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MOLECULAR SYSTEMATICS, HISTORICAL BIOGEOGRAPHY, AND EVOLUTION OF  
SPIDER WASPS (HYMENOPTERA: POMPILIDAE)

by

Juanita Rodriguez

A dissertation submitted in partial fulfillment  
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Biology

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Logan, Utah

2014

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## ABSTRACT

Molecular Systematics, Historical Biogeography, and Evolution of Spider Wasps  
(Hymenoptera: Pompilidae)

by

Juanita Rodriguez, Doctor of Philosophy

Utah State University, 2014

Major Professors: James P. Pitts and Carol D. von Dohlen  
Department: Biology

Spider wasps are solitary parasitoids that use one spider to lay a single egg. Even though their behavior seems homogeneous, the features pertaining to nesting and hunting behavior are diverse for different species. There are approximately 5,000 described species, in 120 genera, but there are probably many undescribed species. The systematics of Pompilidae has been studied in recent years, but only morphological data have been used for this purpose. Because of the morphological homogeneity of spider wasps, molecular data may prove promising for understanding the systematics of the group. Furthermore, dated molecular phylogenies calibrated with fossil data may allow studying the historical biogeography and evolution of the group. I used the nuclear molecular markers elongation factor-1  $\alpha$  F2 copy (EF1), long-wavelength rhodopsin (LWRh), wingless (Wg), RNA polymerase II (Pol2), the D2–D3 regions of the 28S ribosomal RNA (28S), and the mitochondrial Cytochrome C Oxidase I (COI) in a Bayesian and Maximum Likelihood framework, to reconstruct the phylogenies of four main

Pompilidae groups: the subfamily Pompilinae, the tribe Aporini, the genus *Psorthaspis*, and the genus *Drepanaporus*. I also studied the fossil Pompilidae, and used those results to produce time-calibrated phylogenies of Pompilinae, Aporini, and *Psorthaspis*.

Molecular phylogenetic results support the utility of the use of molecular markers for species delimitation and sex-associations in Pompilidae. In addition, the use of dated phylogenies supports the correlation of host use with diversification rate-shifts, the coevolution of mimicry between pompilids and velvet ants, and various biogeographical hypotheses never tested before for spider wasps.

(240 pages)

## PUBLIC ABSTRACT

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The study of the diversity and classification of any group of organisms provides a foundation for further scientific studies in ecology, evolution, and conservation. Insects are among the most diverse organisms that inhabit the planet, but knowledge of their diversity and classification is still limited. One understudied group of insects is spider wasps. These are solitary parasitoids that use one spider to lay a single egg. There are approximately 5,000 described species, and many more to be described. Unfortunately, fewer than 10 scientists worldwide study these insects. One reason the group has not been very well studied is the difficulty in telling species apart. This makes their classification troubling. With the advent of molecular genetics methods, the use of molecular data to understand the classification and evolution of various groups is now possible. My dissertation uses molecular data to understand the classification of spider wasps, as well as their evolutionary relations. The evolutionary trees produced by these analyses are helpful to study the causes of current distributions of species, the diversification and the

evolution of the group. Molecular phylogenetic results support the utility of the use of molecular markers for species delimitation and sex-associations in Pompilidae, the correlation of host use with diversification rate-shifts, the coevolution of mimic pompilids with velvet ants, and various biogeographical hypotheses never tested before for spider wasps.

(240 pages)

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Juanita Rodriguez

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# CHAPTER 1

## INTRODUCTION

Spider wasps (Hymenoptera: Pompilidae) are a widespread group with about 5,000 described species (Pitts et al. 2006) in 120 genera (Wasbauer 1995). Most pompilids are recognized by their continuous wing flicking (Harris 1987). Female pompilids fly short distances searching for prey, but spend most of the time foraging on the ground (Wasbauer 1995). Pompilids are solitary wasps characterized by laying a single egg on one paralyzed spider. For this behavior, they have been classified as predatoids (Evans 1963), or parasitoids (Godfray 1994). The spider wasp hunting and nesting sequence is usually as follows: they hunt and paralyze their prey, carry it to a nesting site, conceal it in a single-celled nest, oviposit on it, and seal the nest (Malyshev 1966). The prey is normally deposited in a nest built by the female or modified from a pre-existing cavity (O'Neill 2001). Even though most of the larvae feed on paralyzed spiders, some species are ectoparasites of active spiders, or cleptoparasites (*i.e.* they take spiders already captured or prepared by other wasps) of other pompilids (Goulet and Huber 1993).

Males are usually only involved in reproduction, but mating behaviors are not well understood. Males are smaller and emerge before the females, spending their time near the ground looking for mates (Wasbauer 1995). The male and female adults are short-tongued and feed on internal or external flower nectaries (Evans 1966; Wasbauer 1995). Females have been observed feeding on spider haemolymph (Wasbauer 1995).

Although the biology of Pompilidae seems uniform in some respects, there is great diversity in several aspects of prey handling and nesting behaviors, such as prey-

carrying mechanisms, choice of nesting habitat, nest structure, and nesting sequences. Prey carrying mechanisms involve pushing, pulling, or flying (Evans and Yoshimoto 1962). In the most extreme cases, the spider's legs are amputated before carrying it in flight (Malyshev 1966).

The location of the nest may vary from underground to aerial, and the number of cells per nest also varies among species. Many pompilids nest in the ground by making their own burrow or using a preexisting one. When a new burrow is dug, excavation by the female is often performed by scraping soil backwards with her forelegs, and the nest is then sealed by using the tip of the abdomen (Evans 1953). A few species nest above the ground in protected places, or by building thick mud walls. Nest building above the ground could have been a pre adaptation for multicellular construction and communal occupation of nests (Evans and Shimizu 1996).

Nesting sequences refer to the order in which behaviors are performed in the hunting and nesting sequence. Many female pompilids prepare the nest cavity after capturing the prey. Other spider wasps prepare the nest before hunting the spider. This last strategy has been proposed as an advantage to reduce the need for prey protection from other predators or cleptoparasites (Evans 1953).

Even though all pompilids use spiders exclusively, many species of Pompilidae use spiders of different, distantly related, spider families. Nonetheless, host use seems to have a pattern when host families are grouped into guilds. This grouping often results in ecological rather than taxonomic specificity. The evolution of host shifts in Pompilidae has not been addressed, but results from various recent studies on parasitoids suggest that

host shifts are correlated with increase in diversification rates (McKenna and Farrell 2006; McLeish et al. 2007; Wheat et al. 2007; Winkler et al. 2009; Fordyce 2010).

Even though taxonomic studies in Pompilidae are scarce, recent advances have been made in the study of localized faunas. Worldwide taxonomical studies, however, are limited and there is a large amount of synonymy at the generic and infra-generic level (Wasbauer 1995). The fauna of North America has been widely addressed, but most of the Central and South American taxa are in need of revision. Few studies have been focused in Neotropical Pompilidae (e.g. Bradley 1944; Evans 1966, 1968; Colomo de Correa 1991, 1998; Snelling and Torres 2004). The South American fauna has not been studied in depth, and the existing studies are mainly focused on species descriptions, which is probably is a result of the lack of reference bibliography, and the difficulty in identifying pompilids (Elliott 2007).

The phylogeny of Pompilidae has been studied using morphological data. Analyses of the whole family began with Shimizu (1994). The phylogeny produced by these data gave the first insight into relationships of spider wasps, but many relationships remained unresolved, and the relations between upper-level groups were not clear (Pitts et al. 2006). Pitts et al. (2006) reanalyzed data from Shimizu (1994) and provided a better understanding of the relationships between these wasps. Further studies that include a greater number of taxa, morphological characters, and DNA sequences are needed to obtain a well-resolved Pompilidae phylogeny.

In light of the lack of knowledge in the systematics of Pompilidae, this dissertation research focused on the use of phylogenetic reconstructions at various taxonomic levels to address evolutionary, biogeographical, and taxonomic questions in

spider wasps. The aims of my dissertation were: 1) to study of fossil Pompilidae in order to establish accurate calibration points for time divergence studies; 2) to study the utility of molecular data for species delimitations and sex-associations in the genus *Drepanaporus*; 3) to test various biogeographic hypotheses using Aporini as a model; 4) to understand the influence of codivergence in the evolution of Müllerian mimicry between velvet ants and *Psorthaspis* spider wasps; and 5) to study the correlation between diversification rate-shifts and host shifts in Pompilinae.

The knowledge of the evolution of any group of organisms involves studying its fossil specimens. Fossil dates are an important tool to use for evolutionary studies, because fossil ages are the most reliable source of calibration points in dating molecular phylogenies (Donoghue and Benton 2007). Studies in fossil Pompilidae are scattered and mostly were done in the 1800s. Therefore they do not fit current classification schemes. Recently, a fossil from Burmese amber was described as the oldest Pompilidae fossil (Engel and Grimald 2006). This is the only amber fossil described to date. Engel and Grimaldi (2006) provided a list of known Pompilidae fossils, but the entire Pompilidae fossil record has never been studied.

Chapter 2 of my dissertation focused on the study of amber fossils from the Oregon State Arthropod Collection. I also studied the previously described *Bryopompilus interfactor* Engel and Grimaldi (2006), which placed Pompilidae in the Cretaceous. Through the analysis of these fossils, I added two genera to the extinct pompilid fauna: *Tainopompilus* gen. nov., and *Paleogenia* gen. nov. I described three new species of fossil spider wasps: *Anoplius planetarius* sp. nov., from Dominican amber (Burdigalian to Langhian); *Paleogenia wahisi* sp. nov., from Baltic amber (Lutetian to Priabonian);

and *Tainopompilus argentum* sp. nov, from Dominican amber (Chattian to Langhian). Through the morphological examination of the holotype of *Bryopompilus interfector*, from Burmese amber (mid-Cretaceous), I determined that this species does not fit the diagnostic characters of Pompilidae. Moreover, it does not fit the diagnosis of any other extant Hymenoptera family. Therefore, I placed it in the new family Bryopompilidae. My results suggest that pompilids originated in the Eocene, not the mid-Cretaceous as previously proposed. This is consistent with a recent estimate based on molecular data, which dated the origin of crown-group Pompilidae to the early Paleogene (Wilson et al. 2013). The sole hosts of Pompilidae, spiders (Araneae), originated in the Carboniferous (Selden et al. 2013), with extant suborders and many sub-lineages diversifying by the Lower Jurassic, ca. 175 Ma (Vollrath and Selden 2007). Thus, the origin and diversification of Pompilidae occurred long after the diversification of their prey. The comparison between the origin of Pompilidae and previous spider diversification studies suggests that spider wasp diversification is probably correlated with an increase in spider familial diversity in the Cenozoic. Chapter 3 of this dissertation was intended to be a thorough taxonomic revision of all the fossil spider wasps. Through this taxonomic study I described a new species of fossil Pompilidae: *Dipogon (Deuteragenia) catalanicus* Rodriguez, Waichert and Pitts. There were various taxonomic changes as follows: *Ceropalites infelix* Cockerell, from the Florissant Fossil Beds (Priabonian), is no longer recognized as Pompilidae. *Agenioideus saxigenus* (Cockerell), from the Florissant Fossil Beds (Priabonian); and *Dipogon wettweri* (Statz), from the Rott deposits (Chattian) are new combinations. This revision studied 21 fossil species of spider wasps. Because of the

lack of morphological characters preserved in compression fossils, ten of the described Pompilidae fossil species had to be declared *nomen dubia*.

Chapter 4 of this dissertation sought to explore the utility of molecular markers for species delimitation and sex-associations in Pompilidae using the genus *Drepanaporus* as a model. *Drepanaporus* Bradley is a genus of dimorphic spider wasps, comprising three species found only in the Antilles. Two of these species had been described previously; the third species, *Drepanaporus bachata* Rodriguez and Pitts sp. nov, was identified and described in this work. *Drepanaporus* females are brightly colored, share a color pattern, and have a higher degree of morphological variation than males. Male external morphology is highly uniform, which makes the taxonomy of *Drepanaporus* complicated, and suggests the need to apply molecular characters for taxonomic purposes. The most widely molecular marker for species delimitation, mitochondrial Cytochrome c Oxidase (COI), has been proposed as the standard molecular barcode for animals (Folmer et al. 1994). This marker has also been used to establish species boundaries (Hou and Li 2010; Dombroskie and Sperling 2012; Navia et al. 2013), and sex-associations (Kurina et al. 2011; Zhang et al. 2013) in various taxa. However, there have been some problems with heteroplasmy (multiple, divergent sequences) in bees (Magnacca and Danforth 2006). Recent studies have used long-wavelength rhodopsin (LWRh), for assessing species boundaries (Derocles et al. 2012). This molecular marker is commonly used in Hymenoptera systematics, and shows high variability at the species level (Hines et al. 2006; Blaimer 2012; Rightmyer et al. 2013). We amplified both molecular markers for both females and males, and reconstructed the phylogeny of *Drepanaporus*. Sequences obtained for COI showed unusually high

divergences and putative introgression, and thus were not used for taxonomic decisions. Taxonomic changes were made based on the LWRh phylogenetic results.

When calibrated phylogenies are produced, there are various research questions that can be asked. One of them is the study of the historical processes that may be responsible for the contemporary geographic distributions of individuals, which is known as historical biogeography. Several pompilid groups have interesting distributions that can be used to test historical biogeography theories. The tribe Aporini is found in the Americas, the Antilles, and the Palearctic, making it an ideal candidate to study dispersal events between these regions. Chapter 5 of my dissertation sought to study the historical biogeography of Aporini spider wasps.

Aporini contains 10 valid genera and 105 species (Table 1.1) (Bradley 1944; Evans 1966). It includes wasps with a characteristic morphology related to the specialization for entering subterranean nests of spiders (Evans 1966). The little information available on the behavior of the group suggests that Aporini is predaceous on various species of *trap-door spiders* (Ctenizidae) (Snelling and Torres 2004), and uses the spider's burrow as a nest.

Aporini are mostly found in the New World. Their distribution ranges from the northeast of the United States to Chile and Argentina, and includes the Antilles (Table 1.1) (Bradley 1944; Evans 1966; Colomo de Correa 1998). Only *Aporus* is found in the Palearctic. The location of geographical areas in the Aporini phylogeny is interesting, giving rise to biogeographic question about disjunct distributions, and its distribution in the Americas, including the Antilles.

Evans (1966) presented an overview of the distribution patterns in Pompilinae, giving some insights into the possible explanations for aporine biogeography. He speculated that the fauna of Mexico and Central America was largely of North American origin. The North American Pompilinae were proposed to belong to an “old North American” (Sonoran) fauna and “new North American” fauna. Also, some of the elements of the West Indies were suggested to have entered through Central America.

Using *Aporini* as a model, I aimed to test the fit of several hypotheses concerning the putative processes underlying the widespread distribution of this group. My molecular data produced a phylogeny of 44 *Aporini* specimens using four nuclear molecular markers, and a lognormal relaxed molecular clock, calibrated with ages from three fossils studied in Chapter 3, was used to estimate lineage divergence times. Biogeographic processes were studied using ancestral area reconstructions. My results suggest that the dispersal from the Nearctic region to the Palearctic occurred through the Bering Land Bridge in the early Miocene, 15–18 Ma (CI = 11.14,23.52). This is consistent with results from previous studies for insects (von Dohlen et al. 2002; Hundsdoerfer et al. 2005; Ohshima et al. 2010; Ren et al. 2013), but is the first time reported for stinging wasps. There were three dispersal events to South America from Mesoamerica, which took place independently. All of these occurred after 18 Ma through the Isthmus of Panama, and are consistent with recent studies that suggest an age for the formation of the Isthmus of Panama ca 15 Ma (Montes et al. 2012a, 2012b) and recent studies for various taxa that dispersed through this area before 7 Ma (Perini et al. 2010; Carvalho and Renner 2012; Pinto-Sanchez et al. 2012; Colston et al. 2013). The Antillean taxa have a Nearctic and Mesoamerican origin. There were three independent over–water dispersal events to the

Antilles from Mesoamerica, and probably the Nearctic, for two genera of the tribe.

Recent molecular divergence dating analyses support the over-water dispersal hypothesis for various taxa (Hedges et al. 1992; Lavin et al. 2003; McDowell et al. 2003; Davalos 2007; Colston et al. 2013), including insects (Oneal et al. 2010). Many of Evans' (1966) hypotheses on the origin of Aporini fauna have been proved in this study.

Dated molecular phylogenies also allow for the study of coevolution by comparing the dates of origin, and the branching patterns of the groups involved in ecological interactions. Some Pompilidae have been found to be putatively involved in Müllerian mimicry systems because of their aposematic coloration. One of the explanations is that their painful sting is a deterrent to predation. *Psorthaspis* spider wasps exhibit a similar coloration to *Dasymutilla* velvet ants, and are found in the same areas where *Dasymutilla* mimics are found. This last group was recently described as part of North America's largest Müllerian mimicry system (Wilson et al. 2012). Chapter 6 of this dissertation sought to study the fit and possible evidence of coevolution between *Psorthaspis* and *Dasymutilla* velvet ants, for which Müllerian mimicry rings have been recently described by Wilson et al. (2012).

*Psorthaspis* includes 28 valid species (Table 1.2) found from the northeastern United States to the northern South America, including the Antilles (Table 1.2). Only one species has been reported for South America, and is located in the northern Sierra Nevada de Santa Marta, Colombia (Bradley 1944; Rodriguez et al. 2010).

The genus is easily identified, showing a characteristic morphology with long pronotum and rounded clypeus for both males and females. The males are very homogeneous morphologically. The identification of North American males is difficult

without the use of genitalia (personal observations). Evans (1966) studied the Central American fauna in detail and provided good keys for the species. The group is in need of revision especially because of the difficulty in male identification and the matching between sexes.

Using *Psorthaspis* molecular data I aimed to determine the fit of *Psorthaspis* spider wasps to the *Dasymutilla* velvet ant Müllerian mimicry rings by performing human perception tests and ordination plots of morphological characters. I also aimed to test for coevolution between *Psorthaspis* and *Dasymutilla* by comparing the branching patterns and date of origin of both groups. For this, I obtained four molecular markers from *Psorthaspis* species and performed a Bayesian divergence dating analysis using a single calibration point for the crown group of *Psorthaspis* obtained from results of Chapter 5. For dates and branching pattern comparison, I used a dated phylogeny of *Dasymutilla* from Williams (2012). The results obtained suggest that *Psorthaspis* belongs to the *Dasymutilla* mimicry ring, but with a low mimetic fidelity. My results also suggest that there is evidence of codivergence between *Psorthaspis* and *Dasymutilla*, therefore there is evidence of coevolution. This large mimicry complex is an intriguing system that should be the focus of further investigations into the evolution of predator avoidance strategies in the temperate regions, the evolution of aposematic coloration, and the evolution of Müllerian mimicry involving unrelated taxa.

Diversification rate-shifts can also be studied with time-calibrated phylogenies, and can be used to correlate to ecological traits such as host shifts. Pompilids use a variety of spider families as hosts, but there is some specificity at the ecological level. Host switching events in parasitoids have been shown to result in rapid species

diversification (Ehrlich and Raven 1964; Cocroft et al. 2008) by environmental differentiation, competition, and specialization, and also antagonistic interactions with hosts (Thompson 1999). One of the most diverse pompilid subfamilies is Pompilinae (Pitts et al. 2006). Members of this family use a variety of spider guilds as hosts, and are an excellent model for the study of the correlation of diversification rate-shifts and host shifts.

The phylogenetic analysis of Pompilinae is also useful to determine the correct classification of the group. This subfamily includes about 2,000 species (almost half of Pompilidae described species). Pompilinae has been established as monophyletic by Shimizu (1994) and Pitts et al. (2006), but the taxonomy of the group has not been explored taking into account the world fauna. The tribal classification of the subfamily has been problematic because many entities have been established for different geographic regions (Evans 1949). The Nearctic Pompilinae are still divided in two tribes: Pompilini and Aporini, as suggested by Evans (1949). According to Evans (1949) a comprehensive revision of the world fauna needs to be performed in order to produce an accurate tribal division that corresponds to natural groups. From this fact emerges the necessity of studying the world fauna from a phylogenetic perspective.

Chapter 7 of my dissertation studied effect of host shifts in diversification in Pompilinae. The classification of the subfamily was also discussed. Diversification rate-shifts have been attributed to niche differentiation in a process known as adaptive radiation (Schluter 2000). This has been shown to have an effect on diversification rates when parasitoid host shifts occur (McKenna and Farrell 2006; McLeish et al. 2007; Wheat et al. 2007; Winkler et al. 2009; Fordyce 2010). In this chapter I aimed to

determine if there is a correlation between host shifts and diversification rate-shifts in pompilid spider wasps. I also aimed to determine if the tribal classification proposed by past authors (Arnold 1937; Priesner 1969; Day 1981) is supported by the phylogeny of the group. To answer these questions I performed a Bayesian molecular phylogenetic analysis of 77 taxa in 36 genera of Pompilinae using four molecular markers. I used a single calibration point obtained by Waichert et al. (submitted) for the Pompilinae crown group and performed a divergence time estimation analysis. I also mapped the host guild use onto the phylogeny using a parsimony and ML approach. Finally I performed two diversification rate shift analyses onto the Pompilinae chronogram. My results suggest that there were multiple host guild shifts throughout the evolutionary history of Pompilinae and that one of them is probably correlated with a switch to the use of ground hunters as hosts. Moreover, none of the tribes previously proposed for the classification of Pompilinae are monophyletic, and neither are some of the genera.

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Table 1.1. Aporini genera showing number of valid species and distribution.

Genus	Number of valid species	Distribution
<i>Allaporus</i>	10	Southern Mexico to Southern United States
<i>Aporus</i>	41	Palaearctic, Nearctic and Neotropical
<i>Aspidaporus</i>	1	Brazil
<i>Drepanaporus</i>	2	Cuba
<i>Rhabdaporus</i>	2	Brazil
<i>Chelaporus</i>	1	Eastern and central Mexico to Texas
<i>Euplaniceps</i>	16	South America
<i>Notoplaniceps</i>	3	Costa Rica to eastern Brazil
<i>Odontaporus</i>	3	Antilles
<i>Psorthaspis</i>	38	Southern United States to eastern Colombia

Table 1. 2. Valid species of *Psorthaspis* and their distributions.

Species	Distribution
<i>Psorthaspis connexa</i>	Northern Mexico to Colombia
<i>P. brimleyi</i>	Eastern United States
<i>P. coelestis</i>	Southern Mexico
<i>P. macronotum</i>	Southern United States to northern Mexico
<i>P. conocephala</i>	Southern United States
<i>P. guatemalae</i>	Costa Rica to Guatemala
<i>P. australis</i>	Southern United States
<i>P. avinoffi</i>	Jamaica
<i>P. laevifrons</i>	Panama to Northern Mexico
<i>P. bequaerti</i>	Northern Colombia
<i>P. bradleyi</i>	Mexico
<i>P. colombiae</i>	Colombia
<i>P. legata</i>	Eastern United States
<i>P. portiae</i>	Arizona to northern Mexico
<i>P. mariae</i>	Eastern to southern United States
<i>P. elegans</i>	Cuba
<i>P. eubule</i>	Costa Rica to Guatemala
<i>P. formosa</i>	Costa Rica to Mexico
<i>P. hispaniolae</i>	Dominican Republic
<i>P. luctuosa</i>	Eastern United States
<i>P. magna</i>	Eastern United States
<i>P. legata</i>	Eastern United States
<i>P. nigriceps</i>	Southern United States
<i>P. purpuripennis</i>	Antilles
<i>P. regalis</i>	Costa Rica to Mexico
<i>P. sanguinea</i>	Eastern United States
<i>P. texana</i>	Texas
<i>P. vicina</i>	Texas

## CHAPTER 2

TWO NEW GENERA AND THREE NEW SPECIES OF FOSSIL POMPILIDAE  
FROM AMBER AND THEIR EVOLUTIONARY IMPLICATIONS<sup>1</sup>

## ABSTRACT

We add two genera to the extinct pompilid fauna: *Tainopompilus* gen. nov., and *Paleogenia* gen. nov. Three new species of fossil spider wasps are described: *Anoplius planetarius* sp. nov., from Dominican amber (Burdigalian to Langhian); *Paleogenia wahisi* sp. nov., from Baltic amber (Lutetian to Priabonian); and *Tainopompilus argentum* sp. nov., from Dominican amber (Chattian to Langhian). *Bryopompilus intersector* Engel and Grimaldi, 2006, from Burmese amber (mid-Cretaceous) is no longer recognized as Pompilidae and is placed in the new family Bryopompilidae. Pompilidae probably originated in the Eocene, not in the mid-Cretaceous as previously proposed. The origin of the spider wasps is probably correlated with an increase in spider familial diversity in the Cenozoic.

**Introduction**

Spider wasps (Hymenoptera: Pompilidae) are solitary ectoparasitoids that show a wide variety of hunting, nesting, and prey-carrying behaviors as adults. Females specialize in hunting spiders, which they typically paralyze permanently, then lay a single egg on their body. The resulting larva consumes the spider host. In several lineages of spider wasps the spider is only temporarily paralyzed and the spider wasp larva feeds on

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it as the spider behaves normally. Cleptoparasitoid pompilids, like *Evagetes* (Wasbauer and Kimsey 1985) and *Poecilagenia* (Shimizu 2000), take the host of another spider wasp and use it as their own host. Although behavior is not recorded for all spider wasp species, a certain degree of ecological or taxonomical host specificity has been reported (Evans and Yoshimoto 1962).

Approximately 5,000 species of Pompilidae are described and are currently classified into four subfamilies (Pitts et al. 2006). Presently there are 21 species of fossil Pompilidae described. The taxonomy of extinct spider wasps is challenging, because many of the descriptions (mostly published from Tertiary compression fossils in the late 1800s and early 1900s) lack necessary details and figures that could facilitate the placement of specimens in appropriate genera (Engel and Grimaldi 2006).

Until recently, the age of Pompilidae was based on the description of a fossil in Burmese amber, which dates from the Albian (mid-Cretaceous) (Engel and Grimaldi 2006). This date conflicts with a recent estimate based on molecular data, which dated the origin of crown-group Pompilidae to the early Paleogene (Wilson et al., 2013). Here we describe two new genera and three new species of spider wasps and provide a discussion on the evolutionary implications of these new fossils.

*Institutional abbreviations.*— Amber fossils from the following collections were studied: AMNH, American Museum of Natural History, New York, New York, USA; OSAC, Oregon State Amber Collection, Oregon State University, Corvallis, Oregon, USA.

*Terminology.*— Abbreviations used in the descriptions are the same as those used by Wasbauer and Kimsey (1985). They are defined as follows: LA3, length of third antennal segment, LC, maximum height of clypeus, WA3, width of third antennal segment, and

WC, width of clypeus, measured between the widest points. Wing venation terminology follows that of Huber and Sharkey (1993, figs. 19–20).

### **Material and methods**

The Dominican amber fossils studied derive from deposits found in mines between the cities of Santiago and Puerto Plata (Dominican Republic). One of the Baltic amber fossils derives from the Kaliningrad region (Russia). The specimens newly described here were preserved in Baltic and Dominican amber. The holotypes are deposited in the Oregon State Arthropod Collection (OSAC), as assigned. The amber fossil of *Bryopompilus interfactor* was obtained on loan from the American Museum of Natural History (AMNH).

The species treated here were assigned to the family Pompilidae based mainly on wing venation features, which are relatively uniform for the family (Day 1988). These were placed in the family Pompilidae based on the following combination of characters: presence of ten closed cells in the forewing, the hind wing with the veins C+Sc+R+Rs fused basally, and the second abscissa of 1A lost. Marginal cell with vein Rs rounded and attached to anterior margin of wing. Vein Rs of cell 1Rs attached to the base of cell 2R1. Costal cell ending on the anterior margin of the wing.

### **Systematic palaeontology**

Order Hymenoptera Linnaeus, 1758

Family Pompilidae Latreille, 1804

Subfamily Pompilinae Latreille, 1804

Genus *Anoplius* Dufour, 1834

*Type species: Sphex fusca* Linnaeus, 1751 (Latreille, 1803), type by subsequent designation; Recent, England.

*Anoplius planetarius* Rodriguez and Pitts **sp. nov.**

Fig. 2.1.

*Etymology:* This species was named in honour of Iomara Arrieta and Francisco Manuel Rodriguez, parents of the first author.

*Type material:* Holotype, complete male inclusion, OSAC Hy-10-45.

*Type locality:* Cordillera Septentrional, between Puerto Plata and Santiago, Dominican Republic.

*Type horizon:* Dominican amber; early Miocene.

*Diagnosis.*— Wings hyaline; maximum width 0.18X its length; 2Rs cell as long as 1Rs; 2m-cu vein slightly curved, meeting 2Rs cell 0.70X distance from base to apex of cell; and 2M cell with an inflection at the base of the vein Cu.

*Description.*— Male. Body length 6.20 mm. Forewing 4.80 mm. Integument dark on head and mesosoma, light on metasoma. Body pubescence short and scattered on entire body. Mandible glabrous. Erect, long setae, present on second half of mandible. Pygidium bare, polished. Punctuation inconspicuous. Antenna elongate; ratio of length of segments two to four 6:15:16; WA3 0.40X LA3; WA4 0.26 LA4. Pronotum short, width 2.35X length, posterior margin slightly angulate; pronotal disc well defined. Length of 2R1 cell 0.71X distance from its margin to wing apex; 2Rs cell as long as 1Rs; 2m-cu slightly curved, meeting 2Rs cell 0.70X distance from base to apex of cell. Tibiae and tarsi with few spines present, short, acute, sparse; pulvillar comb strong; metasoma 1.33X as long as mesosoma.

*Remarks.*— This is the first species of *Anoplius* described from Dominican amber.

We are confident about the placement of this species into *Anoplius* due to the good preservation of the specimen. The characters that place this specimen in *Anoplius* are: the postnotum is a transverse band with parallel anterior and posterior margins, the 2m-cu vein arises on the Cu less than half the distance from the base of the 2M cell to the outer wing margin, the clypeus is emarginated, the strong pulvillar comb, and the claws bifid. The only other genus with which it could be confused is *Arachnospila* Kincaid, 1900, which occurs in the Nearctic region, but *Arachnospila* does not have a strong pulvillar comb. *Anoplius planetarius* sp. nov. does not fit the diagnosis of any of the *Anoplius* subgenera; on the contrary, it shows a combination of characters that belong to many of them. The two subgenera that *Anoplius planetarius* sp. nov. best fits are *Anoplioides* Banks, 1939 and *Arachnoproctonus* Howard, 1901. In the first case, members of the subgenus have a 2Rs cell wider anteriorly than 1Rs, but *A. planetarius* sp. nov. lacks this character. Also, extant members of *Anoplioides* do not show light colouration on the metasoma as does *A. planetarius* sp. nov. The light orange colouration on the metasoma could place *A. planetarius* sp. nov. in the subgenus *Arachnoproctonus*. Nevertheless, members of this subgenus have a fifth tarsomere in the front leg with the inner margin slightly produced, while in *A. planetarius* sp. nov. it is parallel sided. Given these reasons, we are not placing this species in an extant subgenus, rather, this is considered a desiomorph species, i.e., a fossil that possesses morphological characters found in two or more fossil or extant groups. Desiomorphs have been found in amber, and are known in species of Coleoptera, Diptera, Hemiptera, Hymenoptera, and Neuroptera (Poinar 2012).

*Stratigraphic and geographic range.*— This fossil was collected from amber mines in the northern region of Dominican Republic. The age of Dominican amber is controversial, with published dates ranging from 45–30 Ma (Cepek in Schlee 1990) to 20–15 Ma (Iturralde-Vinent and MacPhee 1996). Amber from the northern region of Dominican Republic (where this specimen was collected) has been found to be from 40 to 26 Ma (Lambert et al. 1985), but Iturralde-Vinent and MacPhee (1996) argue that all Dominican amber should be dated to the same age as the deposits bearing it, because evidence suggests that the fragments have not been emplaced by re-deposition. Therefore, Iturralde-Vinent and MacPhee (1996) proposed an age of 20–15 Ma for all Dominican amber, based on biostratigraphic and palaeogeographic data from Hispaniola.

Insect inclusions have also been observed in Dominican copal (Brown 1999). This material is similar to amber in appearance and composition, and for this reason has sometimes been mistakenly reported as Pliocene/Pleistocene amber. Radiocarbon dating has suggested ages of less than 50,000 years for copal, while ambers are not within the radiocarbon age range (Burleigh and Whalley 1983).

Genus *Tainopompilus* Rodriguez and Pitts **gen. nov.**

*Etymology:* The root name comes from Tainos, a pre-columbian indigenous culture that populated the Dominican Republic. The suffix comes from the Latin-*pompilus*, widely used for Pompilidae taxa, which means pilot fish. The gender is masculine.

*Type species:* By monotypy.

*Species included:* *Tainopompilus argentum* Rodriguez and Pitts sp. nov.

*Diagnosis.*— Antennal flagellum crenulate; postnotum is a narrow band, with parallel anterior and posterior margins; metatibia with apical spine-like setae of uniform length, the setae not splayed; 2M cell with an inflection on the base of vein Cu; 2m-cu vein arising on the Cu less than half the distance from the base of the 2M cell to the outer wing margin.

*Remarks.*— This genus resembles *Priochilus* Banks, 1943 in its general morphology. Nevertheless, the presence of spine-like setae of uniform length on the metatibia, and the presence of an inflection at the base of vein Cu of the 2M cell, separate the two genera. *Tainopompilus* gen. nov. is placed in the subfamily Pompilinae by the presence of an inflection at the base of the Cu vein on 2M cell. This is the only genus in the subfamily that has spine-like setae of uniform length on the metatibia.

*Stratigraphic and geographic range.*— The type species specimen was collected in the Dominican Republic from amber mines located between the cities of La Plata and Santiago. The age of Dominican amber is controversial, as discussed previously.

*Tainopompilus argentum* Rodriguez and Pitts **sp. nov.**

Fig. 2.2.

*Etymology:* The epithet *argentum* comes from the Latin and means silver. This species was named in honor of the city Puerto Plata (silver port), close to where the holotype was collected.

*Type material:* Holotype, complete male inclusion, OSAC Hy-10-45.

*Type locality:* Cordillera Septentrional, between Puerto Plata and Santiago, Dominican Republic.

*Type horizon:* Dominican amber; early Miocene

*Diagnosis.*— Wing hyaline; maximum width 0.31X its length; 2Rs cell as long as 1Rs; 2m-cu vein curved, meeting 2Rs cell 0.55X distance from its base to apex of cell; and 2M cell with an inflection at the base of Cu vein.

*Description.*— Male. Body length 3.95 mm. Forewing 2.50 mm. Pubescence sparse and short on entire body including the mandible. Pygidium covered with short pubescence. Punctuation conspicuous on mesosoma. Antennae elongate, crenulate; ratio of segments two to four 6:9:10; WA3 0.8X LA3; WA4 0.4X LA3. Pronotum short, width 8.3X length, posterior margin concave; pronotal disc well defined. Wing long; length of 2R1 cell 0.50X distance from its edge to wing apex; 2Rs cell as long as 1Rs; 2m-cu vein curved, meeting 2Rs cell 0.55X distance from base to apex of cell. Tibiae and tarsi with few short, sharp, sparse spines; metasoma 0.78X as long as mesosoma.

*Remarks.*— This is the only described species of *Tainopompilus* gen. nov.

*Stratigraphic and geographic range.*— The fossil was collected from amber mines in the Dominican Republic. The age of Dominican amber is controversial, as discussed above.

Subfamily Pepsinae Lepeletier, 1845

Genus *Paleogenia* Waichert and Pitts **gen. nov.**

*Etymology.*— The generic epithet has the Greek root *Paleo*, which means ancient. The suffix comes from *Agenia* (an epithet derived from a proper name) widely used for Pepsinae taxa. The gender is feminine.

*Type species:* By monotypy.

*Species included:* *Paleogenia wahisi* Waichert and Pitts sp. nov.

*Diagnosis.*— Antennal segments short; propodeum smooth, with a lateral carina; tibia with apical spine-like setae short, regular; fore, mid and hind tibia not spinose; first metasomal segment with a lateral carina; wing hyaline; forewing with cells short, 2M cell without an inflection on the base of Cu vein; 1Rs and 2Rs about the same size; 1R1 and 1M about the same size; 1M  $\frac{1}{3}$  as wide as long; 2m-cu vein arising on the Cu more than half the distance from the base of the 2M cell to the outer wing margin.

*Remarks.*— This genus is morphologically similar to the cosmopolitan genus *Minagenia* Banks, 1934. These genera resemble each other by having cells 1Rs and 2Rs small and about the same size, a short clypeus, straight stinger, and bulging eyes. However, *Paleogenia* gen. nov. differs from *Minagenia* by having dentate claws, short antennal segments, and subgenital plate S6 not laterally compressed. Additionally, the 2R1 cell in *Paleogenia* gen. nov. is large, with length 2.5X its width, and it almost touches the apical margin of the forewing. Usually in pompilids, the 2R1 cell ends somewhere in the anterior margin of the wing, never the apical margin.

*Paleogenia* gen. nov. is assigned to the subfamily Pepsinae due to the absence of an inflection at the base of the Cu vein in 2M cell and the presence of regular, apical spine-like setae on the tibia. Additionally, *Paleogenia* gen. nov. has the metasomal sternum 2 with a distinct sharp transverse groove. This genus is placed in the tribe Pepsini, because it has a defined carina on the first metasomal segment. This is the only genus in the tribe with short antennal segments. The wing venation also resembles that of *Poecilagenia* and *Nipponodipogon* Ishikawa, 1965 species. However, *Poecilagenia* has an elongated body and punctuated integument that differs from the short body and polished integument shown on *P. wahisi* sp. nov. Besides, *P. wahisi* sp. nov. has a

transversal carina on the first metasomal segment, which is absent on *Poecilagenia* species. *Paleogenia* gen. nov. cannot be placed on *Nipponodipogon*, because it has two apical mandibular teeth, whereas in *Nipponodipogon* three teeth are present. Moreover, *Paleogenia* has hyaline wings, lacking basal or apical fascia on forewing, as present on *Nipponodipogon*.

*Stratigraphic and geographic range.*— Specimens were collected from the Kaliningrad region of Russia, which is the westernmost part of the country, located between Poland and Lithuania along the southeastern coast of the Baltic Sea. Baltic amber deposits have been obtained for more than 100 years, and their age is controversial. Microfaunistic dating of the deposits containing the largest amount of amber suggest they are from the Priabonian, Eocene (37.7 Ma) (Kaplan et al. 1977), whereas radiometrically dated glauconite dates them as Lutetian, Eocene (47.0 to 44.1 Ma) (Ritzkowski 1997). Perkovsky et al. (2007) considered the Ritzkowski (1997) data insufficient to disprove Kaplan et al. (1977), because the former was based on two samples and the latter on seven samples. Novel data indicate that the age of Baltic Amber can be narrowed to 34 to 38 Ma (Aleksandrova and Zaporozhets 2008; Kosmowska-Ceranowicz 2012).

*Paleogenia wahisi* Waichert and Pitts **sp. nov.**

Fig. 2.3.

*Etymology:* This species was named in honor of Raymond Wahis who has greatly contributed to our knowledge of Pompilidae biodiversity.

*Type material:* Holotype, complete male inclusion, OSAC Hy-10-80.

*Type locality:* Kaliningrad Region, Baltic Sea, Russia.

*Type horizon:* Baltic amber; late Eocene

*Diagnosis.*— Wing hyaline; maximum width 0.45X its length; cells short and rounded; 2Rs cell about the same size as 1Rs; 2m-cu vein slightly curved, meeting 2Rs cell 0.5X distance from base to apex of cell; 2R1 ending on apex of the forewing instead of anterior margin; mid and hind tarsi pale brown with apex black; and 2M cell without an inflection at the base of the Cu vein.

*Description.*— Male. Body length 2.55 mm. Forewing 2.04 mm. Integument black; tarsomeres, fore and mid tibia brown; mid and hind tarsi pale brown, apex black; metasoma black. Punctuation inconspicuous. Head with sides convergent ventrally, vertex much broader than frons; clypeus short, trapezoidal; mandible with two sharpened apical teeth. Antennae short; ratio of first four segments 7:5:6:7; WA3 0.8X LA3; WA4 0.8X LA4. Pronotum short, width 2.0X length, posterior margin concave; pronotal disc well defined. Tibiae and tarsi with short sparse spines, almost smooth. Wing long; length of 2R1 cell 0.8X distance from edge to apex of wing; 2Rs as long as 1Rs; 2m-cu vein curved, meeting 2Rs cell 0.4X distance from base to apex of cell. Metasoma 0.9X as long as mesosoma.

*Allotype.* — Female. Body length 3.8 mm. Forewing ~2.5 mm (forewing is folded). Integument black; front and mid tibia and tarsi, hind tarsi pale brown; palpi pale brown. Clypeus, antennae, mesosoma and wing as described for male. Metasoma 1.2X as long as mesosoma; stinger straight.

*Remarks.*— This species was probably a cleptoparasitoid pompilid. It shares characteristics of other pompilid cleptoparasitoids, such as short antennal segments with thick conspicuous setae. No extant species of Pepsini are known to act as cleptoparasites;

the only representatives of the subfamily with this behaviour recorded or suspected are placed within Ageniellini (*Poecilagenia*), Deuterageniini (*Nipponodipogon*) and within Psoropempulini (*Psoropempula* Evans, 1974).

*Stratigraphic and geographic range.*— Five specimens of *P. wahisi* sp. nov. were preserved in Baltic amber. Exemplars were collected from the Kaliningrad region of Russia. The age of Baltic amber is discussed above.

## Discussion

*Accuracy of fossil identification.*— The accuracy of identification of Pompilidae fossil is tenuous at best, especially for compression fossils, for which a thorough revision is needed. Compared to compression fossils, amber-preserved fossils are much easier to identify to genus and even to species. All of the new species from amber described herein are taxonomically determined with confidence. All amber fossils, except one, have been recovered from Eocene or younger deposits. The single known specimen from Burmese amber, *Bryopompilus interfactor* Engel and Grimaldi, 2006, is unusual as it is so much older than all other described Pompilidae fossils. Upon re-examining this specimen, we discovered that it does not have the diagnostic characters of Pompilidae (see Engel and Grimaldi 2006, figs. 1–5). The specimen exhibits a conspicuous, angularly protruding, rounded lobe on the posterior margin of the pronotum, which is absent in Pompilidae. Moreover, the jugal lobe is absent from the wing of this specimen, while present in Pompilidae, and the wing venation greatly differs from that of Pompilidae. In *Bryopompilus interfactor* the Rs vein is not rounded and is attached to the distal wing margin, the costal vein reaches the wing distal margin, and the vein Rs of the cell 1Rs is not attached to the base of the cell 2R1 but to the base of 1R1. The presence of a

mesepisternal groove in *Bryopompilus interfecto*r could be confused with the same structure that defines Pompilidae. Nevertheless, the mesepisternal groove covers the whole mesopleuron in Pompilidae, while in *B. interfecto*r it does not reach the mesopleural margin. The placement of this fossil in extinct or extant Hymenoptera families is dubious. *Bryopompilus interfecto*r is considered herein as member of a new fossil family: Bryopompilidae. This fossil has a rounded posterior lobe on the pronotum, which could be confused with the lobe observed in Apoidea. Nevertheless, this lobe is deeply incised and overlaps the wing base both above and below, whereas in Apoidea it is entire, rounded and somewhat inflated and extremely rarely reaches the wing base and never overlaps it from above. The diagnosis of the family Bryopompilidae is as follows: presence of an angularly protruding, rounded lobe on the posterior margin of the pronotum; the lobe is deeply incised, overlapping the anterior and posterior margins of the wing base. The mesepisternal groove is interrupted, not reaching the mesopleural margin. The fore wing has the Rs vein straight and attached to the distal wing margin; the costal vein reaches the wing distal margin, and the Rs vein of cell 1Rs is attached to the base of 1R1.

*The age and diversification of Pompilidae.* — Given that the Burmese amber specimen dating Pompilidae to the mid-Cretaceous is no longer recognised as a member of the family, we reconsider the age of Pompilidae. The oldest fossils assigned with confidence to the family are from Baltic amber, which is dated from the Eocene; these taxa can be attributed to extant lineages and, thus, represent crown-group Pompilidae. Therefore, the common ancestor of extant (crown-group) Pompilidae must have existed prior to 38 Ma, but more recently than the Cretaceous (because no Cretaceous crown-

group fossils are known). This conclusion is consistent with results from a molecular dating analysis of Aculeata phylogeny, which recovered a maximum age of 55 Ma for crown-group Pompilidae and a mean age of 85 Ma for the divergence of Pompilidae from its sister group (Wilson et al. 2013). Compared to the fossil record of other Hymenoptera (Grimaldi and Engel 2005), crown-group Pompilidae diversified more than 140 Myr after the origin of Hymenoptera and more than 100 Myr after the origin of Aculeata. The sole hosts of Pompilidae, spiders (Araneae), originated in the Carboniferous (360-290 Ma) (Selden et al. 2013), with extant suborders and many sub-lineages diversifying by the Lower Jurassic, ca. 175 Ma (Vollrath and Selden 2005). Thus, the origin and diversification of Pompilidae occurred long after the diversification of their prey.

Pompilidae are unique among Hymenoptera as a diverse, Palaeogene-aged lineage in which all members prey solely on spiders. These wasps most generally attack hunting spiders and sheet-web spiders. Use of spiders occurs only sporadically in younger hymenopteran taxa, e.g., *Trypoxylon* Latreille, 1796 (Crabronidae) wasps. The closest relatives of Pompilidae, Mutillidae and Sapygidae (Pilgrim et al. 2008; Wilson et al. 2013), are predatoids (following the terminology of Evans 1963) of solitary wasp and bee larvae, and occasionally other insects. The ancestral prey type of Aculeata as a whole may have been beetle larvae and probably other concealed insect larvae. Thus, at some point after their divergence from Mutillidae and Sapygidae, pompilids shifted their prey specialization to spiders exclusively. This shift must have been accompanied by specialized behaviour to deal with potentially dangerous (venomous) prey capable of defense, which are often much larger than their attackers (Evans 1953; Evans and Shimizu 1996).

The fossil record of spiders documents an exponential increase in family-level diversity (Penney 2003) since the origin of Araneae in the Carboniferous (Selden et al. 2013). Family-level diversity shows episodes of diversification in both the Mesozoic and Paleogene, and appears to have been unaffected by the Cretaceous-Paleogene extinction event (Penney et al. 2003). Diversity nearly doubled between 65 and 45 Ma. Thus, spiders constituted a ubiquitous, diverse, and abundant source of prey after pompilids diverged from their sister group in the Upper Cretaceous. Once stem-group pompilids evolved the skills to prey on spiders, the continued increase in spider diversity may have played a role in radiation of their pompilid predators in the early Paleogene.

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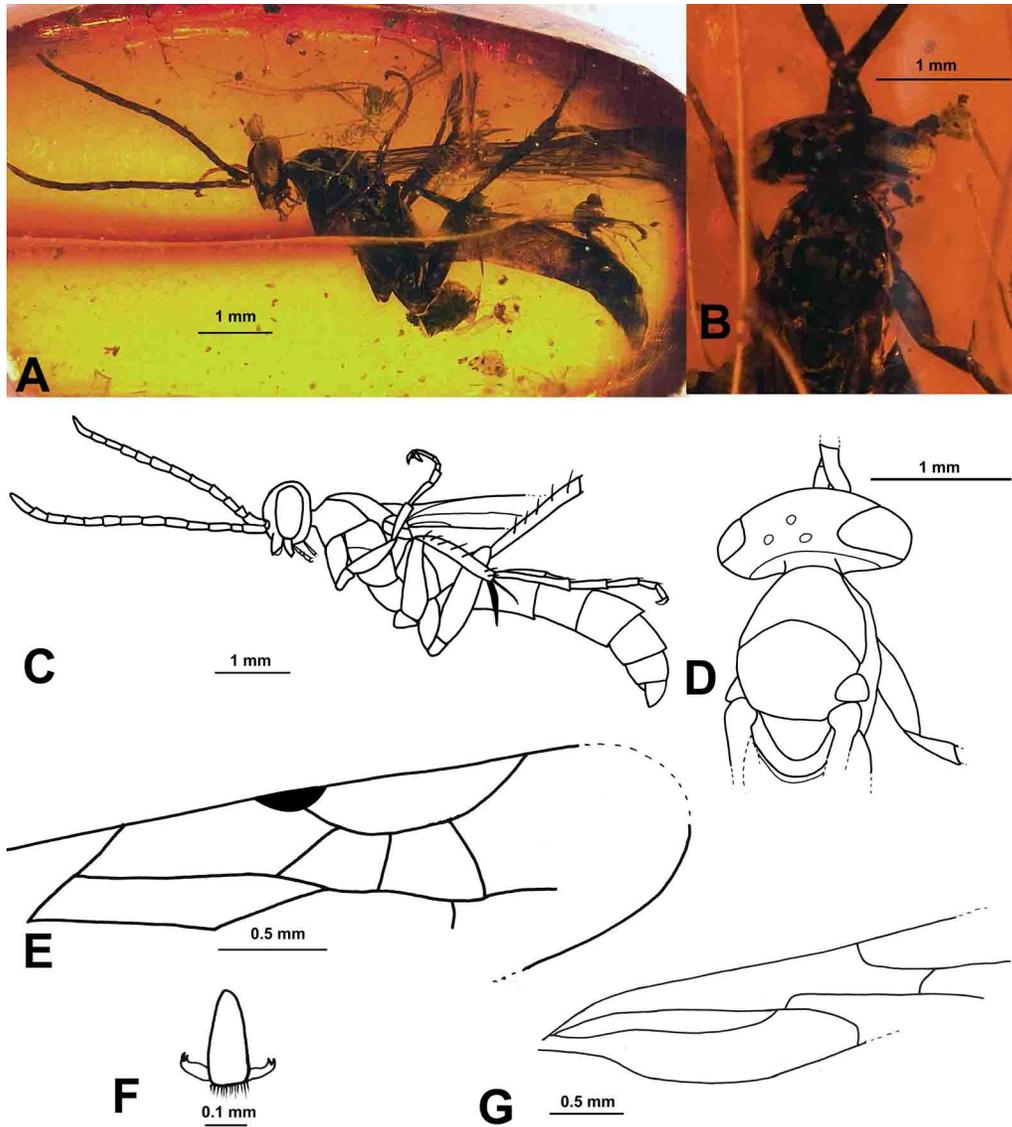


FIGURE 2.1. *Anoplus planetarius* sp. nov. holotype, sp. nov. holotype, OSAC Hy-10-45, male specimen, from early Miocene Dominican amber, Cordillera Septentrional, between Puerto Plata and Santiago, Dominican Republic. **A, B.** Photographs of the holotype. **A.** Habitus, lateral view. **B.** mesosoma dorsal view. **C, D, E, F, G,** Camera lucida illustrations based on the holotype. Lateral view (**C**), mesosoma dorsal view (**D**), forewing (**E**), pulvillus (**F**), hindwing (**G**).

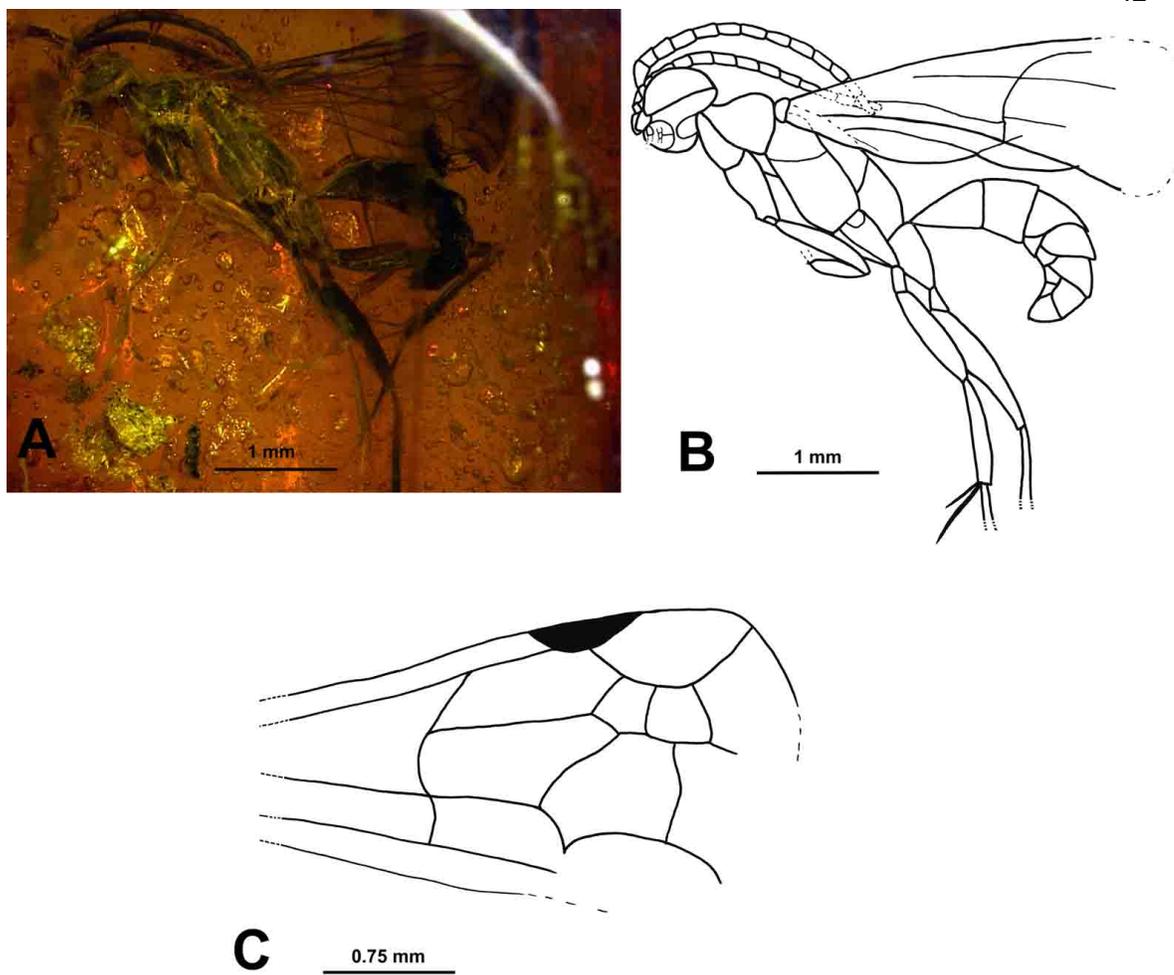


FIGURE 2.2. *Tainopompilus argentum* sp. nov. holotype, sp. nov. holotype, OSAC Hy-10-45, male specimen, from early Miocene Dominican amber, Cordillera Septentrional, between Puerto Plata and Santiago, Dominican Republic. **A**. Photograph in lateral view. **B, C**. Camera lucida illustrations based on the holotype. Lateral view (**B**), forewing (**C**).

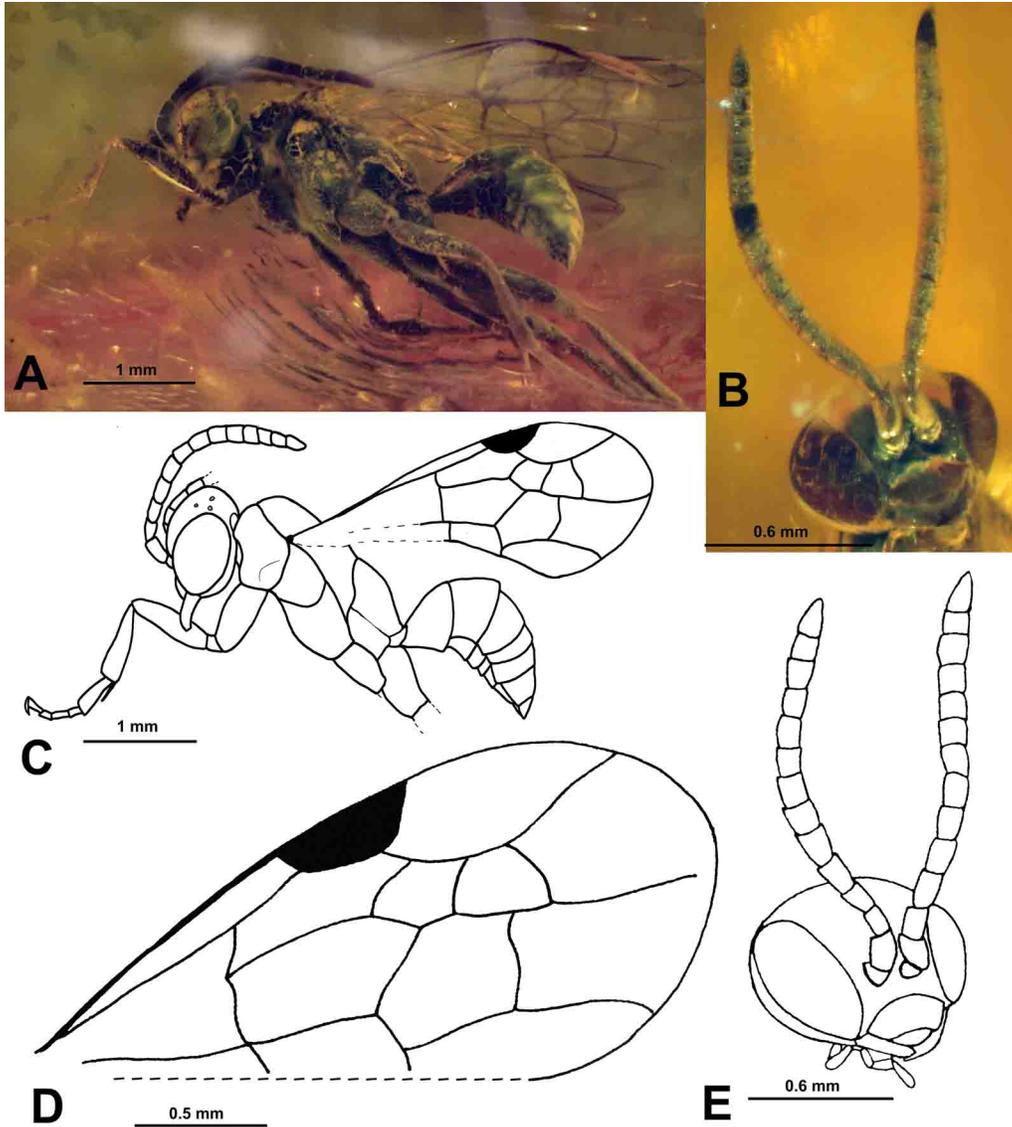


FIGURE 2.3. *Paleogenia wahisi* sp. nov. holotype, OSAC Hy-10-80, male specimen, from late Eocene Baltic amber, Kaliningrad Region, Baltic Sea, Russia. **A, B.** Photographs of the holotype. **A.** Habitus, lateral view. **B.** Head. **C, D, E.** Camera lucida illustrations based on the holotype. Lateral view (**C**), forewing (**D**), head (**E**).

## CHAPTER 3

REVIEW OF FOSSIL SPIDER WASPS (HYMENOPTERA: POMPILIDAE) WITH  
THE DESCRIPTION OF A NEW SPECIES<sup>2</sup>

## ABSTRACT

The known spider wasp (Hymenoptera: Pompilidae) fossils are revised. A new species of fossil spider wasp is described: *Dipogon (Deuteragenia) catalanicus* Rodriguez, Waichert and Pitts. *Ceropalites infelix* Cockerell, from the Florissant Fossil Beds (Priabonian), is no longer recognised as Pompilidae. *Agenioideus saxigenus* (Cockerell), from the Florissant Fossil Beds (Priabonian); and *Dipogon wettweri* (Statz), from the Rott deposits (Chattian) are new combinations. Twenty-one fossil species of spider wasps are now recognised in twelve genera, three of which are extinct.

## INTRODUCTION

SPIDER wasps (Hymenoptera: Pompilidae) are a widespread group with about 5,000 described species (Pitts et al. 2006) in 120 genera (Wasbauer 1995). They are solitary parasitoids wasps characterized by laying a single egg on one paralyzed spider. The resulting larva consumes the spider host. In several lineages of spider wasps the spider is only temporarily paralysed and the spider wasp larva feeds on it as the spider behaves normally. Pompilids show a variety of hunting, nesting, and prey-carrying

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behaviours as adults. There are also cleptoparasitoids, which use the host of another spider wasp as their own hosts. There is ecological or taxonomical host specificity in some species (Evans and Yoshimoto, 1962).

Currently there are 21 species of fossil Pompilidae described (Table 3.1). The taxonomy of extinct spider wasps is challenging, because many of the descriptions (mostly published from Tertiary compression fossils in the late 1800s and early 1900s) are from compression fossils, and lack detail and figures that could facilitate the placement of specimens in appropriate genera (Engel and Grimaldi, 2006).

Until recently, the age of Pompilidae was based on the description of a fossil in Burmese amber, which dates from the Albian (mid-Cretaceous) (Engel and Grimaldi, 2006). However, recent studies place it in the family Bryopompilidae (see Chapter 2). Therefore, spider wasps are not from the mid-Cretaceous, as Engel and Grimaldi (2006) suggested, but are likely younger, possibly originating in the Eocene. This is consistent with recent estimates of the age of spider wasps based on molecular data (Wilson et al., 2013). Here we provide a revision of the existing Pompilidae fossils.

## **MATERIAL AND GEOLOGICAL SETTING**

Compression and amber fossils from various natural history collections were studied. The compression fossils studied belong to six main deposits: the Florissant fossil beds (Florissant, Colorado, USA), the Oeningen deposits (Baden-Württemberg, Germany), the Aix-en-Provence deposits (Bouches-du-Rhône, France), the Rott deposits (Landsberg, Germany), the Terrains sannoisiens du Gard deposits (Gard, France), and the

Bellver deposits (Lleyda, Spain). The Dominican amber fossils studied derive from deposits found in mines between the cities of Santiago and Puerto Plata (Dominican Republic). One of the Baltic amber fossils derives from the Kaliningrad region (Russia). The locality of the second Baltic amber fossil is unknown.

### SYSTEMATIC PALEONTOLOGY

*Terminology.*— Wing venation terminology follows that of Huber and Sharkey (1993, figs 19–20). The kind of material studied is summarised in Table 3.1.

The species treated here were assigned to the family Pompilidae based mainly on wing venation features, which are relatively uniform for the family (Day, 1988). All of the specimens studied have a preserved forewing, and most of them have the hind wing also preserved (Table 3.1). These were placed in the family Pompilidae based on the following combination of characters: presence of ten closed cells in the forewing, the hind wing with the veins C+Sc+R+Rs fused basally, and the second abscissa of 1A lost. Marginal cell with vein Rs rounded and attached to anterior margin of wing. Vein Rs of cell 1Rs attached to the base of cell 2R1. Costal cell ending on the anterior margin of the wing.

*Specimen repositories.*— The collections housing the material used in this study are the following: AMNH, American Museum of Natural History, New York, New York, USA; LACMIP, Los Angeles County Museum of Invertebrate Paleontology, Los Angeles, California, USA; MCZC, Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, USA; MGMM, Museo Geominero de Madrid,

Madrid, Spain; MHNM, Muséum d'histoire naturelle de Marseille, Marseilles, France; MHNN, Muséum d'Histoire naturelle – Nîmes, Nimes, France; MNHN, Muséum National d'Histoire Naturelle, Paris, France; OSAC, Oregon State Arthropod Collection, Oregon State University, Corvallis, Oregon, USA; SMNK, Staatliches Museum für Naturkunde Karlsruhe, Karlsruhe, Germany; UCMC, University of Colorado Museum of Natural History, Boulder, Colorado, USA; USNM, Smithsonian National Museum of Natural History, Washington, District of Columbia, USA.

*Remarks.*— We revised a total of 21 species, of which 17 are compression fossils and five are preserved in amber.

Family POMPILIDAE Latreille, 1804

Subfamily POMPILINAE Latreille, 1804

Genus POMPILUS Latreille, 1796

POMPILUS depressus (Statz, 1936)

1936 *Psammochares depressa* STATZ, p. 283, pl. 12, fig. 34.

1945 *Pompilus depressus* (Statz); ICZN, opinion 166.

*Diagnosis.*— Wing hyaline; maximum width 0.31X its length; 1Rs cell 1.10X as long as 2Rs; 2m-cu slightly curved, meeting 2Rs cell 0.40X distance from base to apex of cell; and Cu vein of 2M cell with an inflection at its base.

*Material.*— Not available.

*Occurrence.*— This specimen was found in the Rott deposit, in Germany. The matrix of this deposit consists mainly of fine-grained paper shales, and thus contains very

well preserved fossils. Its age is controversial, ranging from the Chattian, Oligocene to the Aquitanian, Miocene (Grimaldi and Engel, 2005). Modern publications establish the Chattian as the correct age for these deposits (Fikacek et al., 2010).

*Remarks.*— The generic position of this species is equivocal. We have only examined the wing venation illustrations in Statz (1936, pl. 12, fig. 34), and the characters observed are dubious for determining its taxonomic status. Wing venation characters are not enough to determine the genus of this specimen. An inflection at the base of the Cu vein in the 2M cell is present, which places the species in the subfamily Pompilinae. Further inference would lead to an inaccurate taxonomic placement. We declare *Psammochares depressa* a *nomen dubium*. The original description of this species does not indicate the location of the holotype and none of the natural history museums contacted claimed to have it in their collection.

Genus AGENIOIDEUS Ashmead, 1902

AGENIOIDEUS saxigenus (Cockerell, 1908) **comb. nov.**

Figures 3.1.1, 3.1.2, 3.5.1

1908 *Agenia saxigena* COCKERELL, pp. 229–230, fig. 3.

1912 *Dipogon (Deuteragenia) saxigenus* (Cockerell); SUSTERA, p. 191.

*Diagnosis.*— Forewing hyaline, with two bands that cover 1Rs and 2Rs cells; maximum width 0.31X its length; 1Rs cell almost as long as 2Rs; 2m-cu vein slightly

curved, meeting 2Rs cell 0.95X distance from base to apex of cell; and 2M cell with an inflection at the base of Cu.

*Material*.— Lectotype: USA, Colorado, Florissant Tertiary Shales (UCMC No. 4541A) (Figure 3.1.1). Paralectotype: USA, Colorado, Florissant Tertiary Shales (UCMC No. 4541B) (Figure 3.1.2).

*Occurrence*.— This fossil was collected in the Florissant Lake Beds of Colorado. The formation is a heterolithic accumulation of shale, tuffaceous mudstone and siltstone, tuff, and arkosic, volcanoclastic sandstone and conglomerate (Evanoff et al., 2001). The formation is dated from the Priabonian, Eocene. Epis and Chapin (1975) dated the formation from 34.9 Ma. Later, Evanoff et al. (2001) analyses yielded a range of ages from 34.3 to 33.5 Ma. The most recent study (Prothero and Sanchez, 2004) dated the formation from 33.7 to 34.7 Ma.

*Remarks*.— Two specimens collected from the same locality are found on the type series of this species. *Agenia* is a synonym of *Dipogon* (*Deuteragenia*) Susterer, 1912. This species was placed in that subgenus probably due to the double dark bands on the wings, which entirely covers the 1Rs and 2Rs cells (Fig. 3.1.1, 3.1.2). Nevertheless, the inflection in the vein Cu at the base of the 2M cell identifies this specimen as Pompilinae. We place the species in *Agenioideus*, because the 2m-cu vein of the forewing arises on the Cu vein much more than half the distance from the origin of 2M to the outer wing margin (Fig. 3.5.1). Other genera share this character (i.e. *Priocnemis* Schiodte, 1837; *Balboana* Banks, 1944; *Aplochaes* Banks, 1944; and *Tachypompilus* Ashmead, 1902); this specimen, however, differs from them in other respects. It differs from *Priocnemis* and *Balboana* by the presence of an inflection at the base of the Cu vein in 2M cell; and

from *Tachypompilus* by the absence of irregular contours on the propodeum. Finally, this specimen has a large stigma, whereas in *Aplochaeres* the stigma is reduced.

Genus ANOPLIUS Dufour, 1834

ANOPLIUS *induratus* (Heer, 1849)

Figure 3.1.3

1849 *Pompilus induratus* HEER, pp. 165–166, pl. 13, fig. 10.

1909 *Anoplius induratus* (Heer); Rohwer, p. 28.

*Diagnosis.*— Wing hyaline, maximum width 0.29X its length; 1Rs triangular, small; and 2M cell and 2m-cu vein not visible.

*Material.*— Holotype: Germany, Baden-Württemberg, Wangen im Allgäu, Oeningen (SMNK).

*Occurrence.*— This fossil was collected in the Oeningen region, which had first been reported as belonging to Switzerland. Cockerell (1915) corrected this mistake and located the region in Baden-Württemberg, Germany. Oeningen is one of the richest insect fossil deposits known in the world. It is composed of freshwater limestone deposits that date from the Messinian, Miocene (Grimaldi and Engel, 2005).

*Remarks.*— We are doubtful about the subfamilial and generic classification of this species, because of the poor preservation of the specimen (Fig. 3.1.3). Rohwer (1909) transferred this species to *Anoplius*, but made no comments on the reasons for this decision. Characters that would place it in the subfamily Pompilinae, such as the inflection on the base of Cu vein in the 2M cell or the spines of different lengths on the

apex of the metatibia, cannot be observed. We declare *Anoplius induratus* a *nomen dubium*.

ANOPLIUS planetarius Rodriguez and Pitts

Figures 3.1.4, 3.1.5

*Diagnosis*.— Wings hyaline; maximum width 0.18X its length; 2Rs cell as long as 1Rs; 2m-cu vein slightly curved, meeting 2Rs cell 0.70X distance from base to apex of cell; and 2M cell with an inflection at the base of the vein Cu.

*Material*.— Holotype: DOMINICAN REPUBLIC, Cordillera Septentrional, between Puerto Plata and Santiago (OSAC Hy-10-45).

*Occurrence*.— This fossil was collected from amber mines in the northern region of Dominican Republic. The age of Dominican amber is controversial. According to Rodriguez et al. (see Chapter 2), there are various proposed dates for Dominican Amber (Schlee, 1990; Iturralde-Vinent and MacPhee, 1996). But the age proposed by Iturralde-Vinent and MacPhee (1996) should be used as accurate, because it is based on reliable biostratigraphic and palaeogeographic data from Hispaniola.

Insect inclusions have also been observed in Dominican copal (Brown, 1999), and sometimes been confused with Pliocene/Pleistocene amber. Copal has been dated to 50,000 years (Burleigh and Whalley, 1983).

*Remarks*.— Rodriguez et al. (see Chapter 2) discussed the reasons for placing this species in *Anoplius* with confidence, but mention the impossibility of placing it within any of the extant subgenera.

## Genus TENTHREDINITES Meunier, 1915

TENTHREDINITES *bifasciata* Meunier, 1915

1915 *Tenthredinites bifasciata* MEUNIER, p. 11, fig. 10.

*Diagnosis.*— Forewing with two dark bands, maximum width 0.30X its length.

The wing venation characters are not visible.

*Material.*— Not available.

*Occurrence.*— This fossil was collected from the Oligocene fossil deposits of Aix-en-Provence. The Aix-en-Provence basin is filled mainly with detritic sediments as well as limestone and gypsum (Hippolyte et al., 1993).

*Remarks.*— The holotype of this specimen could not be located. The original description of this species does not mention its location and none of the museums contacted claimed to have it in their collection. Our only source of information is the species description and a blurry photograph included in the original publication. Meunier (1915) mentioned the presence of this specimen in the Natural History Museum of Marseille. However, the curator did not respond to our inquiries concerning this specimen. Theobald (1937) suggested a resemblance of this species to the extant *Pompilus maculipes* Smith, 1870, which is now placed in *Anoplius* (Shimizu and Wahis, 2009). We have no morphological evidence to place this species in *Anoplius* and we keep its original name.

Genus POMPILUS Fabricius, 1798

POMPILUS coquandi Theobald, 1937

Figure 3.2.1, 3.5.2

1937 *Pompilus coquandi* THEOBALD, p. 320, pl. 24, fig. 13, pl. 25, fig. 18.

*Diagnosis.*— Wing hyaline, maximum width 0.37X its length; 2Rs cell as long as 1Rs; 2m-cu vein straight, meeting 2Rs cell 0.50X distance from base to apex of cell; and 2M cell with an inflection at the base of the Cu vein.

*Material.*— Holotype: FRANCE, Bouches-du-Rhône, Aix-en-Provence (MNHN).

*Occurrence.*— This species was collected in the Aix-en-Provence deposits, which dates from the Oligocene (Hippolyte et al., 1993).

*Remarks.*— This species is unquestionably Pompilinae due to the presence of an inflection at the base of the Cu vein on the 2M cell (Fig. 3.5.2). The low quality of specimen preservation hinders an accurate identification to generic level. We declare *Pompilus coquandi* a *nomen dubium*.

POMPILUS fasciatus Theobald, 1937

Figure 3.2.2, 3.5.3

1937 *Pompilus fasciatus* THEOBALD, p. 320, pl. 24, fig 14, pl. 25, fig. 14.

*Material.*— Holotype: FRANCE, Bouches-du-Rhône, Aix-en-Provence (MNHN).

*Diagnosis.*— Wing hyaline, maximum width 0.35X its length; 2Rs cell 1.20X longer than 1Rs; 2m-cu vein slightly curved, meeting 2Rs cell 0.60X distance from base to apex of cell; and 2M cell with an inflection at the base of Cu vein.

*Remarks.*— The general morphology of the specimen is very similar to the description of extant cleptoparasitic Pompilidae by Shimizu et al. (2010). Pompilidae cleptoparasites typically have shortened antennal segments (Fig. 3.2.2), such as in *Aridestus* Banks, 1947, or *Poecilagenia* Haupt, 1927 (Shimizu et al., 2010). Nevertheless, its accurate generic placement is not possible due to the lack of detail in preserved structures. We declare *Pompilus fasciatus* a *nomen dubium*.

*Occurrence.*— This specimen was found in the Oligocene Aix-en-Provence fossil deposits.

#### Genus TAINOPOMPILUS Rodriguez and Pitts

#### TAINOPOMPILUS argentum Rodriguez and Pitts

#### Figure 3.2.3

*Diagnosis.*— Wing hyaline; maximum width 0.31X its length; 2Rs cell as long as 1Rs; 2m-cu vein curved, meeting 2Rs cell 0.55X distance from its base to apex of cell; and 2M cell with an inflection at the base of Cu vein.

*Material.*— Holotype, Cordillera Septentrional, between Puerto Plata and Santiago, Dominican Republic (OSAC Hy-10-45).

*Occurrence.*— The fossil was collected from amber mines in the Dominican Republic. The age of Dominican amber is controversial, as discussed above.

*Remarks.*— This is the only described species of *Tainopompilus*. Rodriguez et al. (submitted) placed it in the subfamily Pompilinae based on the presence of an inflection at the base of the vein Cu on 2M cell (Fig. 3.2.3), but mention that this is the only

Pompilinae genus that has metatibial spine-like setae of equal length and not splayed, and suggest it might belong to a new subfamily.

Subfamily PEPSINAE Lepeletier, 1845

Genus POMPILUS Fabricius, 1798

POMPILUS scelerosus Meunier, 1917

1917 *Pompilus scelerosus* MEUNIER, pp. 181–184.

*Diagnosis.*— Wing hyaline, maximum width 0.26X its length; 2Rs cell 1.29X longer than 1Rs; 2m-cu vein straight, meeting 2Rs cell 0.40X distance from base to apex of cell; and 2M cell without an inflection at the base of Cu vein.

*Material.*— Not available.

*Occurrence.*— This specimen was found in Baltic amber. The exact locality is unknown. Baltic amber deposits have been obtained for more than 100 years, and their age is controversial. Microfaunistic dating of the deposits containing the largest amount of amber suggest they are from the Priabonian, Eocene (37.7 Ma) (Kaplan et al., 1977), whereas radiometrically dated glauconite dates them as Lutetian, Eocene (47.0 to 44.1 Ma) (Ritzkowski, 1997). Perkovsky et al. (2007) considered the Ritzkowski (1997) data insufficient to disprove Kaplan et al. (1977), because the former was based on two samples and the latter on seven samples. Novel data indicate that the age of Baltic Amber can be narrowed to 34 to 38 Ma (Aleksandrova and Zaporozhets, 2008).

*Remarks.*— This is a very small specimen from Baltic amber that does not have an inflection at the base of the Cu vein of the 2M cell, which excludes it from *Pompilus*. The

description and drawings provided by Meunier (1917, figs. 1–3) suggest that this specimen should be placed in Pepsinae. The location of the holotype of this species was not mentioned in the original description and none of the museums contacted claimed to have it in their collection. Because we could not locate the holotype, and the published drawing is inadequate, we cannot make further taxonomic conclusions about this taxon. This name is herein declared a *nomen dubium*.

Genus CHIRODAMUS Haliday, 1837

CHIRODAMUS *avitula* (Cockerell, 1941)

Figure 3.2.4, 3.5.4

1941 *Pepsis avitula* COCKERELL, pp. 355–356, pl. 1, fig. 3.

2005 *Chirodamus avitula* (Cockerell); VARDY, p. 285, fig. 688.

*Diagnosis.*— Wing hyaline, banded; maximum width 0.35X its length. Other wing venation characters are not visible.

*Material.*— Holotype: USA, Colorado, Florissant Tertiary Shales (UCMC No. 19166).

*Occurrence.*— This species was found in the fossil beds of Florissant Colorado (Eocene) (*vide Agenioideus saxigenus* section).

*Remarks.*— Vardy (2005) studied this specimen, and mentioned a number of characters that differentiate it from *Pepsis*. He also mentioned the lack of *Pepsis* species that possess banded wings. Vardy (2005) suggested the proximity of this species to *Chirodamus* Haliday, 1837 and established the new combination. We did not find enough

wing venation characters to place this species in *Chirodamus* (Fig. 3.5.4). Therefore we declare this species a *nomen dubium*.

Genus CRYPTOCHEILUS Panzer, 1806

CRYPTOCHEILUS florissantensis (Cockerell, 1906)

Figure 3.2.5

1906 *Hemipogonius florissantensis* COCKERELL, pp. 52–53.

1914 *Cryptocheilus florissantensis* (Cockerell); COCKERELL, p. 719.

*Diagnosis*.— Wing hyaline with two dark bands, and apex darkened; maximum width 0.20X its length; 2Rs cell slightly shorter than 1Rs; 2m-cu vein slightly curved, meeting 2Rs cell 0.70X distance from base to apex of cell; 2M cell without an inflection at the base of Cu; and hind wing with cu-a ending distinctly before the juncture of M with CU.

*Material*.— Holotype: USA, Colorado, Florissant Tertiary Shales (MCZC No. 2023).

*Occurrence*.— This species was found in Florissant Colorado Fossil Beds, which are dated from the Eocene (*vide Agenioideus saxigenus* section).

*Remarks*.— Unfortunately, the specimen was preserved with wings overlapping (Fig. 3.2.5), which precluded accurate description and illustration of wing venation characters. Nevertheless, Cockerell (1906) provided a good description and several measurements of hind wing cells. The placement of *C. florissantensis* in *Hemipogonius* Cockerell, 1906 was not justified by Cockerell (1906). The same author later placed the

species within *Cryptocheilus* (Cockerell, 1914). The generic position of this species cannot be certain because wing venation characters are not entirely visible. It certainly belongs to Pepsinae, because of the absence of an inflection at the base of the Cu vein in 2M cell. We declare this name *nomen dubium*.

CRYPTOCHEILUS laminarum (Rohwer, 1909)

Figure 3.2.6

1909 *Salius laminarum* ROHWER, pp. 26–27.

1914 *Cryptocheilus laminarum* (Rohwer); COCKERELL, p. 718.

*Diagnosis*.— Wing hyaline with apex darkened; maximum width 0.26X its length; 2Rs cell almost as long as 1Rs; and 2m-cu and 2M cell not observable.

*Material*.— Holotype: USA: Colorado: Florissant, Tertiary shales, Station 14, 1908 (S. A. Rohwer) (UCMC No. 8597).

*Occurrence*.— This species was found in Florissant Colorado Fossil Beds, which are dated from the Eocene (*vide Agenioideus saxigenus* section).

*Remarks*.— Cockerell (1914) placed this species in *Cryptocheilus*. The wing venation is not well preserved, but the robust body, flat metasoma, and the absence on an inflection at the base of the Cu vein of 2M cell likely places it in the tribe Pepsini (Fig. 3.2.6). Characters that might assign it to a particular genus within the tribe are not visible. We declare *Cryptocheilus laminarum* a *nomen dubium*.

CRYPTOCHEILUS *senex* (Rohwer, 1909)

## Figure 3.3.1

1909 *Salius senex* ROHWER, pp. 25–26.

1914 *Cryptocheilus senex* (Rohwer); COCKERELL, p. 718.

*Material*.— Holotype: USA, Colorado, Florissant, Tertiary shales, Station 14, 1908, collector unknown (UCMC. No. 8594).

*Diagnosis*.— Wing hyaline, darkened apically; maximum width 0.30X its length; 2Rs longer than 1Rs; 2m-cu slightly curved, meeting 2Rs slightly before the middle; and 2M cell not observable.

*Occurrence*.— This specimen was collected in the fossil deposits of Florissant Colorado. These deposits are from the Eocene (*vide Agenioideus saxigenus* section).

*Remarks*.— Even though Rohwer (1909) mentions the affinity of *C. senex* with *Anoplius* species, the poor wing preservation precludes a confident designation of this species to *Anoplius*. It is possible, however, that *C. senex* is a junior synonym of *C. florissantensis* (Cockerell) due to the overall body shape, wing colouration, and collection site. Nevertheless, given the poor preservation of the wing venation and absence of legs (Fig. 3.3.1), it is not possible even to assign a subfamily for *C. senex*. A synonymy cannot be well justified and we abstain from taxonomic decisions for now. Herein we declare *C. senex* a *nomen dubium*.

## CRYPTOCHEILUS hypogaeus Cockerell, 1914

## Figure 3.3.2, 3.5.5-3.5.6

1914 *Cryptocheilus hypogaeus* COCKERELL, pp. 718–719.

*Diagnosis.*— Wings hyaline with two transverse dark spots; maximum width 0.33X its length; 2Rs cell longer than 1Rs; 2m-cu vein quite straight, meeting 2Rs slightly after middle; 1m-cu large and long, and 2M cell without an inflection at the base of the Cu vein.

*Material.*— Holotype: USA, Colorado, Miocene (sic) shales of Florissant, Wilson Ranch (H. F. Wickham) (USNM No. 90385).

*Occurrence.*— This fossil was collected in the Florissant shales of Colorado. This deposit dates from the Eocene (*vide Agenioideus saxigenus* section).

*Remarks.*— The wing venation is well preserved in this specimen. The absence of an inflection at the base of the Cu vein in 2M cell places it in the subfamily Pepsinae (Fig. 3.5.5). Furthermore, the robust body and absence of a petiolate appearance in the first metasomal segment suggests that it belongs to the tribe Pepsini (Fig. 3.3.2). Finally, because the 2r-m vein is straight, and the cu-a ends distinctly before the juncture of M with Cu in the hind wing (Fig. 3.5.6), this species can be placed with confidence in the genus *Cryptocheilus*. Venation on forewing fades at the apex, probably because of poor preservation.

## CRYPTOCHEILUS contentus Theobald, 1937

1937 *Criptochilus contentus (sic)* THEOBALD, pp. 129–130.

*Diagnosis.*— Wing hyaline; maximum width 0.31X its length; 2Rs cell 1.5X longer than 1Rs; 2m-cu vein straight, meeting 2Rs cell 0.33X distance from base to apex of cell; and 2M cell without an inflection at the base of the Cu vein.

*Material.*— Holotype: FRANCE, Gard, Célas (MHNN).

*Occurrence.*— This fossil was collected in the terrains sannoisiens du Gard, France. These are fossil deposits dated from the Rupelian, Oligocene (Keen, 1972).

*Remarks.*— This species was described as “*Criptochilus contentus*”, with a misspelling in the generic name, and was not mentioned by Engel and Grimaldi (2006) in their revision. The venation of the hind wing was not illustrated by Theobald (1937), but based on the forewing it belongs unquestionably to Pepsinae. However, the lack of information on the hind wing venation does not allow its accurate generic placement. We declare *Cryptocheilus contentus* a *nomen dubium*.

## Genus DEUTERAGENIA Fox, 1897

DEUTERAGENIA catalanicus Rodriguez, Waichert and Pitts **new species**

## Figure 3.3.3

2001 *Dipogon (Deuteragenia)* sp. ARILLO, pp. 80–82, fig. 5.

*Diagnosis.*— Wing hyaline, with two bands covering cells 1Rs, 2Rs, 2M and most of 2R1; maximum width 0.31X its length; 2Rs cell as long as 1Rs; 2m-cu vein almost

straight, meeting 2Rs cell 0.40X distance from base to apex of cell; and 2M cell without an inflection at the base of Cu vein.

*Types.*— Holotype: SPAIN, Lleyda, Bellver de Cerdanya, deposit of Barranco de Salanca (MGMM No. 2927M).

*Etymology.*— This species is named after the ‘catalanes’, people who inhabit the autonomous community of Catalonia, where the fossil was found.

*Occurrence.*— This species was found in the lacustrine deposits of Bellver, Spain, which are dated from the Messinian, Miocene (Arillo, 2001).

*Remarks.*— This species was first studied by Arillo (2001), who described it, but did not name it. We compared the holotype with the extant and extinct species of *Dipogon*, and name the new species herein. Because this species was found in Miocene deposits (younger than 11 Myr), the specimen was compared to all extant *Deuteragenia* species (Wahis, 1986; Wolf, 1999) to confirm that it is a new species. Characters from wing venation were compared against all the other species. The most conspicuous difference found was the width of the 2R1 cell. *Deuteragenia catalanicus* n. sp. has a 2R1 cell that is 4X as long as wide (Fig. 3.3.3), while in all other species it is less than 3X as long as wide (Arillo, 2001, fig. 2). There are also differences in the distance from the beginning of the 1Rs where the 1m-cu vein is received at the base of 1Rs. Most of the described species do not receive this vein at 0.4X from the beginning of the 1Rs. Also, the distance where the 2m-cu vein is received by 2Rs is not 0.3X its length from the beginning of the cell in most species, as it is in *D. catalanicus* (e.g., 0.2X in *D. vechti* Day, 1979). Moreover, the extent to which the 2R1 cell is covered by dark banding, which in *D. catalanicus* is covered by 0.9X its length, is less in most other species (e.g.

0.3X its length in *D. monticolus* Wahis, 1972, 1.0X its length in *D. subintermedius* [Maggretti, 1886]). Finally, the 1cu-a vein of the forewing meets the M+Cu slightly beyond the origin of the M, while in some species it meets M+Cu well beyond the origin of M (e. g., *D. austriacus* Wolf, 1964). This species can be distinguished from *Priocnemis* fossil species because in *Deuteragenia* the dark region covers the 1Rs and 2Rs cells completely, whereas in *Priocnemis* these are covered only partially (Arillo, 2001).

DEUTERAGENIA *cockerellae* (Rohwer, 1909)

Figure 3.3.4

1909 *Agenia cockerellae* ROHWER, pp. 24–25.

1912 *Dipogon (Deuteragenia) cockerellae* (Rohwer); SUSTERA, p. 191.

2012 *Deuteragenia cockerellae* (Rohwer); LELEJ, p. 7.

*Diagnosis.*— Wing hyaline, with two dark bands; maximum width 0.16X its length; and 2M cell without an inflection at the base of the Cu vein.

*Material.*— Holotype: USA, Colorado, Florissant Tertiary Shales, Station No. 11 (North End of Stump Hill) (UCMC No. 8598).

*Occurrence.*— This specimen was found in Florissant shales of Colorado. This deposit is dated from the Eocene (*vide Agenioideus saxigena* section).

*Remarks.*— This species was placed in the genus *Deuteragenia* based on the wing-banding patterns (Fig. 3.3.4). Many pompilid genera (e.g. *Priochilus* and *Ageniella* Banks, 1912) possess dark areas on the wings, which makes it an ambiguous character.

We conclude that the current generic position of this species is equivocal, and the preservation of the fossil does not allow us to draw any further conclusions. We declare *D. cockerellae* a *nomen dubium*.

DIPOGON wettweri (Statz, 1938) **comb. nov.**

Figures 3.3.5, 3.3.6, 3.5.7

1938 *Priocnemis wettweri* STATZ, pp. 108–109.

*Diagnosis.*— Wing with two bands; maximum width 0.39X its length; 2Rs 1.2X longer than 1Rs; 2m-cu vein straight, meeting 2Rs cell 0.45X distance from base to apex of cell; and 2M cell without an inflection at the base of the Cu vein.

*Material.*— Holotype: GERMANY, Landsberg, Rott (LACMIP No. 3973, LACMIP locality number 2533).

*Occurrence.*— This fossil was collected in the Rott deposits, dated from the Chattian, Oligocene to the Aquitanian, Miocene (Grimaldi and Engel, 2005).

*Remarks.*— The wing venation (Fig. 3.3.5, 3.5.7) of this specimen resembles that of *Dipogon* species rather than *Priocnemis*. The 3r-m vein is curved in *Dipogon* (Fig. 3.5.7), whereas in *Priocnemis* it is straight. Therefore, we propose to include this species in the genus *Dipogon*. We were unable to place the species in a subgenus because of the lack of visible, diagnostic characters.

## Genus PALEOGENIA Waichert and Pitts

## PALEOGENIA wahisi Waichert and Pitts

Figures 3.4.1-3.4.3

*Diagnosis.*— Wing hyaline (Fig. 3.4.3); maximum width 0.45X its length; cells short and rounded; 2Rs cell about the same size as 1Rs; 2m-cu vein slightly curved, meeting 2Rs cell 0.5X distance from base to apex of cell; 2R1 ending on apex of the forewing instead of anterior margin; and 2M cell without an inflection at the base of the Cu vein.

*Material.*— Holotype: RUSSIA, Kaliningrad Region, Baltic Sea (OSAC Hy-10-80).

*Occurrence.*— Exemplars were collected from the Kaliningrad region of Russia. The age of Baltic amber is discussed above (*vide Pompilus scelerosus* section).

*Remarks.*— Rodriguez et al. (submitted) mention the affinity of this species with other pompilid cleptoparasitoids, and the absence of extant Pepsini cleptoparasitoids.

## Genus PRIOCNEMIS Schiodte, 1837

## PRIOCNEMIS aertsii Statz, 1936

Figures 3.4.4, 3.5.8

1936 *Prioncnemis aertsii* STATZ, pp. 283–284.

*Diagnosis.*— Wing hyaline; maximum width 0.27X its length; 2Rs cell 1.20X longer than 1Rs; 2m-cu vein rounded, meeting 2Rs cell 0.33X distance from base to apex of cell; and 2M cell without an inflection at the base of the Cu vein.

*Material.*— Holotype: GERMANY, Landsberg, Rott (LACMIP No. 3972, LACMIP locality number 2533).

*Occurrence.*— This specimen was collected in Germany, on Rott fossil deposits. These are dated from the Chattian, Oligocene to Aquitanian, Miocene (Grimaldi and Engel, 2005).

*Remarks.*— The original description of this species contained drawings of the wing venation and the entire specimen (Statz, 1936, pl. 12, figs 33-34). This species was placed in *Priocnemis* probably due to the presence of the 1cu-a vein extending beyond the M vein by about 0.70 to 1.30 of its length (Fig. 3.5.8), which in other related Pepsini is closer to the M vein. Nevertheless, other Pepsini genera, such as *Entypus* Dahlbom, 1843, *Pepsis*, and *Calopompilus* Ashmead, 1900 also possess this character. The wing venation in *Priocnemis*, however, is distinguished from *Pepsis* by having the 2R1 cell ending straight, not separated apically from the costal margin of the wing; and from *Calopompilus* and *Entypus* by having the 2r-m vein slightly curved. In addition, it can be separated from *Dipogon* by the straight 3r-m, which is curved in *Dipogon*. Schoberlin (1888) mentioned the presence of *Priocnemis* in the Oeningen deposits. However, the author did not provide a species description, or the location of the specimen mentioned.

## Subfamily CTENOCERINAE

Genus CAPUTELUS Waichert and Pitts

CAPUTELUS scudderi (Cockerell, 1906)

## Figure 3.4.5

1906 *Hemipogonius scudderi* COCKERELL, p. 53.

*Diagnosis.*— Wing with two dark bands; maximum width 0.28X its length; 2Rs cell slightly longer than 1Rs; 2m-cu vein curved, meeting 2Rs slightly after its middle; and 2M cell without an inflection at the base of Cu vein.

*Material.*— Holotype, Colorado, Florissant Tertiary Shales, USA (MCZC No. 2024).

*Occurrence.*— This fossil was collected in the Florissant shales of Colorado. This deposit dates from the Eocene (*vide Agenioideus saxigenus* section).

*Remarks.*— This species was placed in Ctenocerinae by Rodriguez et al (submitted), based on the presence of a large antennal pit, and a prolonged vertex, a carinate propodeum and absence of spines on legs. Extant members of this subfamily are found only throughout the Southern Hemisphere (Waichert and Pitts, 2011); therefore, this fossil suggests that the Tertiary distribution of the subfamily extended to the Northern Hemisphere.

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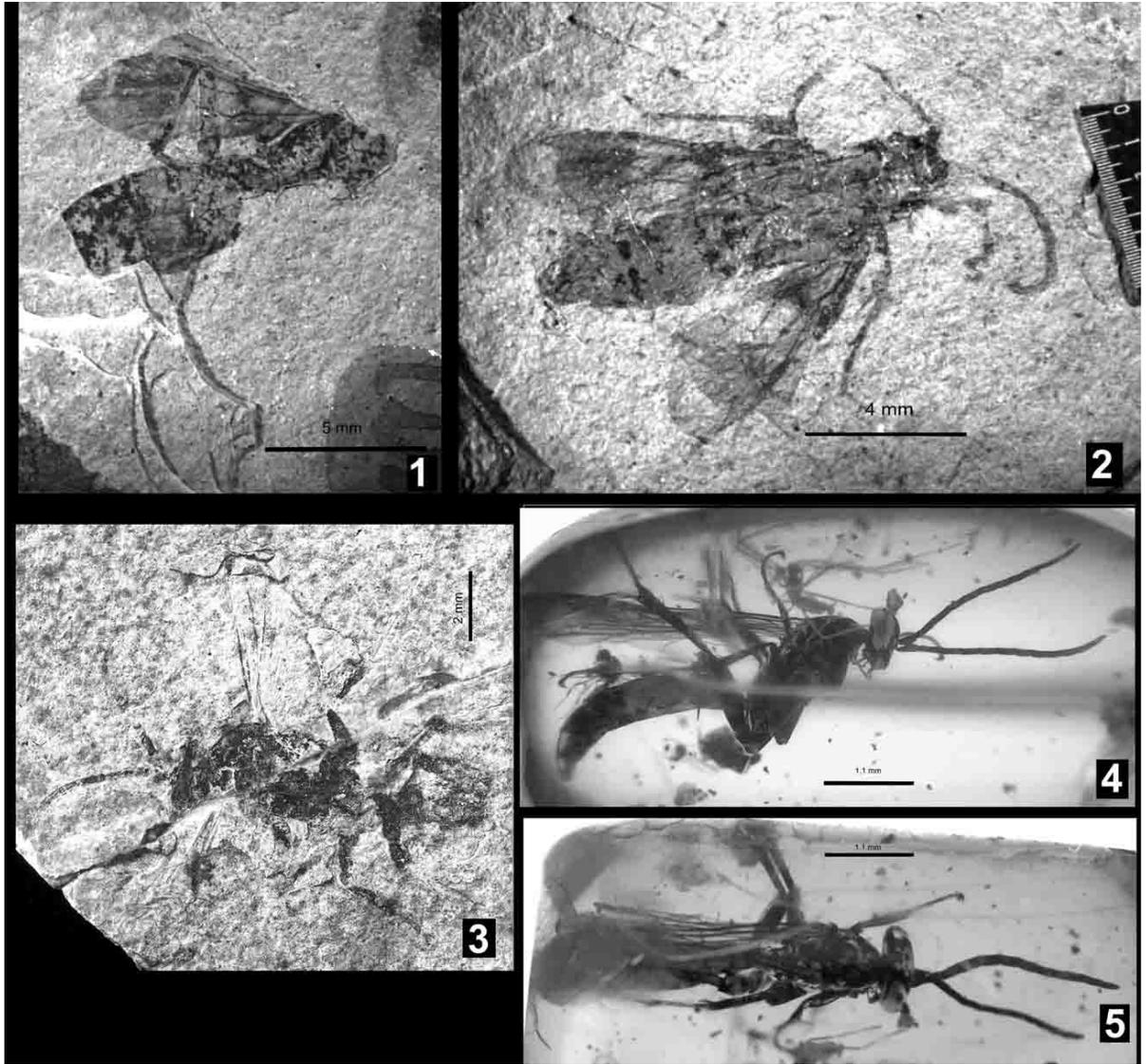
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TABLE 3.1—Species of Fossil Pompilidae

Subfamily	Species name after this revision	Material	Type of fossil	Parts not preserved	Occurrence	Age
Pompilinae	<i>Agenioideus saxigenus</i> (Cockerell, 1908)	Holotype	Compression	Hind wing, antennae	Florissant Fossil Beds	Priabonian
	<b>comb. nov.</b>					
	<i>Anoplius induratus</i> (Heer, 1849)	<b>nom. dub.</b> Holotype	Compression		Oeningen	Messinian
	<i>Tenthredinites bifasciata</i> Meunier, 1915)	Literature	Compression		Aix-en-Provence	Chattian
	<i>Anoplius planetarius</i> Rodriguez and Pitts	Holotype	Amber		Dominican amber	Burdigalian to Langhian
	<i>Psammochares depressa</i> (Statz, 1936)	Literature/ <b>nom. dub.</b> holotype lost	Compression		Rott deposits	Chattian
	<i>Pompilus coquandi</i> (Theobald, 1937)	Holotype	Compression	Part of hind wing	Aix-en-Provence	Chattian
	<i>Pompilus fasciatus</i> (Theobald, 1937)	Holotype	Compression	Mid and fore legs	Aix-en-Provence	Chattian
	<i>Tainopompilus argentum</i> Rodriguez and Pitts	Holotype	Amber		Dominican amber	Burdigalian to Langhian
Pepsinae	<i>Chirodamus avitula</i> Cockerell, 1941	Holotype	Compression	Part of hind wing	Florissant Fossil Beds	Priabonian
	<b>nom. dub.</b>					
	<i>Cryptocheilus florissantensis</i> (Cockerell, 1906)	<b>nom. dub.</b> Holotype	Compression		Florissant Fossil Beds	Priabonian
	<i>Cryptocheilus laminarum</i> (Rohwer, 1909)	<b>nom. dub.</b> Holotype	Compression	Hind wing	Florissant Fossil Beds	Priabonian
	<i>Cryptocheilus senex</i> (Rohwer, 1909)	Holotype	Compression	Hind wing	Florissant Fossil Beds	Priabonian
	<i>Cryptocheilus hypogaeus</i> Cockerell, 1912	Holotype	Compression		Florissant Fossil Beds	Priabonian
	<i>Cryptocheilus contentus</i> (Theobald, 1937)	<b>nom. dub.</b> Literature	Compression	Hind wing	Terrains sannoisiens du Gard	Rupelian

<i>Deuteraenia catalanicus</i> Rodriguez, Waichert and Pitts <b>sp. nov.</b>	Holotype	Compression	Bellver deposits	Messinian
<i>Deuteraenia cockerellae</i> (Rohwer, 1909) <b>nom. dub.</b>	Holotype	Compression	Part of fore and hind wings	Florissant Fossil Beds Priabonian
<i>Dipogon wettweri</i> (Statz, 1938) <b>comb. nov.</b>	Holotype	Compression	Rott deposits	Chattian
<i>Paleogenia wahisi</i> Waichert and Pitts	Holotype	Amber	Baltic amber	Lutetian to Priabonian
<i>Pompilus scelerosus</i> Meunier, 1919 <b>nom. dub.</b>	Literature	Amber	Baltic amber	Lutetian to Priabonian
<i>Priocnemis aertsii</i> Statz, 1936	Holotype	Compression	Hind wing, part of head	Rott deposits Chattian
Ctenocerinae <i>Caputelus scudderi</i> (Cockerell, 1906) <b>comb. nov.</b>	Holotype	Compression	Florissant Fossil Beds	Priabonian

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**FIGURE 3.1**— Pompilidae fossils. 1, 2, *Agenioideus saxigenus* (Cockerell, 1908) from Florissant Tertiary shales, Colorado, US (UCMC No. 4541): 1, Holotype habitus, 2, Paratype dorsal view; 3, habitus of *Anoplius induratus* (Heer, 1849) from Oeningen, Germany (SMNK); 4, 5, *Anoplius planetarius* Rodriguez and Pitts n. sp. from Dominican amber, Dominican Republic (OSAC Hy-10-45): 4, habitus, E5, dorsum.

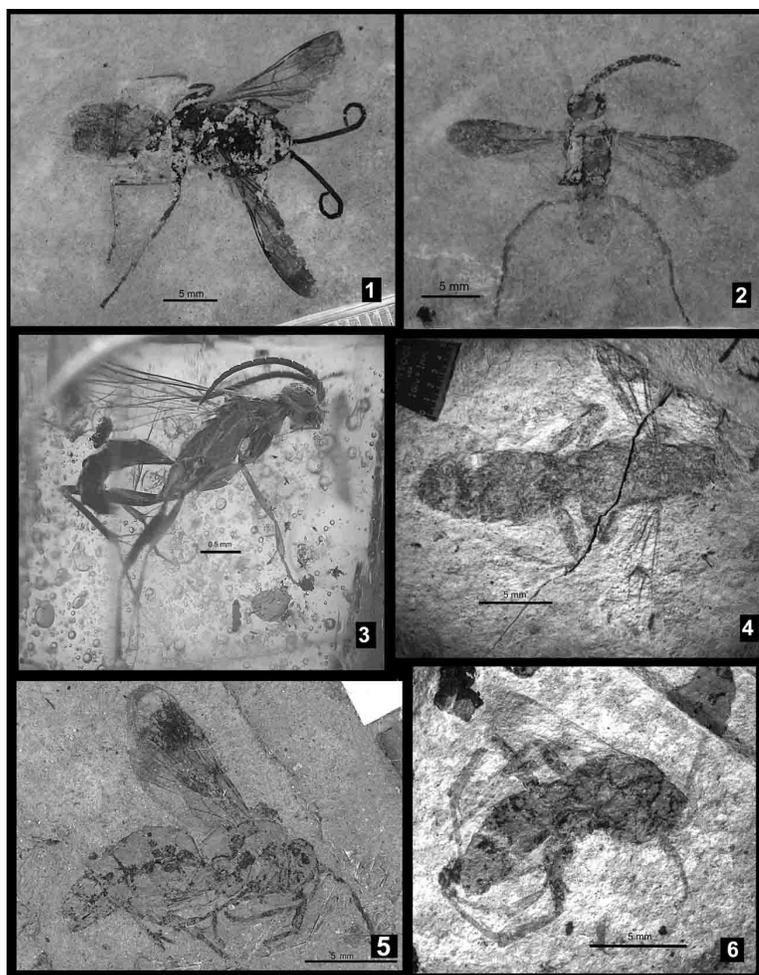
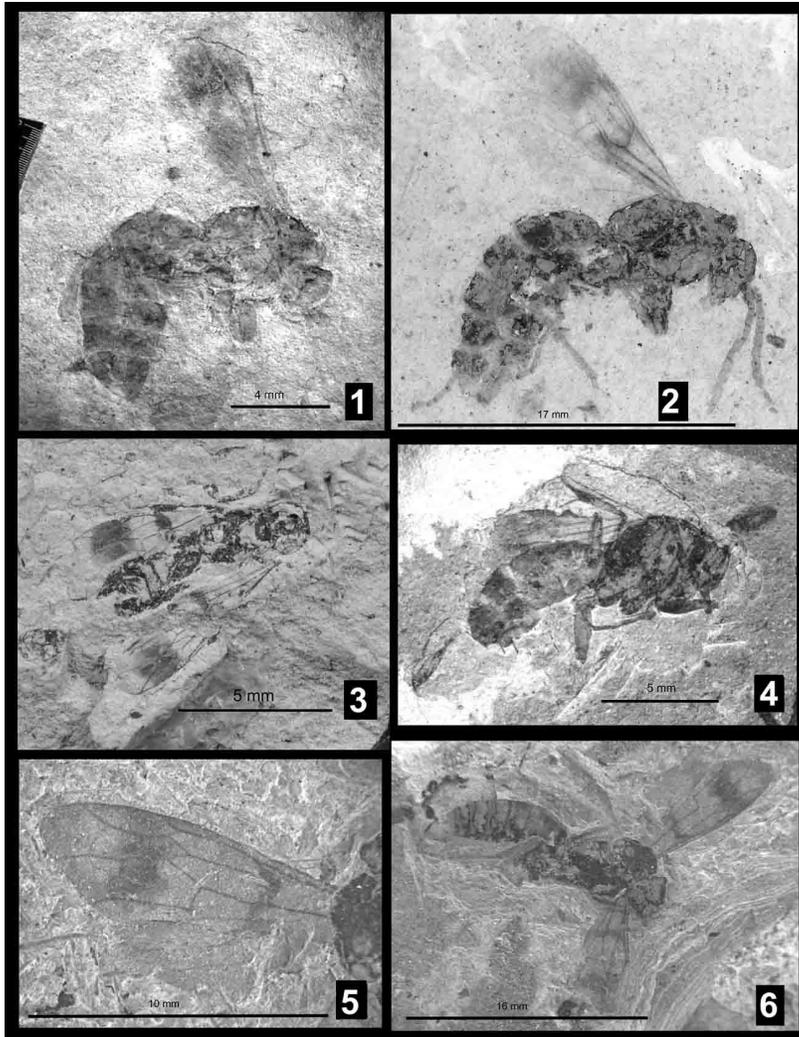
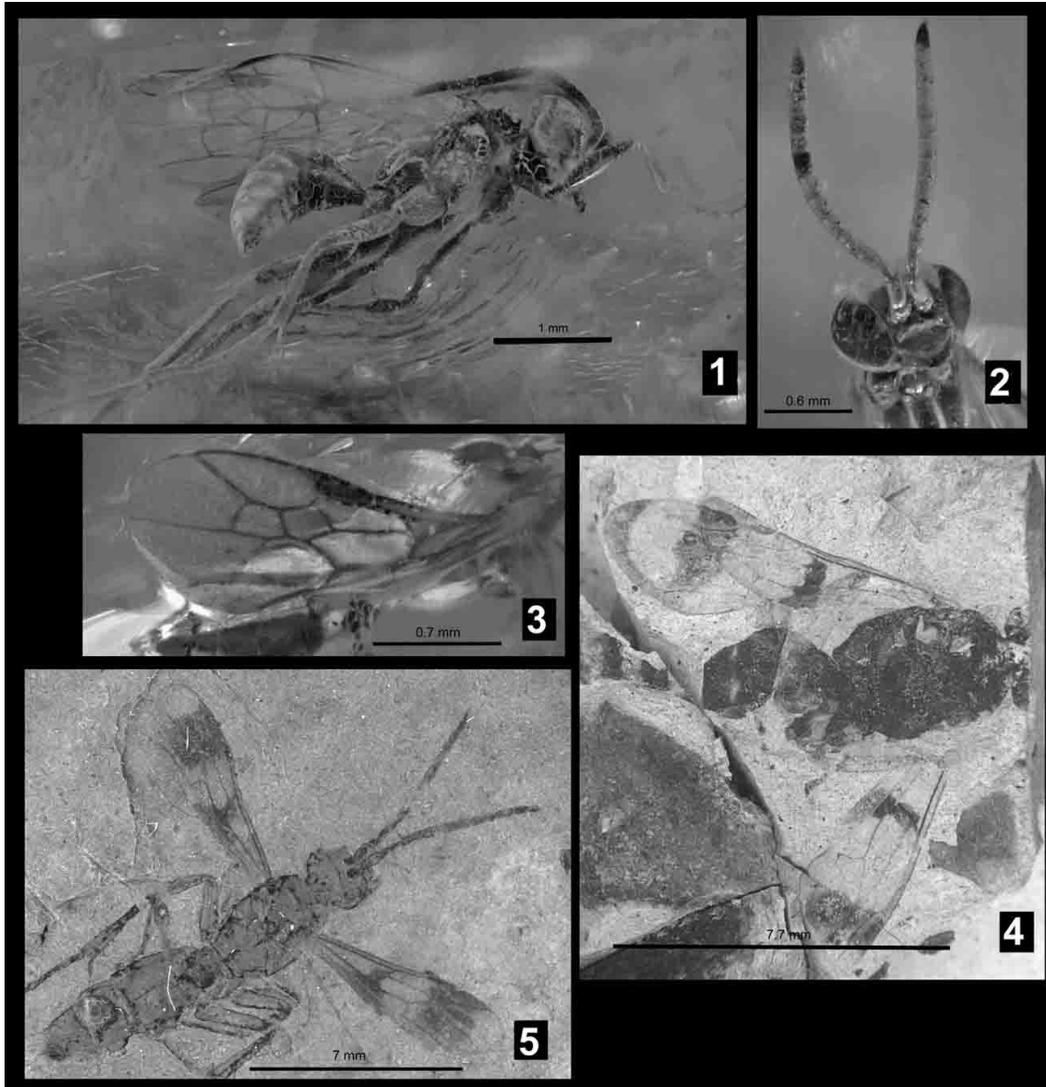


FIGURE 3.2— Pompilidae fossils. 1, dorsal view of *Pompilus coquandi* Theobald, 1937 from Aix-en-Provence deposits (MNHN); 2, dorsal view of *Pompilus fasciatus* Theobald, 1937 from Aix-en-Provence, France (MNHN); 3, habitus of *Tainopompilus argentum* Rodriguez and Pitts n. sp. in Dominican amber, Dominican Republic (OSAC Hy-10-45); 4, dorsal view of *Chirodamus avitula* (Cockerell, 1941) from Florissant Tertiary shales, Colorado, US (UCMC No. 19166); 5, habitus of *Cryptocheilus florissantensis* (Cockerell, 1906) from Florissant Tertiary shales, Colorado, US (MCZC No. 2023); 6, habitus of *Cryptocheilus laminarum* (Rohwer, 1909) from Florissant Tertiary shales, Colorado, US (UCMC No. 8597).



**FIGURE 3.3**— Pompilidae fossils. *1*, habitus of *Cryptocheilus senex* (Rohwer, 1909) from Florissant Tertiary shales, Colorado, US (UCMC. No. 8594); *2*, habitus of *Cryptocheilus hypogaeus* Cockerell, 1914 from Eocene shales of Florissant, Colorado, US (USNM No. 90385); *3*, dorsal view of *Dipogon catalanicus* Rodriguez, Waichert and Pitts n. sp. from lacustrine deposits of Bellver, Spain (MGMM No. 2927M); *4*, habitus of *Dipogon cockerellae* (Rohwer, 1909) from Florissant Tertiary shales, Colorado, US (UCMC No. 8598); *5, 6*, *Dipogon wettveri* (Statz, 1938) from Rott deposits, Germany (LACMIP No. 3973): *5*, fore and hind wings, *6*, habitus.



**FIGURE 3.4**— Pompilidae fossils. 1–3, *Paleogenia wahisi* Waichert and Pitts n. sp. from Baltic Amber, Kaliningrad region of Russia (OSAC Hy–10–80): 1, habitus, 2, head, 3, fore wing; 4, habitus of *Priocnemis aertsii* Statz, 1936 from Rott deposits, Germany (LACMIP No. 3972); 5, dorsal view of *Caputelus scudderi* (Cockerell, 1906) from Florissant Tertiary shales, Colorado, US (MCZC No. 2024).

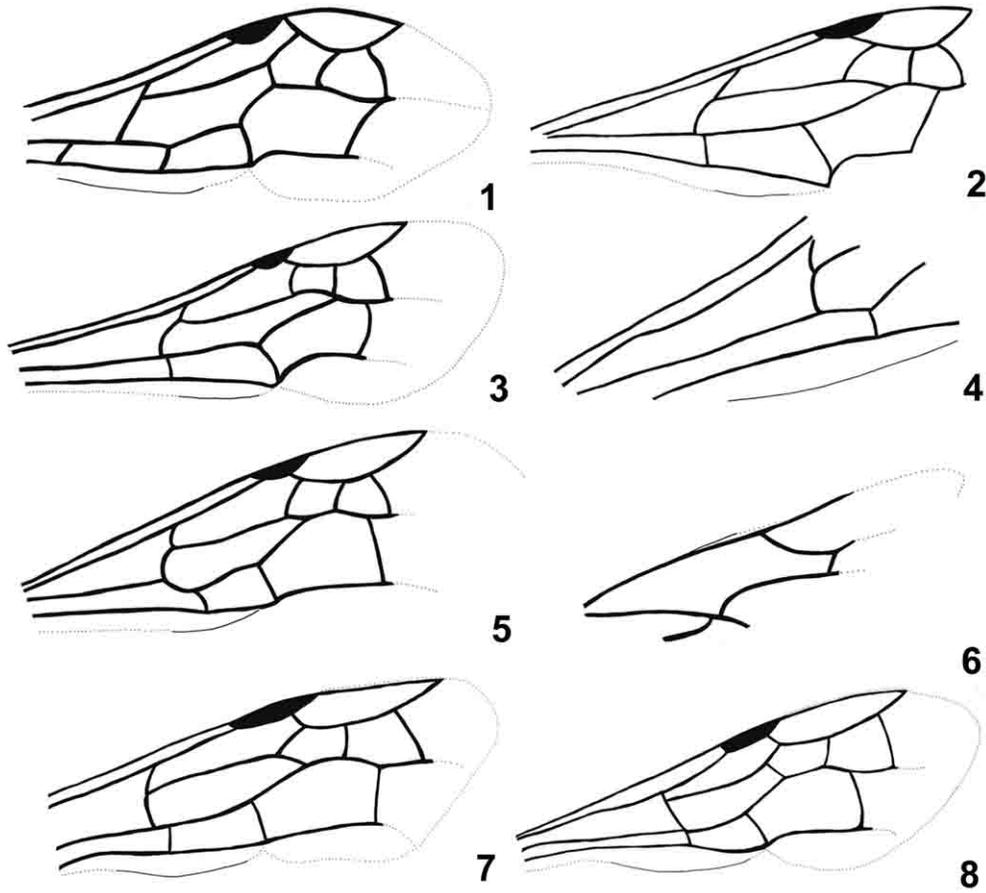


FIGURE 3.5— Wing drawings. 1, fore wing *Agenieoideus saxigenus*; 2, fore wing *Pompilus coquandi*; 3, fore wing *Pompilus fasciatus*; 4, fore wing *Chirodamus avitula*; 5, 6, *Cryptocheilus hypogaeus*: 5, fore wing, 6, hind wing; 7, fore wing *Dipogon wettweri*; 8, fore wing *Priocnemis aertsii*.

## CHAPTER 4

ASSESSING SPECIES BOUNDARIES AND SEX-ASSOCIATIONS IN THE GENUS *DREPANAPORUS* (HYMENOPTERA: POMPILIDAE), WITH COMPARISON OF THE UTILITY OF CYTOCHROME C OXIDASE I AND A NUCLEAR MOLECULAR MARKER, AND THE DESCRIPTION OF A NEW SPECIES OF *DREPANAPORUS*<sup>3</sup>**ABSTRACT**

The taxonomy of the Antillean genus *Drepanaporus* Bradley (Pompilidae) is problematic, due to sexual dimorphism and nearly uniform morphology of males across species. Species limits are not well understood, and sexes are not properly associated in all species. In this study, we reassessed morphology and collected novel molecular data for the purpose of determining species boundaries and establishing sex–associations for all species. Two genes, cytochrome C oxidase (COI) and long–wavelength rhodopsin (LWRh) were amplified for 20 specimens of *Drepanaporus*, from both females and males. Using LWRh (including both introns and exons) and COI sequences the relationships of *Drepanaporus* samples were reconstructed. Sequences obtained for COI showed unusually high divergences and putative introgression, and thus were not used for taxonomic decisions. Taxonomic changes were made based on the LWRh phylogenetic results. A new species of *Drepanaporus* —*D. bachata* sp. nov.— is described herein based on both molecular and morphological characters for both male and female specimens, and a key is provided for the genus for the three species now recognized. We

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<sup>3</sup> This manuscript is formatted for submission to *Annals of the Entomological Society of America*. The authors of the journal article are: Juanita Rodriguez, Carol D. von Dohlen, and James P. Pitts.

also discuss the usefulness of mitochondrial and nuclear markers in Pompilidae for species delimitations and sex–associations.

### **Introduction**

*Drepanaporus* Bradley is a genus of dimorphic spider wasps (Pompilidae), comprising three species found only in the Antilles. Two of these species have been described previously; the third species is identified and described here. The monophyly of *Drepanaporus* has been established by morphological and molecular data (unpublished data). *Drepanaporus* females are brightly colored, share a color pattern, and have a higher degree of morphological variation than males. The uniformity in male external morphology makes the taxonomy of *Drepanaporus* complicated, and suggests the need to apply molecular characters for taxonomic purposes.

One of the most commonly used molecular markers for species delimitation studies is mitochondrial cytochrome c oxidase (COI). More specifically, a 658-bp long fragment of the COI gene, proposed as the standard molecular barcode for animals (Folmer et al. 1994), has been used to establish species boundaries (Hou and Li 2010, Dombroskie and Sperling 2012, Navia et al. 2013) and sex-associations (Kurina et al. 2011, Zhang et al. 2013) in various taxa. However, problems with COI and other mitochondrial genes for such uses have surfaced in certain taxa, in the form of duplicate copies transposed to the nuclear genome, and heteroplasmy (multiple, divergent sequences) among mitochondrial copies. For example, heteroplasmy in COI genes of bees resulted in unusually high divergence distances (Magnacca and Danforth 2006), and even produced different haplotypes for different tissues within the same organism

(Magnacca and Brown 2012). Such anomalies can render COI and other mitochondrial sequences unreliable for species delimitation studies.

In light of the possible problems associated with COI sequences, highly variable nuclear markers have been proposed as potential alternatives. Recent studies have suggested the utility of the nuclear molecular marker, long-wavelength rhodopsin (LWRh), for assessing species boundaries (Derocles et al. 2012). This molecular marker is commonly used in Hymenoptera systematics, and shows high variability at the species level (Hines et al. 2006, Blaimer 2012, Rightmyer et al. 2013).

In this study, we collected molecular data from both COI and LWRh to establish species boundaries and make sex associations in *Drepanaporus*. The COI sequences obtained for *Drepanaporus* showed high divergence levels and evidence of introgression; thus, we ultimately used only LWRh sequences and morphological data to address the taxonomy of the group.

## **Materials and Methods**

### **Taxon sampling**

Females of the two described *Drepanaporus* species were sampled, as well as a female from a putative new species, which was initially recognized with the molecular data presented here (Table 4.1). Males of *Drepanaporus* from various locations in the Dominican Republic were also sampled (Table 4.1). Prior to this study, the only male known was that of *D. collaris* (Cresson 1865). During this study, two other male morphospecies were established using wing venation characters. Waichert et al. (2012) described one of these morphospecies as the male of *D. antillarum* (Bradley 1944). The

other morphospecies is herein described as *D. bachata*. Specimens of *D. collaris* and *D. antillarum* were sampled widely throughout the Antilles, and *D. bachata* was sampled from Dominican Republic. Specimens of a closely related species of spider wasp, *Euplaniceps quadrimaculata* (Smith 1873), were included as an outgroup. The outgroup was chosen as the sister group of the genus as determined by a phylogenetic analysis of the tribe Aporini (unpublished data).

### **Molecular methods**

DNA was extracted from 20 *Drepanaporus* specimens and a single specimen of *E. quadrimaculata* (Table 4.1). PCR took place in 20  $\mu$ L reactions, using 6  $\mu$ L GoTaq Green mastermix (Promega, Madison, WI), 10  $\mu$ L of nuclease free water, 1  $\mu$ M of each primer and approximately 20 ng of template. PCR program included an initial step of 94°C for 150 sec, followed by 35 cycles of 94°C for 30 sec, 46°C (LWRh) or 48°C (COI) for 60 sec, and 72°C for 60 sec, with a final step of 72°C for 10 min. Primers from previous studies were used (Table 4.2). The amplified fragments were visualized, purified and sequenced following the protocol described in Pilgrim and Pitts (2006). All PCR products were sequenced with forward and reverse primers, and were assembled into complete contigs using Sequencher 4.1 (Genecodes, Ann Arbor, MI). All sequences were deposited in GenBank (Table 4.1).

### **Phylogenetic reconstruction**

LWRh (including introns) and COI sequences were aligned in Geneious Pro 5.4 (Drummond et al. 2011, available from <http://www.geneious.com/>). LWRh introns within *Drepanaporus* had no length variation, and thus were easily aligned. Alignment of

introns when the outgroup was included produced gaps in the *Drepanaporus* sequences, but homology of sites was straightforward to infer. Models of molecular evolution were evaluated using PartitionFinder v1.0.1 (Lanfear et al. 2012) for different codon positions and introns. The models used were: GTR+I+G for LWRh 1st codon position and introns, K80+I for LWRh 2nd codon position, SYM+I+G for LWRh 3rd codon position, HKY+I+G for COI 1st codon position, JC for COI 2nd codon position, and HKY+G for COI 3rd codon position.

A Bayesian phylogenetic analysis was performed with MrBayes v3.2.1 (Ronquist et al. 2012) for each gene tree separately, and a distance phylogenetic analysis was performed in PAUP v4.0 (Swofford 2003) through the tree builder plugin in Geneious Pro 5.4 (Drummond et al. 2011). The Bayesian analysis was run with two separate MCMC chains for 30,000,000 generations; convergence diagnostics were assessed with Tracer v1.5 and 10% of the samples were discarded as burn-in. Bayesian and distance trees were visualized in FigTree v1.4.0. Because topologies of the two gene trees conflicted, only the results from *LWRh* were used further for species delimitation (see Results).

The species delimitation plug-in of Geneious (Masters et al. 2011) was used to determine whether members of different clades should be considered different species. We calculated inter- and intraspecific genetic distances based on the K2P model, and compared these to previous studies. We calculated the probability of reciprocal monophyly  $P(AB)$  (Rosenberg 2007), which determines the probability that monophyly of the lineages occurs by chance as an outcome of the random branching of lineages within a single taxon. We also calculated the liberal probability of identification [P

ID(liberal)], which is the mean probability of making a correct identification of an unknown specimen of the focal species using BLAST (best sequence alignment), DNA Barcoding (closest genetic distance), or placement on a tree, with the criterion that it falls sister to or within a monophyletic species. This value is based on simulations, we did not use the strict version of this value given the low sample size of our new putative species.

### **Taxonomic methods**

All the holotypes of the species treated were studied, except for the holotype of *Planiceps cubensis* Cresson 1867 (see *D. collaris* remarks section). Abbreviations used in the descriptions are the same as those used by Wasbauer and Kimsey (1985). They are defined as follows: FD = facial distance; LA3 = length of third antennal segment; MID = middle interocular distance; OOL = ocellocular length; POL = postocellar length; TFD = transfacial distance; UID = upper interocular distance; and WA3 = width of third antennal segment. Measurements of the clypeus are as follow: WC, width of clypeus, measured from the widest points; and LC, highest length of clypeus. Male genital terms follow the terminology by Wasbauer and Kimsey (1985). Wing venation terminology follows that of Huber and Sharkey (1993, figs 19–20). Images were taken with a Jenoptik camera coupled to a dissecting microscope Leica Mz7.5; processed by Auto-Montage™ software; and treated in Adobe Photoshop Elements 9.

The acronyms for the collections used in this study are as follows:

AEIC American Entomological Institute, Gainesville, Florida, USA.

CMNH Carnegie Museum of Natural History, Pittsburgh, Pennsylvania, USA.

EMUS Utah State University Entomology Collection, Utah State University, Logan,  
Utah, USA

FSCA Florida State Collection of Arthropods, Division of Plant Industry, Gainesville, Florida, USA.

MCZC Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, USA.

MPEG Museu Paraense Emilio Goeldi, Belém, Pará, Brazil.

PMAE Provincial Museum of Alberta, Edmonton, Alberta, Canada.

UCFC University of Central Florida – Entomology, Orlando, Florida, USA

USNM National Museum of Natural History, Smithsonian Institution, Washington, USA.

## Results

### Phylogenetic results

**COI sequences.** From the 20 specimens extracted, only 7 amplified successfully for COI. Pairwise Kimura 2-parameter distances (K2P) ranged from 11%–23% between intraspecific samples (Fig. 4.1A). The alignment and translation revealed no insertions, deletions, or stop codons to suggest the possibility of pseudogenes. The topology obtained with this marker differed for certain samples from the nuclear gene phylogeny (Fig. 4.2). One of the *D. collaris* specimens was recovered as a member of the *D. antillarum* clade. Moreover, *D. bachata* was included in the *D. collaris* clade, albeit with a low posterior probability. This pattern suggests the possibility of mitochondrial gene introgression or incomplete lineage sorting. Given these results, the COI data were not used further for taxonomic purposes.

**LWRh sequences.** All 20 specimens yielded LWRh PCR products and sequences. Intraspecific K2P distances ranged from 0–3.4%, and interspecific distances

from 4.1–7.9% (Fig. 4.1B). The Bayesian MCMC analysis for LWRh produced a consensus tree with three major lineages supported by high posterior probabilities (Fig. 4.2). These lineages corresponded to *D. collaris*, *D. antillarum*, and a third lineage that comprised specimens with distinctive morphology, which we designate as a new species of *Drepanaporus* (see Taxonomic results). LWRh K2P distances have been studied in Hymenoptera, where interspecific distances range from 0–34% and intraspecific distances from 0–1% (Derocles et al. 2012). These results were based on sequences where the 5' -end intron was removed. Moreover, the species evaluated by Derocles et al. (2012) were determined *a priori* with morphology. Our results for interspecific distances fall within the range reported by Derocles et al. (2012), but our results show a gap between inter and intraspecific distances (Fig. 4.1B). Rosenberg's P(AB) (Rosenberg 2007) was 0.02 for the lineage of the putative new species. Using the critical values proposed by Rosenberg (2007), the hypothesis that reciprocal monophyly could have been a product of random branching is rejected. The P ID (liberal) of 0.80 for the new putative species suggests a high probability of correctly identifying a specimen to the correct species using molecular data. These results, along with morphological evidence, suggest that samples composing the third lineage should be considered a new species of *Drepanaporus*.

## **Taxonomic results**

### ***Drepanaporus* Bradley 1944**

(Fig. 4.3 – females, Fig 4.4 – males)

Type species *Planiceps collaris* Cresson 1865, by original designation and monotypy.

**Diagnosis.** The female is black except for the following regions: a red band crossing on the streptaulus, and a red band on the posterior margin of the pronotum and the abdomen (Fig. 4.3I). The mandible has a tooth in the inferior margin (Fig. 4.3J). The males have the basal half of parameres forming a broad lamina, where the part beyond the elbow forms a narrow appendage (Figs 4.4A–C).

**Remarks.** This genus was originally separated from other Aporini genera by the presence of a cleft tarsal claw with a truncate tooth. This character is present only in some specimens of *D. collaris* as discussed by Waichert et al. (2012). The male genitalia, (including the subgenital plate) in this genus have high morphological variability. Differences observed in Figs. 4.4A–F (e.g. apex of parameres, shape of digiti) are not consistent across species. The only reliable character is the presence or absence of long setae on the margin of the basal lamina of the paramere, which separates *D. bachata* **n. sp.** from the other two. *D. collaris* and *D. antillarum* can only be separated using wing venation characters.

**Distribution.** This genus is found in the Bahamas, Cuba, Hispaniola, Puerto Rico and the Virgin Islands.

### Key to the species of *Drepanaporus*

#### Females

1 Eyes clothed with dense, rather long setae (Fig. 4.3A); frons with short, coarse upward-directed setae (Fig. 4.3C) ... 2

- Eyes without dense setae (Fig. 4.3B); frons without coarse upward-directed setae (Fig. 4.3D) (Bahamas, Cuba, Haiti, Dominican Republic and Puerto Rico)... *Drepanaporus collaris* (Cresson)

2 Basal third of clypeus transversally concave (Fig. 4.3F); antennal segments 1–4 covered or not with short, coarse, appressed setae (Fig. 4.3H) (Cuba, Dominican Republic and Virgin Islands) ... *Drepanaporus antillarum* (Bradley)

- Basal third of clypeus not transversally concave (Fig. 4.3E); antennal segments 1–8 covered with short, coarse, appressed setae (Fig. 4.3G) (Dominican Republic)...  
*Drepanaporus bachata* Rodriguez and Pitts, **sp. nov.**

#### Males

1 2m-cu vein of fore wing interstitial or slightly basad or distal (less than  $0.15 \times$  height of 1Rs cell) to 2r-m vein (Fig. 4.4G) ...

- 2m-cu vein of fore wing strongly distal (at least  $0.25 \times$  height of 1Rs cell) to 2r-m vein (Fig. 4.4I)... *Drepanaporus collaris* (Cresson)

2 Paramere with long setae on the edge of basal half lamina (Figs 4.4C–D) ...

*Drepanaporus antillarum* (Bradley)

Paramere without long setae on the edge of the basal half lamina (Figs 4.4A–B)...

*Drepanaporus bachata* Rodriguez and Pitts, **sp. nov.**

***Drepanaporus antillarum* (Bradley 1944)**

(Figs. 4.4C, 4.4D, 4.4H, 4.4K)

*Planiceps antillarum* Bradley 1944, Transactions of the American Entomological Society, Philadelphia, vol. 70, p. 107–108 [Holotype: female, HAITI: Port Au Prince or vicinity (MCZC)].

**Diagnosis.** The female has the basal third of the clypeus concave (Fig. 4.3F), setose eyes, frons with short, coarse upward-directed setae, and antennae with short, coarse setae that never surpass segment four. The male is black with silvery pubescence (Fig. 4.4K), has the 2m-cu vein of fore wing interstitial or slightly distal (less than 0.15 × height of 1Rs cell) of the 2r-m vein (Fig. 4.4G), and the basal half of the paramere forms a broad lamina with long setae (Figs 4.4C–D).

**Material examined.** CUBA: Mts. N. of Imias eastern Oriente: 1 female paratype of *Planiceps antillarum* 25–VII–1936 to 28–VII–1936, Darlington, MCZC–26770; DOMINICAN REPUBLIC: Elias Pina: 1 male allotype *Drepanaporus antillarum*, Sierra de Neiba, 9.0 km WSW Hondo Valle, 18° 41' 34'' N, 71° 46' 52'' W, 1843 m, 25–VI–2003, disturbed montane woodland with pine, malaise trap, sample 31382, Rawlins et al., CMNH–370,370; Independencia: 13 females, 10 males, Sierra de Bahoruco, Loma del Toro, 18° 17' 16'' N, 71° 42' 46'' W, 2310 m, 7–8–XI–2002, meadow, pine woods, yellow pan trap, sample 40169, Zanol et al., CMNH–371,199/ 370,984/ 370,856/ 370,114/ 370,577/ 370,012/ 369,464/ 370,089/ 370,963/ 371,963/ 371,020/ 370,800/ 370,411, 369,807/ 369,379/ 369,818/ 371,070/ 369,942/ 370,083/ 370,792/ 369,428/ 369,468/ 370,940; Independencia: 3 females, Sierra de Bahoruco, Loma del Toro, 18° 17' 16'' N, 71° 42' 46'' W, 2310 m, 7–XI–2002 to 8–XI–2002, meadow in pine woods, malaise trap, sample 40189, CMNH–369,819/ 370,167/ 370,587; Independencia: 1

female, Sierra de Neiba just south of crest, 5 km NNW Angel Feliz, 1780 m, 18° 41' N, 71° 47' W, 13–X–1991 to 15–X–1991, cloud forest, J. Rawlins et al., CMNH–370,461; Independencia: 2 females, Sierra de Neiba near crest, 5.5 km NNW Angel Feliz, 18° 41' N, 71° 47' W, 1750 m, 21–VII–1992 to 22–VII–1992, dense cloud forest, J. Rawlins et al., CMNH–369,777/ 370,091/ 370,142/ 370,485; Independencia: 1 male, Sierra de Bahoruco, Loma del Toro, 5.3 km SW El Aguacate, 18° 17' 16'' N, 71° 42' 46'' W, 2316 m, 29–III–2004 to 30–III–2004, Pinus, Garrya montane forest, yellow pan trap, sample 43263, CMNH–370,280; Independencia: 3 males, Sierra de Bahoruco, north slope, 13.5 km SE Puerto Escondido, 18° 12' 24'' N, 71° 30' 54'' W, 1807 m, 24–III–2004 to 25–III–2004, dense broadleaf forest, pine, yellow pan trap, sample 42262, J. E. Rawlins et al., CMNH–370,950/ 370,472/ 369,921; Independencia: 3 males, Sierra de Bahoruco, north slope, 13.5 km SE Puerto Escondido, 18° 12' 18'' N, 71° 31' 08'' W, 1789 m, 24–III–2004 to 26–III–2004, ecotonal Pinus grassland, yellow pan trap, sample 41163, R. Davidson et al., CMNH–371,122/ 369,691/ 370,092; Independencia: 1 male, Sierra de Bahoruco, Loma del Toro, 5.3 km SW El aguacate, 18° 17' 16'' N, 71° 42' 46'' W, 2316 m, 29–III–2004 to 30–III–2004, Pinus, Garrya montane forest, yellow pan trap, sample 43263, C. Young et al., CMNH–369,989; Pedernales: 4 males, 26 km N Cabo Rojo, 18° 06' N, 71° 38' W, 730 m, 19–VII–1990 to 25–VII–1990, wet deciduous forest, intercept trap, L. Masner et al., CMNH–370,037/ 370,177/ 370,117/ 369,911; La Altagracia: 4 males, Punta Cana, Ecological Foundation, Biodiversity Center, old fruit tree grove, 18° 30.847' N, 68° 22.822' W, 11–IX–2008 to 14–IX–2008, MT residue, SEL Hymenoptera, HYM Course, EMUS; La Altagracia: 5 males, Punta Cana, 13–IX–2008 to 14–IX–2008, Sand Hill, M[alaise]T[rap], EMUS; La Altagracia: 1 male, Parque del Este, Caseta

Guaraguao, 4.4 km SE Bayahibe, 18° 19' 59'' N, 68° 48' 42'' W, 3 m, 26–V–2004 to 27–V–2004, semihumid forest near sea, limestone, malaise trap, sample 51184, C. Young et al., CMNH–369,898; Pedernales: 2 females, 4 males, Sierra Bahoruco, 730 m, Cabo Rojo, 26 km W., L. Mesner, PMAE; Duarte: 3 males, 20 km NE San Francisco de Macoris, Loma Quitaespuela, M[alaise]T[rap], 800 m, VI–1991, PMAE; Pedernales: 2 males, 37 km N Cabo Rojo, 1500 m, 18° 09' N, 71° 35' W, 23–IX–1991, grassland with pines, J. Rawlins et al., CMNH–370,112/ 370,401; Pedernales: 1 male, La Abeja, 38 km NNW Cabo Rojo, 18° 09' N, 71° 38' W, 1350 m, 15–VII–1987, J. Rawlins, R. Davidson, CMNH–370,670; Pedernales: 1 male, Upper Las Abejas, 38 km NNW Cabo Rojo, 18° 09' N, 71° 38' W, 1250 m, 22–VII–1990, Davidson R., Rawlins, J., CMNH–370,938; Pedernales: 1 male, 37 km N Cabo Rojo, 18° 09' N, 71° 35' W, 1500 m, 11–VII–1987, Davidson R., Rawlins, J., CMNH–366,651; Pedernales: 1 male, 3.3 km NE Los Arroyos, 18° 15' N, 71° 45' W, 1450 m, 16–VII–1990 to 18–VII–1990, wet montane forest, sweep samples, L. Masner et al., CMNH–370,437; Pedernales: 1 male, Sierra de Bahoruco, Aceitillar, 23.6 km NE Pedernales, 18° 09' 23'' N, 71° 34' 09'' W, 1560 m, 14–VI–2003, open pine forest with grassland, malaise trap, sample 42182, CMNH–370,080; HAITI: Port au Prince or vicinity, 1 female holotype, MCZC; VIRGIN ISLANDS: St. Johns: 1 female, Cinnamon Bay, Oven Trail, 27–VII–1981 to 7–VII–1981, L. Masner, PMAE.

**Distribution.** Cuba, Dominican Republic and Virgin Islands.

**Remarks.** This species was originally described in the genus *Planiceps* Latreille by Bradley (1944). It was placed in the genus *Drepanaporus* by Waichert et al. (2012).

***Drepanaporus collaris* (Cresson 1865)**

(Figs. 4.3B, 4.3F, 4.3H, 4.3J, 4.4E, 4.4F, 4.4H, 4.4L)

*Planiceps collaris* Cresson 1865, Proceedings of the Entomological Society of Philadelphia, vol. 4, p. 132.

[Holotype: female (ANSP)].

*Planiceps cubensis* Cresson 1867, Transactions of the American Entomological Society Philadelphia, vol.

1, p. 136 [Holotype: male, CUBA (IZAC)].

*Pompilus falco* Dalla Torre 1897, Catalogus Hymenopterorum, vol. 8, p. 288 [proposed as new name for

*Planiceps cubensis* Cresson 1867, nec Cresson 1865].

*Pompilus troglodytes* Dalla Torre 1897, Catalogus Hymenopterorum, vol. 8, p. 328 [proposed as new name

for *Planiceps collaris* Cresson 1867, nec *Sphex collaris* Fabricius 1775].

*Odontaporus simulatrix* Bradley 1944, Transactions of the American Entomological Society Philadelphia,

vol. 70, p. 113 [Holotype: female, PUERTO RICO, Jayuya, (USNM)].

**Diagnosis.** The female has the basal third of the clypeus convex (Fig. 4.3J), glabrous eyes, sometimes with vestigial setae (Fig. 4.3B), frons with sparse, long setae (Fig. 4.3D), and antennae glabrous. The male is black with silvery pubescence (Fig. 4.4L), has the 2m-cu vein of fore wing strongly distal (at least 0.25 X height of 1Rs cell) of the 2r-m vein (Fig. 4.4I), and long setae on the base of the widening of the paramere (Figs 4.4E–F).

**Material examined.** BAHAMAS: Eleuthera Rainbow Bay: 5 males, 1–VII–1987, D. B. and R. W. Wiley, malaise trap, EMUS; Eleuthera Rainbow Bay: 2 females, 4 males, 1–VII–1987, D. B. and R. W. Wiley, malaise trap, EMUS; Gorda Cay: 3 males, 26° 05' N, 77° 32' W, 7–VII–1998 to 16–VII–1998, T. Peak, FSCA; Gorda Cay: 1 female, 2 males, 26° 05' N, 77° 32' W, 10–X–1997 to 15–X–1997, Z. Pruzak, FSCA; Gorda Cay: 1 female, 1 male, 26° 05' N, 77° 32' W, 20–VI–1998 to 26–VI–1998, Crew beach mangrove/dune mix, malaise trap, S. Glasscock, UCFC; San Salvador Is[land]: 1

female, 9-V-1982 to 15-V-1982, B. Bowen, PMAE; CUBA: 1 female holotype, ANSP-432; Jaronu: 1 female paratype of *Odontaporus simulatrix*, 27-V-1931, collected on weeds, L. L. Scaramuzza, MCZC-26769; San Blas and vic[inity]: 1 female paratype of *O. simulatrix*, Trinidad m[oun]t[ain]s, 24-IV-1931, MCZC-26769; Santiago Prov.[ince]: 1 female, Gran Piedra, 1100m, 14-XII-1995 to 17-XII-1995, L. Masner, EMUS; Soledad: 1 female paratype of *O. simulatrix*, 16-II-1925, MCZC-26769; DOMINICAN REPUBLIC: Azua: 1 female, east side of crest, Sierra Martin Garcia, 7 km WNW Barrero, 18° 21' N 70° 58' W, 860m, 25-VII-1992 to 26-VII-1992, C. Young et al., CMNH-370,271; La Vega: 1 female, Cordillera Central, Loma Casabito, 16 km NW Bona0, 19° 02' 21'' N, 70° 31' 05'' W, 1487 m, 28-V-2003, J. Rawlins et al., CMNH-370,555; Hato Mayor: 1 female, Parque Los Haitises, 3 km W Cueva de Arena, 19° 04' N 69° 29' W, 20m, 7-VII-1992 to 9-VII-1992, CMNH-370,730; Independencia: 1 female, 3 males, Sierra de Bahoruco, north slope, 13.5 km SE Puerto Escondido, 18° 12' 24'' N, 71° 30' 54'' W, 1807 m, 24-III-2004 to 25-III-2004, dense broadleaf forest, pine, yellow pan trap, sample 42262, J. E. Rawlins et al., CMNH-370,142/ 370,817/ 370,097/370,252; Independencia: 1 male, Sierra de Bahoruco, north slope, 13.3 km SE Puerto Escondido, 18° 12' 33'' N, 71° 30' 47'' W, 1812 m, 24-XI-2004 to 25-XI-2004, *Pinus rubus*, Garrya, open, malaise trap, sample 41385, J. Rawlins et al., CMNH-366,389; Independencia: 1 female, Sierra de Neiba, south slope near summit, 4 km N Angel Feliz, 18° 40' 21'' N, 71° 46' 05'' W, 1825 m, 1-IV-2004 to 2-IV-2004, broadleaf cloud forest without pine, yellow pan trap, sample 34263, J. Rawlins et al., CMNH-370,296; La Altagracia: 1 female, Parque del Este, 2.9 Km SW Boca de Yuma, 18° 21' 51'' N 68° 37' 05'' W, 11m, 28-V-2004, J. Rawlins et al. CMNH-370,945; La

Altagracia: 1 female, 2 km N Bayahibe, 18° 23' N, 63° 51' W, 10m, 3–VII–1992,  
 CMNH–370,471; Pedernales: 1 female, Sierra de Baoruco, Aceitillar, 23.6 km NE  
 Pedernales, 18° 09' 23'' N, 71° 34' 09'' W, 1569 m, 14–VI–2003, open pine forest with  
 grassland, malaise trap, sample 42182, C. Young et al., CMNH–369,993; La Vega: 2  
 females, 2 males, P.N. Armando Bermudez, 1000 m, 17–I–1989, L. Masner, AEIC;  
 Duarte: 4 males, 10 km NE San Francisco de Macoris, Loma Quita Espuela,  
 M[alaise]T[rap], 800 m, VI–1991, PMAE; La Vega: 10 females, 1 male, Cordillera  
 Central Loma Casabito, 15.8 km NW Bonao, 19° 02' 12'' N, 70° 31' 08'' W, 1455 m,  
 28–V–2003, evergreen cloud forest, east slope, yellow pan trap, sample 21262, CMNH–  
 370,630/ 370,799/ 370,268/ 370,161/ 370,994/ 370,387/ 369,505/ 367,265/  
 370,310/370,405; Pedernales: 2 males, 37 km N Cabo Rojo, 18° 09' N, 71° 35' W, 1500  
 m, 11–VII–1987, Davidson R., Rawlins, J., CMNH–370,056/ 371,324; Pedernales: 2  
 males, 23.5 km N Cabo Rojo, 18° 06' N, 71° 38' W, 540 m, 13–VII–1990 to 19–VII–  
 1990, Masner, L., Rawlins, J., Young, C., CMNH–370,715/ 369,757; Pedernales, 1 male,  
 26 km N Cabo Rojo, 18° 06' N, 71° 38' W), 730 m, 19– VII–1990 to 25–VII–1990,  
 Masner, L., Rawlins, J., Young, C., CMNH–371,144; Pedernales: 1 female, 1 male,  
 Sierra de Bahoruco, Aceitillar, 25.4 km ENE Pedernales, 18° 05' 27'' N, 71° 31' 08'' W,  
 1270 m, 14–VI–2003, open pine forest with grassland, yellow pan trap, sample 42162, E.  
 Young et al., CMNH–370,397/370,980; La Vega: 2 females, 1 male, Cordillera Central,  
 4.1 km SW El Convento, 18° 50' 37'' N, 70° 42' 48'' W, 31–V–2003, dense secondary  
 evergreen forest with pine, yellow pan trap, sample 22262, CMNH–369,529/ 370,333/  
 370,639; Pedernales: 2 females, Sierra de Bahoruco, Aceitillar, 25.4 km ENE Pedernales,  
 dense broadleaf forest , pine, yellow pan trap, sample 42262, C. Young et al., CMNH–

371,096/ 370,704/ 369,827; Pedernales: 1 female, Along Rio Mulito, 13 km N Pedernales, 18° 09' N, 71° 46' W, 230 m, 17–VII–1992, riparian woodland, J. Rawlins et al., CMNH–370,849; Pedernales: 1 female, 9.5 km N Cabo Rojo, 18° 02' N, 71° 39' W, 35 m, 13–VII–1990 to 19–VII–1990, desert scrub, intercept trap, L. Masner et al., CMNH–369,623; La Vega: 5 males, Cordillera Central, Loma Casabito, 16 km NW Bonao, 19° 02' 21'' N, 70° 31' 05'' W, 1487 m, 28–V–2003, J. Rawlins et al., CMNH–370,908/ 370,272/ 370,356/ 371,474/ 370,359; Pedernales: 1 female, 1 km S Los Arroyos, 1125m, 18° 14' N 71° 45' W 18–X–1991, R. Davidson et al., CMNH–371,066; Santiago: 1 male, Cordillera Central, Loma Casabito, 16 km NW Bonao, 19° 02' 21'' N, 70° 31' 05'' W, 1487m, 28–V–2003, J. Rawlins et al., CMNH–370,069; Pedernales: 2 females, 19 males, Sierra Bahoruco, 15 km W Cabo Rojo, VIII–1990, 540 m, L. Masner, PMAE; Pedernales Prov[ince]: 1 female, 2 males, 21 km N Cabo Rojo, 19–VI–1976 to 20–VI–1976, R.E. Woodruff and E.E. Grissell, FSCA; HAITI: Kenscoff: 1 female, 1949, G. N. Wolcott, USNM; Trou Forban: 1 female, 9–iv–1944, A. Curtiss, USNM; PUERTO RICO: Jayuya, 1 female holotype of *Odontaporus simulatrix*, USNM; Luquillo Nat[ional] For[est]: 1 female, 1 male, El Verde, 4–X–1968 to 17–X–1968, USNM; El Verde: 3 females, 1 male, Km. 17.5, ruta 186, 14–VII–1993 to 24–VII–1993, C. Porter, FSCA; El Yunque: 1 female, 2000–2100 ft, 15–II–1969 to 24–II–1969, T. and B. Hlavac, L. Herman Jr., MCZC.

**Distribution.** Bahamas, Cuba, Haiti, Dominican Republic and Puerto Rico.

**Remarks.** As mentioned by Waichert et al. (2012) *Odontaporus simulatrix* was originally separated from *D. collaris* by the presence of a cleft tarsal claw in *D. collaris* and a dentate claw in *O. simulatrix* (Bradley 1944). However, tarsal claw shape is

variable and specimens with each of the two types of tarsal claws are found in sympatry throughout both species' distribution. These tarsal claw types are not a synapomorphic character when mapped onto the molecular phylogeny of *Drepanaporus*. All holotypes were examined except for the holotype of *Planiceps cubensis* Cresson 1867, which was revised by Bradley. Bradley provided illustrations of the genitalia of *D. collaris* (1944, plate IV, fig 23), and included *P. cubensis* in the synonym list of *D. collaris* as a result of his revision. We are confident that this belongs to *D. collaris* given the detailed descriptions and illustrations of wing venation and genitalia provided by Bradley (1944).

***Drepanaporus bachata* Rodriguez and Pitts, sp. nov.**

(Figs. 4.3A, 4.3C, 4.3E, 4.3G, 4.3I, 4.4A, 4.4B, 4.4G, 4.4J)

**Diagnosis.** The female has a convex clypeus (Fig. 4.3E), setose eyes (Fig. 4.3A), frons with short, coarse upward-directed setae (Fig. 4.3C), and antennae with short, coarse setae that cover up to segment eight (Fig. 4.3G). The male is black with silvery pubescence (Fig. 4.4J), has the 2m-cu vein of fore wing interstitial to the 2r-m vein (Fig. 4.4G), and the half base lamina of the paramere is glabrous (Fig. 4.4A–B).

**Female.** Holotype. Body length 8.5 mm. Fore wing 6.5 mm; maximum wing width 1.6 mm.

**Coloration.** Head black; clypeus black with dark brown apical margin; mandibular and maxillary palpi pale brown; mandible black from base to half of its length, pale brown apically; antenna black; pronotum black with red spot on anterior margin, and red band on the posterior margin; scutellum black; postnotum black;

propodeum black; metasoma red, except for last segment black; wing translucent brown; veins dark brown; legs black.

**Head** (Fig. 4.3A). Head wide; TFD  $1.05 \times$  FD; MID  $0.70 \times$  FD. Ocelli in acute angle; lateral ocelli closer to compound eyes than to each other; POL  $1.42 \times$  OOL. Mandible wide, with long, sharp apical teeth, and tooth in the inferior margin (Fig. 4.3J); pubescence on mandible short, abundant on first half of length. Clypeus a short band, round, convex; LC  $0.10 \times$  WC; anterior margin polished. Antenna short; width of fourth segment  $0.40 \times$  its length; ratio of the first four antennal segments 34:10:22:25; WA3  $0.45 \times$  LA3; LA3  $0.55 \times$  UID; short thick pubescence abundant on first eight segments (Fig. 4.3G).

**Mesosoma.** Short, silvery pubescence sparse on entire body, pubescence more abundant on propodeum; punctation inconspicuous. Pronotum elongated, width  $1.23 \times$  length, posterior margin semi-angulated; pronotal collar inconspicuous. Notauli present from beginning of mesonotum to scutellum. Postnotum polished. Propodeal disc with long silvery setae, more abundant on lateral and inferior corner. Forewing long, with two Rs cells; length of 1Rs cell  $0.45 \times$  distance from its origin to wing apex; 2Rs cell  $3 \times$  longer than first; 2m-cu vein bent, slightly curved, interstitial with 2r-m vein. Front tibia with three spines on posterior margin; spines on mid tibia, sparse, short, sharp.

**Metasoma.** Metasoma polished, covered by short, abundant setae; pygidium well defined, bare, polished; terminal metasomal sternum with sparse, long setae; metasoma  $1.26 \times$  as long as mesosoma.

**Allotype, Male** (Figs. 4.4A–B, G, J). Body length 3.2 mm. Fore wing 2.2 mm; maximum wing width 1.1 mm.

**Coloration.** Head and mesosoma entirely black; metasoma dark brown; mandibular and maxillary palpi pale reddish brown; mandible black from base to half of its length, light brown apically; antenna dark brown; wing translucent; veins light brown; legs dark reddish brown.

**Head.** Head wide; TFD  $1.1 \times$  FD; MID  $0.70 \times$  FD. Lateral ocelli as close to each other as to compound eyes; POL  $1 \times$  OOL. Mandible wide, with long, sharp apical teeth; silvery pubescence short, abundant on first 0.20 of length. Clypeus wide, rounded; anterior margin somewhat truncate, punctured; LC  $0.78 \times$  WC. Antenna short; width of fourth segment  $1.28 \times$  its length; ratio of first four antennal segments 15:5:5:7; WA3  $1.00 \times$  LA3; LA3  $0.15 \times$  UID.

**Mesosoma.** Pubescence silvery on entire body, more abundant on propodeum. Pronotum elongated, width  $1.60 \times$  length, posterior margin not straight; pronotal collar not differentiated from disc. Postnotum striated. Posterior margin of propodeum with abundant setae at base. Wing long; length of first radial 2 cell  $0.48 \times$  distance from its origin to wing apex; two Rs cells; 2Rs cell  $2.00 \times$  1Rs cell; 2m-cu vein bent, slightly curved, meeting 2Rs cell  $0.90 \times$  distance from base to apex of cell. Front tibia with spines absent on anterior and posterior margins; middle and hind tibiae with sharp, sparse spines present.

**Metasoma.** Metasoma covered by short, abundant pubescence; metasoma  $1.33 \times$  as long as mesosoma.

**Genitalia** (Fig. 4.4A–B). Parapenial lobe split; lobes with finger-like shape, broad, short, its length  $0.44 \times$  total genitalia length; apical lobe semi-angulated, curved; basal portion wider. Digitus narrow, rod-shaped; length  $0.47 \times$  paramere length; setae long, thin, abundant on external surface. Aedeagus thin, long, shorter than parapenial lobes, bilobed apically. Paramere length  $0.80 \times$  total genitalia length; expanded on 0.50 of length from base; apex rounded; setae long, thick, covering 0.33 of length apically. Subgenital plate narrow, rectangular; apex acute; setae apically abundant, short, thick.

**Etymology.** Named in honor of Bachata, a traditional music genre developed in the Dominican Republic. The name is used as a noun in apposition.

**Material examined.** HOLOTYPE: 1 female, La Altagracia, South of Bahahibe [Bayahibe], Dominican Republic,  $18.3433^\circ$  N  $68.8155^\circ$  W, 15–III–2012, S. W. Droege, USGS-DRO, EMUS; ALLOTYPE: 1 male. Pedernales, Sierra de Baoruco, Aceitillar, 25.2 km ENE Pedernales, Dominican Republic,  $18^\circ 05' 29''$  N  $71^\circ 31' 16''$  W, 1272m, 17–vi–2003, dense broadleaf forest, pine, yellow pan trap, C. Young et al., sample 42262, CMNH–371,443; PARATYPE: 1 female, la Altagracia, Parque del Este, Caseta Guaraguao, 4.4 km SE Bayahibe, Dominican Republic  $18^\circ 19' 59''$  N,  $68^\circ 48' 42''$  W, 3 m, 26–V–2004 to 27–V–2004, semihumid forest near sea, limestone, yellow pan trap, sample 51164, C. Young et al., CMNH–370,259.

**Distribution.** Dominican Republic.

**Remarks.** This species shares several morphological similarities with *Drepanaporus antillarum*. Females of the two species have short setae on the eyes, share a common color pattern, and have abundant short setae in the first antennal segments. Differences between the females of these species are the shape of the clypeus and the

amount of setae present on the antenna. Males have almost indistinguishable genitalia, with the only difference being the presence of setae on the base of the parameres in *D. bachata*.

## Discussion

The usefulness of molecular data for species delimitation and sex-associations had not been studied before in Pompilidae. Szafranski (2009) examined COI sequences for a few species in the family, and inferred a high substitution rate from pairwise distances compared to other Hymenoptera (Derocles et al. 2012). However, neither intraspecific comparisons, nor inter- to intraspecific comparisons were studied. Our study shows that the use of universal primers to amplify and sequence the COI barcoding region in Pompilidae may produce misleading phylogenetic results. Given the high sequence variability among individuals within species, and the discordance with the nuclear marker, we were unable to use the COI gene with confidence to establish species boundaries.

The COI phylogeny has very low support values; therefore, it is difficult to make solid conclusions about the unexpected clustering of certain samples. It is important to note, however, that the specimen of *D. collaris* (PO703), which is clustering within *D. antillarum*, was collected in the same locality as one of the *D. antillarum* (PO701) that it is clustering with. This could be evidence of geographic structure within COI. On the other hand, the clustering of *D. bachata* within *D. collaris* in the COI phylogeny and within *D. antillarum* in the LWRh phylogeny is not an unexpected result, given that the

relationship between *D. bachata* with any of the two other species is not supported with high posterior probabilities for any of the two phylogenies (Figure 4.2).

The nuclear gene, LWRh, proved easy to amplify with previously published primers. The sequences were variable between closely related species, and a clear gap between intraspecific versus interspecific pairwise species divergences was observed. This aided in establishing species boundaries, as well as associating sexes within taxa where morphological differences are subtle. Thus, this molecular marker has proved useful for informing taxonomic studies in Pompilidae, as it has in other hymenopteran taxa (Derocles et al. 2012).

Pompilidae contain many groups of closely related species with very similar morphology and confusing intraspecific variation, which are difficult to distinguish on the basis of characters provided in older, published descriptions. Our study demonstrates that the application of molecular data to such cases can help to assign samples to distinct lineages. This greatly facilitates the search for informative, discriminating morphological characters, which can be more effective for species identifications.

Future work in Pompilidae molecular systematics should include the comparison of a wider sample of COI and LWRh sequences in additional genera, in order to determine more broadly their usefulness separately and in conjunction.

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Table 4.1. Identity, voucher code, collection location and GenBank accession numbers for the specimens analyzed for molecular data.

Species	Sex	Voucher ID	Locality	Collection	COI	LWRh
					Accession #	Accession #
<b>Outgroup</b>						
<i>Euplaniceps quadrimaculata</i>		PO832		MPEG	KJ440514	KJ440504
<b>Ingroup taxa</b>						
<i>Drepanaporus bachata</i> n. sp.	Male	PO466	DOMINICAN REPUBLIC: Pedernales, Sierra de Bahoruco	CMNH-371,443	-----	KJ440502
<i>Drepanaporus bachata</i> n. sp.	Female	PO711	DOMINICAN REPUBLIC: La Altagracia, Parque del Este	CMNH-370,259	KJ440511	KJ440503
<i>Drepanaporus antillarum</i>	Female	PO687	DOMINICAN REPUBLIC: Independencia, Sierra de Bahoruco	CMNH-370,587	-----	KJ440493
<i>Drepanaporus antillarum</i>	Male	PO722	DOMINICAN REPUBLIC: Elias Pina, Sierra de Neiba	CMNH-370,370	-----	KJ440492
<i>Drepanaporus antillarum</i>	Male	PO707	DOMINICAN REPUBLIC: Independencia, Sierra de Bahoruco	CMNH-370,280	KJ440509	KJ440491
<i>Drepanaporus antillarum</i>	Male	PO723	DOMINICAN REPUBLIC: Pedernales, Sierra de Bahoruco	PMAE	-----	KJ440494
<i>Drepanaporus antillarum</i>	Male	PO701	DOMINICAN REPUBLIC: Independencia, Sierra de Bahoruco	CMNH-369,468	KJ440508	KJ440490
<i>Drepanaporus antillarum</i>	Male	PO706	DOMINICAN REPUBLIC: Duarte, 20 km NE San Francisco de Macoris	PMAE	-----	KJ463567
<i>Drepanaporus collaris</i>	Female	PO686	DOMINICAN REPUBLIC: Hato Mayor, Parque Los Haitises, 3 km W Cueva de Arena	CMNH-370,730	-----	KJ440505
<i>Drepanaporus collaris</i>	Female	PO688	DOMINICAN REPUBLIC: La Vega, Cordillera Central, Loma Casabito	CMNH	-----	KJ440506
<i>Drepanaporus collaris</i>	Female	PO471	BAHAMAS: Gorda Cay	UCFC	-----	KJ463569
<i>Drepanaporus collaris</i>	Female	PO439	BAHAMAS: San Salvador Island	PMAE	-----	KJ463568
<i>Drepanaporus collaris</i>	Male	PO468	DOMINICAN REPUBLIC: Independencia, Sierra de Bahoruco	CMNH-366,389	KJ440513	KJ440496
<i>Drepanaporus collaris</i>	Male	PO705	DOMINICAN REPUBLIC: Santiago, Cordillera	CMNH-370,069	-----	KJ440499

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Septentrional						
<i>Drepanaporus collaris</i>	Female	PO715	DOMINICAN REPUBLIC: Independencia, Sierra de Bahoruco	CMNH-370,252	-----	KJ440501
<i>Drepanaporus collaris</i>	Male	PO467	DOMINICAN REPUBLIC: Pedernales, Sierra de Bahoruco	CMNH-369,827	KJ440512	KJ440495
<i>Drepanaporus collaris</i>	Male	PO703	DOMINICAN REPUBLIC: La Vega, Cordillera Central	CMNH-370,639	KJ440507	KJ440497
<i>Drepanaporus collaris</i>	Male	PO704	DOMINICAN REPUBLIC: La Altigracia, Parque del Este	CMNH-370,945	-----	KJ440498
<i>Drepanaporus collaris</i>	Male	PO709	DOMINICAN REPUBLIC: La Vega, Cordillera Central	CMNH-370,405	KJ440510	KJ440500

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Table 4.2. Primers used for PCR amplification and sequencing

Marker	Primer name	Primer sequence (5'-3')	Reference
<i>LWRh</i>	PompOps1F	ATTCGACAGATACAACGTAATCG	Pilgrim et al. (2008)
	LwRhR	ATATGGAGTCCANGCCATRAACCA	Mardulyn and Cameron (1999)
<i>COI</i>	LEP-F1	ATTCAACCAATCATAAAGATAT	Herbert et al. (2004)
	LEP-R1	TAAACTTCTGGATGTCCAAAA	Herbert et al. (2004)
	HCO	TTCAGGGTGACCAAAAAATCA	Herbert et al. (2003)
	LCO	TCAACAAATCATAAAGATAT	Herbert et al. (2003)

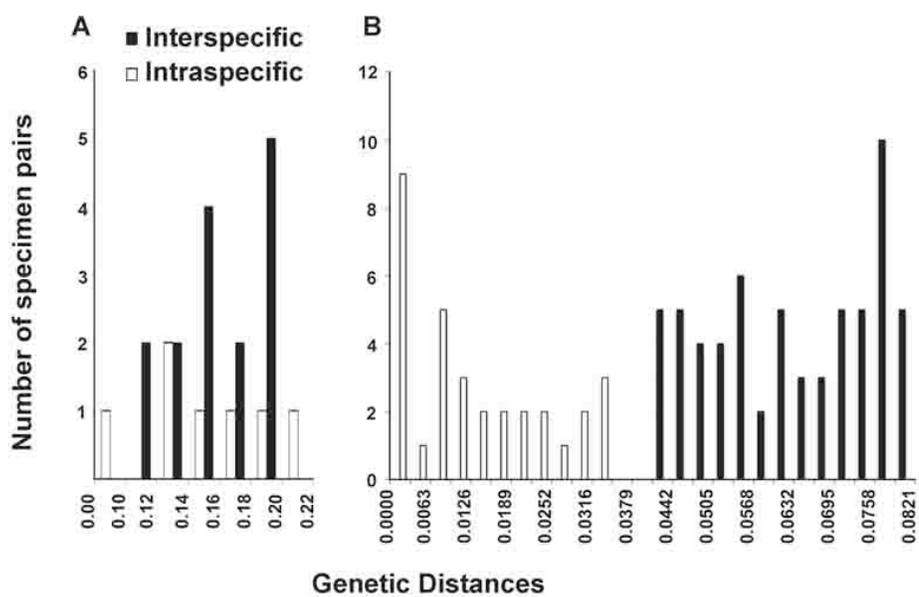


Fig. 4.1. Intraspecific and interspecific distance distribution among *Drepanaporus* species for (A) cytochrome c oxidase I (COI) and (B) long wavelength rhodopsin (LWRh).

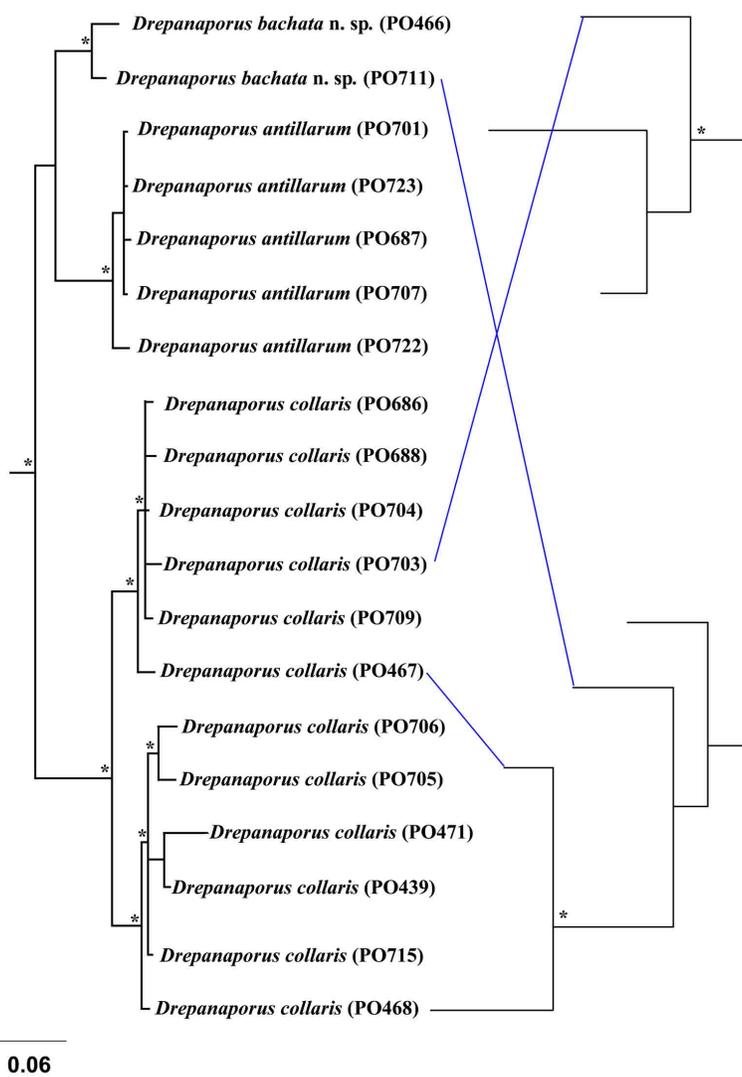


Fig. 4.2. Consensus phylogenetic reconstruction for *Drepanaporus* resulting from 2 Bayesian MCMC runs performed in MrBayes. Nodes with posterior probability of 1.0 are indicated with an asterisk. Left: LWRh reconstruction. Right: COI reconstruction. Blue lines connect identical samples whose positions were incongruent in the COI reconstruction.

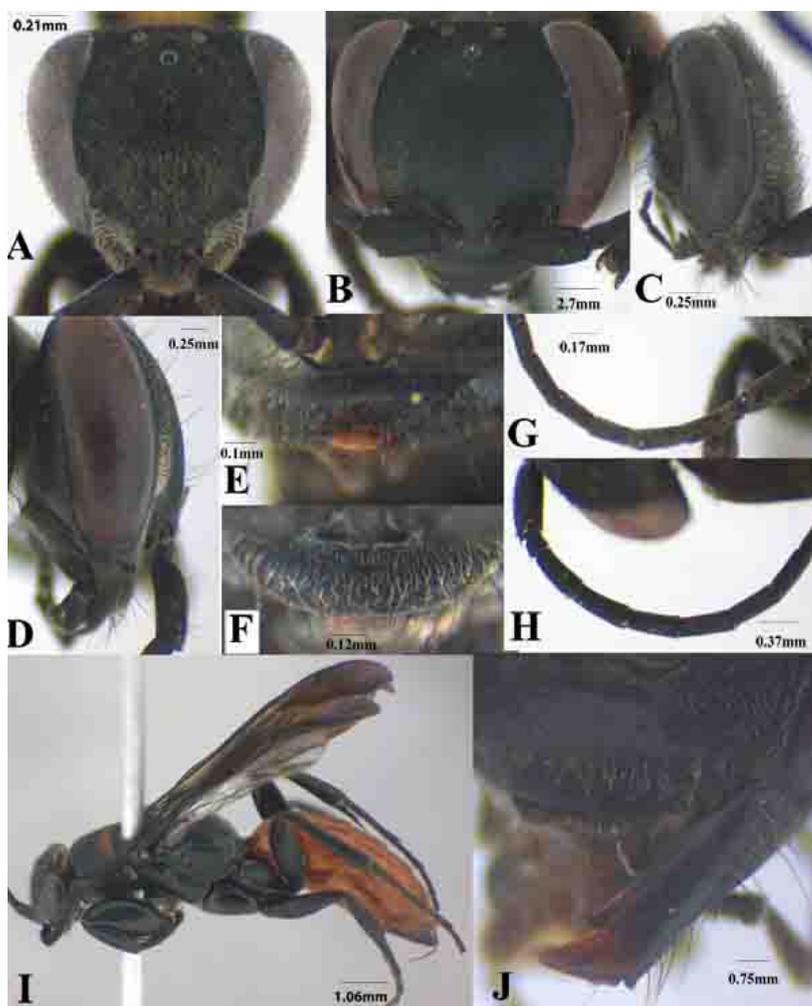


Fig. 4.3. (A) *Drepanaporus bachata* **sp. nov.**: head, dorsal view, female. (B) *Drepanaporus collaris*: head, dorsal view, female. (C) *Drepanaporus bachata* **sp. nov.**: head, lateral view, female. (D) *Drepanaporus collaris*: head, lateral view, female. (E) *Drepanaporus bachata* **sp. nov.**: clypeus, dorsal view, female. (F) *Drepanaporus collaris*: clypeus, dorsal view, female. (G) *Drepanaporus bachata* **sp. nov.**: antenna, female. (H) *Drepanaporus collaris*: antenna, female. (I) *Drepanaporus bachata* **sp. nov.**: habitus, lateral view, female. (J) *Drepanaporus collaris*: mandible, female.

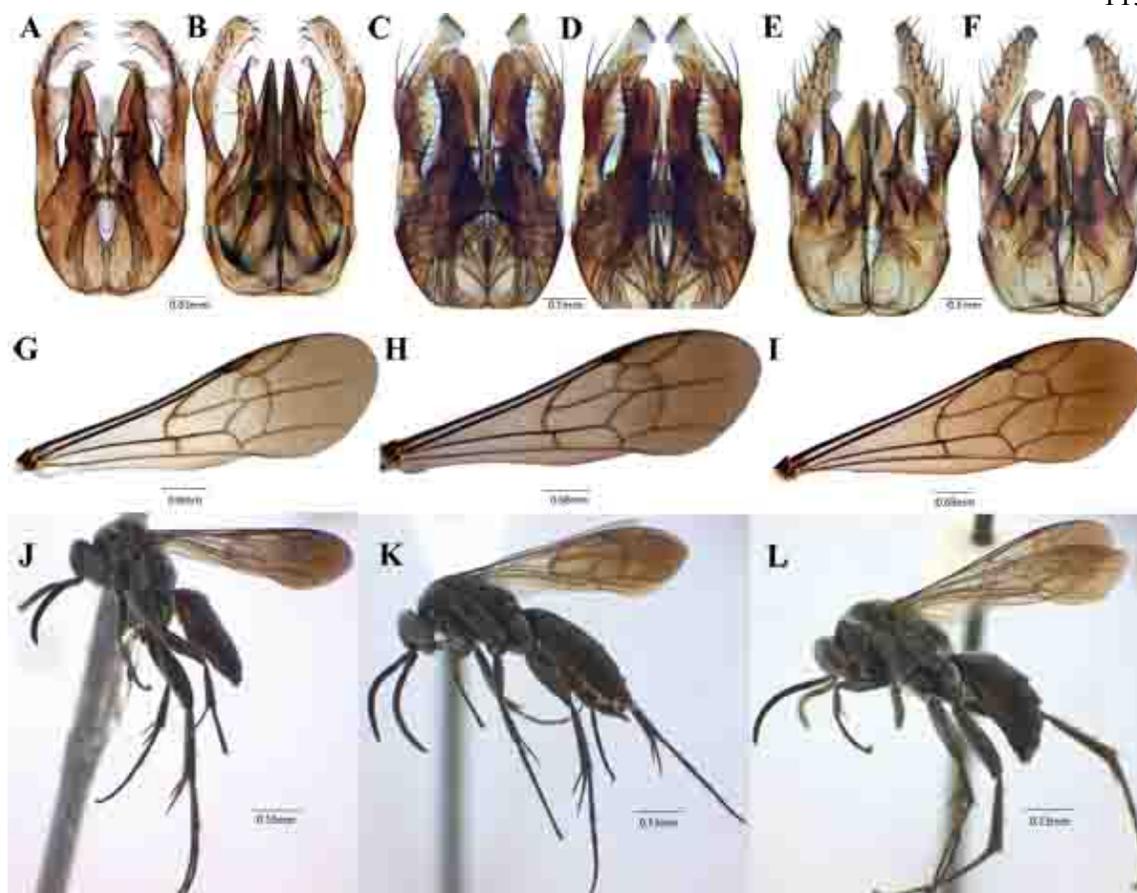


Fig. 4.4. (A–B) *Drepanaporus bachata* **sp. nov.**: genitalia, male: (A) ventral view; (B) dorsal view. (C–D) *Drepanaporus antillarum*: genitalia, male: (C) ventral view; (D) dorsal view. (E–F) *Drepanaporus collaris*: genitalia, male: (E) ventral view; (F) dorsal view. (G) *Drepanaporus bachata* **sp. nov.**: forewing, male. (H) *Drepanaporus antillarum*: forewing, male. (I) *Drepanaporus collaris*: forewing, male. (J) *Drepanaporus bachata* **sp. nov.**: habitus, lateral view, male. (K) *Drepanaporus antillarum*: habitus, lateral view, male. (L) *Drepanaporus collaris*: habitus, lateral view, male.

## CHAPTER 5

HISTORICAL BIOGEOGRAPHY OF THE WIDESPREAD SPIDER WASP TRIBE  
APORINI (HYMENOPTERA: POMPILIDAE)<sup>4</sup>**ABSTRACT**

**Aim** We studied the historical biogeography of Aporini spider wasps. Our aim was to determine the age and area of origin of Aporini and of all its genera. We also aimed to test the fit of several hypotheses concerning the putative processes underlying the widespread distribution of this group.

**Location** The Holarctic and Neotropical areas.

**Methods** A phylogeny of 44 Aporini specimens was produced through Bayesian inference using four nuclear molecular markers (elongation factor-1  $\alpha$  F2 copy, long-wavelength rhodopsin, wingless and the D2–D3 regions of the 28S ribosomal RNA). A lognormal relaxed molecular clock, calibrated with ages from three fossils, was used to estimate lineage divergence times. Biogeographic processes were studied using three methods: i) Statistical–Dispersal–Vicariance Analysis (S–DIVA), ii) Dispersal Extinction Cladogenesis (DEC) Analysis, and iii) Bayesian Binary MCMC (BBM) Analysis.

**Results** Our data suggest an origin of the most recent common ancestor of extant Aporini in the Nearctic region in the Early Miocene, 22.6 Ma (CI= 17.40,28.83). All genera originated in the Miocene, four in the Nearctic region. A constrained DEC analysis, where only dispersal to adjacent regions was allowed, produced the highest likelihood and was mostly congruent with the results from the BBM method.

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<sup>4</sup> This manuscript is formatted for submission to *Journal of Biogeography*. The authors of the journal article are: Juanita Rodriguez, James P. Pitts, and Carol D. von Dohlen.

**Main Conclusions** Dispersal from the Nearctic region to the Palaeartic region occurred through the Bering Land Bridge in the early Miocene, 15–18 Ma (CI = 11.14,23.52), and three dispersal events to South America from Mesoamerica took place independently. All of these occurred after 18 Ma through the Isthmus of Panama. Three independent over-water dispersal events to the Antilles occurred from Mesoamerica, and probably the Nearctic, for two genera of the tribe. The dispersal patterns inferred within the biogeographic history of Aporini agree with several scenarios proposed for other, unrelated taxa.

## INTRODUCTION

Widely separated, disjunct distributions of related taxa often have been attributed to vicariance models, which interpret modern distribution patterns as subdivisions of ancestral areas resulting from climatic, physiographic, physical, or tectonic processes (Rosen, 1975; Nelson & Platnick, 1981; Cooper *et al.*, 2001). The importance of vicariance as the cause of disjunctions, however, has been reconsidered with the increasing use of dating methods in biogeography. Widely separated distributions of recently diverged taxa cannot be attributed to continental drift; thus, models that give a higher weight to these older tectonic events might not be suitable to analyze such cases. Several recent studies of diverse taxa find that vicariance might not be the dominant process behind current distributions; rather, they conclude that dispersal events might explain better the patterns of distribution between closely related organisms (Ree & Smith, 2008; Lomolino *et al.*, 2009).

Spider wasps (Hymenoptera: Pompilidae), are a widespread group with a common ancestor in the Eocene (Wilson *et al.*, 2012). The pompilid tribe Aporini is

widespread, and includes endemic species from the Nearctic, Mesoamerica, South America, the Antilles, and the Palearctic (Fig. 5.1). Its current distribution allows study of the history of disjunctions between the Old and New World, and between North and South America. Because some taxa are endemic to the Antilles, *Aporini* is also a good group to study the biogeographic processes accounting for the origins of fauna on this archipelago. Based on the age of Pompilidae, we assume that *Aporini* could not have originated earlier than approximately 50 Ma and, therefore, major continental drift events are unlikely to have driven the diversification of the group. However, formation and disruption of land bridges in the Tertiary could have been important influences on *Aporini* biogeography.

### **The origin of Old World–New World disjunct distributions**

The events underlying Old World–New World disjunct distributions of recently diverged taxa —i.e., those too young to fit the continental drift vicariance model— have been recently studied (Sanmartín *et al.*, 2001). Two probable Nearctic-Palearctic dispersal routes for groups that radiated in the Cenozoic have been hypothesised: the Bering Land Bridge (BLB) and the North Atlantic Land Bridge (NALB) (Sanmartín *et al.*, 2001). These corridors have been available for dispersal at different and sometimes overlapping times (Tiffney, 1985; Condamine *et al.*, 2013).

The BLB fluctuated with climate changes that occurred over the last 40 Myr. This corridor allowed dispersal in the Early Tertiary (Tiffney, 1985), and was interrupted in the Eocene–Oligocene, causing vicariance of widespread taxa (Sanmartín *et al.*, 2001). Some interchange of cold-adapted taxa, however, was likely until the terminal Eocene event (Tiffney, 1985). Between 14–3.5 Ma only the dispersal of boreal elements was

likely. More recently, glacial cycles in the Pleistocene allowed the dispersal of tundra-adapted groups (Sanmartín *et al.*, 2001).

Although Nearctic–Palaeartic disjunctions have usually been explained as the result of vicariance events splitting ancestral distributions extending across the BLB (Darlington, 1957), trans-Atlantic dispersal–vicariance may have played a more important role in this type of disjunctions (Tiffney, 1985). Palaeozoologic evidence for this connection has been known for some time for mammals (McKenna, 1975) and plants (Tiffney, 1985). The NALB route opened in the Late Cretaceous, but connections existed between the Holarctic areas until the Early Eocene (50 Ma) (Sanmartín *et al.*, 2001). Three North Atlantic land connections have been proposed to exist at different times: the Thulean bridge (55–50 Ma) (McKenna, 1983), the DeGeer Bridge (39 Ma), and the Greenland–Faroës Bridge. This last one was probably present in the Miocene, but has never been considered to be an important dispersal route (Sanmartín *et al.*, 2001) because it probably only allowed the dispersal of tropical elements between 26–23 Ma (Zachos *et al.*, 2001).

### **The origin of Nearctic–South America disjunct distributions**

The geological history of Central America is very complex. Earlier studies proposed that the connection between North and South America through the Isthmus of Panama was established *ca.* 3–7 Ma (e.g. Keigwin, 1978, 1982; Bacon *et al.*, 2013). However, more recent research shows that this connection formed earlier, through a narrowing of the Panama seaway *ca.* 23–25 Ma (Early Miocene) (Farris *et al.*, 2011; Montes *et al.*, 2012a,b), and a complete closure as early as *ca.* 15 Ma (Montes *et al.*, 2012a,b).

The Antilles has been proposed as a probable pathway for dispersal between North and South America (Rosen, 1975; Ramirez *et al.*, 2010; Condamine *et al.*, 2013). Rosen (1975) proposed the South American–Caribbean track as a pattern observed in groups that diversified in South America, dispersed to the Antilles through the Aves Ridge, and then dispersed to Central America with the closure of the Isthmus of Panama.

### **The origin of Antillean taxa**

The origins and patterns of diversification are unclear for most Caribbean groups (Dávalos, 2004). However, hypotheses have been formulated to explain the origin of Antillean endemic taxa. The three main processes suggested are: vicariance of the Protoantilles (Rosen, 1975, 1985), land bridges (land dispersal) (Iturralde-Vinent & MacPhee, 1999), and over-water dispersal (Iturralde-Vinent & MacPhee, 1999). The vicariance model proposes that the Antillean fauna arose in the Late Cretaceous (80–70 Ma) by the fragmentation of the protoantilles, a continuous landmass located between North and South America ca. 65 Ma (Rosen, 1975, 1985). Some ancient and relictual groups are hypothesised to fit this hypothesis, but most Antillean taxa prove too young to fit (Hedges, 2006). Several pieces of evidence reject the Protoantilles hypothesis. The most remarkable are the substantial emergence of the Antillean landmass that occurred after the mid–Eocene (37–49 Ma) (Iturralde-Vinent & MacPhee, 1999), and the asteroid impact in the Yucatan peninsula ca. 65 Ma, that could have removed all the Caribbean fauna (Hedges *et al.*, 1992).

Recently, another vicariance model was proposed for younger Antillean groups, for which taxa from Northern South America are sister to taxa from the Lesser Antilles. This vicariance model asserts a land connection between the Greater Antilles and

northern South America around the Early Oligocene (Iturralde-Vinent & MacPhee, 1999), which allowed continental South American fauna to reach the islands (Dávalos, 2004). This land bridge has been called the Gaarlandia (Greater Antilles and Aves Ridge) land span. Gaarlandia created a 1–2 Myr passage ca. 32 Ma that later fragmented and separated the Antillean and South American fauna once again (Iturralde-Vinent & MacPhee, 1999).

Vicariance models for Antillean biogeography have been challenged mainly by Hedges's (1996) model of dispersal. Many studies—mainly for mammals—have supported the hypothesis of dispersal from South America throughout the Cenozoic (e.g. Hedges *et al.*, 1992). Moreover, over-water dispersal has been proposed to apply to taxa whose divergence dates tend to be younger, spread out, and fit no particular pattern (Hedges, 2006). Another dispersal route suggested for Antillean biota is from Mesoamerica in the middle Eocene (49 Ma), when the Yucatan peninsula and the Antilles coalesced (Pindell, 1994). This hypothesis has been refuted by recent studies (Iturralde-Vinent & MacPhee, 1999).

The analysis of morphological data has yielded various possible models of diversification. The Late Cretaceous vicariance event was supported for leafhoppers (Felix & Mejdalani, 2011), but a post-Gaarlandia vicariance model fit best for weevils and Carabidae (Ball and Shpeley, 2009; Girón & Franz, 2010). Molecular studies also yield different results for different insect groups (Seal *et al.*, 2011; Condamine *et al.*, 2013). Swallowtail butterfly data, for example, suggested over-water dispersal from the Nearctic to the Antilles approximately 20–23 Ma (Condamine *et al.*, 2013).

Many hypotheses used to explain diversification were proposed before the development of divergence–time–estimation methods. To understand the processes producing widespread taxon distributions, a well–resolved, robust phylogeny of the group under study is needed (Ree & Smith, 2008). The phylogeny should also be calibrated in real time to allow comparison of lineage divergence with geological and climatic data (Lomolino et al., 2009). In addition, the data should be analyzed under an event–based method that not only specifies the events, but also their relative or absolute timing to evaluate the histories of ancestral areas (Sanmartin, 2012).

Here, we undertake a study of the historical biogeography of the widespread spider wasp tribe Aporini. We reconstruct a time-calibrated phylogeny using four molecular markers and three fossil calibrations. We further analyze this phylogeny under an event–based method in order to test the hypotheses concerning the processes underlying the widespread distribution of this lineage.

## **MATERIALS AND METHODS**

### **Taxon sampling**

We sampled 44 specimens from six out of nine Aporini genera from all possible geographic distributions. We followed the classification of Bradley (1944) and Evans (1966, 1973) (Table 5.1). Five species from other tribes and subfamilies were used for outgroups. These included taxa from clades that could be calibrated to geological age using the fossil record. Outgroup taxa were: *Anoplius (Lophopompilus) aethiops*, *Allochares azureus*, *Cryptocheillus idoneum birkmanni*, *Dipogon sp.* and *Agenioideus sp.* Dates for calibrations were based on the revision of Pompilidae fossils by Rodriguez

(unpublished data). Vouchers were deposited at the Department of Biology Insect Collection, Utah State University, Logan, Utah (EMUS).

### **Molecular methods**

DNA extraction, amplification and sequencing of elongation factor-1  $\alpha$  F2 copy (EF1), long-wavelength rhodopsin (LWRh), wingless (Wg) and the D2–D3 regions of the 28S ribosomal RNA (28S) followed methods in Pilgrim & Pitts (2006). Primers from previous studies were used, and a reverse primer for a more accurate sequencing of *Aporini* LWRh was developed (Table 5.2). All PCR products were sequenced with forward and reverse primers and were assembled into complete contigs using Sequencher 4.1 (Gene Codes Corp., Ann Arbor, MI).

### **Phylogenetic and dating analyses**

Sequences were aligned using Geneious Alignment (Geneious 6.1) and subsequently refined manually. Intron data was eliminated from the alignment for LWRh. The model of molecular evolution was determined for each gene and by codon position using Partitionfinder 1.01 (Lanfear *et al.*, 2012). Intron data was analyzed as a separate partition for EF1. Single-gene phylogenies were produced through a Bayesian framework implemented in MrBayes 3.2 (Huelsenbeck & Ronquist, 2001) to check for topological incongruence. Single-gene matrices were then concatenated using Geneious 6.1 to produce a combined matrix. Single-gene and combined matrices were run for 10,000,000 generations, with sampling every 1,000 generations. Effective sample size (ESS), trees to remove as burn-in, and graphical examination of chain convergence were examined in Tracer 1.5.

A chronogram was inferred in a Bayesian framework using Beast 1.7.5 (Drummond *et al.*, 2012) under an uncorrelated lognormal relaxed molecular clock model (Drummond *et al.*, 2006; Drummond & Rambaut, 2007). Substitution models were unlinked among partitions with the underlying clock and trees linked. Four calibration points were used for the analysis. Three were obtained from fossil data of Pompilidae species (see Chapter 3), and one from the age of the crown group of Pompilidae inferred by a divergence dating analysis of all stinging wasps (Wilson *et al.*, 2012). The common ancestor of *Anoplius* and *Allochares* was given a normal prior of (mean) 25 Ma (SD=10), based on the fossil of *Anoplius* sp. from Dominican amber, which belongs to the stem group of *Anoplius*. The common ancestor of *Cryptocheilus* and *Dipogon*, as well as the common ancestor of *Agenioideus* and *Allochares* + *Anoplius*, were given a lognormal prior with a mean (in real space) of 33 Ma (LogSD=0.5), based on the fossils of *Cryptocheilus hypogaeus* and *Agenioideus saxigena* found in the Colorado Florissant beds (see Chapter 3). The crown group node of all taxa included in the analysis (family Pompilidae) was assigned a normal prior of (mean) 43 Ma (SD=10), based on Wilson *et al.* (2012). Two separate Markov Chain Monte Carlo (MCMC) searches were performed for 10,000,000 generations. Effective sample size (ESS) and graphical examination of chain convergence were examined in Tracer 1.5. Independent runs were assembled with LogCombiner 1.7.5. Ten percent of generations were discarded as burn-in.

### **Ancestral area reconstruction**

Distribution areas for Aporini were modified from Olson's (2001) areas of endemism. The Neotropical Area was split according to Morrone (2006) into Mesoamerica and South America. The Antilles was considered a separate area given the

high endemism of the Aporini species present there. The areas established were: Nearctic, South America, Mesoamerica, Palaearctic and Antilles (Fig. 5.2). This area delimitation scheme conforms to the distribution of extant Aporini. Each of the taxa studied is distributed in only one of the areas established for this study.

Three event-based methods were implemented to estimate ancestral areas: Statistical-Dispersal-Vicariance Analysis (S-DIVA), Bayesian Binary MCMC (BBM), and Dispersal Extinction Cladogenesis (DEC). S-DIVA (Nylander *et al.*, 2008; Yu *et al.*, 2010), and the BBM algorithm (Yu *et al.*, submitted) were implemented using the program RASP (Yu *et al.*, 2013). These two methods use the DIVA —dispersal-vicariance method that estimates ancestral areas in a parsimony context using a three-dimensional cost matrix (Ronquist, 1996) — method in a statistical context, calculating the probability of ancestral areas over a Bayesian posterior distribution of tree topologies. This method minimises the cost of vicariance events compared to dispersal-extinction events. The estimation of ancestral area marginal probabilities, taking into account phylogenetic uncertainty, has been suggested to reduce uncertainty in the biogeographic reconstruction (Nylander *et al.*, 2008). The DEC method was implemented in the program Lagrange (Ree *et al.*, 2005; Ree & Smith, 2008). This method estimates the maximum likelihood parameters for the rates of dispersal and local extinction, and determines the ancestral areas with a highest-likelihood score in the context of bifurcating range inheritance scenarios, and dispersal constraints set by the researcher. Because Lagrange allows the specification of an instantaneous transition-rate matrix between geographical ranges (Ree *et al.*, 2005; Ree & Smith, 2008; Ree & Sanmartín, 2009), two types of analyses were performed under the DEC method. First, a general analysis (L1)

allowed all dispersal routes with equal probability. Second, a constrained analysis (L2) allowed dispersal from any area other than the Nearctic to the Palaearctic, and vice versa, with probability set to 0.1; the direct dispersal from Nearctic to South America was set to 0.1; ancestral areas that included the Old World must also include the Nearctic, in case a two-area combination was optimised at a node; the area formed by the Nearctic and South America was not allowed. For all analyses, ancestral ranges were assumed to include no more than three areas.

The justification for these settings in the constrained analysis is as follows. Because of the age of crown-group Pompilidae (Wilson *et al.*, 2012), we assumed dispersal between the Palaearctic and any other area to be improbable given the configuration of the continents, which at 43 Ma was similar to the present. Recent dispersals from or to the Palaearctic have been suggested to be through a Northern route, either through the NALB or through the BLB (Sanmartín *et al.*, 2001). The reason for not allowing some ancestral area combinations is that areas younger than 43 Ma cannot include disjunct distributions, because the areas in question have been isolated from each other since the origin of the group.

## RESULTS

### Phylogenetic and dating analyses

The best partitioning strategy for our dataset included five partitions. Overall, we recovered high posterior probabilities (PP) for all nodes (PP>0.95). Our analyses recovered the monophyly of all genera. The genus *Chelaporus* is sister to the rest of Aporini. *Allaporus* and *Psorthaspis* are sister genera, and *Drepanaporus* + *Euplaniceps* is sister to *Aporus* (Fig. 5.1).

### Divergence-time estimation

Our results suggest that *Aporini* had an origin in the Aquitanian, 22.66 Ma (CI= 17.40,28.83). All the genera had origins in the Miocene (Fig. 5.2). Mean ages of nodes and their 95% confidence intervals are shown in Table 5.3.

### Historical biogeography

The constrained DEC analysis (L2) produced higher likelihood scores than the unconstrained analysis (L1); its results are summarised in Fig. 5.2, along with the BBM results. The S-DIVA and BBM results did not recover the same areas as the DEC for all nodes. All analyses recovered an origin for *Aporini* in the Nearctic. The Nearctic was recovered as the ancestral area for *Allaporus* in all analyses. *Psorthaspis* probably originated in the Nearctic as well, as supported by DEC and BBM analyses. S-DIVA recovered a widespread ancestor for *Psorthaspis*, which lived in the Nearctic and Central America. The remaining genera have a more ambiguous ancestral area reconstruction because they are present in more areas, and form sister relationships with lineages comprising multiple areas. The ancestor of *Aporus* + (*Euplaniceps* + *Drepanaporus*) probably lived in the Nearctic, as recovered by DEC and BBM methods. Results for S-DIVA are equivocal for this node. *Aporus* probably had an origin in the Old World, as supported by DEC and BBM. S-DIVA suggests an ancestral area composed of the Nearctic and Palaearctic regions. The ancestor of *Euplaniceps* + *Drepanaporus* was probably in a widespread area comprising Central America and the Antilles, as recovered by DEC. The BBM method suggests an ancestor in the Antilles. *Aporus* ancestors were widespread in the Nearctic and Central America, according to DEC; other methods

recovered Central America, but not necessarily the Nearctic. *Euplaniceps* probably had an origin in a widespread Central–South America area (Fig. 5.2).

## DISCUSSION

### The origin of New World–Old World disjunct distributions

Both the BLB and NALB have been concordant with results from recent divergence time estimation studies, but a comprehensive study of global patterns in diversification in the Holarctic shows a greater frequency of dispersal events between Western Nearctic and Eastern Palaeartic through the BLB (Sanmartín *et al.*, 2001).

According to our analyses, the New World–Old World disjunct distributions in Aporini are the product of a single dispersal event through the BLB. The only aporine genus present in the Palaeartic region is *Aporus* (Fig. 5.2). Because our results suggest a Nearctic origin for Aporini, a dispersal event to the Palaeartic is suggested as the process underlying Old World–New World *Aporus* distribution. The results from the BBM method propose that this event took place 18–15 Ma (CI= 23.52,11.14). Results from the DEC method suggest a more recent dispersal event that occurred 15–12 Ma (CI=20.64,7.83). Taking both results into account, our analyses provide a window of 18–12 Ma (23.52,7.83) for colonization of the Palaeartic, which suggests a dispersal event through the BLB land connection, before it was interrupted in the Late Miocene. Recent studies have proposed the BLB as a dispersal route for plants (e.g. Zhu *et al.*, 2013), fungi (Jeandroz *et al.*, 2008), birds (e.g. Moore *et al.*, 2011; Xu *et al.*, 2010), reptiles (Le & McCord, 2008), amphibians (Carranza *et al.*, 2008), and mammals (Hope *et al.*, 2013). Old World–New World disjunct distributions have been studied for some insect taxa. Various earlier studies that did not include the use of molecular data suggested BLB as a

dispersal route, and subsequent isolating factor, for some Plecoptera (Hynes, 1988), Coleoptera (Liebherr & Schmidt, 2004), and Hymenoptera (Liu *et al.*, 2007). Insect molecular data have also revealed this pattern (Hundsdoerfer *et al.*, 2005; Ohshima *et al.*, 2010; Ren *et al.*, 2013; von Dohlen *et al.*, 2002). To our knowledge, our study is the first to provide evidence for use of this route by stinging wasps (Hymenoptera: Aculeata).

### **Dispersal routes between the Nearctic and South America**

Our analyses suggest two independent dispersal events to South America at similar times, involving *Euplaniceps* and *Aporus* (Fig. 5.2). Combined results from the BBM and DEC analyses show that *Aporus* dispersed to South America between 11 and 5 Ma (CI=2.85,13.65). This date is consistent with recent data suggesting the Isthmus of Panama formed earlier than 7 Ma (Montes *et al.*, 2012a,b). The dispersal of *Euplaniceps* to South America could have occurred through two different routes, according to our results. The BBM analysis suggests dispersal from the Nearctic to the Antilles. The fauna from the Antilles then dispersed back to Central America and then to South America 11 to 9 Ma (CI=15.61,6.60). The DEC method suggests dispersal to South America from Mesoamerica between 18–15 (CI=23.52,11.79) Ma. Because there are no extant relatives of *Euplaniceps* present in the Antillean area, and the BBM results include a lower probability that the ancestral node of *Euplaniceps* + *Drepanaporus* was Mesoamerican rather than Antillean, we propose that the DEC results better explain the distribution of this group. If we take into account both analyses, the time frame of the dispersal event from Mesoamerica to South America would be 18–11 Ma (23.52,6.60). These results are consistent with new data indicating the complete formation of the Isthmus of Panama at 15 Ma (Montes *et al.*, 2012a,b).

Even though recently published molecular phylogenetic studies in insects suggest dispersals at the time of the great biotic interchange (ca. 3 Ma) (Ramirez *et al.*, 2010; Husemann *et al.*, 2013), our data are consistent with accumulating studies suggesting that a number of biotic groups, including plants (Carvalho & Renner, 2012), mammals (Perini *et al.*, 2010), reptiles (Colston *et al.*, 2012), and amphibians (Pinto-Sanchez *et al.*, 2011) dispersed through this area before 7 Ma.

### **The origin of Antillean taxa**

Aporini includes four separate Antillean lineages. Two of these lineages are *Psorthaspis* clades, and all *Drepanaporus* constitutes the third. An additional a group of Antillean taxa for which we were unable to obtain molecular data, might be included in *Aporus*; however, their taxonomic placement is ambiguous, and needs to be studied further in order to be able to make biogeographic conclusions. According to the BBM results, *Drepanaporus* dispersed from the Nearctic region. As discussed earlier, we do not think these results are consistent with the current evidence, because *Euplaniceps*, which is sister to *Drepanaporus*, does not have Antillean relatives, and so we embrace the second-highest probable result for this discussion. The BBM and DEC results combined suggest a dispersal of *Drepanaporus* from Mesoamerica to the Antilles 18–7 Ma (CI=23.52,4.43). This dispersal event is too young to be a result of vicariance via the Protoantilles, which is proposed to have occurred in the Late Cretaceous (Rosen, 1975, 1985). Moreover, the dispersal through Gaarlandia is not supported by our divergence time estimation or ancestral area reconstruction. This land connection existed between South America and the Antilles in the Early Oligocene, approximately 20 Ma earlier than the dispersal event proposed for *Drepanaporus*, which in addition dispersed from

Mesoamerica. The dispersal events that produced the distribution of *Drepanaporus* were probably over-water, from a Mesoamerican ancestor. Recent molecular divergence dating analyses support the over-water dispersal hypothesis for amphibians (e.g. Hedges *et al.*, 1992), reptiles (e.g. Hedges *et al.*, 1992; Colston *et al.*, 2012), bats (Dávalos, 2007), plants (e.g. Lavin *et al.*, 2003; McDowell *et al.*, 2003), and insects (Oneal *et al.*, 2009).

The dispersal of Antillean *Psorthaspis* was probably later, and occurred twice independently. *Psorthaspis elegans* could have dispersed from the Nearctic (BBM method) or Mesoamerica (BBM and DEC methods). Because of its sister relationship to the Mesoamerican *P. variegata*, we conclude that the dispersal was probably from Mesoamerica. The dispersal date obtained for this event is 5–2 Ma (CI=6.63,0.44). The DEC and BBM methods suggest that the same route was taken by *P. hispaniolae*. This dispersal event probably occurred between 7 and 1 Ma (CI=11.02,0.22). Similar to the situation with *P. elegans*, the sister relationship of *P. hispaniolae* to Mesoamerican *P. laevifrons* supports the idea of a dispersal from Mesoamerica. This dispersal pattern had been suggested by earlier studies that proposed a land bridge between Yucatan and western Cuba ca. 49 Ma (Pindell, 1994). This connection, however, is too old to explain the dispersal of Antillean *Psorthaspis* spp. The dispersal of these species occurred mostly in the Pliocene, but could have occurred in the Pleistocene. There is evidence for Pleistocene dispersal for plants from the Nearctic or Mesoamerican region when water levels were low (Gugger & Cavender-Bares, 2011). Ants might have also dispersed via this route in the Pleistocene (Seal *et al.*, 2011). Because there were no land connections to the Antilles from the Nearctic and Mesoamerican regions, *Psorthaspis* dispersal probably

occurred over-water. To our knowledge, there are no other published accounts suggesting biotic dispersal to the Antilles from Mesoamerica in the Pliocene.

## CONCLUSIONS

This is the first study to address biogeographical processes that produced the current distributions of Pompilidae lineages encompassing Old World–New World and Nearctic–South American–Antillean disjunct distributions. In general, our results fit previously suggested hypotheses for the processes underlying these distributions. Dispersal, and subsequent vicariance, over a BLB land bridge in the early Miocene best fits our results for the Old World–New World distribution. The Nearctic–South America distribution is best explained by dispersal through the Isthmus of Panama, and supports recent data that suggest an older age for the formation of this land bridge than the previously accepted late-Miocene–Pliocene age. Over-water dispersal from Mesoamerica appears to explain the diversification of Antillean Aporini. This last route has not been widely reported in the literature.

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Table 5.1. Species studied, distribution, molecular markers amplified, and GenBank accession numbers.

Species	Voucher ID	Locality	28S Accession #	EF1 Accession #	LWRh Accession #	Wg Accession #
<b>Outgroup Taxa</b>						
<i>Anoplius</i> ( <i>Lophopompilus</i> ) <i>aethiops</i>	PO8	USA: AR, Little Rock				
<i>Allochaeres azureus</i>	PO387	USA: AZ 14				
<i>Dipogon</i> ( <i>Deuteraenia</i> ) sp.	PO348	HUNGARY: Borzony Mts, 70 km N Budapest				
<i>Cryptocheillus</i> <i>idoneum</i> <i>birkmanni</i>	PO62	USA: AZ, Coconino co., N side Mormon Lake				
<i>Agenioideus</i> sp.	PO356	Madagascar 19				
<b>Ingroup Taxa</b>						
<i>Aporus</i> ( <i>Aporus</i> ) <i>idris</i>	PO192	MEXICO: Jalisco, Careyes				
<i>Aporus</i> ( <i>Aporus</i> ) <i>idris</i>	PO449	MEXICO: Sonora, 30 km NE Alamos				
<i>Aporus</i> ( <i>Aporus</i> ) <i>concolor</i>	PO435	USA: CA, Luyo Co, White Mts., Wyman Canyon				
<i>Aporus</i> ( <i>Aporus</i> ) <i>luxus</i>	PO6	USA: UT, Cache Co., Blacksmith Fork Canyon				
<i>Aporus</i> ( <i>Neoplaniceps</i> ) <i>umbratilis</i>	PO459	PERU: Cajamarca				
<i>Aporus</i> ( <i>Neoplaniceps</i> ) <i>umbratilis</i>	PO480	PERU: Cajamarca				
<i>Aporus</i> ( <i>Aporus</i> ) <i>planiceps</i>	PO473	ITALY: Liguria, Ortovero				
<i>Aporus</i> ( <i>Aporus</i> ) <i>unicolor</i>	PO434	ITALY: Fauglia, Pisa				
<i>Aporus</i> ( <i>Aporus</i> ) <i>bicolor</i>	PO311	SPAIN: Taradell				
<i>Aporus</i> ( <i>Aporus</i> ) sp.	PO310	SPAIN: Barcelona, Vidra				
<i>Aporus</i> ( <i>Aporus</i> ) sp.	PO333	ISRAEL: Negev				
<i>Drepanaporus</i> <i>collaris</i>	PO467	DOMINICAN REPUBLIC: Pedernales, Sierra de Bahoruco				
<i>Drepanaporus</i> <i>collaris</i>	PO688	DOMINICAN REPUBLIC: La Vega, Cordillera Central				
<i>Drepanaporus</i> <i>collaris</i>	PO468	DOMINICAN REPUBLIC: Independencia, Sierra de				

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		Bahoruco
<i>Drepanaporus antillarum</i>	PO687	DOMINICAN REPUBLIC: Independencia, Sierra de Bahoruco
<i>Drepanaporus bachata</i>	PO466	DOMINICAN REPUBLIC: Pedernales, Sierra de Bahoruco
<i>Euplaniceps saussurei</i>	PO162	CHILE: 13 F. PARKER
<i>Euplaniceps saussurei</i>	PO145	CHILE: 15 F. PARKER
<i>Euplaniceps</i> sp.	PO477	BOLIVIA: Santa Cruz
<i>Euplaniceps evansi</i>	PO290	ARGENTINA: Catamarca
<i>Euplaniceps ornatula</i>	PO460	BRAZIL: Minas Gerais
<i>Euplaniceps</i> sp.	PO261	PERU: Rio Tambopata, Explorer's Inn, Rio Tower
<i>Euplaniceps</i> sp.	PO484	NICARAGUA: Refugia, San Juan
<i>Psorthaspis connexa</i>	PO64	COSTA RICA: San Juan, San Isidro General
<i>Psorthaspis legata</i>	PO33	USA: AL, Escambia Co.
<i>Psorthaspis sanguinea</i>	PO455	USA: AZ, North Little Rock Camp Robinson
<i>Psorthaspis mariae</i>	PO197	USA: FL, Alachua Co., Gainesville
<i>Psorthaspis variegata</i>	PO72	COSTA RICA: Guanacaste, Finca Montezuma, 3 km SE Rio Naranjo
<i>Psorthaspis elegans</i>	PO830	CUBA: Guantanamo Bay
<i>Psorthaspis elegans</i>	PO823	CUBA: Guantanamo Bay
<i>Psorthaspis planata</i>	PO366	USA: CA, San Bernardino Co., 5 mi S Barstow
<i>Psorthaspis portiae</i>	PO456	USA: AZ, Cochise Co.
<i>Psorthaspis formosa</i>	PO457	MEXICO: Sonora
<i>Psorthaspis formosa</i>	PO60	COSTA RICA: Guanacaste, Finca Montezuma
<i>Psorthaspis hispaniolae</i>	PO474	DOMINICAN REPUBLIC: Barahona, Eastern Sierra Bahoruco
<i>Psorthaspis hispaniolae</i>	PO465	DOMINICAN REPUBLIC: Independencia, Sierra de Bahoruco

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<i>Psorthaspis laevifrons</i>	PO56	COSTA RICA: Alajuela, Bijagua, 20 km S Upala
<i>Psorthaspis magna</i>	PO9	USA: AR, Little Rock
<i>Psorthaspis brimleyi</i>	PO195	USA: AR, Little Rock
<i>Allaporus pulchellus</i>	PO432	MEXICO: Sonora, Rancho San Bernardino
<i>Allaporus aurulentus</i>	PO433	MEXICO: Sonora, Rancho San Bernardino
<i>Allaporus rufiventris</i>	PO464	MEXICO: Sonora
<i>Chelaporus anomalus</i>	PO931	USA: TX, Val Verde Co.

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Table 5.2. Primers used for PCR amplification and sequencing

Marker	Primer name	Primer sequence (5'–3')	Reference
EF1	F2for1	GGT TCC TTC AAA TAT GCT TGG G	Pilgrim <i>et al.</i> , 2008
	F2rev1	A ATC AGC AGC ACC TTT AGG TGG	Danforth & Ji, 1998
LWRh	PompOps1F	ATT CGA CAG ATA CAA CGT AAT CG	Pilgrim <i>et al.</i> , 2008
	LwRhR	ATA TGG AGT CCA NGC CAT RAA CCA	Mardulyn & Cameron, 1999
	LwRhRApor	GAG RGA GAT CGT CAT CAA GGC GAC C	This study
Wg	LepWg1for	GAR TGY AAR TGY CAY GGY ATG TCT GG	Brower & DeSalle, 1998
	modLepWg2rev	ACT ICG CRC ACC ART GGA ATG TRC A	Brower & DeSalle, 1998
28S	CF2	TGG TAA CTC CAT CTA AGG CTA AAT A	Pilgrim <i>et al.</i> , 2008
	D5–4625 R	CCC ACA GCG CCA GTT CTG	Schulmeister, 2003
	(D5R)	CTT ACC	

Table 5.3. Nodes recovered by BEAST analysis, their posterior probabilities, their ages, and 95% confidence intervals (highest posterior density (HPD)).

Node	PP	Age	Height 95 HPD range	Taxonomic group
A	1.00	22.66	17.40,28.83	Aporini
B	0.95	20.91	15.89,26.58	
C	1.00	18.22	13.81,23.52	
D	0.99	15.57	11.14,20.64	<i>Aporus</i>
E	1.00	11.19	7.11,15.47	
F	1.00	0.66	0.075,1.80	
G	0.95	9.64	5.82,13.65	
H	1.00	1.30	0.29,3.04	
I	1.00	5.35	2.85,8.58	
J	1.00	0.31	0.010,1.01	
K	0.99	12.31	7.83,17.57	
L	1.00	7.21	4.43,10.85	
M	1.00	2.53	0.75,5.29	
N	0.99	4.66	2.14,8.02	
O	1.00	15.81	11.79,20.74	
P	1.00	7.61	4.43,11.34	<i>Drepanaporus</i>
Q	1.00	2.601	1.03,4.85	
R	1.00	1.72	0.59,3.36	
S	1.00	4.86	2.43,8.00	
T	1.00	11.49	8.17,15.61	<i>Euplaniceps</i>
U	0.99	9.55	6.60,13.06	
V	1.00	6.13	3.12,9.35	
W	1.00	0.43	0.053,1.15	

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X	0.67	8.75	5.92,12.12	
Y	1.00	2.89	1.37,4.92	
Z	0.98	18.14	13.28,23.71	
AA	1.00	12.97	8.76,18.02	<i>Psorthaspis</i>
BB	1.00	6.36	4.17,9.18	
CC	0.97	4.68	2.34,7.43	
DD	1.00	0.82	0.065,2.29	
EE	0.97	5.28	3.33,7.65	
FF	1.00	3.40	1.81,5.38	
GG	0.64	4.37	2.51,6.63	
HH	1.00	1.40	0.44,2.79	
II	1.00	3.40	1.81,5.38	
JJ	0.99	1.75	0.75,3.09	
KK	1.00	8.86	5.92,12.55	
LL	0.92	7.21	3.97,11.02	
MM	1.00	3.01	$5.46 \times 10^4$ ,0.0028	
NN	1.00	0.91	0.22,2.06	
OO	1.00	1.18	0.29,2.66	
PP	1.00	9.19	5.03,14.63	<i>Allaporus</i>
QQ	0.99	1.28	0.023,4.06	

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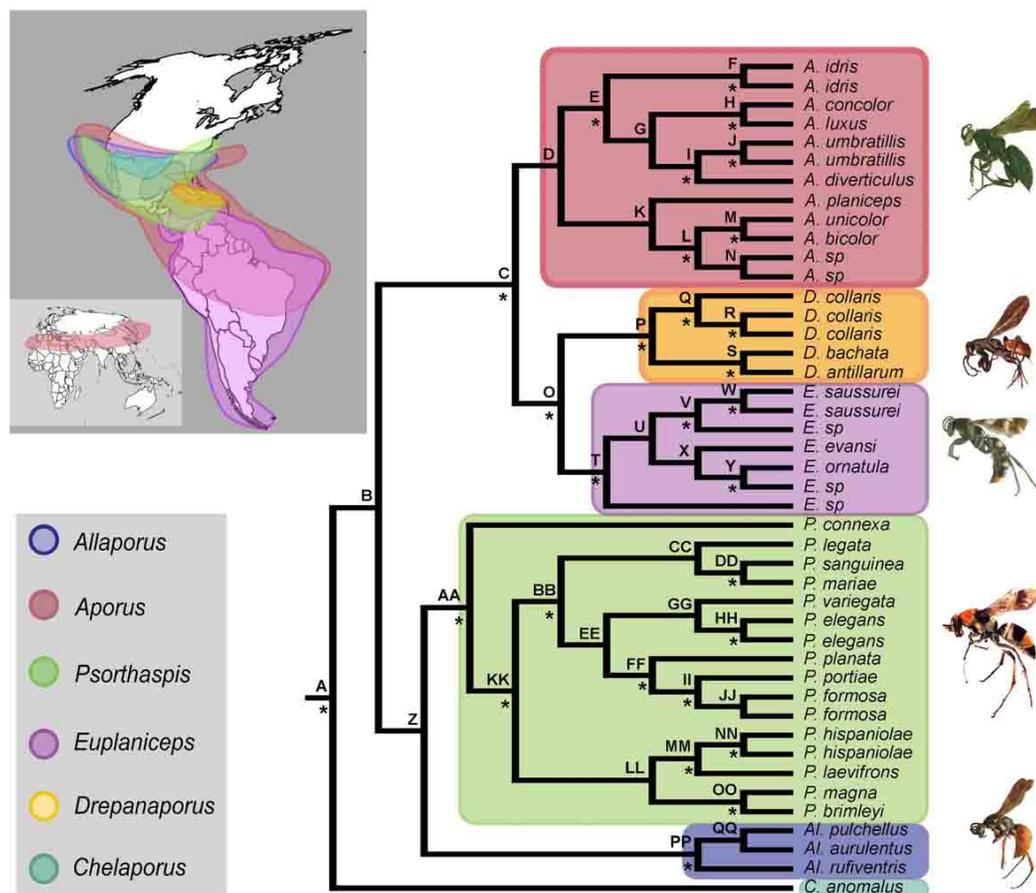


Figure 5.1. Consensus phylogenetic reconstruction for Aporini resulting from two Bayesian MCMC runs performed in BEAST. The bottom left box represents the genera sampled, and their colour-coded distribution. Distribution of each genera is shown in the map (Aitoff projection). Numbers refer to nodes in Table 5.3. All nodes were supported at  $PP \geq 0.95$ ; nodes with  $PP=1.0$  are indicated with asterisks.

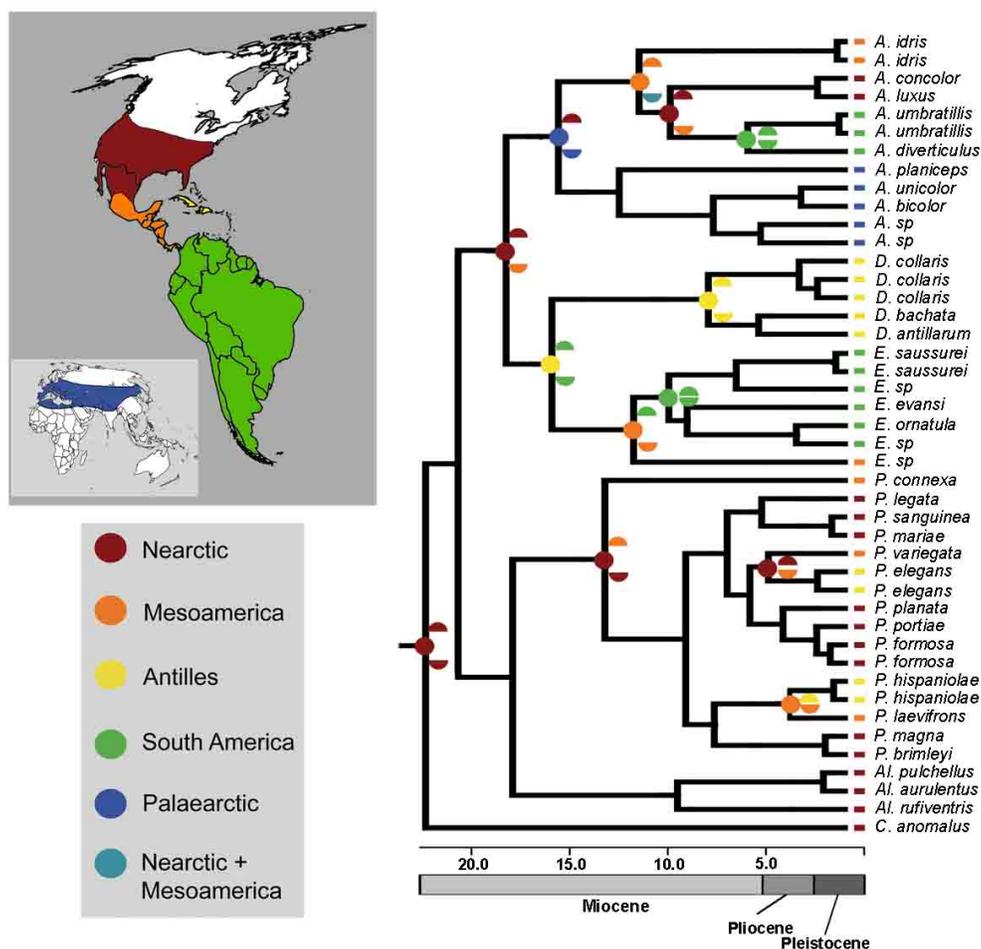


Figure 5.2. Reconstruction of the historical biogeography of Aporini using a dispersal–extinction–cladogenesis (DEC) method, and a Bayesian Binary MCMC (BBM) method. The bottom left box represents the 5 areas assigned in the palaeogeographical model shown in the map (Aitoff projection). For each node, a coloured circle corresponds to the area with highest probability resulting from the BBM analysis, and two coloured semicircles correspond to the daughter areas with highest probability inferred by the DEC method. The time-scale is in millions of years, spanning epochs since 22 Ma.

## CHAPTER 6

MÜLLERIAN MIMICRY AS A RESULT OF CODIVERGENCE BETWEEN VELVET  
ANTS AND SPIDER WASPS<sup>5</sup>

## ABSTRACT

Recent studies have delineated the largest Nearctic Müllerian mimicry complex in *Dasymutilla* velvet ants. *Psorthaspis* spider wasps live in areas where this mimicry complex is found and have similar morphology to *Dasymutilla*. We tested the idea that *Psorthaspis* spider wasps are participating in the *Dasymutilla* mimicry complex and that they codiverged with *Dasymutilla* to do so. We performed morphometric analyses and human perception tests, and tabulated distributional records to determine the fit of *Psorthaspis* to the *Dasymutilla* mimicry complex. We inferred a dated phylogeny using nuclear molecular markers (28S, EF1, opsin and wg) for *Psorthaspis* species and compared it to a dated phylogeny of *Dasymutilla*. We tested for codivergence using two statistical analyses; we further compared divergence dates in the two phylogenies. Our results show that *Psorthaspis* spider wasps are morphologically similar to the *Dasymutilla* mimicry rings. In addition, our tests indicate that *Psorthaspis* and *Dasymutilla* codiverged, and coloration patterns were likely produced through advergent evolution. The origin of mimicry in *Dasymutilla* is estimated to be ca. 5 Ma earlier than that of *Psorthaspis*. This study expands the breadth of the *Dasymutilla* Müllerian mimicry complex and provides insights about how codivergence influenced the evolution

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<sup>5</sup> This manuscript is formatted for submission to *Evolution*. The authors of the journal article are: Juanita Rodriguez, James P. Pitts, Carol D. von Dohlen, and Joseph S. Wilson.

of mimicry in these groups.

## INTRODUCTION

Müllerian mimicry refers to the phenomenon in which sympatric, harmful species share a similar warning signal for mutual benefit (Muller 1879; Benson 1972). This kind of mimicry has been well documented for several tropical groups, such as *Heliconius* butterflies (Benson 1972; Sheppard et al. 1985; Nijhout 1994; Flanagan et al. 2004; Jones et al. 2013) and poisonous Dendrobatidae and Mantellidae frogs (Symula et al. 2001; Toledo and Haddad 2009; Chouteau et al. 2011). Recently, a large Nearctic Müllerian mimicry complex was described in diurnally foraging *Dasymutilla* velvet ants (Hymenoptera: Mutillidae) (Wilson et al. 2012). These aposematic solitary wasps have wingless females that inflict a painful sting, which likely evolved as a defense against predators (Wilson et al. 2012). Although several Batesian mimics of velvet ants have been reported (Edwards 1984; Nentwig 1985; Acorn 1988; Mawdsley 1994; Lanteri and Del Rio 2005), the possibility that other harmful species might be Müllerian mimics of velvet ants has not been investigated.

Various spider wasps in the genus *Psorthaspis* (Pompilidae) closely resemble velvet ant color patterns (Evans 1968), and thus might be participating in the velvet ant mimicry complex. Furthermore, because spider wasps are defended with a sting that invokes some of the most intense, instantaneous pain among stinging insects (Schmidt 2004), these wasps could be Müllerian mimics of velvet ants. However, the resemblance of *Psorthaspis* spider wasps to velvet ants, and the potential fit of these spider wasps to the velvet ant mimicry complex have never been quantified.

In the well-studied *Heliconius* Müllerian mimicry systems, codivergence, or the

parallel divergence of ecologically associated, but unrelated, lineages, has been a major contributor to the development of numerous mimicry rings (Cuthill and Charleston 2012). Codivergence has been proposed as some of the strongest evidence for coevolution (Futuyma and Slatkin 1983; Gilbert 1983; Page 2003; Cuthill and Charleston 2012). Codivergence patterns alone, however, are not enough to demonstrate coevolution in the strict sense (i.e., evolution that occurs in populations of at least two species as the result of reciprocal selective influence) because selective pressures are often not measured between the two groups (Cuthill and Charleston 2012).

As seen in *Heliconius* butterflies, codivergence can sometimes be associated with convergent evolution, with both groups converging on a single phenotype (Cuthill and Charleston 2012). Such phenotypic convergence can occur uniformly between species when traits in each species evolve as a response to traits in the other species, resulting in intermediate convergent phenotypes (Futuyma and Slatkin 1983; Wright 2011).

Alternatively, phenotypic convergence can take place in a more linear, unidirectional fashion (often referred to as advergent evolution) when selective pressures cause the convergence of one species on another, but not vice versa (Brower and Brower 1972; Johnson et al. 2003; Wright 2011). Although codivergence and the associated phenotypic convergence has been tested in some mimicry systems, investigations into the evolution of mimetic patterns in other systems, such as spider wasps and velvet ants, have the potential to better illuminate the role of coevolution in the development of large Müllerian mimicry complexes.

Here, we investigate the phenotypic and phylogenetic similarities of *Dasymutilla* velvet ants and *Psorthaspis* spider wasps to address the following questions. 1) How well

do *Psorthaspis* spider wasps fit in the described velvet ant mimicry rings? 2) Are the color pattern similarities between these wasp groups a result of codivergence—either through reciprocal change in both groups or through advergent evolution?

## METHODS

### Study system

Velvet ants and spider wasps are both classified as stinging wasps (Aculeata: Hymenoptera), and are both solitary parasitoids. Insect parasitoids are a special case of parasitic organisms because they ultimately kill their hosts during development (Tschopp et al. 2013). Velvet ants are usually external parasitoids on the larvae or pupae of bees and solitary wasps. Their females are wingless, while males are typically winged and capable of flight (Williams 2012). Spider wasps (Pompilidae) are parasitoids of spiders. Both males and females are winged. *Psorthaspis* spider wasps use trapdoor spiders of the family Ctenizidae as hosts (Jenks 1938). Even though the venom is primarily used to paralyze the host, the sting of both spider wasps and velvet ants could also be a deterrent to predation (Schmidt 2004; Wilson et al. 2012).

### Morphometric analysis of color patterns

We quantified the color patterns of seven *Psorthaspis* species using digital images following the procedure described by Wilson et al. (2012), with the exception of setal characters, as they are not comparable between velvet ants and spider wasps. Characters included the percent black of the metasoma, integument color, and non-black metasomal color measured in red, green and blue. All area and percentage measurements were made using the program ImageJ (<http://rsb.info.nih.gov/ij/>). Morphological characters were

analyzed together with the data from Wilson et al. (2012) using resemblance matrices, nonmetric multidimensional scaling (NMDS) based on a Bray-Curtis distance matrix, and permutational multivariate analysis of variance (PERMANOVA: Anderson 2005) in R (R development core team 2010) using the *adonis* function in the *vegan* package. The data gathered for this analysis are deposited in Dryad.

#### Human perception of mimetic fidelity

Mimetic fidelity in Müllerian mimicry systems represents how well a given species matches a group of species (i.e., the mimicry ring). To measure mimetic fidelity of spider wasps involved in described Müllerian mimicry rings (Wilson et al. 2012), we used methods outlined by Wilson et al. (2013). We presented slides showing an individual *Psorthaspis* species compared to all members of the velvet ant mimicry ring to which the species was most similar. Volunteers (N = 35) were directed to rank each *Psorthaspis* species on how well it fit into the associated mimicry ring. Rankings were based on a scale of 1 (very poor mimic) to 10 (excellent mimic). All images were presented at magnifications such that all wasps had the same projected body length. The mimetic fidelity of each spider wasp was estimated based on the mean score of a wasp compared to its assigned mimicry ring.

All volunteers participating in this study were students in lower division Biology courses at Utah State University–Tooele. Students were presented with a short presentation introducing the concepts of Batesian and Müllerian mimicry and were then given the option to participate in a survey designed to rank mimetic fidelity of wasps. If students agreed to participate, they were given a link to the website containing the survey. To our knowledge, the volunteers were not experts in insect identification. This

effectively resulted in mimetic fidelity scores that were based on overall resemblance of a mimic to a mimicry ring rather than on preconceived ideas of what specific parts of a mimic should match the ring. All participants were over the age of 18, and no data relating to the volunteers were gathered. No approval from the university was requested for this research because no information about living individuals was collected (i.e., the research did not involve human subjects as per the Code of Federal Regulations 45 CFR part 46). Because of the need to protect the anonymity of our volunteers, no questions were asked regarding any physical characteristics that would affect ranking mimics and models (e.g., colorblindness). While this potentially could influence the reported mimetic fidelity scores, we think any influence of colorblindness would be minimal, due to the nature of aposematic signals in spider wasps and velvet ants. These warning signals primarily result from contrasting black and red or yellow patterns, which would still be visually distinct to colorblind individuals.

#### Estimation of geographical distribution

To determine the distribution of each of the *Psorthaspis* color patterns identified in this study we geo-referenced 1,032 *Psorthaspis* specimens from 13 natural history collections and downloaded data on geo-referenced *Psorthaspis* specimens in the Southwest Collections of Arthropods Network (SCAN 2013). We manually plotted the collection localities of each species on a map using the software Google Earth 5.0 (<http://earth.google.com>) and estimated geographic distributions by drawing a line encompassing all of the collection localities. These estimated distributions were visually compared to the distributions of velvet ant mimicry rings published by Wilson et al. (2012). The datapoints used for this analysis are deposited in Dryad.

## Molecular data and phylogenetic inference

We compiled a data set of four genes (28S, elongation factor 1-alpha, wingless, and long-wavelength rhodopsin) for the 13 *Psorthaspis* species and one outgroup (*Aporus idris*), which were obtained previously by Rodriguez et al. (see Chapter 5). Sequences were aligned using Geneious Alignment (Geneious 5.4: Drummond et al. 2011) and manually refined. The model of molecular evolution used for each gene and by codon position was the same used by Rodriguez et al. (see Chapter 5) except for introns from long-wavelength rhodopsin, for which the model was determined in MrModelTest (Nylander 2004). Single-gene phylogenies were estimated through a Bayesian framework implemented in MrBayes 3.2 (Huelsenbeck and Ronquist 2001) to check for potential conflict between gene trees. Single-gene matrices were then concatenated using Geneious 5.4 to produce a combined matrix, using the best partition scheme used by Rodriguez et al. (see Chapter 5), and an additional partition including long-wavelength rhodopsin introns with the model GTR+I+G. MCMC chains were run for 10,000,000 generations, with sampling every 1,000 generations. Effective sample size (ESS), burn-in, and graphical examination of chain convergence were examined in Tracer 1.5 (Rambaut et al. 2013).

A chronogram of *Psorthaspis* was inferred from the combined matrix in a Bayesian framework using BEAST 1.7.5 (Drummond et al. 2012) under an uncorrelated lognormal relaxed-clock model (Drummond et al. 2006; Drummond and Rambaut 2007). Substitution models were unlinked among partitions; the underlying clock and trees were linked. The crown-group node of all *Psorthaspis* was assigned a normal prior of mean = 12.9 Ma (SD = 10), based on results of Rodriguez et al. (see Chapter 5). Two separate

Markov Chain Monte Carlo (MCMC) searches were performed for 10,000,000 generations. Effective sample size (ESS) and graphical chain convergence were examined in Tracer 1.5. Independent runs were assembled with LogCombiner 1.7.5. and 10% of the generations was discarded as burn-in. Divergence time estimations of *Dasymutilla* were obtained from Williams (2012).

#### Codivergence test

To determine if there was codivergence between *Dasymutilla* and *Psorthaspis* mimicry rings we performed two permutation analyses in R using the phylogenetic trees of both groups. First, an analysis that calculates the Pearson's correlation coefficient (Hommola et al. 2009) was implemented using the correlation between the distances of the two phylogenies. Second, we applied an analysis that calculates the ParaFitGlobal statistic (Legendre et al. 2002), which uses transformed distances derived from the phylogenetic trees into matrices of principal coordinates. Both analyses test the null hypothesis that the two groups are evolving independently. We performed 100,000 simulations for both tests. Additionally, we constructed a tanglegram linking phenotypically similar species between the phylogenies of *Dasymutilla* and *Psorthaspis*.

## RESULTS

### Morphological results

The NMDS and PERMANOVA analyses indicate that *Psorthaspis* spider wasps are morphologically similar to the *Dasymutilla* mimicry rings to which they were assigned *a priori* (Figs. 6.1 and 6.2). The overall effect of the mimicry ring as a categorical variable was  $F = 22.503$ ,  $R^2 = 0.616$ ,  $P < 0.001$ . Despite the overall similarity,

the plot of the NMDS shows that *Psorthaspis* often do not fit tightly with *Dasymutilla* in morphospace, but rather fall out near the periphery of the velvet ant clusters. The sole exception was the Eastern mimicry ring, which fell within the middle of the velvet ant distribution (Fig. 6.2).

Mimetic fidelity reported by volunteers was more variable for spider wasps (Table 6.1) than for velvet ants (Wilson et al. 2013). Although some spider wasps received mimetic fidelity scores comparable to the velvet ants (e.g., the Tropical, Madrean and Eastern mimicry rings), others received much lower scores (e.g., the Western and Texan mimicry rings).

#### Geographical overlap between *Psorthaspis* and *Dasymutilla* mimicry rings

Distributions of *Psorthaspis* spider wasp and *Dasymutilla* velvet ant species putatively involved in the same mimicry rings are largely congruent (Fig. 6.1). In general, *Dasymutilla* mimicry rings have a more widespread distribution than that of spider wasps, particularly in northern latitudes. Distributions of *Psorthaspis* mimicry rings show much greater overlap with each other than do those of *Dasymutilla* velvet ants (Fig. 6.1). This is particularly apparent in the distribution of the *Psorthaspis* Madrean mimicry ring, which is geographically larger than the Madrean ring in *Dasymutilla*. Similarly, the Western *Psorthaspis* ring extends farther south than the Western *Dasymutilla* ring, resulting in a larger overlap between *Psorthaspis* Western and Madrean rings. In addition, the Texan *Psorthaspis* ring seems to be more restricted than its *Dasymutilla* counterpart (Fig. 6.1).

## Phylogenetic relationships, divergence times and codivergence results

The phylogeny of *Psorthaspis* suggests that mimetic species do not compose a monophyletic group. Divergence time estimates suggest that the common ancestor of extant *Psorthaspis* species arose ca. 12.9 Ma (CI = 8.76,18.02). Because taxa composing the sister group to *Psorthaspis* (i. e. species of *Allaporus*) are non-mimics (see Chapter 5), it is probable that mimicry arose in *Psorthaspis* after it diverged from its sister group ca. 18.14 Ma (CI = 13.28,23.71). The origin of *Dasymutilla* was ca. 21 Ma (CI = 18,23), and the divergence from its sister group was 23 Ma (CI = 21,27) (Williams 2012); therefore, the origin of mimicry in *Dasymutilla* was 23 Ma or later. The codivergence tests suggest topological concordance between the phylogenies of *Psorthaspis* and *Dasymutilla* (Pearson's  $p = 0.0027$ , ParaFitGlobal  $p = 0.047$ ). The tanglegram of *Psorthaspis* and *Dasymutilla*, although somewhat complicated by the random order of mimetic color patterns in *Dasymutilla* (Wilson et al. 2012), reveals some similar patterns (Fig. 6.3). For example, the Tropical mimicry ring originates early in both phylogenies, and the Eastern mimicry ring is more phylogenetically conserved in both groups (Fig. 6.3).

## DISCUSSION

### Fit of *Psorthaspis* to the velvet ant mimicry rings

Results of the morphometric analyses and human perception tests indicate that *Psorthaspis* spider wasps likely participate in the *Dasymutilla* velvet ant mimicry complex, albeit with a lower mimetic fidelity than the velvet ant participants. This lower fidelity of the spider wasps is not surprising, given the many morphological differences between the two groups (e.g., wings, setae, etc.). The lower mimetic fidelity might also

be explained by the broad geographic overlap in some *Psorthaspis* mimicry rings. Such overlap between adjacent mimicry rings is correlated with lower mimetic fidelity in velvet ants (Wilson et al. 2013), and likely accounts for lower mimetic fidelity in spider wasps as well.

#### Evidence for coevolution

While not tested directly in this study, our results suggest that coevolution played a role in the development of the large velvet ant and spider wasp mimicry complex. Several lines of evidence support this assertion. First, while it is not immediately evident from the topologies of the *Dasymutilla* and *Psorthaspis* phylogenies (Fig. 6.3), statistical tests show evidence of codivergence between the two wasp families. This suggests that the evolution of mimicry between these wasp groups must have involved convergence at the genetic and phenotypic level, such as has been found for Neotropical butterflies (Hines et al. 2011; Reed et al. 2011).

Furthermore, our results indicate that *Psorthaspis* spider wasps and *Dasymutilla* velvet ants are phenotypically similar, suggesting that either convergent evolution or advergence has taken place. Convergent evolution in Müllerian mimicry complexes produces a shared, intermediate phenotype. Advergent evolution produces phenotypic convergence without reciprocal change, therefore producing a more relaxed pattern (Brower and Brower 1972; Johnson et al. 2003). Distinguishing between convergent and advergent evolution can be difficult, however. Advergence is considered more likely when one species' aposematic signal is established before the arrival of a second species (Gilbert 1983; Chouteau et al. 2011).

Molecular dating estimates suggest that *Dasymutilla* likely evolved approximately

5 Ma earlier than *Psorthaspis*, although there is some overlap in the CI estimates of the two groups. This would suggest that the similar color patterns between *Psorthaspis* spider wasps and *Dasymutilla* velvet ants likely is the result of codivergence through advergent evolution, with the spider wasps converging onto the color patterns of the velvet ants (Fig. 6.1). When Müllerian mimicry evolves via advergence, we expect to find evidence that some members of mimicry rings are models, while others are mimics that adverage to them, similar to the evolution of Batesian mimics (Mallet 1999; Hines et al. 2011). This might explain the low fit, as measured both from morphometrics and mimetic fidelity tests, of *Psorthaspis* mimics compared to *Dasymutilla* models. Interestingly, the low fidelity of spider wasps is not equal across all mimicry rings. For example, *Psorthaspis* participating in the Tropical mimicry ring received higher fidelity scores than many of the mimicry rings in higher latitudes (Table 6.1). This supports the hypothesis that tropical mimics converge on precise mimicry, whereas temperate mimics seem to converge on an “impressionistic” or more relaxed pattern (Merrill and Jiggins 2009).

Coevolution involves reciprocal selective pressures between two groups. While not tested directly, reciprocal selective pressures between *Psorthaspis* spider wasps and *Dasymutilla* velvet ants may indeed be taking place. While *Dasymutilla* velvet ants likely evolved aposematic coloration before *Psorthaspis* spider wasps, once spider wasps converged phenotypically through advergent processes, the aposematic signal of velvet ants would be strengthened because of the presence of harmful, aposematic co-mimics (spider wasps). Likewise, the spider wasp aposematic coloration would also be strengthened through the presence of their harmful aposematic co-mimics (velvet ants). Thus, both groups would be imposing coevolutionary selective pressures on each other,

strengthening the aposematic signal of the mimicry complex as a whole.

#### SUMMARY

We provide evidence that *Psorthaspis* spider wasps participate in velvet ant mimicry rings. Furthermore, we find evidence that the two groups codiverged through adverbent evolution. This study expands the breadth of the largest known North American Müllerian mimicry complex to include spider wasps as well as velvet ants. This large mimicry complex is an intriguing system that should be the focus of further investigations into the evolution of predator avoidance strategies in the temperate regions, the evolution of aposematic coloration, and the evolution of Müllerian mimicry involving unrelated taxa.

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Table 6.1. Human perception tests of mimetic fidelity of *Psorthaspis* species reported by volunteers (N = 35). Average mimetic fidelity of each spider wasp species indicates how well each species matches the velvet ant mimicry ring it was phenotypically and geographically most similar to. Scores are based on a scale of 1 (very poor mimic) to 10 (excellent mimic).

Spider wasp species	Average mimetic		
	fidelity score	SD	Assigned mimicry ring
<i>P. formosa</i>	4.60	2.19	Madrean
<i>P. texana</i>	4.71	3.18	Texan
<i>P. connexa</i>	8.74	1.52	Tropical
<i>P. variegata</i>	6.29	2.53	Tropical
<i>P. legata</i>	8.83	1.69	Eastern
<i>P. mariae</i>	6.74	2.17	Eastern
<i>P. sanguinea</i>	6.63	2.17	Eastern
<i>P. portiae</i>	5.26	2.13	Western
<i>P. nigriceps</i>	5.89	1.91	Western

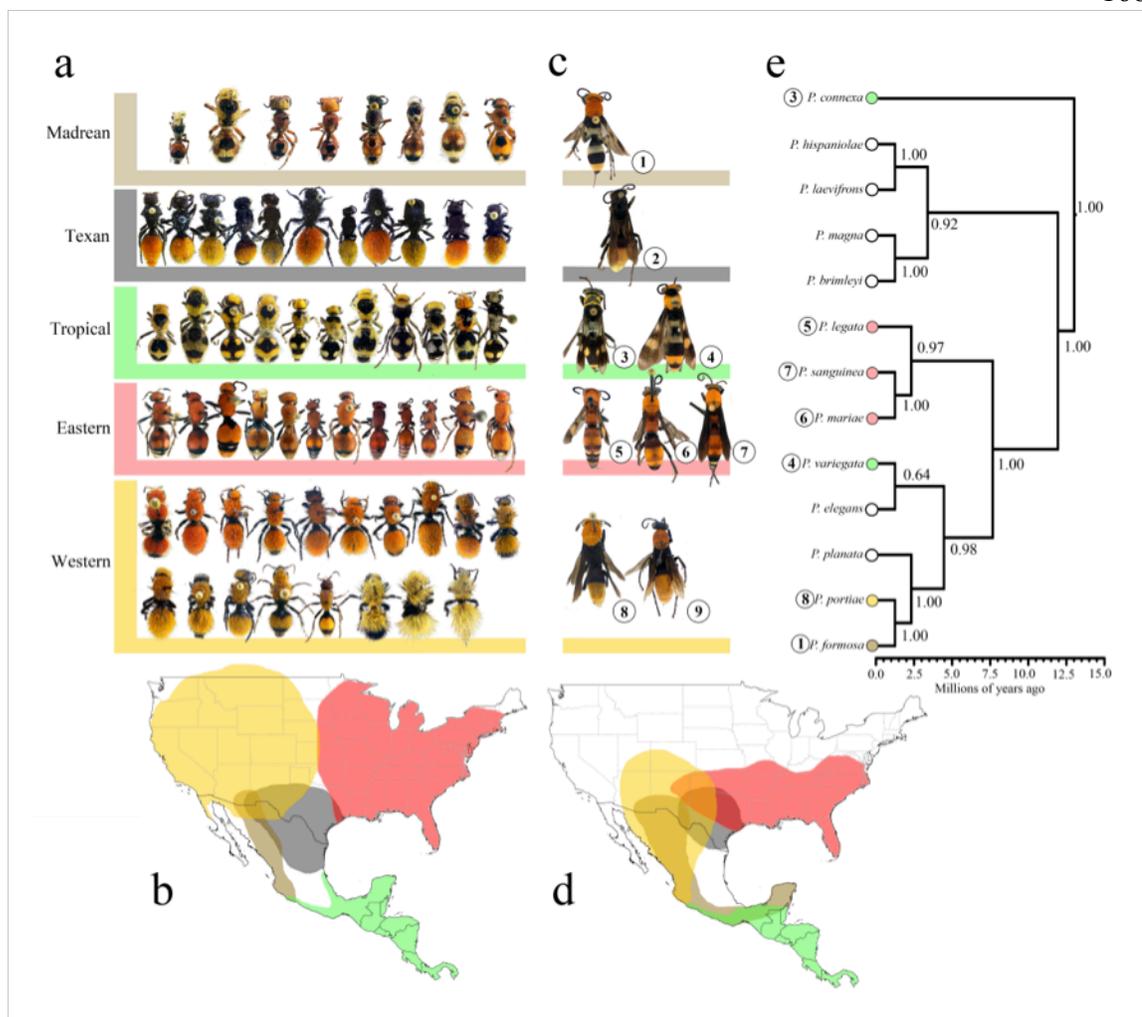


Figure 6.1. (a) Five velvet ant mimicy rings described by Wilson et al. (2012). (b) Geographic distribution of the five velvet ant mimicy rings. (c) Nine *Psorthaspis* species placed into five velvet ant mimicy rings. Numbers under each *Psorthaspis* species correspond to their positions on the phylogenetic tree and in Fig. 2. Species number 2 [*Psorthaspis texana*] did not yield usable DNA samples and was therefore not included in the phylogenetic analysis. (d) Geographic distributions of the *Psorthaspis* spider wasp mimicy rings. (e) *Psorthaspis* spider wasp chronogram. Bayesian posterior probabilities are displayed on nodes.

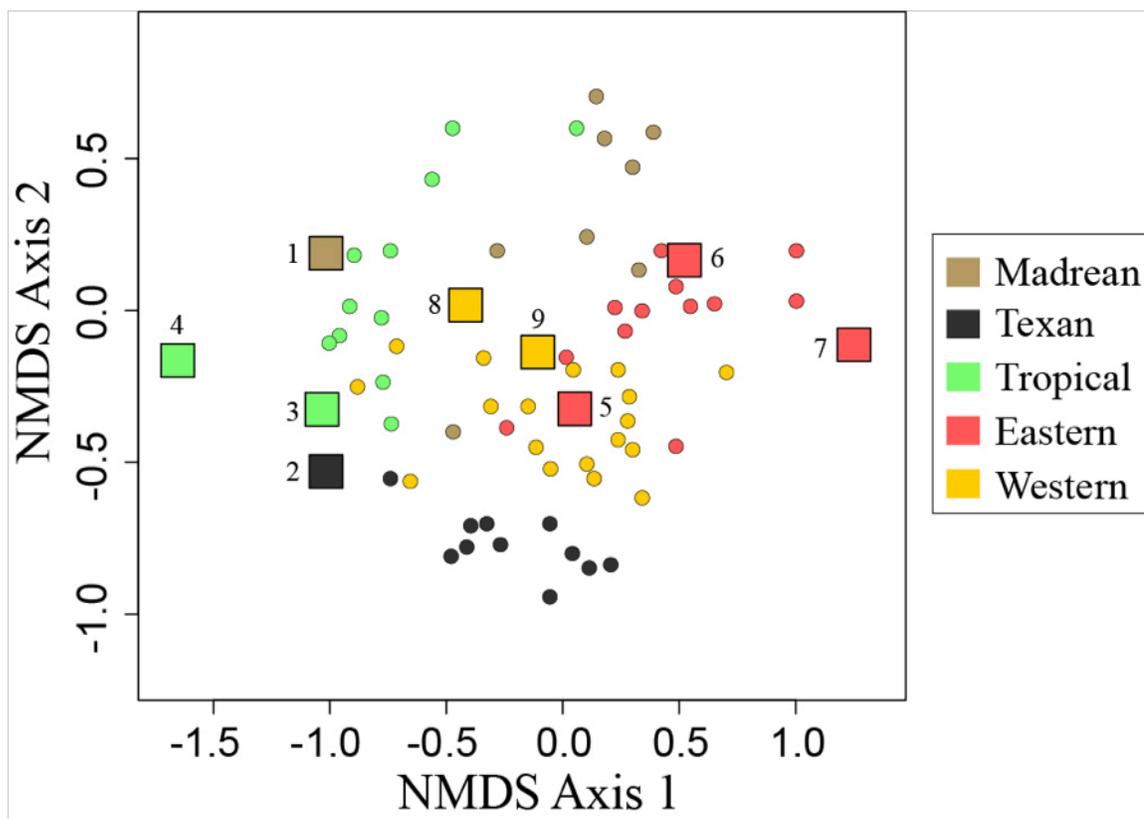


Figure 6.2. NMDS ordination plot based on morphological characters from each *Psorthaspis* and *Dasymutilla* mimicry ring. Circles denote velvet ant data (from Wilson et al. 2012) and squares represent *Psorthaspis* data. Numbers represent *Psorthaspis* species numbered in Figure 6.1.

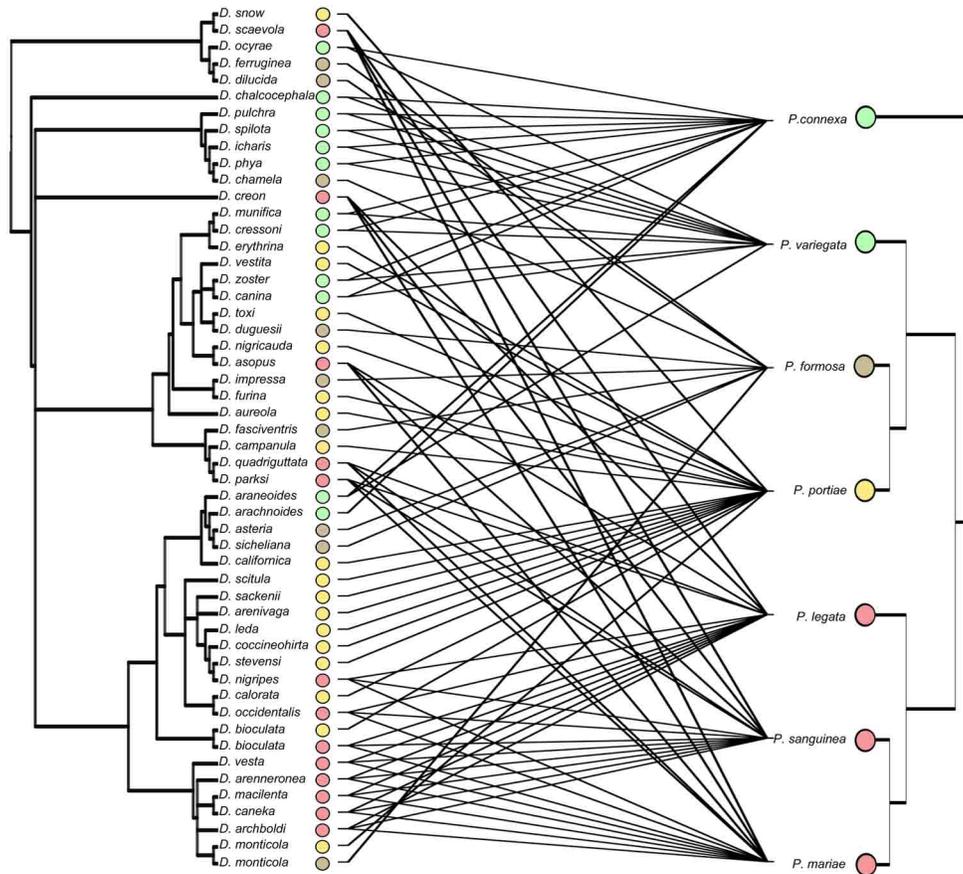


Figure 6.3. Tanglegram of *Psorthaspis* (left topology) and *Dasymutilla* (right topology).

Lines connect between members of the same mimicry rings in the two groups.

## CHAPTER 7

MOLECULAR PHYLOGENY OF POMPILINAE (HYMENOPTERA: POMPILIDAE):  
EVIDENCE FOR RAPID DIVERSIFICATION AND HOST SHIFTS IN SPIDER  
WASPS<sup>6</sup>

## ABSTRACT

Pompilinae are one of the largest groups of spider wasps. Their phylogeny has never been studied with molecular data. Most pompilines are generalist at the spider host family level, but there is some specificity at the ecological level (i. e. host guild). We aimed to test the monophyly of Pompilinae tribes and genera. We also aimed to test whether changes over time in the rate of diversification are associated with host shifts. The first molecular phylogenetic analysis concentrating on Pompilinae spider wasps is presented based on the analysis of five nuclear loci (28S, EF1, LWRh, Wg, Pol2) for 77 taxa in 36 genera. Data were analyzed using maximum likelihood (ML) and Bayesian inference (BI) phylogenetic frameworks. The phylogenetic results were compared with previous hypotheses of tribal classification and generic relationships in the subfamily. The classification of *Pompilus* and *Agenioideus* was also discussed. A Bayesian relaxed molecular clock analysis was used to examine divergence times. Ancestral host family and host guild were reconstructed using parsimony and ML methods. Diversification rate-shifts were tested taking into account taxon sampling bias using ML and BI approaches. None of the tribes proposed by previous authors are monophyletic. Several genera (e.g., *Pompilus*, *Microphadnus*, *Arachnospila*, *Schistonyx* and *Agenioideus*) are

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<sup>6</sup> This manuscript is formatted to be submitted to *Molecular Phylogenetics and Evolution*. The authors of the journal article are: Juanita Rodriguez, James P. Pitts, and Carol D. von Dohlen.

not monophyletic. Divergence dating analyses produced a well-supported chronogram consistent with the BI and ML reconstructions. Ancestral host use reconstructions inferred the use of a guild of spider hunters (other hunters) as the ancestral state for Pompilinae; various switching events to other guilds occurred throughout the evolution of the group. The diversification of Pompilinae shows one main rate-shift that coincides with the use of ground hunters as hosts.

## 1. Introduction

Spider wasps (Hymenoptera: Pompilidae) are solitary wasps that use paralyzed spiders to feed their offspring. It has long been debated whether pompilids should be considered predators or parasitoids (Smit et al., 2002). From a developmental perspective, the ecological niche of the spider wasp larva is that of a parasitoid: the larva needs a single arthropod host to feed on, which is killed at the end of larval development (Godfray, 1994). With respect to host breadth, in a broad sense most pompilids are generalists even at the host family level. Pompilid females' preference for particular spider taxa seems to be related more to ecological factors rather than taxonomic categories.

### 1.1. Systematics of Pompilinae

The subfamily Pompilinae includes approximately 2,000 species. This subfamily is one of the most species-rich and ecologically diverse of Pompilidae (Pitts et al., 2006). Pompilinae has been established as monophyletic by Shimizu (1994) and Pitts et al. (2006); in the latter study *Chirodamus*, *Notocyphus*, and *Priochilus* were included in the subfamily. However, a recent molecular phylogenetic analysis demonstrates that a

monophyletic Pompilinae should exclude *Priochilus*, *Balboana*, *Sericopompilus*, *Chirodamus*, *Cordyloscelis* and *Notocyphus* (Waichert et al., submitted). The monophyly of some genera within the subfamily has been established by molecular methods (see Chapter 5), but more extensive sampling is needed to draw conclusions about the relationships between genera and tribes, as well as their monophyly (Pitts et al., 2006).

The classification within Pompilinae had been poorly understood long before the development of modern phylogenetic analyses. Many erroneous arrangements have been proposed as a result of the difficulties with identification of these wasps, whose morphology displays a considerable degree of inter-specific variation (Evans, 1949). The tribal classification has been particularly problematic. Many names have been proposed but only two have been regularly used, based on species of the New World (Evans, 1949). Ashmead (1902) was the first to propose the subdivision of the subfamily into tribes. Evans (1949) revised the Nearctic fauna, and divided it in two tribes: Pompilini and Aporini. Arnold (1937) divided the Ethiopian Pompilinae into ten tribes. These tribes have never been compared to the Nearctic fauna and remain unused except for classifying the African genera. Bradley (1944) divided the American fauna into seven tribes. Banks (1947) discussed the difficulty of dividing the subfamily into tribes at all. The only tribe that Bradley (1944) and Arnold (1937) have in common is Pompilini. From the tribes proposed in the literature, only Aporini *sensu* Evans (1949) is monophyletic according to the latest morphological phylogeny (Pitts et al., 2006). A comprehensive revision of the world fauna needs to be performed in order to produce an accurate tribal division that corresponds to natural groups (Evans, 1949).

### 1.2. Systematics of *Pompilus*

*Pompilus sensu lato* is a diverse pompilinae genus found in the Americas and the Palearctic region. Its taxonomy has long been conflicting because of the large number of species assigned to it solely by their placement in Pompilidae. The first revision of the genus was by Wilcke (1942), who placed many *Pompilus* species in other genera. Evans (1951) divided the genus in seven subgenera: *Xenopompilus* Evans, *Perissopompilus* Evans, *Xerochaeres* Evans, *Hesperopompilus* Evans, *Arachnospila* Kincaid, *Anoplochaeres* Banks, *Ammosphex* Wilcke, and *Pompilus sensu* Wilcke (1942). Evans's (1951) scheme was followed for some time until Priesner (1969) referred to *Arachnospila* as a genus containing the European subgenera proposed by Evans (1951), excluding *Pompilus sensu stricto*. Day (1981) discussed the taxonomic history of *Pompilus*, restricting it to seven species only found in the Old World, and giving generic status to the subgenera proposed by Evans (1951). *Arachnospila*, *Anoplochaeres* and *Ammosphex* were suggested to be included in the *Arachnospila* genus-group (Day, 1981). The classification of Evans (1951) has been retained by authors because evidence is lacking to support the new classifications of Priesner (1969) and Day (1981) (Wasbauer and Kimsey, 1985).

### 1.3. Host use in Pompilinae

The spiders used by Pompilinae spider wasps are diverse at the taxonomic level, but they are even more diverse at an ecological level. They belong to 21 families, which can be classified into 7 out of the 8 ecological guilds established by Cardoso et al. (2011). Pompilinae are mostly generalist at the host family level. Few species use a single spider family as host. When grouped into ecological guilds, however, pompilines show a greater specificity. Pompilines use the following spider guilds as hosts: ground hunters, other

hunters, ambush hunters, orb web-weavers, sheet web-weavers, space web-weavers and sensing web-weavers.

Spider guilds were determined by Cardoso et al. (2011) using *a posteriori* quantitative methods. The data used to establish guilds was based on ecological characteristics (i.e. foraging strategy, prey range, vertical stratification, and circadian activity). The largest guild generated by these data was ground hunters (26 families) and the smallest was ambush hunters (6 families).

Ground hunter spiders are active hunters that do not build a web, forage on the ground, and are nocturnal. Other hunters also forage on the vegetation in addition to having all the traits found in ground hunters. Ambush hunters have all the traits of other hunters, but they can both be diurnal, or nocturnal, and have an ambush strategy for hunting. Within web-weavers, the main differentiation is the kind of capture web they build, either orb web, space web (tri-dimensional webs), or sheet web. Sensing web-weavers are characterized by the kind of web they build, which usually alerts them on prey movement.

#### *1.4. Evolution of host use in Pompilinae and its correlation with species diversification*

Diversification rate-shifts have been attributed to niche differentiation, in a process known as adaptive radiation (Schluter, 2000). Environmental differentiation (e.g. climate, topography, vegetation), competition, and specialization drive adaptive radiations (Schluter, 2000; Simpson, 1944). Host switching in parasitoids may involve adapting to a new environment, changing the dynamics or avoiding competition, and possibly, specialization, thus providing conditions for adaptive radiations to occur. Similarly, the interactions between hosts and parasitoids have been proposed as

influential in parasitoid diversification processes (Cronin and Abrahamson, 2001). Recent molecular phylogenetic studies have shown a significant increase in the diversification rate with parasitoid host shifts (Fordyce, 2010; McKenna and Farrell, 2006; McLeish et al., 2007; Wheat et al., 2007; Winkler et al., 2009).

Host switching in insect parasitoids can have various ramifications. Parasitoids are a special case of parasitic organisms because they ultimately kill their hosts during development (Tschopp et al., 2013). Idiobionts prevent further development of the host, while koinobionts allow the host to continue development (Quicke, 1997). Pompilids are classified as idiobionts, which tend to be less specialized and more plastic than koinobionts (Shaw, 1994). Therefore, one would expect a relatively high number of host shifts, and low concordance between host and parasitoid phylogenies over the course of their evolutionary history (Althoff, 2008).

In parasitoid wasps, our knowledge of host range evolution is very limited due to a lack of reliable host records in many groups and sound species-level phylogenies (Quicke, 1997; Quicke, 2012). Recent molecular studies have advanced our knowledge of host-use evolution in a phylogenetic framework (Symonds and Elgar, 2013; Taekul et al., 2014; Tschopp et al., 2013), but few studies have specifically addressed the diversification of parasitoids.

Using pompilines as a model, this paper aims to study the correlation between diversification rate-shifts and the evolution of host use. Therefore, our goals were to (1) develop a robust phylogeny of Pompilinae and discuss the classification of the subfamily, and (2) test whether host-guild switches are correlated with diversification rate-shifts.

## 2. Materials and Methods

### 2.1. Taxon sampling

We sampled 70 specimens from 34 Pompilinae genera (Table 7.1). We used the taxonomy of *Pompilus* established by Day (1981). Seven Pompilidae species were used for the out-group, namely, a sample from the probable sister lineage, *Priochilus* + *Balboana* (Waichert *et al.*, submitted) (Table 7.1).

### 2.2. Molecular methods

DNA extraction and amplification of the nuclear genes elongation factor-1  $\alpha$  F2 copy (EF1), long-wavelength rhodopsin (LWRh), wingless (Wg), RNA polymerase II (Pol2) and the D2–D3 regions of the 28S ribosomal RNA (28S) was performed following Pilgrim and Pitts (2006). Primers from previous studies were used (Table 7.2). All PCR products were sequenced with forward and reverse primers and were assembled into complete contigs using Sequencher 4.1 (Gene Codes Corp., Ann Arbor, MI).

### 2.3. Phylogenetic analysis

Sequences were aligned using Geneious Alignment in Geneious 5.4. (Drummond *et al.*, 2011) and then manually refined. Intron data was eliminated from the alignment for LWRh and EF1. The model of molecular evolution was determined for each gene and by codon position using Partition Finder 1.01 (Lanfear *et al.*, 2012). Single-gene phylogenies were produced under Bayesian inference (BI) as implemented in MrBayes 3.2 (Huelsenbeck and Ronquist, 2001). Single-gene matrices were then concatenated using Geneious 5.4 to produce a combined matrix. The model of molecular evolution was determined for the combined matrix using Partition Finder 1.01. The combined matrix

was analyzed in MrBayes 3.2 with partitions by codon position and gene (Table 7.3). Bayesian analyses included four independent runs with three heated chains and one cold chain in each run. The MCMC chains were set for 100,000,000 generations and sampled every 10,000 generations. Convergence diagnostics (e.g., trace plots for visualizing mixing and stationarity; effective sample sizes) were assessed with Tracer 1.5. Trees from the first 10% of the samples were removed as burn-in. The resulting 50% consensus tree was visualized in FigTree 1.4.

A maximum likelihood (ML) analysis was performed using GARLI 2.0 (Genetic Algorithm for Rapid Likelihood Inference; Zwickl, 2006), through the CIPRES gateway (Miller et al., 2010)). The data were partitioned as in BI, above, and bootstrap support levels were calculated by sampling 100 replicates. A 50% consensus tree was generated from the best tree produced by each bootstrap replicate using MI (M-sub-L) methods (Margush and McMorris, 1981) through Consense (Felsenstein, 1989).

#### *2.4. Divergence-time estimation*

A chronogram was inferred in a BI framework using Beast 1.7.5 (Drummond et al., 2012) under an uncorrelated lognormal relaxed-clock model (Drummond et al., 2006; Drummond and Rambaut, 2007). Substitution models were unlinked among partitions with the underlying clock and trees linked. One calibration point was used for our analysis, based on the age of the subfamily obtained by Waichert et al. (submitted). The crown-group node of all Pompilinae taxa included in the analysis was assigned a normal prior of (mean) 27 Ma (SD=10). Two separate Markov Chain Monte Carlo (MCMC) searches were performed for 10,000,000 generations. Convergence diagnostics were

examined in Tracer 1.5, and independent runs were assembled with LogCombiner 1.7.5. Ten percent of the generations were discarded as burn-in.

### *2.5. Ancestral state reconstruction of spider host use*

Spider host guild (Cardoso et al., 2011) was mapped onto the Pompilinae chronogram as a multistate character. The list of known host species of Pompilinae used in our analyses was adopted from data from all published host records (Table 7.4).

We used a ML and a maximum parsimony approach (MP) to map the evolution of host use onto the Pompilinae phylogeny. The ML approach was implemented using the rayDISC command in the package corHMM (Beaulieu et al., 2014) in R (R Development Core Team, 2010). This method allows for multistate characters, unresolved phylogenies, and ambiguities (polymorphic taxa or missing data). Two models of character evolution were evaluated under the ML method; these were: equal rates (ER), and all rates different (ARD). A likelihood-ratio test was performed to determine the significance of the difference in likelihood values for different models of character evolution. Parsimony character mapping was performed in Mesquite ver. 2.7.5 (Maddison and Maddison, 2011) with all character-state changes weighed equally.

### *2.7. Diversification rate-shift analysis*

To determine the best-fit model for Pompilinae diversification, we calculated the Akaike information criterion (AIC) for various models of constant-rate and rate-variable diversification through time with the package laser (Rabosky, 2006) in R. A pure-birth and a birth-death model with constant-rate were tested, as well as pure-birth models with different numbers of rate-shifts: yule2rate, yule3rate. Two models incorporating density-

dependent diversification rates (DDX and DDL) were also tested. To account for bias in taxon sampling we divided the Pompilinae chronogram into two main clades, as indicated in Figure 7.1. The division was based on the time of missing of speciation events within each clade as follows: i) the majority of missing species in clade 1 belong to *Agenioideus*, which had an origin ca. 20.4 Ma (CI = 17.23,23.55) and ii) the majority of missing species in clade 2 belong to *Anoplius* and the clade composed of *Arachnospila*, *Evagetes* and *Aridestus*, which had origins ca. 10.5 (CI=7.85,13.44) and 9.2 (CI=6.08,13.43) Ma, respectively. Missing speciation events equal to the number of missing species were simulated onto both clades 1,000 times starting at the time of origin of the genera containing most species. This simulation generated a dataset of 1,000 trees for each clade, which we refer to as “semi-empirical dataset.” Simulations were performed using the function *corsim* (Cusimano et al., 2012) in the package *TreeSim* (Stadler, 2011) in R. A null distribution was generated separately by simulating 1,000 trees with the total expected number of taxa for each clade individually; we refer to this as the “null distribution”. The difference between the semi-empirical dataset and the null distribution is that the semi-empirical contains information on the “real topology” and the time of missing branching times, whereas the null distribution is a dataset birth-death trees with the same number of taxa generated at random.

We calculated the AIC and  $\Delta AIC_{Crc}$  for the semi-empirical and null distribution datasets. The  $\Delta AIC_{Crc}$  was obtained by subtracting the AIC of the best rate-constant model ( $AIC_{Crc}$ ) from the AIC of the best rate-variable model ( $AIC_{Crv}$ ). The  $\Delta AIC_{Crc}$  is positive when the data best fit a rate-variable model. We then calculated the mean and standard deviation of AIC and  $\Delta AIC_{Crc}$ . We used these values to determine

the model of diversification that best fit our data (lowest AIC), and the fit of our data to a rate-variable versus rate-constant model ( $\Delta AIC_{rc}$ ), according to criteria suggested by Rabosky (2006). We performed a t-test to determine if the  $\Delta AIC_{rc}$  from the null distribution was significantly different than the semi-empirical data (trees with simulated branching events). Our diversification rates results will be expressed in units of speciation events per million years (sp/Myr).

We also performed a Bayesian analysis of diversification in BAMM (Bayesian Analysis of Macroevolutionary Mixtures) (Rabosky, 2014). BAMM uses reversible jump Markov Chain Monte Carlo to explore various models of lineage diversification in order to detect and quantify heterogeneity in evolutionary rates (Rabosky, 2014; Rabosky et al., 2013). We accounted for non-random missing speciation events by quantifying the percentage of taxa sampled per genus and incorporating it into the analysis. The MCMC chain was set for 100,000,000 generations, with sampling every 10,000 generations. Convergence diagnostics were examined using coda (Plummer et al., 2013) in R. Ten percent of the runs were discarded as burn-in. The 95% credible set of shift configurations was plotted in the R package BAMMtools (Rabosky et al., 2014).

### **3. Results**

#### *3.1. Phylogenetic results*

Pompilinae is a well-supported subfamily within Pompilidae, and can be divided in two major clades (Figure 7.1). Within these two clades, various sub-clades were well supported in one or both ML and BI analyses. Clade 1 includes three main sub-clades: *Batozonellus + Episyron*, *Poecilopompilus + Austrochaes*, and the largest clade including *Agenioideus*, *Tachypompilus*, *Ferreola*, *Homonotus*, and *Spuridiophorus*.

Major sub-clades within clade 2 are: *Kyphopompilus* + *Tastiotenia*; Aporini; *Schistonyx*, *Microphadnus*, *Atelostegus* and *Atopompilus*; *Apareia* + *Paracyphononyx*; *Aporinellus*, *Ctenostegus*, *Turneromyia*, *Pompilus*); *Anoplius* (including *Dicranoplius*), and a clade composed of *Xerochares*, *Allochares*, *Arachnospila*, *Evagetes* and *Aridestus*. Within clade 2, *Kyphopompilus* + *Tastiotenia*, *Telostegus*, and *Microphadnus* compose the earliest branching lineages, although the exact positions of the former two lineages is uncertain.

Our results show that none of the tribes previously proposed is monophyletic, except for Aporini *sensu* Evans (1949) (Figure 7.1). Some of the tribes proposed by Arnold (1937) are monophyletic by definition because they contain only one genus (i. e. Cordyloscelini, Spuriophorini and Tachypompilini). Nevertheless, a previous study concludes that *Cordyloscelis* is not included in Pompilinae (Waichert et al., submitted).

Our results reject the monophyly of various pompiline genera and subgenera. The following genera are not monophyletic: *Pompilus sensu* Evans (1951), *Arachnospila*, *Schistonyx*, *Microphadnus*, and *Agenioideus*. The three subgenera of *Arachnospila*, *Arachnospila*, *Ammosphex*, and *Anoplochares*, are recovered in the same clade; however, this clade also includes *Aridestus* and *Evagetes*, whose positions render *Arachnospila* paraphyletic. Further analyses are needed to determine the circumscription of this genus, considering the possibility of synonymizing *Evagetes* and *Aridestus* with *Arachnospila*. *Agenioideus* is also paraphyletic, and might best be redefined by elevating its subgenera to generic status. An expanded analysis with more extensive taxon sampling is also needed to clarify the taxonomy of *Agenioideus*.

### 3.2. *Evolution of host use*

Our results suggest that pompiline wasps are not specialists at the level of host family. Most of the genera parasitize more than one spider family. Nevertheless, when the host family is grouped into guilds, various pompiline genera appear to be specialists at the ecological level (Table 7.4).

The likelihood-ratio test performed on host-guild ancestral-state reconstruction suggests that this character is evolving under the ARD model ( $p = 0.376$ ). The ancestral condition for Pompilinae was the use of other hunters as hosts. The reconstruction of host shifts is consistent between parsimony and ML reconstructions, but the ancestral state of many nodes is equivocal for the parsimony reconstruction (Figure 7.2). From the ancestral use of other hunters there was a shift to use of orb-web weavers in clade 1. In clade 2, there was a shift to ground hunters and then to sensing web-weavers, as well as a reversal back to use of other hunters from ground hunters (Figure 7.2).

### 3.3. *Diversification rate-shift analysis*

DeltaAICrc for semi-empirical *versus* null hypothesis data for clade 1 were significantly different ( $t = 3.50$ ,  $df = 1997.13$ ,  $p = 0.00048$ ), signifying that the diversification of clade 1 deviates from a null hypothesis of rate-constancy. The best-fit model for clade 1 data is a yule3rate model (two rate-shifts; Table 7.5). Clade 2 also deviates from a null hypothesis of rate-constancy ( $t = 23.8082$ ,  $df = 1969.167$ ,  $p\text{-value} = 2.2e-16$ ). The best-fit model for clade 2 data is a yule3rate model (Table 7.6).

For clade 1, the model suggests a shift from a rate of 0.27 (sp/Myr) to 0.78 (sp/Myr) ca 12.80 Ma, and a shift from 0.78 (sp/Myr) to 0.14 (sp/Myr) ca 8.80 Ma. For

clade 2 the model suggests a shift from a rate of 0.12 (sp/Myr) to 0.41 (sp/Myr) ca 10.47 Ma, and from 0.41 (sp/Myr) to 0.20 (sp/Myr) ca 5.16 Ma.

The 95% credibility set of shift configurations of the BAMM analysis shows a higher diversification rate within the clade containing two of the most diverse genera, *Arachnospila* and *Anoplius*, for the two configurations with the highest probability. Both of these show a rate-shift at the node of the MRCA of *Anoplius* and *Arachnospila* (Figure 7.3).

## 4. Discussion

### 4.1. Pompilinae phylogeny and tribal classification

Pompilinae is one of the largest subfamilies of Pompilidae. Its monophyly has been supported by molecular data (Waichert et al., submitted). Pompilinae is divided into two main clades (Figure 7.1). Within these clades, there are various well-supported subclades, but the relationships between many of these are not well supported, especially for clade 2 (Figure 7.2). It is possible that more extensive sampling, and a greater number of molecular loci could improve the phylogenetic resolution, but it is also probable that the ambiguities observed in Pompilidae phylogenies are the result of “hard” polytomies. Hard polytomies are produced when a rapid radiation occurs (Whitfield and Lockhart, 2007), and can not be resolved through phylogenetic methods. Supporting evidence for a rapid radiation of Pompilidae is the morphological homogeneity even at the subfamily level, and the appearance of all subfamilies in a relative short period of time (Waichert et al., submitted).

The topology recovered for Pompilinae is consistent with certain clades reconstructed in previous morphological analyses. For example, Pitts et al. (2006) and

Shimizu (1994) recovered a clade composed of *Episyron*, *Batozonellus*, *Poecilopompilus* and *Austrochares*. The close relationship of *Episyron* and *Poecilopompilus* had also been discussed by Evans (1949). Pitts et al. (2006) also supported the relationship of *Arachnospila* to *Evagetes* and *Xerochares*, along with other genera not recovered for this clade by our analysis. Shimizu (1994) also supported the grouping of *Homonotus* and *Ferreola*. Most of the patterns observed in morphological analyses, however, differ from the results obtained with molecular data.

The tribal classification of Pompilinae has been historically problematic, because of the absence of worldwide revisions. Our data suggest a need for a new tribal classification taking into account the world fauna. This task, however, can only be performed in a phylogenetic framework, incorporating morphological data to assess the synapomorphies of each tribe. This will allow for the inclusion of taxa lacking molecular data in the new tribal classification.

#### 4.2. Pompilinae generic-level classification

At the generic level there are many taxonomic problems to be solved, such as the definition of *Agenioideus* and *Arachnospila*. These are in need of revision at the sub-generic level, for which broader sampling coupled with molecular phylogenetic analyses should be informative. Our results show that the definition of *Pompilus* by Priesner (1969) and Day (1981) was correct. The subgenera established by (Evans, 1951) (i.e. *Xenopompilus*, *Perissopompilus*, *Xerochares*, *Hesperopompilus*, *Arachnospila*, *Anoplochares*, *Ammosphex*, and *Pompilus*), which continued to be used after 1981, are not members of a single clade, and thus should be considered separate genera. Here we give phylogenetic evidence to establish *Xenopompilus*, *Perissopompilus*, *Xerochares*,

*Hesperopompilus*, and *Pompilus* sensu Day (1981) as valid genera. The morphological similarity and probable phylogenetic closeness of *Pompilus* had been discussed by Day (1981). Our analyses show that *Pompilus* and *Aporinellus* are sister taxa, nevertheless, this assemblage does not include *Pompilus* (*Hesperopompilus*) as suggested by Day (1981). *Pompilus* (*Hesperopompilus*) is more closely related to *Pompilus* (*Xenopompilus*) and Aporini.

#### 4.3. Evolution of host use and diversification in Pompilinae

Our results suggest that pompilines are mostly generalists at the host family level, while tending to be specialists at the spider guild level. This can be explained by the host-ecology hypothesis, which assumes that parasitoids can broaden their host range by recruiting new hosts that exist within their own searching niche. Specialization thus takes place on the level of the host's niche instead of its taxonomic or phylogenetic identity (Tschopp et al., 2013; Zaldivar-Riveron et al., 2008).

The diversification rate-shift analysis shows that Pompilinae did not diversify at a constant rate. There is a significant rate-shift in clade 2 supported by both analyses. The rate-shift found in clade 1 was not supported by the BAMM results, which show a slow diversification rate for that clade (Figure 7.3). This result, together with the higher robustness of BAMM, makes the shift in clade 1 not significant for our discussion. The stepwise AIC-based analyses are limited, because they look for a single best model, when many distinct combinations of evolutionary shift regimes could be probable. Rather than identifying a single best model, BAMM samples rate-shift configurations in proportion to their posterior probability. This method is more successful when accounting for non-random species sampling bias (Rabosky, 2014).

The diversification rate-shift supported by both analyses occurs close to the shift of the use of ground hunters as hosts. Other host-shifting events did not show a significant change in diversification rate. The use of other hunters as hosts, however, was maintained in other clades such as the *Apareia* + *Paracyphononyx* and *Pompilus* clade where an increase in diversification rate was not observed (Figure 7.2). The main difference between these two clades is the number of host species used by a single wasp species. Species from the *Anoplius* and *Arachnospila* clade use more than 20 host species, while *Paracyphononyx* and *Pompilus* seem to be specialists at the species level. The ground hunters are the most family-diverse of the guilds (Cardoso et al., 2011). The ability to exploit a greater number of spider species could have made more niches available for the *Anoplius* and *Arachnospila* lineage and spurred a shift in the diversification rate. This may occur through genetic divergence of populations that shift to novel hosts, ultimately leading to reproductive isolation and the formation of new species (Baer et al., 2004).

Host switching has been shown to result in rapid species diversification (Cocroft et al., 2008; Ehrlich and Raven, 1964) by environmental differentiation, competition, and specialization, as well as antagonistic interactions with hosts (Thompson, 1999). In the pompiline scenario, environmental differentiation and competition are the most likely drivers, because specialization does not seem to be the norm in the subfamily. The availability of new niches, along with the capability of using a higher diversity of hosts, probably increased diversification rate in the *Anoplius* and *Arachnospila* clades.

With respect to competition, the only other wasps that use spiders exclusively are various genera of Sphecidae and Crabronidae wasps (Gonzaga and Vasconcellos-Neto,

2005). Sphecids often specialize on older araneid lineages with two-dimensional web-building spiders over derived araneoids with three-dimensional web-building spiders (Blackledge et al., 2003; Uma, 2010), whereas most pompilines specialize on hunter spiders. According to Wilson et al. (2013), the origin of Sphecidae and Crabronidae was earlier than Pompilinae. Therefore, it is possible that Pompilinae diversification was triggered by an ability to use spider guilds not already exploited by other wasps. Our results suggest that the low diversity of the (*Batozonellus* + *Episyron*) + (*Poecilopompilus* + *Austrochares*) clade, which uses orb-web weavers (Figure 7.1), may be explained by competitive exclusion by sphecid wasps. This could have selected for multiple shifts in spider guild use and subsequent diversification of the subfamily.

## 5. Conclusions

Molecular and morphological data yield conflicting phylogenies for Pompilinae. The tribal classification of Pompilinae is in need of thorough revision, especially to circumscribe tribes that apply to all the world fauna and that form monophyletic entities. This is also the case for some genera like *Schistonyx*, *Microphadnus*, *Agenioideus* and *Arachnospila*, for which more extensive sampling, and a phylogenetic framework is needed to understand their taxonomy.

The evidence presented here suggests that, for Pompilinae spider wasps, the interactions with their spider hosts, and occasional shifts among spider ecological guilds, have played an important role in pompiline diversification patterns.

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TABLE 7.1. Outgroups and ingroup species sampled, voucher ID and Genbank accession numbers.

Species	Voucher ID	28S Accession #	EF1 Accession #	LWRh Accession #	Pol2 Accession #	Wg Accession #
<b>Outgroup Taxa</b>						
<i>Balboana sp.</i>	PO394					
<i>Balboana sp.</i>	PO395					
<i>Priochilus sp.</i>	PO398					
<i>Priochilus splendidum.</i>	PO385					
<i>Priochilus sericeifrons</i>	PO260					
<i>Priochilus sp.</i>	PO264					
<b>Ingroup Taxa</b>						
<i>Agenioideus (Agenioideus) humilis</i>	PO141					
<i>Agenioideus (Gymnochaeres) birkmanni</i>	PO191					
<i>Agenioideus (Ridestus) biedermanni</i>	PO189					
<i>Agenioideus decipiens</i>	PO136					
<i>Agenioideus sp.</i>	PO340					
<i>Allochaeres azureus</i>	PO387					
<i>Anoplius (Anopliodes) parsonsi</i>	PO187					
<i>Anoplius (Arachnophroctonus) apiculatus</i>	PO76					
<i>Anoplius (Arachnophroctonus) subfasciatus</i>	PO202					
<i>Anoplius (Lophopompilus) aethiops</i>	PO8					
<i>Anoplius sp.</i>	PO120					
<i>Apareia bellicosa</i>	PO205					
<i>Apareia labialis</i>	PO176					
<i>Aporinellus atristylus</i>	PO43					
<i>Aporinellus fuscatus</i>	PO148					
<i>Aporinellus sinuatus</i>	PO42					

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<i>Aporus (Aporus) concolor</i>	PO435
<i>Aporus (Aporus) niger</i>	PO11
<i>Aporus (Aporus) sp.</i>	PO310
<i>Arachnospila (Ammosphex) eximia</i>	PO147
<i>Arachnospila (Ammosphex) occidentalis</i>	PO7
<i>Arachnospila (Ammosphex) smaragdina</i>	PO153
<i>Arachnospila (Ammosphex) sp.</i>	PO13
<i>Arachnospila (Anoplochaetes) apicatus</i>	PO171
<i>Arachnospila (Arachnospila) scelestus</i>	PO158
<i>Arachnospila sp.</i>	PO211
<i>Aridestus jaffueli</i>	PO144
<i>Atelostegus thrinax</i>	PO342
<i>Atopompilus sp.</i>	PO281
<i>Austrochaetes sp.</i>	PO105
<i>Batozonellus fuliginosus</i>	PO204
<i>Batozonellus madecassus</i>	PO169
<i>Ctenostegus hilli</i>	PO131
<i>Dicranoplius cujanus</i>	PO199
<i>Dicranoplius diphonicus</i>	PO151
<i>Episyron viduus</i>	PO203
<i>Euryzonotulus nigeriensis</i>	PO356
<i>Evagetes nitidulus</i>	PO400
<i>Evagetes padrinus</i>	PO315
<i>Ferreola erythrocephala</i>	PO339
<i>Ferreola saussurei</i>	PO26
<i>Ferreola sp.</i>	PO343
<i>Ferreola symmetria</i>	PO22
<i>Hesperopompilus serrano</i>	PO129
<i>Homonotus sp.</i>	PO224

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<i>Kypopompilus atriventris</i>	PO36
<i>Microphadnus sp.</i>	PO159
<i>Microphadnus sp.</i>	PO278
<i>Paracyphononyx consimilis</i>	PO219
<i>Paracyphononyx consimilis</i>	PO132
<i>Paracyphononyx funereus</i>	PO285
<i>Perissopompilus phoenix</i>	PO70
<i>Perissopompilus sp.</i>	PO121
<i>Poecilopompilus algidus</i>	PO49
<i>Poecilopompilus sp.</i>	PO100
<i>Pompilus cinereus</i>	PO270
<i>Pompilus sp.</i>	PO407
<i>Psorthaspis connexa</i>	PO64
<i>Psorthaspis magna</i>	PO9
<i>Schistonyx aterrimus</i>	PO257
<i>Schistonyx brevispinis</i>	PO346
<i>Schistonyx nyassae</i>	PO353
<i>Spuridiophorus capensis</i>	PO337
<i>Tachypompilus ferrugineus</i>	PO38
<i>Tastiotenia festiva</i>	PO102
<i>Telostegus masrensis</i>	PO329
<i>Turneromyia ahrimanes</i>	PO222
<i>Xenopompilus nugador</i>	PO119
<i>Xenopompilus tarascanus</i>	PO116
<i>Xerochares expulsus</i>	PO54

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TABLE 7.2. Primers used for PCR amplification and sequencing

Marker	Primer name	Primer sequence (5'-3')	Reference
EF1	F2for1	GGT TCC TTC AAA TAT GCT TGG G	Pilgrim <i>et al.</i> , 2006
	F2rev1	A ATC AGC AGC ACC TTT AGG TGG	Danforth & Ji, 1998
LWRh	PompOps1F	ATT CGA CAG ATA CAA CGT AAT CG	Pilgrim <i>et al.</i> , 2006
	LWRhR	ATA TGG AGT CCA NGC CAT RAA CCA	Mardulyn & Cameron, 1999
	LWRhRApor	GAG RGA GAT CGT CAT CAA GGC GAC C	Rodriguez <i>et al.</i> 2014
Wg	LepWg1for	GAR TGY AAR TGY CAY GGY ATG TCT GG	Brower & DeSalle, 1998
	modLepWg2re v	ACT ICG CRC ACC ART GGA ATG TRC A	Brower & DeSalle, 1998
28S	CF2	TGG TAA CTC CAT CTA AGG CTA AAT A	Pilgrim <i>et al.</i> 2006
	D5-4625 R (D5R)	CCC ACA GCG CCA GTT CTG CTT ACC	Schulmeister, 2003

Table 7.3. Best partitioning scheme determined by PartitionFinder, the model of molecular evolution and the loci included in each.

Subset	Best model	Subset partitions
1	SYM+I+G	28S, 3 <sup>rd</sup> codon position LWRh
2	K80+I+G	1 <sup>st</sup> codon position EF1, 2 <sup>nd</sup> codon position EF1, 2 <sup>nd</sup> codon position LWRh
3	SYM+G	3 <sup>rd</sup> codon position EF1
4	K80+G	1 <sup>st</sup> codon position LWRh
5	GTR+I+G	1 <sup>st</sup> codon position Pol2
6	F81	2 <sup>nd</sup> codon position Pol2
7	K80+I+G	3 <sup>rd</sup> codon position Pol2
8	K80+I	1 <sup>st</sup> codon position Wg
9	JC	2 <sup>nd</sup> codon position Wg
	HKY+G	3 <sup>rd</sup> codon position Wg

Table 7.4. Host family and guild for all Pompilinae taxa studied.

Species	Voucher ID	Host family	Host guild	Reference
<i>Batozonellus madecassus</i>	PO169	Araneidae	Orb web-weavers	Endo, 1994
<i>Batozonellus fuliginosus</i>	PO204	Araneidae	Orb web-weavers	Endo, 1994
			Orb web-weavers	Kurczewski & Kurczewski 1968; Kurczewski & Kurczewski 1973; Kurczewski et al. 1987;
<i>Episyron viduus</i>	PO203	Araneidae		Kurczewski & Spofford 1986; Wasbauer & Powell 1962, Kurczewski 1963, Krombein 1955, Krombein 1953a, 1953b; Peckhams 1898; Rau 1922; Evans 1951
<i>Austrochares</i> sp.	PO105	unknown	Orb web-weavers	Cambra et al. 2004; Kurczewski 1981; Kurczewski & Kurczewski 1968; Martins 1991; Krombein 1979
<i>Poecilopompilus algidus</i>	PO49	Araneidae, Nephilidae	Orb web-weavers	Cambra et al. 2004; Kurczewski 1981; Kurczewski & Kurczewski 1968; Martins 1991; Krombein 1979
<i>Poecilopompilus</i> sp.	PO100	Araneidae, Nephilidae		Kurczewski 1981; Kurczewski & Kurczewski 1968; Martins 1991; Krombein 1979
<i>Tachypompilus ferrugineus</i>	PO38	Lycosidae, Pisauridae	Ground hunters, sheet web-weavers	Evans and Yoshimoto 1962; Martins, 1991
<i>Agenioideus decipiens</i>	PO136	unknown		
<i>Agenioideus (Ridestus) biedermani</i>	PO189	Theridiidae, Amaurobiidae	Space web-weavers, sheet web-weavers	Shimizu 1997
<i>Agenioideus (Gymnochares) birkmanni</i>	PO191	Lycosidae, Gnaphosidae, Salticidae	Ground hunters, other hunters	Wilson & Pitts 2007; Krombein 1979; Kurczewski & Spofford 1986
<i>Euryzonotulus nigeriensis</i>	PO356	unknown		
<i>Agenioideus (Agenioideus) humilis</i>	PO141	Segestriidae, Salticidae, Thomisidae, Araneidae	Sensing web-weavers, other hunters, ambush hunters, orb web-weavers	Shimizu 1997

<i>Agenioideus sp.</i>	PO340	unknown		
<i>Spuridiophorus capensis</i>	PO337	unknown		
<i>Homonotus sp.</i>	PO224	Clubionidae	Other hunters	Richards & Hamm, 1939; Day, 1988
<i>Ferreola erythrocephala</i>	PO339	unknown		
<i>Ferreola sp.</i>	PO343	unknown		
<i>Ferreola saussurei</i>	PO26	unknown		
<i>Ferreola symmetria</i>	PO22	unknown		
<i>Kypopompilus atriventris</i>	PO36	unknown		
<i>Tastiotenia festiva</i>	PO102	Theridiidae	Space web-weavers	Evans 1961
<i>Telostegus masrensis</i>	PO329	Thomisidae, Philodromidae; Argiopidae	Ambush hunters, other hunters, orb web-weavers	Gros 1995
<i>Microphadnus sp.</i>	PO159	Lycosidae, Salticidae	Ground hunters, other hunters	Ferton 1897
<i>Perissopompilus phoenix</i>	PO70	unknown		
<i>Perissopompilus sp.</i>	PO121	unknown		
<i>Hesperopompilus serrano</i>	PO129	unknown		
<i>Xenopompilus nugador</i>	PO119	unknown		
<i>Xenopompilus tarascanus</i>	PO116	unknown		
<i>Psorthaspis connexa</i>	PO64	Ctenizidae	Sensing web weavers	Davidson 1915
<i>Psorthaspis magna</i>	PO9	Ctenizidae	Sensing web weavers	Davidson 1915
<i>Aporus (Aporus) sp.</i>	PO310	Idiopidae, Ctenizidae	Sensing web-weavers	
<i>Aporus (Aporus) concolor</i>	PO435	Idiopidae, Ctenizidae	Sensing web-weavers	
<i>Aporus (Aporus) niger</i>	PO11	Idiopidae, Ctenizidae	Sensing web-weavers	
<i>Schistonyx brevispinis</i>	PO346	Ctenizidae, Lycosidae	Sensing web-weavers, ground hunters	Wahis, 2000
<i>Microphadnus sp.</i>	PO278	unknown		
<i>Atelostegus thrinax</i>	PO342	unknown		
<i>Atopompilus sp.</i>	PO281	unknown		
<i>Schistonyx aterrimus</i>	PO257	Ctenizidae, Lycosidae	Sensing web-weavers, ground hunters	Wahis, 2000
<i>Schistonyx nyassae</i>	PO353	Ctenizidae, Lycosidae	Sensing web-weavers, ground hunters	Wahis, 2000
<i>Apareia bellicosa</i>	PO205	unknown		

<i>Apareia labialis</i>	PO176	unknown		
<i>Paracyphononyx funereus</i>	PO285	Lycosidae, Miturgidae	Ground hunters, other hunters	Grout and Brothers 1982; El Hennawy 1998
<i>Paracyphononyx consimilis</i>	PO219	Lycosidae, Miturgidae	Ground hunters, other hunters	Grout and Brothers 1982; El Hennawy 1998
<i>Paracyphononyx consimilis</i>	PO132	Lycosidae, Miturgidae	Ground hunters, other hunters	Grout and Brothers 1982; El Hennawy 1998
<i>Aporinellus atristylus</i>	PO43	Thomisidae, Salticidae, Oxyopidae	Ambush hunters, other hunters	Kurczewski & Kurczewski 1968; Kurczewski & Kurczewski 1973; Wilson and Pitts 2007; Kurczewski et al. 1989
<i>Aporinellus sinuatus</i>	PO42	Thomisidae, Salticidae, Oxyopidae	Ambush hunters, other hunters,	Kurczewski & Kurczewski 1968; Kurczewski & Kurczewski 1973; Wilson and Pitts 2007; Kurczewski et al. 1988
<i>Aporinellus fuscatus</i>	PO148	Thomisidae, Salticidae, Oxyopidae		Kurczewski & Kurczewski 1968; Kurczewski & Kurczewski 1973; Wilson and Pitts 2007; Kurczewski et al. 1987
<i>Ctenostegus hilli</i>	PO131	Lycosidae	Ground hunters	Evans and Mathews 1974
<i>Turneromyia ahrimanes</i>	PO222	unknown		
<i>Pompilus sp.</i>	PO407	Lycosidae, Pisauridae, Clubionidae	Ground hunters, sheet web-weavers, other hunters	
<i>Pompilus cinereus</i>	PO270	Lycosidae, Pisauridae, Clubionidae	Ground hunters, sheet web-weavers, other hunters	
<i>Anoplius (Arachnophroctonus) apiculatus</i>	PO76	Lycosidae	Ground hunters	Wasbauer & Kimsey 1989
<i>Anoplius (Lophopompilus) aethiops</i>	PO8	Lycosidae	Ground hunters	Kurczewski & Kurczewski 1968
<i>Anoplius sp.</i>	PO120	unknown		
<i>Anoplius (Arachnophroctonus) subfasciatus</i>	PO202	Agelenidae, Lycosidae	Sheet web weavers, ground hunters	Wasbauer & Kimsey 1988
<i>Anoplius (Anopliodes) parsonsi</i>	PO187	Ctenidae	Other hunters	Cambra et al 2004

<i>Dicranoplius diphonicus</i>	PO151	unknown		
<i>Dicranoplius cujanus</i>	PO199	unknown		
<i>Xerochares expulsus</i>	PO54	unknown		
<i>Aridestus jaffueli</i>	PO144	unknown		
<i>Arachnospila</i> sp.	PO211	unknown		
<i>Arachnospila (Ammosphex) eximia</i>	PO147	unknown		
<i>Arachnospila (Ammosphex) smaragdina</i>	PO153	unknown		
<i>Evagetes nitidulus</i>	PO400	Cleptoparasite		Evans & Mathews 1973, Kurczewski & Kurczewski 1968, Kurczewski et al. 1987, Kurczewski & Kurczewski 1975
<i>Evagetes padrinus</i>	PO315	Cleptoparasite		Evans & Mathews 1973, Kurczewski & Kurczewski 1968, Kurczewski et al. 1987, Kurczewski & Kurczewski 1975
<i>Arachnospila (Ammosphex) occidentalis</i>	PO7	Lycosidae	Ground hunters	Powell 1957
<i>Arachnospila (Arachnospila) scelestus</i>	PO158	Lycosidae, Salticidae	Ground hunters other hunters	Kurczewski et al 1987, Kurczewski 2010
<i>Arachnospila (Anoplochares) apicatus</i>	PO171	Lycosidae	Ground hunters	Adlerz 1910
<i>Arachnospila (Ammosphex) sp.</i>	PO13	Thomisidae, Gnaphosidae, Salticidae	Ambush hunters, ground hunters, other hunters	
<i>Allochares azureus</i>	PO387	Filistatidae	Sensing web weavers	

Table 7.5. AIC and deltaAIC values for different diversification models for clade 1

Model	AIC (Akaike Information Criterion)				DeltaAICrc (AICrc-AICrv)			
	Empirical		Simulation		Empirical		Simulation	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
yule constant	-739	22.33	-578.53	25.57	<b>13.24</b>	5.12	<b>14.03</b>	5.02
bd constant	-737	22.34	-576.53	25.57	15.23	5.13	16.03	5.02
DDL	-747	18.50	-587.27	22.01	5.69	3.95	4.58	3.82
DDX	-742	20.02	-582.29	23.78	10.56	4.87	10.27	4.58
yule2rate	-750	18.64	-590.05	22.75	2.20	2.28	2.51	2.14
yule3rate	-752	19.14	-592.30	22.43	0.15	0.42	0.26	0.63

Table 7.6. AIC and DeltaAIC values for different diversification models for clade 2

Model	AIC (Akaike Information Criterion)				DeltaAIC <sub>Crc</sub> (AIC <sub>Crc</sub> -AIC <sub>CrV</sub> )			
	Empirical		Simulation		Empirical		Simulation	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
yule constant	-3,946.59	30.45	-2,945.91	43.02	<b>52.85</b>	9.32	<b>43.48</b>	8.25
bd constant	-3,944.59	30.45	-2,943.91	43.02	54.85	9.32	45.48	8.25
DDL	-3,955.18	27.28	-2,980.37	37.09	44.26	6.84	9.02	6.96
DDX	-3,971.40	24.70	-2,958.303	40.25	28.04	6.83	31.09	9.71
yule2rate	-3,977.13	25.17	-2,980.51	38.47	22.31	4.61	8.88	4.23
yule3rate	-3,999.45	24.65	-2,989.16	36.57	0	0	0.23	0.90

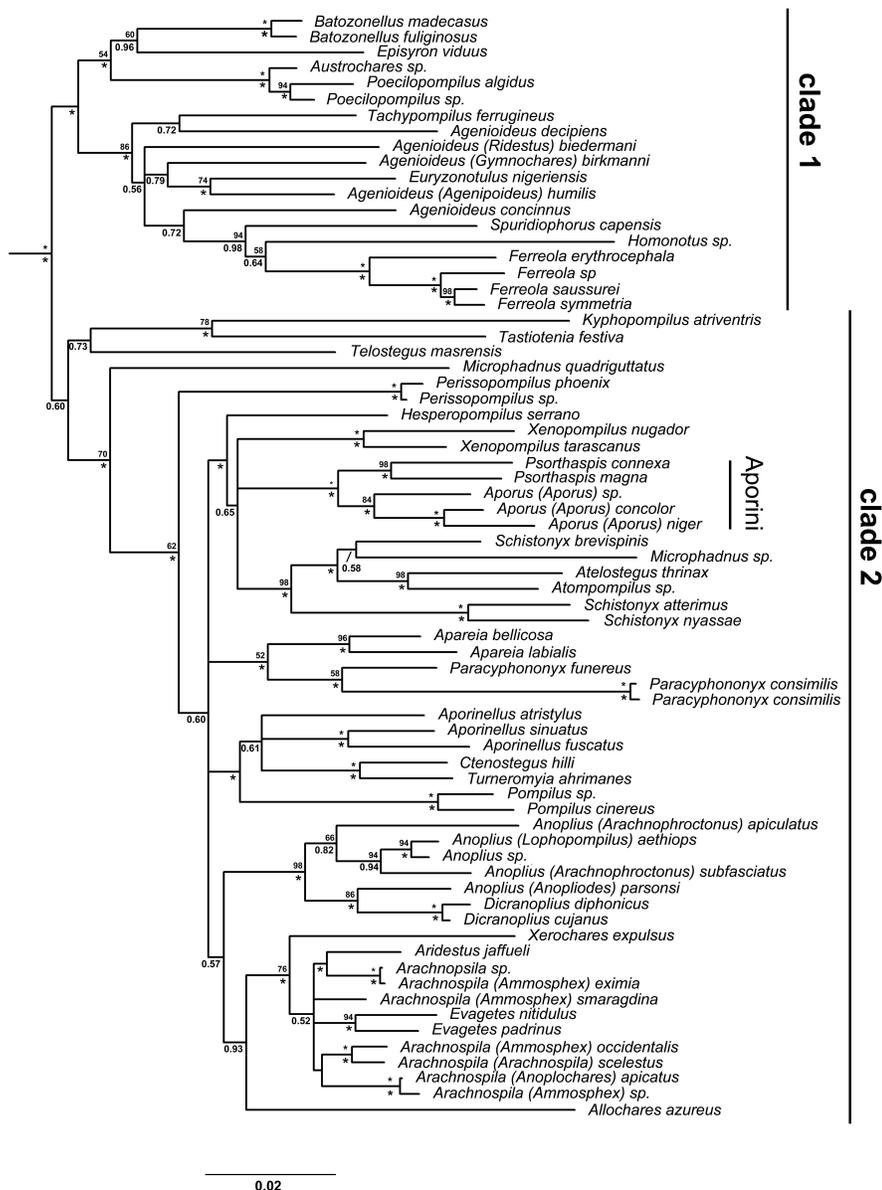


Figure 7.1. Consensus phylogenetic reconstruction for Pompilinae resulting from two Bayesian MCMC runs performed in MrBayes and 100 Bootstrap replicates through a ML search. Bayesian posterior probabilities (PP) are shown below nodes and ML bootstrap support values (BS) are shown above nodes. Nodes with  $PP \geq 0.99$  or  $BS \geq 99$  are indicated with asterisks. Outgroups and support values for nodes with PP or BS < 50 are not shown.

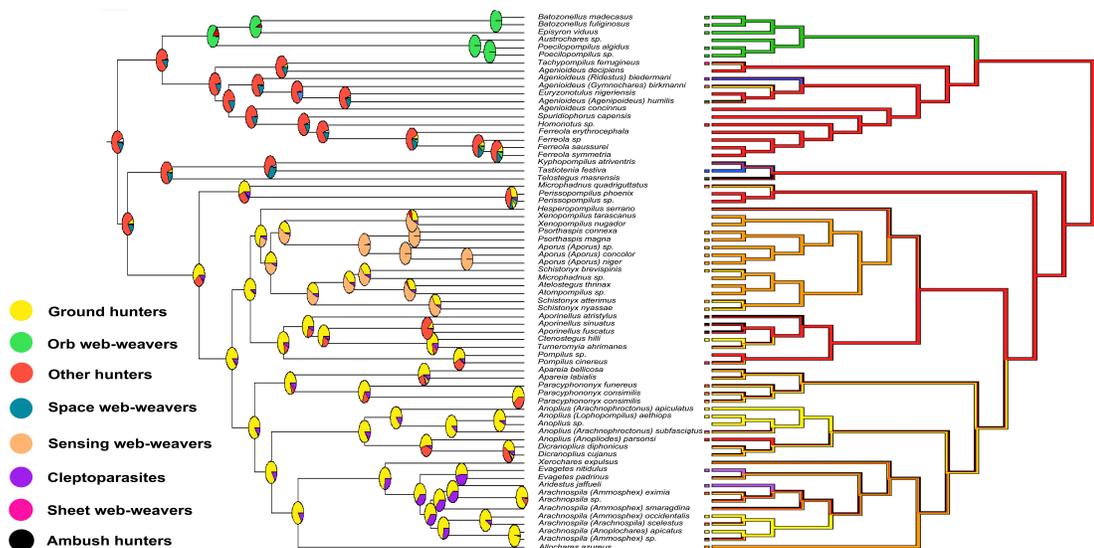


Figure 7.2. Consensus chronogram for Pompilinae resulting from two Bayesian MCMC runs performed in BEAST. Ancestral character mapping by ML is shown on the left with circle areas corresponding to probability of ancestral states. Ancestral character mapping by parsimony is shown on the right with colored lines corresponding to ancestral state.

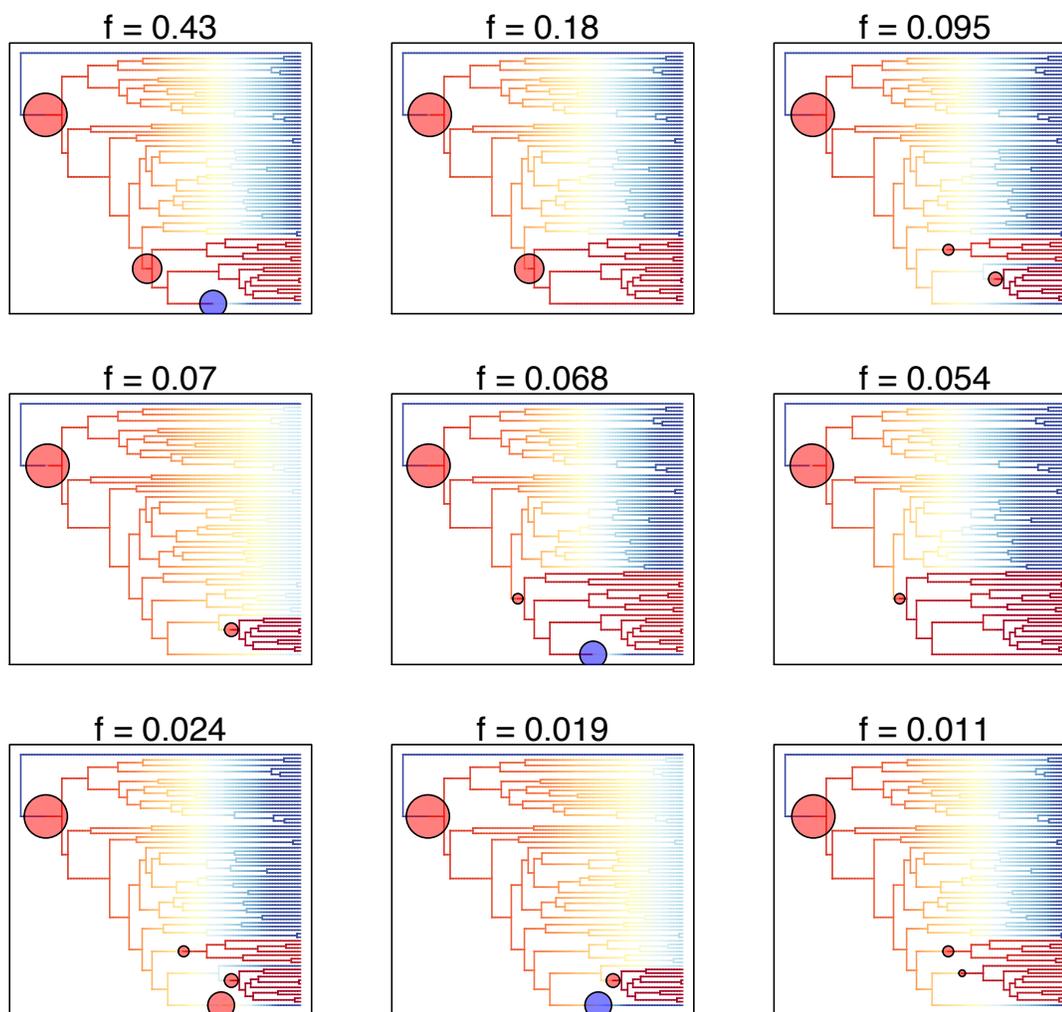


Figure 7.3. Set of distinct diversification rate-shift configurations sampled by BAMM during simulation of the posterior. These are the nine most commonly sampled configurations. Warm colors indicate high diversification rates. Cold colors indicate low diversification rates. Red or blue dots indicate diversification rate-shifts. Larger dots indicate larger diversification rate-shift. The sampling frequency of each diversification scheme is shown over each plot

## CHAPTER 8

## SUMMARY AND CONCLUSIONS

Pompilids are a diverse group of wasps, with an understudied taxonomy, that have a great potential as models for answering compelling evolutionary and biogeographical questions. Some limitations like the lack of knowledge of their natural history, classification and phylogenetics hinder the advancement in these fields. Recently published phylogenetic studies have been based solely of morphological data (Pitts et al., 2006; Shimizu, 1994), which have not proven to be highly informative. In light of the lack of knowledge about the systematics of Pompilidae, this dissertation research focused on the use of molecular data to generate phylogenetic reconstructions at various taxonomic levels to address evolutionary, biogeographical, and taxonomic questions in spider wasps. Chapters 2 and 3 aimed at studying fossil Pompilidae in order to establish accurate calibration points for time divergence studies. Chapter 4 aimed to study the utility of molecular data for species delimitations and sex-associations in the genus *Drepanaporus*. Chapter 5 aimed to test various biogeographic hypotheses using *Aporini* as a model. Chapter 6 aimed to understand the influence of codivergence in the evolution of Müllerian mimicry between velvet ants and *Psorthaspis* spider wasps. Chapter 7 aimed to study the correlation between diversification rate-shifts and host shifts in Pompilinae.

This study expanded our knowledge of the phylogenetics, classification, and evolution of Pompilidae at various hierarchical levels and geological ages. This dissertation revealed, in the first place, that the accurate study of the fossil record is a valuable step for the understanding of the evolutionary history of any group. The results of Chapters 2 and 3 established the correct age of Pompilidae based on the fossil record,

which is almost 50 Myr younger than had been established by previous analyses (i. e. Engel and Grimald, 2006). Moreover, the thorough taxonomic study of Pompilidae fossils established accurate calibration points to be used for divergence time estimation studies.

Molecular studies presented in this work also established the utility of nuclear molecular makers, specifically intron data from LWRh, for species delimitation and sex-associations in pompilids of the genus *Drepanaporus*. Moreover, it was determined that the use of COI for species delimitation and sex associations should be taken with caution, because the sequences can sometimes produce misleading results.

I also established the utility of molecular data to produce time-calibrated phylogenies suitable for the study of biogeographical and evolutionary processes in spider wasps. With the use of molecular data, I tested various biogeographical hypotheses that had never been tested before for Pompilidae. I also expanded North America's largest Müllerian mimicry complex and determined the effect of coevolution in its development. Finally, I tested the hypothesis that host switches have an influence in diversification rate shifts.

Spider wasps are still an understudied group with great potential for evolutionary studies. Because of this, the usage of molecular phylogenetics to understand the diversity and evolution of this group proves promising as a contribution to the knowledge of the diverse insect fauna of the world.

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APPENDICES

Appendix A

Coauthor Permission Letters

22 April, 2014

Juanita Rodriguez has my permission to include the following paper, which has been submitted for publication, of which I was co-author, in her doctoral dissertation.

**Rodriguez, J.,** Waichert, C., von Dohlen, C. D., Poinar Jr, G. & Pitts, J. P. Two new genera and three new species of fossil Pompilidae from amber and their evolutionary implications. *Acta Palaeontologica Polonica*.

*George Poinar* April 22, 2014  
George Poinar  
Corvallis, Oregon

22 April, 2014

Juanita Rodriguez has my permission to include the following paper, which has been submitted for publication, of which I was co-author, in her doctoral dissertation.

**J. Rodriguez, C.D. von Dohlen, J.P. Pitts and Wilson J. S.** Müllerian mimicry as a result of codivergence between velvet ants and spider wasps. *Evolution*.

A handwritten signature in black ink, appearing to read 'Joe Wilson', written in a cursive style.

Joseph Wilson

22 April, 2014

Juanita Rodriguez has my permission to include the following paper, which has been submitted for publication, of which I was co-author, in her doctoral dissertation.

**Rodriguez, J.,** Waichert, C., von Dohlen, C. D., Poinar Jr, G. & Pitts, J. P. Two new genera and three new species of fossil Pompilidae from amber and their evolutionary implications. *Acta Palaeontologica Polonica*.

Cecilia Waichert

22 April, 2014

Juanita Rodriguez has my permission to include the following paper, of which I was co-author, in her doctoral dissertation.

**Rodriguez, J.,** Waichert, C., von Dohlen, C. D. & Pitts, J. P. Review of fossil spider wasps (Hymenoptera: Pompilidae) with the description of a new species.

Cecilia Waichert

**CURRICULUM VITAE****JUANITA RODRIGUEZ****EDUCATION****Ph.D., Biology**, Utah State University, Department of Biology (2008-Present)

Advisors: James P. Pitts &amp; Carol D. von Dohlen

**M.Sc., Biology-Systematics & Taxonomy**, Universidad Nacional de Colombia,  
Department of Biology (2006-2008)

Advisor: Fernando Fernandez

Thesis: Morphological phylogeny of the *Solenopsis* genus-group (Hymenoptera:  
Formicidae)**B.S., Biology**, Universidad Nacional de Colombia, Department of Biology (2000-2005),  
mention of honor.

Advisor: Fernando Fernandez

Thesis: The genus *Odontomachus* (Hymenoptera: Formicidae) in Colombia**PROFESSIONAL EXPERIENCE****2008 - Present**Graduate Research Assistant: Evolution of Behavior and  
Phylogeny of Pompilidae based on morphology and  
molecular data, Utah State University

- 2007 - Present**                      Researcher: Molecular phylogeny of Solenodpsidine ants, Instituto de Ciencias Naturales, Universidad Nacional de Colombia in collaboration with Smithsonian Institution
- 2006 - 2008                            Main researcher: Phylogeny of *Solenopsis* genus-group based on morphology, Universidad Nacional de Colombia.
- 2005-2006                            Main researcher: Revision of genus *Odontomachus* in Colombia, Universidad Nacional de Colombia
- 2006                                      Curatorial assistant for Formicidae: Museo de Historia Natural. Instituto de Ciencias Naturales, Universidad Nacional de Colombia
- 2004 - 2005                            Curatorial assitant for *Odontomachus* (Hymenoptera Formicidae): Instituto de Investigación de Recursos Biológicos Alexander von Humboldt
- 2004 – 2005                            Insect taxonomist: Museo Nacional de Colombia.  
Diagnostic evaluation of insect collections

## PUBLICATIONS

### *Peer Reviewed Journal Articles*

#### **Published**

- Rodriguez, J.**, von Dohlen, C. D. and Pitts, J. P. 2010. The genus *Psorthaspis* (Hymenoptera: Pompilidae) in Colombia. *Caldasia* 32 (2): 407-413.

- Leavengood, J. M., Waichert, C & **Rodriguez, J.** 2011. A distributional checklist of the spider wasps (Hymenoptera: Pompilidae) of Florida. *Insecta Mundi* 0161: 1-8.
- Waichert, C. W., **Rodriguez, J.** & Pitts, J. P. 2012. The Spider Wasps (Hymenoptera: Pompilidae) of the Dominican Republic. *Zootaxa* 3353: 1-47.
- Giraldo, C., **Rodriguez, J.** & Pitts, J. P. 2012. Temporal activity patterns of the spider wasp *Pepsis montezuma* Smith (Hymenoptera: Pompilidae) in a disturbed lower montane rainforest (Manizales, Colombia). *Psyche*, vol. 2012, Article ID 516419, 4 pages, 2012. doi:10.1155/2012/516419.

### **Accepted**

- Rodriguez, J.**, von Dohlen, C. D. & Pitts, J. P. Assessing Species Boundaries and Sex-Associations in the Genus *Drepanaporus* (Hymenoptera: Pompilidae), With Comparison of the Utility of Cytochrome C Oxidase I and a Nuclear Molecular Marker. *Annals of the Entomological Society of America*.

### **In review**

- Rodriguez, J.**, Waichert, C., von Dohlen, C. D., Poinar Jr, G. & Pitts, J. P. Two new genera and three new species of fossil Pompilidae from amber and their evolutionary implications. *Acta Palaeontologica Polonica*.
- Rodriguez, J.**, von Dohlen, C. D. and Pitts, J. P. Historical biogeography of the recently diverged widespread spider wasp tribe Aporini (Hymenoptera: Pompilidae). *Journal of Biogeography*.

**J. Rodriguez, C.D. von Dohlen, J.P. Pitts and Wilson J. S.** Müllerian mimicry as a result of codivergence between velvet ants and spider wasps. *Evolution*.

### **Submitted**

Waichert, C., **Rodriguez, J.**, Wasbauer, M., von Dohlen, C. D. & Pitts, J. P. Molecular phylogeny of spider wasps: redefining subfamily boundaries and the origin of the family. *Zoologica Scripta*.

### ***Book Chapters***

**Rodriguez, J.** 2007. *Odontomachus*, In Jiménez, E., Fernández, F., Arias, T.M. & Lozano-Zambrano, F. H. 2007. Sistemática, biogeografía y conservación de las hormigas cazadoras de Colombia. Instituto de Investigación de Recursos Biológicos Alexander von Humboldt. Bogotá D.C., Colombia 622 p.  
[http://www.humboldt.org.co/publicaciones/uploads/192\\_HORMIGAS\\_CAZADO\\_RAS.pdf](http://www.humboldt.org.co/publicaciones/uploads/192_HORMIGAS_CAZADO_RAS.pdf)

## **FUNDING**

### ***Awarded Grants***

1. Utah State University, Office of Research and Graduate Studies, Dissertation Completion Grant, Juanita Rodriguez, **Aug 2013**, \$20,000.
2. Utah State University Center of Women and Gender Research Grant: Historical Biogeography of *Ceropales* (Hymenoptera: Pompilidae), Juanita Rodriguez PI,

James Pitts Co-PI (Utah State University), Carol von Dohlen Co-PI (Utah State University), **Aug 2013**, \$500.

3. Utah State University Center of Women and Gender Research Grant: Historical Biogeography of the tribe Aporini (Hymenoptera: Pompilidae), Juanita Rodriguez PI, James Pitts Co-PI (Utah State University), Carol von Dohlen Co-PI (Utah State University), **Dec 2010**, \$500.
4. Utah State University Graduate Student Professional Conference Award: Graduate Student Paper Competition Entomological Society of America Annual Meeting, Historical Biogeography of the tribe Aporini (Hymenoptera: Pompilidae), **Dec 2010**, \$300.
5. Universidad Nacional de Colombia Graduate Student Research Award: Phylogeny of *Solenopsis* genus-group based on morphology, **Aug 2006**, \$5,000.

## REGIONAL AND NATIONAL MEETINGS

### Talks

1. **J. Rodriguez**, C.D. von Dohlen and Pitts, J.P. “Is nesting behavior evolving in a step-wise evolution of increasing behavioral complexity in spider wasps? The case of the subfamily Pompilinae (Hymenoptera: Pompilidae)” Entomological Society of America Meeting, Knoxville, TN, 2012. *Awarded 1<sup>st</sup> place in Ph. D. Student Competition.*
2. **J. Rodriguez**, C. Waichert, C.D. von Dohlen and Pitts, J.P. “The origin and diversification of spider wasps (Hymenoptera:Pompilidae)” International Society of

Hymenopterists Meeting, Entomological Society of America Meeting, Knoxville, TN, 2012. *Awarded 2<sup>nd</sup> place in Ph. D. Student Competition.*

3. **J. Rodriguez**, C.D. von Dohlen and Pitts, J.P. “Post-Pangean processes leading to cosmopolitan distribution in Hymenoptera: the case of three widespread spider wasp (Hymenoptera: Pompilidae) taxa” Willi Hennig Society Meeting, Riverside, CA, 2012”.
4. **J. Rodriguez**, C.D. von Dohlen and Pitts, J.P. “Historical Biogeography of the cosmopolitan spider wasp genus *Ceropales* (Hymenoptera: Pompilidae)” Entomological Society of America Meeting, Reno, NV, 2011”.
5. **J. Rodriguez**, C.D. von Dohlen and Pitts, J.P. “Historical Biogeography of the Aporini (Hymenoptera: Pompilidae)” Entomological Society of America Meeting, San Diego, CA, 2010”. *Awarded 1<sup>st</sup> place in Ph. D. Student Competition.*

### Posters

1. **Rodriguez**, C.D. von Dohlen and Pitts, J.P. “The origin and diversification of spider wasps (Hymenoptera:Pompilidae)” Evolution meeting, Snowbird, Utah, 2013
2. **J. Rodriguez**, C.D. von Dohlen and Pitts, J.P. “The genus *Psorthaspis* (Hymenoptera: Formicidae) in Colombia” USU Graduate Research Symposium, Logan, UT, 2010. *Awarded 1<sup>st</sup> place in Biology Student Competition.*
3. **J. Rodriguez**, C.D. von Dohlen and Pitts, J.P. “Historical Biogeography of the Aporini (Hymenoptera: Pompilidae)” Entomological Society of America Pacific Branch Meeting, Boise, ID, 2010. *Awarded 2<sup>nd</sup> Place in Ph.D. Student*

***Competition.***

4. **J. Rodriguez** and Fernandez, F. “Phylogeny of *Solenopsis* genus-group (Solenopsisini: Hymenoptera: Formicidae) based on morphology” II Congreso Colombiano de Zoología, Santa Marta, Colombia, 2006.
5. **J. Rodriguez** and Campos, D. “Morphometric Studies on *Odontomachus haematodus* species complex” V Coloquio de Insectos Sociales IUSI- Sección Bolivariana, Cali, Colombia, 2005.

**PROFESSIONAL SOCIETY MEMBERSHIPS**

American Entomological Society, Systematics, Evolution and Biodiversity Section

(2008-)

International Society of Hymenopterists (2011-)

Society for the Study of Evolution (2013-)

**WORKSHOPS**

1. Getting started as a successful proposal writer and academician, Utah State University, October, 2013.
2. Workshop on molecular evolution, Český Krumlov, Czech Republic, January, 2013.
3. Genomics for phylogenetics and phylogeography, Yale University, January, 2014.

**TEACHING EXPERIENCE**

- 2008 – Present** Graduate Teaching Assistant: Department of Biology, Utah State University. Biology 1610, 1620 honors section. Human Physiology.
- 2007 Biology high school instructor: Newman School, Cajica, Colombia
- 2006 English instructor: Praxis Language School, Bogota, Colombia
- 2004 – 2006 Tutor: Elementary and highschool math, biology, physics, chemistry and English, Bogota, Colombia
- 2003 - 2004 Tutor: Aprender Centro de Estudios, Bogota, Colombia