Molecular phylogeny of Pompilinae (Hymenoptera: Pompilidae): evidence for rapid diversification and host shifts in spider wasps

Juanita Rodriguez ^{a1*}, James P. Pitts ^b, Jaime A. Florez ^c, Jason E. Bond ^d, and Carol D. von Dohlen ^e.

^a Utah State University, Department of Biology, 5305 Old Main Hill, Logan, UT 84322–5305, USA; juanitarodrigueza@gmail.com

^b Utah State University, Department of Biology, 5305 Old Main Hill, Logan, UT 84322–5305, USA; email: jpitts@biology.usu.edu

^c Utah State University, Department of Biology, 5305 Old Main Hill, Logan, UT 84322–5305, USA; email: jaimef69@yahoo.com

^d Auburn University, Department of Biological Sciences, 101 Rouse LSB, Auburn, AL 36849, USA; email: jeb0037@auburn.edu

^e Utah State University, Department of Biology, 5305 Old Main Hill, Logan, UT 84322–5305, USA; email: carol.vondohlen@usu.edu

¹ Present address: Auburn University, Department of Biological Sciences, 101 Rouse LSB, Auburn, AL 36849, USA

*Corresponding author:

Juanita Rodriguez

Utah State University, Department of Biology, 5305 Old Main Hill, Logan, UT 84322–5305, USA; juanitarodrigueza@gmail.com; phone number: 435-797-0358

ABSTRACT

Pompilinae is one of the largest subfamilies of spider wasps (Pompilidae). Most pompilines are generalist spider predators at the family level, but some taxa exhibit ecological specificity (i.e., to spider-host guild). Here we present the first molecular phylogenetic analysis of Pompilinae, towards the aim of evaluating the monophyly of tribes and genera. We further test whether changes in the rate of diversification are associated with host-guild shifts. Molecular data were collected from five nuclear loci (28S, EF1-F2, LWRh, Wg, Pol2) for 76 taxa in 39 genera. Data were analyzed using maximum likelihood (ML) and Bayesian inference (BI). The phylogenetic results were compared with previous hypotheses of subfamilial and tribal classification, as well as generic relationships in the subfamily. The classification of *Pompilus* and *Agenioideus* is also discussed. A Bayesian relaxed molecular clock analysis was used to examine divergence times. Diversification rate-shift tests accounted for taxon-sampling bias using ML and BI approaches. Ancestral host family and host guild were reconstructed using MP and ML methods. Ancestral host guild for all Pompilinae, for the ancestor at the node where a diversification rate-shift was detected, and two more nodes back in time was inferred using BI. In the resulting phylogenies, Aporini was the only previously proposed monophyletic tribe. Several genera (e.g., *Pompilus*, *Microphadnus* and *Schistonyx*) are also not monophyletic. Dating analyses produced a well-supported chronogram consistent with topologies from BI and ML results. The BI ancestral host-use reconstruction inferred the use of spiders belonging to the guild “other hunters” (frequenting the ground and vegetation) as the ancestral state for Pompilinae. This guild had the highest probability for the ML reconstruction and was equivocal for the MP reconstruction;

various switching events to other guilds occurred throughout the evolution of the group. The diversification of Pompilinae shows one main rate-shift coinciding with a shift to ground-hunter spiders, as reconstructed by the BI ancestral character-state analysis.

Keywords: Diversification, classification, host-shift, Pompilinae, spider wasp.

1. Introduction

Spider wasps (Hymenoptera: Pompilidae) are solitary wasps that use spiders to provision their offspring. Typically, a single spider is paralyzed with the stinger and placed in a nest cavity with a single egg. It has been long 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 on which to feed, and the host dies at the end of larval development (Godfray, 1994). With respect to host breadth, most pompilids are generalists even at the spider-host family level. Preference of pompilid females for particular spider taxa seems to be related more to ecological factors rather than taxonomic categories (Evans, 1953).

1.1. Systematics of Pompilinae

The subfamily Pompilinae includes approximately 2,000 species and is one of the most species-rich and ecologically diverse of all 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, other work in progress indicates that Pompilinae should exclude *Chirodamus*, *Cordyloscelis* and *Notocyphus*, and should include *Priochilus* and *Balboana* in Priochilini and *Sericopompilus* in Sericopompilini (Waichert et al., in press). Waichert et al. (in press) suggested that more extensive taxon and gene sampling might advocate raising the latter two tribes to separate subfamilies. The monophyly of some genera within the subfamily has been established with molecular data (Rodriguez et al., 2015), but likewise, more extensive sampling is needed to draw conclusions about the relationships between certain genera and tribes, as well as their monophyly (Pitts et al., 2006; Waichert et al. in press).

The classification within Pompilinae has been historically contentious. Many conflicting arrangements have been proposed as a result of the difficulties with identification of these wasps, whose morphology comprises a considerable degree of interspecific variation (Evans, 1949). The tribal classification has been particularly problematic. Many names have been proposed, but only two have been used consistently, 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 classification of African genera. Bradley (1944) divided the American fauna into seven tribes. Banks (1947) shortly thereafter discussed the difficulty of dividing the subfamily into tribes at all. The only tribe that

Bradley (1944) and Arnold (1937) agreed upon is Pompilini. In the most recent morphological phylogeny (Pitts et al., 2006), only Aporini *sensu* Evans (1949) was recovered as monophyletic. A comprehensive revision of the world fauna is sorely needed to produce a stable tribal scheme that corresponds to natural groups (Evans, 1949).

1.2. Systematics of *Pompilus*

Pompilus sensu lato is a large problematic genus in Pompilinae. Members of this genus are morphologically and behaviorally diverse and distributed in the Americas and the Palearctic region. *Pompilus s.l.* taxonomy has been controversial because of the large number of species assigned to it, solely because they could not be placed reliably elsewhere. The first revision of the genus classified many currently recognized *Pompilus* species in other genera (Wilcke, 1942). Evans (1951) divided the genus into seven subgenera: *Xenopompilus* Evans, *Perissopompilus* Evans, *Xerochaes* Evans, *Hesperopompilus* Evans, *Arachnospila* Kincaid, *Anoplochaes* Banks, *Ammosphex* Wilcke, and *Pompilus sensu* Wilcke (1942). Evans's (1951) scheme was followed for some time until Priesner (1969) referred to *Arachnospila* as comprising the European subgenera proposed by Evans (1951), and excluding *Pompilus sensu stricto*. Day (1981) discussed the taxonomic history of *Pompilus*, restricting it to seven species found only in the Old World, and giving generic status to the subgenera proposed by Evans (1951). *Arachnospila*, *Anoplochaes* and *Ammosphex* were suggested to be included in the *Arachnospila* genus-group (Day, 1981). The classification proposed by Evans (1951) is

used by most authors, because evidence is lacking to support more recent classifications proposed by Priesner (1969) and Day (1981) (Wasbauer and Kimsey, 1985).

1.3. Host use in Pompilinae

Although spider hosts used by Pompilinae represent the breadth of taxonomic diversity, they are even more diverse ecologically. They belong to 21 spider families, which can be classified into seven of eight ecological guilds that Cardoso et al. (2011) defined using *a posteriori* quantitative methods. The data used to delimit guilds were based on multiple ecological characteristics, i.e., foraging strategy, prey range, vertical stratification, and circadian activity. Ground-hunter spiders are active hunters that do not build a web, but rather forage on the ground, and are nocturnal. Other hunters also forage on vegetation in addition to having all the traits found in ground hunters. Ambush hunters have all the traits of other hunters, but they can be either diurnal or nocturnal, and have an ambush strategy for hunting. Web-weavers are differentiated by the kind of capture web they build, either orb web, space web (tri-dimensional webs), or sheet web. Sensing web-weavers build a web that serves to alert them to prey presence and movement. The largest guild identified by Cardoso et al. (2011) was ground hunters (26 families), and the smallest was ambush hunters (6 families).

Individual Pompilinae species are mostly generalists at the host-spider family level, but a few species use a single spider family as hosts. Pompilines show much greater specificity to spider ecological guilds, typically using either ground hunters, other

hunters, ambush hunters, orb web-weavers, sheet web-weavers, space web-weavers, or sensing web-weavers to provision their nests.

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

Diversification rate-shifts have been attributed to niche differentiation, often resulting in a classical adaptive radiation (Schluter, 2000). Environmental differentiation (e.g. climate, topography, vegetation), competition, and specialization can all drive adaptive radiations (Schluter, 2000; Simpson, 1944). Host switching in parasitoids may involve adapting to a new environment, changing the dynamics of competition or avoiding it altogether, 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 significant increases in the diversification rate associated 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). Idiobiont parasitoids prevent further development of the host, while koinobiont parasitoids 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 lack of robust 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 Pompilinae as a model, this study aims to evaluate the correlation between diversification rate-shifts and the evolution of host use. Specifically, our goals were to (1) develop a well-supported phylogeny of Pompilinae and apply it to classification of the subfamily, and (2) use this phylogenetic framework to test whether host-guild switches are correlated with diversification rate-shifts.

2. Materials and Methods

2.1. Taxon sampling

We sampled 84 taxa from 41 ingroup genera (Table 1). We used our interpretation of the taxonomy of *Pompilus* and relatives established by Day (1981). Three Pompilidae species were used for outgroups, namely, a sample from the probable sister lineage Pepsinae (Waichert et al. in press). All specimens used for molecular work were given a unique identifier (voucher ID) and were deposited in the Utah State University

Entomology Collection, Utah State University, Logan, Utah, USA (EMUS). Locality data for voucher specimens are available in figshare:

<http://dx.doi.org/10.6084/m9.figshare.1381882> (Table 1).

2.2. Molecular methods

DNA extraction and amplification of the nuclear genes elongation factor-1 α F2 copy (EF1-F2), 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 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 analyses

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-F2. The model of molecular evolution was determined for each gene and by codon position using PartitionFinder 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 PartitionFinder 1.01. The combined matrix

was analyzed in MrBayes 3.2 with partitions by codon position and gene (Table 3). BI 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, 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 of Pompilinae 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. (in press). 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 generations was 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 (Supplementary Table 1).

We used both 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: 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. The root probability was determined using the procedure described by Maddison et al. (2007) and FitzJohn et al. (2009). The MP character mapping was performed in Mesquite ver. 2.7.5 (Maddison and Maddison, 2011) with all character-state changes weighed equally. To account for phylogenetic uncertainty, we performed an ancestral character reconstruction in MrBayes 3.2 for several nodes of particular interest:

the MRCA of all Pompilinae, the node where a diversification rate- shift was obtained, and two more nodes back in time. This analysis was run at the same time with the BI reconstruction. The character “host guild” was excluded from the topology estimation (see Phylogenetic analysis section 2.3).

2.6. 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 laser package (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 (see Phylogenetic Results, section 3.1) into two main clades, clade 1 and clade 2 (see Figure 1), and excluded Prio chilini and Sericopompilini from the analysis to be able to analyze two large clades. The division was based on the time of missing 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*, *Anoplochares*, *Ammosphex*, *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” dataset. The difference between the semi-empirical and the null distribution is that the semi-empirical dataset contains information on the “real topology” and the time of missing branching times, whereas the null distribution is a dataset of birth-death trees with the same number of taxa generated at random.

We calculated the AIC and ΔAIC_{Crc} for the semi-empirical and null-distribution datasets. The ΔAIC_{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 ΔAIC_{Crc} is positive when the data best fit a rate-variable model. We then calculated the mean and standard deviation of AIC and ΔAIC_{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 (ΔAIC_{Crc}), according to criteria suggested by Rabosky (2006). We performed a t-test to determine if the ΔAIC_{Crc} from the null distribution was significantly different than the semi-empirical data (trees with simulated branching events). Our diversification rates results are expressed in units of speciation events per million years (sp/Myr).

We also performed a BI 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 them into the analysis, as suggested in the BAMM user manual. 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 was 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 analyses

Our phylogenetic analyses resulted in three main lineages: Sericopompilini, Prio chilini and the remaining Pompilinae (Figure 1). The remaining Pompilinae can be divided in two major clades (Figure 1). Within these two clades, various sub-clades are well supported in one or both ML and BI analyses. Clade 1 includes three main sub-clades: *Batozonellus* + *Episyrion*, *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 *Xerochaes*, *Allochaes*, *Arachnospila*, *Ammosphex*, *Anoplochaes*,

Evagetes and *Aridestus*. Within clade 2, *Kyphopompilus* + *Tastiotenina*, *Telostegus*, and *Microphadnus* form the sister group to remaining taxa, although support for their common lineages was weak.

The phylogeny presented shows Aporini as the only monophyletic tribe with high support. The remaining tribes were not supported by our analysis, but it is possible that more sampling and higher support values could render some monophyletic (Figure 1). Some of the tribes proposed by Arnold (1937) are monophyletic by definition, because they contain only one genus (i.e. *Cordyloscelini*, *Spuridiophorini* and *Tachypompilini*). We included representatives of the latter two to test their position in Pompilinae phylogeny. However, a previous study concluded that *Cordyloscelis* is not a member of Pompilinae (Waichert *et al.*, in press); thus, we omitted *Cordyloscelis* from this study.

The Bayesian posterior probabilities (BPP) reject the monophyly of various pompiline genera and subgenera. The following genera are not supported as monophyletic by BPP: *Pompilus sensu* Evans (1951), *Schistonyx* and *Microphadnus*. The three genera of *Arachnospila* genus-group (*Arachnospila*, *Ammosphex*, and *Anoplochaeres*) are recovered in the same clade; this clade also includes *Aridestus* and *Evagetes*, whose positions would render *Arachnospila* genus-group paraphyletic. High BS or BPP values, however, do not support this conclusion, and more data are needed to study the circumscription of this group.

3.2. Evolution of host use

The likelihood-ratio test performed on host-guild ancestral-state reconstruction suggests that the character is evolving under the ARD model ($p = 0.376$). The ancestral condition for Pompilinae was the use of other hunters as hosts in the BI analysis. This guild had the highest probability for the ML reconstruction and was equivocal for the MP reconstruction (Figure 2; Supplementary Table 2). According to the highest probability state in the ML ancestral-state reconstruction, a shift from ancestral use of other hunters to the use of orb-web weavers occurred in clade 1. In clade 2, there was a shift to sensing web-weavers, two shifts to the use of other hunters, and a single shift to cleptoparasitism (Figure 2).

3.3. Diversification rate-shift analysis

DeltaAIC_C 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; Supplementary Table 3). 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 is a yule3rate model (Supplementary Table 4).

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 3). We will refer to this node as DRS-node (diversification rate-shift node) and the clade it defines as DRS-clade (diversification rate-shift clade).

4. Discussion

4.1. Pompilinae phylogeny and tribal classification

The tribe Prioichilini, which was reinstated by Waichert et al. (in press), is here recovered as monophyletic with high posterior probability values (PP=0.98), but low bootstrap support values (BS=68) (Figure 1). Waichert et al. (in press) noted several distinctive morphological traits that would suggest elevating Prioichilini to the subfamily level, but did not do so due to low nodal support. A more extensive analysis is needed to justify this taxonomic change.

Pompilinae, as defined herein, is one of the largest subfamilies of Pompilidae. Its monophyly has been supported by molecular data (Waichert et al., in press). Pompilinae, exclusive of Prioichilini and Sericopompilini, sort into two main clades (Figure 1). Within these clades, various sub-clades are well supported, but the relationships between many of these are not, especially for clade 2 (Figure 2). More extensive sampling, and a greater number of molecular loci will likely improve phylogenetic resolution. The ambiguities

observed in Pompilidae phylogenies may be a consequence of extremely short internodes produced by a rapid radiation (Whitfield and Lockhart, 2007). Supporting evidence for a rapid radiation of Pompilidae is the morphological homogeneity observed even at the subfamily level, the relatively young age of crown-group taxa, and the appearance of all subfamilies in a relative short period of time (Waichert et al., in press).

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 comprising *Episyron*, *Batozonellus*, *Poecilopompilus* and *Austrochares*. The close relationship of *Episyron* and *Poecilopompilus* has also been discussed by Evans (1949). Pitts et al. (2006) also supported a lineage including *Arachnospila*, *Evagetes*, and *Xerochares*, along with other genera not recovered within 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 here with molecular data suggesting a high degree of convergence in morphological characters.

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, is best 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, many taxonomic problems remain to be solved, such as the definition of *Agenioideus* and *Arachnospila*. These are in need of revision with broader sampling and informative molecular phylogenetic analyses. 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*, *Xerochaes*, *Hesperopompilus*, *Arachnospila*, *Anoplochaes*, *Ammosphex*, and *Pompilus*), which continued to be used after 1981, are not members of a single clade; thus, they should be considered separate genera. Here we provide phylogenetic evidence to establish *Xenopompilus*, *Perissopompilus*, *Xerochaes*, *Hesperopompilus*, and *Pompilus sensu* Day (1981) as valid genera. The morphological similarity and probable phylogenetic relatedness of *Pompilus* had been discussed by Day (1981). Our analyses show that *Pompilus* and *Aporinellus* are sister taxa; nevertheless, this assemblage does not include *Hesperopompilus* as suggested by Day (1981). *Hesperopompilus* is more closely related to *Xenopompilus* and *Aporini*.

4.3. Evolution of host use and diversification in Pompilinae

Our results suggest that Pompilinae wasps are not specialists at the level of spider-host family. Most of the genera parasitize more than one spider family. Nevertheless, when the host family is grouped into guilds, various pompiline genera (about half of the species sampled) appear to be specialists at the ecological level (Supplementary Table 1). 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 (Tschopp et al., 2013). Thus, specialization takes place at 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 indicates that Pompilinae did not diversify at a constant rate throughout its history. A significant rate shift in clade 2 is supported by both analytical approaches. The rate shift found in clade 1 by stepwise AIC was not supported by the BAMM results, which recover an overall slow diversification rate for that clade (Figure 3). This result, together with the enhanced robustness of the BAMM analysis, renders 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 even when many distinct combinations of rate 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 approaches is observed in a lineage whose ancestral host is ground hunters according to the BI and ML analyses, and is equivocal between other hunters, cleptoparasites and ambush hunters for the MP analysis. Moreover, the BI analysis shows that a shift from ambush hunters to ground hunters occurred somewhere between the along the stem group of DRS-clade + sister group (Figure 2, Supplementary Table 2). The ground-hunter spiders are the most diverse guild at the family level (Cardoso et al., 2011). The ability to exploit a greater number of spider species may have made more niches available for the *Anoplius* and *Arachnospila*

lineage and spurred the hypothesized shift in the diversification rate. This may occur through genetic divergence of populations shifting 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) as a result of environmental differentiation, competition, and specialization, as well as antagonistic interactions with hosts (Thompson, 1999). For Pompilinae wasps, 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 araneomorph 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 hunting 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 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.

It has been recently proposed that Pompilidae diversification was triggered by spider familial diversification (Wilson et al. 2013). Host-shifts in pompilids, however, do not seem to be temporally correlated with the divergence of spider guilds. Even though ecological guilds are interspersed within the spider phylogeny, the divergence of most extant spider clades containing these guilds took place around 100 Ma (Bond et al. 2014), more than 50 Ma before the origin of Pompilidae.

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 account for all the world fauna and that represent 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 are 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 diversification patterns.

Acknowledgements

We are thankful to Dr. Charles Bell (UNO) for providing his R scripts. We thank various entomological collections for specimen loans. We also thank Dr. Raymond

Wahis for the identification of Pompilinae from the Palearctic. We thank Jill Warnock, Satin Tashnizi, and Tiana Hammer for help with collecting molecular data. We thank Cecilia Waichert (USU) for her assistance in obtaining Pompilinae host records. This work was supported by a Center for Women and Gender Graduate Student Grant, the Utah State University Agricultural Research Station (UAES#8808) and the National Science Foundation (award DEB-0743763 to JPP and CDvD).

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version.

References

- Althoff, D.M., 2008. A test of host-associated differentiation across the 'parasite continuum' in the tri-trophic interaction among yuccas, bogus yucca moths, and parasitoids. *Mol. Ecol.* 17, 3917-3927.
- Arnold, G., 1937. The Psammocharidae (olim Pompilidae) of the Ethiopian Region. Part 7. Subfamily Psammocharinae, Continued. *Ann. Transvaal Mus.* 19, 1-98.
- Ashmead, W., 1902. Classification of the fossorial, predaceous and parasitic wasps of the superfamily Vespoidea. Part 4. *Can. Entomol.* 34, 79-88.
- Baer, C.F., Tripp, D.W., Bjorksten, T.A., Antolin, M.F., 2004. Phylogeography of a parasitoid wasp (*Diaeretiella rapae*): no evidence of host-associated lineages. *Mol. Ecol.* 13, 1859-1869.

- Banks, N., 1947. Studies of South American Psammocharidae. Part 2. Bull. Mus. Comp. Zool. Harv. Coll. 99, 371-486.
- Beaulieu, J.M., Oliver, J.C., O'meara, B., 2014. corHMM: analysis of binary character evolution.
- Blackledge, T.A., Coddington, J.A., Gillespie, R.G., 2003. Are three-dimensional spider webs defensive adaptations? Ecol. Lett. 6, 13-18.
- Bond, J.E., Garrison, N.L., Hamilton, C.A., Godwin, R.L., Hedin, M., Agnarsson, I., 2014. Phylogenomics resolves a spider backbone phylogeny and rejects a prevailing paradigm for orb web evolution. Curr. Bio. 24, 1765-1771.
- Bradley, J.C., 1944. A preliminary revision of the Pompilinae (exclusive of the tribe Pompilini) of the Americas (Hymenoptera: Pompilidae). Trans. Am. Entomol. Soc. 70, 23-157.
- Brower, A.V.Z., DeSalle, R., 1998. Patterns of mitochondrial versus nuclear DNA sequence divergence among nymphalid butterflies: the utility of wingless as a source of characters for phylogenetic inference. Insect Mol. Biol. 7, 73-82.
- Cardoso, P., Pekar, S., Jocque, R., Coddington, J.A., 2011. Global patterns of guild composition and functional diversity of spiders. Plos One 6, e21710.
- Cocroft, R.B., Rodriguez, R.L., E., H.R., 2008. Host shifts, the evolution of communication, and speciation in the *Enchenopa binotata* species complex of treehoppers. In: Tilmon, K.J. (Ed.), Specialization, Speciation and Radiation: The Evolutionary Biology of Herbivorous Insects. University of California Press, Berkeley, CA, pp. 88-100.

- Cronin, J.T., Abrahamson, A.G., 2001. Do parasitoids diversify in response to host-plant shifts by herbivorous insects? *Ecol. Entomol.* 26, 347-355.
- Cusimano, N., Stadler, T., Renner, S.S., 2012. A new method for handling missing species in diversification analysis applicable to randomly or nonrandomly sampled phylogenies. *Syst. Biol.* 61, 785-792.
- Danforth, B.N., Ji, S.Q., 1998. Elongation factor-1 alpha occurs as two copies in bees: implications for phylogenetic analysis of EF-1 alpha sequences in insects. *Mol. Biol. Evol.* 15, 225-235.
- Day, M.C., 1981. A revision of *Pompilus* Fabricius (Hymenoptera: Pompilidae), with further nomenclatural and biological considerations. *Bull. Br. Mus. (Nat. Hist) Entomol.* 42, 1-42.
- Drummond, A.J., Ashton, B., Buxton, S., Cheung, M., Cooper, A., Duran, C., Field, M., Heled, J., Kearse, M., Markowitz, S., Moir, R., Stones-Havas, S., Sturrock, S., Thierer, T., Wilson, A., 2011. Geneious v5.4. Available from <http://www.geneious.com/>.
- Drummond, A.J., Ho, S.Y.W., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. *Plos Biology* 4, 699-710.
- Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *Bmc Evol. Biol.* 7, 214.
- Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular biology and evolution* 29, 1969-1973.
- Ehrlich, P.R., Raven, P.H., 1964. Butterflies and Plants: A Study in Coevolution. *Evolution* 18, 586-608.

- Evans, H.E., 1949. A taxonomic study of the nearctic spider wasps belonging to the tribe Pompilini (Hymenoptera: Pompilidae). Part 1. Trans. Am. Entomol. Soc. 75, 133-270.
- Evans, H.E., 1951. A taxonomic study of the Nearctic spider wasps belonging to the tribe Pompilinae (Hymenoptera: Pompilidae). Part 3. Trans. Am. Entomol. Soc. 77, 203-340.
- Evans, H.E., 1953. Comparative Ethology and the Systematics of Spider Wasps. Syst. Zool. 2, 155-172.
- Felsenstein, J., 1989. PHYLIP -- Phylogeny Inference Package (Version 3.2). Cladistics 5, 164-166.
- FitzJohn, R.G., Maddison, W.P., Otto, S.P., 2009. Estimating trait-dependent speciation and extinction rates from incompletely resolved phylogenies. Biol. 58, 595-611.
- Fordyce, J.A., 2010. Host shifts and evolutionary radiations of butterflies. Proc. R. Soc. B-Biol. Sci. 277, 3735-3743.
- Godfray, H.C.J., 1994. Parasitoids, Behavioral and Evolutionary Ecology. Princeton University Press, Princeton, NJ.
- Gonzaga, M.O., Vasconcellos-Neto, J., 2005. Orb-web spiders (Araneae: Araneomorphae; Orbiculariae) captured by hunting-wasps (Hymenoptera: Sphecidae) in an area of Atlantic Forest in south-eastern Brazil. J. Nat. History 39, 2913-2933.
- Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754-755.

- Lanfear, R., Calcott, B., Ho, S.Y.W., Guindon, S., 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Mol. Biol. Evol.* 29, 1695-1701.
- Maddison, W.P., Midford, P.E., Otto, S.P., 2007. Estimating a binary characters effect on speciation and extinction. *Syst. Biol.* 56, 701-710.
- Maddison, W.P., Maddison, D.R., 2011. Mesquite: a modular system for evolutionary analysis. Version 2.75 <http://mesquiteproject.org>.
- Mardulyn, P., Cameron, S.A., 1999. The major opsin in bees (Insecta: Hymenoptera): a promising nuclear gene for higher level phylogenetics. *Mol. Phylogenetics Evol.* 12, 168-176.
- Margush, T., McMorris, F.R., 1981. CONSENSUS N-TREES. *Bull. Math. Biol.* 43, 239-244.
- McKenna, D.D., Farrell, B.D., 2006. Tropical forests are both evolutionary cradles and museums of leaf beetle diversity. *Proc. Nat. Acad. Sci. U.S.A* 103, 10947-10951.
- McLeish, M.J., Chapman, T.W., Schwarz, M.P., 2007. Host-driven diversification of gall-inducing Acacia thrips and the aridification of Australia. *Bmc Biol.* 5, 3.
- Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Gateway Computing Environments Workshop (GCE)*, 2010, pp. 1-8.
- Pilgrim, E.M., Pitts, J.P., 2006. A molecular method for associating the dimorphic sexes of velvet ants (Hymenoptera : Mutillidae). *J. Kans. Entomol. Soc.* 79, 222-230.

- Pitts, J.P., Wasbauer, M.S., Von Dohlen, C.D., 2006. Preliminary morphological analysis of relationships between the spider wasp subfamilies (Hymenoptera: Pompilidae): revisiting an old problem. *Zool. Scr.* 35, 63-84.
- Plummer, M., Best, N., Cowles, K., Vines, K., Sarkar, D., Almond, R., 2013. coda: Output analysis and diagnostics for MCMC. <http://cran.Rproject.org>
- Priesner, H., 1969. Studien zur taxonomie und faunistik der pompiliden osterreichs. Teil 4. *Nat. Jahrb. Stadt Linz* 15, 77-132.
- Quicke, D.L.J., 1997. Parasitic wasps. Chapman and Hall, London, UK.
- Quicke, D.L.J., 2012. We know too little about parasitoid wasp distributions to draw any conclusions about latitudinal trends in species richness, body size and biology. *Plos One* 7, e32101.
- R development core team, 2010. R: a language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria.
- Rabosky, D.L., 2006. LASER: a maximum likelihood toolkit for detecting temporal shifts in diversification rates from molecular phylogenies. *Evol. bioinform. online* 2, 273-276.
- Rabosky, D.L., Santini, F., Eastman, J., Smith, S.A., Sidlauskas, B., Chang, J., Alfaro, M.E., 2013. Rates of speciation and morphological evolution are correlated across the largest vertebrate radiation. *Nat. Commun.* 4, 1958.
- Rabosky, D.L., 2014. Automatic detection of key innovations, rate shifts, and diversity-dependence on phylogenetic trees. *PLoS ONE* 9, e89543.
- Rabosky, D.L., Grundler, M., Title, P., Anderson, P., Shi, J., Brown, J., Huang, H., 2014. BAMMtools: analysis and visualization of macroevolutionary dynamics on

phylogenetic trees. R package version 1.0.1. <http://CRAN.R-project.org/package=BAMMtools>.

Rodriguez, J., Pitts, J.P., von Dohlen, C., 2015. Historical biogeography of the widespread spider wasp tribe Aporini (Hymenoptera: Pompilidae). *J. Biogeogr.* 42, 495-506.

Schluter, D., 2000. *The Ecology of Adaptive Radiation*. Oxford University Press, New York.

Schulmeister, S., 2003. Simultaneous analysis of basal Hymenoptera (Insecta): introducing robust-choice sensitivity analysis. *Biol. J. Linn. Soc.* 79, 245-275.

Shaw, M.R., 1994. Parasitoid host ranges. In: Hawkins, B.A., Sheehan, W. (Eds.), *Parasitoid community ecology*. Oxford University Press, Oxford, pp. 111-144.

Shimizu, A., 1994. *Phylogeny and classification of the family Pompilidae*. Tokyo Metrop. Univ., Bull. Nat. Hist. 2, 1-142.

Simpson, G.G., 1944. *Tempo and Mode in Evolution*. Columbia University Press, New York.

Smit, J., Nieuwenhuijsen, H., Peeters, T., 2002. Section Hymenoptera of the Netherlands Entomological Society. *Entomol. Ber. (Amst.)* 62, 138-140.

Stadler, T., 2011. Simulating Trees with a Fixed Number of Extant Species. *Syst. Biol.* 60, 676-684.

Symonds, M.R.E., Elgar, M.A., 2013. The evolution of body size, antennal size and host use in parasitoid wasps (Hymenoptera: Chalcidoidea): a phylogenetic comparative analysis. *Plos One* 8, 13.

- Taekul, C., Valerio, A.A., Austin, A.D., Klompen, H., Johnson, N.F., 2014. Molecular phylogeny of telenomine egg parasitoids (Hymenoptera: Platygasteridae s.l.: Telenominae): evolution of host shifts and implications for classification. *Syst. Entomol.* 39, 24-35.
- Thompson, J.N., 1999. What we know and do not know about coevolution: insect herbivores and plants as a test case. In: Olff, H., Brown, V.K., Drent, R.H. (Eds.), *Herbivores: Between Plants and Predators*. Blackwell Science, Oxford, UK, pp. 7-30.
- Tschopp, A., Riedel, M., Kropf, C., Nentwig, W., Klopstein, S., 2013. The evolution of host associations in the parasitic wasp genus *Ichneumon* (Hymenoptera: Ichneumonidae): convergent adaptations to host pupation sites. *BMC Evol. Biol.* 13, 74.
- Uma, D.B., 2010. *Behavioral Ecology of Wasp-Spider Interactions: The Role of Webs, Chemicals, and Deception*. Georgetown University, Washington, D. C.
- Waichert, C., Rodriguez, J., Wasbauer, M.S., von Dohlen, C.D., Pitts, J.P. 2015. Molecular phylogeny and systematics of spider wasps (Hymenoptera: Pompilidae): redefining subfamily boundaries and the origin of the family. *Zoologica Scripta*. In press.
- Wasbauer, M.S., Kimsey, L.S., 1985. California spider wasps of the subfamily Pompilinae (Hymenoptera: Pompilidae). *Bull. Calif. Insect Surv.* 26, 1-130.
- Wheat, C.W., Vogel, H., Wittstock, U., Braby, M.F., Underwood, D., Mitchell-Olds, T., 2007. The genetic basis of a plant-insect coevolutionary key innovation. *Proc. Natl. Acad. Sci. U. S. A.* 104, 20427-20431.

- Whitfield, J.B., Lockhart, P.J., 2007. Deciphering ancient rapid radiations. *Trends Ecol. Evol.* 22, 258-265.
- Wilcke, J., 1942. Het geslacht *Pompilus* F. (*Psammochares* Latr.). *Entomol. Ber. (Amst.)* 11, 23-32.
- Wilson, J.S., von Dohlen, C.D., Forister, M.L., Pitts, J.P., 2013. Family-level divergences in the stinging wasps (Hymenoptera: Aculeata), with correlations to Angiosperm diversification. *Evol. Biol.* 40, 101-107.
- Winkler, I.S., Mitter, C., Scheffer, S.J., 2009. Repeated climate-linked host shifts have promoted diversification in a temperate clade of leaf-mining flies. *Proc. Natl. Acad. Sci. U. S. A.* 106, 18103-18108.
- Zaldivar-Riveron, A., Shaw, M.R., Saez, A.G., Mori, M., Belokoblylskij, S.A., Shaw, S.R., Quicke, D.L.J., 2008. Evolution of the parasitic wasp subfamily Rogadinae (Braconidae): phylogeny and evolution of lepidopteran host ranges and mummy characteristics. *Bmc Evol. Biol.* 8 329.
- Zwickl, D.J., 2006. Genetic Algorithm Approaches for the Phylogenetic Analysis of Large Biological Sequence Datasets Under the Maximum Likelihood Criterion. University of Texas, Austin.

TABLE 1. Outgroup and ingroup species sampled, voucher ID, molecular markers and Genbank accession numbers.

Species	Voucher ID	28S Accession #	EF1-F2 Accession #	LWRh Accession #	Pol2 Accession #	Wg Accession #
Outgroup Taxa						
<i>Macromeris</i> sp.	PO256	KT258345	KT258599	KT258533	KT258365	KT258457
<i>Macromeris violacea</i>	PO518	KT258346	KT258598	KT258534	---	---
<i>Paraclavelia crudelis</i>	PO173	KT258342	KT258596	KT258468	KT258408	KT258453
Ingroup Taxa						
<i>Sericopompilus neotropicalis</i>	PO53	KT258280	KT258594	KT258467	KT258359	KT258463
<i>Balboana</i> sp.	PO394	KT258278	---	KT258495	---	KT258444
<i>Balboana</i> sp.	PO395	KT258279	KT258597	KT258496	---	KT258445
<i>Priochilus</i> sp.	PO398	KT258283	KT258581	KT258526	KT258348	KT258456
<i>Priochilus splendidum</i> .	PO385	KT258282	KT258583	KT258527	KT258350	KT258455
<i>Priochilus sericeifrons</i>	PO260	KT258281	KT258582	KT258525	KT258347	KT258454
<i>Priochilus</i> sp.	PO264	KT258284	KT258584	KT258528	KT258349	---
<i>Agenioideus (Agenioideus) humilis</i>	PO141	KT258294	KT258592	KT258518	KT258405	KT258415
<i>Agenioideus (Gymnochores) birkmanni</i>	PO191	KT258295	KT258593	KT258535	---	KT258417
<i>Agenioideus (Ridestus) biedermani</i>	PO189	KT258289	KT258591	KT258532	---	KT258416
<i>Agenioideus decipiens</i>	PO136	KT258293	KT258585	KT258524	---	---
<i>Agenioideus concinnus</i>	PO340	KT258292	KT258575	KT258520	KT258360	KT258413
<i>Allochores azureus</i>	PO387	KM587052	KM598824	KM587090	KT258403	KM598785
<i>Ammosphex eximia</i>	PO147	KT258307	KT258541	KT258485	KT258376	---
<i>Ammosphex eximia</i>	PO211	KT258308	KT258540	KT258486	KT258377	---
<i>Ammosphex smaragdina</i>	PO153	KT258310	KT258547	KT258489	KT258371	KT258430
<i>Anoplius (Anopliodes) parsonsi</i>	PO187	KT258323	KT258556	KT258514	KT258384	---
<i>Anoplius (Arachnophroctonus) apiculatus</i>	PO76	KT258341	KT258557	KT258475	KT258388	---
<i>Anoplius (Arachnophroctonus) subfasciatus</i>	PO202	KT258320	KT258554	KT258502	---	---
<i>Anoplius (Lophopompilus) aethiops</i>	PO8	KM587027	KM598840	KM587091	KT258382	KM598801
<i>Anoplius</i> sp.	PO120	KT258319	KT258555	KT258500	KT258383	---

<i>Anoplochaeres apicatus</i>	PO13	KT258302	KT258543	KT258487	KT258372	---
<i>Anoplochaeres apicatus</i>	PO171	KT258303	KT258542	KT258488	KT258373	---
<i>Apareia bellicosa</i>	PO205	KT258318	KT258549	KT258515	KT258378	---
<i>Apareia labialis</i>	PO176	KT258317	KT258550	KT258531	KT258380	---
<i>Aporinellus atristylus</i>	PO43	KT258336	KT258553	KT258473	---	KT258436
<i>Aporinellus fuscatus</i>	PO148	KT258313	KT258551	KT258510	---	KT258449
<i>Aporinellus sinuatus</i>	PO42	KT258312	KT258552	KT258469	KT258402	KT258448
<i>Aporus (Aporus) concolor</i>	PO435	KM587060	KM598836	KM587093	---	KM598813
<i>Aporus (Aporus) niger</i>	PO11	KT258314	KT258567	KT258492	KT258393	KT258447
<i>Aporus (Aporus) sp.</i>	PO310	KM587033	KM598856	---	KT258392	KM598804
<i>Arachnospila arctus</i>	PO7	---	KT258545	KT258481	KT258369	KT258422
<i>Arachnospila scelestus</i>	PO158	KT258306	KT258546	KT258483	KT258370	KT258426
<i>Aridestus jaffueli</i>	PO144	KT258309	---	KT258490	KT258374	KT258423
<i>Atelostegus thrinax</i>	PO342	KT258330	KT258562	KT258497	KT258399	KT258451
<i>Atopompilus sp.</i>	PO281	KT258329	KT258563	KT258474	KT258391	KT258450
<i>Austrochaeres sp.</i>	PO105	KT258298	KT258580	KT258523	KT258367	KT258461
<i>Batozonellus fuliginosus</i>	PO204	KT258301	KT258536	KT258465	---	KT258419
<i>Batozonellus madecassus</i>	PO169	KT258300	KT258537	KT258466	KT258352	KT258418
<i>Ctenostegus hilli</i>	PO131	KT258337	KT258574	KT258470	KT258400	KT258432
<i>Dicranoplius cujanus</i>	PO199	KT258322	KT258558	KT258530	KT258385	KT258431
<i>Dicranoplius diphonicus</i>	PO151	KT258321	KT258559	KT258529	---	KT258429
<i>Episyrus viduus</i>	PO203	KT258343	KT258538	KT258511	KT258368	---
<i>Euryzonotulus nigeriensis</i>	PO356	KM587035	KM598818	KM587111	KT258406	KT258420
<i>Evagetes nitidulus</i>	PO400	KT258305	KT258548	KT258482	KT258375	KT258425
<i>Evagetes padrinus</i>	PO315	KT258304	---	KT258484	---	KT258424
<i>Ferreola erythrocephala</i>	PO339	KT258288	KT258589	KT258478	KT258356	KT258412
<i>Ferreola saussurei</i>	PO26	KT258285	KT258588	KT258479	KT258355	KT258410
<i>Ferreola sp.</i>	PO343	KT258286	KT258587	KT258480	KT258354	KT258411
<i>Ferreola symmetria</i>	PO22	KT258287	---	KT258477	KT258353	KT258409
<i>Hesperopompilus serrano</i>	PO129	KT258333	KT258569	KT258501	KT258381	---
<i>Homonotus sp.</i>	PO224	KT258290	KT258590	KT258509	KT258407	KT258458
<i>Kypopompilus atriventris</i>	PO36	---	KT258579	KT258471	KT258361	---
<i>Microphadnus quadriguttatus</i>	PO159	---	KT258576	KT258513	KT258363	KT258452

<i>Microphadnus</i> sp.	PO278	KT258331	KT258568	KT258472	KT258396	KT258440
<i>Paracyphononyx</i> <i>consimilis</i>	PO219	KT258325	KT258577	KT258507	KT258364	---
<i>Paracyphononyx</i> <i>consimilis</i>	PO132	KT258324	KT258578	KT258506	KT258351	---
<i>Paracyphononyx</i> <i>funereus</i>	PO285	KT258326	---	KT258508	KT258379	KT258446
<i>Perissopompilus</i> <i>phoenix</i>	PO70	KT258339	KT258571	KT258498	KT258386	KT258442
<i>Perissopompilus</i> sp.	PO121	KT258340	KT258570	KT258499	KT258387	KT258443
<i>Poecilopompilus</i> <i>algidus</i>	PO49	KT258296	---	KT258521	---	KT258462
<i>Poecilopompilus</i> sp.	PO100	KT258297	---	KT258522	KT258366	KT258460
<i>Pompilus cinereus</i>	PO270	KT258334	KT258572	KT258493	KT258394	KT258438
<i>Pompilus</i> sp.	PO407	KT258335	---	KT258494	KT258395	KT258439
<i>Psorthaspis connexa</i>	PO64	KM587041	KM598862	KM587073	KT258390	KM598798
<i>Psorthaspis magna</i>	PO9	KM587040	KM598850	KM587077	KT258389	---
<i>Schistonyx aterrimus</i>	PO257	KT258327	KT258561	KT258503	KT258397	KT258434
<i>Schistonyx</i> <i>brevispinis</i>	PO346	KT258332	KT258564	KT258505	---	KT258441
<i>Schistonyx nyassae</i>	PO353	KT258328	KT258560	KT258504	KT258398	KT258435
<i>Spuridiophorus</i> <i>capensis</i>	PO337	---	---	---	KT258357	KT258414
<i>Tachypompilus</i> <i>ferrugineus</i>	PO38	KT258291	KT258586	KT258519	KT258358	KT258421
<i>Tastiotenia festiva</i>	PO102	KT258344	KT258595	KT258476	KT258362	---
<i>Telostegus masrensis</i>	PO329	KT258299	KT258539	KT258464	KT258404	KT258459
<i>Turneromyia</i> <i>ahrimanes</i>	PO222	KT258338	KT258573	KT258512	KT258401	KT258433
<i>Xenopompilus</i> <i>nugador</i>	PO119	KT258316	KT258565	KT258517	---	KT258428
<i>Xenopompilus</i> <i>tarascanus</i>	PO116	KT258315	KT258566	KT258516	---	KT258427
<i>Xerochares expulsus</i>	PO54	KT258311	---	KT258491	---	KT258437

TABLE 2. Primers used for PCR amplification and sequencing

Marker	Primer name	Primer sequence (5'–3')	Reference
EF1-F2	F2for1	GGT TCC TTC AAA TAT GCT	Pilgrim et al., 2006
		TGG G	
	F2rev1	A ATC AGC AGC ACC TTT	Danforth and Ji, 1998
		AGG TGG	
LWRh	PompOps1F	ATT CGA CAG ATA CAA	Pilgrim et al., 2006
		CGT AAT CG	
	LWRhR	ATA TGG AGT CCA NGC	Mardulyn and Cameron, 1999
		CAT RAA CCA	
	LWRhRApor	GAG RGA GAT CGT CAT	Rodriguez et al. 2015
		CAA GGC GAC C	
Wg	LepWg1for	GAR TGY AAR TGY CAY	Brower and DeSalle, 1998
		GGY ATG TCT GG	
	modLepWg2rev	ACT ICG CRC ACC ART GGA	Brower and DeSalle, 1998
		ATG TRC A	
28S	CF2	TGG TAA CTC CAT CTA	Pilgrim et al. 2006
		AGG CTA AAT A	
	D5–4625 R (D5R)	CCC ACA GCG CCA GTT	Schulmeister, 2003
		CTG CTT ACC	

Table 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 rd codon position LWRh
2	K80+I+G	1 st codon position EF1-F2, 2 nd codon position EF1-F2, 2 nd codon position LWRh
3	SYM+G	3 rd codon position EF1-F2
4	K80+G	1 st codon position LWRh
5	GTR+I+G	1 st codon position Pol2
6	F81	2 nd codon position Pol2
7	K80+I+G	3 rd codon position Pol2
8	K80+I	1 st codon position Wg
9	JC	2 nd codon position Wg
10	HKY+G	3 rd codon position Wg

FIGURE LEGENDS

Figure 1. Consensus phylogenetic reconstruction for Pompilinae resulting from two Bayesian MCMC runs performed in MrBayes. Bayesian posterior probabilities (BPP) are shown below nodes and ML bootstrap support values from 100 bootstrap replicates (BS) are shown above nodes. Nodes with $PP \geq 0.99$ or $BS \geq 99$ are indicated with asterisks. Support values for nodes with PP or BS < 50 are not shown.

Figure 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 MP is shown on the right with colored lines corresponding to ancestral state. BI ancestral character reconstructions are indicated by an outer red circle and are shown for the ancestor of all Pompilinae, the DRS -node, the MRCA of the DRS -node and its sister clade and the MRCA of the DRS-node and *Hesperopompilus serrano*. A black star indicates the DRS -node.

Figure 3. Set of distinct diversification rate-shift configurations sampled by BAMM during simulation of the posterior. The six most commonly sampled configurations are shown. Warm colors indicate high diversification rates and cold colors indicate low diversification rates. Red or blue dots indicate diversification rate-shifts. Larger dots indicate larger diversification rate-shifts. The sampling frequency of each diversification scheme is shown over each plot.

Figure1

Figure2

Figure3