

**PHYLOGENETIC STUDIES OF APOID WASPS (HYMENOPTERA: APOIDEA)
WITH INSIGHTS INTO THE EVOLUTION OF COMPLEX BEHAVIORS**

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By

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Ansel Payne, M.S.

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ABSTRACT

The wasp superfamily Apoidea – a group composed of more than 20,000 species of solitary, cleptoparasitic, and social bees, as well as a paraphyletic grade of more than 9,600 species of predatory and cleptoparasitic wasps – has played an outsized role in the history of behavioral research. Favorite subjects of field naturalists and ethologists for more than two hundred years, these insects have evolved a tremendous diversity of behavioral strategies, each one an equally successful variation on a shared ancestral groundplan. Understanding the course of these evolutionary derivations and innovations is an important part of understanding insect behavior *in toto*, and one that requires a phylogenetically informed, comparative approach.

As a contribution to ongoing efforts in apoid phylogenetic systematics – and by extension to the study of behavioral evolution within the group – the current work presents four phylogenetic studies of apoid taxa, with an additional fifth study examining the placement of Apoidea within Hymenoptera as a whole. Each provides some insight into the evolution of a complex behavioral syndrome, namely the development of predatory behavior from within a parasitoid wasp clade (Chapter II), the origins of cleptoparasitism in apid bees (Chapter III), trends in prey choice among philanthine wasps (Chapters IV and V), and innovations in nesting behavior within thread-waisted wasps (Chapter VI).

In the first of these studies, I use a combination of direct optimization phylogeny reconstruction and clade sensitivity analysis to re-examine a previously published total evidence dataset based on 111 taxa from across Hymenoptera. This new analysis simultaneously reveals and formalizes deep topological instabilities within this important insect order, and shows how such instability can complicate back-of-the-envelope reconstructions of behavioral evolution (e.g., the origins of aculeate predatory behavior from within a paraphyletic "Parasitica").

In the second, I demonstrate once again the importance of combining multiple classes of phylogenetically informative characters through a simultaneous reanalysis of the bee family Apidae. By merging previously published datasets based on molecular, behavioral, and adult and larval morphological characters (and by providing new adult and larval character codings for taxa previously represented by molecular data alone), I add qualified support to a recently published, nucleotide-derived hypothesis concerning the origins of cleptoparasitism. This hypothesis – that the trait evolved fewer times than previously supposed, with the nomadine and "melectine" lineages sharing a common cleptoparasitic ancestor – is corroborated under a variety of different transformation cost parameters and appears relatively robust to the addition of morphological and behavioral data.

The next two studies present the results of the most taxonomically comprehensive phylogenetic analyses of the digger wasp subfamily Philanthinae (Apoidea: Crabronidae) to date. While Chapter IV represents the first molecular analysis of the group to include species level terminals from all eight genera and all four tribes, Chapter V expands that work to include 66 newly coded morphological and behavioral characters. Although basal

relationships among the four tribes remain either ambiguous or poorly supported, monophyly of the hyperdiverse, cosmopolitan genus *Cerceris* is strongly suggested for the first time – a finding that challenges previous notions concerning the evolution of prey choice within the "beetlewolf" tribe Cercerini.

Finally, the last study briefly examines relationships among the so-called "thread-waisted wasps" of the family Sphecidae *sensu stricto* as a prelude to a larger study of nest evolution within the group. While maximum parsimony analysis of 16 nest-related behavioral characters produces a largely unresolved topology, cladistic analysis of a three-gene dataset reveals new cases of paraphyly at both the tribal and generic levels. I briefly discuss the implications of this latter topology for our understanding of nest evolution within the group.

*To Burton and Joseph Payne,
who walked high steel
so the author wouldn't have to*

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Producing these chapters took me halfway around the world, from a coffee farm high above the Nicaraguan countryside to a desert wash in eastern Oregon; from a cowboy bar in New Mexico to a hidden wadi in the West Bank. There were only a few times I thought I might get hurt. Mostly it was an exciting opportunity to see some of the world's hidden places and meet some of her interesting, generous people. To all the men, women, and wasps I met along the way: Thanks.

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Non mihi non tibi sed nobis, babe.

Chacun a ses ruses de guerre, ses procédés d'attaque, sa façon de tuer.

– J. H. Fabre (*in* Legros 1913)

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CHAPTER I

GENERAL INTRODUCTION

Background I: Phylogeny and Behavior

In 1963, in an influential article dedicated to his friend and colleague Konrad Lorenz, Niko Tinbergen laid out the four essential aims of modern ethology (Tinbergen 1963): To fully understand behavioral phenomena, he wrote, one must simultaneously address: a.) their mechanistic causation, b.) their immediate survival value, c.) their specific ontogenetic trajectories, and d.) their unique evolutionary histories. While each of these research aims represents a different way of asking the same question – "Why does behavior *X* exist?" – none on its own can lead to a complete and comprehensive answer (Tinbergen 1963; Sherman 1988). Since observable behavioral traits result from a combination of all four causative pathways, fully understanding their origins necessarily requires a multidimensional, multimodal approach (Sherman 1989).

Given the equal importance of these four "levels of analysis" (Sherman 1988), one might expect to see each represented equally in the animal behavior literature. Unfortunately, even a quick review of that literature (see, for instance, last year's issues of *Ethology* or *Animal Behaviour*) shows just how wrong that expectation can be. By far, the lion's share of effort over the last fifty years has gone toward devising Darwinian (i.e., fitness-based, or "survival value") explanations for given behaviors, followed closely by studies uncovering the physiological mechanisms behind them ("mechanistic causation"). Studies of behavioral development ("ontogeny," e.g., Holmes and Sherman 1982; Wenzel 1993) and those that attempt to place behavioral evolution within its historical, phylogenetic context ("evolutionary history," e.g., Evans 1962, 1966a, 1966b) are by

comparison few and far between (see discussion in Dobson 1985; Lauder 1986; Prum 1990; Wenzel 1992). The fact is that modern animal behavior researchers are much more likely to know why a given behavior might be selected for in the present than we are to understand the evolutionary course it might have taken in the past.

This bias toward mechanistic and survival value studies is not entirely surprising given some of their real advantages in terms of methodological tractability. While phylogenetically informed behavioral research requires a broad understanding of group characters and putative homologies, survival value studies often focus solely on compartmentalizable and autapomorphous behaviors (Wenzel 1992). A single season study testing the effects of nesting strategy on breeding female fitness can be planned with a high degree of geographic and taxonomic focus: One chooses a *model organism*, constructs one or more testable hypotheses, locates the organism in the field, and conducts the necessary experimental tests or observations (see, e.g., Alexander 1986; O'Neill and O'Neill 2003; Payne et al. 2011). These tests do not always go smoothly, of course, and the success or failure of an entire field season may hinge on any number of uncontrollable variables (among them weather, political instability, or the mysterious failure of the organisms to appear in the right place at the right time). The point is merely that Darwinian studies may allow for a more precise delimitation – in a single species, population, location, or time horizon – of a behavioral phenomenon of interest, a delimitation that we might respectfully call the "microscopic" approach to behavioral research.

By contrast, the "macroscopic approach to behavior focuses on making sense of the general patterns that emerge from comparisons of many taxa" (Wenzel 1993). Such

comparisons require both broad sampling of the group of interest and a nuanced understanding of phylogenetic relationships within that group, neither of which may be particularly easy to come by. Among the major obstacles facing the phylogenetically minded ethologist are serious problems associated with large scale data collection, especially for students of taxonomically rich and/or poorly explored insect taxa (see, for example: Lobl and Leschen 2014; Bocak et al. 2014 on Coleoptera); the incompleteness (Tinbergen 1963; Prum 1990) and occasional untrustworthiness (e.g., Frish 1940, vis-à-vis Peckham and Peckham 1898) of the ethological record; and, of course, ongoing and deeply contentious debates over best practices in phylogenetic inference (Hull 1988; Nixon and Carpenter 1993, 1996, 1997, 2012, 2013; Ryan 1996; Felsenstein 2004; Wheeler 2012).

The benefits, however, are well worth the effort. By incorporating tree-thinking into our studies of behavioral phenomena, we come closer to a complete picture of how these phenomena arise, persist, and diversify through the combined forces of natural selection and historical momentum (Brooks and McLennan 1991). Behavioral traits may be used as phylogenetically informative characters in their own right, defining groups and revealing patterns of common descent (Prum 1990; de Queiroz and Wimberger 1993; Wenzel 1993; Zyzkowski and Prum 1999; Bosch et al. 2001; Noll 2002; Cap et al. 2008), or they may be incorporated into simultaneous (i.e., total evidence) analyses of multiple character sets (Nixon and Carpenter 1996), thus enriching the evidentiary basis for phylogenetic classifications (Bosch et al. 2001; Pickett and Carpenter 2010; Caetano and Machado 2013; Payne 2014). In addition, behaviors may be analyzed *a posteriori*, as one or more character states optimized on an analytically derived phylogeny (preferably one

derived at least in part from those characters), and thus reveal explicit patterns of historical innovation, loss, and/or modification (Packer 1991; Winkler and Sheldon 1993; Danforth et al. 1999; Emlen 2006; Rasmussen and Camargo 2008; Cardinal and Danforth 2011; Litman et al. 2011; Hosner and Moyle 2012; Sedivy et al. 2013).

The five independent studies included in this dissertation together provide preliminary phylogenetic scaffolds for studying the evolution of a handful of interesting behavioral characters – nesting strategies, patterns of prey choice, and cleptoparasitism – in selected apoid wasp taxa. As such, the author hopes the current work might be considered as a small contribution to ethology *sensu* Tinbergen (1963), or what we might just as easily call ethology *sensu lato*.

Background II: Apoidea, an Unsettled Taxon

This dissertation's taxonomic parameters are centered around and within the hymenopteran superfamily Apoidea, a somewhat diverse (~30,000 species) and tremendously important aculeate clade that includes both critical pollinators (the bees, Apiformes or Anthophila) and their predatory wasp relatives (Sphecidae *sensu* Bohart and Menke 1976, "Spheciformes" *sensu* Brothers 1975; now commonly referred to as *apoid wasps*). Approximately two-thirds of the included species (or about 20,000 spp.; Ascher and Pickering 2014) are bees: solitary, cleptoparasitic, or social pollenivores held together as a natural group by a number of conspicuous and unchallenged synapomorphies (Michener 2007, pp. 60-62). The remainder constitute a somewhat more heterogeneous and presumably paraphyletic assemblage of approximately 9,600 species (Pulawski 2014) of predatory and cleptoparasitic wasps.

Hymenopterists have traditionally treated Apoidea as one of the three main

branches (along with Chrysidoidea and Vespoidea) of the aculeate, or stinging, wasps (Hymenoptera: Aculeata) (Melo 1999). While many recent studies have cast doubts on the monophyly of Vespoidea (Sharkey 2007; Pilgrim et al. 2008; Heraty et al. 2011; Debevec et al. 2012; Sharkey et al. 2012; Payne et al. 2013), and a handful have done the same for Chrysidoidea (Vilhelmsen et al. 2010; Heraty et al. 2011; Payne et al. 2013), Apoidea's monophyletic status has never been seriously contested. (The group is united by a number of conspicuous characters [Prentice 1998; Melo 1999; Michener 2007], perhaps the most prominent of which is a posterior pronotal lobe that is distinct and *usually well separated from the tegula* [Michener 2007; Bohart and Menke 1976]).

While the superfamily itself is well supported as a natural group in both morphological and molecular studies, relationships among its major constitutive lineages remain far from settled. For instance, although entomologists have long appreciated the close affiliation of some apoid wasps with bees (Michener 2007; but see Lanham 1980), the precise nature of this relationship remains a major current controversy in the systematics literature (Alexander 1992a; Prentice 1998; Melo 1999; Ohl and Bleidorn 2006; Michener 2007; Debevec et al. 2012). The identity of the basal lineage or lineages is also unclear, although most studies have pointed either to the cockroach wasps of the family Ampulicidae (Bohart and Menke 1976; Melo 1999; Debevec et al. 2010; see also Ohl and Spahn 2010), to the bizarre and rarely collected Heterogynaidae (Prentice 1998; although see Ohl and Bleidorn 2006; Debevec et al. 2012), or to a clade uniting the two (Prentice 1998; Melo 1999). Beyond these basic questions, much uncertainty also surrounds subfamilial and tribal relationships within the two largest families, Sphecidae *s. str.* (the "thread-waisted wasps," i.e., mud-daubers and their allies) and Crabronidae,

the latter likely paraphyletic with respect to bees (Prentice 1998; Ohl and Bleidorn 2006; Debevec et al. 2012).

While a thorough review of the history of apoid classification is beyond the scope of the current work, the reader is encouraged to begin with Bohart and Menke's (1976) landmark monograph *Sphecid Wasps of the World*. Although preliminary and non-analytically derived, those authors' hypotheses of subfamilial relationships nevertheless represent the starting point for all subsequent work, and the book as a whole remains the basic reference for systematic and taxonomic studies within non-bee Apoidea. Important other studies include Evans's (1959) work on larval characters and their relationship to classification; Alexander's formal cladistic analyses (1992a) of characters proposed by Bohart and Menke (1976); Prentice's (1998) extended cladistic treatment of internal and external anatomical characters; Melo's (1999) analyses of 139 morphological characters; and more recent molecular work by Ohl and Bleidorn (2006), Lohrmann et al. (2008), and Debevec et al. (2012).

Background III: Select Topics in Apoid Behavioral Evolution

Apoid wasps and bees have played important roles in the modern study of animal behavior and were among the favorite subjects of both popular naturalists (Fabre 1891; Peckham and Peckham 1898; Rau and Rau 1918; reviewed in Evans 1966a) and influential early ethologists (e.g., Tinbergen 1932, among others). Short of reviewing the entire history of apoid behavioral studies – a task well beyond the scope of this general introduction – I here briefly point out some features of the group's behavioral evolution that are most pertinent to the present work.

Cleptoparasitism in bees: While the public notion of a "bee" is often strongly

linked with the idea of advanced social evolution (Wilson 1971; Michener 2007), the highly eusocial lifestyle is actually quite rare among these insects and found within just two tribes of corbiculate Apidae: the Apini and the Meliponini (Michener 2007). While less highly developed forms of eusociality are present in various other bee groups (e.g., the primitively eusocial *Bombus* spp., various lineages of communally nesting halictids), the overall number of social species is dwarfed by the combined totals of solitary and *cleptoparasitic* species – the latter an implicit focus of this dissertation's third chapter.

Cleptoparasites invade the nests of other species and oviposit on the provisions stored by their host, in effect stealing both the material resources and the labor of the parasitized individual (hence *cleptoparasitism*). According to Michener (2007), most of these species have an obligate, rather than opportunistic, pattern of resource appropriation, and in the majority of cases lack the anatomical adaptations necessary for pollen gathering and nest building. As such, the cleptoparasitic lifestyle would appear to be an evolutionary one-way street, with reversal to a pollen-gathering lifestyle extremely unlikely. These observations, along with questions regarding evolutionary relationships between host and parasite species (e.g., the interesting and unsettled case of *Tetrapedia* and *Coelioxys*, see Chapter III), have made the evolution of cleptoparasitism a subject of great interest within the bee systematics community. In this dissertation, I examine recent claims about the origin of the behavior within the family Apidae (Straka and Bogusch 2007; Cardinal et al. 2011) by testing those claims against an expanded evidentiary base and a broad series of parsimony weighting schemes.

Prey choice evolution in apoid wasps: While bees are unified by their use of vegetable material (pollen) for provisioning their larvae, the apoid stock from which they

presumably arose employs a wide variety of arthropod prey, with a range covering almost all of the major insect orders (Evans 1966a), as well as Collembola and Araneae (Bohart and Menke 1976). But while the superfamily as a whole displays an impressive diversity of prey choices, its individual species, genera, and even some tribes are considerably more specific (Polidori et al. 2012); in fact, prey preference characteristics are so reliable and so often constrained by phylogenetic history that they serve a valuable role in taxonomic classification throughout Apoidea (Bohart and Menke 1976).

Given that predator-prey interactions lead *by their very nature* to evolutionary arms races between hunters and the hunted, we might expect these efficient predatory wasps to display a high level of anatomical and behavioral adaptation to specific prey types. Indeed, decades of observational and anatomical studies seem to bear this out, with individual lineages developing extraordinary adaptations suited to the pursuit and capture of preferred prey taxa (see, e.g., Evans [1962] on the evolution of morphological adaptations for prey-carriage; Uma and Weiss [2010] on chemical mediated prey recognition systems; Andrietti [2011] on adaptations in stinging behavior; Polidori et al. [2012] on the correlation between antennal sensillar morphology and prey preference).

But if prey preferences are, in fact, so strongly reinforced by the ratcheting mechanisms of natural selection, why do we observe so much prey choice diversity in the first place? When and how did critical prey preference transitions take place? And what are the morphological or behavioral pre-adaptations that make "breakthroughs" in prey niche utilization possible? These kinds of questions are particularly well suited to the methodology of phylogenetic inference and to the phylogenetically informed approach to behavioral study outlined in *Background I*, above. As such, I devote two chapters of this

dissertation to the construction of a phylogenetic scaffold for studying prey choice in the subfamily Philanthinae, a large clade of bee-, beetle-, and ant-hunting wasps.

Nesting behavior: The construction of a long-term shelter for offspring and their associated provisions represents a major breakthrough in aculeate evolution, and one that may have been a necessary prerequisite for the advanced forms of eusociality found within the apoid and vespoid lineages (Evans and West Eberhard 1970; Wilson 2008). But beyond their evolutionary importance, their comparative ease of study, and the access they provide to little studied immature life stages, it is the extraordinary phenotypic diversity of wasp and bee nests that have made them a favorite topic of field naturalists and behavioral ecologists since at least the time of Fabre (Fabre 1891; Evans 1958; Brockmann 1980; Wenzel 1993; Rozen et al. 2010).

Within Apoidea, three main nest "types" are common: a.) the *cavity nest*, in which pre-existing niches or tunnels are modified (sometimes more so, sometimes less) to create a suitable habitat for larval development; b.) the *free-standing constructed nest*, in which externally harvested materials (usually either mud or plant resin) are used as building materials, with the nest built *de novo* on some suitable substrate; and c.) the most common and perhaps most primitive form, the *fossorial nest*, consisting of one to many tunnels dug directly into the soil. But while each of these types is found repeatedly throughout the superfamily – and while each obviously represents an evolutionarily successful strategy for the protection of larvae and their stores from predators and parasites – the evolutionary connections *between* forms are not immediately obvious.

Within a single apoid family (Sphecidae, s. str., 728 spp.; Pulawski 2014), all three of these forms exist, with few obvious evolutionary patterns suggested by the group's

current classification. In this dissertation, I briefly examine the diversification of nest types within this group using a phylogenetically informed, comparative approach.

Background IV: A Short Introduction to the Data Chapters

In many ways, the current work is typical of recent trends in dissertation structure, in which traditional monographs have given way to thematic collections of published or soon-to-be-submitted manuscripts. Here, for instance, I present five distinct analyses linked primarily by their taxonomic focus (Apoidea) and by their relationship (either implicit or explicit) to ongoing problems in comparative wasp behavior. While each of these studies is introduced in more detail at the start of its respective chapter, what follows are brief *précis*, linked to the themes of this general introduction.

Chapter II: Lanham's (1980) observation that aculeate phylogenetic systematics is "an inherently frustrating subject, in the manner of all phylogenetic studies" certainly rings true vis-à-vis recent attempts to resolve higher order hymenopteran relationships (Heraty et al. 2011; Sharkey et al. 2012). Despite these coordinated efforts, many details of the group's phylogeny remain as unclear as ever – an unfortunate situation for students of deep behavioral evolution in this economically important insect order.

In Chapter II (recently published as Payne et al. 2013), my colleagues and I present a small contribution to ongoing debates surrounding hymenopteran relationships, the status of the order's twenty-two currently recognized superfamilies, and the origins of the predatory aculeate clade (Aculeata) from within the ranks of the hyperdiverse "Parasitica." By subjecting a recently published total evidence dataset to multiple, parallel direct optimization parsimony analyses (each using a different set of transformation cost parameters), we formalize deep instabilities within the hymenopteran

tree of life; at the same time, we also demonstrate strong support for the monophyly of the majority of currently recognized superfamilies.

Chapter III: In the second study (recently published as Payne 2014), I bring many of these same analytical tools to bear on the systematics of the bee family Apidae. While this large and important taxon contains familiar species such as the bumblebees (*Bombus* spp.) and the honeybee (*Apis mellifera*, among the most intensely studied of all insects), it is also home to a large number of cleptoparasitic bees, traditionally divided into several lineages defined by convergent behavioral evolution (Roig-Alsina and Michener 1993).

While several previous studies have attempted to infer the phylogeny of the family (Roig-Alsina and Michener 1993; Straka and Bogusch 2007; Cardinal et al. 2010), each has taken a different approach in terms of phylogenetic character sets (adult and larval morphology, larval morphology, and sequence data, respectively). Operating under the principle that a broader evidentiary base leads to a more robust phylogenetic hypothesis, I combined these datasets (along with new adult morphological character codings for 48 genera, and new larval codings for 22) to deliver the first total evidence, direct optimization-based sensitivity analysis of apid bee relationships. In doing so, I also address ongoing debates regarding the number of origins of cleptoparasitism within the group.

Chapters IV and V: (*N.B.* – The third and fourth studies included in this dissertation are closely related, synergistic analyses of prey choice evolution in the subfamily Philanthinae, and I introduce them here as a coherent unit.) The philanthine wasps (Crabronidae: Philanthinae *sensu* Alexander 1992a,b) are charismatic predators of other holometabolous insects and "some of the most beautiful species in the tribe of fossorial

Hymenoptera" (Cresson 1865); as such, they have been a favorite subject of study for generations of behavioral ecologists and ethologists. Despite this interest, however, the phylogenetic relationships among the four tribes – as well as the monophyletic status of at least three of the eight currently recognized genera – remain largely unclear. In these two studies, I attempt to uncover phylogenetic patterns of prey choice within the group through joint analyses of newly developed molecular and morphological datasets.

Of particular interest here are what we might call *disjunct prey patterns* within presumably well-defined genera: cases in which certain species differ greatly from their close relatives in terms of their prey preferences. For instance, while two of the four species currently assigned to the genus *Aphilanthops* are highly specialized predators of alate *Formica* queens, a third species (*Aphilanthops hispidus*) appears to hunt exclusively for bees and other wasps, the presumably primitive behavior within the subfamily. This pattern is made even more interesting by the prey preferences of the closely related genus *Clypeadon*, a group of behaviorally and morphologically specialized predators on *Pogonomyrmex* workers. Without phylogenetic clarity regarding the relationships of these individuals species to one another, tracing the evolution of their prey preferences remains a highly speculative exercise.

Chapter VI: In the final empirical chapter, I briefly address what Jane Brockmann (1980) has called "[one] of the mysteries of sphecid wasp evolution: How have such extremely diverse nesting patterns evolved within very closely related groups?" As a prelude to a more extensive future study of nest evolution in thread-waisted wasps (i.e., the family Sphecidae *sensu stricto*), I present the results of two preliminary phylogenetic analyses: the first based exclusively on sixteen behavioral characters related to nest

construction and provisioning, the second based on a newly expanded three gene molecular dataset covering all but one of the family's 19 genera.

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CHAPTER II

DIRECT OPTIMIZATION, SENSITIVITY ANALYSIS, AND THE EVOLUTION OF THE HYMENOPTERAN SUPERFAMILIES

Adapted from Payne, Ansel, Phillip M. Barden, Ward C. Wheeler, and James M. Carpenter. 2013. "Direct Optimization, Sensitivity Analysis, and the Evolution of the Hymenopteran Superfamilies." *American Museum Novitates* 3789: 1-19.

Abstract

Even as recent studies have focused on the construction of larger and more diverse datasets, the proper placement of the hymenopteran superfamilies remains controversial. In order to explore the implications of these new data, we here present the first direct optimization-sensitivity analysis of hymenopteran superfamilial relationships, based on a recently published total evidence dataset. Our maximum parsimony analyses of 111 terminal taxa, four genetic markers (18S, 28S, COI, EF-1 α), and 392 morphological/behavioral characters reveal areas of clade stability and volatility with respect to variation in four transformation cost parameters. While most parasitican superfamilies remain robust to parameter change, the monophyly of Proctotrupoidea sensu stricto is less stable; no set of cost parameters yields a monophyletic Diaprioidea. While Apoidea is monophyletic under eight of the nine parameter regimes, no set of cost parameters returns a monophyletic Vespoidea or Chrysidoidea. The relationships of the hymenopteran superfamilies to one another demonstrate marked instability across parameter regimes. The preferred tree (i.e., the one that minimizes character incongruence among data partitions) includes a paraphyletic Apocrita, with (Orussoidea + Stephanoidea) sister to all other apocritans, and a monophyletic Aculeata. "Parasitica" is rendered paraphyletic by the aculeate clade, with Aculeata sister to (Trigonaloidea + Megalyroidea).

Introduction

Despite being one of the most diverse, well-studied, and economically important groups of insects (Goulet and Huber, 1993; Grimaldi and Engel, 2005), the Hymenoptera (ants, bees, wasps, and sawflies) still present major problems for phylogenetic systematists. While certain aspects of the group's phylogeny – the paraphyly of the "Symphyta," the monophyly of the aculeate wasps, and the rise of the Aculeata from within a paraphyletic "Parasitica" – are relatively uncontroversial, the proper placement of the order's 22 extant superfamilies (Sharkey, 2007) remains elusive.

While recent efforts associated with the Hymenoptera Tree of Life project (HymAToL: e.g., Vilhelmsen et al., 2010; Heraty et al., 2011; Sharkey et al., 2012; Klopstein et al., 2013) represent major advances in taxon sampling and character scoring, the results of those studies still point to a deep instability among higher-order hymenopteran relationships. In an effort to further explore the implications of these new data, and to more precisely define regions of topological instability, we here present the first direct optimization-sensitivity analysis of hymenopteran superfamilial relationships, based on a reanalysis of the most recently published total evidence dataset (Sharkey et al., 2012).

Background I: General Outline of Hymenopteran Phylogeny

A long list of synapomorphies – including a unique hamulus-based wing-joining mechanism, protibial antennal cleaners, and a haplodiploid sex determination system (among others, see Sharkey, 2007) – clearly unites the hyperdiverse membership of the Hymenoptera as a natural group (Goulet and Huber, 1993; Grimaldi and Engel, 2005). In addition, the general outline of the order's higher-level relationships are more or less

clear: a basal grade, the "Symphyta," comprising no more than 5% of hymenopteran diversity, leads to an extremely diverse suborder, the Apocrita, united by the evolution of the wasp waist (Vilhelmsen et al., 2010) and a series of highly successful developments in the parasitic lifestyle.

The Apocrita, or true wasps, are further subdivided into two groups, the "Parasitica" (12 superfamilies) and the Aculeata (three superfamilies), the latter defined by an unambiguous synapomorphy in the form of a complex ovipositor based sting apparatus. No readily apparent morphological character unites the extremely diverse parasitican superfamilies (Sharkey et al., 2012), and the results of many phylogenetic studies have pointed to an aculeate origin from within the group (Rasnitsyn, 1988; Dowton and Austin, 1994; Downton et al., 1997; Carpenter and Wheeler, 1999; Vilhelmsen et al., 2010; Heraty et al., 2011; Sharkey et al., 2012; but see Ronquist et al., 1999; Dowton and Austin, 2001).

While this basic outline (Figure 2.1) is relatively uncontroversial (Sharkey, 2007), the details of the superfamilial relationships, and especially of the exact position of the aculeate clade within "Parasitica," are far from settled. Among the more acute problems facing the higher order hymenopteran systematist are: (1) establishing the monophyly of each of the 22 superfamilies proposed by Sharkey (2007); (2) establishing the basal most lineage within the order: either Xyeloidea or some combination of Xyeloidea + other symphytan clades; (3) resolving the phylogenetic structure of the Vespina (Orussoidea + Apocrita) and determining whether or not the symphytan Orussoidea renders Apocrita paraphyletic (as suggested by Heraty et al., 2011); and finally, (4) inferring the position of the Aculeata among the parasitican lineages and establishing the identity of the group's

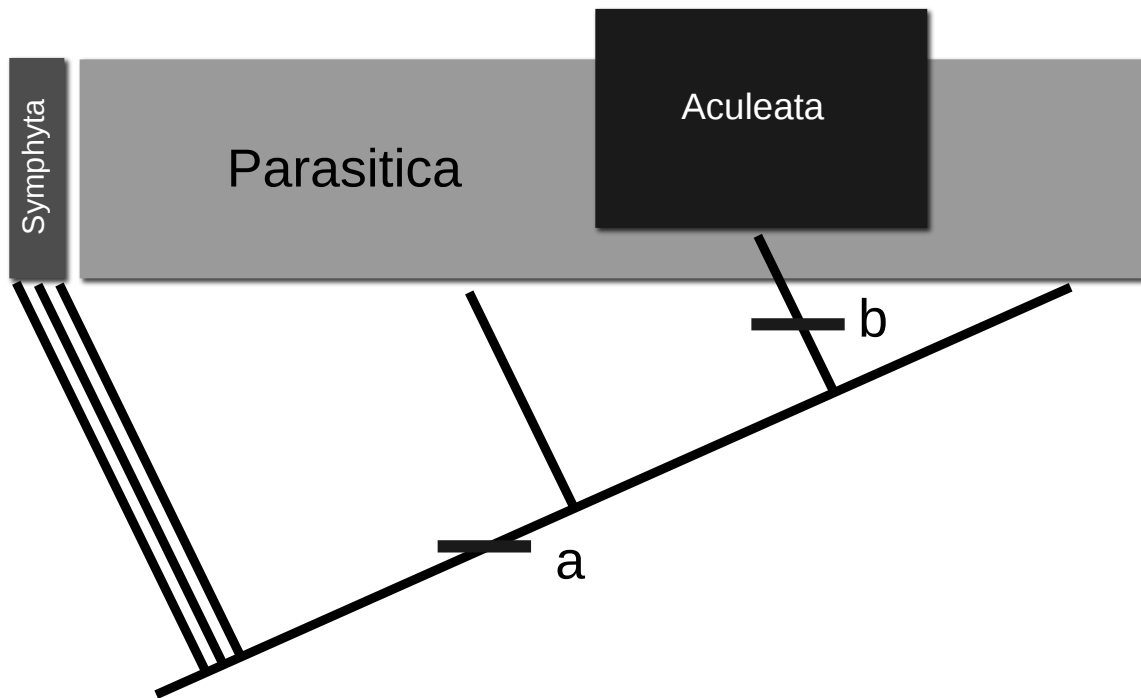


Figure 2.1. Schematic representation of hymenopteran relationships. Box size proportional to estimated species diversity, based on conservative estimates in Goulet and Huber (1993): "Symphyta": ~ 15,000 sp.; Aculeata: ~ 92,000 sp.; "Parasitica": 200,000 sp. Bars represent key synapomorphies: the wasp-waist (a) and the defensive sting apparatus (b). Some estimates of species diversity within "Parasitica" are much higher; see, for instance, the 375,000 to 500,000 chalcidoids predicted by Heraty and Darling (2009).

sister taxon.

In order to contribute to these efforts, we bring a powerful set of analytical tools — direct optimization and sensitivity analysis — to bear on a slightly expanded version of a recently published total evidence dataset. Sharkey et al. (2012) examined 111 taxa, including three outgroups and 84 generic exemplars within Apocrita, using 392 morphological and behavioral characters, along with eye-aligned sequence data from four genes. While this represents the most extensive phylogenetic study of the Hymenoptera to date, their total evidence analysis returned only weak support for a number of important clades and did not address issues of parametric contingency in parsimony analysis (Wheeler, 1995; Giribet, 2003). The current study was designed to expand upon the previous work's findings, and to further explore the implications of the newly available HymaToL data.

Background II: Direct Optimization

When analyzing molecular sequence characters, conventional phylogenetic methods require two separate and sequential optimization procedures: an initial multiple sequence alignment (MSA), followed by some form of character optimization and tree search. Sequence alignment is a necessary first step given that variations in sequence length, which presumably reflect long series of historical insertion and deletion events, are a pervasive feature of comparative molecular datasets.

MSAs are methods for “correcting” this length heterogeneity through the insertion of gaps, placeholders that stand in for absent homologous nucleotides. In doing so, they establish putative homologies among nucleotide base positions across terminal taxa, and at the same time present a visible manifestation of that homology in the form of neat

columns of molecular characters. Once these putative homologies are established, researchers can treat sequence based datasets just as they would any other set of phylogenetically informative static characters (Wheeler, 2001). Firmly embedded in a static matrix, aligned nucleotide characters can reveal the patterns of state change and stasis that form the backbone of phylogenetic analysis.

There are, however, problems associated with this separate and sequential approach. Given that true multiple sequence alignment is computationally prohibitive for all but the most trivial of datasets (Schulmeister et al., 2002), all of the currently implemented MSA optimization methods rely on some form of heuristic search: most often a “binary ‘guide’ tree” that points the way, via a series of simpler pairwise alignments, toward an approximation of the global optimum (Wheeler, 2001). Unfortunately, different guide trees can produce vastly different optimum alignments, which in turn may result in vastly different phylogenetic outcomes. In a worst-case, but probably common scenario, the optimum cladogram for a given alignment will not represent the lowest cost cladogram that *could have been* generated from the same sequence data given a different static alignment.

Wheeler’s (1996) optimization alignment (i.e., direct optimization) algorithm solves this problem by combining the sequence alignment and character optimization/tree search steps. Putative homologies are no longer determined a priori via a separate and prior MSA, but rather with reference to each unique cladogram encountered during a given tree search. Homologies are thus “dynamically determined and uniquely tailored to each topology...” (Wheeler, 2001), with direct optimization based cladograms routinely obtaining lower costs than cladograms derived from conventional analyses (Wheeler,

2001). For an introduction to the mechanics of the optimization alignment algorithm, see Wheeler (1996); for an extended discussion of its advantages in total evidence analysis, see Schulmeister et al. (2002).

Background III: Sensitivity Analysis

At its most basic, the phylogenetic implementation of maximum parsimony is a method for determining the minimum amount of character change demanded by a.) a given dataset, b.) the assumption of common descent, and c.) Hennig's auxiliary principle (Hennig, 1966). As a test of the null hypothesis that putative homology reflects final homology, it does nothing more than minimize the number of ad hoc hypotheses of evolutionary convergence required to explain patterns present within the data.

Despite this logical simplicity, parsimony methods cannot escape the need to assign a priori costs to the various character transformations we seek to optimize (Wheeler, 1995; Donoghue and Ackerly, 1996). While changes in the relative magnitudes of these costs can have a dramatic effect on the outcome of phylogenetic analyses, no empirical, extraphylogenetic methods exist for determining "realistic" cost assignments.

Sensitivity analysis (sensu Wheeler, 1995) allows for a liberal exploration of the effects of varying cost parameters on the outcome of parsimony analyses. By choosing an expanded set of transformation cost regimes and using them as the basis for multiple parallel analyses of the same character data, we can explore the sensitivity of a given clade or clades to changes in those cost parameters (Wheeler, 1995; Schulmeister et al., 2002). Clades that hold together regardless of changes in the relative costs of transitions, transversions, insertion/deletion events, or morphological changes may be considered more stable or more "robust" than those that exist only under one or a few cost regimes

(Giribet, 2003); such robustness may justify more confidence in the clade, and thus function as a form of clade support (Schulmeister et al., 2002).

Of course, the many trees produced by even a small scale sensitivity analysis still leave us with the dilemma of choosing a "best" tree from among the phylogenetic hypotheses derived from competing cost regimes. Wheeler (1995) suggested using one of two measures of congruence, either taxonomic (based on topological agreement) or character based (a measure of character conflict among constituent datasets, e.g. the incongruence length difference of Mickevich and Farris, 1981). Whichever set of cost parameters minimizes the chosen incongruence measure yields the preferred phylogenetic hypothesis.

Materials and Methods

Taxa and characters

Our dataset was nearly identical to the one analyzed by Sharkey et al. (2012); it contained the same 111 genus-level terminals (108 ingroup, 3 outgroup), the same genetic markers (18S, 28S, COI, EF-1 α), and the same 392 morphological/behavioral characters. However, ours also included fragments of 23 additional sequences downloaded from GenBank and used to fill in gaps in the molecular data matrix (accession numbers in Table 2.1). In some cases, these sequences provided molecular characters for genera (*Orgilus*, *Plumarius*, *Spalangia*, and *Urocetus*) that were previously represented by morphology alone (Sharkey et al., 2012). All other sequence, morphological, and behavioral data were obtained directly from one of the previous study's authors (JMC).

Sequences were initially aligned by eye using Geneious Pro version 5.5

Table 2.1. Additional sequences. Sequences downloaded from GenBank and added to the Sharkey et al. (2012) dataset, with accession numbers.

Taxon	18S	28S	COI	EF1- α
<i>Aleiodes</i> sp.	--	--	FJ413811	--
<i>Anacharis zealandica</i>	--	--	AF379981	--
<i>Exallonyx obsoletus</i>	--	--	AF380021	--
<i>Isostasius</i> sp.	--	--	DQ888408	--
<i>Orgilus</i> sp.	--	--	HQ107656	--
<i>Phaenoserphus viator</i>	--	--	AF380022	--
<i>Plumarius</i> sp.	--	EU367150	--	EU367208
<i>Spalangia cameroni</i>	--	AY855173	--	--
<i>Sparasion</i> sp.	DQ888559	AF379927	AF380003	--
<i>Urocerus gigas</i>	AY621143	EF032265	EF032232	--
<i>Urosigalphus</i> sp.	AJ307454	AJ302923	--	--
Outgroups:				
<i>Myrmelon formicularis</i> (Neuroptera composite)	--	--	--	AY620194
<i>Panorpa striata</i>	--	--	--	AF423866
<i>Micropterix</i> spp. COI: <i>M. calthella</i> EF1- α : <i>M. wockei</i>	--	--	HM424688	GU828950, GU829241, & AF436596

(Drummond et al., 2010). This temporary alignment facilitated the identification of nonoverlapping sequence regions (e.g., leading and trailing gaps), and allowed for the partitioning of sequences into shorter homologous fragments (14 subfragments in 18S; 21 in 28S; 6 in COI; 10 in EF-1 α). All gaps were removed prior to the direct optimization phylogenetic analyses described below.

Phylogenetic analyses

Nine total evidence maximum parsimony analyses were performed simultaneously using POY version 4.1.2.1 (Varón et al., 2010). These nine analyses differed only in terms of the costs assigned to four classes of character transformations: insertion/deletion events, transversion substitutions, transition substitutions, and morphological/behavioral changes (Table 2.2). "Neuroptera" was designated as the outgroup for all analyses.

Each analysis began with a 15 hour tree search using POY's default search command on four processors:

```
search(max_time:00:15:00)
```

The trees produced by these nine simultaneous searches were concatenated into a single file that served as the input tree file for the next round of heuristic search. Subsequent tree search iterations each performed 1000 rounds of tree fusing followed by swapping on unique trees:

```
fuse(iterations:1000) select() swap(trees:10) select()
```

The best trees from all nine analyses were again concatenated and used as input for subsequent rounds of fusing and swapping; this procedure continued iteratively until the costs of all nine output tree sets equaled the costs of all nine input tree sets for three consecutive rounds (in this case, after four rounds of tree fusing and swapping).

Table 2.2. Transformation cost parameter regimes examined in this study.

Cost regime	Description
1 : 1 : 1 : 1	equal weights parsimony
1 : 1 : 1 : 2	morphological/behavioral changes twice all others
2 : 1 : 1 : 1	indel events twice all others
2 : 1 : 1 : 2	indels equal to morphological/behavioral changes, twice all others
2 : 2 : 1 : 1	indels equal to transversions, twice all others
2 : 2 : 1 : 2	transitions one half all other changes
4 : 1 : 1 : 1	indels four times all other changes
4 : 1 : 1 : 2	indels twice morphological/behavioral changes, four times substitutions
4 : 2 : 1 : 1	indels twice transversions, four times all other changes

In addition to the total evidence analyses, all four individual gene partitions and the morphological/behavioral dataset were analyzed separately, using the same iterative procedure described above. Analyses were terminated once the input and output tree costs were the same for at least two consecutive rounds of tree search (five rounds each for the 18S, 28S, COI, and EF-1 α partitions; three rounds for the morphology/behavior partition).

The preferred tree was chosen after calculating the incongruence length difference (ILD; Mickevich and Farris, 1981; Wheeler, 1995; Schulmeister et al., 2002) for each total evidence tree and selecting the parameter set that minimized the statistic. The ILD here represents a measure of character incongruence, i.e., the character conflict created by the combination of multiple data partitions.

Clade sensitivities for groups within the preferred tree were calculated and visualized using Cladescan version 1.0 (Sanders, 2010). Bremer supports were calculated using POY version 5.0.1 alpha (Varón et al., 2011) and based on exhaustive enumeration of the TBR neighborhood of the preferred tree:

```
swap(tbr,all,visited:"bremertrees.tre") report(graphsupports:bremer:"bremertrees.tre")
```

Results

Each of the nine total evidence analyses returned a set of one or more most parsimonious trees (Figures 2.2-2.4); of these, the fully resolved tree generated by the 2:2:1:1 parameter set (indels equal to transversions, twice transitions and morphological/behavioral changes) resulted in the lowest ILD score and was thus chosen as the preferred phylogenetic hypothesis (Table 2.3). Details of this minimum ILD (mILD) tree, including Bremer supports and major clade sensitivities, are shown in Figures 2.5 and 2.6,

Figure 2.2. Strict consensus trees produced by four transformation cost parameter sets (1:1:1:1, 1:1:1:2, 2:1:1:1, 2:1:1:2) and simplified, when possible, to the superfamilial level. Total tree lengths, as well as the number of most parsimonious trees, are shown at the bottom left of each tree. Monophyletic Aculeata highlighted.

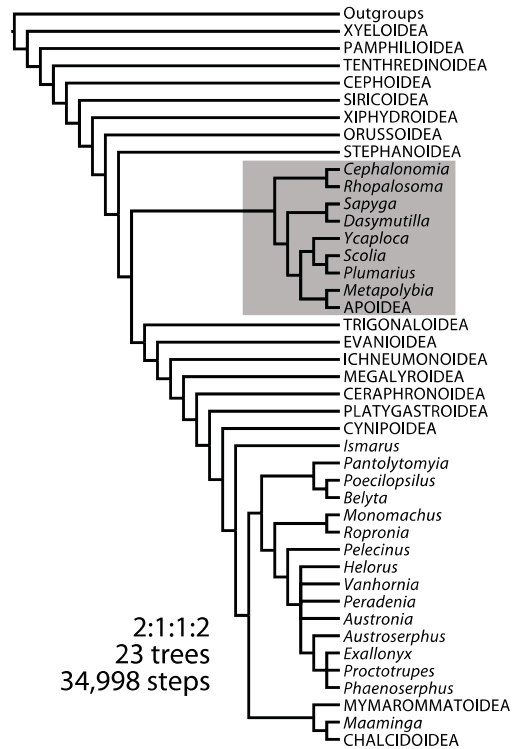
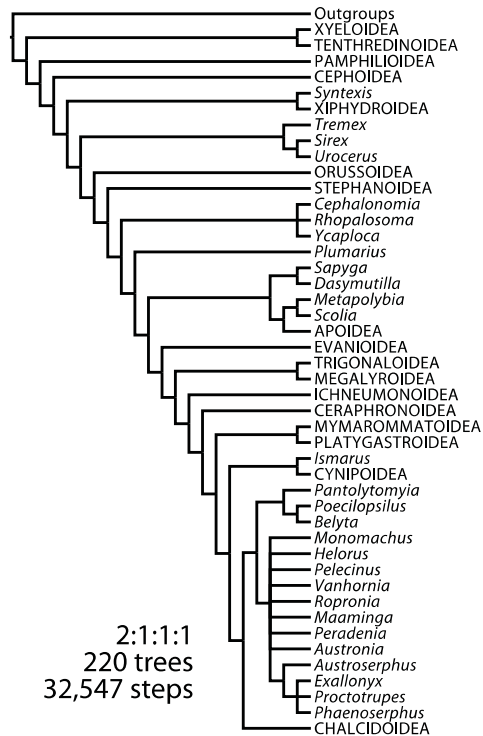
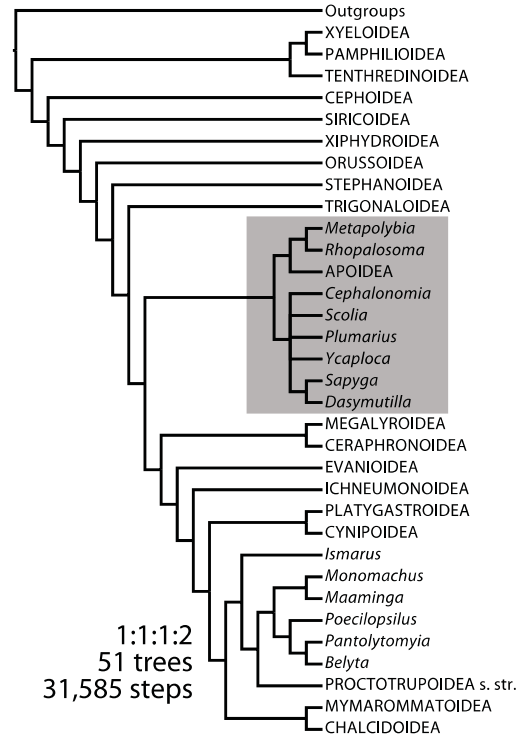
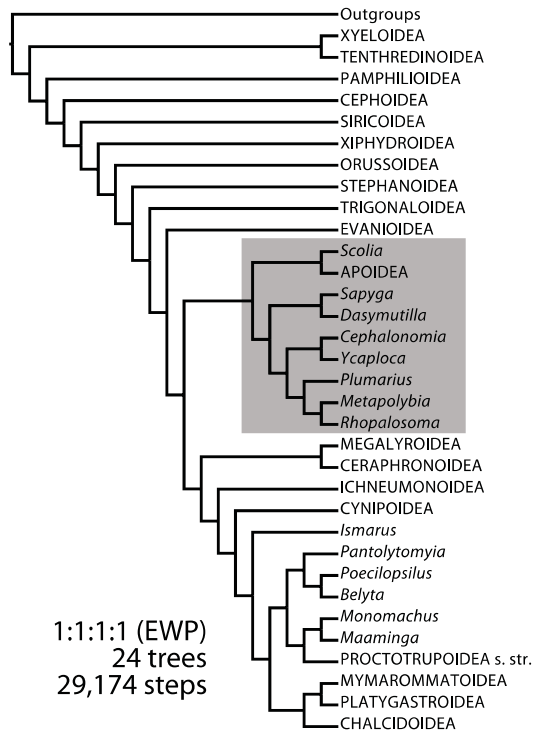
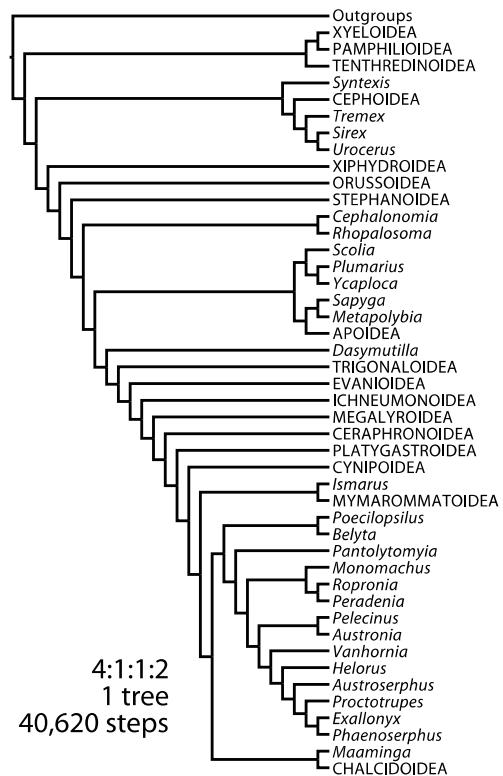
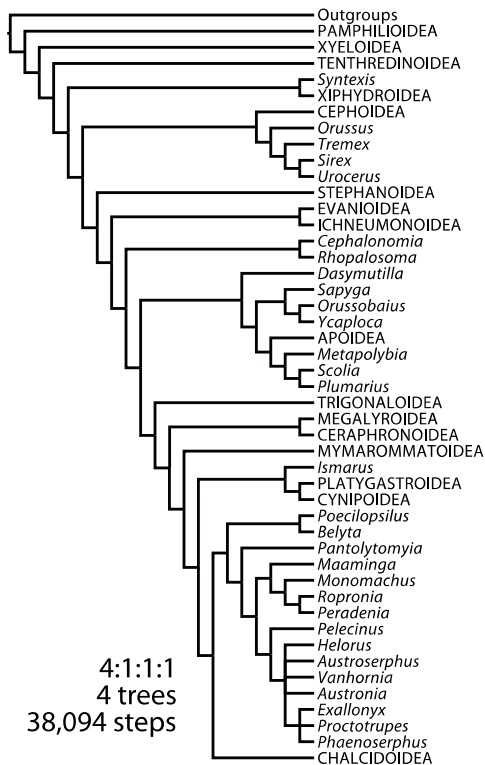
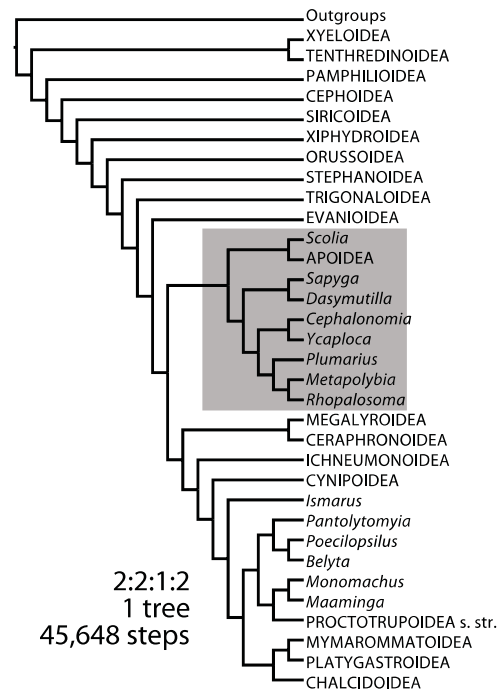
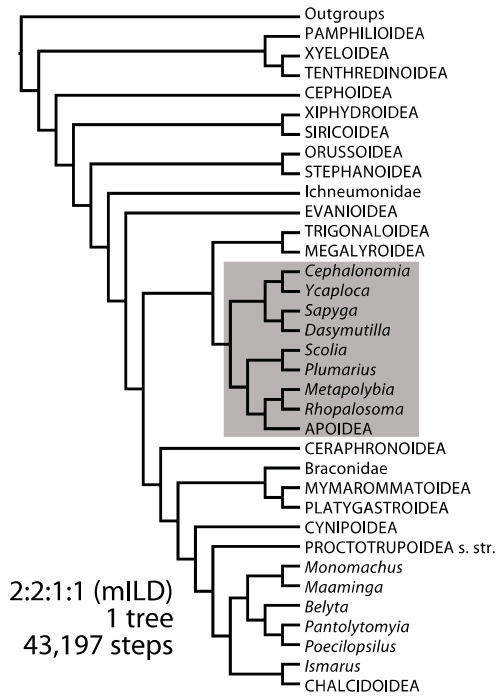


Figure 2.3. Strict consensus trees produced by four additional transformation cost parameter sets (2:2:1:1, 2:2:1:2, 4:1:1:1, 4:1:1:2) and simplified, when possible, to the superfamilial level. Total tree lengths, as well as the number of most parsimonious trees, are shown at the bottom left of each tree. Monophyletic Aculeata highlighted.



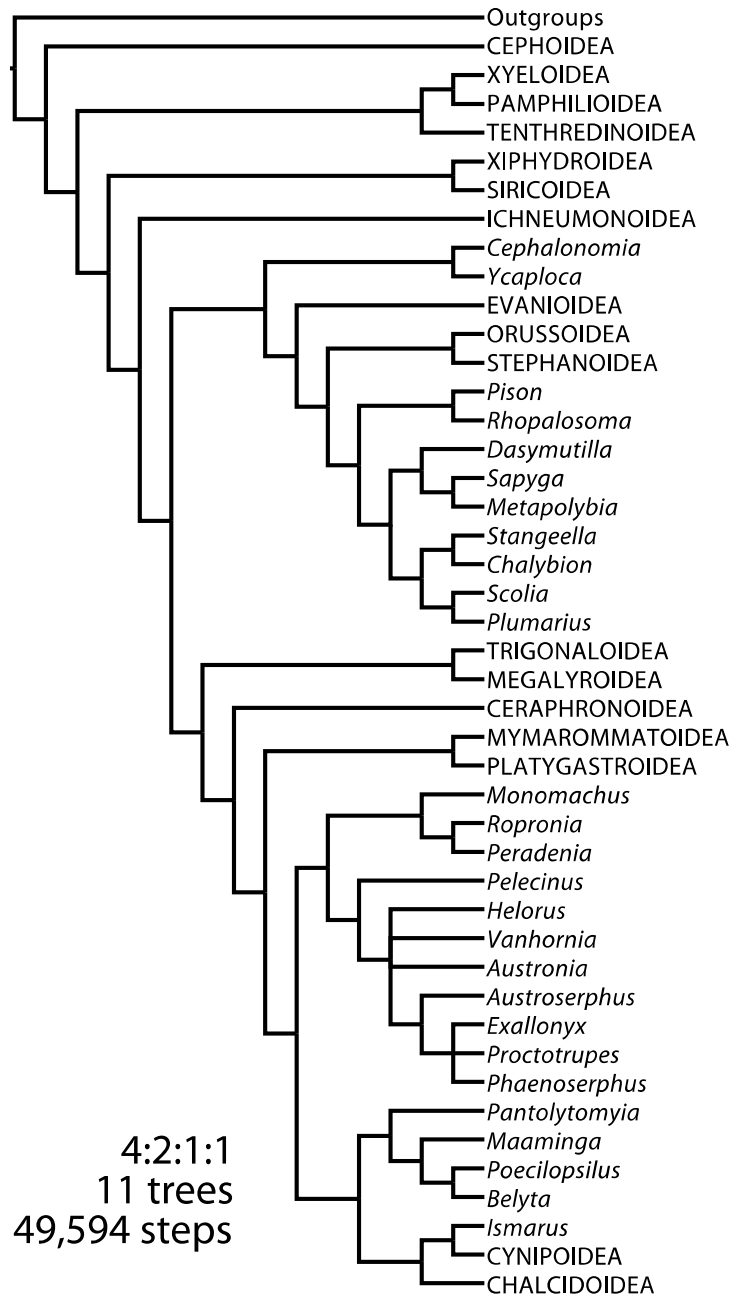


Figure 2.4. Strict consensus tree produced by the 4:2:1:1 cost parameter set and simplified, when possible, to the superfamilial level. Total tree length, as well as the number of most parsimonious trees, at bottom left.

Table 2.3. Partition costs, total evidence tree cost, and incongruence length difference (ILD). Values associated with each of the transformation cost parameter regimes examined in this study. The preferred (mILD) parameter set, 2:2:1:1, is highlighted in gray.

Cost regime	18S	28S	COI	EF1- α	morphology/ behavior	total evidence analysis	ILD
1 : 1 : 1 : 1	2637	8979	7743	6466	2336	29174	0.03472
1 : 1 : 1 : 2	"	"	"	"	4672	31585	0.03445
2 : 1 : 1 : 1	3096	11425	7886	6484	2336	32547	0.04056
2 : 1 : 1 : 2	"	"	"	"	4672	34998	0.04100
2 : 2 : 1 : 1	3810	13981	12779	8830	2336	43197	0.03382
2 : 2 : 1 : 2	"	"	"	"	4672	45648	0.03453
4 : 1 : 1 : 1	3855	15305	7968	6484	2336	38094	0.05633
4 : 1 : 1 : 2	"	"	"	"	4672	40620	0.05751
4 : 2 : 1 : 1	4675	18513	13008	8836	2336	49594	0.04488

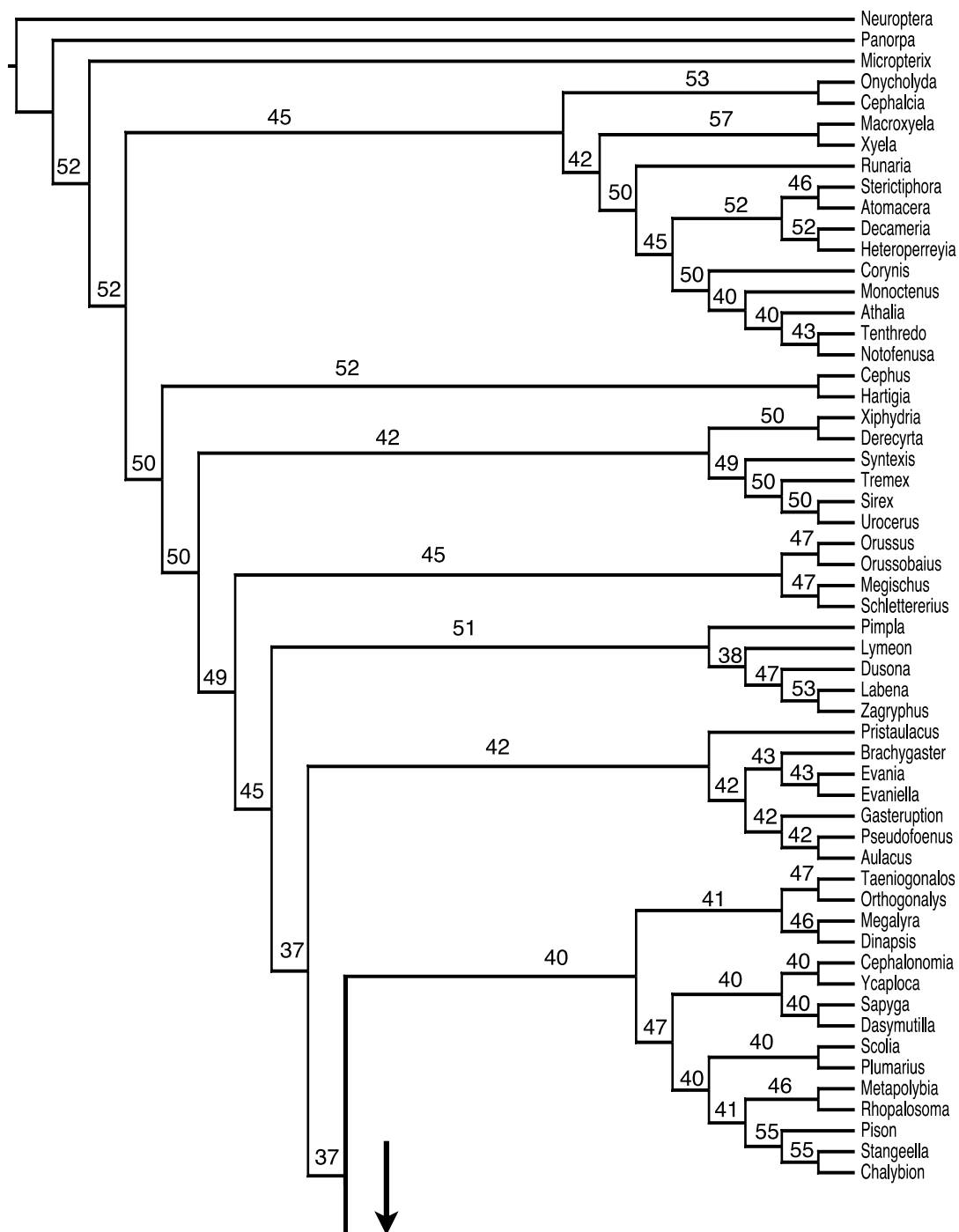
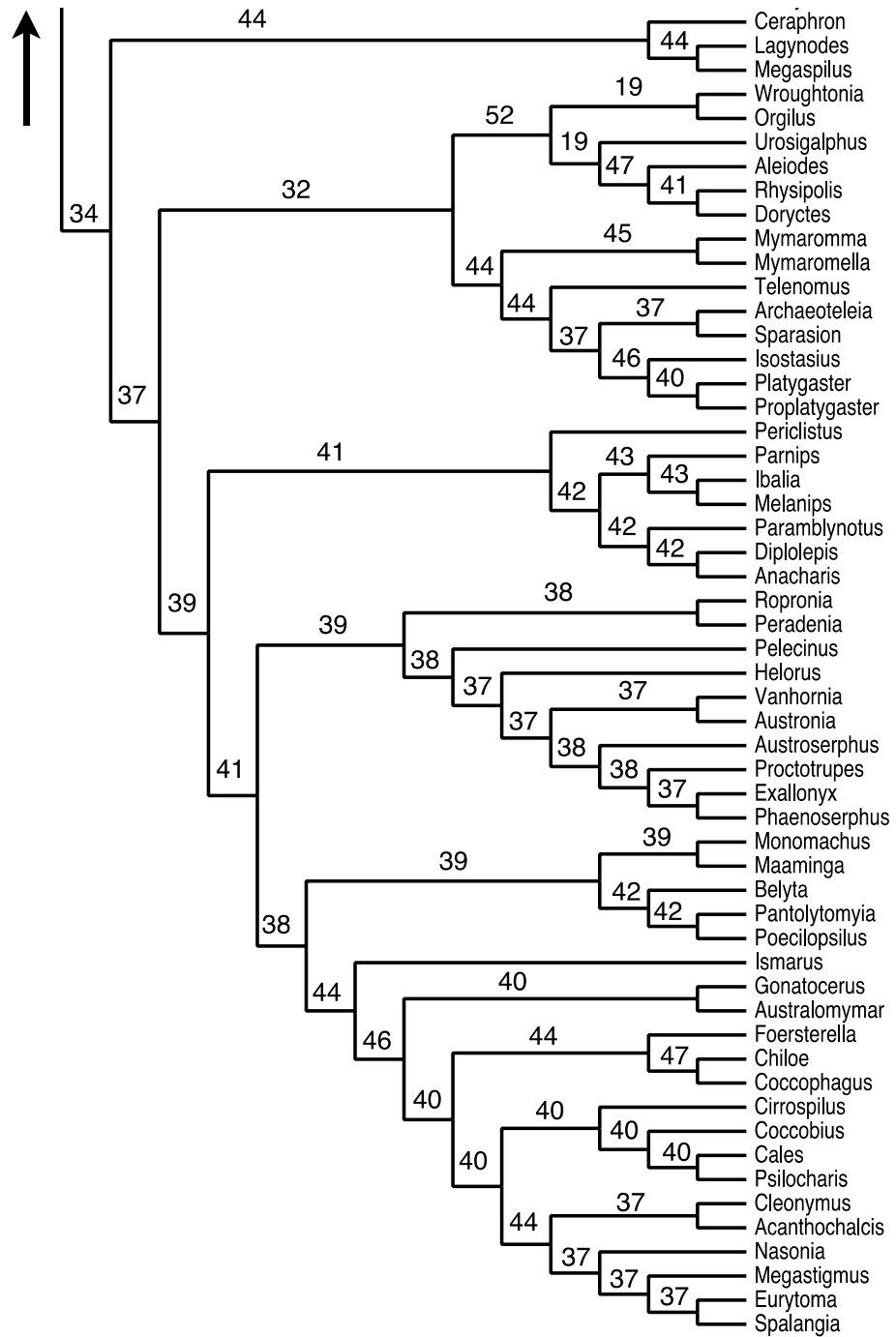


Figure 2.5. Fully resolved genus-level cladogram produced by the 2:2:1:1 (mILD) transformation cost parameter set, with Bremer supports. *Figure extends to two pages.*



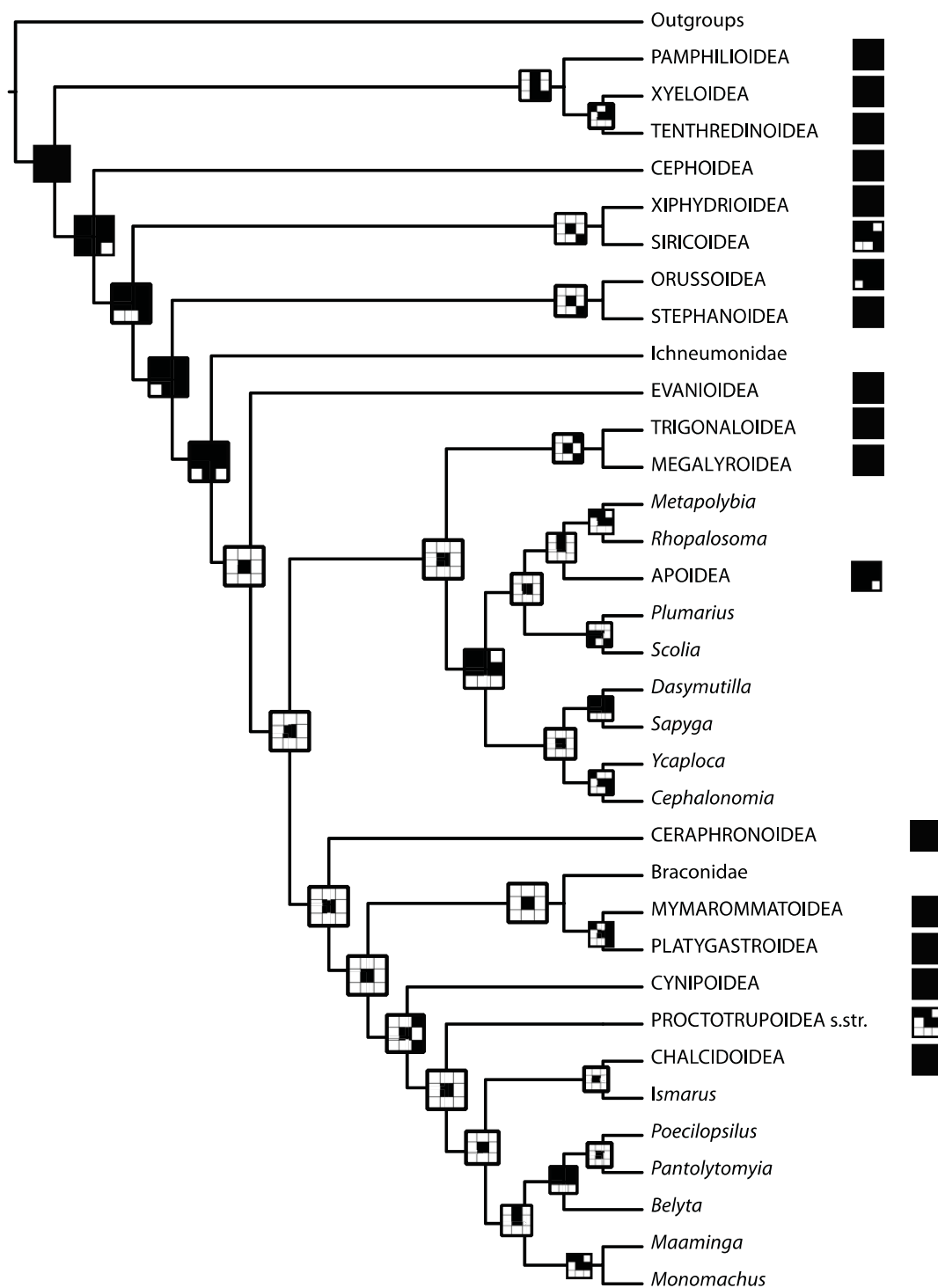


Figure 2.6. Simplified 2:2:1:1 (mILD) tree with sensitivity plots for each node.
 Superfamily sensitivity plots are shown to the right of each superfamilial terminal.

respectively.

The mILD tree differed in a number of respects from the equal weights parsimony (EWP) consensus tree; Figure 2.7 shows a side-by-side comparison of these trees. A simplified version of the EWP tree, with sensitivity plots for major clades superimposed, is shown in Figure 2.8.

Discussion

The results of this study reveal and formalize deep instabilities among higher order hymenopteran phylogenetic relationships, at least with respect to variation in four key transformation cost parameters. Such instability is consistent with a history of competing, mutually incompatible phylogenetic hypotheses (reviewed in Sharkey, 2007; see also Vilhelmsen et al., 2010; Heraty et al., 2011; Sharkey et al., 2012), and serves as a reminder of the difficulties facing hymenopteran systematists. What follows are notes on some of the major implications of our results:

On the mILD tree vs. the EWP tree

Two of our final consensus trees, the mILD (2:2:1:1) and EWP (1:1:1:1) cladograms, deserve special attention: the former, because it maximizes an objective optimality criterion (in this case the minimization of the ILD statistic), and the latter because it is the tree most consistent with an agnostic, equal weights approach to parsimony that also minimizes the overall number of transformations.

Of the two, the mILD tree deviates the most from a traditional and intuitive classification of the Hymenoptera. The most dramatic of these deviations is almost certainly a polyphyletic Ichneumonoidea, with its closely related families Braconidae and Ichneumonidae placed far apart on the tree (Figure 2.7); given the long list of

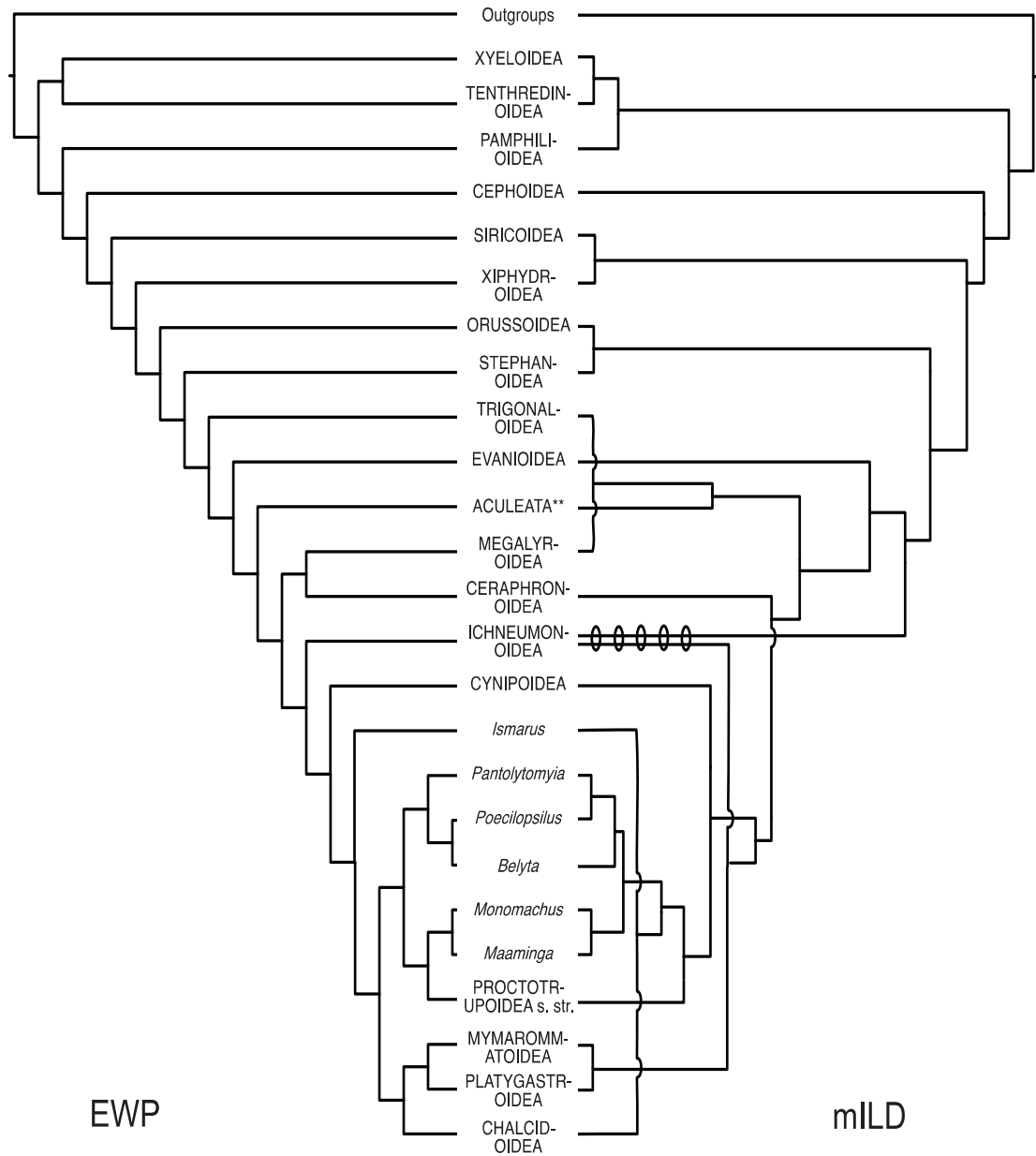


Figure 2.7. Direct comparison of the 2:2:1:1 (mILD) and 1:1:1:1 (EWP) topologies (simplified to superfamilial level). Note the polyphyletic Ichneumonoidea in the mILD tree.



Figure 2.8. Simplified consensus of 24 trees produced by the 1:1:1:1 (EWP) cost parameter set, with sensitivity plots for each node. Superfamily sensitivity plots are shown to the right of each superfamilial terminal

synapomorphies uniting these families (Goulet and Huber, 1993; references therein), this result seems unlikely to reflect actual phylogenetic relationships. In addition, the mILD tree also renders Apocrita paraphyletic with respect to Orussoidea, as discussed below.

These features contrast with the more traditional scheme found in the EWP tree, which also returns the largest proportion of extant superfamilies as monophyletic groups. The two cladograms also differ on many of the details of apocritan relationships.

While we designate the mILD tree as the "preferred" phylogenetic hypothesis, we recognize the value of the EWP tree as an alternate hypothesis and discuss the results of both analyses below.

On the monophyly of the hymenopteran superfamilies

Of the 22 superfamilies evaluated here, 14 (Xyeloidea [S], Tenthredinoidea [S], Pamphilioidea [S], Cephoidea [S], Xiphydroidea [S], Stephanoidea [P], Evanioidea [P], Trigonaloidea [P], Megalyroidea [P], Ceraphronoidea [P], Mymarommatoidea [P], Platygastroidea [P], Cynipoidea [P], and Chalcidoidea [P]; S = "Symphyta," P = "Parasitica," and A = Aculeata) were stable across all nine transformation cost parameter sets. Three more (Orussoidea [S], Ichneumonoidea [P], and Apoidea [A]) were monophyletic in eight out of nine analyses. To the extent that a clade's robustness to parametric change may function as a form of clade support (Giribet, 2003), we consider these groups well-supported by the sensitivity analysis.

Siricoidea, composed of the symphytan families Anxyelidae and Siricidae, was a monophyletic group in six of nine analyses, while Proctotrupoidea sensu stricto [P] (i.e., sensu Sharkey, 2007: Austroniidae + Heloridae + Pelecinidae + Peradeniidae + Proctotrupidae + Proctorenyxidae + Roproniidae + Vanhorniidae) was only monophyletic

in four. The remaining three superfamilies: the parasitican Diaprioidea (again sensu Sharkey, 2007: Diapriidae + Monomachidae + Maamingidae) and the aculeate Chrysidoidea and Vespoidea, did not appear as natural groups under any of the cost regimes.

Diaprioidea is a relatively new concept (Sharkey, 2007), and while the group appeared in both the total evidence parsimony tree of Sharkey et al. (2012) and in the maximum likelihood, Bayesian, and eye-aligned parsimony trees of Heraty et al. (2011), it was not necessarily well-supported (MP tree: bootstrap < 50% [Heraty et al., 2011]; MP tree: symmetric resampling frequency difference = 0 [Sharkey et al., 2012]). In the current study, the group breaks up in a variety of ways depending on the cost parameters investigated; however, one consistent feature is the excision of the diapriid genus *Ismarus* from the rest of Diapriidae and its relocation elsewhere within Proctotrupomorpha. In the EWP tree, *Ismarus* is sister to a clade composed of ((Chalcidoidea + (Mymarommatoidea + Platygastroidea)) + (Diapriidae + (Proctotrupeoidea sensu stricto + (Maamingidae + Monomachidae)))). In the mILD tree, the topology is ((Chalcidoidea + *Ismarus*) + the remaining Diaprioidea). This wayward *Ismarus* and its relationship to the rest of the Diapriidae were anticipated in part by Sharkey (2007), who doubted the latter's monophyly; Vilhelmsen *et al.* (2010) reached a similar conclusion based on morphological data alone, while Sharkey *et al.* (2012) raised the Ismarinae to family status, Ismaridae.

Serious doubts about the monophyly of Vespoidea have been building for some time (Sharkey, 2007; Pilgrim et al., 2008; Heraty et al., 2011; Debevec et al., 2012; Sharkey et al. 2012) and the current study supports that notion. At the moment, the more

interesting question is not whether Vespoidea constitutes a paraphyletic group, but rather how, exactly, other aculeates render that paraphyly. In the current study, we see a variety of vespoid deconstructions: In the EWP tree, *Scolia* is sister to Apoidea, while a polyphyletic Chrysidoidea shows up twice among the remaining Vespoidea. In the mILD tree, Apoidea is sister to *Metapolybia* + *Rhopalosoma*, while *Scolia* is sister to the chrysidoid genus *Plumarius*, and *Sapyga* + *Dasymutilla* is sister to the rest of the chrysidoids. No clear picture of aculeate relationships emerges, with the possible exception of a clade composed of Sapygidae + Mutillidae (in six out of nine analyses), and, of course, the monophyly of Apoidea.

The meaning of a paraphyletic or polyphyletic Chrysidoidea is much harder to gauge. The group has traditionally been considered to be a well established clade, united by a number of key synapomorphies (enlarged female femora, reduction of the Cu₂ vein of the forewing, et cetera [Grimaldi and Engel, 2005]). Among the recent HymaToL studies, Vilhelmsen *et al.* (2010) and Heraty *et al.* (2011) both recovered a nonmonophyletic Chrysidoidea, while Sharkey *et al.* (2012) united their three chrysidoid genera (*Plumarius* [Plumariidae], *Cephalonomia* [Bethylinidae], and *Ycaploca* [Scolebythidae]; the same taxa used in the current study). The true nature of chrysidoid relationships, both within the group and with the other aculeates, is thus unclear.

On the basal most lineage of the Hymenoptera

That the "Symphyta" form a paraphyletic grade at the base of the hymenopteran tree has never really been in doubt (Schulmeister *et al.*, 2002); instead, debate has centered on the precise nature of the relationships of the symphytan superfamilies (Xyeloidea, Pamphilidoidea, Tenthredinoidea, Siricoidea, Cephoidea, Xyphidoidea, and

Orussoidea) to one other (reviewed in Schulmeister et al., 2002) and to the considerably more speciose and economically important Apocrita.

Most recent analyses (Schulmeister et al., 2002; Schulmeister, 2003; Vilhelmsen, 2010; Sharkey et al., 2012,) place Xyeloidea, with its single small and geographically restricted family, in the basal most position within Hymenoptera, a placement bolstered in part by the group's ancient fossil record (Goulet and Huber, 1993; Grimaldi and Engel, 2005). That said, Heraty et al. (2011) united Xyeloidea with Tenthredinoidea as the basal lineage of the order, an arrangement found in three of our nine analyses, including the EWP tree. Four of the nine analyses, including the preferred mILD tree, produced a basal lineage composed of Pamphilioidea + (Xyeloidea + Tenthredinoidea); only two of our cladograms place Xyeloidea alone as the basal lineage.

Of these hypotheses, the last is the most intuitive. The remaining Hymenoptera (the so-called Neohymenoptera; Grimaldi and Engel, 2005) share a number of putative morphological synapomorphies including certain details of wing venation and postspiracular mesothoracic sclerites, among others. Unfortunately, our analyses do little to resolve this debate, except to confirm a place for Xyelidae within the basal lineage; whether or not that relict family is joined by Tenthredinoidea and Pamphilioidea is unclear.

On the phylogenetic structure of Vespina [i.e. Orussoidea + Apocrita]

While some authors have challenged apocritan monophyly through the unification of Orussoidea and Stephanoidea (reviewed in Schulmeister et al., 2002; Heraty et al., 2011), support for this clade has never been particularly strong, and in fact requires the reversal of the wasp waist constriction on the lineage leading to modern orussids. (The

close relationship of Orussoidea to Apocrita, of course, has never been in doubt).

The traditional and intuitive arrangement of Vespina (= Euhymenoptera of Grimaldi and Engel, 2005) is Orussoidea + (Stephanoidea + all other apocritans); this topology allows for a single origin of parasitoid behavior on the branch leading to Vespina, followed by a single, unreversed origin of the wasp waist constriction in the branch leading to Stephanoidea + the other apocritans. In fact, this arrangement is present here in six out of nine analyses, including the EWP tree; only the mILD tree has the Orussoidea + Stephanoidea clade as sister to the rest of Apocrita.

On the position of Aculeata within "Parasitica"

Deciphering the precise relationships among Aculeata and the other apocritan lineages is probably the most challenging issue facing hymenopteran systematists. From the mostly unresolved tree of Königsman (1978 in Whitfield, 1992) to the more-or-less resolved, but poorly supported, total evidence cladogram of Sharkey et al. (2012), a variety of aculeate sister-group hypotheses have been proposed including, but not limited to:

1. Aculeata sister to Ichneumonoidea (= Ichneumonomorpha; Rasnitsyn, 1988; Dowton and Austin, 1994; Dowton et al., 1997; Sharkey, 2007; Vilhelmsen, 2010)
2. Aculeata sister to a monophyletic Parasitica (Ronquist et al., 1999; Dowton and Austin, 2001)
3. Aculeata sister to Evanioidea (Sharkey et al., 2012)
4. Aculeata sister to Trigonoidea or Trigonoidea + Megalyroidea (Heraty et al., 2011)
5. Aculeata sister to all apocritans except Stephanoidea (Vilhelmsen, 2010)

Our trees present a wide range of possible sister groups (Figures 2.2-2.4),

underlining the topological instabilities inherent in apocritan relationships, at least given the current state of taxon and character sampling. In our mILD tree, Aculeata is sister to Trigonoidea + Megalyroidea, while the EWP tree has Aculeata sister to all other apocritans excluding Stephanoidea, Trigonoidea, and Evanioidea. The important point is that these relationships demonstrate too much instability to allow for confident statements regarding final relationships within the true wasps.

On the deep structure of hymenopteran phylogeny

Besides the monophyly of Hymenoptera itself, none of the order's deepest and oldest relationships were unanimously supported across all parameter sets; nevertheless, two important clades were present in eight out of nine analyses: Unicalcarida (all Hymenoptera with the exception of Xyeloidea, Tenthredinoidea, and Pamphilioidea) and Proctotrupomorpha (Platygastroidea + Cynipoidea + Proctotrupeoidea sensu stricto + Diaprioidea + Mymaromatoidea + Chalcidoidea). The symphytan lineages as a whole clearly form a basal grade relative to Apocrita, which may or may not include the orussids as sister to Stephanoidea.

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CHAPTER III

RESOLVING THE RELATIONSHIPS OF APID BEES (HYMENOPTERA: APIDAE) THROUGH A DIRECT OPTIMIZATION SENSITIVITY ANALYSIS OF MOLECULAR, MORPHOLOGICAL, AND BEHAVIOURAL CHARACTERS

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Abstract

Phylogenetic analyses that incorporate the most character information also provide the most explanatory power. Here I demonstrate the value of such an approach through a direct optimization sensitivity analysis of apid bee phylogeny. Whereas prior studies have relied solely on one class of data or the other, this analysis combines previously published molecular, morphological, and behavioural characters into a single supermatrix. The final dataset includes 191 ingroup and 30 outgroup taxa, and includes data from seven unaligned gene sequences (18S, 28S, wingless, EF- α , polIII, NaK, LW rhodopsin), 209 adult and larval morphological characters, and two behavioural characters. Nine different sets of transformation cost parameters are evaluated, along with their relative degrees of character incongruence. The preferred parameter set returns a strict consensus tree somewhat similar to, but more resolved than, a previous parsimony tree based on molecules alone. I also describe the effects of including EF-1 α and LW rhodopsin intron sequences on the outcome of the direct optimization analysis. By accounting for more evidence, this study provides the most comprehensive treatment yet of apid phylogenetic relationships.

Introduction

Proponents of cladistic methods have long advocated for simultaneous analyses, i.e. studies that incorporate all available evidence in the construction of phylogenetic hypotheses (Kluge, 1989; Eernisse and Kluge, 1993; Chavarría and Carpenter, 1994; Bremer, 1996; Kluge, 1996; Nixon and Carpenter, 1996; Carpenter and Wheeler, 1999; Schulmeister *et al.*, 2002; Meier and Lim, 2009; Pickett and Carpenter, 2010). These “total evidence” analyses maximize explanatory power by accounting for an entire body of evidence, and thus provide the “best approach to phylogenetic inference...the one that best applies parsimony” (Nixon and Carpenter, 1996). To the extent that multiple character partitions – e.g., behavioral, morphological, molecular, or ontogenetic datasets – are readily available via *de novo* coding or literature search, including them can only result in more strongly corroborated phylogenetic hypotheses (Kluge, 1996).

At the moment, however, simultaneous analyses are by no means standard operating procedure among systematic biologists, even among those who specialize in maximum parsimony studies. Many recently published datasets have been limited to single classes of character data, most often in the form of pre-aligned nucleotide sequences, and even when the data used represent a small fraction of potential or actual characters. While such limitations often result from unavoidable constraints on time, expertise, or the availability of study material (and not necessarily, as Pickett and Carpenter (2010) have suggested, from a culture of “data chauvenism”), total evidence still represents a best practice in phylogenetic analysis.

Here I examine the effects of combining readily available character information on the outcome of a direct optimization sensitivity analysis of the relationships of apid bees

(Hymenoptera: Apidae). In doing so, I demonstrate once again the advantages of simultaneous analysis and data exploration for phylogenetic studies.

Background: Previous studies

There have been several attempts in the last twenty years to infer the phylogeny of the bee family Apidae through separate analyses of morphological or molecular datasets (Roig-Alsina and Michener, 1993; Straka and Bogusch, 2007; Cardinal *et al.*, 2010); these studies thus offer an excellent opportunity to explore the effects of simultaneous analysis on previously published phylogenetic hypotheses. Apidae is a large family with over 5,000 species of solitary, cleptoparasitic, and social bees (Michener, 2007; Ascher and Pickering, 2012), including the familiar honey bee, *Apis mellifera*. Michener (2007) divided the group into three subfamilies, the Apinae, the Xylocopinae, and the Nomadinae, based on the first large-scale phylogenetic study of long-tongued bees by Roig-Alsina and Michener (1993). Those authors scored 66 ingroup and 16 outgroup taxa for 131 adult morphological characters and modified McGinley's (1981) larval data to produce a larval matrix featuring 59 taxa (22 outgroup, 37 ingroup) and 77 characters. They then subjected these data to several maximum parsimony analyses, some of which involved the ad hoc removal of characters believed to be associated with cleptoparasitism.

In 2007, Straka and Bogusch revisited apid phylogeny with a study based on 78 larval characters in 54 genus-level taxa. The authors modified Roig-Alsina and Michener's larval dataset through the addition and removal of several taxa, the re-scoring of some characters, and the addition of a new one. They then used both maximum parsimony and Bayesian analyses to recover rooted and unrooted trees that revealed far

fewer independent origins of cleptoparasitism than previously supposed (Straka and Bogusch, 2007). However, despite their familiarity with Roig-Alsina and Michener's work, the authors did not combine their modified larval dataset with the previously published adult matrix; instead, they used conflicts between the datasets to test taxonomic congruence and to offer what they called "an alternative to the current opinion."

In 2010, Cardinal and colleagues published a taxonomically comprehensive phylogeny of the Apidae based on 190 (30 outgroup, 160 ingroup) taxa and seven genes. The methods used were extensive and diverse, and included maximum parsimony, maximum likelihood, and Bayesian analyses, as well as fossil-calibrated divergence time estimates and a Bayesian ancestral state reconstruction of the evolution of cleptoparasitism. The authors made a point, however, of excluding morphological characters in order to produce "a phylogenetic hypothesis that is independent of possible morphological convergence in the cleptoparasites" (Cardinal *et al.*, 2010). As a result, their final phylogenetic hypotheses were based on only a subset of the available informative data, and may represent suboptimal solutions given additional information in the form of previously published morphological and behavioral characters.

The current study evaluates phylogenetic relationships within the Apidae using both classes of previously published data, as well as a limited number of behavioral characters. It does so through a direct optimization (Wheeler, 1996, 2000) sensitivity analysis (Wheeler, 1995; Schulmeister *et al.*, 2002) approach that also provides valuable information regarding the robustness (*sensu* Giribet, 2003) of clades to changes in transformation cost parameters.

Materials and methods

Characters and taxa

The 131 adult morphological characters are the same as those in Roig-Alsina and Michener's (1993) published dataset. While that study also included a second matrix with 77 mature larval characters, later work by Straka and Bogusch (2007) modified many of the original character state assignments, added and excluded several taxa, and incorporated one additional character. In light of those updates and modifications, the 1993 paper's adult matrix was merged with the 78 character larval matrix from 2007. In addition, larval data from Roig-Alsina and Michener (1993) and from McGinley's earlier study (1981) were used to code characters for taxa not included by Straka and Bogusch. When possible, additional larval characters were newly coded based on published descriptions (Appendix A); however, the fragmentary nature of many of these descriptions meant that only a fraction of characters could be coded with confidence.

Some genus-level adult morphological assignments were also coded *de novo* by the author using specimens available in the Invertebrate Zoology collection of the American Museum of Natural History, New York, USA. Characters that were newly coded represent a subset of the complete Roig-Alsina and Michener character set, and include unambiguous and easily diagnosed exoskeletal characters of the head, legs, and metasoma, as well as wing venation characters. For a complete list of newly coded genera, see Appendix A.

Cardinal *et al.* (2010) assigned states for a single behavioral character, the presence or absence of cleptoparasitism, to each of their 190 terminal taxa as part of a Bayesian ancestral state reconstruction; however, they did not use that character in their

phylogenetic analyses. Operating under the assumption that more information yields results with greater explanatory power, I incorporated that character into the current analysis with two states, nest-building (0) and cleptoparasitism (1). I also included a second behavioral character, degree of social development, coded as (0) solitary, (1) primitively social, and (2) eusocial (Cardinal and Danforth, 2011).

With one exception, the molecular data for all seven genes – ribosomal 18S and 28S, as well as the protein coding genes RNA polymerase II (polII), wingless, long-wavelength rhodopsin (LW rhodopsin), sodium potassium adenosine triphosphate (Nak), and elongation factor-1 alpha (EF-1 α) – are the same as those used by Cardinal and colleagues (2010). The current study also includes previously published partial EF-1 α , 28S, and LW rhodopsin sequences from the meliponine bee *Partamona testacea* (accession numbers FJ042339; FJ042063; FJ042441). All 1,318 sequences used by Cardinal *et al.* (2010; see paper for accession numbers), as well as the three *P. testacea* sequences, were downloaded from GenBank and temporarily eye-aligned using Geneious Pro version 5.5 (Drummond *et al.*, 2010). Temporary alignment allowed for the identification of introns and/or non-overlapping sequence regions.

Two intron regions were identified in the LW rhodopsin sequences and one in the EF-1 α sequences; these were treated as separate partitions and included or excluded as outlined below. When individual gene partitions contained non-overlapping regions (e.g., leading/trailing gaps), those sequences were partitioned into shorter homologous sequence fragments (3 fragments in 18S; 6 in 28S; 3 in polII; 2 in wingless; 6 in LW rhodopsin; 5 in Nak; 3 in EF-1 α ; see also Schulmeister *et al.* 2002). Gaps were subsequently removed, and all phylogenetic analyses performed using unaligned

sequences.

The final supermatrix included 209 morphological characters, two behavioral characters, seven unaligned gene sequences divided into 28 sequence partitions, and the same 190 taxa used by Cardinal *et al.* (2010) plus one more species, *Partamona testacea*.

Terminal mismatch, extrapolation, and taxonomic change

Simultaneous analyses often present problems of “terminal mismatch,” a lack of one-to-one correspondence between the OTUs of component matrices (Nixon and Carpenter, 1996). In this study, morphological characters were coded at the generic level, while behavioral and molecular characters were coded for individual species. Following precedent (Chavarria and Carpenter, 1994; Carpenter and Wheeler, 1999; Dowton, 2001; see also Nixon and Carpenter, 1996), generic characters were extrapolated to cover each species contained within that genus. For example, all nine *Xylocopa* species included in this analysis received the same morphological character state assignments, despite belonging to no fewer than seven recognized subgenera (Michener, 2007). The extent to which such extrapolation is justified in this particular case is unclear (although see Roig-Alsina and Michener, 1993: "...we believe that in most cases the characters listed for a species are those of its genus and its tribe, etc... the use of exemplars is more practical and probably better..."); following Nixon and Carpenter (1996), the absence of counter-evidence serves as a reasonable methodological criterion. (For a different perspective, see Malia *et al.*, 2003).

In some cases, morphological data existed for taxa that were not represented in the molecular dataset; these taxa were excluded from this analysis in an effort to minimize problems associated with vast amounts of missing sequence data. Some terminal names

were also adjusted to account for taxonomic changes occurring between the publication of the first data matrix (Roig-Alsina and Michener, 1993) and the present study; specific changes are included in Appendix A.

Phylogenetic analyses

All phylogenetic analyses were carried out using direct optimization (Wheeler, 1996) as implemented in POY version 4.1.2 (Váron *et al.*, 2010). The first simultaneous analysis (“exons-only total evidence,” or ETE) used all available character data, with the exception of EF-1 α and LW rhodopsin intron regions. The second simultaneous analysis (“introns-included total evidence,” ITE) used all available character data including introns. Separate analyses were carried out for each gene partition, as well as for the combined morphology plus behavior dataset (in order to calculate the incongruence length difference statistic, see below). In order to assess clade sensitivity (*sensu* Wheeler, 1995), each of these ten analyses was repeated nine times using nine different sets of transformation cost parameters. Costs were assigned to insertion/deletion events, nucleotide transversions, nucleotide transitions, and morphological/behavioral transformations as in Table 3.1.

Both the ETE and ITE analyses began with 24 hour tree searches using POY’s default search command, *search(max_time:00:24:00)*, which includes several rounds of tree building, swapping using TBR, perturbation using ratchet, and tree fusing, for each of the nine sets of transformation cost parameters. For both ETE and ITE, the trees produced by all nine analyses were concatenated into a single tree file that provided the starter trees for the next round of heuristic search.

Subsequent tree search iterations each performed 1,000 rounds of tree fusing

Table 3.1. Transformation cost parameter regimes as implemented in the sensitivity analysis.

Cost parameters	Description
1 : 1 : 1 : 1	equal weights parsimony (EWP)
1 : 1 : 1 : 2	morphology/behavior twice all others
1 : 1 : 1 : 8	morphology/behavior eight times all others
2 : 1 : 1 : 1	indels twice all other changes
2 : 1 : 1 : 2	indels equal to morphology/behavior; twice substitutions
2 : 2 : 1 : 1	indels equal to transversions; twice transitions and morphological/behavioral changes
4 : 1 : 1 : 1	indels four times all others
4 : 1 : 1 : 2	indel events twice morphology and behavior; four times nucleotide substitutions
4 : 2 : 1 : 1	indel events twice transversions; four times transitions and morphological/behavioral changes

followed by swapping on unique trees – *fuse(iterations:1000) select() swap(trees:10) select()* – ultimately followed by concatenation of the lowest cost output trees (which again served as starter trees for subsequent iterations; Figure 3.1). Analyses were terminated when the set of input trees matched the set of output trees in both cost and number of optimum cladograms (6 iterations for ETE, 3 for ITE).

Individual partition analyses followed a similar heuristic search routine, but were terminated once the cost of the output trees equaled the cost of the input trees for three consecutive iterations, regardless of the number of optimum cladograms in each set.

Of the nine strict consensus trees produced by the nine separate ETE analyses, one was chosen as the “preferred tree” based on its minimization of the incongruence length difference statistic (ILD, Mickevich and Farris, 1981; Wheeler, 1995), as follows:

$$ILD = ((\text{cost of simultaneous tree}) - \sum(\text{cost of each partition tree})) / (\text{cost of simultaneous tree})$$

As a test of morphological support for Cardinal *et al.*'s (2010) cleptoparasitic clade, all 209 morphological characters were optimized on the preferred consensus tree using both accelerated transformation (ACCTRAN) and delayed transformation (DELTRAN) parsimony algorithms, as implemented in PAUP * version 4.0 beta (Swofford, 2002).

Results

Each of the nine maximum parsimony ETE analyses returned a distinct consensus topology (simplified to tribal level in Figures 3.2 and 3.3). The most parsimonious trees (cost = 49,955 steps) associated with the 2:2:1:1 parameter set (indels equal to transversions and double all other changes) were chosen as the optimal trees based on their minimization of the ILD (Table 3.2); for the strict consensus of those two trees,

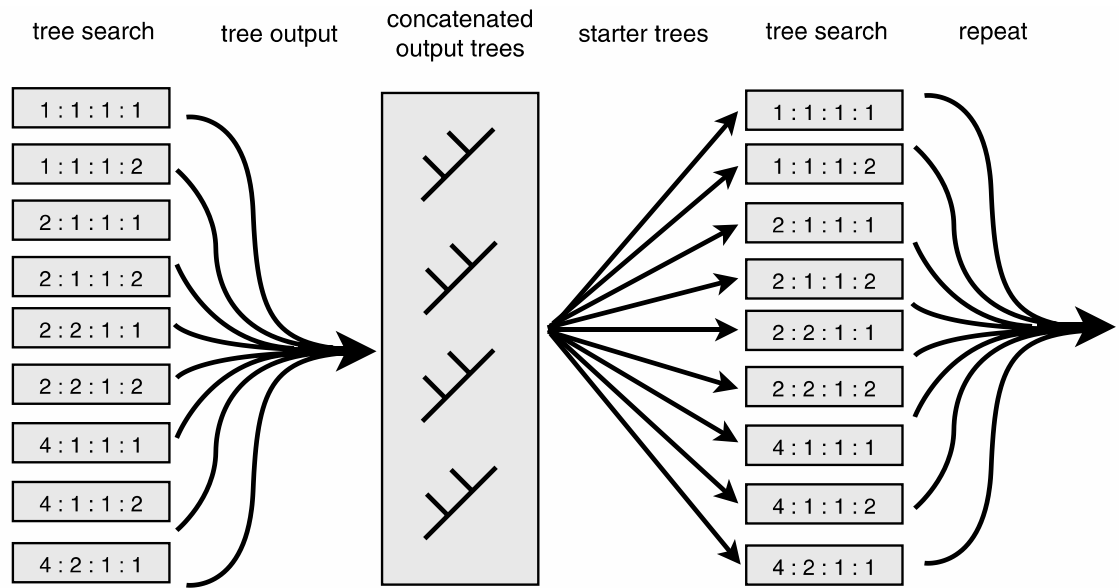


Figure 3.1. Graphical representation of one tree search cycle. Output trees based on each transformation cost parameter set were concatenated into a single tree file that then served as the starter tree set for subsequent rounds. These cycles continued until the set of input (starter) trees matched the set of output trees.

Figure 3.2. Strict consensus trees generated by the exons-only total evidence analyses under four different transformation cost regimes (1:1:1:1, 1:1:1:2, 1:1:1:8, 2:1:1:1), and simplified to tribal level. Genera that have escaped from their traditional tribal classifications are marked with an asterisk; black dots highlight Cardinal et al.'s (2010) cleptoparasitic apid clade.

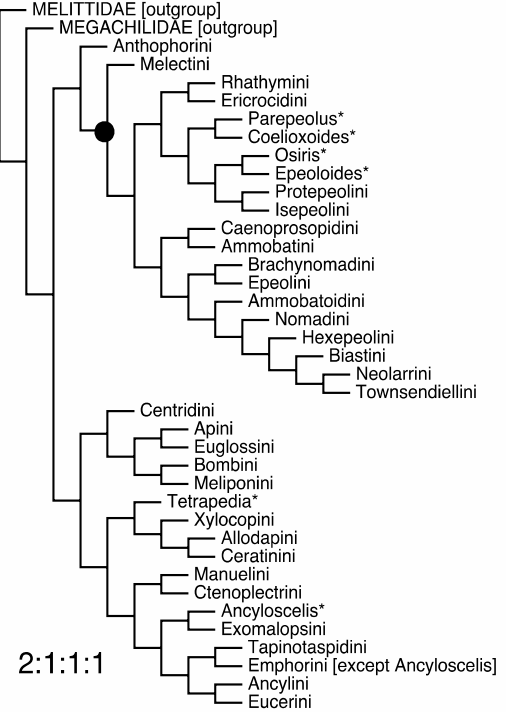
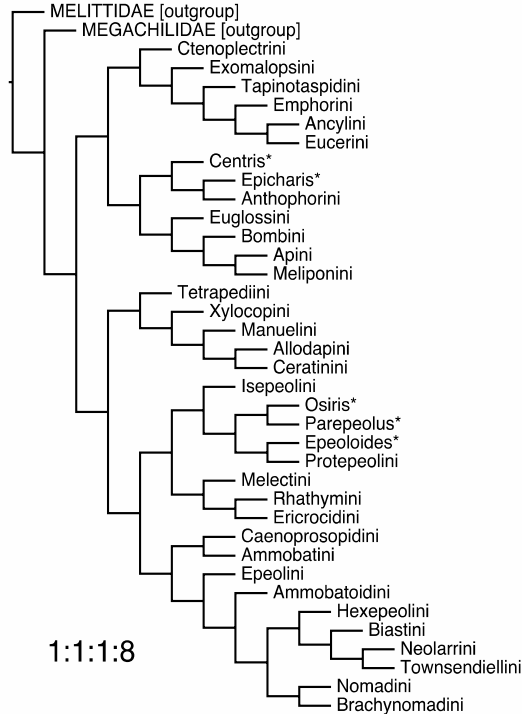
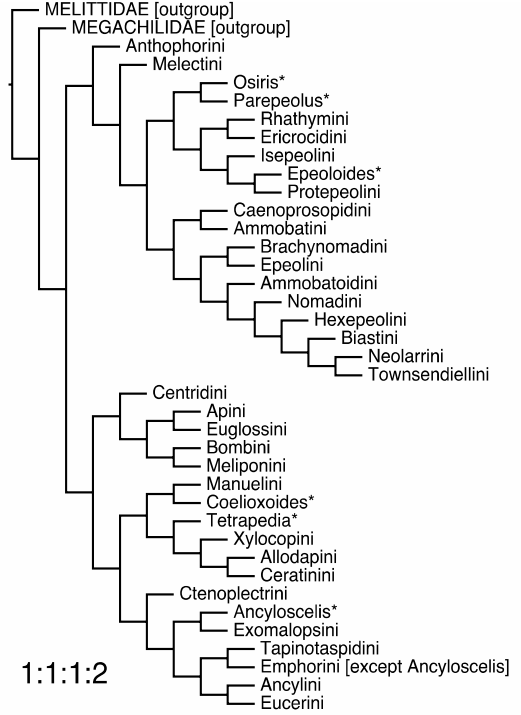
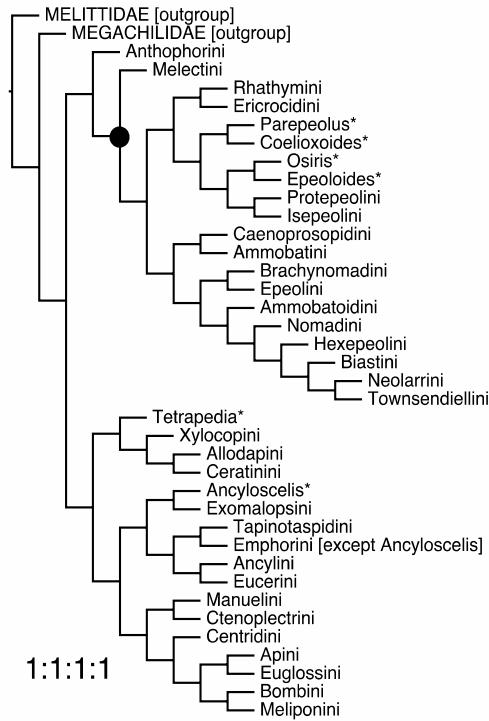
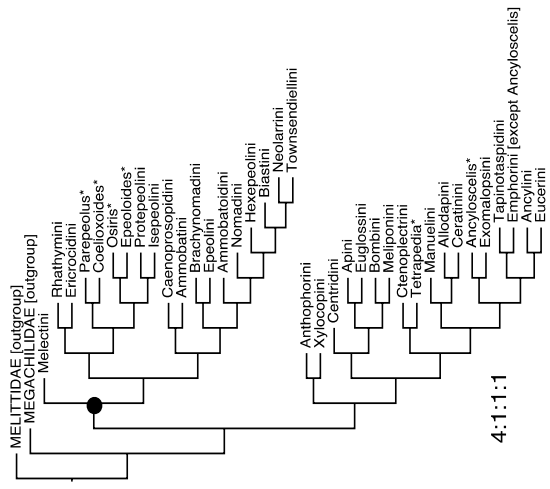
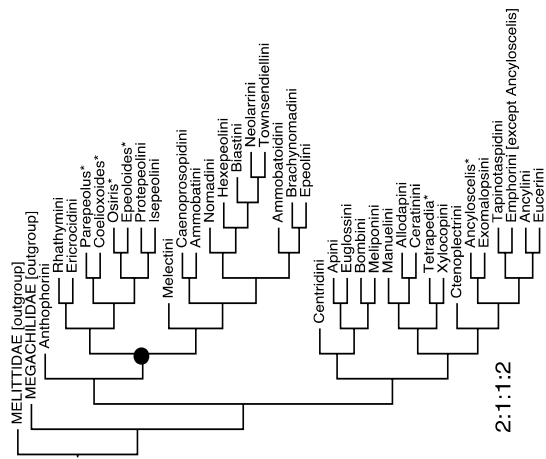
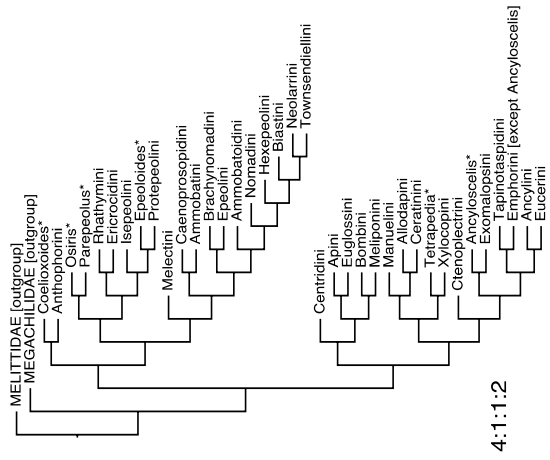
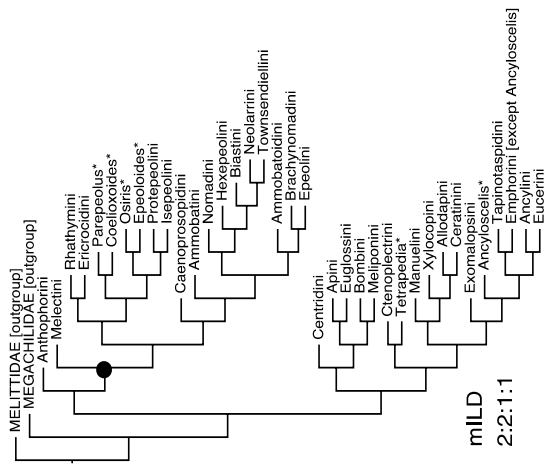
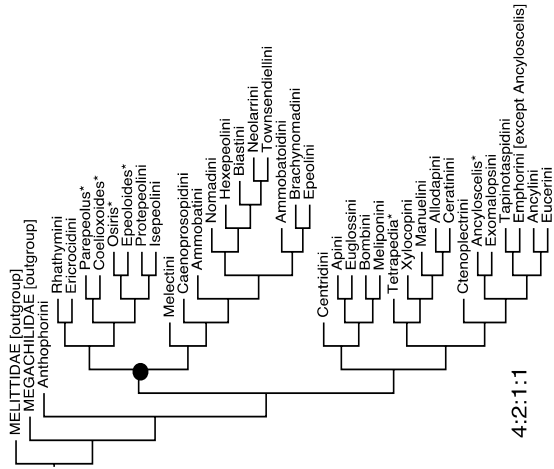


Figure 3.3. Strict consensus trees generated by the exons-only total evidence analyses under five additional transformation cost regimes (2:1:1:2, 2:2:1:1, 4:1:1:1, 4:1:1:2, 4:2:1:1), and simplified to tribal level. Genera that have escaped from their traditional tribal classifications are marked with an asterisk; black dots highlight Cardinal et al.'s (2010) cleptoparasitic apid clade.



see Figure 3.4. The sensitivities of the 2:2:1:1 ETE and 1:1:1:1 ETE (equal weights parsimony, or EWP) clades to changes in parameter costs were determined using Cladescan version 1.0 (Sanders, 2010), and are detailed in Figures 3.5 and 3.6, respectively.

Each of the nine ITE analyses returned a single maximum parsimony cladogram; two of those trees (simplified to tribe) are contrasted with their counterpart ETE cladograms in Figure 3.7. The length of the most parsimonious 2:2:1:1 ITE cladogram was 73,024 steps (over 23,000 steps more than were required for the ETE dataset under the same cost parameters). No unique, unreversed morphological synapomorphies were found to support the Cardinal *et al.*'s cleptoparasitic clade.

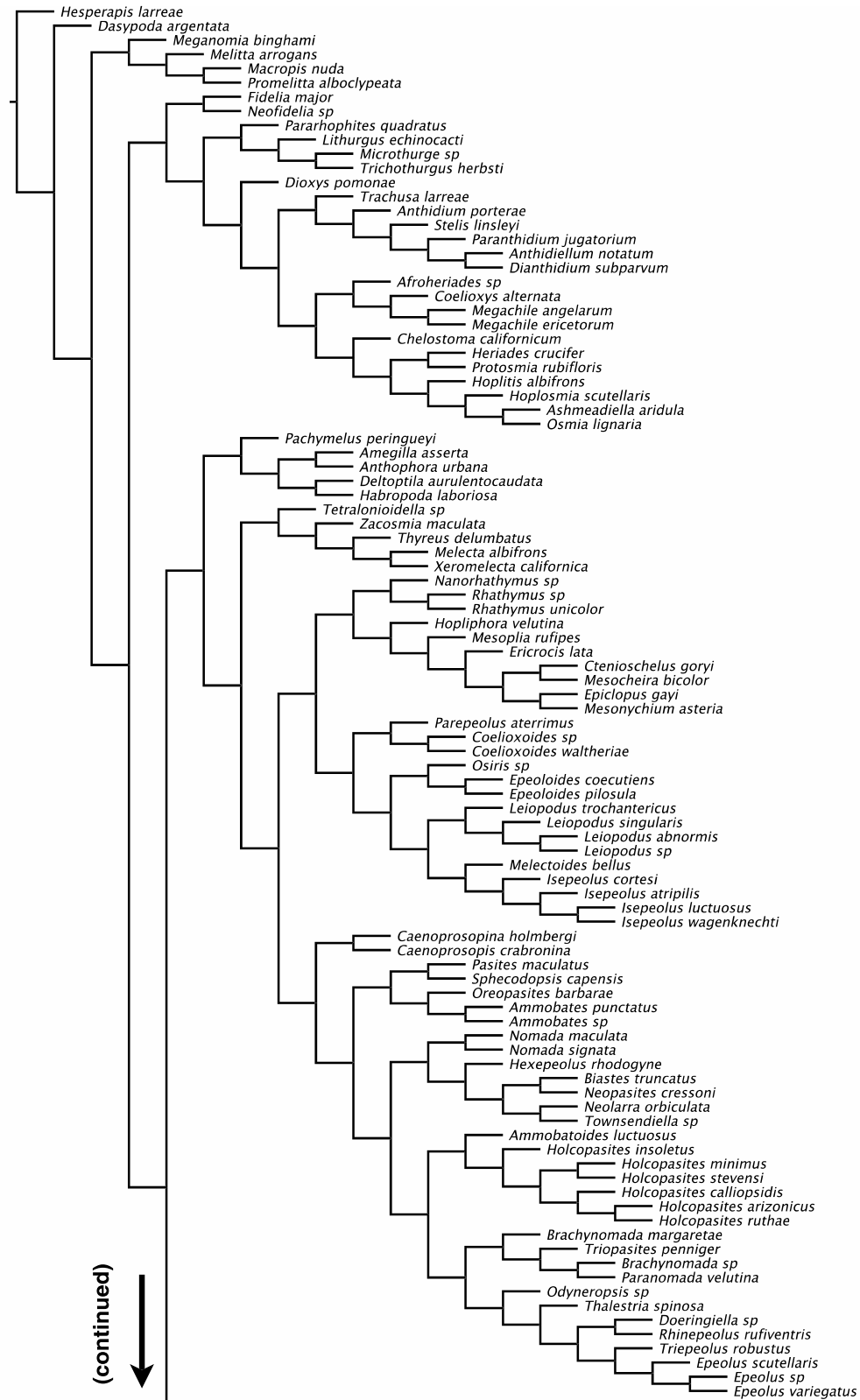
Discussion

The results of the current study lend support to Cardinal and colleagues' (2010) hypothesis of a much reduced number of cleptoparasitic origins within the Apidae. Those authors' maximum parsimony analyses returned a set of 32 equally parsimonious trees, the strict consensus of which revealed a large, exclusively cleptoparasitic clade composed of the Nomadinae plus various cleptoparasitic apine tribes (Ericrocidini, Rhathymini, Isepeolini, Protepeolini, Osirini, Melectini), as well as the cleptoparasitic component (*Coelioxoides*) of the tribe Tetrapediini. This radical re-shuffling of apid phylogeny reduced the number of hypothesized origins of cleptoparasitism from six (Straka and Bogusch [2007], itself a reduction from the eleven proposed by Roig-Alsina and Michener [1993]) to four; more importantly, it united the so-called "melectine line" (*sensu* Straka and Bogush, 2007: Ericrocidini, Rhathymini, Isepeolini, Protepeolini, Osirini, Melectini) with the Nomadinae (Ammobatoidini, Neolarrini, Biastini,

Table 3.2. Partition tree costs, simultaneous analysis tree cost, and incongruence length difference (ILD) associated with each transformation cost regime.

Cost regime	18S	28S	EF1- α	Nak	Ops	PolII	wng	Morphology/behavior	Simultaneous analysis	ILD
1 : 1 : 1 : 1	560	5270	4600	11758	3964	6064	2746	1479	38296	0.048438
1 : 1 : 1 : 2	"	"	"	"	"	"	"	2958	39957	0.05098
1 : 1 : 1 : 8	"	"	"	"	"	"	"	4437	49508	0.204189
2 : 1 : 1 : 1	631	6590	4609	11765	3975	6068	2774	1479	39851	0.049183
2 : 1 : 1 : 2	"	"	"	"	"	"	"	2958	41510	0.051554
2 : 2 : 1 : 1	765	7989	5653	15518	5208	7251	3815	1479	49955	0.045581
4 : 1 : 1 : 1	732	8845	4611	11766	3976	6070	2828	1479	42562	0.052982
4 : 1 : 1 : 2	"	"	"	"	"	"	"	2958	44225	0.05515
4 : 2 : 1 : 1	882	10495	5663	15525	5222	7253	3869	1479	52904	0.047558

Figure 3.4. The mILD (minimum incongruence length difference) tree. Strict consensus of two most parsimonious trees generated by the 2:2:1:1 transformation cost parameter regime. Tree cost = 49,955 steps. *Figure runs to two pages.*



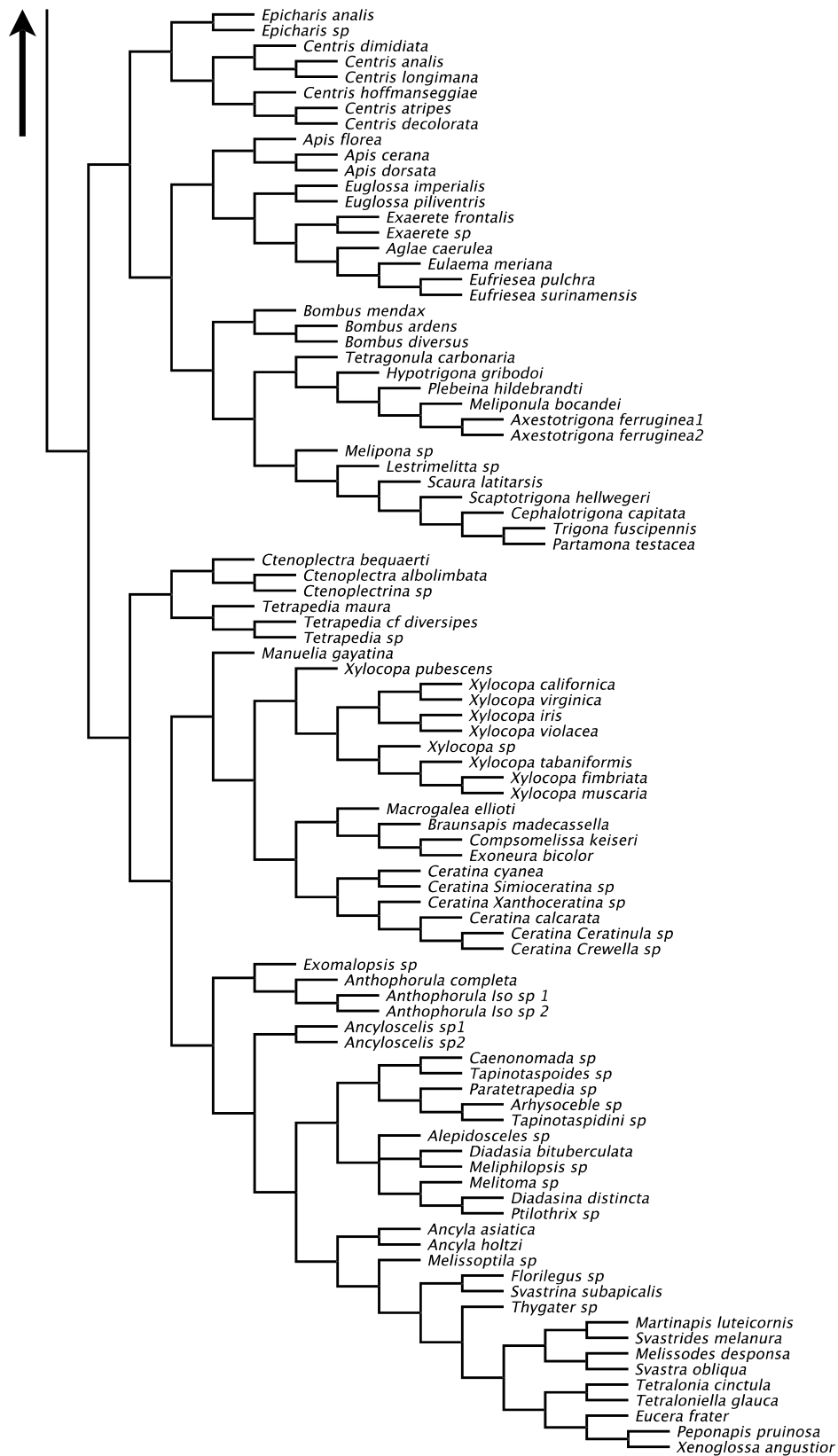


Figure 3.5. Sensitivity plots superimposed on the mILD (minimum incongruence length difference) (2:1:1:1) exons-only total evidence consensus tree (simplified to genus level). Nodes without annotation were present under all nine cost regimes.

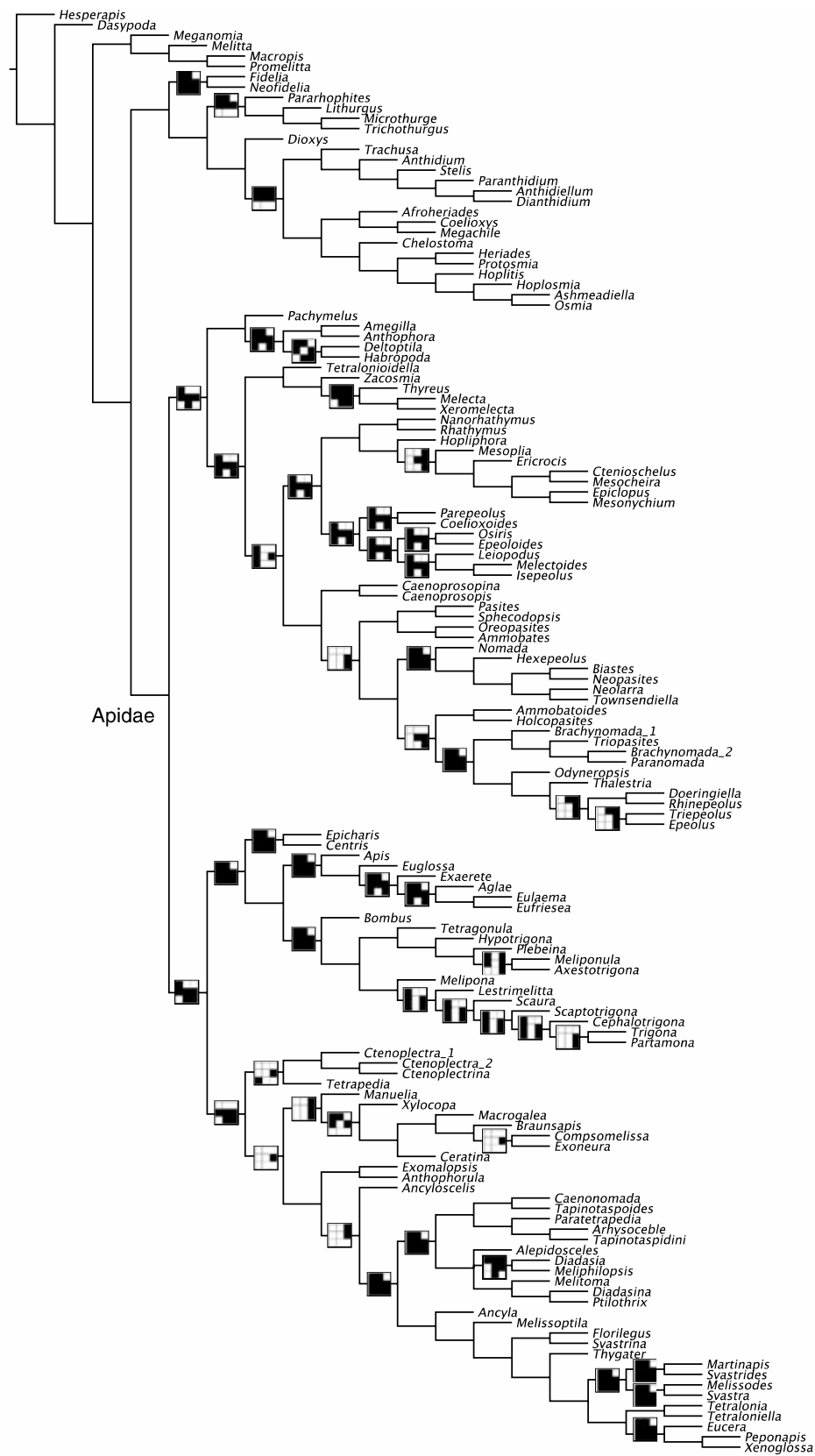
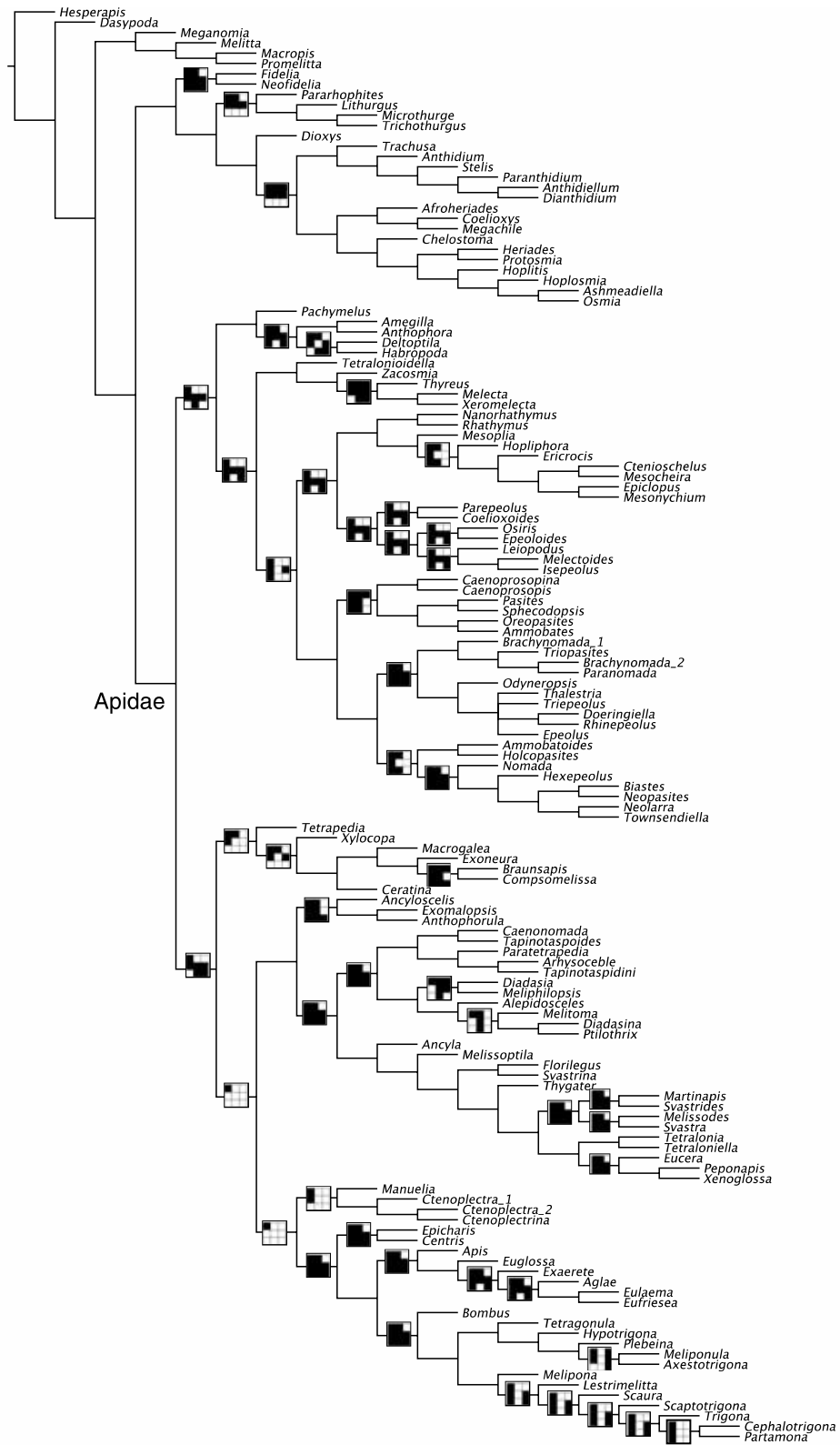


Figure 3.6. Sensitivity plots superimposed on the equal weights parsimony (1:1:1:1) exons-only total evidence consensus tree (simplified to genus level). Nodes without annotation were present under all nine cost regimes.



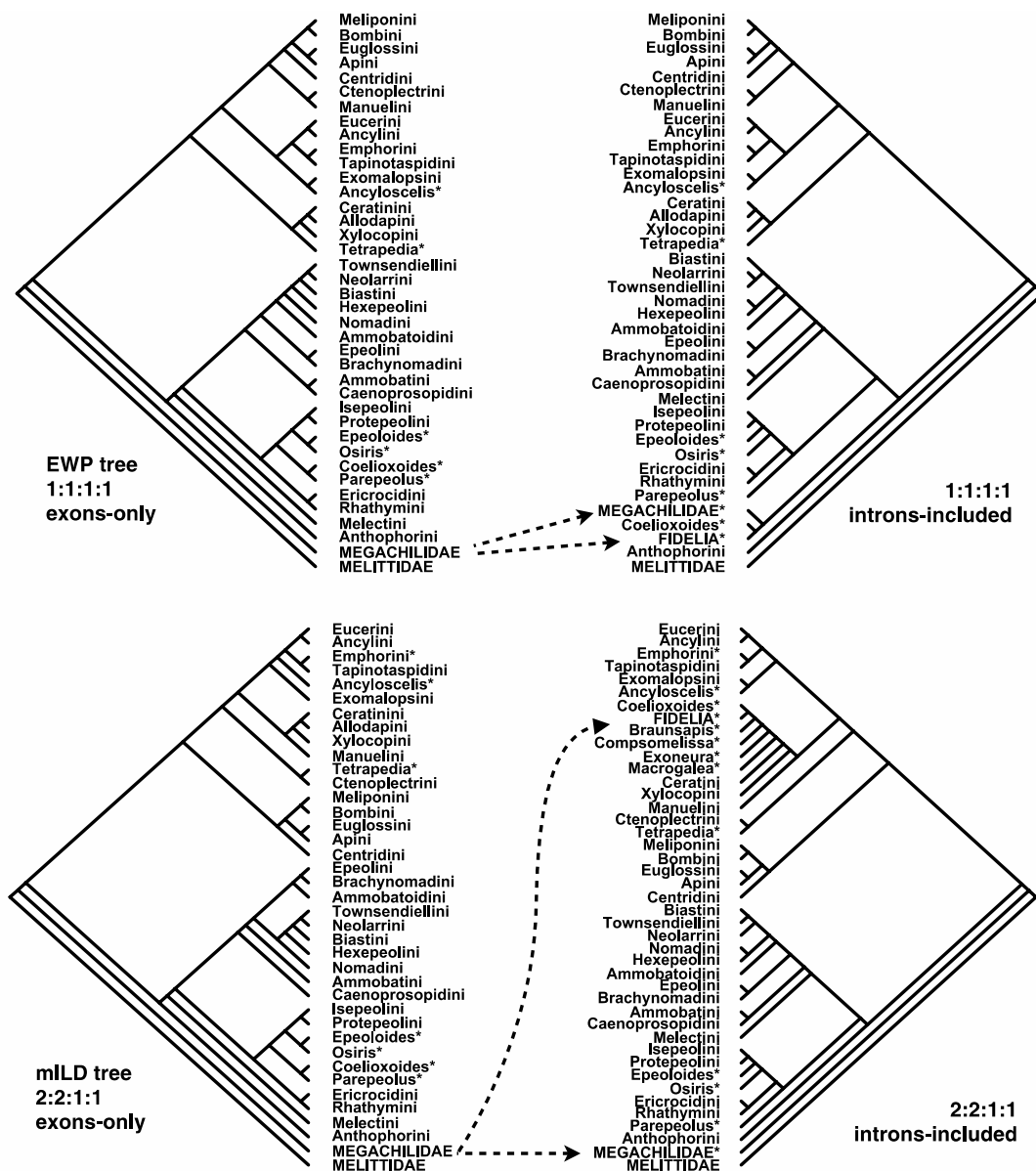


Figure 3.7. The effects of intron inclusion in direct optimization parsimony analysis. Side-by-side comparison of optimal cladograms (simplified to tribal level) demonstrate topological differences resulting from inclusion of intron regions within EF-1 α and LW rhodopsin sequences. Genera that have escaped from their traditional tribal classifications are marked with an asterisk. Note the position of *Fidelia* with respect to the rest of the Megachilidae and to the apid genus *Coelioxoides*.

Townsendiellini, Hexepeolini, Nomadini, Brachynomadini, Epeolini, Ammobatini, Caenoprosopidini) for the first time. However, bootstrap support for the MP tree's cleptoparasitic clade was low ($< 50\%$), and its basal relationships largely unresolved.

In the present study, Cardinal *et al.*'s cleptoparasitic clade is relatively robust to cost parameter change, appearing under six of the nine cost parameter regimes (Figures 3.2 and 3.3). In the three cases where the clade does not appear, the loss of monophyly is caused only by the removal of *Coelioxoides*; the unification of the "melectine line" and the Nomadinae remains present under all nine cost regimes.

The current analysis also results in much higher resolution among the basal lineages of the cleptoparasitic clade (Figure 3.4). In the preferred tree (2:2:1:1), the Melectini occupy the most basal position within the group; the Nomadinae then form the sister group to a clade composed of the rest of the "melectine" lineages (Ericrocidini, Rhathymini, Isepeolini, Protepeolini, Osirini) plus *Coelioxoides*. Osirini appears to describe a paraphyletic assemblage from within which *Coelioxoides*, the Protepeolini, and the Isepeolini evolved (an arrangement that appears in six out of the nine analyses).

In the preferred tree, the large cleptoparasitic clade is sister to the Anthophorini. Together, they form a clade that is sister to all other apids. The Centridini are sister to the corbiculate apids, here as (Apini + Euglossini) + (Bombini + Melectini), an arrangement represented in eight out of nine analyses (Figure 3.5). The overall topology of the mILD tree is shown in Figures 3.3 and 3.4.

The present study does little to place the genus *Coelioxoides* with any confidence: While six out of nine topologies show it as sister to the cleptoparasitic osirine genus *Parepeolus*, alternative placements include within the Xylocopinae (1:1:1:2), sister to

Anthophorini (4:1:1:2), and in its traditional role as sister group to *Tetrapedia* (1:1:1:8). With the exception of this last case, Tetrapediini here appears to be an artificial grouping (as it was in Cardinal *et al.* [2010]; although see both Straka and Bogusch [2007] and Roig-Alsina and Michener [1993]). While *Tetrapedia* and *Coelioxoides*, the sole members of the tribe, share both a host-parasite relationship and a handful of morphological features, they are nevertheless "very different" (Michener, 2007) and may not be as closely related as previously believed.

Unlike in Cardinal and colleagues' MP tree, in which *Manuelia* was positioned as sister to the Anthophorini, the Xylocopinae here appear as a monophyletic group (in three of nine analyses). Within the so-called "eucerine line" (*sensu* Michener 2007: Ancylini, Emphorini, Eucerini, Exomalopsini, Tapinotaspidini), all tribes appear as monophyletic groups, with the exception of Emphorini, which is rendered unnatural by the loss of *Ancyloscelis*.

One interesting result of this study points to the effects of introns or other difficult-to-align sequence fragments on the outcome of direct optimization parsimony analyses. As Figure 3.7 demonstrates, including even a few of these fragments can have a profound outcome on the topology of the resulting cladogram; see, for instance, the unusual placement of the megachilid genus *Fidelia* deep within the apid clade. Post hoc examination of the LW rhodopsin sequences reveals that both *Fidelia* and *Coelioxoides*, its sister group in the ITE analyses, share particularly elongated second intron sequences (1,067 bp in *Fidelia major*, 466 bp in *Coelioxoides waltheriae*, and 465 bp in *Coelioxoides* sp., contrasted with an average sequence length much closer to 90 bp). If this grouping is based solely on unusual and non-homologous length extensions (what

Lecointre and Deleporte [2004] might call "aberrant rates of change"), then intron sequences may well qualify as "misleading data" (*sensu* Lecointre and Deleporte, 2004) that violate a strict interpretation of the total evidence principle. Researchers are thus advised to pay special attention to intron regions, and to run parallel analyses with the regions both included and excluded in order to evaluate their effects on final phylogenetic hypotheses.

The results of the current study lend support to the hypothesis that most cleptoparasitic behavior within the bee family Apidae is the result of a single major origin (exceptions include convergences within the Ctenoplectrini and the Euglossini, as well as a possible origin in the lineage giving rise to *Coelioxoides*). Together, the results also constitute one more argument for the simultaneous analysis approach to maximum parsimony, and for the thorough exploration of available data through sensitivity analysis.

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CHAPTER IV

A PRELIMINARY MOLECULAR PHYLOGENY FOR THE PHILANTHINE WASPS (APOIDEA: CRABRONIDAE: PHILANTHINAE), WITH AN EMPHASIS ON NORTH AMERICAN BEETLEWOLVES (CERCERINI)

Adapted from: Payne, Ansel, Manuela Sann, and Michael Ohl. In prep. "A Preliminary Molecular Phylogeny for the Philanthine Wasps (Apoidea: Crabronidae: Philanthinae), with an Emphasis on North American Beetlewolves (Cercerini)."
For submission to *Systematic Entomology*.

Abstract

Despite over a hundred years of interest, the comparative study of philanthine wasp behavior remains hindered by phylogenetic uncertainty. In order to advance comparative work on the group's behavioral evolution, we here present the first molecular phylogeny to include each of the subfamily's four tribes and eight genera, as represented by 77 ingroup terminals. Using both maximum likelihood and maximum parsimony methods, we analyzed nucleotide sequence data from four loci (18S, 28S, EF-1 α F2, and COI), together accounting for over 3,700 aligned bp. While previous morphological work has consistently placed Pseudoscoliini as the sister group to Cercerini, our analyses return *Pseudoscolia* as the basal philanthine; however, bootstrap and symmetric resampling support for this arrangement is low. While the sister taxa *Eucerceris* and *Cerceris* each represent well-supported monophyletic genera, *Philanthus* is rendered paraphyletic by the neotropical genus *Trachypus*. The aphilanthopine tribe is monophyletic; however, *Aphilanthops* may be paraphyletic with respect to a monophyletic and well-supported *Clypeadon*. Our MP and ML analyses return optimal trees that disagree on fundamental relationships among the tribes: While all MP topologies are consistent with Pseudoscoliini + (Philanthini + (Cercercini + Aphilanthopini)), the ML topology is

Pseudoscoliini + (Cercercini + (Aphilanthopini + Philanthini)). Support values are low for several key clades in both analyses, pointing to continued uncertainty in philanthine relationships. We briefly discuss the implications of these competing topologies for the evolution of prey choice within the group.

Introduction

With over 1,100 species in eight genera (Pulawski 2014), the wasp subfamily Philanthinae accounts for a major component (~12%) of non-bee apoid wasp diversity. Globally distributed, conspicuous, and commonly encountered across a range of habitat types (Bohart and Grissell 1975; Bohart and Menke 1976), these predatory wasps are well represented in natural history collections and have long been a favorite of both naturalists and ethologists (see, e.g., Fabre 1891; Tinbergen 1951, 1958; Evans and O'Neill 1988). As a result, few groups of apoid wasps are as well documented in terms of natural history data, with a wealth of prey records (Scullen and Wold 1969; Evans and O'Neill 1988), nest descriptions (Evans 1962a; Evans 1971; Evans and O'Neill 1988), and hunting observations (Evans 1962b) available for many species worldwide.

While these charismatic insects have been the subjects of numerous observational and experimental ethological studies – including Tinbergen's much cited papers on flight orientation in the European beewolf, *Philanthus triangulum* (1932, 1935; Tinbergen and Kruyt 1938; Tinbergen and van der Linde 1938) and Polidori's studies of hunting behavior in *Cerceris* (Polidori et al. 2005, 2006, 2007, 2011; Polidori 2011) – comparative work on the group's behavioral evolution has been hindered by the lack of a resolved and well-supported phylogenetic hypothesis. In order to lay the groundwork for such studies, we here present the first molecular phylogeny to include representatives of

each of the subfamily's four tribes and eight genera. By sampling extensively within New World Cercerini, we also provide the first ever phylogenetic analysis of relationships within the diverse beetlewolf clade (*Cerceris* + *Eucerceris*).

Systematics of Philanthinae and previous phylogenetic hypotheses

While the philanthine wasps together make up one of the largest subfamilies of non-bee Apoidea (surpassed in species number only by the Bembicinae, ~1,700 spp., and the Crabroninae, ~4,600 spp.; Pulawski 2014), the majority of the group's diversity is found within its two largest genera: *Philanthus*, with 137 Afrotropical, Indomalayan, and Holarctic species, and *Cerceris*, with 870 species distributed worldwide. The other six genera are more locally distributed and considerably less diverse: *Trachypus* (31 spp.) is limited to the Neotropics, while the rarely collected *Philanthinus* (4 spp.) and *Pseudoscolia* (47 spp.) are found only in Central Asia, North Africa, and the Middle East. Three genera (*Eucerceris* [41 spp.]; *Aphilanthops* [4 spp.], and *Clypeadon* [9 spp.]) are limited to North and Central America, with their highest diversity concentrated in the arid regions of the U.S. southwest and northern Mexico (Bohart 1966; Scullen 1968; Bohart and Menke 1976; Pulawski 2014).

At present, these eight genera are placed within four tribes: Philanthini (*Philanthus*, *Philanthinus*, and *Trachypus*), Aphilanthopini (*Aphilanthops* and *Clypeadon*), Cercerini (*Cerceris* and *Eucerceris*), and the monotypic Pseudoscoliini (*Pseudoscolia*). With a number of important exceptions, these tribes show a high degree of unity in terms of prey choice: The Philanthini (with the possible exception of the ethologically undescribed *Philanthinus*) are efficient hunters of bees and other apoid wasps, the source of their common sobriquet, the *beewolves* (Tinbergen 1932; see discussion in Evans and O'Neill

1988). Most aphilanthopine species, on the other hand, are highly specialized predators of ants: *Aphilanthops frigidus* and *A. subfrigidus* on the alate queens of *Formica*; *Clypeadon* species on *Pogonomyrmex* workers (Evans 1962a; 1977a). (The critical exception is *Aphilanthops hispidus*, a bee hunter [Evans 1977b]; the prey of *A. foxi* is unknown.). Members of the tribe Cercerini mostly hunt beetles (hence *beetlewolves*, the neologism of our title), with many species specializing on weevils (Coleoptera: Curculionidae); however, some species of Old World *Cerceris* are, like *Philanthus* and *Trachypus*, bee hunters. At present, pseudoscoliine prey preferences are definitively known from only two species: *martinezi* (a predator of bees; Asís et al. 1991) and *simplicornis* (a predator of *Cataglyphis* ants; Kazenas 2001).

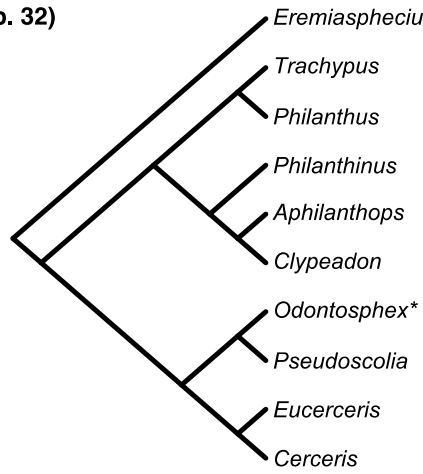
The relationships among, and to a lesser extent within, these tribes represent the central problem of philanthine systematics. In their much cited worldwide revision of apoid wasps, Bohart and Menke (1976) included two non-analytically derived, and in fact mutually exclusive, dendrograms depicting probable relationships within the group. In the first of these (1976: p. 32; see our Figure 4.1), Aphilanthopini (*sensu* Bohart and Menke, i.e. including *Philanthinus*) is sister to the subtribe Philanthina (*Trachypus* + *Philanthus*), and the clade thus formed sister to a group composed of (*Odontosphe* + *Pseudoscolia*) + Cercerini. In their second dendrogram (p. 556), Aphilanthopini + *Philanthinus* is sister to a clade composed of *Odontosphe* + (*Pseudoscolia* + Cercerini), with the combined clade in turn sister to Philanthina.

Bohart and Menke offered no explanation for these conflicting figures in their accompanying text (see Alexander 1992), and the simultaneous inclusion of two different hypotheses probably represents simple editorial oversight. That said, the conflict does

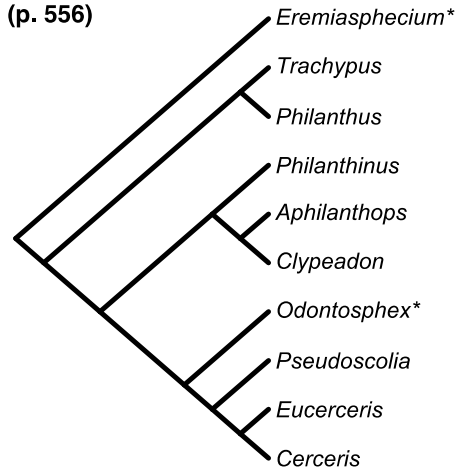
Figure 4.1. Previously published phylogenetic hypotheses for philanthine wasps.

Bohart and Menke (1976) considered Philanthinae in the loose sense, with the inclusion of two genera, *Odontosphex* and *Eremiasphecium*, subsequently removed by Alexander (1992a,b) and now placed in the Pemphredoninae and the Eremiapheciinae, respectively (Pulawski 2014). Both the Prentice (1998) and the Debevec et al. (2012) topologies were taken from larger phylogenetic studies of Apoidea and Aculeata, respectively. Kaltenpoth et al. (2014) focused their sequencing effort almost exclusively on the tribe Philanthini; in their analyses, *Clypeadon*, *Aphilanthops*, and Cercerini (*Cerceris*/*Eucerceris* composite) were represented by a single terminal each. Side-by-side comparison of these topologies clearly reveals Aphilanthopini's pendulum-like oscillation.

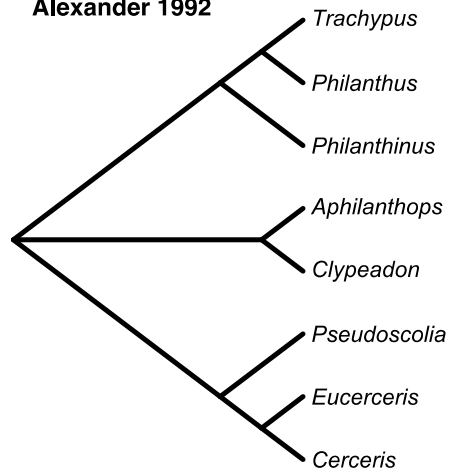
Bohart & Menke 1976
(p. 32)



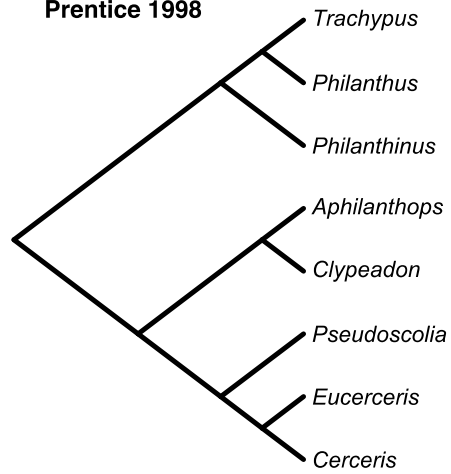
Bohart & Menke 1976
(p. 556)



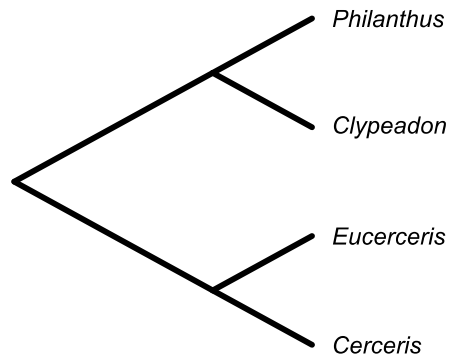
Alexander 1992



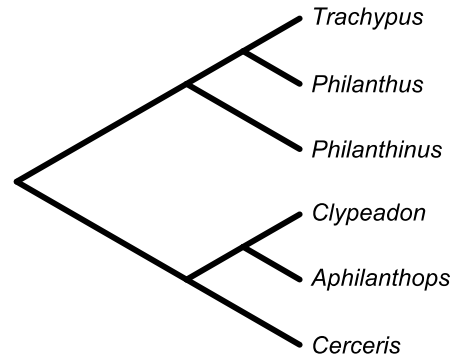
Prentice 1998



Debevec et al. 2012



Kaltenpoth et al. 2014



provide us with the earliest example of what we here call the *aphilanthopine pendulum*: the unstable oscillation of Aphilanthopini between, on the one hand, a sister group relationship with Philanthini, and on the other, a position closer to Cercerini + Pseudoscoliini. Subsequent phylogenies have been defined largely by their adherence to one of these two schemata (Figure 4.1).

In 1992, Alexander published the first methodologically rigorous cladistic analysis of the Philanthinae (this time excluding *Eremiasphecium* and *Odontospheex*) using 33 adult morphological characters, one larval character, and three behavioral characters, each coded for genus level terminals. (The four *Aphilanthops* species were coded separately.) While his maximum parsimony analyses revealed a number of topologies that differed based on methodological approach, we here reproduce the basally unresolved strict consensus topology produced by successive approximation weighting (Figure 4.1). Most important for our purposes, Alexander's study returned *Philanthinus* to a well-supported position within Philanthini; maintained *Pseudoscolia* in a sister group relationship to *Cerceris* + *Eucerceris*; cast explicit doubt on the monophyly of *Aphilanthops* and implicit doubt on that of *Philanthus* and *Cerceris*; and formally delimited the aphilanthopine ambiguity discussed above.

Prentice's 1998 doctoral dissertation presented an extensive exploration of tribal relationships throughout Apoidea, based on a set of maximum parsimony analyses using 182 morphological characters. That study included terminals for each of the four philanthine tribes, coded using exemplars from all eight genera, and thus provided the most extensive morphological treatment of the group's relationships to date. The preferred superfamily phylogeny included the topology shown in Figure 4.1, where the

Aphilanthopini have returned as sister group to Pseudoscoliini + Cercerini. However, Prentice pointed out that this relationship is "not supported by particularly strong evidence," and offered as an alternative arrangement Aphilanthopini + (Philanthini + (*Pseudoscolia* + Cercerini)) (1998, p. 860).

In a recent effort to uncover the sister group to the bees, Debevec et al.'s (2012) four locus molecular treatment of Aculeata included seven philanthine terminals (three species of *Philanthus*, two species of *Clypeadon*, and one each of *Cerceris* and *Eucerceris*) among 226 other aculeates. Both their maximum likelihood and Bayesian topologies placed the aphilanthopine lineage closer to Philanthini (Figure 3.1), with moderate support (ML bootstrap = 78%; Bayesian posterior probability = 0.82) for the Aphilanthopini + Philanthini clade.

Most recently, Kaltenpoth et al. (2014) investigated relationships within the tribe Philanthini as part of an ongoing effort to understand the origin and function of the tribe's antennal gland actinobacterial symbiosis (Kaltenpoth et al. 2006, 2010, 2012). Their maximum parsimony, maximum likelihood, and Bayesian analyses of a six gene molecular dataset each returned Philanthini + (Cercerini + Aphilanthopini), although bootstrap support values for the Cercerini + Aphilanthopini clade were low (both ML/MP < 50%) and sampling within these tribes extremely limited (i.e., *Aphilanthops foxi*, *Clypeadon laticinctus*, and a *Cerceris/Eucerceris* composite terminal). However, Kaltenpoth et al. did provide the first independent molecular support for Alexander's (1992a) suggestion that *Philanthus* is paraphyletic with respect to *Trachypus*: In each of their analyses, Philanthini is arranged as *Philanthinus* + (a grade of Old World *Philanthus* + (*Trachypus* + New World *Philanthus*)).

Here we provide the latest entry in the philanthine phylogenetics literature, through maximum likelihood and maximum parsimony analyses of four genes (COI, EF-1 α F2, 18S, and 28S) for 77 philanthine taxa. Given that the evolutionary relationships within Cercerini are almost completely unexplored, our study provides the first analytically rigorous insight into the relationship between *Cerceris* and *Eucerceris*. We also show how our results lead to a more nuanced understanding of prey choice evolution within the subfamily as a whole.

Materials and methods

Taxonomic sample and outgroups

In preparation for sequencing, we acquired whole adult specimens representing 71 ingroup and five outgroup taxa collected at various sites located throughout the Americas, Europe, and the Middle East. Five of these were recently dried (= post-2008) pinned specimens from the entomological collections of the American Museum of Natural History (AMNH); all others (with the exception of a loaned Peruvian *Trachypus* specimen) were field collected by the authors directly into 95% ethanol. We also included six additional ingroup and five outgroup species represented solely by sequences downloaded from GenBank (accession numbers in bold, Table 4.1).

The final combined taxonomic sample included 77 ingroup terminals, representing all four tribes and all eight genera of philanthine wasps, as well as 10 outgroup taxa from across Apoidea. While the ingroup sample was heavily biased toward Nearctic species (58), some exemplars from the Palearctic (8), Neotropical (9), Afrotropical (1), and Indomalayan (1) fauna were also included. For collection locations and GenBank accession numbers, see Table 4.1.

Table 4.1. Specimen sources and GenBank accession numbers. Superscripts: * = additional specimen sequenced; *d* = dried specimen. Previously published sequences that were downloaded from GenBank and included in the final data matrix are listed in bold. (Collection localities for previously published sequences were inferred from original publications and are listed in parentheses.)

Taxon	Collection location(s)	COI (906 bp)	EF-1 α (753 bp)	18S (794 bp)	28S (1088 bp)
Aphilanthopini					
<i>Aphilanthops foxi</i> Dunning	(USA)	JQ040298	—	—	JN674301
<i>Aphilanthops hispidus</i> W. Fox	USA: CA	xxxxxxx	xxxxxxx	xxxxxxx	—
<i>Clypeadon haigi</i> (R. Bohart)	USA: AZ	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Clypeadon laticinctus</i> (Cresson)	USA: CO; USA: AZ*	xxxxxxx	xxxxxxx	xxxxxxx*	xxxxxxx
<i>Clypeadon sculleni</i> (R. Bohart)	USA: AZ	xxxxxxx	xxxxxxx	xxxxxxx	—
<i>Clypeadon taurulus</i> (Cockerell)	USA: AZ	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Clypeadon utahensis</i> (Baker)	USA: AZ	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
Cercerini					
<i>Cerceris acanthophila</i> Cockerell	USA: AZ	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Cerceris aequalis</i> Provancher	USA: CA	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Cerceris arenaria</i> (Linnaeus)	Germany	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Cerceris californica</i> Cresson	USA: CA	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Cerceris calochorti</i> Rohwer	USA: CA	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Cerceris compacta</i> Cresson	USA: AZ	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Cerceris confrons</i> Mickel	USA: UT	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Cerceris convergens</i> Viereck and	USA: CA	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Cerceris crotonella</i> Viereck and Cockerell	USA: AZ	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Cerceris dilatata</i> Spinola	USA: AZ	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Cerceris dione</i> Fritz	Argentina	xxxxxxx	—	xxxxxxx	xxxxxxx
<i>Cerceris echo</i> Mickel	USA: UT	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Cerceris femurrubrum</i> Viereck and Cockerell	USA: CA	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Cerceris fumipennis</i> Say	USA: NY	xxxxxxx	xxxxxxx	xxxxxxx	—
<i>Cerceris halone</i> Banks	USA: NY	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Cerceris huachuca</i> Banks	USA: AZ	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Cerceris intricata graphica</i> F. Smith	Nicaragua	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Cerceris isolde</i> Banks	USA: CA	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Cerceris kennicottii kennicottii</i> Cresson	Nicaragua	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Cerceris</i> cf. <i>marginula</i> Dalla Torre	Nicaragua	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Cerceris mimica</i> Cresson	USA: AZ	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Cerceris nigrescens</i> F. Smith	USA: WY	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Cerceris panama</i> Scullen	Nicaragua	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Cerceris quinquefasciata</i> (Rossi)	Germany	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Cerceris rufopicta</i> F. Smith	USA: NE	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Cerceris rybyensis</i> (Linnaeus)	Germany	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Cerceris tepaneca</i> de Saussure	USA: AZ	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Cerceris vierecki</i> Banks	USA: AZ	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx

Table 4.1 continued.

Taxon	Collection location(s)	COI (906 bp)	EF-1 α (753 bp)	18S (794 bp)	28S (1088 bp)
<i>Eucerceris arenaria</i> Scullen	USA: AZ	xxxxxxx	—	xxxxxxx	—
<i>Eucerceris bitruncata</i> Scullen	USA: NM	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Eucerceris canaliculata</i> (Say)	USA: CA; USA: AZ*	xxxxxxx	xxxxxxx	xxxxxxx*	xxxxxxx
<i>Eucerceris</i> cf. <i>conata</i> Scullen	USA: CO	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Eucerceris cressoni</i> (Schletterer)	USA: WY; USA: AZ*	xxxxxxx*	xxxxxxx	xxxxxxx	xxxxxxx
<i>Eucerceris flavocincta</i> Cresson	USA: CA	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Eucerceris lacunosa</i> Scullen	USA: AZ	xxxxxxx	xxxxxxx	xxxxxxx	—
<i>Eucerceris melanovittata</i> Scullen	USA: AZ	xxxxxxx	xxxxxxx	xxxxxxx	—
<i>Eucerceris montana</i> Cresson	USA: AZ	xxxxxxx	xxxxxxx	xxxxxxx	—
<i>Eucerceris nevadensis</i> (Dalla Torre)	USA: NV	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Eucerceris pimarum</i> Cockerell and	USA: AZ	xxxxxxx	—	xxxxxxx	—
<i>Eucerceris provancheri</i> (Dalla Torre)	USA: CA	xxxxxxx	xxxxxxx	xxxxxxx	—
<i>Eucerceris similis</i> Cresson	USA: WY	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Eucerceris superba</i> Cresson	USA: WY	xxxxxxx	xxxxxxx	xxxxxxx	—
<i>Eucerceris tricolor</i> Cockerell	USA: AZ	xxxxxxx	xxxxxxx	xxxxxxx	AY654460
<i>Eucerceris vittatifrons</i> Cresson	USA: OR	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
Philanthini					
<i>Philanthinus quattuordecimpunctatus</i> F. Morawitz	(Turkey)	JQ04297	—	—	JN674300
<i>Philanthus albopilosus</i> Cresson	USA: NV	xxxxxxx JQ040264	xxxxxxx	xxxxxxx	xxxxxxx
<i>Philanthus barbatus</i> F. Smith	USA: ID	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Philanthus barbiger</i> Mickel	USA: WY	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Philanthus basalis</i> F. Smith	(India)	JQ040267	—	—	JN674254
<i>Philanthus basilaris</i> Cresson	USA: WY	JQ040268	xxxxxxx	xxxxxxx	xxxxxxx
<i>Philanthus bicinctus</i> (Mickel)	USA: WY	—	xxxxxxx	xxxxxxx	xxxxxxx
<i>Philanthus coarctatus</i> Spinola	Oman	xxxxxxx	—	xxxxxxx	xxxxxxx
<i>Philanthus crabroniformis</i> F. Smith	USA: CA	JQ040271	xxxxxxx	xxxxxxx	JN674261
<i>Philanthus crotoniphilus</i> Viereck and	USA: AZ	xxxxxxx	xxxxxxx	xxxxxxx	JN374855
<i>Philanthus gibbosus</i> (Fabricius)	USA: NE USA: AZ*	—	xxxxxxx	xxxxxxx*	xxxxxxx
<i>Philanthus gloriosus</i> Cresson	USA: AZ	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Philanthus inversus</i> Patton	USA: AZ	xxxxxxx	xxxxxxx	xxxxxxx	JN674267
<i>Philanthus melanderi</i> Arnold	(S. Africa)	JQ040276	—	—	JN674270
<i>Philanthus multimaculatus</i> Cameron	USA: AZ; USA: UT*	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx*
<i>Philanthus pacificus</i> Cresson	USA: UT	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Philanthus pulchellus</i> Spinola	(Spain)	JQ040282	—	—	JN674277
<i>Philanthus pulcher</i> Dalla Torre	USA: CO	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Philanthus triangulum</i> (Fabricius)	Germany	xxxxxxx	—	xxxxxxx	JN674288

Table 4.1 continued.

Taxon	Collection location(s)	COI (906 bp)	EF-1 α (753 bp)	18S (794 bp)	28S (1088 bp)
<i>Philanthus ventilabris</i> Fabricius	USA: NY	xxxxxxx JQ040291	xxxxxxx	xxxxxxx	xxxxxxx
<i>Trachypus boharti</i> Rubio-Espina	(Brazil)	JQ040293	—	—	JN674294
<i>Trachypus</i> cf. <i>mexicanus</i> de Saussure	Nicaragua	xxxxxxx	xxxxxxx	xxxxxxx	—
<i>Trachypus</i> sp. Peru	Peru	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
Pseudoscoliini					
<i>Pseudoscolia dewitzi</i> (Kohl)	Israel	xxxxxxx	—	xxxxxxx	—
Outgroups					
<i>Ampulex compressa</i> (Fabricius)		GQ374639	GQ410718 JN374864	GQ410619	JN374845
<i>Anthidium manicatum</i> (Linnaeus)	USA: MA	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Apis mellifera</i> Linnaeus		AF214668	AF015267	AY703484	AY703551
<i>Bembix americana</i> Fabricius		—	AY585168	AY995580	AY654459
<i>Chalybion californicum</i> (de Saussure)	USA: WV	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Crabro</i> sp.	USA: NY	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx
<i>Pison chilense</i> Spinola		GQ374629	GQ410710 JQ519595	GQ410608	GQ374715
<i>Sceliphron caementarium</i> (Drury)	USA: AZ	xxxxxxx	JF927440	xxxxxxx	xxxxxxx
<i>Stangeella cyaniventris</i> (Guérin-Ménéville)		JF927358	GQ410716 JQ519596	GQ410616	GQ374723
<i>Stizoides foxi</i> Gillasp	USA: AZ	xxxxxxx	xxxxxxx	xxxxxxx	xxxxxxx

Sequence acquisition and alignment

For the majority of samples, we extracted total genomic DNA from either the legs or, in the case of minute but easily identified specimens, the heads using the DNeasy Blood & Tissue Kit (Qiagen). In one case (*Pseudoscolia dewitzi*), we used a non-destructive, whole body lysis bath in order to preserve the integrity of the pinned specimen *in toto*: Rather than destroy valuable morphological structures, the entire insect was suspended in lysis buffer and incubated overnight at 54°C. All subsequent extraction steps followed standard protocols.

We amplified newly extracted genomic DNA at four phylogenetically informative loci – the nuclear non-coding ribosomal subunit genes 18S and 28S; the F2 copy of the nuclear protein-coding gene elongation factor 1-alpha (EF-1 α F2); and the mitochondrial protein-coding locus cytochrome oxidase I (COI) – using the polymerase chain reaction (PCR), optimized for the primer pairs in Table 4.2. PCR routines were the same as those in Cardinal et al. (2010; for H17F/H35R; A-28S-For/Mar-28S-Rev; HaF2For1/F2-Rev-1) and Field et al. (2011; for LCO1490/H7005). Amplified PCR products were purified using the Agencourt AMPure XP system (Beckman Coulter), then cycle-sequenced with the BigDye 3.1 Terminator Reaction Kit on an ABI 3730xl DNA analyzer sequencing core (Applied Biosystems) located at the Sackler Institute for Comparative Genomics (AMNH).

In order to establish reading frames, intron boundaries, and/or non-overlapping sequence regions (i.e., leading/trailing gaps), we assembled and temporarily eye-aligned all sequences using Geneious version 6.0.5 (BioMatters). Comparison to an *Apis mellifera* reference translation (GenBank accession: AF015267) combined with the

Table 4.2. Primer pairs for amplification and sequencing reactions, with source publications. For information on PCR conditions, see text.

Target locus	Primer pairs	Sequence	Source
COI	LCO1490	5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3'	Folmer et al. 1994
	H7005	5'-CCG GAT CCA CNA CRT ART ANG TRT CRT G-3'	Hafner et al. 1994
EF-1 α F2	HaF2For1	5'-GGG YAA AGG WTC CTT CAA RTA TGC-3'	Danforth et al. 1999
	F2-Rev-1	5'-A ATC AGC AGC ACC TTT AGG TGG-3'	Danforth et al. 1999
18S	H17F	5'-AAA TTA CCC ACT CCC GGC A-3'	Heraty et al. 2004
	H35R	5'-TGG TGA GGT TTC CCG TGT T-3'	Heraty et al. 2004
28S	A-28S-For	5'-CCC CCT GAA TTT AAG CAT AT-3'	Ward and Brady 2003
	Mar-28S-Rev	5'-TAG TTC ACC ATC TTT CGG GTC CC-3'	Mardulyn and Whitfield 1999

identification of canonical splicing sites revealed one common intron region (ingroup lengths: 200-218 unaligned bp) in EF-1 α F2, corresponding to the 753/4-position intron in the *A. mellifera* reference sequence (Danforth and Ji 1998). The fragment of 28S used in the present study covers a region that extends roughly from the H235 to D3-3 stems of the *A. mellifera* large subunit rRNA secondary structure model (Gillespie et al. 2006); however, since a number of sequences did not amplify for the first ~340 bp, we split this 28S dataset into two sub-fragments (H235 to H15 and H15 to D3-3), each of which we aligned separately (see below). The 18S fragment used here extends roughly from the H367 stem to the H960 stem of the small subunit rRNA secondary structure model (Gillespie et al. 2006).

While nucleotide alignment was trivial for the EF-1 α F2 exon regions (no indels) and COI (3bp deletion in the outgroup taxon *Stizoides foxi*, located using Geneious's translation alignment algorithm), our 18S and 28S sequences presented the usual rDNA alignment difficulties (see, e.g., Klopstein et al. 2013). In the absence of an objective method for aligning these loci with respect to published secondary structure models, we instead adopted an agnostic, sequence-based approach, namely the E-INS-i algorithm as implemented in MAFFT version 7.154. (Katoh et al. 2002; Katoh and Standley 2013). Ingroup EF-1 α F2 intron sequences were also aligned using MAFFT (E-INS-i) and were included in the final dataset.

Prior to phylogenetic analysis, we concatenated all aligned sequences into final data files using SequenceMatrix version 1.7.8 (Vaidya et al. 2010). The complete dataset thus arranged contained 3,776 nucleotide positions (of which either 1,312 [gaps as fifth state] or 1,180 [gaps as missing] were parsimony informative). Altogether, gaps accounted for

3.54% of the final matrix; completely undetermined characters, 14.13%

Phylogenetic analyses

We performed two sets of phylogenetic analyses, each employing a different optimality criterion, either maximum likelihood (ML), as implemented in RAxML version 8.0 (Stamatakis 2014), or maximum parsimony (MP), as implemented in TNT version 1.1 (Goloboff et al. 2008). *Ampulex compressa* was designated as the outgroup under both criteria.

ML analysis: Prior to analysis, the full molecular dataset was divided into nine separate partitions: 18S, 28S, the EF-1 α F2 intron region, and one for each codon position in each of the two protein-coding genes. RAxML runs were performed using the default hill-climbing search algorithm with 5,000 rapid bootstrap pseudoreplicates and a GTR + gamma model of nucleotide substitution ('-f a - 5000 -m GTRGAMMA')

While *post hoc* inspection revealed strong A-T bias in the third codon position of COI (93%), a separate ML analysis of this partition alone produced a structured topology suggesting valuable phylogenetic signal (Appendix B, Figure S4.1a). In light of these findings, we chose to retain these data in the final analysis. We also performed separate analyses of each gene as a stand alone dataset, using the same procedures as above (but with only 100 rapid bootstrap pseudoreplicates each).

MP analyses: We carried out two analyses of the complete dataset under the equal weights parsimony criterion: the first with gaps treated as a fifth state ('nstates GAPS;'), the second with gaps as missing data ('nstates NOGAPS;'). Both analyses proceeded as follows: holding 100,000 trees in memory ('mxram 100; hold 100000'), we conducted a tree search using 200 random addition sequences with TBR (holding 100 trees per

replicate), and implementing 200 rounds of parsimony ratchet (upweight probability = 8%; downweight = 4%; Nixon 1999) with 30 iterations of default drift. (Command structure: 'ratchet: iter 200 upfactor 8; mult = replic 200 hold 100 ratchet drift;'). After this initial search, we performed branch swapping using trees in memory with the command 'bbreak;'. Clade supports for both sets of MP trees were estimated using 1,000 pseudoreplicates of symmetric resampling, reported as GC scores (Goloboff et al. 2003) on the strict consensus topologies. Single gene topologies were also inferred using TNT with gaps treated as a fifth state and using the heuristic search routine outlined above.

All trees from both the ML and MP analyses were visualized using the packages *ape*, *geiger*, and *apTreeshape* in R version 3.1.0 ("Spring Dance"; R Core Team 2014).

Results

The results of the ML, MP gaps-as-fifth-state, and MP gaps-as-missing analyses are shown in Figures 4.2-4.4, respectively. (For trees generated by the ML and MP single gene analyses, see Appendix B, Figures S4.1 and S4.2, respectively.)

Agreements among optimal topologies: While our limited sample size precluded any formal test of pseudoscoliine monophyly, all three polytypic philanthine tribes (Philanthini, Aphilanthopini, and Cercerini) appear as very well supported natural groups (BS = 100%; GC $\geq$ 95%) (Figures 4.2-4.4). In addition, *Trachypus*, *Clypeadon*, *Cerceris*, and *Eucerceris* are all recovered as monophyletic genera, the latter three with very high support (BS = 100%; GC = 100%); *Eucerceris lacunosa* and *Cerceris mimica* are positioned as the basalmost lineages within their respective genera, the latter with very high support (BS = 100%; GC = 100%). *Philanthus* (*sensu lato*) is paraphyletic with respect to *Trachypus*, although the precise nature of this paraphyly differs (see

Figure 4.2. Maximum likelihood tree based on RAxML rapid bootstrap analysis of all four genes plus ingroup intron regions, as a cladogram (left) and with branch lengths (right). Bootstrap support values $\geq 50\%$ (based on 5,000 pseudo-replicates) are shown just below and to the left of each node.

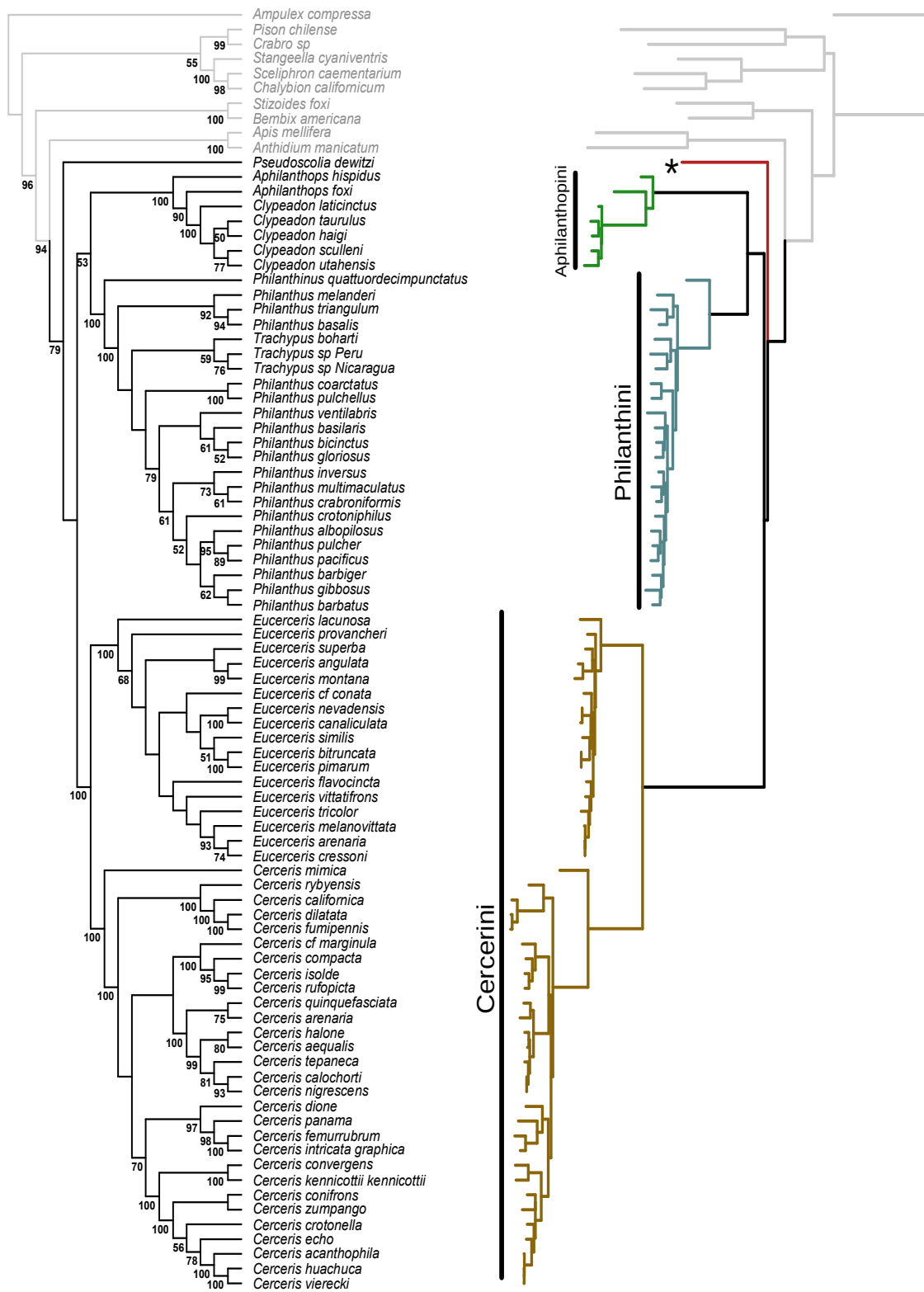


Figure 4.3. Strict consensus of 12 most parsimonious trees (7,815 steps; CI = 0.360; RI = 0.778) based on the complete dataset (four genes plus ingroup intron regions), with gaps treated as a fifth state. Symmetric resampling support values (reported as GC scores and based on 1,000 pseudoreplicates) are shown just below and to the left of each node. Average group support = 59.0

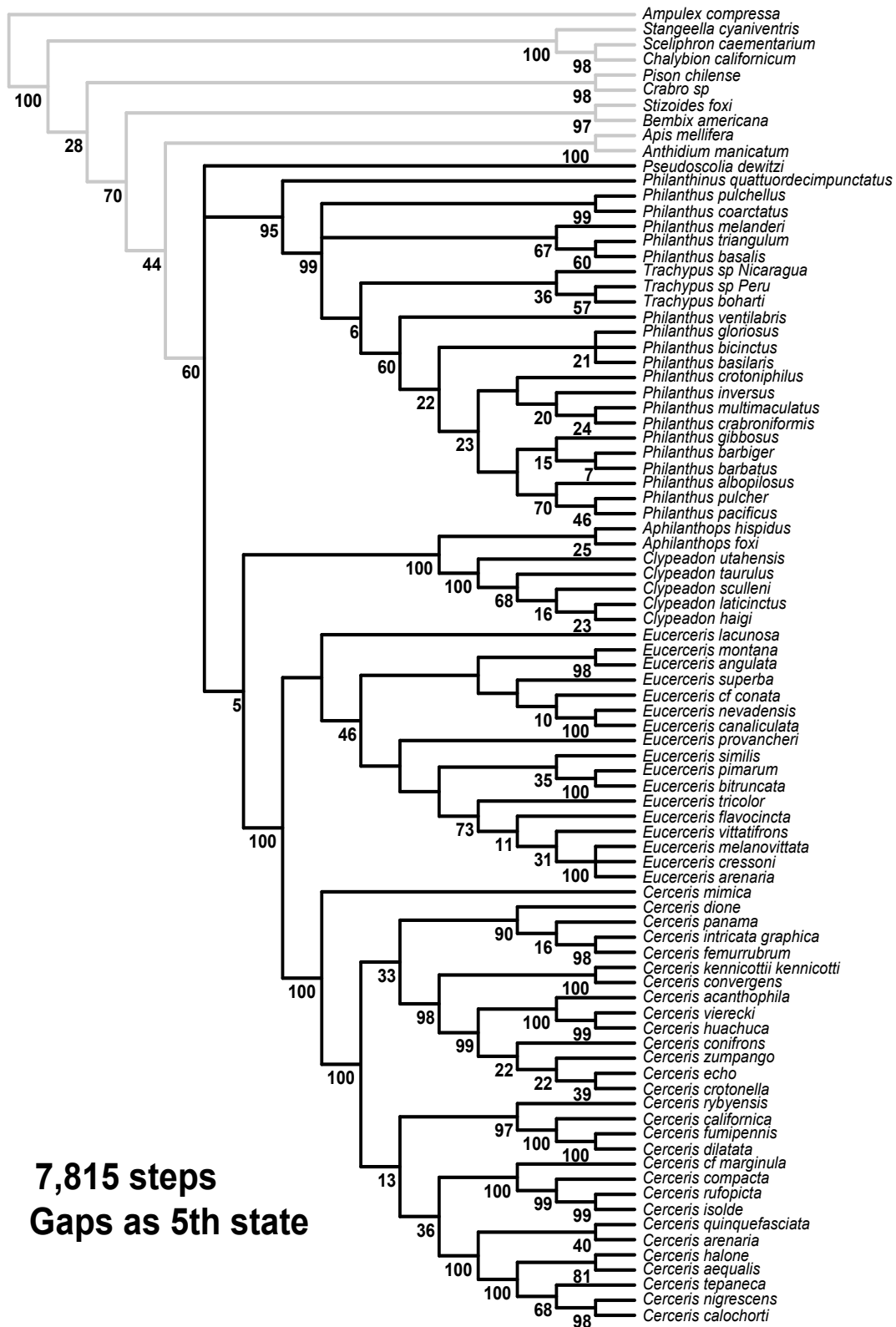
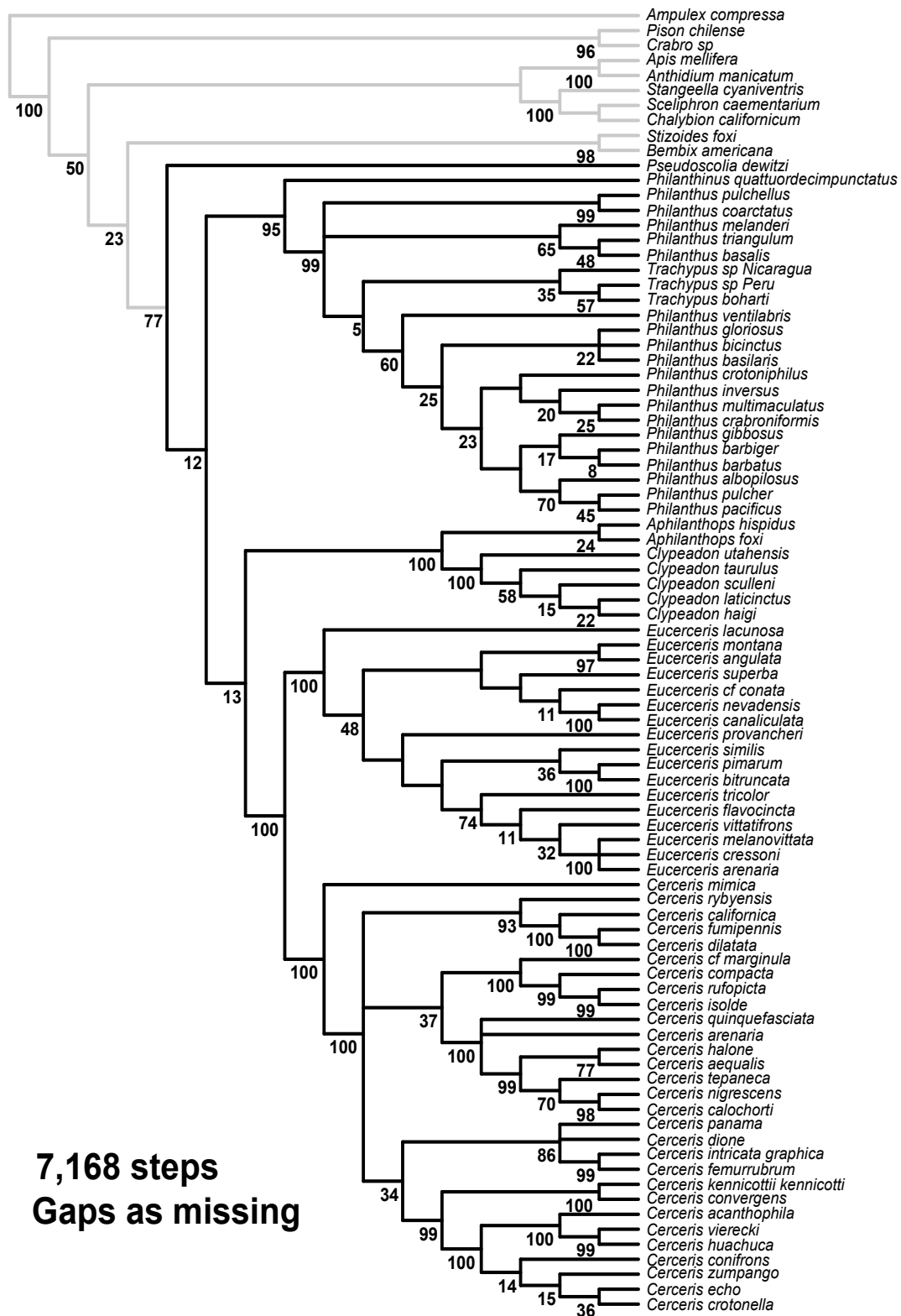


Figure 4.4. Strict consensus of 48 most parsimonious trees (7,168 steps; CI = 0.325; RI = 0.743) based on the complete dataset (four genes plus ingroup intron regions), with gaps treated as missing data. Symmetric resampling support values (reported as GC scores and based on 1,000 pseudoreplicates) are shown just below and to the left of each node. Average group support = 58.0.



Discussion, below). All three optimal topologies are consistent with a basal position for *Pseudoscolia* (unresolved in the MP gaps-as-fifth-state topology), although support values for this arrangement are low.

Disagreements among optimal topologies: While the MP gaps-as-fifth state analysis returns a basal polytomy (*Pseudoscolia* + Philanthini + (Cercerini + Aphilanthopini)), these relationships are resolved in the MP gaps-as-missing and ML topologies, with *Pseudoscolia* positioned at the base of the tree in both cases.

Both MP trees disagree with the ML topology in terms of tribal relationships, with each optimality criterion producing a different swing of the *aphilanthopine pendulum*: In the MP trees, Aphilanthopini is sister to Cercerini, while the ML topology places the group sister to Philanthini. Support values are low (BS = 53%; GC $\leq$ 13%) for both arrangements.

Discussion

The current study presents the first molecular phylogeny to include representatives of all eight genera and all four tribes of philanthine wasps. While Kaltenpoth et al. (2014) recently presented a phylogenetic analysis of species relationships within the tribe Philanthini, ours is the first molecular study to include an exemplar of the geographically restricted and rarely collected genus *Pseudoscolia*, as well as the first formal analysis of any kind to explore intrageneric relationships within *Cerceris* and *Eucerceris*.

Although this preliminary dataset was limited in scope – with fewer than 4,000 nucleotide characters, and terminals representing less than 10% of the group's described diversity – our analyses still point toward important considerations for future philanthine workers. We discuss those considerations in more detail below.

Relationships among the tribes: Ambiguity and conflict

The unexpected and unprecedented placement of *Pseudoscolia* in both our MP and our ML trees renders those topologies incompatible with all previously published phylogenetic treatments of the group. However, very low support for the clade composed of all Philanthinae except Pseudoscoliini, combined with our limited sampling within the latter group (one specimen with mixed amplification success), provide little confidence in this result.

Pseudoscolia does represent a somewhat unusual philanthine lineage, with a number of morphological traits (e.g., thick hair combs on the basal third of the male mandibles [Prentice and Pulawski 2004], a pointed glossa similar to that of some long-tongued bees [Michener 2005]), that appear to be unique among apoid wasps. However, similarities in larval (Asís et al. 1991) and adult morphology (Bohart and Menke 1976, Alexander 1992a,b; Prentice 1998) have traditionally been interpreted as strong evidence for a close relationship with the Cercerini. Given early reports of divergent prey preferences (Asís et al. 1991; Kazenas 2001) and the interesting morphological diversity found within *Pseudoscolia* (Bohart and Menke 1976), we look forward to increased molecular sampling from within this rarely collected genus.

Setting aside the placement of the Pseudoscoliini, our trees still do little to resolve the *aphilanthopine pendulum* discussed above. While our MP topologies consistently place Aphilanthopini as sister to Cercerini, the ML tree positions it as sister to Philanthini. Neither position is strongly supported, however, and the results of the current study thus offer little help in arranging the philanthine tribes into natural groupings – a problem that clearly calls for greater sampling across a broad range of phylogenetically

informative characters.

*Paraphyly of *Philanthus* Fabricius 1790 and of *Aphilanthops* Patton 1881*

While the current study represents the third formal analysis to suggest a paraphyletic *Philanthus* (Alexander 1992a; Kaltenpoth et al. 2014), this idea dates back at least as far as Bohart and Menke's (1976, p. 561) entry on Philanthina in *Sphecid Wasps of the World*: "While there is a clear separation among New World species [of *Philanthus* and *Trachypus*] on the basis of the sessile versus pedunculate or petiolate gaster, the same cannot be said for the Old World." Likewise, Evans and O'Neill (1988, p. 2) describe *Trachypus* as a "closely related derived" genus vis-à-vis *Philanthus*. The well supported and intuitively appealing biogeographic scenario proposed by Kaltenpoth et al. (2014) – a paraphyletic grade of Old World *Philanthus*, leading to a clade composed of a monophyletic *Trachypus* + a monophyletic clade of New World *Philanthus* – accords well with the results of our MP analyses (although not our ML analysis). In either case, all of our trees support the notion that *Philanthus* is rendered unnatural by the derived Neotropical *Trachypus*.

The monophyletic status of *Aphilanthops* is less clear, both because of disagreements between our ML and MP topologies and due to our incomplete sampling from within the genus. In his 1966 revision of *Aphilanthops* sensu lato, Bohart raised the subgenus *Clypeadon* (along with *Listropygia*, subsequently synonymized by Alexander [1992b]) to generic status based primarily on unique and unreversed modifications of the female pygidium (Evans 1962a). However, all three of the morphological features that Bohart used to united the remaining *Aphilanthops* species – the simple female pygidium, the apical hair fringe on the male sternum IV, and the lack of a post-scutellar angular

lamina – are almost certainly symplesiomorphic within Philanthinae. (Alexander [1992a] appears to have reached the same conclusion based on his morphological study.)

While the prey preferences of *A. foxi* are currently unknown, the two species not sampled here – *A. frigidus* and *A. subfrigidus* – are highly specific predators on the alate queens of *Formica* ants (Evans 1962a). It seems quite possible, then, that these myrmecophagous species are more closely related to the ant specialists in *Clypeadon*, than either are to their putative congener *A. hispidus*, a predator of bees (Evans 1977b; Alcock 2009). Unfortunately, a test of that hypothesis will have to await a more complete sample from within the Aphilanthopini.

Eucerceris Cresson 1865 and *Cerceris* Latreille 1802 as sister taxa

With their great disparity in terms of species number, behavioral diversity, and geographic scope, it has long seemed reasonable to assume that the genus *Eucerceris* arose from within a paraphyletic *Cerceris*. Indeed, Alexander (1992a) reached that same conclusion based on his inability to identify definitive adult synapomorphies uniting the latter and excluding the former. In addition, the existence of presumably primitive prey preferences (hymenopterophagy) in some palearctic *Cerceris* species has led many (e.g., Gess 1980; Evans and O'Neill 1988) to speculate that beetle hunting is a derived character linking only a subset of *Cerceris* species with the *Eucerceris* clade.

Given this history, it is somewhat surprising to find both genera so strongly supported as natural groups in the topologies presented here. Nevertheless, both our ML and MP trees (as well as most individual gene analyses, see Figures S4.1-S4.2) agree with the concept of *Eucerceris* and *Cerceris* as well-defined monophyletic sister taxa. In addition, the otherwise unremarkable North American weevil-hunter *C. mimica* is

strongly supported as a basal lineage vis-à-vis the other *Cerceris* sampled here.

One obvious objection is that our results are based on only a handful of *Cerceris* species, with a clear bias toward Nearctic representatives. This is certainly a fair criticism, and in fact our dataset includes sequences from no more than 5% of currently described *Cerceris* species. Nevertheless, that sample does appear to cover much of the described morphological and behavioral diversity found within the group, both in the Nearctic and around the world. In other words, no subset of unsampled *Cerceris* (with the possible exception of the Mediterranean species *C. histerisnica* [Bohart and Menke 1976; see below] or the neotropical *C. binodis*) stands out as a particularly likely sister group to a clade composed of our *Cerceris* sample + *Eucerceris*. In fact, Bohart and Menke seem to have anticipated our conclusions in their discussion of second submarginal cell evolution within the Cercerini:

It seems to us that the genera must have arisen independently and both from ancestors with sessile submarginal cell II. Thus, *Cerceris histerisnica* (sometimes placed in the separate genus *Nectanebus*) would be closest to the ancestral type of that genus on the basis of the sessile second cell. In *Eucerceris*, the generalized types would be *lacunosa*, *velutina*, *violaceipennis*, and *punctifrons* in which cell II is sessile in both sexes. (Bohart and Menke 1976, p. 590)

Our preliminary molecular results partly bear this out, including the placement of *E. lacunosa* at the base of the *Eucerceris* tree. (The placement of *C. histerisnica* will have to await further molecular sampling.)

Species groups within Cerceris

For the most part, the taxonomic affiliations first delimited by Scullen (1965, 1972) and later revisited by Bohart and Grissell (for California species; 1975) are supported by the current results. For instance, most of Scullen's Group I species (= *finitima* group of

Bohart and Grissell) – here represented by *acanthophila*, *conifrons*, *convergens*, *crotonella*, *echo*, *huachuca*, *kennicottii*, *marginula*, *vierecki*, and *zumpango* – hold together as a well supported clade in both the ML (BS = 100%) and MP (GC $\geq$ 98%) trees. (The exception is *C. marginula*, a rarely collected neotropical species that our trees place as the sister species to Group III, below.)

As represented by *C. californica*, *dilatata*, and *fumipennis*, Scullen's buprestid-hunting Group II (= *californica* group of Bohart and Grissell 1975) is also well supported in both our ML (BS = 100%) and MP (GC = 100%) topologies. In addition, *C. rybyensis* – a Eurasian species that specializes in hunting bees – is strongly supported as the sister to this clade, a somewhat unsurprising result given a shared female clypeal morphology (*sans* projections) and the somewhat concave shape of the female fifth sternum.

Group III (Scullen 1965, 1972; = *compacta* group of Bohart and Grissell 1975) is also present here, with *compacta*, *isolde*, and *rufopicta* forming a well supported clade (BS = 95%; GC $\geq$ 99%) united by a distinctly lamellate apical margin on the female clypeal projection.

The rather small Group IV (= *graphica* group of Bohart and Grissell 1975) contains just two species, *intricata* and *femurrubrum*, both of which hunt tenebrionid prey and both of which were represented in our taxonomic sample (the former by the common subspecies *graphica*). Our results place these species as strongly supported (BS = 100%; GC $\geq$ 98%) sister taxa in all trees.

Finally, Bohart and Grissell's (1975) *nigrescens* group – here represented by *aequalis*, *calochorti*, *nigrescens*, and *tepaneca* – is infiltrated by a previously ungrouped species, *halone* (not present in California, and thus not treated by Bohart and Grissell).

Together, this group of weevil-specialists is sister to a clade composed of two Eurasian weevil-hunters, *C. arenaria* and *C. quinquefasciata*.

Some implications for prey choice evolution

Our results support the traditional idea of an ancestral preference for bee and wasp prey within the subfamily Philanthinae. While myrmecophagy appears to have evolved at least twice (in the Aphilanthopini and *Pseudoscolia*) and possibly three times (separate origins in *Aphilanthops* and *Clypeadon*), the topologies presented here clearly support a single origin of coleopterophagy in the ancestor to all Cercerini, with the ancestral preference presumably linked to the weevil family Curculionidae. The well supported position of a Eurasian bee-hunting *Cerceris* (i.e., *rybyensis*) deep within the genus and sister to a clade of buprestid hunters (*C. dilatata*; *C. californica*; *C. fumipennis*) implies convergent evolution/reversal and not, as suggested by previous authors, a symplesiomorphic prey preference (see Evans and O'Neill [1988, p. 254]: "Although most Cercerini use beetles, several Eurasian species of *Cerceris* prey on bees, suggesting that beewolf behavior may have been characteristic of the original stock of this now very large genus."). This confusion of symplesiomorphy with homoplasy in prey choice may be a major factor in long-standing doubts concerning the monophyly of *Cerceris* – doubts that appear increasingly unfounded in light of our phylogenetic results.

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CHAPTER V

ON THE EVOLUTION OF PREY CHOICE IN PHILANTHINE WASPS: FIRST INSIGHTS FROM A TOTAL EVIDENCE PHYLOGENETIC ANALYSIS

Abstract

Phylogenetic hypotheses play a key role in the comparative study of behavior. As one more illustration of this principle, I here present the first total evidence cladistic analysis of the wasp subfamily Philanthinae (Hymenoptera: Crabronidae) as a tool for understanding the evolution of prey choice within the group. Through equal weights and implied weights parsimony analyses of a new dataset based on recently developed sequence data (COI, EF-1 α F2, 18S, 28S) combined with 66 newly coded morphological and behavioral characters, I show that the tribes Philanthini, Aphilanthopini, and Cercerini are all highly supported monophyletic groups. While *Eucerceris* and *Cerceris* are both monophyletic sister taxa, *Philanthus* is rendered paraphyletic with respect to *Trachypus*. *Aphilanthops* is paraphyletic with respect to *Clypeadon*. Overall, the results of this study accord well with those based on the molecular data alone; however, in the trees generated by the morphology/behavior dataset alone and in the implied weights total evidence topology, *Pseudoscolia* is returned to its traditional sister group relationship with the Cercerini. Parsimony based ancestral state reconstruction also yields the first phylogenetically rigorous insight into the evolution of philanthine prey preferences. As predicted, hymenopterophagy is the ancestral state within the group; however, contrary to previous speculation, bee hunting is *not* ancestral for the mostly coleopterophagous Cercerini. Instead, the use of bee prey appears to have evolved convergently at least once from within a derived beetle hunting clade.

Introduction

To fully understand why an animal behaves a certain way, researchers must simultaneously address four different aspects of behavioral causation, corresponding roughly to what Tinbergen (1963) called the *physiological*, *survival value*, *ontogenetic*, and *evolutionary* (i.e. phylogenetic) aims of ethology. While each aim represents a different way of asking the same question ("Why does behavior *x* exist?"), none on its own can lead to a complete and comprehensive answer (Sherman 1988, 1989).

At present, such an holistic approach remains relatively rare within the animal behavior literature, where survival value and mechanistic studies seem to exist largely independent of comparative, phylogeny-based analyses. This situation persists despite ample evidence that phylogenetically informed behavioral studies – here broadly construed as ancestral state reconstructions (e.g. Winkler and Sheldon 1993; Branham and Wenzel 2001; Cardinal et al. 2011), phylogenies based exclusively on behavioral characters (e.g. de Queiroz and Wimberger 1993; Zyskowski and Prum 1999, Noll 2002), and total evidence phylogenies that include behavioral data (e.g. Bosch et al. 2001; Pickett and Carpenter 2010) – can illuminate key aspects of behavioral evolution (Dobson 1985; Wenzel 1992; Emlen 2006).

This need for phylogenetic and comparative insight is readily apparent in the case of the charismatic digger wasps of the subfamily Philanthinae (Apoidea: Crabronidae; Figure 5.1), a possible sister group to the bees (Ohl and Bleidorn 2006; Debevec et al. 2012) and taxonomic home to *Philanthus triangulum*, a major model organism in the history of ethology (Tinbergen 1932, 1935; Tinbergen and Kruyt 1938; Tinbergen and van der Linde 1938; see discussion in Burkhardt 2005). Despite decades of interest from

Figure 5.1. Male exemplars of the eight genera currently assigned to the subfamily Philanthinae (Apoidea: Crabronidae): A. *Clypeadon haigi*; B. *Aphilanthops frigidus*; C. *Eucerceris ferruginosa*; D. *Cerceris dione*; E. *Philanthinus integer*; F. *Philanthus crabroniformis*; G. *Trachypus mexicanus*; H. *Pseudoscolia dewitzi*.



prominent field biologists (see, among others, Evans 1955, 1962a,b, 1964b,c, 1966, 1970a,b, 1971, 1973a,b, 1974, 1975, 1977a,b, 1982, 1983, 1988, 1992, 1996, 2000), researchers have only recently attempted to place the group's evolution in historical context through the use of explicit and analytically derived phylogenetic hypotheses (Alexander 1992; Kaltenpoth et al. 2014; Chapter IV of this dissertation) – a necessary first step toward a truly comparative study of philanthine behavior.

Of particular interest from the comparative standpoint is the story of how prey preferences have evolved and diversified within the group. These insects attack a diverse range of "formidable prey" (Evans and O'Neill 1988), and in many cases must have overcome substantial evolutionary obstacles during critical prey preference transitions (Andrietti 2011). Even though successfully attacking an adult weevil obviously requires an entirely different set of approach strategies, recognition systems, and stinging techniques compared to attacking a honeybee worker or an alate ant queen, all three predatory strategies have evolved within the group (Bohart and Menke 1976). Given that "[t]he adaptation of a particular wasp to its prey presents one of the most intriguing problems in the study of behavior" (Evans and West Eberhard 1970), the philanthine wasps represent a promising opportunity to apply tree-based thinking to an important question in comparative ethology.

In broad outline, philanthine prey preferences fall along tribal divisions, with the Philanthini (at least *Philanthus* and *Trachypus*; the prey of *Philanthinus* is unknown) and Pseudoscoliini (*Pseudoscolia*) provisioning their nests with apoid wasps and bees; the Cercerini (*Eucerceris*, *Cerceris*) mostly hunting a variety of beetle species; and the Aphilanthopini (*Aphilanthops*, *Clypeadon*) developing highly specialized, sometimes

species-specific, preferences for ants (Bohart and Menke 1976). This simple picture is complicated, however, by a number of important exceptions (e.g. *Cerceris* species that hunt sweat bees [Bohart and Menke 1976], an *Aphilanthops* that preys on bees and other apoid wasps [Evans 1977a], a *Pseudoscolia* species reported to take ants [Kazenas 2001]), as well as persistent doubts about the monophyly of the two largest genera (*Philanthus*, with 137 species, and *Cerceris*, with 870 species; Pulawski 2014). (For a detailed review of philanthine systematics and previously published hypotheses, see Chapter IV.)

In this study – the second in a series aimed at uncovering philanthine phylogenetic relationships – I attempt to place the group's predatory behavior in its proper evolutionary context through a total evidence maximum parsimony analysis of molecular, morphological, and behavioral characters. As such, the 66 newly coded morphological and behavioral characters used here complement earlier work (Chapter IV of this dissertation) based on sequence data alone, with the total evidence results representing the most extensive treatment of philanthine relationships to date.

Materials and methods

Taxon set

In order to take full advantage of the molecular dataset developed in Chapter IV of this dissertation, I here used the same ingroup sample employed in that study, but with the addition of four new ingroup terminals. Of these, two (*Aphilanthops frigidus*, *A. subfrigidus*) were coded for morphology/behavior alone, while the other two (*Trachypus denticollis*, *T. elongatus*) were coded for morphology/behavior *and* were represented by newly available molecular data previously published elsewhere (Kaltenpoth et al. 2014).

While most terminals were coded at the species level, two (*Pseudoscolia* and *Philanthinus*) were treated as genus level composite terminals (*sensu* Nixon and Carpenter 1996), with their respective molecular and morphological data merged from different sources. In the case of *Pseudoscolia*, molecular data were from *P. dewitzi*, while morphological and behavioral character codings were based on a combination of *P. dewitzi*, *P. theryi*, and relevant literature sources. For *Philanthinus*, molecular data were from *P. quattuordecimpunctatus*, while morphological data were based on *P. integer* and the literature. The use of composite terminals obviously rules out any study of variation within these two groups, and the current analysis makes no attempt to resolve their intrageneric relationships.

New outgroup terminals were drawn from a potential sister taxon to Philanthinae, Anthophila (Debevec et al. 2012), and from the more distantly related subfamily Bembicinae. Two bees (*Apis mellifera* and *Anthidium manicatum*) and the bembecine *Stizoides foxi* were chosen primarily based on the availability of molecular data for the loci used here (Table 5.1). While these outgroups obviously represent only a small sample of the tremendous morphological diversity found within the bees (Michener 2007) and within Apoidea generally (Bohart and Menke 1976), they should be sufficient for the determination of ingroup relationships in accordance with Nixon and Carpenter's (1993) *simultaneous outgroup analysis* method. The final taxon set thus arranged contains three outgroup and 81 ingroup terminals, the latter representing all eight genera and all four tribes of philanthine wasp.

Molecular data

The combined character matrix includes the same molecular data used in Chapter

Table 5.1. Outgroup molecular exemplars and newly downloaded ingroup sequences. *A. manicatum* and *A. mellifera* sequences were also used as part of the outgroup sample in Chapter IV.

OTU	GenBank accession	Source publication
<i>Outgroup</i>		
<i>Stizoides foxi</i>	xxxxxxx (COI)	Payne et al. (in prep)
	xxxxxxx (EF1- α)	"
	xxxxxxx (28S)	"
	xxxxxxx (18S)	"
<i>Apis mellifera</i>	AF214668 (COI)	Tanaka et al. 2001
	AF015267 (EF1- α)	Danforth et al. 2006
	AY703551 (28S)	Ward & Downie 2005
	AY703484 (18S)	"
<i>Anthidium manicatum</i>	xxxxxxx (COI)	Payne et al. (in prep)
	xxxxxxx (EF1- α)	"
	xxxxxxx (28S)	"
	xxxxxxx (18S)	"
<i>Ingroup</i>		
<i>Aphilanthops foxi</i>	KJ556973 (EF1- α)	Kaltenpoth et al. 2014
<i>Philanthinus</i>	KJ556972 (EF1- α)	"
<i>Philanthus basalis</i>	KJ556927 (EF1- α)	"
<i>Philanthus coarctatus</i>	KJ556931 (EF1- α)	"
<i>Philanthus melanderi</i>	KJ556942 (EF1- α)	"
<i>Philanthus pulchellus</i>	KJ556949 (EF1- α)	"
<i>Philanthus triangulum</i>	KJ556960 (EF1- α)	"
<i>Trachypus boharti</i>	KJ556966 (EF1- α)	"
<i>Trachypus denticollis</i>	KJ556967 (EF1- α)	"
	JN674295 (28S)	"
	JQ040294 (COI)	"
<i>Trachypus elongatus</i>	KJ556968 (EF1- α)	"
	JN674296 (28S)	"

IV, with the addition of 13 newly available sequences downloaded from GenBank (GenBank accession numbers in Table 5.1). All four loci (COI, EF-1 α F2, 18S, 28S) were realigned with respect to the new taxon set using the E-INS-i algorithm as implemented in MAFFT v. 7.154 (Katoh et al. 2002; Katoh and Standley 2013); as in the previous study, non-coding EF-1 α F2 regions were included for ingroup taxa only and were aligned independent of their flanking coding regions. The complete molecular dataset thus included 3,744 aligned nucleotide basepairs, of which 1,213 were parsimony informative when gaps were treated as a fifth state.

Morphological and behavioral characters

Two previous morphological studies were of particular value in the development of the current dataset: Alexander's (1992) cladistic analysis of the Philanthinae, and Prentice's voluminous (1998) study of tribal relationships within Apoidea. In the former, Alexander investigated relationships among philanthine genera using 37 morphological and behavioral characters, some of which were repurposed and recoded here. Meanwhile, Prentice's doctoral dissertation provided a more global view of philanthine synapomorphies and tribal characters, including several internal traits that appear to unite the Philanthinae. (Two of these were used here, as characters 1 and 29.)

While both of those works were valuable in determining generic and tribal relationships, no previous morphological analysis (with the exception of Alexander's [1992] treatment of *Aphilanthops* species as separate terminals) has addressed subgeneric relationships within the group. With that in mind, the current character set emphasizes traits of subgeneric value within the beetlewolves *Cerceris* and *Eucerceris* (i.e., characters 3-5, 10-12, 15, 20, 29-30, 49, and 51) and, to a lesser extent, within *Philanthus*

(character 9) and *Aphilanthops* (characters 23, 35, 37).

A total of 55 adult and three larval morphological characters, as well as eight behavioral characters, were coded through a combination of literature search (see Table 5.2) and reference to pinned adult specimens located at the American Museum of Natural History (AMNH) insect collections. Characters coded directly from the literature (larval and internal morphology, as well as behavioral characters) are annotated as such in the character list below (see **Results**). In one case (*Cerceris zumpango*), male characters could not be coded as the sex remains unknown.

While apoid larvae tend to be fairly homogeneous at the generic level (Evans and Lin 1955), I generally avoided extrapolating larval character codings from a single species to all other members of its genus. The reasons for this were both practical (i.e., the unclear status of *Cerceris*, *Philanthus*, and *Aphilanthops* as monophyletic groups) and philosophical (i.e., the desire to avoid *a priori* assumptions of character stasis); however, the dataset does contain two important exceptions: *Pseudoscolia*, treated here as a composite taxon for all characters, and *Trachypus*, in which the only detailed larval description is for a species (*T. petiolatus*) not included in the current taxon set. Behavioral characters were never extrapolated, and represent unique observations for each species coded. Despite potentially strong arguments for transformation series in some characters, all multistate characters were here coded as non-additive.

Phylogenetic analyses

All phylogenetic analyses were performed under the maximum parsimony criterion using either equal or implied weighting schemes (Goloboff 1993), as implemented in TNT version 1.1 (Goloboff et al. 2008). All implied weights analyses were performed

Table 5.2. Literature sources consulted for larval and behavioral character coding.

Species	Larval characters	Behavioral characters
<i>Aphilanthops frigidus</i>	Evans 1957	Evans 1962a
<i>Aphilanthops hispidus</i>	–	Evans 1977a; Alcock 2009
<i>Aphilanthops subfrigidus</i>	–	Evans 1962a; Evans 1970a; O'Neill 1990
<i>Clypeadon haigi</i>	–	Evans 1962a (as <i>A. haigi</i>)
<i>Clypeadon laticinctus</i>	Evans 1964a	Evans 1962a (as <i>A. laticinctus</i>); Evans 1977b
<i>Clypeadon sculleni</i>	–	Evans 1962a (as <i>A. sculleni</i>)
<i>Clypeadon taurulus</i>	–	Evans 1962a (as <i>A. taurulus</i>)
<i>Clypeadon utahensis</i>	–	Evans 1962a (as <i>A. concinnula</i>); Evans 1977b
<i>Cerceris acanthophila</i>	–	Hook 1987
<i>Cerceris arenaria</i>	–	Polidori et al. 2011
<i>Cerceris californica</i>	–	Linsley & MacSwain, 1956; Evans & Rubink, 1978; Hook 1987
<i>Cerceris confrons</i>	–	Evans & Rubink 1978
<i>Cerceris crotonella</i>	–	Evans & Rubink 1978
<i>Cerceris dilatata</i>	–	Hook 1987
<i>Cerceris echo</i>	–	Evans 1971; Evans & Rubink, 1978; Hook 1987
<i>Cerceris femurrubrum</i>	–	Hook 1987
<i>Cerceris fumipennis</i> Say	Evans 1957	Evans 1971; Evans & Rubink, 1978; Matthews & Matthews, 2005
<i>Cerceris halone</i> Banks	–	Evans 1971; Byers 1978
<i>Cerceris huachuca</i> Banks	–	Hook, 1987
<i>Cerceris intricata</i> graphica F. Smith	–	Alcock 1974 (as <i>simplex macrostictia</i>); Evans & Rubink, 1978 (as <i>simplex macrostictia</i>); Hook 1987.
<i>Cerceris mimica</i> Cresson	–	Evans 2000
<i>Cerceris nigrescens</i> F. Smith	–	Evans 1971 (as <i>nigrescens nigrescens</i>)
<i>Cerceris quinquefasciata</i> (Rossi)	Evans 1957	Polidori et al. 2012
<i>Cerceris rufopicta</i> F. Smith	Evans 1957 (as <i>robertsonii</i>)	Evans 1971 (as <i>robertsonii</i>); Kurczewski & Miller, 1984; Alexander & Asís, 1997
<i>Cerceris rybyensis</i> (Linnaeus)	–	Polidori et al. 2012
<i>Cerceris tepaneca</i>	–	Alcock 1974 (as <i>C. morata</i>)
<i>Eucerceris biruncata</i>	Evans 1964a (as <i>tricolorata</i>)	Krombein 1960 (as <i>tricolorata</i>)

Table 5.2. continued

Species	Larval characters	Behavioral characters
<i>Eucerceris cressoni</i>	—	Evans 1970 (as <i>fulvipes</i>)
<i>Eucerceris flavocincta</i>	Evans 1957	Bohart & Powell 1956; Evans 1970
<i>Eucerceris superba</i>	—	Scullen & Wold 1969 (as <i>E. superba bicolor</i>)
<i>Philanthus albopilosus</i>	—	Evans & O'Neill 1988
<i>Philanthus barbatus</i>	—	Evans & O'Neill 1988
<i>Philanthus barbiger</i>	—	Evans & O'Neill 1988
<i>Philanthus basalis</i>	—	Evans & O'Neill 1988
<i>Philanthus basilaris</i>	—	Evans & O'Neill 1988
<i>Philanthus bicinctus</i>	—	Evans & O'Neill 1988
<i>Philanthus crabroniformis</i>	—	Evans & O'Neill 1988
<i>Philanthus crotoniphilus</i>	—	Evans & O'Neill 1988
<i>Philanthus gibbosus</i> (Fabricius)	Evans 1957	Evans & O'Neill 1988
<i>Philanthus inversus</i> Patton	—	Evans & O'Neill 1988
<i>Philanthus multimaculatus</i> Cameron	—	Evans & O'Neill 1988
<i>Philanthus pacificus</i> Cresson	—	Evans & O'Neill 1988
<i>Philanthus pulchellus</i> Spinola	Asis et al. 1996	Asis, Tormos, and Gayubo 1996
<i>Philanthus pulcher</i> Dalla Torre	—	Evans & O'Neill 1988
<i>Philanthus triangulum</i> (Fabricius)	Evans 1957	Evans & O'Neill 1988
<i>Philanthus ventilabris</i> Fabricius	—	Alcock & Gamboa, 1975; Evans & O'Neill 1988
<i>Trachypus boharti</i> Rubio-Espina	—	Koedam et al. 2009
<i>Trachypus denticollis</i> Spinola	Evans & Matthews 1973 (extrapolated from <i>T. petiolatus</i>)	Polidori et al. 2009
<i>Trachypus elongatus</i> (Fabricius)	Evans & Matthews 1973 (extrapolated from <i>T. petiolatus</i>)	Bristowe 1925 (as <i>T. gomesii</i>)
<i>Pseudoscolia</i>	Evans & Matthews 1973 (extrapolated from <i>T. petiolatus</i>)	Asis et al. 1991 (<i>P. martinezi</i>); Kazenas 2001 (<i>P. simplicornis</i>)

using the default value of K (=3) as invoked by the *piwe* command. All tree searches were performed using the following commands after holding 100,000 trees in memory and setting *ratchet: iter 200 upfactor 8*;

mult = replic 200 hold 100 ratchet drift; bbreak;

Support values were determined using 1,000 pseudoreplicates of symmetric resampling (Goloboff et al. 2003) and were reported as frequency differences (GC values) on the strict consensus topologies. Prey choice characters (59-61) were optimized on the final preferred topology using PAUP* 4.0 (Swofford 2003).

All tree visualizations were carried out using the *ape*, *geiger*, and *apTreeshape* packages implemented in R version 3.1.0 ("Spring Dance"; R Core Team 2014).

Results

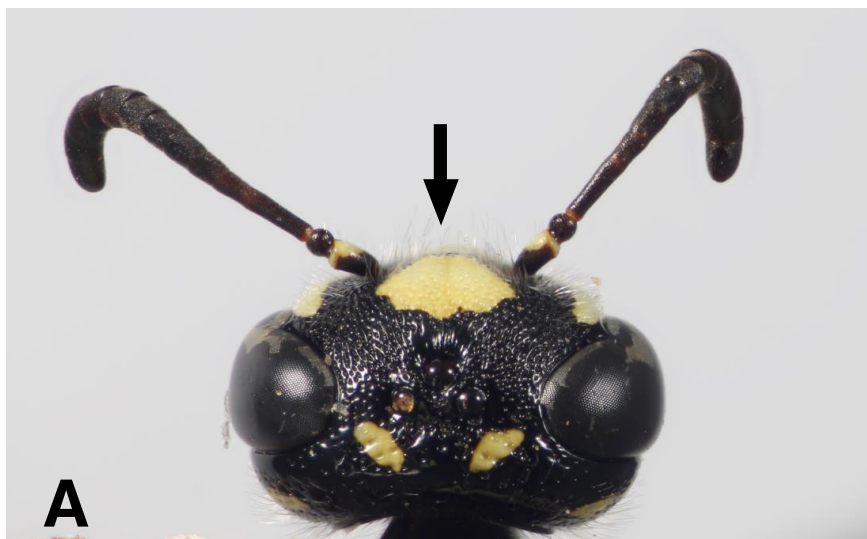
Annotated morphological and behavioral characters

The 66 morphological and behavioral characters are described below, along with relevant annotations. Characters first defined in previously published generic- or tribal-level studies (e.g., Alexander 1992; Prentice 1998) and *used verbatim* are here set in italics, with their source publication listed first in brackets. For the complete character matrix, see Appendix C, Table S5.1.

01. Internal antennal socket ridge: (0) not expanded; (1) expanded [Prentice 1998: Fig: 4a, character 1]. Prentice (1998) identified the expanded state as a unique, unreversed synapomorphy for the Philanthinae. He also suggested that the internal ridges, which allow for a recessed antennal socket and antennal membrane, may have evolved as a defense against the stinging attacks of aculeate prey. *This character was coded from the literature.*

02. Prominent interantennal carina: (0) absent (Figure 5.2a); (1) present, rounded, with depressed frontal line (Figure 5.2b); (2) present, sharply keeled, with an opaque to semi-translucent crest (Figure 5.2c). While interantennal carinae are found within the outgroup (see, e.g., workers of *Apis mellifera*), they are neither *prominent*, nor shaped as in 1 or 2. State 1 is characteristic of *Eucerceris*, state 2 of *Cerceris*. Compare to Alexander's (1992) binary character 6.
03. Male flagellomere XI strongly inflexed beneath: (0) no (Bohart and Grissell 1975: Fig. 99); (1) yes (*Ibid*: Fig. 96). State 1 is found in many *Cerceris* species and in *Eucerceris lacunosa*.
04. Male flagellomere XI with hairlike setae beneath: (0) no (Bohart and Grissell 1975: Fig. 99); (1) yes (*Ibid*: Fig. 87). State 1 is characteristic of the *Cerceris nigrescens* group.
05. Male flagellomere XI sharply truncate (Bohart and Grissell 1975: Fig. 82): (0) no; (1) yes. State 1 is characteristic of *Trachypus*, but is also found in some *Cerceris* species.
06. Subantennal sutures: (0) *more or less parallel below, forming a right angle with the epistomal suture between the subantennal sutures*; (1) *not parallel below, forming an oblique angle with the epistomal suture between the subantennal sutures* [Modified from Alexander 1992: character 3]. The subantennal sutures are not readily apparent in *Philanthus ventilabris* and are almost completely absent in *Pseudoscolia* (in the latter case due to the low placement of the antennal sockets). These taxa are coded *inapplicable*.
07. Antennal sockets less than 1/2 socket diameter from the epistomal suture: (0) no

Figure 5.2. Interantennal area in A. *Philanthus gibbosus*; B. *Eucerceris tricolor*, C. *Cerceris aequalis*. Note the absence of the interantennal carina (character 2, state 0) in *Philanthus*, the rounded, centrally furrowed carina (character 2, state 1) in *Eucerceris*, and the sharp, keel-like crest (character 2, state 2) in *Cerceris*.



(Alexander 1992: Fig. 8); (1) yes (*Ibid* Figs. 6, 7). Within the ingroup, state 1 is found only in *Pseudoscolia*.

08. Compound eyes emarginate within: (0) no (Figures 5.3 and 5.5b); (1) yes (Figures 5.4 and 5.5A). [Alexander 1992: character 5; see also Prentice 1998: character 80]. Within the ingroup, state 1 is characteristic of *Philanthus* and *Trachypus*.
09. Eyes strongly convergent above: (0) no (Figure 5.4a); (1) yes (Figure 5.4b). State 1 is characteristic of the *Philanthus zebratus* group (Bohart and Grissell 1975; Ferguson 1983) – represented here by *basilaris*, *bicinctus*, and *gloriosus* – as well as of certain *Pseudoscolia* species (Bohart and Menke 1976: Fig. 189).
10. Female with a single, prominent coniform to nasiform process located near the middle of the medial clypeal disc (Bohart and Grissell 1975: Figs. 109-110): (0) no; (1) yes. Found multiple times throughout the Cercerini, state 1 also appears to unite the *Cerceris intricata graphica* group.

N.B. – Diverse elaborations of the female clypeus are found throughout the Cercerini, where they seem to play an important role in prey transport (Byers 1978: Fig. 2). While their absence may unify certain groups of buprestid- and bee-hunting *Cerceris* species (e.g., Scullen's [1965] "Group II"), the diversity of clypeal morphologies found within the Cercerini defies simple coding; rather than homologize *clypeal elaboration* as such, I here take a conservative approach, treating readily recognizable forms as separate presence/absence characters (e.g., characters 10-12).

11. Female with a broad, prominent clypeal projection with an apical *deflected*

Figure 5.3. Male facial portraits of A. *Cerceris dione*; B. *Eucerceris canaliculata*. Arrows point to the *short and broad clypeal brushes* (character 13, state 1). In many *Cerceris* species, the brushes are both *waxy* (character 14, state 1) and *adherent, forming a distinct subrectangular to rectangular comb* (character 15, state 1); in *Eucerceris*, by contrast, the brushes are *relatively sparse* (character 14, state 0), *forming a somewhat adherent subtriangular brush* (character 15, state 0).



Figure 5.4. Male facial portraits of two *Philanthus* species: A. *crabroniformis*; B. *basilaris*. Arrows point to the *mesally directed clypeal brushes* (character 13, state 2). Note the strongly convergent upper margins of the compound eyes (character 9, state 1) in *P. basilaris* (B), as well as the *emarginate* inner eye margins (character 8, state 1) in both species.

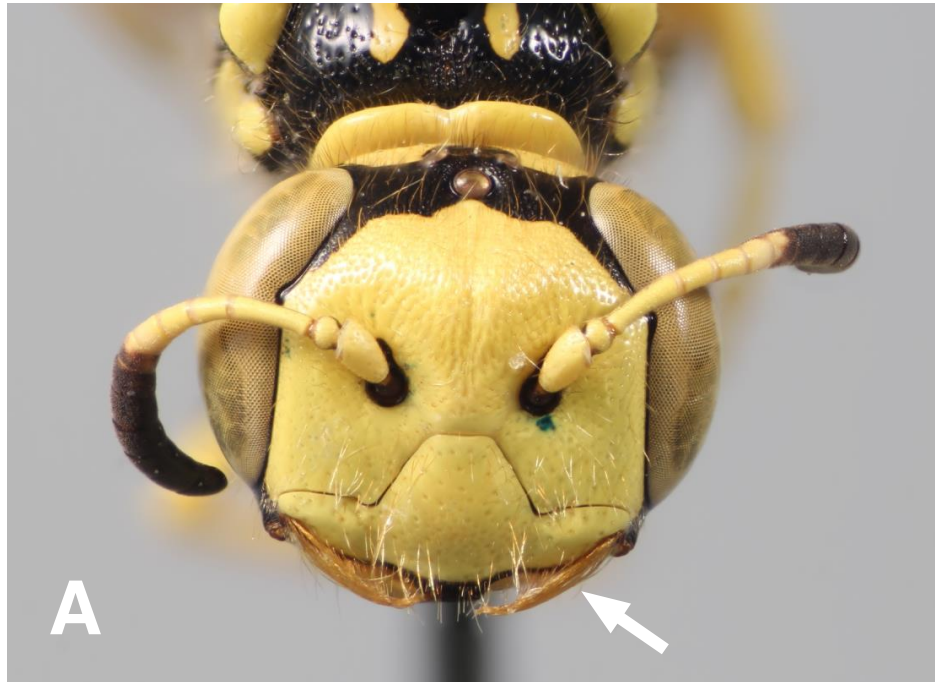


Figure 5.5. Male facial portraits of A. *Trachypus mexicanus*, B. *Clypeadon haigi*. In A., note the emarginate inner eyes (character 8, state 1) and the *clypeus with a complete row of upturned hairs, distinct from the lateral clypeal brushes* (character 16, state 1); in B., note that while the clypeal brush appears *waxed, easily distinguished from facial setae* (character 14, state 1), it only forms a *somewhat adherent subtriangular brush* (character 15, state 0).



membrane (Bohart and Grissell 1975: Fig. 138): (0) no; (1) yes. This membrane corresponds to Scullen's (1965; 1972) *clypeal lamella*. State 1 is characteristic of the *Cerceris compacta* group.

12. Female with a broad, prominent clypeal projection, its apical margin smooth, but strongly convex below and scooplike (Scullen 1965: Figs. 169a, 176a): (0) no; (1) yes. State 1 is found in the *Cerceris nigrescens* group.
13. *Male clypeal brush*: (0) *absent*; (1) *present: short and broad* (Figures 5.3 and 5.5B); (2) *present: long and narrow, with apex directed mesad* (Figure 5.4 and 5.5A). [Alexander 1992: character 4; see Prentice 1998: character 75] The presence of a well-defined clypeal brush is characteristic of philanthine males, with the notable exception of the North American species *Philanthus albopilosus*, where the loss appears to be associated with a change in male territorial scent marking behavior (Evans and O'Neill 1988; Prentice 1998). According to Prentice, the derived is also found in the unrelated apoid genera *Hoplisoides* and *Plenoculus*.
14. If clypeal brush short and broad, then hairs: (0) relatively sparse, difficult to distinguish from facial setae (Figure 5.3B); (1) appearing waxed, easily distinguished from facial setae (Figure 5.3A, 5.5B). State 0 is characteristic of *Eucerceris*, *Pseudoscolia*, *Philanthinus*, and *Aphilanthops*; state 1 is common in *Cerceris* and *Clypeadon*.
15. If clypeal brush short and broad, then hairs: (0) forming a diffuse to somewhat adherent subtriangular brush (Figures 5.3B, 5.5B; see also Bohart and Grissell 1975: Figs. 88, 92, 100); (1) strongly adherent, forming a distinct subrectangular to rectangular comb (Figure 5.3A; see also Bohart and Grissell Figs. 81, 86, 89, 94,

- 95, 97, 98). State 1 is found in many *Cerceris* species.
16. Apical margin of male clypeus with a complete row of upturned hairs, distinct from the lateral clypeal brushes (if present) (Figure 5.5A): (0) no; (1) yes. These hairs are similar in texture and color to those of the clypeal brush. State 1 is found only in *Trachypus* species.
 17. Female mandibles simple and edentate: (0) no; (1) yes.
 18. Apex of the glossa: (0) truncate or bilobed; (1) acute. According to Michener (2005; 2007), state 1 is found in the bee families Melittidae, Andrenidae, Halictidae, the long-tongue bees, and in certain male colletids. Within the ingroup (and in fact all crabronid wasps), state 1 is unique to *Pseudoscolia*. *This character was coded primarily from the literature.*
 19. Lateral [carinate] ridge on pronotum: (0) absent or very weakly developed; (1) present. [Alexander 1992: character 8, Fig. 9a]. Both states occur in *Pseudoscolia*.
 20. Female mesopleuron with a distinct, tooth-like projection: (0) no; (1) yes. This is Scullen's (1965) *mesosternal process/tubercle* and Bohart and Grissell's (1975) *mesosternal tooth*. State 1 is found in many cercerine species.
 21. Episternal sulcus: (0) sulcus present, extending ventrad to pronotum; (1) present, but not extending ventrad as far as pronotum; (2) absent [Alexander 1992: character 9, Figs. 9-10, 18]. .
 22. Scrobal sulcus: (0) absent; (1) present, distinct, but not extending anterad to contact pronotum; (2) present as a broad groove extending anterad to contact pronotum [Alexander 1992: character 11]. .
 23. Subalar carina: (0) without a lamellate process angled downward over subalar

fossa; (1) *sinuate*, with a *lamellate process angled downward over subalar fossa*.

[Alexander 1992: character 17, Figs. 9a,b, 18]. State 1 is found only in

Aphilanthops frigidus and *subfrigidus*.

24. *Lateral flange or lamellate process on the metanotum*: (0) *absent*; (1) *present*.

[Alexander 1992: character 16]. State 1 is found only in *Clypeadon*.

25. *Form of the anterior propodeal rim*: (0) *well developed and recessed*; (1) *absent*, not recessed [Prentice 1998: character 3]. Within Apoidea, state 1 is found only in Philanthinae and in the unrelated bembicine subtribe Heliocausina (Prentice 1998). *This character was coded from the literature*.

26. *Metasternum*: (0) *apophyseal pit more or less central and sternum broad anteriorly, so that middle coxae are well separated*; (1) *as in state (0), but sternum narrow anteriorly, so that middle coxae are not widely separated*; (2) *apophyseal pit near posterior margin and sternum narrow anteriorly, with a distinct median longitudinal carina*. [Alexander 1992: character 12; compare Prentice 1998: character 17].

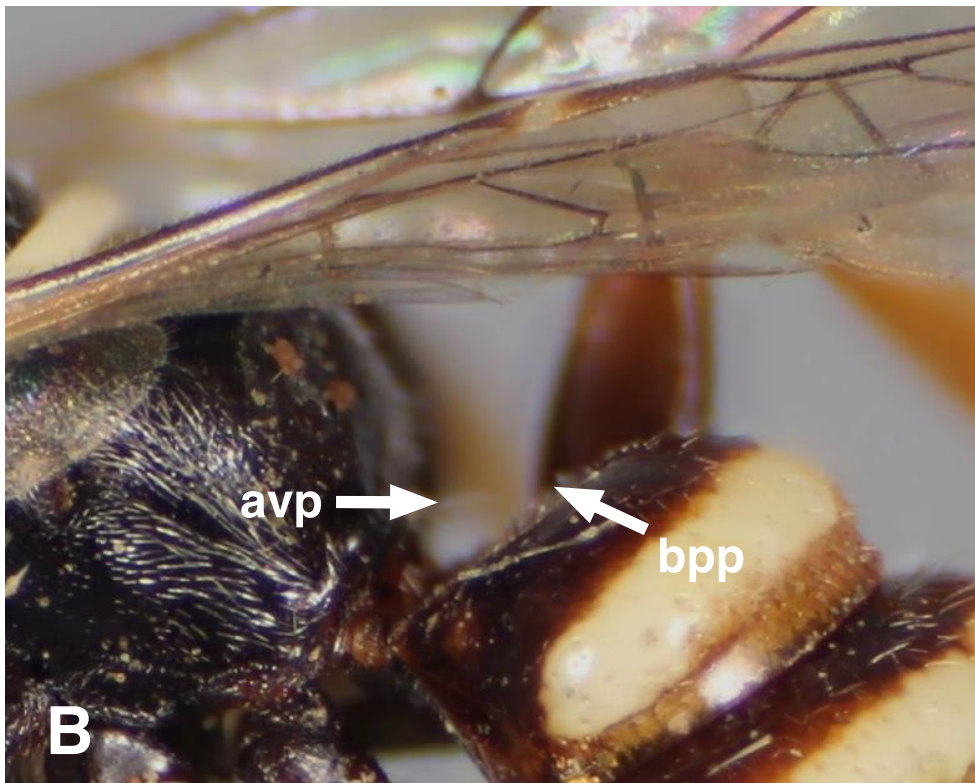
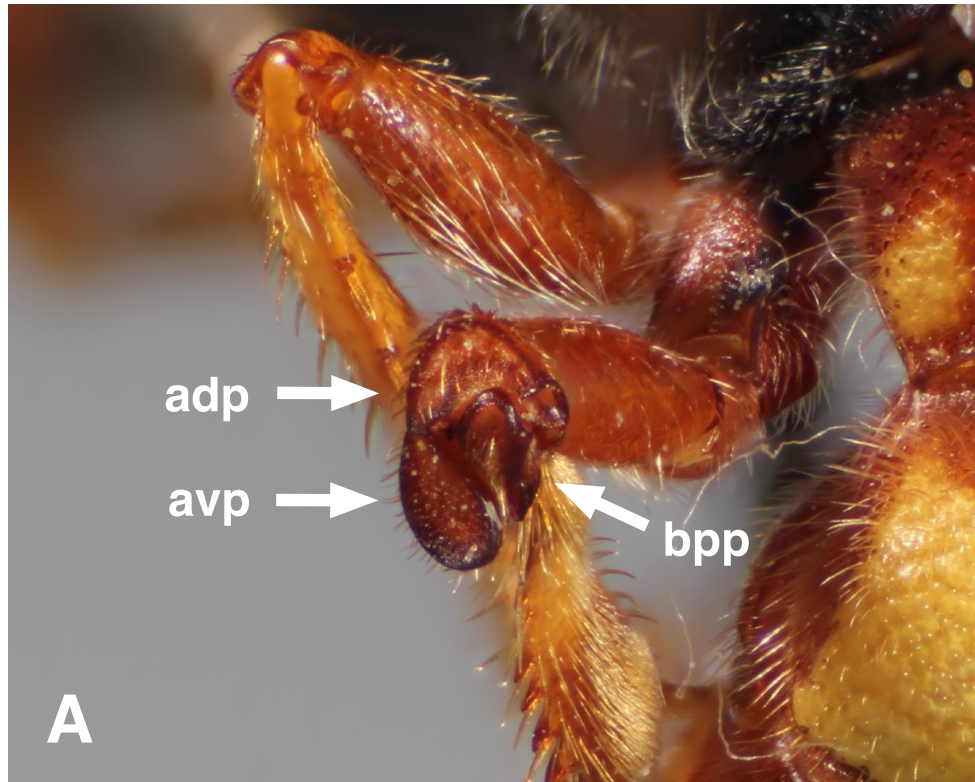
27. *Apex of marginal cell*: (0) *pointed, ending on costal margin of wing*; (1) *narrowly rounded, not ending on costal margin*; (2) *very broadly rounded or truncate*. [Alexander 1992: character 20].

28. *Forewing vein 2 r-m*: (0) *received in basal three-quarters of marginal cell* (Bohart and Menke 1976: Figs. 184b-d,i); (1) *received in apical quarter of marginal cell* (Figure 5.1C; see also Bohart and Menke 1976: Figs. 184e,f). [Alexander 1992: character 21]. State 1 is diagnostic for *Eucerceris*.

29. *Second submarginal cell of the male forewing*: (0) *not petiolate* (Bohart and Menke

- 1976: Fig.184b); (1) petiolate (Bohart and Menke 1976: Fig. 184d).
30. Second submarginal cell of the female forewing: (0) not petiolate; (1) petiolate.
- N.B.* – Characters 38 and 39 appear to evolve independently within *Cercerini*, and so are treated separately.
31. *Hindwing vein M*: (0) *diverging from Cu at or before intersection with cu-a*; (1) *diverging from Cu well beyond intersection with cu-a*. [Alexander, 1992: character 21].
32. Hindwing jugal lobe: (0) much more than half as long as anal area (Bohart and Menke 1976: Fig. 184h,i); (1) less than half anal area (Bohart and Menke 1976: Fig. 184b-d).
33. Midtibia with a single apical spur: (0) no; (1) yes.
34. Tarsal plantulae: (0) absent; (1) present. [Modified from Alexander 1992: character 13]
35. Base of hind tibia with *a distinct, short longitudinal carina*: (0) no; (1) yes.
Compare to Alexander's (1992) character 14.
36. Base of hind tibia with *a distinct flattened region* (the basoposterior plate), bordered, if present, by the short longitudinal carina mentioned above: (0) no; (1) yes (Figure 5.6: **bpp**). Compare to Alexander's (1992) character 14.
37. *Apex of hind femur bearing a narrow auriculate or spatulate process on the anterior (inner) face* (Alexander 1992: Fig.15b): (0) no; (1) yes. State 1 unites *Aphilanthops frigidus* and *A. subfrigidus*. While Alexander incorporated this as one state of a multi-state femoral apex character, the spatulate process is distinct

Figure 5.6. The hindfemoral apex in A. *Cerceris mimica*, B. *Pseudoscolia dewitzi*. *bpp* = *basoposterior plate* of the hind tibia (character 36, state 1); *avp* = *apicoventral plate* of the hindfemoral apex (character 38; state 1); *adp* = *apicodorsal plate* of the hindfemoral apex (not present in B).



from the femoral truncation found in *Eucerceris* and *Cerceris*, itself distinct from that of *Pseudoscolia* (Alexander treated the form of the cercerine femur and that of the pseudoscoliine femur as putatively homologous). Characters 37-39 here represent a deconstruction of Alexander's (1992) character 15.

38. Apex of hind femur bearing a greatly expanded *apicoventral* plate, surrounded at least in part by a carinate ridge: (0) no; (1) yes (Figure 5.6). Much has been made of the "truncate hindfemur" shared between *Pseudoscolia* and the Cercerini. However, close inspection reveals that these two taxa have truncate hindfemora in distinctive ways: while the *Pseudoscolia* truncation is composed of a single apicoventral plate (Alexander 1992: Fig. 16b), the cercerine apex is composed of the apicoventral plate *and* an apicodorsal plate (see character 39, below; Alexander 1992: Fig. 15b).
39. Apex of hind femur bearing a greatly expanded *apicodorsal* plate, surrounded at least in part by a carinate ridge: (0) no (Figure 5.6B); (1) yes (Figure 5.6A). See discussion above.
40. Hindcoxae with a prominent posterior apicodorsal lobe: (0) no (Figure 5.7C); (1) yes (Figure 5.7D). State 1 is characteristic of the Cercerini.
41. Strong longitudinal serrations ("shark fins") arising on the dorsal surface of the hind tibia, between tibial spines: (0) absent (Figure 5.7A); (1) present. (Figure 5.7B) State 1 is characteristic of Cercerini and, like character 40, is most apparent in females.
42. Terga with transverse mesal depressions: (0) no (Figure 5.8B); (1) yes (Figure 5.8C). State 1 is diagnostic for the genus *Eucerceris*.
43. First metasomal segment: (0) sessile, more than 2/3 width of segment II; (1)

Figure 5.7. Selected leg characters: A. hind tibia normal (character 41, state 0), *Philanthus ventilabris*; B. hind tibia with *strong longitudinal serrations* (character 41, state 1), *Cerceris mimica*; C. hind coxae normal (character 40, state 0), *Philanthus gloriosus*; D. *hindcoxae with a prominent posterior apicodorsal lobe* (character 40, state 1), *Eucerceris tricolor*. Compare also the strongly truncate apex of the cercerine hindfemur (B), with its normal counterpart (A).

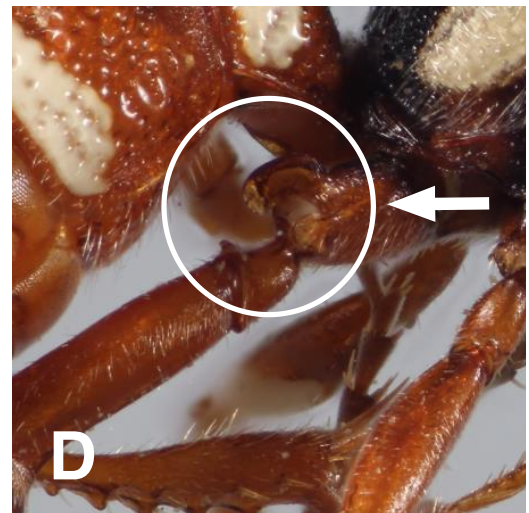
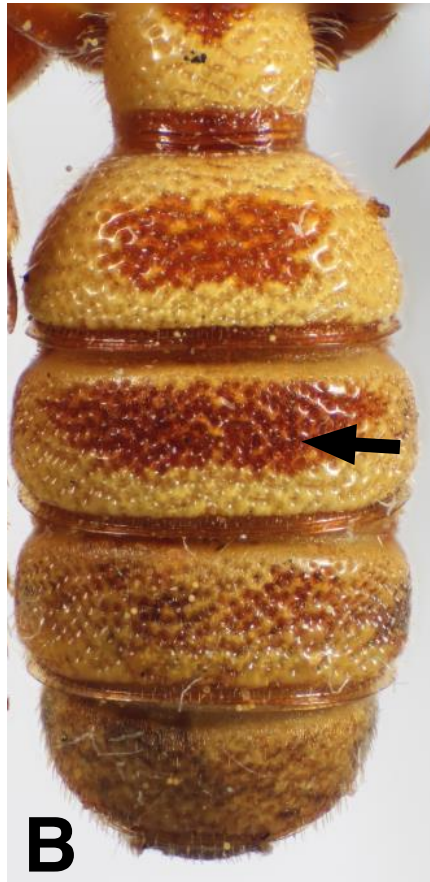


Figure 5.8. Selected metasomal characters in Cercerini: A. male pygidial plate, *Eucerceris superba* (arrow points to the *prominent, well-defined lateral denticles* [Character #47, state 1]); B. metasomal terga, *Cerceris intricata graphica* (without *transverse mesal depressions*; Character# 42, state 0); C. metasomal terga, *Eucerceris superba* (with *transverse mesal depressions*; Character #42, state 1).



somewhat to strongly pedunculate, less than or equal to 2/3 width of segment II, distinct; (2) narrowly petiolate and more than twice as long as broad (Figure 5.1g). Within the ingroup, state 1 is found throughout Cercerini and in some Old World *Philanthus*; state 2 is characteristic of *Trachypus*, although also present in a less extreme form in some *Cerceris* species (e.g., *C. marginula*). .

44. Female pygidial plate: (0) absent or greatly reduced; (1) present. Compare with Alexander's (1992) character 22.
45. Apical abdominal segments of the female metanotum modified into an "ant-clamp" (Evans 1962a: Fig. 3, 4b,c,d): (0) no; (1) yes. State 1 is a unique, unreversed synapomorphy for *Clypeadon*.
46. Female pygidial area surround by a ring of stiff fibriae: (0) no; (1) yes. State 1 is characteristic of Cercerini. While *Pseudoscolia* is here coded as state 1, the hairs are considerably less pronounced (Prentice and Pulawski 2004: Fig. 4c).
47. Male with pygidial plate ending in two prominent, well-defined lateral denticles (Figure 5.8A): (0) no; (1) yes. State 1 is diagnostic of *Eucerceris*.
48. Prominent median longitudinal carina on sternum I: (0) absent; (1) present.
49. Female sternum V distinctly concave: (0) no; (1) yes. State 1 is characteristic of buprestid- and bee-hunting *Cerceris* species.
50. Female sternum VI *bifid or deeply notched*: (0) no; (1) yes. Compare with Alexander's (1992) character 23.
51. Male sternum II *with a bulging, basal, sometimes platform-like swelling which is usually delimited by a curving or angular transverse declivity* (Bohart and Grissell, 1975): (0) no; (1) yes. State 1 is characteristic of some *Cerceris* species.

52. *Furcula of sting apparatus*: (0) all three arms more or less equal; (1) basal arms much shorter than median arm. [Alexander 1992: character 25] *N.B. – Characters 51-65 were coded primarily from the literature.*
53. *Sting shaft*: (0) gently downcurved, evenly tapering in lateral view; (1) very sharply downcurved, with slight swelling beyond which shaft is abruptly narrowed; (2) very long and slender, evenly tapering. [Alexander 1992: character 26]. .
54. *Digitus of volsella*: (0) more or less cylindrical; (1) distinctly flattened and bladelike. [Alexander 1992: character 27]
55. *Volsella*: (0) with articulating digitus and cuspis; (1) digitus and cuspis fused into a single cylindrical column. [Alexander 1992: character 28]
56. *Lacinial area of larval maxilla*: without a digitiform process; (1) with a distinct digitiform process. [Alexander 1992: character 1]
57. Larval mandibles: (0) bidentate; (1) tridentate; (2) quadridentate.
58. Larval spinnerets: (0) shorter than or not much longer than labial palpi; (1) greatly surpassing the labial palpi (Evans 1957).
59. Larval provisions: (0) pollen; (1) thrips (2) bees and wasps; (3) ants; (4) beetles. [Modified from Alexander's (1992) character 34].
60. Ant prey: (0) alate *Formica* gynes; (1) *Pogonomyrmex* workers. This character is only applicable within Aphilanthopini.
61. Beetle prey by family: (0) Curculionidae; (1) Tenebrionidae; (2) Buprestidae; (3) Chrysomelidae; (4) Phalacridae. This character accounts for changes occurring within the Cercerini *after* the evolution of coleopterophagy, and is inapplicable in non-coleopterous taxa.

62. Nest temporarily closed while foraging: (0) no; (1) yes. Compare with Alexander's (1992) character 35.
63. Nest entrance with tumulus: (0) no; (1) yes. Compare Alexander's (1992) character 36.
64. *Burrow orientation*: (0) *oblique*; (1) *vertical*. [Alexander, 1992: character 37]
65. Prey flown to the nest: (0) mainly held by the mandibles, but with partial support from the middle legs (Evans's [1962b] *mandibular mechanism type 3*); (1) held entirely by the middle legs, and without mandibular support (Evans's [1962b] *pedal mechanism type 1*); (2) by a specialized appendage on the end of the metasoma (Evans's [1962b] *abdominal mechanism type 2*)
66. Endosymbiotic antennal streptomycetes: (0) absent; (1) present. In a series of recent papers (2006; 2010; 2012; 2014), Kaltenpoth et al. explored the endosymbiotic relationship between members of the tribe Philanthini and a clade of *Streptomyces* bacteria that live in the adult wasps' antennomeres. Neither *Pseudoscolia* nor *Eucerceris* have been investigated for bacterial presence/absence, and members of those genera are scored as unknown in the matrix.

Phylogenetic results

Both the equal (Figure 5.9) and implied weights (Figure 5.10) analyses of the morphology/behavior dataset returned topologies that were largely unresolved at the intrageneric level. The trees produced by the total evidence analyses (Figures 5.11-5.12) were, on the other hand, both highly resolved and relatively well supported (equal weights average group support = 57.6; implied weights average group support = 64.6). The rest of the results discussed here refer to the total evidence analyses only.

Figure 5.9. Strict consensus of 100,000 most parsimonious trees (131 steps; CI = 0.618; RI = 0.961) generated by an equal weights analysis of the morphology + behavior dataset. Symmetric resampling support values (reported as GC scores and based on 1,000 pseudoreplicates) are shown just below and to the left of each resolved node. Average group support = 18.3. Note the well supported position of *Pseudoscobia*, sister to the Cercerini.

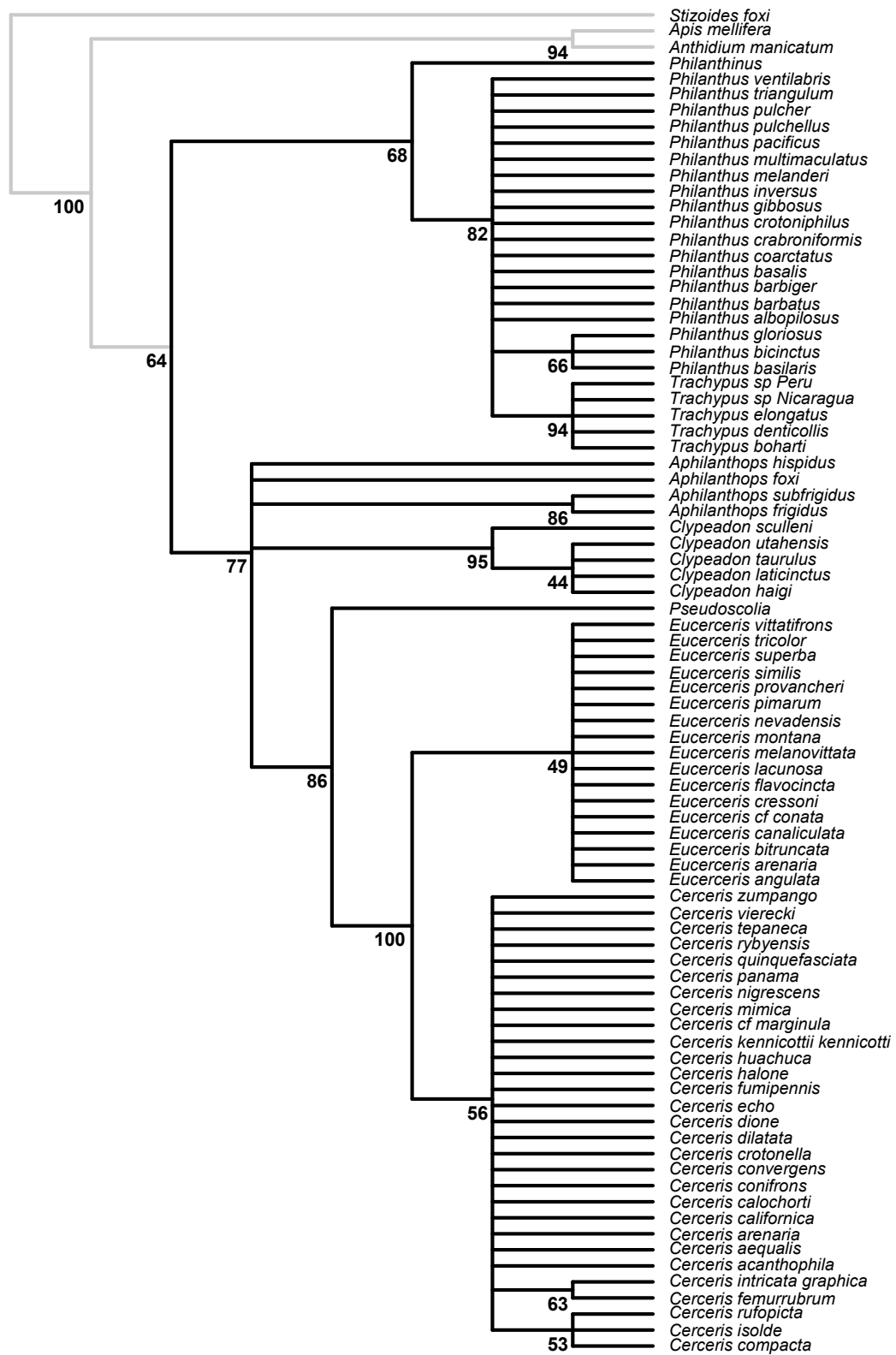


Figure 5.10. Strict consensus of 100,000 most parsimonious trees (fit = 9.625) generated by an implied weights ($K = 3$) analysis of the morphology + behavior dataset. Symmetric resampling support values (reported as GC scores and based on 1,000 pseudoreplicates) are shown just below and to the left of each node. Average group support = 19.7. Note the well supported position of *Pseudoscobia*, sister to the Cercerini

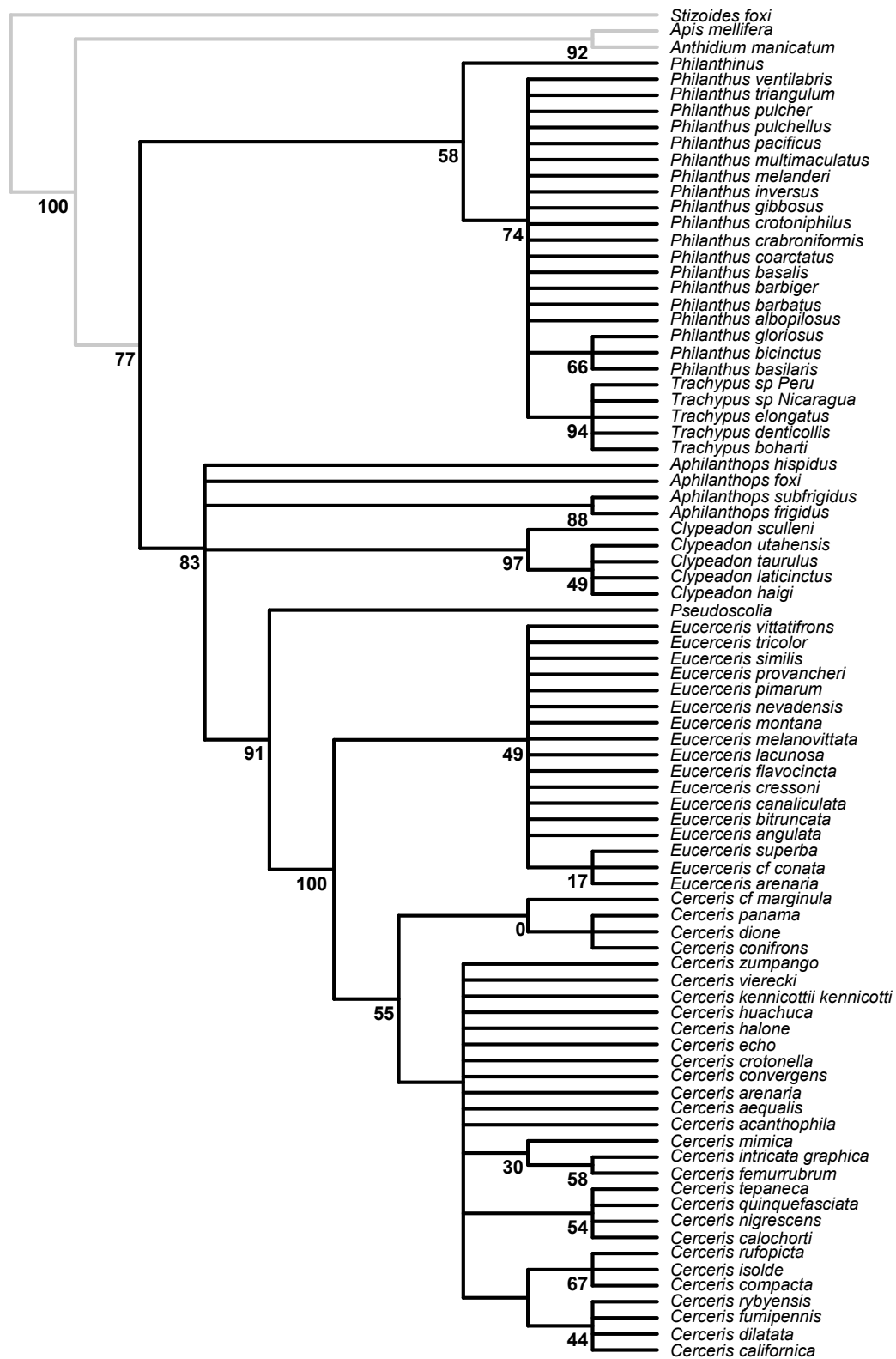
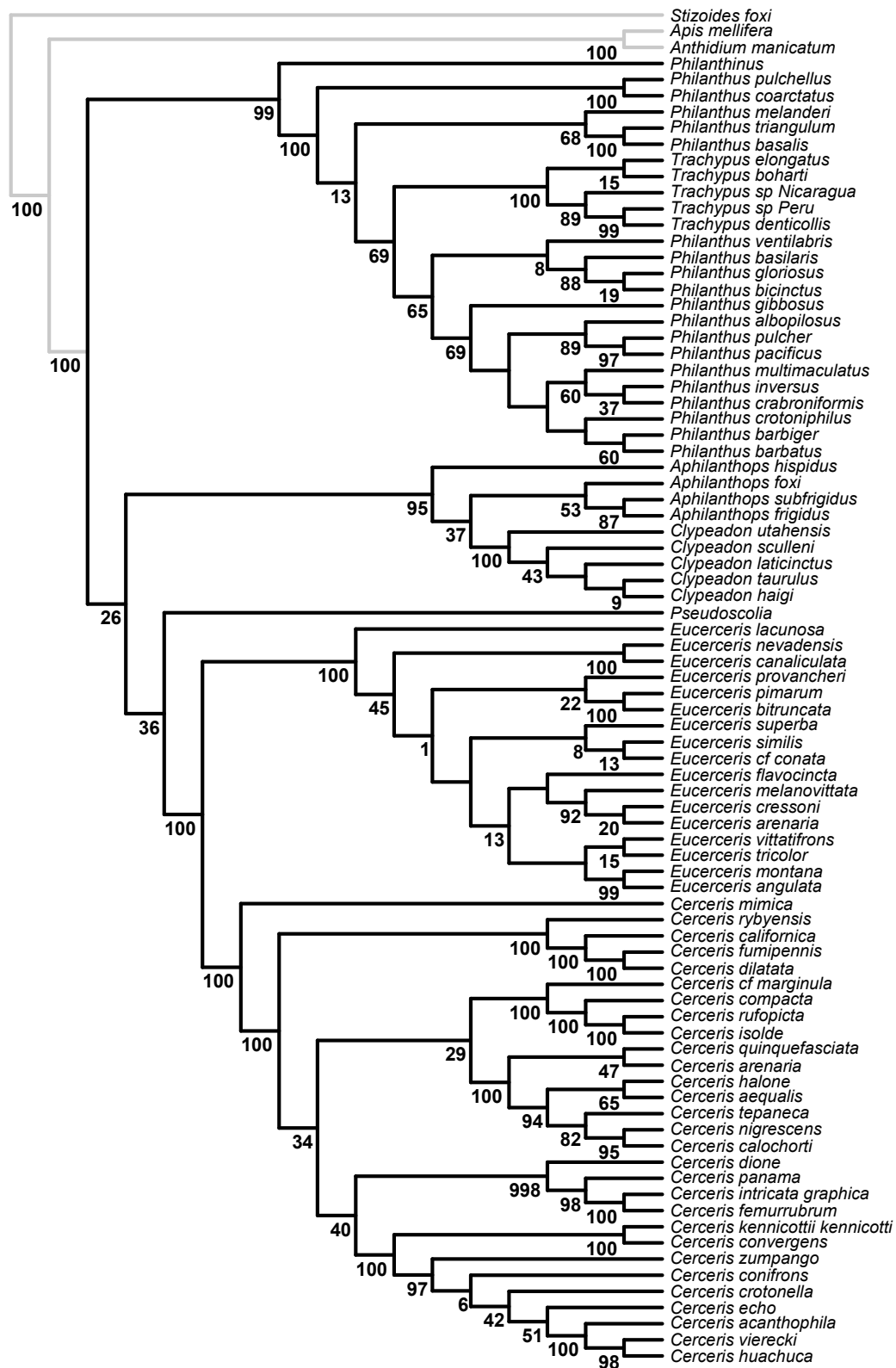


Figure 5.11. Strict consensus of 360 most parsimonious trees (6,666 steps; CI = 0.376; RI = 0.817) generated by an equal weights analysis of the total evidence dataset. Symmetric resampling support values (reported as GC scores and based on 1,000 pseudoreplicates) are shown just below and to the left of each node. Average group support = 57.6.



Figure 5.12. The preferred phylogenetic hypothesis for philanthine relationships.
The single most parsimonious tree (score = 444.08106) generated by an implied weights ($K = 3$) analysis of the total evidence dataset. Symmetric resampling support values (reported as GC scores and based on 1,000 pseudoreplicates) are shown just below and to the left of each node. Average group support = 64.6. Note the position of *Pseudoscobia*, sister to the Cercerini.



While the equal (Figure 5.11) and implied (Figure 5.12) weighting schemes produced broadly similar topologies, there were critical differences in terms of *Pseudoscobia*'s placement, and with respect to the monophyletic status of the genus *Aphilanthops*. While the equal weights analysis placed *Pseudoscobia* at the base of the philanthine tree, the implied weights analysis placed it sister to Cercerini; meanwhile, the implied weights tree returned *Aphilanthops* as a paraphyletic group with respect to *Clypeadon*, with the tribal topology: *A. hispidus* + (all other *Aphilanthops* + *Clypeadon*).

Ancestral state reconstructions for two prey choice characters (59 and 61) are shown in Figures 5.13-5.14.

Discussion

The current study represents a continuation and natural extension of the analyses first carried out in Chapter IV of this dissertation. Whereas that study analyzed a strictly sequence-based dataset, this chapter utilized an additional 66 newly coded morphological and behavioral characters to produce the most extensive phylogenetic analysis of philanthine relationships to date.

For the most part, the results presented here agree with the maximum parsimony topologies returned by the previous molecular analysis: just as before, the tribes Philanthini, Cercerini, and Aphilanthopini are all strongly supported monophyletic clades; the genera *Eucerceris*, *Cerceris*, *Trachypus*, and *Clypeadon* appear as strongly supported natural groups; and *Philanthus* is paraphyletic with respect to *Trachypus*, which is sister to a clade composed of all New World *Philanthus* species.

Pseudoscoliini + *Cercerini*

The most important difference between the preferred tree (i.e., the fully resolved

Figure 5.13. Most parsimonious reconstruction of character 59 ('larval provisions'), as optimized in PAUP* 4.0. Black = hymenopterous prey; blue = ant prey; red = beetle prey; gray = unknown. The genus *Pseudoscolia* is polymorphic for this character, with fragmentary reports of both hymenopterophagy and myrmecophagy.



Figure 5.14. Most parsimonious reconstruction of character 61 ('beetle prey by family'), as optimized in PAUP* 4.0. Gray = unknown; dark green = Curculionidae; gold = Burprestidae; blue = Chrysomelidae; light green = Tenebrionidae; purple = Phalacridae; red = Hymenoptera



cladogram produced by the total evidence, implied weights analysis; Figure 5.12), and the the topologies shown in Chapter IV, concerns the placement of the pseudoscoliine lineage. Here, *Pseudoscolia* is returned to its traditional sister group relationship with Cercerini, with the philanthine tribes arranged thus: Philanthini + (Aphilanthopini + (Pseudoscoliini + Cercerini)). While this topology accords well with previously published trees based on morphological evidence (Prentice 1998), support values for Aphilanthopini + (Pseudoscoliini + Cercerini) and for Pseudoscoliini + Cercerini remain relatively low (Figure 5.12). The results of this study – much like those presented in Chapter IV – thus call for an agnostic stance regarding basal philanthine tribal relationships, pending new analyses based on greatly expanded taxonomic and character sampling.

The evolution of myrmecophagy in Aphilanthopini

The equal weights total evidence tree shown here (Figure 5.11) presents a difficult scenario for the evolution of prey preferences within Aphilanthopini, with either a single switch to ant prey at the base of the tribe, followed by a secondary loss on the branch leading to *A. hispidus*, or else convergent evolution of myrmecophagy in the branches leading to *Clypeadon* and to *Aphilanthops frigidus* + *A. subfrigidus*. Myrmecophagy is relatively rare among apoid wasps, with only a few other instances described in the Crabronini (in the genera *Encopognathus*, *Lindenius*, and *Trachelioides*; Bohart and Menke 1976) and in one species of *Pseudoscolia* (*simplicornis*, a predator of *Cataglyphis* ants; Kazenas 2001). That this habit would evolve twice in the Aphilanthopini *and* that that development would be accompanied by a high degree of genus or species-level specificity (alate *Formica* gynes in *A. frigidus* and *A. subfrigidus*; *Pogonomyrmex*

workers in *Clypeadon*) seems highly unlikely. On the other hand, the return to a generalist hymenopterophagous habit on the branch leading to *A. hispidus* might present an equally challenging set of evolutionary transformations and reversals (although see *Cerceris rybyensis*, below).

The preferred tree (Figure 5.12) presents a more intuitively appealing scenario (formalized in Figure 5.13). In this reconstruction, a broadly hymenopterophagous preference is primitive for the Philanthinae and is still found in *A. hispidus*, the basal lineage of the Aphilanthopini. Myrmecophagy evolves just once, on the branch leading to all other *Aphilanthops* + *Clypeadon*, with subsequent prey specializations (on *Formica* queens and *Pogonomyrmex* workers, respectively) each occurring just once. Given this scenario, we might predict some form of myrmecophagy will be found in the poorly known species *A. foxi* (Figure 5.13), a rarely collected wasp known only from oases in California's Coachella Valley (Bohart and Grissell 1975). As such, descriptive natural history studies of *A. foxi* should be a high priority for workers interested in understanding more about aphilanthopine prey preference evolution.

The diversification of coleopterophagy in Cercerini

The current study also presents the first analytically rigorous reconstruction of coleopterous prey preference evolution within the tribe Cercerini (Figure 5.14). Contrary to earlier speculation (see, e.g., Evans and O'Neill 1988), hymenopterophagy is not ancestral in the clade; rather, the use of weevil prey (Curculionidae; dark green in Figure 5.14) appears to be the primitive state for the group. All ethologically described species of *Eucerceris* persist in this habit, as do basal *Cerceris* species such as *C. mimica*. Members of the *C. nigrescens* group, the palearctic species *C. arenaria* and *C.*

quinquefasciata, and the nearctic *C. conifrons* (among many other species not included here) are also weevil hunters.

As reconstructed on the most parsimonious topology, a preference for chrysomelid prey (blue in Figure 5.14) has evolved twice within the tribe, once in the ancestor of the *C. compacta* species group, and once again within Bohart and Grissell's *C. finitima* group. *C. echo* (purple) represents an unusual case of specialization on phalacrid beetles (Evans 1971; Evans and Rubink 1978; Hook 1987). Meanwhile, the use of tenebrionid prey (light green) unites the two members of the *C. femurrubrum* group.

Finally, it is worth noting here that the actual origins of hymenopterophagy among Eurasian *Cerceris* lineages (here represented by *C. rybyensis*) are nested deep within the coleopterous habit, on a lineage most closely related to a clade of buprestid specialists, represented here by the New World species *dilatata*, *californica*, and *fumipennis*.

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CHAPTER VI

THE HOUSE PLAN AS *BAUPLAN* PART I: A PRELIMINARY INVESTIGATION OF NEST EVOLUTION IN SPHECID WASPS (APOIDEA: SPHECIDAE *S. STR.*)

Abstract

Wasps of the family Sphecidae *sensu stricto* (i.e., "thread-waisted" wasps) are well known for their diverse approaches to nest construction, maintenance, and provisioning; however, the origins of this behavioral diversity remain obscured by phylogenetic uncertainty. As a preliminary contribution to the comparative study of sphecid nesting behavior, I here present the most taxonomically comprehensive molecular phylogeny for Sphecidae *s. str.* to date, with nucleotide data from three protein-coding loci (COI, EF-1 α F2, LW rhodopsin) and 70 ingroup terminals (including representatives of 61 species and all but one of the family's 19 genera). Maximum parsimony analysis reinforces previous suggestions regarding paraphyly of the subfamily Sphecinae with respect to Ammophilinae: The sphecine tribe Prionychini is sister to the ammophiline clade. The tribe Sceliphhrini is also paraphyletic with respect to Podiini, with *Chalybion* more closely related to the podiine clade. The overall topology for the family is: (Chloriontinae + Sceliphhrinae) + (Stangeellinae + (Sphecini + (Prionychini + Ammophilinae))). In addition to the molecular topology, I also present a largely unresolved maximum parsimony tree based on 16 newly developed behavioral characters coded from the literature. The current work is the first installment in a series of studies dedicated to the evolution of sphecid nesting behavior, and also represents the first molecular analysis of family relationships to include representatives of two neotropical podiine genera: *Trigonopsis* and the rarely collected *Dynatus*.

Introduction

While the so-called "thread-waisted" wasps of the family Sphecidae *sensu stricto* (Hymenoptera: Apoidea; ~725 spp, Pulawski 2014) are united by a number of unambiguous morphological traits (Bohart and Menke 1976), their diverse approaches to nest construction, maintenance, and provisioning present an impressive array of evolutionary strategies. Indeed, in just three of the more familiar North American species (the great golden digger, *Sphex ichneumoneus*; the grass-carrying wasp, *Isodontia mexicana*; and the common black-and-yellow mud-dauber, *Sceliphron caementarium*), we find nearly all of the major nest types described within apoid wasps: While the first species excavates branching tunnels in loose soil (Brockmann 1979) and the second fills abandoned wood cavities with a dried grass lining (O'Neill and O'Neill 2009), the third takes an entirely different approach, building fully exposed "adobe" mud nests on the sides of buildings and exposed rock outcroppings (Bohart and Menke 1976).

Within the family as a whole, the range of imported construction materials includes plant resins (in some Podiini), mud (in Scelphrini and Podiini), grasses and other fibrous plant materials (*Chilosphex*, *Isodontia*), pebbles and debris (*Hoplammophila*, some *Ammophila* species), and even uric acid scavenged from bat feces (some *Chalybion* species; Gess and Gess 1980). Nests may consist of single brood cells bearing single offspring, or they may contain multiple cells, in series, in parallel, or in some other spatial arrangement. Some *Isodontia* (e.g., *auripes*; see Krombein 1970) even create a single large, shared brood chamber containing multiple larvae, an apparently unique phenotype within apoid wasps (Bohart and Menke 1976). In addition, various other traits related to nesting and provisioning behavior (e.g., prey preferences, the presence or

absence of temporary nest closures, details of prey carriage, and mass versus progressive provisioning) vary widely throughout the group (Bohart and Menke 1976).

While the family's nesting behaviors have been studied extensively from a descriptive standpoint (see **References**, below), few studies have attempted to devise explanatory frameworks (either functional or historical) for the group's behavioral diversity; the origins of this *extended phenotypic* diversity (Dawkins 1982) thus continue to represent what Jane Brockmann (1980) has called "[one] of the mysteries of sphecoid wasp evolution: How have such extremely diverse nesting patterns evolved within very closely related groups?"

The current study presents the results of a preliminary attempt to frame this question through two separate, but complementary analytical approaches: 1.) the expansion and phylogenetic analysis of a three-locus molecular dataset for Sphecidae *s. str.*, and 2.) a phylogenetic analysis of behavioral characters sampled from the sphecoid nesting behavior literature. As a prelude to a more extensive planned study of nest evolution in the Sphecidae, these findings provide a basic phylogenetic backbone for future work.

Materials and methods

Molecular characters and taxonomic sample

The molecular dataset analyzed here represents an expansion of a previously published matrix (Field et al. 2011) focused largely on evolutionary relationships within the sphecoid subfamily Ammophilinae. In order to take full advantage of those pre-existing data, I here sequenced 39 additional ingroup taxa (Table 6.1, in red) for the same three protein-coding genes (mitochondrial cytochrome oxidase I [COI], nuclear

elongation factor 1-alpha [EF-1 α F2], and nuclear long-wavelength rhodopsin [LW rho]) used in that study. Between these newly generated sequences and previously published data downloaded from GenBank, the final taxonomic sample included 70 ingroup terminals, representing 61 species and 18 genera of sphecid wasp, as well as 10 outgroup terminals drawn from across Apoidea (Table 6.1). The final data matrix also included the first phylogenetic sequence data for two podiine genera, *Trigonopsis* and the rarely collected neotropical *Dynatus*. The only sphecid genus not represented in the current molecular sample was the Old World sphecine taxon *Chilosphex*.

Sequence acquisition and alignment

Total genomic DNA was extracted from hind- and mid-leg muscle tissues using the DNeasy Blood & Tissue Kit (Qiagen) and amplified for the three protein-coding loci detailed above using the polymerase chain reaction (PCR). Primer pairs and primer routines were the same as those in Field et al. (2011). Amplified PCR products were purified using the Agencourt AMPure XP system (Beckman Coulter) and cycle-sequenced using the BigDye 3.1 Terminator Reaction Kit on an ABI 3730xl DNA analyzer sequencing core (Applied Biosystems) located at the American Museum of Natural History's Sackler Institute for Comparative Genomics.

All sequences were aligned using the translation alignment algorithm implemented in Geneious version 6.0.5 (BioMatters). Intron regions in both the EF-1 α F2 and LW rho sequences were identified by comparison with *Sceliphron caementarium* reference sequences (GenBank accession numbers: JF927440 and JF927398, respectively), and removed prior to phylogenetic analysis.

The final aligned sequences were concatenated into a single TNT file using

Table 6.1. Taxonomic sample used in the molecular analysis. Previously published GenBank sequences are listed in **bold**. Newly added taxa are highlighted in **red**.

Taxon	Collection location	COI (993 bp)	EF-1 α (750 bp)	LW rho (489 bp)
AMMOPHILINAE				
<i>Ammophila aberti</i> Haldeman	–	JF927311	JF927402	JF927360
<i>Ammophila azteca</i> Cameron	–	JF927312	JF927403	JF927361
<i>Ammophila ferrugineipes</i> Lepeletier	–	JF927317	JF927408	JF927366
<i>Ammophila laeviculis</i> Ed. André	–	JF927321	JF927412	JF927370
<i>Ammophila pictipennis</i> Walsh	–	JF927325	JF927416	JF927374
<i>Ammophila sabulosa</i> (Linnaeus)	–	JF927329	JF927418	JF927377
<i>Ammophila urnaria</i> Dahlbom	–	JF927332	JF927421	JF927380
<i>Ammophila vulcania</i> du Buysson	–	JF927333	JF927422	JF927381
<i>Ammophila wrightii</i> (Cresson)	–	JF927334	JF927423	JF927382
<i>Eremnophila aureonotata</i> (Cameron)	–	JF927337	JF927426	JF927385
<i>Eremnophila binodis</i> (Fabricius)	–	JF927338	JF927427	JF927386
<i>Eremochares dives</i> (Brullé)	–	JF927339	JF927428	JF927387
<i>Hoplammophila armata</i> (Illiger)	–	JF927340	JF927429	JF927388
<i>Hoplammophila clypeata</i> (Mocsáry)	–	JF927341	JF927430	JF927389
<i>Parapsammophila herero</i> (Arnold)	–	JF927343	–	–
<i>Parapsammophila turanica</i> F. Morawitz	–	JF927344	JF927432	–
<i>Podalonia affinis</i> (W. Kirby)	–	JF927346	JF927434	JF927392
<i>Podalonia hirsuta</i> (Scopoli)	–	JF927348	JF927435	JF927393
<i>Podalonia melaena</i> (Murray)	–	JF927351	JF927436	JF927394
<i>Podalonia valida</i> (Cresson)	–	JF927353	JF927437	JF927395
<i>Podalonia tydei</i> (Le Guillou)		xxxxxxx	xxxxxxx	xxxxxxx
<i>Podalonia</i> sp.		xxxxxxx	xxxxxxx	–
CHLORIONTINAE				
<i>Chlorion aerarium</i> Patton	–	JF927336	JF927425	JF927384
<i>Chlorion funereum</i> Gribodo	Oman	xxxxxxx	xxxxxxx	xxxxxxx
<i>Chlorion lobatum</i> (Fabricius)	Malaysia	xxxxxxx	xxxxxxx	xxxxxxx
<i>Chlorion lobatum</i> (Fabricius)	Vietnam	xxxxxxx	–	xxxxxxx
<i>Chlorion strandi</i> Willink	Argentina	xxxxxxx	xxxxxxx	xxxxxxx
SCELIPHRINAE				
Podiini				
<i>Dynatus burmeisteri</i> (Burmeister)	Argentina	xxxxxxx	–	–
<i>Penepodium</i> sp.	–	JF927345	JF927433	JF927391
<i>Podium rufipes</i> Fabricius	–	JF927354	JF927438	JF927396
<i>Trigonopsis rufiventris</i> (Fabricius)	Ecuador	xxxxxxx	xxxxxxx	xxxxxxx

Table 6.1 continued.

Taxon	Collection location	COI (993 bp)	EF-1 α (750 bp)	LW rho (489 bp)
<i>Trigonopsis cameronii</i> (Kohl)	Ecuador	xxxxxxx	xxxxxxx	xxxxxxx
Sceliphriini				
<i>Chalybion californicum</i> (de Saussure)	West Virginia	xxxxxxx	xxxxxxx	EF013561
<i>Chalybion zimmermanni aztecum</i> (de Saussure)	–	JF927335	JF927424	JF927383
<i>Sceliphron assimile</i> 1 (Dahlbom)	Br. Virgin Islands	xxxxxxx	xxxxxxx	xxxxxxx
<i>Sceliphron assimile</i> 2 (Dahlbom)	Nicaragua	xxxxxxx	xxxxxxx	xxxxxxx
<i>Sceliphron caementarium</i> 1 (Drury)	Arizona	xxxxxxx	–	xxxxxxx
<i>Sceliphron caementarium</i> 2 (Drury)	–	JF927356	JF927440	JF927398
<i>Sceliphron funestum</i> Kohl	Greece	xxxxxxx	xxxxxxx	xxxxxxx
<i>Sceliphron spirifex</i> (Linnaeus)	Greece	xxxxxxx	–	xxxxxxx
SPHECINAE				
Prionychini				
<i>Palmodes californicus</i> R. Bohart and Menke	California	xxxxxxx	xxxxxxx	xxxxxxx
<i>Prionyx atratus</i> (Lepeletier)		xxxxxxx	xxxxxxx	xxxxxxx
<i>Prionyx crudelis</i> (F. Smith)	Oman	xxxxxxx	xxxxxxx	xxxxxxx
<i>Prionyx globosus</i> 1 (F. Smith)	Australia	xxxxxxx	xxxxxxx	xxxxxxx
<i>Prionyx globosus</i> 2 (F. Smith)	Australia	xxxxxxx	–	xxxxxxx
<i>Prionyx kirbii</i> (Vander Linden)	–	JF927355	JF927439	JF927397
<i>Prionyx parkeri</i> Bohart and Menke	Arizona	xxxxxxx	xxxxxxx	xxxxxxx
<i>Prionyx saevus</i> (F. Smith)	Australia	xxxxxxx	xxxxxxx	–
<i>Prionyx subatratus</i> 1 (R. Bohart)	Arizona	–	xxxxxxx	xxxxxxx
<i>Prionyx subatratus</i> 2 (R. Bohart)	–	–	JN374861	JN374887
<i>Prionyx</i> sp.	Ecuador	xxxxxxx	xxxxxxx	xxxxxxx
Sphecini				
<i>Isodontia apicalis</i> (F. Smith)	Louisiana	xxxxxxx	xxxxxxx	xxxxxxx
<i>Isodontia auripes</i> (Fernald)	West Virginia	xxxxxxx	xxxxxxx	xxxxxxx
<i>Isodontia elegans</i> (F. Smith)	New Mexico	xxxxxxx	xxxxxxx	xxxxxxx
<i>Isodontia mexicana</i> (de Saussure)		JF927342	JF927431	JF927390
<i>Isodontia philadelphica</i> (Lepeletier de	California	xxxxxxx	–	xxxxxxx
<i>Sphex argentinus</i> Taschenberg	Argentina	xxxxxxx	xxxxxxx	xxxxxxx
<i>Sphex ashmeadi</i> 1 (Fernald)	–	xxxxxxx	xxxxxxx	xxxxxxx
<i>Sphex ashmeadi</i> 2 (Fernald)	–	xxxxxxx	xxxxxxx	xxxxxxx
<i>Sphex ashmeadi</i> 3 (Fernald)	–	–	JN374863	JN374899

Table 6.1 continued.

Taxon	Collection location	COI (993 bp)	EF-1 α (750 bp)	LW rho (489 bp)
<i>Sphex ermineus</i> Kohl	Australia	xxxxxxx	xxxxxxx	xxxxxxx
<i>Sphex ichneumoneus</i> 1 (Linnaeus)	—	xxxxxxx	xxxxxxx	xxxxxxx
<i>Sphex ichneumoneus</i> 2 (Linnaeus)	—	JF927357	JF927441	JF927397
<i>Sphex jamaicensis</i> (Drury)	Florida	xxxxxxx	xxxxxxx	xxxxxxx
<i>Sphex lucae</i> 1 de Saussure	Arizona	xxxxxxx	xxxxxxx	xxxxxxx
<i>Sphex lucae</i> 2 de Saussure	—	xxxxxxx	xxxxxxx	xxxxxxx
<i>Sphex sericeus</i> (Fabricius)	India	xxxxxxx	xxxxxxx	xxxxxxx
<i>Sphex subtruncatus</i> Dahlbom	India	xxxxxxx	xxxxxxx	xxxxxxx
<i>Sphex</i> sp.	Papua New Guinea	xxxxxxx	xxxxxxx	xxxxxxx
STANGEELINAE				
<i>Stangeella cyaniventris</i> (Guérin-Méneville)	—	JF927358	JF927442	JF927400
Outgroups				
<i>Ampulex compressa</i> (Fabricius)	—	GQ374639	GQ410718	JN374888
<i>Anacrabro ocellatus</i> Packard	—	—	AY585160	DQ116700
<i>Tachysphex yolo</i> Pulawski	—	—	AY585171	DQ116707
<i>Clypeadon utahensis</i> (Baker)	Arizona	xxxxxxx	xxxxxxx	—
<i>Philanthus pulcher</i> Dalla Torre	Colorado	xxxxxxx	xxxxxxx	—
<i>Lasioglossum athabascense</i> (Sandhouse)	—	—	AF435390	AF448867
<i>Anthidium manicatum</i> (Linnaeus)	Massachusetts	xxxxxxx	xxxxxxx	xxxxxxx
<i>Apis mellifera</i> Linnaeus	—	GU056175	AF015267	AF091732
<i>Hesperapis regularis</i> (Cresson)	—	GQ374630	AY585151	DQ116692
<i>Macropis nuda</i> (Provancher)	—	FJ582306	AY585155	DQ116686

SequenceMatrix version 1.7.8 (Vaidya et al. 2010). The final character matrix included 2,232 nucleotide basepair positions (COI: 993 bp; EF-1 α F2: 750 bp; LW rho: 489 bp), with gaps accounting for 0.00094% (missing data = 12.32%).

Behavioral characters and taxonomic sample

In order to investigate the phylogenetic potential of frequently reported sphecids nesting behaviors, I here coded 16 newly developed behavioral characters for the 65 ingroup and three outgroup taxa listed in Table 6.2. (All codings were taken directly from the literature; for source publications, see Table 6.2). While the characters used here by no means constitute a comprehensive set of variable nesting traits, they do represent a preliminary effort to accommodate both intrageneric behavioral variation and the inherent inconsistencies (in detail, comprehensiveness) found throughout the ethological literature.

For the complete behavioral character matrix, see Appendix D, Table S6.1.

Terminal mismatch and a compromise

Little overlap existed between the taxonomic sample available for molecular sequencing (Table 6.1) and the subset of sphecids wasp species for which behavioral characteristics have been adequately described in the literature. While the combined analysis of different data types represents a best practices approach to phylogenetic inference (Nixon and Carpenter 1996; Payne 2014), differential taxonomic sampling among datasets can cause serious problems for simultaneous analyses. Although various methods have been proposed for dealing with this "terminal mismatch" problem (e.g., *ambiguity coding*, *terminal fusion*, and *extrapolation*; see Nixon and Carpenter 1996), none proved suitable for the current study, where a.) intrageneric variation in both molecular and behavioral characters suggested potential phylogenetic utility, and b.) the

Table 6.2 Literature sources for behavioral character codings used here.

Taxon	Behavioral references
AMMOPHILINAE	
<i>Ammophila azteca</i> Cameron	Evans 1965, 1970
<i>Ammophila dysmica</i> Menke	Evans 1970; Rosenheim 1987
<i>Ammophila harti</i> (Fernald)	Hager and Kurczewski 1986
<i>Eremnophila aureonotata</i> (Cameron)	Evans 1959 (as <i>A. aureontata</i>)
<i>Eremnophila binodis</i> (Fabricius)	Buys 2009
<i>Eremnophila opulenta</i> (Guérin-Méneville)	Richards 1937
<i>Eremochares dives</i> (Brullé)	Kazenas 1970
<i>Hoplammophila aemulans</i> (Kohl)	Iwata 1938; Tsuneki 1963, 1968; reviewed in Bohart and Menke 1976
<i>Parapsammophila erythrocephala</i> (Fabricius)	Misra 1984 (as <i>A. erythrocephala</i>)
<i>Podalonia atriceps</i> (F. Smith)	Evans 1970 (as <i>P. communis</i>)
CHLORIONTINAE	
<i>Chlorion aerarium</i> Patton	Peckham and Kurczewski 1978; Lechner 2006, 2007
<i>Chlorion cyaneum</i> Dahlbom	Hook 2004
<i>Chlorion lobatum</i> (Fabricius)	Bohart and Menke 1976
<i>Chlorion maxillosum</i> (Poiret)	Bohart and Menke 1976
SCELIPHRINAE	
Podiini	
<i>Dynatus nigripes</i> (Westwood)	Kimsey 1978 (on <i>D. n. spinolae</i>); Cooper 1980
<i>Penepodium gorianum</i> (Lepeletier)	Garcia and Asis 1993 (as <i>P. goryanum</i>)
<i>Penepodium haematogastrum</i> (Spinola)	Williams 1928
<i>Penepodium latro</i> (Kohl)	Buys 2006a
<i>Penepodium luteipenne</i> (Fabricius)	Buys 2012
<i>Podium denticulatum</i> F. Smith	Richards 1937; Ribeiro and Garófalo 2010
<i>Podium fulvipes</i> Cresson	Genaro 1994
<i>Podium luctuosum</i> F. Smith	Krombein 1967
<i>Podium rufipes</i> Fabricius	Krombein 1967, 1970.
<i>Trigonopsis cameronii</i> (Kohl)	Williams 1928 (as <i>T. abdominale</i> var. <i>cameronii</i>); Richards 1937;
<i>Trigonopsis rufiventris</i> (Fabricius)	Williams 1928
Sceliphriini	
<i>Chalybion californicum</i> (de Saussure)	Bohart and Menke 1976
<i>Chalybion japonicum</i> (Gribodo)	Barthélemy 2011

Table 6.2 continued.

Taxon	Behavioral references
<i>Chalybion spinolae</i> (Lepeletier)	Gess et al 1982
<i>Chalybion tibiale</i> (Fabricius)	Gess and Gess 1980
<i>Chalybion zimmermani</i> Dahlbom	Ward 1970
<i>Sceliphron asiaticum</i> (Linnaeus)	Bohart and Menke 1976
<i>Sceliphron assimile</i> (Dahlbom)	Freeman and Johnston 1978
<i>Sceliphron caementarium</i> (Drury)	Bohart and Menke 1976
<i>Sceliphron curvatum</i> (F. Smith)	Gepp and Bregnant 1987
<i>Sceliphron spirifex</i> (Linnaeus)	Polidori et al. 2005
SPHECINAE	
Prionychini	
<i>Chilosphex argyrius</i>	Berland 1958; Gogala 1997
<i>Palmodes carbo</i> Bohart and Menke	Evans 1970
<i>Palmodes dimidiatus</i> (de Greer)	Krombein 1953 (as <i>Sphex daggyi</i>)
<i>Palmodes laeviventris</i> (Cresson)	Gwynne and Dodson 1983
<i>Palmodes occitanicus</i> (Lepeletier)	Bohart and Menke 1976
<i>Prionyx atratus</i> (Lepeletier)	Evans 1958 (as <i>Priononyx atratus</i>)
<i>Prionyx bifoveolatus</i> (Taschenberg)	Evans 1958 (as <i>Priononyx striatulus</i>)
<i>Prionyx chilensis</i> (Spinola)	Evans 1958 (as <i>Priononyx spinolae</i>)
<i>Prionyx crudelis</i> (F. Smith)	Bohart and Menke 1976
<i>Prionyx fervens</i> (Linnaeus)	Evans 1958 (as <i>Priononyx striatus</i>); Buys 2006b
<i>Prionyx kirbii</i> (Vander Linden)	Gess 1981; Tormos et al. 1994
<i>Prionyx parkeri</i> Bohart and Menke	Evans 1958 (as <i>Priononyx pubidorsus</i>)
<i>Prionyx subfuscatus</i> (Dahlbom)	Evans 1958 (as <i>Priononyx subfuscatus</i>)
<i>Prionyx thomae</i> (Fabricius)	Evans 1958 (as <i>Priononyx thomae</i>)
Sphecini	
<i>Isodontia auripes</i> (Fernald)	Lin 1966; Krombein 1967
<i>Isodontia costipennis</i> (Spinola)	Lin 1966; Soares et al. 2001, Tunes Buschini and Woiski 2006
<i>Isodontia diodon</i> (Kohl)	Barthélemy 2010
<i>Isodontia elegans</i> (F. Smith)	Krombein 1967; O'Neill and O'Neill 2007
<i>Isodontia mexicana</i> (de Saussure)	Lin 1966; Krombein 1967; O'Neill and O'Neill 2003, 2009
<i>Isodontia pelopoeiformis</i> (Dahlbom)	Gess and Gess 1982
<i>Isodontia simoni</i> (de Buysson 1898)	Gess and Gess 1982

Table 6.2 continued.

Taxon	Behavioral references
<i>Isodontia stanleyi</i> (Kohl)	Gess and Gess 1982
<i>Sphex argentatus</i> Fabricius	Belavadi and Mohanraj 1996
<i>Sphex cognatus</i> F. Smith	Ribi and Ribi 1979
<i>Sphex ichneumoneus</i> (Linnaeus)	Brockmann 1979
<i>Sphex jamaicensis</i> (Drury)	Genaro 1998
<i>Sphex lucae</i> de Saussure	Cazier and Mortenson 1965 (as <i>Fernaldina lucae</i>)
<i>Sphex opacus</i> Dahlbom	Buys 2005
<i>Sphex tepanecus</i> de Saussure	Gillaspy 1962
STANGEELINAE	
<i>Stangeella cyaniventris</i> (Guérin-Méneville)	Janvier 1926 (as <i>Sphex cyaniventris</i>)
Outgroups	
<i>Ampulex compressa</i> (Fabricius)	Bohart and Menke 1976; Keasar et al. 2006; Fox et al. 2009
<i>Ampulex denticollis</i> (Cameron)	Gess 1981
<i>Dolichurus corniculus</i> (Spinola)	Bonelli 1991

monophyletic status of some sphecid genera (e.g. *Prionyx*, *Podalonia*) was not initially clear.

As a compromise solution, I chose to forgo any simultaneous analysis of the molecular and behavioral data, and instead treated those datasets separately. While neither of the phylogenetic hypotheses generated can thus claim to account for the totality of available evidence, these preliminary analyses can serve as exploratory investigations of sphecid wasp relationships and of the phylogenetic utility of basic behavioral characters.

Phylogenetic analyses

Both the molecular and the behavioral phylogenetic analyses were carried out using the maximum parsimony optimization criterion as implemented in TNT version 1.1 (Goloboff et al. 2008). Holding 100,000 trees in memory, I conducted tree searches using 200 random addition sequences with TBR and holding 100 trees per replicate. Two hundred rounds of parsimony ratchet (upweight probability = 8%; downweight = 4%; Nixon 1999) were also used, along with 30 rounds of default drift. (Command structure: 'ratchet: iter 200 upfactor 8; mult = replic 200 hold 100 ratchet drift;').

For the molecular phylogeny, support values were determined using 1,000 pseudoreplicates of symmetric resampling (Goloboff et al. 2003) and were reported as GC values on the strict consensus topology.

Results

Annotated behavioral characters

Details of the 16 nest-related characters used in the behavioral phylogenetic analysis are given below, along with relevant notes and references.

01. Nests: (0) with all cells constructed inside pre-existing cavities; (1) built *de novo* via fossorial excavation; (2) built *de novo* as free-standing aerial structures. Non-additive. **Notes:** Taxa that construct nest cells within pre-existing cavities (such as the cracks between rocks, hollow plant stems, extant insect tunnels, or previously constructed mud nests), and which do so as an *obligate* behavioral pattern, are coded as 0. The derived states occur in those taxa that create their own nest cavities, either via soil excavation (1) or through the construction of free-standing mud nests (2). The outgroup taxa nest exclusively in pre-existing cavities.

The genus *Chlorion* demonstrates an interesting behavioral grade with respect to this character. While *C. maxillosum* is capable of digging, it does so only to find the burrow of its prey, which subsequently provides the nesting cavity. *C. lobatum* also employs the prey burrow. *C. aerarium* and *C. cyaneum*, on the other hand, dig their own nests *de novo* (see discussion in Bohart and Menke 1976). In the case of *C. aerarium*, the excavated nest may begin within and branch away from a pre-existing insect burrow (Peckham and Kurczewski 1978); however, since the bulk of the nest, including the sequential brood chambers, is dug by the wasp, this species is coded here as 1.

Some nest building taxa appear to adopt pre-existing cavities as a conditional *tactic* within a larger fossorial or mud-nesting *strategy* (Maynard Smith 1982). For example, Gess (1981) reported both nest digging and facultative cavity utilization in *Prionyx kirbii* in South Africa. Similarly, Buys (2012) reports facultative use of pre-existing, conspecific burrows by females of *Penepodium luteipenne*; he also reports brood parasitism in this species, and facultative cavity nesting may well be

part of the same emergent behavioral complex. Females of *Trigonopsis cameronii* may also re-use previously built nests (Eberhard 1974). In each of these cases, taxa are coded as either 1 or 2, and not as 0 (which requires an obligate cavity nesting *strategy*, and not an opportunistic cavity nesting *tactic*). Some cavity nesters routinely modify their pre-existing burrows before provisioning and oviposition (see, e.g., Kimsey 1978 and Cooper 1980, on *Dynatus nigripes*), but these species are nevertheless coded here as 0.

The behavior of the ammophiline genus *Parapsammophila* is known in the literature from a single fragmentary, and somewhat unusual, report (Misra 1984); however, the description accords well with suggestions of fossorial nesting as inferred from foretarsal morphology (Bohart and Menke 1976), and that species is here coded as 1.

02. Nest located or constructed: (0) only after prey obtained; (1) before. **Notes:** Bohart and Menke (1976) described this dichotomy as *prey-niche* versus *niche-prey*. In the outgroup, females invariably locate their prey first, only securing a suitable cavity nest after the target cockroach has been subdued. However, the primitive state is rare among sphecids, most notably occurring among certain Sphecinae. There is some controversy surrounding the status of this character in a few species, presumably due to a.) the difficulty of observing prey stashes in certain prey-niche taxa, and b.) the highly camouflaged temporary closures found in a few niche-prey wasps (see, e.g., Bohart and Menke 1976 on *Palmodes occitanicus*; Evans 1958 on *Prionyx fervens*).
03. Number of eggs laid per nest: (0) invariant, with no more than a single egg and

single brood cell per nest; (1) variable, often multicellular, with clearly demarcated brood cells, each containing a single egg; (2) variable, but with the divisions among brood cells weak, occasionally resulting in multiple eggs/larvae per cell; (3) invariably with a single large brood chamber containing multiple eggs/larvae.

Additive. **Notes:** The elaborate wording of this character is due to the special situation found in certain *Isodontia* (*Murrayella*) species, which either a.) lay more than one egg inside a single, expanded brood chamber (e.g., *I. auripes*), or else b.) build nests with such flimsy partitions that larvae routinely break through into adjoining cells (e.g., *I. mexicana*). A simple binary nest character (for instance, unicellular versus multicellular) fails to differentiate between the large brood chambers of *I. auripes* and the genuinely unicellular/unilarval nests found in the Ammophilini and other taxa.

In the case of *Chilosphex argyrius*, Gogala (1997) reported finding two egg-bearing prey sharing a single undifferentiated brood cell in Slovenia; previous reports by Berland (1958), however, described a unicellular/unilarval nest. Given the extremely limited nature of these descriptions, it seems prudent to provisionally code this species as 0 pending further study.

Frisch (1940) reported bi-cellular nests in *Ammophila urnaria*, but the vast majority of subsequent reports for this species and its congeners indicate strict unicellularity. In the outgroup, Bohart and Menke (1976) describe *Ampulex compressa* nests as occasionally multicellular; however, among other ampulicids unicellularity appears to be the rule.

04. Brood cells: (0) mass provisioned; (1) progressively provisioned. **Notes:** In most

sphecids, the full complement of prey is allocated shortly after or shortly before oviposition. This *mass provisioning* appears to be primitive within Apoidea, while *progressive provisioning*, the continual stocking of prey items throughout some period of larval development, remains relatively rare. A transition between the two strategies has been cited as a probable milestone on the path to sociality (Eberhard 1974; Field 2005; Wilson 2008).

05. Brood cells: (0) without added linings; (1) with linings, walls, and/or partitions built from imported dirt, pebbles, or debris; (2) built from imported plant materials; (3) built from imported mud. Non-additive. **Notes:** In the outgroup, cavity nests are largely unmodified except for the addition of a terminal plug (see below). Most unicellular/unilarval sphecids employ the same minimalist modification strategy. The closures of individual brood cell tunnels in multicellular, branching, fossorial nests (such as those found in *Sphex*) are functionally analogous to intercellular partitions and are treated as such here.
06. Terminal plug constructed from: (0) soil or debris, including pebbles; (1) plant materials; (2) mud. Non-additive. **Notes:** While the two are clearly related, the terminal plug of the nest is functionally, and often structurally, different from the partitions between brood cells. *Isodontia pelopoeformis*, for instance, combines strictly plant-based cell partitions with a mixed plant- and debris-based terminal plug (Gess and Gess 1982).

Eremnophila species (specifically *binodis*, *aureonotata*, *opulenta*) appear to use miscellaneous debris, some of which specifically derives from plant materials, in both the temporary and final closures of the nest (Evans 1959; Buys 1999).

However, given that this seems to be part of a "debris" pattern rather than a plant pattern, this character is here coded as 0. Both *Podium rufipes* and *Hoplammophila aemulans* use a combination of debris and "wet sand," which practically speaking works very much like a mud closure (Krombein 1967, 1970; Bohart and Menke 1976). The parasitoid-like *Chlorion* species do not close their nests, and this character is coded as non-applicable for these taxa.

07. When plant material used, that material is primarily composed of: (0) whole grasses and stems; (1) lighter, fibrous, pubescent materials such as trichomes, pappus, or bark fibers. **Notes:** This character is non-applicable outside *Isodontia* and *Chilosphex*.
08. Terminal plug: (0) without a sealing layer of added material different from the rest of the plug; (1) sealed with resin; (2) sealed with a white layer of uric acid derived from vertebrate feces. Non-additive. **Notes:** State 1 of this character is found only in certain species within the Podiini. In a fragmentary report, Cooper (1980) noted female *Penepodium albovillosum* "collecting resin from a sap flow in the forest near the *Dynatus* nest"; the character seems to break down along species group lines within *Penepodium*. State 2 is found in those *Chalybion* (*Chalybion*) species that use vertebrate feces – most often bird dropping – as a final seal. Gess and Gess (1980) poetically described such nests as "whited sepulchres."
09. Nest: (0) left open during prey search; (1) temporarily closed. **Notes:** While *Podium denticulatum* does temporarily close the nest at night, the nest is left open during active prey search (Ribeiro and Garófalo 1993), and the species is coded 0. The same is apparently true for *Trigonopsis* species, including the extensively

described *T. cameronii* (Eberhard 1974). For a discussion of this character in *Chlorion aerarium*, see Peckham and Kurczewski (1978).

10. Use of water in nest construction: (0) absent; (1) present, with water transported to nest site; (2) present, with pre-mixed mud transported to nest site. Non-additive.

Notes: Among some free-standing mud nesters and even among some fossorial taxa water is transported to the nest site during nest construction. Taxa that carry water are coded as 1; pre-mixed mud transport is coded as 2. The literature is unclear on how *Trigonopsis* species should be coded for this character; however, given the detail of Eberhard's (1974) contribution, I am inclined to trust his report over that of Williams (1928). Likewise, Richards (1937) contradicts Williams on *T. rufiventris*. This character is non-additive because the direction of mud transport evolution is unclear. *Hoplammophila aemulans* transports water in order to wet the sand on site (Bohart and Menke); *Podium rufipes* and *Eremochaeres dives* transport damp sand (Krombein 1967, 1970; Kazenas 1970).

11. Prey exclusively: (0) Blattaria; (1) Orthoptera; (2) immature holometabola, most often Lepidoptera; (3) Araneae; (4) Mantodea and Phasmatodea. Non-additive.

Notes: Among *Ampulex* species, all Podiini, certain *Isodontia*, and at least one *Chlorion* species, prey choice is restricted to cockroaches (Blattaria). Evans (1970) reports *Ammophila azteca* as taking the immatures of sawflies as well as lepidopteran caterpillars. The observation (Hook 2004) of cockroach prey in *Chlorion cyaneum* may be supported by an observation from Girard (1879, in Richards 1937) that *C. viridicoeruleum* shares this preference. *Eremnophila dives* represents an interesting departure from most other ammophilines in its use of

Orthoptera (Kazenas 1970). State 4 is found only in the monotypic genus *Stangeella* (Bohart and Menke 1976).

12. Oviposition occurs: (0) inside the nest; (1) outside. **Notes:** In the outgroup, oviposition occurs only after the prey has been brought inside the nest cavity. Once again, coding is difficult in *Chlorion maxillosum*, where egg-laying sometimes occurs outside the prey's burrow (ultimately the brood chamber). *Podium denticulatum* apparently uses both tactics (Ribeira and Garofalo 2010).
13. Paralyzed prey: (0) dragged backwards to the nest; (1) straddled and carried across the ground; (2) flown to the nest. Non-additive. **Notes:** In the outgroup, prey are dragged to the nest by the antennae (Bohart and Menke 1976). Intrageneric variation for this character in *Ammophila* is likely correlated with the weight of the preferred prey species.
14. During transport to the nest, prey held: (0) dorsum up; (1) venter up. **Notes:** In the outgroup, prey are dragged to the nest dorsum up. In most *Ammophilini*, the prey are carried ventral side up.
15. Prey: (0) pulled backward into cell; (1) carried into cell; (2) pushed into the cell. **Notes:** The method of moving a large prey item into the nest appears to be fixed at the generic, or in some cases, the tribal level. The most common method, a backwards pulling of the prey into the nest, is also found in the outgroup.
16. Amputation of prey appendages before deposition in brood cell: (0) present; (1) absent. **Notes:** Most reports do not address this phenomenon directly; however, when it does occur, the behavior appears to be conspicuous and noteworthy. Antennal amputation is present throughout the outgroup.

Behavioral results

Equal weights parsimony analysis of the behavioral characters alone returned a set of 30,276 equally parsimonious trees (cost = 72 steps), the strict consensus of which was largely unresolved (Figure 6.1). Of the 12 genera represented in the dataset by multiple terminals, only three (*Isodontia*, *Sceliphron*, and *Trigonopsis*) were returned as monophyletic groups. In addition, only one of the family's higher order taxa (the subfamily Sceliphrinae) was monophyletic.

Molecular results

In contrast to the behavioral results, the molecular analysis returned a set of 68 most parsimonious trees (cost = 7,994 steps), the strict consensus of which (Figure 6.2) was mostly resolved, relatively well-supported (average group support = 63.9), and more in keeping with both traditional classifications (Bohart and Menke 1976) and previously published analytical hypotheses of group relationships (Ohl 1996a,b; Lohrmann et al. 2008; Field et al. 2011). The family as a whole was returned as a well supported (GC = 100%) monophyletic group, with the subfamilies and tribes arranged thus: (Chloriontinae + Sceliphrinae) + (Stangeellinae + (Sphecini + (Prionychini + Ammophilinae))).

Within the subfamily Sceliphrinae, the tribe Sceliphriini (composed of the mostly spider hunting genera *Chalybion* and *Sceliphron*) is paraphyletic with respect to the neotropical tribe Podiini, with the subfamilial relationships arranged thus: *Sceliphron* + (*Chalybion* + (*Trigonopsis* + (*Podium* + (*Dynatus* + *Penepodium*))))). However, support for the *Chalybion* + Podiini clade is low (GC = 31%).

The subfamily Sceliphrinae (composed of *Isodontia*, *Sphex*, *Prionyx*, *Palmodes*, and the unsampled genus *Chilosphex*) is here paraphyletic with respect to the subfamily

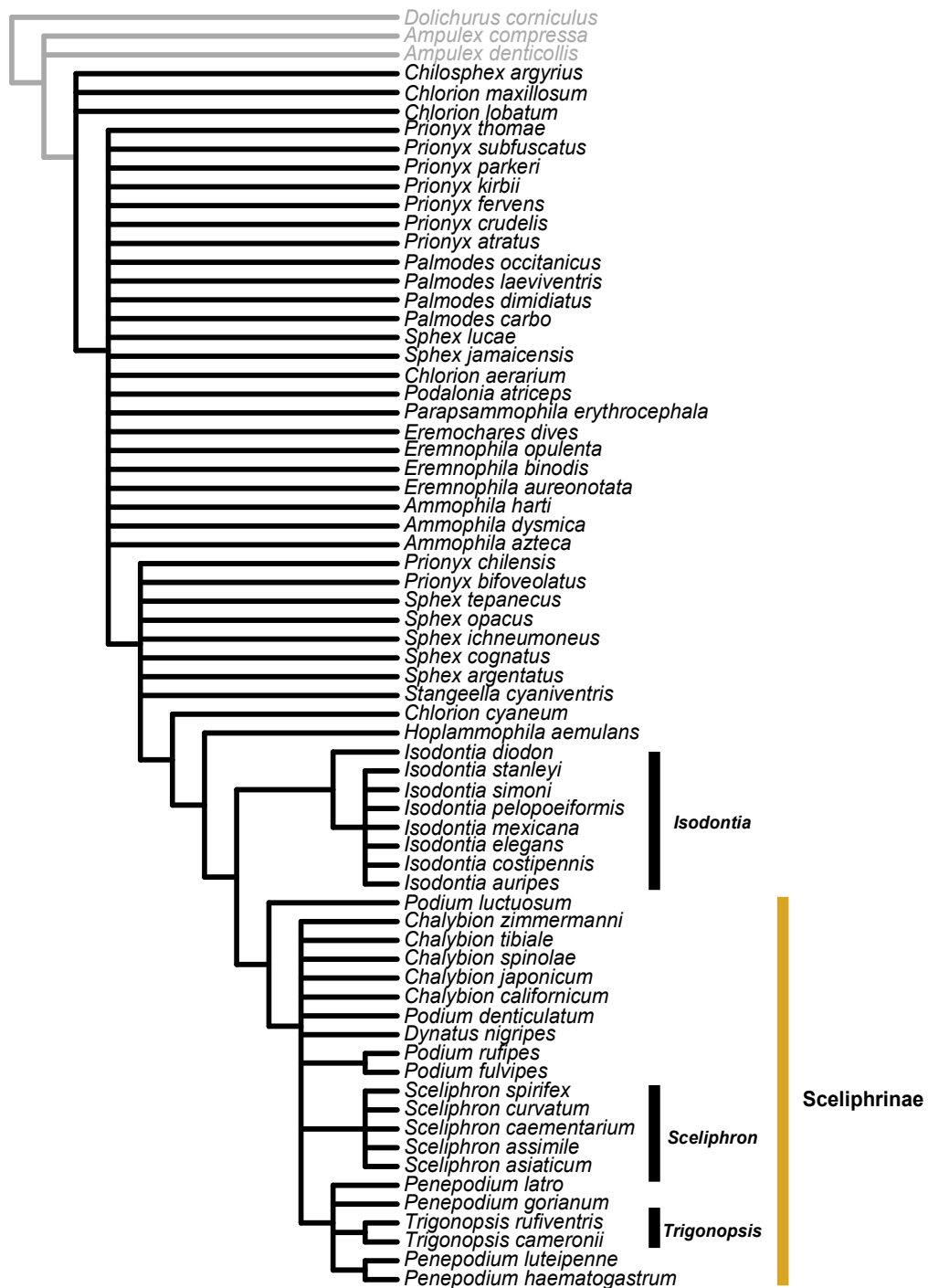
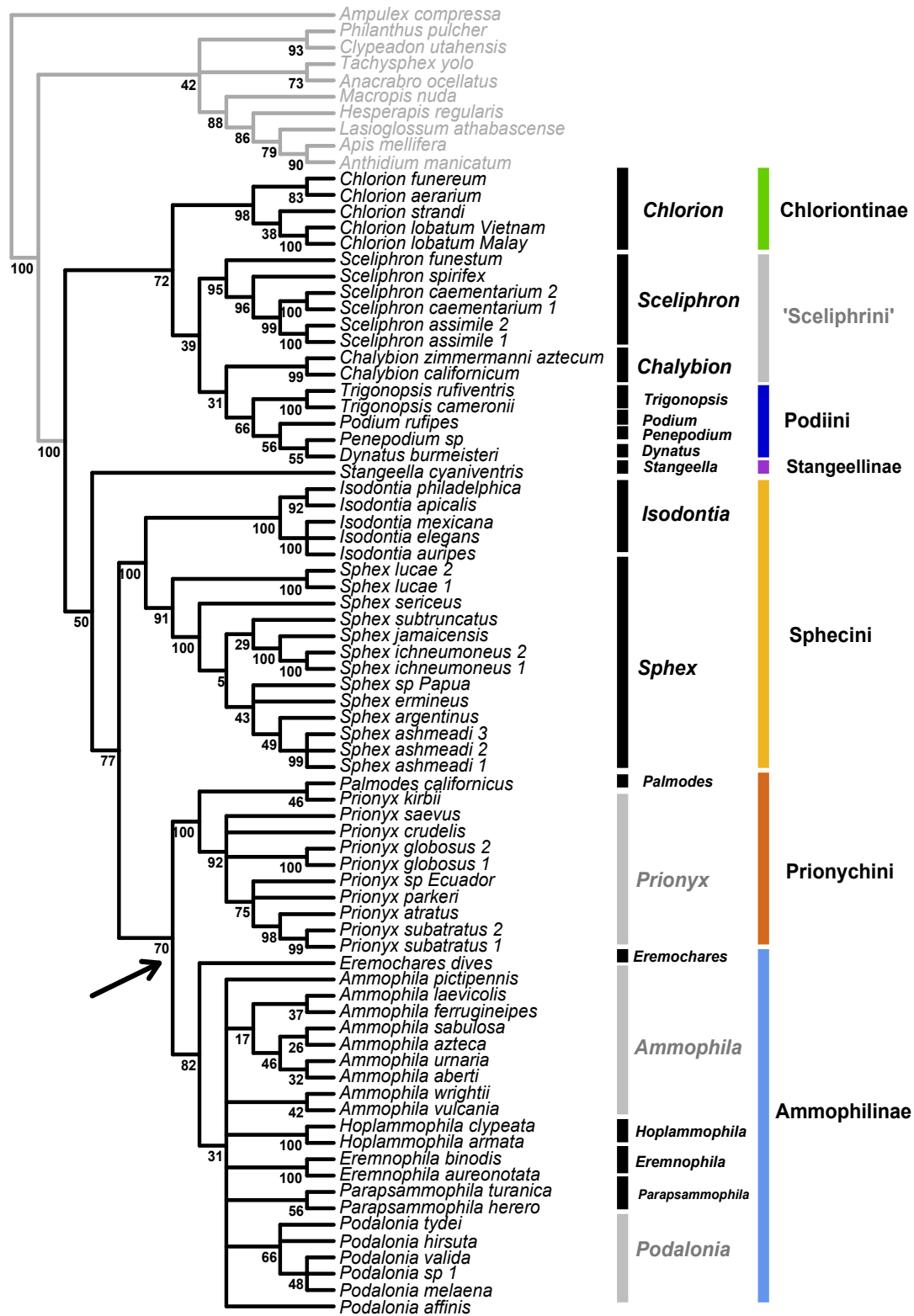


Figure 6.1. Strict consensus of 30,276 most parsimonious trees (73 steps) generated by an equal weights analysis of the sixteen character behavioral dataset.

Figure 6.2. Strict consensus of 68 most parsimonious trees (7,994 steps; CI = 0.225; RI = 0.555) generated by an equal weights analysis of the three gene molecular dataset. Symmetric resampling support values (reported as GC scores and based on 1,000 pseudoreplicates) are shown just below and to the left of each node. Average group support = 63.9. Clade names in gray are either paraphyletic (*Prionyx*, the tribe Sceliphrynini) or else are of unclear status (i.e., *Ammophila* and *Podalonia*). Note the position of the sphecine tribe Prionychini as sister to the subfamily Ammophilinae (the clade marked by an arrow).



Ammophilinae, with the sampled members of the tribe Prionychini (*Palmodes* and *Prionyx*) more closely related to the ammophiline wasps (GC = 70%). *Prionyx* is paraphyletic with respect to *Palmodes* (*Prionyx kirbii* is weakly supported as the sister taxon to *Palmodes californicus* (GC = 46%)). Meanwhile, *Isodontia* and *Sphex* are well-supported sister taxa, with *Sphex lucae* (previously assigned to the genus *Fernaldina*) positioned as the well-supported (GC = 100%) sister to all other *Sphex*.

Interestingly, relationships within the Ammophilinae are largely unresolved, even though most of the data for that group came from the previous study by Field et al. (2011). Nevertheless, *Eremochares dives* still appears as the basalmost ammophiline lineage, although support for this arrangement is low (GC = 31%). In addition, the genera *Hoplammophila*, *Parapsammophila*, and *Eremnophila* are each returned as relatively well-supported monophyletic groups. While the position of *Podalonia affinis* is ambiguous, all other *Podalonia* are moderately well-supported (GC = 66%) as a monophyletic clade.

Discussion

The results of the current molecular analysis are largely consistent with those proposed by Field et al. (2011), the study on which the present dataset was based. However, while the previous study focused mostly on the subfamily Ammophilinae, the current work expanded its taxonomic sample to include representatives of the genera *Palmodes*, *Trigonopsis*, and *Dynatus*, as well as more exemplars from within both the sphecine and sceliphrine lineages. In addition, the present study used equal weights maximum parsimony analyses instead of the exclusively statistical methods employed by Field et al. (2011).

On the utility of behavioral characters in sphecid phylogenetics

While the use of behavioral characters in phylogenetic reconstruction has been supported by numerous previous studies (e.g., Wenzel 1993; Noll 2002; Cap et al. 2008) the results of the current analysis cast some doubt on the utility of nesting characters alone for the reconstruction of sphecid wasp relationships. Sphecids are well known for having evolved an extraordinarily diverse set of nesting behaviors (Bohart and Menke 1976) and it would seem that convergent evolution is a common theme throughout the group. Many of the characters developed here – including cavity nesting and mud use – appear multiple times in apparently unrelated groups. As such, future work will have to be increasingly discriminating in its choice of characters – a sometimes difficult task considering the inconsistent quality of published ethological data.

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CHAPTER VII

GENERAL CONCLUSIONS

Overview: The Current Work in Context

At its most basic, the comparative study of animal behavior is the study of descent with modification. As with any other biological trait, behavioral syndromes are the observable outcomes of ancient historical processes – processes that have left detectable patterns scattered throughout the phenotypes of modern organisms. The job of the comparative ethologist is to discover these patterns, and by doing so, to learn more about the historical processes that created them.

This idea of pattern and process points to an essential element of comparative ethological work, namely its fundamental reliance on informative phylogenetic hypotheses. Phylogenetic trees are, in a sense, the pipelines through which evolutionary changes flow, and the shapes of these pipelines place important constraints on the course of behavioral evolution. If two seemingly identical behaviors (e.g., the hymenopterophagy of *Philanthus* species and that found in *Cerceris rybyensis*; Chapter V) are observed on two widely separated branches of the tree of life, does it make sense to conclude that these behaviors are, in fact, the *same*? And if they aren't the same (i.e., they aren't homologous, or *identical by descent*), then what does that tell us about the Darwinian forces that generate convergence? Phylogenies may not always help us to answer those questions, but without a solid foundation in tree-based thinking, we would never know to ask them in the first place.

While the need for *historical context* in our assessment of behavioral evolution is abundantly clear – and has been for some time (see, among others, Evans and O'Neill

1988; Brooks and McLennan 1991; Harvey and Pagel 1991; Blackledge et al. 2009) – cultural differences between systematists and behavioral ecologists have sometimes given the impression of what one might call (after Gould 1997) *nonoverlapping magisteria*. Brooks and McLennan (1991) have provided a thoughtful examination of the historical trends behind this perceived separation (what they poetically call "the eclipse of history in ethology"), and it seems unnecessary to revisit that story here. The point is merely that more collaboration between these sometimes disparate fields can only result in stronger hypotheses, both for systematists and for behavioral ecologists.

In Chapter I, I suggested that this work should be viewed as a contribution at this somewhat neglected intersection between behavioral ecology and phylogenetic systematics. While no study is perfect, and no thesis ever truly finished (see "Shortcomings of the current work," below), the author hopes that this dissertation will still serve to advance the phylogenetically informed study of apoid wasp behavior. Through the five data-driven research chapters presented here, I show that:

- (1) maximum parsimony phylogenetic analyses may be highly sensitive to changes in fundamental transformation cost parameters, and that this sensitivity may have a major influence on our understanding of behavioral evolution within a group of interest (Chapters II and III);
- (2) a broader evidence base leads to a more strongly corroborated phylogenetic hypothesis, an important consideration when making strong claims about behavioral evolution (Chapters III and V);

- (3) the phylogenetic analysis of molecular, morphological, and/or behavioral characters can shed new light on trends in the evolution of prey preferences among philanthine wasps (Chapters IV and V); and
- (4) while the phylogenetic analysis of a small set of nesting characters alone does not recover relationships within the wasp family Sphecidae, a phylogeny based on molecular data can still provide a framework for future studies of nest evolution within the group (Chapter VI).

A Brief Review of Each Data Chapter

In Chapter II (p. 21; recently published in *American Museum Novitates* as Payne et al. 2013) I presented an analytically rigorous reevaluation of an existing total evidence hymenopteran dataset (from Sharkey et al. 2012) with the explicit goal of uncovering areas of clade sensitivity with respect to variation in basic transformation cost parameters. While most of the hymenopteran superfamilies were robust to such parameter variation, the relationships *among* the major lineages were not: Different cost parameter sets produced wildly different higher order relationships among the superfamilies. The results of the study pointed to the need for further work on higher level hymenopteran systematics, with the ultimate goal of understanding more about major behavioral innovations (e.g., the switch from parasitoid to predatory behavior) within the group.

In Chapter III (p. 54; recently published in *Cladistics* as Payne 2014), I subjected recently published claims regarding the origins of cleptoparasitism in apid bees (Cardinal et al. 2010) to new phylogenetic tests based on a greatly expanded evidence base. While previous authors had provided phylogenetic hypotheses for the family Apidae

(Hymenoptera: Anthophila) based exclusively on either adult and larval morphology (Roig-Alsina and Michener 1993), larval morphology (Straka and Bogusch 2007), or nucleotide sequence data (Cardinal et al. 2010), I combined (Nixon and Carpenter 1996) those pre-existing datasets into a single supermatrix, provided new morphological character codings for genera previously represented by molecular data only, and subjected the newly merged character set to an extensive direct optimization sensitivity analysis. As in Chapter II, nine transformation cost parameter sets were used as the basis for nine parallel analyses (although in this case a second set of nine additional analyses were also performed with the intron regions of elongation factor 1- α included). While the results largely supported Cardinal et al.'s sequence-based finding of a much reduced number of origins of cleptoparasitism within the group (with the "melectine" and nomadine lines consistently combined into a single large clade), the position of the cleptoparasitic genus *Coelioxoides* was revealed to be highly unstable.

In Chapter IV (p. 86), I presented the results of the first molecular phylogenetic analysis of the digger wasp subfamily Philanthinae to include representatives of all eight genera and all four tribes, as well as the first phylogenetic analysis of any kind to explore relationships within the beetlewolf genera *Cerceris* and *Eucerceris*. While both the molecular sample (four loci, 3,776 basepairs) and the taxonomic sample (less than 10% of described species) were relatively limited, the results still revealed new insights into the group's evolutionary history: Both the maximum likelihood and maximum parsimony trees presented a strong case for the monophyly of the large genus *Cerceris* with respect to its much less diverse and geographically restricted sister clade *Eucerceris*. At the same time, the study presented the case for deeper taxonomic and character sampling in order

to resolve the ambiguous relationships among the groups major lineages.

In Chapter V (p. 122), I revisited the philanthine wasps as part of the first total evidence phylogenetic analysis of the group. By combining the molecular dataset developed in Chapter IV with 66 newly coded morphological and behavioral characters, I carried out the most comprehensive investigation of philanthine relationships to date. In order to focus on the history of prey choice within the group, I also performed a set of ancestral state reconstructions aimed at identifying major shifts in prey utilization. The results of the study showed that the ancestral prey choice in the tribe Cercerini was almost certainly not hymenopterophagy, but rather a preference for curculionid beetles (weevils). The use of bee and wasp prey by certain Old World *Cerceris* species thus represents an evolutionary reversal, rather than a symplesiomorphic preference.

Finally, in Chapter VI (p. 184), I presented the results of a preliminary study of sphecid wasp (Hymenoptera: Apoidea: Sphecidae *sensu stricto*) phylogenetics as the foundation for future work on the evolution of nesting behaviors within the group. Two complementary phylogenetic analyses – the first based on a newly expanded three-locus molecular dataset, the second on 16 newly coded behavioral characters derived from the literature – returned highly disparate results: While the molecular phylogeny was relatively well-resolved, well-supported, and compatible with previously published hypotheses (e.g. Ohl 1996: Field et al. 2011), the behavioral phylogeny was poorly resolved, with only three genera (*Isodontia*, *Trigonopsis*, and *Sceliphron*) returned as monophyletic groups.

Shortcomings of the Current Work

In the tradition of scientific self criticism, I address some of the current work's more

important shortcomings below.

While the analytical scope of the second and third chapters was relatively extensive, those studies might have benefited from a less strict adherence to the direct optimization implementation of maximum parsimony and from the inclusion of a second set of sensitivity analyses using static alignments followed by traditional parsimony tree searches. As pockets of substantial opposition to direct optimization exist within the phylogenetic community, such an expanded methodology might have provided more convincing evidence of clade instability to a wider segment of interested researchers. Beyond these merely sociological motivations, an expanded set of analyses would also have been in keeping with the our stated desire for a more "thorough exploration of available data."

By far the biggest shortcoming of the fourth and fifth chapters is their relatively limited taxonomic sample, especially with regard to the diverse beetlewolf genus *Cerceris*. Given that *Cerceris* is one of the largest (if not particularly morphologically diverse) genera of apoid wasps, an argument for monophyly based on a sample of less than 5% of the described species diversity inevitably invites skepticism. However, given the time frame for the completion of this dissertation, the taxon set used here represents the best available sample that could be acquired over the course of two years of directed field work and, at any rate, represents a fairly robust sample of known morphological diversity within the genus. Nevertheless, future work should focus on expanding taxonomic coverage to include much greater species diversity, especially among Australasian and Afrotropical groups. In addition, more intense sampling of potentially informative molecular loci should be a priority for future workers.

The preliminary nature of Chapter VI also leaves many questions unanswered and should be considered an exploratory effort in preparation for future studies of sphecids systematics and behavioral evolution.

Future Directions

While this dissertation suggests multiple avenues for future research, perhaps the most promising is a deeper and more thorough investigation into the nature of cercerine phylogenetic systematics. The behavior, morphology, and biogeographic distribution of both *Cerceris* and *Eucerceris* present a fascinating range of questions for students interested in the evolution of digger wasps in general and the Philanthinae in particular. For instance, if *Eucerceris* and *Cerceris* are in fact monophyletic sister groups, then why have they enjoyed such different levels of evolutionary success (as measured by geographic distribution, species diversity, and behavioral diversity)? If, in fact, *Cerceris mimica* and its close relatives represent a basal *Cerceris* lineage (as strongly implied by molecular data), what morphological synapomorphies unite the rest of the genus? What are the presumably prey-carriage and prey-choice related forces that drive the highly labile development of the clypeal elaborations in females, and why do those elaborations appear to have been lost within the bee-hunting and buprestid-hunting lineage that includes *Cerceris rybyensis*, *C. dilatata*, *C. californica*, and others?

The strongly supported scenario of a secondary return to hymenopterophagy is also of particular interest, especially given the well documented adaptive aculeate venom immunity demonstrated by members of the primitively bee-hunting genus *Philanthus* (reviewed in Evans and O'Neill 1988). Does the bee-hunting *C. rybyensis* share this

immunity? Or did that hymenopterophagous lineage's origins within a derive beetle-hunting clade necessitate a different set of strategies for dealing with stinging prey?

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APPENDIX A:

**SUPPLEMENTARY MATERIAL FOR CHAPTER III:
RESOLVING THE RELATIONSHIPS OF APID BEES (HYMENOPTERA: APIDAE)
THROUGH A DIRECT OPTIMIZATION SENSITIVITY ANALYSIS OF MOLECULAR,
MORPHOLOGICAL, AND BEHAVIOURAL CHARACTERS**

Table S3.1. Sources of morphological character data used in the present study along with relevant taxonomic notes.

Genus	Adult characters	Larval characters
<i>Afroheriades</i>	N/A	N/A
<i>Aglae</i>	<i>de novo</i> coding (<i>A. caerulea</i>)	N/A
<i>Alepidosceles</i>	<i>de novo</i> coding (<i>A. filitarsis</i>)	N/A
<i>Amegilla</i>	N/A	Cardale, 1967 (<i>A. pulchra</i>)
<i>Ammobates</i>	<i>de novo</i> coding (<i>A. punctatus</i>); Rozen, 2003 (ovarioles)	Straka & Bogusch, 2007
<i>Ammobatoides</i>	<i>de novo</i> coding (<i>A. luctuosus</i>); Rozen, 2003 (ovarioles)	N/A
<i>Ancyla</i>	<i>de novo</i> coding	Straka & Rozen, 2012 (<i>A. asiatica</i>)
<i>Ancyloscelis</i>	Roig-Alsina & Michener, 1993	N/A
<i>Anthidiellum</i>	<i>de novo</i> coding (<i>A. notatum</i>)	N/A
<i>Anthidium</i>	Roig-Alsina & Michener, 1993	Straka & Bogusch, 2007
<i>Anthophora</i>	Roig-Alsina & Michener, 1993	Straka & Bogusch, 2007
<i>Anthophorula</i>	Roig-Alsina & Michener, 1993 (<i>A. niveata</i> , listed as <i>Isomalopsis niveata</i>)	Straka & Bogusch, 2007 (<i>A. chionura</i> , listed as <i>Examolopsis chionura</i>)
<i>Apis</i>	Roig-Alsina & Michener, 1993	Straka & Bogusch, 2007
<i>Arhysoceble</i>	Roig-Alsina & Michener, 1993 (<i>A. picta</i> , listed as <i>Paratetrapedia (A.) melampoda</i>)	N/A
<i>Ashmeadiella</i>	<i>de novo</i> coding (<i>A. aridula</i>)	Roig-Alsina & Michener, 1993
<i>Axestotrigona</i>	N/A	N/A
<i>Blastes</i>	Roig-Alsina & Michener, 1993	Rozen <i>et al.</i> , 2009 (<i>B. emarginatus</i>)
<i>Bombus</i>	Roig-Alsina & Michener, 1993	Straka & Bogusch, 2007
<i>Brachynomada</i>	Roig-Alsina & Michener, 1993	Roig-Alsina & Michener, 1993 (<i>B. sidaefloris</i> , listed as <i>Melanomada sidaefloris</i>)
<i>Braunsapis</i>	Roig-Alsina & Michener, 1993	N/A
<i>Caenonomada</i>	Roig-Alsina & Michener, 1993	N/A
<i>Caenoprosopina</i>	<i>de novo</i> coding (<i>C. holmbergi</i>); Rozen, 2003 (ovarioles)	Rozen & Roig-Alsina, 1991 (<i>C. holmbergi</i>)
<i>Caenoprosopis</i>	Roig-Alsina & Michener, 1993; Rozen, 2003 (ovarioles)	Rozen & Roig-Alsina, 1991 (<i>C. crabronina</i>)
<i>Centris</i>	Roig-Alsina & Michener, 1993	Straka & Bogusch, 2007
<i>Cephalotrigona</i>	N/A	N/A
<i>Ceratina</i>	Roig-Alsina & Michener, 1993 (<i>C. calcarata</i> ; <i>C. rupestris</i>)	Straka & Bogusch, 2007
<i>Chelostoma</i>	<i>de novo</i> coding (<i>C. californicum</i>)	N/A

Table S3.1. continued

Genus	Adult characters	Larval characters
<i>Coeloxoides</i>	Roig-Alsina & Michener, 1993; Rozen, 2003 (ovarioles)	Straka & Bogusch, 2007
<i>Coeloxys</i>	Roig-Alsina & Michener, 1993; Rozen, 2003 (ovarioles)	Straka & Bogusch, 2007
<i>Compsomelissa</i>	<i>de novo</i> coding	N/A
<i>Ctenioschelus</i>	N/A	N/A
<i>Ctenoplectra</i>	Roig-Alsina & Michener, 1993	Roig-Alsina & Michener, 1993 (<i>C. armata</i>)
<i>Ctenoplectrina</i>	N/A	N/A
<i>Dasypoda</i>	Roig-Alsina & Michener, 1993	Straka & Bogusch, 2007
<i>Deltoptila</i>	Roig-Alsina & Michener, 1993	N/A
<i>Diadasia</i>	Roig-Alsina & Michener, 1993	Roig-Alsina & Michener, 1993 (<i>D. diminuta</i>)
<i>Diadasina</i>	Roig-Alsina & Michener, 1993	N/A
<i>Dianthidium</i>	<i>de novo</i> coding (<i>D. subparvum</i>)	Roig-Alsina & Michener, 1993
<i>Dioxys</i>	<i>de novo</i> coding (<i>D. pomonae</i>); Rozen, 2003 (ovarioles)	Roig-Alsina & Michener, 1993
<i>Doeringiella</i>	<i>de novo</i> coding (<i>D. baeri</i>); Rozen, 2003 (ovarioles)	Roig-Alsina & Michener, 1993 (<i>D. pomonae</i>)
<i>Epeoloides</i>	<i>de novo</i> coding (<i>E. coecutiens</i>); Rozen, 2003 (ovarioles)	N/A
<i>Epeolus</i>	Roig-Alsina & Michener, 1993; Rozen, 2003 (ovarioles)	Straka & Bogusch, 2007
<i>Epicharis</i>	Roig-Alsina & Michener, 1993	Straka & Bogusch, 2007
<i>Epiclopus</i>	<i>de novo</i> coding (<i>E. gayi</i>); Rozen, 2003 (ovarioles)	Straka & Bogusch, 2007
<i>Ericrocis</i>	Roig-Alsina & Michener, 1993; Rozen, 2003 (ovarioles)	N/A
<i>Euclera</i>	Roig-Alsina & Michener, 1993	Straka & Bogusch, 2007
<i>Eufriesea</i>	Roig-Alsina & Michener, 1993	Miliczky, 1985 (<i>E. hamata</i> , listed as <i>Tetralonia hamata</i>)
<i>Euglossa</i>	Roig-Alsina & Michener, 1993	Straka & Bogusch, 2007
<i>Eulaema</i>	<i>de novo</i> coding (<i>Eulaema</i> sp.)	N/A
<i>Exaerete</i>	<i>de novo</i> coding (<i>E. frontalis</i>); Rozen, 2003 (ovarioles)	Zucchi <i>et al.</i> , 1970 (<i>E. nigrita</i>)
<i>Exomalopsis</i>	Roig-Alsina & Michener, 1993	N/A
<i>Exoneura</i>	<i>de novo</i> coding (<i>E. bicolor</i>)	N/A
<i>Fidelia</i>	<i>de novo</i> coding (<i>F. (Fidelopsis) major</i>)	N/A
<i>Florilegus</i>	<i>de novo</i> coding (<i>Florilegus</i> sp.)	Straka & Bogusch, 2007
<i>Habropoda</i>	Roig-Alsina & Michener, 1993	LaBerge & Ribble, 1966. (<i>F. condignus</i>)
		Straka & Bogusch, 2007

Table S3.1. continued

Genus	Adult characters	Larval characters
<i>Heriades</i>	<i>de novo</i> coding (<i>Heriades</i> spp.)	N/A
<i>Hesperapis</i>	Roig-Alsina & Michener, 1993	Roig-Alsina & Michener, 1993
<i>Hexepeolus</i>	Roig-Alsina & Michener, 1993; Rozen, 2003 (ovarioles)	Rozen, 1996 (<i>H. rhodogyne</i>)
<i>Holcopasites</i>	Roig-Alsina & Michener, 1993; Rozen, 2003 (ovarioles)	Straka & Bogusch, 2007
<i>Hopliphora</i>	<i>de novo</i> coding (<i>Hopliphora velutina</i>)	N/A
<i>Hoplitis</i>	Roig-Alsina & Michener, 1993	Straka & Bogusch, 2007
<i>Hoplosmia</i>	<i>de novo</i> coding (<i>Hoplosmia</i> spp.)	N/A
<i>Hypotrigona</i>	N/A	N/A
<i>Isepeolus</i>	Roig-Alsina & Michener, 1993	Straka & Bogusch, 2007
<i>Leiapodus</i>	Roig-Alsina & Michener, 1993	Straka & Bogusch, 2007
<i>Lestrimelitta</i>	N/A	N/A
<i>Lithurgus</i>	Roig-Alsina & Michener, 1993 (<i>Lithurge apicalis</i>)	Straka & Bogusch, 2007
<i>Macrogalea</i>	Roig-Alsina & Michener, 1993	N/A
<i>Macropis</i>	Roig-Alsina & Michener, 1993	Straka & Bogusch, 2007
<i>Manuelia</i>	Roig-Alsina & Michener, 1993	N/A
<i>Martinapis</i>	<i>de novo</i> coding (<i>M. luteicornis</i>)	N/A
<i>Megachile</i>	Roig-Alsina & Michener, 1993	Straka & Bogusch, 2007
<i>Meganomia</i>	Roig-Alsina & Michener, 1993	Straka & Bogusch, 2007
<i>Melecta</i>	<i>de novo</i> coding (<i>M. albifrons</i>); Rozen, 2003 (ovarioles)	Straka & Bogusch, 2007
<i>Melectoides</i>	Roig-Alsina & Michener, 1993	N/A
<i>Meliphiopsis</i>	<i>de novo</i> coding (<i>M. melanandra</i>)	Straka & Bogusch, 2007
<i>Melipona</i>	Roig-Alsina & Michener, 1993	N/A
<i>Meliponula</i>	N/A	McGinley, 1981 (<i>M. pallidignata</i>)
<i>Melissodes</i>	Roig-Alsina & Michener, 1993	N/A
<i>Melissoptila</i>	<i>de novo</i> coding (<i>Melissoptila</i> sp.)	Straka & Bogusch, 2007
<i>Melitoma</i>	Roig-Alsina & Michener, 1993	Straka & Bogusch, 2007
<i>Melitta</i>	Roig-Alsina & Michener, 1993	N/A
<i>Mesocheira</i>	<i>de novo</i> coding (<i>M. bicolor</i>)	

Table S3.1. continued

Genus	Adult characters	Larval characters
<i>Mesonychium</i>	Roig-Alsina & Michener, 1993	N/A
<i>Mesoplia</i>	Roig-Alsina & Michener, 1993	Rozen, 1969 (<i>M. rufipes</i>)
<i>Microthurge</i>	<i>de novo</i> coding (<i>Microthurge</i> spp.)	N/A
<i>Nanorhathymus</i>	<i>de novo</i> coding (<i>Nanorhathymus</i> sp.)	N/A
<i>Neofidelia</i>	Roig-Alsina & Michener, 1993	Straka & Bogusch, 2007
<i>Neolarra</i>	Roig-Alsina & Michener, 1993	Straka & Bogusch, 2007
<i>Neopasites</i>	<i>de novo</i> coding (<i>N. cressoni</i>); Rozen, 2003 (ovarioles)	Straka & Bogusch, 2007
<i>Nomada</i>	Roig-Alsina & Michener, 1993	Straka & Bogusch, 2007
<i>Odyneropsis</i>	<i>de novo</i> coding (<i>O. gertschi</i>)	N/A
<i>Oreopasites</i>	Roig-Alsina & Michener, 1993	Straka & Bogusch, 2007
<i>Osirix</i>	Roig-Alsina & Michener, 1993	N/A
<i>Osmia</i>	Roig-Alsina & Michener, 1993	Roig-Alsina & Michener, 1993 (<i>O. lignaria</i>)
<i>Pachymelus</i>	N/A	N/A
<i>Paranomada</i>	<i>de novo</i> coding (<i>P. velutina</i>); Rozen, 2003 (ovarioles)	Straka & Bogusch, 2007
<i>Paranthidium</i>	<i>de novo</i> coding (<i>P. jugatorium</i>)	N/A
<i>Pararhophites</i>	Roig-Alsina & Michener, 1993	Straka & Bogusch, 2007
<i>Paratetrapedia</i>	Roig-Alsina & Michener, 1993 (as <i>P. (Paratetrapedia)</i> sp.)	Straka & Bogusch, 2007
<i>Parepeolus</i>	Roig-Alsina & Michener, 1993	Straka & Bogusch, 2007
<i>Partamona</i>	Roig-Alsina & Michener, 1993 (<i>P. cupira</i>)	Roig-Alsina & Michener, 1993 (<i>P. testacea</i> , listed as <i>Trigona testacea</i>)
<i>Pasites</i>	<i>de novo</i> coding (<i>P. maculatus</i>); Rozen, 2003 (ovarioles)	Roig-Alsina & Michener, 1993 (<i>P. histrio</i> , listed at <i>Morgania histrio transvaalensis</i>)
<i>Peponapis</i>	Roig-Alsina & Michener, 1993	Straka & Bogusch, 2007
<i>Plebeina</i>	N/A	N/A
<i>Promeliita</i>	<i>de novo</i> coding (<i>P. alboclypeata</i>)	N/A
<i>Protosmia</i>	<i>de novo</i> coding (<i>P. rubifloris</i>)	N/A
<i>Ptilothrix</i>	Roig-Alsina & Michener, 1993 (<i>P. tricolor</i>)	Michener, 1953 (<i>P. bombyformis</i> , listed as <i>Emphor bombyformis</i>)
<i>Rhathymus</i>	Roig-Alsina & Michener, 1993	Straka & Bogusch, 2007
<i>Rhinepeolus</i>	<i>de novo</i> coding (<i>R. rufiventris</i>)	N/A

Table S3.1. continued

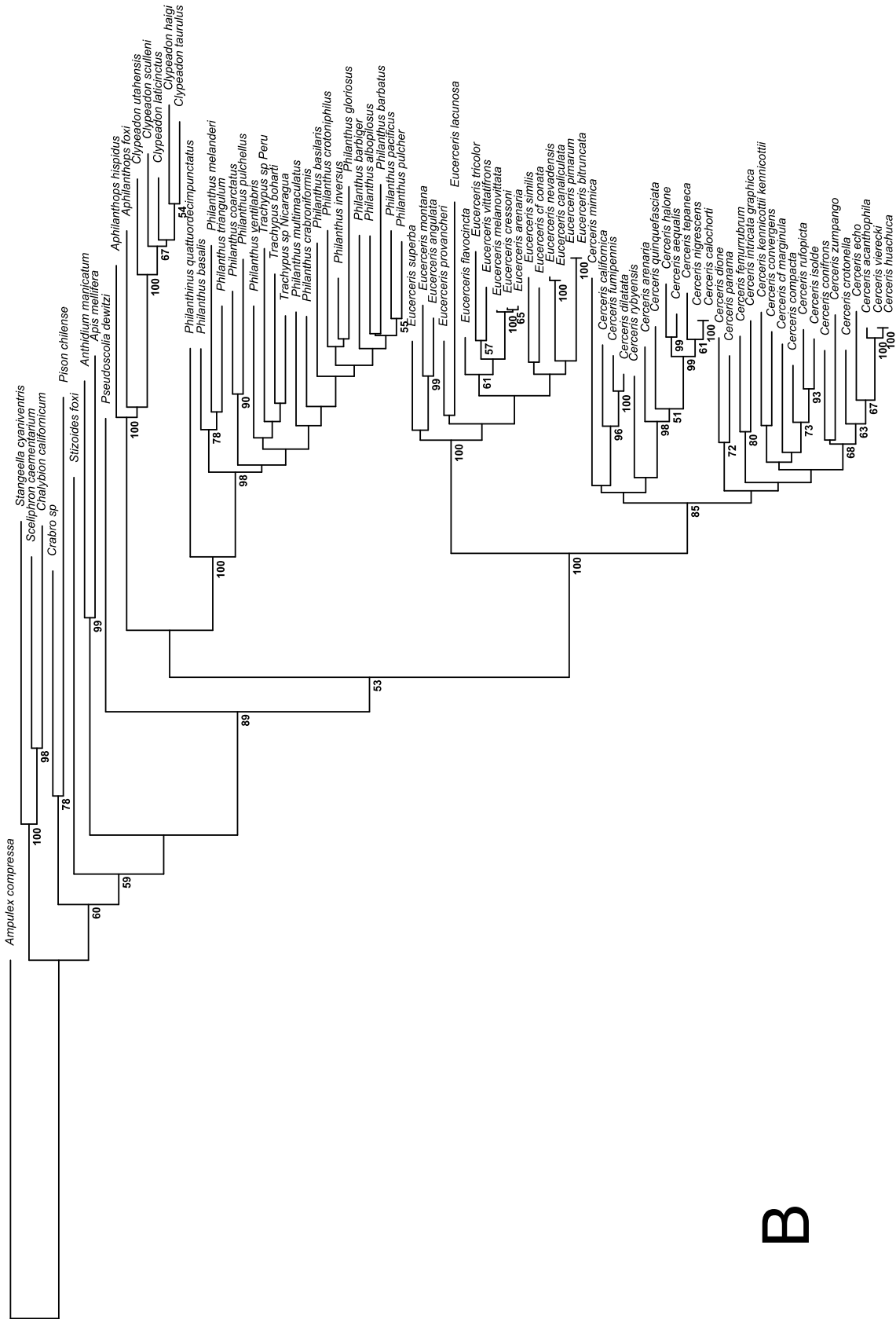
Genus	Adult characters	Larval characters
<i>Scaptotrigona</i>	N/A	N/A
<i>Scaura</i>	N/A	N/A
<i>Sphecodopsis</i>	<i>de novo</i> coding (<i>S. capensis</i>)	Roig-Alsina & Michener, 1993 (<i>S. fumipennis</i> , listed as <i>Pseudodichroa fumipennis</i>)
<i>Stelis</i>	<i>de novo</i> coding (<i>S. linsleyi</i>); Rozen & Hall, 2011 (ovarioles)	Rozen & Hall, 2011 (<i>S. ater</i>)
<i>Svaestra</i>	Roig-Alsina & Michener, 1993	McGinley, 1981 (<i>S. obliqua</i>)
<i>Svastrides</i>	<i>de novo</i> coding (<i>S. melanura</i>)	N/A
<i>Svastrina</i>	N/A	N/A
<i>Tapinotaspoides</i>		N/A
<i>Tetragonula</i>	N/A	N/A
<i>Tetralonia</i>	<i>de novo</i> coding (<i>T. cinctula</i>)	N/A
<i>Tetraloniella</i>	N/A	N/A
<i>Tetralonioidella</i>	N/A	N/A
<i>Tetrapedia</i>	Roig-Alsina & Michener, 1993	Straka & Bogusch, 2007
<i>Thalestria</i>	<i>de novo</i> coding (<i>T. spinosa</i>)	N/A
<i>Thygater</i>	<i>de novo</i> coding (<i>Thygater</i> sp.)	Packer, 1987
<i>Thyreus</i>	Roig-Alsina & Michener, 1993	Straka & Bogusch, 2007
<i>Townsendiella</i>	<i>de novo</i> coding (<i>T. pulchra</i>);	Rozen & McGinley, 1991 (<i>T. pulchra</i>)
<i>Trachusa</i>	Roig-Alsina & Michener, 1993	Roig-Alsina & Michener, 1993 (<i>T. perdita</i>)
<i>Trichothurgus</i>	<i>de novo</i> coding (<i>T. aterrimus</i>)	Rozen, 1973 (<i>T. dubius</i>)
<i>Triepeolus</i>	Roig-Alsina & Michener, 1993	Roig-Alsina & Michener, 1993
<i>Trigona</i>	N/A	N/A
<i>Triopasites</i>	<i>de novo</i> coding (<i>T. penniger</i>); Rozen, 2003 (ovarioles)	Rozen, 1977 (<i>T. penniger</i>)
<i>Xenoglossa</i>	<i>de novo</i> coding (<i>Xenoglossa</i> sp.)	Straka & Bogusch, 2007
<i>Xeromelecta</i>	Roig-Alsina & Michener, 1993	Michener, 1953 (<i>X. californica</i> , listed as <i>Melecta (Melectomorpha) californica</i>)
<i>Xylocopa</i>	Roig-Alsina & Michener, 1993 (<i>X. virginica</i>)	Straka & Bogusch, 2007
<i>Zacosmia</i>	Roig-Alsina & Michener, 1993	Straka & Bogusch, 2007

APPENDIX B:

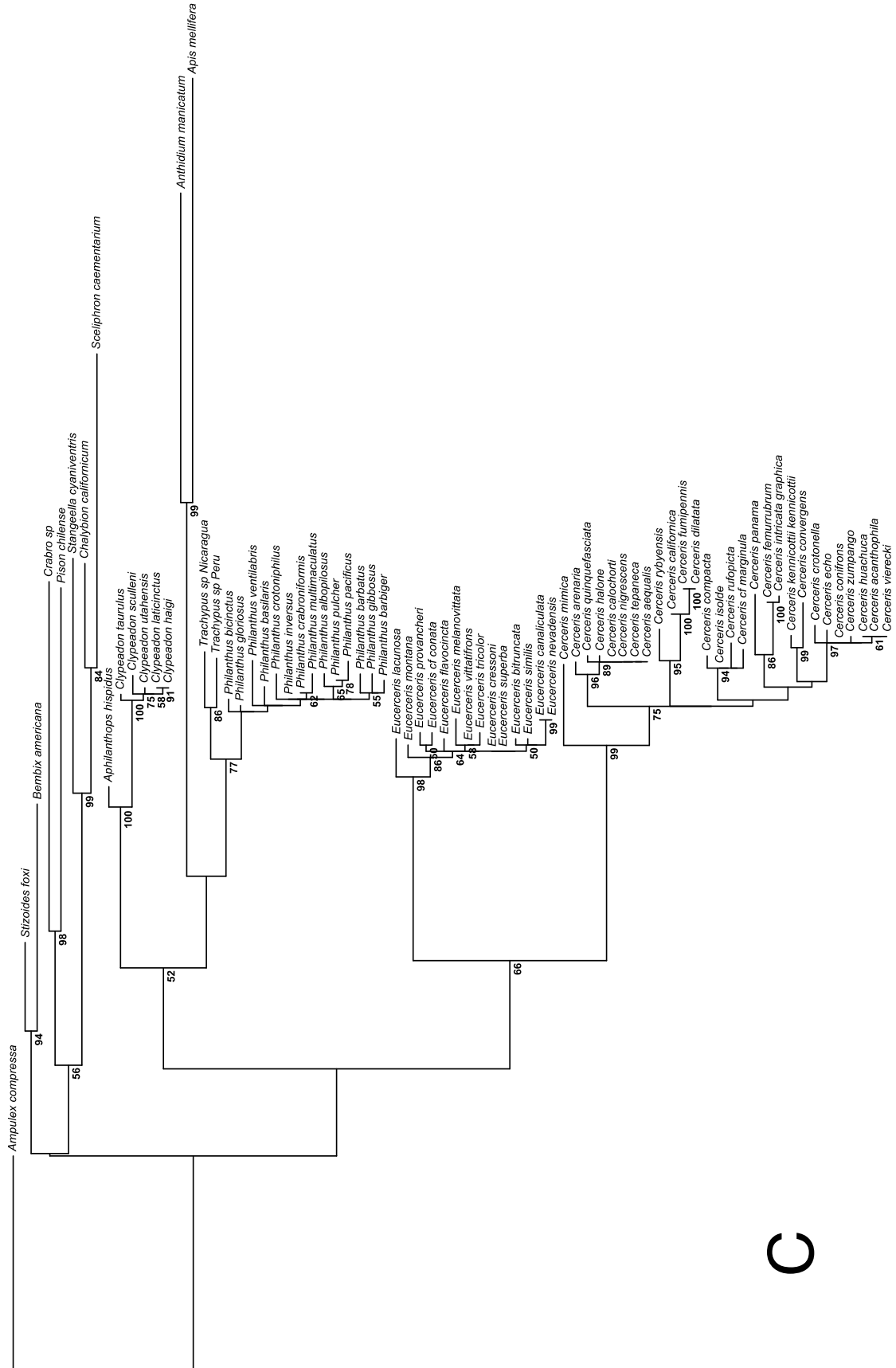
**SUPPLEMENTARY MATERIAL FOR CHAPTER IV:
A PRELIMINARY MOLECULAR PHYLOGENY FOR THE PHILANTHINE WASPS
(APOIDEA: CRABRONIDAE: PHILANTHINAE), WITH AN EMPHASIS ON NORTH
AMERICAN BEETLEWOLVES (CERCERINI)**

Figure S4.1. Maximum likelihood trees based on RAxML rapid bootstrap analyses of five nucleotide datasets. Bootstrap support values $\geq 50\%$ (based on 100 pseudo-replicates) are shown just below and to the left of each node.

- A.** COI codon position III
- B.** COI, all codons
- C.** EF-1 α F2 coding regions
- D.** 18S
- E.** 28S.



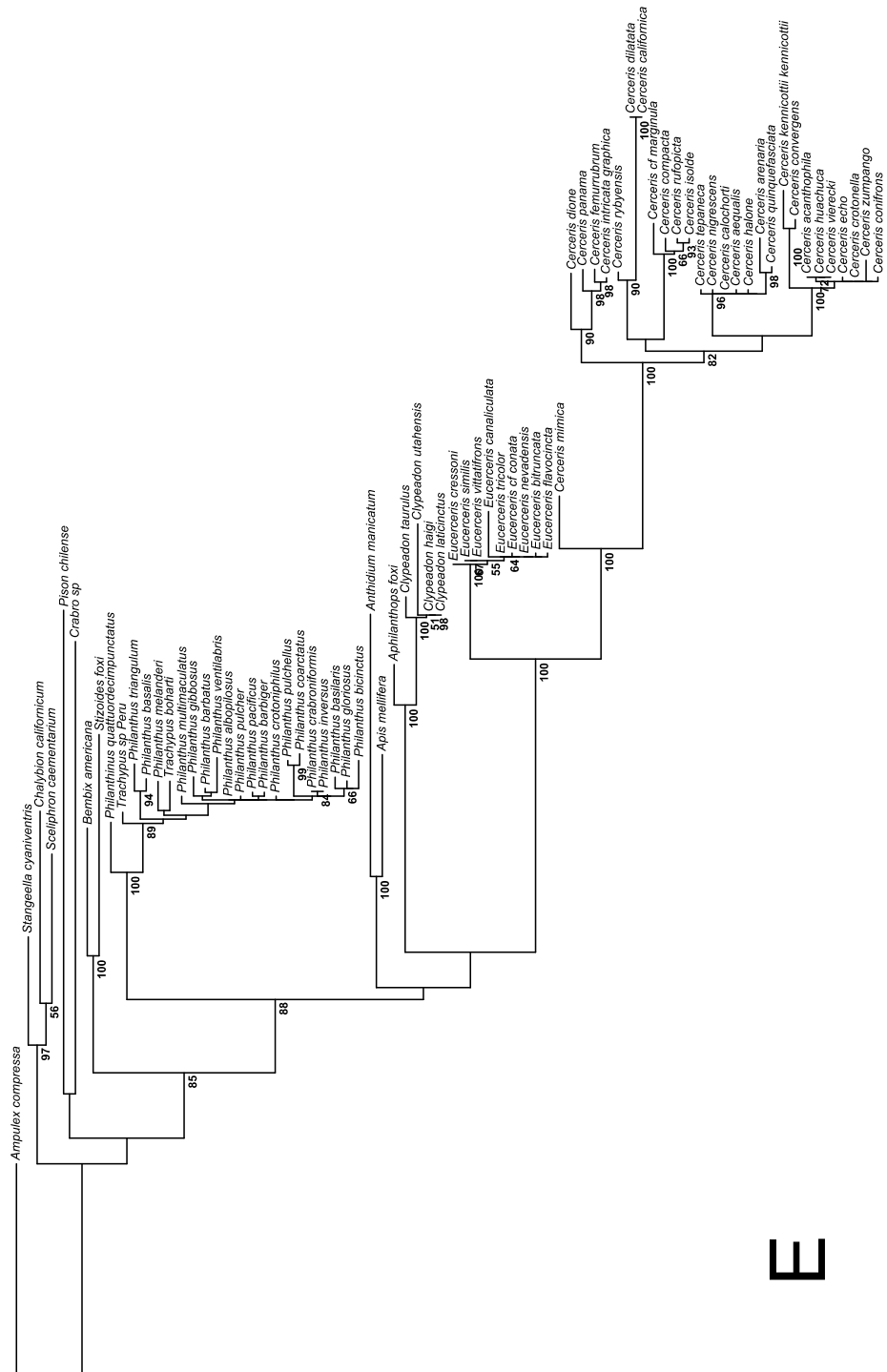
B



C



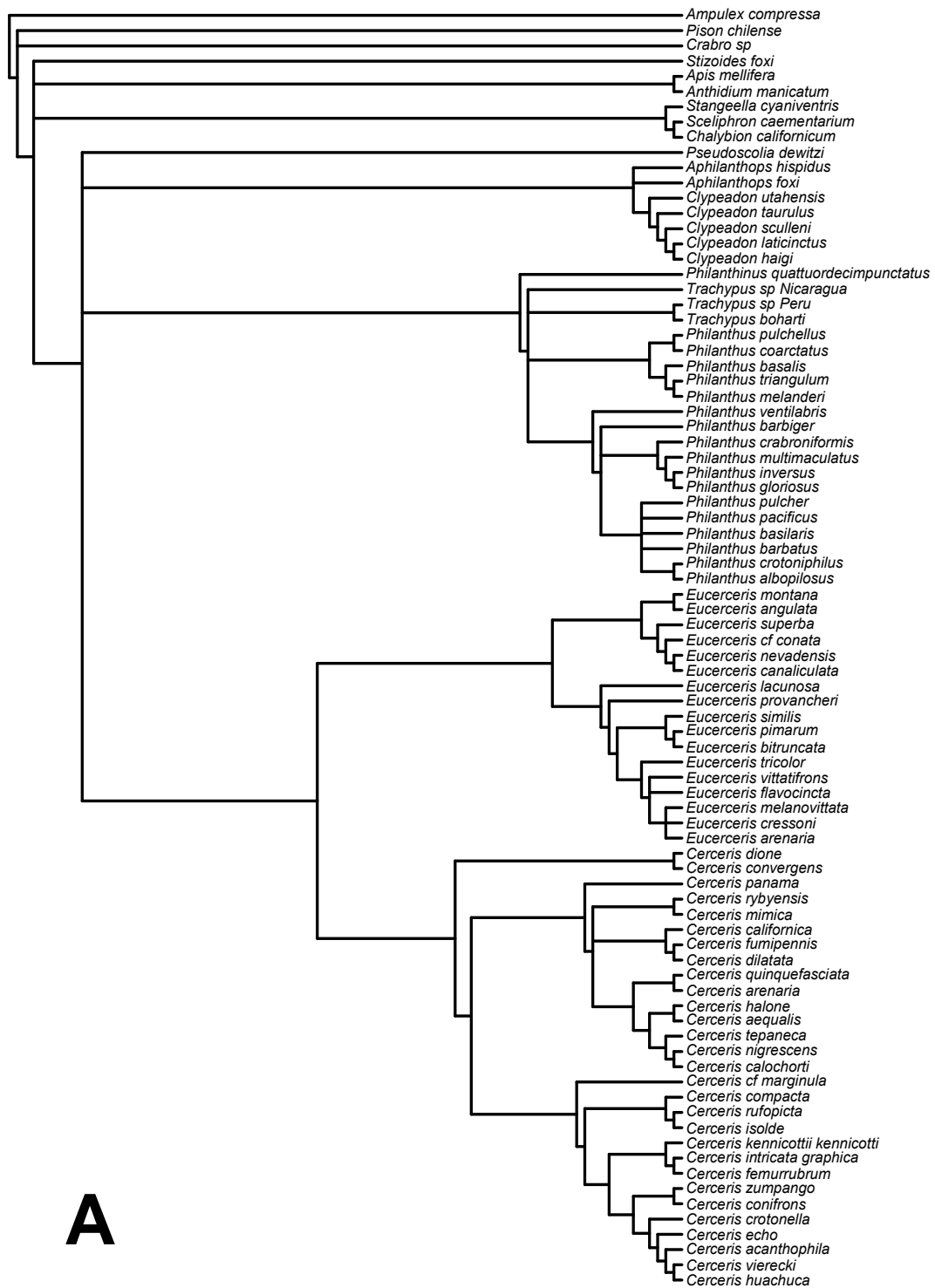
D

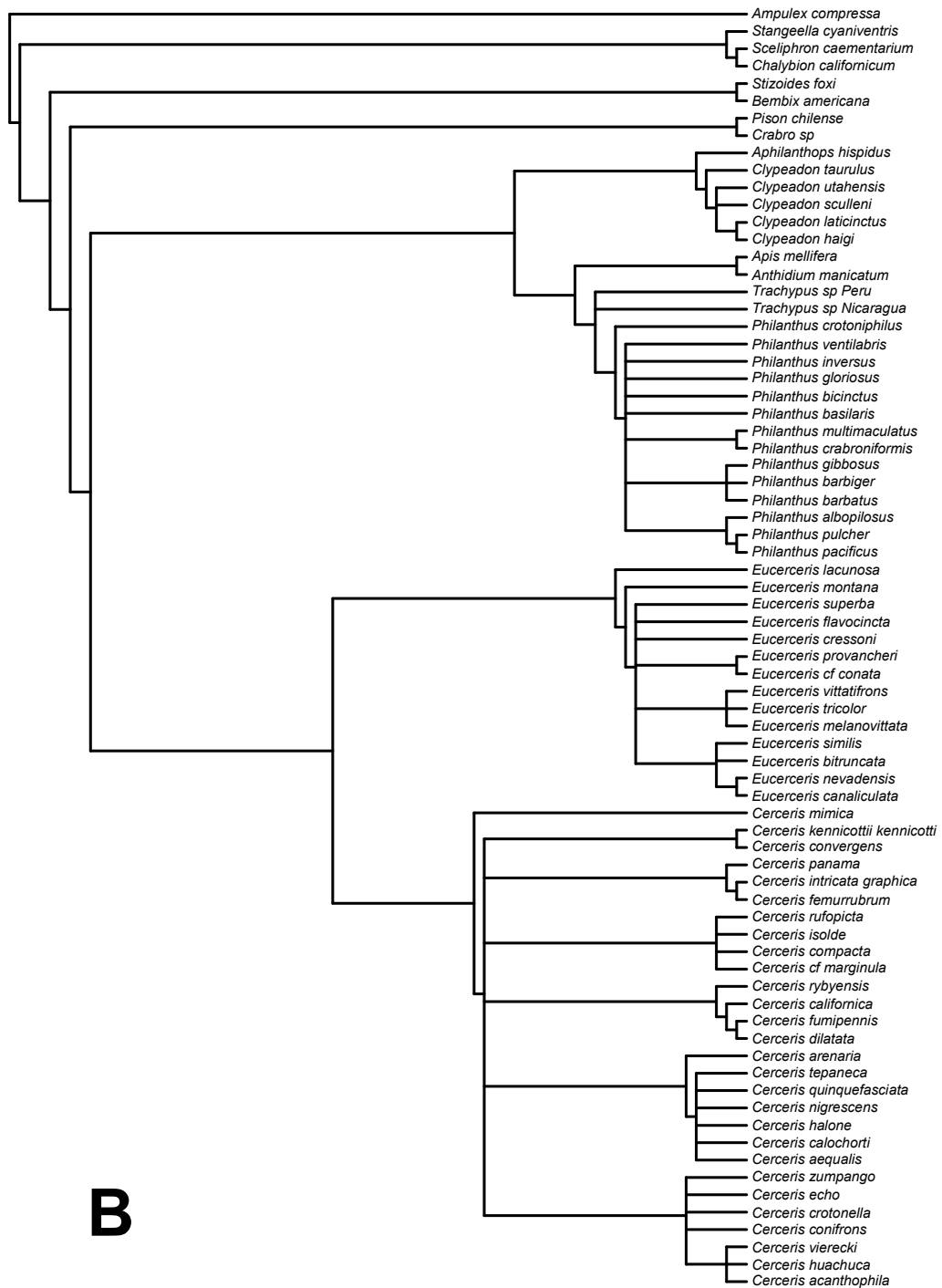


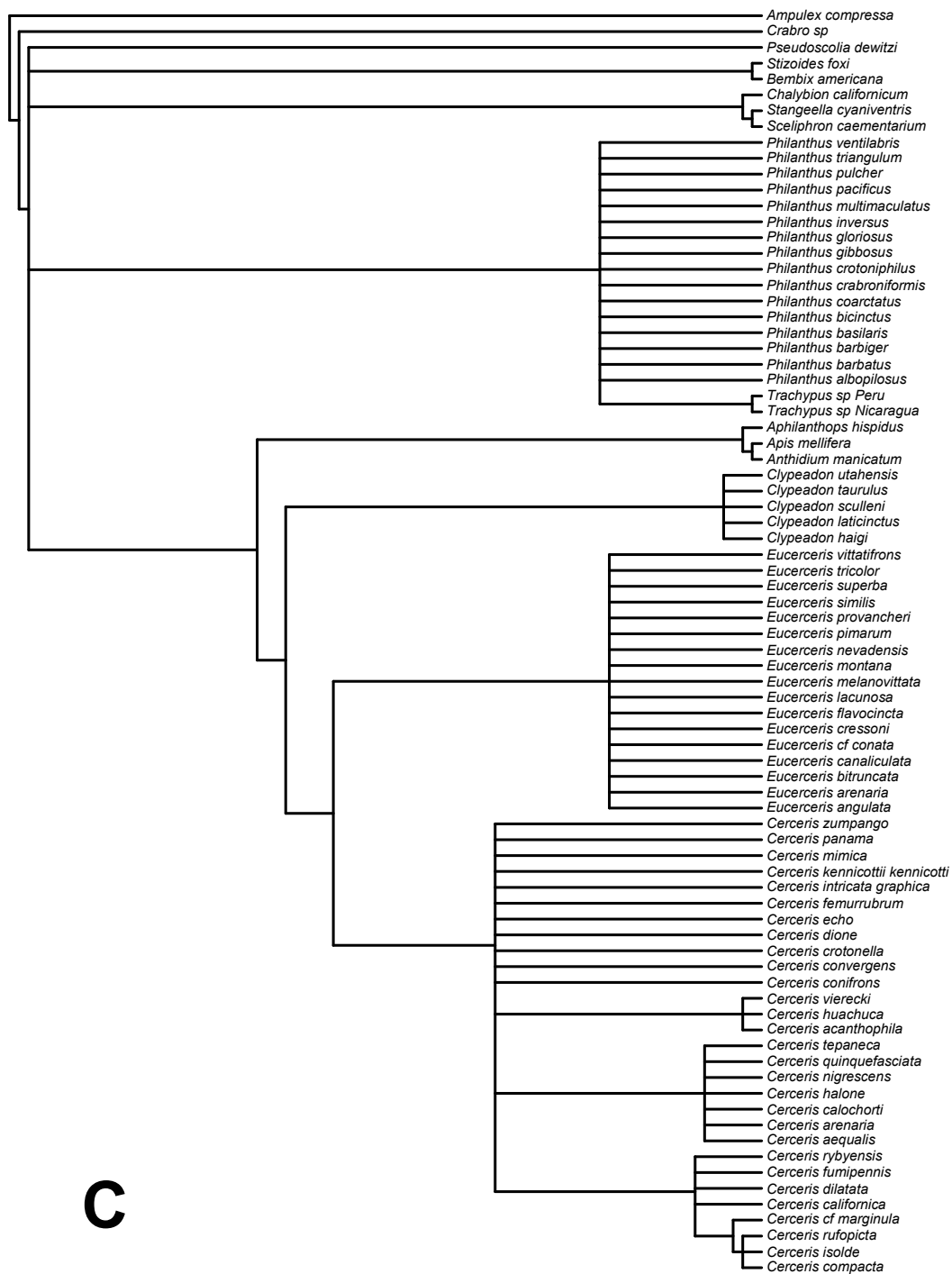
E

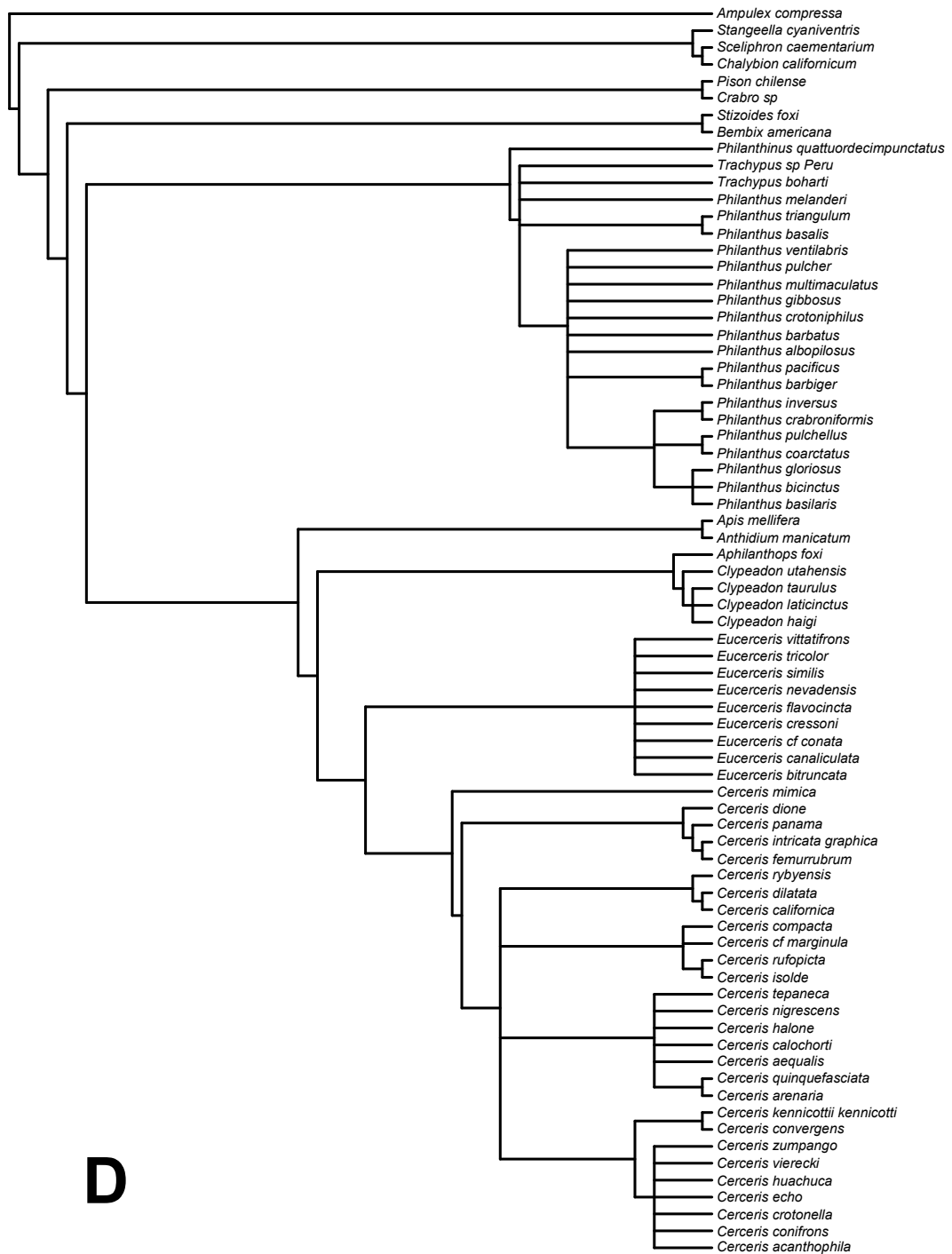
Figure S4.2. Strict consensus trees produced by equal weights maximum parsimony analyses of the individual loci (with gaps treated as a fifth state):

- A.** COI, all codon positions (24 trees; length = 4,310 steps; CI = 0.203; RI = 0.561)
- B.** EF-1 α F2 coding regions (38 trees; length = 852 steps; CI = 0.428; RI = 0.832)
- C.** 18S (40 trees; length = 161 steps; CI = 0.727; RI = 0.961)
- D.** 28S (2,160 trees; length = 1,826 steps; CI = 0.619; RI = 0.908)









APPENDIX C:

**SUPPLEMENTARY MATERIAL FOR CHAPTER V:
ON THE EVOLUTION OF PREY CHOICE IN PHILANTHINE WASPS:
FIRST INSIGHTS FROM A TOTAL EVIDENCE PHYLOGENETIC ANALYSIS**

Table S5.1. Morphological and behavioral character matrix used in Chapter V. Polymorphisms are designated as follows: [01] = &; [2,3] = %.

	1	2	3	4	5	6
	1234567890	1234567890	1234567890	1234567890	1234567890	123456
<i>Stizoides foxi</i>	0000010000	000---1000	1100010000	0000000000	?000000100	00000??--
<i>Anth. manicatum</i>	0000010000	000--00100	21000001000	11100000000	00000000000	00000??0-
<i>Apis mellifera</i>	00000-10&0	000--00100	11000001000	10100000000	00000000000	00000??0-
<i>Aph. foxi</i>	1000000000	00100001000	01000111000	10111000000	00010000100	00000???-
<i>Aph. frigidus</i>	1000000000	00100001000	01100111000	10111010000	00010000100	0000012030
<i>Aph. hispidus</i>	1000000000	00100001000	01000111000	10110000000	00010000100	00000??2-
<i>Aph. subfrigidus</i>	1000000000	00110001000	01100111000	10111010000	00010000100	00000??30
<i>Clyp. haigi</i>	1000000000	00110001010	11011120000	10110000000	00011000101	00100??31
<i>Clyp. laticinctus</i>	1000000000	00110001010	11011120000	10110000000	00011000101	0010011031
<i>Clyp. sculleni</i>	1000000000	00100001010	11011120000	10110000000	00011000101	00100??31
<i>Clyp. taurulus</i>	1000000000	00110001010	11011120000	10110000000	00011000101	00100??31
<i>Clyp. utahensis</i>	1000000000	00110001010	11011120000	10110000000	00011000101	00100??31
<i>C. acanthophila</i>	1200000000	00111000011	22000102011	11100110111	10110101010	11201??4-
<i>C. aequalis</i>	12110000001	00111000010	22000102011	11100110111	10110101010	01201???-
<i>C. arenaria</i>	12110000000	00110000011	22000102011	11100110111	10110101010	01201???-
<i>C. californica</i>	12000000000	00111000010	22000102011	11100110111	10110101011	01201??4-
<i>C. calochorti</i>	12110000000	01111000010	22000102011	11100110111	10110101010	01201???-
<i>C. compacta</i>	12000000000	10111000010	22000102011	11100110111	10110101010	01201??4-
<i>C. confirons</i>	12000000001	00111000011	22000102011	11100110111	10110101010	0000000
<i>C. convergens</i>	12000000000	00111000011	22000102011	11100110111	10110101010	01201???-
<i>C. crotonella</i>	12000000000	00110000010	22000102011	11100110111	10110101010	01201??4-
<i>C. dilatata</i>	12000000000	00111000010	22000102011	11100110111	10110101011	01201??4-
<i>C. dione</i>	12000000001	00111000010	22000102011	11100110111	10110101010	01201???-
<i>C. echo</i>	12000000000	00111000011	22000102011	11100110111	10110101010	01201??4-
<i>C. femurrubrum</i>	12002000001	00111000010	22000102011	11100110111	10110101010	01201??4-
<i>C. fumipennis</i>	12000000000	00111000010	22000102011	11100110111	10110101011	012010014-
<i>C. halone</i>	12110000000	00111000010	22000102011	11100110111	10110101010	01201??4-
<i>C. huachuca</i>	12000000000	00111000011	22000102011	11100110111	10110101010	11201??4-

Table S5.1. continued.

	1	2	3	4	5	6
	1234567890	1234567890	1234567890	1234567890	1234567890	123456
<i>C. i. graphica</i>	1200200001	0011100010	2200102011	1110110111	1011010101	01201???4- 101100
<i>C. isolde</i>	1200000000	1011100010	2200102011	1110110111	1011010101	01201???- ????0
<i>C. k. kennicottii</i>	1200000000	0011000011	2200102011	1110110111	1011010101	11201???- ????0
<i>C. marginula</i>	1200000001	0011000010	2200102011	1110110111	1021010101	01201???- ????0
<i>C. mimica</i>	1200200000	0011000010	2200102011	1110110111	1011010101	01201???4- 000100
<i>C. nigrescens</i>	1211000000	0111000010	2200102011	1110110111	1011010101	01201???4- 001100
<i>C. panama</i>	1200000001	0011100010	2200102011	1110110111	1011010101	01201???- ????0
<i>C. quinquefasc</i>	1211000000	0111100010	2200102011	1110110111	1011010101	01201???4- 0???0
<i>C. rufopicta</i>	1200000000	1011100011	2200102011	1110110111	1011010101	012010014- 301100
<i>C. rybyensis</i>	1200000000	0011100010	2200102011	1110110111	1011010101	11201???2- -???0
<i>C. tepaneca</i>	1211000000	0111100010	2200102011	1110110111	1011010101	01201???4- 0?1?0
<i>C. vierecki</i>	1200000000	0011100011	2200102011	1110110111	1011010101	11201???- ????0
<i>C. zumpango</i>	12???00000	00???00011	22001020?1	1110110111	1011010101	?1201???- ????0
<i>Eu. angulata</i>	1100000000	0010000010	2200102111	1110110111	1111011101	01201???- ????0
<i>Eu. arenaria</i>	1100000001	0010000010	2200102101	1110110111	1111011101	01201???- ????0
<i>Eu. bitruncata</i>	1100000000	0010000011	2200102101	1110110111	1111011101	012010004- 0?0?0
<i>Eu. canaliculata</i>	1100000000	0010000011	2200102101	1110110111	1111011101	01201???- ????0
<i>Eu. conata</i>	1100000001	0010000011	2200102101	1110110111	1111011101	01201???- ????0
<i>Eu. cressoni</i>	1100000000	0010000010	2200102101	1110110111	1111011101	01201???4- 00110?
<i>Eu. flavocincta</i>	1100000000	0010000011	2200102101	1110110111	1111011101	012010004- 0?0???
<i>Eu. lacunosa</i>	1110000000	0010000011	2200102100	1110110111	1111011101	01201???- ????0
<i>Eu. melanovittata</i>	1100000000	0010000010	2200102101	1110110111	1111011101	01201???- ????0
<i>Eu. montana</i>	1100000000	0010000010	2200102111	1110110111	1111011101	01201???- ????0
<i>Eu. nevadensis</i>	1100000000	0010000010	2200102101	1110110111	1111011101	01201???- ????0
<i>Eu. pimarum</i>	1100000000	0010000010	2200102101	1110110111	1111011101	01201???4- 0???0
<i>Eu. provancheri</i>	1100000000	0010000010	2200102101	1110110111	1111011101	01201???- ????0
<i>Eu. similis</i>	1100000000	0010000010	2200102101	1110110111	1111011101	01201???- ????0
<i>Eu. superba</i>	1100000001	0010000010	2200102101	1110110111	1111011101	01201???4- 0???0

Table S5.1. continued.

	1	2	3	4	5	6
	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890
<i>Eu. tricolor</i>	1100000000	0010000010	2200102111	1110110111	1111011101	01201???- ??????
<i>Eu. vittatifrons</i>	1100000000	0010000010	2200102111	1110110111	1111011101	01201???- ??????
<i>Philanthinus</i>	1000010000	0011001000	1100120000	0011000000	0000000001	00010???- -????1
<i>Phil. albopilosus</i>	1000010100	000--01000	0100120000	0011000000	0000000000	00010???2- -11011
<i>Phil. barbatus</i>	1000010100	002--01000	0100120000	0011000000	0000000000	00010???2- -?1011
<i>Phil. barbiger</i>	1000010100	002--01000	0100120000	0011000000	0000000000	00010???2- -11011
<i>Phil. basalis</i>	1000010100	002--01000	0100120000	0011000000	0000000000	00010???2- -????1
<i>Phil. basilaris</i>	1000010110	002--01000	0100120000	0011000000	0000000000	00010???2- -11011
<i>Phil. binctus</i>	1000010110	002--01000	0100120000	0011000000	0000000000	000100102- -11011
<i>Phil. coarctatus</i>	1000010100	002--01000	0100120000	0011000000	0000000000	00010???- -????1
<i>Phil. crabronif</i>	1000010100	002--01000	0100120000	0011000000	0000000000	00010???2- -11011
<i>Phil. crotoniph</i>	1000010100	002--01000	0100120000	0011?00000	0000000000	00010???2- -????1
<i>Phil. gibbosus</i>	1000010100	002--01000	0100120000	0011000000	0000000000	000100102- -11011
<i>Phil. gloriosus</i>	1000010110	002--01000	0100120000	0011000000	0000000000	00010???- -????1
<i>Phil. inversus</i>	1000010100	002--01000	0100120000	0011000000	0000000000	00010???2- -01011
<i>Phil. melanderi</i>	1000010100	002--01000	0100120000	0011000000	0000000000	00010???- -????1
<i>Phil. multimagul</i>	1000010100	002--01000	0100120000	0011000000	0000000000	00010???2- -01011
<i>Phil. pacificus</i>	1000010100	002--01000	0100120000	0011000000	0000000000	00010???2- -10011
<i>Phil. pulchellus</i>	1000010100	002--01000	0100120000	0011000000	0000000000	000100102- -11011
<i>Phil. pulcher</i>	1000010100	002--01000	0100120000	0011000000	0000000000	00010???2- -10011
<i>Phil. triangulum</i>	1000010100	002--01000	0100120000	0011000000	0000000000	000100102- -&1011
<i>Phil. ventilabris</i>	10000-0100	002--01000	0100120000	0011000000	0000000000	00010???2- -11011
<i>Tr. boharti</i>	1000210100	002--11000	0100120000	0011000000	0000000000	000100102- -????1
<i>Tr. denticollis</i>	1000210100	002--11000	0100120000	0011000000	0000000000	000100102- -??0?1
<i>Tr. elongatus</i>	1000210100	002--11000	0100120000	0011000000	0000000000	000100102- -????1
<i>Tr. sp. Nicaragua</i>	1000210100	002--11000	0100120000	0011000000	0000000000	00010010?- -????1
<i>Tr. sp. Peru</i>	1000210100	002--11000	0100120000	0011000000	0000000000	00010010?- -????1
<i>Pseudoscobia</i>	10000-10&0	00100011&0	2000100000	01111110100	0001010&00	00000011% -&00??

APPENDIX D:

**SUPPLEMENTARY MATERIAL FOR CHAPTER VI:
THE HOUSEPLAN AS *BAUPLAN* PART I: A PRELIMINARY INVESTIGATION OF
NEST EVOLUTION IN SPHECID WASPS (APOIDEA: SPHECIDAE S. STR.)**

Table S6.1. Behavioral character matrix used in Chapter VI. Polymorphisms are designated as follows: [01] = &; [12] = @; [13] = %; [02] = #.

			111	1111
	1234	5678	9012	3456
<i>Dolichurus corniculus</i>	0000	00-0	0000	0000
<i>Ampulex denticollis</i>	0000	00-0	0000	0000
<i>Ampulex compressa</i>	0010	00-0	0000	0000
<i>Ammophila azteca</i>	1101	00-0	1020	2101
<i>Ammophila dysmica</i>	1100	00-0	1020	1101
<i>Ammophila harti</i>	1101	00-0	1020	1101
<i>Eremnophila aureonotata</i>	1100	00-0	1020	1101
<i>Eremnophila binodis</i>	1100	00-0	1020	1101
<i>Eremnophila opulenta</i>	1100	00-0	1020	1101
<i>Eremochares dives</i>	1101	00-0	1210	2001
<i>Hoplammophila aemulans</i>	0110	%#-0	1120	11?1
<i>Parapsammophila erythrocephala</i>	1100	00-0	1020	1101
<i>Podalonia atriceps</i>	1000	00-0	0020	1101
<i>Chlorion aerarium</i>	1110	00-0	1010	1000
<i>Chlorion cyaneum</i>	1110	10-0	1000	1001
<i>Chlorion lobatum</i>	0000	0--0	0010	--0?
<i>Chlorion maxillosum</i>	0000	0--0	0011	---?
<i>Stangeella cyaniventris</i>	1110	10-0	1040	2????
<i>Dynatus nigripes</i>	0100	02-0	020?	2011
<i>Penepodium gorianum</i>	0100	02-1	020?	??21
<i>Penepodium latro</i>	0110	02-1	0101	??21
<i>Penepodium haematogastrum</i>	1100	02-0	0101	2021
<i>Penepodium luteipenne</i>	1100	02-0	0101	@121
<i>Podium denticulatum</i>	0110	32-1	020&	201?
<i>Podium luctuosum</i>	0110	10-1	020?	????
<i>Podium fulvipes</i>	0100	0#-0	020?	???0
<i>Podium rufipes</i>	0100	0#-1	0200	2010
<i>Trigonopsis cameronii</i>	2111	32-0	0101	2021
<i>Trigonopsis rufiventris</i>	211?	32-0	010?	21?1
<i>Chalybion californicum</i>	01?0	32-0	0130	2011
<i>Chalybion japonicum</i>	0110	32-2	0130	2011
<i>Chalybion spinolae</i>	1100	32-0	0130	2011
<i>Chalybion tibiale</i>	0110	32-2	0130	2011
<i>Chalybion zimmermanni</i>	0110	32-2	0230	2011
<i>Sceliphron asiaticum</i>	2110	32-0	0230	2011
<i>Sceliphron assimile</i>	2110	32-0	0230	2011
<i>Sceliphron caementarium</i>	2110	32-0	0230	2011
<i>Sceliphron curvatum</i>	2110	32-0	0230	2011
<i>Sceliphron spirifex</i>	2110	32-0	0230	2011
<i>Isodontia auripes</i>	0130	2100	1010	2000
<i>Isodontia costipennis</i>	0120	2110	1010	200?
<i>Isodontia diodon</i>	0110	2110	1000	2001

<i>Isodontia elegans</i>	0110	2100	1010	2001
<i>Isodontia mexicana</i>	0120	2100	1010	2001
<i>Isodontia pelopoeiformis</i>	0120	2&&0	1010	2001
<i>Isodontia simoni</i>	0110	2110	1010	2001

Table S6.1. continued.

	111		1111	
	1234	5678	9012	3456
<i>Isodontia stanleyi</i>	0110	2100	1010	2001
<i>Sphex argentatus</i>	1110	10-0	1010	2001
<i>Sphex cognatus</i>	1110	10-0	1010	2001
<i>Sphex ichneumoneus</i>	1110	10-0	1010	200?
<i>Sphex jamaicensis</i>	1101	10-0	1010	200?
<i>Sphex lucae</i>	1100	00-0	1010	2000
<i>Sphex opacus</i>	1110	10-0	1010	2001
<i>Sphex tepanecus</i>	1110	10-0	1010	2000
<i>Chilosphex argyrius</i>	0100	2110	0010	1000
<i>Palmodes carbo</i>	1100	00-0	1010	1001
<i>Palmodes dimidiatus</i>	1100	00-0	1010	1001
<i>Palmodes laeviventris</i>	1110	00-0	0010	1001
<i>Palmodes occitanicus</i>	1100	00-0	0010	1001
<i>Prionyx atratus</i>	1000	00-0	0010	1001
<i>Prionyx bifoveolatus</i>	1110	10-0	1010	1001
<i>Prionyx chilensis</i>	1110	10-0	1010	2001
<i>Prionyx crudelis</i>	1000	00-0	0010	1001
<i>Prionyx fervens</i>	1000	00-0	0010	1001
<i>Prionyx kirbii</i>	1100	00-0	1010	1001
<i>Prionyx parkeri</i>	1000	00-0	0010	1001
<i>Prionyx subfuscatus</i>	1000	00-0	0010	1001
<i>Prionyx thomae</i>	1000	00-0	0010	1001

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On Jun 12, 2014, at 7:55 AM, Ansel Payne wrote:

To whom it may concern:

I'll soon be completing a doctoral dissertation at the Richard Gilder Graduate School at the American Museum of Natural History entitled: "Phylogenetic studies of apoid wasps (Hymenoptera: Apoidea) with insights into the evolution of complex behaviors," with an anticipated publication date of June 2014. I'm writing to request permission to reprint in my dissertation excerpts from the following or the following in its entirety as originally published:

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Sincerely,

Ansel Payne

CURRICULUM VITAE

ANSEL PAYNE

Current address: 1306 Greenway Rd, Charlottesville, VA 22903

Education

- **M.S. Biology**, Tufts University, 2011. "Further aspects of the reproductive biology of the European wool-carder bee, *Anthidium manicatum*." Advisor: Dr. Philip T. Starks.
- **B.A. Biology**, Harvard University, 2004.

Research interests

Behavioral evolution and systematics of apoid wasps, theory of systematics, theory of recognition systems, the public understanding of science.

Peer-reviewed publications

- **Payne, Ansel**. 2014. "Resolving the Relationships of Apid Bees (Hymenoptera: Apidae) Through a Direct Optimization Sensitivity Analysis of Molecular, Morphological, and Behavioral Characters." *Cladistics* 30: 11-25.
- **Payne, Ansel**, Phillip M. Barden, Ward C. Wheeler, and James M. Carpenter. 2013. "Direct Optimization, Sensitivity Analysis, and the Evolution of the Hymenopteran Superfamilies." *American Museum Novitates* 3789: 1-20
- Brown, Ronald, **Ansel Payne**, Kelsey K. Graham, and Philip T. Starks. 2012. "Prey Capture and Caste-Specific Payload Capacities in the European Paper Wasp *Polistes dominulus* (Hymenoptera: Vespidae)." *Insectes sociaux* 59: 519-525.
- Rosenfeld, Jeffrey A., **Ansel Payne**, and Rob DeSalle. 2012. "Random Roots and Lineage Sorting." *Molecular Phylogenetics and Evolution* 64: 12-20.
- **Payne, Ansel**, Dustin A. Schildroth, and Philip T. Starks. 2011. "Nest Site Selection in the European Wool-Carder Bee, *Anthidium manicatum*, with Methods for an Emerging Model Species." *Apidologie* 42: 181-191.
- **Payne, Ansel**, and Philip T. Starks. 2010. "Recognition Systems in the Social Insects." In *The Encyclopedia of Animal Behavior*, volume III, edited by Michael D. Breed and Janice Moore. Oxford Academic Press.
- **Payne, Ansel**, Aviva E. Liebert, and Philip T. Starks. 2010. "Kin Recognition and Genetics." In *The Encyclopedia of Animal Behavior*, volume II, edited by Michael D. Breed and Janice Moore. Oxford Academic Press.

Funding and awards

- Theodore Roosevelt Memorial Grant (\$2,500). 2013. "The Evolution of Prey Choice in North American Beewolf Wasps and Their Relatives (Hymenoptera: Philanthinae)."
- Willi Hennig Society Marie Stopes Student Travel Award (\$500). 2012. Entomological Society of America (Systematics, Evolution, and Biodiversity Section) Student Travel Award (\$1,000). 2012. "Tracing the Evolution of Hunting Behavior Through a Total Evidence Phylogeny of the Philanthine Wasps (Apoidea: Crabronidae: Philanthinae)."
- Theodore Roosevelt Memorial Grant (\$1,700). 2011. "House Plan as *Bauplan*: The Evolution of Nest Architecture in Sphecid Wasps (Hymenoptera: Sphecidae s. str.)."
- Tufts University Graduate Student Research Award (\$410). 2010. "Further Studies of Nest Site Selection in the European Wool-Carder Bee."
- Tufts University Graduate Student Travel Fund (\$200). 2008.

Selected talks and posters

- 2012 Annual Meeting of the Willi Hennig Society: "Direct Optimization Sensitivity Analysis, and the Evolution of the Hymenopteran Superfamilies." Contributed talk.
- 2012 Israel Taxonomy Initiative Bee Taxonomy Workshop: "The *Anthidium* Condominium: A Case Study in Enclosure-Based Behavioral Ecology." Invited talk.
- 2012 Israel Taxonomy Initiative Bee Taxonomy Workshop: "Phylogenetic Methods and Uncertainty: Using Sensitivity Analysis to Explore the Evolutionary Relationships of Bees." Invited Talks
- 2011 Annual Meeting of the Entomological Society of America: "Resolving the Relationships of Apid Bees Through the Simultaneous Analysis of Molecular, Morphological, and Behavioral Characters." Student competition poster.
- 2011 Joint Conference of the Animal Behavior Society and the International Ethological Congress: "Nest Site Selection in the Wool-Carder Bee *Anthidium manicatum*." Contributed poster.
- 2011 Annual Richard Gilder Graduate School Symposium: "Sensitivity Analysis and the Evolution of the Hymenopteran Superfamilies." Invited talk.
- 2009. Tufts University Biology Department Seminar Series: "Morphological Plasticity Among Invasive Paper Wasps (*Polistes versicolor*) on the Galápagos Islands." Invited talk.

Teaching positions and courses taught

- Teaching assistant and lecturer, Systematics and Biogeography, Richard Gilder Graduate School, AMNH, fall 2012.
- Teaching assistant, Israel Taxonomy Initiative Bee Taxonomy Workshop, Tel Aviv University, 2012.
- Teaching assistant and lecturer, Insect Taxonomy, Richard Gilder Graduate School, AMNH, spring 2012.

- Teaching assistant, Biology: Cells and Organisms, Tufts University, fall 2008, fall 2009.
- Teaching assistant, Biology: Organisms and Populations, Tufts University, spring 2009, spring 2010.
- Graduate writing consultant, Tufts University, 2009-2010.
- Guest lecturer, Animal Behavior, Tufts University, spring 2009.
- Graduate mentor, NSF REU program, Tufts University, summer 2009, summer 2010.

Other research and work experience

- Staff science teacher, Calhoun County High School, Mt. Zion WV and Roane County High School, Spencer WV, 2006-2008.
- Microbiologist and diagnostic immunologist, West Virginia Department of Health and Human Resources, 2004-2006.
- Research assistant, Cavanaugh Lab at Harvard University, 2002-2004.

Field experience

- Field work in Dominican Republic (2004), Arizona and New Mexico (2010, 2011, 2012), the central Appalachians (2011), Israel (2012), Mexico (2012), California (2012, 2013), Nicaragua (2013), Colorado (2013), Oregon (2013), Utah (2013) Wyoming (2013)
- The Bee Course. AMNH Southwestern Research Station. Portal, AZ. 2010.

Professional affiliations and professional service

- Linnean Society of London, since 2011 (**Fellow, 2011-present**)
- Cambridge Entomological Club, since 2008 (**vice president, 2009-2010**)
- International Society of Hymenopterists, since 2012
- Willi Hennig Society, since 2012
- West Virginia Entomological Society, since 2005
- Referee for *Ethology*, *Mitochondrial DNA*.