ABSTRACT

Spider wasps (Hymenoptera: Pompilidae) constitute a monophyletic family supported by numerous morphological and behavioral traits. The subfamilial and tribal classifications, however, have a history of conflicting and confusing designations and nomenclature. Here, we reconstruct a molecular phylogeny of Pompilidae from Bayesian and maximum-likelihood analyses of four nuclear molecular markers (elongation factor–1 α F2 copy, long–wavelength rhodopsin, RNA polymerase II, and 28S ribosomal RNA). A Bayesian divergence-time estimation was performed using four calibration points. An ancestral-area reconstruction was performed with a Bayesian binary Markov chain Monte Carlo method. New relationships are recovered, and new subfamilial delimitations are proposed and discussed based on the phylogeny. The origin of Pompilidae was ca. 43.3 Ma, probably in the Nearctic region. Most of the extant subfamilies originated during the late Eocene through Oligocene, and their current distributions are the product of various dispersal events that occurred over the course of ~40 Ma. This is the first phylogenetic reconstruction of Pompilidae from molecular characters, with broad geographic and taxonomic sampling. The following subfamilies and relationships are recognized: Ctenocerinae + (Ceropalinae + Notocyphinae) + Pompilinae + Pepsinae. We revalidate Notocyphinae, which contains only *Notocyphus*, and define a new tribe in Pompilinae: Sericopompilini. Priochilini is reinstated. Sericopompilini contains *Sericopompilus* as the sole representative; Priochilini contains *Priochilus* and *Balboana*. *Epipompilus* and *Chirodamus* are now classified as Pepsinae.

KEYWORDS

Ceropalinae – Ctenocerinae – Eocene – Notocyphinae – Pepsinae – Pompilinae – Systematics

**Molecular phylogeny and systematics of spider wasps (Hymenoptera: Pompilidae): redefining subfamily boundaries and the origin of the family**

## INTRODUCTION

Spider wasps (Hymenoptera: Pompilidae) are solitary, predatory insects that provision their offspring with spiders as the sole food source. The family contains approximately 4,855 described species grouped into 125 genera (Aguiar *et al.*, 2013) and four subfamilies (Pitts, Wasbauer & von Dohlen, 2006). Although the family has a cosmopolitan distribution, species diversity is highest in tropical regions (Wasbauer, 1995).

Spider wasps exhibit a wide array of nesting and foraging behavior. Females hunt spiders in short flights or while crawling along trails. They usually nest in burrows prepared by scraping soil backward with their forelegs (Evans & Shimizu, 1996; Kurczewski, 2010; Kurczewski & Edwards, 2012), but some species use spider burrows (Williams, 1928), pre-existing cavities (Kurczewski, 1981), or construct aerial nests from mud (Evans & Shimizu, 1996; Barthélémy & Pitts, 2012). Prey-carrying mechanisms also vary considerably throughout the family; these include pulling, pushing, carrying, or flying with the spider to the nest (Evans & Yoshimoto, 1962).

Pompilidae are unquestionably a monophyletic family (Shimizu, 1994; Fernández, 2006; Pitts *et al.*, 2006; Pilgrim, von Dohlen & Pitts, 2008; Debevec, Cardinal

& Danforth, 2012), distinguished morphologically by presence of a straight transverse carina on the mesopleuron, dividing it into upper and lower regions (Townes, 1957), and behaviorally by provisioning nest cells exclusively with a single spider. Divergence-time estimation (Wilson *et al.*, 2013) and the fossil record (Rodriguez *et al.*, 2015) suggest that stem-group Pompilidae appeared in the Upper Cretaceous and crown-group taxa diversified in the early Eocene.

## PHYLOGENETIC POSITION OF POMPILIDAE WITHIN ACULEATA

Historically, there has been disagreement regarding the relationship of Pompilidae to other families of aculeate (stinging) Hymenoptera (reviewed in Brothers, 1999; Pilgrim *et al.*, 2008). Pompilidae has been proposed as the sister group to (1) Rhopalosomatidae (Brothers, 1975, 1999); (2) Sapygidae + Mutillidae (Brothers & Carpenter, 1993); (3) Mutillidae + (Sapygidae + Myrmosinae) (Pilgrim *et al.*, 2008); (4) Mutillidae (excluding Myrmosinae) (Debevec *et al.*, 2012); and (5) Chrysididae (Heraty *et al.*, 2011). More recently, a phylogenomic study recovered Pompilidae as sister to Mutillidae in a clade composed of (Pompilidae + Mutillidae) + a paraphyletic Bradynobaenidae (Johnson *et al.*, 2013). However, this study did not include representatives of Myrmosinae or Sapygidae. The superfamily Pompiloidea was proposed by Pilgrim *et al.* (2008) to include the families Pompilidae, Mutillidae, Sapygidae, and Myrmosidae.

## PHYLOGENETIC RELATIONSHIPS IN POMPILIDAE

The internal classification of Pompilidae has remained unsettled (see Fig. 1 in Pitts *et al.*, 2006). The family and its component subfamilies and tribes have had different names throughout their taxonomic history. Süstera (1912) was the first to group

Pompilidae into subfamilies, dividing the family into three: Pepsinae, Ceratopalinae (=Ceropalinae) and Psammocharinae (=Pompilinae). After Süstera (1912), as many as eight authors have proposed conflicting subfamilial and tribal classifications (e.g., Haupt, 1927, 1930; Arnold, 1932a,b, 1934, 1935, 1936a,b, 1937; Banks, 1912, 1934; Bradley, 1944; Priesner, 1955; Townes, 1957; Shimizu 1994; Pitts *et al.* 2006). Townes' (1957) scheme has been the classification used most often. He suggested three subfamilies: Pepsinae, Pompilinae and Ceropalinae, with Ceropalinae composed of three tribes: Notocyphini, Minageniini and Ceropalini. This last tribe was elevated to subfamily status based on cladistic analyses in subsequent studies (Shimizu, 1994; Pitts *et al.*, 2006). More recently, two studies proposed subfamilial boundaries in Pompilidae based on maximum-parsimony analyses of morphology. Shimizu (1994) proposed six subfamilies: Ceropalinae + (Notocyphinae + (Pepsinae + Pompilinae + Ctenocerinae + Epipompilinae)), and Pitts *et al.* (2006) proposed four subfamilies: Ceropalinae + (Pepsinae + (Ctenocerinae + Pompilinae)).

Tribal classification of Pompilidae has been similarly contentious, with no consensus reached as yet. Some tribes have had as many as seven different names in the past, and the monophyly of most tribes has never been tested. For example, Bradley (1944) divided Pompilinae into seven tribes: Aporini, Ctenocerini, Epipompilini, Pompilini, Pedinaspini, Allocharini, and Allocyphononychini. Allocharini and Allocyphononychini were transferred to Pompilini by Evans (1951). Ctenocerini included taxa currently classified as both Aporini and Ctenocerinae, while Epipompilini was elevated to subfamily level by Shimizu (1994) and transferred to Ctenocerinae by Pitts *et al.* (2006). Similar problems abound in other subfamilies, and the taxonomic confusion

extends to the generic level. Fernández (2006) suggested that several genera in Pompilidae are probably not natural groups and are in need of taxonomic revisions.

The majority of problems and disagreements in Pompilidae classification likely stem from the homogeneous morphology of many spider wasp species. In addition, authors working in different zoogeographical regions have used different upper-level classifications. This discordance between authors at tribal and generic levels has generated a plethora of names, causing further confusion. Some higher classifications of Pompilidae were proposed based on characters that are either non-apomorphic or are probably homoplasious (Shimizu, 1994), which has contributed to unstable taxa. Informative, homologous characters in pompilids are usually subtle and often less conspicuous than the convergent features developed in different clades (Shimizu, 1994).

Herein, we conducted a molecular phylogenetic study to address the lack of consensus in higher-level Pompilidae classification. This work is based on a comprehensive sampling of genera and geographic areas, and four nuclear molecular markers. Our aim was to 1) determine the phylogenetic relationships of major lineages within Pompilidae, 2) estimate the ages and ancestral areas of these lineages, and 3) test the validity of prior subfamily classifications. In addition, we briefly discuss the generic classification of Pompilidae and point to areas needing further studies.

## MATERIAL AND METHODS

### TAXON SAMPLING

We sampled 150 specimens representing 74 Pompilidae genera (Support Information, Table S1). Specimens were selected from a variety of genera, in an effort to cover the breadth of morphological and geographical variation in the family. Based on

the subfamilies defined by Pitts *et al.* (2006), we sampled six genera of the previously defined Ctenocerinae, including *Epipompilus* Kohl that was tentatively placed in this subfamily; the two representatives of Ceropalinae; 38 genera of Pompilinae, including questionable pompiline taxa as *Chirodamus* Haliday, *Notocyphus* Smith, and *Balboana* Banks; and 28 genera of Pepsinae. Samples were obtained on loan from various entomological collections (Table S1) and field collecting trips. Vouchers are deposited as indicated in Table S1.

Outgroup taxa were chosen based on previous studies indicating (Sapygidae + Mutillidae) (Brothers & Carpenter, 1993; Pilgrim *et al.*, 2008) and (Pompilidae + Mutillidae) + a paraphyletic Bradynobaenidae (Johnson *et al.*, 2013) as sister taxa of Pompilidae. Taxa selected were: *Ephuta grisea* Bradley and *Timulla divergens* Mickel (Mutillidae); *Typhoctoides aphelonyx* Brothers (Chyphotidae); and *Sapyga centrata* Say and *Sapyga pumila* Cresson (Sapygidae).

## DNA EXTRACTION, PCR AMPLIFICATION AND SEQUENCING

DNA was extracted from the entire individual after puncturing the top of the mesosoma (small-medium specimens), or from 2-3 legs (large individuals). Extractions were performed with the Roche High Pure PCR Template Purification Kit (Roche Diagnostics Corp., Indianapolis, IN) following the manufacturer's protocol.

The nuclear genes elongation factor–1 α F2 copy (EF), long–wavelength rhodopsin (LWRh), RNA polymerase II (Pol2) and the D2–D3 regions of the 28S ribosomal RNA (28S) were amplified from each individual with the polymerase chain reaction (PCR. Double-stranded amplifications were performed with 20 μL reaction volume containing genomic DNA (10 ng), 1.5 mm $MgCl_2$, 0.2 mM of each dNTP, 1 μM

primer of each primer, 2 units of Qiagen taq (Qiagen, Valencia, CA), and buffer supplied by the manufacturer. In some reactions, GoTaq (Promega, Madison, WI) was used in the following amounts: 6 µl of $ddH_2O$, 10 µl of GoTaq Green Master Mix, and 1 mM of each primer. The optimal cycling parameters varied for each primer pair used.

Molecular markers were chosen based on phylogenetic investigations in other Hymenoptera families (e.g. Pilgrim *et al.*, 2008; Danforth, Fang & Sipes, 2006). Primers from previous studies and modified primers were used (Table 1). All PCR products were sequenced in forward and reverse directions at Utah State University's Center for Integrated Biosystems and were assembled into complete contigs using Sequencher 4.1 (Gene Codes Corp., Ann Arbor, MI).

## PHYLOGENETIC ANALYSES

Sequences were aligned using Geneious Alignment (Geneious 6.1) followed by manual refinement. Introns of LWRh and EF markers were removed from the alignment. The model of molecular evolution was determined for each gene by codon position using Partition Finder 1.01 (Lanfear *et al.*, 2012). Single-gene phylogenies were estimated in a Bayesian framework implemented in MrBayes 3.2 (Ronquist *et al.*, 2012) to check for topological incongruences. Single-gene matrices were then concatenated using Geneious 6.1 to produce a combined-gene matrix. The models of molecular evolution were determined for the combined data by gene and codon position using Partition Finder 1.01 (Lanfear *et al.*, 2012), and then analyzed in MrBayes 3.2 (see partitions and models in Table 2). 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. Trace plots and effective sample size (ESS) were

examined in Tracer v1.5 to determine MCMC mixing and convergence. Trees from the first 25% of the samples were removed as burn-in. A consensus of the post-burnin trees was visualized in FigTree v1.3.1.

Maximum-likelihood analysis (ML) was performed using RAxML, under the GTRCAT model carried out at the CIPRES website (Stamatakis, 2006; Stamatakis, Hoover & Rougemont, 2008). For this analysis, the combined alignment was partitioned by gene. Rapid-bootstrap heuristic searches were calculated to estimate support levels, from 100 replicates.

## DIVERGENCE TIME ESTIMATION

A chronogram was inferred 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 & Rambaut, 2007). Best-fit 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 reliable fossil data of Pompilidae species (Rodriguez *et al.*, in press), and one from the age of the crown group of Pompilidae as inferred by a dating analysis of all stinging wasps (Wilson *et al.*, 2013). The common ancestor of *Anoplius* Dufour + *Dicranoplius* Haupt was given a lognormal prior of 25 Ma (mean in real space) (LogSD=0.5) based on the fossil of *Anoplius* sp. n. (Rodriguez *et al.*, in press) from Dominican amber, which belongs to the stem group of *Anoplius*. The common ancestor of *Cryptocheilus* Panzer + (*Entypus* Dahlbom + (*Diplonyx* Saussure + (*Hemipepsis* + (*Leptodialepis* Haupt + *Dinosalius* Banks)))), as well as the common ancestor of *Agenioideus* Ashmead+ (*Homonotus* Dahlbom + *Ferreola* Lepeletier), were given a lognormal prior, with mean in real space,

of 33 Ma (LogSD=0.5) based on the fossils of *Cryptocheilus hypogaeus* Cockerell and *Agenioideus saxigenus* (Cockerell) found in the Colorado Florissant beds (Cockerell, 1908, 1914). The crown group node of Pompilidae was assigned a normal prior of (mean) 43 Ma (SD=10), based on the data published by Wilson *et al.* (2013). Two separate Markov Chain Monte Carlo (MCMC) searches were performed for 100,000,000 generations. Effective sample sizes (ESS), mixing, and graphical chain convergence were examined in Tracer 1.5. Independent runs were combined with LogCombiner 1.7.5. Twenty-five percent of samples was discarded as burn–in.

## ANCESTRAL AREAS RECONSTRUCTION

The possible ancestral ranges of the family and its main lineages were reconstructed on the Pompilidae chronogram. We used a Bayesian binary MCMC approach (BBM; Markov chain Monte Carlo (MCMC)) implemented in RASP 2.1b (Yan, Harris & Xingjin, 2012). We scored the area of occurrence at the genus-level, to minimize sampling bias (see Table S2). The number of maximum areas allowed at the nodes was six, which corresponded to Wallace's zoogeographic realms (Wallace, 1876) and were coded as follows: Australian region (A); Oriental region (B); Ethiopian region (C); Neotropical region (D); Nearctic region (E); and Palearctic region (F). Two MCMC chains were run simultaneously for 5,000,000 generations, sampled every 1000 generations. The model used was a fixed JC+G (Jukes-Cantor+Gamma).

# RESULTS

## PHYLOGENETIC ANALYSES

The concatenated sequence alignment of four molecular markers included 2,931 bp after trimming. GenBank accession numbers for all markers are indicated in Table S1. Bayesian and ML analyses produced congruent topologies, displaying only minor differences in resolution and topology (Supporting Information, Fig. S1). Both approaches recovered Pompilidae as a well-supported monophyletic group (posterior probability (PP)=1.0; bootstrap (BS)=100%). However, none of the approaches was able to support relationships among the deeper lineages. These earliest-branching lineages mostly correspond to previously recognized, major subfamilies, but with some differences (explained below). The BEAST analysis increased PPs of nodes overall and found support for monophyly of several major clades. Such "relaxed" phylogenetic approaches typically produce more accurate and precise topologies than do unrooted and strict-clock methods (Drummond *et al.*, 2006; Pybus, 2006). Thus, we use the topology resulting from the relaxed-clock analysis (Fig. 1) as our most accurate estimate of Pompilidae phylogeny in the discussion below.

We recovered four, large, well-supported clades (A, B, C, and D; Fig. 1). Within these four major clades, two contained additional lineages that are supported by morphology, behavior, and/or by phylogenetic support measures (E, F, G, H, and I; Fig. 1), as presented below.

The basal split in Pompilidae is formed by the African species of Ctenocerinae, clade A (*sensu* Arnold, 1932b) *versus* all remaining taxa. African Ctenocerinae, here represented by *Trichosalius* (Arnold), *Ctenocerus* Dahlbom, *Paraclavelia* Haupt, and *Pseudopedinaspis* Brauns, were well supported as monophyletic (PP=0.99); however, their position as sister group to remaining Pompilidae was weak (PP=0.72). The

Neotropical and Australian Ctenocerinae genera (*Lepidocnemis* Haupt and *Maurillus* Smith, respectively) were independently nested among Pepsinae genera.

The second major split is between clade B and the remaining pompilids. Clade B is composed of *Notocyphus* Smith, *Ceropales* Latreille, and *Irenangelus* Schulz. This clade is further divided into two well-supported lineages: E (*Notocyphus*) (PP=1.0) and F (*Irenangelus* + *Ceropales*) (PP=0.93).

The remaining pompilids are split into two large, well-supported lineages, clades C and D. Clade C (PP=1.0) comprises species of Pompilinae, as defined by Pitts *et al.* (2006), but excluding *Chirodamus* Holiday. We recognize three major lineages within clade C: clades G, H, and I. The sister relationship of clade G and H is poorly supported (PP=0.51); clade G is monotypic and includes only *Sericopompilus*, whereas clade H is formed by (*Balboana* + *Priochilus*) (PP=0.82). Clade I (PP=1.0) includes most of the Pompilinae *sensu stricto* taxa.

Clade D (PP=0.93) includes most of the Pepsinae (*sensu stricto*) genera and some taxa traditionally treated separately (e.g. *Epipompilus* Kohl, *Chirodamus* Haliday, *Lepidocnemis*). The internal relationships in this group are somewhat uncertain, with only few genera recovered as monophyletic with high support (e.g. *Psoropempula* Evans, *Pepsis* Fabricius). Some larger genera were monophyletic with less-than-significant support, such as *Epipompilus* Kohl (PP = 0.88), or rendered paraphyletic by the inclusion of only one or two other taxa, such as *Auplopus* Spinola and *Ageniella* Banks. One large clade within clade D was recovered with high support: clade J. Within this lineage we further recognize two well-supported clades: K, containing *Priocnessus* Banks +

(*Cryptocheilus* + (*Entypus* + (*Diplonyx* + (*Hemipepsis* + (*Leptodialepis* + *Dinosalius*)))) (PP=1.0), and L, containing *Cyphononyx* Dahlbom + Ageniellini genera (PP=1.0).

## DIVERGENCE-TIME ESTIMATION

The estimated age of crown-group Pompilidae was recovered as 43.3 Ma (95% highest posterior probability density [HPD]=112.2–27.1), i.e. in the mid Paleogene – Eocene (Fig. 1). The internal age estimates indicate that extant species of the most diverse groups, e.g. Pepsinae and Pompilinae, began to diverge during the late Eocene, about 38.6 Ma (HPD=65.1–19.4). The diversification of extant Ctenocerinae (clade A) began around 29.8 Ma (HPD=53.3–12.2), similar to Ceropalinae (31.0 Ma, HPD=54.8–14.7), (*Sericopompilus* + *Balboana* + *Priochilus*) (31.3 Ma, HPD=52.7–15.3), and Pompilinae *sensu stricto* (28.8 Ma, HPD=52.7–15.3) (Table 3). Crown-group *Notocyphus* emerged more recently (25.5 Ma, HPD=45.4–11.3), whereas crown-group Pepsinae emerged earlier (34.7 Ma, HPD=58.3–17.0), as compared to other major clades (Table 3).

## ANCESTRAL AREAS RECONSTRUCTION

The combined results of the BBM analysis indicated the Nearctic region as the most probable ancestral area for crown-group Pompilidae (Fig. 2 and Supporting Information, Fig. 2). The Ethiopian region was recovered as the ancestral area for Ctenocerinae (clade A) (Fig. 2). The ancestor of *Notocyphus*, *Ceropales*, and *Irenangelus* (clade B) more likely had a range including the Neotropical and Nearctic regions, which is the same as the current and ancestral distribution of *Notocyphus* (clade E) (Fig. 2). The ancestor of Ceropalinae (clade F) dispersed to and occupied all other zoogeographic regions, except for the Palearctic. The ancestral range of clade C (Pompilinae) was

ambiguous; it was equally likely to be the New World or the Neotropical region only (Fig. 2). Within this group, the ancestral area of clade I could not be reconstructed with confidence. The ancestry of clade D (most of Pepsinae) was reconstructed as ranging from the Neotropical to Nearctic regions (Fig. 2).

## DISCUSSION

The diverse family Pompilidae is a well-supported monophyletic group of aculeate wasps. With the application of molecular data to the problem of Pompilidae phylogenetics, many internal lineages are well supported as monophyletic, yet certain relationships remain somewhat ambiguous. However, morphological and behavioral characteristics, coupled with phylogenetic signal, justify the taxonomic decisions we present here concerning subfamily delimitations and nomenclatural changes. We recognize the following subfamilies and their relationships: Ctenocerinae + ((Ceropalinae + Notocyphinae) + Pompilinae + Pepsinae) (Fig. 2; Table 4). Our delimitations differ from previous phylogenetic studies in number, structure, and relationship of subfamilies. Shimizu (1994) proposed six subfamilies: Ceropalinae + (Notocyphinae + (Pepsinae + Pompilinae + Ctenocerinae + Epipompilinae)); whereas Pitts *et al.* (2006) proposed four subfamilies: Ceropalinae + (Pepsinae + (Ctenocerinae + Pompilinae)). We propose five subfamilies, with Ctenocerinae as the sister group to all other pompilid taxa. This is a major departure from the previous schemes derived from morphology, which proposed Ceropalinae as the sister group to all other pompilid wasps (Shimizu, 1994; Pitts *et al.*, 2006). In agreement with Shimizu (1994), however, our analyses favor reinstatement of Notocyphinae.

The position of Ctenocerinae as emerging from the basal node of Pompilidae—rather than Ceropalinae as in previous schemes—has implications for the evolution of spider wasp nesting behavior. It has been suggested that nesting behavior in Pompilidae has evolved in a step-wise fashion of increasing complexity. The secondary loss of some of the steps, such as transporting the host and building a nest, has been proposed to descend from some of the most complex nesting sequences (Evans, 1953). Similarly, cleptoparasitism has been suggested as a case of secondary loss from an ancestral, more complex state (Evans, 1953). Previous phylogenetic schemes reconstructing cleptoparasitic Ceropalinae at the base of Pompilidae (Shimizu, 1994; Pitts *et al.*, 2006) might imply that cleptoparasitism was an ancient strategy not descended from complex behavior, and possibly represents the ancestral behavior of the family. In contrast, our results suggest that cleptoparasitism is likely not ancestral, as discussed below.

The biology of most ctenocerine species remains unknown, but morphology suggests that they are parasitoids of trap-door spiders (Waichert & Pitts, 2011). In addition, a female Ctenocerinae has been collected from the nest of a trap-door spider (Arnold, 1932a), and Ctenocerinae specimens have been reared from trap-door spiders in the laboratory (Evans, 1972). Furthermore, ctenocerines have converged on morphology similar to Aporini (Pompilinae), a group known to parasitize trap-door spiders. Aporini spider wasps have been observed using the spider burrow as a nest (Jenks, 1938), thus reducing the nesting sequence by eliminating carrying and nest building steps.

Our reconstruction of the basal Pompilidae node is consistent with the idea that ancestral pompilids used a generalist strategy involving attacking and paralyzing spiders in their own nest. Cleptoparasitism—such as observed in Ceropalinae—as an ancestral

strategy is logically inconsistent, as (a) it is a highly specialized behavior, and (b) it requires the prior existence of pompilid lineages with more complex behavior from which to steal prey (e.g., other females that leave prey unattended while digging nests). A generalist ancestral strategy of attacking spiders in their own nest could conceivably evolve from the unspecialized wasp behavior of capturing any arthropod prey. We do not necessarily suggest that the earliest pompilid ancestors were trap-door spider specialists. It is more logical to propose that ctenocerine trap-door spider specialists concentrated on trap-door spiders after their evolutionary origin, and their specialized morphology followed. A more detailed discussion on the evolution of behavior in the family will require comparative phylogenetic analyses and quantitative ancestral state reconstruction of behavioral traits. This is beyond the scope of this particular paper, but will be addressed in future publications.

## SUBFAMILIAL DIVERGENCE TIMES AND ANCESTRAL AREAS RECONSTRUCTION

The age of crown-group Pompilidae inferred here is consistent with the date proposed by Wilson *et al.* (2013) of ~47 Ma. Our findings support the origin of spider wasps in the mid-Paleogene, and possibly in the Nearctic region. Wilson *et al.* (2013) suggested that the increased diversity of spider families at the beginning of the Paleogene (Penney, 2004) might have driven the diversification of Pompilidae. Our results, however, show that most of the subfamilies diverged around 25–35 Ma in the late Paleogene. These results are puzzling, however, given that the cooling temperatures at the Eocene-Oligocene boundary were thought to have affected biodiversity negatively (Katz *et al*., 2008; Zhonghui *et al*., 2009). Neotropical floras, for example, show a decrease in diversity at this time (Jaramillo, Rueda & Mora, 2006). Nevertheless, abiotic

factors, such as high volcanic and tectonic activity in Southeast Asia, could have provided refugia for certain taxa, which may have triggered diversification in some groups (Buerki *et al.*, 2013). It is possible that local climatic and geological changes such as these might have affected pompilid diversification.

Because of the recent divergence of Pompilidae lineages, their current distribution patterns cannot be attributed to continental drift. Therefore, the current geographic distribution of spider wasps appears to have resulted from several dispersal events at different geological times, rather than as a consequence of vicariant processes. Recent historical biogeography analyses of more recently diverged spider wasp groups support this pattern (Rodriguez *et al.*, 2015). Spider wasp dispersal events occurred during a time span of ~40 Ma and expanded spider wasp distribution from a single biogeographic area to a cosmopolitan distribution.

Pompilinae, the most diverse subfamily, originated around 34 Ma, possibly in the Neotropical and/or Nearctic region. The diversification of most of the clades apparently occurred between 13–29 Ma during the late Oligocene to early Miocene. Pepsinae taxa show a similar range of diversification dates and similar geographic origin, but origins of more genera in this subfamily appear to have occurred earlier in the history of the subfamily.

## CTENOCERINAE

This subfamily was first proposed by Haupt (1929), as Claveliinae, to separate its members from Pepsinae; it includes two genera in the Neotropics, four in Australia and 11 in Africa. The name was changed to Ctenocerinae (Shimizu, 1994), but the composition of this subfamily remained mostly stable, except for a suggestion to include

*Apinaspis* Banks and *Epipompilus* (Pitts *et al.*, 2006). *Epipompilus* is discussed below (see Pepsinae section), whereas *Apinaspis* is an Oriental monotypic genus (Banks, 1938) and has characteristics similar to the Australian genera described by Evans (1972). We support the classification of *Apinaspis* in Pepsinae, as proposed by Shimizu (1994) and Banks (1938), until further analyses suggest otherwise. Although these African, Neotropical and Oriental/Australian taxa share several morphological features – a large antennal scrobe, a transverse groove on the second sternite that is usually prolonged to vertex, and a hind tibia with short spines directed straight backwards – these may be adaptations for preying on trap-door spiders (Evans, 1972) that were independently acquired. More information on behavior is needed, as the natural history of these taxa remains poorly understood.

Our analyses did not recover the monophyly of Ctenocerinae. The Neotropical *Lepidocnemis* and the Australian *Maurillus* are nested within different non-ctenocerine lineages with high support. The morphological similarities of these and the African ctenocerine genera must now be interpreted as convergent traits. Four Australian taxa assigned to Ctenocerinae by Evans (1972) (*Cteniziphontes* Evans*, Apoclavelia* Evans, *Maurillus*, *Austroclavelia* Evans) and the three genera discussed by Waichert & Pitts (2011) (*Abernessia* Arlé, *Lepidocnemis*, *Hypoferreola* Ashmead) are herein transferred to Pepsinae on the basis of the molecular phylogeny and of morphology.

The monophyly of African Ctenocerinae (clade A) was recovered in all analyses. While support for this clade was low in the unconstrained analyses, it was high in the clock-constrained analysis. We redefine Ctenocerinae as the lineage represented by clade A, as it includes the nominal genus, *Ctenocerus.* The 11 Afro-tropical genera recognized

by Arnold (1932b), with distribution extending into Java and India, should retain their classification as Ctenocerinae until further analyses are performed. Males of all 11 Ctenocerinae genera designated by Arnold (1932b) are distinguished from Pepsinae by having flagellum uni- or biramous, or crenulate antennae. These character states are not observed in Pepsinae. The subfamily is now recognized by 1) the metasomal sternum 2 with a distinct sharp transverse groove; 2) the mesofemur and the metafemur without subapical spine-like setae set in grooves or pits; 3) the metatibia without scale-like spines or serrate carina and with short, subequal spines directed straight backwards; and 4) the fore wing with vein Cu1 simple at base, without any definite downward deflection; 5) the clypeus plate-like in shape; and 6) males with crenulate antennae. As far as we know, ctenocerine spider wasps prey on trap-door spiders.

## CEROPALINAE

Ceropalinae was first erected by Haupt (1929) to comprise only two genera, *Ceropales* and *Irenangelus*. Townes (1957) later included several genera that have been transferred since to Pepsinae and Notocyphinae. Our analyses are congruent with those of Shimizu (1994) and Pitts *et al.* (2006) in recovering Ceropalinae as monophyletic (clade F), and we confirm that *Ceropales* and *Irenangelus* are the sole representatives of Ceropalinae. Although this lineage was poorly supported in the unconstrained analyses, support in the relaxed-clock analysis was high. The position of this group in the family, however, diverges from results of previous authors. Shimizu (1994) and Pitts *et al.* (2006) recovered Ceropalinae as the sister group to all other Pompilidae. In our study, Ceropalinae is strongly supported as the sister group to Notocyphinae. Shimizu (1994) and Pitts *et al.* (2006) defined the subfamily by a set of non-unique homoplasies,

including a reniform compound eye, the inner margin of eye converging below, and females with a straight stinger. However, Ceropalinae shares a large and exposed labrum and a compressed subgenital plate with its sister group, Notocyphinae. The exposed labrum is present in other spider wasp genera (e.g. *Paracyphononyx* Gribodo and *Pepsis*), but the extended labrum observed in Ceropalinae and Notocyphinae distinguishes them from other genera by being large and almost as long as the clypeus, which gives the clypeus+labrum a diamond shape. Ceropalines are distinguished by their mode of cleptoparasitism specialized on other pompilid species.

## NOTOCYPHINAE

*Notocyphus*, the sole representative of Notocyphinae, was elevated to subfamily status by Haupt (1929), Banks (1934), and Shimizu (1994). The morphological analyses conducted by Pitts *et al.* (2006) did not support this subfamily. Townes (1957) moved *Notocyphus*, along with *Minotocyphus* Banks, into the tribe Notocyphini within Ceropalinae. Pitts *et al.* (2006) considered *Notocyphus* (and so Notocyphinae) to be a member of Pompilinae. Our molecular analyses recover *Notocyphus* (and therefore Notocyphinae; clade E) as monophyletic with high support, and sister to Ceropalinae. Morphological and behavioral characters confirm the status of *Notocyphus* as a subfamily. Distinguishing morphology of Notocyphinae includes the sting curved downward, the claws bifid in both sexes and the eyes subparallel along the internal margin. Behaviorally, *Notocyphus* are parasitoid wasps, paralyzing their prey temporarily without constructing a nest. In contrast, all Ceropalinae are cleptoparasitic on other pompilid species. For these reasons we abstain from merging these two subfamilies. Instead, Notocyphinae is revalidated and Ceropalinae is maintained.

Notocyphinae is monotypic and defined by the character states discussed above. The other genus included in Notocyphini by Townes (1957), *Minotocyphus*, is a small Oriental group with morphological resemblance to *Notocyphus* (Townes, 1957; Wahis, 1981). Wahis (1981) discussed several character states that separate *Minotocyphus* from *Notocyphus*, such as having the fore wing with the vein Cul deflected downward at the base and the second sternite with a sulcus with the end curved towards the apex of metasoma. *Minotocyphus* is currently placed in Pompilinae (Wahis, 1981); we were not able to obtain suitable samples for this study.

## POMPILINAE

Pompilinae has been historically the most diverse group in Pompilidae. Although several diagnostic character states apparently define this group, its classification and taxonomic composition have been a continuing topic of discussion for systematists. *Notocyphus* and *Chirodamus* were previously included in Pompilinae (Pitts *et al.*, 2006). *Epipompilus* was previously classified as Pompilinae (Harris, 1987), until it was elevated to Epipompilinae (Shimizu, 1994), and then transferred to Ctenocerinae (Pitts *et al.*, 2006). *Cordyloscelis* Arnold was also considered a member of Pompilinae (Arnold, 1935).

*Sericopompilus* Howard + *Priochilus* Fabricius + *Balboana* form an early-branching lineage (clades G and H) within the pompilines *sensu lato*. Although the placement of this lineage with respect to clade I (remaining Pompilinae) was uncertain, clade I is a well-supported, separate lineage (Fig. 1). The taxa of clades G and H have unique morphology and behavior among the Pompilinae, which would justify elevating both clades to subfamily level. However, we abstain from defining these as different

subfamilies until further data are available; instead, we propose the tribes Sericopompilini and Priochilini. It is possible that future studies will provide the necessary support to consider these taxa as subfamilies with unique evolutionary histories.

Our analyses recovered a lineage (clade I) composed of most of the genera traditionally placed in Pompilinae. The large pompiline lineage excluded several contentious genera, namely, *Cordyloscelis*, *Chirodamus*, *Notocyphus* and *Epipompilus*. Our analyses placed *Chirodamus* and *Cordyloscelis* within Pepsinae. Several clades within the large pompiline lineage received high support and could be good candidates for tribal revisions.

Pompilinae are herein characterized by: 1) the metatibia with apical spine-like setae long, of irregular lengths and spacing, the setae distinctly splayed (except in species of *Balboana* and some species of *Priochilus*); 2) the fore wing with vein Cul usually distinctly deflected downward at base (second discal cell (2D) with a posterior "pocket") (except in species of *Balboana* and *Priochilus*); 3) the mesofemur and metafemur usually with 1 or more distinct subapical dorsal spine-like setae set in grooves or pits, but rarely without such setae; and 4) the tarsomere 5 (last tarsal segment of hind leg) with ventral preapical setae often forming a distinct median row, but the setae sometimes absent. Not all pompilines have spiny legs. Some have smooth legs that could mislead subfamilial classification, for example, in the African genus *Kyphopompilus* Arnold and the genera of Aporini. Nesting behavior within this group is variable and contains most of the states observed in Pompilidae, such as nesting in pre-existing cavities, using the spider’s burrow, digging a burrow on the ground, and cleptoparasitism.

## SERICOPOMPILINI (NEW RANK)

Three species of *Sericopompilus* are found in North America and one in Australia (Evans 1950). Evans (1950) suggested that the disjunct distribution and lack of morphological specialization indicate that *Sericopompilus* is an old lineage within Pompilinae. Evans (1966) further proposed, without formal cladistic analysis, that *Sericopompilus* was related to *Poecilopompilus* Howard and *Episyron* Schiödte, but had retained "ancestral conditions" compared to these genera. Shimizu (1994) placed *Sericopompilus* as sister to (*Austrochares* Banks + *Parabatozonus* Yasumatsu + *Poecilopompilus* + *Batozonellus* Arnold + *Episyron* Schiødte). Later, Shimizu (1997) concluded that *Agenioideus* Ashmead should be considered sister to *Sericopompilus,* a conclusion supported by Pitts *et al.* (2006). Our analyses suggest that *Sericopompilus* are possibly an old lineage within this subfamily (clade G), as suggested by Evans (1950).

*Sericopompilus* have slender bodies, long wings (Wasbauer, 1995) and are distinguished from Pompilinae by having the apical tarsal segments without spines beneath and all claws of both sexes dentate (Evans, 1966). Little is known about hunting and nest behavior of *Sericopompilus* but *S. apicalis* (Say) have been observed nesting in holes in the ground (Evans, 1950).

## PRIOCHILINI (REINSTATED)

*Priochilus* and *Balboana* are morphologically enigmatic genera; consequently, their classification has varied according to author. Both genera exhibit a Neotropical distribution. Two aspects of their characteristic morphology have also been historically associated with pepsines and ctenocerines – a sharp transverse groove on the second

metasomal sternite and the fore wing with vein Cu1 not deflected downward at base. Another character state is shared with pompilines – the metatibia with apical spine-like setae of irregular lengths and spacing. This morphological similarity has generated conflicting classifications. Both genera were classified in Cryptocheilinae (Pepsinae) by Banks (1944, 1946). Haupt (1959) included *Priochilus* in Macromerinae (currently Ageniellini (Pepsinae)). Both *Priochilus* and Ageniellini species have slender bodies, a petiolate metasoma, and build nests using mud. Evans (1966) considered the morphological features as convergences associated with the unusual mud-nesting behavior, and placed *Priochilus* in Pompilinae.

*Priochilus* and *Balboana* are smaller genera, with only 21 and 6 described species, respectively (F. Fernandez pers. comm.). However, this is likely an underestimate, based on our qualitative assessment of the diversity of unassigned specimens present in collections. Priochilini is distinguished by 1) lacking malar space; 2) having the propodeum with an angled declivity; and 3) having males with short pronotum, which slopes abruptly. The natural history of *Balboana* remains unknown, while *Priochilus* species use mud pellets to build aerial nests (Evans & Shimizu, 1996; Auko, Silvestre & Pitts, 2013) similar to those of Ageniellini (Pepsinae).

## PEPSINAE

Pepsinae is also a diverse group with a conflicting history of classification, and several genera of uncertain membership. For example, *Epipompilus* was previously considered a monotypic subfamily (Shimizu, 1994), and then transferred to Ctenocerinae (Pitts *et al.*, 2006). More recently, cladistic morphological analyses with qualitative and quantitative characters suggested *Epipompilus* to be the sister to *Minagenia* Banks (E. F.

Santos pers. comm.). *Minagenia* has suffered similar inconsistencies. *Minagenia* species are morphologically homogeneous, but difficult to assign to a subfamily (Dreisbach, 1953). Townes (1957) placed *Minagenia* in Ceropalinae; Haupt (1959), Evans (1973), and Pitts *et al.* (2006) considered it a member of Pepsinae. Another example concerns the variable *Chirodamus* Haliday. Roig Alsina (1989) split *Chirodamus* into six Neotropical genera: *Chirodamus s.s.*, *Plagicurgus* Roig Alsina, *Calopompilus* Ashmead, *Pompilocalus* Roig Alsina, *Aimatocares* Roig Alsina, and *Anacyphononyx* Banks. *Chirodamus s.s.* was placed in Pompilinae by Pitts *et al.* (2006), but the other genera of *Chirodamus s.l.* have been considered Pepsinae.

Our results recovered a monophyletic Pepsinae in the relaxed-clock analysis, only, with good support. Most of the deeper relationships within this clade were not supported, while several lineages of more recent origin were highly supported. The molecular phylogeny supports the assignment of the controversial genera, discussed above, as members of Pepsinae. *Epipompilus* is monophyletic, although its position within Pepsinae is ambiguous. It has a disjunct distribution, with species found in the Neotropics and Australasia. In both our molecular phylogeny and a morphological phylogenetic study (E. F. Santos pers. comm.), *Epipompilus* is recovered as two major clades, one Neotropical and the other Australasian. *Epipompilus* hunt spiders inside their burrows and permanently paralyze them before oviposition (Pollard, 1982).

Our analyses also support *Minagenia* and *Chirodamus s.l.* as members of Pepsinae. *Minagenia* is strongly supported as monophyletic, but its position within Pepsinae is uncertain. Species of *Minagenia* differ from other Pepsinae by having a straight stinger, a compressed metasoma, bifid claws and the cells 2 r-m and 3 r-m

continuously curved outward and with similar appearance. They are ectoparasitoids, paralyzing their prey only temporarily. Our results also confirm Roig Alsina's (1989) division of *Chirodamus* into several genera, to the extent that we have sampled these taxa.

Among Pepsinae tribes, the most morphologically and behaviorally diverse is Ageniellini (clade L, excluding *Cyphononyx*). The monophyly of Ageniellini was recovered by Shimizu (1994), Pitts *et al.* (2006), and Shimizu, Wasbauer & Takami (2010), but this tribe is made paraphyletic in our analyses by the position of *Melanagenia*. *Melanagenia* was recently described by Wahis, Durand & Villemant (2009), and was defined and placed in Ageniellini by having the metasoma petiolate and by the first tergite lacking a transverse carina. Our results indicate that *Melanagenia* is unrelated to other Ageniellini. Rather, it emerges as sister to *Sphictostethus*, with which *Melanagenia* shares states of facial characters (lacking of malar space with eyes touching mandibles and a clypeus somewhat rectangular and convex), pronotal characters (rounded with a deep sulcus laterally), and wing-venation characters. However, since *Melanagenia* species lack a carina on the first tergite and have a petiolate metasoma, these two character states–although useful in identifying Ageniellini taxa–can no longer be considered unique synapomorphies of the tribe. The observation that *Phanagenia* Banks (Ageniellini) possesses a carina on the first metasomal segment further undermines the diagnostic value of this metasomal character. *Melanagenia* is herein removed from Ageniellini and placed in Pepsini. As discussed above (see *Ctenocerinae*), *Lepidocnemis* is sister to *Pompilocalus* and *Aimatocares*, within a larger lineage including *Sphictostethus* and *Melanagenia. Lepidocnemis* is the only representative of

Neotropical Ctenocerinae in our study and is herein transferred to Pepsinae. Pepsini and the other tribes are in dire need of further studies and redefinition of most of their taxa. Our samples and analyses are not sufficient to make further nomenclatural decisions regarding tribes.

Pepsinae (clade D) are now defined by: 1) the metasomal sternum 2 with a distinct sharp transverse groove; 2) the mesofemur and the metafemur without subapical spine-like setae set in grooves or pits; 3) the metatibia with apical spine-like setae of uniform length, the setae not splayed; and 4) the fore wing with vein Cu1 simple at base, without any definite downward deflection, such that the second discal cell (2D) is without a "pocket" posterior. A broad range of nesting behavior occurs within this subfamily, including nesting in pre-existing cavities, using the spider's burrow, digging a burrow in the ground, building nests of mud, and behaving as true parasitoids and cleptoparasites.

## GENERIC RELATIONSHIPS IN POMPILIDAE

Several genera represented in our analyses were not recovered as monophyletic. In Pompilinae, both *Agenioideus* and *Arachnospila* Kincaid are paraphyletic. Generic validation and phylogenetic relationships of Pompilinae will be discussed in more detail elsewhere (Rodriguez *et al.* unpubl. data). In Pepsinae, *Hemipepsis* is paraphyletic, with a Neotropical clade nesting within *Epipompilus* and *Minagenia*, and an Old World clade sister to *Leptodialepis. Caliadurgus, Priocnemis* and *Sphictostesthus* have species nesting within different clades; in addition, *Auplopus* and *Ageniella* are paraphyletic. The relationships and the status of genera in Ageniellini will be discussed in detail elsewhere (Waichert *et al.* unpub. data).

*Dipogon* was divided into five genera by Lelej & Loktionov (2012): *Dipogon*, *Deuteragenia*, *Nipponodipogon* Ishikawa, *Stigmatodipogon* Ishikawa, and *Winnemanella* Krombein. The divisions were based on morphological phylogenetic analyses of 13 species. Our study included only representatives of *Deuteragenia* and *Dipogon*; the latter genus nested within *Deuteragenia*. Thus, we did not recover *Deuteragenia* as a monophyletic genus, as suggested by Lelej & Loktionov's (2012) analyses.

## CONCLUSION

Five subfamilies are now recognized for Pompilidae. Pompilidae has accumulated a plethora of names over the years, mostly due to specialists in different regions having worked on different groups, and a lack of worldwide catalogues, revisions, and keys to several genera. Spider wasps share a number of morphological features that must be interpreted as examples of convergence between unrelated lineages. Such convergence is likely due to ecological factors that have driven similar morphology in different groups of spider wasps in distinct geographic areas. Spider wasps that hunt and nest in similar ecological niches are likely to evolve similar morphological adaptations (e.g. Ctenocerinae genera, Aporini genera in Pompilinae, and *Lepidocnemis* and *Abernessia* Arlé in Pepsinae). Moreover, it is apparent that several groups have not accumulated sufficient morphological differences to distinguish them reliably. These results suggest that morphological features should be evaluated very carefully when defining and classifying pompilid taxa. Geographical characters can help in delimiting genera and certain tribes and subfamilies, as many such lineages are restricted to one or a few zoogeographic regions. Crown-group Pompilidae originated in the middle Paleogene (ca. 43 Ma) in the Nearctic region, and appear to have experienced various dispersal events

and episodes of rapid diversification (Rodriguez *et al*. unpubl. data). It is possible that the increased diversity of spider families at the beginning of the Paleogene helped to drive the later diversification of Pompilidae (Penney, 2004; Wilson *et al.*, 2013).

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FIGURE LEGENDS

**Figure 1.** Consensus phylogenetic reconstruction for Pompilidae resulting from two Bayesian MCMC runs performed in BEAST. BEAST Posterior Probability (PP) values are displayed on nodes. Colors indicate subfamilial boundaries proposed.

**Figure 2.** A summarized phylogeny from Fig. 1 transformed to show newly proposed subfamilial relationships in Pompilidae; dashed line represents PP below 90%. For each node a color circle corresponds to the area with highest probability resulting from the BBM analysis. Ranges with probability less than 10% are reported as black. The bottom circles represent the areas code and ancestral area range assigned in the model: A, Australian; B, Oriental; C, Ethiopian; D, Neotropical; E, Nearctic; F, Palearctic.

TABLES

**Table 1**. Primers used for PCR amplification and sequencing processes.

| Primer name | Primer sequence | Reference |
|---|---|---|
| **28S** | | |
| CF2 | TGG TAA CTC CAT CTA AGG CTA AAT A | Campbell *et al.* 2000 |
| CF | CGTGTTGCTTGATAGTGCAGC | Heraty *et al.* 2004 |
| D5R | CCC ACA GCG CCA GTT CTG CTT ACC | Schulmeister 2003 |
| **EF-1α** | | |
| F2for1 | GGTTCCTTCAAATATGCTTGG | Pilgrim *et al.* 2008 |
| F2for4 | CGT GGT ATC ACG ATC GA | Danforth & Ji 1998 |
| F2for2 ?? | GCCGAACGTGAGCGTGG | Modified from Pilgrim *et al.* 2008 |
| F2rev4 | GCT TCG TGG TGC ATT TC | Pilgrim *et al.* 2008 |
| F2rev1 | AATCAGCAGCACCTTTAGGTG | Danforth & Ji 1998 |
| **LWRh** | | |
| LWRhR | ATA TGG AGT CCA NGC CAT RAA CCA | Mardulyn & Cameron 1999 |
| MutiOpsin1F | ACG CGA TGT GCG GTT CAC TGT TCG G | Pilgrim *et al.* 2008 |
| **Pol2** | | |
| Polfor2a | AAYAARCCVGTYATGGGTATTGTRCA | Danforth *et al.* 2006 |
| PL758R | ACGACCATAGCCTTBAGRTTR | Wild & Maddison 2008 |
| Polfor5 | AACAACCCGGTCATGGGTATTGTGCA | Modified from Danforth *et al.* 2006 |
| Pol2rev5 | GAATTCTCGACGAATCCTCT | Modified from Danforth *et al.* 2006 |
| **Wg** | | |
| LepWg1 for | GAR TGY AAR TGY CAY GGY ATG TCT GG | Brower & DeSalle 1998 |
| LepWg2 | ACTGCGCARCACCARTGGAATGTGCA | Modified from Pilgrim *et al.* 2008 |
| modLepWg2 rev | ACT ICG CRC ACC ART GGA ATG TRC A | Brower & DeSalle 1998 |
| Wg290F | GCW GTR ACT CAC AGY ATC GC | Pilgrim *et al.* 2008 |

**Table 2.** Best partitioning scheme determined by PartitionFinder, with the corresponding model of molecular evolution and the loci included in each.

| | PartitionFinder | MrBayes |
|---|---|---|
| **28S** | SYM+I+G | nst=6 rates=invgamma statefreqpr=fixed(equal) |
| **EF-1α** | codon1=K80+I+G<br>codon2=SYM+I+G<br>codon3= SYM+G | codon1= nst=2 rates=invgamma statefreqpr=fixed(equal) codon2= nst=6 rates=invgamma statefreqpr=fixed(equal) codon3=nst=6 rates=gamma statefreqpr=fixed(equal) |
| **LWRh** | codon1=SYM+I+G<br>codon2=SYM+I+G<br>codon3=GTR+I+G | codon1= nst=6 rates=invgamma statefreqpr=fixed(equal) codon2= nst=6 rates=invgamma statefreqpr=fixed(equal) codon3= nst=6 rates=invgamma |
| **Pol2** | codon1=JC+G<br>codon2=K80+I+G<br>codon3=GTR+I+G | codon1=nst=1 rates=gamma codon2= nst=2 rates=invgamma statefreqpr=fixed(equal) codon3= nst=6 rates=invgamma |

**Table 3.** Age estimates and mean (in Myr) from BEAST for subfamilies of Pompilidae.

| Subfamily | Mean age | Range (HPD 95%) | Clade (Figure 1) |
|---|---|---|---|
| Ceropalinae | 28.4 | 54.8–14.7 | F |
| Ctenocerinae | 27.9 | 53.3–12.2 | A |
| Notocyphinae | 23.1 | 45.4–11.3 | E |
| Pepsinae | 31.4 | 58.3–17.0 | D |
| Pompilinae | 30.5 | 52.7–15.3 | C |

**Table 4.** Newly proposed subfamilial groups, clades in Fig. 1, number of genera, and biological traits.

| Subfamily | Clade in Fig. 1 | Number Genera | Life History |
|---|---|---|---|
| Ceropalinae | F | 2 | Cleptoparasite* of other pompilids |
| Ctenocerinae | A | 11 | Likely idiobiont* ectoparasitoid of trap-door spiders |
| Notocyphinae | E | 1 | Koinobiont* ectoparasitoid of Theraphosidae spiders |

| | | | |
|---|---|---|---|
| Pepsinae | D | ~34** | Cleptoparasite of other pompilids; idiobiont or koinobiont ectoparasitoid of various spider families nesting in pre-existing cavity, self-constructed burrow, or in a mud nest |
| Pompilinae | C | ~42** | Cleptoparasite of other pompilids; idiobiont ectoparasitoid of various spider families nesting in pre-existing cavity, self-constructed burrow, or in a mud nest |

***Cleptoparasite=**takes its host from another wasp; **idiobiont=**parasitoid that prevents further development of the host; **koinobiont=**parasitoid that allows further development of the host.

**There are likely more genera than the number presented here in geographical areas where the taxa are understudied.

## SUPPORTING INFORMATION

**Figure S1.** Consensus phylogenetic reconstruction for Pompilidae resulting from two Bayesian MCMC runs performed in MrBayes and 100 Bootstrap replicates through a ML search. Bayesian Posterior Probabilities (PP) of nodes shown as the first value, Maximum Likelihood Bootstrap pseudoreplicates (BP) shown as the second value. Asterisk (*) indicates nodes not recovered in the analysis. Only BP> 50% and PP> 0.5 are displayed on nodes.

**Figure S2.** Chronogram for Pompilidae derived from a Bayesian analysis employing a relaxed molecular clock (bottom). Asterisks below branches indicate calibration nodes. Branch lengths are drawn proportional to time and 95% intervals for the ages of select nodes are indicated by horizontal bars.

**Figure S3.** Ancestral area reconstruction obtained from a Bayesian binary Markov Chain Monte Carlo (BBM) approach. The bottom right box represents the areas code and ancestral area range assigned in the model: A, Australian; B, Oriental; C, Ethiopian; D, Neotropical; E, Nearctic; F, Palearctic. For each node a color circle corresponds to the area with highest probability resulting from the BBM analysis. Ranges with probability

less than 10% are reported as black. Asterisks designate nodes used to calibrate the beast analysis.

**Table S1.** Voucher and collection information for specimens used in the molecular analyses, and GenBank accession number for sequences.

| | | | | | GenBank Accession Numbers | | | |
|---|---|---|---|---|---|---|---|---|
| **Subfamily** | **Species name** | **ID** | **Locality** | **Collec.** | **28S** | **EF-1α** | **LWRh** | **Pol2** |
| Ceropalinae | *Ceropales tenuatus* Turner | PO227 | Australia | EMUS | | | | |
| | *Ceropales pacifica* Townes | PO233 | U.S.A | EMUS | | | | |
| | *Ceropales* sp. | PO232 | Argentina | EMUS | | | | |
| | *Irenangelus furtiva* Evans | PO262 | Peru | EMUS | | | | |
| | *Irenangelus* sp. | PO392 | Argentina | EMUS | | | | |
| Ctenocerinae | *Ctenocerus klugi* Dahlbom | PO165 | South Africa | EMUS | | | | |
| | *Ctenocerus klugi* Dahlbom | PO326 | South Africa | EMUS | | | | |
| | *Paraclavelia crudelis* (Smith) | PO164 | South Africa | EMUS | | | | |
| | *Paraclavelia crudelis* (Smith) | PO173 | South Africa | EMUS | | | | |
| | *Pseudopedinaspis* sp. | PO277 | Madagascar | EMUS | | | | |
| | *Trichosalius* sp. | PO336 | South Africa | EMUS | | | | |
| Notocyphinae | *Notocyphus bipartitus* Banks | PO987 | Colombia | EMUS | | | | |
| | *Notocyphus dorsalis* Cresson | PO27 | U.S.A | EMUS | | | | |
| | *Notocyphus sp.* | PO28 | Costa Rica | EMUS | | | | |
| | *Notocyphus* sp. | PO289 | Argentina | EMUS | | | | |
| Pompilinae | *Ageniodeus* (*Ridestus*) *biedermani* (Banks) | PO189 | U.S.A | EMUS | | | | |
| | *Ageniodeus* (*Gymnochares*) *birkmanni* (Banks) | PO191 | U.S.A | EMUS | | | | |
| | *Agenioideus* (*Agenioideus*) *humilis* (Cresson) | PO141 | U.S.A | EMUS | | | | |
| | *Agenioideus* sp. | PO340 | Madagascar | EMUS | | | | |
| | *Allochares azureus* (Cresson) | PO387 | U.S.A | EMUS | | | | |
| | *Ammosphex occidentalis* (Dreisbach) | PO7 | U.S.A | EMUS | | | | |
| | *Anoplius* (*Lophopompilus*) *aethiops* (Cresson) | PO8 | U.S.A | EMUS | | | | |
| | *Anoplochares apicatus* Provancher | PO171 | U.S.A | EMUS | | | | |
| | *Aporinellus atristylus* (Saussure) | PO43 | Madagascar | EMUS | | | | |
| | *Aporinellus fuscatus* (Kohl) | PO148 | Chile | EMUS | | | | |
| | *Aporinellus sinuatus* Evans | PO42 | U.S.A | EMUS | | | | |
| | *Aporus bicolor* Spinola | PO333 | Israel | EMUS | | | | |

| | | | |
|---|---|---|---|
| *Aporus bicolor* Spinola | PO310 | Spain | EMUS |
| *Aporus luxus* (Banks) | PO6 | U.S.A | EMUS |
| *Aporus niger* (Cresson) | PO11 | U.S.A | EMUS |
| *Aporus unicolor* Spinola | PO311 | Spain | EMUS |
| *Arachnospila scelestus* (Cresson) | PO158 | U.S.A | EMUS |
| *Arachnospila* (*Ammosphex*) *smaragdina* (Herbst) | PO153 | Chile | EMUS |
| *Aridestus jaffueli* (Herbst) | PO144 | Chile | EMUS |
| *Atelostegus thrinax* Kohl | PO342 | Madagascar | EMUS |
| *Atopopompilus* nr. c*arinatus* (Radoszkowski) | PO281 | Madagascar | EMUS |
| *Atopopompilus nefas* (Dalla Torre) | PO32 | Madagascar | EMUS |
| *Batozonellus fuliginosis* (Klug) | PO204 | South Africa | EMUS |
| *Batozonellus madecassus* (Saussure) | PO169 | Madagascar | EMUS |
| *Ctenostegus hilli* Turner | PO131 | Australia | EMUS |
| *Dicranoplius cujanus* (Holmberg) | PO199 | Argentina | EMUS |
| *Dicranoplius diphonicus* (Spinola) | PO151 | Chile | EMUS |
| *Entomobora crassitarsis* (Costa) | PO312 | Spain | EMUS |
| *Epiclinotus* sp. | PO352 | South Africa | EMUS |
| *Euplaniceps saussurei* (Kohl) | PO145 | Chile | EMUS |
| *Euplaniceps sima* Bradley | PO290 | Argentina | EMUS |
| *Euryzonotulus nigeriensis* Arnold | PO356 | Madagascar | EMUS |
| *Evagetes* nr. *argenteodecoratus* (Cameron) | PO349 | South Africa | EMUS |
| *Evagetes nitidulus* (Guérin) | PO400 | Chile | EMUS |
| *Ferreola erythrocephala* (Guérin) | PO339 | Madagascar | EMUS |
| *Ferreola saussurei* (Banks) | PO26 | Madagascar | EMUS |
| *Ferreola* sp. | PO343 | South Africa | EMUS |
| *Homonotus* sp. | PO224 | Australia | EMUS |
| *Homonotus* sp. | PO388 | Thailand | EMUS |
| *Kyphopompilus atriventris* Wahis | PO36 | Madagascar | EMUS |
| *Microphadnus* sp. | PO278 | Madagascar | EMUS |
| *Microphadnus* sp. | PO159 | Madagascar | EMUS |
| *Perissopompilus phoenix* (Evans) | PO70 | U.S.A | EMUS |
| *Perissopompilus* sp. | PO121 | U.S.A | EMUS |
| *Poecilopompilus algidus* (Smith) | PO49 | Costa Rica | EMUS |
| *Pompilus cinereus* (Fabricius) | PO270 | Madagascar | EMUS |
| *Psorthaspis connexa* (Cresson) | PO64 | Costa Rica | EMUS |

| | | | | |
|---|---|---|---|---|
| | *Schistonyx aterrimus* Arnold | PO257 | Namibia | EMUS |
| | *Schistonyx* sp. | PO346 | Madagascar | EMUS |
| | *Schistonyx nyassae* (Dalla Torre) | PO353 | Madagascar | EMUS |
| | *Tachypompilus ferrugineus* Say | PO38 | U.S.A | EMUS |
| | *Telostegus* sp. | PO329 | Israel | EMUS |
| | *Turneromyia ahrimanes* (Turner) | PO222 | Australia | EMUS |
| | *Turneromyia wiluna* (Evans) | PO220 | Australia | EMUS |
| | *Xenopompilus tarascanus* Evans | PO116 | Costa Rica | EMUS |
| | *Xenopompilus nugador* (Evans) | PO119 | Mexico | EMUS |
| | *Balboana* sp. | PO395 | Bolivia | EMUS |
| | *Priochilus captivum* (Fabricius) | PO964 | Brazil | UFES |
| | *Priochilus sericeifrons* (Fox) | PO260 | Peru | EMUS |
| | *Priochilus* sp. | PO398 | Guyana | EMUS |
| | *Priochilus* sp. | PO264 | Bolivia | EMUS |
| | *Priochilus* sp. | PO347 | Bolivia | EMUS |
| | *Priochilus splendidum* (Fabricius) | PO385 | Guyana | EMUS |
| | *Sericopompilus neotropicalis* (Cameron) | PO53 | U.S.A | EMUS |
| Pepsinae | *Ageniella* (*Ageniella*) *accepta* (Cresson) | PO52 | U.S.A | EMUS |
| | *Ageniella* (*Cyrtagenia*) *fallax* Arlé | PO535 | Brazil | UFES |
| | *Ageniella* (*Ageniella*) *coronata* Banks | PO75 | U.S.A | EMUS |
| | *Ageniella* (*Priophanes*) *faceta faceta* (Cresson) | PO354 | U.S.A | EMUS |
| | *Ageniella* (*Priophanes*) *sanguinolenta* (Smith) | PO812 | Brazil | UFES |
| | *Ageniella* (*Alasagenia*) *sartoriana* (Cresson) | PO288 | Mexico | EMUS |
| | *Ageniella* (*Priophanes*) sp. | PO526 | Peru | EMUS |
| | *Ageniella* (*Ameragenia*) *zeteki* Banks | PO512 | Nicaragua | EMUS |
| | *Aimatocare longula* (Banks) | PO263 | Bolivia | EMUS |
| | *Auplopus adjunctus* (Banks) | PO78 | U.S.A | EMUS |
| | *Auplopus mellipes* (Say) | PO2 | U.S.A | EMUS |
| | *Auplopus smithi* (Dalla Torre) | PO265 | Peru | EMUS |
| | *Auplopus* sp. | PO20 | Madagascar | EMUS |
| | *Auplopus* sp. | PO16 | Madagascar | EMUS |
| | *Auplopus* sp. | PO293 | Papua New Guinea | EMUS |
| | *Auplopus* sp. | PO350 | Madagascar | EMUS |
| | *Auplopus* sp. | PO302 | Papua New Guinea | EMUS |

| | | | |
|---|---|---|---|
| *Caliadurgus cinereus* (Fox) | PO161 | Chile | EMUS |
| *Caliadurgus* sp. | PO320 | Australia | EMUS |
| *Calopompilus feroculis* (Banks) | PO284 | U.S.A | EMUS |
| *Calopompilus pyrrhomelas* (Walker) | PO57 | U.S.A | EMUS |
| *Chirodamus hirsutulus* (Spinola) | PO168 | Chile | EMUS |
| *Cordyloscelis* sp. | PO338 | South Africa | EMUS |
| *Cryptocheilus idoneum birkmanni* Banks | PO62 | U.S.A | EMUS |
| *Cryptocheilus terminatus terminatus* (Say) | PO283 | U.S.A | EMUS |
| *Cyphononyx vitiensis* Turner | PO875 | Fiji | EMUS |
| *Diplonyx campanulatus* Saussure | PO970 | Madagascar | EMUS |
| *Deuteragenia sayi* (Banks) | PO81 | Madagascar | EMUS |
| *Deuteragenia sericea* (Banks) | PO5 | U.S.A | EMUS |
| *Deuteragenia* sp. | PO348 | Hungary | EMUS |
| *Dipogon graenicheri* Banks | PO77 | U.S.A | EMUS |
| *Dinosalius flavifrons* (Cameron) | PO301 | Malaysia | EMUS |
| *Epipompilus bushi* Evans | PO317 | Australia | EMUS |
| *Epipompilus incompletus* Evans | PO163 | Australia | EMUS |
| *Epipompilus insularis* Kohl | PO304 | New Zealand | EMUS |
| *Epipompilus tucumanus* Evans | PO213 | Bolivia | EMUS |
| *Epipompilus* sp. | PO389 | Colombia | EMUS |
| *Entypus unifasciatus* (Say) | PO184 | U.S.A | EMUS |
| *Hemipepsis australasiae* (Smith) | PO221 | Australia | EMUS |
| *Hemipepsis* nr. *capensis* | PO24 | Madagascar | EMUS |
| *Hemipepsis ustulata ochroptera* Stal | PO30 | U.S.A | EMUS |
| *Herbstellus pachylopus* (Kohl) | PO149 | Chile | EMUS |
| *Lepidocnemis antiquus* Haupt | PO402 | Argentina | EMUS |
| *Leptodialepis* (*Nyctalosalius*) sp. | PO300 | India | EMUS |
| *Machaerothrix* sp. | PO672 | Thailand | EMUS |
| *Macromeris* sp. | PO256 | Papua New Guinea | EMUS |
| *Maurillus australis* Smith | PO404 | Australia | EMUS |
| *Maurillus* sp. | PO405 | Australia | EMUS |
| *Maurillus* sp. | PO406 | Australia | EMUS |
| *Maurillus* sp. | PO225 | Australia | EMUS |
| *Melanagenia* sp. | PO1003 | New Caledonia | RW |
| *Minagenia julia* (Brimley) | PO230 | U.S.A | EMUS |
| *Minagenia* sp. | PO274 | Madagascar | EMUS |
| *Minagenia* sp. | PO973 | India | EMUS |

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|---|---|---|---|---|---|---|---|---|
| | *Minagenia* sp. | PO967 | South Africa | EMUS | | | | |
| | *Pepsis formosa* (Say) | PO360 | U.S.A | EMUS | | | | |
| | *Pepsis pallidolimbata* Lucas | PO358 | U.S.A | EMUS | | | | |
| | *Phanagenia bombycina* (Cresson) | PO916 | U.S.A | UFES | | | | |
| | *Pompilocalus caupolican* Roig Alsina | PO150 | Chile | EMUS | | | | |
| | *Priocnemella micans* (Fabricius) | PO545 | French Guyana | EMUS | | | | |
| | *Priocnemis minorata* Banks | PO34 | U.S.A | EMUS | | | | |
| | *Priocnemis pertubator* (Harris) | PO313 | Hungary | EMUS | | | | |
| | *Priocnemis parvula* Dahlbom | PO309 | Spain | EMUS | | | | |
| | *Priocnemis* sp. | PO201 | South Africa | EMUS | | | | |
| | *Priocnemis* sp. | PO321 | Australia | EMUS | | | | |
| | *Priocnessus nuperus* (Cresson) | PO286 | U.S.A | EMUS | | | | |
| | *Priocnessus* sp. | PO66 | Costa Rica | EMUS | | | | |
| | *Psoropempula erythrostethus* (Smith) | PO200 | Australia | EMUS | | | | |
| | *Psoropempula perpulchra* (Turner) | PO223 | Australia | EMUS | | | | |
| | *Sphictostethus fugax* (Fabricius) | PO296 | New Zealand | EMUS | | | | |
| | *Sphictostethus xanthopus* (Spinola) | PO167 | Chile | EMUS | | | | |
| Outgroup | *Chyphotes mellipes* (Blake) | Chyphotes | U.S.A | EMUS | Pilgrim et al. 2008 | Pilgrim et al. 2008 | Pilgrim et al. 2008 | |
| | *Dasymutilla chiron* (Blake) | JP256 | U.S.A | EMUS | Pilgrim et al. 2008 | Pilgrim et al. 2008 | Pilgrim et al. 2008 | Pilgrim et al. 2008 |

**Table S2.** Genera of spider wasps used in the analyses and current range distribution. Areas are coded as followed: Australian region (A); Oriental region (B); Ethiopian region (C); Neotropical region (D); Nearctic region (E); and Palearctic region (F).

| **Taxon** | **Distribution** |
|---|---|
| *PO262_Irenangelus_furtiva* | ABCD |
| *PO392_Irenangelus_sp.* | ABCD |
| *PO232_Ceropales_sp.* | ABCDE |
| *PO233_Ceropales_pacifica* | ABCDE |
| *PO227_Ceropales_tenuatus* | ABCDE |
| *PO289_Notocyphus_sp.* | DE |
| *PO28_Notocyphus_sp.* | DE |
| *PO27_Notocyphus_dorsalis* | DE |

| | |
|---|---|
| *PO987_Notocyphus_bipartitus* | DE |
| *PO284_Calopompilus_feroculis* | DE |
| *PO57_Calopompilus_pyrrhomelas* | DE |
| *PO338_Cordyloscelis_sp.* | C |
| *PO201_Priocnemis_sp.* | ABCDEF |
| *PO200_Psoropempula_erythrostethus* | A |
| *PO223_Psoropempula_perpulchra* | A |
| *PO81_Deuteragenia_sayi* | BCDEF |
| *PO348_Deuteragenia_sp.* | BCDEF |
| *PO77_Dipogon_graenicheri* | DE |
| *PO5_Deuteragenia_sericea* | BCDEF |
| *PO313_Priocnemis_pertubator* | ABCDEF |
| *PO34_Priocnemis_minorata* | ABCDEF |
| *PO309_Priocnemis_parvula* | ABCDEF |
| *PO296_Sphictostethus_fugax* | AD |
| *PO1003_Melanagenia_sp.* | B |
| *PO402_Lepidocnemis_antiquus* | D |
| *PO150_Pompilocalus_caupolican* | D |
| *PO263_Aimatocare_longula* | D |
| *PO163_Epipompilus_incompletus* | AD |
| *PO304_Epipompilus_insularis* | AD |
| *PO317_Epipompilus_bushi* | AD |
| *PO389_Epipompilus_sp.* | AD |
| *PO213_Epipompilus_tucumanus* | AD |
| *PO149_Herbstellus_pachylopus* | D |
| *PO321_Priocnemis_sp.* | ABCDEF |
| *PO405_Maurillus_sp.* | A |
| *PO406_Maurillus_sp.* | A |
| *PO404_Maurillus_australis* | A |
| *PO225_Maurillus_sp.* | A |
| *PO320_Caliadurgus_sp.* | DEF |
| *PO168_Chirodamus_hirsutulus* | D |
| *PO358_Pepsis_pallidolimbata* | D |
| *PO360_Pepsis_formosa* | D |
| *PO167_Sphictostethus_xanthopus* | AD |
| *PO161_Caliadurgus_cinereus* | DEF |
| *PO24_Hemipepsis_nr_capensis* | ABCDE |
| *PO30_Hemipepsis_ustulata* | ABCDE |
| *PO286_Priocnessus_nuperus* | DE |
| *PO300_Leptodialepis_sp.* | A |
| *PO301_Dinosalius_flavifrons* | A |
| *PO221_Hemipepsis_australasiae* | ABCDE |
| *PO970_Diplonyx_campanulatus* | AB |
| *PO184_Entypus_unifasciatus* | D |
| *PO62_Cryptocheilus_idoneum* | ACDE |
| *PO283_Cryptocheilus_terminatus* | ACDE |
| *PO672_Machaerothrix_sp.* | B |
| *PO916_Phanagenia_bombycina* | BE |
| *PO52_Ageniella_accepta* | DE |
| *PO812_Ageniella_sanguinolenta* | DE |
| *PO545_Eragenia_micans* | D |
| *PO535_Ageniella_fallax* | DE |
| *PO66_Priocnessus_sp.* | DE |
| *PO288_Ageniella_sartoriana* | DE |

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|---|---|
| *PO512_Ageniella_zeteki* | DE |
| *PO526_Ageniella_sp.* | DE |
| *PO354_Ageniella_faceta* | DE |
| *PO75_Ageniella_coronata* | DE |
| *PO2_Auplopus_mellipes* | ABCDEF |
| *PO78_Auplopus_adjunctus* | ABCDEF |
| *PO256_Macromeris_sp.* | AB |
| *PO302_Auplopus_sp.* | ABCDEF |
| *PO293_Auplopus_sp.* | ABCDEF |
| *PO16_Auplopus_sp.* | ABCDEF |
| *PO20_Auplopus_sp.* | ABCDEF |
| *PO265_Auplopus_smithi* | ABCDEF |
| *PO350_Auplopus_sp.* | ABCDEF |
| *PO875_Cyphononyx_vitiensis* | BF |
| *PO274_Minagenia_sp.* | BCDEF |
| *PO973_Minagenia_sp.* | BCDEF |
| *PO967_Minagenia_sp.* | BCDEF |
| *PO230_Minagenia_julia* | BCDEF |
| *PO53_Sericopompilus_neotropicalis* | EA |
| *PO964_Priochilus_captivum* | D |
| *PO347_Priochilus_sp.* | D |
| *PO264_Priochilus_sp.* | D |
| *PO260_Priochilus_sericeifrons* | D |
| *PO385_Priochilus_splendidum* | D |
| *PO398_Priochilus_sp.* | D |
| *PO395_Balboana_sp.* | D |
| *PO169_Batozonellus_madecassus* | BCF |
| *PO204_Batozonellus_fuliginosis* | BCF |
| *PO43_Aporinellus_atristylus* | BCDEF |
| *PO42_Aporinellus_sinuatus* | BCDEF |
| *PO148_Aporinellus_fuscatus* | BCDEF |
| *PO222_Turneromyia_ahrimanes* | A |
| *PO220_Turneromyia_wiluna* | A |
| *PO131_Ctenostegus_hilli* | A |
| *PO270_Pompilus_cinereus* | ABCF |
| *PO116_Xenopompilus_tarascanus* | DE |
| *PO119_Xenopompilus_nugador* | DE |
| *PO281_Atopopompilus_nr_carinatus* | C |
| *PO342_Atelostegus_thrinax* | C |
| *PO346_Schistonyx_sp.* | CF |
| *PO278_Malgaporus_sp.* | C |
| *PO257_Schistonyx_aterrimus* | CF |
| *PO353_Schistonyx_nyassae* | CF |
| *PO352_Epiclinotus_sp.* | C |
| *PO32_Atopopompilus_nefas* | C |
| *PO145_Euplaniceps_saussurei* | D |
| *PO290_Euplaniceps_sima* | D |
| *PO310_Aporus_bicolor* | DEF |
| *PO311_Aporus_unicolor* | DEF |
| *PO6_Aporus_luxus* | DEF |
| *PO11_Aporus_niger* | DEF |
| *PO64_Psorthaspis_connexa* | DE |
| *PO333_Aporus_bicolor* | DEF |
| *PO70_Perissopompilus_phoenix* | E |

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|---|---|
| *PO121_Perissopompilus_sp.* | E |
| *PO312_Entomobora_crassitarsis* | F |
| *PO171_Anoplochares_apicatus* | EF |
| *PO158_Arachnospila_scelestus* | EF |
| *PO7_Ammosphex_occidentalis* | E |
| *PO153_Arachnospila_smaragdina* | EF |
| *PO144_Aridestus_jaffueli* | D |
| *PO349_Evagetes_nr_argenteodecoratus* | BCDEF |
| *PO400_Evagetes_nitidulus* | BCDEF |
| *PO387_Allochares_azureus* | DE |
| *PO199_Dicranoplius_cujanus* | D |
| *PO151_Dicranoplius_diphonicus* | D |
| *PO8_Anoplius_aethiops* | ABCDEF |
| *PO159_Microphadnus_sp.* | ACF |
| *PO329_Telostegus_sp.* | AF |
| *PO36_Kyphopompilus_atriventris* | C |
| *PO49_Poecilopompilus_algidus* | DE |
| *PO141_Agenioideus_humilis* | ABCDEF |
| *PO38_Tachypompilus_ferrugineus* | BCDE |
| *PO191_Ageniodeus_birkmanni* | ABCDEF |
| *PO356_Euryzonotulus_nigeriensis* | C |
| *PO340_Agenioideus_sp.* | ABCDEF |
| *PO388_Homonotus_sp.* | FC |
| *PO224_Homonotus_sp.* | FC |
| *PO339_Ferreola_erythrocephala* | F |
| *PO26_Ferreola_saussurei* | F |
| *PO343_Ferreola_sp.* | F |
| *PO189_Ageniodeus_biedermani* | ABCDEF |
| *PO336_Trichosalius_sp.* | C |
| *PO326_Ctenocerus_klugi* | C |
| *PO165_Ctenocerus_klugi* | C |
| *PO277_Pseudopedinaspis_sp.* | C |
| *PO164_Paraclavelia_crudelis* | C |
| *PO173_Paraclavelia_crudelis* | C |
| *Sapyga_centrata* | EF |
| *Sapyga_pumila* | EF |
| *Typhoctoides_aphelonyx* | D |
| *Timulla_divergens* | E |
| *Ephuta_grisea* | DE |