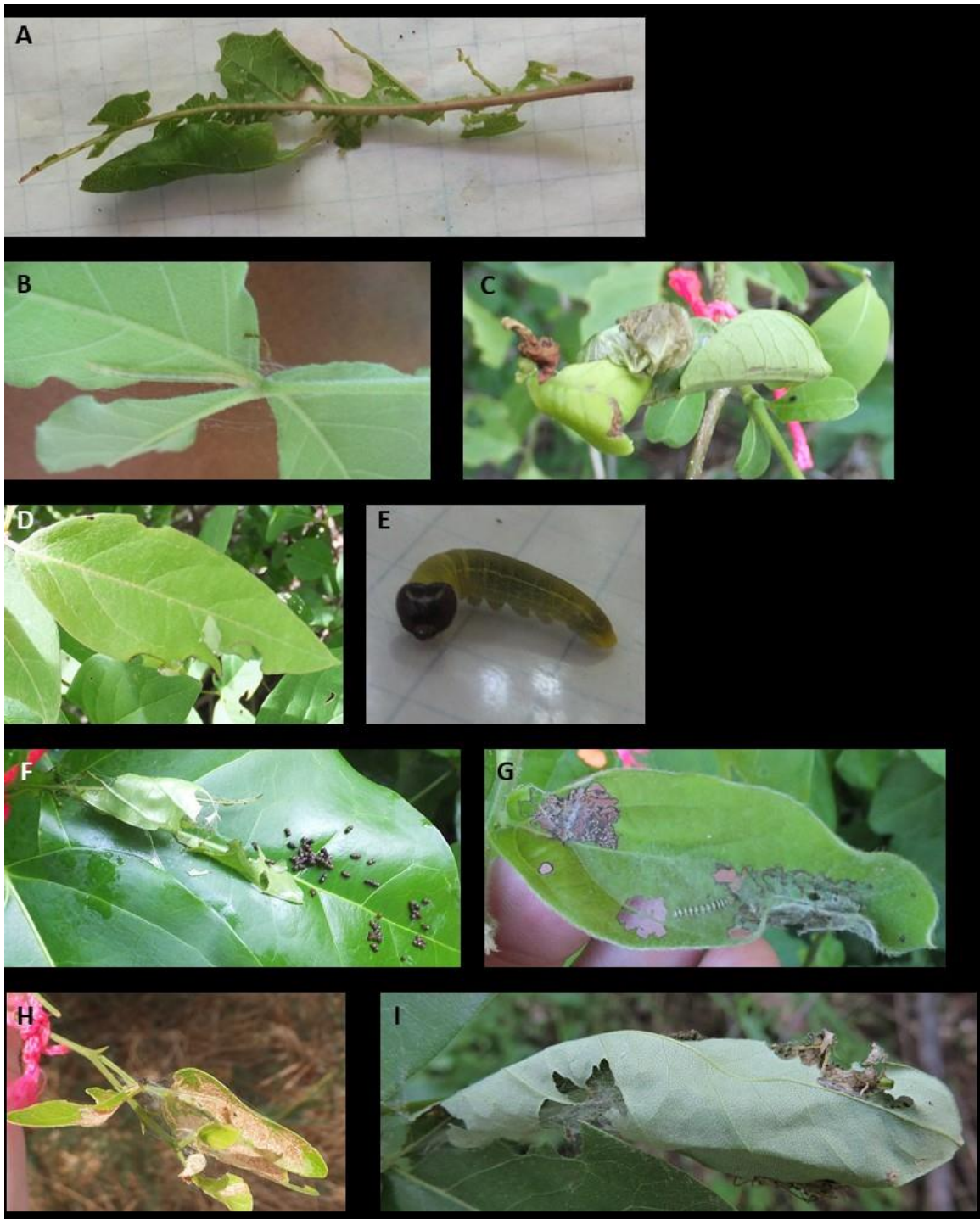
Adaxial leaf web. Feeding by scraping and cutting inside and outside the shelter. The shelter and damage look similar to *Conchylodes salamisalis*'s shelter and damage, although there's less frass on the web and the caterpillars look quite different. All three caterpillars disappeared in the field.

**Unidentifiable sp. 4**, (field code = C43) on *Pithecellobium lanceolatum*:  
Small sample (1): PV15-0019

Open web surrounding leaves and stem. No frass accumulation. Feeding by scraping and cutting. Caterpillar disappeared in field.

**Unidentifiable sp. 5**, unidentified species (field and lab code = C68) on *Gliracidia sepium*:  
Small sample (3): PV16-0478, -0479, -0480

Gregarious web with dense webbing pulling first one, then several leaves together. Feeding by cutting at the edges of the leaf and on the interior. Some accumulation of frass in the webbing. All caterpillars disappeared in the field.



**Figure 8.** A. *Syllepte amando* shelter; B. *Symphysa lepidaria* shelter with caterpillar; C. Unidentifiable sp. 1 shelter; D-E. *Timochares trifasciata* shelter and caterpillar; F. Unidentifiable sp. 2 shelter; G. Unidentifiable sp. 3 shelter; H. Unidentifiable sp. 4 shelter; I. Unidentifiable sp. 5 shelter.

**Unidentifiable sp. 6**, (field code = C72) on *Byttneria aculeata*:  
Small sample (1): PV16-0294

Open web on leaves and stem, partially folding leaves. No frass accumulation and feeding by cutting inward from the edges of the webbed leaves. Caterpillar disappeared in the field.

**Unidentifiable sp. 7**, (field code = C77) on *Coccoloba caracasana*:  
Small sample (1): PV17-0385

A small web parallel to the surface of the leaf. Frass and feeding details were difficult to determine since the shelter was near a large Pyralidae sp. 1 shelter. Caterpillar lost during rearing.

**Unknown sp. 1**, BOLD:AAF0316 (field and lab code = C21) on *Coccoloba guanacastensis* and *C. caracasana*:

A complex leaf web built on either surface of a leaf. An irregularly-shaped loose outer web covers a portion of the leaf. Within the outer web, there is an oval area covered by sheet webbing. The caterpillar rests under the sheet webbing and the resting area usually has a second layer of sheet webbing directly on the leaf surface. The caterpillar feeds by cutting leaf material from the area between the outer and inner webs. Feeding may progress to the point that the resting area is only attached to the rest of the leaf by a thin bridge, usually formed by a major vein. Frass pellets are sometimes caught in the outer webbing, although they do not match the amount of leaf material removed.

**Unknown sp. 2**, a previously unbarcoded species (field and lab code = C23) on *Crateva palmeri*:

Leaf tie with guy lines, often between leaflets of a single leaf. The tie usually has a pleat on the top leaf (made with additional guy lines). The caterpillar appears to rest in the pleat when not feeding. The leaf edges are flush to each other (closed) but not sealed, and some frass accumulates in the tie. Feeding by scraping.

See Chapter 1, Figure 1B for shelter photo.

**Unknown sp. 3**, not yet identified (field and lab code = C24) on *Trixis inula*:  
Small sample (5): PV14-0329, -0417, -0418, -0419, -0424

Leaf fold parallel to the midvein, open and free of frass. Feeding by scraping and cutting inside and outside the shelter. No caterpillars reared to pupation.

**Unknown sp. 4**, not yet identified (field and lab code = C37) on *Bursera simaruba*:  
Small sample (2): PV14-0256, -0257

Web on the abaxial surface to one side of the midvein with frass forming a tunnel. First feeding by scraping, then feeding by cutting, all approximately within the web.

**Unknown sp. 5**, not yet identified (field and lab code = C38) on *Lasiacis ruscifolia*:  
Small sample (1): PV14-0454

Leaf fold parallel to the midvein, involving the whole leaf. Shelter somewhat open, but containing frass. Feeding by scraping.

Pupation occurred in a sealed cut-and-fold shelter.

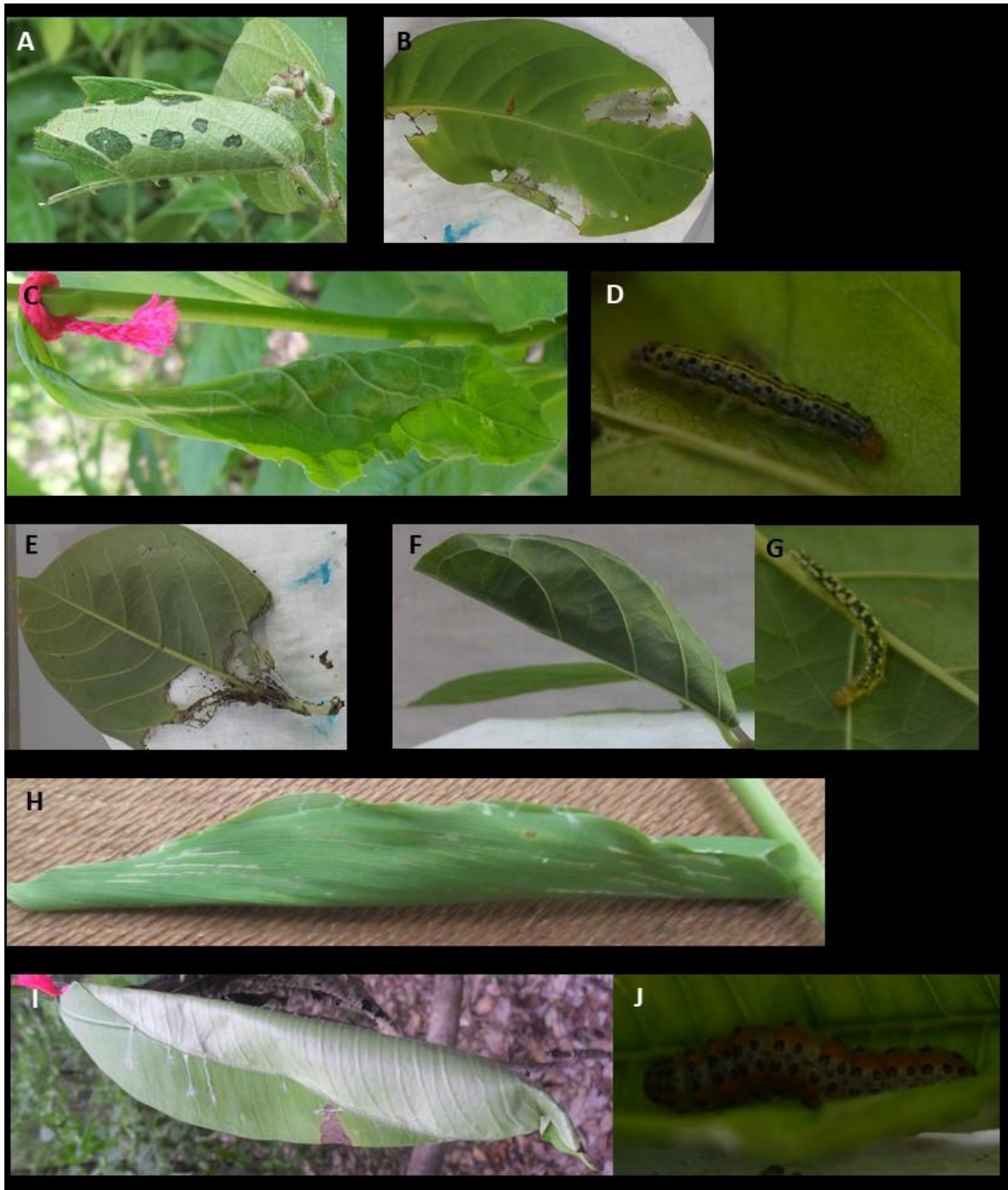
**Unknown sp. 6**, not yet identified (field and lab code = C39) on an unidentified Malpighiaceae:  
Small sample (1): PV14-0460

Leaf fold parallel to the midvein, involving the whole leaf. Shelter somewhat open, but containing frass. Feeding by scraping.

**Unknown sp. 7**, not yet identified (field and lab code = C40) on *Plumeria rubra*:  
Small sample (1): PV14-0481

Leaf fold parallel to midvein with guy lines and sealing silk. The one sample was collected two days before pupation, and there was neither feeding damage nor frass in the shelter.





**Figure 9.** A. Unidentifiable sp. 6 shelter; B. Unknown sp. 1 shelter; C-D. Unknown sp. 3 shelter and caterpillar; E. Unknown sp. 4 shelter; F-G. Unknown sp. 6 shelter and caterpillar; H. Unknown sp. 5 shelter; I-J. Unknown sp. 7 shelter and caterpillar.



**Unknown sp. 8**, not yet identified (field and lab code = C41) on *Vachellia collinsii*:  
Small sample (1): PV14-0492

Dense, disorganized web containing rachii, leaflets (alive and dead), and frass.

**Unknown sp. 9**, not yet identified (field and lab code = C56) on *Samea saman*:  
Small sample (5): PV16-0011, -0033, -0034, -0035, -0036

Shelters are ties of opposite leaflets similar to those of Gelechiidae sp. 3, although the larger leaflets of this host plant correspond to the larger size of these caterpillars. This species does not seal its shelters, but some frass still accumulates in the shelter. Initial feeding is by scraping, followed by cutting.

**Unknown sp. 10**, not yet identified (field and lab code = C57) on *Combretum farinosum*?:  
Small sample (3): PV16-0049, -0050, -0084

At first, expanding leaves (which begin development folded along the midvein) are secured with guy lines to form folds, with feeding by scraping inside the shelter. There can be some frass accumulation, although other shelters were clean. Caterpillars continue building folds throughout development, but feed more and more by cutting.

**Unknown sp. 11**, not yet identified (field and lab code = C58) on *Dispilus acapulcensis*:  
Small sample (2): PV16-0324, -0325

A web that either pulls two leaves together or pulls one leaf over on itself, with feeding by scraping within the shelter. Some frass accumulation. Looks similar, but not identical, to *Conchylodes salamisalis* shelters.

**Unknown sp. 12**, not yet identified (field and lab code = C59) on *Maclura tinctoria*:  
Small sample (8): PV16-0331, -0332, -0333, -0334, -0335, -0379, -0391

Adaxial or abaxial leaf folds. In addition to guy lines, some edging silk is used, creating a closed shelter that accumulates frass. Feeding by cutting inward from the edges of the shelter.

**Unknown sp. 13**, not yet identified (field and lab code = C61) on *Dicliptera sexangularis*:  
Small sample (1): PV16-0394

A web beginning at the newest leaves on the stem and incorporating successive pairs of leaves into the shelter as necessary. Feeding by cutting both from the edges of the shelter and on the interior. There is some frass accumulation.

**Unknown sp. 14**, not yet identified (field and lab code = C62) on *Guazuma ulmifolia*:  
Small sample (2): PV16-0412, -0413

A web between leaves at the branch tip. Feeding by scraping, with some frass accumulation. The shelter is similar to that of the *Stenoma* sp., but no structure is formed with the frass, and the adults are different.

**Unknown sp. 15**, not yet identified (field and lab code = C63) on *Maclura tinctoria*:  
Small sample (1): PV16-0230

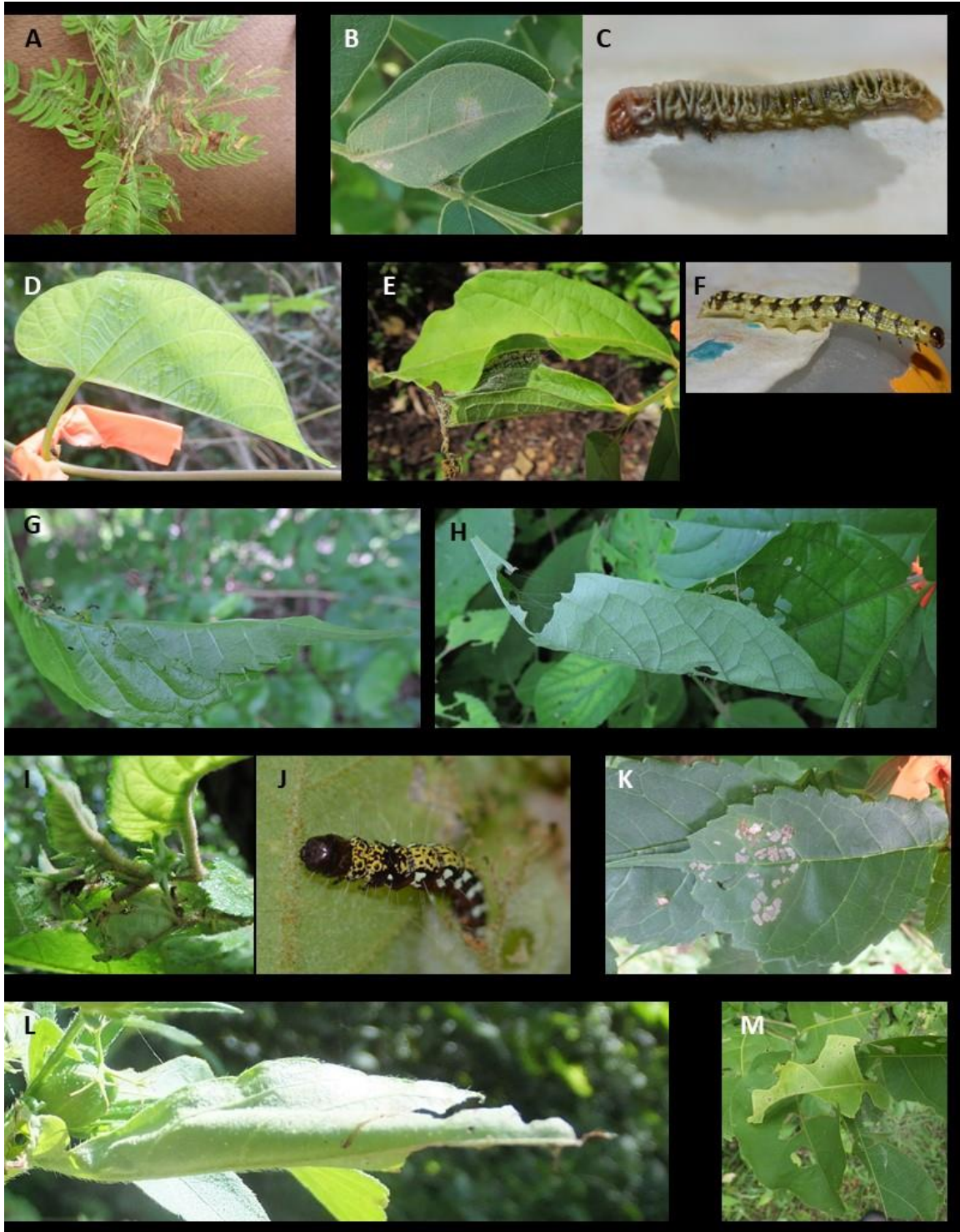
Tie, with a silk and frass enclosure inside. Feeding by scraping in shelter.

**Unknown sp. 16**, not yet identified (field and lab code = C65) on *Astraea labata*:  
Small sample (1): PV16-0242

Adaxial leaf folds with guy lines at the edge of the shelter and webbing inside. Folds are open with little to no frass inside. Feeding by cutting outside the shelter and at the edges. Pupal shelter is a cut-and-fold shelter with guy lines and webbing.

**Unknown sp. 17**, not yet identified (field and lab code = C67a) on *Combretum farinosum*:  
Small sample (1): PV16-0262

Open cobwebby web between several leaves, with no frass accumulation. Feeding by cutting inward from the edges of the webbed leaves.



**Figure 10.** A. Unknown sp. 8 shelter; B-C. Unknown sp. 9 shelter and caterpillar; D. Unknown sp. 10; E-F. Unknown sp. 11 shelter and caterpillar; G. Unknown sp. 12 shelter; H. Unknown sp. 13 shelter; I-J. Unknown sp. 14 shelter and caterpillar; K. Unknown sp. 15 shelter; L. Unknown sp. 16 shelter; M. Unknown sp. 17 shelter.

**Unknown sp. 18**, not yet identified (field and lab code = C69) on *Astronium graveolens*:  
Small sample (4): PV16-0495, -0496, PV16-0508, PV16-0546

Open web between leaves with frass accumulation. Feeding by scraping and cutting.

**Unknown sp. 19**, not yet identified (field and lab code = C70) on *Calycophyllum candidissimum*:  
Small sample (2): PV16-0514, -0515

Leaf roll secured with guy lines, with rolling usually beginning from the leaf tip, but occasionally rolled parallel to the midvein. The ends of the rolls are open, but frass usually accumulates inside. Feeding by cutting inward from the edges. Pupal shelter is a sealed cut-and-fold shelter secured with guy lines and edging silk, with webbing inside.

**Unknown sp. 20**, not yet identified (field and lab code = C71) on *Alvaradoa amorphoides*:  
Small sample (3): PV16-0516, PV17-0450, -0451

Web of stripped leaflets around the rachis. 2016 adult released because it looked like *Pococera sabbasa*, but the host plant is a different family. Needs barcoding.

**Unknown sp. 21**, not yet identified (field and lab code = C75) on *Coccoloba caracasana*:  
Small sample collected (1), but many seen in the field: PV17-0237

Abaxial leaf roll parallel to the midvein, sometimes incorporating up to half the leaf. Secured with guy lines, ends open. Frass accumulation not recorded.

**Unknown sp. 22**, not yet identified (field and lab code = C78) on *Petivera alliacea*:  
Small sample (2): PV17-0667, -0668

Open-ended rolls parallel to the midvein, secured with guy lines. Feeding inside by scraping and cutting, frass accumulates inside.

**Unknown sp. 23**, not yet identified (field and lab code = C79) on *Rivinia humilis*:  
Small sample (2): PV17-0669, -0670

Open-ended abaxial rolls beginning at the leaf tip, secured with guy lines. Feeding and frass accumulation details not recorded.

**Unknown sp. 24**, not yet identified (field and lab code = C80) on *Luehea speciosa*:  
Small sample (4): PV17-0671, -0672, -0673, -0674

Open-ended abaxial rolls parallel to the midvein, secured with guy lines. Feeding and frass accumulation details not recorded.

**Unknown sp. 25**, not yet identified (field and lab code = C82) on *Coccoloba caracasana*:  
Small sample (1): PV17-1138

A small web parallel to the surface of the leaf. Frass and feeding details difficult to determine since the shelter was near a large Pyralidae sp. 1 shelter. The caterpillar looked different from Unidentifiable sp. 7.

*Urbanus dorantes* on *Desmodium glabrum*, *D. barbatum*, *D. procumbens*:

Early shelters are most commonly shriveled leaf folds (Type 1) created by partially cutting the petiole and securing it with silk. A small fold within the shriveled leaf may be further defined and secured with silk. These shelters are open and do not accumulate frass and are built on still-expanding, vertically oriented leaflets. If an early-instar caterpillar is on a horizontal leaflet, it will construct a rectangular or triangular cut-and-fold shelter with a flat awning (Type 5). These shelters are also clean and open.

Later shelters are either leaf folds or ties (Types 1 and 2) on *D. glabrum*, and leaf ties on *D. barbatum* and *D. procumbens*, as their leaves are too small to make effective leaf folds for mid- or late-instar caterpillars. Pupation occurs in the last shelter, which is still somewhat open.

See Chapter 3 for shelter and caterpillar photos.

***Urbanus esta*** on *Desmodium glabrum*:

Small sample (1): PV13-0048

Cut-and-fold skipper shelter with a long narrow hinge and a very acutely triangular awning (Type 5). Guy lines secure the awning, which is given a slightly peaked shape by folding one edge in half. The presence or absence of resting silk was not recorded. Feeding by cutting occurred outside the shelter, and the shelter was free of frass. This single shelter appears to have a more acutely triangular awning than *U. proteus* shelters, but with only one sample, whether this is diagnostic or not is impossible to say.

***Urbanus evona*** on *Centrosema sagittatum*:

Small sample (1): PV13-0211

A later instar shelter: either a fold, or possibly a cut-and-fold shelter (Type 1 or Type 5). A roughly rectangular flap folded in two using guy lines was surrounded by feeding by cutting outside the shelter. The fold contained resting silk inside and was free of frass.

***Urbanus proteus*** on *Desmodium glabrum*, *D. procumbens*, *D. incanum*, *Centrosema sagittatum*:

Early shelters are adaxial cut-and-fold shelters with roughly triangular flaps (Type 5). One cut side of the flap is constricted with silk so that the flap forms a peaked awning. The cone is not pulled completely flush to the leaf surface but hangs over it.

Later shelters are leaf folds or ties, depending on plant architecture (Types 1 and 2). Pupation occurs in the last shelter, which may be more tightly pulled together with guy lines than previous ties, but is not closed or sealed. The pupa is lightly coated with a white waxy secretion similar in appearance to *C. ethlius*', but it does not usually extend to the shelter interior.

Shelters also previously described by Greeney and Sheldon (2008).

See Chapter 3 for shelter and caterpillar photos.

***Xenophanes tryxus*** on *Malvaviscus arboreus*, *Elytaria imbricata*, and *Malachra alceifolia*:

Early shelters are adaxial cut-and-fold shelters with roughly semicircular flaps (Type 5). They are secured by guy lines and have resting silk inside, usually on the awning. Feeding is by cutting outside the shelter, often creating a narrow vein-based bridge connecting the shelter to the rest of the leaf.

Later shelters are adaxial leaf folds (Type 1). Caterpillars feed by cutting inward from the shelter margins, ultimately producing a semicircular shelter with a vein-based bridge to the rest of the leaf similar to an early shelter. In later shelters, the resting silk more frequently occurs on the floor of the shelter, or on both the ceiling and floor of the shelter. The pupal shelter is a fold or cut-and-fold shelter which may be pulled more tightly together than previous shelters but is not sealed.





**Figure 11.** **A.** Unknown sp. 18 shelter; **B.** Unknown sp. 19 shelter; **C.** Unknown sp. 20 shelter; **D.** Unknown sp. 21 shelter; **E.** Unknown sp. 22 shelter; **F.** *Urbanus esta* shelter; **G-H.** *U. evona* shelter and caterpillar; **I-J.** *Xenophanes tryxus* shelter and caterpillar.

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