MIAMI BLUE BUTTERFLY LARVAE (LEPIDOPTERA: LYCAENIDAE) AND ANTS (HYMEOPTERA: FORMICIDAE): NEW INFORMATION ON THE SYMBIONTS OF AN ENDANGERED TAXON

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Abstract

Historical, anecdotal records of the state-endangered Miami blue butterfly, *Cyclargus thomasi bethunebakeri* (Comstock & Huntington) (Lepidoptera), have mentioned larval associations with the Florida carpenter ant, *Camponotus* sp. Recent population studies confirm that *C. t. bethunebakeri* larvae associate with *Camponotus floridanus* (Buckley) as well as another member of the genus, *Camponotus planatus* (Roger). Additionally, caterpillars have been observed tended by *Crematogaster ashmeadi* (Emery), *Forelius pruinosus* (Roger), and *Tapinoma melanocephalum* (Fab.). Field surveys of remaining Miami blue habitat and recent butterfly reintroduction sites reveal other potential ant associates, *Paratrechina longicornis* (Latreille) and *Paratrechina bourbonica* (Forel), and a host of possible predaceous ant species. The corresponding conservation implications are discussed. Detailed information is also presented about larval ant-associated organs and their mediation of this facultative symbiosis.

Key Words: Ant organs, butterfly-ant relationship, facultative symbiosis

Resumen

Registros históricos y anecdóticos de la mariposa en peligro de extinción Miami blue, *Cyclargus thomasi bethunebakeri* (Comstock y Huntington) (Lepidóptera) mencionan la asociación de sus larvas con la hormiga carpintera, *Camponotus* sp. Estudios recientes poblacionales confirman que las larvas de *C. t. bethunebakeri* están asociadas con *Camponotus floridanus* (Buckley), como también con otro miembro del género, *Camponotus planatus* (Roger). Adicionalmente, se observaron orugas atendidas por *Crematogaster ashmeadi* (Emery), *Forelius pruinosus* (Roger), y *Tapinoma melanocephalum* (Fabricius). Análisis de campo del hábitat remanente de la mariposa Miami blue y de localidades de reintroducciones recientes, revelaron asociaciones potenciales con otras especies de hormigas, *Paratrechina longicornis* (Latreille) y *Paratrechina bourbonica* (Forel), y un hospedero de posibles especies de hormigas depredadoras. Las implicaciones de conservación son discutidas en este artículo. Así mismo, se presenta información detallada sobre los órganos involucrados en la asociación larva-hormiga y su intervención en esta simbiosis facultativa.

Translation provided by the authors.

Introduction

The Miami blue, *Cyclargus thomasi bethunebakeri* (Comstock & Huntington) (Lycaenidae: Polyommatinae), represents one of Florida's rarest endemic butterflies and is currently listed as state-endangered. Once commonly found in tropical coastal hammocks, beachside scrub, and tropical pine rocklands from the southern Florida mainland south through the Florida Keys to Key West and the Dry Tortugas, the species' overall distribution and numerical abundance has been reduced to a single remaining metapopulation within the boundaries of Bahia Honda State Park in the Lower Keys (Klots 1964; Kimball 1965; Lenczewski 1980; Minno & Emmel 1993; Ruffin & Glassberg 2000; Calhoun et al. 2002). Developing larvae of *C. t. bethunebakeri* have been shown to be tended by ants in the genus *Camponotus* but the extent of the relationship remains poorly understood (Minno & Emmel 1993). Recent population studies of the butterfly at Bahia Honda State Park and additional reintroduction sites within Everglades National Park confirm a continued association.

Over 75 percent of lycaenid larvae with known life histories associate with ants (Pierce et al. 2002). Such myrmecophilous relationships may be mutualistic to varying degrees or even parasitic whereby larvae are predatory in ant nests (Pierce & Mead 1981; Fiedler & Maschwitz 1988; New 1993). The resulting communication between larvae and ants is mediated by a complex array of tactile, chemical, and often audible signals (DeVries 1990). Specifically, larvae possess highly specialized organs that can extrude alarm, reward, or appeasement chemicals. In response, tending ants often protect the surrounding larvae from a variety of natural predators and parasitoids, and thus can potentially provide a benefit for survival (Thomas 1980; Webster & Nielson 1984; Pierce & Easteal 1986; Savignano 1994). Cushman & Murphy (1993) suggest that ant associations also may play an important role in the persistence of lycaenid populations. They additionally propose that species with a dependence on ants, whether facultative or obligatory, display an increased sensitivity to environmental change, and thus are more susceptible to endangerment than species that lack ant associations. Here, we identify additional ant associates and potential predatory ant species and discuss the corresponding implications for the conservation and recovery of the Miami Blue butterfly, a critically imperiled butterfly.

Materials and Methods

Field surveys of ant species were conducted at Bahia Honda State Park and the Flamingo Campground, Rowdy Bend Trail, and Bear Lake Road sites in southern portions of Everglades National Park during daylight hours on 24-27 May, 2004 and 31 July-2 August, 2004. These areas contain low numbers of *Cyclargus thomasi bethunebakeri*, either as part of a remaining natural metapopulation or as reintroduced individuals. Hand-collecting and baiting were used to survey ants on and around patches of the butterfly's larval host, *Caesalpinia bonduc* (L.) Roxb. (Fabaceae). Sugar baits consisting of index cards with approximately 10 g of crushed pecan cookie were placed along transects at the base of *C. bonduc* plants. Baits were left in the field for one hour, at which time all cards were collected in Ziploc-style plastic bags. Additionally, when *C. t. bethunebakeri* larvae were found in association with ants, 1-2 ant specimens were collected from the tended larvae.

Finally, to provide additional detail on the structure of the larval ant organs, three *C. t. bethunebakeri* larvae from a captive colony maintained at the University of Florida were preserved and used for SEM and Auto-Montage photographic analysis. Larvae were placed in near boiling water for 60 seconds, transferred to 25% ethanol for two hours, 50% ethanol for another two hours, and stored in 75% ethanol before being photographed. No additional preparation or gold coating was done to prepare specimens.

Results

Eighteen ant species were collected in Everglades National Park and Bahia Honda State Park (Table 1). Of these, *Camponotus floridanus*, *Camponotus planatus*, *Crematogaster ashmeadi*, *Forelius pruinosus*, and *Tapinoma melanocephalum* were confirmed to tend larvae of *Cyclargus thomasi bethunebakeri*. Late instars were always found in association with ants but early instars, prepupae, and pupae were frequently found without ants present. *Camponotus floridanus* tended larvae for the majority of the observations and all other ants were encountered 1-2 times, with no two species tending larvae simultaneously. Two ants typically tended a larva at a time, with the exception of *Crematogaster ashmeadi* which often tended in higher numbers (Fig. 1).

We name two additional species, *Paratrechina longicornis* and *Paratrechina bourbonica*, as potential ant associates. The former species was found in proximity to *C. t. bethunebakeri* larvae and appeared to tend them although encounters were brief. The latter species was observed tending larvae of another lycaenid, *Strymon martialis* (Herrich-Schäffer), on *Caesalpinia bonduc* at Bahia Honda State Park. No predation by these ants was observed.

Details of the ant organs of *C. t. bethunebakeri* are shown in Fig. 2. Second through fifth instars possess a dorsal nectary organ (=honey gland) with associated perforated cupola organs on abdominal segment A7 and a pair of eversible tentacular organs on abdominal segment A8. Abdominal segments A7 and A8 are fused dorsally. Tentacular organs were observed to evert independently in the field when stimulated by attendant ants, and liquid droplets from the dorsal nectary organ were actively imbibed by all species of ants. *Camponotus floridanus* became excited and agitated, evidenced by increased body and antennal movements, when the tentacular organs were everted.

Discussion

This study documents *Camponotus floridanus* to be the primary ant species attending Miami blue larvae. *Camponotus floridanus* is a native ant species primarily active at night throughout Florida; they are commonly found foraging on *C. bonduc* and tending *C. t. bethunebakeri* larvae in both the Everglades and Bahia Honda locations. *Camponotus planatus* is a diurnal species, but is not commonly encountered in association with larvae, having never been found tending larvae in the Everglades and only once in Bahia Honda. It is possible that in higher densities *C. planatus* may more regularly tend larvae and could potentially be important at protecting larvae during the day. Buckley & Gullan (1991) have shown that more aggressive ants provide better

TABLE 1. ANTS OF EVERGLADES NATIONAL PARK AND BAHIA HONDA STATE PARK COLLECTED IN PROXIMITY TO THE ENDANGERED MIAMI BLUE BUTTERFLY *CYCLARGUS THOMASI BETHUNEBAKERI* WITH INFORMATION ON TROPHIC INTERACTIONS.

	Park Location		
	Everglades	Bahia Honda	Ant Status
Subfamily Pseudomyrmicinae			
Pseudomyrmex elongatus (Mayr)	1	2	P
Pseudomyrmex gracilis (Fab.)	1	-	P
Pseudomyrmex simplex (Smith)	-	1	P
Subfamily Myrmicinae			
Crematogaster ashmeadi (Emery)	-	1, 2	S
Monomorium floricola (Jerdon)	1	2	u
Pheidole dentata Mayr	1	2	u
Pheidole floridana Emery	1	1	u
Solenopsis invicta Buren	-	1, 2	P
Solenopsis geminata (Fab.)	1	1, 2	u
Tetramorium simillimum (F. Smith)	1	-	u
Wasmannia auropunctata (Roger)	1	1, 2	P
Subfamily Dolichoderinae			
Forelius pruinosus (Roger)	1	1, 2	S
Tapinoma melanocephalum (Fab.)	-	1	S
Subfamily Formicinae			
Brachymyrmex obscurior Forel	1	1, 2	u
Camponotus floridanus (Buckley)	1, 3	1, 2	S
Camponotus planatus (Roger)	1	1	S
Paratrechina bourbonica (Forel)	-	1	pS
Paratrechina longicornis (Latreille)	1	1, 2	pS

Collectors/Authors: 1 = present study; 2 = Deyrup et al. (1988), 3 = Ferster and Prusak (1994).
S = confirmed symbiont of *C. thomasi bethunebakeri* (present study); pS = potential symbiont; u = unknown; P = potential predator (noted as a predaceous ant in included literature).

protection for soft scales and mealybugs, and as a corollary the large and potentially aggressive *Camponotus* species may prove effective in deterring predators and parasitoids (Axén 2000).

Crematogaster ashmeadi were observed tending individual larvae in Bahia Honda but have not yet been observed with larvae in the Everglades. While not commonly found tending larvae, interactions involving *C. ashmeadi* were characterized by a minimum of five individuals. This behavior demonstrates the quality of their trailing and recruitment signals. Other *Crematogaster* species have been found worldwide to tend lycaenid larvae and this genus seems predisposed to lycaenid symbioses (Atsatt 1981; Fiedler 1991; Pierce et al. 2002; Saarinen 2005). These ants are equipped with a flexible abdomen and attached sting; despite their small size they are potentially capable of defending larvae from other ants or harmful invertebrates.

Both *Forelius pruinosus* and *Tapinoma melanocephalum* may be opportunistically imbibing food rewards from *C. t. bethunebakeri* larvae. Field observations suggest that their behavior offers little or no protection for the larvae they tend;

Fig. 1. *Crematogaster ashmeadi* ants tending a late instar *Cyclargus thomasi bethunebakeri* larva. Several other *C. ashmeadi* ants were present but not visible in this photo. Photo by Jaret Daniels.

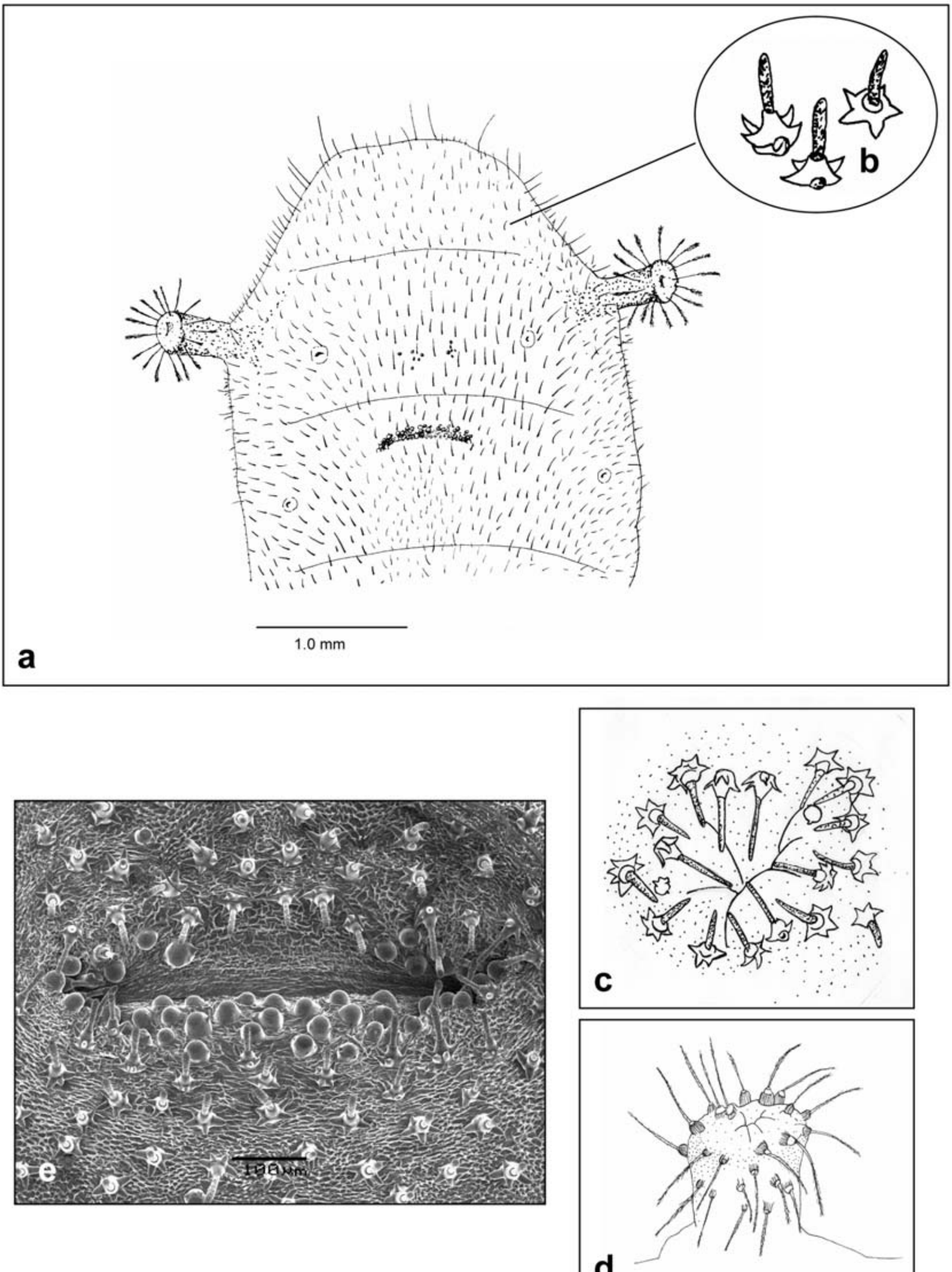

Fig. 2. Details of *Cyclargus thomasi bethunebakeri* fifth instar. a, dorso-posterior abdominal segments (A7-A8) showing ant organs; b, detail of cuticular setae; c, tentacular nectary organ inverted; d, tentacular nectary organ everted (cuticular setae omitted); e, dorsal nectary organ bordered by perforated cupolas. Figures by Emily Saarinen.

however merely their presence may deter predators. Both *Paratrechina longicornis* and *P. bourbonica*, along with *T. melanocephalum*, have been referred to as "tramp ants" (Passera 1994). While such species may not provide demonstrative protection for larvae, at the very least they tolerate nearby larvae and coincidentally tend instars feeding on *C. bonduc* flowers and buds adjacent to where the ants are also gathering nectar.

The facultative ant associations of *C. t. bethunebakeri* encompass four genera (five including *Paratrechina*) and three subfamilies; Formicinae, Myrmicinae, and Dolichoderinae. These lycaenid larvae may secrete "non-specific" ant semiochemicals as attractants to various ant species, as proposed by Henning (1983). These chemicals, primarily from the tentacular organs and potentially from the perforated cupola organs, may serve to alarm, excite, or appease ants. Further study into the chemical secretions of all ant organs may clarify the "intentions" of the larvae in their emissions. Further comparisons of each ant species' alarm and attractant pheromones with those isolated from lycaenid volatiles may further elucidate ant-larval relationships, including if certain ants are chemically targeted and if others are simply opportunistic tenders.

No interactions between other identified ant species and *C. t. bethunebakeri* larvae were observed. Several of these ants, however, may be predating larvae at other times. All three *Pseudomyrmex* species may be predators, possibly excepting *P. simplex* (Smith) due to its small size. Miami blue larvae are always found in proximity to abundant colonies of *Camponotus floridanus* and further field observations, especially at night when *C. floridanus* are most active, need to be carried out to assess interactions within the ant mosaic of symbionts and predators.

This study also shows the persistence of *Wasmannia auropunctata* (Roger) on Bahia Honda State Park (first recognized there by Deyrup et al. 1988). This invasive tramp ant is native to the New World tropics and its presence in the Florida Keys may be a cause for concern. *W. auropunctata*, also known as the little fire ant is an opportunistic feeder that forages day and night and bears a painful sting. Both *W. auropunctata* and the red imported fire ant, *Solenopsis invicta* Buren, have been implicated in the displacement of endemic species, resulting in a loss of biodiversity (Meier 1994; Wojcik 1994). Neither ant has been found near *C. t. bethunebakeri* in the Everglades, nor have they been observed harvesting immatures or predating adults in Bahia Honda. *Solenopsis invicta* mound density is perhaps not high enough to impact the *C. t. bethunebakeri* metapopulation on Bahia Honda because they do not appear as large or extensive in area as those found in more disturbed habitats (personal observation). Further field work will need to examine predation rates by ants and the specific impact that these invasive ants may have on endemic butterfly species, especially species of special concern.

Ant attendance may be critical to the long-term survival of lycaenid taxa by impacting larval development time, larval weight gain, and other developmental responses (Robbins 1991; Wagner 1993). The presence of an ant guard has led to larger, more fecund adults in the related butterfly *Hemiargus isola* (Reakirt) (Wagner 1993). However in the Australian species *Jalmenus evagoras* (Donovan), ant-tended larvae pupate at a smaller size, pupate for a shorter duration, and develop into smaller adults (Pierce et al. 1987). In an assessment of potential ant partners, it was shown that *Tapinoma sessile* (Say) is a "neutral partner" for the widely distributed North American lycaenid *Glaucopsyche lygdamus* (Doubleday), providing no significant cost or benefit (Fraser et al. 2001). Researchers of the critically imperiled European lycaenid butterfly *Maculinea rebeli* (Hirschke) have repeatedly emphasized "the importance of identifying local host ant species prior to further management conservation strategies in order to avoid failure of management programs or even damage to populations on the edge of extinction" (Steiner et al. 2003). Ant attendance, obligate or facultative, is not trivial; it can have profound effects on the length of time individuals spend in vulnerable immature stages as well as the resulting fecundity of adults. Both symbiotic ant partnerships and the negative impacts of predaceous ants should be addressed in management plans for the conservation of endangered lycaenid taxa.

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