Ancient butterfly–ant symbiosis: direct evidence from Dominican amber

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SUMMARY

Although symbiotic association with ants is pervasive in the butterfly families Lycaenidae and Riodinidae the age of these symbioses has never been estimated explicitly. Here we report the first known fossil riodinid caterpillar. This fossil can be dated minimally between 15 and 20 Ma old, and confidently placed in the extant genus *Theope*. Differing little from modern day *Theope*, this fossil from Dominican amber provides direct evidence that secretory and acoustical organs used by modern caterpillars to mediate symbioses with ants have been highly developed at least since the Miocene. This fossil therefore becomes the point of reference for future studies using molecular clock methods for dating these symbioses within the riodinid butterflies. Modern evidence, and the abundance of dolichoderine ants in Dominican amber (now extinct in the West Indies) imply that specialized symbiotic relationships between *Theope* caterpillars and these ants were likely in existence at least 15 Ma ago. The current distribution of neotropical riodinid butterfly and ant faunas indicates the extinction in the West Indies of at least two unrelated taxa that formed a tightly linked symbiotic association, which persisted to the present elsewhere.

1. INTRODUCTION

Among butterflies the ability to form intimate associations with ants has evolved only in the lycaenoids—the families Lycaenidae and Riodinidae (Cottrell 1984; Pierce 1987; DeVries 1991*a*, 1997; Fiedler 1991). The fundamental nature of these symbiotic associations is that caterpillars provide ants with food secretions in exchange for protection against predators (Cottrell 1984; Pierce 1987; Pierce *et al.* 1987; DeVries 1988, 1997; DeVries & Baker 1989). Experimental studies on both groups have demonstrated that when they are found by insect predators and parasitoids, caterpillars without ant symbionts have little chance of survival, whereas caterpillars with ant symbionts are provided significant protection against these natural enemies (e.g. Pierce & Mead 1981; Pierce *et al.* 1987; DeVries 1991*b*; Cushman *et al.* 1994). By virtue of the strong influence on caterpillar fitness the maintenance of a constant guard of ants is at a premium, and this is achieved through the use of specialized secretory organs that not only produce food secretions to ants, but may also produce semiochemicals that modify ant behaviours (Cottrell 1984; DeVries 1988, 1991*a*, 1997; Fiedler & Suefert 1996).

Vibrational communication is prevalent among ants (Hölldobler & Wilson 1990), and studies suggest that these signals are used by some ants as part of colony communication and recruitment (Markl & Hölldobler 1978; Baroni-Urbani *et al.* 1988; Roces *et al.* 1995). It has recently been established that the ability to produce substrate-borne acoustical calls is a widespread trait among lycaenid and riodinid caterpillars that form symbioses with ants (DeVries, 1990, 1991*c*), and that these calls bear similarities to ant vibrational signals (DeVries *et al.* 1993; DeVries, unpublished data). Thus, lycaenid and riodinid caterpillar calls are considered to function in concert with secretory organs to achieve and maintain ant symbionts (DeVries 1990, 1991*c*, 1997; DeVries *et al.* 1993). Although the mechanisms for caterpillar call production in all lycaenids and members of the riodinid tribe Eurybiini remain unknown, it has been demonstrated that riodinid caterpillars in the tribes Lemoniini and Nymphidiini produce calls by using paired organs, termed vibratory papillae (DeVries 1990, 1991*c*, 1997).

Despite the prevalence of riodinid caterpillars in the tribes Eurybiini, Lemoniini and Nymphidiini to form intimate associations with ants (Harvey 1987; DeVries 1997; DeVries *et al.* 1994), the age of these symbiotic interactions is unknown. The origin of riodinid–ant associations could potentially be estimated indirectly using molecular clock methods with a phylogenetic hypothesis (Kimura 1983; Li & Graur 1991), or directly through the fossil record. However, the age of this phenomenon has never been estimated by molecular clock methods, and no pertinent fossil evi dence exists.

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Dominican amber fossils have been extremely important to our understanding of insect evolution and biogeography (e.g. Baroni-Urbani 1980; Wilson 1985*a*,*b*; Poinar 1992, 1996; Grimaldi 1991, 1996*a*), but fossil inclusions of butterflies in Dominican amber are exceedingly rare. Three adult butterflies of the same species are known from Dominican amber, all of which possess systematic affinities with the mainland neotropical genus *Napaea*, but not to the only extant West Indian riodinid, *Dianesia carteri* (Harvey 1987; Grimaldi 1996*a*; DeVries 1997). As neither present day *Napaea* caterpillars and its relatives, or caterpillars of *Dianesia* and its close relatives possess specialized organs to form associations with ants (see summaries in Harvey 1987; DeVries 1997), riodinid–ant symbioses are unknown from the West Indies. Here we report the first fossil riodinid caterpillar in Dominican amber that not only provides the first accurate benchmark for dating the antiquity of riodinid–ant symbioses, but it contributes further evidence for the historical extinction of major groups of fauna in the Dominican Republic.

2. RESULTS AND DISCUSSION

The unique amber piece containing the riodinid caterpillar originated from the La Toca group of mines in the northern mountain ranges of the Dominican Republic located in the El Mamey Formation (Upper Eocene), which is composed of shale-sandstone interspersed with a conglomerate of well-rounded pebbles (Eberle *et al.* 1980). Recent biostratigraphic and palaeostratigraphic evidence indicates that all Dominican amber was formed in a single sedimentary basin dated from 15–20 Ma old (Iturralde-Vincent & MacPhee 1996), whereas previous chemical and fossil evidence has suggested an age of 30–40 million years old (Schlee 1990).

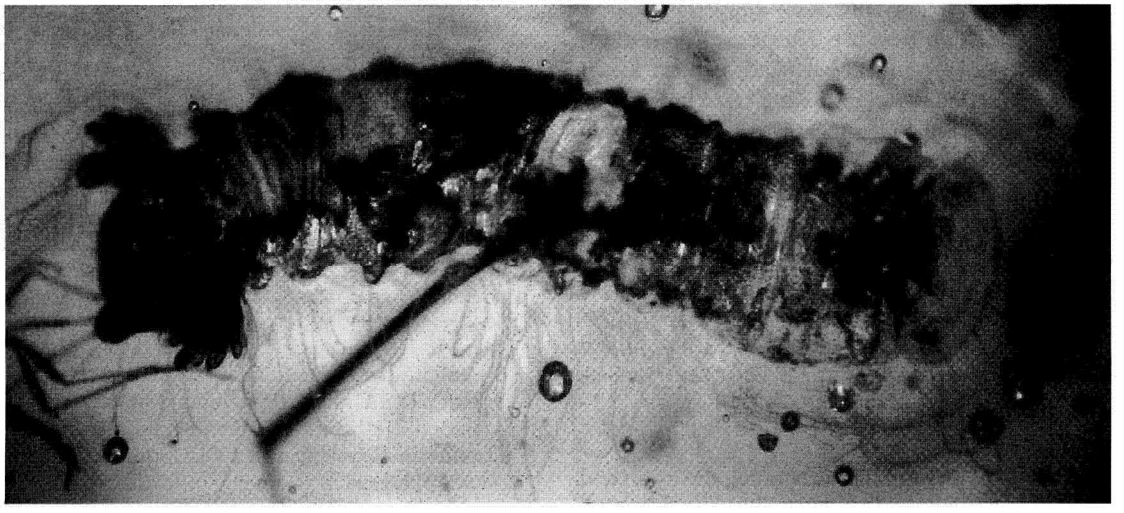
The combined presence of tentacle nectary organs, vibratory papillae, and balloon setae are found only on riodinid caterpillars in the tribe Nymphidiini, and nowhere else among the Lepidoptera (Cottrell 1984; Harvey 1987; DeVries 1997). Thus, the possession of all three of these specialized organs by this fossil (figure 1) place it unquestionably as a riodinid caterpillar that formed symbioses with ants. Possession of exaggerated balloon setae along the anterior margin of the first thoracic segment is typical of caterpillars in the genus *Theope*, although less exaggerated balloon setae are found in the close relative *Nymphidium* (Harvey 1987; DeVries 1997). Present day *Theope* caterpillars possess strongly developed ventro-lateral flanges on all body segments, long plumose lateral body setae, and absence of anterior tentacle organs, traits that are not shared by *Nymphidium* caterpillars (Penz & DeVries, unpublished data). A detailed comparison on contemporary caterpillars of four species of *Theope* (*T. matuta*, *T. guillaumei*, *T. virgilius*, *T. lycaenina*), and four *Nymphidium* species (*N. mantus*, *N. balbinus*, *N.* nr *baeotia*, *N. cachrus*), not only showed that the fossil may be confidently placed in the genus *Theope* (probably in the fourth, and penultimate instar), but that it falls within the overall variation observed among different species of present day *Theope* caterpillars (see illustrations in DeVries (1997)). This report therefore provides the first direct evidence that secretory and acoustical organs used by riodinid butterfly caterpillars to mediate symbioses with ants were highly developed at least 15–20 Ma ago, and serves to constrain future studies that approximate the age of riodinid–ant symbioses using a molecular clock.

There are currently about 50 species in the genus *Theope*, with members occurring in lowland forests throughout Mexico, Central and South America, and on Trinidad and Tobago (Seitz 1916–1920; Barcant 1970; Harvey 1987; Bridges 1988), but they are absent from the West Indies. The contemporary West Indian riodinid fauna consists of a single species, *Dianesia carteri*, and this taxon is confined entirely to Cuba and the Bahamas (Spencer-Smith *et al.* 1994). The fossil *Theope* reported here, together with the other Dominican amber fossil riodinid species (see Grimaldi 1996*a*; DeVries 1997), therefore establishes the extinction of at least two riodinid taxa from the West Indies.

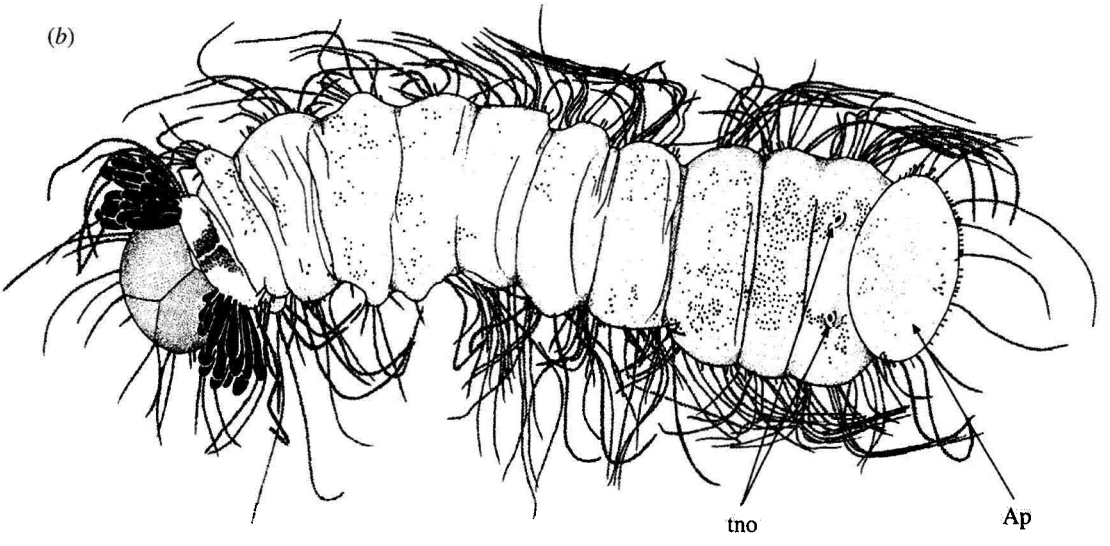
Two points suggest that the palaeoriodinid fauna of the West Indies included more than two taxa. First, it is likely that the composition of the Domincan palaeoentomofauna was similar to the composition of entomofaunas found presently in Central and South America (Baroni-Urbani & Saunders 1980; Wilson 1985*a*,*b*). Analyses of 11 contemporary Central and South American riodinid faunas indicate that approximately 30% of the total species richness at each site is accounted for by taxa that form symbioses with ants (see DeVries 1997). Based on these estimates the Dominican palaeo-riodinid fauna would be predicted to include a minimum of three to four species. Second, the palaeo-entomofauna in Dominican amber remains largely unstudied, and future work will undoubtedly increase the number of taxa above what is currently known (Poinar 1992, unpublished data), including rare taxa like riodinid butterflies. Therefore both comparative estimates based on present day faunas, and the quantity of Dominican amber material yet to be examined, suggest a richer palaeo-riodinid fauna than established by fossil evidence.

A wide range of fossil evidence indicates that ancient symbiotic associations have been remarkably persistent through evolutionary time (reviewed in Boucot 1990; Poinar 1992). For example, Wasmann (1932) described a reduviid bug with its *Dolichoderus* ant prey from Baltic amber, and noted that it differed little from modern day ancestors who remain apparent specialists on these same ants. Other examples are found in Dominican amber (Poinar 1992), and one of the most spectacular is an alate *Acropyga* ant queen carrying a symbiotic scale insect in her mandibles (Grimaldi 1996*b*). Excepting the single record of *Theope guillaumei* being tended by *Solenopsis* ants (while feeding on the *Azteca*-inhabited ant-plant *Cecropia*), all other observations indicate that present day *Theope* caterpillars typically form obligate symbioses with dolichoderine ants, especially the genera *Azteca* and *Dolichoderus* (DeVries *et al.* 1994; DeVries 1997, unpublished data). In these obligate symbioses the exaggerated balloon setae surrounding the head of *Theope* caterpillars (figure 1) appear important in mediating associations with dolichoderine ants by providing semiochemical stimuli to ant symbionts (DeVries 1997).

(*a*)



(*b*)



(*c*)

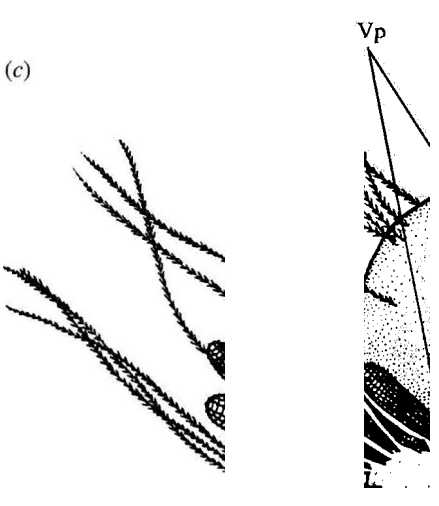


Figure 1. Fossil *Theope* caterpillar in Dominican amber. (*a*) Photograph of the fossil in dorsal view. (*b*) Illustration of the entire fossil in dorsal view showing features characteristic of riodinid caterpillars that form symbioses with ants: conspicuous corona of balloon setae on thoracic segment one, the well-developed anal plate (Ap) on abdominal segments nine and ten, and the external orifices of the tentacle nectary organs (tno) on abdominal segment eight. (*c*) Illustration of head and first thoracic segment detailing the placement of vibratory papillae (Vp) on anterior edge of thoracic segment one. The vibratory papillae function in the production of substrate borne acoustical signals that enhance modern riodinid caterpillar–ant symbioses.

The abundance of *Azteca* and *Dolichoderus* ants in Dominican amber (Wilson 1985*a*,*b*) suggests that a specialized relationship between *Theope* and these dolichoderine ants is likely to have existed at least 15–20 Ma ago. Moreover, as is the case for *Theope* butterflies, neither *Azteca* or *Dolichoderus* are part of the living Dominican Republic ant fauna, but these ant genera form principal elements of mainland neotropical forest habitats (Wilson 1985*a*; Hölldobler & Wilson 1990). Thus, our report not only provides evidence for the extinction of *Theope* from the Dominican Republic, but points to the possible correlated extinctions of two unrelated species that acquired, and maintained a tightly linked symbiotic association that has survived to the present in Central and South America.

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